# Molecular Essentials Guide

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About this guide

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Lab & Production Materials

# Molecular Essentials Guide

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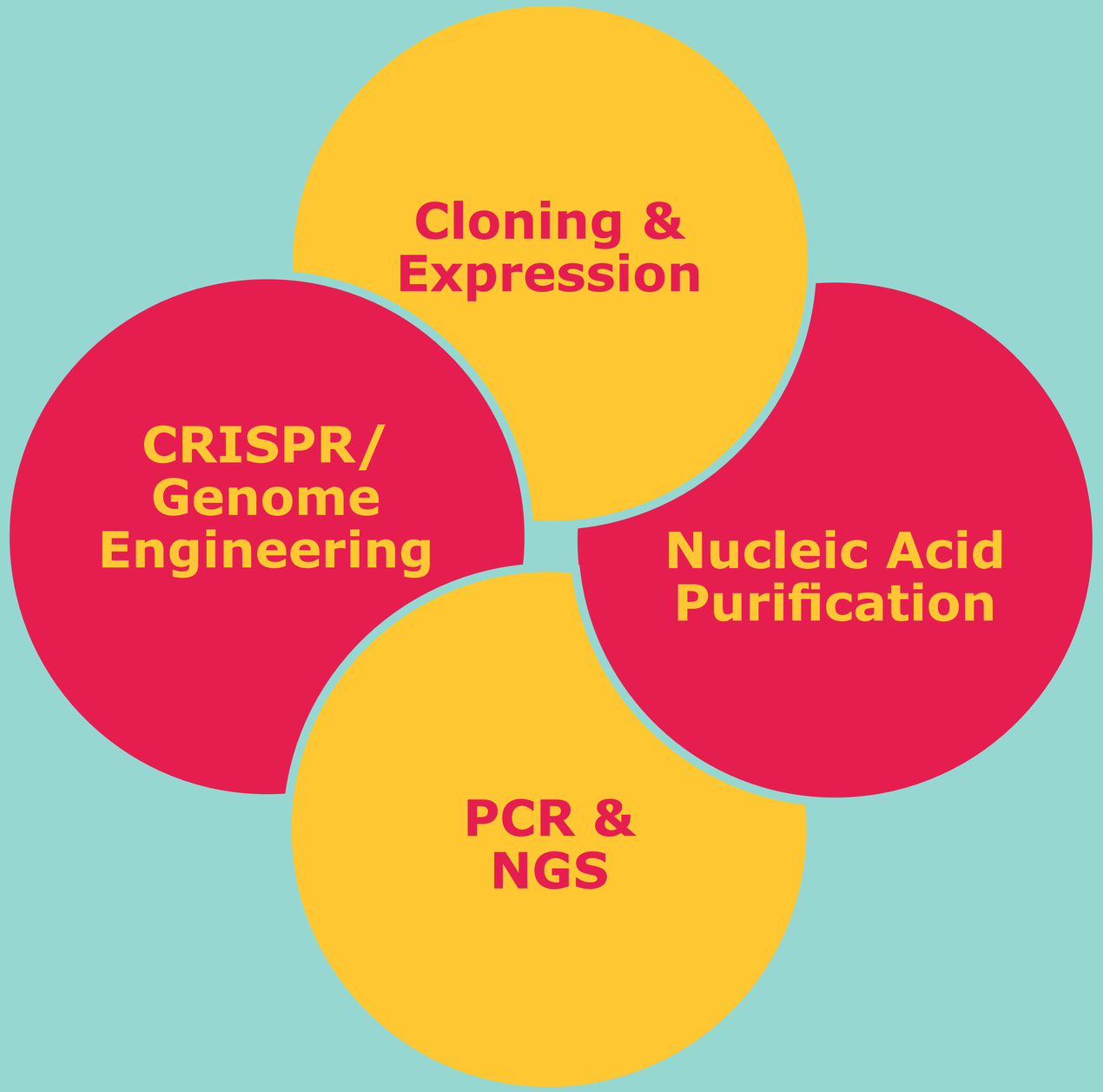


# MOLECULAR ESSENTIALS GUIDE

Our Molecular Essentials Guide is meticulously assembled for the academic researcher or industry professional whose molecular workflow requires easy access to a comprehensive portfolio of consistent, precise, ready-to-use reagents and technical support. From routine cloning, rapid nucleic acid purification, reliable PCR amplification, and novel CRISPR gene editing tools, our global team of technical experts remain committed to offering the time and resource-saving benefits of our molecular tools that are ready when you are. Our highly proven molecular reagents provide a range of “grab-and-go” tools so that you can select from a variety of gold-standard offerings and solutions that scale from bench-top R&D to production-level needs. Explore our robust offering of cloning, expression, nucleic acid purification, PCR, gene editing, and NGS reagents.

***For incremental innovations, day after day, choose molecular essentials that are the foundation of great work, not a variable that undermines it.***

# Molecular Essentials Guide Workflow



## Cloning & Expression

- Expression Vectors
- Modifying Enzymes
- Selection Essentials
- Transfection Reagents
- Expression Essentials
- Molecular Biology Essentials

# Cloning & Expression

## Expression Vectors

### Novagen® pET System

Driven by powerful bacteriophage T7 promoter and translation signals, the pET System is the gold standard for cloning and expression of recombinant proteins. The Novagen® pET System has been used to express thousands of different proteins in host cells expressing the T7 polymerase. With a variety of pET vector types, host strains, and complementing products, the pET system provides you with the flexibility to design and optimize your cloning and protein expression needs.

| Cat. No. | Single Expression Vectors | Key Features & Application |
|---|---|---|
| **69436** | pET-11a | Basic pET vectors offer single BamHI cloning site in three frames |
| **69439** | pET-11d | |
| **69660** | pET-14b | Basic cleavable N-terminal His•Tag® fusion vectors, single frame with three cloning sites |
| **69661** | pET-15b | |
| **69662** | pET-16b | |
| **69677-M** | pET-19b | |
| **69739** | pET-20b(+) | Signal sequence fusion to facilitate export of target proteins to the periplasm. Signal sequence cleaved by signal peptidase upon export |
| **69744** | pET-22b(+) | |
| **69753-M** | pET-25b(+) | |
| **69862** | pET-26b(+) | |
| **69863-M** | pET-27b(+) | |
| **69740** | pET-21a-d(+) | Combination of N-terminal T7•Tag® epitope and optional C-terminal His•Tag® sequence. Multiple cloning sites in three frames |
| **69745-M** | pET-23a-d(+) | |
| **69749** | pET-24a-d(+) | |
| **69864** | pET-28a-c(+) | Cleavable N-terminal fusion tags and optional C-terminal His•Tag® sequence. Multiple cloning sites in three frames |
| **69871** | pET-29a-c(+) | |
| **69909** | pET-30a-c(+) | |
| **69952** | pET-31b(+) | High yield bioproduction of peptides and small proteins |
| **69015-M** | pET-32a(+) | Production of soluble, active target proteins in *E. coli* |
| **69016** | pET-32b(+) | |

| Cat. No. | Single Expression Vectors | Key Features & Application |
|---|---|---|
| **70090-M** | pET-39b(+) | Dsb tags for export and periplasmic folding of target proteins |
| **70091-M** | pET-40b(+) | |
| **70556** | pET-41a(+) | Popular GST fusion tags for enhanced production and solubility |
| **70557-M** | pET-41b(+) | |
| **70561** | pET-42a(+) | |
| **70562** | pET-42b(+) | |
| **70939** | pET-43.1a(+) | Cloning and high-level expression of polypeptide sequences fused with the 495 aa NusA (Nus•Tag™) protein |
| **70940-M** | pET-43.1b(+) | |
| **71122** | pET-44a(+) | Nus•Tag™ sequence plus N- and C-terminal His•Tag sequences |
| **71327** | pET-45b(+) | His•Tag™ sequence and minimal extraneous sequences |
| **71335** | pET-46 Ek/LIC Kit | Ligation-independent cloning, with amino-terminal His•Tag™ sequence (more available) |
| **71461** | pET-47b(+) | HRV 3C Protease cleavage site for efficient fusion tag removal |
| **71462** | pET-48b(+) | |
| **71463-M** | pET-49b(+) | |

Explore the full list of offerings here

# Cloning & Expression

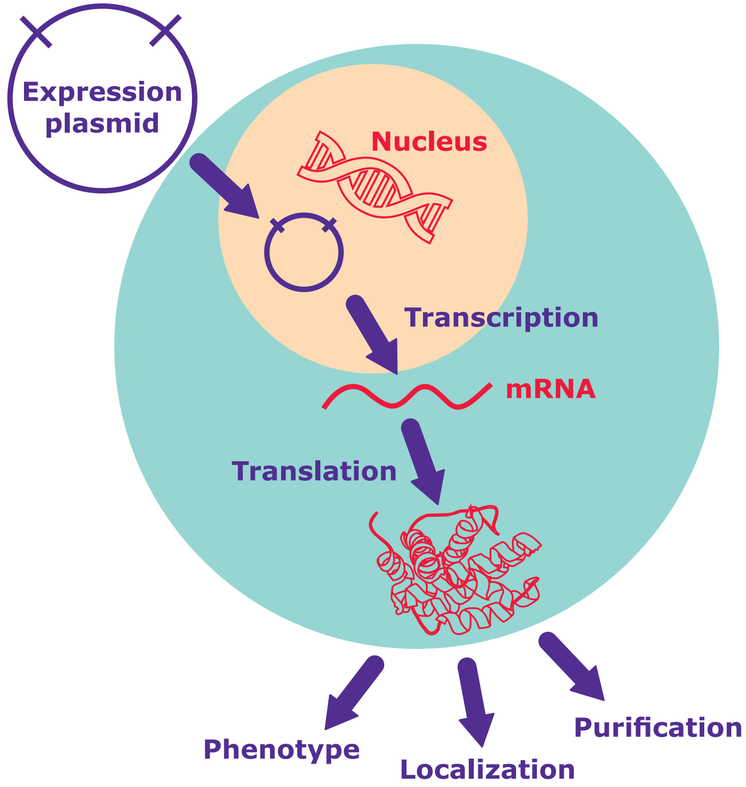
## Expression Vectors

### Novagen® pET System

The pET System is the most powerful system for the cloning and expression of recombinant proteins in *E. coli*. Target genes are cloned in pET plasmids under control of strong bacteriophage T7 transcription and (optionally) translation signals; expression is induced by providing a source of T7 RNA polymerase in the host cell. The pET System offers numerous pET vector types, different host strains, and many other companion products designed for efficient detection and purification of target proteins.

| Cat. No. | Single Expression Vectors | Key Features & Application |
|---|---|---|
| **70608** | pETBlue™-1 | Identify recombinants by traditional blue/white screening (more available) |
| **70609** | pETBlue™-2 | |
| **71129** | pETcoco-1 | Precise control of the expression of toxic proteins (more available) |
| **71363-M** | pRSF-1b | Express N-terminal His•Tag® fusion proteins containing minimal extraneous sequences (more available) |
| **71330-M** | pCDF-1b | |

| Cat. No. | Co-Expression Vectors | Key Features & Application |
|---|---|---|
| **71146** | pETDuet™-1 | Express multiple target proteins in *E. coli* |
| **71147** | pACYCDuet™-1 | |
| **71341** | pRSFDuet™-1 | |
| **71340-M** | pCDFDuet™-1 | |
| **71406-M** | pCOLADuet™-1 | |




Find the full list of offerings here

# Cloning & Expression

## Modifying Enzymes

Traditional cloning remains the most popular way to insert your GOI into an expression vector for protein expression in the target cell, whether that is an insect, mammalian, or microbial cell. We carry a variety enzymes and kits dependent on your research needs.

Additionally, we offer a wide variety of high-fidelity **PCR reagents**.

| Cat. No. | Cloning Reagents | Application |
|---|---|---|
| **LIG2** | QuickLink™ DNA Ligation Kit | Reagents necessary to perform DNA ligation reactions at room temperature using blunt or sticky ends |
| **D2886** | DNA Ligase from T4-infected Escherichia coli | Ligation of cloning vector and restriction insert fragments in a buffered aqueous solution |
| **70099-M** | T4 DNA Polymerase, LIC-qualified | Qualified for ligation-independent cloning (LIC), to insert DNA fragments into plasmid vectors without the need for traditional restriction enzyme digestion and ligation |
| **Cat. No.** | **Additional Reagents** | **Application** |
| **SCR508** | TAT-CRE Recombinase | Cell-permeant fusion Cre-recombinase protein known to catalyze the site-specific recombination event between two loxP DNA sites |

**Polymerases**

**Colony PCR**

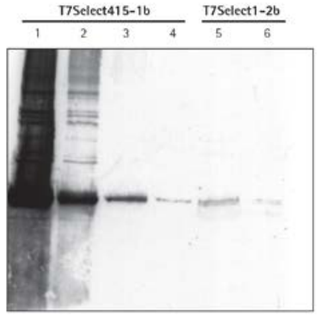
### T7Select® Phage Display

The T7Select® Phage Display System is based on bacteriophage T7. This novel phage display system has the capacity to display small peptides in high copy number, and larger peptides or proteins in low-to- mid-copy number range. In contrast to filamentous phage assembly, peptides or proteins displayed on the surface of T7 do not need to be capable of secretion through the cell membrane. Instead, phage assembly takes place in the *E. coli* cytoplasm and mature phage are released by cell lysis. Available in select kit sizes.

| Kit | Use | Display Number Per Phage | Amino Acid Display Limit | Host |
|---|---|---|---|---|
| T7Select® 1-1 Cloning Kit | Peptides or Proteins | 0.1 – 1 | 900 – 1200 aa | BLT5403 and BLT5615 strains |
| T7Select® 10-3 Cloning Kit | Peptides or Proteins | 5 – 15 | 1200 aa | |
| T7Select® 415-1 Cloning Kit | Peptides | 415 | 40 – 50 aa | BL21 |

### T7Select® vectors enable varying copy numbers of displayed peptides.

Phage particle proteins were analyzed by Western blot using the HSV•Tag® monoclonal antibody. Lanes 1 and 5 represent the equivalent of 25 µL lysate for the indicated vectors. Lanes 2-4 and lane 6 represent 10-fold serial dilutions. Data confirm that T7Select® 415-1b vector is ideal for high copy number phage display, while T7Select® 1-2b is appropriate for low copy number phage display, (Data courtesy of A. Rosenberg, Brookhaven National Laboratories).




**Explore the full list of offerings here**

**Agarose Gel Electrophoresis**

# Cloning & Expression

## Selection Essentials

### Competent Cells, Induction, and Selection Reagents

Novagen® competent cells come in a variety of strains for chemical transformation. Optimized for high transformation efficiency, incorporation of Novagen® competent cells into transformation protocols ensures superior yields of stable, high-quality plasmid DNA and recombinant proteins. For related products, explore our plasmid preparation and additional kits for **nucleic acid research**.

