Twist Oligo Pools Amplification Protocol

Twist Oligo Pools are diverse collections of single-stranded oligonucleotides synthesized using a silicon-based DNA writing technology. The synthesis platform enables massively parallel production of hundreds of thousands of high-quality, accurate oligos per run. Oligo sequences are available from 20–350 nucleotides and pool sizes start at 2,000 sequences with no maximum. Twist synthesizes highly accurate Oligo Pools with error rates of 1:2,500 nt. Sequences in the pools have excellent representation and uniformity, with over 90% of oligos present within <2.5x of the mean.

PROTOCOL COMPONENTS

Please read the product packaging and storage recommendations carefully and store components as recommended immediately upon arrival.

NAME	DESCRIPTION	STORAGE
Twist Oligo Pool	Highly diverse collections of single-stranded oligonucleotides	4°C for 12 months. -20°C for 24 months. -80°C for long-term storage.

For Research Use Only. *Not intended for use in diagnostic procedures.*

LEGAL

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MATERIALS SUPPLIED BY USER

The following materials or their equivalent are required to generate libraries using the Twist Oligo Pools Amplification Workflow.

PRODUCT	SUGGESTED SUPPLIER
REAGENTS AND CONSUMABLES	
Molecular biology grade water	—
Forward primer	—
Reverse primer	—
Twist TrueAmp Polymerase Mix (See catalog numbers below this table)	Twist Bioscience
DNA purification beads	Omega Bio-tek (M1378-02)
Ethanol (optional; only if using purification beads)	—
Qubit dsDNA Broad Range Quantification Assay	Thermo Fisher Scientific
Agilent Bioanalyzer DNA 1000 Kit	Agilent Technologies
EQUIPMENT	
Pipettes and tips	—
Vortex mixer	—
Benchtop mini centrifuge for 0.2-ml tubes	—
Thermal cycler (96-well) with heated lid	—
Fluorometer (Qubit 3.0)	Thermo Fisher Scientific
2100 Bioanalyzer	Agilent Technologies

Use the following catalog numbers to order the Twist TrueAmp Polymerase Mix:

116471: Twist TrueAmp Polymerase Mix, 16 reactions
116472: Twist TrueAmp Polymerase Mix, 96 reactions

GENERAL NOTES AND PRECAUTIONS

Wear appropriate protective equipment (lab coat, gloves, and protective glasses or goggles) at all times when performing this protocol.

For best results, read this document before performing the protocol and follow the instructions provided. Twist cannot guarantee the performance of the Twist Oligo Pools Amplification Protocol if modifications are made to the protocol.

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, in which 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 - 350 nt	12 - 14 cycles

Another important factor is the 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 the Twist TrueAmp Polymerase Mix.

For any troubleshooting or other technical support, please first refer to Appendix A and B.

FOR ADDITIONAL TECHNICAL SUPPORT, CONTACT CUSTOMERSUPPORT@TWISTBIOSCIENCE.COM.

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. The total yield in ng is printed on the shipping tube label.

Reagents Required

- Input DNA template
- Twist TrueAmp Polymerase Mix
- Forward primer
- Reverse primer
- Molecular biology grade water
- DNA Purification Beads (OMEGA BIOTEK M1378-02 or equivalent)

Before You Begin

- Thaw all reagents completely and vortex to ensure complete mixing.
- 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).

AMPLIFICATION

1

Program a thermal cycler with the following conditions. Set the temperature of the heated lid to 105°C.

