Your pathway toward CRISPR workflows.

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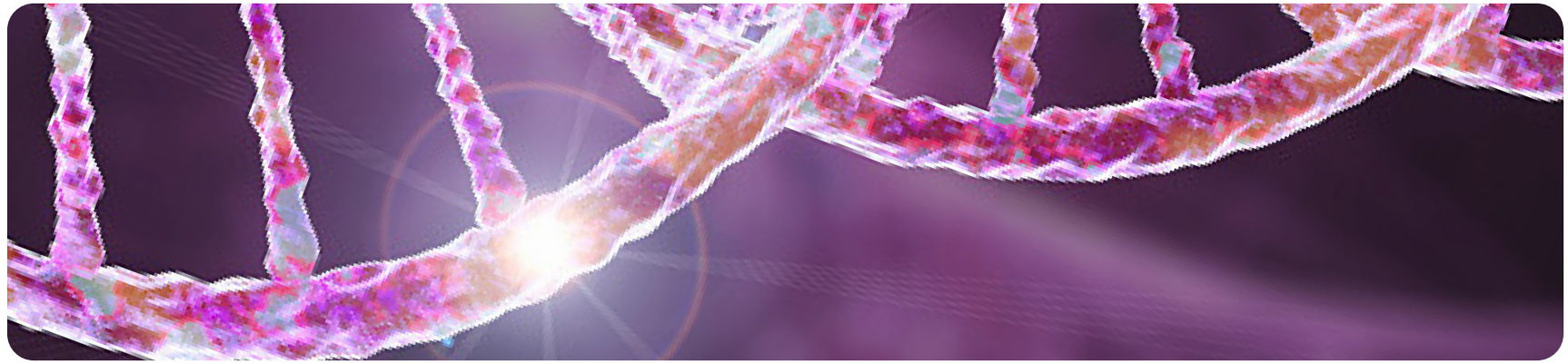
CRISPR workflow solutions



CRISPR functional genomic screening sets a new standard

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 an alternative approach to RNAi functional genomic screening because it suffers from fewer off target effects. The two main CRISPR screening approaches are:

- Pooled screening, whereby editing is performed with a mixture of guide RNA (gRNA) and thus a high number of perturbations occur. The mixed cell population is then exposed to a treatment and analysed by NGS for deconvolution with an optional sorting step before. This approach is very cost effective, however only macro effects can be detected.
- 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 and gene therapy

- Reverse endogenous disese-causing mutations
- Editing immune cells to develop next generation immunotherapies

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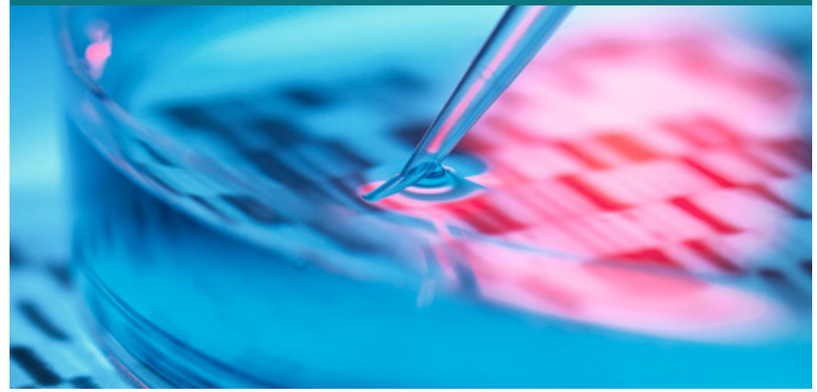
CRISPR types

Permanent Gene Editing

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.

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

Bi-directional Gene Modulation



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 essential genes or those that are amplified.

