

Prepare for discovery

Molecular biology workflow solutions



Molecular biology solutions fit for discovery

In your pursuit to advance science, every experiment matters. There's no time to start again.

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

Set the foundation and explore solutions fit for discovery, from sample preparation to reverse transcription, PCR, and cloning. With this guide, there's no need to wonder if the products you choose will set you back or propel you forward.

Find additional information at thermofisher.com/pcrandcloning.

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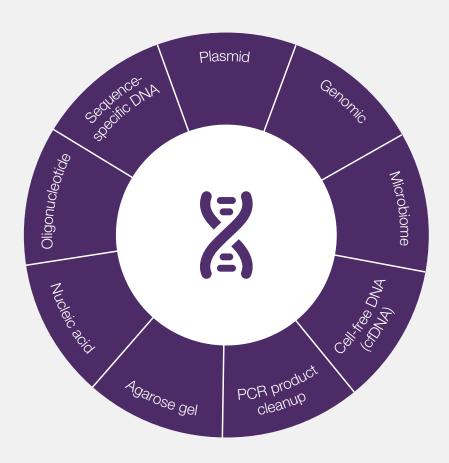


Nucleic acid isolation is a crucial first step in the molecular biology workflow, whether you are isolating genomic DNA (gDNA) or RNA. Selecting nucleic acid purification products that are optimized to provide maximum yield, purity, and integrity from virtually any sample type and application is important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

Advance your research at thermofisher.com/kingfisher

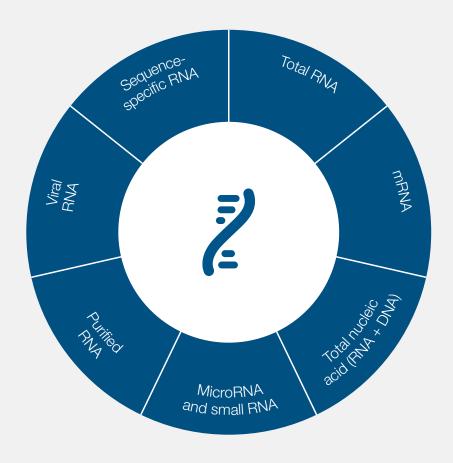


Portfolio of solutions for your nucleic acid isolation



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

Learn more at thermofisher.com/kingfisherkits



Common nucleic acid isolation methods



Automated purification instruments: automated processing of magnetic particles in a microplate format (e.g., Thermo Scientific™ KingFisher™ 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/run
- 24- or 96-well plates for different input volumes
- Easily edit, modify, or create new protocols
- All benefits of magnetic beads (below)



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, 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



Learn more at thermofisher.com/sampleprep





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



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



Learn more at thermofisher.com/sampleprep



Automation platform: Find a model that meets your needs

Optimize and automate your DNA and RNA purification workflow 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. Learn more and request a demo at **thermofisher.com/kingfisher**.









KingFisher instrument:	Duo Prime	Flex	Apex	Presto
Instrument size	Compact benchtop	Benchtop	Benchtop	Benchtop—integrates with robotic liquid handler
Throughput level	Low to medium	High	High	Ultrahigh
Processing volume range	 50–1,000 μL: 12-pin magnet head 200–5,000 μL: 6-pin magnet head 	 PCR plate (20–100 μL*), skirted 20–200 μL: 96-well plate 50–1,000 μL: 96 deep-well plate 200–5,000 μL: 24 deep-well plate 	 15–1,000 μL: 96 deep-well plate 15–200 μL: 96-well KingFisher standard plate 10–80 μL: 96-well PCR plate 30–5,000 μL: 24 deep-well plate 30–200 μL: 96 storage tubes 200–1,000 μL: 24 storage tubes 	 50–1,000 μL: 96 deep-well plate 200–5,000 μL: 24 deep-well plate KingFisher 96 plate: 50–150 μL
Samples per run	6 or 12	24 or 96	24 or 96	24 or 96
Customizable protocols	Yes	Yes	Yes, with touchscreen or PC software	Yes
Heating/cooling	 10°C to 75°C (plate row block A) 4°C to 75°C (elution strip block) 	From 5°C above ambient temperature to 115°C	 From 4°C above ambient temperature to 100°C Cooling down to 4°C 	From 5°C above ambient temperature to 115°C
Ultraviolet lamp	8 watts (up to 16 hr)	No	2 UV lamps, max 23 h 59 min	No
Additional details	For Research Use Only	For Laboratory Use	For Laboratory Use	For Laboratory Use

^{*} Or similar skirted PCR plate.

Typical run with a KingFisher instrument









Press start



Run time

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



Resource

Use our selection tool to find the right magnetic bead–based kit for your automated sample preparation.

Find out more at

thermofisher.com/kingfisherkits



Learn more at thermofisher.com/kingfisher



^{**} Can vary depending on application and instrument.

Selecting the right DNA isolation kits for your downstream research

Cancer research: Applied Biosystems[™] MagMAX[™] cell-free nucleic acid kits on KingFisher instruments are ideal for liquid biopsy research. They are optimized specifically for enrichment of cfDNA and total nucleic acid (cfTNA), not gDNA, which means increased recovery and lower starting volumes.

