Assembling designer genomes

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Centre for Synthetic Biology and Innovation

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Assembly

1. The scales and methods of modular DNA assembly

2. Synthetic yeast chromosome construction

DNA Assembly

Sequential   Parallel - Ordered   Combinatorial
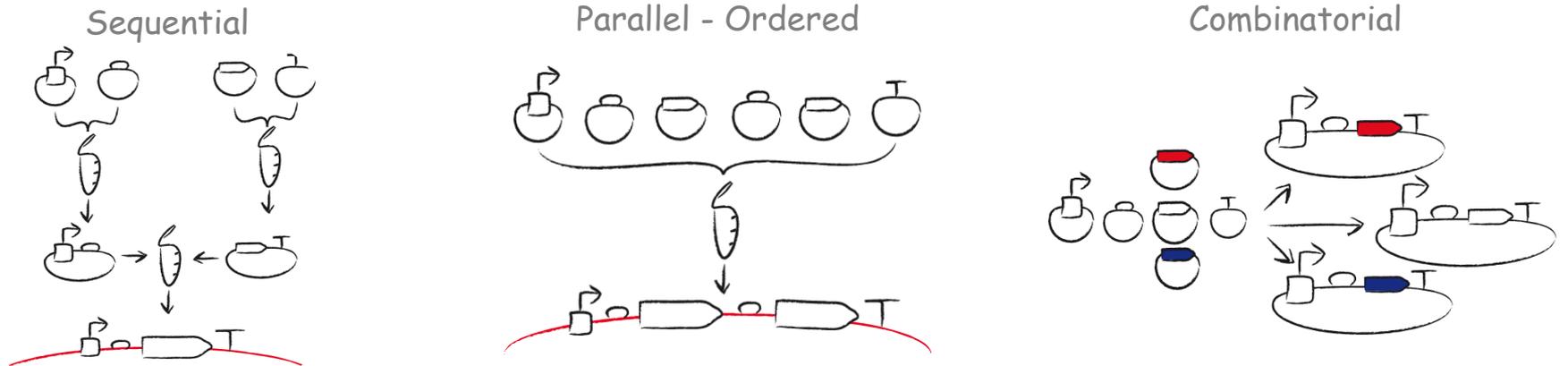
DNA assembly for synthetic biology: from parts to pathways and beyond

Ellis, Adie and Baldwin
Integrative Biology April 2011

DNA assembly from base to genome

DNA sequence	1
Parts	~100
Genes/Operons	1000+
Pathways and Circuits	10000+
Clusters/Regions	100000+
Chromosomes	1000000+
Genome	10000000+

