APPENDIX

Preparation of Reagents in the Experiment

Preparation of Metaphase Chromosomes

1). Growth medium

RPMI 1640 powder (Biochrom AG)	10.42	g.
Sodium bicarbonate ($NaHCO_3$)	2	g.
Streptomycin	100	mg.
Ampicillin	30	mg.
Distilled water	1000	ml.

Dissolve and mix by magnetic stirrer with adjust pH 7.2, and then filter through sterilized filter. Before use, add fetal calf serum 20 ml in growth medium 80 ml.

2). Phytohemagglutinin (PHA)

PHA (L-form) lyophilized (Biochrom AG)

Sterile distilled water 5 ml.

Dissolve the lyophilized in sterile distilled water and store at 4-8°C.

3). Phosphate Buffer Saline (1 x PBS)

Sodium chloride (NaCl)	8	g.
Potassium chloride (KCl)	0.2	g.
Sodium hydrogen phosphate dibasic ($Na_2HPO_42H_20$)	1.15	g.
Potassium dihydrogen phosphate (KH_2PO_4)	0.2	g.
Distilled water	1000	ml.

Dissolve and mix by magnetic stirrer, autoclaving before used and store at room temperature.

4). 0.075 M Potassium Chloride (0.56% KCl)

Potassium chloride (KCl)	5.62	g.
Distilled water	1000	ml.

Dissolve and mix by magnetic stirrer, autoclaving before used and store at room temperature.

5). Sorensen's buffer

Potassium dihydrogen phosphate (KH_2PO_4)	5.26	g.
Disodium hydrogen phosphate dibasic ($Na_2HPO_42H_2O$)	8.65	g.
Distilled water	1000	ml.

Dissolve and mix by magnetic stirrer, autoclaving before used and store at room temperature.

6). 10% Giemsa Staining

Gurr's Giemsa stain (Merck)	2.5	ml.
Sorensen's buffer	25	ml.

Dilute Giemsa stain and store at room temperature.

7). 0.9% Sodium chloride (NaCl)

Sodium chloride (NaCl)	9	g.
Distilled water	1000	ml.

Dissolve and mix by magnetic stirrer, autoclaving before used and store at room temperature.

8). 2.5% Trypsin

Trypsin powder (Seromed)	2.5	g.
0.9% Sodium chloride (NaCl)	100	ml.

Dissolve and mix by magnetic stirrer and aliquot, 1 ml in each microcentrifuge tubes. Store at -20°C.

Polymerase Chain Reaction (PCR) and Agarose Gel Electrophoresis

9). 10 x PCR Buffer (Perkin Elmer)

100 mM Tris-HCl, pH 8.3

500 mM KCl

25 mM $MgCl_2$

0.1 mg/ml Gelatin

The kit is stable at 4°C. Repeated freezing and thawing should be avoided. The kit can also be stored at -20°C.

10). Deoxynucleotide triphosphate (dNTPs) 4 x 25 µmol (250 µl)

Form: Sodium salts, solution, 100 mM, pH 8.3

Contain individual vials of dATP, dCTP, dGTP, and dTTP; 250 µl each.

Aliquot and dilute to 10 mM of each dNTP with deionized sterile water into 1.5 ml microcentrifuge tubes. Store at -15°C to -25°C until ready to use.

11). AmpliTaq Gold 5 u/µl

Aliquot about 20 µl of this reagent into 0.5 ml microcentrifuge tubes. Store at -20°C in a constant temperature freezer.

12). DOP-PCR Primer 22 bp, HPLC grade, 1000 µM/µl conc.

Sequence: 5'-CCGACTCGAGNNNNNNATGTGG-3'

Aliquot and dilute to 40 µM/µl with deionized sterile water into 0.5 ml microcentrifuge tubes. Store at -20°C

13). 1 Kb Plus DNA LADDER, 0.1 µg/µl conc.

1 Kb Plus 10 µl.

TE buffer 90 µl.

Vortex and store at -20°C.

14). 5 x Tris-Borate-EDTA Buffer (TBE buffer), pH 8.0

Tris borate	108	g.
Boric acid	55	g.
0.5 M EDTA	40	ml.
Distilled water	960	ml.

Dissolve and mix by magnetic stirrer and adjust the solution to pH 8.0. Store at room temperature. Dillute to 0.5 x TBE when using.

15). Gel Loading Buffer (6 x Dye)

Bromophenol blue	0.25%
Xylene cyanol FF	0.25%
Glycerol	30%

Dissove and mix in distilled water by magnetic stirrer. Store at 4°C in aliquot.

16). 1% Agarose gel

Agarose powder (Sigma)	1	g.
0.5 x TBE buffer (pH 8.0)	100	ml.

Dissolve and melt over heater before use. Store at room temperature.

17). 0.5 μg/ml Ethidium Bromide Solution (working solution)

Ethidium bromide	0.5	g.
Distilled water	50	ml.

Dissolve and mix well for several hours with shaker. Store stock solution at room temperature in a dark bottle to prevent exposure to light. For working, dilute 10 μl of stock into 200 ml distilled water.

Fluorescence *in Situ* Hybridization

18). 2% AES (3-Aminopropyltriethoxysilane)

AES	2	ml.
Acetone	98	ml.

Prepare this solution for coating slides (1" x 3"). At first, immerse these slides in cleaning solution for overnight or until ready to use. Then, rinse slides with tap water and then 3 times in distilled water. Store in 70% ethanol at least one day.

When coating, leave these slide to dry. Then, coat in 2% AES and immerse in acetone 3 times, rinse with distilled water for 1 minute each. After drying, store the coated slide in the storage case slide at room temperature.

