FM 4-64 Labeling
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1. Grow cells to early log (1.0 OD/ml).

2. Spin down 1-1.5 ml of culture in a microfuge tube.

3. Remove medium until 100µl remains.

4. Resuspend pellet by pipeting.

5. Add 0.5 to 1.0 µl of FM 4-64 (Molecular Probes: dilute FM 4-64 in 100 µl DMSO and store at –20°C; concentration = 16 mM)

6. Incubate at 30°C for 30 to 60 min. Agitate the cells every 10 min.

7. Wash cells 2x with 1ml of medium (SMD).

8. Resuspend in 1ml of SMD “chase” and incubate at 30°C for 1 hr in microfuge tube with occasional agitation. Alternatively, you can transfer cells to Falcon 2059 tubes and incubate them on a shaker.

9. Spin and resuspend in 1/10 volume and mount on slides.