

AACR 2018



Harnessing the Power of CRISPR Cas-9 Technology for Cancer Research

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Field Application Scientist

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Agenda

- **Speaker Introduction & Quick Audience Poll**
- **Horizon Discovery Functional Genomic Products & Services Overview**
- **Introduction to CRISPR Screening Approaches**
 - CRISPRko
 - CRISPRi
 - CRISPRa
- **Considerations for Optimal CRISPR Screening**
- **Pooled & Arrayed CRISPR Screening Examples**
- **Summary**
- **Q & A**

Vicki Racicot | Background

- **20 years of combined experience spanning:**
 - Assay development for early stage oncology drug discovery in large pharma (10 years).
 - Agricultural gene discovery.
 - HTS/Microarray/FISH product development.
 - Applications support for high content imaging, bioproduction, and microarray scanner instrumentation.
- **M.S. in Plant Cell Biology (Purdue University); B.A. in Biology/Biotechnology (Assumption College),.**
- **Enjoys world travel, live music, gourmet cooking & mixology.**
- **Lives in a vintage tiny house in beautiful Alameda, CA with her cat Cassidy.**



Who has used our products (Dharmacon, Cell Lines, Diagnostics Reference Standards)?

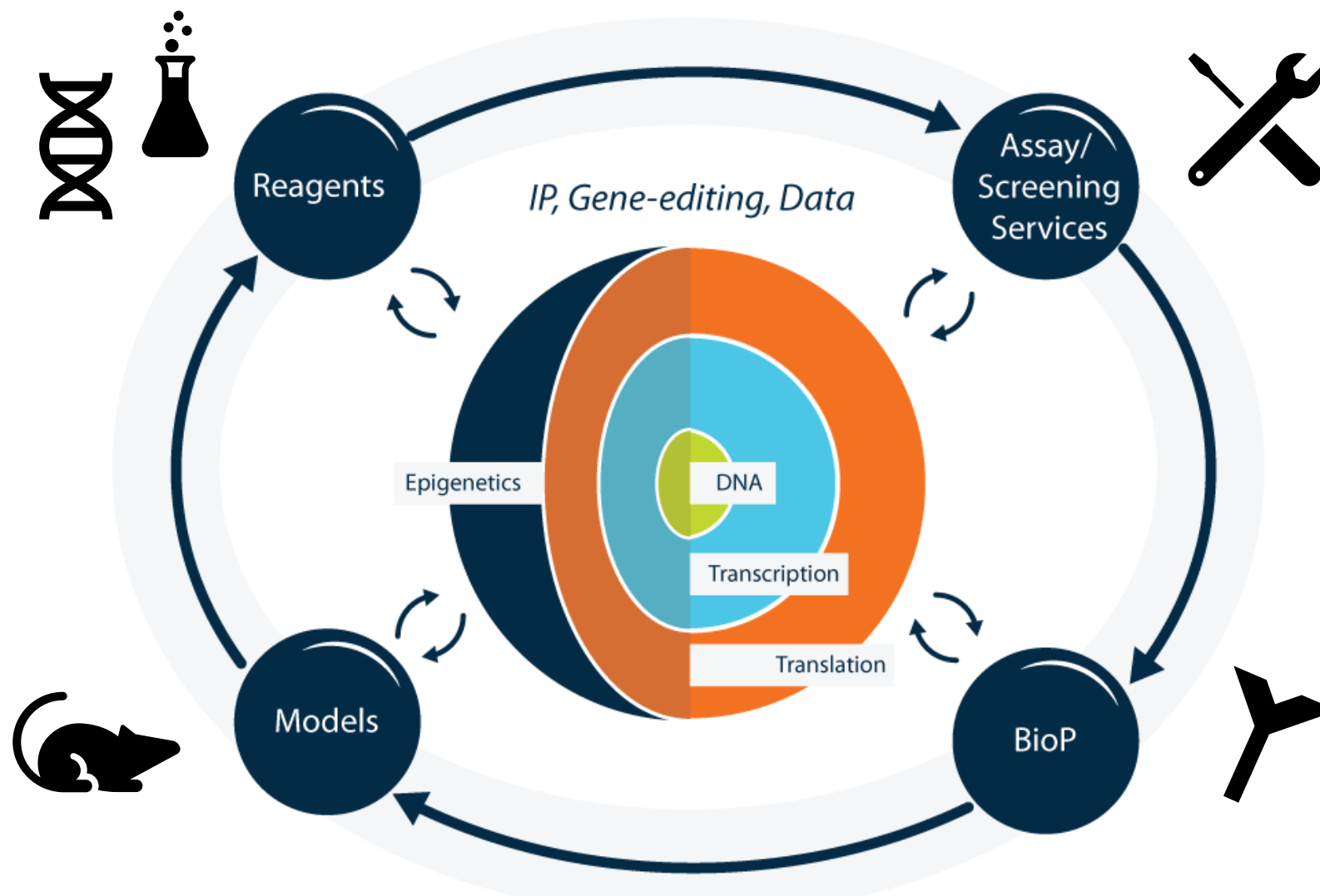
Who is aware of Horizon's Functional Genomic Screening products & services?

Who has worked with us previously as a service provider?

Who has used CRISPR-Cas9 tools for their research?

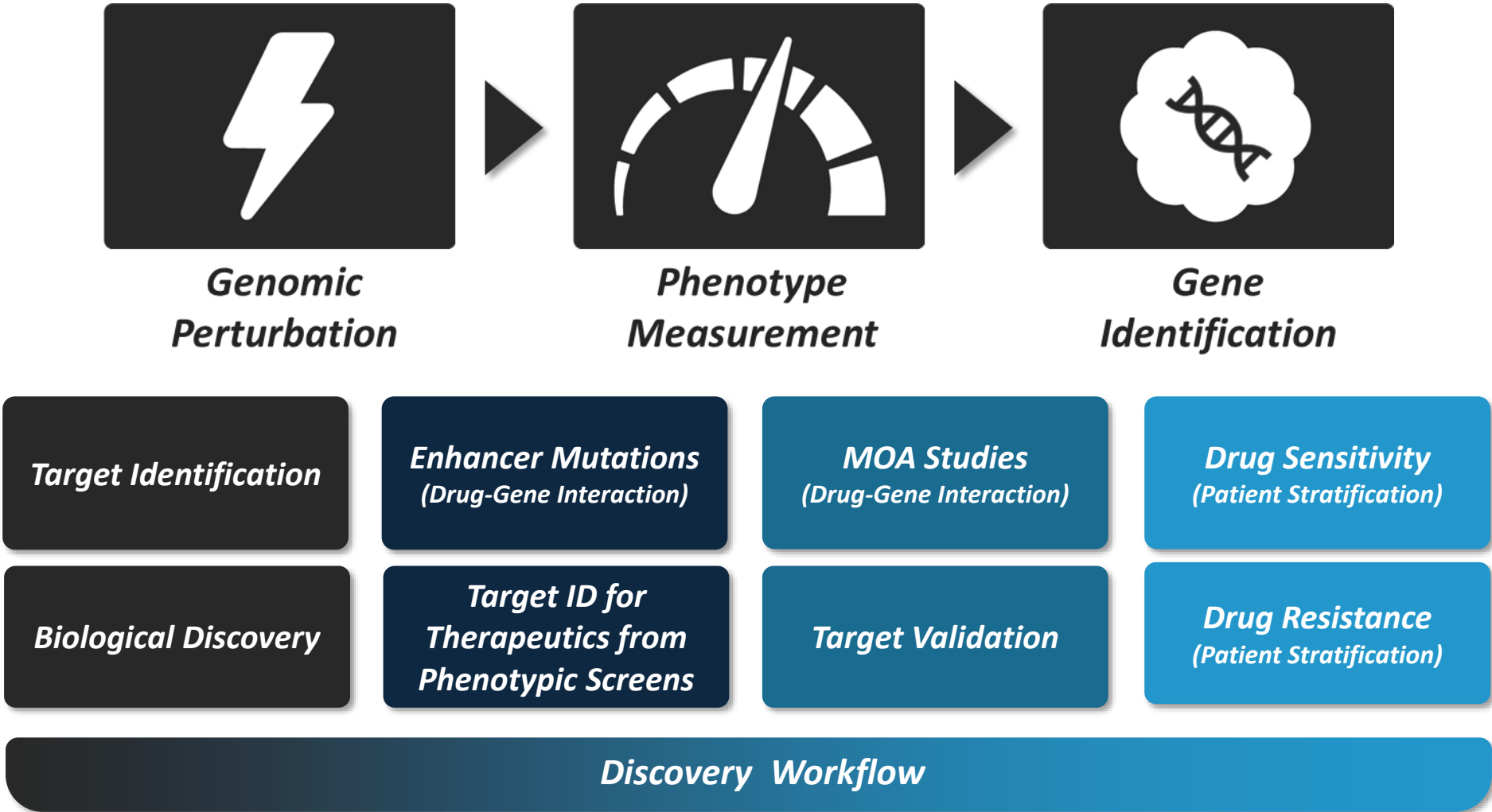
Who has conducted CRISPR-based pooled or arrayed screening?

Who plans to conduct CRISPR screening?



Horizon's Mission: Facilitating Functional Genomics and Translational Medicine

Functional Genomic Screening in Research



CRISPR Screening Technology Has Developed Very Rapidly

Genome-Scale CRISPR-Cas9 Knockout Screening in Human Cells

Ophir Shalem,^{1,2*} Neville F. Sanjana,^{1,2*} Ella Hartenian,¹ Xi Shi,^{1,3} David A. Scott,^{1,2} Tarjei Mikkelsen,¹ Dirk Heckl,⁴ Benjamin L. Ebert,⁴ David E. Root,¹ John G. Doench,¹ Feng Zhang^{1,2†}

Genome-wide recessive genetic screening in mammalian cells with a lentiviral CRISPR-guide RNA library

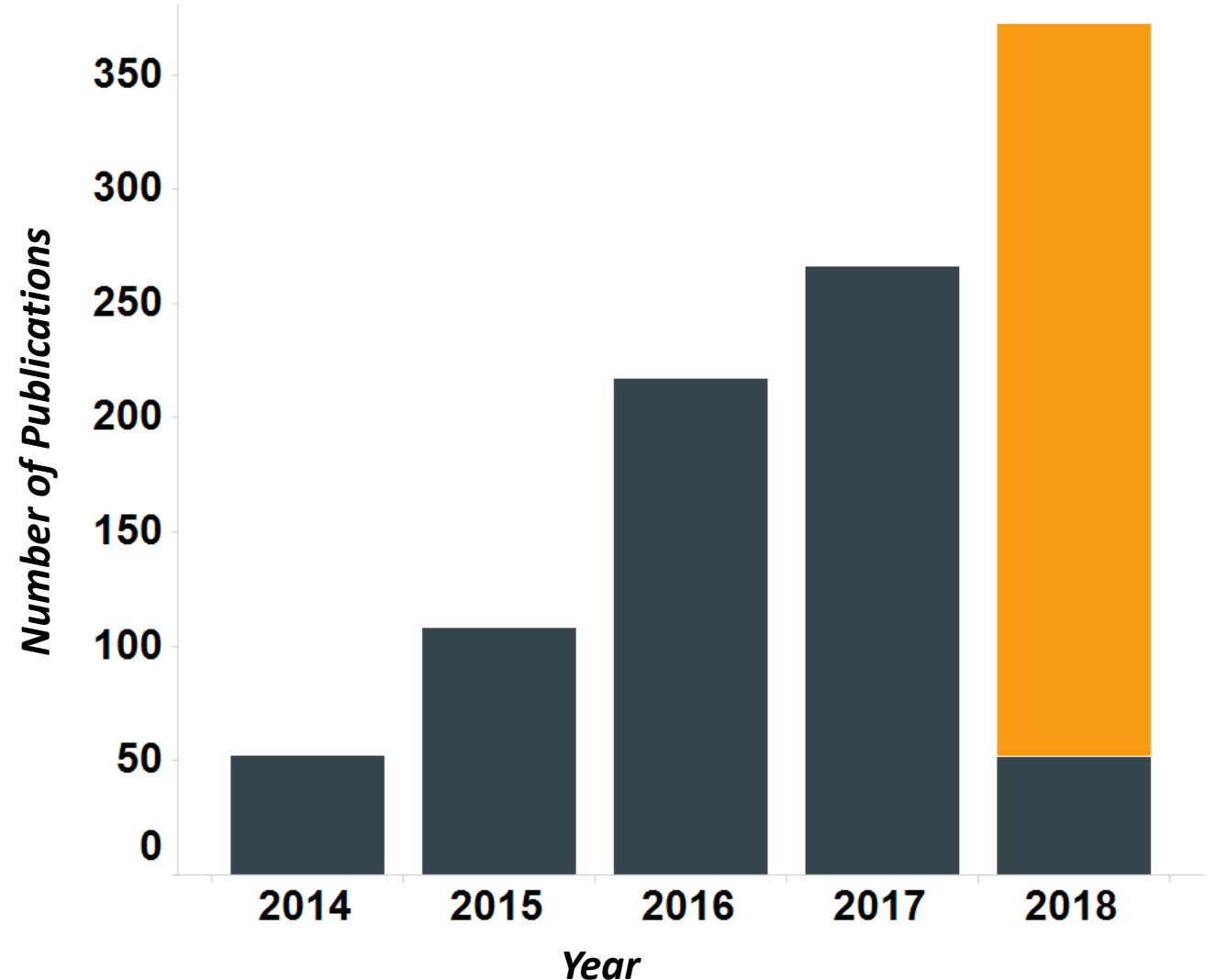
Hiroko Koike-Yusa^{1,2}, Yilong Li^{1,2}, E-Pien Tan¹, Martin Del Castillo Velasco-Herrera¹ & Kosuke Yusa¹