	STEP	TEMPERATURE	TIME	NUMBER CYCLES
1	Initialization	98°C	45 seconds	1
2	Denaturation	98°C	15 seconds	*Variable depending on nucleotide length. See General Notes and Precautions section and the table below.*
	Annealing	60°C	15 seconds	
	Extension	68°C	45 seconds	
3	Final Extension	68°C	1 minute	1
4	Final Hold	4°C	HOLD	–

OLIGO POOL LENGTH	# PCR CYCLES
20 - 100 nt	6 - 10 cycles
100 - 150 nt	10 - 12 cycles
151 - 350 nt	12 - 14 cycles

2 Calculate the number of reactions required and prepare an amplification mix as indicated below.

COMPONENT	FINAL CONCENTRATION	PER 50 µl REACTION
Twist TrueAmp Polymerase Mix	1x	25 µl
Forward primer (10 µM)	0.3 µM	1.5 µl
Reverse primer (10 µM)	0.3 µM	1.5 µl
Oligo pool (20 ng/µl)	20 ng	1 µl
Water	–	21 µl
Total	–	**50 µl**

3 Mix the components well by gentle pipetting and then pulse-spin for 2 seconds. Immediately place the reactions in the thermal cycler. Start the program.

4 Once the program is complete, purify the PCR reactions with DNA purification beads (OMEGA BIOTEK M1378-02 or equivalent).
NOTE: If preferred, pools of oligos >120 nt can be purified with DNA purification beads using a high bead-to-DNA ratio (1.8x for 120-300 nt and 1.2x for 300-500 nt).

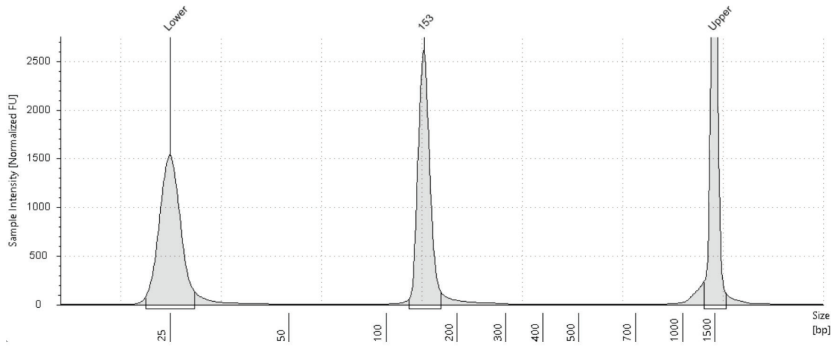
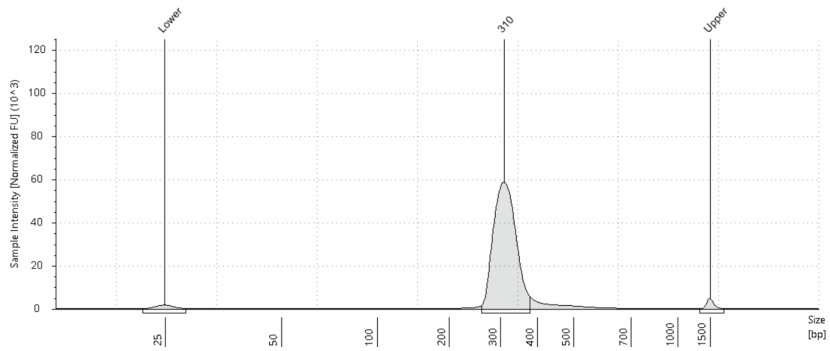
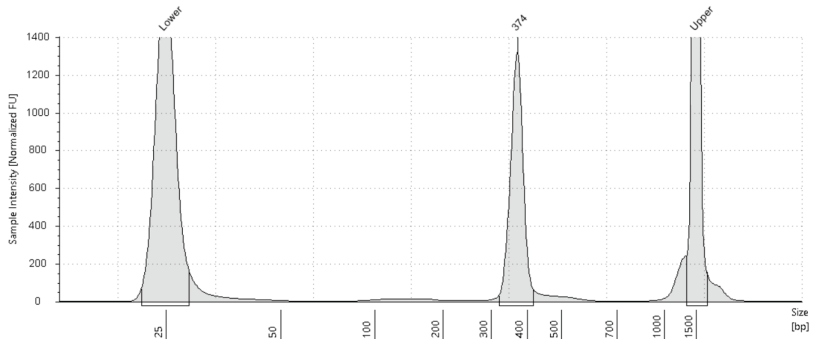
5 Quantify and validate the size of the pool with an Agilent Bioanalyzer DNA 1000 chip. Please refer to Appendix A for details on expected results.

