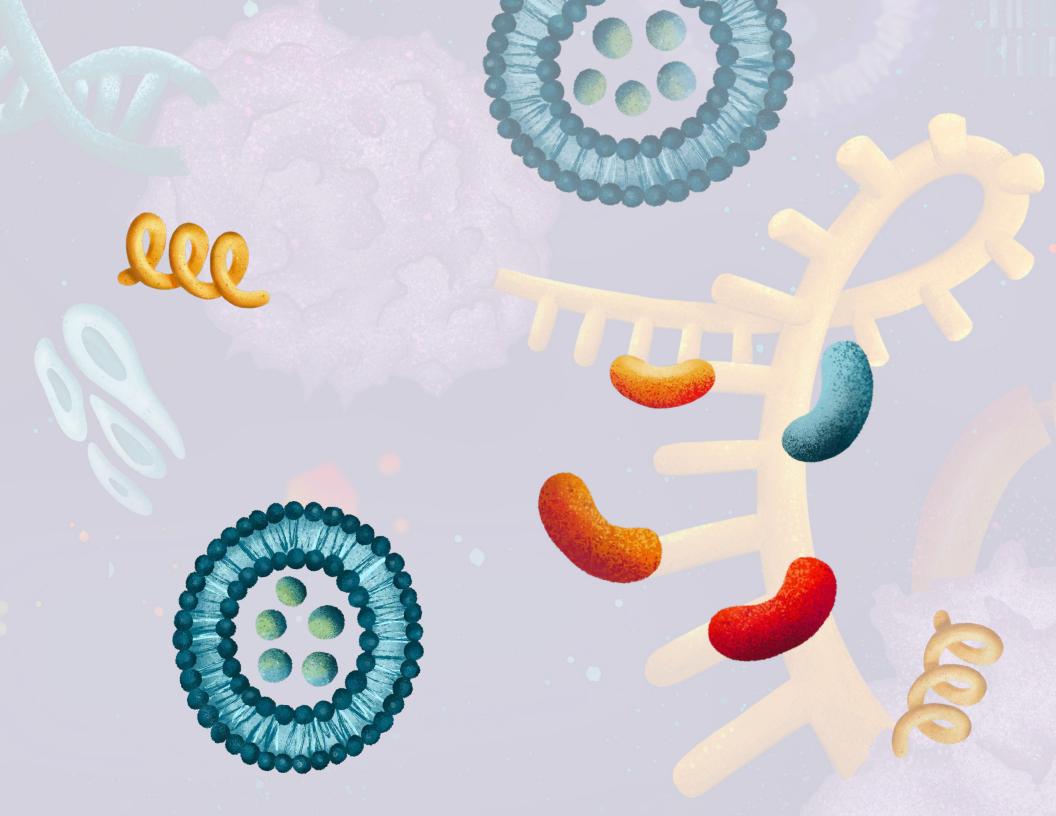
Molecular biology

# Prepare for discovery Molecular biology workflow solutions

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invitrogen

DNA

#### Unlocking scientific discovery: making molecular biology accessible to all

From the beginning, our scientists have set out to commercialize tools to help advance molecular biology. In the early 1990s, Invitrogen<sup>™</sup> SuperScript<sup>™</sup> Reverse Transcriptase and TOPO<sup>™</sup> cloning technology were introduced to the market. TOPO cloning technology was followed by many other products to speed up and simplify previously complex techniques. Over the decades, we have continued to develop new products and improve old favorites.

Helping our customers apply these tools to solve new research challenges is our purpose. Here we provide foundational information and introduce the latest tools to aid your research.

Find additional information at thermofisher.com/molecularmasterpiece.

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# Sample preparation

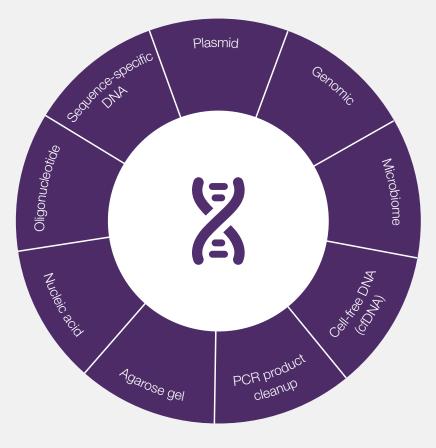


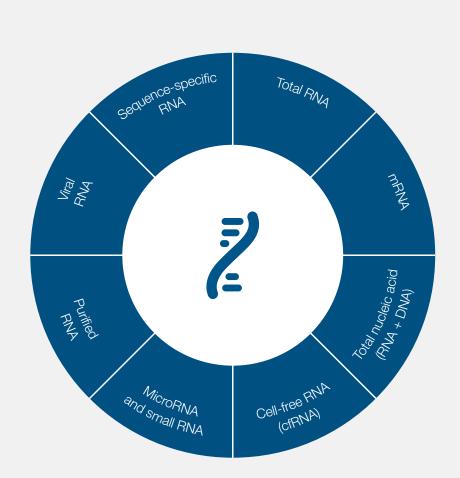
Nucleic acid isolation is a crucial first step in the molecular biology workflow, whether you are isolating genomic DNA (gDNA) or RNA. Selecting nucleic acid purification products that are optimized to help provide maximum yield, purity, and integrity from virtually any sample type and application is important for your research success.

Advance your research at thermofisher.com/kingfisher



## Portfolio of solutions for your nucleic acid isolation





#### DNA type

For gDNA extraction, cfDNA isolation, plasmid isolation, and DNA cleanup

#### **RNA** type

For purification of total RNA, cell-free RNA (cfRNA), transcriptome RNA, messenger RNA (mRNA), microRNA (miRNA) and other small RNA, and sequence-specific RNA capture



## Understanding common nucleic acid isolation methods

Automated purification instruments: automated processing of magnetic particles in a microplate format (e.g., Thermo Scientific<sup>™</sup> KingFisher<sup>™</sup> purification systems)



Samples are processed by moving magnetic beads (not liquid). The system utilizes magnetic rods covered with a disposable, specially designed tip comb and plates. The instrument functions without any dispensing or aspiration parts or devices. Before the run, samples and reagents, including magnetic particles, are dispensed into plates according to default protocols that are installed on the instrument.

#### Benefits:

- Process 6-96 samples per run
- 24- or 96-well plates for different input volumes
- Easily edit, modify, or create new protocols
- All the benefits of magnetic beads (below)

Magnetic beads: 0.5–1.0 μm particles with a paramagnetic core and modified shell (e.g., Applied Biosystems<sup>™</sup> MagMAX<sup>™</sup> kits and Invitrogen<sup>™</sup> Dynabeads<sup>™</sup> magnetic beads)

Samples are lysed in solution and allowed to bind nucleic acid to magnetic particles based on specific surface modifications. Application of an external magnetic field rapidly collects the particles. Rounds of release, wash, and recapture enable purification of the desired nucleic acid.

#### Benefits:

- No risk of clogging
- Increased target capture efficiency
- Rapid collection and concentration of sample
- Specialized equipment not required
- Scalability



#### Helpful tip

Invitrogen<sup>™</sup> Dynabeads<sup>™</sup> magnetic beads are suitable for a variety of applications in addition to nucleic acid purification.



Visit <u>thermofisher.com/dynabeads</u> to learn about additional applications, including cell isolation, immunoprecipitation, and chromatin immunoprecipitation (ChIP).



Learn more at thermofisher.com/sampleprep





**Spin columns:** Glass fiber, derivatized silica, or ion exchange membrane in column (e.g., Thermo Scientific<sup>™</sup> GeneJET<sup>™</sup> and Invitrogen<sup>™</sup> PureLink<sup>™</sup> kits)

Samples are lysed and passed through the membrane using centrifugal or vacuum force. Wash and elution solutions are subsequently passed through the membrane, and the sample is collected into a tube by centrifugation.

#### **Benefits:**

- Convenience
- Ease of use
- Throughput flexibility
- Specialized equipment not required



**Organic extraction:** Phenol-chloroform solution (e.g., Invitrogen<sup>™</sup> DNAzol<sup>™</sup> and TRIzol<sup>™</sup> Reagents)

After homogenizing the sample with TRIzol Reagent, chloroform is added and the mixture separates into a clear upper aqueous layer containing RNA, an interphase layer, and a pink lower organic layer containing the DNA and protein. RNA is precipitated from the upper aqueous layer with isopropanol. DNA is precipitated from the interphase and organic layers with ethanol. Protein is precipitated from the phenol–ethanol supernatant with isopropanol.

#### Benefits:

- Efficient lysis of cells and tissue
- Rapid denaturation of nucleases
- Stabilization of nucleic acids
- Great for fatty and cartilaginous samples



## Automation platforms: Find a model that meets your needs

Optimize and automate your DNA and RNA, cell, and protein purification workflows with KingFisher systems. When used with compatible bead-based reagents such as MagMAX and Dynabeads products, these versatile instruments enable the automation of DNA, RNA, protein, and cell isolation procedures.





The Thermo Scientific<sup>™</sup> KingFisher<sup>™</sup> PlasmidPro<sup>™</sup> Maxi Processor uses a cartridgebased system to automate the preparation of endotoxin-free plasmid suitable for transfection. Learn more and request a demo at <u>thermofisher.com/kingfisher</u>.





Resource

Find out more at

Use our selection tool to find the right

magnetic bead-based kit for your

thermofisher.com/kingfisherkits

automated sample preparation.



KingFisher instrument:	Duo Prime	Flex	Apex	Presto	PlasmidPro
Instrument size	Compact benchtop	Benchtop	Benchtop	Benchtop—integrates with robotic liquid handler	Benchtop
Throughput level	Low to medium	High	High	Ultrahigh	Low
Processing volume range	<ul> <li>50–1,000 μL: 12-pin magnet head</li> <li>200–5,000 μL: 6-pin magnet head</li> </ul>	<ul> <li>20–100 μL: skirted PCR plate</li> <li>20–200 μL: 96-well plate</li> <li>50–1,000 μL: 96 deep-well plate</li> <li>200–5,000 μL: 24 deep-well plate</li> </ul>	<ul> <li>15–1,000 μL: 96 deep-well plate</li> <li>15–200 μL: 96-well KingFisher standard plate</li> <li>10–80 μL: 96-well PCR plate</li> <li>30–5,000 μL: 24 deep-well plate</li> <li>30–200 μL: 96 storage tubes</li> <li>200–1,000 μL: 24 storage tubes</li> </ul>	<ul> <li>50–1,000 μL: 96 deep-well plate</li> <li>200–5,000 μL: 24 deep-well plate</li> <li>50–150 μL: KingFisher 96 plate</li> </ul>	<ul> <li>150 mL overnight bacterial culture up to 6 OD or 10<sup>6</sup> cells/mL in LB medium</li> </ul>
Samples per run	6 or 12	24 or 96	24 or 96	24 or 96	One
Customizable protocols	Yes	Yes	Yes, with touchscreen or PC software	Yes	No
Heating and cooling	<ul> <li>10°C to 75°C (plate row block A)</li> <li>4°C to 75°C (elution strip block)</li> </ul>	From 5°C above ambient temperature to 115°C	<ul> <li>From 4°C above ambient temperature to 100°C</li> <li>Cooling down to 4°C</li> </ul>	<ul> <li>From 5°C above ambient temperature to 115°C</li> </ul>	NA
Ultraviolet lamp	8 W (up to 16 hr)	No	2 UV lamps, max 23 hr 59 min	No	No
Additional details	For Research Use Only. Not for use in diagnostic procedures.	For Laboratory Use	For Laboratory Use	For Laboratory Use	For Research Use Only. Not for use in diagnostic procedures. Designed for automated endotoxin-free maxi preps of plasmid DNA.

#### Typical run with a KingFisher instrument



\*\* Can vary depending on application and instrument.

The graphic shows the expected run time for sample preparation when using a KingFisher instrument and prefilled plates. It takes less than 15 minutes to prep plates, 1 minute to select the program or protocol, and 1 minute to load plates. You can then press start and walk away for 25–120 minutes, depending on the sample and analyte type.

#### Learn more at thermofisher.com/kingfisher



## Selecting the right DNA and RNA isolation kits for your downstream research

Cancer research: Applied Biosystems<sup>™</sup> MagMAX<sup>™</sup> cell-free nucleic acid kits on KingFisher instruments are excellent for liquid biopsy research. They are optimized specifically for enrichment of cfDNA and cell-free total nucleic acid (cfTNA), enabling highly reproducible recovery, flexible input volumes, and efficient protocols. Learn more about <u>sample preparation solutions for liquid biopsy</u>.

Application	Automation-ready extraction kit and reagents	Cat. No.
	MagMAX Cell-Free DNA Isolation Kit	A29319
	MagMAX Cell-Free Total Nucleic Acid Isolation Kit	A36716
	MagMAX mirVana Total RNA Isolation Kit	A27828
	MagMAX FFPE DNA/RNA Ultra Kit	A31881
	MagMAX DNA Multi-Sample Ultra 2.0 Kit	A36570
	MagMAX Sequential DNA/RNA Kit	A65309
	Dynabeads FlowComp Human CD3 Kit	11365D
Cancer research	Dynabeads FlowComp Human CD4 Kit	11361D
research	Dynabeads FlowComp Human CD8 Kit	11362D
	Dynabeads Untouched Human T Cells	11344D
	Dynabeads Untouched Human CD4 T Cells Kit	11346D
	Dynabeads CD15*	11137D
	Dynabeads CD4	11145D
	Dynabeads Epithelial Enrich	16102
	Dynabeads MyOne CD45 Leukocyte Depletion	11170D

\* Available in 2 mL sizes with automation scripts. Learn more about <u>cell isolation products</u>.

**DNA research:** Using the Applied Biosystems<sup>™</sup> MagMAX<sup>™</sup> DNA Multi-Sample Ultra 2.0 Kit and KingFisher instruments, you can isolate gDNA from 50 µL to 2 mL of whole blood, saliva, buffy coat, buccal swabs, or other biological samples. The resulting purified gDNA is ideal for many downstream molecular biology applications such as real-time PCR (qPCR), next-generation sequencing (NGS), microarray analysis, and other applications.

Application	Automation-ready extraction kit and reagents	Cat. No.
	MagMAX DNA Multi-Sample Ultra 2.0 Kit	A36570
Genomics	MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit	A48383R
	MagMAX mirVana Total RNA Isolation Kit	A27828
NGS	Dynabeads Streptavidin for Target Enrichment	65606D
NGS	MagMAX Pure Bind Beads	A58521





## Selecting the right DNA isolation kits for your downstream research

Infectious disease research: Applied Biosystems<sup>™</sup> MagMAX<sup>™</sup> viral/pathogen kits on KingFisher instruments provide a sensitive and simple method for nucleic acid extraction from samples containing viruses or other pathogens. Learn more at thermofisher.com/mvpprime.

Application	Automation-ready extraction kit and reagents	Cat. No.
Infectious disease research	MagMAX Viral/Pathogen Nucleic Acid Isolation Kit for HIV-1 dried blood spots	A53770
	MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit	A48383R
	MagMAX Microbiome Ultra Nucleic Acid Isolation Kit, with bead tubes	A42358
	MagMAX DNA Multi-Sample Ultra 2.0 Kit	A36570
	MagMAX Prime Viral/Pathogen NA Isolation Kit	A58145
	Dynabeads Intact Virus Enrichment	10700D



Plasmid purification: Choose from our wide range of high-performing, cost-effective Thermo Scientific<sup>™</sup> and Invitrogen<sup>™</sup> kits for plasmid DNA isolation designed to isolate plasmid DNA at the purity and scale you need. Learn more at <u>thermofisher.com/plasmid</u>.

