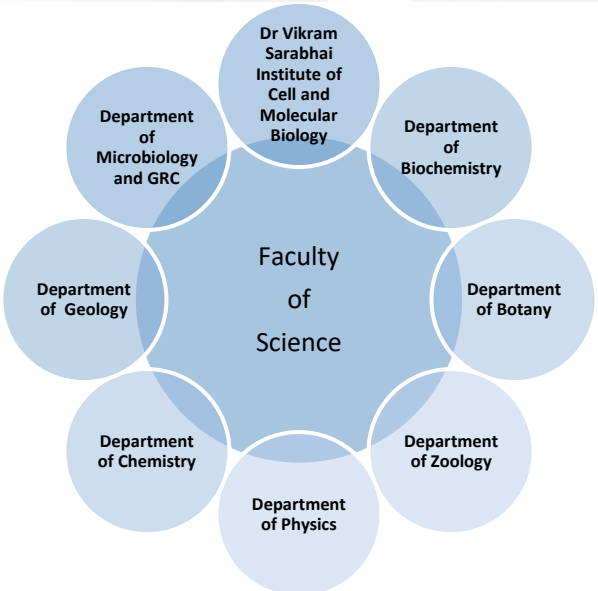
CIF-FoS-MSU

Central Instrumentation Facility of the Faculty of Science

Standard Operating Procedures (SOPs)



Faculty of Science
The Maharaja Sayajirao University of Baroda
Vadodara-390 002, Gujarat, India

List of Instruments			
S No	**Instruments**	**Funding Agency**	**Page**
	Dr Vikram Sarabhai Institute of Cell and Molecular Biology		
1	QuantStudio 12K Flex Real Time PCR System	DBT - MSUBILSPARE	
2	Beckman Coulter Optima L-100XP Ultracentrifuge	DBT - MSUBILSPARE	
3	Zeiss LSM 710 Confocal Microscope	DBT - MSUBILSPARE	
4	BD FACS Aria III Flow Cytometry System	DBT - MSUBILSPARE	
5	AB Sciex 3200 QTRAP LC-MS/MS System	DBT - MSUBILSPARE	
6	Sykam S500 or S600 HPLC System	DBT - MSUBILSPARE	
7	Protean i12 Isoelectric Focusing System	DBT - MSUBILSPARE	
8	Zeiss Axio Observer A1 Fluorescence Inverted Microscope	DBT - MSUBILSPARE	
9	VilberLourmat Fusion SL 2 400 Gel and Blot Documentation System	GOG	
10	Birthhold Tristar² Multimode plate Reader	DBT - MSUBILSPARE	
11	Cell Culture Facility	DBT - MSUBILSPARE	
	Department of Biochemistry		
12	Ettan IPGphor 3 Isoelectric Focusing System	UGC DRS-I	
13	Nikon Eclipse Ti-2E Inverted Fluorescent Microscope for Live-Cell Imaging	DST-FIST	
14	Bio-Rad QX200 Droplet Digital PCR System	DST-FIST	
15	Labconco FreeZone Benchtop Freeze Dry System		
16	Thermo Scientific UltiMate 3000 Basic Manual HPLC System	UGC DRS-I	
17	BioTek Synergy HTX Multi-Mode Microplate Reader	DST-FIST	
	Department of Botany		
18	Shimadzu LC 20AT HPLC System	DST-FIST	
19	Shimadzu GC-2010 Gas Chromatography	DST-FIST	
20	UVP GelDoc-It2 Imaging System	DST-FIST	
	Department of Microbiology & Bharat Chattoo Genome Research Centre		
21	Fermentor with Accessories (BiosatB)	DBT-AJD	
22	Waters High Performance Liquid Chromatography	UGC-DRS	
23	High Speed Refrigerated Centrifuge (Kubota)	DST-FIST (P-II)	
24	Gel Documentation System (Alphaimager-HP)	DST-FIST (P-II)	
25	New Brunswick Scientific Incubator Shaker	DST-FIST (P-II)	
26	Real time PCR, step one cycler applied biosystems	UGC-DRS	
27	Refrigerated shaking incubator- Lab Tech	UGC XII PLAN	
28	UV-1800 Shimadzu Spectrophotometer	DST-FIST (P-III)	
29	Gas Chromatography Shimadzu	UGC-DRS	
30	Real time PCR, Agilent Technologies	DST-FIST (P-III)	
31	Zeiss Confocal Microscope LSM 700	DBT	
32	Fast Real-Time PCR System, Applied Biosystems	DBT	
33	BioTek Synergy HT Multimode Microplate Reader	DBT	
34	Beckman Coulter Optima Max-XP ultracentrifuge	DBT	
35	Nikon Eclipse 80i Fluorescence Microscope	DBT	
36	Nikon Eclipse TS100 Inverted Microscope	DBT	
37	Affymetrix GeneChip Fluidics Station 450	GSBTM	
38	Beckman-Coulter DNA Sequencer CEQ8000	DBT	
	Department of Zoology		
39	Cryocut Cold microtome	COSIST	
	Department of Geology		
40	Rock section cutting machine	DST FIST	
41	Stereozoom Microscope; Model- Nikon SMZ	DST PURSE	

42	SediGraph III 5120	DST FIST	
43	Scanning Electron Microscope; Model- Hitachi SU1510	DST FIST	
44	Energy-dispersive X-ray spectroscopy (EDS)	DST PURSE	
45	Ground Penetrating Radar (GSSI); Model – SIR-20	DST	
46	AGICO MFK1B Kappa bridge	DST-FIST	
	Department of Physics		
47	Impedance /Gain Phase Analyzer 1260- solartrom	Cosist Prog	
48	2182 Nanovoltmeter-Keithly 220E programmable current	Cosist Prog.	
49	FTIR-4100 spectrometer with spectra manager	DRS-III	
50	Thermal Analysis system.(DSC)	DRS-III	
51	Kithley Electrometer	DST Project	
52	LCR Meter	DST Project	
53	High Performance Computer Cluster HPCL	DST FIST	
54	High Performance Computer Cluster	SERB Project	
55	Raman Spectrophotometer	DST PURSE	
	Department of Chemistry		
1	Nuclear Magnetic Spectrophotometer,BRUKER Avance III 400MHz	DST-FIST I	
2	IR Spectro-photometer(FTIR), Bruker Alpha AXT	XII Plan	
3	Thermal Analyser TG/DTA, SIINT - TG-DTA 6300	UGC CAS	
4	Gas Chromatograph-Mass Spectrometer, Thermofisher, Thermo Trace DSQII GC-MS	UGC CAS I	
5	Liquid Cryogenic Plant StirLITE	XI Plan	
6	Single Crystal X-ray Diffractometer system, Agilent *Xcalibur CCD area Detector*	DST-PURSE	
7	Polarising Microscope with Hot Stage, LEICA- DM 2500P	DST-FIST I	
8	Differential Scanning Calorimeter, Mettler Toledo - *DSC 822*	UGC DSAIII	
9	Atomic Absorption Spectrophotometer, Perkin Elmer Analyst 90 Plus	DST Project	
10	BET Surface Area and Porosity Analyser	DST-FIST I	

QuantStudio 12K Flex Real Time PCR System

Name of the facility / activity: Real Time PCR

Instrument Incharge: Technical Assistant

Department: Dr Vikram Sarabhai Institute of Cell and Molecular Biology

Name, Model of Instrument: QuantStudio 12 K Flex

Software name: Quant Studio software

Introduction & Principle: In real-time polymerase chain reaction we can monitors the amplification of a targeted DNA molecule during the PCR, not at its end, as in conventional PCR. In real-time PCR, the amount of DNA is measured after each cycle via fluorescent dyes that yield increasing fluorescent signal in direct proportion to the number of PCR product molecules (amplicons) generated. Using sequence-specific primers, the number of copies of a particular DNA sequence can be determined. Quantification of amplified product is obtained using fluorescent probes or fluorescent DNA-binding dyes and real-time PCR instruments that measure fluorescence while performing the thermal cycling needed for the PCR reaction.
In Real Time PCR we can measure the amount of amplified product at each stage during the PCR cycle, quantification is possible. If a particular sequence (DNA or RNA) is abundant in the sample, amplification is observed in earlier cycles; if the sequence is scarce, amplification is observed in later cycles.

Applications: Real-time polymerase chain reaction (real-time PCR) is commonly used to measure gene expression. In field of oncology, gene therapy, microbiology it has become the standard method for disease detection. Real-time PCR has been shown to be extremely useful for quantification of viruses and bacteria.

Calibration status: Yearly done by both operator and service engineer

Requirement of Sample preparation: Chemistry of experiment plates should be match with instrument block. Sample should be properly sealed with adhesive film. No air bubbles in samples.

Procedure for instrument startup:

- Room temperature should not be more than 20 °C.
- Switch on the Power of instrument, computer.
- After instrument start open software.
- Feed all information according steps.
- Start the experiment.
- After complete run export data in excel & PDF file.

Do's and Don'ts:
Beware with pipetting errors. Plate should not touch from bottom side. Prepare Sample in sterile condition on Ice pack or on below temp 4 and in dark place.

Operating schedule: (gap b/w two experiments or time being experiment): At least 1hour
Data collection / analysis: Excel file and PDF file provided to users.

Documentation: Maintaining complete records for the calibration and performance checks of all equipment and instruments.

QuantStudio 12K Flex Real Time PCR System

Beckman Coulter Optima L-100XP Ultracentrifuge

Name of the facility / activity: Ultracentrifuge

Instrument Incharge: Technical Assistant

Department: Dr Vikram Sarabhai Institute of Cell and Molecular Biology

Name, Model of Instrument: – Optima L 100 XP – Backman Coulter

Software name: NA

Introduction & Principle: Ultracentrifugation is best tool for Biochemical research. Through Ultracentrifugation rapid spinning imposes high centrifugation force on suspended particles or even molecules in solution and causes separation of such matter on the basis of differences in weight.
Applications: Ultracentrifuge is used for sedimentation of several cellular, subcellular and nano- formulations. All the operations of handling the sample filled tubes, rotors, startup, program setting, ending of centrifuge and removal of the tubes from rotors

Calibration status: NA

Procedure for instrument startup:

1. There are 2 swinging rotors Sw-Ti-60 and Sw-Ti-90 which run at maximum of 60,000.RPM and 90,000 RPM respectively.
2. There are two fixed angle rotors which are 40Ti and 70Ti which can run upto maximum speed of 40,000 RPM and 70,000 RPM respectively.
3. Ultracentrifuge will be operated only if the sample has to be spun $\geq$ 30000 RPM or 1 lakh g force.
4. Respective allocated tubes should be only used during ultracentrifugation. The details of the tubes required for use can be obtained from the operators or from Beckman coulter website.
5. The tubes should be filled full upto the Beckman Coulter mark embossed on the tube. If full volume cannot be filled then either sample has to be diluted or mineral oil should be overlaid and the tube should be filled upto the mark mentioned on the tube.
6. All the buckets should be kept in the all the rotors. Tubes should be of equal volume and should be balanced with the opposite tubes. Respective numbered buckets should be hanged in respective rotors only.
7. Utmost care should be taken while putting the tubes, buckets, rotors in the centrifuge.

Do's and Don'ts: As this centrifuge requires 10KVA power no other heavy instruments like FACS ARIA III or Confocal microscope should be switched on as all the equipments could be at risk. Before starting the machine, power and UPS system should be checked. If rotors are needed to be externally cooled, operators should be reminded one day prior the operation of the centrifuge. Users should proper discard their samples and clean the working bench before leaving the working space. Proper usage entries should be made in the Log Books. Both user and operator should sign and proper remarks should be mentioned by the operator in the log book. No chemicals and consumables will be given from the institutes. Users should make their own arrangements.
Operating schedule: According schedule

Caution: Proper placement of tubes, buckets, rotors into the centrifuge. Vacuum should be checked.

Shut down procedure: Proper shutdown procedure should be followed.

Data collection/ analysis: According to sample

Documentation: Maintaining complete records for the log book, Requisition form and performance checks of all equipment and instruments.

Beckman Coulter Optima L-100XP Ultracentrifuge

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Zeiss LSM 710 Confocal Microscope

Name of the facility / activity: Confocal Microscope

Instrument Incharge: Technical Assistant

Department: Dr Vikram Sarabhai Institute of Cell and Molecular Biology

Name, Model of Instrument: LSM 710 – Carl Zeiss

Software name: ZEN 10

Introduction &Principle: Similar to the wide field microscope, the confocal microscope uses fluorescence optics. Instead of illuminating the whole sample at once, laser light is focused onto a defined spot at a specific depth within the sample. This leads to the emission of fluorescent light at exactly this point. A pinhole inside the optical pathway cuts off signals that are out of focus, thus allowing only the fluorescence signals from the illuminated spot to enter the light detector.

Applications: Protein localization, Protein protein interaction, 3D imaging, Z stack, co localization

Calibration status: Calibrated by service engineers annually

Requirement of Sample preparation: 1mmx1mm thick glass coverslip of glass bottom dish. Mounting, staining or antibody should be properly stained in dark. Excitation and emission of the fluorochrome used be known. Animal or plant tissue sections should preferably not more than 20um.

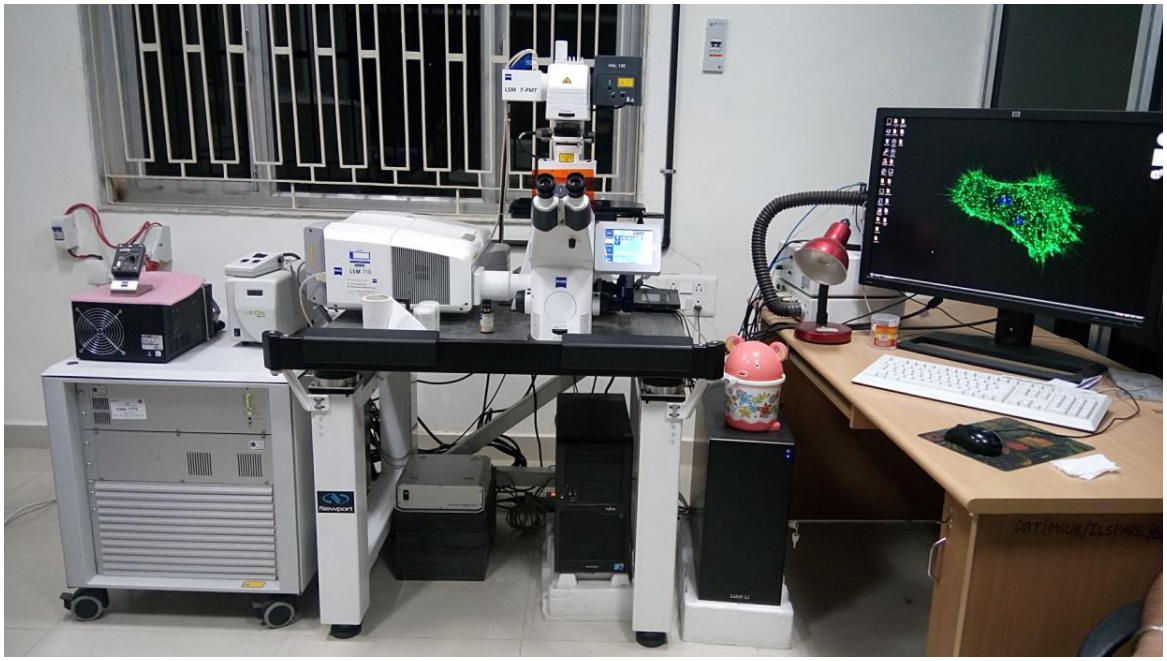
Procedure for instrument start up:
Instrument should be on by switching on all power buttons and laser powers. Software should be on and instrument would be undergoing initialization step. After the system gets auto checked and stabilized it should be operated.

- This confocal microscope can resolve the sample through glass bottom 1mmX1mm thick cover slip only. So specified dimension cover slips or dishes with this thickness should only be used.
- Mounting media should properly be added to protect the cells/ tissues from drying or spreading of the fluorescence.
- Users should provide proper information regarding the fluorochrome used with their excitation and emission range.
- For animal tissues, plant tissues or other thick sections, it is highly recommended that the section should be around 20um or less thick for proper resolution.
- If CO_2 incubator or CO_2 cylinder are required for live cell imaging then it has to be informed 3-4 days in advance as it may take time to get them functional and available to the user.

Do's and Don'ts: Confocal microscope will only be operated by the operator. Experiments should be discussed properly with the operator for successful acquisition of the images of the samples. User should specifically mention number of sections to be visualized. If sample acquisition takes longer time then, the users are requested to take another appointment in order to continue visualizing the slides. No two appointments will be clubbed or no other individual's samples would be acquired on any other person's appointment or extra samples would not be acquired. Users should discard their coverslips, slides, samples etc. properly before leaving the room. Data will be given in CD or DVD.

Operating schedule: (gap b/w two experiments or time being experiment): 4-6 hours continuous. Fluroscence Lamp and Lasers should not be switched off at small intervals.

Data collection / analysis: Data provide to users in CD
Documentation: Maintaining complete records for all equipment and instruments.



Zeiss LSM 710 Confocal Microscope

BD FACS Aria III Flow Cytometry System

Name of the facility / activity: Fluorescence Activated Cell Sorter FACS

Instrument Incharge: Technical Assistant

Department: Dr Vikram Sarabhai Institute of Cell and Molecular Biology

Name, Model of Instrument: FACS ARIA III – BD Bioscience

Software name: Diva

Introduction &Principle: The basic principle of flow cytometry is the passage of cells in single file in front of a laser so they can be detected, counted and sorted. Cell components are fluorescently labelled and then excited by the laser to emit light at varying wavelengths detected by the detectors.

Applications:

- Cell counting.
- Cell sorting.
- Determining cell characteristics and function.
- Detecting microorganisms.
- Biomarker detection.
- Diagnosis of health disorders such as blood cancers.

Calibration status: Every 15 days and by Service engineer every 3 months

Requirement of Sample preparation: Experiments should be discussed properly with the operator for successful acquisition of the samples.

Procedure for instrument startup:
Power should be on. Instrument and Software should be configured. Fluidics startup needs to be done. Lasers should be on and stabilized. Stream should be stabilized.

Minimum 0.5 to 1 million cells per tube are advisable to be present in the sample f.or acquisition of 10,000 events. Samples specifically animal tissues, plant tissues, cells should be properly digested and single cell suspension should be prepared. The processed samples should be passed through specific dimension cell strainers like 40uM, 70uM etc.

Minimum 350uL volume of sample should be there in each tube. If samples are not clear and if debris damage the instrument, immediately sample acquisition on FACS ARIA III would be stopped and no such samples would be further acquired. Also if tubes have extremely low number of cells and acquisition takes longer time then such samples would not also be continued acquiring.

If samples are hazardous and contagious, then operator should also be informed and gloves and masks (if required) should be worn both by the user and operator while handling the samples.

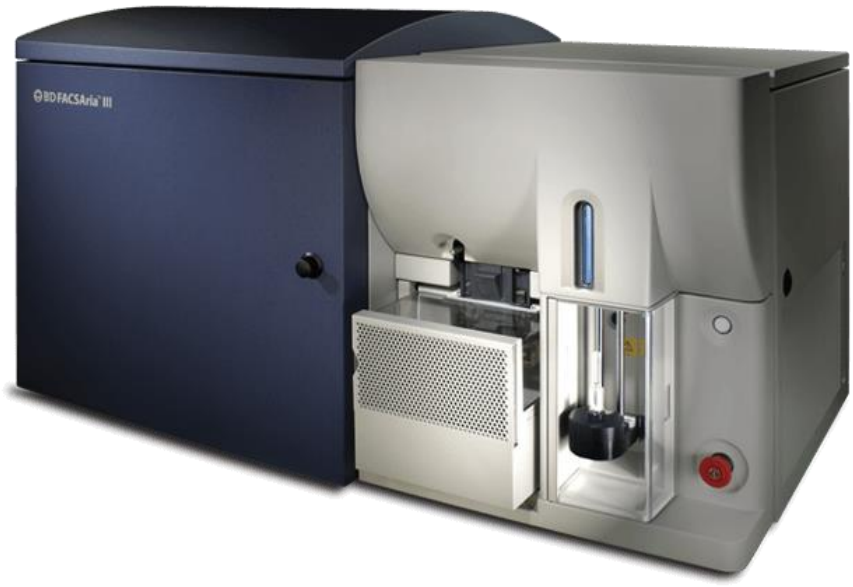
For sorting the samples, operator should be informed very well in advance. For sorting number of cells required for sorting the cells would depend on the % of population present and intensity of the fluorochrome through which sample would be sorted. As machine has to be specifically configured for sorting, operator would require specific time and schedule to configure FACS ARIA III.

Do's and Don'ts: FACS ARIA III will be only operated by the operator. Number of fluorochromes, number of samples and number of events per sample to be acquired should be informed to the operator prior to the experiments. Users should proper discard their samples and clean the working bench before leaving the working space. Samples should be passed through strainer properly because it may be block the instrument.

Operating schedule: (gap b/w two experiments or time being experiment):

Data collection/ analysis: Data will be provided to users in CD.

Documentation: Maintaining complete records for all equipment and instruments.



BD FACS Aria III Flow Cytometry System

AB Sciex 3200 QTRAP LC-MS/MS System

Name of the facility / activity: LC–MS/MS

Instrument In charge: Technical Assistant

Department: Dr Vikram Sarabhai Institute of Cell and Molecular Biology

Name, Model of Instrument: QTRAP 3200 - ABSciex

Software name: Analyst software

Introduction &Principle: Liquid Chromatography with tandem mass spectrometry (LC-MS-MS) is a powerful analytical technique that combines the separating power of liquid chromatography with the highly sensitive and selective mass analysis capability of triple quadrupole mass spectrometry.

Applications: The 3200 series of instruments are best suited to three specific applications:

- Quantitative analysis of small molecules: This application involves measurement of specific molecular weight compounds, such as a drug or metabolite in a liquid sample, and their resulting fragment ions for determining the exact quantity of the compound in the sample of interest.
- Qualitative analysis of small molecules: This application involves MS analysis of low molecular weight (typically less than 1000 Da) compounds. This is for the purpose of identification and structural characterization of the chemical compounds in a liquid sample.
- Qualitative analysis of proteins and peptides: This is for the purposes of identification of the compounds or for structural characterization. Qualitative analysis of proteins and peptides is used when the researcher wants to know what molecular weight species and what sequences of peptides or proteins are in the sample.

Calibration status: As per requirement by service engineers

Requirement of Sample preparation: As per your protocol or developed method.

Procedure for instrument startup: Turn on the roughing pump, if it was turned off.

Make sure that all gas supplies are flowing correctly to the system

Turn on the mass spectrometer switch & Turn on the computer, if it was turned off, and then start the Analyst software.

The Instrument Status lights indicate the status of the mass spectrometer vacuum. When the operational vacuum conditions are satisfied and the mass spectrometer is in analysis mode, the Ready light (green) is illuminated and the Fault light (red) is extinguished

Create a hardware profile, Create projects to store data & optimize the mass spectrometer.
To analyze samples, create an acquisition method for the mass spectrometer and any LC devices. An acquisition method indicates which peripheral devices to use, when to use them to acquire data, and the associated parameters.

After creating an acquisition method, run samples by creating an acquisition batch and submitting the batch to the Acquisition Queue.

Acquire data:
In Explore mode, many tools are available for viewing and processing the acquired data. Graphs can be customized with peak labels and captions, contour plots can be displayed.

Do's and Don'ts: Always wear clean, powder-free gloves for the cleaning procedures.

Do not use cleaning supplies other than those specified in this procedure.

Prepare cleaning solutions just before beginning the procedure, if possible.

Prepare and store all organic solutions and organic-containing solutions in very clean glassware only. Never use plastic squirt bottles. Contaminants can leach from these bottles and further contaminate the mass spectrometer.

To avoid contaminating the solution, pour the solution on the wipe or swab.

Only dampen the wipe or swab slightly when applying water or cleaning solution. Water, more so than organic solvents, may cause the wipe to deteriorate, leaving residue on the mass spectrometer.

Operating schedule: (gap b/w two experiments or time being experiment): According to prior appointment.

Data collection/ analysis: Data will be provided to users in CD.

Documentation: Maintain complete records for all equipment and instruments.

