APPENDIX A

Peripheral blood culture for high resolution

Culturing

1. Aliquot 10 ml of complete medium (MEM supply with 10% FCS, 0.3 ml PHA, phytohemagglutinin) into labeled tube.
2. Add 0.5 ml of whole blood, mix well.
3. Incubate the culture at 37°C (72 hours culture)
4. On the day prior to harvest, add 0.1 ml of 10^{-5} mol/L of methotrexate (MTX) to culture and incubate overnight
5. Next day, Centrifuge culture at 1,200 rpm for 10 minutes and discard supernatant.
6. Resuspend pellets in 10 ml of MEM plain media, mix as well
7. Centrifuge culture at 1,200 rpm for 10 minutes, discard supernatant.
8. Resuspend pellets in 10 ml of supplemented MEM medium.
9. Add 0.1 ml of Thymidine to culture; incubate at 37°C for 3 hours and 10 minutes.
10. After that add Ethidium Bromide 0.1 ml, incubate at 37°C for 1 hour and 45 minutes.

Harvesting and Slide preparation

1. Add 0.2 ml of colcemid (concentration 10μg/ml); incubate at 37°C for 15 minutes.
2. Pellet cultured cells by centrifugation at 1,200 rpm for 10 minutes, discard supernatant.
3. Resuspend pellet in 10 ml 0.075M KCl, incubate at 37°C for 15 minutes.
4. Add 3 ml of fresh fixative (3:1 v/v Methanol:Glacial Acetic Acid)
5. Pellet cells by centrifugation and resuspend by fresh fixative 10 ml, gentle mix.
6. Repeat step 5 again.
7. Kept pellet at -20°C at least one day or overnight before drop sample onto glass microscope slide.

Trypsin G-banding

1. Heat slide at 60-65°C 1 day or overnight before banding.

2. Incubate the slides for 5-10 seconds in 0.025% of trypsin in saline (may be vary by the quality of chromosome)
3. Immerse slide in 1% Fetal calf serum 30 seconds (To stop trypsin reaction)
4. Stain for 5 minutes in 4% Giemsa solution in Sorrensen buffer, pH 6.8
5. Rinse the slide in a stream of distilled water and air dry.
6. Observe slide under light microscopy

Preparation of Plasmid DNA by alkaline lysis with SDS: Minipreparation

(Sambrook and Russel, 2001)

Preparation of cells

1. Inoculate 2 ml of rich medium (LB, YT, or terrific broth) containing the appropriate antibiotic with a single colony of transformed bacteria. Incubate the culture overnight at 37°C with vigorous shaking.
2. Pour 1.5 ml of the culture into a microcentrifuge tube. Centrifuge at maximum speed for 30 seconds at 4°C.
3. When centrifugation is complete, remove the medium.
4. Resuspend the bacterial pellet in 100 μl of ice-cold Alkaline lysis solution I by vigorous vertexing.
5. Add 200 μl of freshly prepared Alkaline lysis solution II, mix and store the tube on ice.
6. Add 150 μl of ice-cold Alkaline lysis solution III, inverting the tube several times and store on ice for 3-5 minutes.
7. Centrifuge the bacterial lysate at maximum speed for 5 minutes at 4°C. Transfer the supernatant to a fresh tube.
8. (Optional) Add an equal volume of phenol:chloroform. Mix the organic and aqueous phases by vortexing and then centrifuge the emulsion at maximum speed for 2 minutes at 4°C in a microfuge. Transfer the aqueous upper layer to a fresh tube.

Recovery of Plasmid DNA

9. Precipitate nucleic acids from the supernatant by adding 2 volumes of ethanol at room temperature

10. Centrifugation at maximum speed for 5 minutes at 4°C.
11. Remove the supernatant. Add 1 ml of 70% ethanol to the pellet and invert the closed tube several times.
12. Centrifugation at maximum speed for 2 minutes at 4°C. Disard supernatant.
13. Remove any beads of ethanol that form on the sides of the tube. Store the open tube at room temperature until the ethanol has evaporated and no fluid is visible in the tube.
14. Dissolve the nucleic acids in 50 µl of TE (pH 8.0) containing 20 µg/ml DNase-free RNase A (pancreatic RNase). Vortex the solution gently for a few second. Store the DNA solution at -20°C.

Nick translation

Add reation below into microcentrifuge tube

Nuclease-free water	17.5- X	µl
1 µg DNA	X	µl
0.2 mM spectrum green-dUTP or spectrum orange-dUTP	2.5	µl
0.1 dTTP	5	µl
dNTP mix	10	µl
10X nick translation buffer	5	µl
nick translation enzyme	10	µl
Total volume	50	µl

Incubate at 15°C for 16 hours and stop reaction at 72°C for 10 minute and kept at 4°C or -20°C

Precipitating the Probe

1. Pipette 5 µl of the nick translation reaction mixture into microcentrifuge tube.
2. Add 1 µg COT-1 DNA, 2 µg human placental DNA and 4 µl purified water to the tube.
3. Add 1.2 µl (0.1 volume) 3M sodium acetate, then add 30 µl (2.5 volume) of 100% EtOH to precipitate the DNA. Vortex briefly and place on dry ice for 15 minutes.