Application	Automation-ready extraction kit and reagents	Cat. No.
	MagMAX Cell-Free DNA Isolation Kit	A29319
	MagMAX mirVana Total RNA Isolation Kit	A27828
	MagMAX FFPE DNA/RNA Ultra Kit	A31881
	MagMAX DNA Multi-Sample Ultra 2.0 Kit	A36570
	Dynabeads FlowComp Human CD3 Kit	11365D
Cancer research	Dynabeads FlowComp Human CD4 Kit	11361D
	Dynabeads FlowComp Human CD8 Kit	11362D
	Dynabeads Untouched Human T Cells Kit	11344D
	Dynabeads Untouched Human CD4 T Cells Kit	11346D
	Dynabeads CD15	11137D
	Dynabeads CD14	11145D

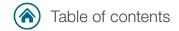
DNA research: Using Applied Biosystems™ MagMAX™ DNA Multi-Sample Ultra 2.0 Kits and KingFisher instruments, you can isolate gDNA from 50 µL to 2 mL of whole blood, saliva, buffy coat, buccal swabs, or other biological samples. The resulting purified gDNA is ideal for many downstream molecular biology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

Application	cation 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	





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



Selecting the right DNA isolation kits for your downstream research

Infectious disease research: Applied Biosystems™ MagMAX™ viral/pathogen kits on KingFisher instruments provide a sensitive and simple method for nucleic acid extraction from samples containing viruses or other pathogens.

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

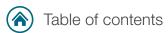
Plasmid purification: Choose from our wide range of high-performing, cost-effective Thermo Scientific™ and Invitrogen™ kits for plasmid DNA isolation designed to isolate plasmid DNA at the purity and scale you need.

Application	Cat. No.	
	GeneJET Plasmid Miniprep Kit	K0502
	PureLink HiPure Plasmid Filter Maxiprep Kit	K210016
Plasmid purification	PureLink Fast Low-Endotoxin Midi Plasmid Purification Kit	A35892
	GeneJET Endo-Free Plasmid Maxiprep Kit	K0861
	PureLink Expi Endotoxin-Free Maxi Plasmid Purification Kit	A33073









Selecting the right DNA isolation kits for your downstream research

Wastewater DNA and RNA for disease surveillance: Applied Biosystems™ MagMAX™ 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 is ideal for use in a variety of downstream applications such as quantitative qPCR, digital PCR, or NGS.

Application	Automation-ready extraction kit and reagents	Cat. No.		
0	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		

Streptavidin: Utilization of Invitrogen™ Dynabeads™ streptavidin magnetic beads offers the best 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)).

Application	Automation-ready extraction kit and reagents Cat. No.			
	Dynabeads Streptavidin for Target Enrichment	65606D		
	Dynabeads M-270 Streptavidin	65305		
IP / 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[™] and Invitrogen[™] 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 _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	Blood, plants, saliva, urine, stool, soil, plasma, serum*	Cultured cells
Starting material	100 mg of tissue or 10 ⁷ cells	10° cells, 200 mg of tissue, 250 mg of plant tissue, 0.2 mL of blood, 5 x 10° yeast, 10° bacteria	Variable depending on sample	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	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.



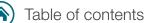
Helpful tip

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



Learn more at thermofisher.com/rnapreps







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

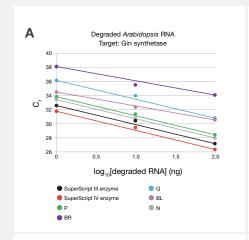
Thermostable reverse transcriptases allow reactions to occur at higher temperatures, which help denature RNA with complex secondary structures or high GC content, for generation of longer cDNA, higher cDNA yields, and better coverage of RNA populations in the cDNA.

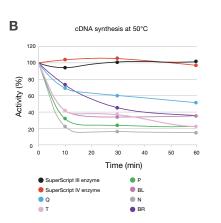
Processivity

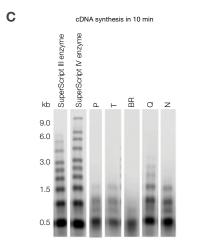
Processivity is the enzyme's ability to add consecutive nucleotides without releasing the template. Highly processive reverse transcriptases allow synthesis of longer cDNA strands in a shorter reaction time, and overall better efficiency in making full-length cDNA.

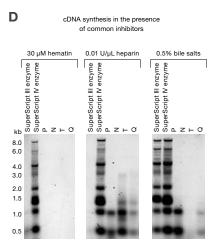
Inhibitor tolerance

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.





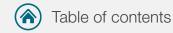




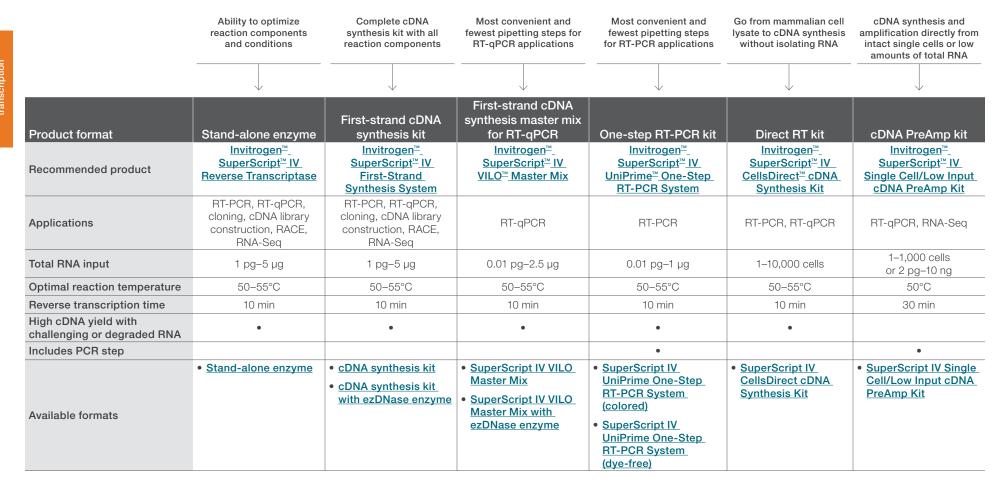
(A) Sensitivity, (B) thermostability, (C) processivity, and (D) inhibitor tolerance of reverse transcriptases can affect the quantity and length of cDNA.



