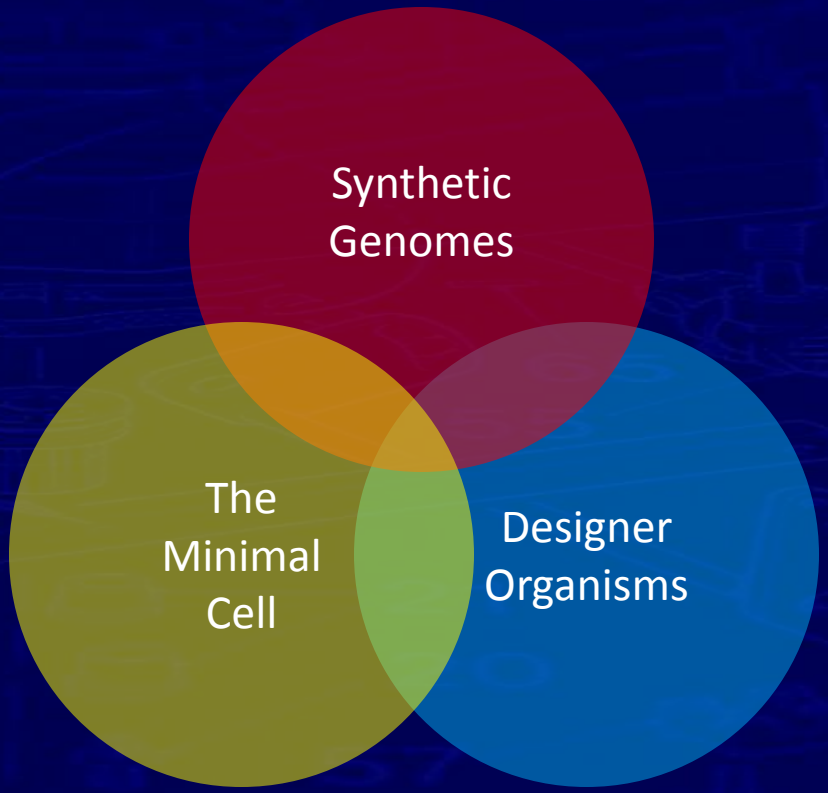
Synthetic Biological Systems

2. Synthetic Life and Genome Engineering

The construction of synthetic organisms

Synthesising biological life is already a reality



Fascinating big-ticket projects

Making the minimal cell

- Bottom-up approach to build from parts
- Top-down approach to reduce natural cells

Building the first synthetic organism

- J. Craig Venter Institute

Re-factoring a genome

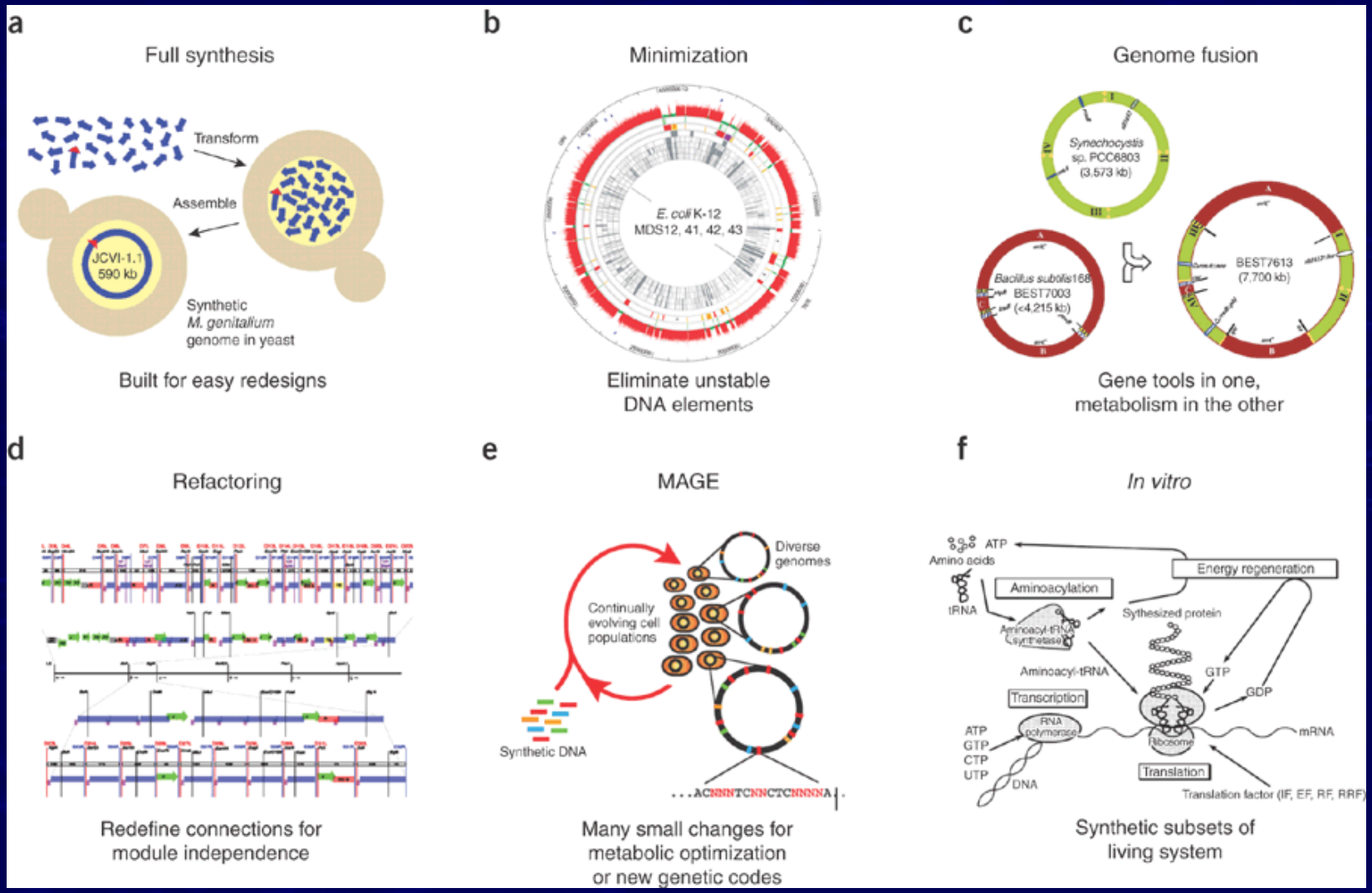
- Re-write the genetics of a cell to suit our needs
- “Genomic Engineering”

Lecture Content

In this lecture we'll learn about:

1. Craig Venter's first cell made with a synthetic genome
2. The DNA assembly techniques used to build a genome
3. Rival attempts at genome-scale engineering
4. The top-down approach to creating a minimal cell
5. The bottom-up approach to creating a minimal cell
6. Examples of minimal cells in nature
7. Refactoring genomes and techniques for editing genomes
8. How to go about designing a new genome
9. The many applications of minimal cells and engineered genomes

Genome Engineering



Two major groups are engineering synthetic life

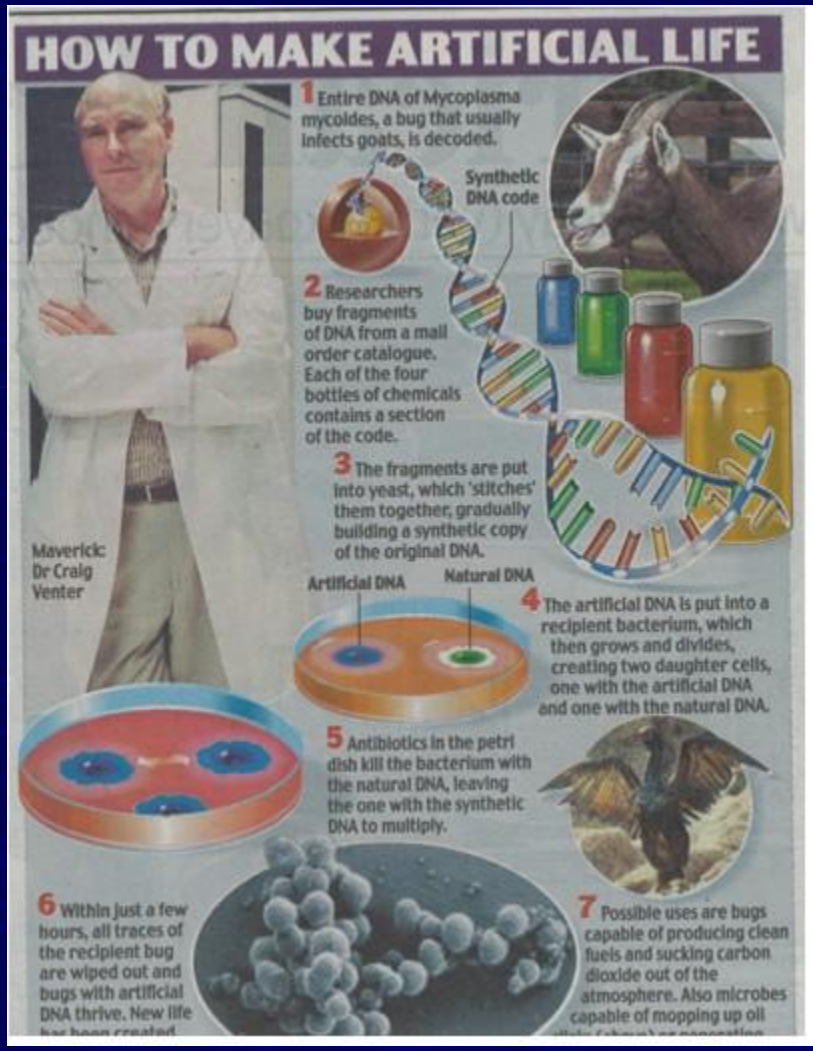
LS9, INC.

