Using the Pinner to transfer the Yeast Deletion Collection to new plates

Materials needed:
- Plates to pin from – donor plates
- Plates to pin to – recipient plates
- 1 384 pin pinner
- 2 guide trays
- 1 empty Omni-Tray
- YPD (~ 150 ml)
- one pinner cover/ethanol bath
- 2 tip lid boxes for water
- 95% Ethanol
- 1 Sonicating water bath – Branson 2150
- 1 Hair dryer
- permanent marker to label plates

About the pinner

The pins must remain clean in order to deliver a consistent volume/amount each time. The pins can get dirty from finger oils, lint from papers (kim wipes or paper towels) or from yeast. Don’t touch the pins or blot them on paper and always clean after use.

If the holes (through which the pins float) get wet: the pins will stick. Don’t get these holes wet if at all possible and dry them thoroughly if you do. Only wet the tips of the pins to minimize this problem.

To wash the pins, triple dip the pins in the liquid. It is not enough to just dip the pins in once. In the following instructions, wash means triple dip.

The rate at which you remove the pins from liquid determines how much liquid is retained on the pin.

- If you pull out of the water FAST – MORE liquid is on the pins
- If you pull out of the water SLOW – LESS liquid is on the pins

Between successive wash baths, make sure to remove pins slowly to minimize carryover between baths.

Wetting the pins aids in even transfer of yeast from one plate to the next. We use YPD to wet the pins because water alone has a very high surface tension and does not stick to the pins consistently.

Set up:

1. Wipe down your bench with ethanol before you start (use ethanol squirt bottles)
2. Fill the sonicating water bath (all the way to the Operating level) , plug it in and place it towards the back of your bench.
3. Fill two tip lid boxes with DI water, place in front of sonicating bath.
4. Fill a sterile, empty Omni-Tray with YPD. Replace lid when not in use.
5. Just before starting, fill the pinner lid box with 95% Ethanol.
6. Label your plates on the front of the bottom of the plate (like the donor plates). The minimum amount of information you should include when labeling these plates are: Plate code (DA --DZ or DSG), what’s in the plate (YPD, drug), date and group number.

Cleaning and sterilizing the pinner

1. Dip TIPS of pinner into sonicating bath for 20-30 seconds, depending on how dirty the pins are. This cleans pins and washes yeast away.
2. Wash tips of pins in two successive water baths to wash away water from sonicating bath. Dip pinner slowly each time and remove slowly last time.
3. Wash pins in 95% Ethanol solution
4. To remove ethanol, DO NOT flame pins. Instead, use a hair dryer to dry pins until the ethanol is evaporated. Do not dry longer than this or the pins get too hot. You may want to check that the pins are free floating by flipping the pinner upside down and back upright again. If they are stuck, see an instructor.
5. Cool pins before using.

Copying plates

1. Place guide trays over plates so that the notched edge of the plate and the single holes of the guide are both at the bottom. The orientation of plates must be consistent because you will need to compare them to one another to interpret the results of your screening. Place the lid of the plate over the guide to keep the plate covered while not in use.
2. Check to make sure that the guide tray is completely flush with each plate. You can do this by wiggling the guide tray while the plate is underneath.
3. Sterilize pinner.
4. Dip tips of pins into sterile YPD (in an empty Omni Tray) and remove slowly, being careful not to get excess liquid on the pins. Never use a non-sterile pinner in this YPD. If you do, you must re-sterilize the pinner, change the Omni Tray and get new YPD. If you always use a sterile pinner you do not need to use new YPD each time.
5. Holding the pinner over the guide tray and donor plate, align the guide pins so they will go into the single holes at the bottom of the tray. The pins must be totally vertical to prevent the guide pins from lodging in the guide holes.
6. As you lower the pinner into the guide try, visually inspect the alignment of the pins with the spots on the plates. This is a second check that the guide tray is fit properly to the plate.
7. When properly aligned, set the pinner down into the plate. It is not necessary to push down for the pins to pick up yeast.
8. Lift the pinner up and check to see that the pins are all down again. This is important so that the cells are all transferred to the donor plate.
9. Repeat steps 5-9 with the recipient plate.
10. To pin to multiple recipient plates from one donor plate, you have to switch the plate under the guide try between pinnings.

Other notes about copying strains

1. We make a donor dilution plate (see diagram) when copying the collection because often the number of cells transferred from the master plate is too high. Starting out with too many cells can make scoring sensitive strains difficult. We have found that if you make a new donor plate with less cells in each spot, then the spots are easier to screen.
2. When copying a plate, we use the following sequence of events:

   #- pin from original donor to new donor
   • sterilize pins
   • pin from new donor to drug plate
   • pin from new donor to YPD plate