DOP-PCR Labeling (SKY)

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National Institutes of Health

Reagents

Agarose, Ultrapure
Gibco BRL, Cat. 15510-027
Ampli Taq polymerase 5 U/μl
Perkin Elmer, Part No. N808-0158
Buffer, 10X and 25 mM $MgCl_2$
Perkin Elmer, Part No. N808-00
Loading buffer, 5X
Quality Biological, Cat. 51-026-030
Ethidium Bromide
Research Genetics, Cat.750007
Primer "UN1"
Midland Certified Reagent Co. Telenius 6MW
[5'-CCGACTCGAGNNNNNNATGTGG-3']
10X TAE buffer
Advanced Biotechnologies, Cat. 08-514-001
Template DNA
Use 400 – 600 ng for each reaction
100 mM dNTP nucleotides

- **dGTP**
  Boehringer Mannheim, Cat. 1051466
- **dCTP**
  Boehringer Mannheim, Cat. 1051458
- **dATP**
  Boehringer Mannheim, Cat. 1051440
- **dTTP**
  Boehringer Mannheim, Cat. 1051482

x – dUTP:

- **Rhodamine 110 (100 μM)**
  Perkin Elmer, Cat. 401896
- **Spectrum orange (1 mM)**
  Vysis, Cat. 30-803000
- **Texas red (1 mM)**
  Molecular Probes, Cat. C-7631
- **Biotin (50 mM)**
  Boehringer Mannheim, Cat. 1093070

Digoxygenin (50 mM)
Boehringer Mannheim, Cat. 1558706
Water, sterile (H_20)
Molecular grade sterile distilled water

Materials and Equipment

PCR Thermocycler
MJ – Research, Inc., Model PTC - 100
Gel system and power source
PCR tubes
PGC Scientifics, Cat. 502-075

Preparation

1X TAE buffer
Dilute 10X TAE with dH_20

1% agarose gel
Dilute 1 g of agarose into 100 ml of 1X TAE buffer, heat until dissolved

Stock dNTP 2mM

	μl	mM final
dGTP	10	0.2
dCTP	10	0.2
dATP	10	0.2
dTTP	7.5	0.15
dH_20	462.5	-
Total	500	

x – dUTP's
Spectrum Orange (SpO) is diluted 1:5 in sterile H_20 (14 μl SpO + 56 μl H_20).
Texas Red (TR) is diluted 1:5 in sterile H_20 (13 μl TR + 52 μl H_20).
Rhodamine 110 is used undiluted.

Microcentrifuge tubes, pipette tips, and pipettes
Autoclave PCR microcentrifuge tubes (0.5 ml size), 2 ml microcentrifuge tubes, and pipette 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 TC hood).

Workspace
Before starting make sure that the work space is cleaned with ethanol and there is a steady air flow; if not, then use the tissue culture hood to maintain sterility.

Template DNA
400 – 600 ng (see note 1)

Procedure

Use sterile techniques throughout, especially when handling the DNA samples.

1. Label PCR tubes according to labeling scheme (note 2) (e.g. 1A, 1E, etc.) plus a zero control for each color (master mix with no DNA sample).

2. Defrost template DNA at 37^0 C, vortex and spin briefly prior to aliquoting out the DNA. Aliquot 4 μl of the appropriate DNA into each PCR tube, seal lid, and temporarily place at 4^0C.

3. Label five 2 ml microcentrifuge tubes (A – E) and make the master mix for each fluorochrome according to the table (table 1) below and put on ice. The order for pipetting the master mix is as follows: dH_20, buffer, $MgCl_2$, dNTP, and primer.

4. Dilute the TR and SpO dUTP's immediately before use. Vortex the labeled fluor dUTP's, spin down briefly and add each to the appropriate master mix and place back on ice.

5. Mix the Taq enzyme carefully (tap with finger), spin down, then add appropriate amounts to each master mix. Vortex the master mix tubes, spin and put back on ice.

6. Put PCR tubes with aliquoted DNA in fluorochrome order (all A's in one row, all B's in the next, etc.)

7. Carefully open each tube in row A by handling only the outside of the tube. Vortex the master mix for SpO, pipet 96 μl into each tube (change tips between each tube) and put on ice. Repeat the same procedure for each remaining x-dUTP.

8. Vortex each tube, spin down briefly and put them on ice again.

9. Start the PCR machine and select the PCR program (note 3), arrange the tubes in the PCR machine, close lid, and start the program.

10. After completion, remove the tubes from the PCR machine, vortex, spin down, and place on ice. In a round bottom Elisa plate, place 0.8 µl of 5X DNA loading buffer in each well to which you will be adding a 2 µl aliquot from each reaction to run on a 1% agarose gel. The resulting smear migrates to around 500 bp (note 4).

Table 1.

	Spec Orange **A**	Texas Red **B**	Bio (Cy5) **C**	Rh 110 **D**	DIG (Cy5.5) **E**
10X PCR buffer	130	120	140	150	130
$MgCl_2$ (25 mM)	104	96	112	120	104
1-dNT (2 mm)	65	60	70	75	65
sterile dH20	819	756	910	945	845
Primer (100 uM)	52	48	56	60	52
PE AmpliTaq polymerase	13	12	14	15	13
x-dUTP	65 (dil.1:5)	60 (dil.1:5)	42	75	39
No.reactions	1248:96=13	1152:96=12	1344:96=14	1440:96=15	1248:96=13

Notes

1. The stock DNA used for labeling PCR reactions is initially amplified as described in the primary DOP-PCR protocol. This amplified product can then be amplified again as described in the secondary DOP-PCR protocol. It is this secondary amplification product that is then used as the starting material for the SKY labeling PCR reactions.

2. Labeling scheme:
   A-Spectrum Orange
   B-Texas Red
   C-Biotin
   D-Rhodamine 110
   E-Digoxygenin

   chromosome 1-AE
   2-E
   3-CDE
   4-BC

5-ABDE
6-BE
7-CD
8-D
9-ADE
10-CE
11-A
12-BCDE
13-AD
14-B
15-ABC
16-BCD
17-C
18-ABD
19-AC
20-ACD
21-DE
22-ABCE
X-BD
Y-ACDE

3. PCR program :

Step	Temperature (°C)	Minutes
1	94	1
2	56	1
3	72	3 with addition of 1 sec/cycle
4	repeat steps 1-3, 29 times	
5	72	10
6	4	∞

4. The 1% agarose gels migrate to about 500 bp.