CROWN BIOSCIENCE

Data Science and Bioinformatics Services

Unlocking Potential: Data-driven Insights for Informed Decision-making

A JSR Life Sciences Company

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In the realm of preclinical drug discovery, bioinformatics stands as a vital catalyst. Leveraging computational analysis and data-driven insights, it accelerates target identification, disease and drug mechanism of action (MoA) discovery, and biomarker discovery, reducing costs and late-stage risks. Crown Bioscience's in-house bioinformatics team excels in harnessing 'omics' big data, unlocking innovation-driven insights. By leveraging bioinformatics, we maximize data value, enabling informed decisions, including biomarker-driven clinical trial design, derisking drug development, and offering cost savings. Our commitment to innovation drives us to bring promising drugs to the clinical stage, advancing healthcare and improving outcomes.

Our Advantages:

In-house Expertise with Proven Publication History:

Our dedicated in-house bioinformatics team has a proven history of contributing to peer-reviewed publications, bringing specialized knowledge to your projects.

Vast 'OMICS' Data Expertise:

With deep expertise in handling and interpreting 'omics' big data, we harness its full potential to drive meaningful insights.

Customized Solutions:

Our services are tailored to meet your unique research needs, offering flexibility and adaptability.

Collaborative Partnership:

We work closely with you, enhancing the success of your research endeavors.

Rapid Response:

Known for our prompt communication and efficient project management, we deliver timely results.

Cost-effective Solutions:

We optimize study design and resource usage to provide cost-effective solutions.

Our Bioinformatics Services at a Glance

- **Preclinical Biomarker Discovery:**
  Uncover insights for cell line, organoid, and mouse screening studies.
- **OMICS Data Analysis:**
  Harness extensive 'OMICS' datasets, including Genomics, Transcriptomics, and Proteomics.
- **Unique NGS Services:**
  Innovative NGS offerings, such as Mouse Immuno-oncology RNA-Seq and Patented Cell Line and Biosample Authentication with Deep Sequencing.
- **Drug Combination Analysis:**
  Enhance *in vitro* and *in vivo* combination studies.
- **General Bioinformatics and Statistical Consulting:**
  Tailored support for your research and development needs, including expert statistical guidance.
- **NGS Data Purchase/Transfer:**
  Secure and ethical data procurement is facilitated through Material Transfer Agreements (MTAs).

Crown Bioscience Bioinformatics Services

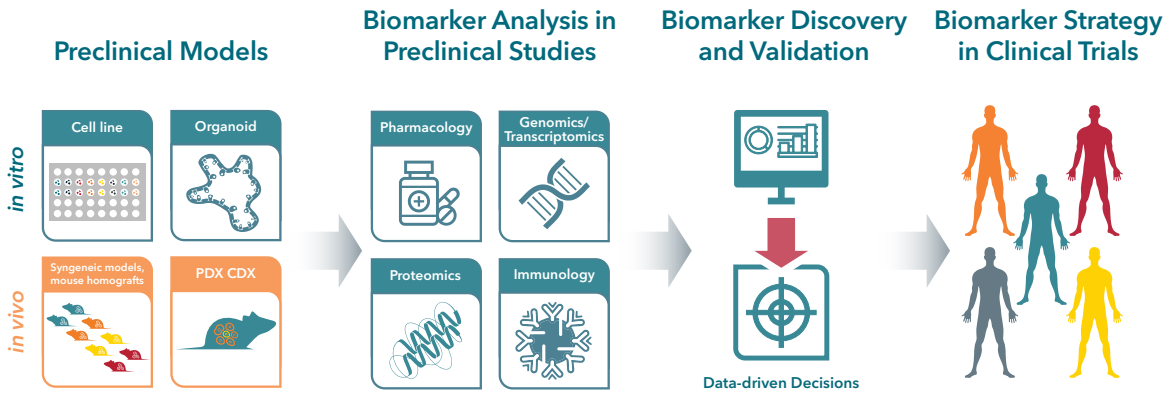
Preclinical Biomarker Discovery

Preclinical biomarker discovery strategies help to identify potential therapeutic targets, assess the safety and effectiveness of new compounds, identify subgroups of patients who are likely to respond to a particular therapy and inform the design of clinical trials. If introduced early in preclinical studies, they can play a key role in reducing the cost and time required for drug development.

Utilize our services to make informed decisions:

- Large-collection of *in vivo* and *in vitro* preclinical models and screening services
- Comprehensive multiomics biomarker analysis
- Complementary databases covering baseline profiling data and historical data of our in-house models
- Experimental design aids
- Powerful bioinformatics data processing capabilities to maximize the value of your data

Preclinical Biomarker Discovery with Crown Bioscience



Biomarker Discovery Via *In Vitro* Screening

Utilize time- and cost-efficient *in vitro* screening to explore/validate your drug MoA, identify genetic features associated with drug response on a set of cell lines/organoids, develop single or composite biomarkers, and provide advice and guidance on future *in vivo* model selection.

Cell line and organoid collections: Over 500 validated and well characterized cell lines for drug response screening and the only CRO service provider of oncology adult stem cell-derived organoids.

Omni**Screen**™ **Cell-Based Screening Service:**
Biomarker discovery streamlined with Omni**Screen**

- Well-validated, large-scale cell panel screening platform
- Focused screening panels
- By cancer type
- Mutation of interest
- Availability of matched *in vivo* models
- Quarterly enrollment
- Cost effective: positive drug control and cell line revival costs are included

Biomarker Discovery Via *In Vivo* Screening

In vivo screening using mouse models can mimic phase II clinical trials to provide accurate measurement of drug efficacy and is powerful for biomarker discovery due to its unique study designs.

Animal model collection: the world's largest commercial collection of patient-derived xenografts (PDX) models, cell line-derived xenografts (CDX), syngeneic models, humanized models, immunocompetent chimeric mouse models, tumor homografts, and more

***In vivo* screening via mouse clinical trial:** preclinical population studies that help stratify patients for clinical trials

***In vivo* screens for evaluating immunotherapies:** Mu**Screen**™

- Cost-effective and time-efficient screen to fast-track *in vivo* preclinical immunotherapy development
- Screen compounds across well-validated panels of syngeneic and tumor homograft models
- Benefit from Crown Bioscience covering the cost of the vehicle group for all models

Multiomics Biomarker Analysis

Comprehensive multiomics biomarker analysis allows researchers to gain a more complete understanding of the molecular changes that underlie a particular disease or during the response to certain treatment.

