CRISPR Workflow Solutions

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CRISPR FGS Formats Set the Standard for Screening Technology

Generally, functional genomic screening (FGS) employs two gene interrogation technologies: RNAi and CRISPR, where RNAi represses gene expression at the mRNA level (knockdown), while CRISPR works at the DNA level and can permanently knockout, modulate, or knock-in genes.

In recent years CRISPR has become the gold standard screening technology because there are fewer off-target effects than with RNAi. There are two main FGS formats:

- **Pooled screening,** whereby editing is performed with a mixture of gRNA and thus a high number of perturbations occur. The mixed cell population is then mostly sorted by FACS, followed by NGS for deconvolution. This approach is very cost effective, however there is only a binary read-out.
- **Arrayed screens,** where each CRISPR-based perturbation occurs in a separate well of a multi-well plate, offering a clear phenotype to genotype correlation. The main read-out is imaging, which could be combined with detection or sequencing enabling a multi-parametric analysis. They are also well-suited for more advanced cell models like primary cells or 3D cell cultures.

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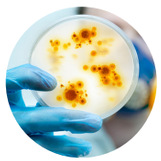
A Better Understanding Starts Here

APPLICATION:



Disease Models
- Use CRISPR knockout, CRISPR activation, and CRISPR interference to understand specific gene contribution to a disease.
- Use gene knock-in to insert mutations mimicking disease or wild-type biology for better validated targets or compound identification.

Pathway Analysis
- Understand the function of genes and how they contribute to biological processes and diseases.

Target Identification
- Identify relevant targets or hits from phenotypic screening
- Assess the contribution of each gene in the genome to compound activity in a full genome screen loss-of-function screen

Target Validation
- Verify targets in an arrayed secondary screen (smaller set of genes) and eliminate false positives
- Investigate gene knockout or modification in a variety of cell types to determine if a gene target provides the same phenotype in multiple cell types

Cell & Gene Therapy
- Reverse disease-causing mutations
- Edit CAR-T cells to attack cancer cells

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The Various Types of CRISPR

Permanent and Heritable Gene Editing

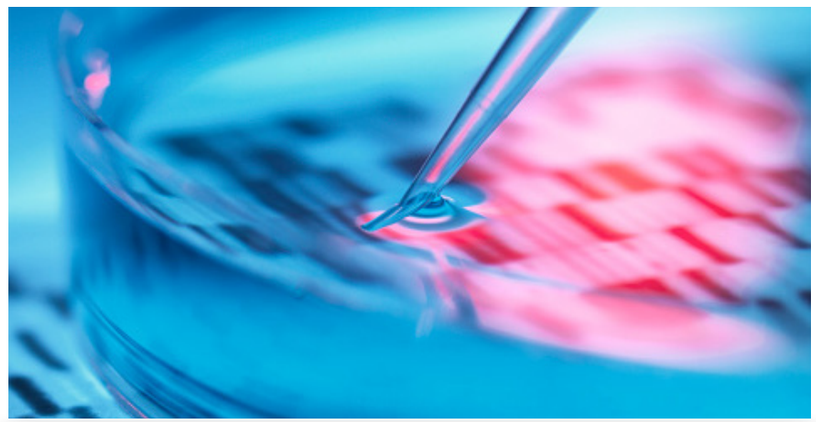
Down Regulation

CRISPR Knockout: Enables the loss of function studies: screening with complete loss of gene expression provides the maximal window for phenotypic effect and high statistical power for hit discovery.

Up regulation

CRISPR Knock-in: This homology-directed repair (HDR) introduces or corrects a SNP mutation, or adds a reporter tag to an endogenous gene.

Reversible Gene Modulation



Down Regulation

CRISPRi Screening: Represses expression rather than completely knocking out the target gene and is ideally suited to study drugability and to evaluate the function of genes that when knocked-out are essential and those that are amplified.

Up regulation

CRISPRa Screening: Amplifies gene expression in its endogenous context and enables for the first time the ability to study activation-linked responses on a genome-wide level.

