

Anneal complementary pairs of oligonucleotides

TR0045.1

Introduction

Many methods can be used to anneal complementary strands of nucleic acids. In each case, the goal is to denature the complementary strands to remove any secondary structure and then allow the strands to hybridize. Two factors that influence the efficiency of oligonucleotide hybridization are salt concentration and the rate of temperature decrease. Annealing occurs most efficiently when the temperature is slowly decreased after denaturation, especially when the oligonucleotides have high GC content or form hairpin structures.

Stock solutions of oligonucleotides are most stable when kept at a high concentration (1-100 pmol/ μ l) and working dilutions prepared as needed. For example, prepare and store probe at a concentration of 1 pmol/ μ l and then dilute the stock solution to 10 fmol/ μ l immediately before performing an electrophoretic mobility shift assay (EMSA) with the Thermo Scientific LightShift™ Chemiluminescent EMSA Kit (Product No. 20148).

General Procedure

1. Mix concentrated complementary oligonucleotides together at a 1:1 molar ratio in a microcentrifuge tube.
2. Dilute oligonucleotide mixture to a final concentration of 1 pmol/ μ l with a Tris or phosphate buffer containing salt; for example, 10 mM Tris, 1 mM EDTA, 50 mM NaCl (pH 8.0) or 100 mM sodium phosphate, 150 mM NaCl, 1 mM EDTA (pH 7.5).
3. Anneal oligonucleotides using one of the annealing methods described below.
4. Aliquot and store probe at -20°C. Alternatively, the double-stranded DNA probe may be stored at 4°C for several weeks if protected from nucleases.

Alternative 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 thermocycler

A temperature thermocycler enables convenient and reproducible annealing of oligonucleotides. Use Table 1 as a guide to program a thermocycler for either a simple or advanced protocol. The notation “-1°C/cycle” indicates that the temperature of the heating block will decrease 1°C per cycle. Consult the thermocycler Owner’s Manual or manufacturer for information about programming individual machines.

Table 1. Thermocycler programs for annealing complementary oligonucleotides.

		Cycles	Temperature	Time
Simple Protocol	Step 1:	1	95°C	5 min
	Step 2:	70	95°C (-1°C/cycle)	1 min
	Step 3:		4°C	HOLD
Advanced Protocol (example in which the oligonucleotide pair has a T _m of 55°C)*	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 2 and 4 depends on the T_m of the oligonucleotides to be annealed.

Related Thermo Scientific Products

20148 **LightShift® Chemiluminescent EMSA Kit**, sufficient components for 100 binding reactions and detection reagents for 8 mini-blot

89818 **Biotin 3' End DNA Labeling Kit**, sufficient components for 20 labeling reactions

Current versions of product instructions are available at www.thermo.com/pierce. For a faxed copy, call 800-874-3723 or contact your local distributor.

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