

# Basic Microbiological Techniques

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

Microbiology: the study of “simple” microorganisms

Bacteria

Fungi

Viruses/bacteriophage

Tom Randall tarandall@gmail.com





## An Institution for the Do-it-Yourself Biologist

DIYbio.org was founded in 2008 with the mission of establishing a vibrant, productive and safe community of DIY biologists. Central to our mission is the belief that biotechnology and greater public understanding about it has the potential to benefit everyone.

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### Latest news

#### DIY+iGEM

November 6th, 2013

The International Genetically Engineered Machines Competition (iGEM) is opening to DIY

#### Funding Models for DIY biologists

December 14th, 2012

Over at Nature's SpotOn, Rayna Stamboliyska asks the question: "How do we make DIYBio

#### Hardware: Algae microscope and cell-picker

June 12th, 2012

Draft entry Developer: Urs Gaudenz AKA



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31 of 4652 topics (99+ unread) ☆ [8+1](#)



Welcome to the DIYbio discussion forum. If you are new to this discussion group, your first post may not appear immediately

Also see our website at [www.DIYbio.org](http://www.DIYbio.org)

**Ask a Biosafety Expert: A new service from DIYbio.org to get some free advice from biosafety experts** (32)  
By Jason Bobe - 32 posts - 916 views

**Promising comp cell protocol for Bacillus** (6)  
By Koeng - 6 posts - 26 views

**Transgenic CCD resistant honeybee - need help** (18)  
By Mega [Andreas Stuermer] - 18 posts - 30 views

**Making peptone** (5)  
By Joffrey - 5 posts - 19 views

**Interesting bio statistics programming course for free** (1)  
By alexander hollins - 1 post - 11 views

**Need eukaryote expression help - two promoters and just one terminator ok??** (2)  
By Mega [Andreas Stuermer] - 2 posts - 11 views

**The Open Discovery Institute(ODIN) is Opening a DIYBio Store Soon!** (33)  
By Josiah Zayner - 33 posts - 85 views

**Advice on cheap tentative toxicity tests for diacetyl substitutes in butter flavorings** (2)  
By Martin Baldan - 2 posts - 4 views

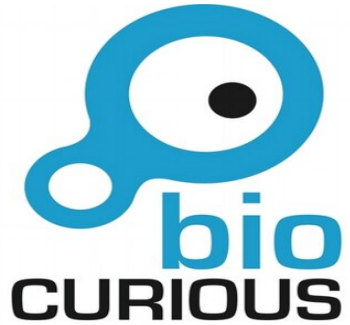
**Realities of Biohacking in a UK home lab** (5)  
By Dave Jenkins - 5 posts - 64 views

