Applications of Advanced Synthetic Biology

Genome Engineering

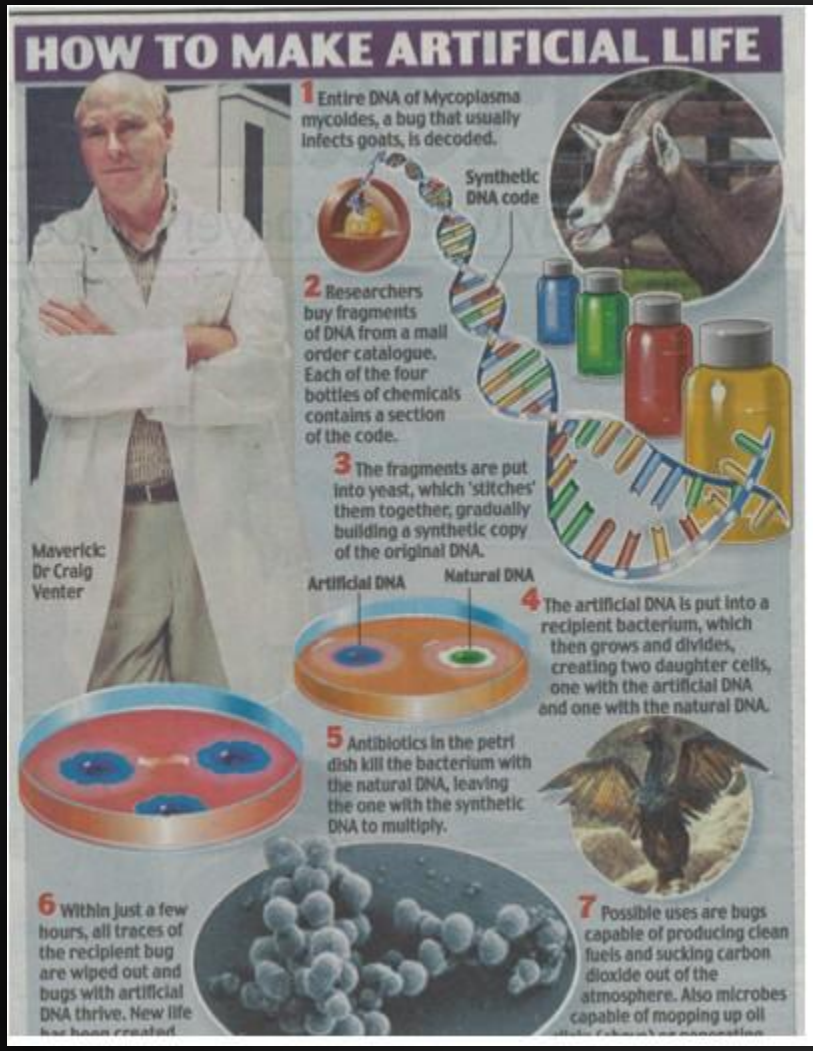
Genome Engineering - big-ticket projects

JCVI synthetic cell

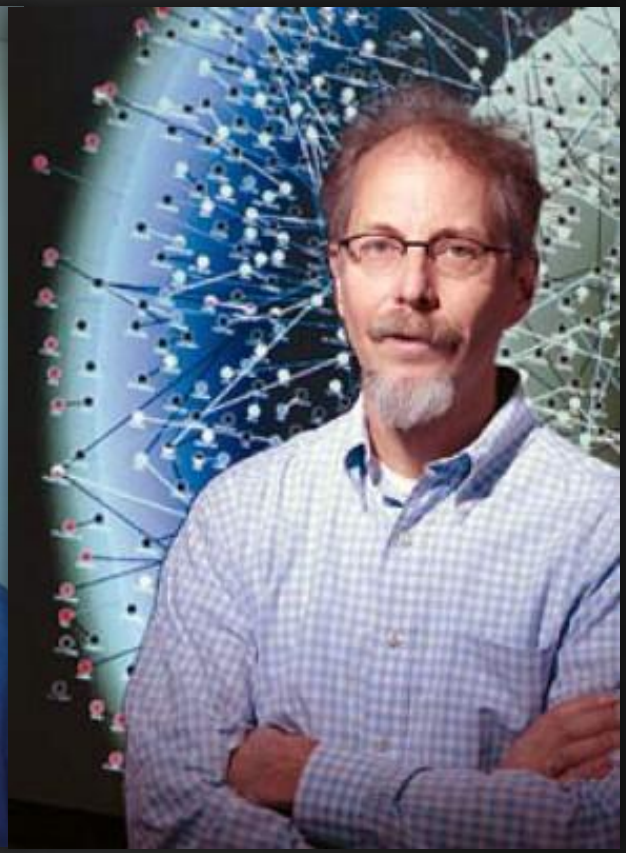
10+ years
30+ people

>$50 million

The first synthetic cell – 'Synthia'



Three major groups are leading synthetic life



George Church
Harvard
E. coli

J. Craig Venter
JCVI
Mycoplasma

Jef Boeke
JHU
Yeast

Lecture Content

In this lecture we'll cover:

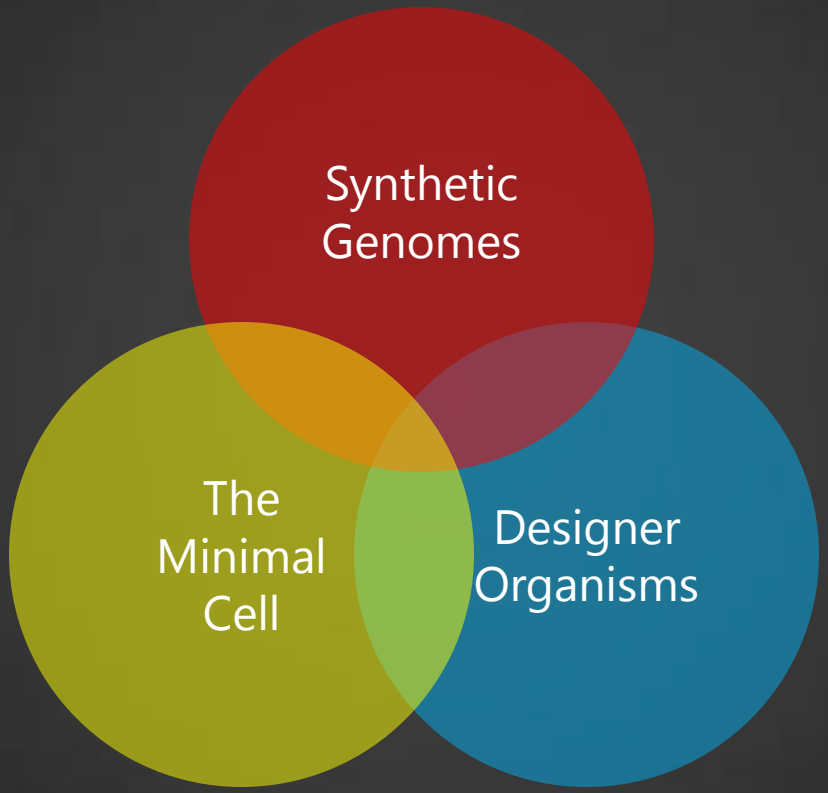
1. Minimal cells
2. Craig Venter's *first cell made with a synthetic genome*
3. The DNA assembly techniques used
4. SC2.0 – a synthetic yeast genome
5. Design rules for engineered genomes
6. Techniques for editing genomes
7. RE.coli
8. Applications of minimal cells and engineered genomes

Learning Objectives

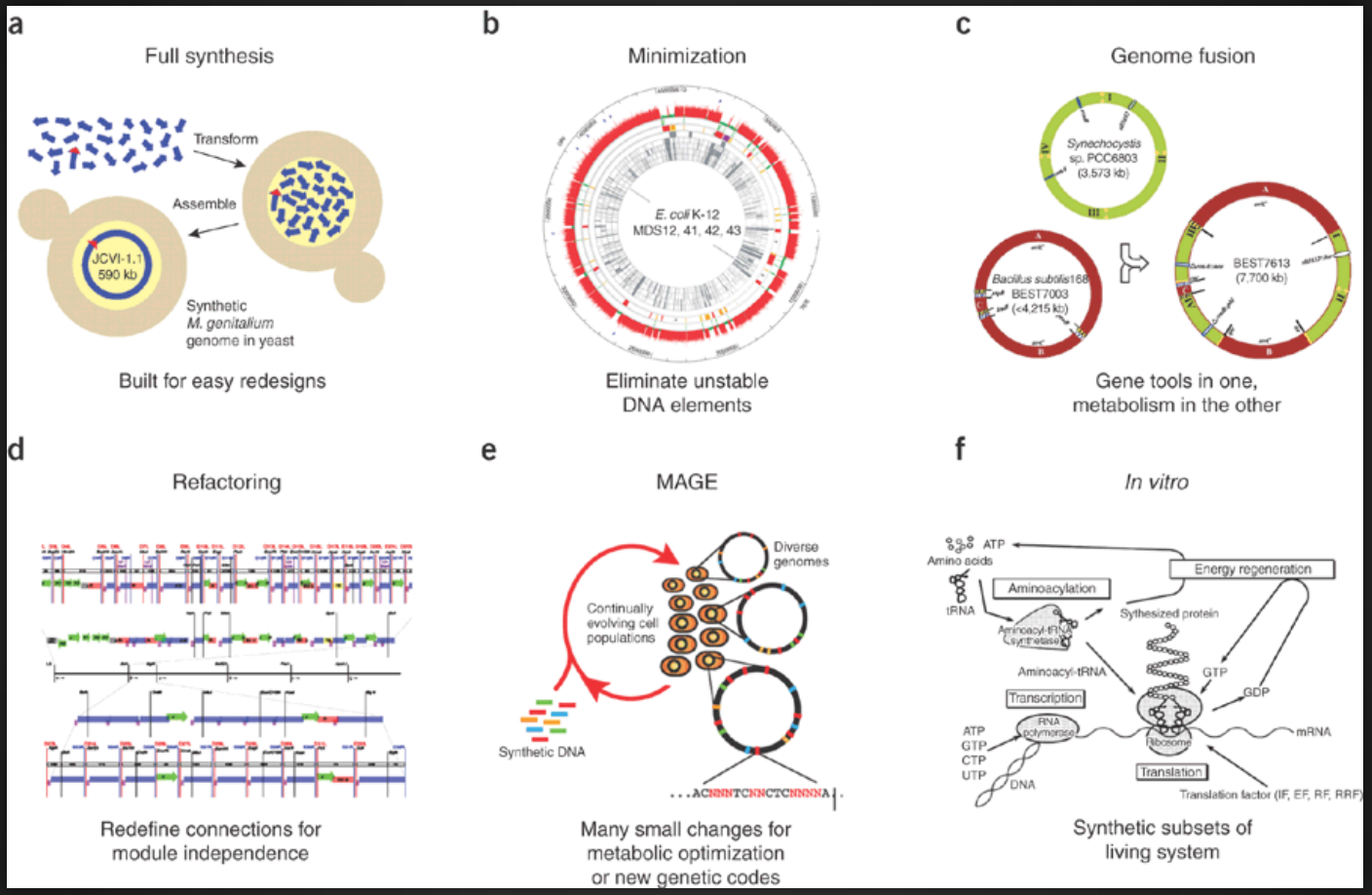
In this lecture you will learn:

1. Examples of natural and synthetic minimal cells
2. DNA Assembly techniques to allow genome-scale work
3. Examples of genome engineering projects
4. Examples of synthetic changes to make to genomes
5. Synonymous codons and their use in synthetic biology
6. Applications of minimal cells and engineered genomes

The construction of synthetic organisms



Genome Engineering



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

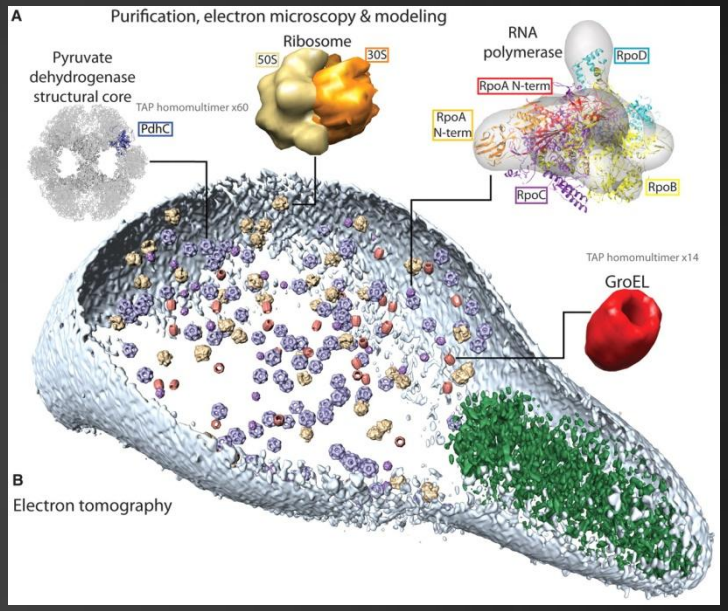
Natural Minimal Cells

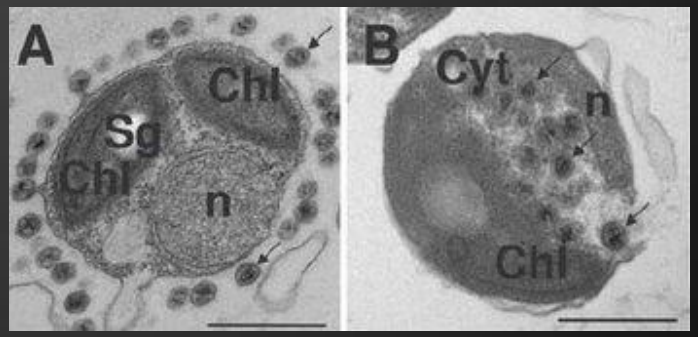
M.genitalium, Pelagibacter ubique, Nanoarchaeum equitans
Carsonella ruddii (213 genes – 160 kbp)
Hodgkinia cicadicola (188 genes – 144 kbp)

