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DE L'INNOVATION AGRONOMIQUE

Nouvelles approches d'ingénierie pour la biologie de synthèse

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Laboratoire d'Ingénierie des Système Biologiques et des Procédés.

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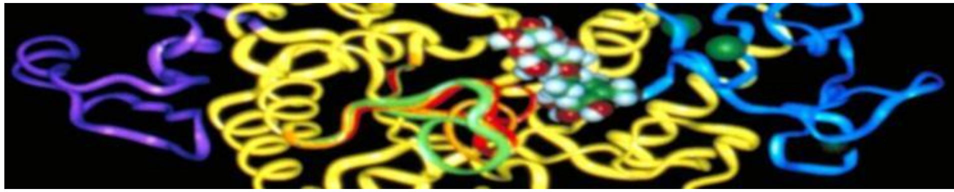
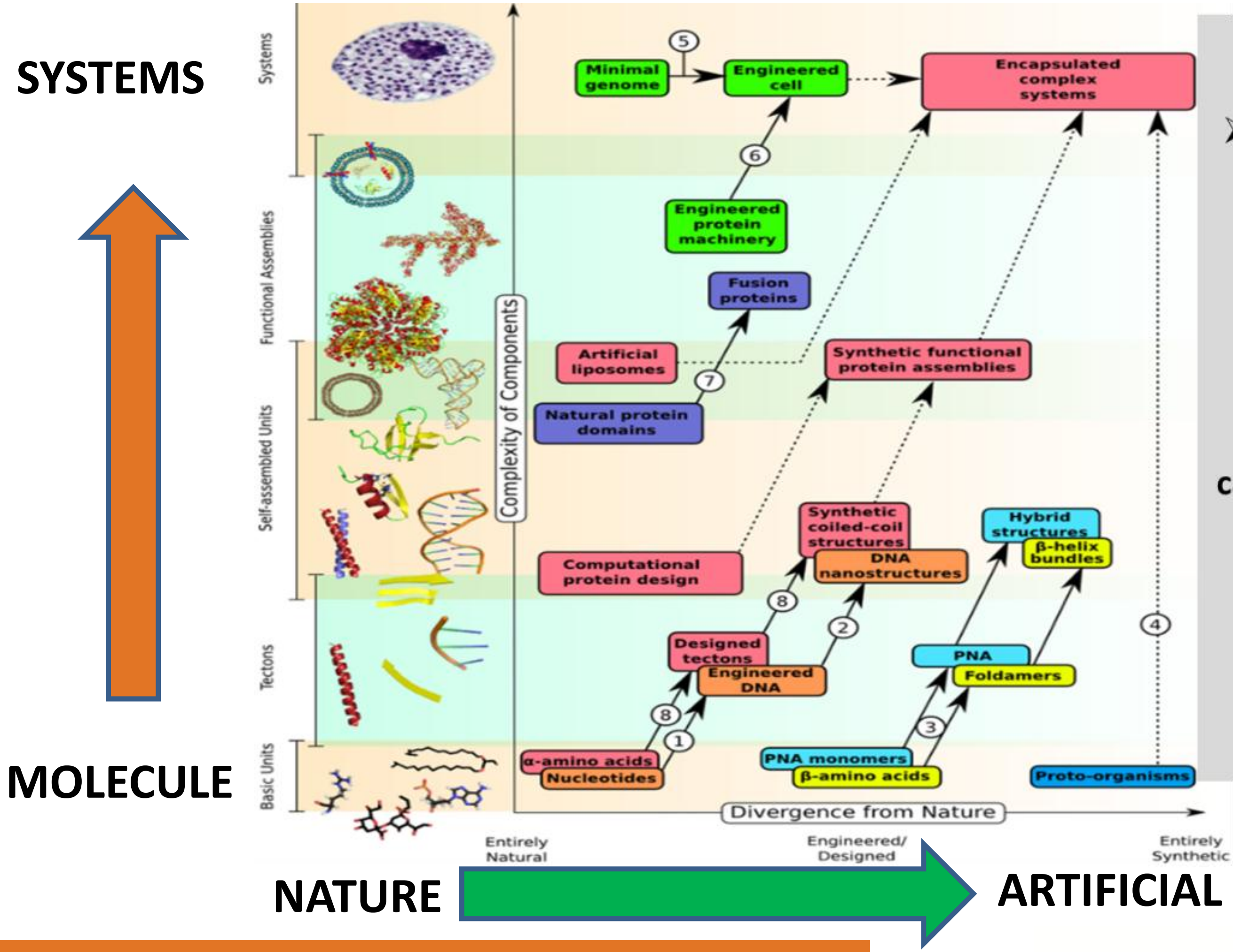


Synthetic Biology overview

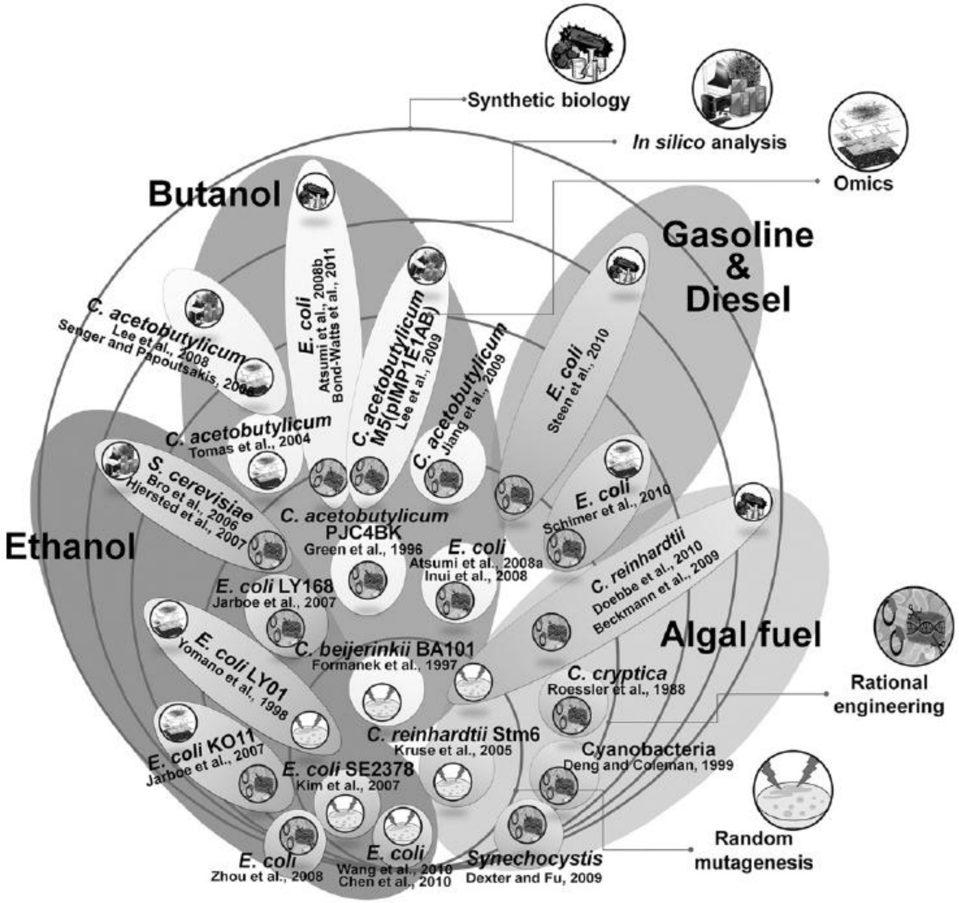


Synthetic biology :

- ➢Use life building blocks as a construction game
- ➢Cover a wide range of complexity levels from molecule to systems
- ➢Expands from nature carbon copy to fully *de novo* designs
- ➢Break barriers between living and non living materials / systems

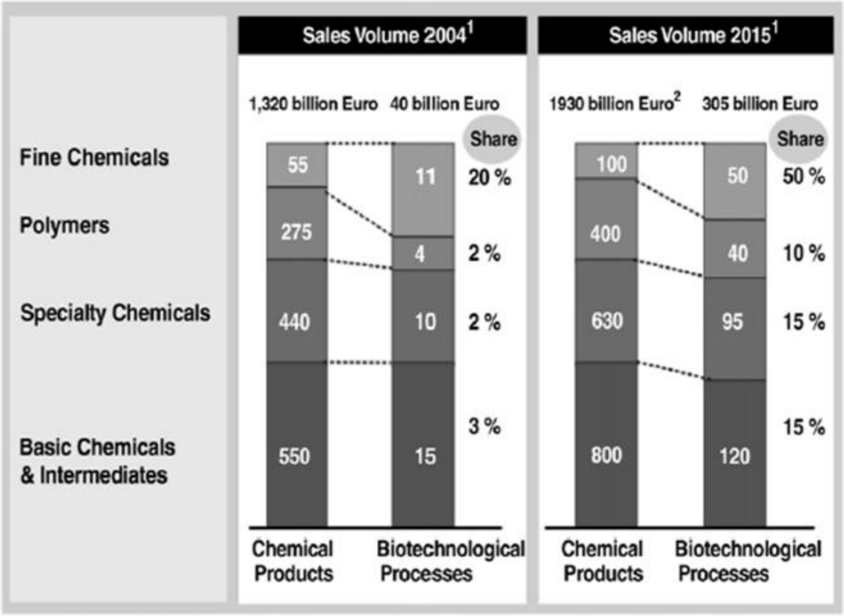
Adapted from Bromley et al. (2008) ACS Chem Biol 3:38-50

Metabolic engineering



Jang Y.S. et al., Biotechnol Adv (2011)

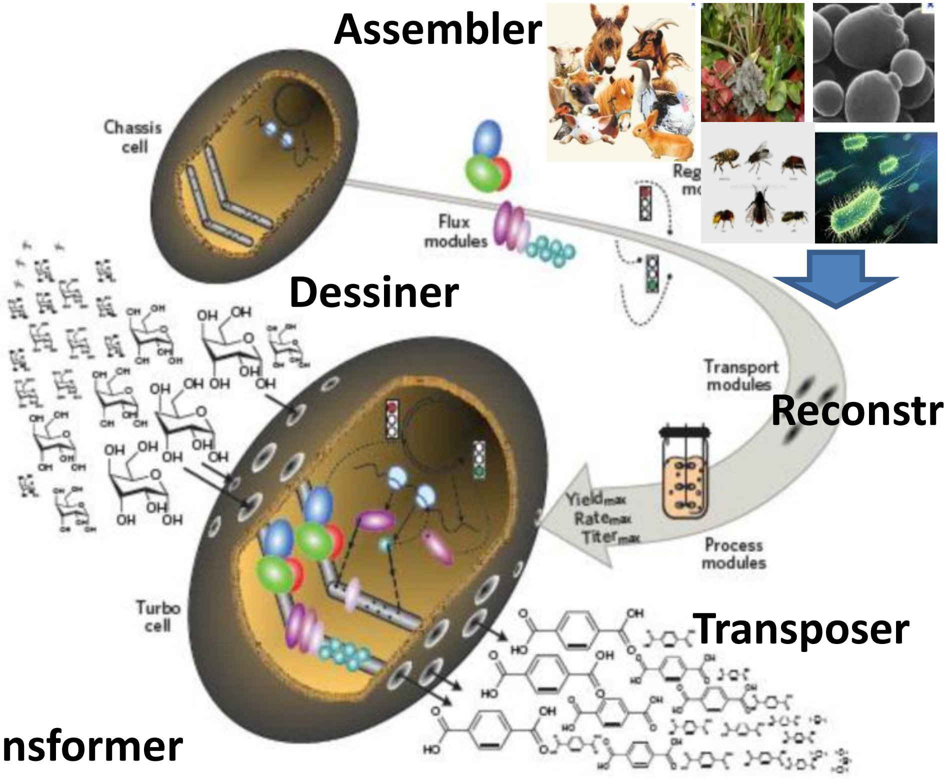
Market of bioprocess for chemical synthesis



Saxena R.K. *et al.* (2009) Biotechnology Advances 27 895–913

Une vision modulaire de l'ingénierie métabolique

S'inspirer ou réutiliser les briques moléculaires du vivant avec une logique différente