AB Sciex 3200 QTRAP LC-MS/MS System

Sykam S600 HPLC System

Name of the facility / activity: HPLC (High Performance Liquid Chromatography)

Instrument Incharge: Technical Assistant

Department: Dr Vikram Sarabhai Institute of Cell and Molecular Biology

Name, Model of Instrument: Sykam HPLC System

Software name: Data system clarity chromatography software

Introduction & Principle: High Performance Liquid Chromatography (HPLC) is a form of liquid chromatography to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by injecting a plug of the sample mixture onto the column. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase. Instrumentation

Applications: Solvents must be degassed to eliminate formation of bubbles. The pumps provide a steady high pressure with no pulsating, and can be programmed to vary the composition of the solvent during the course of the separation. The liquid sample is introduced into a sample loop of an injector with a syringe. When the loop is filled, the injector can be inject the sample into the stream by placing the sample loop in line with the mobile phase tubing. The presence of analytes in the column effluent is recorded by detecting a change in refractive index, UV-VIS absorption at a set wavelength, fluorescence after excitation with a suitable wavelength, or electrochemical response.

Calibration status: As per requirement by service engineers

Requirement of Sample preparation: As per your protocol or developed method.

Procedure for instrument startup:

Do's and Don'ts: Always wear clean, powder-free gloves for the cleaning procedures.

Do not use cleaning supplies other than those specified in this procedure.

Prepare cleaning solutions just before beginning the procedure, if possible.

Prepare and store all organic solutions and organic-containing solutions in very clean glassware only. Never use plastic squirt bottles. Contaminants can leach from these bottles and further contaminate the mass spectrometer.

Operating schedule: (gap b/w two experiments or time being experiment): According to prior appointment.

Data collection / analysis: Data will be provided to users in CD.

Documentation: Maintain complete records for all equipment and instruments.

Sykam S600 HPLC System

Protean i12 Isoelectric Focusing System

Name of the facility/ activity: 2 D Gel Electrophoresis

Instrument Incharge: Technical Assistant

Department: Dr Vikram Sarabhai Institute of Cell and Molecular Biology

Name, Model of Instrument: Protean i12 IEF - Bio Rad

Software name: PD Quest software

Introduction & Principle: Two dimensional gel electrophoresis is the primary technique for proteomics work. It separates the complex mixture of two different proteins using two different properties. In the first dimension proteins are separated on the basis of their charge (isoelectric focusing, IEF) and then by relative molecular weight (MW). A 2D-PAGE gel image is captured and image analysis is done to find the number of proteins expressed in a particular tissue.

Applications: 2 D Gel electrophoresis is a powerful and widely used method for the analysis of complex protein mixtures extracted from cells, tissues or other biological samples. It is the method available which is capable of simultaneously separating thousands of proteins.

First dimension is isoelectric focusing (IEF), which separates proteins according to their isoelectric points (pl). Second dimension is SDS-polyacrylamide gel electrophoresis (SDS-PAGE) which separates proteins according to their molecular weight (MW).

Calibration status: As per requirement by service engineers

Requirement of Sample preparation: As per protocol.

Procedure for instrument startup: steps for 2D Gel electrophoresis

1. sample preparation
2. Isoelectric focusing (first dimension)
3. SDS-PAGE (second dimension)
4. Visualization of proteins spots
5. Identification of protein spots

Do's and Don'ts: Always wear clean, powder-free gloves for the cleaning procedures.
Do not use cleaning supplies other than those specified in this procedure.
Prepare cleaning solutions just before beginning the procedure, if possible.
Prepare and store all organic solutions and organic-containing solutions in very clean glassware only. Never use plastic squirt bottles. Contaminants can leach from these bottles and further contaminate the mass spectrometer.

Operating schedule: (gap b/w two experiments or time being experiment): According to prior appointment.

Data collection/ analysis: Data will be provided to users in CD.

Documentation: Maintain complete records for all equipment and instruments.

PROTEAN i12 IEF system

Zeiss Axio Observer A1 Fluorescence Inverted Microscope

Name of the facility / activity: Fluorescence Microscope

Instrument Incharge: Technical Assistant

Department: Dr Vikram Sarabhai Institute of Cell and Molecular Biology

Name, Model of Instrument: Axio Observer A1-Carl Zeiss

Software name: ProgRes capture Pro v2.8.0

Introduction & Principle: Fluorescence describes a phenomenon where light is emitted by an atom or molecule that has absorbed light or electromagnetic radiation from another source. In absorption, high energy light excites the system, promoting electrons within the molecule to transition from the ground state, to an excited state. Here, the electrons quickly relax to the lowest available energy state. Once this state is achieved and after a fluorescence lifetime, the electrons will relax back to ground state, releasing their stored energy as an emitted photon. Usually, the emitted light has lower energy than the absorbed radiation. Although all fluorescence microscopy technologies are generally based on the excitation of a fluorophore with a specific range (band) of wavelengths and subsequent detection of the emitted photons on a camera system, they differ by their specimen illumination and signal-detection strategies. The specimen is illuminated with light of a specific wavelength (or wavelengths) which is absorbed by the fluorophores, causing them to emit light of longer wavelengths (i.e., of a different color than the absorbed light).

Applications: Although fluorescence microscopy permeates all of cell and molecular biology, most biologists have little experience with the underlying photophysical phenomena. Fluorescence microscopy useful in Immunology, Cell and Molecular Biology, Diagnostic of disease, Labeling of cells, Protein interactions, Gene expression and protein localization patterns. Labeling of organs and organisms to capture morphogenesis

Calibration status: As per requirement by service engineers

Requirement of Sample preparation: As per protocol.

Procedure for instrument startup:

1. Both light and fluorescent mode should not be used simultaneously.
2. Continuous usage of bright field bulb and fluorescent bulbs are not advisable. Please switch off the bulbs after 1 hour of continuous usage as to avoid heating and other issues for the bulbs or lamps.
3. Once the fluorescent lamp is off then it can be switched on only after atleast 30 minutes as it may damage the bulb and its life. Fluorescent bulbs should be used very judiciously.
4. Microbial cultures should be sealed in the slide and then visualized on the microscope.

Do's and Don'ts: Fluorescence microscope is kept in the cell culture room. Users should operate the microscope only in presence of trained official or operator of the institute. Minimize no. of fluorescent unit on/off rapidly. Room temperature should not be more than 20 C.

Operating schedule: (gap b/w two experiments or time being experiment): According to prior appointment.

Data collection/ analysis: Data will be provided to users in CD.

Documentation: Maintain complete records for all equipment and instruments.

Zeiss Axio Observer A1 Fluorescence Inverted Microscope

VilberLourmat Fusion SL 2 400 Gel and Blot Documentation System

Name of the facility/ activity: Gel Doc

Instrument Incharge: Technical Assistant

Department: Dr Vikram Sarabhai Institute of Cell and Molecular Biology

Name, Model of Instrument: Fusion SL 2 400- VilberLourmat

Software name:

Introduction & Principle: Principle of fluorescence with fluorescent staining of nucleic acids, a fluorescent substance that has bound to nucleic acids is excited by ultraviolet irradiation and emits fluorescent light. It is widely used in Molecular Biology laboratories for imaging and documentation of nucleic acid and Poly acrelamide or agarose gels typically stained with ethidium bromide or fluorophore as SYBER Green. Generally Gel doc is composed of ultra violet (UV) light transilluminator a hood to shield external light sources and CCTV camera for image processing.

Applications: Gel documentation, or gel imaging, systems are used to record and measure labeled nucleic acid and protein in various types of media such as agarose, acrylamide or cellulose. Systems come in a variety of configurations depending on throughput and sample type.

Applications:

- Monoclonal and polyclonal antibody binding affinities
- Gel and blot imaging
- Colony counting
- Immunoassay
- Multiplex Protein Detection
- Post-Translational Modification Characterization
- 2D Electrophoresis
- Protein Quantitation

Calibration status: As per requirement by service engineers

Requirement of Sample preparation: As per protocol.

Procedure for instrument startup:

1. This instrument is used both for chemi- luminescence and UV based band visualization.
2. This system should be strictly used under the supervision of trained official of the institute.
3. Software and the instrument should be properly initialized and stabilized.
4. Cooling camera should be observed for its proper functioning.
5. Cooling camera should not be kept on for a longer duration of time if the samples are not ready.
6. Continuous use more than 1.5 hours is not advisable.
7. Frequent switching on and off the Chemi-Doc system is not advisable.
8. For visualization in U.V. range (agarose gel), protection shield should be used and door over the gel tray should be gently used.

Do's and Don'ts:

Operating schedule: (gap b/w two experiments or time being experiment): According to prior appointment.

Data collection/ analysis: Data will be provided to users in CD.

Documentation: Maintain complete records for all equipment and instruments.

VilberLourmat Fusion SL 2 400 Gel and blot documentation system

Birthhold Tristar² Multimode plate Reader

Name of the facility/ activity: Multimode Plate Reader

Instrument Incharge: Technical assistant

Department: Dr Vikram Sarabahai Institute of Cell and Molecular Biology

Name, Model of Instrument: Multimode Plate Reader, Birthold Tristar2

Software name: ICE software

Introduction & Principle: The microplate reader is a multimodal instrument that allows for a variety of experiments to be performed and measured simultaneously. Microplate readers can make absorbance, fluorescence and luminescence measurements. Multiwell plates are integral to the microplate reader and allow for many experiments to be performed at once. Regardless of the assay type, experiments on the plate reader utilize a standard curve to determine the experimental values. This curve uses samples of known concentration to generate a line of best fit or standard curve. Experimental values are then extrapolated to the curve or are calculated using the equation from the linear regression. Besides standards and samples being run on the multiwall plate, the blank along with positive and negative controls are also used in the assay to ensure it is working correctly. Multiplate readers are used to quantify protein, gene expression and various metabolic processes such as reactive oxygen species and calcium flux.

A **microplate reader** detects light signals produced by samples which have been pipetted into a **microplate**. The optical properties of these samples are the result of a biological, chemical, biochemical or physical reaction.
Applications: Microplate readers can make absorbance, fluorescence and luminescence measurements. Multiwell plates are integral to the microplate reader and allow for many experiments to be performed at once.

The range of applications for multi-mode plate readers is extremely large. Some of the most common assays are:

- ELISAs
- Protein and cell growth assays
- Protein:protein interactions
- Reporter assays
- Nucleic acid quantification
- Enzyme activity
- Cell toxicity, proliferation, and viability
- ATP quantification
- Immunoassays

Calibration status: As per requirement.

Requirement of Sample preparation: compatible chemicals and consumables, type of sample, Microplates. Microplates are available in opaque white, opaque black or translucent. Opaque plates are used to enhance detection technologies. Generally white assay plates are for luminance assays and black assay plates are for florescence assays. The bottom material of an opaque plate may be clear to support bottom reads and colorimetric (absorbance) assays.

Switch on the instrument and computer power on at least 15 min before using the instrument.
Open the software, select assay (Fluorescence, Absorbance, Chemi- luminescence) . Feed the parameters according to assay. Click on finish.

Do's and Don'ts: Put the sample plate in respective slot in instrument without lid during sample reading.

Operating schedule: As per pre booking and requirement of users.

Caution:

Shut down procedure: Proper shutdown procedure should be followed.

Data collection /analysis: Type of data acquisition / File format which we can provided to users.

Documentation: Maintain complete records for instrument.

Birthhold Tristar² Multimode plate Reader

Cell Culture Facility

Name of the facility/ activity: Cell Culture facility

Instrument Incharge: Technical assistant

Department: Dr Vikram Sarabahai Institute of Cell and Molecular Biology

Name, Model of Instrument: Cell Culture facility

Cell Culture facility at CMB:
Cell culture facility of CMB is equipped with high quality instruments like Biosafety cabinet, CO2 incubator, Fluorescence Inverted Phase Contrast microscope, Refrigerated Cooling Centrifuge and Water Bath. User has to take prior appointment and timings for working in Cell Culture facility. It is a common facility for everyone therefore with mutual concern the user should work and maintain the cell culture facility and should preferably work during official hours. Payment of cell culture facility is usage/week.

1: Biosafety Cabinet: Thermo Fisher

Conditions: Well maintained and regular service.

Biosafety cabinet is used for culturing of Mammalian Cell culture experiments only and no microbial cultures should be handled to avoid contamination.

1. Power of Biosafety cabinet should be on.
2. UV should be kept on for 20'.
3. Hood should be opened till the marking and sensor of the cabinet.
4. After use the cabinet should be cleaned properly with 70% isopropanol and waste materials (all types of biomaterials, consumables, plastics etc.) should be disposed off through Biosafety Rules only.
5. Biosafety power Button should be long press and switched OFF and then main power plug should be off.

2: Cooling Refrigerated Centrifuge: Thermo Fisher

Conditions: Well maintained and regular service.

1. This centrifuge is used for separation of samples under cooling conditions. This instrument should be used by trained or knowledgeable individuals. No graduate or masters students are allowed to operate it individually.
2. Centrifuge can be used upto maximum of 15000/17000 RPM.
3. It has 1.5ml/2ml fixed angle rotor, 15ml/50ml fixed angle rotor, bucket rotor, plate rotor and card rotor.
4. Tubes or samples should be properly balanced.
5. If cooling is required then the centrifuge should be operated carefully. The lid of the centrifuge should not be kept open when instrument is on. Also during intermittent centrifugation cooling should not be kept on continuously. It is advisable not to use the centrifuge below 4°C. Samples should not be spilled. As the cooling centrifuge is kept in the cell culture room, bacterial or any microbial samples should be placed under sealed condition. Spillage or opening of the microbial samples are not allowed. If samples are spilled users should aseptically clean them.
6. The lid of the cooling centrifuge should be kept open when the machine is switched off.

3: CO_2 Incubator: Thermo Fisher

Conditions: Well maintained and regular service.

1. CO_2 Incubator is used for growing cells. Prior usage of CO_2 Incubator users should verify that all the requisites like HEPA filters, line filters, CO_2 cylinder etc. should be functional.
2. Users should take proper care of the instrument. Frequent closing and opening of the incubator should be avoided. The water in the tray should be sterile and should be replenished after 10 days or as per requirement. Sterile RO water should be filled upto adequate level to maintain temperature and CO_2 levels of the incubator.
3. Hands should be properly cleaned, gloves and mask should be worn. Due precautions and care should be taken to avoid mishandling and contamination.

4. During monsoon, it is advisable that you should enter cell culture lab and handle cultures only if you are dry and clean.
5. No microbial cultures or treated cells would be allowed to be kept in this CO_2 incubator as they will contaminate the mammalian cells or cell lines.
6. If there is any contamination or cells are dead due to some reason, plates, dishes or flasks should not be opened in the incubator and no media should be spilled which can cause contamination.
7. Decontamination procedures should be followed as per ethical protocols.
8. It is advisable to keep a spare filled cylinder which can be replaced if the working cylinder gets exhausted. For refilling the cylinders, the users should take help of the institute officials.
9. For any damage or errors please inform the officials of the institute for getting them rectified.
10. If the usage of the incubator is over, it highly recommended that the CO_2 incubator should be switched off and the CO_2cylinder also.

4. Inverted Phase Contrast Fluorescence Microscope AXIO A1- Carl Zeiss

Condition: Functional

1. Fluorescence microscope is kept in the cell culture room. Users should operate the microscope only in presence of trained official or operator of the institute.
2. Both light and fluorescent mode should not be used simultaneously.
3. Continuous usage of bright field bulb and fluorescent bulbs are not advisable. Please switch off the bulbs after 1 hour of continuous usage as to avoid heating and other issues for the bulbs or lamps.
4. Once the fluorescent lamp is off then it can be switched on only after atleast 30 minutes as it may damage the bulb and its life. Fluorescent bulbs should be used very judiciously.
5. Microbial cultures should be sealed in the slide and then visualized on the microscope.
6. The samples, slides, coverslips, dishes, flask etc. should be discarded properly.

If any problem occurs during operation of the equipment in the Cell culture facility please take help of CMB officials.

Ettan IPGphor 3 Isoelectric Focusing System

Name of the facility/ activity: 2-D Gel Electrophoresis

Principle Investigator: Dr Laxmipriya

Department: Department of Biochemistry

Name, Model of Instrument: Ettan IPGphor 3 Isoelectric Focusing System

Software name: IQTL Security 8.1, node locked license
Software Set IQTL 8.1 and IQTL SecurlTy 8.1

Introduction &Principle: 2DGE is used for protein separation from complex tissue. This process involves separation of protein in 2 dimensions. This technique can identify proteins that vary in their Isoelectric point but have same molecular weight. Therefore becomes a sensitive method for separating proteins. Method involves first dimensional separation on the basis of isoelectric point wherein slight change in charge can be separated. Then followed by second dimension wherein SDS PAGE is employed wherein based on molecular weight, proteins are separated. Thus, increasing the sensitivity of the method.

Applications: Protein separation and identification

Calibration status: 2013

Requirement of Sample preparation:
In order to characterize specific proteins in a complex protein mixture, the proteins of interest must be completely soluble under electrophoresis conditions The effectiveness of solubilization depends on the choice of cell disruption method, protein concentration and dissolution method, choice of detergents, and composition of the sample solution. Proteases may be liberated upon cell disruption, thus the protein sample should be protected from proteolysis if one of the methods described in this section is to be used. It is generally preferable to disrupt the sample material directly into a strongly denaturing lysis solution to rapidly inactivate proteases and other enzymatic activities that may modify proteins.

Gentle lysis methods
Gentle lysis methods are generally employed when the sample of interest consists of easily lysed cells (such as tissue culture cells, blood cells, and some microorganisms). Gentle lysis methods can also be employed when only one particular subcellular fraction is to be analyzed. These include

a. Osmotic Lysis
b. Freeze-thaw lysis
c. Detergent lysis

Vigorous lysis Methods are available. These include a. Sonication, b. Grinding, c. French Press

To prepare proteins from tissues that are dilute sources of protein and contain high levels of interfering substances (e.g. plant tissues), the following procedure is recommended. This method produces protein solutions substantially free of salts, nucleic acids, and other contaminants: 1. Grind tissue in mortar and pestle with liquid nitrogen. 2. Suspend powder in 10% TCA with 0.3% DTT in acetone. 3. Keep at -18 ºC overnight and centrifuge. Wash pellet with acetone. 4. Dry and resuspend in 9 M urea, 2% CHAPS, 1% DTT, 2% Pharmalyte 3–10.

- ***Protecting against proteolysis***
  When cells are lysed, proteases are often liberated or activated. Degradation of proteins through protease action greatly complicates the analysis of 2-D electrophoresis results, so measures should be taken to avoid this problem. If possible, inhibit proteases by disrupting the sample directly into strong denaturants such as 8 M urea, 10% TCA, or 2% SDS.
- ***Precipitation procedures*** Protein precipitation is an optional step in sample preparation for 2-D electrophoresis. Precipitation, followed by resuspension in sample solution, is generally employed to selectively separate proteins in the sample from contaminating species such as salts, detergents, nucleic acids, lipids, etc., that would otherwise interfere with the 2-D result. Precipitation followed by resuspension can also be employed to prepare a concentrated protein sample from a dilute source (e.g. plant tissues, urine).

2-D Clean-Up Kit from GE Healthcare can be used to remove contaminating substances and improve the 2-D electrophoresis pattern. Proteins are precipitated with a combination of precipitation reagents while the interfering substances, such as nucleic acids, salts, lipids, or detergents, remain in solution. Samples can be resuspended in the desired denaturing solution for IEF. Each kit can process 50 samples of up to 100 µl each. Section 1.4.1 describes the kit and provides a protocol for use.

- 2-D Clean-Up Kit produces a protein pellet. When using cup loading**, resuspend the pellet in sample preparation solution**
- Rehydration solution containing 8 M urea Use solution C. This all-purpose solution gives clean, sharp 2-D separations. 2. Rehydration solution containing 7 M urea and 2 M thiourea Use solution D in appendix I. This is a more strongly solubilizing solution that results in more spots in the final 2-D pattern. Any other components added to the rehydration solution must either be uncharged or present at a concentration of less than 5 mM. The addition of salts, acids, bases, and buffers is not recommended. 3. DeStreak Reagent Use for basic strips.
- **The volume of rehydration** solution used to resuspend the sample depends on the sample loading method and the length of the ImmobilineDryStrip gel used for the first-dimension separation. If using EttanIPGphor 3 and the sample is to be loaded onto the ImmobilineDryStrip gel using a sample cup, the sample volume should not exceed 150 µl. If the sample is to be loaded onto the ImmobilineDryStrip gel by rehydration

Procedure for instrument start up:

- Ensure that the Strip Holders are properly positioned on the EttanIPGphor 3 platform.
- Use the guide marks along the sides of the platform to position each Strip Holder and check that the pointed end of the Strip Holder is over the anode (pointing to the back of the unit) and the blunt end is over the cathode. (Please refer to the EttanIPGphor 3 user manual for complete details.)
- Check that both external electrode contacts on the underside of each Strip Holder make metal-to-metal contact with the platform.
- Before closing the safety lid, insert the lid adaptor (an accessory included with IPGphor 3) such that the pressure pads press gently against the cover of each Strip Holder to ensure contact between the electrodes and the electrode areas.
- Begin IEF. 64 80-6429-60 AD As isoelectric focusing proceeds, the bromophenol blue tracking dye migrates toward the anode. Note that the dye front leaves the ImmobilineDryStrip gel well before focusing is complete, so clearing of the dye is no indication that the sample is focused. If the dye does not migrate, no current is flowing.
- If this occurs, check the contact between the external face of the Strip Holder electrodes and the electrode areas on the instrument, and between the rehydrated gel and the internal face of the electrodes.
- Table 19 lists guidelines for running ImmobilineDryStrip gels on EttanIPGphor 3. It is possible that the programmed maximum voltage will not be reached when using shorter ImmobilineDryStrip gels or with samples having high conductivity.
- The final step of focusing should be run in volt-hours to ensure reproducibility from run to run. The following protocols are suitable for first-dimension isoelectric focusing of proteins run on EttanIPGphor 3 Isoelectric Focusing Unit. Preparative sample loads often increase the electroosmotic pumping of water
- . Excess free water on the gel surface contributes to streaky results and should be absorbed with electrode pads. This technique is standard when using the EttanIPGphor 3 Manifold; for standard Strip Holders this technique is described in section 2.7.
- The focusing times below are guidelines only, based on well-prepared samples. Times may vary with the nature of the sample and how the sample is applied.
- Using crude samples with high protein and salt content or using paper-bridge loading, the run time in total kiloVolt-hours should be increased by 10%.
- For Immobiline Dry Strip pH 6.2–7.5, 6–9, 6–11, and 7–11 NL, loading the sample anodically in a sample cup is recommended. For preparative sample loads with these basic strips, paper-bridge loading is recommended. If using the Manifold and 18- and 24-cm strips, the maximum voltage is 10 000 V. With these two strip lengths and standard Strip Holders, the maximum allowed voltage is 8000 V. With all other strips and regardless of whether the Manifold is being used, the maximum voltage is 8000V.

- ***Second-dimension SDS-PAGE using vertical electrophoresis systems***
  After IEF, the second-dimension SDS-polyacrylamide gel electrophoresis (SDS-PAGE) can be performed on various vertical or flatbed systems. SDS-PAGE consists of four steps:
  1) Preparing the system for second-dimension electrophoresis
  2) Equilibrating the ImmobilineDryStrip gel (s) in SDS equilibration buffer

Pour off buffer from above step and add the appropriate volume of SDS equilibration buffer (+iodoacetamide) to each strip. Again cap or seal the tubes with flexible paraffin film and place them on their sides on a rocker for the equilibration process. Equilibrate for an additional 15 min.

3) Placing the equilibrated ImmobilineDryStrip gel on the SDS gel

4) Electrophoresis

Equilibrating ImmobilineDryStrip gels. After IEF it is important to proceed immediately to gel equilibration, unless the IPG strip is being frozen (at -60 °C or below) for future analysis. Equilibration is always performed immediately prior to the second-dimension run, never before storage of the ImmobilineDryStrip gels. The second-dimension gel itself should be prepared and ready to accept the ImmobilineDryStrip gel before beginning the equilibration protocol

Equilibration solution components. The equilibration step saturates the ImmobilineDryStrip gel with the SDS buffer system required for the second dimension separation.

The equilibration solution contains buffer, urea, glycerol, reductant, SDS, and dye. An additional equilibration step replaces the reductant with iodoacetamide.

