APPENDIX A

List of the chemicals and materials were used in the study

Chemicals / Materials	Source
Absolute ethanol	E. Merk, Germany
Agarose gel	Sigma chemical Co., USA.
Amidoblack B	Sigma chemical Co., USA.
Ammonium sulphate	E. Merk, Germany
Beta mercaptoethanol	Sigma chemical Co., USA.
Boric acid	E. Merk, Germany
Bovine serum albumin	Gibco, USA.
Brilliant cresyl blue	Sigma chemical Co., USA.
Bromophenol blue	Sigma chemical Co., USA.
Deoxynucleotide triphosphates	Promega, Medison WI, USA
Dimethyl sulfoxide	Sigma chemical Co., USA.
Dioxan	Labscan , Ireland
Disodium hydrogenphosphate	E. Merk, Germany
Ethidium bromide	Amarican resesch product Co., USA
Ethylenediaminetetra-acetic acid	E. Merk, Germany
Glycerol	E. Merk, Germany
Hydrochloric acid	J.T. Baker, England
Isopropanol	Riedel-dehaen, Germany
Magnesium chloride	E. Merk, Germany
Oligonucleotide primer	Gibco, USA.
Ponceau S	Sigma chemical Co., USA.
Potassium cyanide	E. Merk, Germany
Potassium hexacyanoferate (III)	E. Merk, Germany
Proteinase K	Amarican resesch product Co., USA
Sodium citrate	E. Merk, Germany
Sodium chloride	E. Merk, Germany

Sodium dodesyl sulphate	Sigma chemical Co., USA.
Sodium hydrogencarbonate	E. Merk, Germany
Sodium hydroxide	E. Merk, Germany
Sodium sulphate	E. Merk, Germany
Sucrose	E. Merk, Germany
Tag DNA polymerase	Promega, Medison WI, USA
Trichloroacetic acid	E. Merk, Germany
Tris (hydroxymethyl) aminomethane	E. Merk, Germany

APPENDIX B

List of instruments used in the study

Instrument	**Model**	**Source**
Centrifuge	202MC	Sigma, Germany
DNA thermal cycle	2000	Perkin-Elmer cetus Ins, USA
High speed microcapillary hematocrit centrifuge	D-7200	Hettich,Germany
pH meter	PHI 34	Backman, USA
Power supply		
- for agarose gel electrophoresis	250	BRL, USA.
- for cellulose acetate electrophoresis	4063	Titan Helena, USA
Refrigerator (-20 °C)	SF-C95	Sanyo, Japan
Spectrophotometer	spectronic20	Miltonroy Co, USA.
UV-detector and Gel doc thermal printer	Gel Doc1000	Bio-Rad, USA

APPENDIX C

Lists of solutions and buffers were used in the study

1C. Solutions and buffers for DNA extraction

1. Red cells lysis buffers

1.0 M Tris(pH 7.65)	2.5	ml
1.0 M $MgCl_2$	1.25	ml
5.0 M NaCl	0.5	ml
distilled water to	250	ml

Sterized by autoclaving and store at 4°C.

2. Nuclei lysis buffer

1.0 M Tris (pH 8.0)	12.5	ml
0.5 M EDTA(pH 8.0)	0.5	ml
5.0 M NaCl	5.0	ml
distilled water to	500	ml

Sterized by autoclaving and store at roomtemperature.

3. Proteinnase K solution

0.5 M EDTA(pH 8.0)	40	µl
10% SDS	1.0	ml
proteinase K	20	mg
distilled water to	10	ml

Dispense into aliquots and store at -20°C.

4. Tris-EDTA (TE) buffer

1.0 M Tris (pH 8.0)	5	ml
0.5 M EDTA(pH 8.0)	100	µl
deionized water to	500	ml

Sterized by autoclaving and store at room temperature

2C. Solutions and buffers for agarose gel electrophoresis

1. 10X Tris-borate (TBE) buffer

Tris base	54	g
boric acid	27.5	g
0.5 M EDTA(pH 8.0)	20	ml
distrilled water to	1,000	ml

Mix throughly and store at room temperature.

2. Gel-loading buffer

bromophenol blue	0.25	%
sucrose in water	40	%(W/V)

Mix throughly and store at 4°C.

3. Stock ethidium bromide (10 mg/ml)

ethidium bromide	100	mg
distilled water	10	ml

Mix throughly until dissolve. Store at room temperature in a dark bottle.

3C. Solutions and buffers for hemoglobin electrophoresis in cellulose acetate

1.Tris-EDTA-Borate buffer pH 8.6

Tris	6.05	g
EDTA	0.78	g
distilled water	1,800	ml

Adjust pH to 8.6 with concentrated HCL. Adjust the volume to 2,000 ml with distilled water. Mix throughly and store at room temperature.

2.Boric acid-NaOH buffer pH 9.0

Boric acid	18.55	g
NaOH	5	g

distilled water	900	ml

Adjust pH to 9.0 with concentrated HCL. Adjust the volume to 1,000 ml with distilled water. Mix throughly and store at room temperature.

3. Ponceau S

Ponceau S	0.2	g
TCA	3.0	g
distilled water	100	ml

Mix throughly and store at room temperature.

4. Reagent for membrane transparency

isopropanol	30	ml
dioxan	70	ml

Mix throughly and store at room temperature.

4C. Solutions and buffers for Hematological examination

1. Drabkin's solution

$K_3Fe(CN)_6$	0.1	g
KCN	0.025	g
$NaHCO_3$	0.5	g
distilled water to	500	ml

Mix throughly and store in a brown bottle at room temperature only one month.

2. Saline's solution for osmotic fragility test

Na_2HPO_4	1.1359	g
$NaH_2PO_4.H_2O$	0.2318	g
NaCl	2.812	g
37% glycerol	15.668	ml
distilled water to	1,000	ml

Stable for several months at room temperature and kept well stoppered.

3. Grower's solution

$NaSO_4$	12.5	g
glacial acetic acid	33.3	ml
distilled water to	200	ml

Mix throughly and store at room temperature.

4. 1% Brilliant cresyl blue

Brilliant cresyl blue	1	g
Sodium citrate	0.4	g
0.85% NaCl	100	ml

Mix throughly until dissolve. Store at room temperature.

5. Amidoblack B.0.1% Amidoblack B.

Amidoblack B	0.25	g
80% ethyl alcohol (pH 2)	250	ml

Mix throughly until dissolve. Store at room temperature.

VITA

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