

Syllabus

Protein Expression Boot Camp - Summer 2013

Part I - Expression in E.coli

Day 1: 1PM- lecture

The Big Picture about Expressing Proteins in Pro and Eucaryotes:
Hosts - Pros and Cons
Choosing a system
Introduction to plasmid Vectors
Transformation- Introduction of plasmid DNA into bacteria

2:30 PM – laboratory exercise

Transformation by temperature shock.
Media production: LB, LB +agar, Magic Media (Terrific Broth plus supplements)
Streak transformed bacteria onto ampicillin LB agar plates/Incubate at 37 C overnight

Day 2: (10 AM) 15 min break from PBF class)- check overnight colonies; pick to 5 ml culture

1 PM lecture

pET vectors
inducible vectors
vectors for fusion proteins
solubility
purification

2 PM – laboratory exercise

supplement Magic Media and pre-warm
make lysis buffers and electrophoresis buffers for next day
transfer mini-culture from AM to maxi cultures for o/n growth

Day 3: 1 PM - laboratory exercise

Harvest cultures by centrifugation; resuspend in lysis buffer; break open pelleted cells with micro fluidizer; remove cellular debris

Part II Purification of Proteins

Day 4: 1 PM

Introduction to purification- AKTA exercise-
Gel of day 1 to 3
Lecture - Selecting methods based on your protein

Day 5: 1 PM

AKTA purification on Phenyl Sepharose 6b column
Acrylamide gel of all fractions days 2 -5

Conclusion of Expression and Purification in E.coli Workshop

BACULOVIRUS EXPRESSION starts in 1 week