Application	Technologies for plasmid DNA isolation	Cat. No.
	GeneJET Plasmid Miniprep Kit	K0502
	GeneJET Endo-Free Plasmid Maxiprep Kit	K0861
	PureLink Fast Low-Endotoxin Midi Plasmid Purification Kit	A35892
Plasmid purification	PureLink HiPure Plasmid Filter Maxiprep Kit	K210016
	PureLink Expi Endotoxin-Free Maxi Plasmid Purification Kit	A33073
	MagMAX Pro HT NoSpin Plasmid Miniprep Kit	A58309
	KingFisher PlasmidPro Maxi Processor Endotoxin-Free Cartridge	A54072





#### Helpful tip

Select the best plasmid preparation kit for your application with the **Plasmid Purification Selection Tool**.

Go directly to our kit selection tool at thermofisher.com/kingfisherkits



## Selecting the right DNA isolation kits for your downstream research

Wastewater DNA and RNA for disease surveillance: Applied Biosystems<sup>™</sup> MagMAX<sup>™</sup> wastewater kits offer an efficient and simple method for extracting high-quality nucleic acids from wastewater, sewage, or sludge samples for disease surveillance workflows. The purified DNA and RNA are excellent for use in a variety of downstream applications such as quantitative PCR, digital PCR, and NGS.

Application	Automation-ready extraction kit and reagents	Cat. No.
	MagMAX Wastewater Ultra Nucleic Acid Isolation Kit with Virus Enrichment	A52610
	MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit	A48383R
Surveillance	MagMAX mirVana Total RNA Isolation Kit	A27828
	MagMAX Microbiome Ultra Nucleic Acid Isolation Kit, with bead tubes	A42358
	MagMAX Prime Viral/Pathogen NA Isolation Kit	A58145

Streptavidin: Utilization of Invitrogen<sup>™</sup> Dynabeads<sup>™</sup> streptavidin magnetic beads offers an exceptional balance of capacity and yield, reproducibility, purity, and cost for smaller-scale isolation of specific proteins (e.g., immunoprecipitation (IP)) and protein complexes (co-immunoprecipitation (co-IP)) and other applications.

Application	Automation-ready extraction kit and reagents Cat		
	Dynabeads Streptavidin for Target Enrichment	65606D	
	Dynabeads M-270 Streptavidin	65305	
IP and co-IP	Dynabeads M-280 Streptavidin	11205D	
	Dynabeads MyOne Streptavidin T1	65601	
	Dynabeads MyOne Streptavidin C1	65001	

Go directly to our kit selection tool at thermofisher.com/kingfisherkits



## Selecting the right DNA and RNA isolation kits

For the quality and performance you need, a full suite of products for DNA and RNA isolation is available for a wide range of sample types, throughputs, and input quantities. To use our online kit selection guide, go to **thermofisher.com/rnaselection**.









#### Applied Biosystems<sup>™</sup> and Invitrogen<sup>™</sup> technologies for DNA and total RNA isolation

Capabilities	Process a large amount of tissue	Fast isolation of RNA from a variety of samples	High-throughput purification of RNA and DNA	Process cells for gene expression		
Kits	TRIzol reagents	PureLink kits	MagMAX kits	Cells-to-C <sub>7</sub> kits		
Prep time	30–60 min	<20 min	45 min	≤10 min		
Sample types	Most samples, particularly those more difficult to lyse	Bacteria, liquid, blood, cells, yeast, plants, tissue				
Starting material	100 mg of tissue or 10 <sup>7</sup> cells	10 <sup>8</sup> cells, 200 mg of animal tissue, 250 mg of plant tissue, 0.2 mL of blood, 5 x 10 <sup>8</sup> yeast, 10 <sup>9</sup> bacteria	Variable depending on sample	1–100,000 cells		
Yield	10 <sup>6</sup> epithelial cells: 8–15 μg; 100 mg tobacco leaf: 73 μg (variable depending on sample)	Up to 350 µg	to 350 µg Variable depending on sample			
High throughput-compatible	No	Yes	Yes	Yes		
Technology	Organic extraction	Silica membrane spin column/filter plate	Magnetic beads	Crude lysate		

\* Specialty kits with optimized chemistry are available for extraction of cell-free DNA/RNA, total RNA, gDNA, and total nucleic acid.



#### Helpful tip

If you are not ready to process your RNA sample, simply store it in Invitrogen<sup>™</sup> RNA*later*<sup>™</sup> Stabilization Solution for use at a later time. Visit **thermofisher.com/stabilizerna**.

#### Learn more at thermofisher.com/rnapreps



## Reverse transcription

Reverse transcription is the synthesis of complementary DNA (cDNA) from an RNA template using reverse transcriptase enzymes. The cDNA can be used as a template for PCR amplification, cDNA library construction, RNA sequencing, and more. Selecting the right reverse transcriptase is critical to detecting low-abundance RNAs in a sample and obtaining high yields of full-length cDNA.

Find technical resources on reverse transcription at **thermofisher.com/rteducation** 

## **Considerations for selecting** the right reverse transcriptase

Sensitivity, thermostability, processivity, and inhibitor tolerance of reverse transcriptases all affect the quantity and length of cDNA synthesized.

#### Sensitivity

The ability of a reverse transcriptase to generate cDNA from the least amount of input RNA is an important attribute when working with low-copy genes or difficult sample sources where RNA may have already degraded.

#### Thermostability

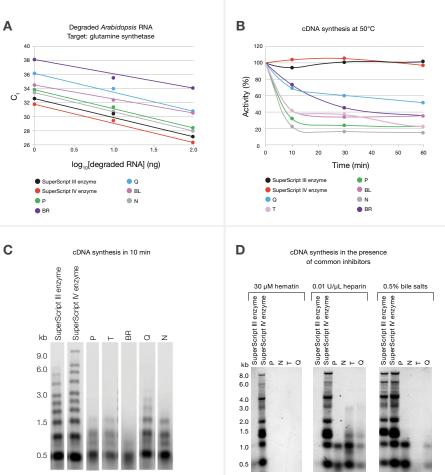
Thermostable reverse transcriptases allow reactions to occur at higher temperatures, which help denature RNA with complex secondary structures or high GC content, for generation of longer cDNA, higher cDNA yields, and better coverage of RNA populations in the cDNA.

#### Processivity

Processivity is the enzyme's ability to add consecutive nucleotides without releasing the template. Highly processive reverse transcriptases allow synthesis of longer cDNA strands in a shorter reaction time, and overall better efficiency in making full-length cDNA.

#### Inhibitor tolerance

Compounds that have inhibitory effects on reverse transcriptases are common in RNA samples even after purification. Their sources include reagents used for RNA isolation and contaminants carried over from biological samples. Reverse transcriptases resistant to common inhibitors help minimize inconsistent or suboptimal results in cDNA-based assays.



(A) Sensitivity, (B) thermostability, (C) processivity, and (D) inhibitor tolerance of reverse transcriptases can affect the quantity and length of cDNA. P, BR, Q, BL, T, and N represent enzymes from other suppliers.

Reverse transcription





## Reverse transcription reagent selection guide

Ability to optimize

	reaction components and conditions	synthesis kit with all reaction components	and fewest pipetting steps for RT-qPCR applications	
Product format	Stand-alone enzyme	First-strand cDNA synthesis kit	First-strand cDNA synthesis master mix for RT-qPCR	
Recommended product	<u>Invitrogen<sup>™</sup> SuperScript<sup>™</sup> IV</u> <u>Reverse Transcriptase</u>	Invitrogen <sup>™</sup> SuperScript <sup>™</sup> IV First-Strand Synthesis System	<u>Invitrogen<sup>™</sup> SuperScript<sup>™</sup> IV</u> <u>VILO<sup>™</sup> Master Mix</u>	
Applications	RT-PCR, RT-qPCR, cloning, cDNA library construction, RACE, RNA-Seq	RT-PCR, RT-qPCR, cloning, cDNA library construction, RACE, RNA-Seq	RT-qPCR	
Total RNA input	1 pg–5 µg	1 pg–5 µg	0.01 pg-2.5 µg	
Optimal reaction temperature	50-55°C	50–55°C	50–55°C	
Reverse transcription time	10 min	10 min	10 min	1.1
High cDNA yield with challenging or degraded RNA	•	•	•	
Includes PCR step				
Available formats	<u>Stand-alone enzyme</u>	<ul> <li><u>cDNA synthesis kit</u></li> <li><u>cDNA synthesis kit</u> with ezDNase enzyme</li> </ul>	SuperScript IV VILO Master Mix     SuperScript IV VILO Master Mix     with ezDNase enzyme	

Complete cDNA

Most convenient



#### Did you know?

These products are free of OPE and NPE, making them safer for aquatic life.

Learn more at thermofisher.com/superscript



Most convenient and fewest pipetting steps for RT-PCR applications Optimized template switching for RACE and RNA-Seq applications cDNA synthesis and amplification directly from intact single cells or low amounts of total RNA Go from mammalian cell lysate to cDNA synthesis without isolating RNA

	One-step RT-PCR kit	Template switching master mix	cDNA PreAmp kit	Direct RT kit	
	<u>Invitrogen<sup>™</sup> SuperScript<sup>™</sup> IV</u> <u>UniPrime<sup>™</sup> One-Step RT-PCR System</u>	Invitrogen <sup>™</sup> SuperScript <sup>™</sup> IV Template Switching RT Master Mix	Invitrogen <sup>™</sup> SuperScript <sup>™</sup> IV Single Cell/Low Input cDNA PreAmp Kit	Invitrogen <sup>™</sup> SuperScript <sup>™</sup> IV CellsDirect <sup>™</sup> cDNA Synthesis Kit	
	RT-PCR	RT-PCR, RACE, RNA-Seq	RT-qPCR, RNA-Seq	RT-PCR, RT-qPCR	
	0.01 pg–1 µg	1–1,000 cells or 2 pg–10 ng	1–1,000 cells or 2 pg–10 ng	1–10,000 cells	
	50–55°C	50°C 50°C		50–55°C	
	10 min	30 min	30 min	10 min	
	•	•		٠	
÷	•		•		
	SuperScript IV UniPrime One-Step RT-PCR System (colored)     SuperScript IV UniPrime One-Step RT-PCR System (dye-free)	<ul> <li>Invitrogen<sup>™</sup> SuperScript<sup>™</sup> IV Template Switching RT Master Mix</li> </ul>	SuperScript IV Single Cell/Low Input <u>cDNA PreAmp Kit</u>	SuperScript IV CellsDirect cDNA Synthesis Kit	



4

The standard enzyme format is incompatible with lyophilization because of the glycerol in the storage buffer. The lyo-ready (lyophilization-ready) format of SuperScript reverse transcriptases has a glycerol content below 0.1% and offers greater stability for lyophilized molecular assay kits. Learn more at <u>thermofisher.com/lyoreadyenzymes</u>.



## **RNase inhibitors**

To ensure your RNA template is protected and reverse transcription is successful, it's necessary to include RNase inhibitors. This step is crucial for preserving the integrity of the sample and achieving accurate and reproducible results.

Note: All SuperScript IV kit formats either contain an RNase inhibitor or are supplied with one.

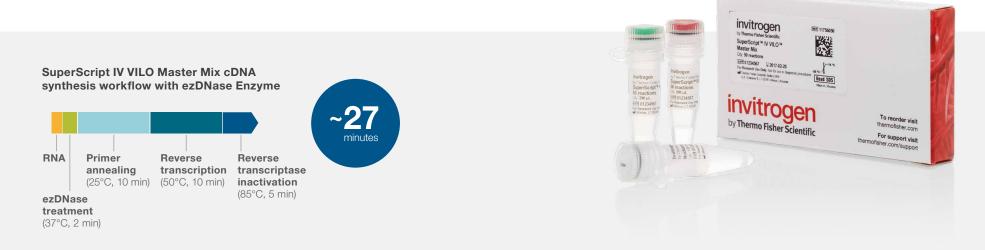


Product	Invitrogen <sup>™</sup> SUPERase∙In <sup>™</sup> _ RNase Inhibitor	Invitrogen <sup>™</sup> RNaseOUT <sup>™</sup> Recombinant Ribonuclease Inhibitor	Applied Biosystems <sup>™</sup> RNase Inhibitor	Invitrogen <sup>™</sup> Ambion <sup>™</sup> RNase Inhibitor, cloned
Mechanism	Protein-based inhibitor of nonhuman origin that noncovalently binds to and inhibits the most common and troublesome RNases, including RNase A, B, C, 1, and T1	Noncompetitive inhibitor of pancreatic-type ribonucleases such as RNase A	50 kDa recombinant enzyme used to inhibit RNase activity	Recombinant human protein produced in <i>E. coli</i> and inhibitor of neutral pancreatic RNase A-type enzymes
Sizes available	• <u>2,500 units</u>	• <u>5,000 units</u>	• <u>2,000 units</u>	• <u>1,000 units</u>
	• <u>10,000 units</u>			• <u>2,500 units</u>
				• <u>10,000 units</u>

## **Genomic DNA removal**

RNA purification methods, including protocols with DNase digestion on column, often fail to completely remove gDNA. Amplification of contaminating gDNA can cause inaccurate results. Moreover, traditional gDNA decontamination protocols with DNase I include time-consuming DNase inactivation steps under conditions that can damage RNA and affect results.

Invitrogen<sup>™</sup> SuperScript<sup>™</sup> IV VILO<sup>™</sup> Master Mix is available in a format with the novel dsDNA-specific Invitrogen<sup>™</sup> ezDNase<sup>™</sup> Enzyme, which enables efficient, fast, and gentle gDNA removal from RNA samples to help ensure high confidence in RT-PCR and RT-qPCR results.



#### Traditional cDNA synthesis workflow with DNase I



#### Learn more at thermofisher.com/4vilo

### **Reverse transcription primers**

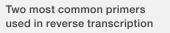
The priming strategy you choose for reverse transcription is important for cDNA synthesis efficiency, consistency, and yield. Each primer type has its benefits and drawbacks, depending on the individual target RNA.

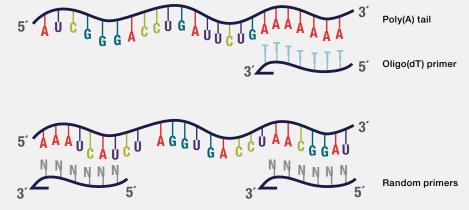
**For full-length first-strand cDNA synthesis,** oligo(dT) primers are recommended because of their specificity for eukaryotic mRNA, and they allow many different targets to be studied from the same cDNA pool. Typically, oligo(dT) primers are strings of 12–20 deoxythymidines. We offer oligo(dT) in different lengths and formats for flexibility in your reverse transcription experiments.

**For target mRNA containing strong transcriptional pauses,** random primers are better suited because they anneal throughout the target molecules. They are also an ideal choice for nonpolyadenylated RNA, such as bacterial RNA.

**For two-step RT-PCR,** a mixture of oligo(dT) and random primers is often used to achieve the benefits of both primer types. SuperScript IV VILO master mix is provided with an optimized ratio of primers.







•Q.

#### Helpful tip

To avoid poly(A) slippage during priming, anchored oligo(dT) primers can be used to anneal to the 5' end of the poly(A) tail of mRNA and prevent priming within the poly(A) tail. Learn more about selection of primers for reverse transcription at thermofisher.com/rteducation.

#### Learn more at thermofisher.com/rtprimers





The polymerase chain reaction (PCR) amplifies DNA, allowing its detection from very low sample inputs. The process involves 3 steps that are repeated over multiple cycles:

**1) Denaturation:** double-stranded DNA is heated to separate the strands

**2) Annealing:** primers, short DNA molecules, bind to specific regions of the DNA

3) Extension: DNA polymerase extends the primers

Find technical and educational resources about PCR at **thermofisher.com/pcreducation**.

