molecular biology

applied biosystems invitrogen

Prepare for discovery

Molecular biology workflow solutions

Find it at **fishersci.com**

fisher scientific
part of Thermo Fisher Scientific


AmpliFly
AmpliFly

Prepare for discovery with molecular biology

In molecular biology research, you may take different routes to reach your destinations. Choices can often be confusing, and the path you take may impact the experience and overall success of your experiments.

This handbook is intended to guide you by providing technical information and clear choices across the molecular biology workflow to help you soar in your research. Applied Biosystems™ and Invitrogen™ products incorporate the latest innovations to enable quicker results, more assurance, and less optimization in your lab studies.

Prepare to step into discovery, from sample preparation to reverse transcription, PCR, and cloning. Explore this handbook for more direct routes and a first-class experience in your research from sample to result.

Find additional information at **thermofisher.com/amplifly**

Contents

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 provide maximum yield, purity, and integrity from virtually any sample type and application is important for your research success.

Find technical resources on nucleic acid isolation at
thermofisher.com/prepon

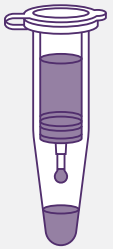
Benefits and underlying principles of common nucleic acid isolation methods

Organic extraction: Phenol-chloroform solution (e.g., Invitrogen™ DNAzol™ and TRIzol™ 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

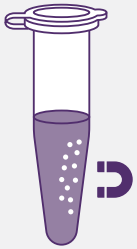


Spin columns: Glass fiber, derivatized silica, or ion exchange membrane in column (e.g., Thermo Scientific™ GeneJET™ and Invitrogen™ PureLink™ kits)

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



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

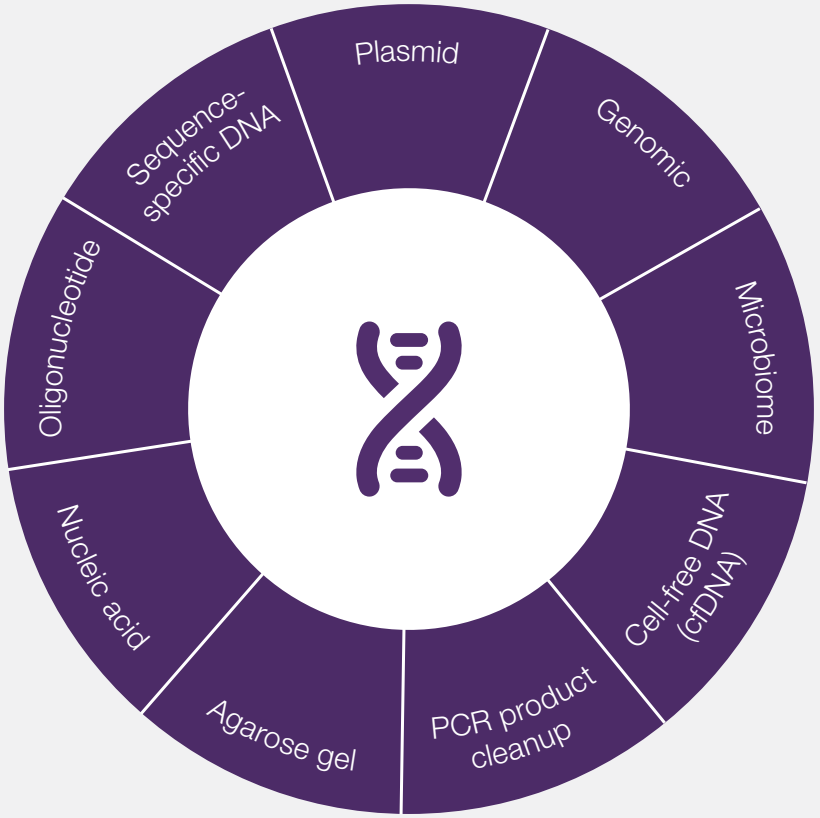
Samples are lysed in solution and allowed to bind nucleic acid to magnetic particles based on specific surface modifications. Application of an external magnetic field rapidly collects the particles. Rounds of release, washes, and recapture enable purification of the desired nucleic acid.

Benefits:

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

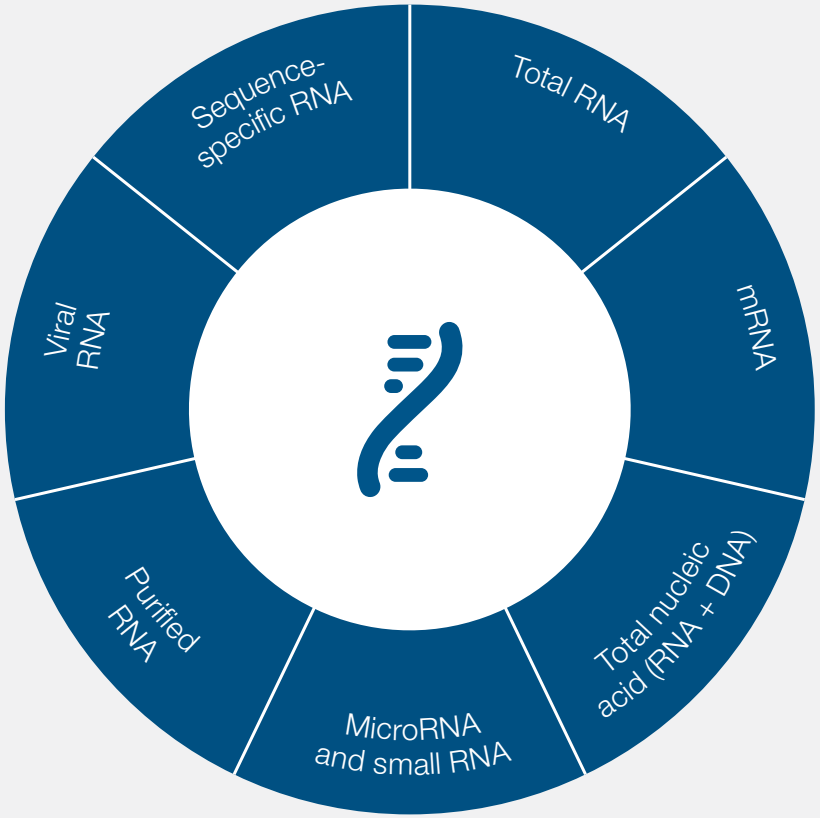
Find out more at **thermofisher.com/sampleprep**

Tools for success in nucleic acid isolation



DNA type

For gDNA extraction, plasmid isolation, and DNA cleanup



RNA type

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

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

Selecting the right DNA isolation kits

To suit your specific DNA isolation needs, a comprehensive portfolio is available offering three highly developed purification technologies: silica membrane, anion-exchange resin, and switchable surface charge. Refer to the following tables to determine which purification kit is appropriate for your DNA type and sample type. To use our online kit selection guide, go to **thermofisher.com/dnaselection**

Applied Biosystems™ and Invitrogen™ technologies for gDNA isolation

Throughput/format	Low throughput or manual (organic)	Medium throughput and spin-column technology	High throughput and 96-well filter plate	High throughput and magnetic bead technology
Kits	**DNAzol reagents**	**PureLink kits**	**PureLink *Pro* gDNA kits**	**MagMAX DNA kits and Dynabeads products**
Tissue	DNAzol Reagent	PureLink Genomic DNA Mini Kit	PureLink *Pro* 96 Genomic DNA Purification Kit	MagMAX DNA Multi-Sample Kit
Cells	DNAzol Reagent	PureLink Genomic DNA Mini Kit	PureLink *Pro* 96 Genomic DNA Purification Kit	MagMAX DNA Multi-Sample Kit
Blood	DNAzol BD Reagent	PureLink Genomic DNA Mini Kit	PureLink *Pro* 96 Genomic DNA Purification Kit	MagMAX DNA Multi-Sample Ultra 2.0 Kit
Plant	Plant DNAzol Reagent	PureLink Genomic Plant DNA Purification Kit	PureLink *Pro* 96 Genomic DNA Purification Kit	MagMAX Plant DNA Isolation Kit
Buccal swabs	Not recommended	PureLink Genomic DNA Mini Kit	PureLink *Pro* 96 Genomic DNA Purification Kit	MagMAX DNA Multi-Sample Ultra 2.0 Kit
Bacteria	DNAzol Reagent	PureLink Microbiome DNA Purification Kit	PureLink *Pro* 96 Genomic DNA Purification Kit	MagMAX DNA Multi-Sample Ultra 2.0 Kit, Dynabeads MyOne Streptavidin products
Virus	Not recommended	PureLink Viral RNA/DNA Mini Kit	PureLink *Pro* 96 Viral RNA/DNA Purification Kit	MagMAX Viral Nucleic Acid Kit, Dynabeads MyOne Streptavidin products
Compatibility				
Scalable and automatable			•	•
Thermo Scientific™ KingFisher™ instrument				•
qPCR	•	•	•	•
NGS	•	•	•	•

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

Selecting the right DNA purification kits (cont.)

Comparison of DNA cleanup solutions

DNA cleanup application	PCR cleanup	PCR cleanup	PCR cleanup	Gel extraction	PCR cleanup and gel extraction	PCR cleanup	Sequencing reaction cleanup
Kit	**PureLink PCR Purification Kit**	**PureLink Pro 96 PCR Purification Kit**	**PureLink PCR Micro Kit**	**PureLink Quick Gel Extraction Kit**	**PureLink Quick Gel Extraction Kit and PCR Purification Combo Kit**	**ChargeSwitch-Pro PCR Clean-Up Kit**	**Centri-Sep Spin Columns**
Format	Silica spin/ vacuum column	96-well silica plate	Silica spin column	Silica spin/ vacuum column	Silica spin/ vacuum column	Derivatized spin/ vacuum column	Spin column
Product size	• 50 preps • 250 preps	• 4 plates (4 x 96 rxns)	• 10 preps • 50 preps • 250 preps	• 50 preps • 250 preps	• 50 preps	• 10 preps • 50 preps • 250 preps	• 100 columns • 32 columns
Time	<15	20	≤10	<30	10–30	<10	<5
Elution volume	50	50–150	5–20	30–100	30–100	50	20
Recovery	>80%	>70%	>80%	Up to 95%	Gel cleanup: >80% PCR cleanup: >95%	NA	NA
Primer removal	>99%	NA	>95%	NA	>99%	NA	>98%

