Protocol: Induction of ASKA strains and Imaging

Materials:

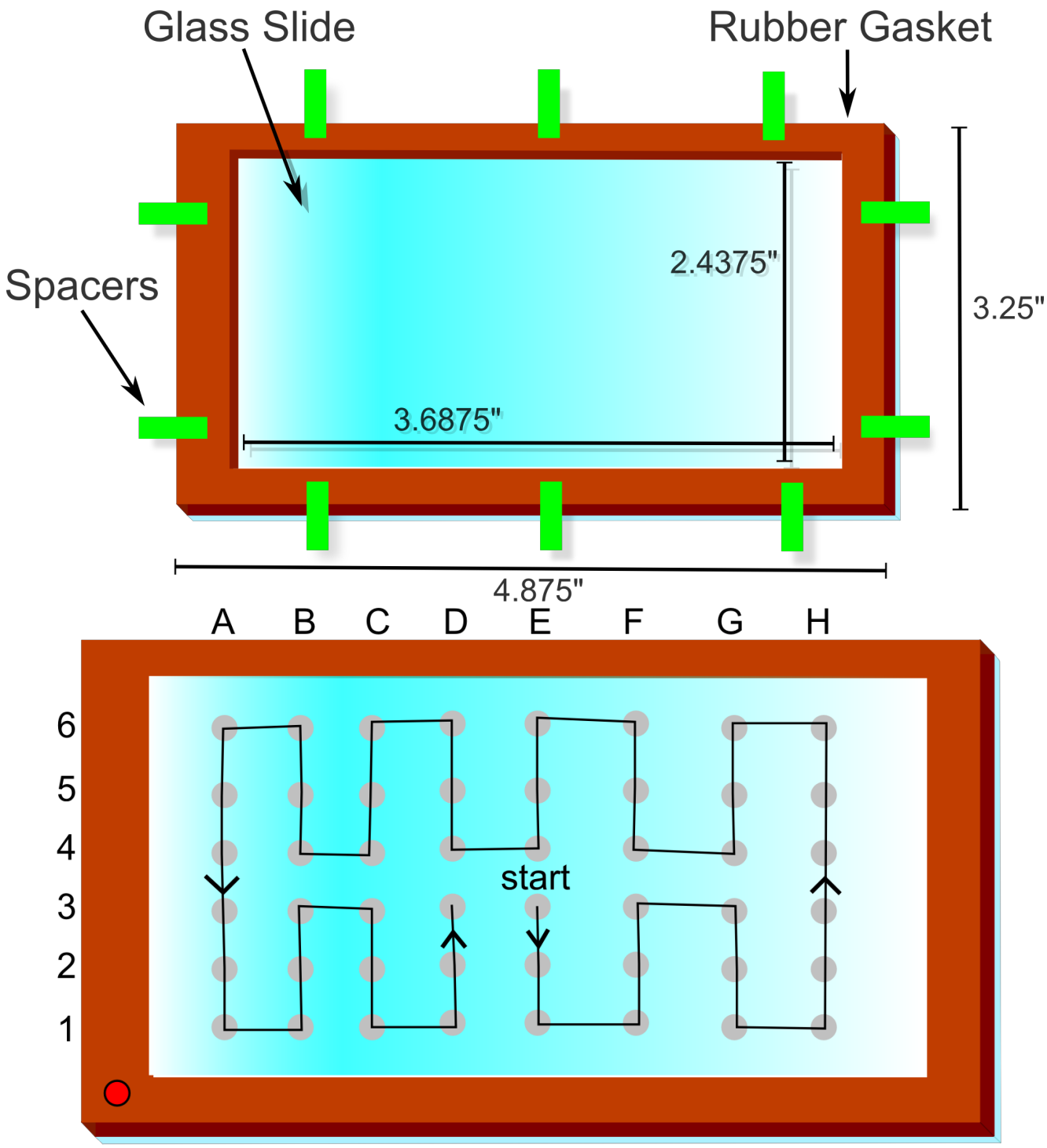
Material	Description
LB	Luria Broth
M9 ASKA	M9 Minimal Media: 1X M9 Salts, 2 mM MgSO4, 0.1 mM CaCl2, 0.2% glucose, 10 μg/mL Thiamine HCl 0.2% Casamino Acids
Cm34	Chloramphenicol, 34 μg/mL
IPTG	Isopropyl β-D-1-thiogalactopyranoside, 50-500 μM
VaLP	1:1:1 (by weight) vaseline, lanolin, paraffin wax
Rubber Gasket	1.58 mm rubber gasket plumbing material

Time Total	4 hours (prep) + 24 hours (growth)

Protocol:

