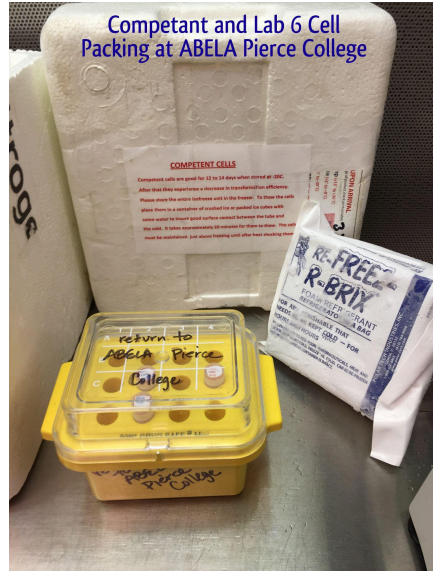


# Competent Cell Hacks and Best Practices

The ABE *Foundations of Biotech* labs can require competent cells depending on which sequence of labs is implemented. And, we have all experienced challenges with ensuring that competent cells are maintained and work for transformation. Here we present a series of “hacks” and best practices to ensure the best results. A lot of the “hacks” involve good planning, no special equipment. You could have the fanciest portable  $-80$  freezer in the world, but it doesn't matter if a teacher takes out all their cells in the morning and leaves them on the bench top all day!

- **Confirm pickup/drop off time for cells 24+ hours in advance**
  - Ensure teachers know where to go for the materials pickup
  - Ensure teachers knows exactly what to do with the cells after they pick them up and the time limit for each step
- **Confirm teacher understanding of transformation procedure, especially common “pain points”**
  - Teachers should perform the transformation lab within 48 hours of receipt of the cells
    - If stored in a  $-20^{\circ}\text{C}$  freezer, the efficiency of transformation decreases dramatically after 48 hours
    - Teachers should place the isofreeze unit in the back of the freezer, surrounded with ice packs. They should **not** place the cells in the door of the freezer where they may be jostled.
  - The cells can be kept on ice (outside freezer) for about 30 minutes on transformation day
  - Cells should be thawed on ice
  - Teachers should pipette up and down gently to resuspend cells prior to aliquoting
  - Thawed cells should **not** be refrozen
  - Competent cells should **not** be centrifuged
  - Frozen cells should **not** be taken out of the freezer for multiple classes at once; only enough cells for each class should be taken out of the freezer at a time since they can only be out of the freezer (on ice) for  $\sim 30$  minutes
  - If using dry ice for transporting/storing cells, teachers should **not** put the dry ice in the freezer as it will cause the freezer to shut off and may damage its thermostat
- **Pack cells for transportation**
  - Fill the bottom of a styrofoam box (that has a lid) with ice. Consider labeling the lid with a reminder for the teacher to place the cells in the freezer.
  - Bring a second styrofoam box (with lid) filled with ice and a plastic bag to your  $-80^{\circ}\text{C}$  freezer.

- Place cell tube(s) in the plastic bag and seal the bag.
- Place the bag and tubes into the second container.
- Place a  $-80^{\circ}\text{C}$  cold pack (such as the [Polar Tech Re-Freez R-Brix](#) that are safe to use at ultra cold temperatures) into the second styrofoam box, and top it with more ice before closing the lid.
- Deliver to teachers at designated pickup locations.



**Competent cells are only good for 12 days when stored in a regular  $-20^{\circ}\text{C}$  freezer. They MUST STAY FROZEN to remain competent. Put the isofreeze unit in the back of the freezer. Thaw the cells in a container of packed crushed ice. This will take about 20 minutes. The cells must remain just above freezing until the heat shock step.**

- **Best practices for transporting competent cells**

- A combination of using dry ice and multiple coolers is usually the most feasible and cheapest option for transporting competent cells
  - “Nest” the coolers inside each other
  - If you aren’t able to use coolers, consider a thermos large enough to hold the cells and some [reusable ice cubes](#)
    - Place both thermos and ice cubes into the  $-80^{\circ}\text{C}$  freezer  $\sim 24$  hr prior to pickup to help keep cells frozen
  - Consider using [freezer bricks](#)
- $-20^{\circ}\text{C}$  portable freezers
  - These can sometimes be plugged into car outlets, then brought into the school upon arrival
  - Can fit two cryoboxes
- $-80^{\circ}\text{C}$  shuttle freezer such as [Stirling Portable Freezer](#)
  - Fits 6 boxes
  - This is the most expensive option
- Consider splitting the cost of shuttle between geographically close schools, for shared usage during the school year to transport cells from site to schools and between schools.
- Consider investing in reusable freezer bricks

- **Other tips and techniques**
  - Cells *can* be stored for 12–13 days in a *frost-free freezer*, provided that the cells were transported directly from the  $-80^{\circ}\text{C}$  freezer to the on-site frost-free freezer without thawing (e.g. in a benchtop cooler that maintained the  $-20^{\circ}\text{C}$  temperature during transport)
  - Making competent cells at a higher concentration and volume could help with transformation success
  
- Useful resources:
  - [FAQs for ABE Foundations of Biotech Transformation Lab](#)
  - [ABE Teacher FAQs](#)
  - [GoldBio Competent Cell FAQs](#)