Table of contents

## **Thermal cyclers**

Thermal cyclers, which automate the heating and cooling cycles required to amplify DNA, play a critical role in the success of PCR. The following are things to consider when selecting a thermal cycler.

#### Precise temperature control

Thermal cyclers with precise temperature control enable you to quickly and accurately determine optimal annealing temperatures. Several block technologies, including gradient and Applied Biosystems<sup>™</sup> VeriFlex<sup>™</sup> Blocks temperature control, are available. A VeriFlex Block employs a separate heating and cooling element in each temperature zone, allowing better control and precision of temperatures. Learn more about the technology at <u>thermofisher.com/veriflextechnology</u>.

#### Reliability

Thermal cyclers should be able to withstand repeated use, environmental stress, and shipping conditions. Component reliability can be tested using robotic assemblies for repeated testing of frequently used instrument components such as the heated lid, touchscreens, and temperature cycling modules. Applied Biosystems<sup>™</sup> thermal cyclers adhere to stringent reliability criteria, which are reported at **thermofisher.com/thermalcyclerreliability**.

#### **Temperature accuracy**

Thermal cycler temperature accuracy is a key factor in the success or failure of a PCR reaction. It is particularly important during annealing temperature optimization, which requires both accuracy and consistency in the thermal cycler block. If the temperature set point of the instrument does not correspond to the actual temperature of the block, further temperature optimization could be required. Review a study of temperature accuracy in a number of models, available at thermofisher.com/thermalcycleraccuracy.

#### **Features**

A variety of Applied Biosystems thermal cyclers are available to fit your applications and budget. Certain features may be important to you, depending on your needs. If you perform PCR optimization frequently, you will likely benefit from an instrument with a VeriFlex Block. If you would like to run optimized assays on a new or different thermal cycler, you can save re-optimization time by using a simulation mode.

If you want remote access to your instrument, you will appreciate the convenience of cloud-enabled thermal cyclers. They allow you to design and share protocols, schedule an instrument, start or stop a run, and check run status from anywhere, on any mobile device or desktop computer.

#### **Fleet control**

If you manage multiple thermal cyclers and users, you may benefit from a single interface for viewing all instruments at a glance and setting custom permissions by instrument, user, and method. Learn more at thermofisher.com/fleetcontrol.



#### Helpful tip

Using the right PCR plastics for your application and instrument can improve the reliability of your PCR results. Go to **thermofisher.com/findplastics** to determine the right PCR plastics for you.

#### VeriFlex Block temperature zones

Applied Biosystems<sup>™</sup> Thermal Cycler Fleet Control Software



PCF

## Select the Applied Biosystems thermal cycler that's right for you

	Cloud-enabled	Cloud-enabled
	ProFlex™ PCR System	VeritiPro <sup>™</sup> Thermal Cycler
Questions for customers	<ul> <li>&gt; Do you share the device with colleagues?</li> <li>&gt; Do you expect your throughput needs to change?</li> <li>&gt; Do you want to access your instrument remotely?</li> </ul>	<ul> <li>Do you perform a lot of optimizations?</li> <li>Do you want to access your instrument remotely?</li> </ul>
Key benefits	Ultimate flexibility and throughput	Ultimate performance
Max sample throughput	768 reactions (2 x 384-well block) 480,000 reactions (flat block)	384 reactions
Max block ramp rate	6.0°C/sec	6.0°C/sec
Temperature optimization	6-zone VeriFlex Block on 96-well system 2-zone VeriFlex Block on 3 x 32-well system	6-zone VeriFlex Block on 96-well system
Compatible with Fleet Control Software	Yes	Yes
3D tour	thermofisher.com/proflex3dtour	thermofisher.com/veritipro3dtour

Looking for a CE-IVD-labeled thermal cycler? Learn more about VeritiPro<sup>™</sup> Dx Thermal Cycler at thermofisher.com/veritiprodx

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#### MiniAmp<sup>™</sup> Thermal Cycler

- > Do you want an instrument with just the features needed for routine PCR?
- > Do you want to access your instrument remotely?

Routine PCR, elevated

96 reactions

3.5°C/sec

3-zone VeriFlex Block on MiniAmp Plus model

Yes



#### Automated Thermal Cycler

> Do you want to place your instrument on a robotic platform now or in the future?

Designed for easy robotic integration
384 reactions
3.5°C/sec
None
Yes
thermofisher.com/atc3dtour

Find out more at **thermofisher.com/thermalcyclers** 



## PCR and qPCR plastics, seals, and accessories

Since PCR is a sensitive detection method, PCR plastics must be of high quality and free of contaminants and inhibitors to enable optimal performance. Regardless of the plastics format, proper fit and uniform heat transfer during thermal cycling are essential.

#### Manufacturing guality control

Applied Biosystems<sup>™</sup> PCR and qPCR plastic consumables are manufactured in world-class facilities dedicated to the production of high-guality molecular biology-grade plastics. After manufacturing, all plastics undergo strict quality control.

Integrity testing: Each well of every plate is visually inspected and tested for leaks. This thorough quality control screening verifies every well is intact to protect all reactions.

Evaporation testing: Samples are run through PCR to test sealing performance. Well liquid volumes are analyzed post-PCR to verify seal integrity. This helps ensure that every production lot conforms to strict tolerances.

Biological testing: All PCR plastics from Thermo Fisher Scientific are tested and guaranteed to be free of DNA, RNases, nuclease inhibitors, and PCR inhibitors. PCR certificates are available for convenience and documentation purposes, upon request.

#### Materials

Applied Biosystems<sup>™</sup> MicroAmp<sup>™</sup> optical microplates are made of polypropylene for optimal transfer of thermal energy for efficient PCR. A select medical-grade polypropylene is utilized for its exceptional

#### Applied Biosystems<sup>™</sup> MicroAmp<sup>™</sup> EnduraPlate<sup>™</sup>

biocompatibility and inert properties.

microplates are constructed with a polycarbonate frame. This rigid design prevents damage from robotic grippers and provides better tolerance of rapid heating and cooling, while retaining thin-walled polypropylene wells for efficient heat transfer to the reaction mixture.

#### ⑦ Thermo Scientific<sup>™</sup> Sustain<sup>™</sup> Series reduce the environmental impact of your lab

Explore PCR plastics from the Sustain Series. Manufactured under ISO 9001 guidelines within a Class 100,000 cleanroom from a biobased medical-grade virgin polypropylene that is chemically and molecularly identical to existing Thermo Scientific<sup>™</sup> PCR plastics, these products are certified by the International Sustainability and Carbon Certification (ISCC+) system. This production approach is more sustainable and is equivalent to 1.96x\* lower CO<sub>2</sub> equivalent emissions per kg of biobased polypropylene resin compared to traditional fossil fuelbased polypropylene resin.

thermofisher.com/sustainplastics



Polycarbonate (hard shell) frame Skirt

Polypropylene wells

EnduraPlate microplate for high-throughput robotic applications

\* Compared to equivalent fossil fuel-derived polypropylene resin emissions, cradle to gate (kg CO2e)

#### Find out more at thermofisher.com/pcrplastics



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PCB

Applied Biosystems PCR and qPCR plastics are validated and tested for reliability and optimal performance. They are "Engineer Approved" for use with all Applied Biosystems thermal cyclers and qPCR instruments, and are available in a variety of 32-, 48-, 96-, and 384-well plates; tube strips; single tubes; caps; and seals. The table below provides a detailed comparison of each product. Easily find the PCR and qPCR plastics compatible with your instrument using the online selection tool at <u>thermofisher.com/findplastics</u>.

	Small-scale experiments with a few samples	Daily experiments	Reliable and sustainable PCR plastics	Complete-workflow experiments- ideal for automation	Automation-compatible
PCR plastics product	Single tubes, strips, caps, adhesive film, and accessories	MicroAmp <sup>™</sup> optical microplates	Sustain Series products	MicroAmp <sup>™</sup> EnduraPlate <sup>™</sup> optical microplates	MicroAmp <sup>™</sup> EnduraPlate <sup>™</sup> optical reaction plates
Formats	Single tubes	• 32-well	• 96-well (multiple)	• 96-well	96-well
	<ul> <li>Single tubes with caps</li> </ul>	• 48-well Fast	• 384-well	• 96-well Fast	96-well Fast
	<ul> <li>8-strip tubes with caps</li> </ul>	• 96-well*	Strip tubes	• 384-well	• 384-well
	• 12-strip caps	<ul> <li>96-well Fast*</li> </ul>	<ul> <li>Strip tube caps</li> </ul>	96-well full-skirted	
		• 384-well*	<ul> <li>Strip tubes with attached caps</li> </ul>		
DNA-, RNase-, PCR inhibitor-free			Yes		
Colors available	Clear, or mixed packs containing red, orange, blue, and green	Clear	Clear Single-color packs (red, blue, green yellow, or clear) and 5-plate sample (one of each color)		Clear
Barcode available	No	Yes (1 or 2 sides)	No	Yes (3 sides)	Yes (3 sides)
Automation- compatible	No	Yes (for those with * above)	Yes	Yes	Yes









### ?

Did you know?

Low-profile plastics, also referred to as "Fast" tubes or plates, are generally required for fast (0.1 mL) thermal blocks. Fast plastics utilize lower volumes (0.1 mL) than the standard (0.2 mL) tubes or plates. The low profile minimizes the air space above the reaction, helping reduce the effects of evaporation and enhancing thermal conductivity. Learn more about PCR and qPCR plastics at <u>thermofisher.com/pcrplastics-education</u>.



### **PCR reagents**

DNA polymerase is an essential component for PCR because of its key role in synthesizing new DNA strands. Because of the sensitive and specific nature of PCR, it is important to choose high-quality enzymes and reagents to produce optimal results. The following are things to consider when choosing PCR enzymes.

#### Specificity

Nonspecific amplification is one of the major hurdles in PCR, since it can drastically impact the yield and sensitivity of target amplification. One way to help reduce nonspecific amplification is through the use of a hot-start DNA polymerase, which utilizes an antibody or chemical modification so that the polymerase becomes active only at the high temperature of the denaturation step. In addition to improving specificity, a hot-start DNA polymerase increases yield and allows convenient roomtemperature setup for high-throughput applications.

#### Thermostability

Since thermal cycling is a key feature of the conditions that enable the repetitive chain reaction of amplifying DNA, thermostability of the DNA polymerase to be used is also an important feature. Highly thermostable DNA polymerases are recommended for amplifying GC-rich or long templates that often require prolonged high-temperature reactions.

#### Fidelity

The fidelity, or proofreading capability, of a DNA polymerase is based on its 3' to 5' exonuclease activity, which corrects misincorporated nucleotides. This function is critical in applications such as cloning, sequencing, and site-directed mutagenesis, for accurate replication of DNA sequences.

#### Processivity

A DNA polymerase's processivity is defined as the number of nucleotides being incorporated in a single binding event. This property often reflects synthesis rate and speed, as well as affinity for its substrates. Therefore, highly processive DNA polymerases are beneficial to amplify challenging templates such as long, GC-rich, or inhibitor-containing DNA.

#### Primer annealing temperature

The primer annealing temperature of each DNA fragment to be amplified often needs optimization when designing a PCR protocol. To help simplify annealing and enable co-cycling of PCR assays, consider a DNA polymerase with a reaction buffer that allows a universal annealing temperature of 60°C for primers.



#### Did you know?

The residual bacterial DNA in recombinant PCR enzymes poses challenges in microbial genome analysis, such as accurately detecting bacterial strains by 16S rRNA gene sequences. To enable confidence and success in microbial PCR assays, choose PCR enzymes with controlled low levels of residual bacterial and human genomic DNA.

Find out more at thermofisher.com/broad-range-pcr.



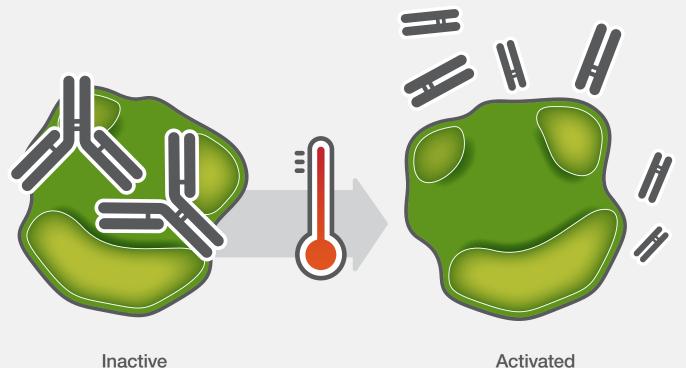
#### Helpful tip

Direct PCR is a way to help simplify PCR experiments, save time, and prevent sample loss in the workflow. Direct PCR allows you to amplify target sequences directly from the samples without the need to first isolate and purify the DNA.

Find out more at thermofisher.com/direct-pcr.



Antibody-based hot-start DNA polymerase and its activation in PCR for enhanced specificity



Activated



#### Helpful tip

One of the most common PCR troubleshooting issues is the presence of unwanted bands, or nonspecific amplification. To reduce nonspecific amplification:

- Use hot-start PCR
- Optimize annealing temperature
- Check primer design
- Prevent DNA cross-contamination
- Decrease template and/or primer concentration
- Optimize Mg<sup>2+</sup> concentration



#### Did you know?

High-quality dNTPs are essential for successful PCR reactions. Thermo Fisher is one of the few primary manufacturers of nucleotides. Our high-quality nucleotides include dNTPs, NTPs, and modified nucleotides to support PCR and related applications. Building your own master mix? Learn more about our offerings and robust quality testing.

Find out more at thermofisher.com/dNTPs.

## Choose the right PCR reagent for your research needs

A comprehensive portfolio of PCR enzymes and master mixes is available with the high performance and consistency you need. Start with the selection guide below to find the best enzyme for common PCR applications.

DNA polymerase	Invitrogen <sup>™</sup> Platinum <sup>™</sup> SuperFi <sup>™</sup> II DNA Polymerase	Invitrogen <sup>™</sup> Platinum <sup>™</sup> II <i>Taq</i> Hot-Start DNA Polymerase	Applied Biosystems <sup>™</sup> AmpliTaq Gold <sup>™</sup> 360 DNA Polymerase	Invitrogen <sup>™</sup> Platinum <sup>™</sup> Direct PCR Universal Master Mix
PCR type	High-fidelity PCR	Hot-start PCR	Hot-start PCR	Direct PCR
Capabilities	Highly accurate amplicon sequences, universal primer annealing, robust amplification of difficult targets	Universal primer annealing, fast DNA synthesis, detection of low-abundance targets	Chemical hot start	Detection of target DNA without genomic DNA purification
Technical specifications				
Fidelity compared to Taq polymerase	>300x	1x	1x	1x
Target length	Up to 20 kb*	Up to 5 kb	Up to 5 kb	Up to 8 kb
Hot-start modification	Antibody-mediated	Antibody-mediated	Chemical modification	Antibody-mediated
Speed	15–30 sec/kb	15 sec/kb	60 sec/kb	20 sec/kb
Universal primer annealing	Yes	Yes	No	Yes
Inhibitor tolerance	Yes	Yes	No	Yes
Blunt or 3'-A end	Blunt	З´-А	З´-А	З´-А
Compatible with Applied Biosystems <sup>™</sup> TaqMan <sup>™</sup> probes	No	Yes	Yes	No
Certified low level of bacterial gDNA	Yes	Yes	Yes	No
Applications				
Cloning and subcloning	•			
Site-directed mutagenesis	•			
GC-rich amplification	•	•	•	•
Template generation for sequencing	•	•	•	•
High-throughput PCR	•	•		•
Long PCR (up to 20 kb)	•			
Genotyping	•	•	•	•
Amplification of samples with suboptimal purity	•	•		•
Colony PCR	•	•	•	•
Multiplex PCR	•	•	•	•
Fast PCR	•	•		•

\* Amplification of up to 40 kb fragment sizes is possible, but may require additional optimization of reaction conditions and primer design.