Thermo Scientific™ and Invitrogen™ technologies for plasmid DNA isolation

Purity grade	Molecular	Transfection	Transfection	Advanced transfection
Kits	**GeneJET kits**	**PureLink HiPure kits**	**PureLink Fast Low-Endotoxin kits**	**PureLink Expi Endotoxin-Free kits**
Endotoxin level	Standard (>10 EU/µg)	Low endotoxin (1–10 EU/µg)	Low endotoxin (0.1–1 EU/µg)	Endotoxin-free (<0.1 EU/µg)
Yield	20 µg–1 mg	20 µg–15 mg	0.4 mg (midi), 1.5 mg (maxi)	1.5–15 mg
Technology	Silica membrane	Anion exchange (resin)	Advanced silica membrane	Anion exchange (membrane)
Total protocol time	15–60 min	30–120 min	30 min	90–120 min
Prep size	Mini, midi, and maxi	Mini, midi, maxi, and giga	Midi and maxi	Maxi, mega, and giga
Downstream application	• PCR • Nucleic acid labeling • Cloning (digestion, ligation) • Sequencing	• Standard transfection • All molecular biology applications • *In vitro* transcription	• Standard transfection • Transfection of certain sensitive cell lines	• Primary and stem cell transfection • Gene therapy and vaccine (*in vivo*) research • Microinjection • All molecular biology applications

Selecting the right RNA isolation kits

For the quality and performance you need, a full suite of products for 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™ and Invitrogen™ technologies for 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_T 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	Cells, blood, plants	Cultured cells
Starting material	100 mg of tissue or 10^7 cells	10^8 cells, 200 mg of tissue, 250 mg of plant tissue, 0.2 mL of blood, 5 x 10^8 yeast, 10^9 bacteria	100 mg of tissue or 5 x 10^6 cells	1–100,000 cells
Yield	10^6 epithelial cells: 8–15 µg 100 mg tobacco leaf: 73 µg (Variable depending on sample)	Up to 350 µg	Variable depending on sample	NA
High-throughput compatible		Yes	Yes	Yes
Technology	Organic extraction	Silica membrane spin column/filter plate	Magnetic beads	Crude lysate

Helpful tip

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

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

Automation platform

Optimize and automate your DNA and RNA purification workflow with Thermo Scientific™ KingFisher™ purification systems. When used with compatible bead-based reagents, such as MagMAX kits and Dynabeads products, these instruments enable versatile automation of DNA and RNA isolation procedures. Learn more and request a demo at **thermofisher.com/kingfisherdemo**

Thermo Scientific™ KingFisher™ Duo Prime Purification System

Automated nucleic acid purification of 6 or 12 samples using magnetic bead technology.

Thermo Scientific™ KingFisher™ Flex Purification System

Highly versatile and automated nucleic acid purification of 24 or 96 samples per run using magnetic bead technology.

Thermo Scientific™ KingFisher™ Presto Purification System

Automated nucleic acid purification of 24 to 96 samples per run using magnetic bead technology. This instrument is specifically designed to be paired with a liquid handler for high-throughput, fully automated nucleic acid purification.

Resources

Navigate through the DNA and RNA support categories below to obtain relevant technical information, view tips and tricks when starting an experiment, and find answers to everyday problems.

thermofisher.com/napsupport

thermofisher.com/technicalresources

thermofisher.com/prepforsuccess

thermofisher.com/rnabasics

thermofisher.com/rnahandlingtips

thermofisher.com/magmax

thermofisher.com/dynabeads

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

Reverse transcription

Reverse transcription is the synthesis, by reverse transcriptase, of complementary DNA (cDNA) using single-stranded RNA as a template. 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 lowest 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

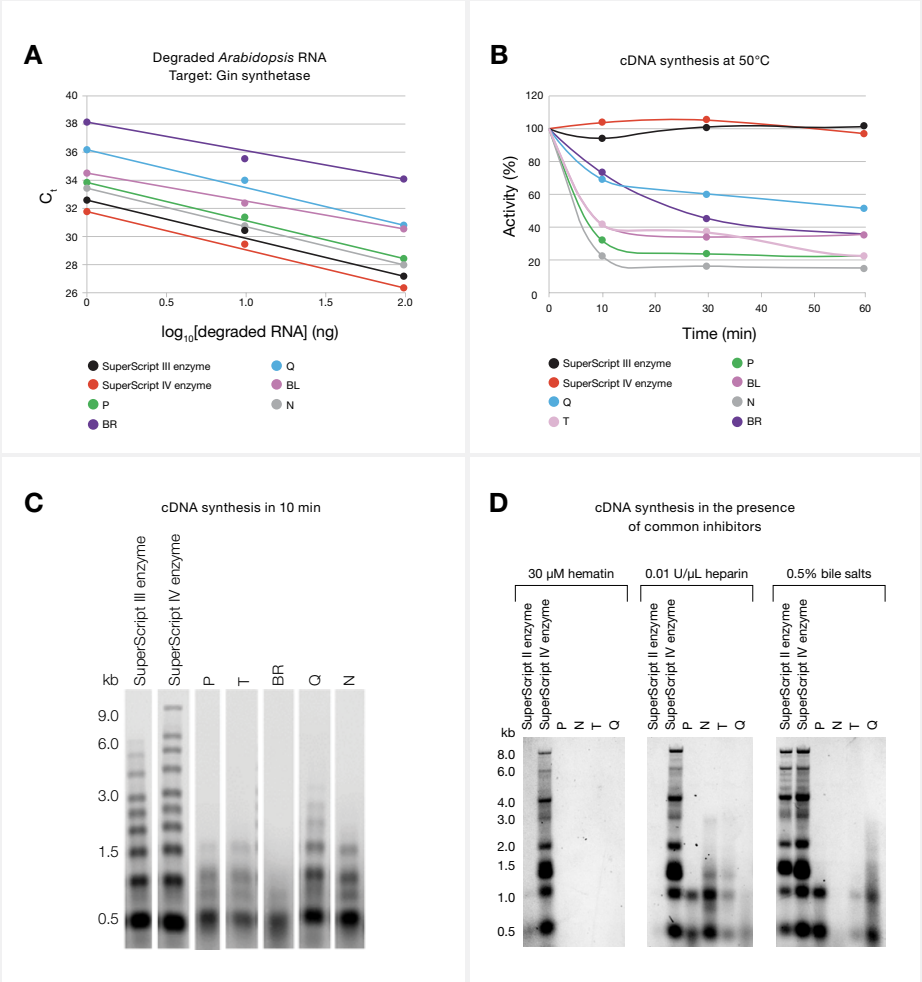
Thermostable reverse transcriptases allow reactions to occur at higher temperatures, which help denature RNA with strong secondary structure or high GC content, for generation of longer cDNA, higher cDNA yields, and better coverage of RNA populations in the cDNA.

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.

Find out more at **thermofisher.com/reverse-transcription**

Reverse transcription reagent selection guide

	Ability to optimize reaction components and conditions	Complete kit with all cDNA synthesis reaction components	Most convenience and fewest pipetting steps for RT-qPCR applications	Most convenience and fewest pipetting steps for RT-PCR applications	Go from mammalian cell lysate to cDNA synthesis without having to isolate RNA
Product format	**Stand-alone enzyme**	**First-strand cDNA synthesis kit**	**First-strand cDNA synthesis master mix for RT-qPCR**	**One-step RT-PCR kit**	**Direct RT kit**
Recommended product	Invitrogen™ SuperScript™ IV Reverse Transcriptase	Invitrogen™ SuperScript™ IV First-Strand Synthesis System	Invitrogen™ SuperScript™ IV VILO™ Master Mix	Invitrogen™ SuperScript™ IV One-Step RT-PCR System	Invitrogen™ SuperScript™ IV CellsDirect™ cDNA Synthesis Kit
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	RT-PCR	RT-PCR, RT-qPCR
Input total	1 pg–5 µg	1 pg–5 µg	0.01 pg–2.5 µg	0.01 pg–1 µg	1–10,000 cells
Optimal reaction temperature	50–55°C	50–55°C	50–55°C	50–55°C	50–55°C
Reverse transcription time	10 min	10 min	10 min	10 min	10 min
High cDNA yield with challenging or degraded RNA	•	•	•	•	•

Did you know?

The standard enzyme format is incompatible for 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 **thermofisher.com/lyoreadyenzymes**

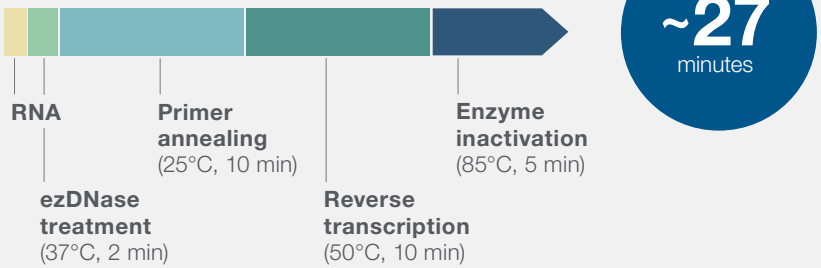
Find out more at **thermofisher.com/superscript**

Genomic DNA removal

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

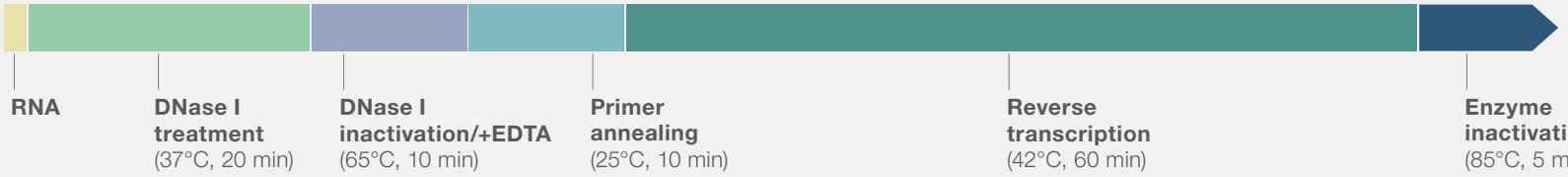
Both the SuperScript IV One-Step RT-PCR System and SuperScript IV VILO Master Mix are available in a format with the novel dsDNA-specific Invitrogen™ ezDNase™ enzyme, which enables efficient, fast, and gentle (<5 min at 37°C) gDNA removal from RNA samples to help ensure high confidence in RT-PCR and RT-qPCR results.