George Church
Harvard

J. Craig Venter
JCVI

SYNTHETIC GENOMICS

The first synthetic cell – ‘Synthia’



The first synthetic organism – JCVI Project

The 1st synthetic organism – life made from a chemically synthesized genome

tRNA gene synthesized – Nobel Prize for Khorana

Phage/Virus genomes synthesized – synthesis of polio virus 2002

Viruses re-factored – separate each gene in a human-designed logical way

2010... Bacteria

A big two-part project by the J Craig Venter Institute

Part 1: Can a complete DNA genome be synthesized from chemicals	2008
Part 2: Can a cleaned DNA genome boot-up a cell	2007
Synthetic Organism = Parts 1 and Parts 2 combined	2010

The first synthetic organism: (2) Booting-up

AIM: Genome A into Cell B → turn Cell B into Cell A

A: *M.mycodies* B: *M.capricolum* *different but compatible biology

C Lartigue *et al*. Science 2007

Comparable to nuclei-switch experiments in *In Vitro* Fertilisation

Genomes are fragile to handle in the lab – maintain in agarose plugs

How to get DNA into cell B? – incredibly inefficient, requires cell fusions (no cell wall)

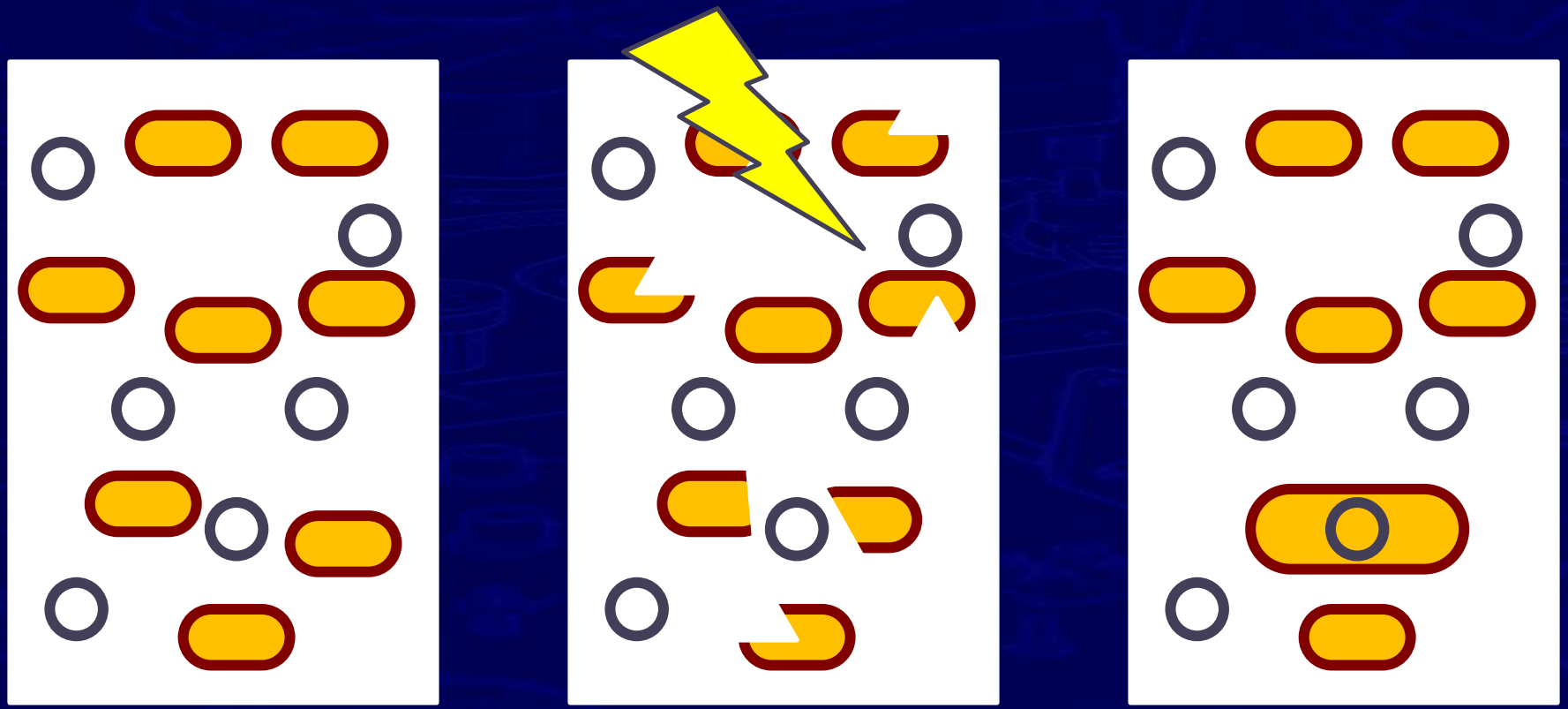
What happens to genome of cell B? – doesn't have antibiotic resistance

Verify with sequencing, proteomics and phenotyping - Expensive

The first synthetic organism: (2) Booting-up

Successful cell fusion is a very rare event for bacteria

Works with Mycoides but would be tough with bacteria with cell walls



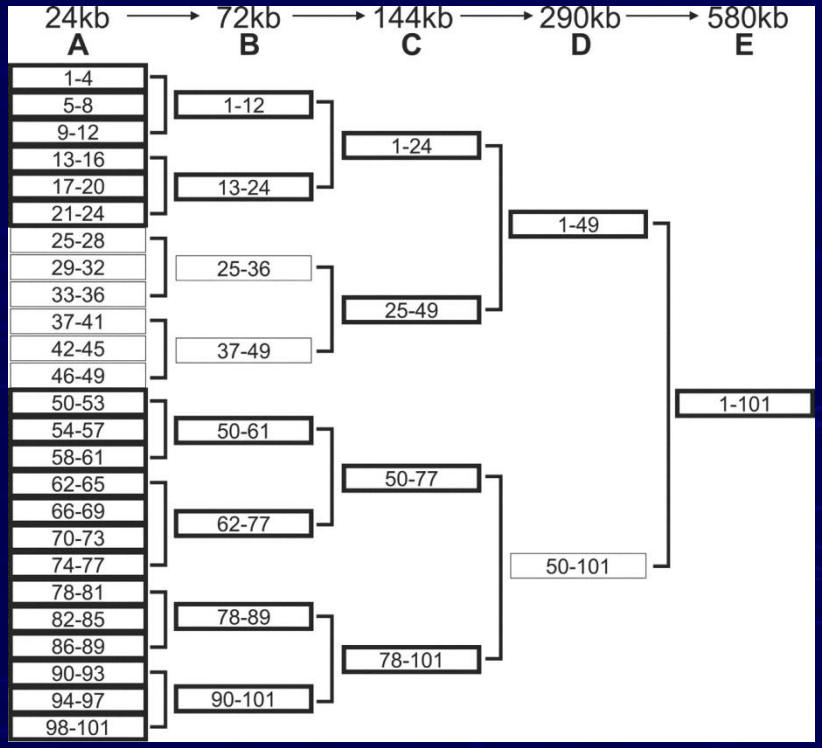
The first synthetic organism: (1) Synthesis

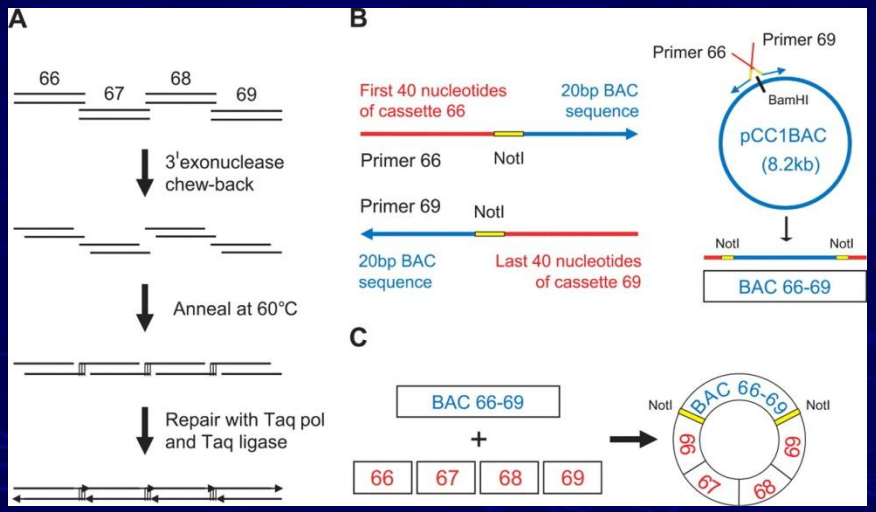
AIM: Synthesize ~10^4 DNA 50-base oligomers and assemble into a complete error-free 582970 bp *M.genitalium* genome (watermarks)

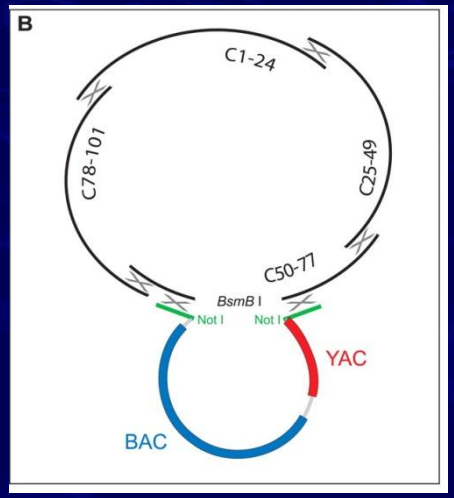
1. Companies synthesise 101 pieces of 5 to 7 kb from overlapping oligos (e.g. Blue Heron and GeneArt)
2. 101 pieces recombined using *in vitro* enzymes to make 24 big pieces
3. 24 big pieces maintained in BACs in *E.coli* and recombined to make even bigger pieces
4. Big pieces all inserted into yeast and whole circular genome is made by recombination using native yeast genetics (using a YAC)
5. DNA sequencing used to check fidelity throughout process

