Nucleus Protocols

Assemble Base Cytosol

1. Cytosol Reaction Setup

Component	Cytosol +deGFP DNA (µL)	Cytosol -deGFP DNA (µL)
SMix	10.5	10.5
Water	7	8.75
RNAse Inhibitor	1.75	1.75
PMix	4.2	4.2
tRNA	3.5	3.5
Ribosomes	6.3	6.3
p0pen-deGFP DNA template	1.75	0
Total master mix volume (µL)	35	35

2. Assemble Cytosol Reactions

- ☐ Thaw reagents above on ice.
- ☐ Chill two (2) PCR tubes on ice to assemble reaction master mixes.
- ☐ Resuspend each component according to the following instructions:
    - ☐ Vortex SMix aggressively until visibly clear. Alternate 10s vortex / 10s rest on ice to maintain cool temperature. SMix should be transparent with no visible precipitate when ready.
    - ☐ Vortex or pipette mix tRNA.
    - ☐ Pipette mix PMix.
    - ☐ **Do NOT vortex** ribosomes! *Gently* pipette mix or flick the tube.
- ☐ For each reaction, assemble reaction master mix in a chilled PCR tube by adding each reagent in the order and volume listed in the table above.
- ☐ Mix the master mix thoroughly by pipette (6-10x) until visibly homogeneous.
- ☐ Hold assembled reactions on ice or at 4°C until ready for measurement.
- ☐ On a 384-well optical plate, array 10 µL of each reaction master mix into three (3) wells and note their locations.
- ☐ In a plate reader set to 37°C, measure deGFP expression using the standard green fluorescence channel (ex: 485 nm, em: 515 nm).

3. Return reagents to their appropriate storage locations

- ☐ Mark the lid of each Cytosol component that you used. The number of dots indicates how many freeze–thaw cycles each component has gone through.