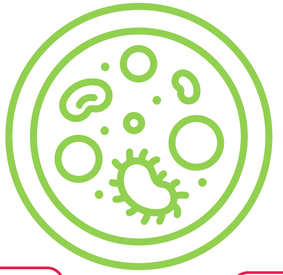
| Cat. No. | Induction Reagents | Key Features & Application |
|---|---|---|
| **70527** | 100 mM IPTG Solution | Blue/White screening,<br>Induction of protein expression with pET system |
| **I1284** | Isopropyl β-D-thiogalactopyranoside solution | Ready-made formulation for Blue/White screening |
| **Cat. No.** | **Selection Reagents** | **Key Features & Application** |
| **B2904** | Bluo-Gal | Designed to replace X-Gal in Blue/White screening of recombinant bacterial colonies |
| **B3928** | Blue-White Select™ Screening Reagent | Ready-made formulation with intense color contrast for easy colony selection |
| **71077** | X-Gal Solution | Convenient 40 mg/mL concentrate in DMSO for Blue/White screening |

**BL21 Cells**

The gold standard for protein expression from target genes cloned in pET vectors

**NovaBlue Cells**

For routine molecular cloning applications, blue/white screening and plasmid preparation



**ROSETTA™ Cells**

To enhance the expression of eukaryotic proteins that contain codons rarely used in *E. coli*

**ORIGAMI™ Cells**

For proper disulfide bond formation and increasing yields of folded, soluble protein.

*Need cells that are manufactured free of animal-derived media and components?*
*Veggie™ Competent Cell versions are available for several strains*

**Microbial Media**

# Cloning & Expression

## Selection Essentials

### BL21 Competent Cells

***The gold standard for protein expression from target genes cloned in pET vectors.***

BL21 has been the gold standard for protein expression since it was first introduced in 1990. Deficient in lon and ompT proteases, BL21 and its derivatives are high-yielding and ideal for many applications.

DE3 indicates that the host is a lysogen of λDE3, and therefore carries a chromosomal copy of the T7 RNA polymerase gene under control of the lacUV5 promoter. Such strains are suitable for production of protein from target genes cloned in pET vectors by induction with IPTG.

pLysS strains express T7 lysozyme, which further suppresses basal expression of T7 RNA polymerase prior to induction, thus stabilizing pET recombinants encoding target proteins that affect cell growth and viability.

| Cat. No. | Host Strains | Derivation | Guaranteed Efficiency | Packaging Format | Resistance | Key Features & Application |
|---|---|---|---|---|---|---|
| **69449** | BL21 Cells | B834 | >2.0 x $10^7$ | Standard | None | Routine protein expression, control non-expression host |
| **69450-M**<br>**70235-M**<br>**71012** | BL21(DE3) Cells | B834 | >2.0 x $10^7$ | Standard, Singles™, HT96™ | None | General purpose expression host |
| **69451-M**<br>**70236-M** | BL21(DE3) pLysS Cells | B834 | >2.0 x $10^7$ | Standard, Singles™ | Cam | High-stringency expression |

Explore

# Cloning & Expression

## Selection Essentials

### NovaBlue Competent Cells

***For routine molecular cloning applications, blue/white screening and plasmid preparation.***

For routine cloning, time-tested NovaBlue Competent Cells are ideal. NovaBlue is a K-12 strain ideally suited as an initial cloning host due to its high transformation efficiency, blue/white screening capability (with appropriate plasmids), and recA endA mutations, which result in high yields of excellent quality plasmid DNA.

The DE3 lysogen of NovaBlue is potentially useful as a stringent host due to the presence of the lacIq repressor encoded by the F episome. Blue/white screening is not possible with NovaBlue(DE3) due to the presence of the lacZ α-peptide coding sequences in the lysogenic phage. NovaBlue T1R Competent Cells have the added benefit of being resistant to T1 and T5 phage.

| Cat. No. | Host Strains | Derivation | Guaranteed Efficiency (cfu/µg) | Packaging Format | Resistance | Key Features & Application |
|---|---|---|---|---|---|---|
| **69825-M** | NovaBlue Cells | K-12 | >1.5 x $10^8$ | Standard, Singles™ | Tet | Non-expression host, general purpose cloning host, plasmid preps |
| **69284** | NovaBlue(DE3) Cells | K-12 | >1.0 x $10^8$ | Standard | Tet | Stabilizing target plasmids |
| **71227** | NovaBlue GigaSingle™ Cells | K-12 | >1.0 x $10^8$ | Singles™ | Tet | Non-expression host, high-efficiency cloning |
| **71251-M** | Veggie™ NovaBlue Cells | K-12 | >1.5 x $10^8$ | Singles™ | Tet | Non-expression host, general purpose cloning host, plasmid preps with non-animal origin components |
| **71318-M** | NovaBlue T1R Cells | K-12 | >1.5 x $10^8$ | Singles™ | Tet | Non-expression host, general purpose cloning, plasmid preps, T1 and T5 phage resistant |

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# Cloning & Expression
## Selection Essentials

### Origami™ Competent Cells

***For proper disulfide bond formation and increasing yields of folded, soluble protein.***

Origami™ 2 and Origami™ B strains have mutations in glutathione reducatase (gor) and thioredoxin reductase (trxB), facilitating proper disulfide bond formation. These strains also include the lon and ompT deficiencies of BL21, which increases protein stability.

DE3 indicates that the host is a lysogen of λDE3, and therefore carries a chromosomal copy of the T7 RNA polymerase gene under control of the lacUV5 promoter. Such strains are suitable for production of protein from target genes cloned in pET vectors by induction with IPTG.

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| Cat. No. | Host Strains | Derivation | Guaranteed Efficiency (cfu/µg) | Packaging Format | Resistance | Key Features & Application |
|---|---|---|---|---|---|---|
| **71344-M** | Origami™ 2 Cells | K-12 | >2 x $10^6$ | Standard | Tet + Str | Control non-expression host; kanamycin sensitive |
| **71345-M** | Origami™ 2(DE3) Cells | K-12 | >2 x $10^6$ | Standard, Singles™ | Tet + Str | General expression host; two mutations in cytoplasmic disulfide reduction pathway enhance disulfide bond formation in *E. coli* cytoplasm; kanamycin sensitive |
| **71346-M** | Origami™ 2(DE3) pLysS Cells | K-12 | >2 x $10^6$ | Standard | Tet + Str + Cam | High-stringency expression host; two mutations in cytoplasmic disulfide reduction pathway enhance disulfide bond formation in *E. coli* cytoplasm; kanamycin sensitive |
| **70836-M** | Origami™ B Cells | Tuner™ (B strain) | >2 x $10^6$ | Standard | Kan + Tet | Control non-expression host |
| **70837** | Origami™ B(DE3) Cells | Tuner™ (B strain) | >2 x $10^6$ | Standard | Kan + Tet | General expression host; contains Tuner™ lac permease mutation and trxB/gor mutations for cytoplasmic disulfide bond formation |
| **70839** | Origami™ B(DE3) pLysS Cells | Tuner™ (B strain) | >2 x $10^6$ | Standard | Kan + Tet + Cam | High-stringency expression host; contains Turner™ lac permease mutation and trxB/gor mutations for cytoplasmic disulfide bond formation |

# Cloning & Expression

## Selection Essentials

### Rosetta™ Competent Cells

***To enhance the expression of eukaryotic proteins that contain codons rarely used in E. coli.***

Rosetta™ and Rosetta™ 2 host strains are BL21 derivatives designed to enhance the expression of eukaryotic proteins that contain codons rarely used in *E. coli*.

If you're studying a eukaryotic protein, its open reading frame (ORF) may contain codons that are rarely employed in *E. coli*. Rosetta™ and Rosetta™ 2 strains include a chloramphenicol-selectable plasmid bearing tRNAs for codons that are infrequently used in *E. coli*, thus conferring "universal" translation.

DE3 indicates that the host is a lysogen of λDE3, and therefore carries a chromosomal copy of the T7 RNA polymerase gene under control of the lacUV5 promoter. Such strains are suitable for producing protein from target genes cloned in pET vectors by induction with IPTG.

| Cat. No. | Host Strains | Derivation | Guaranteed Efficiency (cfu/µg) | Packaging Format | Resistance | Key Features & Application |
|---|---|---|---|---|---|---|
| **70953** | Rosetta™ Cells | BL21 | >2.0 x $10^6$ | Standard | Cam | Control non-expression host |
| **70954** | Rosetta™(DE3) Cells | BL21 | >2.0 x $10^6$ | Standard | Cam | General expression host; provides six rare codon tRNAs |
| **70956-M** | Rosetta™(DE3) pLysS Cells | BL21 | >2.0 x $10^6$ | Standard | Cam | High-stringency, expression host; provides six rare codon tRNAs |
| **70920** | Rosetta™(DE3) pLacI Cells | BL21 | >2.0 x $10^6$ | Standard | Cam | - |
| **71402-M** | Rosetta™ 2 Cells | BL21 | >2.0 x $10^6$ | Standard | Cam | Control non-expression host |
| **71397** | Rosetta™ 2(DE3) Cells | BL21 | >2.0 x $10^6$ | Standard, Singles™ | Cam | General expression host; provides seven rare codon tRNAs |
| **71403-M** | Rosetta™ 2(DE3) pLysS Cells | BL21 | >2.0 x $10^6$ | Standard, Singles™ | Cam | High-stringency, expression host; provides seven rare codon tRNAs |
| **71404-M** | Rosetta™ 2(DE3) pLacI Cells | BL21 | >2.0 x $10^6$ | Standard | Cam | - |

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# Cloning & Expression

## Selection Essentials

### Microbial Media

Microbial culture is widely used in molecular biology for protein expression in different species, such as bacteria, yeast, or viruses. We offer the microbial media you need for fast and easy growth that leads to reproducible results and high protein yield.

Our culture medium expertise, and stringent quality standards enable us to offer media products with:

- Versatile formulations for different component needs
- Multiple easy-to-use formats (powder, liquid, and plates)
- High-quality media
- Lot-to-lot consistency

| Cat. No. | Microbial Media | Packaging Format | Application |
|---|---|---|---|
| **L3522** | LB Broth | Powder | Granulated medium for the cultivation of *E. coli* on scales ranging from small cultures to fermentation. Available in Miller and Lennox formulations. |
| **L3022** | | Liquid | |
| **L2542** | | | |
| **L7275** | | Tablet | |
| **L5542** | | Plate | |
| **T5574** | Terrific Broth | Powder | Highly enriched granulated medium to improve the yield of plasmid DNA from *E. coli*. |
| | | Liquid | |
| **Y2377** | 2xYT Broth | Powder | Powdered medium for the enrichment of *E. coli*. |
| **Y1003** | | Liquid | |
| **M6030** | M9 Minimal Salts | Powder | Suitable for non-selective cultivation of *E. coli* strains for cloning, production of DNA, plasmid DNA and recombinant proteins. |
| **M9956** | | Liquid | |
| **H8032** | SOB and SOC Medium | Powder | Enables the high efficiency transformation of competent cells. |
| **S1797** | | Liquid | |
| **N3643** | NZCYM Broth | Powder | Suitable for non-selective cultivation of lambda bacteriophage and *E. coli* strains for cloning, production of DNA, plasmid DNA and recombinant proteins. |
| **Y1250** | Yeast Nitrogen Base | Powder | Highly-referenced growth medium used for the cultivation of yeast. The nutrient-rich microbial broth contains nitrogen, vitamins, trace elements and salts. Available with or without amino acids. |
| **Y0626** | | | |

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# Cloning & Expression

## Selection Essentials

## Microbial Media Supplements

We offer a wide array of components, additives, and supplements for your specialized culture needs.

| Cat. No. | Microbial Supplements | Packaging Format | Application |
|---|---|---|---|
| **MBD0055** | MediaBoost | Liquid | A ready-to-use, non-animal, protein-free supplement that amplifies growth of certain gram-positive bacteria, gram-negative bacteria, and yeast species in minimal or standard microbial growth media. |
| **MBD0056** | Trace Elements Ready Made Solution | Liquid | A solution comprised of vital minerals to support critical bacterial development. Based on Wolin and Wolfe's recipe, it is suitable for culturing anaerobic bacteria of the human microbiome. |
| **MBD0063** | Vitamin Mixture Solution | Liquid | A solution of vitamins, additives, and mineral supplements used for special culturomics growth media to mimic the natural environment of bacteria and fungi for high throughput culturing applications. |
| **Y1625** | Yeast Extract | Powder | Used as a microbial media component for a variety of microorganisms, molecular genetics applications, and complex media for industrial fermentations. |
| **Y1626** | | | |
| **71279** | Veggie™ Yeast Extract and Veggie™ Peptone | Powder | Animal-free media components that can be used as direct replacements for tryptone and yeast extract in microbial growth media. |
| **71280** | | | |
| **Y1771** | Yeast Synthetic Drop-Out Medium Supplements | Powder | Supplements for rich medium formulations. Used to increase yield, growth rate, and the probability of successful yeast transformations in screening libraries and genetic knock-outs. Available in a variety of formulations. |
| **Y1896** | | | |
| **Y2021** | | | |
| **Y2146** | | | |

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## Application Data

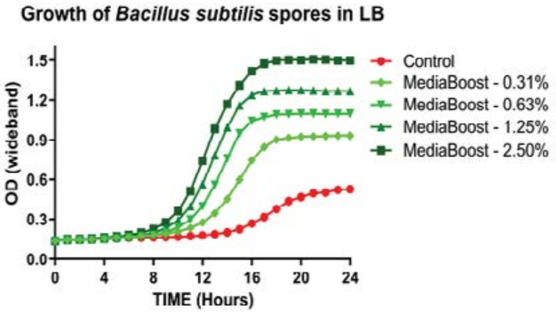
### Compatibility of MediaBoost with Relevant Species and Culture Media.

(✓) indicates that a relative increase in growth was observed when compared to media without MediaBoost, and (x) indicates that no growth improvement was observed with MediaBoost. Colored cells indicate media and species not tested together with MediaBoost.

| Microbe/ Media Type | LB | 1/3 LB | BM2 | M9 | IMDM | DMEM | YPD | 1/3 YPD | MRS | 1/3 MRS |
|---|---|---|---|---|---|---|---|---|---|---|
| *Bacillus subtilis* | ✓ | | | | ✓ | ✓ | | | | |
| *Lactobacillus rhamnosus* | ✓ | ✓ | x | x | | ✓ | | | ✓ | ✓ |
| *Lactobacillus reuteri* | | | | | ✓ | | | | ✓ | ✓ |
| *Lactobacillus salivarius* | | | | | | | | | ✓ | ✓ |
| *Bacillus coagulans* | | | | | ✓ | | | | | |
| *Lactococcus lactis* | | | | | ✓ | | | | | |
| *Escherichia coli* | x | | | | ✓ | | | | | |
| *Saccharomyces cerevisiae* | x | | ✓ | ✓ | ✓ | | ✓ | ✓ | | |
| *Pseudomonas aeruginosa* | x | | | ✓ | | | | | | |

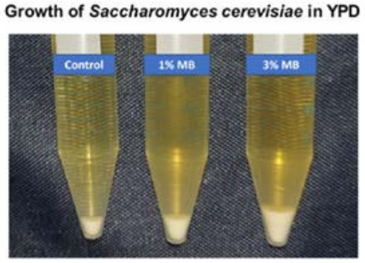
### Growth of *Bacillus subtilis* Spores in LB

*Bacillus subtilis*, a gram-positive species, were cultured in LB media with increasing concentrations of MediaBoost. An untreated neat culture served as a negative control. Data points represent means of triplicate cultures and S.D. did not exceed 10% of mean.




### Growth of *Saccharomyces cerevisiae* in YPD

*Saccharomyces cerevisiae* were cultured in YPD media for 24 hours at 32°C in a shaker flask, with 1% (middle tube) or 3% (right tube) MediaBoost (MB). An untreated neat culture served as a negative control (left tube). Aliquots were removed and tubes were centrifuged at high speed to obtain pellets.



