

# Labeled Primers

One of the STR primer pairs can be synthesized with a 5'-IRDye label. In this manner one strand of the resulting PCR product is labeled during amplification. The method is very robust and no purification is required prior to gel analysis. Custom primers are available from LI-COR.

## Protocol

1.	Enter the <i>Standard</i> program, or a custom program on the thermocycler (given earlier in this Section).																																				
2.	Add 20-50 ng of genomic DNA to a microcentrifuge tube or a microplate well.																																				
3.	Determine the quantity of each component required using the following table, based on the total number of reactions. Substitute water for the other primers if only a single locus is being run. If MgCl <sub>2</sub> is not premixed in the PCR buffer, account for MgCl <sub>2</sub> as necessary.																																				
	<table border="1"> <thead> <tr> <th>Component</th> <th>Stock Concentration</th> <th>Qty Per Reaction</th> </tr> </thead> <tbody> <tr> <td>10X Buffer</td> <td></td> <td>1.0</td> </tr> <tr> <td>dH<sub>2</sub>O</td> <td></td> <td>3.8</td> </tr> <tr> <td>dNTPs</td> <td>2.0 mM</td> <td>1.0</td> </tr> <tr> <td>Primer 1 labeled</td> <td>1.0 pmol/μl</td> <td>0.5</td> </tr> <tr> <td>Primer 1 unlabeled</td> <td>1.0 pmol/μl</td> <td>0.5</td> </tr> <tr> <td>Primer 2 labeled</td> <td>1.0 pmol/μl</td> <td>0.5</td> </tr> <tr> <td>Primer 2 unlabeled</td> <td>1.0 pmol/μl</td> <td>0.5</td> </tr> <tr> <td>Primer 3 labeled</td> <td>1.0 pmol/μl</td> <td>0.5</td> </tr> <tr> <td>Primer 3 unlabeled</td> <td>1.0 pmol/μl</td> <td>0.5</td> </tr> <tr> <td>Taq</td> <td>5.0 unit</td> <td>0.2</td> </tr> <tr> <td><b>Total Volume</b></td> <td></td> <td><b>9.0 μl</b></td> </tr> </tbody> </table>	Component	Stock Concentration	Qty Per Reaction	10X Buffer		1.0	dH <sub>2</sub> O		3.8	dNTPs	2.0 mM	1.0	Primer 1 labeled	1.0 pmol/μl	0.5	Primer 1 unlabeled	1.0 pmol/μl	0.5	Primer 2 labeled	1.0 pmol/μl	0.5	Primer 2 unlabeled	1.0 pmol/μl	0.5	Primer 3 labeled	1.0 pmol/μl	0.5	Primer 3 unlabeled	1.0 pmol/μl	0.5	Taq	5.0 unit	0.2	<b>Total Volume</b>		<b>9.0 μl</b>
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4.	Pipette gently to mix.																																				
5.	Add 9 μl of the STR mixture from above to each tube or well and pipette gently to mix.																																				
6.	If the thermocycler does not have a heated lid, add one drop of mineral oil to each well.																																				
7.	Place the tubes or plate in the thermocycler. Start the cycling program.																																				
8.	After completion of the program, add 2 μl of stop buffer to each tube or well and mix gently.																																				
9.	Heat the samples at 95°C for 3 minutes and snap cool on ice before loading.																																				
10.	Load gel (volume depends on comb and gel thickness).																																				

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