GenTitan™ Oligo Pool User Guide and PCR Amplification Guidelines

GenScript's GenTitan™ Oligo Pool leverages our high-density Complementary Metal Oxide Semiconductor (CMOS) technology to achieve unparalleled precision, uniform distribution, and minimal batch-to-batch variability. This technology significantly enhances the capability to synthesize numerous individual sequences simultaneously. However, since each synthesis site measures only in microns with the yield from each location amounts to the femtomoles level, it is recommended that users design the oligo pool with common priming sequences for PCR amplification prior to usage.

Note: GenTitan™ Oligo pools are purified before shipment. No extra purification process is needed, unless for specific purposes.

Storage of GenTitan™ Oligo Pools:
GenScript's oligo pools are suspended in TE buffer and ready to use. For long term storage, it is recommended to store at -20°C under limited freeze-thaw cycles.

PCR Amplification Guide of GenTitan™ Oligo Pools

To PCR amplify GenTitan™ oligo pools, **include primer binding sites in your oligo design for successful pool amplification.** Priming regions of **20-30 bases** are commonly used.

GenScript has compared multiple DNA Polymerases, each with differing fidelities and processivities and found that KAPA HiFi DNA polymerase (Roche) performance provides robust yields of high-quality amplified oligo pools. This guideline explains the two-round PCR (PCR1 and PCR2) protocol using the KAPA HiFi PCR kit yielding ~500ng-1µg of high-purity, full-length double-stranded DNA (dsDNA). To achieve higher PCR product yield from your oligo pool, minimize potential amplification artifacts, and increase oligo pool diversity, we recommend performing multiple PCR amplifications of the oligonucleotide pool.

Disclaimer/Note: *This PCR guideline uses oligo pools ranging from 80-170 bases, flanking the design with a 20-base priming region. Yields may vary based on primer design, length of priming region, DNA polymerase, and DNA purification method. See* ***Technical Note: Oligo Amplification Priming Design for more details (bottom of the document).***

Round-1 PCR (PCR1): Enrichment PCR

1. Prepare PCR1 Reaction Mixture for each oligo pool you wish to amplify.

KAPA HiFi Recipe	Volume per sample (µl)	Final Concentration
5x Polymerase Buffer	5 µl	1x
HiFi Polymerase	0.5 µl	1 U
10mM dNTP	0.75 µl	300 µM
10µM Forward Primer	0.75 µl	300 nM
10µM Reverse Primer	0.75 µl	300 nM
Oligo Pool Template	x	See 2. below
Molecular-grade H2O	Fill to 25 µl	-

2. Oligo pool template input is dependent on the GenScript synthesis chip type.

Chip Type	Oligo Pool Input into PCR1 reaction*
12k chip	10 ng
GenTitan™ (92k Chip)	10 ng
GenTitan™ HD Chip	50 ng

** Oligo pool input has been optimized for maximal PCR yield and purity.*

3. PCR cycling conditions:

Cover Temperature: 105°C			
Step	Temperature	Reaction time	Cycle **
Pre-denature	98 °C	30 sec	
Denature	98 °C	10 sec	Pools between 80 – 120 bases: **10-13x**
Annealing	Tm of design*	10 sec	Pools between 120 – 140 bases: **14-16x**
Extension	72 °C	15 sec	Pools between 140 – 170 bases: **16x-18x**
Final extension	72 °C	5 min	
Storage	4 °C	Hold	

** The ideal primer Tm is between 55°C – 70°C. KAPA HiFi PCR conditions typically raise the annealing temperature by 5-8°C from typical primer Tm calculations. See **Technical Note: Oligo Amplification Priming Design (bottom of document).***

*** When amplifying a sub-pool within the oligo pool, PCR cycling conditions may require adjusting based on pool stoichiometry and primer length. See **Technical Note: PCR Cycle Adjustment for sub pool Amplification (bottom of document).***

4. Post PCR verification (Optional): Perform agarose gel electrophoresis by loading 1-2ul of the PCR1 product. Confirm the expected product size before purification. PCR1 products may be faint but should be present with no smears.
5. Perform DNA purification, and elute PCR1 in 20µl of Molecular-grade H2O or Low TE buffer (10mM Tris/0.1mM-EDTA).
6. Quantify the purified DNA using a fluorescent-based assay (e.g., Qubit HS dsDNA Assay/Quant-IT (Invitrogen)) as yields may be below the 10 ng range (<0.5ng/µl).

