

	<b>Step</b>	<b>Duration</b>
<b>I</b>	Bacterial liquid cultures	2 days
<b>II</b>	Glass tubes cleaning after culture	
<b>III</b>	Glass tubes wash and sterilization	1 day

I. Bacterial liquid cultures

1. The tubes are stored below qPCR machine next to bacterial bench. Take appropriate number and label (do not use red marker – it is hard to clean from glass).
2. Turn on the flame (fire on the bacterial bench).
3. Using sterile conditions take off the cap and quickly rotate the open end of the glass tube in the flame to maintain sterile edge.
4. Add LB medium with antibiotic.
  - a. For clone testing use 3.5mL.
  - b. For plasmid amplification you can use up to 9mL.
5. Inoculate a single colony from an agar plate into a glass tube.
6. Quickly rotate the open end of the glass tube in the flame and close it with the cap tightly.
7. Incubate the culture overnight at 30°C or 37°C with vigorous aeration (250 rpm).
8. The next day transfer 1.5mL of the bacterial culture to an eppendorf tube.
9. Centrifuge at max speed for 2 minutes.
10. Decant the supernatant.
11. Repeat steps 8-10 if necessary:
  - a. For clone testing do 2x (centrifuge the bacteria from one glass tube in the same eppendorf tube). The leftover 0.5mL keep in the fridge till you get positive sequencing results. You can store these tubes up to two weeks. After confirmation you can add 9mL of LB with antibiotic and amplify more plasmid.
  - b. For plasmid amplification do 6x or spin down in glass tube (centrifuge at 4000 rpm for 10 minutes).
12. Isolate plasmids using Qiagen MiniPrep Kit.

II. Glass tubes cleaning after culture

1. Remove the caps to the bucket by the sink next to the bacterial bench.
2. Aspirate the remaining bacterial liquid culture to Wescodyne flask.
3. Discard the tip used to inoculate single colony in bacteriology waste box.
4. Clear the labels with 70% EtOH.
5. Spray Cavicide1 inside of the glass tube, wait one minute.
6. Wash tube with water in the sink.
7. Put upside down in the washing rack (with the holes in the bottom).

III. Glass tubes wash and sterilization

1. Tubes are washed on the same settings as other glass/plasticware.
2. The caps are best washed by hand. Use LiquiNox detergent and a brush. Rinse with tap water 3X, then MilliQ 3X. Turn upside down in racks to dry.
3. Once dry, turn tubes right side up and add caps (make sure the caps are tight on the tubes). Tubes and caps need to be dry before sterilizing. Sterilize using the Dry, 121°C autoclave program.