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



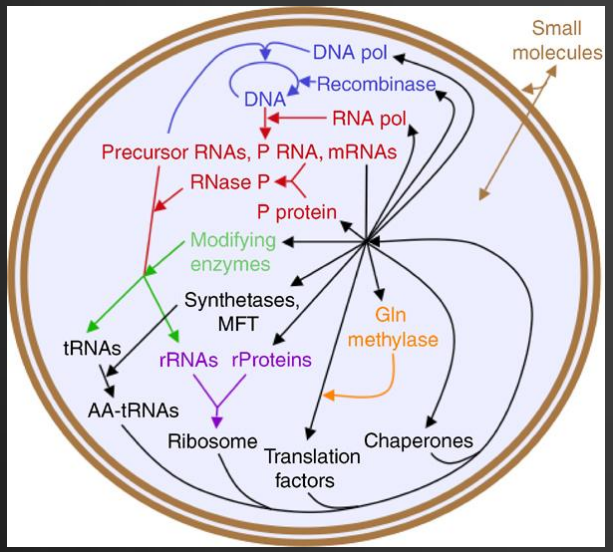


The minimal cell: bottom-up approach

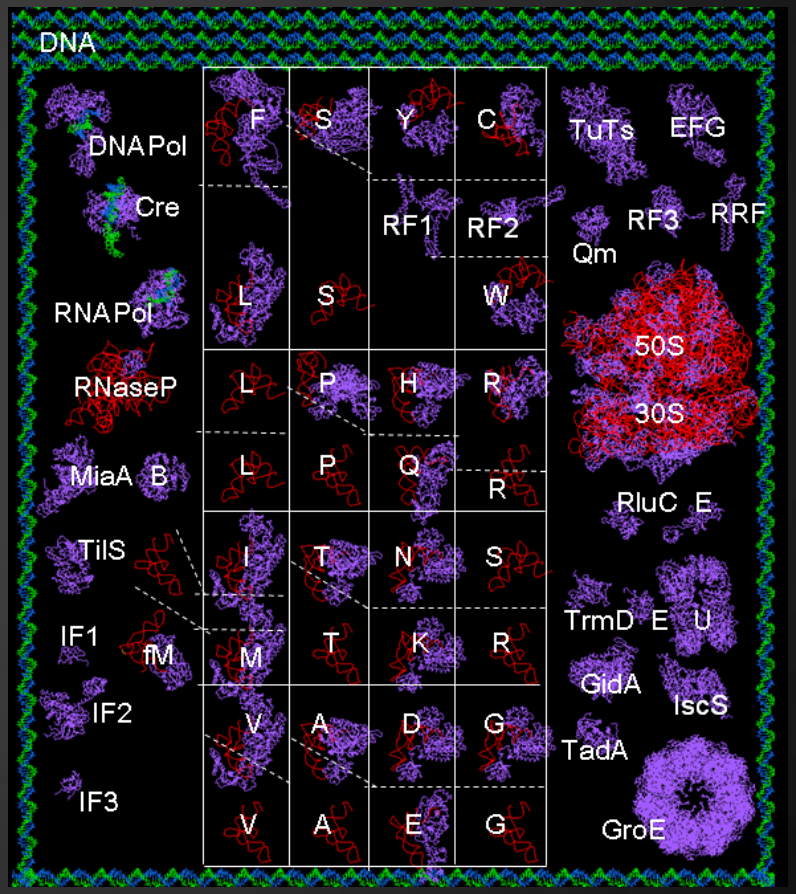
"We know enough about a cell to identify the essential molecules and build our own from scratch" - hardcore synthetic biology

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

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

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

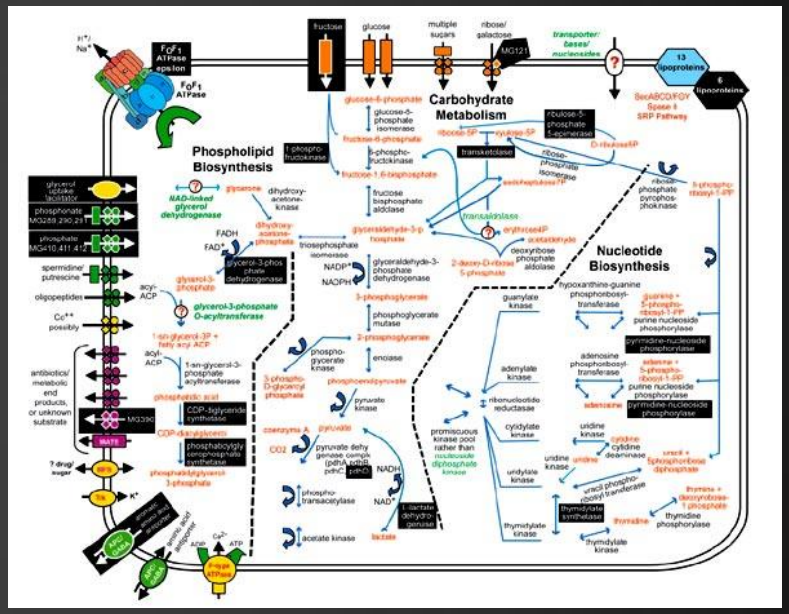
Working minimal cell – capable of self-evolution

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?

The minimal cell: top-down methods

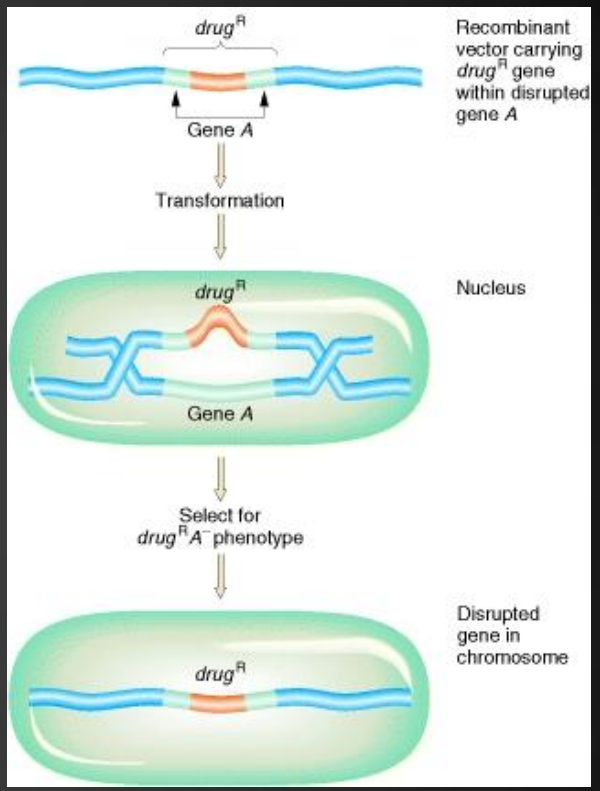
In vivo reduction

- Traditionally done using transposons and recombinases (e.g. Cre)
- 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 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

2010... Bacteria

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

Part 1: Can a complete DNA genome be synthesized from chemicals
Completed in 2008

Part 2: Can a cleaned DNA genome boot-up a cell
Completed in 2007

Synthetic Organism = Parts 1 and Parts 2 combined 2010

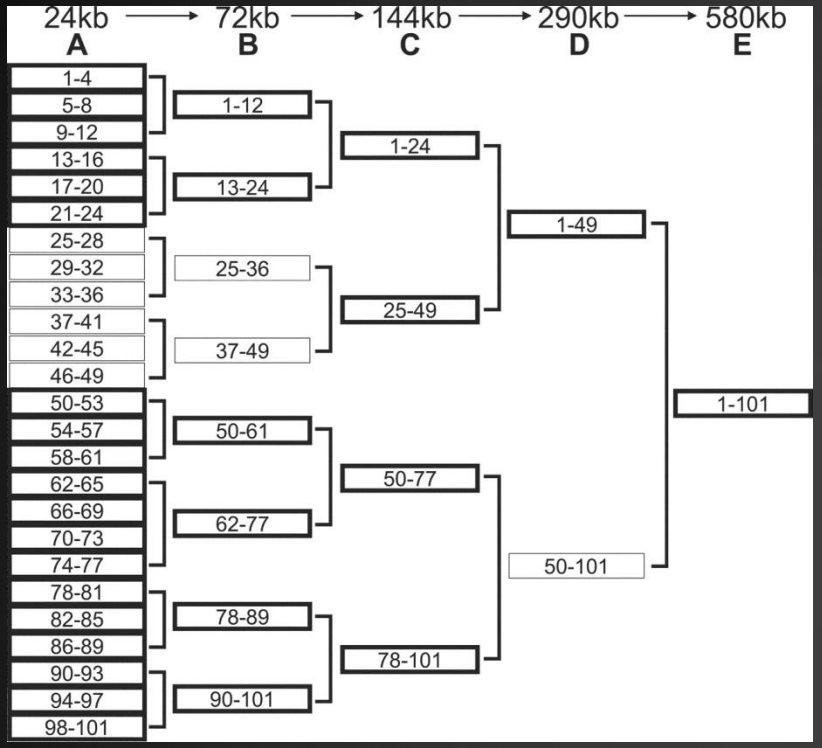
The first synthetic organism: (1) Synthesis

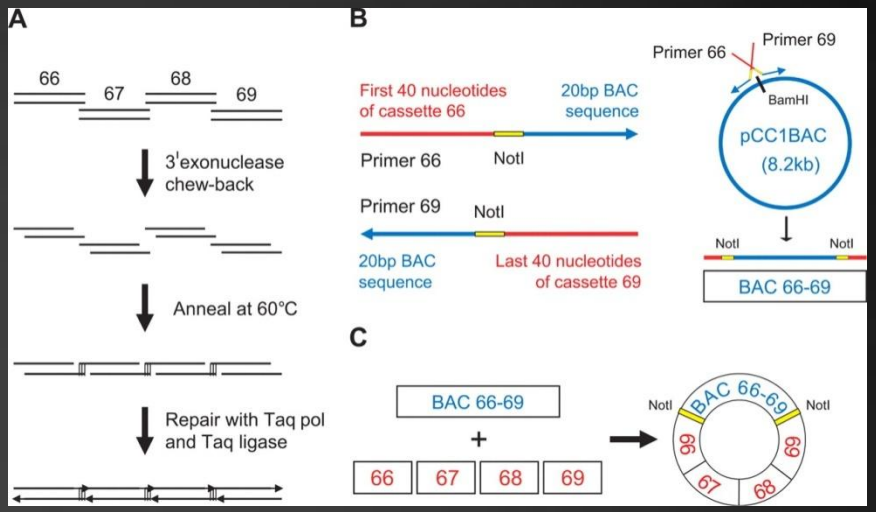
AIM: Synthesize ~10^4 DNA 50-base oligomers and assemble into a complete error-free 582970 bp *M.genitalium* genome (with watermarks)

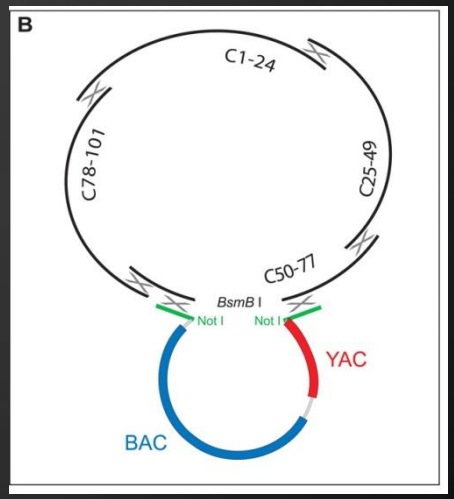
1. Companies synthesise 101 pieces of 5 to 7 kb from overlapping oligos (e.g. Blue Heron and GeneArt)
2. 101 pieces recombined using *in vitro* enzymes to make 24 big pieces
3. 24 big pieces maintained in BACs in *E.coli* and recombined to make even bigger pieces
4. Big pieces all inserted into yeast 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

