invitrogen

Every step counts

Molecular biology workflow solutions

ThermoFisher SCIENTIFIC

Every step counts

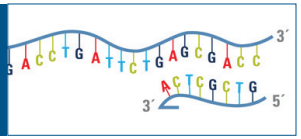
In the journey to scientific discoveries, every step plays a critical role in reaching your goals. As a partner for success in your molecular biology experiments, we offer complete workflow solutions to facilitate each step of your research.

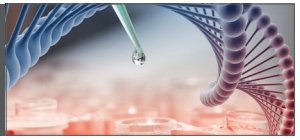
Molecular biology involves investigation of nucleic acids for their structure, expression, and functions. Genomic DNA and total RNA are routinely isolated from cells and tissues for downstream applications such as reverse transcription, PCR, and cloning. Cloning can be performed not only with restriction enzymes and a DNA ligase, but also with techniques such as PCR, DNA recombination, and gene synthesis. Electrophoresis is essential for verifying purity, specificity, and quantity of the nucleic acid samples for experimental success.

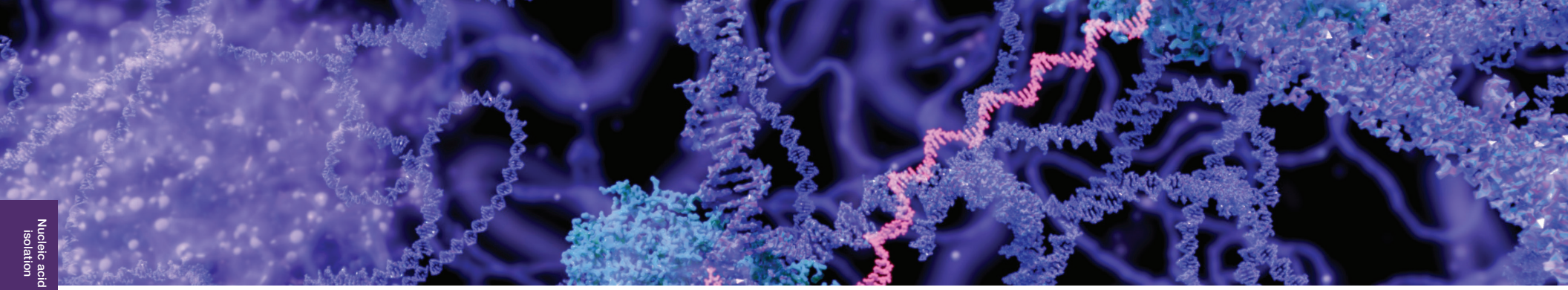
Regardless of the PCR and cloning steps you take, our Invitrogen™ and Applied Biosystems™ molecular biology products enable you to discover with quicker results, more assurance, and less optimization. From nucleic acid isolation to cloning, we are here to support you at every stride of your journey.

In this booklet, find the research tools that can advance your science. For additional information, please visit **thermofisher.com/everystepcounts**

Contents







Nucleic acid isolation

Nucleic acid isolation is a crucial first step in your molecular biology workflow, whether you are starting with RNA or genomic DNA (gDNA). Table 1 describes the benefits and challenges of common isolation methods.

Table 1. Methods for nucleic acid isolation.

	Organic extraction	Filter-based membranes	Magnetic particles
Description	Phenol-chloroform solution	Glass fiber, derivatized silica, or ion exchange membrane in column	0.5–1 µm particles with a paramagnetic core and modified shell
Benefits	• Rapid denaturation of nucleases • Stabilization of nucleic acids	• Convenience • Ease of use • Throughput flexibility • Ability to automate	• No risk of clogging • Increased target capture efficiency • Rapid collection and concentration of sample • Ability to automate • Scalable
Drawbacks	• Use and associated waste of organic reagents • Manually intensive processing	• Propensity to clog • Fixed binding capacity within a manufactured format • Automation requires vacuum or centrifugation systems	• Potential carry-through of particles into sample • Slow migration of particles in viscous solutions • Difficulty automating large volumes
Process	Samples are homogenized in a phenol-containing solution and then centrifuged. During centrifugation, the sample separates into three phases: an organic phase, an aqueous phase that contains nucleic acids, and a phase between them that contains denatured proteins.	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 or vacuum.	Samples are lysed in solution and allowed to bind to magnetic particles based on specific surface modifications. Application of an external magnetic field rapidly collects the particles. Rounds of release, resuspension, and recapture isolate the desired nucleic acid.

Clone collections

We offer several types of clone collections—comprising Ultimate™ ORF clones, Mammalian Gene Collection (MGC) full-length clones, bacterial artificial chromosome (BAC) and P1-derived artificial chromosome (PAC) clones, and yeast deletion and GFP clones, among others.

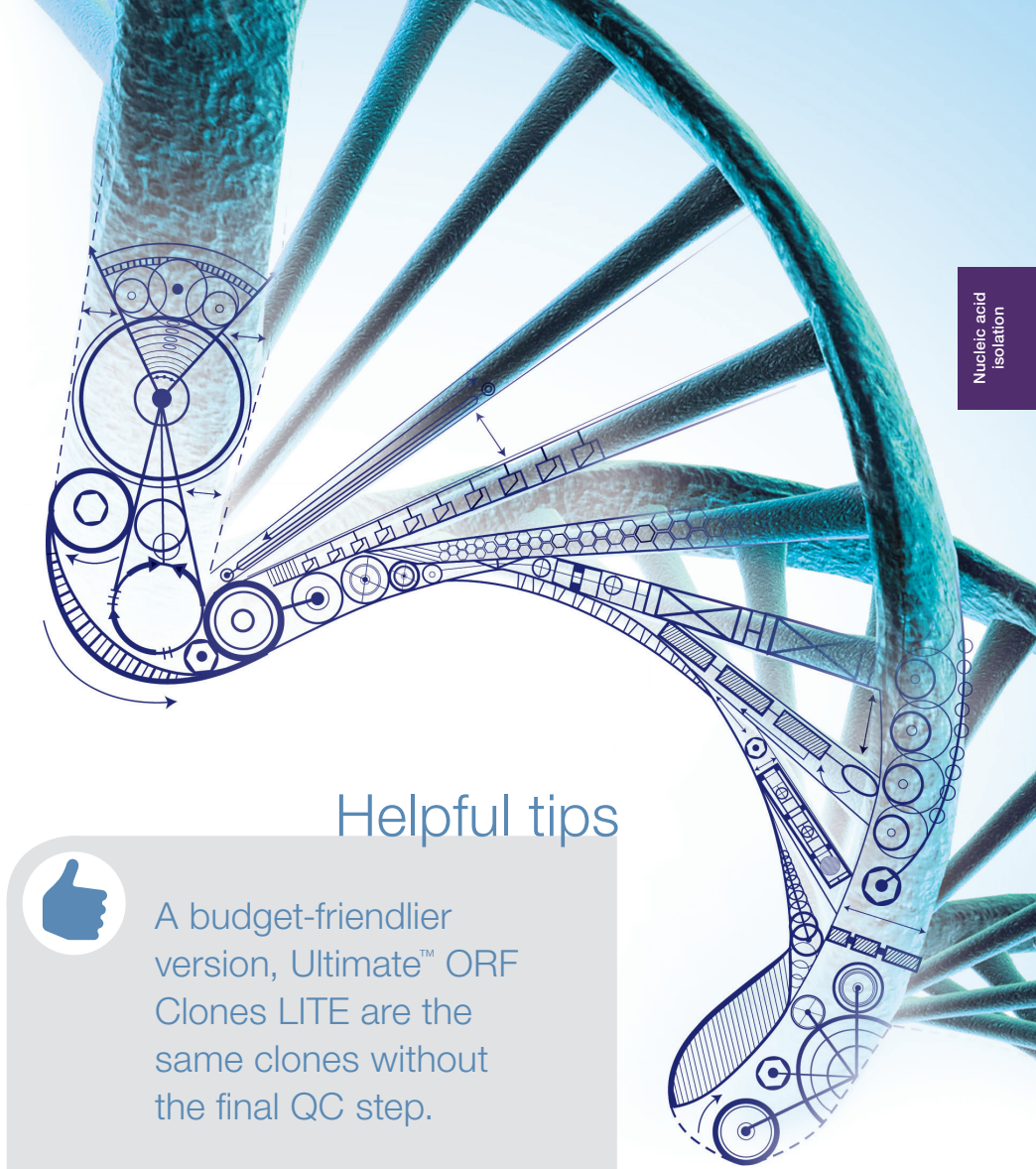
Check out the full selection of clone collections at
thermofisher.com/clonecollectionselection

Ultimate ORF clones

Ultimate ORF clones are fully sequenced open reading frames (ORFs) of human and mouse clones in a Gateway™ entry vector, offering complete versatility for protein expression in multiple expression systems.

Fast track to expression and analysis

- **Vast selection**—over 16,000 Ultimate™ Human ORF Clones and over 2,500 Ultimate™ Mouse ORF Clones
- **Easy ordering**—using the online Ultimate™ ORF Browser
- **Gateway™ pENTR™221 format**—1-hour recombinatorial cloning into expression vectors gets you to expression and analysis faster
- **100% amino acid match tested**—sequence verification against GenBank™, Ensembl™, and Swiss-Prot™ databases. Before shipping, the clone culture must pass a stringent QC test that includes end sequencing to verify the identity of the clone



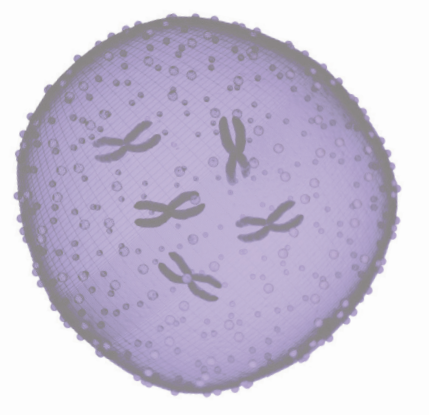
Helpful tips

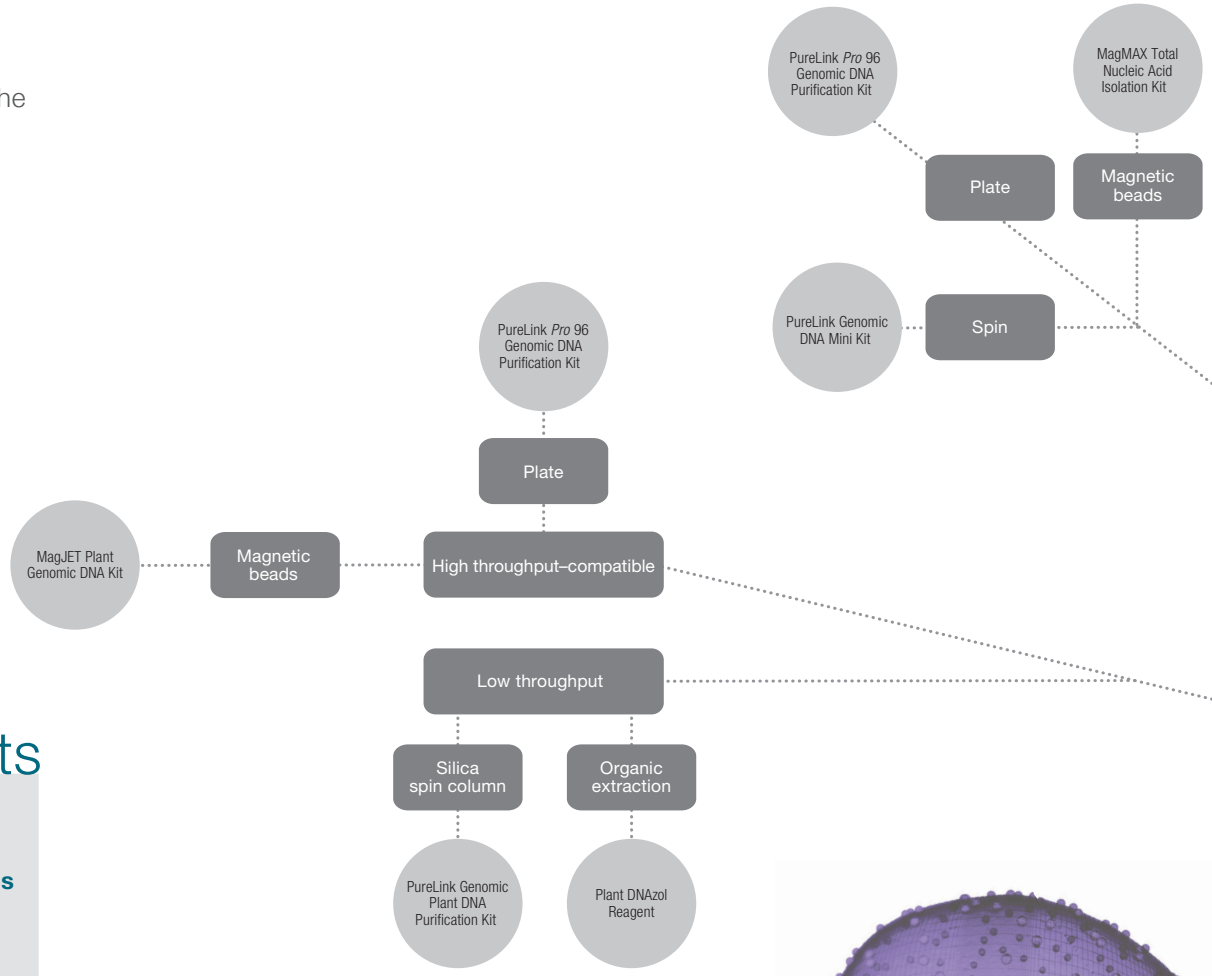
A budget-friendlier version, Ultimate™ ORF Clones LITE are the same clones without the final QC step.