Equilibration buffer (75 mMTris-HCl, pH 8.8) maintains the ImmobilineDryStrip gel in a pH range appropriate for electrophoresis. Urea (6 M) together with glycerol reduces the effects of electroendosmosis by increasing the viscosity of the buffer

Electroendosmosis is due to the presence of fixed charges on the ImmobilineDryStrip gel in the electric field and can interfere with protein transfer from the ImmobilineDryStrip gel to the second-dimension gel. Glycerol (30%) together with urea reduces electroendosmosis and improves transfer of proteins from the first to the second dimension (3).

Dithiothreitol (DTT) preserves the fully reduced state of denatured, unalkylated proteins. Sodium dodecyl sulfate (SDS) denatures proteins and forms negatively charged protein-SDS complexes.

Iodoacetamide alkylates thiol groups on proteins, preventing their reoxidation during electrophoresis. Protein reoxidation during electrophoresis can result in streaking and other artifacts. Iodoacetamide also alkylates residual DTT to prevent point streaking and other silver-staining artifacts.

Iodoacetamide is introduced in a second equilibration step. The second equilibration with iodoacetamide minimizes unwanted reactions of cysteine residues (i.e. when mass spectrometry is to be performed on the separated proteins). Tracking dye (bromophenol blue) allows monitoring of the progress of electrophoresis.

Do's and Don'ts:

- EttanIPGphor 3 is a high-voltage instrument that can cause fatal electrical shock if the safety features are disabled. As such, the safety lid must be properly latched before instring a protocol, otherwise voltage will not be applied. Exceeding the recommended current limit of 75 µA per IPG strip can cause the strip to burn and may damage the instrument.
- During isoelectric focusing, do not lean on the safety lid, do not apply excess pressure or uneven weight to the lid, and do not place any items on the lid. Such pressure could cause arcing between the Strip Holder electrodes and the electrode areas, damaging the instrument.
- The Strip Holders and Manifold trays are made of ceramic and should be handled carefully. Always wear gloves when handling IPG strips and the equipment that comes in contact with them. This will help minimize protein contamination, which can result in artifactual spots in the resulting 2-D spot patterns. Clean Strip Holders and Manifold with the Strip Holder cleaning solution provided or the protective coating will be compromised.
- Clean all other components that come in contact with the IPG strip or the sample with a detergent designed for glassware. Rinse well with distilled water.
- Use the appropriate rehydration volume for the IPG strip length (refer to appropriate protocol). Do not heat any solutions containing urea above 30 ºC as isocyanate, a urea degradation product, will carbamylate the proteins in the sample, thus changing their isoelectric points.
- All chemicals should be of the highest purity (electrophoresis grade or better), and water should be double distilled or deionized.

Operating schedule: (gap b/w two experiments or time being experiment)

Caution: Proper GLP should be followed. Glows should be worn. Hazardous samples should be handled carefully. Care should be taken for sample spillage, cleaning, sample and relevant material disposal procedures.

Shut down procedure: Proper shutdown procedure should be followed.

Data collection /analysis: Type of data acquisition / File format which we can provided to users.

Documentation: Maintain complete records for the calibration and performance checks of all equipment and instruments.

Ettan IPGphor 3 IEF System

Nikon Eclipse Ti-2E Inverted Fluorescent Microscope for Live-Cell Imaging

Name of the facility/ activity: Live Imaging Microscopy System

Instrument In-charge: Prof. Rajesh Singh

Department: Department of Biochemistry

Name, Model of Instrument: Nikon Ti-2E Inverted Fluorescent Microscope Live Imaging Microscope with 10X (Phase), 20X (Phase) 40X (DIC) and 60X (DIC) objectives.

Software name: Nikon NIS-Elements AR-6D with Deconvolution

Introduction & Principle:
Fluorescence microscopy is a very powerful analytical tool that combines the magnifying properties of light microscopy with visualization of fluorescence. Fluorescence is a phenomenon that involves absorbance and emission of a small range of light wavelengths by a fluorescent molecule known as a fluorophore. Fluorescence microscopy is accomplished in conjunction with the basic light microscope by the addition of a powerful light source, specialized filters, and a means of fluorescently labeling a sample. This video describes the basic principles behind fluorescence microscopy including the mechanism of fluorescence, the Stoke's shift, and photo bleaching. It also gives examples of the numerous ways to fluorescently label a sample including the use of fluorescently tagged antibodies and proteins, nucleic acid fluorescent dyes with, and the addition of naturally fluorescent proteins to a specimen. The major components of the fluorescence microscope including a xenon or mercury light source, light filters, the dichroic mirror, and use of the shutter to illuminate the sample are all described. Finally, examples of some of the many applications for fluorescence microscopy are shown.
(REFERENCE: JoVE Science Education Database. General Laboratory Techniques. Introduction to Fluorescence Microscopy. JoVE, Cambridge, MA, 2019.)

Applications:
- Imaging structural components of small specimens, like mammalian, plant and bacterial cells, chromosomes, Etc.
- Conducting viability studies on cell populations, scratch assay, bright field imaging of small specimens.
- Fluorescence imaging of live cells using TOKAI-HIT incubation system for longer time period.
- Viewing specific cells within a larger population with techniques such as FISH

Calibration status: Calibrated and under 5 year AMC (2019-2024)

Requirement of Sample preparation eg. Filtered, properly sealed coverslips, compatible chemicals and consumables, type of sample, precaution:
Read literature and discuss with technical expert before performing the experiment.
1. Use appropriate mounting media for fluorescence imaging.
2. Live Imaging Can be performed only with 20X and 40X objectives. Live imaging adapters supports only $3cm^2$, $6cm^2$ culture dish, any multi-well culture plates and slides.
3. For imaging with 60X (oil) objective, coverslip with ≤0.17mm thickness must be used.
4. There are three filter cubes in the system for fluorescence detection: FITC (GREEN), DAPI (BLUE) and TRITC (RED).
5. Qi2 is a monochrome camera for efficient fluorescence imaging and Fi3 is a color camera for the imaging of colored specimen (eg. H&E staining of tissues, Gram staining of Bacteria, etc)

Procedure for instrument startup:
1. AC always ON & FANS always OFF
2. Switch-ON computer (Not NIS-AR software)
3. Switch-ON main power button and within 10 seconds Switch-ON microscope power button, Then NIS-AR Software (Select Camera in software-Any One or Both).
4. Switch-ON power of required camera only. Don't Switch-ONboth camera (Fi3 and Qi2) if not required.

Do's and Don'ts:
1. Confirm time slot from google form to issue keys from office.
2. Put entries for Microscopy use, Live imaging equipment useand also for data analysis in the Register.

3. Get acquainted with all features (microscope/Software) before use for the best imaging of your experiments. (Use PFS only if required)
4. Use only appropriate camera for capturing
5. Discuss your experiments with trained scholars/Technical Expert to setup the instrument and software properly.
6. Extra precaution for live imaging setup and accessories.
7. Capture Images in TIFF/ND2 File format only. (Preferably ND2**)**
8. Supervision of lab's instrumentation operation in-charge is compulsory. Contact NIKON's technical expert if needed.
   NOTE: Last user will be responsible for the loss/damage of accessories. In such case, he/she and lab's instrumentation operation in-charge/s will be responsible for the replacement of damaged/lost parts.

Operating schedule: (gap b/w two experiments or time being experiment)
Monday to Friday 10am – 7pm, 1-hour gap between two experiments.

Caution: Proper GLP should be followed. Glows should be worn. Hazardous samples should be handled carefully. Care should be taken for sample spillage, cleaning, sample and relevant material disposal procedures.

Shut down procedure: Proper shutdown procedure should be followed.
1. Turn off Software first, Then Camera and Fluorescence Unit Power Button
2. Turn off microscope power.
3. Retrieve your data and turn off Computer system.
4. Cover Microscope and table properly.

Data collection / analysis: Type of data acquisition / File format which we can provided to users.
1. Define a Folder in your lab's data folder in appropriate drive except C Drive/Desktop. You may lose your data during maintenance if found on Desktop/C Drive.
2. Pen-drive will be issued from the office with a register entryand return it to office after use.
3. CDs/DVDs can be used for data transfer.
4. Insert the pen-drive and "Quick Format" it in microscope computer every time before transferring your data to pen-drive.(Format pen-drive once you transferred it to your system)
5. Last user has to replace the pen-drive if lost.(Make sure of returning pen-drive to office)
   NOTE: Taking pen-drive to Lab/Personal use is prohibited.

Documentation: Fill up Google form (get it filled from any instrument operating in-charge of the Biochemistry Department) for prior booking the time slot and date. Make an entry in Register on the day of instrument use and data analysis. Maintain complete records for the calibration and performance checks of all equipment and instruments.

Nikon Eclipse Ti-2E Inverted Fluorescent Microscope

Bio-Rad QX200 Droplet Digital PCR System

Name of the facility / activity: Droplet Digital PCR

Instrument Incharge: Prof. Rajesh Singh

Department: Department of Biochemistry

Name, Model of Instrument: QX200 (Bio-Rad)

Software name: QuantaSoft Analysis Pro Software

Introduction & Principle:
Droplet Digital polymerase chain reaction (ddPCR™) was developed to provide high- precision, absolute quantification of nucleic acid target sequences with wide-ranging applications for both research and clinical diagnostic applications. ddPCR measures absolute quantities by counting nucleic acid molecules encapsulated in discrete, volumetrically defined water-in-oil droplet partitions.

ddPCR has the following benefits for nucleic acid quantification:

- Unparalleled precision — the massive sample partitioning afforded by ddPCR enables small fold differences in target DNA sequence between samples to be reliably measured
- Increased signal-to-noise — enrich for rare targets by reducing competition that comes from high-copy templates
- Removal of PCR efficiency bias — error rates are reduced by removing the amplification efficiency reliance of PCR, enabling accurate quantification of targets
- Simplified quantification — a standard curve is not required for absolute quantification

Workflow: Bio-Rad's QX100 or QX200 ddPCR System combines water-oil emulsion droplet technology with microfluidics. The QX200 Droplet Generator partitions samples into 20,000 droplets. PCR amplification is carried out within each droplet using a thermal cycler. After PCR, droplets are streamed in single file on a QX200 Droplet Reader, which counts the fluorescent positive and negative droplets to calculate target DNA concentration.

Droplet Generation
Before droplet generation, ddPCR reactions are prepared in a similar manner as real-time PCR reactions that use TaqMan hydrolysis probes labeled with FAM and HEX (or VIC) reporter fluorophores, or an intercalating dye such as EvaGreen®.
ddPCR must be performed with the proprietary reagents developed specifically for droplet generation by Bio-Rad. Reagent mixes include the ddPCR Supermix for Probes and QX200™ ddPCR™ EvaGreen® Supermix to partition DNA, and the One-Step RT-ddPCR Advanced Kit for Probes.
Samples are placed into a QX100 or QX200 Droplet Generator, which uses specially developed reagents and microfluidics to partition each sample into 20,000 nanoliter-sized droplets. Target and background DNA are distributed randomly into the droplets during the partitioning process.
Droplet generation produces uniform droplets for the sample, enabling precise target quantification.

PCR Amplification
Droplets are transferred to a 96-well plate for PCR in a thermal cycler. The C1000 Touch™ Thermal Cycler with 96–deep well reaction module for PCR is recommended. This high-performance thermal cycler has excellent temperature uniformity and settling across all 96 wells to help ensure successful PCR.

Droplet Reading
Following PCR amplification of the nucleic acid target in the droplets, the plate containing the droplets is placed in a QX100 or QX200 Droplet Reader, which analyzes each droplet individually using a two-color detection system.
The autosampler of the droplet reader picks up the droplets from each well of the PCR plate.
Droplets are spaced out individually for fluorescence reading by the droplet reader. Fluorescence in two channels is then measured for individual droplets.
Positive droplets, which contain at least one copy of the target DNA molecule, exhibit increased fluorescence compared to negative droplets.

ddPCR Data Analysis

Droplet Digital PCR data can be viewed as a 1-D plot with each droplet from a sample plotted on the graph of fluorescence intensity vs. droplet number.

Applications:
Sample partitioning allows the sensitive, specific detection of single template molecules as well as precise quantification. It also mitigates the effects of target competition, making PCR amplification less sensitive to inhibition and greatly improving the discriminatory capacity of assays that differ by only a single nucleotide. Digital PCR offers the benefits of absolute quantification and greatly enhanced sensitivity. Therefore, its application in the following areas is growing:

- Absolute quantification — ddPCR provides a concentration of target DNA copies per input sample without the need for running standard curves, making this technique ideal for target DNA measurements, viral load analysis, and microbial quantification
- Genomic alterations such as gene copy number variation (CNV) — CNVs result in too few or too many dosage-sensitive genes responsible for phenotypic variability, complex behavioral traits, and disease. ddPCR enables measurement of 1.2x differences in gene copy number
- Detection of rare sequences — researchers must amplify single genes in a complex sample, such as a few tumor cells in a wild-type background. ddPCR is sensitive enough to detect rare mutations or sequences
- Gene expression and microRNA analysis — ddPCR provides stand-alone absolute quantification of expression levels, especially low-abundance microRNAs, with sensitivity and precision
- Next-generation sequencing (NGS) — ddPCR quantifies NGS sample library preparations to increase sequencing accuracy and reduce run repeats. Validate sequencing results such as single nucleotide polymorphisms or copy number variations with absolute quantification
- Single cell analysis — the high degree (10- to 100-fold) of cell-cell variation in gene expression and genomic content among homogeneous post-mitotic, progenitor, and stem cell populations drives a need for analysis from single cells. ddPCR enables low copy number quantification
- Genome edit detection — ddPCR enables fast, precise, and cost-effective assessment of HDR (Homology directed repair) and NHEJ (Non-homologous end joining) generated by CRISPR-Cas9 or other genome editing tools

Calibration status: Calibrated on installation

Requirement of Sample preparation eg. Filtered, properly sealed coverslips, compatible chemicals and consumables, type of sample, precaution:
As with any PCR-based technology, assay design and sample preparation are important for obtaining high-quality data. Before running a Droplet Digital PCR (ddPCR™) experiment, know the goal or possible expected outcomes of the experiment because different types of experiments require different controls, sample preparation, amounts of DNA or RNA, and data analysis.
The amplification reaction of target molecules in ddPCR workflows follows similar principles of real-time PCR.

- Plan to amplify a 60–200 bp product
- Avoid regions that have secondary structure when possible
- Choose a region that, ideally, has a GC content of 40–60%

<u>Designing Primers</u>
Widely accepted quantitative PCR (qPCR) design guidelines apply to ddPCR primer design. Important criteria for single primers include melting temperature (Tm), length, base composition, and GC content. In addition, because primers are used in pairs, ensure that paired primers do not exhibit significant complementarity between 3' ends because this can result in primer-dimers. Extensive primer-dimer formations can significantly decrease or prevent amplification. The QX200™ Droplet Digital PCR System will support both hydrolysis probe (TaqMan) and DNA binding dye (EvaGreen®) assays.

When designing primers for a target sequence, follow these guidelines:

- Design primers that have a GC content of 50–60%
- Strive for a Tm between 50 and 65°C.
- Avoid secondary structure and adjust primer locations so they are outside the target sequence secondary structure, if required.
- Avoid repeats of Gs or Cs longer than 3 bases.
- Place Gs and Cs at the 3' nucleotide of primers when possible.
- Check forward and reverse primer sequences to ensure no 3' complementarity (avoid primer-dimers).

<u>Sample Preparation</u>

The quality of the nucleic acid preparation from the sample of interest can impact ddPCR results. An optimized protocol should be used to extract the DNA or RNA from the raw material you are testing. Ensure that the sample has not been degraded, for example by heating above 60°C.

Adding DNA to the Reaction Mix

The recommended dynamic range of the QX100 or QX200 System is from 1 to 120,000 copies/20 µl reaction.To estimate the number of copies/ng of DNA for your organism you must know the mass or the number of base pairs in the genome.

If the experiment entails quantifying samples known to have extremely high amounts of target molecules (such as next-generation sequencing [NGS] libraries), plan to reduce the starting sample accordingly. If the target copy number/genome is unknown, we recommend that you determine the optimal starting amount by doing four tenfold dilution series of each sample at the expected digital range. By assaying the four data points above and below the expected digital range, you ensure that one of the data points is within the optimal digital range.

For sample DNA loading, follow these guidelines:

- Assess input DNA/RNA concentration using A260 spectroscopy to ensure the target DNA/RNA concentration is being loaded within the dynamic range of detection
- Add no more than 1 µg of digested DNA to the 20 µl reaction (final concentration of 50 ng/µl)
- Intact DNA requires restriction digestion for optimal performance, especially at a concentration above 3 ng/µl (60 ng/20 µl reaction). For copy number and absolute quantification, always do restriction digestion at all DNA concentrations unless you want to access proximal replicate sequences
- Do not perform a restriction digestion of the DNA sample within the amplicon sequence
- Fragmented DNA may not need restriction digestion. However, some assays and/or targets require digestion for optimal target detection regardless of fragmentation
- cDNA does not require restriction digestion

Droplet Generation

For droplet generation, transfer 20 µl of a PCR reaction containing sample nucleic acid, primers (and probes for a TaqMan experiment), and the appropriate Bio-Rad ddPCR supermix to the middle rows of a DG8™ Cartridge for a QX100 or QX200 Droplet Generator. We recommend creating an initial reaction pool that is slightly more than 20 µl (22–25 µl) to ensure that 20 µl of mixture is transferred to the DG8 Cartridge. Reaction mixes should be combined and well mixed in a separate tube and not in the droplet generator cartridge. Reaction mixes should then be transferred to the DG8 Cartridge already preloaded in the DG8 Cartridge Holder.

Note: Each DG8 Cartridge generates eight wells of droplets. Any unused wells on the cartridge must be filled with 1x ddPCR buffer control.

The Bio-Rad ddPCR supermixes have been formulated specifically to work with the droplet chemistry. Altering the components used in the QX200 Droplet Generator or using a different supermix will negatively impact results. A 1x final concentration of supermix must be used for proper droplet formation and proper target quantification.After loading a 20 µl PCR reaction, load 70 µl of Droplet Generation Oil into the bottom wells of the DG8 Cartridge. Attach a gasket across the top of the DG8 Cartridge and place it into the QX200 Droplet Generator. The Droplet Generator produces about 20,000 droplets per sample in about 2.5 min for eight samples.Droplets shouldbe transferred to a 96-well PCR plate by pipetting gently.

PCR

After generating droplets in the DG8 Cartridge, pipet the droplets from the top wells of the cartridge into a PCR plate. The PCR plate should be heat sealed using Bio-Rad's PX1™ PCR Plate Sealer and pierceable foil heat seal.

Note: Using an alternative seal with glue can damage the Droplet Reader.

After heat sealing, place the PCR plate in a thermal cycler for PCR using the following guidelines.

- Use a recommended thermal cycling protocol
- Use a 2.5°C/sec ramp rate to ensure each droplet reaches the correct temperature for each step during the cycling
- 40 cycles of PCR is enough for an optimized ddPCR assay. Do not exceed 50 cycles
- After PCR, the plate can be left in the thermal cycler overnight at 10°C or stored
- at 4°C. Do not store the plate for more than 3–4 days before running it in a QX100 or QX200 Droplet Reader.

Setting Up an Experiment in QuantaSoft™ Software

From the computer attached to the droplet reader, open QuantaSoft Software in the setup mode and design a new plate with a layout according to your experimental design. Detailed instructions for how to set up a new experiment and interpret ddPCR data can be found in the user manual.

Double click on a well in the plate layout to open the Well Editor dialog box. Designate the sample name, experiment type, and which assays correspond to which channels, such as FAM and HEX. You can select several contiguous wells at one time using shift + double click or select non-contiguous wells using Ctrl + double click. Either selection will bring up the labeling menu. In the Well Editor dialog box, input sample names and use the dropdown menu to designate the experiment type.

There are three types of experiments that can be selected for each well:

- ABS — absolute quantification
- RED — rare target sequence detection (rare event detection)
- CNV — copy number variation to measure the concentration of target relative to the concentration of a reference.

Select Apply to load the wells and when finished select OK. Once the plate layout is complete, select Run to begin the droplet reading process.

Droplet Reading

Following PCR amplification of the nucleic acid target in the droplets, place the PCR plate in a QX100 or QX200 Droplet Reader. The Droplet Reader and QuantaSoft Software count the PCR-positive and PCR-negative droplets to provide absolute quantification of target DNA.

Droplet reading considerations are as follows:

- Before a run, the instrument can be set to interrogate droplets either in rows or columns
- Ensure there is enough Droplet Reader Oil in the instrument and the waste is empty before a run
- Each sample is processed individually and interrogated for both FAM and HEX (or VIC) fluorescence
- Data from 12,000–16,000 droplets are used in concentration calculations
- The reader measures fluorescence intensity of each droplet and detects the size and shape as droplets pass the detector; droplets are excluded if they do not meet quality metrics

Do's and Don'ts/ Troubleshooting:

Positive Droplets in No Template Control Wells

Digital PCR can detect very low levels of target DNA so it is important to prevent template/ amplicon contamination and to run no template controls (NTCs). Positive droplets in NTC wells that are at intensities equal to those of positive droplets in sample wells are typically caused by template or PCR product (amplicon) contamination in the reagents. Having a clean environment and clean NTC wells (that is, no positive droplets) is imperative when the application is rare sequence detection (wells with a low number of positives).

If positive droplets in NTC wells occur, make sure that good laboratory practices for PCR are being followed in the laboratory

.

Suggested guidelines are as follows:

- Wipe down pipets, tip boxes, and benchtops with 5–10% bleach
- Prepare master mixes in a template-free environment, add samples and generate droplets in an amplicon-free environment, perform PCR, and read droplets in a room separate from the sample preparations
- Do not reuse DG8™ Droplet Generator Cartridges, oils, gaskets, plates, or pipet tips
- Wear appropriate personal protective equipment that is discarded or confined to appropriate locations (that is, template-free room for master mix assembly, amplicon-free room for template addition and droplet generation, and PCR and post-PCR rooms for droplet reading)

High Mean Fluorescence Amplitude Intensity

If the fluorescence amplitude of negative droplets is excessively high such that they are all considered positive and therefore concentration cannot be determined, it is possible the sample's target concentration is so high that every droplet contains DNA target and no negative droplets exist. When there are no negative droplets, Poisson correction cannot be applied and it is not possible to calculate a concentration.

No or Few Positive Droplets

If a new, never-before-tested assay fails to give positive droplets, consider the following:

1. The selected restriction enzyme may have cut within the target locus.
- Recommendation: test the assay against DNA digested with a different restriction enzyme as well as undigested DNA
2. The target locus resides in a region that contains secondary structure.
- Recommendation: use restriction enzymes to cut the sequences surrounding the region to be amplified in order to limit the number of possible interactions with nearby nucleotides
3. The assay does not work at the predicted temperature.
- Recommendation: first perform an annealing/extension temperature gradient to determine the temperature at which the assay works
4. The ddPCR reaction mix was not assembled correctly or the probe/primers were not ordered correctly.
5. One of the assay components was designed incorrectly or a mistake was made during synthesis.

No or Low Total Droplet Count

To determine your droplet count, select the well in setup, click Analyze, then click the Events tab and make sure total is selected. If the total accepted events or droplet counts are less than 10,000 consider the following recommendations:

- Use the recommended concentration of primer (900 nM), probe (250 nM), and 1x master mix. The QX100™ and QX200™ Droplet Digital PCR Systems are compatible only with Bio-Rad's ddPCR supermixes. Using less than the recommended concentration of any of these components may lower your droplet count
- Load the DG8 cartridge with the appropriate volumes of sample and droplet generation oil (20 µl and 70 µl, respectively). If less than 20 µl of sample is loaded, fewer droplets will be generated. Be sure to load the sample before the oil
- Use only purified nucleic acids. Any particulate matter (for example, residual fibers from sample preparation columns or beads) in the sample should be removed before assembling the ddPCR reaction mixture because these particulates can clog the DG8
- Cartridge's microfluidic channels and disrupt droplet generation. To remove particulates from purified nucleic acids, spin the sample at 10,000 x g for 1 min and transfer the supernatant to a clean tube
- Do not exceed the recommended DNA load (66 ng/well undigested DNA or 1,500 ng/well digested DNA)
- Use only approved plates (ddPCR 96-Well Plates, Bio-Rad catalog #12001925 and Eppendorf twin.tec Semi-skirted 96-well Plates, catalog #951020362) with approved pierceable foil heat seals (Bio-Rad catalog #1814040)
- Properly seal the 96-well plate. Under- or over-sealed plates result in oil evaporation during thermal cycling and compromise droplet data quality. If using the PX1™ PCR Plate Sealer (Bio-Rad catalog #1814000), seal plates at 180°C for 5 sec. Do not use the PX1 sealing protocol twice on the same plate because this often disrupts the original seal
- Ensure that the full volume of the generated droplets is transferred into the 96-well plate by inspecting the DG8 Cartridge after transfer
- Use only approved pipet tips for droplet generation and droplet transfer. Rainin and Eppendorf tips are approved for use

For sample loading, use P-20 pipet tips and slowly dispense the sample into the bottom of the DG8 well rather than pipetting at the top edge of the well. Then dispense 70 µl of oil into the oil wells. Begin droplet generation within 2 min of oil loading.