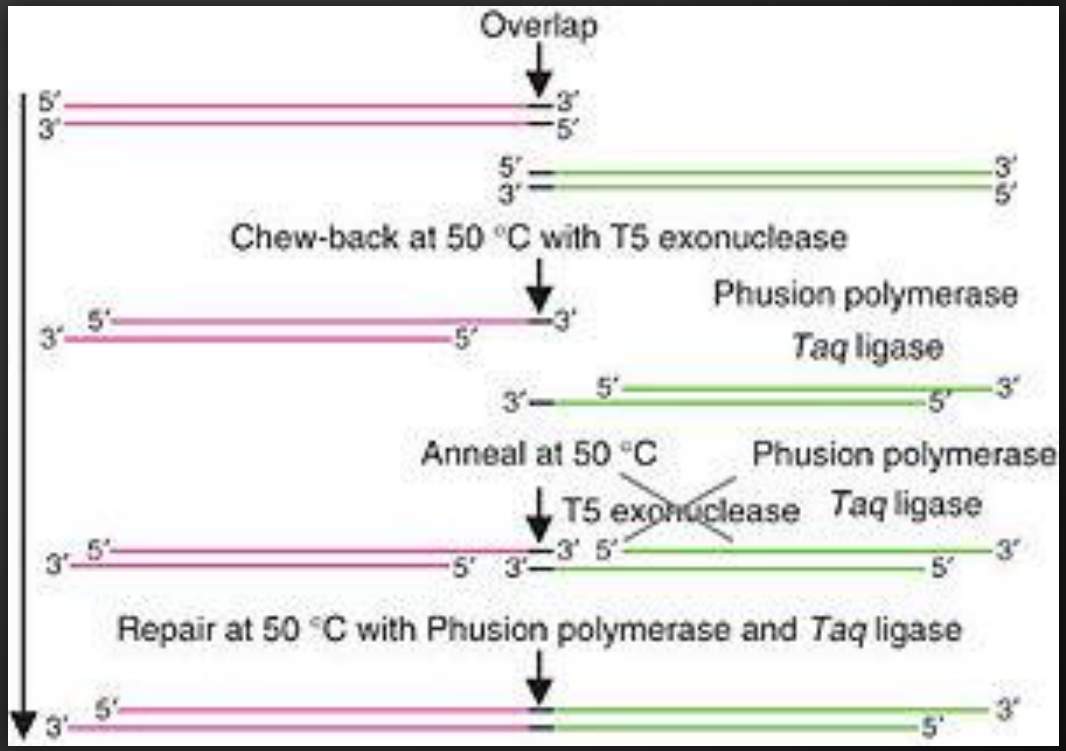
Make and store a master-mix for months

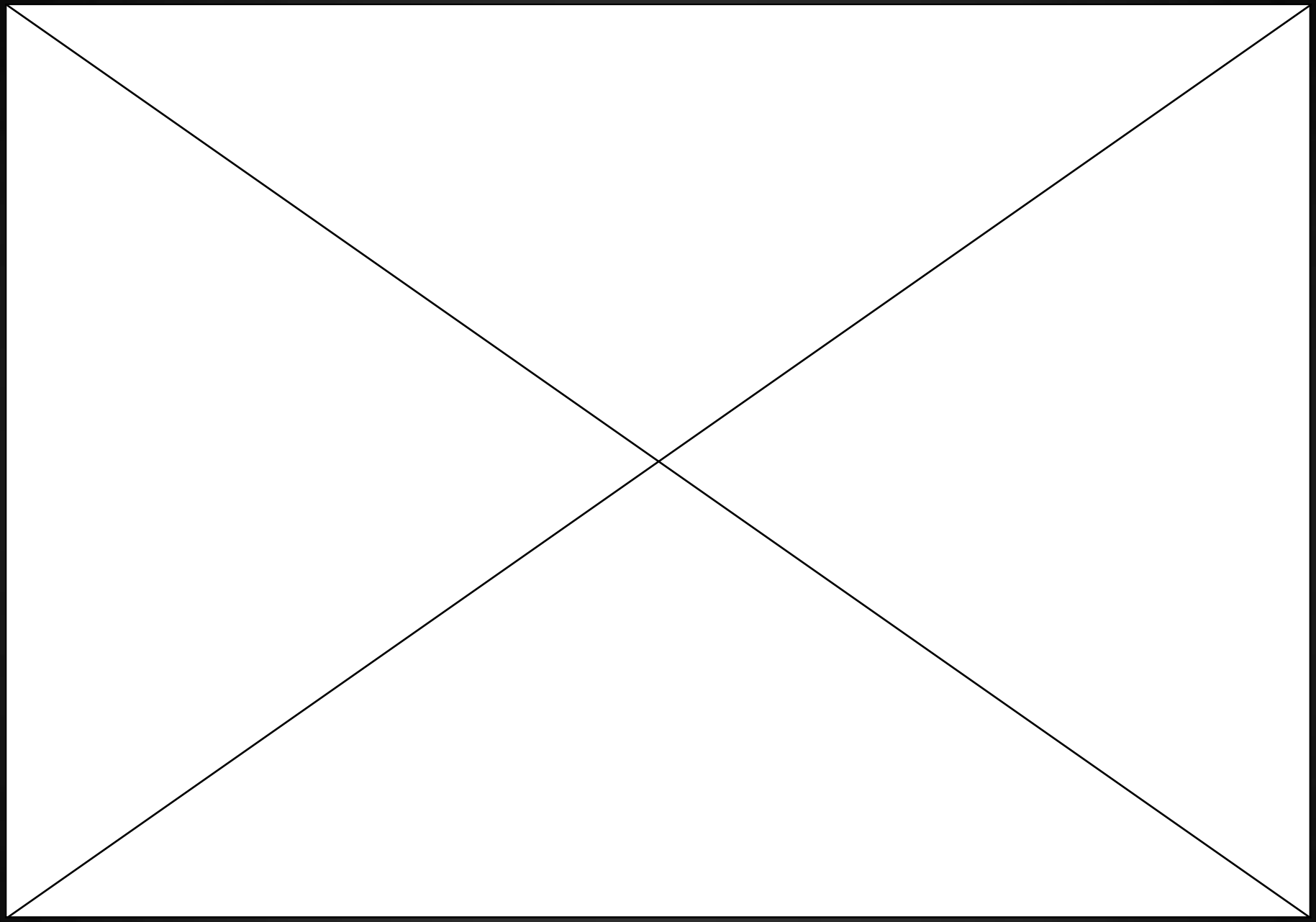
Takes 30 minutes at 50 degrees C

Very simple and scalable

In vitro and automatable

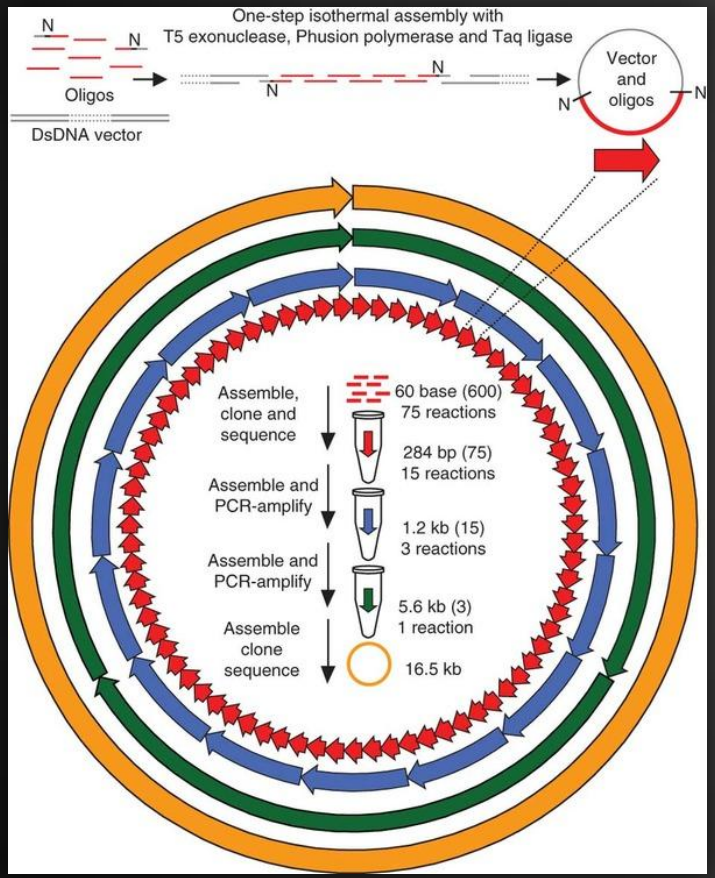
Requires overlapping DNA

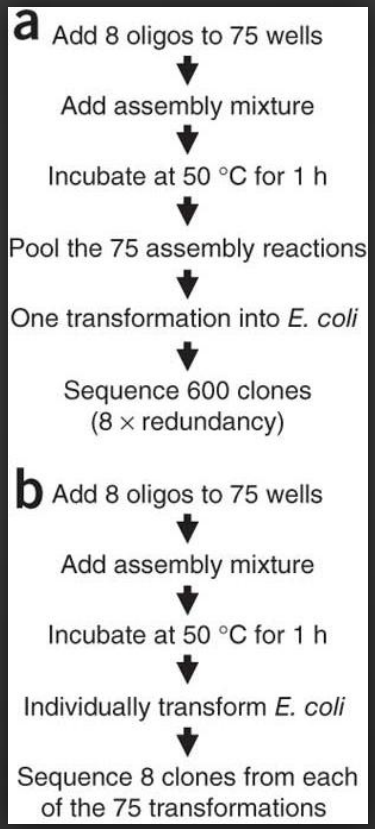


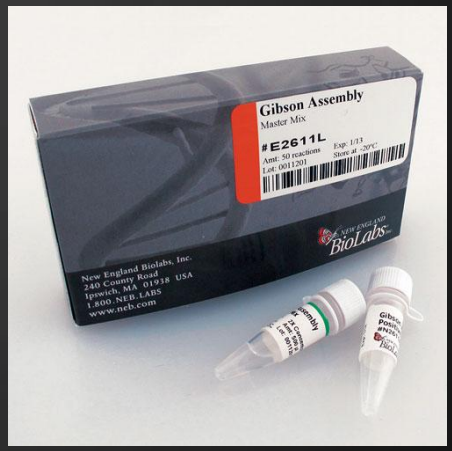


Gibson Isothermal Assembly (2)

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







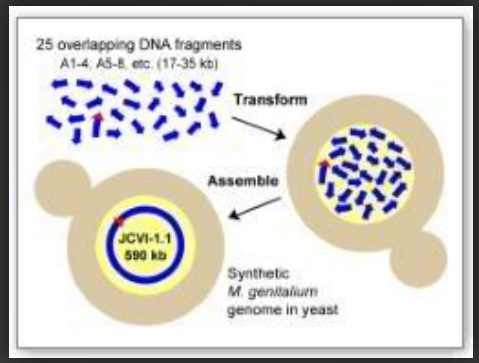
Yeast Assembly (TAR cloning)

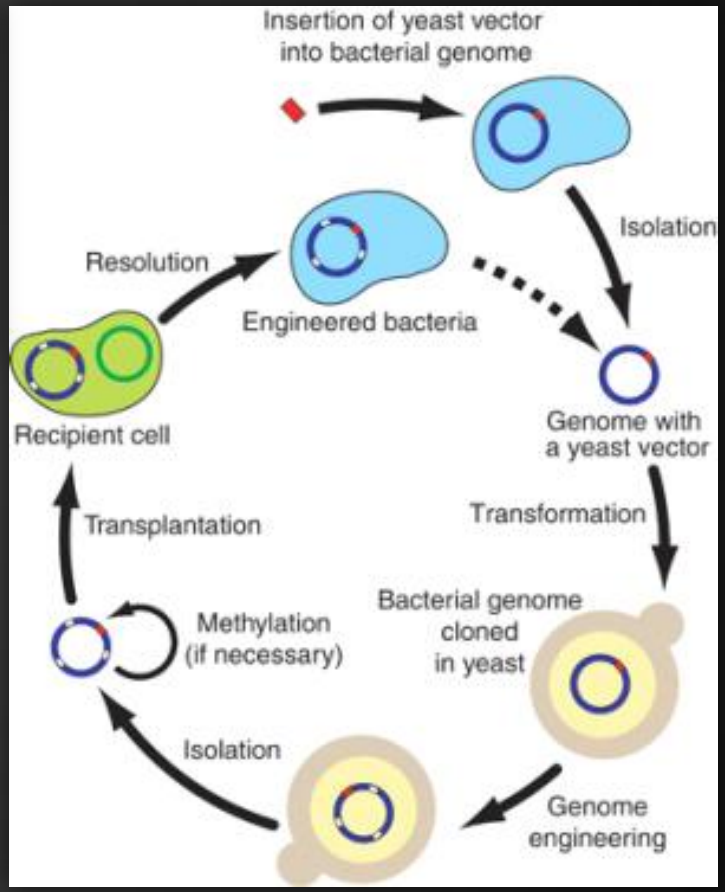
Yeast can be transformed with DNA and will assemble overlapping DNA

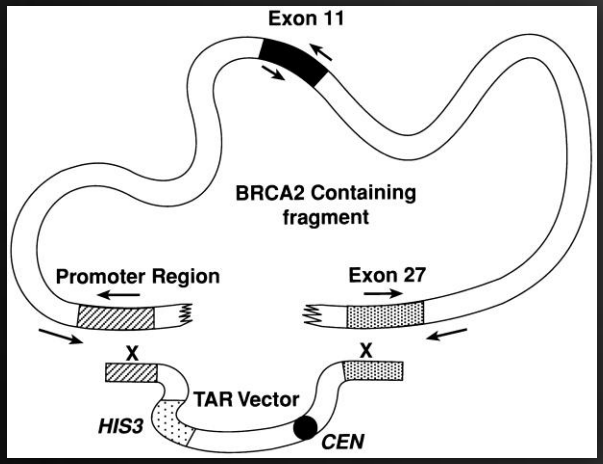
Quite an old method 'TAR Cloning'

Overlaps need to be about 40 bp or more

Yeast can take >2.5 Mbp







All you need is yeast!

