Lambda Packaging in PLK-A

- from a single colony inoculate 5mls of LB supplemented w/ MgSO4 and Maltose and grow O/N at 37°C w/ 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 O.D.
- pellet bacteria at 2000 rpms 10 mins. Table top 10mins 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 Gigapak II XL extracts and place on ice immediately.
2- Aliquot DNA 1-4µl (0.1-5µg DNA) in tube. Add contents of 10µl (Red/blue) tub. to DNA tube and pipette up and down to mix and place on ice.
3- Quickly add 15mls of (yellow/white) tube to tube w/ 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 hours
* DO NOT EXCEED 2 HOURS (9 MINS OPTIMUM).
5- Add 500µl SM Buffer and 10µl $CHCl_3$. Mix contents of tube gently. Centrifuge to sediment debris briefly (30secs).
6- Store supernatant w/ virus at 4°C for use.

Infection:

- Bacteria (PLK-A) resuspend in MgSO4 10mM 200µl - 25µl phage
200µl - 50µl phage (up to 100µl of phage)
- mix contents in each tube and incubate at 37°C for 20 mins.
- Add 1ml LB 5mg + maltose. Shake at 37°C for 1 hour.
- plate on ampicillin plates. Incubate O/N 37°C

Lambda Packaging in PLK-A

- From a single colony inoculate 5 mls of LB supplemented in $MgSO_4$ and Maltose and grow overnight at 37°C @ Shaking.
- Dilute Culture 1/10 in LB mg + Maltose into 5 mls and grow for 4-6 hours or until density at 600nm ≃ 0.4-0.6 O.D.
- Pellet bacteria at 2000rpms 10 mins. Table top for 10 mins R.T.
- Resuspend cells in half original volume 10mM $MgSO_4$

* Can be stored for 2-3 days before use.

Packaging Protocol

1. Remove appropriate number of packaging tubes Red & yellow or blue & white for Gigapack II XL extracts and place on ice immediately
2. Aliquot DNA 1-4µl (0.1-5µg DNA) in tube. Add contents of (10µl) Red (or blue) tube to DNA and pipette up & down to mix and place on ice
3. Quickly add 15µl of yellow (white) tube to DNA tube and pipette up and down to mix. Microfuge briefly to get rid of bubbles (3-5 secs)
4. Incubate tube at R.T. 22°C for 2 hours * DO NOT EXCEED 2 HOURS. 90 mins optimum.
5. Add 500µl SM Buffer and 10µl chcl3. Mix contents of tube gently. Centrifuge to sediment debris.
6. Store Supernatant at 4°C for use.

Infection

1. Bacteria Resuspend in $MgSO_4$ 10mM

Bacteria	phage
- 200µl	25µl
- 200µl	50µl
- 200µl	~~75~~µl
- 200µl	100µl

- Mix contents in each tube.

2. Incubate at 37°C for 20 minutes. Add 1 ml of LB mg + Maltose Medium. Shake at 37°C for 1 hour
3. Plate on LB ampicillin plates. Incubate O/N 37°C

① Recipe For Antifade

pH to 8.0

1X PBS – 4 mls

Phenylenediamine HCl – 40mg

Glycerol – 36mls

1M bicarb/carb – 1.5mls pH 9.5

② $MgSO_4$ for Phage Packaging

1.0mls – 1M $MgSO_4$

90 mls – ddH_2O

Filter Sterilize. Volume 100mls.

③ LB w $MgSO_4$ and Maltose

10g – Bacto tryptone

5g – Yeast Extract

5g – NaCl

1000mls – ddH_2O autoclave for 20minutes at 121°C 15lbs.

10mls – 1M $MgSO_4$

20mls – 10% Maltose } add after cooling by filter sterilizing thru 0.2μM Filter.

④ SM Buffer

5.8g – NaCl

2.0g – $MgSO_4 \cdot 7H_2O$

50.0mls – 1M Tris. pH 7.5

5.0mls – 2% w/v Gelatin

1 liter – dd H_2O autoclave