Genomic DNA isolation

Our gDNA isolation technology guide will help you identify the right product for your sample type and particular workflow.

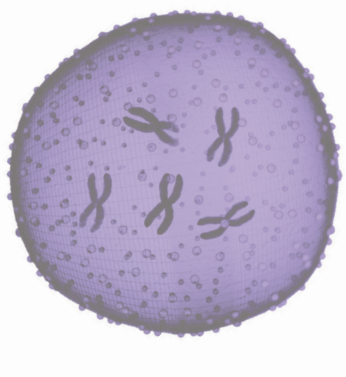
Learn more at **thermofisher.com/gdnaprep**

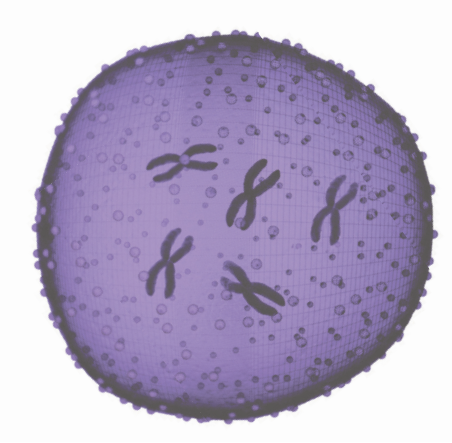


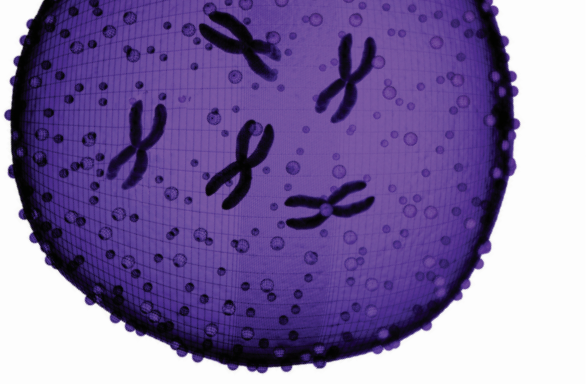


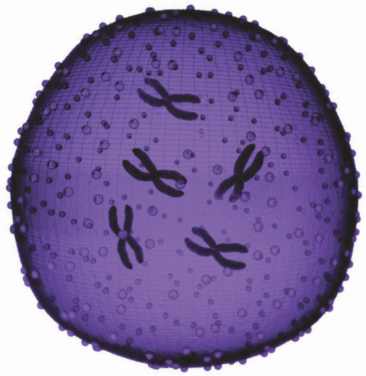
Facts

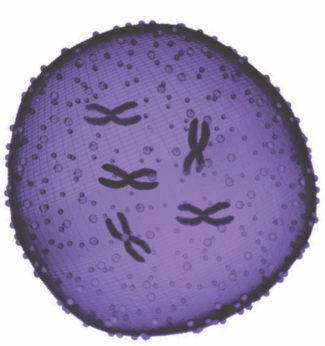
- View our protocol video library at **thermofisher.com/nucleicacidisolationvideos**
- Your DNA isolations can be automated—learn more at **thermofisher.com/kingfisher**
- In many cases vortexing during nucleic acid isolations is not recommended because it can shear DNA, causing contamination or compromised final sample. Read the protocol carefully.











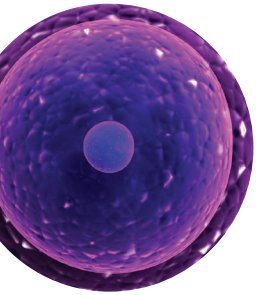
Start here
(choose sample type)

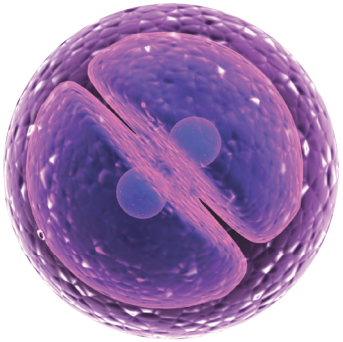
- Blood/serum
  - Low throughput
    - Silica spin column → PureLink Genomic DNA Mini Kit
    - Organic extraction → DNAzol BD Reagent
  - High throughput–compatible (plate format)
    - Magnetic beads → MagMAX-96 DNA Multi-Sample Kit
    - Silica → PureLink *Pro* 96 Genomic DNA Purification Kit
- Buccal
  - Magnetic beads → MagMAX DNA Multi-Sample Kit
  - Column → PureLink Genomic DNA Mini Kit
  - Plate → PureLink *Pro* 96 Genomic DNA Purification Kit
- Virus
  - High throughput–compatible (plate format) → Magnetic beads → PureLink *Pro* 96 Viral RNA/DNA Purification Kit
  - MagJET Viral DNA and RNA Kit
  - Low throughput (silica spin column) → PureLink Viral RNA/DNA Mini Kit
- Tissue and cells
  - High throughput–compatible (silica spin column)
    - Magnetic beads → MagMAX DNA Multi-Sample Kit
    - Silica → PureLink *Pro* 96 Genomic DNA Mini Kit
  - Low throughput
    - Multi-sample → MagMAX DNA Multi-Sample Kit
    - Organic extraction → DNAzol Reagent
    - Silica spin column → PureLink Genomic DNA Mini Kit
- Bacteria
- Plant

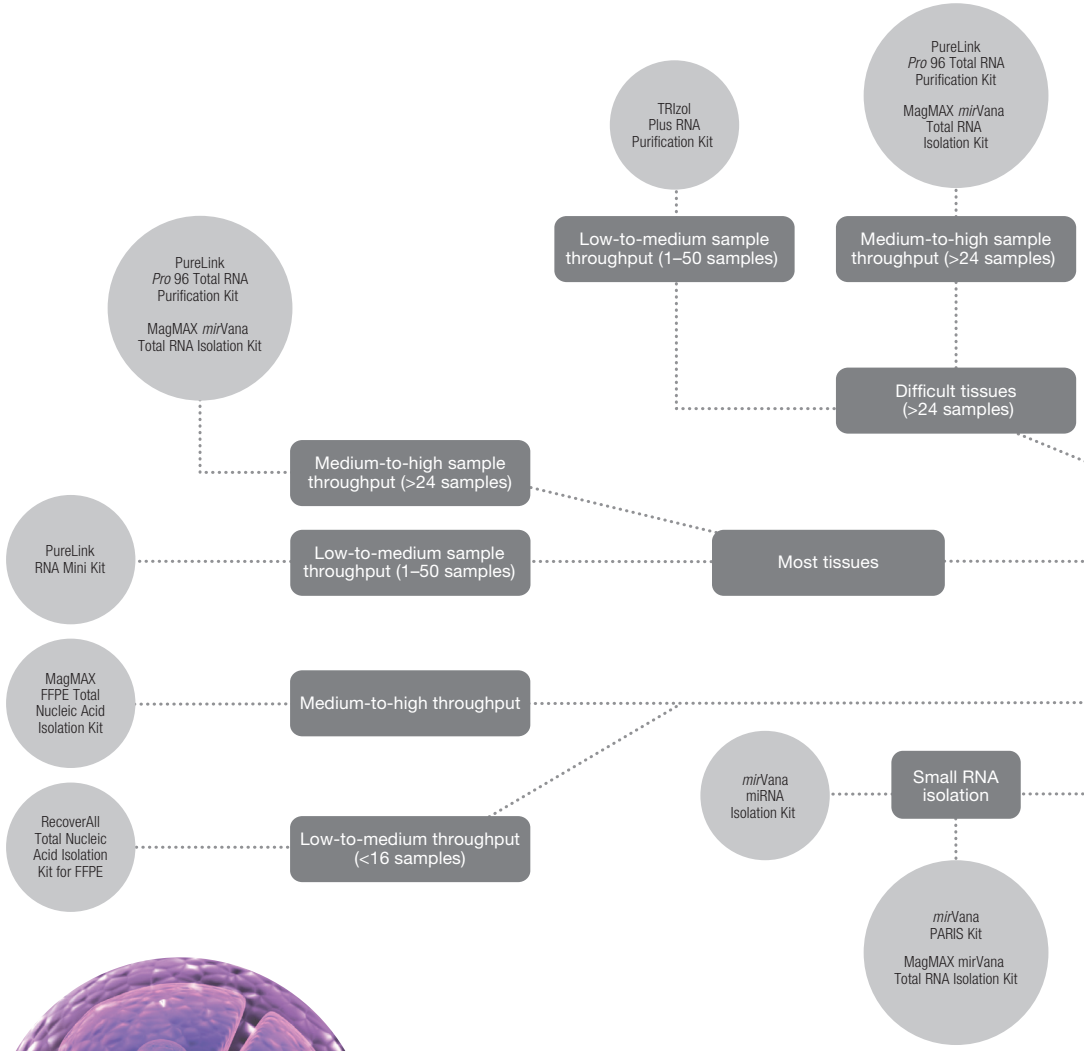
RNA isolation

Our RNA isolation technology guide will help you identify the right product for your sample type and specific workflow.

