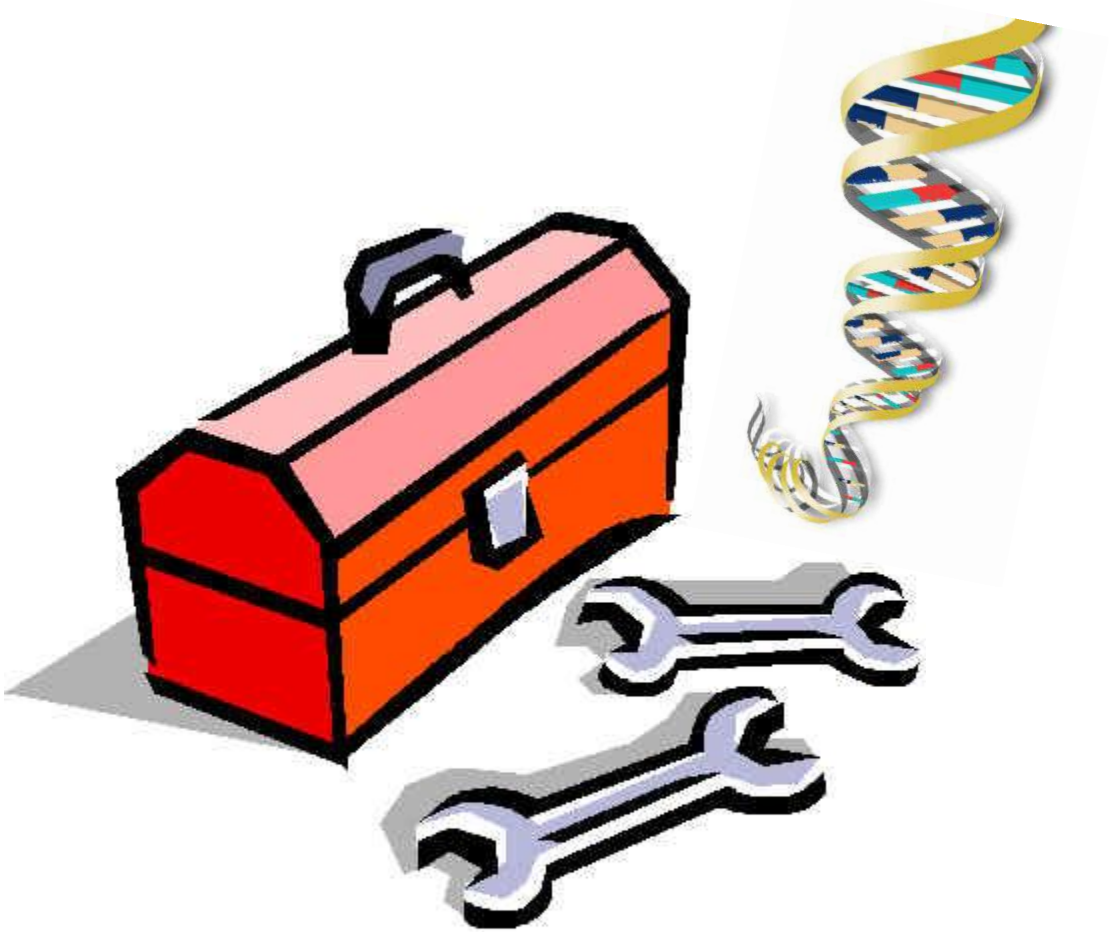
A Brief History of Biotech

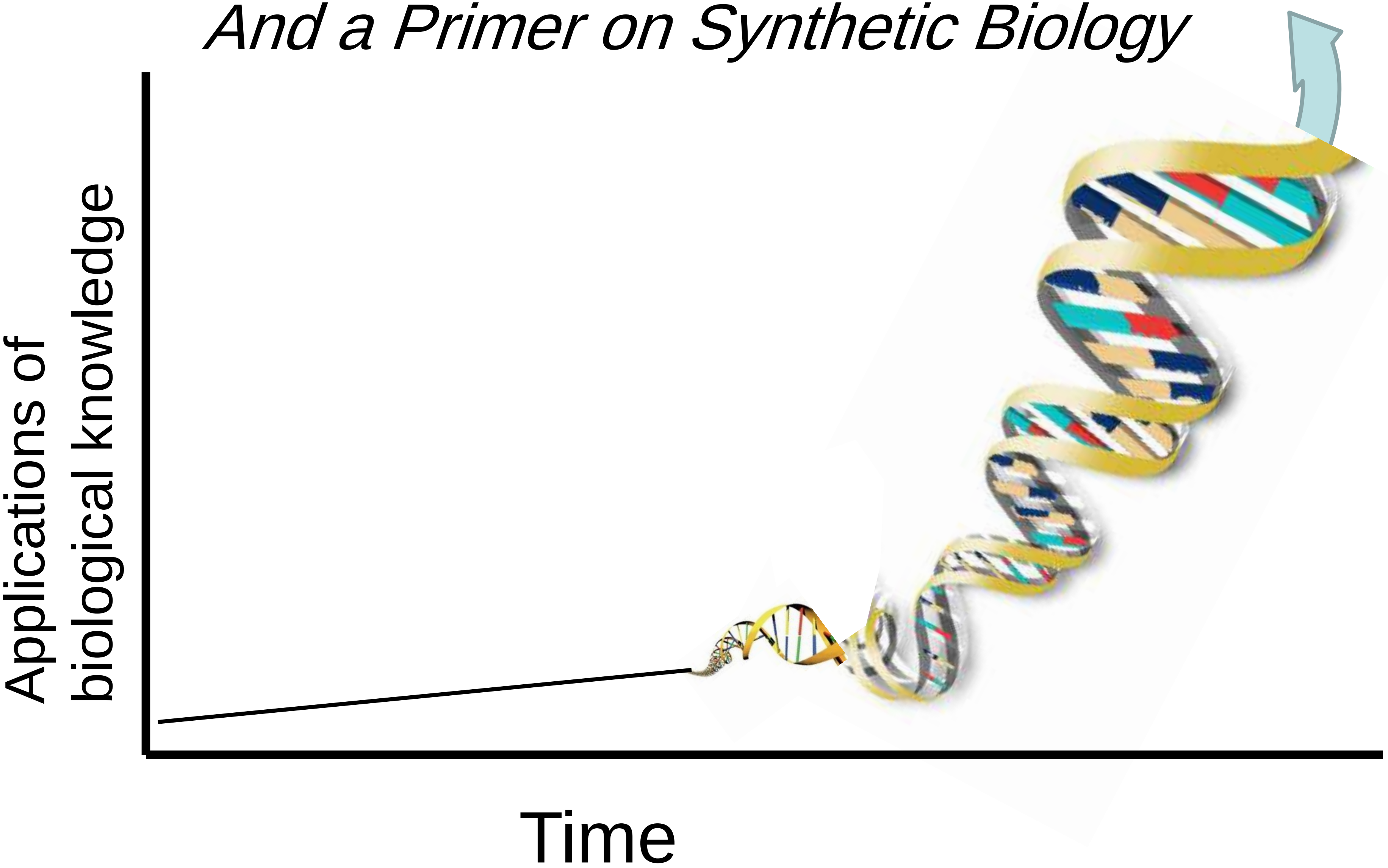
And a Primer on Synthetic Biology



Presenter:
Damon Tighe

A Brief History of Biotech

And a Primer on Synthetic Biology



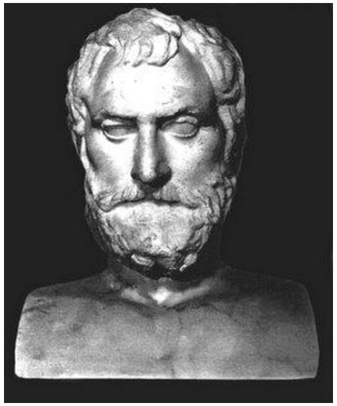
History of biology and biotechnology

(highly abbreviated and biased)

Sumerian Beer Recipe
3000BC

Kyui and Kimchi
6000BC

Wine in Armenia
6000BC

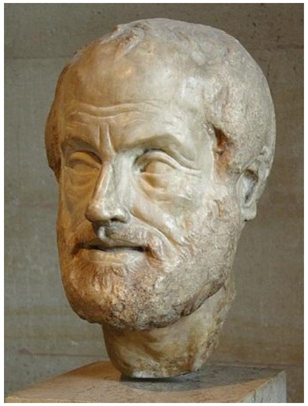


Thales of Melitus
624-546BC

Father of Philosophy

Olive Press fortune

Battle of Halys



Aristotle
384-322BC

Father of Biology

Categorical –Observation

Ethics

Teleology

History of biology and biotechnology

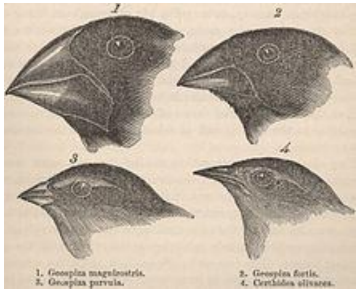
(highly abbreviated and biased)

Birth of Microbiology
~1650s – 1850s

Anton van Leeuwenhoek

Louis Pasteur

Robert Koch



Natural Selection
~1850s – 1900s

Charles Darwin

Alfred Russel Wallace

Gregor Mendel

Molecular Biology
~1900s – 1950s

Fridrich Miescher

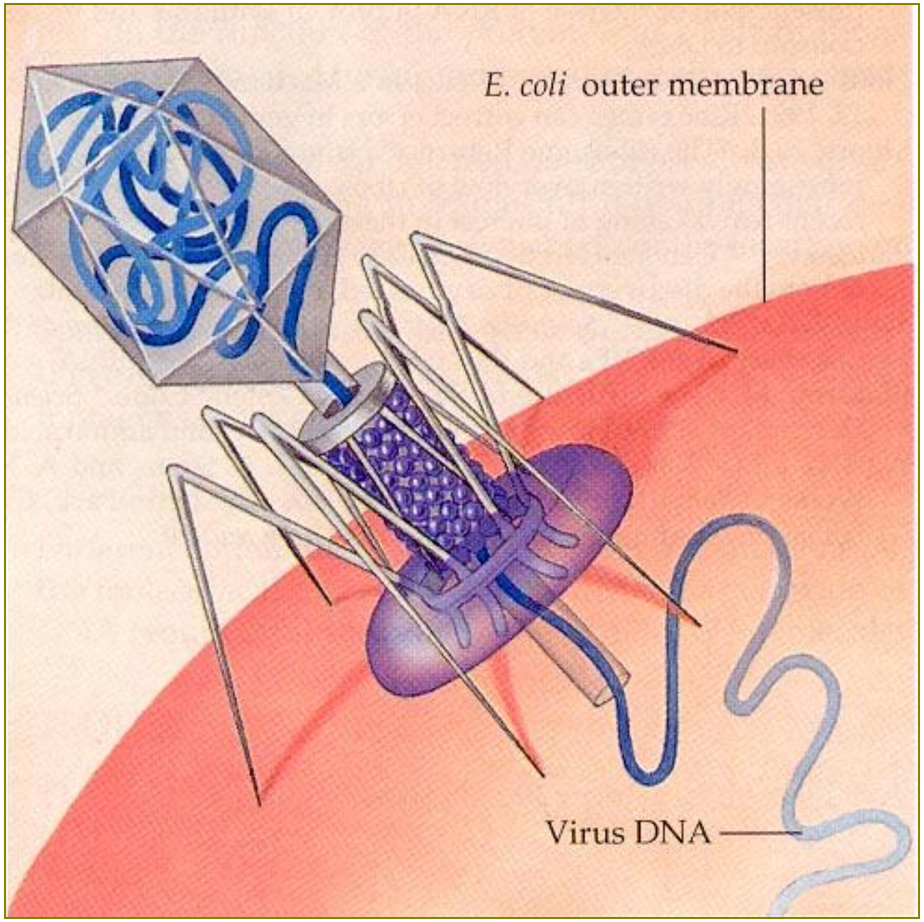
Frederick Griffith et al.

Watson, Crick, Franklin

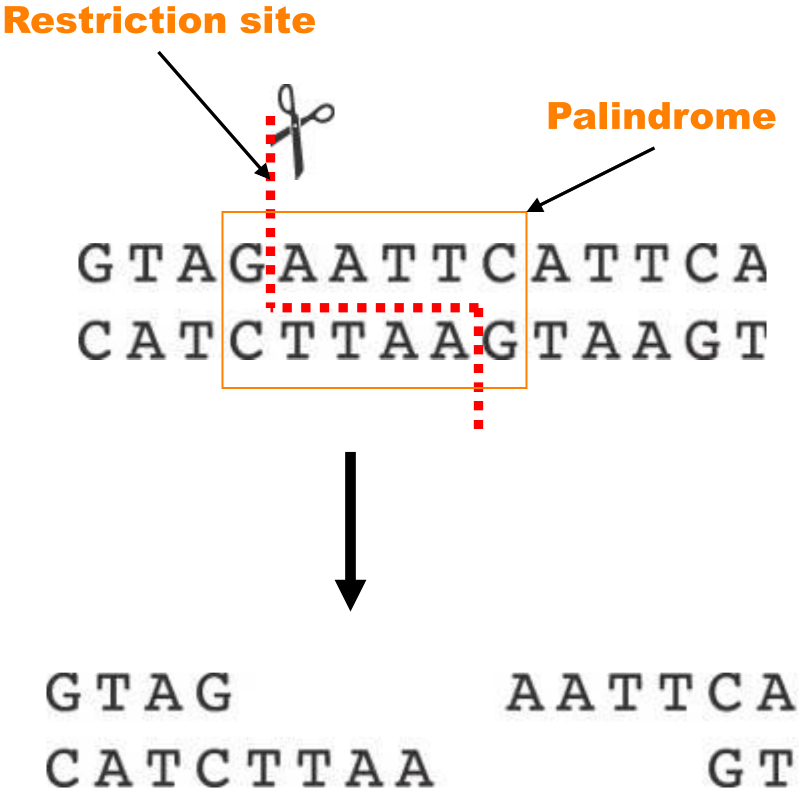
Tools: Restriction Enzymes

Borrowed from Bacterial Innate Immunity System

Allows bacteria to recognize common foreign DNA and cut it up



Endonucleases



Restriction site

Palindrome

GTAGAATTCATTCA
CATCTTAAGTAAGT

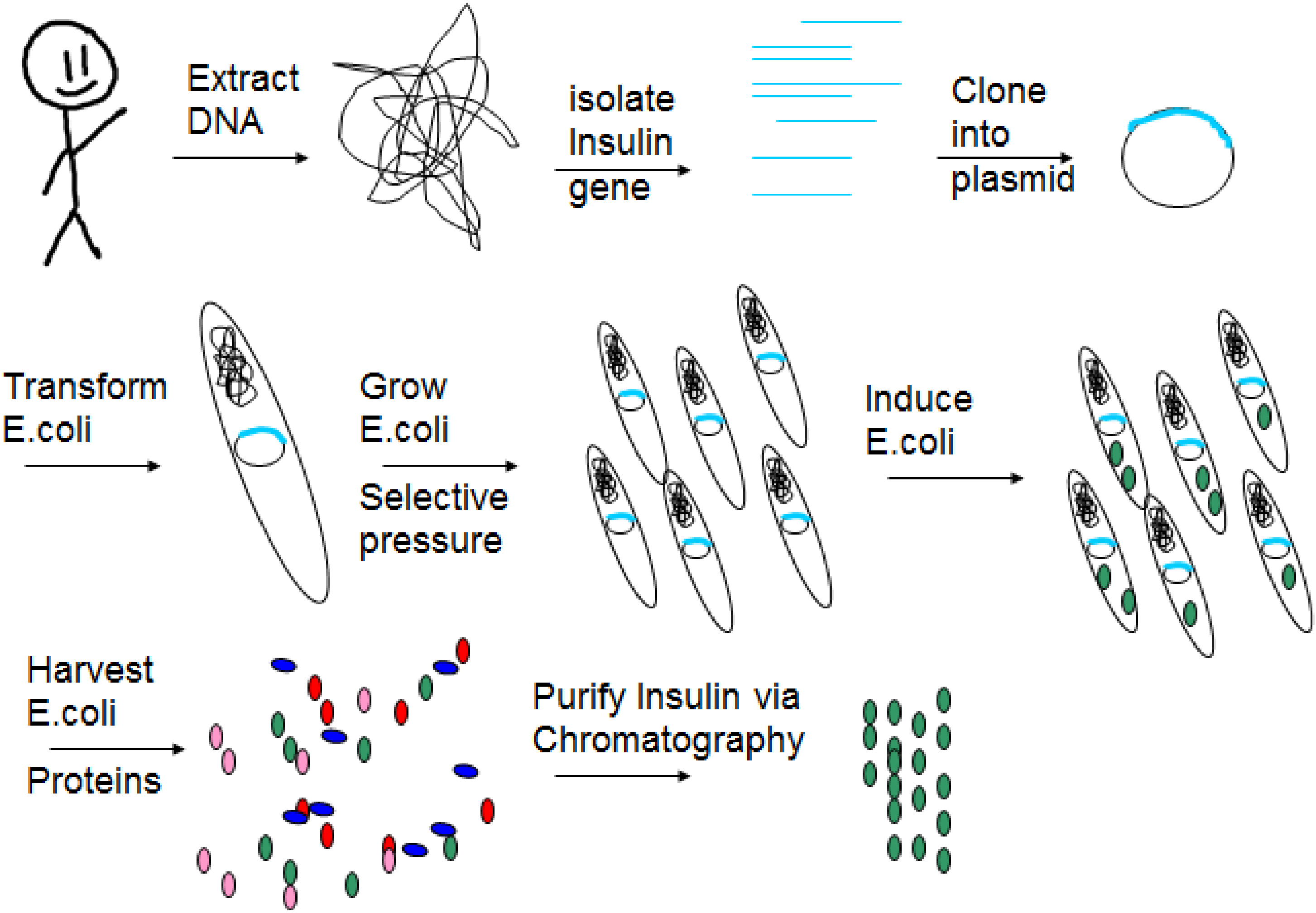
GTAG AATTCA
CATCTTAA GT

1950s - Allows scientist to cut and paste DNA → **Recombinant DNA technology**

History of biology and biotechnology

(highly abbreviated and biased)

Recombinant DNA technology applied to Insulin Production 1970s



Extract DNA

isolate Insulin gene

Clone into plasmid

Transform E.coli

Grow E.coli

Selective pressure

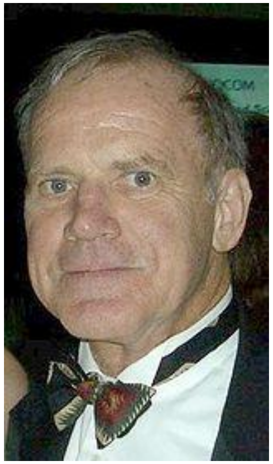
Induce E.coli

Harvest E.coli

Proteins

Purify Insulin via Chromatography

History of biology and biotechnology (highly abbreviated and biased)



PCR

Polymerase chain reaction

Kary Mullis

1983

Target and amplify specific regions of DNA

Human Genome Project

1990-2003

Ari Patrinos

Francis Collins

Craig Venter

Cheap Sequencing

Allows for understanding the code of many organisms, which is useful for finding new tools, bio-parts, and a more systematic approach to understanding biological complexity

History of biology and biotechnology

Sequencing Technologies Evolution

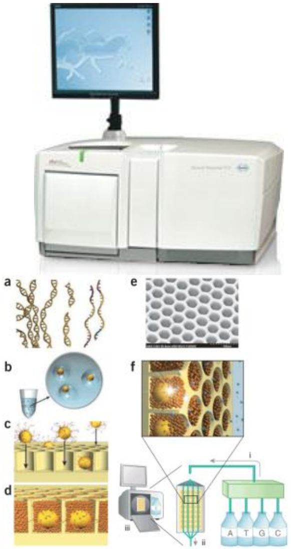
Capillary

1990s

ABI 3730

$3 billion and 10 years with 400+ people working 24 hours a day 7 days a week produced the first human genome

2003
Human Genome
Project Published



Pyro

2005

454

1 human genome = 3 months $1-2 million

Cluster-SBS

2007

illumina

Technology continually evolved to reach 3 day, $1,000 human genome in 2015

Ligation

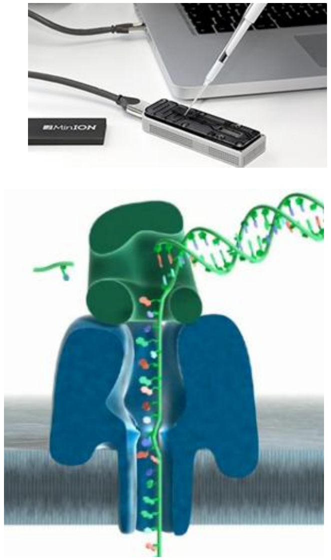
2008

ABI SOLiD

1 human genome = 2 months, ~$1M. Techonolgy didn’t scale well and was not adopted widely