Custom Synthesis

DNA Assembly

DNA assembly methods

L. Kahl & D. Endy
J. Bio Eng May 2013

Different methods for different scales

DNA sequence	1
Parts	~100
Genes/Operons	1000+
Pathways and Circuits	10000+
Clusters/Regions	100000+
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Custom Synthesis
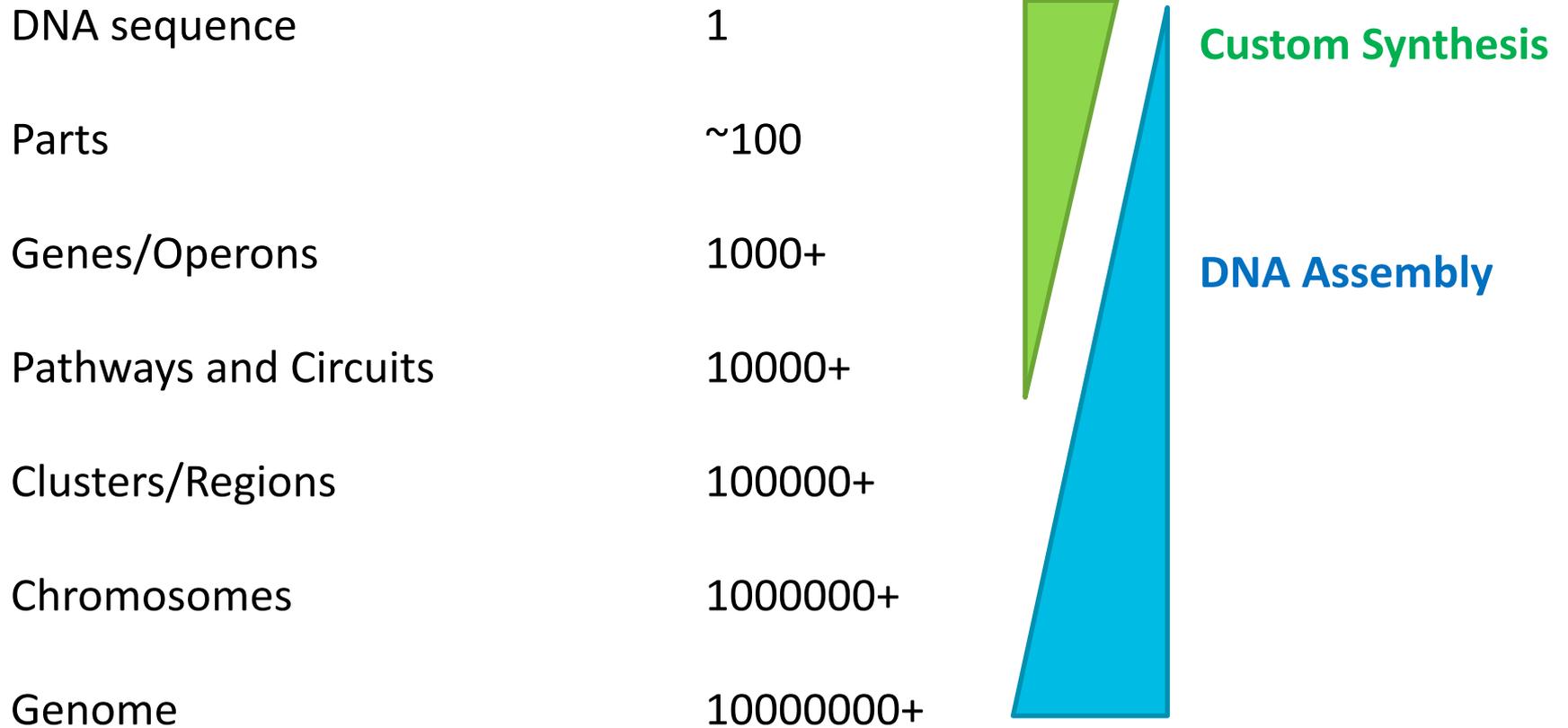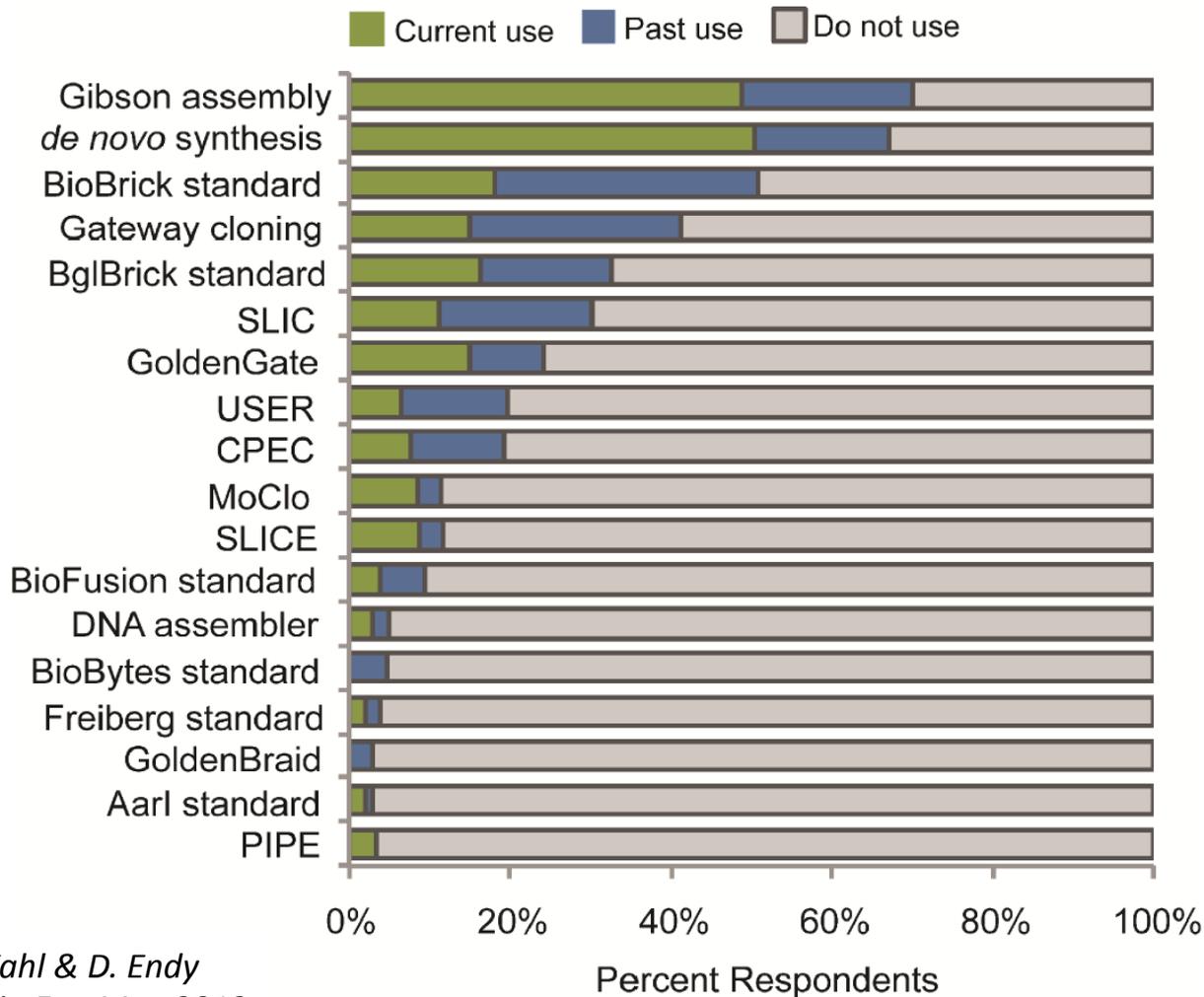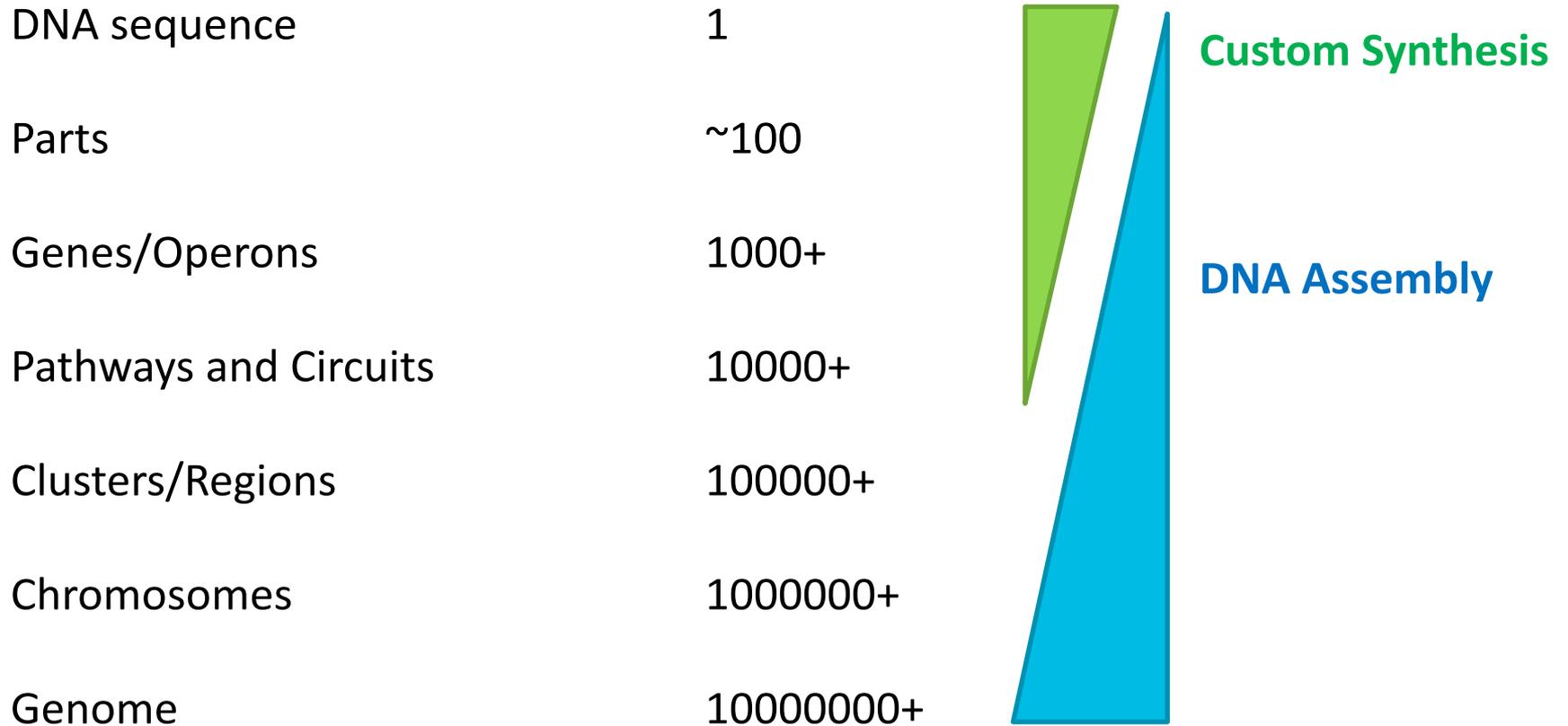
DNA Assembly

