Evaluation of linear synthetic DNA fragments from separate suppliers

GeneArt™ Strings™ DNA Fragments vs. gBlocks™ Gene Fragments

Introduction

Gene synthesis has become an indispensable tool and reliable source of genetic material for the research community. The process of *de novo* gene synthesis involves assembly and PCR amplification of chemically synthesized oligonucleotides. Since this chemistry is not entirely perfect, a small amount of sequence error occurs during oligonucleotide synthesis that remains through the downstream assembly process. Cloning and sequencing, therefore, is a reasonable way to screen for error-free molecules and discard those with mutations.

After screening, synthetic genes are typically delivered in a cloned and sequenced plasmid to ensure 100% sequence accuracy. However, many scientists prefer to directly obtain linear synthetic DNA fragments to perform cloning and sequencing on their own or for a variety of other applications, including direct assembly and cloning for protein expression, CRISPR-based genome editing, *in vitro* transcription and translation, or real-time PCR (Figure 1).

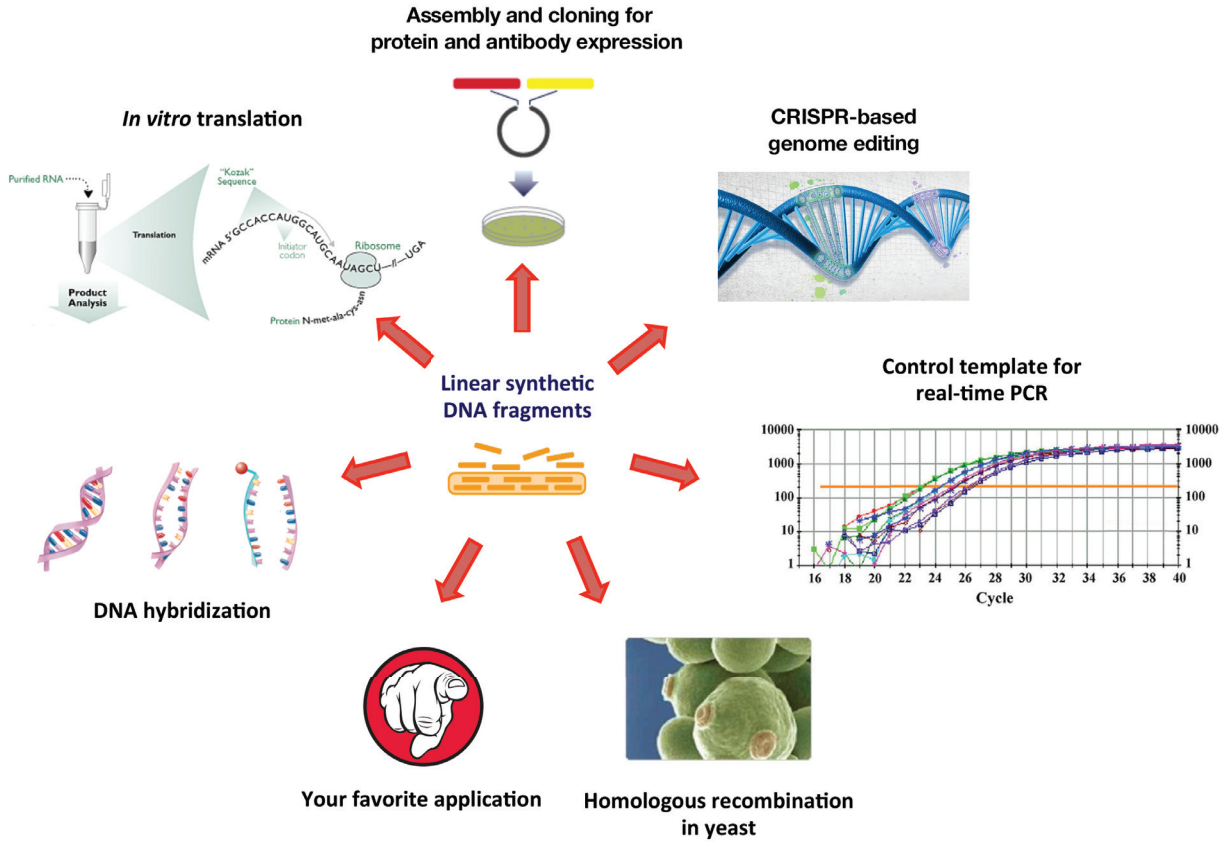

Figure 1. Possible applications for linear synthetic DNA fragments.