At Crown Bioscience, we continually invest in cutting-edge technologies, and validate and develop new assays, to provide our customers with a comprehensive range of multiomics laboratory services.

Genomics and Services Transcriptomics

- Standard Services: sample preparation, PCR, qPCR, Sanger Sequencing
- High throughput methods: WGS, WES, RNA-Seq, WGBS, LncRNA-Seq, single cell sequencing, microbiome sequencing, NanoString services, etc.
- Mouse I/O RNA-Seq panel, cell line authentication with deep sequencing

Proteomics

- Immunoassays: ELISA, MSD, Luminex®, Western Blot, 2D gel, and ELISpot
- Mass spectrometry-based proteomics: 4D-DIA quantitative Proteomics, 4D phosphoproteomics and post-translational modification analysis
- Sample preparation and protein separation

Spatial Biology and Digital Pathology

- Digital pathology, IHC, IF, *in-situ* hybridization/ RNAScope
- NanoString spatial multiomics services
- Tumor tissue microarray
- Rare cell analysis
- High content imaging

Immune Monitoring

- Mouse I/O RNA-Seq Panel
- Flow cytometry
- Cytokine and chemokine profiling
- ELISA and multiplex immunoassays

Bioanalysis

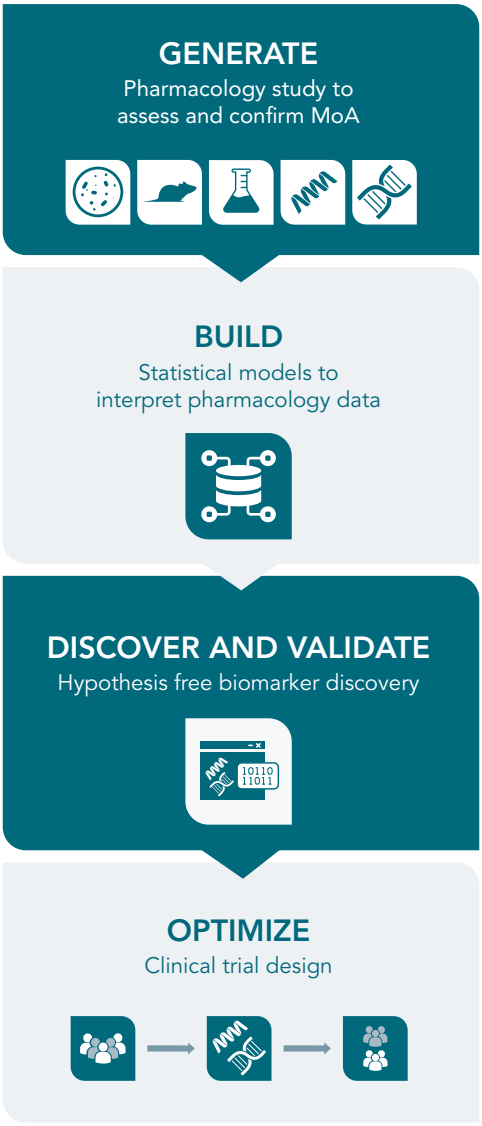
- DMPK
- ELISA, MSD, HPLC, LC-MS/MS based bioanalysis
- Biofluid test
- Flow cytometry
- Cytokine and chemokine profiling

Bioinformatics Data Analysis for Biomarker Discovery

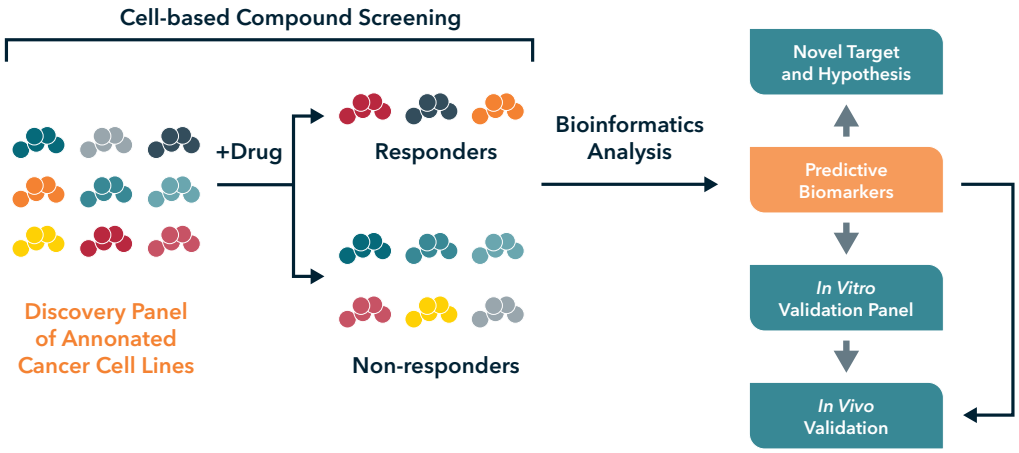
Trust our extensive experience and powerful bioinformatics capabilities to maximize the value of your preclinical data, and de-risk your drug development through early identification of candidate biomarkers.