4. Centrifuge at 12,000 rpm for 30 minutes at 4°C to pellet the DNA.
5. Remove the supernatant and then add 70% EtOH, centrifuge at 12,000 rpm for 30 minutes at 4°C to pellet the DNA.
6. Remove the supernatant and dry the pellet for 10-15 minutes under vacuum at ambient temperature.
7. Resuspend the pellet in 20 µl of hybridization buffer, incubate at 37°C 1-2 hours

Hybridizing the probe to the target metaphase

Prehybridization

1. Immerse slides into 2XSSC/0.05% Tween 20 at 37°C for 30 minutes.
2. Dehydrate slides by through an ethanol series (2 minutes each at 70%, 80% and 100%), allow slide to dry.

Hybridization

3. Denature probe at 75°C 5 minutes before place probe on slide, apply a cover slip and seal with rubber solution glue and allow drying completely.
4. Denature target DNA at 72°C for 2 minutes.
5. Place slides in a humid, lightproof container, incubate in water bath at 37°C overnight.

Posthybridization

6. Remove coverslip.
7. Immerse slide in 0.4XSSC at 68°C for 1 minute.
8. Immerse slide in 2XSSC at room temperature for 2 minutes. Drain excess solution.
9. Apply 20 µl of the DAPI antifade. Apply a coverslip and allow colour to develop in the dark for 10 minutes.

Detection

10. View with a fluorescence microscope

APPENDIX B

20XSSC (stock)

NaCl	175.2 g
NaCitrate	88.2 g

Double distill water to 1 L, adjust pH = 7.4, kept at room temperature

2XSSC (500 ml.)

20 X SCC	50 ml
Double distill water	450 ml

Mix and kept at room temperature

2XSSC/0.05 % Tween 20 (500 ml.)

20 X SCC	50 ml
Double distill water	450 ml
5% Tween 20	5 ml

Mix and kept at room temperature

0.4XSSC (500 ml.)

2XSSC	100 ml
Double distill water	400 ml

Mix and kept at room temperature

Working DAPI

4,6-Diamino-2-Phenyl-indole(DAPI)	100 µl
Double distill water	900 µl
Antifade	200 µl

Mix and kept at -20°C

Antifade

p-Phenylenediamine	3-10 mg
PN buffer	5-10 ml
Glycerol	5-10 ml

Mix and kept at -20°C

Hybridization solution

50% Formamide

10% Dextran Sulfate

2X SSC

LB Medium Plate

Tryptone	10	g
Yeast extract	5.0	g
NaCl	10	g

Alkaline Lysis Solution I

50 mM glucose

25 mM Tris-Cl (pH 8.0)

10 mM EDTA (pH 8.0)

stocks 100 ml, sterilization, kept at 4°C

Alkaline Lysis solution II

0.2 N NaOH

1 % (w/v) SDS

Kept at room temperature

Alkaline Lysis Solution III

5 M potassium acetate	60.0 ml
glacial acetic acid	11.5 ml
Double distill water	28.5 ml

Kept at 4°C

0.2 mM Spectrum Green, Spectrum Orange, Spectrum Red-dUTP

1mM dUTP	10 µl
nuclease-free water	40 µl

Mix and kept at -20°C

0.1 mM dTTP

0.3 mM dTTP	10 µl
nuclease-free water	20 µl

Mix and kept at -20°C

0.1 dNTP mix

each of 0.3 mM dATP, 0.3 mM dCTP, 0.3 mM dGTP	10 µl

Mix and kept at -20°C

3M Sodium acetate (pH 7.0)

Sodium acetate $3H_2O$	408.3 g
Double distill water	800 ml

Adjust pH to 7.0 and adjust volume to 1 L, Sterilization and kept at room temperature

APPENDIX C

Table 12 List of BAC/PAC clones used in subtelomeric-specific FISH.

Chromosome	Clone	location from TEL(Mb)
1p	RP11-465B22	1.05
1q	RP11-438F14	0.26
2p	RP11-1N7	0.16
2q	RP11-341N2	0.05
3p	RP11-306H5	0.16
3q	RP11-114F20	2
4p	GS-36-P21	0
4q	RPCI-11-463J17	0.19
5p	RP11-811I15	0.07
5q	RP11-451H23	0.25
6p	RP3-416J7	0.1
6q	RP1-140C12	0.44
7p	GS-164-D18	0
7q	GS-3K-23	0
8p	RP11-1116K10	1.02
8q	RP11-152B5	0.07
9p	RP11-174M15	0.12
9q	RP11-885N19	0.01
10p	RP11-486H9	0.29
10q	RP11-108K14	0.13
11p	RP11-326C3	0.21
11q	RP11-116B3	2.9
12p	RP11-598F7	0.04
12q	GS-221-K18	0
13q	RP11-139P6	1.32
14q	RP11-417P24	0.93

Table 12 (continued)

Chromosome	Clone	location from TEL(Mb)
15q	RP11-89K11	0.22
16p	RP11-243K18	0.27
16q	RP4-597G12	0.32
17p	GS-202-L17	0.01
17q	GS-36-K4	0
18p	RP11-705O1	0.14
18q	RP11-154H12	0.43
19p	GS-546-C11	0
19q	GS-48-O23	0
20p	RP11-530N10	0.01
20q	RP11-11M20	0.28
21q	GS-2-H14b	0.09
22q	RP4-579N16	0.29
XpYp	RP13-465B17	0.31
Xq	RP11-954J6	0.36
Yq	RP11-242E13	0.44