SuperScript IV and SuperScript IV VILO Master Mix cDNA synthesis workflow with ezDNase enzyme



~27 minutes

Traditional cDNA synthesis workflow with DNase I



~105 minutes

Find out more at **thermofisher.com/ssiv-onestep** and **thermofisher.com/4vilo**

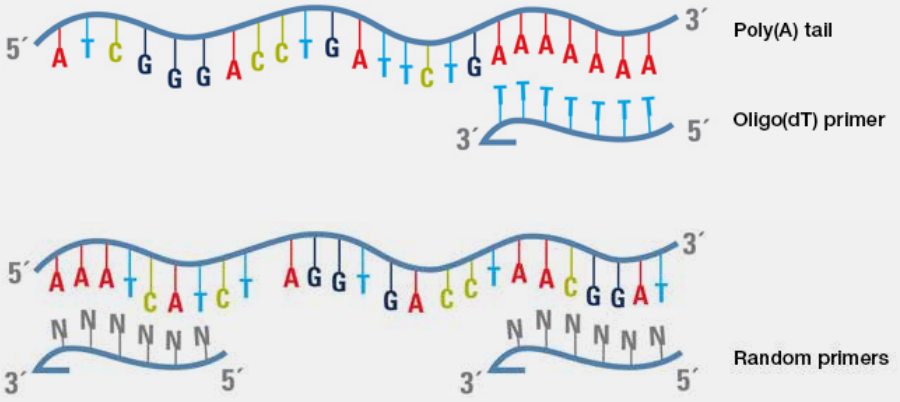
Reverse transcription primers

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. Oligo(dT) primers typically 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 ideal for nonpolyadenylated RNA, such as bacterial RNA.

Two most common primers used in reverse transcription

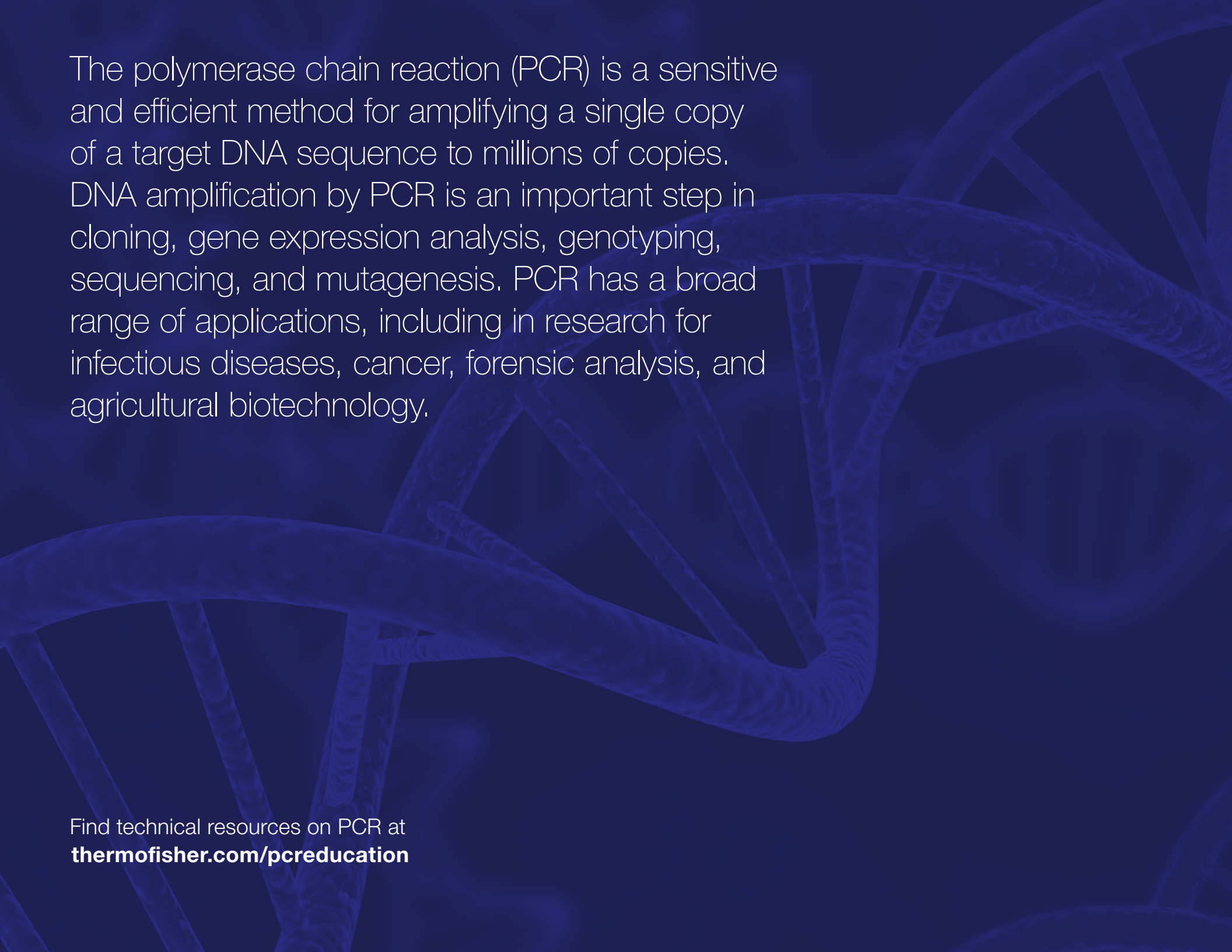


Helpful tip

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**

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


PCR



The polymerase chain reaction (PCR) is a sensitive and efficient method for amplifying a single copy of a target DNA sequence to millions of copies. DNA amplification by PCR is an important step in cloning, gene expression analysis, genotyping, sequencing, and mutagenesis. PCR has a broad range of applications, including in research for infectious diseases, cancer, forensic analysis, and agricultural biotechnology.

Find technical resources on PCR at
thermofisher.com/pcreducation

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™ VeriFlex™ 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 **thermofisher.com/veriflextechnology**

Reliability

Thermal cyclers should be able to withstand repeated use, environmental stress, and shipping conditions. Component reliability can be tested using robotic assemblies in repeated testing of frequently used instrument components such as the heated lid, touchscreens, and temperature cycling modules. Applied Biosystems™ 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.

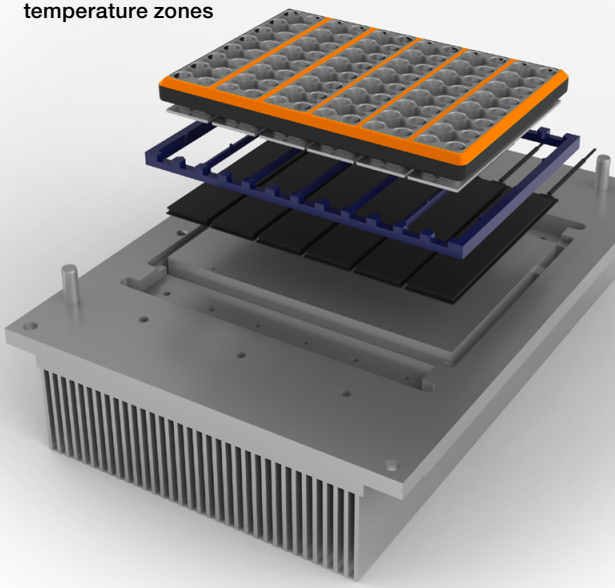
If you want remote access to your instrument, you will appreciate the convenience of cloud-enabled thermal cyclers. They allow you to design and share protocols, schedule an instrument, start or stop a run, and check run status from anywhere, on any mobile device or desktop computer.

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



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

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

Cloud-enabled

	ProFlex™ PCR System	Veriti™ Thermal Cycler
	› Do you share the device with colleagues? › Do you expect your throughput needs to change? › Do you want to access your instrument remotely?	› Do you perform a lot of optimizations? › Do you require an FDA Class 1/CE-IVD labeled device?
Key benefits	Ultimate flexibility and throughput	Proven reliability, precise PCR optimization
Max sample throughput	480,000 reactions	384 reactions
Max block ramp rate	6.0°C/sec	5.0°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	

Do you require an FDA Class 1/CE-IVD labeled thermal cycler?
Visit **thermofisher.com/veritidx**

Cloud-enabled

SimpliAmp™ Thermal Cycler

- Do you need an intuitive interface?
- Do you train new technicians often?
- Do you want to access your instrument remotely?

Cloud-enabled

MiniAmp™ Plus and MiniAmp™ Thermal Cyclers

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

Automated Thermal Cycler

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

Elegantly simple and precise	Routine PCR, elevated	Designed for easy robotic integration
96 reactions	96 reactions	384 reactions
4.0°C/sec	MiniAmp Plus: 3.5°C/sec; MiniAmp: 3.0°C/sec	3.5°C/sec
3-zone VeriFlex Block on 96-well system	MiniAmp Plus: 3-zone VeriFlex Block on 96-well system MiniAmp: none	None
Yes	Yes	Yes

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 help enable optimal performance. Regardless of the plastics format you select, proper fit and uniform heat transfer during thermal cycling are essential.

Manufacturing quality control

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

Integrity testing: Every well of every plate is visually inspected and leak tested. This thorough 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.

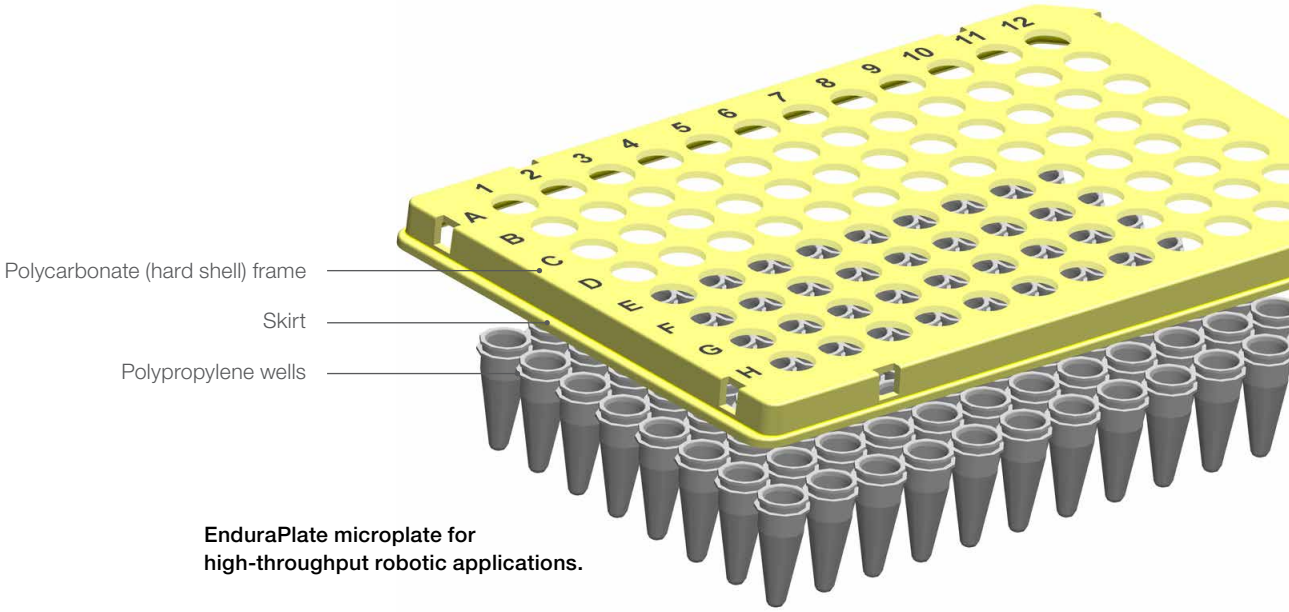
Biological testing: Our plastics are biologically tested to certify them as free of DNA, RNase, and PCR inhibitors. We offer General Purpose Laboratory Equipment (GPLE) plastics that are provided with a PCR certificate for your convenience and documentation.