Use our biomarker discovery services to advance your preclinical and clinical therapeutic candidates with:

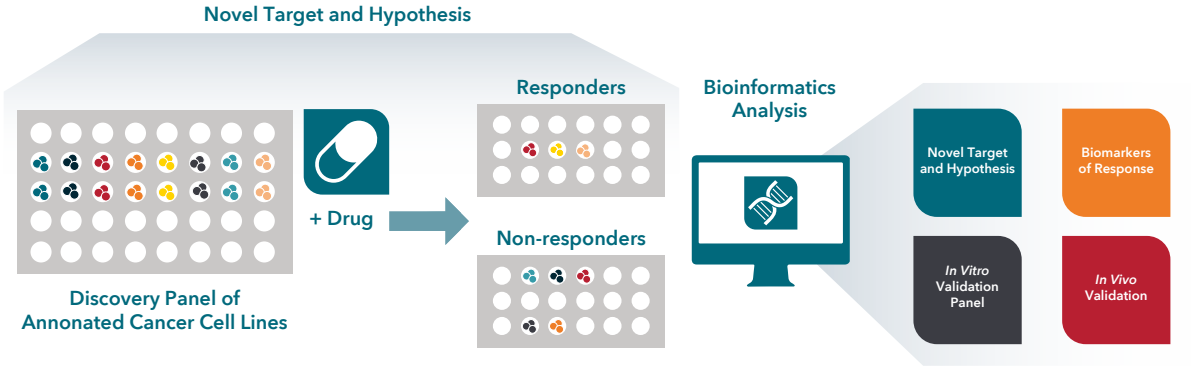
- In-depth biological insight into mechanisms of action and pharmacodynamics
- Enhanced trial design and patient stratification through data-informed trial decisions
- Improved clinical trial success using predictive or prognostic biomarkers



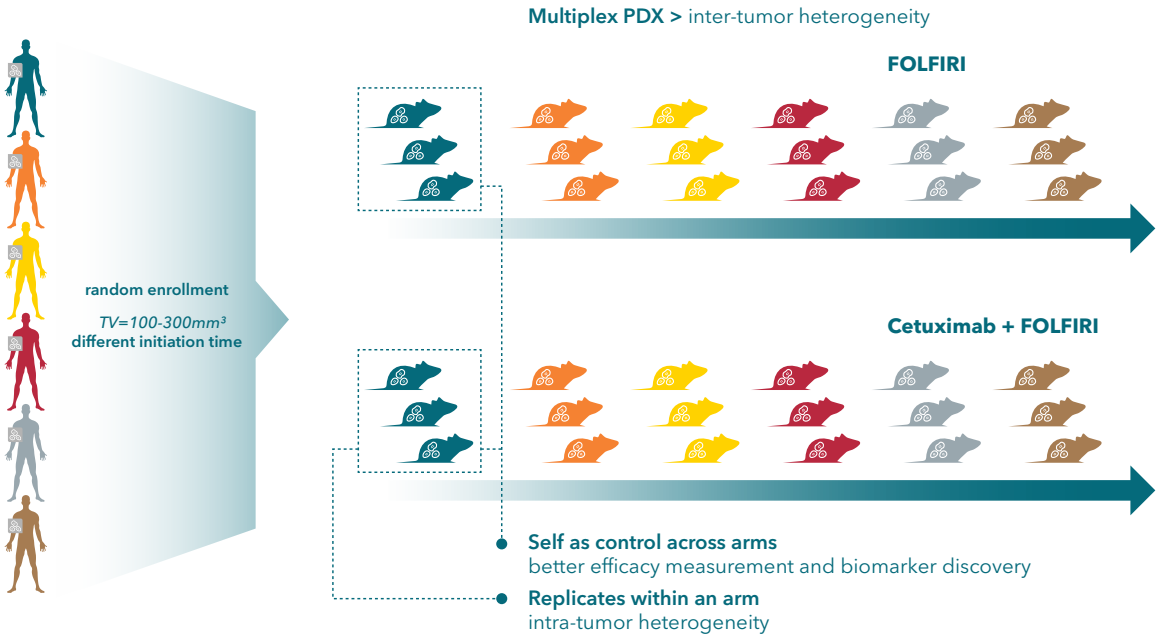
Biomarker discovery can be conducted using data from cell line and organoid screens.



OmniScreen™ Cell based screening service.
Well-validated *in vitro* screening program for rapid and cost-effective screening of over 500 cell lines.



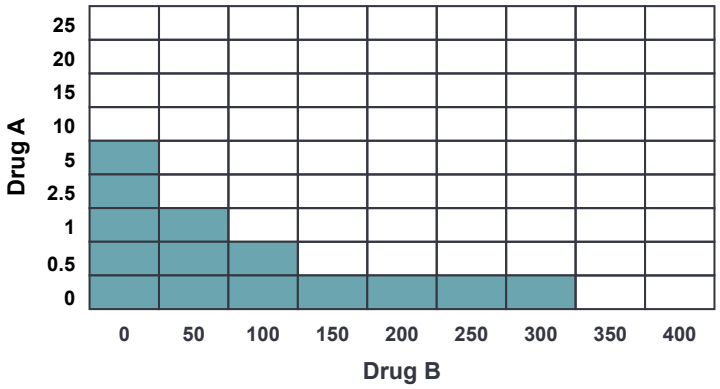
Mouse clinical trials. Design and advantages with inter-tumor heterogeneity.



Crown**Syn™ Drug Combination Analysis**

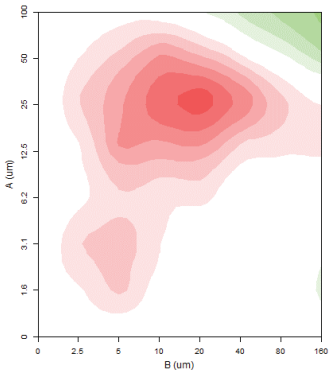
Evaluate and quantify two-drug combination effects of synergistic, antagonistic, and additive cell-based assays with Crown**Syn** algorithm-based reports.

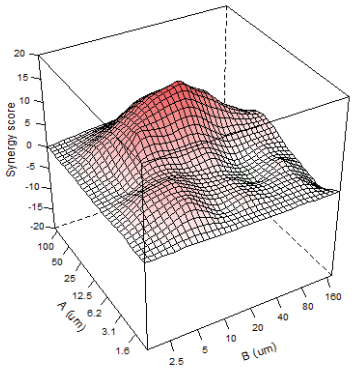
Use your dosing data from your existing assays or work with our experts to design a study, taking advantage of our well-validated cell lines and variety of assay options.