DNA assembly levels
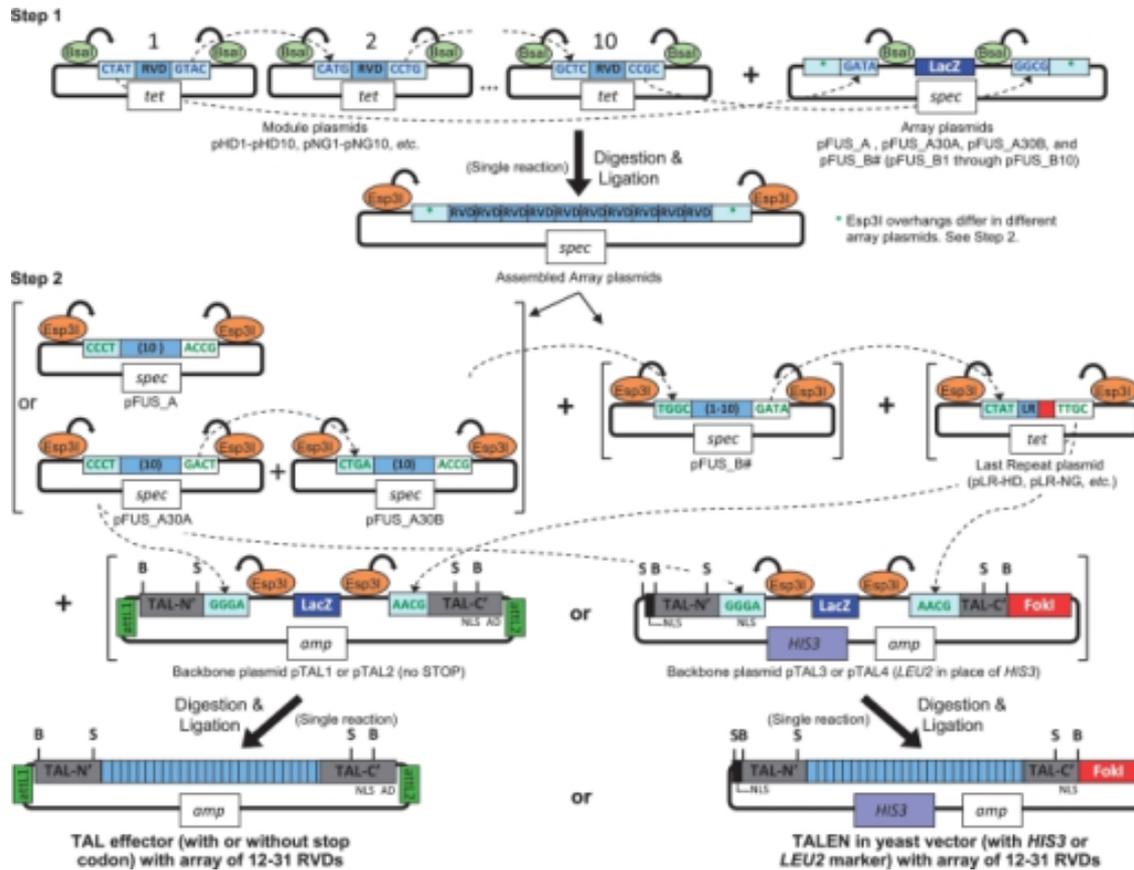
Golden Gate
TALEN kit