Learn more at thermofisher.com/reverse-transcription



Reverse transcription reagent selection guide



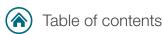


Did you know?

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 thermofisher.com/lyoreadyenzymes.



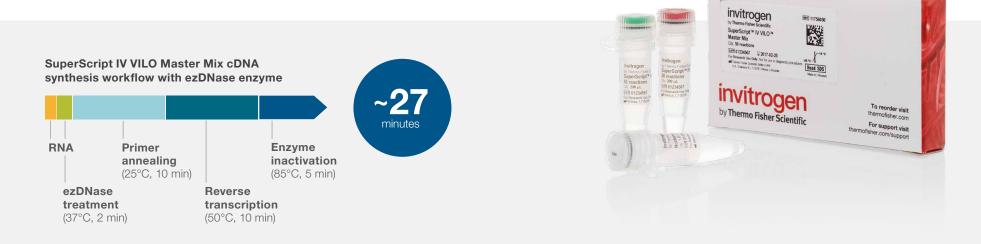
Learn more at thermofisher.com/superscript

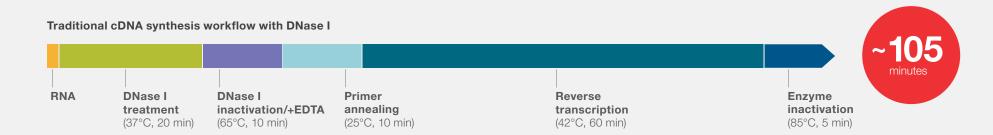


Genomic DNA removal

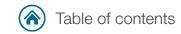
RNA purification methods, including protocols with DNase digestion on column, often fail to completely remove gDNA. Amplification of contaminating gDNA can cause inaccurate results. Traditional gDNA decontamination protocols with DNase I include time-consuming DNase inactivation or gDNA removal steps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

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





Learn more at thermofisher.com/4vilo



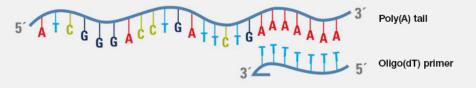
Reverse transcription primers

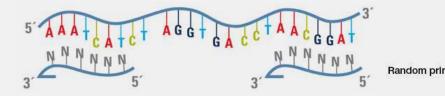
The priming strategy you choose for reverse transcription is important for cDNA synthesis efficiency, consistency, and yield. Each primer type has its benefits and drawbacks, depending on the individual target RNA.

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 ideal for nonpolyadenylated RNA, such as bacterial RNA.

Two most common primers used in reverse transcription







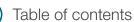
Helpful tip

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.



Learn more at thermofisher.com/rtprimers







The polymerase chain reaction (PCR) is a scientific method used to make many copies of a specific piece of DNA. The process involves the following steps:

- 1) Denaturation: The double-stranded DNA is heated to separate the strands into single strands.
- 2) Annealing: Primers, which are short DNA molecules, bind to specific regions of the target DNA.

3) Extension: DNA polymerase is used to extend the primers along the single strands, creating new copies of the target DNA. This step is performed in a thermal cycler, alternating between high and low temperatures, making millions of copies of the target DNA.

PCR is a powerful tool for various applications in molecular biology.

Find technical and educational resources about 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™ 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 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.

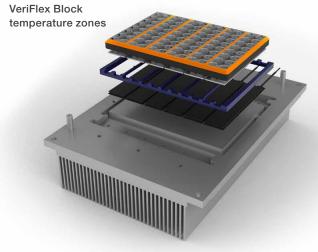
Fleet control

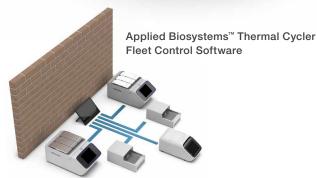
If you manage multiple thermal cyclers and users, you may benefit from a single interface for viewing all instruments at a glance and setting custom permissions by instrument, user, and method. Learn more at thermofisher.com/fleetcontrol.



Helpful tip

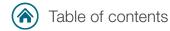
Using the right PCR plastics for your application and instrument can improve the reliability of your PCR results. Go to **thermofisher.com/findplastics** to determine the right PCR plastics for you.







Learn more at thermofisher.com/thermalcyclers



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







	ProFlex [™] PCR System	VeritiPro [™] 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 want to access your instrument remotely?
Key benefits	Ultimate flexibility and throughput	Ultimate performance
Max sample throughput	480,000 reactions	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











SimpliAmp [™] Thermal Cycler	MiniAmp™ Thermal Cycler	Automated Thermal Cycler
> Do you need an intuitive interface?> Do you train new technicians often?> Do you want to access your instrument remotely?	Do you want an instrument with just the features needed for routine PCR?Do you want to access your instrument remotely?	> 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	3.5°C/sec	3.5°C/sec
3-zone VeriFlex Block on 96-well system	3-zone VeriFlex Block on MiniAmp™ Plus model	None
Yes	Yes	Yes





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.