#### Did you know?

These products are free of OPE and NPE, making them safer for aquatic life.

#### Learn more at thermofisher.com/pcrenzymes



## **Innovations for superior PCR**

To help your research move faster, we have continually improved our PCR enzymes and reagents. For example, the latest Invitrogen<sup>™</sup> Platinum<sup>™</sup> DNA polymerases are designed with the following key innovative features.

#### More robust and versatile

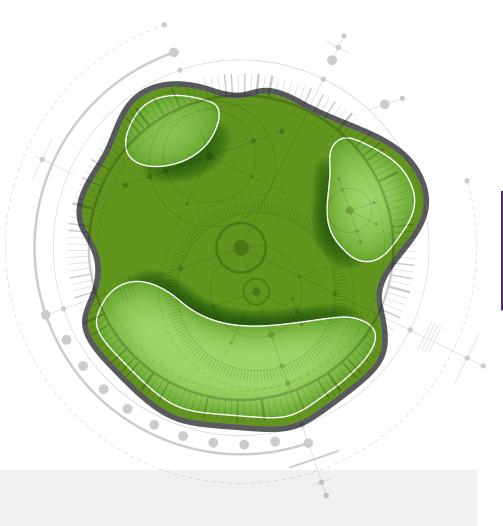
Advanced enzymatic engineering and methodology provide DNA polymerases with fast cycling, high tolerance of PCR inhibitors, and efficient amplification of challenging DNA like GC-rich sequences. These features help you amplify DNA targets confidently with speed and simplicity.

Find out more at thermofisher.com/platinumenzymes.

#### Universal primer annealing

The innovative Invitrogen<sup>™</sup> Platinum<sup>™</sup> PCR buffers enable universal primer annealing at 60°C. This design allows you to co-cycle different PCR assays (instead of running them sequentially), drastically reducing tedious optimization steps and saving time.

Find out more at thermofisher.com/universalannealing.



#### **Direct gel loading**

The latest Invitrogen<sup>™</sup> Platinum<sup>™</sup> DNA polymerase master mixes are available in a green buffer format that allows direct gel loading and eliminates tedious steps of dye addition, helping to reduce pipetting errors. DNA migration is easily tracked with two dyes (blue and yellow) that are readily visible during electrophoresis (depicted in the lanes for 5 and 15 min in the figure to the right).





PCF

## **Custom DNA oligos**

With a complete range of custom-synthesized oligonucleotide primers, probes, and genes, we recognize the need for quality, reliability, and convenience.

Invitrogen<sup>™</sup> custom DNA oligos are synthesized on state-of-the-art automated systems to help increase performance, speed, and capacity. Available in a range of synthesis scales, purification options, and modifications, oligos are analyzed by mass spectrometry to help ensure the quality of all end products. This means you will receive high-quality custom DNA oligos quickly and efficiently.

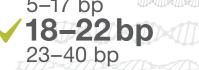
Choose the right oligos and purification methods for your applications at thermofisher.com/oligos.

#### Best practices for primer design

Good primer design is essential for a successful PCR assay. For design tips, review the infographic below or go to thermofisher.com/primerdesign.

#### Length 5–17 bp

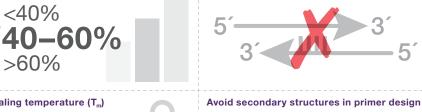
GC content (%)





Avoid low-complexity sequences in the template and primer, and runs of the same nucleotide

#### Avoid self-dimers (complementarity of >3 bases



Annealing temperature (T<sub>m</sub>) <55°C ✓55–65°C >65°C



Find out more at thermofisher.com/oligos



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#### Primer design made easy

Whether you're performing PCR, cloning, or capillary electrophoresis (CE) sequencing, take advantage of the benefits offered by our robust and easy-to-use Primer3-based Invitrogen<sup>™</sup> OligoPerfect<sup>™</sup> Designer.

- Speed up-design primers for up to 50 genes at the same time
- Store your data-ability to save your projects
- Work smarter-recognizes .txt and .fasta file types
- Order with ease-select and add primers directly to your online cart from the design tool\*

Try the OligoPerfect Designer at **thermofisher.com/oligoperfect** or visit the oligo utility hub for our full suite of tools and calculators at **thermofisher.com/oligotools**.



#### Did you know?

Thermo Fisher Scientific offers scales beyond 10 µmol up to kilograms, and employs a team of manufacturing scientists dedicated to custom method development for unique modifications.

For information on large-scale and complex project capabilities, visit <u>thermofisher.com/largescaleoligos</u>.

Purification					25	50	200	1	10
method	Description	Benefit	Applications		nmol	nmol	nmol	µmol	µmol
<b>Desalt</b> 25 nmol, 10–100 nt; 50 nmol, 5–100 nt	Oligos are processed through a normal-phase chromatography column, which removes salts but not truncated sequences	A salt-free DNA solution, ready to use; suitable for many PCR and sequencing applications without further purification	<ul><li>Endpoint PCR</li><li>Isothermal sequencing</li><li>Fluorescent sequencing</li></ul>	<ul> <li>Microarrays</li> <li>Amplified fragment length polymorphism (AFLP) analysis</li> </ul>	•	•	•	•	•
<b>Cartridge</b> 50 nmol–1 µmol, 7–55 nt	Based on reverse-phase chromatography; removes truncated sequences from the completed synthesis	Provides full-length sequences necessary for some applications	<ul> <li>Antisense oligos (ASO)</li> <li>First-strand cDNA synthesis for generation</li> </ul>	• PCR using oligos with critical 5' sequences (e.g., restriction	NA	•	٠	•	NA
HPLC ≥50 nmol, 10–55 nt; long oligo HPLC available	Reverse-phase high-performance liquid chromatography (HPLC) removes truncated sequences or unincorporated labels the same way as cartridge purification	Ensures highly purified primer required in some applications (≥85% full length)	<ul><li>of libraries</li><li>Fluorescent sequencing</li><li>Gel shift assays</li></ul>	<ul> <li>endonuclease sites, RNA polymerase promoters)</li> <li>Production of cloning adapters</li> </ul>	NA	۰	۲	۲	•
<b>PAGE</b> ≥200 nmol, 7–100 nt	Polyacrylamide gel electrophoresis (PAGE) is a method used to differentiate full- length product from truncated sequences based on size and conformation	Provides the highest percentage of full-length oligos (≥85%) required for certain demanding applications such as mutagenesis or adapter production		Site-directed     mutagenesis	NA	NA	•	•	•

\* Pricing and "Add to cart" feature may vary by geographic region. For questions, please contact your local office or distributor at thermofisher.com/contactus.

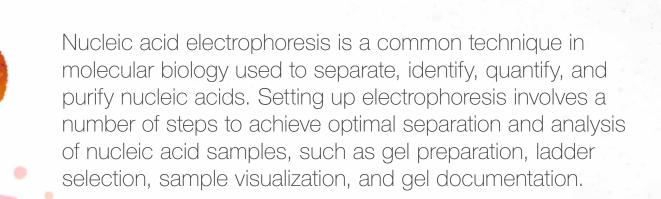
## Electrophoresis

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Find technical resources on nucleic acid electrophoresis at **thermofisher.com/na-electrophoresis-education** 

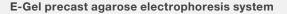
## **Nucleic acid electrophoresis**

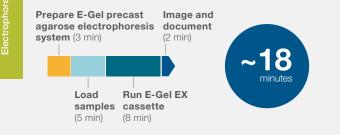
Choosing the right tools for nucleic acid electrophoresis can significantly improve and accelerate results, enabling you to address downstream applications sooner.

Determining the appropriate gel type and gel concentration is an essential step that will help streamline the separation of nucleic acids. Learn more about convenient reagents for agarose gel electrophoresis, including hassle-free precast Invitrogen<sup>™</sup> E-Gel<sup>™</sup> Agarose Gels and pour-your-own Invitrogen<sup>™</sup> UltraPure<sup>™</sup> Agarose reagents, in this section.

If you need	Convenience, rapid results, and a safer workflow	High-quality reagents, a versatile workflow, and cost savings
Product	E-Gel Agarose Gels	UltraPure Agarose
Product format	Precast agarose cassettes	Powder
Buffer	Dry-none*	TBE or TAE
Protocol time (approx.)	18 min	120 min
Ready to use	Yes	No
Get more information at	thermofisher.com/egel	thermofisher.com/ultrapure

\* Note: This is a dry precast electrophoresis system.





#### Traditional DNA electrophoresis workflow



#### Find out more at thermofisher.com/electrophoresis



## Simplify electrophoresis with E-Gel precast agarose cassettes

#### E-Gel precast gels

Using precast agarose gels can simplify the nucleic acid electrophoresis workflow. Invitrogen<sup>™</sup> E-Gel<sup>™</sup> precast gels are self-contained and ready for use with the agarose, electrodes, and DNA stain packaged inside a disposable cassette. There are no gels to pour, buffers to make, staining or destaining steps to perform, or gel boxes to assemble. Just load your samples and run.

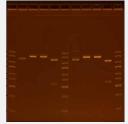
E-Gel precast gels offer excellent resolution and clarity in ≤18 minutes and are ideal for analyzing PCR products, restriction digests, plasmid preparations, and genotyping products. To help simplify cloning workflows, Invitrogen<sup>™</sup> E-Gel<sup>™</sup> CloneWell<sup>™</sup> II gels use a double-comb design to enable recovery of purified DNA for downstream applications, without the need for additional purification kits or steps.

Find out more at thermofisher.com/egel.

#### E-Gel precast gels-faster and safer workflows

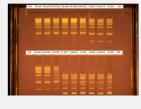
#### **Restriction digest analysis:**

• Fast and easy preparation of vectors for cloning experiments using FastDigest restriction enzymes and the Invitrogen<sup>™</sup> E-Gel<sup>™</sup> Power Snap Plus Electrophoresis System



#### **RNA** analysis:

 Optimization of loading conditions of RNA markers on an Invitrogen<sup>™</sup> E-Gel<sup>™</sup> system





Convenient and accurate

such as universal target

fragments, for genotyping

visualization of DNA fragments,

Genotyping:



Load

#### Helpful tips

E-Gel precast gels are available in a variety of formats for routine and high-throughput applications, with different stains and agarose percentages (0.8%, 1%, 2%, and 4%). To find the right gel for your needs, see the selection guide at thermofisher.com/egelselection.

2 Run

Choose Invitrogen<sup>™</sup> E-Gel<sup>™</sup> DNA ladders for precise electrophoresis band analysis with exceptional DNA fragment purity and quality, reduced dye masking, and improved ladder migration on E-Gel precast agarose gels. Find out more at thermofisher.com/egel-ladders.

**3** Analyze



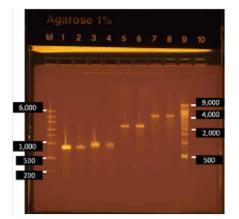


## **E-Gel application examples**

#### Fast and accurate analysis of RNA with E-Gel EX agarose gels

As RNA is the starting material for many workflows, its quality is important. Because RNA tends to form secondary structures and is prone to degradation, traditional methods of agarose gel electrophoresis are challenging. Traditional methods require additional time for buffer preparation, hand-pouring of gels, and prolonged run times. Because denaturing agents need to be added, gels are run in a fume hood.

With Invitrogen<sup>™</sup> E-Gel<sup>™</sup> EX Agarose Gels, several labor-intensive steps are removed from this process. The use of toxic denaturing reagents in the gel is no longer required. To denature the RNA, samples are treated with 50% formamide and heated at 70°C for 10 min, and then chilled on ice prior to loading on the E-Gel EX gel. The figure to the right shows clear, crisp bands from a diverse collection of RNA samples. Lanes 1–8 are samples, lanes M and 9 are ladders.



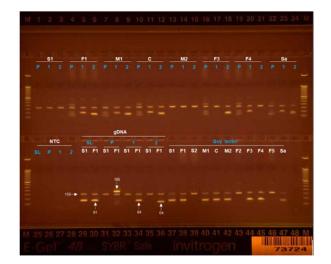
#### Genotyping detection of GMOs in soy products

Invitrogen<sup>™</sup> E-Gel<sup>™</sup> 48 and 96 Agarose Gels are useful for the rapid testing of larger numbers of samples. In this example, store-bought foods with unknown GMO status were tested. Multiplex PCR was used to amplify a known GMO sequence and an internal control. A sample with a known genetic modification was used as a positive control (F1). The PCR products were separated and visualized on an E-Gel 48-well 2% agarose gel with Invitrogen<sup>™</sup> SYBR<sup>™</sup> Safe stain. Read the **application note** for more details. Two bands indicate the presence of genetic modification in many of the samples.



#### Helpful tips

Improve your nucleic acid electrophoresis. View our <u>webinar</u> Nucleic Acid Agarose Gel Electrophoresis: Techniques, Innovations, and Common Errors



## Select your E-Gel electrophoresis device

Integrate your electrophoresis running and imaging into a single small device

Enjoy speed and convenience





	Invitrogen <sup>™</sup> E-Gel <sup>™</sup> Power Snap Plus Electrophoresis System	Invitrogen <sup>™</sup> E-Gel <sup>™</sup> Power Snap Electrophoresis System
Which system is right for you?	<ul> <li>&gt; Do you use electrophoresis often?</li> <li>&gt; Would you like to store your gel images on internal servers or the cloud?</li> <li>&gt; Do you want to perform quantitative analysis of your gels?</li> <li>&gt; Do you value speed?</li> <li>&gt; Would you like to store, share, and analyze gel images online?</li> </ul>	<ul> <li>&gt; Do you run less than 25 samples at a time?</li> <li>&gt; Do you want a simple and fast solution for your electrophoresis?</li> <li>&gt; Do you need to save bench space?</li> </ul>
Key difference	Low- to high-throughput analysis	Low-throughput analysis
Memory	64 GB	32 GB
Connectivity	USB drive, ethernet, Wi-Fi, printer	USB drive
Sample throughput	Up to 96 samples	Up to 22 samples
Applications	Genotyping, fast PCR analysis, routine electrophoresis, and cloning	Routine electrophoresis and cloning
Software	Invitrogen™ iBright™ Analysis Software	NA
Find out more	thermofisher.com/powersnapplus	thermofisher.com/powersnap



## **Electrophoresis reagents**

Choose high-quality agarose to pour your own gels. Select a ladder of the correct size range and an improved DNA stain for best detection and size estimation of DNA fragments.

#### **DNA** stains

Detection of nucleic acid samples in gels can be improved using fluorescent dyes that are safer or more sensitive than ethidium bromide. Invitrogen<sup>™</sup> SYBR<sup>™</sup> Safe and SYBR<sup>™</sup> Gold stains provide greater safety or sensitivity with lower background fluorescence than the conventional ethidium bromide stain.

SYBR Safe stain is specifically formulated to be less hazardous than ethidium bromide and reduces your exposure to UV light.

Find out more at <u>thermofisher.com/stains</u> and <u>thermofisher.com/sybrsafe</u>.

#### UltraPure reagents for electrophoresis

Invitrogen<sup>™</sup> UltraPure<sup>™</sup> reagents are specifically formulated to help meet your nucleic acid analysis and purification needs. Invitrogen<sup>™</sup> UltraPure<sup>™</sup> Agarose and other reagents are made from highly pure biochemicals for maximum reliability and superior performance.

