APPENDICES

APPENDIX A

Reagents and buffers preparation

1. LB broth (Luria-Bertani broth)

Trypton	10 g
Yeast extract	5 g
NaCl	10 g
Distilled H_2O	1 L

Sterillize by autoclaving

2. LB with amplicilin agar

Trypton	10 g
Yeast extract	5 g
NaCl	10 g
Agar	15 g
Distilled H_2O	1 L

Sterillize by autoclaving

Ampicillin 20 mg/ml

3. LB with Kanamycin agar

Trypton	10 g
Yeast extract	5 g
NaCl	10 g
Agar	15 g
Distilled H_2O	1 L

Sterillize by autoclaving

Kanamycin 50 mg/ml

4. SOC medium (Super optimal broth)

Tryptone	20 g
Yeast Extract	5 g
5M NaCl	2 ml
1M KCl	2.5 ml
1M $MgCl_2$	10 ml
1M $MgSO_4$	10 ml
1M glucose	20 ml
Distilled H_2O	1 L

Sterillize by autoclaving

5. 0.1 M $CaCl_2$

1M $CaCl_2$	100 ml
Distilled H_2O	900 ml

6. 0.1 M $CaCl_2$+ 15% glycerol

1M CaCl2	100 ml
50 % glycerol	300 ml
Distilled H2O	600 ml

7. Reagent for electrophoresis

1.5 % agarose gel

LE Agarose	1.5 g
0.5X TAE buffer	100 ml

Heat the solution in microwave

50X Tris-aceiate buffer (TAE)

Tris base	242 g
Glacial acetic acid	57.1 g
0.5 M EDTA (pH 8.0)	100 ml

Dissolve in 1,000 ml distilled water

Sterilization by autoclaving and store at room temperature

APPENDIX B

Instruments

Instruments	Source
Centrifuge	Eppendorf, North America
Chromo4thReal-time PCR detector	Bio-Rad, USA
Shaking incubator	N-Biotek, Korea
UV spectrophotometry	Shimadzu, North America
Water bath	Shel Lab, USA

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