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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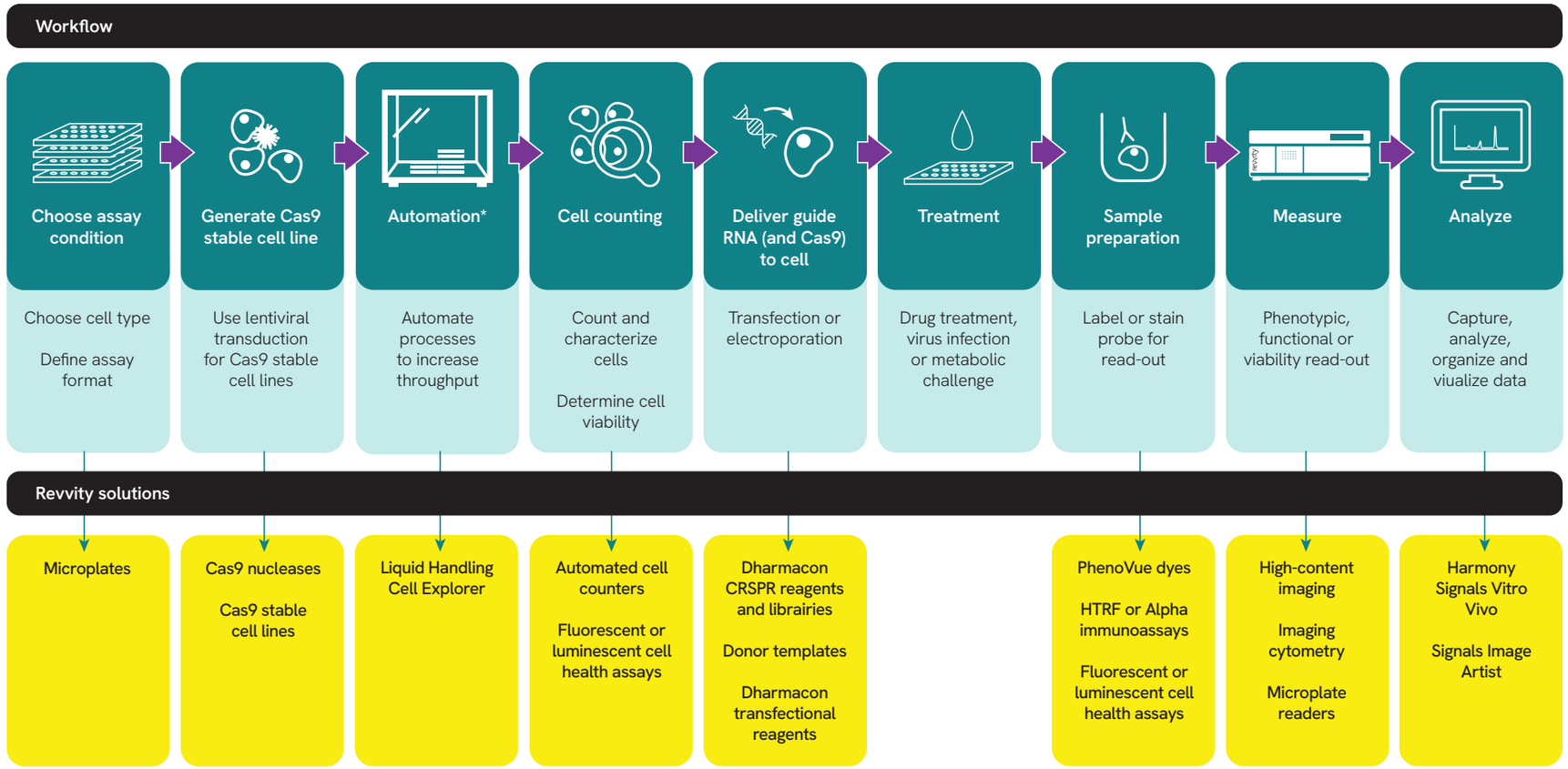
Take steps toward a better workflow

Arrayed screening workflow



*Automation can be used at different steps of the process.

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 we can support your experimental needs.

- The **Cas9 nuclease** reagents are available as Cas9 protein, mRNA, plasmid or Lentiviral Cas9. For optimization and enrichment versions co-expressing a fluorescent tag are available. "CRISPR-ready" **Cas9-expressing stable cells** can help you shorten your editing workflow.
- Dharmacon™ 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 ~ 50nt 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

