Protein Production in Avidity Atum Vectors

The yield of target proteins with different promoters is difficult to predict and varies with the individual proteins being produced. Avidity offers the choice of the T5 promoter (IPTG inducible) and Rha_{BAD} (rhamnose inducible) both of which work well in Avidity's BirA-overproducing *E. coli* strains (AVB100, AVB101).

Rhamnose Induction Protocol

The rhaBAD vector can be used in either of Avidity's BirA overproducing strains (AVB100, AVB101). The rhaBAD promoter is tightly regulated and tunable using glucose (repression) and L-Rhamnose (induction). Unlike IPTG inducible systems, protein expression levels within each cell can be increased by using higher L-Rhamnose concentrations.

1) Grow cells overnight in LB plus kanamycin (50 micrograms/ml).
2) Dilute 1/100 into fresh LB (Kn50) or rich media (minus glucose, Kn50) and grow to mid-log (A_{600} 0.4-0.6 in LB; OD600 0.6-0.8 in rich media)
3) Induce by adding rhamnose to a final concentration to 0.2 % (1/100 of 20% w/v stock solution provided), and grow for an additional 4-8 hours. More or less Rhamnose may be optimal Titration of L-Rhamnose concentrations allows optimal conditions to be identified. If the culture is grown at lower temperatures (16C-30C) to increase solubility of the expressed protein, up to 24 hours may be required to achieve optimal expression.

Rha_{BAD} Auto-induction Protocol

Cells will preferentially use glucose until it is exhausted, whereupon rhamnose will be utilized inducing the Rha_{BAD} promoter. Optimal conditions for expression of soluble protein, including growth temperature, length of induction, and concentration of rhamnose should be determined empirically for each target protein.

1) From a plate, take a single colony, or from an overnight culture (1/100 dilution), inoculate LB media or rich media containing 50 µg/mL kanamycin plus 0.15% D-glucose and 0.2% L-rhamnose. The timing of induction can be altered by adding less (0.05%) or more (up to 0.2%) of D-glucose. [A timecourse experiment is recommended to determine optimal induction times. Take 1 ml aliquots, spin 12k for 1 min in a microfuge, resuspend pellet in 50 microliters gel loading buffer].
2) Grow until late log phase (OD_{600} of 0.8 in LB, OD_{600} 1.2 in rich media) at 37C, or overnight (up to 24 hours) at reduced temperatures (16C-30C).