Shantani™

Advancing Technologies and Applications of Proteome Analysis

Overview of Company and Capabilities

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Company Overview – Inception & Growth

Protein Chemistry & Proteomics Based Biotechnology Company

Established Year 2010
Seed-Funds from Technology Incubation Park

April 2012
Top Indian Private VC firms Fuels the growth

August 2014
Disease Diagnostic Tools & Technologies Development

December 2011
Small Business Innovation Grants for New Target Discovery in T2D

July 2012
- Target ID Technology Commercialization
- Initiation of Discovery Programs

Bioanalytical and Proteomics Services @ Shantani

Shantani™

Advancing Technologies and Applications of Proteome Analysis

Shantani Services Portfolio

Characterization of Biologics

Structure of active substance

- Primary structure analysis
- Secondary structure analysis
- Tertiary structure analysis
  - Isoforms of active substance
  - Purity of active substance
  - Host and process related impurities
  - Drug product characterization
  - Lipid and Carbohydrate Analysis

HPLC based Analysis

- Method development
- Method validation
- Sample analysis
- Stability studies

Proteomics Services

- Labelled and label-free protein Mass-Spectrometry
- Differential proteomics
- De Novo peptide sequencing
- Peptide mass fingerprinting using single and multi-enzyme digestion
- MS analysis of intact proteins and peptides

Additional Basic Protein Related Services

- Protein quantification in various biological samples
- 1-D/2-D Gel electrophoresis
- Protein Expression and purification
- Method development and purification of antibodies

For more details visit : www.shantani.com

Outline : Case Studies

Characterization of Biologics : Structural Analysis of Active Substance

Primary Structure Analysis: This analysis typically involves intact mass-assessment of therapeutic protein by **SDS-PAGE, LC-ESI / MALDI-TOF and Peptide Mapping**

Secondary Structure Analysis: To confirm the secondary structure **Far UV Circular Dichroism (CD) Spectroscopy, Fourier Transformed Infra-Red Spectroscopy (FTIR), Fluorescence Spectroscopy** (Steady-State, Time-Resolved, Quenching) and **Sulfhydryl groups and Di-Sulfide Bridge Analysis** are utilized in protein specific manner

Tertiary Structure Analysis: Tertiary structure of the protein is analyzed using fluorescence Spectroscopy, **Near UV-visible spectroscopy, Differential Light Scattering (DLS) and Electron Microscopy (EM / TEM).**

Case-Study-1 : Biophysical Characterization of an Antigen - Client - An Indian Vaccine Company

Challenges:

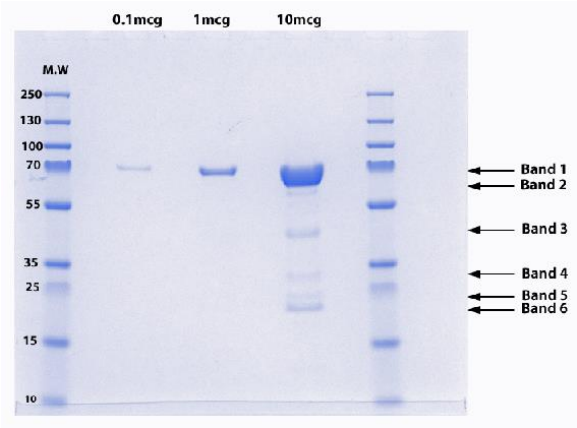
- Primary and secondary structural analysis of purified antigen.
- Confirmation of antigen component purity, identity and integrity in the given sample.

Timeline: 4 weeks

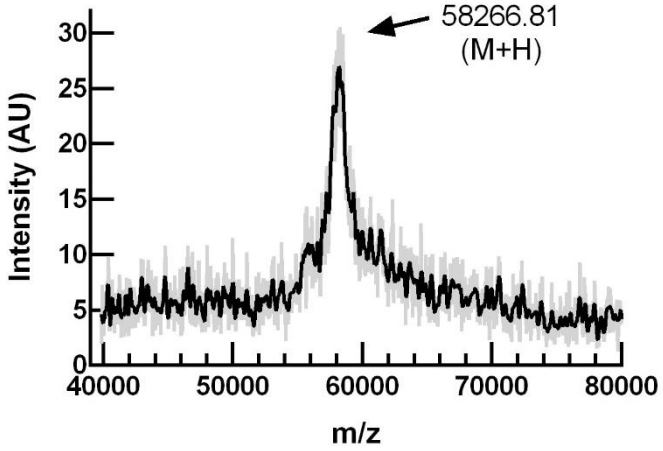
Project Plan:

- Calculate Intact mass of the protein using **SDS-PAGE and Mass-Spectrometry** based methods.
- Analyze complete primary amino-acid sequence of protein by digesting it with three different proteases followed by **Mass-Spectrometry based Peptide Mapping**.
- Analyze secondary structure of the protein using **Fluorescence and CD spectroscopy**.