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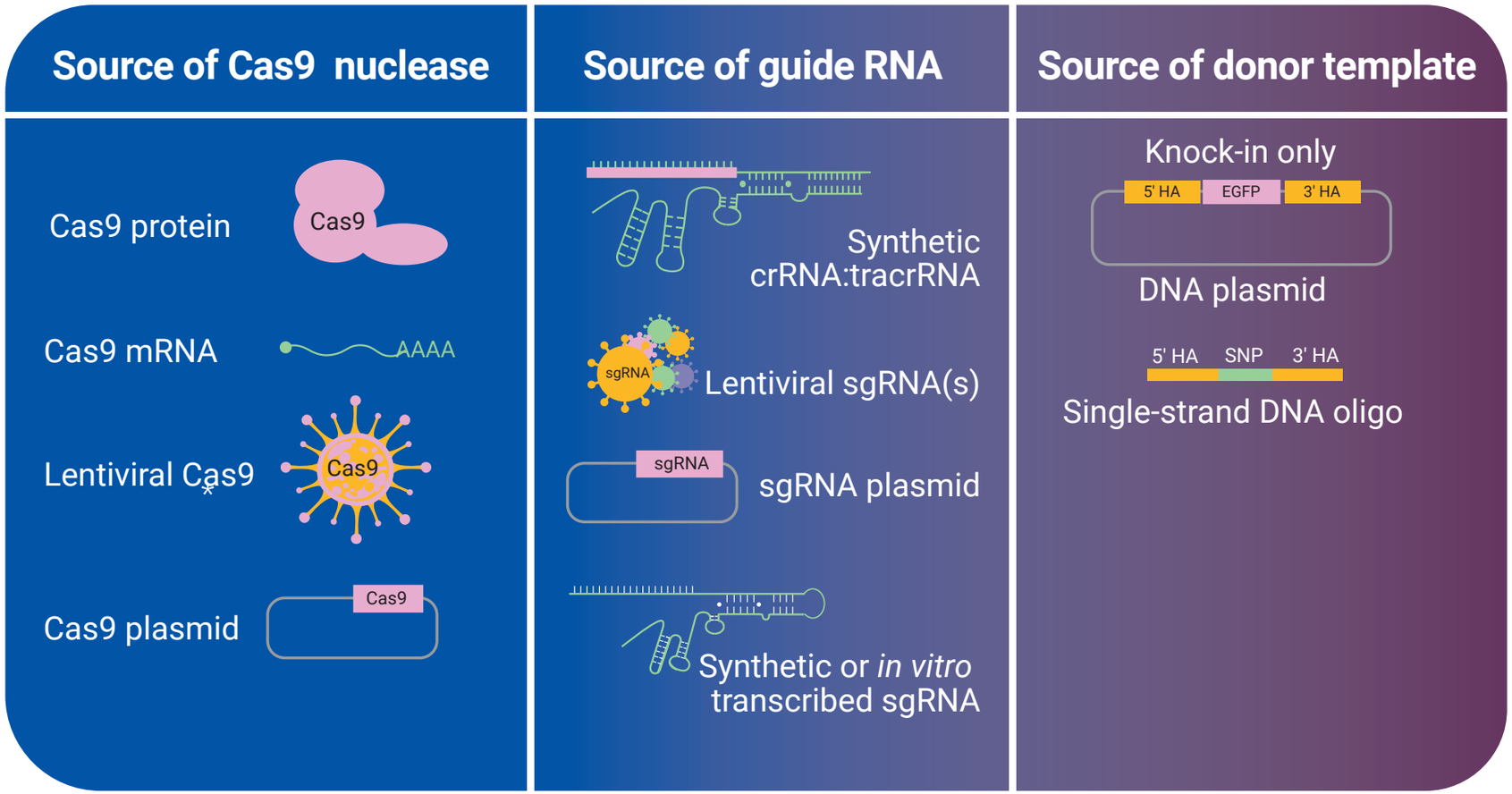

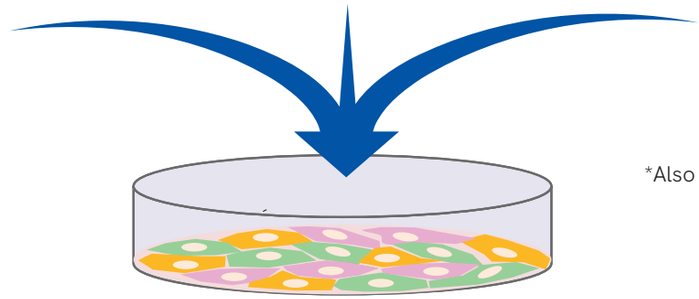

*Also available as all-in-one lentiviral format.