Analyze dosing data from two common types of drug combination experimental designs:

- **Fixed Ratio:** constant concentration ratio
- **Matrix Ratio:** variable concentration ratios





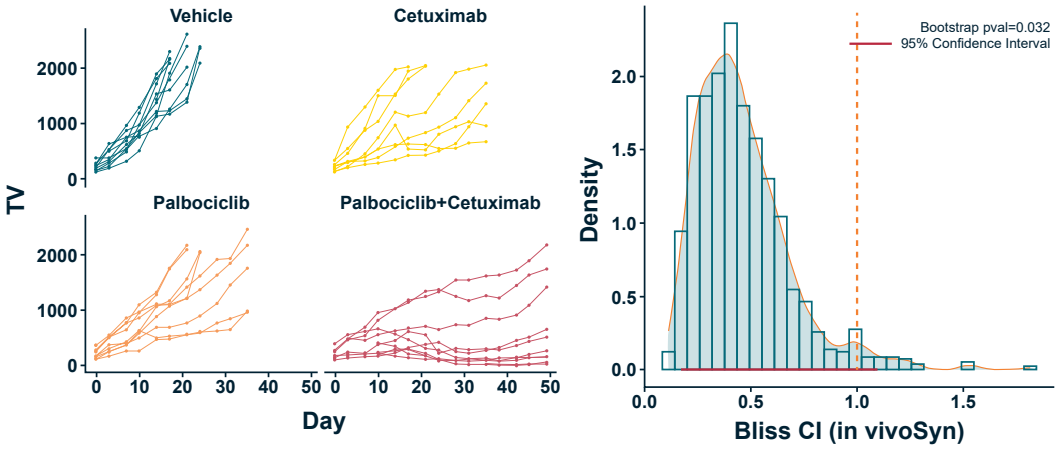
Quantitative Data Visualization

- Dose-response curve graphs
- Combination index graphs
- Inhibition heat maps
- 2D contour maps
- 3D response surface plots

Validated • Accurate • Reproducible • Robust • Reliable

To optimize your Crown**Syn** service, use our extensive cell-based assay options to produce your dosing data, learn more at www.crownbio.com/crownsyn

A standard 4-arm *in vivo* 2-drug combination study.



Proteomics Services and Data Analysis

Services Offered

- 4D-DIA quantitative proteomics
- 4D phosphoproteomics
- Post-translational modification (PTM) analysis

Applications

- Global proteomics profiling of cells or tissues, with/without treatment
- Proteomics biomarker discovery and validation
- Drug mechanism of action and toxicity studies
- Disease mechanism studies
- Target identification and validation
- Complementary analysis and correlation approaches for other omics analysis

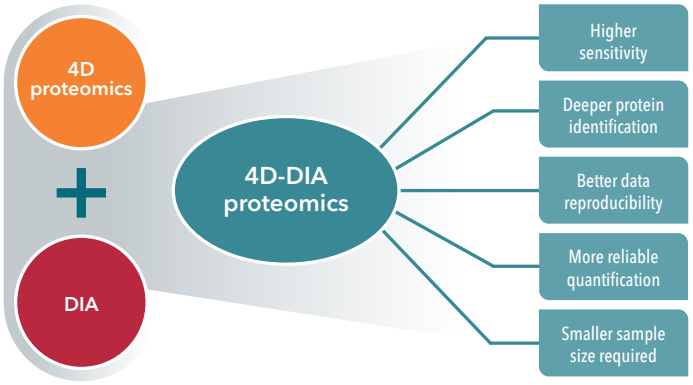
Key Advantages

- **Cost-effective and fast turnaround time, with no compromise on quality**
- **Enhanced sensitivity:** ion mobility can improve the sensitivity of MS by reducing the background signal and increasing the signal-to-noise ratio. This is particularly useful for identifying and quantifying low-abundance proteins.
- **Superior data quality and reproducibility:** DIA provides a more comprehensive analysis of all ions in the sample, rather than just a select few in data dependent acquisition (DDA).
- **Improved PTM identification:** ion mobility mass spectrometry can separate isobaric species and provide improved resolution of PTMs, to facilitate the identification and characterization of PTMs in proteins.
- **Suitable for large sample cohort studies**
- **Proteomics-based biomarker discovery capability:** customized bioinformatic analysis available for large cohort studies

4D-DIA Quantitative Proteomics

Rapid, Unbiased and Deep Proteome Profiling

A new generation of 4D-DIA proteomics technology combines 4D proteomics, which added ion mobility as the fourth separation dimension to traditional LC-MS/MS (retention time, mass-to-charge ratio (m/z) and MS/MS fingerprint), with data independent acquisition (DIA) strategy, which avoids data imbalance caused by randomness by realizing "lossless acquisition" of all possible data.



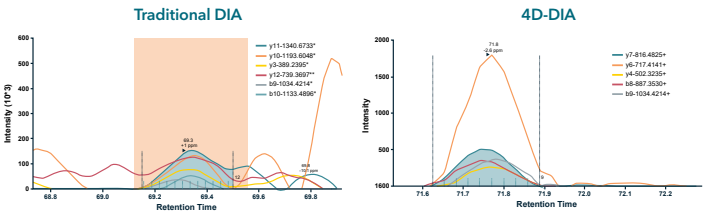
Technical Strengths of 4D-DIA Quantitative Proteomics

4D Alignment for Better Identification

Adding ion mobility separation to the traditional LC-MS/MS adds an extra dimension for calibration, so it can accurately discriminate the specific peptide signals from the mixed spectra of DIA, effectively reducing spectra complexity and improving the detection accuracy and reliability of DIA.

Mass Spectrometry Facility

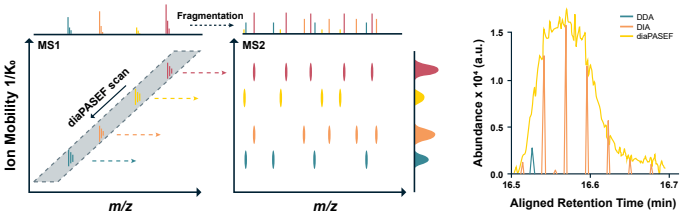
4D-DIA technology effectively reduces the complexity of the spectrum



The chromatograms of EVGSHFDDFVTNLIEK peptide after calibration. There is a high interference signal in the traditional DIA method (left), while the signal background in the 4D-DIA (right) is very clean.

