Synthetic Genomics and its Applications to Bacterial Infectious Diseases

Sanjay Vashee
JCVI

JCVI-NIAID-NIH Genomics and Bioinformatics Training Workshop
ILRI, Nairobi, Kenya
August 26-28, 2013

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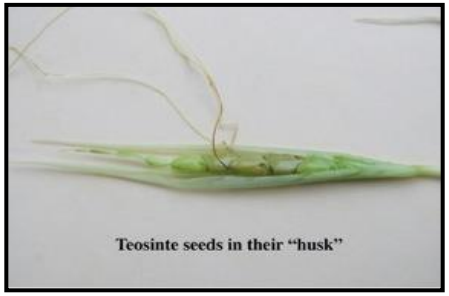
Early Synthetic Biology – Domestication of Maize



Teosinte

Natural Variation

Artificial Selection

Maize

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Early Synthetic Biology – World-Wide Domestication of Plants

Note: The pointer locations indicate general regions where crops are believed to have first been domesticated. In some cases, the center of origin is uncertain. Other geographic regions also harbor important genetic diversity for these crops.

Source: This map was developed by the General Accounting Office using data provided by the National Plant Germplasm System's Plant Exchange Office.

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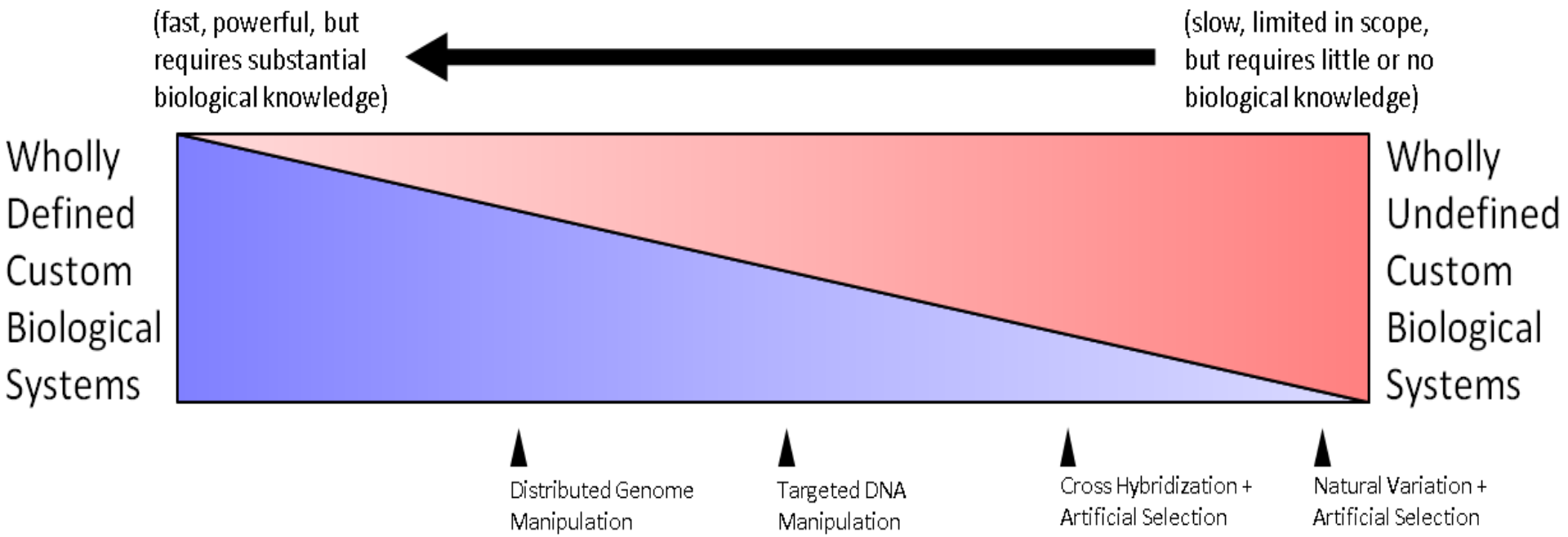
More Knowledge → Better Engineering Approach

Progress in Synthetic Biology is defined by the shifting of life-manipulation from the undefined to defined products and techniques.



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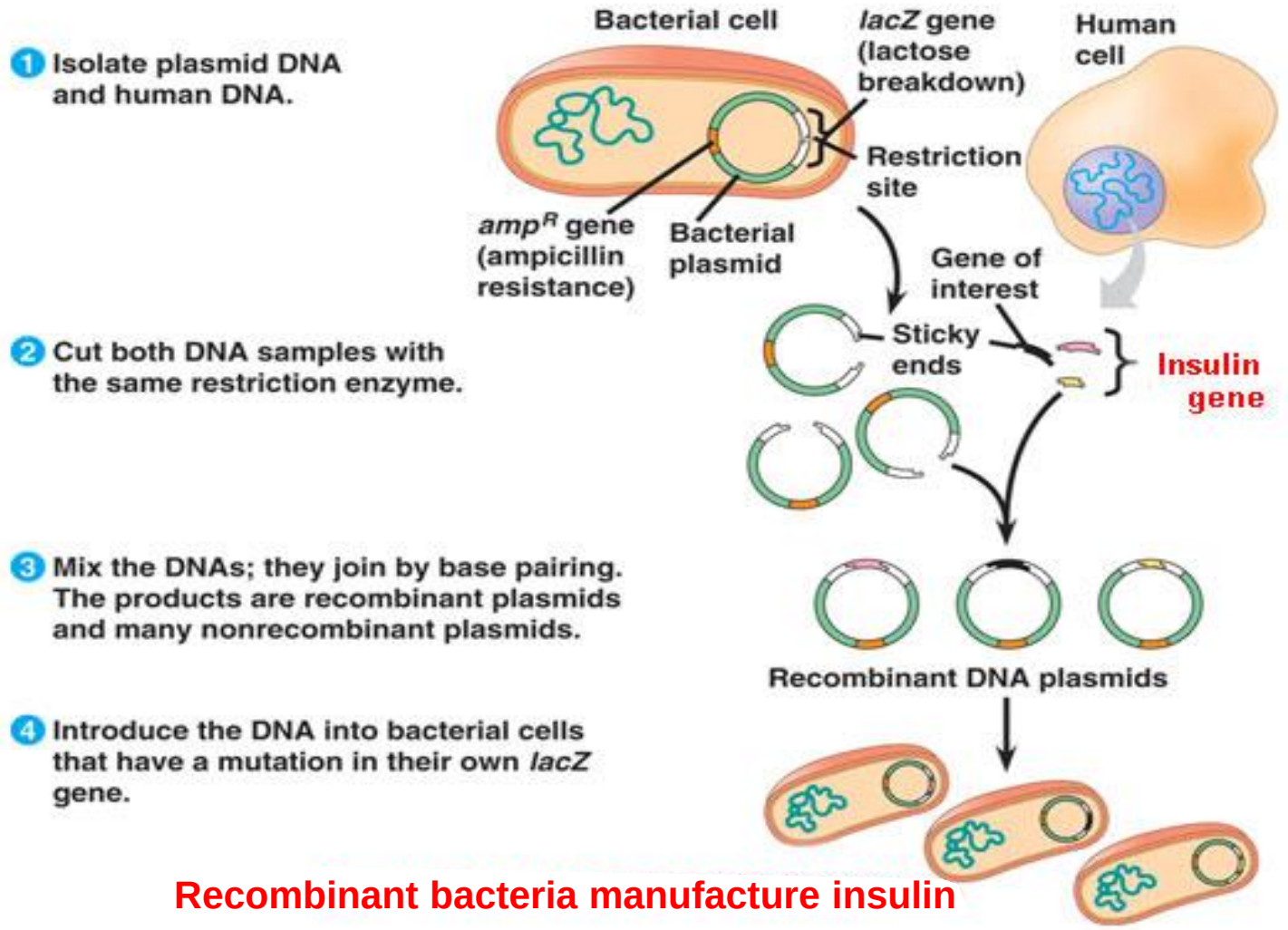
Human Insulin: Synthetic Biology's 1st drug



Recombinant bacteria manufacture insulin

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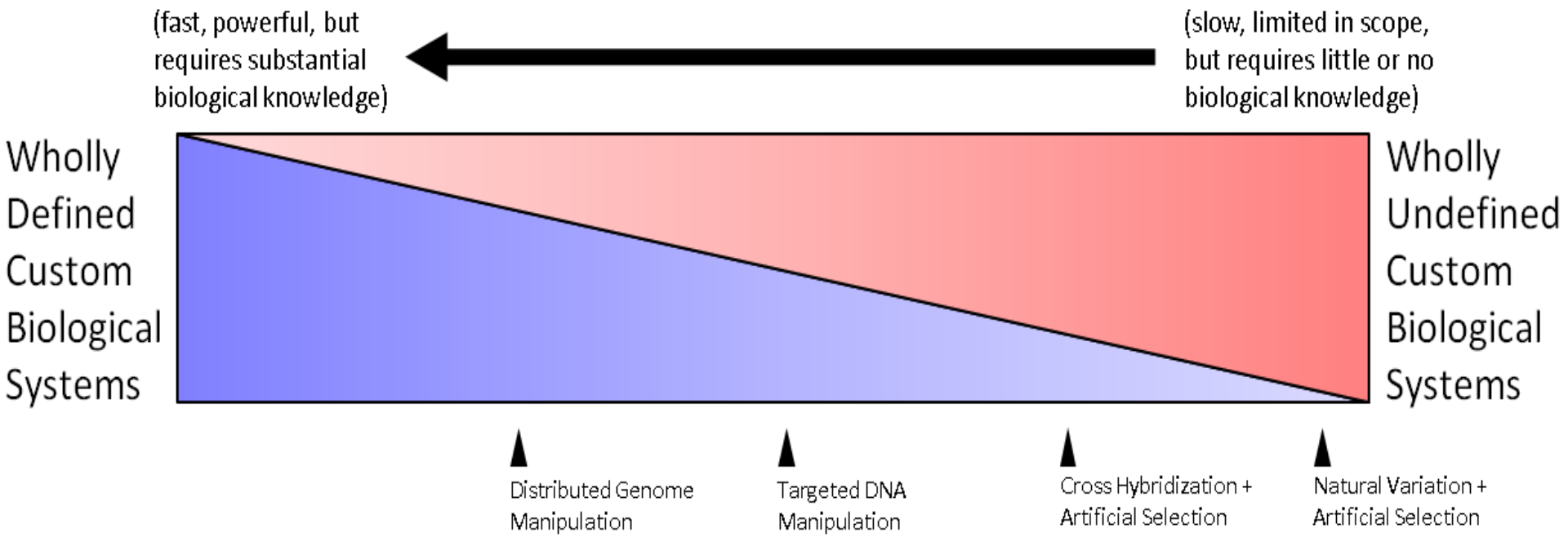

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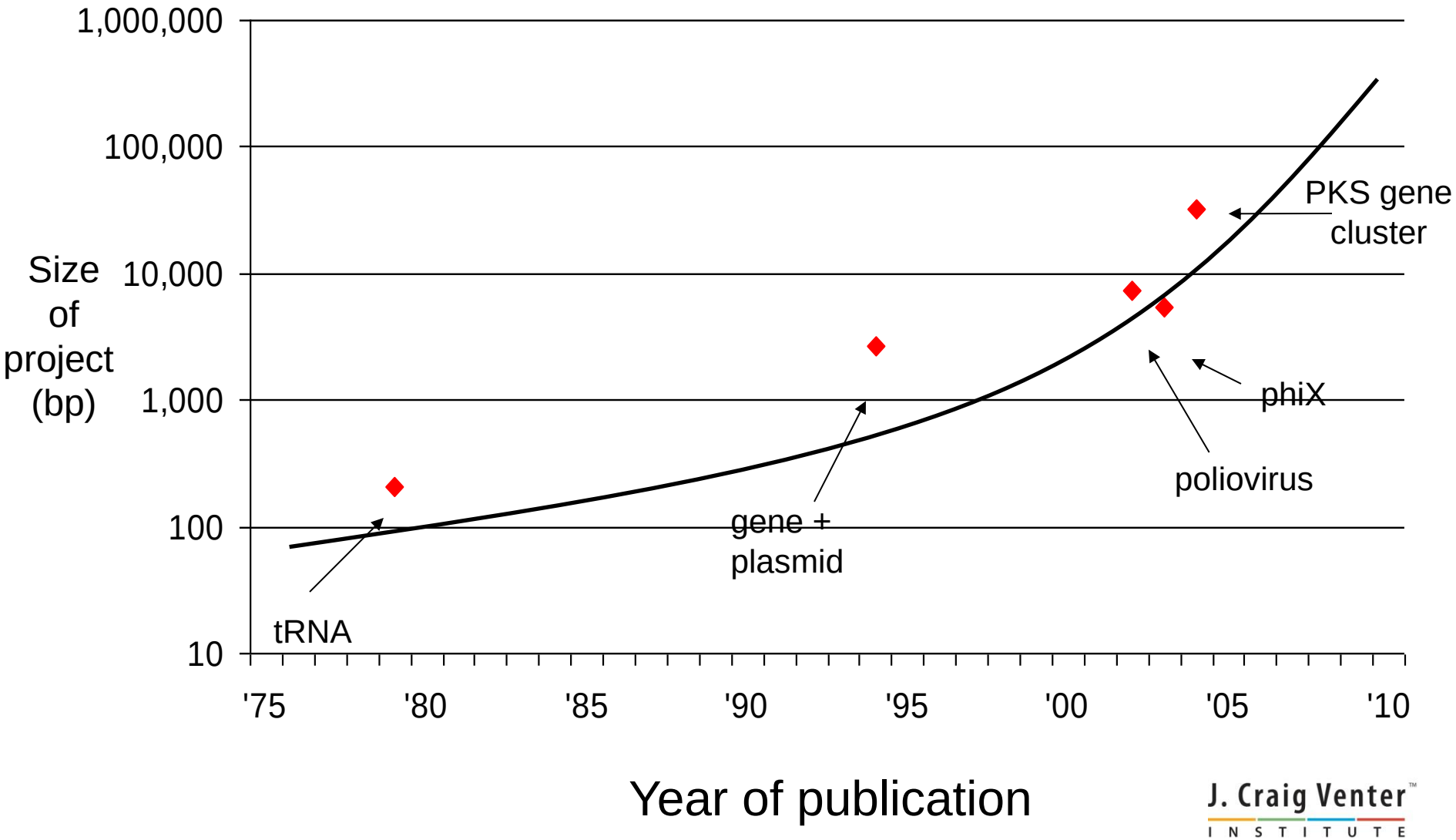
Gene Synthesis is Getting Easier, Cheaper



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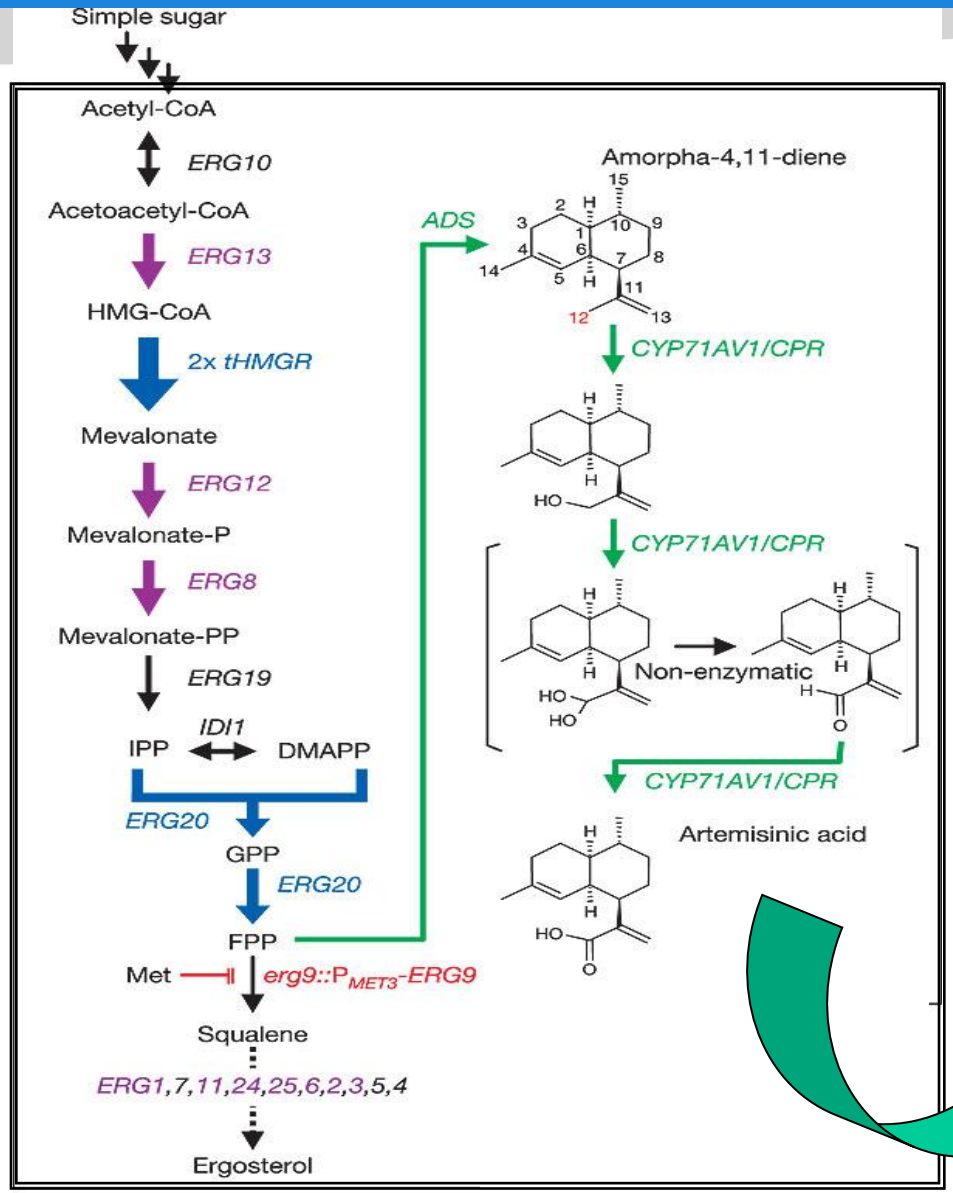
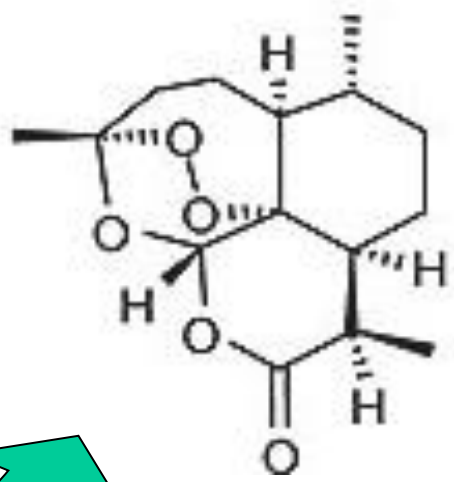
Production of Artemisinin Precursor in Yeast



"Production of the antimalarial drug precursor artemisinic acid in engineered yeast "

ZJ.D. Keasling et al. *Nature* **440**, 940-943 (13 April 2006)



Artemisinin

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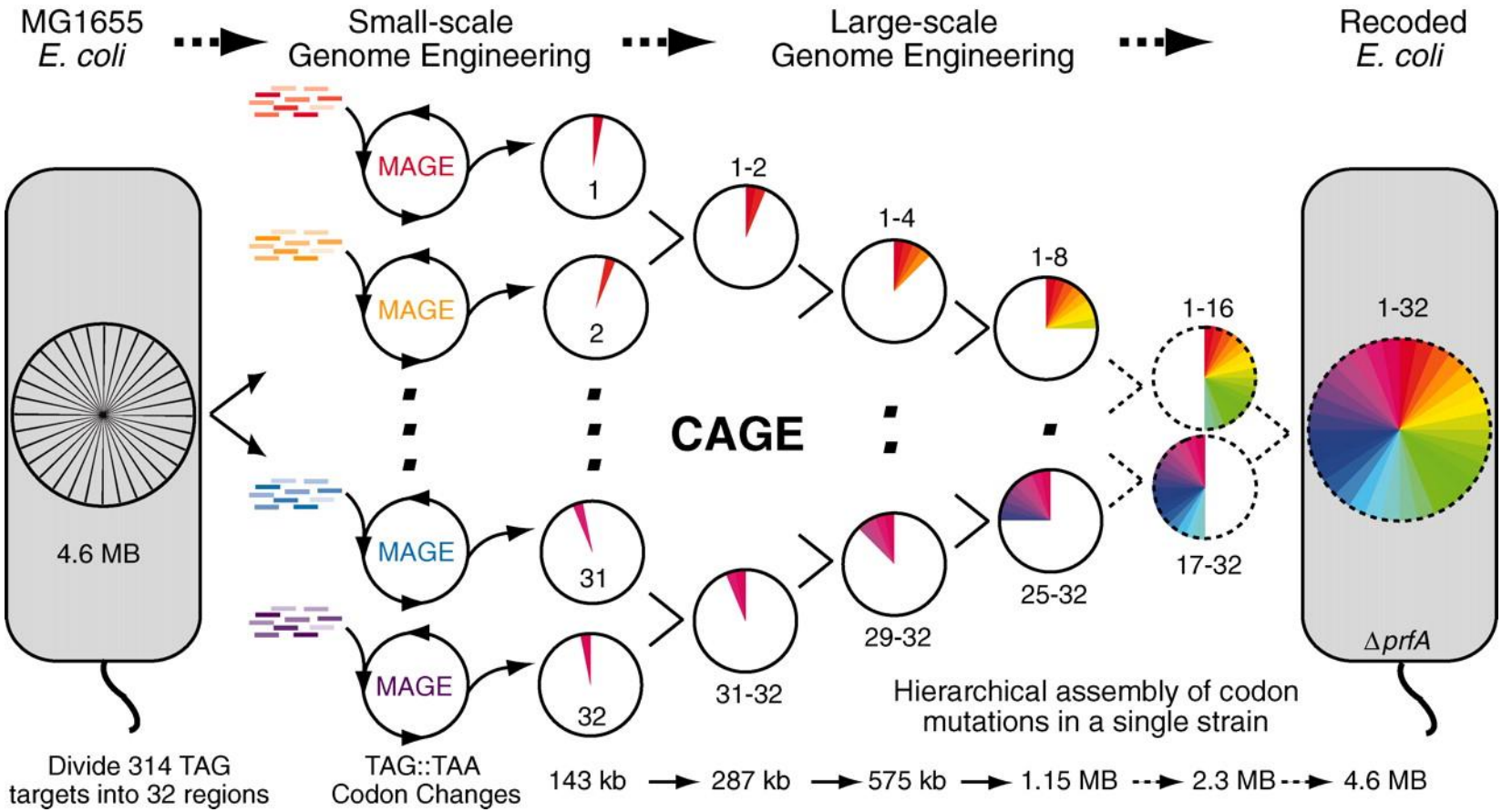
Whole Genome Engineering

Strategy for reassigning all 314 TAG codons to TAA in E. coli.



F J Isaacs et al. Science 2011;333:348-353 (Church Lab)

Science AAAS

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Application of Engineering Principles to Synthetic Biology

Tom Knight, Randy Rettberg, Drew Endy….

Construct biological systems that have medical, industrial and scientific applications via engineering principles.