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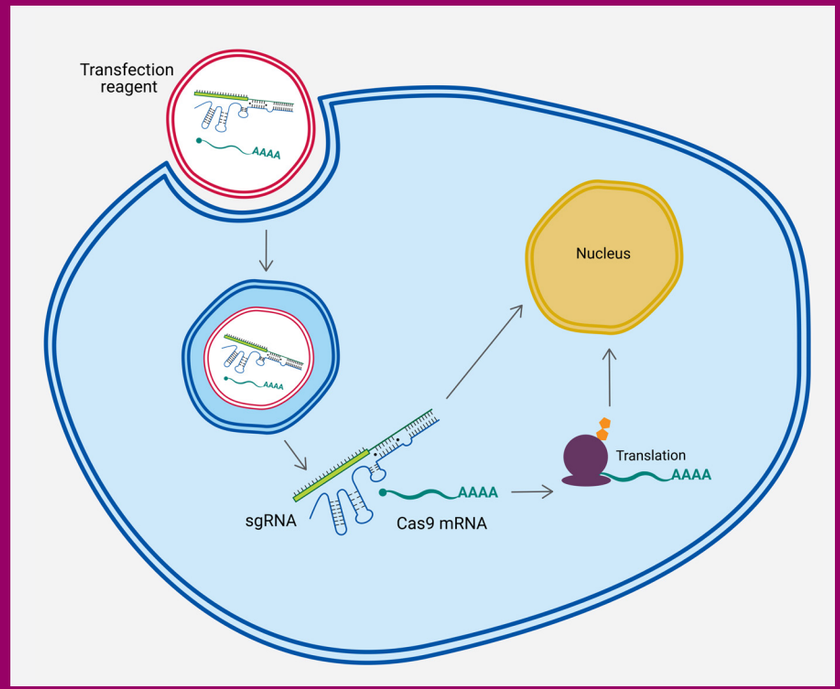
Maximize editing efficiency

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, consequently, 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**



gRNA and Cas9 are delivered 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.

Our solutions

- DharmaFECT™ transfection reagents can provide efficient and reliable transfection at low RNA or plasmid reagent concentrations with minimal cellular toxicity.
- It is possible to either co-transfect Cas9 and synthetic CRISPR gRNA, or to transfect CRISPR gRNA into stably 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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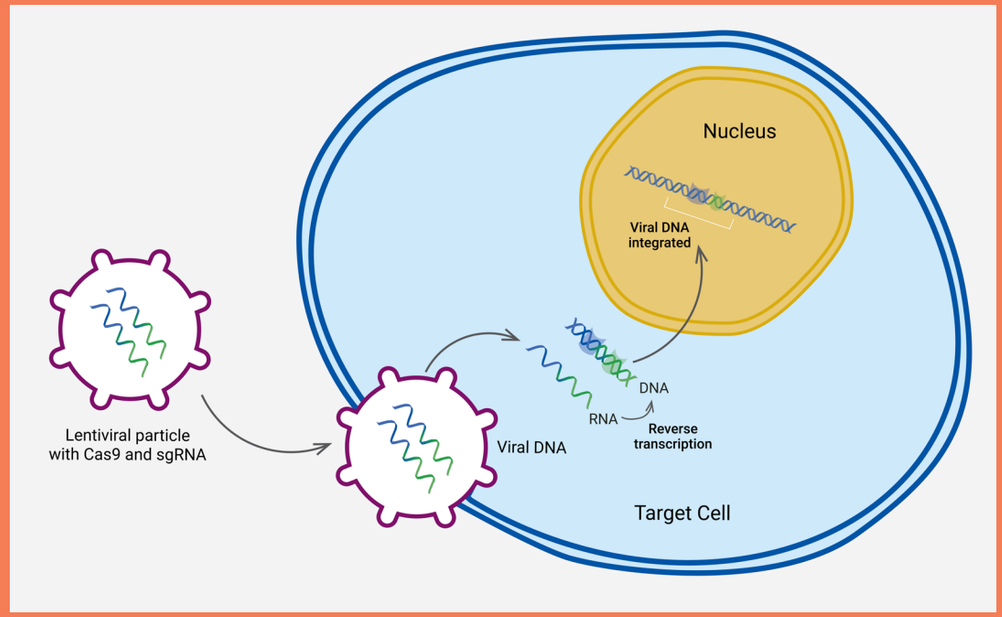

Lentiviral particles carrying a gene-specific sgRNA and/or Cas9 nuclease into the cell.