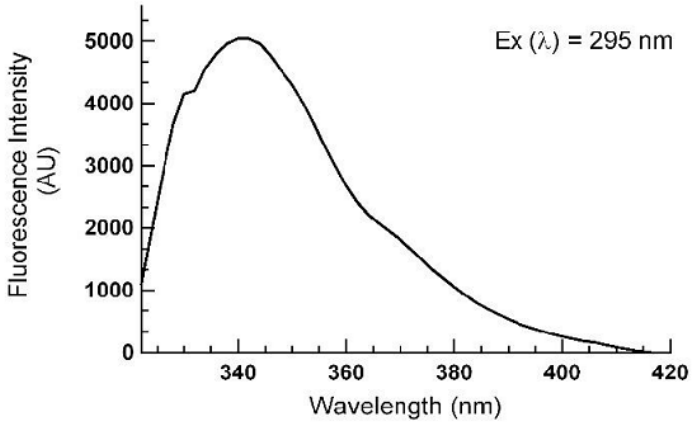
Molecular Weight and Purity Analysis Using 1-D SDS-PAGE



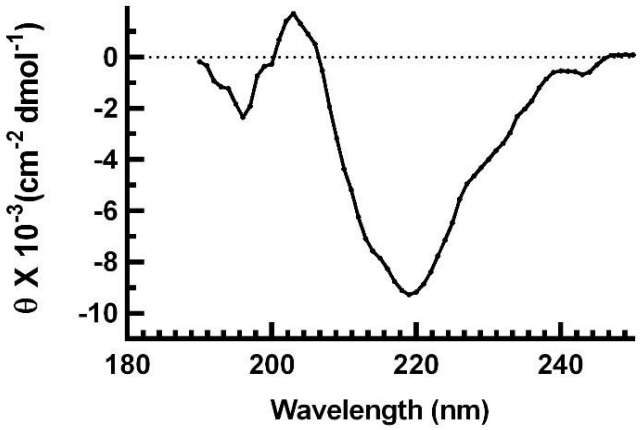
Intact Mass Analysis Using MALDI-TOF Mass-Spectroscopy



Secondary Structure Analysis using Fluorescence Spectroscopy



Secondary Structure Analysis using CD Spectroscopy



Results

- Intact mass-analysis using SDS-PAGE based method identified one prominent protein band at 67.8 kDa, however the intact mass of the primary protein analyzed using Mass-Spectrometry was 58.26 kDa. Values obtained were in agreement with the information available in literature.

- Densitometry based analysis suggested that percentage contribution of the most prominent band in the sample was > 80%.

- Peptide sequences identified (confidential) through peptide mapping over-lapped with > 76% of the known protein sequence of the protein and provided a confirmatory evidence of the protein in the given sample.

- Secondary structure of the protein analyzed using Fluorescence and CD spectroscopy based methods was found to be predominantly (> 90%) is in β-structure form.

Characterization of Biologics : Isoform and Purity Analysis of active substance

Isoforms of active substance: We provide analysis of Glycoforms and other modifications like Phosphorylation, acetylation, myrsitoylation, PEGylation, esterification using varieties of methods such as **HPLC & LC-ESI-MS / MALDI-TOF,** charge variants by **Iso-electric focusing and N-terminal and C-Terminal sequence Analysis**

Purity of active substance: Purity of the therapeutically active proteins is analyzed using **SDS-PAGE and Western Blot Analysis** and varieties of other methods including **RP-HPLC, SE-HPLC, IE-HPLC, IEF / CE-IEF**

Host and process related impurities: Host and process related impurities are analyzed by confirming absence or presence of host-Cell DNA and Protein by **Electrophoresis and Mass-Spectrometry** based analysis. Pyrogen contents are also analyzed similarly.

Case-Study-2 : Protein Purity Analysis - Client – Largest Indian Biopharma Company.

