

## 11 ANNEXES

### Annex 1 Chemicals and buffers

#### Stock Phosphate-buffer Saline solution (PS)

16,90 g	Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O
0,78 g	NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O
4,25 g	NaCl
1000 ml	distilled water

The pH-value is adjusted to pH 7.3 with 5% H<sub>3</sub>PO<sub>4</sub> and the solution is autoclaved.

#### Stock Phosphate-buffer Saline Glucose solution (PSG)

400 ml	PS
600 ml	distilled water
25 g	Glucose

The pH-value is adjusted to pH 7.3 with 5% H<sub>3</sub>PO<sub>4</sub> and the buffer is sterilized and stored in the refrigerator.

Three parts of 100% Glycerol solution is mixed with seven parts of PSG to obtain 30% glycerol solution.

#### Lysis buffer (pH 7.7)

0.32 M Sucrose	10.9 g
Tris (0.01 M)	0.121 g
MgCl <sub>2</sub>	0.1 g
Triton X-100 (1%)	1.0 ml
Distilled Water	100 ml

The pH values were adjusted to 7.5 using either 1 M HCl or NaOH, and stored at room temperature.

10 x PCR buffer (pH 8.3)

100 mM Tris	1.211 g
500 mM KCl	3.728 g
Triton X-100 (1%)	1.0 ml
DNase and RNase free water	100 ml

The pH value was adjusted to 8.3, the buffer was aliquated in 2 ml tubes and stored at room 4°c.

10 x Electrophoresis buffer (PH 8) stock solution

0.36 M Tris	43.6 g
0.30 M NaH <sub>2</sub> PO <sub>4</sub>	41.4 g
0.01M EDTA	3.72 g
Distilled water	1000 ml

The working solution (1x) was prepared by diluting the stock solution (10x) 1/10 diluted (1x). Bothe the stock and working solutions were stored at 4°c.

Ethidium bromide stock solution

Ethidium bromide	10.0 mg
DNase and RNase free water	1.0 ml

The solution is stored at room temperature.

Proteinase-K

Proteinase-K	10.0 mg
DNase and RNase free water	1.0 ml

The solution is aliquated and stored at room temperature.

Sample buffer

1 x Electrophoresis buffer	5.0 ml
50% Glycerin	5.0 ml
100 mM EDTA	0.37 g
0.05% Bromophenolblue	5.0 mg

The solution is stored at room temperature.

DNA Marker

DNA Marker 50 or 100 bp	50.0 $\mu$ l
1 x Electrophoresis buffer	400.0 $\mu$ l
Sample buffer	50.0 $\mu$ l

The solution is aliquated and stored at - 20°C.

Agarose Gel (2%)

Agarose	2.0 g
1 x Electrophoresis buffer	100.0 ml
Ethidium bromide stock solution	10.0 $\mu$ l

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**Annex 2      Equipment and consumables**

Adjustable Pipettes (10, 100, 1000 $\mu$ l)	Eppendorf, Hamburg, Germany
Balance	Sartorius GmbH, Göttingen, Germany
Centrifuge	Heraeus Spatech GmbH, Osterode, Germany
DE-52-Cellulose	Whatman, Springfield, England
Thermocycler	Trio-Thermoblock <sup>TM</sup> (Whatman Biometra, Göttingen, Germany)
Electrophoresis chamber	Whatman Biometra, Göttingen, Germany
Freezer	Bosch, Germany
Gene Quant Calculator	Amersham Pharmacia Biotech, Freiburg, Germany
Minicentrifuge	Roth GmbH, Karlsruhe, Germany
Pipettes tips (10, 100, 1000 $\mu$ l)	Biozyme Hessisch Oldendorf, Germany
Polaroid film	Polaroid GmbH, Offenbach, Germany
Silicone membrane	Wacker Chemie GmbH, Munich, Germany
Transilluminator	Whatman Biometra, Göttingen, Germany
Vortex	Heidolph Instruments GmbH, Seelbach, Germany
Water bath	Julabo Labortechnik GmbH, Seelbach, Germany
Whatman filter paper No. 1	Whatman Biometra, Göttingen, Germany

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**Annex 3 Reagents**

Agarose	Oncor Appligene, Heidelberg, Germany
Ampli Taq Gold <sup>®</sup> DNA polymerase	Applied Biosystems, GmbH, Darmstadt, Germany
Boric acid	Sigma Chemie GmbH, Deisenhofen, Germany
Bromophenol blue	Pharmacia LKB, Uppsala, Sweden
DNA Marker (100 bp)	Gibco, Life Technologies, Karlsruhe, Germany
DNase and RNase free water	Sigma Chemie GmbH, Deisenhofen, Germany
dNTP's	Amersham Pharmacia Biotech, Freiburg, Germany
DTT (Dithiothreitol)	Sigma Chemie GmbH, Deisenhofen, Germany
Ethidium bromide	Sigma Chemie GmbH, Deisenhofen, Germany
KCl	Merck KGaA, Darmstadt, Germany
MgCl <sub>2</sub>	Sigma Chemie GmbH, Deisenhofen, Germany
MgCl <sub>2</sub> x 6H <sub>2</sub> O	Sigma Chemie GmbH, Deisenhofen, Germany
NaOH	Merck KGaA, Darmstadt, Germany
Oil (mineral oil)	Sigma Chemie GmbH, Deisenhofen, Germany
Proteinase-K	Merck KGaA, Darmstadt, Germany
Restriction enzymes	New England BioLabs Inc.*,
Sucrose	Merck KGaA, Darmstadt, Germany
Tris	Sigma Chemie GmbH, Deisenhofen, Germany
Triton X-100	ma Chemie GmbH, Deisenhofen, Germany