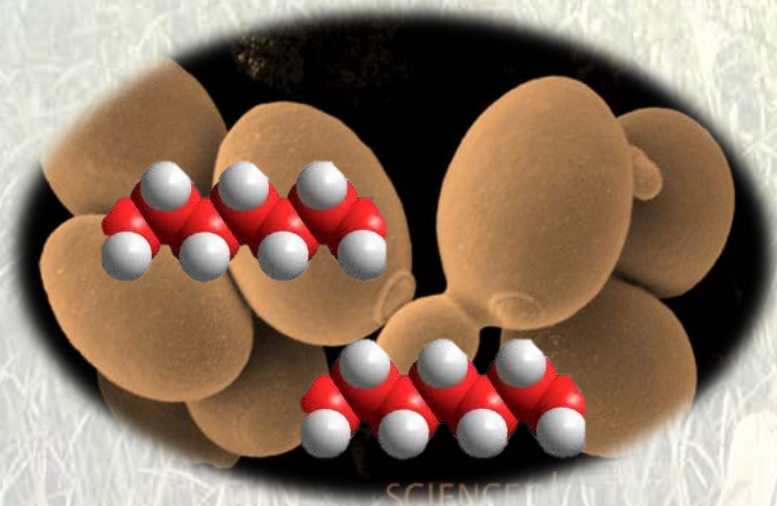


Challenges and Opportunities in Synthetic Biology

Huimin Zhao

Department of Chemical and Biomolecular Engineering
Department of Chemistry
Department of Biochemistry
Department of Bioengineering
Institute for Genomic Biology



Building Blocks of Biotechnology

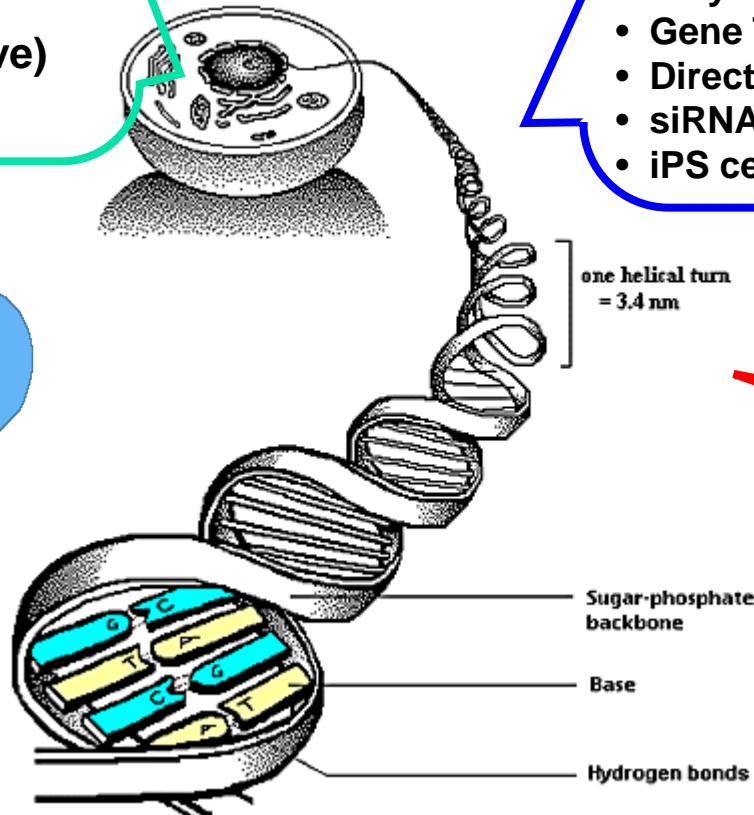
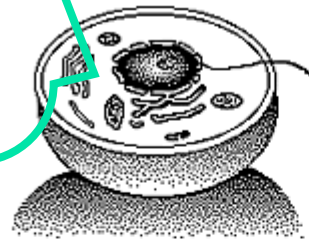
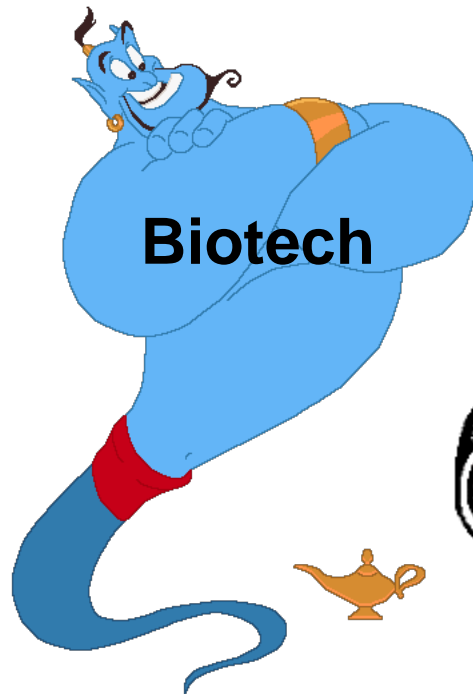


Applications

- **Medical (RED, 1st wave)**
(1970s-present)
- **Agricultural (GREEN, 2nd wave)**
(1990s-present)
- **Industrial (WHITE, 3rd wave)**
(2000s-present)

Enabling Components

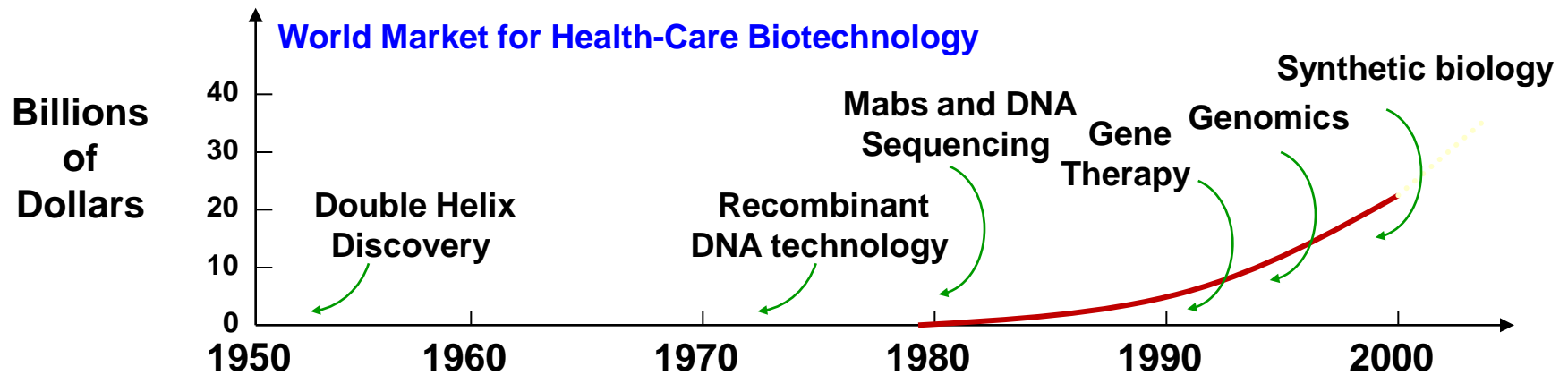
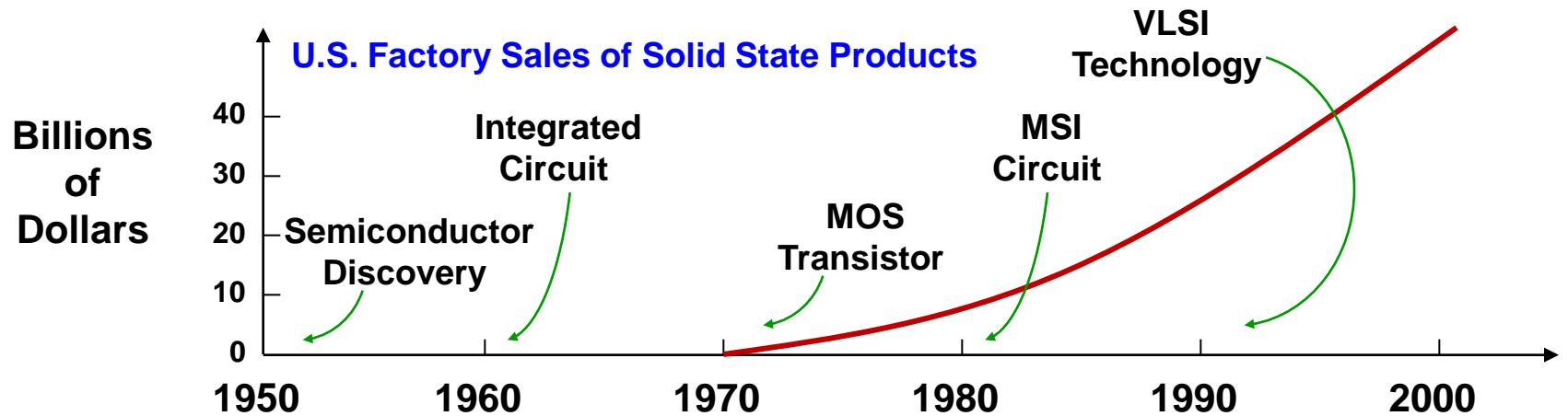
- Recombinant DNA Technology (1973)
- DNA Sequencing (1977, 1987)
- Monoclonal Antibodies (1977)
- Site-directed Mutagenesis (1982)
- Polymerase Chain Reaction (1983)
- Gene Therapy (1981,1990-)
- Directed Molecular Evolution (1994)
- siRNA Technology (1998)
- iPS cells (2006)



Systems and Processes

- Expression Systems
- Metabolic Control
- Fermentor Design
- Downstream Processing

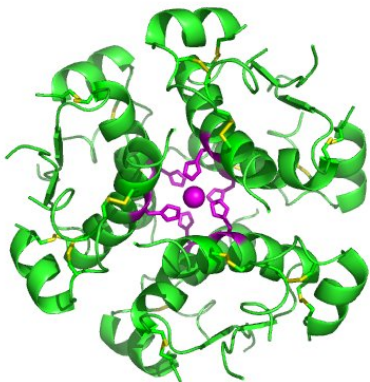
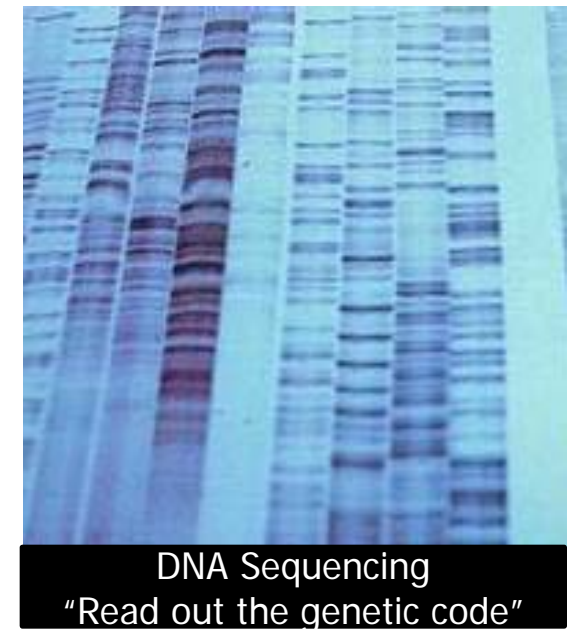
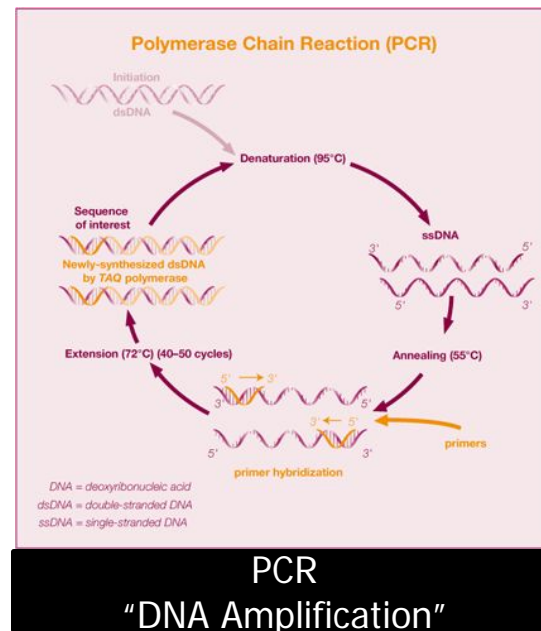
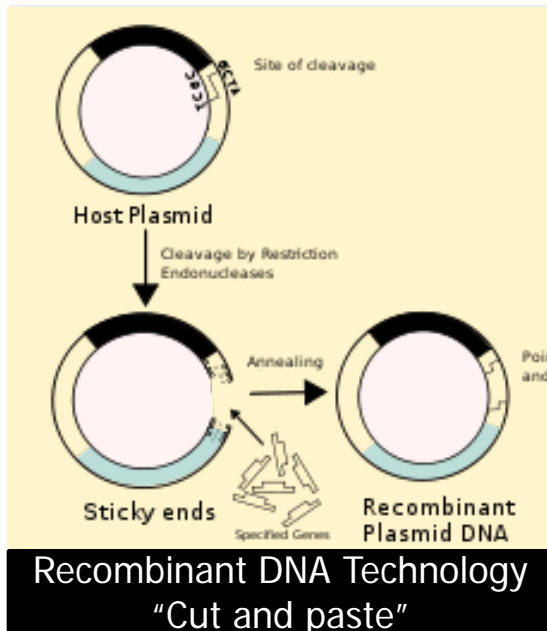
Microelectronics and Biotechnology



Tools Driving Biotechnology



First Generation Biotech



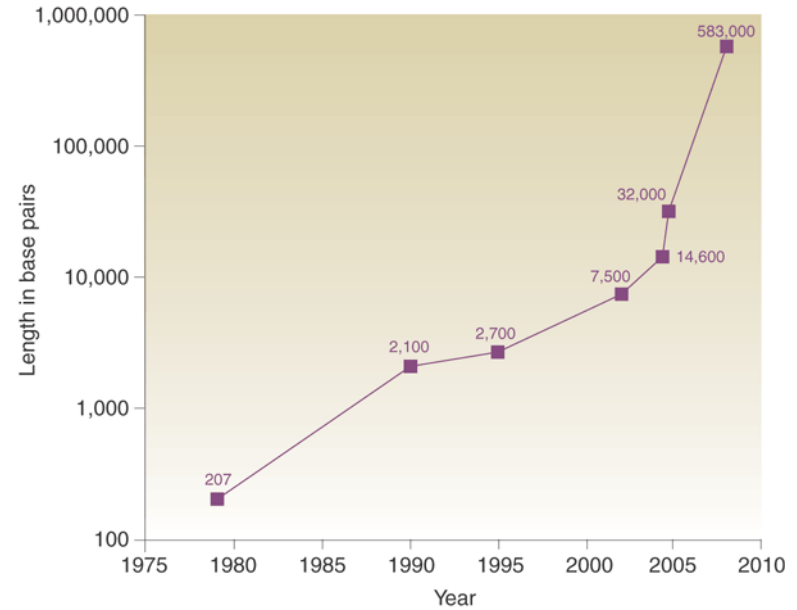
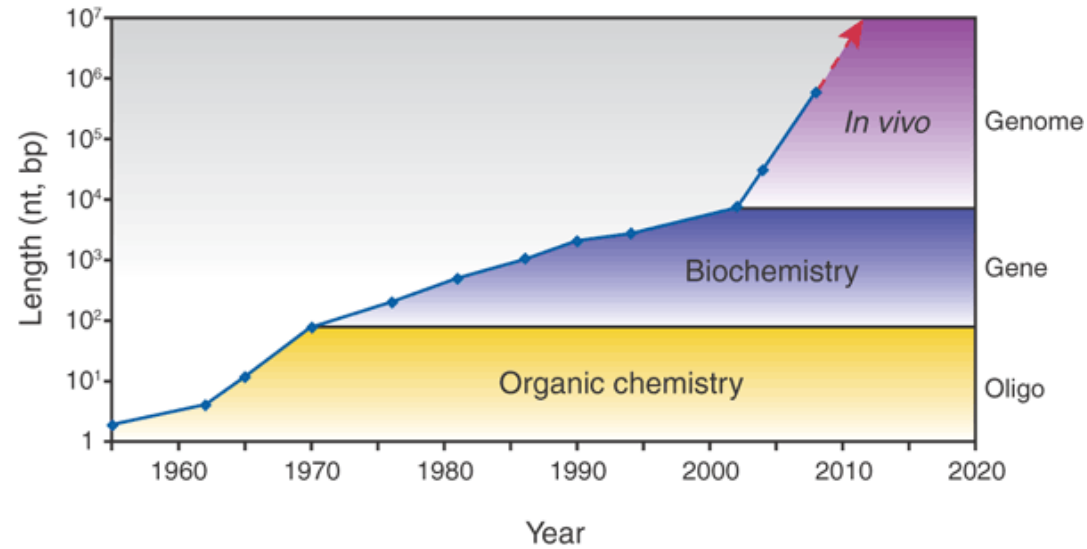
human insulin

First product: human insulin, produced in *E. coli* in 1978.

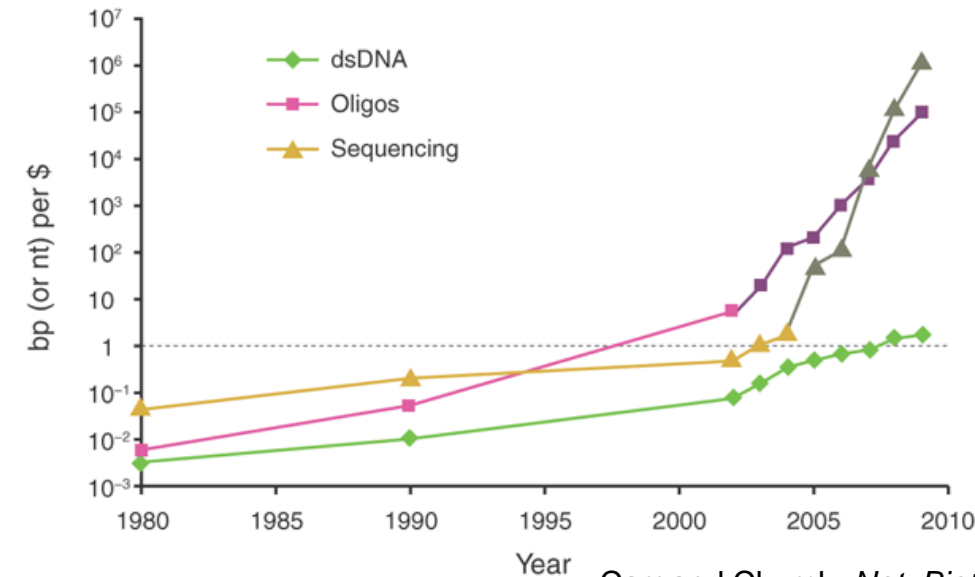
- Recombinant human growth hormone
- Recombinant blood clotting factor VIII
-

Global market size for recombinant proteins: ~\$60B in 2009

Transformative Advances in DNA Sequencing and Synthesis



Carlson, *Nat. Biotech.* 27, 1091 (2009)



Carr and Church, *Nat. Biotech.* 27, 1151 (2009)

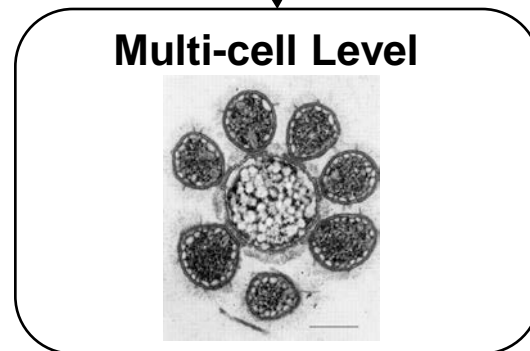
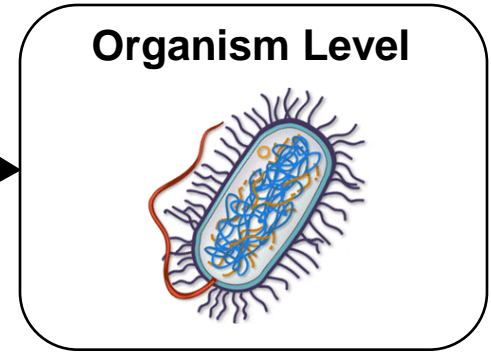
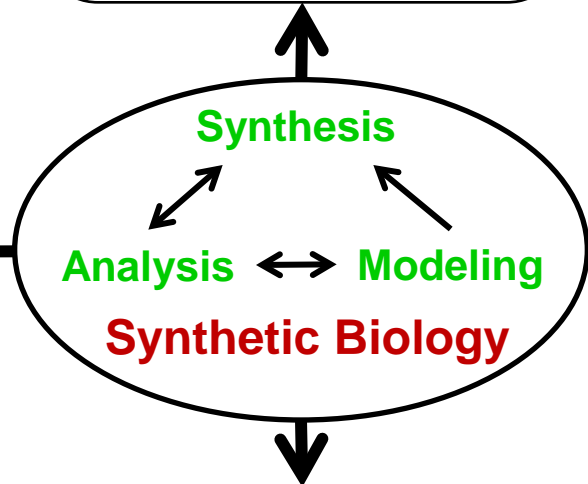
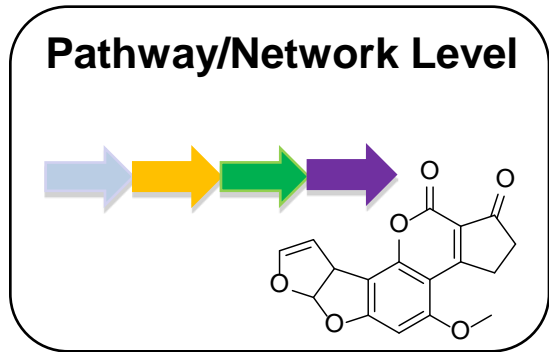
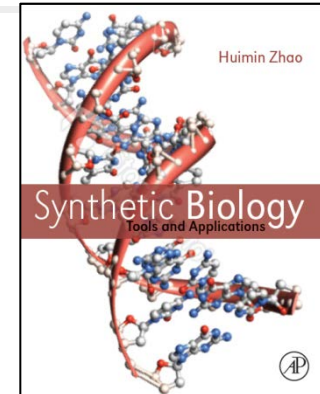
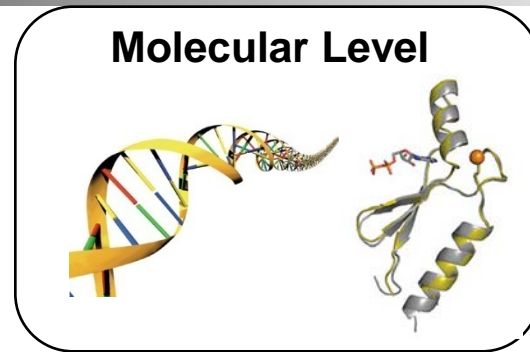
10-1000's genes
 complex chemicals
 and materials
 organisms as
 products

...