Find out more at thermofisher.com/ultrapure.

#### **DNA ladders**

Invitrogen<sup>™</sup> DNA ladders are available in a wide variety of size ranges (10 to 48,502 bp) and formats for different applications. To create DNA ladders of exceptional quality, each fragment is purified individually using proprietary chromatography-based technology. Our DNA ladders are stable during prolonged storage at room temperature and after multiple freeze-thaw cycles.

Find out more at thermofisher.com/ladders.





#### Fluorescent nucleic acid gel stains

	Safer detection	Ultimate detection
	SYBR Safe stain	SYBR Gold stain
Sensitivity (dsDNA)	Sensitive (>3 ng)	Ultrasensitive (>0.1 ng)
Less hazardous and more environmentally friendly	•	
Improved cloning efficiency	•	•

#### Did you know?

Chromatographically purified nucleic acid fragments are considered the trusted standard for ladders since the technology provides high control over quality, banding pattern, intensity, and quantity for ladder composition.

#### Learn more at

thermofisher.com/na-electrophoresis-education.



## Cloning

Molecular cloning and synthetic DNA assembly are essential techniques in biotechnology for DNA sequence assembly. Restriction enzymes, TOPO PCR cloning, Gibson Assembly cloning, Golden Gate cloning, and Gateway cloning enable precise assembly of simple and complex constructs.

Find technical resources on molecular cloning at thermofisher.com/cloningeducation

## **Cloning and gene synthesis**

From restriction enzymes to gene synthesis, a large portfolio of tools and resources is available to help you obtain high-quality cloned DNA for your next discovery.

	Move fragments from one vector to another	Clone PCR fragments	Express genes in multiple systems		e inserts from fragments	Assemble or clone multiple fragments	Sequenced, cloned genes
		$\checkmark$	$\checkmark$	,			
Method Key benefits	<ul> <li>Thermo Scientific<sup>™</sup></li> <li>FastDigest<sup>™</sup> restriction enzymes</li> <li>Familiarity, flexibility, convenience, time savings</li> <li>Universal protocol and complete digestion in 5–15 minutes in one buffer</li> <li>100% buffer compatibility with downstream applications</li> <li>Direct loading on gels</li> </ul>	Invitrogen <sup>™</sup> TOPO <sup>™</sup> cloning • >95% efficiency, 5-minute PCR cloning • Available in a variety of formats and sizes	Invitrogen <sup>™</sup> Gateway <sup>™</sup> cloning • High-throughput and high-efficiency shuttling among multiple expression vectors	<ul> <li>Invitrogen<sup>™</sup> GeneArt<sup>™</sup> seamless cloning and GeneArt<sup>™</sup> Gibson Assembly<sup>™</sup> cloning kits</li> <li>Seamless multifragment assembly by homologous recombination</li> <li>Directional cloning of up to 15 fragments</li> <li>Up to 95% efficiency and 15-minute cloning</li> </ul>	<ul> <li>Invitrogen<sup>™</sup> GeneArt<sup>™</sup></li> <li>Type IIS assembly</li> <li>One-tube seamless multifragment assembly by simultaneous restriction digestion and ligation</li> <li>Directional cloning of up to 8 fragments, for up to 20 kb total</li> <li>Efficient for repetitive and very small sequences</li> </ul>	Invitrogen <sup>™</sup> GeneArt <sup>™</sup> Strings <sup>™</sup> DNA Fragments • Synthesized DNA fragments ready to clone via the method of your choice • No starting DNA required • Pool sequence-verified	<ul> <li>Invitrogen<sup>™</sup> GeneArt<sup>™</sup> Gene Synthesis</li> <li>Custom-cloned genes in your choice of vector</li> <li>Sequence-verified</li> <li>Can be optimized for a specific host for maximal protein expression</li> <li>No starting DNA required</li> </ul>
Technology basics	Restriction digestion and ligation	Topoisomerase-based, ligase-free cloning	<ul> <li>Single-step, directional, and site-specific DNA recombination</li> <li>Restriction enzyme- and ligase-free</li> </ul>	<ul> <li>End-terminal homology recombination using overlapping sequences</li> <li>Transformation- associated recombination (TAR) in Saccharomyces cerevisiae</li> </ul>	Type IIS restriction and ligation in a single reaction	<ul> <li>Linear dsDNA assembled from pooled synthetic oligonucleotides</li> <li>200–3,000 bp, also available in library format with randomized bases</li> </ul>	<ul> <li>DNA of interest cloned in vector</li> <li>100% sequence-verified with quality assurance documentation</li> </ul>
Online tools available	FastDigest selection tool	TOPO PCR selection tool	Vector list	<u>Invitrogen</u> Primer and Cons	<u><sup>≝</sup> GeneArt™</u> struct Design Tool	<u>Invitrogen™</u> Instant Designer for d	<u>' GeneArt™</u> esign and optimization
Needs DNA source material (gene in plasmid, library, etc.)	•	•	•	•	•		
Use your own vector	•		*	٠	•	•	•

\* Vector needs to be converted with Invitrogen<sup>™</sup> Gateway<sup>™</sup> Vector Conversion System with One Shot<sup>™</sup> *ccdB* Survival<sup>™</sup> 2 T1<sup>R</sup> Competent Cells.



## **Restriction enzyme cloning**

Found naturally in bacteria, restriction enzymes recognize and cleave specific DNA sequences. Cleavage results in "sticky" ends (5' or 3' protruding ends) or blunt ends that allow DNA inserts to be cloned into vectors with compatible ends. Star activity, buffer compatibility, and varying protocols for complete digestion are some common hurdles in restriction digestion.

#### FastDigest restriction enzymes

To simplify cloning, we offer FastDigest enzymes—an advanced line of restriction enzymes that share buffer compatibility with downstream modifying enzymes. Benefits include:

- Complete digestion in 5–15 min
- Universal buffer for multiple digestions for any combination of enzymes
- No sequential digestions and buffer changes
- 176 unique enzymes
- Direct loading of reaction mixture on gels

See our enzyme selection tool at thermofisher.com/fastdigest.

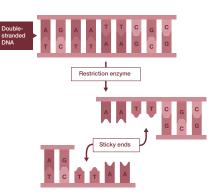
#### **DNA/RNA-modifying enzymes**

Complete your cloning reaction with modifying enzymes that have 100% activity in FastDigest and FastDigest Green buffers. These include:

- Thermo Scientific<sup>™</sup> T4 DNA Ligase—used to join the ends of DNA fragments and vectors in a fast 10 min reaction
- Thermo Scientific<sup>™</sup> FastAP<sup>™</sup> Thermosensitive Alkaline Phosphatase—used for the dephosphorylation of cloning vectors to prevent recircularization. Fast 10 min reaction with complete inactivation in a 5 min reaction
- Thermo Scientific<sup>™</sup> T4 Polynucleotide Kinase phosphorylates PCR products, oligonucleotides, and other DNA prior to ligation reactions
- Thermo Scientific<sup>™</sup> DNA polymerases—used for DNA blunting by filling in 5'-overhangs and second-strand synthesis of cDNA
- Invitrogen<sup>™</sup> CorrectASE<sup>™</sup> enzyme—utilized to enhance the accuracy of synthetic gene and DNA fragment assembly by removing mismatches





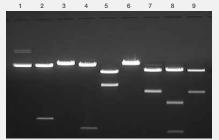


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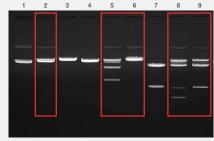
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Plasmid DNA digested with supplier N's enzymes



Plasmid DNA digested with FastDigest enzymes



1: Undigested	6: FastDigest XmaJI	1: Undigested	6: AvrII
plasmid DNA	7: FastDigest Eco31I	plasmid DNA	7: Bsal
2: FastDigest Bcul	8: FastDigest Eco52I	2: Spel-HF	8: Eaql-HF
3: FastDigest Xbal	9: FastDigest BgIII	3: Xbal	9: Ball
4: FastDigest Ndel	5. I datbigeat byin	4: Ndel	J. Dgn
5: FastDigest Sall		5: Sall-HF	

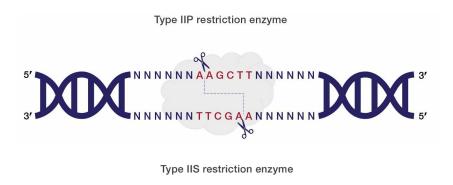
Comparison of digestion efficiencies of restriction enzymes. FastDigest restriction enzymes (A) digest plasmid DNA much more efficiently than supplier N's enzymes (B). In this experiment, 1 µg each of plasmid DNA was digested for ~15 min with the indicated restriction enzymes, using supplier N's protocol. Incomplete digestion is shown in red boxes.

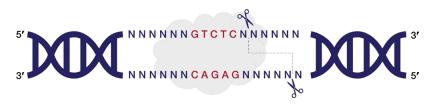
#### Type IIS restriction enzymes

A specific group of restriction enzymes called Type IIS endonucleases cleave DNA outside of their recognition sequences. In combination with DNA ligase, Type IIS restriction enzymes are utilized to drive the insertion of one or several DNA fragments into a recipient vector without the inclusion of residual restriction enzyme sites and other unwanted DNA sequences at fragment junctions (scarless cloning). Commonly referred to as Golden Gate cloning, this method is useful for the construction of large DNA fragments.

Find FastDigest Type IIS enzymes at thermofisher.com/fastdigesttypeiis.

For GeneArt Type IIS Assembly Kits, go to thermofisher.com/typeiis.







#### Did you know?

Both Gateway and Type IIS restriction enzyme (Golden Gate) cloning technologies have been used for CRISPR-based genome editing in plants. View an article and references <u>here</u>.



## PCR cloning

PCR cloning is a method in which double-stranded DNA fragments amplified by PCR are ligated into a vector. With PCR amplification, this cloning technique requires much less starting material for the insert sequence and allows introduction of new restriction and/or recombination sites to the 5' end of the inserts.

#### **TOPO** cloning

TOPO PCR cloning technology was developed to help improve cloning efficiency, simplify protocol setup, and accommodate a wide range of PCR insert sizes. TOPO cloning vectors are linearized by the activity of topoisomerase I (which also has a ligase function) that is covalently bound to the 3' phosphate on each end (see figure below). This system enables the vectors to be joined to PCR inserts with compatible ends (with up to 95% efficiency), without the need for additional ligation steps, in 5 minutes.

Find out more at thermofisher.com/topo.



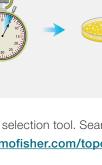
To shuttle a DNA fragment of interest (insert) among vectors, the Gateway cloning system offers site-specific, recombinase-based cloning. It maintains the insert's proper orientation and reading frame during shuttling using the Gateway vectors. Once a gene is cloned into an entry clone, you can then move the DNA fragment into one or more destination vectors simultaneously.

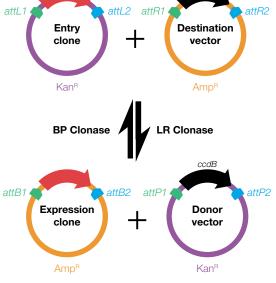
ccdB

Find out more at thermofisher.com/gatewaycloning.



Quickly find your TOPO cloning kit with our interactive selection tool. Search by application, vector, or desired competent cells at thermofisher.com/topoguide.





Gateway cloning system reactions. The scheme shows the four types of plasmids and enzyme mixes involved in Gateway cloning reactions. Red arrow represents the fragment of interest. Adapted from Katzen F (2007) Expert Opin Drug Discov 2(4):571-589.

#### Did you know?

The Invitrogen<sup>™</sup> TOPO<sup>™</sup> XL-2 Complete PCR Cloning Kit provides all the necessary elements for highly efficient cloning of extra-long PCR products from 1 to 13 kb. thermofisher.com/topoxl2



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Cloning

#### GeneArt Gibson Assembly and seamless cloning kits

Several methods are available to assemble multiple PCR or synthesized DNA fragments. These include seamless cloning, Type IIS restriction enzyme (Golden Gate) cloning, and Gibson Assembly cloning. Using our kits, cloning can typically take place within half a day.

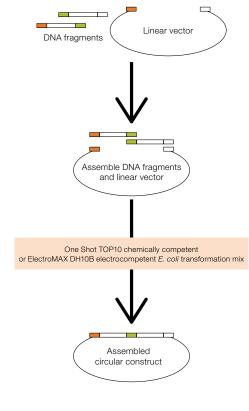
Invitrogen<sup>™</sup> GeneArt<sup>™</sup> Gibson Assembly<sup>™</sup> kits allow for the simultaneous assembly of up to 15 very large DNA fragments (up to a total of 100 kb) to create precise and seamless constructs with no additional sequences, in highly efficient reactions. This cloning method circumvents the need for multiple rounds of restriction enzyme analysis and digestion, DNA end repair, dephosphorylation, ligation, enzyme inactivation, and cleanup, and is a powerful tool in synthetic biology.

GeneArt Gibson Assembly kits offer these benefits:

- Assembly of up to 15 fragments to build seamless clones
- Cloning efficiencies up to >95%
- Choice of complete kits with competent cells or master mixes

Invitrogen<sup>™</sup> GeneArt<sup>™</sup> Seamless Cloning and Assembly Kits allow the assembly of up to 4 DNA fragments. The Invitrogen<sup>™</sup> GeneArt<sup>™</sup> Seamless PLUS Cloning and Assembly Kit comes with two linearized vectors for expression in either *E. coli* or *Saccharomyces cerevisiae*.

#### Comparison of seamless cloning kits





#### Did you know?

The Gibson Assembly method has been referenced in thousands of peer-reviewed publications and is a powerful method that can be used to seamlessly construct synthetic and natural genes, genetic pathways, and entire genomes [1].

Invitrogen product	<u>GeneArt<sup>™</sup> Seamless</u> <u>Cloning and Assembly Kit</u>	<u>GeneArt<sup>™</sup> Seamless PLUS</u> <u>Cloning and Assembly Kit</u>	<u>GeneArt<sup>™</sup> Type IIS</u> <u>assembly kits</u>	<u>GeneArt<sup>™</sup> Gibson Assembly™</u> <u>HiFi and EX Kits</u>
Number of fragments for simultaneous assembly	Up to 4	Up to 4	Up to 8	Up to 6 (HiFi) and 15 (EX)
Reaction time	30 min	15–60 min	5–60 min	15–60 min
Homologous overlaps	15 nt	15–80 nt	4–6 nt	>20 nt
Ligase in the master mix	No	No	Yes	Yes
Vector included	Yes (pUC19L)	Yes (pYES7L and pUC19L)	Yes (pType IIS recipient vector)	No
Mutagenesis protocol	Yes	Yes	No	Yes

1. Gibson DG et al. (2009) Enzymatic assembly of DNA molecules up to several hundred kilobases. Nat Methods 6(5):343–345.



## **Cloning with synthetic DNA**

Save time by ordering your gene cloned into a vector of interest. Your gene can also be delivered as a DNA fragment similar to a PCR product. The only starting material required is the sequence.

