Basic Microbiological Techniques

- Sterile technique
- Pouring plates and media supplements
- Inoculating and streaking cultures

Microbiology: the study of “simple” microorganisms
- Bacteria
- Fungi
- Viruses/bacteriophage

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diybio.org
Mackenzie Cowell, Jason Bobe

https://groups.google.com/forum/?hl=en#!forum/diybio

4796 members (Mar 2017)
In reality…

DIYbio is:

Community based labs

Home based labs

*Doing their own thing, independently and with no governing oversight*

Basic scientific research

Building low cost equipment

Developing low cost reagents/strains

Outreach/education to public/schools
Triangle DIY Biology: Community Citizen Science and DIYBio Group of the NC Triangle

http://www.tridiybio.org/
Previously...

Session I

Jan 15 @ SplatSpace: An introduction to using pipettemen and pouring agarose gels.

Session II
Feb 19 @ SplatSpace: A continuation of hands-on Introductory training in two basic molecular biology techniques, using pipettemen and running an agarose gel.

Session III (tonight)

Introduction to Microbiological Techniques
Future??

DNA preparations (genomic and plasmid)
Restriction digests
PCR Analysis
Transformation of *E. coli*

In silico analysis tools for molecular biology

CRISPR – gene editing in *E. coli*

Microscopy
Introduction to Microbiological Techniques

The culturing and manipulation of bacteria and other microorganisms is a fundamentally important technique underlying all the developments of modern biotechnology. Today we will cover the basics of sterile technique. This will include transferring and streaking of bacterial cultures as well as the preparation and pouring of selective media in petri dishes. Hands-on participation will be encouraged.
Microorganism – microscopic life forms of any kind

Virus – infectious entity that reproduces solely within an organism; infect all known forms of life
not considered to be a true living organism (cannot reproduce on its’ own)

Bacteria – single cell, prokaryotic, asexual reproduction; fission or budding

Bacteriophage – viruses that infect bacteria

Fungi – single or filamentous, eukaryotic, sexual and asexual reproduction

**Prokaryote** – less internal structure

**Eukaryote** – sophisticated internal structure
organelles – nucleus, mitochondria, more
examples – plants, animals, fungi, more
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**Prokaryote** – less internal structure

**Eukaryote** – sophisticated internal structure organelles – nucleus, mitochondria, more examples – plants, animals, fungi, protozoa, more
Collapse of Aztec society linked to catastrophic salmonella outbreak
DNA of 500-year-old bacteria is first direct evidence of an epidemic — one of humanity’s deadliest — that occurred after Spanish conquest.

Ewen Callaway
Nature 452; 16 February 2017
Chipotle to reopen 43 restaurants after *E. coli* all-clear

2011 Germany *E. coli* O104:H4 outbreak

The agriculture minister of Lower Saxony identified an organic farm in Bienenbuttel, Lower Saxony, Germany, which produces a variety of sprouted food, as the likely source of the *E. coli* outbreak. The farm was shut down. In all, 3,950 people were affected and **53 died**, 51 of whom were in Germany.

**Presence and characterization of *Campylobacter jejuni* in organically raised chickens in Quebec**


Further monitoring and characterization of isolates originating from organic chickens is of interest since this type of production might represent another source of exposure of consumers to a variety of the foodborne pathogen *C. jejuni*.

**Louis Pasteur** Pasteur discovered that heating beer and wine was enough to kill most of the bacteria that caused spoilage, preventing these beverages from turning sour. This was achieved by eliminating pathogenic microbes and lowering microbial numbers to prolong the quality of the beverage. Today, the process of pasteurization is used widely in the dairy and food industries for microbial control and preservation of the food consumed.
Plate streaking
Goal is to begin to identify what is in a biological culture

Each sequence of streaks dilutes culture

A single colony represents a single founding bacterium

*A clonal culture*

Is the culture pure?
Is the culture mixed?