Use a manual P-50 pipet with a normal bore P-200 tip (not wide or narrow bore) to transfer droplets. Angle the P-200 tip in the well to prevent the droplets from having to squeeze between the pipet tip and well bottom (angle the tip position such that it is not vertical in the well). Slowly draw 40 µl of droplets into the pipet tip over ~5 sec. Typically ~5 µl of air will be pulled into the tip, which helps prevent the oil from leaking out.

Position the pipet tip (containing the droplets) near the bottom of the well and dispense the sample, ensuring ample room between the well and the pipet tip so that the droplets do not shear upon dispensing.

Inconsistent Concentration Results

Technical replicates of the same sample should yield concentration estimates that are within the Poisson confidence error bars 95% of the time. If the concentration estimates between technical replicates are not close, the most common causes are poorly mixed reaction mixtures or poor thermal cycler temperature uniformity.

Insufficient Mixing

When creating technical replicates, thoroughly mix the reaction mixture (master mix, sample, and assay) by pipetting the reaction mixture up and down ten times, using 90% volume strokes. Alternatively, pulse vortex the

reaction mixture for 15 sec followed by spinning the sample down. Do not assemble or mix reaction mixtures in the DG8 Cartridge.

Effects of Poor Cycler Uniformity

If the reaction mixtures used to create technical replicates are thoroughly mixed but there is wide variation in concentration estimates, consider the uniformity performance of your thermal cycler. Generally, this effect is observed only when a temperature-sensitive assay is used on a thermal cycler with poor uniformity. Uniformity at both the denaturation and annealing/extension temperatures is important. Bio-Rad's C1000 Touch™ Thermal Cycler with 96–Deep Well Reaction Module has excellent thermal uniformity. To test the module's uniformity, use the temperature-sensitive assay that has concentration variability and create droplets from the same reaction mixture for the entire plate. Check the entire plate for a discrepancy in concentration that exceeds the 95% confidence bounds for the wells. If one of the block's Peltier devices is broken or underperforming, a drop in concentration will be consistently observed in the same quadrant(s) of the block.

If it is suspected that the variation in concentration is due to thermal cycler performance, consider:

- Increasing the hot start from 94°C for 10 min to 96°C for 10 min
- Raising the denaturation temperature from 94 to 96°C for the first 5 cycles
- Purchasing Bio-Rad's C1000 Touch Thermal Cycler with 96–Deep Well Reaction Module
- If drops in concentration estimates are consistently confined only to a quadrant(s) of the block, contact the manufacturer and request thermal-couple uniformity analysis and,
- if necessary, repair

Concentrations Consistently Lower than Predicted

If concentrations measured in ddPCR are consistently lower than predicted, consider poor target accessibility, poor or incorrect assay design, or the presence of PCR inhibitors in samples.

It is possible that the reference concentration measurement that suggests ddPCR concentration calls are low is, in fact, in error and is reporting a higher than actual concentration. ddPCR gives a concentration measurement of intact DNA targets while spectroscopic measurements typically do not distinguish between degraded and intact nucleic acids.

Also consider the following options:

- Make sure the ddPCR assay has been optimized by running a temperature gradient experiment
- Amplicons longer than 150 nucleotides may require longer annealing times during PCR
- If duplexing 2 assays together for the first time, test them in a singleplex assay using the same sample to confirm that the assays are not interfering with one another
- Verify that the fluorophore is not conjugated to a G residue
- Add the recommended primer (900 nM) and probe (250 nM) concentration

Additional Tips

No Concentration Calls on Some Wells

If a concentration estimate fails to appear in the concentration chart in QuantaSoft, this indicates the software could not auto-analyze or assign droplets to positive or negative populations using its auto-analysis algorithm, or the well had an unusually low droplet count (<10,000). Low total droplet counts indicate a problem with the assembly of the reaction mix, poor preparation of the sample, or poor handling. Manually set a threshold and QuantaSoft Software will calculate a concentration, which will appear in the concentration chart.

Target Accessibility

Strong or excessive secondary structure can prevent a DNA target from being amplified. Human gDNA and plasmid DNA can usually be restriction digested to remove inhibiting secondary structure, thereby rescuing detection. RNA secondary structure is best addressed by changing the location of the assay, if possible, or reverse transcribing the assay at a warmer temperature.

High-Fluorescence Amplitude Droplets

Droplet coalescence can create droplets that are much higher in fluorescence amplitude than the other positive droplets. This can be caused by poor droplet transfer technique or extended storage of the droplets pre– or post–thermal cycling. Adjust the scale on the 1-D or 2-D amplitude charts in order to set the thresholds in these cases.

Operating schedule: 9:00 am to 7:00 pm

Caution: Proper GLP should be followed. Gloves should be worn. Care should be taken for sample spillage, cleaning, sample and relevant material disposal procedures.

Shut down procedure: Proper shutdown procedure should be followed.

Data collection/ analysis: A separate pen drive only for the purpose of transferring data will be provided to transfer the data file from the instrument's laptop to your personal laptop. Software has to be downloaded for data analysis and graph may be exported from the software.

Documentation: Prior entry for using the instrument will have to be made in the register book of the instrument.

BioRad QX200 Droplet Digital PCR System

Labconco FreeZone Benchtop Freeze Dry System

Name of the facility / activity: Lyophilizer

Department: Department of Biochemistry

Instrument Incharge:

Name, Model of Instrument: Lyophilizer (Free Zone 2.5 Liter Freeze Dry Systems)
Labconco: 7670081

Software name:

Introduction & Principle: This instrument is used for freeze-drying by pre-freezing the material and then under low temperature and pressure, the frozen water in the material is directly converted to gaseous phase from the solid phase by a process called sublimation.

Applications: Lyophilized/freeze dried products retain their original properties without changing their composition. It is used for drying of biological materials such as microbial cultures, concentration and recovery of reaction product etc.

Calibration status: Service done on 24/09/2019

Requirement of Sample preparation: The samples should be in pre-freezed condition before lyophilization. Sealing of the sample container by parafilm is must.

Precaution:

- Chemicals causing corrosion of the vacuum pump and the instrument should be avoided.
- The adjusting plates of the instrument should be dust free.
- All the valve positions should be check to create appropriate vacuum.

Procedure for instrument start-up:

1. Switch on the Main power supply followed by instrument (right side upside).
2. Press Manual refrigeration button and Let the temperature rise to -80^{o} C
3. Press vacuum button. Monitor screen will initially indicate high.
4. Later on it will slowly reduce from 4 to 3, 2, 1, 0.2 mBar to .01 mBar. The lyophilization process will start.

Shut down procedure:

1. To switch off the instrument, Press the vacuum button.
2. Slowly release the vacuum by pulling out the black outlet valve.
3. Put the temperature off by pressing the refrigeration button.
4. Let the temperaturefall from -80 to normal temperature.
5. Switch off the instrument from the mains.

Do's and Don'ts:

- During operation, care should be taken to prevent sample spillage onto the instrument
- Finally, wipe the water using tissue paper such that no water enters the outlet valve.
- In case of Excess of water formed inside the instrument, it should be drained fromthe outlet valve

Operating schedule: Overnight operating of the instrument should be avoided.

Caution:

- Proper GLP should be followed.
- Carefully monitor the temperature and vacuum pump oil movement.
- In case of sudden power supply cut off, switch off the whole system or contact the concerned person.
- In case of alarm, press menu button to look for message.

Data collection/ analysis: The lyophilized product can be provided to users.

Documentation: Maintain complete records for the calibration and performance checks of all equipment and instruments.

Labconco FreeZone Benchtop Freeze Dry System

Thermo Scientific UltiMate 3000 Basic Manual HPLC System

Name of the facility: Basic Manual HPLC System

Instrument Incharge: Dr. Laxmipriya P. Nampoothiri

Department: Department of Biochemistry

Name & Model of Instrument: UltiMate™ 3000 Basic Manual HPLC System, Thermo Fisher Scientific

Software name: CHROMELEON® CDS Software

Introduction & Principle: The Thermo Scientific™ UltiMate™ 3000 Basic Manual system offers unique Liquid chromatography (LC) and entry level ultra-high-performance liquid chromatography (UHPLC) capabilities without compromising on quality and robustness. High Performance Liquid Chromatography (**HPLC**) is a form of column chromatography that pumps a sample mixture or analyte in a solvent (known as the mobile phase) at high pressure through a column with chromatographic packing material (stationary phase). UltiMate™ 3000 HPLC System consists of Dionex Ultimate™ 3000 Pump (Thermo Scientific), Dionex Ultimate™ 3000 VWD Variable Wavelength Detector (Thermo Scientific) and Acclaim™ 120 (Thermo Scientific) C18 column of 4×250mm, 120 Å pore diameter and 5 µm particle size. A Four-solvent channel pump for versatile gradient methods along with integrated 4-channel degasser present to simplify operation. Typical application areas of UltiMate™ 3000 HPLC Basic Manual system are teaching laboratories within general academic research and quality control laboratories with low sample throughput requirements.

Procedure for instrument run:

- 'Turn ON' the mains using the switches located outside the departmental mammalian cell-culture-room facility. Turn on the switches for the Dionex Ultimate™ 3000 Pump and Dionex Ultimate™ 3000 VWD Variable Wavelength Detector, located behind the pumps and detector respectively. Also, 'Turn ON' the computer workstation and launch the CHROMELEON CDS software.

- **Sample Preparation**: The samples should be dissolved in Methanol/Ethanol or Acetonitrile or a combination of these depending upon the best solubility of compound of interest and they can be sonicated using a bath type sonicator. The samples should be filtered prior to injection using 0.2µm filters. All the samples should be at room temperature before injecting them. Complex samples such as plasma/serum/crude plant extracts cannot be injected as such. They need to be prepared as per the literature. Depending on your compound properties and limit of quantification you want to reach, you could determine the suitable sample preparation.

- **Mobile phase Preparation:** HPLC grade solvents should be sonicated for 15 minutes using a bath type sonicator and the channels can be kept inside respective solvent bottles. [Note- Channel B should be used for Acetonitrile only]. Unused channels can be stored in another bottle containing 70% isopropanol.

- **Opening the CHROMELEON CDS Software**: Turn on PC and wait for windows to start. Click the Chromeleon icon on the desktop.
  a. A control panel often just called 'PANEL'–allows you to control an entire chromatography system from your PC.
  b. Connect the 'PUMP', open the purge valve (located in the front panel of Dionex Ultimate™ 3000 Pump. Initiate 'PURGE'. After completion of the purge, close the purge valve. [Note- Column should not be attached in the purging state].
  c. Connect the Dionex Ultimate™ 3000 VWD Variable Wavelength Detector, ignite the detector and wait for it to turn green from red. Wait for 5-10 minutes to ensure that the lamp of the detector is ready.

- **Preparing the Program:** Use Chromeleon Program Wizard to create a basic program structure.
  a. Select 'NEW' on the 'FILE' menu.
  b. Select 'PROGRAM FILE' from the list and click 'OK' to start the Program Wizard.
  c. Depending on the installed instruments, the Program Wizard provides various steps for setting up the type of run-isocratic /gradient, composition of solvent, flow rate, the run- time, wavelength etc.
  d. Clicking 'NEXT' takes you to the next wizard page. Click 'FINISH' to generate a program with the settings selected.
  e. Select 'SAVE AS' on the 'FILE' menu to save the PGM File under a descriptive name.

- **Preparing the machine to sequence**: To allow a chromatographic system to process several samples one after another without interruption, you have to define the order and the program to be used for sample processing. The easiest way to create a new sequence is to use the Sequence Wizard.
  a. Select 'NEW' on the 'FILE' menu.
  b. From the list, select 'SEQUENCE (USING WIZARD)'.
  c. Click 'OK' to start the Sequence Wizard
  d. Enter the name and the directory of the program file 'PGM File' and the quantification file 'QNT File' to be used.
  e. Specify the name for the sequence to create and where to save it.
  f. Enter any name in the 'SEQUENCE NAME' field and select, for example, the local data <USERNAME_LOCAL> from the Datasource field. Generate a separate sequence directory by specifying a directory name in the Directory field.
  g. On the last Wizard page, click 'DONE' to complete your input.

- **Starting the analysis:**
  a. Select a sequence, click on 'BATCH' and click 'START' to start the analysis.

- **Disassembly of the UltiMate™ 3000 Basic Manual HPLC System:** After your sequences have finished:
  a. Make sure the sequences created in the sequence wizard have completed their runs. After completion of the batch run, manually go the pump module in PANEL HPLC and change the flow rate to 0 ml/min. In case, you want to stop the batch run suddenly, click on BATCH in the toolbar and then click END BATCH.
  b. Rinse the column with the mobile phase for 30 min (minimum) - incase you have more samples to be analysed the following day. However, if you are not planning any more experiments or you wish to change to a different solvent, then rinse the system with acetonitrile for 1 hour (minimum). And store the column in acetonitrile only.
  c. Disconnect and disable the pump as well as the detector after completion.

- **Quantification of peaks:** Used to define a method for peak detection and recognition, quantification and evaluation. Before peaks can be identified and quantified, they have to be detected. Based on default values for the peak recognition algorithm, Chromeleon is able to detect even the smallest peaks.
  a. Parameters such as Retention time, Minimum Area, Minimum Height, Minimum Width, Maximun Width, Maximum Peak Height can be studied.
  b. The Peak Table is used to assign peak names to all peaks of interest in a chromatogram. Peaks are typically identified by the retention time.
  c. Enter the names of all peaks to be identified in the peak name column. Assign the expected Retention time to each peak in the Retention Time column. If a peak is detected at the specified time, the name is assigned automatically.
  d. You can use standard samples to quantify unknown samples.

- **Printing Results:** The Printer Layout window displays custom print templates. To open a print template for a specific sample:
  a. Double-click a sample name in the Browser. Chromeleon displays the chromatogram in the Integration plot window.
  b. Select 'PRINTER LAYOUT' on the 'VIEW' menu to change from the integration plot to the Printer Layout. Save the results in .xls format and can be viewed in your private PC/Laptops.

Do's and Don'ts:

1. Do not modify any critical calibration parameters of the instrument without permission.
2. Use only HPLC grade solvents and degas them using a bath-type sonicator prior to run.
3. For cleaning the channels, remove the column and run the system with luke-warm 70% isopropanol for 1 hour (minimum), followed by luke-warm acetonitrile for 1 hour (minimum). The unused channels can be stored in 70 % isopropanol.
4. Purging is mandatory for every fresh batch of solvent.
5. All the samples should be reconstituted in HPLC grade Methanol/Ethanol/ Acetonitrile only (based upon the best solubility of the compound of interest) and passed through 0.2µm filters. Unfiltered samples should be strictly avoided. C18 column in not compatible with any other organic solvents.
6. Avoid using water for sample preparation or in the mobile phase (it should not be passed through the C18 column under any condition). If the analysis requires the usage of water, then discuss with the in-charge prior to initiation of the analysis.

7. After completion of the experiment, the column should be rinsed for 1 hour (minimum) and stored in acetonitrile (the last solvent to be run through the column should be acetonitrile only). Make sure to detach the column and close its end caps properly post usage. Return the column to the in-charge.
8. Make sure that the solvent bottles are properly cleaned, dried and then returned to the overhead of the instrument. The channels when not in used should be stored in 70% isopropanol.

Operating schedule: Please ensure availability of instrument at required time before setting up any experiments.

Caution: Proper GLP should be followed. Gloves should be worn. Hazardous samples should be handled carefully. Care should be taken for sample spillage, cleaning and material disposal procedures.

Data collection: Use formatted pen drive or rewritable CD to retrieve the data from the PC and use it for further analysis.

Documentation: Maintain complete records.

UltiMate 3000 Basic Manual HPLC System

BioTek Synergy HTX Multi-Mode Microplate Reader

Name of the facility: Multi-Mode Microplate Reader

Instrument Incharge: Dr. Ravi Vijayvargia

Department: Department of Biochemistry

Name & Model of Instrument: Synergy HTX Multi-Mode Reader-BioTek Instruments

Software name: Gen5

Introduction & Principle:
Synergy™ HTX Multi-Mode Microplate Reader is a compact, affordable system for 6- to 384-well microplates and Take3 Micro-Volume Plates. It's unique dual optics design provides superior performance for UV-Vis absorbance, fluorescence, luminescence and AlphaScreen®/ AlphaLISA® workflows. Incubation and shaking plus a dual reagent injector module to meet all your laboratory's assay requirements now and in the future. Synergy HTX is controlled by the easy-to-use, yet powerful, Gen5 Software for data collection, analysis, exporting and reporting.

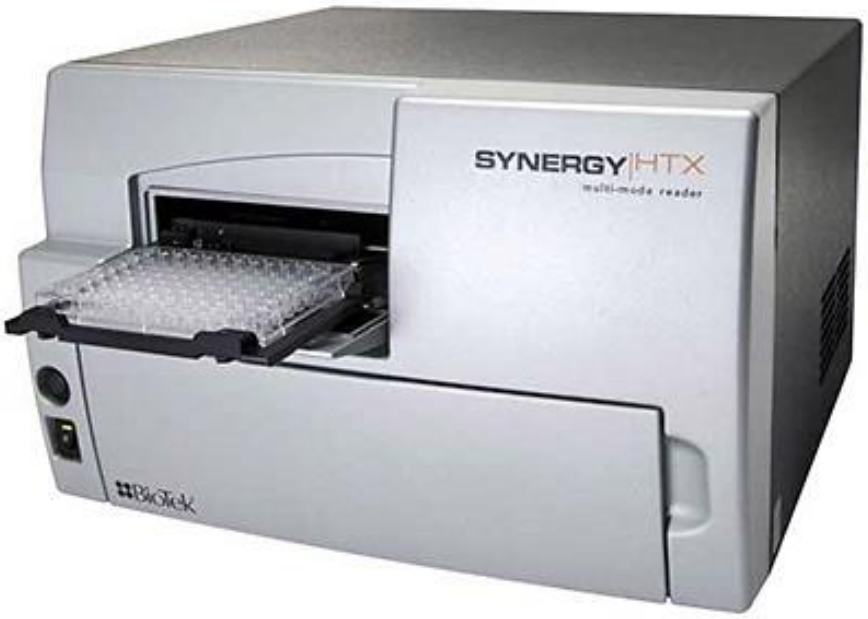
Procedure for instrument run:
1. 'Turn ON' the mains using the switch located on the instrument. Also, 'Turn ON' the computer workstation and launch the Gen5 Software.
2. Wait for 5 minutes while the instrument is being calibrated. After successful calibration, a final beep sound will come. Now, put a plate on the plate stand.
3. Select Absorbance, Chemiluminescence or Fluorescence as per your requirement and set an appropriate wavelength.
4. Select plate layout. You can also select plate layout manually.
5. Set shaking parameters if required (10s in linear mode is recommended).
6. RUN the protocol and wait until it is completed.
7. Save the file in .xpt format only in your folder. You can export into excel whenever required.
8. Once your experiment gets completed a. Remove the sample plate from the machine. b. Close the Gen5 software window. c. 'Turn OFF' the machine as well as the computer workstation.

Do's and Don'ts:
A. Only those are allowed to use the instrument who have taken hands on training from the technical person. Others also can use but they need to take help from trained persons. B. Do not keep plate until instrument gets completely calibrated. **C.** Wait for final beep sound. **D.** While using Take3 Micro-Volume Plate for DNA and/or RNA quantification, wipe the plate with Kimwipes only. Do not use tissue paper. Also take extra care for this plate as it's sensitive and costly too. **E. Do not modify any critical calibration parameters of the instrument without permission.**

Operating schedule: Please ensure availability of instrument at required time before setting up any experiments. **Caution:** Proper GLP should be followed. Gloves should be worn for Take3 plate. Hazardous samples should be handled carefully. Care should be taken for sample spillage, cleaning and material disposal procedures.

Data collection: Use formatted pen drive to retrieve the data from the PC and use it for further analysis.

Documentation: Maintain complete records in log book.



Synergy™ HTX Multi-Mode Microplate Reader

Shimadzu LC 20AT HPLC System

Name of the facility/ activity: HPLC

Instrument Incharge: Dr PS Nagar

Department: Department of Botany

Name, Model of Instrument: Shimadzu LC 20AT HPLC system

Software name: Class VP 5.0

Introduction & Principle: Relevant background information, methods of the specific instrument to be used. Chromatography is a technique to separate mixtures of substances into their components on the basis of their molecular structure and molecular composition. This involves a stationary phase (a solid, or a liquid supported on a solid) and a mobile phase (a liquid or a gas). The mobile phase flows through the stationary phase and carries the components of the mixture with it. Sample components that display stronger interactions with the stationary phase will move more slowly through the column than components with weaker interactions.

All chromatographic separations, including HPLC operate under the same basic principle; separation of a sample into its constituent parts because of the difference in the relative affinities of different molecules for the mobile phase and the stationary phase used in the separation.

Applications: The PDA UV-Vis spectrophotometer has a multi-channel detector, controlled by a microprocessor, which can collect spectral data for many wavelengths simultaneously. Photometric diode array with measurement wavelength range from 190nm to 900nm and resolution of 1nm. HPLC with PDA detector is good for the analysis of Alkaloids, phenols and most of the Pesticides.

Calibration status: Calibrated

Requirement of Sample preparation eg. Filtered, properly sealed coverslips, compatible chemicals and consumables, type of sample, precaution: Samples should be filtered without any particulate matter and chemicals should be of HPLC grade. Apart from sugars, it is good for the analyses of alkaloids, phenols, pesticides.

Procedure for instrument startup:

- Switch on the Power of all the LC units and System Controller.
- Switch on the Power of Computer, Monitor and Printer.
- Click <Start>, <Programs>, <Chromatography>, and <CLASS-VP 5.0>
- Shimadzu CLASS-VP Main menu will be opened. Double-click <Instrument 1>.
- Type your User name. Click and type your Password.
- [Default: - User Name: - <System> and Password: - <2001>.]
- Wait for the beep sound from the SCL-10Avp.
- Create new method file by going in <New> <File>.

Do's and Don'ts:

Flush the column properly atleast for 2hr and check the line for bubbles. The pump should never run out of solvent. The solvents must be of HPLC grade and the final sample preparation should also in HPLCgrade solvent. Minimum use of buffers.

Operating schedule: (gap b/w two experiments or time being experiment) : Atleast 24hrs

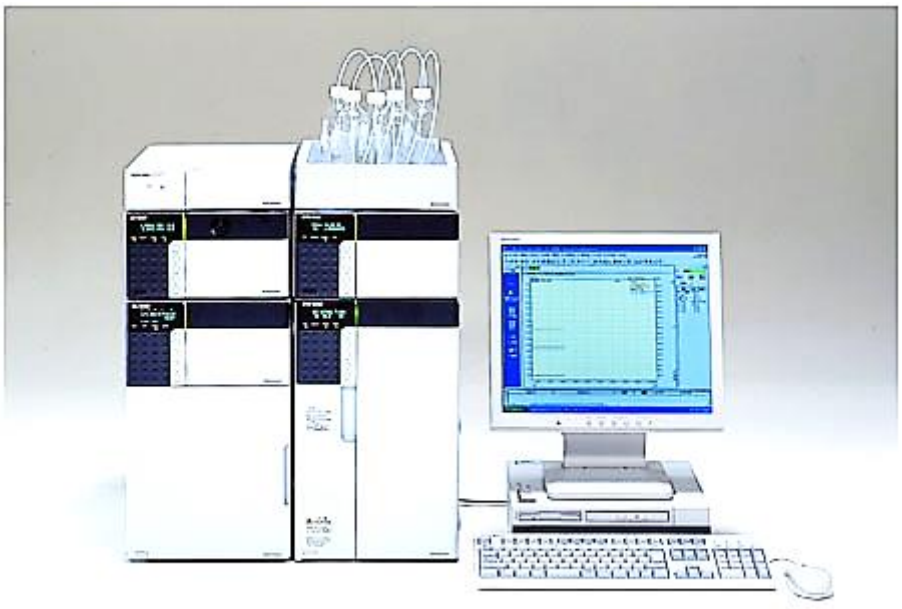
Caution: Proper GLP should be followed. Glows should be worn. Hazardous samples should be handled carefully. Care should be taken for sample spillage, cleaning, sample and relevant material disposal procedures.