# Cloning & Expression

## Transfection Reagents

High-efficiency transfection in nearly any cell type is achievable with the wide variety of reagents that we offer. Our portfolio covers a variety of popular transfection reagents, including our Escort™ lipid reagents, RiboJuice™ reagents , and GeneJuice® transfection reagents.

| Cat. Number | Transfection Reagent | Application |
|---|---|---|
| **70967** | GeneJuice® Transfection Reagent | Non-lipid based chemical transfection reagent optimized for maximum transfection efficiency, ease-of-use, and minimal cytotoxicity on a wide variety of mammalian cells. |
| **TR-1013** | RiboJuice™ mRNA Transfection Kit | Formulated specifically for the delivery of larger RNA species into mammalian cells with high efficiencies and low toxicities. |
| **71115** | RiboJuice™ siRNA Transfection Kit | Efficiently delivers siRNA into a wide range of mammalian cell lines for targeted gene suppression. |
| **71281** | ProteoJuice™ Protein Transfection Reagent | An effective reagent for the transfection of intact functional protein and peptides into mammalian cells with minimal toxicity and broad cell specificity. |
| **72181** | 293-Free™ Transfection Reagent | Animal-free polycationic liposomal transfection reagent optimized for the transfection of HEK293 cells grown in suspension culture. |

| Cat. Number | Transfection Reagent | Application |
|---|---|---|
| **L3037** | Escort™ III Transfection Reagent | Unique formulation of a proprietary polycationic lipid and a neutral non-transfecting lipid. This liposome-forming compound is used for transfection of nucleic acids into primary cells. |
| **L3287** | Escort™ IV Transfection Reagent | Unique formulation of a proprietary polycationic lipid and a neutral non-transfecting lipid. This liposome-forming compound is used for transfection of nucleic acids into a wide variety of eukaryotic cell types. |
| **TR-1003** | Polybrene Transfection Reagent | A cationic polymer that can greatly enhance the efficiency of the retroviral or lentiviral infection to mammalian cells. |
| **71259** | Insect GeneJuice® Transfection Reagent | Optimized for maximal transfection efficiency of Sf9 insect cells for baculovirus protein expression. |

Transfection

# Cloning & Expression

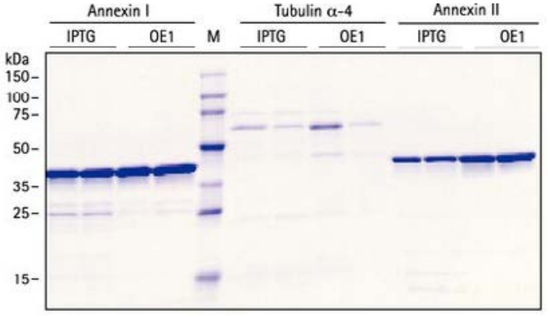
## Expression Essentials

## Overnight Express™ Platform

Simplify bacterial protein expression and increase soluble protein yields using the Overnight Express™ Autoinduction System. Simply prepare, inoculate, incubate, and harvest. With Overnight Express™, save time and drive your protein research forward. LB, TB, and complete systems are available.

- Effortless protein expression in *E. coli* without the need for monitoring or induction
- Convenient for routine expression of proteins in multiple cultures or for high-throughput parallel analysis
- High cell densities and protein expression levels

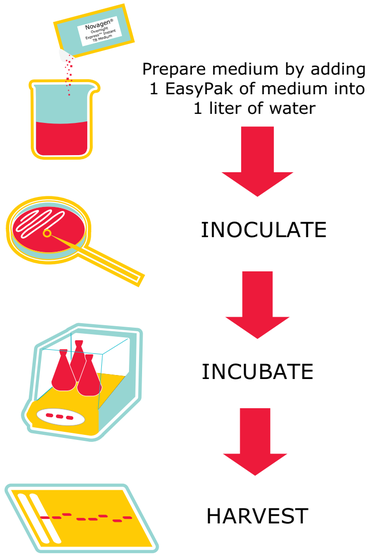
| Cat. No. | Product Name | Application |
|---|---|---|
| **71300-M** | Overnight Express™ Autoinduction System 1 | Allows the induction of protein expression without monitoring cell density and without conventional induction with isopropyl β-D-1-thiogalactopyranoside (IPTG). |
| **71757-M** | Overnight Express™ Instant LB Medium | A complete granulated autoinduction culture medium for high-level protein production in pET and other IPTG-inducible bacterial expression systems. |
| **71491** | Overnight Express™ Instant TB Medium | A complete granulated autoinduction culture medium for high-level protein production in pET and other IPTG-inducible bacterial expression systems. |




| Lanes | Sample volume |
|---|---|
| Annexin I | 4 µL |
| Tubulin a-4 | 4 µL |
| Annexin II | IPTG, 8 µL; OE1, 4.5 µL |
| **Lanes** | **Sample** |
| IPTG | IPTG induction |
| OE1 | Overnight Express™ Autoinduction System 1 |
| M | Perfect Protein™ Markers, 15-150 kDa |

Better yields, better nights' sleep with Overnight Express™ autoinduction. pET recombinants encoding the indicated His Tag® fusion proteins were transformed into BL21(DE3) cells. For Overnight Express System 1 induction, 5 mL medium was inoculated with a single colony and incubated overnight (~16 h) at 30 °C with shaking. For IPTG induction, 5 mL medium was inoculated with a single colony and incubated at 16 °C with shaking to an average $OD_{600}$ of 1.0. IPTG was added to 1 mM final concentration and incubated an additional 16 h. Proteins were purified and then analyzed by SDS-PAGE and Coomassie® blue staining.

**Overnight Express™ Protocol**



# Cloning & Expression

## Molecular Biology Essentials

From setup to clean up, and every step in between, we've got you covered.

| Cat. No. | Molecular Biology Reagents | Key Features & Application |
|---|---|---|
| **R6513** | Ribonuclease A from bovine pancreas | Highly stable endoribonuclease suitable for removal of RNA, RNA sequencing, and DNA purification applications |
| **P1585** | PMA | Activator of protein kinase C (PKC) used in a variety of molecular and cellular biology research |
| **D8418** | Dimethyl Sulfoxide | A highly polar, aprotic organic solvent with many applications in organic chemistry and molecular biology |
| **I9616** | 2-Propanol | Suitable for use in DNA precipitation using standard protocols and as a solvent for making solutions for molecular biology applications |
| **W4502** | Water | 0.1 µm filtered, suitable for molecular biology applications. Analyzed for the absence of nucleases and proteases and has undergone bioburden analysis |

| Cat. No. | Molecular Biology Reagents | Key Features & Application |
|---|---|---|
| **B0300** | Betaine solution | PCR enhancing reagent that is widely used for improving the yield and specificity of PCR products, especially targets rich in GC content or those that form secondary structures resulting in poor yield |
| **R2020** | RNaseZAP™ reagent | Purifying agent used for eliminating RNase contamination from glassware, plastic surfaces, reaction vessels, countertops and pipettors |
| **D9542** | DAPI | Cell permeable, fluorescent dye that binds to DNA, several times more sensitive than ethidium bromide for agarose gel staining |
| **F3917** | Forskolin | Cell-permeable diterpenoid that possesses anti-hypertensive, positive inotropic, and adenylyl cyclase activating properties |
| **F2637** | Ficoll® 400 reagent | Non-ionic synthetic polymer of sucrose used for cell separation and organelle isolation |

# Molecular Essentials Guide Workflow



## Nucleic Acid Purification

The purity, quality, and quantity of your nucleic acid samples depend on reliable nucleic acid purification reagents and kits. We offer a variety of suitable nucleic acid purification essentials for your sample needs, including reagents and kits for gDNA, RNA, and plasmid purification, single spin purification, nucleic acid gel extraction, PCR clean-up, and more. Explore our comprehensive offering to maximize the reproducibility of your results and ensure that your purification needs are met day after day.

- Essentials
- Gold-Standard Kits
- Sustainable Solutions
- Gel Electrophoresis Essentials

# Nucleic Acid Purification

## Essentials

### For General Molecular Needs:

**Sample stabilization and isolation: Protect your precious samples and optimize your yield.**

| Product | Application |
|---|---|
| RNAaseZAP™ Reagent | Keep your surfaces, racks and other implements clean by removing RNases. |
| Rnase AWAY® Reagent | Remove RNases from surfaces with this ready-to-use solution |
| **Save it for later! Keep your samples in top shape until you can process them.** | |
| RNAlater® Reagent | Stabilize and protect RNA with immediate RNAse inactivation |
| GenElute™-E Tissue Stabilizer | Stabilize your tissue samples prior to DNA or RNA purification |

**Keep it clean! Decontamination reagents can save your experiments.**

### Breaking it down: Proteinase K options to fit your needs

| Proteinase K | Product Name | Cat. No. | Concentration or Activity | Key Features |
|---|---|---|---|---|
| Liquid Formulations | Proteinase K from *Tritirachium album* | **P5568** | ≥10 mg/mL<br>≥500 units/mL | |
| | Proteinase K from *Tritirachium album* | **P4850** | ≥10 mg/mL<br>≥800 units/mL | Tested for DNAse, RNAse, endonuclease and nickase |
| | Proteinase K from *Tritirachium album* | **SRE0005** | ≥10 mg/mL protein<br>≥800 units/mL | Tested for DNAse, RNAse, endonuclease and nickase<br>Designed under ISO13485 |
| | Proteinase K | **71049-M** | ≥ 600 mAnsonU/mL | Tested for DNAse, RNAse, endonuclease, nickase, and bioburden<br>Highest Quality Level |
| Lyophilized Powders | Proteinase K from *Tritirachium album* | **P2308** | ≥30 units/mg protein | Tested for DNAse, RNAse, endonuclease and nickase |
| | Proteinase K from *Tritirachium album* | **P6556** | ≥30 units/mg protein | Water soluble |
| | Proteinase K from *Tritirachium album* | **SRE0047** | ≥ 30.0 mAnsonU/mg | Tested for DNAse, RNAse, endonuclease and nickase<br>Highest Quality Level |
| | Proteinase K | **70663** | ≥ 30.0 mAnsonU/mg | Tested for DNAse, RNAse, endonuclease and nickase |

# Nucleic Acid Purification

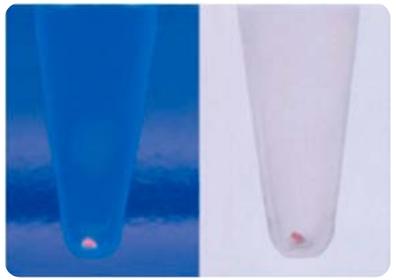
## Essentials

## For General Molecular Needs:

**Make the most of your samples: TRI Reagent® allows for isolation of DNA, RNA & protein from a single sample, allowing for more data and less effort. RNAzol® isolates small RNA.**

| Cat. No. | Product Name | Sample Type |
|---|---|---|
| **T9424** | TRI Reagent® solution - For processing tissues, cells cultured in monolyer or cell pellets | Tissues & Cells |
| **93289** | TRI Reagent® solution - For DNA, RNA and protein isolation | Various: cells, tissue, yeast, plant, etc. |
| **T3809** | TRI Reagent® BD solution - For processing whole blood plasma or serum | Blood, Plasma and Serum |
| **T3934** | TRI Reagent® LS solution - For processing fluid samples such as cell suspensions, CSF, and amniotic fluid | Fluid Samples |
| **R4533** | RNAzol® RT solution - For processing total and small RNA from human, animal, plant, bacterial, and viral samples | Total small RNA from various samples |

| Cat. No. | Product Name | Process | Key Features |
|---|---|---|---|
| **70748** | Pellet Paint® NF Co-Precipitant | Carrier for Nucleic Acid Precipitation | Non-fluorescent visible DNA co-precipitant |
| **P2069** | Phenol:Chloroform:Isoamyl Alcohol 25:24:1 Saturated with 10 mM Tris, pH 8.0, 1 mM EDTA | P:C:I for RNA and DNA isolations | pH 6.5-6.9 without buffer, pH 7.8-8.2 with buffer |
| **P3803** | Phenol:Chloroform:Isoamyl Alcohol 25:24:1, Saturated with 10 mM Tris, pH 8.0, 1 mM EDTA | | pH 6.5-6.9 |
| **C0549** | Chloroform:Isoamyl alcohol 24:1 | Chloroform:Isoamyl Alcohol for DNA isolations and more | 0.6-1.0% ethanol as stabilizer |
| **P4557** | Phenol solution | Phenol solutions for DNA extractions, plasmid isolations, RNA preps and more | pH 7.7-8.1 equilibrated |
| **P4682** | Phenol solution | | pH 4.1-4.5 equilibrated |



Pellet Paint® NF Co-Precipitant non-fluorescent visible
DNA co-precipitant for automated sequencing applications

# Nucleic Acid Purification

## Sustainable Solutions

### GenElute™-E Single Spin Isolation Kits Selection Guide

Reduce hands-on time from 1 hour or more, to just 3 minutes. GenElute™-E single spin purification kits offer up to a 55% reduction in plastic waste for greener purifications.

| Viral RNA/DNA | Blood | | Tissue | Cells | Plant | | Clean Up | |
|---|---|---|---|---|---|---|---|---|
| EC810/EC848/EC896#* | EC100/EC196* | EC200 | EC300/EC396* | EC400 | EC500/EC596* | EC600 | EC700 | EC800 |
| Nasopharyngeal swabs in transport media | Blood | Blood high yield | Tissue | Cell Culture | Plant | DNA | DNA with organic solvents | RNA |

**Safer use***

No hazardous liquid waste by eliminating bind and wash steps containing ethanol and chaotropic salts.

**Sustainable packaging**

Box and insert with sustainable forestry certification and more than 70% of recycled content. Starch-based, compostable bags for kit components.

**Better usability**

Simplified workflow with fewer steps.

**Waste prevention**

55% reduction of the consumption of plastic consumables (tubes, pipet tips).

Comparison of waste generation of traditional silica-based kits (top) and GenElute™-E kits (bottom).

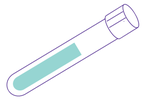
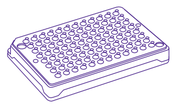
#Viral RNA/DNA kit has some chaotrophic salts

*High-throughput options available for increased efficiencies.