1 week = 20+ part
modular assembly

one-pot, ordered

large number of
plasmids

Different methods for different scales

DNA sequence   1

Parts   ~100

Genes/Operons   1000+

Pathways and Circuits   10000+

Clusters/Regions   100000+

Chromosomes   1000000+

Genome   10000000+
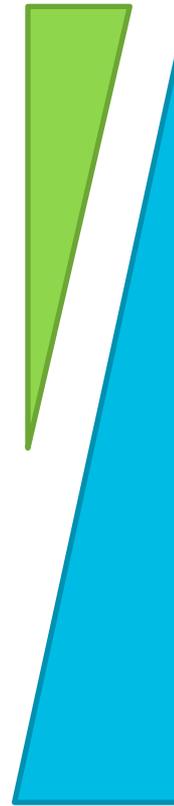
Custom Synthesis

DNA Assembly

# Moving up to the next level
Genome Synthesis and Design

Synthetic Yeast 2.0

Jef Boeke, Johns Hopkins University, USA
http://biostudio.bme.jhu.edu/sc2/

A major international collaboration

Goals:
- Learn by building
- Enable new phenotypes by design
- Open access for everyone

# 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.

Sc2.0 – A global project

Chromosome	Lab	Size	MADE	INT
I	JHU	229,237	20%	0%
II	BGI	813,138	48%	8%
III	JHU	315,339	100%	100%
IV	JHU/JGI	1,531,974	0%	0%
V	Tianjin U	576,870	90%	8%
VI	JHU/GenScript	270,148	100%	70%
VII	BGI	1,090,936	0%	0%
VIII	JHU	562,638	20%	0%
IX	JHU	439,885	100%	95%
X	BGI	745,440	0%	0%
XI	Imperial College London	666,448	13%	3%
XII	Tsinghua U	1,078,172	75%	22%
XIII	BGI	924,430	0%	0%
XIV	Hong Kong Univ?	784,328	0%	0%
XV	CSIR, India?	1,091,283	0%	0%
XVI	Pondicherry U, India?	948,61	0%	0%

