7. Appendix
7.1. Solutions for preparing Resolving Gels for Tris-glycine SDS-PAGE

For native PAGE gel was prepared excluding SDS in the gel.

<table>
<thead>
<tr>
<th>Components</th>
<th>Volume (ml) of Components required to cast gels of indicated volumes and concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gel Volume</td>
</tr>
<tr>
<td>10 % gel</td>
<td></td>
</tr>
<tr>
<td>H$_2$O</td>
<td>1.9</td>
</tr>
<tr>
<td>30 % Acrylamide mix</td>
<td>1.7</td>
</tr>
<tr>
<td>1.5 M Tris(pH 8.8)</td>
<td>1.3</td>
</tr>
<tr>
<td>10 % SDS</td>
<td>0.05</td>
</tr>
<tr>
<td>10 % Ammonium persulfate</td>
<td>0.05</td>
</tr>
<tr>
<td>TEMED</td>
<td>0.002</td>
</tr>
<tr>
<td>12 % gel</td>
<td></td>
</tr>
<tr>
<td>H$_2$O</td>
<td>1.6</td>
</tr>
<tr>
<td>30 % Acrylamide mix</td>
<td>2.0</td>
</tr>
<tr>
<td>1.5 M Tris(pH 8.8)</td>
<td>1.3</td>
</tr>
<tr>
<td>10 % SDS</td>
<td>0.05</td>
</tr>
<tr>
<td>10 % Ammonium persulfate</td>
<td>0.05</td>
</tr>
<tr>
<td>TEMED</td>
<td>0.002</td>
</tr>
<tr>
<td>15 % gel</td>
<td></td>
</tr>
<tr>
<td>H$_2$O</td>
<td>1.1</td>
</tr>
<tr>
<td>30 % Acrylamide mix</td>
<td>2.5</td>
</tr>
<tr>
<td>1.5 M Tris(pH 8.8)</td>
<td>1.3</td>
</tr>
<tr>
<td>10 % SDS</td>
<td>0.05</td>
</tr>
<tr>
<td>10 % Ammonium persulfate</td>
<td>0.05</td>
</tr>
<tr>
<td>TEMED</td>
<td>0.002</td>
</tr>
</tbody>
</table>
### 7.2. Solutions for preparing 5% Stacking Gels for Tris-glycine SDS-PAGE

<table>
<thead>
<tr>
<th>Components</th>
<th>Volume (mL) of Components required to cast gels of indicated volumes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gel Volume 1ml</td>
</tr>
<tr>
<td>H₂O</td>
<td>0.58</td>
</tr>
<tr>
<td>30% Acrylamide mix</td>
<td>0.17</td>
</tr>
<tr>
<td>1.0 M Tris (pH 6.8)</td>
<td>0.13</td>
</tr>
<tr>
<td>10% SDS</td>
<td>0.01</td>
</tr>
<tr>
<td>10% Ammonium persulfate</td>
<td>0.01</td>
</tr>
<tr>
<td>TEMED</td>
<td>0.001</td>
</tr>
</tbody>
</table>

### 7.3. Composition of important buffers, reagents and media

**Luria Bertani Agar (LB) (g/L)**

- Casein enzymic hydrolysate: - 10.00
- Yeast extract: - 5.00
- Sodium chloride: - 10.00
- Agar: - 15.00
- Final pH (at 25°C): - 7.5 ± 0.2

**Luria Bertani Broth (LB) (g/L)**

- Casein enzymic hydrolysate: - 10.00
- Yeast extract: - 5.00
- Sodium chloride: - 10.00
- Final pH (at 25°C): - 7.5 ± 0.2

**King's B medium**

- Peptone: - 20 g
- Magnesium sulphate: - 1.5 g
- Dipotassium hydrogen phosphate: - 1.5 g
- Glycerol: - 10 ml
- Distilled water: - 1000 ml

**Tris EDTA (pH 8.0)**

- Tris Cl (pH 8.0): - 10 mM
- EDTA (pH 8.0): - 1 mM
10X PBS
NaCl - 8g
KCl - 0.2g
Na$_2$HPO$_4$ - 1.44g
KH$_2$PO$_4$ - 0.24g
Distilled water - 100ml

Cryopreservation medium (1ml)

800µL LB broth +% Glycerol (Final concentration of glycerol=10 %)

TAE 50X
Tris base - 242g
0.5M EDTA (pH 8.0) - 100ml
Glacial acetic acid - 57.1ml
Water to make up to - 1000ml

242g of EDTA was dissolved in 750ml of water followed by glacial acetic acid and EDTA. The pH is adjusted to 8.0 the final volume is made up to 100ml and autoclaved.