History of biology and biotechnology

Sequencing Technologies Evolution

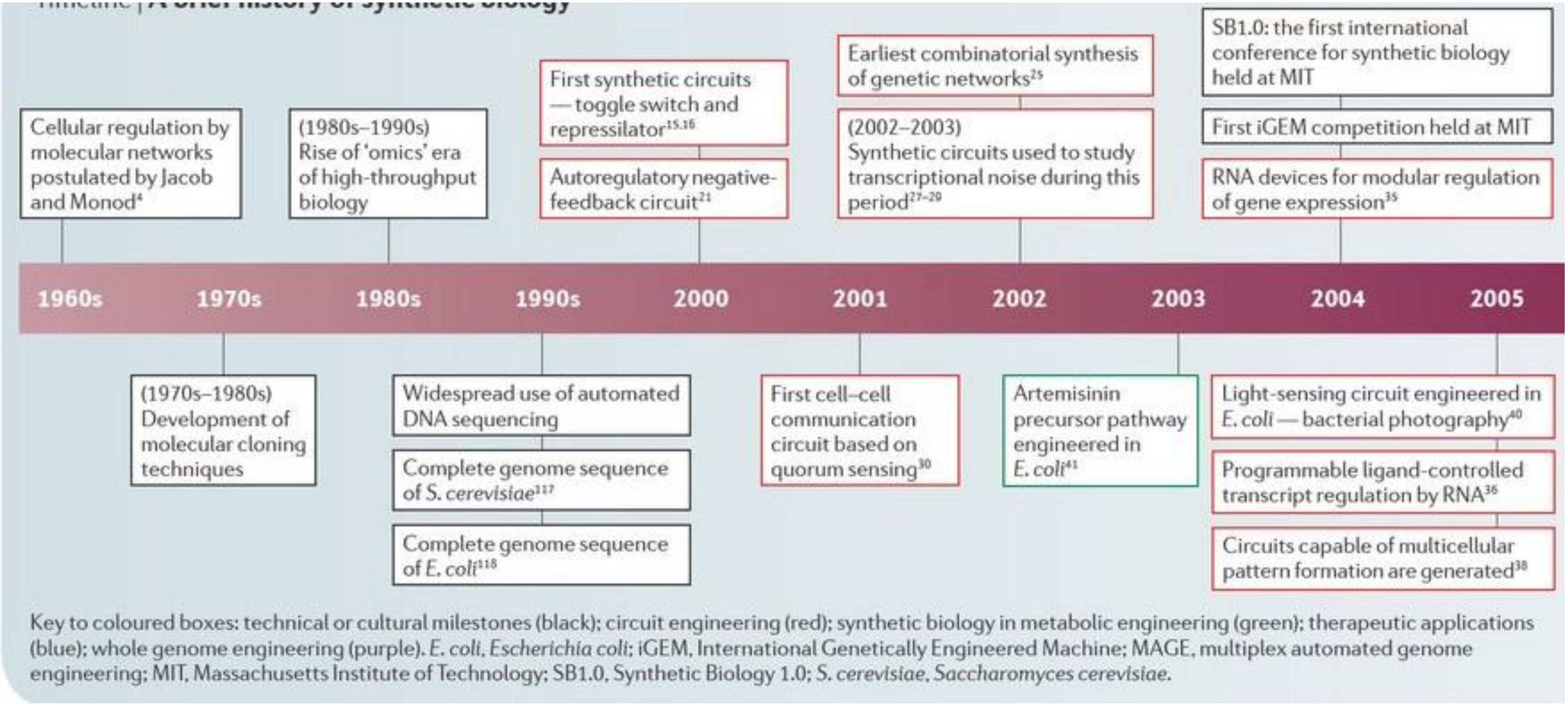


pH	Heliscope	ZMWG	Nanopore
2010	2008	2011	2014
Ion torrent	Helicos Biosciences	Pacific Biosciences	Oxford Nanopore
Non-optical	Single Molecule Sequencing		
1 human, 1 day Cost is below $5,000. Error prone	Too sensitive to vibration. FAILED	Long reads (60kb), error prone, but getting better	USB stick format, long reads (80kb+), error prone

Synthetic Biology

GGTCCTCGCGCCA
GCTTAAGACGCTAA
TCCCTAACTGCTGG
CGGAAAAGATGTGA
CAGACGCGACGGC

Timeline | A brief history of synthetic biology



Cellular regulation by molecular networks postulated by Jacob and Monod[4]

(1970s–1980s) Development of molecular cloning techniques

(1980s–1990s) Rise of 'omics' era of high-throughput biology

Widespread use of automated DNA sequencing

Complete genome sequence of *S. cerevisiae*[117]

Complete genome sequence of *E. coli*[118]

First synthetic circuits — toggle switch and repressilator[15,16]

Autoregulatory negative-feedback circuit[21]

First cell–cell communication circuit based on quorum sensing[30]

Earliest combinatorial synthesis of genetic networks[25]

(2002–2003) Synthetic circuits used to study transcriptional noise during this period[27–29]

Artemisinin precursor pathway engineered in *E. coli*[41]

SB1.0: the first international conference for synthetic biology held at MIT

First iGEM competition held at MIT

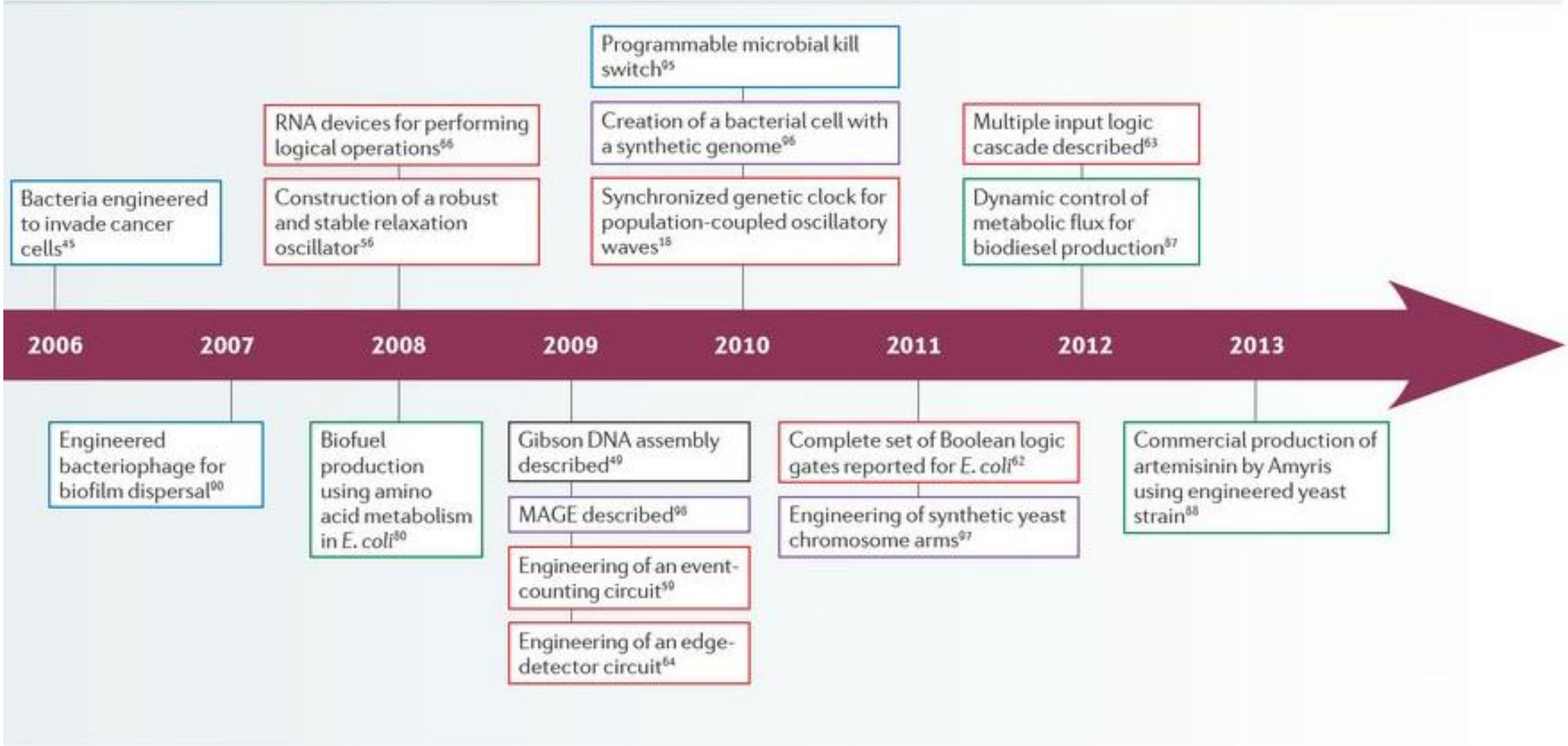
RNA devices for modular regulation of gene expression[35]

Light-sensing circuit engineered in *E. coli* — bacterial photography[40]

Programmable ligand-controlled transcript regulation by RNA[36]

Circuits capable of multicellular pattern formation are generated[38]

Key to coloured boxes: technical or cultural milestones (black); circuit engineering (red); synthetic biology in metabolic engineering (green); therapeutic applications (blue); whole genome engineering (purple). *E. coli*, *Escherichia coli*; iGEM, International Genetically Engineered Machine; MAGE, multiplex automated genome engineering; MIT, Massachusetts Institute of Technology; SB1.0, Synthetic Biology 1.0; *S. cerevisiae*, *Saccharomyces cerevisiae*.



2006: Bacteria engineered to invade cancer cells[45]

2007: Engineered bacteriophage for biofilm dispersal[90]

2008: RNA devices for performing logical operations[66]

2008: Construction of a robust and stable relaxation oscillator[56]

2008: Biofuel production using amino acid metabolism in *E. coli*[80]

2009: Gibson DNA assembly described[49]

2009: MAGE described[98]

2009: Engineering of an event-counting circuit[59]

2009: Engineering of an edge-detector circuit[64]

2010: Programmable microbial kill switch[95]

2010: Creation of a bacterial cell with a synthetic genome[96]

2010: Synchronized genetic clock for population-coupled oscillatory waves[18]

2011: Complete set of Boolean logic gates reported for *E. coli*[62]

2011: Engineering of synthetic yeast chromosome arms[97]

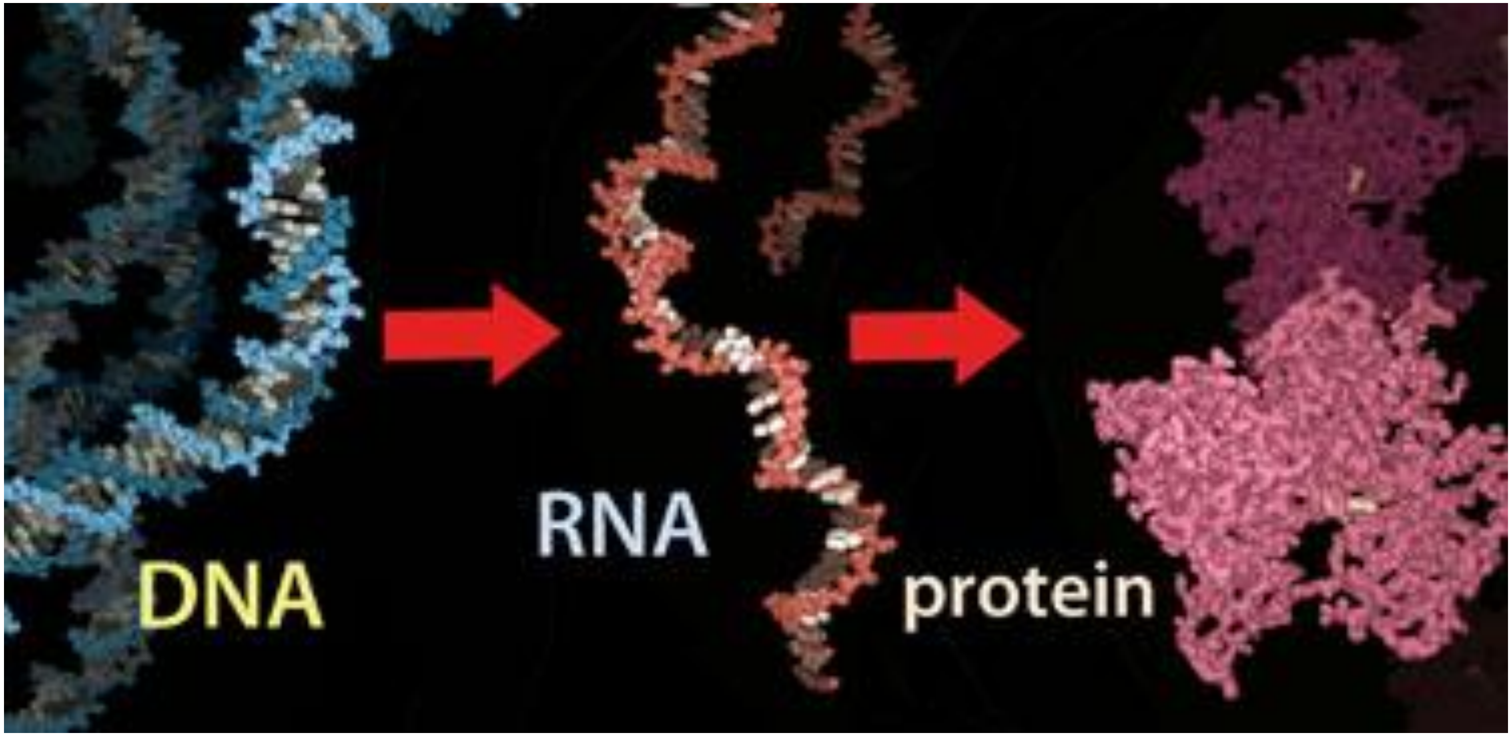
2012: Multiple input logic cascade described[63]

2012: Dynamic control of metabolic flux for biodiesel production[87]

2013: Commercial production of artemisinin by Amyris using engineered yeast strain[88]

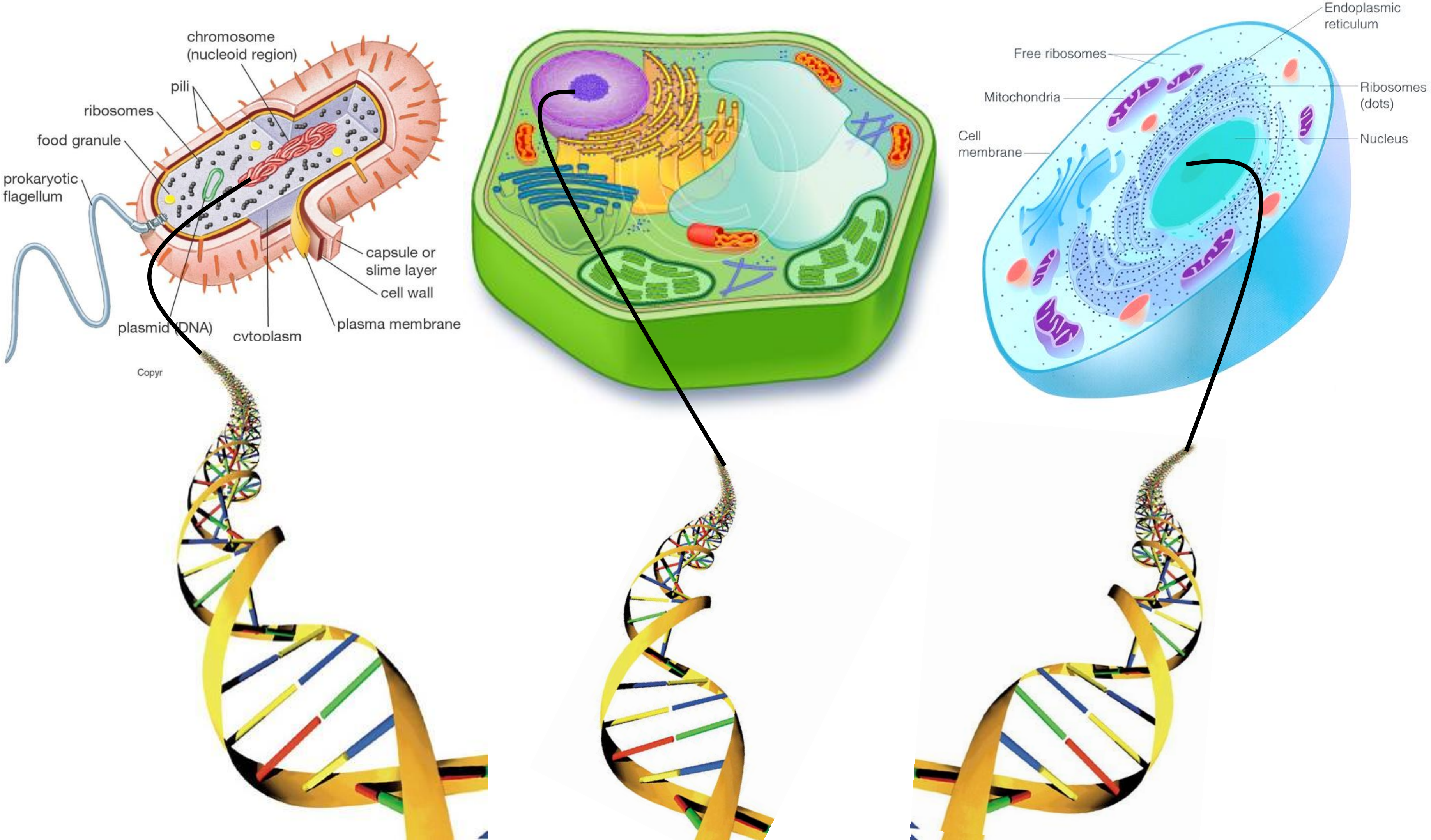
Central Dogma of Molecular Biology

Flow of information



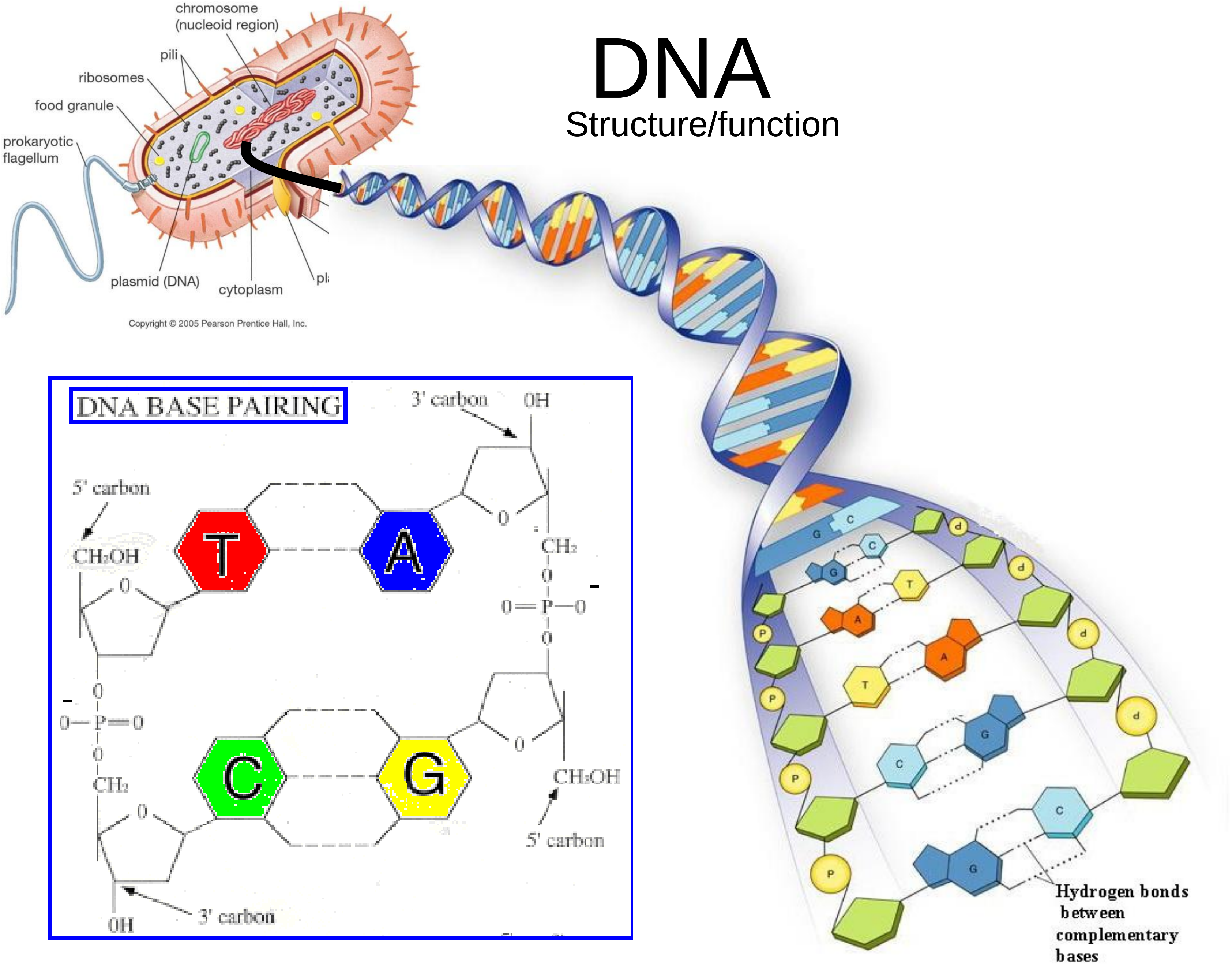
DNA

macromolecule of information



DNA

Structure/function



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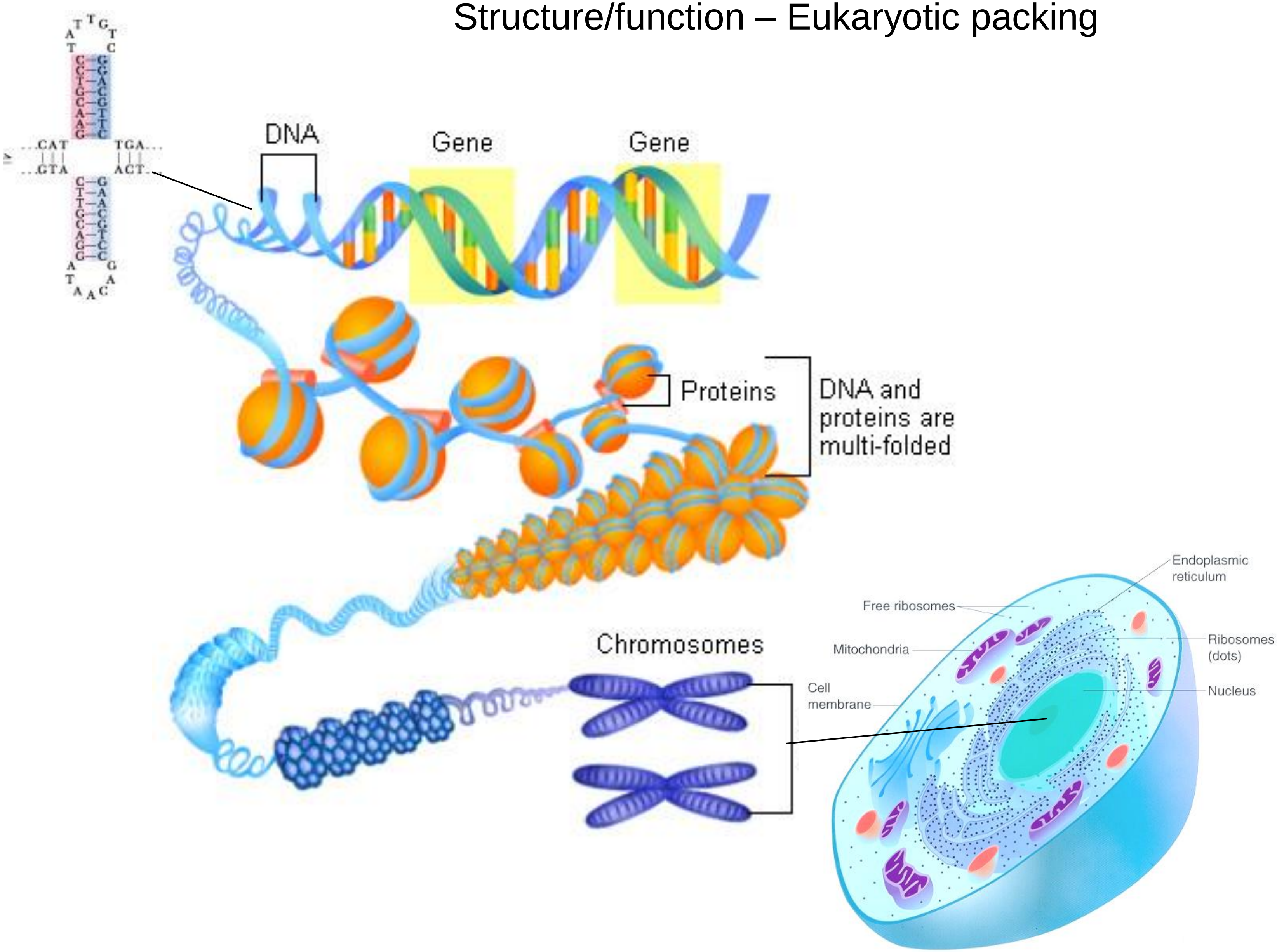
DNA

