

## **Preparation of Alpha-satellite DNA by PCR**

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

### **Reagents**

#### **Biotin-16-dUTP**

Boehringer, Mannheim, Cat. 1093 070

#### **Cot 1 DNA (1 mg/ml)**

Gibco BRL, Cat. 15279-011

#### **Cy5-dUTP**

Amersham, Cat. PA 55022

#### **Digoxigenin-11-dUTP**

Boehringer, Mannheim, Cat. 1558 706

#### **Dextran sulfate (50%)**

Intergen, Cat. S4030

#### **Template DNA**

DNA from somatic cell hybrids containing single human chromosomes  
(see table at the end of the protocol)

#### **Ethanol**

#### **Formamide (deionized)**

#### **PCR Buffer I**

Perkin Elmer

#### **Degenerate Primers 1 and 2**

**#1: GAAGCTTA(A/T)(C/G)T(C/A)ACAGAGTT(G/T)AA**

**#2: GCTGCAGATC(A/C)C(A/C)AAG(A/T/C)AGTTTC**

Custom order from Midland Certified Reagent Company, Texas

#### **Ampli Taq Polymerase (5 U/μl)**

Perkin Elmer, Cat. N808-0107

#### **dNTP:**

**dATP** Cat. 1051 440

**dCTP** Cat. 1051 458

**dGTP** Cat. 1051 466

**dTTP** Cat. 1051 482

Boehringer, Mannheim

#### **Salmon sperm DNA**

Sigma, Cat. D7656

#### **Sodium acetate, 3 M, pH 5.2**

Advanced Biotechnologies, Columbia, MD, Cat. 08-512-100

#### **2X SSC**

## Preparation

<b>Master Mix</b>		<b><u>final conc.</u></b>
Dextran sulfate, 50%	40 ml	20%
20X SSC, pH 7.0	20 ml	4X SSC
Sterile dH <sub>2</sub> O	40 ml	
<b>Total</b>	<b>100 ml</b>	

Vortex solution and place tube on a shaking platform overnight to insure proper mixing.

Aliquot, and store at -20°C.

## Procedure

### Primary PCR for amplification of alpha satellite sequences

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

Template DNA	200 ng
10x PCR buffer	10 µl
dNTP (2 mM)	12.5 µl
Primer 1 (20 µM)	5 µl
Primer 2 (20 µM)	5 µl
Ampli Taq Polymerase	1 µl
dH <sub>2</sub> O, add to a final volume of	100 µl

2. Carry out the PCR according to the following profile:

<b><u>Step</u></b>	<b><u>Temperature</u></b>	<b><u>Minutes</u></b>
1	94°C	2 min
2	94°C	40 sec
3	50°C	1 min
4	72°C	2 min
Repeat steps 2-4, 25 cycles		
5	72°C	5 min

2. Take 5 µl of the PCR product, check on a 1% agarose gel; if there are products one should see multiple bands--(171)n, proceed to PCR II.

### Secondary PCR for labeling of alpha satellite sequences

1. Carry out second PCR reaction mixture using product from first PCR (final volume is 50  $\mu$ l)

DNA template from primary PCR product	2 $\mu$ l
10X PCR buffer	5 $\mu$ l
dATP, dCTP, dGTP mixture (2.5 mM each)	8 $\mu$ l
dTTP (1mM)	5 $\mu$ l
Biotin-16-dUTP (1mM)	
<u>or</u> Digoxigenin-11-dUTP (1mM)	
<u>or</u> Cy5-dUTP (1mM),	5 $\mu$ l
Primer 1 (20 $\mu$ M)	2.5 $\mu$ l
Primer 2 (20 $\mu$ M)	2.5 $\mu$ l
Ampli Taq polymerase	0.5 $\mu$ l
dH <sub>2</sub> O	19.5 $\mu$ l

2. PCR reaction profile: same as step I.

### Precipitation of PCR products

1. Precipitate the secondary DNA product and redissolve in 20  $\mu$ l formamide and 20  $\mu$ l Mastermix.

### FISH hybridizations of alpha satellite DNA probes

1. Use 1  $\mu$ l of probe prepared above to perform FISH (1  $\mu$ l + 4.5  $\mu$ l formamide and 4.5  $\mu$ l Mastermix with or without other probe).  
You may adjust the probe concentration according to the signal intensity (cross hybridization can be seen if the probe concentration is too high).

### Hamster and mouse hybrid cell lines containing one single human chromosome

	Chr No	Cell Line	Other Name	Background	t(r;h)	Hum Frag	Selection
A9neo1-4	1	A9 Neo(1)	A9+1	Mouse	5%		G418
A9CH2	2	GM 11712	A9+2	Mouse			G418
A9+3	3	GM 11713	A9+3	Mouse			G418
GM10115	4	GM 11687	A9+4	Mouse			G418
A9+5	5	GM 11714	A9+5	Mouse			G418
	6	GM 11580		Hamster			Histidinol
1HL11G	7	GM 10791	1HL11g	Hamster			None
GM10897	8	GM 10156		Hamster			None
7K87-9	9	GM 10611	PK87-9	Hamster	13%	9%	Histidinol
A9+10	10	GM 11688		Mouse			G418
GM10481-7A4	11	GM 10481	PA4	Mouse			G418
A9+12	12	GM 13259	A9+12	Mouse			G418
GM00898	13	GM 10898	HHW725	Hamster	19%		None
		GM 11689		Mouse			G418
MP1D2	14	GM 10479	HDM-5	Mouse			G418
A9+15	15	GM 11715	A9+15	Mouse	5%		G418
Mouse+16	16	GM 10567	CY 18	Mouse	5%		AAT
Anna	17	GM 10498	MH22-6	Mouse			HAT
Homol+16	18	GM 11010	E2B3	Hamster		?	None
GM10449	19	GM 10449	5HL94	Hamster			None
HHW690	20	GM 13260	A9+20	Mouse		4%	G418
N08854	21	GM 08854		Mouse	5%	5%	None
A9+22	22	GM 13258	A9+22	Mouse			G418
1771A3	X	GM 10324		Mouse	8%		HAT
A9+Y	Y	GM 06317	853g30	Hamster	5%	90%	None
Control			A9	Mouse			
Control			RJK88	Hamster			