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)

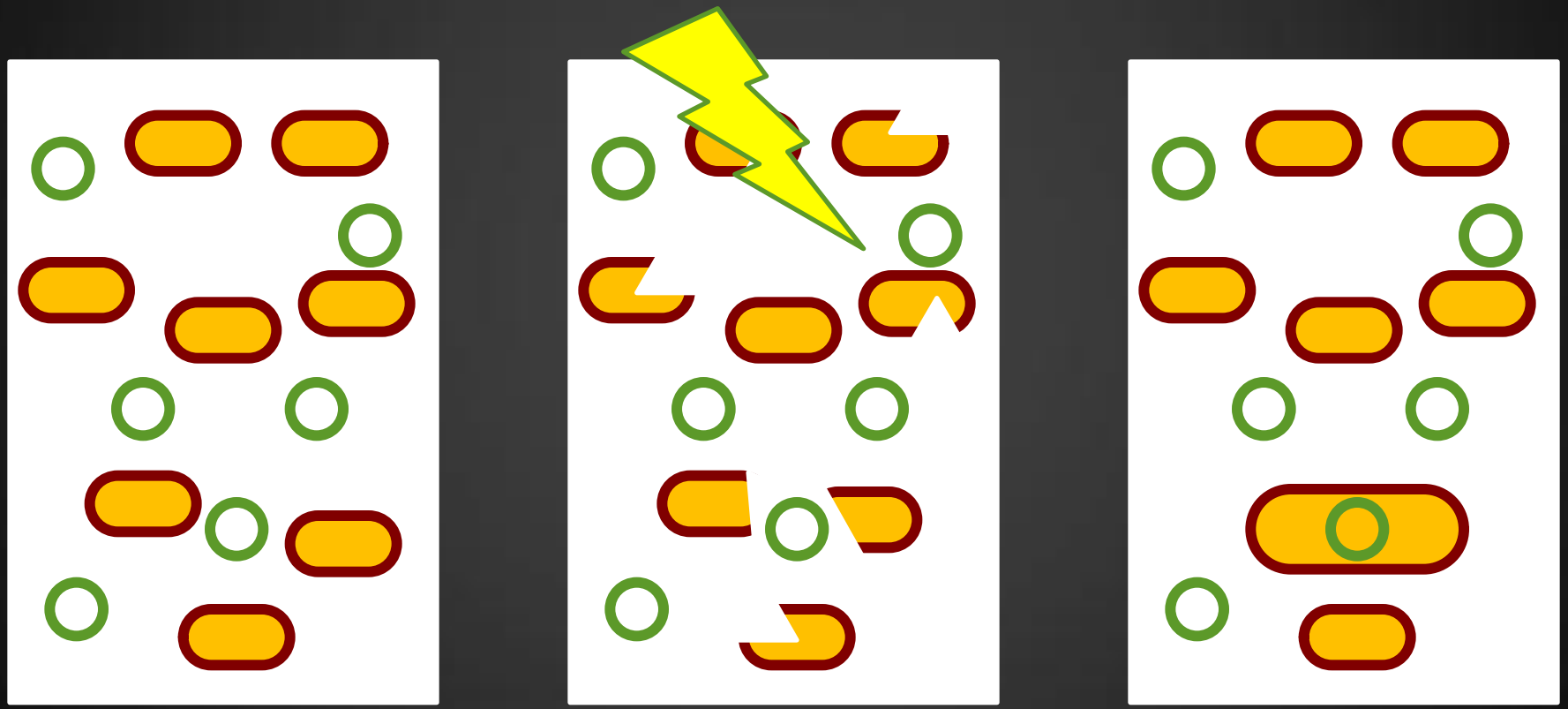
What happens to genome of cell B? – doesn't have antibiotic resistance

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



Finally making Synthia- published in 2010

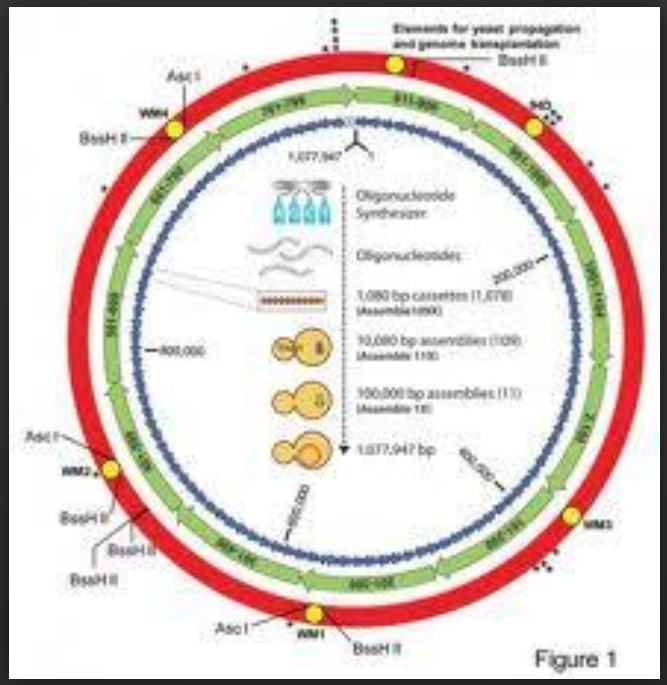
They got it to work: D.G. Gibson *et al* 2010, Science 329 (5987): 52-56

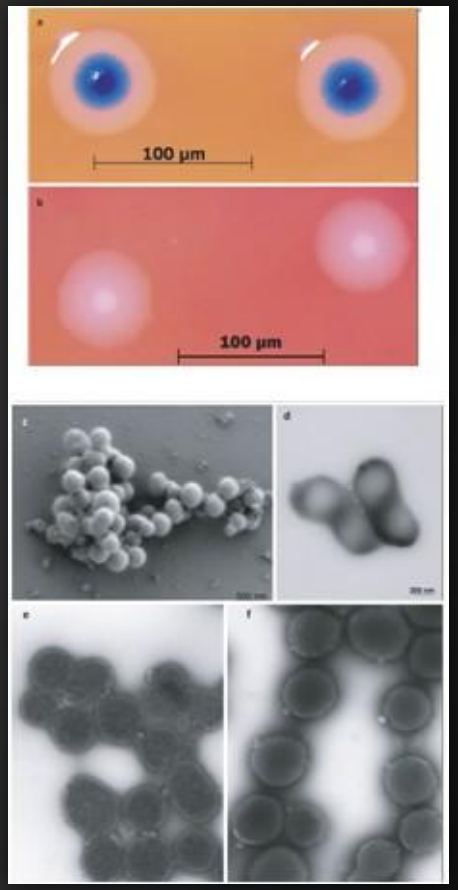
Methylation and restriction enzymes caused trouble in *M.genitalium* – 'natural immune system for bacteria'

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





Genome Engineering can be done by students too!

Jef Boeke and others at John's Hopkins University, USA

Project = Synthetic Yeast 2.0 http://biostudio.bme.jhu.edu/sc2/

Undergrad course & iGEM 2008 = JHU Build-A-Genome Project

Now international (China, India, USA and UK)

Jessica S. Dymond *et al.* **Synthetic chromosome arms function in yeast and generate phenotypic diversity by design**. *Nature*, 2011; DOI: 10.1038/nature10403

JHU iGEM 2009

http://2009.igem.org/Team:Johns_Hopkins-BAG

Team:Johns Hopkins-BAG/B-A-G course

JHU Build A Genome

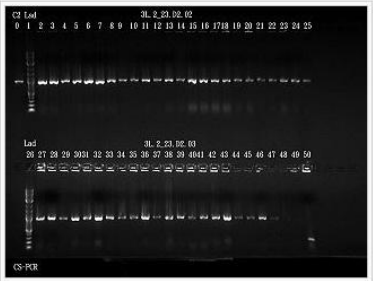
Moodle Synthetic Yeast Wiki Protocols References Acknowledgements Contact

- Home
- Team
- Synthetic Yeast Genome
- B-A-G course
- Building Block synthesis
- A New Standard
- Software Tools

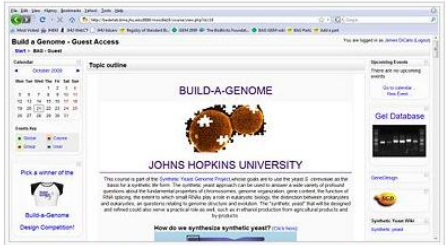
B-A-G Course

The Build-a-Genome course offers a cool environment in which undergraduate students can participate in cutting-edge interdisciplinary research. In pursuing the synthesis of Saccharomyces cerevisiae Sc2.0 chromosomes, students gain hands-on experience in synthetic biology research. The de novo synthesis of genetic information offers the promise of a deeper understanding of chromosome structure, gene function, and gene order. The ultimate goal of this class is the development of students into skilled, independent researchers with well-developed troubleshooting skills and experience and familiarity with molecular biology and computational approaches to genomics problems.

The Build-a-Genome course consists of lectures, "Molecular Biology Boot Camp", and eventually, independent research. The lectures offered in the course reflect the many-faceted underpinnings of synthetic biology, ranging in topics from fundamentals of genetics (such as nucleic acid structure and function, chromosome structure, and genome organization) to bioinformatics, to central concepts of synthetic biology (such as recombinant DNA technology, gene synthesis, synthetic circuitry and of course iGEM!). After introductory lectures are complete, students go through eight sessions of molecular biology "boot camp" that serve as a period to review lecture topics, master lab techniques, and learn the methods used in this project. Graduation from the boot camp requires students to submit assignments that verify proficiency in each step of the gene synthesis protocol – such as PCR, agarose gel electrophoresis, molecular cloning, sequence analysis, etc.



An example of a student's colony screening PCR to check for correct inserts after transformation



A screen shot of Build-a-Genome Moodle(Modular Object-Oriented Dynamic Learning Environment) course management software

JHU iGEM 2009 http://2009.igem.org/Team:Johns_Hopkins-BAG

Build-a-Genome Course

1. Intro Lectures
2. Molecular Biology Boot Camp (lab practicals on yeast, PCR, recombination etc.)
3. Each student is assigned 12-16 building blocks (~10,000 bp) to build
4. Integration of synthetic DNA into yeast to build the synthetic genome

Building blocks are constructed by the students from 60-80 base oligos.

a) Use 'overlap assembly' PCR to generate ~800 bp blocks from synthetic oligos

b) Sequence verification and quality control needed

c) Building blocks are linked together *in vitro* using Gibson or USER Assembly (Uracil Specific Excision Reagent)

