

Bio 368
Lecture 2

Grubb's test for Outliers $Z = \frac{|\text{mean} - \text{value}|}{\text{StdDev}}$

Sterilization:

Methods of Sterilization:

- Autoclave Machine - Heats Steam heats under high pressure
 - ↳ More effective than dry oven, Kills everything
- Glassware, Metals, heat resistant plastics, Heat resistant Liquids, Bio-hazard Materials
- Filter
- Filter Sterilization - Liquid is passed through small (0.22 μ m or less) pores in a sterile disposable filter which removes any bacteria/particles
 - Liquids, non-heat resistant
- Gamma ray Sterilization -
 - Plastics (Non-heat resistant)

Aseptic techniques:

↳ Techniques to prevent contamination of sterile equipment & pure cultures

- Clean environment with disinfectant → Wash hands with soap
 - ↳ Wipe work area with 80% EtOH
- Work near a flame → creates an upwards airflow & minimizes chance of particles landing on sample
 - ↳ Flame ~~tips~~ ^{mouths} of tubes & glass bottles before using & between uses
- Biosafety Cabinet - Provides better airflow than a flame & is a better technique

Hilroy

Single colony isolation

Streaking → Technique to isolate single colony ^{from} a single cell

- ↳ Can use tooth pick or loop → Must flame loop btwn streaks
- ↳ Change tooth picks btwn streaks

Bacterial cell count

- Direct counting (Hemocytometer) → Counting by microscope
 - ↳ Normally counts dead cells to
 - ↳ Cell Viability Staining can differentiate live or dead cells
- OD_{600} Optical Density - Estimate cell # by measuring turbidity
 - ↳ Does Not differentiate dead cells from live ones
 - ↳ Fastest & easiest method

Viable Cell Count - Estimate # of colonies the culture can form (CFU)

- ↳ Serial dilution of culture is necessary to obtain well formed colonies
- ↳ Select a plate with ~100-300 colonies for accurate reading
- ↳ Requires plating
 - ↳ Dip spreader in etOH & flame

Lecture 3

Biosafety Levels

LVL 1 - ~~Most~~ Biological Materials used pose no threat ^{or} little threat to health

LVL 2 - Pathogens that don't cause serious hazard to health
↳ Treatment & preventative measures available

LVL 3 - Pathogens that cause serious risk hazard to health
↳ Treatment available, & minimal risk of transmission

LVL 4 - Pathogens that cause serious risk to health
↳ Often untreatable & High risk of transmission

Biosafety Cabinet

Class II - Air is directed into exhaust system & Low sash

Class III - Cabinet is completely sealed, manipulation via gloves, user never exposed to the material

Containment LVL 4 - Not discussed

Viable Count Calculation

Units (cfu/mL)

$$\frac{(\# \text{ of colonies}) (\text{Dilution factor})}{(\text{Volume in mL})} = \text{Viable count}$$

Phage Titre

Units (pfu/mL)

$$\frac{(\# \text{ colonies of phages}) (\text{Dilution factor})}{(\text{Volume in mL})} = \text{viable phage titre}$$

Sugar Metabolites Mutants

ara - arabinose

mal - maltose

xyl - xylose

gal - galactose

Bacteriophage

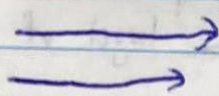
Subclass of viruses that infect bacterial cells $\sim (20-250 \text{ nm})$

↳ SPO1, T4, Lambda

↳ Lytic or Lysogenic?

Cells lysed by phages form plaques on plate

Confluent lysis
(Many Plaques)



Bacteria Lawn
(No plaques)

Deletion & Insertion

Deletion - Δ

ex NR6042 (Δ *gpt-lacS*)

↳ Location of deletion

Insertion - $::$

ex ~~NR6042~~ CAG18475 (*mutC162* **Tn10**)

↳ Transposon

Antibiotic Resistance

Tn10 → Carries **tetracycline resistance** gene

Tn10 Kan → carries **kanamycin resistance** gene

rpsL mutation → ~~rpsL~~ carries a gene coding for a **ribosomal protein** that is the target of streptomycin → Makes it **streptomycin resistance**

Transposons or transposable elements

↳ Small piece of DNA that can insert itself elsewhere in the genome

How to test Lac Phenotype

1) Min Lactose Media + Control (Lac⁻ Mutants won't grow on Min Lac plate)