Construction materials

Applied Biosystems™ MicroAmp™ optical microplates are made of polypropylene for optimal transfer of thermal energy for efficient PCR. A select medical-grade polypropylene is chosen for its exceptional biocompatibility and inert properties.

Applied Biosystems™ EnduraPlate™ microplates are constructed with a stronger polycarbonate frame to resist distortion caused by robotic grippers and to better tolerate rapid heating and cooling, while retaining thin-walled polypropylene wells for efficient heat transfer to the reaction mixture. The polycarbonate frames of the plates are available in multiple colors to help with organization and visual monitoring of assays in a high-throughput setting.

MicroAmp optical microplates.



EnduraPlate microplate for high-throughput robotic applications.

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

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 real-time PCR 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 **thermofisher.com/findplastics**.

	Small-scale experiments with a few samples	Daily experiments	Complete-workflow experiments—ideal for automation	Diagnostic procedures and automation compatible
	Single tubes, strips, caps, adhesive film, and accessories	**MicroAmp™ optical microplates**	**MicroAmp™ EnduraPlate™ optical microplates**	**EnduraPlate™ optical GPLE* reaction plates**
Formats	• Single tubes • Single tubes with caps • 8-strip tubes with caps • 12-strip caps	• 32-well • 48-well Fast • 96-well • 96-well Fast • 384-well	• 96-well • 96-well Fast • 384-well • 96-well full skirted	• 96-well • 96-well Fast • 384-well
DNA-, RNase-, PCR inhibitor–free	Yes	Yes	Yes	Yes
Colors available	Clear, or mixed packs containing red, orange, blue, and green	Clear	Single-color packs (red, blue, green, yellow, or clear) and 5-plate sampler (one of each color)	Clear
Barcode available	No	Yes (1 or 2 sides)	Yes (3 sides)	Yes (3 sides)
Automation compatible			Yes	Yes

* Each lot of reaction plates is certified in an ISO 13485–registered facility to be free of DNA, RNase, and PCR inhibitors. Ideal for use in diagnostic procedures.

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 **thermofisher.com/pcrplastics-education**

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 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 room temperature 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

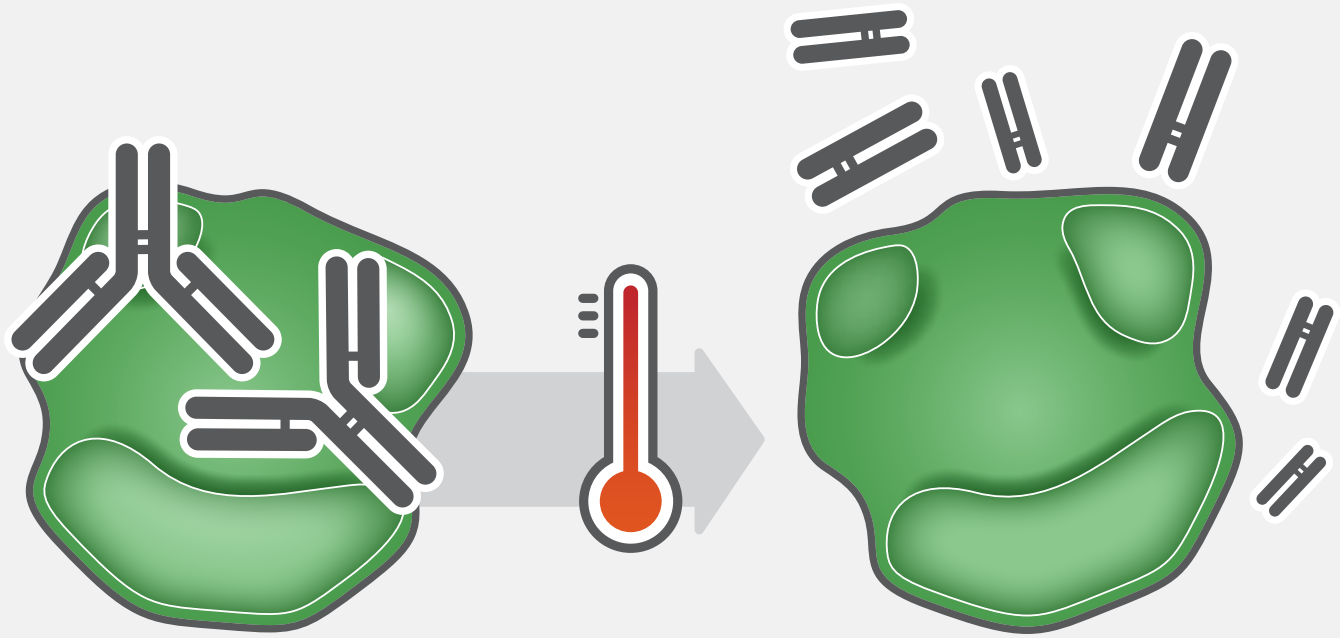
Helpful tip

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

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

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



Inactive **Activated**

Helpful tip

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

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

Choose the right PCR reagent for your research needs

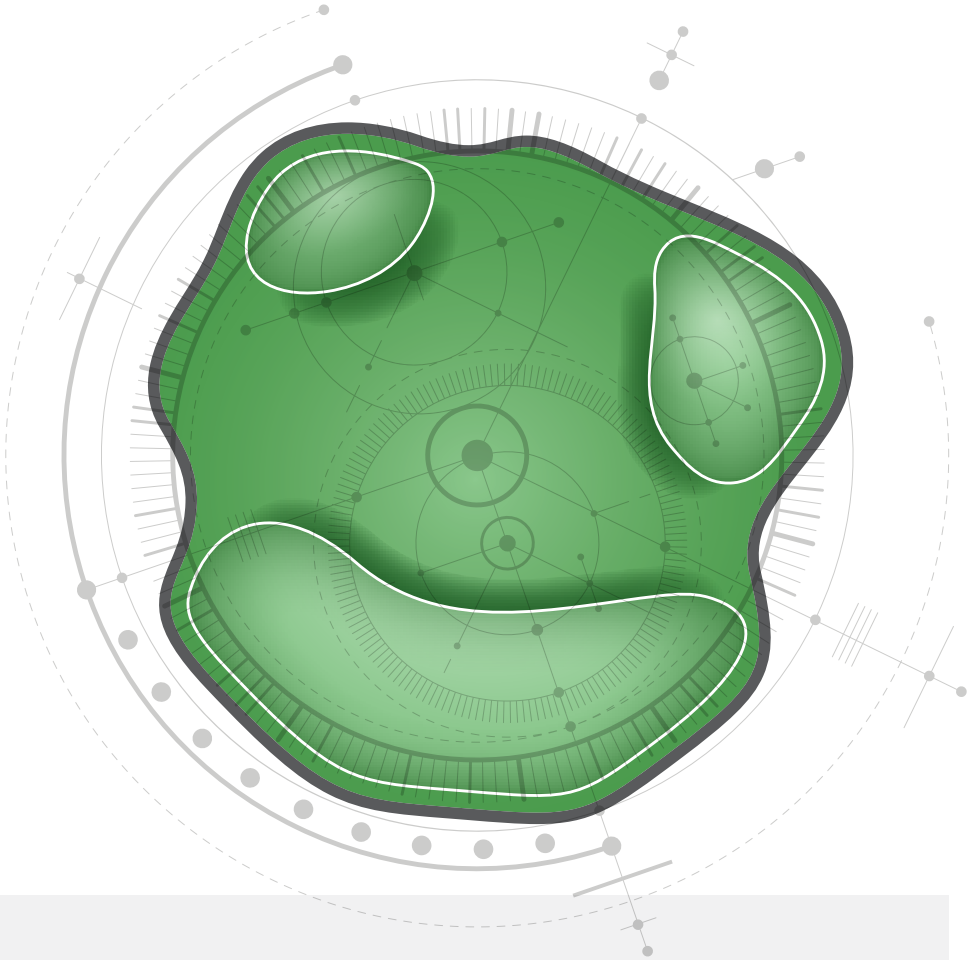
A comprehensive portfolio of PCR enzymes and master mixes is available with the high performance and consistency you need. Start with the selection guide below to find the best enzyme for common PCR applications.

DNA polymerase	Invitrogen™ Platinum™ SuperFi™ II DNA Polymerase	Invitrogen™ Platinum™ II *Taq* Hot-Start DNA Polymerase	Applied Biosystems™ AmpliTaq Gold™ 360 DNA Polymerase	Invitrogen™ Platinum™ 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		Yes
Inhibitor tolerance	Yes	Yes		Yes
Blunt or 3´-A end	Blunt	3´-A	3´-A	3´-A
Compatible with Applied Biosystems™ TaqMan® probes		Yes	Yes	
Certified low level of bacterial gDNA	Yes	Yes	Yes	
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.

Innovations for superior PCR

PCR enzymes and reagents are continually being improved to help you get to your research destination faster. For example, the latest Platinum DNA polymerases are designed with the following key innovative features.



More robust and versatile

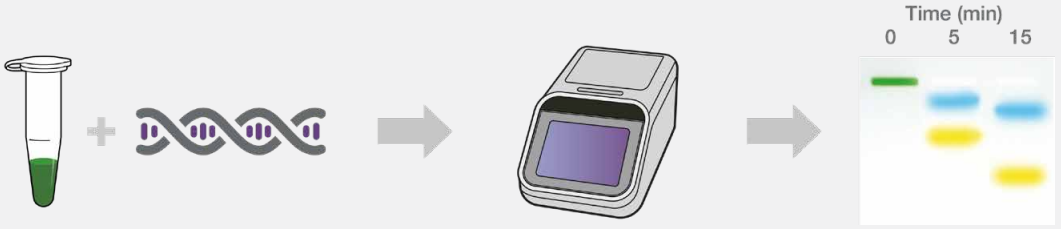
Advanced enzymatic engineering and methodology provide DNA polymerase with fast cycling, high tolerance of PCR inhibitors, and efficient amplification of challenging DNA like GC-rich sequences. These features help you amplify DNA targets confidently with speed and simplicity.
Find out more at **thermofisher.com/platinumenzymes**

Universal primer annealing

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



PCR primers and DNA oligos

Good design (i.e., good sequence selection) and high-quality primers are critical to your PCR reactions. In general, a length of 18–30 nucleotides for primers is optimal. The melting temperatures (T_m) of the primers should be between 65°C and 75°C, and within 5°C of each other.

