

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.

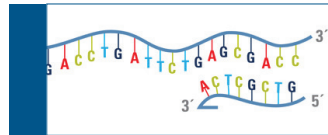
In this booklet, find the research tools that can advance your science. For additional information, please visit [thermofisher.com/everystepcounts](https://www.thermofisher.com/everystepcounts)

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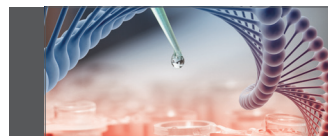
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# Nucleic acid isolation

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  |
|--------------------|---|--|---|
| <b>Description</b> | Phenol-chloroform solution  | Glass fiber, derivatized silica, or ion exchange membrane in column  | 0.5–1 $\mu\text{m}$ particles with a paramagnetic core and modified shell   |
| <b>Benefits</b>    | <ul style="list-style-type: none"> <li>• Rapid denaturation of nucleases</li> <li>• Stabilization of nucleic acids</li> </ul>   | <ul style="list-style-type: none"> <li>• Convenience</li> <li>• Ease of use</li> <li>• Throughput flexibility</li> <li>• Ability to automate</li> </ul>  | <ul style="list-style-type: none"> <li>• No risk of clogging</li> <li>• Increased target capture efficiency</li> <li>• Rapid collection and concentration of sample</li> <li>• Ability to automate</li> <li>• Scalable</li> </ul>   |
| <b>Drawbacks</b>   | <ul style="list-style-type: none"> <li>• Use and associated waste of organic reagents</li> <li>• Manually intensive processing</li> </ul>   | <ul style="list-style-type: none"> <li>• Propensity to clog</li> <li>• Fixed binding capacity within a manufactured format</li> <li>• Automation requires vacuum or centrifugation systems</li> </ul>                              | <ul style="list-style-type: none"> <li>• Potential carry-through of particles into sample</li> <li>• Slow migration of particles in viscous solutions</li> <li>• Difficulty automating large volumes</li> </ul>   |
| <b>Process</b>     | 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](https://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



Nucleic acid  
isolation

## Helpful tips



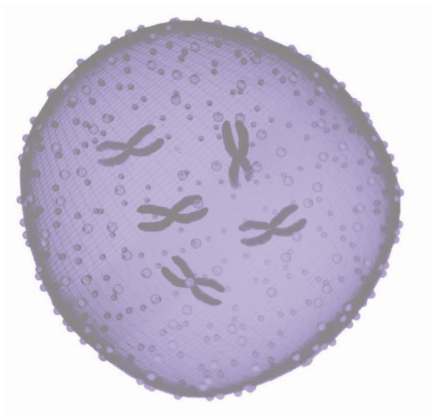
A budget-friendlier version, Ultimate™ ORF Clones LITE are the same clones without the final QC step.

# 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](https://thermofisher.com/gdnaprep)

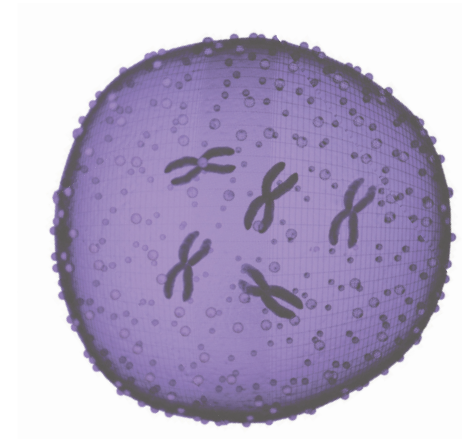
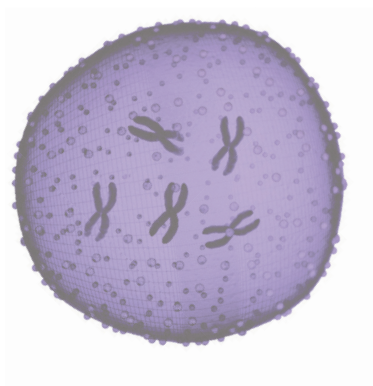
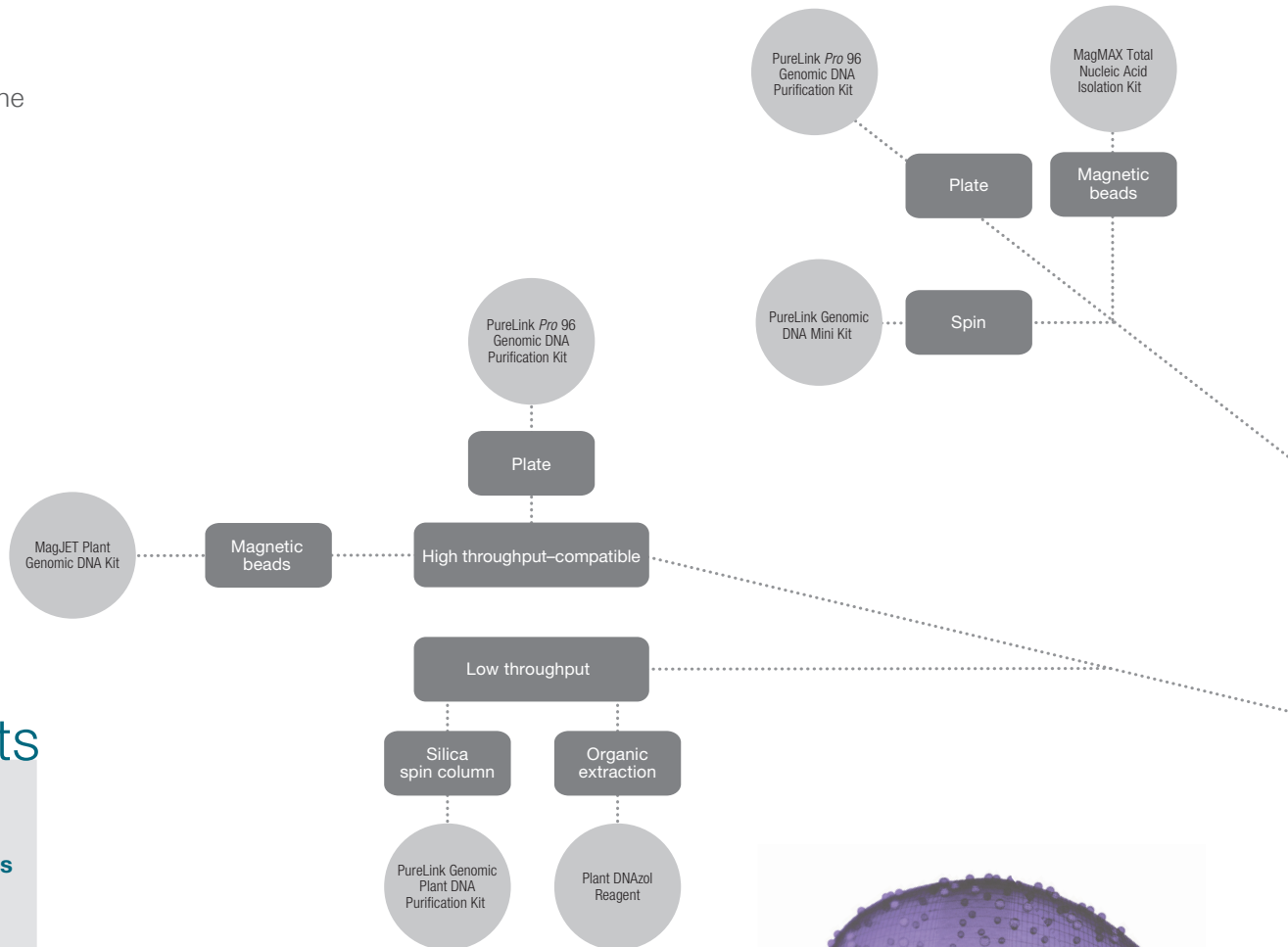
Nucleic acid isolation