TBE 10X
Tris - 108g
0.5 M EDTA (pH 8.0) - 40ml
Boric acid - 55g
Water - 1000ml

6X Loading dye

2.5 % Ficoll 400 (60 % glycerol)

11 mM EDTA

3.3 mM Tris-HCl

0.017 % SDS
0.015 % BromophenolBlue
0.015 % XylenecyanolFF
pH 8.0 @ 25°C

Tris Glycine Buffer (5X pH 8.3) (1000ml)
Tris - 15.1g
Glycine - 94g
10 % SDS - 50ml
The 1x working solution is 25 mM Tris-Cl/250 mM glycine/0.1 % SDS
The solution can be stored at room temperature for several months

**Activation of Dialysis Bag**
Prepare 2 % Sodium bicarbonate and boiled it. To this added 10mM EDTA. Put the dialysis bag in this and boiled for 20 minutes. Decanted the solution and rinsed with hot water. Kept in refrigerator at 10 % alcohol.

**Colloidal Coomassie G250 (6X)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium sulphate</td>
<td>10 %</td>
</tr>
<tr>
<td>Coomassie G250</td>
<td>0.1%</td>
</tr>
<tr>
<td>Orthophosphoric acid</td>
<td>3 %</td>
</tr>
<tr>
<td>Ethanol</td>
<td>20%</td>
</tr>
<tr>
<td>Sterile Water</td>
<td>Up to 1000ml</td>
</tr>
</tbody>
</table>

Make sure the Ammonium Sulfate is dissolved before adding the Coomassie G-250 solution to a final of 0.1 %. Bring final volume up to 1L with H₂O. Store at room temperature with a tightly sealed lid

**TE Buffer (pH 8.0)**
10mM Tris HCl (pH 8.0)
1mM EDTA

**Kanamycin** - 25 mg of kanamycin was dissolved in 1 ml of sterile double distilled water.

**Ethidium Bromide (10 mg/mL)**
Add 1 g of ethidium bromide to 100 mL of water. Stir on a magnetic stirrer for several hours to ensure that the dye has dissolved. Wrap the container in aluminium foil or transfer the solution to a dark bottle and store at room temperature.

**Reagents for Catalase assay**

1. Phosphate buffer 0.1 M, pH7
2. H₂O₂ - 30μl 30 % H₂O₂/10 ml buffer
Reagents used for plasmid isolation (Sambrook et al, 1989)

- **Solution 1 (TEG)**

  Tris (pH 8) − 25 mM  
  EDTA (pH 8.0) − 10 mM  
  Glucose − 50 mM

  After adding Tris and EDTA, solution was autoclaved and to that filter sterilized glucose was added.

- **Solution 2 (Alkaline SDS)**

  Sodium hydroxide (NaOH) − 0.2 N (freshly diluted from 1 N NaOH Stock)  
  Sodium dodecyl sulphate (SDS) − 1 %

  This was freshly prepared during the experiment.

- **Solution 3 (KAc)**

  Potassium acetate (5M) − 60 ml  
  Glacial acetic acid − 11.5 ml  
  Distilled water − 28.5 ml

  The resulting solution is 3M with respect to potassium and 5 M with respect to acetate.

- **70 % Ethanol**

  70 ml of absolute ethanol dissolved in 30 ml of milliQ.

Reagents used for MgCl$_2$ method

- **Lysis buffer**

  SDS − 1%  
  EDTA − 10mM (diluted from 0.5M EDTA stock at pH 8.0)  
  NaOH − 0.1 N
Reagents used for boiling lysis method

- Lysis buffer (STET)
  - Tris – HCl - 10 mM
  - NaCl - 0.1M
  - EDTA - 1 mM
  - Triton X 100 - 5 % (v/v)
- Lysozyme - 50 mg/ml stock

Reagents used for Kado Liu method

- **E buffer**
  - Tris- acetate - 40mM
  - Sodium EDTA - 2mM

  Tris adjusted to pH 7.9 with glacial acetic acid.

- **Lysis solution**
  - SDS - 3 %
  - Tris - 50mM (pH 12.6)

  The solution was made 12.6 by adding 1.6 ml of 2N NaOH.

