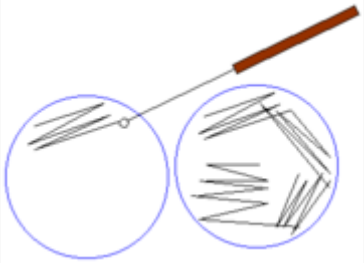
Streaking from agar stabs-

estimated time: 15 min. active, 16-24 hrs. incubation

material needed:

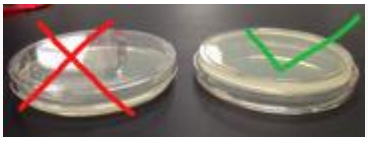
- 70% ethanol
- Paper towels
- Lab marker/Sharpie
- Agar Stab: Part A - BBa_J04500 (kit)
- Agar Stab: Part B - BBa_J04650 (kit)
- Inoculating loops (kit)
- LB agar plates – Amp/Kan (kit)

Protocol:

1. Clean the lab bench by wiping down with 70% ethanol and paper towels.
2. **Part A** (BBa_J04500) and **Part B** (BBa_J04650) are both maintained on pSB1AK3 plasmid backbones, which means they are ampicillin- and kanamycin-resistant. Label the agar plates with the names of **Part A** and **Part B**.
3. 

Notice how each zig-zag overlaps with the previous one just a little, and only at the end.

Use an inoculating loop to transfer some cells from the **Part A** agar stab to the appropriately labeled Amp/Kan agar plate. There is a hole in each agar stab from where it was inoculated. Dip an inoculating loop into the stab at the same location, and streak the bacteria onto the agar plate in a zig-zag pattern. Using a fresh inoculating loop, streak onto the agar plate again creating a new zig-zag pattern that overlaps the first. This will help ensure that you will have single colonies to pick from. Streak gently, and try not to puncture the agar.

4. Repeat step 4 for **Part B**..
5. 

> This prevents other bacteria from settling, and growing, on your agar plate.

Place the agar plates into the incubator with the agar side facing up, lid facing down (see insert). Incubate the agar plates at 37°C for 14-16 hours. Alternately, incubate at room temperature for 24-30 hours.

6. Once your agar plates have grown up you can store them in your fridge (4°C) until you're ready to grow up your cell culture.
    1. Plates can be stored at 4°C for up to 3 weeks.

Growing up cell culture-

estimated time: 30 min. active, 16 hrs. incubation

material needed:

- 70% ethanol
- paper towels
- Lab marker/Sharpie
- 14ml culture tubes (kit)
- 10ml of LB broth - Amp/Kan (kit)
- Inoculating loops (kit)
- Agar plate: Part A – BBa_J04500 (see previous step)
- Agar plate: Part B – BBa_J04650 (see previous step)
- Rotator/Shaker

Protocol:

1. Clean the lab bench by wiping down with 70% ethanol and paper towels.
2. Remove the agar plates for **Part A** and **Part B** from the incubator or 4°C fridge.
3. Label one 14ml culture tube for each Part. Add 5ml of LB broth (with ampicillin and kanamycin) to each culture tube.
4. Use an inoculating loop to pick a single colony from each agar plate and inoculate the LB broth, in the appropriately labeled culture tube. Do not use the same inoculating loop more than once! Press lightly on the snap caps of the 14ml tubes, the caps should be a bit loose to allow for air flow.
5. Incubate for 16 hours at 37°C, in a rotator or shaker. Rotation helps the cells grow faster, and prevents them from settling at the bottom.
6. After incubation, the cell culture should be cloudy. You can now firmly press down on the snap caps to seal the tubes and store the cell culture at 4°C until you're ready to move onto the next step.