



Thermo Sequenase® Labeled Primer Cycle Sequencing Protocol for USB® Kits

Template Recommendations

<u>Template</u>	<u>Amount (fmoles)</u>
Plasmid	200-500
PCR products	20-50
ssDNA	100-200
Cosmids	50 (1.5 µg)

Reagents

<u>Item</u>	<u>Supplier</u>	<u>Catalog Number</u>
USB Thermo Sequenase Cycle Sequencing Kit	USB	78500
2.5 mM dNTP mix (with 7-deaza dGTP)*	Any Supplier	
IRDye labeled Primer (1 pmol/µl)	LI-COR	4000-28B or 4200-28
IR ² Stop Solution	LI-COR	830-04997

* Prepare a solution of: 2.5 mM dATP, 2.5 mM dCTP, 2.5 mM dTTP, 2.5 mM 7-deaza dGTP.

Reactions

- 1) Program the thermal cycler as follows:
 - 92°C for 2 minutes
 - 92°C for 30 seconds
 - 50°C for 30 seconds**
 - 72°C for 1 minute
 - Repeat steps 2 to 4 for a total of 30 cycles
 - 4°C soak

** 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) Combine these reagents in a 0.2 ml tube:

	<u>Your Reaction</u>
Template DNA	— µl
IRDye labeled primer (1.0 pmol/µl)	2.0 µl
Thermo Sequenase Reaction Buffer	2.0 µl
2.5 mM dNTP nucleotide mix	1.0 µl
Thermo Sequenase DNA Polymerase	2.0 µl
<u>ddH₂O to bring final volume to 17.0 µl</u>	<u>— µl</u>
TOTAL VOLUME	17.0 µl

- 3) Mix well. **Tip:** Add water last and mix the components well by pipetting the reaction up and down several times with the same tip. Simply tapping the tube to mix is not sufficient.

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Reactions (continued)

- 4) Label a set of four 0.2 ml tubes A, T, G and C for each template/primer combination.
- 5) Add 4.0 μ l of each Thermo Sequenase[®] Termination Mix to each specific tube from Step 4. (i.e., ddGTP termination mix to the G tube(s), the T reagent to the T tube(s), etc.
- 6) Add 4.0 μ l of the appropriate template/primer combination 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. Tightly capped or sealed tubes may be used with a heated lid. Place tubes in the thermal cycler and start the cycling program as in Step 1.
- 8) At the completion of the cycling program, add 3 μ l of IR² stop solution to each tube.
- 9) Remove oil (if used) from samples. Denature samples at 92°C for 3 minutes and place on ice.
- 10) Load gel using 1.0-1.5 μ l, depending on the comb used in loading.

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.

<u>Insert Size</u>	<u>Template Amounts</u>
300 - 600 bp	50 - 100 fmol
600 - 1200 bp	125 - 225 fmol
1300 - 1800 bp	250 - 300 fmol
>1800 bp	300 - 500 fmol

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