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

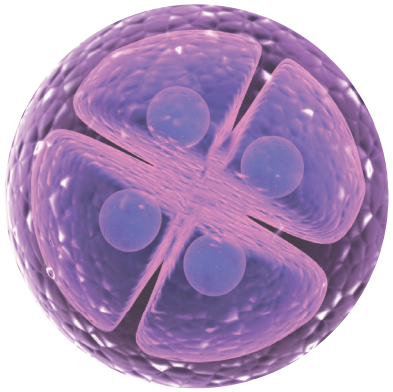


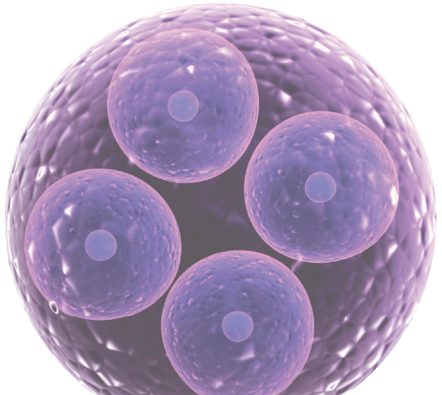


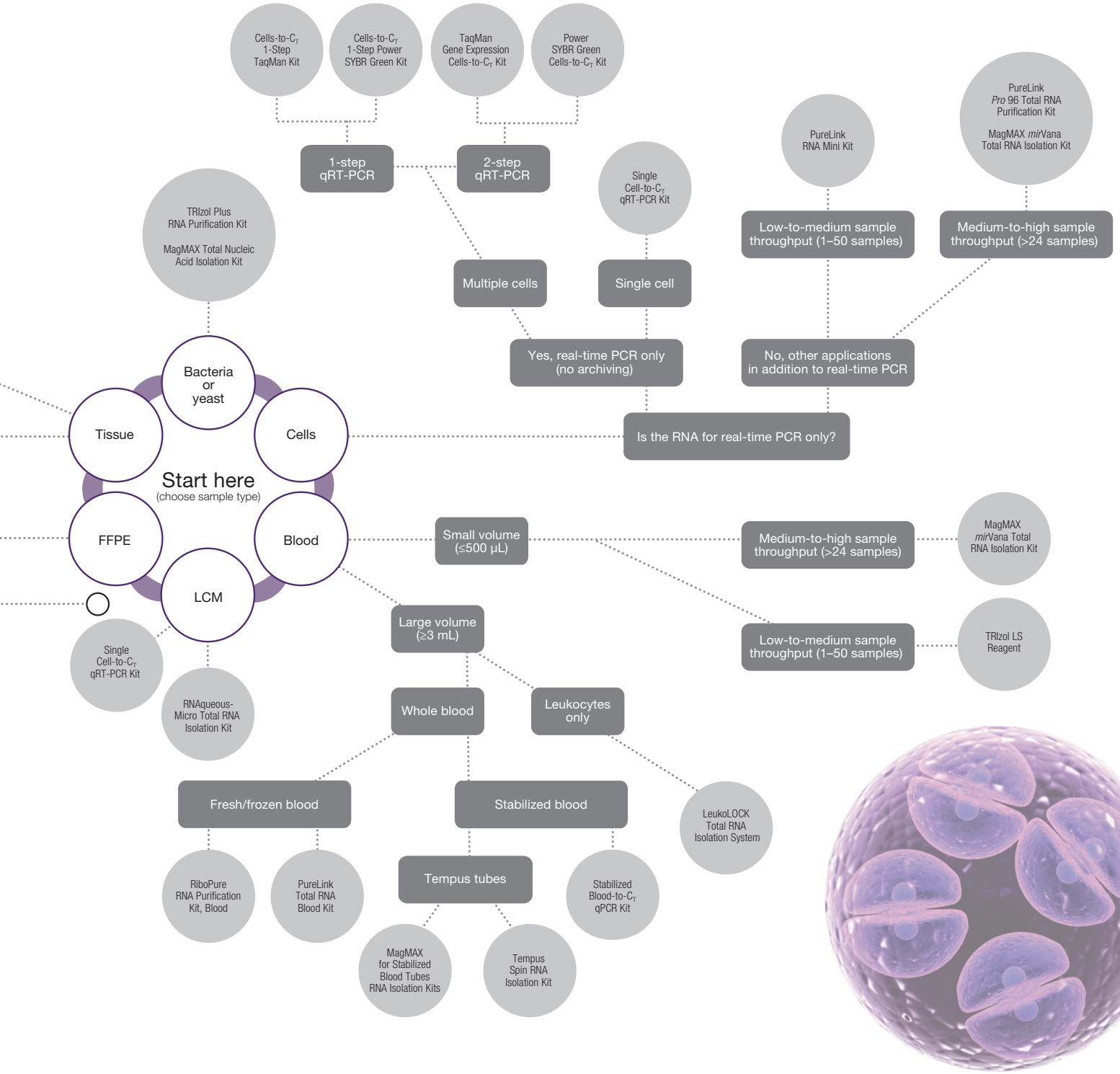


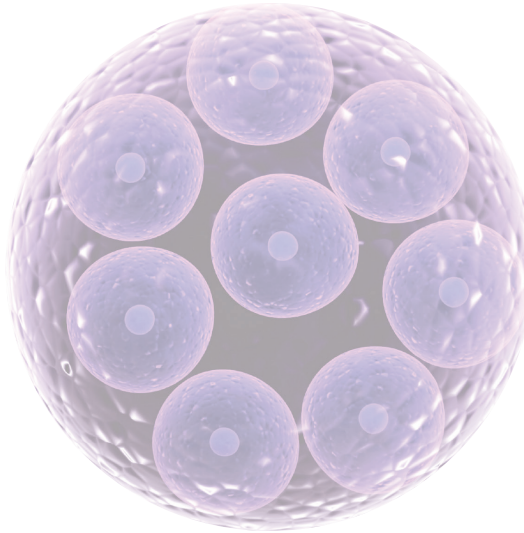
Facts

- Your RNA isolations can be automated—learn more at **thermofisher.com/kingfisher**
- View our protocol video library at **thermofisher.com/nucleicacidisolationvideos**
- Working with RNA can be challenging. Learn all about controlling RNases in this free webinar: **thermofisher.com/rnabasicswebinar**
- If you are not ready to process your RNA sample, simply store it in Invitrogen™ RNA*later*™ Stabilization Solution for use at any time. Go to **thermofisher.com/stabilizerna**






Cells-to-C_T 1-Step TaqMan Kit
Cells-to-C_T 1-Step Power SYBR Green Kit
TaqMan Gene Expression Cells-to-C_T Kit
Power SYBR Green Cells-to-C_T Kit
1-step qRT-PCR
2-step qRT-PCR
Single Cell-to-C_T qRT-PCR Kit
PureLink RNA Mini Kit
PureLink *Pro* 96 Total RNA Purification Kit
MagMAX *mir*Vana Total RNA Isolation Kit
TRIzol Plus RNA Purification Kit
MagMAX Total Nucleic Acid Isolation Kit
Low-to-medium sample throughput (1–50 samples)
Medium-to-high sample throughput (>24 samples)
Multiple cells
Single cell
Yes, real-time PCR only (no archiving)
No, other applications in addition to real-time PCR
Bacteria or yeast
Tissue
Cells
Start here
(choose sample type)
FFPE
Blood
LCM
Is the RNA for real-time PCR only?
Small volume (≤500 µL)
Medium-to-high sample throughput (>24 samples)
MagMAX *mir*Vana Total RNA Isolation Kit
Low-to-medium sample throughput (1–50 samples)
TRIzol LS Reagent
Large volume (≥3 mL)
Single Cell-to-C_T qRT-PCR Kit
RNAqueous-Micro Total RNA Isolation Kit
Whole blood
Leukocytes only
Fresh/frozen blood
Stabilized blood
LeukoLOCK Total RNA Isolation System
RiboPure RNA Purification Kit, Blood
PureLink Total RNA Blood Kit
Tempus tubes
Stabilized Blood-to-C_T qPCR Kit
MagMAX for Stabilized Blood Tubes RNA Isolation Kits
Tempus Spin RNA Isolation Kit



Reverse transcription

Reverse transcription is the reverse transcriptase (RT enzyme)–mediated synthesis of single-stranded DNA (complementary DNA or cDNA) using single-stranded RNA as a template. The cDNA can be used as a template for PCR amplification, or for cDNA library construction. Selecting the right reverse transcriptase for cDNA synthesis is critical to detecting low-abundance RNAs in a sample and obtaining high yields of full-length cDNA. Here are a few considerations for selecting the right reverse transcriptase:

Sensitivity

The ability of reverse transcriptase to generate cDNA from the lowest amount of input RNA is an important attribute when looking for low copy genes or working with increasingly difficult sample sources where RNA becomes degraded during purification process (Figure 1A).

Thermostability

The secondary structure of RNA can interfere with cDNA synthesis, and many commercially available RTs can anneal nonspecifically to the strong secondary structures of these RNA targets. Nonspecific cDNA products derived from such mispriming can impair overall RT efficiency and reduce the yield of full-length cDNA. To minimize these effects, RT reactions should be performed at higher temperatures so that the RNA secondary structures are partially or completely denatured (Figure 1B).

Processivity

Processivity is the ability of a polymerase to perform consecutive nucleotide additions without releasing the template. The more processive an RT, the longer the synthesized cDNA and the more efficient the enzyme is in making full-length cDNA (Figure 1C).

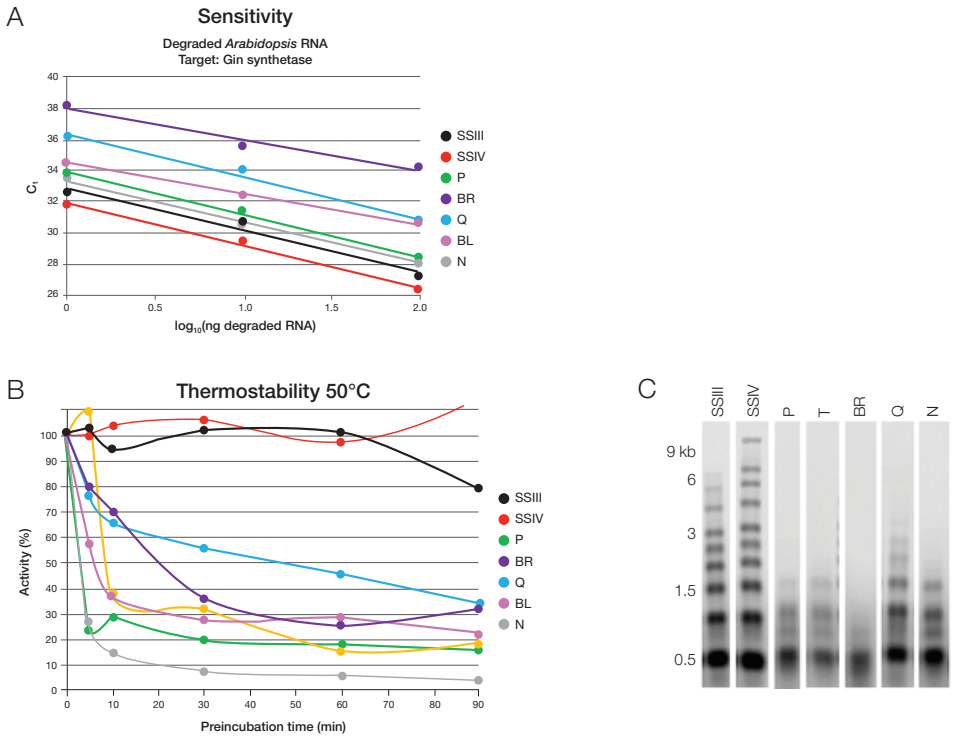


Figure 1. Choosing the right reverse transcriptase. Sensitivity **(A)**, thermostability **(B)**, and processivity of SuperScript™ IV (SSIV) all affect the quantity and length of cDNA **(C)**.

Facts

- Major sources of variation in gene expression analysis by RT-qPCR include pipetting, biological degradation of RNA, reverse transcriptases, and more. View the free webinar "Overcoming RT-qPCR common mistakes and challenges" at **thermofisher.com/molbiowebinars**
- The processivity of wild type Moloney murine leukemia virus (MMLV) RT is 30 nucleotides, and that of SuperScript IV RT is 1,500 nucleotides.

Reverse transcriptase selection guide

	Goals		
	Maximize data	**Reduce pipetting variability and easy to use**	**Select my own primers and components**
Product	SuperScript™ IV First-Strand Synthesis System	SuperScript™ VILO™ Master Mix	SuperScript™ IV Reverse Transcriptase
Sensitivity	10 pg–2 µg	1 pg–2 µg	10 pg–2 µg
Optimal reaction temperature	50°C	42°C	50–55°C
Reaction time	10 min	60 min	10 min
cDNA yield with challenging or degraded RNA	High	Good	High

To learn more about SuperScript™ reverse transcriptases, go to **thermofisher.com/superscript**

Did you know

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.

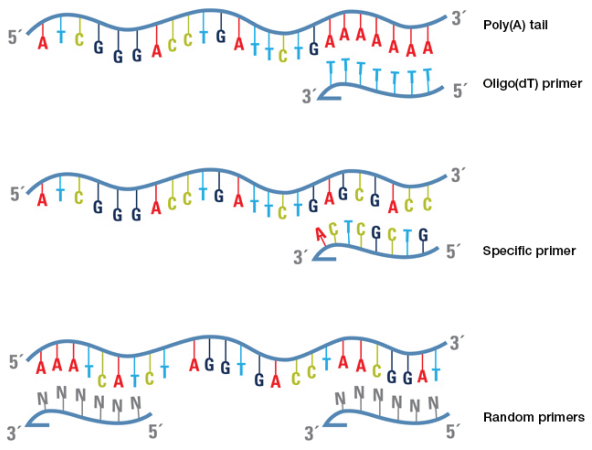
RT primers

The priming strategy chosen for your reverse transcription is important for cDNA synthesis efficiency, constancy, and yield. Each primer type has its benefits and drawbacks depending on individual target RNA.

For full-length first-strand cDNA synthesis, oligo(dT) primers are recommended because of their specificities to mRNA and they allow many different targets to be studied from the same cDNA pool. Oligo(dT) primers typically contain strings of 12–20 deoxythymidines.

For target mRNA containing strong transcriptional pauses, we suggest random primers to anneal throughout the target molecules. They are also ideal for non polyadenylated RNA, such as bacterial RNA.

For the complete offering of RT primers, go to **thermofisher.com/primers**



PCR

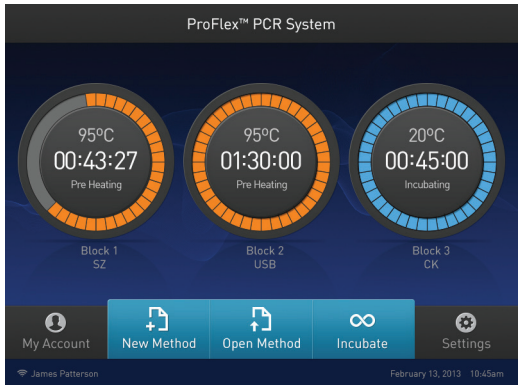
The polymerase chain reaction (PCR) is a technique that is central to molecular biology research. Developed by Kary Mullis in 1983, this method revolutionized genetic research, opening many doors to new applications in medicine and biotechnology. PCR applications include cloning, gene expression analysis, genotyping, sequencing, and mutagenesis. PCR is also used in research for infectious diseases, cancer, and forensic analysis. It is also a critical tool in agricultural biotechnology—in numerous steps from discovery to applications, such as plant pathogen detection and quality control purposes.

PCR instruments—system innovations

Since the introduction of our first thermal cycler in 1987, Thermo Fisher Scientific engineers have continued to design, develop, and support innovative PCR instruments to empower your research.

Our Applied Biosystems™ ProFlex™, Veriti™, and SimpliAmp™ thermal cyclers have updated color touch-screens for easy programming and monitoring of your run status at the bench (Figure 2). Simulation modes allow easy transition from previous thermal cyclers by simulating the ramp speed of your old instrument.

