
APPENDIX A

Appendix B: Buffer and chemical preparation

1x Red cell lysis Buffer

0.32 M sucrose	54.77 g
75%T Triton-x-100	3.75 ml
5mM Magnesium chloride(MgCL ₂)	2.5 ml
1mM Tris-Hcl; PH 7.5	5 ml
Distilled Water (dh ₂ O) to	500 ml

Sucros was dissolved in 450 ml of dh₂O; Triton-x-100, MgCL₂ and Tris-Hcl were added and the final volume was topped up to 500 ml. The resolution solution was stored in 4⁰c.

10 x proteinase k Buffer

0.375 M sodium chloride (Nacl)	10ml
0.12 EDT	10ml

The solution above were added and the resultant solution was stored in room tempreture.

20% (W/V) SODIUM Dodecyl sulphate (SDS)

SDS	20 g
Distilled water to (dh ₂ O)	100 ml

AS SDS was hard to to dissolve ,it was added in dh₂O little by little. The final volume was topped up to 100 ml and the solution was stored in room temperture.

6M sodium chloride (Nacl)

Nacl	35.0 g
Distilled Water (dh ₂ O) to	100ml

Nacl was dissolved in dh₂O and the final volume was made up to 100 ml. Nacl was stored in room temperture.

Phenol-chloroform (1:1; w/v)

Phenol	250 ml
Chloroform	250 ml

Equal volume of phenol and chloroform were mixed. As the chemicals were photosensitive, the bottle for the mixture was wrapped with aluminum foil. The resultant solution was stored at 4⁰c to ensure the mixture mixed well.

70% (v/v) Ethanol

Ethanol (100%)	70 ml
Distilled Water (dh ₂ O)	30 ml

Ethanol and Distilled Water were mixed and the resulting 70% ethanol was stored in 4⁰c.

TE buffer; PH 8.0

10 m M Tris -Hcl	50ml
1Mm EDTA	5 ml
Distilled Water (dh ₂ O) to	500 ml

Tris-Hcl and EDTA were mixed, PH of resulting solution was adjusted to 8.0 and its final volume was made up to 500 ml. TE buffer was stored in RT.

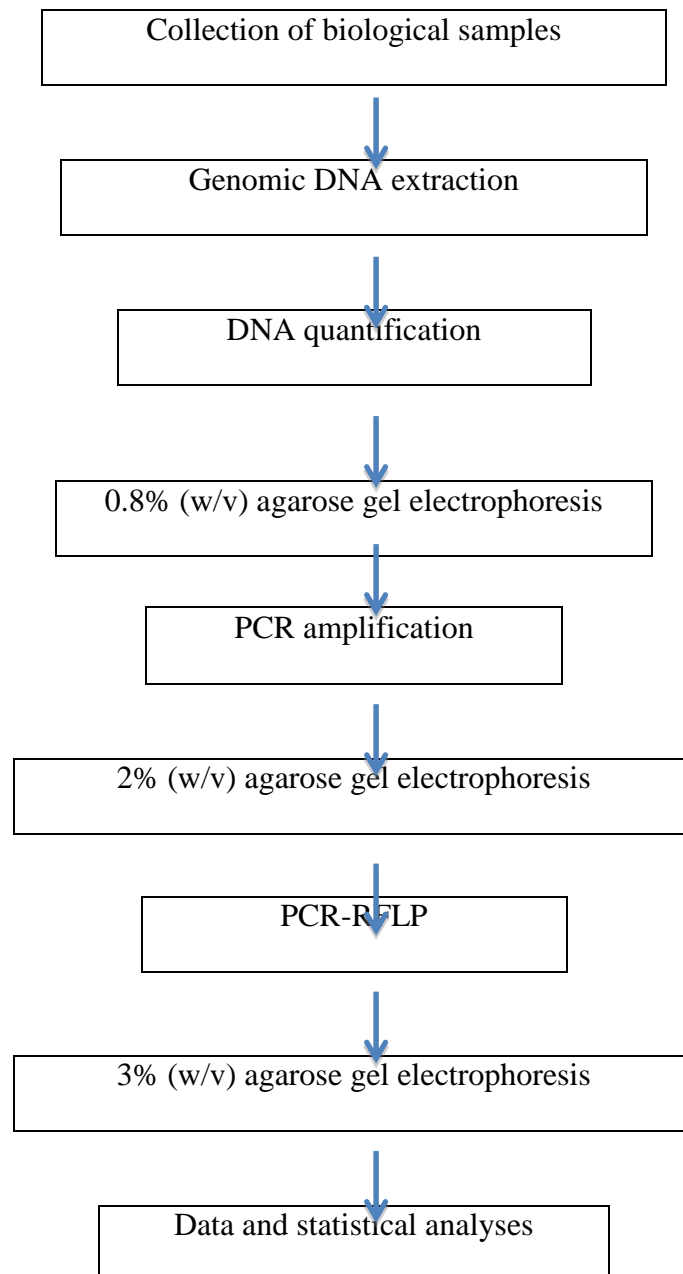
5X TBE

Tris base	49 g
Boric acid	27.5 g
EDTA	3.72 g
Distilled Water (dh ₂ O) to	1L

EDTA was adjusted to PH 8.0 befor mixing it with tris base and boric acid. Distilled water aws added to top up to the final volume of 1 L. TBE was stored in RT and diluted 1x befor usage.

APPENDIX B

Flowchart of procedures which carried out in this study.



APPENDIX C

Sequence of IL10 gene which include -1082 and -819 SNPs

SNP IL10819:

gnl|dbSNP|rs1800871|allelePos=401|totalLen=801|taxid=9606|snpclass=1|alleles='C/
T'|mol=Genomic|build=132

CCTTCCCCAGGTAGAGCAACACTCCTCGCCGCAACCCAACTGGCTCCCCTTACCTTCTACA
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TGGAATCCTGACTTCTTTTCTTGTATTTCAACTTCTTCCACCCCATCTTTTAACTTTAGACTCCA
GCCACAGAAGCTTACAATAAAAGAACTCTAAGGCCAATTTAATCCAAGGTTTCATTCTATGTGC
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GTAAAATGGAAGGGCTGCTTGGGAACTTTGAGGATATTTAGCCCACCCCTCATTCTTACTTGGGG
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SNP IL101082 :

gnl|dbSNP|rs1800896|allelePos=414|totalLen=913|taxid=9606|snpclass=1|alleles='A/
G'|mol=Genomic|build=132

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