Challenge:

- To characterize a recombinant protein sample by two-dimensional electrophoresis and Western blotting.

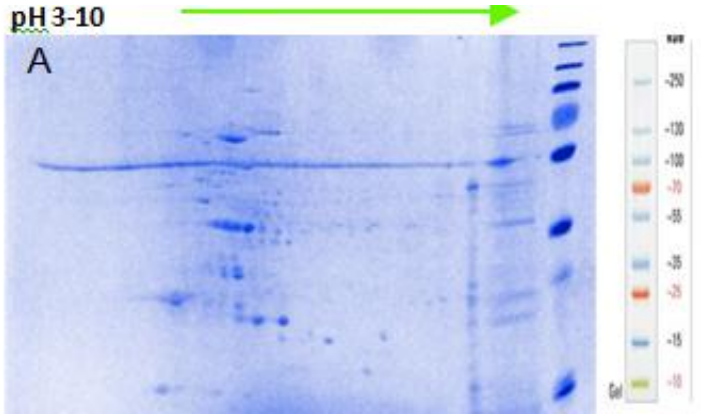
Timeline: 2 weeks

Project Plan:

- Protein Precipitation and Iso-electric focussing
- Second dimension polyacrylamide gel electrophoresis
- Western blot analysis

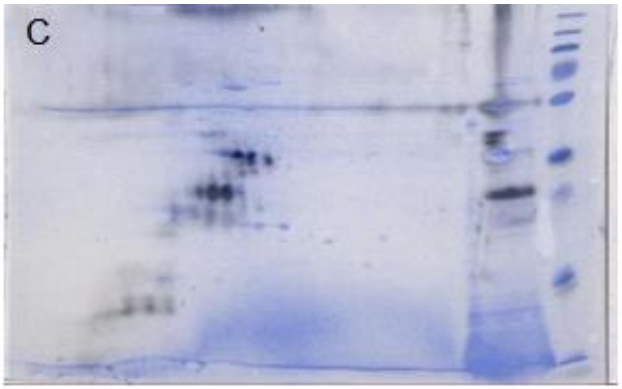
2D gel electrophoresis - Western blotting of protein sample for the detection of Protein using monoclonal antibody.

Coomassie Stained Gel

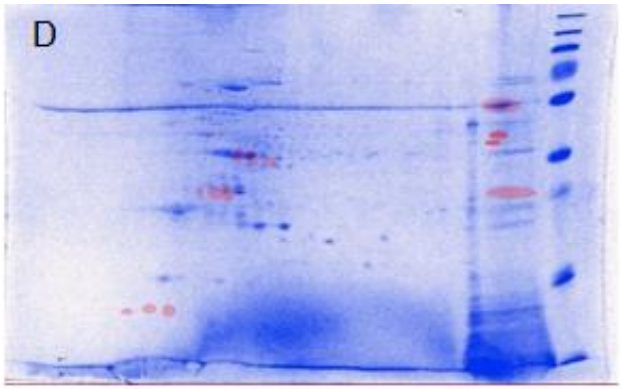


Western Blot Analysis of Proteins

CBB-Western Overlaid Image



Western Reactivity Comparison



Results

The western-blot and 2D gel electrophoresis analysis of the given proteins revealed following information:

- The protein bands at ~55 Kda were confirmed to be of recombinant protein.

- CBB stains established the presence of several proteins in the sample that have molecule weight above and below of 55 Kda. Western Blot analysis confirmed that a few of these proteins are derived degradation/multimer) from source Protein.

Characterization of Biologics : Lipid/Carbohydrate Analysis and Drug Product Characterization.

Lipid and Carbohydrate Analysis:

For the purpose of purity and efficacy batch-to-batch consistency analysis of protein associated lipids and carbohydrates is essential. We utilize variety of techniques including **TLC, LC-MS, GC-MS, FACS** for characterization of biologics on case by case basis.

Drug Product Characterization:

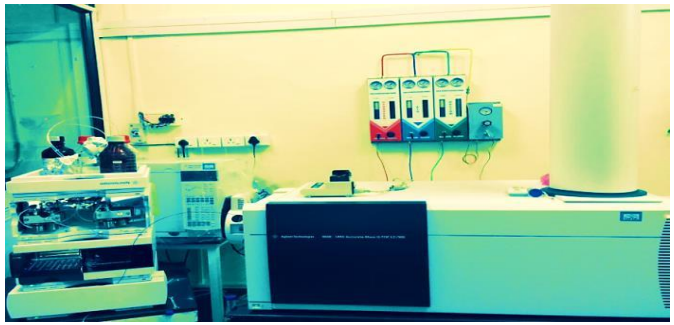
Based on the formulation protein Content, Appearance, pH, Osmolarity, Composition of Key excipient including stabilizer and visible and sub-visible particles in the samples are analyzed by variety of methods.