Challenges in Synthetic Biology



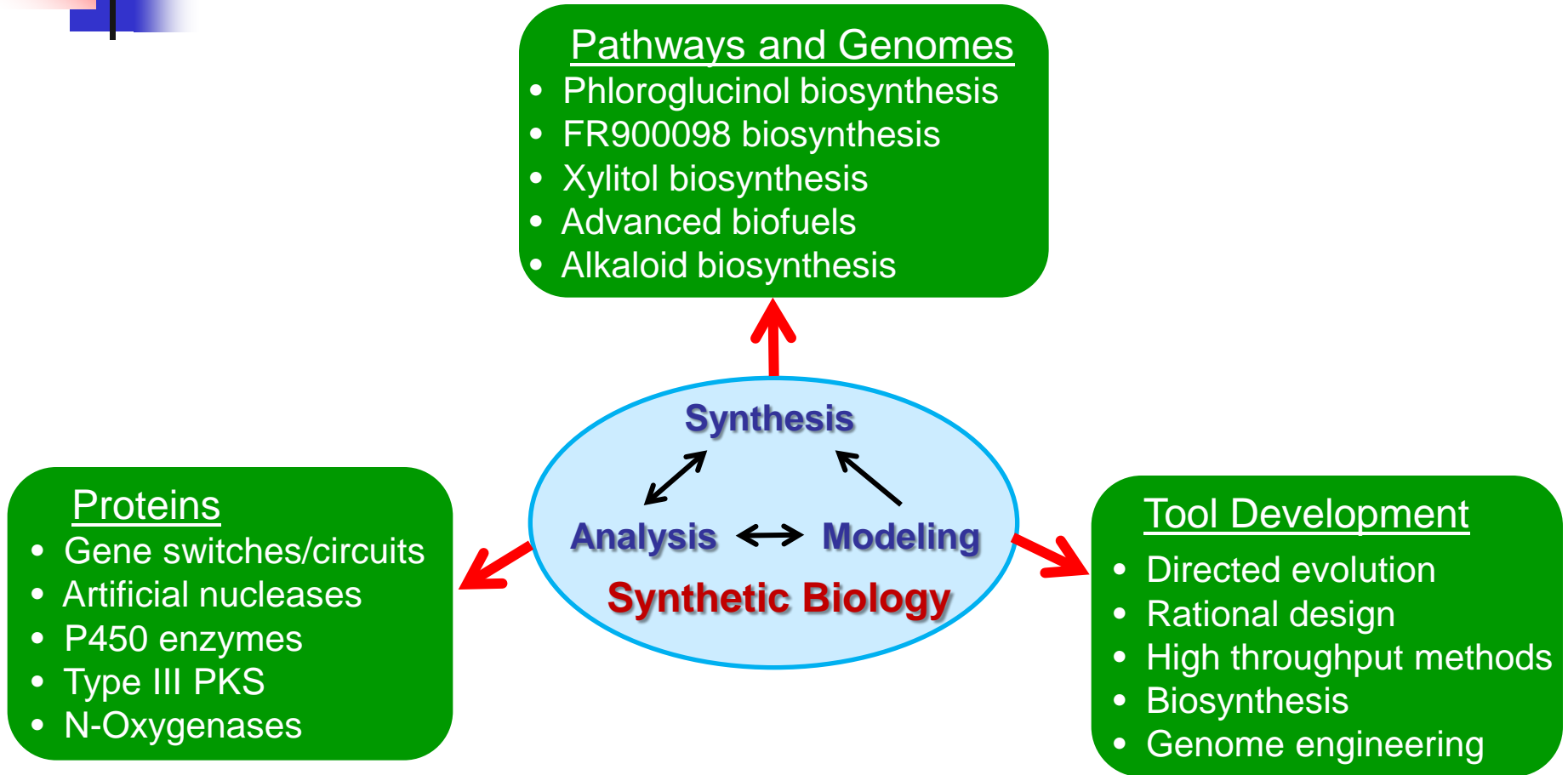
Synthetic Biology: deliberate design of improved or novel biological systems that draws on principles elucidated by biologists, chemists, physicists, and engineers.



- **Standardization**
- **Modularization**
- **System integration**

Liang, Luo, and Zhao. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, 2010.

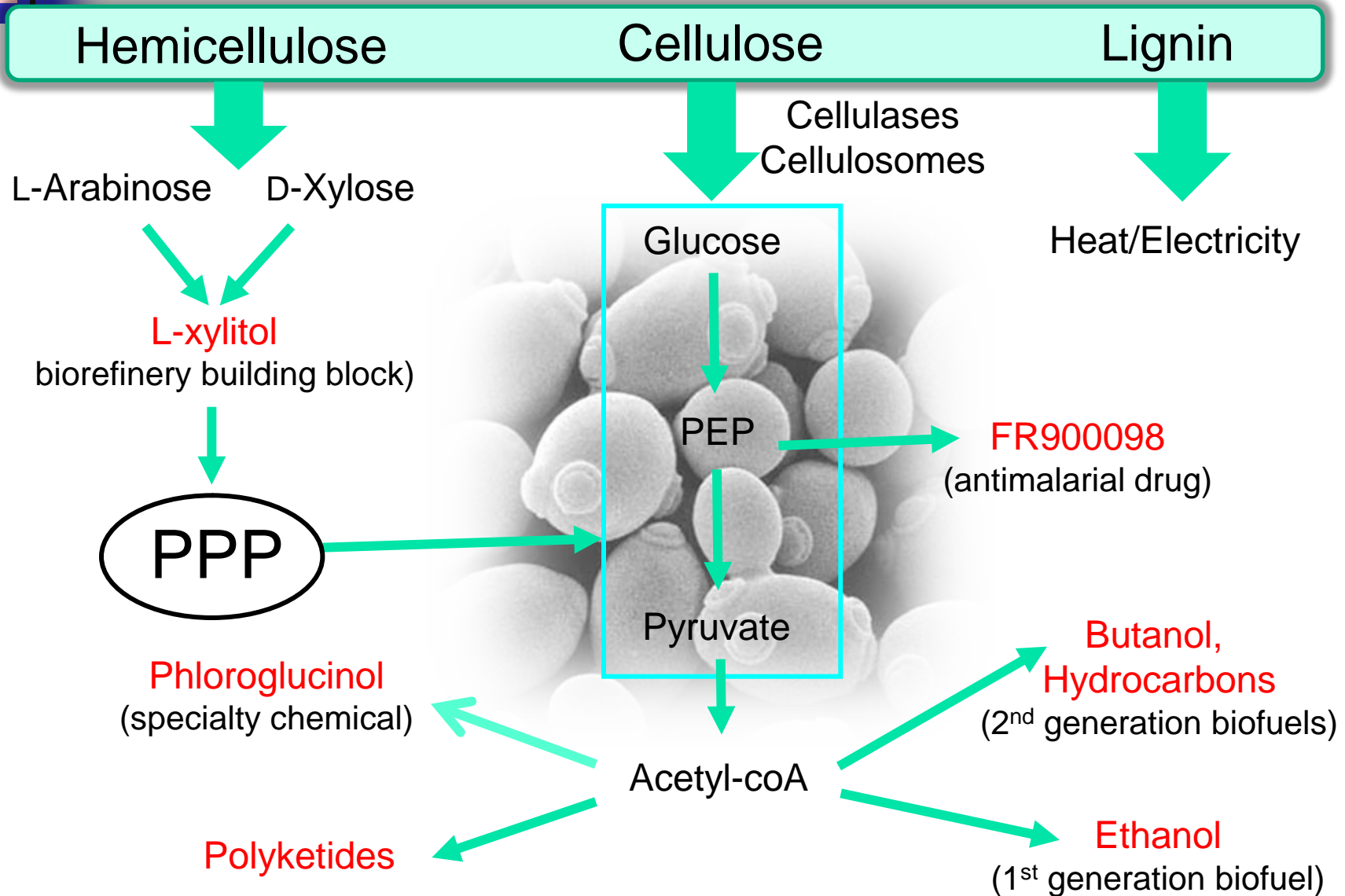
Research Interests in Zhao Group



Grand Challenge #1 (Energy & Sustainability): *Urgent need for oil replacement*
→ Use renewable feedstocks to produce fuels, chemicals, and drugs

Grand Challenge #2 (Health): *Need for new therapeutics*

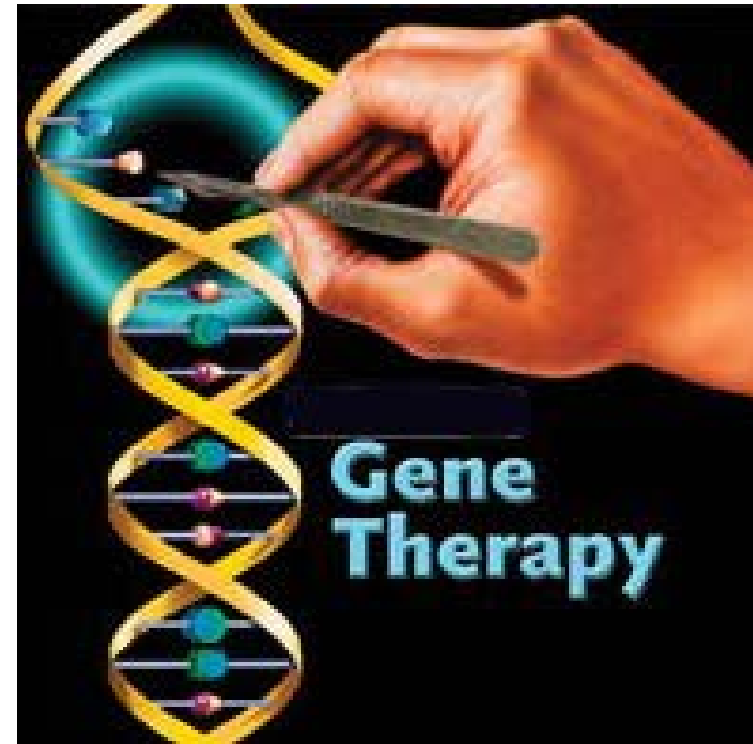
#1. Engineering Microbial Factories (Fuels and Natural Products @ UIUC)



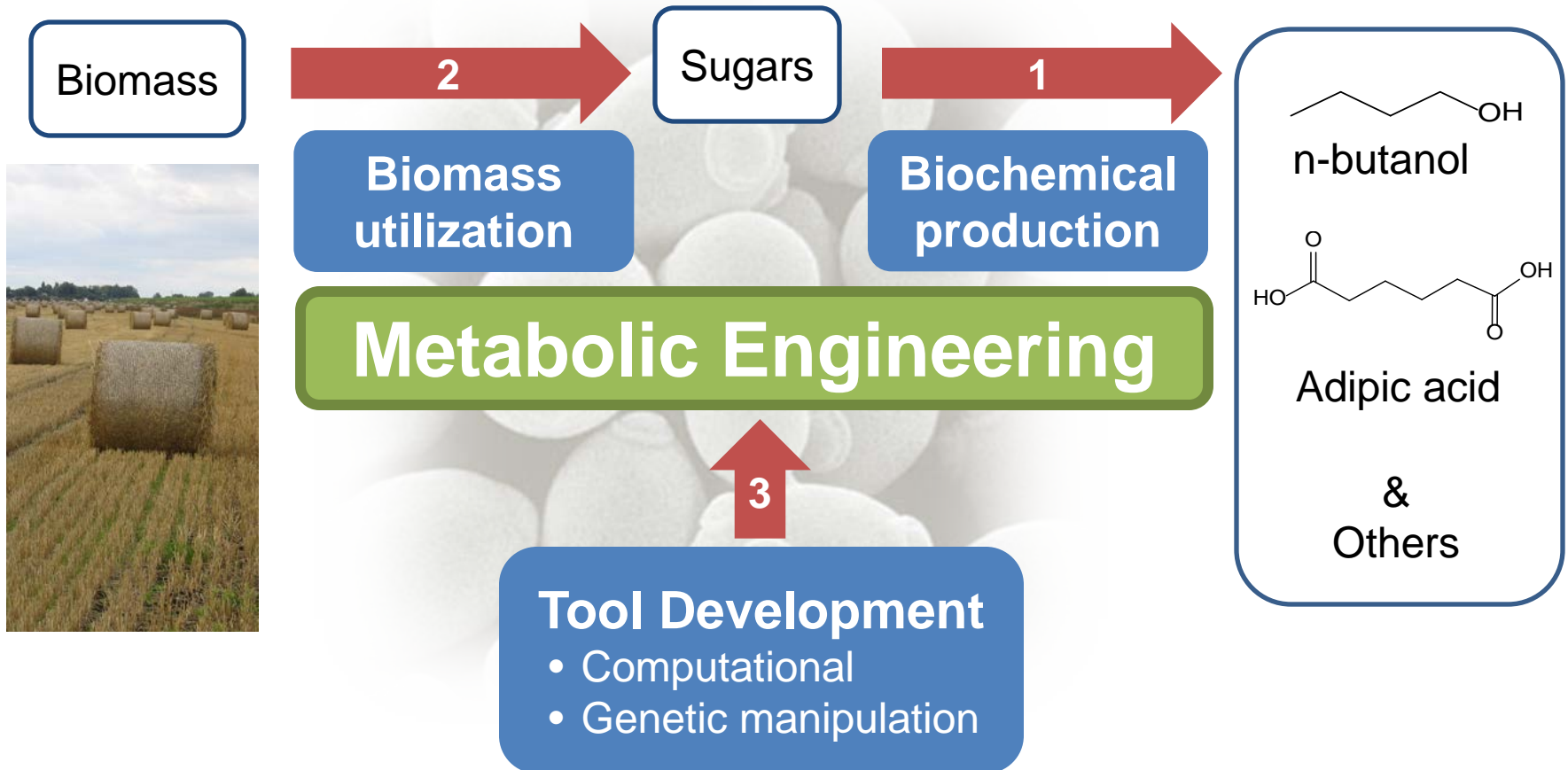
#2: Developing New Therapeutic Tools and Agents



- Gene switches
 - Small molecule regulated gene expression systems
- Gene scissors
 - Artificial nucleases

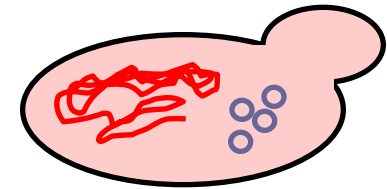
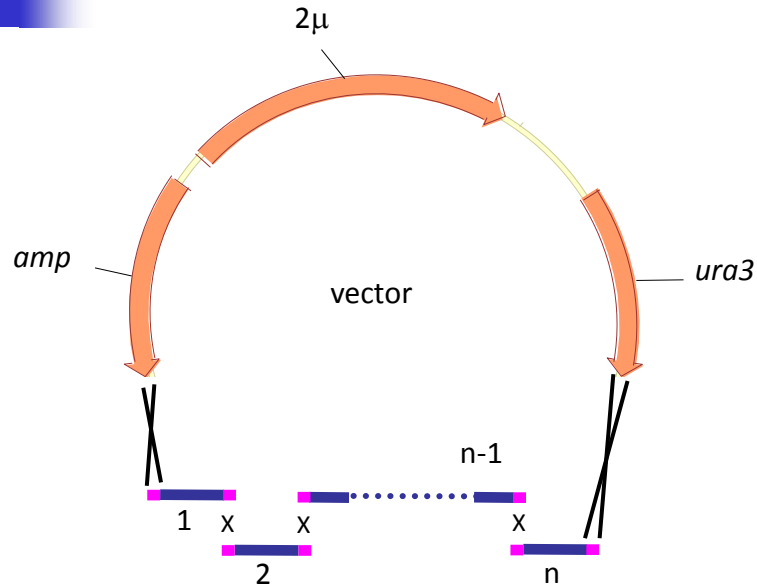


Metabolic Engineering Research Lab (MERL) @ Singapore

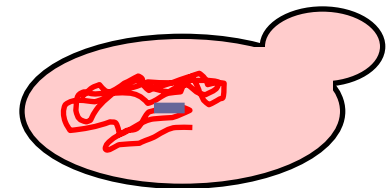
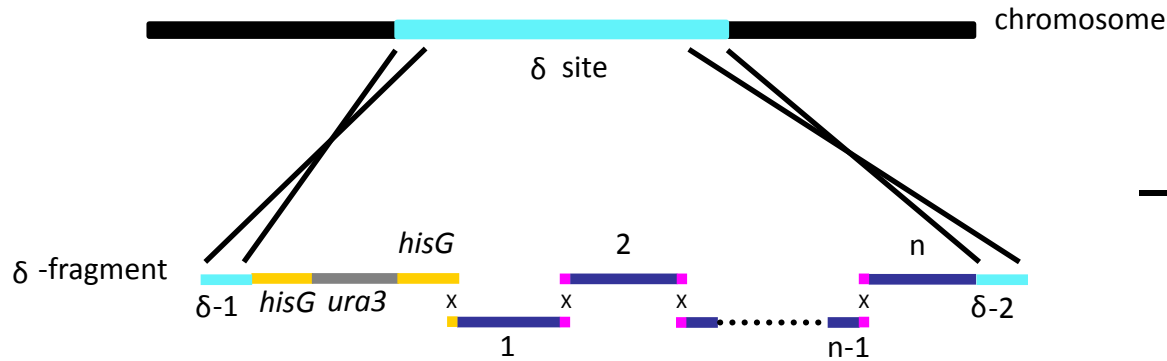


Overall Goal: Develop and apply systems and synthetic biology approaches to engineer microorganisms capable of cost-effectively producing industrial chemicals from renewable feedstocks.

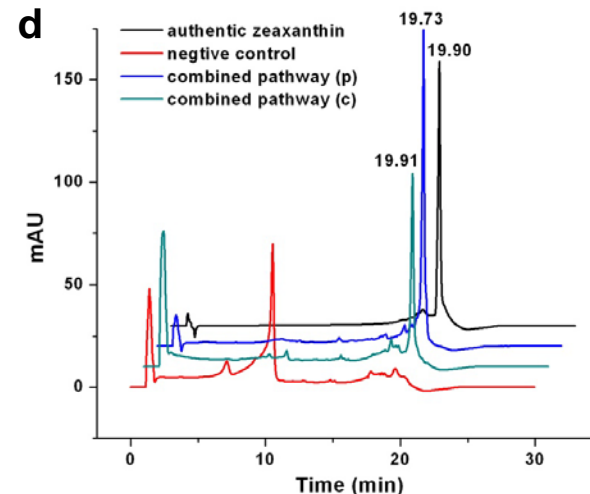
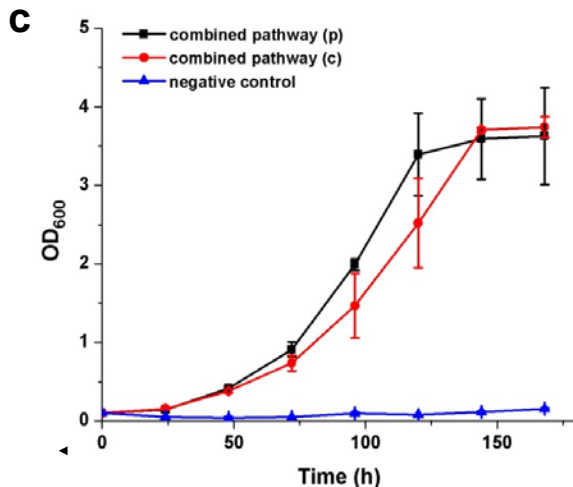
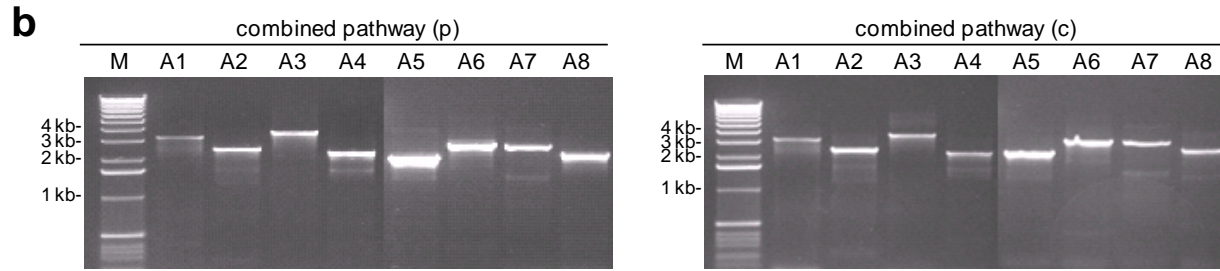
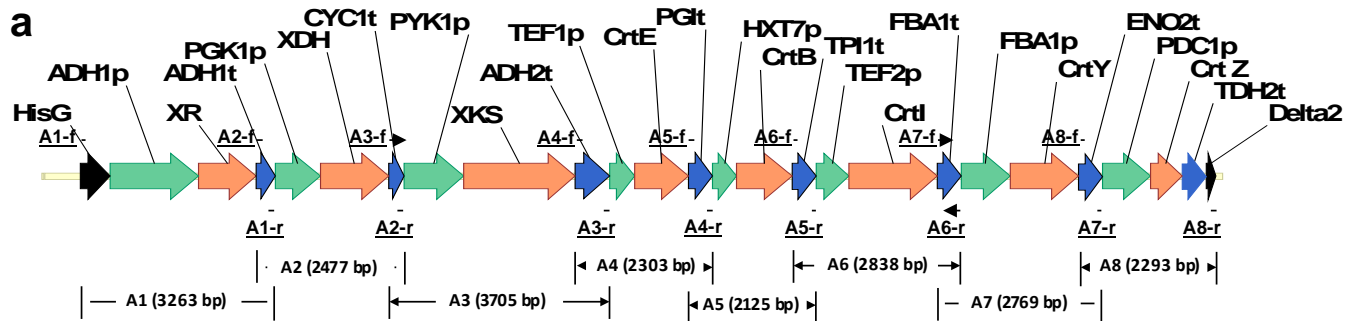
Building Large DNA Molecules via One-step DNA Assembler



