PROTOCOL FOR GENOMIC DOUBLE STRANDED DNA AMPLIFICATION FOR RAPDs

1. Thaw all master mix components but Taq polymerase on ice. (See instructions for master mix preparation under Recipes section)

2. While master mix components are thawing, pipette 11 µl of autoclaved nanopure water into autoclaved, labeled PCR tubes on ice, and add 3 µl of template DNA .

3. Denature the DNA by placing the PCR tubes into the thermalcycler at 94˚ C for three minutes.

4. Immediately transfer the tubes into an ice/water slurry (speed is essential to keep the DNA from renaturing).

5. Add Taq polymerase to the master mix, vortex and centrifuge for 10 seconds.

6. Pipette 14 µl of master mix into each of the tubes containing the denatured DNA. Mix each tube and centrifuge for 10 seconds.

7. Overlay the solution with 2-3 drops of PCR mineral oil to avoid evaporation during amplification. (we use Sigma mineral oil for molecular biology)

8. Place the tubes in the thermalcycler for 45 cycles programmed as follows: 1 minute at 94˚ C, 1 minute at 36˚ C, and 2 minutes at 72˚ C to denature the DNA, anneal the primer, and extend the sequence, respectively.

RECIPES SECTION FOR DNA AMPLIFICATION

(All Solutions Must be Made with Autoclaved Nanopure Water)

A. dNTPs- 100 mM each

Mix dNTPs in 1:19 ratios for final concentrations of 5mM.

We use Promega dNTPs. Aliquot into small amounts to avoid repeated freezing and thawing. Store at -20˚ C.

B. Primer- 0.5 OD units = 16.5 µg/ml

Example, G12 with 15.5 µg/tube dry and MW=2988 g is diluted in 0.9394 ml water for a final concentration of 5 µm.

We use Operon 10-mer primer kits. Aliquot into small amounts to avoid repeated freezing and thawing. Store at -20˚ C.

C. Master Mix:

component	storage temp.	volume/reaction	final concentration
10X Stoffel buffer	-20˚ C	2.5 µl	2.5 mM Tris-HCL, pH 8.3; 2.5 mM KCl
dATP (5 mM)	-20˚ C	1 µl	200 µM
dCTP (5 mM)	-20˚ C	1 µl	200 µM
dGTP (5mM)	-20˚ C	1 µl	200 µM
dTTP (5mM)	-20˚ C	1 µl	200 µM

Primer (5 µM)	-20˚ C	4 µl	0.8 µM
$MgCl_2$ (25 mM)	-20˚ C	3.5 µl	3.5 mM

4. Stoffel Fragment Mix:

component	storage temp.	volume/reaction	final concentration
Stoffel Fragment (10 units/ µl)	-20˚ C	0.2 µl	2 units/ 25 µl

We use Perkin Elmer Amplitaq DNA Polymerase, Stoffel Fragment with 10X Stoffel Buffer and 25 mM $MgCl_2$ Solution. Stoffel Fragment Taq Polymerase is more expensive than other Taq Polymerase, so it may be beneficial to find the right Taq to fit your own needs and budgets.