19). TE buffer

(contain 10 mM Tris-HCl, pH 8.0 and 1 mM EDTA)

Tris-HCL	158	mg.
EDTA	37	mg.
Distilled water	100	ml.

Dissolve and mix well by magnetic stirrer with adjusting to pH 8.0. Store at room temperature.

20). 3 M Sodium Acetate (pH 5.5)

Sodium acetate	40.8	g.
Distilled water	80	ml.

Dissolve and adjust the solution to pH 5.5 with glacial acetic acid. Add distilled water to a final volume of 100 ml, and store at room temperature.

21). 1 M $MgCl_2$ (Molecular weight 203.3 g)

$MgCl_2$	10.165	g.
Distilled water	50	ml.

Dissolve and mix by magnetic stirrer. Store at room temperature.

22). 0.5 M EDTA, pH 8.0

($C_{10}H_{14}N_2Na_2O_8.2H_2O$: M = 372.24 g/mol)

EDTA	18.612	g.
Distilled water	100	ml.

Dissolve and mix by magnetic stirrer. Adjust the pH to 8.0 with NaOH. EDTA will not dissolve completely until pH is greater than about 7. Adjust the final volume to 100 ml and autoclave. Store at room temperature.

23). Hybridization mixture

(contain 50% formamide, 2 x SSC, and 10% dextran sulphate)

Deionized formamide	500	µl.
20 x SSC	100	µl.
Distilled water	400	µl.
Dextran sulphate	0.1	g.

Vortex until dextran sulphate is dissolved. Store at -20°C.

24). Dig-Nick Translation Mix (digoxigenin-11-dUTP)

One vial containing 160 µl., 5 x conc.

Stabilized reaction buffer in 50% glycerol (v/v) and DNA polymerase I, DNase I, 0.25 mM dATP, 0.25 mM dCTP, 0.25 mM dGTP, 0.17 mM dTTP, and 0.08 mM DIG-11-dUTP.

The solution is stable at –15 to -25°C. Repeated freezing and thawing should be avoided. To avoid contamination we recommend to aliquot and store in 2-3 portions. (Roche)

25). Anti-digoxigenin-rhodamine, Fab fragment, 200 µg.

The lyophilized conjugates are stable at 4°C when stored protected from light. Dissolving the lyophilisate in 1 ml distilled water results in a concentration of 200 µ g/ml. The reconstituted solutions are stable at 4°C for 2 months, when stored protected from light. Stored in aliquots at -20°C; avoid repeated freezing and thawing. (Roche)

26). Biotin-Nick Translation Mix (Biotin-16-dUTP)

One vial containing 160 µl, 5 x conc.

Stabilized reaction buffer in 50% glycerol (v/v) and DNA polymerase I, DNase I, 0.25 mM dATP, 0.25 mM dCTP, 0.25 mM dGTP, 0.17 mM dTTP, and 0.08 mM biotin-16-dUTP.

The unopened vial is stable at –15 to 25°C through the expiration date printed on the label. Repeated freezing and thawing should be avoided. To avoid contamination we recommend to aliquot and store in 2-3 portions. (Roche)

27). Avidin-Fluorescein, 1 mg.

The lyophilized conjugate is stable at 2-8°C when stored dry and protected from light.

Dissolve the lyophilisate in 1 ml distilled water. This results in a 1 mg/ml stock solution. The reconstituted solution should be stored in aliquots at –15 to -25 ° C protected from light. Avoid repeated freezing and thawing. (Roche)

28). 10 mg/ml RNase

RNase	50	mg.
Distilled water	5	ml.

Mix well and store in aliquots at -20°C

29). Post Fixation Treatment

Formaldehye	1	µl.
1 M $MgCl_2$	5	µl.
1 x PBS	94	µl.

100 µl. of the solution is used per slide. The solution should be prepared fresh before using.

30). 10% Pepsin

Pepsin	0.5	g.
Distilled water	5	ml.

Mix well and store in aliquots at -20°C

31). 70% formamide in 2 x SSC (Denature solution)

Deionized formamide	70	µl.
20 x SSC	10	µl.
Distilled water	20	µl.

100 µl of the solution is used per slide. Warm the solution at 75°C at least 30 minutes before using.

32). 50% formamide in 2 x SSC (Posthybridization washes solution)

Deionized formamide	15	µl.
20 x SSC	3	µl.
Distilled water	12	µl.

Mix well by magnetic stirrer. Warm at 42°C at least 30 minutes before using. Store at 4°C.

33). Cot-1 DNA, Human, 500 µg. (500 µl)

(concentration : 1 mg/ml, 10 mM Tris-HCl, 1 mM EDTA, pH 7.4)

Store at –15 to -20°C until use. Repeated freezing and thawing does not deteriorate the product.

34). 0.1% tween 20 in PBS

Tween 20	1	ml.
1 x PBS	999	ml.

Mix wells by magnetic stirrer and adjust to pH 7.0. Autoclave and store at room temperature.

35). 10% Blocking reagent

Blocking reagent (powder)	0.5	g.
Maleic acid buffer	5	ml.

Dissolve in maleic acid buffer (100 mM Maleic acid, 150 mM NaCl, pH 7.5 (20°C), adjusted with conc. or solid NaOH, sterile) with shaking and heating either on a heating block or in a microwave oven. This stock solution is autoclaved and stored at 2-8°C or –15 to -25°C subsequently. (Roche)

36). DAPI, 5 mg/ml conc.

DAPI (lyophilized)	10	mg.
Distilled water	2	ml.

Mix well and aliquot to microcentrifuge tubes. Store at -20°C protected from light. Dilute to 0.5 μg/ml when using.

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