Shut down procedure: Proper shutdown procedure should be followed.

After the analyses, give washing to the column atleast for 1hr with minimum flow rate. Stop the flow rate and pump, close the software and monitor and then the LC system.

Data collection/ analysis: Type of data acquisition / File format which we can provided to users.
PDF and Word file in virus free pendrive.

Documentation: Maintain complete records for the calibration and performance checks of all equipment and instruments.



Shimadzu LC 20AT HPLC System

Shimadzu GC-2010 Gas Chromatography

Name of the facility/ activity: Gas Chromatography

Instrument In charge: Dr. PS Nagar and Prof Aruna Joshi

Department: Department of Botany

Name, Model of Instrument: Shimadzu GC-2010 Gas Chromatography

Software name: GC Solution

Introduction &Principle: Relevant background information, methods of the specific instrument to be used.
Gas chromatography runs on the **principle of** partition chromatography for separation of components. The stationary phase is a liquid layer supported over a stationary phase while the mobile phase is an inert and stable gas. Hence the name as Gas-Liquid chromatography (GLC).

Applications:
An FID typically uses a Hydrogen/Air flame into which the sample is passed to oxidise organic molecules and produces electrically charged particles (ions). The ions are collected and produce an electrical signal which is then measured. Essential Oil, all kinds of oils (esterified), and also used for petrochemical, pharmaceutical and natural gas markets.

Calibration status: calibrated

Requirement of Sample preparation eg. Filtered, properly sealed coverslips, compatible chemicals and consumables, type of sample, precaution:
Samples should be filtered and should be easily vaporized when injected in GC, injected sample boiling point should not be above than 350˚c and chemical should be GC grade. Essential Oil and all kinds of oils (esterified).

Procedure for instrument startup:
1. Switch on the Power of all the GC units and System Controller.
2. Switch on the Power of Computer, Monitor and Printer.
3. Open the gas flow knob one by one; Nitrogen→ Air→ Hydrogen
4. Click <Start>, <Programs>, and <GC Solution>
5. Double-click the icon to start up the program. The following <Login> screen will appear. Enter your user ID and password

6. Just click the [OK] button without entering a password when you start up the program for the first time, for no password has been assigned at that time.
7. To log into the program, wait for the beep sound from the instrument.
8. Click the [Configuration and Maintenance] then click the [Configuration] icon on the Assistant Bar to set up the GC devices configuration such as the column information. You need not set up [Configuration] every time you run the program because the previous configuration has been saved. (Change the configuration only when it is necessary such as that the column has been replaced or any component has been changed.)

Do's and Don'ts:
Open the gas flow knob in a sequence Nitrogen→ Air→ Hydrogen. Check the gas filters, always take injection port temperature higher than the column temperature. Always check the gas leakage by using sops solution. Sample must be properly filtered

Operating schedule: (gap b/w two experiments or time being experiment)

Caution: Proper GLP should be followed. Glows should be worn. Hazardous samples should be handled carefully. Care should be taken for sample spillage, cleaning, sample and relevant material disposal procedures.

Shut down procedure: Proper shutdown procedure should be followed.
Once the analysis is done set the new program to lower down the column temperature near 40˚C after this close the software and then shut the GC system after that close the monitor then at end close all the gas valves.

Data collection / analysis: Type of data acquisition / File format which we can provided to users.

PDF and word file in virus free pen drive.

Documentation: Maintain complete records for the calibration and performance checks of all equipment and instruments.

Shimadzu GC-2010 Gas Chromatography

UVP GelDoc-It^2 Imaging System

Name of the facility / activity: Gel documentation unit

Instrument Incharge: Dr Nagesh Chirumamilla

Department: Department of Botany

Name, Model of Instrument: UVP GelDoc-It^2 Imaging System

Software name: Image capture and analysis is done using VisionWorks Acquisition and Analysis software on an external computer.

Introduction & Principle: This equipment is widely used in molecular biology laboratories for the imaging and documentation of nucleic acid and protein suspended within polyacrylamide or agarose gels. (Lee et al., 2012). These gels are typically stained with ethidium bromide or other nucleic acid stains such as GelGreen. Generally, a gel doc includes an ultraviolet (UV) light transilluminator, a hood or a darkroom to shield external light sources and protect the user from UV exposure, and a CMOS camera for image capturing.

Applications: This is an upgradeable system for imaging and quantitation of DNA gels, protein gels, blue light gels, Coomassie Blue, GelGreen, SYBR Green and more.

Calibration status: Camera focus features and positions are calibrated at every six month interval.

Requirement of Sample preparation eg. Filtered, properly sealed coverslips, compatible chemicals and consumables, type of sample, precaution: Lab gloves must be used for handling of EtBr stained gels

Procedure for instrument startup: Switch on instrument, switch on external computer, open VisionWorks acquisition and Analysis software and capture image

Do's and Don'ts:

Operating schedule: (gap b/w two experiments or time being experiment): not applicable

Caution: Proper GLP should be followed. Glows should be worn. Hazardous samples should be handled carefully. Care should be taken for sample spillage, cleaning, sample and relevant material disposal procedures.

Shut down procedure: Proper shutdown procedure should be followed: Save captured image in JPEG/ TIF format, switch of software, switch off instrument and external computer

Data collection/ analysis: Type of data acquisition / File format which we can provided to users.

Documentation: Maintain complete records for the calibration and performance checks of all equipment and instruments.

UVP GelDoc-It2 Imaging System

BiosatB Fermenter with Accessories

Name of the facility/activity: Fermenter

Instrument Incharge: Prof G. Archana/ Mr. Mihir sarang, Mr. Ajinkya Thakare

Department: Department of Microbiology

Name, Model of Instrument: BiosatB

Software name: Not applicable

Introduction &Principle: A fermenter refers to a device or system that supports a biologically active environment. The fermenter is a vessel in which a chemical process is carried out which involves organisms or biochemically active substances derived from such organisms. This process can either be aerobic or anaerobic. These bioreactors are commonly cylindrical, ranging in size from litres to cubic metres, and are often made of stainless steel.

Applications: The fermenter can be used for variety of purpose such as growth of bacterial biomass, production of bioactive compounds, growth of microalgae etc.

Calibration status: Not applicable

Requirement of Sample preparation: Before operation the fermenter must be thoroughly washed and autoclaved at 121 °C for 15 mins, along the desired media for growth.

Procedure for instrument startup:

1. Prepare the required media for the growth of your organism and pour it in the fermenter.
2. Autoclave the fermenter along with the media at 121 °C for 15mins.
3. Once the fermenter is autoclaved it must be allowed to cool at room temperature.
4. The biomass to be inoculated in the fermenter must be first grown at shake flask level and scaled up to attain required cell density.
5. Once the inoculum is ready, inoculate in the fermenter in sterile conditions. A small cotton ball dipped in kerosene can be ignited near the sample inlet valve during inoculation.
6. Set the desired RPM of the impeller using controller.
7. Samples can be withdrawn from the fermenter, using a sterile syringe – inserted in sample collection pipe.
8. Once the run is complete, collect all the sample through sample collection pipe.
9. Thoroughly wash the fermenter and perform decontamination in autoclave.

Do's and Don'ts:

- Maintain the pH and appropriate dissolved oxygen concentration while operating the fermentor.
- Avoid inoculation of the sample if the media inside the fermenter is hot. During inoculation of sample, precaution must be taken while igniting the cotton dipped in kerosene.

Operating schedule: No particular schedule

Caution: Care should be taken to prevent culture spillage. Proper treatment of spillage should be done at the site of origin. Care should be taken while igniting the cotton dipped in kerosene.

Shut down procedure: once the run is complete, the fermenter must thoroughly washed and be re-autoclaved for proper decontamination.

Data collection/analysis: Samples can be withdrawn from fermenter using as sterile syringe.

Documentation: Maintain complete records for the use and performance checks of the instrument. Put details in the Log book after each use.

BiosatB Fermenter

Waters High Performance Liquid Chromatography

Name of the facility/activity: High Performance Liquid Chromatography (HPLC)

Instrument Incharge: Prof G. Archana/Mr. Ashtaad Vesuna, Mr. Ajinkya Thakare

Department: Department of Microbiology

Name, Model of Instrument: Waters HPLC (Binary HPLC pump, UV/ Visible detector, Temp. control module)

Software name: Breeze

Introduction & Principle: High-performance liquid chromatography (HPLC; formerly referred to as high-pressure liquid chromatography) is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column.

Applications: High-performance liquid chromatography (or high-pressure liquid chromatography, HPLC) is a chromatographic technique that can separate a mixture of compounds and is used to identify, quantify and purify the individual components of the mixture. Samples sure as carbohydrates, proteins etc can be detected.

Calibration status: Done during each run.

Requirement of Sample preparation – Filter and dissolve the sample in organic solvent like methanol, ethanol, ethyl acetate (HPLC grade only). Mobile and stationary phase solvents required to run the sample will be as per the individual experiment requirements.

Procedure for instrument startup: Turn on switches: (in order) - Computer, UV/Visible Detector, column box, Binary HPLC pump, Temperature control module II. Turn on PC and start Breeze software to begin work.

Do's and Don'ts:

- User must know the solvents and their ratios required to prepare the mobile phase.
- User must know about the column required for the run.
- User must know the operating conditions for the run as to: wavelength, temperature.
- Use only HPLC grade solvents.
- Samples must be filtered to remove suspended particles.
- Always follow proper protocol for shutting down the system.
- Do not use contaminated solvents.
- Do not use the instrument without prior knowledge of its hardware or software.

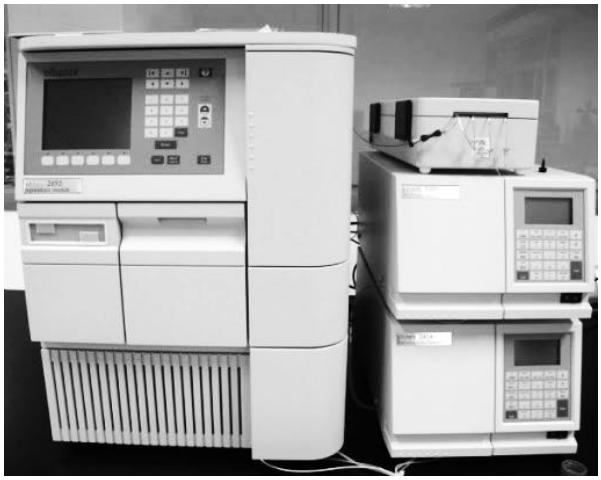
Operating schedule: Give the machine a rest of 1 hours after 4 hours

Caution: Proper GLP should be followed. Gloves should be worn. Hazardous samples should be handled carefully. Care should be taken for sample spillage, cleaning, sample and relevant material disposal procedures.

Shut down procedure: Close the Breeze application with proper procedure. Shut down the PC. Switch off (in order) - Computer, UV/Visible Detector, column box, Binary HPLC pump, Temperature control module II.

Data collection/analysis: Graphs and data in PDF format

Documentation: Maintain complete records for the use and performance checks of the instrument. Put details in the Log book after each use.



Waters HPLC System

Kubota High Speed Refrigerated Centrifuge 6500

Name of the facility/activity: High speed refrigerated centrifuge

Instrument Incharge: Prof G. Archana/ Mr. Ashtaad Vesuna, Ms. Prachi Gandhi

Department: Department of Microbiology

Name, Model of Instrument: Kubota High Speed Refrigerated Centrifuge 6500

Software name: Not applicable

Introduction & Principle: Refrigerated centrifuge works on the concept of sedimentation principle by holding up the sample tubes or bottles with a capacity of 40ml and 250ml, respectively in rotation around a fixed axis. In this, the centripetal force causes the denser substances to separate out along the radial direction in the bottom of the centrifuge tube. The rate of the centrifugation is calculated by the acceleration applied to the sample and it is typically measured in revolution per minute (RPM) or relative centrifugal force (RCF). The particle's settling velocity during centrifugation depends on the function of their size and shape, centrifugal acceleration, the volume fraction of solids present, the density difference between the particle and the liquid, and the viscosity.

Applications: This equipment is extensively used in chemistry, biology, and biochemistry for isolating and separating suspensions. It additionally provides the cooling mechanism to maintain the uniform temperature throughout the operation of the sample.

Calibration status: None required.

Requirement of Sample preparation – Sample should be put in appropriate centrifuge tubes or bottles compatible with the instrument. The tubes/bottles should be weighed with the samples and it should be made sure that they are balanced before starting the centrifuge.

Procedure for instrument startup: Turn on the centrifuge. Set the temperature if required.

Do's and Don'ts:

- The samples should always be weighed and balanced.
- Clean the machine and rotors properly after use.
- Do not exceed the Maximum Operating Speed (MOS). MOS for centrifuge tubes (40ml) is 15000 rpm at room temperature and 12000 rpm at 4°C. MOS for centrifuge bottles (200-250ml) is 11000 rpm at room temperature and 10000 rpm at 4°C.
- Do not use imbalanced bottled.
- Do not fill the sample till the top of the bottle
- Do not screw the tube/bottle cap very tight.

Operating schedule: Give the machine rest for 2hrs after 1 hour of use.

Caution: Proper GLP should be followed. Glows should be worn. Hazardous samples should be handled carefully. Care should be taken for sample spillage, cleaning, sample and relevant material disposal procedures.
Shut down procedure: Remove rotor, clean the rotor and the instrument and switch off the instrument from the power source.

Data collection/analysis: Not applicable

Documentation: Maintain complete records for the use and performance checks of the instrument. Put details in the Log book after each use.

Kubota High Speed Refrigerated Centrifuge 6500

Alphaimager-HP Gel Documentation System

Name of the facility/activity: Gel Documentation System

Instrument Incharge: Prof G. Archana/ Ms. Shubhangi Pandey, Ms. Prachi Gandhi, Mr. Jitendra Gosai

Department: Department of Microbiology

Name, Model of Instrument: Alphaimager-HP

Software name: Alphaimager HP

Introduction & Principle: The instrument houses a stages for placing agarose or polyacrylamide gels and has UV as well white light bulbs for both, incident and transilluminating paths. The instrument has a rotating filter which is (exclusively) controlled by software in the accompanying computer and can be set depending on the light source and purpose of experiment. The image can be captured using the inbuilt CCD camera and is viewed on and stored in the accompanying computer.

Applications: The Gel documentation instrument can be used to view or photograph agarose gels and polyacrylamide gels containing DNA or proteins.

Calibration status: Calibrated.

Requirement of Sample preparation eg. Filtered, properly sealed coverslips, compatible chemicals and consumables, type of sample, precaution: Must wear latex/plastic gloves when the agarose gels with etBr staining is used. The gloved hands must not touch other places such as keyboard or mouse.

Procedure for instrument startup: Turn the power supply on to the computer as well the machine. Turn the switch at the back of machine down to on position. Allow the PC to boot and launch the software. Let the filters reposition themselves (this makes some sound). Place the gel on the desired platform (commonly, the glass platform is used for agarose gels while the plastic platform is used for polyacryleamide gels). Default exposure and zoom settings give the best results and must not need to be adjusted unless specifically required. Select the filter and light source from the user-friendly software UI. Click on preview or acquire to just view or photograph the gel. This image can be processed or saved in a number of file formats from File>Save as (or Save modified if image was edited for clarity etc.) including .tif (recommended) or .jpg *et cetera*. The gels can then be removed and PC and machine be turned off.

Do's and Don'ts:

- Agaorse gels containing EtBr must be handled with gloves and the gloved hands must not touch at any other place than the door of the machine and the platform.
- The platform must be wiped before and after the use ensuring lints/pieces are not scattered inside or around machine.

- Making an entry in the logbook with all the details is must.
- Images should be saved only in a designated folder in D:/Data/'your folder' and should not be saved anywhere else.
- The machine and PC must be turned off after the use.

Operating schedule: No particular schedule.

Caution: Proper GLP should be followed. Glows should be worn. Hazardous samples should be handled carefully. Care should be taken for sample spillage, cleaning, sample and relevant material disposal procedures.

Shut down procedure: Once the imaging is done, the gel documentation machine must be turned off using the switch at its back, and then powered off from the switch board. The PC should be shut down and powered off.

Data collection/analysis: Image files are saved in .tif or .jpg format.

Documentation: An entry in the logbook is must, please contact any of the incharges in case of any query or malfunction.

Alphaimager-HP Gel Documentation System

New Brunswick Scientific Incubator Shaker

Name of the facility/activity: Incubator Shaker

Head/Instrument Incharge: Prof G. Archana/ Mr. Anand Dave, Ms. Shubhangi Pandey

Department: Department of Microbiology

Name, Model of Instrument: New Brunswick Scientific Incubator Shaker

Introduction & Principle: A shaker is a piece of laboratory equipment used to mix, blend, or agitate substances in a tube or flask by shaking them. It is mainly used in the fields of chemistry and biology. A shaker contains an oscillating board that is used to place the flasks, beakers, or test tubes. Although the magnetic stirrer has lately come to replace the shaker, it is still the preferred choice of equipment when dealing with large volume substances or when simultaneous agitation is required. An orbital shaker has a circular shaking motion with a slow speed (25-500 rpm). It is suitable for culturing microbes, washing blots, and general mixing. Moreover, it can be modified by placing it in an incubator to create an incubator shaker due to its low temperature and vibrations.

Applications: For providing sterile enclosed environment to conduct sensitive scientific work, which could be a potential harmful to the user and surrounding environment**.**

Calibration status: Not applicable

Requirement of Sample preparation: No specific requirement

Procedure for instrument startup:

1. Put your sample material in an acceptable container with a lid.
2. Gently press the container in one of the spring housings until it is securely in place.
3. Close the lid of the incubator and turn on the machine using the power switch to the right-hand side. The LED display will momentarily show the model number. (NOTE: the shaker will not operate of the lid is open).
4. Once the machine is powered on, the incubator will start running. Pressing the start/stop button will cause the shaking to stop.
5. Press the select button until the RPM indicator is illuminated on the left-hand side of the control panel.
6. Use the arrow keys to set the RPM of the shaker. A value from 50 to 400 RPM is available. The number will set when no buttons are pressed.
7. Press the select key until the ºC INDICATOR illuminates.
8. Set the temperature using the arrow keys. Temperature range is from 4º to 60ºC
9. Press the select key until the HRS INDICATOR is illuminated.
10. Use the arrow keys to set the TIME of the shaker. This can be a value from .1 to 99.9. The number will set when no buttons are pressed. If a continuous run time is desired, simply press the START/STOP button.
11. Press the START/STOP key. The shaker will start in untimed mode.
12. Press the START/STOP key again. The shaker will stop and the display will read OFF.
13. Press the START/STOP key a third time; the time indicator will light and the shaker will now start the timed run.
14. The machine will come to a stop once the timed run has ended. If running in untimed mode, the START/STOP key can be pressed at any desired time.

Shutdown procedure

1. Make sure the machine has come to a complete stop and open the lid.
2. Remove any samples you need. Use a hot glove if high temperatures were set.
3. Turn off the power by flipping the switch on the right side of the machine.

Do's and Don'ts:

- Wear appropriate personal protective equipment (PPE) before starting to work on BSC.
- Perform surface decontamination on the work area before and after using BSC.
- Place the waste container (biohazard bag) inside the BSC work area.

- Place all items and apparatus in the safe working area
- Minimize room activities, since these external airflow disturbances may affect the BSC's internal flow.
- Work as far back in the BSC as possible- at least 150 mm (6 inches)
- It is recommended that the BSC be operated continuously in order to achieve optimal containment and cleanliness.

Operating schedule: Please ensure availability of instrument at required time before setting up any experiments.

Caution: Do not change any parameters of the instrument without permission.

Shut down procedure: Proper shutdown procedure should be followed.

1. Turn OFF the fan by pressing the FAN button. Input the FAN if asked. This will start the post purge procedure (default: 0min). All buttons are disabled during post purge period.
2. Lower the sash to the fully closed position (the display will show UV MODE). The sash can be lowered immediately after turning off the fan as it will not interrupt the post purge procedure. NOTE: When Quickstart mode is selected, fan will turn off without pressing the fan button.
3. Turn ON the UV lamp to decontaminate the work area by pressing the UV button. Leave the UV lamp on to make sure the decontamination is done effectively. The UV lamp can only be turned ON after the post purge procedure is finished.
4. Put OFF the main power button for BSC.

Documentation: Maintain complete records for the use and performance checks of the instrument. Put details in the log book after each use.

Applied Biosystems StepOne Real-Time PCR System

Name of the facility/activity: Real time PCR

Instrument Incharge: Prof G. Archana/Ms. Roshni Patel, Ms. Juliya Thomas, Ms. Abhi shah

Department: Department of Microbiology

Name, Model of Instrument: Real time PCR, StepOne cycler applied biosystem

Software name: Step one

Introduction & Principle: Real-time PCR is used for sensitive, specific detection and quantification of nucleic acid targets. The StepOnePlus™ Real-Time PCR is a 48 well Real-Time PCR instrument perfect for performing gene quantification in real time. Utilizing robust LED based 4-color optical recording, the StepOnePlus™ Real-Time PCR System is designed to deliver precise, quantitative Real-Time PCR results for a variety of genomic research applications. The StepOnePlus™ Real-Time PCR System utilizes a long-life LED-based optical system that can record fluorescence from FAM™/SYBR® Green, VIC®/JOE™, NED™/TAMRA™, and ROX™ dyes. This cost-effective, 4-color, 48-well system delivers precise, quantitative Real-Time PCR results and saves data from all filters in every run without depending on a computer or plate setup. It can discriminate between 2 populations of 5,000 and 10,000 template copies of a TaqMan® assay with 99.7% confidence.

Principle: The principle of real-time PCR relies on the use of fluorescent dye. The amount of the nucleic acid present in the sample is quantified using the fluorescent dye or using the fluorescent labelled oligos. Two types of chemicals are available for the real-time quantitative PCR:
1) DNA binding dye (Intercalating dye-based method)
2) Sequence-specific probe (Hydrolysis Probe-based detection method)

Melting curve analysis:
Once the amplification reaction is completed and the fluorescence signals are recorded, the template is melted for the determination of the non-specific bindings.
The template is melted using heating, the dye dissociates and the fluorescence signals are reduced.
The decreased transition of a wide range fluorescence is reported for the specific product while different heat transition recorded for different short non-specific bands.

Specifications of the instrument:
Capacity: 1 x 48-well plate, 48 x 0.1 ml tubes
Detection Method: Primer-Probe Detection, SYBR
Dimensions: 24.6 cm/9.7 in (W) x 42.7 cm/16.8 in (D) x 51.2 cm/20.2 in.(H)
Dynamic Range: Linear Dynamic Range greater than 9 log units (detection)
For use With (Equipment): StepOne™
Format: 0.1 ml tubes, 48-well Plate
Green Features: Energy efficient, fewer resources used and less waste

High Throughput Compatibility: Multiplexing
Optics: 3 emission filters, LED, photodiode
Passive Reference Dye: No ROX, ROX (Pre-mixed), ROX (Separate Tube)
Peak Block Ramp Rate: 4.6°C⁄sec
Product Line: StepOne™
Product Size: 1 instrument
Reaction Speed: Fast,Standard
Reaction Volume Range: 10-30 µl (Standard curve experiments: 40 µl in standard mode is validated)
Run Time: <2 hrs⁄run (Standard Mode),<40 min⁄run (Fast Mode)
Sample Ramp Rate: Fast mode: ± 2.2°C⁄sec, Standard mode: ± 1.6°C⁄sec
Sensitivity: 1 copy
Temperature Accuracy: 0.25°C (35 to 95°C) of display temperature
Temperature Range (Metric): 4-100°C
Temperature Uniformity: 0.25°C (35 to 95°C) of setpoint/display temperature
Thermal Cycling System: Peltier-Based System

Applications: SNP Genotyping, Gene Expression Analysis, MicroRNA Expression, Protein Expression, Translocation Analysis, Gene Detection, Viral Load Analysis

Calibration status: Not applicable

Requirement of Sample preparation: SYBR green master mix or TaqMan probe reaction system

Consumable: 96well plates.