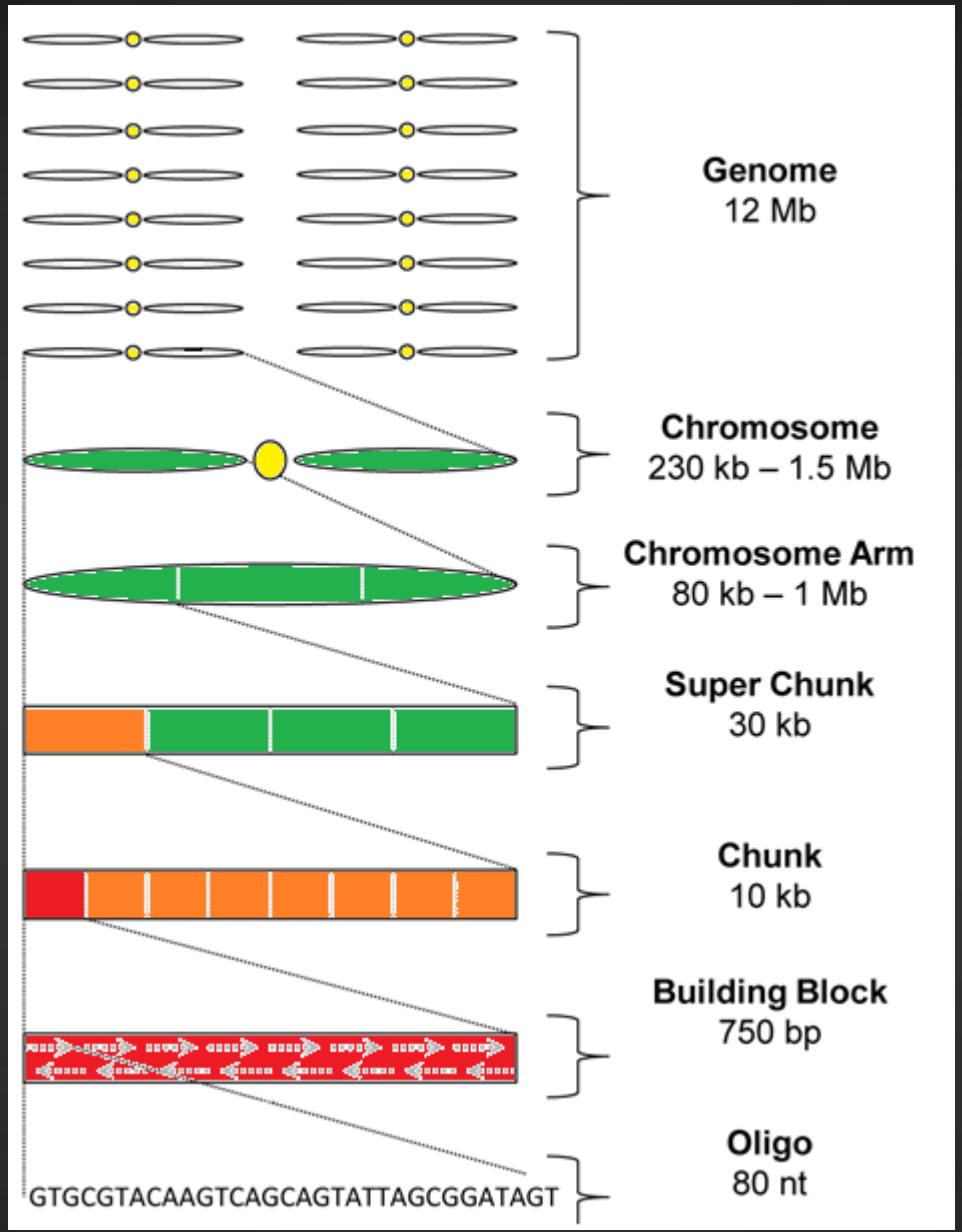
DNA ASSEMBLY

Yeast native recombination

Restriction enzyme digest and Ligate

Gibson/USER

PCA: Polymerase Chain Assembly



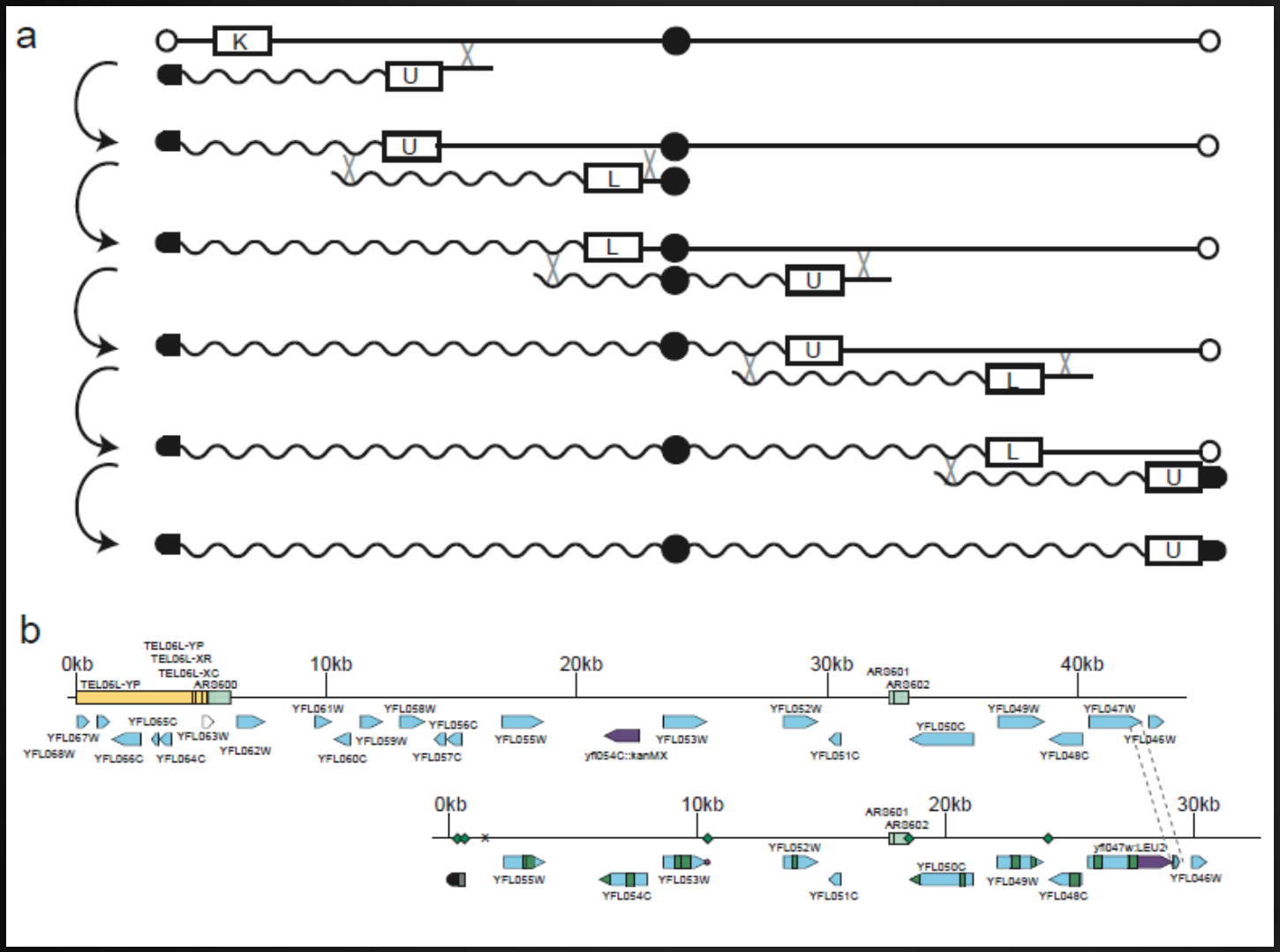
DNA SOURCE

CHUNKS (10 kb)
Buy from Genscript, GeneArt, DNA2.0, etc.

BLOCKS (0.8 kb)
Buy from IDT, Gen9 etc.

OLIGOS (80 to 120 nt)
Buy from IDT, Agilent, and many others.

How to replace native sequence with synthetic in yeast



Reiterative Recombination Method

Requires two selectable markers

Makes use of yeast's ability to recombine matching sequences

SERIAL process

Two chromosome arms working by 2011

2011 Nature Paper (make sure to also look at the Supplementary materials)

Jessica S. Dymond et al. Synthetic chromosome arms function in yeast and generate phenotypic diversity by design. Nature, 2011; DOI: 10.1038/nature10403

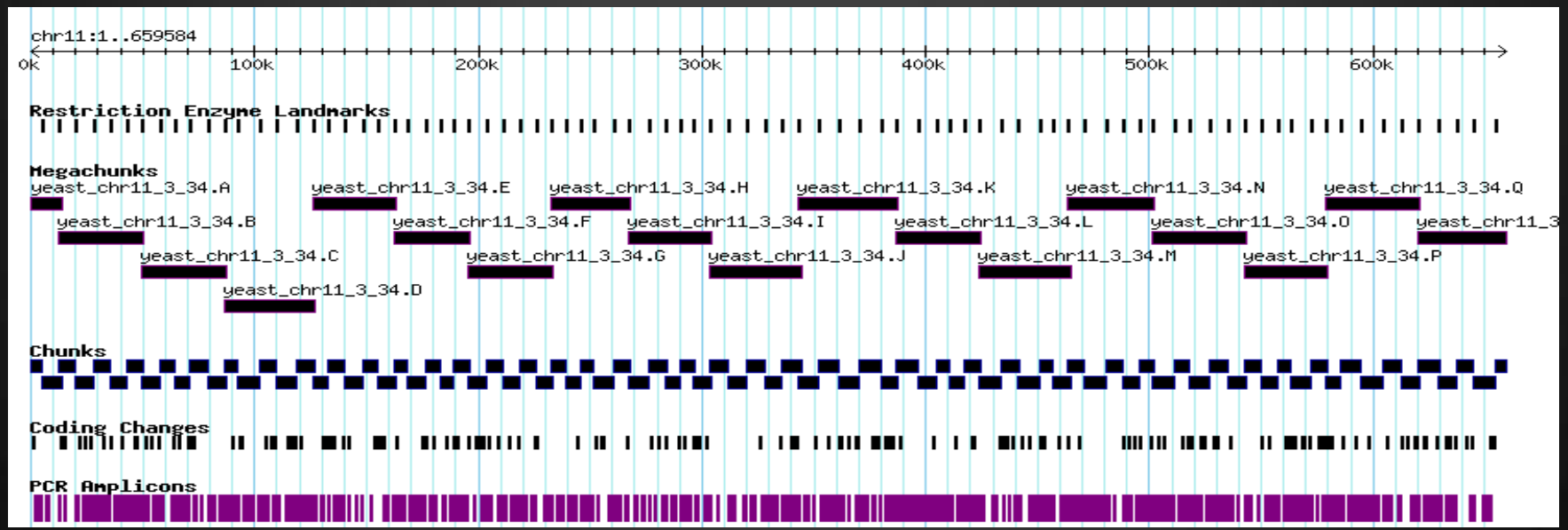
Same basic idea as the iGEM 2009 project and the Build-A-Genome Course

1. synIXR Chromosome 9 right arm added in one go

   made by commercial gene synthesis and assembled into a BAC (bacterial artificial chromosome) by the company Codon Devices.

2. semi-synVIL Chromosome 6 left arm 4 chunks added serially

   4 big chunks (up to 10,000 bp) were assembled from synthetic oligos by Epoch Biolabs


chr11:1..659584
0k
100k
200k
300k
400k
500k
600k
Restriction Enzyme Landmarks
Megachunks
yeast_chr11_3_34.A
yeast_chr11_3_34.E
yeast_chr11_3_34.H
yeast_chr11_3_34.K
yeast_chr11_3_34.N
yeast_chr11_3_34.Q
yeast_chr11_3_34.B
yeast_chr11_3_34.F
yeast_chr11_3_34.I
yeast_chr11_3_34.L
yeast_chr11_3_34.O
yeast_chr11_3_34.C
yeast_chr11_3_34.G
yeast_chr11_3_34.J
yeast_chr11_3_34.M
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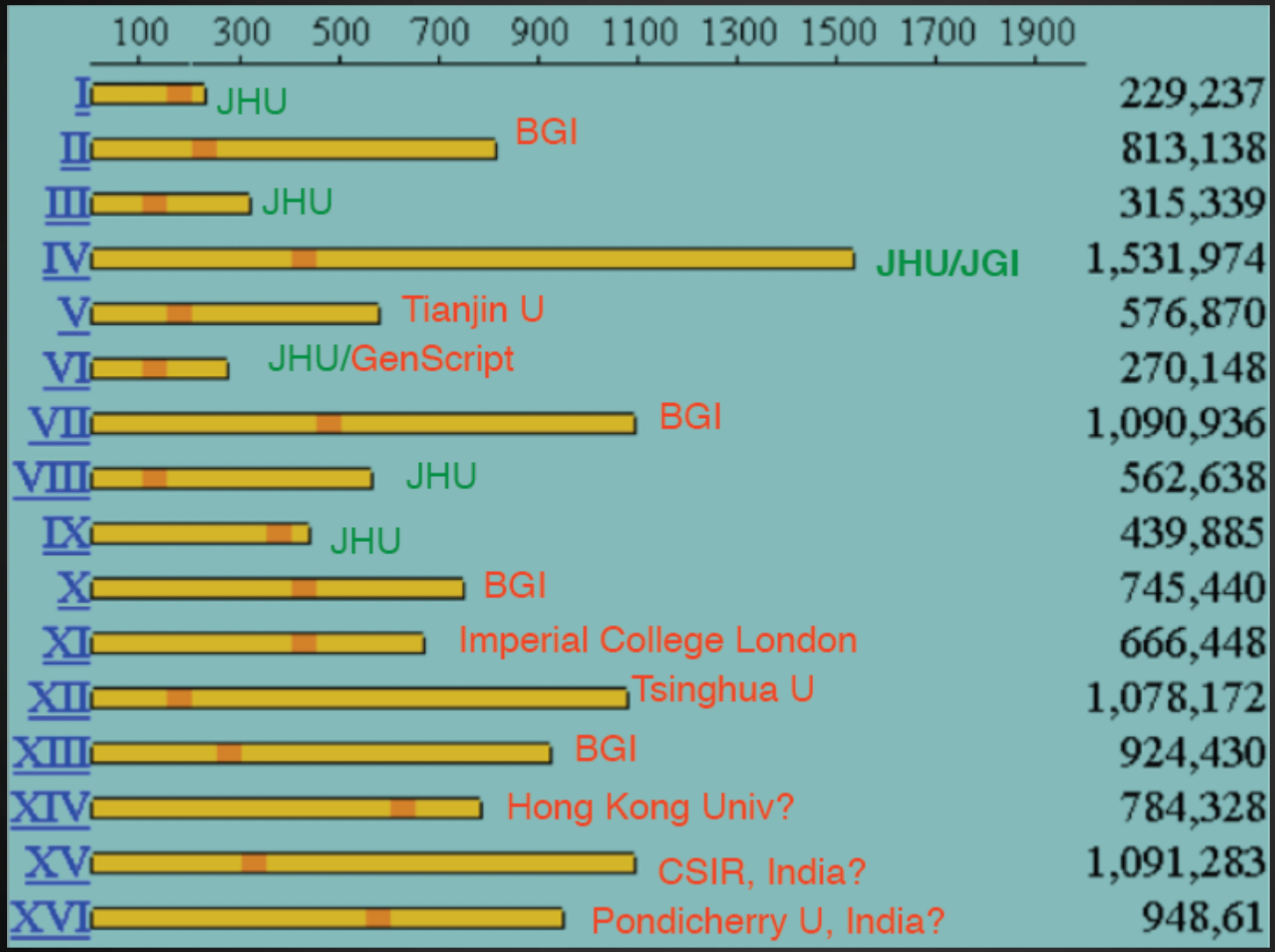
Chunks
Coding Changes
PCR Amplicons

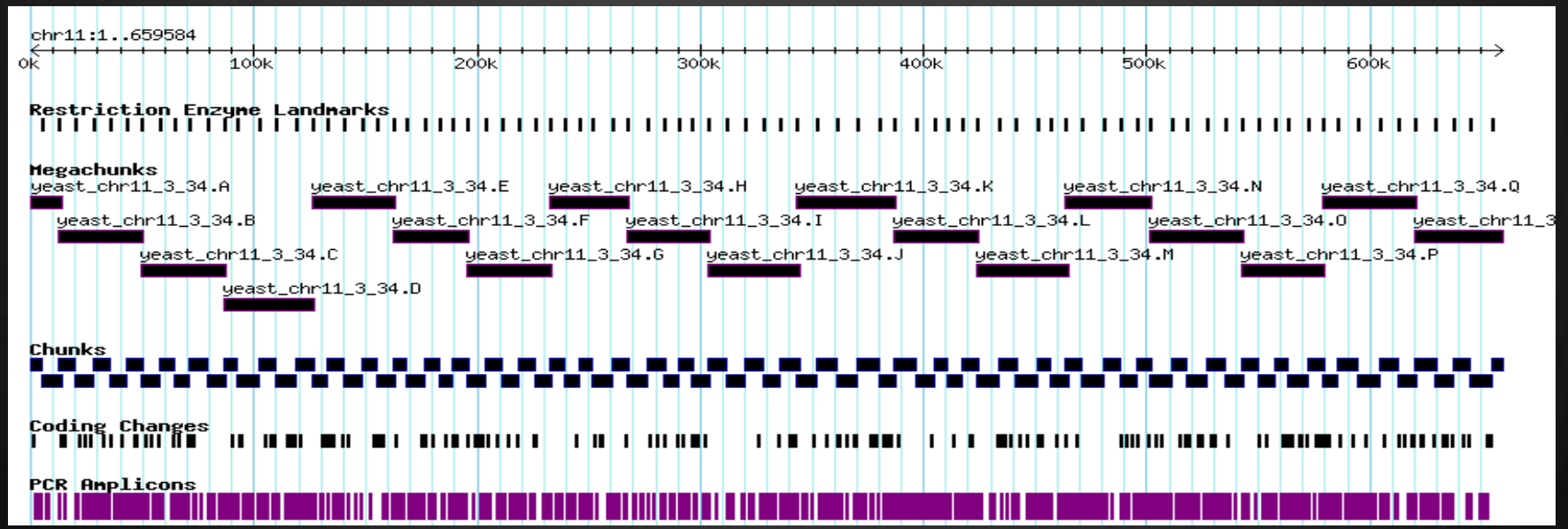
Synthetic Yeast Goes Global



	Dec 2012 status MADE	INT
229,237	20%	0%
813,138	48%	8%
315,339	100%	100%
1,531,974	0%	0%
576,870	90%	8%
270,148	100%	70%
1,090,936	0%	0%
562,638	20%	0%
439,885	100%	95%
745,440	0%	0%
666,448	13%	0%
1,078,172	75%	22%
924,430	0%	0%
784,328	0%	0%
1,091,283	0%	0%
948,61	0%	0%

