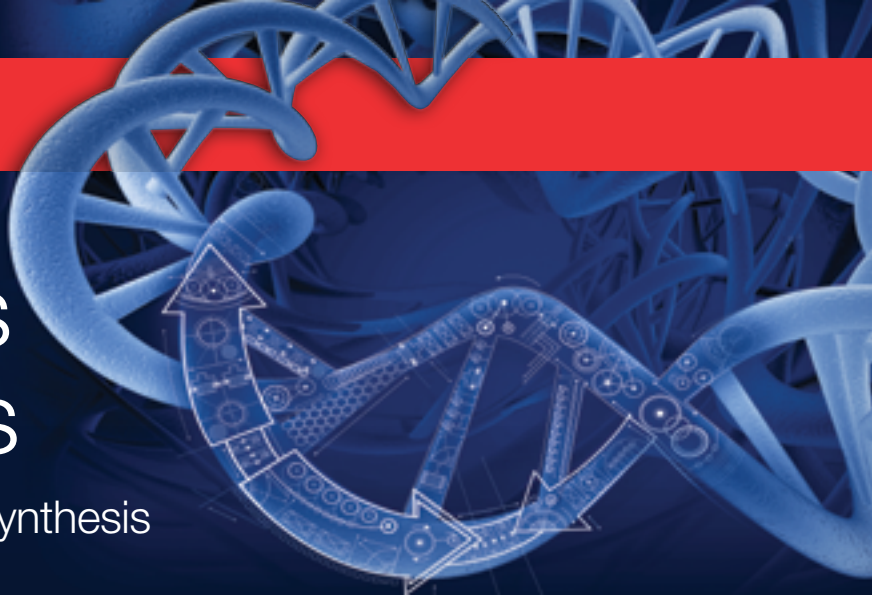
invitrogen

GeneArt Strings DNA Fragments

Fast and affordable custom gene synthesis



- Linear, dsDNA fragments up to 3kb.
- 100% pool-sequence verified.
- Optimize, obtain a quote and order online via the Invitrogen™ GeneArt™ portal.
- Ready to clone using the method of your choice.
- A variety of applications served, such as protein production, antibody engineering and CRISPR-based genome editing.
- A fast alternative to PCR-based cloning.
- An affordable alternative to complete gene synthesis.
- Also available as **Invitrogen™ GeneArt™ Strings™ DNA Libraries** containing mixed, randomized bases using the full IUPAC code of DNA nucleotides.

Introduction

Invitrogen™ GeneArt™ Strings™ DNA Fragments are custom-made synthetic double-stranded DNA fragments that are ready for cloning. They are less expensive alternatives to complete Invitrogen™ GeneArt™ Gene Synthesis, yet provide the same benefits, including design flexibility, gene optimization and quick turnaround time. We offer custom DNA fragments up to 3kb, as well as an online project portal. Ordering is easy—you can directly enter, edit, optimize and order your sequence in the GeneArt portal. You don't need a physical template; just design and order your desired sequence.

GeneArt Strings DNA Fragments can be cloned into the vector of your choice using any standard cloning method. We offer 11 categories of Strings DNA Fragments (Table 1) that are shipped within 5 to 8 business days, depending on length (Strings DNA Libraries have a longer production time). They are bulk-sequenced to guarantee that your desired sequence is represented in the DNA population that you receive. In gel analysis, well-defined bands corresponding to Strings DNA Fragments and Libraries demonstrate the robustness of the oligo assembly process (Figure 1).

Table 1. Production time and pricing for Strings DNA Fragments.

Length	Production time†	List price
150–600 bp	5	$109
601–750 bp	5	$139
751–1,000 bp	5	$159
1,001–1,250 bp	8	$239
1,251–1,500 bp	8	$279
1,501–1,750 bp	8	$319
1,751–2,000 bp	8	$369
2,001–2,250 bp	8	$419
2,251–2,500 bp	8	$479
2,501–2,750 bp	8	$529
2,751–3,000 bp	8	$579
IUPAC mixed bases	10–15	$82.58 ea

† Production time can vary depending on the sequence, and is listed in business days. After production, delivery time varies depending on location.

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Usage instructions

GeneArt Strings DNA Fragments can be used directly in many applications, such as cloning for protein or antibody expression, CRISPR-based genome engineering, or as in vitro transcription/translation and RT-PCR controls. See Table 2 for guidelines on how to use Strings DNA Fragments with various cloning methods.

