

## Oligo Pool User Manual

Oligo pools are a collection of thousands of oligonucleotides that are simultaneously synthesized on GenScript's advanced semiconductor-based oligo synthesis platform. They can be used for a variety of downstream applications, including construction of libraries and high-throughput screening experiments.

The yield of each oligo within an oligo pool is low, typically at the femtomole (fmol) level. Unless otherwise specified, PCR amplification is recommended to increase total amount and remove truncated oligonucleotides. Downstream cloning and assembly can be performed with the amplified products.

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

### Storage of 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 of Oligo Pools

1. High-fidelity polymerases such as Phusion, Q5, KAPA, are recommended for PCR amplification. Taq polymerase is not recommended for use in amplification of oligo pools. Please follow the recommendations of the polymerase supplier for optimal PCR amplification conditions. A typical PCR reaction procedure for amplification is as follows:

Step	Cycles	Temperature (°C)	Time
Initial Denaturation	1	98	30 s
Denaturation		98	15 s
Annealing	20-30	55-72	30 s
Extension		72	30 s
Final extension	1	72	5 min
Hold	1	4	Hold

2. The concentration of an oligo pool is generally ng/μl. For PCR amplification, we recommend adding 5-100 ng of template. The amount of template added is related to the length and number of oligo sequences, and the number of sub-pools. It is recommended to increase the amount of template and PCR cycles in order to amplify more sub-pools per reaction.

3. The PCR amplification conditions need to be matched with the specific downstream applications. It is recommended to carry out pilot experiments to determine the optimal reaction conditions. For example:

- Determine optimal PCR conditions by altering different parameters, such as template amount and cycle number.
- For applications that require higher quality PCR products, it is recommended to increase the amount of template added and reduce the number of PCR cycles.

4. PCR products can be cloned into target vectors for library construction.