Synthetic Yeast Goes Global

UK are doing chromosome XI (666,000 bp)



Dymond *et al* 2011 - 2 synthetic chromosome arms in yeast

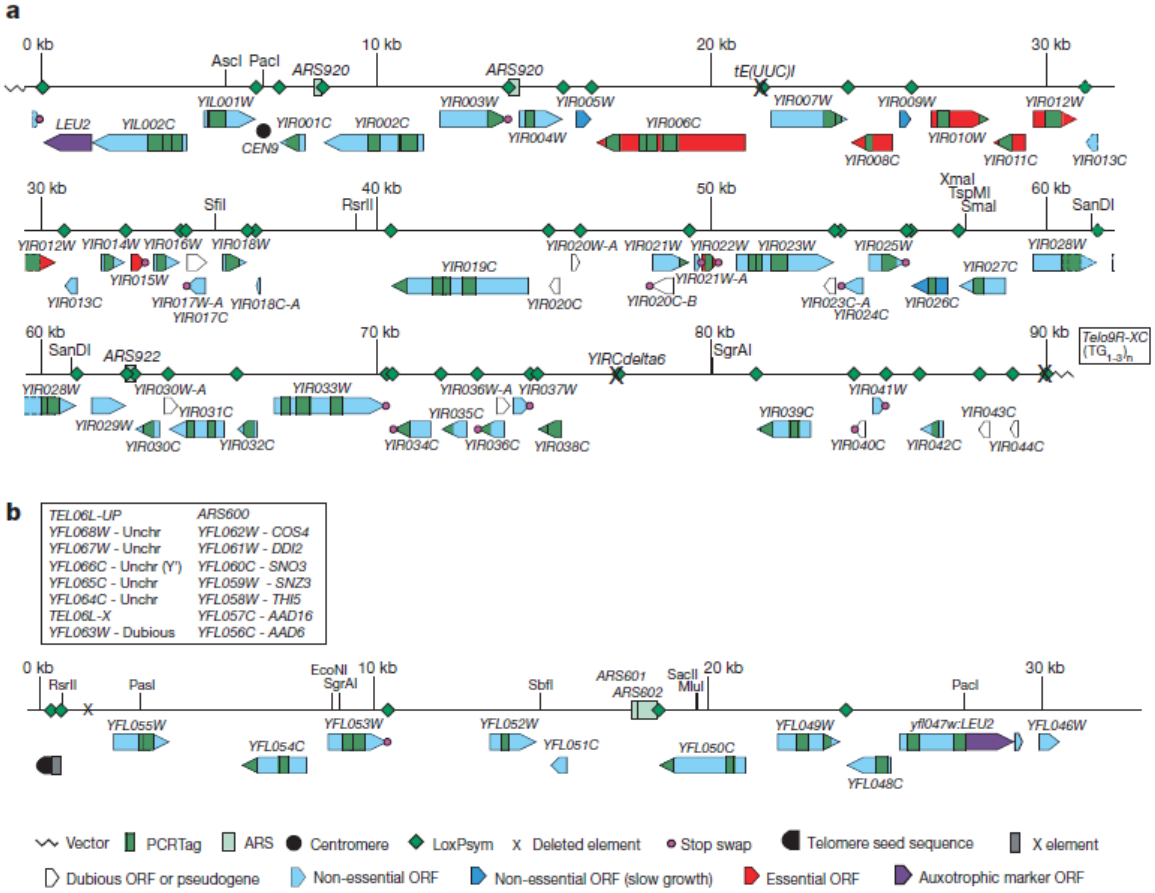


Figure 1 | Maps of synIXR and semi-synVIL. Boxed text indicates elements deleted in the synthetic chromosomes. Vertical green bars inside ORFs indicate PCRTag amplicons; only sequences at the outside edges of these are recoded. ARS, autonomously replicating sequence. a, SynIXR. Vector is circular. b, Semi-synVIL.

Dymond *et al* 2011 – Synthetic Design

BOX 1

Modifications in synthetic sequence

Elements removed

Retrotransposons: The *S. cerevisiae* genome contains both active retrotransposons and retrotransposon-derived sequences. These highly repetitive sequences are known to contribute to genome instability[22]. Because retrotransposons are presumed to be nonessential in yeast, we are eliminating these sequences from the synthetic genome.

Subtelomeric repeats: Two major types of subtelomeric repeats, Y′ and X elements, reside in the genome. Y′ elements are of unknown function, and are present at some, but not all, *S. cerevisiae* chromosome ends[23]. In contrast, X elements are present in a single copy at all *S. cerevisiae* chromosome ends; they are more highly divergent, and function in telomeric silencing and possibly in chromosome segregation[23]. To create a more streamlined genome, all Y′ elements will be deleted from the synthetic genome; extant X elements will be replaced with the consensus core X-element sequence, as in semi-synVIL.

Introns: The yeast genome is estimated to contain approximately 285 introns. Based on a previous intron-deletion study[24] we do not anticipate that removal of introns will result in fitness defects; however, in some cases these introns house small non-coding RNAs (snoRNAs) that can be expressed ectopically in the synthetic yeast.

Elements relocated to extrachromosomal array

tRNA genes: tRNA genes (tDNAs) are highly redundant, with 275 nuclear tDNAs encoding only 42 tRNA species[25]. In addition, these genes are known regions of genome instability[8,9]. They will therefore be relocated to a dedicated chromosome to contain any instability resulting from their presence.

Elements replaced

TAG stop codons replaced by TAA: Removal of the TAG stop codon from the synthetic genome will allow future genetic code manipulation. The 'free' codon may be used to incorporate artificial amino acids[11,12]; alternatively, the TAG codon may be placed in essential genes, and, exploiting an engineered orthogonal synthetase/tRNA pair, specify a non-genetically encoded amino acid, thereby providing a mechanism of reproductive isolation and an additional level of control over the synthetic yeast.

Individual synonymous codons: The synthetic genome is fabricated in fragments as small as 750 bp[26]. Unique restriction sites are necessary within the synthetic fragment to facilitate construction of these building blocks into large contigs of up to 100 kb. Short stretches of fewer than four codons may therefore be synonymously recoded to introduce or eliminate restriction sites.

Strings of synonymous codons: Although several modifications exist between the native and synthetic genomes, the presence of a dedicated mechanism to distinguish between the two sequence types is invaluable. Short stretches of fewer than ten codons are therefore recoded to generate 'PCRTags', synonymous sequences used as the basis for PCR primer design to amplify selectively from wild-type or synthetic genomes.

Elements introduced

LoxPSym sites: Symmetrical loxP sites[13] are inserted in the 3′ UTR of all non-essential genes, as well as at synthetic landmarks. LoxPsym sites lack the directionality of canonical loxP sites, and can therefore align in two orientations. As a result, both inversions and deletions are predicted at equal probability. These loxPsym sites and an inducible Cre recombinase[15] form the basis of the SCRaMbLE toolkit.

Elements not changed

Gene order: Gene order is preserved in the synthetic yeast to prevent incorporation of a non-permissible configuration in the design phase. Induction of SCRaMbLE results in changes in gene order and chromosome structure; all recovered SCRaMbLEd yeast have viable genome structures.

Noncoding regions: Except where noted, noncoding regions have not been modified. The yeast genome is well annotated; however, it is of paramount importance that the synthetic yeast be as fit as wild type until SCRaMbLE is induced. We therefore eschewed changes of noncoding regions to avoid disrupting unannotated critical elements. The few modifications that are made in noncoding sequence are kept to a minimum.

Genome Engineering – what to change?

Venter – added watermark sequences at intergenic sites using a cryptic code
scientists' names, famous quotes, email address (rest stays the same)

SC2.0 – many more changes…

1. Remove unwanted elements
   Retrotransposons, subtelomeric repeats, introns (285 in yeast)

2. Relocate essential elements
   Move tRNA genes to a dedicated chromosome

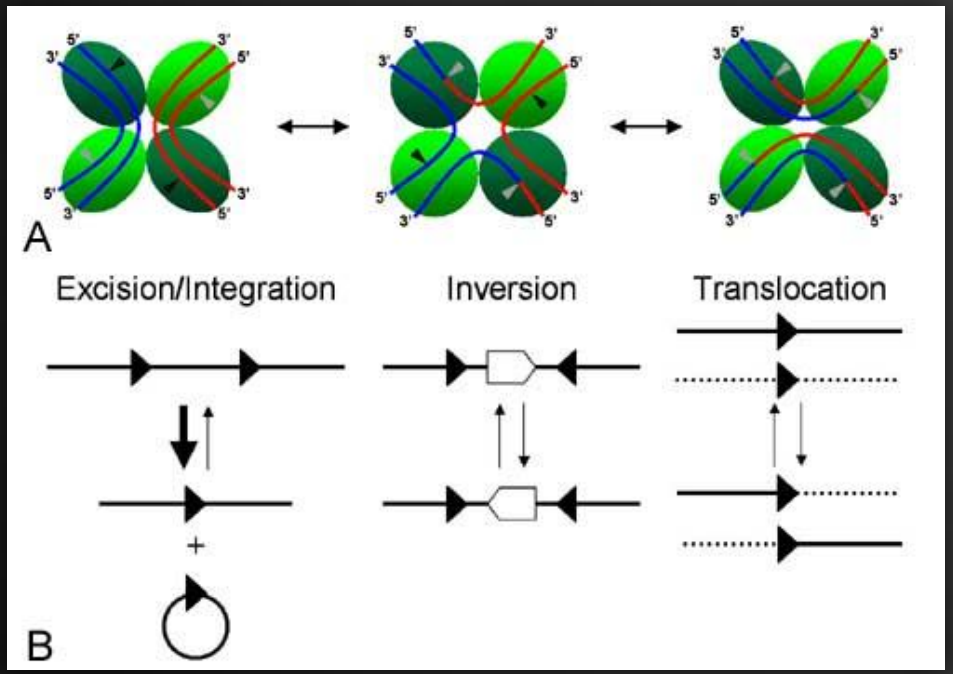
3. Introduce new elements
   Symmetrical loxP sites inserted in the 3'UTR of all non-essential genes, and at synthetic landmarks. This generates the SCRaMbLE toolkit.

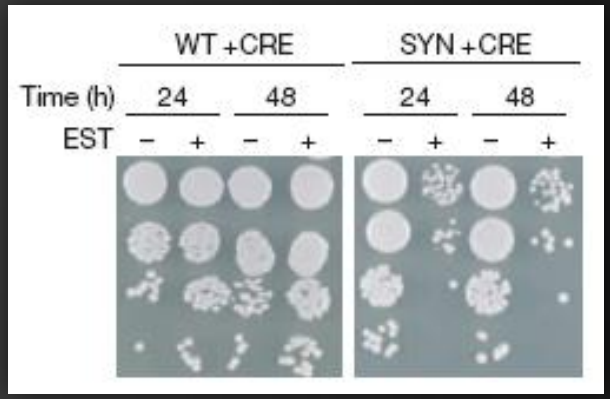
4. Recode existing elements
   With DNA synthesis it is possible to 'silently' change protein coding sequence by using synonymous codons – change all TAG stop codons to TAA codons, incorporate unique sequence tags for PCR and remove some restriction sites.

Automated refactoring of genomes

SC2.0

Symmetrical loxP sites inserted in the 3'UTR of all non-essential genes, and at synthetic landmarks. This generates the SCRaMbLE toolkit.
LoxPsym sites are cut and moved around by Cre recombinase
SC2.0 has inducible Cre expression. Add oestradiol = whole genome shuffle





Automatic refactoring of genome – un-needed genes will be lost

Genome Engineering – what to change?

