Appendix A

Cloning strategies
A.1 *Disfp* knockout construct #1 cloning strategy

- Similar strategy was used for pCR421, 422, pDN6 and pDN7 – *diacps* knockout construct #1.
A.2 *Disfp* knockout construct #2 cloning strategy

- Similar strategy was used for pDN10, pDN11 and pDN12, pDN13 *diacps* knockout construct #2 and *dipks37* knockout construct respectively.
A.3 *Disfp* cloning strategy
A.4 *Diacps* cloning strategy

**PCR Product**
Exon 1 + Exon 2
A.5 *pk{s}12 ACP* cloning strategy

• Similar strategy was used for pCR655 and pDN19—*Dipks{16} ACP*.
A.6 *dia*cp cloning strategy
A.7 \textit{dipks1 C2930A} cloning strategy

Appendix A

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Appendix B

Buffers and Media
1. Composition of culture media and buffers

**HL5 Medium (1L)**

- Protease peptone-14.3 gm
- Yeast extract-7.15 gm
- Glucose -16 gm
- Na₂HPO₄-0.626 gm
- KH₂PO₄- 0.48 gm
- MQ- up to 1L
- pH-6.5

**NNA agar (100 mL)**

- Agar-2 gm
- 1X KK₂ buffer- 100 mL

**10X KK₂ Buffer (1L)**-

- KH₂PO₄- 22.5 gm
- K₂HPO₄-6.2 gm
- MQ- up to 1L
- pH-6.2

**HF-50 buffer (100 mL)**-

- HEPES- 4.76 gm
- KCl- 3.728 gm
- NaCl- 0.584 gm
MgSO₄ - 0.246 gm
NaHCO₃ - 0.420 gm
NaH₂PO₄ - 0.138 gm

**Healing Solution**

CaCl₂ - 100 mM
MgCl₂ - 100 mM

**Luria-Bertani Broth**

Tryptone: 10 gm
Yeast Extract: 10 gm
Sodium Chloride: 5 gm

Dissolved in 1L milli-Q water (resistivity of 18.2 MΩcm⁻¹). pH adjusted to 7.0. Autoclaved and stored at room temperature.

**Luria-Bertani Agar**

Tryptone: 10 gm
Yeast Extract: 10 gm
Sodium Chloride: 5 gm
Agar: 15 gm

Dissolved in 1 L milli-Q water, resistivity of 18.2 MΩcm⁻¹ (MQ). pH adjusted to 7.0. Autoclaved and stored at room temperature.
DH5 Medium

Bactotryptone: 20 gm

Yeast Extract: 5 gm

MgSO₄: 5 gm

Dissolved in 1L MQ water, pH adjusted to 7.6 with 1M KOH. Autoclaved and stored at room temperature.

2. Buffers for preparing chemical competent cells

TF1 buffer

30 mM potassium acetate

100 mM KCl

10 mM CaCl₂

50 mM MnCl₂

15% glycerol

Solution in MQ water, pH was adjusted to 5.8 with acetic acid and the solution was filter sterilized.

TF2 buffer

10 mM MOPS

10 mM CaCl₂

15% glycerol

Solution in MQ water, pH was adjusted to 6.5 with potassium hydroxide and the solution was filter sterilized.
3. Buffers for plasmid extraction from E. coli

**Resuspension Buffer (P1)**
Tris.Cl: 50 mM  
EDTA: 10 mM  
Rnase A: 100 µg/mL  
pH to 8.0 with HCl  
Solution in MQ water, pH was adjusted to 8.0 with HCl and buffer was autoclaved. 10 mL Rnase A (10mg/ml stock) was added and stored at 4°C.

**Lysis Buffer (P2)**
NaOH: 200 mM  
1%SDS  
Solution in MQ water, filter sterilized and stored at room temperature.

**Neutralizing Buffer (P3):**
3 M Potassium Acetate, pH 5.5.  
Solution in MQ water, pH adjusted to 5.5 with glacial acetic acid. Autoclaved and stored at room temperature.

**Tris-EDTA (TE) buffer**
Tris.Cl: 10 mM  
EDTA: 1 mM
Solution in MQ water, pH adjusted to 8.0 with HCl.

Autoclaved cooled and stored at room temperature.

