Experimental Procedures

Steps		Procedure Details
1	 **Dilute and mix oligonucleotides**	1. Dilute oligonucleotides with Tris buffer (10 mM Tris, 0.1 mM EDTA, 50 mM NaCl, pH 8.0) or phosphate buffer (100 mM sodium phosphate, 150 mM NaCl, 0.1 mM EDTA, pH 7.5 or 8.0). * **Note:** Some salts are required for the hybridization of oligonucleotides. 2. Mix the two sequences together in equal molar amounts. * **Note:** If different amounts are used, there will always be single-stranded sequences left over.
2	 **Option 1. Anneal with a water bath or heating block**	3. Incubate oligonucleotides at 95°C for 5 min. 4. Turn off the hotplate of water bath (or heating block) and allow oligonucleotides to slowly cool to room temperature. 5. Store at -20°C in small aliquots.
	 **Option 2. Anneal with a thermal cycler**	3. Perform the reaction under the following conditions. • Simple protocol

Step	Temperature	Time	Cycles
Step 1	95°C	5 min	1 cycle
Step 2	95°C (-1°C/cycle)	1 min/cycle	70 cycles
Step 3	4°C	Hold	

• Advanced protocol (Example in which the oligonucleotide pair has a Tm of 55°C)

Step	Temperature	Time	Cycles
Step 1	95°C	5 min	1 cycle
Step 2	95°C (-1°C/cycle)	1 min/cycle	40 cycles*
Step 3	55°C	30 min	1 cycle
Step 4	55°C (-1°C/cycle)		20 cycles*
Step 5	4°C	Hold	

* The number of cycles in step 2 and 4 can be according to Tm value of oligonucleotides to be annealed.

4. Store at -20°C in small aliquots.