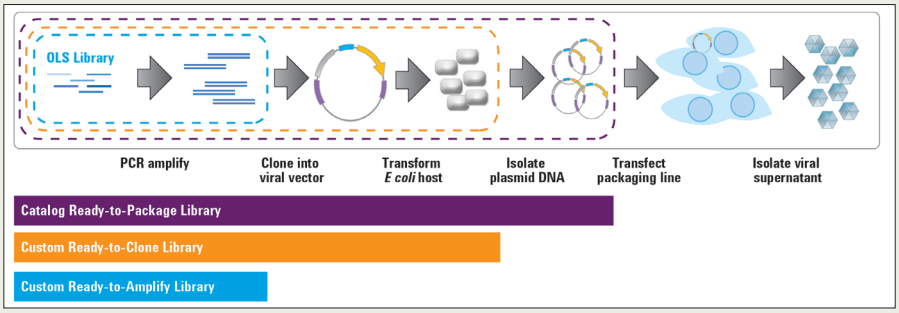
Product Focus: CRISPR/Cas9

Optimizing Workflow and Reducing Costs of Targeted Genome Editing

The potential for CRISPR (clustered regularly interspaced short palindromic repeats) technologies in genome editing has exploded recently, sparking an exponential growth in research interest and development of new techniques and products. The development of CRISPR is unlikely to slow down, but there are challenges to overcome, such as delivery of Cas9, precision of target effects and fully understanding how genome modifications have an affect on the phenotype. Targeted genome editing technologies are prevailing as tools for studying biology and disease, with the applications spanning multiple fields. In contrast to manipulating individual genes, large-scale screening methods are now under development.

Genome screening

An ongoing difficulty in DNA synthesis for CRISPR is fidelity: during the synthesis process, errors are introduced through base deletions, insertions and altered identities. The rate at which these errors occur is determined by fidelity. **Agilent** has created the **SureGuide CRISPR Library,** enabling exome-wide knock-down screening which obtains superior fidelity, improving functional results and reducing screening time. CRISPR libraries are complex mixtures of numerous DNA sequences and are designed around obtaining perfect sequence input, with errors being eliminated in the screening process as the guides are typically selected by phenotype and sequenced. Agilent's GeCKO CRISPR catalog libraries are available in three formats; ready to package, ready to clone and ready to amplify. All exonic sequences are targeted in the human and mouse genome and are ideal for genome-wide functional screening, containing fewer missed guides and more uniform representation across the library.



SureGuide CRISPR Library from Agilent

Workflow optimization

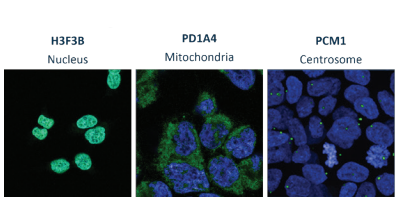
Genome editing, especially the CRISPR-Cas9 method, is part of the most common workflows in modern cell biology and molecular biology labs. Due to the high efficiency and versatility of the CRISPR/Cas9 method, the throughput in standard applications and workflows, such as NGS, increases steadily. An automated liquid handling workstation can help to process large quantities of highly-reproducible samples in a short time period. The **epMotion 5075m** from **Eppendorf** offers pre-programmed, optimized methods in combination with dedicated reagent kits for nucleic acid purification.

The experimental workflow of genome editing can be laborious and susceptible to human error.

Dharmacon™ Edit-R™ CRISPR-Cas9 genome-wide and druggable screening libraries from **GE Healthcare** leverage a powerful design algorithm for high functionality and superior specificity. Arrayed, ready-to-use synthetic CRISPR RNA libraries support a wide range of assays with a one-well-per-gene format. Choose from arrayed glycerol stock or pooled lentiviral sgRNA libraries for unbiased, powerful loss-of-function screens.

Reducing costs and improving throughput

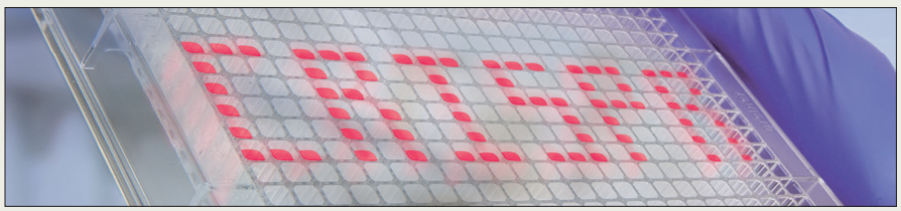
The new range of **Tagged Organelle** cell lines from **Horizon Discovery** is ideal for studying protein localization in live or fixed assays, avoiding concerns of overexpression systems or non-specific antibodies. These cell lines are ready-to-go, without the need for molecular biology skills or resource, and enable scientists to quickly advance their theories. Many labs find gene editing to be costly in terms of time and resources, even though the technologies are now readily available. Horizon's products are designed to provide researchers with access to the latest gene editing technologies, effectively democratizing gene editing. This is the latest extension to Horizon's Endogenous Tagged Protein portfolio, which is engineered using a novel self-releasing CRISPR plasmid and Horizon's proprietary HAP1 cell line.



