

## 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**

**Master Mix**

Dextran sulfate, 50%      40 ml

20X SSC, pH 7.0      10 ml

Sterile dH<sub>2</sub>O      50 ml

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>Step</b>	<b>Temperature</b>	<b>Minutes</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) or Digoxigenin-11-dUTP (1mM) or 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	3.	A9 Neo(1)	A9+1	Mouse	5%		G418
A9CH2	4.	GM 11712	A9+2	Mouse			G418
A9+3	5.	GM 11713	A9+3	Mouse			G418
GM10115	6.	GM 11687	A9+4	Mouse			G418
A9+5	7.	GM 11714	A9+5	Mouse			G418
	8.	GM 11580		Hamster			Histidinol
1HL11G	9.	GM 10791	1HL11g	Hamster			None
GM10897	10.	GM 10156		Hamster			None
7K87-9	11.	GM 10611	PK87-9	Hamster	13%	9%	Histidinol
A9+10	12.	GM 11688		Mouse			G418
GM10481-7A4	13.	GM 10481	PA4	Mouse			G418
A9+12	14.	GM 13259	A9+12	Mouse			G418
GM00898	15.	GM 10898	HHW725	Hamster	19%		None
		GM 11689		Mouse			G418
MP1D2	16.	GM 10479	HDM-5	Mouse			G418
A9+15	17.	GM 11715	A9+15	Mouse	5%		G418
Mouse+16	18.	GM 10567	CY 18	Mouse	5%		AAT
Anna	19.	GM 10498	MH22-6	Mouse			HAT
Homol+16	20.	GM 11010	E2B3	Hamster		?	None
GM10449	21.	GM 10449	5HL94	Hamster			None
HHW690	22.	GM 13260	A9+20	Mouse		4%	G418
N08854	23.	GM 08854		Mouse	5%	5%	None
A9+22	24.	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			