

## Thawing of the 96 Well Gene Trap Plates

We use 96 tube well plates from Continental Lab products (cat# 2600mini) for freezing down our clones. The individual tubes hold a total of 600ul. We usually freeze each clone in a total of 300ul. The plates can not be stored directly in liquid nitrogen but can be placed in the vapour phase of nitrogen tanks. Sanyo also makes a really good -150 freezer that does not require liquid nitrogen (only for back up purposes).

**For best results/recovery, all clones should be thawed onto feeders.**

To thaw the whole plate:

The easiest way to thaw the whole plate is by popping off the bottom of the plate and then immersing the tubes, still in the rack, into warm water, trying to thaw them quickly. We then spin the whole plate at 1000 RPM for at least 5 minutes. Aspirate off the freezing media. Resuspend the cells in 200ul of ES media and transfer to a regular 96 well plate. Incubate at 37 degrees overnight, changing the media on the next day.

To thaw individual clones:

Thaw the individual tubes quickly in warm water. You can either add the contents of the tube directly to one well of a 24 well plate (with feeders) or you can remove the DMSO by adding the contents of the tube to 2mls of media and then spinning down the cells. I've never had a problem thawing the cells directly to the plate as long as you have at least 1.5mls of media in the well and the media is changed the next morning to remove any remaining DMSO.