TWIST TIPS

AMPLIFYING TWIST OLIGO POOLS

Twist Oligo Pools are diverse collections of single-stranded oligonucleotides synthesized using our silicon-based DNA writing technology. Our synthesis platform enables massively parallel production of hundreds of thousands of high-quality, accurate oligos per run. Oligo sequences are available from 20–300 nucleotides and pool sizes start at 2,000 sequences with no maximum. Twist synthesizes highly accurate Oligo Pools with error rates of 1:2,000 nt. Sequences in the pools have excellent representation and uniformity, with over 90% of oligos present within <2.5x of the mean.

WHY DO I NEED TO AMPLIFY TWIST OLIGO POOLS?

Twist uses phosphoramidite chemistry for oligo synthesis. Compared to conventional column-based synthesizers, our synthesis platform miniaturizes the synthesis process, which reduces reaction volumes and increases throughput. Because the resulting oligos are single-stranded and present at femtomole levels, they may need to be converted to double-stranded DNA or amplified for certain applications via PCR. PCR amplification also enriches the amounts of full-length oligo sequences. **Therefore, we recommend including primer-binding sites in your oligo design and performing PCR amplification of the pool prior to usage.**

WHAT DO I NEED TO KNOW ABOUT AMPLIFYING TWIST OLIGO POOLS?

Factors such as **PCR primer specificity** and the **number of amplification cycles** are important for effective oligo pool amplification. Overamplification caused by factors such as GC content, regions of small homology, and non-specific primer binding, introduces bias, wherein particular sequences are preferentially amplified over others. To avoid overamplification, use a high amount of input template (~10 ng) for PCR and minimize the number of amplification cycles. Refer to the table below for recommended PCR cycles for different oligo pool lengths.

Recommended Number of PCR Cycles

OLIGO POOL LENGTH	# PCR CYCLES
20–100 nt	6–10 cycles
100–150 nt	10–12 cycles
151–300 nt	12–14 cyles

Another important factor is **choice of polymerase**. A high-fidelity polymerase with minimal amplification bias is needed to maintain the quality of the oligo 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 Oligo Pools are delivered as a lyophilized product pooled in a single tube. Total yield in ng is printed on the shipping tube label.

1. Prepare a stock solution of your Oligo Pool by resuspending in 10 mM Tris buffer, pH 8.0 to a concentration of at least 20 ng/µl.

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
5x KAPA HiFi Fidelity Buffer	1x	5.0 µl
10 mM each dNTP Mix	0.3 mM each dNTP	0.75 µl
10 µM Forward Primer	0.3 µM	0.75 µl
10 µM Reverse primer	0.3 µM	0.75 µl
Twist Oligo Pool (20 ng/µl)	0.4 ng/µl	0.5 µl
KAPA HiFi HotStart DNA Polymerase (1 U/µl)	0.5 U/reaction	0.5 µl
PCR grade water	—	Fill to 25 µl

PCR Reaction Conditions

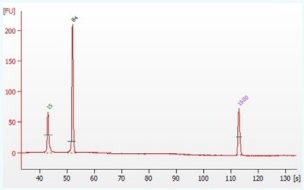
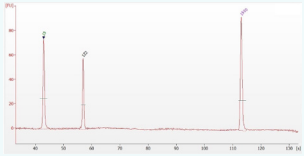
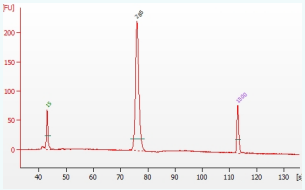
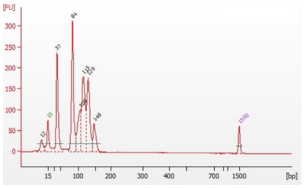
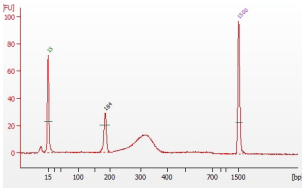
	CYCLING STEP	TEMPERATURE	DURATION
1	Initialization Denaturation	3 min at 95°C	1x
2	Denaturation	20 sec at 98°C	6–12 Cycles**
3	Annealing	15 sec at optimum temperature*	
4	Extension	15 sec at 72°C	
5	Final Extension	1 min at 72°C	1x

** Annealing temperature depends on primer sequences and must be optimized accordingly.*
*** PCR cycles can vary, depending on the length of oligos; see chart above for recommendations.*

3. Purify the PCR reactions with spin column-based purification kits. If preferred, pools of oligos >120 nt can be purified with SPRI® magnetic beads using a high bead-to-DNA ratio (1.8x).

QUALITY ANALYSIS AND TROUBLESHOOTING

When analyzed by capillary electrophoresis [for example, with an Agilent® 2100 Bioanalyzer® (Agilent Technologies®, Waldbronn, Germany)], an optimized PCR-amplified oligo pool yields a strong band/peak at the correct size. The following oligo pools were amplified with the protocol above, and quality was assayed with an Agilent Bioanalyzer DNA 1000 chip.

#	OLIGO POOL LENGTH	ELECTROPHEROGRAM IMAGE	INTERPRETATION	TROUBLESHOOTING
1	80 nt		A clean peak at the expected size indicates effective oligo pool amplification	—
2	115 nt			
3	281 nt			
4	142 nt		Multiple side peaks indicate non-specific amplification	Repeat PCR with higher annealing temperature to increase specificity, or re-design PCR primers
5	187 nt		The presence of a hump after the peak of interest indicates heteroduplexes, a result of over-amplification.	Re-try PCR with lower number of cycles