A



B

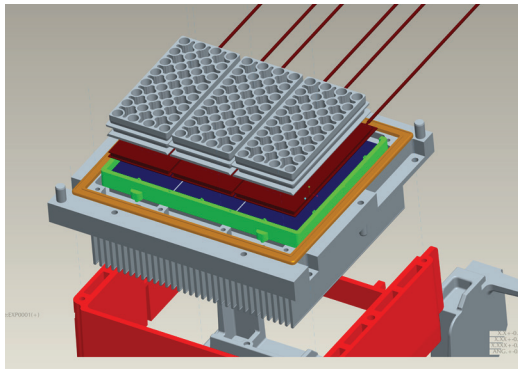


Figure 2. Applied Biosystems™ thermal cyclers. (A) Color touch-screens for easy programming. **(B)** VeriFlex technology for simple and accurate optimization.

Facts

- Better-than-gradient VeriFlex™ Blocks temperature control (Figure 2) allows for a true linear temperature slope across metal blocks, with the ability to set up to six different temperatures. The user can set each zone to a unique temperature for precise control.
- The ProFlex™, Veriti™, and SimpliAmp™ systems feature VeriFlex™ Blocks for enhanced PCR functionality. Separate Peltier blocks provide maximal versatility and flexibility.

Helpful tips

Tired of water baths? Incubate samples at up to six different temperatures simultaneously for enzyme activation studies, restriction digests, or sequencing library preps.

ProFlex PCR System

High-performance thermal cycler with advanced flexibility and control

- **Multi-user accessibility**—run 3 experiments at once with three independently controlled blocks
- **Flexible block configuration**—accepts 5 different block formats for optimization and throughput, including the triple 32-well block with independent control
- **Convenient remote access**—connect to your instrument from anywhere with a free mobile app

Veriti Thermal Cycler

Easy-to-program, robust thermal cycling

- **6 temperature zones for PCR optimization**—features VeriFlex Blocks for precise temperature control
- **Easy to operate**—easy-to-use graphical interface, fast protocol setup, and convenient protocol transfer with a USB memory stick

SimpliAmp Thermal Cycler

Elegant design, smart choice

- **Compact design**—helps save bench space
- **Easy to operate**—large, color LCD touch-screen that simplifies operations
- **Three independent temperature zones for PCR optimization**—features VeriFlex Blocks for precise temperature control

Want to see a thermal cycler now? Request a demo at **thermofisher.com/thermalcyclers**

PCR plastics

Applied Biosystems™ MicroAmp™ plastics consumables offer excellent PCR and qPCR performance in formats developed to meet your experimental needs. All of our plastics are validated with Applied Biosystems™ instruments for optimal fit and performance.

We offer a variety of 96-well plates, 384-well plates, tube strips, single tubes, caps, and seals. Use our online interactive selection guide, or download the compatibility table, to find the right products for your instrument.

For more information, visit **thermofisher.com/plastics**

Table 2. PCR tubes, caps, and accessories.

	Small-scale experiments with a few samples	Daily experiments	Complete-workflow experiments—ideal for automation	High throughput, automation
	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	• 48-well Fast • 96-well • 96-well Fast • 384-well	• 96-well • 96-well Fast • 384-well	• 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	No	Yes (1 or 2 sides)	Yes (3 sides)	Yes (3 sides)
Multiple application	No	No	Yes	Yes
Optical compatibility	Yes (applicable for optical version)	Yes	Yes	Yes

PCR reagents

PCR is highly efficient and specific, generating millions of copies of target DNA from just a few molecules. Due to the sensitive and specific nature of the PCR process, it is important to choose high-quality PCR products to produce optimal results. As early innovators of PCR enzymes and reagents, we continue to develop new PCR enzymes and master mixes with the highest performance and quality.

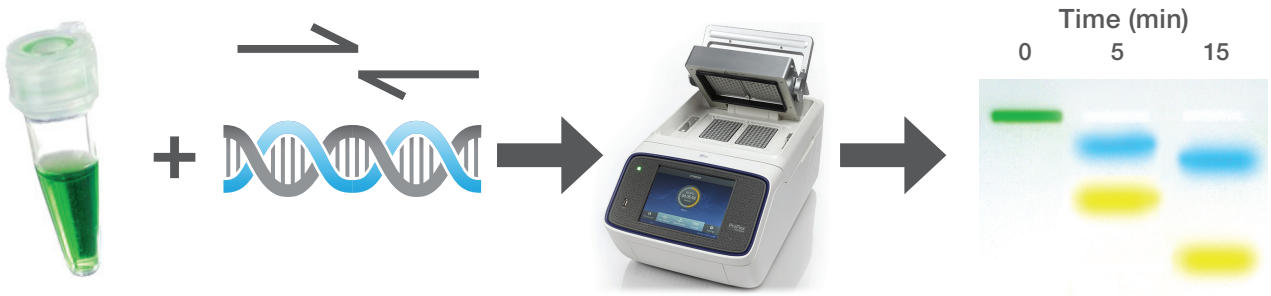
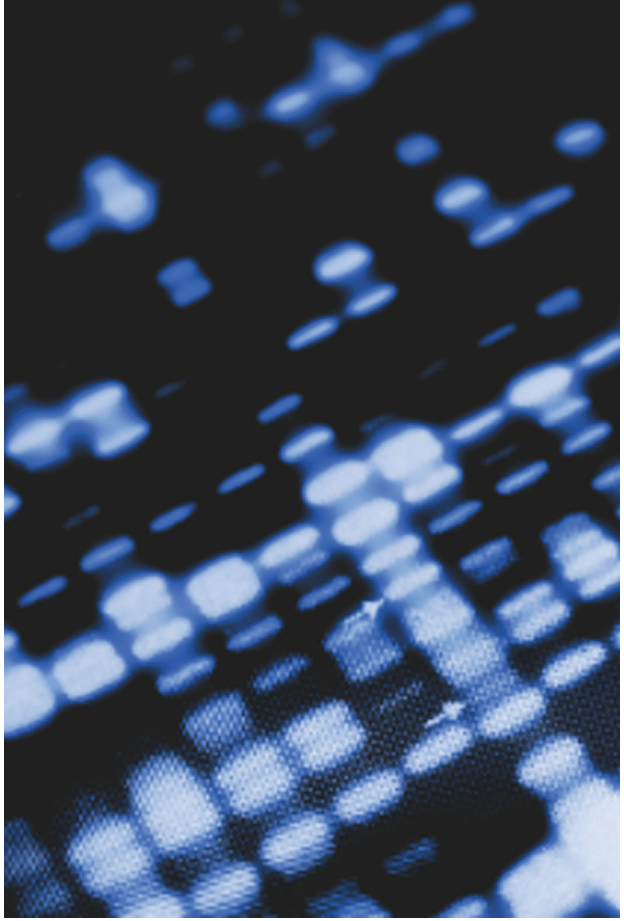


Figure 3. Simplified PCR workflow. Direct gel loading of PCR products eliminates tedious steps of dye addition. Left lane of gel shows PCR reaction mixture prior to electrophoresis. Right two lanes show gel dye migration following 5 and 15 minutes of electrophoresis.



Did you know

You can save time and reduce plastic waste with direct gel loading of PCR products.

Helpful tips

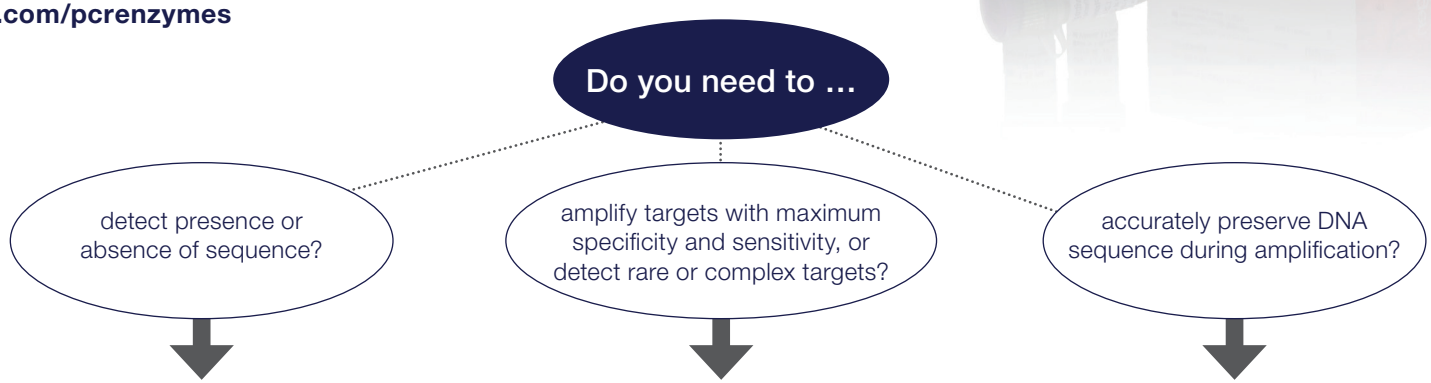
One of the most common PCR troubleshooting issues is the presence of unwanted bands or nonspecific amplification. To reduce nonspecific amplification:

1. Optimize annealing temperature
2. Check primer design
3. Perform hot-start PCR
4. Prevent DNA cross-contamination
5. Decrease template and/or primer concentration
6. Optimize Mg^{2+} concentration

Choose the right PCR reagent for your research needs

We offer a comprehensive portfolio of PCR enzymes and master mixes with the high performance and consistency you need. Standard *Taq* DNA polymerase reagents provide reliable amplification for routine PCR applications. Hot-start PCR reduces nonspecific amplification, increases the yield of the target amplicons, and allows for convenient room-temperature reaction setup. High-fidelity PCR is required for applications where sequence accuracy is crucial.

Start with the selection guide below to find the best enzyme for common PCR applications.
Find more options at **thermofisher.com/pcrenzymes**



PCR type	Standard PCR	Hot-start PCR	High-fidelity PCR
Recommended DNA polymerase	Invitrogen™ *Taq* DNA Polymerase	Invitrogen™ Platinum™ *Taq* DNA Polymerase	Invitrogen™ Platinum™ SuperFi™ DNA Polymerase
Applications	Routine PCR, genotyping, colony PCR	Routine PCR, high-throughput PCR, GC-rich templates, colony PCR, multiplex PCR	High-fidelity PCR, cloning, site-directed mutagenesis, GC-rich templates, template generation for sequencing, high-throughput PCR, long PCR, fast PCR
Blunt or 3′-A end	3′-A	3′-A	Blunt
Target length	Up to 5 kb	Up to 5 kb	Up to 20 kb**
Hot start	No	Yes	Yes
Fidelity vs. *Taq* polymerase	1x	1x	>100x
Formats available			
Stand-alone enzyme	Colorless	Colorless/green*	Colorless/green*
Master mix	Colorless	Colorless/green*	Colorless/green*

*Direct gel loading with green buffer options.
**Amplification of >20 kb fragment sizes is possible (up to 40 kb), but may require additional optimization of reaction conditions and primer design.

PCR primers

Good design (i.e., good sequence selection) and high quality of 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.

For more tips on primer design, go to **thermofisher.com/primerdesign**

Helpful tips

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.

DNA oligos

Backed by over 20 years of customer service experience, custom DNA oligos at Thermo Fisher Scientific are synthesized on a highly automated, computer-controlled system followed by rigorous quality control, such as mass spectrometry for short and capillary electrophoresis (CE) for long oligos, to ensure the quality of the process and end product.

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:

Application	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 additional to standard delivery, next-day delivery is also available. For more on ordering information, yield guarantees, designing tools, technical resources, protocols, and FAQs, please visit **thermofisher.com/oligos**

Did you know

- Want a fixed price on oligos? Get value oligos, available at 25 nmol and 50 nmol scales. Pay the same price whether you need a 5-mer, 40-mer, or anything in between. Learn more at **thermofisher.com/valueoligos**
- Need ASR/GMP oligos manufactured in compliance with US FDA? For details, go to **thermofisher.com/oligos**
- The newly enhanced ordering portal makes ordering a lot easier and faster.

PCR purification

PCR products are routinely purified to remove short primers, nonspecific amplicons, unincorporated dNTPs, enzymes, failed PCR products, and salts that may interfere with downstream applications. For example, unincorporated primers, enzymes, or products of nonspecific annealing may disrupt cloning. PCR products may be directly purified (if specific amplification occurs) or subjected to electrophoresis for gel extraction of the desired product.