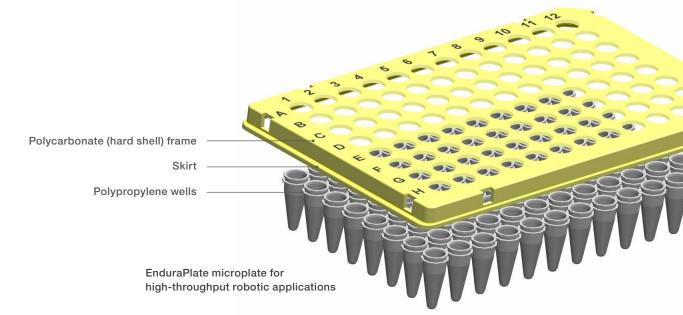
Biological testing: Our plastics are biologically tested to certify them as free of DNA, RNase, and PCR inhibitors. We offer plastics for laboratory use that are provided with a PCR certificate for your convenience and documentation.

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™ MicroAmp™ 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.







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 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 thermofisher.com/findplastics.

	Small-scale experiments with a few samples	Daily experiments	Complete-workflow experiments—ideal for automation	Automation-compatible
	Single tubes, strips, caps, adhesive film, and accessories	MicroAmp optical microplates	MicroAmp EnduraPlate optical microplates	MicroAmp EnduraPlate optical reaction plates
Formats	Single tubes	• 32-well	• 96-well	• 96-well
	Single tubes with caps	• 48-well Fast	96-well Fast	96-well Fast
	8-strip tubes with caps	• 96-well*	• 384-well	• 384-well
	• 12-strip caps	96-well Fast*	96-well full skirted	
		• 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	No	Yes (for those with * above)	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 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.



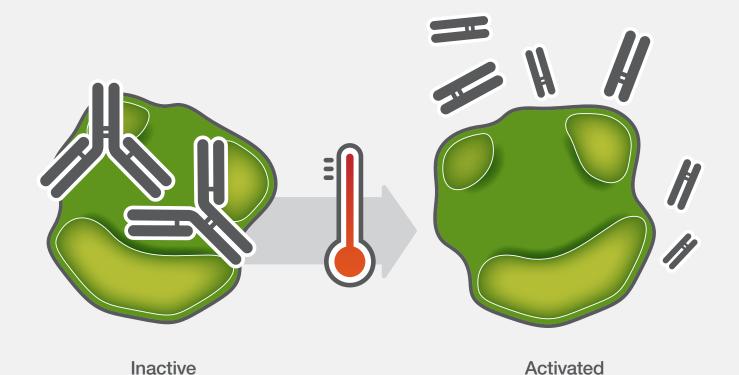
Find out more at thermofisher.com/direct-pcr







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





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 Mg²⁺ 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 <i>Taq</i> 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	No	Yes		
Inhibitor tolerance	Yes	Yes	No	Yes		
Blunt or 3'-A end	Blunt	3´-A	3´-A	3′-A		
Compatible with Applied Biosystems [™] TaqMan [™] 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.

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).



Custom DNA oligos

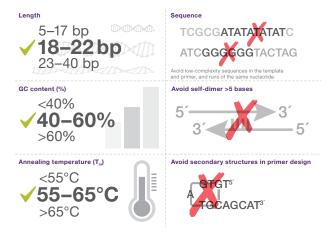
With a complete range of custom-synthesized oligonucleotide primers, probes, and genes, we recognize the need for quality, reliability, and convenience.

Invitrogen™ custom DNA oligos are synthesized on state-of-the-art, automated systems to increase performance, speed, and capacity. Available in a range of synthesis scales, purification options, and modifications, oligos are analyzed by mass spectrometry or capillary electrophoresis to help ensure the quality of the process and 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**.



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™ 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 or visit the oligo utility hub for our full suite of tools and calculators at thermofisher.com/oligotools.

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

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 **thermofisher.com/largescaleoligos**.







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



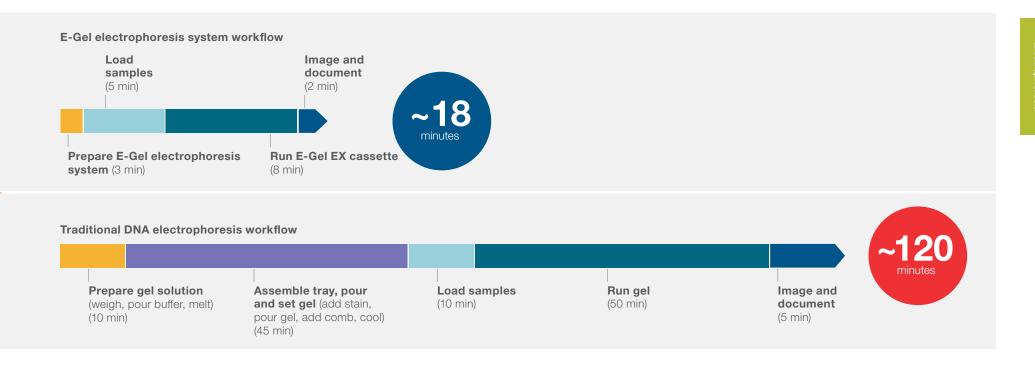
Nucleic acid electrophoresis

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
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.