Genetic Screens in Human Cells Using the CRISPR/Cas9 System

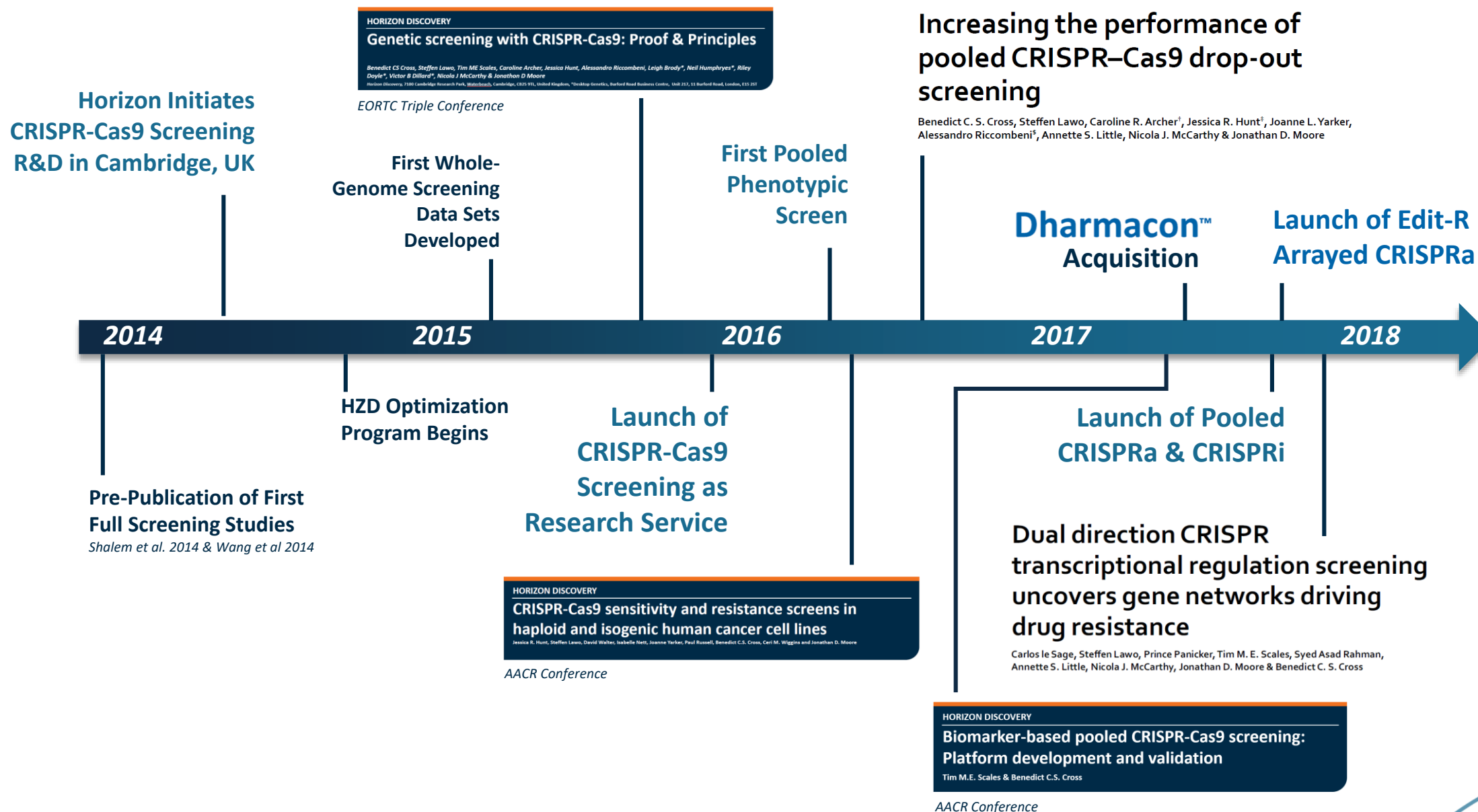
Tim Wang,^{1,2,3,4} Jenny J. Wei,^{1,2} David M. Sabatini,^{1,2,3,4,5*}† Eric S. Lander^{1,3,6*}†

High-throughput screening of a CRISPR/Cas9 library for functional genomics in human cells

Yuxin Zhou^{1*}, Shiyong Zhu^{1*}, Changzu Cai^{1*}, Pengfei Yuan¹, Chunmei Li², Yanyi Huang² & Wensheng Wei¹



Horizon: Early Adopters of CRISPR Screening



CRISPR_a

CRISPR activation: Screen for **gain-of-function** mutations by targeting the **endogenous promoter site** with **transcriptional activators**.

CRISPR_i

CRISPR interference: Inactivate gene function by **site-specific transcriptional silencing** at the TSS.

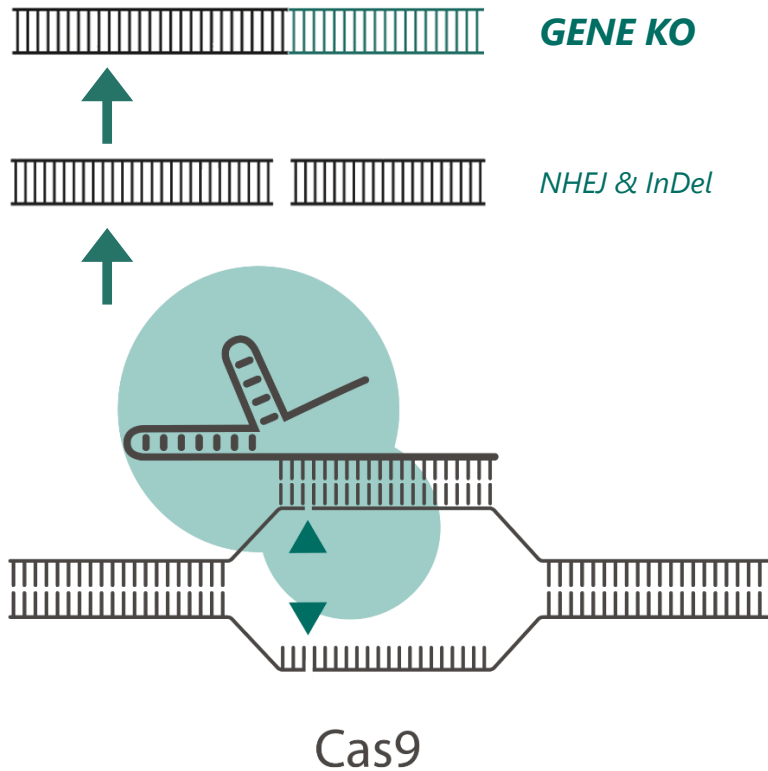
CRISPR_{ko}

CRISPR knock-out: Loss-of-function screening using irreversible **gene editing** to provide a robust phenotypic response.

CRISPR Screening: Not Just Knock-Outs!