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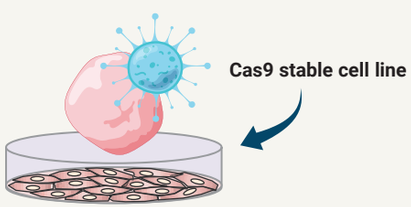
Take Steps Towards a Better Workflow

Efficiency At Every Step of The Workflow

1 Choose cell type and choose CRISPR and Cas9 protein

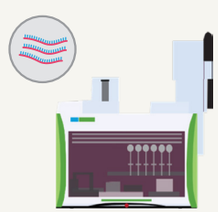
2 Generate Cas9 stable cell line using lentivirak transduction (optional)



3 Reagent processing and cell seeding

Liquid handling and automation

- Handle CRISPR reagents
- Automated cell seeding
- Staining

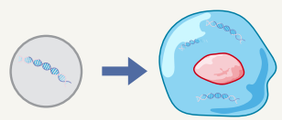


Cell counting and viability

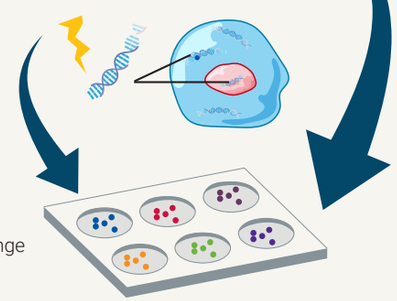
4 Deliver guide RNA (and Cas9 plasmid) to cell

Transfection

Liposome-gRNA complex



Short electrical pulse



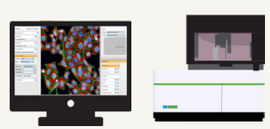
5 Treatment

- One edit per well
- Drug treatment, virus infection, metabolic challenge

6 Read-out

Functional and viability read-out

Phenotypic read-out



Live cell imaging for assay development and QC

7 Analysis

Image analysis and management

Data management and analysis for all modalities

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Choose from a Variety of CRISPR Reagents Formats

Selecting the appropriate format of CRISPR reagents is the first critical step. before starting a screen. We offer a variety of formats, so that regardless of the scientific question, we can support your experimental needs.

- For the **Cas9 nuclease**, you can also shorten your editing workflow with "CRISPR-ready" **Cas9-expressing stable cells** in a variety of popular cell types. Cas9 reagents are also available in a version co-expressing a fluorescent tag for optimization and enrichment.
- Edit-R CRISPR **guide RNA** are available in pools or as individual reagents, as predefined CRISPR knockout libraries, or a library can be designed using the cherry-pick library tool. There is also a CRISPR design tool for custom guide RNA.
- There are two options for **donor template** (only for knock-ins): a single-stranded DNA donor oligo for short insertions or alterations of ~ 50 nt or less, or a donor plasmid that allows for large insertions.

Notable Studies

Effectiveness of CRISPR-Cas9 using pools of synthetic crRNAs in high-content analysis screening experiments

LEARN MORE

Fluorescent tagging of an endogenous gene by homology-directed repair using Dharmacon™ Edit-R™ CRISPR-Cas9 reagents

LEARN MORE

Gene Editing of Primary Immune Cells to Aid Next-Generation Cellular Immunotherapies (WP WIP)

