# Day 2: subculture and induction of gst-dhfr-his protein expression report example...

Art & Culture

Day 1 - Cell Culture: At First an LB/amp plate was labeled with initials, the date, the name of the bacterial strain to be used (BL21) and the plasmid (pDHFR). Then 10 uL of reconstituted BL21-pDHFR was pipetted onto an LB/amp plate using sterile tip. The bacteria was streaked into four quadrants of the plate and after this it was covered immediately to avoid contamination. The plate was placed upside down inside the incubator overnight at 37°C and once colonies have grown to ~1 mm in diameter, they may be stored in a sealed bag upside down at 4º C for up to one month.
- Overnight Cell Culture: At first a 50 mL conical tube was labeled with initials, the date, and the name of the E. coli strain (BL21) and plasmid (pDHFR). Then 150 µL sterile 20% glucose is added to the 3 mL of sterile LB/amp broth. Using a sterile pipet tip, a single colony was picked from the starter plate and dropped it into the LB/amp/glucose. Then the tube was recapped loosely and the culture was incubated by shaking, at 37°C for 12-18 hours.

At first Uninduced SDS-PAGE sample was prepared from overnight culture in a 1. 5 mL microcentrifuge tube. Then the tube was heated at 95°C for 5 minutes and the sample was stored at –20°C until ready to analyze via SDS-PAGE analysis. Cell Density of overnight culture was then measured. After this, a subculture with a starting OD600 of 0. 3 was prepared. Then OD600 of subculture was measured and GST-DHFR-His expression was induced. In the next steps, preparation of Induced SDS-PAGE Sample and pelleting of induced cells were carried out.

## Day 3: Lysing cells and performing protein purification.

At first 250 µl Lysis buffer 1 was added to each of two Induced Cell Pellet tubes and cell pellets were resuspended by pipetting or vortexing as if there are no cell clumps remaining in both of the tubes. After performing lysing process, soluble parts from Insoluble Induced Cell Fractions were separated and SDS-PAGE Sample was prepared by Affinity and Size exclusion (Desalting) Purification of GST-DHFR –His protein (eluate fraction)

## Day 4: SDS-PAGE analysis of fractions and Protein Quantitation.

SDS-PAGE samples were reheated at 95˚C for 2 minutes to redissolve any precipitated detergent and samples were centrifuged at 16, 000 x g for 2 minutes . Then a SDS-PAGE gel was loaded and run at 200 V for 30 minutes or until the dye front reaches the bottom of the gel. After this, the gel was removed from the cassette. Bradford Protein Assay was performed to quantitate the concentration and yield of protein in eluate desalted eluate sample. In the net step, the fraction to be tested was determined for enzyme activity.

## Day 5: DHFR Enzymatic Activity Assay

At first spectrophotometer was set to read at 340 nm and a stopwatch arranged to measure time intervals. for reading samples every 15 seconds for 150 seconds (11 readings total). After arranging Blank with 985 uL 1X PBS, a UV compatible cuvette was taken out of the spectrophotometer and saved. In next step enzyme sample of GST-DHFR-His was added with DHFR’s cofactor, NADPH, to the 1x PBS. Then the enzymatic reaction with GST-DHFR-His, NADPH (cofactor) and DHF (substrate) were allowed to take place. As the

NADPH cofactor was oxidized to NADP+, its absorbance at 340 nm decreased. So by measuring the reduction in absorbance of a blank (NADPH, GST-DHFR-His, but no DHF substrate) and also the reduction in absorbance of the enzyme reaction (NADPH, GST-DHFR-His, and DHF), the conversion of NADPH to NADP+ attributing to the enzymatic activity of DHFR was evaluated.