DG Gibson *et al*. Science 2008

The first synthetic organism: (1) Synthesis







101 synthesized fragments with overlap
Stepwise *in vitro* DNA assembly using a new method
Final assembly using yeast as the vector

Gibson Isothermal Assembly (1)

Daniel Gibson's PCR-free method of annealing overlapping DNA sequences

- Overlapping sequences need to be 30 or more bp
- Requires a cocktail of T5 Exonuclease, Taq Ligase and Phusion Polymerase

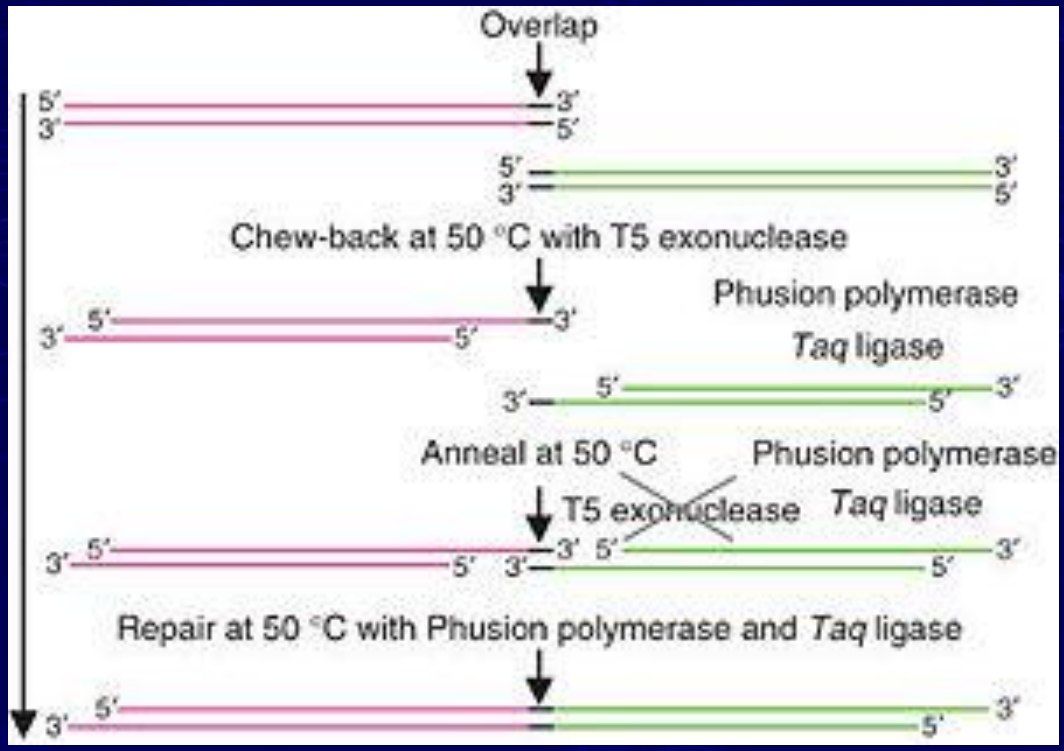
Make and store a master-mix for months

Takes 30 minutes at 50 degrees C

Very simple and scalable

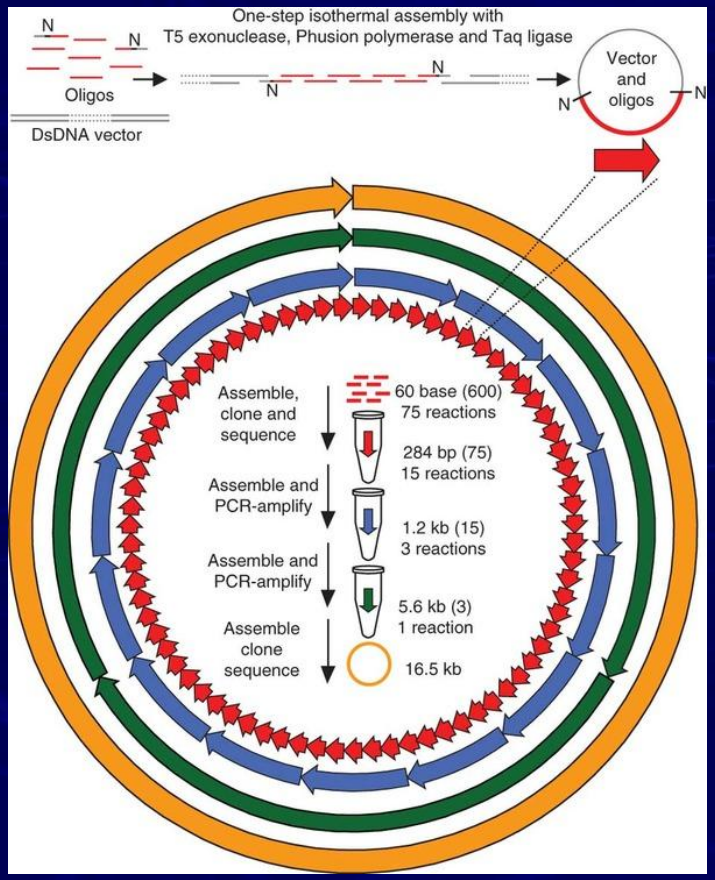
In vitro and automatable

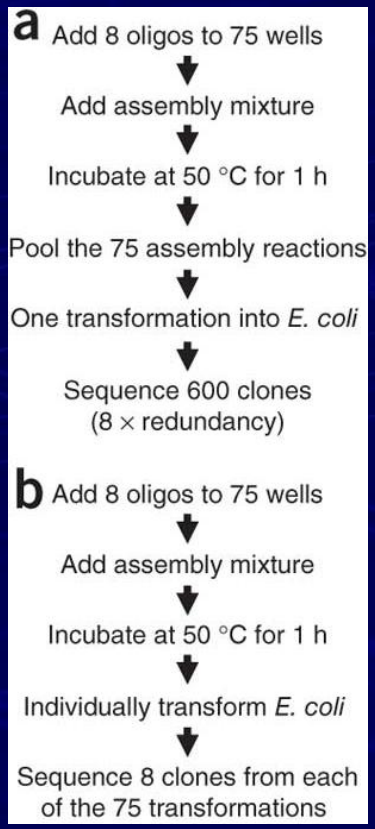
Requires overlapping DNA



Gibson Isothermal Assembly (2)

Gibson Isothermal Assembly used as the only method to create a whole Mouse Mitochondrial Genome. DG Gibson *et al.* Nature Methods 2010





GIBTHON

GENEART
THE GENE OF YOUR CHOICE

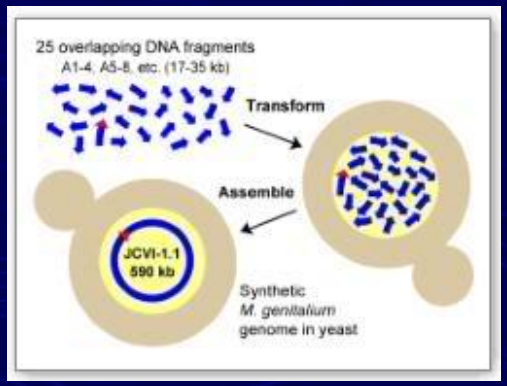
Yeast Assembly (TAR cloning)

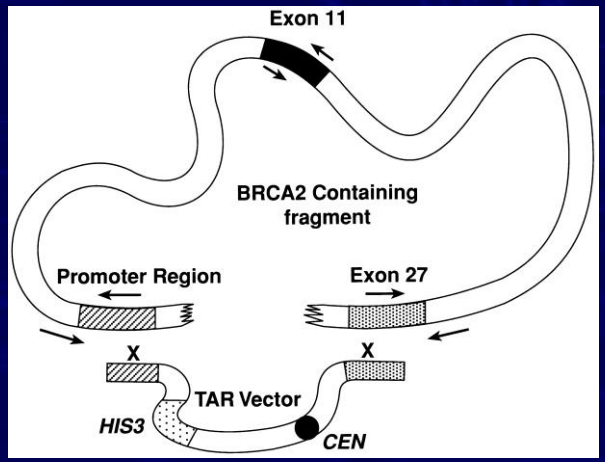
Yeast can be transformed with DNA and will assemble overlapping DNA

Quite an old method
'TAR Cloning'

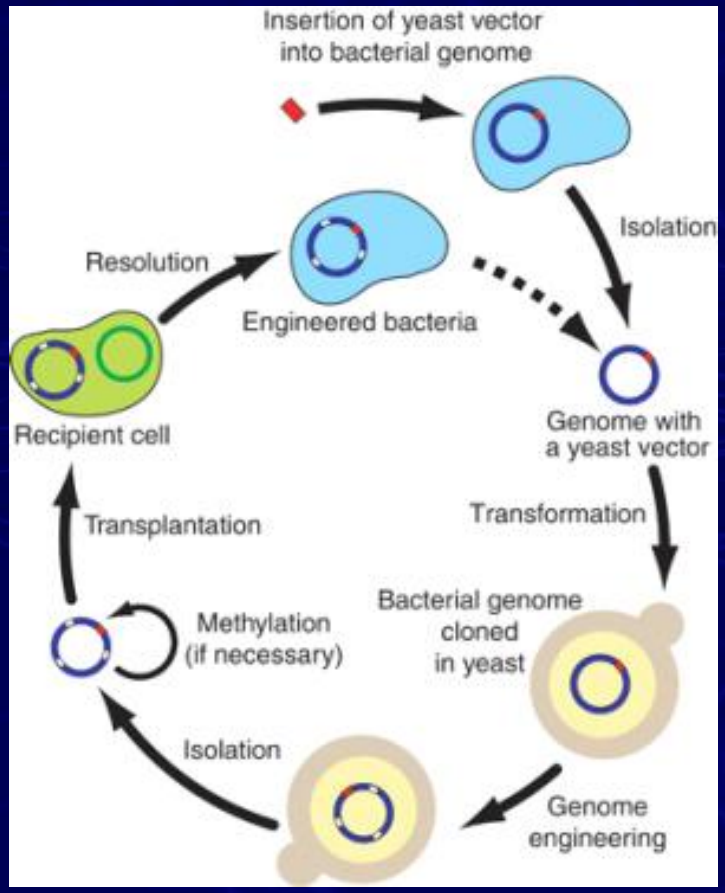
Overlaps need to be about 40 bp or more

Yeast can take >2.5 Mbp





All you need is yeast!



