

SequiTherm EXCEL™ II Labeled Primer Sequencing

The SequiTherm EXCEL™ II kit comes with all reagents needed except the labeled primer. This kit can be used for routine sequencing and difficult templates.

Template Recommendations

Template	Amount (fmol [*])
Plasmid	200-400
PCR products	100-200
M13	100-200
Cosmids	50 (1.5 µg)

* fmol to µl conversion equations for DNA templates are listed in the Appendix. 1 fmol = 10⁻¹⁵ mole.

Reagents


Item	Supplier	Catalog Number
SequiTherm EXCEL™ II DNA Sequencing Kit - LC (for 25 and 41 cm gels).	Epicentre	SE9101LC
SequiTherm EXCEL™ II DNA Sequencing Kit - LC (for 66 cm gels).	Epicentre	SE9202LC
IRD-labeled M13 Primer (1 pmol/µl)	LI-COR	4000-20B or 4200-20

Reactions


1. Program the thermal cycler as follows:

Program:

Step	Temperature (°C)	Time
1.	92	2 minutes
2.	92	30 seconds
3.	54 **	30 seconds
4.	70	1 minute
5.	4	hold

 **30 cycles total**

** The annealing temperature may be adjusted depending on the T_m of the primer used. A good place to start is 5°C below the T_m of the primer.

2.	<p>Add the following components to a 0.2 ml tube to prepare the reaction mix (add the largest volume first, then follow the order below):</p> <table> <tr> <td>Template DNA</td> <td>— μl</td> </tr> <tr> <td>IRD-labeled Primer (1.0 pmol/μl)</td> <td>1.5 μl</td> </tr> <tr> <td>Sequencing Buffer</td> <td>7.2 μl</td> </tr> <tr> <td>EXCEL II DNA polymerase</td> <td>1.0 μl</td> </tr> <tr> <td>Distilled water</td> <td>— μl</td> </tr> <tr> <td colspan="2"><hr/></td> </tr> <tr> <td>TOTAL VOLUME</td> <td>20 μl</td> </tr> </table>	Template DNA	— μ l	IRD-labeled Primer (1.0 pmol/ μ l)	1.5 μ l	Sequencing Buffer	7.2 μ l	EXCEL II DNA polymerase	1.0 μ l	Distilled water	— μ l	<hr/>		TOTAL VOLUME	20 μ l
Template DNA	— μ l														
IRD-labeled Primer (1.0 pmol/ μ l)	1.5 μ l														
Sequencing Buffer	7.2 μ l														
EXCEL II DNA polymerase	1.0 μ l														
Distilled water	— μ l														
<hr/>															
TOTAL VOLUME	20 μ l														
3.	<p>Mix well.</p>  <p>Mix the components by pipetting the reaction up and down. Simply tapping the tube to mix is not sufficient.</p>														
4.	Label a set of four 0.2 ml tubes A, T, G and C for each template/primer combination.														
5.	Add 2.0 μ l of the A termination mix to the A tube(s), the T termination mix to the T tube(s), etc.														
6.	Add 4.0 μ l of the reaction mix to each A, T, G, and C tube and mix well.														
7.	For thermal cyclers without heated lids, add a drop of mineral oil to each tube.														
8.	Carefully seal the tubes, place them in the thermal cycler, and start the program.														
9.	At the completion of the cycling program, add 4 μ l of Stop Solution to each tube.														
10.	Remove oil (if used). Denature samples at 92°C for 3 minutes and place on ice. Load gel (volume depends on comb and gel thickness).														

SBS™ Modifications

The amount of template used in SBS™ reactions is based on the size of the insert between the forward and reverse priming sites. The table below gives guidelines for an SBS™ reaction.

Insert Size (bp)	Template Amounts (fmol*)
300-600	50 - 100
600-1200	125-225
1300-1800	250 - 300
>1800	300 - 500

* 1 fmol = 10^{-15} mole

1.	<p>Add the following components to a 0.2 ml tube to prepare the reaction mix for each template (add the largest volume first, then follow the order below):</p> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 80%;">ds DNA</td> <td style="text-align: right;">— μl</td> </tr> <tr> <td>IRD700 Fwd primer (1.0 pmol/μl)</td> <td style="text-align: right;">1.5 μl</td> </tr> <tr> <td>IRD800 Rev primer (1.0 pmol/μl)</td> <td style="text-align: right;">1.5 μl</td> </tr> <tr> <td>Sequencing Buffer</td> <td style="text-align: right;">7.2 μl</td> </tr> <tr> <td>EXCEL II DNA polymerase</td> <td style="text-align: right;">1.0 μl</td> </tr> <tr> <td>Distilled water</td> <td style="text-align: right;">— μl</td> </tr> <tr> <td style="border-top: 1px solid black;">TOTAL VOLUME</td> <td style="text-align: right; border-top: 1px solid black;">20 μl</td> </tr> </table>	ds DNA	— μ l	IRD700 Fwd primer (1.0 pmol/ μ l)	1.5 μ l	IRD800 Rev primer (1.0 pmol/ μ l)	1.5 μ l	Sequencing Buffer	7.2 μ l	EXCEL II DNA polymerase	1.0 μ l	Distilled water	— μ l	TOTAL VOLUME	20 μ l
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Distilled water	— μ l														
TOTAL VOLUME	20 μ l														
2.	Label a set of four 0.2 ml tubes A, T, G and C for each template/primer combination.														
3.	Add 2.0 μ l of the A termination mix to the A tube(s), the T termination mix to the T tube(s), etc.														
4.	Add 4.0 μ l of the reaction mix to each A, T, G, and C tube and mix well.														
5.	For thermal cyclers without heated lids, add a drop of mineral oil to each tube.														
6.	Carefully seal the tubes, place them in the thermal cycler, and start the program.														
7.	At the completion of the cycling program, add 4 μ l of Stop Solution to each tube.														
8.	Remove oil (if used). Denature samples at 92°C for 3 minutes and place on ice. Load gel (volume depends on comb and gel thickness).														

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