#### GeneArt gene synthesis and cloned genes

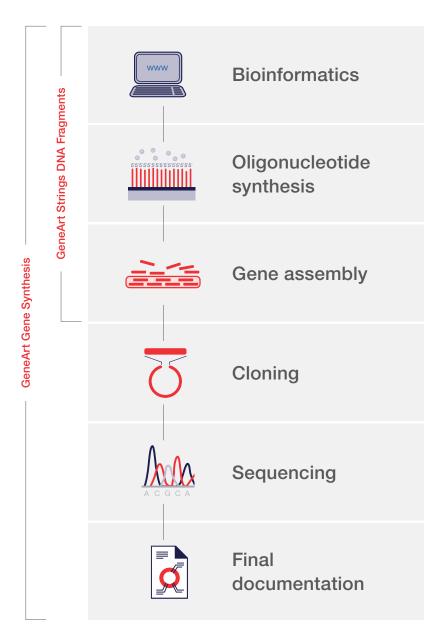
Using the Invitrogen<sup>™</sup> GeneArt<sup>™</sup> Custom Gene Synthesis service is a reliable and cost-effective way to obtain customized DNA constructs with 100% sequence accuracy. The GeneArt gene synthesis service offers a variety of options, including accelerated production, cloning into your vector of choice, and plasmid preparation.

- An industry leader for reliable delivery and performance
- Proprietary, peer-reviewed, and widely used Invitrogen<sup>™</sup> GeneArt<sup>™</sup> GeneOptimizer<sup>™</sup> algorithm optimizes genes for maximum protein expression, at no additional cost
- High-quality production with ISO 9001:2015 certification
- Delivery in tube format with a minimum of 5  $\mu g$  DNA

#### **GeneArt Strings DNA fragments**

Invitrogen<sup>™</sup> GeneArt<sup>™</sup> Strings<sup>™</sup> DNA fragments are an economical and time-saving alternative to PCR. They are made by the same high-quality process used for gene synthesis, and they can be prepared quickly for cloning.

- Up to 3 kb available with fast production
- Can be optimized with the GeneArt GeneOptimizer algorithm
- Delivered dried, ready for resuspension and cloning with any method

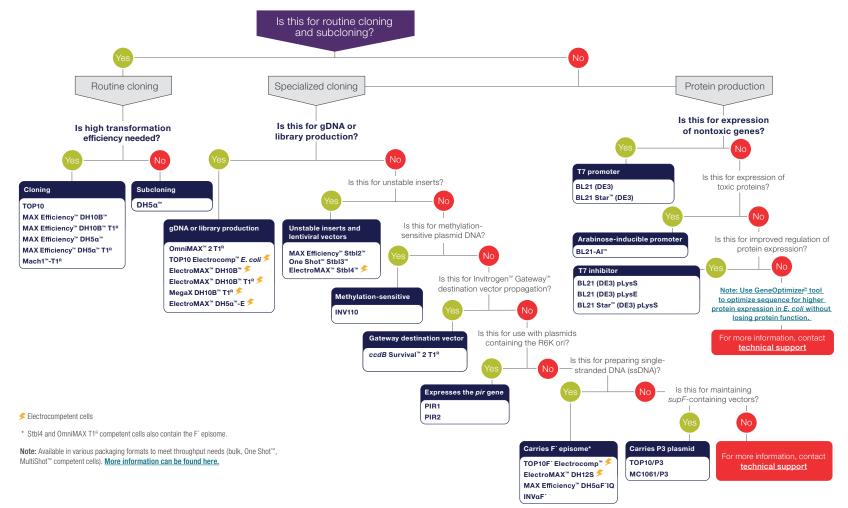




### **Transformation**

Once the DNA fragment is cloned into a vector, transformation into bacteria is performed to enable propagation of sufficient quantities of the cloned DNA for downstream experiments. Selection of competent cells for transformation depends upon the transformation methods, strain genotypes, plasmid characteristics, and desired applications. Visit <u>thermofisher.com/compcells-education</u> for technical resources on competent cells.





#### Find out more at thermofisher.com/compcells



## **Transformation (cont.)**

#### Medium- and high-throughput transformation

Performing bacterial transformations one by one can be very time-consuming and create a bottleneck in your experimental workflow. There are times when medium- and high-throughput transformation options are desired. Invitrogen<sup>™</sup> MultiShot<sup>™</sup> chemically competent cells provide three flexible product formats to meet your throughput needs.

#### Find out more at thermofisher.com/multishot



#### StripWell format

- Medium-throughput option
- Twelve 8-tube strips
- Suitable for 1–96 transformations
- Five *E. coli* strains available

#### Bacterial growth media

Sterile, quality growth media and plates are essential for selection and growth of transformed cells. Premade solutions offer convenience and consistency for your cloning workflows. Featured products include:



#### • Invitrogen<sup>™</sup> One Shot<sup>™</sup> LB Agar Plates

with or without Antibiotics—ready-to-use pre-poured plates with a longer shelf life of 3.5 months

- <u>Gibco<sup>™</sup> LB Broth (1X)</u> and <u>Gibco<sup>™</sup> Terrific Broth</u>—popular growth media, available as convenient ready-to-use liquids
- Invitrogen<sup>™</sup> MagicMedia<sup>™</sup> E. coli Expression Medium</u>—autoinduction medium for protein expression in E. coli

Other available media include Invitrogen<sup>™</sup> S.O.C., 2-YT Broth, M9 Minimal Salts, and Gibco<sup>™</sup> Bacto<sup>™</sup> CD Supreme Fermentation Production Medium (FPM). Visit <u>thermofisher.com/growthmedia</u> to learn more.

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#### Did you know?

Invitrogen competent cells can be provided in custom configurations per your request. Large and custom volumes as well as multiple formats are at your fingertips. Simply email us at customorders@thermofisher.com.



#### Did you know?

One Shot LB Agar Plates are available with a 3.5-month shelf life. Learn more at thermofisher.com/growthmedia.

#### FlexPlate format

- High-throughput option
- 96-well plate separates into 12 x 8-well segments
- Manual and automated platform transformations
- Six *E. coli* strains available





#### 96-well plate

- Highest-throughput option
- Five 96-well plates
- Available with the TOP10 strain
- Stable replication of high copy number plasmids

Cloning

# Isothermal amplification

Isothermal amplification is a technique that utilizes enzymes, typically strand-displacing polymerases, to amplify nucleic acid sequences at a constant temperature. This results in continuous and exponential amplification that is not limited by the constraint of thermal cycling.

Find technical and educational resources about isothermal amplification at thermofisher.com/isothermal



### Isothermal amplification overview

Isothermal nucleic acid amplification techniques (INAATs) are fast alternatives to PCR that enable exponential amplification of nucleic acids at constant temperatures. PCR requires repeated denaturation steps at 95°C to separate the DNA strands for the primers to bind. In contrast, INAAT uses DNA polymerases with strand-displacing properties. This eliminates the need for temperature cycling, resulting in reaction times that are as short as 15 min. There are many known INAATs, including loop-mediated amplification (LAMP), multiple displacement amplification (MDA), whole genome amplification (WGA), recombinase polymerase amplification (RPA), and more. Different methods use specific enzymes and reaction conditions. Once assembled, isothermal reactions can be run on a heat block, thermal cycler, or real-time PCR instrument. Using a real-time PCR instrument also enables fluorescent detection of reaction products. Alternatively, positive results can be detected visually using DNA-binding dyes like Invitrogen<sup>™</sup> SYBR<sup>™</sup> Green Nucleic Acid Gel Stain. Many instruments, like microplate readers, can be also used to measure the dye-labeled reaction products. Reactions can be further analyzed by agarose gel electrophoresis.

#### Comparison of seamless cloning kits

Technology	Reaction temperature	Reaction time	Detection method
Loop-mediated isothermal amplification (LAMP)	60-65°C	15–60 min	Fluorescence, colorimetry, turbidity, lateral flow
Multiple displacement amplification (MDA)	30-40°C	60–180 min	Fluorescence, colorimetry
Whole genome amplification (WGA)	30-40°C	60–180 min	Fluorescence, colorimetry
Recombinase polymerase amplification (RPA)	37°C	30–60 min	Fluorescence, lateral flow
Rolling circle amplification (RCA)	30-65°C	60–90 min	Fluorescence, colorimetry, turbidity

## amplificati

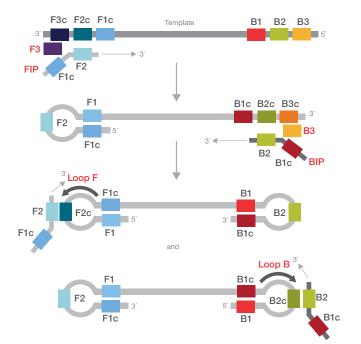


## Loop-mediated isothermal amplification (LAMP)

#### LAMP overview

One commonly used isothermal amplification technique is loop-mediated isothermal amplification (LAMP), which utilizes a set of four to six primers and a strand-displacing polymerase, such as Invitrogen<sup>™</sup> Lyo-ready Bst DNA Polymerase, to amplify target DNA at a constant temperature. The LAMP reaction produces a large amount of DNA amplicons, with a characteristic ladder-like pattern that can be visualized by gel electrophoresis, or that can be detected by turbidity, fluorescence, or colorimetry.

Loop-mediated isothermal amplification (LAMP)



The amplification process begins with the invasion of an inner primer into the target nucleic acid sequence, followed by extension via a strand-displacing DNA polymerase. As the extension proceeds, the first product is displaced, and an outer primer anneals to the newly synthesized strand, forming a self-hybridizing loop structure. This structure contains multiple sites for amplification initiation and serves as a seed for exponential LAMP reactions.

Find out more at thermofisher.com/lamp.

#### SuperScript IV RT-LAMP Master Mix

Invitrogen<sup>™</sup> SuperScript<sup>™</sup> IV RT-LAMP Master Mix is a reverse transcription loop-mediated isothermal amplification (RT-LAMP)-based solution for fast and simple detection of various pathogens, including influenza virus, measles virus, *S. enterica*, *S. aureus*, SARS-CoV-2, and others. Our master mix reagents provide maximum flexibility to help optimize and accelerate your pathogen research and surveillance.

#### **Product highlights**

- Fast—RNA and DNA target detection in as little as 5 minutes with evolved Bst DNA polymerase
- Efficient—one-step reaction for reverse transcription of RNA to cDNA with Invitrogen<sup>™</sup> SuperScript<sup>™</sup> IV Reverse Transcriptase
- Sensitive-greater sensitivity and specificity utilizing Invitrogen<sup>™</sup> RNaseOUT<sup>™</sup> Recombinant Ribonuclease Inhibitor and an optimized buffer
- Simple-streamlined workflow: single-tube format, only requires a 65°C heating block
- Flexible—several options for evaluating results, including real-time and endpoint detection methods



Find out more about SuperScript IV RT-LAMP Master Mix here.

#### Lyo-ready Bst DNA Polymerase

Lyo-ready Bst DNA Polymerase is an engineered version of the Bst DNA polymerase large fragment, which shows a significantly faster reaction speed, increased sensitivity, and tolerance to inhibitors.

Lyo-ready Bst DNA Polymerase provides maximum flexibility to optimize your LAMP reaction and works with various types of pathogens, including human adenovirus, measles virus, SARS-CoV-2, and other pathogens.

#### **Product highlights**

- Fast—amplifies targets in as little as 10 minutes
- Sensitive-provides sensitivity down to 50 copies
- **Robust**—amplifies even from inhibitor-containing RNA/DNA samples
- Flexible—provides the ability to optimize your LAMP or RT-LAMP reaction

#### Tips:

- 1. Did you know? The term "lyo-ready" refers to an enzyme that is provided in a liquid formulation without glycerol, making it compatible with microfluidics-based systems and various downstream applications such as lyophilization. Furthermore, it maintains the necessary stability and activity levels for direct enzymatic reactions.
- 2. To minimize nonspecific amplification in LAMP, follow these steps:
  - a. **Prevent cross-contamination:** Use uncontaminated reagents and maintain a clean work environment.
  - b. Enhance primer design: Optimize primer sequences for improved specificity.
  - c. **Optimize reaction conditions:** Adjust Lyo-Ready Bst DNA Polymerase amount and reaction time.







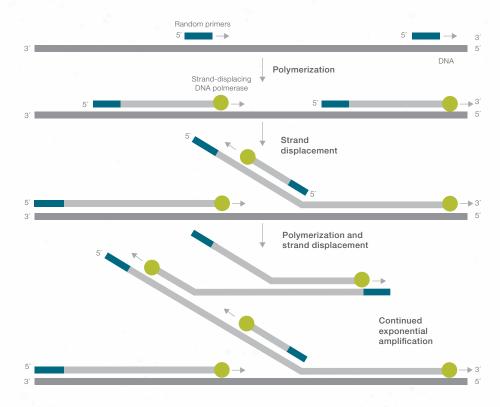
## Rolling circle amplification (RCA) and whole genome amplification (WGA)

WGA is a popular technique used to amplify small amounts of DNA, as little as that of a single cell, to obtain large quantities of product for downstream applications like next-generation sequencing. This method is based on multiple displacement amplification (MDA). When the starting material is circular, RCA is utilized. Both MDA-WGA and RCA use a strand-displacing DNA polymerase. Thermo Fisher Scientific offers two types: the wild-type Thermo Scientific<sup>™</sup> phi29 DNA Polymerase, and the engineered Thermo Scientific<sup>™</sup> EquiPhi29<sup>™</sup> DNA Polymerase, which has exceptional performance compared to phi29 DNA Polymerase.

## Multiple displacement amplification-whole genome amplification (MDA-WGA)

The process of MDA-WGA starts with the addition of random hexamer primers to the DNA sample, which hybridize to the template and initiate DNA synthesis. A strand-displacing polymerase then synthesizes DNA strands along the template from the multiple priming positions.

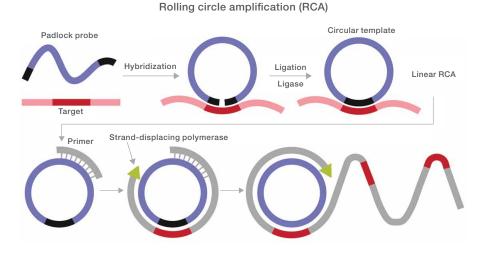




Learn more about rolling circle amplification at thermofisher.com/rca

#### Rolling circle amplification (RCA)

The process of RCA uses a circular DNA template, such as a plasmid or circularized oligonucleotide. DNA polymerase continuously synthesizes new DNA strands as it moves around the template. This leads to exponential amplification and the generation of concatemers containing numerous tandem repeats that are complementary to the circular template.



#### EquiPhi29 DNA Polymerase

EquiPhi29 DNA Polymerase is a proprietary phi29 DNA Polymerase mutant developed through *in vitro* protein evolution. This enzyme is significantly improved over phi29 DNA Polymerase in protein thermostability, reaction speed, product yield, and amplification bias. At the same time, it retains all the benefits of the wild-type enzyme, including high processivity (>70 kb), strong strand-displacing activity, and 3' to 5' exonuclease (proofreading) activity. EquiPhi29 DNA Polymerase is also available as a convenient kit with all required components for RCA and MDA-WGA.

#### **Product highlights**

- Fast-amplifies target in less than 2 hours
- Sensitive-provides sensitivity down to 1 fg of DNA
- High yield-yields up to 17 µg amplified DNA
- Variety of applications—compatible with cell-free DNA enrichment, and cell-free protein expression
- Availability-available in lyo-ready format

#### Did you know?

These products are free of OPE and NPE, making them safer for aquatic life.



Find out more about EquiPhi29 DNA Polymerase at thermofisher.com/equiphi29.