LEARN MORE

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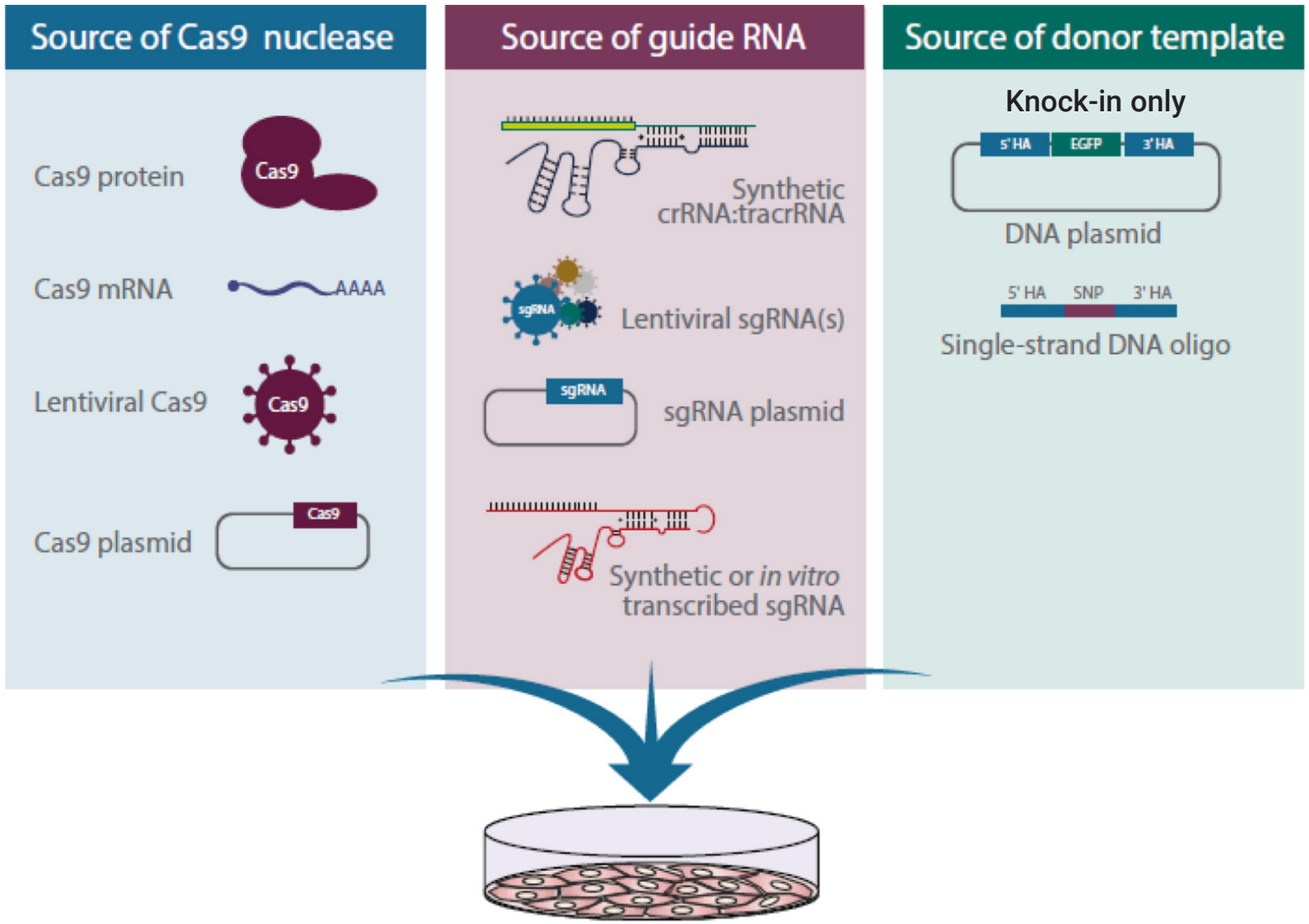

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Maximize Editing Efficiency

A key factor in any gene-editing experiment is the successful introduction of guide RNA and Cas9 protein into the cell. Transfection, transduction and electroporation optimization is therefore important to ensure high editing efficiency. In particular, the Cas9 transfection can be a challenging step. Therefore, we offer a range of stably expressing Cas9 cell lines to support your research needs.

Transfection | **Transduction** | **Electroporation**

IMAGE TO COME

- Deliver gRNA and Cas9 to cells using a chemical transfection reagent. This method relies on electrostatic interactions to bind with nucleic acids and to target cell membranes using compounds like calcium phosphate, polycations, or liposomes.
- DharmaFECT transfection reagents provide efficient and reliable transfection at low RNA or plasmid reagent concentrations with minimal cellular toxicity.
- Either co-transfection of Cas9 and synthetic CRISPR gRNA or transfection of CRISPR guide RNA into stable Cas9 expressing cells.

