6. Protein Expression

After checking which clones were correct and preparing plasmid from the clone, we need to start characterizing the biobricks. One of the very first way to characterize is to overexpress the protein, and a common is called sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). On a SDS-PAGE gel it is at least possible to trace if the biobrick was well expressed. In order to express the protein after T7-promoters, IPTG (isopropyl β-D-1-thiogalactopyranoside), a galactose homolog, will be used to induce the expression of the proteins.

Materials

BL21 cells
Ice
Agar plates with ampicillin
Alcohol lamps
LB
Ampicillin
SDS-PAGE equipment
Separating buffer
Stacking buffer
40% 1:19 acrylamide
TEMED (tetramethylethylenediamine)
1% APS (ammonium Persulfate)
10X SDS buffer
SDS dye
IPTG
Coomassie Blue solution
Gel boxes

Procedures

Transformation

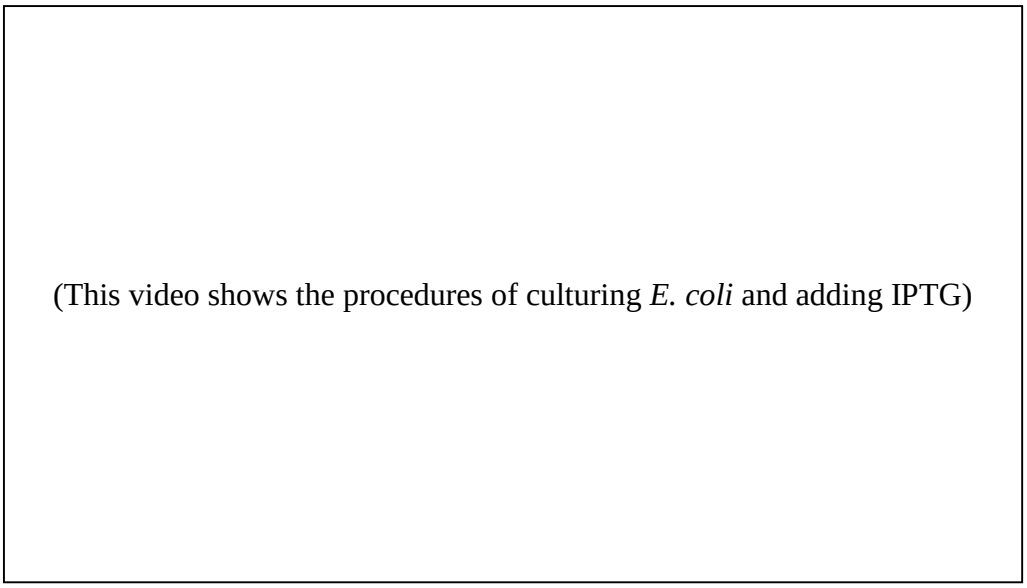
**REMINDER: All steps in this protocol is to be performed under aseptic conditions!*

1. Thaw competent cells (*BL21*) on ice until totally melted.

2. Aliquot 1 ng of DNA into the 100 ul of cells.
3. Store on ice for 30 min.
4. Heat shock the cells at 42 ℃ for 2 min sharp.
5. Cool on ice for 2 min.
6. Aliquot 800 ul of LB/SOB/SOC/KB (king's broth) into a 1.5 ml centrifuge tube. Add all cells into the tube. Shake in 37 ℃ and 250 rpm for 45 min to 1.5 h.
7. Centrifuge at top speed briefly and remove 800 ul of supernatant.
8. Re-suspend the cells in the remaining medium.
9. Spread cells on agar plates with ampicillin.
10. Incubate the plate in 37 ℃ overnight.

Expression

1. Pick a single colony into 5 ml LB with ampicillin and grow in 37 ℃ overnight.
2. 1% inoculation into 50 ml LB with ampicillin in 200 ml flask. Shake in 37 ℃ and 250 rpm.
3. Track OD 600 every 30 min until OD reaches ~0.5 A. This takes about 3-4 h depending of strains.
4. Collect 0.5 ml un-induced sample and record the OD, centrifuge at top speed for 1 min, and re-suspend in 50 ul of water. Add 50 ul of SDS-dye, and boil in 95 ℃ for 15 min.
5. Add 0.1% IPTG into culture, and shake in 37 ℃ and 250 rpm for 4h.
6. Take 0.1 ml induced sample and record OD. Dilute appropriately, and then add 50 ul of SDS-dye, and boil in 95 ℃ for 15 min.



SDS-PAGE

1. Cast 4 ml separating 12.5% SDS gel with 1.7 ml water, 1ml 4×separating buffer, 1.25 ml 1:19 40% acrylamide, 4 ul TEMED, and 50 ul 1% APS.
2. Add a few drops of isopropanol on top, and wait for 10 min.
3. Pour isopropanol, absorb remaining isopropanol with filter paper stripes.
4. Cast 2 ml stacking gel on top with 1 ml water, 0.22 ml 1:19 40% acrylamide, 0.45 ml 4× stacking buffer, 4 ul TEMED, 25 ul 1% APS. Put on comb quickly
5. Run SDS-PAGE with 4 ul markers, 10 ul of un-induced, and induced samples at 50 mA for 30 min.
6. Stain gel with Coomassie Blue solution for 15 min after heating.
7. Destain for a few hours after heating.