Sc2.0 - Yeast Genome Engineering

Step-by-step assembly of a synthetic yeast genome

DNA ASSEMBLY METHOD

DNA SOURCE

Genome
12 Mb

Chromosome
230 kb – 1.5 Mb

Chromosome Arm
80 kb – 1 Mb

Yeast native recombination

Super Chunk
30 kb

Restriction enzyme digest and Ligate

Chunk
10 kb

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

Gibson/USER

Building Block
750 bp

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

PCA: Polymerase Chain Assembly

Oligo
80 nt

GTGCGTACAAGTCAGCAGTATTAGCGGATAGT

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

DNA synthesis is changing
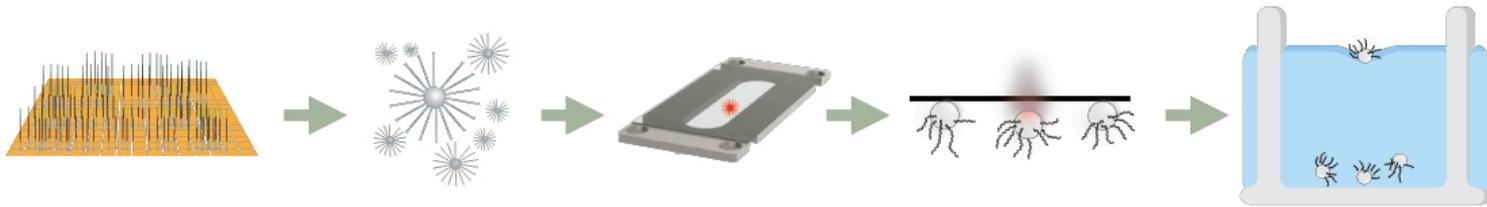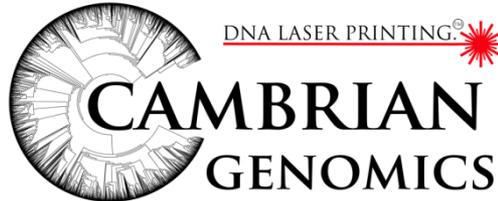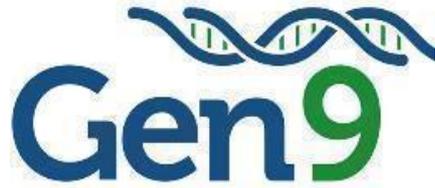
| Synthesize 10^6 oligos | Emulsion PCR onto beads | Sequence 10^9 beads | Laser Eject 10^6 beads in 3 hours | PCR and Assemble 25,000 genes |

Step-by-step assembly of a synthetic yeast genome

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Replacing native sequence with synthetic in yeast
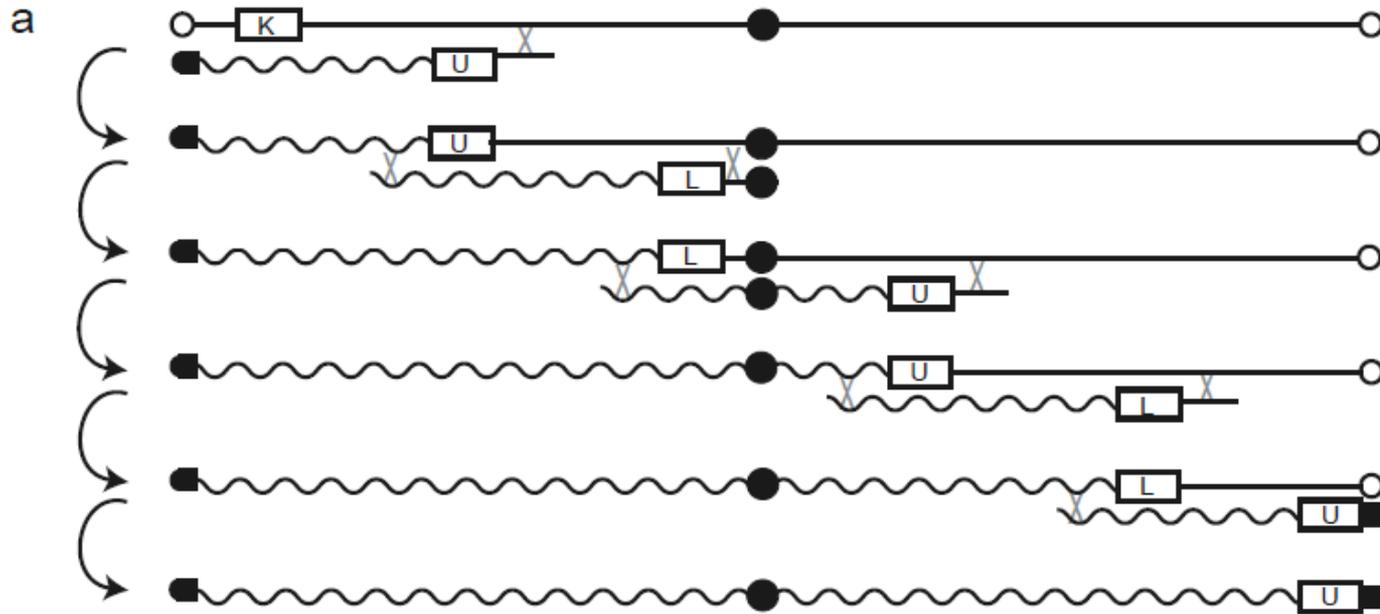
Reiterative Recombination Method