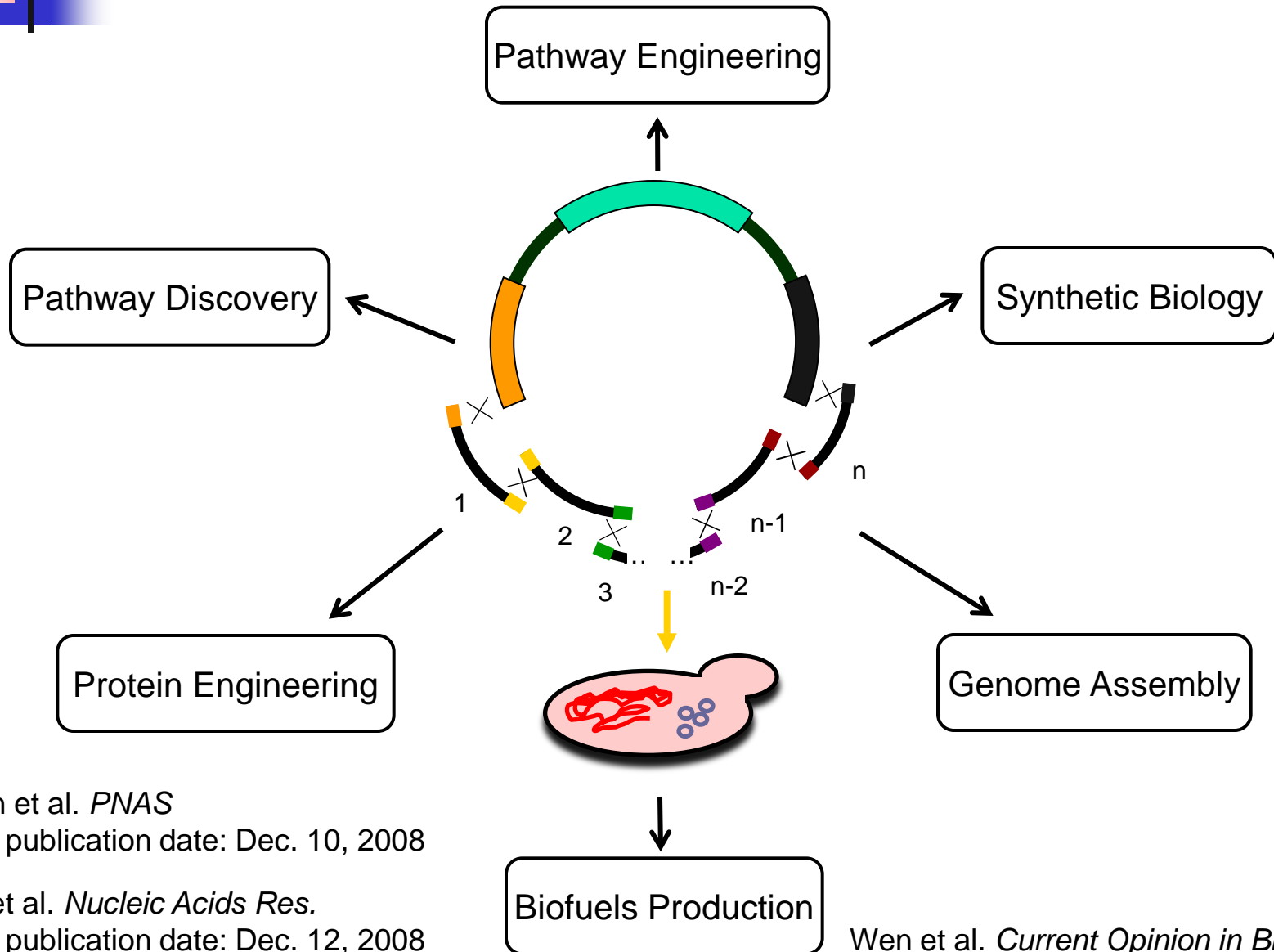
in vivo assembly of DNA fragments in yeast



Eight-gene Pathway: A Combined Xylose and Zeaxanthin Pathway



Broad Application of DNA Assembler

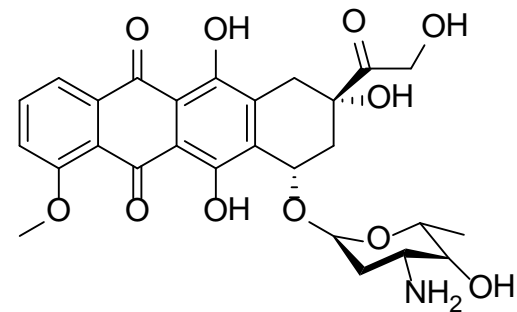
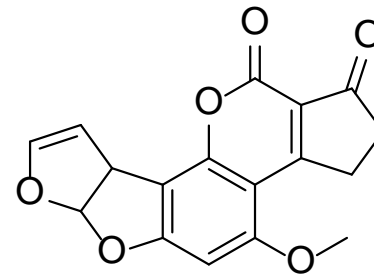


Gibson et al. *PNAS*
Online publication date: Dec. 10, 2008

Shao et al. *Nucleic Acids Res.*
Online publication date: Dec. 12, 2008

Wen et al. *Current Opinion in Biotech.* (2009)

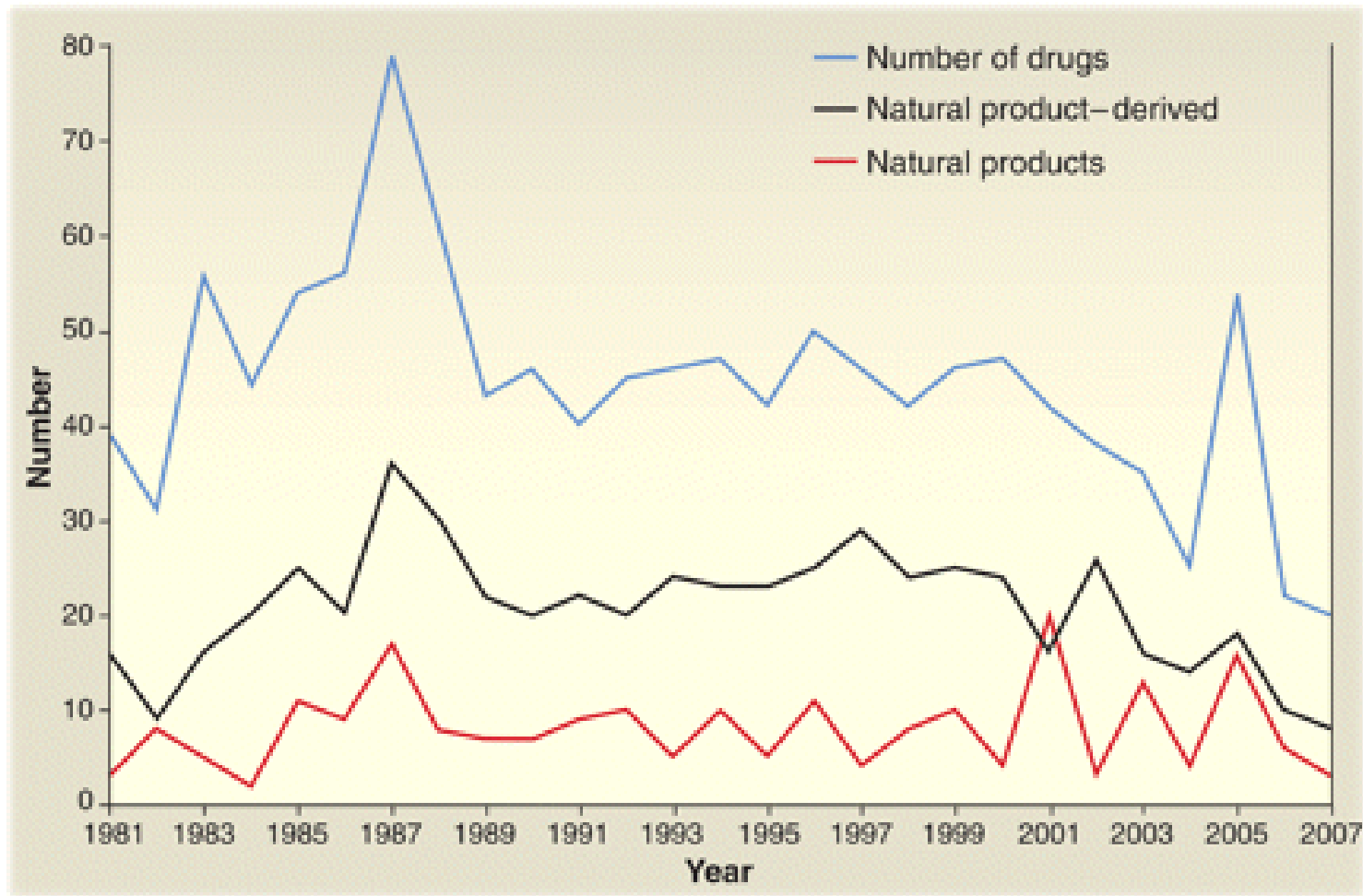
Discovering New Drugs



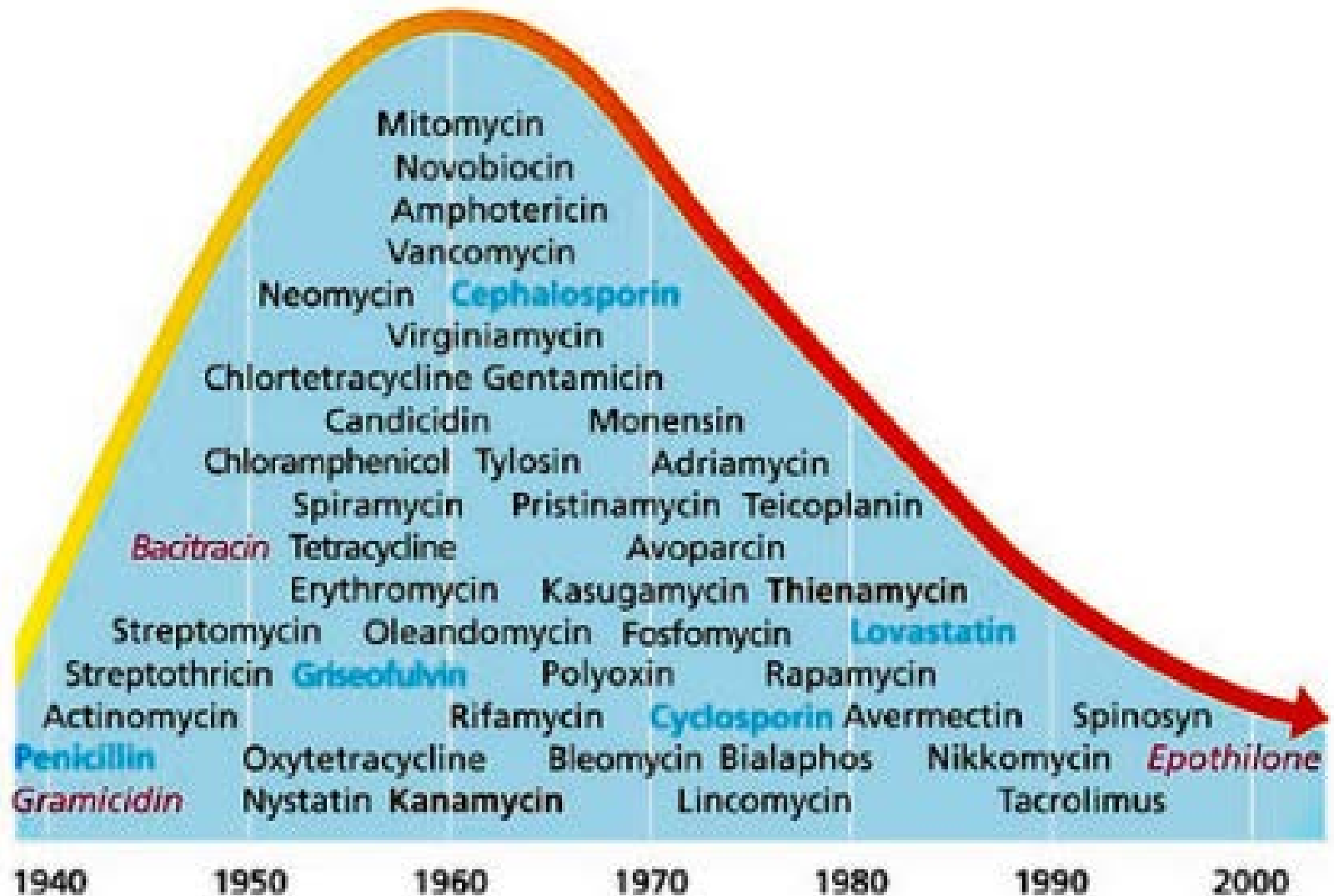
Natural Products and Drug Discovery



Number of drugs approved in the United States from 1981 to 2007



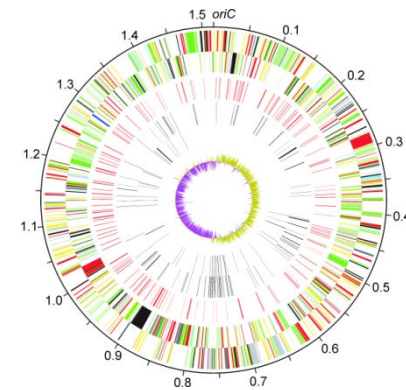
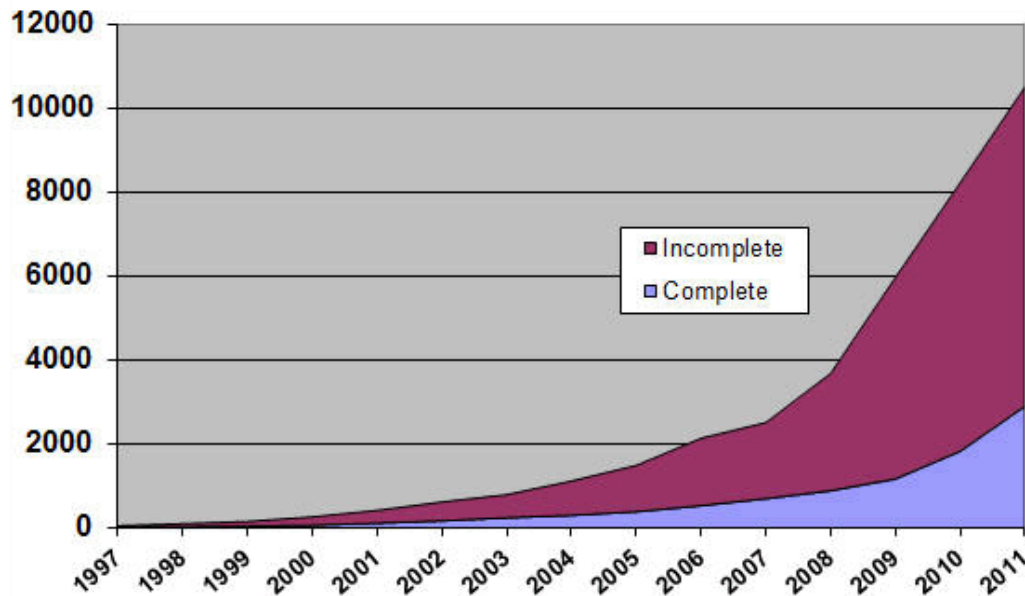
Natural Products and Drug Discovery



Underexplored Biosynthetic Treasures: Cryptic Pathways



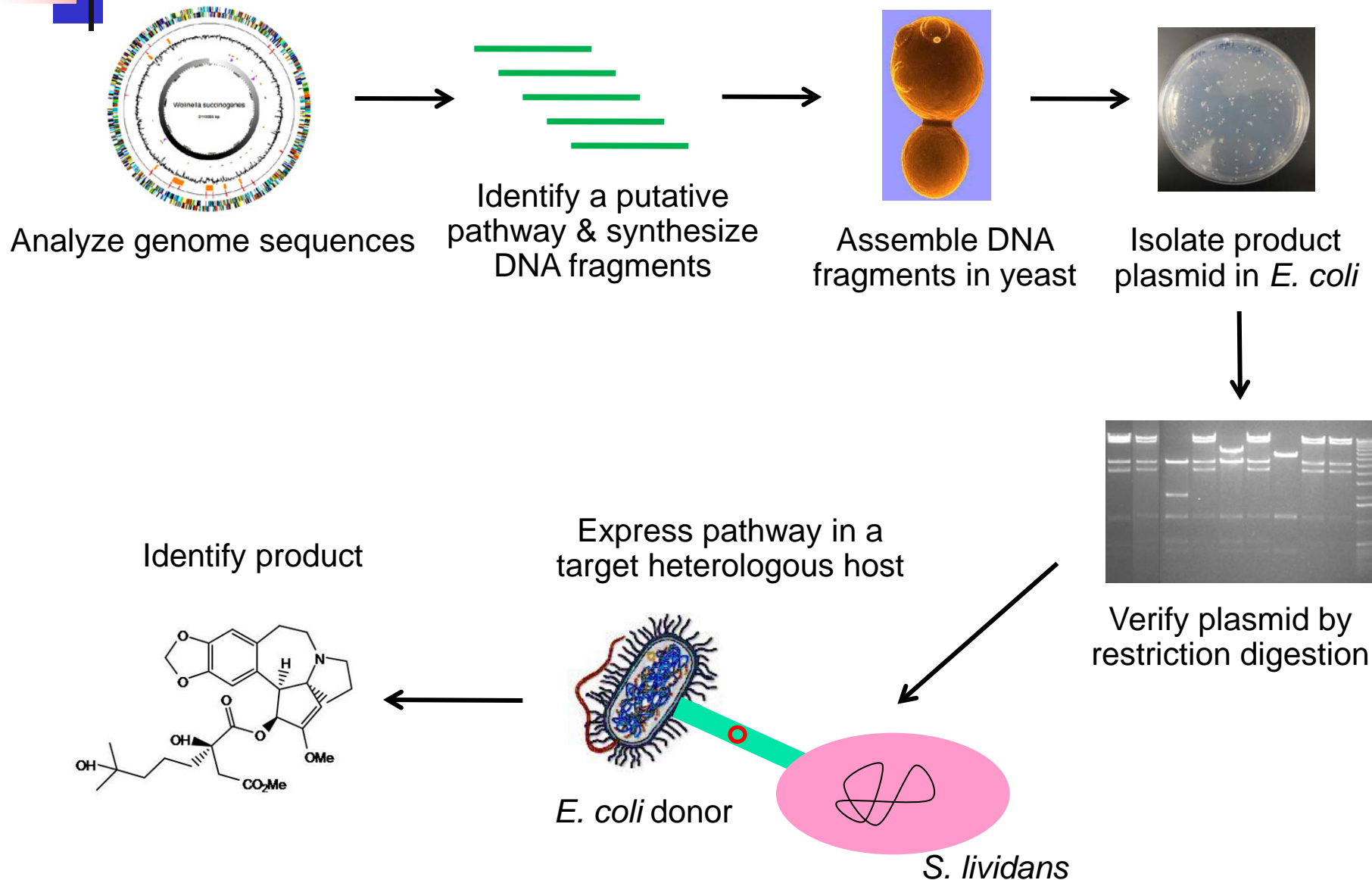
- Over 2000 organisms have been sequenced, representing a rich source for discovery of new genes and pathways.



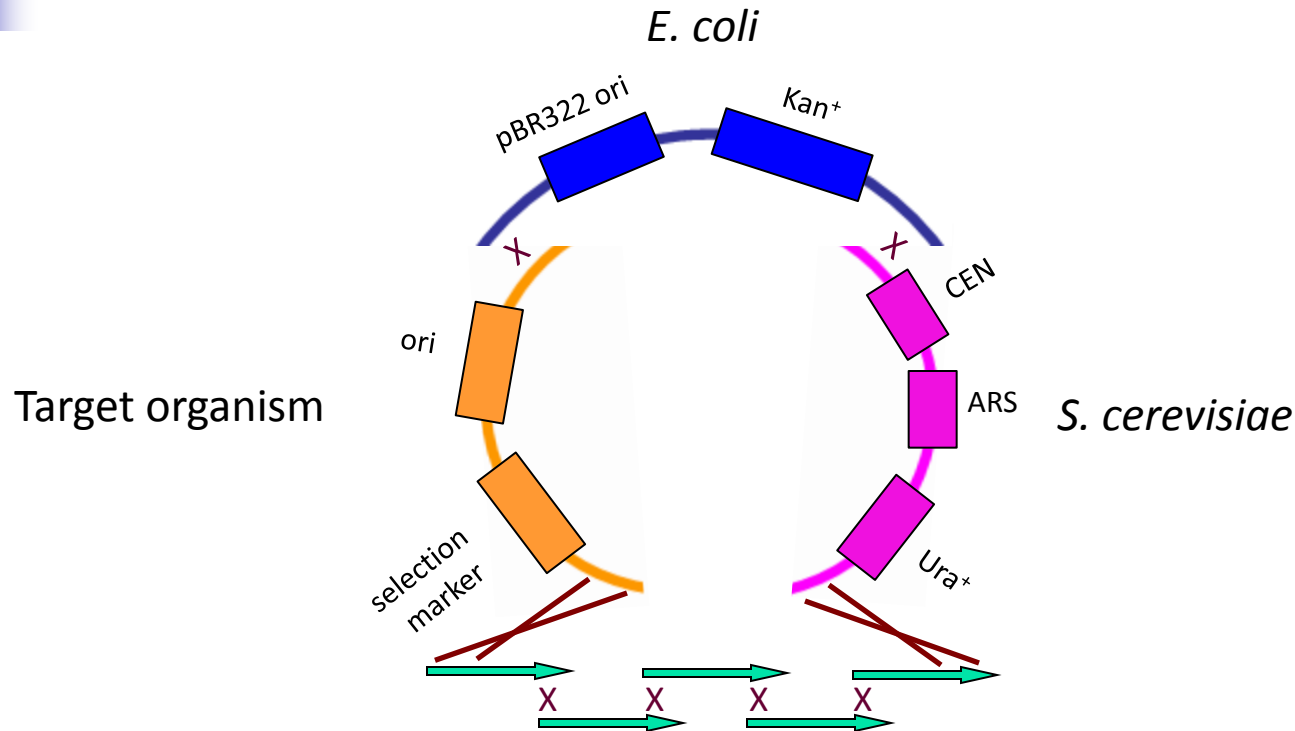
<http://www.genomesonline.org/gold.cgi>

- Potential secondary metabolite gene clusters far outnumber known secondary metabolites. For example,
 - ❑ *Streptomyces griseus*: 34 clusters, 6 known secondary metabolites
 - ❑ *Streptomyces coelicolor*: 23 clusters, 5 known secondary metabolites

DNA Assembler-based Approach (Bottom-up)



DNA Assembler-based Approach (Bottom-up)

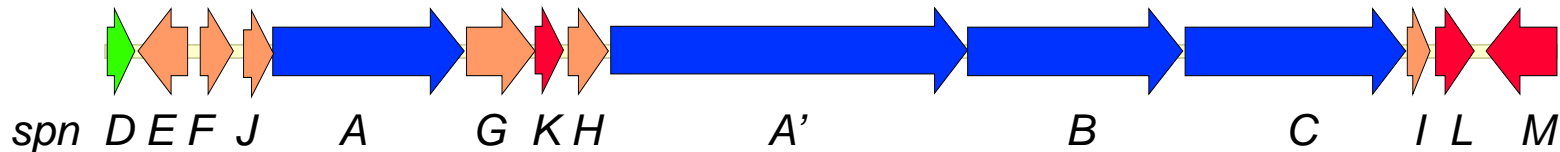


- Enables facile heterologous expression of a biochemical pathway in any desired organism
- A useful tool for studying the biosynthetic mechanism
- A useful tool for enzyme discovery and engineering
- A useful tool for pathway engineering and combinatorial biosynthesis

The Spectinabilin (Spn) Gene Cluster

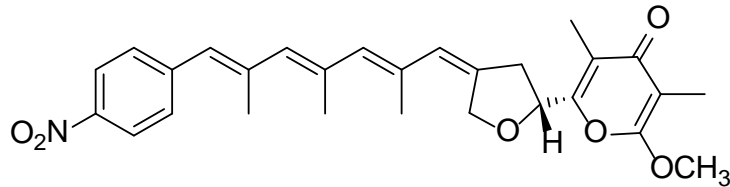


- We isolated a spectinabilin gene cluster from *S. spectabilis*. Spectinabilin is nitrophenyl containing polyketide that exhibits antiviral and antimalarial activities.