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

2 Run

3 Analyze

E-Gel gels—faster and safer workflow

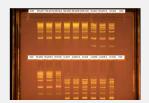
Pathogen detection:

• Detection of potato spindle tuber viroid (PSTVd) RNA using Invitrogen™ SuperScript™ IV RT-LAMP Master Mix and E-Gel gels



RNA analysis:

 Optimizing loading conditions of RNA markers on an Invitrogen™ E-Gel™ system



Mouse genotyping:

• E-Gel electrophoresis systems can aid in genotyping by providing a convenient and accurate platform for visualizing DNA fragments, such as universal target fragments





Helpful tips

E-Gel precast gels are available in a variety of formats for routine and high-throughput applications, with different stains (see page 38) 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.

Choose Invitrogen™ E-Gel™ 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.







Select your E-Gel device

Integrate your electrophoresis running and imaging into a single small device.

Enjoy speed and convenience.





	Invitrogen [™] E-Gel [™] Power Snap Plus Electrophoresis System	Invitrogen [™] E-Gel [™] Power Snap Electrophoresis System
	 > Do you use electrophoresis often? > Would you like to store your gel images on internal servers or the cloud? > Do you want to perform quantitative analysis of your gels? > Do you value speed? > Would you like to store, share, and analyze gel images online? 	 > Do you run less than 25 samples at a time? > Do you want a simple and fast solution for your electrophoresis? > Do you need to save bench space?
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, cloning
Software	Invitrogen™ iBright™ analysis software	NA
Find out more	thermofisher.com/powersnapplus	thermofisher.com/powersnap

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 and SYBR™ Gold stains provide greater safety and/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. SYBR Safe stain also carries the ACT™ label.

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

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 to 48,502 bp) 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

	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 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





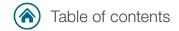
Cloning and gene synthesis

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	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 and complete digestion in 5–15 minutes in one buffer 100% buffer compatibility with downstream applications Direct loading on gels 	>95% efficiency, 5-minute PCR cloning Compatible with many other cloning systems	High-throughput and high-efficiency shuttling among multiple expression vectors	Seamless multifragment assembly by homologous recombination Directional cloning of up to 15 fragments Up to 95% efficiency and 15-minute cloning	One-tube seamless multifragment 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 200–3,000 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.





Restriction enzyme cloning

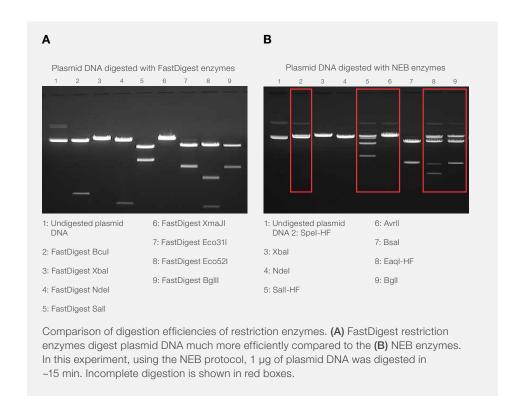
Found naturally in bacteria, restriction enzymes recognize and cleave specific DNA sequences, resulting in sticky ends (5' or 3' protruding ends) or blunt ends, enabling DNA inserts to be cloned into vectors with compatible ends. Star activity, buffer compatibility, and varying protocols for complete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