END OF WORKFLOW

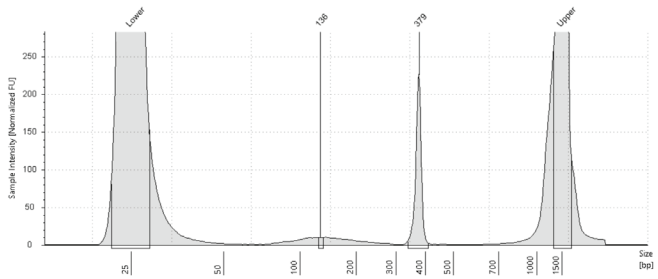
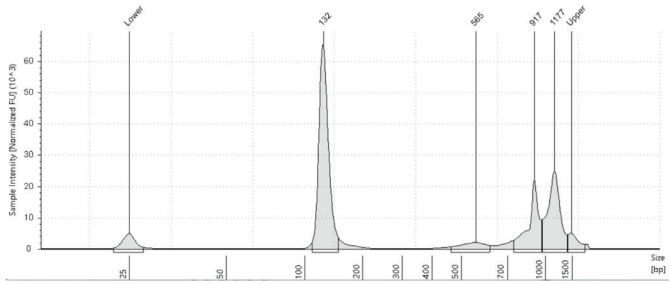
APPENDIX A: QUALITY ANALYSIS AND TROUBLESHOOTING

When analyzed by capillary electrophoresis (e.g., 4200 TapeStation), an optimized PCR-amplified oligo pool yields a strong band/peak at the expected size. The following oligo pools were amplified with the protocol above, and quality was assayed with an Agilent Bioanalyzer DNA 1000 chip.

EXAMPLES

#	OLIGO POOL LENGTH	ELECTROPHEROGRAM IMAGE	INTERPRETATION
1	150 nt		A clean peak at the expected size indicates effective oligo pool amplification
2	300 nt		
3	350 nt		

TROUBLESHOOTING

#	OLIGO POOL LENGTH	ELECTROPHEROGRAM IMAGE	INTERPRETATION	TROUBLESHOOTING
1	350 nt		Target peak is weak and some by-product in the middle.	Repeat PCR with higher annealing temperature to increase specificity, or re-design PCR primers.
2	150 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.

APPENDIX B: FREQUENTLY ASKED QUESTIONS

1. FOR THE INPUT OLIGO POOL AMOUNT, DO WE HAVE TO ADD 20 NG PER 50 µL OF REACTION?

No, 20 ng is the optimized number to secure good uniformity and a low dropout rate. If necessary, using 1 amol of each oligo would still give good uniformity and low dropout rate.

2. WHAT IS THE RELATIONSHIP BETWEEN UNIFORMITY AND THE PCR CYCLE NUMBER?

More PCR cycles would lead to worse uniformity. Keeping PCR cycles in the ranges outlined in the General Notes and Precautions section is recommended.

3. COULD WE USE OTHER POLYMERASES?

Yes, other polymerases could also amplify the oligo pool. Since the Twist TrueAmp polymerase is optimized for oligo pools, there may be worse uniformity and less yield with the same PCR cycle numbers when comparing with alternative polymerases.

4. DO I NEED TO CARE ABOUT GC% WHEN ORDERING OLIGO POOLS?

Yes, PCR amplification efficiency has a strong correlation with the GC%. The higher the GC%, the less efficient PCR amplification is in the same oligo pool. We recommend keeping the GC% in the range 35% and 65% to avoid bad uniformity and high dropout.

END OF APPENDIX