Type of sample: cDNA for Gene expression study, DNA for Gene quantification

Procedure for instrument startup:
1. Switch on the instrument and the connected PC.
2. Once it's switched on, instrument starts it's calibration while which it should not be interrupted
3. Open the StepOne software from the PC
4. Open a new file and go to advanced setup
5. Input all the reaction setup starting from the status, plate setup, sample name, target name, amplification cycle and volume
6. Once all the setup is done, press start and save the method

Do's and Don'ts:
- Remove shoes outside the real time room to keep it dust free.
- Wear gloves while dealing with real time plates or tubes as the fingerprints can hamper the fluorescence quenching.
- AC should be switched on while the reaction is going on.

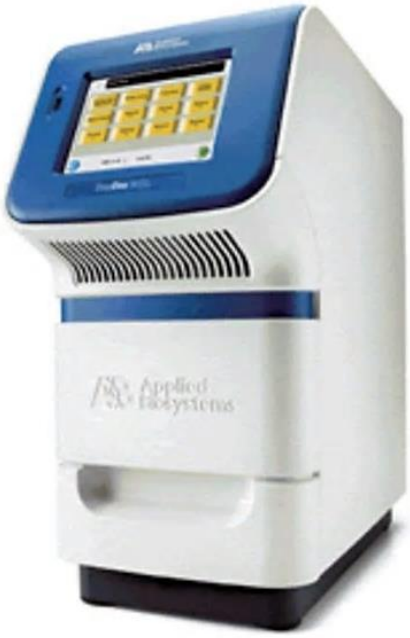
Operating schedule: 2-3 hours

Caution: Proper GLP should be followed. Gloves should be worn. Hazardous samples should be handled carefully. Care should be taken for sample spillage, cleaning, sample and relevant material disposal procedures.

Shut down procedure: Once the run is finished, ensure that the document file is saved properly. Close the software and shut down the PC. Once the PC is off, shut the main switch.

Data collection /analysis: Data can be collected as Excel sheet.

Documentation: Maintain complete records for the use and performance checks of the instrument. Put details in the log book after each use.



Applied Biosystems StepOne Real-Time PCR System

LabTech Refrigerated shaking incubator

Name of the facility/activity: Refrigerated shaker Incubator

Instrument Incharge: Prof G. Archana/ Mr. Anand Dave, Ms. Jaswinder Kaur Saini, Mr. Jitendrapuri Gosai

Department: Department of Microbiology

Name & Model of Instrument: LabTech shaker Incubator

Software name: Not applicable

Introduction & Principle: The Labtech Shaker Incubator is a floor unit that is used for microbial growth in liquid cultures. The power switch is located on the right side of the machine, near the front. A lamp switch, safety knob are next to the power switch. The operation is run entirely from the control panel on the front of the machine. Buttons are used to control speed and temperature and time of incubation. It is capable of running maximum of 300 rpm. Inside is a platform with Sample holding Clamps into which Erlenmeyer flasks can be easily accommodated. There is also a lamp in the incubator that allows to view samples from the clear glass. The functioning of the incubator automatically comes to halt when the lid is opened.

The control panel consists of a display to visualize the current settings. Temperature, rpm and COOL buttons are used to switch on an off those parameters. SET button on the panel is used to toggle between rpm, temperature and timer settings. AT button is used to toggle between the digits of the shown parameters. DSP button is used to change the blinking digit between 0-9.
This equipment is designed to mix components by horizontal plane at set rotary motion and at set temperatures. The incubator is intended to ensure optimum growth conditions for the microbial cultures

Applications: LabTech shaker Incubator is used to grow microbial cultures, incubate samples at their optimal temperatures and provides a rotatory effect to get a homogenized culture.

Calibration status: Not applicable

Requirement of Sample preparation:
Only Flasks of 250mL to 100mL can be kept in the shaker incubator. For flasks with smaller volume (below 250ml), a rubber band is to be used to tighten the clamp to the flask to avoid movement of the sample during rotation. Test tubes can also be incubated by keeping them in a beaker, and tightening the beaker to the sample clamp.

Procedure for instrument startup:
1. Turn on the power switch located above the instrument.
2. The display turns on and is available.
3. Ensure that the chamber door is properly closed.
4. Press SET button on the panel, it displays the present parameter that is to be changed
5. Use AT button to toggle between the blinking Digits.
6. Press DSP to change the blinking digit between 0-9
7. Use the SET button again to toggle to the next two parameters and change your values using AT and DSP as mentioned.
8. Press SET again to confirm and Lock the settings
9. Make sure the Temperature, rpm and timer settings are as per your requirement**.**

Do's and Don'ts:
- Do not change any parameters of the instrument without permission.
- Always Keep the lid closed
- Always connect the Incubator to a UPS

Operating schedule: Please ensure availability of instrument at required time before setting up any experiments.

Caution: Proper GLP should be followed. Gloves should be worn. Care should be taken for sample spillage, cleaning and relevant material disposal procedures. Be cautious when using the apparatus with high speed. Always

cover your Containers to avoid Spillage. Never use caustic cleaning agents such as soap suds, phosphoric acid, bleaching solutions or scrubbing power for cleaning the platform.

Shut down procedure: Proper shutdown procedure should be followed.

1. Switch the Power off from the panel and Switch the main power off.
2. Remove Flasks, beakers gently from the instrument and close the chamber door.
3. Remove any dust or other foreign objects from the incubator platform with a soft towel or cloth.
4. Ensure that the main power switches are turned off before leaving.
5. Remember to make an entry in the log book after every use.

Data collection/analysis: Not applicable

Documentation: Make an entry in the log book after each use.

Shimadzu UV-1800 UV-VIS spectrophotometer

Name of the facility/activity: UV-VIS spectrophotometer

Instrument Incharge: Prof G. Archana/ Ms. Mansi Agarwal, Ms. Dipeksha Hansoti, Ms. Prachi Gandhi

Department: Department of Microbiology

Name and Model of Instrument: Shimadzu UV-1800 Spectrophotometer

Software name: UV Probe

Introduction & Principle: UV spectroscopy obeys the Beer-Lambert law, which states that: when a beam of monochromatic light is passed through a solution of an absorbing substance, the rate of decrease of intensity of radiation with thickness of the absorbing solution is proportional to the incident radiation as well as the concentration of the solution. The expression of Beer-Lambert law is

$A = \log (I0/I) = \varepsilon c l$

Where, A = absorbance, I0 = intensity of light incident upon sample cell, I = intensity of light leaving sample cell, c = molar concentration of solute, l = length of sample cell (cm), ε = molar absorptivity. From the Beer-Lambert law it is clear that greater the number of molecules capable of absorbing light of a given wavelength, the greater the extent of light absorption. This is the basic principle of UV spectroscopy.

Applications: One-wavelength and Multi-wavelength measurement, Spectrum measurement, Quantitation of calibration curve, Kinetics and Kinetics Rate Measurement, Time Scan, Multi-component Quantitation, Bio-method for DNA and protein concentrations

Procedure for instrument startup:

All switches are marked for ease of use.

1. Switch ON the main switch on switchboard behind spectrophotometer marked UV-1800 for instrument. For use of computer, switch ON the switches marked CPU and monitor.
2. Switch ON the instrument power on right bottom of instrument.
3. When the instrument is turned ON, the UV-1800 starts executing various checks and initializations. After that, [Login screen] will be displayed.
4. Press ‘Enter’ and display will switch to the [Mode menu] screen. To use appropriate function, press button number associated with it on instrument.
5. To set up the desirable wavelength, press button ‘GOTOWL’ and write wavelength then press ‘Enter’.
6. Use ‘START/STOP’ for measuring readings.
7. Launch UV Probe Software using Desktop icon.

Do’s and Don’ts:

- Before turning ON the power switch of UV-1800, ensure that nothing is placed in the sample compartment and cell holder.
- Keep the sample compartment cover closed during measurement.

Operating schedule: A half an hour gap between two experiments is required.

Caution: Proper GLP should be followed. Gloves should be worn. Hazardous samples should be handled carefully. Care should be taken for sample spillage, cleaning and relevant material disposal procedures.

Shut down procedure: Proper shutdown procedure should be followed.

1. After work is done, press ‘RETURN’ till [Login screen] is displayed.
2. Switch OFF the instrument switch and main switches after use.

Data collection/ analysis: Type of data acquisition / File format which can be provided to users: Table data file has to be saved with extension *.pho and Curve data file has to be saved with extension *.spc and *.tmc. Save your data in path computer/ new volume (E:)/ USER DATA.

Documentation: An entry after every use has to be made in the logbook. Any remarks or problems encountered during usage should be mentioned in the logbook.

Shimadzu UV-1800 UV-VIS spectrophotometer

Shimadzu GC-2014 Gas Chromatography

Name of the facility/activity: Gas chromatography

Instrument Incharge: Prof G. Archana/ Mr. Mihir Sarang, Ms. Shubhangi Pandey, Ms. Roshni Patel

Department: Department of Microbiology

Name, Model of Instrument: Shimadzu GC-2014

Software name:

Introduction & Principle:
Gas HYPERLINK https://www.sciencedirect.com/topics/materials-science/chromatography \o "Learn more about Chromatography from ScienceDirect's AI-generated Topic Pages" chromatography (GC) is a separation technique capable of separating highly complex mixtures based primarily upon differences of boiling point/ HYPERLINK https://www.sciencedirect.com/topics/chemistry/vapor-pressure \o "Learn more about Vapor Pressure from ScienceDirect's AI-generated Topic Pages" vapor pressure and of HYPERLINK https://www.sciencedirect.com/topics/chemistry/polarity \o "Learn more about Polarity from ScienceDirect's AI-generated Topic Pages" polarity. It utilizes an inert gaseous mobile phase and a liquid stationary phase. It used for the analysis and quantification of volatile compounds. Compounds are injected onto the gas chromatograph and flash evaporated onto the column. This can be done using a packed or capillary column. The column is the stationary phase and the gas is the mobile phase. The compounds are separated on the column by how they interact with these phases; heat also moves the compounds along the column. After leaving the column, the compounds can be detected by various detectors.

Applications: It is used in the separation and identification of lipids, carbohydrates and proteins. It is hence very commonly used in food industries to determine their nutritional value and also the presence of pesticides. It can also be used to determine the presence of pollutants like formaldehyde, carbon monoxide, benzene or DDT etc. In laboratories, it can be used to analyze the alcohol content in blood. Thus, any organic compound which is volatile or can be derivatized into one, can be separated and identified using GC.

Calibration status: Not applicable

Requirement of Sample preparation: The sample must be filtered properly sealed. Avoid the presence of water in samples as this can damage the GC column.

Procedure for instrument startup:

Do's and Don'ts:
- Avoid presence of water in sample.
- Wait for the GC column to attain the desired temperature before introducing the sample.
- Flammable solvents must be handled with care.

Operating schedule:
Caution: Proper GLP should be followed. Gloves should be worn. Hazardous samples should be handled carefully. Care should be taken for sample spillage, cleaning, sample and relevant material disposal procedures. The sample should not have any water, else will damage the column. Care must be taken while dealing with hydrogen cylinder.

Shut down procedure: Proper shutdown procedure should be followed. The cylinder knobs (Hydrogen, Oxygen and air) should be properly closed to avoid any leakage.

Data collection/analysis: Type of data acquisition / File format which we can provided to users.

Documentation: Maintain complete records for the use and performance checks of the instrument. Put details in the Log book after each use.

Shimadzu GC-2014 Gas Chromatography

Agilent's Real-Time PCR System

Name of the facility/activity: Real Time PCR

Instrument Incharge: Prof G. Archana/ Ms. Roshni Patel, Ms. Juliya Thomas

Department: Department of Microbiology

Name, Model of Instrument: Real time PCR, Agilent Technologies

Software name: Aria Software

Introduction & Principle: Real-time PCR (RT-PCR) is also called quantitative PCR or qPCR. The key feature in RT-PCR is that amplification of DNA is detected in real time as PCR is in progress by the use of fluorescent reporter. The fluorescent reporter signal strength is directly proportional to the number of amplified DNA molecules. There are two detection methods of RT-PCR, the first is based on sequence-specific probe such as TaqMan probe, molecular beacon; the second is based on generic non-sequence specific double-stranded DNA-binding dye such as SYBR green. RT-PCR is a very sensitive and powerful DNA analysis tool. RT-PCR can be divided into four stages: linear ground phase, early exponential phase, linear exponential phase (log phase) and plateau phase. In the first phase, PCR is just starting, fluorescent signal has not risen above background. The second phase is where fluorescent signal just rise significantly above background, the cycle at which occurs is called *cycle threshold* (Ct). In linear exponential phase, PCR is in its optimal amplification stage with doubling PCR products in every cycle. The last phase is when substrates are exhausted and Taq DNA polymerase is in its end of life, fluorescent signal will no long increase.

Applications: Gene Expression and gene quantification.

Calibration status: Calibrated

Requirement of Sample preparation: SYBR green master mix or TaqMan probe reaction system**.**

Consumable: 96well plates.

Type of sample: cDNA for Gene expression study, DNA for Gene quantification

Procedure for instrument startup:

1. Switch on the instrument and the connected PC.
2. Once it's switched on, instrument starts it's calibration during this procedure it should not be interrupted.
3. Open the Aria software from the PC.
4. Open a new file.
5. Input all the reaction setup starting from the status, plate setup, sample name, target name, amplification cycle and volume.
6. Once all the setup is done, press start and save the method

Do's and Don'ts:

- Remove shoes outside the real time room to keep it dust free
- Wear gloves while dealing with real time plates or tubes as the fingerprints can hamper the fluorescence quenching.
- AC should be switched on while operating the instrument.

Operating schedule: 2-3 hours gap b/w two experiments.

Caution: Proper GLP should be followed. Glows should be worn. Care should be taken for sample spillage, cleaning, sample and relevant material disposal procedures.

Shut down procedure: Once the run is finished, ensure that the document file is saved properly.
Close the software and shut down the PC
Once the PC is off, shut the main switch off

Data collection/analysis: Data will be generated in the form of pdf and JPG.

Documentation: Maintain complete records for the use and performance checks of the instrument. Make an entry in the log book after each use.

Zeiss Confocal Microscope LSM 700

Name of the facility/activity: Confocal Laser Scanning Microscope

/Instrument Incharge: Prof G. Archana/ Dr. Rajesh Patkar

Department: Department of Microbiology and GRC

Name & Model of Instrument: Zeiss Confocal Microscope LSM 700

Software name: ZEN 2010

Introduction & Principle: Confocal microscopy offers several advantages over conventional optical microscopy, including elimination of out-of-focus glare, and the ability to collect serial optical sections from thick specimens. A major application of confocal microscopy involves imaging either fixed or living cells and tissues that have usually been labeled with one or more fluorescent probes.
Coherent light emitted by the laser system (excitation source) passes through a pinhole aperture that is situated in a conjugate plane (confocal) with a scanning point on the specimen and a second pinhole aperture positioned in front of the detector (a photomultiplier tube). As the laser is reflected by a dichromatic mirror and scanned across the specimen in a defined focal plane, secondary fluorescence emitted from points on the specimen (in the same focal plane) pass back through the dichromatic mirror and are focused as a confocal point at the detector pinhole aperture. By having a confocal pinhole, the microscope is really efficient at rejecting out of focus fluorescent light. The practical effect of this is that your image comes from a thin section of your sample (you have a small depth of field). By scanning many thin sections through your sample, you can build up a very clean three-dimensional image of the sample.

Applications: Capture Z-stack, Tile Scan, Live cell imaging.

Calibration status: Calibrated

Requirement of Sample preparation: Please ensure that the sample is ready for imaging (i.e. the required stain has been added and incubated for the required time) on properly sealed glass slides or multi-well plates. Ensure that the slides/plates are clean. No consumables will be provided.

Procedure for instrument startup: All switches are marked for ease of use.

1. Switch ON the main switches on extension board behind microscope table marked No. 1(computer) and No. 2 (microscope)
2. Switch ON two switches on stabilized Power supply 232 to microscope (near monitor)-No. 3 and 4 (Green light = ON)
3. For LSM (only if required), Turn key on laser chamber (No. 5) to 3'o clock position to switch on laser. (Orange light = ON)
4. For reflective light illumination (only if required), turn on the X-Cite lamp Series 120(No. 6).
5. Switch on power button on CPU (No. 7) to start computer
6. Launch **ZEN 2010** Software using Desktop icon and select Start System option.

7. Allow system to connect and align before selecting any light path or objectives.

For image processing:

1. Only turn on switch Nos. 1 and 6.
2. Launch ZEN 2010 Software using Desktop icon and select Processing option.

FIND YOUR SAMPLE

1. Click Ocular tab for direct observation through the eyepieces.
2. Pull back the head of the microscope carefully and place your sample (slide faced down). Bring down the "tilt back piece'' to start viewing.
3. Select Brightfield/DIC option for viewing.
4. Under Ocular tab select your desired objective.
5. Using the joystick move your sample to correct location and focus on your sample with the coarse and fine focus knobs. (Knobs are below the stage and on the side of the microscope).

6. Select a filter to view fluorescent samples (DAPI, GFP, DSRED)-once you have selected your filter the fluorescence shutter should automatically turn ON and the shutter to the transmitted light should close.

CONFIGURE SETTINGS

1. Click Acquisition tab
2. Turn on the lasers that you need and keep all others switched off.
3. Click Smart Setup
4. Choose your dyes of interest.
5. Under Proposals choose the configuration that best suits your sample- Fastest/ Best signal
6. Click apply to use selected settings

Do's and Don'ts:

- Please discuss experiment and applications with incharge prior to system use.
- Please book your date and time of use in advance.
- All waste generated must be removed and disposed of in the lab of origin. There are no waste disposal facilities provided in the facility.
- Confocal microscope uses intense laser & illumination light. Don't look directly at the light beam coming out of the objective.
- Take sufficient breaks when excessive use is required.
- Avoid direct contact with immersion oil and wash hands after use.

Operating schedule: A gap of 30 minutes between two experiments is required.

Caution: Proper GLP should be followed. Gloves should be worn. Hazardous samples should be handled carefully. Care should be taken for sample spillage, cleaning and relevant material disposal procedures.

Shut down procedure: Proper shutdown procedure should be followed.

1. Move the objective to an empty position
2. Switch back the laser turnkey. Allow the laser to cool off.
3. Exit ZEN2009 software
4. Follow starting procedure in reverse order to switch off all switches step by step.
5. Please clean after yourself! Do not leave any slides, coverglass, etc. lying around after use. (DO NOT CLEAN OBJECTIVES! ONLY the incharge person will clean objectives!)

Data collection/analysis: Type of data acquisition/ File format which we can provided to users. File storage format is '**.lsm**'. Files can be opened in imageJ or Fiji. User is requested to collect data immediately after use.

Documentation: Maintain complete records. Please make detailed entry in log book including information of user, host lab (dept.), samples visualised, duration of use, whether laser or lamp used and specify which laser used. Please mention any problems you encountered.

Zeiss Confocal Microscope LSM 700

Applied Biosystems 7900HT Fast Real-Time PCR System

Name of the facility/activity: Real Time PCR

Instrument Incharge: Prof G. Archana/ Mr. Anand Parnandi, Mr.Divya Purohit

Department: Department of Microbiology

Name & Model of Instrument: Applied Biosystems 7900HT Fast Real-Time PCR System

Software name: SDS

Introduction & Principle:
The 7900HT Fast Real-Time PCR System is a real-time quantitative PCR system that combines 384-well plate compatibility and the TaqMan® Low Density Array. Real-time quantitative PCR offers researchers a powerful tool for the quantitation of target nucleic acids.
Methods for relative quantitation of gene expression allow you to quantify differences in the expression level of a specific target (gene) between different samples. The data output is expressed as a fold-change or a fold-difference of expression levels.

Applications: Key applications include gene expression quantitation and the detection of single nucleotide polymorphisms (SNPs) using the fluorogenic 5' nuclease assay, measure gene expression levels, detect and quantitate pathogens, perform allelic discrimination (SNP genotyping) assays as well as score the presence of gene sequences.

Calibration status: Calibrated

Requirement of Sample preparation: System accepts only 384 well plate format. The plate should be sealed properly and the surface of the plate should be clean before insertion into the instrument. No consumables will be provided.

Procedure for instrument startup:
1. Turn on main power switches for the instrument as well as the PC.
2. The instrument requires at least 5 minutes to warm up.
3. After the PC has started, allow the instrument to finish its warmup procedure before running the **SDS** software.
4. When instrument warmup is complete, launch the **SDS** software and create the protocol for the real time run.
5. After protocol has been created, ensure that the protocol is valid before keeping the plate in the instrument tray.
6. Run the protocol.
7. Once protocol is finished, export the result into an Excel file.
8. Use formatted pen drive to retrieve the data from the PC. (Data will only be stored in the PC for three months before it is deleted.)

Do's and Don'ts: Do not modify any critical calibration parameters of the instrument without permission.

Operating schedule: Please ensure availability of instrument at required time before setting up any experiments.

Caution: Proper GLP should be followed. Gloves should be worn. Hazardous samples should be handled carefully. Care should be taken for sample spillage, cleaning and relevant material disposal procedures.

Shut down procedure: Proper shutdown procedure should be followed.
1. Export data and ensure that it is saved before closing the **SDS** software.
2. Remove plate from the instrument tray before closing the door and turn off the instrument.
3. Shut down the PC.
4. Ensure that the main power connections have been switched off before leaving.
5. Remember to make an entry in the log book after every use.

Data collection/analysis: You can export this run data for use in a spreadsheet program such as Microsoft Excel. The data, which consists of the set and actual parameters, is saved in **.sds** format, which can be exported to Excel.

Documentation: Maintain complete records for the use and performance checks of the instrument. Make an entry in the log book after each use.

Applied Biosystems 7900HT Fast Real-Time PCR System

BioTek Synergy HT Multimode Microplate Reader

Name of the facility/activity: Multimode Microplate Reader

Instrument Incharge: Prof G. Archana/Mr. Anand Parnandi, Mr. Divya Purohit

Department: Department of Microbiology

Name & Model of instrument: BioTek Synergy HT Multimode Microplate Reader

Software name: Gen5

Introduction & Principle:
The Synergy HT is a single-channel absorbance, fluorescence, and luminescence microplate reader. It is computer-controlled using BioTek's Gen5™ PC software for all operations including data reduction and analysis.

When making fluorescence determinations, the Synergy HT uses a tungsten quartz halogen lamp with interference filters for wavelength specificity in conjunction with a photomultiplier (PMT) tube detector. The Synergy HT has both top and bottom probes for fluorescence measurements. The top probe can be adjusted vertically for the correct reading height.
Luminescence is measured by the low-noise PMT detector through an empty filter position in the Emission filter wheel. A filter can also be left in place if light filtering is necessary. Absorbance measurements are made by switching to a xenon flash lamp and a monochromator for wavelength selection. The use of a xenon flash lamp allows for both UV and visible light absorbance measurements. The monochromator provides wavelength selection from 200 to 999 nm in 1-nm increments.

The Synergy HT has a 4-Zone™ temperature control from 4°C, over ambient to 50°C that ensures superior temperature uniformity necessary for kinetic assays. Internal plate shaking is also supported. Synergy HT support the reading of 6-, 12-, 24-, 48-, 96-, and 384-well microplates with standard 128 x 86 mm geometry.

Absorbance mode reads plates up to 0.8" (20.3 mm) in height; fluorescence mode reads plates up to 1.25" (31.75 mm).

Applications: The system can detect absorbance, luminescence and fluorescence. A multi-mode reader has many applications ranging from ELISAs to nucleic acid and protein quantitation.

Calibration status: Calibrated

Requirement of Sample preparation: 96-well plates (or other multi-well plates) must be cleaned from outside with alcohol before insertion into the instrument. No consumables will be provided.

Procedure for instrument startup:
1. Turn on main power switches for the instrument as well as the PC.
2. The instrument requires at least 3 minutes to warm up.
3. After the PC has started, allow the instrument to finish its warmup procedure before running the **Gen5** software.
4. When instrument warmup is complete, launch the **Gen5** software and create the protocol for the plate reader.
5. After protocol has been created, ensure that the protocol is valid before keeping the plate in the instrument tray.
6. Run the protocol.
7. Once protocol is finished, export the result into an Excel file.
8. Use formatted pen drive to retrieve the data from the PC. (Data will only be stored in the PC for three months before it is deleted.)

Do's and Don'ts: Please ensure availability of instrument at required time before setting up any experiments.