- Heterologously expressed the cluster in *S. lividans*

Spectinabilin



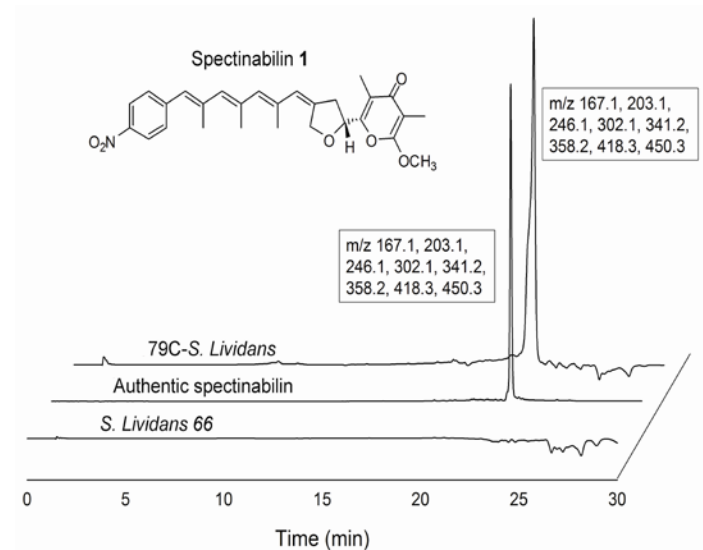
Chemical Formula: $C_{28}H_{31}NO_6$

Exact Mass: 477.22

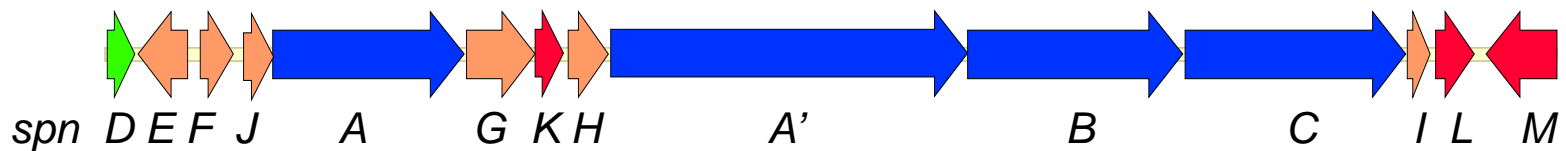
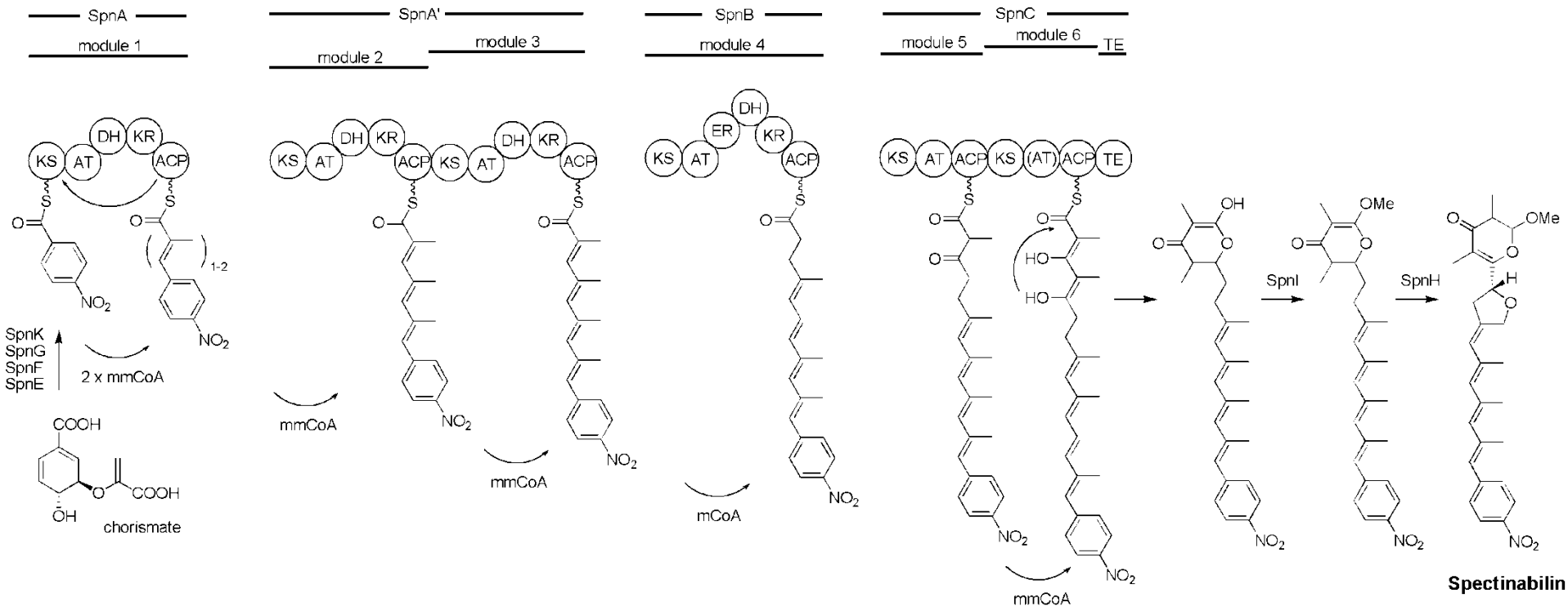
Molecular Weight: 477.55

m/z: 477.22 (100.0%), 478.22 (30.9%), 479.22 (5.9%)

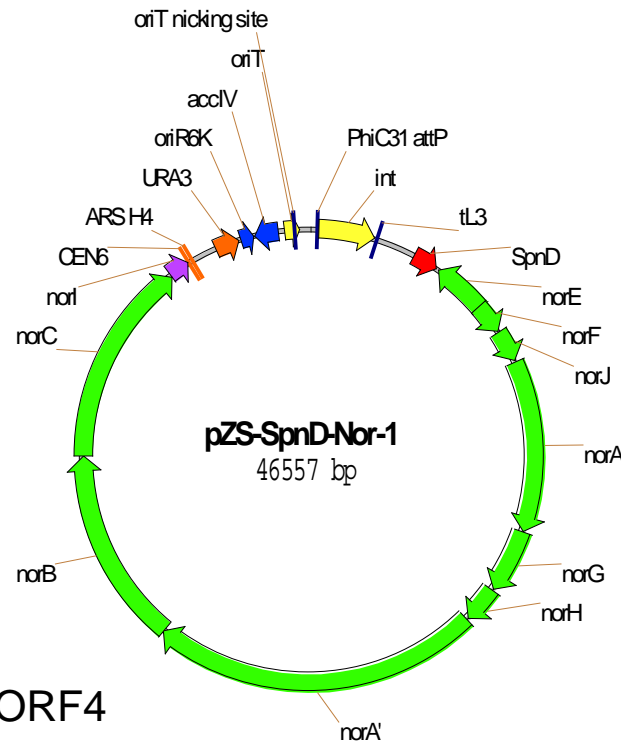
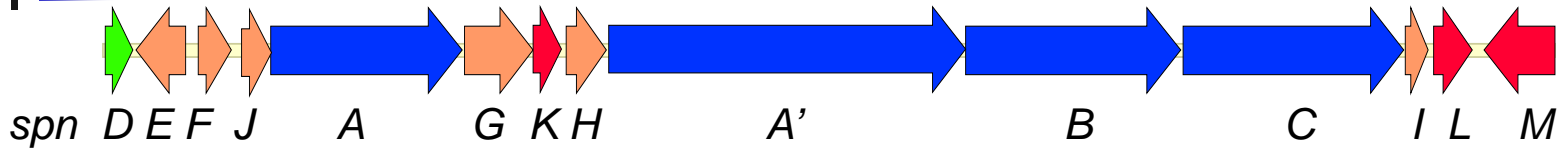
Elemental Analysis: C, 70.42; H, 6.54; N, 2.93; O, 20.10



Biosynthetic Mechanism

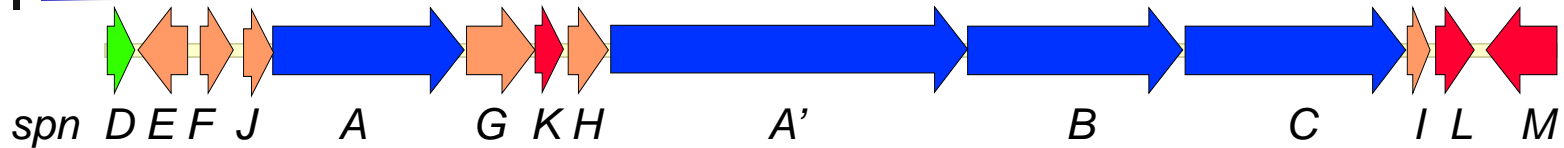


Versatility in Pathway Engineering

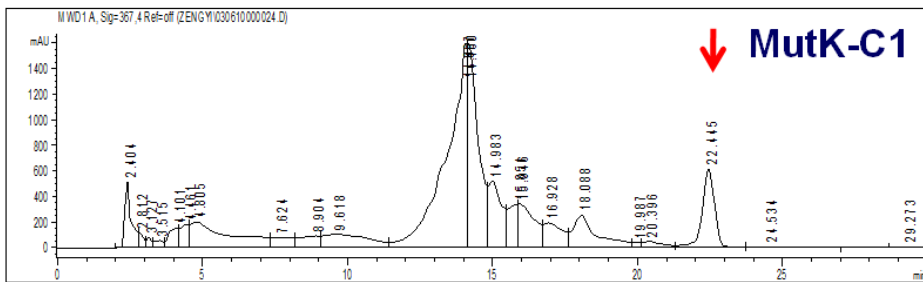


- Deletion: SpnL and SpnM
- Point mutation: SpnK
- Insertion: *ermE** and *actI/actII-ORF4*
- Replacement: NorD → SpnD
- New derivatives:
 - removing SpnA' to produce aureothin
 - introducing mutations to the active sites of the DH, KR or ER domains of SpnA' and SpnB

Versatility in Pathway Engineering

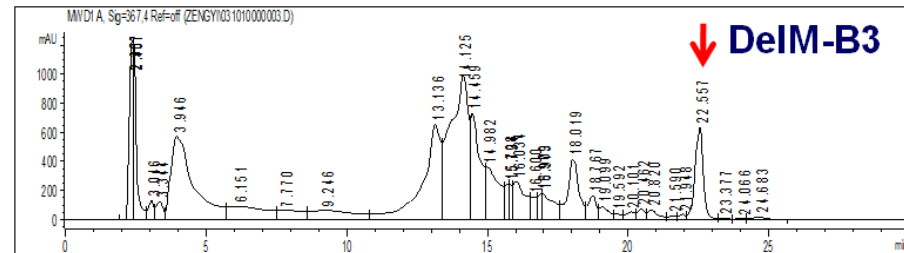


- Point mutation:
 - introducing a stop codon into SpnK

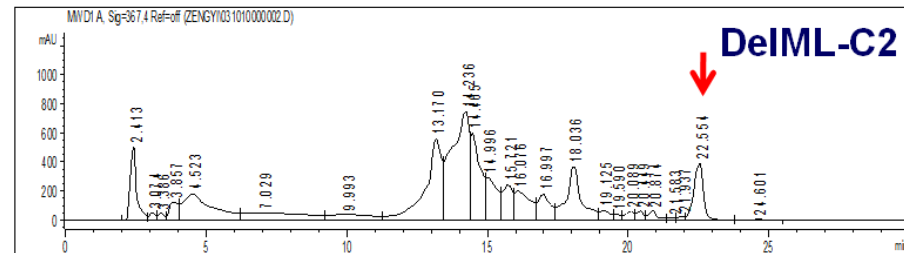


SpnK, L and M are not essential genes for Spn biosynthesis

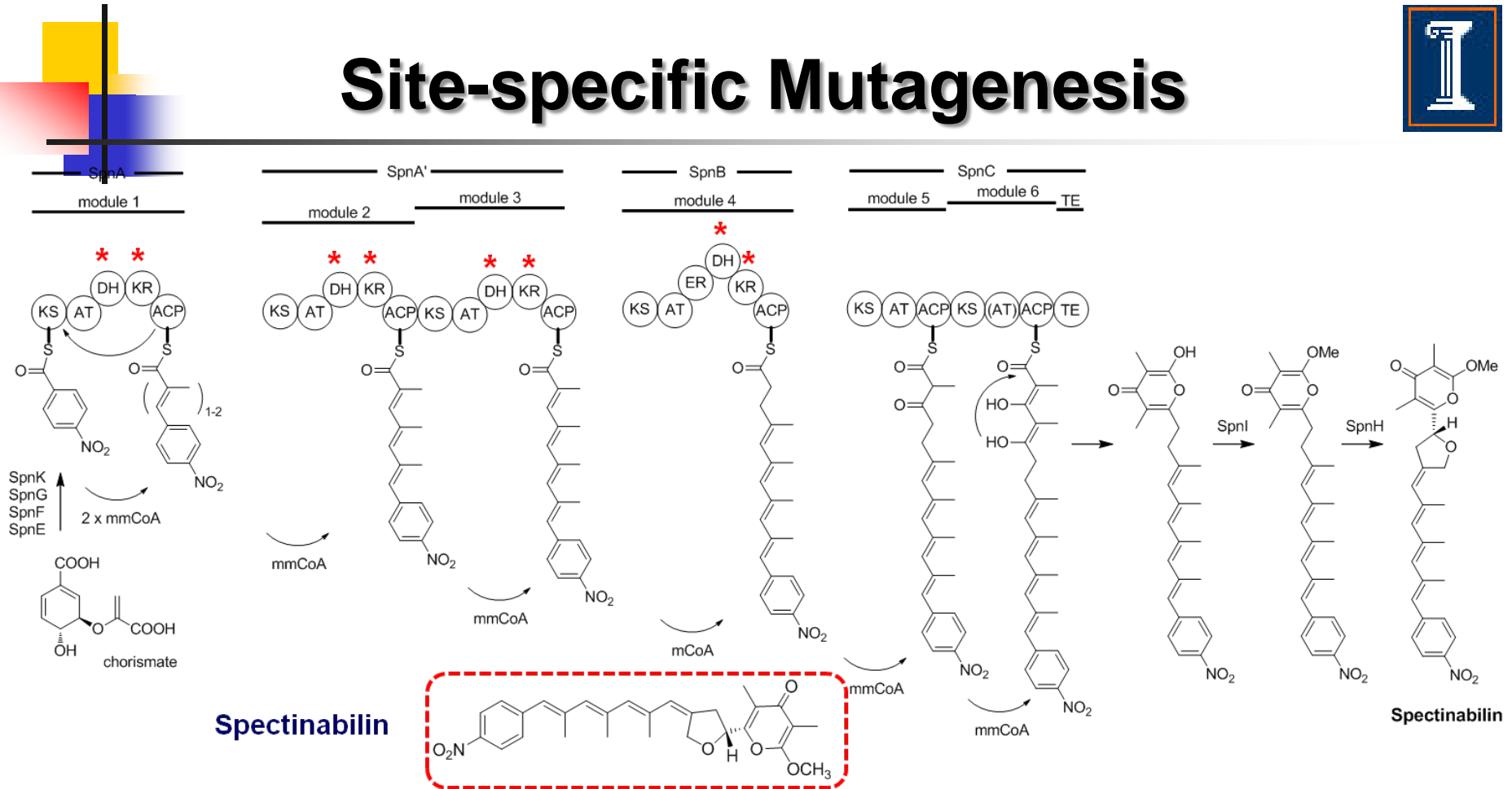
- Deletion
 - deleting SpnM



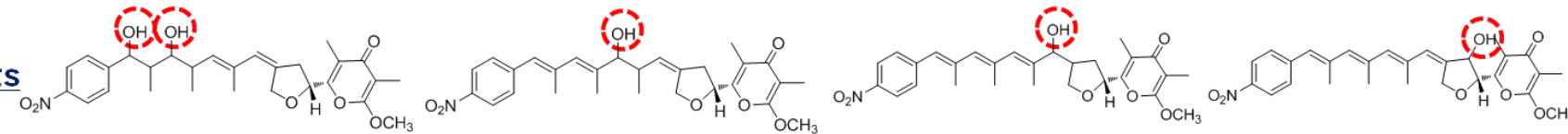
- deleting SpnM+L



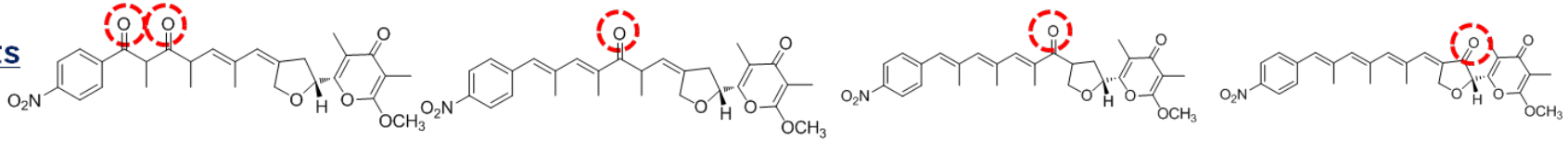
Site-specific Mutagenesis



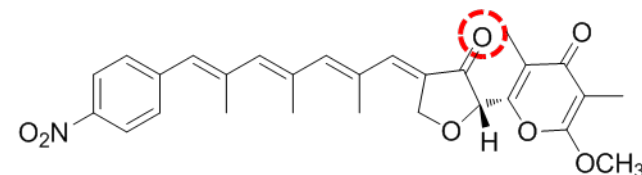
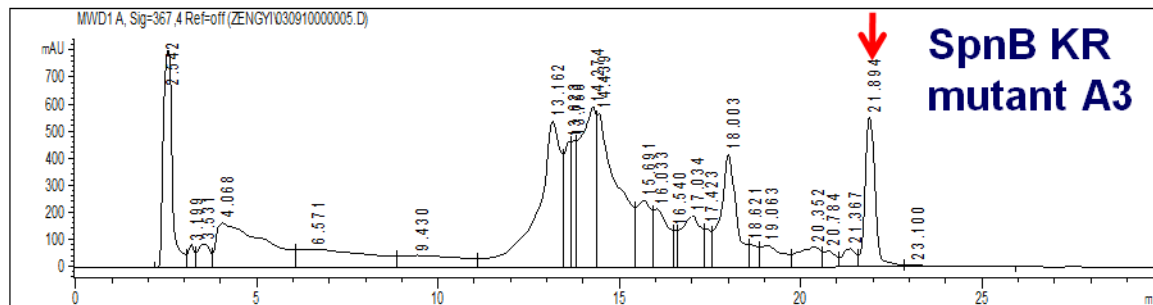
DH mutants



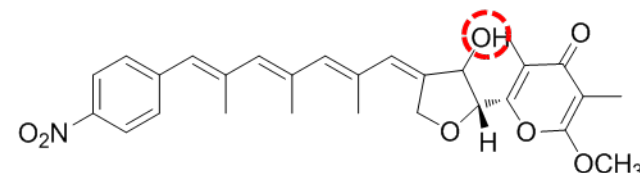
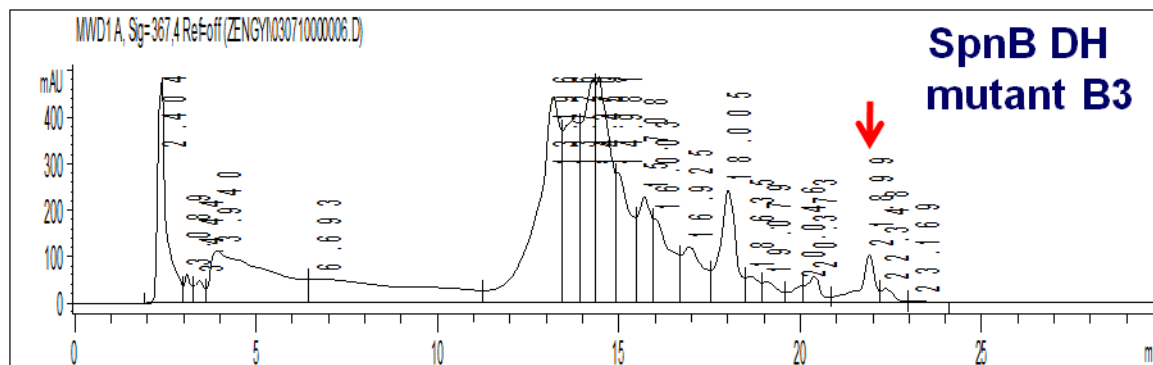
KR mutants



