Plating of yeast cells using a pin-frogger (serial dilutions)

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Use:

Quick and uniform plating of serial dilutions of yeast cultures
(better results than using multi-channel pipette or grid pattern)

Requirements:
empty Petri dish with EtOH; agar (e.g. YPD plate); sterile 96 well microtiter plate; 48 pin or 96 pin frogger w/ round pins of 4 mm diameter

1. dilute mid-log phase grown yeast cultures (in YPD or selective media) to OD_{600} = 0.1 (equals 1.5-2 x10^6 cells per ml) with sterile water
   (alternatively, a stationary culture may also be used)
2. transfer 250 µl of each diluted cell suspension to first column of 96-well microtiter plate
3. add into the following 5 columns 200 µl sterile water
4. make serial dilutions 1:5 using a multi-channel pipette transferring 50 µl from each column to the following
5. plate diluted cell suspension series using a pin frogger (stamp, pin diameter 0.3 cm):
   a. sterilize pin frogger by EtOH/flame
   b. place sterilized pin frogger on a YPD (or any other plate) for 1-2 min to let it cool down
   c. dip pin frogger briefly into the cell suspension wells of the microtiter plate
   d. lift up frogger quickly to avoid loosing cell suspensions drops from the pins
   e. place frogger carefully on the plate on which dilution series should be analyzed and leave it there for 20-25 seconds to ensure that the drops have been come off efficiently from the pins
      (shorter plating times will result in small and uneven droplets; note: the diameter of the droplet depends also on the humidity of the plate – if results don't look satisfying, try freshly poured plates)
   f. repeat step c-e for further plates to be analyzed in parallel
      (sterilizing frogger is not necessary when using the same cultures/wells)
   g. sterilize frogger by repeating steps a and b

After use, clean the pins by first rinsing/dipping the frogger in water or EtOH, then subsequently by flaming off the EtOH; otherwise when flaming directly remnants of media and cells will be "baked" on the surface of the pins, which is hard to remove.