OligoPerfect primer designer

Whether your are performing PCR, cloning, or capillary electrophoresis (CE) sequencing, take advantage of the benefits offered by our robust and easy-to-use Primer3-based Invitrogen™ OligoPerfect™ 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**—seamlessly integrates with the Invitrogen™ ordering portal

Try the OligoPerfect Designer at **thermofisher.com/oligoperfect-designer**

Custom DNA oligos

Invitrogen™ custom DNA oligos are synthesized on a highly automated, computer-controlled system, followed by rigorous quality control. Mass spectrometry and capillary electrophoresis are performed for short and long oligos, respectively, to help ensure the quality of the process and end products.

The appropriate synthesis scale and purification for your application depend on the nature of your downstream applications. Choose the right oligos and purification methods for your applications.

Helpful tip

If the T_m of your primer is very low, try to find a sequence with higher GC content; alternatively, the length of the primer can be extended. For more tips on primer design, go to **thermofisher.com/primerdesign**

Processing option	Desalted	HPLC	PAGE
Oligos	• 25 nmol–10 µmol • 5–100 bp	• 50 nmol–10 µmol • 7–55 bp • >85% full-length sequence	• 50 nmol–10 µmol • 7–100 bp • >90% full-length sequence
Standard PCR	•		
Specialty PCR		•	•
Cloning		•	•

In addition to standard delivery, next-day delivery is also available in most regions where local oligo manufacturing exists. For more on ordering information, yield guarantees, designing tools, technical resources, protocols, and FAQs, go to **thermofisher.com/oligos**

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

Electrophoresis

Nucleic acid electrophoresis is a common technique in molecular biology to separate, identify, quantify, and/or purify nucleic acids. Setting up electrophoresis involves a number of steps to achieve optimal separation and analysis of nucleic acid samples, such as gel preparation, ladder selection, sample visualization, and gel documentation.

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

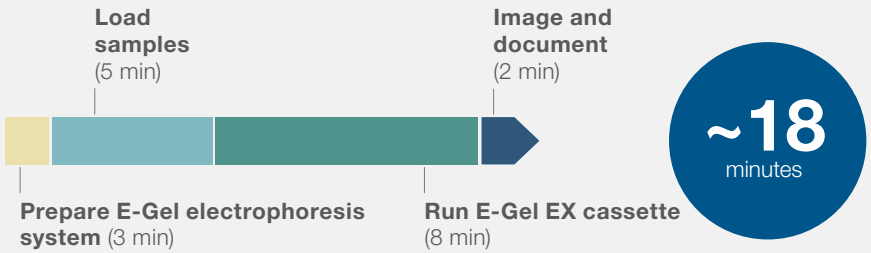
Nucleic acid electrophoresis

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

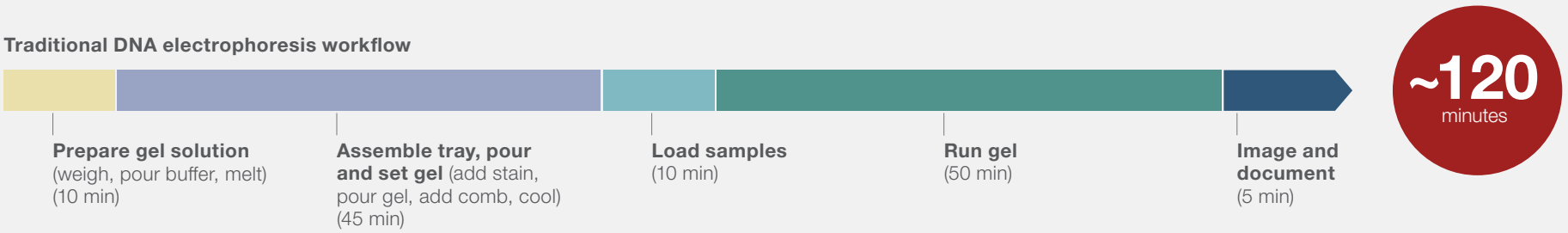
Determining the appropriate gel type and gel concentration is an essential step that will help streamline the separation of nucleic acids. Learn more about convenient reagents for agarose gel electrophoresis, including hassle-free precast Invitrogen™ E-Gel™ agarose gels and pour-your-own Invitrogen™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

If you need …	Rapid results, quality control, and a safer workflow	High-quality reagents, a versatile workflow, and cost savings
Product	E-Gel precast agarose gels	UltraPure Agarose
Product format	Precast agarose cassettes	Powder
Protocol time (approx.)	18 min	120 min
Ready to use	Yes	No
Get more information at	**thermofisher.com/egel**	**thermofisher.com/ultrapure**

E-Gel electrophoresis system workflow



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. E-Gel precast gels are self-contained and ready for use with the agarose, electrodes, and the 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™ E-Gel™ CloneWell™ 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**

Load Run Analyze

E-Gel DNA ladders

Accurate analysis of electrophoresis bands often depends on the DNA ladder you choose for your gel run. For optimal performance on E-Gel precast agarose gels, Invitrogen™ E-Gel™ DNA ladders are formulated with chromatography-purified DNA fragments in ready-to-use loading buffer. The chromatography-based purification method results in exceptional purity and quality of the DNA fragments, while an optimal buffer formulation reduces dye masking and helps improve ladder migration for more accurate analysis.

Find out more at **thermofisher.com/egel-ladders**

Helpful tip

E-Gel precast gels are available in a variety of formats for routine and high-throughput applications, with different stains (see page 36) and agarose percentages (0.8%, 1.2%, 2%, and 4%). To find the right gel for your needs, see the selection guide at **thermofisher.com/egelselection**

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

E-Gel Power Snap Electrophoresis System

To help reduce user errors and workflow time, the Invitrogen™ E-Gel™ Power Snap Electrophoresis System integrates rapid, real-time nucleic acid analysis with high-resolution image capture. The system offers:

Faster analysis— go from sample loading to image capture in as little as 18 minutes

Simple operation— intuitive user interface with large touchscreen and integrated operating system

Safer workflow— minimize handling of hazardous chemicals

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

E-Gel system for high-throughput electrophoresis

High-throughput electrophoresis is often performed for high-volume analysis of PCR products, plasmid preparations, and restriction digests. Gel runs and analysis can be accelerated using the E-Gel *48* and E-Gel *96* precast gels and expandable Invitrogen™ E-Base™ Electrophoresis System. The integrated design of the E-Gel™ Mother E-Base™ and Daughter E-Base™ devices saves space and allows up to 384 samples to run at one time.

Find out more at **thermofisher.com/egel-highthroughput**

Electrophoresis reagents

For pouring your own agarose gels, choosing high-quality agarose, optimized DNA ladders, and improved DNA stains can help you achieve optimal electrophoresis results.

DNA stains

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

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

UltraPure reagents for electrophoresis

Invitrogen™ UltraPure™ reagents are specifically formulated to meet your nucleic acid analysis and purification needs. UltraPure agarose and reagents are made from highly pure biochemicals for maximum reliability and superior performance.

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

DNA ladders

Invitrogen™ DNA ladders are available in a wide variety of size ranges (10 bp to 15 kb) and formats for different applications. To create DNA ladders of superior 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

	Standard detection	Safer detection	Enhanced detection	Ultimate detection
	UltraPure ethidium bromide	SYBR Safe stain	SYBR Green I stain	SYBR Gold stain
Sensitivity (dsDNA)	Sensitive (1 ng)	Sensitive (1 ng)	Highly sensitive (>60 pg)	Ultrasensitive (>25 pg)
Less hazardous and more environmentally friendly		•		
Improved cloning efficiency		•	•	•

Did you know?

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

Learn more at **thermofisher.com/na-electrophoresis-education**

Cloning

Molecular cloning involves recombinant DNA technologies that insert a DNA sequence of interest into a vector to generate a large number of copies. Traditionally, cloning has been carried out with restriction enzymes and a DNA ligase to form a new vector capable of expressing the gene of interest. In the case of gene synthesis, researchers can obtain their desired DNA directly in a specified vector with just sequence information. Other cloning methods, such as PCR cloning, Invitrogen™ TOPO™ cloning, ligation-independent cloning, and gene assembly are commonplace, exploiting unique characteristics of other DNA-modifying enzymes.

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.

Method	Thermo Scientific™ FastDigest™ restriction enzymes	Invitrogen™ TOPO™ cloning	Invitrogen™ Gateway™ cloning	Invitrogen™ GeneArt™ seamless cloning and GeneArt™ Gibson Assembly® cloning kits	Invitrogen™ GeneArt™ Type IIs assembly	Invitrogen™ GeneArt™ Strings™ DNA Fragments	Invitrogen™ GeneArt™ Gene Synthesis
Key benefits/ description	• Familiarity, flexibility, convenience, time savings • Universal protocol with 15 min digestion in one buffer	• >95% efficiency, 5 min PCR cloning • Compatible with many other cloning systems	• High-throughput and high-efficiency shuttling among multiple expression vectors	• Seamless multi-fragment assembly by homologous recombination • Directional cloning of up to 15 fragments • Up to 95% efficiency and 15 min cloning	• One-tube seamless multi-fragment assembly by simultaneous restriction digestion and ligation • Directional cloning of up to 8 fragments, for up to 20 kb total • Efficient for repetitive and very small sequences	• Synthesized DNA fragments ready to clone via the method of your choice • No starting DNA required • Pool sequence-verified	• Custom-cloned genes in your choice of vector • Sequence-verified • Can be optimized for a specific host for maximal protein expression
Technology basics	• Restriction digestion and ligation	• Topoisomerase-based, ligase-free cloning	• Single-step, directional, and site-specific DNA recombination • Restriction enzyme– and ligase-free	• End-terminal homology recombination using overlapping sequences • Transformation-associated recombination (TAR) in *Saccharomyces cerevisiae*	• Type IIs restriction and ligation in a single reaction	• Linear dsDNA assembled from pooled synthetic oligonucleotides • 150–3,000 bp, also available in library format with randomized bases	• DNA of interest cloned in vector • 100% sequence-verified with quality assurance documentation
Needs DNA source material (gene in plasmid, library, etc.)	•	•	•	•	•		
Use your own vector	•		*	•	•	•	•

* Vector needs to be converted with Invitrogen™ Gateway™ Vector Conversion System with One Shot™ *ccdB* Survival™ 2 T1^{R} Competent Cells.