# Nucleic Acid Purification

## Gold-Standard Kits

### GenElute™ Kits, Spectrum™ Kits, and Montage® Kits: Classic Solutions

| | Plasmid | DNA | RNA | Clean-up |
|---|---|---|---|---|
| |  |  |  |  |
| **Mini** |  |  |  |  |
| **Midi** |  | | | |
| **Maxi** |  | | | |
| **Plate** |  | | | |

# Nucleic Acid Purification

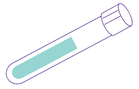
## Gold-Standard Kits

## GenElute™ Kits and Montage® Kits: Classic Solutions

### Plasmid

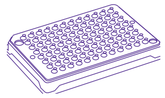
**Mini**

**Midi**



**Maxi**

**Plate**



| Plasmid Prep Scale | Product Name | Cat. No. | Key Features |
|---|---|---|---|
| Mini | GenElute™ Plasmid Miniprep Kit | **PLN70/PLN350** | 70 or 350 purification kits available<br>Yield: up to 15 µg |
| | GenElute™ HP Plasmid Miniprep Kit | **NA0150/NA0160** | Ultra-fast processing<br>70 or 350 purification kits available<br>Yield: up to 25 µg |
| | Montage® Plasmid Miniprep96 kit | **LSKP096** | 96 well plate format<br>Plasmids & BAC isolation |
| | GenElute™ HP 96-Well Plasmid Miniprep Kit | **NA9604** | 4 96-well purifications<br>Yield: up to 10 µg |
| Midi | GenElute™ Plasmid Midiprep Kit | **PLD35** | 35 purifications<br>20-40 mL prep<br>Yield: up to 300 µg |

| Plasmid Prep Scale | Product Name | Cat. No. | Key Features |
|---|---|---|---|
| Maxi | GenElute™ Plasmid Maxiprep Kit | **PLX15** | 15 purifications<br>25-200 mL prep<br>Yield: up to 1.2 mg |
| | GenElute™ HP Plasmid Maxiprep Kit | **NA0300/NA0310** | 10 or 25 purification kits available<br>Rapid lysate clearing step<br>150 mL prep<br>Yield: up to 1.2 mg |
| | GenElute™ Endotoxin-free Plasmid Maxiprep Kit | **PLEX15** | 15 purifications<br>5-40 mL prep<br>Yield: up to 250 µg with endotoxin ≤0.1EU/µg |
| | GenElute™ HP Endotoxin-Free Plasmid Maxiprep Kit | **NA0400/NA0410** | 10 or 25 purification kits available<br>150 mL prep<br>Yield: up to 1.2 mg with endotoxin ≤0.1EU/µg |
| Mega | GenElute™ HP Endotoxin-Free Plasmid Megaprep Kit | **NA0600** | 5 purifications<br>600 mL-1.2 L prep<br>Yield: up to 15 mg with endotoxin ≤0.1EU/µg |
| Giga | GenElute™ HP Select Plasmid Gigaprep Kit | **NA0800** | 5 purifications<br>200 mL-1 L prep<br>Yield: up to 5 mg with endotoxin ≤0.1EU/µg |

# Nucleic Acid Purification

## Gold-Standard Kits

### GenElute™ Kits: Classic DNA Solutions

#### Genomic DNA

#### Mini

| Sample Type | Product Name | Cat. No. | Key Features |
|---|---|---|---|
| Mammalian: cells, tissues, blood | GenElute™ Mammalian Genomic DNA Miniprep Kits | **G1N70/G1N350** | 70 or 350 preparation kits available<br>Yield: up to 25 µg ( from 2 x $10^6$ cultured cells; 30 µg from 25 mg of tissue) |
| Blood | GenElute™ Blood Genomic DNA Kit | **NA02010/NA2020** | 70 or 350 preparation kits available<br>Yield: up to 10 µg from 200 µL |
| Bacterial Genomic | GenElute™ Bacterial Genomic DNA Kits | **NA2110/NA2120** | 70 or 350 preparation kits available<br>Yield: up to 20 µg from 1.5 mL of culture |
| Plant | GenElute™ Plant Genomic DNA Miniprep Kit | **G2N70/G2N350** | 70 or 350 preparation kits available<br>Yield: up to 20 µg from 100 mg plant tissue |

# Nucleic Acid Purification

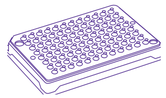
## Gold-Standard Kits

### GenElute™ Kits and Spectrum™ Kits: Classic RNA Solutions

**RNA**

**Mini**

**Plate formats**



| Sample Type | Product Name | Cat. No. | Key Features |
|---|---|---|---|
| Mammalian | GenElute™ Mammalian Total RNA Miniprep Kit | **RTN70/RTN350** | 70 or 350 preparation kits available<br>Isolate from as few as 100 cells up $10^7$ or 40 mg tissue<br>Yield: up to 150 µg |
| | GenElute™ 96 Well Total RNA Purification Kit | **RTN9604** | 4 96-well purifications<br>Yield: up to 100 µg (dependent on sample type) |
| Viral: Saliva, viral transport media, cell suspension, cell culture media. | GenElute™ Viral RNA Miniprep Kit | **RNV100** | 70 or 350 preparation kits available<br>For Viral RNA purification |
| Plants | Spectrum™ Plant Total RNA Kit | **STRN50/ STRN250** | 50 or 250 preparation kits available<br>Yield: up to 60 µg from 100 mg of tissue<br>30 minute or less prep<br>Works with difficult plant tissues |

# Nucleic Acid Purification

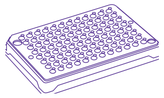
## Gold-Standard Kits

### GenElute™ Kits and Montage® Kits: Classic Clean-Up Solutions

**Clean-Up**

**Mini**

**Plate formats**



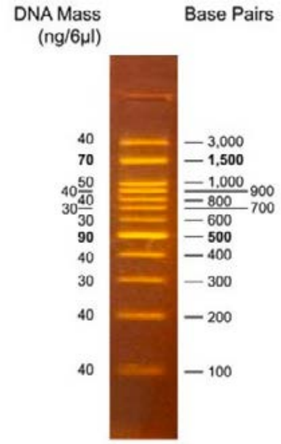
| Sample Type | Product Name | Cat. No. | Key Features |
|---|---|---|---|
| PCR Clean-Up | GenElute™ PCR Clean-Up Kit | **NA1020** | 70 preparations<br>Yield: Purifies up to 100 µL or 10 µg of PCR amplified DNA<br>Recovers up to 95% of PCR products between 100 bp and 10 kb |
| | | **LSKMPCR** | |
| | | **MSNU030** | |
| | GenElute™ 96 Well PCR Clean-Up Kit | **PCR9604** | 4 96-well purifications<br>Recovers 75-90% of of PCR products between 100 bp and 10 kb |
| Gel Extraction | GenElute™ Gel Extraction Kit | **NA1111** | 70 preparations<br>Yield: Binds up or 10 µg of DNA<br>Recovers up to 80% of DNA in gel slices up to 3.5g |
| | Montage® Gel Extraction Kit | **LSKGEL050** | 50 preparations<br>Recovers up to 75%<br>Unique gel nebulizer extracts DNA |
| Sequencing | Montage® SEQ96 Sequencing Reaction Cleanup Kit | **LSKS096** | 1, 4 and 24 preparation plate kits available |

# Nucleic Acid Purification

## Gel Electrophoresis Essentials

### Solutions To Save Time For Quick Results

| Electrophoresis Need | Product Name | Cat. No. | Key Features |
|---|---|---|---|
| Safe Gel Stains | GelRed® Nucleic Acid Stain 3X Water | **SCT121** | Sensitive, stable, and environmentally friendly |
| | GelRed® Nucleic Acid Stain 10000X DMSO | **SCT122** | Sensitive, stable, and environmentally friendly |
| | GelRed® Nucleic Acid Stain 10000X Water | **SCT123** | Sensitive, stable, and environmentally friendly |
| | GelGreen® Nucleic Acid Stain 10000X DMSO | **SCT124** | Sensitive, stable, and environmentally friendly |
| | GelGreen® Nucleic Acid Stain 10000X Water | **SCT125** | Sensitive, stable, and environmentally friendly |
| Ready-to-Use DNA Ladders | DirectLoad™ Plus 100bp High DNA Ladder | **DPLUS100H** | 12 fragments<br>From 3000bp to 100bp<br>Reference bands at 500bp and 1500bp |
| | DirectLoad™ Plus 100bp High DNA Ladder with fluorescent DNA stains | **DPLUS100HS** | 12 fragments<br>From 3000bp to 100bp<br>Reference bands at 500bp and 1500bp |
| | DirectLoad™ Plus 100bp DNA Ladder | **DPLUS100** | 11 fragments<br>From 1500bp to 100bp<br>Reference bands at 500bp and 1500bp |
| | DirectLoad™ Plus 1kb DNA Ladder | **DPLUS1K** | 12 fragments<br>From 10,000bp to 100bp<br>Reference bands at 1000bp and 3000bp |
| | DirectLoad™ Plus 1kb DNA Ladder with fluorescent DNA stains | **DPLUS1KS** | 12 fragments<br>From 10,000bp to 100bp<br>Reference bands at 1000bp and 3000bp |




1.5 % TBE agarose gel

DPLUS100HS DirectLoad™ Plus 100bp High DNA Ladder with fluorescent DNA stains.

**Explore our gel reagents and casting systems here**

# Gel Electrophoresis Essentials

## DirectLoad™ Electrophoresis Systems: CAST LOAD RUN

### Gel Systems – what's included

**Loading Guides**

Adhesive stickers can be attached to the tank for increased visibility

**Gel Tray**

Different sizes available:

Mini: 7x7 or 7x10 cm

Midi: 15x7, 15x10 or 15x15 cm

**Easy-Click Lid**

Single orientation assembly

Auto-disconnects power supply upon removal


**Gel Tank**

Color-coded for single orientation assembly

Mini and Midi sized tank options

**Gel Combs**

Height adjustable; invert to use as loading guide.

**Gel Tray Dams**

Leak-proof design

Gel electrophoresis is used for separating molecules, such as protein, DNA, and RNA, by their size. Enhance your nucleic acid gel electrophoresis workflow with our DirectLoad™ Mini and Midi Horizontal Electrophoresis Systems.

Buffers, ladders, loading dyes, and stains complement a compellingly economical solution for agarose gel electrophoresis. Featuring unprecedented sample throughput and experimental versatility, explore the full list of offerings within the DirectLoad™ lineup of products and optimize your nucleic acid research.

Millipore®
Preparation, Separation,
Filtration & Monitoring Products

# Gel Electrophoresis Essentials

## DirectLoad™ Electrophoresis Systems

### Gel Electrophoresis: Modular Electrophoresis Systems and components

| | | DirectLoad™ Mini Horizontal Electrophoresis Systems DMINI | DirectLoad™ Midi Horizontal Electrophoresis Systems DMIDI |
|---|---|---|---|
| **Product Description** | | **Replacement Cat. No.** | **Replacement Cat. No.** |
| Tank (with wired electrodes) |  | DMS7-TANK | DMS15-TANK |
| Lid |  | DMS7-LID | DMS15-LID |
| Trays |  | 7x7 cm: DMS7-TR7<br>7x10 cm: DMS7-TR10 | 15x7 cm: DMS15-TR7<br>15x10 cm: DMS15-TR10<br>15x15 cm: DMS15-TR15 |

| | | DirectLoad™ Mini Horizontal Electrophoresis Systems DMINI | DirectLoad™ Midi Horizontal Electrophoresis Systems DMIDI |
|---|---|---|---|
| **Product Description** | | **Replacement Cat. No.** | **Replacement Cat. No.** |
| Gel Tray Dams |  | DMS7-DAMS | DMS15-DAMS |
| Combs |  | Multiple Sizes available at **SigmaAldrich.com** | |
| Loading Guides (stickers) | | DMS7-LG | DMS15-LG |
| Electrophoresis cables (same for both) |  | DMS-CABLES | DMS-CABLES |

Millipore®
Preparation, Separation,
Filtration & Monitoring Products

Explore all of our gel reagents and casting systems here

# Molecular Essentials Guide Workflow

Cloning & Expression | Nucleic Acid Purification | PCR & NGS | CRISPR/Genome Engineering

## PCR & NGS

- PCR Essentials
- RT-PCR Essentials
- NGS Essentials
- Isothermal Amplification Essentials

**Key to Icons:** Home Page | Respective Page | External weblink | More Information | Close

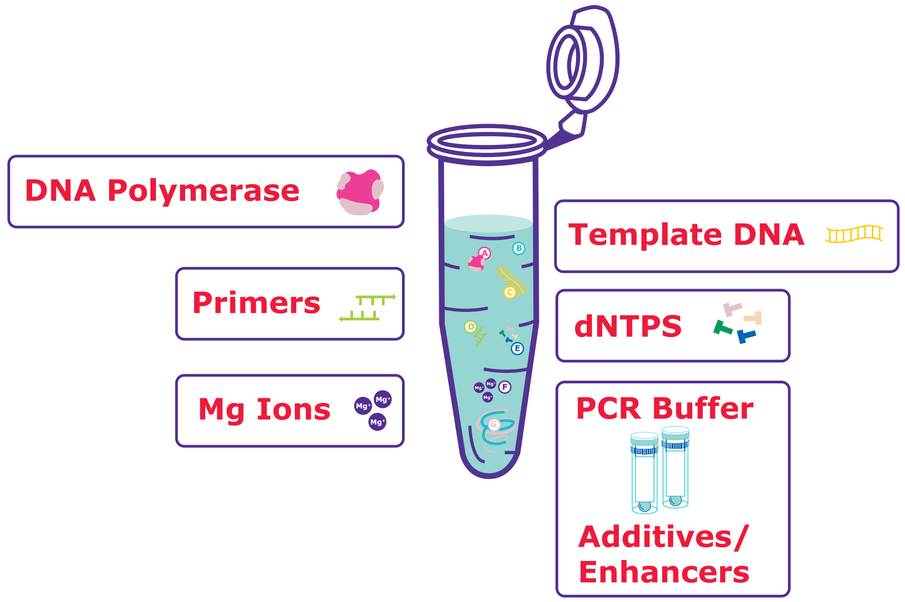
# PCR & NGS

## PCR Essentials

### Nucleic Acid Amplification

PCR consists of repeated cycles of denaturation, annealing, and extension where target sequences are amplified exponentially. A typical PCR reaction mix includes components such as the template, the forward and reverse primers, the polymerase enzyme with a preferred reaction buffer, dNTPs, and optional additives to help boost your PCR. It is important to note that all of these components and the cycle conditions can determine the success of a PCR. Explore our comprehensive PCR essentials and select the right components while designing your assay to achieve optimum results. Click on each element to learn more.

### PCR Ecosystem



# PCR & NGS

## PCR Essentials

### Our broad PCR portfolio offers a variety of application options.

| | Type of PCR | Customer Need | Application |
|---|---|---|---|
| S Standard | Standard | Simple detection (Yes/No) | Cloning, colony PCR screening |
| HS Hot Start | Hot Start | Room temp set-up without non-specific amplification | Genotyping, clinical applications where non-specific amplification needs to be reduced |
| HF High Fidelity | High Fidelity | Target DNA must be accurate after amplification | Cloning, mutation detection, NGS |
| GC GC-rich | GC Rich | Amplify targets with high GC content | Analysis of certain clinical samples, environmental samples and plant samples with high GC content |
| L Long-range | Long PCR | Synthesize long fragments >20kb | Amplification of eDNA, certain genomic and mitochondrial DNA for biodiversity studies, phylogenetic studies, population genetics etc |
| M Multiplex | Multiplex PCR | Amplify multiple targets at one go | Amplification of multiple DNA targets in a single reaction tube, saving time and resources |
| D Direct | Direct PCR | Sample-to-answer without purification | Crude sample analysis such as blood, saliva, plant samples. Save time, cost & effort. |

Based on the type of application, we offer a broad portfolio of PCR products to meet various research needs. For example, standard PCR, or end-point PCR, is used for simple detection to get a Yes or No answer and is widely used for applications such as gene cloning. Hot start PCR, wherein the Taq DNA polymerase is inactive at room temperature, allows room temperature reaction setup and prevents non-specific amplification. High-fidelity PCR is useful when accuracy is paramount, especially in applications such as NGS. Similarly, we have variations of the DNA polymerase or the buffer formulation to address the need for long PCR where templates greater than >20kb are amplified. Additionally, we offer reagents to enable multiplex PCR, where multiple targets can be amplified simultaneously to save time and resources. Our direct PCR products also address the need for crude sample analysis, where the DNA/RNA is extracted is ready to be amplified directly without any purification step.

