Picking colonies after DNA transfer

Colonies of cells must be trypsinized and removed individually. For this we use "cloning rings". These are small glass cylinders (autoclaved) which are placed over the colony and held in place with autoclaved silicone grease, so that a drop of trypsin can be applied to just the cells of that particular colony.

1. Mark the positions of the colonies to be picked by drawing circles on the bottom of the dish. Check under the microscope that the cells look OK.
2. Remove the medium, wash with 5ml PBS, remove.
3. Pick up a cloning ring with sterile forceps, touch one end into the silicone grease and place it over the colony based on the circle drawn on the bottom of the dish. Put them on all the colonies before adding the trypsin.
4. Place one drop of trypsin from a pasteur pipette into each cloning ring.
5. Incubate at 37oC for 1 to 2 mins. Check under the microscope that the cells have detached.
6. Place 1ml selection medium into each well of a 24 well dish (one for each colony).
7. Take 150ul medium and remove the cells from within a cloning ring by pipetting up and down a few times, place the cells into the well in the 24 well dish. Repeat until you have picked all the colonies on that dish.
8. If you want to incubate the master plate further, remove the cloning rings and add back selection medium.
9. Wash the cloning rings - they are not disposable - and autoclave in a glass petri dish.

From here the cell lines can be transferred into larger dishes. When you have enough cells growing freeze at least 1 vial so that you don't have a whole incubator of dishes to keep passaging and then assay for expression of your gene.