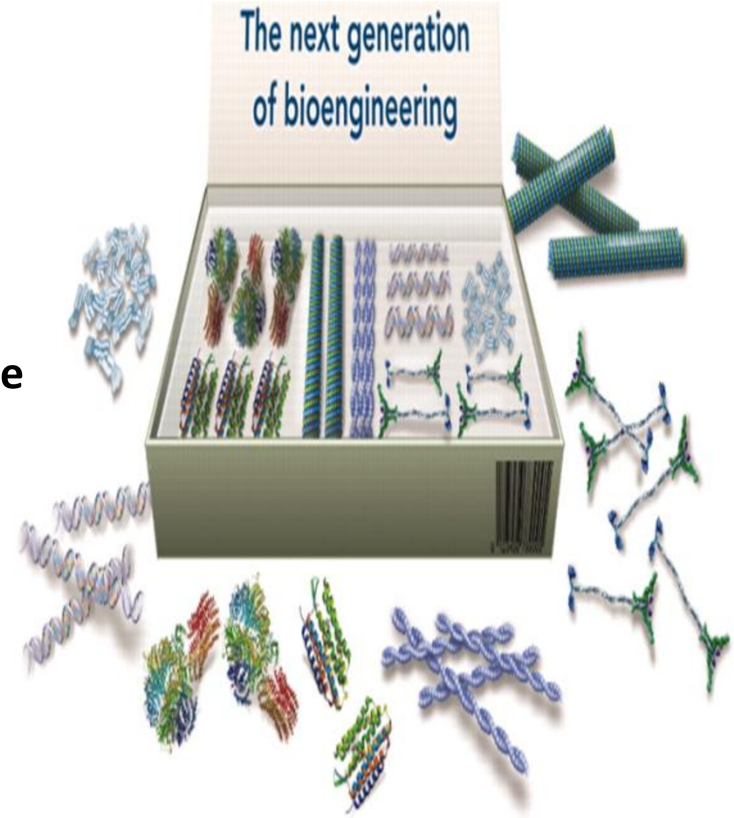
Assembler

Dessiner

Reconstruire

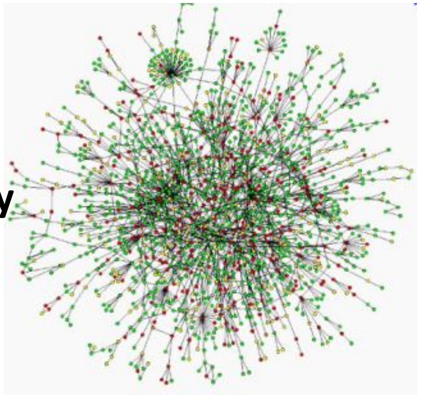
Transposer

Transformer



Une vision modulaire

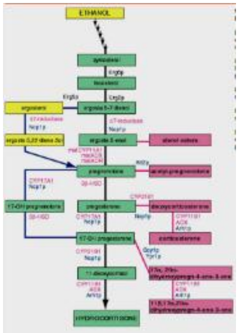
Redesigning
Metabolic networks with a bio-engineer vision

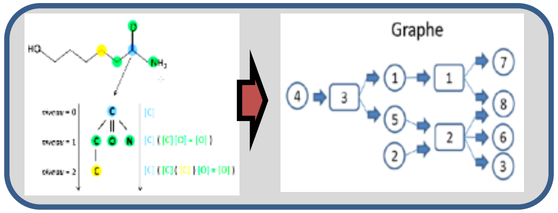


From the complexity of nature

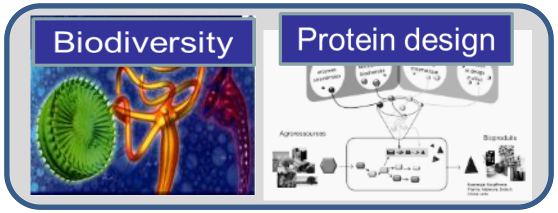
to

Efficient & durable synthetic bioprocesses

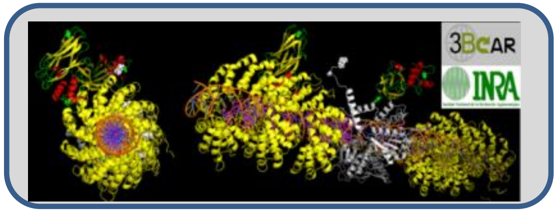




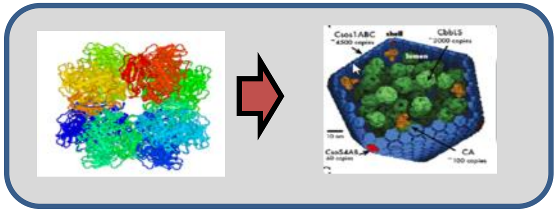
***In silico* design of non-natural biosynthesis**



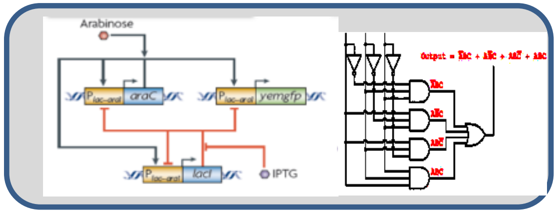
Biodiversity recovery and *de novo* catalyst designs



Synthetic molecular assemblies



Synthetic subcellular compartments

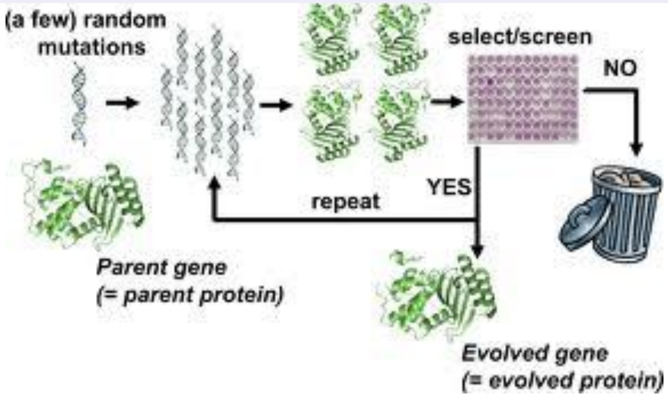


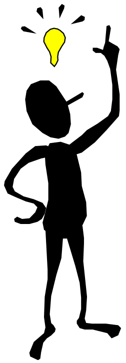
Synthetic regulations

Metabolic engineering drivers

Take profit of the biodiversity of natural catalysts

Create / evolve biocatalysts toward new functions

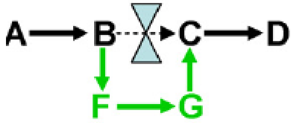




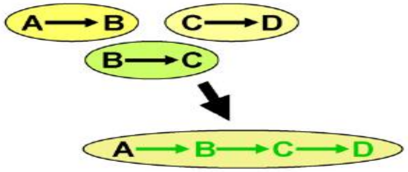
Escape nature logic to meet biotechnology requirements

Disrupt paths

Create alternate paths

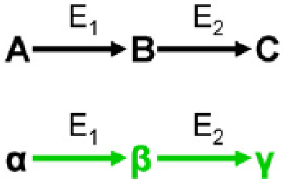


Composing Hybrid

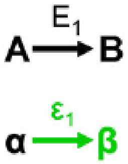


Inter-species hybrid pathways

Exploit promiscuous activities



Combine natural & artificial biocatalysts



In silico design of non-natural biosynthetic pathways (escape nature designs)

Integrate

- Thermodynamic constraints
- Know natural solutions and bottlenecks

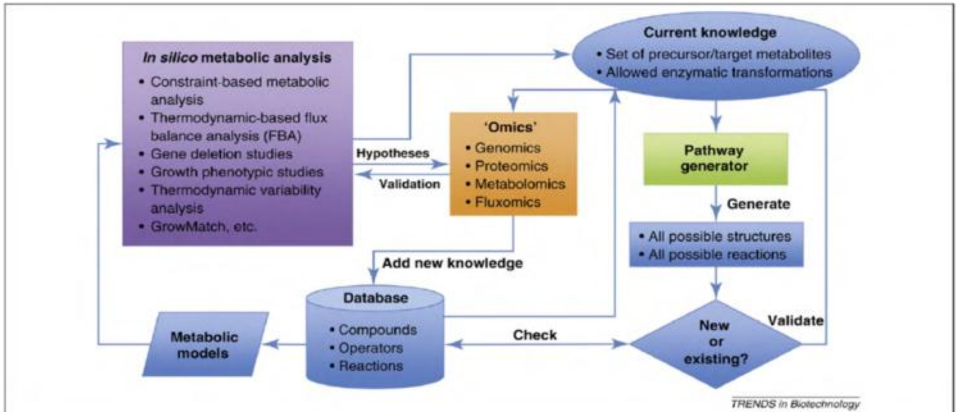
- Scan natural catalyst biodiversity
- Predict catalyst activity promiscuities
- Search for non natural intermediates

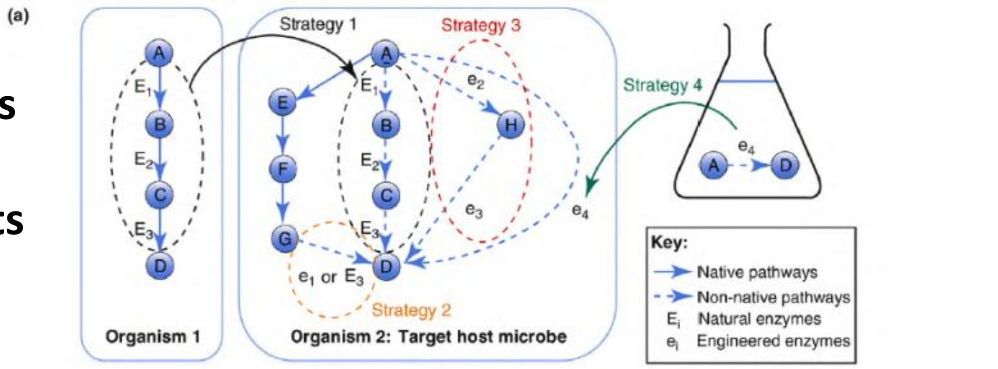
Design

- Generate all possible alternate pathways
- Rank generated pathways
- Identify lacking or non –optimal catalysts
- Select optimal scaffold organism(s)

Validate

- Validate critical steps for best candidates
- Set-up assembly strategy



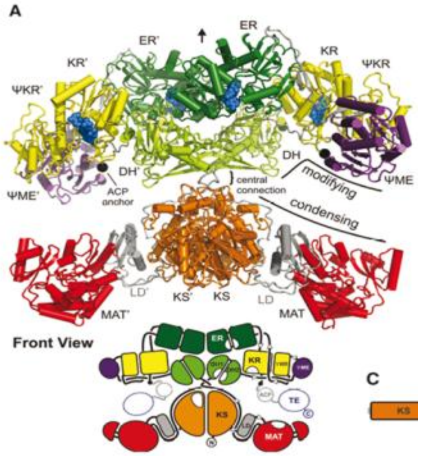


Modified from DREAMS of metabolism . Soh KC et al., (2010) Trends in Biotechnology (28) 501–508

Directing biosynthesis through enzyme scaffolding

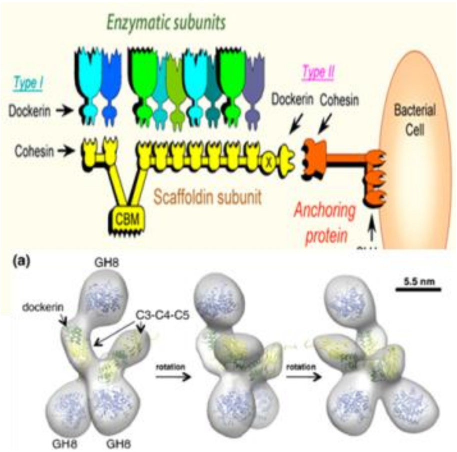
Natural assemblies

Fatty acid synthase



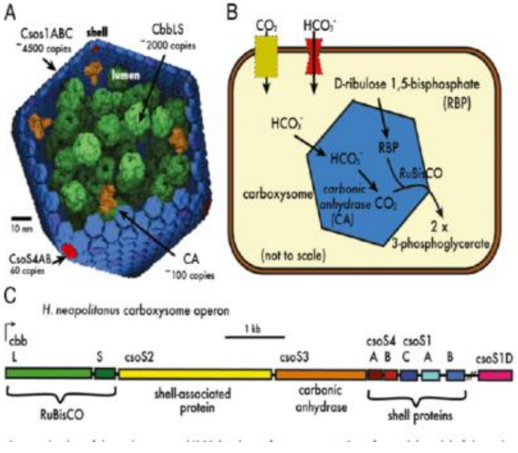
Maier T et al., (2008)

Cellulosome



Alvarez BG et al., (2011)

Carboxysome



Bonaci W et al., (2012) , J

Synthetic assemblies

Some synthetic tuning for alternate function of natural scafolds

- ➢ Simplified scafold (mini-cellulosome)
- ➢ Tuning of matrix specificity
- ➢ Assembly of unrelated activities
- ➢ Display technologies

- ➢ Transfer in alternate host
- ➢ Alternate enzyme content
- ➢ Engineering selective permeability
- ➢ Toxic compound segregation

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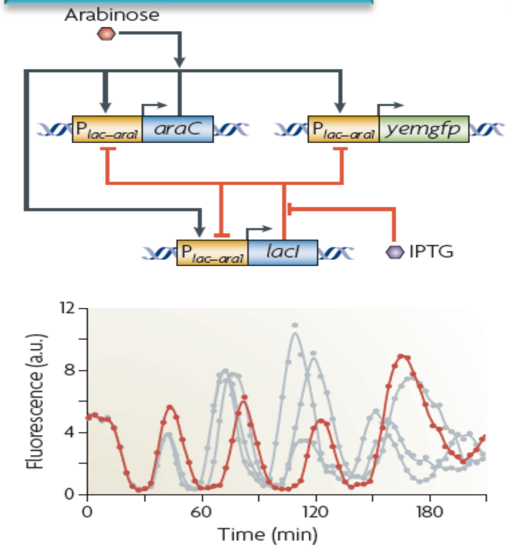
Synthetic regulations

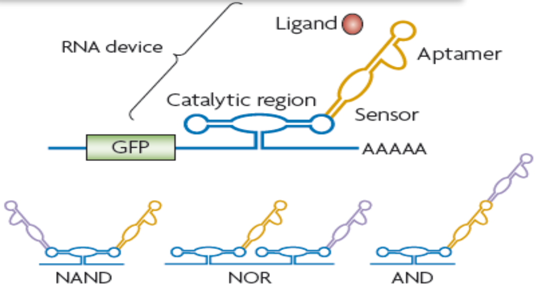
- Natural regulations have been selected by nature to optimize the global metabolic network of a cell.
- They are generally not adapted to metabolism reprograming involving specific input and output,
- Standard promoter sets used to rebuild artificial biosynthesis are not designed for specific regulation