Finally making Synthia- published in 2010

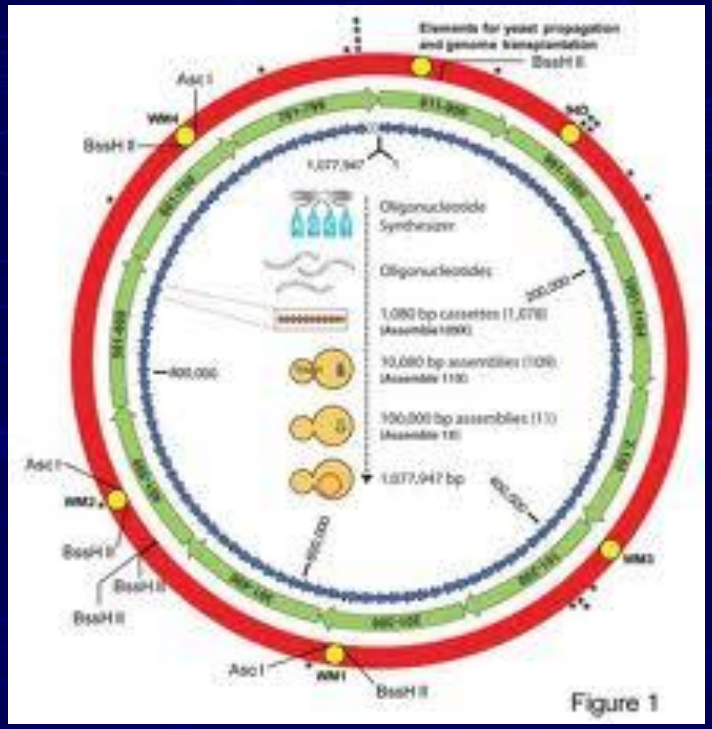
How they got it to work: D.G. Gibson *et al* 2010, Science 329 (5987): 52-56

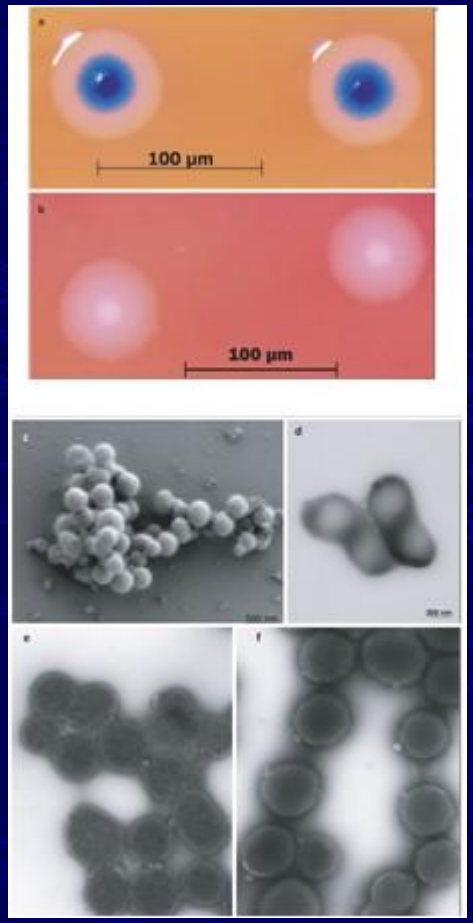
Methylation and restriction enzymes were causing trouble in *M.genitalium* – natural immune system for bacteria

Switched to a different cell *M. Mycoides* (worked before!)

M. Mycoides not a minimal genome cell

Single-base error in synthesis set them back by months





Other genome-scale projects

M Itaya *et al.* 2005 PNAS

Fused together the complete genome of 3.5Mbp *Synechocystis* into the 4.2 Mbp *B.subtilis* genome (Megacloning)

- Used a method to slowly add in the DNA – inchworm method
- Create a bacillus that can do photosynthesis?
- No – bacillus just silences almost all of the foreign DNA

Holt Lab: build *H.influenzae* genome as BACs in *E.coli* – incompatible
Only about half of the genes cloned in expressed at all

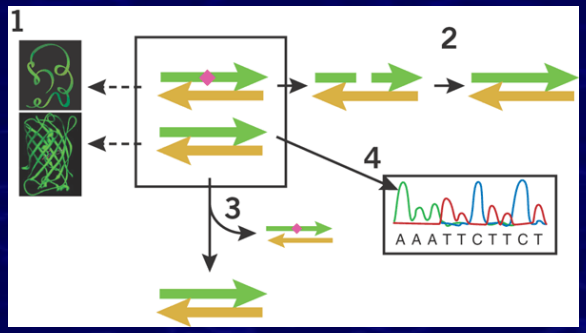
RL Warren *et al.* 2008 Genome Res

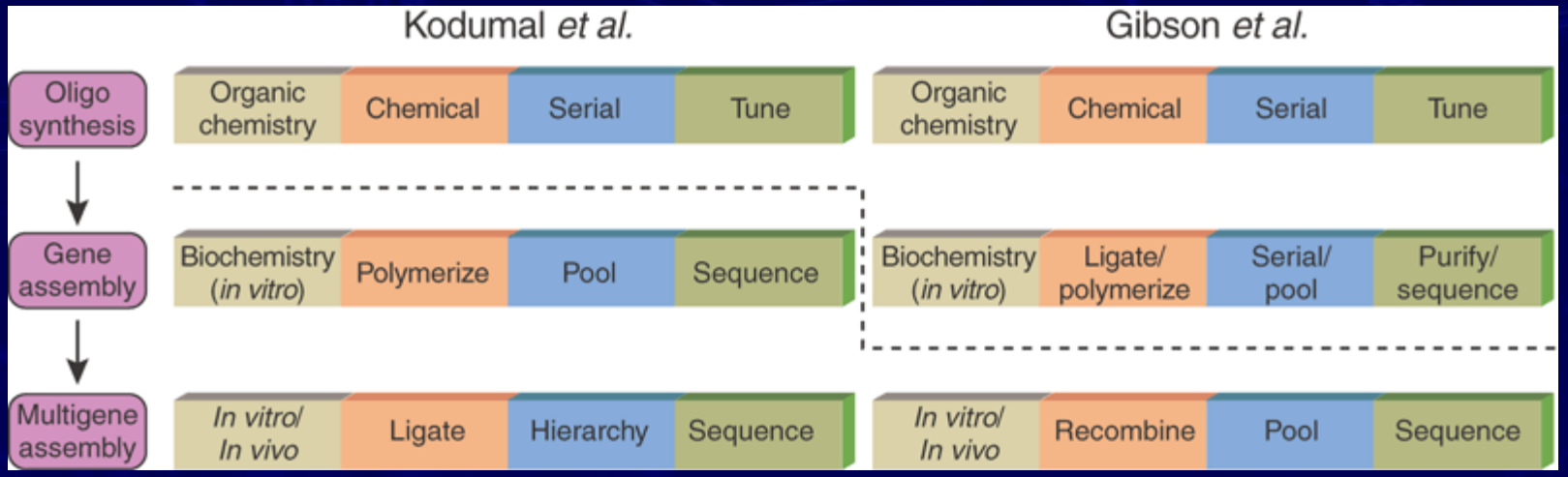
S Kodumal *et al.* 2004 PNAS Total synthesis of long DNA sequences: Synthesis of a contiguous 32-kb polyketide synthase gene cluster

- Famous example of a large DNA construct synthesised and working in cells

Genome Engineering Stages and Technologies

1. Synthesis — traditional oligo chemistry, on-chip polymerisation, cell factories
2. Joining — chemical, ligation, polymerisation, recombination
3. Assembly — serial, heirarchical, parallel, pooling
4. Error control — selection, tuning, repair, purification, sequencing





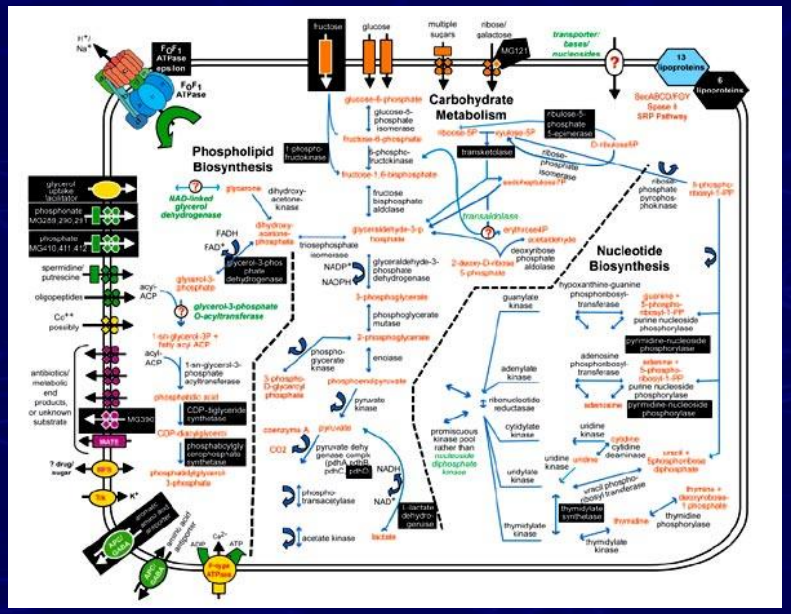
The minimal cell: top-down approach

Smallest natural genomes = 500 genes 500000 bps of DNA (e.g. *M.genitalium*)

But... not all genes are required for lab-based growth

How many essential genes?