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GeneArt Strings DNA Fragments and gBlocks Gene Fragments

GeneArt Strings DNA Fragments were introduced in 2013 to meet this growing demand for less expensive alternatives to complete gene synthesis, while providing the same benefits of design flexibility and sequence optimization. They are uncloned, double-stranded, sequence-verified linear DNA fragments 150–3,000 bp in length, assembled from synthetic oligonucleotides using the same high-quality process developed for GeneArt™ Gene Synthesis. Since no 5´ or 3´ sequence constraints apply, GeneArt Strings DNA Fragments can be cloned using any technology to obtain a clone with no sequence errors for subsequent experimental use (see References).

In this paper, we compare the performance of GeneArt Strings DNA Fragments from Thermo Fisher Scientific to gBlocks Gene Fragments from IDT, which is another well-established synthetic DNA product. gBlocks Gene Fragments are also uncloned, double-stranded, sequence-verified linear DNA fragments, but have a more limited size range of 125–2,000 bp.

We show that GeneArt Strings DNA Fragments have better sequence accuracy than gBlocks Gene Fragments, resulting in reduced screening effort to obtain a correct clone, which can lead to time and cost savings for researchers. The low error rate of GeneArt Strings DNA Fragments is achieved with an enzymatic error-correction step during production, allowing synthesis of larger DNA fragments up to 3 kb.

Error rate analysis of linear synthetic DNA fragments

To analyze the impact and benefit of the additional enzymatic error-correction step, we performed a comparative study of GeneArt Strings DNA Fragments and gBlocks Gene Fragments.

Eleven gBlocks Gene Fragments (173–1,991 bp) were resuspended and cloned into pUC19-derived vectors using either traditional restriction and ligation cloning or homology-based cloning via the GeneArt™ Seamless Cloning and Assembly Kit (Cat. No. A13288). Clones were analyzed by colony PCR and Sanger sequencing. For restriction enzyme cloning of gene fragments, we obtained an average cloning efficiency [(colonies with full-length DNA)/(total analyzed colonies)] of 80%, and for homology-based cloning we obtained an average efficiency of 70%. For each gene fragment, 16 colonies were further analyzed by Sanger sequencing of purified full-length colony PCR product. Sequence deviations (insertions, deletions, and substitutions) were evaluated, and the average error rate of gBlocks Gene Fragments was determined to be **1 error per 1,329 bp**.

Likewise, we analyzed 24 GeneArt Strings DNA Fragments (153–2,986 bp) and determined cloning efficiencies and error rates. While efficiencies using restriction enzyme and ligation cloning or the GeneArt Seamless Cloning and Assembly Kit were comparable to the results from gBlocks Gene Fragments, the mean sequence error rate of GeneArt Strings DNA Fragments was 5x lower than gBlocks Gene Fragments, with **1 error per 6,757 bp**.