# PCR & NGS

## PCR Essentials

## Selector guide based on the type of PCR


### Simple end-point detection (Yes/No)

| | Cat. No. | Easy $MgCl_2$ Optimization | Direct Load | Mastermix | Fidelity compared to Std Taq | Amplicon Size* | Includes separate dNTP mix |
|---|---|---|---|---|---|---|---|
| Taq DNA Polymerase | **D1806** | | | | 1× | 0.1 to >3 (5) | |
| Taq DNA Polymerase without $MgCl_2$ | **D4545** | ✓ | | | 1× | 0.1 to >3 (5) | |
| REDTaq® DNA Polymerase | **D4309** | | ✓ | | 1× | 0.1 to >3 (5) | |
| REDTaq® DNA Polymerase SuperPak™ Reagent | **D6063** | | ✓ | | 1× | 0.1 to >3 (5) | ✓ |
| ReadyMix™ Taq PCR Reaction Mix | **P4600** | | | ✓ | 1× | 0.1 to >3 (5) | |
| REDTaq® ReadyMix™ PCR Reaction Mix with $MgCl_2$ | **R2523** | | ✓ | ✓ | 1× | 0.1 to >3 (5) | |

*Two values are provided for the indicated amplification length. The range provided refers to the average length achieved from complex genomic targets, while the number in parentheses refers to lengths routinely achieved with less complicated targets such as plasmid or lambda phage DNA.

# PCR & NGS

## PCR Essentials

## Selector guide based on the type of PCR


### Room temp set-up without non-specific amplification

| | Cat. No. | Easy $MgCl_2$ Optimization | Direct Load | Mastermix | Fidelity compared to Std Taq | Amplicon Size* | 3′→5′ Exonuclease Activity | Lyo-Ready |
|---|---|---|---|---|---|---|---|---|
| JumpStart™ Taq DNA Polymerase | **D9307** | | | | 1× | 0.1 to >3 (10) | | |
| JumpStart™ Taq DNA Polymerase without $MgCl_2$ | **D4184** | ✓ | | | 1× | 0.1 to >3 (10) | | |
| JumpStart™ REDTaq® DNA Polymerase | **D8187** | | ✓ | | 1× | 0.1 to >3 (10) | | |
| JumpStart™ Taq ReadyMix™ | **P2893** | | | ✓ | 1× | 0.1 to >3 (10) | | |
| JumpStart™ REDTaq® ReadyMix PCR Reaction Mix | **P0982** | | ✓ | ✓ | 1× | 0.1 to >3 (10) | | |
| JumpStart™ REDTaq® ReadyMix For High Throughput PCR | **P1107** | | ✓ | ✓ | 1× | 0.1 to >3 (10) | | |
| JumpStart™ AccuTaq™ LA DNA Polymerase | **D5809** | | | | up to 6.5× | 0.1 to >20 (40) | ✓ | |
| JumpStart™ REDAccuTaq™ LA DNA Polymerase | **D1313** | | ✓ | | up to 6.5× | 0.1 to >20 (40) | ✓ | |
| Glycerol-free JumpStart™ Taq DNA Polymerase | **D9310** | | | | 1× | 0.1 to >3 (10) | | ✓ |

*Two values are provided for the indicated amplification length. The range provided refers to the average length achieved from complex genomic targets, while the number in parentheses refers to lengths routinely achieved with less complicated targets such as plasmid or lambda phage DNA.

# PCR & NGS

## PCR Essentials

## Selector guide based on the type of PCR


### Highly accurate amplification

| | Cat. No. | Direct Load | Mastermix | Fidelity compared to Std Taq | Amplicon Size (kb)* | 3′→5′ Exonuclease Activity | Includes dNTPs | Ultra-fast |
|---|---|---|---|---|---|---|---|---|
| AccuTaq™ LA DNA Polymerase | **D8045** | | | up to 6.5× | 0.1 to >20 (40) | ✓ | | |
| REDAccuTaq® LA DNA Polymerase | **D4812** | ✓ | | up to 6.5× | 0.1 to >20 (40) | ✓ | | |
| JumpStart™ AccuTaq™ LA DNA Polymerase | **D5809** | | | up to 6.5× | 0.1 to >20 (40) | ✓ | | |
| JumpStart™ REDAccuTaq™ LA DNA Polymerase | **D1313** | ✓ | | up to 6.5× | 0.1 to >20 (40) | ✓ | | |
| KOD Xtreme™ Hot Start DNA Polymerase | **71975-M** | | | 10× | 0.1 to >24 (40) | ✓ | ✓ | |
| KOD Hot Start DNA Polymerase | **71086** | | | 80× | 0.1 to >12 (21) | ✓ | ✓ | |
| KOD Hot Start Master Mix | **71842** | | ✓ | 80× | 0.1 to >12 (21) | ✓ | ✓ | |
| KOD One™ PCR Master Mix | **KMM-101NV** | | ✓ | 80× | 0.1 to 40 | ✓ | ✓ | ✓ |
| KOD One™ PCR Master Mix -BLUE | **KMM-201NV** | ✓ | ✓ | 80× | 0.1 to 40 | ✓ | ✓ | ✓ |

*Two values are provided for the indicated amplification length. The range provided refers to the average length achieved from complex genomic targets, while the number in parentheses refers to lengths routinely achieved with less complicated targets such as plasmid or lambda phage DNA.

# PCR & NGS

## PCR Essentials

## Selector guide based on the type of PCR


### Amplify targets with high GC content

| | Cat. No. | Direct Load | Mastermix | Fidelity compared to Std Taq | Amplicon Size (kb)* | 3′→5′ Exonuclease Activity | Includes dNTPs | GC content |
|---|---|---|---|---|---|---|---|---|
| KOD Xtreme™ Hot Start DNA Polymerase | **71975-M** | | | 10x | 0.1 to >24 (40) | ✓ | ✓ | Up to 90% |
| KOD One™ PCR Master Mix | **KMM-101NV** | | ✓ | 80x | 0.1 to 40 | ✓ | ✓ | Up to 70% |
| KOD One™ PCR Master Mix -BLUE | **KMM-201NV** | ✓ | ✓ | 80x | 0.1 to 40 | ✓ | ✓ | Up to 70% |

*Two values are provided for the indicated amplification length. The range provided refers to the average length achieved from complex genomic targets, while the number in parentheses refers to lengths routinely achieved with less complicated targets such as plasmid or lambda phage DNA.

# PCR & NGS

## PCR Essentials

## Selector guide based on the type of PCR


### Synthesize long fragments (> 20kb)

| | Cat. No. | Direct Load | Mastermix | Fidelity compared to Std Taq | Amplicon Size (kb)* | 3′→5′ Exonuclease Activity | Includes dNTPs | Ultra-fast |
|---|---|---|---|---|---|---|---|---|
| AccuTaq™ LA DNA Polymerase | **D8045** | | | up to 6.5× | 0.1 to >20 (40) | ✓ | | |
| REDAccuTaq® LA DNA Polymerase | **D4812** | ✓ | | up to 6.5× | 0.1 to >20 (40) | ✓ | | |
| JumpStart™ AccuTaq™ LA DNA Polymerase | **D5809** | | | up to 6.5× | 0.1 to >20 (40) | ✓ | | |
| JumpStart™ REDAccuTaq™ LA DNA Polymerase | **D1313** | ✓ | | up to 6.5× | 0.1 to >20 (40) | ✓ | | |
| KOD Xtreme™ Hot Start DNA Polymerase | **71975-M** | | | 10x | 0.1 to >24 (40) | ✓ | ✓ | |
| KOD XL DNA Polymerase | **71087** | | | 3x | 0.1 to 30 | | ✓ | |
| KOD One™ PCR Master Mix | **KMM-101NV** | | ✓ | 80x | 0.1 to 40 | ✓ | ✓ | ✓ |
| KOD One™ PCR Master Mix -BLUE | **KMM-201NV** | ✓ | ✓ | 80x | 0.1 to 40 | ✓ | ✓ | ✓ |

*Two values are provided for the indicated amplification length. The range provided refers to the average length achieved from complex genomic targets, while the number in parentheses refers to lengths routinely achieved with less complicated targets such as plasmid or lambda phage DNA.

# PCR & NGS

## PCR Essentials

## Selector guide based on the type of PCR


### Amplify multiple targets at one go

| | Cat. No. | Direct Load | Mastermix | Fidelity compared to Std Taq | Amplicon Size (kb)* | Mlutiplexing | Includes dNTPs |
|---|---|---|---|---|---|---|---|
| JumpStart™ Taq ReadyMix™ | **P2893** | | ✓ | 1x | 0.1 to >3 (10) | ✓ | ✓ |
| JumpStart™ Taq DNA Polymerase | **D9307** | | | 1x | 0.1 to >3 (10) | ✓ | |
| SYBR® Green JumpStart™ Taq ReadyMix™ | **S4438** | | ✓ | 1x | 0.1 to >3 (10) | ✓ | ✓ |

*Two values are provided for the indicated amplification length. The range provided refers to the average length achieved from complex genomic targets, while the number in parentheses refers to lengths routinely achieved with less complicated targets such as plasmid or lambda phage DNA.

# PCR & NGS

## PCR Essentials

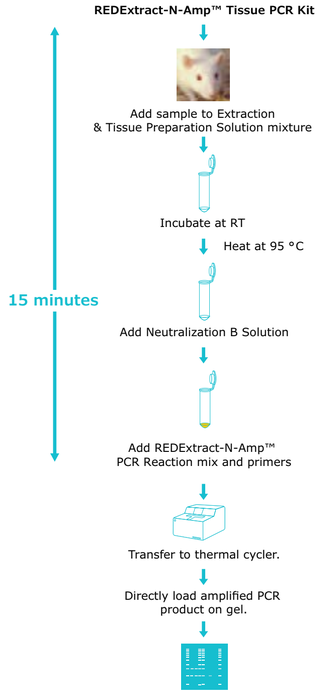
## Selector guide based on the type of PCR

Standard | Hot Start | High Fidelity | GC-rich | Long-range | Multiplex | Direct

### Sample-to-answer without purification

| | Cat. No. | Sample Type | Direct Load | Mastermix | Kit | Includes dNTPs | Amplicon Size (kb)* |
|---|---|---|---|---|---|---|---|
| Direct Tissue PCR | | | | | | | |
| Extract-N-Amp™ Tissue PCR Kit | **XNAT2** | • Mouse-tails | | | ✓ | ✓ | 0.1 to >3 (10) |
| | **XNAT2R** | • Hair | | | ✓ | | |
| REDExtract-N-Amp™ Tissue PCR Kit | **XNAT** | • Animal Tissue | ✓ | | ✓ | ✓ | 0.1 to >3 (10) |
| Extract-N-Amp™ PCR ReadyMix™ | **E3004** | • Saliva | | ✓ | | ✓ | 0.1 to >3 (10) |
| REDExtract-N-Amp™ PCR ReadyMix™ | **R4775** | • Buccal Swabs | ✓ | ✓ | | ✓ | 0.1 to >3 (10) |

*Two values are provided for the indicated amplification length. The range provided refers to the average length achieved from complex genomic targets, while the number in parentheses refers to lengths routinely achieved with less complicated targets such as plasmid or lambda phage DNA.



# PCR & NGS

## PCR Essentials

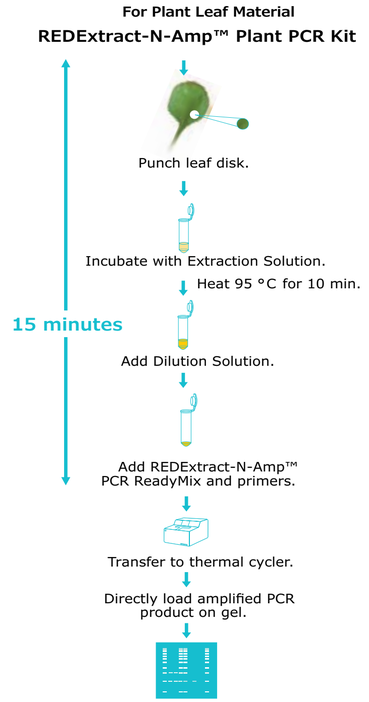
## Selector guide based on the type of PCR

Standard | Hot Start | High Fidelity | GC-rich | Long-range | Multiplex | Direct

### Sample-to-answer without purification

| | Cat. No. | Sample Type | Direct Load | Mastermix | Kit | Includes dNTPs | Amplicon Size (kb)* |
|---|---|---|---|---|---|---|---|
| **Direct Plant PCR** | | | | | | | |
| Extract-N-Amp™ Plant PCR Kit | **XNAP2** | Plant leaves | | | ✓ | ✓ | 0.1 to >3 (10) |
| | **XNAR** | | | | | | |
| | **XNAP2E** | | | | | | |
| REDExtract-N-Amp™ Plant PCR Kit | **XNAPR** | | ✓ | | ✓ | ✓ | 0.1 to >3 (10) |
| | **XNAP** | | | | | | |
| | **XNAPS** | | | | | | |
| | **XNAPE** | | | | | | |
| Extract-N-Amp™ PCR ReadyMix™ | **E3004** | | | ✓ | | ✓ | 0.1 to >3 (10) |
| REDExtract-N-Amp™ PCR ReadyMix™ | **R4775** | | ✓ | ✓ | | ✓ | 0.1 to >3 (10) |

*Two values are provided for the indicated amplification length. The range provided refers to the average length achieved from complex genomic targets, while the number in parentheses refers to lengths routinely achieved with less complicated targets such as plasmid or lambda phage DNA.



# PCR & NGS

## PCR Essentials

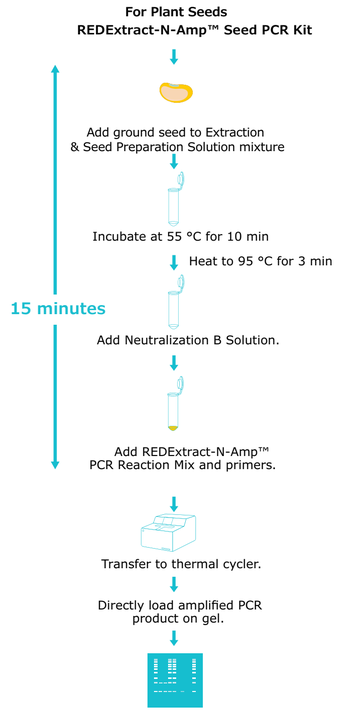
## Selector guide based on the type of PCR

Standard | Hot Start | High Fidelity | GC-rich | Long-range | Multiplex | Direct

### Sample-to-answer without purification

| | Cat. No. | Sample Type | Direct Load | Mastermix | Kit | Includes dNTPs | Amplicon Size (kb)* |
|---|---|---|---|---|---|---|---|
| **Direct Seed PCR** | | | | | | | |
| Extract-N-Amp™ Seed PCR Kit | **XNAS2** | Plant seeds | | | ✓ | ✓ | 0.1 to >3 (10) |
| REDExtract-N-Amp™ Seed PCR Kit | **XNASS** / **XNAS** | Plant seeds | ✓ | | ✓ | ✓ | 0.1 to >3 (10) |
| Extract-N-Amp™ PCR ReadyMix™ | **E3004** | Plant seeds | | ✓ | | ✓ | 0.1 to >3 (10) |
| REDExtract-N-Amp™ PCR ReadyMix™ | **R4775** | Plant seeds | ✓ | ✓ | | ✓ | 0.1 to >3 (10) |

*Two values are provided for the indicated amplification length. The range provided refers to the average length achieved from complex genomic targets, while the number in parentheses refers to lengths routinely achieved with less complicated targets such as plasmid or lambda phage DNA.