**RE: [DIYbio] IP free fluorescent protein** (5)  
By Sebastian - 5 posts - 16 views

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

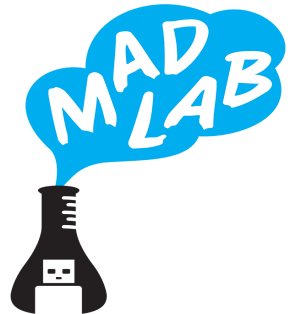
Basic scientific research

Building low cost equipment

Developing low cost reagents/strains

Outreach/education to public/schools

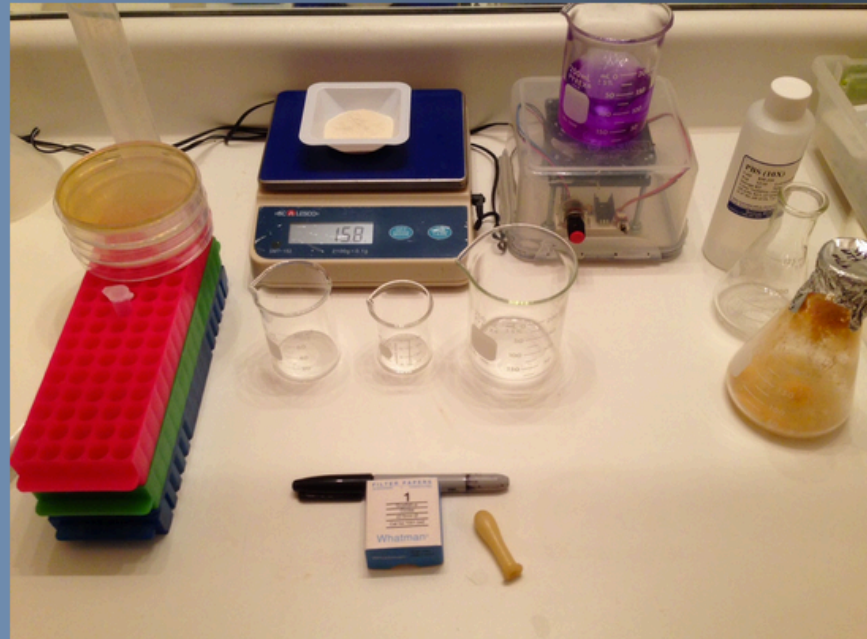
GENSPACE



CHARLOTTESVILLE  
Open Bio Labs

**Ronin Genetics**

# Triangle DIY Biology: Community Citizen Science and DIYBio Group of the NC Triangle



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About Us

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Upcoming Meetings

Safety

Resources



## Learning

Triangle DIY Biology provides one-on-one tutoring in Do It Your-self Biology. Future directions include iGEM Projects and more! [read more](#)



## DIY Biology Labwork

- Genomics
- Upstream
- Downstream
- Analysis and Purification



## Projects

Triangle DIY Biology members are informed and experienced. Many work in a Biology or Bio-tech industry/academia [read more](#)

# Previously...

## Session I

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

## Session II

Feb 19 @ SplatSpace: A continuation of hands-on Introductory training in two basic molecular biology techniques, using pipettes 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.

# Terminology

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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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.



**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.

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

Can J Vet Res. 2011 Oct; 75(4): 298–307.

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*.

# Plate streaking

Goal is to begin to identify what is in a biological culture

Each sequence of streaks dilutes culture

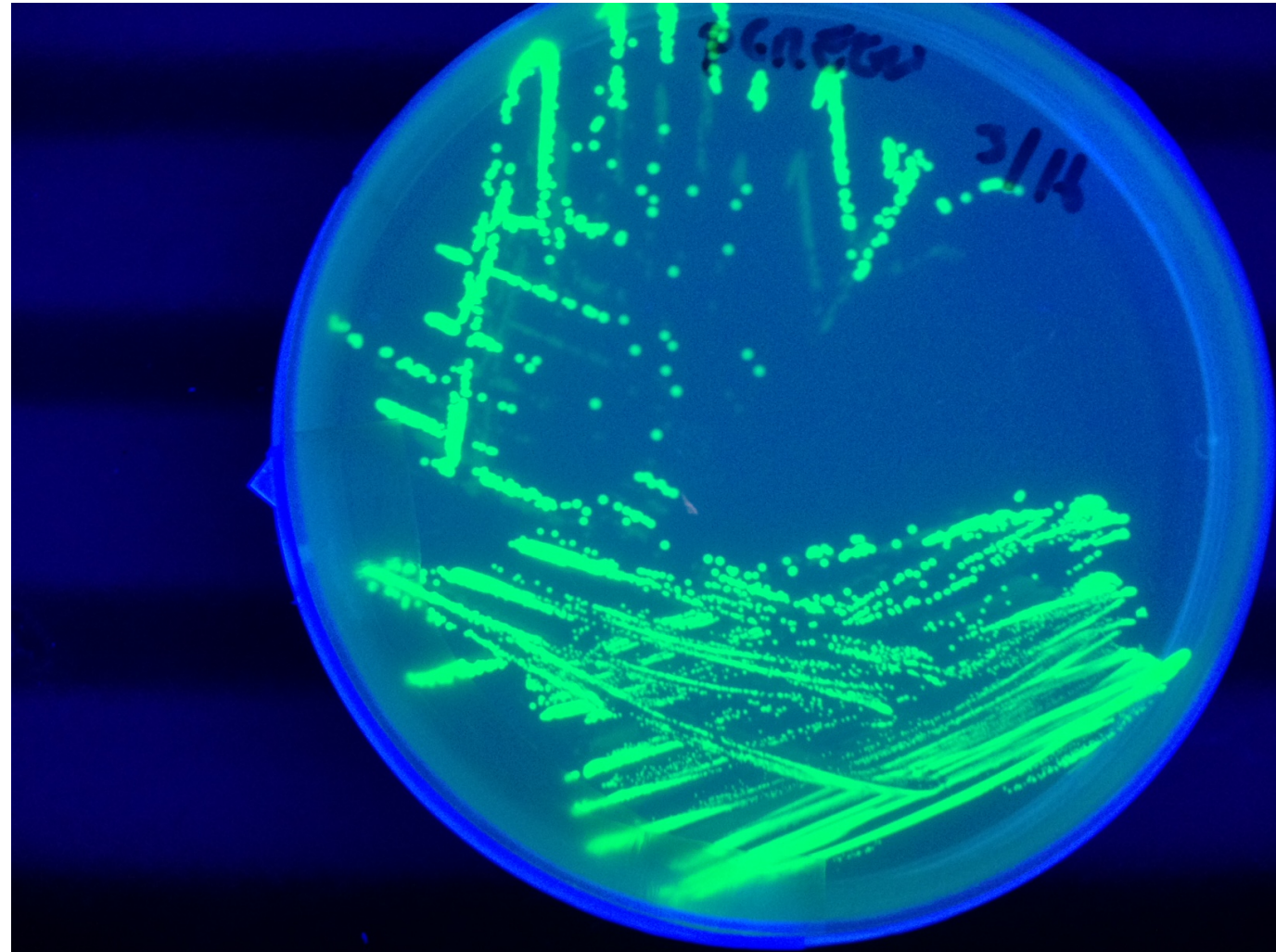
A single colony represents a single founding bacterium