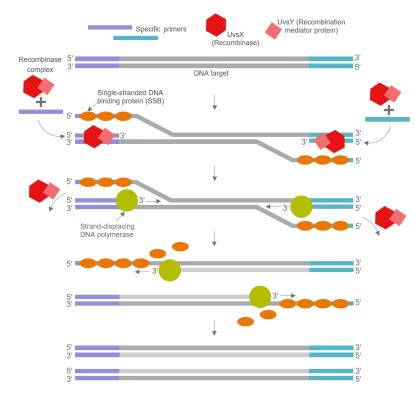


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## **Recombinase polymerase amplification (RPA)**

RPA is a sensitive, low-temperature (39–42°C) isothermal DNA amplification technique that is initiated with two primers and carried out using a mixture of recombinase enzymes, a single-stranded DNA-binding protein (SSB), and a strand-displacing DNA polymerase such as Bst DNA polymerase. To detect RNA targets, a reverse transcriptase can be incorporated into the RPA reaction. Amplified DNA can be detected using various detection methods such as endpoint detection, real-time analysis, lateral flow assays, and CRISPR-Cas9 systems.

As shown below, RPA uses a recombinase complex made of UvsX and UvsY proteins, which binds to the primers in the presence of ATP. This complex seeks a sequence on the DNA homologous to the primer, and promotes strand opening and invasion by the primer. The SSB stabilizes the resulting D-loop structure, allowing the DNA polymerase to extend the primer while displacing the DNA strand.



#### Lyo-ready RPA Kit

The Invitrogen<sup>™</sup> Lyo-ready RPA Kit includes all required components in a glycerol-free format for isothermal RPA, enabling high reaction sensitivity, specificity, and tolerance to inhibitors. The Lyo-ready RPA Kit contains separate reagents that provide maximum flexibility to optimize the RPA reaction and work with various types of pathogens (RNA or DNA), including *S. aureus*, *P. aeruginosa*, influenza and measles viruses, and others. Components for RPA and RT-RPA reactions are also available in a stand-alone format.

#### **Product highlights**

- Sensitive-provides sensitivity down to as low as one copy of target
- Specific-detects and amplifies a single target in a mixture of RNA and DNA
- Fast-amplifies target in as little as 20 minutes
- Robust-amplifies even from inhibitor-containing RNA and DNA samples



Find out more about the Lyo-ready RPA Kit here.

Learn more about RPA at thermofisher.com/rpa.

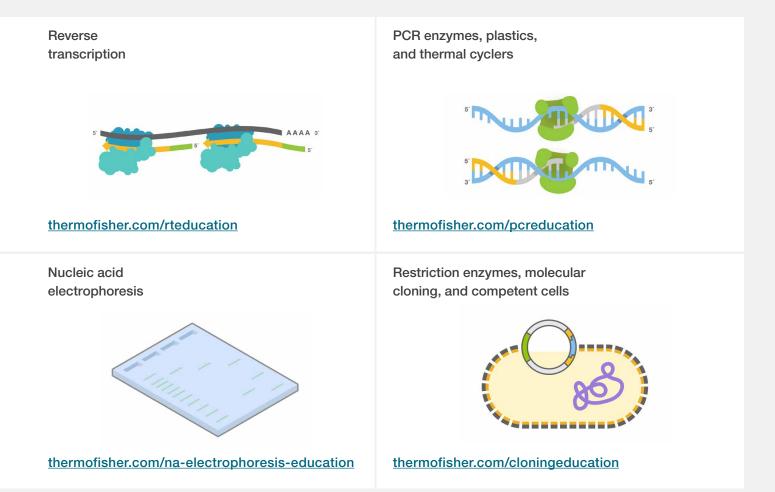
## Resources



We have compiled educational resources, mobile apps, frequently asked questions, and other information to help you achieve success in your research.

## **Educational resources**

Suitable for new and experienced molecular biologists alike, our free online educational resources are designed to help you review the basics, build your expertise, or discover our latest innovative technologies. Explore our educational resources in the following areas of molecular biology.





#### Resources

Webinars: Watch live and recorded webinars for in-depth understanding of molecular and synthetic biology techniques and tools to help elevate your research.

#### thermofisher.com/mbwebinars

Videos: Experience entertaining and visual learning with our educational videos on molecular biology techniques, how-to guides, tips and tricks, and more. thermofisher.com/mbvideos

Application notes: Read white papers and application notes from our R&D scientists on our product innovations. thermofisher.com/mbliterature

Online tools: Use our interactive online tools for PCR annealing temperature, restriction enzyme information, product selection, and more. thermofisher.com/mbtools



## **Podcast: Speaking of Mol Bio**



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This podcast helps scientifically curious people—from all scientific and nonscientific backgrounds—understand how modern molecular biology applications can help push the boundaries in medicine, science, drug discovery, and in the cure and treatment of diseases.

Listen now at thermofisher.com/molbiopodcast.

### Mobile apps

DailyCalcs—science calculator The DailyCalcs app turns your phone into a science calculator to help simplify everyday tasks in the lab. The app features eight calculators: molarity, dilution, formula weight, transfection, unit conversions, culture vessel data, media conversions, and specific productivity. Find it in your preferred mobile application store.



invitrogen

#### Instrument Connect-remote monitoring

Instrument Connect allows you to view instrument status, monitor or schedule a run, and more on any cloud-enabled instrument, including the Applied Biosystems<sup>™</sup> ProFlex<sup>™</sup>, SimpliAmp<sup>™</sup>, and MiniAmp<sup>™</sup> PCR instruments. Access at <u>thermofisher.com/connect</u>.

#### PCR Quest-match-3 lab game

Test your PCR knowledge with our lab game—PCR Quest—where you travel from lab to lab crushing the world's toughest diseases. Download at <u>thermofisher.com/pcrquest</u>.

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## **Frequently asked questions**

Below are some common questions and answers to help you start or troubleshoot molecular biology experiments.

#### Sample preparation

#### Which kit should I use to isolate nucleic acids from my sample?

Choosing the right product is fundamental to ensuring proper lysis of cells and tissue, as well as sufficient yield and quality of isolated nucleic acids. Look to our selection guides (**see pages 11–14**) to help you decide according to nucleic acid type, sample source, experimental throughput, and format as well as downstream applications.

#### What are the key steps to preventing RNA degradation?

The basic lab precautions listed below can help minimize RNA degradation and avoid experimental inconsistency and failure.

- Use nuclease-free pipette tips and tubes
- Use nuclease-free water and reagents
- Regularly decontaminate work surfaces
- Properly stabilize RNA sources before storage

For more tips and troubleshooting advice on sample prep, visit thermofisher.com/rnabasics and thermofisher.com/napsupport.

#### **Reverse transcription**

How do I improve the efficiency of cDNA synthesis when working with challenging samples (e.g., low-abundance, degraded, inhibitor-containing, or GC-rich RNA)?

When working with challenging RNA samples, select a reverse transcriptase that is highly sensitive, processive, thermostable, and resistant to common inhibitors, to help you obtain the highest cDNA yield (see pages 17–19).

## What are the benefits of using random primers, oligo(dT) primers, gene-specific primers, or oligo(dT)/random mixed primers in reverse transcription?

- Random primers are good to use with degraded RNA, RNA with high secondary structure, nonpolyadenylated RNA, or prokaryotic RNA.
- Oligo(dT) primers are an optimal choice for synthesis of full-length cDNA from eukaryotic mRNA. Applications include cDNA cloning, cDNA library construction, and 3' rapid amplification of cDNA ends (3' RACE).
- Gene-specific primers are designed based on known sequences of the target RNA. These primers offer the most specific priming and are commonly used in one-step RT-PCR.
- A mixture of oligo(dT) and random primers is often used in two-step RT-PCR to achieve the benefits of each primer type (see page 22).

For more tips and troubleshooting advice on reverse transcription, visit **thermofisher.com/rteducation**.

#### **PCR** amplification

#### How can I optimize primer annealing for PCR?

Traditionally, gradient thermal cyclers have been used to simultaneously assess multiple temperatures around the theoretical annealing point. Compared to gradient thermal cyclers, instruments with VeriFlex technology allow more precise temperature control for faster optimization of primer annealing (**see pages 26–27**).

Tedious optimization steps may be circumvented using the novel Invitrogen<sup>™</sup> Platinum<sup>™</sup> DNA polymerases. Their innovative buffers enable specific annealing at 60°C for most primers when they are designed following general primer design rules (**see pages 30–33**).



## Frequently asked questions (cont.)

#### What do I need to run fast PCR?

PCR amplicons shorter than 1 kb can be amplified in as little as 40 minutes using "fast" enzymes (high processivity; **see page 32**), "fast" plastics (low profile and ultra-thin walls; **see page 29**), and "fast" thermal cyclers (fast ramp rate; **see pages 26–27**).

## How can I prevent sample evaporation during PCR?

Proper sealing of your reactions will help prevent evaporation during PCR.

- When using adhesive film to seal a plate, be sure to properly align the seal to cover all wells and press firmly along all edges of the plate using an applicator tool.
- When sealing a plate using cap strips, ensure that the cap strips are compatible with the plate and thermal cycler being used. Be sure to align cap strips with each well of the plate and place firmly across the plate for a secure fit.
- Use the applicator tool (Cat. No. 4333183 or 4330015) or other comparable sealing tools as needed.

For more tips and troubleshooting advice on PCR, visit <u>thermofisher.com/pcreducation</u> and <u>thermofisher.com/pcrsupport</u>.

#### Nucleic acid electrophoresis

Why is it important to choose the right ladder when using E-Gel precast agarose gels?

Accurate analysis of electrophoresis bands often depends on the DNA ladder chosen for your gel run. E-Gel DNA ladders are formulated with ready-to-use buffers unique for E-Gel precast agarose gels, and DNA standards designed for optimal separation (**see page 42**).

### Are there safer alternatives to ethidium bromide for staining nucleic acids in gel electrophoresis?

SYBR Safe DNA Gel Stain is a safer alternative to ethidium bromide and is commonly used in gel electrophoresis. SYBR Safe DNA stain is not classified as hazardous waste or as a pollutant under US federal regulations (see page 42).

#### Do I need a buffer to run the E-Gel system?

No. The E-Gel electrophoresis system does not require electrophoresis buffers like TBE or TAE. E-Gel cassettes already contain everything you will need and are classified as dry electrophoresis.

For more tips and troubleshooting advice on nucleic acid electrophoresis, visit <u>thermofisher.com/na-electrophoresis-education</u> and <u>thermofisher.com/na-electrophoresis-support</u>.

#### Cloning

## Do you have a buffer compatibility chart for restriction enzymes?

All FastDigest restriction enzymes are 100% active in one universal FastDigest buffer (**see page 46**). Hence, there is no buffer compatibility chart for FastDigest restriction enzymes.

#### What is the main difference between GeneArt Strings DNA Fragments and GeneArt Gene Synthesis?

GeneArt Strings DNA Fragments are custom-made, linear DNA fragments that are uncloned and double-stranded. GeneArt Gene Synthesis is a service offered for chemical synthesis, cloning, and sequence verification of genetic sequences (**see page 50**).

## What are some key considerations for choosing competent cells for my cloning applications?

Genotype, transformation efficiency, growth rate, and throughput format are important factors in choosing competent cells for cloning. The genotype of a cell strain may determine growth conditions and suitability for transformation with specific DNA types (**see page 51**).

For more tips and troubleshooting advice on cloning, visit <u>thermofisher.com/cloningeducation</u> and <u>thermofisher.com/cloningsupport</u>.



#### Ordering information

	Quantity	Cat. No.
Nucleic acid isolation		
PureLink Quick Plasmid Miniprep Kit	50 preps	K210010
PureLink HiPure Plasmid Filter Midiprep Kit	25 preps	K210014
PureLink HiPure Plasmid Filter Maxiprep Kit	10 preps	K210006
PureLink Pro Quick96 Plasmid Purification Kit	4 x 96 preps	K211004A
PureLink Quick Gel Extraction Kit	50 preps	K210012
TRIzol Plus RNA Purification Kit	50 preps	12183555
PureLink RNA Mini Kit	10 preps	12183020
PureLink Genomic DNA Mini Kit	10 preps	K182000
PureLink Pro 96 Genomic DNA Purification Kit	4 x 96 preps	K182104A
PureLink Pro 96 Viral RNA/DNA Purification Kit	4 plates	12280096A
PureLink Viral RNA/DNA Mini Kit	50 preps	12280050
PureLink Genomic Plant DNA Purification Kit	50 preps	K183001
MagMAX Microbiome Ultra Nucleic Acid Isolation Kit, with bead tubes	100 preps	A42358
MagMAX Microbiome Ultra Nucleic Acid Isolation Kit, with bead plate	100 preps	A42357
MagMAX Cell-Free DNA Isolation Kit	1 kit	A29319
MagMAX Cell-Free Total Nucleic Acid Isolation	1 kit	A36716
MagMAX FFPE DNA/RNA Ultra Kit	1 kit	A31881
MagMAX mirVana Total RNA Isolation Kit	96 reactions	A27828
MagMAX Sequential DNA/RNA Kit	1 kit	A65309
MagMAX Prime Viral/Pathogen NA Isolation Kit	Up to 600 reactions	A58145
KingFisher PlasmidPro Maxi Processor Endotoxin-Free Cartridge	4 cartridges	A54072
PureLink PCR Purification Kit	50 preps	K310001
PureLink Quick Gel Extraction and PCR Purification Combo Kit	50 preps	K220001
PureLink Quick Gel Extraction Kit	50 preps	K210012
	5 mL	A58521
MagMAX Pure Bind Beads	50 mL	A58522
	250 mL	A58523
TagMan Fast Advanced Calle to C. 1/4	40 reactions	A35374
TaqMan Fast Advanced Cells-to-C <sub>T</sub> Kit	100 reactions	A35377
Dynabeads M-270 Streptavidin	2 mL	65305
Dynabeads MyOne Streptavidin C1	2 mL	65001

	Quantity	Cat. No.
KingFisher instruments – For Laboratory Use.		
KingFisher Apex Purification System with 96 PCR Head	1 system	5400910
KingFisher Apex Purification System with 96 Combi Head	1 system	5400920
KingFisher Apex Purification System with 96 Deep-Well Head	1 system	5400930
KingFisher Apex Purification System with 24 Combi Head	1 system	5400940
KingFisher Flex Purification System with 96 PCR Head	1 system	5400610
KingFisher Flex Purification System with 96 KingFisher Plate	1 system	5400620
KingFisher Flex Purification System with 24 Deep-Well Head	1 system	5400640
KingFisher Flex Purification System with 96 Deep-Well Head	1 system	5400630
KingFisher Presto Purification System with 24 Deep-Well Head	1 system	5400840
KingFisher Presto Purification System with 96 Deep-Well Head	1 system	5400830

KingFisher instruments – For Research Use Only. Not for use in diagnostic procedures.			
KingFisher Duo Prime Purification System	1 system	5400110	
KingFisher PlasmidPro Maxi Processor	1 instrument	A66427	



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	Quantity	Cat. No.
Reverse transcription		
	2,000 units	18090010
SuperScript IV Reverse Transcriptase	10,000 units	18090050
	50 reactions	18091050
SuperScript IV First-Strand Synthesis System	200 reactions	18091200
	50 reactions	11756050
SuperScript IV VILO Master Mix	500 reactions	11756500
	50 reactions	11766050
SuperScript IV VILO Master Mix with ezDNase Enzyme	500 reactions	11766500
	25 reactions	12594025
SuperScript IV One-Step RT-PCR System	100 reactions	12594100
SuperScript IV UniPrime One-Step RT-PCR System	100 reactions	12597100
	50 reactions	11750150
SuperScript IV CellsDirect cDNA Synthesis Kit	500 reactions	11750350
SuperScript IV CellsDirect Lysis Reagents	500 reactions	11750550
	48 reactions	11752048
SuperScript IV Single Cell/Low Input cDNA PreAmp Kit	96 reactions	11752096
	192 reactions	11752192
	48 reactions	A65423
SuperScript IV Template Switching RT Master Mix	96 reactions	A65424
	5 x 96 Reactions	A65425
NIPERana In PNana Inhibitar (2011/11)	2,500 units	AM2694
SUPERase-In RNase Inhibitor (20 U/µL)	10,000 units	AM2696
RNaseOUT Recombinant Ribonuclease Inhibitor	5,000 units	10777019
Nase Inhibitor	2,000 units	N8080119
mbion RNasa Inhibitar alanad 40 U/ul	2,500 units	AM2682
Ambion RNase Inhibitor, cloned, 40 U/µL	10,000 units	AM2684
Ribonuclease H	30 units	18021014
Random Hexamers (50 µM)	5 nmol	N8080127