We offer the Invitrogen™ PureLink™ PCR Purification Kit for rapid and efficient removal of primers, dNTPs, enzymes, and salts. The kit has a high binding capacity (up to 40 µg) and a convenient protocol that can be completed in less than 10 minutes, with no need for extra pH adjustment. Learn more at **thermofisher.com/pcrcleanup**

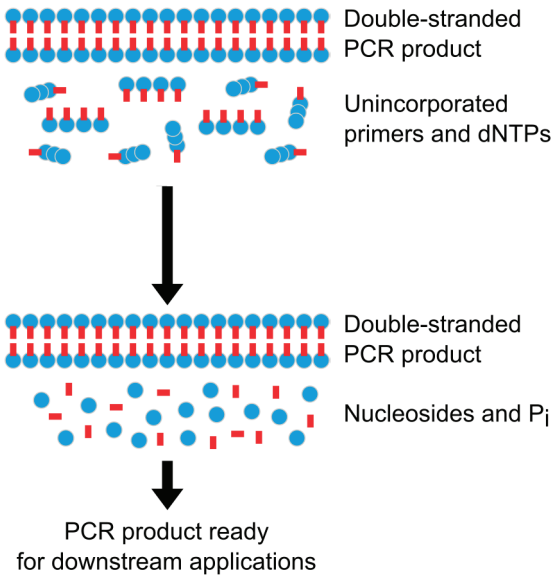


Figure 4. PCR purification workflow with CleanSweep™ reagent.

Helpful tips

- Increase the yield of your clean-up prep by 10–20%, by incubating the elution buffer for >1 min and eluting the DNA off the column a second time.
- Protocol time can be reduced by utilizing a vacuum manifold.

Nucleic acid electrophoresis

Electrophoresis is a common lab technique used to identify, quantify, and purify nucleic acid fragments. Samples are loaded into wells of an agarose or polyacrylamide gel and subjected to an electric field, moving the negatively charged nucleic acids towards the positive electrode. Shorter DNA fragments travel more rapidly, whereas longer fragments move more slowly, resulting in separation based on size.

Did you know

You can turn your routine agarose gel electrophoresis into an automated, high-throughput operation. Run 48 to 96 samples per gel and more with the E-Gel™ high-throughput DNA electrophoresis system.

For more information, go to **thermofisher.com/highthroughputegels**

Nucleic acid gels

We offer convenient reagents for agarose gel electrophoresis including hassle-free precast Invitrogen™ E-Gel™ agarose gels and UltraPure™ reagents for pouring your own agarose gels. We also provide precast polyacrylamide gels in multiple formats for applications that require very high resolution of DNA or RNA fragments, such as synthetic oligonucleotide analysis and purification, RNase protection, and gel retardation assays. Select the right gels for your applications:

	Pour-your-own gel reagents	Broadest range of precast bufferless gels	Ultimate speed and sensitivity	High-resolution gel in precast format
	UltraPure reagents	**E-Gel agarose gels**	**E-Gel™ EX Agarose Gels**	**Polyacrylamide gels**
Prep time	30 min	Ready-to-use	Ready-to-use	5–10 min
Run time	60 min	15–30 min	10 min	Variable
Sensitivity	Variable	Highly sensitive (>5 ng)	Ultrasensitive (>1 ng)	Variable
Gel percentages available	Variable	0.8%, 1.2%, 2%, and 4%	1%, 2%, and 4%	Multiple, including gradients
Number of lanes	Variable	12–18	11	10–15
Nonhazardous and environmentally friendly	Variable	Yes	No	Variable

E-Gel precast agarose gel electrophoresis system

The E-Gel™ system is a bufferless system for agarose gel electrophoresis, viewing, and documentation. Samples are run in E-Gel™ precast agarose gels that include electrodes, and are packaged in a dry, disposable, and UV-transparent cassette. E-Gel agarose gels are run in a base that includes a combined electrophoresis chamber and power supply. The precast agarose gels contain Invitrogen™ SYBR™ Safe stain, ethidium bromide, proprietary fluorescent DNA stain, or no DNA stain.

For more information, go to **thermofisher.com/egels**

Gel extraction

Gel extraction is a technique to isolate and purify a DNA fragment of a desired size from an agarose gel after separation by electrophoresis. Similar to PCR purification, gel extraction removes unincorporated primers, enzymes, salts, and other undesirable impurities that could interfere with downstream applications. It is commonly used in cloning workflows to isolate the desired DNA fragments after restriction digestion or PCR.

Learn more at **thermofisher.com/gelextraction**

E-Gel CloneWell agarose gels

Invitrogen™ E-Gel™ CloneWell™ agarose gels are unique double-comb gels designed to enable you to gel-purify DNA with ultimate convenience.

- Gel-purify your DNA in 3 simple steps
- Get improved cloning efficiencies
- View bands in real time and minimize DNA damage
- Collect multiple DNA bands from a single lane

To purify your DNA, simply load it, select the desired run protocol, and retrieve DNA ready for cloning. No additional purification kits or steps are required. Use the Invitrogen™ E-Gel™ iBase™ Power System and E-Gel™ Safe Imager™ Real-Time Transilluminator to run and visualize E-Gel CloneWell agarose gels.

With E-Gel CloneWell gels, by eliminating UV damage, you get improved cloning efficiency compared to conventional methods (Figure 5).

For more information, visit **thermofisher.com/clonewell**

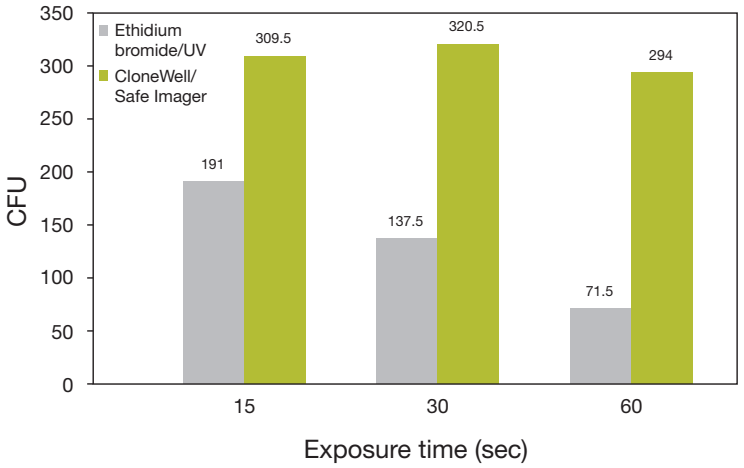
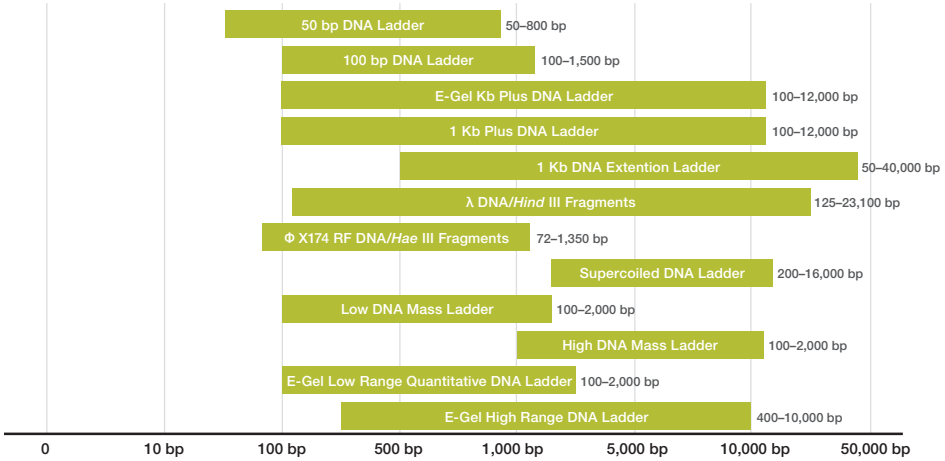


Figure 5. Results obtained using Invitrogen™ TOPO™ TA Cloning™ Kits after E-Gel CloneWell gel purification, compared to a traditional gel purification method.

DNA ladders

We offer unique DNA ladders and markers for a wide variety of size ranges, applications, and formats. The key to accurate band analysis is to use the correct marker or standard for your particular application. Use the selection chart to help you choose the ladder with the most appropriate size range for your application. Some of our ladders are also available optimized for our convenient E-Gel system. For quantitative estimation, we recommend you choose from our DNA mass ladders.

Double-stranded nucleic acid markers



DNA stains

Our fluorescent Invitrogen™ SYBR™ Gold, SYBR™ Green I, and SYBR™ Safe stains are highly sensitive reagents for staining DNA in electrophoresis gels (Table 3). These gel stains provide greater sensitivity with lower background fluorescence than the conventional ethidium bromide stain.

For more information, go to **thermofisher.com/stains**

Table 3. Fluorescent nucleic acid gel stains.

	Detection of nucleic acids in agarose gels and CsCl gradients	Safer, smarter alternative to ethidium bromide	Ultimate sensitivity for real-time PCR and capillary electrophoresis	Ultimate sensitivity for DNA detection
	UltraPure Ethidium Bromide	**SYBR Safe Stain**	**SYBR Green I Stain**	**SYBR Gold Stain**
Sensitivity (dsDNA)	Sensitive (1 ng)	Sensitive (3 ng)	Highly sensitive (>60 pg)	Ultrasensitive (>25 pg)
Nonhazardous and environmentally friendly		✔		
Improved cloning efficiency		✔	✔	✔

Did you know

- A variety of these ladders are available in ready-to-load Invitrogen™ TrackIt™ format. No need to heat, mix, or dilute prior to loading sample on your gel.
- Some DNA ladders are now shipped at ambient temperature without impacting quality and stability—learn more at **thermofisher.com/ladders**

Facts

Invitrogen™ SYBR™ Safe DNA Gel Stain exhibited very low mutagenicity compared to ethidium bromide, when tested by an independent, licensed testing laboratory. SYBR Safe stain is not classified as hazardous waste or as a pollutant under US federal regulations. In addition, our scientists have demonstrated a vast improvement in cloning efficiency of DNA fragments stained with SYBR Safe DNA Gel Stain and visualized using our Safe Imager™ blue-light transilluminator, compared to the conventional method.

Learn more at **thermofisher.com/sybrsafe**

Cloning

For over 25 years, Thermo Fisher Scientific has provided the latest tools for DNA cloning—continuously improving old technologies and developing new ones. From restriction enzymes to gene synthesis, we have a large portfolio of tools and resources to help you achieve high-quality cloned DNA for your next discovery (Table 4).

Did you know

We have over 200 vectors available for applications ranging from basic subcloning to inducible mammalian expression. To select the most suitable vector for your application, visit **thermofisher.com/vectors**. Custom vectors are also available through the Invitrogen™ GeneArt™ Elements™ service.

Table 4. Tools and associated features for DNA cloning.

	Restriction enzyme cloning	Invitrogen™ TOPO™ cloning	Invitrogen™ Gateway™ cloning	Invitrogen™ GeneArt™ Seamless Cloning	Invitrogen™ GeneArt™ Type IIs Assembly Kits	Invitrogen™ GeneArt™ Strings DNA Fragments	Invitrogen™ GeneArt™ Gene Synthesis
Key benefits	• Flexible and economical	• >95% efficiency, 5 min PCR cloning • Compatible with many other cloning systems	• Shuttling ORF among multiple expression systems	• Seamless directional cloning of ≤4 fragments for up to 40 kb total	• Seamless directional cloning of ≤8 fragments for up to 20 kb total • Efficient for repetitive and very small sequences	• Synthesized linear DNA fragments ready to clone via the method of your choice • Pool sequence-verified	• Custom-cloned genes in vector • Sequence-verified • Can be optimized for maximal protein expression
Technology basics	• Restriction digestion and ligation	• Topoisomerase-based, ligase-free cloning	• Single-step, directional site-specific DNA recombination • Restriction enzyme- and ligase-free	• End-terminal homology recombination using overlapping sequences	• Type IIs restriction and ligation in a single reaction	• 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 Gateway™ Vector Conversion System with One Shot™ *ccdB* Survival Cells.

Restriction enzyme cloning

Where would modern molecular biology research be without restriction enzymes? Found naturally in bacteria, restriction enzymes recognize and cleave specific DNA sequences, resulting in sticky ends (5′ or 3′ protruding ends) or blunt ends, allowing for DNA inserts to be cloned into vectors with compatible ends. This traditional method for cloning DNA can be divided into three main steps: digestion, modifcation, and ligation of DNA. Each step requires high-quality, reliable enzymes to ensure success.

Some restriction enzymes can exhibit star activity, or decreased specificity for their DNA recognition site, with prolonged digestions. Star activity results in nonspecific cleavage of DNA and can occur under suboptimal reaction conditions such as high glycerol content or presence of Mg^{2+}. The Invitrogen™ Anza™ restriction enzymes, in conjunction with the Anza™ 10X buffer, have been optimized for flexibility in digestion times without having to worry about star activity (Figure 6).

The Invitrogen™ Anza™ Restriction Enzyme Cloning System comprises 128 restriction enzymes and 5 DNA-modifying enzymes that all work compatibly and are fully functional in a single Anza™ buffer, for beautifully simple cloning.