Time	Activity
Day before	**Start LB culture in 96 deep-well plate (plate #1)** • Fill the 48 left wells with **500 µL LB Cm34** • Use 48 tyne pinner to transfer cells from freezer plate into LB wells • Cover with microporous sealing film. • Shake @ 30°C for ~24 hours. **Prepare Minimal Media and Induction deep-well plate (plate #2)** • Fill the left 48 with **1000 µl M9 ASKA Cm34** • Fill the right 48 with **200 µl M9 ASKA + IPTG** (0, 50, or 500 μM) • Cover with microporous sealing film. • Shake @ 30°C to bring up to temperature
3-4 h before induction	**Inoculate M9 Cultures** • Using the multi-channel pipetter, transfer **25 µL** from each well of LB cell culture (plate #1) to corresponding 1 mL M9 (plate#2). Pipette up and release 200 µL before transferring each 8-well row to ensure mixing. Incubate at 30°C in shaker for 3-4 hours.
2-3 h before induction	**Slide Prep:** Heat blocks and hot plates: • Turn on heat block ('high' setting) • Turn on VaLP hot plates to marked setting (140°C) • Turn on Water Bath hot plates to 'high' setting wait ~30 minutes for blocks and hot plate to heat. **Make pad agarose. For each pad, mix:** • 25 mL M9 ASKA • 0.5 g Low Melt Agarose (2%) in a 50 ml conical Falcon tube. Vortex, and melt in boiling water bath for ~30 min (ensure lid is loose while in water bath to prevent blow out). **Meanwhile, make slides:**

	• *See figure below for example slide.* • Select two 4.875” by 3.25” glass slides • Cut/Clean Rubber Gasket to slide size with 2.4375” x 3.6875” hole cut out of middle. • Clean slides with water. Remove excess water with napkin. • Clean slides with acetone. Remove excess acetone with kimwipe. • Clean slides with 70% ethanol. Remove excess with kimwipe. • VaLP the Rubber Gasket onto one glass slide. • Add single layer lab tape spacers around the edge of the Rubber Gasket to ensure constant pad height • Pour agarose into slide and cover carefully with the second clean slide, forcing out any bubbles, and place a weight on top while the agarose sets. Wait at least 30 minutes for proper cooling. The slides can be left at room temperature up to 2 hours while covered with the glass slide. **Sterilize Pinner** • Dip and swish the 48 tyne pinner in 70% Etoh • Place on clean napkin or flame and allow time to dry or cool, respectively.
- (0 hr)	**Induction** • Check OD600. Hopefully, it's .1-.2. If not, keep waiting, and let t=0 be the time when the OD enters this range. • Transfer **200 µL of culture** from the 1 mL wells into the 100 µL inducing media wells, and return to the shaker. (Use the 8-tip pipette, and be fast, as this can easily take 5 minutes.)
+ 25 min	**Dry Agaose Pad:** • Slowly slide the glass cover horizontally from the agarose pad, being careful not to score the pad surface by dragging excess agarose chunks over it. Carefully remove tape risers and excess agarose using razor blade. • Use a permanent marker to mark the lower left corner of the slide. This will be the register for the 1-A well on the 96-well slide.
+ 40 min	**Pin Cells onto Pad** • Use the 48-pin spotter to spot approximately 1-2 μL of each strain onto the pad. • Align the pinner such that the top-left corner of the plate (1-A) is near the lower-left corner of the slide, previously marked with a permanent marker. • Allow spots and slide to dry for 5 min longer than it takes all the pinner spots to disappear (~15-30 min). **Cover with the cover slip** • Use canned air to remove any dust from the coverslip surface • Starting from one corner, slowly lower coverslip onto the pad, pushing air bubbles out. • Seal the coverslip with VaLP. • Acclimate the slide at 30°C for ~1 hr before imaging to reduce thermal drift.
+ ~2hr	**Imaging** • Make sure there’s enough disk space. An 8-hour run can yield 3-4k images, so you’ll need 10-15 GB+. • Replace standard slide mount with 96-well format mount. • Visit each position in pattern (*shown below*) to minimize stage movement between points and adjust stage set screws to make the slide height as constant as possible. • Once the grid is set up, go to each point and adjust your focus and position (staying in the neighborhood of the guessed point) to find an ideal spot to image. • Exposure times: Phase - autoexpose, GFP - 700 ms with ND = 8 (inteselight) + 4 (slider). • Load the autofocus2 script in the ‘XY’ tab of the ND-Acquisition window. • Start automated imaging at 5 minute intervals for 8 hours.


Glass Slide
Rubber Gasket
Spacers
2.4375"
3.25"
3.6875"
4.875"
A B C D E F G H
6
5
4
3
2
1
start