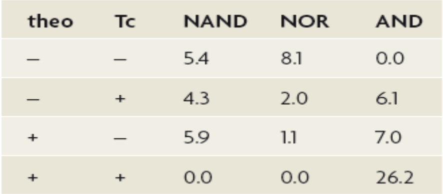
Transcription based



Oscillating circuitry
by suitable assembly of transcriptional repressor *(lacI), activator (araC) and a* reporter (GFP) *downstream a* Plac–ara1 *promoter ,*

Translation based

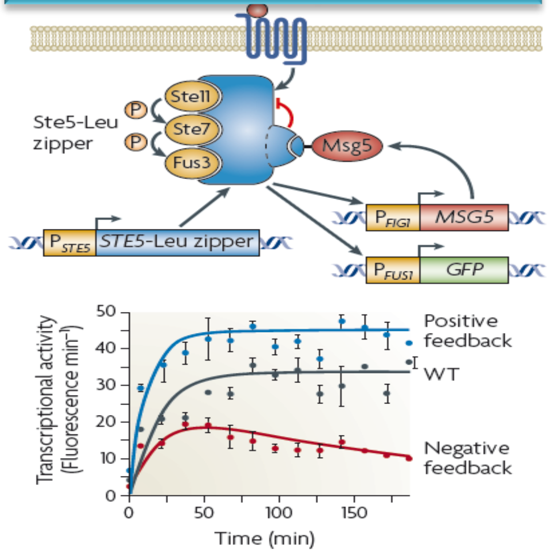




theo	Tc	NAND	NOR	AND
–	–	5.4	8.1	0.0
–	+	4.3	2.0	6.1
+	–	5.9	1.1	7.0
+	+	0.0	0.0	26.2

Logic gate simulations
by translation efficiency control through ligand sensing aptamers

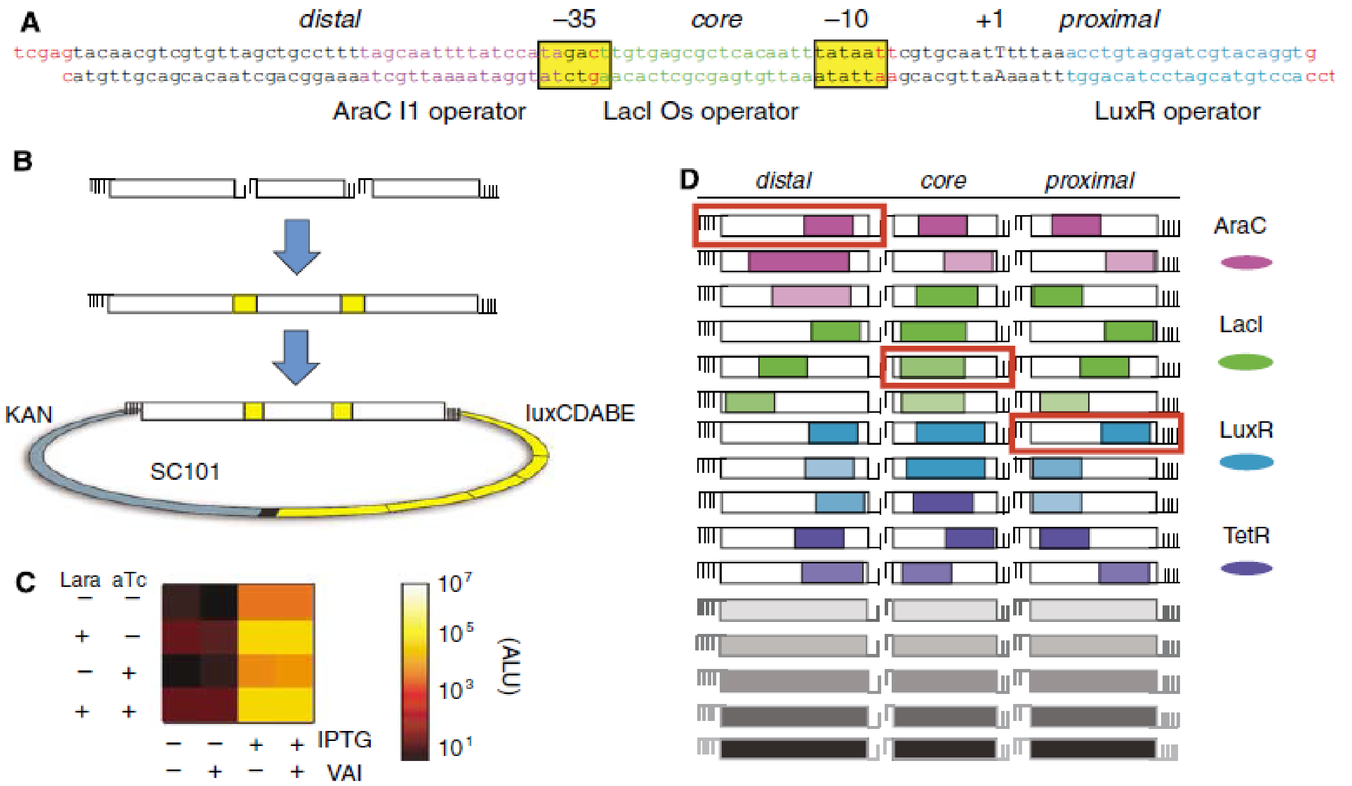
Post-translationaly based



Feeback regulation
by formation of synthetic complexes and allosteric effects

Adapted from *Purnick P. and Weiss R.*, 2009, *Nature* 410)

Transcription control through a family of synthetic promoters covering a wide range of expression levels

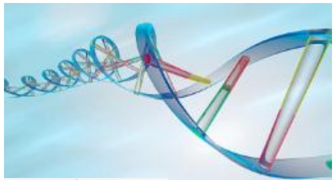


Programming gene expression with combinatorial promoters

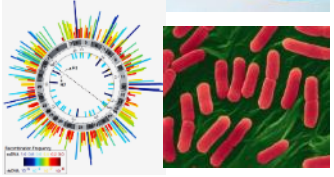
Robert Sidney Cox III[1], Michael G Surette[2] and Michael B Elowitz[1,3,*]

Molecular Systems Biology 3; Article number 145; doi:10.1038/msb4100187
Citation: *Molecular Systems Biology* 3:145

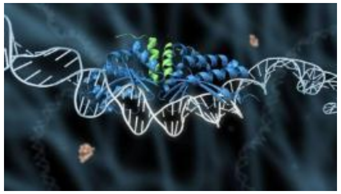
Re-encoding genetic information and genomes



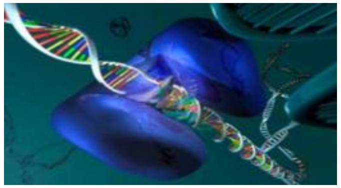
Synthetic genomes & new gene assembly technologies



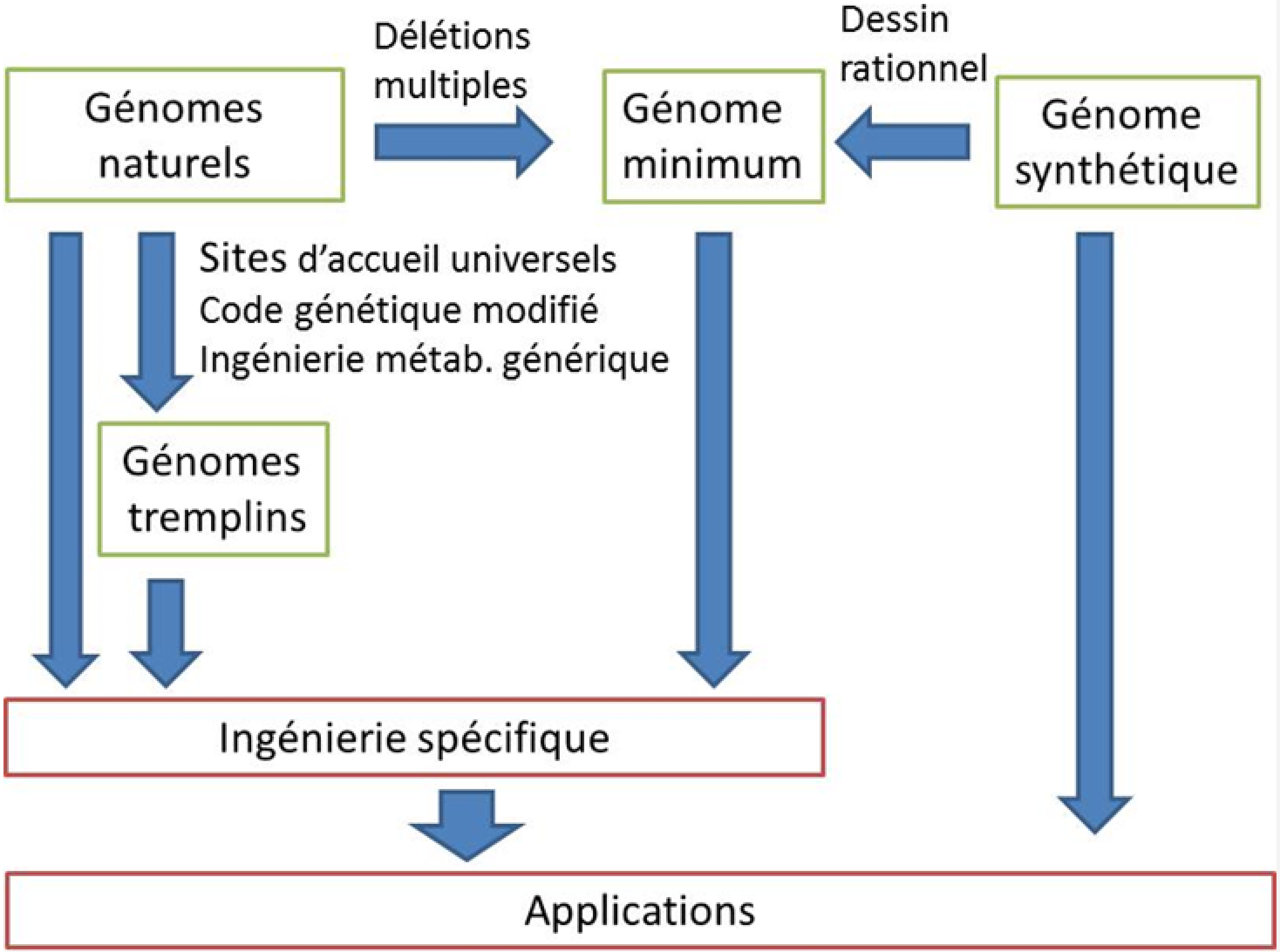
Massive genome editing & alternate genetic codes



Automated synthetic gene assemblies based on homologous recombination



Controlled genome shuffling



Génomes naturels

Délétions multiples

Génome minimum

Dessin rationnel

Génome synthétique

Sites d'accueil universels
Code génétique modifié
Ingénierie métab. générique

Génomes tremplins

Ingénierie spécifique

Applications

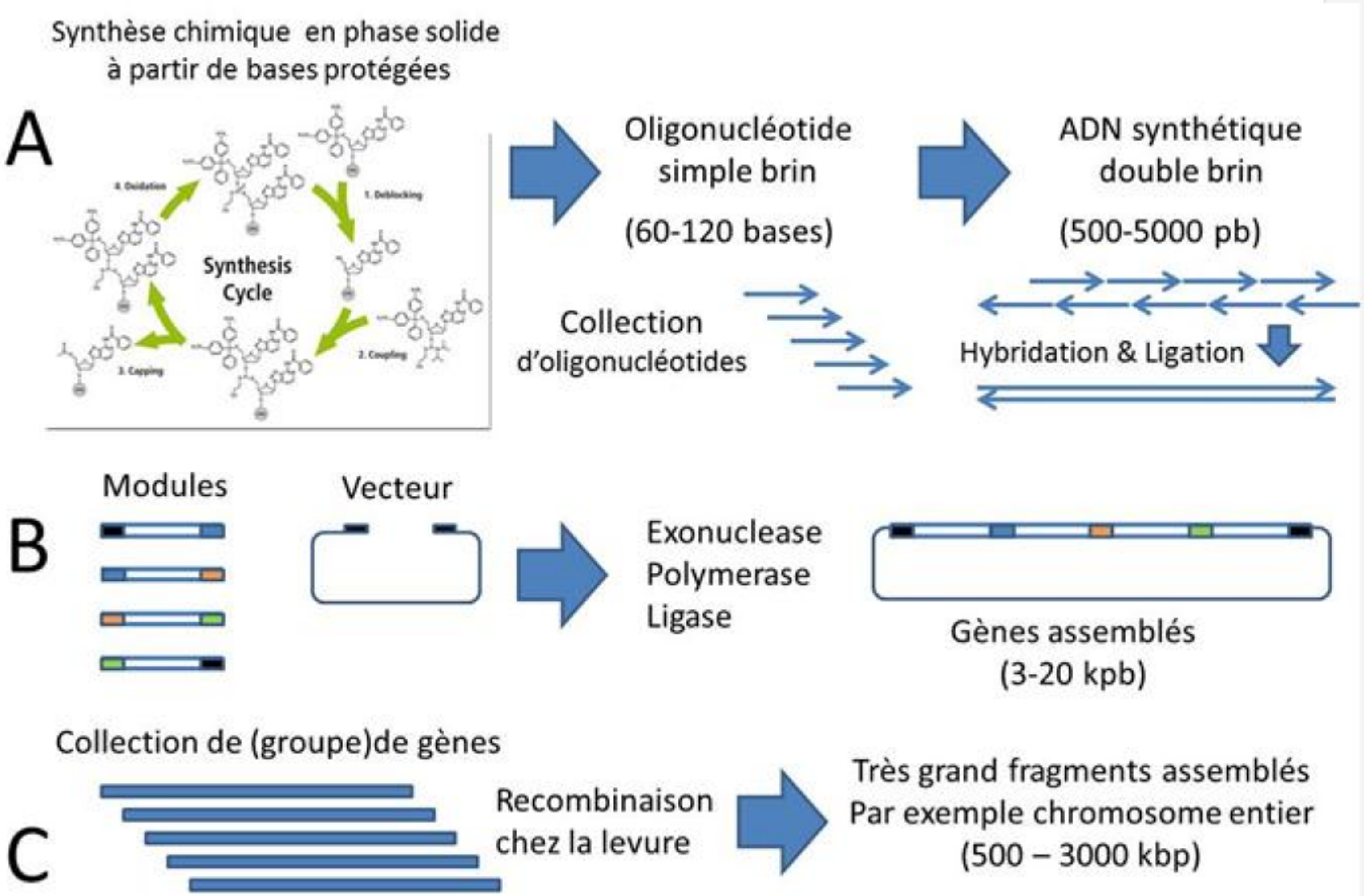
Genome down-sizing

Minimizing the genome of *Mycoplasma mycoides* JCVI-syn1.0 using two different approaches:

Top down approach involving **iterative deletion** of non-essential large gene clusters (phase I) and followed by deletion of small clusters and individual non-essential genes (phase II).