Our solutions

- 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.
- There are multiple lentiviral strategies to support either pooled or arrayed high-throughput screens.

On the web

Lentiviral Cas9 Nuclease Reagents

Learn more

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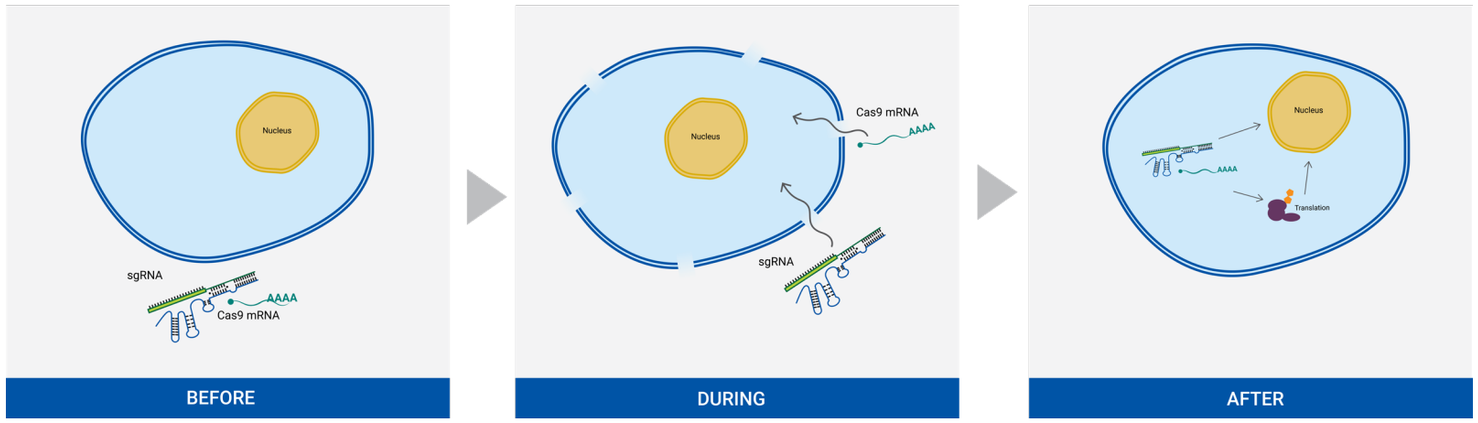


- Cells are exposed to a short electric pulse, as a result the cell membrane forms temporary pores and the gRNA and Cas9 nuclease to enter the cell.
- Electroporation can be performed in high-throughput arrayed format to support large scale screens.
- Often preferred when using difficult-to-transfect cells and primary cells.

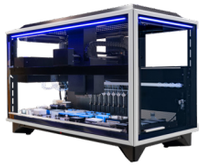
Notable study

Optimized HDR-mediated fluorescent protein knock-in in K-562 cells using Edit-R™ CRISPR-Cas9 reagents and electroporation **Learn more**

Innovative, easy-to-use, efficient: our liquid handlers and cell counters automate and improve every step of the workflow

Liquid handling and integrated automation solutions

These types of assays are often labor intensive and require many steps including re-suspending the CRISPR reagents, cell transfer, compound addition, and preparing the samples for detection read-outs. To help you accelerate your assay, we offer a full range of liquid handling and laboratory automation solutions.



Fontus liquid handler

Fontus liquid handler helps to simplify your workflows and improve turnaround times offering easy-to-use software, optimized deck access, verified protocols, and reformatting capabilities.

Learn more

explorer™ G3 workstation

The explorer G3 integrated workstation provides innovative application-focused laboratory automation solutions which simplify microplate handling, liquid handling, and detection.

Learn more

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 can 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

Learn more

Microplates deliver superior data

Choosing the right microplate is a critical, often overlooked, part of an assay. The right microplate helps provides valuable data, whereas the wrong microplate can lead to inaccurate data leading to missed project timelines and ultimately higher costs.

Imaging microplates

We've developed and validated a range of microplates optimized for high-content imaging including the PhenoPlate™ microplates. These microplates have been engineered to deliver superior images and high quality data due to excellent flatness of the plate bottom and cyclic olefin imaging surface. They are also available with different coatings to suit your application.

