**Electroporation of Agrobacterium**
 **Prepare Agrobacterium competent cells.**
Reagent
Autoclave the following:
YEP/LB + Rifampicin 10µg/ml    500 ml in  2L flask
10% glycerol 300 ml
450 ml centrifuge tube x 2
35 ml centrifuge tube x 2
1.5 ml eppendorf tube (many)

1. Prepare 2 tubes of LB/YEP + 10µg/ml Rif (in case of GV3101 derivatives) and inoculate with glycerol stock of Agrobacterium.  Inoculate large piece of ice.  Do it in the morning.  Shake at 28-30°C.
2. Next evening, innoculate 5ml culture to 500 ml.  Shake overnight at 20-30°C.
3. Put altoclaved tubes and 10% glycerol in cold room.  Keep them in 4°C overnight.
4. Check the culture first thing in the morning.  Dependent of the growth, decide when to start the preparation.  If OD600>0.6, start immediately.
5. Cool culture on ice for 15-30 min.
6. Transfer the culture to prechilled 450ml centrifuge tubes.
7. Centrifuge at 5000rpm for 5 min in 4°C.
	* From here, work in the cold room.
8. Remove supernatant (autoclave before discarding).  Gently resuspend the pellet in 100 ml ice-cold 10% glycerol, do not vortex.
9. Centrifuge at 5000rpm for 5 min in 4°C.  Remove supernatant (autoclave before discarding).  Gently resuspend the pellet in 5 ml ice-cold 10% glycerol, do not vortex.  Transfer and combine the suspension to a new prechilled 35 ml centrifuge tubes.  Rince the 450 ml tubes with 5 ml of 10% glycerol once or twice and combine with the suspension.
10. Centrifuge at 10,000 rpm for 5 min at 4°C.
11. Remove supernatant (autoclave before discarding) with 5 ml pipet.  Centrifuge again for 1 min and remove as much solution quickly as possible.(it is usual to have some (about 0.5 ml) solution left)
12. Add equal (pellet) volume of ice cold 10% glycerol.  It is usually 1-2 ml.
13. Gently resuspend the pellet (do not vortex).
14. Dispense the suspension to chilled 1.5 ml tubes on ice.  For single use competent cells, dispense 20µl/tube.  Set the pipet to 23µl.  This will make 20µl actual volume when pipeting repeatedly.  For larger stock, dispense 43µl/tube or 100µl/tube.
15. Freeze the tubes by putting at -20°C for 1 hour (Do not use Liquid N2), then transfer to -80°C for storage.

**Electroporation**

Two electroporation equipments are available.
     In Bourlog Center, BioRAD Genepulser II can be found in tissue culture lab.  Use 1mm cubette and use 180kV (in other words, use like E. coli transformation).  1 microL of miniprep DNA and plate 100 microL/plate (out of 500 microL LB).
     In Bourlog Center, BRL cell porator is available.  This transforms with higher efficiency.  Use 380kV with 2mm cubette.  Use 1 microL of miniprep and plate 100 microL or less per plate.

Always keep competent cells on ice, do not warm them up above 0°C.

1. Take tubes of LB or YEP media from -20°C and thaw at room temperature or 28-30°C.  label the tube with the DNA sample name,
	* It is the best if the media is at 28-30°C when recovering the cells after electroporation.
2. Label 2 (LB/YEP + antibiotics plates)/DNA sample.  Put them in the 28-30°C incubator face up with their lids slightly open to dry the surface.
3. Put DNA samples, electroporation cubette, and competent cells on ice (you will need same number of these as DNA samples).  Label the tubes of cells with sample names.
	* Make sure competent cells contain 20µl.  If using 40µl or 100µl stock, dispense to prechilled new 1.5 ml tube to prepare 20µl aliquots.
4. Quickly thaw the competent cells in hand, and bring them back to ice.  Put 2 µl miniprep DNA (1x-100x dilution) to a labeled tube containing 20µl competent cells.  Mix briefly by tapping.
5. Bring the DNA-cell mixtures, cubettes (these should be on ice) and LB/YEPmedia (this should not be on ice) to CBC Cell Porator.
6. Check the setting of porator
	* Capacitance: 330
	* High Ω
	* Charge rate: fast
	* Voltage Booster: 4 kΩ
7. Put ice in the chamber of the Cell Porator, turn the Porator on (2 switches)
8. Set cubette (without cells) to the chamber position to make the hole in the ice.
9. Put DNA-cell mix to the cubette (between the two knobs inside the cubette).  Be careful not to introduce bubbles.
10. Set the cubette to the chamber, close the lid and make sure the dial knobs on the lid and the position of the cubette match.  Connect the cable to the chamber.
11. Set the dial knob to "CHARGE" position.  Press "UP" button to increase the voltage.  Bring the voltage to around 4.00.
12. When voltage reached 4.00 release the "UP" button and quickly change the dial knob to "ARM" position.
	* The light will change from green to red. this will connect the Porator to the chamber.  If voltage dropped less than 3.80, set the dial back to "CHARGE" and re charge using "UP" button.
13. When voltage dropped 3.80, press "TRIGGER" (red button) to apply pulse to the cells.
	* The reading of Voltage Booster should be aroun 1.7-2.0.  When the solution contain more salts (such as ligation mix), the reading become lower.  If the reading is above 2.0, it may indicate that the Porator and Chamber was not connected, or dial knob position of the chamber was wrong.
14. Open the chamber and recover cells from chamber using 200µl pipette.  Transfer the cells to the matching tube of LB/YEP media.
	* You may observe very tiny bubbles on in the cell suspension that indicate the successful application of pulese to the cells.  However, this may not apparent when using very clean DNA solution such as low concentration of DNA in DW.
15. Incubate 30°C for 1-2 hour (with or without shaking).
16. Put 3 autoclaved glass beads to the first plate.  Put 100-200 µl DW to the second plate.
17. Pour or pipette all (for most ligations) or part of 0.5 ml culture to the first plate.  Shake the plate holizontally to move around the glass beads on the surface untill the solution is evenly spread.
18. Transfer the glass beads (now coated with cell culture) to the second plate of same lebel (with DW), and shake the plates to spread water.  This will dilute the cells on second plate.
19. Transfer the glass beads to recycle bottle containing 70% ethanol.
20. Put the plate in 30°C incubator.  If the surface is wet, put them face up with their lids slightly open to dry the surface.  When dried, close the lid, wrap with parafilm and put them face down.  Incubate at 30°C for 2-3 overnight.

Media can be YEP or LB (liquid and plate).
Incubation is 30°C for 1 hour before plating.
Seal the plates and leave for 3 days.