Yeast Protein Expression System 2min

Sunday, 29 May 2016   12:23 PM

Yeast Protein Expression System
- Produce large amounts of protein
- Expression systems:
  - Saccharomyces cerevisiae
  - Bacillus gender
  - Pichia pastoris:
    - Methylotrophic yeast (methyl used as carbon source)
    - Stable and lasting production of proteins
    - Commercial kits
    - High yield
    - P. pastoris expression vectors integrated into genome
- Advantages:
  - Eukaryotic system
  - Cost effective easy to manipulate and grow
  - Tightly regulated promotors
  - Variety of selectable markers
  - Grown in chemically defined media
  - High density production of proteins
  - Available strains for secreted proteins that are glycosylated

Budding Yeast Cell Cycle
- Results in two cells of unequal size:
 - Mother and daughter cell
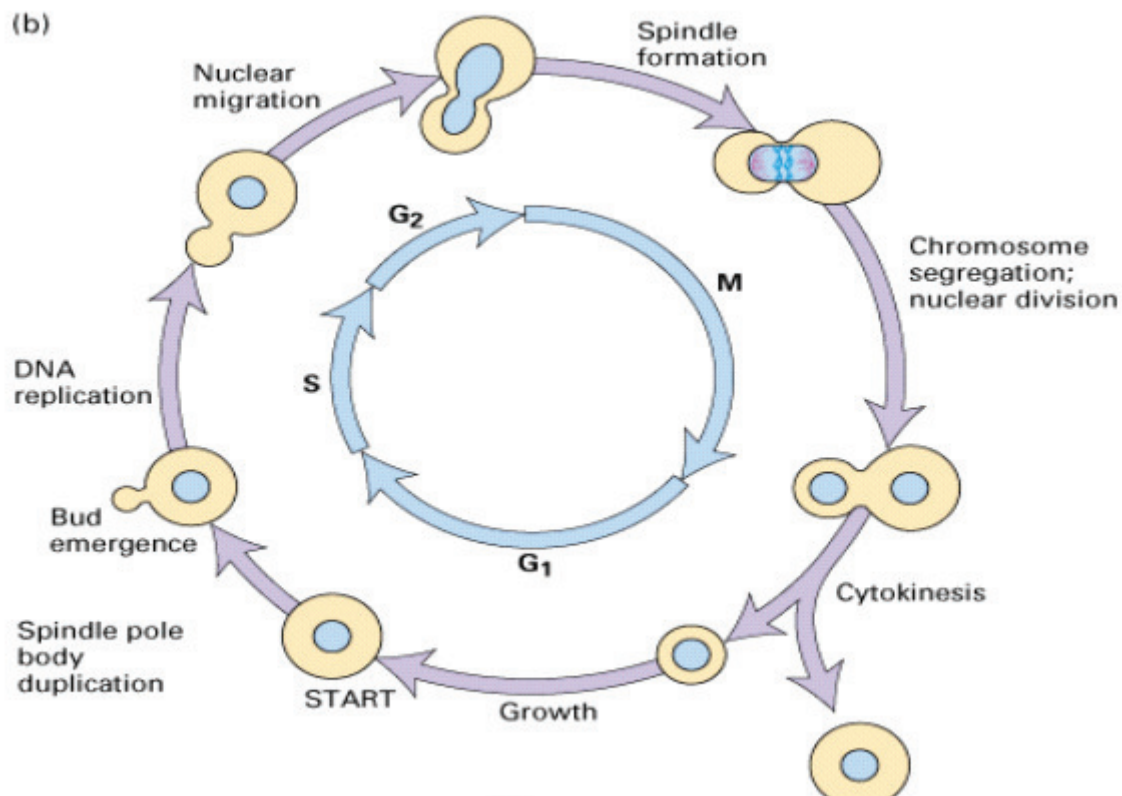
Saccharomyces cerevisiae
- Budding yeast
- Most common yeast
- Model system
- Episomal or plasmid vectors
  - Commonly use episomal expression vectors

- Shuttle vectors
- Integrating vectors
- Yeast artificial chromosomes (YACs)

Shuttle Vectors
- Yeast episomal plasmid:
  - Denoted by Yep
  - Backbone of E. coli vector such as pBR322, pUC19, pBLUESCRIPT
  - Yeast selection markers such as URA3, HIS3, TRP1, LEU2
  - Yeast replication origin of yeast 2micron plasmid
  - Expression controlled by GAL1 or GAL10 promotor which responds to glucose
  - High copy number of 20-50 per cell
  - Grown in glucose: Immediate transcription
  - Grown in galactose: High level transcription in 3-5 hours
  - Grown in glucose and galactose: Immediate transcription using glucose then galactose starts as glucose is preferred for metabolism
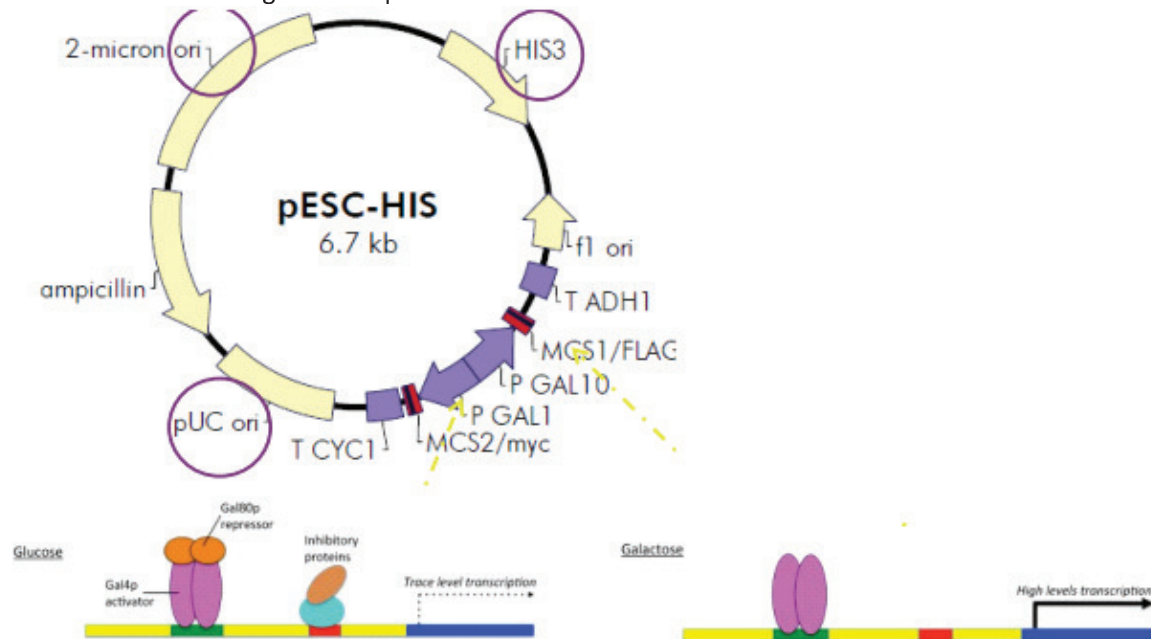
- Yeast integrated plasmid:
  - Denoted by Yip
  - Lack yeast replication origin
    - Have to integrate into yeast genome via homologous recombination to replicate