Questions from last lecture?
Why study bacteria?

(Why do we need bioengineers to study bacteria?)
“We’re here. We’re in the post-antibiotic era. There are patients for whom we have no therapy, and we are literally in a position of having a patient in a bed who has an infection, something that five years ago even we could have treated, but now we can’t.”

-Arjun Srinivasan, Associate Director at the CDC
A class of bacteria commonly found in the guts of people—and rodents—appears to keep mice safe from food allergies, a study suggests. The same bacteria are among those reduced by antibiotic use in early childhood. The research fits neatly into an emerging paradigm that helps explain a recent alarming increase in food allergies and other conditions, such as obesity and autoimmune disease, and hints at strategies to reverse the trend.”

Couzin-Frankel, Science, 2014
Next Generation: Souped-up Probiotics Pinpoint Cancer

Genetically engineered commensal bacteria help researchers detect cancer metastases in mouse livers.

By Kate Yandell | May 28, 2015

“Researchers at MIT and the University of California, San Diego, have programmed a probiotic Escherichia coli strain to detect cancer metastases in the liver.”

Bacterial cultures

- Liquid cultures
  - Nutrient media
  - Aeration (shaking)
  - Temperature

- Solid cultures
  - Agar with nutrient media
  - Temperature
Plating bacteria from liquid culture

- Bacteria “colonies” arise from an individual cell
- A “lawn” is when individual colonies are not distinguishable
Bacteria growth phases (liquid culture)

- **Lag phase**
- **Exponential (log) phase**
- **Stationary phase**
- **Death phase**

![Graph showing the growth phases of bacteria](image-url)
Working with bacteria

• Bacteria are EVERYWHERE (so are molds, viruses)...and you don’t want them to contaminate your experiments

• We need aseptic technique!
Aseptic technique: minimize contamination

• Decontaminate work area and other surfaces with 70% ethanol
• Do not move over open tubes/containers/plates
• Do not place caps on bench top
• Keep lids on bottles when not in use
Importance of aseptic technique!
Technical point: Starting a bacteria culture

- Streaking a plate from liquid culture or frozen stock ("glycerol stock")
Technical point: Plating bacteria cultures

• Pipette liquid culture directly on to LB agar plate
• Spread culture evenly across plate using cell spreader
• Allow liquid to absorb into plate before turning upside down and placing in the incubator
• This will be demonstrated by the TA in lab
How do we count bacteria?

- Bacteria range in size from less than 0.5μM to ~2μM in length.
- Bacteria are hard to identify at less than 1000X magnification.

Scale bar 10μM, 1152X

How do we count bacteria?

- Counting bacteria on a microscope is equivalent to counting the population of the state of IL by looking at an area the size of Memorial Stadium.
Optical density as a growth measurement

- Most often read at 600nm
- Varies from instrument to instrument (path length, etc)
- RELATIVE measurement

\[ OD = c \log_{10} \left( \frac{R}{R_0} \right) \]
Optical density as a growth measurement

The Jensen Lab in BIOE has projects and a startup company designing plate readers...and is recruiting students! 😊

Biotek Synergy HT plate reader
**E. coli**

- Rod-shaped bacterium 2μM in length
- Found in the lower intestine of warm-blooded mammals
- Widely studied model and a major tool in genetic engineering
- *E. coli* HB101 K-12 is a nonpathogenic (BSL1) strain
**Technical Point: Serial Dilutions**

- Used to generate accurate dilutions of cell cultures, drugs, etc
- Identify a range (to single cells, toxicity of a drug)
- Importance of good pipetting skills!

**Example of 10-fold serial dilution**

1. **Sample**: 90 mL diluent + 10 mL sample
2. **Dilution**: 90 mL diluent + 10 mL dilution #1
3. **Mix well**: 10 mL
4. **Dilution**: 90 mL diluent + 10 mL dilution #2
   - ...
Technical Point: Error Propagation

• Each pipette has % error associated with it (larger for larger pipettes)
• Error propagates with each transfer
• You do not need to calculate error propagation in 202 reports, but you should consider the implications as you run experiments!
Bacterial Cell Culture Lab Overview

Optical Density
*Relative* measurement

Goal: map CFUs/ml to optical density

Colony forming units (CFUs)
*Absolute* measurement

2-fold dilutions

10-fold dilutions

Plate

10-fold dilutions

1 2 3 4 5 6 7 8 9 10 11 12

A B C   D
E F G   H
How do we engineer bacteria?