- **Hierarchical Design**
- **Modular Reusable Parts**
- **Isolation of Unrelated Functions**
- **Standard Interfaces**

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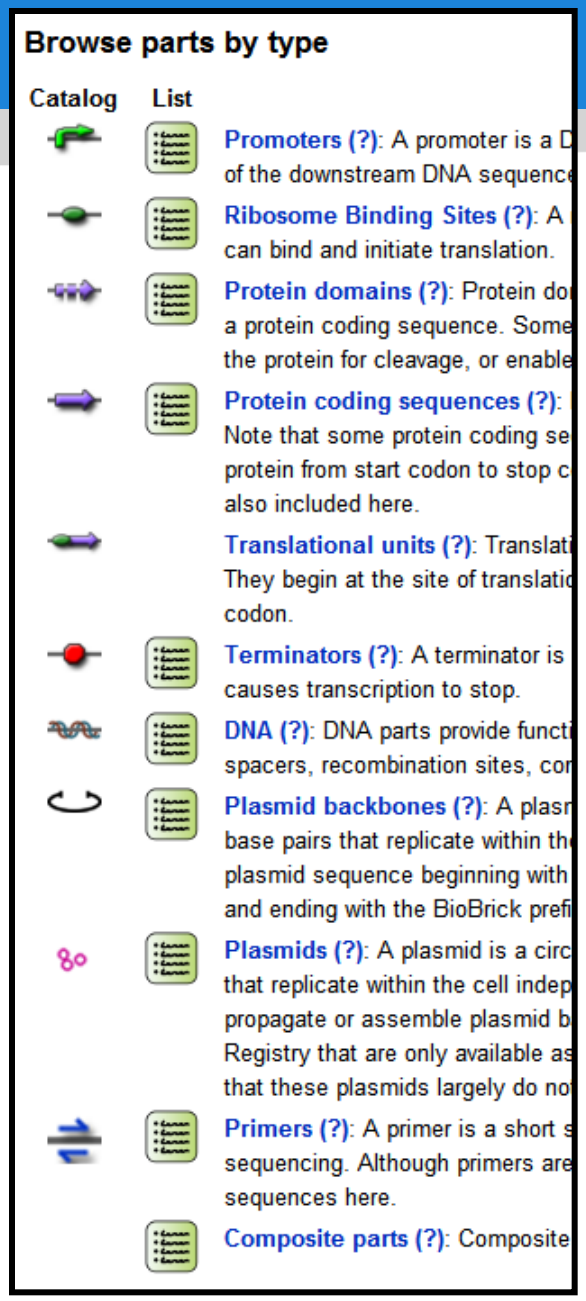
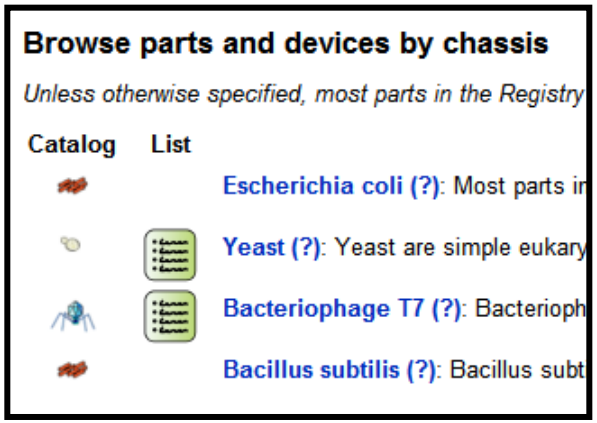
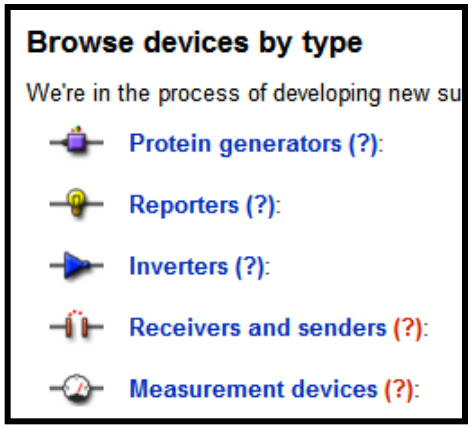
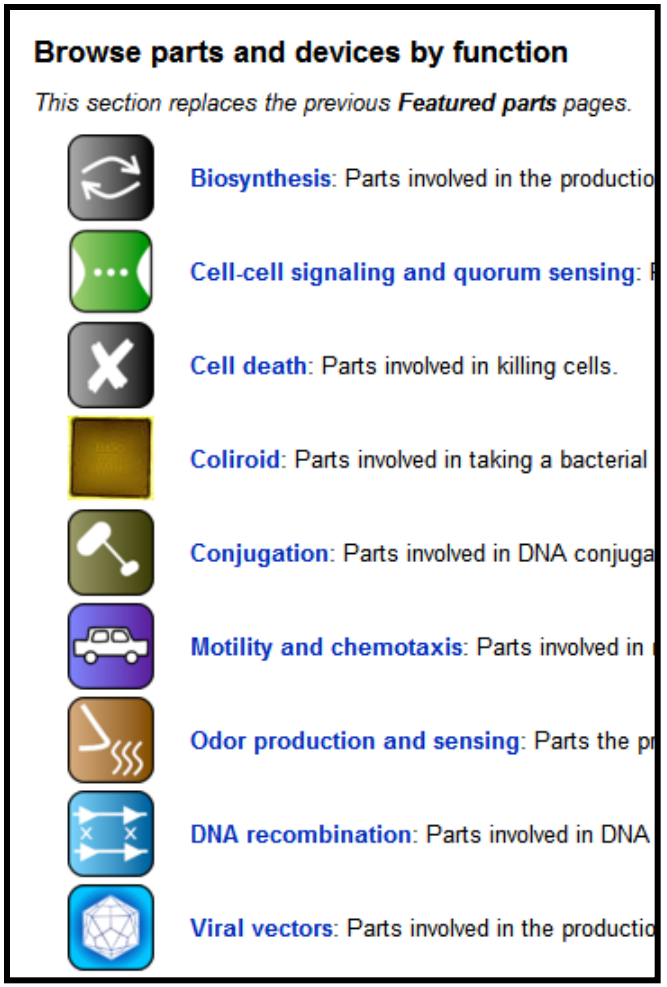
Registry of Standard Biological Parts









Tom Knight, Randy Rettberg, Drew Endy
BioBricks Foundation (http://partsregistry.org/Catalog)

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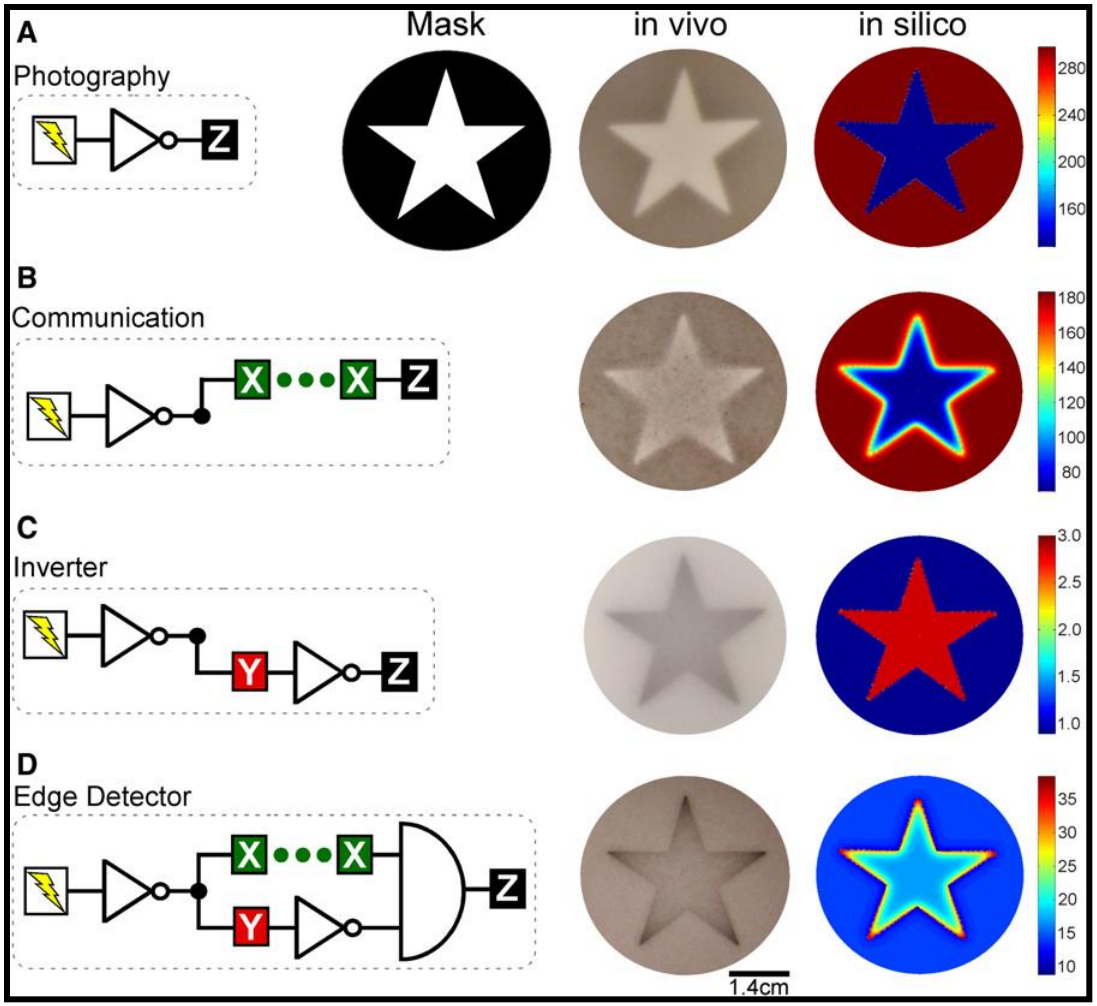
Synthetic Genetic Edge Detection



Tabor *et al.* (2009) Cell *137*, 1272-1281

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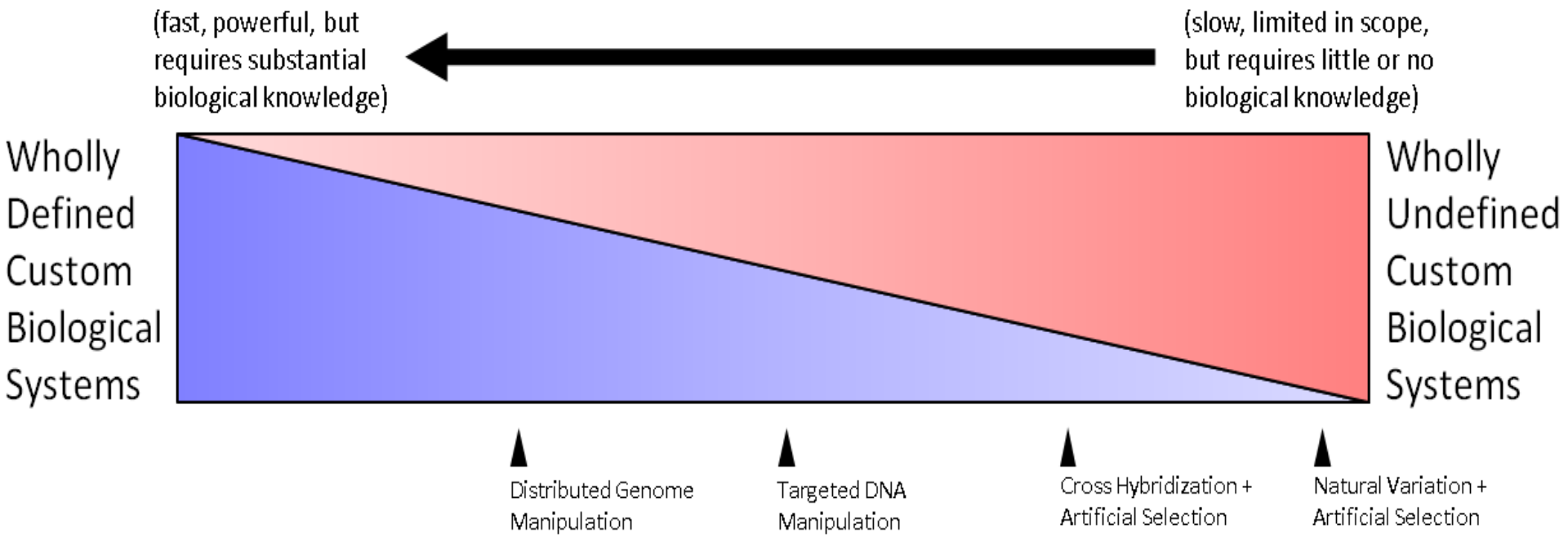

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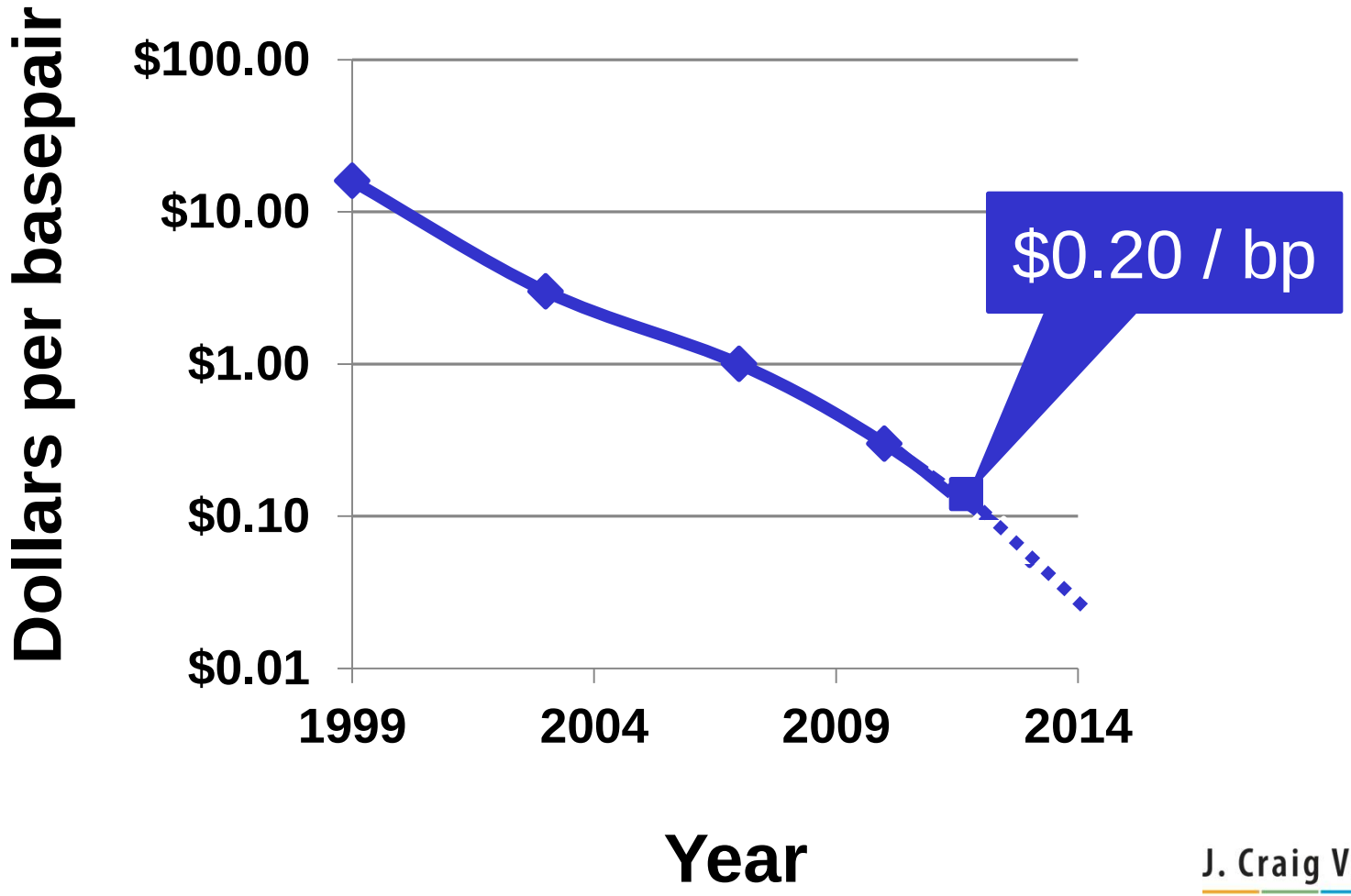
DNA synthesis is getting easier, faster, and cheaper



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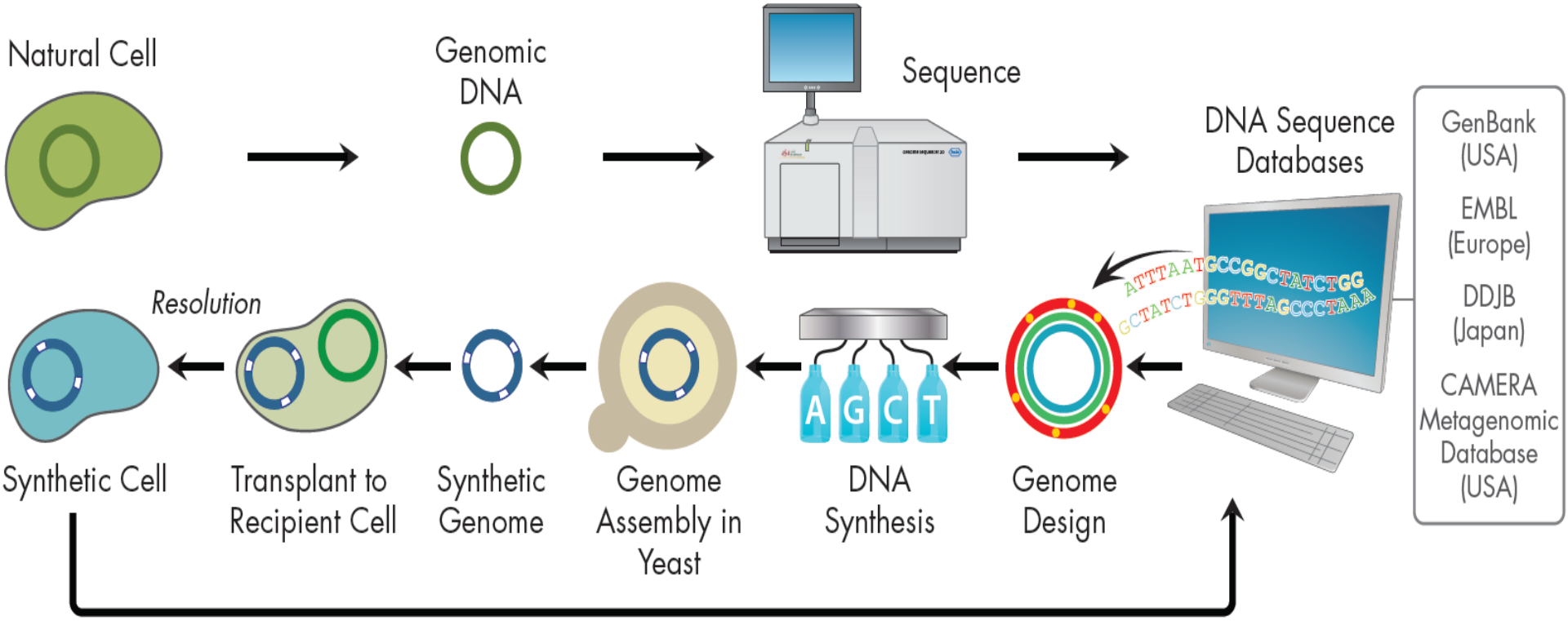
Moving Life into the Digital World and Back

Synthetic Genomics



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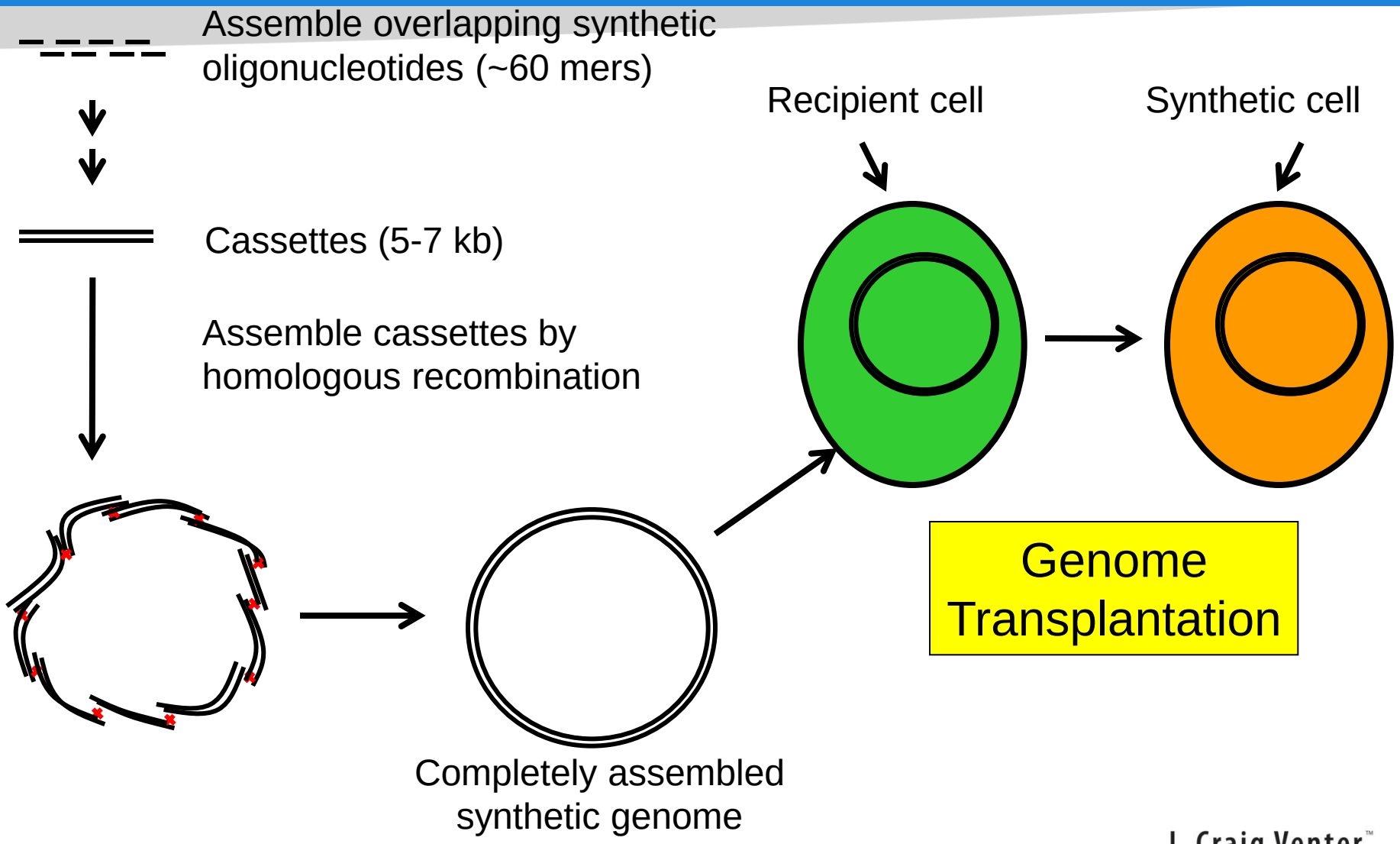
Approach Used to Create a Synthetic Cell



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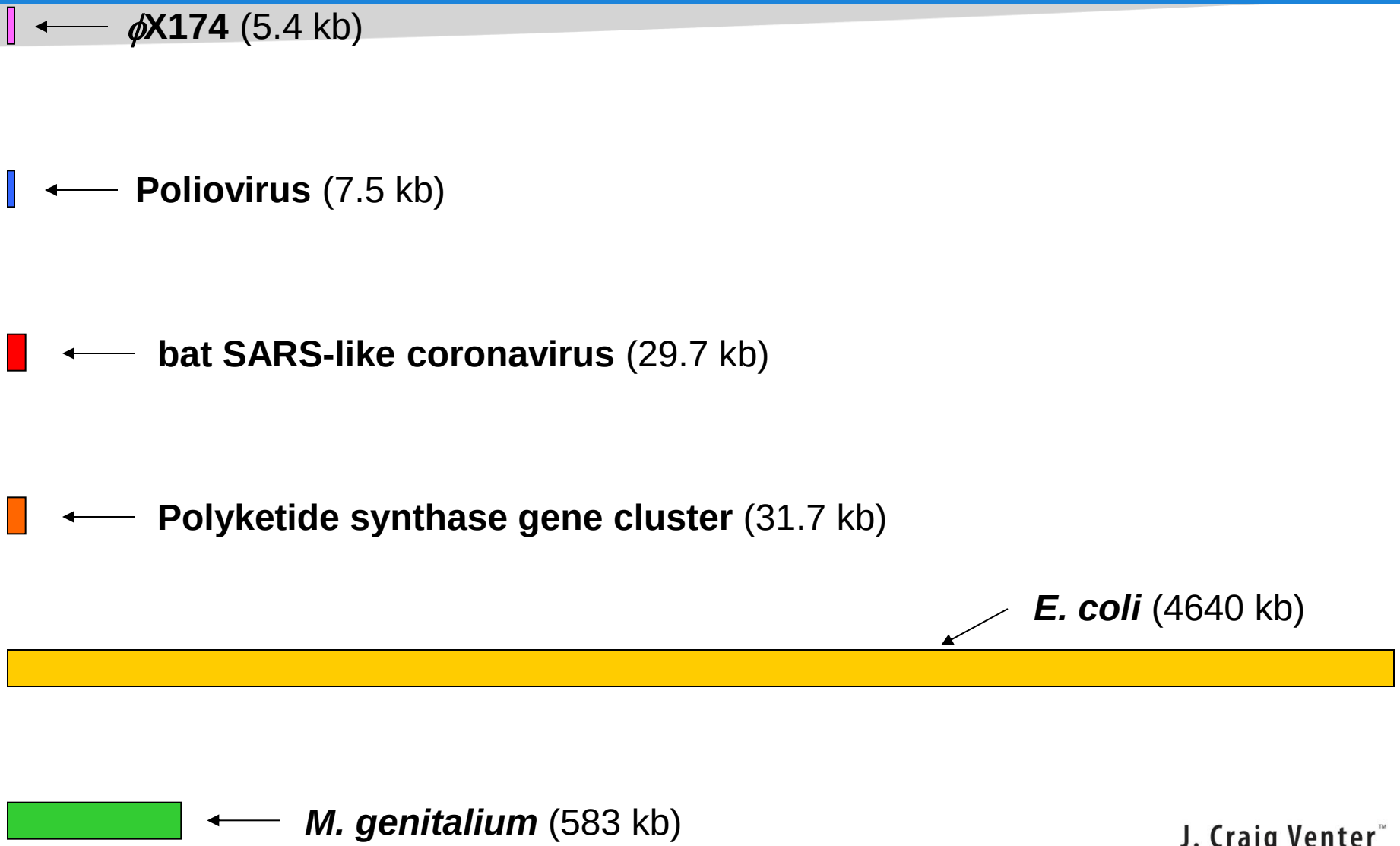
It Makes Sense to Start with a Natural Genome