Venter – added watermark sequences at intergenic sites using a cryptic code
scientists' names, famous quotes, email address (rest stays the same)

SC2.0 – many more changes…

1. Remove unwanted elements
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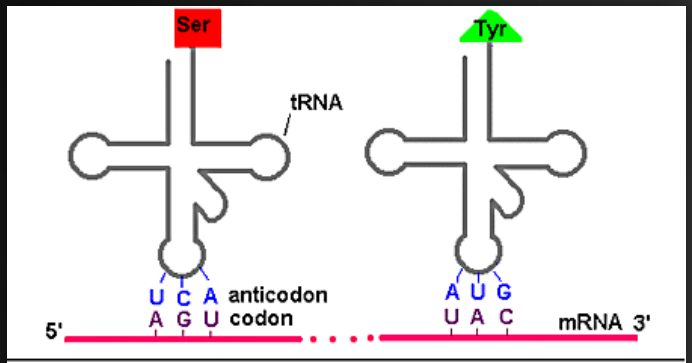
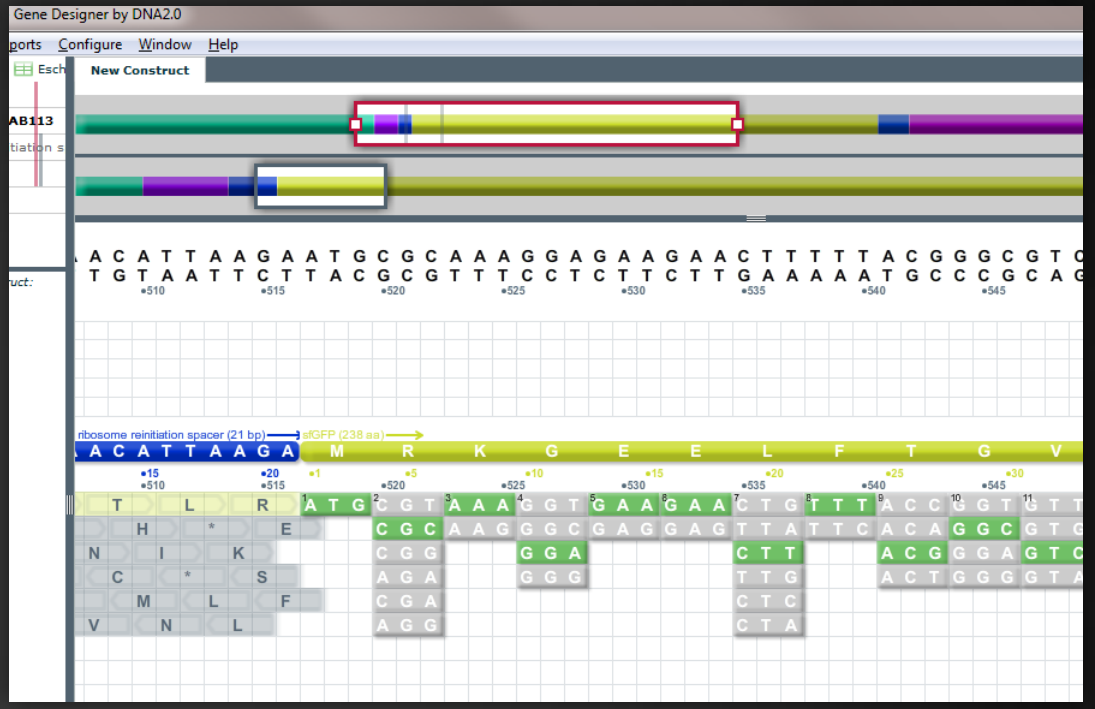
Synonymous Codons



2nd base in codon

1st base in codon	U	C	A	G	3rd base in codon
U	Phe Phe Leu Leu	Ser Ser Ser Ser	Tyr Tyr STOP STOP	Cys Cys STOP Trp	U C A G
C	Leu Leu Leu Leu	Pro Pro Pro Pro	His His Gln Gln	Arg Arg Arg Arg	U C A G
A	Ile Ile Ile Met	Thr Thr Thr Thr	Asn Asn Lys Lys	Ser Ser Arg Arg	U C A G
G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G

The Genetic Code



Gene Designer
Synthetic Biology Design Software
DNA2.0 ltd.

Codon Optimisation for Synthetic Biology

UUU F 0.57	UCU S 0.11	UAU Y 0.53	UGU C 0.42
UUC F 0.43	UCC S 0.11	UAC Y 0.47	UGC C 0.58
UUA L 0.15	UCA S 0.15	UAA * 0.64	UGA * 0.36
UUG L 0.12	UCG S 0.16	UAG * 0.00	UGG W 1.00
CUU L 0.12	CCU P 0.17	CAU H 0.55	CGU R 0.36
CUC L 0.10	CCC P 0.13	CAC H 0.45	CGC R 0.44
CUA L 0.05	CCA P 0.14	CAA Q 0.30	CGA R 0.07
CUG L 0.46	CCG P 0.55	CAG Q 0.70	CGG R 0.07
AUU I 0.58	ACU T 0.16	AAU N 0.47	AGU S 0.14
AUC I 0.35	ACC T 0.47	AAC N 0.53	AGC S 0.33
AUA I 0.07	ACA T 0.13	AAA K 0.73	AGA R 0.02
AUG M 1.00	ACG T 0.24	AAG K 0.27	AGG R 0.03
GUU V 0.25	GCU A 0.11	GAU D 0.65	GGU G 0.29
GUC V 0.18	GCC A 0.31	GAC D 0.35	GGC G 0.46
GUA V 0.17	GCA A 0.21	GAA E 0.70	GGA G 0.13
GUG V 0.40	GCG A 0.38	GAG E 0.30	GGG G 0.12

[Codon/a.a./fraction per codon per a.a.]
E. coli K12 data from the Codon Usage Database

E. coli

UUU F 0.46	UCU S 0.19	UAU Y 0.44	UGU C 0.46
UUC F 0.54	UCC S 0.22	UAC Y 0.56	UGC C 0.54
UUA L 0.08	UCA S 0.15	UAA * 0.30	UGA * 0.47
UUG L 0.13	UCG S 0.05	UAG * 0.24	UGG W 1.00
CUU L 0.13	CCU P 0.29	CAU H 0.42	CGU R 0.08
CUC L 0.20	CCC P 0.32	CAC H 0.58	CGC R 0.18
CUA L 0.07	CCA P 0.28	CAA Q 0.27	CGA R 0.11
CUG L 0.40	CCG P 0.11	CAG Q 0.73	CGG R 0.20
AUU I 0.36	ACU T 0.25	AAU N 0.47	AGU S 0.15
AUC I 0.47	ACC T 0.36	AAC N 0.53	AGC S 0.24
AUA I 0.17	ACA T 0.28	AAA K 0.43	AGA R 0.21
AUG M 1.00	ACG T 0.11	AAG K 0.57	AGG R 0.21
GUU V 0.18	GCU A 0.27	GAU D 0.46	GGU G 0.16
GUC V 0.24	GCC A 0.40	GAC D 0.54	GGC G 0.34
GUA V 0.12	GCA A 0.23	GAA E 0.42	GGA G 0.25
GUG V 0.46	GCG A 0.11	GAG E 0.58	GGG G 0.25

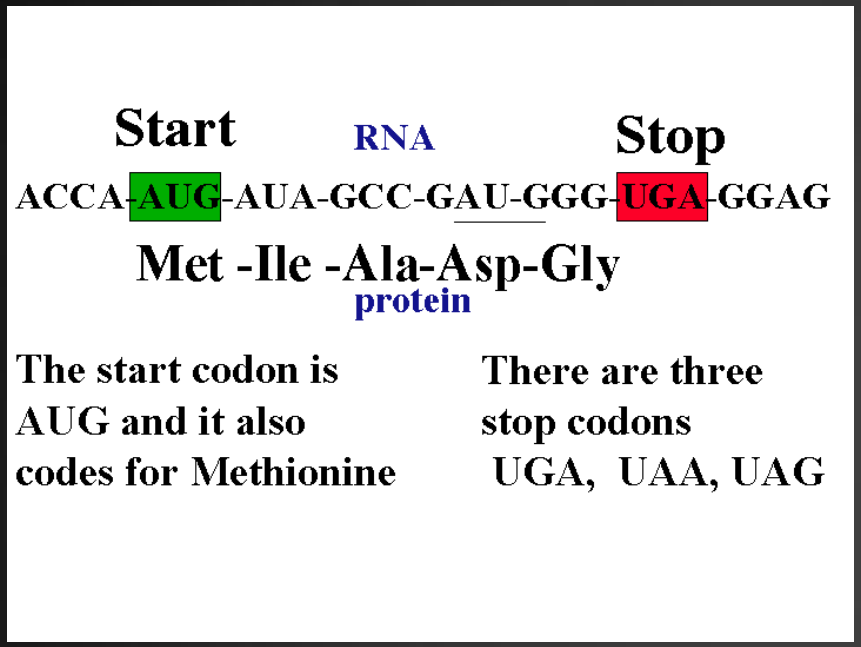
[Codon/a.a./fraction per codon per a.a.]
Homo sapiens data from the Codon Usage Database

Human

Codon optimisation – algorithms that use these tables so that genes can be transferred from one organism to another.

Different synthesis companies use different tables and algorithms

Stop Codon Swapping



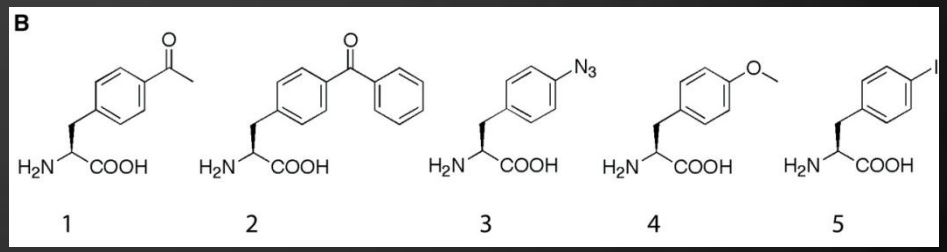
The rarest codon in most genomes is the TAG stop codon

Swapping all of these for TAA codon will 'free-up' an unused codon

Previous work by Peter Schultz's Lab has shown that unnatural amino acids can be programmed into yeast proteins by hijacking the TAG codon.

SC2.0 Yeast with 21 amino acids?

An Expanded Eukaryotic Genetic Code
Jason W. Chin et al. Science 2003



Genome Editing *en masse* - RE.Coli

Genome Engineering of *E. coli*

George Church Lab – Harvard

Remove all TAG stop codons on the natural genome (326)

Free-up further codons in the future

NOT using DNA synthesis

Future:
Swap over codons?
Serine → Arginine

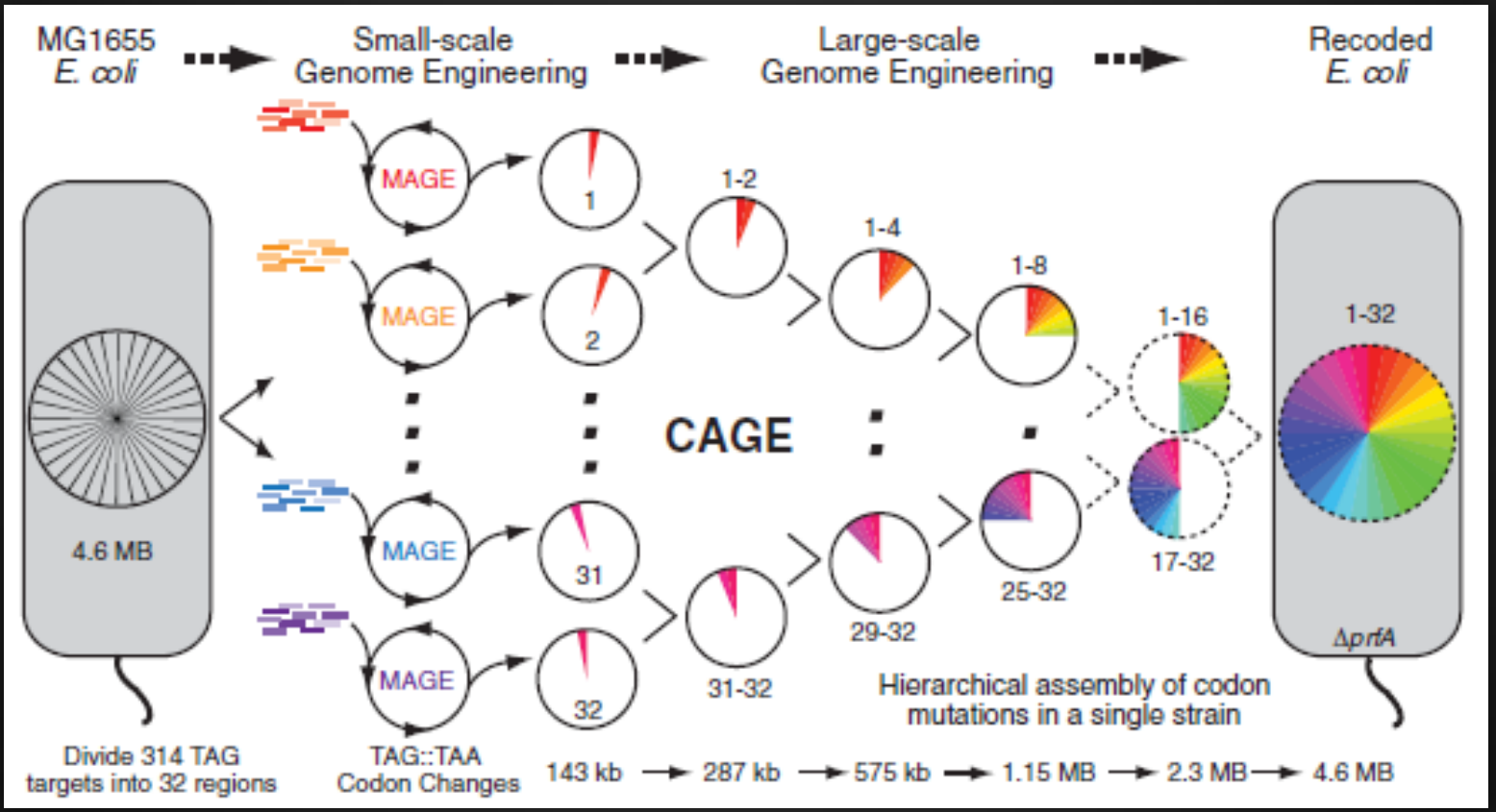
TTT	F	30362	TCT	S	11495	TAT	Y	21999	TGT	C	7048
TTC		22516	TCC		11720	TAC		16601	TGC		8816
TTA	L	4 18932	TCA		9783	TAA	STOP	2703	TGA	STOP	1256
TTG		18602	TCG		12166	TAG	STOP	1 326	TGG	W	20683
CTT	L	15002	CCT	P	9559	CAT	H	17613	CGT	R	28382
CTC		15077	CCC		7485	CAC		13227	CGC		29898
CTA		5314	CCA		11471	CAA	Q	20888	CGA		4859
CTG		71553	CCG		31515	CAG		39188	CGG		7399
ATT	I	41309	ACT	T	12198	AAT	N	24159	AGT	S	3 11970
ATC		34178	ACC		31796	AAC		29385	AGC		21862
ATA		5967	ACA		9670	AAA	K	45687	AGA	R	2 2896
ATG	M	37915	ACG		19624	AAG		14029	AGG		1692
GTT	V	24858	GCT	A	20762	GAT	D	43719	GGT	G	33622
GTC		20753	GCC		34695	GAC		25918	GGC		40285
GTA		14822	GCA		27418	GAA	E	53641	GGA		10893
GTG		35918	GCG		45741	GAG		24254	GGG		15090

Total number of codons in *E. coli* genome

RE.coli - Large-Scale Editing of Genomes

Last update: Farren J. Isaacs *et al*. Science 15 July 2011: Vol. 333 pp. 348-353

2013… done but not yet published



Status of 3 main projects in 2013

Mycoplasma Mycoides
1 million bp
Completed
100% synthetic genome

Changes:
A few watermark regions

Team CRAIG VENTER
Private bio-entrepreneur

Special Powers: Minimal
- Good starting chassis for synthetic biology
- Investigate what is need for life?