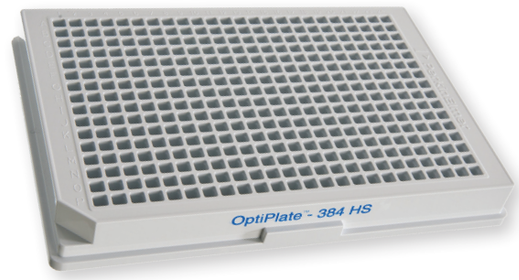
Learn more

Detection microplates

Our detection microplates are designed to give you optimal performance, whether you are working with fluorescence, luminescence or absorbance-based assays.

They are available with a selection of treatments and coatings that fit all your research needs. Choose from our OptiPlates™, AlphaPlate™, or ProxiPlates™, available in black, gray and white.

Learn more



Phenotypic drug discovery

Primary read-out of an arrayed CRISPR screen is imaging - from simple phenotypic to multiparametric high content analysis - which assists you in determining expression levels during assay optimization, cell morphology or localization of target genes. To monitor processes like differentiation or migration over an extended period, you can turn to live cell imaging. We provide a complete portfolio of high-content screening products, and live cell imaging instruments, software, and fluorescent probes.

PhenoVue cellular imaging reagents

Optimized and verified our PhenoVue™ suite includes:

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

Learn more

High-content imaging

Configure the systems to suit your lab requirements

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

Learn more

High-content imaging and analysis software

Analyze data in context

Harmony™ software supports the user in controlling every aspect of a high-content analysis or screening experiment through a single workflow-based user interface.

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

Learn more

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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 help you keep your costs down, we provide cell viability and toxicity assays based on bioluminescent or fluorescent detection chemistries that facilitate endpoint assays in a high-throughput format.

Fluorescent reagents

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

Learn more

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. When paired with a system from our plate reader family, you'll see reliability and inspiration come together.

Learn more

Multimode plate readers

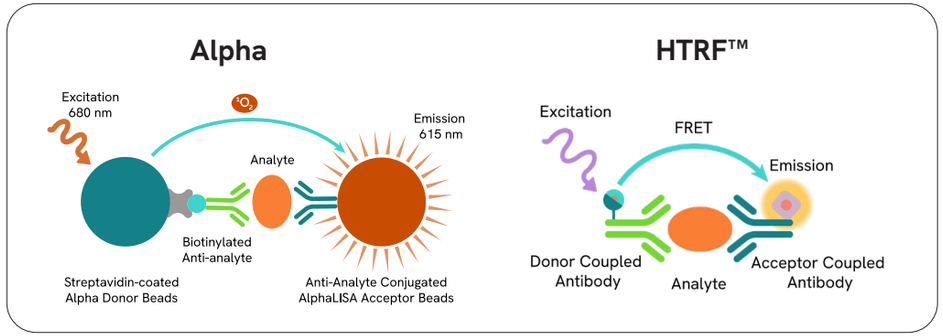
Our portfolio of microplate readers is equipped with multiple detection modes to support the diverse assay requirements of today's laboratories. With various models, configurations, and accessories, a Revvity plate reader can match a lab's throughput needs and meet budgetary constraints.

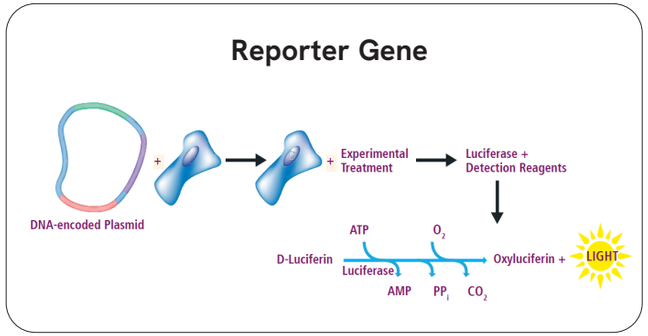
Learn more

Functional assays help you gain insight

Functional assays can help to gain complementary insights to imaging data in different steps of a CRISPR screening campaign. During assay development and optimization, they can assist to quantify Cas9 expression in the cells. They can also be used later in the workflow to confirm knock-out efficiency or to identify pathways by quantifying protein levels.

- 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 our family of plate reader systems.