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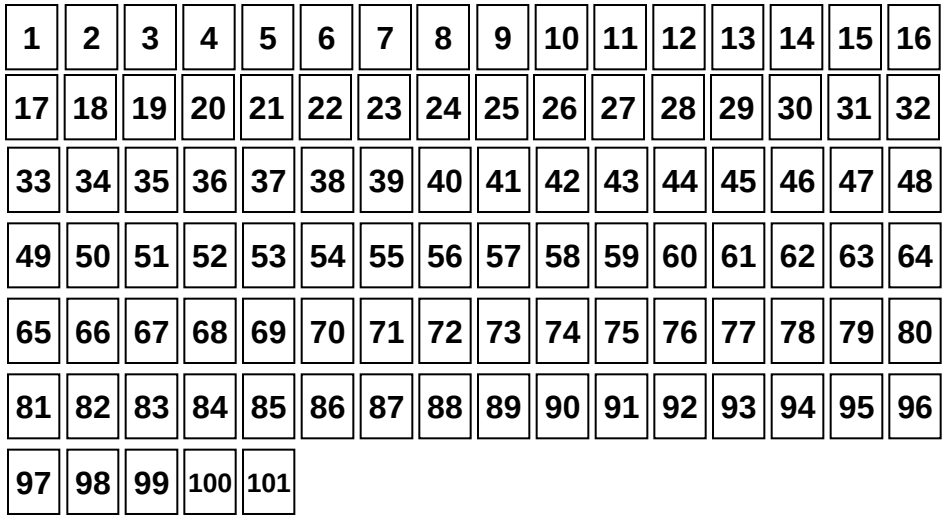
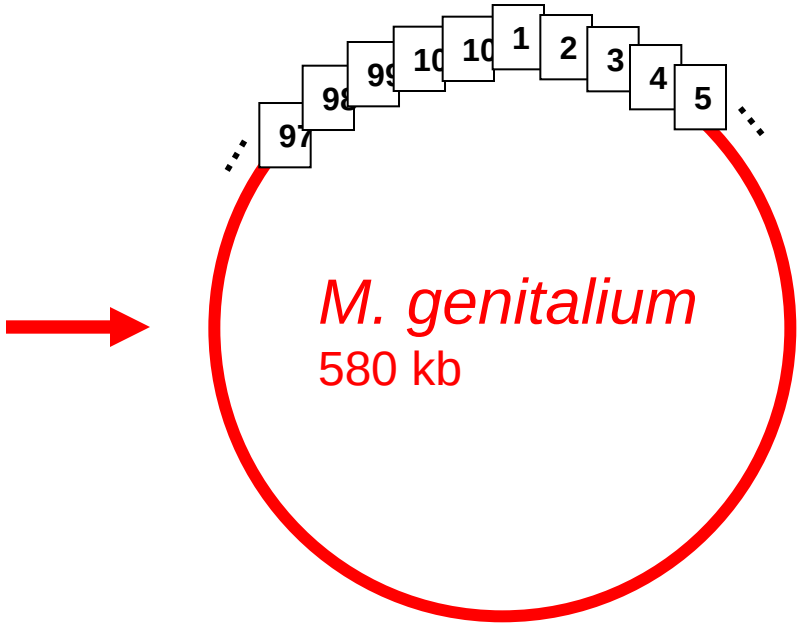
Assembly of a Synthetic *M. genitalium* Chromosome

small pieces of DNA (50 nts) → genome (580 000 bp)





Start

101 cassettes

Each ~6 kb

Made commercially

End

Complete genome

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In Vitro Genomic Assembly

Many short segments of DNA with overlapping ends

Add:
- T5 exonuclease
- Phusion DNA polymerase
- Taq Ligase
- Phusion buffer + dNTPs + PEG

Incubate 50 °C 30 minutes

One large target sequence

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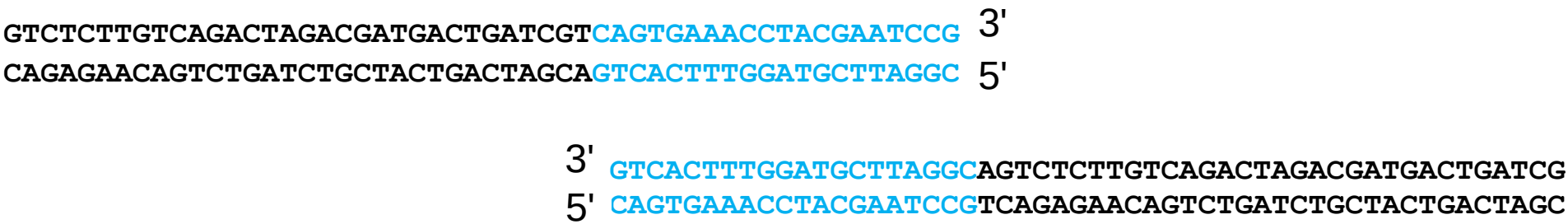
In Vitro Genomic Assembly



T5 Exonuclease Degrades 5' ends

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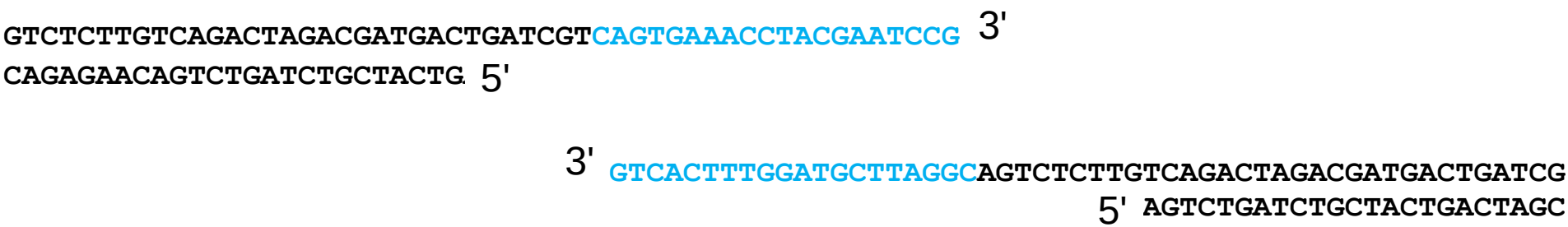
In Vitro Genomic Assembly



single-stranded 3' ends can now anneal

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In Vitro Genomic Assembly

"*The Gibson Assembly Song*"
The Cambridge iGEM Team for 2010
http://www.cambridgeigem.org
http://www.gibthon.org
http://www.youtube.com/watch?v=WCWjJFU1be8

GTCTCTTGTCAGACTAGACGATGACTGATCGTCAGTGAAACCTACGAATCCGTCAGAGAACAGTCTGATCTGCTACTGACTAGC
CAGAGAACAGTCTGATCTGCTACTGACTAGCAGTCACTTTGGATGCTTAGGCAGTCTCTTGTCAGACTAGACGATGACTGATCG

Phusion DNA polymerase extends the 3' ends to fill in the single stranded region.

Taq Ligase closes the remaining knicks.

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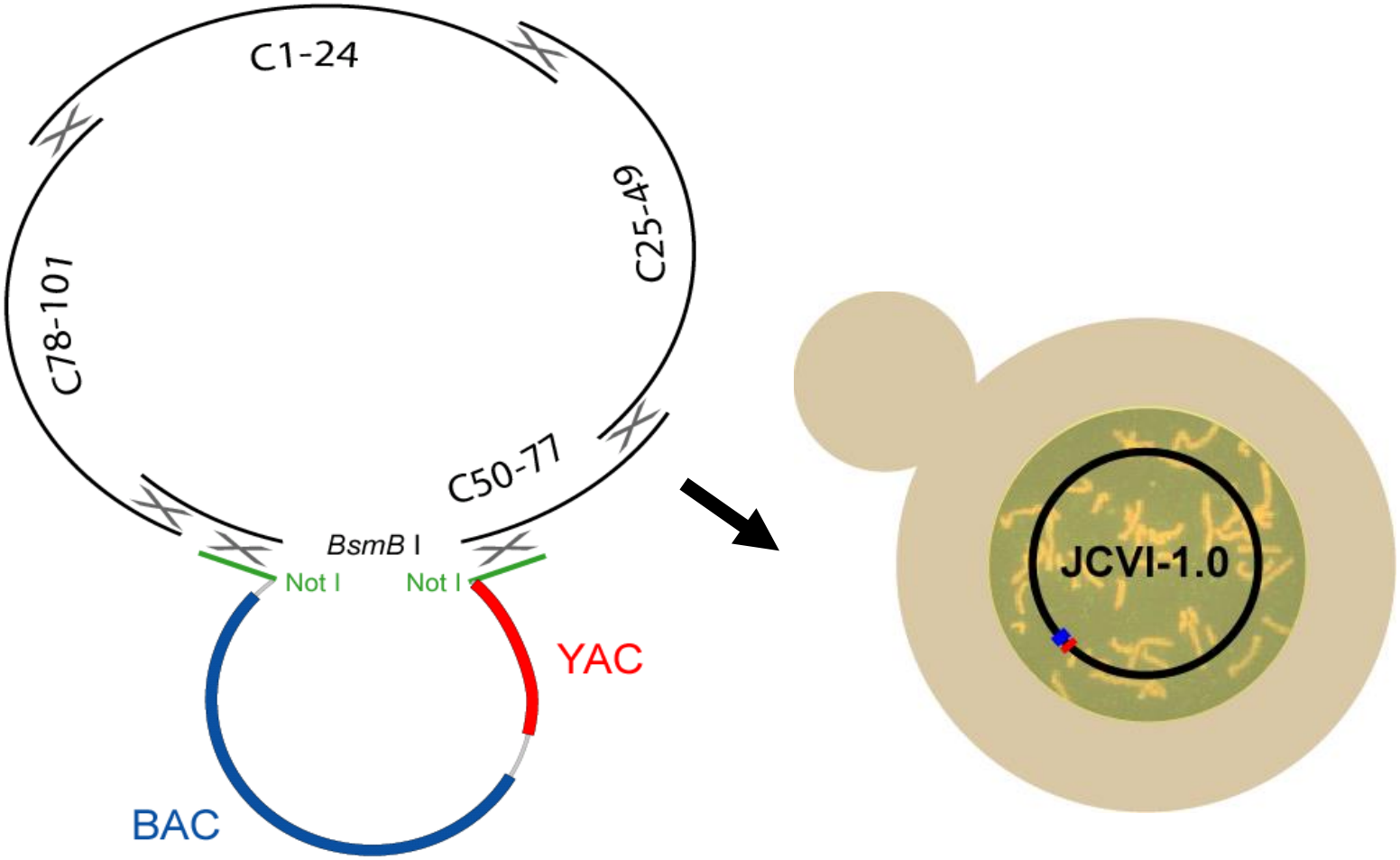
In Vivo Genomic Assembly

TAR Cloning in Yeast (Larionov, NIH)



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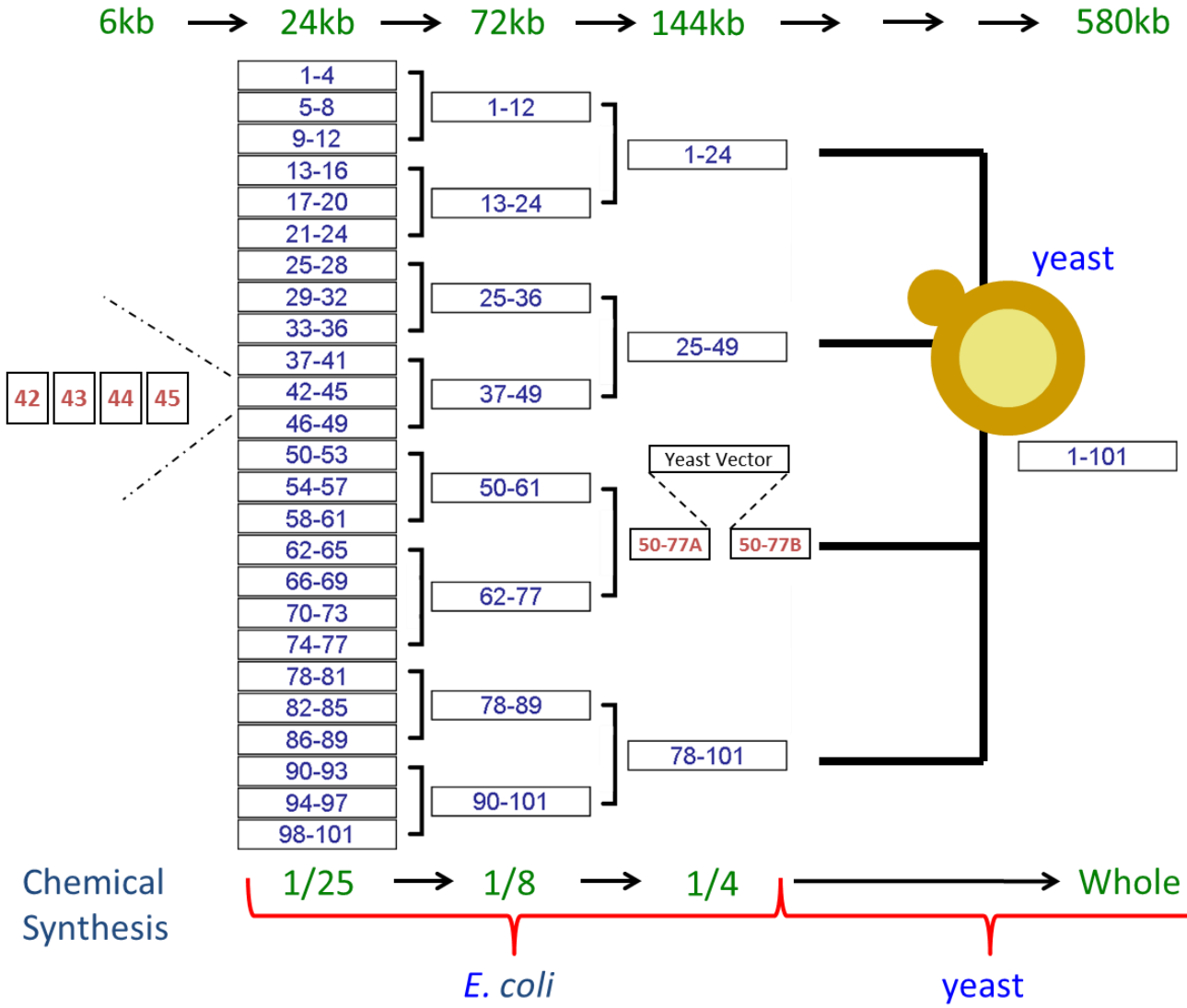
Assembly of *M.genitalium Genome*



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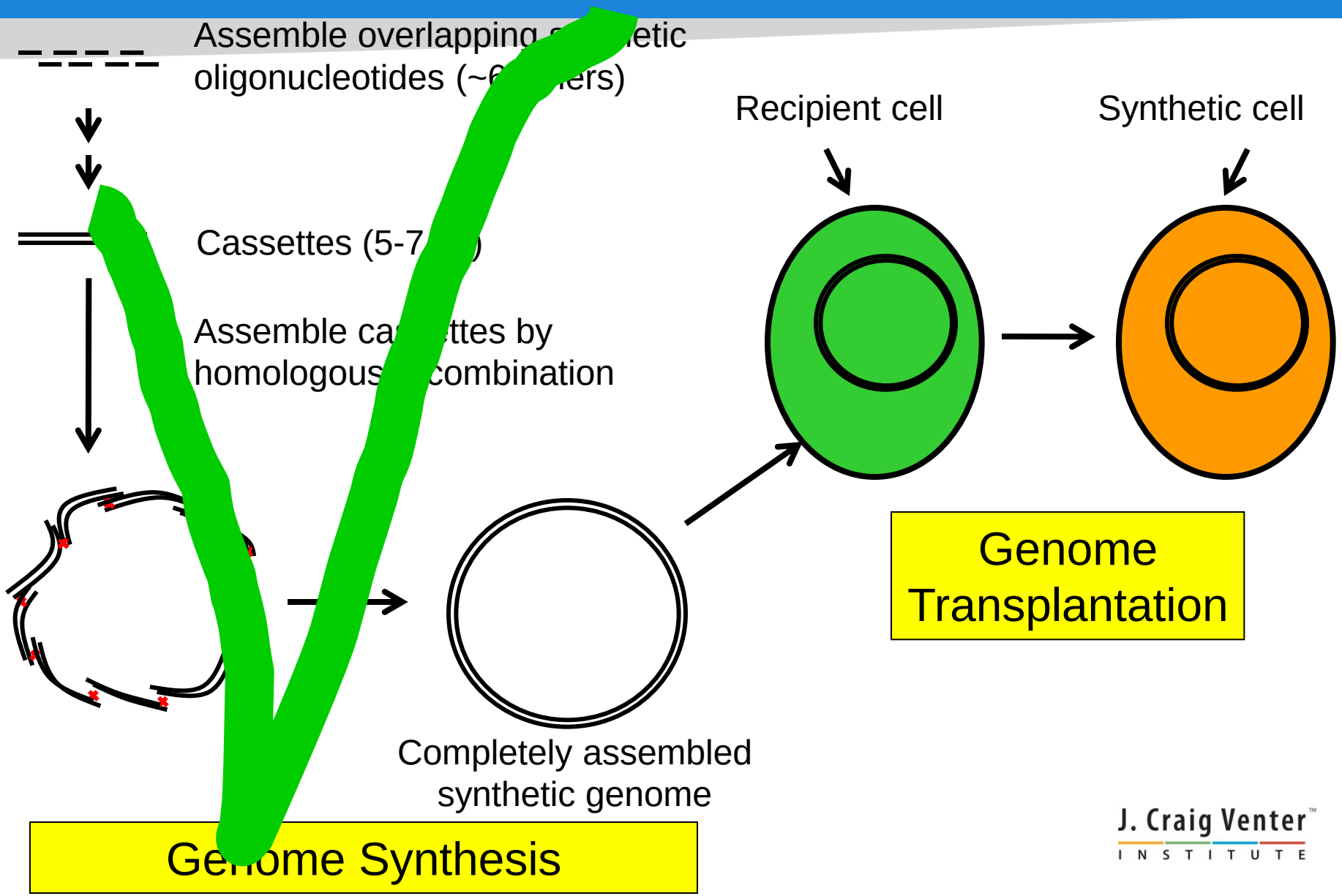
Approach Used to Create a Synthetic Cell



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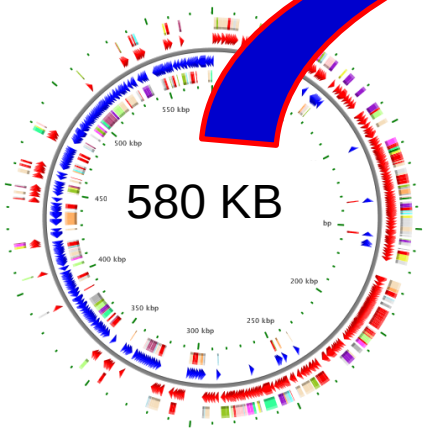
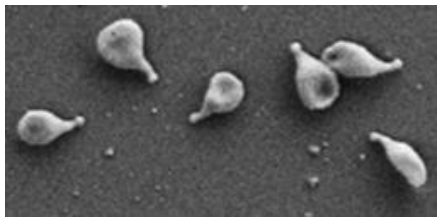
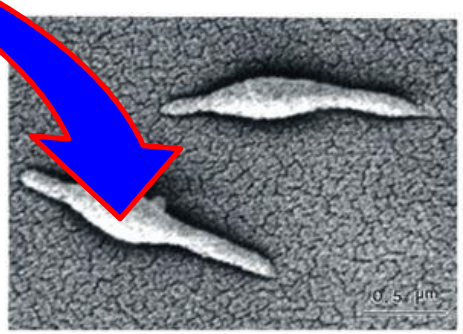
Whole Genome Transplantation





Mycoplasma genitalium



Mycoplasma pneumoniae

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Whole Genome Transplantation

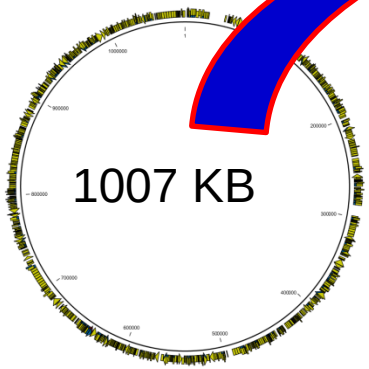
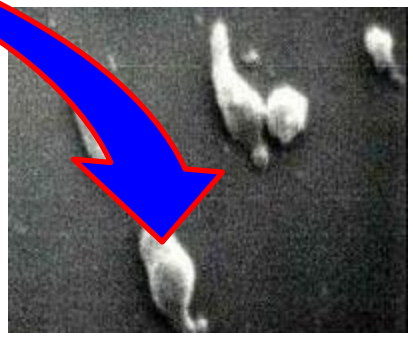
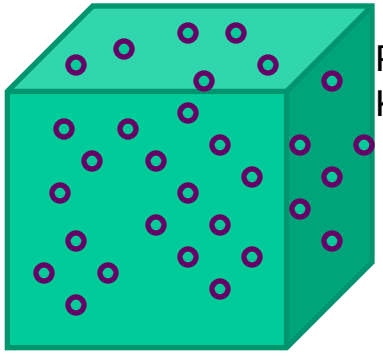


Photo: F. Chris Minion

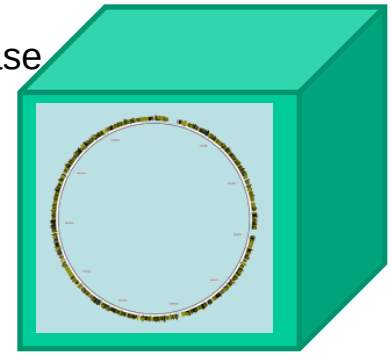


Mycoplasma mycoides LC(capri) *Mycoplasma capricolum*



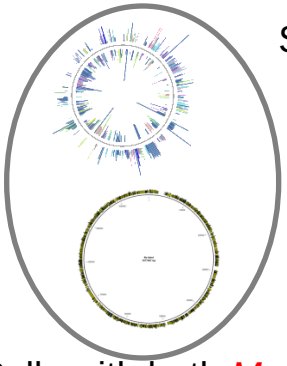
M. mycoides cells suspended agarose plug

→ Proteinase K



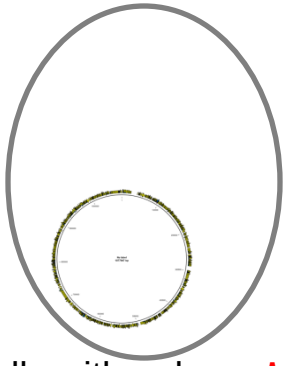
Naked *M. mycoides* genomes suspended agarose plug

→ Melt plug, and incubate *M. mycoides* DNA with *M. capricolum* cells in PEG and Ca++



Cells with both *M. mycoides* and *M. capricolum* genomes

→ Selection



Cells with only an *M. mycoides* genome

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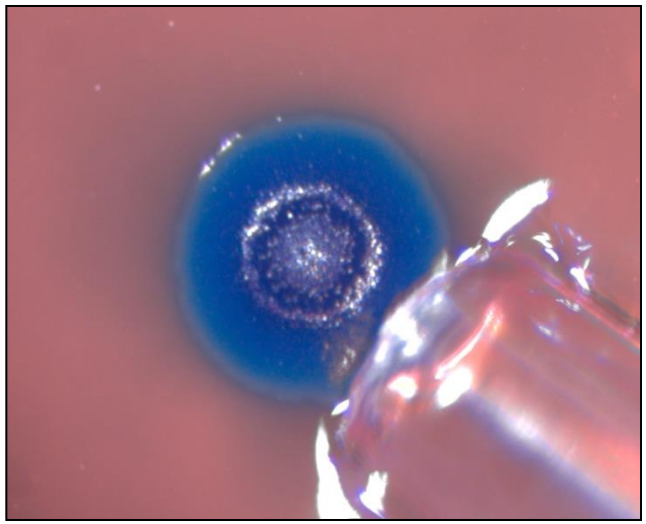
Putative Transplant Phenotype

colony tetR, blue, diameter ~1mm, after 3 to 5 days at 37ºC



→

***M. mycoides* colonies?**

Successful transplants!!