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

Restriction enzyme cloning

Found naturally in bacteria, restriction enzymes recognize and cleave specific DNA sequences, resulting in sticky ends (5′ or 3′ protruding ends) or blunt ends, enabling 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

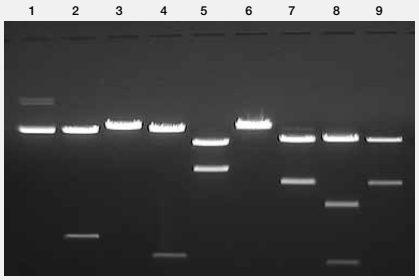
To simplify cloning, we offer FastDigest enzymes—an advanced line of restriction enzymes that share buffer compatibility with downstream modifying enzymes. Its benefits include:

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

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

A

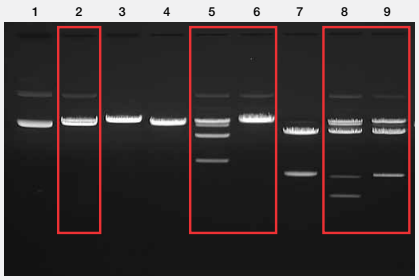
Plasmid DNA digested with FastDigest enzymes



1: Undigested plasmid DNA
2: FastDigest BcuI
3: FastDigest XbaI
4: FastDigest NdeI
5: FastDigest SalI
6: FastDigest XmaJI
7: FastDigest Eco31I
8: FastDigest Eco52I
9: FastDigest BglII

B

Plasmid DNA digested with NEB enzymes



1: Undigested plasmid DNA
2: SpeI-HF
3: XbaI
4: NdeI
5: SalI-HF
6: AvrII
7: BsaI
8: EagI-HF
9: BglI

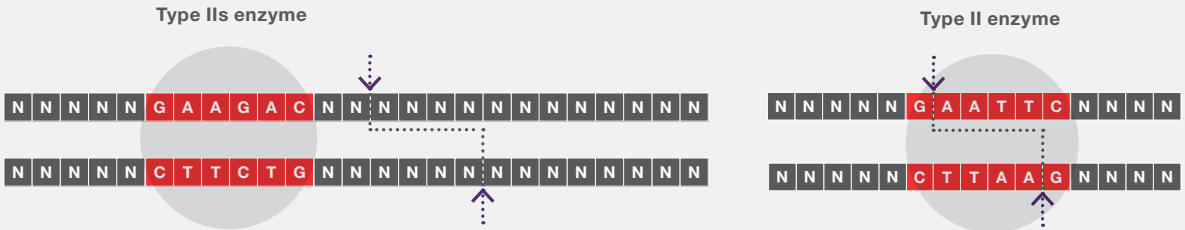
Comparison of digestion efficiencies of restriction enzymes. (A) FastDigest restriction enzymes digest plasmid DNA much more efficiently compared to the **(B)** NEB enzymes. In this experiment, using the NEB protocol, 1 µg of plasmid DNA was digested in ~15 min. Incomplete digestion is shown in red boxes.

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).

Find FastDigest Type IIs enzymes at
thermofisher.com/fastdigesttypeiis

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



Recognition site and cleavage site of Type IIs versus Type II restriction enzymes.

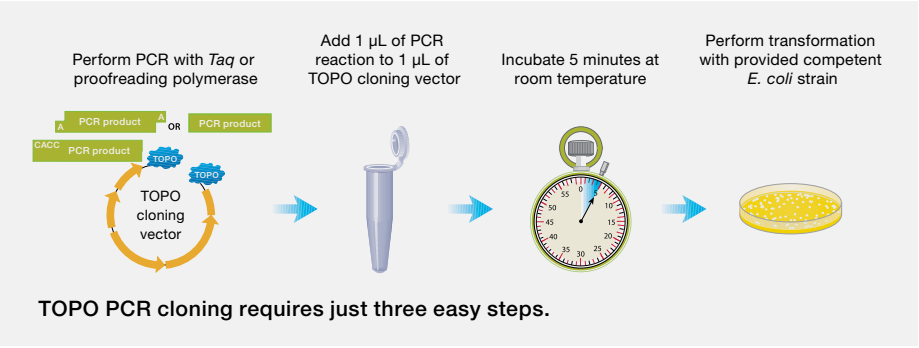
PCR cloning

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 readily 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**

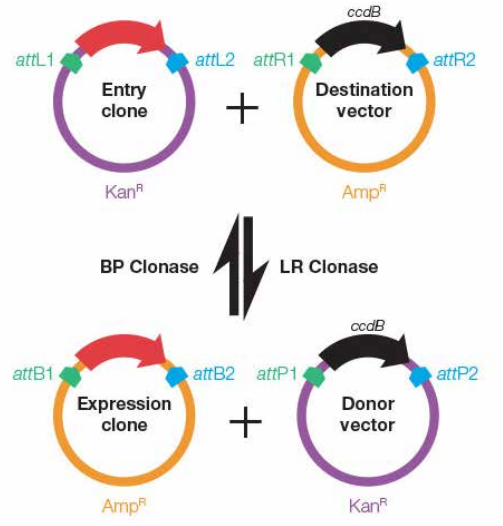


TOPO PCR cloning requires just three easy steps.

Gateway cloning

To shuttle a PCR 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.

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



Did you know?

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

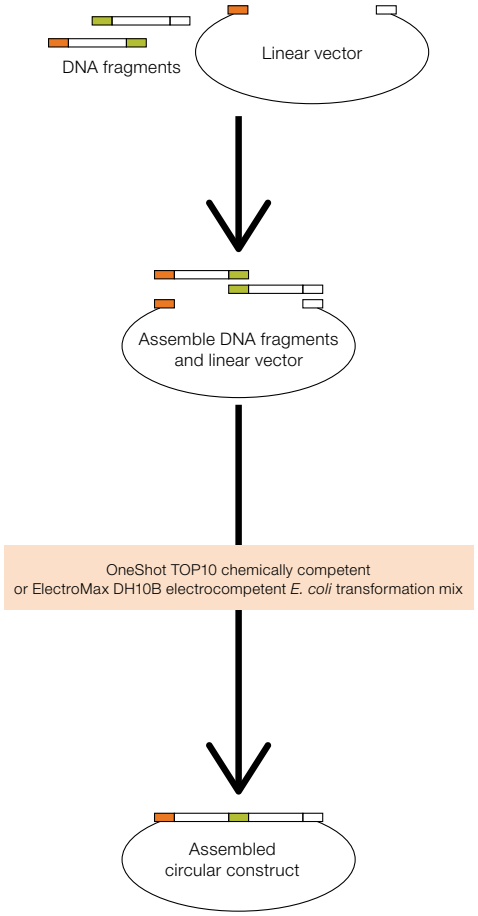
Seamless cloning and GeneArt Gibson Assembly cloning kits

To assemble multiple PCR fragments by end-terminal homologous recombination, several seamless cloning technologies are available for scarless and directional cloning into any vector. GeneArt seamless cloning kits offer the option of building constructs using *E. coli* and *Saccharomyces cerevisiae*.

Invitrogen™ GeneArt™ Gibson Assembly® kits allow for the simultaneous assembly of up to 15 very large DNA fragments to create precise 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



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]

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

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

Cloning with synthetic DNA

If you lack the time to generate and clone insert DNA, including optimization and troubleshooting, our synthetic DNA fragments and cloning service might be right for you. GeneArt Strings DNA Fragments and GeneArt Gene Synthesis offer genes analogous to optimized, error-free PCR products.

GeneArt Strings DNA Fragments

A time-saving alternative to PCR, GeneArt Strings DNA Fragments are available in lengths up to 3 kb and are compatible with any downstream cloning method of choice, providing:

- Synthetic, ready-to-use DNA fragments
- DNA with your specified ends to facilitate the cloning method of choice
- No starting DNA required
- Free optimization of gene with Invitrogen™ GeneArt™ GeneOptimizer™ software for maximum protein expression
- Option of Strings DNA Libraries with mixed, randomized nucleotides using full IUPAC code

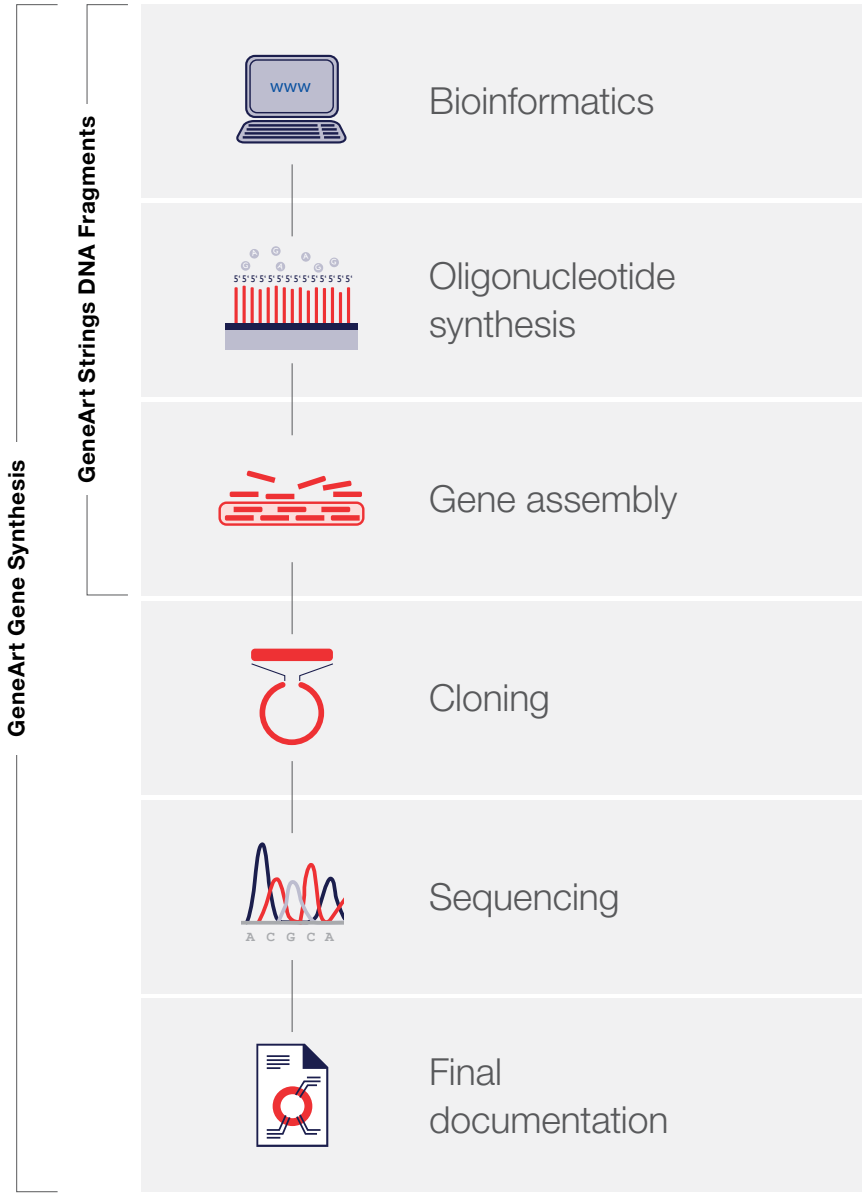
Find out more at **thermofisher.com/strings**