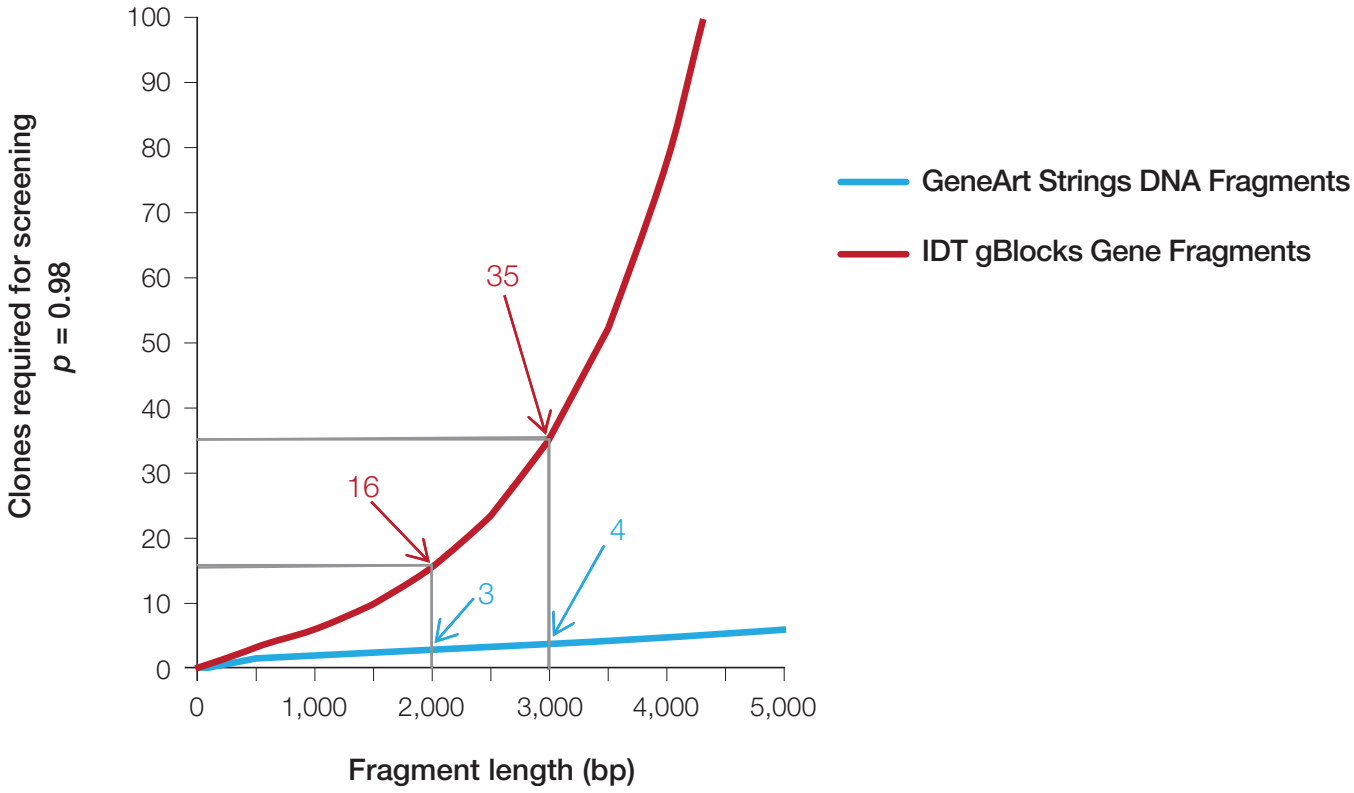

Figure 2. Error rates for gBlocks Gene Fragments vs. GeneArt Strings DNA Fragments.

Figure 2 illustrates the number of clones that need to be screened in order to find (with a 98% likelihood) at least one correct fragment when using gBlocks Gene Fragments or GeneArt Strings DNA Fragments. Since this number exponentially increases with the total size of the DNA fragment, differences in error rate become particularly substantial as fragments get longer.

Given the determined error rate of 1/1,329 bp for gBlocks Gene Fragments, 16 colonies containing a 2 kb fragment would have to be screened to find at least one correct clone. In theory, the equivalent screening effort would comprise 35 colonies for a gBlocks Gene Fragment of 3 kb, which would take considerable time and effort. (Note that gBlocks Gene Fragments are not actually available up to 3 kb.)

The substantial impact of sequence accuracy on downstream screening is apparent when doing the same calculation with GeneArt Strings DNA Fragments. With an error rate of 1/6,757 bp, the screening effort for a 2 kb fragment is reduced to only 3 colonies, and a correct 3 kb fragment can be identified by sequencing only 4 colonies. Since this trend continues beyond 3 kb, the error-correction technology also allows for direct assembly of two or more GeneArt Strings DNA Fragments to build larger constructs, which can then be screened and sequenced.

Conclusion

With their high sequence accuracy (1/5 of the error rate of gBlocks Gene Fragments), length availability of up to 3 kb, and an easy-to-use online ordering portal that includes expression optimization for various host organisms, GeneArt Strings DNA Fragments are the better choice for linear synthetic gene fragments for your research.

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