Notable study

CRISPR can identify regulators of alzheimer's-related protein tau

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 more

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Data for all modalities in one platform

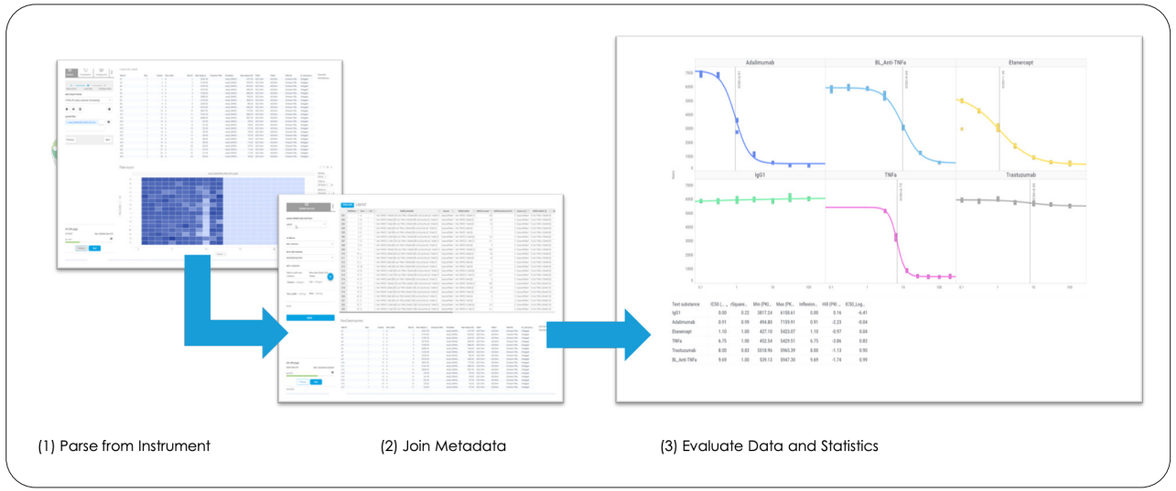
Signals VitroVivo is an intuitive, configurable flexible screening workflow processor coupled with the unparalleled data visualization and analysis capabilities of TIBCO® Spotfire®. It is flexible enough for one-off assay work during assay development, comprehensive enough for more sophisticated assays, 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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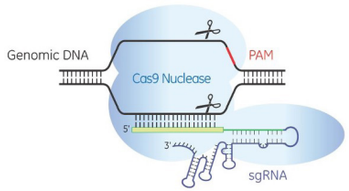
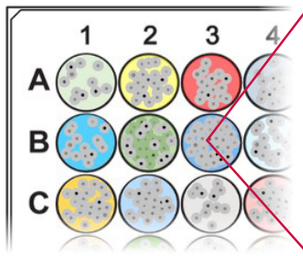
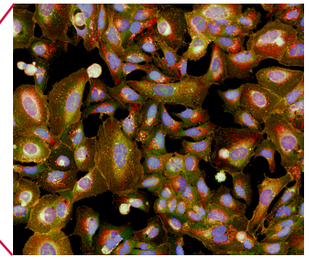
Case Study

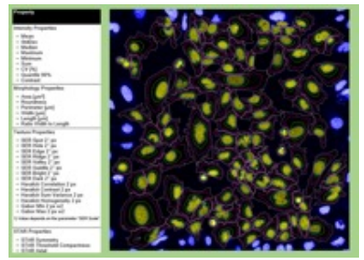
Studying cell cycle regulation in an arrayed CRISPR gene knockout screen using cell painting

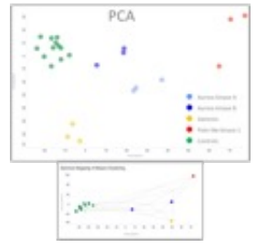
Gene editing → Staining → Image acquisition → Feature extraction → Computational analysis











