Agilent SurePrint Oligo Pools Amplification Guidelines

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

Please note that SurePrint Oligo Pools are custom research tools with a wide variety of applications. As such, Agilent does not provide specific protocols for the product.

Materials and storage

Oligo pool yield 10 pmol of linear, unamplified, pooled oligonucleotides

Resuspension buffer TE, pH 8

Oligo pool storage Store the oligo pool at –20°C. The oligonucleotides (lyophilized or resuspended) are stable for at least 24 months when stored at –20°C. Long term storage at –80°C is also appropriate. Agilent recommends resuspending the oligo pool prior to storage. If you anticipate needing to repeatedly amplify the oligo pool, aliquot the resuspended oligo pool into working volumes prior to storage to avoid multiple freeze/thaw cycles.

Resuspension of the dried oligo pool

Agilent recommends resuspending the dried oligo pool in 100 µL of TE buffer using the instructions below.

1. Briefly spin down the dried oligo pool in a benchtop microcentrifuge.
2. Carefully add 100 µL of TE buffer.
3. Vortex the solution for 5 to 10 seconds followed by another brief spin in the microcentrifuge.
4. Incubate the oligo pool at room temperature for 15 minutes.
5. Repeat **step 3** through **step 4** an additional 4 more times over a period of an hour to ensure complete resuspension of the dried oligonucleotides.
6. Spin down the final resuspended solution prior to use.

Following resuspension, quantify the total DNA using a fluorescent dye-based quantification method, such as the Qubit ssDNA Assay Kit (Thermo Fisher Scientific p/n Q10212).

Agilent does not recommend using a spectrophotometric measurement at 260 nm to quantify resuspended, unamplified oligo pools.

Amplification guidelines

Most applications require PCR amplification of the oligo pool for use in downstream applications.

Optimization of the PCR protocol is needed to retain oligo pool quality and ensure high-quality amplicons. When optimizing PCR conditions, consider the following guidelines.

- Agilent recommends using higher amounts of oligo pool template (200 pM) while minimizing the total number of amplification cycles (≤15 cycles). These conditions help ensure adequate representation of all the sequences in the pool.
- Over-amplification can lead to high molecular weight artifacts. If you observe this issue, try reducing the number of cycles in the PCR cycling protocol and/or the concentration of the oligo pool in the PCR reaction.

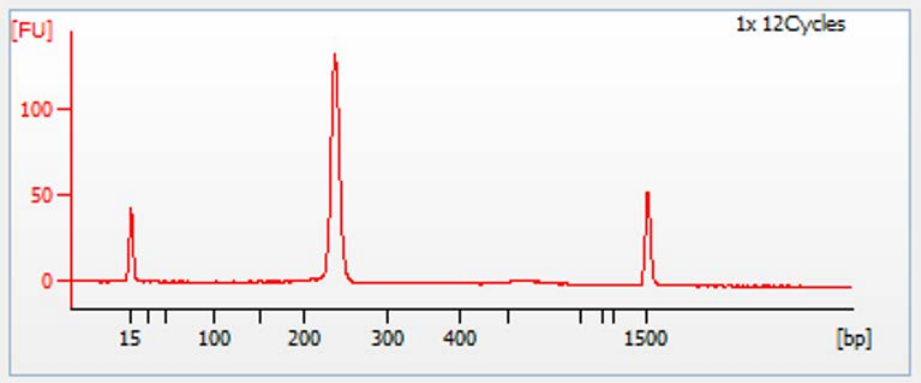

Figure 1. DNA 1000 BioAnalyzer electropherogram of the PCR product from a 230mer oligo pool with optimal PCR conditions. The DNA 1000 assay produced a single 230bp amplicon peak.

- Keep the final volume of the PCR reaction at 50 µl or less, and increase the number of reaction tubes to increase yield.
- Use high-quality PCR primers for the downstream application.
- Use a high-fidelity polymerase, such as Agilent's Herculase II Fusion DNA Polymerase and accompanying 5× Herculase II Reaction Buffer (Agilent p/n 600675, 600677, 600679). *Do not include DMSO in the reactions.*
- After PCR, purification of the amplicons is recommended prior to use in a downstream application. Suggested purification methods for amplicons >100 bp include silica-based columns, SPRI beads, or a comparable method.
- The tables below offer suggested starting points for PCR optimization of reactions that use Herculase II Fusion DNA Polymerase. A well-optimized reaction yields a robust band of the correct size on an Agilent BioAnalyzer DNA 1000 assay, Agilent TapeStation D1000 assay, or comparable electrophoresis-based analysis method.

Table 1 Suggested reagent concentrations for PCR with Herculase II Fusion DNA Polymerase

Reagent	Final concentration in the PCR reaction
Oligo pool	200 pM
dNTP mix	1 mM (250 µM of each NTP)
Forward primer	250 nM
Reverse primer	250 nM

Table 2 Suggested cycling conditions for PCR with Herculase II Fusion DNA Polymerase

Number of cycles	Temperature	Duration
1	95°C	2 minutes
15	95°C	20 seconds
	55°C	20 seconds
	72°C	30 seconds
1	72°C	3 minutes

Notes

Excess oligo input into the PCR reaction or over-amplification may lead to the formation of a heteroduplex peak that migrates larger than the expected product amplicon peak of 230 bp, as indicated by the arrow in the example electropherogram shown below.

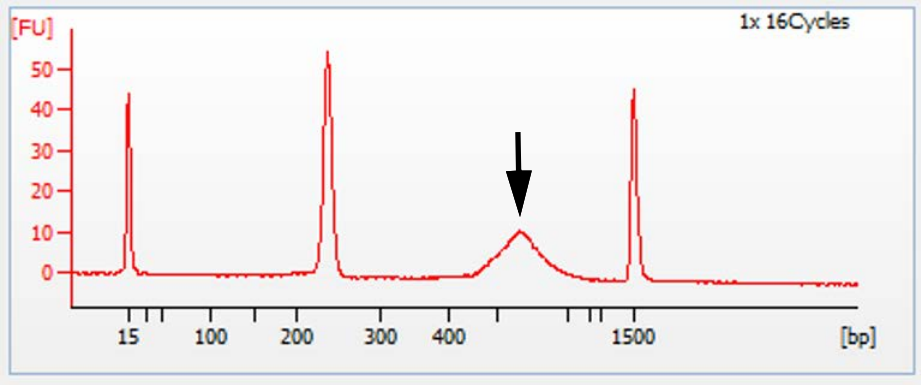

Figure 2. DNA 1000 BioAnalyzer electropherogram of the PCR product from a 230mer oligo pool illustrating the impact of excess PCR cycles. Addition of too much oligo pool will yield a similar profile.

Primer annealing temperature for the PCR reaction is dependent on PCR primer length and sequence.

www.agilent.com

Agilent Worldwide Technical Support

For US and Canada

Call (800)227-9770 (option 3, 4, 3)
Or send an email to Genome.engineering@agilent.com

For all other regions
Agilent's world-wide Sales and Support Center contact details for your location can be obtained at www.agilent.com/en/contact-us/page.

Revision B0, March 2024

G7263-90000