

Standard Operating Procedure for Hazardous Chemicals

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Chemical(s)	Calcium chloride (<u>CaCl₂</u>), liquid nitrogen (<u>LN₂</u>).
Process	Calcium Chloride Preparation of <i>Agrobacterium</i> Competent Cells
Specific Hazards <i>referred to MSDSs for more detailed information</i>	<u>CaCl₂</u> is hazardous as a skin/eye/respiratory irritant and may cause burns. It may also irritate or burn the digestive tract if swallowed. May cause cardiac disturbances. Reaction with water evolves heat. <u>LN₂</u> is a simple asphyxiant and can cause cryogenic burns.
Personal protective equipment	Must wear 3-5 mil nitrile gloves; cryogloves should be used for pouring large volumes of <u>LN₂</u> . Chemical safety goggles and lab coat should be worn when splash potential exists.
Engineering/ventilation controls	None applicable for the quantities needed for this procedure.
Special handling procedures and storage requirements	Store <u>CaCl₂</u> on bench top in a tightly closed container. Minimize exposure to moisture and open air. Incompatible with bromine trifluoride and 2-furanpercarboxylic acid. Store minimal volumes of <u>LN₂</u> in covered Styrofoam containers for procedural use.
Spill and accident procedures <i>for hazardous chemicals only</i>	<u>Skin exposure:</u> Rinse affected skin with plenty of water while removing contaminated clothing/shoes. Rinse for > 15 minutes. <u>Eye exposure:</u> Wash eyes for > 15 minutes. <u>Inhalation:</u> Remove to fresh air immediately. If not breathing give artificial respiration (NOT mouth-to-mouth). <u>Ingestion:</u> Do not induce vomiting. If victim is alert, give 2-4 cupfuls of milk or water. For all cases, seek medical attention immediately.
	<u>Small</u> (< 2L): Vacuum or sweep up material and place into a suitable disposal container, avoiding generation of dust. Notify PIs.
	<u>Large</u> (> 2L): Evacuate the room, notify PIs and call 2-5801 to request emergency spill assistance from the Environmental Safety Division.
Waste disposal	No special procedures needed.
Special approval	No special authorization needed after SOP training & reading MSDSs.
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Reviewed by	Name/date:

Calcium Chloride Preparation of *Agrobacterium* Competent Cells

This method is effective for preparing *Agrobacterium tumefaciens* to be used for genetic transformation.

Solutions and Reagents needed

Luria-Bertani broth (LB)	10 g/L tryptone, 10 g/L sodium chloride, 5 g/L yeast extract
20 mM Calcium chloride	sterilized by passing through a 20 um filter before use
Liquid nitrogen	

Precautions: Keeping cells cool throughout steps 4-9 is critical to the transformation efficiency of your cells. Utilize standard sterile technique procedures when working with bacteria to avoid contamination.

Procedure

1. Streak an LB plate with the desired strain of *Agrobacterium* (use selection antibiotic as appropriate) and grow for 2 d at 28°C.
2. Prepare a 5 ml tube of LB and add a single colony from the plate. Grow overnight at 28°C on a rotating shaker.
3. Prepare 50 ml LB in a 125 ml Erlenmeyer flask and add 500 ul (1:100 dilution) of culture from the tube. Grow overnight at 28°C on a rotating shaker.
4. Chill flask in ice water (**0°C**) for 30 min.
5. Transfer 45 mL of the chilled culture into a pre-chilled Falcon centrifuge tube (DO NOT over-fill the tube) kept in ice water, then centrifuge for 10 min at a low speed (4,000 rpm) at 4°C.
6. Remove the supernatant and gently resuspend the pellet in 5.0 mL **ice cold** 20 mM calcium chloride, holding the tube in ice water as you work.
7. Centrifuge the tubes for 5 min at 4,000 rpm, 4°C. Remove the supernatant and gently resuspend the pellet in 1.0 mL **ice cold** 20 mM calcium chloride, holding the tube in ice water as you work. Aliquot 200 ul to pre-chilled 1.7 ml microcentrifuge tubes.
8. At this point you may proceed directly to transformation or snap freeze the tubes in liquid nitrogen and store at -80°C.

Alternatively a **mini-scale preparation** could be made using 5-ml overnight culture, starting from Step 2:

- 3b. Chill tube in ice water (**0°C**) for 30 min.
- 4b. Transfer 1.5 mL of chilled culture into a pre-chilled Eppendorf tube kept in ice water, then centrifuge for 10 min at a low speed (4,000 rpm) at 4°C.
- 5b. Remove the supernatant and gently resuspend the pellet in 1.0 mL **ice cold** 20 mM calcium

chloride, holding the tube in ice water as you work.

- 6b. Centrifuge the tubes for 5 min at 4,000 rpm, 4°C. Remove the supernatant and gently resuspend the pellet in 200 µL **ice cold** 20 mM calcium chloride, holding the tube in ice water as you work.
- 7b. At this point you may proceed directly to transformation or snap freeze the tubes in liquid nitrogen and store at -80°C.

Freeze-Thaw Transformation: (*Note: It is often useful to run a control transformation to determine transformation efficiency before using a batch of cells for experimental transformations*)

9. Add 500 ng plasmid DNA to 1 tube of competent cells. Hold on ice for 15-30 min if using frozen cells, or 5 min if using freshly prepared cells.
10. Freeze in liquid nitrogen for 5 min.
11. Heat shock in 37°C water bath for 5 min, then return to ice for ~5 minutes.
12. Add 1.0 ml of LB to the tube, then incubate on a 28°C rotating shaker for 3-4 hrs.
12. Plate out 50-200 µl of culture on an LB plate containing an appropriate selection agent. Remaining culture may be held at 4°C for up to 14 days in case you wish to plate out more later.
13. Grow up plates for 2 d at 28°C and proceed to colony PCR to confirm transformation.

References:

Holsters M, De Waele D, Depicker A, Messens E, van Montagu M, Schell J (1978) Transfection and transformation of *Agrobacterium tumefaciens*. *Mol Gen Genet* 163: 181-187