**Rnase A stock solution (10 mg/mL)**

Dissolved 0.02gm Rnase A in 2 mL of a buffer [10 mM Tris pH 7.5 + 15 mM NaCl ].

Aliquoted into microfuge tubes and heated at 100°C for exactly 15 min. Cooled at room temperature and stored at −20°C.

4. dNTP mix for PCR

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Required concentration</th>
<th>Stock concentration</th>
<th>Amount of nucleotide per 100 μL</th>
</tr>
</thead>
<tbody>
<tr>
<td>dATP</td>
<td>2.5 mM</td>
<td>100 mM</td>
<td>2.5 μL</td>
</tr>
<tr>
<td>dCTP</td>
<td>2.5 mM</td>
<td>100 mM</td>
<td>2.5 μL</td>
</tr>
<tr>
<td>dTTP</td>
<td>2.5 mM</td>
<td>100 mM</td>
<td>2.5 μL</td>
</tr>
<tr>
<td>dGTP</td>
<td>1.25 mM</td>
<td>100 mM</td>
<td>1.25 μL</td>
</tr>
<tr>
<td>7-deaza GTP</td>
<td>1.25 mM</td>
<td>5 mM</td>
<td>1.25 μL</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>33.75 μL</strong></td>
</tr>
</tbody>
</table>

Sterile milli-Q water to make volume to 100 μL. Stored at −20°C.
5. Solutions for Agarose Gel Electrophoresis

**Agarose Gel Sample Buffer (6X)**
Sucrose: 4 gm  
Bromophenol Blue: 2.5 mg  
Dissolved in 10 L TE buffer. Stored at room temperature.

**Ethidium Bromide (10 mg/mL)**
Ethidium Bromide: 1 gm  
Dissolved in 100 ml autoclaved MQ water. Stored in amber coloured bottle at 4°C.

**50X TAE**
Tris base: 242 gm  
Na₂EDTA₂H₂O: 37.2 gm  
Dissolved in 900 mL MQ water. Added 57.1 mL glacial acetic acid. Volume made up to 1L with MQ water. Autoclaved and stored at room temperature.

**1Kb DNA Ladder Stock**
1Kb DNA Ladder: 10 μL  
Agarose Gel Sample Buffer (6X): 10 μL  
Autoclaved MQ water: 30 μL  
Stored at -20°C.
6. Buffers FOR SDS-PAGE

**30% Acrylamide Mix: 100 mL**

Acrylamide: 29 gm

N,N-methylene bis acrylamide: 1 gm

Volume made upto 100 mL with MQ water and stored at 4°C in amber coloured bottle.

**5X Tris Glycine Electrophoresis Buffer (1L)**

Tris base: 15.1 gm

Glycine: 94.0 gm

Dissolved in 900 mL MQ water. Added 50 mL 10% (w/v) stock solution of SDS. Volume made up to 1000 ml and stored at room temperature.

**1M Tris (pH 6.8):100 mL**

Tris base: 12.11 gm

Adjusted pH to 6.8 with conc. HCl (few mL). Autoclaved and stored at room temperature.

**1.5M Tris (pH 8.8):250 mL**

Tris base: 45.417 gm

pH adjusted to 8.8 with HCl. Autoclaved and stored at room temperature.

**10% SDS Stock Solution**

Dissolved 5 gm SDS in 50 ml MQ water and stored at room temperature.
10% Ammonium Persulfate (APS)
Dissolved 0.1 gm APS in 1 mL MQ water and stored at room temperature.

4X SDS Gel Loading Buffer (10 mL)
2.5 mL 1M Tris (pH 6.8)
2 mL β-mercaptoethanol
0.8 gm SDS
1.7 mg Bromophenol Blue
4 mL Glycerol
Dissolved in MQ water and stored at -20°C.

Coomassie Brilliant Blue Stain
Coomassie Brilliant Blue R250/G250: 1.25 gm
Methanol: 450 mL
MQ water: 450 mL
Glacial acetic acid: 100 mL
Stored at room temperature.

Destain
Methanol: 450 mL
MQ water: 450 mL
Glacial Acetic Acid: 100 mL
Stored at room temperature.