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## Anneal complementary pairs of oligonucleotides.

### 1. General Procedure

1. Mix the concentrated complementary oligonucleotides together at 1:1 molar ratio in a micro centrifuge tube.
2. Dilute the oligonucleotide mixture to a final concentration of 1 pmol/μl with Tris or phosphate buffer containing salts, e.g. 10mM Tris, 0.1 mM EDTA, 50 mM NaCl (pH 8.0) or 100 mM sodium phosphate, 150 mM NaCl, 0.1 mM EDTA (pH 7.5 or 8.0).
3. Anneal the oligonucleotides using one of the annealing methods described below.
4. Aliquot and store at -20°C. The double-stranded DNA probes may be stored at 4°C for several weeks, given that care is taken to protect the probes from nuclease degradation.

### 2. Annealing Methods

#### • Option 1: Anneal with a heating block

1. Incubate the oligonucleotides at 95°C for 5 minutes.
2. Gradually reduce the heat until the oligonucleotides have reached room temperature.

#### • Option 2: Anneal with a water bath

1. Boil 400 ml of water in a large glass beaker on a hotplate.
2. Incubate the tube of oligonucleotides in the boiling water for 5 minutes.
3. Turn off the hotplate, leaving the oligonucleotides in the beaker on the hotplate to slowly cool to room temperature.

#### • Option 3: Anneal with a thermal cycler

A thermal cycler allows for convenient and reproducible annealing of oligonucleotides.

Use Table1 as a guide to program your thermal cycler for either a simple or advanced protocol.

The notation “-1°C/cycle” indicates a 1°C decrease in temperature per cycle.

Refer to your thermal cycler’s Operation Manual or consult the manufacturer for information

about programming your particular instrument.

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**Table1. Thermo cycler programs for annealing complementary oligonucleotides.**

| Simple protocol  |        | Cycles | Temperature         | Time   |
|--|--------|--------|---------------------|--------|
|  | Step 1 | 1      | 95 °C               | 5 min  |
|  | Step 2 | 70     | 95 °C (-1 °C/cycle) | 1 min  |
|  | Step 3 |        | 4 °C                | HOLD   |
| Advanced Protocol<br>(example in which the<br>Oligonucleotide pair<br>has a Tm of 55 °C) |        | Cycles | Temperature         | Time   |
|  | Step 1 | 1      | 95 °C               | 5 min  |
|  | Step 2 | 40*    | 95 °C (-1 °C/cycle) | 1 min  |
|  | Step 3 | 1      | 55 °C               | 30 min |
|  | Step 4 | 20*    | 55 °C (-1 °C/cycle) | 1 min  |
|  | Step 5 |        | 4 °C                | HOLD   |

\* The number of cycles in step and 4 depends on the Tm of the oligonucleotides to be annealed.