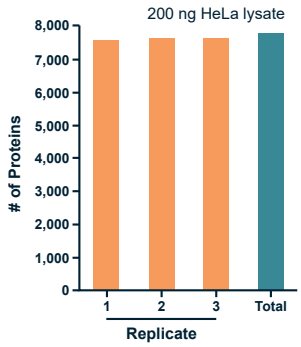
Nearly 100% Ion Utilization, Maximized Detection Sensitivity

The ion-mobility-related collisional cross-section (CCS) value correlates well with m/z. This feature allows the Quadrupole to scan gradually to collect nearly 100% of the ion signals, greatly enhancing the sensitivity and depth of detection.



Significant Improvement in Detection Depth

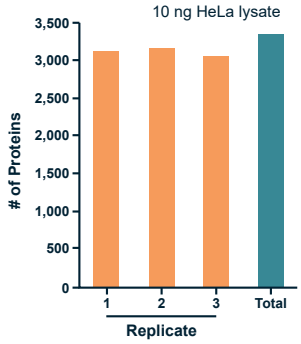
4D-DIA identifies and quantifies low-abundant proteins more accurately than traditional methods, thereby increasing the depth of proteomics detection.



>7,500 proteins can be identified from a single injection of 200 ng of HeLa lysate (120-minute run time), and 6,974 proteins can be quantified with 96% data completeness in triplicate runs. In comparison, conventional proteomics usually requires µg-level samples to detect about 5,000 proteins.

Better Quantitative Integrity

4D-DIA technology further pushes the limits of sensitivity with advanced instrument performance and upgraded acquisition methods.



>3,000 protein can be identified from a single injection of 10 ng HeLa lysate (120-minute run time), and 3,323 proteins can be identified by triplicated runs.

4D Phosphoproteomics

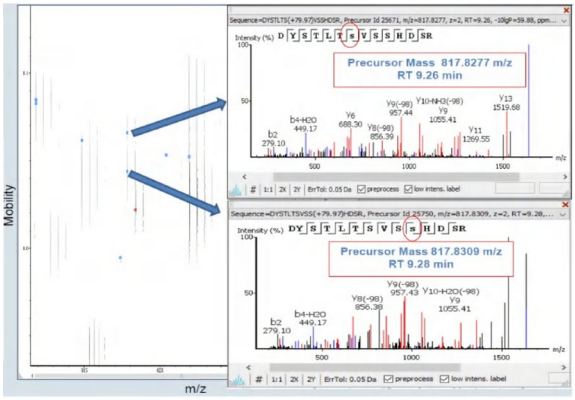
Rapid, Deep and Accurate Phosphoproteome Analysis

- Immobilized metal affinity chromatography (IMAC) strategy: using proprietary targeted antibodies to enrich phosphopeptides, to reduce sample complexity
- Additional ion mobility separation enables more reliable and deeper coverage for phosphorylation
- Strict dual quality control to remove low confident data: false discovery rate (FDR, 1%); false localization rate (FLR, 0.75)
- Upgraded bioinformatic analysis available: kinase prediction, signaling analysis, and data mining

Technical Strengths of 4D Phosphoproteomics

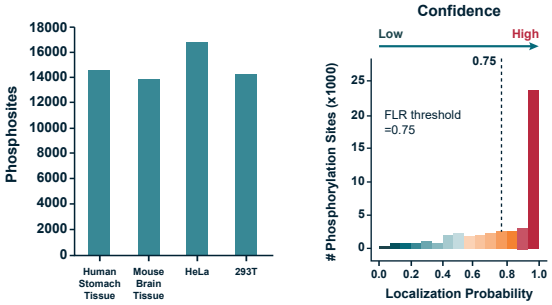
More Reliable Modification Identification Results

4D phosphoproteomics resolves the issue of isomerization in PTM through ion mobility separation, which ensures more reliable identification of PTMs.



Deeper Coverage of Phosphorylation Site Identification

>10,000 phosphorylation sites (localization probability > 0.75) were identified with high confidence in various cell tissues, 50% higher than the traditional method.



Genomics, Transcriptomics Services and NGS Data Analysis

Our Genomics and Transcriptomics Services

Genomics and transcriptomics have transformed drug discovery and development by illuminating disease mechanisms and potential drug targets. We invest in cutting-edge equipment and streamlined workflows to ensure precision and reproducibility. Our technology platforms include:

- **Next-Generation Sequencing (NGS):** utilizing MGISEQ-2000 and Illumina NovaSeq 6000 platforms for cost-efficient, high-quality sequencing across various applications.
- **PacBio Long-read Sequencing:** employing PacBio third-generation sequencing for de novo genome assembly, structural variation detection, and microbiome sequencing.
- **Bionano Optical Genome Mapping:** leveraging the Bionano Saphyr system for high-resolution structural variation detection and genome assembly.
- **NanoString:** with nCounter and GeoMx platforms, we enable high-throughput gene expression analysis and spatial biology.
- **10x Genomics:** utilizing the 10x Genomics platform, including chromium single cell for high-throughput single-cell transcriptomics, and visium spatial for comprehensive spatial gene expression insights. (Indivumed Services, a Crown Bioscience Company)


Illumina NovaSeq 6000


PacBio SEQUEL II SYSTEMS

Bionano Saphyr


NanoString GeoMx


10x Genomics Chromium

10x Genomics Visium

We offer a diverse range of advanced genomics and NGS services, including:

- **RNA-Seq**
- **Whole Exome Sequencing (WES)**
- **Whole Genome Sequencing (WGS)**
- **Whole Genome Bisulfite Sequencing (WGBS)**
- **Long Non-coding RNA Sequencing (lncRNA-Seq)**
- **NanoString Services:** nCounter, spatial genomics and proteomics services.
- **10X Genomics Services:** single cell sequencing and spatial transcriptomics.
- **Microbiome Sequencing:** this includes full-length 16S sequencing, metagenomics sequencing, and metatranscriptomics sequencing.
- **PacBio Long Read Sequencing**
- **Bionano Structural Variation Detection**