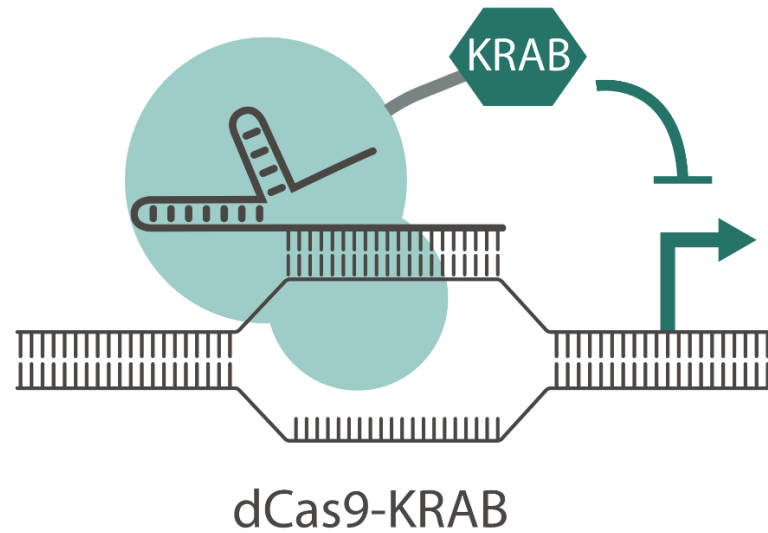
CRISPRko

Genome editing



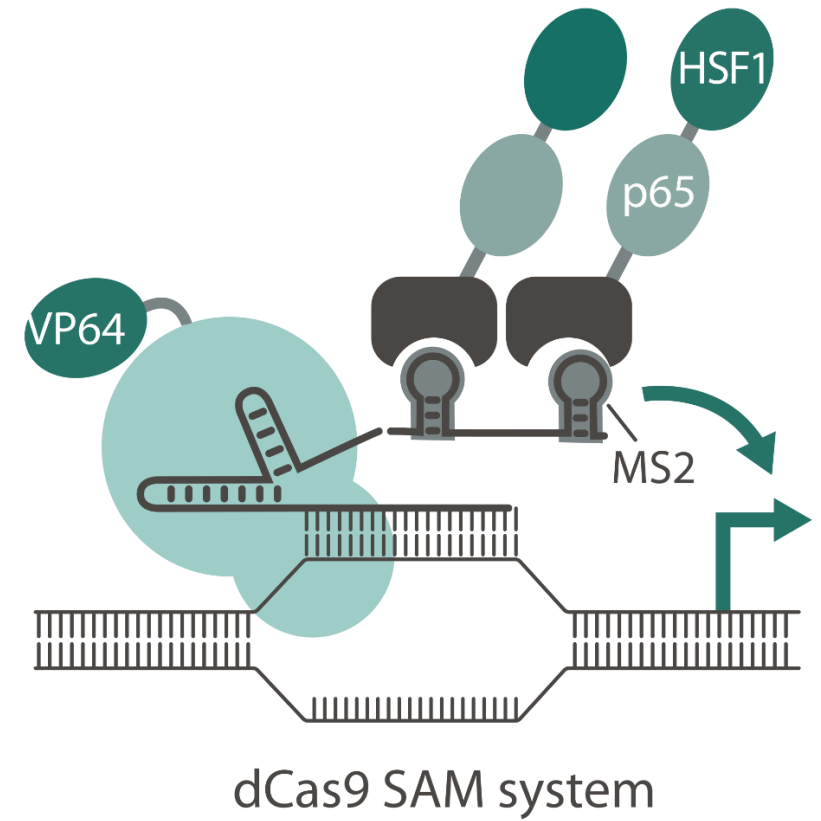
CRISPRi

transcriptional repression

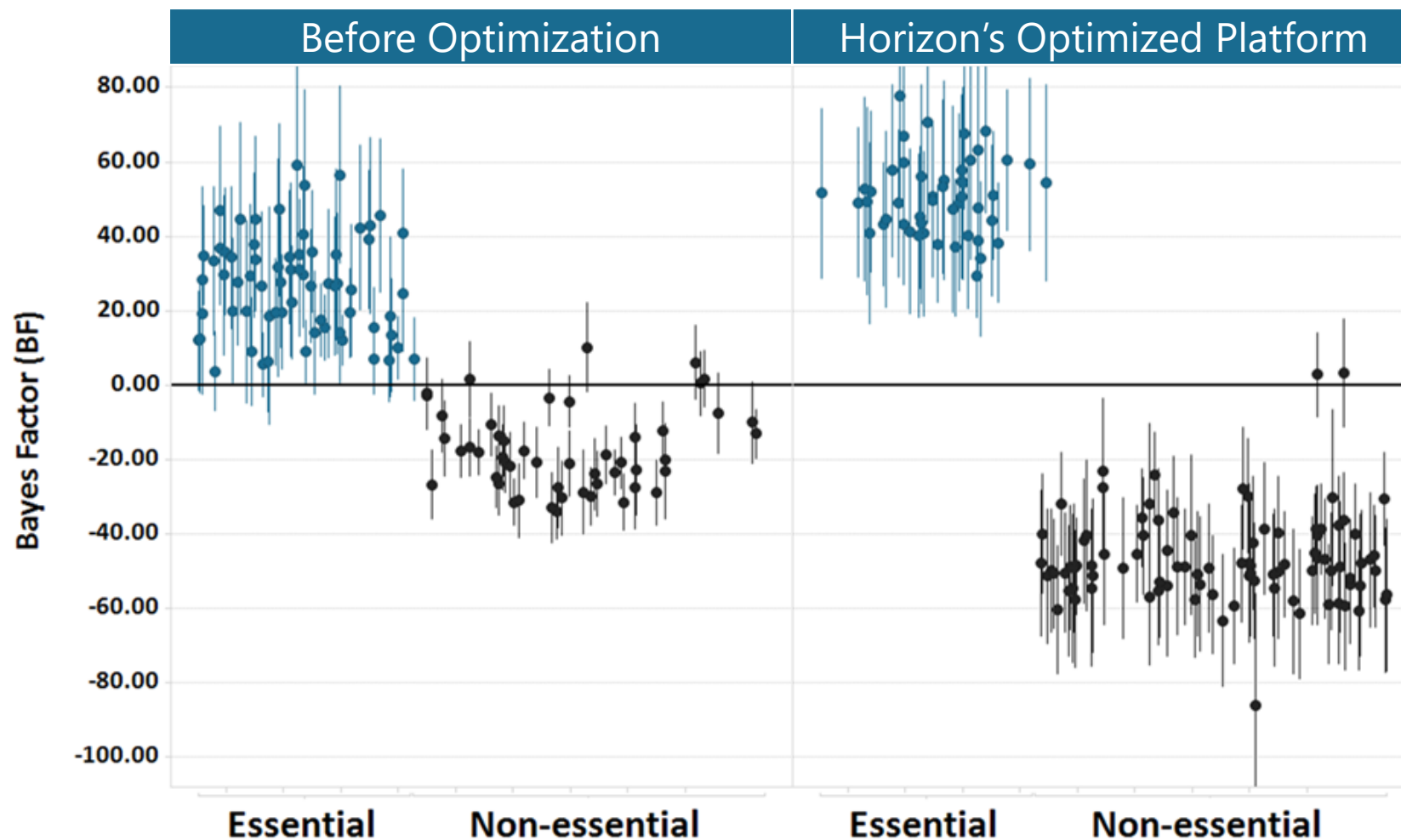


CRISPRa

transcriptional activation

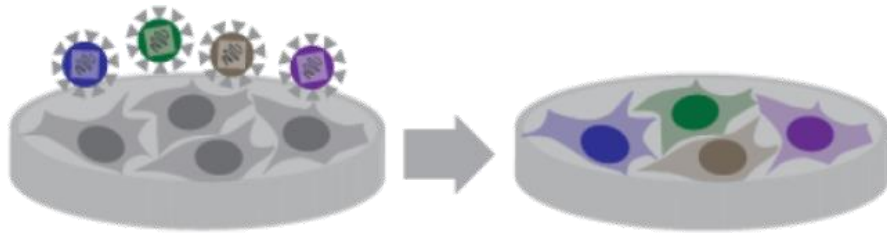


Horizon's Optimized CRISPRko Platform: Improved Performance



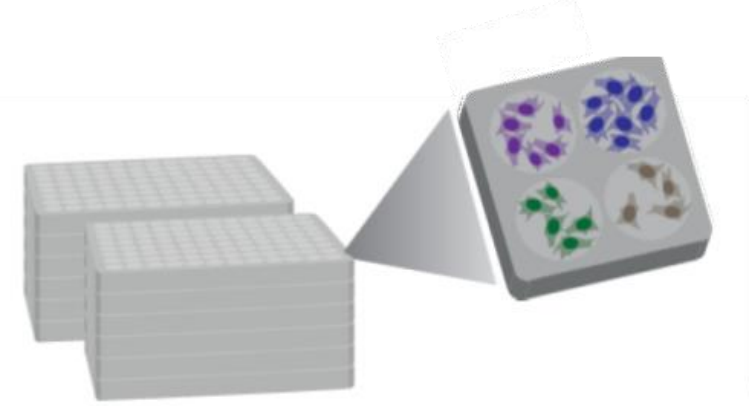
Cross *et al*, (2016)

POOLED SCREENING



- Up to whole-genome level screening.
- Choose a proliferation or phenotypic (i.e. cell sorting) readout.
- NGS-Linked Readouts
- Longer Assay Time Points Possible

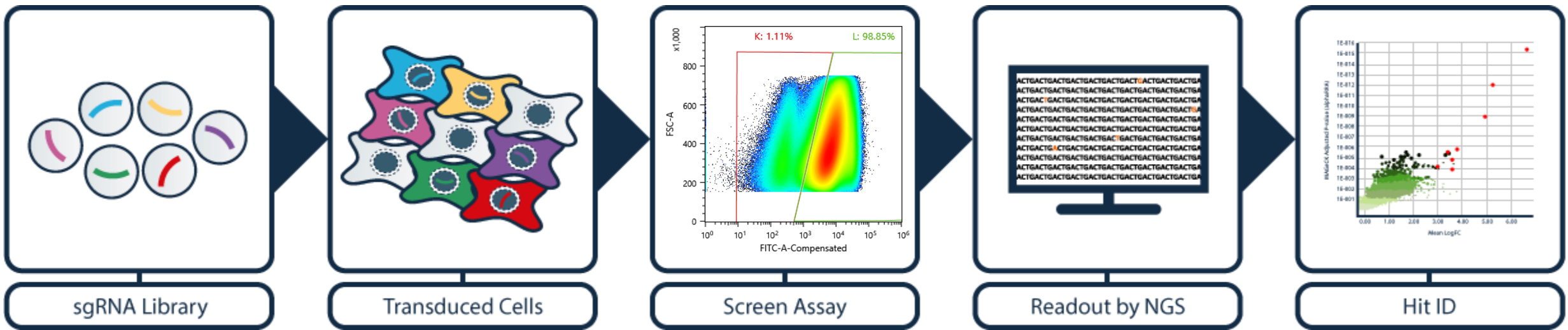
ARRAYED SCREENING



- From very low to high throughput.
- Multiple and Multiplexed Readouts
- Complex growth models (i.e. 3D, co-culture) possible.
- Shorter Assay Time Points (48-144hrs)

Horizon provides both **Products** and **Services** for Functional Genomic Screening

Pooled Screening: How Does It Work?



CRISPR_{ko}
CRISPR_i
CRISPR_a

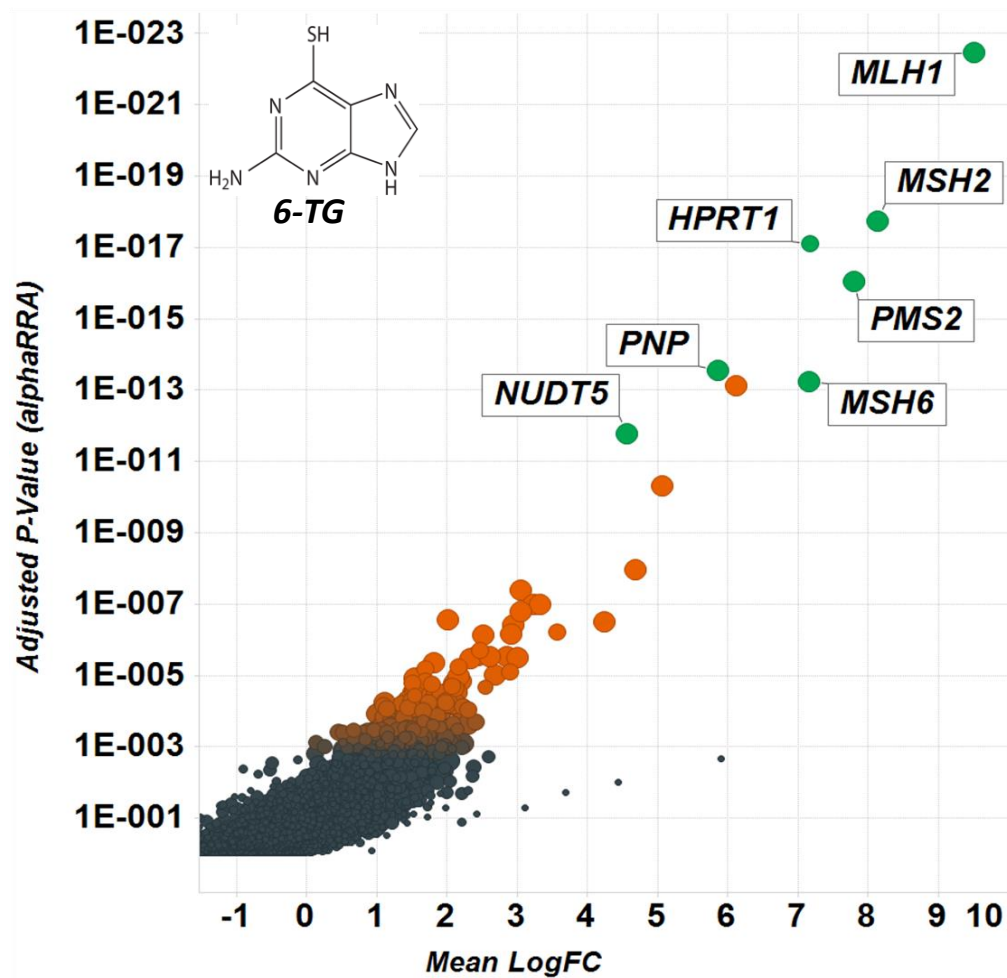
Optimized for
CRISPR Screening
(>100 at Horizon)

Choose Cell Proliferation
OR
Cell Sorting Endpoint

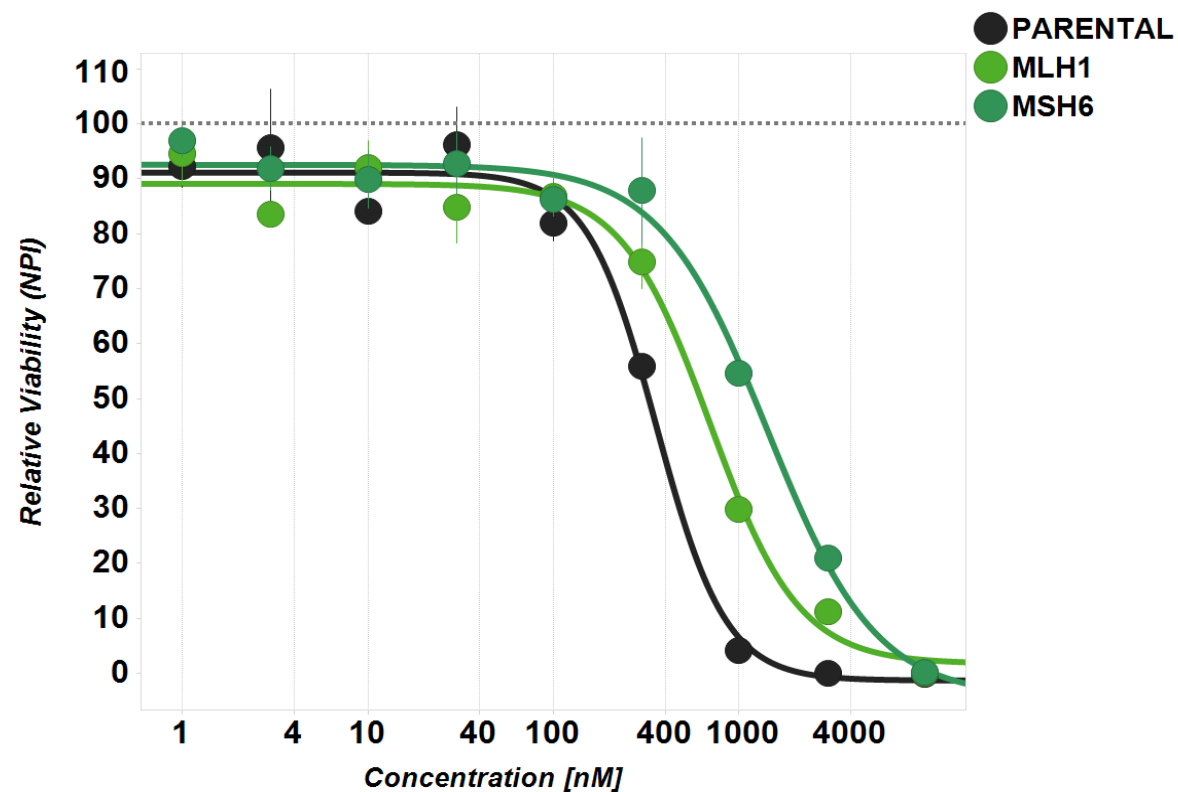
Rigorous Internal QC & Bioinformatics
Expertise for Robust Hit Selection