Notable Study

Optimization of reverse transfection of Dharmacon™ Edit-R™ synthetic crRNA and tracrRNA components with DharmaFECT™ transfection reagent in a Cas9-expressing cell line **Learn More**

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Transfection | **Transduction** | **Electroporation**

IMAGE TO COME

- Lentiviral particles carrying sgRNA vector that contains a gene-specific sgRNA and/or Cas9 nuclease
- For arrayed screen we recommend to use lentiviral transduction to create a stable Cas 9 cell line. The synthetic RNA is then transfected in a second step.

Notable Studiey

Easier, faster CRISPR-Cas9 gene editing with the All-in-one sgRNA lentiviral vector.

Learn More

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Transfection | **Transduction** | **Electroporation**

IMAGE TO COME

- Cells are exposed to a short electric pulse, as a result the cell membrane forms temporary pores and allows charged molecules to enter the cell.
- Electroporation allows to transfect a large number of cells in minutes and is ideal for larger-scale screens
- Also applicable to difficult-to-transfect cells and primary cells

Notable Study

App Note: Optimized HDR-mediated fluorescent protein knock-in in K-562 cells using Edit-R™ CRISPR-Cas9 reagents and electroporatio **Learn More**

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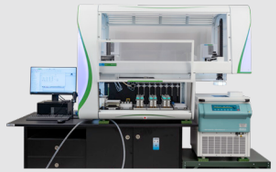
Automate Your Sample Prep

Safe, easy-to-use, and efficient—these liquid handlers and cell counters improve every step of the workflow.

Liquid Handling

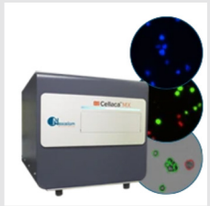


JANUS® G3

The JANUS G3 automated workstations include a portfolio of precision liquid handling solutions that provide adaptability in throughput, plate capacity, and dynamic volume range to meet your current and future automation needs.

Cell Counters

Determination of cell concentration is an essential step in any cell-based assay to ensure reproducibility and quality. We offer automated cell counters that reduce time and variations compared to manual counting. They are available in slide or high-throughput plate-based versions and include cell cycle analysis features



Cellaca MX High-throughput Cell Counter

- As little as 25 µl of a cell sample required
- Multiple fluorescent filter options with autofocus function
- Perform cell-based assays, including viability, vitality, and apoptosis

Cellometer K2 Flourescent Cell Counter

Makes viewing, analyzing, and reporting complex and messy samples even more accessible.

- As low as 10 µl of a cell sample required
- Generate counts, concentration, viability, and size in less than 60 seconds

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Phenotypic Drug Discovery

Arrayed CRISPR screening technology is versatile in both methodology and analysis and offers a variety of phenotypic assays. We provide a portfolio of high-content screening products, live cell imaging instruments, software, and fluorescent probes. We provide a portfolio of high-content screening products, live cell imaging systems, including imaging instruments, software, and fluorescent probes.

PHENOVUE CELLULAR IMAGING REAGENTS

Optimized and Validated Our PhenoVue™ Suite Includes:

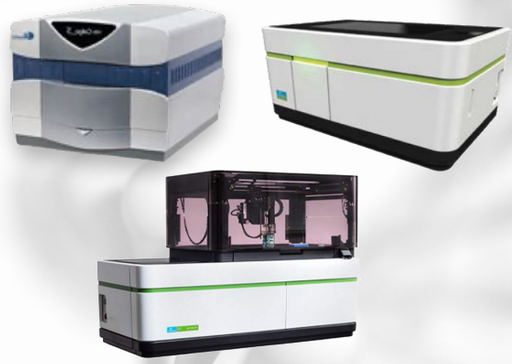
- **Organelle and cell compartment probes** for high-quality images
- **Fluorescenct secondary antibodies** for multiplexed immunofluorescence experiments
- **Cell painting kits** with a set of six fluorescent probes. **LEARN MORE**