Bottom up approach involving **design of a minimal genome** based on the totality of our information on viable single gene transposon knockouts and viable single or multiple cluster deletions. The designed minimal genome will then be **chemically synthesized**.

La synthèse de gènes de l’oligonucléotide au génome



Synthèse chimique en phase solide
à partir de bases protégées

A

Oligonucléotide
simple brin
(60-120 bases)

ADN synthétique
double brin
(500-5000 pb)

Collection
d’oligonucléotides

Hybridation & Ligation

B

Modules

Vecteur

Exonuclease
Polymerase
Ligase

Gènes assemblés
(3-20 kpb)

C

Collection de (groupe)de gènes

Recombinaison
chez la levure

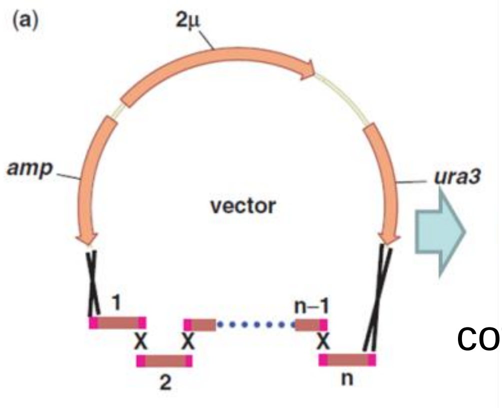
Très grand fragments assemblés
Par exemple chromosome entier
(500 – 3000 kbp)

New gene assembly technologies greatly accelerate engineering

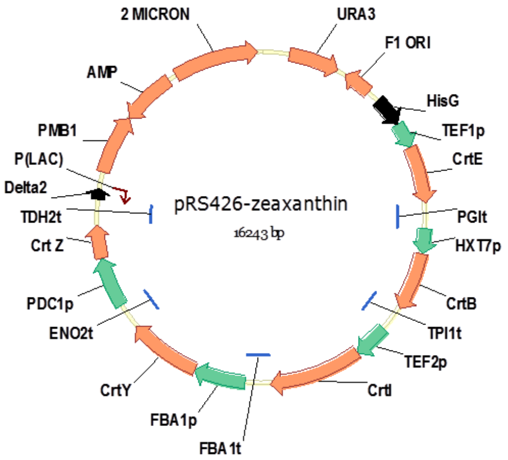
Gene assembly by multiple *in vivo* recombination in yeast

Single step reconstruction of
zeaxantine biosynthesis
(5 genes of *Erwinia uredovora*)

by recombination in *S. cerevisae*
(**Shao et al. 2009**)



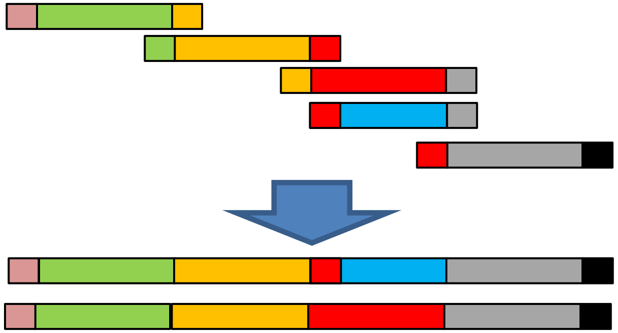
cotransformation

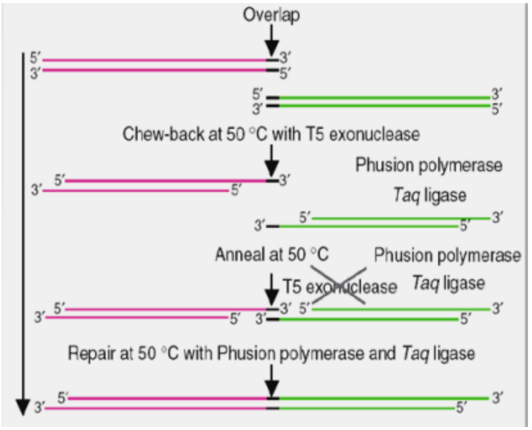


Gene assembly by multiple *in vitro* recombination

Sequence assembly
by the chew-back anneal
Gibson's method.

Single pot generation of
defined or combinatorial
assemblies
from few to ~200 kbp





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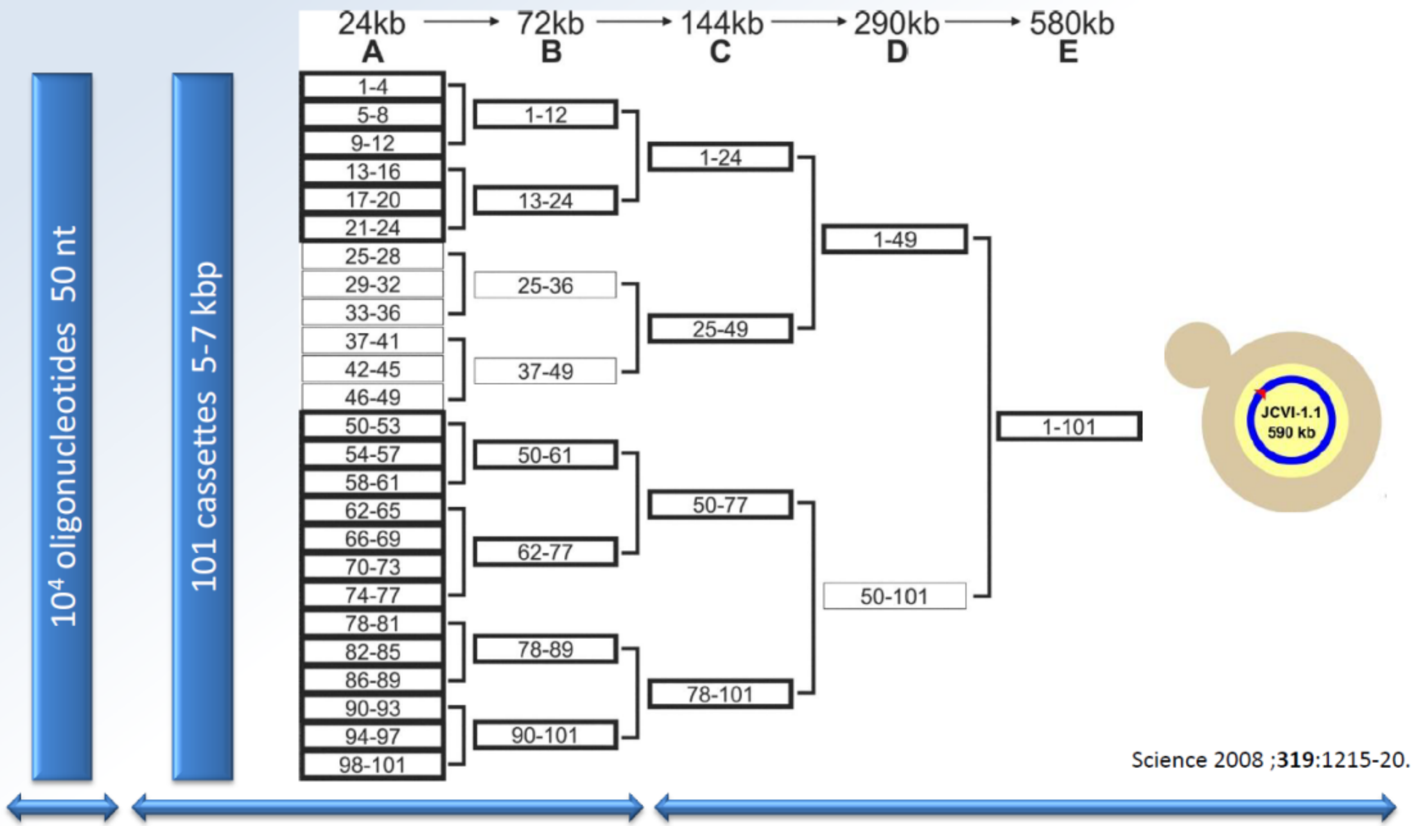
M. genitalium genome synthesis and assembly



10^4 oligonucleotides 50 nt

101 cassettes 5-7 kbp

24kb → 72kb → 144kb → 290kb → 580kb

A B C D E

A: 1-4, 5-8, 9-12, 13-16, 17-20, 21-24, 25-28, 29-32, 33-36, 37-41, 42-45, 46-49, 50-53, 54-57, 58-61, 62-65, 66-69, 70-73, 74-77, 78-81, 82-85, 86-89, 90-93, 94-97, 98-101

B: 1-12, 13-24, 25-36, 37-49, 50-61, 62-77, 78-89, 90-101

C: 1-24, 25-49, 50-77, 78-101

D: 1-49, 50-101

E: 1-101

JCVI-1.1
590 kb

Science 2008 ;**319**:1215-20.

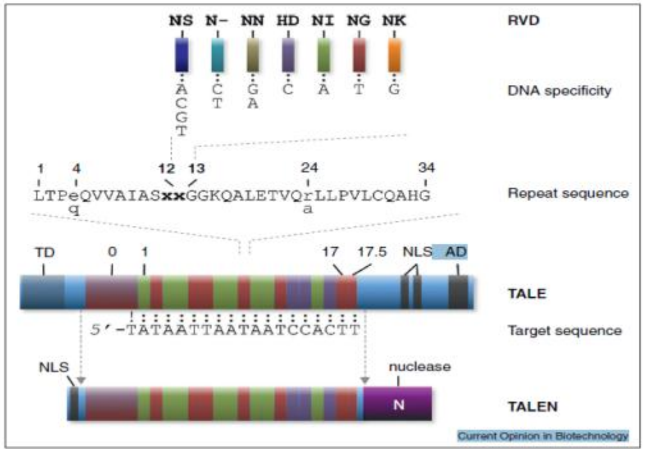
Chemical synthesis

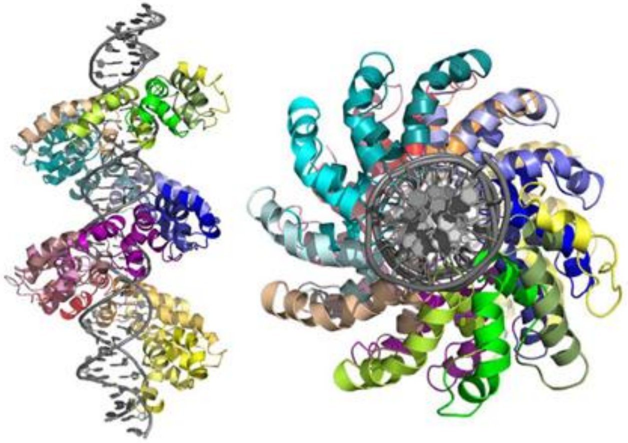
In vitro assembly
Cloning into *E. coli*

Final steps by homologous recombination in yeast

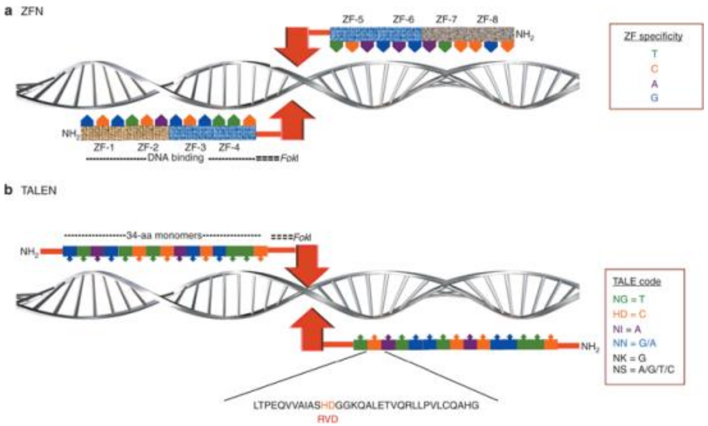
TALE domain derived technologies: a scalpel for genome surgery

