Safe Operating Procedure (SOP)	School of Molecular & Biomedical Science	
Prepared by: Nicholas Eyre		**Date:** 21/9/08
Procedure: Propagation of plasmid DNA		
Approved by:	**Signature:**	

PPE required: Gown, closed shoes, glove & eye protection-Centrifuge & Spill Procedures may apply

Materials

- Plasmid DNA
- Chemically competent *E.coli* cells (e.g XL-10 Blue, XL-10 Gold, DH5α)
- Spectrophotometer.
- Bacterial culture reagents (Luria broth, chemically competent cells, selective antibiotics, sterile glassware, sterile plasticware, disposable plasticware).
- Heating block
- Benchtop centrifuge.
- Centrifuge (eg. Beckman-Avanti J-25I)
- 37 ºC controlled temperature (CT) room with shaking platform.
- 12.5% (w/v)sodium hypochlorite solution.
- 0.85% (w/v) saline.
- Ethanol.
- 80% (v/v) ethanol solution.
- Isopropanol.
- Plasmid MINI-prep and MAXI-prep kits (Qiagen).
- Bunsen burner, flame loop and flame spreader.

Methods

Plasmid DNA transformation

1. Thaw an aliquot of competent cells (from -80 ºC freezer) on ice. If necessary further aliquot cells into chilled microcentrifuge tubes (100 μl/tube).
2. Add appropriate quantity of plasmid DNA (approximately 10 ng), mix gently and incubate on ice for 30 minutes.
3. 'Heat-shock' cells by incubation (on heating block) at 42 ºC for 30 seconds.
4. Return cells to ice and incubate for 2 minutes.
5. Add 1ml of Luria broth and incubate cells at 37 ºC on a shaking platform for 45 minutes.
6. Pellet cells by centrifugation (18,000×G for 1 minute) and resuspend pellet in 70 μl of 0.85% (w/v) saline.
7. Plate onto Luria agar (1.5% [w/v]) plates containing appropriate selection antibiotic (e.g. ampicillin 100 μg/ml, kanamycin 50 μg/ml).
8. Label plates with your name and room number, *E.coli* strain, date and transformed plasmid name and incubate at 37 ºC overnight.

Small-scale plasmid DNA preparation (Qiagen MINIprep)

1. Before starting, (if not already done) add RNase solution (provided) to buffer P1 (provided) and store at 4 ^{o}C (final concentration of RNAse A is 100 μg/ml). Also add 30 ml ethanol to buffer PE (provided).
2. Innoculate a single bacterial colony into a tube containing 10 ml of sterile (autoclaved) Luria broth.
3. Add appropriate antibiotic (ampicillin to 100 μg/ml, kanamycin to 50 μg/ml).
4. Incubate cultures at 37 ^{o}C overnight on shaking platform (CT room).
5. Transfer 5 ml of culture to a disposable plastic centrifuge tube and centrifuge at 18,000×G for 5 minutes.
6. Tip supernatants into waste container. Add 12.5% (w/v) sodium hypochlorite solution to liquid culture waste to a final concentration ≥2.5% (w/v).
7. Resuspend bacterial pellets in 250 μl of buffer P1 (provided). Transfer suspension to 1.5 ml microcentrifuge tube.
8. Add 250 μl of buffer P2 and invert tubes 6 times.
9. Add 350 μl of buffer P3 and invert tubes 6 times.
10. Centrifuge samples at 18,000×G for 10 minutes.
11. Transfer supernatant to spin column (provide).
12. Centrifuge samples at 18,000×G for 1 minute. Tip out flow-through (into liquid waste container).
13. Add 0.5 ml buffer PB (provided)and centrifuge at 18,000×G for 1 minute. Tip out flow-through (into liquid waste container).
14. Add 0.75 ml buffer PE (provided) and centrifuge at 18,000×G for 1 minute. Tip out flow-through (into liquid waste container).
15. Centrifuge column at 18,000×G for 1 minute and transfer column to a new 1.5 ml microcentrifuge tube.
16. Apply 50 μl buffer EB (provided) and centrifuge at 18,000×G for 1 minute.
17. Dispose of column. Flow-through contains plasmid DNA. Plasmid DNA can be stored at 4°C (short-term) or -20 ^{o}C (long-term) and is suitable for many downstream applications (restriction digest, sequencing, agarose gel electrophoresis, *in vitro* RNA transcription etc.)