The system offers:

- One buffer for all restriction enzymes
- One digestion protocol for all DNA types
- Complete digestion in 15 minutes
- Overnight digestion without star activity

To learn more, visit **thermofisher.com/anza**

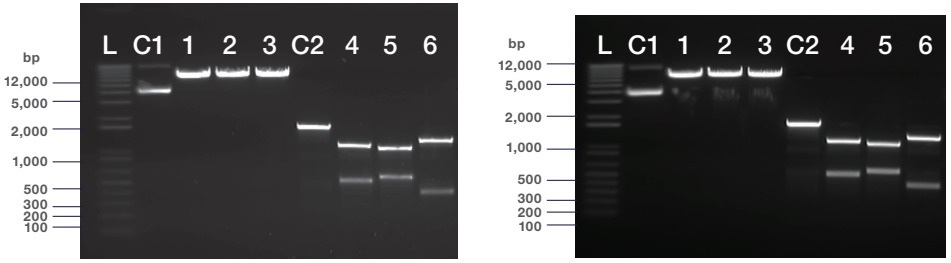


Figure 6. Anza restriction enzymes show complete digestion in 15 minutes with no star activity after overnight digestion. Plasmid DNA (6,215 bp) and purified PCR product (1.6 kb) were digested using Anza 11 EcoRI, Anza 12 XbaI, and Anza 1 NotI restriction enzymes. Reaction mixtures included 1 µg of DNA and 1 µL of restriction enzyme in a total volume of 20 µL, following the recommended protocol. Incubation was done at 37°C for 15 minutes or 16 hours.

L - 1 Kb Plus DNA Ladder
C1 – Undigested plasmid DNA
1 – Anza 11 EcoRI enzyme
2 – Anza 12 XbaI enzyme
3 – Anza 1 NotI enzyme
C2 – Undigested PCR fragment
4 – Anza 11 EcoRI enzyme
5 – Anza 12 XbaI enzyme
6 – Anza 1 NotI enzyme

Did you know

The Invitrogen™ Anza™ Starter Kits include 5 or 10 of the most common Anza™ restriction enzymes, Anza 10X buffer, and selected Anza DNA-modifying enzymes, so you can be on your way to simpler cloning.

Helpful tips

The CloningBench mobile app now features Anza restriction enzymes and modifying tools. Find the right Anza restriction enzyme for your research using simple and single search functionality. View datasheets and add Anza restriction enzymes to your cart to share via email or immediate checkout.

Download for free at
thermofisher.com/cloningbench

TOPO cloning

PCR cloning is a technique used to directly insert PCR products into a plasmid vector. Invitrogen™ TOPO™ cloning technology allows for a quick, simple, and efficient way to PCR clone. The key to TOPO cloning is the enzyme DNA topoisomerase I, which has a ligase function. TOPO™ cloning vectors are provided linearized with topoisomerase I covalently bound to the 3′ phosphate on each end, enabling the vectors to readily ligate DNA sequences with compatible ends and eliminating the need for additional ligation steps.

TOPO cloning technology is:

- **Efficient**—up to 95% of clones contain desired insert
- **Fast**—5-minute, room temperature reaction
- **Easy**—simple 3-step procedure
- **Proven**—over 20,000 citations
- **Flexible**—available with or without competent cells, in multiple reaction sizes

Select a TOPO cloning solution whether you are performing general subcloning, sequencing, TA cloning, blunt-end cloning, long-fragment cloning, expression vector cloning, directional cloning, or using the Gateway™ system.

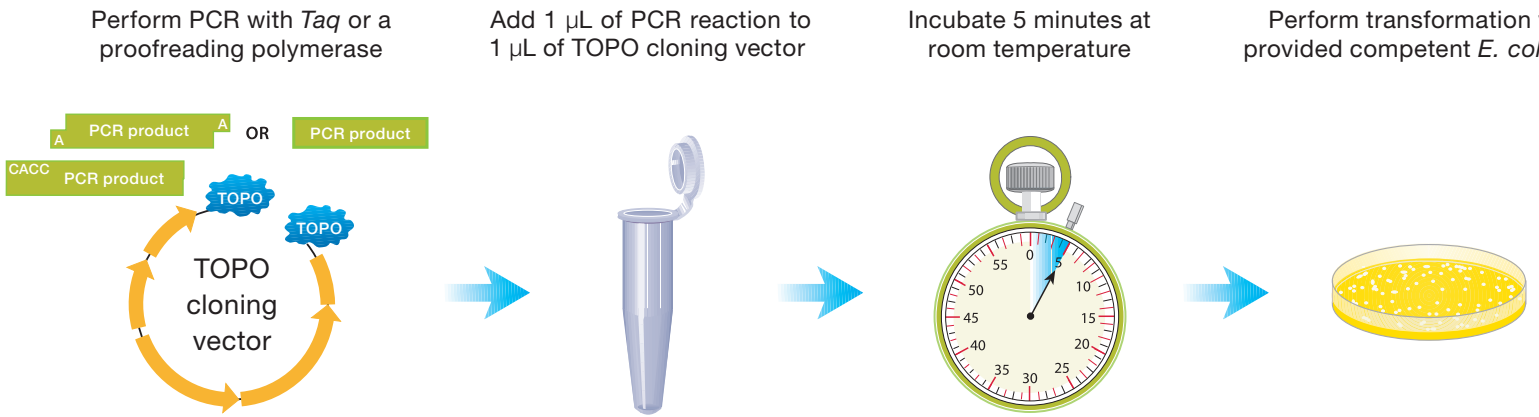


Figure 7. TOPO PCR cloning requires just three easy steps. Simply combine your PCR product and a TOPO cloning vector in the provided reaction buffer, wait 5 minutes, then transform *E. coli*. With TOPO cloning, the additional time, steps, and reagents required for ligase-mediated cloning are eliminated.

To learn more, visit **thermofisher.com/topo**

Gateway cloning

Gateway cloning technology is the easy-to-use choice for cloning into multiple expression systems. There's no need for subcloning or spending hours to screen and resequence countless colonies.

Gateway cloning offers:

- **Easy solution**—no need for restriction enzymes or ligation to maintain orientations and reading frames for expression-ready clones
- **Convenient workflow**—no resequencing required; use the same clone from target identification to validation for consistency
- **Versatile technology**—easily shuttle DNA material/insert from vector-to-vector, and select from *E. coli*, yeast, insect, or mammalian destination vectors
- **Fast reactions**—1-hour room-temperature cloning reactions
- **Accurate results**—cloning reactions achieve >95% efficiency to deliver the clone you need

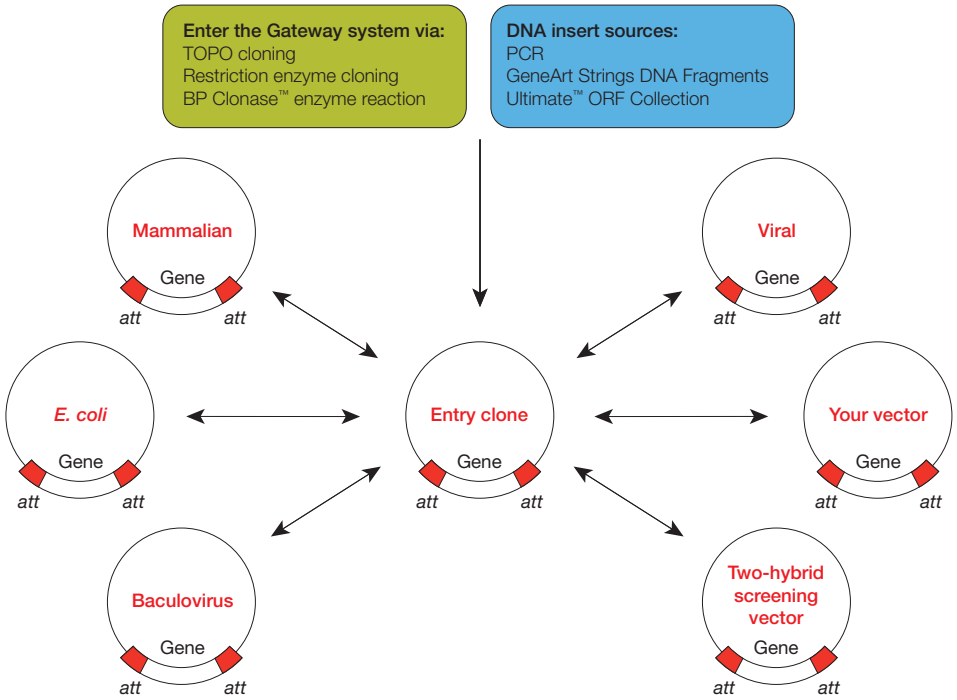


Figure 8. Gateway technology facilitates cloning of genes into and back out of multiple vectors via site-specific recombination. Once a gene is cloned into an entry clone you can then move the DNA fragment into one or more destination vectors simultaneously.

Helpful tips

A single-step BP/LR Clonase reaction protocol is available. Download the *BioTechniques* PDF at **thermofisher.com/gateway**

To learn more, visit **thermofisher.com/gateway**

GeneArt Seamless Cloning and Assembly Kit

Invitrogen™ GeneArt™ Seamless Cloning and Assembly Kit enables *in vitro* cloning of up to 4 DNA fragments simultaneously into virtually any linearized vector, typically in 30 minutes, without extra DNA sequences, restriction endonucleases, or ligation. With potential construct sizes of up to 40 kb, our kits offer researchers the flexibility and convenience to complete basic, standard, and advanced cloning and assembly protocols.

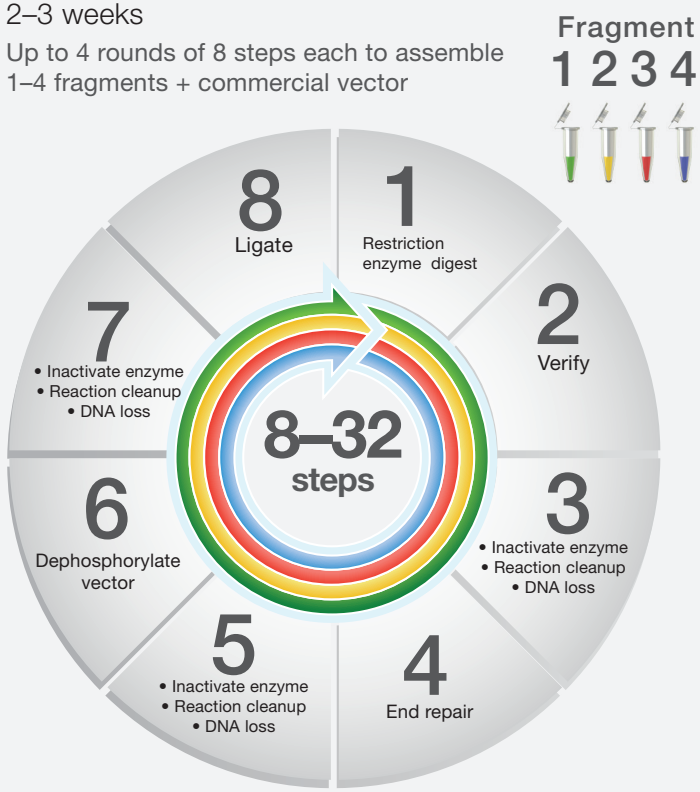
The GeneArt Seamless Cloning and Assembly Kit is:

- **Flexible**—use any vector of your choice
- **Precise**—no scars; clone what you want and where you want
- **Efficient**—cloning efficiency >90%

Traditional cloning*

2–3 weeks

Up to 4 rounds of 8 steps each to assemble
1–4 fragments + commercial vector



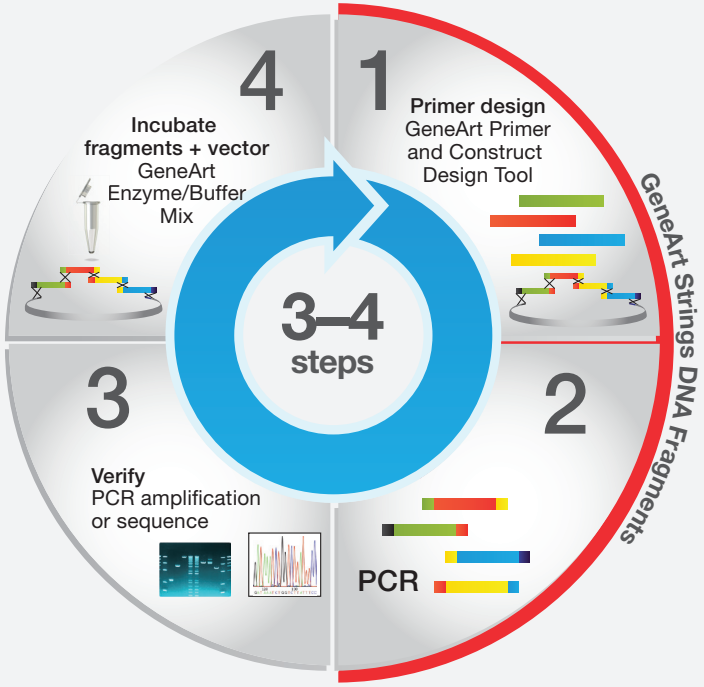
* Lower cloning efficiency with traditional cloning means more colonies need to be screened to find your correct clone.

