Bio 368
Lecture 2

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

Sterilization:
Methods of Sterilization:
- Autoclave Machine - ~~Heats~~ Steam heats under high preasswre
  ↳ 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 µm 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 enviroment 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 ~~Ends~~ Mouths of tubes & glass bottles before using & between uses
- Biosafety Cabinet - Provides better airflow than a flame & is a better technique

Single colony isolation

Streaking → Technique to isolate single colony (desired) 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 too
↳ Cell Viability Staining can differentiate live or dead cells

- OD_{600} Optical Density – Estimates cell # by measuring turbidity
↳ Does Not differentiate dead cells from live ones
↳ Fastest & easiest method

Viable Cell Count – Estimates # 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 – ~~Vector~~ 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 completly 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)

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

Hilroy

Sugar Metabolites Mutants

ara - arabinose
mal - maltose
xyl - xylose
gal - galactose

Bacteriophage

Subclass of viruses that infect bacterial cells ~(20-250 nm)
(↳ SP01, T4, Lambda
↳ Lytic or Lysogenic?

Cells lysed by phage form plaques on plate

Confluent lysis (Many Plaques) ⟶ Bacteria Lawn
⟶ (No plaques)

Deletion & Insertion

Deletion - Δ

ex NK6042 (Δ(gpt-lac)5)

↳ Location of deletion

Insertion - ::

ex ~~Hfr~~ CAG18475 (metC 162 :: Tn10)

↳ Transposon

Antibiotic Resistance

Tn10 → Carries tetracycline resistance gene

Tn10 Kan → carries Kanamycin resistance gene

rpsL mutation → ~~Str~~ 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

$$= \frac{1000 \times (OD_{420} - 1.75(OD_{550})}{t \times V \times OD_{600}}$$

Hilroy

OD_{550} = Cell debris

OD_{600} = Cell density

OD_{420} = Yellow from ONPG + Cell debris

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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 glnV44 mutation converts Amber (UAG) stop into glu

Mutations

1. Microscopic Scale : Point Mutation
   - Base Substitution
   - Base deletion or insertion

2. Macroscopic :
   - Insertion, deletion, duplication, Inversion, Transposition

Base Sub.

Synonomous ~~mutation~~ sub. → Silent mutation → No change in A.A.

Non synonomous Sub.
- Missense Mutation → Change to A.A produced
- Nonsense Mutation → Introduces a stop codon

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

Kilroy

Sources of Mutation

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

Mutagens:

1) Chemical: Base Analogs ex. 2AP is Adenine analog
In protonated form 2AP ~~binds~~ pairs with C in non-protonated it pairs with T

2) Alkylating Agents: ~~Resul~~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 → 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

Foward
- Changes from WT to mutant

Screening Vs Selection

Selection - Growth conditions where only ~~spef~~ specific strains can grow

Screening - Growth conditions where all strains grow but mutant alleles 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 :

Transposable genetic elements ~~(Transposons)~~
2 types in prokaryotes; IS (Insertion Sequence) & Transposon (Tn)

i) 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 gene + 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
↳ ~~tsa~~ 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 ~~Ati~~Antibiotic res. cells

Mutation Frequency = $\frac{\text{\# of mutant colonies}}{\text{\# of cells plated}}$ → = $\left(\frac{\text{viable count} \times \text{volume plated}}{\text{Dilution factor}}\right)$

Volume plated

* 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