Upon receipt of your GeneArt Strings DNA Fragments, the following steps are recommended:

1. Briefly centrifuge the tube before opening, as the pellet can become displaced during shipping. Strings DNA Fragments are provided dried (≥200ng; the actual amount will be printed on the label).
2. Add nuclease-free water to adjust the DNA to the desired concentration. Strings DNA Fragments are dried in 10mM Tris (pH 8.5), so water is recommended for resuspension to minimize the final salt concentration.
3. Incubate for 1 hour at room temperature or 4°C overnight (for Strings DNA Libraries, do both), then carefully resuspend by gently pipetting up and down.
4. For long-term storage, aliquot and store at –20°C. Avoid multiple freeze/thaw cycles.

For cloning and downstream protein expression, colony PCR with vector-specific primers can be performed to quickly identify full-length clones. The probability of identifying a correct clone is typically >90% if you sequence:

- 2–4 full-length clones for Strings DNA Fragments ≤1kb.
- 3–5 full-length clones for Strings DNA Fragments 1–2kb.
- 4–8 full-length clones for Strings DNA Fragments 2–3kb.

For improved success in the cloning step we recommend high-efficiency competent cells such as Invitrogen™ One Shot™ TOP10 Chemically Competent *E. coli* cells (Cat. No. C404003).

To assess cloning efficiency, the success rate of restriction enzyme cloning was evaluated on GeneArt Strings DNA Fragments (Figure 2). Different size ranges of DNA were cloned, and colonies were analyzed by colony PCR to determine cloning efficiency. Fragments with the expected size were then sequenced. A high probability of obtaining the correct clone was observed when two to four full-length clones were sequenced.

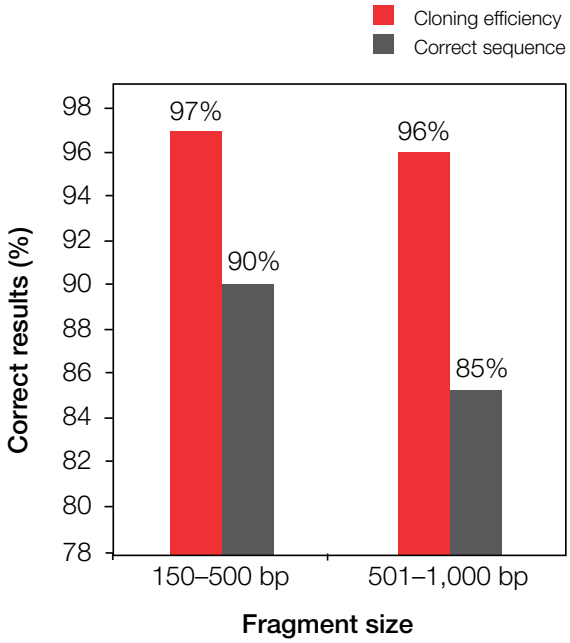

Figure 2. Cloning efficiency of GeneArt Strings DNA Fragments. The graph shows the frequency of obtaining at least one correct clone out of four analyzed colonies (for fragments 150–500 bp) or six analyzed colonies (for fragments 501–1,000 bp). The percentages of clones correct for insert size (cloning efficiency) and for insert sequence are shown.

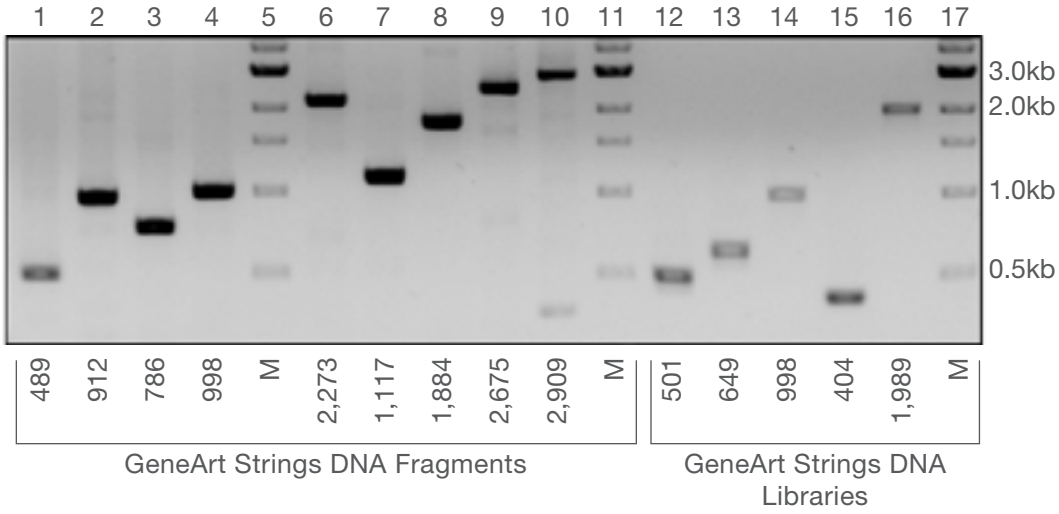

Figure 1. Strings DNA Fragments (lanes 1–4 and 6–10) and Strings DNA Libraries (lanes 12–16) after gel electrophoresis on a 1% agarose gel. DNA marker **(M)** is a 1kb DNA ladder.

