Amplifying Twist Multiplexed Gene Fragments

For Research Use Only (RUO). Not for use in diagnostic procedures.

Twist Multiplexed Gene Fragments (MGF) are diverse collections of double-stranded gene fragments synthesized using our silicon-based DNA writing technology. Our synthesis platform enables massively parallel production of hundreds of thousands of high-quality, accurate fragments per run. Multiplexed Gene Fragment sequences are available from 301-500 nucleotides and pool sizes start at 1,000 sequences with no maximum. Twist synthesizes highly accurate Multiplexed Gene Fragments with error rates of 1:2,000 nt. Sequences in the pools have excellent representation and uniformity, with over 90% of fragments present within <3x of the mean*.

*Uniformity may vary depending on sequence features. Data was collected from a pool of 10,000 gene fragments of 450 nt length with approximately 50% GC content.

QUESTIONS?
Get in touch at **sales@twistbioscience.com** or learn more at **twistbioscience.com**

CAN I (SHOULD I) AMPLIFY TWIST MULTIPLEXED GENE FRAGMENTS?

Twist uses phosphoramidite chemistry for synthesis. Compared to conventional column-based synthesizers, our synthesis platform miniaturizes the synthesis process, which reduces reaction volumes and increases throughput. PCR amplification on the initial synthesis material is very challenging; to mitigate the risk of poor amplification results, Twist is performing this step for you. If you need more DNA mass for cloning, are amplifying out subpools, or are adding a tailed primer to this pool, it is possible to do further amplification on the provided fragments. However, please note that there is an inherent risk in performing further amplification. Overamplifcation may introduce various undesired effects into the pool such as skewed uniformity, high % chimera molecules, or potential truncation of molecules containing repeats. While the following guidelines will help mitigate these risks, every pool will behave differently.

WHAT DO I NEED TO KNOW ABOUT AMPLIFYING TWIST MULTIPLEXED GENE FRAGMENTS?

Two factors, **template input mass** and **the number of amplification cycles**, are important for effective pool amplification to achieve the minimal byproduct introduction while maintaining high uniformity. Overamplification caused by excessive template mass or high number of PCR cycles may lead to undesired molecules (e.g. chimera, truncation), skewed uniformity, or increasing errors from the polymerase. To avoid overamplification, input template should not exceed 0.5 ng for the PCR reaction, and the number of amplification cycles should be minimized to fewer than 8. If a certain target mass is needed, it is recommended to set up multiple PCR replicates following these guidelines to reach the target yield.

Another important factor is choice of polymerase. A high-fidelity polymerase with minimal amplification bias is needed to maintain the quality of the MGF pool. Twist has evaluated a variety of high-fidelity polymerases, and we recommend using KAPA® HiFi HotStart Polymerase (Roche, Catalog #KK2502) and its associated PCR Protocol.

RECOMMENDED PCR AMPLIFICATION PROTOCOL

The protocol below offers a starting point for PCR amplification. Twist Multiplexed Gene Fragments are delivered as a lyophilized product pooled in a single tube. Total yield in ng is printed on the tube label.

1 Prepare a stock solution of your Multiplexed Gene Fragments by resuspending in 10 mM Tris buffer, pH 8.0 to any concentration. A final concentration of 0.5 ng/µl is needed for the PCR Reaction. Stock solution concentration (ng/µl) = Total yield (ng) / resuspension volume (µl)

2 Use the KAPA HiFi HotStart PCR Kit (Catalog #KK2502) to perform PCR.

PCR REACTION COMPONENTS

COMPONENT	FINAL CONCENTRATION	PER 25 µL REACTION
2X KAPA HiFi HotStart ReadyMix (contains dNTP and Polymerase)	1x	12.5 µl
10 µM Forward Primer	0.3 µM	0.5 µl
10 µM Reverse primer	0.3 µM	0.5 µl
Twist Multiplexed Gene Fragments (template input should be no more than 0.5 ng)	0.5 ng/µl	1.0 µl
PCR grade water	—	10.5 µl

PCR REACTION CONDITIONS

	CYCLING STEP	TEMPERATURE	DURATION
1	Initialization Denaturation	2 min at 95°C	1x
2	Denaturation	20 sec at 98°C	8 Cycles
3	Annealing	15 sec at 60°C or optimum temperature*	
4	Extension	1 min at 72°C	
5	Final Extension	2 min at 72°C	1x
6	Hold	Hold at 4°C	1x

**Annealing temperature depends on primer sequences and must be optimized accordingly.*

3 Purify the PCR reactions with SPRI magnetic beads using a low bead-to-DNA ratio (0.8X).

QUALITY ANALYSIS AND TROUBLESHOOTING

1. I tried amplifying the pool using the recommended guidelines and my pool did not amplify.

A. In addition to following the amplification conditions provided, please also follow the recommended primer design guidelines according to our Multiplexed Gene Fragments Design Guide. Note that using tailed primers may also reduce primer binding, resulting in low yield of the desired amplicon and off-target priming events especially when the tail is long.

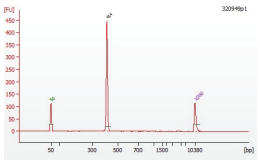
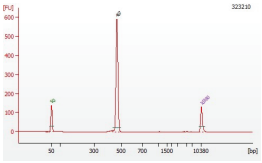
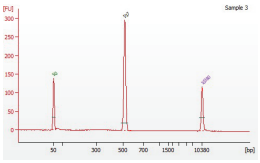
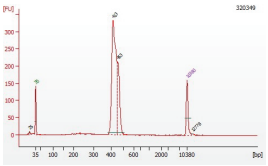
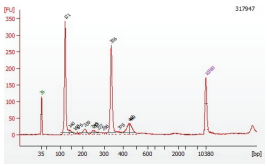
2. After amplification of the pool, my sequencing demonstrated a skew in uniformity greater than what was expected.

A. Reducing the number of cycle counts will help keep the uniformity within the expected specification of 95/5 < 3. Note that pools with length variation >10% and with wide GC-distribution will typically have a wider distribution of fragment frequency.

3. After amplification of the pool, I noticed a truncated population after running a gel.

A. Even with optimal amplification conditions, there will be cases when the sequences contain direct repeats, which can lead to truncated amplificons. Our amplification protocol has been optimized to minimize such outcomes. However, to further remove undesired truncated molecules, lower SPRI bead-to-DNA ratio (0.6x) can be helpful.

When analyzed by capillary electrophoresis [for example, with an Agilent® 2100 Bioanalyzer® (Agilent Technologies®, Waldbronn, Germany)], an optimized PCR-amplified pool yields a strong band/ peak at the correct size. The following Multiplexed Gene Fragment pools were amplified with the protocol above, and quality was assayed with an Agilent Bioanalyzer DNA HS chip.

#	FRAGMENT LENGTH	ELECTROPHEROGRAM IMAGE	INTERPRETATION	TROUBLESHOOTING
1	400 bp		A clean peak at the expected size indicates effective fragment pool amplification	—
2	450 bp			
3	500 bp			
4	410 bp - 450 bp		A broader peak within the expected size range indicates effective and unbiased pool amplification	—
5	334 bp		Multiple peaks including truncation due to repeats and heteroduplex molecules due to overamplification	Repeat PCR with fewer cycles and higher annealing temperature