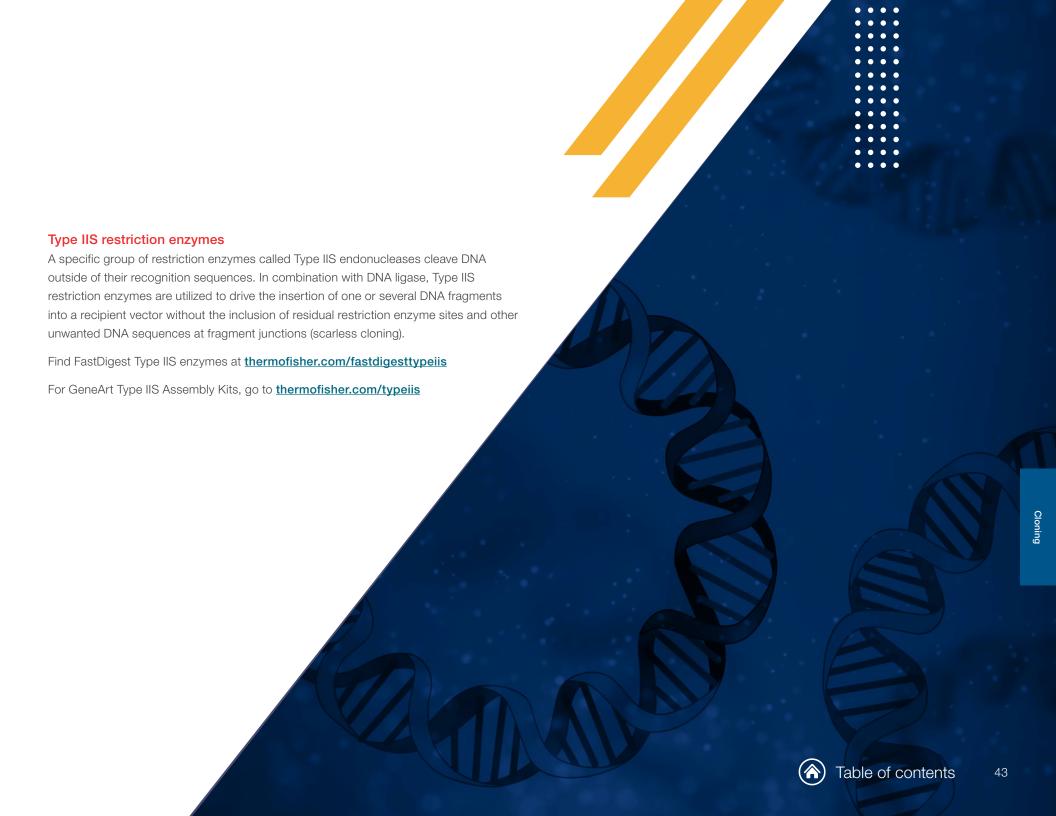
FastDigest restriction enzymes

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

- Complete digestion in 5–15 min
- Universal buffer allows for multiple digestions 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





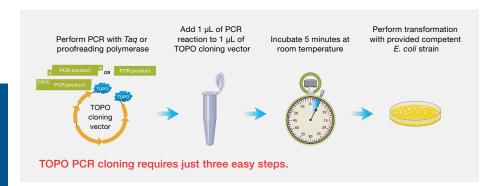
PCR cloning

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



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

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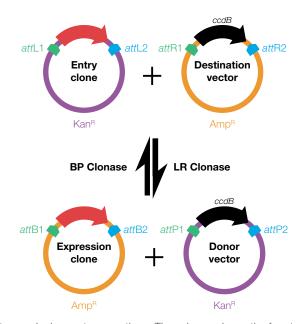
Did you know?

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

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



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

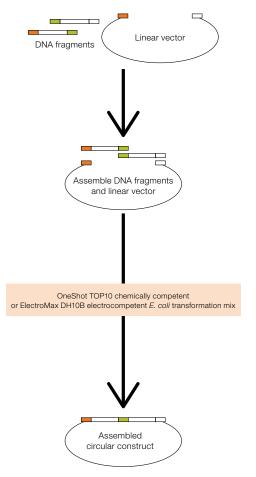
Seamless cloning and GeneArt Gibson Assembly cloning kits

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

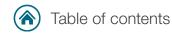




Did you know?

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].





^{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:

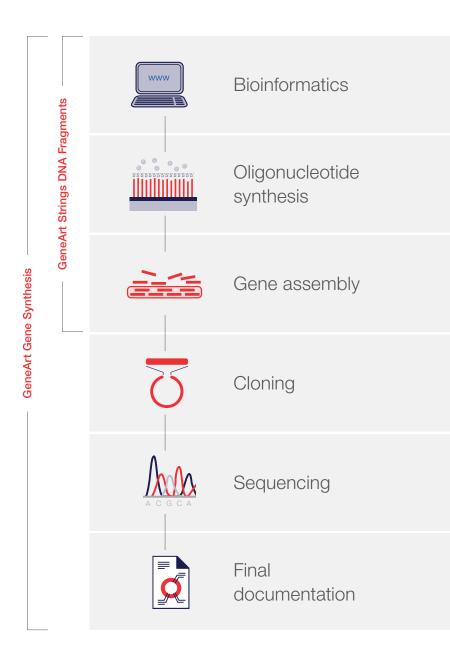
- Synthetic, ready-to-use DNA fragments
- DNA with your specified ends to facilitate the cloning method of choice
- No starting DNA required
- Free optimization of gene with Invitrogen™ GeneArt™ GeneOptimizer™ software for maximum protein expression
- · Option of Strings DNA Libraries with mixed, randomized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





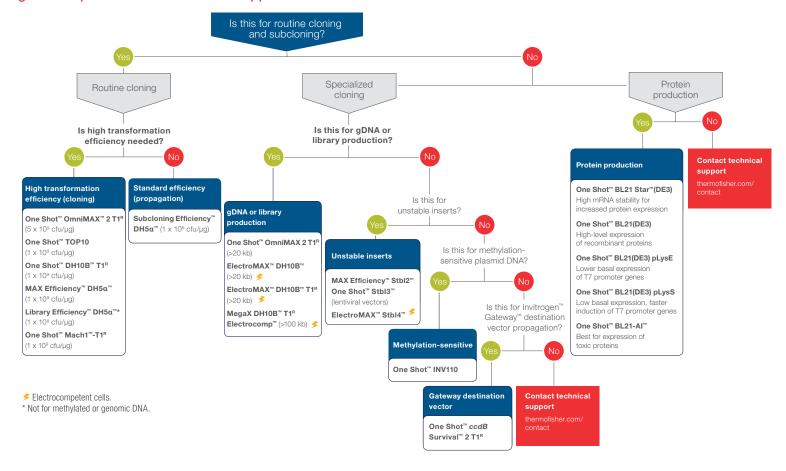
Find out more at thermofisher.com/genesynthesis



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 https://documents.org/length/ transformation for technical resources on competent cells.

Choosing Invitrogen[™] competent cells based on the application



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.