Table 2. Suitable cloning systems for GeneArt Strings DNA Fragments.

Cloning system	Description/How to use with Strings DNA Fragments	Technical resources
Restriction enzymes	Cloning by restriction enzyme digestion and ligation is a simple and easy way of moving a fragment of double-stranded DNA from one plasmid to another. Strings DNA Fragments must have the restriction enzyme sites incorporated at the ends of the sequence, plus stuffer nucleotides. Continue cloning via standard restriction enzyme and DNA ligase technology.	**thermofisher.com/restriction**
GeneArt Type IIs Assembly Kits	Assembly with Type IIs restriction enzymes and DNA ligase, also known as Golden Gate cloning, can be used to assemble up to eight DNA fragments. It is highly efficient and is not based on homologous recombination. Consequently, it is less prone to unwanted rearrangements due to repetitive or homologous sequences and minimizes the need for sequence confirmation of your final construct. Strings DNA Fragments must have appropriate restriction enzyme sites incorporated at the ends (either *Aar*I, *Bsa*I or *Bbs*I, depending on the kit), plus stuffer nucleotides. For more detailed information, refer to the GeneArt Type IIs Assembly Kit manual, "Guidelines for Generating DNA Inserts" (Cat. Nos. A15916, A15917 and A15918).	**thermofisher.com/typeiis**
GeneArt Seamless Cloning and Assembly Kits	Seamless Cloning and Assembly technology is a highly efficient, vector-independent system for the simultaneous and seamless assembly of up to four DNA inserts between 100 bp and 2–10kb and any vector, totaling up to 13–40kb in length (depending on the kit). This technology relies on homologous recombination to assemble adjacent DNA inserts sharing end-terminal homology. Strings DNA Fragments must have a 15–80 bp sequence overlap with the adjacent insert (including the cloning vector), and the length of the overlap depends on the size of the insert and the total size of the final construct. For detailed information, refer to the GeneArt Seamless Cloning and Assembly Kit manual, "Preparing DNA Inserts by PCR" (Cat. No. A13288), or the GeneArt Seamless PLUS Cloning and Assembly Kit manual, "Guidelines for Generating DNA Inserts" (Cat. No. A14603).	**thermofisher.com/seamless**
Gateway technology	Gateway technology is a universal cloning method based on the site-specific recombination properties of bacteriophage lambda. It provides a rapid and highly efficient way to move DNA sequences into multiple vector systems for functional analysis and protein expression. The most typical method of Gateway vector construction is to first create an entry clone by recombining your DNA insert with a pDONR vector. To do so, Strings DNA Fragments must have *att*B sites, and then are recombined with pDONR in a recombination reaction mediated by BP Clonase enzyme. For more detailed information, refer to the Gateway Technology with Clonase II manual, "Designing *att*B PCR Primers" (Cat. Nos. 12535029 and 12535037).	**thermofisher.com/gateway**
Zero Blunt TOPO PCR Cloning Kits	Zero Blunt TOPO PCR Cloning provides a highly efficient 5-minute, one-step cloning strategy for the direct insertion of blunt-ended DNA fragments into a plasmid vector. Primers containing specific sequences are not required. Strings DNA Fragments are blunt-ended, and therefore no further manipulation is required. However, we recommend that you add an additional 5–10 nucleotides of random stuffer DNA to both ends of the DNA fragment because small terminal truncations can occur on linear DNA fragments. For more detailed information, refer to the Zero Blunt PCR Cloning Kit manual, "Clone into pCR-Blunt" (Cat. No. K270020).	**thermofisher.com/topo**
TA Cloning and TOPO TA Cloning methods	TA Cloning and TOPO TA Cloning methods provide a quick, one-step cloning strategy for the direct insertion of *Taq* polymerase–amplified PCR products (or DNA fragments with A-overhangs) into a plasmid vector. Primers containing specific sequences are not required. Strings DNA Fragments are blunt-ended, and therefore further manipulation is required to add end-terminal A-overhangs. For more detailed information, refer to the TA Cloning Kit manual, "Add 3´ A-Overhangs" (Cat. No. K200001), or the TOPO TA Cloning Kit manual, "Adding 3´ A-Overhangs Post-Amplification" (Cat. No. K450001).	**thermofisher.com/tacloning** **thermofisher.com/topo**