How do we use bacteria to engineer mammalian cells, proteins, etc?
Plasmids

• Small DNA molecule (usually <15kb) that is separate from chromosomal DNA and replicates on its own

• Plasmids can be engineered to code genes of interest that may or may not be modified

• Plasmids are usually found in bacteria but can be present in archaea and eukaryotes too!

http://www.asbmb.org/asbmbtoday/asbmbtoday_article.aspx?id=15152
Plasmid features

- Promoter-governs expression
- ORI-origin of replication
- Gene(s) of interest
- Selectable marker-allow for isolation of cells carrying plasmid (usually antibiotic resistance gene)
- Restriction enzyme sites-recognition sequences for enzymes to cut DNA
- Transcription regulator-gene that controls promoter
- Engineered plasmids are made to prevent bacteria to bacteria transfer—and the spread of antibiotic resistance!
How can we introduce DNA into bacteria?

- Uptake of exogenous DNA into bacteria is called *transformation*

- Note: Uptake of exogenous DNA into mammalian cells is called *transfection*
Competence

• Types of competence
  – Natural competence
  – Induced competence

• Induced competence is a stressed state

Streptococcus pneumoniae
How can we introduce DNA into bacteria?

• Heat shock
  – Rapid temperature change
  – “chemically competent cells”

• Electroporation
  – Electrical field transiently applied to cells
  – “electrocompetent cells”
pGLO plasmid

- ori: origin of replication
- GFP gene
- araC: transcription regulator
- bla: antibiotic resistance
GFP as a tool in molecular biology

- Green fluorescent protein (GFP)
- First isolated from the jellyfish Aequorea victoria
- Exhibits fluorescence when exposed to light in the blue to UV range
- 2008 Nobel prize for the discovery and development of GFP
- More on fluorescence later this semester!

http://www.conncoll.edu/ccacad/zimmer/GFP-ww/GFP-1.htm

Nathan Shaner
Arabinose operon
Arabinose operon in pGLO
Transformation efficiency

Transformation efficiency = \# transformants/DNA
Units CFUs/μg
Variables Affecting Transformation efficiency

• Quality of DNA
• Growth phase of bacteria
• Transformation method
• Plasmid size
Nanodrop Spectrophotometer

• Measures small volumes (2 μL)
• Nucleotides, RNA, ssDNA, and dsDNA all absorb at 260nm
DNA Purity Measured by Nanodrop

- 260/280 ratio
  - Protein and phenol absorb at 280 nm
- 260/230 ratio
  - EDTA, carbohydrates, and phenol absorb at 230 nm
Technical Point: How to “pick” colonies

• Colonies can be “picked” with an inoculation loop or micropipette tip
• Carefully “pick” the colony without removing agar from the plate
• Mix colony into media
• This will be demonstrated in lab.
Reminder: Lab Archives ELNs

- Lab notebooks are important for documenting experiments, protecting IP, etc. and can have legal consequences

These are all the notebooks from my research in grad school!
Reminder: Lab Archives ELNs

• Example
• **Experiment Description:** An *E. coli* HB101 K-12 culture will be diluted and the optical density will be measured using a plate reader. Dilutions of the culture will be plated on LB agar plates and grown overnight to determine the number of cells in the culture.

• **Experiment Purpose:** The purpose of this experiment is to map an optical density value to CFUs/ml for *E. coli* HB101 K-12. This measurement can be used for future experiments.
Announcements

- Special Office Hour: Plotting in Matlab
  - TA Mohammad Zahid
  - Monday, Feb. 1st at 8pm in DCL 1265
- Critical Thinking Questions: A great way to study for the written exam! Some of these EXACT questions or variations of them will be on your exam.
- Quizzes will not be returned. You may view your quiz by appointment with Dr. Jensen
- Stop by my office ANY TIME or email me to make an appointment
- Grades will be posted on Compass
  https://compass2g.illinois.edu
Schedule and logistics

- Lab next week: Bacteria Transformation Week 1
- There will be a quiz at Main Lab only.