Requires two selectable markers

Makes use of yeast's ability to recombine matching sequences
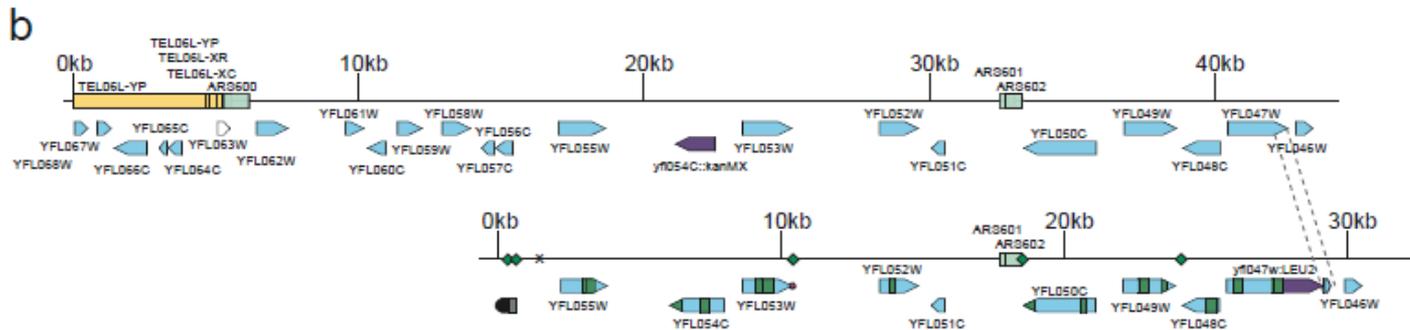
SERIAL process

Sc2.0 design changes

Craig 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
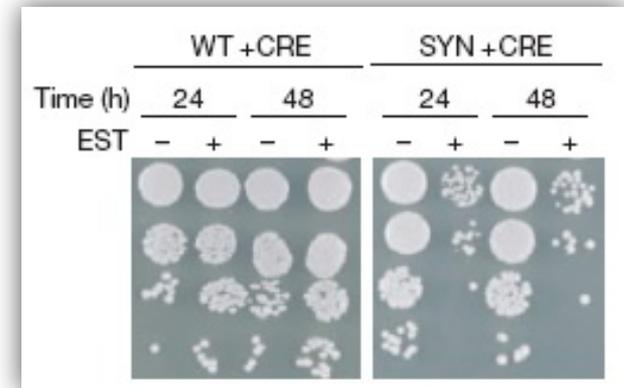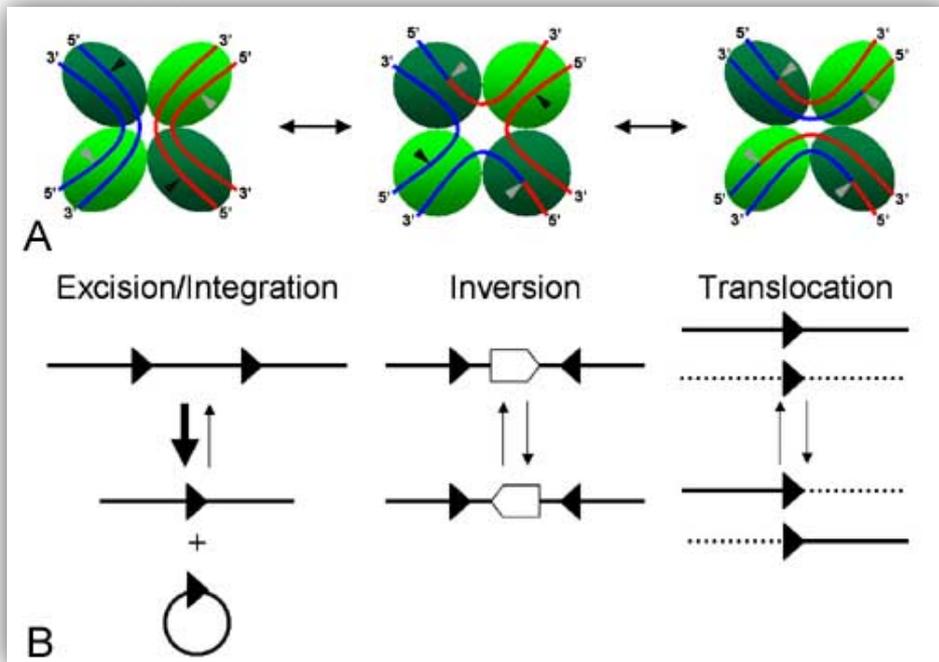
**Automatic refactoring of genome Un-needed genes will be lost**

Sc2.0: can we understand genome layout rules?