Modular TALE DNA recognition domains



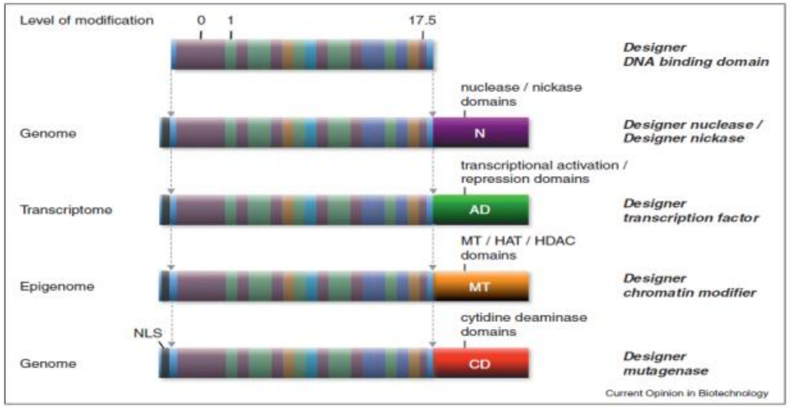


TALEN initiate targeted recombination



Other TALE based genomic tools

Current Opinion in Biotechnology 2012, 23:644–650



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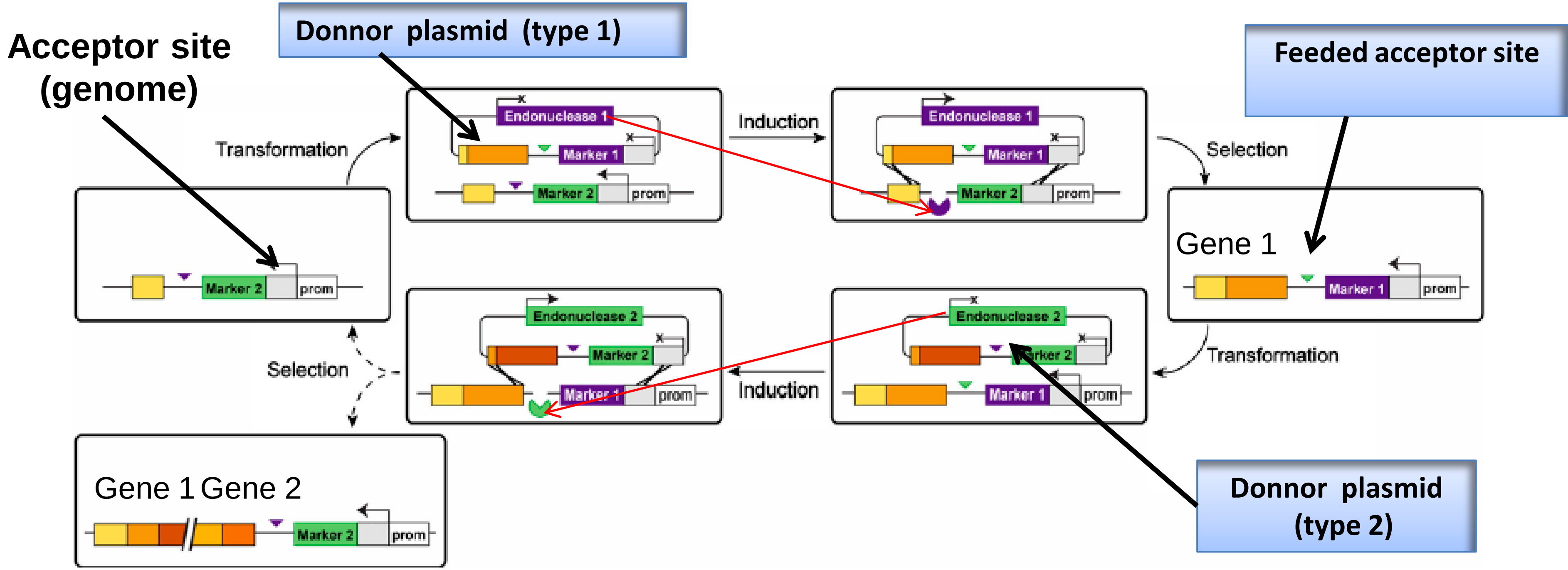
Recursive construction of a synthetic gene cluster at a specific locus using TALEN induced recombination.



Alternate use of two orthogonal mega-nuclease induces sequential recombination events adds one new expression unit at each cycle to the acceptor site bearing alternating selection markers.

Laura M. Wingler and Virginia W. Cornish (PNAS, 2011)

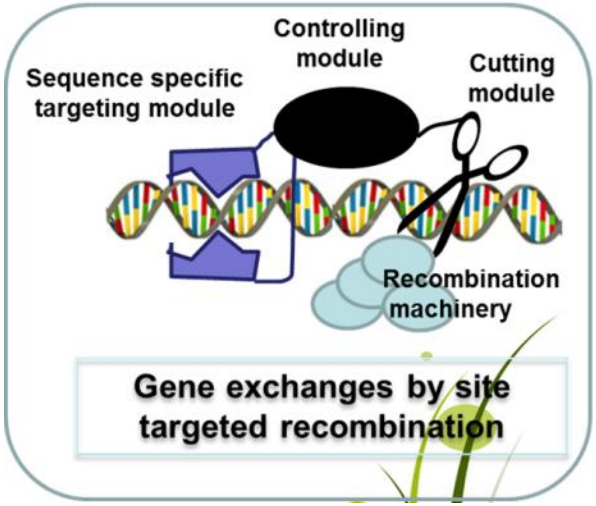
Targeted recombination (shuffling) of genomes

During **sexual crossing** parental genes are **randomly** selected to build the genome of the daughter organism

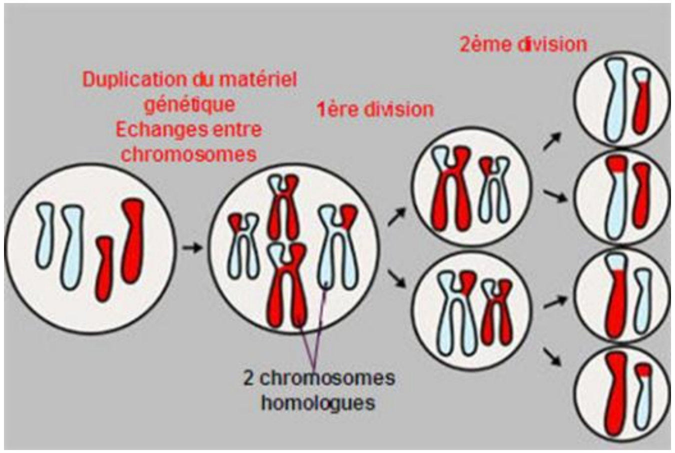
This results from **exchanges** of DNA segments between homologous parental chromosomes through **meiotic recombination**

Points of exchanges and thus exchanged genes are defined by initial events of DNA **double strand breaks** that initiate recombination.

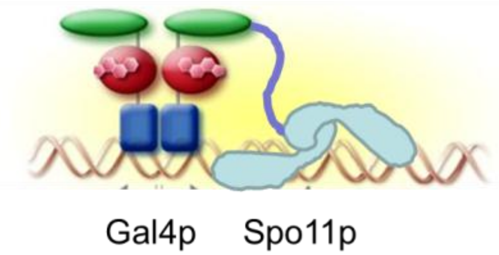
Controlling the position of such initiating events allows to control in turn **which genes** thus which **functional properties** will be exchanged.



Gene exchanges by site targeted recombination



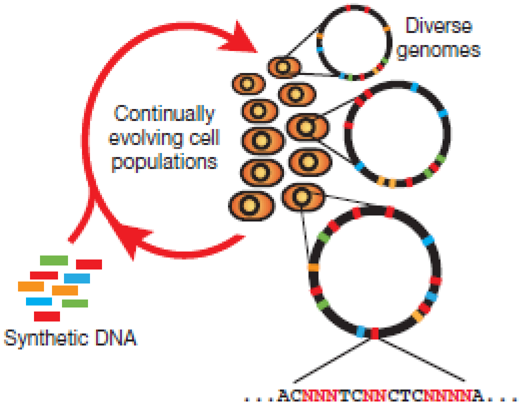
Synthetic Gal4-Spo11 fusion



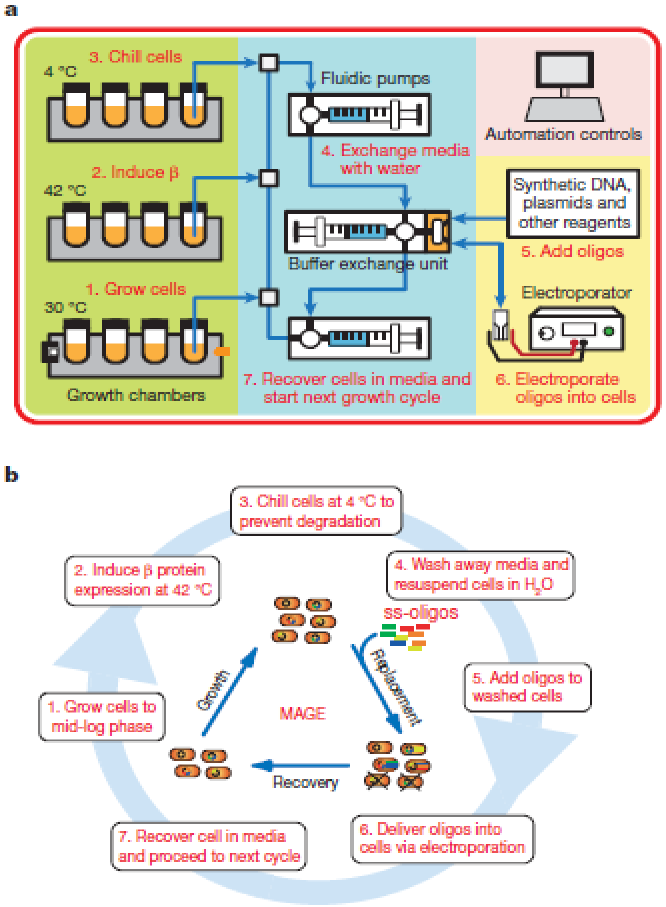
Longhese M.P. et al., DNA Repair 8 (2009) 1127–1138

Massive genome editing by automated multiplex targeted mutagenesis

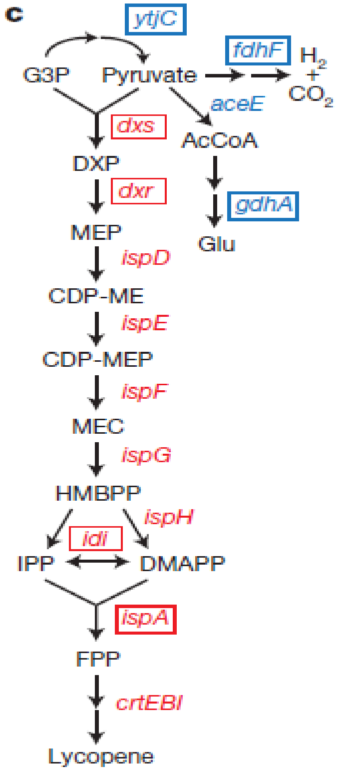
Programming cells by multiplex genome engineering and accelerated evolution



MAGE automation



Application to optimisation of lycopene biosynthesis



Harris H. Wang et al., (2009) Nature Letters Vol 460

Massive genome-wide editing for extended genetic code

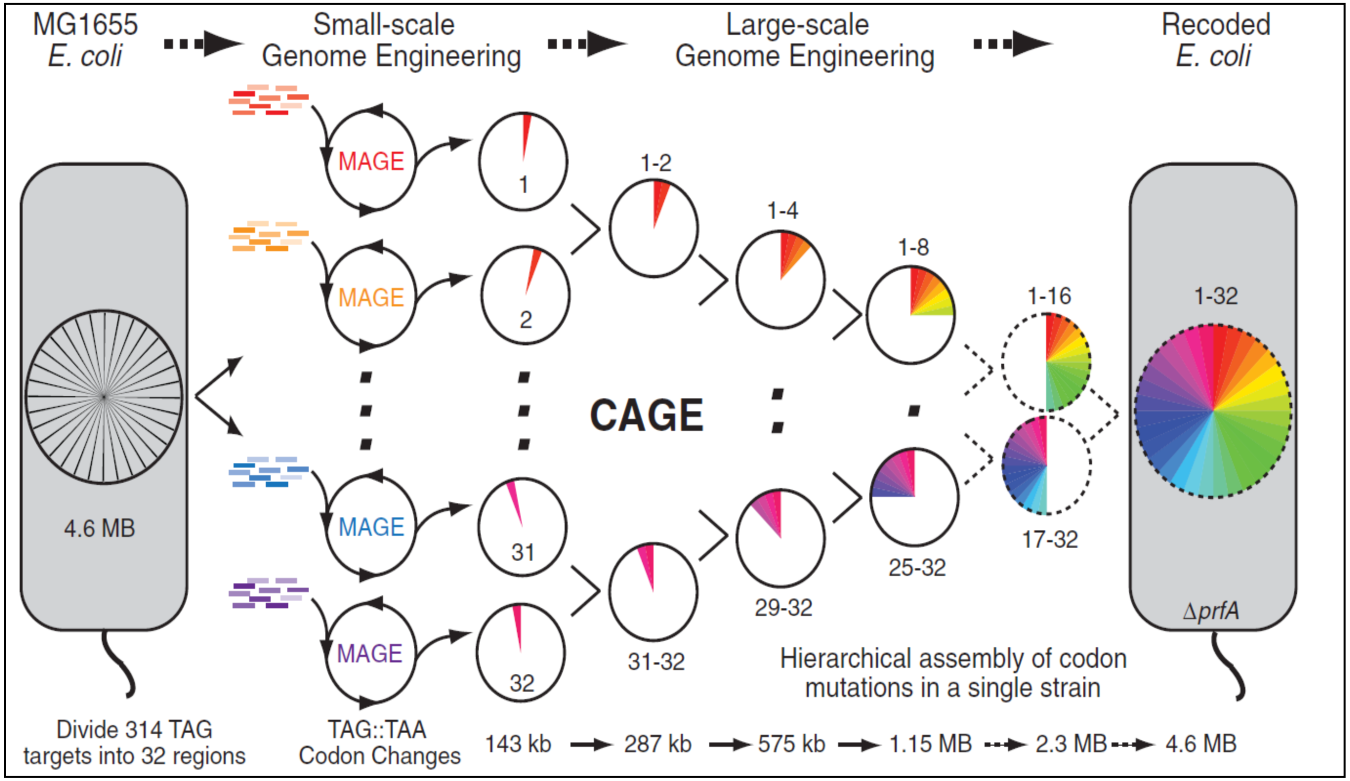
Substitution of 314 TAG stop codons with synonymous TAA codons in parallel across 32 *Escherichia coli* strains