***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

Peptone – 17 g  
Proteose peptone – 3 g  
Lactose – 10 g  
Bile salts – 1.5 g  
Sodium chloride – 5 g  
Neutral red – 0.03 g  
Crystal violet – 0.001 g  
Agar – 13.5 g  
Water – add to make 1 liter; adjust pH to 7.1 +/- 0.2



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)

## 1941



George W. Beadle (1903-1989) and Edward L. Tatum (1909-1975) show how genes direct the synthesis of enzymes that control metabolic processes

In addition to governing the expression of hereditary characteristics, genes direct the manufacture of proteins that control the basic metabolic functions, which characterize life itself. This insight, with profound consequences for molecular biology, was experimentally confirmed in 1941 by George W. Beadle and Edward L. Tatum.



George W. Beadle  
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Beadle, a geneticist, initially worked with the fruit fly *Drosophila* in the laboratory of Thomas Hunt Morgan at Columbia University. By 1935 he had developed suggestive evidence that eye color, known to be inherited, represents a series of genetically determined chemical reactions. His work over the next six years, much of it with Edward L. Tatum, a biochemist, furthered this hypothesis. But the complexity of *Drosophila* proved a drawback to developing experiments that would demonstrate a link between specific genes and their chemical products.

In 1941, Beadle and Tatum turned to a simpler creature, in which specific products of metabolism could be directly studied. A bread mold, *Neurospora crassa*, proved ideal. *Neurospora* can be cultured together with sugar, inorganic salts, and the vitamin biotin. This fungus has a short life cycle, and reproduces sexually and replicates asexually—that is, sexual reproduction gives rise to spores. In addition, *Neurospora* possesses only one set of unpaired chromosomes, so that any mutation is immediately expressed. This much was known, mainly through the work of Bernard O. Dodge, when Beadle and Tatum began their research.

In what became a celebrated experiment, Beadle and Tatum first irradiated a large number of *Neurospora*, and thereby produced some organisms with mutant genes. They then crossed these potential mutants with non-irradiated *Neurospora*.

Normal products of this sexual recombination could multiply in a simple growth medium. However, Beadle and Tatum showed that some of the mutant spores would not replicate without addition of a specific amino acid—arginine. They developed four strains of arginine-dependent *Neurospora*—each of which, they showed, had lost use of a specific gene that ordinarily facilitates one particular enzyme necessary to the production of arginine.