Using Pooled Screening to Define Drug Mechanism of Action

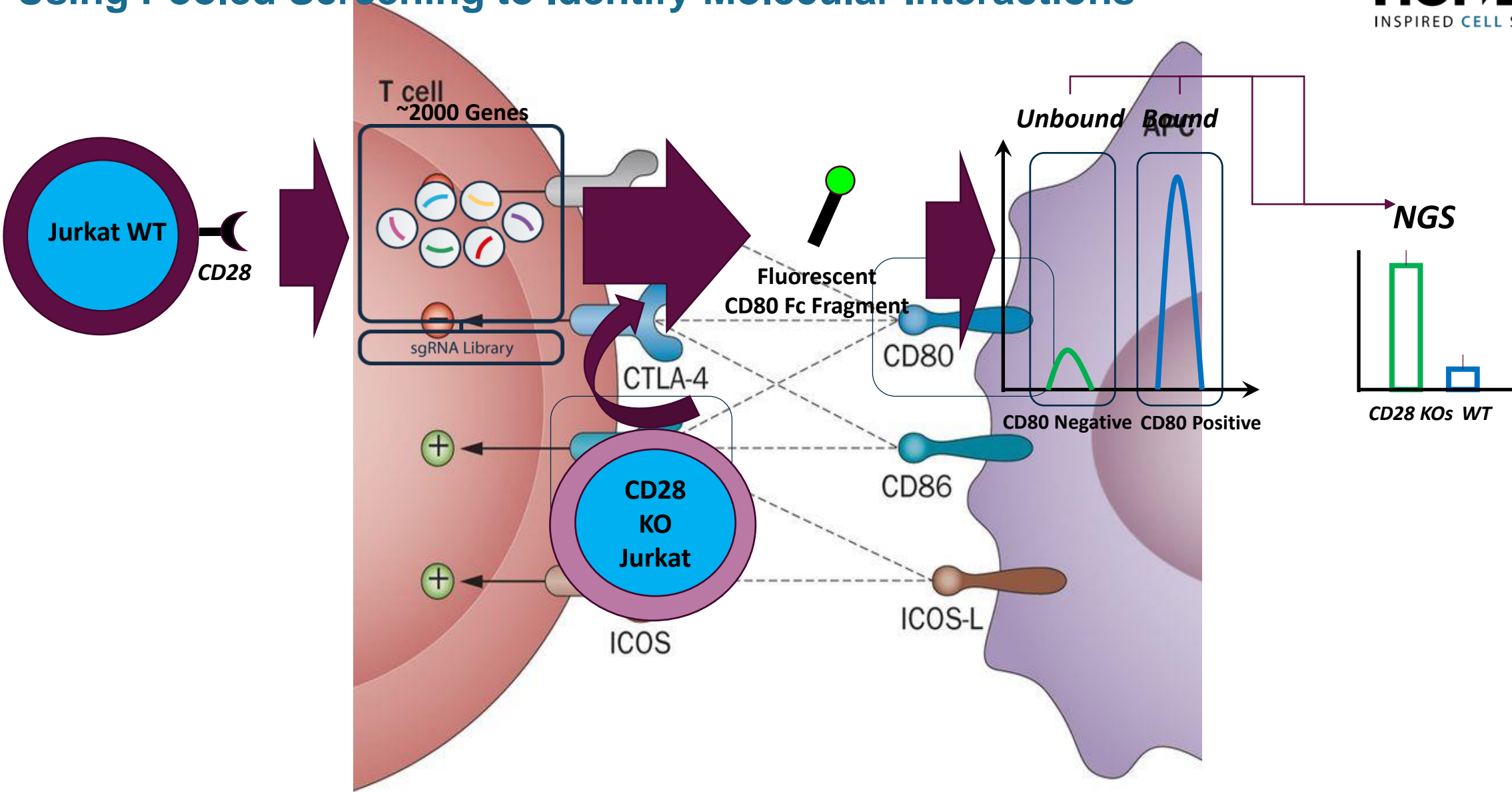
Whole Genome Screen Prosecco Plot



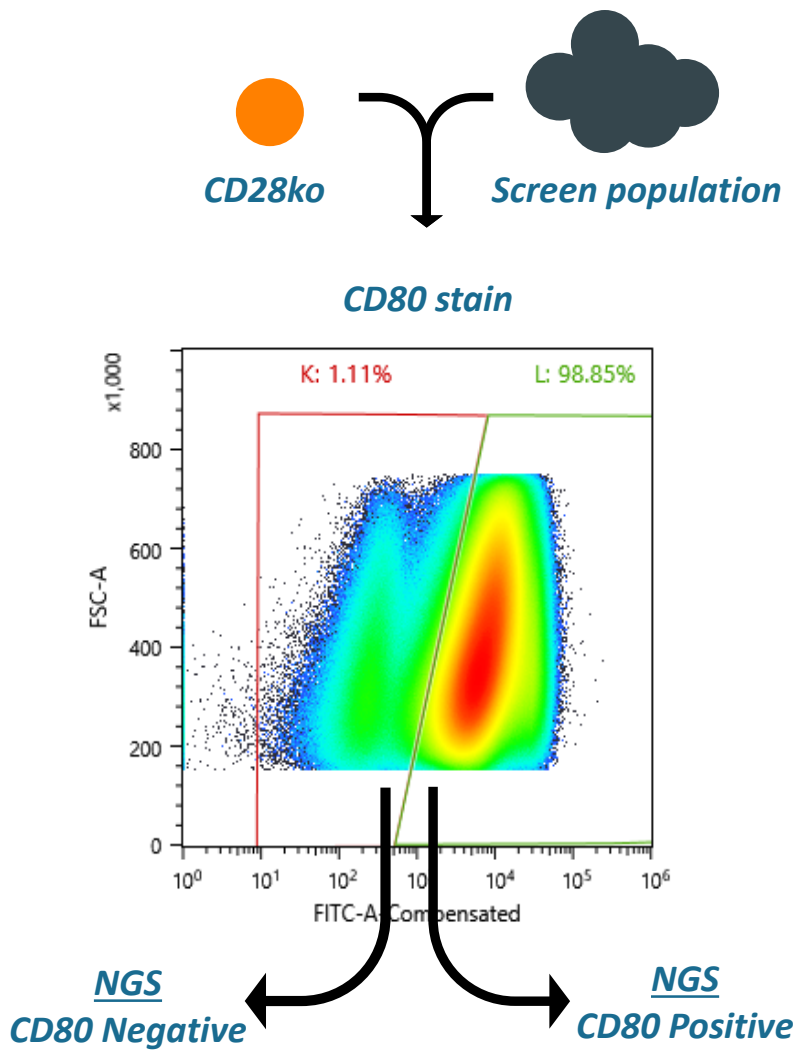
Validation of Top Hits with Engineered KO Cell Lines



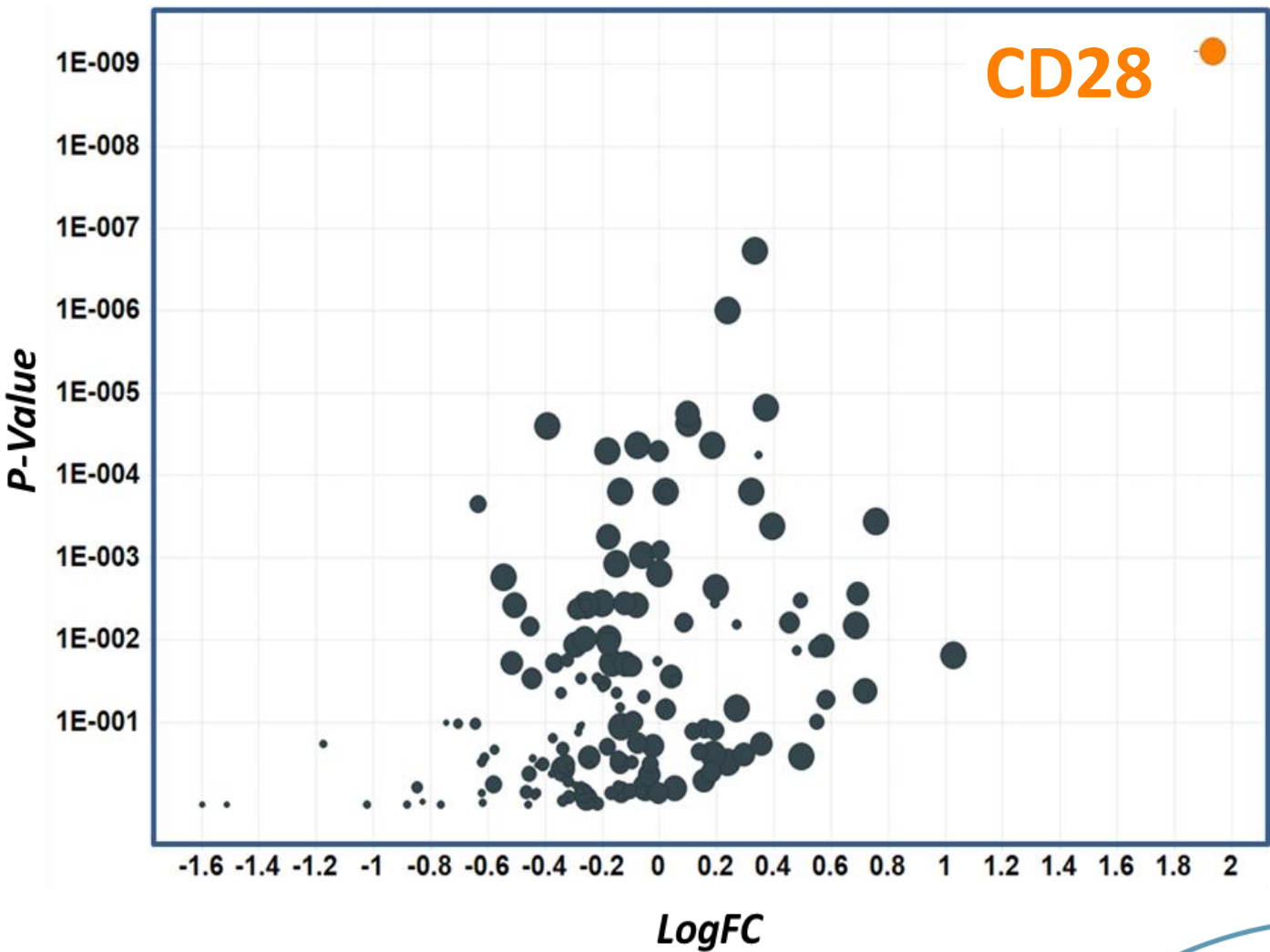
Using Pooled Screening to Identify Molecular Interactions



Using Pooled Screening to Identify Molecular Interactions



Focused Gene Library KO Prosecco Plot



Using multiple platforms for screening increases the sensitivity of screening.

CRISPR_a

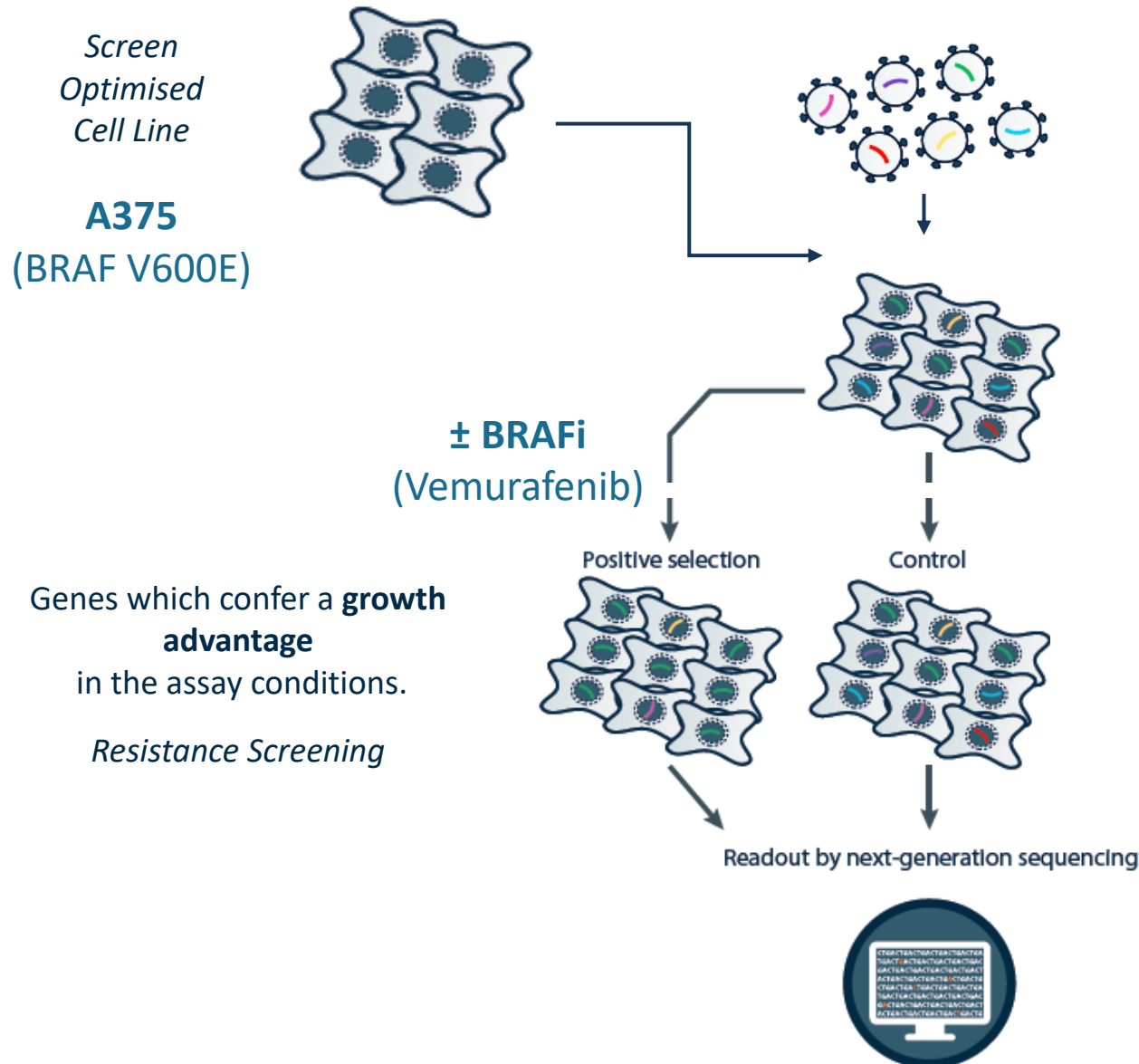
CRISPR_i

Parallel Screens



- ***Identify genes within the same pathway with opposing effects.***
- ***Connect gene networks for novel hit identification.***
- ***Cross-validate hits with two independent datasets.***
- ***Determine effect of gene drop-out with high sensitivity via enrichment in opposite function***