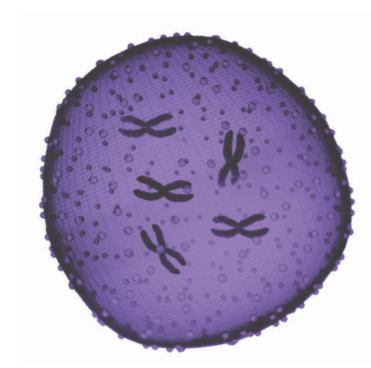
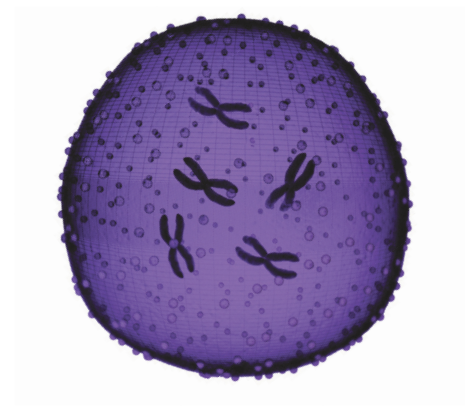
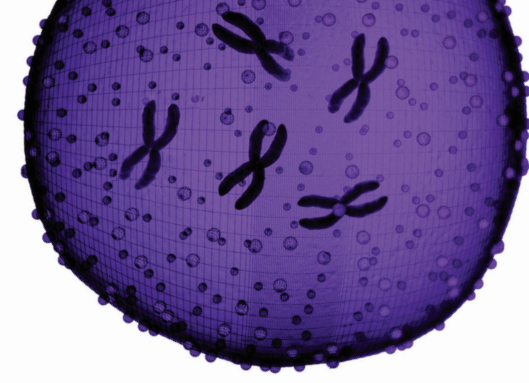
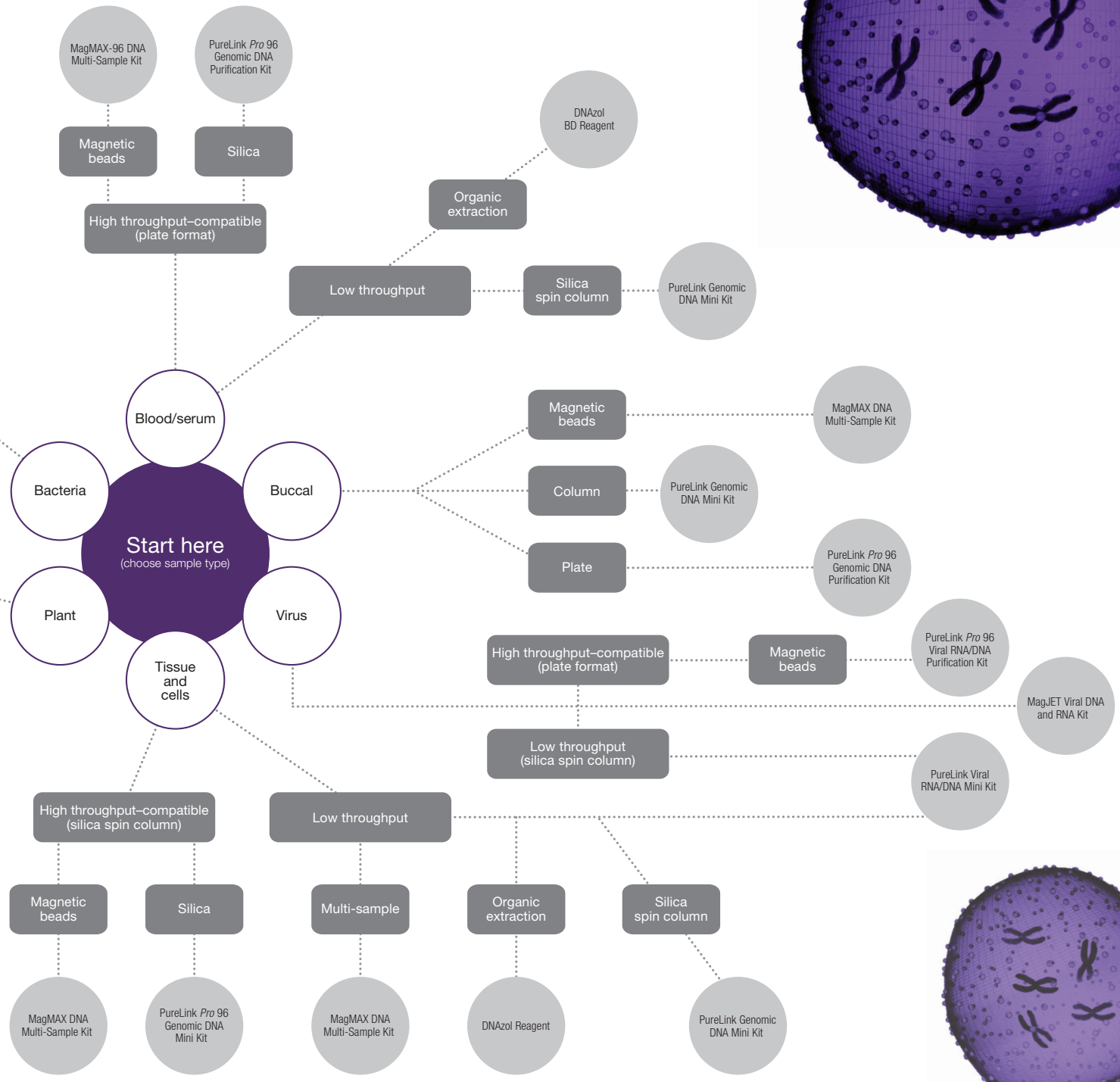


## Facts



- View our protocol video library at [thermofisher.com/nucleicacidisolationvideos](https://thermofisher.com/nucleicacidisolationvideos)
- Your DNA isolations can be automated—learn more at [thermofisher.com/kingfisher](https://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.



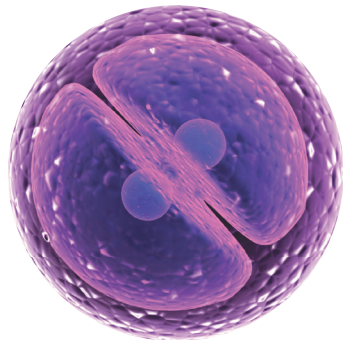
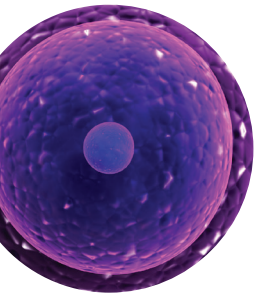


# RNA isolation

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](https://thermofisher.com/rnapreps)