HIGH-CONTENT IMAGING

System Can Be Configured to Suit Lab Requirements

Opera Phenix® Plus and Operetta™ CLS high-content imaging systems high-content imaging features confocal spinning disk technology for exceptional image quality. Our live cell imaging systems Nexcelom Celigo enable studying dynamic processes in real time over more extended periods.
LEARN MORE

HIGH-CONTENT IMAGING AND ANALYSIS SOFTWARE



Analyze Data in Context

Harmony® software enables the user to control every aspect of a high-content analysis or screening experiment through a single workflow-based user interface. **LEARN MORE**

Access, analyze, store, re-analyze, and share your organization's image data from PerkinElmer and other HCS and cellular imaging systems with **Signals Image Artist™.**

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The Early Analysis of Cell Health

Cell health is one of the most critical parameters during any cell-based screening assay and should be analyzed as early as possible.. To keep your costs down, we provide cell viability and toxicity assays based on bioluminescent or fluorescent detection chemistries that enable endpoint assays in a high-throughput format.

APPLICATION:

Luminescent Reagents
Our ATPLite™ and ATPLite 1step reagents kits for quantitative evaluation of cell viability and proliferation provide a simple, robust protocol for ATP-content endpoint measurements. In addition, they work best on one of our systems of our plate reader family

On the Web

Multimode Plate Reader
Our portfolio of microplate readers is equipped with multiple detection modes to meet the diverse assay requirements of today's laboratories. With various models, configurations, and accessories, a PerkinElmer plate reader can match a lab's throughput needs and meet budgetary constraints **On the Web**

Fluorescent Reagents
Our range of fluorescent reagents and kits enable the measurement of cell health parameters like viability, vitality, and are optimized. They are optimized to work with our cellometers or the Celigo imaging system. **On the Web**

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Functional Assays Help You Gain Insights

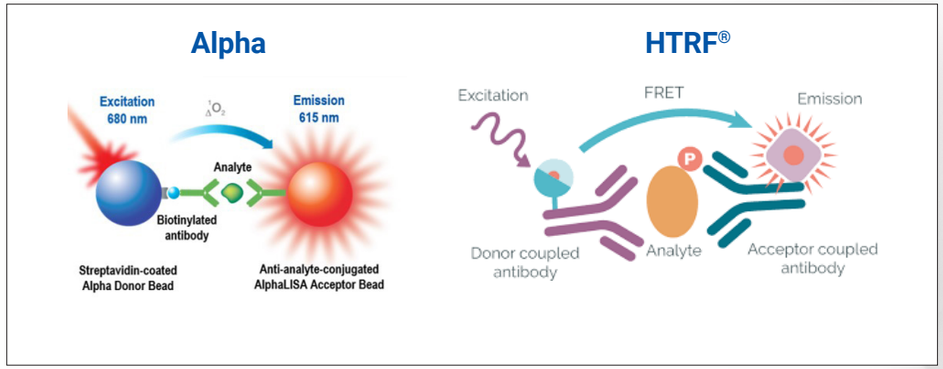
During assay development and optimization, Immunoassays can help to quantify Cas9 expression in the cells and other important complementary insights to imaging data. They can also be used later in the workflow for functional assays to confirm knock-out efficiency or to identify pathways by quantifying protein levels either in the supernatant or after cell lysis.