Positive Selection Screening for Drug Resistance Factors



PoC CRISPR screening paradigm: *Vemurafenib resistance*

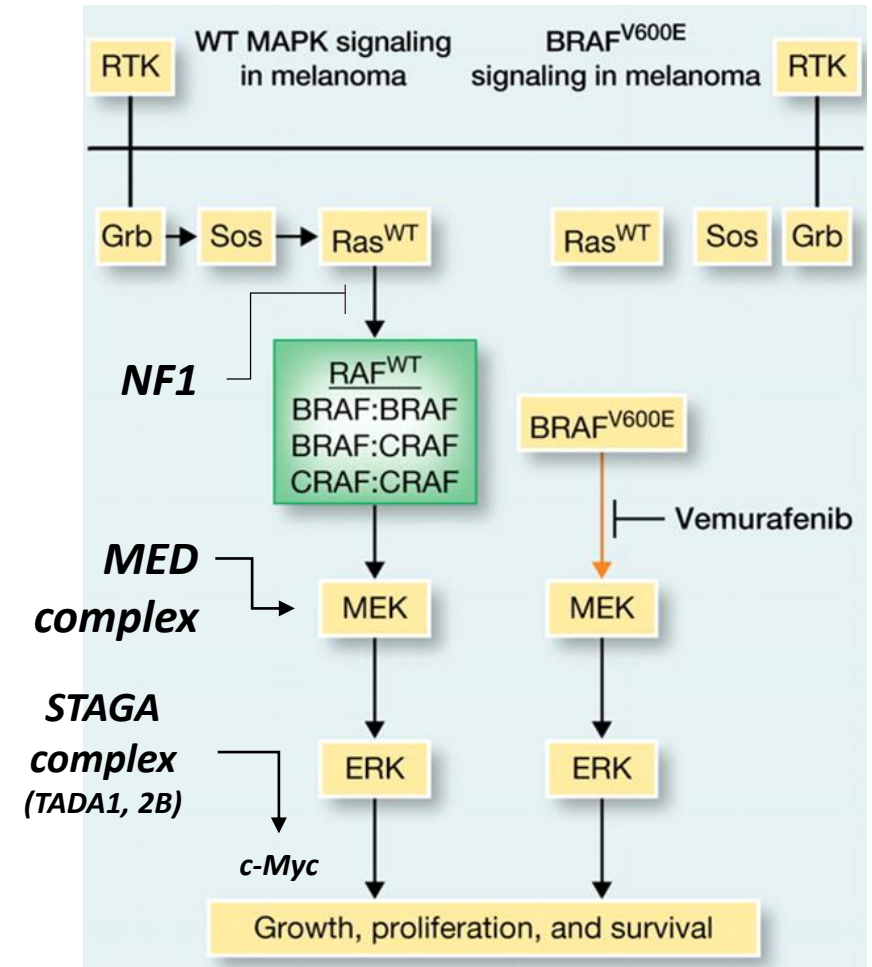
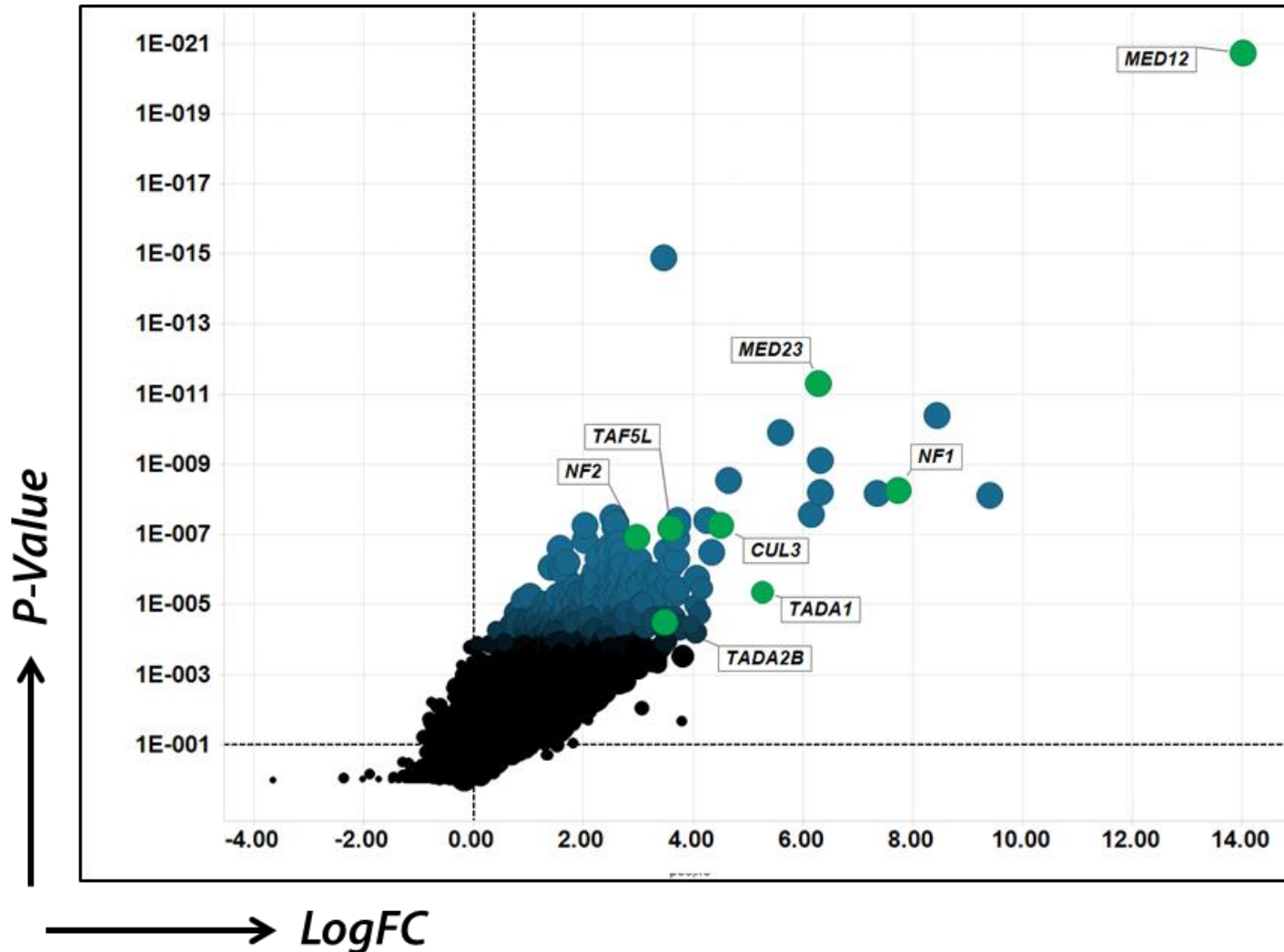
This BRAF V600E inhibitor has a number of known resistance mechanisms which have been discovered and validated using both CRISPR KO, CRISPRa and RNAi.

CRISPR_a

CRISPR_i

CRISPR_{ko}

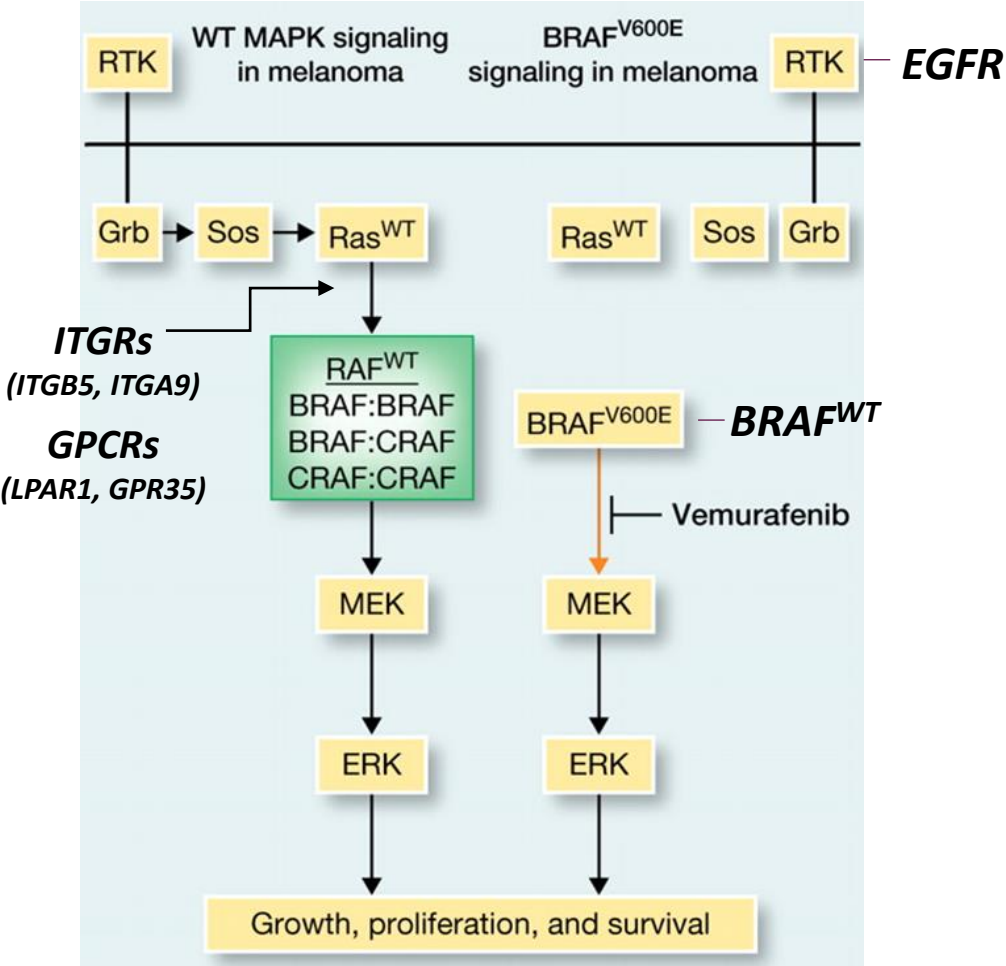
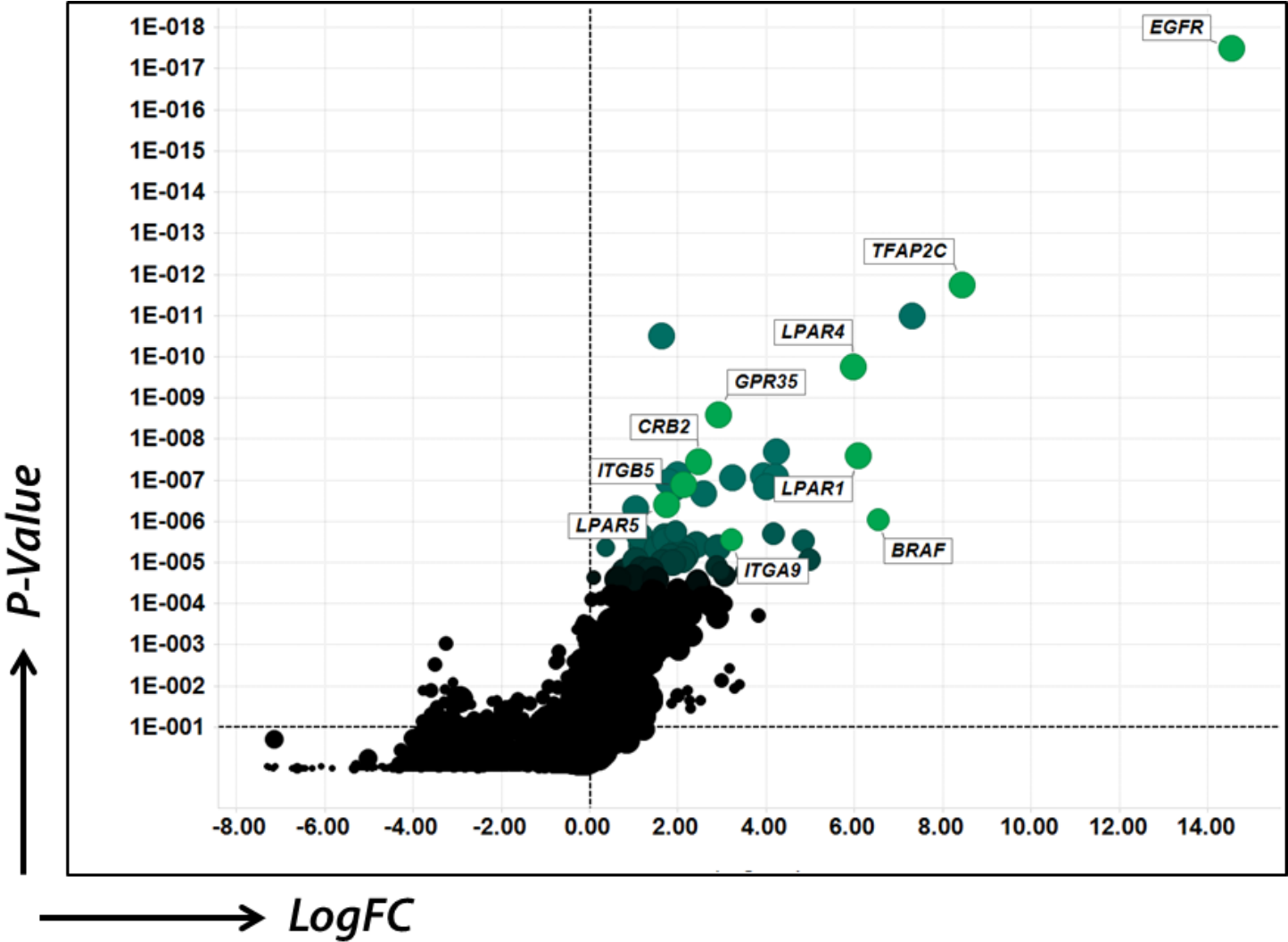
Pooled CRISPRi Screen Identifies Genes Driving Drug Resistance