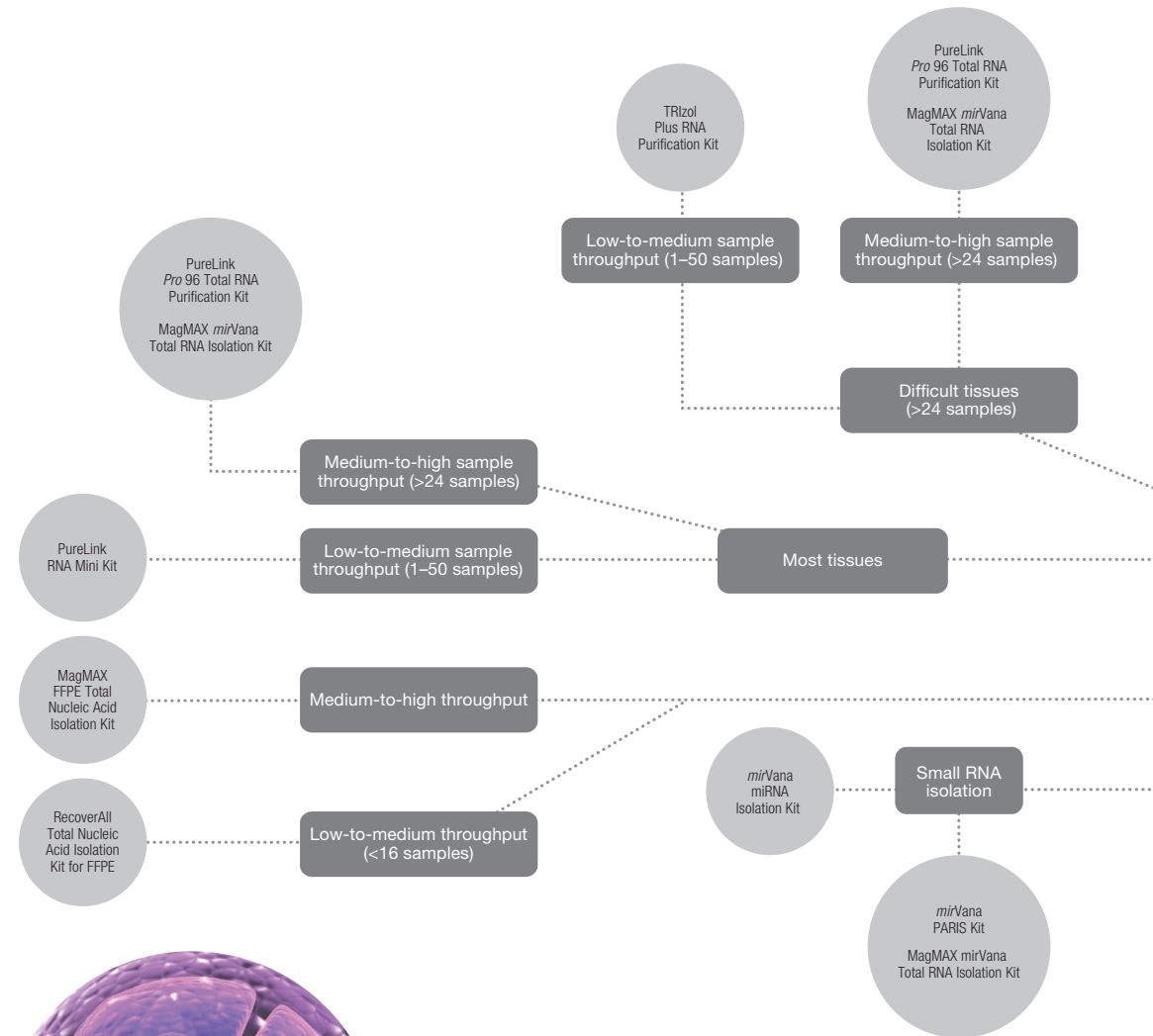
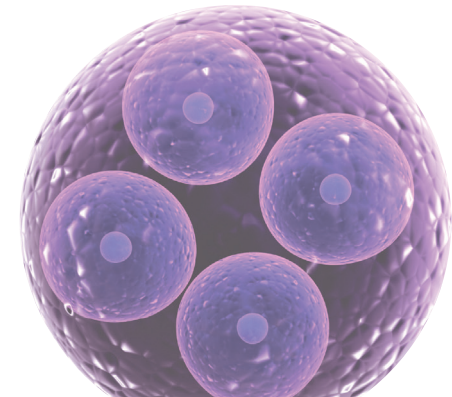
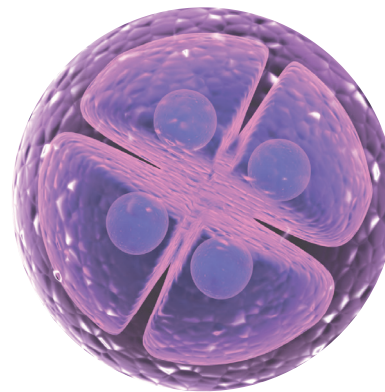
Nucleic acid isolation



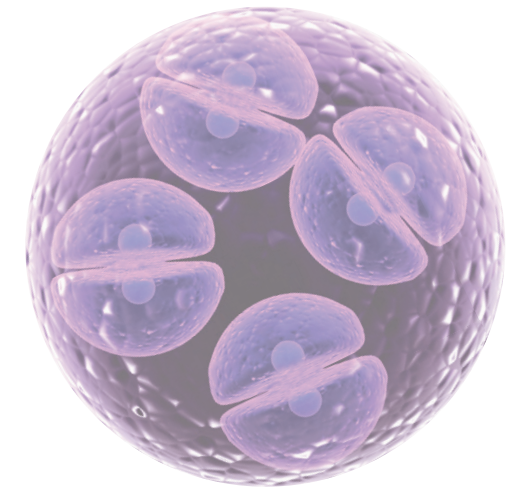
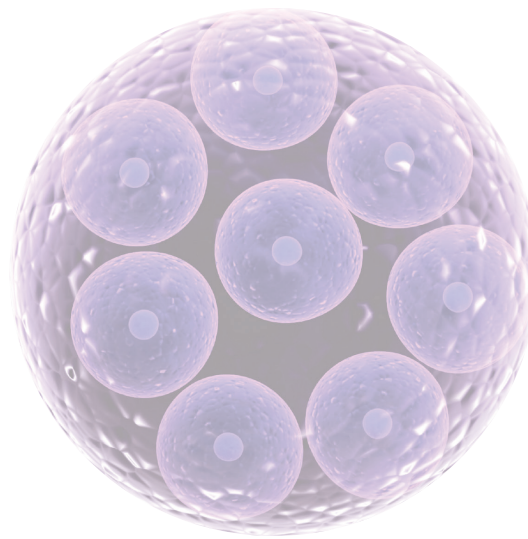
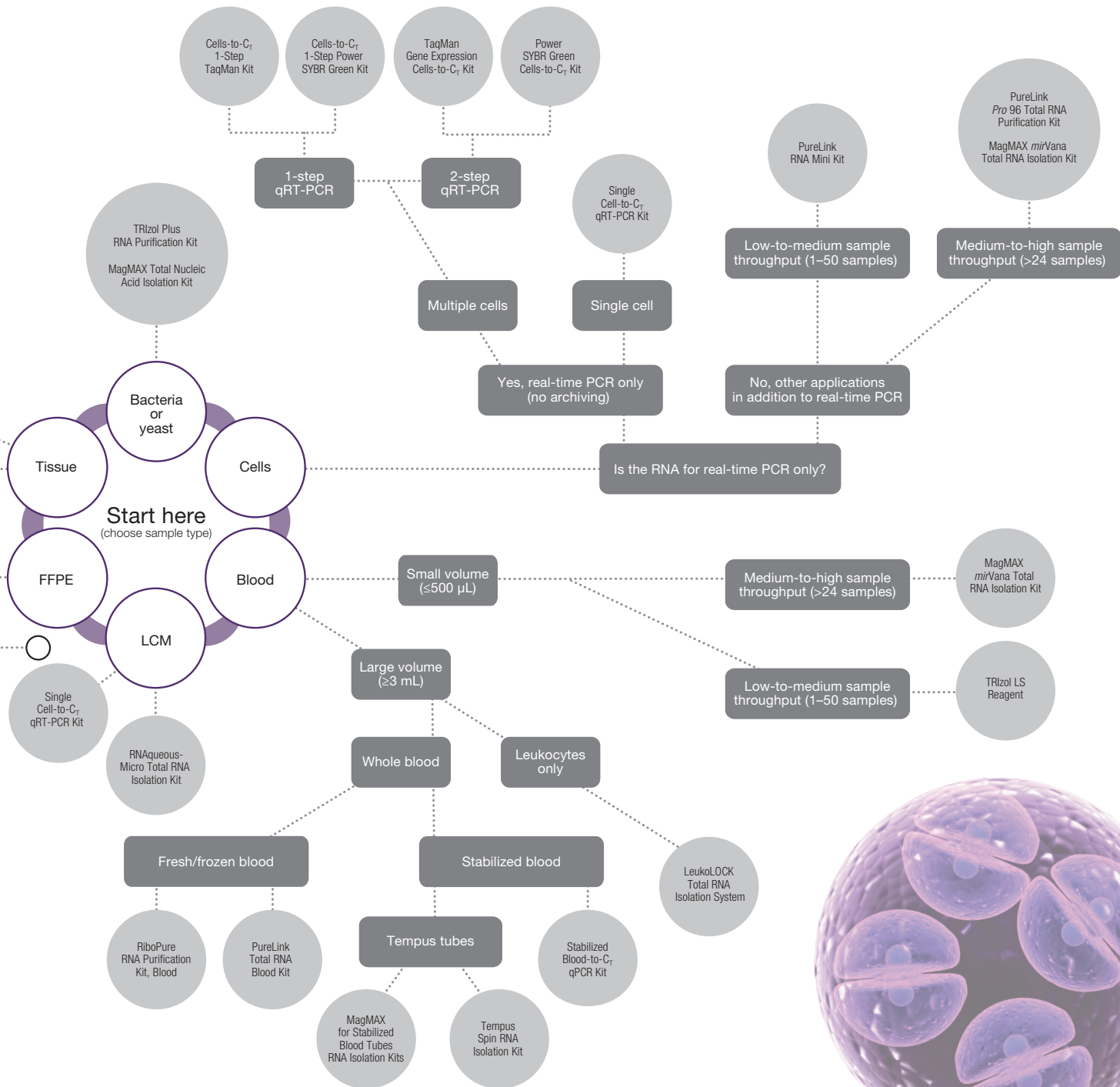
## Facts