Proteomics Services

Our team is highly experienced and specializes in high resolution mass spectrometry. We routinely use Mass spectrometry to provide rapid and best plausible solution for a range of bio-analytical problems.

We can provide solutions through :

- Labelled and label-free Protein Mass-Spectrometry
- Differential Proteomics
- De Novo peptide sequencing
- Peptide mass fingerprinting using single and multi-enzyme digestion
- MS analysis of intact proteins and peptides



Case-Study-3 : Quantitative Proteomic Study– Client – A World Health Organization Unit

Challenge:

- To identify and quantitate the up and down regulated proteins in different treatment conditions using mass spectrometry

Timeline: 3 weeks

Project Plan:

- Protein Extraction, Trypsin Digestion
- TMT labelling of peptides, Purification and Fractionation of TMT labelled peptides by strong cation exchange chromatography
- Mass Spectrometry Analysis of Proteins

Results

Protein ID	Protein Description	D	M	CD
Upregulated				
AAEL017134	Pyruvate dehydrogenase	✓	×	✓
AAEL013613	AAEL017134-PA	×	✓	✓
Downregulated				
AAEL010464	Glutamate dehydrogenase	✓	×	✓
AAEL004294	Dihydrolipoamide acetyltransferase	✓	✓	×
AAEL012359	Nucleoside-diphosphate kinase NBR-A, putative	✓	✓	✓
AAEL003011	NADH dehydrogenase, putative	✓	×	✓
AAEL017320	AAEL017320-PA	×	✓	✓

HPLC based Analysis

High-Performance Liquid Chromatography offers selectivity through separation and sensitivity through UV-Visible/Fluorescence detection and over the last few decades has become synonyms of chemical and biochemical analysis. We utilize HPLC for varied analysis needs of our clients in multiple industries including food, nutraceutical and agro sciences.

We offer our services for:

- Method development
- Method validation
- Sample analysis
- Degradation study

Case-Study-4: Method Development for mAB digestion for MS analysis - Client - US based Biotech

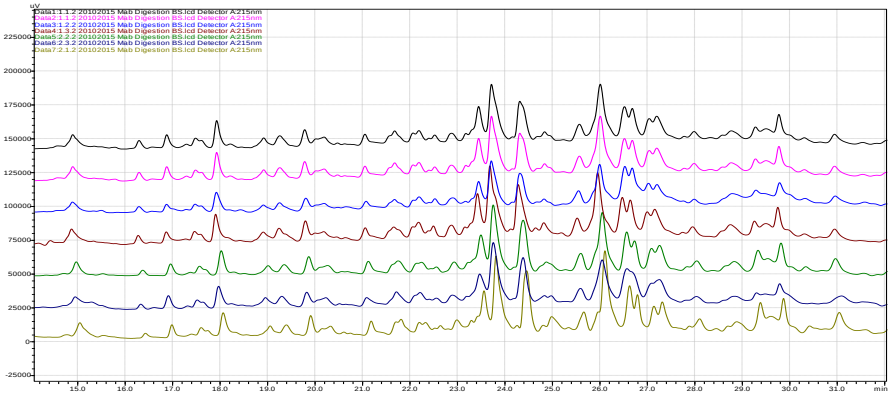
Challenge :

- To develop a mAB Digestion Protocol using HPLC based method that can provide consistent results
- Establish Intraday and Inter-day variation Parameters.