Reagents used for modified Kado Liu method

- **Lysis solution**
  - SDS - 3 %
  - Tris - 50mM (pH 12.6)

  The solution was made 12.6 by adding 1.6 ml of 2N NaOH

- **TBE buffer**
Reagents for competent cell preparation

TFB (Transformation Buffer) - 1 [100 ml]

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium acetate</td>
<td>30 mM</td>
<td>0.294 g</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>10 mM</td>
<td>0.147 g</td>
</tr>
<tr>
<td>Manganese chloride</td>
<td>50 mM</td>
<td>0.989 g</td>
</tr>
<tr>
<td>Rubidium chloride</td>
<td>100 mM</td>
<td>1.209 g</td>
</tr>
<tr>
<td>Glycerol</td>
<td>15 %</td>
<td>15 ml</td>
</tr>
</tbody>
</table>

Adjusted pH to 5.8 with 1M acetic acid. Made up to 100 ml with water. Filter sterilized (0.2 µm filter) and store at 4°C.

TFB (Transformation Buffer) – 2 [100ml]

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOPS or PIPES*, pH 6.5 (10 mM)</td>
<td></td>
<td>0.2082 g</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>75 mM</td>
<td>1.1025 g</td>
</tr>
<tr>
<td>Rubidium chloride</td>
<td>10 mM</td>
<td>0.1209 g</td>
</tr>
<tr>
<td>Glycerol</td>
<td>15 %</td>
<td>15 ml</td>
</tr>
</tbody>
</table>

Adjusted pH to 6.5 with 1M Potassium hydroxide. Made up to 100 ml. Filter sterilized and store at 4°C.

* MOPS – 3-(N-morpholine) propane sulfonic acid.

PIPS – Piperazine – N, N'-bis (2-ethanesulfonic acid)

7.4. Protocols

GFX PCR DNA and gel band purification kit (GE Healthcare)

a) gel band purification

- The slice with the desired band was cut from the agarose gel using a scalpel and placed inside a 1.7 ml tube and its weight was determined.
- 10 µl of capture buffer 1 was added per 10 mg of the slice and was kept at 60°C for 10 min; inverting the tube every three minutes, until the slice got fully dissolved in the buffer.
• This was added onto an GFX MicroSpin column, was kept for a minute, and spun at 16,000 x g for 30 s. The flow through was discarded.
• 600 μl of wash buffer type 2 was added to the column and spun at 16,000 x g for 30s. The flow through and the elution tube were discarded.
• The column was kept on a fresh nuclease free 1.7 ml tube and 20 μl of elution buffer was added to the column; was kept for a minute and spun at 16,000 x g for a minute.

b) PCR DNA purification

• Add 500 μl Capture buffer type 3 to up to 100 μl sample
• Mix thoroughly.
• Check that the Capture buffer type 3-sample mix is yellow or pale orange in color. If the color of the binding mixture is dark pink or red, add a small volume (~ 10 μl) of 3 M sodium acetate pH 5.0 and mix. Ensure that the binding mixture turns a yellow or pale orange color before loading onto the GFX Microspin column.
• For each purification that is to be performed, place one GFX MicroSpin column into one Collection tube.
• Centrifuge Capture buffer type 3-sample mix briefly to collect the liquid at the bottom of the tube.
• Load the Capture buffer type 3-sample mix onto the assembled GFX MicroSpin column and Collection tube.
• Spin the assembled column and Collection tube at 16 000 x g for 30 seconds.
• Discard the flow through by emptying the Collection tube. Place the GFX MicroSpin column back inside the Collection tube
• Add 500 μl Wash buffer type 1 to the GFX MicroSpin column
• Spin the assembled column and Collection tube at 16 000 x g for 30 seconds.
• Discard the Collection tube and transfer the GFX MicroSpin column to a fresh DNase-free 1.5 ml microcentrifuge tube
• Add 10–50 μl Elution buffer type 4 OR type 6 to the center of the membrane in the assembled GFX MicroSpin column and sample Collection tube.
• Incubate the assembled GFX MicroSpin column and sample Collection tube at room temperature for 1 minute.
• Spin the assembled column and sample Collection tube at 16 000 × g for 1 minute to recover the purified DNA.
• Proceed to downstream application. Store the purified DNA at -20°C.

c) Protein desalting protocol

• The top cap was removed from the column and the column storage solution was poured off. The sealed end of the column at the notch was cut.
• The column was filled up with about 25 ml equilibration buffer (PBS) and allowed to enter the packed bed completely. The flow through was discarded.
• A maximum of 2.5 ml of the sample was added to the column and let it enter the packed bed completely. The flow through was discarded.
• The sample was eluted with 3.5 ml buffer and the eluate was collected under the column.
7.5) 16S rDNA sequence of S15 (1422 bp) Accession no JN377436 of NCBI gene bank