1. Compare DNA throughout nature to identify essential genes
   Estimates: 50 to 380
2. Knock-out (delete) genes of small genomes to see what is needed
   Estimate: 430



Around a quarter of genes identified by these screens have unknown function

How do we really know that a gene is essential and not just playing many roles in a network?

Natural Minimal Cells

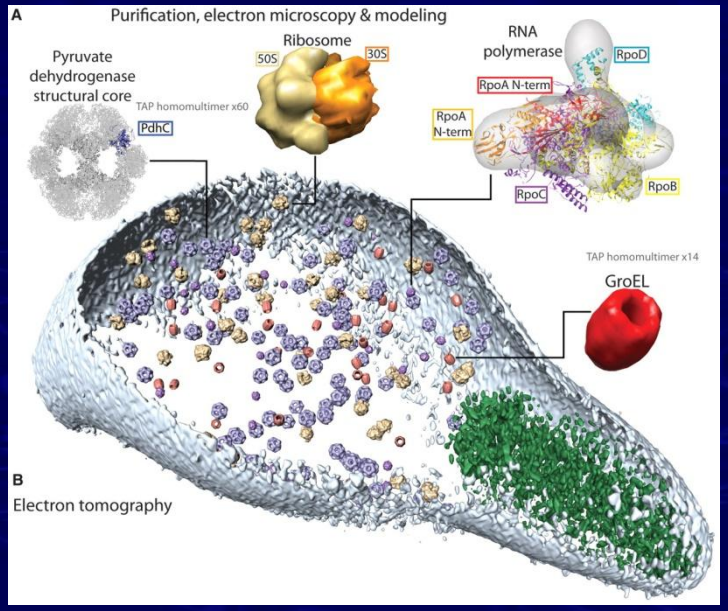
M.genitalium, Pelagibacter ubique, Nanoarchaeum equitans
Carsonella ruddii (213 genes – 160 kbp)
Hodgkinia cicadicola (188 genes – 144 kbp)

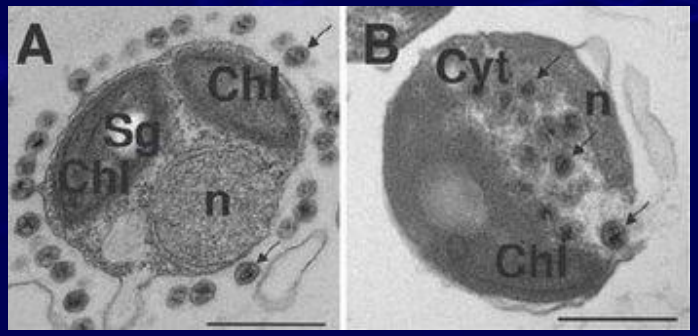
Many are not free-living but either parasites or symbionts

Mycoplasma pneumoniae has recently been studied in detail
Science: 27th November 2009
Guell *et al.* – Systems biology study
Yus *et al.* - Metabolism study
Kunhner *et al.* - Proteome study

Ostreococcus tauri, the smallest known free-living photosynthetic eukaryote.

12.56-Mb genome with high gene density

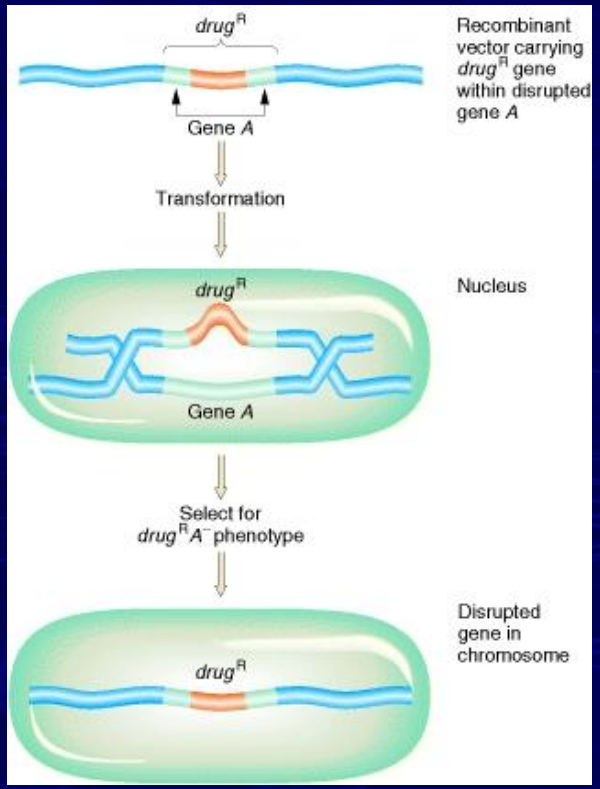




The minimal cell: top-down methods

In vivo reduction

- Traditionally done using transposons and recombinases
- Knock-out genes at random and work out which aren't essential



- Venter's plan = *Mycoplasma laboratorium*
- Synthesise a version of *M.genitalium* with only the essential genes

Venter 2010: "We can ascribe no function to almost 100 of approximately 370 [essential] genes in *M.genitalium*"

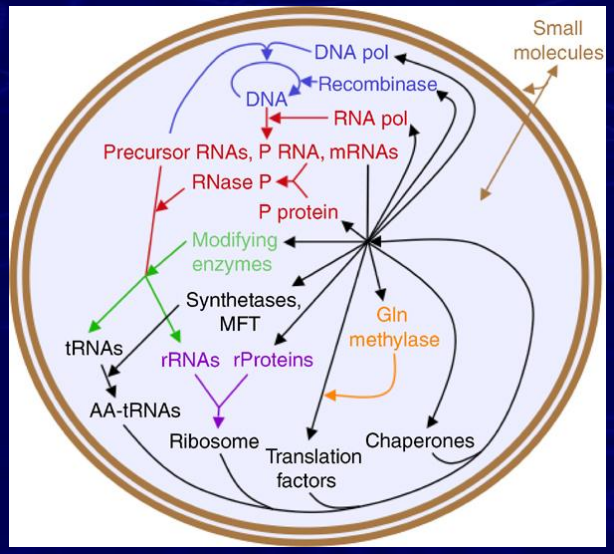
M.Genitalium naturally has 525 genes

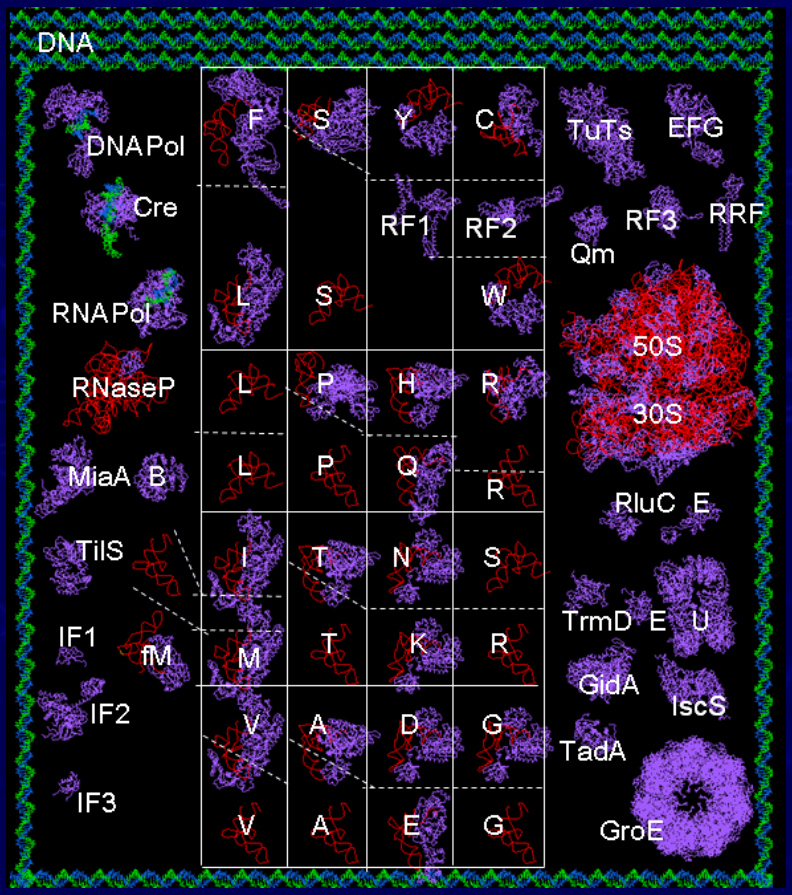
The minimal cell: bottom-up approach

"We know enough about a cell to identify the essential molecules and build our own from scratch" - hardcore synthetic biology