Visit our website at **thermofisher.com/strings** for more application examples with multiple Strings DNA Fragments and Libraries and with other cloning methods, including Invitrogen™ GeneArt™ Type IIs Assembly and Invitrogen™ GeneArt™ Seamless Cloning and Assembly kits. Note that cloning efficiency depends on the quality of the DNA and proteins used.

Design and ordering

The fastest and easiest way to edit, optimize and order GeneArt Strings DNA Fragments is online through the GeneArt portal found at **thermofisher.com/strings** (Figure 3).

Strings DNA Fragments may require certain specific sequence ends, depending on the cloning method of choice; refer to Table 2. You can adjust the 5′ and 3′ termini with the required cloning sequences through the Web portal (e.g., restriction enzyme sites for restriction enzyme/ligase cloning or GeneArt Type IIs Assembly kits, homologous sequences for GeneArt Seamless Cloning and Assembly kits or *att* sites for Invitrogen™ Gateway™ cloning). By their very nature, linear DNA fragments are not entirely free of small terminal truncations. Therefore, if blunt cloning methods (such as Invitrogen™ Zero Blunt™ TOPO™ cloning) are used, or to maintain restriction sites, we recommend that you add an additional 5–10 nucleotides of random stuffer DNA to both ends of the fragment, preserving functional DNA elements needed for downstream applications. Finally, if Invitrogen™ TA Cloning™ or TOPO™ TA Cloning™ methods are to be used, you can add A-overhangs to your Strings DNA Fragment upon receipt.

If the desired sequences have high- or low-GC content, unusual sequence complexity or long repeats, synthesis of GeneArt Strings DNA Fragments might not be possible. If this is the case, you will be informed

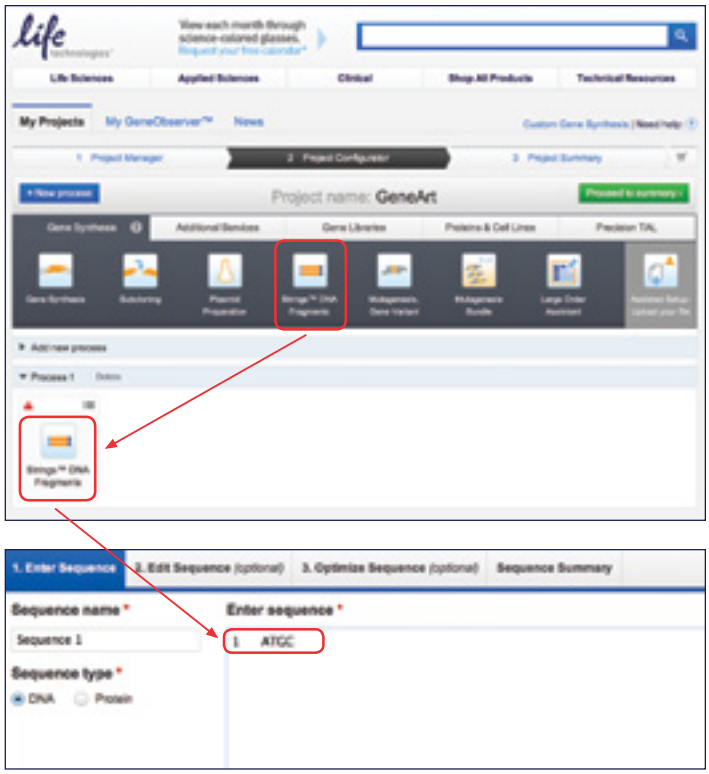

Figure 3. Screenshots of the online GeneArt portal for ordering Strings DNA Fragments.

within the GeneArt portal, and sequence optimization is recommended using the integrated Invitrogen™ GeneArt™ GeneOptimizer™ tool. After optimization is complete, you can add the sequence to the online cart for ordering. To check the status of your Web order(s) in the manufacturing process, simply use the Invitrogen™ GeneArt™ GeneObserver™ module within the GeneArt portal, available 24 hours per day.

You can also manually edit your sequence and order by email. Download the Sequence Submission Form from the website as an Excel™ spreadsheet, and send your request via email to **geneartsupport@lifetech.com** (additional handling fees apply).

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

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