E. coli *(RE.coli)*
4.6 million bp
Done but not published
Oligo-edited genome

Changes:
All TAG codons to TAA

Team GEORGE CHURCH
Famous Harvard scientist

Special Powers: Recoded
- Non-natural amino acids can be added
- Potential xenobiology

S. cerevisiae yeast *(SC2.0)*
12 million bp
5 arms out of 32 (16 Chr)
~90% synthetic genome

Changes: no introns
loxPsyms, no TAG codons

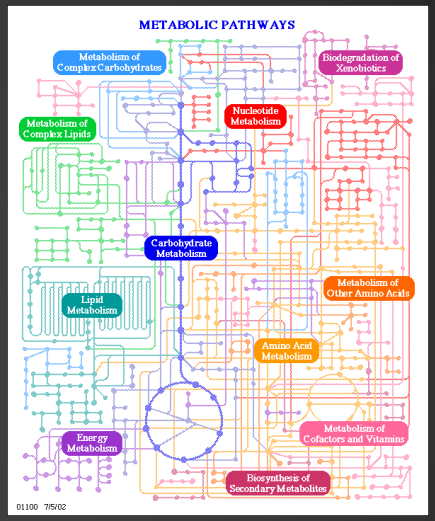
Team BOEKE and.... iGEM undergrads, China, UK

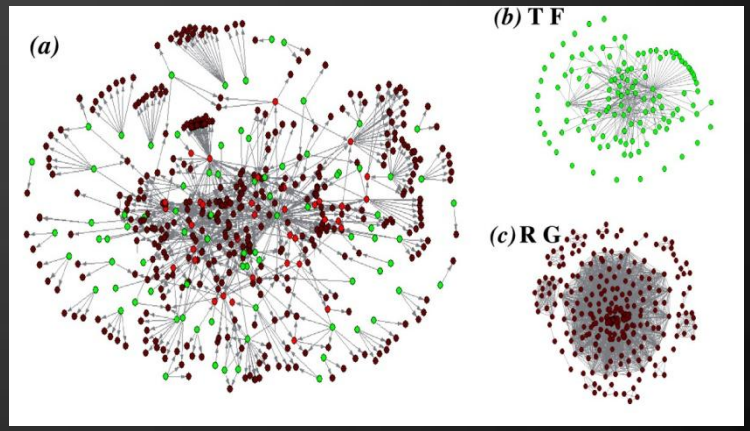
Special Powers: Shuffling
- Automated way to probe gene order
- Automated removal of unneeded genes

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

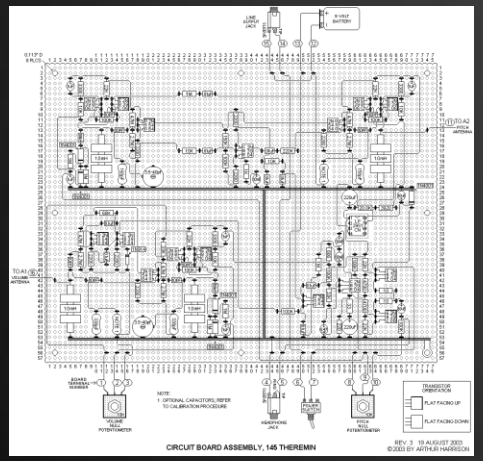


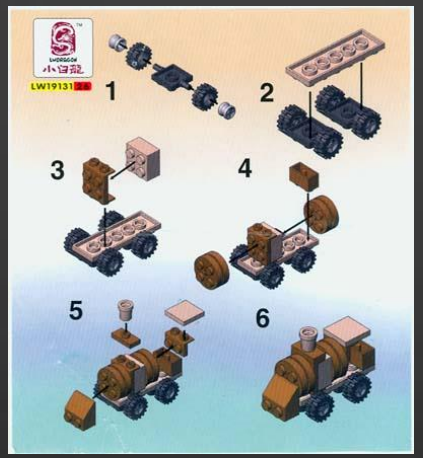


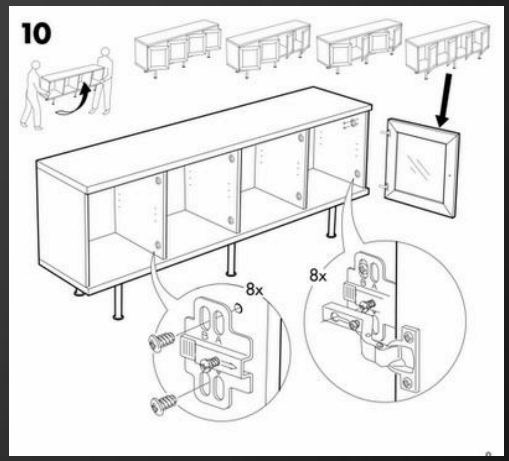
Why do genome engineering?

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





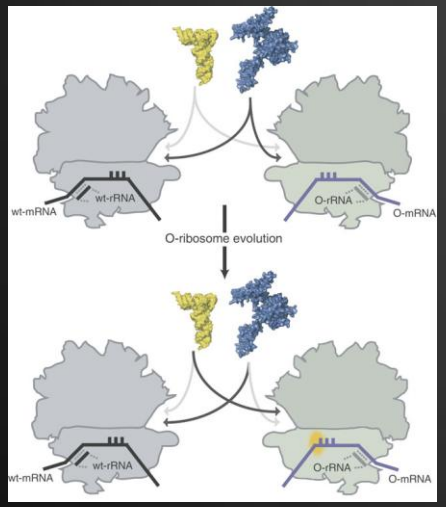


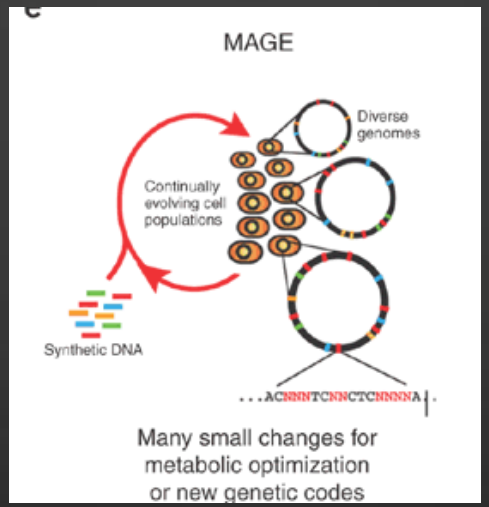
Why do genome engineering?

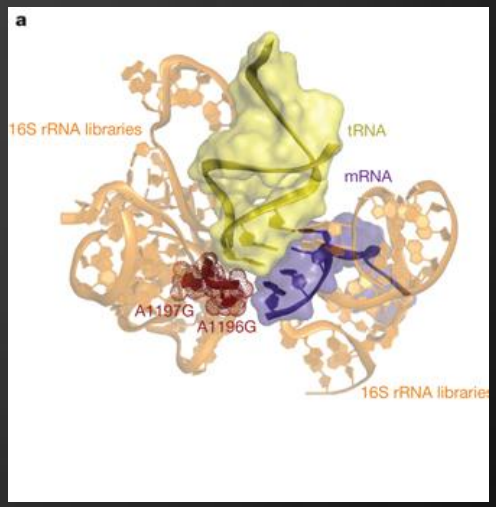
2. Provides for safer synthetic biology

- Cell can be designed to only survive in lab conditions
- Cell could be made orthogonal so that its biology doesn't interact with nature – a.k.a *xenobiology*

  examples: change codon usage or change stereochemistry
- Better predictability from bottom-up design



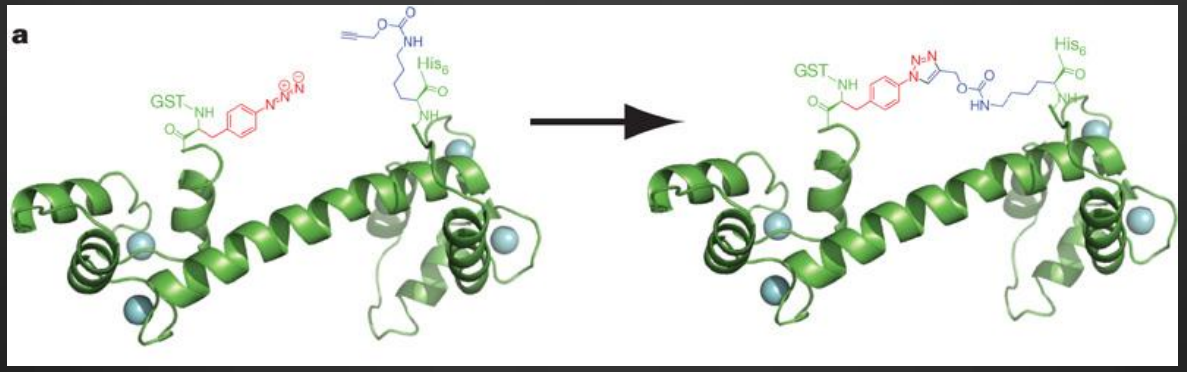




Why do genome engineering?

3. Custom synthesis of products

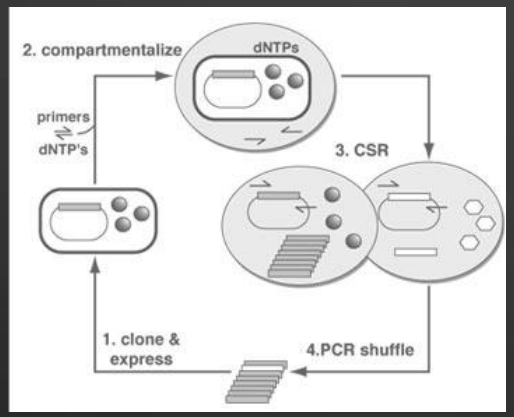
- Cells could be designed to produce non-natural proteins and sugars using synthetic building blocks
- Minimal cells would only use resources to make the desired products and so be more efficient
- Very cheap production of DNA could be engineered
- Synthesis of molecules that are toxic to produce in normal cells
- Recoded cells may not be attached by viruses and phages

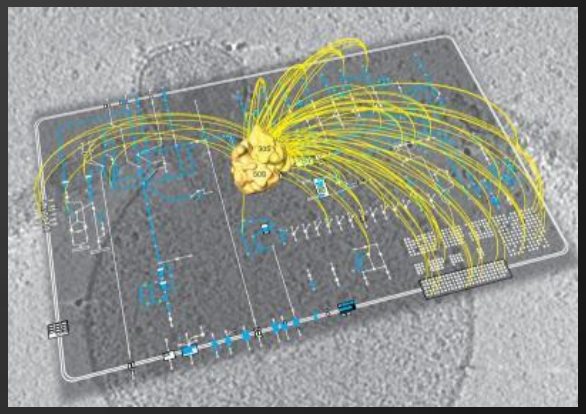


Why do genome engineering?

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. Examples of minimal cells in nature
2. Top down versus bottom-up minimal cells
3. How JCVI made the first cell with a synthetic genome
4. The DNA assembly methods for assembling genomes
5. How to make synthetic yeast chromosomes
6. Re-factoring genomes rationally and automatically
7. Synonymous codons and recoding the genetic code
8. Editing bacterial genomes – how and why?
9. Design and modelling for genome-scale engineering
10. Applications for minimal cells and engineered genomes

Reading – Key Research 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.

Synthetic chromosome arms function in yeast and generate phenotypic diversity by design
- JS Dymond et al. Nature 477 (22 September 2011) pp. 471–476

ALSO take a look at the Supplementary Materials for this paper

And also there is: http://2009.igem.org/Team:Johns_Hopkins-BAG

http://biostudio.bme.jhu.edu/sc2/

Precise manipulation of chromosomes in vivo enables genome-wide codon replacement.
- FJ Isaacs et al. Science Vol. 333 (15 July 2011) pp. 348-353

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

Reading – Further Research Papers

METHODS FOR GENOME ASSEMBLY AND TRANSPLANTATION BY VENTER GROUP

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

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