How does genomic and nuclear location matter for regulated gene networks and metabolic pathways?
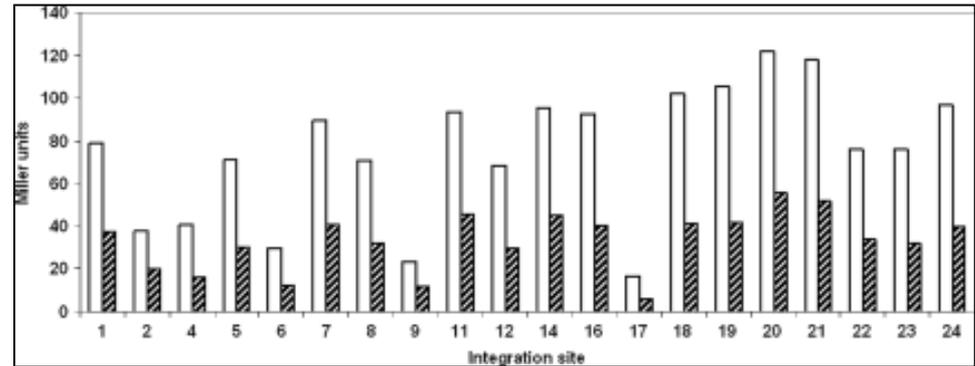
Flagfeldt et al. Yeast 2009

• Gene expression has been shown to be significantly affected by genomic location

• Metabolic pathways often cluster in plants and filamentous fungi.
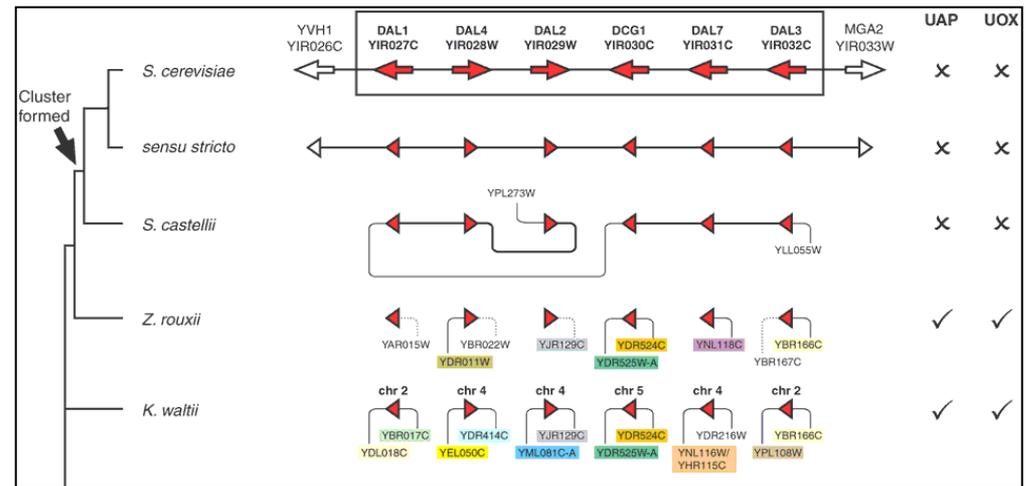
Evolution of DAL cluster *Wong et al. Nat Genetics 2005*