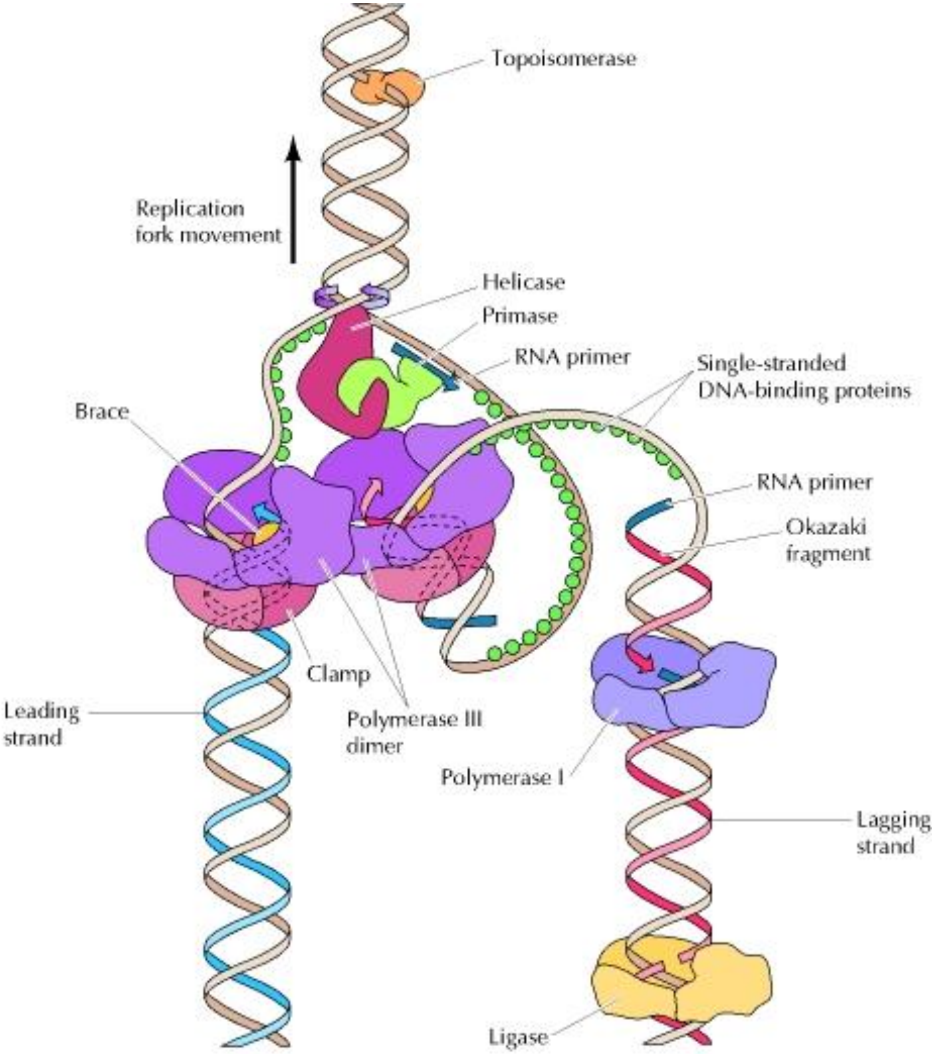
Structure/function – Eukaryotic packing



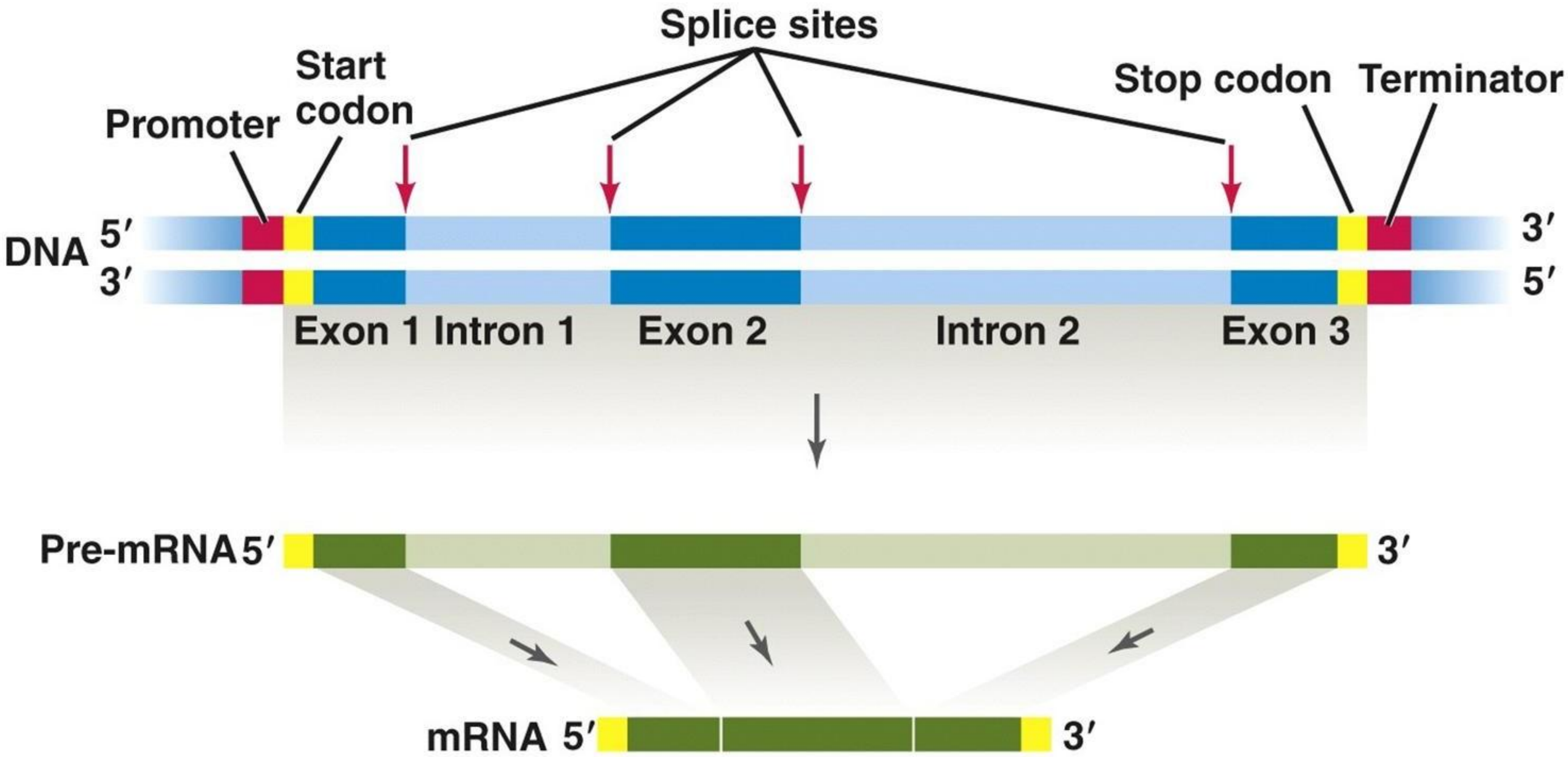
DNA

Replication

5’ to 3’



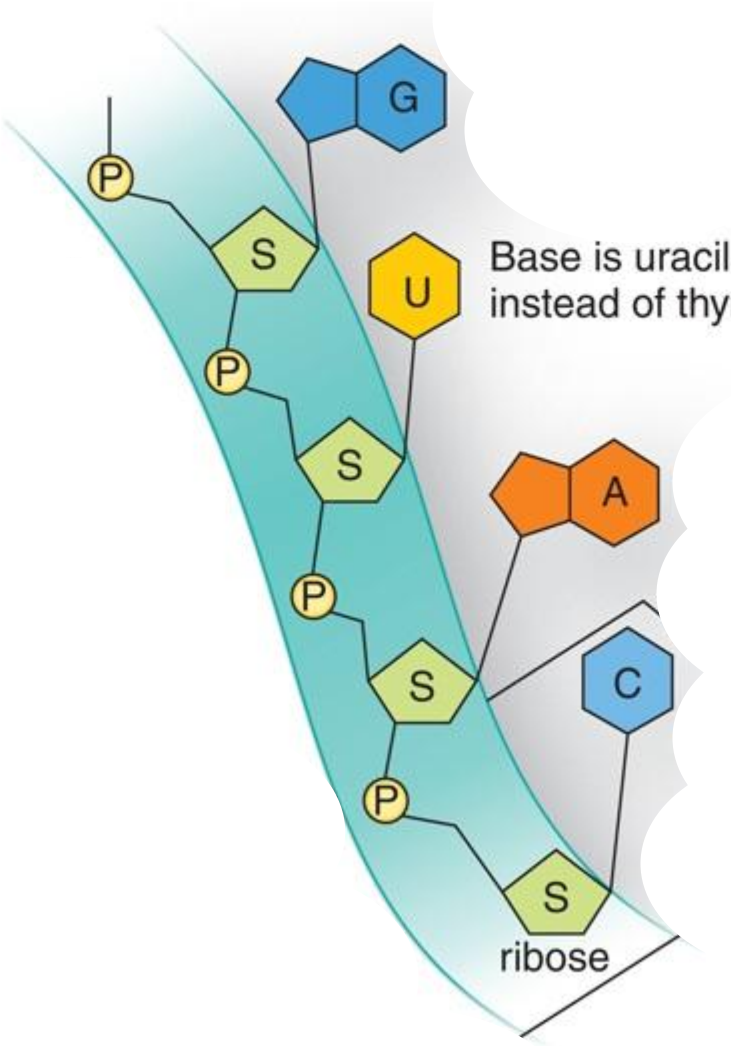
DNA -> RNA



PRINCIPLES OF LIFE, Figure 10.6 (Part 2)

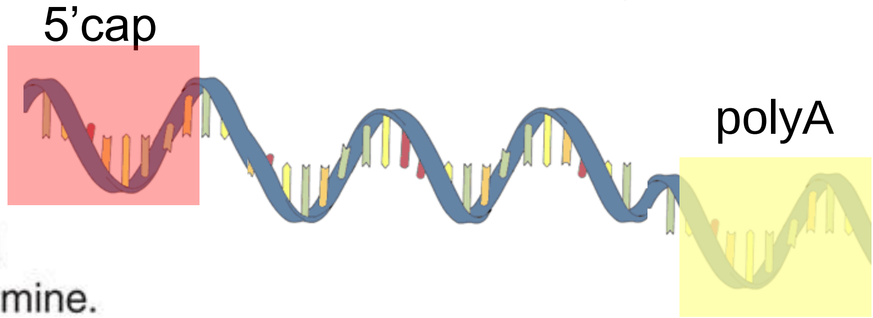
RNA

Structure/function



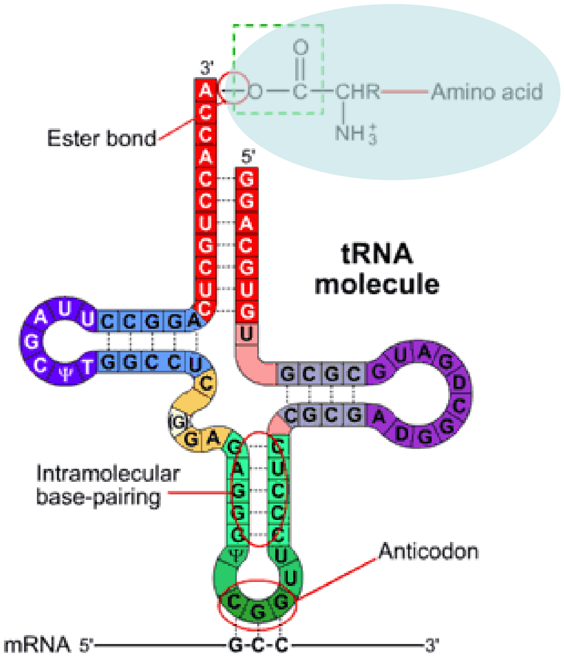
Base is uracil instead of thymine.

mRNA – messenger RNA


5'cap

polyA

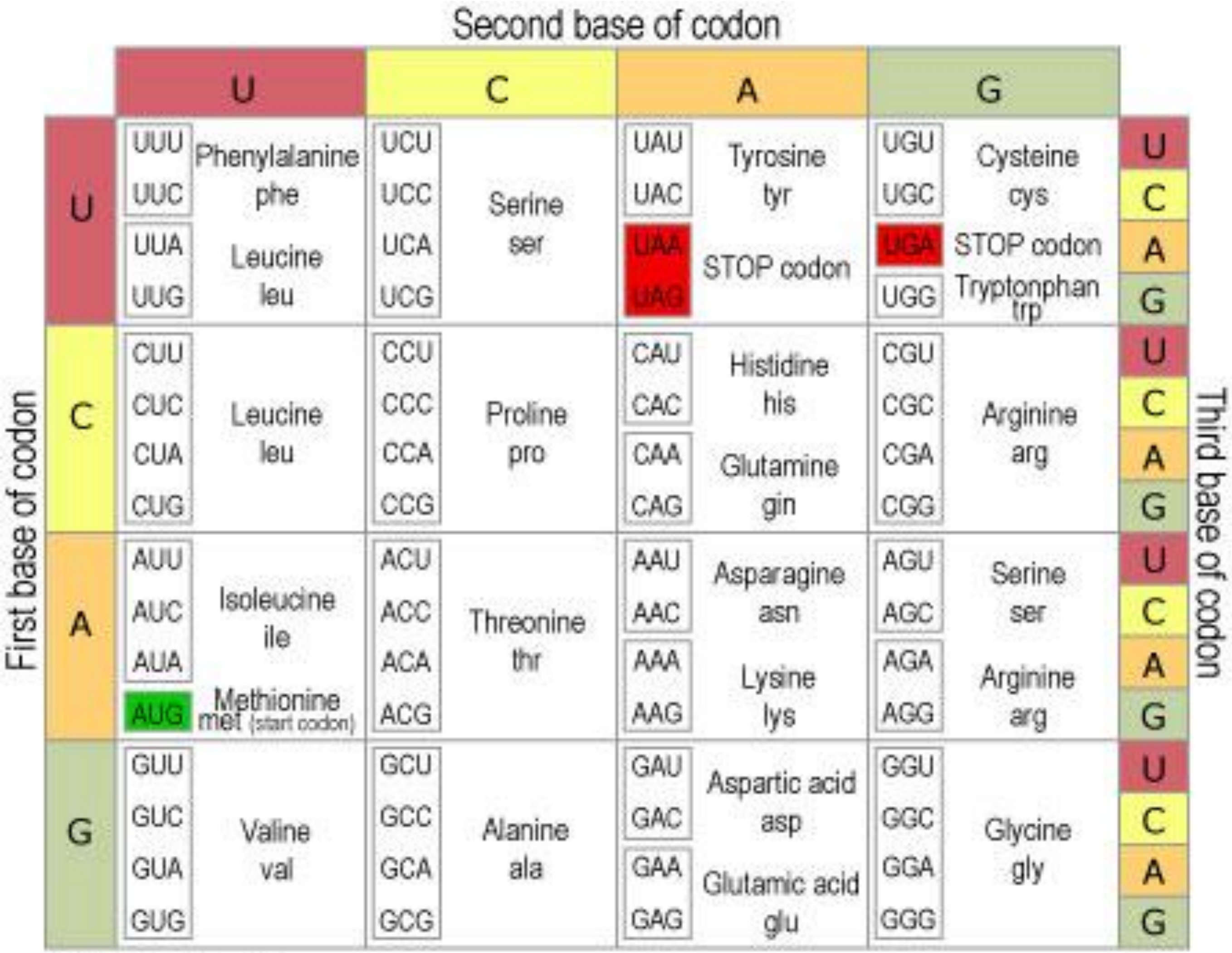
tRNA – transfer RNA



DNA

Transcription and Translation

The Protein Code (Translation of RNA to Amino Acids)



	Second base of codon								
First base of codon	U		C		A		G		Third base of codon
U	UUU	Phenylalanine phe	UCU	Serine ser	UAU	Tyrosine tyr	UGU	Cysteine cys	U
	UUC		UCC		UAC		UGC		C
	UUA	Leucine leu	UCA		UAA	STOP codon	UGA	STOP codon	A
	UUG		UCG		UAG		UGG	Tryptonphan trp	G
C	CUU	Leucine leu	CCU	Proline pro	CAU	Histidine his	CGU	Arginine arg	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Glutamine gln	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Isoleucine ile	ACU	Threonine thr	AAU	Asparagine asn	AGU	Serine ser	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	Lysine lys	AGA	Arginine arg	A
	AUG	Methionine met (start codon)	ACG		AAG		AGG		G
G	GUU	Valine val	GCU	Alanine ala	GAU	Aspartic acid asp	GGU	Glycine gly	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	Glutamic acid glu	GGA		A
	GUG		GCG		GAG		GGG		G

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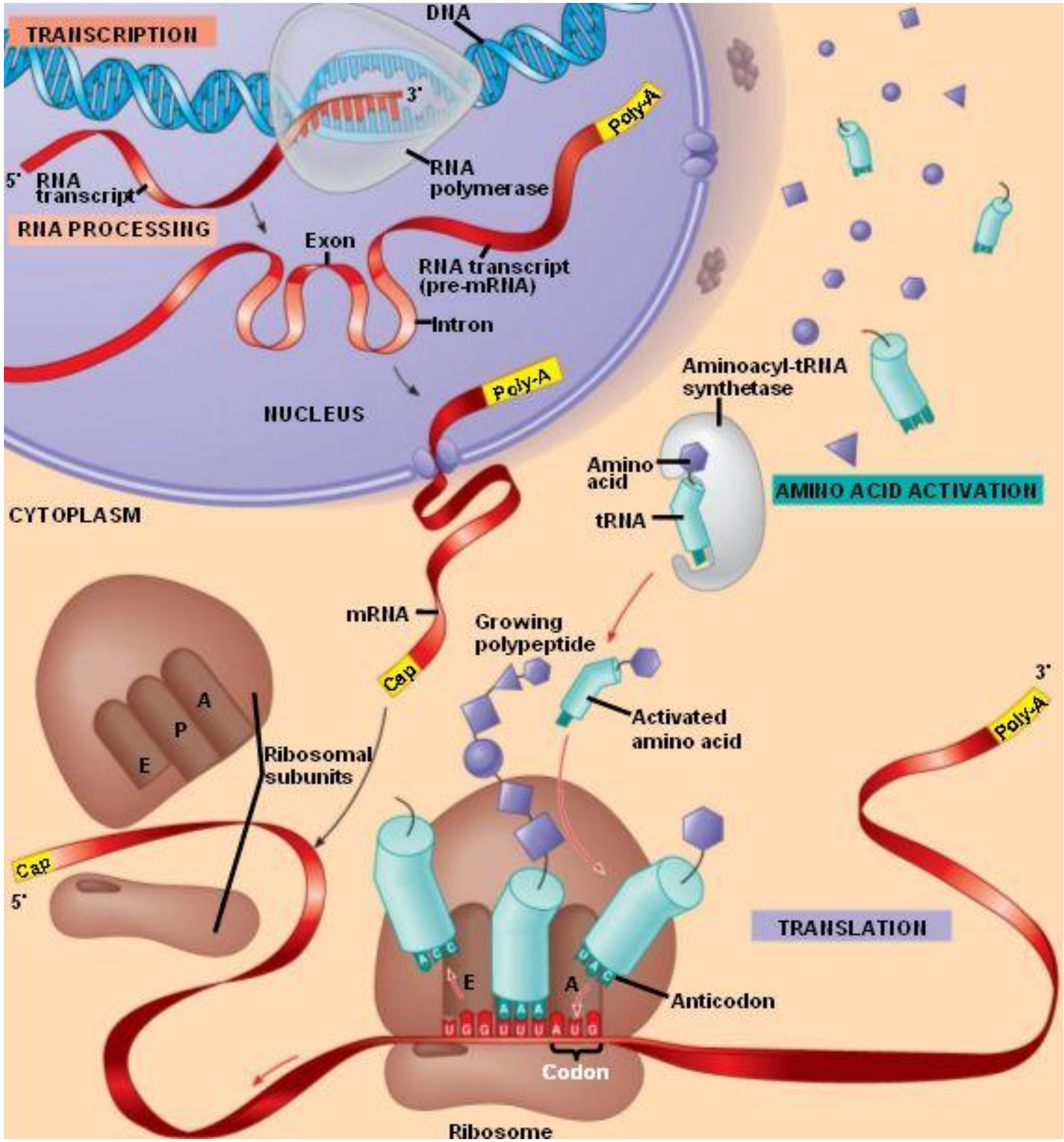
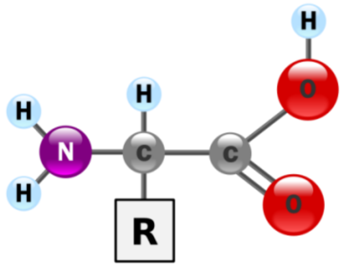
DNA