Edward L. Tatum

# *Escherichia coli*

## **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>)

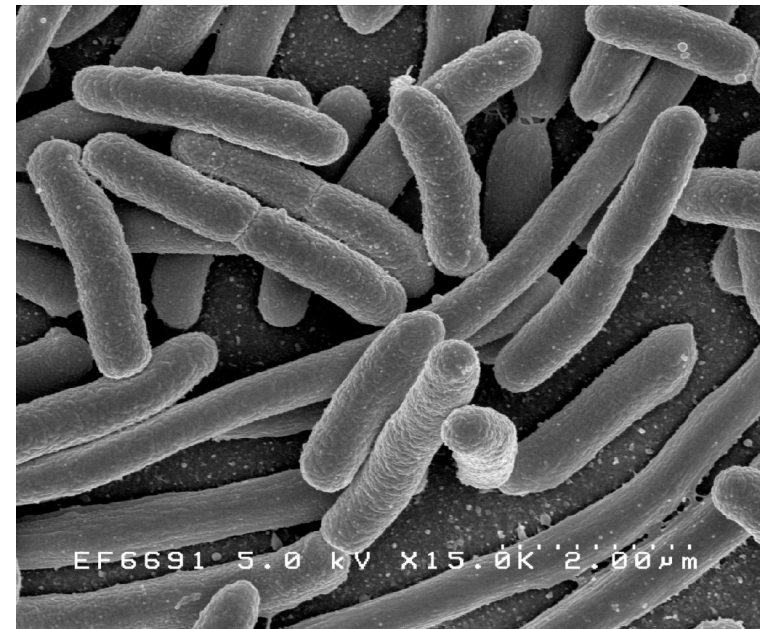
*E. coli* DH5  $\alpha$  genotype: F<sup>-</sup>,  $\Phi$ 80*lacZ* $\Delta$ M15,  $\Delta$ (*lacZYA-argF*), U169, *recA1 endA1, hsdR17* (rK<sup>-</sup>, mK<sup>+</sup>), *phoA, supE44,  $\lambda$ -*, *thi-1, gyrA96, relA1*

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

M9 salts

LB (Luria Broth)

Minimal media

Na<sub>2</sub>HPO<sub>4</sub>-7H<sub>2</sub>O     4 g  
KH<sub>2</sub>PO<sub>4</sub>            15 g  
NaCl                  2.5 g  
NH<sub>4</sub>Cl                5 g  
2 ml 1M MgSO<sub>4</sub>  
100 ml 1 M CaCl<sub>2</sub>  
20 ml 20 % Glucose

(per liter)

For plates, use 1.5% agar

Rich media

Tryptone            1.0%  
(tryptic digest of casein)  
Yeast extract      0.5%  
(autolysed, powdered yeast;  
vegemite, marmite are variants)  
NaCl                 0.5%

(per liter)

For plates, use 1.5% agar

**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 agaropectin

Neither are common carbon sources for microbes

– Agar is a neutral surface, very little grows on 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.”

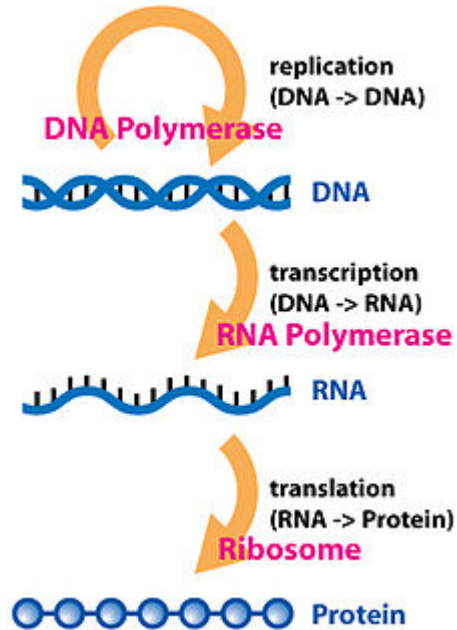
David Connett, The Independent

<http://www.independent.co.uk/news/science/seaweed-shortage-prompts-calls-to-ration-use-of-vital-scientific-resource-a6794291.html>