Site-specific Mutagenesis



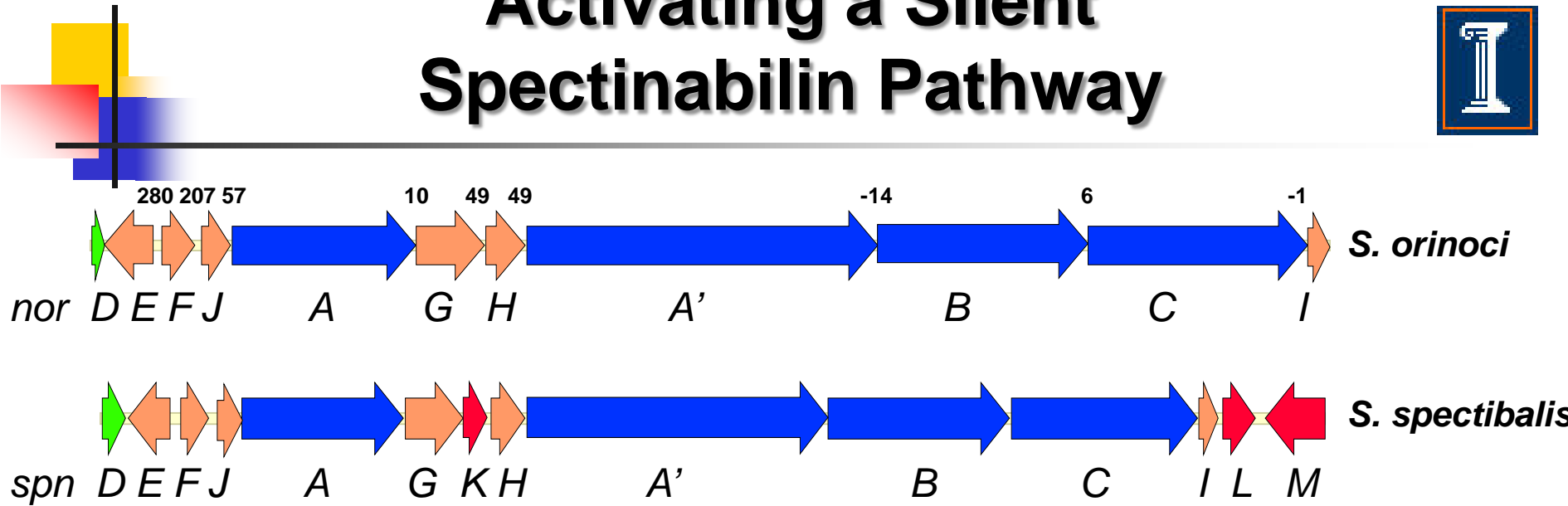
Chemical Formula: C₂₈H₂₉NO₇
Exact Mass: 491.19
Molecular Weight: 491.53



Chemical Formula: C₂₈H₃₁NO₇
Exact Mass: 493.21
Molecular Weight: 493.55

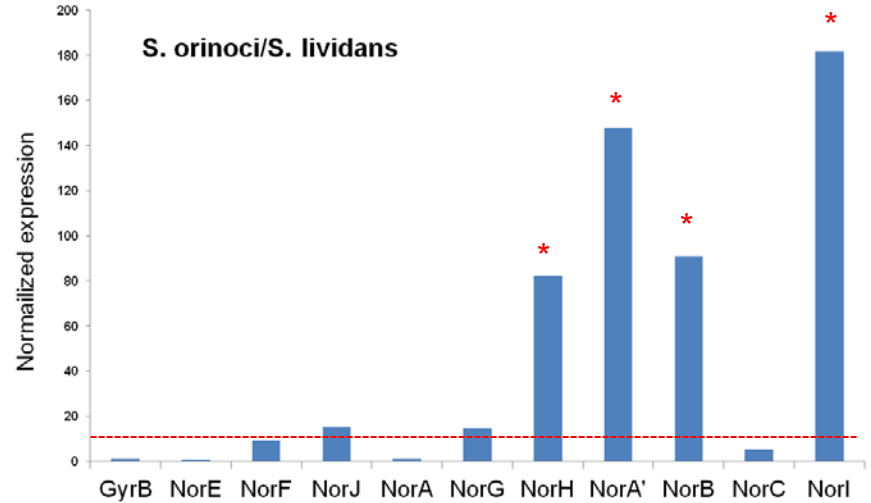
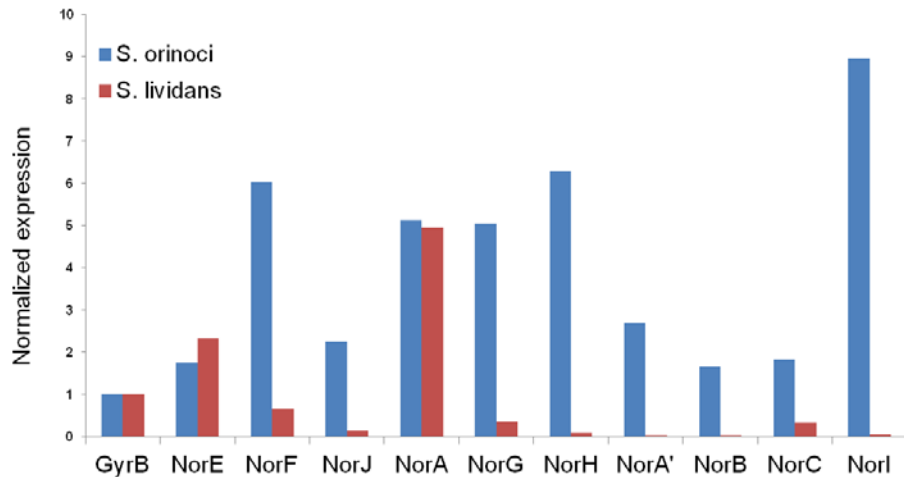
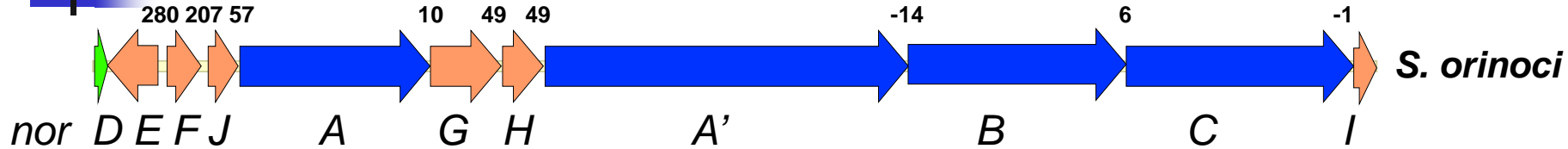
- Elution time: Spn (22.5 min); new peak (21.9 min)
- Used MS to confirm the structures of these new compounds.

Activating a Silent Spectinabilin Pathway



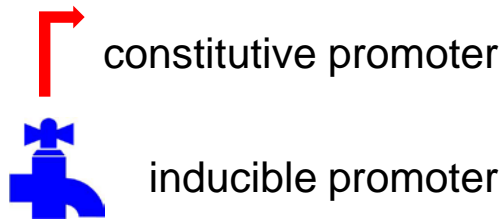
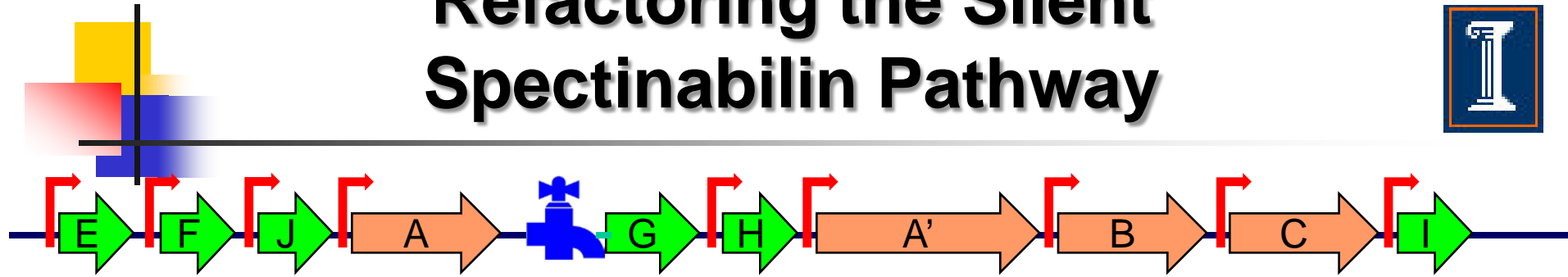
- The spectinabilin gene cluster from *Streptomyces orinoci* did not produce spectinabilin when it was heterologously expressed in *S. lividans*.
- Possible repression reasons:
 - Other inducers (effectors, proteins) in the native host can derepress NorD
 - Need a second activator available in the native host to activate the cluster

Activating a Silent Spectinabilin Pathway

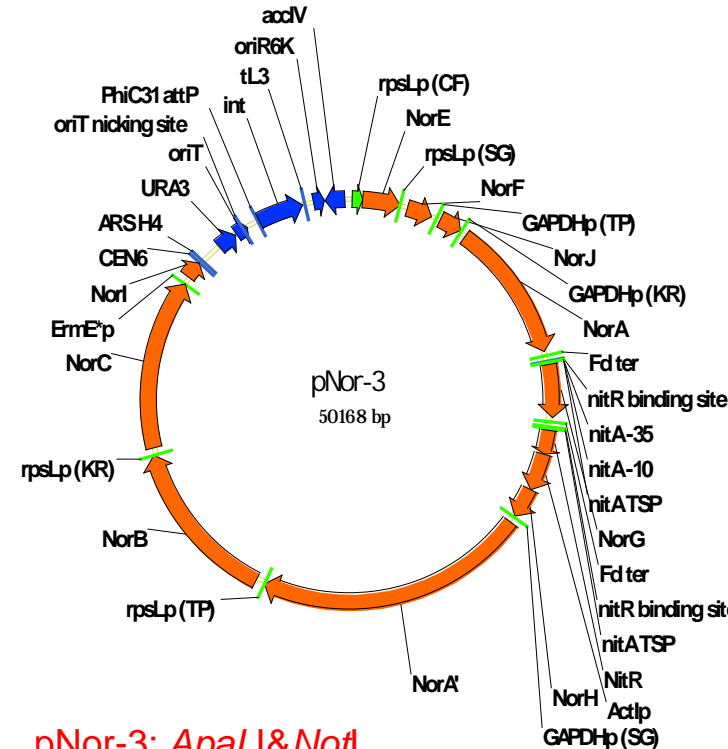
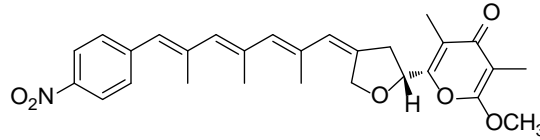


➤ The expression of the *Nor* genes in *S. lividans* is extremely low.

Refactoring the Silent Spectinabilin Pathway

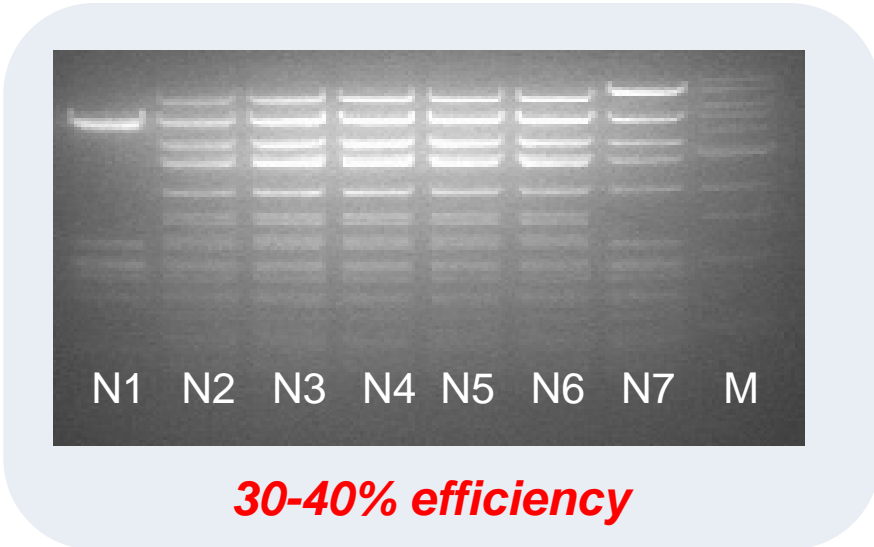


spectinabilin



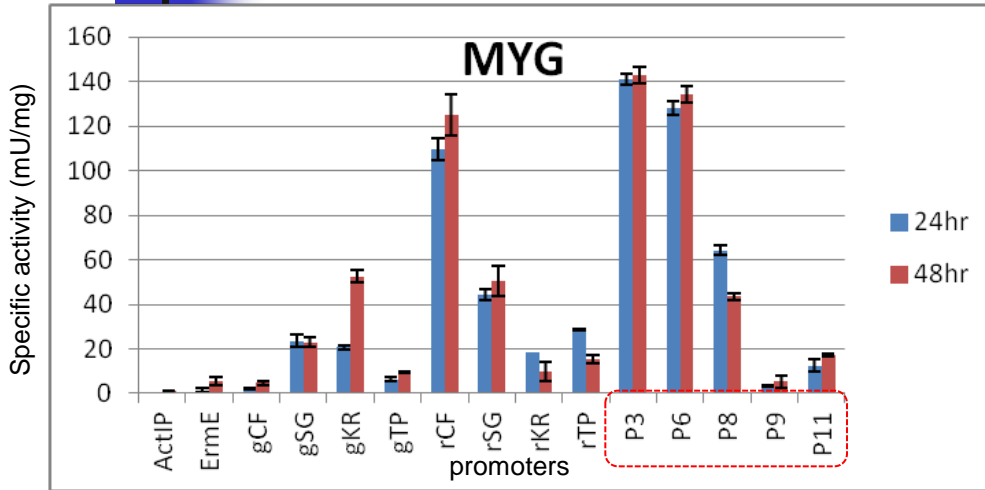
pNor-3: *Apa*LI&*Not*I

55, 77, 119, 326, 332, 391, 443,
447, 491, 578, 702, 722, 862, 944,
1011, 1185, 1233, 1442, 1553, 1956,
1969, 2658, 2709, 2832, 3346,
3470, 4812, 4856, 6779

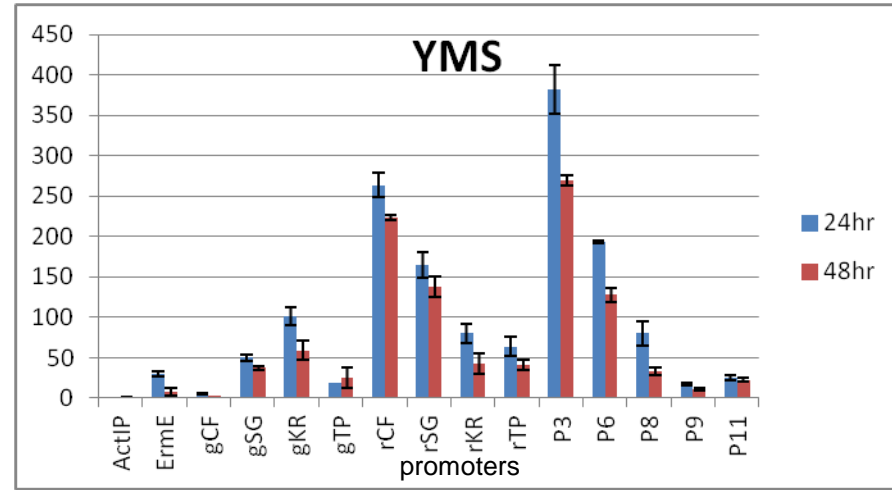


30-40% efficiency

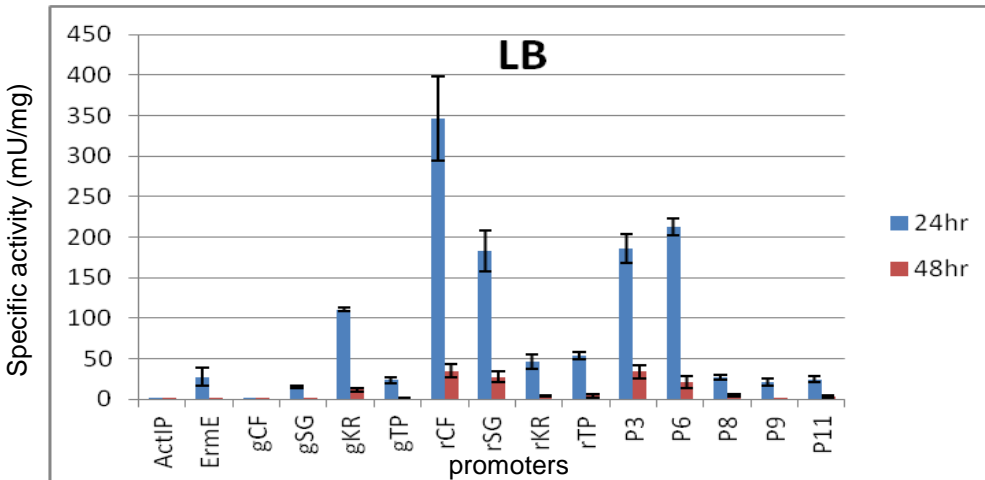
Cloning and Characterization of New Constitutive Promoters



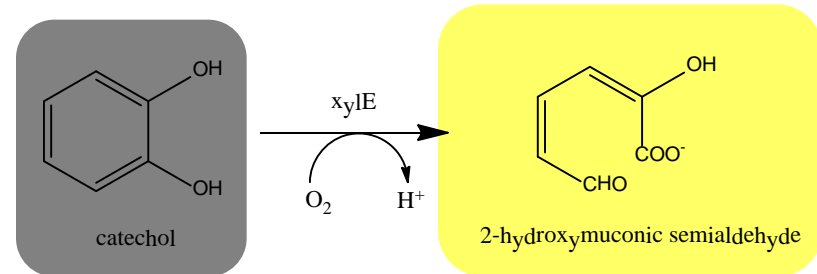
malt extract 10 g/L; yeast extract 4 g/L; glucose 4 g/L