Transplant characterization

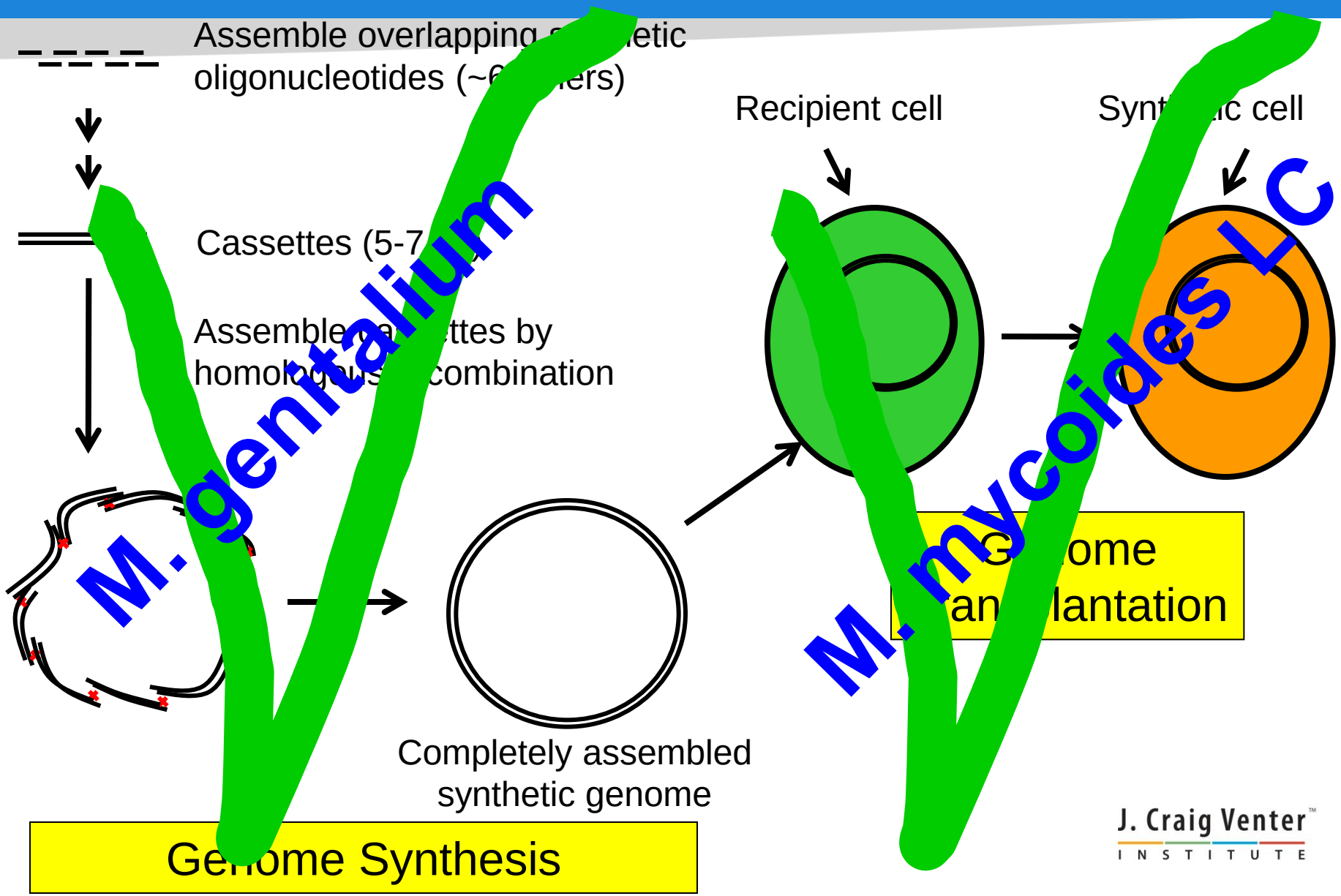
Phenotypic Analysis

- Blue tet^R colonies
- Colony-blot
- 2-Dimentionel gel electrophoresis

Genotypic Analysis

- PCR
- Southern blots
- Genome sequencing

Approach Used to Create a Synthetic Cell



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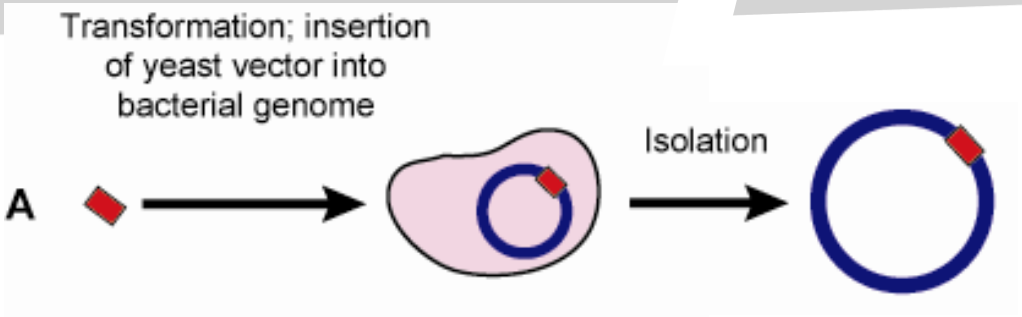
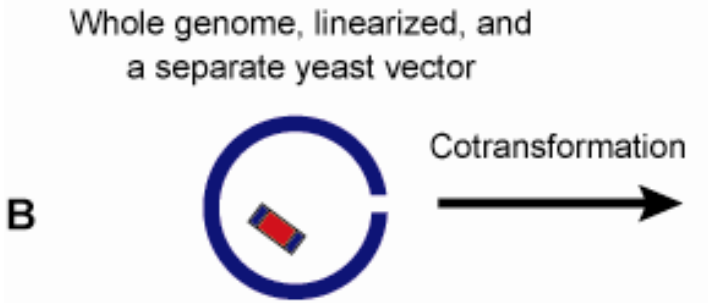
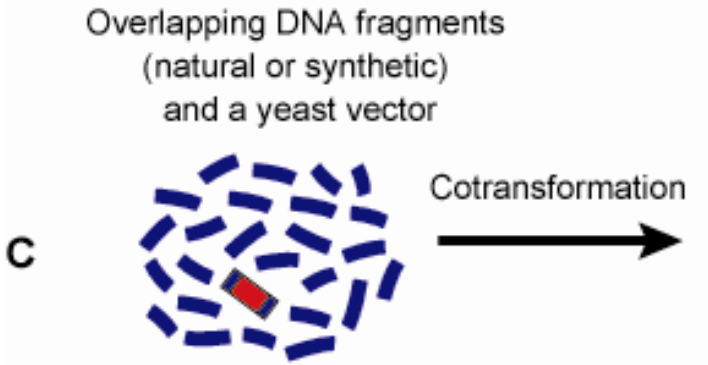
Methods for Cloning Bacterial Genomes in Yeast





M. genitalium



M. genitalium **6 pieces**
25 pieces

Succesful Examples

1. ***M. genitalium***
2. ***M. mycoides* LC**
3. ***M. pneumoniae***
4. ***M. genitalium* & *M. mycoides* LC**

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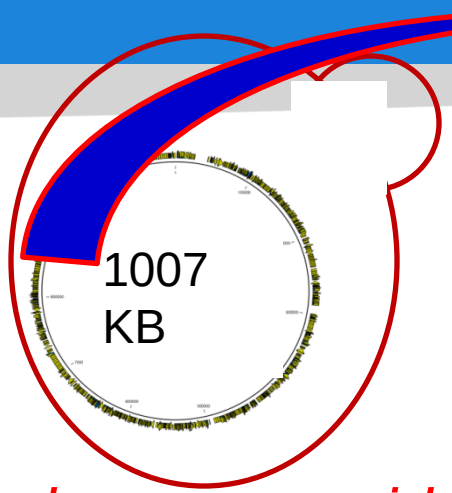
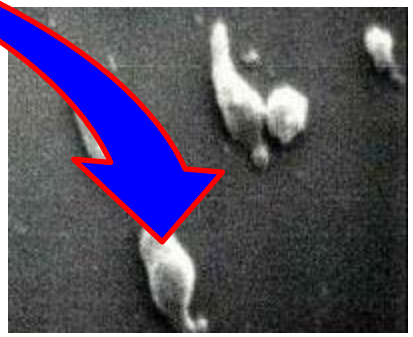
Whole Genome Transplantation

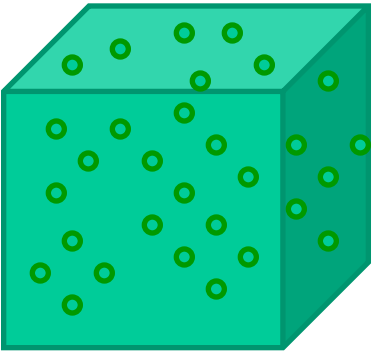


Mycoplasma mycoides capri...

IN YEAST

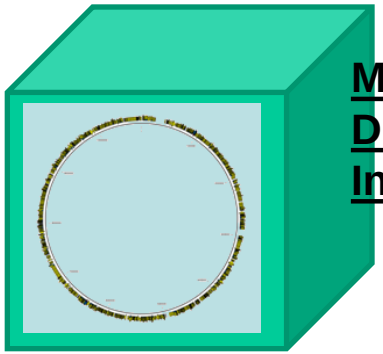


Mycoplasma capricolum



M. mycoides cells suspended agarose plug

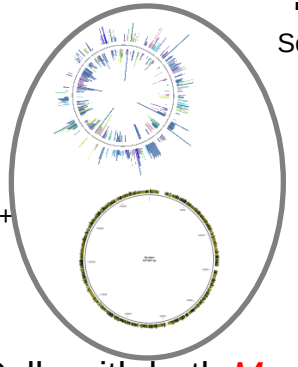
→ Proteinase K & Degrade Yeast DNA



Naked *M. mycoides* genomes suspended agarose plug

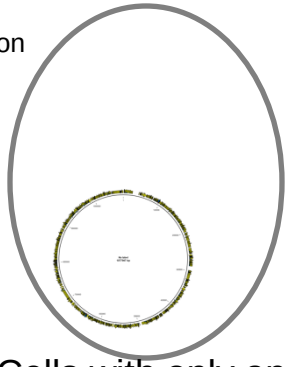
→ **Methylate DNA In Plug**

→ Melt plug, and incubate *M. mycoides* DNA with *M. capricolum* cells in PEG and Ca++



Cells with both *M. mycoides* and *M. capricolum* genomes

→ Selection



Cells with only an *M. mycoides* genome

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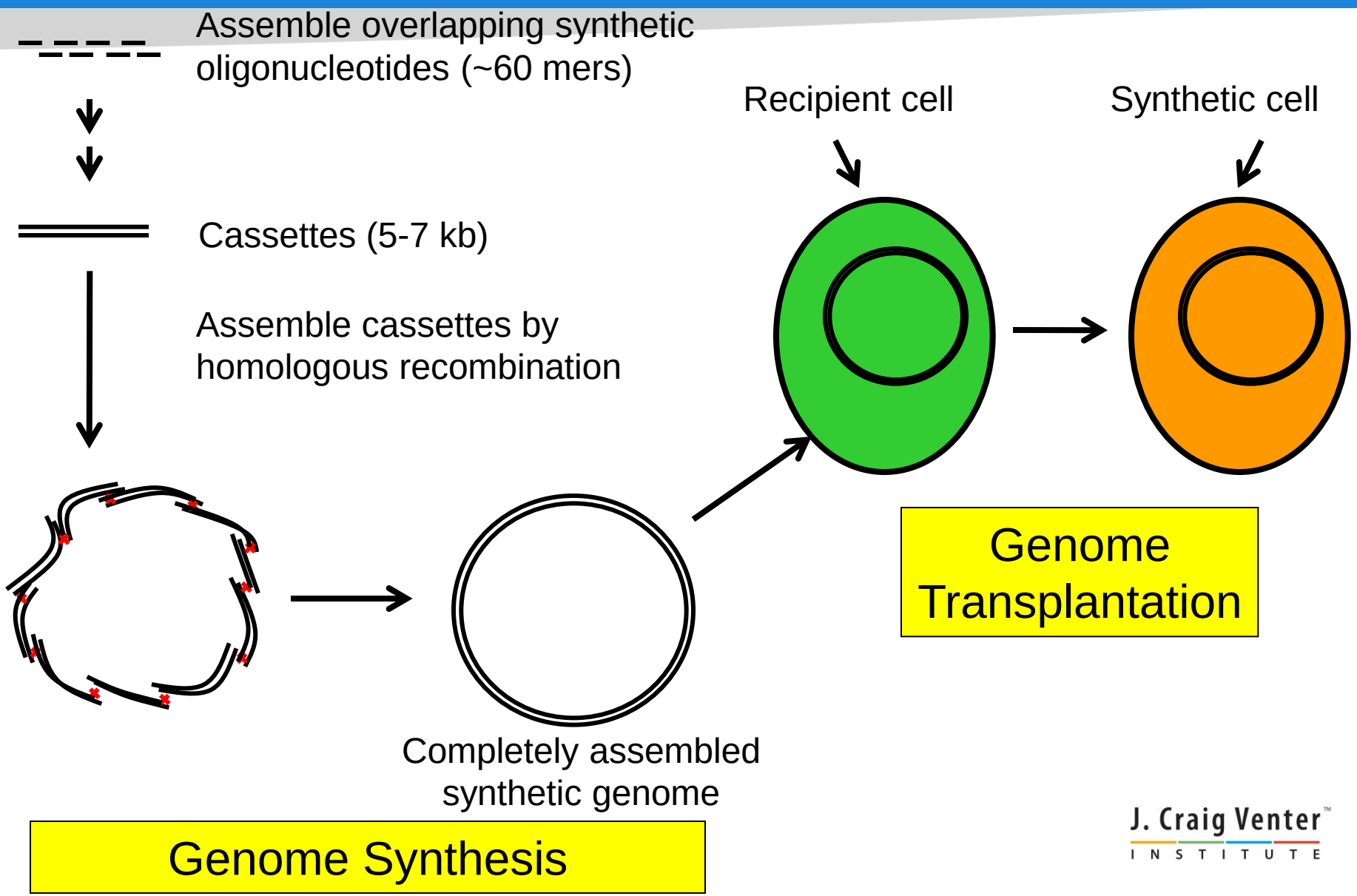
Approach Used to Create a Synthetic *M.mycLC* Cell



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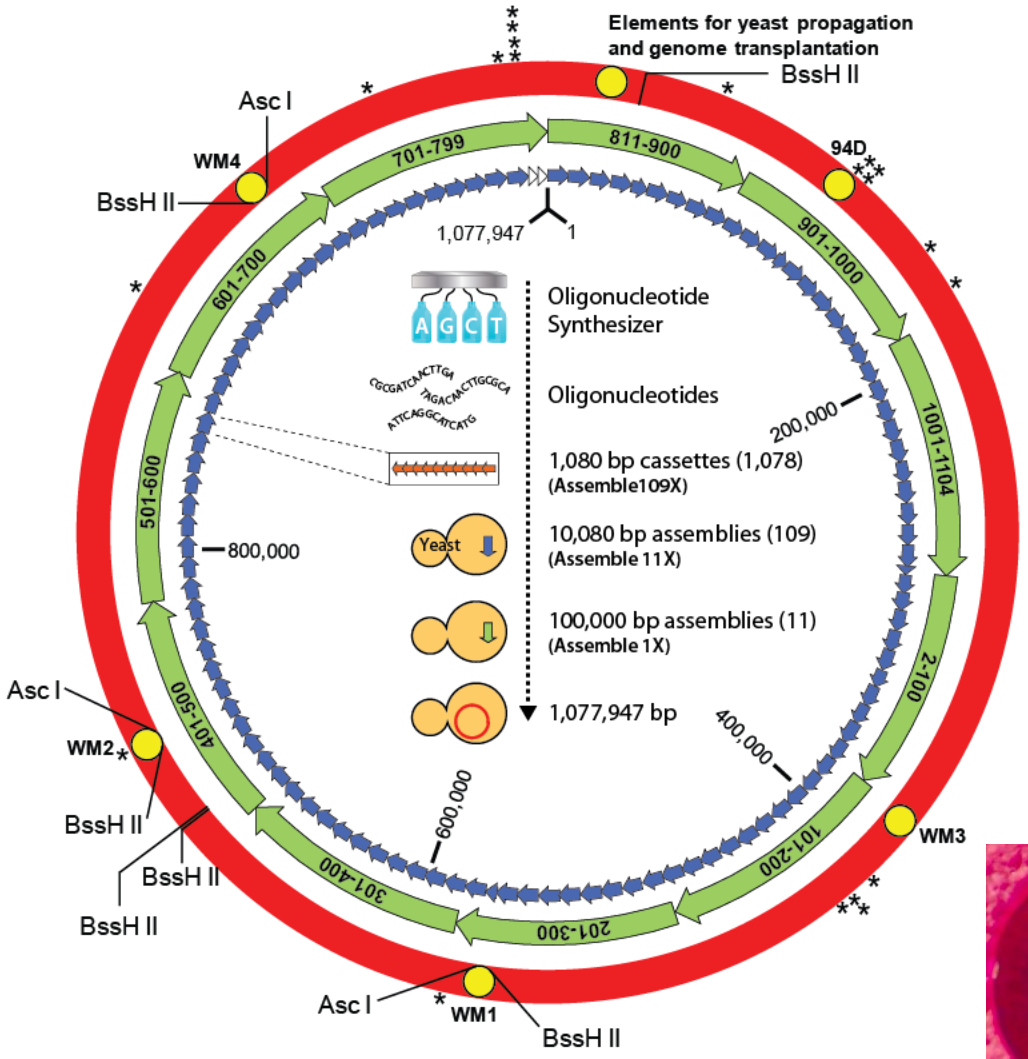
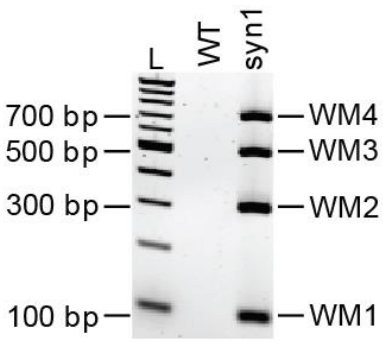
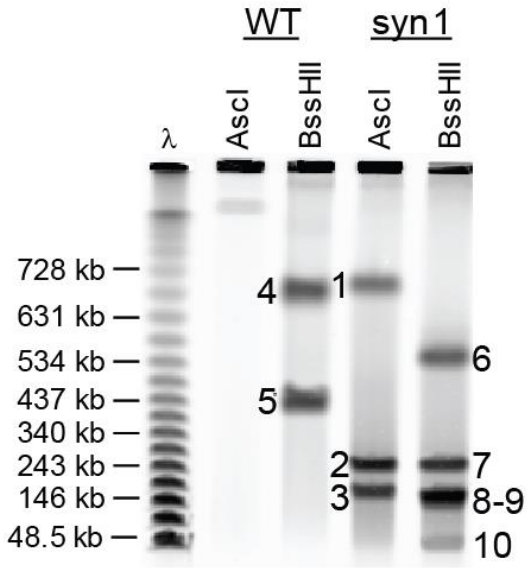
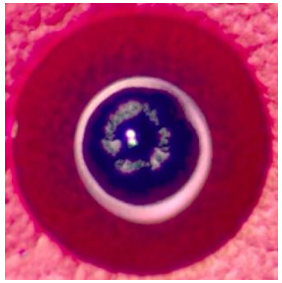
Creation of Synthetic *M.mycLC* Cell









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Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

Daniel G. Gibson,[1] John I. Glass,[1] Carole Lartigue,[1] Vladimir N. Noskov,[1] Ray-Yuan Chuang,[1] Mikkel A. Algire,[1] Gwynedd A. Benders,[2] Michael G. Montague,[1] Li Ma,[1] Monzia M. Moodie,[1] Chuck Merryman,[1] Sanjay Vashee,[1] Radha Krishnakumar,[1] Nacyra Assad-Garcia,[1] Cynthia Andrews-Pfannkoch,[1] Evgeniya A. Denisova,[1] Lei Young,[1] Zhi-Qing Qi,[1] Thomas H. Segall-Shapiro,[1] Christopher H. Calvey,[1] Prashanth P. Parmar,[1] Clyde A. Hutchison III,[2] Hamilton O. Smith,[2] J. Craig Venter[1,2]*

We report the design, synthesis, and assembly of the 1.08–mega–base pair *Mycoplasma mycoides* JCVI-syn1.0 genome starting from digitized genome sequence information and its transplantation into a *M. capricolum* recipient cell to create new *M. mycoides* cells that are controlled only by the synthetic chromosome. The only DNA in the cells is the designed synthetic DNA sequence, including "watermark" sequences and other designed gene deletions and polymorphisms, and mutations acquired during the building process. The new cells have expected phenotypic properties and are capable of continuous self-replication.

In 1977, Sanger and colleagues determined the complete genetic sequence of phage φX174 (*1*), the first DNA genome to be completely sequenced. Eighteen years later, in 1995, our team was able to read the first complete genetic sequence of a self-replicating bacterium, *Haemophilus influenzae* (*2*). Reading the genetic sequence of a wide range of species has increased exponentially from these early studies. The ability to rapidly digitize genomic information has increased by more than eight orders of magnitude over the past 25 years (*3*). Efforts to understand all this new genomic information have spawned numerous new computational and

We developed a strategy for assembling viral-sized pieces to produce large DNA molecules that enabled us to assemble a synthetic *M. genitalium* genome in four stages from chemically synthesized DNA cassettes averaging about 6 kb in size. This was accomplished through a combination of in vitro enzymatic methods and in vivo recombination in *Saccharomyces cerevisiae*. The whole synthetic genome [582,970 base pairs (bp)] was stably grown as a yeast centromeric plasmid (YCp) (*7*).

Several hurdles were overcome in transplanting and expressing a chemically synthesized chromosome in a recipient cell. We needed to improve crude *M. mycoides* or *M. capricolum* extracts, or by simply disrupting the recipient cell's restriction system (*8*).

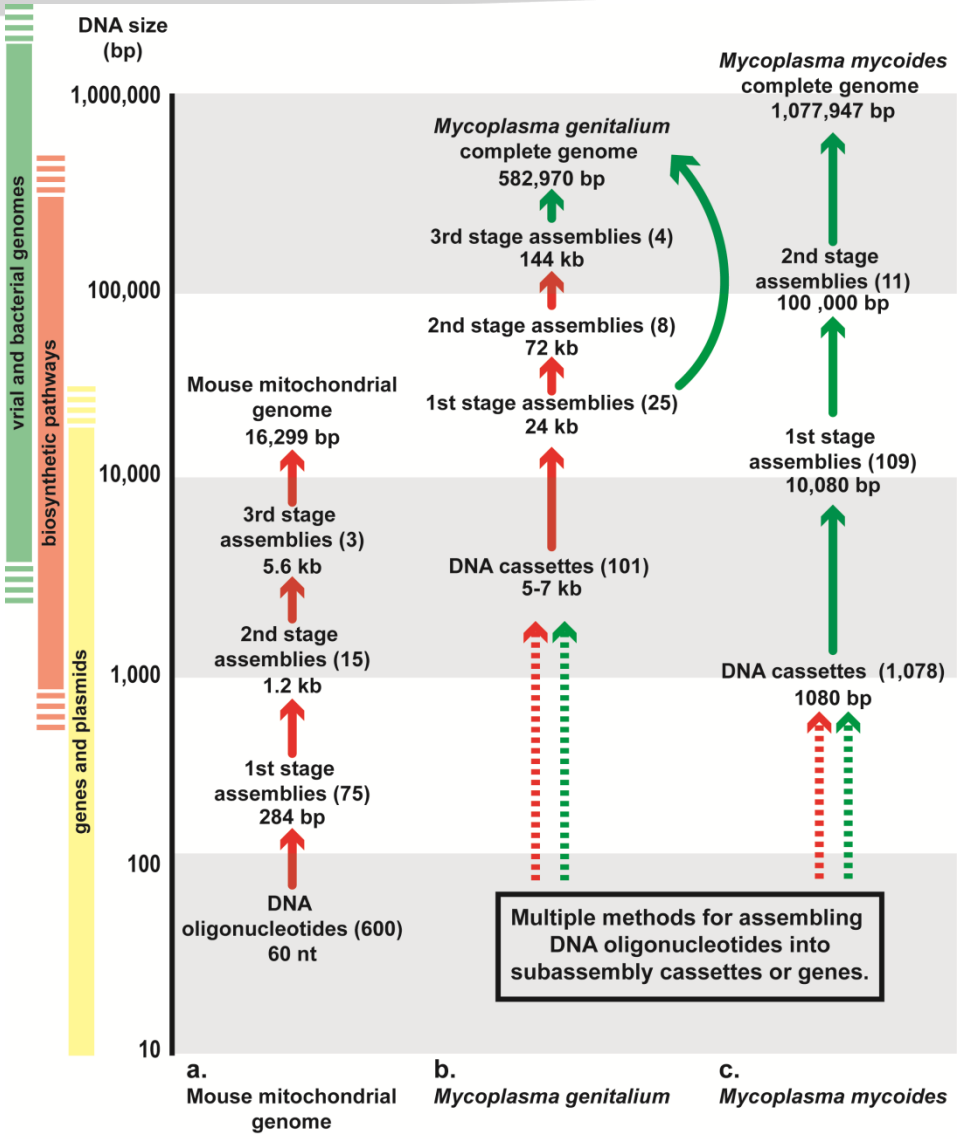
We now have combined all of our previously established procedures and report the synthesis, assembly, cloning, and successful transplantation of the 1.08-Mbp *M. mycoides* JCVI-syn1.0 genome, to create a new cell controlled by this synthetic genome.

Synthetic genome design. Design of the *M. mycoides* JCVI-syn1.0 genome was based on the highly accurate finished genome sequences of two laboratory strains of *M. mycoides* subspecies *capri* GM12 (*8*, *9*, *11*). One was the genome donor used by Lartigue *et al.* [GenBank accession CP001621] (*10*). The other was a strain created by transplantation of a genome that had been cloned and engineered in yeast, YCpMmyc1.1-Δ*typeIIIres* [GenBank accession CP001668] (*8*). This project was critically dependent on the accuracy of these sequences. Although we believe that both finished *M. mycoides* genome sequences are reliable, there are 95 sites at which they differ. We began to design the synthetic genome before both sequences were finished. Consequently, most of the cassettes were designed and synthesized based on the CP001621 sequence (*11*). When it was finished, we chose the sequence of the genome successfully transplanted from yeast (CP001668) as our design reference (except that we kept the intact *typeIIIres* gene). All differences that appeared biologically significant between CP001668 and previously synthesized cassettes were corrected to match it exactly (*11*). Sequence differences between our synthetic cassettes and CP001668 that occurred at 19 sites appeared harmless and so were not corrected. These provide 19 polymorphic

Synthetic Biology/Synthetic Genomics Summary

- Synthetic Biology is not really a new field
- Several approaches for Synthetic Biology
- More easy manipulation of whole genome
- Has potential dual use

Technologies Used/Developed for Synthetic Cell



Assembly Tools

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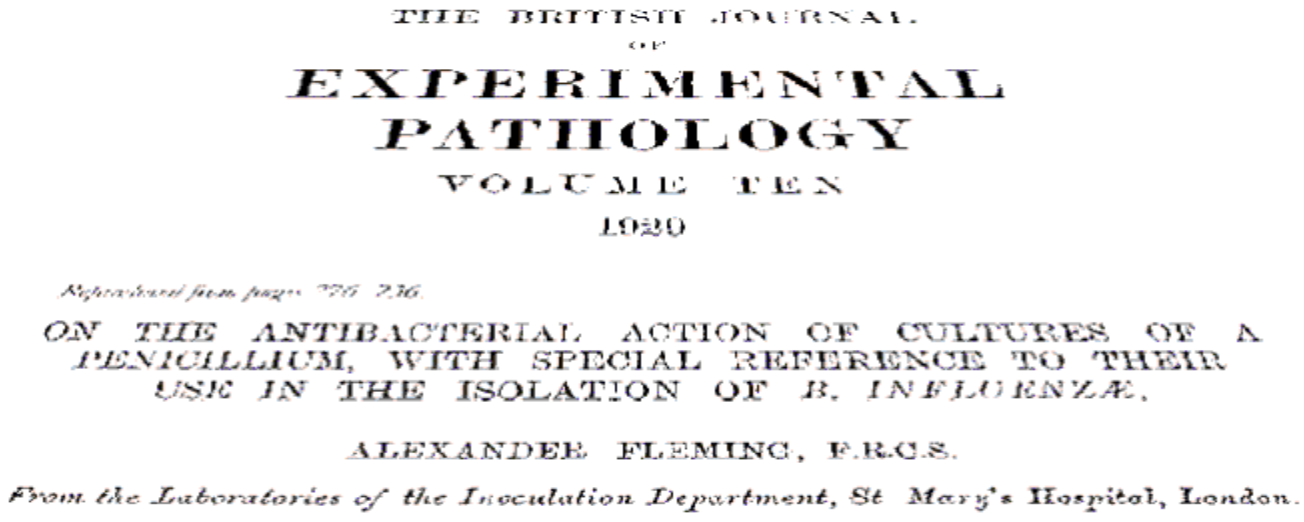
Secondary Metabolite Clusters

Natural Product Drugs from Organisms

Organism → Extract → Assay



THE BRITISH JOURNAL
OF
EXPERIMENTAL
PATHOLOGY
VOLUME TEN
1929

Reproduced from pages 226–236.

ON THE ANTIBACTERIAL ACTION OF CULTURES OF A *PENICILLIUM*, WITH SPECIAL REFERENCE TO THEIR USE IN THE ISOLATION OF *B. INFLUENZÆ*.