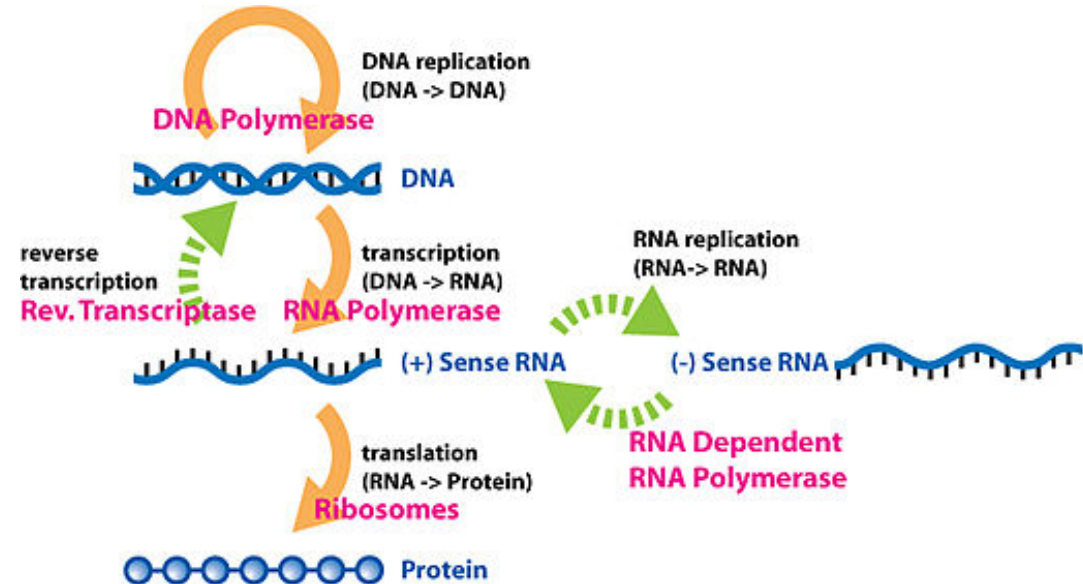


# Microbiology -> Molecular Biology

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



Central Dogma; more current

Alternative splicing  
Epigenetics (methylation, etc.)  
Prions

# *E. coli* vs Linux

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

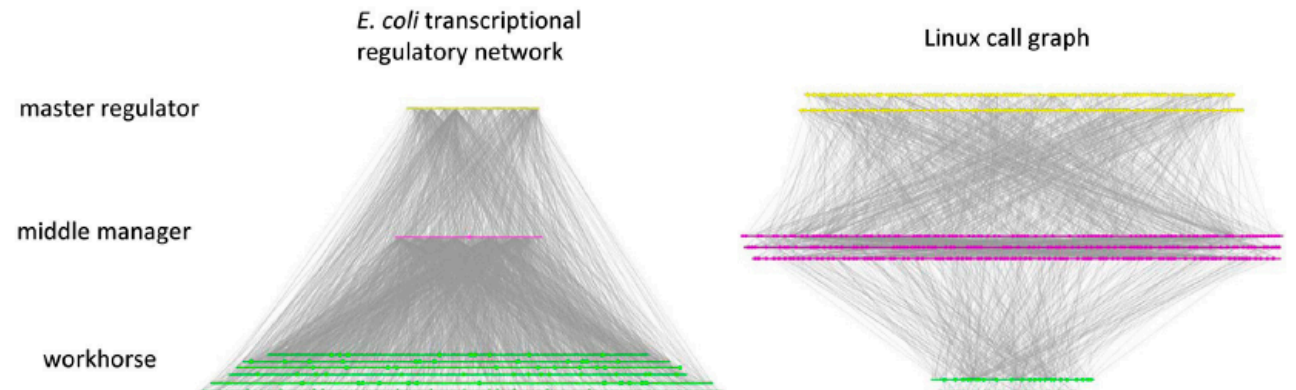
PNAS 107 9186

Koon-Kiu Yan<sup>a</sup>, Gang Fang<sup>a</sup>, Nitin Bhardwaj<sup>a</sup>, Roger P. Alexander<sup>a</sup>, and Mark Gerstein<sup>b,a,c,1</sup>

<sup>a</sup>Program in Computational Biology and Bioinformatics, <sup>b</sup>Department of Molecular Biophysics and Biochemistry, and <sup>c</sup>Department of Computer Science, Yale University, Bass 432, 266 Whitney Avenue, New Haven, CT 06520

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

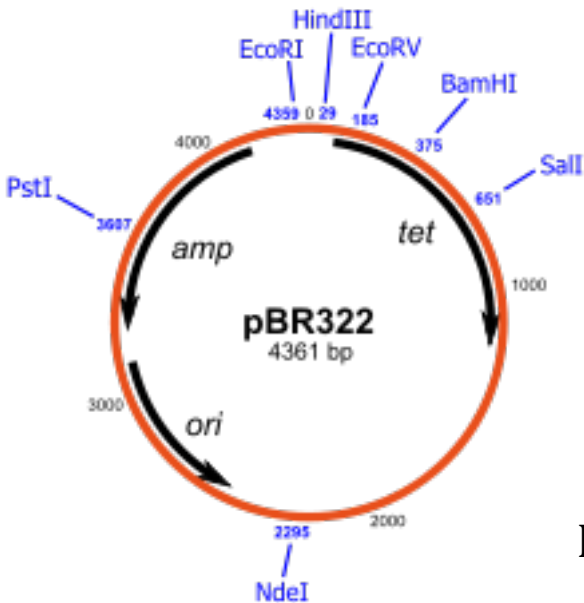
Often carry a selectable marker (antibiotic, virulence)

*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)