Luke et al. CCR (2012)

Enrichment of selected **CRISPRi** hits after compound treatment

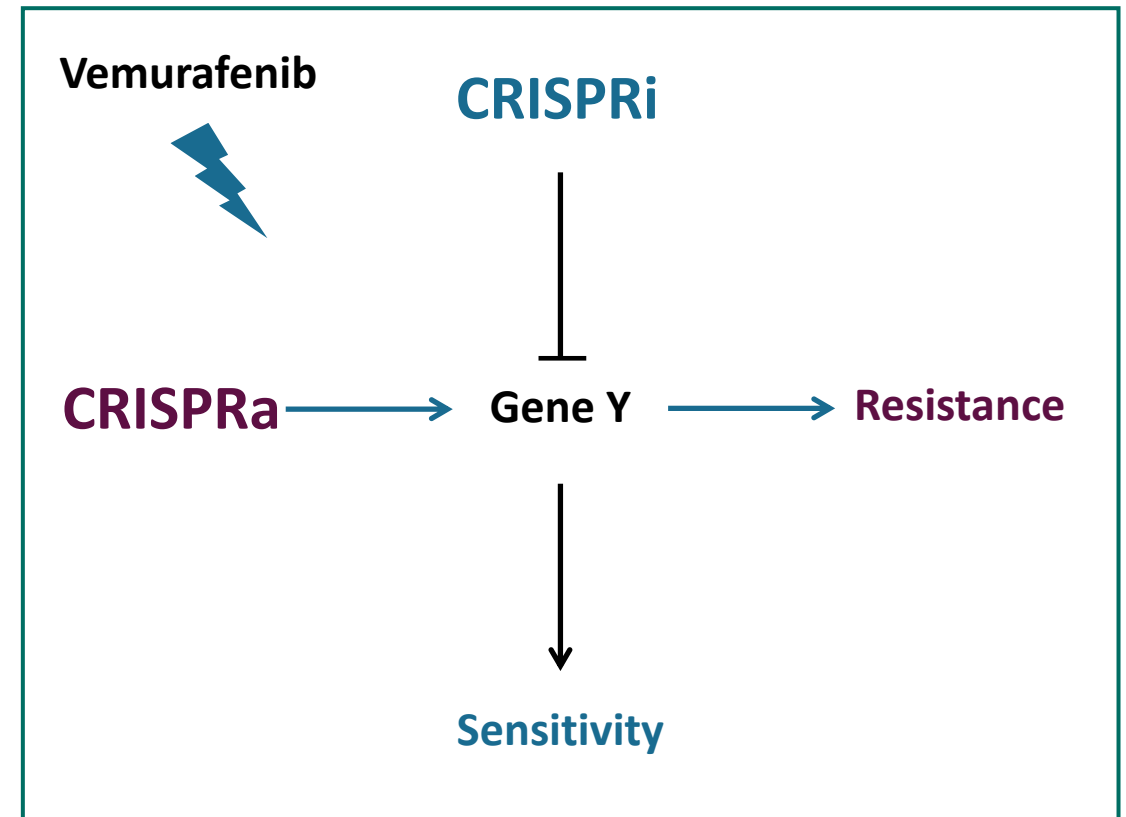
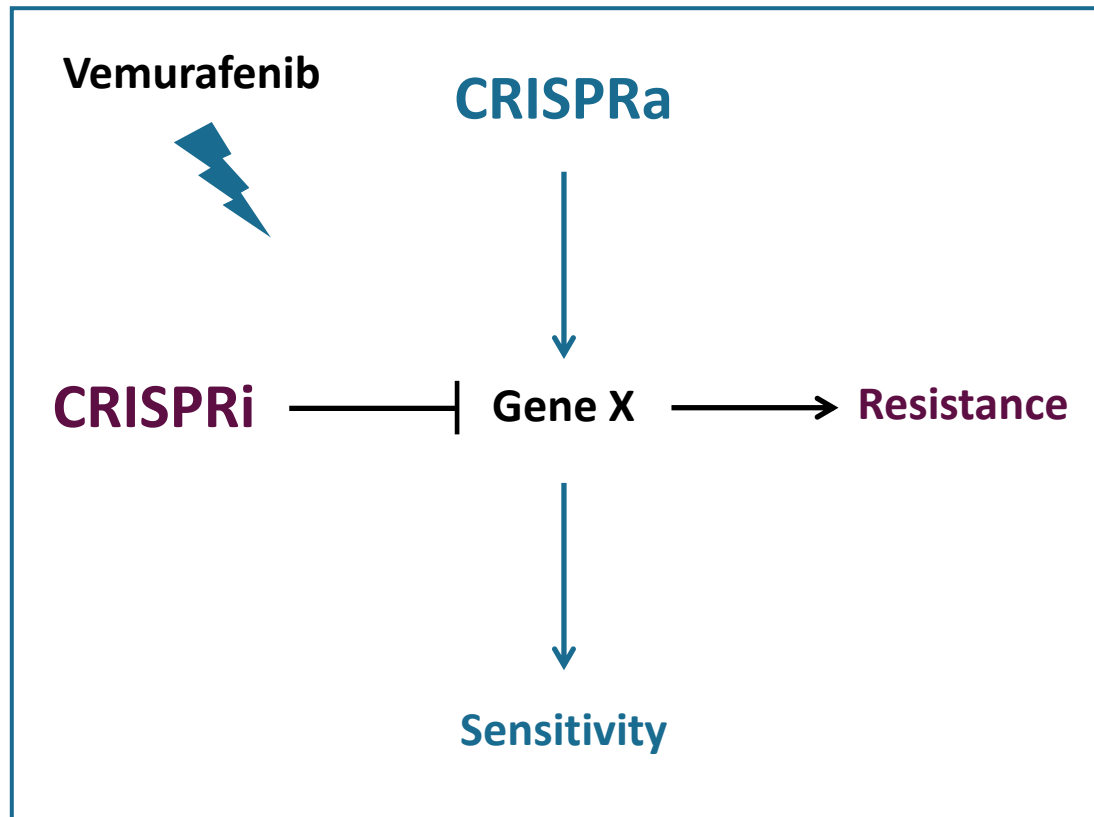
CRISPRa Screen Identified Activated Genes Driving Drug Response



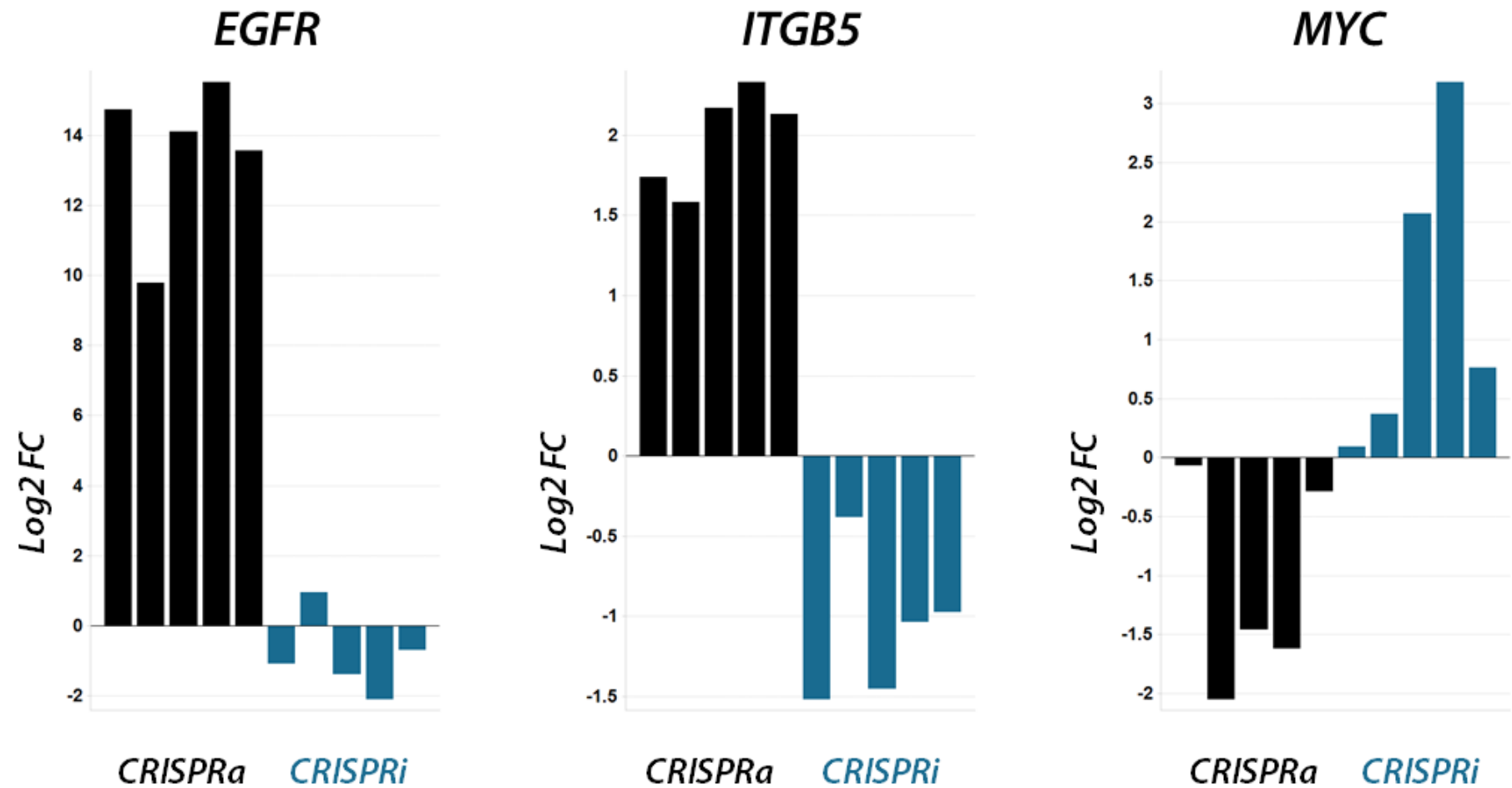
Enrichment of selected **CRISPRa** hits after compound treatment

Dual CRISPR Screening Identified Gene Opposing Effects

Hit overlay from of the **CRISPRi** and **CRISPRa** screens identified 'switch' like genes that display opposing effects when activated or inhibited in the presence of Vemurafenib.