ALEXANDER FLEMING, F.R.C.S.

From the Laboratories of the Inoculation Department, St Mary's Hospital, London.

Received for publication May 10th, 1929.

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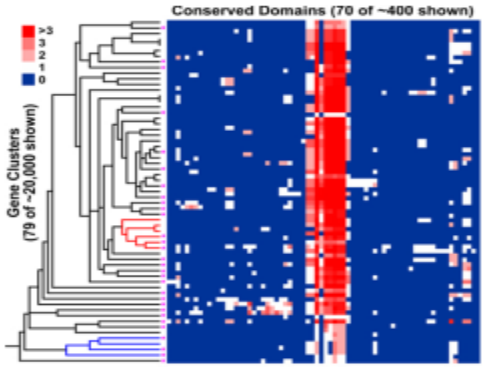
Orphan Clusters

For 'Sanger' genomes alone
~20,000 clusters with no metabolite



Known products
SCO5071–5092
SCO5877–5898
SCO3210–3249
SCO2782–2785

~ predictable products
SCO0489–0499
SCO7681–7691
SCO5314–5320
SCO1206–1208
SCO0185–0191
SCO6759–6771
SCO0124–0129
SCO6073
SCO6266

Unpredictable products
SCO6429–6438
SCO6273–6288
SCO6826-6827
SCO7669–7671
SCO7222
SCO5222–5223
SCO5799–5801
SCO1265–1273
SCO0381–0401

Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2)

S. D. Bentley*, K. F. Chater†, A.-M. Cerdeño-Tárraga*, G. L. Challis†‡, N. R. Thomson*, K. D. James*, D. E. Harris*, M. A. Quail*, H. Kieser†, D. Harper*, A. Bateman*, S. Brown*, G. Chandra†, C. W. Chen§, M. Collins*, A. Cronin*, A. Fraser*, A. Goble*, J. Hidalgo*, T. Hornsby*, S. Howarth*, C.-H. Huang§, T. Kieser†, L. Larke*, L. Murphy*, K. Oliver*, S. O'Neil*, E. Rabbinowitsch*, M.-A. Rajandream*, K. Rutherford*, S. Rutter*, K. Seeger*, D. Saunders*, S. Sharp*, R. Squares*, S. Squares*, K. Taylor*, T. Warren*, A. Wietzorrek†, J. Woodward*, B. G. Barrell*, J. Parkhill* & D. A. Hopwood†

NATURE | VOL 417 | 9 MAY 2002 | www.nature.com

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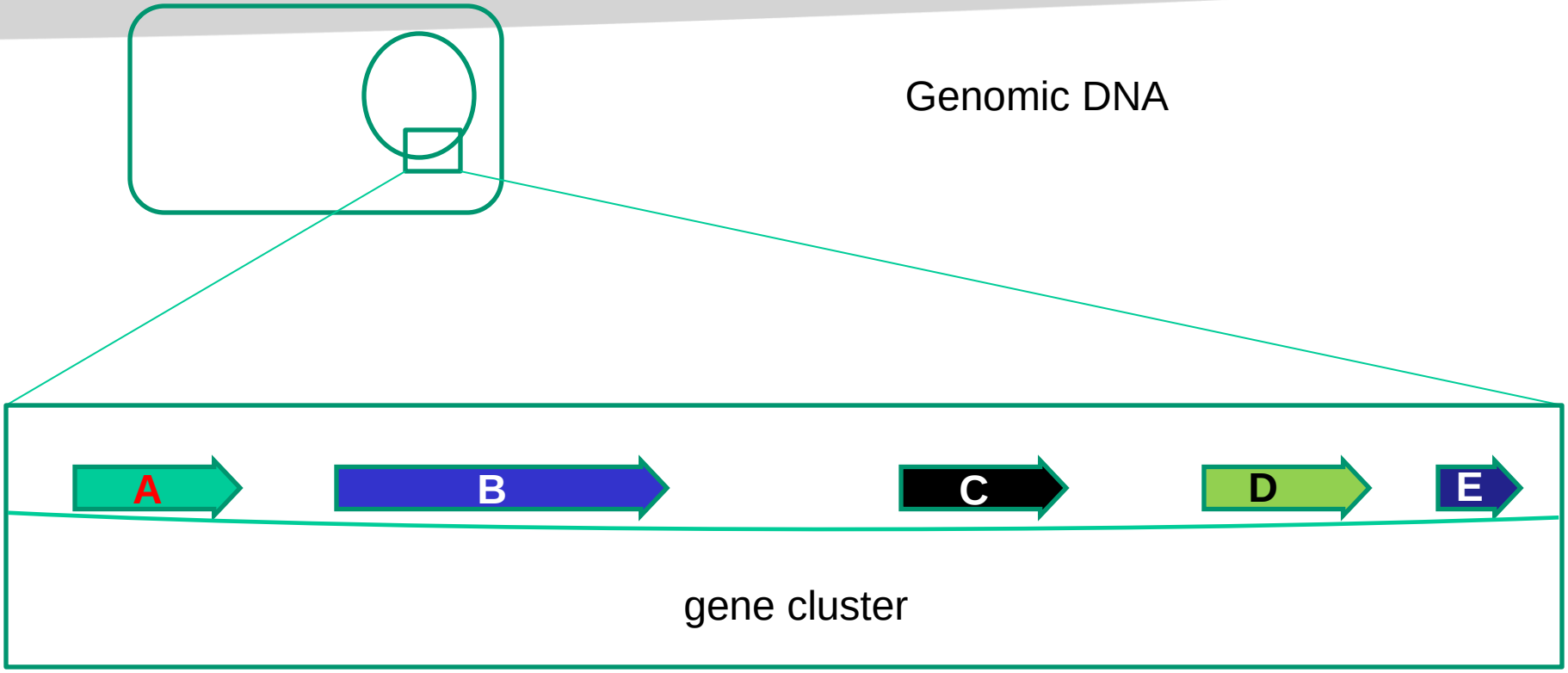
Structural Genes



PCR

ATG to stop

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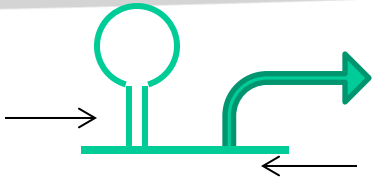
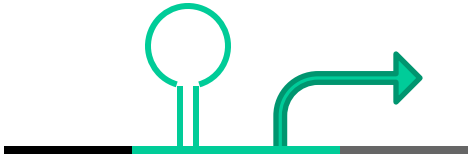
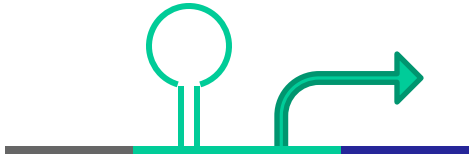
Transcription Promoters and Terminators



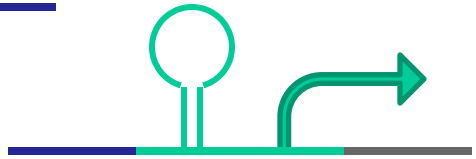
PCR



Plasmid & A homology added



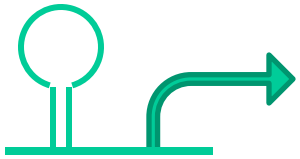
A&B



B&C

…

Stock templates



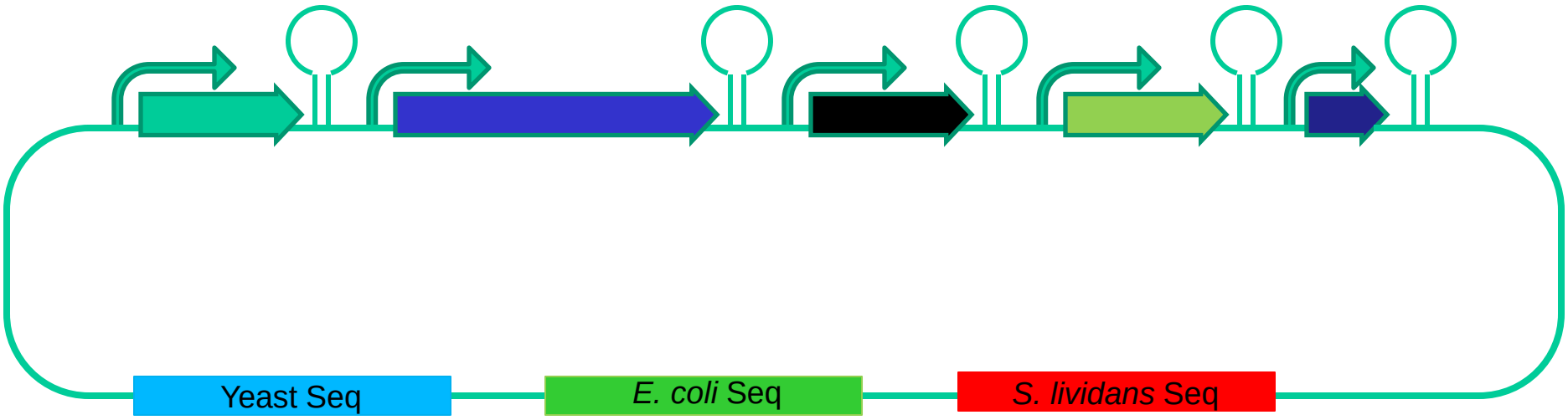
~400 synthetic terminator & promoter combinations (streptomyces)

ATG to stop

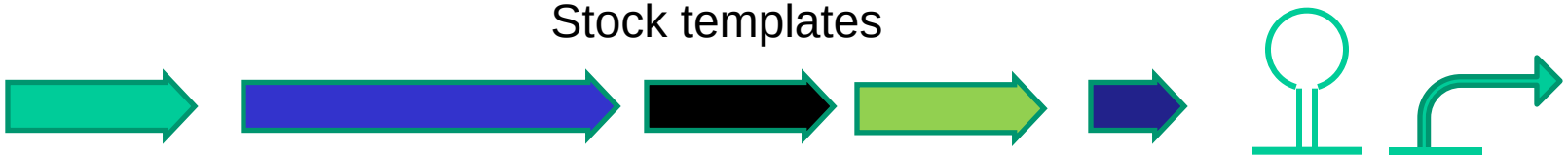
J. Craig Venter INSTITUTE

One/two Step Assembly

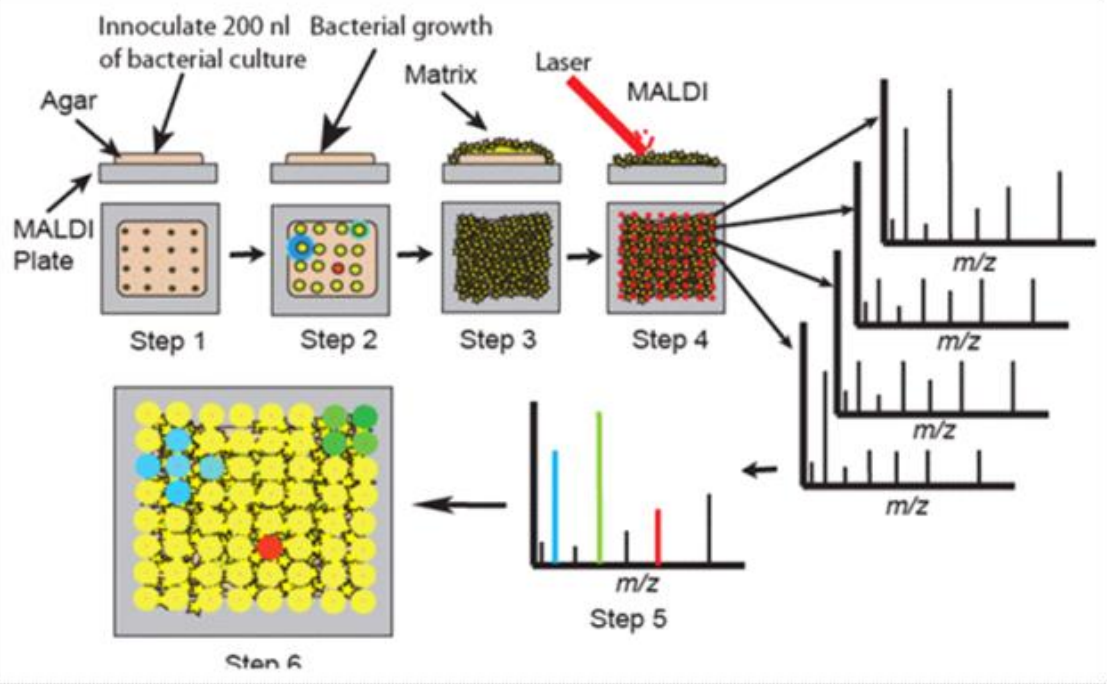
(mate with *S. lividans*, insert at phage attachment site, induce expression)



Stock templates



Evaluation of Product

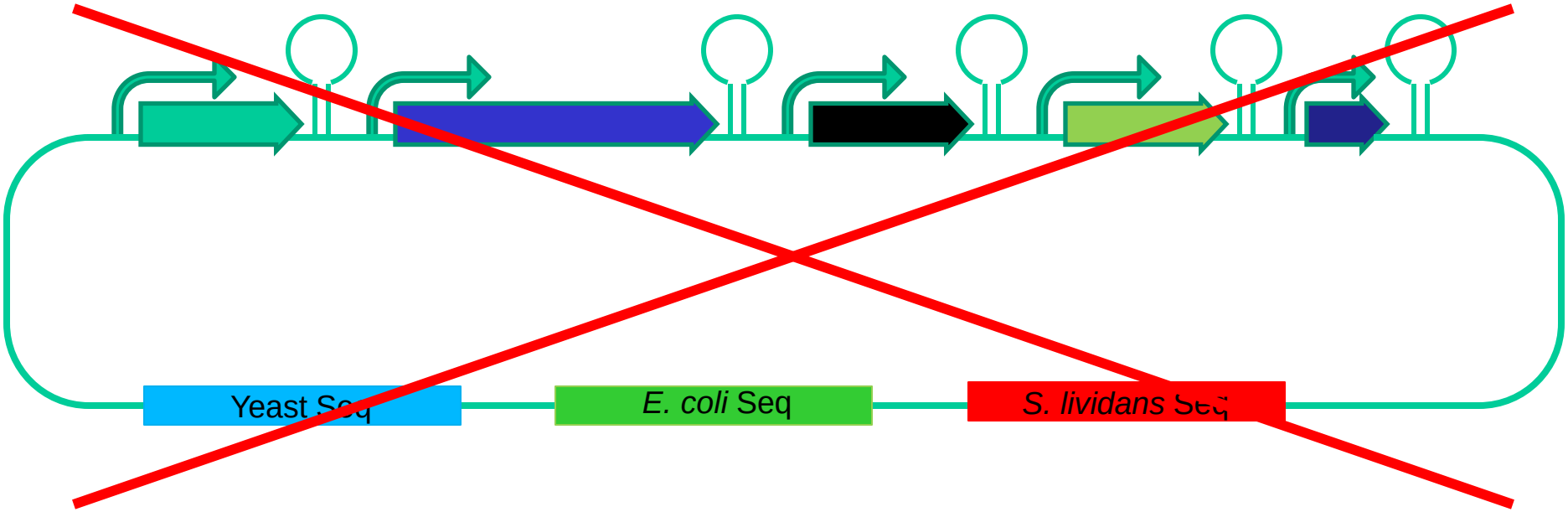


Screen colonies for metabolite production by MALDI-IMS UCSD

J. Craig Venter INSTITUTE

One/two Step Assembly

(mate with *S. lividans*, insert at phage attachment site, induce expression)

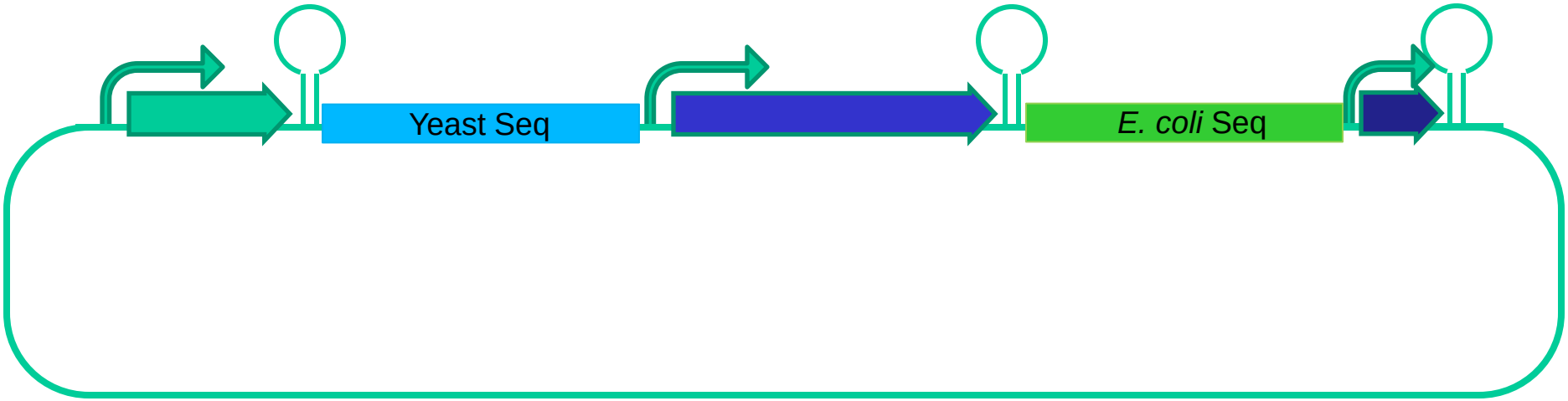


Initial attempts showed mis-assemblies

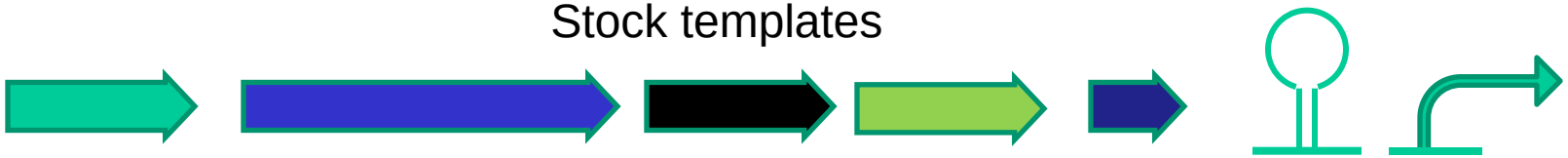
One/two Step Assembly

(mate with *S. lividans*, insert at phage attachment site, induce expression)

Solution



Stock templates



Yeast Seq

S. lividans Seq

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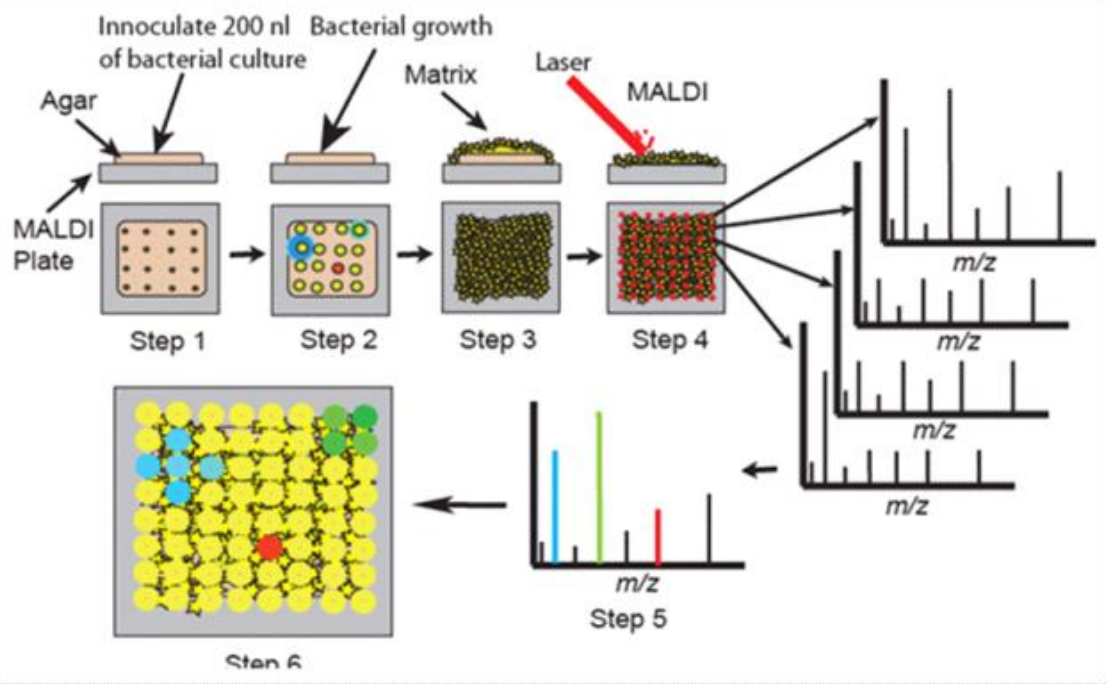
Evaluation of Product



Screen colonies for metabolite production by MALDI-IMS UCSD

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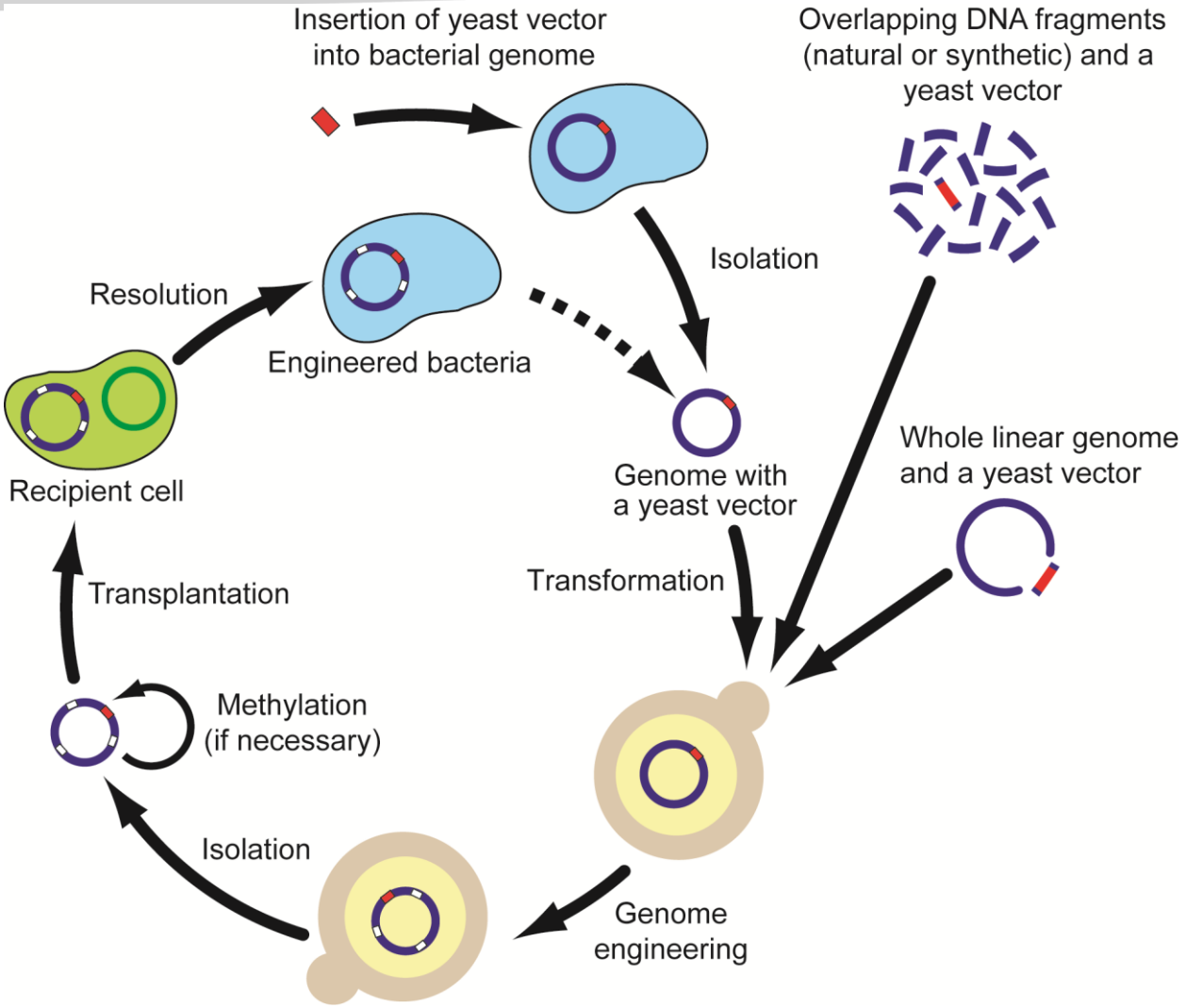
Technologies Used/Developed for Synthetic Cell



Genome Transplantation

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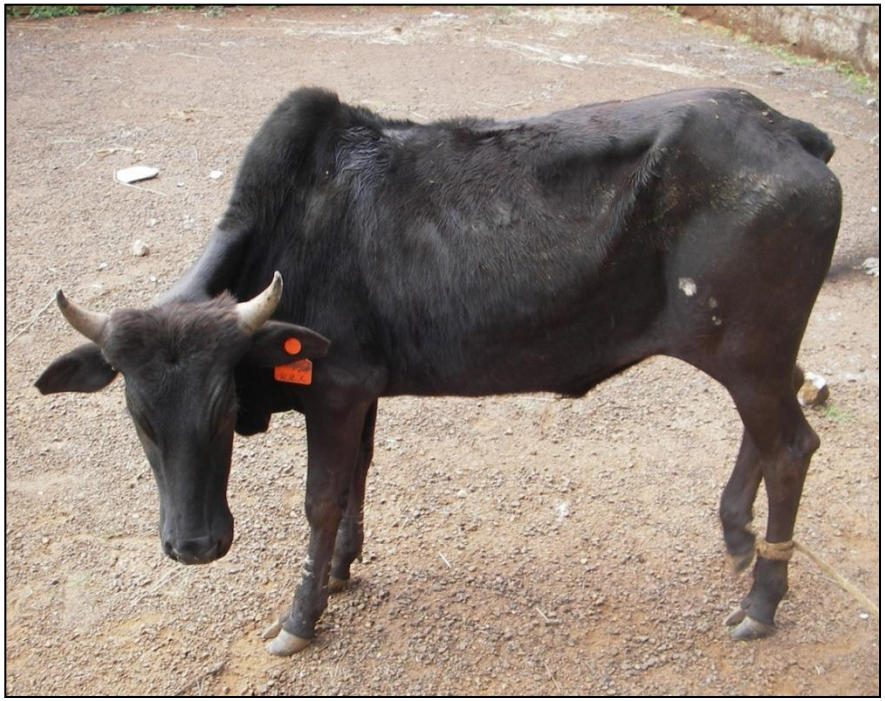
CBPP – Main Bacterial Cattle Disease in Africa

Clinical symptoms of CBPP



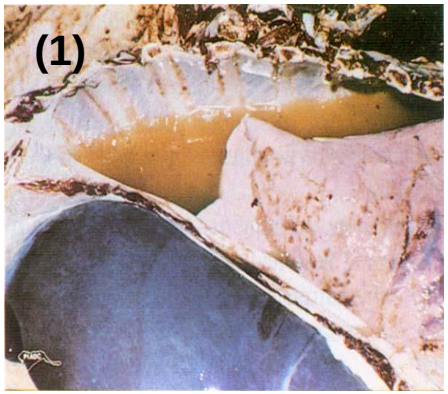
Animals depressed, painful and difficult breathing (dyspnea), fever, cough, nasal discharge and anorexia

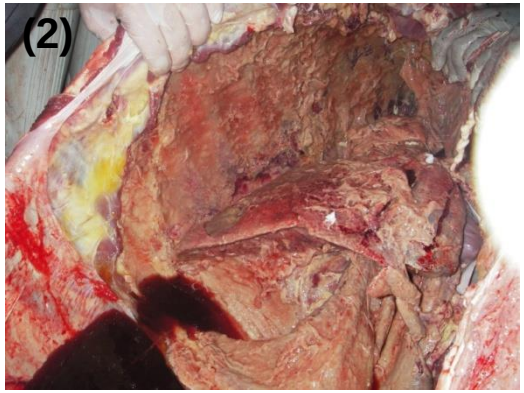
Post-mortem lesions:

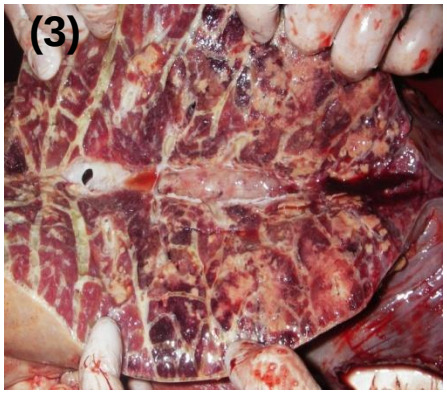
(1) Fluid in the thorax,

(2) Fibrinization of Lung

(3) Marmorization of Lung







http://www.fao.org/docrep/003/t0756e/T0756E03.htm

CBPP is a highly infectious disease that affects cattle. It is transmitted mostly by direct contact from droplets emitted by coughing animals, saliva and urine."