**Pseudomonas aeruginosa S15**

TGCAGTCGAGCGGATGAAGGGAGCTTGCTCCTGGATTCCAGCGGGCGGAGCGG
GTGGTATGCTGCTGGAAGGCTTGCTCTGGATGTTGGGGAATACGCTGCCCCGAAA
CGGGCGCTACTACCCGATACGCTCCTGAGGGAGAACTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGGATTAGCTAG
TTGGTGGGGTA
AAGGGCTTCAAGCGGCGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGG
AATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAA
GAAGGGTATGTTGCCATTCTGGAATACGCGCAAGGGGCGTCGACCTACTACTGAGTACGGG
GAGGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAA
GGGAACACCACTGCAAGCGGCAAGGGCCGACCACTGCCGATGACTGAGCACTGCGT
GGGAAAGCGCTGGGAGCAAAACAGGATTAGATACCTTGATGTTCCACCGCG
TTAAGCGATGTCGACTACGCGGCTTGGAATCTTGAGATCTTAGTGGCCGACG
TAAGCGATAAATCGGACCGGCTGCGGAGTACGGCGGCGAACAGGTTAAAACCTC
AAATGAACTGAGGGGCGCCAGCAACGCGGTGAGCTGCTGTAATTCAACTC
GAAGCAACGCAGGAACCTTACCTGGCTTGCATGCACATAGCTGAAGACTTTCCA
GAGATTGCTTGCTGCTCTCGCAACTACGCAAGGGGCAGCTGCAAGACTCTGT
TGCTAGCTGTGTGAGGTTAGTGTGGTTAAGTCCGGAACGCCGCCACGACCC
CCTTGCTCTTAGTTACCAAGCACCCTCGGATTGGGACTCAAGGAGACTGCG
GGTGACAAAACGGAAGGTTGGGAGTAGCTAGTAAGATCACTATAGCTGGCCCTT
ACGCGCAGACGCTACACAGGCTGCTACAATGTTGGCTGACCA
GCCGCGAGGTGGAGCTAATCCATATAACGGATCGTATCGCAGTGTCAG
TCTGCAACTCGAGTCTGGAAGTGGCAATGCCTATGTAATCGTGAATAGA
ATGTCACGCGTGAAATACGTTCCGCGGCTTCCGTTACAGCCGCCGCGTCGAC
ATGGGAGGTGGGTGCTCCAGAATGACTAGTCTAACCAGCAAGGGGACGG
TTACCCACCGAGCTTACATGAC
7.6) 1.2 kb segment nucleotide sequence

GTGATGATCGAGGCGCCCTGGCGGTGTAGACGGTGGTCTATCTGCGCTCCGTGGTGGTGGCAGTC
AAGCAGATCGCAACGCTGGGCTGCGGACGATCGGCAGGAGCTTCTCGGAGATTCTTCTCGGCACTCGGCG
CGGCGGCGAGAGATCTGGAGTCCCTGGAGCCGTCTCAGGCTGGCACACATCCGGAG
GTCCAGTGTGATGGTGACCCGCGCGGCGACGAGCGGGGTCTCTAGGGTGGGCTGGGCGACAGCGTACCGC
CATGAGTTGGTCTAGCTAGGTGCTAGTCTGTGGCAACACACAGGAATACAACTCTCTATAGC
ATCCCATCAGGAACCTGGGAGCTGGGATCAAGCGACGGTAGTCTTTCTGCTGAAACCTGTTGCGGA
TCCTCGGTTCGGTGCGAATCCCGACGCGCCTGTTGCGGACGGAGCTGACGCTCATGGGAAGGACAA
TACCGTCTACATCGGCGGCGCTCAGAAGCATGTCATTGCAGATCGAGACACAACATCGCCGAG
GGCGTTCTCACAACAGCTGATACCACCTTCCATGGCGGAGCCGCGGGCGGTGTGCTGCT
TCGGCGGCCCGAACGAGGTACCGGGATCATGGCTGGACGAGGGCTGCTGACGGTGCACGCTG
TTCATGGACCTGGCCAGCGGCCGGTCAAGGTGGATGACCTTGAGAGGTGACATCGCT
GACCTGCTGCGGCGACGGAGCTGGGATCCCGCTGCACGTGCGCAGCATGCCCGG
CTTCATGGACCTGGCCAGCGGCCGGTCAAGGTGGATGACCTTGAGAGGTGACATCGCT
GACCTGCTGCGGCGACGGAGCTGGGATCCCGCTGCACGTGCGCAGCATGCCCGG
CTTCATGGACCTGGCCAGCGGCCGGTCAAGGTGGATGACCTTGAGAGGTGACATCGCT
GACCTGCTGCGGCGACGGAGCTGGGATCCCGCTGCACGTGCGCAGCATGCCCGG
CTTCATGGACCTGGCCAGCGGCCGGTCAAGGTGGATGACCTTGAGAGGTGACATCGCT
GACCTGCTGCGGCGACGGAGCTGGGATCCCGCTGCACGTGCGCAGCATGCCCGG
CTTCATGGACCTGGCCAGCGGCCGGTCAAGGTGGATGACCTTGAGAGGTGACATCGCT
GACCTGCTGCGGCGACGGAGCTGGGATCCCGCTGCACGTGCGCAGCATGCCCGG
CTTCATGGACCTGGCCAGCGGCCGGTCAAGGTGGATGACCTTGAGAGGTGACATCGCT
GACCTGCTGCGGCGACGGAGCTGGGATCCCGCTGCACGTGCGCAGCATGCCCGG