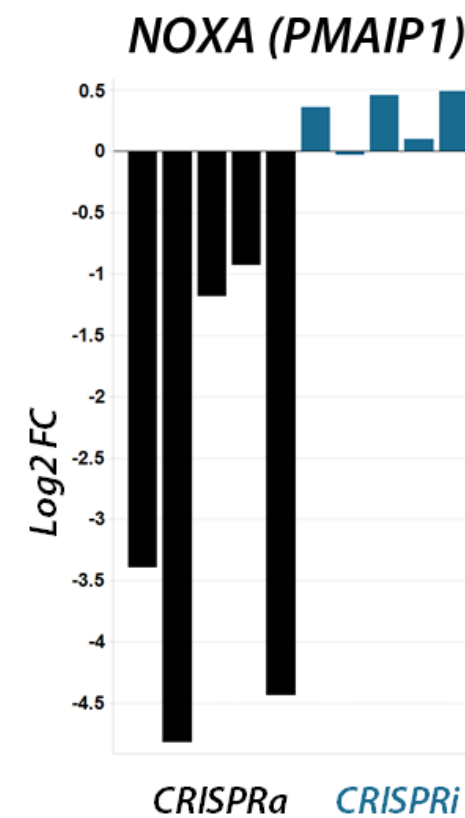
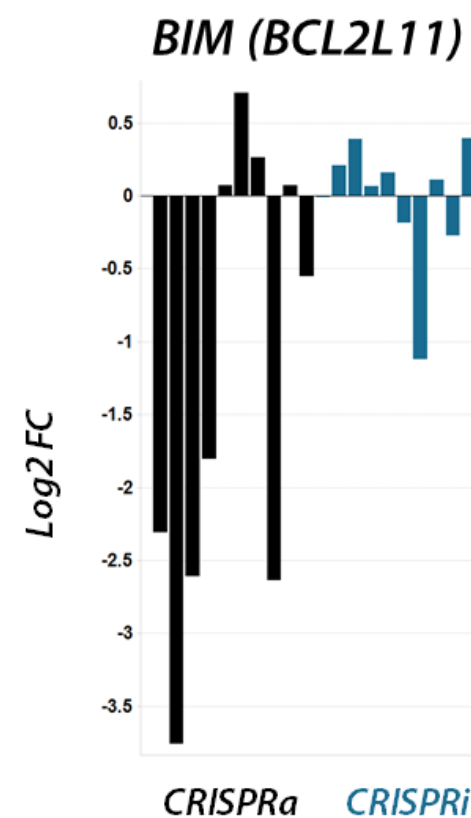
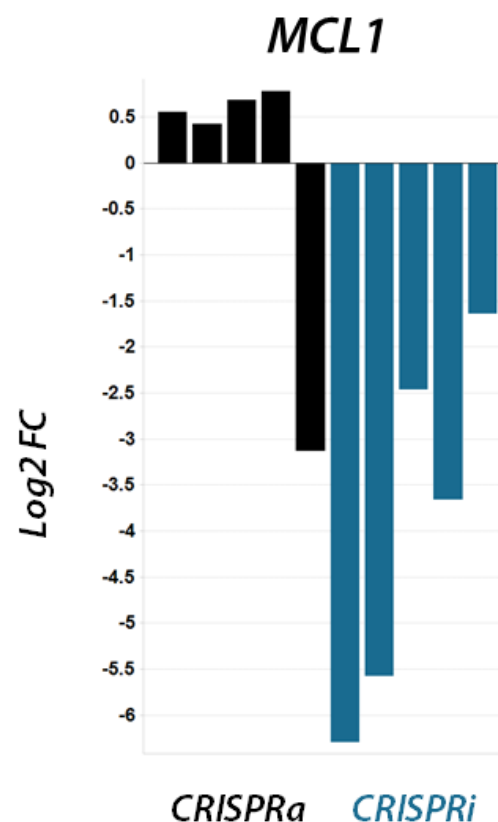
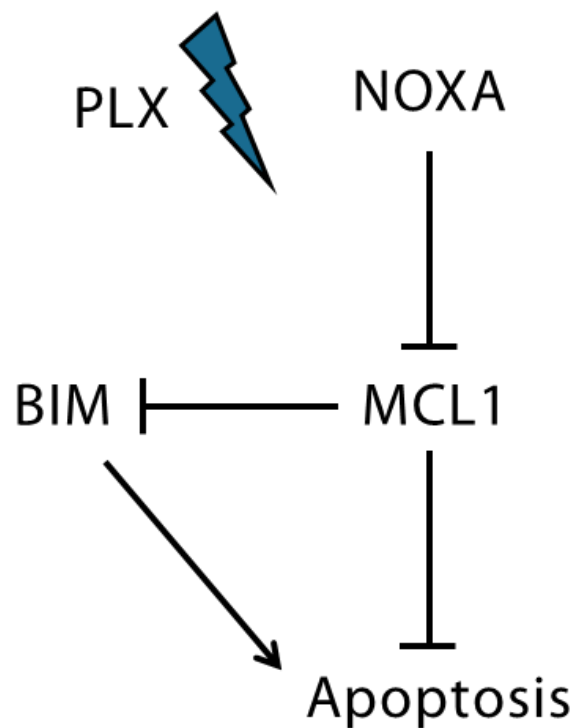


Dual CRISPR Screening Identifies Gene Opposing Effects



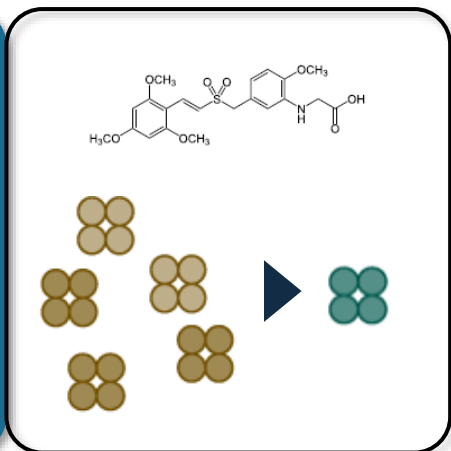
The True Power of CRISPR Dual Screening

Dual screening approach reveals interacting and opposing effects within pathways

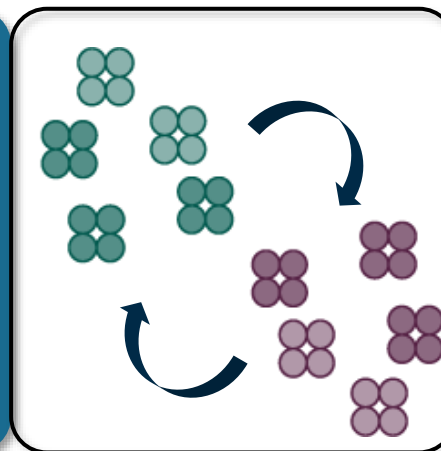


Common Applications of Pooled CRISPR Screening

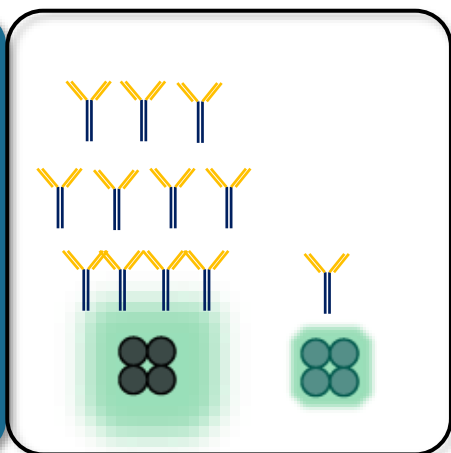
DRUG-GENE INTERACTIONS



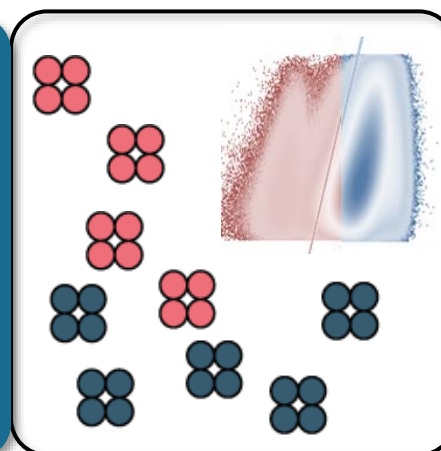
GENETIC INTERACTIONS

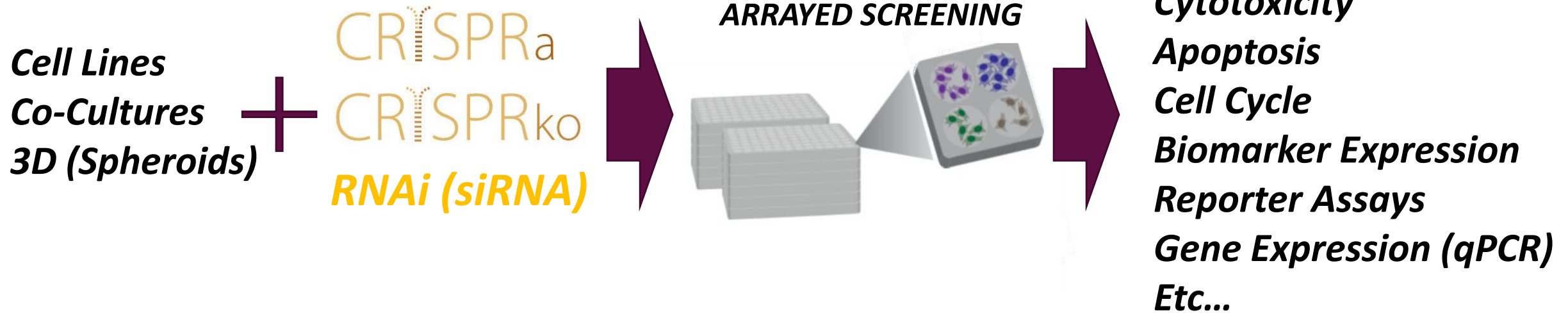


BIOMANUFACTURE SCREENING

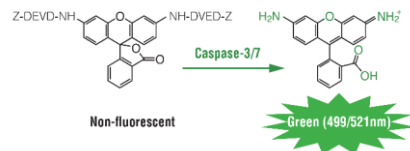
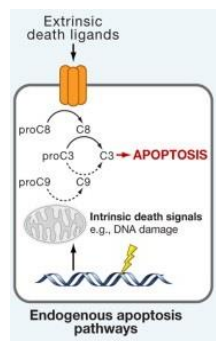


POOLED PHENOTYPIC ANALYSIS



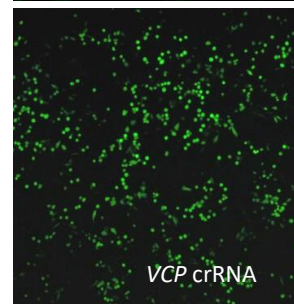
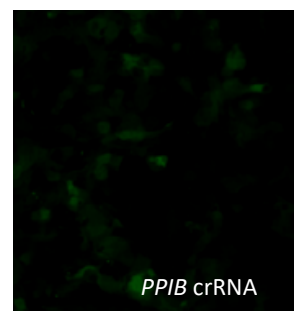


Enzymatic Assay

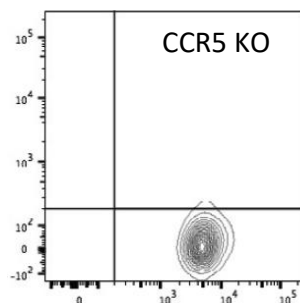
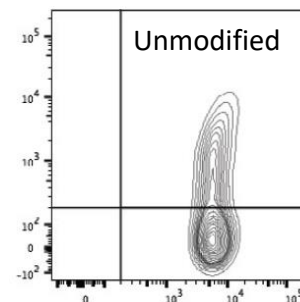


Apo-ONE™
apoptosis assay

Reporter Assays



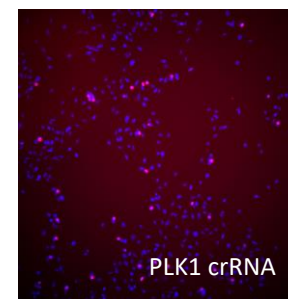
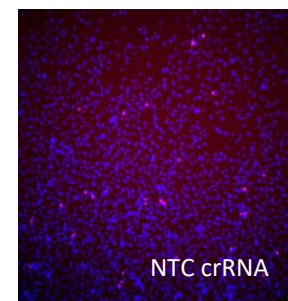
Proteasome reporter
assay; U2OS cells



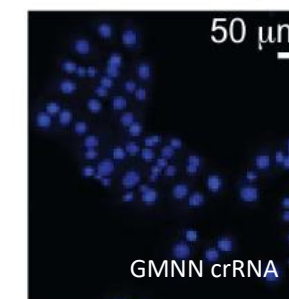
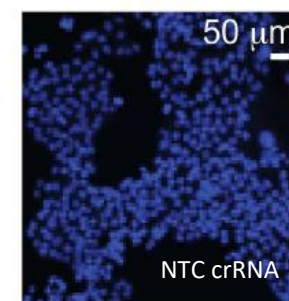
HIV infectivity;
primary T cells

Hultquist, J. et. al. 2016

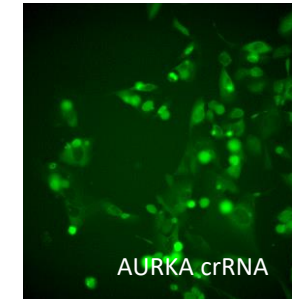
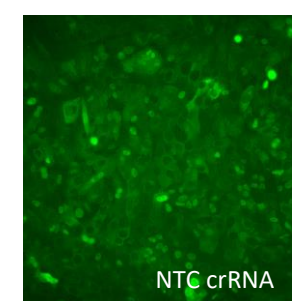
High-Content Assays