Can use a single colony to Identify genus and species
Selective media

Enrichment/inhibition

MacConkey agar is a selective and differential culture medium for bacteria designed to selectively isolate Gram-negative and enteric (normally found in the intestinal tract) bacilli and differentiate them based on lactose fermentation. The crystal violet and bile salts inhibit the growth of gram-positive organisms which allows for the selection and isolation of gram-negative bacteria. Enteric bacteria that have the ability to ferment lactose can be detected using the carbohydrate lactose, and the pH indicator neutral red. (Wikipedia)

*E. coli* ferment lactose – pink colonies
Salmonella spp do not ferment lactose – white colonies

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>17 g</td>
</tr>
<tr>
<td>Proteose peptone</td>
<td>3 g</td>
</tr>
<tr>
<td>Lactose</td>
<td>10 g</td>
</tr>
<tr>
<td>Bile salts</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5 g</td>
</tr>
<tr>
<td>Neutral red</td>
<td>0.03 g</td>
</tr>
<tr>
<td>Crystal violet</td>
<td>0.001 g</td>
</tr>
<tr>
<td>Agar</td>
<td>13.5 g</td>
</tr>
<tr>
<td>Water</td>
<td>Add to make 1 liter; adjust pH to 7.1 +/- 0.2</td>
</tr>
</tbody>
</table>

MacConkey agar

Blood agar
Escherichia coli as a model organism

In 1946, Joshua Lederberg and Edward Tatum first described the phenomenon known as bacterial conjugation using *E. coli* as a model bacterium.

Conjugation: horizontal transfer of DNA between bacteria – not sexual reproduction but the closest bacteria get to it.

- Short generation time
- Production of abundant progeny
- Low cost maintenance
- Ease of maintaining stocks of strains
- Wide spread community/sharing
- Established stock center
- Established genetic/molecular genetic methods
- Genome(s) available
- Not a pathogen (unless that is the point)
Appendix C-II. (of the NIH Guidelines)
Experiments which use *Escherichia coli* K-12 host-vector systems, with the exception of those experiments listed in Appendix C-II-A, are exempt from the *NIH Guidelines*...

([http://www.tridiybio.org/safety.html](http://www.tridiybio.org/safety.html))

*E. coli* DH5 α genotype: F−, Φ80lacZΔM15, Δ(lacZYA-argF), U169, recA1 endA1, hsdR17 (rK−, mK+), phoA, supE44, λ−, thi-1, gyrA96, relA1

PubMed (Title/abstract): 111,233 articles mention *E. coli*
All Pubmed articles: 347,829
Oliver Smithies (Author): 328

The bacterium can be grown and cultured easily and inexpensively in a laboratory setting, and has been intensively investigated for over 60 years. *E. coli* is the most widely studied prokaryotic model organism, and an important species in the fields of biotechnology and microbiology, where it has served as the host organism for the majority of work with recombinant DNA. Under favorable conditions, it takes only 20 minutes to reproduce. (Wikipedia)
**Defined media vs Complex/rich media**

<table>
<thead>
<tr>
<th>M9 salts</th>
<th>LB (Luria Broth)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimal media</td>
<td>Rich media</td>
</tr>
<tr>
<td><strong>Na$_2$HPO$_4$·7H$_2$O</strong> 4 g</td>
<td>Tryptone 1.0%</td>
</tr>
<tr>
<td><strong>KH$_2$PO$_4$</strong> 15 g</td>
<td>(tryptic digest of casein)</td>
</tr>
<tr>
<td><strong>NaCl</strong> 2.5 g</td>
<td>Yeast extract 0.5%</td>
</tr>
<tr>
<td><strong>NH$_4$Cl</strong> 5 g</td>
<td>(autolysed, powdered yeast;</td>
</tr>
<tr>
<td><strong>2 ml 1M MgSO$_4$</strong></td>
<td>vegemite, marmite are variants)</td>
</tr>
<tr>
<td><strong>100 ml 1M CaCl$_2$</strong></td>
<td><strong>NaCl</strong> 0.5%</td>
</tr>
<tr>
<td><strong>20 ml 20% Glucose</strong></td>
<td>(per liter)</td>
</tr>
<tr>
<td>(per liter)</td>
<td>For plates, use 1.5% agar</td>
</tr>
</tbody>
</table>