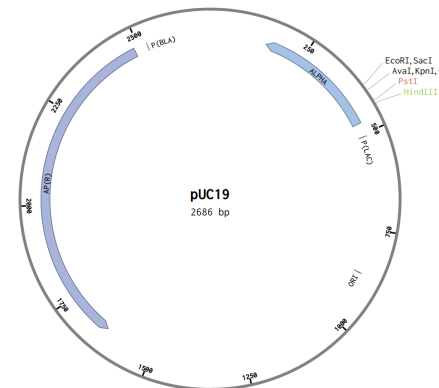
pUC19 – 2.7 kb



pBR322 – 4.3 kb

B=Bolivar

R=Rodriguez



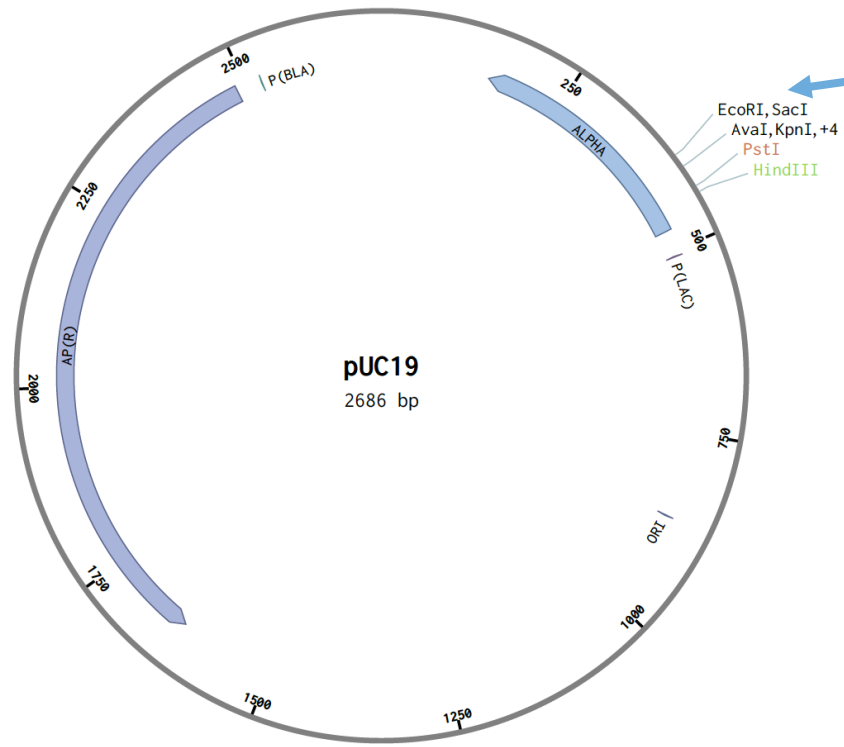
pUC19 – 2.7 kb

UC=Univ of California

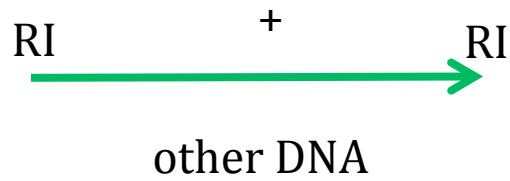
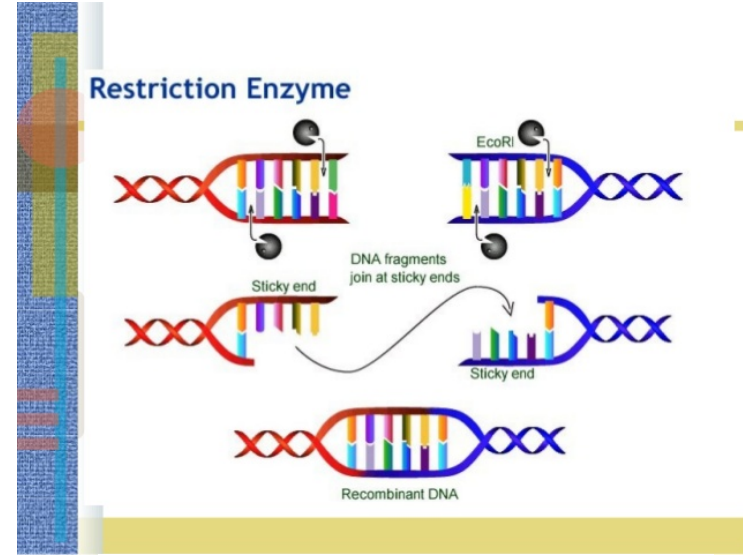
High copy number > 100/cell

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*