Genomics/Transcriptomics Bioinformatics Data Analysis

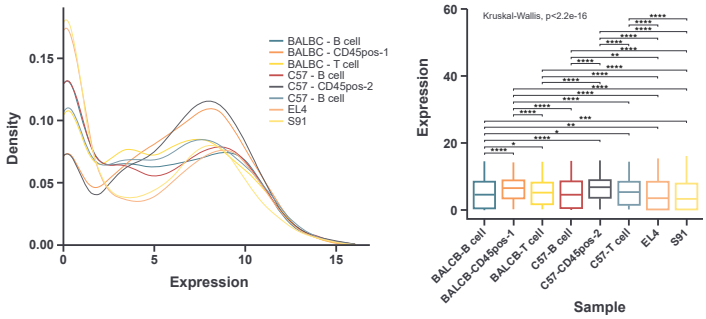
Our expertise merges programming, big data analysis, and biological knowledge to empower you in fully understanding and interpreting your genomics data. Whether as an add-on to sequencing services or a stand-alone option, our bioinformatics services help you maximize data value:

- Data Visualization
- Statistical and Comparative Analysis
- Biomarker Discovery
- Drug Mechanism of Action Understanding
- Complex Sample Analysis

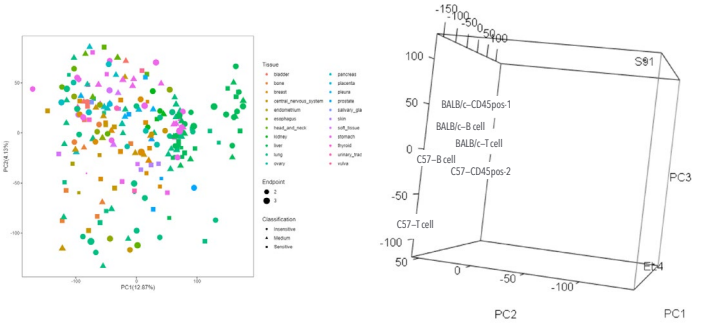
Examples of Analysis (not limited to):

- Data Distribution Analysis
- Dimensional Reduction (Clustering Analysis)
- Differential Expression Analysis
- Gene Ontology Enrichment Analysis
- Pathway Enrichment Analysis
- Pathway View
- Protein-Protein Interaction Network (STRING)
- Co-expression Network Analysis
- Deconvolution Analysis for Cell Fractions Identification
- Genomic and Transcriptomic Variants Analysis

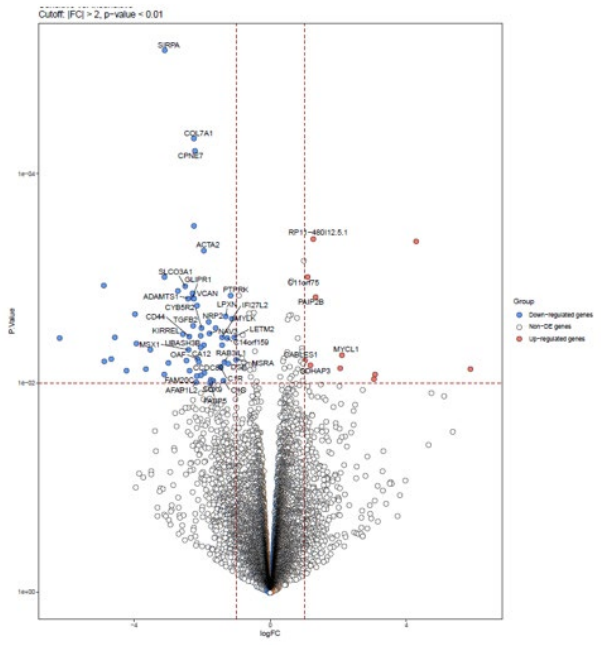
Data Distribution Analysis



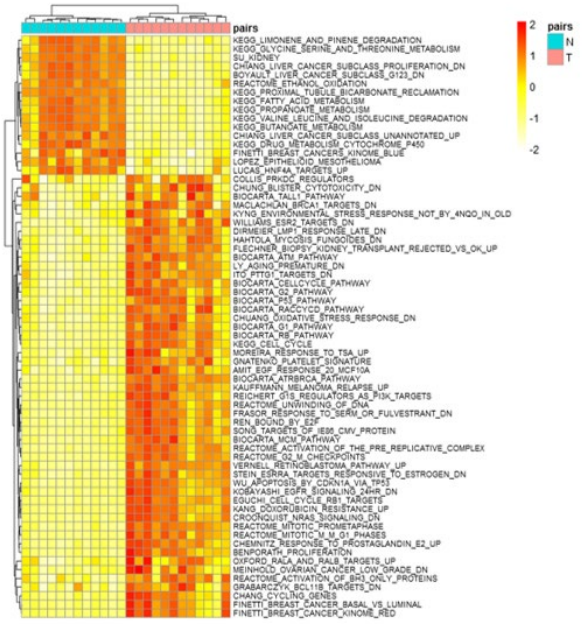
Dimensional Reduction (Clustering Analysis)



Volcano Plot: Differential Expression on Single Gene Level



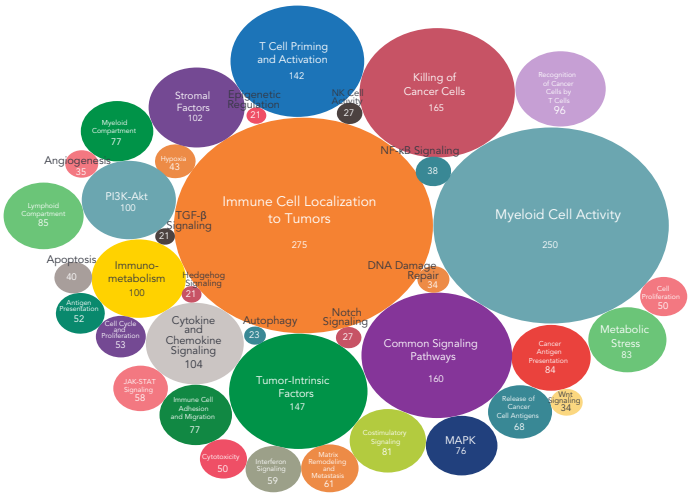
Heatmap: Differential Expression on Gene Set/Pathway Level



Mouse I/O RNA-Seq Panel

- Comprehensive profiling of genes associated with tumor immunity by measuring 1080 mouse transcripts from a single sample
- Rapid transcriptomic insights into key immune cell populations and I/O pathways and process in the TME