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







# 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

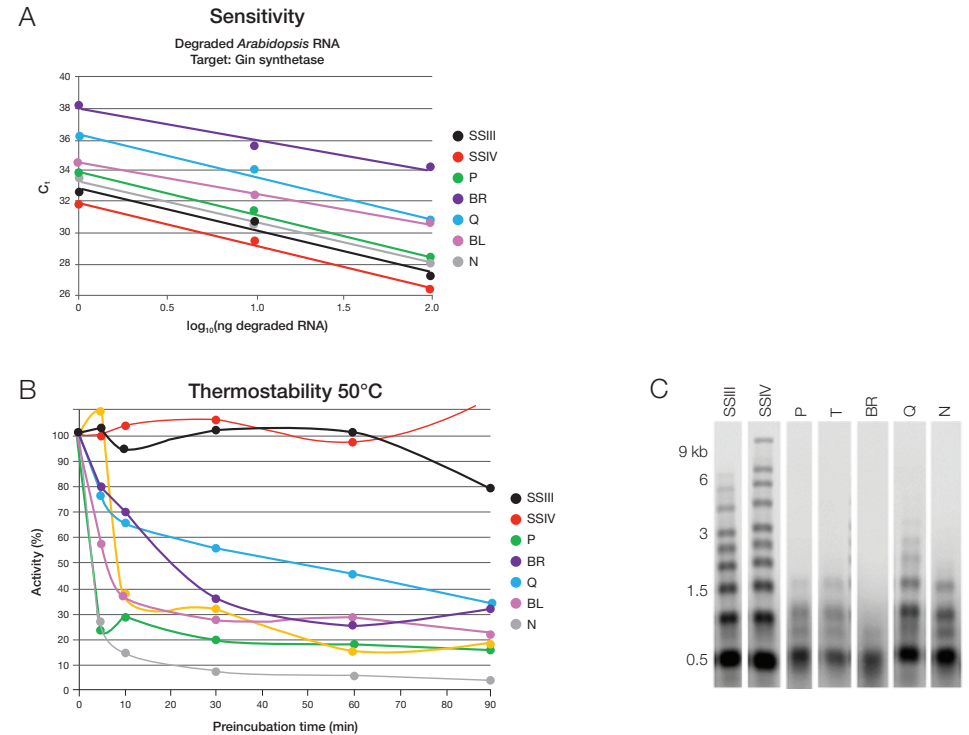
The ability of reverse transcriptase to generate cDNA from the lowest amount of input RNA is an important attribute when looking for low copy genes or working with increasingly difficult sample sources where RNA becomes degraded during purification process (Figure 1A).

## 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](https://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™ IV VILO™ Master Mix             | SuperScript™ IV Reverse Transcriptase |
| Sensitivity                                 | 10 pg–2 µg                                    | 1 pg–2.5 µg                                  | 10 pg–2 µg                            |
| Optimal reaction temperature                | 50°C  | 50°C   | 50–55°C                               |
| Reaction time                               | 10 min  | 10 min                                       | 10 min                                |
| cDNA yield with challenging or degraded RNA | High  | High   | High                                  |

To learn more about SuperScript™ reverse transcriptases, go to [thermofisher.com/superscript](https://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.

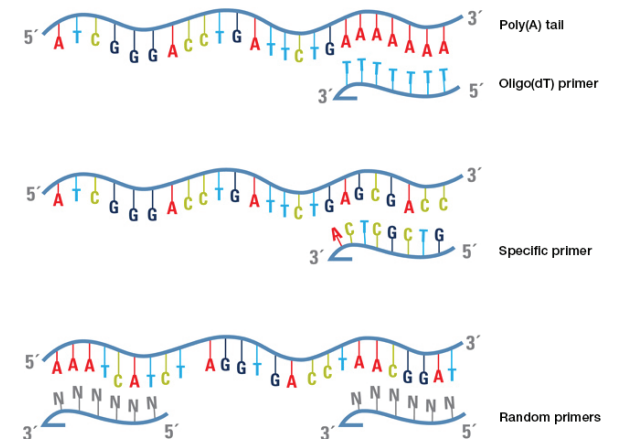
## RT primers

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](https://thermofisher.com/primers)



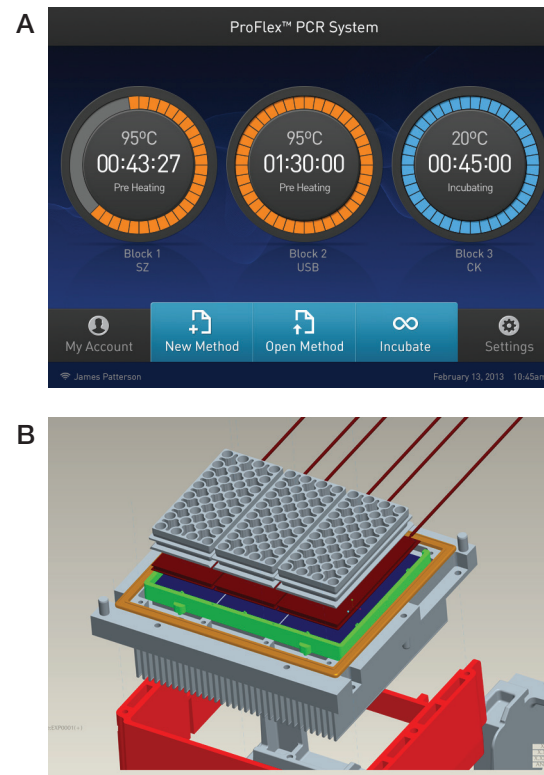
# PCR

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.