Biochemistry identifies the essential molecules that make cellular life

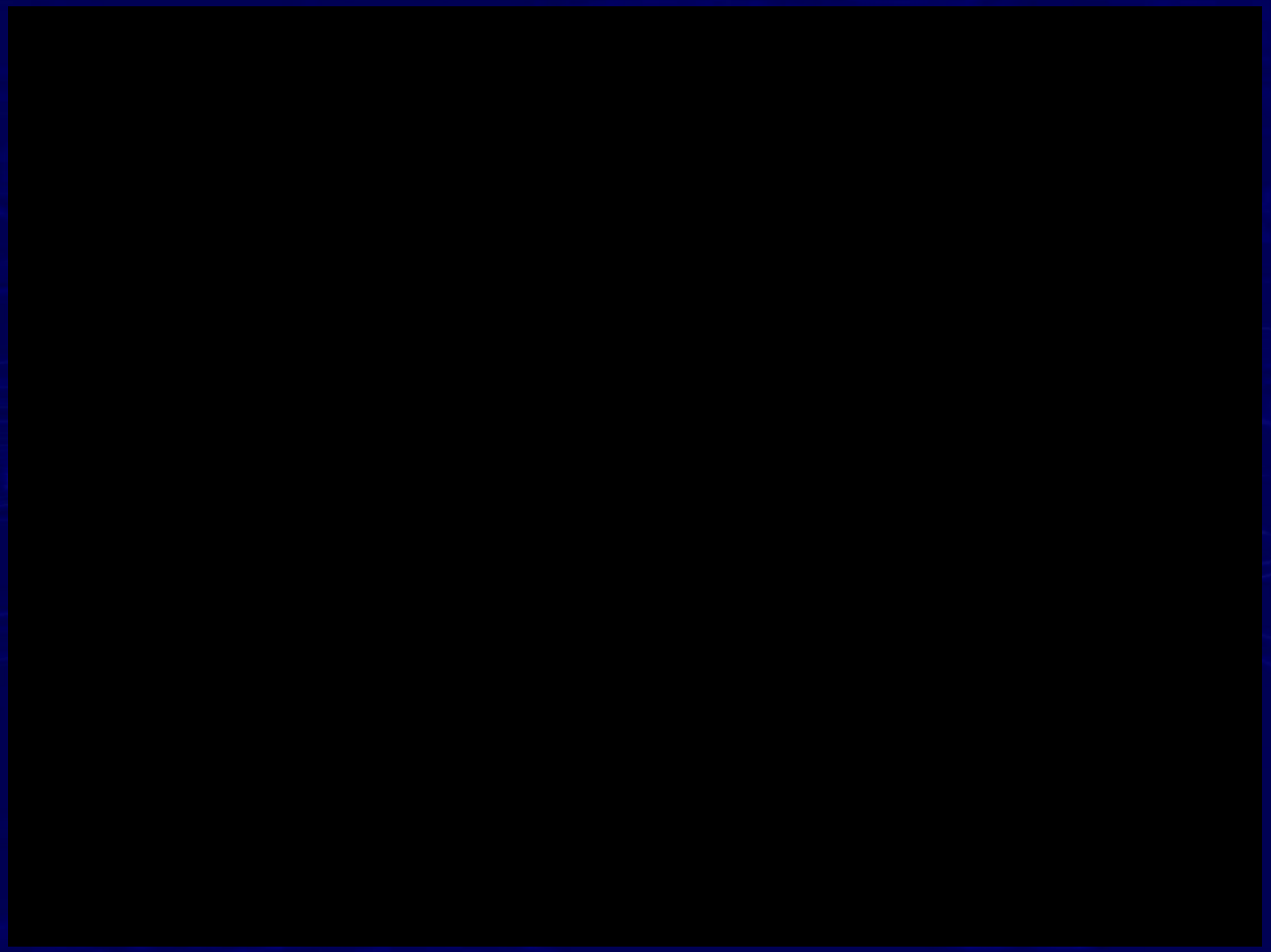
DNA → RNA → Protein





AC Forster & GM Church. Mol Sys Biol 2006

The minimal cell: bottom-up approach



The minimal cell: bottom-up approach

Just how many genes for a bottom-up minimal cell?

- Estimate: 151 genes = 38 RNAs + 113 proteins (~200 is a better guess)

Basic DNA replication 2	Chaperones 2	Ribosome 63
RNA transcription 1	RNA Processing 3	tRNA set 33
Translation Factors 11	AA-tRNA synthetases 21	tRNA modifiers 15

Would require all metabolites (eg. NTP) to be provided – no metabolism

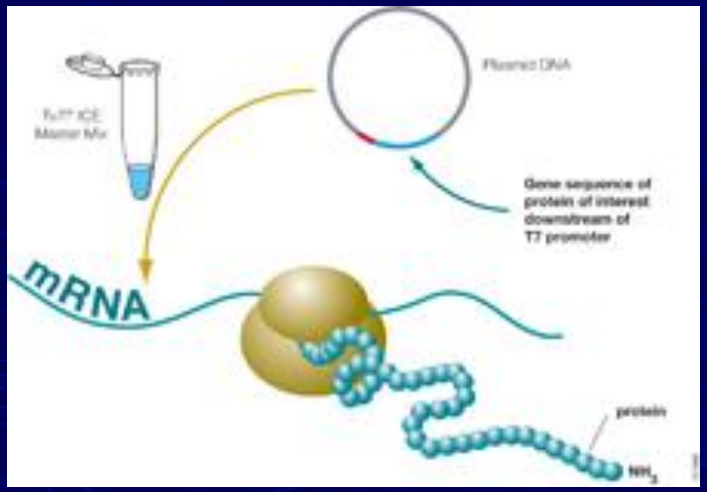
Would have no control over compartmentalisation – no membrane synthesis

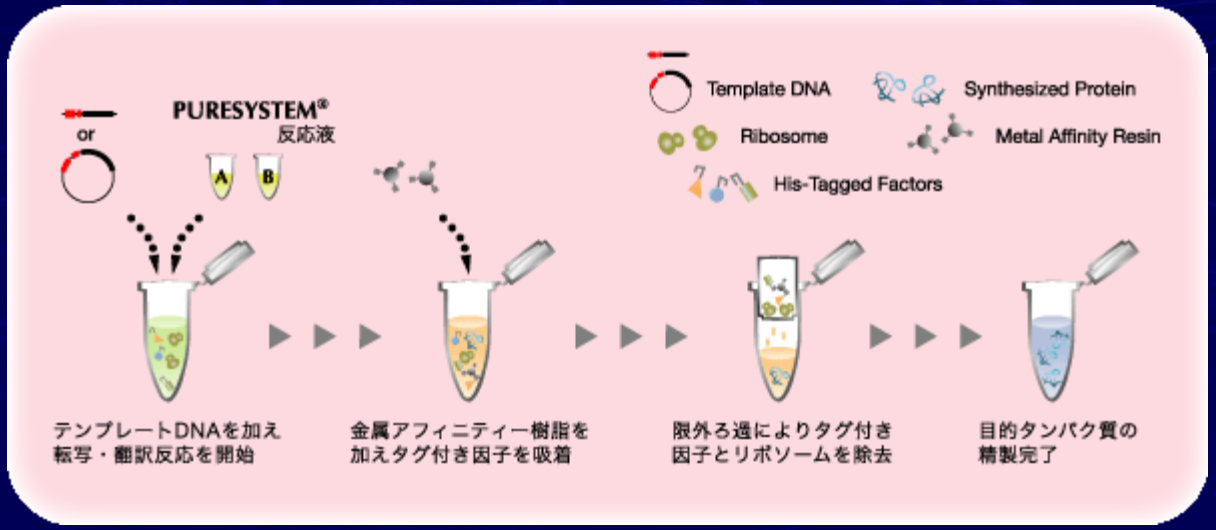
Really minimal cell – fragile *in vitro* system

Add metabolism, add lipid-synthesis for membranes, add proteins to control cell division, pores and transporters for sugar-import

Working minimal cell – capable of self-evolution

Existing *In Vitro* Transcription/Translation





Cell-free systems

Existing use:

- Protein synthesis for research and screening

Planned use:

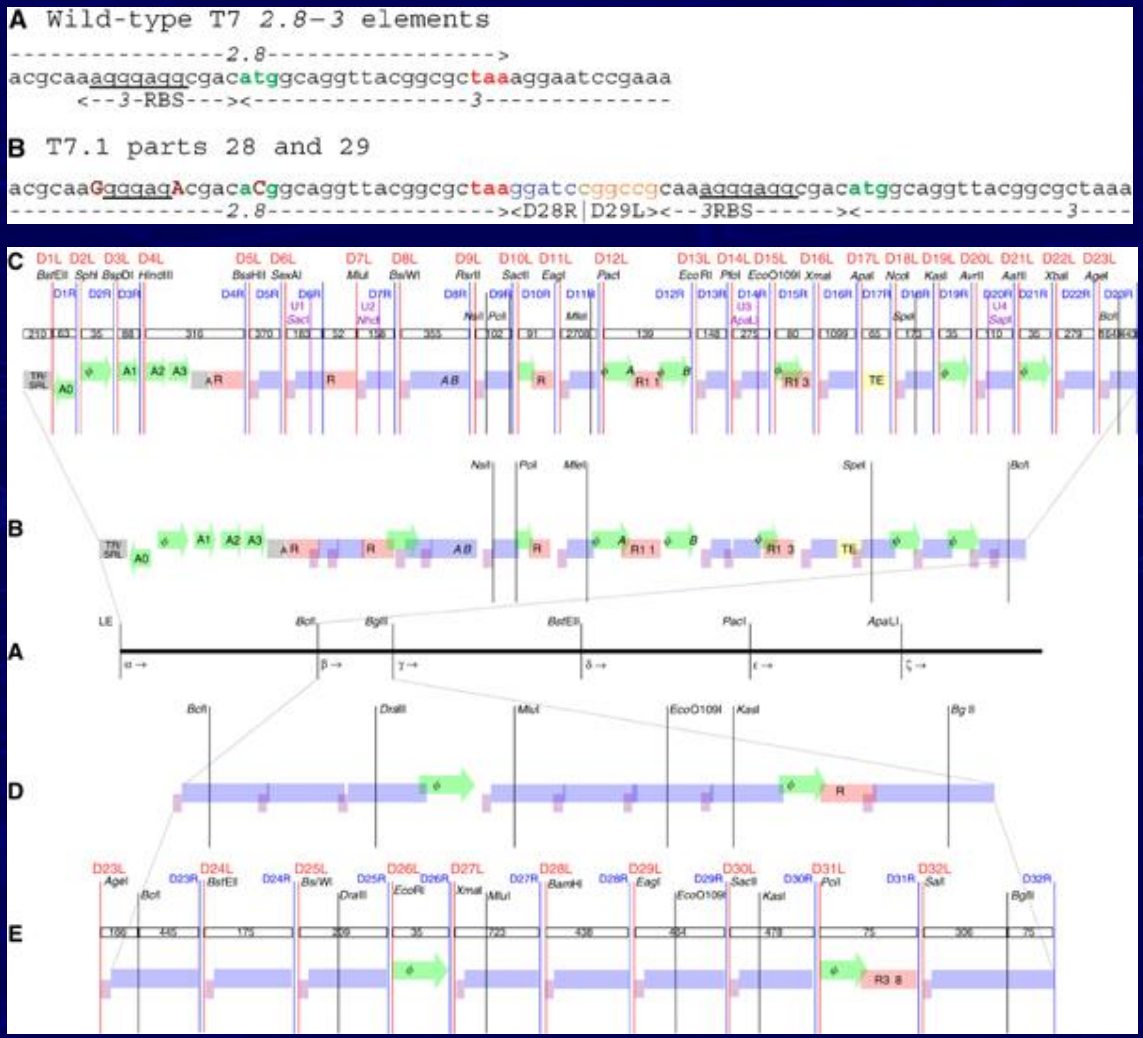
- Microfluidic systems - e.g. lab-on-a-chip
- Fast mutation and evolution of DNA

Re-factored genomes

Can we logically re-arrange a genome?