Hypothetical coded protein sequence

MIEAPCPCGWSSMALRAPATSWLHRHCSVSGRCCRVWRSTMTSRSP
TGSSPVCISIPAKHIRQLIDETGAEVQLIALPSATRARRREILSELESFPFLHLVRSMPGMFMDLASGKVDDLDQEVDIALD LLGRDSVAPRKEL
LERCIRQVVMTCAGGSIGSELRCQIMCSCPSVLILFEHSEYNLYS
IHQELERIRIKRELSVNLPLLPGSVRNPRLVDMRTWDKNTVYHAAAYKHVPIVEHNIAEGVLMN VIGTLHNAVQA AAVQ VGVQNFVLIST
D KAVRPTNVMGSTKRLAEMLVQLASNESAPVLFGD KDV VH V N K
TRFTMVRFONGVNLSSGSVIPFLREQIKRGGPVTVTHPSITR
7.7) ABC transporter obtained by PCR analysis

GTGATGATCGAGCGGCGGTGAAAAACAGGAGGAACCTACCAGTTGAGTCCGAGTGCGGA
AGTTGAACTGGTGATCGAGCCGGCGGTGAAAAAGATGTGCGTCCTGCGGGGCCTGGCCTGAGATT
ATGTCAGCCACCCATGAGAAGAATTCGCCCGCATTTGCTCTGGGCACCCCTTATATCGGGA
TGCTCAAGGTGGCGGCGGCGATGGCTCAAGATGACCATATATGTGACGCTG
ATGCTCGGCGCGGCGATCACGTAAATAT

Hypothetic protein coded

D H V N I A P V S P P E I E D E A V L E V R A S R H K P K L E D I S F T L R R G E V L G I A
G L L G A G R L P N H

7.8) ABC transporter obtained by BfuCl analysis (KC245089)

TCTAGAGGATCCGACGCCAGCGCGCTAGCGTCCGGGCTGCAAGCTGCGTAGCTGCGACGGCGG
CGCGCGATCGATCGAGCTCGCCGATCGCCGATCGCTCGAGACAGATGACATGCTCTGCCGGGCTGCGACGACTGG
TGATCGGCGCGCGATAGGTATCGCTGCAGCCAGAGATGCTCTGCCGGGCTGCGACGACTGG
TGATCGGCGCGCGATAGGTATCGCTGCAGCCAGAGATGCTCTGCCGGGCTGCGACGACTGG

Hypothetic Predicted protein

F A A K Q A V F G I Q T A L K L L A G Q A P E H E K D G V V E T P V E L V T A P
7.9) Proof of culture deposition

<table>
<thead>
<tr>
<th>Accession number</th>
<th>Name of organism</th>
<th>Strain designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCC2035</td>
<td>Pseudomonas aeruginosa</td>
<td>S15</td>
</tr>
</tbody>
</table>

Dear Sir/ma’am,

The following strain(s) sent by you for general deposit has been processed and assigned the following accession number(s):

Please note that this culture is now accessible to public for teaching and/or scientific research.

Sincerely yours,

Avinash Sharma
Curator (General Deposits)

December 10, 2012