Timeline : 2 weeks

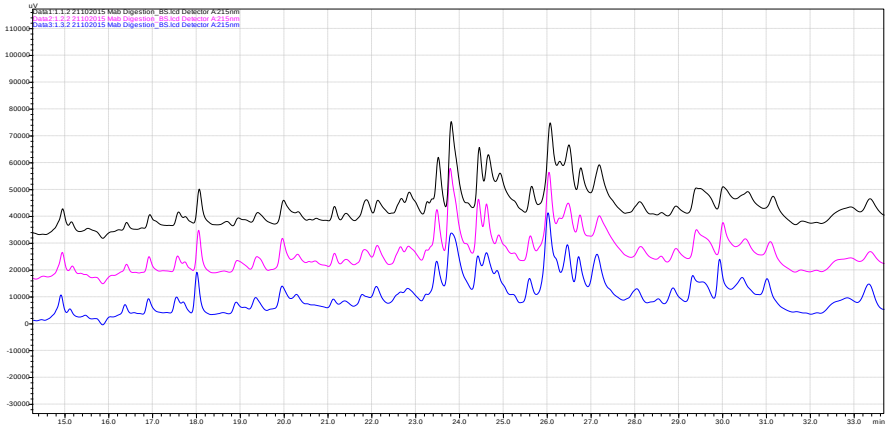
Method Reproducibility

Intra-day variability study:

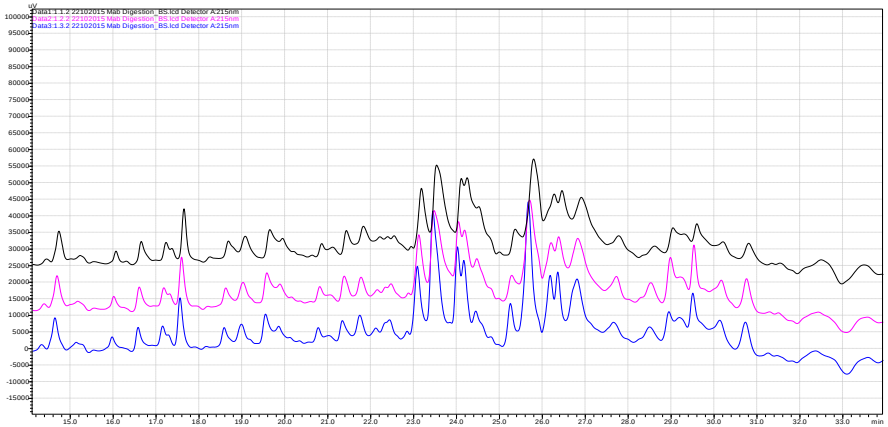


Base shifted raw chromatogram obtained from studies on Day-1

Inter-day variability study:



Base shifted raw chromatogram obtained from studies on Day-2



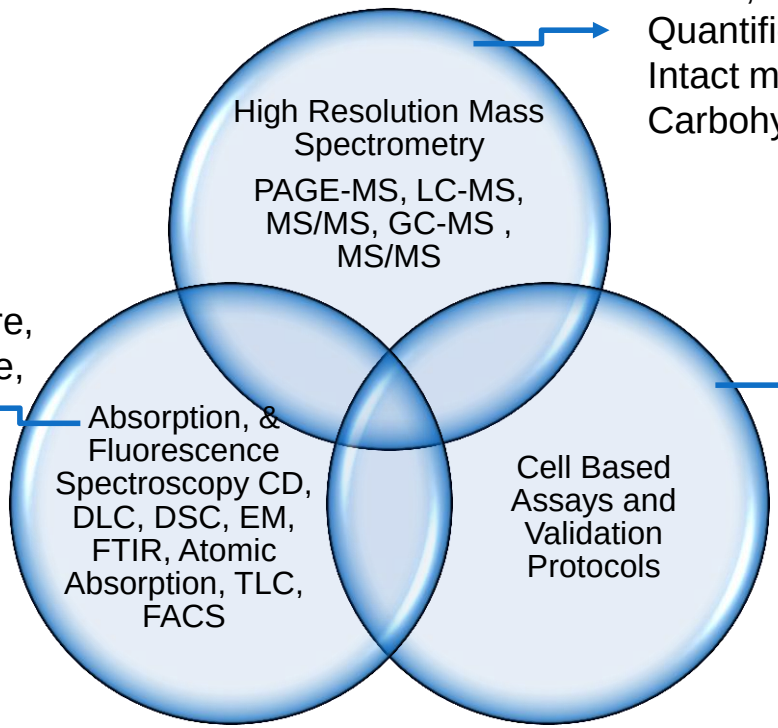
Base shifted raw chromatogram obtained from studies on Day-3

Results

- Variation in three experiments performed in triplicate on three different days (inter-day variation) were less than or equal to 9%, 16% and 13% when respectively peak area-to-height, peak area and peak height was used as the criteria.