# PCR & NGS

## PCR Essentials

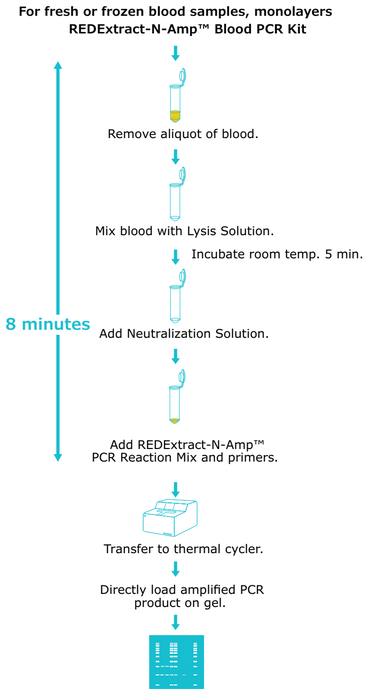
## Selector guide based on the type of PCR

Standard | Hot Start | High Fidelity | GC-rich | Long-range | Multiplex | Direct

### Sample-to-answer without purification

| | Cat. No. | Sample Type | Direct Load | Mastermix | Kit | Includes dNTPs | Amplicon Size (kb)* |
|---|---|---|---|---|---|---|---|
| **Direct Blood PCR** | | | | | | | |
| Extract-N-Amp™ Blood PCR Kit | **XNAB2** | • Whole blood<br>• Whole blood dried on a blood card<br>• Cultured mammalian cells | | | ✓ | ✓ | 0.1 to >3 (10) |
| | **XNAB2R** | | | | | | |
| REDExtract-N-Amp™ Blood PCR Kit | **XNABE** | | ✓ | | ✓ | ✓ | 0.1 to >3 (10) |
| | **XNAB** | | | | | | |
| | **XNABS** | | | | | | |
| | **XNABR** | | | | | | |
| Extract-N-Amp™ PCR ReadyMix™ for Blood | **P8115** | | | ✓ | | ✓ | 0.1 to >3 (10) |
| REDExtract-N-Amp™ PCR ReadyMix™ for Blood | **P8240** | | ✓ | ✓ | | ✓ | 0.1 to >3 (10) |

*Two values are provided for the indicated amplification length. The range provided refers to the average length achieved from complex genomic targets, while the number in parentheses refers to lengths routinely achieved with less complicated targets such as plasmid or lambda phage DNA.



# PCR & NGS

## PCR Essentials

## Selector guide based on the type of PCR


### Sample-to-answer without purification

| | Cat. No. | Sample Type | Stand-alone Reagent | Compatibility | Sensitivity Range |
|---|---|---|---|---|---|
| **Direct RNA Extraction & Stabilization** | | | | | |
| Extract-N-Amp™ Cellular RNA Lysis Buffer | **XNACRL** | Adherent and non-adherent mammalian cell lines | ✓ | qRT-PCR, RT-LAMP, and NGS Library Prep | 10-100k cells |
| Viral RNA Extraction Buffer | **VRE100** | Enveloped viral particles in saliva, saline, Amies medium, and viral transport medium | ✓ | qRT-PCR and RT-LAMP | $1.5x10^3$ in saline to $5x10^4$ in saliva (particles per mL) |

# PCR & NGS

## PCR Essentials

Depending on your assay conditions, choose the right kind of PCR additive to boost your PCR results.

### Essential components for a successful PCR

| | Cat. No. | Key Features |
|---|---|---|
| 10 mM Deoxynucleotide Mix | **D7295** | Building block |
| 25 mM Deoxynucleotide Mix | **D7297** | Building block |
| 100 mM Deoxynucleotide Set (0.25 mL each) | **DNTP100** | Building block |
| 100 mM Deoxynucleotide Set (1 mL each) | **DNTP100A** | Building block |
| Betaine solution (5 M) | **B0300** | Reduces duplex stability and facilitates the GC-rich amplification |
| Magnesium chloride solution (25 mM) | **M8787** | Enhances PCR specificity and yield |
| Magnesium chloride solution (1 M) | **M1028** | Enhances PCR specificity and yield |
| Amplification Grade DNase 1 | **AMPD1** | Digestion of DNA during isolation and purification of RNA |
| JumpStart™ Taq Antibody | **A7721** | Adds hot-start capabilities to any Taq DNA Polymerase |
| Dimethyl sulfoxide | **D8418** | Reduces secondary structures in GC-rich templates |
| Mineral oil | **M8662** | Prevents evaporation of reaction mix |
| Single-strand Binding Protein | **S3917** | Enhances PCR specificity |
| Heat-labile Cod Uracil-DNA Glycosylase | **SRE0111** | Prevents false-positives by eliminating carry-over contamination |
| Heat-labile Cod Uracil-DNA Glycosylase (Glycerol-free) | **SRE0112** | Prevents false-positives by eliminating carry-over contamination |
| Water | **W4502** | Nuclease-free water for dilution |
| 10X PCR Buffer | **P2192** | Optimized for routine PCR with $MgCl_2$ included |
| 10X PCR Buffer without $MgCl_2$ | **P2317** | Optimized for routine PCR |

# PCR & NGS

## PCR Essentials

## PCR Master Mix Calculator

PCR Master Mix Calculator

Performing calculations for large scale PCR reactions can be cumbersome and tedious. Ensure your success of scaled up reactions by using the PCR Master Mix Calculator. This online tool will calculate the amounts of components needed to create your PCR master mix.

**PCR Master Mix Calculator**

| Composition of PCR reaction | | | PCR MasterMix Formulation for ☐ PCR reactions | |
|---|---|---|---|---|
| Template DNA | | µl | Template DNA | µl |
| PCR Buffer | | µl | PCR Buffer | µl |
| Forward Primer | | µl | Forward Primer | µl |
| Reverse Primer | | µl | Reverse Primer | µl |
| dNTP mix | | µl | dNTP mix | µl |
| DNA Polymerase | | µl | DNA Polymerase | µl |
| PCR grade Water | | µl | PCR grade Water | µl |
| Total Number of Reactions | | | | |
| | | | Total PCR Reaction Volume | µl |
| | | | TOTAL VOLUME | µl |

Reset | Calculate | Print

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# PCR & NGS

## PCR Essentials

### Specialty Enzymes – KOD DNA Polymerase and Master Mix

| Product Name | Cat. No. | Fidelity | Efficiency | Velocity (Extension time) | Target size | Crude sample | Hot-start | Key Applications | Key Feature |
|---|---|---|---|---|---|---|---|---|---|
| KOD One™ PCR Master Mix | **KMM-101NV** | 80-fold<br>++++ | +++ | 5 sec/kb<br>++++ | ~40 kb | ++++ | ✓ | • Crude samples<br>• Long targets<br>• Templates with high GC content | Ultra-fast; High Fidelity |
| KOD One™ PCR Master Mix - Blue | **KMM-201NV** | | | | | | | | |
| KOD Hot Start Master Mix | **71842** | 80-fold<br>++++ | ++ | 1 min/kb<br>+ | ~12 kb | + | ✓ | • Cloning: Plasmid DNA up to 21 kbp<br>• cDNA amplification including GC-rich regions | High fidelity |
| KOD Hot Start DNA Polymerase | **71086** | | | | | | | | |
| KOD Xtreme™ Hot Start DNA Polymerase | **71975-M** | 11-fold<br>+++ | +++ | 1 min/kb<br>+ | ~24 kb | +++ | ✓ | • Crude samples<br>• Long targets<br>• Difficult and GC-rich targets (up to 90% GC content) | High success-rate DNA polymerase (GC-rich and crude sample) |
| KOD XL DNA Polymerase | **71087** | 3-fold<br>+ | +++ | ≤0.5 min/kb<br>+++ | ~18 kb | ++ | - | • Crude samples, multiplex, incorporation of derivatized dNTPs | High Speed & Efficient DNA polymerase |
| KOD DNA Polymerase | **71085** | 3-fold<br>+ | ++ | 1 min/kb<br>+ | ~6 kb | - | - | • Cloning<br>• cDNA amplification | 2X higher elongation rate and 3X higher fidelity than *Taq* DNA polymerase |

++++: Best, +++: Excellent or Strong, ++: Good or Moderate, +:Satisfactory, -: Not recommended, ✓: Applicable

KOD DNA Polymerase is an ultra-high-fidelity, thermostable DNA polymerase. Numerous independent studies have also verified the superior high-fidelity of KOD DNA Polymerase compared to other thermophilic polymerases. In addition to a low mutation frequency, the fast extension rate and high processivity of KOD polymerase results in higher yields of full-length product in fewer reaction cycles. Combined, these make KOD DNA polymerases the PCR enzyme of choice when speed and fidelity matter. Explore our new KOD One™ PCR Master Mix, a ready-to-use 2x PCR master mix containing a novel, genetically modified KOD DNA polymerase (UKOD), along with a new elongation accelerator, enabling fast PCR with an extension time of 5 sec/kb for template DNA <10kb.

# PCR & NGS

## RT-PCR Essentials

To amplify mRNA, use reverse transcription PCR (RT-PCR). This method involves converting RNA into complementary DNA (cDNA) by using a reverse transcriptase enzyme. Standard PCR then uses the cDNA as a template to produce double-stranded DNA (dsDNA) of the target sequence for analysis.

| Category | Product | Cat. No. | Features |
|---|---|---|---|
| Stand-alone Reverse Transcriptase | M-MLV Reverse Transcriptase | **M1302** | Thermostable reverse transcriptase active at 37 °C<br>Generates first strand cDNA up to 7 kb |
| | Enhanced Avian Reverse Transcriptase [eAMV™ RT] | **A4464** | Greater sensitivity for low abundance mRNA |
| | | | Efficient generation of full-length cDNA, up to 14.1 kb |
| cDNA Synthesis Kit | Enhanced Avian First Strand Synthesis Kit | **STR1** | Produces high quality full-length cDNA from total RNA or poly(A)+ RNA |
| | | | Enhanced ability to transcribe through difficult secondary structure at elevated temperatures (up to 65 °C) |
| | ReadyScript® cDNA Synthesis Mix | **RDRT** | Sensitive and easy-to-use solution for two-step RT-PCR |
| | | | ReadyScript™ Enzyme is an RNase H (+) modified M-MuLV RT |

# PCR & NGS

## RT-PCR Essentials

### qPCR and RT-qPCR Kits

We offer a broad array of qPCR kits for all detection chemistries, including probe-based or SYBR® Green-based applications, and instrument platforms.

Choose between SYBR® Green and probe-based detection, and select from the following formats:

- Standard qPCR – Flexibility to optimize RT and PCR reactions separately
- One-step RT-qPCR – Combine the effect of reverse transcriptase with hot-start Taq DNA Polymerase in convenient master mix formats.

# PCR & NGS

## RT-PCR Essentials

| Product Name | Cat. No. | SYBR® Green Reagent | Probe Based | ROX™ Reagent | Instrument Compatibility |
|---|---|---|---|---|---|
| KiCqStart® SYBR® Green qPCR ReadyMix™ Reagent | KCQS00 | ✓ | | | Bio-Rad, Cepheid, Eppendorf, Illumina, Corbett, and Roche systems |
| | KCQS01 | | | Low ROX™ Reagent | ABI and Stratagene instruments |
| | KCQS02 | | | With ROX™ Reagent | ABI instruments |
| | KCQS03 | | | | iQ™ technology, with fluorescein for Bio-Rad systems |
| KiCqStart® Probe qPCR ReadyMix™ Reagent | KCQS04 | | ✓ | | For Bio-Rad, Cepheid, Eppendorf, Illumina, Corbett, and Roche systems |
| | KCQS05 | | | Low ROX™ Reagent | with Low ROX for ABI and Stratagene instruments |
| | KCQS06 | | | With ROX™ Reagent | with ROX for ABI instruments |
| KiCqStart® One-Step Probe RT-qPCR ReadyMix™ Reagent | KCQS07 | | ✓ | | for Bio-Rad, Cepheid, Eppendorf, Illumina, Corbett, and Roche systems |
| | KCQS08 | | | Low ROX™ Reagent | ABI and Stratagene instruments |
| | KCQS09 | | | With ROX™ Reagent | ABI instruments |
| LuminoCt® qPCR ReadyMix™ Reagent | L6544 | ✓ | | Separate reference dye | Compatible with any qPCR instrument. Select the proper final ROX concentration based on your instrument. |
| | L6669 | | ✓ | | |
| SYBR® Green JumpStart™ Taq ReadyMix™ Reagent | S5193 | ✓ | | Separate reference dye | Compatible with any qPCR instrument. Select the proper final ROX concentration based on your instrument. |
| | S4438 | | | | |
| JumpStart™ Taq ReadyMix™ for Quantitative PCR | D7440 | | ✓ | Separate reference dye | Compatible with any qPCR instrument. Select the proper final ROX concentration based on your instrument. |
| SYBR® Green Quantitative RT-qPCR Kit | QR0100 | ✓ | | Separate reference dye | Compatible with any qPCR instrument. Select the proper final ROX concentration based on your instrument. |
| Quantitative RT-PCR ReadyMix™ | QR0200 | | ✓ | Separate reference dye | Compatible with any qPCR instrument. Select the proper final ROX concentration based on your instrument. |

# PCR & NGS

## Next-Generation Sequencing Essentials

### Whole Genome Amplification

Genomic testing and characterization have become an important tool for understanding biological systems. Often, such analysis is hampered by the number of samples examined and the availability of sufficient quantities of genomic DNA. This challenge is particularly challenging for rare and archived sources of DNA. The GenomePlex® Whole Genome Amplification (WGA) kits are a high-throughput system for the rapid and highly representative amplification of genomic DNA from trace amounts of starting material. These kits are suitable for microarray, qPCR and cloning.

The GenomePlex® Single Cell Whole Genome Amplification Kit (**WGA4**) amplifies the genome of a single cell, resulting in a million-fold amplification yielding microgram quantities of genomic DNA.

The SeqPlex™-I DNA Amplification Kit for whole genome amplification (WGA) facilitates Illumina® next-generation sequencing (NGS) from minuscule quantities or degraded/highly fragmented DNA.

### Choosing a Whole Genome Amplification Kit

| Starting material | Suitable for microarray, qPCR and cloning | Compatible with any sequencing platform | Compatible with Illumina® sequencers |
|---|---|---|---|
| Fragmented DNA | | SeqPlex™ Reagent (SEQXE) | SeqPlex™-I Reagent (SEQXI) |
| Intact genomic DNA | GenomePlex® Reagent (WGA2) | SeqPlex™ Reagent (SEQXE) | SeqPlex™-I Reagent (SEQXI) |
| Single cell; low concentration template | GenomePlex® Reagent for Single Cells (WGA4) | SeqPlex™ Reagent (SEQXE) | SeqPlex™-I Reagent (SEQXI) |

# PCR & NGS

## Next-Generation Sequencing Essentials

### Whole Transcriptome Amplification

Whole transcriptome amplification (WTA) kits have been developed to generate sufficient transcript targets from minute amounts of RNA. Successful WTA requires accurate replication of representative transcripts present in a sample – without dropout or bias of specific mRNAs. Our WTA kits provide a precise, fast, and simple method of amplifying total RNA from a variety of sources including blood, fixed and frozen tissue, cell culture, FACS-sorted cells, plants, and microorganisms. Additionally, the WTA (**WTA1**, **WTA2**, **SeqR**) kits provide rapid amplification of total RNA in less than 4 hours without 3′-bias. Amplified RNA (cDNA) is suitable for qPCR, microarray analysis, and traditional cloning. The SeqPlex™-I RNA Amplification Kit (SeqRI) for whole transcriptome amplification (WTA) is designed to facilitate Illumina® next-generation sequencing (NGS) from extremely small quantities or degraded/highly fragmented DNA and RNA.

