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Genetics Day 1 Recitation Notes
(Eric Sullivan)

Sterile Techniques:

Using media:

Glass containers - use a flame on it before and after
Whatever enters the media must be sterile as well
Lids should never be stored face up, and never touch anything if possible.
Tilt jar, so that only pipetman tip touches insides

Sticks and Picks:

Idea: Quickly dilute a colony.
Always drag - pushing punctures agar
Pushing down hard does *not* make it work better
Spread onto whole surface
If inoculating several plates, use the same pick **(show good example w/ colors at end)**

Spreading Plates:

Flaming spreader – use ethanol, *briefly* in flame
How to cool spreader – on agar or inside of lid.
Spread to dryness – will becomes more resistant and look dry
Try to avoid talking into open plates, lids face down, etc…
Gloves? Your choice, but they are flammable…

Organization of lab bench:

Don't move non-sterile objects over sterile ones (liquids over tip box…)
Ethanol is *not* underneath where the flaming spreader will be
When using eppendorfs, systematically move them for each step.

Other:

Close tip boxes when not used.
Keep pipettes horizontal.

Media:

Minimal – synthetic. M9 salts include phosphorous, nitrogen source, other elements and cofactors (see appendix). Auxotrophs also require metabolites to grow (e.g. leucine). Need to add a carbon source (like a sugar). From these, organism builds everything it needs.

Q: what is the minimal media you'll use today? A: M9 (check who read protocol)

Q: will WT *E. coli* grow in just M9 salts? A: No, requires carbon source too.

Rich – complex; made of yeast extract; has amino acids, nucleic acids, etc. already in it for cell to use to grow (these are sources of carbon, nitrogen, phosphorous, etc.)

Q: What rich media(s) will you use today? A: Lauria-Bertani (LB) and MacConkey

Q: In which media will cells grow fastest? A: Rich, don't need to spend time synthesizing their own amino acids. nucleic acids if they're scavenged from the media.

Growth Curve:

Lag Phase – slow growth. Adaptation to the new environment or from stationary phase.
Log Phase – rapid division.
Stationary Phase – cell death = cell growth -> no net growth. Limited nutrients, toxin buildup.
(Death Phase) – also logarithmic, cells are no longer viable

Q: Where could you find definitions of these terms? The appendix!

Q: What two ways will we determine cell count? A: OD_{550} and viable titer
Viable titer – number of cells/ml capable of growing (i.e. CFU/ml)
Q: Why are you diluting into saline? (What kind of media is it?) A: to stop growth

Data Organization:

Example of bad table / good table

.05	.07
.07	.12
.15	.22

you should be able to look at your data year later and still understand it…
Q: why ignore highlighted? A: only .1->1.0 is in linear range of spec

Q: what did you put on your tables for today? You did create them ahead of time right?
A: (Media) & Title
Time point | water bath temp | start time | stop time | actual time | OD 550

(if you only record start times you won't know how long something was left on a bench
i.e. you have 1:30, 2:30, 3:00 - was the first an hour incubation or was it left on the bench)

Labeling scheme: initials, bench, section
Tape on bags, everything else sharpies. Ethanol washes off sharpies, so can be used to label glassware

Indicator Plates: (how to read them)

1st media composition – minimal require carbon source and metabolites.
- LB – Luria-Bertani (a rich media)
- Mac – MacConkey (a rich media)
- M9 – a minimal media

2nd carbon source – on minimal, the bacteria must be able to use it to grow. On rich, it may function as an indicator or an inducer
- Ara – Arabinose
- Lac – Lactose
- Glu – Glucose

Metabolites (like amino acids) – Auxotrophs requiring it need it provided in the media to grow, all others grow fine without it
- Leu – leucine (an amino acid)

Indicator Plates: (how to read them) (continued)

Indicator –confers a visible phenotype

Xgal – LacZ+ indicator, turns blue

Mac – contains a pH sensitive dye; colonies turn red if provided sugar is used as a carbon source (lowers pH), white if they cannot (because they use amino acids, breakdown of which raises pH)

Antibiotic – Can only grow if they have resistance to the antibiotic

Kan - kanamycin

Cm – chloramphenicol

Example:

H33 Ara^-, Lac^-, $LacZ^+$-constitutive, Kan^R, Leu^-, Cm^R

Plate	Phenotype	Explanation
M9 Leu Kan	no growth	no carbon source provided
M9 **Glu** Leu Kan	growth	carbon source and required metabolite (leu) are provided
Mac Ara Kan	growth, white colonies	cells grow because Mac is a rich media; cells are Ara- (cannot use arabinose as an energy source) and thus are white
Mac Lac Kan	growth, white colonies	cells grow because Mac is a rich media; cells are Lac- (cannot use lactose as an energy source) and thus are white
LB Kan	growth	cells are resistant to kanamycin (KanR)
LB Cm	growth	cells are resistant to chloramphenicol (CmR)
LB Ara Xgal Kan	growth, blue colonies	cells have lacZ expressed constitutively (all the time). The produce of the lacZ gene, B-galactosidase, can break down Xgal to produce a blue product