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APPENDIX A

BUFFERS AND REAGENT

1. Lysis Buffer 1

Sucrose	109.54	g
1.0 M Tris – HCl (pH 7.5)	10	ml
1.0 M $MgCl_2$	5	ml
Triton X – 100 (pure)	10	ml
Distilled water to	1,000	ml

Sterilize the solution by autoclaving and store in a refrigerator (at $4^{0}C$).

2. Lysis Buffer 2

5.0 M NaCl	15	ml
0.5 M EDTA (pH 8.0)	48	ml
Distilled water to	1,000	ml

Sterilize the solution by autoclaving and store at room temperature.

3. 10% SDS solution

Sodium dodecyl sulfate	10	g
Distilled water to	100	ml

Mix the solution and store at room temperature.

4. 20 mg/ml Proteinase K

Proteinase K	2	mg
Distilled water to	1	ml

Mix the solution and store in a refrigerator (at -20^{0}C).

5. 1.0 M Tris – HCl

Tris base	12.11	g

Dissolve in distilled water and adjusted pH to 7.5 with HCl

Distilled water to	100	ml

Sterilize the solution by autoclaving and store at room temperature.

6. 0.5 M EDTA (pH 8.0)

Disodium ethylenediamine tetraacetate.2H	186.6	g

Dissolve in distilled water and adjusted pH to 8.0 with NaOH

Distilled water to	1,000	ml

Sterilize the solution by autoclaving and store at room temperature.

7. 1.0 M $MgCl_2$ solution

Magnesium chloride.6H_2O	20.33	g
Distilled water to	100	ml

Dispense the solution into aliquots and sterilize by autoclaving.

8. 5 M NaCl solution

Sodium chloride	29.25	g

Distilled water to	100	ml

Dispense the solution into aliquot and sterilize by autoclaving.

9. 10X Tris borate buffer (10X TBE buffer)

Tris – base	100	g
Boric acid	55	g
0.5 M EDTA (pH 8.0)	40	ml

Adjust volume to 1,000 ml with distilled water. The solution was mixed and store at room temperature.

10. 6X loading dye

Bromphenol blue	0.25	g
Xylene cyanol	0.25	g
Glycerol	50	ml
1M Tris (pH 8.0)	1	ml
Distilled water until	100	ml

Mixed and stored at $4^{0}C$

11. 7.5 M Ammonium acetate (CH_3COONH_4)

Ammonium acetate	57.81	g
Distilled water	80	ml

Adjust volume to 100 ml with distilled water and sterilize by autoclaving.

12. 25:24:1 (v/v) Phenol-chloroform-isoamyl alcohol

Phenol	25	volume
Chloroform	24	volume
Isoamyl alcohol	1	volume

Mix the reagent and store in a sterile bottle kept in a refrigerator.

13. 12% Non-denature acrylamide gel (w/v)

40%acrylamide: Bis (19:1)	3	ml
5X TBE	1	ml
10% ammoniumpersulfate	105	µl
TEMED	8	µl
H_2O	6	ml

Dissolve by heating in microwave oven and occasional mix.

14. TE buffer

Tris base	1.21	g
5M EDTA	200	µl

Adjust pH to 7.5 with conc.HCL and adjust volume to 1.0 litre with H_2O.

APPENDIX B

Sequence of LINE-1 and primer

Human transposon L1.2 ACCESSION M80343 (5′UTR)

BEFORE BISULFITE

Gggggaggagccaagatggccgaataggaacagctccggtctacagctcccagcgtgagcgacgcagaa
gacggtgatttctgcatttccatctgaggtaccgggttcatctcactagggagtgccagacagtgggcgcaggcca
gtgtgtgtgcgcaccgtgcgcgagccgaagcagggcgaggcattgcctcacctgggaagcgcaagggggtcag
ggagttccctttctgagtcaaagaaaggggtgacggtcgcacctggaaaatcgggtcactcccacccgaatattgc
gcttttcagaccggcttaagaaacggcgcaccacgagactatatcccacacctggctcggagggtcctacgccc
acggaatctcgctgattgctagcacagcagtctgagatcaaactgcaaggcggcaacgaggctgggggaggggg
cgcccgccattgcccaggcttgcttaggtaaacaaagcagc

AFTER BISULFITE

GggggaggagTTaagatggTCGaataggaaTagTtTCGgtTtaTagTtTTagCGtgagCGaCG
TagaagaCGgtgatttTtgTatttTTatTtgaggtaTCGggttTatTtTaTtagggagtgTTagaTagtgggC
GTaggTTagtgtgtgtgCGTaTCGtgCGCGagTCGaagTagg

FCORBA LINE-1

gCGaggTattgTTtTaTTtgggaagCGTaaggggtTagggagttTTTtttTtgagtTaaagaa

TasI TaqI

AggggtgaCGgtCGTaTTtggaaaatCGggtTaTtTTTaTTCGaatattgCGTttttTaga

RCORBA LINE-1

TCGgTttaagaaaCGgCGTaTTaCGagaTtatatTTTaTaTTtggTtCGgagggtTTtaCGTTTaC
GgaatTtCGTtgattgTtagTaTagTagtTtgagatTaaaTtgTaaggCGgTaaCGaggTtgggggagg
ggCGTTCGTTattgTTTaggTttgTttaggtaaaTaaagTagT

F COBRALINE-1 = ccgTaaggggtTagggagttTTT

R COBRALINE-1 = RTAAAACCCTCCRAACCAAATATAAA

Total amplicon = 160 bp and TasI = 63+ 97 and TaqI = 80+80

BIOLOGRAPHY

Miss Krisanee Chalitchagorn was born in Nakornsawan in1979. She graduated from Faculty of Science, Chulalongkorn University in Biochemistry program and then attended to participate in Medical Science program in Faculty of Medicine for her master degree.