Mitotic index;
U2OS, PC3



Alteration in nuclear
area; HCT116 cells



G1S Cell cycle phase
reporter assay

Tan, J. et. al. 2016

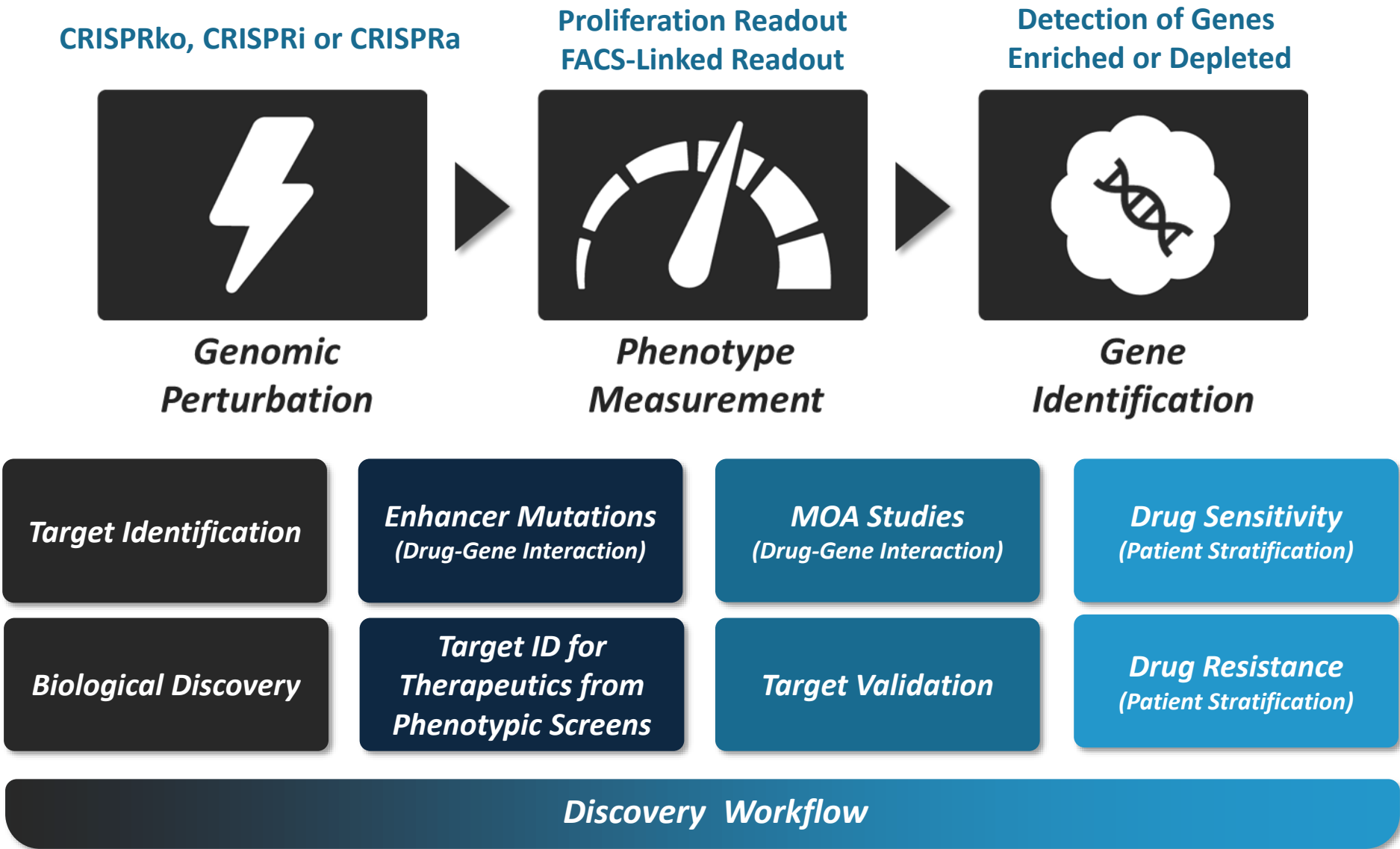
Strezoska et. al. 2017

Edit-R crRNA Libraries for CRISPRko & CRISPRa

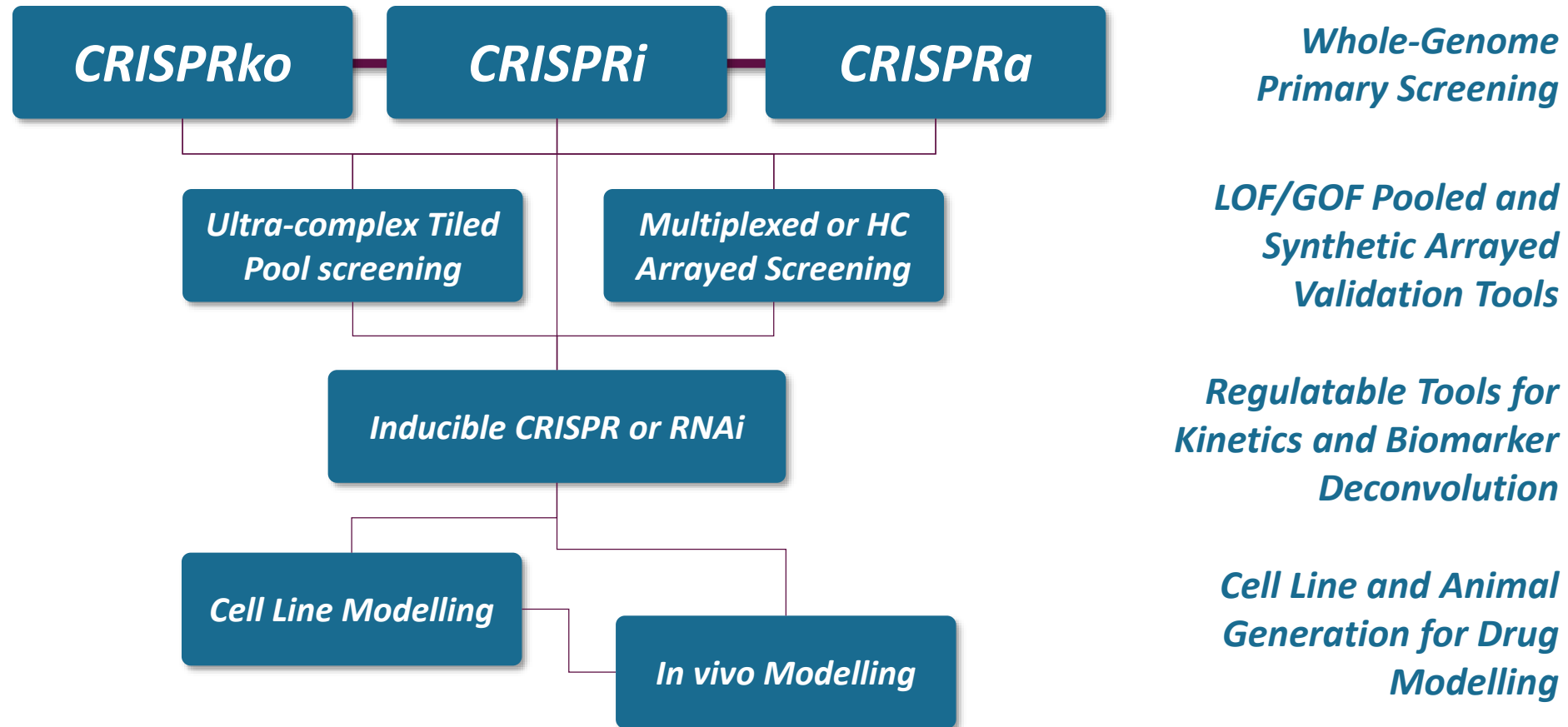
- **Catalog libraries of predefined gene family collections for human and mouse.**
 - Pools or a Set of 4 individuals
- **The only whole human genome arrayed CRISPR libraries for activation.**
- **Cherry-pick crRNA libraries based on your gene list:**
 - Pilot studies prior to larger screen
 - Follow-up of pooled CRISPRa screening hits
 - Custom gene targets for CRISPRa screens

Catalog Edit-R library	Human	Mouse
Tyrosine Kinases	✓	✓
Deubiquitinating Enzymes	✓	✓
Cytokine Receptors	✓	✓
Membrane Trafficking	✓	✓
Cell Cycle Regulation	✓	✓
DNA Damage Response	✓	
Phosphatases	✓	✓
Ion channels	✓	✓
G Protein-Coupled Receptors	✓	✓
Proteases	✓	✓
Ubiquitin Enzymes	✓	✓
Protein Kinases	✓	✓
Epigenetics	✓	✓
Transcription Factors	✓	✓
Drug Targets	✓	
Druggable Genome	✓	
Genome	✓	

Functional Genomic Screening in Research



Complete Discovery Workflow with the Horizon CRISPR Toolbox



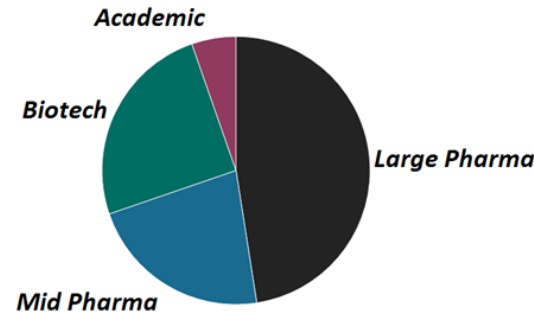
Application of both aligned and orthologous tools for target ID, validation & production.

Horizon's Extensive CRISPR Screening Experience

Why Work with Horizon?

- Outstanding platform success
- Strong IP position
- Excellent customer support
- Continuous evolution
- Multiple unique offerings

Horizon Customer Partnerships

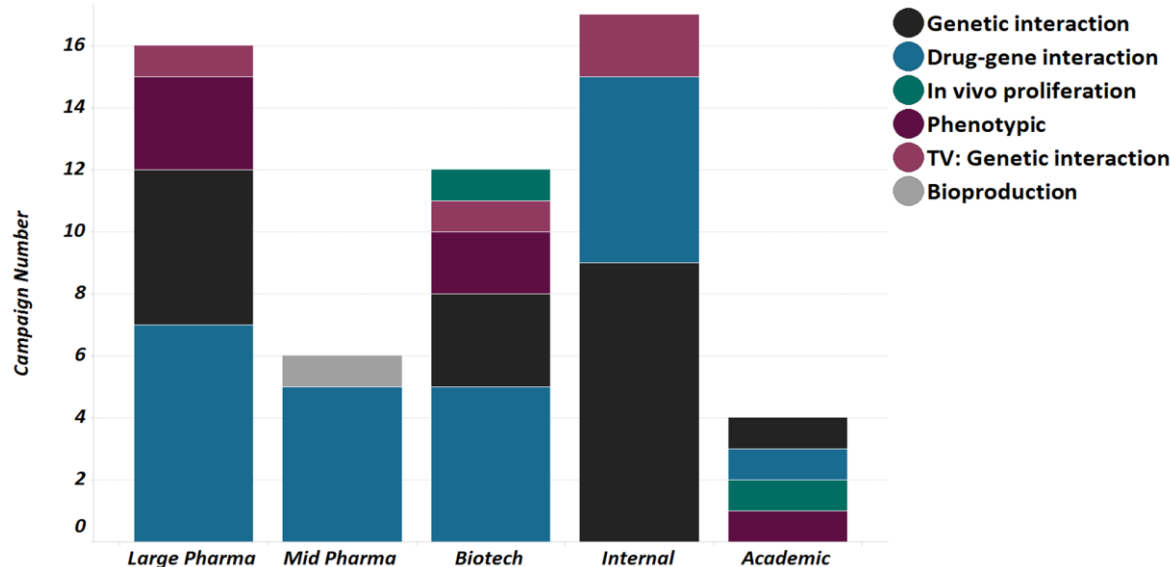


Customer Testimonials

"The team at Horizon worked in a very collaborative way, and we were able to have detailed scientific and technical discussions in real time during the process. We felt that the team listened to our suggestions and concerns in a very sincere way. The time lines and any small deviations were clearly communicated. An interactive discussion about the data analysis lead to our further internal validation project."

Director, Large Pharmaceutical, 30 parallel targeted CRISPR screens

CRISPR Screening Experience



"We are very pleased with the service provided by Horizon Discovery. From the initial optimization experiments to the final data analysis, Horizon Discovery scientists were diligent and highly collaborative, ensuring a successful screen. Thanks again, Horizon."

Oncology Project Leadership, Large Biotech, two parallel whole-genome screens

"Thanks a lot for the nice report and for the excellent work. Having a little bit of functional screening experience myself I'm really impressed by the quality of the service provided. It was done with great thoroughness (lots of controls!) and done within a very acceptable time-line. So a big thank you to all of you!"

Scientist, Large Biotech, three parallel whole-genome screens

Acknowledgements

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Ceri Wiggins
David Walter
Paul Russell
Annette Little
Asad Rahman
Nicola McCarthy
Jon Moore



CRISPR_a
CRISPR_i
CRISPR_{ko}



horizon

INSPIRED CELL SOLUTIONS

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