SOP Name: Bacterial CFPS Cell Extract Preparation Procedure
Date: 22/05/17
Author: Bradley Brown
Source(s): Adapted from: Kwon & Jewett 2015 (DOI: 10.1038/srep08663), Kelwick *et al.* 2016 (DOI: 10.1016/j.ymben.2016.09.008)
Time Required: 3-4 days

Notes:

- 200 mL culture yields approximately 1,600 µL cell extract (~100 reactions at 50 µL)
- Once the cells have been harvested, keep everything on ice
- For *E. coli* harvest time is at OD_{600nm} ~2.5, and at OD ~2.0 for *B. subtilis* 168
- Resuspension of cell pellets during the wash stage can be difficult due to the pellet becoming dislodged – try re-suspending in 1 mL per gram wet cell pellet of wash buffer first, and then adding the remainder once the pellet is mostly suspended.
- Total energy input during the sonication stage can be calculated using:

$$Total\ energy\ input\ (joules) = sonication\ time\ (seconds) \times watts$$

Materials:

- 200 mL LB broth
- 2 L conical flask
- Cells to be used as the extract source (e.g. *E. coli* BL21)
- CFPS wash buffer + 2 mM DTT

Procedure:

1. Inoculate 4-5 mL LB broth with your cells of choice and incubate overnight at 37°C and 250 RPM
2. Inoculate 200 mL LB in a 2 L conical flask with 2 mL of the liquid culture
3. Incubate at 37°C and 250 RPM until the cells reach late exponential phase
4. Transfer 100 mL of culture into two pre-weighed 50 mL falcon tubes (50 mL each)
5. Harvest by centrifuging at 4,500 RPM and 4°C for 20 mins
6. Discard the supernatant and split the remaining culture between the two falcon tubes and centrifuge at 4,500 RPM and 4°C for 20 mins
7. Discard the supernatant and weigh the tubes to determine the wet cell pellet weight
8. Store the pellets at -20°C (max 48 hours)
9. Defrost the pellets on ice (30-60 mins)
10. Add DTT to CFPS wash buffer (on ice) such that the final concentration is 2 mM (0.3 mg mL^{-1})
11. Re-suspend the pellets in 10 mL wash buffer per gram of wet cell pellet and centrifuge at 4,500 RPM for 20 mins at 4°C
12. Discard the supernatant and repeat steps 11-12 twice more
13. Re-suspend the pellets in 1 mL wash buffer per gram wet cell pellet
14. Aliquot cells to 1 mL in 2 mL tubes
15. Lyse the cells using sonication with cycles of 40 seconds on, 60 seconds off and an amplitude of 20%
    - The total energy input required depends on the cells being used – for *E. coli* BL21 432.5 J is recommended and for *B. subtilis* 168 1,200 J is recommended (Kwon & Jewett 2015, Kelwick *et al.* 2016)
16. Centrifuge the lysed cells at 12,000g for 10 mins, transfer the supernatant to fresh 2 mL tubes, flash freeze in liquid nitrogen and store at -80°C