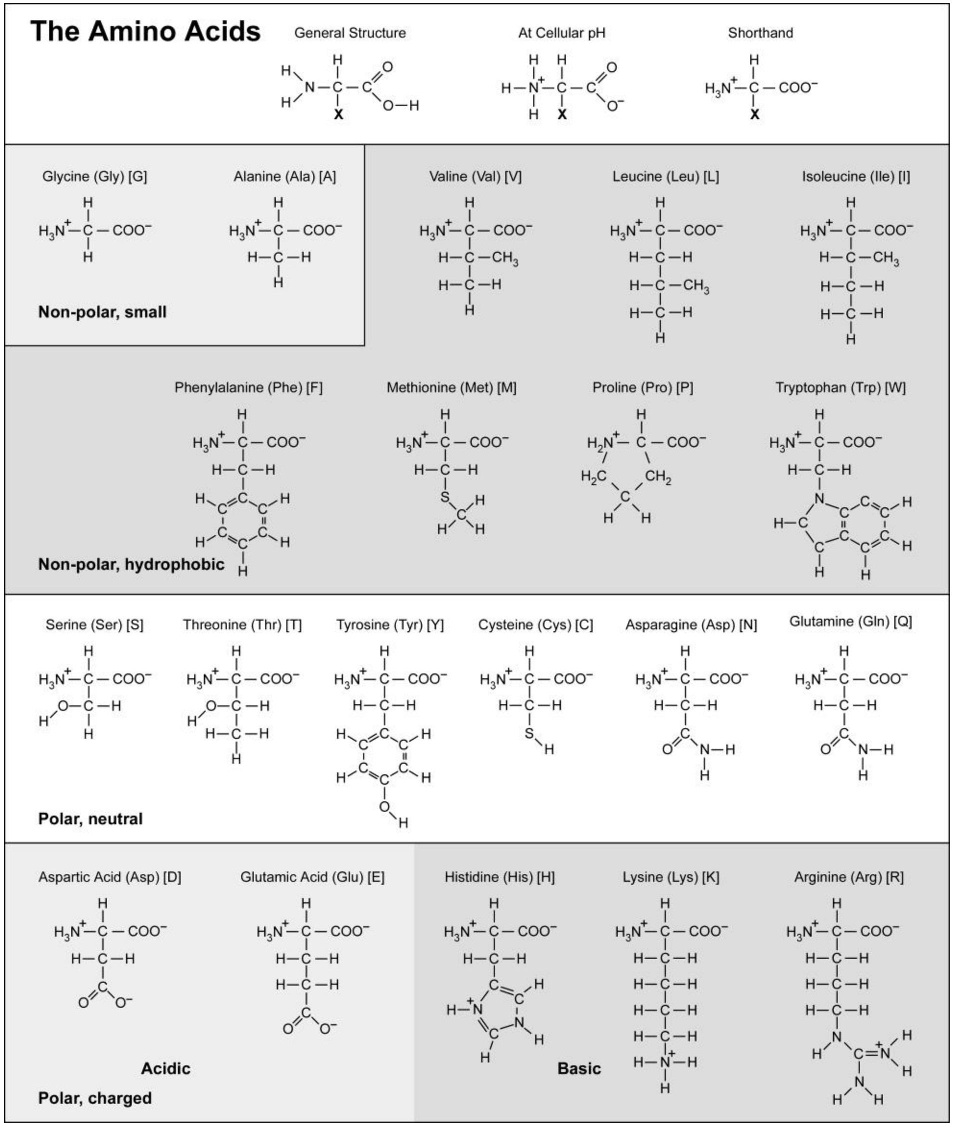
Transcription and Translation

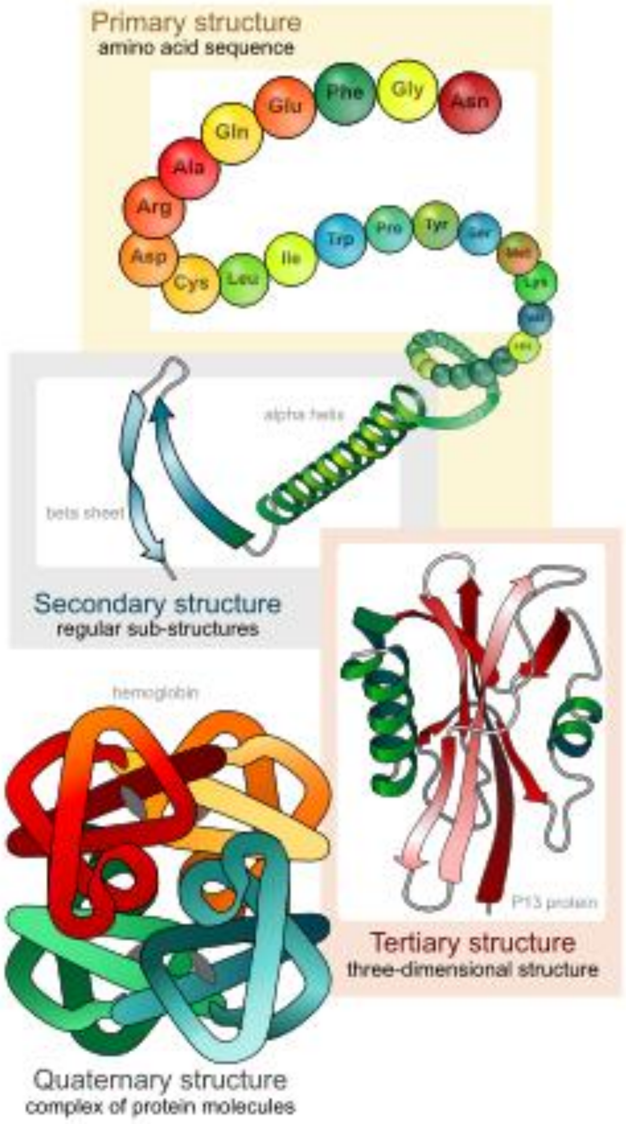


Protein

Structure/function



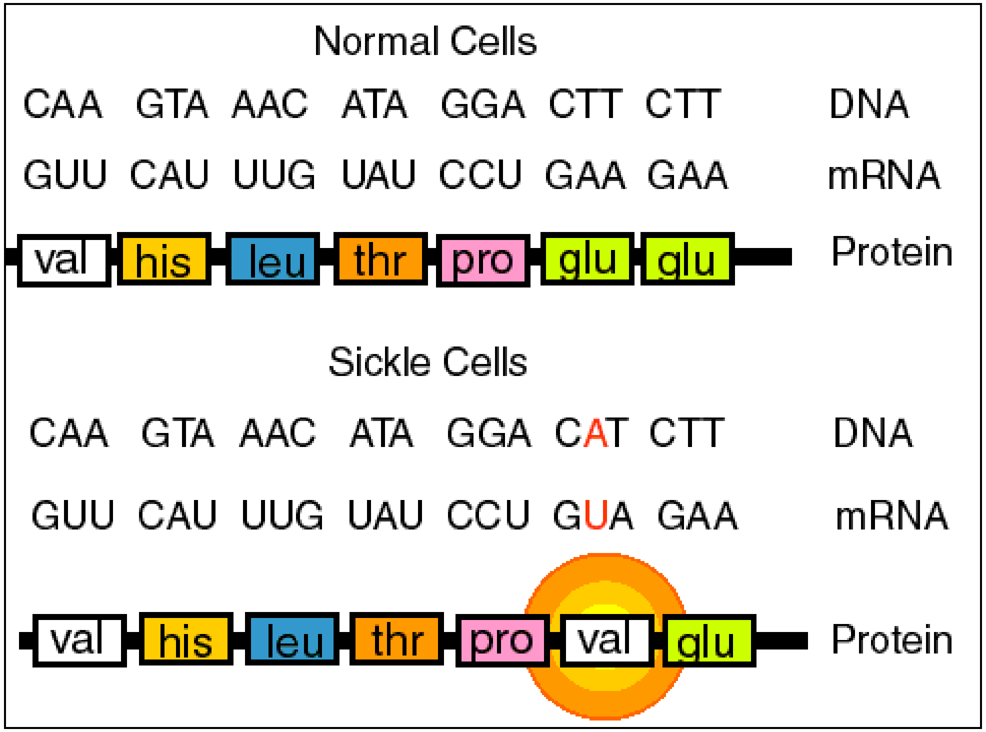




Protein

Structure/function

Sickle Cell Anemia



Normal Cells

CAA GTA AAC ATA GGA CTT CTT DNA

GUU CAU UUG UAU CCU GAA GAA mRNA

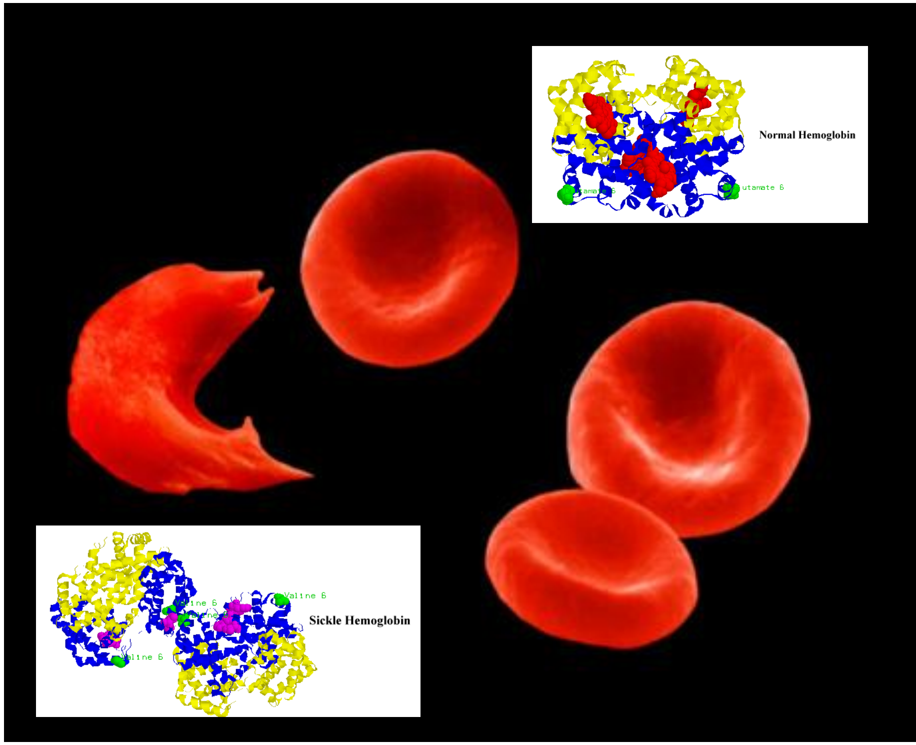
val his leu thr pro glu glu Protein

Sickle Cells

CAA GTA AAC ATA GGA CAT CTT DNA

GUU CAU UUG UAU CCU GUA GAA mRNA

val his leu thr pro val glu Protein



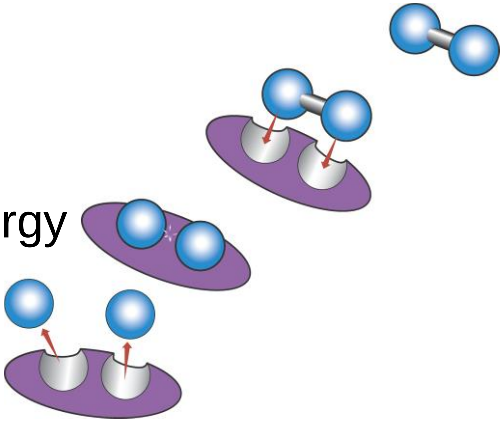
Single nucleotide mutation changes protein code, changes protein shape and function

Protein

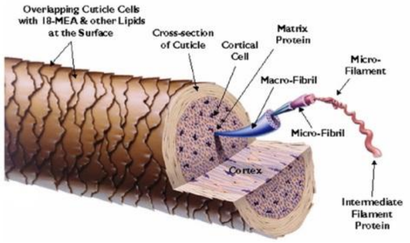
function

Form defines Function:

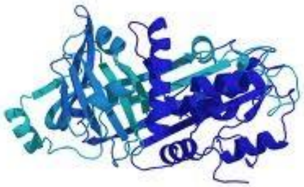
Enzymes – catalyze reactions by lowering activation energy



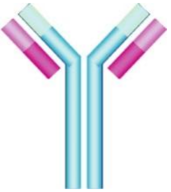
Structural- collagen, keratin, etc



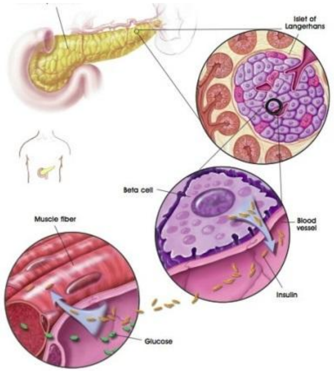
Storage – act as a reservoir of amino acids – ovalalbumin



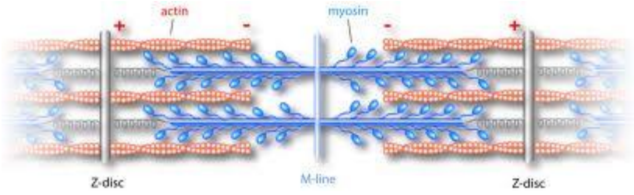
Antibodies – immune system ability to pin point antigens



Hormones – signaling molecules that travel long distance in the body



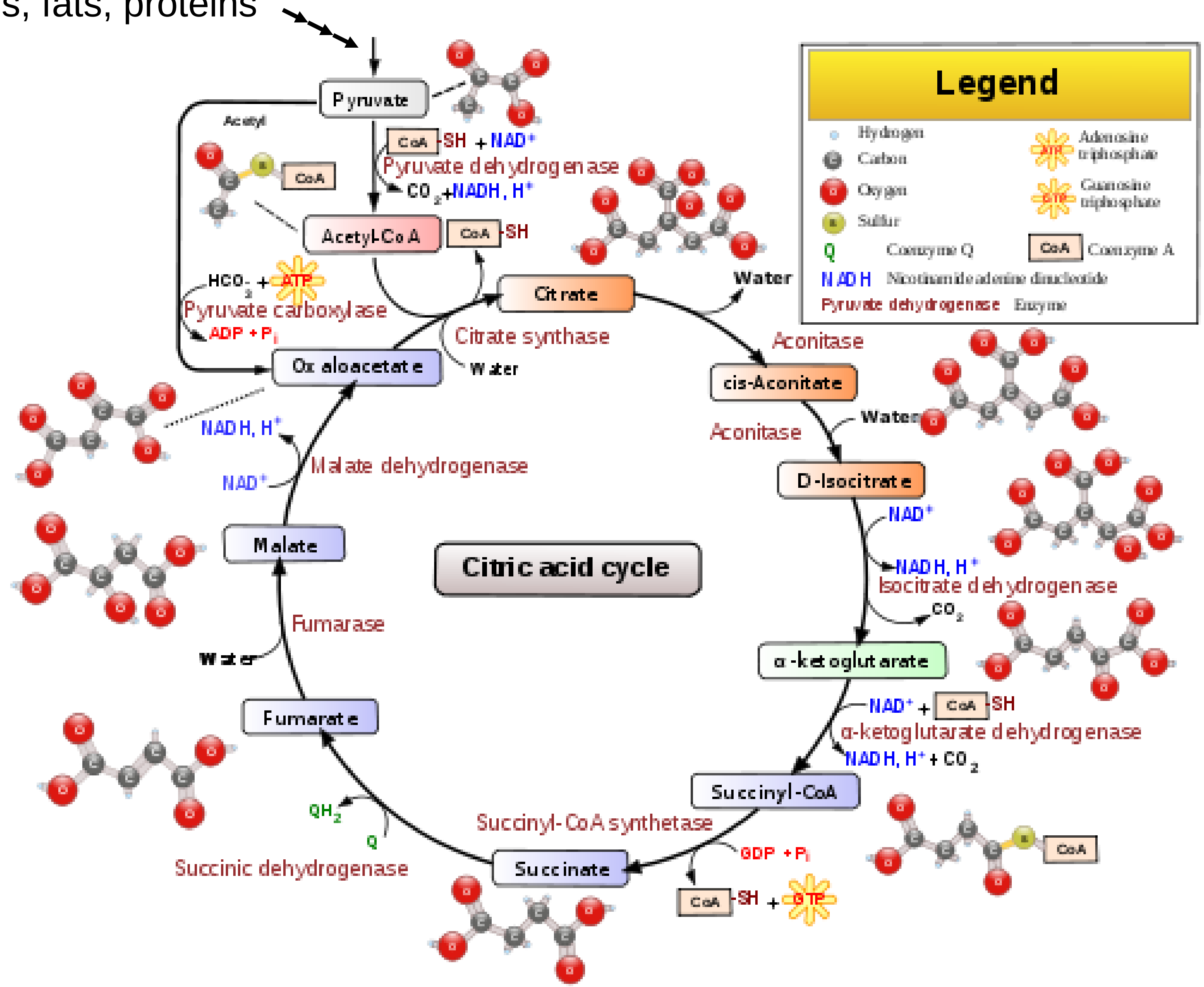
Contractile – myosin, muscle fibers

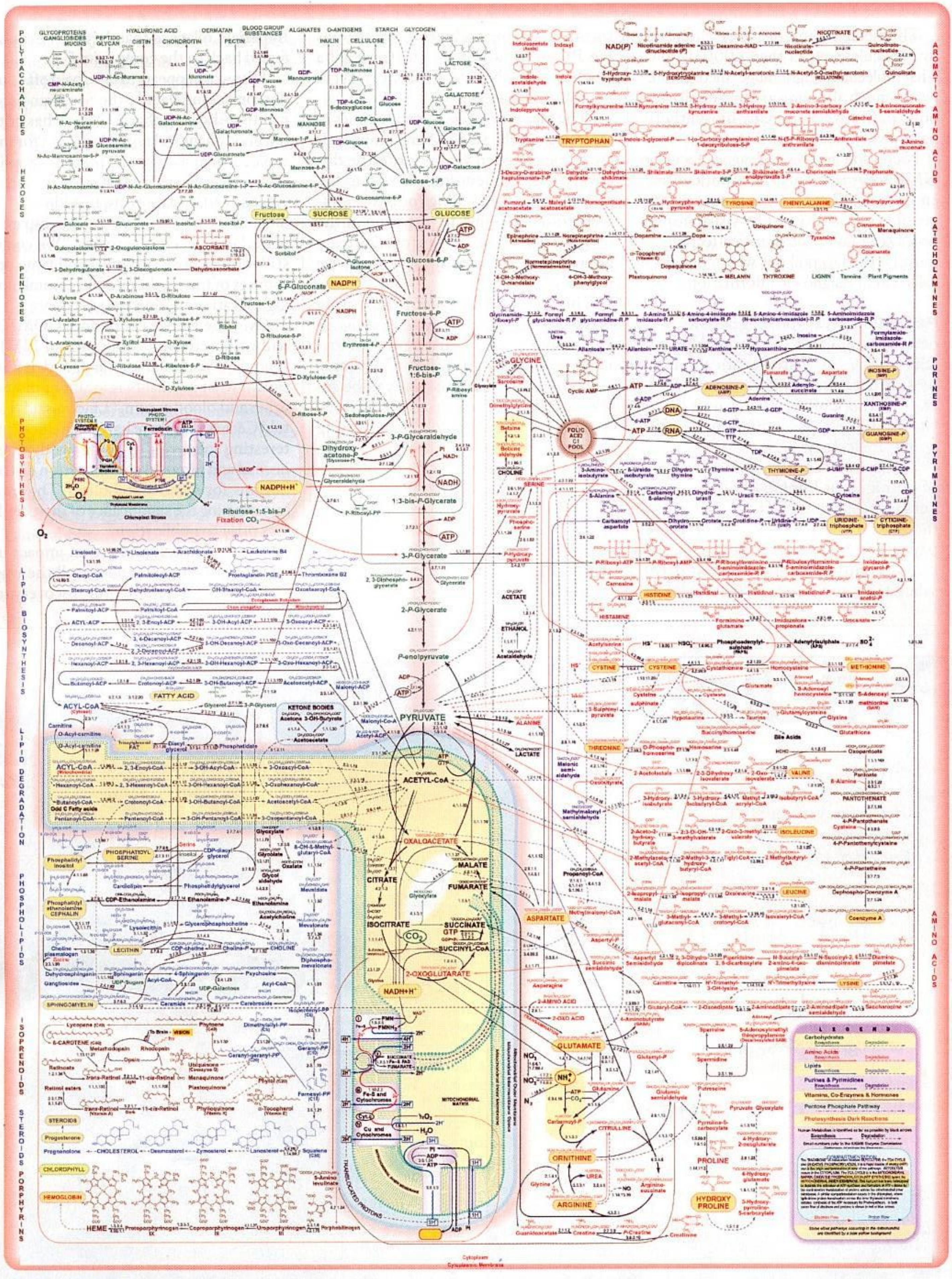


Protein

Enzymatic Pathways

Carbs, fats, proteins





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Synthetic Biology

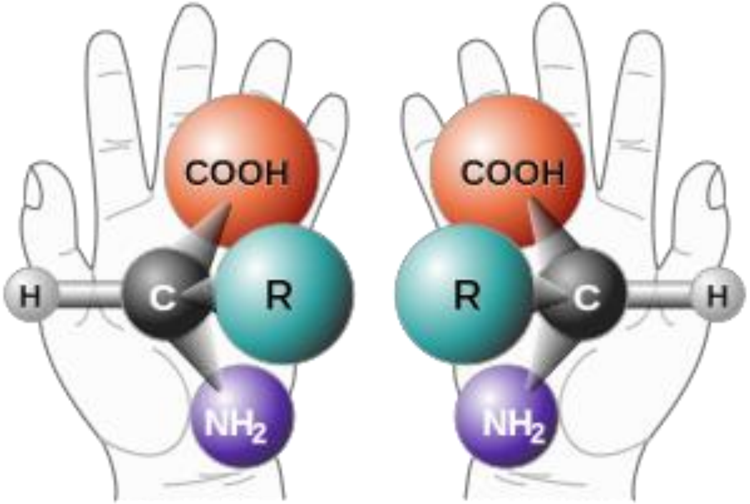
What does it encompass?