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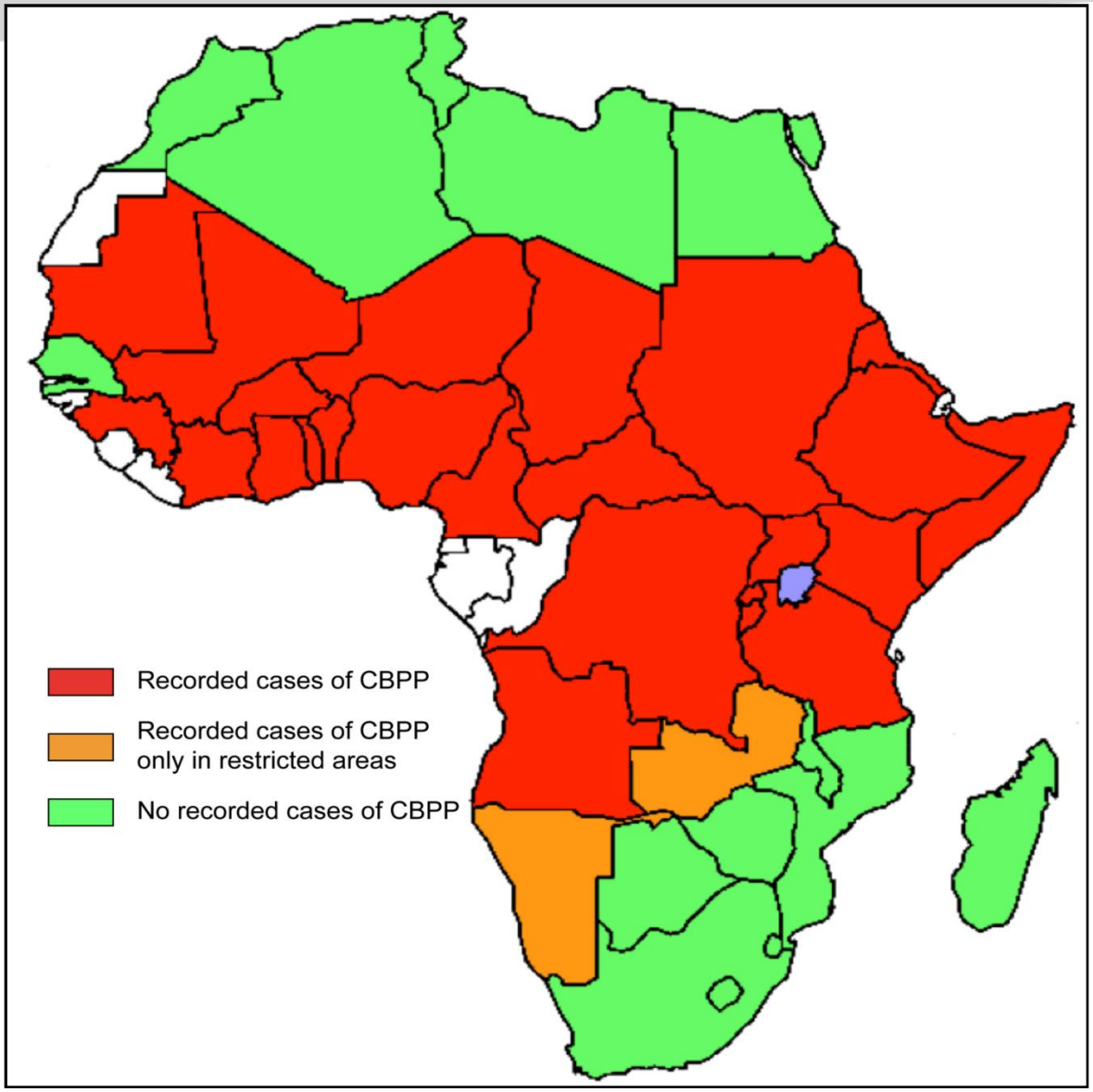
Distribution of CBPP in Africa



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Control of CBPP

- On-farm quarantine of exposed animals
- Slaughter of infected and exposed animals
- Proper disposal of animals and contaminated material

Method was effective for eliminating CBPP in developed countries but not really possible in developing countries

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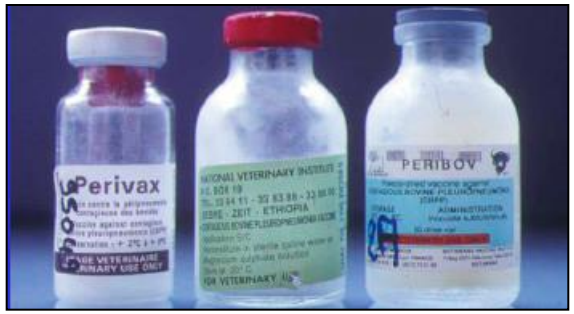
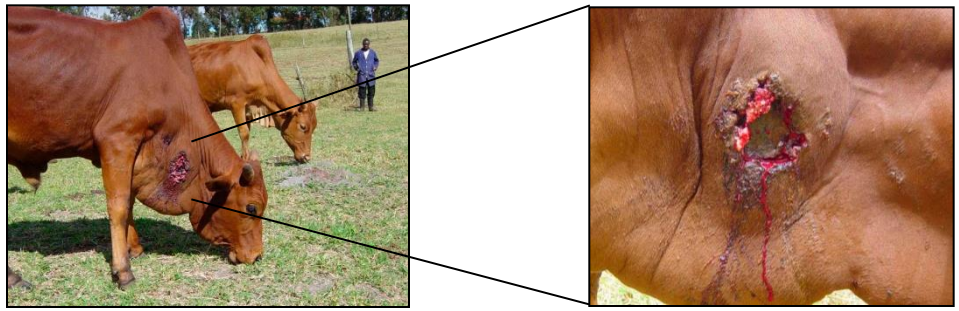
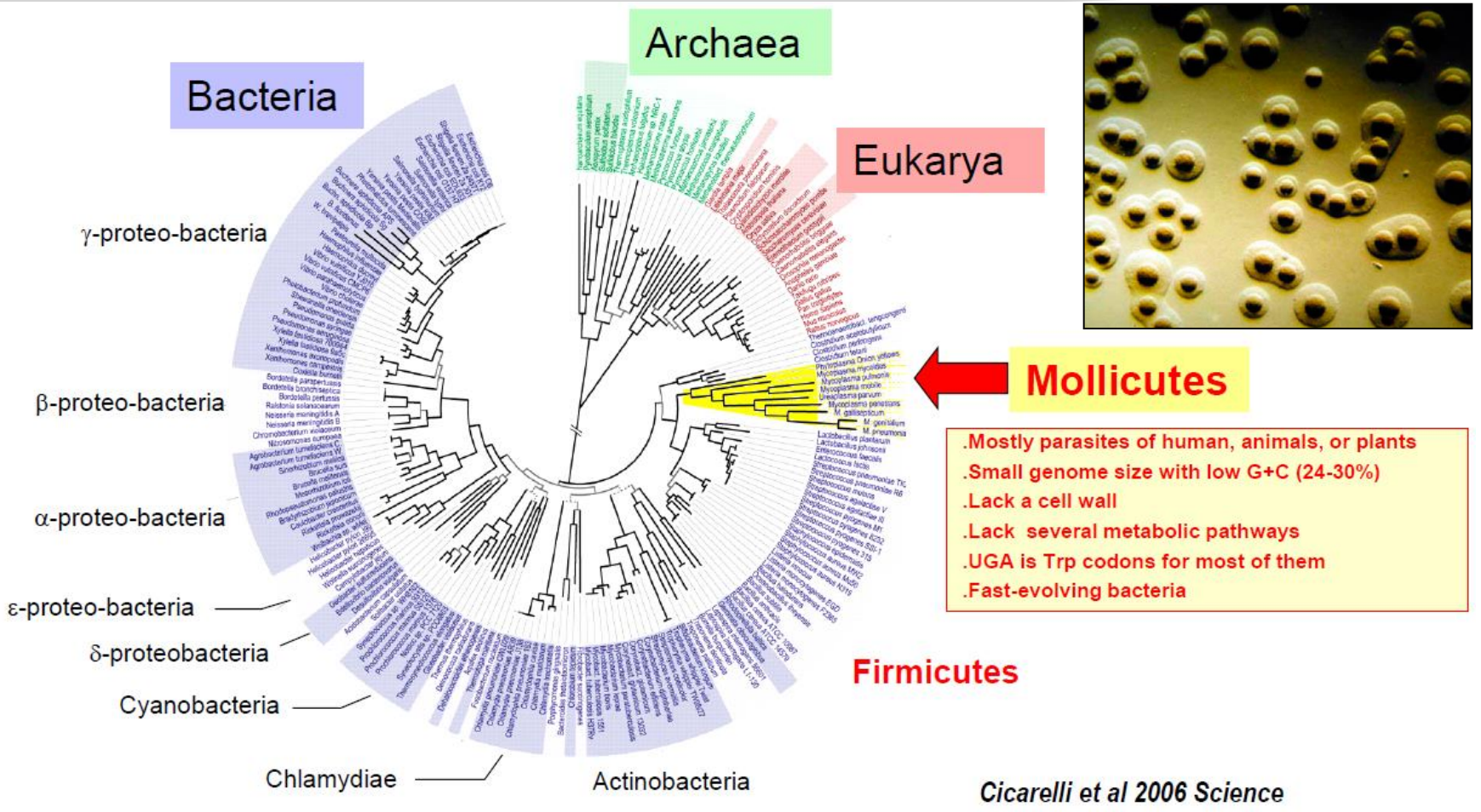
Control of CBPP

- **Vaccination**
  - Low efficacy, protection for short periods of time
  - Adverse reactions e.g. lesions, loss of tail
  - Possibility of reverting to pathogenic strain (T1/44)





CBPP CAUSAL AGENT: *Mycoplasma mycoides subsp. mycoides* (Mmm) (Mollicutes Class)



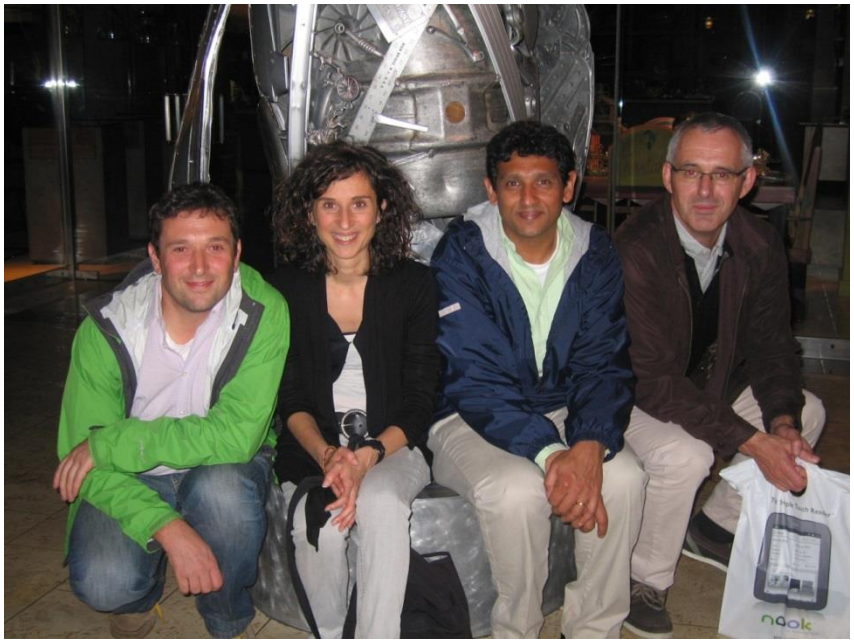
Mollicutes have evolved from gram positive bacteria. These fast-evolving organisms are mostly parasites of humans, animals and plants

J. Craig Venter INSTITUTE

BREAD: Toward Development of an Effective Vaccine for Contagious Bovine Pleuropneumonia (CBPP)

Sanjay Vashee (PI)[1], Carole Lartigue (Co-PI)[2], Joerg Jores (Co-PI)[3], Alain Blanchard[2], Vishvanath Nene[3], Pascal Sirand-Pugnet[2], John Glass[1]

[1] J. Craig Venter Institute, Rockville, MD 20850 USA, [2] National Institute for Agronomical Research, Bordeaux, France, [3] International Livestock Research Institute, Nairobi, Kenya



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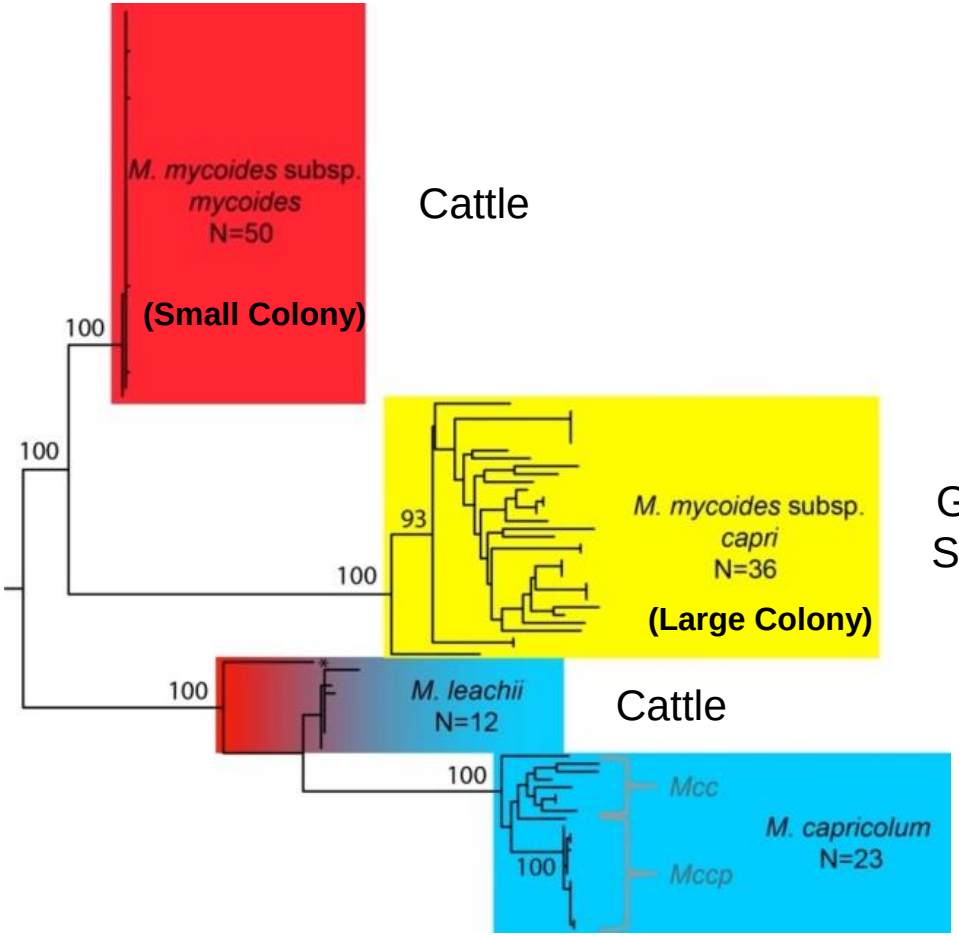
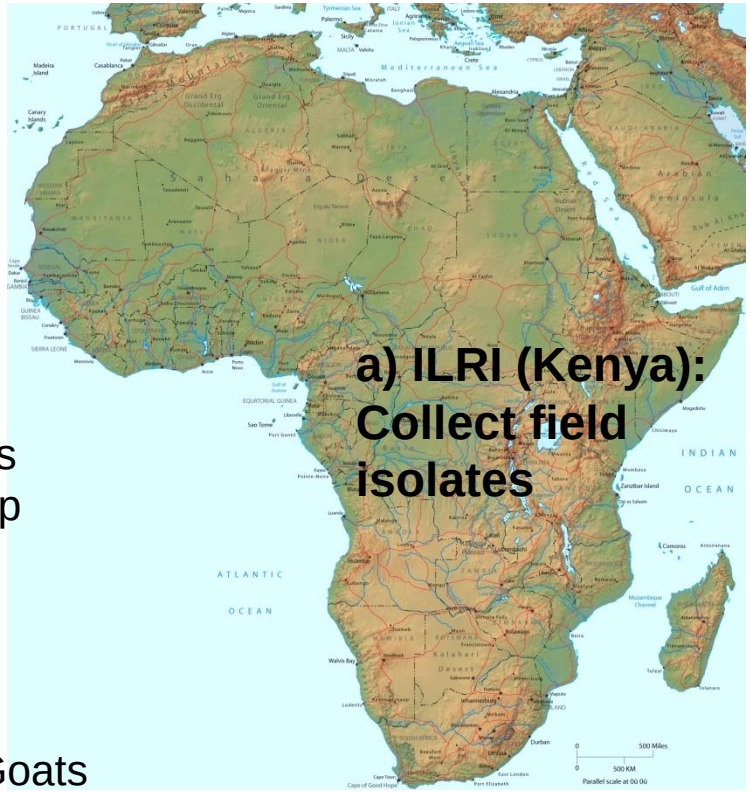
Aim 1. Characterize the pan genome of the mycoides cluster to identify target virulence genes.

Mycoides Cluster: Species Infecting Ruminants





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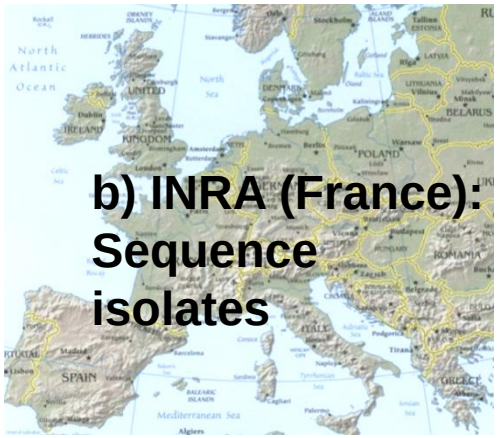
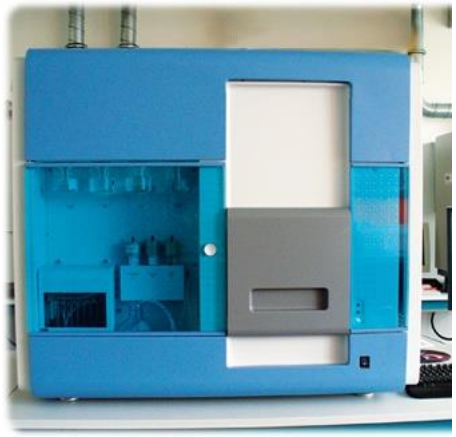
Aim 1. Characterize the pan genome of the mycoides cluster to identify target virulence genes.

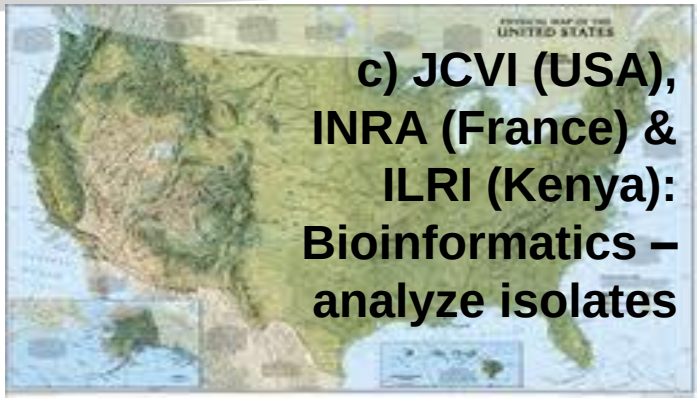
Genome sequencing using Next Generation technologies



b) INRA (France): Sequence isolates



Illumina Sequencer



c) JCVI (USA), INRA (France) & ILRI (Kenya): Bioinformatics – analyze isolates

Strain	Year of isolation	Country	Host	Supplier	Cloning	gDNA isolation
Mmm:						
V5	1936	Australia	vaccine strain	FLI	filter cloning	12.06.12
B66	2000	Kenya	cattle	KARI	filter cloning	12.06.12
95014	1995	Tanzania	cattle	FLI	filter cloning	12.06.12
Fatick	1968	Senegal	cattle	FLI	filter cloning	12.06.12
C11	1962	Chad	cattle	FLI	filter cloning	12.06.12
L2	1993	Italy	cattle	FLI	filter cloning	12.06.12
Matapi	2004	Namibia	cattle	FLI	filter cloning	12.06.12
Mmc:						
Capri L	1975	France	goat	FLI	filter cloning	ongoing
Kombolcha	1975	Ethiopia	goat	FLI	filter cloning	ongoing
Y-goat	1956	Australia	goat	FLI	filter cloning	ongoing
G1313.94	1994	Germany	Barbary sheep	FLI	filter cloning	ongoing

Virulence factors

Host specificity

Understand Organism (host-pathogen interaction)