Round-2 PCR (PCR2): PCR1 Amplification

1. Prepare PCR2 Reaction Mixture for each sample. Perform multiple reactions to increase yield and minimize potential amplification artifacts.

KAPA HiFi Recipe	Volume per sample (µl)	Final Concentration
5x Polymerase Buffer	10 µl	1x
HiFi Polymerase	1 µl	1U
10mM dNTP	1.5 µl	300µM
10µM Forward Primer	1.5 µl	300nM
10µM Reverse Primer	1.5 µl	300nM
PCR 1 Product (2ng) *	x	2 ng
Molecular-grade H2O	Fill to 50 µl	-

Based on the quantification of the PCR 1 product, load **2ng of template for each 50µl PCR reaction.*

2. PCR2 cycling conditions.

Cover Temperature: 105 °C			
Step	Temperature	Reaction time	Cycle
Pre-denature	98 °C	30 sec	
Denature	98 °C	10 sec	9x
Annealing	Tm of design*	10 sec	
Extension	72 °C	15 sec	
Final extension	72 °C	5 min	
Storage	4 °C	Hold	

** The ideal primer Tm is between 55°C – 70°C. KAPA HiFi PCR conditions typically raise the annealing temperature by 5-8°C from typical primer Tm calculations. See **Technical Note: Oligo Amplification Priming Design (bottom of document).***

3. Post PCR verification (Optional): Perform agarose gel electrophoresis by loading 1-2ul of the PCR2 product. Confirm the expected product size before purification.
4. Perform DNA purification, and elute PCR2 in 20µl of Molecular-grade H2O or Low TE (10mM Tris/ 0.1mM-EDTA).
5. Quantify the purified DNA with the Qubit HS dsDNA Assay/Quant-IT (Invitrogen) or Nanodrop.

High-yield, full-length double-stranded DNA (dsDNA) of the oligonucleotide pool is now ready for downstream applications. In Figure 1, three oligo pools synthesized at lengths of 120, 140, and 160 bases followed the PCR1 and PCR2 processes outlined above. DNA recovery was performed via solid-phase reversible immobilization (SPRI) with paramagnetic particles of the PCR2 product, recovering distinct products at expected sizes.

