Tailed Primers

In this approach, one of the unlabeled STR primers is synthesized with an M13 forward or reverse primer sequence on the 5'-end. An IRDye-labeled M13 Primer is included in the PCR reaction. The M13 primer is added to the PCR product during the first few cycles of amplification. The labeled M13 primer is incorporated in subsequent cycles, thus labeling the PCR product. This method requires no purification prior to gel analysis.

Important: When using tailed primers to amplify a locus that will be analyzed with Saga^{GT}, the modified minimum and maximum locus boundaries in Saga's Locus Manager should be increased by the length of the primer incorporated in the PCR product.

The M13 primer sequence can be used on both 800 and 700 channels. LI-COR M13 forward and reverse primer sequences are as follows:

4000-20B Forward (-29)/IRDye 800-labeled Primer, 19-mer: 5' - CACGACGTTGTAAAACGAC - 3'

4000-21B Reverse/IRDye 800-labeled Primer, 20-mer: 5' - GGATAACAATTTCACACAGG - 3'

Protocol

1. Enter the *Standard* 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 $MgCl_2$ is not premixed in the PCR buffer, account for $MgCl_2$ as necessary.

Component	Stock Concentration	Qty Per Reaction
10X Buffer		1.0
dH_2O		3.3
dNTPs	2.0 mM	1.0
Primer 1fwd	1.0 pmol/µl	0.5
Primer 1rev	1.0 pmol/µl	0.5
Primer 2fwd	1.0 pmol/µl	0.5
Primer 2rev	1.0 pmol/µl	0.5
Primer 3fwd	1.0 pmol/µl	0.5
Primer 3rev	1.0 pmol/µl	0.5
M13f-29 primer	1.0 pmol/µl	1.0
Taq	5.0 unit	0.2
Total Volume		**9.0 µl**

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 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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