

CELL BIOLOGY

Overview

Target Validation

- Cell Line Selection for Target Validation
- siRNA-Mediated Gene Knockdown
- shRNA-Mediated Gene Knockdown
- CRISPR-based Gene Knockout
- CRISPR-based Pooled Library Screen
- Stable Cell Line Generation with Lentivirus
- Rescue to Prove On-Target Effect
- *In Vivo* Target Validation

Cell-based Assays

- Cell functional assays
 - Proliferation (2D and 3D growth assay)
 - Cytotoxicity
 - Migration
 - Apoptosis
 - Cell Cycle
- Cell signaling assays
- Cell metabolite assays

Cancer Cell Line Panel Screen

- 800+ cell line collection
- Mycoplasma tested and STR verified
- Various assay formats to choose from
- Dedicated team with 12 years experience
- Assay validated by uniformity test and test/retest
- Stringent in-study QC

Assay Platforms

- Absorbance-based assays
- Luminescence-based assays
- Fluorescence-based assays
- High content analysis
- Flow cytometry & cell sorting
- Quantitative PCR
- Western blot, ELISA, Luminex,
- AlphaScreen, HTRF, Reporter Assay

Cell-based Assay Service

Cellular Functional Assays

- Stable cell line generation and characterization
- Cell proliferation assay (CTG, CyQuant, cell counting)
- Long-term proliferation
- 2D clonogenic assay
- 3D growth assays (Soft agar, Matrigel, ULA)
- Apoptosis assay
- Cell cycle analysis
- PROTAC related assays
- DNA damage response assays
- RNA modifying enzyme assays
- High content screen (Acumen eX3, INCELL2000, Operetta)
- Drug combination studies

Signal Transduction Assays

- Cellular protein phosphorylation by Western blot, ELISA, AlphaScreen® SureFire®, HCS
- Protein nuclear translocation

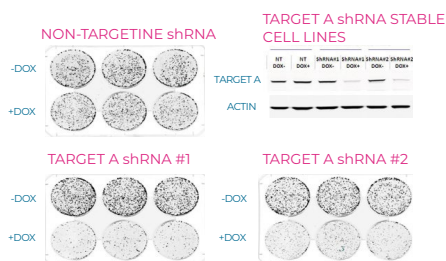
Cell-based Metabolite Assays

- To support cancer metabolism programs
- Use LC/MS to quantify metabolites

Target Validation

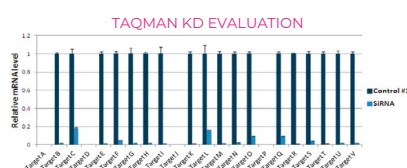
Full Spectrum Target Validation Platform

- siRNA-Mediated Gene Knockdown
- shRNA-Mediated Gene Knockdown
- CRISPR Target Gene Knockout
- Stable Cell Line Generation with Lentivirus
- Rescue to Prove On-Target Effect
- Pooled shRNA or CRISPR Library Screen
- In Vivo Target Validation



RNAI-Mediated Target Gene Knockdown

- siRNA transient transfection
- Lentivirus-based shRNA knockdown
- Evaluate knockdown efficiency
 - RT-Taqman for mRNA
 - Western blot for protein
- Evaluate cellular phenotypes by target gene knockdown
 - 2D cell growth assay
 - 3D cell growth assay
 - Migration, apoptosis, and other functional assays



CRISPR Target Gene Knockout

- Transient Transfection & Lentivirus Infection
- One Vector System & Two Vector System
- Evaluation of Cleavage Efficiency
- Stable KO Clone Generation & Validation
- Functional Assays with Various Readouts
- Pooled CRISPR Library Screen

VALIDATE TARGET GENE KO BY WESTERN BLOT IN SINGLE KO CLONES



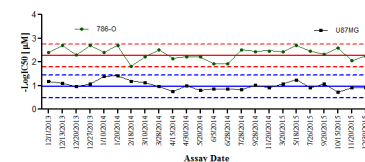
Cancer Cell Line Panel Screen

Tumor Type	Number of Cell Lines
Lung	153
Brain	50
Leukemia	49
Breast	48
Lymphoma	45
Melanoma	38
Colon	37
Pancreas	31
Liver	31
Ovary	25
Esophagus	25
Kidney	20
Stomach	19
Bone	17
Bladder	12
Tongue	12
Myeloma	11
Prostate	7
Others	73

Asian (JCRB/RIKEN/KCLB/SIBS)	Number of Cell Lines
Lung	21
Brain	18
Esophagus	13
Stomach	12
Liver	11
Ovary	9
Pharynx	7
Leukemia	6
Adrenal Gland	4
Tongue	4
Kidney	3
Pancreas	3
Endometrial	2
Skin	2
Others	12

Primary Cancer Lines/PDCs	Number of Cell Lines
Liver	14
Pancreas	20
Others	10+

Human Normal Cells/Cell Lines	Number of Cell Lines
Kidney	2
Skin	2
Breast	1
Umbilical Cords	1



800+ CANCER CELL LINES READY FOR SCREENING

- >80% of the cell lines are in CCLE panel with genetic annotations
- Representative of genomic diversity, tissue and ethnic origins
- High quality cell panel guaranteed by STR verification and mycoplasma testing

STRINGENT QC AT DIFFERENT LEVELS

