Amplifying Twist Multiplexed Gene Fragments

For Research Use Onl (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

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

RECOMMENDED PCR AMPLIFICATION PROTOCOL

The protocol below o□ers a starting point for PCR amplification. Twist Multiplexed Gene Fragments are deliv8d Gs wd GvS

1 Prepare a stock solution of your Multiplexed Gene Fragments by resuspending in 10 mM Tris bu□er, 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.