VS.

GeneArt Seamless Cloning and Assembly

4–5 days

Up to 4 steps to assemble
1–4 fragments + any vector simultaneously



To learn more, visit **thermofisher.com/seamless**

GeneArt Type IIs Assembly Kit

Are you assembling more than 4 fragments? Invitrogen™ GeneArt™ Type IIs Assembly kits are perfect for simultaneously assembling up to 8 fragments in any order; you can assemble Invitrogen™ GeneArt™ Strings DNA Fragments or Libraries, GeneArt™ TALs, gene variants, and repetitive or small sequences.

With GeneArt Type IIs kits you can:

- Avoid homologous recombination and associated rearrangements when cloning homologous or repetitive sequences
- Create your own cloning and expression vectors with custom vector elements
- Perform your restriction/ligation reaction using any one of three Type IIs enzymes (AarI, BsaI, and BbsI); each 10-reaction kit contains all-in-one enzyme mix, cloning vector, and cloning controls

To learn more, visit **thermofisher.com/typeiis**

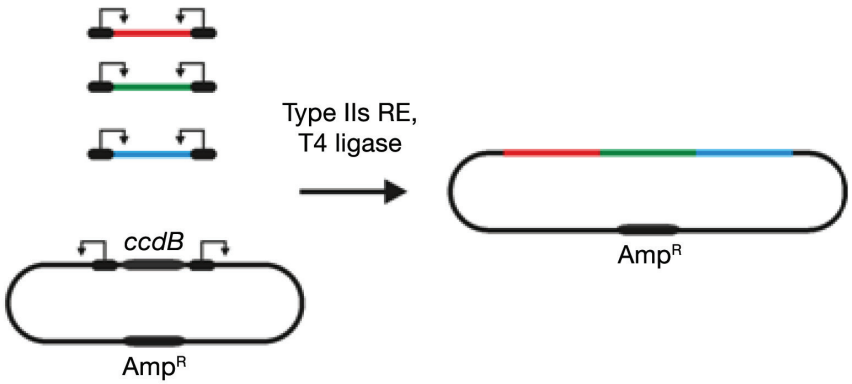


Figure 9. An example of cloning 3 DNA fragments into a single vector using Type IIs assembly. The black arrows indicate the orientation of the Type IIs restriction enzyme sites, pointing towards the cleavage sites.

Helpful tips

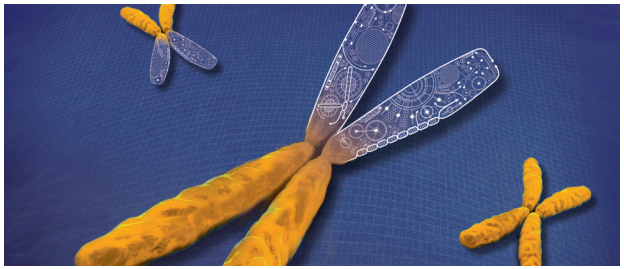
- Use the free online web tool to design oligos and assemble DNA molecules *in silico* for both types of GeneArt cloning and assembly kits found here: **thermofisher.com/order/oligodesigner**
- Invitrogen™ GeneArt Site-Directed Mutagenesis System is also available.
- Read the GEN publication from our R&D team: **genengnews.com/gen-articles/one-step-cloning/5002/**

GeneArt Gene Synthesis

Have you ever lacked the time to clone your favorite gene? Conventional PCR and cloning techniques require optimization and troubleshooting, which take up valuable lab time and resources. Invitrogen™ GeneArt™ Gene Synthesis makes your favorite gene analogous to an optimized, error-free PCR reaction.

Did you know

We also offer custom GeneArt™ services—from custom cell lines and protein production to cloning, mutagenesis, plasmid prep, and directed evolution services.



GeneArt Gene Synthesis

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

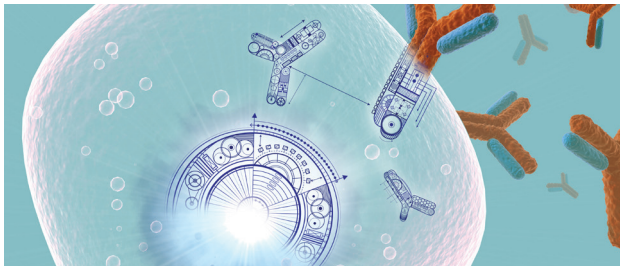
- The GeneArt™ Portal for easy online editing and ordering
- Outstanding quality—ISO 9001:2008 certification and responsive project management
- GeneOptimizer™ software for gene optimization for reliable maximum protein expression up to 15X*
- GeneObserver™ Interface for 24-hour order tracking status

* Fath et al. (2011) A Standardized Tool to Assess and Enhance Autologous Mammalian Gene Expression. *PLoS ONE* 6(3): e17doi:10.1371/journal.pone.0017596

GeneArt Strings DNA Fragments

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

- 100% pool-sequence verification
- Ready-to-clone DNA fragments using the method of your choice
- An economical solution that maintains the gene synthesis benefits of both flexibility and performance
- Easy ordering—you can directly enter, edit, optimize, and order your sequence through the online GeneArt Portal



GeneArt Strings DNA Libraries

GeneArt Strings DNA Libraries are custom-made, synthetic double-stranded GeneArt Strings DNA Fragments that contain randomized nucleotides and are ready for cloning. They are an affordable alternative to complete gene synthesis or combinatorial libraries. The GeneArt Strings DNA Libraries offer:

- Linear dsDNA fragments from 200 bp to 2 kb (≥500 ng, dried)
- Full IUPAC code of mixed, randomized DNA nucleotide options
- Up to 3 blocks of randomization, with up to 30 mixed bases in each block

Learn more about the GeneArt Gene Synthesis products and services at **thermofisher.com/genesynthesis**

Transformation

Once the DNA fragment is cloned into a vector, transformation is performed to enable propagation, within a bacterial culture, of sufficient quantities of your cloned DNA for downstream experiments. Transformation is a naturally occurring process in which bacterial cells take up foreign DNA at a low frequency. In molecular biology applications, this process is enhanced and exploited to propagate plasmids inside bacteria that have been made competent (permeable) for more efficient DNA uptake.

We offer a variety of competent cells, selection of which depends upon the transformation methods, characteristics of the plasmid, and desired applications (Table 5).

Invitrogen™ libraries of chemically competent and electrocompetent cells are designed to be:

- **Innovative**—advanced strains that grow faster, produce more colonies, and offer more protection against phage contamination and DNA recombination
- **Reliable**—years of proven performance and documented lot-to-lot consistency
- **Flexible**—available in a wide range of packaging formats and transformation efficiencies

Table 5. Selection guide for competent cells.

Routine cloning	High-efficiency cloning	Protein expression	Cloning unstable DNA
One Shot™ OmniMAX™ 2 T1^R Chemically Competent *E. coli*	ElectroMAX™ DH10B™ T1^R Competent Cells	One Shot™ BL21(DE3) Chemically Competent *E. coli*	ElectroMAX™ Stbl4™ Competent Cells
One Shot™ MAX Efficiency™ DH5α T1^R *E. coli*	MegaX™ DH10B™ T1^R Electrocomp Cells	One Shot™ BL21(DE3)pLysS Chemically Competent *E. coli*	MAX Efficiency™ Stbl2™ Competent Cells
One Shot™ MAX Efficiency™ DH10B T1^R *E. coli*	One Shot OmniMAX 2 T1^R Chemically Competent *E. coli*	One Shot™ BL21 Star (DE3) Chemically Competent *E. coli*	**Single-stranded DNA production**
One Shot™ TOP10 Chemically Competent *E. coli*	**High-throughput cloning**	BL21-A1™ One Shot™ Chemically Competent *E. coli*	ElectroMAX™ DH12S™ Cells
Library Efficiency™ DH5α™ Competent Cells	MultiShot™ StripWell TOP10 Chemically Competent *E. coli*		**Propagating unmethylated DNA**
Subcloning Efficiency™ DH5α™ Competent Cells	MultiShot™ TOP10 Chemically Competent *E. coli*		One Shot™ INV110 Chemically Competent *E. coli*
	MultiShot™ StripWell Mach1™ T1^R Chemically Competent *E. coli*		**Recombinant baculo-virus production**
			MAX Efficiency™ DH10Bac™ Competent Cells

For a more comprehensive selection guide for competent cells, download a copy at **thermofisher.com/compcells** or download the CloningBench mobile app at **thermofisher.com/cloningbench**

Facts

Applications and features of popular competent cell strains:

- TOP10 cells (subcloning, and high-throughput)
- Stbl3™ cells (cloning unstable DNA)
- One Shot BL21 Star cells (protein expression)
- One Shot OmniMax 2 T1^R cells (highest-efficiency subcloning)
- Mach1™ T1^R cells (fast-growing, subcloning)
- DH5α™ cells (subcloning, increased yield)
- DH10B™ cells (general subcloning)

To learn more, visit **thermofisher.com/compcells**

Plasmid DNA isolation

Low plasmid DNA recovery and impurities such as protein, salts, RNA, or genomic DNA can adversely affect cloning and PCR reactions. Different purity grades result from different plasmid DNA isolation technologies and are appropriate at different points in the cloning workflow.

Purity grade	Advanced transfection	Transfection	Molecular
Endotoxin classification	Endotoxin-free	Low-endotoxin	Standard
Endotoxin level	<0.1 EU/µg	0.1–1 EU/µg	1–10 EU/µg
Application	Transfection in sensitive cell lines (i.e., primary cells)	• Transfection in traditional cell lines • *In vitro* transcription • All molecular-grade applications	• Cloning • Nucleic acid labeling • PCR • Sequencing • Transformation

We recommend using molecular-grade plasmid DNA for cloning and vector verification (sequencing, enzyme digestion) and transfection-grade plasmid DNA for the final vector. Molecular-grade plasmid DNA is not recommended for transfection due to relatively high levels of endotoxin and RNA contamination, which can decrease cell viability and expression.

Sufficient amounts (<50 µg) of molecular-grade plasmid DNA can be purified using silica-membrane mini columns (i.e., Invitrogen™ PureLink™ Quick Plasmid Miniprep Kit) for molecular biology applications. Once a vector is validated, larger quantities (500 µg–1 mg) of required transfection-grade plasmid DNA can be purified using anion exchange resin (i.e., Invitrogen™ PureLink™ HiPure Midiprep or Maxiprep Kit).

Learn more about plasmid isolation kits at **thermofisher.com/plasmidprep**

Helpful tips

Once your plasmid has been validated and is ready to be scaled up for transfection experiments, keep the following in mind:

1. Larger quantities are needed for transfection of mammalian cell lines and thus require larger preps (midi-, maxi-, mega-, or gigaprep).
2. Kits for purification of transfection-grade or advanced transfection-grade plasmid should be used.
3. RNA contamination can interfere with transfection.
4. Using PureLink HiPure plasmid purification kits address all of the concerns listed above.

Frequently asked questions

One resource for all your support needs.
Browse our support centers for useful resources, tips and tricks for when you start an experiment, or troubleshooting help.

Cloning: **thermofisher.com/cloningsupport**
Nucleic acid purification and analysis: **thermofisher.com/napsupport**
PCR and cDNA synthesis: **thermofisher.com/pcrsupport**

Frequently asked questions from our support centers:
What are the best ways to improve my RNA isolation?
These are the top 10 ways to improve your RNA isolation results:

- Immediately inactivate endogenous, intracellular RNases.
- Use proper cell or tissue storage conditions.
- Thoroughly homogenize samples.
- Pretreat homogenate before RNA isolation to remove interfering compounds.
- Choose the best RNA isolation method for your sample.
- Include a DNase treatment.
- Reduce exposure to environmental RNases.
- Precipitate appropriately for the downstream application.
- Resupend the RNA properly.
- Store the RNA properly after isolation.

How can I determine if an RNA sample has genomic DNA contamination? What will the A_{260}/A_{280}, A_{260}/A_{230}, and 28S/18S ratios indicate about an RNA sample and why are these values useful?
RNA and DNA absorb at 260 nm, while protein absorbs at 280 nm. UV/Vis spectrophotometry will give you an RNA/DNA absorbance ratio, therefore indicating the purity of your sample. For RNA, the A_{260}/A_{280} ratio should be approximately 2.0. If the ratio is lower, this indicates protein and/or DNA contamination of your sample. The A_{260}/A_{230} values are a measure for pure nucleic acids, with an expected range between 2.0 and 2.2. If the ratio is much lower than this, contaminants are present in your sample (typically phenol, guanidine, or ethanol).

The 28S and 18S bands are indicative of intact RNA. On a gel, the 28S and 18S bands should be present in an approximately 2:1 ratio.