2) **MacConkey + Lactose** → Mac Medium has pH indicator & turns red in low pH

↳ **Lac⁺ → Red** → **fermentation of Lactose produces acidic Metabolites**

Lac⁻ → Colourless

3) β -gal activity via X-Gal + IPTG → X-Gal turns blue in presence of β -gal
↳ needs Lac-operon inducer → IPTG to produce β -gal

4) ONPG + (chloroform & SDS) (Quantitative Method)

↳ Turns Yellow ↳ Extracts protein

$$= 1000 \times (OD_{420} - 1.75 OD_{550})$$

↳ $\epsilon \times V \times OD_{600}$ **Hilroy**

OD₅₅₀ = Cell debris

OD₆₀₀ = Cell density

OD₄₂₀ = Yellow from ONPG + Cell debris

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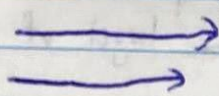
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Lecture 4

Amber Mutation

- Mutation caused by premature introduction of amber stop codon (UAG)
UAG → Amber
UAA → Ochre
UGA → Opal

Amber suppressor mutation

A mutation in tRNA gene that converts the Amber stop codon (UAG) into an Amino Acid codon.

ex gln V44 mutation converts Amber (UAG) stop into gln

Mutations

1. Microscopic Scale: Point Mutation

- Base Substitution
- Base deletion or insertion

2. Macroscopic:

- Insertion, deletion, duplication, inversion, Transposition

Base Sub.

Synonymous ~~mutation~~ Sub. → Silent mutation → No change in A.A.

Non Synonymous Sub.

- Missense Mutation → Change to A.A produced
- Nonsense Mutation → Introduce a stop codon

Frame Shift Mutation → Insertion or deletion of bps to shift the reading frame

Source of Mutation

- Spontaneous mutations - DNA Poly errors ($10^{-6} - 10^{-8}$)
- Oxidative DNA damage
 - ↳ Depurination & deamination

Mutagens:

- 1) Chemical: Base Analogs ex. 2 AP is Adenine analog
In protonated form 2 AP binds pairs with C in non-protonated it pairs with T
- 2) Alkylating Agents: Results in mispairing
Alkylated G pairs with T & Alkylated T pairs with G
- 3) Intercalating Agents: Mimic bps & can slip btwn the stacked N bases at core of DNA - double helix
- Results in insertion or deletion of nucleotide \rightarrow frame shift mutation
ex. Ethidium bromide which is used in DNA staining

Forward VS Reverse Mutations

Reverse mutation (reversion)

- Changes from mutant back to WT

Forward

- Changes from WT to mutant

Screening vs Selection

Selection - Growth conditions where only spc specific strains can grow

Screening - Growth conditions where all strains grow but mutant allele can be detected visually or by additional analysis

Insertional Mutagenesis

Insertion of DNA into a gene that causes disruption to gene function

Types of insertions :

1) Transposable genetic elements (~~Transposons~~)

2 types in prokaryotes; IS (Insertion Sequence) & Transposon (Tn)

1) IS - Short transposable element composing of a Transposable gene & IR (Inverted repeat) sequences at both ends

↳ Mechanism of transposition

Alignment of IR sequences result in loop formation → Transposase cuts & pastes DNA to & from the insertion site

2) Transposon (TN) - Consists of antibiotic resistance genes + IS elements on both ends + Transposase (located in IS element)

Insertional Mutagenesis via Tn10 Transposon

- Mutations are induced at random locations in the chromosome

- To deliver Tn10 into E-coli, cells are infected with phage containing Tn10

Lecture 5

Lambda phage - Used as a method of DNA delivery

↳ λ is a lysogenic phage

- Is integrated by site specific recombination

Dilemma → Lambda is site specific so all transposons will be inserted into the same location

Solution → Delete attachment site on the lambda phage → Random insertion

↳ Lambda will not insert itself into E-coli chromosome

Insertional events can be monitored by the # of Antibiotic res. cells

$$\text{Mutation Frequency} = \frac{\text{\# of mutant colonies}}{\text{\# of cells plated}} \rightarrow = \frac{\text{viable count} \times \text{V}_{\text{plate}}}{\text{Dilution factor}}$$

Note: A circled label "Volume plated" has an arrow pointing to the V_{plate} term in the denominator of the second equation.

* Dilution cancels out as you dilute both equally (I think it just says that Phage dilutions are irrelevant)

Limit of detection * (If viable count = 0 must calculate)
1 CFU / # cells plated