Lysis Method Comparison

Goal: Compare various lysis methods for the determination of intracellular NADPH and NADH in *E. Coli BL21* with and without the zwf gene.

Background:

Last week, we compared various sonication methods by determining the fluorescence of mCherry protein before and after sonication. However, when the methods were applied to tests for NADPH and NADH, the levels of each were negligible. We believe that the sonication may have destroyed the NADPH and NADH in the solution.

Comments:

- All samples should be kept on ice at all time.
- Readings should be done immediately.

Protocol:

1. Prepare starters of *E. Coli* BL21 with plasmid pSB1C3 containing pT7 and RBS, as well as *E. Coli* BL21 with plasmid pSB1C3 containing pT7, RBS, and the *zwf* gene with 5 µl CM antibiotics! Incubate at 37^{o}C in the shaker, overnight.

2. Dilute the starters at a ratio of 1:100 and incubate in the shaker until O.D. at 600 nm is 0.6.

3. Pipette 1 ml of samples into 27 halicots (3 lysis techniques x 3 settings x 3 samples).

4. Centrifuge at the maximum speed for 5 minutes. Discard supernatant.

5. Quench cells twice with methanol on liquid nitrogen.

6. Resuspend cells in 0.5 ml cold BA**.**

7. Freeze in liquid nitrogen until use.

8. Conduct the relevant lysis method (as listed in the tables below).

9. Read wells in fluorescence reader with 340 nm excitation and 460 nm emission.

Freeze-thawing:

Sample #	Time in -30 C freezer (min)	Time in liquid nitrogen (min)	Time in water bath (min)	Water bath Temperature (C)	Repeats
1	10	0	10	30	1
2	0	3/4	15	30	3
3	10	0	10	30	2

Bead Vortex:

Sample #	Amount of Beads	Agitation Time (min)	Cooling Time (min)	Repeats
4	50%	1	1	2
5	50%	1/2	1	2
6	50%	2	1	1

Comment: Recover the lysate by centrifuging the mixture for 2 min at max speed.

Sonication:

Sample #	Amplitude	Sonication Time (sec)	Cooling Time (sec)	Repeats
7	20%	4	30	5
8	25%	4	30	3
9	20%	10	30	3

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