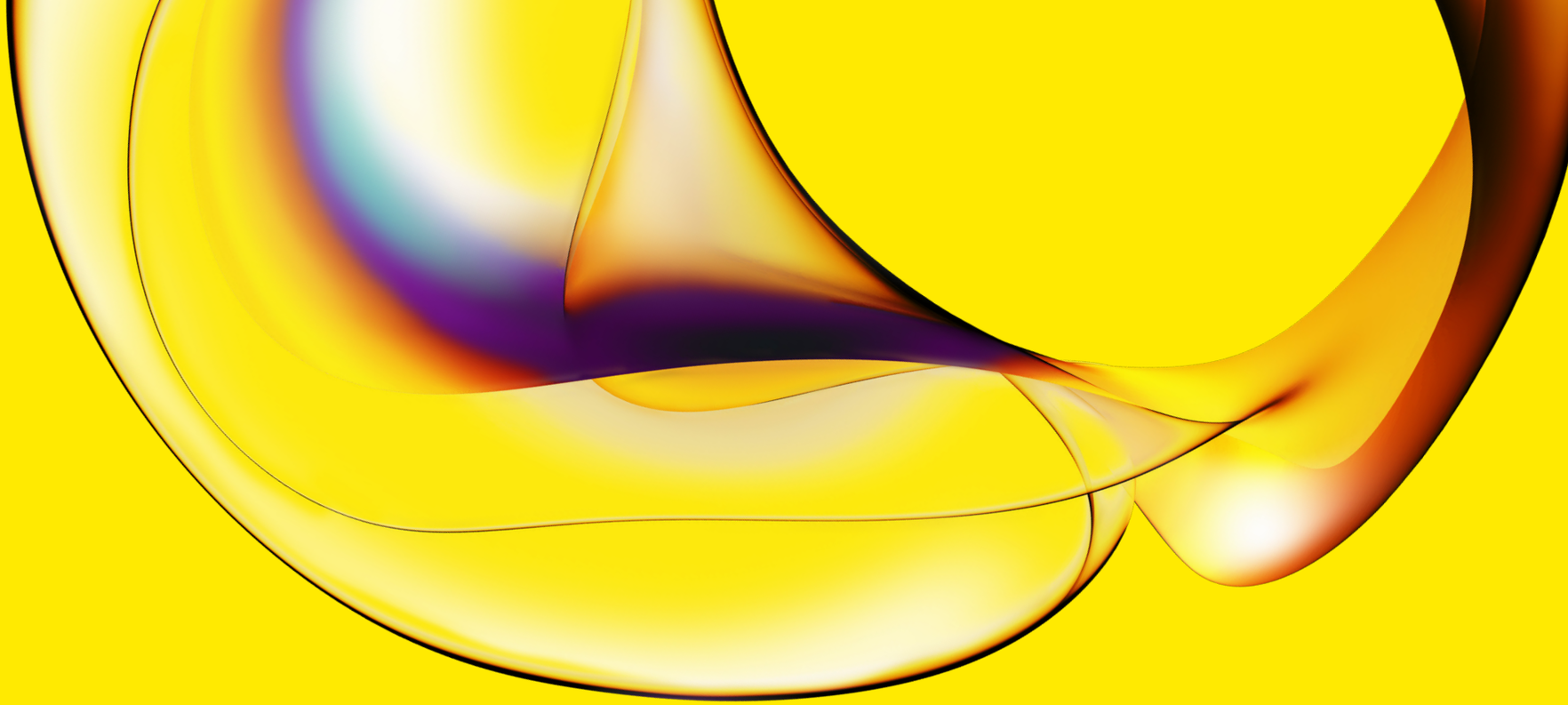
In this case study U2OS cells stably expressing Cas9 were seeded into sterile and tissue culture-treated PhenoPlate™ microplates. Four different cell cycle regulators (Aurora kinase A and B, Geminin and Polo-like kinase 1) were knocked out using Dharmacon™ Edit-R™ CRISPR knockout synthetic sgRNAs (pool of three sgRNAs per target, one target per well).

Cells were stained using PhenoVue™ Cell Painting Kit which comprises 6 verified, pre-optimized fluorescent probes for labelling the nucleus, ER, Golgi apparatus, actin, nucleoli and mitochondria.

Imaging was performed on the Opera Phenix™ Plus High-Content Screening system, 5 channels were acquired sequentially in confocal mode using a 20x water objective. The PhenoVue Cell Painting Kits are verified and optimized for use with this system.

Even complex phenotypes can be easily quantified with Harmony™ high-content analysis software which contains a dedicated cell painting analysis building block.

Use Signals VitroVivo for visualization and phenotypic clustering. In a Principal Component Analysis (PCA) controls and knock-out wells cluster together according to the target. Phenotypic clustering can also be analysed by unsupervised clustering and visualization via a Sammon connections network.



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