2004

Primary Amplification of Genomic DNA using DOP - PCR

Section of Cancer Genomics, Genetics Branch, NCI
National Institutes of Health

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
$MgCl_2$, 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_20)
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_20, 1:10 to make 1X TAE
Dissolve 1 g of agarose in100 ml 1X TAE buffer by warming the solution

Stock dNTP 2mM

	µl	mM final
dGTP	10	0.2
dCTP	10	0.2
dATP	10	0.2
dTTP	10	0.2
dH_20	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_20, buffer, $MgCl_2$, 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
$MgCl_2$ 25mM	8 μl
dNTP	10 μl
Sterile dH_20	X μl (Note 2)

4. Pipette 96 μl 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 μl (for each 100 μl) (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 μl of 5X DNA loading buffer with a 2 μl 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.

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 μl.

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):

Step	Temperature (°C)	Minutes
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	∞

5. 1% agarose gel