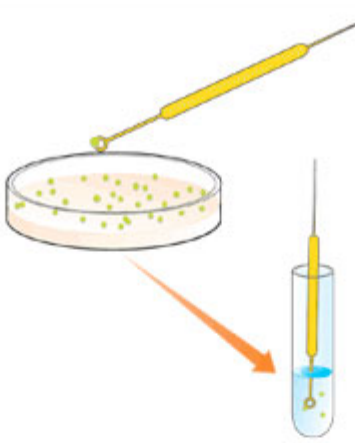
Start with bacterial colonies transformed with pGLO plasmid DNA

Pick a single fluorescent green colony from the agar plate using a sterile inoculation loop



Inoculate into nutrient broth containing ampicillin and arabinose

Grow overnight at 32°C or 2 days at room temperature with shaking

Lab 1

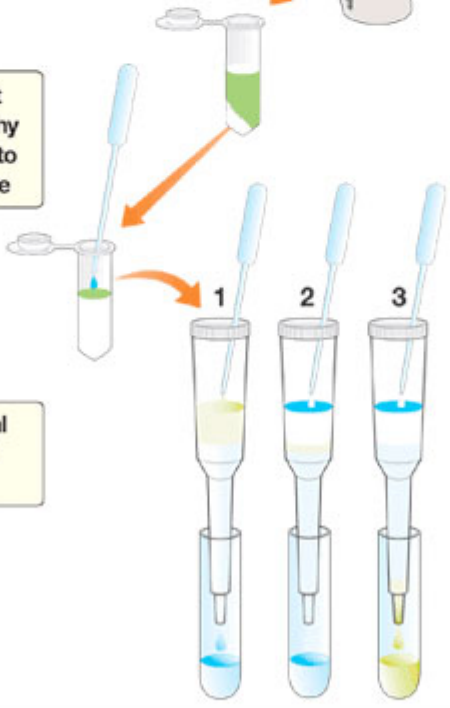
Transfer cell culture to micro test tube, then centrifuge and pellet cells

Resuspend cells, add lysozyme, and freeze overnight to rupture cell membranes

Lab 2

Centrifuge bacterial lysate to pellet membranes and debris

Add high-salt chromatography binding buffer to bacterial lysate



Load bacterial lysate onto columns

1. GFP binds to chromatography matrix in high-salt buffer

2. Add medium-salt buffer to wash bacterial proteins from column

3. Add low-salt buffer to elute GFP

Collect three fractions

Separate GFP from bacterial proteins

Lab 3

Extension: Use protein gel electrophoresis to conduct quantitative and qualitative analysis of fractions.