Operating schedule: No gaps required between short reads. After longer reads, gap of at least 12 hours recommended.

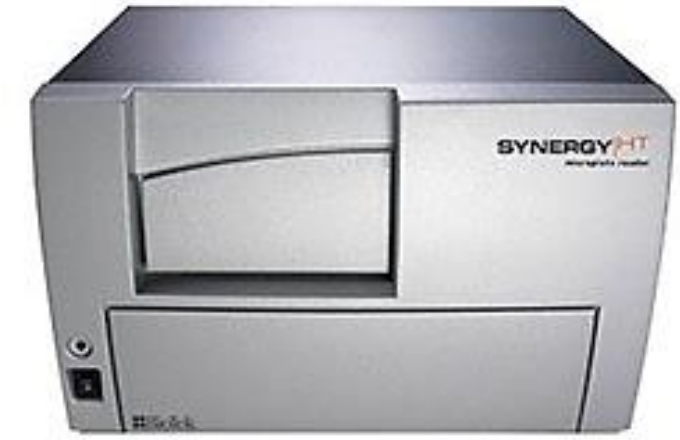
Caution: Proper GLP should be followed. Gloves should be worn. Hazardous samples should be handled carefully. Care should be taken for sample spillage, cleaning and relevant material disposal procedures.

Shut down procedure: Proper shutdown procedure should be followed.

1. Export data and ensure that it is saved before closing the **Gen5** software.
2. Remove plate from the instrument tray before closing the door and turn off the instrument.
3. Shut down the PC.
4. Ensure that the main power connections have been switched off before leaving.
5. Remember to make an entry in the log book after every use.

Data collection/analysis: File type generated - .xlsx

Documentation: Maintain complete records for the use and performance checks of the instrument. Make an entry in the log book after each use.



BioTek Synergy HT Multimode Microplate Reader

Beckman Coulter Optima Max-XP ultracentrifuge

Name of the facility/activity: Ultracentrifuge

Instrument Incharge: Prof G. Archana/ Mr. Anand Parnandi

Department: Department of Microbiology

Name & Model of Instrument: Beckman Coulter Optima Max-XP ultracentrifuge

Software name: Not applicable

Introduction & Principle: The Optima MAX-XP microprocessor-controlled tabletop ultracentrifuge generates high centrifugal forces for a variety of applications. The ultracentrifuge design features a variable frequency induction drive, thermoelectric temperature control system, self-purging vacuum system, rotor overspeed identification system, user login feature, program memory that contains multiple five-step programs, and a choice of acceleration and deceleration rates.

Manual and programmed operations are available from the integrated touchscreen interface.

- In manual operation, you enter the individual run parameters before beginning each run.
- In programmed operation, you can duplicate runs quickly and accurately by selecting previously entered programs and running them again.

Additionally, a pulse feature allows you to manually accelerate the rotor for sample preparation.

Applications: The Optima MAX-XP Ultracentrifuge is used for applications requiring high force fields that are capable of separating and isolating small particles (virus, bacteria, and subcellular components like mitochondria) and large molecules (peptides, DNA, proteins). The samples can be derived from a variety of natural and synthesized components.

Calibration status: Calibrated

Requirement of Sample preparation:
Samples will have to be pre-sealed in the ultracentrifuge compatible sealing tubes. No consumables will be provided.

Procedure for instrument startup:

1. Turn on the power switch located behind the instrument.
2. The touchscreen interface display turns on and is available.
3. Ensure that the chamber door is properly closed.
4. Press the SPEED button, then enter the run speed.
5. Press the TIME button, then enter the run time.
6. Press the TEMPERATURE button, then enter the required run temperature (0-40°C).
7. Place the pre-sealed tubes into the slots and close the chamber door.
8. Press the START button to start the run.

Do's and Don'ts: Do not change any parameters of the instrument without permission.

Operating schedule: Please ensure availability of instrument at required time before setting up any experiments.

Caution: Proper GLP should be followed. Gloves should be worn. Hazardous samples should be handled carefully. Care should be taken for sample spillage, cleaning and relevant material disposal procedures.

Shut down procedure: Proper shutdown procedure should be followed.

1. When the run stops and the time reaches 0, press the VACUUM button to turn off the vacuum system and vent the vacuum.
2. Remove tubes gently from the instrument and close the chamber door.
3. Log out of the touchscreen application before switching off the instrument.
4. Ensure that the main power switches are turned off before leaving.
5. Remember to make an entry in the log book after every use.

Data collection/analysis: The ultracentrifuge automatically saves data associated with each run. You can export this run data for use in a spreadsheet program such as Microsoft Excel. The data, which consists of the set and actual parameters, user information, and rotor type and serial number, is saved in **.csv** format.

Documentation: Maintain complete records for the use and performance checks of the instrument. Make an entry in the log book after each use.

Beckman Coulter Optima Max-XP ultracentrifuge

Nikon Eclipse 80i Fluorescence Microscope

Name of the facility/activity: Fluorescence microscope

Instrument Incharge: Prof G. Archana/ Ms. Khyati Mehta

Department: Department of Microbiology

Name & Model of Instrument: Nikon Eclipse 80i Microscope

Software name: NIS-Elements D

Introduction & Principle: Designed to truly harness the power of Nikon's CFI60 optics, the Eclipse 80i delivers remarkably high signal to noise ratios, producing fluorescence images that reveal more than ever before. Never has there been a research microscope so optimized for digital microscopy. The state-of-the-art design includes a vast array of advanced features, including Nikon's proprietary "fly-eye" technology, VC Plan Apo objectives and both intelligent and motorized digital imaging heads for the ultimate digital imaging microscope system. The 80i has a bright illumination system with a 12v-100w halogen lamp and "fly-eye" to enhance digital image lighting. It also comes with a noise terminator, which directs stray light away from the light-collection path, increasing image contrast and clarity.
Other elements unique to this model include:

- Excitation balancer, for use with multi stained specimens
- 6-filter turret
- All-in-one digital imaging unit
- Alumite stage, creating a hard, smooth enduring surface
- Auto-detection

Applications: The Eclipse 80i, with its many advanced and powerful features, can be applied in an array of settings, including clinical, industrial and educational, and science fields such as histology, microbiology, genetics, cell biology, pathology etc.

Calibration status: Calibrated

Requirement of Sample preparation: No consumables will be provided.

Procedure for instrument startup:

1. Power on the instrument and switch on UV lamp as well in case of epi-fluorescence microscopy.
2. Push the optical path-switching lever to direct the full optical path toward the binocular section.
3. Rotate the excitation method switchover turret to set it at position depending on the method of microscopy.
4. Move the filter cube for the excitation method to be used into the optical path in case of epi-fluorescence microscopy.
5. Open the shutter for epi-illumination attachment and center the lamp.
6. Switch to the desired objective and view the specimen.
7. In case of usage of camera for capturing microscopic images, Push the optical path-switching lever to direct the full optical path toward the camera section.
8. Launch the software NIS-Elements D for image acquisition and ensure that the images are saved in the proper format in the selected folder.
9. Turn off power after completing observations.

Do's and Don'ts:

- Use non-fluorescent glass slide.
- Use non-fluorescent immersion oil.
- To keep specimen colours from fading, keep the shutter closed when not performing microscopy.
- Please restore the original settings of microscope if it has been changed during the experiment.

Operating schedule: Please ensure availability of instrument at required time before setting up any experiments. Please at least keep half an hour gap between the two sessions of Epi-fluorescence microscopy.

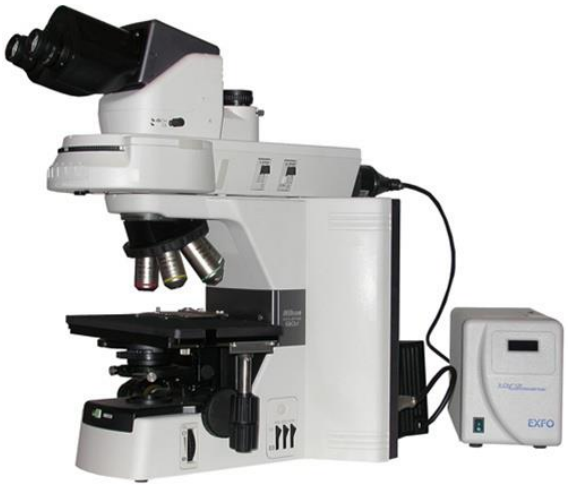
Caution: Proper GLP should be followed. Gloves should be worn. Hazardous samples should be handled carefully. Care should be taken for sample spillage, cleaning and relevant material disposal procedures.

Shut down procedure: Proper shutdown procedure should be followed.

1. Remove sample from the specimen holder and wipe the stage with clean tissue.
2. Ensure that images are saved before closing the **NIS-Elements D** software.
3. Transfer images to a formatted USB drive and proceed to shut down the PC.
4. Turn off the light switch of the microscope as well as the power switch of the UV lamp.
5. Ensure that the main power switches are turned off before leaving.
6. Remember to make an entry in the log book after every use.

Data collection/analysis: Images are saved in **.png, .jpg** and **.tiff**

Documentation: Maintain complete records for the use and performance checks of the instrument. Make an entry in the log book after each use.



Nikon Eclipse 80i Fluorescence Microscope

Nikon Eclipse TS100 Inverted Microscope

Name of the facility/activity: Inverted microscope

Instrument Incharge: Prof G. Archana/ Mr. Anand Parnandi

Department: Department of Microbiology

Name & Model of Instrument: Nikon Eclipse TS100 Inverted Microscope

Software name: NIS-Elements D

Introduction & Principle: The compact high-performance inverted Nikon Eclipse TS100 Microscope uses Eco-illumination, a newly developed LED illumination. Eco-illumination provides enough brightness for phase contrast and NAMC observations on the microscope. With a fly-eye lens, uniform brightness is provided in the entire field of view.

Nikon's CFI60 optical system is used, providing flat, sharp and clear images, while achieving longer working distances and higher numerical apertures.

Applications: The Nikon Eclipse TS100 is ideal for applications such as cell and tissue culture imaging, plaque measurement and blood typing.

Calibration status: Calibrated

Requirement of Sample preparation: Please ensure that the sample is ready for imaging (i.e. the required stain has been added and incubated for the required time) on properly sealed glass slides or multi-well plates. Ensure that the slides/plates are clean. No consumables will be provided.

Procedure for instrument startup:

1. Turn on the main power switches and allow the microscope to warm up for at least 5 minutes. Meanwhile, turn on the PC and camera unit.
2. If fluorescence microscopy is to be performed, ensure that the UV lamp has been switched on and allowed to warm up for at least 15 minutes before use.
3. Launch the **NIS-Elements D** software for image acquisition.
4. Place sample in the specimen holder and select the required objective.
5. Ensure that the images are saved in the proper format in the selected folder.

Do's and Don'ts: Do not change any parameters of the instrument without permission. If the condenser and diaphragm settings are changed, restore them to their original settings before turning off the instrument.

Operating schedule: Please ensure availability of instrument at required time before setting up any experiments.

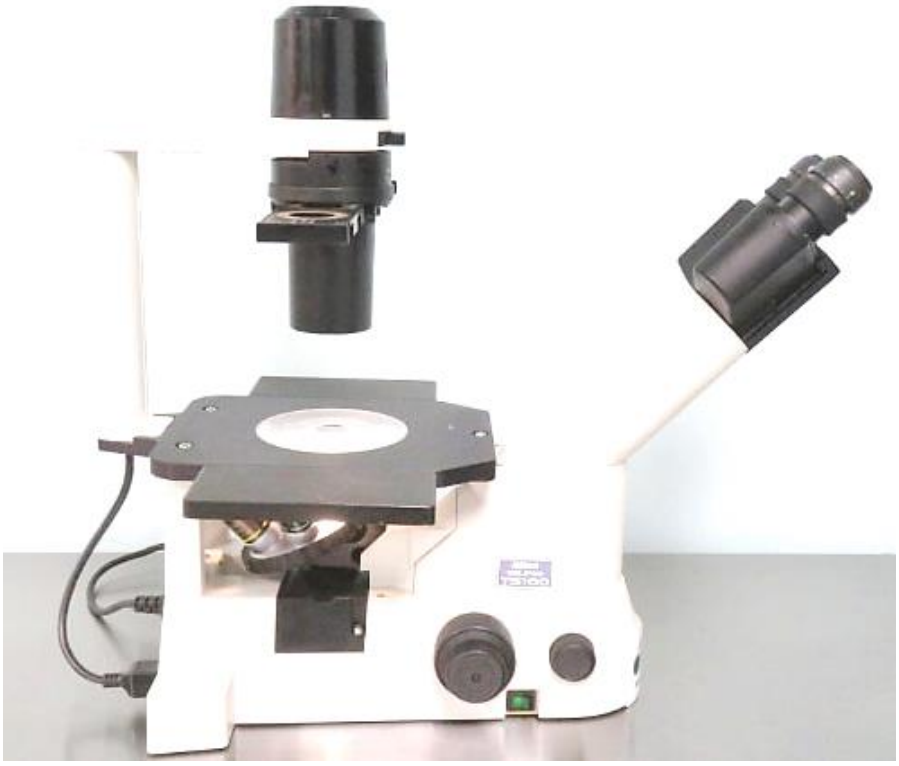
Caution: Proper GLP should be followed. Gloves should be worn. Hazardous samples should be handled carefully. Care should be taken for sample spillage, cleaning and relevant material disposal procedures.

Shut down procedure: Proper shutdown procedure should be followed.

1. Remove sample from the specimen holder and wipe the stage with clean tissue.
2. Ensure that images are saved before closing the **NIS-Elements D** software.
3. Transfer images to a formatted USB drive and proceed to shut down the PC.
4. Turn off the light switch of the microscope as well as the power switch of the UV lamp.
5. Ensure that the main power switches are turned off before leaving.
6. Remember to make an entry in the log book after every use.

Data collection/analysis: Images are saved in **.png, .jpg** and **.tiff.**

Documentation: Maintain complete records for the use and performance checks of the instrument. Make an entry in the log book after each use.



Nikon Eclipse TS100 Inverted Microscope

Affymetrix GeneChip Fluidics Station 450

Name of the facility: Affymetrix GeneChip Fluidics Station (DNA Microarray)

Instrument Incharge: Dr. Rajesh Patkar

Department: Department of Microbiology & Bharat Chattoo Genome Research Centre

Name & Model of Instrument: Affymetrix GeneChip Fluidics Station 450

Software name: Affymetrix GeneChip Command Console (AGCC)

Introduction & Principle:

The GeneChip Fluidics Station 450 is used for the wash and stain operations for DNA microarray (Genechip®) experiments. GeneChip probe arrays are manufactured using technology that combines photolithography and combinatorial chemistry. Tens to hundreds of thousands of different oligonucleotide probes are synthesized on each array. Each oligonucleotide is located in a specific area on the array called a probe cell. The hybridized probe array is stained with streptavidin phycoerythrin conjugate and scanned by the GeneChip® Scanner 3000, or the GeneArray® Scanner. The amount of light emitted at 570 nm is proportional to the bound target at each location on the probe array.

Steps for Genechip Expression Analysis:

1. Target Preparation, 2. Target Hybridization, 3. Experiment and Fluidics Station Setup, 4. Probe Array Washing and Staining, 5. Probe Array Scan and 6. Data Analysis

Procedure for instrument run:

1. 'Turn ON' the mains using the switch located on the left of the fluidics machine. Also, 'Turn ON' the computer workstation and launch the AGCC software.

2. **Fluidics system Priming**: In the AGCC Launcher, click the AGCC Fluidics Control Icon; or Click Programs Affymetrix → Command Console → AGCC Fluidics Control. The Master Control dialog box appears. In the Select Protocols section of the Master controls. Select Prime_450 from the Protocol drop-down list. Select the modules to be primed. Click Copy to Selected Modules. The selected protocol (Prime_450) is applied to the selected stations and modules. Fill the intake buffer reservoirs A and B with the appropriate priming buffer. (Refer to the appropriate GeneChip® probe array package insert). Empty the waste bottle and fill the water reservoir with deionized water. Load an empty, standard 1.5 mL microcentrifuge tube in the sample holder of each module to be primed. Click the Run All button; or Select Start → Run All Modules Selected on Master Page. Follow the prompts in the Status window (also shown in the module LCD window). The Status window and the module LCD window display the status of the procedure. The fluidics station is ready to use when priming is completed and Priming done, 'Ready' appears in the module LCD window.

3. **Running a Protocol on a Probe Array**: After you have primed the fluidics station, it is ready to run a fluidics protocol. Click the Run button to begin the protocol. The protocol will begin. The LCD window on the fluidics station and the Fluidics Station dialog box in the AGCC application will indicate the status of the protocol as it progresses. Follow the instructions on the LCD window or in the Fluidics Station dialog box as displayed in AGCC application. If prompted to "Remove Vials," remove the vials from the sample holder of the fluidics station. If prompted to "Load Cartridge," open the cartridge holder by pressing down on the cartridge lever to open the cartridge loading door. Flip the cartridge lever up to engage the cartridge septa needles into the septa. Proper engagement of the washblock with the cartridge is indicated by a change in the message on the LCD. If prompted to "Load Vials 1-2-3," place the three 1.5 mL vials containing the proper reagents into the sample holders 1, 2, and 3 on the fluidics station. When you have loaded the vials, gently but firmly press down on the needle lever to insert the needles into the vials. The run will commence automatically. As the run progresses, check to ensure that the cartridge is filling properly and that bubbles are not forming. When the staining and washing are complete, eject the cartridge by pushing down on the cartridge lever. Remove the cartridge and run the Cleanout protocol on the particular module. Fill the cartridge manually with the last buffer used by inserting a pipette tip or syringe needle through the bottom septum and by using a second

pipette tip or syringe needle in the top septum to permit air to escape. The fluidics station will automatically perform a Cleanout protocol.

4. **Shutting Down**: You should perform the Shutdown protocol at the end of a session. The shutdown protocol requires three 1.5 mL vials for each module. After removing a probe array from the probe array holder, the LCD window displays the message ENGAGE WASHBLOCK. Engage the washblock by gently pulling up on the probe array lever to the up position. When the fluidics station LCD window indicates REMOVE VIALS, the Cleanout protocol is complete. 5. Remove the vials from the sample holder, place the wash lines into a bottle filled with deionized water. Choose Shutdown_450 for all modules from the drop-down Protocol list in the Fluidics Station dialog box. Click the Run button for all modules. After the Shutdown protocol is complete, flip the ON/OFF switch to the OFF position.

Do's and Don'ts: Do not modify any critical calibration parameters of the instrument without permission.

Operating schedule: Please ensure availability of instrument at required time before setting up any experiments.

Caution: Proper GLP should be followed. Gloves should be worn. Hazardous samples should be handled carefully. Care should be taken for sample spillage, cleaning and material disposal procedures.

Data collection: Use formatted pen drive to retrieve the data from the PC and use it for further analysis.

Documentation: Maintain complete records.

Affymetrix GeneChip Fluidics Station 450

Beckman-Coulter DNA Sequencer CEQ8000

Name of the facility: DNA Sequencer

Instrument Incharge: Dr. Rajesh Patkar

Department: Department of Micorbiology & Bharat Chattoo Genome Research Centre

Name & Model of Instrument: Beckman-Coulter DNA Sequencer CEQ8000

Software name: CEQ8000

Introduction & Principle:

CEQ8000 machine is a capillary-based DNA sequencer that essentially based on Sanger's dideoxy chain termination DNA sequencing principle. The crosslinked polyacrylamide filled capillary columns provide high resolution separation and automated sequencing platform show striking increase in separation and analysis speed (5-fold) in comparison with conventional polyacrylamide slab gel electrophoresis for the separation of a fluorescein-labeled DNA.

Procedure for instrument run:

1. 'Turn ON' the mains using the switch located on the left of the fluidics machine. Also, 'Turn ON' the computer workstation and launch the AGCC software.

2. **Sample Preparation**: Reactions should be put in 0.2 mL thin wall tubes or 96 well microplates. To the tubes, add the appropriate amount of template DNA and water up to 5 µL. Incubate the DNA at 95 °C for 1 minute. In the following order, add to the DNA: 1 µL of 3.2 µM primer, 4 µL of the DTCS Quick Start Master Mix.

3. Thermal cycling program should be set as, 30 cycles of: 96 °C 20 seconds, 50 °C 20 seconds, 60 °C 4 minutes, 4 °C hold followed by Ethanol precipitation. Resuspend the sample in 40 µL of Sample Loading Solution (provided with the kit).

4. **Preparing sample plates**: Overlay 1 drop of mineral oil to each sample. Prepare a buffer plate using the flat-bottomed culture plates. Fill the wells corresponding to samples in your sample plate 2 /3 – 3 /4 full of buffer. After the reaction, samples can be kept at -20 °C and re-run on the machine if necessary.

5. **Opening the CEQ8000 Software**: Turn on PC and wait for windows to start. Click the CEQ8000 icon on the desktop. 'Sample Setup' – used to set up the sample plates. 'Run' – used to interact with the sequencing machines, and actually running your sequences. 'Sequence Analysis' – used to look at your results. 'Databases' – manages where data is stored and 'Exit' – exits the program

6. **Preparing the machine to sequence**: 1. Wetting tray a. From the CEQ Analysis Software window or the Shortcut Bar, click on the Run icon b. Click Replenish / Replace Wetting Tray c. Open the sample cover, when prompted by the software. Remove the wetting tray, empty any water in the tray and fill with distilled water up to the bottom of the letters, NOT TO THE FILL LINE.
7. **Running the samples:** a. Then go to Direct Control, click purge manifold. b. After manifold is purged click under Direct Control, gel capillary fill c. After it is filled, also under the same window, hit optical alignment d. Then go to Run, Monitor Baseline, when its dialog box appears, check enable monitor baseline 1. If any baselines are above 6000, try another optical alignment, or clean off the array, or purge the manifold e. Then under the Direct Control window, click Load sample plates. f. Load in your sample and buffer plates in the appropriate place. g. Then go into Run, start sample plate to begin your run. Make sure that the correct cells are highlighted 1. The columns each take between 90 and 110 minutes to run.

8. **Disassembly of the CEQ8000:** After your sequences have finished a. Remove the sample and buffer plates from the machine b. Rinse the wetting tray with distilled water and refill it c. Remove the gel cartridge, using a Kimwipe to remove the excess gel from the end. Replace the cartridge with the plug.

Do's and Don'ts: Do not modify any critical calibration parameters of the instrument without permission.

Operating schedule: Please ensure availability of instrument at required time before setting up any experiments.

Caution: Proper GLP should be followed. Gloves should be worn. Hazardous samples should be handled carefully. Care should be taken for sample spillage, cleaning and material disposal procedures.

Data collection: Use formatted pen drive to retrieve the data from the PC and use it for further analysis.

Documentation: Maintain complete records.

Beckman-Coulter DNA Sequencer CEQ8000

CRYOCUT MICROTOME

Name of the facility / activity: Cryocut microtome **(for fresh frozen sections of tissues)**

Instrument Incharge: Dr. R.V. Devkar

Department: Zoology

Name, Model of Instrument: IEC Minotome plus

Microtome: Motorized

Introduction &Principle: The IEC Minotome Plus is an automatic cryocut from Minot microtome technology. The system carries an easy-to-use motorized specimen advance-and-retract feature which offers the ultimate speed, convenience and safety. This cryocut also keeps operators' hands out of the chamber entirely, except when placing the specimen on the holder or adjusting the micron setting. The Minotome Plus features cool-down to -30°C with accurate temperature control at the knife edge. A knife safety guard offers the operator further protection from injury. The IEC Minotome Plus includes knife guards, heat extractor and anti-roll assembly. Additional accessories include disposable blade holder, heavy-duty disposable blades, two specimen plates (concentric circle and cross-hatched, 26 mm), lubricating oil, freezing spray, freezing medium, dyes for specimen samples, 3 brushes (1/8", 1/4", 3/8" W bristles), specimen holder shelf and protective mat.

Requirement of Sample preparation: Tissue mounting medium,

Procedure for instrument start up :

- Switch on the air conditioner of the lab cubicle
- Switch on the Power of instrument
- Wait till the digital display shows temperature between -18 to -22^{o}C.
- Mount Pre-freeze tissue samples on the Cryo-chuck.
- Cut about 10-15 micron sections and collect it on slides.
- Keep the slides inside the cryocut
- Transfer the slides to -20 deg or storage cask.
- Switch off the instrument leaving the lid open.
- Wipe the instrument dry after about 1h and then close the lid

Do's and Don'ts:
Instrument takes at least 2 h to reach -20 deg on winter days and about 3h during summer days. Instrument can be left on overnight if needed for next day use. Blades and microtome has to be wiped dry before final shut off.