I'm setting up my RT reaction and am trying to decide whether I should use random primers, oligo(dT) primer, gene-specific primer, or oligo(dT)/random mix primers. What would you suggest?
Random primers are the best choice for degraded RNA, RNA with heavy secondary structure, non-polyadenylated RNA, or prokaryotic RNA. It is recommended only for two-step RT-PCR, and typically gives the highest yields, although the cDNA may not necessarily be full-length. Oligo(dT) primers are good to use when trying to recover full-length cDNA from 2-step RT-PCR. The reaction is influenced by secondary structure and RNA quality. Gene-specific primers should be used for very specific, mainly one-step RT-PCR reactions.

My PCR tubes got deformed after thermal cycling. What is wrong?
The heated thermal cycler lid is designed to make sure that optimized pressure is applied on the microplate for an efficient PCR reaction. When using PCR tubes, excess pressure on the tubes from the heated lid can cause deformation of the tubes. To avoid this, we recommend using the tray/retainer set, as shown below:

- Cat. No. 4381850, Applied Biosystems™ MicroAmp™ 96-Well Tray/Retainer Set for Veriti™ Systems—this tray is for use with MicroAmp™ Strip Tubes (or MicroAmp™ Reaction Tubes without Caps, 0.2 mL), with the Veriti™ Thermal Cycler, SimpliAmp™ Thermal Cycler, and ProFlex™ PCR System.
- Cat. No. 403081, Applied Biosystems™ MicroAmp™ 96-Well Tray/Retainer Set—this tray is for use with MicroAmp Strip Tubes (or MicroAmp Reaction Tubes without Caps, 0.2 mL), with the 7000 System, 2720 Thermal Cycler, and GeneAmp™ PCR System 9700.
- Cat. No. 4379983, Applied Biosystems™ MicroAmp™ 96-Well Tray for VeriFlex™ Blocks—this tray is for use with MicroAmp Strip Tubes (or MicroAmp Reaction Tubes with Caps, 0.2 mL), with the Veriti Thermal Cycler, Veriti Fast Thermal Cycler, SimpliAmp Thermal Cycler, and ProFlex PCR System.

I'm getting no bands from my PCR product. What could cause this?
Here are some possible causes:

- Suboptimal template quality or quantity: poor integrity, poor purity, and/or too little or too much template
- Incorrect primer design
- Suboptimal cycling conditions: e.g., annealing temperature, number of cycles
- Suboptimal reaction conditions: insufficient amount of polymerase, primers, or Mg^{2+}; inhibition by dUTP if high-fidelity DNA polymerase is used
- Difficult template: GC-rich or long amplicons may need more powerful enzyme
- Activation step for hot-start polymerase is not at a high enough temperature, or time is not long enough

How can I get better separation of my bands after nucleic acid gel electrophoresis?
First check the percentage of your agarose gel. A higher percentage will help you to resolve smaller fragments, while a lower percentage will help you to resolve larger fragments.

What is the best ratio of insert:vector to use for cloning? Is there an equation to calculate this?
You may have to try different ratios ranging from 1:1 to 15:1 insert:vector.

Equation:

$$\frac{\text{length of insert (bp)}}{\text{length of vector (bp)}} \times \text{ng of vector} = \text{ng of insert needed for 1:1 insert:vector}$$

For calculations at your fingertips, check out our CloningBench moblie app.
thermofisher.com/cloningbench

Are there any limitations on the insert length in Gateway cloning?
There is no theoretical size limitation. PCR products between 100 bp and 11 kb have been readily cloned into a Gateway™ pDONR™ vector. Other DNA pieces as large as 150 kb with *att* sites will successfully recombine with a Gateway–compatible vector. Overnight incubation is recommended for large inserts.

What are some tips you can give me to obtain the highest transformation efficiency for my competent cells?
Some suggestions that will help you to obtain the highest transformation efficiency are:

- Thaw competent cells on ice instead of room temperature; do not vortex cells.
- Add DNA to competent cells once thawed.
- Ensure that the incubation times are followed as outlined in the competent cell protocol for the strain you are working with; changes in the length of time can decrease efficiency.
- Remove salts and other contaminants from your DNA sample; DNA can be purified before transformation using a spin column, or phenol-chloroform extraction and ethanol precipitation can be employed.

I'm getting no or low yields from my column-based plasmid purification experiment. What do you suggest I try?
Here are some suggestions:

- Make sure the binding of the plasmid is being done at room temperature (RT). Temperature affects the pH of the binding solution. Make sure all other solutions were also warmed to RT.
- Verify that the centrifugation immediately following the neutralization step was not done at 4°C. If it was, the supernatant must be warmed to RT before binding on the column. We find that the DNA binds to the matrix of the columns better if the lysate is at room temperature.
- Low copy number plasmid may have been used. Check plasmid.
- Not all the medium may have been removed at the cell harvesting step, so the pH of the subsequent steps was affected.
- The cell pellet may not have been thoroughly resuspended in the resuspension step.
- Purified DNA may have been overdried after isopropanol precipitation and ethanol wash. Only air dry the pellet.
- Pellet may have been lost during the isopropanol precipitation and ethanol wash. Be careful at this step, as the pellet tends to be slippery. It is best to pipette off alcohol solutions rather than pour them off.
- Try elution of DNA with heated elution buffer. For plasmids less than 10 kb, no heating is required. For 10–30 kb, heating (65–70°C) is optional, and may increase elution efficiency by ~20%. For plasmids >30 kb, heating is recommended, and may increase elution efficiency by ~50%. Perform an additional elution to increase yield by up to 10%.
- If there is some insoluble material in the eluted DNA, it could be resin particles (resin fines). These are inert and can be removed by a centrifugation at 12,000 x *g* for 1 minute at RT.

Ordering information

	Quantity	Cat. No.
Nucleic acid isolation		
PureLink Quick Plasmid Miniprep Kit	50 preps	K2100-10
PureLink HiPure Plasmid Filter Midiprep Kit	25 preps	K2100-14
PureLink HiPure Plasmid Maxiprep Kit	10 preps	K2100-06
PureLink *Pro* Quick96 Plasmid Purification Kit	4 x 96 preps	K211004A
PureLink Quick Gel Extraction Kit	50 preps	K2100-12
TRIzol Plus RNA Purification Kit	50 preps	12183-555
PureLink RNA Mini Kit	10 preps	12183020
PureLink Genomic DNA Mini Kit	10 preps	K1820-00
PureLink *Pro* 96 Genomic DNA Mini Kit	4 x 96 preps	K182104A
PureLink *Pro* 96 Viral RNA/DNA Purification Kit	4 plates	122800-96A
PureLink Viral RNA/DNA Mini Kit	50 preps	12280-050
PureLink Genomic Plant DNA Purification Kit	50 preps	K1830-01
MagMAX DNA Multi-Sample Ultra Kit	500 preps	A25597
Reverse transcription		
SuperScript IV Reverse Transcriptase	2,000 units	18090010
	10,000 units	8090050
SuperScript IV First-Strand Synthesis System	50 reactions	18091050
	200 reactions	18091200
SuperScript VILO Master Mix	50 reactions	11755050
	250 reactions	11755250
SuperScript III Reverse Transcriptase	2,000 units	18080093
	10,000 units	18080044
RNaseOUT Recombinant Ribonuclease Inhibitor	5,000 units	10777-019
Ribonuclease H	30 units	18021-014

	Quantity	Cat. No.
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
PureLink PCR Purification Kit	50 preps	K3100-01
PureLink Quick Gel Extraction and PCR Purification Kit	50 preps	K2200-01
Taq DNA Polymerase, recombinant	500 units	10342-020
	3 x 500 units	10342-046
Platinum *Taq* DNA Polymerase	120 reactions	10966-018
	600 reactions	10966-034
Platinum *Taq* Green Hot Start DNA Polymerase	200 reactions	11966-018
	1,000 reactions	11966-034

Ordering information (continued)

	Quantity	Cat. No.
PCR (continued)		
Platinum Hot Start PCR 2X Master Mix	200 reactions	13000-013
	1,000 reactions	13000-014
Platinum Green Hot Start PCR 2X Master Mix	200 reactions	13001-013
	1,000 reactions	13001-014
Platinum SuperFi DNA Polymerase	100 units	12351010
	500 units	12351050
Platinum SuperFi Green DNA Polymerase	100 units	12357010
	500 units	12357050
Platinum SuperFi PCR Master Mix	100 reactions	12358010
	500 reactions	12358050
Platinum SuperFi Green PCR Master Mix	100 reactions	12359010
	500 reactions	12359050
dNTP Set (100 mM)	4 x 250 µL	10297-018
	8 x 1.25 mL	10297-117
ProFlex 3 x 32-well PCR system	1 instrument	4484073
ProFlex 96-well PCR system	1 instrument	4484075
ProFlex 2 x flat PCR System	1 instrument	4484078
SimpliAmp Thermal Cycler	1 instrument	A24811
Veriti 96-Well Thermal Cycler	1 instrument	4375786
Veriti 384-Well Thermal Cycler	1 instrument	4388444
Veriti 96-Well Fast Thermal Cycler	1 instrument	4375305
HID Veriti 96-Well Thermal Cycler	1 instrument	4479071
2720 Thermal Cycler	1 instrument	4359659
MicroAmp EnduraPlate Optical 96-Well Fast Multicolor Reaction Plates with Barcode	5 plates	4483493

	Quantity	Cat. No.
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
Nucleic acid separation and analysis		
UltraPure 10 mg/mL Ethidium Bromide	10 mL	15585011
PureLink Quick Gel Extraction Kit	50 preps	K2100-12
SYBR Safe DNA Gel Stain	400 µL	S33102
UltraPure Agarose	100 g	16500100
TrackIt 1 Kb Plus DNA Ladder	100 applications	10488085
UltraPure TAE Buffer, 10X	4 L	15558026
E-Gel CloneWell Agarose Gels with SYBR Safe DNA Gel Stain, 0.8%	18 gels	G6618-08
E-Gel Agarose Gels with SYBR Safe DNA Gel Stain, 2%	18 gels	G5218-02
E-Gel EX Agarose Gels, 2%	10 gels	G401002
E-Gel 1 Kb Plus DNA Ladder	100 applications	10488-090
E-Gel Sample Loading Buffer, 1X	4 x 1.25 mL	10482-055
E-Gel EX Agarose Gels Starter Kit, 2%	1 kit	G6512ST
E-Gel CloneWell Agarose Gels with SYBR Safe DNA Gel Stain, 0.8% + E-Gel iBase Power System + E-Gel Safe Imager Transilluminator Starter Kit	1 each	G6500ST
E-Gel *48* Agarose Gels, 2%	8 gels	G8008-02
E-Gel *96* Agarose Gels, 2%	8 gels	G7008-02
Safe Imager 2.0 Blue-Light Transilluminator	1 each	G6600

	Quantity	Cat. No.
Cloning		
Anza 10-Pack Starter Kit	1 kit	IVGN300-6
Anza 5-Pack Starter Kit	1 kit	IVGN300-4
Anza 10X Buffer Set	2,000 reactions	IVGN200-8
Anza T4 DNA Ligase Master Mix	200 reactions	IVGN2108
	50 reactions	IVGN2104
Anza Alkaline Phosphatase	2,000 reactions	IVGN220-8
	50 reactions	IVGN220-4
Anza T4 PNK Kit		IVGN230-4
Anza DNA Blunt End Kit		IVGN240-4
Anza DNA End Repair Kit		IVGN2504
TOPO TA Cloning Kit for Subcloning, without competent cells		450641
Zero Blunt TOPO PCR Cloning Kit, without competent cells		450245
pENTR/D-TOPO Cloning Kit, with One Shot TOP10 Chemically Competent *E. coli*		K240020
pcDNA 6.2/V5-PL-DEST Mammalian Expression Vector		12537162
LR Clonase II Plus Enzyme		12538-120
One Shot TOP10 Chemically Competent *E. coli*		C404003
One Shot Stbl3 Chemically Competent *E. coli*		C737303
MAX Efficiency DH5α Competent Cells		18258-012
ElectroMAX DH10B Cells		18290-015
MAX Efficiency Stbl2 Competent Cells		10268-019
GeneArt Seamless Cloning and Assembly Enzyme Mix		A14606
GeneArt Seamless Cloning and Assembly Kit		A13288
GeneArt Seamless PLUS Cloning and Assembly Kit		A14603

	Quantity	Cat. No.
GeneArt Type IIs Assembly Kit, AarI		A15916
GeneArt Type IIs Assembly Kit, BsaI		A15917
GeneArt Type IIs Assembly Kit, BbsI		A15918
Gateway BP Clonase II Enzyme Mix		11789-020
Gateway LR Clonase II Enzyme Mix		11791-020
Gateway Vector Conversion System with One Shot *ccdB* Survival Cells		11828-029
PCR Cloning System with Gateway Technology with pDONR 221 and OmniMAX 2 Competent Cells		12535-029
PCR Cloning System with Gateway Technology with pDONR/Zeo and OmniMAX 2 Competent Cells		12535-037
Gateway pDONR 221 Vector		12536-017
pENTR/D-TOPO Cloning Kit, with One Shot TOP10 Chemically Competent *E. coli*		K240020
pCR 8/GW/TOPO TA Cloning Kit with One Shot TOP10 *E. coli*		K250020

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