FACULTY OF ENGINEERING &TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

PROTEIN EXPRESSION

Work Flow

- Choose an expression vector
- Insert the target gene
- Transfer the cloned DNA into the host strain
- Express the protein
- Evaluate protein yield, solubility and/or activity

Choose an Expression Vector

Choice of promoter:

- Inducible or constitutive?
- Requires engineered expression strain?

Selection marker:

- • Ampicillin selection prone to satellite colonies
- Carbenicillin or kanamycin less prone to satellites

Replication origin:

- • High or low-copy
- • Inducible copy number

- Fusion tags:

- • Purification or detection
- • Enhance expression/solubility
- • Reporter

PROTEIN EXPRESSION

Key Variables that Determine Success:

The DNA sequence

- ❖Codon optimized for E. coli
- ❖Optimized for secondary structure
- ❖Target truncation

Activity of promoter

Cloning & host strain capabilities

- ❖Toxic genes, repetitive structures
- ❖“Leaky” expression

Growth & induction conditions

- ❖Cell density at time of induction
- ❖Length and temperature of induction
- ❖Concentration of inducing agent