Bio-Engineering BE	Biomimetic Chemistry BC	Genetic Customization GC
Control logic & circuits oscillators, memory, transistors, cell-cell communication	**Non-natural nucleic acids & proteins, the left-handed cell**	Biomedicine Pathogen hunters, gene drive, epigenetic switches
Standardized parts, decoupling, abstraction, orthogonality, BioBricks	Cell free factories DNA and RNA catalysts, aptamers	Environmental & metagenomic surveys how does nature get it done?
Systems biology **metabolic modeling, pathway**	*in vitro* evolution chemical self-replication of	**Synthetic genes & genomes**

Adapted from Sarah Richardson / LBL

Biological Chirality

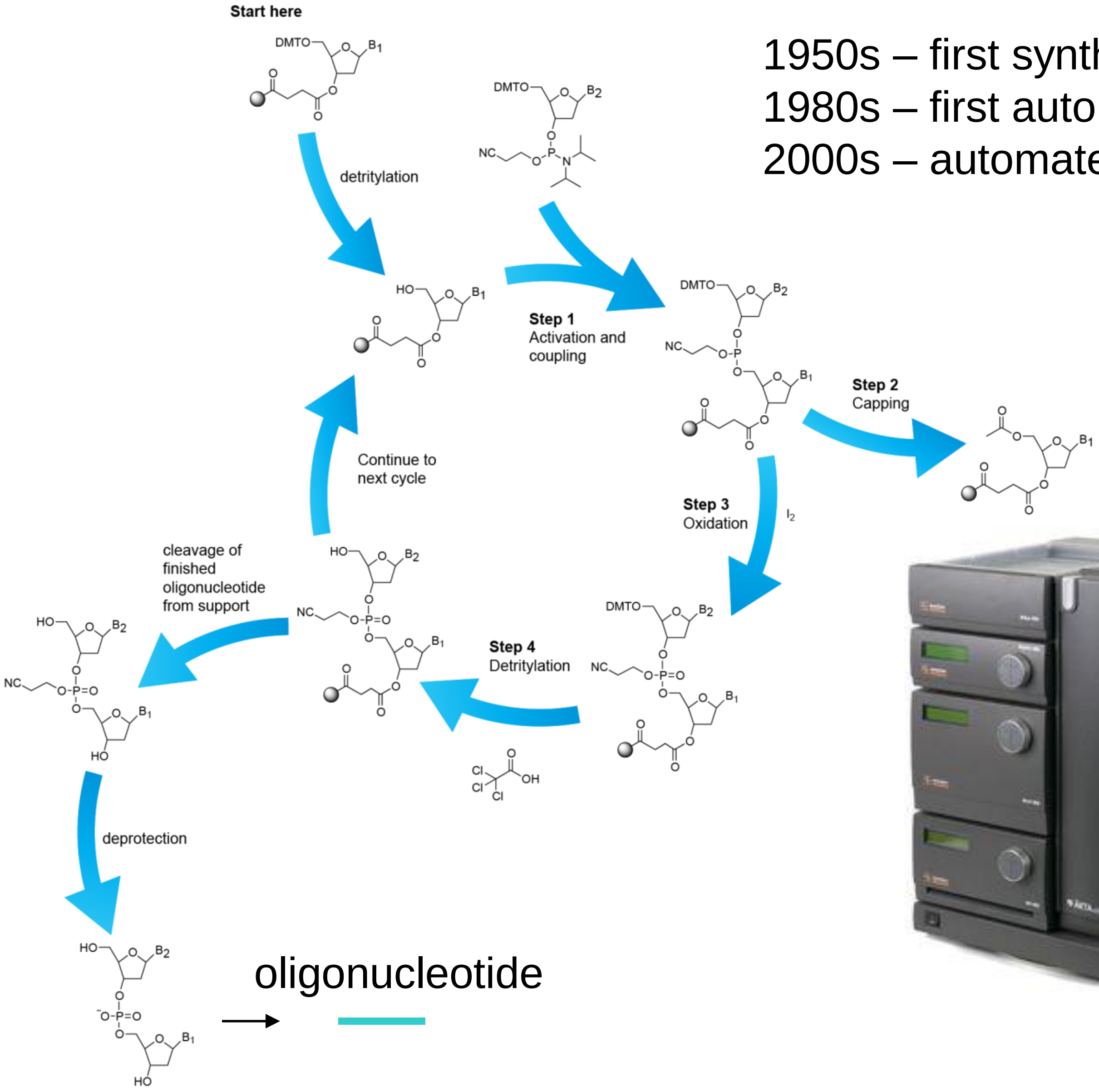
Molecules can have the same composition but be mirror images of each other, which can effect molecular interactions



Biological molecules tend to be of one chirality:
Left-handed amino acids and Right-handed sugars

Building biological molecules of the opposite chirality and ultimately cell components and cells could confer non-escapable synthetic life forms as they'd need to be fed their chiral inputs or make them scarily non-attackable.

Tools: Oligo (DNA) Synthesis



1950s – first synthetic oligonucleotides
1980s – first automated systems
2000s – automated to a send out service

Tools: Oligo (DNA) Synthesis

BioXp 3200
Synthetic Genomics

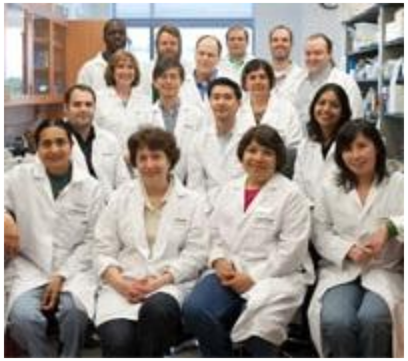
Current DNA synthesizers:
DNA fragment assembly from 400 bp to 1.8 kb in length

The First Synthetic Genome: *first steps*

J. Craig Venter™

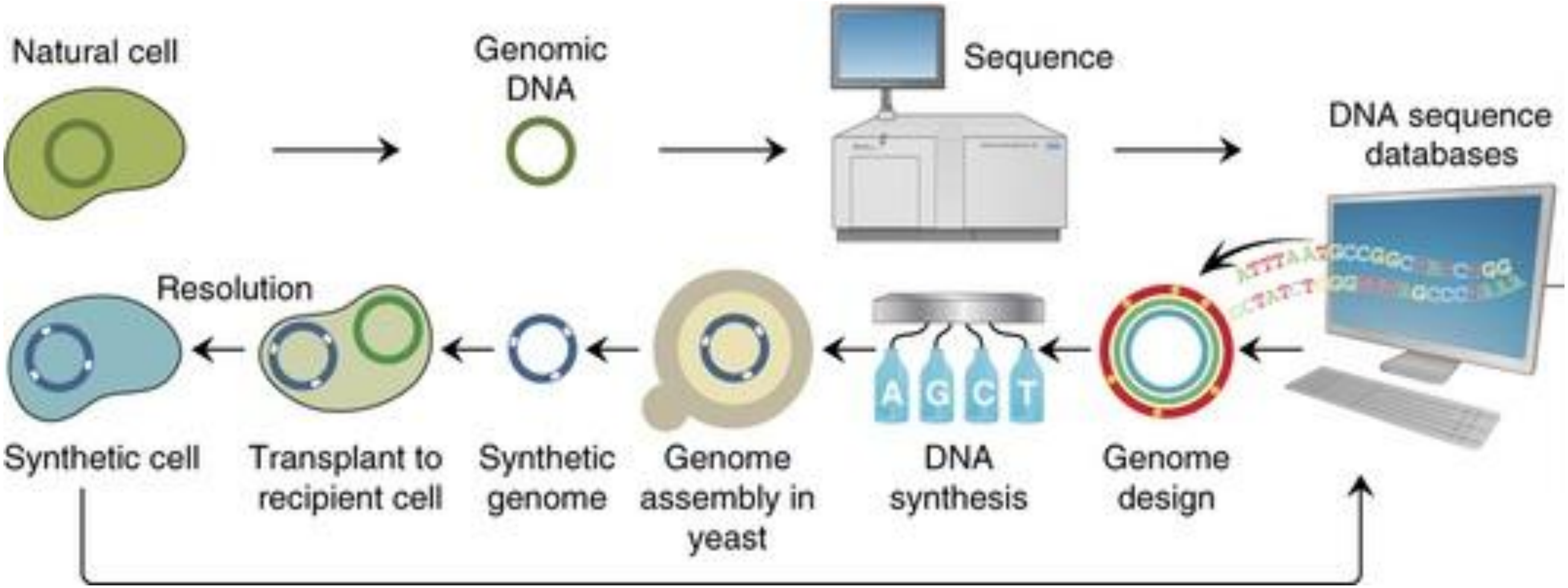
INSTITUTE

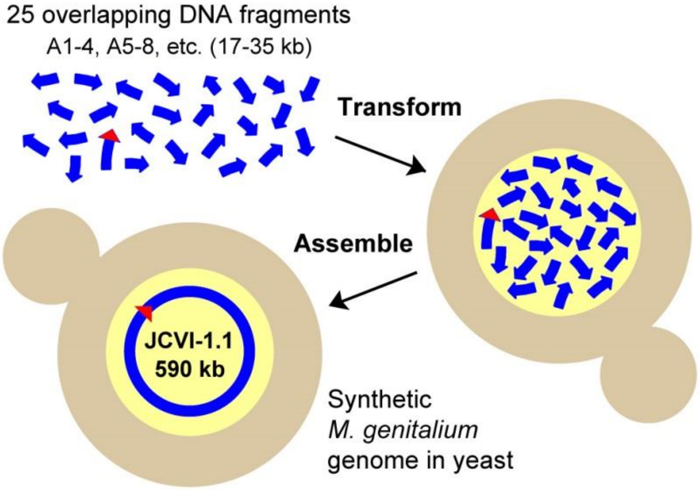
SYNTHETIC GENOMICS®



- **1995 -** Sequence *Mycoplasma genitalium*
- **2007 -** Chromosomal transplant of *M. mycoides* into *M. capricolum*
- **2008 -** Synthesize *Mycoplasma genitalium* genome
- **2009 –** Choose a faster growing *Mycoplasma*

JCVI approach to synthetic genomes





GC

The First Synthetic Genome: *JCVI-syn1.0*

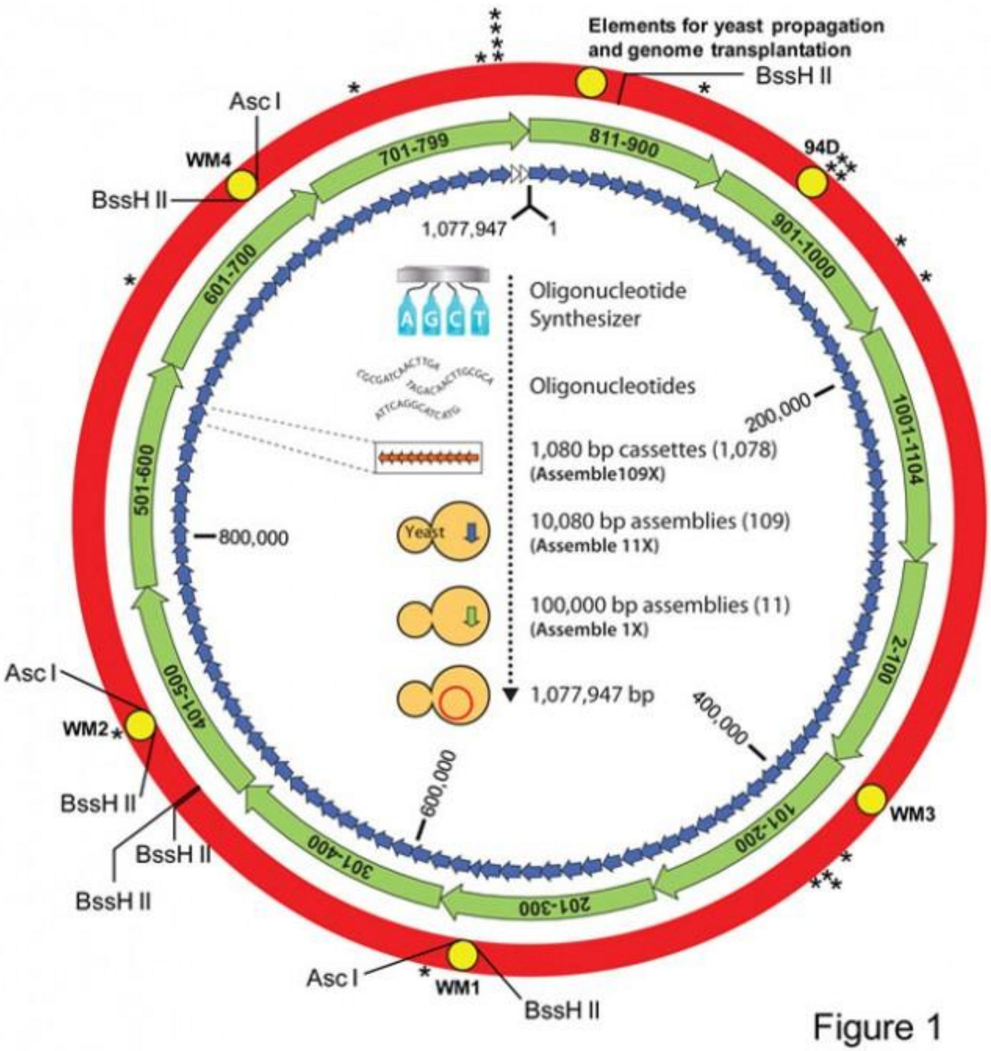
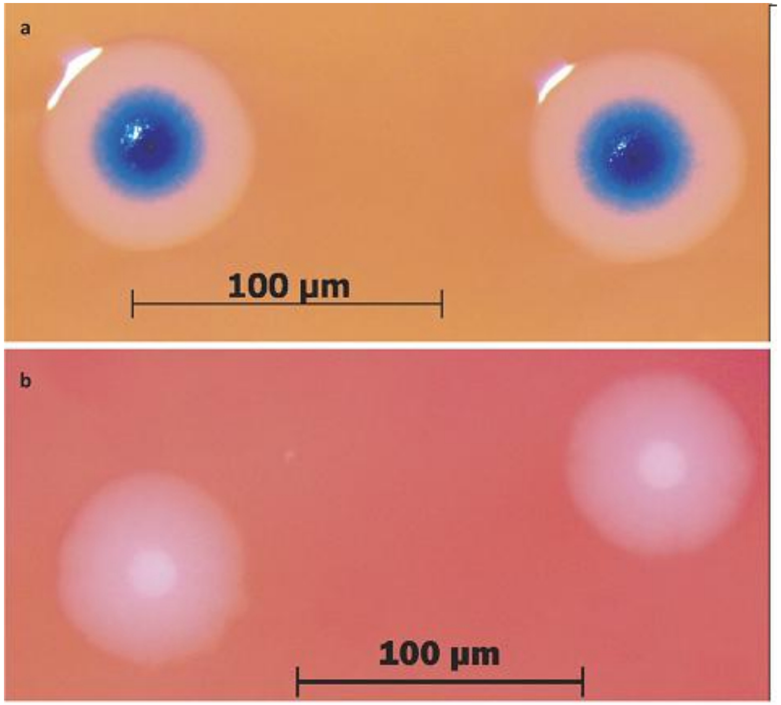


Figure 1

M. mycoides – JCVI-syn1.0



M. capricolum

Gibson DG, Glass JI, Lartigue C, Noskov VN, Chuang RY, Algire MA, et al. Creation of a bacterial cell controlled by a chemically synthesized genome. Science. 2010;329:52–56

The First Synthetic Genome: *Beyond*

- **the Minimal Genome Project**
  - An attempt to make a synthetic genome containing the lowest number of genes necessary for life

  - *Mycoplasma laboratorium* is the proposed synthetic genome containing only 387 genes and 43 structural RNAs that JVCI is interested in making

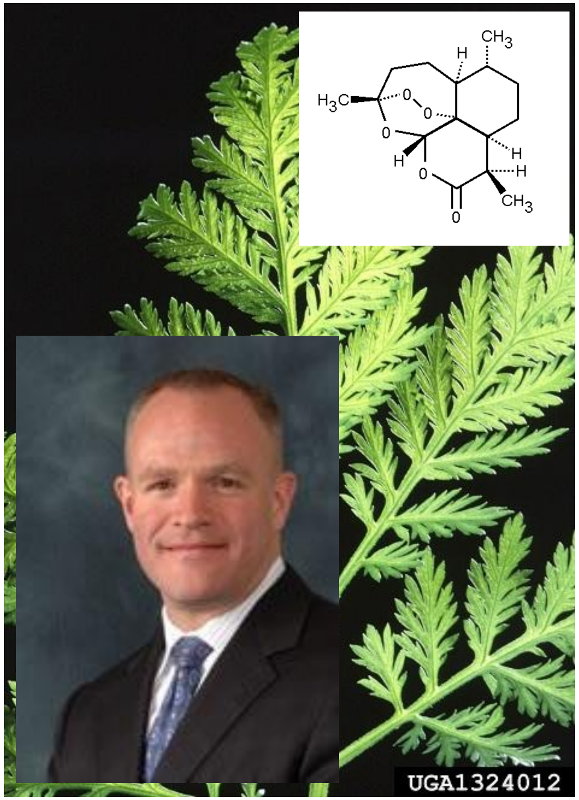
  - A minimal Genome would provide a chassis upon which to build many different useful genomes

BE

Semi-Synthetic Artemisinin

Malaria- the worlds largest deadly communicable parasitic disease. In 2013 according the World Health Organization (WHO) – 2 million infections and 500,000 deaths.

***Plasmodium falciparum* –** causative agent passes from mosquitoes to humans



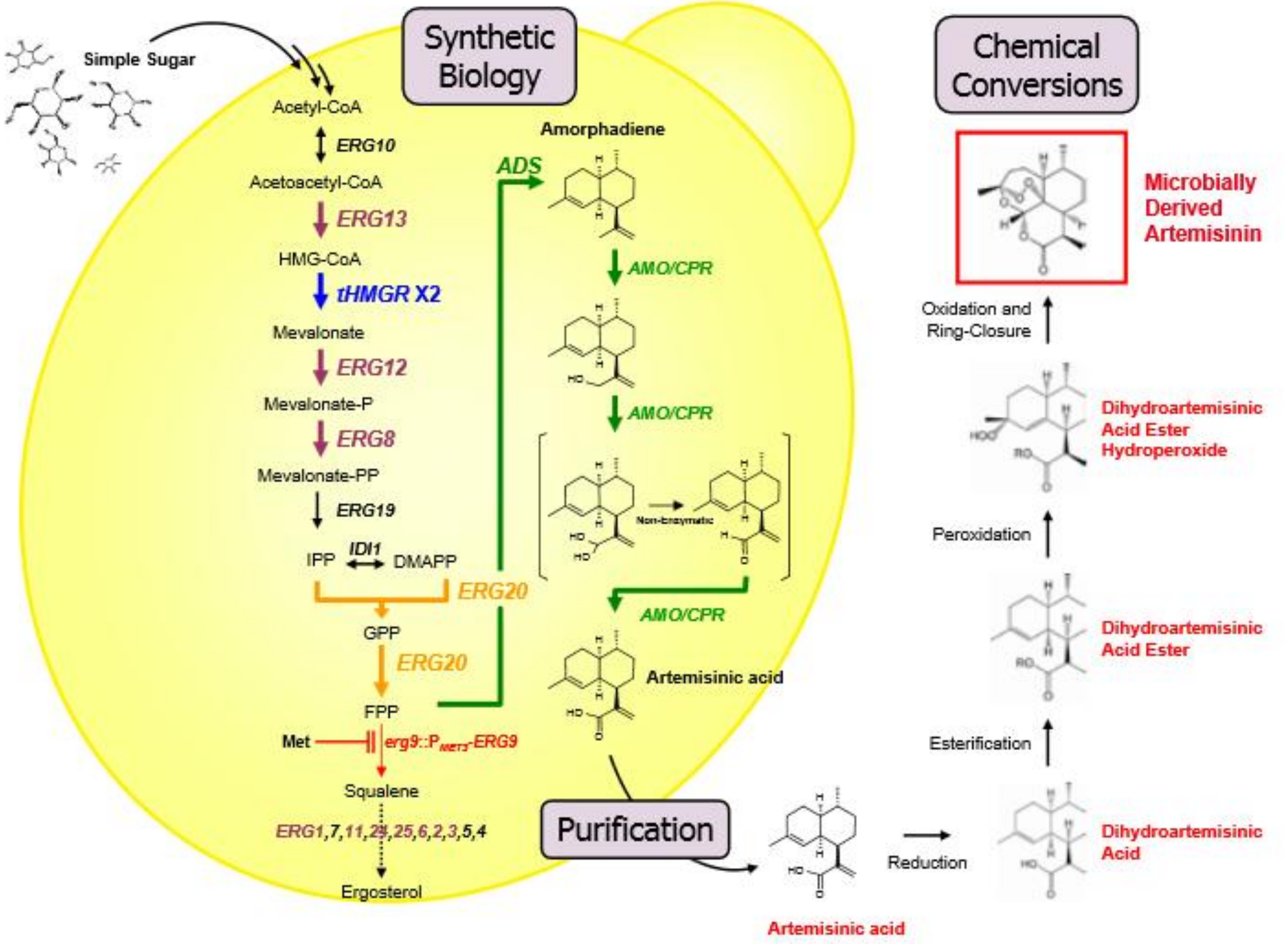
Artemisinin - effective anti-malarial derived from sweet wormwood plant (***Artemisia annua***). WHO recommends its use as a Artemisinin combined therapy (ACT) with other derivatives to keep *Plasmodium* from developing resistance.