Applying Sc2.0 to Secondary Metabolism
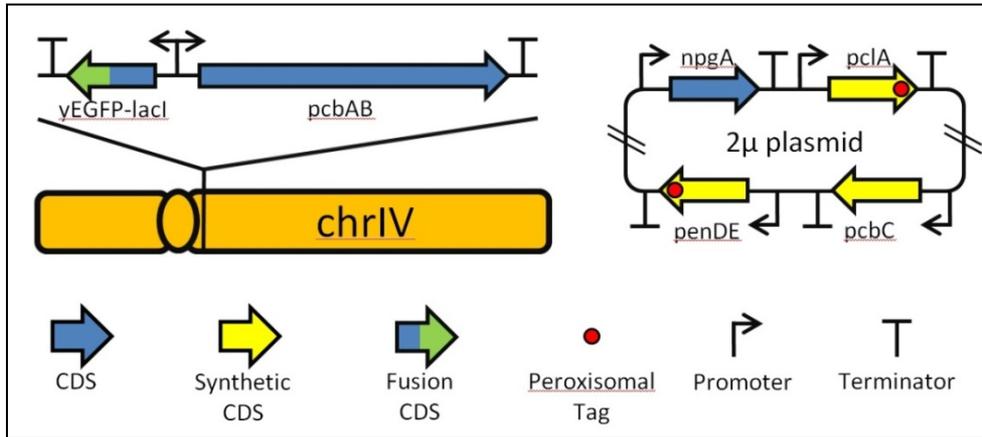
Antibiotic Production
+ Sc2.0 Genome
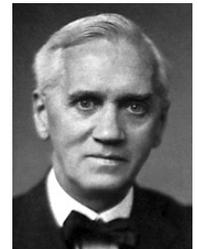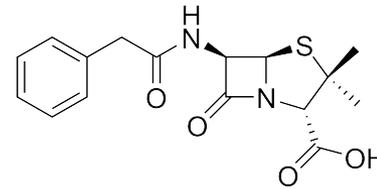
Penicillin Biosynthesis encoded into synthetic yeast chromosomes

Can yields be improved by genomic rearrangement?

Sc2.0 - Yeast Genome Engineering (SUGER)
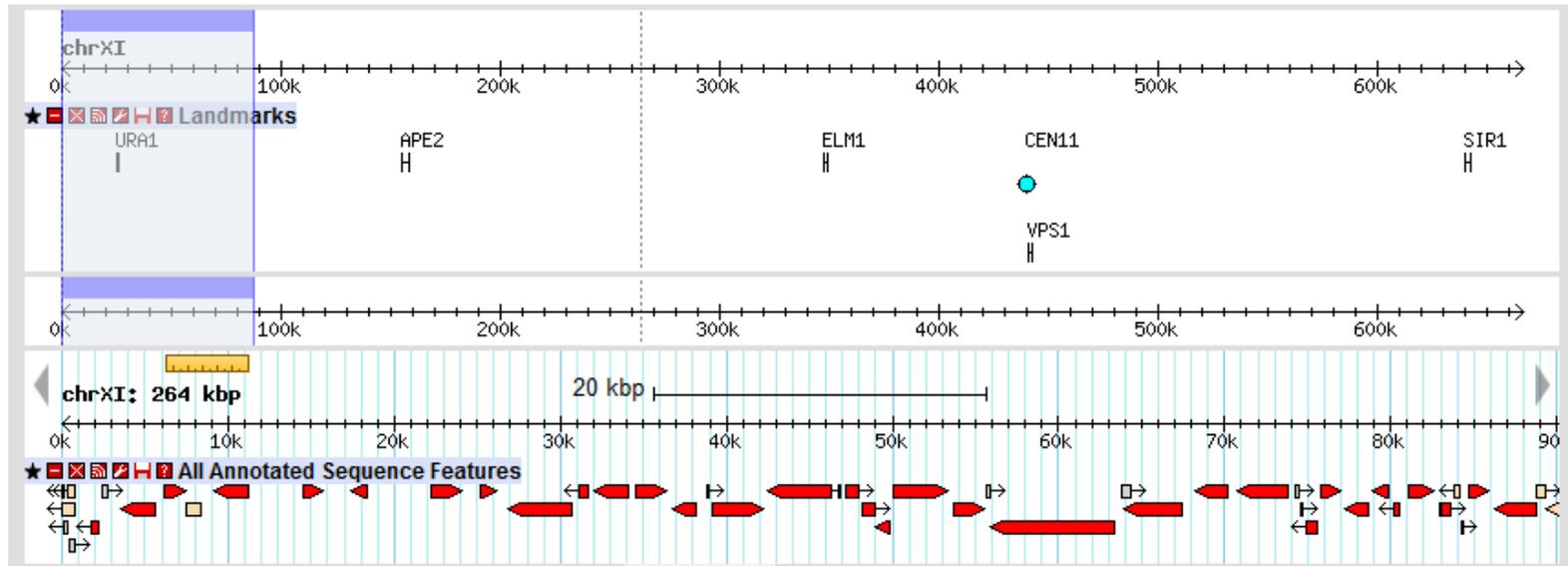
UK Chromosome
SynChr XI – 0.67 Mbp

SUGER and resources

- Access to synthetic and hybrid yeast strains and DNA building blocks

- High-throughput characterisation data of hybrid yeast strains

- Online repository: data, methods, software

- Improved DNA assembly methods for genome synthesis beyond yeast

- Software tools for genome synthesis beyond yeast

- Protocols for genome synthesis using super-cheap synthetic DNA

- Annual workshop for genome synthesis and genome engineering

UNIVERSITY OF CAMBRIDGE   THE UNIVERSITY of EDINBURGH   Imperial College London   BBSRC bioscience for the future

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http://syntheticyeast.eventbrite.com/