Mouse I/O RNA-Seq Panel Benefits	
Comprehensive	• Expression level of 1080 genes from a single sample • Wide coverage in tumors, TME, and immune response
Flexible Sample Types	• Extracted RNA • Multiple tissues: tumor, blood, immune cells etc.
High-Throughput	• Suitable for large-scale immunotherapy screening, as well as examining multiple tissues in parallel
High Sensitivity High Accuracy	• >3 times the sequencing depth of RNA-Seq • Avoid biases on non-protein coding RNAs like mitochondrial RNAs
Easy Integration	• As standalone service or streamline with preclinical *in vivo* studies • Complementary with FACS, IHC and Western Blot
Cost-Effective Fast Turnaround	• Lower cost per sample • 3-week turnaround time



Assay Comparison		
	Mouse I/O RNA-Seq Panel	Microarray I/O profiling
Technology	Targeted deep NGS	Target mRNA (cDNA) hybridize with DNA probes
Target Molecules	mRNAs	mRNAs
Throughput	High (1080 genes)	High (hundreds to thousands of genes, depending on vendor)
Accuracy	High	Low-medium
Sensitivity on detecting low expressing genes	High	Low-medium
Mouse strain discrimination	Yes	No
Turnaround time	2-3 weeks	3-10 weeks depending on vendor
Cost	Low	Medium

Panel Includes Key Immune Cell Signatures

- B cells*
- Dendritic cells*
- Tolerogenic DCs
- Macrophages*
- Tumor-associated macrophages
- G-MDSCs*
- M-MDSCs*
- Neutrophils
- Natural killer cells*
- CD4 T cells*
- CD8 T cells*
- Naïve T cells
- Memory T cells
- Th1 cells
- Th2 cells
- Th17 cells
- T_{reg} cells
- iT_{reg} cells

*Strain specific gene signatures available for C57BL/6 and BALB/c

Streamline Your *In Vivo* Study Sample Analysis Workflow

3 Weeks

Sample generation

- Integrate into existing syngeneic, tumor homograft (MuPrime™), and human target knock-in model (HuGEMM™/HuCell™) studies
- In-house or externally generated murine samples
- Suitable for large-scale immunotherapy screening such as MuScreen™

Sample submission

- Accepted murine samples types include*:
  - Extracted RNA (recom. 2 µg, Min. 500 ng)
  - Frozen tissue
  - Whole blood
  - Immune cell pellets

* See sample Collection and Preparation Guide for more detail

Sequencing

- Targeted RNA-Seq of 1080 genes
- ≥ 2 GB data

Data analysis

- Raw and normalized data
- Comprehensive report with up to 6 comparisons
- Additional comparisons available
- Consultation for follow up studies

Cell Line and Biosample Authentication with Deep Sequencing (Patented)

Outperforms Conventional PCR-based STR/SNP Assays

- Increased Accuracy
- Increased Sensitivity
- Higher Throughput
- Extensive Information
- Lower Cost Per Sample
- Rapid Turnaround

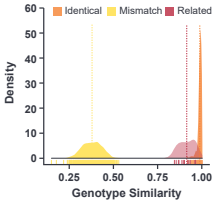
Achieve Three-Level Authentication

Level 1: Match your sample to a reference sample (e.g., a standard cancer cell line)

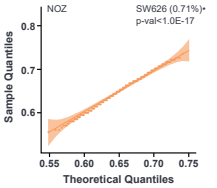
Level 2: Detect interspecies and intraspecies contamination

Level 3: Identify contaminant and estimate contamination ratio

Definite Authentication



Ultra-Sensitive Contaminant Detection



Suitable Sample Types:
Human and mouse samples
(cell lines, tissues, organoids, and xenografts)

Use Cases	
Catalogue new samples with SNP fingerprints	As a standard QC during experiments
Authenticate samples across multiple species	Simultaneously check for contamination and mycoplasma infection

Cell Line Authentication (CLA) Assay Comparison	CLA with Deep Sequencing	CLA with PCR-based STR Assay
Technology	Barcode Deep Sequencing	Multiplex PCR & capillary electrophoresis
# of DNA sites detected	600+	Usually 9 to 24, depending on the vendors
Readout Type	Digital (clean, near-zero quantification error)	Analog (noisy, high quantification error)
Contamination-Detecting Sensitivity	High (1%)	Low to medium (5-20%)
Accuracy	High	Low to medium
Throughput	Yes	Low
Human Sample Authentication	Yes	Yes
Mouse Sample Authentication	Yes	Limited
Mycoplasma Detection	Yes	No
Viral Infection Detection	Yes	No
Quantification of Contamination Ratio	Yes	No
Interspecies Contamination Detection	Yes	Limited
Intraspecies Contamination Detection	Yes	Limited
Population Structure Inference for Human Samples	Yes	No
Gender Detection for Human Samples	Yes	No
Suitable for Large Biobanks	Yes	No
Tracing Genetic Drift And Constructing Phylogeny of Samples	Yes	No
Suitable for Detecting Contamination for Samples w/o Reference	Yes	No

Additional information:

- List of authenticatable human and mouse cell lines at https://qc.crownbio.com
- NAR Genomics and Bioinformatics, Volume 2, Issue 3, September 2020, lqaa060, https://doi.org/10.1093/nargab/lqaa060

Additional service information:

- https://www.crownbio.com/technologies/genomics/cell-line-model-authentication

General Bioinformatics Services and Statistical Consultant

Our in-house team of bioinformatics experts is at your disposal to facilitate a range of general bioinformatics services and consultancy. Leveraging a rich background in bioinformatics, we tailor our offerings to meet the distinct needs of your research, providing a service characterized by adaptability and flexibility. From data analysis to the integration of various bioinformatics tools and databases, our team crafts solutions that can drive your research forward, offering insight and expertise at every step of the process. Moreover, we hold a firm belief in the power of collaborative efforts, and warmly welcome opportunities for academic collaborations.