Conventional production –
Plants grown in SE China, Vietnam or Africa
Hexane extraction from dried leaves to get drug
Huge fluctuation in price $120 - $1200 (2005-2008)

Semi-Synthetic production goal-
Stabilize volume, price, and quality
Make ACT more affordable and accessible to those in need

BE

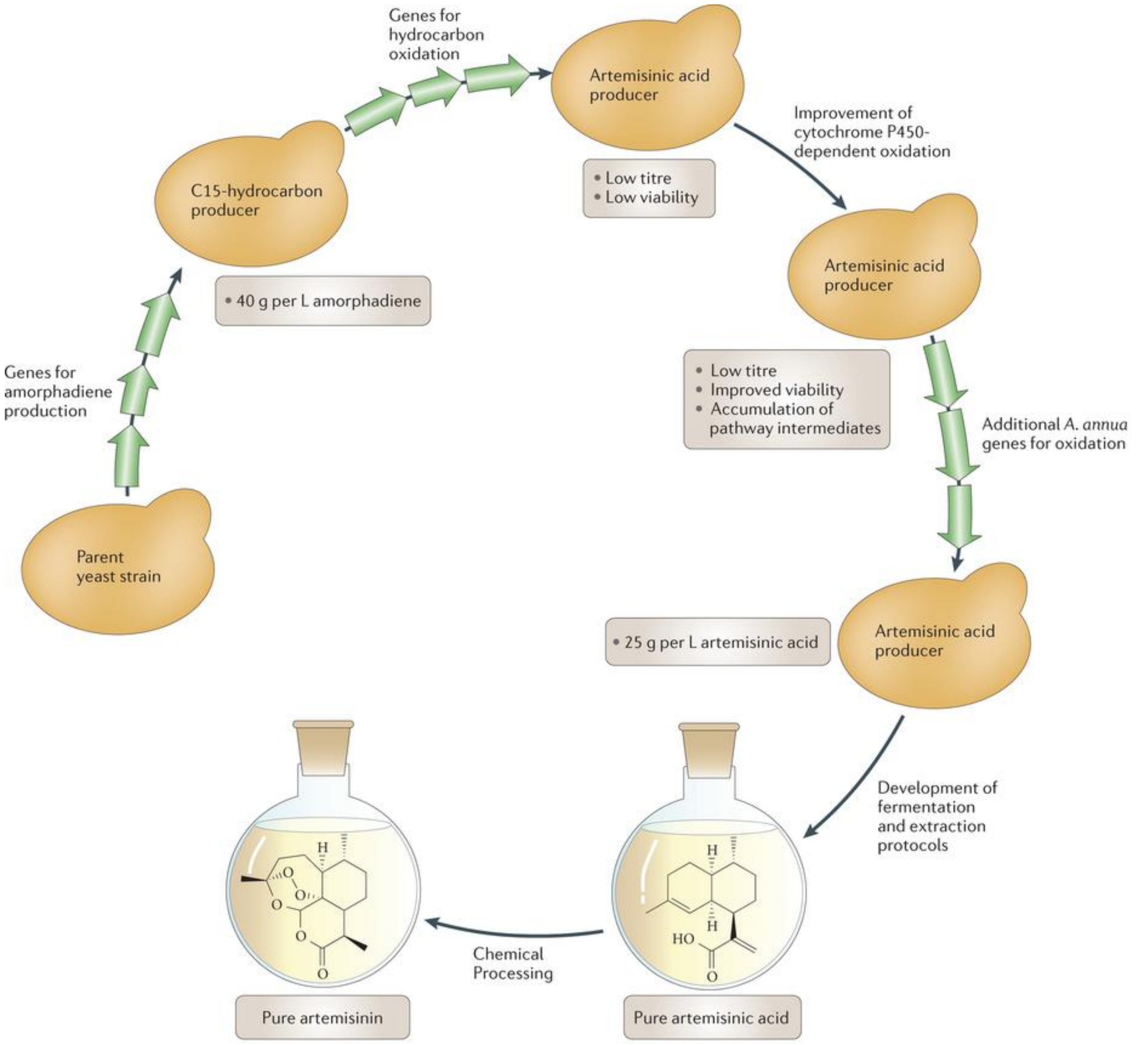
Semi-Synthetic Artemisinin



REPORT of the 2008 Artemisinin Enterprise Conference, October 8-10 York UK

BE

Semi-Synthetic Artemisinin



Nature Reviews | Microbiology

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Semi-Synthetic Artemisinin

Timeline (>10 years)
2003 – Keasling first publication
2005-2008 – Development of technology
2009-20013 – Industrialization, Scaling, Validation
2014 – 1st ACT from Semi-Synthetic Artemisnin to countries

Outcomes (2014):
$400 kg
55 tons of Semi-Synthetic Artemisnin in 2014
Reaction time as low as 3 months
Much more stable market
Will it change the effect of Malaria on 3rd world countries?

SANOFI

BILL & MELINDA GATES foundation

Berkeley
University of California

Tools: CRISPR/Cas9

This genome editing tool is the most powerful new tool in synthetic biology, especially for BioEngineering, because it allows an easy process for site specific recognition and cutting which can allow scientist to delete or insert sequences.

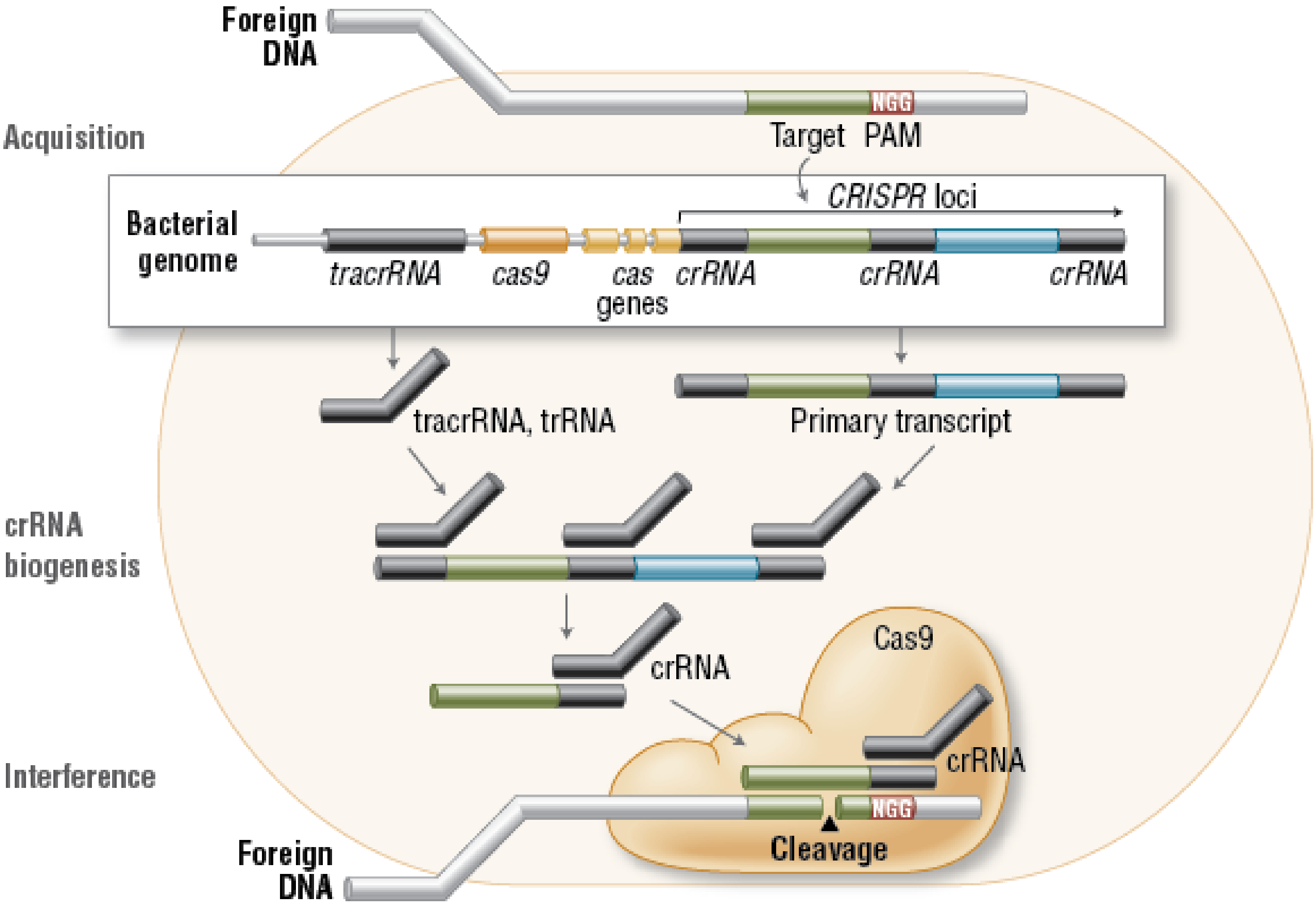
CRISPR = Clustered Regularly Interspaced Short Palindromic Repeats

Cas = CRISPR-associated

The Bacterial Adaptive Immunity systems function was discovered in 2007 by Dr. Rodolphe Barrangou at North Carolina State University. Bacteria use it to recognize foreign DNA and quickly destroy it. Primarily the type II CRISPR system is what is used in synthetic biology because of its low complexity, requiring only one Cas protein is required (Cas9)

Tools: CRISPR/Cas9

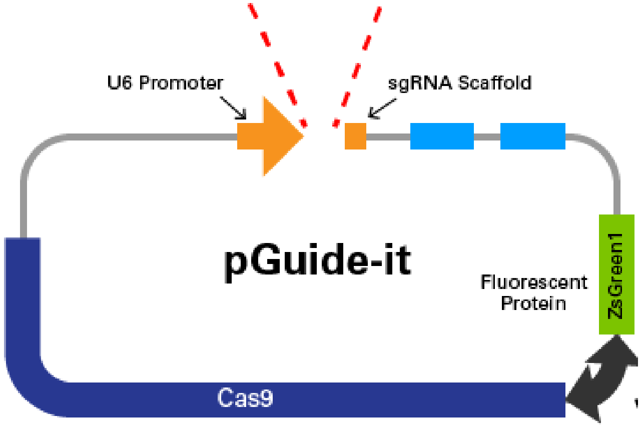
Bacterial Adaptive Immunity system:



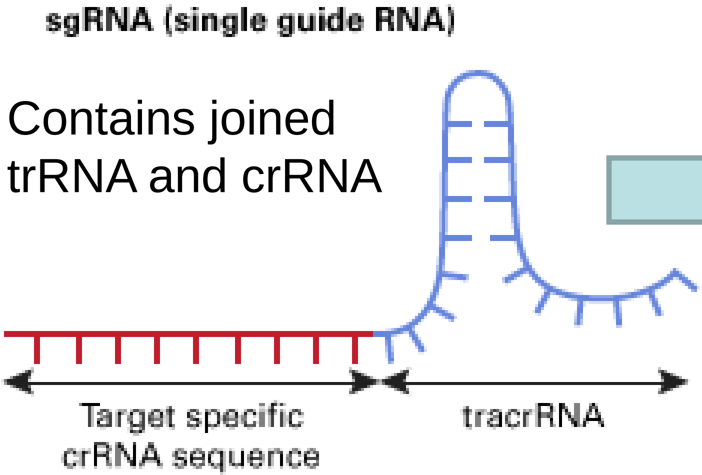
Tools: CRISPR/Cas9

Synthetic Single Guide RNA (sgRNA)

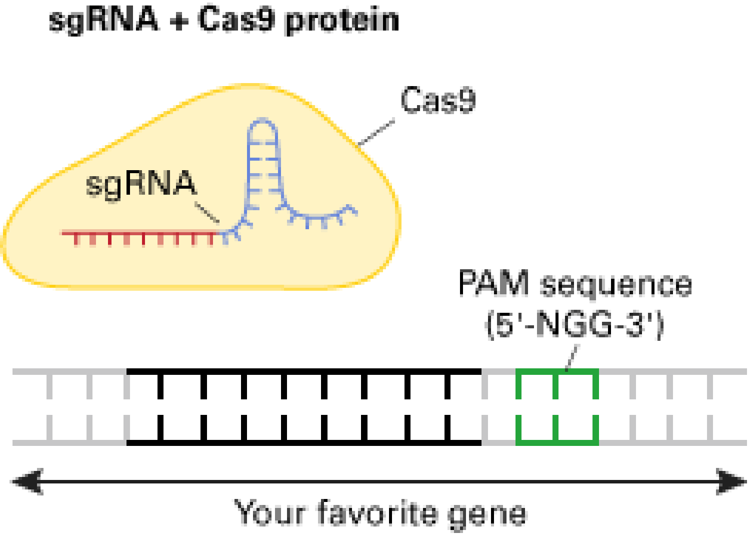
Insert your target sequence



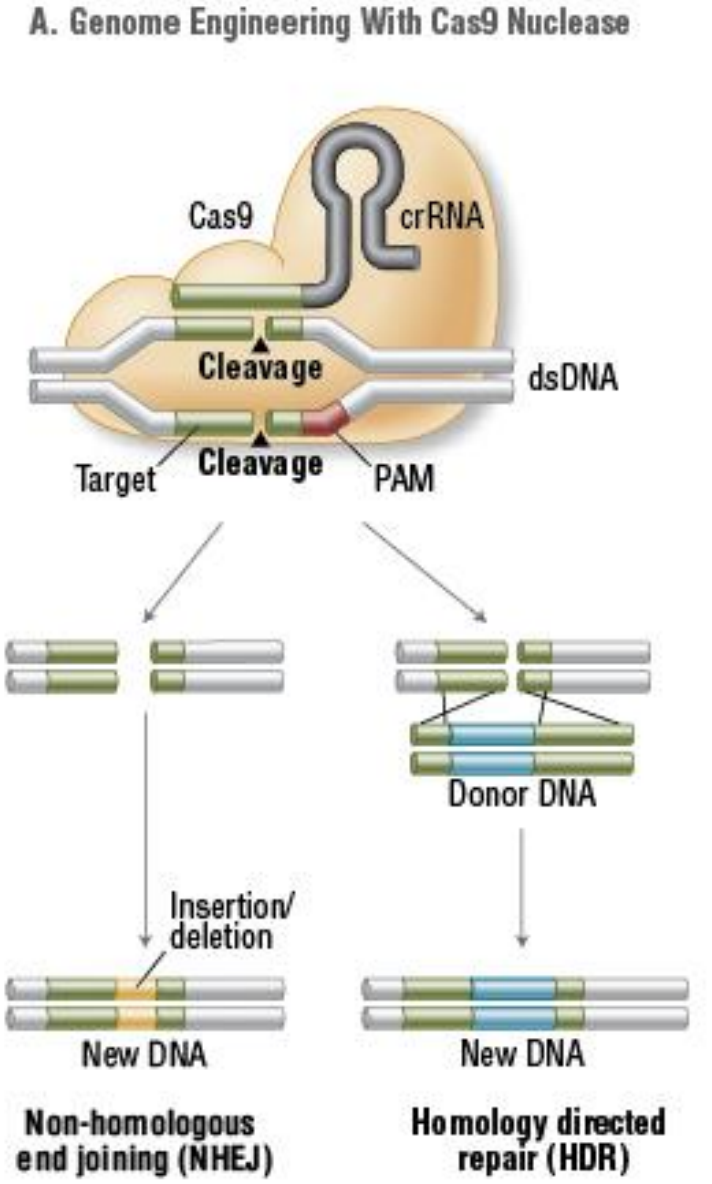
CRISPR/Cas9
Vector for sgRNA



Contains joined
trRNA and crRNA



Tools: CRISPR/Cas9

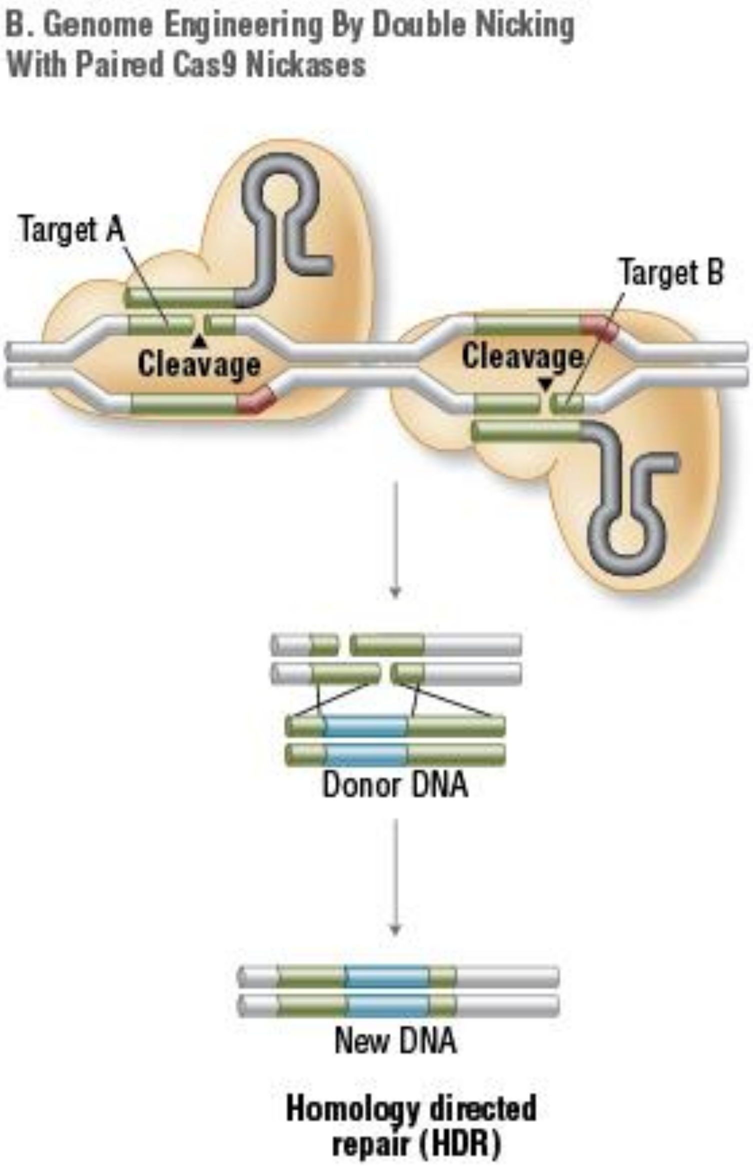


sgRNA sticks to specific location in genome that you've picked and crRNA arm recruits Cas9

Cas9 causes Double stranded break and activates Non-Homologous End Joining (NHEJ) pathway that can lead to insertions or deletions

If a homologous template is provided then Homology directed repair (HDR) can also take place, allowing for insertions

Tools: CRISPR/Cas9D10A



Mutant version of Cas9 that only nicks DNA (causing single strand break)

Does not activate Non-Homologous End Joining (NHEJ) pathway

Homologous repair template can be used for high-fidelity HDR pathway, greatly reducing indels and giving a robust way to insert DNA into specific genome locations

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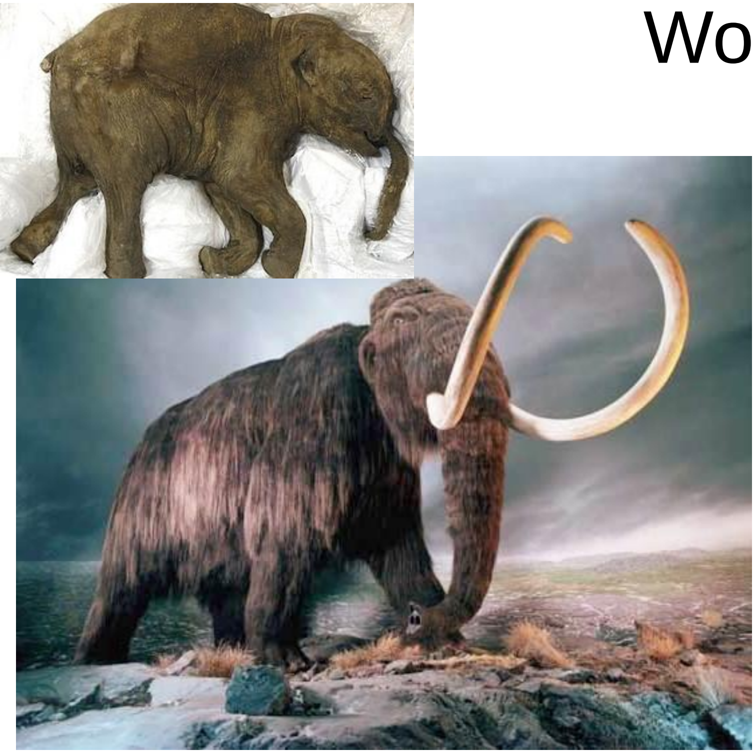
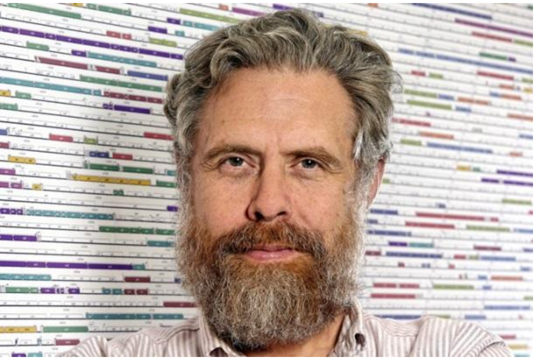
The De-Extinctionists

Wooly Mammoth



Mammuthus primigenius – small near artic elephant that went extinct 5,000 years ago. Keystone species that transformed the environment through expansion of northern grasslands. Some people want to bring it back and as a way to deal with quickly thawing tundra into carbon capturing grasslands

Other De-Extinction projects also center around re-storing lost ecosystems



George Church, Harvard

14 genes moved to Asian elephant to make cold addapted

Beth Shapiro, UC Santa Cruz

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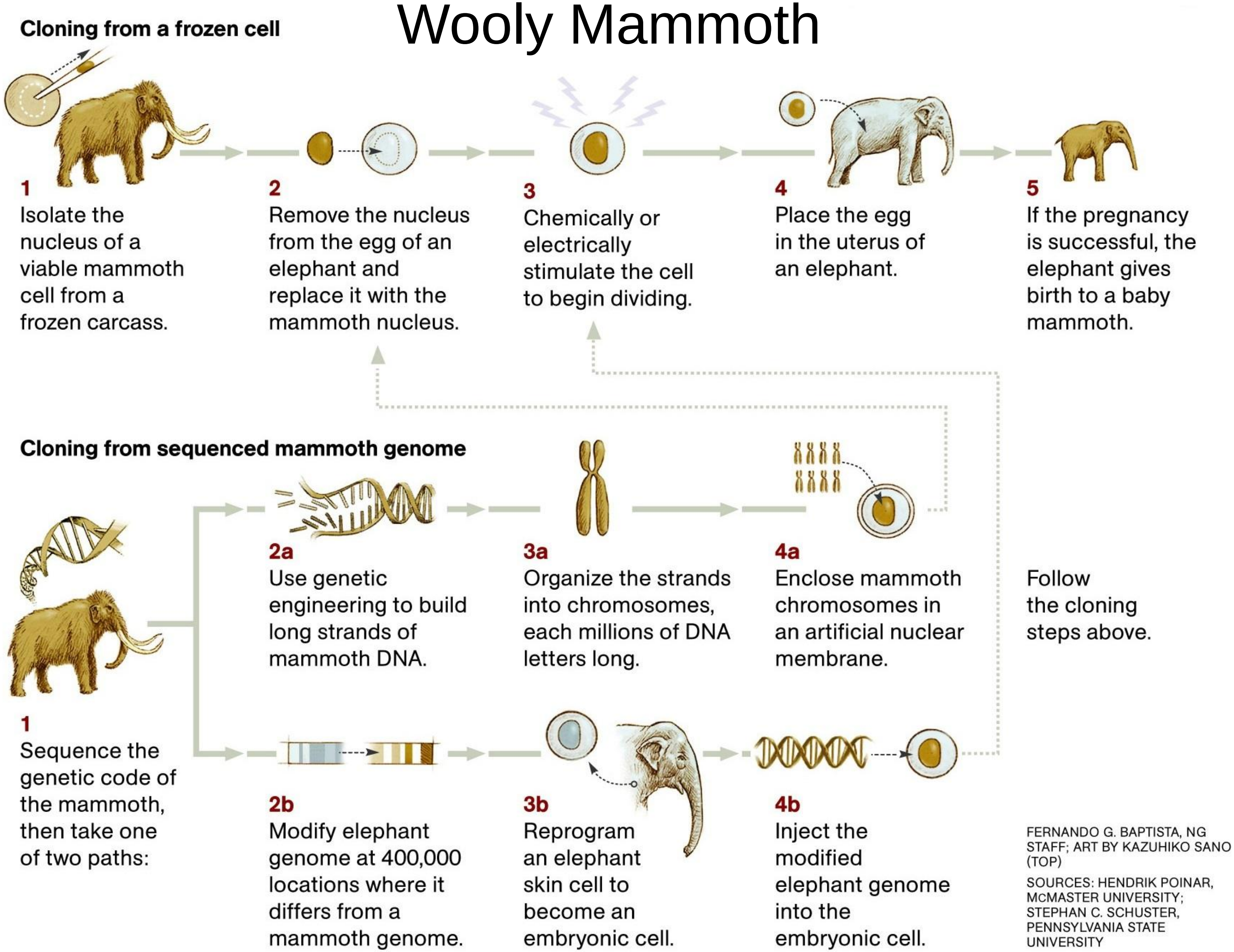
The De-Extinctionists

Wooly Mammoth



Cloning from a frozen cell

1 Isolate the nucleus of a viable mammoth cell from a frozen carcass.

2 Remove the nucleus from the egg of an elephant and replace it with the mammoth nucleus.

3 Chemically or electrically stimulate the cell to begin dividing.

4 Place the egg in the uterus of an elephant.

5 If the pregnancy is successful, the elephant gives birth to a baby mammoth.

Cloning from sequenced mammoth genome

1 Sequence the genetic code of the mammoth, then take one of two paths:

2a Use genetic engineering to build long strands of mammoth DNA.