**Auxotrophic mutation** – deficiency in a biochemical pathway (requires an amino acid, vitamin, other due to a mutation in a gene producing a specific enzyme)
Agar and agarose

Both used to make a gel

Agarose used as a matrix for size selection of DNA/RNA molecules
Commonly used concentrations: 0.7 % – 2 %
expensive

Agar used as a solid surface to support a growth medium – not sensitive enough to resolve sizes between DNA, RNA, and protein
Commonly used concentrations: 1.5 % - 2 %
Cheap

Agar: historically a gelling agent used in asian desserts
A vegetarian form of gelatin (in jello)
Extract from seaweed or algae (prior to WWII Japan was major source, now Morocco)
Composed of two polysaccharides (sugars); agarose and agarpectin
Neither are common carbon sources for microbes
– Agar is a neutral surface, very little grows an unsupplemented agar (water agar)

So...
Agarose is a purified form of agar
Seaweed shortage prompts calls to ration use of vital scientific resource

Red algae seaweed, which produces high-quality agar used to cultivate micro-organisms in Petri dishes, is in short supply

“The highest-quality agar is obtained from red algae hand-harvested by divers in a few areas, notably Spain, Morocco, Japan (where agar is a popular food ingredient), Mexico and South Africa.”

David Connett, The Independent
Microbiology -> Molecular Biology

Understanding any of the various functions of living organisms by studying the macromolecules that make up an organism – mainly DNA, RNA, and proteins

Central Dogma; Crick

Alternate splicing
Epigenetics (methylation, etc.)
Prions

Central Dogma; more current
E. coli vs Linux

Comparing genomes to computer operating systems in terms of the topology and evolution of their regulatory control networks

Koon-Kiu Yan, Gang Fang, Nitin Bhardwaj, Roger P. Alexander, and Mark Gerstein

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Edited by Gregory A. Petsko, Brandeis University, Waltham, MA, and approved April 2, 2010 (received for review December 20, 2009)

The genome has often been called the operating system (OS) for a living organism. A computer OS is described by a regulatory control network termed the call graph, which is analogous to the transcriptional regulatory network in a cell. To apply our firsthand knowledge of the architecture of software systems to understand cellular design principles, we present a comparison between the transcriptional regulatory network of a well-studied bacterium (Escherichia coli) and the call graph of a canonical OS (Linux) in terms of topology and evolution. We show that both networks have a fundamentally hierarchical layout, but there is a key difference: The transcriptional regulatory network possesses a few global regulators at the top and many targets at the bottom; conversely, the call graph has many regulators controlling a small set of generic functions. This top-heavy organization leads to highly overlapping functional modules in the call graph, in contrast to the relatively independent modules in the regulatory network. We further develop a way to measure evolutionary rates comparably between the two networks and explain this difference in terms of network evolution. The process of biological evolution via random mutation and subsequent selection tightly constrains the evolution of regulatory network hubs. The call graph, however, exhibits rapid evolution of its highly connected generic components, made possible by designers' continual fine-tuning. These findings stem from the design principles of the two systems: robustness for biological systems and cost effectiveness (reuse) for software systems.
Plasmids

Double stranded DNA molecules; usually circular
Non-chromosomal
Often multicopy
Originally found naturally occurring in bacteria
Often carry a selectable marker (antibiotic, virulence)

*E. coli* – genome ~ 4.6 Mb (megabase; 1 million bp)
F plasmid – 100 kb (fertility factor for conjugation) either extrachromosomal or integrated
pBR322 – 4.3 kb (kilobase; 1000 bp)
pUC19 – 2.7 kb

pBR322 – 4.3 kb
B=Bolivar
R=Rodriguez

pUC19 – 2.7 kb
UC=Univ of California
High copy number > 100/cell

Lower copy number, no MCS

Multiple cloning site (MCS)
Restriction Enzymes
EcoRI cuts asymmetrically at GAATTC
Leaves 4 bp overhang – sticky end

Incubate fragments with DNA ligase
Transform into E. coli