Precise Manipulation of Chromosomes in Vivo Enables Genome-Wide Codon Replacement

MAGE
Multiplex genome engineering

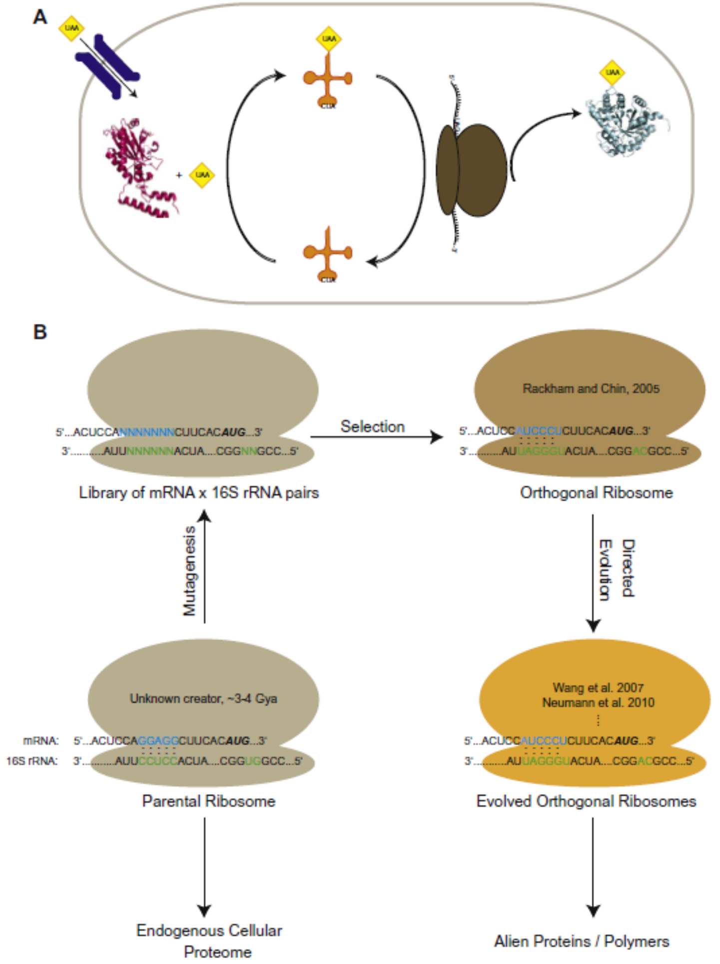
+

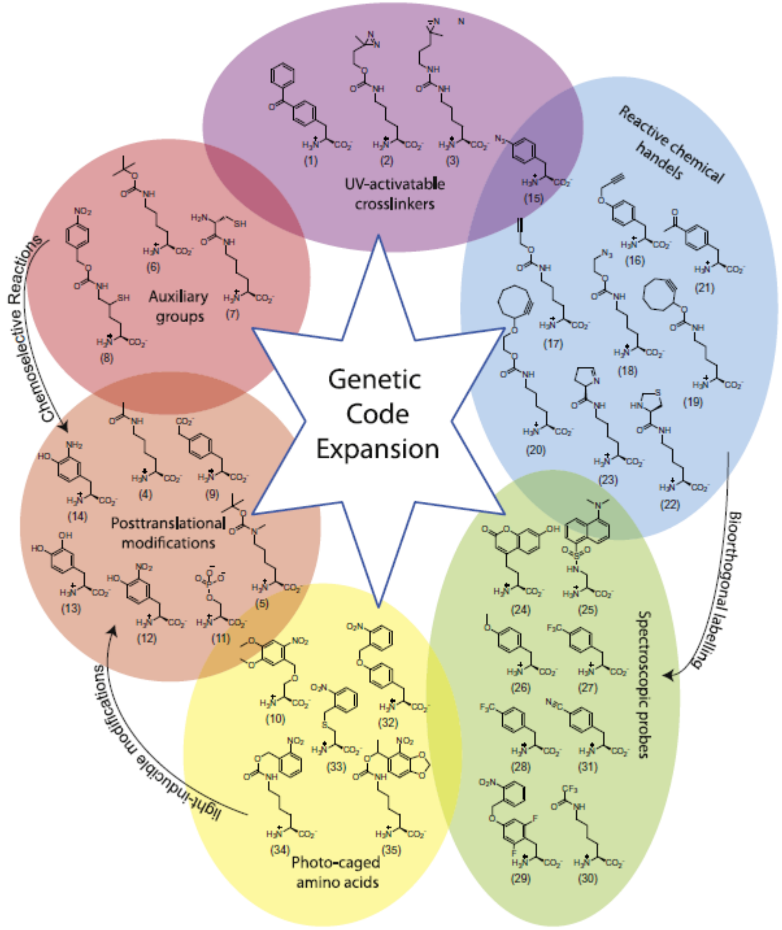
CAGE
Hierarchical conjugative assembly genome engineering



Farren J. Isaacs et al., (2011) Science vol. 333

Expended genetic code available by reprograming alternate codons



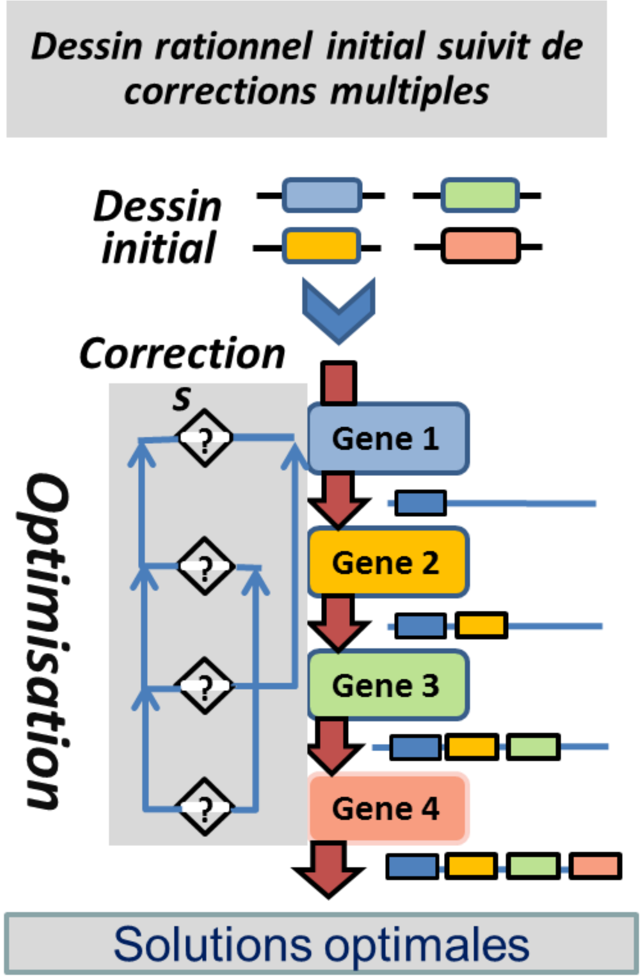


Rewiring translation – Genetic code expansion and its applications

Heinz Neumann*

FEBS Letters 586 (2012) 2057–2064

Combinatorial metabolic engineering: time saving and diversity



Dessin rationnel initial suivit de corrections multiples

Dessin initial

Corrections

Optimisation

Gene 1

Gene 2

Gene 3

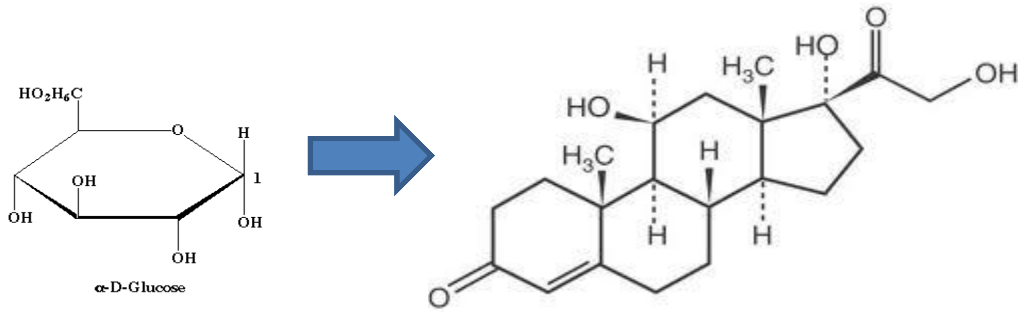
Gene 4

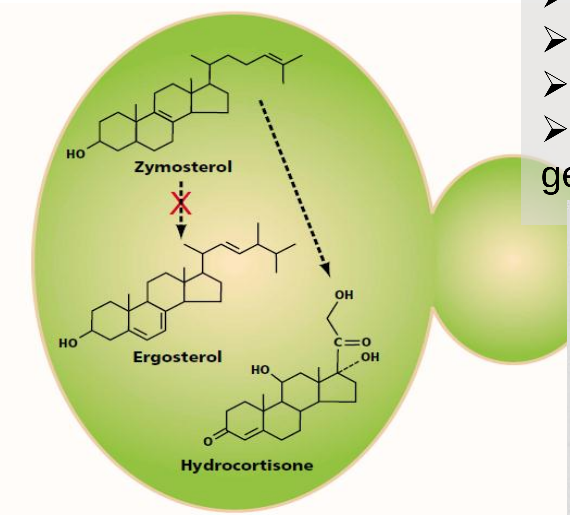
Solutions optimales

Metabolic engineering of complex pathways: a highly combinatorial problem inefficiently addressed by classical trial& error approaches

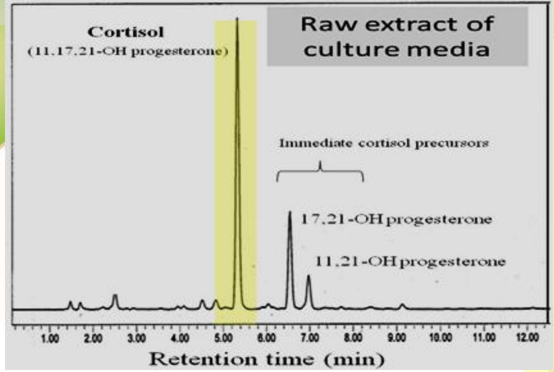
- Serial reconstruction approaches (intermediates availability)
- Alternate choice of catalysts in biodiversity for a same step.
- Alternate relative levels of expression in the absence of regulation
- Case by case managing of side reactions
- Host metabolism / heterologous metabolism cross-talk
- Partial optimization of individual step might lead to poor global optimization

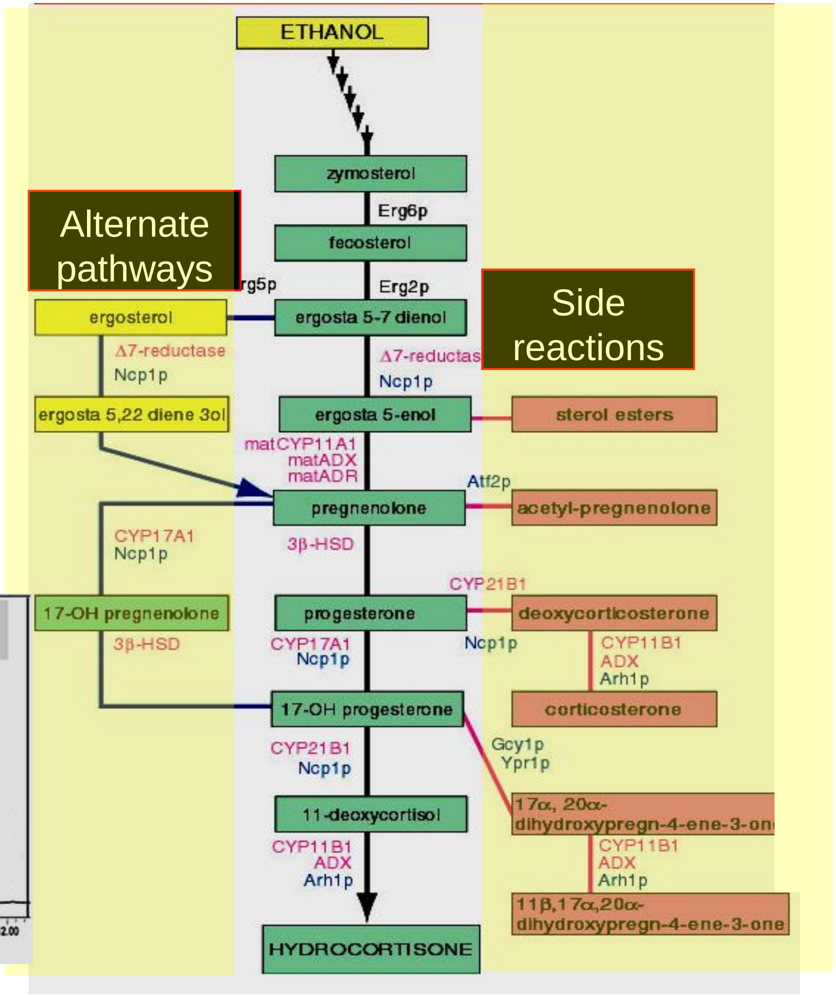
Un exemple d'approche classique
la synthèse autonome d'hydrocortisone par des levures