**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](https://www.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](http://thermofisher.com/plastics)



**Table 2. PCR tubes, caps, and accessories.**

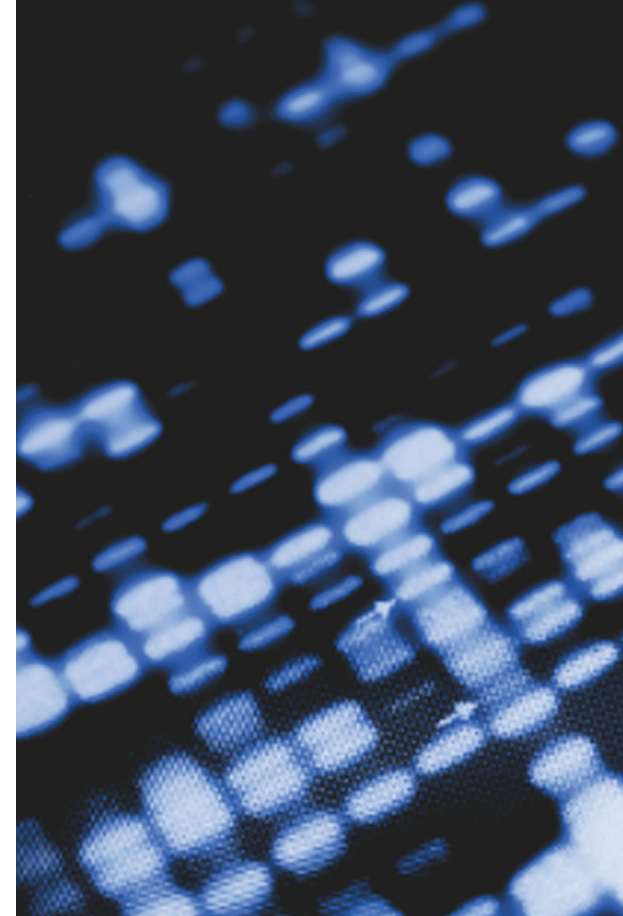
|                                  | Small-scale experiments with a few samples   | Daily experiments   | Complete-workflow experiments —ideal for automation   | High throughput, automation   |
|----------------------------------|--|---|---|---|
|                                  | Single tubes, strips, caps, adhesive film, and accessories   | MicroAmp™ optical microplates   | MicroAmp™ EnduraPlate™ optical microplates  | EnduraPlate™ optical GPLE reaction plates   |
| Formats                          | <ul style="list-style-type: none"> <li>• Single tubes</li> <li>• Single tubes with caps</li> <li>• 8-strip tubes with caps</li> <li>• 12-strip caps</li> </ul> | <ul style="list-style-type: none"> <li>• 48-well Fast</li> <li>• 96-well</li> <li>• 96-well Fast</li> <li>• 384-well</li> </ul> | <ul style="list-style-type: none"> <li>• 96-well</li> <li>• 96-well Fast</li> <li>• 384-well</li> </ul> | <ul style="list-style-type: none"> <li>• 96-well</li> <li>• 96-well Fast</li> <li>• 384-well</li> </ul> |
| 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.



PCR

## 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

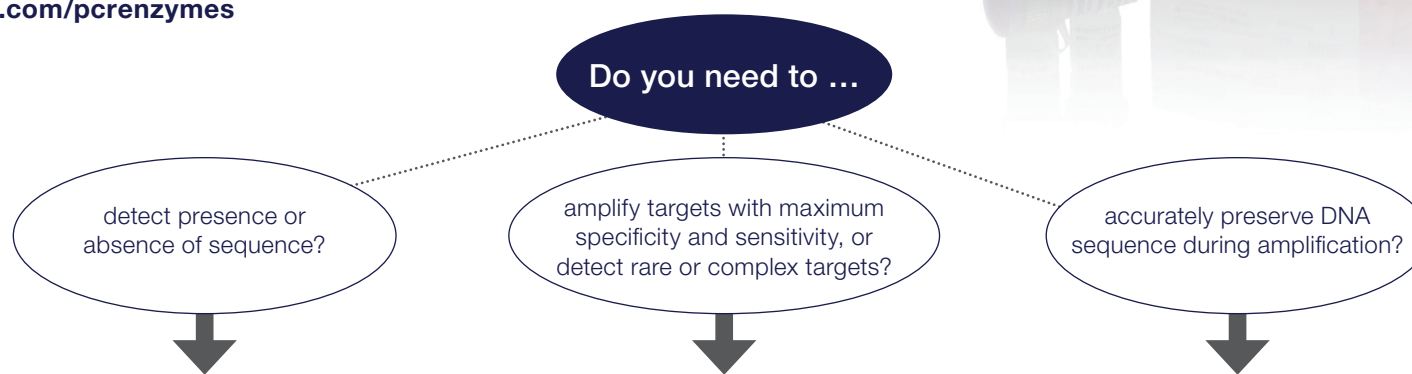
We offer a comprehensive portfolio of PCR enzymes and master mixes with the high performance and consistency you need. Standard *Taq* DNA polymerase reagents provide reliable amplification for routine PCR applications. Hot-start PCR reduces nonspecific amplification, increases the yield of the target 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](http://thermofisher.com/pcrenzymes)



PCR



| PCR type                           | Standard PCR                          | Hot-start PCR  | High-fidelity PCR   |
|------------------------------------|---------------------------------------|--|---|
| Recommended DNA polymerase         | Invitrogen™ <i>Taq</i> DNA Polymerase | Invitrogen™ Platinum™ <i>Taq</i> 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. <i>Taq</i> 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](https://www.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        | <ul style="list-style-type: none"><li>• 25 nmol–10 <math>\mu</math>mol</li><li>• 5–100 bp</li></ul> | <ul style="list-style-type: none"><li>• 50 nmol–10 <math>\mu</math>mol</li><li>• 7–55 bp</li><li>• &gt;85% full-length sequence</li></ul> | <ul style="list-style-type: none"><li>• 50 nmol–10 <math>\mu</math>mol</li><li>• 7–100 bp</li><li>• &gt;90% full-length sequence</li></ul> |
| Standard PCR  | ✓   |   |  |
| Specialty PCR |   | ✓   | ✓  |
| Cloning       |   | ✓   | ✓  |

In addition 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](https://www.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](https://www.thermofisher.com/valueoligos)
- Need ASR/GMP oligos manufactured in compliance with US FDA? For details, go to [thermofisher.com/oligos](https://www.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](https://www.thermofisher.com/pcrcleanup)

PCR

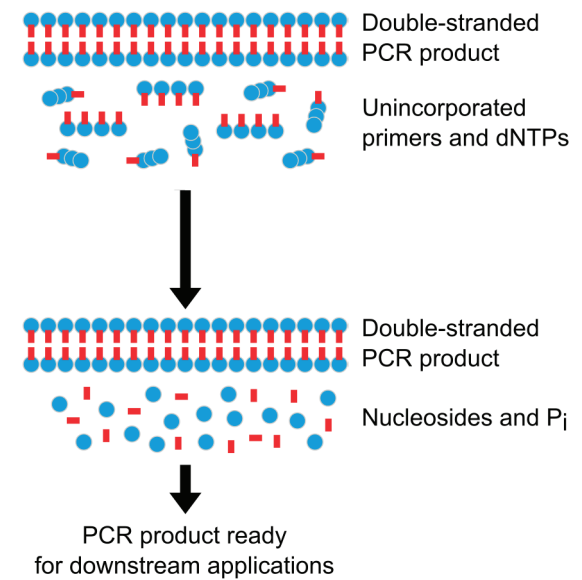


Figure 4. PCR purification workflow with CleanSweep™ reagent.