OMICS Data Purchase and Transfer

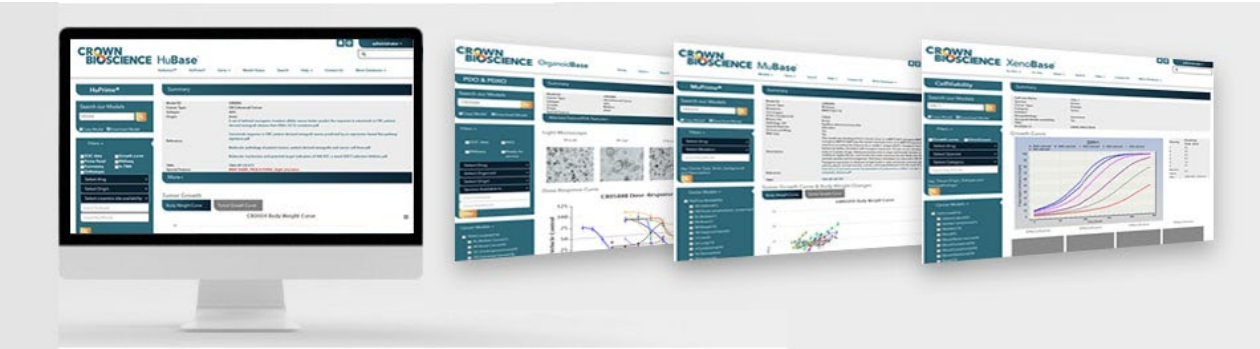
Crown Bioscience is pleased to offer access to our extensive repository of in-house data, encompassing detailed genomics, transcriptomics, and proteomics datasets crafted through meticulous research and analysis. These valuable resources, generated utilizing our sophisticated in-house models, are readily available for purchase to fuel your research and analysis needs. The acquisition of this data is facilitated through a structured fee system designed to provide a seamless transfer of knowledge and information.

We encourage interested parties to reach out to us for further details regarding the specifics of the transfer process, pricing, and to explore how our datasets can complement and enhance your research endeavors.

Our dedicated team is on standby to assist you in navigating the range of data available, ensuring you secure the data most pertinent to your requirements. Let Crown Bioscience become your partner in research excellence, providing you with the data foundation necessary for groundbreaking discoveries.

Cancer Model Databases

Quickly Find Preclinical Models to Fit Your Research Needs



Choosing the right preclinical models is vital for successful oncology drug discovery, avoiding failures and ensuring accurate predictions for human trials. By utilizing our free cancer model databases, researchers gain a significant advantage in their preclinical oncology studies with time and cost savings. Access to diverse and well-characterized tumor models, along with extensive profiling and pharmacologic data, empowers researchers like you to make confident and well-informed decisions during oncology drug discovery.

Our Complimentary Cancer Model Databases: A Comprehensive Collection of Tumor Models and Data

We are proud to present our free databases, specifically designed to empower researchers with a diverse and extensive range of tumor models for preclinical oncology studies. Our databases are supported by a wealth of profiling and pharmacologic data, providing you with a valuable resource to accelerate your research efforts.

Our databases go beyond merely providing models. They include a wealth of existing data essential for comprehensive research. This data encompasses genomics and proteomics profiling, growth characteristics, information on standard of care treatments, and patient treatment history, among other critical details. With access to such comprehensive information, researchers can make informed decisions and select models that best represent the patient populations they aim to study.

Hu**Base™ - The World's Largest Collection of PDX Data**

Access a diverse collection of over 2,400 (and growing) well-characterized and ready-for-service PDX models from US, European, and Asian populations, with genomic and proteomic profiling PDX data.

Organoid**Base™ - A Unique Collection of Patient-Relevant Organoid Models**

The only commercially available 3D *in vitro* models derived using IP-protected Hubrecht Organoid Technology (HUB) protocols.

Mu**Base® - Multiple Immuno-Oncology Murine Models**

Search across murine immunity and human target expressing models including;

- Syngeneic models
- GEMM and carcinogen-induced primary mouse tumor models
- Murine tumor homografts (Mu**Prime™**)
- Humanized target Hu**GEMM™** models

Xeno**Base® - A Large Selection of Cell Lines for *In Vitro* and *In Vivo* Applications**

Well-curated and detailed profiles for more than 1000 cancer cell lines and 200 validated *in vivo* cell line derived xenograft (CDX) models.

North America

Global Headquarters
San Diego
16550 West Bernardo Drive
Building 5, Suite 525
San Diego, CA 92127
USA
Tel: +1 858 622 2900

Indivumed Services
7210 Corporate Court
Ste. E
Frederick, MD 21703
USA
Tel: +1 301 228 9739

Europe

United Kingdom
Hillcrest, Dodgeford Lane
Belton, Loughborough
LE12 9TE
UK
Tel: +44 870 242 2900
Fax: +44 870 166 6233

The Netherlands
Leiden
J.H. Oortweg 21
2333 CH Leiden
The Netherlands
Tel: +31 713322871

Indivumed Services GmbH
Falkenried 88
Bldg. D
20251 Hamburg
Germany
Tel: + 49 40 69 63 572 0
Fax: +49 40 41 33 83 14

APAC

Beijing
6th Floor, Yilan Building
No.28 Huojü Street
Zhong Guan Cun Science Park
Changping District
Beijing
P.R. China 102200
Tel: +86 10 5633 2600

Shanghai
3rd Floor, Building 10
Changtai Square
Zhangjiang Hi-Tech Park
Pudong New Area
Shanghai
P.R. China 201203
Tel: +86 21 8016 8666

Singapore
21, #02-03 Biopolis Road
Singapore
138567
Tel: +65 86963941

Suzhou
Room 303, Building A6
No. 218 Xinghu Street
Industrial Park
Suzhou City
Jiangsu Province
P.R. China 215000
Tel: +86 512 6799 3717

Taicang
Science & Technology
Innovation Park
No.6 Beijing West Road
Taicang City
Jiangsu Province
P.R. China 215400
Tel: +86 512 5387 9999
Fax: +86 512 5387 9999

Taipei
333 Keelung Road
Xinyi District
Taipei 11012
Taiwan
Tel: +886 2 7718 1690

Tokyo
Crown Bioscience & MBL
2-11-8 Shibadaimon
Minato-ku
Tokyo 105-0012
Japan
Tel: +81 3 4363 1361

Get in touch

Sales
busdev@crownbio.com
www.crownbio.com

Science
consultation@crownbio.com