GeneArt Gene Synthesis

A reliable and cost-effective method for obtaining customized DNA constructs with 100% sequence accuracy, GeneArt Gene Synthesis offers:

- Synthetic, ready-to-transfect genes
- Cloning into several available vectors (custom options available)
- 100% sequence-verified and ready for downstream applications
- No starting DNA required
- Free optimization of gene with GeneOptimizer software for maximum protein expression

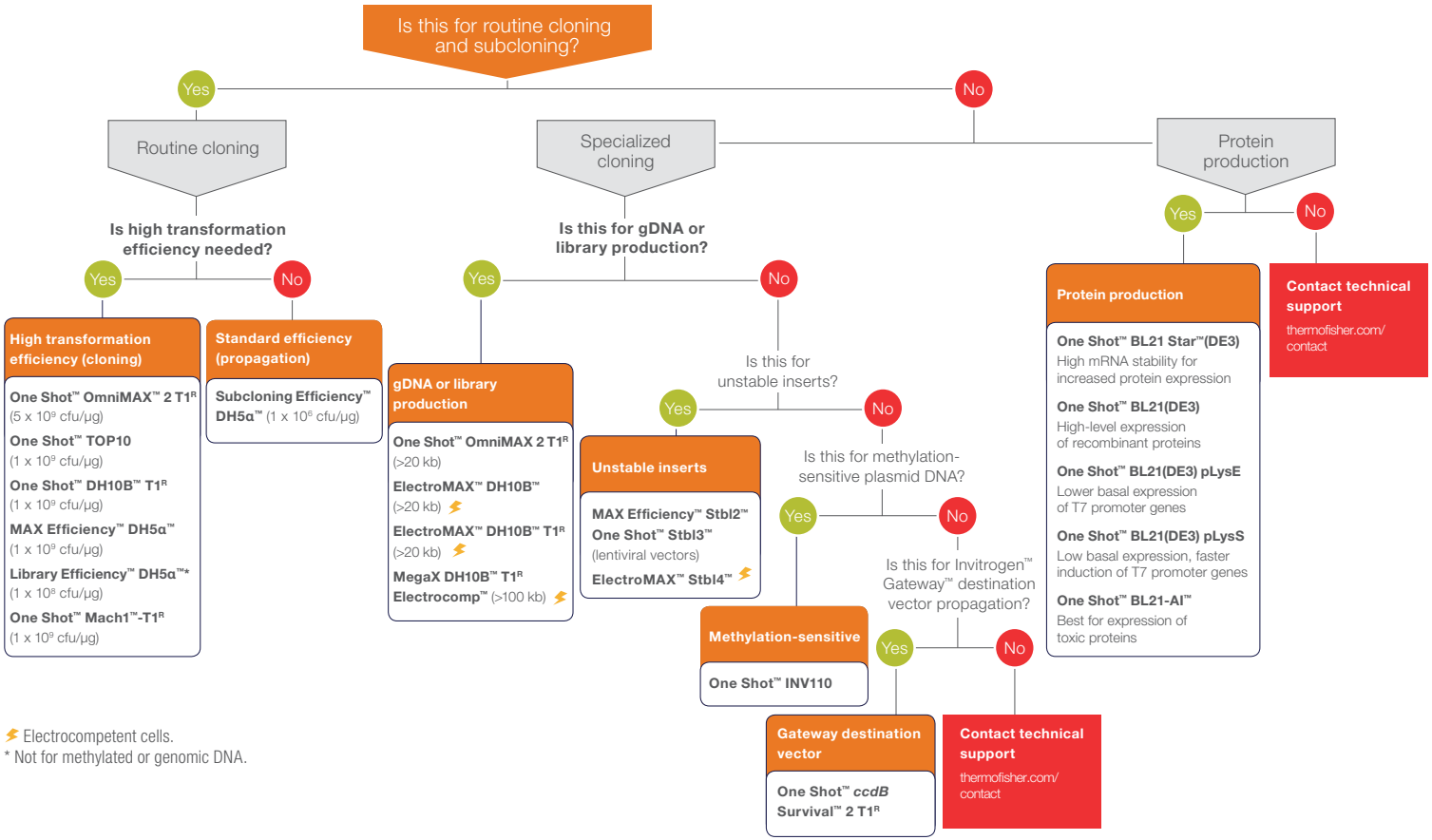
Find out more at **thermofisher.com/genesynthesis**



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 **thermofisher.com/compcells-education** for technical resources on competent cells.

Choosing Invitrogen™ competent cells based on the application



⚡ Electrocompetent cells.
* Not for methylated or genomic DNA.

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™ MultiShot™ 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

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

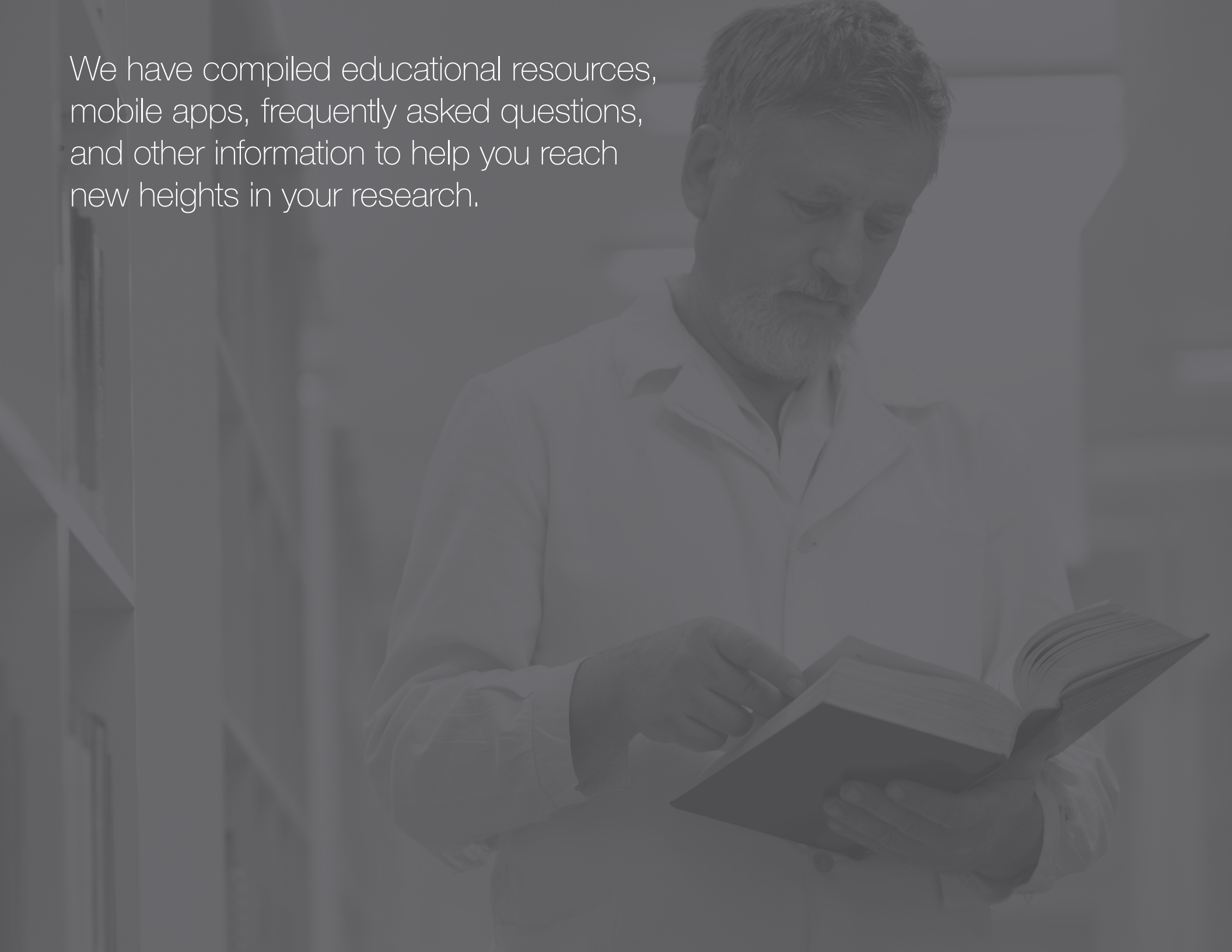
96-well plate

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

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**

Resources

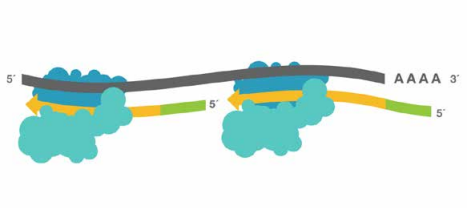


We have compiled educational resources, mobile apps, frequently asked questions, and other information to help you reach new heights in your research.

Educational resources

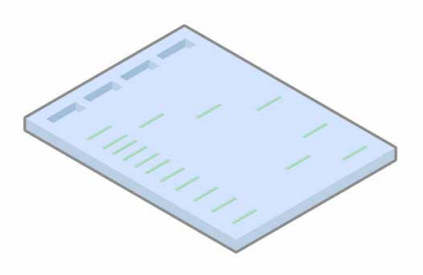
Suitable for new and experienced molecular biologists alike, our free online education resources are designed to help you review the basics, build your expertise, or discover our latest innovative technologies. Explore our technical resources in the following areas of molecular biology.

Reverse transcription

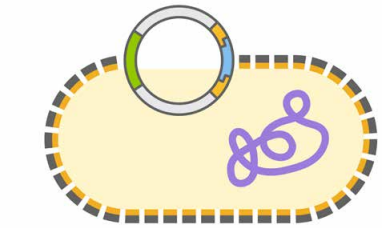


PCR enzymes, plastics, and thermal cyclers

Nucleic acid electrophoresis



Restriction enzymes, molecular cloning, and competent cells



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-tos, 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

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

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.

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 ProFlex, SimpliAmp, and MiniAmp PCR instruments.

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 **thermofisher.com/pcrquest**

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

Custom and OEM solutions

As a leading supplier of molecular biology reagents, plastics, and instruments, we offer customizable manufacturing solutions used by companies in developing next-generation molecular assays. Regardless of where you are in your assay development, we have off-the-shelf or custom solutions to help you achieve your goals. Partner with an experienced supplier that understands both raw materials and new technologies—a market leader with a dedicated diagnostics partnering business that brings value beyond products.

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- Risk and liability management

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

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 9–11) 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 page 15).