## 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

## 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/highthroughputgels](http://thermofisher.com/highthroughputgels)

## 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                              |

Nucleic acid electrophoresis



## E-Gel precast agarose gel electrophoresis system

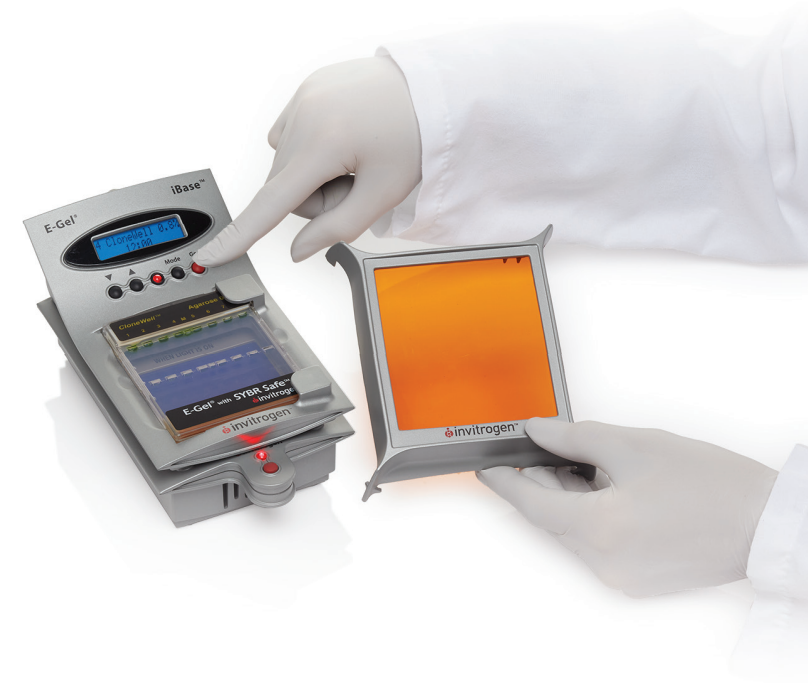
The E-Gel™ system is a bufferless system for agarose gel electrophoresis, viewing, and documentation. Samples are run in E-Gel™ precast agarose gels that include electrodes, and are packaged in a dry, disposable, and UV-transparent cassette. E-Gel agarose gels are run in a base that includes a combined 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](http://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](https://www.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](https://www.thermofisher.com/clonewell)

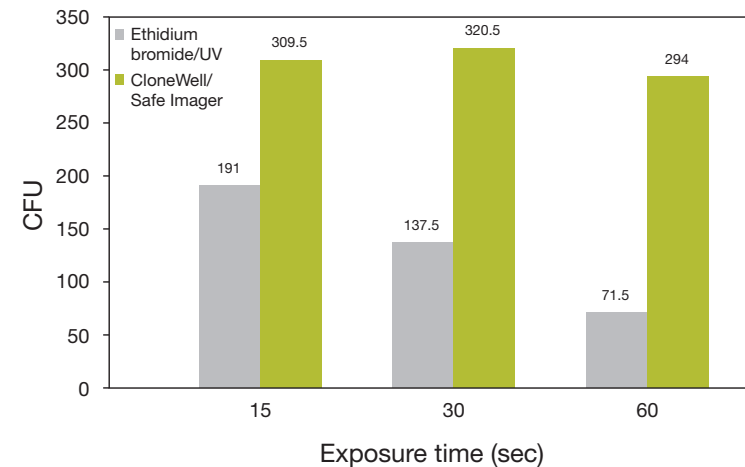
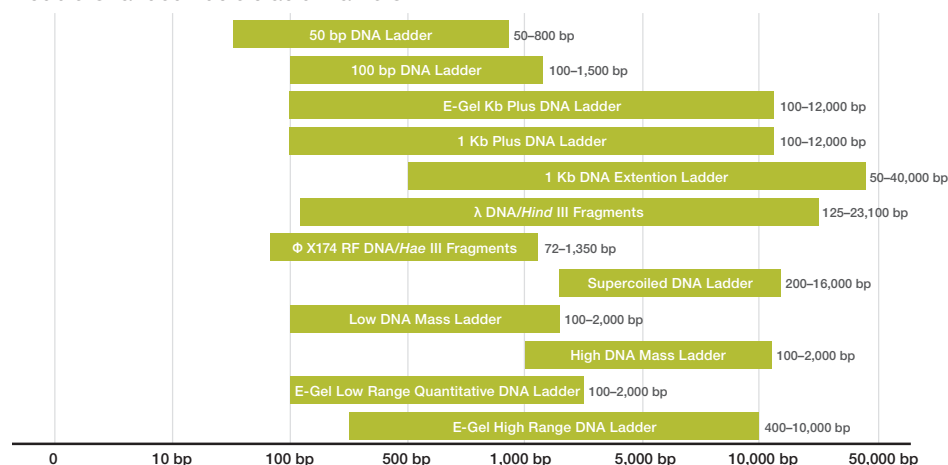


Figure 5. Results obtained using Invitrogen™ TOPO™ TA Cloning™ Kits after E-Gel CloneWell gel purification, compared to a traditional gel purification method.

## 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



## 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](https://thermofisher.com/ladders)

## 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](https://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               |   | ✓  | ✓  | ✓                                      |

Nucleic acid electrophoresis

## 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](https://thermofisher.com/sybrsafe)

# Cloning

## 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](https://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   |
|---|--|---|---|--|---|---|---|
| <b>Key benefits</b>   | <ul style="list-style-type: none"> <li>Flexible and economical</li> </ul>            | <ul style="list-style-type: none"> <li>&gt;95% efficiency, 5 min PCR cloning</li> <li>Compatible with many other cloning systems</li> </ul> | <ul style="list-style-type: none"> <li>Shuttling ORF among multiple expression systems</li> </ul>   | <ul style="list-style-type: none"> <li>Seamless directional cloning of ≤4 fragments for up to 40 kb total</li> </ul> | <ul style="list-style-type: none"> <li>Seamless directional cloning of ≤8 fragments for up to 20 kb total</li> <li>Efficient for repetitive and very small sequences</li> </ul> | <ul style="list-style-type: none"> <li>Synthesized linear DNA fragments ready to clone via the method of your choice</li> <li>Pool sequence-verified</li> </ul>                   | <ul style="list-style-type: none"> <li>Custom-cloned genes in vector</li> <li>Sequence-verified</li> <li>Can be optimized for maximal protein expression</li> </ul> |
| <b>Technology basics</b>  | <ul style="list-style-type: none"> <li>Restriction digestion and ligation</li> </ul> | <ul style="list-style-type: none"> <li>Topoisomerase-based, ligase-free cloning</li> </ul>  | <ul style="list-style-type: none"> <li>Single-step, directional site-specific DNA recombination</li> <li>Restriction enzyme- and ligase-free</li> </ul> | <ul style="list-style-type: none"> <li>End-terminal homology recombination using overlapping sequences</li> </ul>    | <ul style="list-style-type: none"> <li>Type IIs restriction and ligation in a single reaction</li> </ul>  | <ul style="list-style-type: none"> <li>Assembled from pooled, synthetic oligonucleotides</li> <li>150–3,000 bp, also available in library format with randomized bases</li> </ul> | <ul style="list-style-type: none"> <li>DNA of interest cloned in vector</li> <li>100% sequence verified with quality assurance documentation</li> </ul>             |
| <b>Needs DNA source material (gene in plasmid, library, etc.)</b> | ✓  | ✓   | ✓   | ✓  | ✓   |   |   |
| <b>Use your own vector</b>  | ✓  |   | *   | ✓  | ✓   | ✓   | ✓   |

\* 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, modification, 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).

