Plating subclones for fluorescent screening

For every 12 colonies picked, you will need:

	21ml appropriate maintenance media
1	TC-treated 12-well plate
1.5	8 well chambered coverglass slides
1	10ml serological pipet
	serological pipetor
15	200 ul pipet tips (3 rows of 12)
	200 ul pipetor
	waste box for pipet tips
	pen

1. Choose 12 wells on the 96-well plate that contain one fluorescent colony. Choose from the lowest density plate first.

2. Remove media from selected wells, rinse once with 200ul PBS, then add 50 ul trypsin. Watch to see when cells detach.

3. While cells are trypsinizing:
   put 1 ml media in each well of 12-well plate
   put 0.5ml media into each of the 12 chambers
   mark each well and chamber with name of a chosen well from the 96 well plate

4. Once cells detach, to each well
   add 200ul of media
   triturate 5x to suspend cells and remove 200ul suspension
   plate one drop of suspension in appropriately marked chamber
   place the rest of the cells in appropriate well of 12 well plate

5. Add 200 ul media to picked wells of 96-well plate, this makes a backup from straggling cells.

6. Place cells in incubator.

7. Check chambered slides for fluorescence 1-2 days later.

8. Plate upto 6 good colonies in T-75, using all cells in the 12 well plate when confluency is 50-90%.

9. Freeze when T-75 is 50-90% confluent.