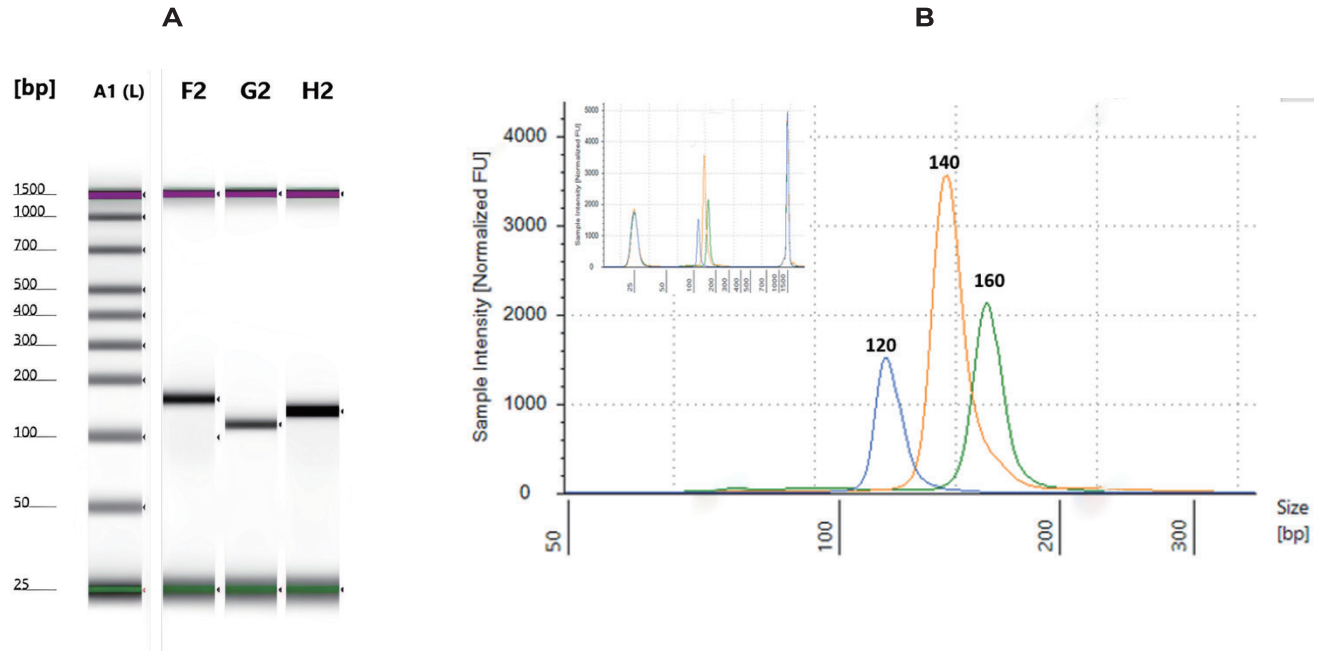

Figure 1. Visualization of post-PCR amplification of three unique PCR2 products from oligonucleotide pools of 120, 140, and 160 bases using the Agilent TapeStation System (DNA High-Sensitivity (HS) D1000 ScreenTape). All three pools were synthesized independently on GenTitan (92k) chips. A. Electronic gel electrophoresis image: Lane 1 -- HS D1000 DNA Ladder, Lane F2 – 160bp PCR amplification products, Lane G2 – 120bp PCR amplification product, Lane H2 – 140bp PCR amplification product. B. Overlays of 120bp PCR2 product (blue curve), 140bp PCR2 product (yellow curve), and 160bp PCR2 product (green curve).

Technical Notes

Here, we highlight tips and tricks to help optimize oligo pool designs and PCR amplification conditions maximizing yield while maintaining quality, purity, and diversity for downstream applications.

Oligo Amplification Priming Design:

Recommended primer binding site lengths are 20-35 bases, and we do not recommend lengths <18 bp. General primer design rules apply as highlighted below:

1. Avoid consecutive G in the first 5 bp of the primer.
2. Avoid high ΔG for primer dimers and possible secondary structures
   a. We suggest using Mfold algorithms to check for ΔG and possible secondary structure of the primers: http://www.unafold.org/mfold/applications/dna-folding-form.php
   b. Another useful tool is Primer3 – a widely used program for designing PCR primers as well as checking for secondary primer interactions: https://primer3.org
3. BLAST or BLAT primers against your reference design for specificity.
4. The primers should have a melting temperature (Tm) that matches the annealing temperature of the PCR, generally between 55°C-70°C.

Depending on the length of your priming region, PCR cycling efficiency may also vary. We have observed that longer primers tend to have much higher PCR yield. In general, if primers are >30 bases, PCR cycles decreases as shown in table below.

Pool	Synthesis Length (bases)	Priming Region (bases)	Recommended modification to PCR1 Cycles
Pool 1	130 bp	33 bp	-2x
Pool 2	170 bp	40 bp	-2x
Pool 3	80 bp	20 bp	Same

PCR Cycle Adjustment for Sub pool Amplification

PCR cycles recommended in this protocol are based on pools designed with one universal Amplification Priming Site (APS). Sub pools refer to Oligo pools with greater than one APS. As a guideline, when amplifying sub pools, adjust the PCR cycles recommended by the percentage of the specific sub pool within the Oligo Pool:

Pool	Oligo Count	Oligo Stoichiometry	Recommended modification to PCR1 Cycles recommendation (Section A3).
Sub Pool 1 (Primer A)	4,500	42%	+ 1 cycle
Sub Pool 2 (Primer B)	1,200	11%	+ 3-4 cycles
Sub Pool 3 (Primer C)	5,000	47%	+ 1 cycle
Total	**10,700**	**100%**	-

Agarose gel electrophoresis of PCR1 (prior to purification) as a qualitative confirmation is recommended.

Additional PCR adjustments

Helpful procedural modifications and tweaks to address differences in reagents, instrumentation, and workflows include:

1. PCR cycle count - If the option is available, we recommend a fluorescent-based endpoint PCR (SyBR Green) using a real-time PCR instrument (RT-PCR) with available PCR1 and/or PCR2 reagents. This strategy determines the Cycle Threshold (Ct) value for the specific reagents used in the reaction and is a good surrogate to determine PCR cycles required for amplification.
2. Annealing Temperature (Ta) – If using KAPA HiFi PCR conditions, optimal annealing temperature tends to be 5-8°C higher than the typical primer Tm calculations. Target the highest Ta permissible can help minimize non-specific artifacts. If available, performing RT-PCR will also be advantageous for further Ta optimization.