3a Organize the strands into chromosomes, each millions of DNA letters long.

4a Enclose mammoth chromosomes in an artificial nuclear membrane.

2b Modify elephant genome at 400,000 locations where it differs from a mammoth genome.

3b Reprogram an elephant skin cell to become an embryonic cell.

4b Inject the modified elephant genome into the embryonic cell.

Follow the cloning steps above.

FERNANDO G. BAPTISTA, NG STAFF; ART BY KAZUHIKO SANO (TOP)

SOURCES: HENDRIK POINAR, MCMASTER UNIVERSITY; STEPHAN C. SCHUSTER, PENNSYLVANIA STATE UNIVERSITY

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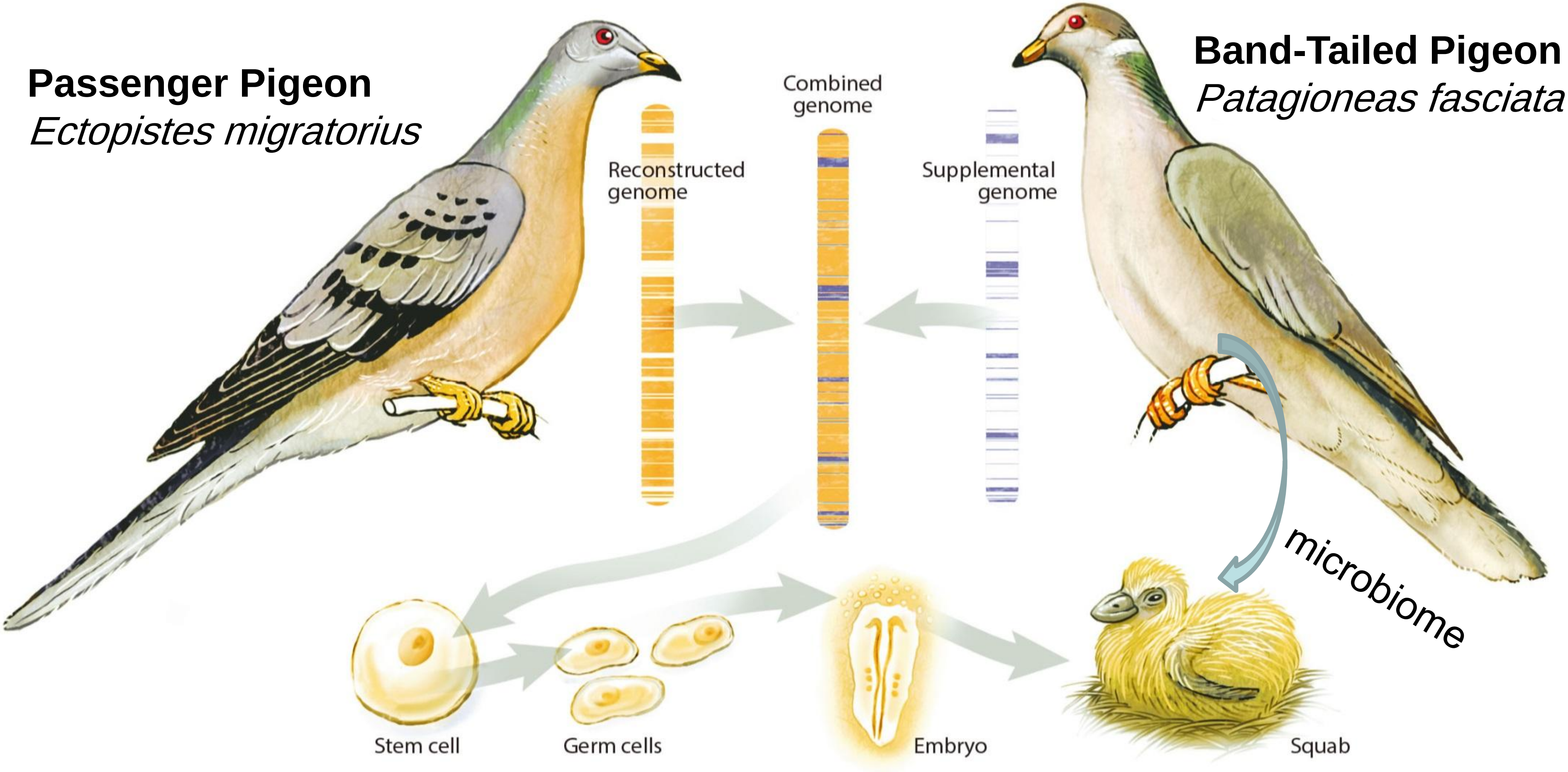
The De-Extinctionists

Passenger Pigeon

Passenger Pigeon
Ectopistes migratorius

Band-Tailed Pigeon
Patagioneas fasciata



Patterson Clark/the Washington Post

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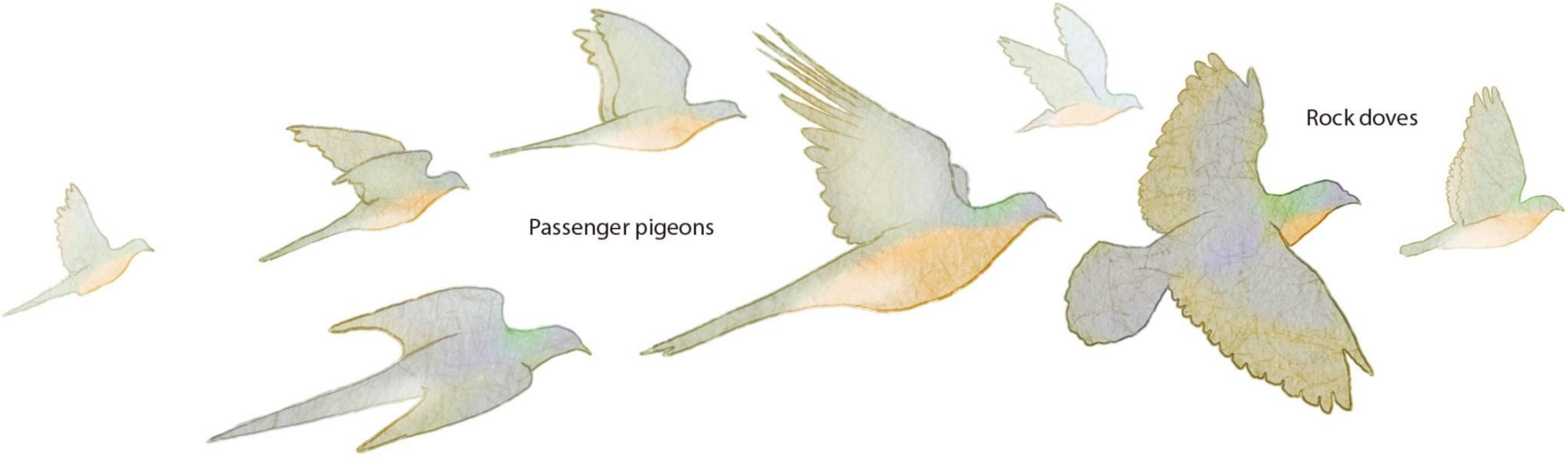
The De-Extinctionists

Passenger Pigeon

Passenger Pigeon v2.0 would learn migratory routes from trained rock doves



Patterson Clark/the Washington Post

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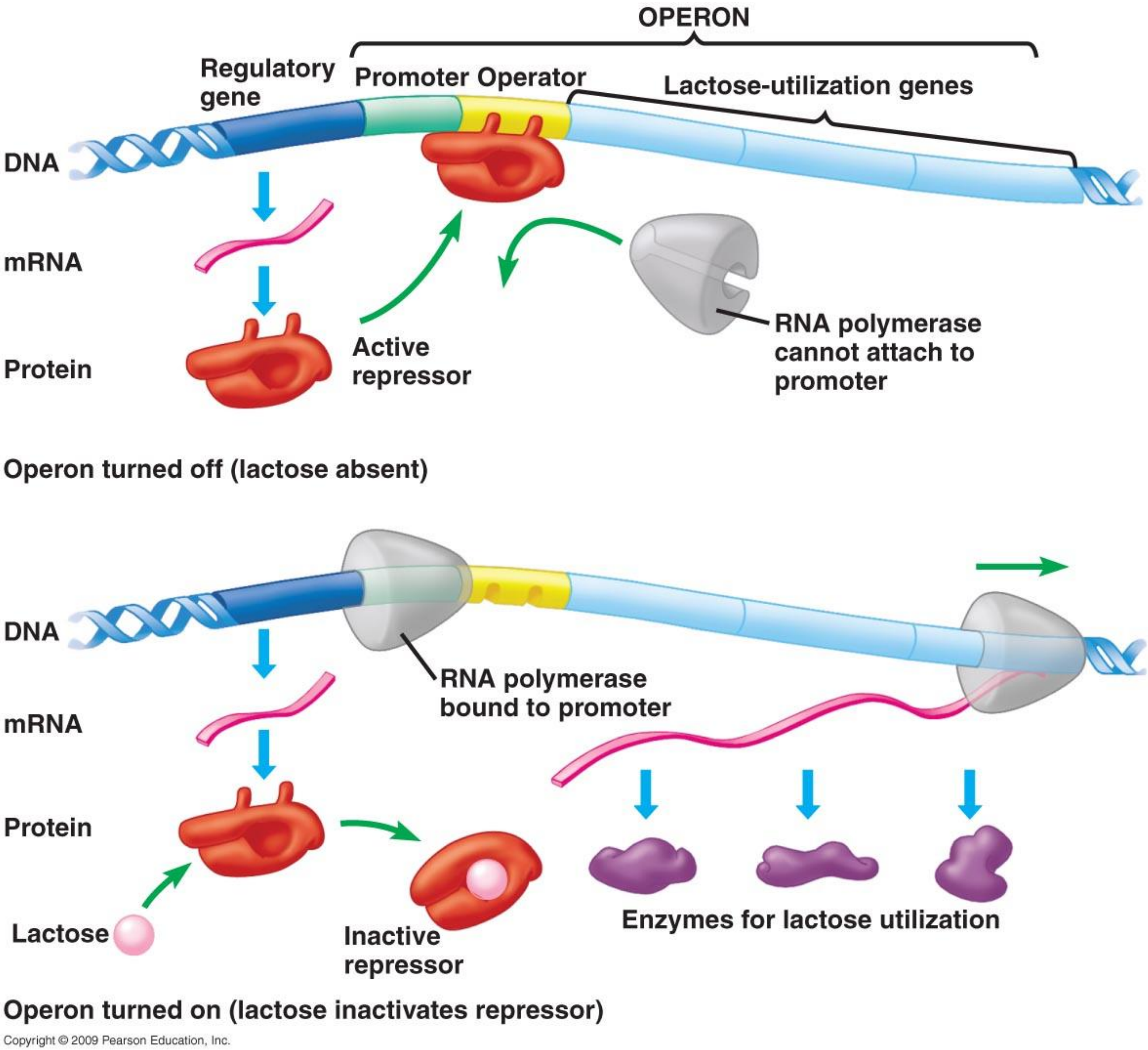
Circuits

Lac Operon, the classic regulatory circuit



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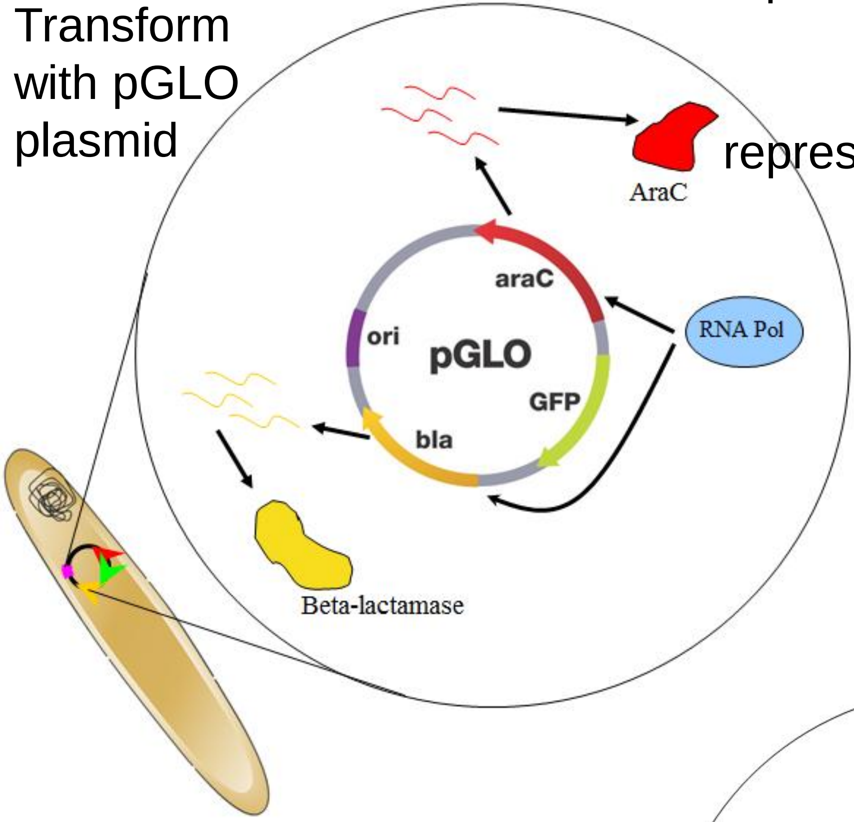
Circuits

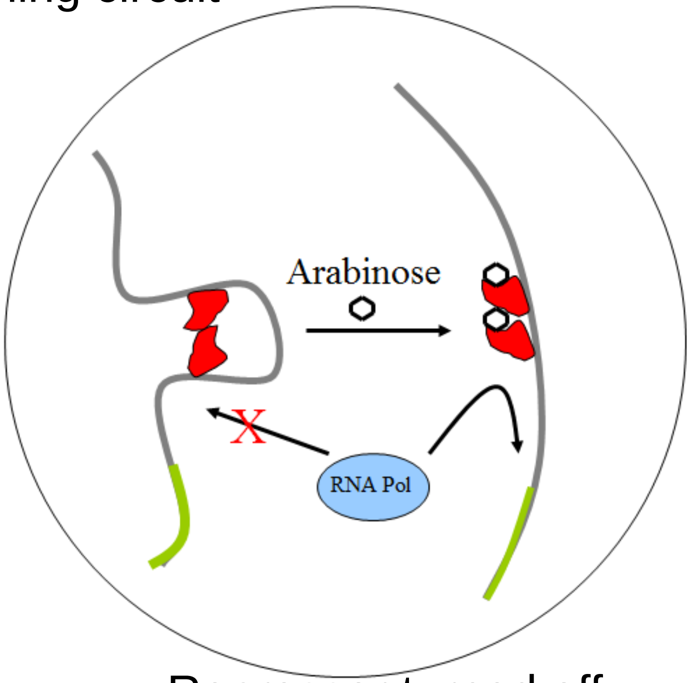
Bio-Rad pGLO: teaching circuit

Transform with pGLO plasmid

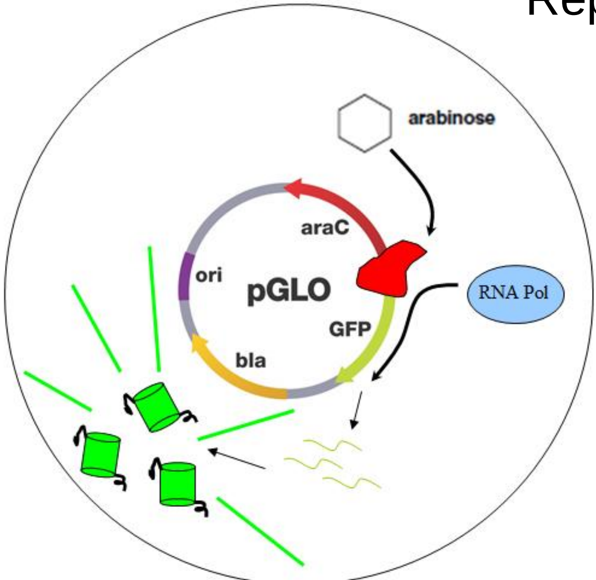


repressor

E.coli



Repressor turned off



Glowing Bacteria

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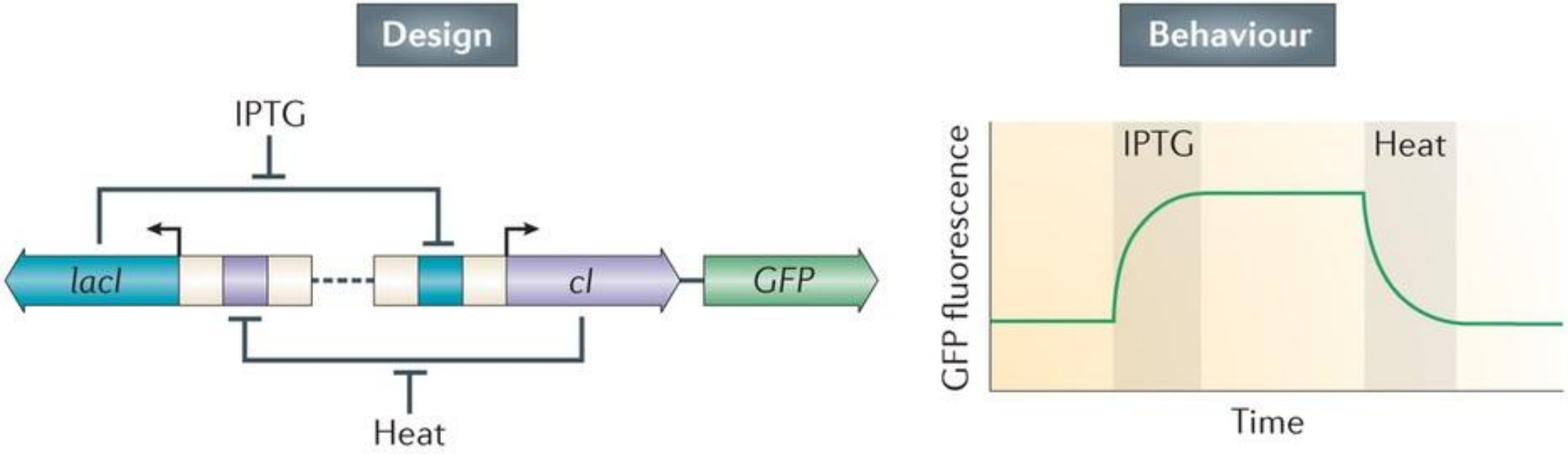
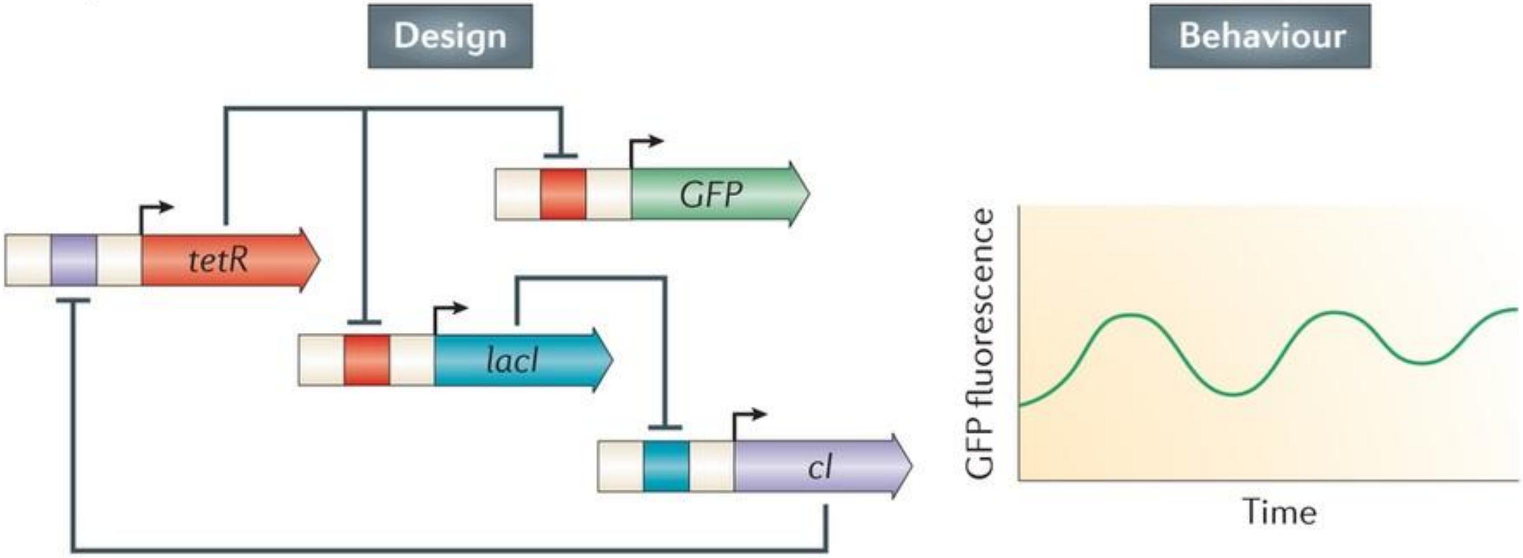
Circuits

