APPENDIX C

Reagents for LPS, CT, and TcpA preparation

1. $NaHCO_3$ (5%)

This solution was prepared by dissolving 5 g $NaHCO_3$ in 100 ml DW.

2. NaCl (0.85%)

This solution was prepared by dissolving 8.5 g NaCl in 1,000 ml DW.

3. Tris-HCl (1 M, pH 8.6)

Tris base (hydroxymethyl aminomethane) (121.1 g) was dissolved in 800 ml distilled water. The pH was adjusted to 8.6 with concentrated hydrochloric acid and the volume was made up to 1,000 ml.

4. NaCl (0.85% in 10 mM Tris-HCl, pH 8.6)

Ten ml of 1 M Tris-HCl was diluted with 990 ml 0.85% NaCl and the pH was checked and adjusted to 8.6 with 1 N NaOH.

5. Tris-HCl (50 mM, pH7.4)

Tris base (hydroxymethyl aminomethane) (6.06 g) was dissolved in 800 ml DW. The pH was adjusted to 7.4 with concentrated hydrochloric acid and the volume was made up to 1,000 ml.

6. TEAN buffer, pH 7.4

The TEAN buffer consisted of the following:

NaCl	11.76 g
NaN_3	1.95 g
EDTA (ethylenediamine tetraacetic acid tetrasodium salt)	3.80 g

All ingredients were dissolved in 1,000 ml 50 mM Tris-HCl, pH 7.4.

7. D-galactose (1 M in TEAN buffer, pH 7.4)

D-galactose 180.16 g was dissolved in 1,000 ml TEAN buffer, pH 7.4.

8. LB medium (with ampicillin)

The LB medium consisted of the following:

Tryptone	10 g
Yeast extract	5 g
NaCl	5 g

All ingredients were dissolved in 950 ml deionized water. The pH was adjusted to 7.5 with 5 M NaOH and the volume was made up to 1,000 ml and medium was autoclaved at 15 lb/inch^2 for 20 min. The solution was allowed to cool to about 55°C. Ampicillin was added to a final concentration of 50 μg/ml. The medium was stored at 4°C. It was stable for only 1-2 weeks.

9. LB agar plates (with ampicillin)

The LB agar plates consisted of the following:

Tryptone	10 g
Yeast extract	5 g
NaCl	5 g
Agar	15 g

All ingredients were dissolved in 950 ml deionized water. The pH was adjusted to 7.5 with 5 M NaOH then the volume was made up to 1,000 ml and the preparation was autoclaved at 121°C, 15 lb/inch^2 for 20 min. It was allowed to cool to about 55°C. Then ampicillin was added to a final concentration of 50 μg/ml. The medium was poured into petridishes. The agar in the plates was allowed to solidify, then the plates were inverted, and stored at 4 °C. The medium containing ampicillin was stable for 1-2 weeks. The agar surface was dried before use.

10. 6 M Guanidinium Lysis Buffer, pH 7.8

The 6 M Guanidinium Lysis Buffer consisted of the following:

Guanidine HCl	95.53 g
NaH_2PO_4	2.76 g
NaCl	29.22 g

All ingredients were dissolved in 500 ml deionized water. The pH was adjusted to 7.8 and then the volume was made up to 1,000 ml.

11. Denaturing Binding Buffer, pH 7.8

The Denaturing Binding Buffer consisted of the following:

Urea	480.48 g
NaH_2PO_4	2.76 g
NaCl	29.22 g

All ingredients were dissolved in 500 ml deionized water. The pH was adjusted to 7.8 and then the volume was made up to 1,000 ml.

12. Denaturing Wash Buffer, pH 6.0

The Denaturing Wash Buffer consisted the following:

Urea	480.48 g
NaH_2PO_4	2.76 g
NaCl	29.22 g

All ingredients were dissolved in 500 ml deionized water. The pH was adjusted to 6.0 and then the volume was made up to 1,000 ml.

13. Denaturing Wash Buffer, pH 5.3

Denaturing Wash Buffer, pH 5.3 was prepared by using a 10 ml aliquot of the Denaturing Wash Buffer, pH 6.0, adjusted the pH o 5.3 by using HCl.

14. Denaturing Elution Buffer, pH 4.0

The Denaturing Elution Buffer consisted the following:

Urea	480.48 g
NaH_2PO_4	2.76 g
NaCl	29.22 g

All ingredients were dissolved in 500 ml deionized water. The pH was adjusted to 4.0 and then the volume was made up to 1,000 ml.