Amplification of Genomic DNA using Alu PCR

Reagents

DNA fragment block from gel or YAC DNA
dATP, dTTP, dGTP, dCTP
Boehringer Mannheim, Cat. 105 1440, 105 1458, 105 1466, 105 1482
Ethanol, absolute
Sigma, Cat. E7023
$MgCl_2$ (25 mM)
(comes with Perkin Elmer buffer)
10X PCR Buffer II (without $MgCl_2$)
Perkin Elmer, Cat. N808-0010
Primer CL1 (50 µM)
(5'- TCCCAAAGTGCTGGGATTACAG -3')
Primer CL2 (50 µm)
(5' -CTGCACTCCAGCCTGGG -3')
Sodium chloride (NaCl) (5 M)
Quality Biologicals, Cat. 351-036-100
Taq Polymerase: Ampli Taq (5 U/µl)
Perkin Elmer, Cat. N808-0158
Tris-HCl (1 M), pH 7.5
Quality Biologicals, Cat. 351-006-100
H_2O

Preparation

DNA
Cut desired band from agarose gel (block) or prepare DNA.
Wash gel block with 1M Tris-HCl for 3 x 45 min.
Incubate gel block with 1X PCR-buffer in order to remove EDTA.
Melt gel block in 70°C water bath for 5 min.

dNTP :Nucleotides (Total Concentration 100mM)
dATP, 100 µl for a final concentration of 25mM
dTTP, 100 µl for a final concentration of 25mM
dGTP, 100 µl for a final concentration of 25mM
dCTP, 100 µl for a final concentration of 25mM
Mix and store at –20°C.

Procedure

1**.** Prepare the PCR reaction mix (final volume 100 µl)

DNA (100ng),	**X**µl
10X PCR Buffer II(without $MgCl)_2$,	10 µl
$MgCl_2$ 25mM	6 µl
dNTP :Nucleotides (25mM),	1µl
Primer CL1 (50 µM)	0.5 µl
Primer CL2 (50 µm)	0.5 µl
Sterile H_2O	82.5 µl – **X** µl
*Taq Polymerase	0.5 µl

*Add Taq after the first 3 min step

2**.** Carry out PCR according to the following profile (Program called "DOPMS"):

Step	**Temperature (°C)**	**Minutes**
1	96	3
2	96	1
3	37	0.5
4	72	6
5	repeat steps 2-4, 29 times	
6	72	5
7 (cooling)	4	∞

Purification (steps 3-8)

3. Check 10 µl of PCR product on a 1.2% agarose gel.
4. Precipitate the DNA using 1/25 vol. 5 M NaCl, 1/50 vol. 0.5 M $MgCl_2$, and 2.5 vol. absolute ethanol for at least 10 min at -80°C.
5. Centrifuge for 30 min at 13,000 rpm.
6. Pour off supernatant and wash with 400 µl 70% ethanol.
7. Dry in the speed vac.
8. Dissolve DNA in 50 µl sterile water.