malt extract 10 g/L; yeast extract 4 g/L; starch 4 g/L

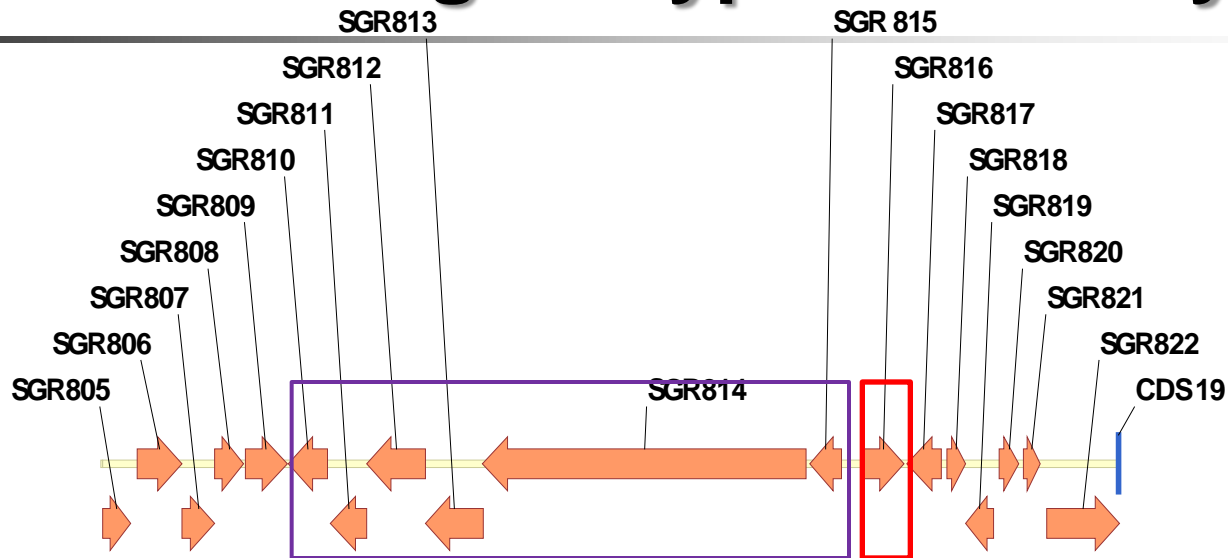


trypton 10 g/L; yeast extract 5 g/L; NaCl 5 g/L; glucose 1 g/L



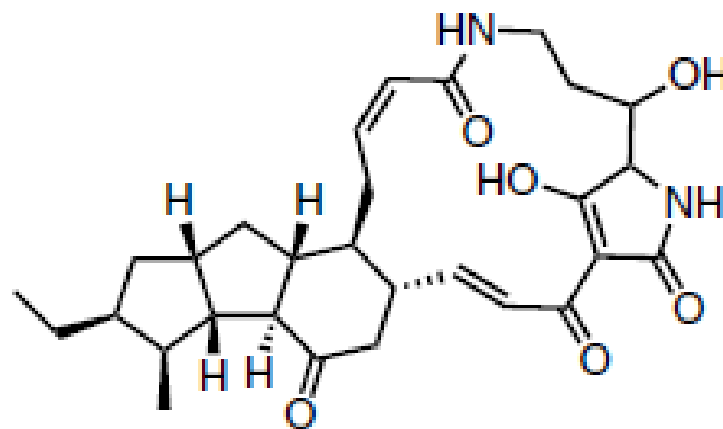
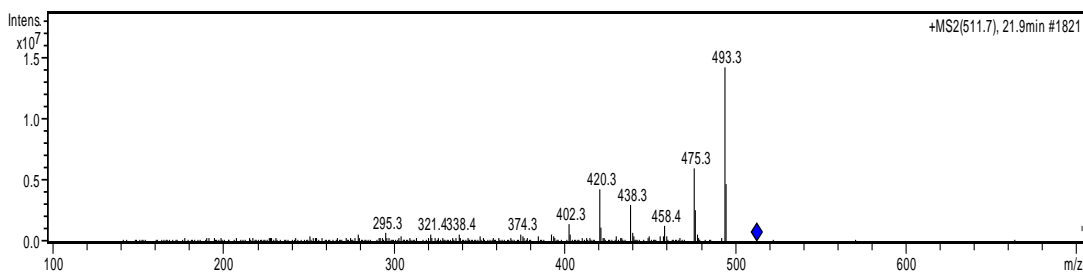
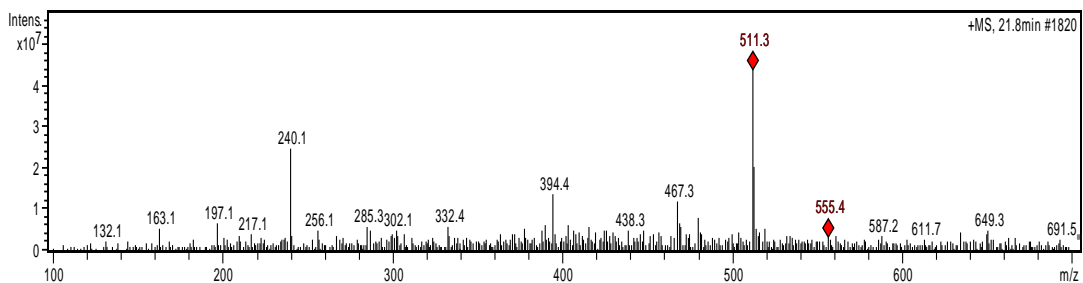
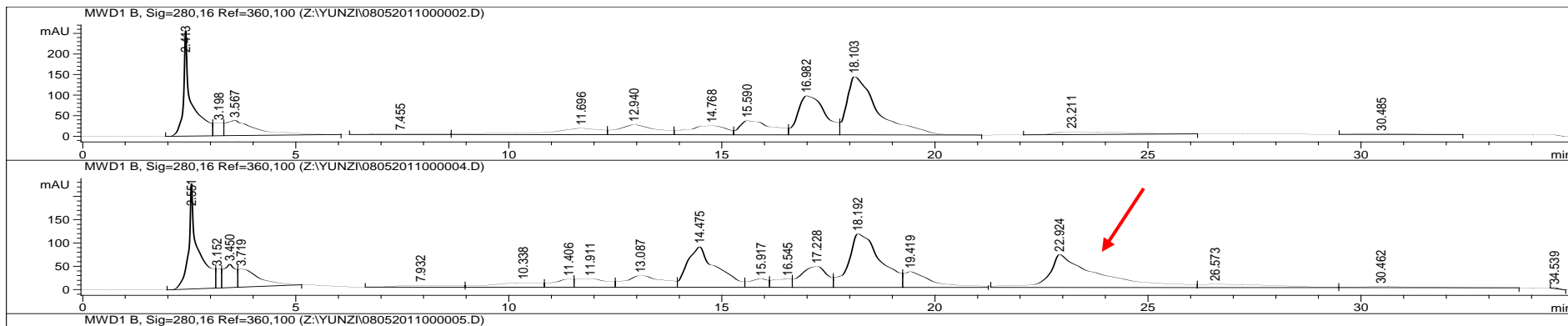
- active in all the media
- medium-dependent
- much stronger than ermE*p

Activating a Cryptic Pathway



Position	Predicted Function	Position	Predicted Function
SGR805	GntR family transcriptional regulator	SGR814	putative NRPS-type-I PKS fusion protein
SGR806	putative ABC transporter substrate-binding protein	SGR815	fatty acid hydroxylase/Sterol desaturase
SGR807	putative ABC transporter permease protein	SGR816	putative aminotransferase
SGR808	putative ABC transporter permease protein	SGR817	MerR family transcriptional regulator
SGR809	putative starvation sensing protein	SGR818	hypothetical protein
SGR810	putative cytochrome P450	SGR819	hypothetical protein
SGR811	putative oxidoreductase	SGR820	hypothetical protein
SGR812	putative FAD-dependent oxidoreductase	SGR821	MarR family transcriptional regulator
SGR813	putative FAD-dependent oxidoreductase	SGR822	putative transmembrane transport protein

Activating a Cryptic Pathway

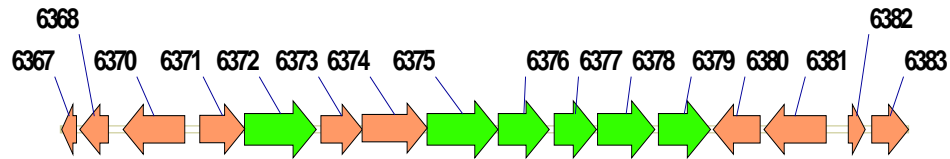


Similar compound

Activating Additional Cryptic Pathways

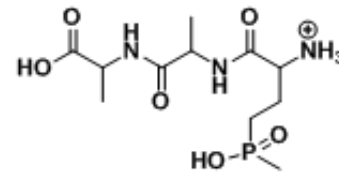


Expressing gene clusters from actinobacteria in *S. lividans*



Frankia alni
16678 bp

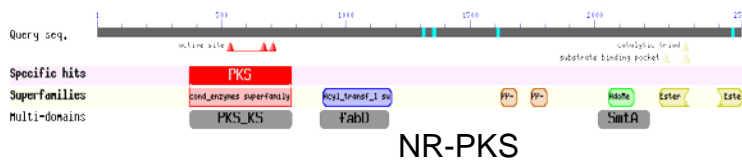
- six enzymes sharing high homology with those from PTT gene clusters
- possibly encodes a novel phosphonate gene cluster



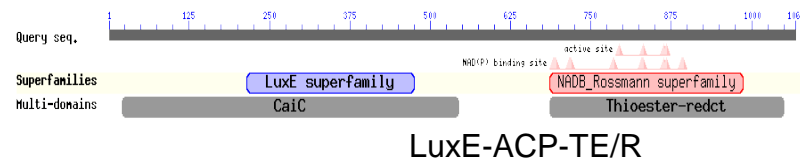
PTT

Expressing gene clusters from fungi in *S. cerevisiae*

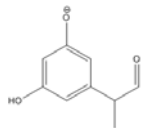
Aspergillus terreus



NR-PKS

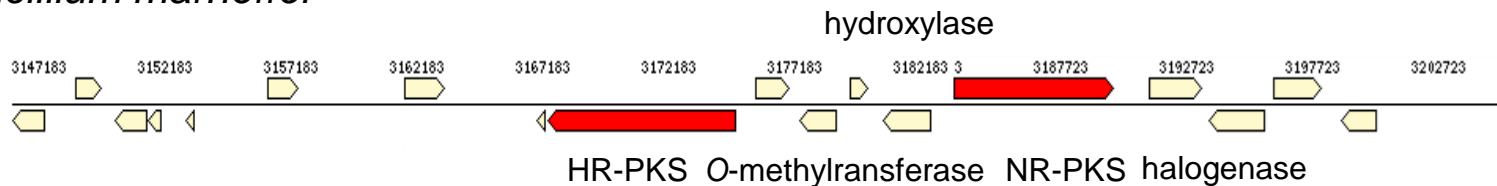


LuxE-ACP-TE/R

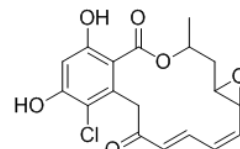


$C_9H_9O_3$
(165.06)

Penicillium marneffei



HR-PKS O-methyltransferase NR-PKS halogenase

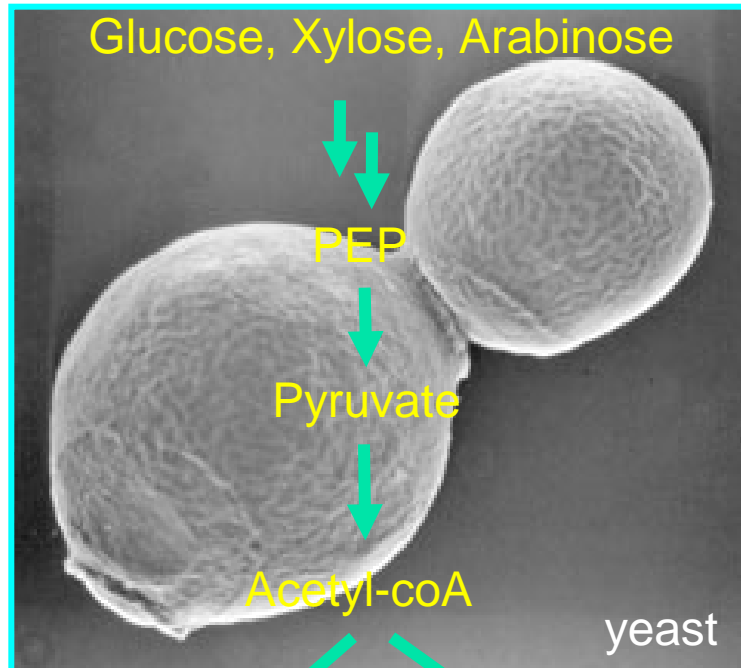


radicicol

Engineering a Microbial Factory for Advanced Biofuels Production



Hemicellulose/Cellulose



Ethanol

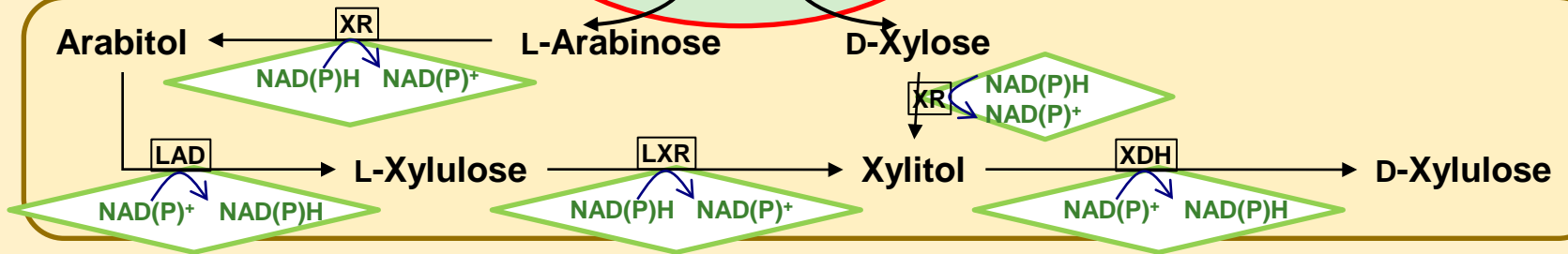
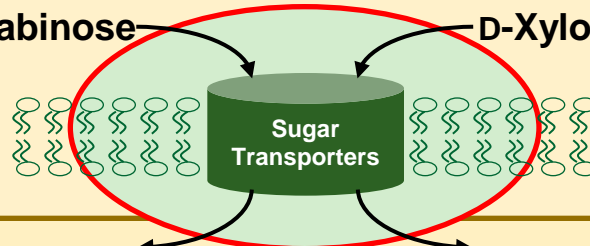
Advanced biofuels
(Butanol, Hydrocarbons)



Pentose Utilization in Yeast



L-Arabinose D-Xylose

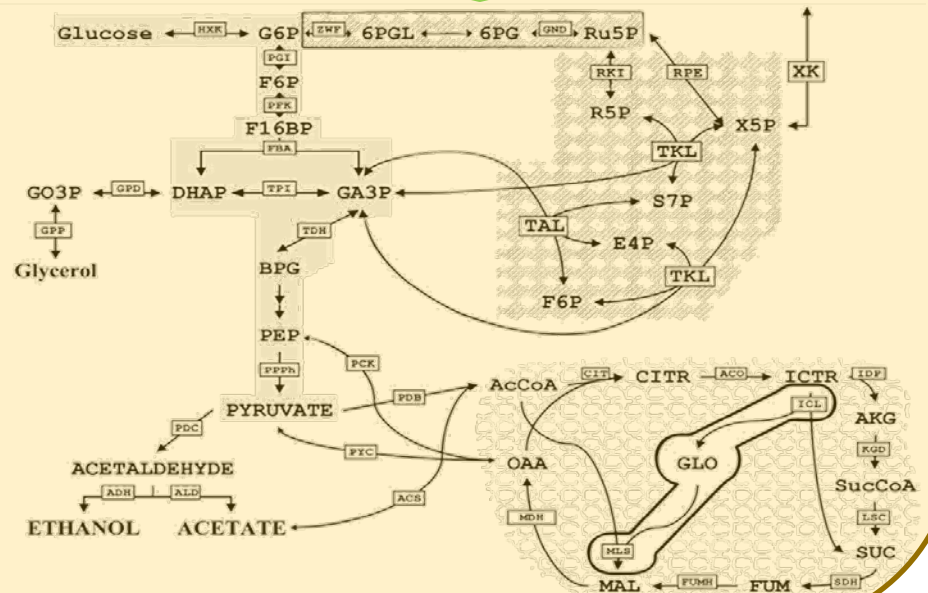


Sugar Uptake

Heterologous Pathway

Redox Imbalance

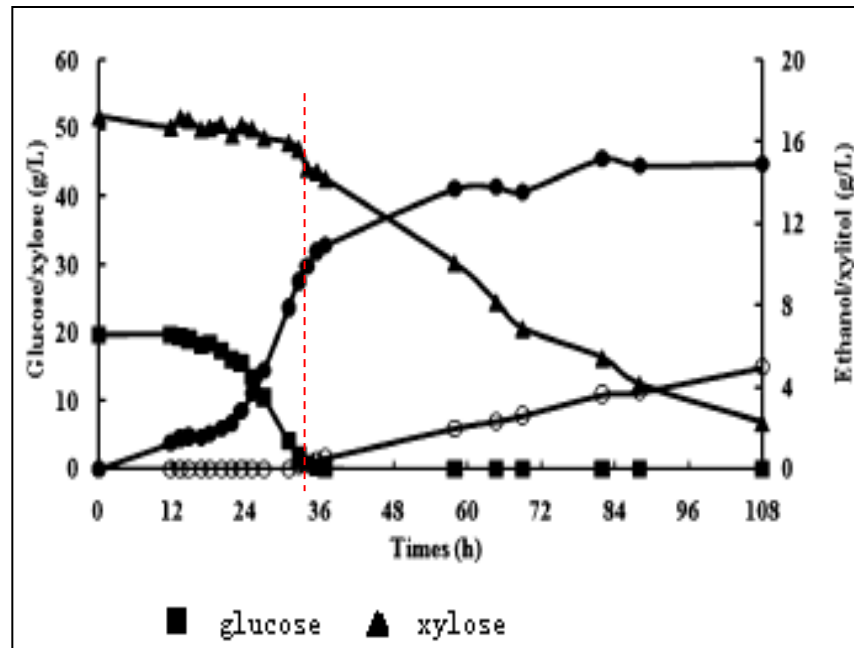
Metabolic Flux



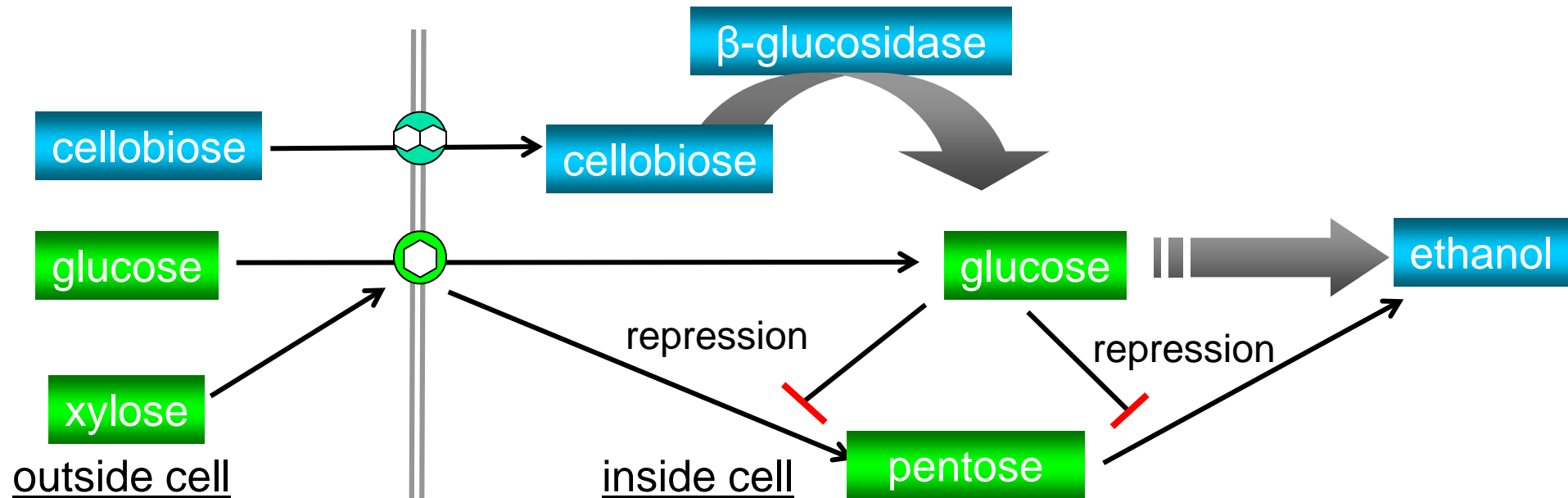
Glucose Repression in Mixed Sugar Fermentation



- Glucose repression occurs in *S. cerevisiae*
- Alternative carbon source fermentation is inhibited in the presence of glucose
- Lag time in xylose and arabinose consumption curve



Coexpression of Cellobiose Transporter and β -Glucosidase

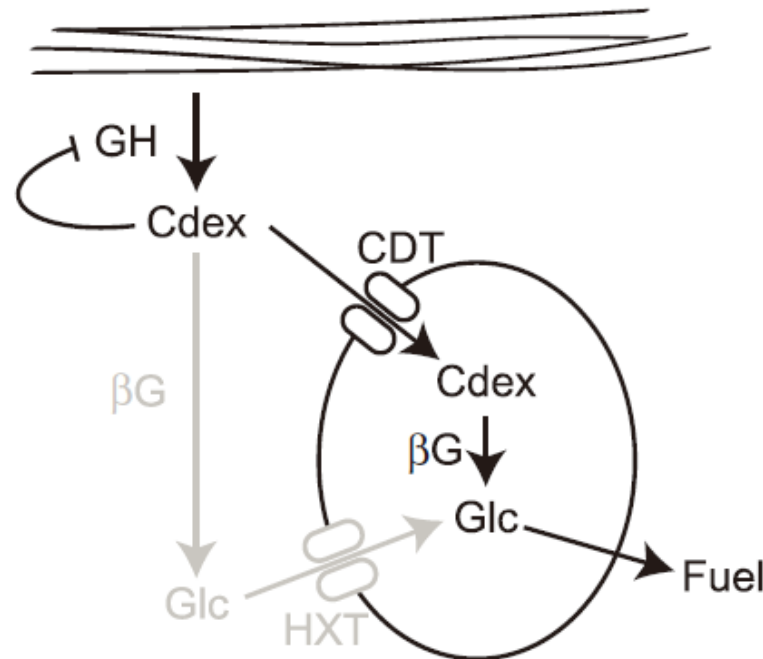


Coexpression of Cellobiose Transporter and β -Glucosidase



- Cellodextrin transport system from *Neurospora crassa*

- Cellodextrin transporters: NCU00801 (*cdt1*), NCU00809, NCU08114(*cdt2*)
- β -glucosidase: NCU00130 (*gh1-1*)



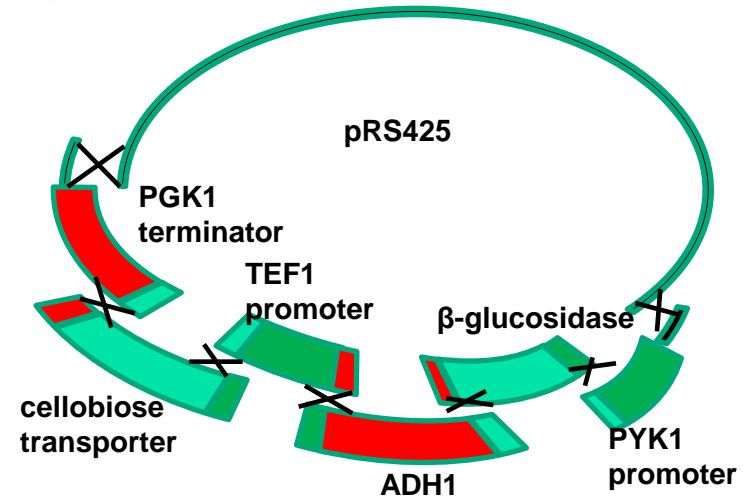
- *S. cerevisiae* with a heterologous cellodextrin transport system showed improved growth rate.

