Polysialic Acid (PSA) Fluorescence Microscopy:

For this experiment, prepare three separate cultures:

Culture 1: XL1-Blue *E. coli* transformed with pBAD (control) in standard LB media w/ glucose
Culture 2: XL1-Blue *E. coli* transformed with pSR23 (PSA) in standard LB media w/ glucose
Culture 3: XL1-Blue *E. coli* transformed with pSR23 (PSA) in LB supplemented with 14 mg/mL D-xylose and 17.7 mg/mL L-proline

1. Grow cultures for 8 hours and measure their OD600. To standardize each culture, dilute them with sterile PBS until each culture's OD600 is approximately 0.5 - 0.6.

Note: For fluorescence microscopy, the ideal OD600 is approximately 0.2 - 0.3. However, some of the culture will be lost in subsequent wash steps.

2. Fix the bacteria by adding approximately 20 ul culture to the slide, followed by 4% paratormaldehyde and 0.02% glutaraldehyde. Let sit for 15 minutes at room temperature and 30 minutes in a 4°C fridge, and then wash each slide or well with sterile PBS about 5 times.

Note: When it's unknown what dilution of antibody works best for a certain sample, prepare multiple slides/wells for differing dilutions. Also, be sure to include an antibody control slide/well where only primary antibodies are added, not secondary.

3. Take the slides out of the 4°C fridge and let air dry. Once dry, treat them for 5 min with methanol (chilled to - 20°C), and 30 sec with acetone (chilled to - 20°C) at room temperature.

4. Treat the slides with 2% (w/v) bovine serum albumin for 15min at room temperature in PBS prior to incubation with the primary antibodies.

5. Incubate the samples at 48°C overnight, adding a 1:5000, 1:2000 or 1:1000 dilution of PSA-specific primary antibody. Add only enough primary antibody to cover the sample.

6. On the next day, wash the samples with sterile PBS about 5 times. Once washed and dried, add the fluorescein-conjugated secondary antibodies at a 1:300 dilution. Incubate for 2 hrs in the dark.

7. Mount slide coverslips with Slow Fade containing 50% glycerol.