Large-scale plasmid DNA preparation (Qiagen MAXIprep)

1. Before starting, (if not already done) add RNase solution (provided) to buffer P1 (provided) and store at 4 ^{o}C (final concentration of RNAse A is 100 μg/ml). Pre-chill buffer P3 at 4 ^{o}C.
2. Pick a single colony from an agar plate and inoculate into a culture of 5 ml Luria broth containing the appropriate selective antibiotic (ampicillin to 100 μg/ml, kanamycin to 50 μg/ml). Culture overnight at 37 ^{o}C overnight on shaking platform (CT room).
3. Sub-culture starter culture by transferring 200 μl of into 200 ml of Luria broth containing the appropriate selective antibiotic (ampicillin to 100 μg/ml, kanamycin to 50 μg/ml). Culture overnight at 37 ^{o}C overnight on shaking platform (CT room).
4. Transfer bacterial culture to large centrifuge tube and pellet bacteria by centrifugation at 6000×G for 15 minutes at 4 ^{o}C.
5. Tip supernatants into waste container. Add 12.5% (w/v) sodium hypochlorite solution to liquid culture waste to a final concentration ≥2.5% (w/v).
6. Resuspend bacterial pellets in 10 ml of buffer P1 (provided). Transfer suspension to a sterile mid-size centrifuge tube (~40 ml).
7. Add 10 ml of buffer P2 and invert tubes 6 times. Incubate at room temperature for 5 minutes.
8. Add 10 ml of buffer P3 and invert tubes 6 times. Incubate on ice for 20 minutes.

9. Centrifuge at 20,000×G for 30 minutes at 4 ºC. Meanwhile equilibrate QIAGEN-tip 500 column by addition of 10 ml of buffer QBT (provided) and allowing the column to empty by gravity flow (collect waste into 50 ml conical centrifuge tube for disposal).
10. Following centrifugation collect plasmid DNA-containing supernatant and apply to equilibrated QIAGEN-tip 500 column and allow the column to empty by gravity flow (collect waste into 50 ml conical centrifuge tube for disposal).
11. Wash the column by addition of 30 ml buffer QC (provided) and allow the column to empty by gravity flow (collect waste into 50 ml conical centrifuge tube for disposal).
12. Repeat above wash step.
13. Transfer column to a new 50 ml conical centrifuge tube. Elute plasmid DNA by addition of 15 ml of buffer QF (provided). Allow the column to empty by gravity flow and collect plasmid DNA-containing supernatant.
14. Add 10.5 ml of isopropanol, mix by inversion and immediately transfer to a sterile mid-size centrifuge tube. Centrifuge at 20,000×G for 30 minutes at 4 ºC.
15. Remove ~95% of supernatant and resuspend plasmid DNA pellet in remaining volume of supernatant.
16. Transfer suspension to a microcentrifuge tube and centrifuge at 18,000×G for 10 minutes. Aspirate supernatant.
17. Resuspend pellet in 70% ethanol (v/v) and centrifuge at 18,000×G for 10 minutes. Aspirate supernatant.
18. Dry plasmid DNA pellet in 37 ºC heating block for 2 minutes.
19. Resuspend plasmid DNA pellet in 100 μl of buffer EB (provided).
20. Quantify DNA concentration using the spectrophotometer. Plasmid DNA can be stored at 4ºC (short-term) or -20 ºC (long-term) and is suitable for many downstream applications (restriction digest, sequencing, agarose gel electrophoresis, *in vitro* RNA transcription, transfection etc.).

Important safety notes: ***All used disposable plasticware must be disposed of in autoclave bags. When full, seal bags and transfer in locked biohazard 'wheelie' bins to outside containment area (lockable) for collection for incineration. All unwanted culture fluids and liquid waste from the above processes must be treated with sodium hypochlorite solution to >2.5% (w/v) (final concentration) for at least 15 minutes before tipping down the sink with running water. Alternatively unwanted culture medium contained within sealable glass containers may be sterilized by autoclaving before dispoal as above. Spills of potentially contaminated materials should be wiped with sodium hypochlorite solution to >2.5% (w/v) (final concentration) and then 80% (v/v) ethanol. Full length laboratory gowns and gloves should be worn. Care should be taken to ensure that centrifuges are balanced before centrifugation and that centrifuge O-rings and seals are intact. Tubes should be inspected before use for signs of cracking. Care should be taken when working with open flames. Before and after work surfaces should be wiped with 80% (v/v) ethanol. Hands must be washed before and after handling bacterial cultures.***