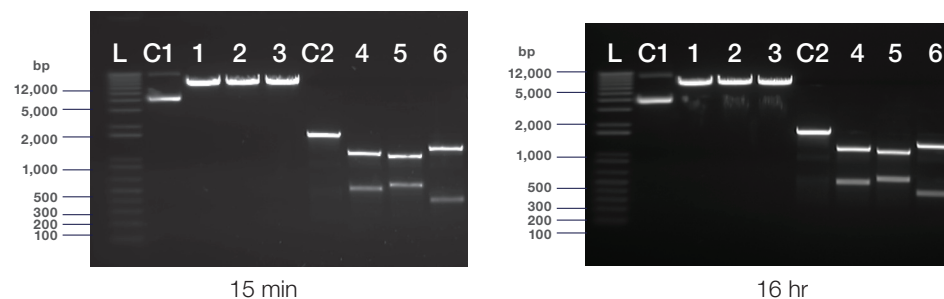
The Invitrogen™ Anza™ Restriction Enzyme Cloning System comprises 128 restriction enzymes and 5 DNA-modifying enzymes that all work compatibly and are fully functional in a single Anza™ buffer, for beautifully simple cloning.



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](http://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  $\mu$ g of DNA and 1  $\mu$ L of restriction enzyme in a total volume of 20  $\mu$ L, 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](http://thermofisher.com/cloningbench)

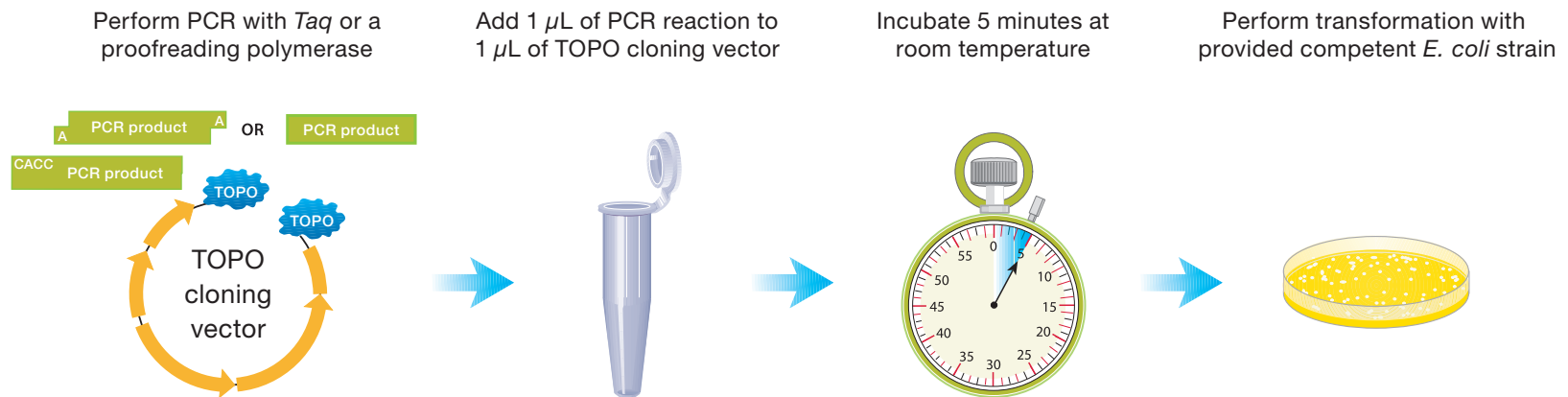
## TOPO cloning

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](https://thermofisher.com/topo)



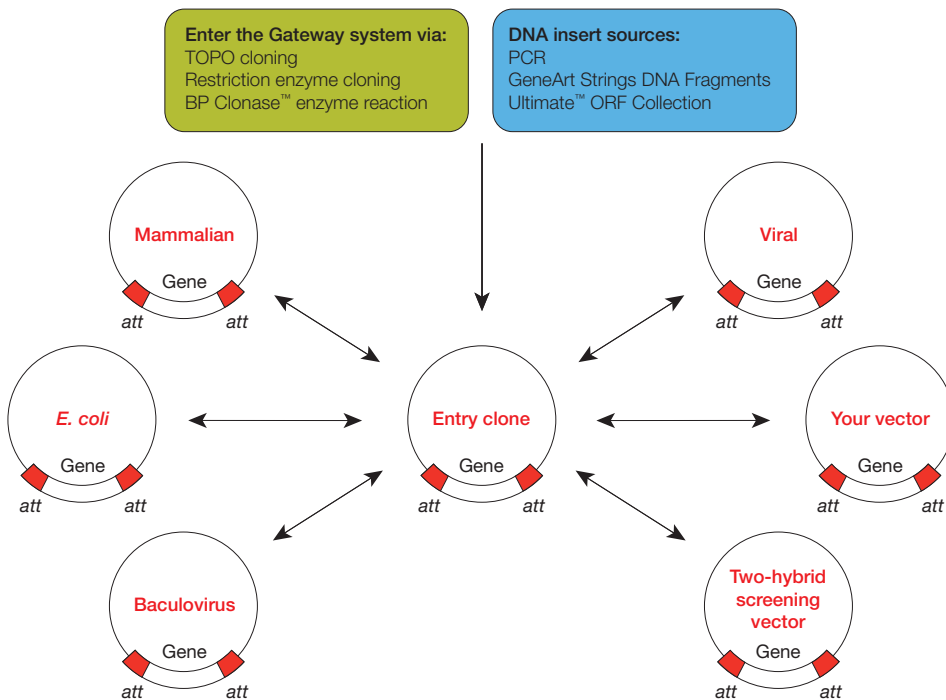
## Gateway cloning

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.

To learn more, visit [thermofisher.com/gateway](https://thermofisher.com/gateway)

## Helpful tips



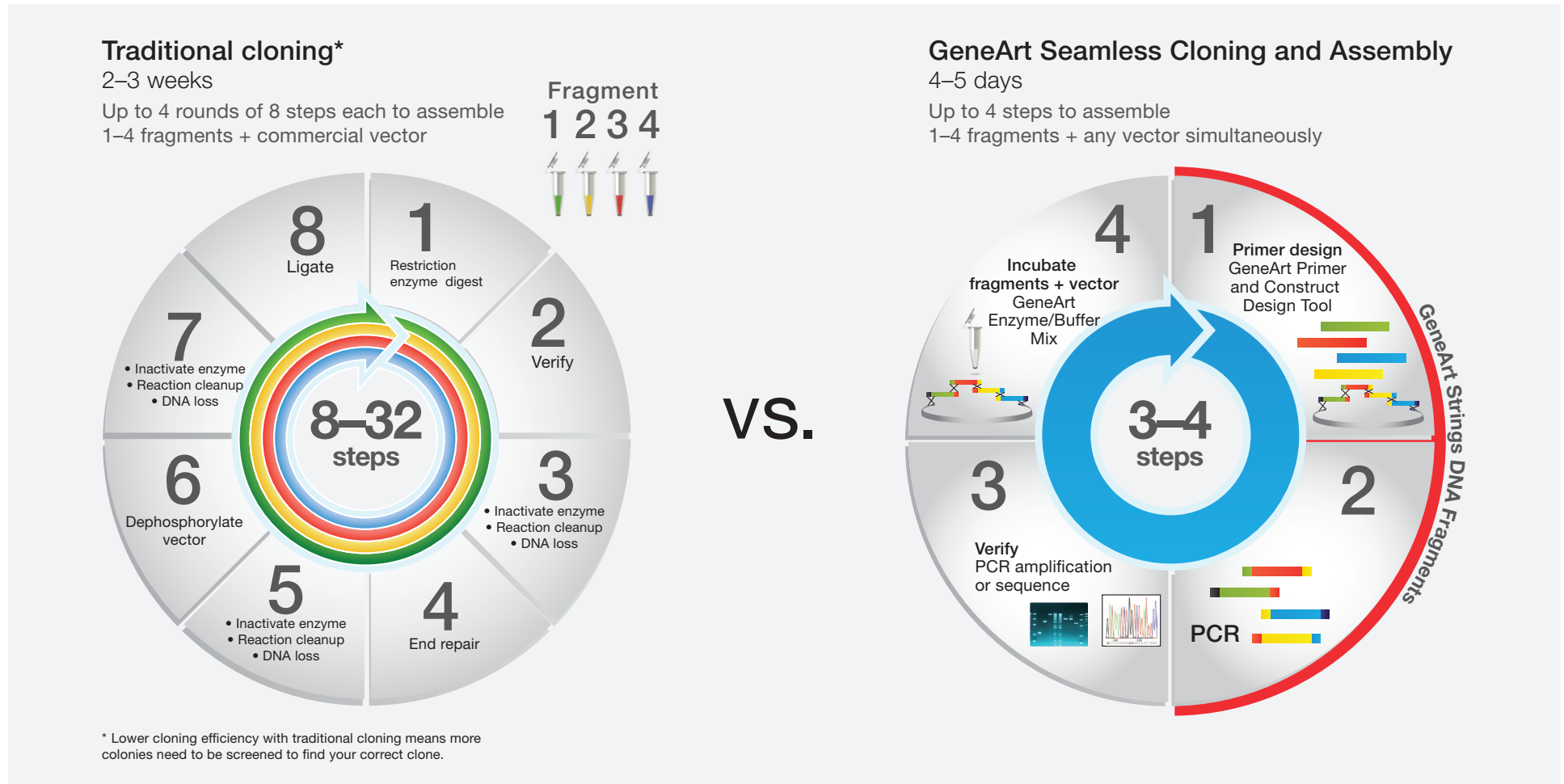
A single-step BP/LR Clonase reaction protocol is available. Download the *BioTechniques* PDF at [thermofisher.com/gateway](https://thermofisher.com/gateway)

