**Steady-state microfluidic chemostat experiments**

Last edited by Jen Nguyen, 2020 Feb 2

**Description:** steady nutrient supply in straight channels, with E. coli attached to glass surface of microfluidic device. This protocol sets up three nutrient concentrations of diluted LB, which can of course be substituted for whatever nutrient source you choose.

**Word of caution:** Jen initial**l**y performed experiments with glucose as the sole carbon source and did not get reproducible results! Juani has since performed experiments with alternative sources…

**Items to prepare in advance (day before experiment, or earlier):**

1. PDMS microchannel (bonded to glass slide)
2. Book the scope (one with a CAGE incubator)
3. Book a syringe pump (any Harvard Apparatus pumps, I tend towards programmable)
4. Overnight culture the evening before microscope experiment

**Items to make sure you have**:

1. frozen stock of E. coli
2. MOPs minimal media (I store 10x medium at -4°C)
3. Glucose and potassium phosphate supplement (100x stored at room temp)
4. Stock solution of LB (Lysogeny broth, aka: Luria-Bertani), 1x
   * **Note**: because LB is an undefined medium, batches can differ. Thus, all published experiments are best compared to the same stock solution of LB.
5. Equimolar (to LB) NaCl solution, autoclaved the morning of experiment
   * Same day autoclaving has been the only method I have found to avoid long-term bubble formation
6. 10 mL syringes (plastic)
7. Syringe tips
8. 1.5 mm outer diameter tubing (I punch devices with 1.5 mm inlets/outlets)

**DAY ONE**: Pre-experiment preparations

* **Book scope and syringe pump (often good to do several days early)**
* **Heat-up CAGE incubator to 37 C**
* **Start overnight cultures (two: in case one doesn’t work out):**
  + Thaw 1x MOPS medium (Teknova Product # M2101)
  + Mix: 6 mL 1x MOPS, 60 ul glucose (20%), 60 ul K2HPO4 stock
  + Separate culture medium into 2 culture tubes, 3 ml each
  + Inoculate each tube with frozen stock of *E. coli* NCM3722 *delta-motA*
  + Incubate tubes at 37C, shaking: \_\_\_\_\_\_\_\_\_ (time)
* **Bond channels to glass slide:** 
  + Wash slides by rubbing with isopropanol and kimwipe(s), then rinse with DI water. Dry completely, leaving no dust.
  + Plasma treat glass and PDMS for 1 min each.
  + Place treated sides together and rest overnight on 80°C hot plate. (One hour on hot plate is fine, if in a rush.)

**DAY TWO**: Experiment day!

* **Prepare LB dilutions (Clow, Cave and Chigh):**
  + Full LB stock made on: \_\_\_\_\_\_\_\_\_\_\_\_\_
  + Dissolve 2.5 g NaCl in 250 mL milliQ water.
  + Autoclave on liquid cycle #1. Allow to cool before mixing (to avoid burning nutrients).
  + Dilution sequence:
    - Chigh (2% LB) = 1 mL full LB + 49 mL equimolar NaCl
    - Clow (0.1% LB) = 2.5 mL Chigh + 47.5 mL equimolar NaCl
    - Aliquot 10 mL of Chigh and Clow for pH-ing
    - Initial pH of Chigh: \_\_\_\_\_\_\_\_\_
    - Initial pH of Clow: \_\_\_\_\_\_\_\_\_
    - Volume NaOH (100 mM) added to Chigh: \_\_\_\_\_\_
    - Volume NaOH (100 mM) added to Clow: \_\_\_\_\_\_\_
    - Final pH Chigh: \_\_\_\_\_\_\_\_
    - Final pH Clow: \_\_\_\_\_\_\_
    - Volume NaOH to add to Chigh:
      * Tube 1: 37.5 ml \* (vol added to aliquot) /10 ml = \_\_\_ x 3.75 =
    - Volume NaOH to add to Clow:
      * Tube 1: 40 ml \* (vol added to aliquot)/10 ml = \_\_\_ x 4 =
  + Load 10 mL syringes, one for each condition
    - Vacuum for 10 mins and remove air, place in CAGE incubator
* **Dilute o/n culture**:
  + OD600 at \_\_\_\_\_\_\_ am: \_\_\_\_\_\_\_\_
  + Mix: 9 ml 1x MOPS, 90 ul glucose (20%), 90 ul K2HPO4
  + Dilution of O/N by \_\_\_\_\_\_\_\_\_\_ for OD600 = 0.00 (tubes: 1A, 1B)
  + Stronger dilution of O/N by \_\_\_\_\_\_\_\_\_\_ (tube: 2A). Both in shaker at \_\_\_\_\_\_.
* **Poly-lysine treatment of channels**
  + Remove channels and allow to cool for 10+ mins
  + Dilute poly-lysine stock: 10 ul stock in 90 ul milliQ water
  + Incubate EVERY OTHER channel with diluted poly-lysine for 15 min
  + Remove poly-lysine, then wash channels with milliQ water
  + Remove water. Let rest to dry at room temp for 2+ hours
* **Prepare waste collectors and tubing**
  + Two 50 ml conicals: 2 for stable channels flow through
* **Vacuum devices for 10+ mins. Check culture OD:**
  + Goal: seed cells into channel between OD600 0.07 and 0.1
  + At \_\_\_\_\_\_\_\_, OD600 =
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  + At \_\_\_\_\_\_\_\_, seed channels with culture \_\_\_\_\_\_ of OD600 \_\_\_\_\_\_\_\_, leaving excess culture over inlets to prevent bubbles.
* **Set-up image acquisition:**
  + Hook up waste, vial and syringe tubing to device. Establish flow.
  + Remove EPI shutter and Piezo stage from device manager (this is for scope 5).
  + Select positions for imaging:
    - 1-10 (Clow)
    - 11-20 (Cave)
    - 21-30 (Chigh)

**DAY THREE** clean-up and data transfer