Microscopy Protocol

Day 1.
Grow Overnight cultures of desired strains LB (or MH)
Treat cover slips with poly-L-lysine (see below)

Day 2.
Dilute overnights 1:20 in 150µl fresh media
Incubate 90 minutes at 37C
Add chemical treatment (ie, peptide, etc.)
    Peptide concentrations vary, segregation effects seen beginning at 10-100ug/ml depending on the strain, media, blah, blah...
Incubate another 90 minutes at 37C
Transfer to eppies
Spin down, resuspend in 10µl of PBS + flurophore
    i.e. for 10µg/ml DAPI: 900µl PBS
    20µl 500µg/ml DAPI
    80µl (~2 drops?) 1x Slo-Fade
Fluorophores:
    DAPI (~5-10µg/ml)
    PI (~50µg/ml)
    FM4-64 (~10µg/ml)

Staining is virtually instantaneous, but fluorophores can be added prior to the end of the second incubation, if they are protected from light (this may reduce background if it is a problem, although it hasn't for me).

I keep the cells on ice until right before mounting, and I usually mount one sample at a time, and view them immediately (I take the Ice bucket, slides, cover slips, etc to the EM facility).

Poly-L-Lysine Immobilization
Suitable for methods that require cells and membranes to be intact

Clean cover slips
Dilute poly-L-lysine 1:10 with sterile DI or DDI water (as directed in PI of Poly-l-lysine)
    (poly-l-lysine solution should only be placed in plastic containers.)
Immerse cover slips in poly-L-lysine for 5 minutes
Dry slips at 60C for one hour or at room temperature overnight

Drop culture on clean microscope slide
Cover with coated cover slip
    I've noticed that the cells adhere to the cover slip better if given a few minutes to adhere.

OR

Methanol Fixation
Suitable for methods that do not require the membranes to be intact, or cells to be alive.
(*In other words, don't use this method unless your cells are already fixed!*)

Clean slides
Smear sample over slide surface
Air dry
Immerse in methanol for 5 minutes
Air dry