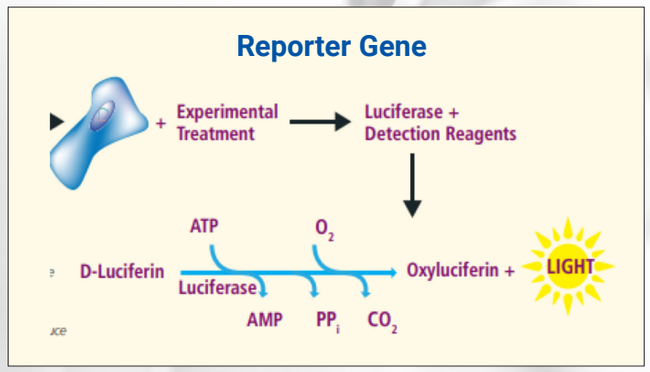
- Our proprietary detection technologies, HTRF (homogeneous time-resolved fluorescence) and Alpha (amplified luminescent proximity homogeneous assay), provide a wider dynamic range, expanded signal stability, increased sensitivity and the option for no-wash assays compared to traditional immunoassays. Both technologies avoid background effects from the samples usually visible in fluorescent assays.
- Reporter gene assays enable high sensitivity measurement of gene expression and cell signaling through the addition of bioluminescent genes into target cells. Use our assays to characterize the strength of promoters and enhancers, define the role of transcription factors, or assess transfection efficiency.
- These assays are optimized to work best on one of our systems from our plate reader family.

Notable Studies

Read how genes and pathways involved in the modulation of endogenous tau levels were identified using biotinylated anti-Tau antibody HT7 and Anti-Tau BT2 conjugated AlphaLISA acceptor beads

Learn how CRISPR Can Identify Regulators of Alzheimer's-related Protein Tauv





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Data for ALL Modalities in ONE Platform

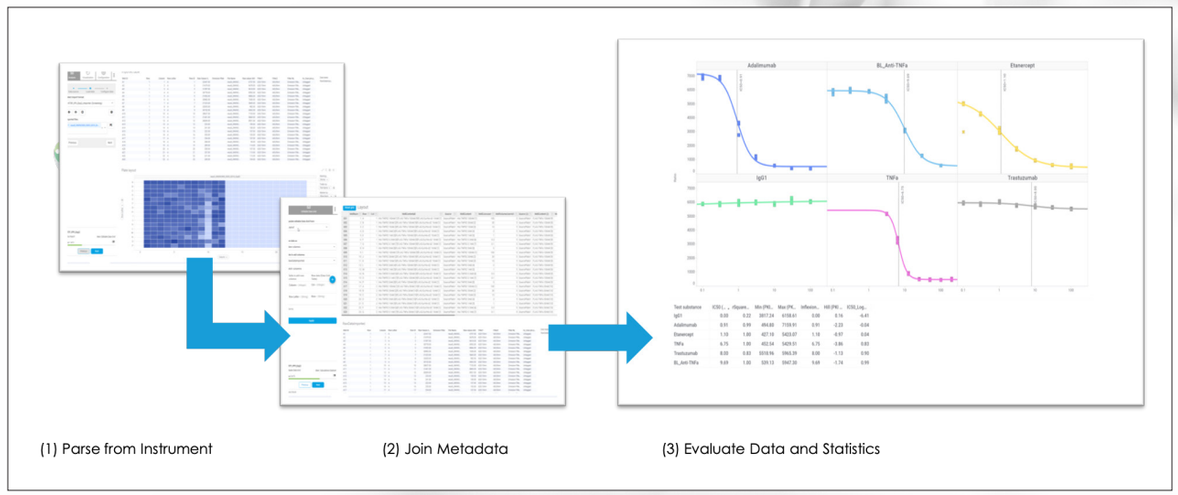
Signals VitroVivo is an intuitive, configurable flexible screening workflow processor coupled with the unparalleled data visualization and analysis capabilities of TIBCO Spotfire. It is flexible enough for one-off assay work during assay development, comprehensive enough for more sophisticated assays, and diverse enough to support a long and growing list of techniques scalable all the way to ultra-high data volumes.

Scientists can now leverage a consistent, repeatable pattern for data acquisition as well as the data processing protocols themselves.

More features:

- Intuitive Data Capture
- Configurable Calculation Engine
- Store and Search All Assay Parameters
- Unique Data Handling for In vivo/ DMPK
- Signals Image Artist is fully integrated

LEARN MORE



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