### Choosing a Whole Transcriptome Amplification Kit

| Starting material | Suitable for microarray, qPCR and cloning | Compatible with any sequencing platform | Compatible with Illumina® sequencers |
|---|---|---|---|
| • Total RNA blood, fixed and frozen tissue, cell culture, FACS sorted cells, plants and microorganisms | TransPlex® Reagent (WTA2) | SeqPlex™ Reagent (SEQR) | SeqPlex™-I Reagent (SEQRI) |
| • Intact or fragmented RNA samples<br>• FFPE RNA<br>• RNA immunoprecipitation (RIP) samples | | SeqPlex™ Reagent (SEQR) | SeqPlex™-I Reagent (SEQRI) |

# PCR & NGS

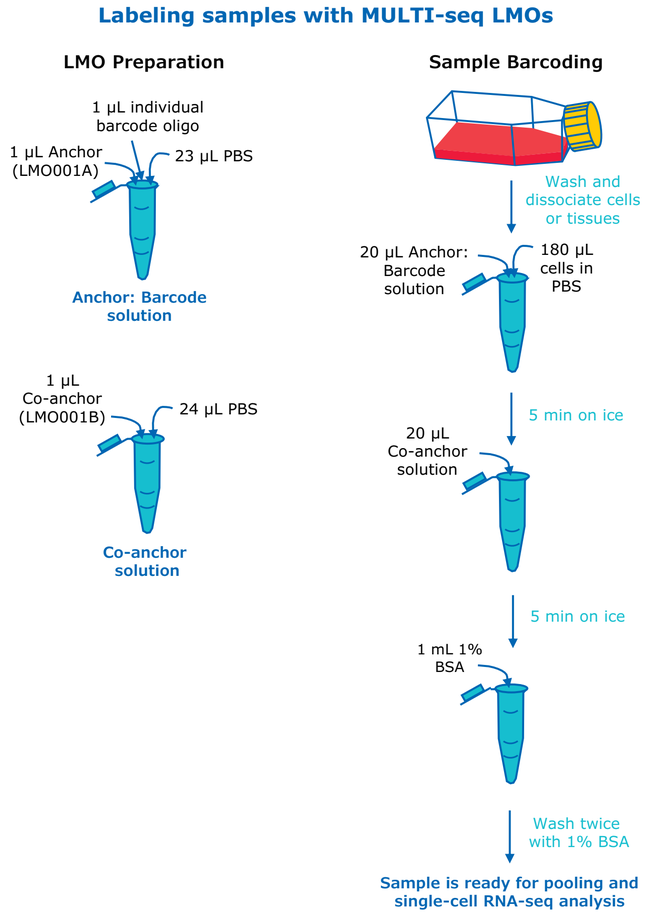
## Next-Generation Sequencing Essentials

## Single Cell Analysis - MULTI-seq Sample Multiplexing

Single-cell RNA-seq (scRNA-Seq) is a powerful but relatively low-throughput tool to analyze gene expression at the single-cell level. MULTI-seq was developed to multiplex sample types using lipid-modified oligonucleotides (LMOs) complexed with unique DNA sample barcodes, allowing for multiple samples to be pooled together in the same single-cell analysis workflow. Sample multiplexing reduces associated costs and provides the additional power of identifying artifacts such as cell doublets in single-cell sequencing and single-nucleus sequencing (snRNA-Seq) applications.

To learn more about scRNA-seq and snRNA-seq sample multiplexing using lipid-tagged indices, click on the product below.

| Cat. No. | Product Name | Description |
|---|---|---|
| **LMO001** | MULTI-seq Lipid-Modified Oligos | For Single Cell and Single Nucleus Multiplexing |



# PCR & NGS

## Isothermal Amplification Essentials

### Isothermal Amplification - Bst Max DNA Polymerase

Loop-mediated isothermal amplification (LAMP) has emerged as a key alternative to polymerase chain reaction (PCR)-based techniques for amplifying nucleic acids. While conventional PCR employs a thermal cycler for repetitive cycling temperatures when amplifying, isothermal amplification techniques such as LAMP occur at a single and fixed temperature, allowing the use of simple, portable, and more robust instruments for fast and exponential amplification.

The Bst Max DNA polymerase is an in-silico designed homolog of Bst DNA Polymerase (large fragment) suitable for DNA amplification at elevated temperatures with an optimum of 65 °C. Bst Max DNA polymerase is a salt-tolerant recombinant polymerase (optimal salt concentration 50-350 mM) with more than two-fold enhanced strand-displacement activity and processivity compared to Bst polymerase. The Bst Max enzyme is active from 25 to 65 °C. It is ideal for isothermal applications such as LAMP and RT-LAMP for its superior amplification performance and robustness.


| Product Name | Cat. No. | Application | Key Features |
|---|---|---|---|
| Bst Max DNA Polymerase | **SRE0113**-1600U | LAMP | • Strong strand displacement activity<br>• High salt and inhibitor tolerance<br>• Ideal for amplification of most sample types, especially small and impure samples |

# Molecular Essentials Guide Workflow



## CRISPR/Genome Engineering

- RNAi and CRISPR Essentials
- shRNA Essentials
- CRISPR Screening Essentials
- CRISPR Gene Editing Essentials
- Guide RNA Essentials

# CRISPR/Genome Engineering

## RNAi and CRISPR Essentials

### Functional Genomics

CRISPR gene editing technology is a multicomponent system that accomplishes specific and targeted changes to a DNA sequence through two main molecular components: a guide RNA (gRNA), and a bacterially-derived nuclease (Cas9). The CRISPR system is often used to add, remove or modify DNA. Our CRISPR reagents and support offer reliable genome engineering tools and services to ensure reliable results. Additionally, our lentivirus-based shRNA libraries deliver unparalleled coverage and are available in multiple formats that include whole-genome, individual RNAi clones/vectors, and gene family sets. These innovative products are available in standard glycerol format or higher quality DNA and lentiviral formats. Whether you are looking to knockout, knock-in, knockdown, or overexpress your targets, our comprehensive suite of CRISPR gene-editing tools, and our shRNA library essentials are designed to ensure reliable results when your work demands them.

# CRISPR/Genome Engineering

## RNAi and CRISPR Essentials

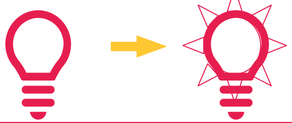
### Functional Genomics

**Knock-down:** reduce gene expression

- shRNA libraries and shRNA clones
- CRISPRi libraries including the Dolcetto Library, 10X Compatible clones, and more on our website

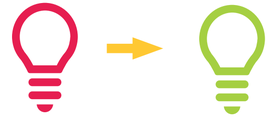
**Knock-out:** eliminate gene expression

- Design your gRNA
- Order our trusted pre-designed gRNAs or your own custom sequences
- Find your Cas editor and increase your efficiency with PEXBUF



**Activator:** increase gene expression

- CRISPRa Calabrese genome library Human
- CRISPRa Caprano whole genome library Mouse
- More options available on our website



**Knock-in:** insert new genetic information

- Design your gRNA
- Order our trusted pre-designed gRNAs or your own custom sequences
- Find your Cas editor and increase your efficiency with PEXBUF

# CRISPR/Genome Engineering

## shRNA Essentials: Foundational Tools for Experimental Success

### Expert Bioinformatics Analysis to enable your discovery

#### Analysis solutions: Deconvolution Services for shRNA and CRISPR pooled libraries

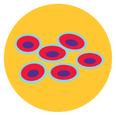
| Sample Type | Product Name | Cat. No. | Key Features |
|---|---|---|---|
| shRNA libraries | MISSION® LentiPlex® Complete Human Pooled shRNA Library | **SHPHLIBR** | Featuring rapid, convenient genome-wide shRNA screens with 125,000 shRNA constructs from the TRC collection targeting 20,000+ human genes. |
| | MISSION® LentiPlex® Human Pooled shRNA Library | **SHPH01** | "Featuring rapid, convenient genome-wide shRNA screens with 75,000 shRNA constructs from the TRC collection targeting 15,000+ human genes. |
| | | | Full Selection of shRNA libraries here.<br>Custom options also available in pooled and arrayed formats. |
| shRNA clones | | Glycerol Stocks<br>Purified DNA<br>Lentiviral Particles | Convenient glycerol, DNA and lentiviral formats available, with shRNAs and gRNAs predesigned for human and mouse.<br>Many controls available |
| Packaging Mix | MISSION® Lentiviral Packaging Mix | **SHP001** | Optimized plasmid formulation to allow for lentiviral packaging and pseudo-typing. This product is suitable for use in conjunction with transfer vectors. |

# CRISPR/Genome Engineering

## CRISPR Screening Essentials

### The Researcher's Guide to CRISPR Screening

## STEP 1



### Determine Cell Line

**Identify which cell lines will work for your requirements.**

1. Ensure the cell line is a good model in terms of relevance, biological process & genotype
2. Do you need a primary, transformed or stem cell platform?
3. Determine if the cell line can be adapted to your workflow
4. Consider the doubling time and ploidy of the cell line

## STEP 2

### Design/Choose a gRNA Library and Screening Strategy

**CRISPR libraries typically contain thousands of plasmids and multiple gRNAs per target gene. First you need to select an appropriate library.**

1. Are you interested in the whole genome or a more focused pathway?
2. Lentivirus or ribonucleoprotein?
3. Pooled or arrayed?
   - Pooled: maximize the number of gRNA per gene target
   - Arrayed: optimize the gRNA design
4. Controls: use non-targeting guides and consider controls for enrichment and depletion depending on your screening approach
5. Use optimal designs for gRNA and—if designing your own libraries—spread them to avoid clusters in inaccessible genomic regions

## STEP 3

### Determine Optimal Conditions

**Low transduction efficiency can result in insufficient representation of the modified cell population.**

1. Perform a kill curve to determine the concentration of selection antibiotic needed to kill the untransfected or untransduced cells
2. Determine the functional titer in your intended cell line using
   - A colony forming unit assay based on antibiotic resistance, or
   - A vector containing a fluorescence marker like GFP
3. Use a control vector to optimize the multiplicity of infection (MOI). Use the lowest MOI that offers one gRNA per cell

## STEP4

### Evaluate Your Cas9 Source

**Establish a Cas9-expressing cell line or provide in an "all-in-one" vector.**

1. Cas9 expressing cell lines: perform clonal isolation or use the mixed population of Cas9 expressing cells for screening.
2. All-in-one vectors: deliver both the Cas9 effector and gRNA by introducing one construct
3. Considerations for the optimal Cas9 source:
   - Ensures constant expression levels in a uniform genetic background
   - Eliminates concerns about co-transduction of gRNAs
   - Supports high-throughput sgRNA applications

## STEP 5

### Perform Your Screen

**Pooled and arrayed screens have similar workflows with some differences:**

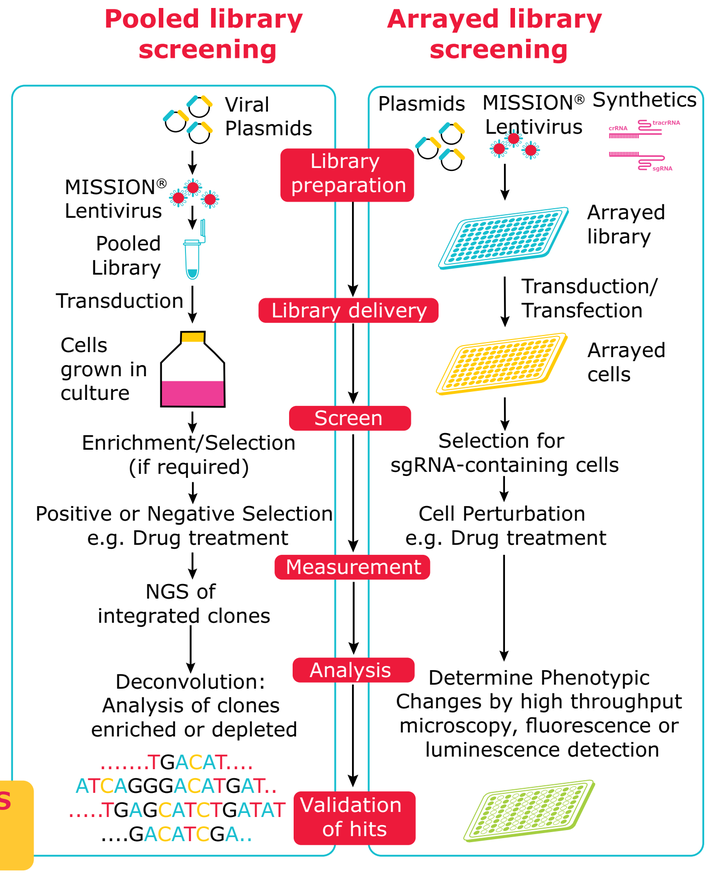
| STEP | POOLED | ARRAYED |
|---|---|---|
| Library Preparation | 1000s gRNAs | per tube 1 gRNA per well |
| Library Delivery | Lentivirus required | Multiple feasible formats |
| Screen Duration | Efficient whole genome screening | Time to screen increases with the number of clones |
| Screen Capability | *in vivo* screening possible | *in vivo* screening not possible |
| Analysis | Deep sequencing/ deconvolution required to analyze data/ identify hits | NGS is not required to understand results |
| Readout | Limited options (e.g. cell death or proliferation) but can be coupled with single cell analysis | Multiple options e.g. fluorescence, luminescence, high content, live cell imaging |

# CRISPR/Genome Engineering

## CRISPR Screening Essentials

### Pooled

- 1000s of gRNAs in one tube
- Lentivirus required
- Whole genome can be screened efficiently
- *In vivo* screening possible
- Deconvolution/NGS required to analyze data/identify hits
- Limited options for phenotype/readout e.g., cell death or proliferation




### Arrayed

- 1 gRNA per well
- Multiple format options
- Time to screen increases with # of clones
- *In vivo* screening not possible
- No NGS required to understand results
- Multiple options for phenotype/readout e.g., fluorescence, luminescence, high content imaging