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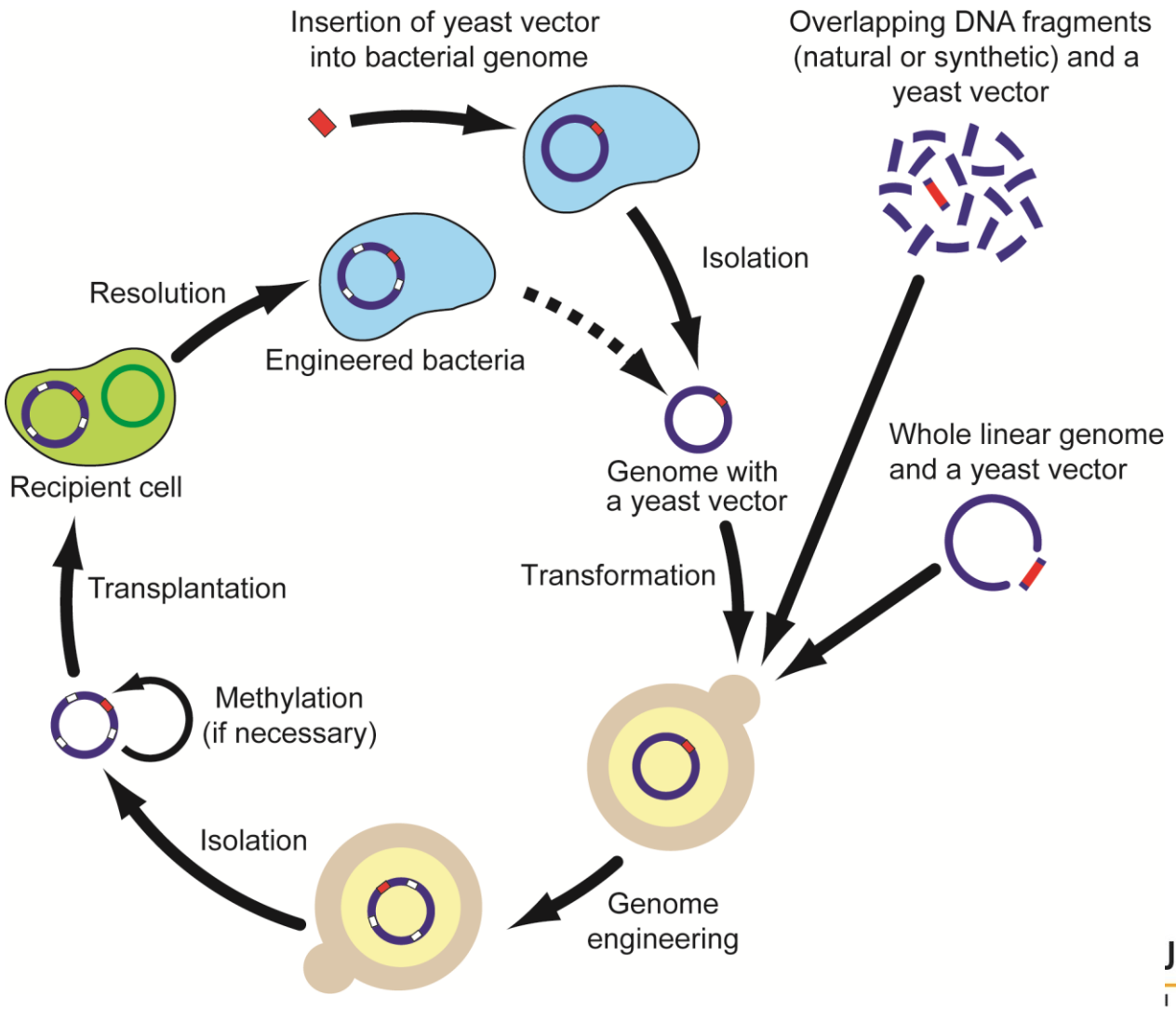
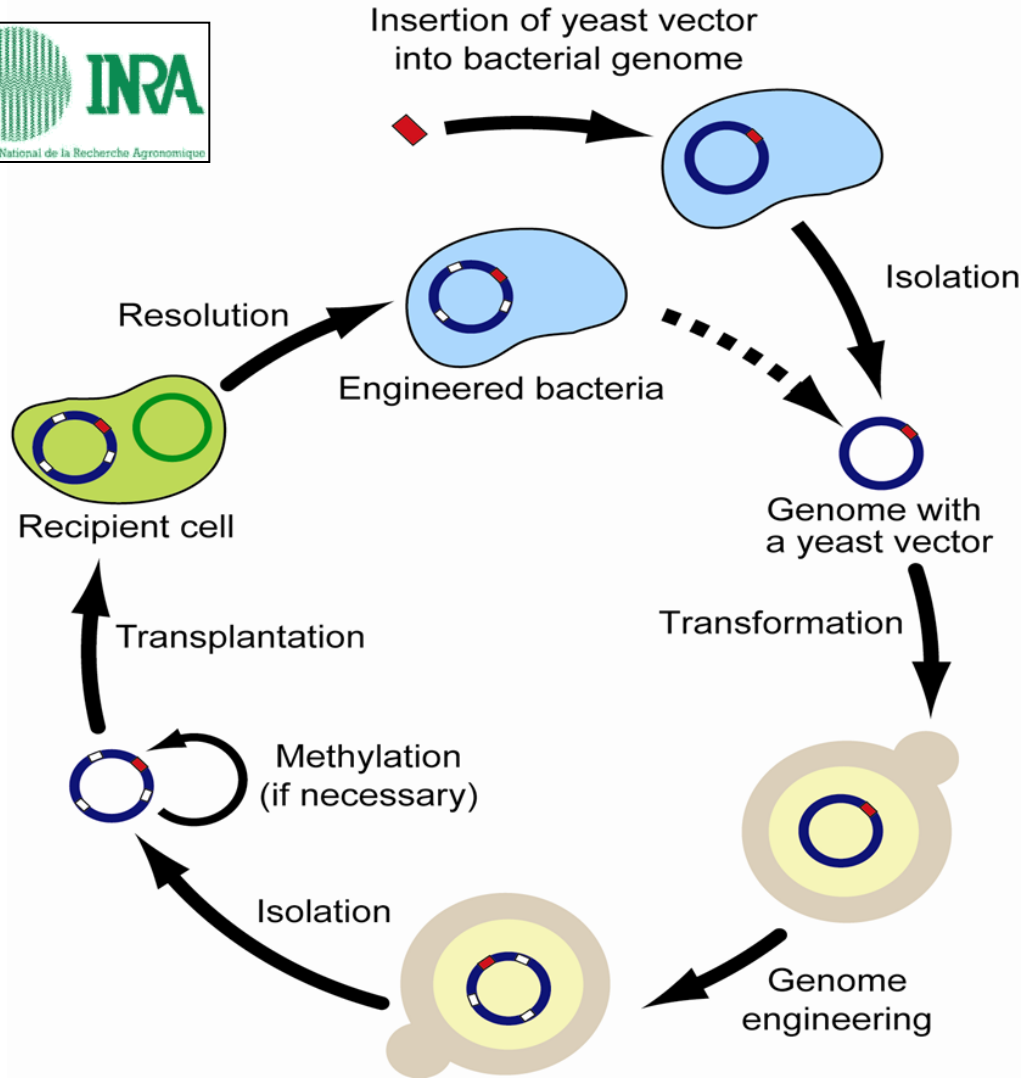
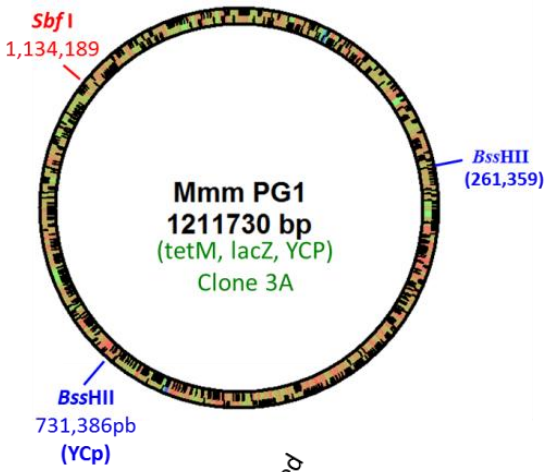
Aim 2. Adapt the JCVI synthetic biology tools to Mmm at INRA and transfer the technology to ILRI in Africa.

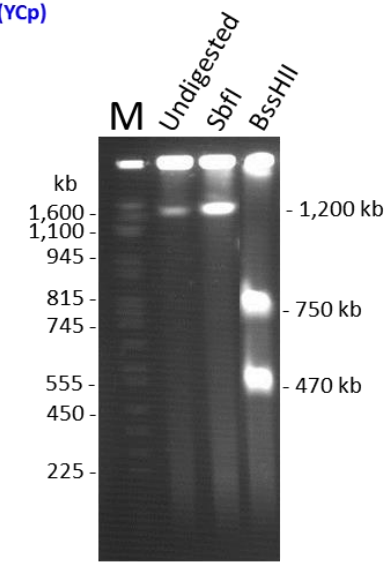


Aim 2. Adapt the JCVI synthetic biology tools to Mmm at INRA and transfer the technology to ILRI in Africa.

Cloning Mmm Genome in Yeast







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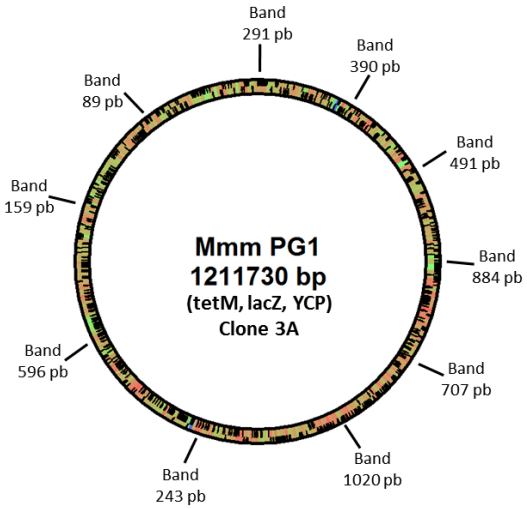
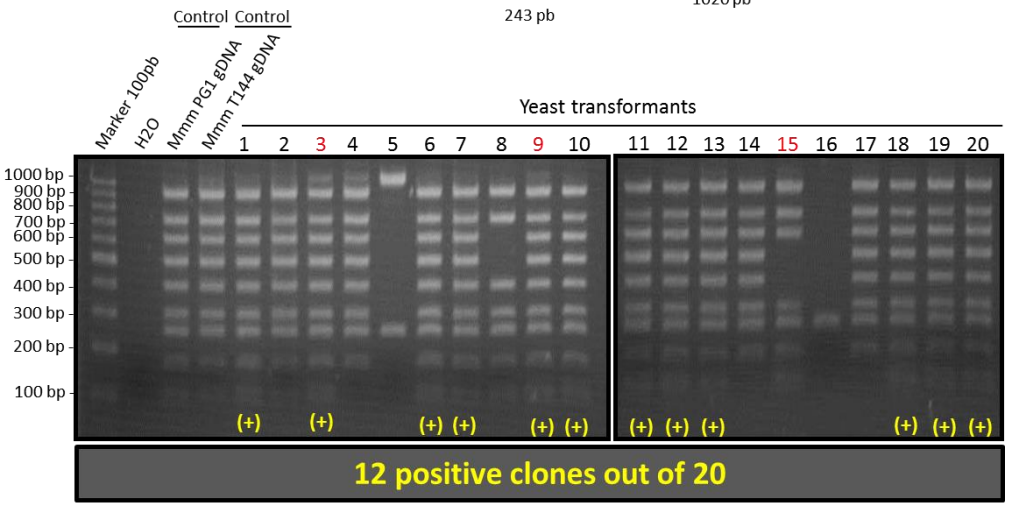
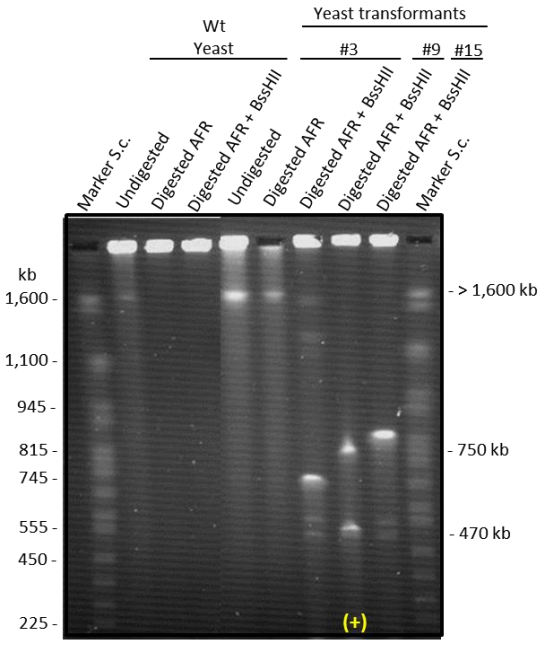
Aim 2. Adapt the JCVI synthetic biology tools to Mmm at INRA and transfer the technology to ILRI in Africa.

Cloning Mmm Genome in Yeast

Analysis of Yeast Clones by Multiplex PCR and PFGE



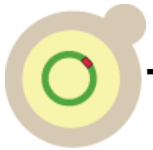




Notes:
- **Marker S.c.: marker Saccaromyces cerevisae**
- **AFR**: a cocktail of 3 enzymes (AsiSI, FseI, RsrII) that cut yeast gDNA but not Mmm genome
- **BssHII**: enzyme that cut Mmm genome twice

Current Mmm Strains in Yeast

PG1



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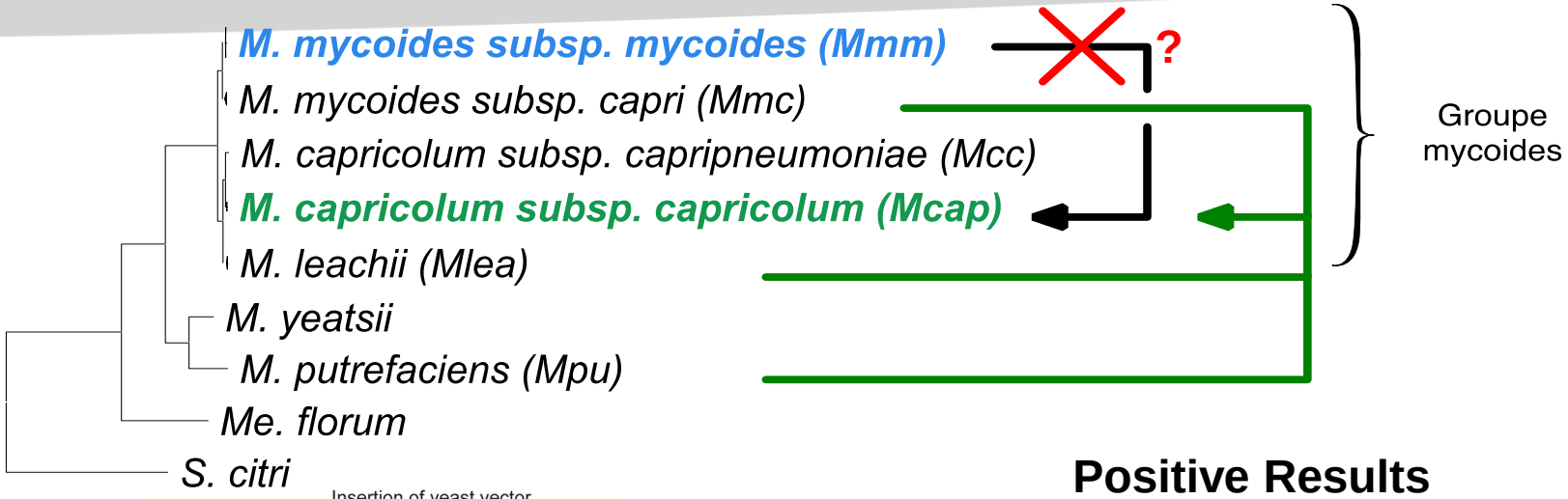
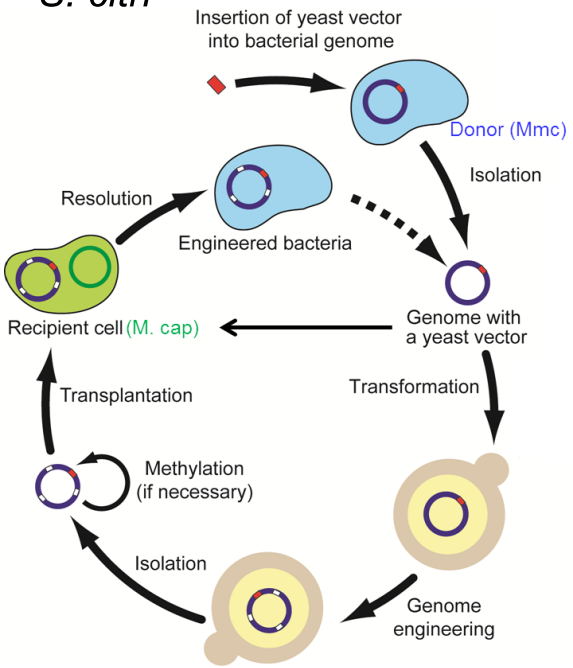
Genome Transplantation





Positive Results

Donor DNA	Recipient cell
Mmc	M. cap
M. leachii	M. cap
M. putrefaciens	M. cap
Mmc	M. leachii

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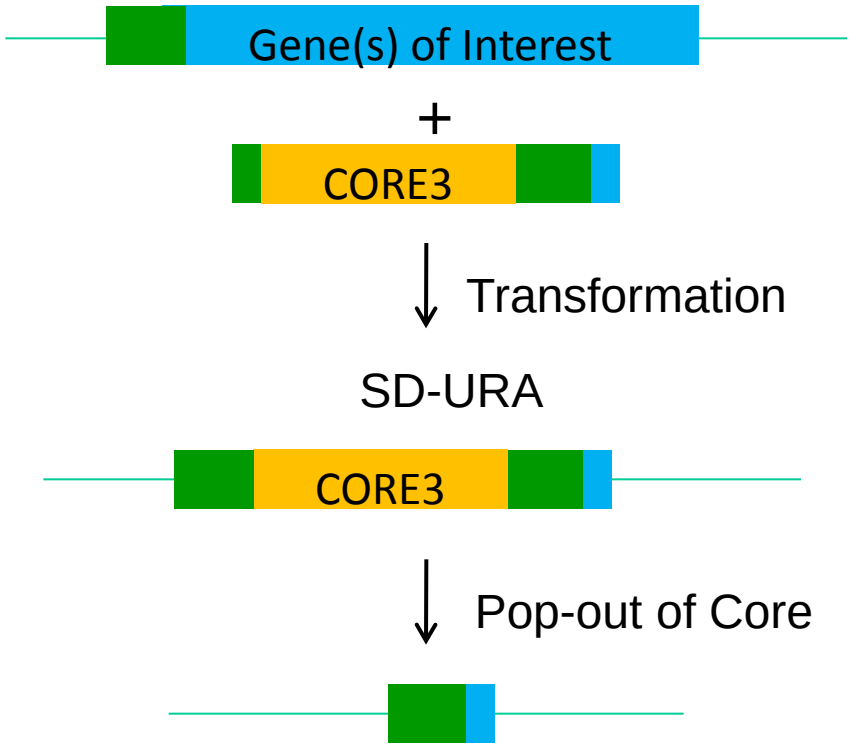
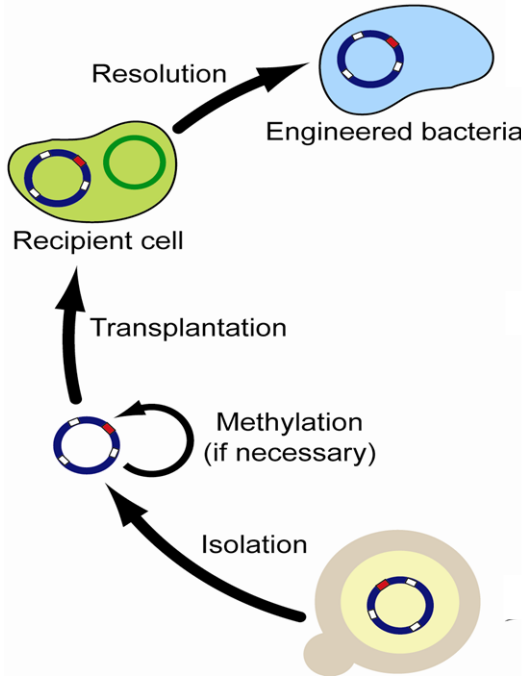
Aim 3. Establish a caprine model for pulmonary mycoplasma infections using the closely related pathogen Mmc.

→ Mutagenesis of *Mmc* virulence genes, characterization of *Mmc* mutants *in vitro*

→ *In vivo* testing of *Mmc* mutants using a goat infection model

Use yeast genetic tools on  Mmc





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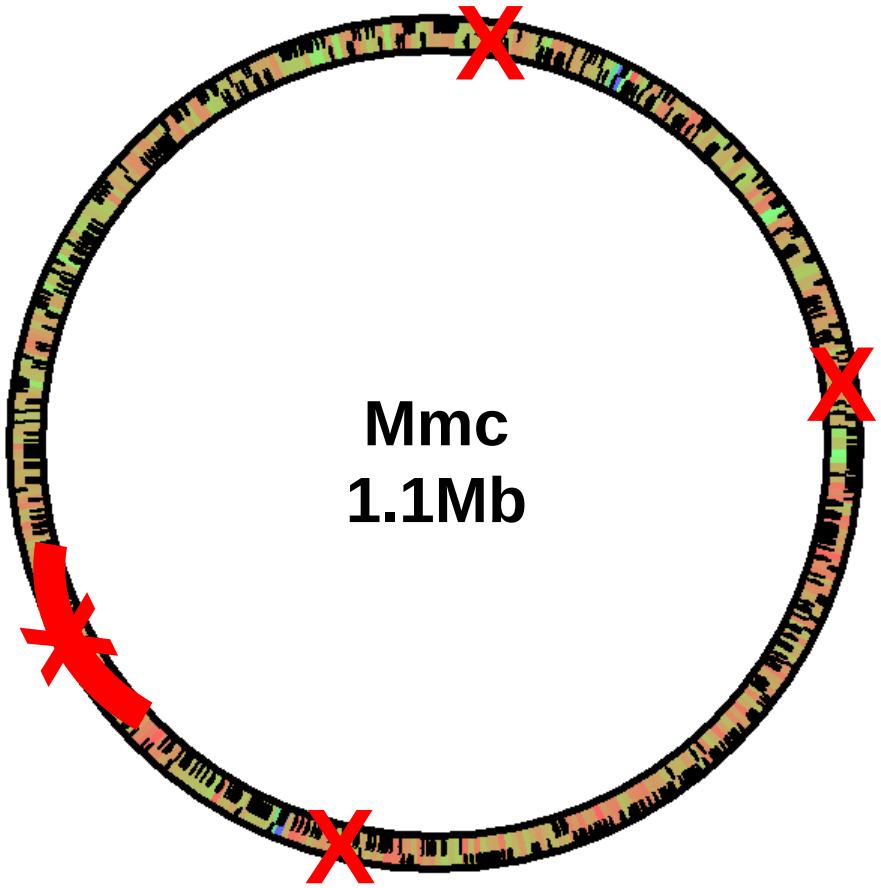

Current Status

- Over 40 genes removed so far from Mmc.

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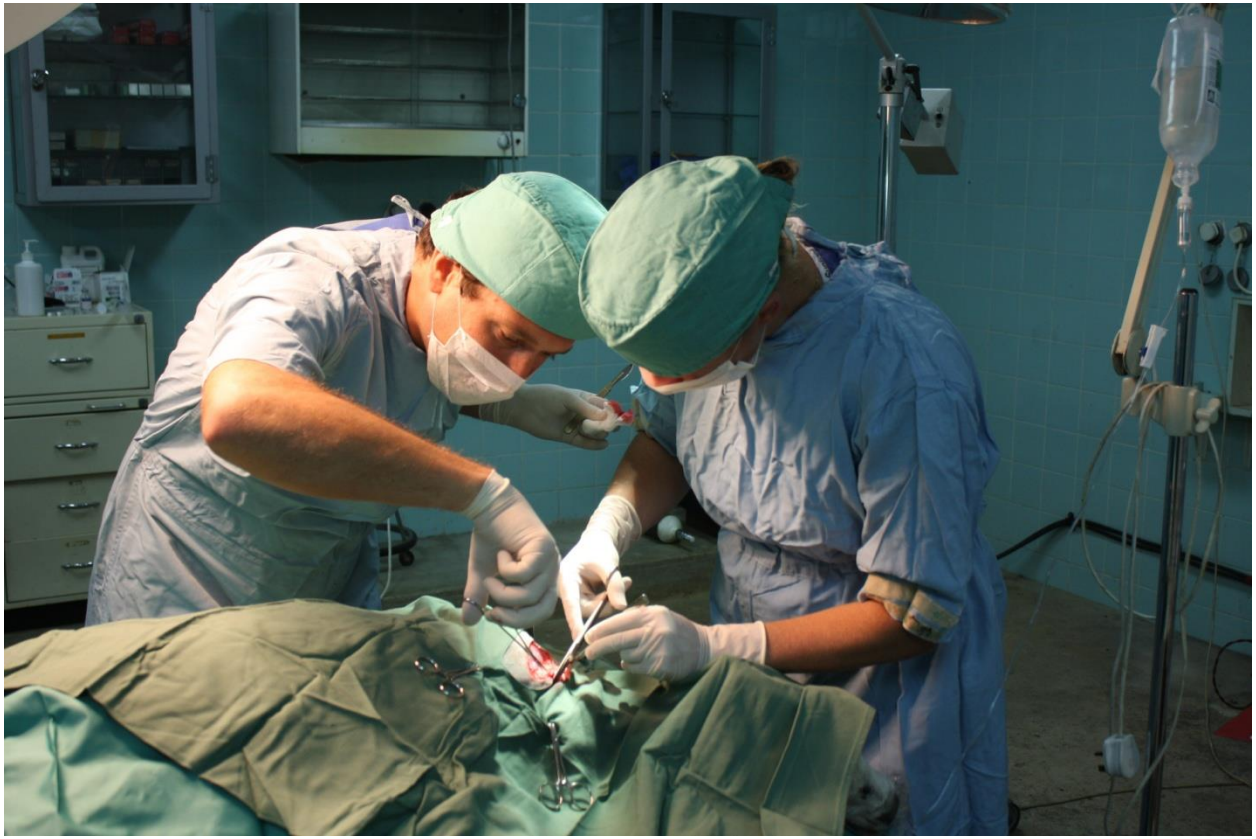

ILRI
INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE

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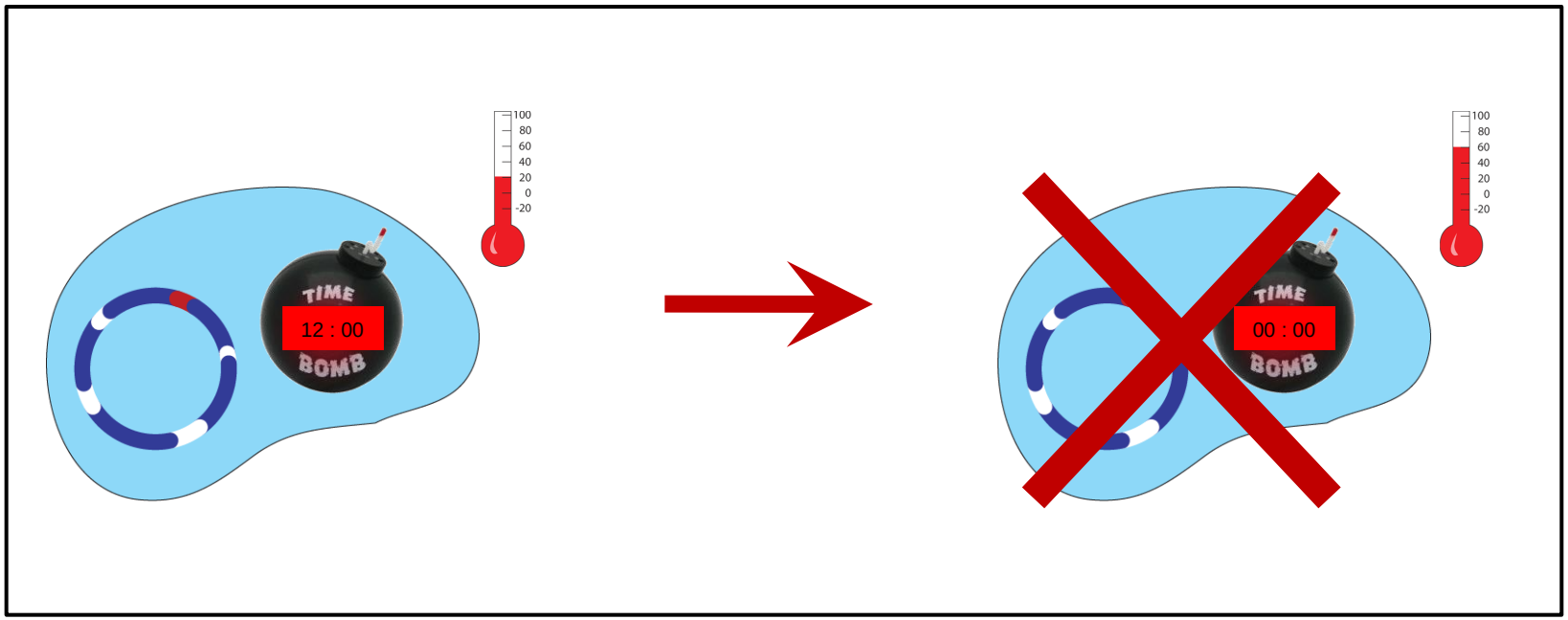
Aim 4. Expand mycoplasma toolbox using Mmc as a model to enhance our capacity to produce modern Mmm vaccines.