Add spacers, cut sites
Remove redundant DNA
Separate overlaps

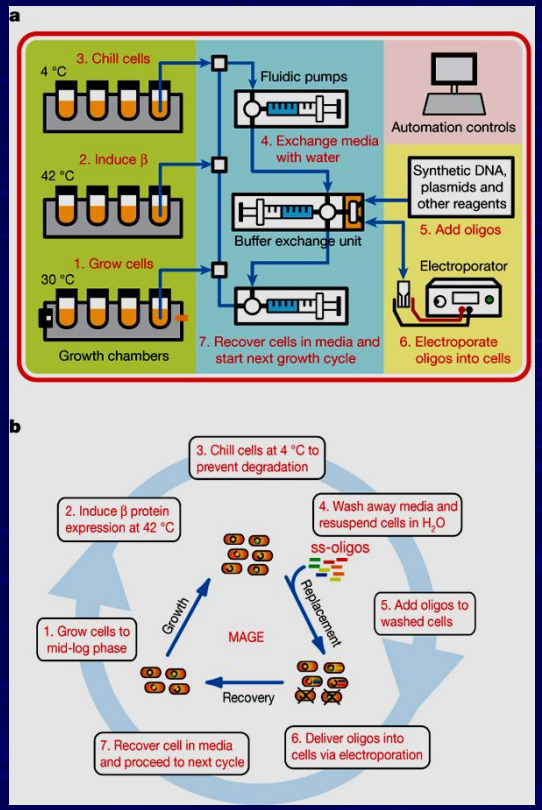
- M13 phage
- T7 phage
- Yeast chromosome (part of the synthetic yeast genome project)

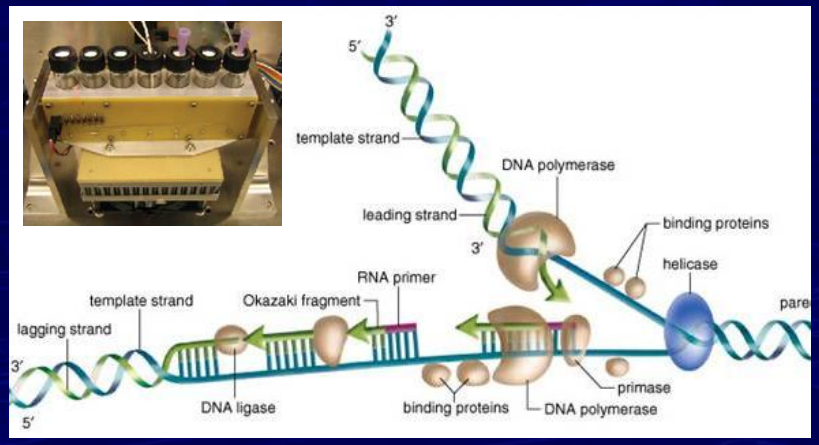


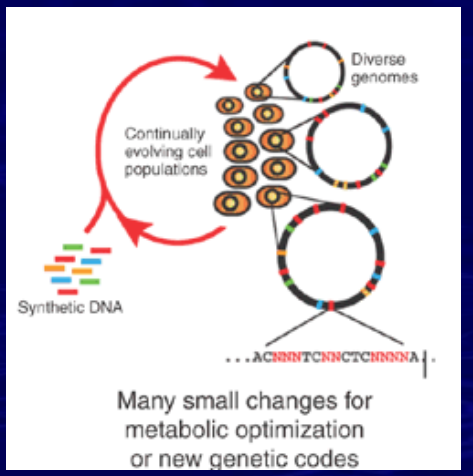
Large-Scale Editing Genomes

MAGE - multiplex automated genome engineering

HH Wang *et al.* 2009 Nature

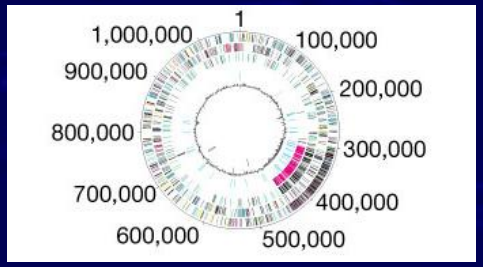


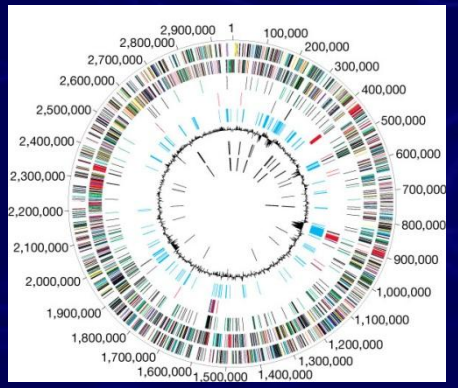




Recombinases – shuffle natural DNA
e.g. *Vibrio Cholera*

or can use to direct DNA removal or insertion
e.g. Red system

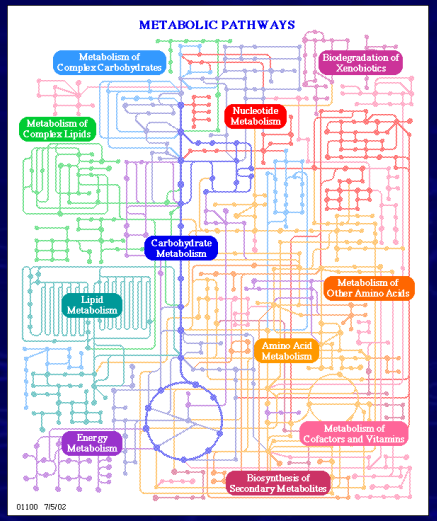


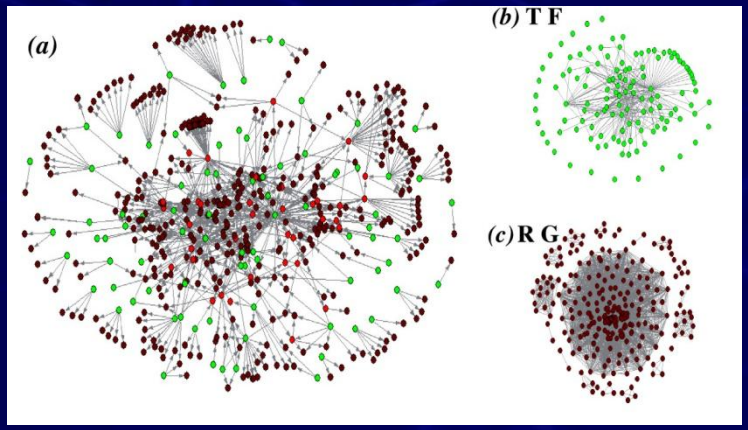


Modeling & Genome Engineering

A computational platform to design genomes : needs large-scale bottom-up models

1. Model the central core life functions – Replication, Transcription and Translation
2. Model metabolic networks and enzymes involved
3. Add regulation: a global transcriptional model
4. Improve the models with *in silico* directed evolution
5. Use the models to choose the organisation of genes on the genome
6. Try building versions and testing these

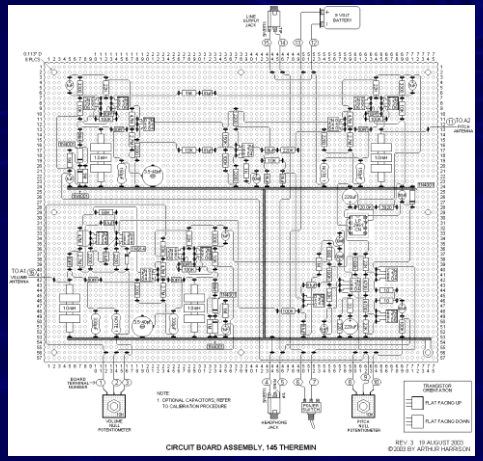


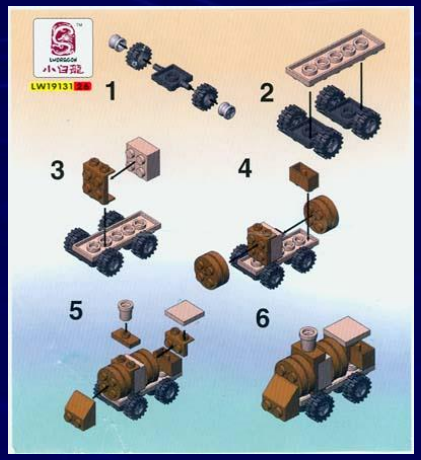


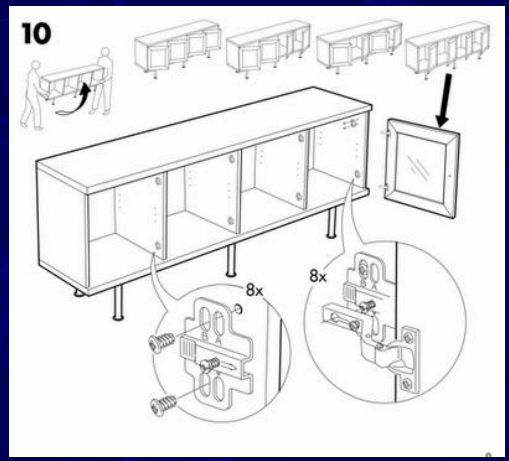
Why do genome engineering?