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 18).

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

PCR amplification

How can I optimize primer annealing for PCR?
Traditionally, gradient thermal cyclers have been used to simultaneously assess a number of temperatures around the theoretical annealing point. Compared to gradient thermal cyclers, instruments with the VeriFlex technology allow more precise temperature control for faster optimization of primer annealing (see page 21).

Tedious optimization steps may be circumvented using the novel Platinum 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 28–29).

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 28), "fast" plastics (low profile and ultra-thin walls; see page 25), and "fast" thermal cyclers (fast ramp rate; see pages 22–23).

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 **thermofisher.com/pcreducation** and **thermofisher.com/pcrsupport**

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 34).

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 gel stain is not classified as hazardous waste or as a pollutant under US federal regulations (see page 36).

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

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 40). 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, uncloned, double-stranded linear DNA fragments. GeneArt Gene Synthesis is a service offered for chemical synthesis, cloning, and sequence verification of genetic sequences (see page 44).

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 45).

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

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 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 Mini 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 DNA Multi-Sample Ultra Kit	500 preps	A25597
KingFisher Flex Purification System with 96 Deep-Well Head	1 system	5400630
KingFisher Duo Prime Purification System	1 system	5400110
PureLink PCR Purification Kit	50 preps	K310001
PureLink Quick Gel Extraction and PCR Purification Kit	50 preps	K220001
PureLink Quick Gel Extraction Kit	50 preps	K210012
Dynabeads M-270 Streptavidin	2 mL	65305
Dynabeads MyOne Streptavidin C1	2 mL	65001
Reverse transcription		
SuperScript IV Reverse Transcriptase	2,000 units	18090010
	10,000 units	18090050
SuperScript IV First-Strand Synthesis System	50 reactions	18091050
	200 reactions	18091200
SuperScript IV VILO Master Mix	50 reactions	11756050
	500 reactions	11756500
SuperScript IV VILO Master Mix with ezDNase Enzyme	50 reactions	11766050
	500 reactions	11766500
SuperScript IV One-Step RT-PCR System	25 reactions	12594025
	100 reactions	12594100
SuperScript IV One-Step RT-PCR System with ezDNase Enzyme	25 reactions	12595025
	100 reactions	12595100

	Quantity	Cat. No.
Reverse transcription (cont.)		
SuperScript IV CellsDirect cDNA Synthesis Kit	50 reactions	11750150
	500 reactions	11750350
SuperScript IV CellsDirect Lysis Reagents	500 reactions	11750550
RNaseOUT Recombinant Ribonuclease Inhibitor	5,000 units	10777019
Ribonuclease H	30 units	18021014
Random Hexamers (50 µM)	5 nmol	N8080127
Random Primers	9 A_{260} units	48190011
Oligo(dT)$_{12–18}$ Primer	25 µg	18418012
Oligo(dT)$_{20}$ Primer	15 µg	18418020
DNase I, Amplification Grade	100 units	18068015
PCR		
DNA Oligo, Desalted, Dry	25 nmol	A15612
DNA Oligo, Desalted, Dry, next-day (ordered before 1 PM Eastern Time)	25 nmol	A15613
DNA Oligo, Desalted, Liquid	25 nmol	A15611
DNA Oligo, Desalted, Dry	50 nmol	A15610
DNA Oligo, Desalted, Liquid	50 nmol	A15609
DNA Oligo, Cartridge, Dry	50 nmol	A15614
DNA Oligo, Cartridge, Liquid	50 nmol	A15608
DNA Oligo, HPLC, Dry	50 nmol	A15607
DNA Oligo, HPLC, Liquid	50 nmol	A15606
DNA Oligo, PAGE, Dry	50 nmol	A15605
DNA Oligo, PAGE, Liquid	50 nmol	A15604
Platinum II *Taq* Hot-Start DNA Polymerase	100 reactions	14966001
	500 reactions	14966005
Platinum II Hot-Start PCR Master Mix (2X)	50 reactions	14000012
	200 reactions	14000013
Platinum II Hot-Start Green PCR Master Mix (2X)	50 reactions	14001012
	200 reactions	14001013
AmpliTaq Gold 360 DNA Polymerase	100 units	4398813
	250 units	4398823
AmpliTaq Gold 360 Master Mix	1 mL	4398876
	5 mL	4398881

Ordering information (cont.)

	Quantity	Cat. No.
PCR (cont.)		
Platinum SuperFi II DNA Polymerase	100 units	12361010
	500 units	12361050
Platinum SuperFi II PCR Master Mix	100 reactions	12368010
	500 reactions	12368050
Platinum SuperFi II Green PCR Master Mix	100 reactions	12369010
	500 reactions	12369050
Platinum Direct PCR Universal Master Mix	100 reactions	A44647100
	500 reactions	A44647500
dNTP Set (100 mM)	4 x 250 µL	10297018
	8 x 1.25 mL	10297117
ProFlex 3 x 32-Well PCR System	1 instrument	4484073
ProFlex 96-Well PCR System	1 instrument	4484075
SimpliAmp Thermal Cycler	1 instrument	A24811
Veriti 96-Well Thermal Cycler	1 instrument	4375786
Veriti 384-Well Thermal Cycler	1 instrument	4388444
MiniAmp Plus Thermal Cycler	1 instrument	A37835
MiniAmp Thermal Cycler	1 instrument	A37834
Automated Thermal Cycler, 96-well	1 instrument	A31486
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

	Quantity	Cat. No.
Nucleic acid separation and analysis		
UltraPure Ethidium Bromide, 10 mg/mL	10 mL	15585011
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
UltraPure Agarose	100 g	16500100
TrackIt 100 bp Plus DNA Ladder	100 applications	10488058
UltraPure TAE Buffer, 10X	4 L	15558026
E-Gel Agarose Gels with SYBR Safe stain	10 gels	A42135
E-Gel Double Comb Agarose Gels with SYBR Safe stain	10 gels	A42348
E-Gel EX Double Comb Agarose	10 gels	A42346
E-Gel CloneWell II Agarose Gels with SYBR Safe DNA Gel Stain, 0.8%	18 gels	G661818
E-Gel Agarose Gels with SYBR Safe DNA Gel Stain, 2%	18 gels	G521802
E-Gel EX Agarose Gels, 2% with SYBR Gold DNA stain	20 gels	G402002
E-Gel 1 Kb Plus DNA Ladder	100 applications	10488090
E-Gel Sample Loading Buffer, 1X	4 x 1.25 mL	10482055
E-Gel Power Snap Electrophoresis System Starter Kit, EX 2%	1 kit	G8342ST
E-Gel Power Snap Electrophoresis Device Starter Kit, CloneWell II 0.8% with SYBR Safe gel stain	1 kit	G8168ST
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
Cloning and gene synthesis		
FastDigest BamHI	800 reactions	FD0054
	2,500 reactions	FD0055
FastDigest BcuI	20 reactions	FD1253
	50 reactions	FD1254
FastDigest BshTI	20 reactions	FD1464
FastDigest DpnI	50 reactions	FD1703
	100 reactions	FD1704
FastDigest EcoRI	800 reactions	FD0274
	2,500 reactions	FD0275
FastDigest KpnI	300 reactions	FD0524

	Quantity	Cat. No.
Cloning and gene synthesis (cont.)		
FastDigest NotI	20 reactions	FD0593
	50 reactions	FD0594
	150 reactions	FD0595
	250 reactions	FD0596
FastDigest SalI	200 reactions	FD0644
FastDigest XbaI	300 reactions	FD0684
	750 reactions	FD0685
FastDigest XhoI	400 reactions	FD0694
	1,200 reactions	FD0695
FastDigest Esp3I (BsmBI) (IIs class)	20 reactions	FD0454
FastDigest BpiI (BbsI) (IIs class)	20 reactions	FD1014
FastDigest Eco31I (BsaI) (IIs class)	50 reactions	FD0293
	100 reactions	FD0294
TOPO TA Cloning Kit for Subcloning, without competent cells	25 reactions	450641
Zero Blunt TOPO PCR Cloning Kit, without competent cells	25 reactions	450245
pENTR/D-TOPO Cloning Kit, with One Shot TOP10 Chemically Competent *E. coli*	20 reactions	K240020
pcDNA 6.2/V5-PL-DEST Mammalian Expression Vector	6 µg	12537162
One Shot TOP10 Chemically Competent *E. coli*	20 reactions	C404003
One Shot StbI3 Chemically Competent *E. coli*	20 x 50 µL	C737303
MAX Efficiency DH5α Competent Cells	200 µL	18258012
ElectroMAX DH10B Cells	100 µL	18290015
MAX Efficiency StbI2 Competent Cells	5 x 200 µL	10268019
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
MultiShot FlexPlate TOP10 Chemically Competent *E. coli*	1 plate	C4081201
MultiShot FlexPlate DH5α T1^{R} Chemically Competent *E. coli*	1 plate	C4481201
MultiShot FlexPlate StbI3 Chemically Competent *E. coli*	1 plate	C7381201

	Quantity	Cat. No.
Cloning and gene synthesis (cont.)		
GeneArt Gibson Assembly HiFi Cloning Kit, chemically competent cells	10 reactions	A46624
GeneArt Gibson Assembly EX Cloning Kit, chemically competent cells	10 reactions	A46633
GeneArt Seamless PLUS Cloning and Assembly Kit	20 reactions	A14603
GeneArt Type IIs Assembly Kit, AarI	10 reactions	A15916
GeneArt Type IIs Assembly Kit, BsaI	10 reactions	A15917
GeneArt Type IIs Assembly Kit, BbsI	10 reactions	A15918
GeneArt High-Order Genetic Assembly System	10 reactions	A13285
Gateway BP Clonase II Enzyme Mix	20 reactions	11789020
Gateway LR Clonase II Enzyme Mix	20 reactions	11791020
MultiSite Gateway Pro Plus	20 reactions	12537100
LR Clonase II Plus Enzyme	20 reactions	12538120
Gateway Vector Conversion System with One Shot *ccdB* Survival Cells	1 kit	11828029
PCR Cloning System with Gateway Technology with pDONR 221 and OmniMAX 2 Competent Cells	20 reactions	12535029
PCR Cloning System with Gateway Technology with pDONR/Zeo and OmniMAX 2 Competent Cells	20 reactions	12535037
Gateway pDONR 221 Vector	6 µg	12536017
pENTR/D-TOPO Cloning Kit, with One Shot TOP10 Chemically Competent *E. coli*	20 reactions	K240020
pCR 8/GW/TOPO TA Cloning Kit with One Shot TOP10 *E. coli*	20 reactions	K250020

GeneArt Gene Synthesis **thermofisher.com/genesynthesis**

GeneArt Strings DNA Fragments **thermofisher.com/strings**

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