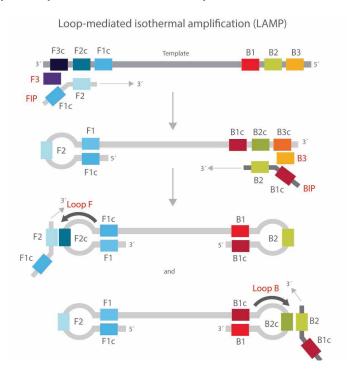




Loop-mediated isothermal amplification

LAMP overview

One commonly used isothermal amplification technique is loop-mediated isothermal amplification (LAMP), which utilizes a set of four to six primers and strand-displacing polymerase, such as 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 detected by turbidity, fluorescence, or colorimetry.



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

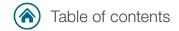
SuperScript IV RT-LAMP Master Mix is a reverse transcription loop-mediated isothermal amplification (RT-LAMP)—based solution for faster and simpler detection of various pathogens, including influenza virus, measles virus, *S. enterica*, *S. aureus*, SARS-CoV-2, and other pathogens. Our master mix reagents provide maximum flexibility to optimize and accelerate your pathogen research and surveillance.

Product highlights

- Fast—pathogen detection in as little as 5 minutes with evolved Bst DNA polymerase
- Efficient—one-step reaction for reverse transcription of RNA to cDNA with SuperScript IV Reverse Transcriptase
- Sensitive—greater sensitivity and specificity utilizing Invitrogen™ RNaseOUT™ 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 here



Lyo-ready Bst DNA Polymerase

Invitrogen™ Lyo-ready Bst DNA Polymerase is an engineered version of 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—achieves 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.



Find out more here





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.

Reverse transcription



thermofisher.com/rteducation

Nucleic acid electrophoresis



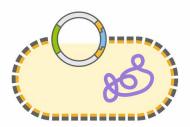
thermofisher.com/na-electrophoresis-education

PCR enzymes, plastics, and thermal cyclers



thermofisher.com/pcreducation

Restriction enzymes, molecular cloning, and competent cells



thermofisher.com/cloningeducation



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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 11–13) 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 17).

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 20).

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 23).

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 30–31).

Resource

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 30**), "fast" plastics (low profile and ultra-thin walls; **see page 27**), and "fast" thermal cyclers (fast ramp rate; **see pages 24–25**).

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 36).

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 38).

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

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 42). 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 46).

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 47).

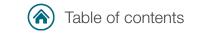
For more tips and troubleshooting advice on cloning, visit thermofisher.com/cloningsupport.

thermofisher.com/cloningsupport.

Ordering information

Quantity	Cat. No.
50 preps	K210010
25 preps	K210014
10 preps	K210006
4 x 96 preps	K211004A
50 preps	K210012
50 preps	12183555
10 preps	12183020
10 preps	K182000
4 x 96 preps	K182104A
4 plates	12280096A
50 preps	12280050
50 preps	K183001
500 preps	A25597
100 preps	A42358
100 preps	A42357
1 system	5400630
1 system	5400110
50 preps	K310001
50 preps	K220001
50 preps	K210012
2 mL	65305
2 mL	65001
1 system	5400910
1 system	5400920
1 system	5400930
1 system	5400940
1 system	5400610
1 system	5400620
1 system	5400640
1 system	5400630
1 system	5400110
1 system	5400840
1 system	5400830
	50 preps 25 preps 10 preps 4 x 96 preps 50 preps 10 preps 10 preps 10 preps 10 preps 10 preps 4 x 96 preps 50 preps 50 preps 50 preps 50 preps 500 preps 100 preps 100 preps 100 preps 100 preps 2 mL 2 mL 1 system

	Quantity	Cat. No.
Reverse transcription		
SuperSeriet IV Poverse Transcriptore	2,000 units	18090010
SuperScript IV Reverse Transcriptase	10,000 units	18090050
Cupar Cariat IV First Strand Cunthasia Custom	50 reactions	18091050
SuperScript IV First-Strand Synthesis System	200 reactions	18091200
SuperScript IV VILO Master Mix	50 reactions	11756050
Supersoript in AITO Master Mix	500 reactions	11756500
SuperScript IV VILO Master Mix with ezDNase Enzyme	50 reactions	11766050
Superscript iv VILO iviaster iviix with ezdinase Enzyme	500 reactions	11766500
SuperScript IV One-Step RT-PCR System	25 reactions	12594025
Superscript in Otte-step n1-Fon System	100 reactions	12594100
SuperScript IV UniPrime One-Step RT-PCR System	100 reactions	12597100
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
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



^{**} For Laboratory Use.

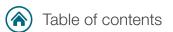
esonices

Ordering information (cont.)

