





Experimental Procedures

Steps		Procedure Details															
	 Precautions	<ul style="list-style-type: none"> Dried RNA oligonucleotides can be safely stored at -20°C for up to 6 months. RNA is highly susceptible to degradation by exogenous RNases introduced during handling. Therefore, it is essential that all handling steps are conducted under sterile and RNase free conditions. RNase free reagents, barrier pipette tips, and tubes should be used and handled with gloved hands. 															
1	 Dilute and mix oligonucleotides	<ol style="list-style-type: none"> Dissolve RNA oligonucleotides with DEPC-D.W. (provided) at the desired concentration (recommended at 100 µM). This stock solution should be stored at -20°C. Dilute each RNA oligonucleotide using an annealing buffer [30 mM HEPES-KOH (pH 7.4), 100 mM KCl, 2 mM MgCl₂, 50 mM NH₄Ac] to a final concentration of 50 µM. Mix 30 µl of each RNA oligonucleotide solution and 15 µl of annealing buffer to a total volume of 75 µl. * Note: Final concentration of siRNA duplex is 20 µM. 															
2	 Option 1. Anneal with a water bath or heating block	<ol style="list-style-type: none"> Incubate oligonucleotides mixture in a water bath (or heating block) at 90°C for 1 min. Turn off the hotplate of water bath (or heating block) and allow oligonucleotides to slowly cool to room temperature (over a period of ca. 45 min). Store at -20°C in small aliquots and avoid repeated freeze and thaw cycles. 															
	 Option 2. Anneal with a thermal cycler	<ol style="list-style-type: none"> Perform the reaction under the following conditions. <table border="1" data-bbox="376 1110 1046 1228"> <thead> <tr> <th>Step</th> <th>Temperature</th> <th>Time</th> <th>Cycles</th> </tr> </thead> <tbody> <tr> <td>Step 1</td> <td>90°C</td> <td>1 min</td> <td>1 cycle</td> </tr> <tr> <td>Step 2</td> <td>90°C (-1°C/cycle)</td> <td>1 min/cycle</td> <td>65 cycles</td> </tr> <tr> <td>Step 3</td> <td>4°C</td> <td></td> <td>Hold</td> </tr> </tbody> </table> Store at -20°C in small aliquots and avoid repeated freeze and thaw cycles. 	Step	Temperature	Time	Cycles	Step 1	90°C	1 min	1 cycle	Step 2	90°C (-1°C/cycle)	1 min/cycle	65 cycles	Step 3	4°C	
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