- ➢10 heterologous enzymes
- ➢3 genes disruptions
- ➢1 overexpression
- ➢Subcellular retargeting of 2 gene products





ETHANOL

zymosterol

Erg6p

fecosterol

Alternate pathways

rg5p

Erg2p

ergosterol

ergosta 5-7 dienol

Side reactions

Δ7-reductase
Ncp1p

Δ7-reductas
Ncp1p

ergosta 5,22 diene 3ol

ergosta 5-enol

sterol esters

matCYP11A1
matADX
matADR

Atf2p

pregnenolone

acetyl-pregnenolone

CYP17A1
Ncp1p

3β-HSD

CYP21B1

17-OH pregnenolone

progesterone

deoxycorticosterone

3β-HSD

CYP17A1
Ncp1p

Ncp1p

CYP11B1
ADX
Arh1p

17-OH progesterone

corticosterone

CYP21B1
Ncp1p

Gcy1p
Ypr1p

11-deoxycortisol

17α, 20α-dihydroxypregn-4-ene-3-on

CYP11B1
ADX
Arh1p

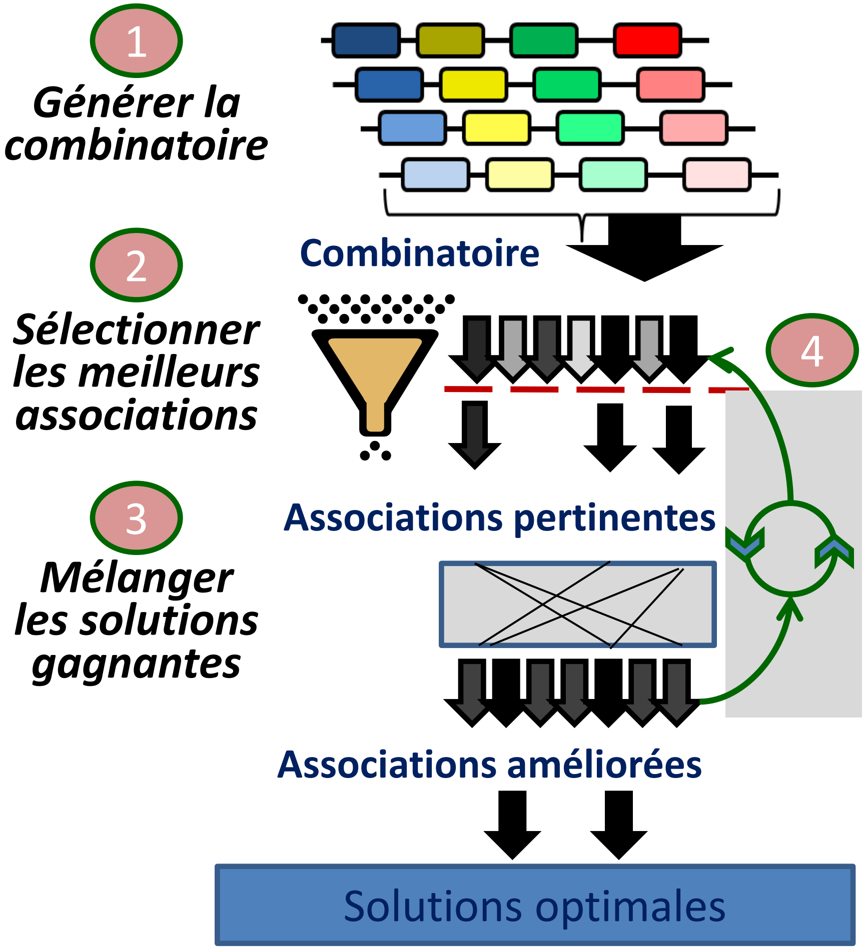
CYP11B1
ADX
Arh1p

11β,17α,20α-dihydroxypregn-4-ene-3-one

HYDROCORTISONE

Ménard Szczebara et al.(2003) *Nature Biotechnology*

Dessin initial d'une combinatoire de solutions alternatives pour chaque étape



1

Générer la combinatoire

Combinatoire

2

Sélectionner les meilleurs associations

4

Associations pertinentes

3

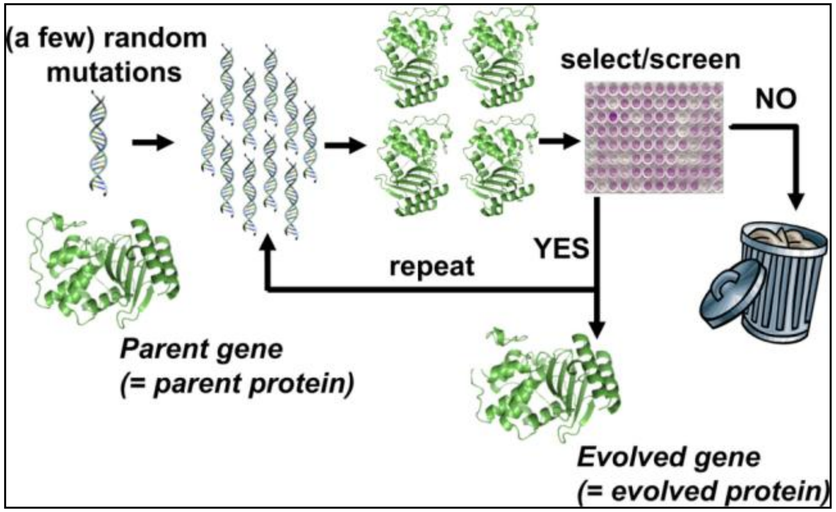
Mélanger les solutions gagnantes

Associations améliorées

Solutions optimales

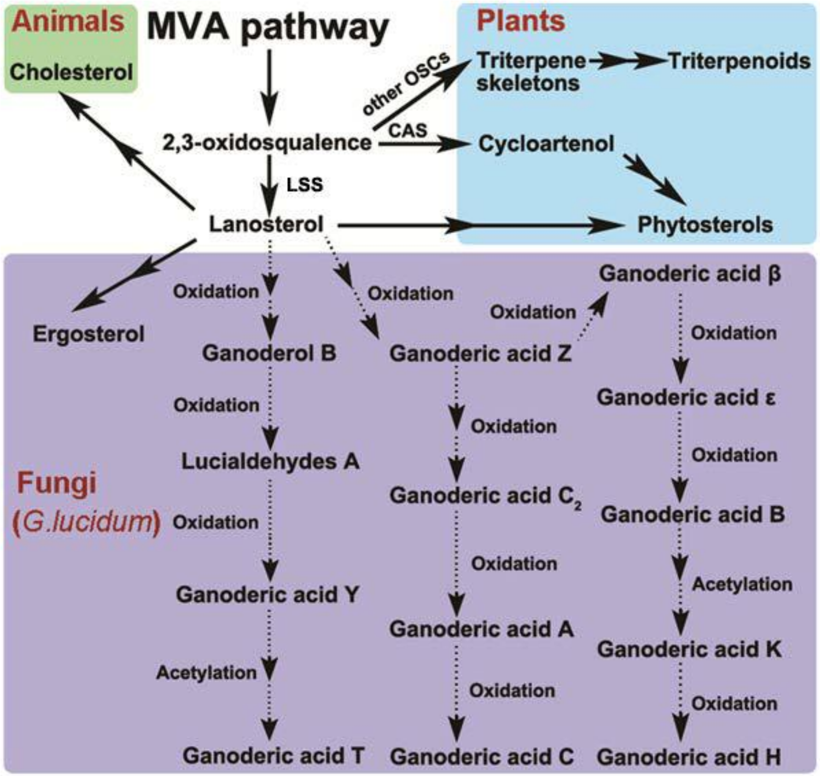
Une approche combinatoire de l'ingénierie métabolique similaire à celle actuellement bien validée pour l'optimisation des protéines apparait comme un enjeu majeur

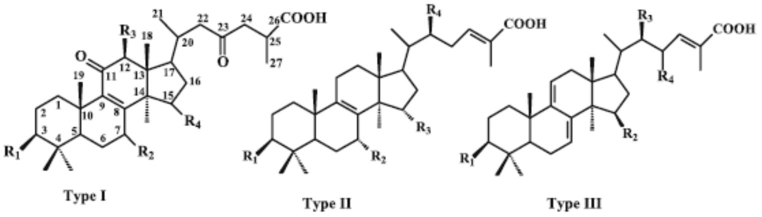
Evolution dirigée des protéines

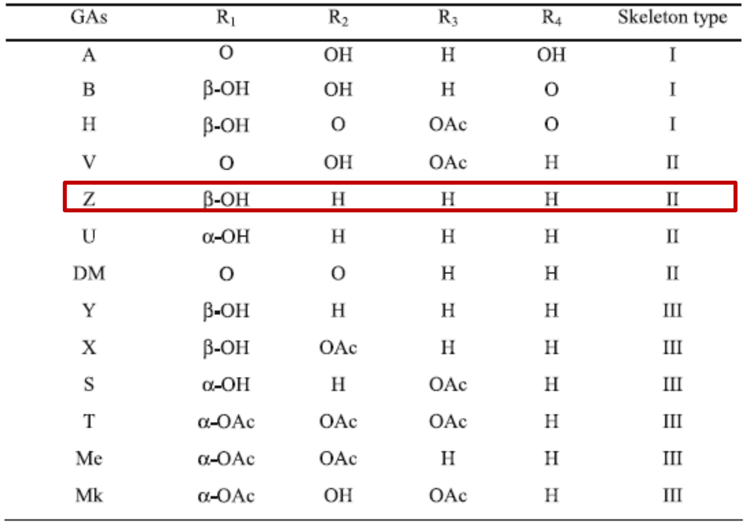


Generation of synthetic molecular diversity as an alternative to combinatorial chemistry constitutes a second important goal for combinatorial biology.

Triterpenoid biosynthetic pathway in *G. lucidum and compound of potential therapeutic interest*.