	Quantity	Cat. No.
PCR		
Thermal cyclers		
ProFlex 3 x 32-Well PCR System	1 instrument	4484073
ProFlex 96-Well PCR System	1 instrument	4484075
SimpliAmp Thermal Cycler	1 instrument	A24811
VeritiPro Thermal Cycler, 96 well	1 instrument	A48141
MiniAmp Plus Thermal Cycler	1 instrument	A37835
MiniAmp Thermal Cycler	1 instrument	A37834
Automated Thermal Cycler, 96 well	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 enzymes		
Platinum II Tag Hot-Start DNA Polymerase	100 reactions	14966001
Thatman hay not otall brown olymorase	500 reactions	14966005
Platinum II Hot-Start PCR Master Mix (2X)	50 reactions	14000012
Tradition in tot-otal tri ortiviaster with (2A)	200 reactions	14000013
Platinum II Hot-Start Green PCR Master Mix (2X)	50 reactions	14001012
Tradition in 10t-Start Green Fortiviaster with (2A)	200 reactions	14001013
AmpliTag Cold 360 DNA Polymorago	100 units	4398813
AmpliTaq Gold 360 DNA Polymerase	250 units	4398823
AmpliTag Gold 360 Master Mix	1 mL	4398876
Ampiray dolu 300 Master Mix	5 mL	4398881
Platinum SuparEi II DNA Polymoraec	100 units	12361010
Platinum SuperFi II DNA Polymerase	500 units	12361050

	Quantity	Cat. No.
Platinum SuperEi II DCD Meater Mix	100 reactions	12368010
Platinum SuperFi II PCR Master Mix	500 reactions	12368050
Distincton Consulti II Ocean DOD Master Mic	100 reactions	12369010
Platinum SuperFi II Green PCR Master Mix	500 reactions	12369050
Platinum Direct PCR Universal Master Mix	100 reactions	A44647100
Platinum Direct PCR Universal Master Mix	500 reactions	A44647500
ANTO Cat (400 ann)	4 x 250 μL	10297018
dNTP Set (100 mM)	8 x 1.25 mL	10297117
Oligos		
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
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
UltraPure Agarose	100 g	16500100
Tracklt 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 Cal Dawar Span Diva Floatrapharagia System	1 avatam	G9301*
E-Gel Power Snap Plus Electrophoresis System	1 system	G9311**

^{**} Asia Pacific, Japan, Latin America, and greater China.



^{*} North America, Europe, Middle East, and Africa.

	Quantity	Cat. No.
Nucleic acid separation and analysis (cont.)		
F. Cal Dawer Chan Dive Floatronbarraia Custom Starter Lit 40 year 10/	4 154	G9341*
E-Gel Power Snap Plus Electrophoresis System Starter kit, 48-well, 1%	1 kit	G9331**
Col Device Cree Dive Fleetreshouse Content Observation 1:4 40 col 100/	4 1.54	G9342*
E-Gel Power Snap Plus Electrophoresis System Starter kit, 48-well, 2%	1 kit	G9332**
F. Col Dower Chan Dive Flootrophorosis Custom Starter Lit 06 well 10/	1 kit	G9391*
E-Gel Power Snap Plus Electrophoresis System Starter kit, 96-well, 1%	I KIL	G9381**
E Cal Dawar Span Diva Floatropharosia System Starter kit 06 yeall 20/	1 kit	G9392*
E-Gel Power Snap Plus Electrophoresis System Starter kit, 96-well, 2%	I KIL	G9382**
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
Cloning and gene synthesis		
Fast Digest RemUI	800 reactions	FD0054
FastDigest BamHI	2,500 reactions	FD0055
FastDigest Bcul	20 reactions	FD1253
FastDigest DCui	50 reactions	FD1254
FastDigest BshTl	20 reactions	FD1464
FastDigest Dpnl	50 reactions	FD1703
rasibigest opiii	100 reactions	FD1704
FastDigest EcoRI	800 reactions	FD0274
Tasibigest Loom	2,500 reactions	FD0275
FastDigest Kpnl	300 reactions	FD0524
	20 reactions	FD0593
FastDigest NotI	50 reactions	FD0594
Fasibigest Noti	150 reactions	FD0595
	250 reactions	FD0596
FastDigest Sall	200 reactions	FD0644
East Diggest Vhal	300 reactions	FD0684
FastDigest Xbal	750 reactions	FD0685
East Digget Vhol	400 reactions	FD0694
FastDigest Xhol	1,200 reactions	FD0695
FastDigest Esp3I (BsmBI) (IIS class)	20 reactions	FD0454
FastDigest Bpil (Bbsl) (IIS class)	20 reactions	FD1014
FastDigest Eco31l (Bsal) (IIS class)	50 reactions	FD0293

^{**} Asia Pacific, Japan, Latin America, and greater China.

	Quantity	Cat. No.
Cloning and gene synthesis (cont.)		
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 <i>E. coli</i>	20 reactions	K240020
One Shot TOP10 Chemically Competent E. coli	20 reactions	C404003
One Shot Stbl3 Chemically Competent E. coli	20 x 50 μL	C737303
MAX Efficiency DH5a Competent Cells	200 μL	18258012
ElectroMAX DH10B Cells	100 μL	18290015
MAX Efficiency Stbl2 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 <i>E. coli</i>	1 rack	C609601
MultiShot FlexPlate TOP10 Chemically Competent E. coli	1 plate	C4081201
MultiShot FlexPlate DH5α T1R Chemically Competent E. coli	1 plate	C4481201
MultiShot FlexPlate Stbl3 Chemically Competent E. coli	1 plate	C7381201
GeneArt Gibson Assembly HiFi Master Mix	50 reactions	A46628
GeneArt Gibson Assembly EX Master Mix	50 reactions	A46636
GeneArt Seamless Cloning and Assembly Enzyme Mix	20 reactions	A14606
GeneArt Type IIS Assembly Kit, Aarl	10 reactions	A15916
GeneArt Type IIS Assembly Kit, Bsal	10 reactions	A15917
GeneArt Type IIS Assembly Kit, Bbsl	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 <i>E. coli</i>	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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