**Deconvolution Services: Expert Bioinformatics Analysis to enable your discovery**

**CRISPR Products and Services**

# CRISPR/Genome Engineering

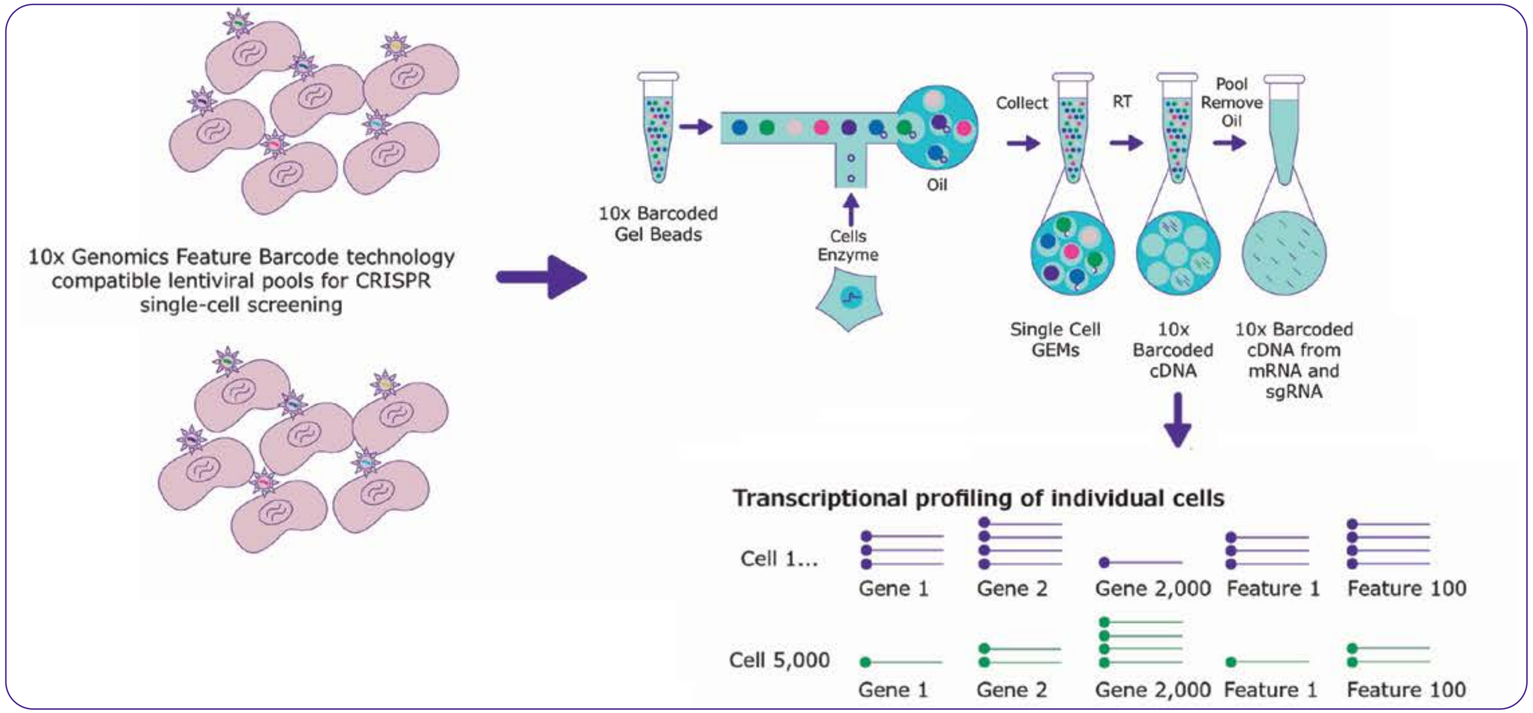
## Single Cell Analyses

### 10x Reagents Feature Barcode Technology

#### 10X Genomics Compatible Products*

| | 10X CRISPRi Feature Barcode Optimization Kit Includes Positive and Negative Controls | Individual Clones | Custom Pools (20-2000 clones) |
|---|---|---|---|
| Viral Titer (by p24) | 1 x $10^6$ | 1 x $10^6$ | 5 x $10^8$ |
| Volume | 20 µL | 200 µL | 200 µL |

*All products contain Feature Barcode technology



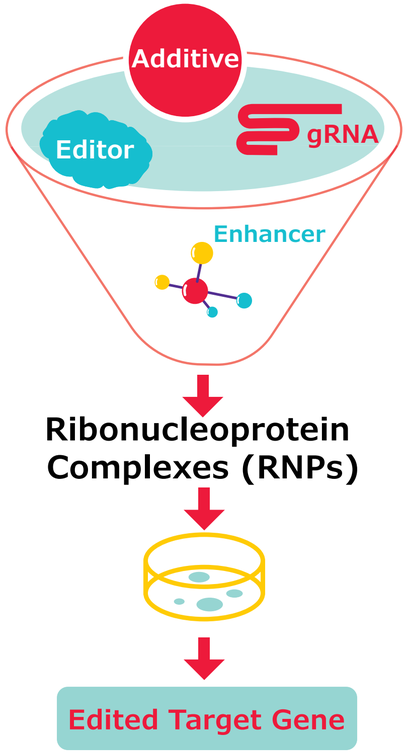

10x GENOMICS Compatible Products

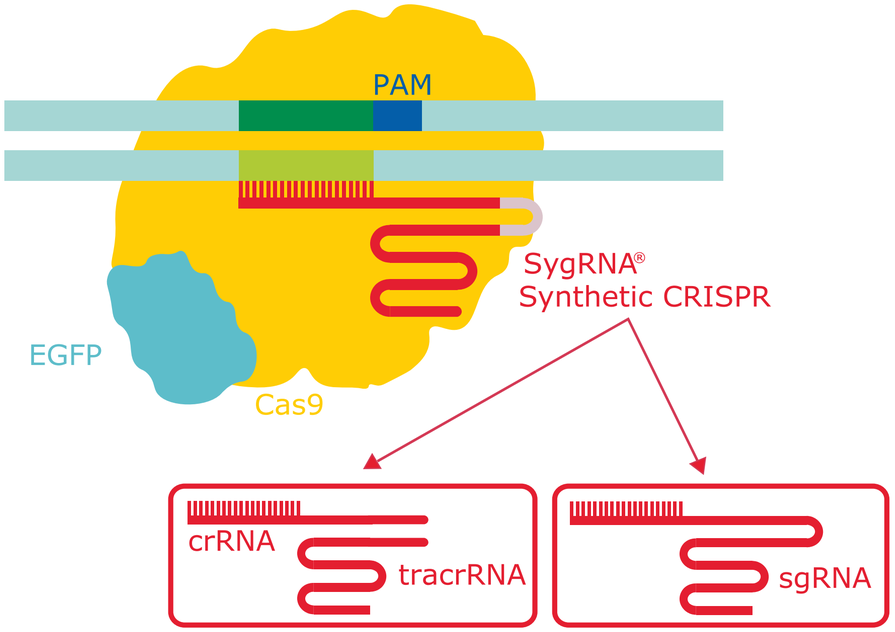
10xCRISPRpools

# CRISPR/Genome Engineering

## CRISPR Gene Editing Essentials

### Ribonucleoprotein (RNP) complexes for enhanced safety and efficacy




| Workflow components | Recommended Reagents and Tools | | |
|---|---|---|---|
| gRNA | **Order our pre-designed gRNAs here** | **Design your own gRNA here** | **Order your own gRNA design here** |
| Editor | Best-in-class editor: **PURedit® Cas9 Protein reagents** | spCas9 standard: **Cas9 Protein** | spCas9 GFP fusion: **Cas9-GFP Protein** |
| Additive | Transfection Reagent: **GeneJuice® Transfection Reagent** | Increase Transfection Efficiency: **PEXBUFF Transfection Enhancer** | |
| Detect your edit | Check your editing events by looking for indels without NGS: **T7 Endonuclease Detection Assay** | | |

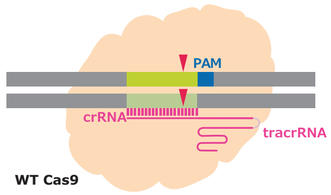
# CRISPR/Genome Engineering

## CRISPR Gene Editing Essentials

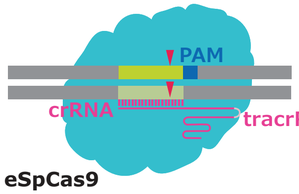
## Cas9 & sgRNA Essentials – Options To Optimize

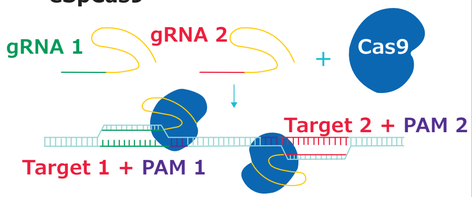
### Cas9 Cutting

**Cas9 Protein (CAS9PROT)**



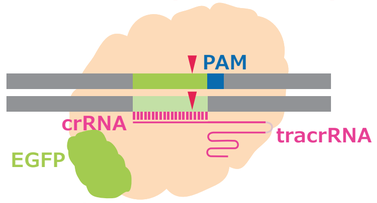

**Cas9 Plus Protein (CAS9PL)**
**PURedit® Cas9 Protein (PECAS9)**
**eSpCas9 Protein (ESPCAS9PRO)**



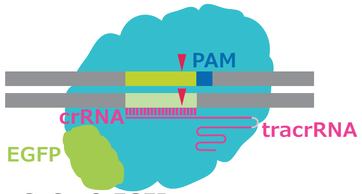

### Cas9 Tracking

**Cas9-GFP Protein (CAS9GFPPRO)**




**SpCas9-EGFP Fusion**

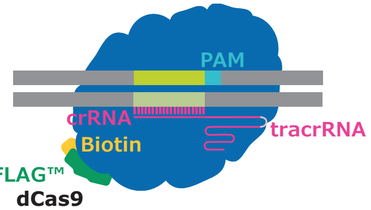
**eSpCas9-GFP Protein (ECAS9GFPPR)**



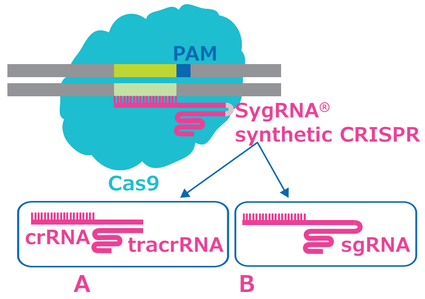

**eSpCas9-EGFP Fusion**

### Cas9 Recognizing

**dCas9-3XFLAG™-Biotin Protein (DCAS9PROT)**




**sgRNA and crRNA/SygRNA™ Cas9 Synthetic tracrRNA (trcrRNA) – custom and pre-designed**




**Product Guarantee**

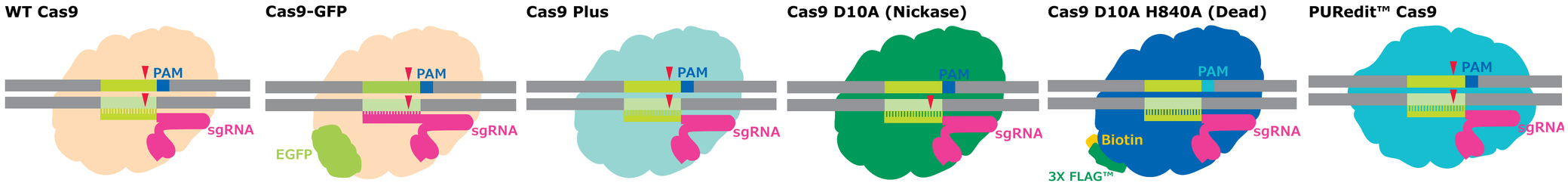
We are so confident in the performance of our SygRNA® products, that we fully guarantee the quality and performance of any gRNA we produce, including custom sequences. If your crRNA or sgRNA do not yield detectable cleavage at the intended target site, we will provide you a one-time replacement, free of charge.

To qualify for this guarantee, please send an image or sequencing data from a single experiment demonstrating detectable cleavage using one of our positive controls, side-by-side with the negative results from your SygRNA® gRNA. To receive your replacement, simply email **oligotechserv@milliporesigma.com** and include sample data from a representative experiment (T7E1, TIDE, or NGS).

# CRISPR/Genome Engineering

## CRISPR Gene Editing Essentials

### Cas9 Protein Essentials



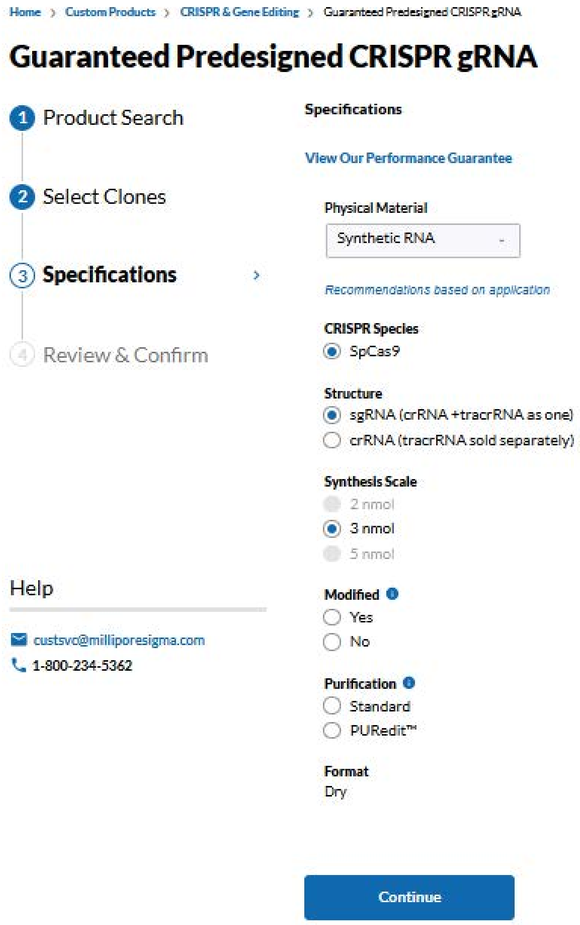

| | WT Cas9 | SpCas9-EGFP Fusion | Cas9 Plus | CAS9D10A | dCas9 | PUREdit™ Cas9 |
|---|---|---|---|---|---|---|
| **Description** | WT SpCas9 | WT SpCas9 fused to GFP | SpCas9 protein with both high editing activity and on-target specificity | Cas9 mutated to nick one DNA strand | Cas9 with mutation inhibiting cutting but not DNA binding | SpCas9 with highest activity and specificity; enhanced quality control |
| **Recommended Use** | Early evaluation of gRNA targets | Optimizing conditions and new delivery mechanisms | Experiments that cannot compromise activity or specificity | HDR applications; requires 2 target sites for modification | Activation/inhibition of target expression | Pre-clinical and early clinical research |
| **Features** | 1x NLS | 3x NLS<br>Enhanced GFP | Activity- and specificity-enhancing mutations<br>1x NLS | D10A mutation<br>1x NLS | D10A and H840A mutations<br>1x NLS 3x FLAG™-biotin tag | Activity- and specificity-enhancing mutations<br>Higher MQ rating 1XNLS |
| **Gene Editing Activity** | +++ | +++ | +++ | ++ | N/A | ++++ |
| **On-Target Specificity** | ++ | ++ | ++++ | +++ | N/A | ++++ |

# CRISPR/Genome Engineering

## Guide RNA Essentials

## Formats and Purity For Success

| Feature | Standard sgRNAs | PURedit™ sgRNAs |
|---|---|---|
| Differentiators | Lowest price in the market with higher activity than competitors; Useful in variety of applications | High purity sgRNAs manufactured under higher quality standards |
| Purification | Standard (RP1) | PURedit™ sgRNAs (HPLC) |
| Gene Editing Activity | +++ | ++++ |
| Stabilizing Modifications Available | Yes | Yes |
| Quality Standard | ISO 9001 | ISO 9001 with enhanced quality control required for preclinical & early clinical research |



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**SigmaAldrich.com/molecularbiology**



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