Converting ssDNA oligos to dsDNA with T4 DNA polymerase V.1

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We use this protocol and it's working

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ABSTRACT

This protocol allows one to convert ssDNA to dsDNA oligos. In principle, one can buy two complementary oligos and anneal them. However, there are a few cases where randomized bases are desired, and therefore complementary oligos can not be ordered. The protocol works similarly to PCR, except that the reaction is not performed at the high temperature of typical thermocycling, because the oligos are short and their melting temperature can be in the order of the extension temperature of high-fidelity polymerases.

Primer design

1 Design your target single-stranded DNA oligo such that the 3' end contains the reverse complement of an extending oligonucleotide. For example, appending "GTCATAGCTGTTTCCTG" to the end of your oligo will allow an oligonucleotide matching the M13 Reverse (-27) primer to extend it (5'-CAGGAAACAGCTATGAC-3').

2 Resuspend your oligonucleotides in 1/10th TE (10mM Tris, 0.1mM EDTA pH 8) to a final concentration of 100uM to form your stock oligonucleotide solutions.

3 Make working oligonucleotide solutions by diluting the stock oligonucleotides to a final concentration of 10uM.

Oligo annealing

4 Mix in a PCR tube 5 µL of a [M] 10 micromolar (µM) stock of the target oligo with 10 µL of a [M] 10 micromolar (µM) stock of the extending oligo. Add 27ul of molecular biology grade water.

5 Add 5 µL of NEBuffer 2.1 - 5.0 ml **New England Biolabs Catalog #B7202S**. Mix well by vortexing.

6 Place the tube in a thermocycler and run the following protocol: 15m
1. 95 degrees for 10 seconds
2. Decrease by 1 degrees
3. Repeat step 2 every 10 seconds, for 90 cycles.
4. Hold at 4 degrees.

Oligo extension

7 Add 2.5 µL dNTPs **Contributed by users** ([M] 10 millimolar (mM) stock) and mix well by vortexing.

Go to the thermocycler and prepare the reaction cycle. When ready, start the protocol and pause 40m

8 when the block reaches 0 degrees.

1. 5 minutes at 0 degrees C.
2. 5 minutes at 22 degrees C.
3. 30 minutes at 37 degrees C.
4. Hold at 0 degrees.

9 Add 0.5 µL of T4 DNA Polymerase - 750 units **New England Biolabs Catalog #M0203L** and mix well.

10 Place the tube in the thermocycler and unpause the run.

Oligonucleotide cleanup

10m

11 As soon as the reaction is completed, add EDTA (0.5 M), pH 8.0 **Life Technologies Catalog #AM9260G** to [M] 10 millimolar (mM).

12 Use a PCR purification kit to purify the oligo. Ensure the kit is able to purify short oligonucleotides if needed (a kit such as Monarch PCR and DNA Cleanup Kit - 250 preps **New England Biolabs Catalog #T1030L** can be suitable).

10m