PREPARING & ISOLATING ACTIVE RIBOSOMES

Patricia L. Clark

last updated: 12/7/01

PREPARING

- Inoculate 100 ml of minimal media with 2 ml of a fresh O/N culture of *Salmonella* strain 7136.
- Grow at 30°C until $2x10^8$ cells/ml
- Infect with 5-/13- phage to moi=10
- Continue to culture at 30°C for 1.5 hr
- Quickly chill cultures on ice, adding 2 8 ml R buffer ice cubes to each culture to speed chilling; pour into pre-chilled SS34 or GSA bottles; spin @ 5K for 10 min
- Resuspend pellet in 0.5 ml R+OG buffer, split into two microfuge tubes
- Freeze/store in -85°C; or, freeze cells in liq.N_2 and store @ -30°C indefinitely

ISOLATING

- Make 17 ml sucrose gradients: 16 ml linear gradient of 10-30% sucrose in R buffer, with 0.75 ml 60% sucrose cushion (plus ~0.25 ml sample)
- Thaw frozen cells in RT H_2O bath to initiate lysis (can add 75 µl RNase + 75 µl 0.5 M EDTA to destroy ribosomes, if desired…incubate 10 min at RT for complete destruction)
- Spin in microfuge @ 14 K for 1.5 min
- Layer lysate supernatant onto gradients
- Spin 18 K rpm for 18 hr in SW28.1 rotor, or some other combination of speed/time to give ω^2/sec=$2.3x10^{11}$ (max speed = 28 K rpm, for 7.25 hr)
- Fractionate gradients using peristaltic pump (pumping from bottom, at 1 ml/min) making thirty (30) 0.57 ml fractions
- Check A_{260} of fractions: make 12.5-fold dilutions (420 µl R buffer + 80 µl fraction) and measure with Cary UV spec; good A_{260} for 50*S* peak ~ 0.6