	Quantity	Cat. No.
Random Primers	100 µL	48190011
Oligo(dT) <sub>12-18</sub> Primer	50 µL	18418012
Oligo(dT) <sub>20</sub> Primer	50 µL	18418020
DNase I, Amplification Grade	100 units	18068015

Isothermal amplification		
	1,200 units (6 U/μL)	A56655
Lyo-ready Bst DNA Polymerase	6,000 units (6 U/µL)	A56656
	1,200 units (40 U/µL)	A56657
	100 reactions	A51801
SuperScript IV RT-LAMP Master Mix	400 reactions	A51802
	1,000 reactions	A51803
EquiPhi29 DNA Polymerase 250 units 1,000 units 5,000 units	A39390	
	1,000 units	A39391
	5,000 units	A39392
FauiDhioo DNA Amplification Kit	100 reactions	A65393
EquiPhi29 DNA Amplification Kit	500 reactions	A65394
	250 units	EP0091
phi29 DNA Polymerase (10 U/µL)	1,000 units	EP0092
	5,000 units	EP0094
	100 reactions	A72127
Lyo-ready RPA Kit	500 reactions	A72128
Lyo-ready T4 UvsX Protein	120 µg	A72124
Lyo-ready T4 UvsY Protein	120 µg	A72125
Lyo-ready T4 Gene 32 Protein	1,600 µg	A72123
	200 pmol	A40001796
L.b. Cas12a Nuclease	2,000 pmol	A40001795

	Quantity	Cat. No.
PCR		
Thermal cyclers		
ProFlex PCR System, 3 x 32-well	1 instrument	4484073
ProFlex PCR System, 96-well	1 instrument	4484075
VeritiPro Thermal Cycler, 96-well	1 instrument	A48141
MiniAmp Plus Thermal Cycler	1 instrument	A37835
MiniAmp Thermal Cycler	1 instrument	A37834
Automated Thermal Cycler (ATC), 96-well, laptop	1 instrument	A31486
Plastics		
MicroAmp EnduraPlate Optical 96-Well Fast Multicolor Reaction Plates with Barcode	5 plates	4483493
MicroAmp Optical Adhesive Film	100 covers	4311971
MicroAmp Optical 96-Well Reaction Plate	10 plates	N8010560
MicroAmp Optical 8-Cap Strips	300 strips	4323032
MicroAmp Fast Optical 96-Well Reaction Plate, 0.1 mL	10 plates	4346907
MicroAmp Fast Reaction Tube with Cap, 0.1 mL	1,000 tubes	4358297
MicroAmp EnduraPlate Optical 384-Well Multicolor Reaction Plates with Barcode	5 plates	4483316
MicroAmp EnduraPlate Optical 96-Well Clear Reaction Plates with Barcode	20 plates	4483354
MicroAmp TriFlex 3 x 32-Well PCR Reaction Plate	20 plates	A32811
MicroAmp 8-Tube Strip with Attached Domed Caps, 0.2 mL	125 strips	A30589
MicroAmp EnduraPlate Optical 96-Well Full-Skirted Plates with Barcode, clear	50 plates	A31728
PCR Plate, 384-well, standard, Sustain Series	50 plates	AB1384SS
PCR Plate, 96-well, non-skirted, Sustain Series	25 plates	AB0600SS
PCR Plate, 96-well, segmented, semi-skirted, Sustain Series	25 plates	AB0900SS
Tubes and Flat Caps, strips of 8, Sustain Series	250 strips	AB1182SS

	Quantity	Cat. No.
PCR enzymes		
Platinum II Tag Llat Start DNA Dalumaraaa	100 reactions	14966001
Platinum II Taq Hot-Start DNA Polymerase	500 reactions	14966005
Platinum III Lat Ctart DCD Maatar Mir (0V)	50 reactions	14000012
Platinum II Hot-Start PCR Master Mix (2X)	200 reactions	14000013
Distingues II Liet Chart Organ DOD Master Mir (OV)	50 reactions	14001012
Platinum II Hot-Start Green PCR Master Mix (2X)	200 reactions	14001013
	100 units 4398	4398813
AmpliTaq Gold 360 DNA Polymerase	250 units	4398823
		4398876
AmpliTaq Gold 360 Master Mix		4398881
	100 units         4398813           250 units         4398823           1 mL         4398876           5 mL         4398881           100 units         12361010           500 units         12361050	
Platinum SuperFi II DNA Polymerase	500 units	12361050
	500 reactions         1496600           50 reactions         1400001           200 reactions         1400001           200 reactions         1400101           100 units         4398820           1 mL         4398876           5 mL         4398887           100 units         1236101           500 reactions         1236801           500 reactions         1236801           500 reactions         1236805           100 reactions         1236905           100 reactions         1236905           100 reactions         1236905           100 reactions         A446475           500 reactions         A446475	12368010
Platinum SuperFi II PCR Master Mix	500 reactions	12368050
	50 reactions         14001012           200 reactions         14001013           200 reactions         14001013           100 units         4398813           250 units         4398823           1 mL         4398876           5 mL         4398881           100 units         12361010           500 units         12361010           500 units         12361050           100 reactions         12368050           100 reactions         12368050           100 reactions         12369010	12369010
Platinum SuperFi II Green PCR Master Mix	500 reactions	12369050
	100 reactions	A44647100
Platinum Direct PCR Universal Master Mix	500 reactions	A44647500
	4 x 250 μL	10297018
dNTP Set (100 mM)	8 x 1.25 mL	10297117



	Quantity	Cat. No.
Nucleic acid separation and analysis		
SYBR Safe DNA Gel Stain	400 µL	S33102
SYBR Gold Nucleic Acid Gel Stain	500 µL	S11494
UltraPure DNase/RNase-Free Distilled Water	500 mL	10977015
Ø UltraPure Agarose	100 g	16500100
Tracklt 100 bp DNA Ladder	100 applications	10488058
UltraPure TAE Buffer, 10X	4 L	15558026
E-Gel Agarose Gels with SYBR Safe DNA Gel Stain	10 gels	A42135
E-Gel Double Comb Agarose Gels with SYBR Safe DNA Gel Stain	10 gels	A42348
E-Gel EX Double Comb Agarose	10 gels	A44889
E-Gel CloneWell II Agarose Gels with SYBR Safe DNA Gel Stain, 0.8%	10 gels	G661818
E-Gel Agarose Gels with SYBR Safe DNA Gel Stain, 2%	2 x 10 gels	A45204
E-Gel EX Agarose Gels, 2%, with SYBR Gold DNA Stain	20 gels	G402022
E-Gel 1 Kb Plus DNA Ladder	100 applications	10488090
E-Gel Sample Loading Buffer, 1X	4 x 1.25 mL	10482055
N Dower Span Dlue Electropherecia System	1 ovotom	G9301*
E-Gel Power Snap Plus Electrophoresis System	2 x 10 gels         A           20 gels         G           100 applications         10           4 x 1.25 mL         10           1 system         G           1 kit         G	G9311**
E-Gel Power Snap Plus Electrophoresis System Starter Kit,	4 L         1555802           10 gels         A42135           10 gels         A42348           10 gels         A42348           10 gels         A42348           10 gels         A44889           10 gels         G661818           2 x 10 gels         G40202           100 applications         1048805           4 x 1.25 mL         1048205           1 system         G9301*           1 kit         G9341*           1 kit         G9342*           1 kit         G9332**           1 kit         G9391*	G9341*
48-well, 1%	I KIL	G9331**
E-Gel Power Snap Plus Electrophoresis System Starter Kit,	10 gels         A42348           10 gels         A44389           10 gels         A44889           10 gels         G661818           2 x 10 gels         A45204           20 gels         G40202           100 applications         1048808           4 x 1.25 mL         1048208           4 x 1.25 mL         1048208           1 system         G9301*           G9301*         G9331**           1 kit         G9331**           1 kit         G9332**           1 kit         G9391*           G9381**         G9382**	G9342*
48-well, 2%		G9332**
E-Gel Power Snap Plus Electrophoresis System Starter Kit,	4 1.44	G9391*
96-well, 1%	I KIL	G9381**
E-Gel Power Snap Plus Electrophoresis System Starter Kit,	1 1214	G9392*
96-well, 2%	1 kit	
E-Gel Power Snap Electrophoresis System Starter Kit, EX 2%	1 kit	G8342ST
E-Gel 48 Agarose Gels with SYBR Safe DNA Gel Stain, 2%	8 gels	G820802
E-Gel 96 Agarose Gels with SYBR Safe DNA Gel Stain, 2%	8 gels	G720802
E-Gel 48 Agarose Gels with SYBR Safe DNA Gel Stain, 4%	8 gels	G820804

	Quantity	Cat. No.
Cloning and gene synthesis		
	800 reactions	FD0054
FastDigest BamHI	2,500 reactions	FD0055
FootDirect David	20 reactions	FD1253
FastDigest Bcul	50 reactions	FD1254
FastDigest BshTl	20 reactions	FD1464
	50 reactions	FD1703
FastDigest DpnI	100 reactions	FD1704
	800 reactions	FD0274
FastDigest EcoRI	2,500 reactions	FD0275
FastDigest Kpnl	300 reactions	FD0524
	20 reactions	FD0593
	50 reactions	FD0594
FastDigest Notl	150 reactions	FD0595
	250 reactions	FD0596
FastDigest Sall	200 reactions	FD0644
	2,500 reactions         FD0055           20 reactions         FD1253           50 reactions         FD1254           20 reactions         FD1254           20 reactions         FD1264           20 reactions         FD1703           100 reactions         FD1704           800 reactions         FD0274           2,500 reactions         FD0275           300 reactions         FD0594           20 reactions         FD0594           150 reactions         FD0594           150 reactions         FD0594           200 reactions         FD0594           150 reactions         FD0594           150 reactions         FD0596           200 reactions         FD0684           300 reactions         FD0684           300 reactions         FD0694           1,200 reactions         FD0694           1,200 reactions         FD0694           1,200 reactions         FD0694           1,200 reactions         FD0694           20 reactions         FD0694           20 reactions         FD0694           20 reactions         FD0694           20 reactions         FD0293           100 reactions	FD0684
FastDigest Xbal	750 reactions	FD0685
	50 reactions         FD1254           20 reactions         FD1464           50 reactions         FD1703           100 reactions         FD1704           800 reactions         FD0274           2,500 reactions         FD0274           2,500 reactions         FD0524           20 reactions         FD0593           50 reactions         FD0593           50 reactions         FD0594           150 reactions         FD0595           250 reactions         FD0596           200 reactions         FD0694           300 reactions         FD0684           750 reactions         FD0684           750 reactions         FD0694           1,200 reactions         FD0695           20 reactions         FD0694           1,200 reactions         FD0695           20 reactions         FD0694           1,200 reactions         FD0695           20 reactions         FD0104           50 reactions         FD0293           100 reactions         FD0293           100 reactions         FD0294           200 units         EL0014           1,000 units         EL0014	FD0694
FastDigest Xhol		FD0695
FastDigest Esp3I (BsmBI) (IIS class)	20 reactions	FD0454
FastDigest Bpil (Bbsl) (IIS class)	20 reactions	FD1014
	50 reactions	FD0293
FastDigest Eco31I (Bsal) (IIS class)	100 reactions	FD0294
	200 units EL00	EL0014
NA Ligase (5 U/µL)	1,000 units	EL0011
	300 units	EF0654
FastAP Thermosensitive Alkaline Phosphatase (1 U/ $\mu$ L)	1,000 units	EF0651

Resources



	Quantity	Cat. No.		Quantity	Cat. No
	500 units	EK0031	GeneArt Gibson Assembly HiFi Master Mix	50 reactions	A46628
T4 Polynucleotide Kinase (10 U/µL)	2,500 units	EK0032	GeneArt Gibson Assembly EX Master Mix	50 reactions	A46636
	500 units	EP0041	GeneArt Seamless Cloning and Assembly Enzyme Mix	20 reactions	A14606
DNA Polymerase I (10 U/µL)	2,500 units	EP0042	GeneArt Type IIS Assembly Kit, Aarl	10 reactions	A15916
	300 units	EP0051	GeneArt Type IIS Assembly Kit, Bsal	10 reactions	A15917
Klenow Fragment (10 U/µL)	1,500 units	EP0052	GeneArt Type IIS Assembly Kit, Bbsl	10 reactions	A15918
	1 mL of 5X	EP0061	GeneArt High-Order Genetic Assembly System	10 reactions	A13285
T4 DNA Polymerase (5 U/μL)	reaction buffer 2 x 1 mL of 5X		Gateway BP Clonase II Enzyme Mix	20 reactions	1178902
	reaction buffer	EP0062	Gateway LR Clonase II Enzyme Mix	20 reactions	11791020
T7 DNA Polymerase (10 U/μL)	300 units	EP0081	MultiSite Gateway Pro Plus	20 reactions	1253710
CorrectASE Enzyme	50 reactions	A14972	LR Clonase II Plus Enzyme	20 reactions	1253812
TOPO TA Cloning Kit for Subcloning, without competent cells	25 reactions	450641	Gateway Vector Conversion System with One Shot ccdB	1 kit	1182802
Zero Blunt TOPO PCR Cloning Kit, without competent cells	25 reactions	450245	Survival Cells		
pENTR/D-TOPO Cloning Kit, with One Shot TOP10 Chemically Competent E. coli	20 reactions	K240020	PCR Cloning System with Gateway Technology with pDONR 221 and OmniMAX 2 Competent Cells	20 reactions	1253502
One Shot TOP10 Chemically Competent E. coli	21 x 50 μL	C404003	PCR Cloning System with Gateway Technology with pDONR/Zeo and OmniMAX 2 Competent Cells	20 reactions	1253503
One Shot Stbl3 Chemically Competent E. coli	21 x 50 µL/tube	C737303	Gateway pDONR 221 Vector	6 µg	1253601
MAX Efficiency DH5a Competent Cells	5 x 200 μL	18258012	pENTR/D-TOPO Cloning Kit, with One Shot TOP10	20 reactions	K240020
ElectroMAX DH10B Cells	5 x 100 μL	18290015	Chemically Competent E. coli		
MAX Efficiency Stbl2 Competent Cells	5 x 200 μL	10268019	pCR 8/GW/TOPO TA Cloning Kit with One Shot TOP10 E. coli	20 reactions	K250020
MultiShot TOP10 Chemically Competent E. coli	5 plates	C40005	-		
MultiShot StripWell TOP10 Chemically Competent E. coli	1 rack	C409601	-		
MultiShot StripWell BL21 Star (DE3) Chemically Competent E. coli	1 rack	C609601	Order custom cloned genes or DNA fragments at		
MultiShot FlexPlate TOP10 Chemically Competent E. coli	1 plate	C4081201	thermofisher.com/geneartdashboard.		
MultiShot FlexPlate DH5a T1R Chemically Competent E. coli	1 plate	C4481201	-		
MultiShot FlexPlate Stbl3 Chemically Competent E. coli	1 plate	C7381201	-		



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