Human designed

a Toggle switch



b Repressilator



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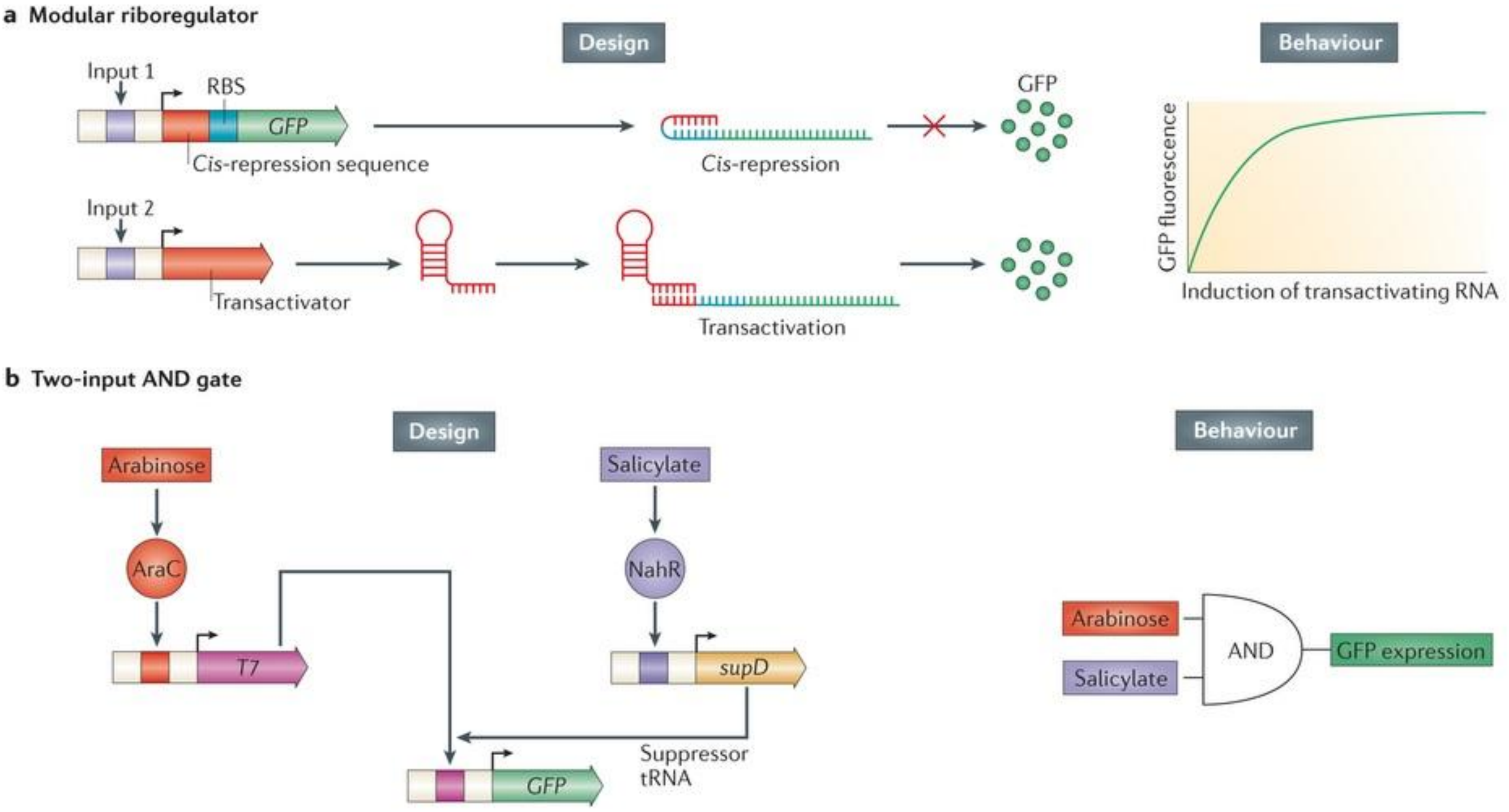
Circuits

Human designed



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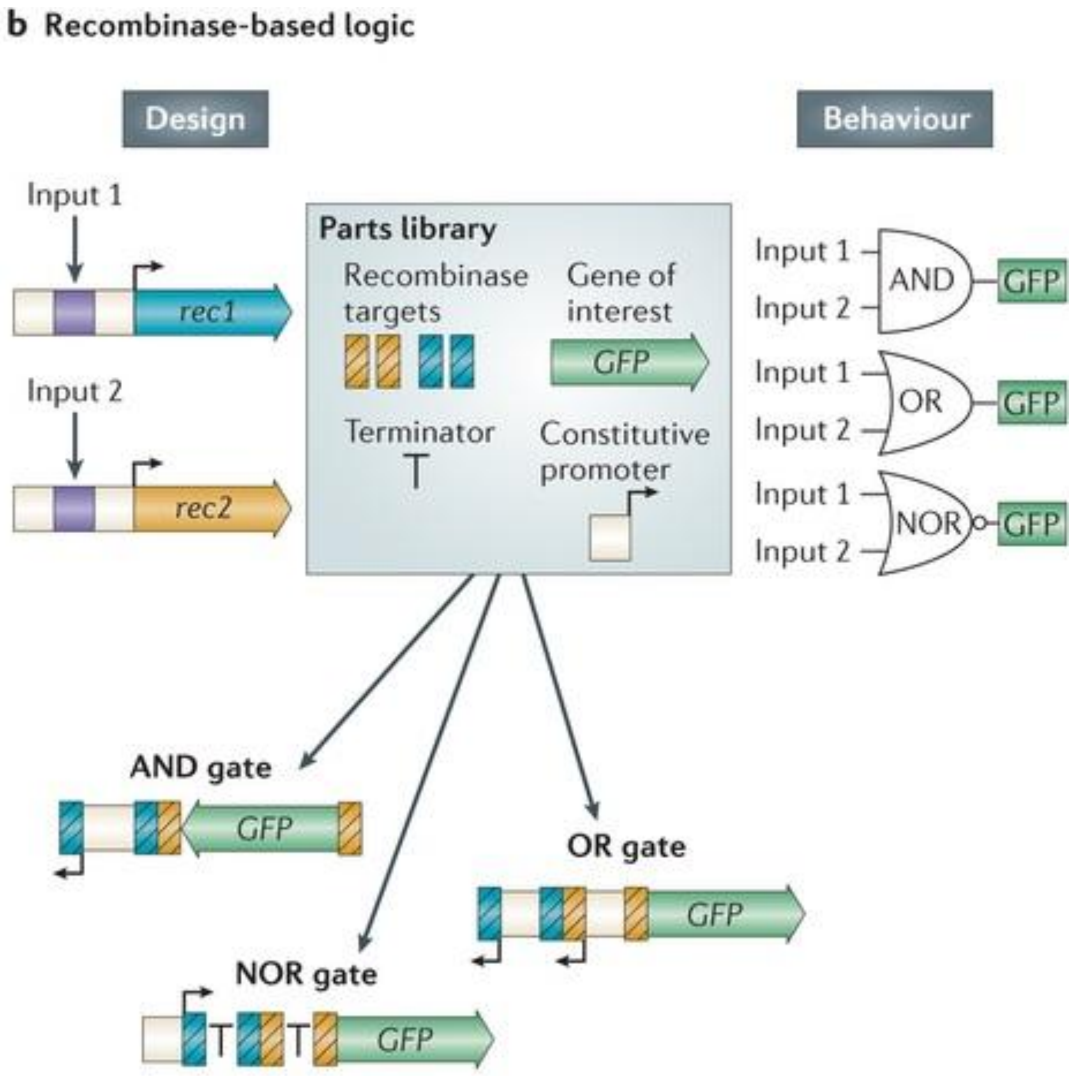
Circuits

Human designed



iGEM

INTERNATIONAL
GENETICALLY
ENGINEERED
MACHINE

Undergraduate Synthetic Biology Competition

2004 – First year, 5 teams, grew out of MIT
2015 – 280 teams, Colleges, High Schools and entrepreneurs

Teams use the Registry of Standardized Biological Parts to create a machine of their interest. Open platform.

Focus on thinking about biological systems with a high level of abstraction and applying engineering processes

Input ⇨ Device ⇨ output

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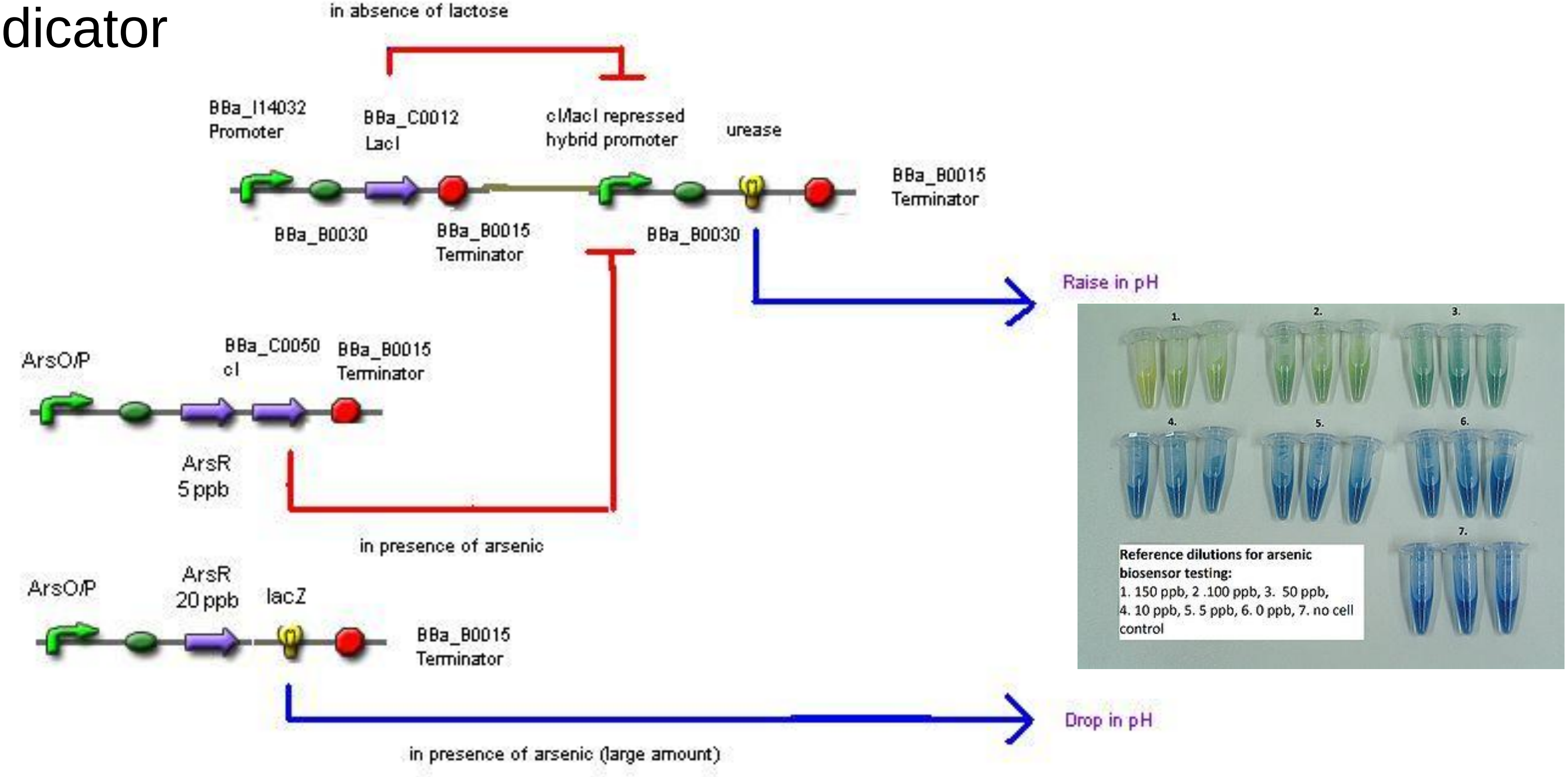
INTERNATIONAL
GENETICALLY
ENGINEERED
MACHINE

Notable projects:

Arsenic Biosensor – Arsenic concentration dependent switch regulates lactose degradation in presence of urea and pH indicator



Reference dilutions for arsenic biosensor testing:
1. 150 ppb, 2 .100 ppb, 3. 50 ppb,
4. 10 ppb, 5. 5 ppb, 6. 0 ppb, 7. no cell control

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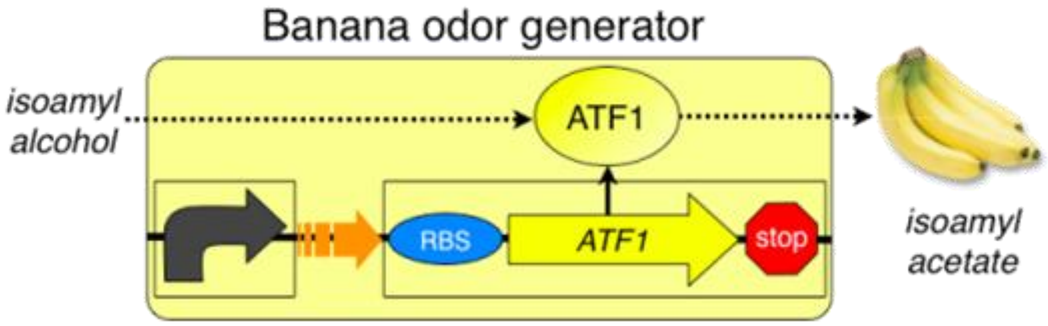
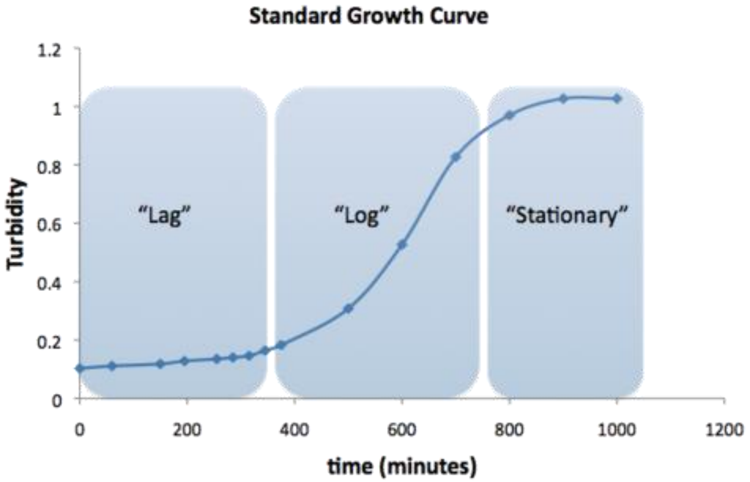
iGEM INTERNATIONAL GENETICALLY ENGINEERED MACHINE → BioBuilder

Popular iGEM projects have been bundled for teaching with BioBuilder





Sample 1-1. Banana odor device
Sample 1-2. Banana odor device with an inverter between the promoter & RBS.
Sample 1-3. Banana odor device linked to the log phase promoter
Sample 1-4. A strain of *E. coli* that has no smell generating devices.

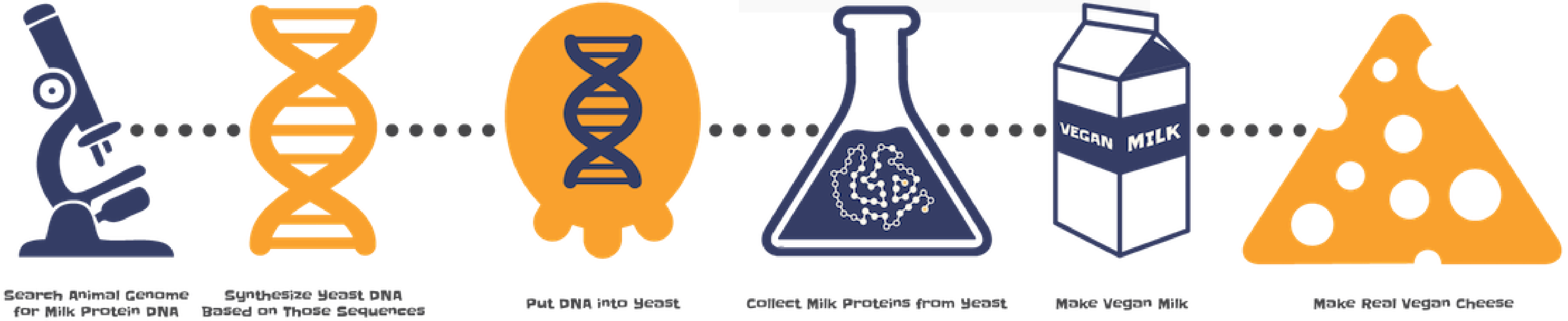
DIYBIO / BioHackers

These are spaces open to anyone in the community to use all of the tools involved in biotech / Synthetic Biology

These spaces will be at the more creative end of Synthetic Biology, but will be limited by funding.

http://diybio.org/local/

bioCURIOUS & Counter Culture Labs



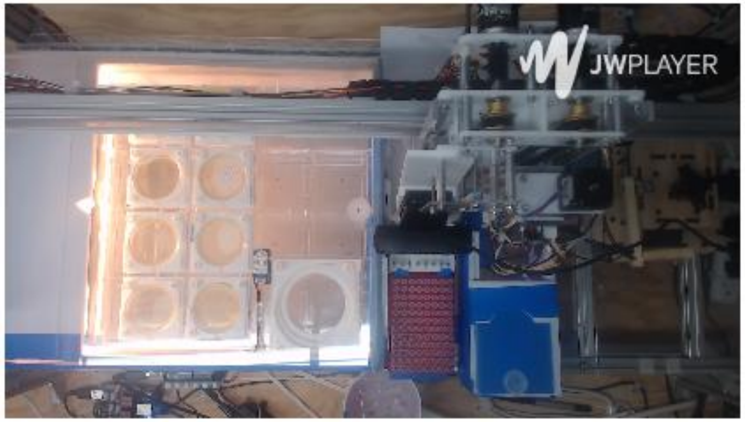
Using Synthetic Biology to make Vegan Cheese

Synthetic Bio Access and Automation

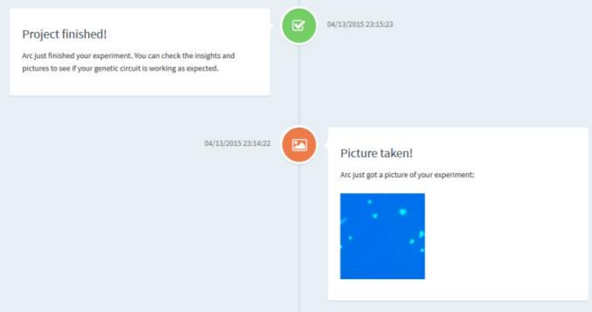
Arcturus BioCloud

@ INDIE BIO
ACCELERATING BIOLOGY

Design construct online



Automated remote construction



Project finished!

Arc just finished your experiment. You can check the insights and pictures to see if your genetic circuit is working as expected.

04/13/2015 23:15:23

04/13/2015 23:14:22

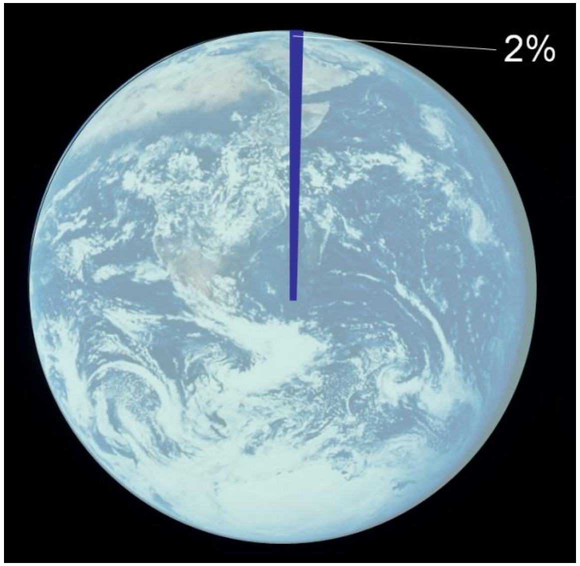
Picture taken!

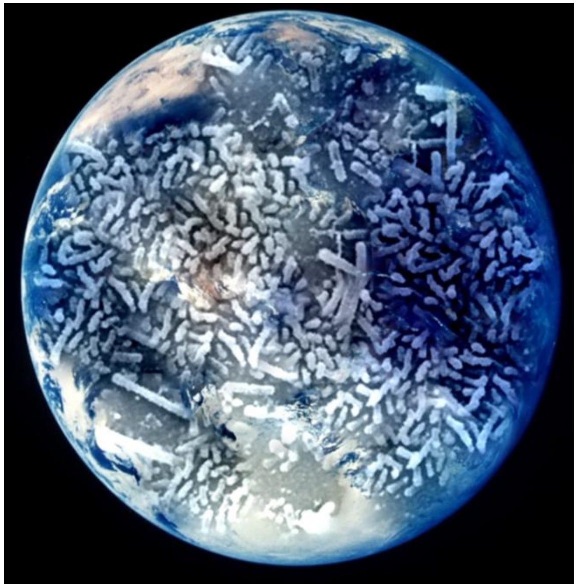
Arc just got a picture of your experiment:

Automated updates

Design Delivery

Free Code and Creativity





We have only cataloged approximately 2% of the organisms on the planet

The majority of organisms are microbes that we can’t culture and thus haven’t been able to study

DNA Sequencing technologies (metagenomic and single cell approaches) are allowing us access to these organisms for the first time

Free Code and Creativity

GGTCCTCGCGCCA
GCTTAAGACGCTAA
TCCCTAACTGCTGG
CGGAAAAGATGTGA
CAGACGCGACGGC

As we sequence more organisms and study their inner workings, we will inherit more biological parts / code and have at least the catalog to make and harness many of the processes carried out on the planet by biological life forms

Artificial Intelligence and Synthetic Biology

The interface between Synthetic Biology and Artificial Intelligence is quickly taking shape as biology is translated into formats where standard AI routines can be applied:

Synthetic Biology Open Language (SBOL)

This will lead to more programs like BioCompiler, MatchMaker etc. that will allow more AI topic areas to be applied to biological constructs.

In the far far future potentially an automated construct and analysis AI/robot could be used to find the best constructs for defined outputs.

http://web.mit.edu/jakebeal/www/Publications/AAAI-SynBio-2013.pdf

BioEthics

With every technology comes the ability to do great levels good and harm. How do we choose wisely?

Paying attention to history is a good guide:

- Eugenics
  - the tools of synthetic biology will eventually be applied to greatly extend human life and make decisions about what those lives look like
- Weaponization of old technologies
  - In the same way that we protect against the proliferation nukes, how will we handle disease codes etc?
- biological control agents
  - Often times we try to use biology via the introduction of an organism to an area to solve a problem, but usually the system is more complex then we imagined with outcomes that were unforeseen.

BioEthics

Continued Public discussion of the applications of Synthetic Biology is the best way to keep it as safe as possible

As a world we make these decisions and need to figure out how to bring everyone to the conversation