DOP-PCR Secondary (SKY)

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

Reagents

Agarose, Ultrapure
Invitrogen Corp., Cat. 15510-027
10X Buffer, and 25 mM $MgCl_2$
Perkin Elmer, Part No. N808-0010
Template DNA
Use 50 – 100 ng for each reaction
Ethidium Bromide
Research Genetics, Cat. 750007
5X Loading buffer
Quality Biological , Cat. 51-026-030
100 mM dNTP nucleotides
- **dGTP**
  Boehringer Mannheim, Cat. 1051466
- **dCTP**
  Boehringer Mannheim, Cat. 1051458
- **dATP**
  Boehringer Mannheim, Cat. 1051440
- **dTTP**
  Boehringer Mannheim, Cat. 1051482

Primer "UN1"
Midland Certified Reagent Co. Telenius 6 MW
[5'-CCGACTCGAGNNNNNNATGTGG-3']
Ampli Taq polymerase 5 U/μl
Perkin Elmer, Part No. N808-0158
10X TAE buffer
Advanced Biotechnologies , Cat. 08-514-001
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 the 10X TAE with dH_2O

1% agarose gel
Dissolve 1g of agarose in 100 ml of 1X TAE buffer and heat 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	

Microcentrifuge tubes, pipet tips, and pipettes

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 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
150-200 ng (see note 1)

Procedure

1. Label each 0.5 ml tube with the chromosome number and date (1 to Y and a control tube lacking any DNA).

2. Thaw template DNA at 37˚C, vortex and spin briefly in picofuge prior to aliquoting out the DNA. Aliquot 2 µl of the appropriate DNA into each PCR tube and place closed PCR tubes at 4˚C temporarily.

3. Since you will have a total of 25 reactions you will use two 2 ml microcentrifuge tubes for putting together the master mix. One tube will contain enough master mix for 13 reactions and the other will contain enough for 12.

4. The master mix for one reaction is as follows (multiply each by number of reactions) and specifically in this order:

sterile dH_2O	65 μl		845
10X buffer	10 μl		130
$MgCl_2$	8 μl		104
dNTP	10 μl		130
Primer	4 μl	for 13 reactions	52
Taq	1 μl	(Note 2)	13

5. Vortex the two tubes, spin them down and place on ice. At this point take out the Taq enzyme, mix 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 order and open each tube by handling only the outside of the tube. Pipette 96 μl of master mix into each tube (change tips between each tube) and put on ice.

7. Vortex each tube, spin down briefly and put them all into the PCR machine. Make sure that the correct PCR program (Note 3) is selected.

8. 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 (see note 4).

Notes

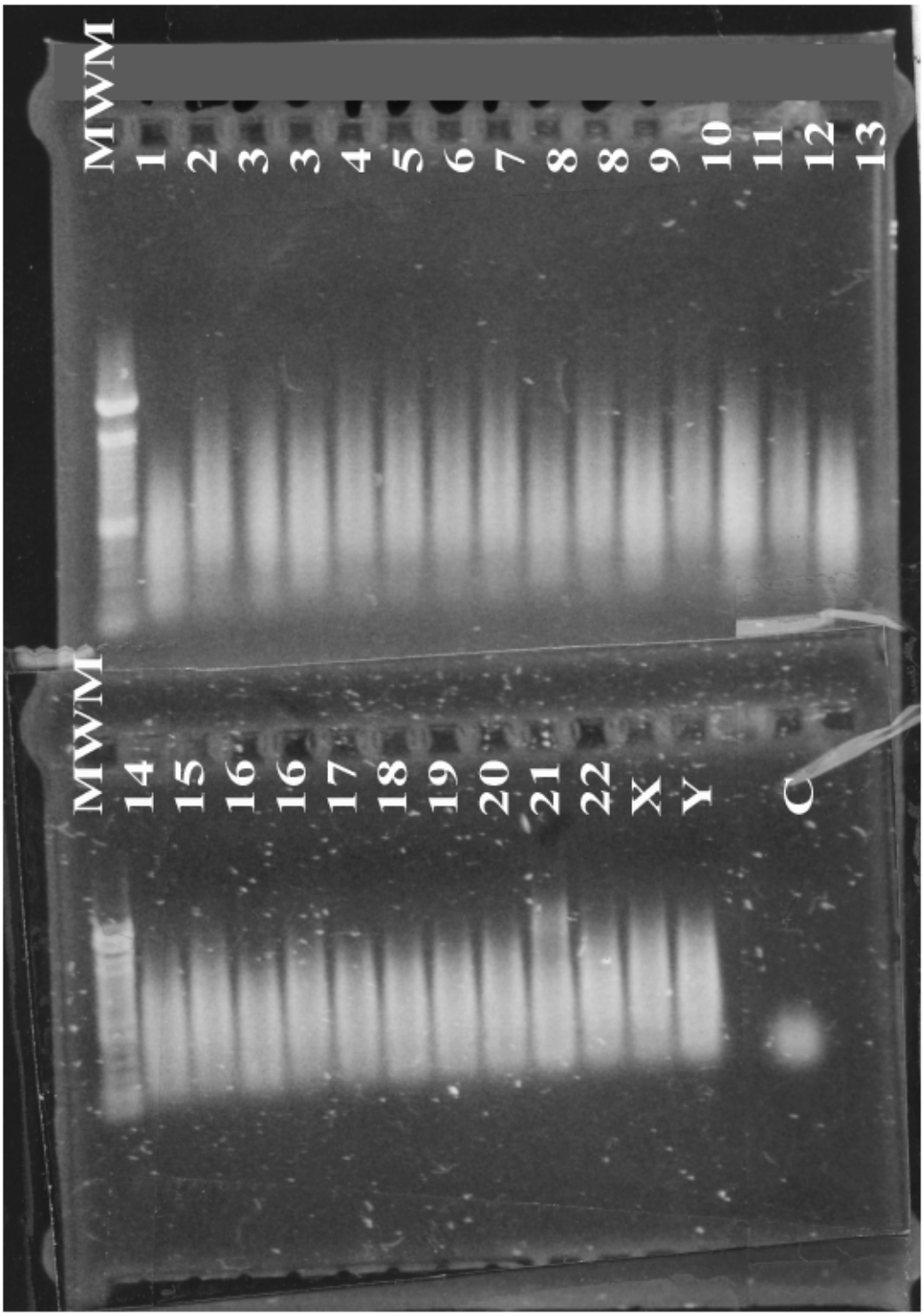
1. The stock DNA used for this secondary amplification is derived from the primary amplification as described in the primary DOP-PCR protocol. If the primary amplification yield is 50 µl and you use 2 µl per secondary amplification then you should have enough starting material for 25 secondary PCR reactions.

2. 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.

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. 1% agarose gel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