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Hybridization of DNA oligos

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ABSTRACT

This protocol is used to hibridate two complementary DNA chains.

MATERIALS TEXT

Equipment

- Thermocycler
- Vortex
- Centrifuge

Reagents

- 1.0 M NaCl
- Sterile H2O

DNA

- Promoters 5'
- Promoters 3'

SAFETY WARNINGS

Lab coat and gloves should be weared throughout the whole experiment. All working surfaces must be clean and all the reactives should be treated following manufacturer's instructions.

Annealing of oligonucleotides

1 Add the required volume of H_2O to the lyophilized oligonucleotides to obtain a concentration of 100 µM.
Vortex both tubes for 30 s and incubate them at RT for 5 min to dissolve them.

2 Prepare the annealing mix by adding into a PCR tube:
> 45.5 µl of H_2O
> 2.5 µl of 1.0 M NaCl
> 1 µl of oligo 5' (100 µM)
> 1 µl of the oligo 3' (100 µM)

3 Place the PCR tube with the mix in a thermocycler with the following annealing programme:
- 5 min at 95°C
- 1 min at 95°C
- ramp down 1°C per cycle for 72 cycles
- end by keeping the temperature at 10°C

4 Take 10 µl of the annealed oligonucleotides and dilute it with 90 µl of H_2O to obtain a 0.2 µM concentration.

5 The annealed oligonucleotides stocks can be stored at −20°C for future use.