Coexpression of Cellobiose Transporter and β -Glucosidase



Genes

- 3 transporters: *cdt-1*, *cdt-2*, *NCU00809*
- 2 β -glucosidases:
gh1-1 from *N. crassa*,
bgl1 from *A. aculeatus*



Plasmids

- Use DNA assembler method to integrate genes into pRS425 plasmid

Strains

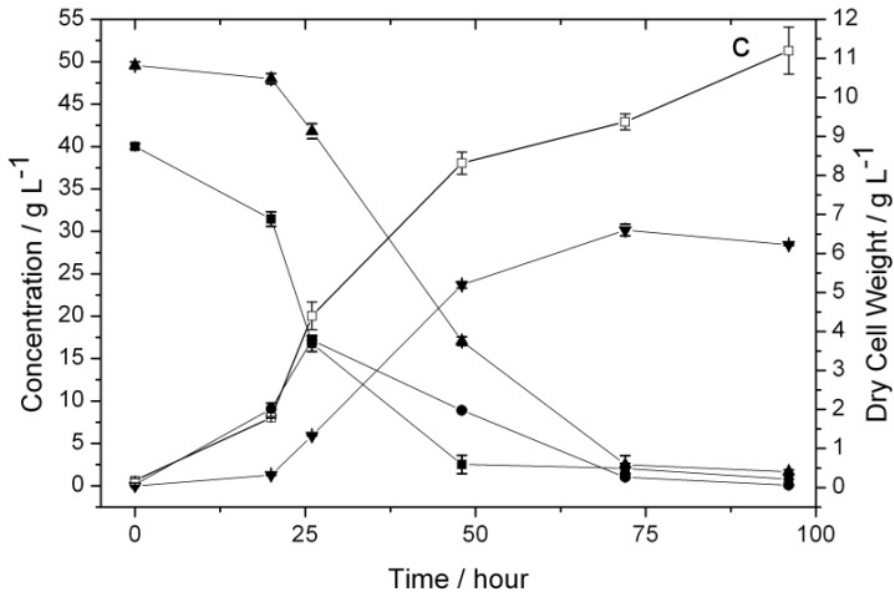
- 6 plasmids constructed were transformed into *S. cerevisiae* strain with an integrated xylose utilization pathway

Strain	Transporter	β -glucosidase
SL01	<i>cdt1</i>	<i>gt1-1</i>
SL02	<i>NCU00809</i>	<i>gt1-1</i>
SL03	<i>cdt2</i>	<i>gt1-1</i>
SL04	<i>cdt1</i>	<i>bgl1</i>
SL05	<i>NCU00809</i>	<i>bgl1</i>
SL06	<i>cdt2</i>	<i>bgl1</i>
SL00	-	-

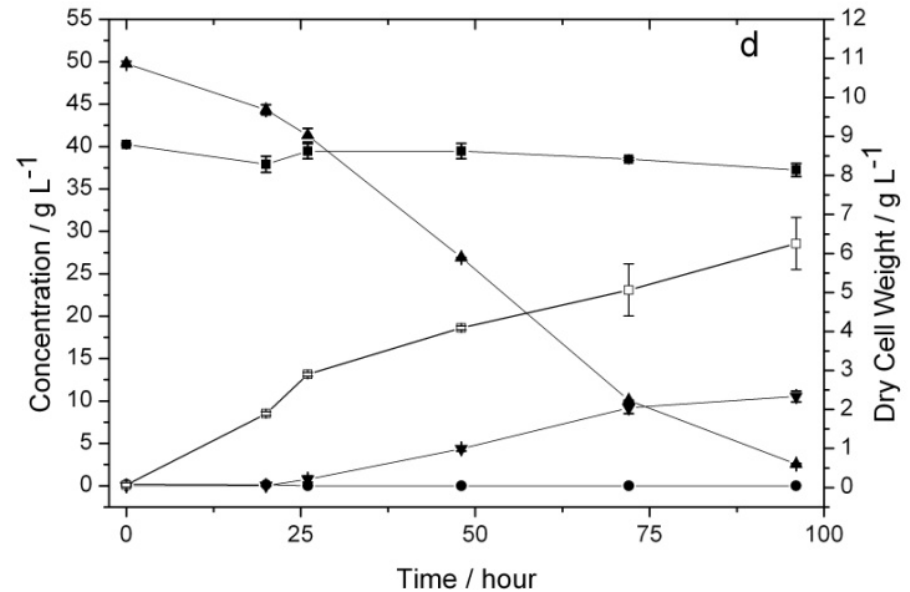
Mixed Sugar Cultivation in Bioreactor: Cellobiose+Xylose



SL01



SL00



cellobiose (■) , xylose (▲), glucose(●), ethanol (▼) , Dry cell weight (□)

	SL01	SL00
Yield _{ethanol}	0.39	0.24
Productivity _{ethanol} (g/(L h))	0.49	0.09

Balancing Metabolic Flux Remains a Big Challenge

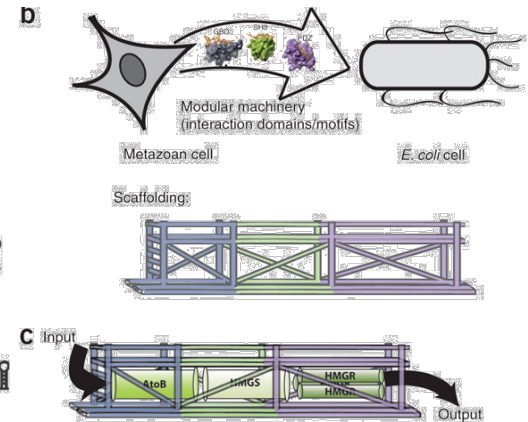
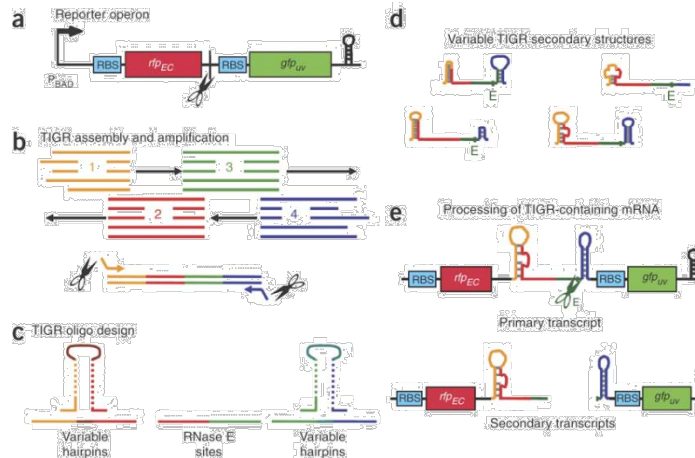
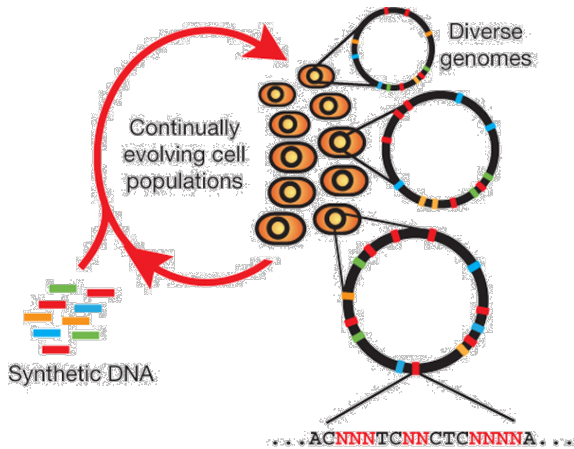
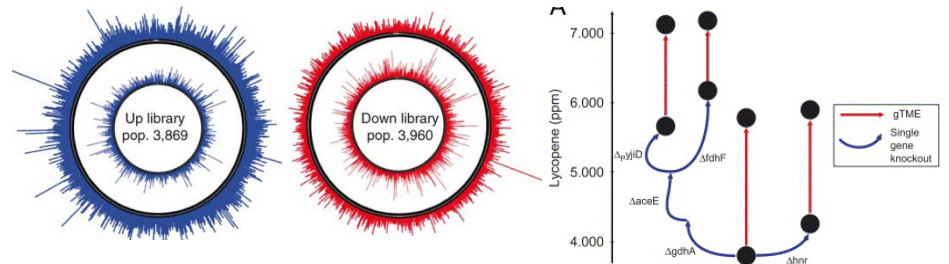


- Production of value-added compounds usually requires introduction of multi-step metabolic pathways
- Metabolic flux in multistep metabolic pathways need to be optimized to avoid metabolic burden
 - Overexpression of certain genes,
 - Redox imbalance from unmatched cofactor specificity
 - Accumulation of unstable or toxic intermediates
- Traditional approaches
 - Overexpression and deletion of certain genes in metabolic pathways
 - Modulating the expression levels of individual enzymes
 - Protein engineering to improve performance of rate limiting enzymes
 - Targeting a specific enzyme instead of the overall pathway
- Simultaneous optimization of multiple metabolic genes remains a big challenge

Balancing Metabolic Flux Remains a Big Challenge



- Perturbation of global transcription machinery
- Genome-scale mapping of fitness altering genes
- Multiplex genome engineering
- Balance metabolic flux within the target pathway
 - Strengths of promoters
 - Ribosome binding sites
 - Intergenic regions
 - Synthetic scaffolds



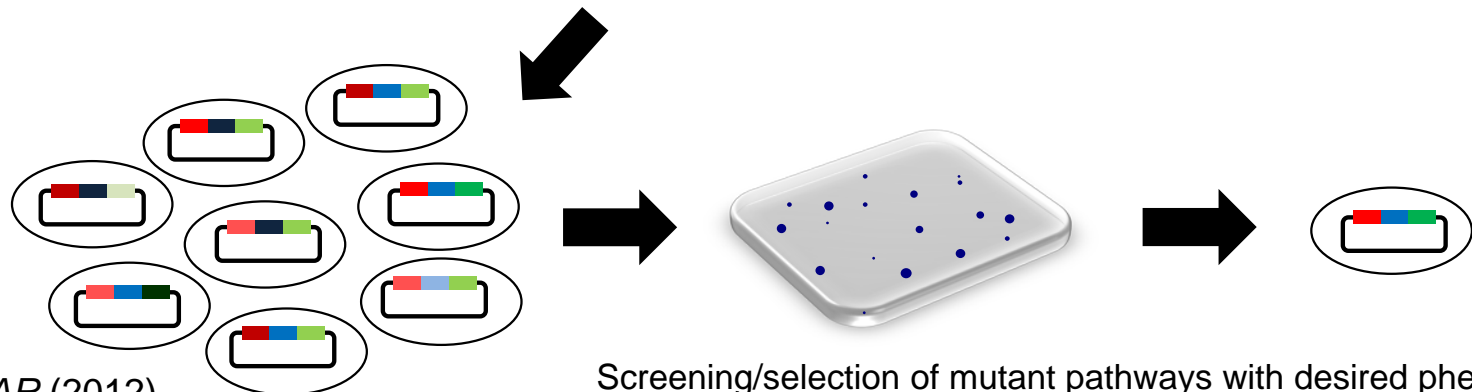
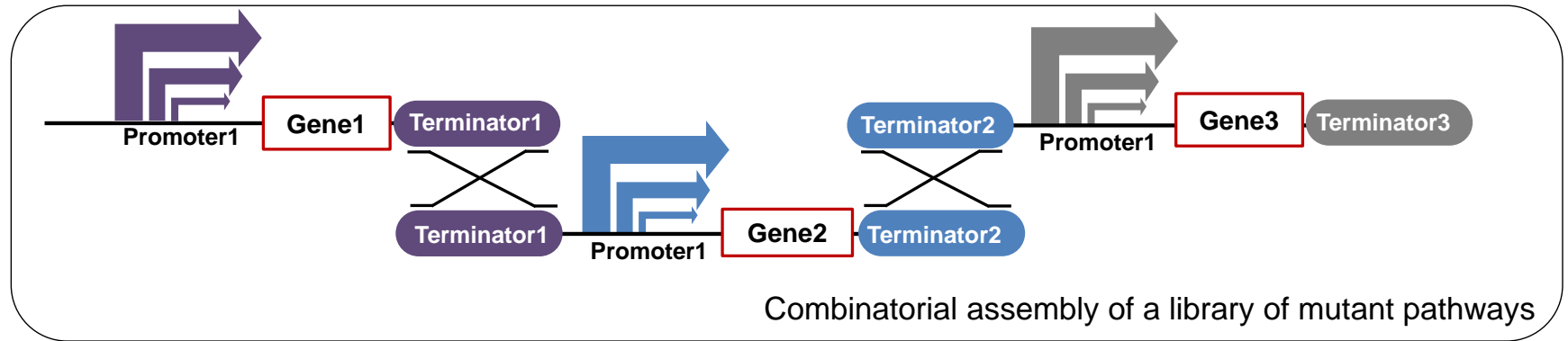
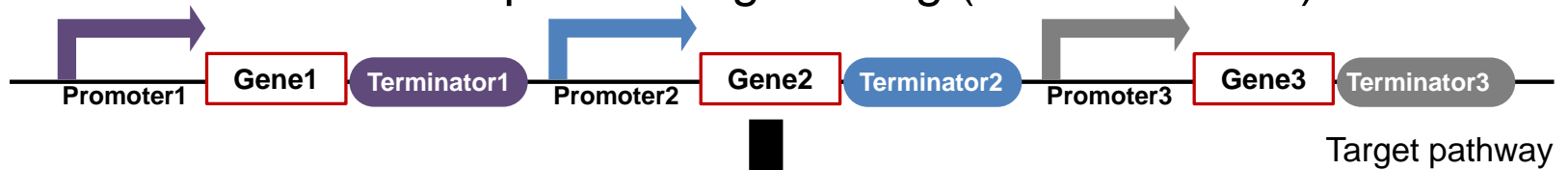
Warner et al., Nature Biotechnology 28, 856 (2010)
 Wang et al., Nature 460, 894 (2009)
 Salis et al., Nat Biotechnol 27, 946 (2009)
 Alper et al., PNAS 102, 12678 (2005)

Pfleger et al., Nat Biotechnol 24, 1027 (2006)
 Dueber et al., Nat Biotechnol 27, 753 (2009)
 Alper et al., Metab Eng 9, 258 (2007)
 Warnecke et al., Metab Eng 12, 241 (2010)

Pathway Optimization by COMPACTER



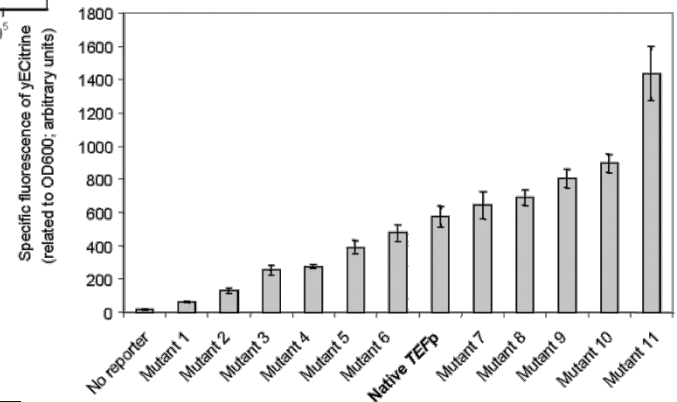
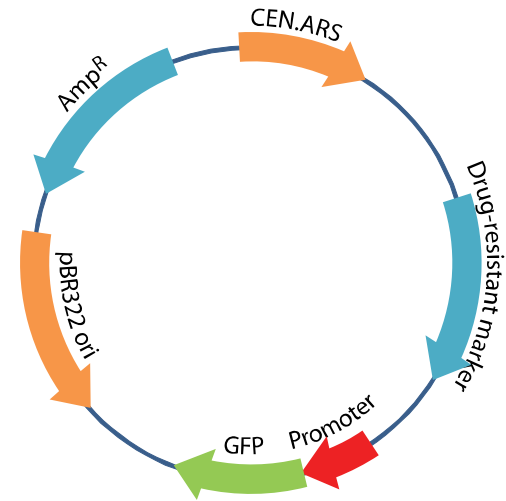
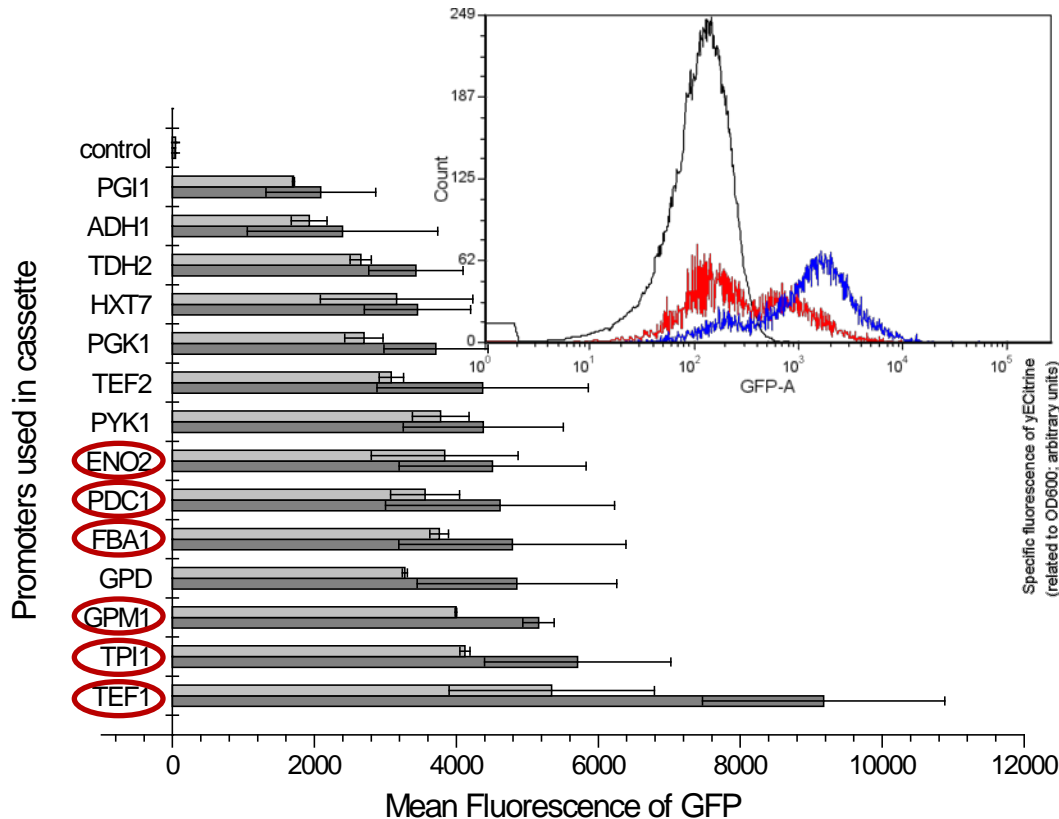
Customized Optimization of Metabolic Pathways by Combinatorial Transcriptional Engineering (COMPACTER)



Promoter Mutants with Varying Strength



- Selected 6 yeast promoters
- Nucleotide analogue mutagenesis
- Isolating promoter mutants via FACS

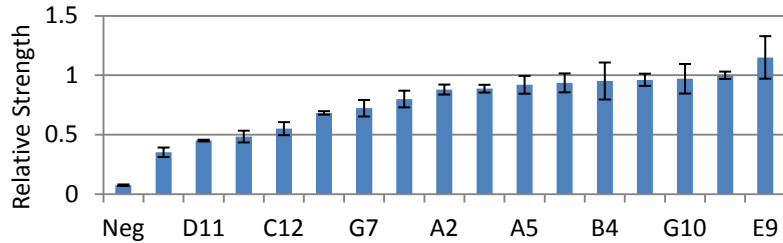


Alper et al. *PNAS* (2005)
Sun et al. *B&B* (2012)

