

## Primary Amplification of Genomic DNA using DOP - PCR

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### Reagents

**Agarose, Ultrapure**

Gibco, BRL, Cat. no. 15510-027

**10X Buffer, and**

Perkin Elmer (Part no. N808-0010)

**Template DNA**

Use 50-100 ng for each reaction

**Ethidium Bromide**

Research Genetics, Cat. no. 750007

**Loading buffer, 5X**

Quality Biological, Cat. no. 51-026-030

**MgCl<sub>2</sub>, 25 mM**

Perkin Elmer (Part no. N808-0010)

**100 mM dNTP nucleotides**

**dGTP**

Boehringer Mannheim, 1051466

**dCTP**

Boehringer Mannheim, 1051458

**dATP**

Boehringer Mannheim, 1051440

**dTTP**

Boehringer Mannheim, 1051482

**Primer “UN1”**

Midland Certified Reagent Co. Telenius 6MW  
[5'-CCGACTCGAGNNNNNNATGTGG-3']

**Taq DNA polymerase, 5 U/μl**

Fermentas Life Sciences, Cat. Epo282 500U

**TAE buffer, 10X**

Advanced Biotechnologies, Cat. no.08-514-001

**Sterile water (H<sub>2</sub>O)**

Molecular grade sterile distilled water

### Materials and Equipment

PCR Thermocycler

Gel system and power source

PCR tubes

## Preparation

### 1X TAE buffer

Dilute the 10X TAE with dH<sub>2</sub>O, 1:10 to make 1X TAE

Dissolve 1 g of agarose in 100 ml 1X TAE buffer by warming the solution

### Stock dNTP 2mM

	<u>μl</u>	<u>mM final</u>
dGTP	10	0.2
dCTP	10	0.2
dATP	10	0.2
dTTP	10	0.2
dH <sub>2</sub> O	460	-
total	500	

- Autoclave PCR microcentrifuge tubes (0.5 ml size), 2 ml microcentrifuge tubes, and pipet tips (10, 200, and 1000 μl).
- Sterilize pipettes (using UV) to be used for PCR and only use that set for PCR (can use Stratalinker or UV light source from tissue culture hood).
- Before starting, make sure that the work space is cleaned with ethanol and that it is located in a low traffic area. Use the tissue culture hood to maintain sterility if you cannot find a corner to work in.

## Procedure

1. Label each 0.5 ml tube, being careful to only handle the outside of the tube.
2. Add appropriate volume of DNA to each PCR tube, close, and set aside into ice or 4°C until the reaction mix is ready to aliquot
3. The order for pipetting the reaction mix is as follows: dH<sub>2</sub>O, buffer, MgCl<sub>2</sub>, dNTP, and primer. The reaction mix for one reaction is as follows (multiply each by the number of reactions):

Primer	4 μl
Genomic DNA	Y μl (Note 1)
10X buffer	10 μl
MgCl <sub>2</sub> 25mM	8 μl
dNTP	10 μl
Sterile dH <sub>2</sub> O	X μl (Note 2)

4. Pipette 96  $\mu$ l of the reaction mix into each PCR tube (change tips between each tube) and put on ice.
5. Vortex the tubes, spin quickly (few seconds, not higher than 5000 rpm), and place on ice. At this point take out the Taq enzyme, mix carefully (tap with finger), spin quickly, then add 1  $\mu$ l (for each 100  $\mu$ l) (Note 3) to each reaction. Vortex the tubes, spin quickly, and put back on ice.
6. Vortex each tube, spin quickly, and put them all into the PCR machine and run using the appropriate PCR program (Note 4).
7. After completion, remove the tubes from the PCR machine, vortex, spin quickly, and place on ice. Mix 0.8  $\mu$ l of 5X DNA loading buffer with a 2  $\mu$ l aliquot from each reaction to run on a 1% agarose gel. The resulting smear migrates around 500 bps (Note 5).

### Notes

1. The volume of genomic DNA depends on the concentration that you have available, keeping in mind that you need at least 50 ng.
2. The volume of water to add depends on the volume of DNA that is added, and the total reaction volume should be equal to 100  $\mu$ l.
3. The Taq polymerase should always be added last, and since it is more viscous and sticky, it needs to be mixed well before each use. Use the pipet tip you are about to draw with to gently stir the contents as you draw up the enzyme.
4. PCR program (runs approximately 7 hours):

<b>Step</b>	<b>Temperature (°C)</b>	<b>Minutes</b>
1 (initial denaturation).	93	10
2	94	1
3	30	1.5
4	ramp 30-70	3
5	72	3
6 repeat steps 2-5, 4 times		
7	94	1
8	62	1
9	72	3 + 1 second/cycle
10 repeat steps 7-9, 34 times		
11	72	10
12	4	$\infty$

5. 1% agarose gel

