Lambda Packaging in PK-A

- From a single colony, inoculate 5ml of LB supplemented with MgSO4 and Maltose and grow O/N at 37°C with shaking.
- Dilute culture 1/10 in LB Mg+Maltose in 5mls, and grow for 4-6 hours or until density at 600nm = 0.4-0.6 OD.
- Pellet bacteria at 2000rpm for 10 mins. Table top 10mini R.T.
- Resuspend Cells in half original volume 10mM MgSO4.

+ Cells here can be stored 2-3 days before use.

Packaging Protocol:

1. Remove appropriate number of packaging tubes (Red/Yellow) or (blue and white) from Stratagene Giga pack II XL extracts and place on ice immediately.

2. Aliquot DNA 1-4ml (0.5-5ug DNA) in tube, add contents of 1ml (red/blue) to DNA tube and pipette up and down to mix and place on ice.

3. Quickly add 15mL of (yellow/white) tube to tube in mixture and pipette up and down to mix. Microfuge briefly to get rid of bubbles (5 secs).

4. Incubate tube at R.T. 22°C for 2 hrs.

* Do Not Exceed 2 hours (9 mins optimum).


6. Store Supernatant to viro at 4°C for use.

Infection:
- Bacterial (PK-A) resuspend in MgSO4 10m
  20ul - 25ul phage
  20ul - 50ul phage (up to 100ul)

- Mix contents in each tube and incubate at 37°C for 20 mins.
- Add 1ul binding mixture. Shake at 37°C for 1hour.
- Plate on ampicillin plates. Incubate O/N 37°C.
Lambda Packaging in PLK-A

- From a single colony, incubate 5 ml of LB supplemented in M9, Sp4Y and Maltose and grow overnight at 37°C in Shaker.
- Dilute culture 1:10 in LB and maltose into 5 ml and grow for 4-6 hours or until density at 660 nm = 0.8-0.6 OD.
- Pellet bacteria at 2000 rpm 10 mins. Table top for 10 mins R.T.
- Resuspend cells in half original volume room AB5044
  A can be stored for 2-3 days before use.

Packaging Protocol

1. Remove appropriate number of packaging tubes. Red = yellow or blue; white for Lambda II X L extract and make on ice immediately.

2. At least DNA 1-4 µl (0.1-5ng DNA) in tube. Add contents of (10 µl) Red (~blue) tube to DNA and pipette up and down 3 times to mix and place on ice

3. Quickly add 50 µl of yellow (white) tube to DNA tube and pipette up and down to mix. Cap centrifuge briefly to get rid of bubbles (8-10 secs)

4. Incubate tube at R.T. 22°C for 2 hour or Do Not Exceed 2 hours. 90 mins optimum.


6. Store Supernatant at 4°C for use.

Infect:

1. Bacteria resuspended in AB5044 10X

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Phage</th>
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<tbody>
<tr>
<td>200 µl</td>
<td>25 µl</td>
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<tr>
<td>200 µl</td>
<td>50 µl</td>
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<tr>
<td>200 µl</td>
<td>75 µl</td>
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<tr>
<td>200 µl</td>
<td>100 µl</td>
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</tbody>
</table>

- Mix contents in each tube.

2. Incubate at 37°C for 30 minutes. Add 1 ml of LB agar + Maltose Medium. Shake at 37°C for 1 hour

3. Plate on LB ampicillin plates. Incubate 0/10 37°C
1. **Recipe For Antifade**

   PH to 8.0

   1 x PBS - 4 mls

   Phenylethedianiline HCl - 40 mg

   Glycerol - 36 mls

   1mM bicarb/carb - 1.5 mls pH 9.5

2. **MgSO4 for Phage Packaging**

   10 mls - 1mM MgSO4

   90 mls - ddH2O

   Filter Sterilize. Volume 100 mls.

3. **LB to MgSO4 and Maltose**

   10g - Bacto tryptone

   5g - Yeast Extract

   5g - NaCl

   100 mls - ddH2O, autoclave for 20 minutes at 121°C 15 lbs.

   10 mls - 1mM MgSO4

   90 mls - 10% Maltose \{ add after cooling by filter sterilizing then 0.2 um Filter \}

4. **SMM Buffer**

   5.8g - NaCl

   2.0g - MgSO4.7H2O

   50.0 mls - 1mM Tris, pH 7.5

   5.0 mls - 2% w/v Gelatin

   1 Liter - dd H2O, autoclave