Tagged organelle markers from Horizon Discovery

These reporter lines can be multiplexed with antibodies or intracellular dyes, and are ideal for providing accuracy and confidence in high-throughput screening approaches, such as assay development to establish time points.

The Dharmacon™ Edit-R™ CRISPR-Cas9 platform from GE Healthcare



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Genome editing workflow offers from Eppendorf

Battling off-target effects

Editing efficiency and consistency can be compromised by increased impurities, which cause off-target effects. **CRISPRevolution**, a portfolio of synthetic guide RNA designed for CRISPR genome editing, has been developed by **Synthego** to deliver full-length 100-mer synthetic single guide RNA (sgRNA) for highly efficient CRISPR genome editing at high volume and an economical scale. With editing efficiencies of up to 90%, Synthego synthetic sgRNA is well suited for all cell types including primary and stem cells. Synthego synthetic sgRNA is higher in quality, available at up to five times lower cost, and has a faster turnaround time of 4X. Compared to traditional plasmid and *in vitro* transcription methods for generating CRISPR guide RNA, Synthego synthetic sgRNA eliminates countless hours and days of cloning and construction, has extremely high editing efficiencies and consistencies, and arrives ready for transfection. Furthermore, there is no risk of foreign DNA incorporation as Synthego synthetic sgRNA is 100% DNA-free, which is critical for applications such as CRISPR therapeutics and plant genomics.

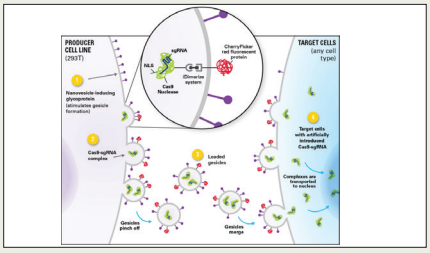
CRISPRevolution RNA Kit from Synthego

CRISPR/Cas9 technology has revolutionized the field of cell biology by introducing efficient and straightforward genome editing into the toolbox of laboratories. While CRISPR/Cas9 is a powerful technique for genome manipulation, two significant challenges remain: obtaining efficient delivery of Cas9 and gene-specific single guide RNAs (sgRNA) to a broad range of cell types, and achieving fewer off-target effects. To begin addressing these challenges, **Takara Bio USA, Inc**. has developed a technology based on cell-derived nanovesicles called gesicles, which are produced using the **Guide-it™ CRISPR/Cas9 Gesicle Production System**. Gesicles made using this kit contain active Cas9 protein, along with sgRNA specific to a gene of interest, and express a glycoprotein on their surface which mediates binding and fusion with the membrane of target cells. These features enable gesicles to knockout genes in a wide range of cell types, including human induced pluripotent stem cells (hiPSCs), often with higher efficiency than using plasmid-based delivery methods. Additionally, this nanoparticle-based method allows for tight control of the dose and duration of the Cas9-sgRNA complex in the cell, which decreases off-target effects.

Cas9 proteins

ToolGen provides a comprehensive range of CRISPR products and services including recombinant Cas9 protein, customized guide RNAs, and genome-edited cell lines. ToolGen's **Recombinant Cas9 protein with a NLS tag** for eukaryotic genome editing, has been used successfully in a wide variety of systems from human primary cells/cell lines, insects, mammals and plants (Arabidopsis to fruits). It can be provided in both purified protein and plasmid form.

Customized gRNA services include a design of right gRNAs with the consideration of an off-target site analysis for a target genome and synthesis of gRNA in both forms of mRNA and plasmid-based vectors. The surrogate reporter is a plasmid-based reporter system that can be used to observe the activity of CRISPR/Cas9 in a cell and to enrich genome-edited cells during cell line development processes.



Guide-it™ CRISPR/Cas9 Gesicle Production System from Takara Bio USA, Inc.

With its expertise in genome editing process, ToolGen is also providing gene knock-out and knock-in services for immortalized/transformed cell lines.

Improving the workflow method, optimizing delivery of Cas9 and reducing off-target effects caused by impurities are all challenges which are gradually being overcome in the CRISPR gene editing industry. The boom in popularity of this cost-efficient method is driving research forwards in targeted genome editing to help understand the basis of diseases.

Recombinant Cas9 protein from ToolGen

Companies mentioned in this Product Focus

Agilent Technologies	www.agilent.com
Eppendorf	www.eppendorf.com
GE Healthcare	www3.gehealthcare.com
Horizon Discovery	www.horizondiscovery.com
Synthego	www.synthego.com
Takara Bio USA, Inc.	www.takara-bio.com
ToolGen	http://toolgen.com

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