Validation Type	Peak Retention Time	Peak Area / Height Ratio	Peak Area	Peak Height
Intraday Variation (Two experiments on same day in triplicates)	0%	4%	11%	3%
Interday Variation (Three experiments on three different days in triplicates)	0%	9%	16%	13%

Matrix of Protein Analysis at Shantani



Protein Identification, Peptide Maps PTMs, Host-Cell Impurity, Protein Quantification, Differential Proteomics, Intact mass, Isoform, Lipids & Carbohydrates

Secondary and Tertiary Structure, Protein Folding, di-sulfide bridge, Particle Size, Metal Impurity, Lipid & Carbohydrate Characterization

Immunogenicity, Toxicity, Mechanism of Action, siRNA, Overexpression, Purification

For more details visit : www.shantani.com

Analytical Tools Available at Shantani

- Amino acid sequence and modifications: MS, Peptide Mapping, Chromatography
- Glycosylation: Anion Exchange, Enzymatic Digestion, Peptide Mapping, MS Mapping
- Folding: MS, S-S Bridge Determination, Calorimetry, Circular Dichroism, FT-IR and Fluorescence
- PEGylation & isomers: Chromatography, Peptide Mapping
- Aggregation: Size-exclusion Chromatography, Light Scattering, Microscopy
- Proteolysis: Electrophoresis, Chromatography, MS
- Impurities: Proteomics, Immunoassays, Metal & Solvents Analysis
- Subunit interactions: MS, Chromatography
- Heterogeneity of size, charge, hydrophobicity: Chromatography; gel & capillary electrophoresis, Light Scattering

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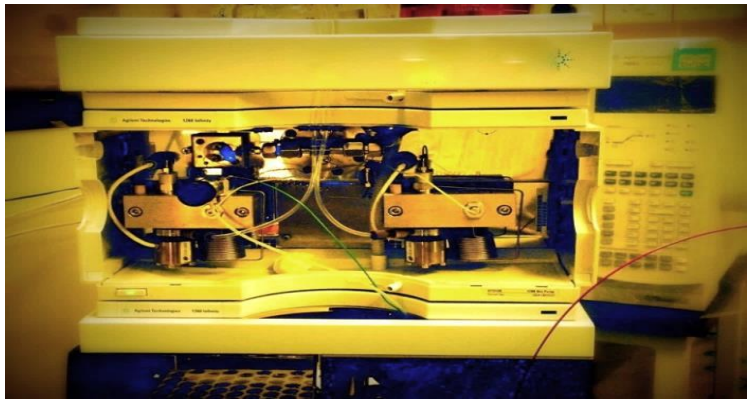
Our Key Strengths

- Highly Customized R&D Services
- Fast-turnaround time and affordable rates
- Globally Competitive Science
- High Ethical and Professional Standards
- Networked Operational Model for Cost-Effectiveness



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Our Team

- Shantani collectively has four decades of experience and expertise in protein chemistry and bioanalytical testing
- 50% of our work force are Ph.D.'s with 10+ years of experience. Their expertise is underpinned by cutting-edge techniques and technologies
- Enthusiastic and committed to assist you with innovative ideas and cost-effective partnership.

How do we work with you – Steps involved

Steps	Activities
1	Interaction with Partner (Client / Collaborator)
2	Project Consultation (Problem Definition)
3	Shantani proposes solution / experiment work-plan (Technical Plan)
4	Client agree / modify and finalize the technical plan
5	Shantani provides cost and time-line estimation (Final Plan)
6	Mutual agreement on final plan
7	Shantani carry out studies and submit results as report (Deliverables)
8	Shantani Receives Payment as per the Final Plan, Project considered closed
9	Data storage and 'on-demand' access provided at no cost for 12 months

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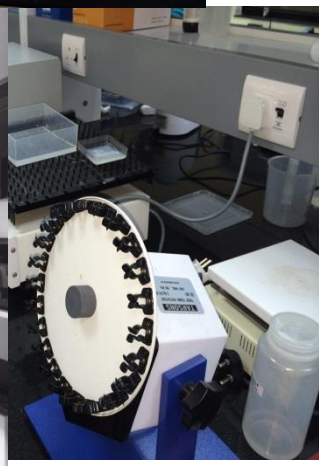
Shantani R&D Center @ Innovation Park



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Some of our clients

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Thank You.

Connect with us…

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