1. Bottom-up synthetic biology

- Adding genes and devices should be more predictable
- Creating a whole-cell model should be easier and allow better predictions of behaviour
- Provides a route to designing the chassis cell fit for a specific application
- Removal of unstable / recombination elements



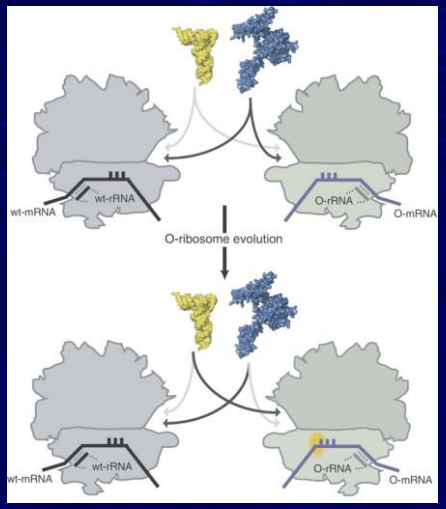


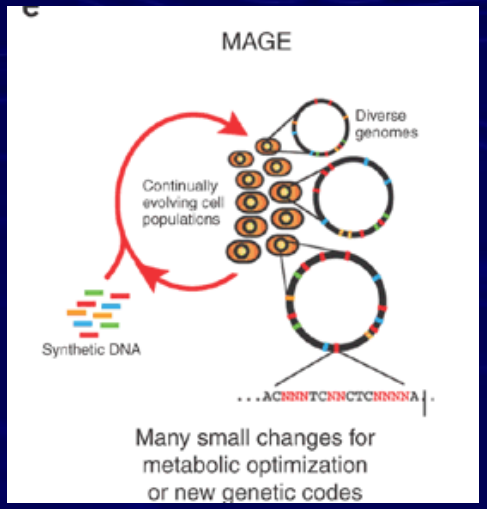


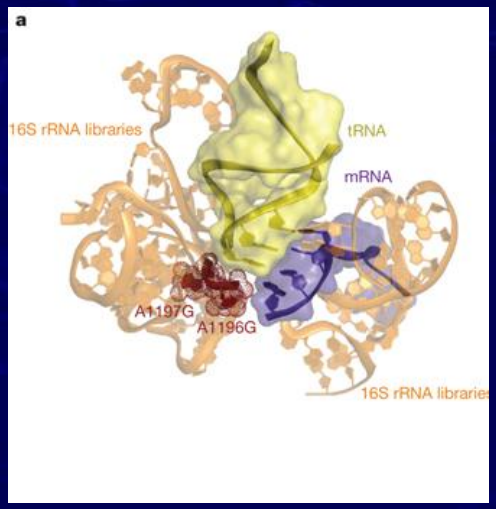
Why do genome engineering?

2. Provides for safer synthetic biology

- Cell can be designed to only survive in lab conditions
- Cell could be made " orthogonal" so that its biology doesn't interact with nature
- examples: change codon usage or change stereochemistry
- Better predictability from bottom-up design



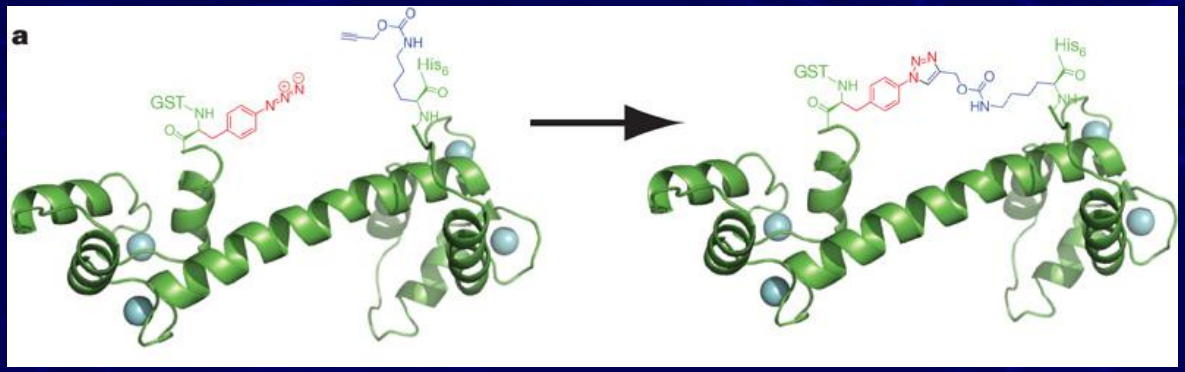




Why do genome engineering?

3. Custom synthesis of products

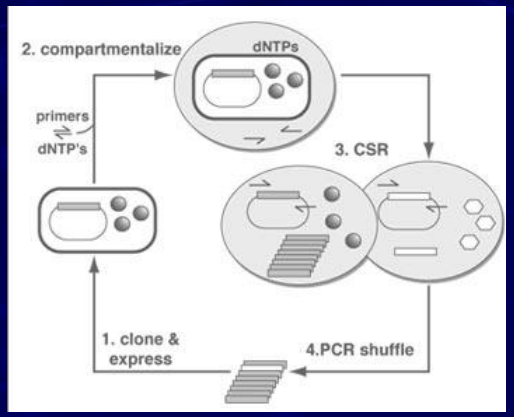
- Cells could be designed to produce non-natural proteins and sugars using synthetic building blocks
- Minimal cells would only use resources to make the desired products and so be more efficient
- Very cheap production of DNA could be engineered
- Synthesis of molecules that are toxic to produce in normal cells

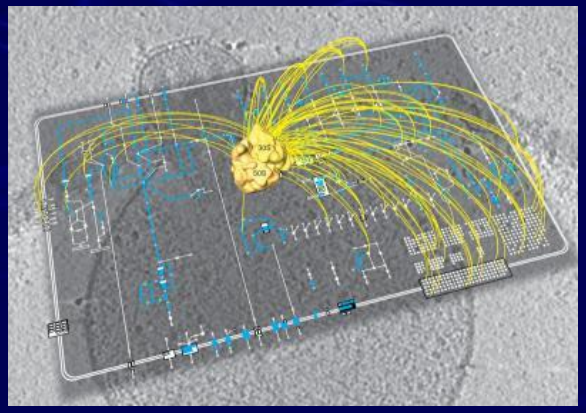


Why do genome engineering?

4. Other areas

- Minimal cell gives us a chance to study the origins of cellular life and potentially exobiology
- Fast evolution can be engineered to rapidly produce new enzymes
- Minimal cells would be easier to integrate into life-on-a-chip systems – e.g. a small screening device that sequences DNA, then synthesizes all the proteins from that DNA and compares their affinity to an antigen





What you should now know and read up on!

You could get exam questions on...

1. How JCVI made the first cell with a synthetic genome
2. How DNA can be constructed using *Gibson* or *Yeast* assembly
3. Rival attempts at genome-scale engineering
4. What a top-down minimal cell is and how big they are
5. The parts required for a bottom-up minimal cell
6. Examples of minimal cells in nature
7. Re-factoring genomes for predictability
8. Using MAGE to edit genomes of bacteria
9. Using modelling & software in genome-scale engineering
10. Applications for minimal cells and engineered genomes

Reading – Useful Reviews & Perspectives

Genome Engineering – PA Carr and GM Church
Nature Biotechnology, Vol. 27, No.12. (12 December 2009), pp. 1151-1162

Update on designing and building minimal cells – MC Jewett and AC Forster
Current Opinion in Biotechnology, Vol. 21, Issue 5 (October 2010), pp. 697-703

Towards Synthesis of a Minimal Cell – AC Forster and GM Church
Molecular Systems Biology, Vol. 2 (22 August 2006)

Excavating the Functional Landscape of Bacterial Cells - H Ochman and R Raghavan
Science, Vol. 326 no. 5957 (27 November 2009), pp. 1200-1201 Minimal Cell Model

Artificial assembly of a minimal cell – G Murtas
Mol. BioSyst., Vol. 5, No. 11. (2009), pp. 1292-1297. Artificial Cells

Towards the automated engineering of a synthetic genome – J Carrera, G Rodrigo and A Jaramillo
Mol. BioSyst., Vol. 5, No. 7. (July 2009), pp. 733-743. Modeling/Software

Reading – Key Papers

Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome
- DG Gibson et al. Science Vol. 329 no. 5987 (2 July 2010), pp. 52-56

Complete Chemical Synthesis, Assembly, and Cloning of a Mycoplasma genitalium Genome -
- DG Gibson et al. Science Vol. 319 no. 5867 (29 February 2008), pp. 1215-1220.

Enzymatic assembly of DNA molecules up to several hundred kilobases
- DG Gibson et al. Nature Methods 6, 343 - 345 (2009)

Chemical synthesis of the mouse mitochondrial genome
- DG Gibson et al. Nature Methods 7, 901–903 (2010)

Genome Transplantation in Bacteria: Changing One Species to Another
- C Lartigue et al. Science Vol. 317 no. 5838 (3 August 2007), pp. 632-638

Programming cells by multiplex genome engineering and accelerated evolution
- HH Wang et al. Nature 460, 894-898 (13 August 2009)

The minimal cell: bottom-up approach