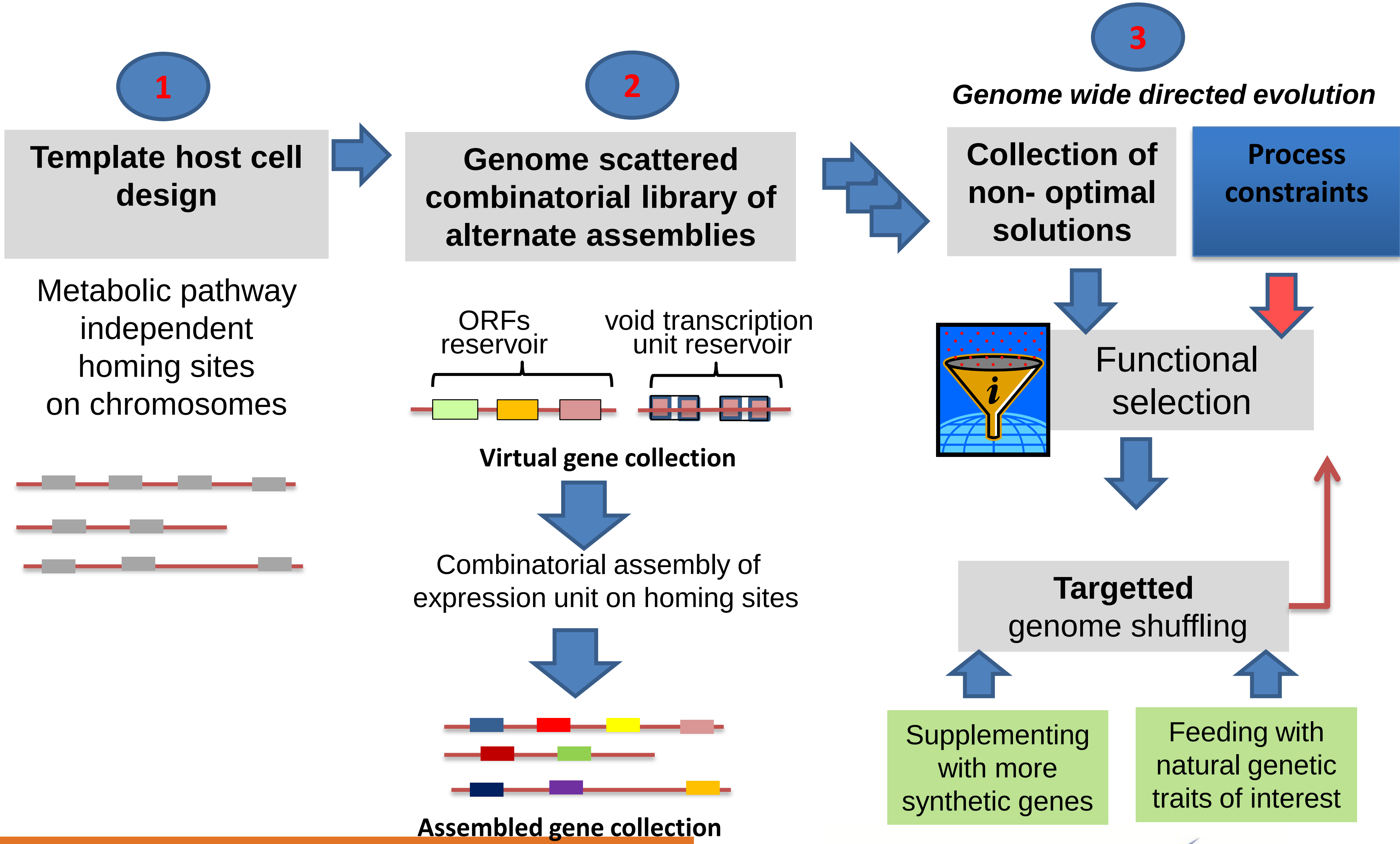






GAs	R_1	R_2	R_3	R_4	Skeleton type
A	O	OH	H	OH	I
B	β-OH	OH	H	O	I
H	β-OH	O	OAc	O	I
V	O	OH	OAc	H	II
Z	β-OH	H	H	H	II
U	α-OH	H	H	H	II
DM	O	O	H	H	II
Y	β-OH	H	H	H	III
X	β-OH	OAc	H	H	III
S	α-OH	H	OAc	H	III
T	α-OAc	OAc	OAc	H	III
Me	α-OAc	OAc	H	H	III
Mk	α-OAc	OH	OAc	H	III

Combinatorial approach to metabolic engineering



1

Template host cell design

Metabolic pathway independent homing sites on chromosomes

2

Genome scattered combinatorial library of alternate assemblies

ORFs reservoir

void transcription unit reservoir

Virtual gene collection

Combinatorial assembly of expression unit on homing sites

Assembled gene collection

3

Genome wide directed evolution

Collection of non- optimal solutions

Process constraints

Functional selection

Targetted genome shuffling

Supplementing with more synthetic genes

Feeding with natural genetic traits of interest

Un bilan de l’ingénierie génétique en biologie de synthèse

Génome synthétiques

Quel que soit le caractère spectaculaire, voir médiatique de la synthèse complète de génomes, l’approche et loin d’être mature. La notion de génome minimal reste à définir tant au niveau de ses concepts que de l’utilisation qui pourrait en être faite. On sait reproduire, certainement pas encore créer…

Ingénierie massive des génomes

L’approche est beaucoup plus mure et va bénéficier des progrès de la robotisation. Il s’agit d’une alternative réaliste à la synthèse pouvant cibler les même objectifs avec des ressources plus limitées. De nombreux domaines d’applications sont possibles, celui de l’utilisation de codes génétiques étendus étant probablement un des plus prometteurs.

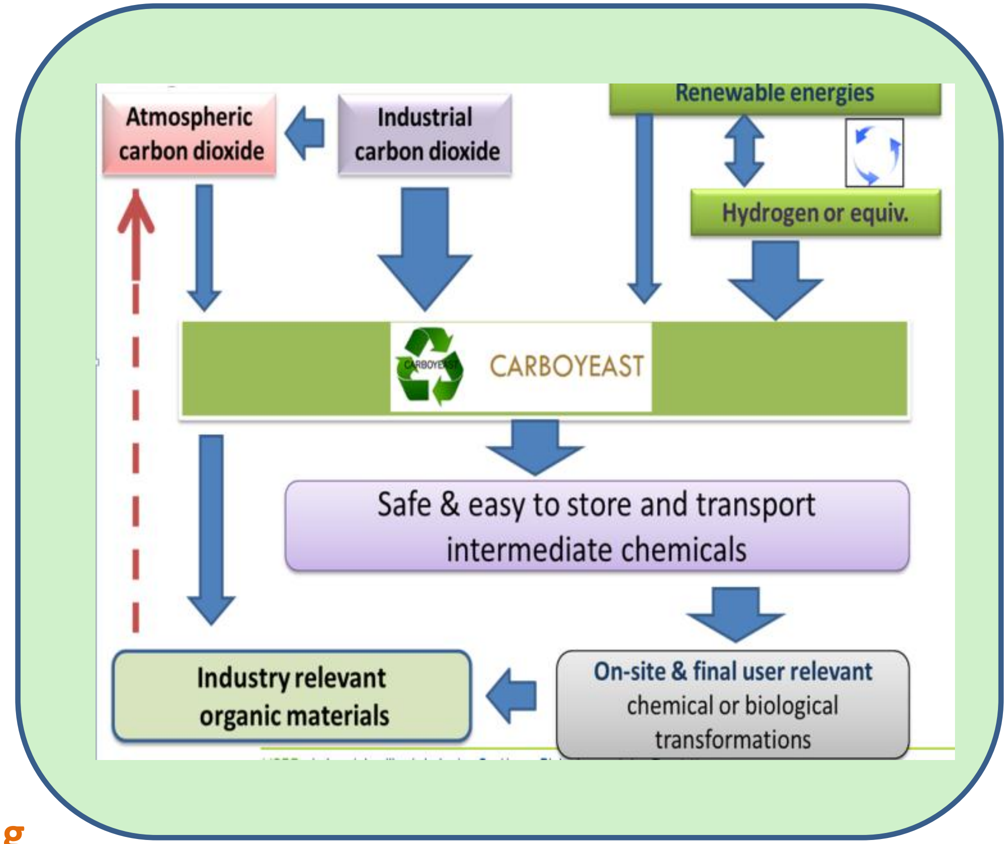
Les nouvelles technologies de synthèse et d’assemblage de séquences

Le gain est massivement quantitatif, plus que qualitatif, mais permet de réduire de manière majeure les ressources et fortement le temps nécessaire. La qualité des méthodes d’assemblage par recombinaison permet d’envisager une automatisation de beaucoup d’approches.

Les approches combinatoires d’ingénierie métabolique

Le gain potentiel est qualitatif et quantitatif mais la technologie n’est pas encore mure au-delà des premiers démonstrateurs. Les approches amonts restent critiques pour maitriser les mécanismes de maintenance des génomes. Néanmoins, comme dans les autres domaines l’optimal proviendra de l’association du rationnel et du combinatoire.

An example combining a wide range of synthetic biology approaches for sustainable & biomass independent organic carbon for industry



Atmospheric carbon dioxide

Industrial carbon dioxide

Renewable energies

Hydrogen or equiv.

CARBOYEAST

Safe & easy to store and transport intermediate chemicals

On-site & final user relevant chemical or biological transformations

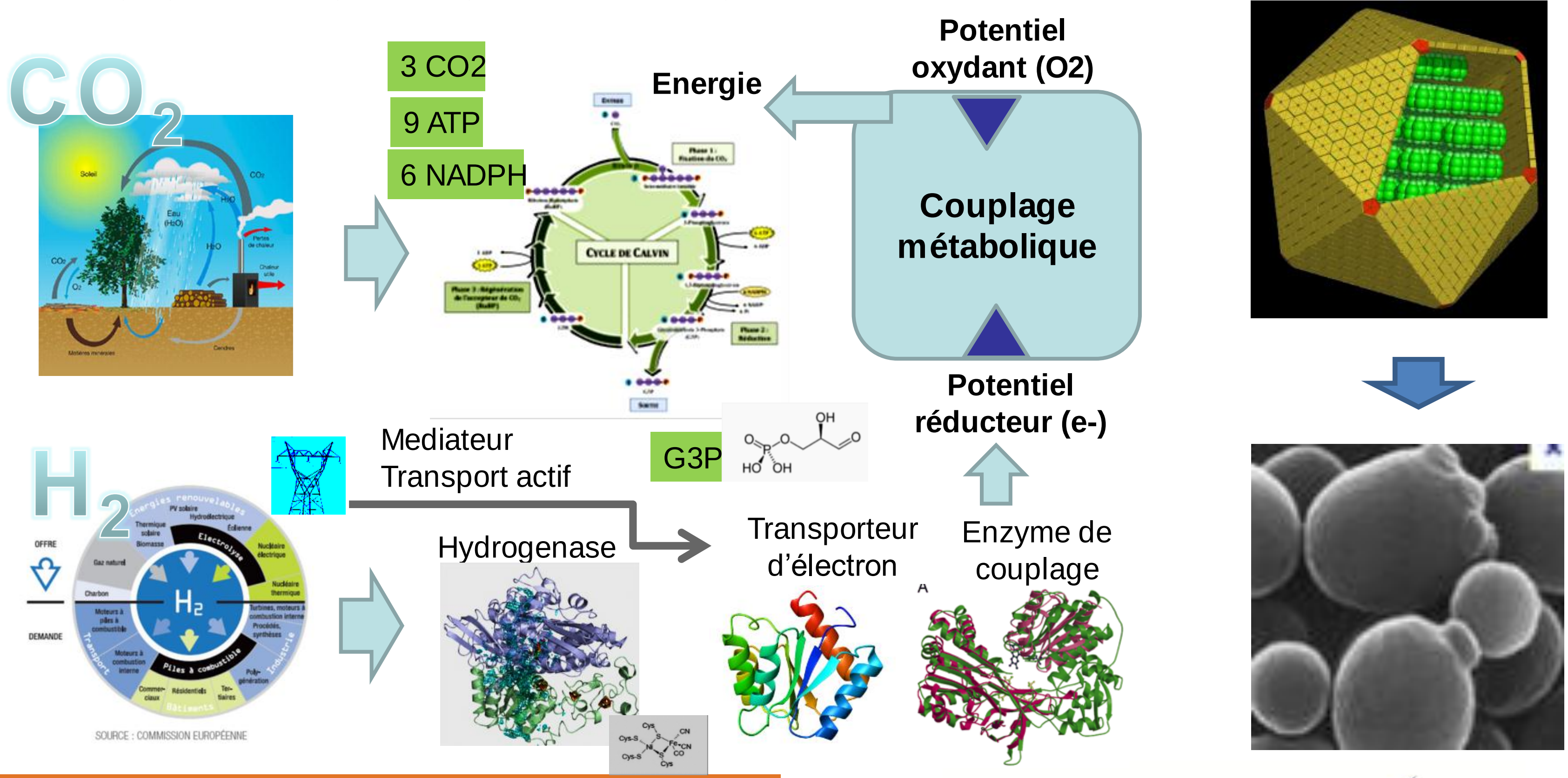
Industry relevant organic materials

Build a synthetic microorganism permitting :

- Efficient use & chemical storage of non photosynthetic renewable energy.
- To create a closed carbon cycle for potentially any organic molecule
- To reuse most of current bio-engineering

CARBOYEAST

- Reconstruction of photosynthetic cyanobacteria carbon dioxide capture mechanism in yeast
- Carbon free energy feeding through direct electron transfer or hydrogen oxidation
- Use of artificial intracellular nano-compartments to secure incompatible metabolic paths
- Very large genetic engineering (> 25 artificial genes)



CO_2

3 CO2

9 ATP

6 NADPH

Energie

Potentiel oxydant (O2)

Couplage métabolique

Potentiel réducteur (e-)

H_2

Mediateur
Transport actif

G3P

Hydrogenase

Transporteur d'électron

Enzyme de couplage

SOURCE : COMMISSION EUROPÉENNE

Pour en savoir plus aller voir le document d’accompagnement

Nouvelles approches d’ingénierie pour la biologie de synthèse.

D. Pompon, P. Urban, T. Lautier, M. Carquet, G. Truan

Table 1. Quelques exemples d’ingénierie métabolique relevant de la biologie de synthèse

Composé cible	**Organisme**	**Approche**	**Reference**
Polykétides	*E. coli*	Combinatoire	Horinouchi, 2009
Médicaments	*E. coli*	Combinatoire	Julsing, 2006
Flavonoïdes non naturels	*E. coli*	Mimétique	Katsuyama, 2007
Métabolisme xylose	*S. cerevisiae*	Rationnelle	Kim, 2013
Terpénoïdes	*E. coli*	Souches plateformes	Martin, 2003
Isoprenoïdes	*S. cerevisiae*	Transfert organismes	Maury, 2008
Flavanones	*E. coli*	Cluster gènes synthétiques	Miyahisa, 2005
Diversité chimique	*S. cerevisiae*	Combinatoire	Naeby, 2009
Artemisine	*S. cerevisiae*	Rationnelle	Ro, 2006
Hydrocortisone	*S. cerevisiae*	Rationnelle	Szczebara, 2003
Stilbénoïdes	*S. cerevisiae*	Rationnelle	Trantas, 2009
Amorphadiene	*E. coli*	Rationnelle	Tsuruta, 2009
Caroténoïdes	*E. coli* / levure	Mimétique	Wang, 2007
Générique	levure	Combinatoire/ robotique	Wingler, 2011
Carotene	*E. coli*	Combinatoire	Yoon, 2009

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CARREFOURS DE L’INNOVATION AGRONOMIQUE

Merci de votre attention

Questions