A. Expression of heterologous genes in Mmc and Mmm to enhance vaccine potential.

B. Design an Mmc strain that has a defined life-span or a kill switch.



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Construction of TS Bacterial Vaccines

Essential genes from Arctic bacteria used to construct stable, temperature-sensitive bacterial vaccines

Barry N. Duplantis[a], Milan Osusky[a], Crystal L. Schmerk[a], Darrell R. Ross[a], Catharine M. Bosio[b], and Francis E. Nano[a,1]

[a]Department of Biochemistry and Microbiology, University of Victoria, Victoria, BC, V8W 3P6 Canada; and [b]Laboratory of Intracellular Parasites, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT 59840

All bacteria share a set of evolutionarily conserved essential genes that encode products that are required for viability. The great diversity of environments that bacteria inhabit, including environments at extreme temperatures, place adaptive pressure on essential

that leads to lower enzyme stability, such as fewer salt bridges between protein domains, can be found in psychrophilic enzymes.

The introduction of mutations that make an essential gene product temperature-sensitive (TS) renders the host organism TS.

- Replace ligase, select cell division or molecular chaperone gene of target organism with counterpart gene from psychrophilic organism.

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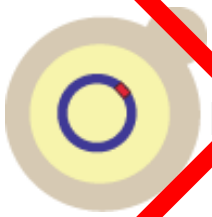
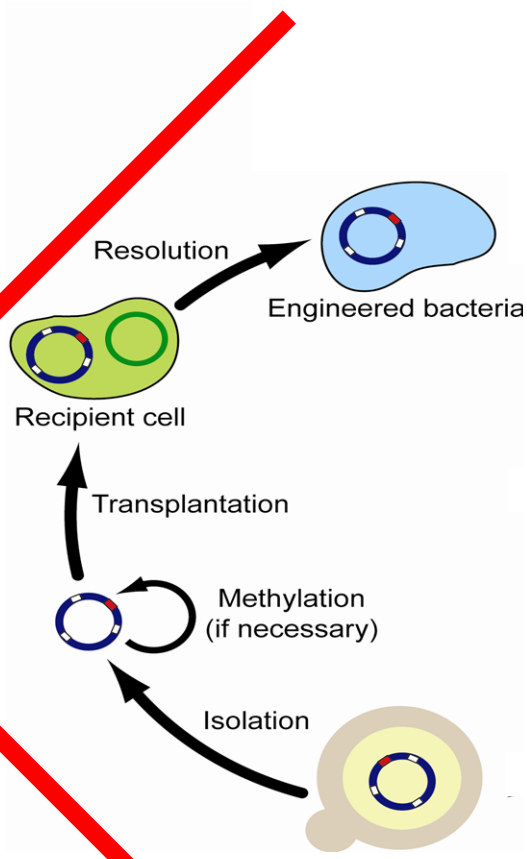
Construction of TS Bacterial Vaccines

Use yeast genetic tools on 



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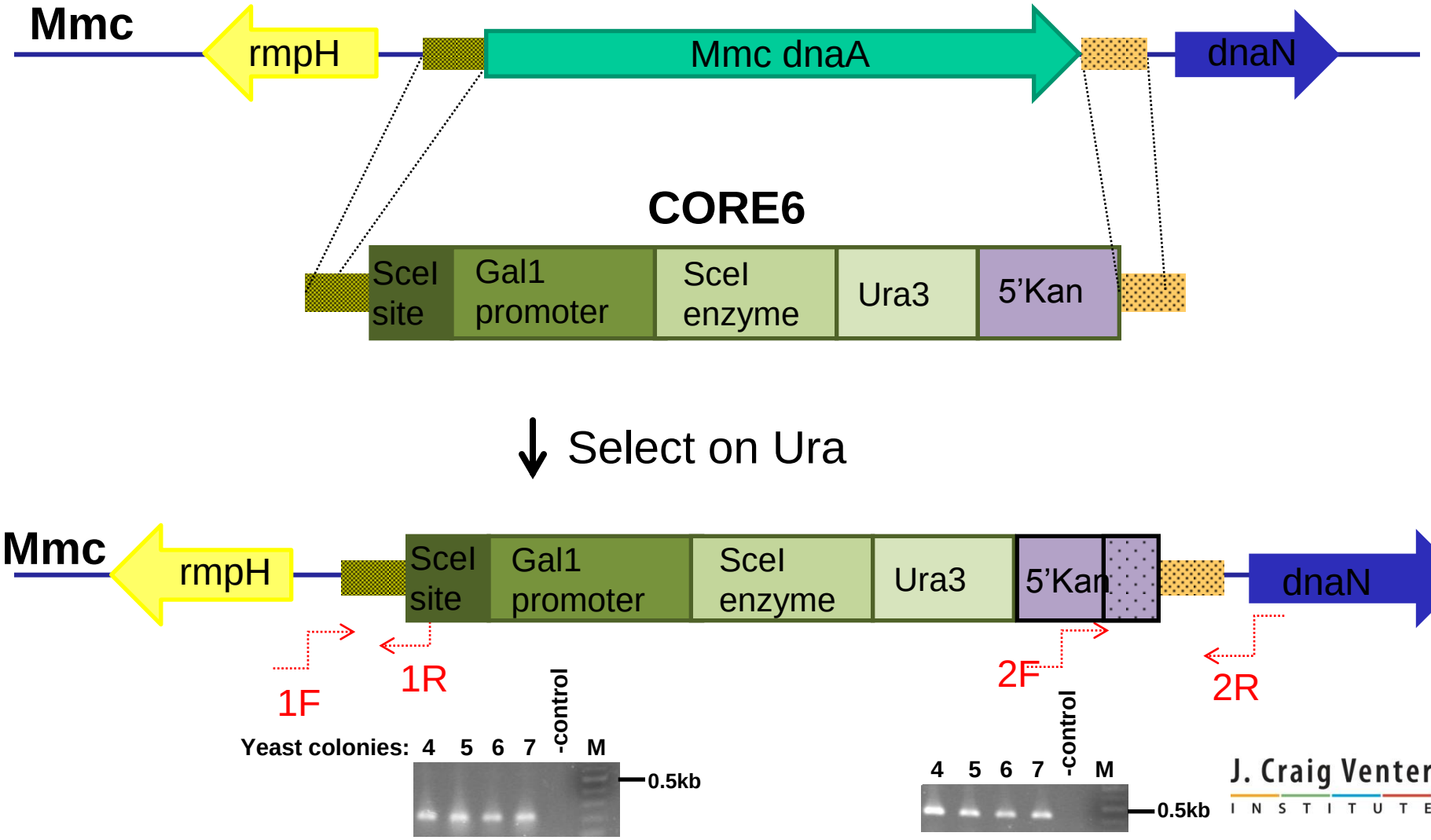
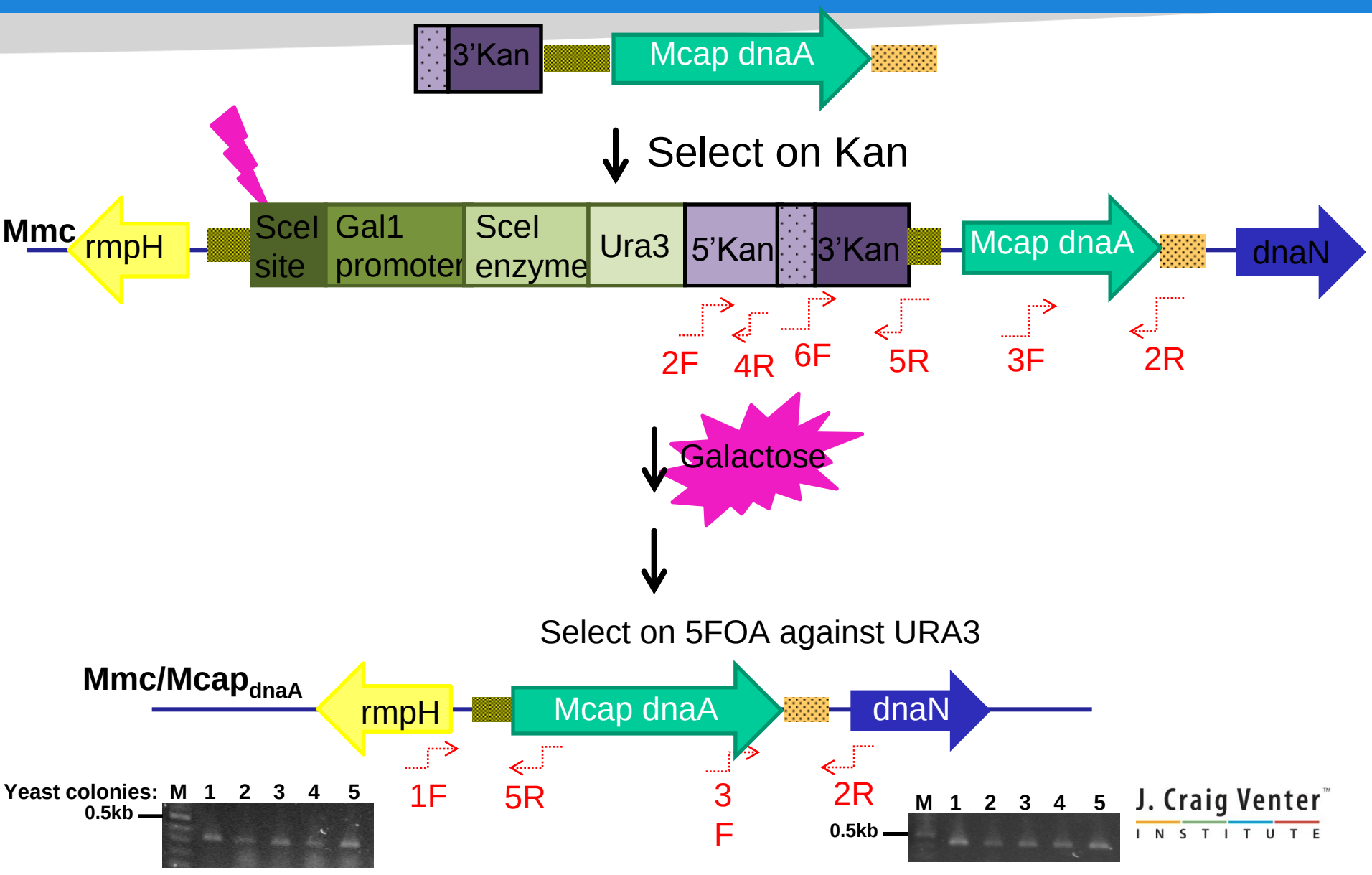
Method: Modified TREC

- Example: Replacement of Mmc dnaA gene with that of M.cap dnaA

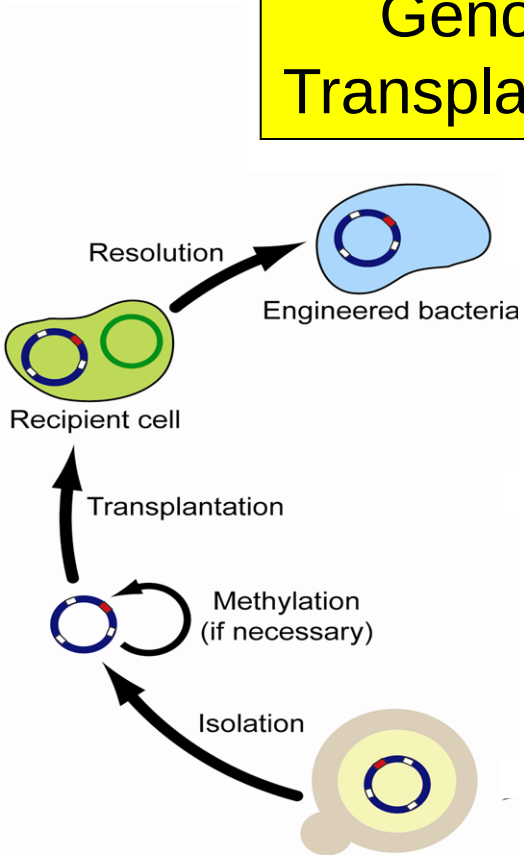


Method: Modified TREC (cont'd)

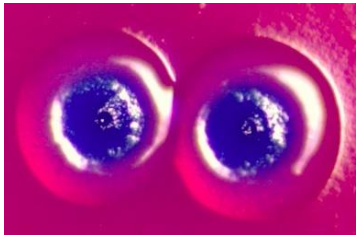


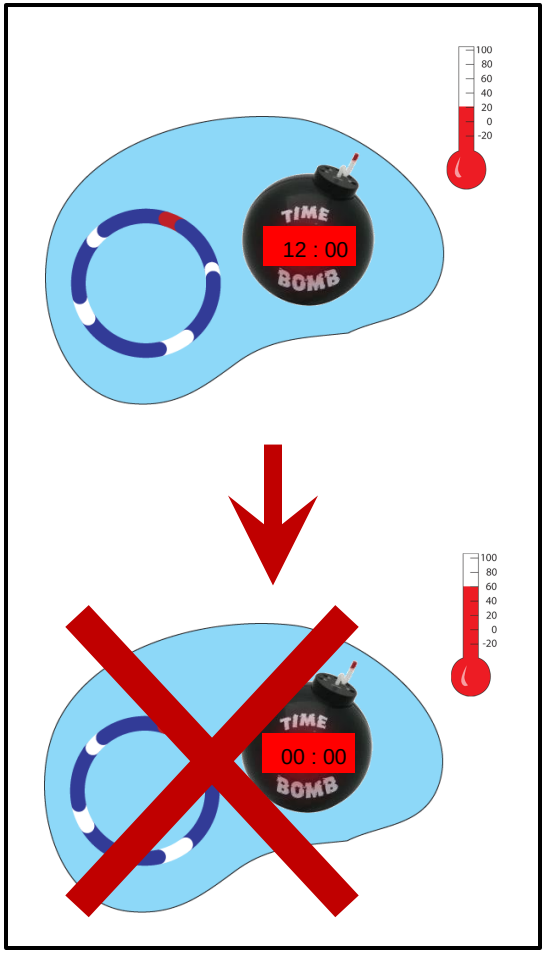
Method: Modified TREC (cont'd)

Genome Transplantation



Mmc/Mcap$_{dnaA}$





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Aim 4. Expand mycoplasma toolbox using Mmc as a model to enhance our capacity to produce modern Mmm vaccines.

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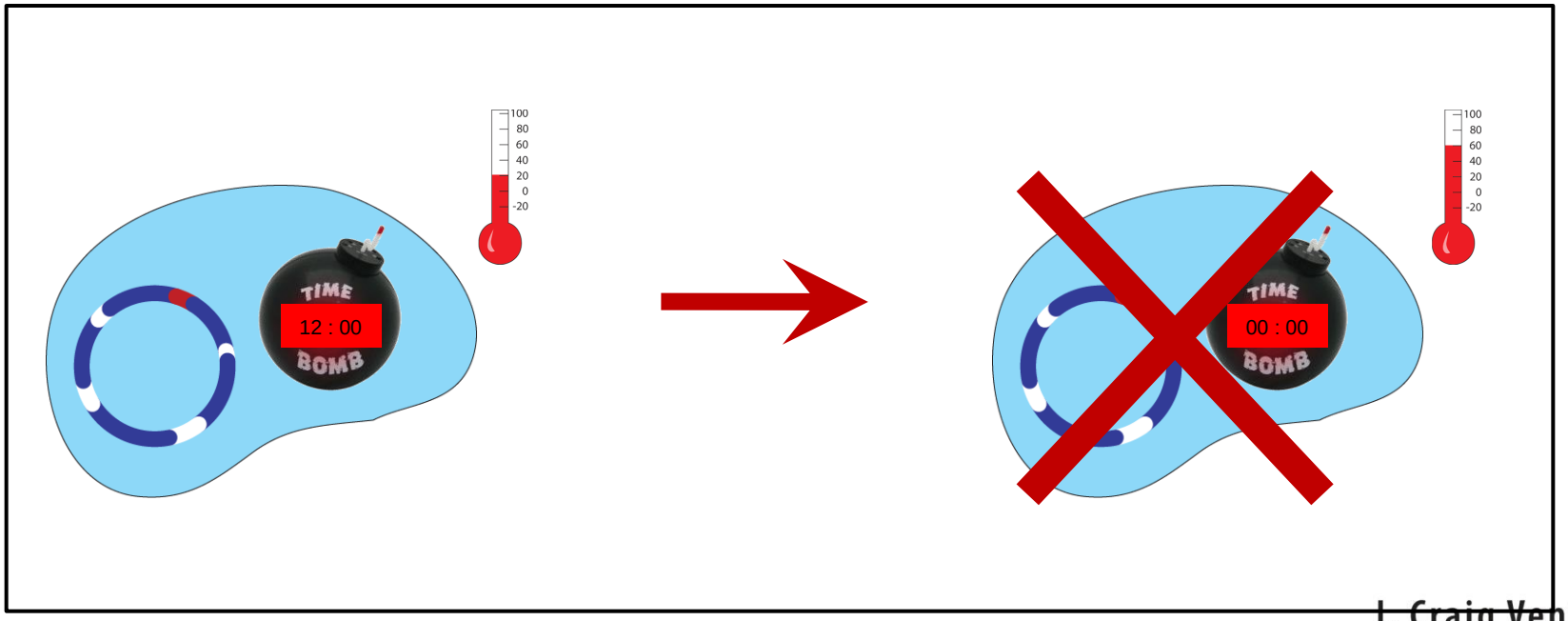

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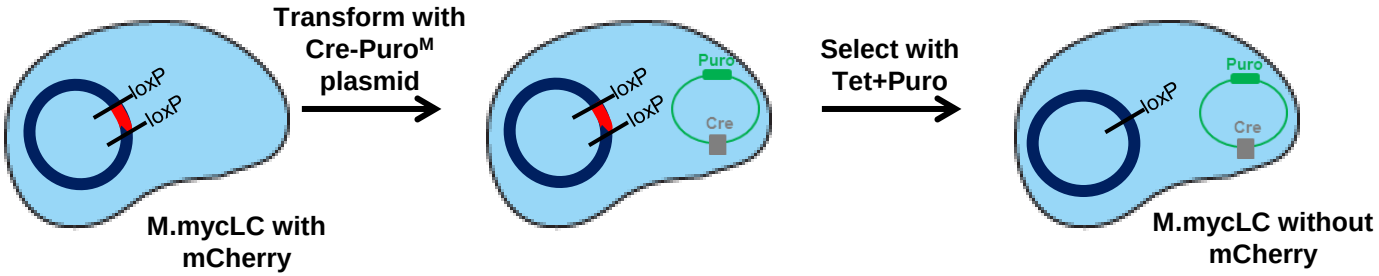
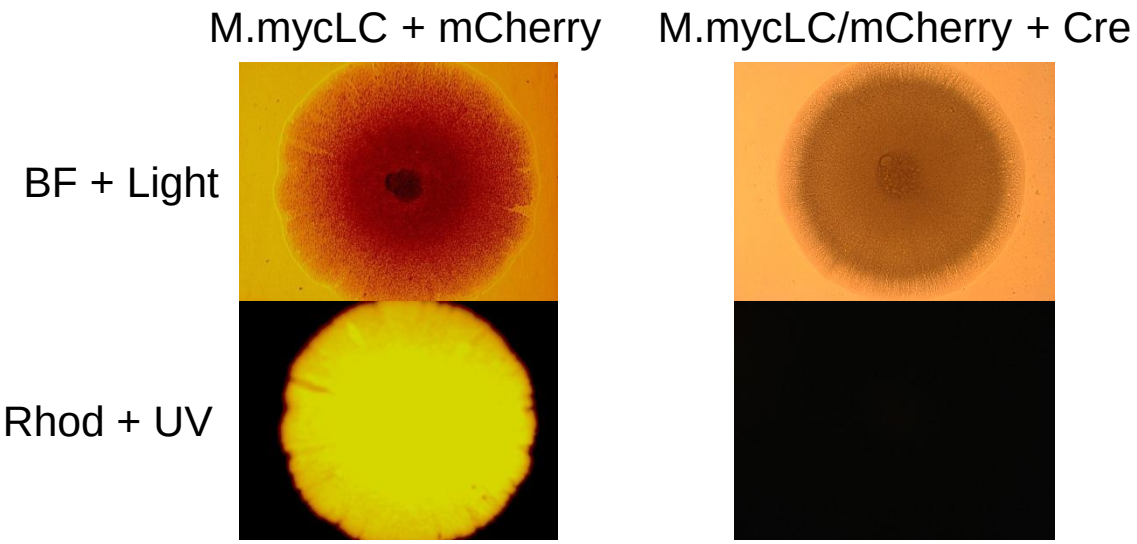
Aim 4. Expand mycoplasma toolbox using Mmc as a model to enhance our capacity to produce modern Mmm vaccines.

- **Cre-Lox system: test in Mmc**





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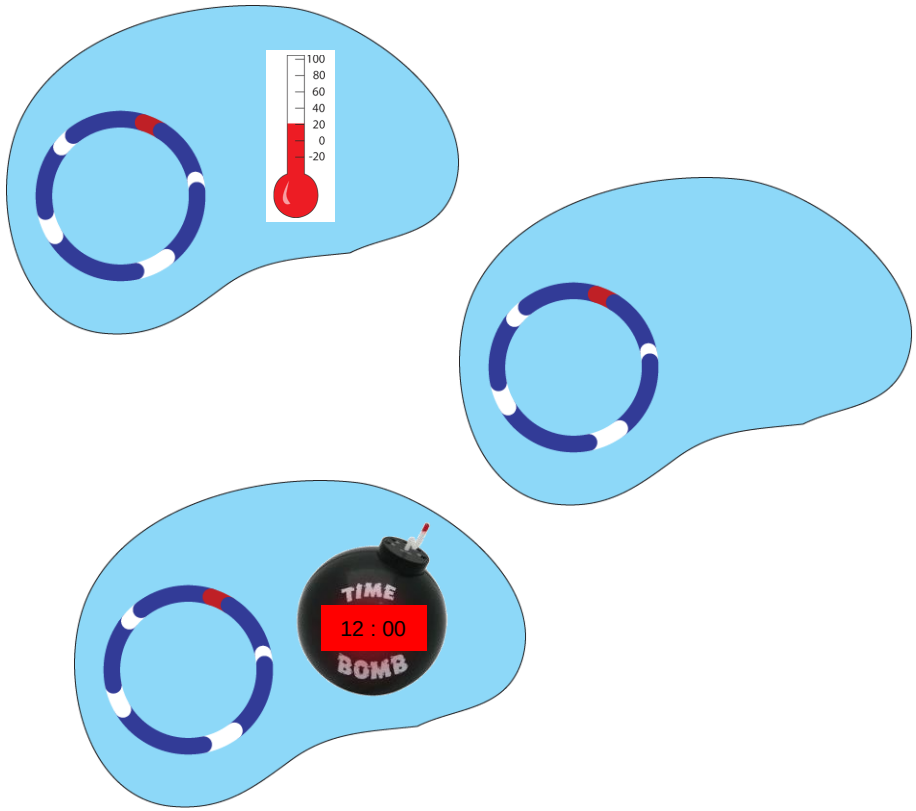
BREAD Recap



- Rational approach using newly developed technologies to produce a number of candidate vaccine strains

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Final Points

- Synthetic Biology is a powerful approach against infectious diseases.
- It can be used to identify new antimicrobials.
- There are applications for vaccines, both animal and human
- Work on computational tools for genome and pathway design is urgently needed.
- There has never been a more exciting time to be a biologist.

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It Takes a Village to Create a Cell

- Algire, Mikkel
- Alperovich, Nina
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- Dai, Jianli
- Denisova, Evgeniya
- Galande, Amit
- Gibson, Daniel
- Glass, John
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- Brownley, Anushka
- Parmar, Prashanth
- Pieper, Rembert
- Stockwell, Tim
- Sutton, Granger
- Viswanathan, Lakshmi
- Yooseph, Shibu

Ethical Considerations

- Michele Garfinkel
- Robert Friedman

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JCVI
DOE GTL program

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Clusters Personnel

JCVI (Clusters)
Chuck Merryman
Carissa Grose
Monica Gonzalez
Mikkel Algire

NIAID
Maria Giovanni

J. Craig Venter™ INSTITUTE

BREAD Personnel

JCVI (MD, USA)

- Suchismita Chandran
- Sanjay Vashee
- Ray-Yuan Chuang
- Li Ma
- Nacyra Assad-Garcia
- Sheetala Vijaya
- Caitlyn Whiteis
- John Glass

INRA (France)

- Carole Lartigue
- Anne Lebaudy
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- Pascal Sirand-Pugnet

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- Elise Schieck (BMZ)
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