Amplification of Flow Sort DNA using DOP-PCR (SKY)

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National Institutes of Health

Reagents

Agarose, Ultrapure
Gibco, BRL, Cat. no. 15510-027
5X Buffer D, (contains $MgCl_2$)
Invitrogen, Cat. K1220-02D
Template DNA
Ethidium Bromide
Research Genetics, Cat. 750007
5X Loading buffer
Quality Biological, Cat. 51-026-030
10 mM dNTP nucleotide mix
Invitrogen , comes with buffer D above
Primer "UN1"
Midland Certified Reagent Co. Telenius 6MW
[5'-CCGACTCGAGNNNNNNATGTGG-3']
Super Taq polymerase 15U/µl
CPG, Part No. STAQ050H-500U
TAE buffer, 10AX
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 into dH_20

1% agarose gel

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

Sterile Techniques

Sterile techniques are extremely crucial since you will be re-amplifying the product from this reaction and any contaminants will also be amplified.

1. Autoclave molecular grade water using liquid cycle program.

2. Autoclave all tubes using dry cycle program with slow drying time to prevent condensation.

3. Thoroughly wipe down UV hood with 70% ethanol including walls, ceiling, and work area.

4. UV expose all equipment used including the hood, pipets, pipet tips, tube racks, microcentrifuge tubes, autoclaved water, waste container, etc. for at least 20 minutes.

5. Use sterile gloves.

Flow Sorted Chromosomes

Flow sorted chromosomes should be kept tightly wrapped and sealed at 4^0C until they are ready to be used. Be aware that each 50 μl reaction will be carried out in the original tube that is sent carrying the flow sorted chromosomes.

Procedure

1. Vortex each tube well in order to detach chromosomes from the inner walls. Spin them at 13,000 rpm for at least 3 min.

2. Combine the following for each reaction (make sure to change tips every time):

Buffer D	10 μl
dNTP	4 μl
primer	1.5 μl
dd H_20	2.0 μl
Super Taq	0.25 μl

(Note: Do not mix up and down with pipet.)

3. Vortex each tube lightly and spin at 13,000 rpm for 30 sec.

4. IMMEDIATELY transfer to PCR machine.

5. Run the PCR program as described (Note 1 and 2).

6. When the program is completed run a 2 µl aliquot (dissolved in place 0.8 µl of 5X DNA loading buffer) from each reaction on a 1% agarose gel to initially determine the efficiency of the amplification reaction. The resulting smear migrates to around 500 bp (Note 3).

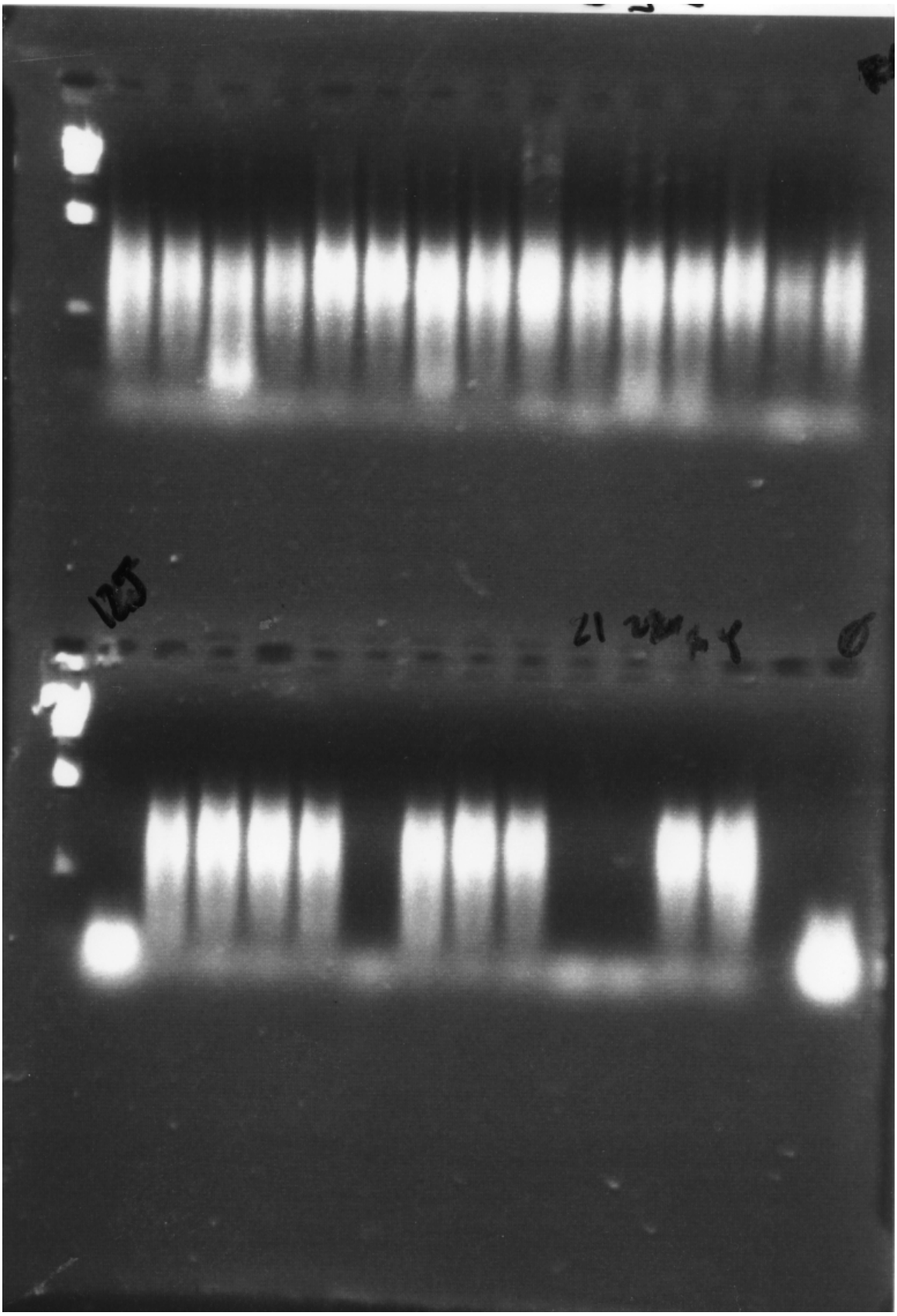
Notes

1. PCR program:

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
	4	∞

2. After running the PCR program it is time to take out the samples and evaluate the quality of your amplification. Make sure that you still maintain sterile techniques with this amplified product. You will be using this product as starting material later for secondary amplification or for a labeling PCR. Therefore do not touch the tubes without gloves and only open tubes in the hood to decrease chances of contamination.

3. 1% agarose gel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