



RAMA
UNIVERSITY

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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:

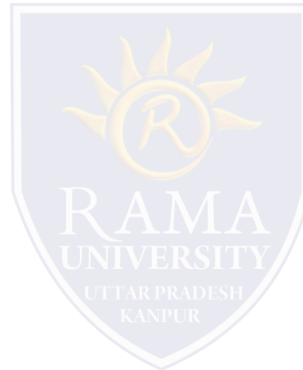
- ❖ Inducible or constitutive?
- ❖ Requires engineered expression strain?

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