# 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%



To learn more, visit [thermofisher.com/seamless](http://thermofisher.com/seamless)

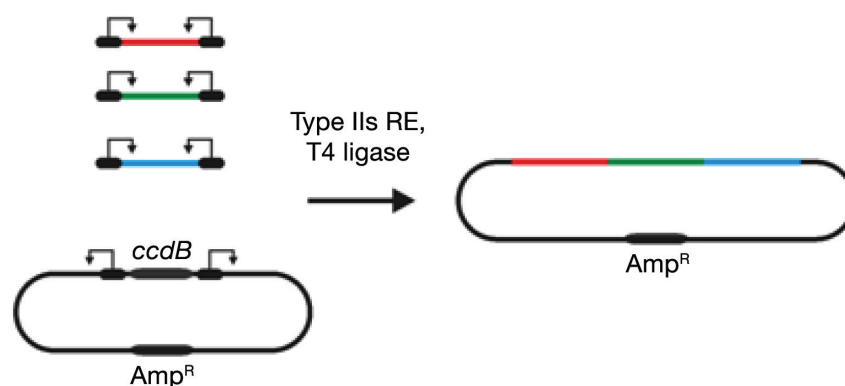
## GeneArt Type IIs Assembly Kit

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](https://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](https://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/](https://genengnews.com/gen-articles/one-step-cloning/5002/)

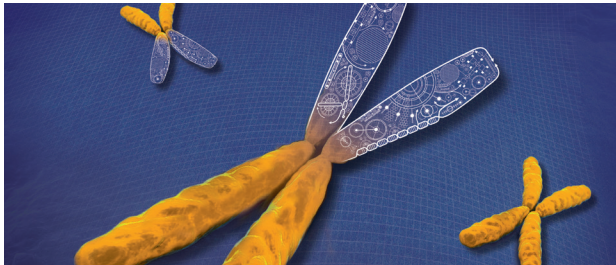
## GeneArt Gene Synthesis

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

### 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

\* 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

Learn more about the GeneArt Gene Synthesis products and services at [thermofisher.com/genesyntesis](http://thermofisher.com/genesyntesis)

## 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).

**Table 5. Selection guide for competent cells.**

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

For a more comprehensive selection guide for competent cells, download a copy at [thermofisher.com/compcells](http://thermofisher.com/compcells) or download the CloningBench mobile app at [thermofisher.com/cloningbench](http://thermofisher.com/cloningbench)

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

## 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<sup>R</sup> cells (highest-efficiency subcloning)
- Mach1™ T1<sup>R</sup> cells (fast-growing, subcloning)
- DH5α™ cells (subcloning, increased yield)
- DH10B™ cells (general subcloning)

To learn more, visit [thermofisher.com/compcells](http://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) | <ul style="list-style-type: none"> <li>• Transfection in traditional cell lines</li> <li>• <i>In vitro</i> transcription</li> <li>• All molecular-grade applications</li> </ul> | <ul style="list-style-type: none"> <li>• Cloning</li> <li>• Nucleic acid labeling</li> <li>• PCR</li> <li>• Sequencing</li> <li>• Transformation</li> </ul> |

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).

## 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.

Learn more about plasmid isolation kits at [thermofisher.com/plasmidprep](https://www.thermofisher.com/plasmidprep)

# 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](https://www.thermofisher.com/cloningsupport)  
Nucleic acid purification and analysis: [thermofisher.com/napsupport](https://www.thermofisher.com/napsupport)  
PCR and cDNA synthesis: [thermofisher.com/pcrsupport](https://www.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.
- Resuspend 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<sup>2+</sup>; 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 mobile app.

[thermofisher.com/cloningbench](http://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.   |
|---|---------------|------------|
| <b>Nucleic acid isolation</b>                         |               |            |
| 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 <i>Pro</i> 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 <i>Pro</i> 96 Genomic DNA Mini Kit           | 4 x 96 preps  | K182104A   |
| PureLink <i>Pro</i> 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     |
| <b>Reverse transcription</b>                          |               |            |
| 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 IV VILO Master Mix                        | 50 reactions  | 11756050   |
|   | 500 reactions | 11756500   |
| SuperScript IV VILO Master Mix with ezDNase           | 50 reactions  | 11766050   |
|   | 500 reactions | 11766500   |
| 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 <sub>260</sub> units | 48190 011 |
| Oligo(dT) <sub>12-18</sub> Primer                                     | 25 µg                    | 18418012  |
| Oligo(dT) <sub>20</sub> Primer  | 15 µg                    | 18418020  |
| DNase I, Amplification Grade  | 100 units                | 18068015  |
| <b>PCR</b>  |                          |           |
| 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  |
| <i>Taq</i> DNA Polymerase, recombinant                                | 500 units                | 10342-020 |
|   | 3 x 500 units            | 10342-046 |
| Platinum <i>Taq</i> DNA Polymerase                                    | 120 reactions            | 10966-018 |
|   | 600 reactions            | 10966-034 |
| Platinum <i>Taq</i> Green Hot Start DNA Polymerase                    | 200 reactions            | 11966-018 |
|   | 1,000 reactions          | 11966-034 |

## Ordering information (continued)

|   | Quantity        | Cat. No.  |
|---|-----------------|-----------|
| <b>PCR (continued)</b>  |                 |           |
| 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   |
| <b>Nucleic acid separation and analysis</b>   |                  |           |
| 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.  |
|---|-----------------|-----------|
| <b>Cloning</b>  |                 |           |
| 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 <i>E. coli</i> |                 | K240020   |
| pcDNA 6.2/V5-PL-DEST Mammalian Expression Vector                                  |                 | 12537162  |
| LR Clonase II Plus Enzyme   |                 | 12538-120 |
| One Shot TOP10 Chemically Competent <i>E. coli</i>                                |                 | C404003   |
| One Shot Stbl3 Chemically Competent <i>E. coli</i>                                |                 | 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 <i>ccdB</i> 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 <i>E. coli</i>       |          | K240020   |
| pCR 8/GW/TOPO TA Cloning Kit with One Shot TOP10 <i>E. coli</i>                         |          | K250020   |

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