Operating schedule: (gap b/w two experiments or time being experiment) : Nil

Data collection / analysis: Slides to be carried by the end user

Documentation: Calibration not required

Rock section cutting machine

Name of the facility / activity: Science

Principal Investigator: Prof. D. M. Maurya

Department Head: Prof. H.R.Kataria

Name, Model of Instrument: Rock section cutting machine;
Discoplan-TS, CitoVac, LaboForce-Mi

Introduction &Principle: This instrument is used for making thin sections of rock samples for microscopic studies. Instrument is an assembly of multiple units. Here, Discoplan-TS can be used for re-sectioning of specimen, LaboForce-Mi for grinding, lapping and polishing processes that includes final polishing with oxide polishing suspensions.

Applications: Thin section cutting and polishing

Requirement of Sample preparation: Rock samples

Procedure for instrument startup: Switching on the individual units.

Do's and Don'ts: Protect your eyes with a protecting glasses while grinding and polishing

Operating schedule: Can be operated continuously without any delay

Caution: Proper GLP should be followed. Glows should be worn. Hazardous samples should be handled carefully. Care should be taken for sample spillage, cleaning, sample and relevant material disposal procedures.

Shut down procedure: Switching off the individual units.

Data collection /analysis: Final outcome is Glass slides. These glass slides are later used for microscopic studies.

Documentation: Maintain complete records for the calibration and performance checks of all equipment and instruments.

Figure 1 LaboForce-Mi

Name of the facility / activity : Science

Principal Investigator: Prof. D. M. Maurya

Department Head: Prof. H.R.Kataria

Name, Model of Instrument: Stereozoom Microscope; Model- Nikon SMZ

Software name: NIS element BR
Introduction & Principle: Developed for standard research applications such as analysis and photo documentation. NIS-Elements BR is features with four-dimensional acquisition and advanced device control capabilities.

Applications: Capturing quality images, Metallography

Calibration status: Auto calibration

Requirement of Sample preparation eg. Filtered, properly sealed coverslips, compatible chemicals and consumables, type of sample, precaution: Any sample other than liquids and chemicals

Procedure for instrument startup: Switch on the camera and the light source. Open the NIS Element software in the desktop.
Operating schedule: Can be operated continuously without any delay

Caution: Proper GLP should be followed. Gloves should be worn. Hazardous samples should be handled carefully. Care should be taken for sample spillage, cleaning, sample and relevant material disposal procedures.

Shut down procedure: Exit the NIS Element software window and then switch off the camera and light source

Data collection / analysis: JPEG,GIF,TIFF,PNG
Documentation: Maintain complete records for the calibration and performance checks of all equipment and instruments.

Figure 2 Stereozoom Microscope

Name of the facility / activity : Science

Principal Investigator: Prof. D. M. Maurya

Department Head: Prof. H.R.Kataria

Name, Model of Instrument: SediGraph III 5120

Software name: Sedigraph III
Introduction & Principle: The SediGraph 5120 is a particle size analyser. This system consists of up to two particle size analysers and a multi-function computer. The particle size analyser is mounted on an adjustable feet that contains a level indicator, so that the accuracy of the analysis is maintained. It is designed for completely automatic operation; however, a sliding transparent door on the front panel provides convenient observation. The sedigraph works on the principle of Stoke's Law.
Applications: The size range of particles and the distribution of mass in each size class can analyzed.

Calibration status: Automatic calibration before an experiment

Requirement of Sample preparation eg. Filtered, properly sealed coverslips, compatible chemicals and consumables, type of sample, precaution:
The sample should be mixed with distilled water and sodium hexametaphosphate solution.
Solution should be ultra-sonicated before loading in the instrument.

Procedure for instrument startup:
Switch on the UPS, CPU and the Sedigraph. Turn on the X ray by turning the X ray key from stand.
Do's and Don'ts:
Load the sample immediately after mixing.
Operating schedule:18-20 min for an experiment
Caution: Proper GLP should be followed. Glows should be worn. Hazardous samples should be handled carefully. Care should be taken for sample spillage, cleaning, sample and relevant material disposal procedures.

Shut down procedure: Proper shutdown procedure should be followed.
1) Close the sample analysis window
2) Turn off the X ray key
3) Switch off the Sedigraph machine

Data collection / analysis: Supports all file format.
Documentation: Maintain complete records for the calibration and performance checks of all equipment and instruments.

Figure 3 SediGraph III 5120

Scanning Electron Microscope; Model- Hitachi SU1510

Name of the facility / activity: Science

Principal Investigator: Prof. D. M. Maurya

Department Head: Prof. H.R.Kataria

Name, Model of Instrument: Scanning Electron Microscope; Model- Hitachi SU1510

Software name: PC-SEM
Introduction & Principle: SEM is a microscopic imaging technique in which a particle surface is scanned with beam of electrons and finally an out image is produced.

Applications: Used in geology, chemistry, bio-chemistry, metallurgy, etc.

Calibration status: Calibrated

Requirement of Sample preparation eg. Filtered, properly sealed coverslips, compatible chemicals and consumables, type of sample, precaution: powdered and unpowdered sample can be used. Make sure that the sample is properly mounted on the SEM stage with carbon tape.

Procedure for instrument startup: Procedure from switching on the mains till the final steps should be properly followed**.**

Do's and Don'ts: Mishandling of equipment should be avoided. Should be handled by someone who knows to operate.

Operating schedule: Can be operated continuously without any delay.

Caution: Proper GLP should be followed. Glows should be worn. Hazardous samples should be handled carefully. Care should be taken for sample spillage, cleaning, sample and relevant material disposal procedures.

Shut down procedure: Proper shutdown procedure should be followed**.**

Data collection / analysis: JPEG

Documentation: Maintain complete records for the calibration and performance checks of all equipment and instruments.

Figure 4 Scanning Electron Microscope (SEM)

Energy-dispersive X-ray spectroscopy (EDS)

Name of the facility / activity: Science

Principal Investigator: Prof. D. M. Maurya

Department Head: Prof. H.R.Kataria

Name, Model of Instrument: Energy-dispersive X-ray spectroscopy (EDS)
Model- Oxford instruments - 51-ADD0053

Software name: Oxford

Introduction & Principle: EDS is an accessory instrument attached to SEM in which a surface of a material is sequentially scanned and composition of material is analyzed.

Applications: Used in geology, chemistry, bio-chemistry, metallurgy, etc.

Calibration status: Calibrated

Requirement of Sample preparation eg. Filtered, properly sealed coverslips, compatible chemicals and consumables, type of sample, precaution: Sample can be given in powdered or unpowdered form. The sample is to be properly mounted on the SEM stage with carbon tape.

Procedure for instrument startup: Procedure from switching on the mains unit till the final steps is to be properly followed as prescribed in the instrument manual**.**

Do's and Don'ts: Mishandling of equipment should be avoided. Should be handled by someone who knows to operate.

Operating schedule: Can be operated continuously without any delay.

Caution: Proper GLP should be followed. Glows should be worn. Hazardous samples should be handled carefully. Care should be taken for sample spillage, cleaning, sample and relevant material disposal procedures.

Shut down procedure: Proper shutdown procedure as prescribed in the instrument manual is to be followed**.**

Data collection / analysis: JPEG, PDF

Documentation: Maintain complete records for the calibration and performance checks of all equipment and instruments.

Figure 5 Energy-dispersive X-ray spectroscopy (EDS)

Ground Penetrating Radar (GSSI); Model – SIR-20

Name of the facility / activity : Science

Principal Investigator: Prof. L.S.Chamyal
Department Head: Prof. H.R.Kataria

Name, Model of Instrument: Ground Penetrating Radar (GSSI); Model – SIR-20

Software name: Radan
Introduction & Principle: Ground Penetrating Radar (GPR) is used to image the geology of the shallow subsurface. This nondestructive method uses electromagnetic radiation in the microwave band (UHF/VHF frequencies). The nstrument acquires reflected signals from subsurface to produce an image of underlying geological features. The data acquired in the field is processed using RADAN software for further analysis and interpretation

Applications:. GPR has applications in a variety of areas that includes mapping of rock, soil, ice, fresh water, pavements, structures utilities in the shallow subsurface.
Calibration status: Calibrated

Requirement of Sample preparation eg. Filtered, properly sealed coverslips, compatible chemicals and consumables, type of sample, precaution: Used for on site geophysical surveys

Procedure for instrument startup: The instrument comprises various components viz. the control unit, Antenna, Receiver, Survey wheel, cables and others. These are to be connected properly to the main unit (Sir System- 20). The main unit is connected to a laptop or tough book. Switch on the tough book and start the Radan software for viewing the acquired image.
Do's and Don'ts: Rough handling or mishandling of equipment can damage the equipment. Trained and experienced person is needed to carry out the surveys and data interpretation.

Operating schedule: Can be operated at all times except during inclement weather conditions.

Caution: The surveys should not be carried out near heavy machineries or power lines.

Shut down procedure: Close the Radan software, and disconnect various components.
Data collection / analysis:

Documentation: Data/profiles stored in digital format in RADAN software.

Figure 6 Ground Penetrating Radar shielded Antenna

AGICO MFK1B Kappa bridge

Name of the facility / activity: Magnetic susceptibility measurement

Instrument In-charge: Prof. N. P. Bhatt

Department: Geology

Name, Model of Instrument: AGICO MFK1B Kappa bridge

Introduction & Principle:

The Kappabridge apparatus consists of the Pick-Up Unit, Control Unit and Computer. In principle the instrument represents a precision fully automatic inductivity bridge. It is equipped with automatic zeroing system and automatic compensation of the thermal drift of the bridge unbalance as well as automatic switching appropriate measuring range. The measuring coils at frequency F1 are designed as 6th-order compensated solenoids with a remarkably high field homogeneity. Special diagnostics was embedded in MFK1 Kappabridges, which monitors important processes during measurement with MFK1 and also with CS4 or CSL unit. The digital part of the instrument is based on micro-electronic components, with two microprocessors controlling all functions of the Kappabridge. The instrument has no control knobs, it is fully controlled by external computer via serial channel RS- 232C. The output signal from pick-up coils is amplified, filtered and digitalized, raw data are transferred directly to the computer which controls all the instrument functions. The MFK1-B versions measure the AMS of a static specimen fixed in the manual holder. In the static method, the specimen susceptibility is measured in 15 different orientations following rotatable design. From these values six independent components of the susceptibility tensor and statistical errors of its determination are calculated. The specimen positions are changed manually during measurement.

Requirement of Sample preparation: Loose sediments. Rock cylinder with 25.4 mm diameter and 22 mm height. Rock cube 20 mm sides.

Procedure for instrument start up :

- Switch on the air conditioner of the lab cubicle
- Switch on the Power of instrument
- Switch on the attached computer
- Open main menu of SAFYR in computer
- Follow the instructions given in user manual for measurement of AMS in static specimen in 15 directions
- Save the data file and take copy in CD.
- Switch off the instrument and computer
- Switch off the Room air conditioner and other electrical.

Do's and Don'ts: Please do not use your USB drive in the computer attached with the instrument. It is not having anti-virus software.

Operating schedule: N.A.

Data collection / analysis: In CD only.

Documentation: Nil

Figure 7 Kappa bridge

NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROMETER

Name of the facility / activity : Nuclear Magnetic Resonance (NMR) Spectrometer

Department Head / Instrument Incharge : Prof. Shailesh R Shah, Prof. Shibhangi S Soman, Dr. Vinay K Singh, Dr. Arpita S. Desai

Name , Model of Instrument: Bruker, Avance-400 MHz

Software name : Topspin

Introduction & Principle: NMR is a spectroscopic technique used for characterization of small organic molecules. The sample is placed in a magnetic field and the NMR signal is produced by excitation of the nuclei sample with radio waves into nuclear magnetic resonance, which is detected with sensitive radio receivers. The intramolecular magnetic field around an atom in a molecule changes the resonance frequency, thus giving access to details of the electronic structure of a molecule and its individual functional groups.

Applications: NMR is a spectroscopic technique used for characterization of small organic molecules, organometallic complexes along with interaction involved in small molecules such as host guest binding studies, interaction with chiral compounds.

Calibration status: Calibration is carried out once in six month.

Requirement of Sample preparation:
NMR Sample Tubes, Deuterated solvents such as CDCl3, DMSO-d6
Sample is placed in NMR tube and dissolved in deuterated solvent based on solubility. NMR tube containing solution of sample is used for analysis. NMR tubes must be properly cleaned, dried for analysis. Sample should have good solubility in deuterated solvent.

Procedure for instrument startup :
1. Start the AC (Air-conditioner) to maintain proper cooling and air circulation in the room. Need to start chiller first and allow it to stabilize until it shows reading below 10.0 ^{o}C. Once it has reached to that reading start the compressor. After starting compressor wait until pressure reaches to maximum pressure.
2. Start the computer, software and open the lead on instrument for insert and eject the sample.
3. Run the sample under automation set up.

Do's and Don'ts: NMR room's temperature is maintained below 22 ^{o}C for 24 hours by running AC all time. Continuous power supply is required by UPS or generator.

NMR Instrument is maintained at below 22 ºC for minimizing evaporation liquid nitrogen and hence that of liquid Helium. All items containing iron or magnetic susceptibility should be kept away from instrument. NMR should be operated by only experienced person.
Only use properly fitting NMR Tubes. Sample should not contain any paramagnetic species.

Operating schedule: Time of experiment depends on type of experiment and concentration of sample. Simple proton NMR of sample takes 5 min for collecting data on instruments and further processing of it. 13C-NMR of concentrated samples (20-25 mg amount) takes 1h while dilute samples (8-10 mg/ or with low solubility) 12-15 h. 2DNMR takes 5 h or more than that (depends on number of scans).

Caution: Temperature is maintained below 22 ºC 24 x 7 in NMR facility room.
Continuous power supply is required (which is maintained by UPS or generator)
Filling of Liquid nitrogen should be carried out every week.
Proper GLP should be followed. Care should be taken for handling NMR tubes, NMR spinner.

Shut down procedure: NMR instrument is kept on. It is only shut down during maintenance or emergency (When there is power cut or problem with power supply)

Data collection / analysis :
Data are collected and processed using Topspin software. Print out of fourier transform spectrum containing all details of experiment, signals and values can be given as hard copy. Processed data can also be given only in new or blank CD.

Documentation :

(1) Requisition form for analysis of samples

(2) Log book for samples analysed from department

(3) Log book for external samples analysed

(4) Log book for Nitrogen filling

(5) Log book for Helium feeling

(6) Log book for Engineer's visit

Maintain complete records for the calibration and performance checks of all equipment and instruments.

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FTIR – FOURIER TRANSFORM INFRARED SPECTROSCOPY

Name of the facility / activity : FTIR

Department Head / Instrument Incharge : Prof. Ashutosh Bedekar

Name , Model of Instrument : Brucker-Alpha

Software name : OPUS

Introduction & Principle :

FTIR stands for Fourier Transform Infrared. IR spectroscopy is based on the absorption principle, it is the interaction between IR radiations with molecule. When IR radiation absorbed by the molecule it causes excitation of a molecule to higher vibration or rotation level and generate the IR spectra of a molecule which contains functional group region and fingerprint region. IR spectrum (graph) is plot between intensity (% transmittance) on vertical axis and wavenumber on horizontal axis. Wavenumber is a reciprocal of wavelength, hence its unit is cm^{-1}.

Applications: Identification of the functional groups in unknown sample.

Calibration status: -

Requirement of Sample preparation : 5 mg sample to 100 mg of KBr

Procedure for instrument startup :

Switch on the stabilizer connected with the instrument, switch for the instrument and the PC connected with the instrument. Open the IR software (OPUS) on the PC. Let the yellow light on IR instrument turn green.

Do's and Don'ts:

Always use the clean and dry mortar pestle for sample preparation to avoid contamination.

Do not allow to test the corrosive and strong acid or base samples as it damage the sample holder.

After each operation clean the sample holder and instrument properly and discard the sample in a waste bin.

Operating schedule:

One can make KBr pallet as blank and run it. Then, make KBr pallet containing sample, crush it on a mortar and pestle. Place the powdered sample in KBr in palletizer. Apply pressure upto 10 on pressure guage for around a minute. Remove it and place pallet in sample holder of instrument.

In the menu bar click on sample measurement, put in all the requirements i.e. range(4000-400 cm^{-1}). Put in the sample name, put in the location for data recovery that is in D drive, FTIR data.

Caution:

Do not locate the FTIR instrument in an environment with condensing moisture and excessive dust.

Shut down procedure:

After completion of analysis, logout from the OPUS software. Shut down the PC. Switch off the PC, instrument and stabilizer.

Data collection / analysis :

To be integrate the data, open the OPUS data file from D drive. Make baseline correction from menu bar from the spectra, then label peak intensity. After all data is integrated, save the data file as save pdf in the folder created in D drive.

Documentation :

1. **Requisition form for sample analysis**
2. **Maintenance of Log-Book.**

   Maintain complete records for the calibration and performance checks of all equipment and instruments.

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THERMOGRAVIMETRY/ DIFFERENTIAL THERMAL ANALYZER (TG-DTA)

Name of the facility / activity: Thermogravimetry/ Differential Thermal Analyzer (TG-DTA)

Department Head / Instrument Incharge: Dr. Prasanna Ghalsasi and Dr. Kiran Nakum

Name, Model of Instrument: Stage II INCARP EXSTAR 6300

Software name: TA 7000 Standard Analysis

Introduction & Principle: Thermogravimetric analysis (TGA) measures weight changes in a material as a function of temperature (or time) under a controlled atmosphere. A TGA analysis is performed by gradually raising the temperature of a sample in a furnace as its weight is measured on an analytical balance that remains outside of the furnace. In TGA, mass loss is observed if a thermal event involves loss of a volatile component. Chemical reactions, such as combustion, involve mass losses, whereas physical changes, such as melting, do not. The weight of the sample is plotted against temperature or time to illustrate thermal transitions in the material

Applications:

1. Testing thermal stability of material.
2. Monitoring mass changes of materials under controlled gas atmosphere and temperature: volatiles, reactive gas evaluation, filler content, compositional analysis, material identification
3. Phase transitions of metals and alloys.
4. Qualitative analysis of phase transitions: melting, Tg, crystallization
5. Determining the effect of oxidative or reductive atmospheres on materials
6. Analysis of polymers, organic and inorganic materials

Calibration status: Calibrated

Requirement of Sample preparation: Solid samples ground to fine powder must be placed in pan for the measurements.

Procedure for operating the instrument:

1. Turn on the N_2 gas (gas cylinder) ENSURESURE THAT N2 supply is sufficient carry out the analysis – IT IS MANDATORY FOR SMOOTH OPERATION OF THE INSTRUMENT.
2. Turn on the instrument; the switch is in the lower front left corner. Click on the "MEASURE" icon to start the software. Click on "File" and select "Open Communication Port" select "Open COM Port (O)" and then click on "COM1".
3. Once the link between the instrument and software is established place "reference pan" and "sample pan" in the respective pan holders and click on "ZERO" to tare the pan. This will zero the weight and the weight will be displayed as "0.000". Remove the sample pan and add 2-6 mg of sample and put it back in the instrument and click on "AUTOLOADING" to record the sample weight.
4. In the "condition editor" tab, under the "Sample Conditions" enter the sample details like Sample Name and PAN selected for the analysis. Under the Data File give path to save the data in "D drive". Be careful not to overwrite existing files.

5. Mention proper method to be used for carrying out the analysis under the “Method” tab in the “condition editor” by choosing details in the “Temperature Program” tab. Click “OK” to select the method.
6. Click on “START” button to begin the measurement.

Do’s and Don’ts:

- The heater and the furnace are hot during and after the analysis and must not be touched until cooled.
- The analysis of materials generating toxic fumes upon degradation must be avoided.

Operating schedule: (gap b/w two experiments or time being experiment)
The heater and furnace must be brought to room temperature before the initiation of new measurement**.**

Caution: Proper GLP should be followed. Gloves should be worn. Hazardous samples should be handled carefully. Care should be taken for sample spillage, cleaning, sample and relevant material disposal procedures.

Shut down procedure: Proper shutdown procedure should be followed.

Data collection/analysis: Data is acquired in STA file format. These are converted to “WORD” and “EXCEL” output and to users.

Documentation:

1. **Requisition form for sample analysis**
2. **Maintenance of Log-Book.**

Maintain complete records for the calibration and performance checks of all equipment and instruments.

GAS CHROMATOGRAPH-MASS SPECTROMETER (GC-MS)

Name of the facility / activity: Gas chromatograph-Mass Spectrometer **(GC-MS)**

Department Head: Prof. (Dr.) Anjali Patel

Instrument Incharge: Prof. P. Padmaja Sudhakar/ Prof. A.V Bedekar

Name, Model of Instrument: Thermo Scientific DSQ II

Software name: Xcaliber

Introduction & Principle: The Gas Chromatography-Mass Spectrometry (GC-MS) instrument separates chemical mixtures (the GC component) and identifies the components at a molecular level (the MS component). It is one of the most accurate tools for analyzing samples. The GC works on the principle that a mixture will separate into individual substances when heated. The heated gases are carried through a column with an inert gas (such as helium). As the separated substances emerge from the column opening, they flow into the MS. Mass spectrometry identifies compounds by the mass of the analyte molecule.

Applications: GC-MS is used in research and development and quality control. It is used in separation and identification of individual components and impurities present in the reaction mixture under study.

Calibration status: Calibrated. (It requires monthly calibration)

Requirement of Sample preparation: Prepared in volatile GC grade solvents like chloroform, acetone, MDC etc., Filtered and properly sealed.

Type of sample: Volatile organic samples with boiling point lower than 250^{o}C

Precaution: Concentration of the prepared sample should be less than 1ppm

Procedure to start an instrument:

1. Switch on Helium Gas.
2. Room temperature should be 22-24 ^{o}C.
3. Switch on line connected through UPS.
4. Switch on GC.
5. Wait for initial stabilization of GC display.
6. Switch on MS.
7. Wait for 10 min to achieve stable vacuum conditions.
8. Start software.

Operating schedule: Four days a week
Caution: Proper GLP should be followed. Gloves should be worn. Hazardous samples should be handled carefully. Care should be taken for sample spillage, cleaning, sample and relevant material disposal procedures.

Shut down procedure: Proper shutdown procedure should be followed.

Data collection / analysis: Chromatogram and Mass spectrum of the analysed sample will be given as a print-out.

Documentation:

1. **Requisition form for sample analysis**
2. **Maintenance of Log-Book.**

Maintain complete records for the calibration and performance checks of all equipment and instruments.

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LIQUID NITROGEN PLANT

Name of the facility / activity : Liquid Nitrogen Plant

Department Head / Instrument Incharge : Prof. Shailesh R Shah (University Central facility)

Name , Model of Instrument: 'StirLITE J0593', Stirling cryogenics (The Netherlands)
The instrument is made of three units (1) chiller (2) Kaeser compressor (recently replaced- under warranty) (3) Cryogenerator

Software name : Inbuilt

Introduction & Principle: Liquid nitrogen is produced from air via complex filtration, high pressure, solenoid valves by stirling cycle, in a heavy duty instruments
Applications: NMR is a spectroscopic technique used for characterization of small organic molecules, organometallic complexes along with interaction involved in small molecules such as host guest binding studies, interaction with chiral compounds.

Calibration status: Daily (currently break down)

Requirement of Sample preparation: Inbuilt in machine
Two exhaust fans and ceiling fans should be functional. Surrounding room should be cleansed at least once a week. No dust, cement dust should be entering the premises. Window, front door should be always open with exhaust fans on.
Chiller to be cleansed with air blower every two months. Water level to be maintained with addition of (ethylene glycol) radiator coolant added in that

Procedure for instrument startup : Inbuilt in machine

Do's and Don'ts: Proper stabilized power supply to be ensured and readings to be noted regularly (8-10 status)
Operating schedule: Non-stop, auto-controlled, liquid nitrogen level should be above 80 %

Caution: Safety glasses, glows for inexperienced individuals

Shut down procedure: No shut down. Automatically controlled
Data collection / analysis :
Everyday normally 8-10 reading to be noted daily in log sheet
Documentation :

(1) Log book for Liquid Nitrogen generated

(2) Log book for Liquid nitrogen distributed

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