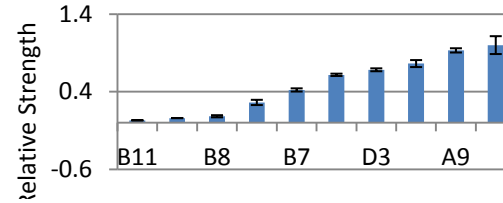
Promoter Mutants with Varying Strength



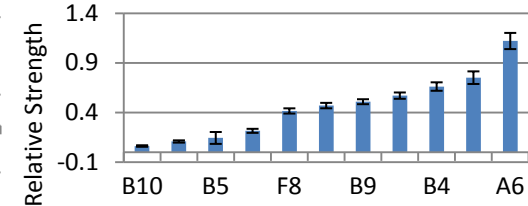
TEF1p Mutants



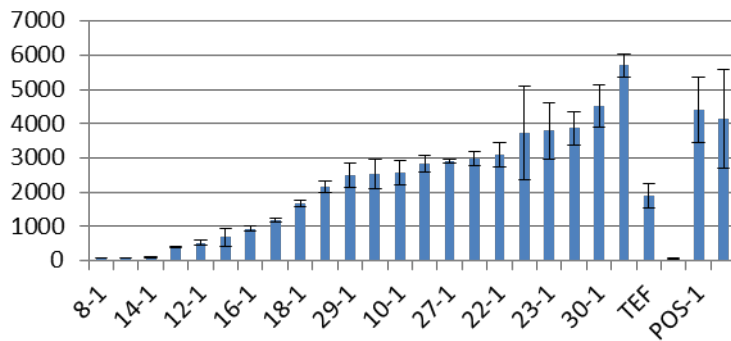
PDC1p mutants



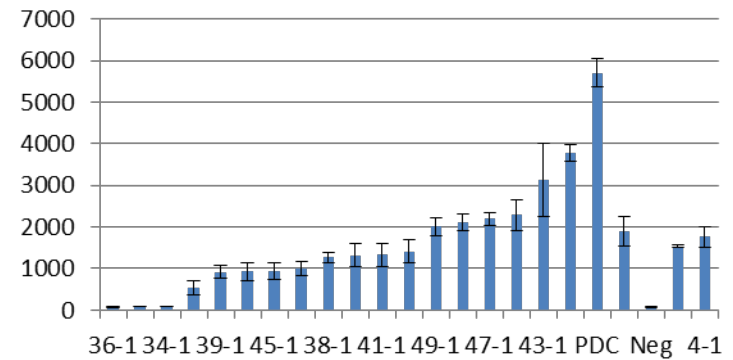
ENO2p mutants



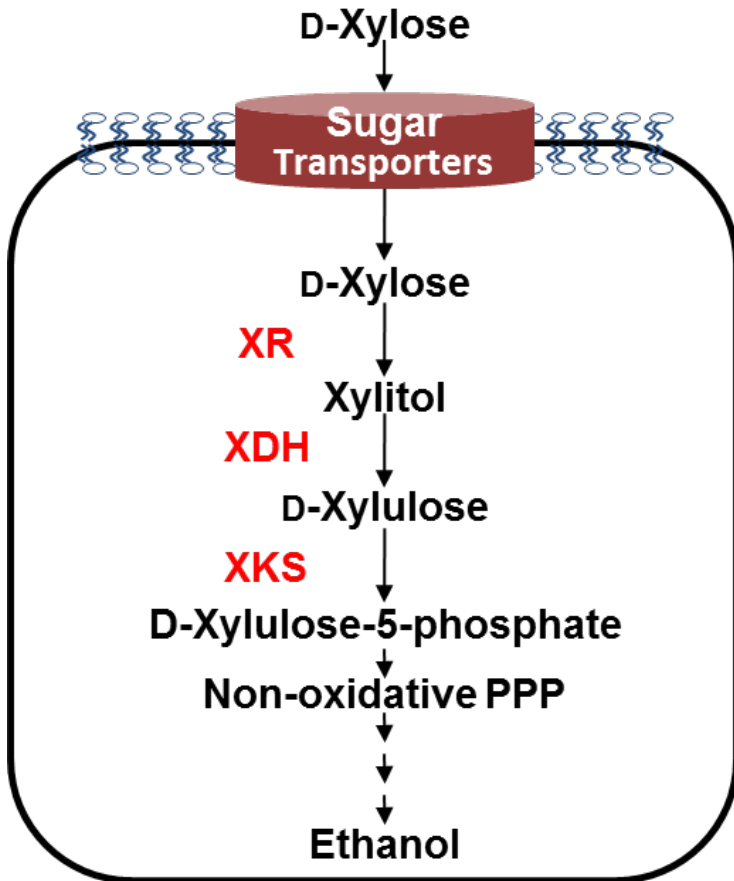
FBA mutants



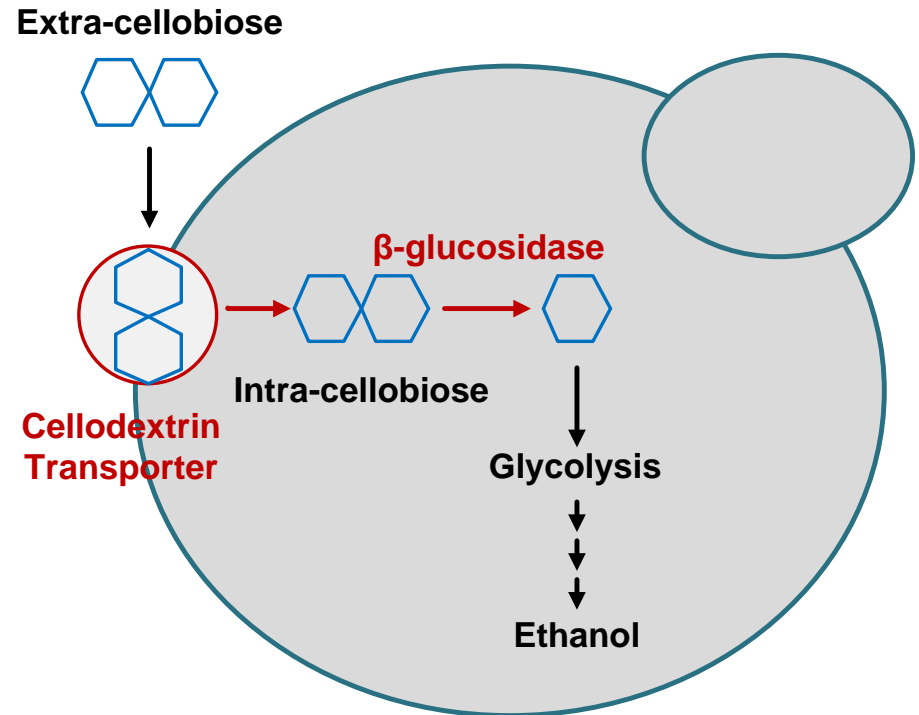
GPM mutants



Pathway Optimization by COMPACTER



Xylose Utilizing Pathway



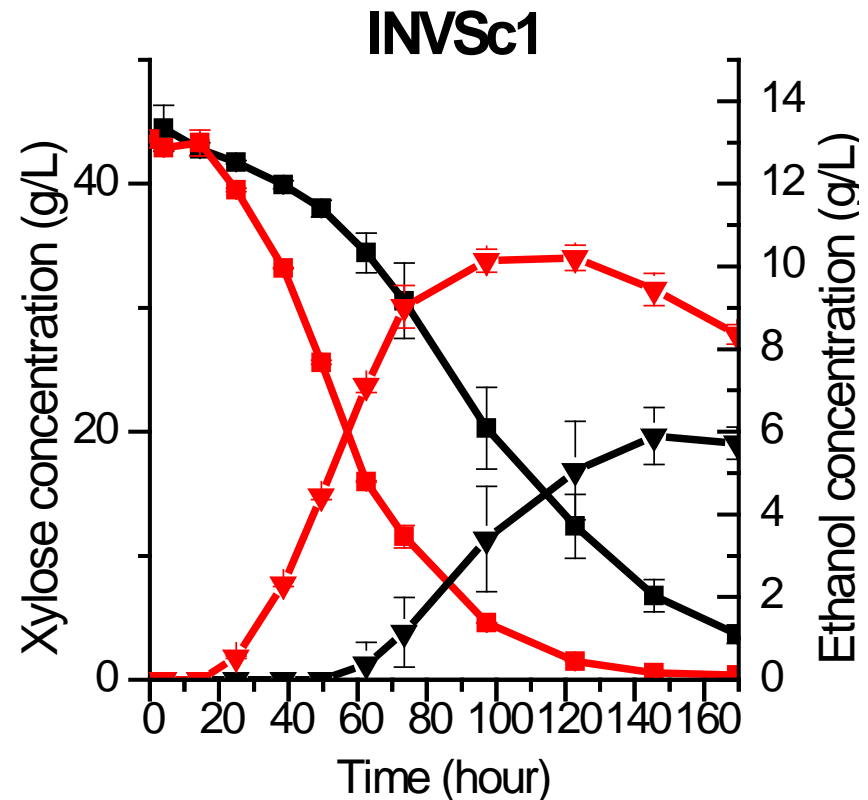
Cellobiose Utilizing Pathway

Optimization of the Xylose Utilizing Pathway in the INVSc1 Strain



- Host strain: INVSc1 (Invitrogen)
 - Diploid, auxotrophic mutation available
- Control
 - pRS416-PDC1p(WT)-csXR-TEF1p(WT)-ctXDH-ENO2p(WT)-ppXKS
- Backbone: pRS416
 - Single copy shuttle vector
- Library size: $10^4 \sim 10^5$
- Fermentation:
 - Initial OD~1
 - Oxygen limited condition
 - YP media

	WT	S3	Unit
Xylose consumption rate	0.24	0.40	g/L/hr
Ethanol production rate	0.04	0.10	g/L/hr
Ethanol yield	0.16	0.25	g/g xylose

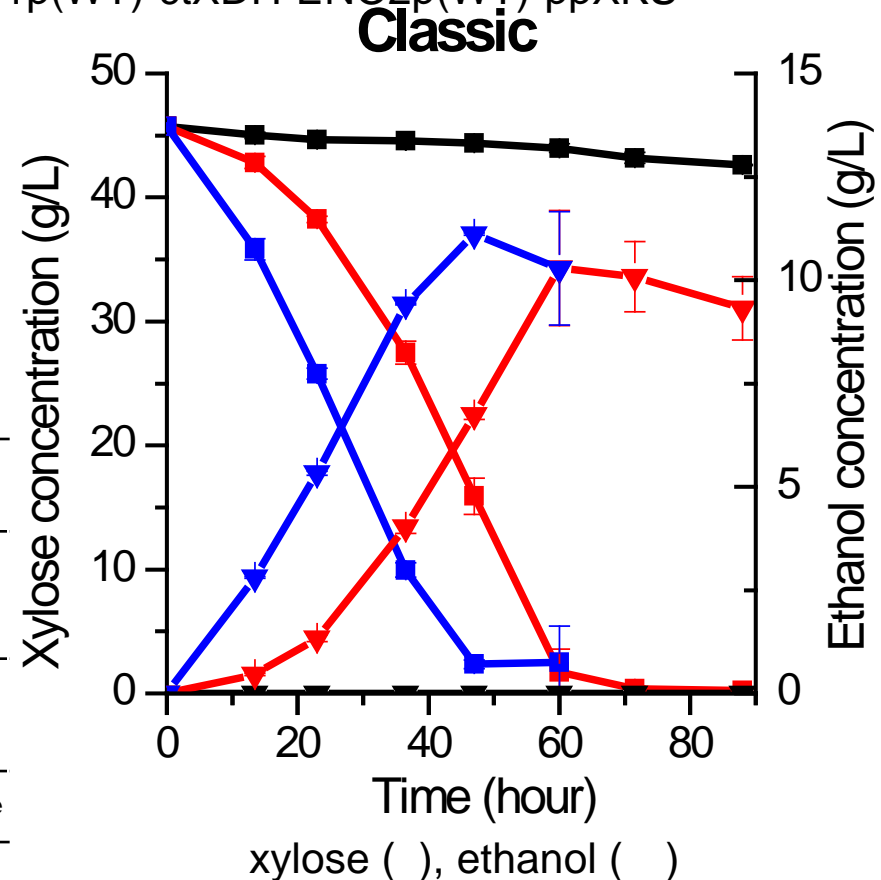


Optimization of the Xylose Utilizing Pathway in an Industrial Strain



- Host Strain
 - Still Spirits (Classic) Turbo Distiller's Yeast
- Control
 - pRS-KanMX-PDC1p(WT)-csXR-TEF1p(WT)-ctXDH-ENO2p(WT)-ppXKS
- Backbone:pRS-KanMX
 - Single copy shuttle vector
- Library size: $10^3 \sim 10^4$
- Fermentation:
 - Initial OD~10
 - Oxygen limited condition
 - YP media

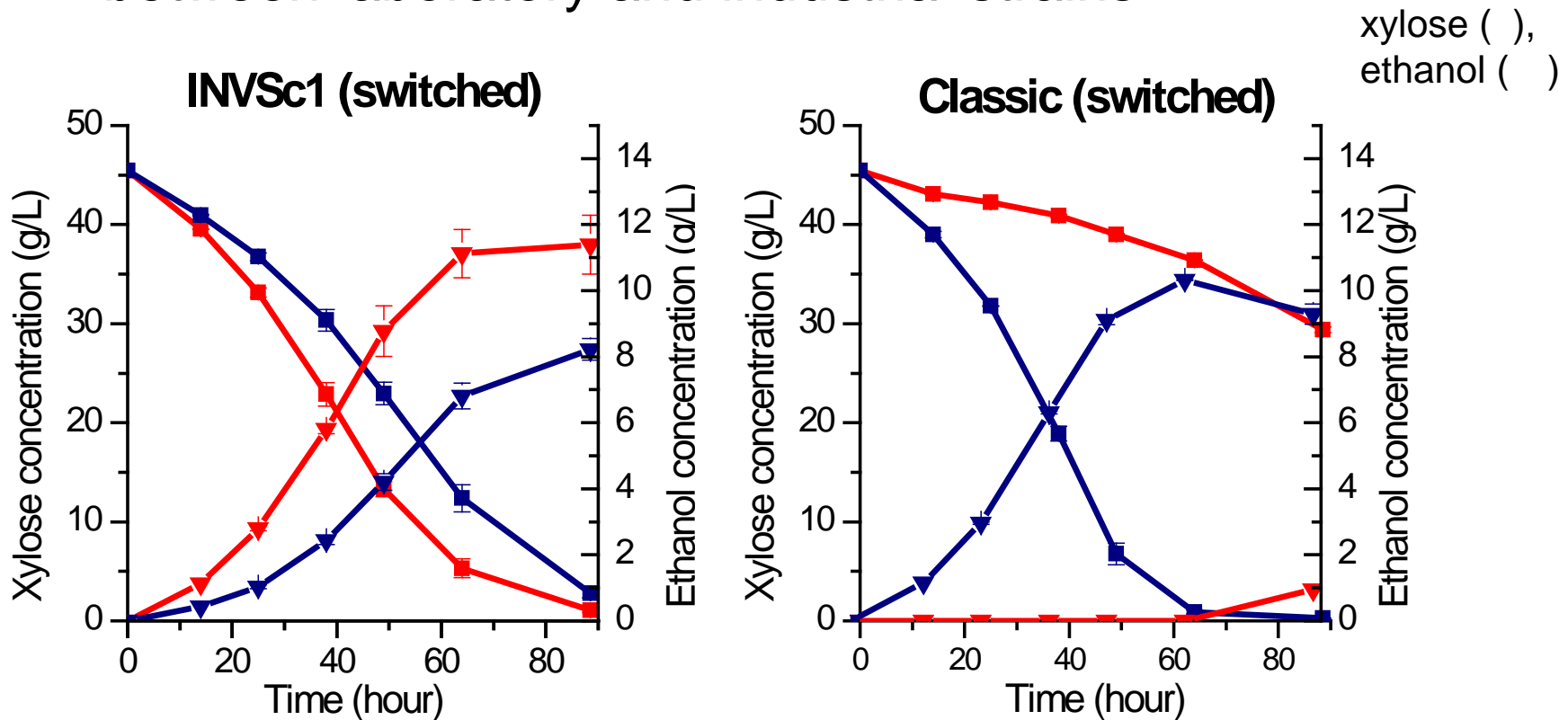
	YPD seed		YPX seed	Unit
	Classic WT	Classic S7	Classic S7	
Xylose consumption rate	0.06	0.74	0.92	g/L/hr
Ethanol production rate	0	0.17	0.24	g/L/hr
Ethanol yield	0	0.24	0.26	g/g xylose



Host-specific Pathway Optimization

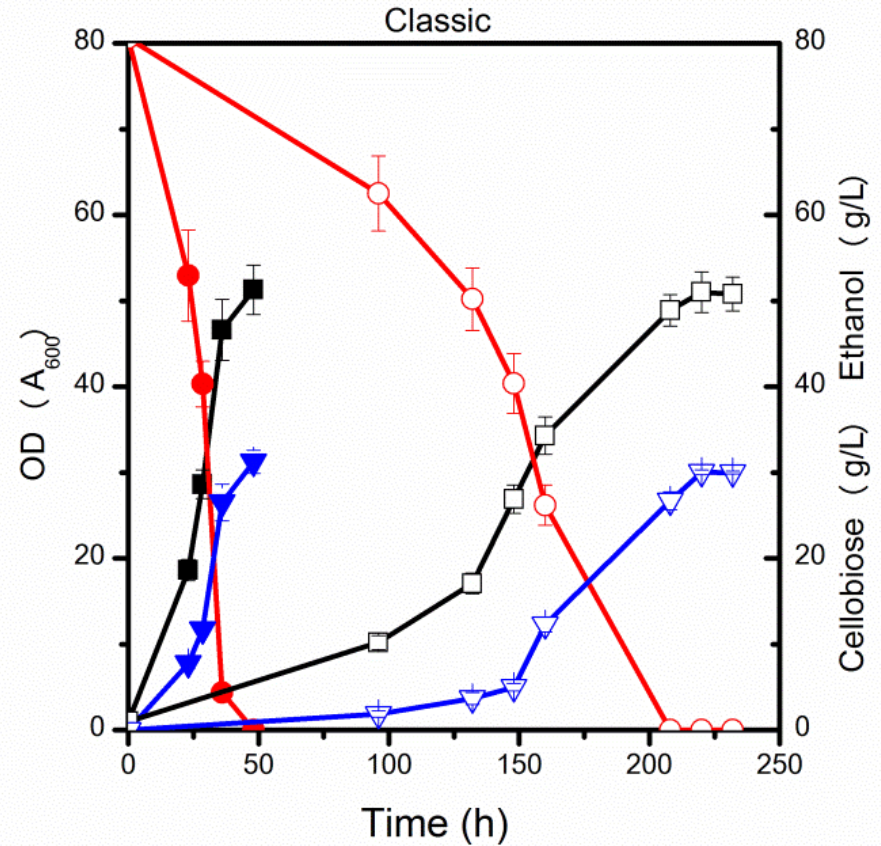
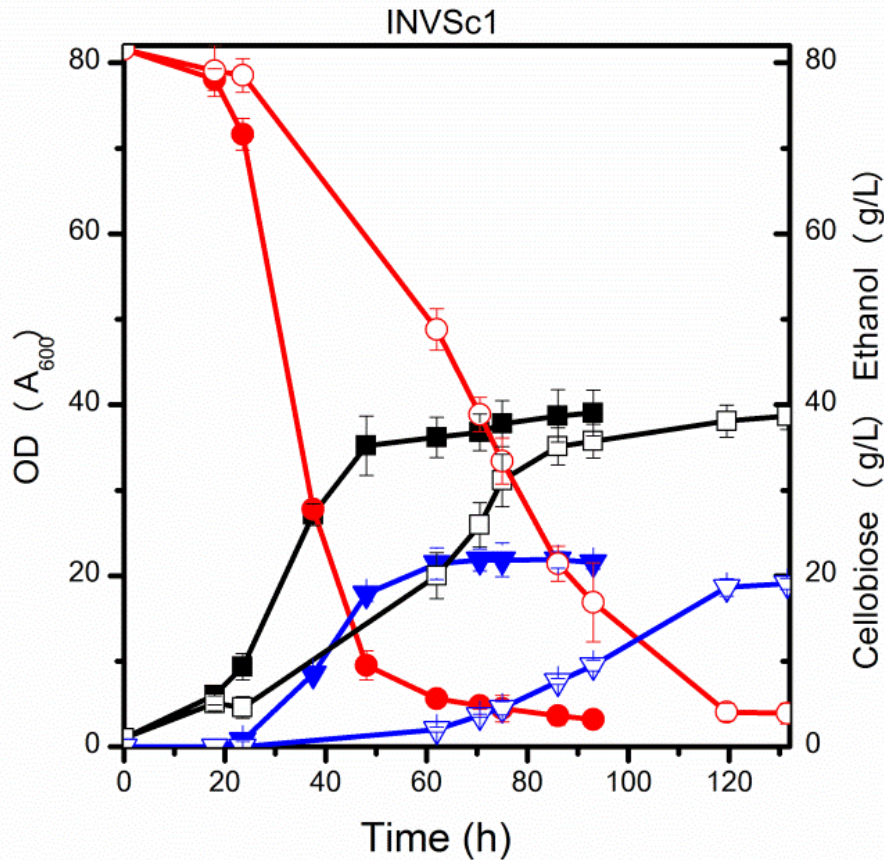


- Switching optimized xylose utilizing pathways between laboratory and industrial strains

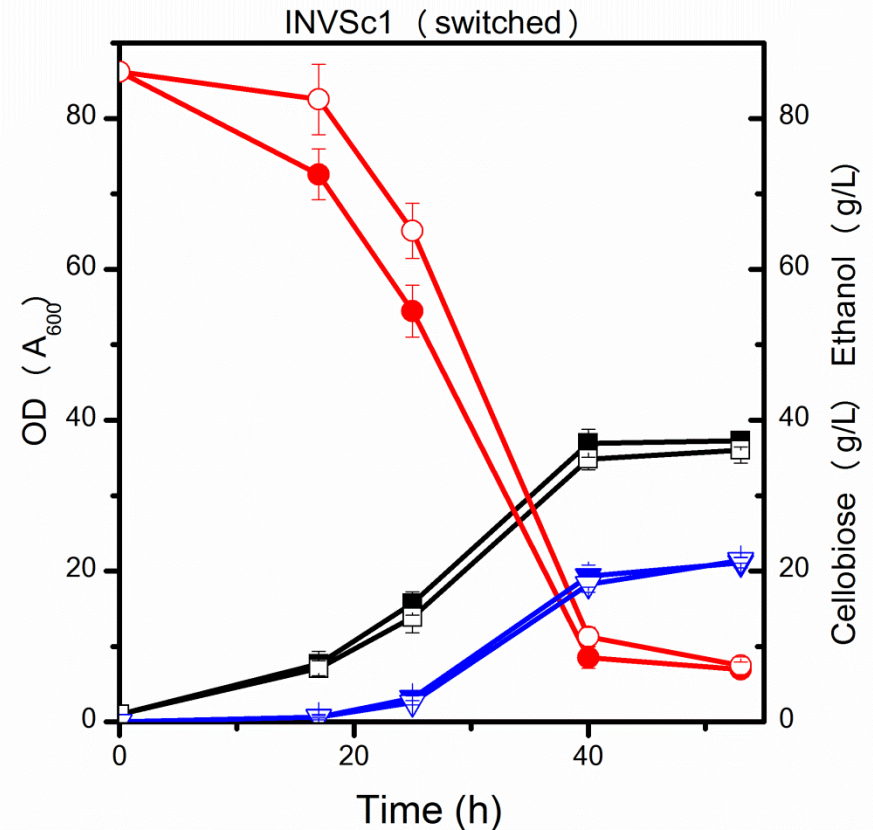
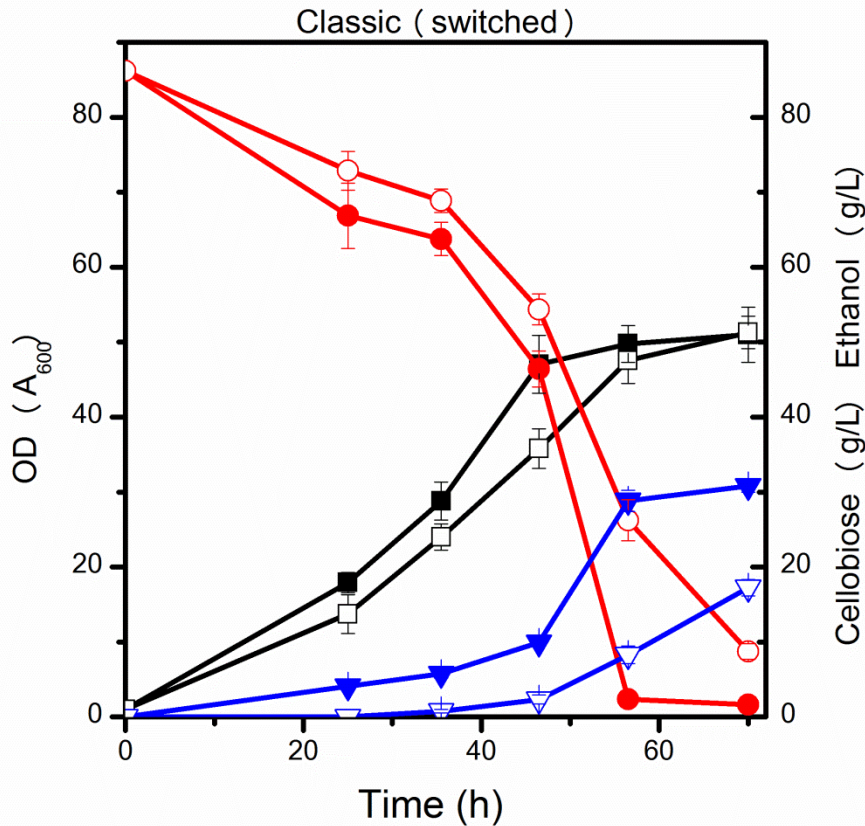


This finding highlighted one of the biggest challenges in synthetic biology: the **context-dependence** issue.

Optimization of the Cellobiose Utilizing Pathway

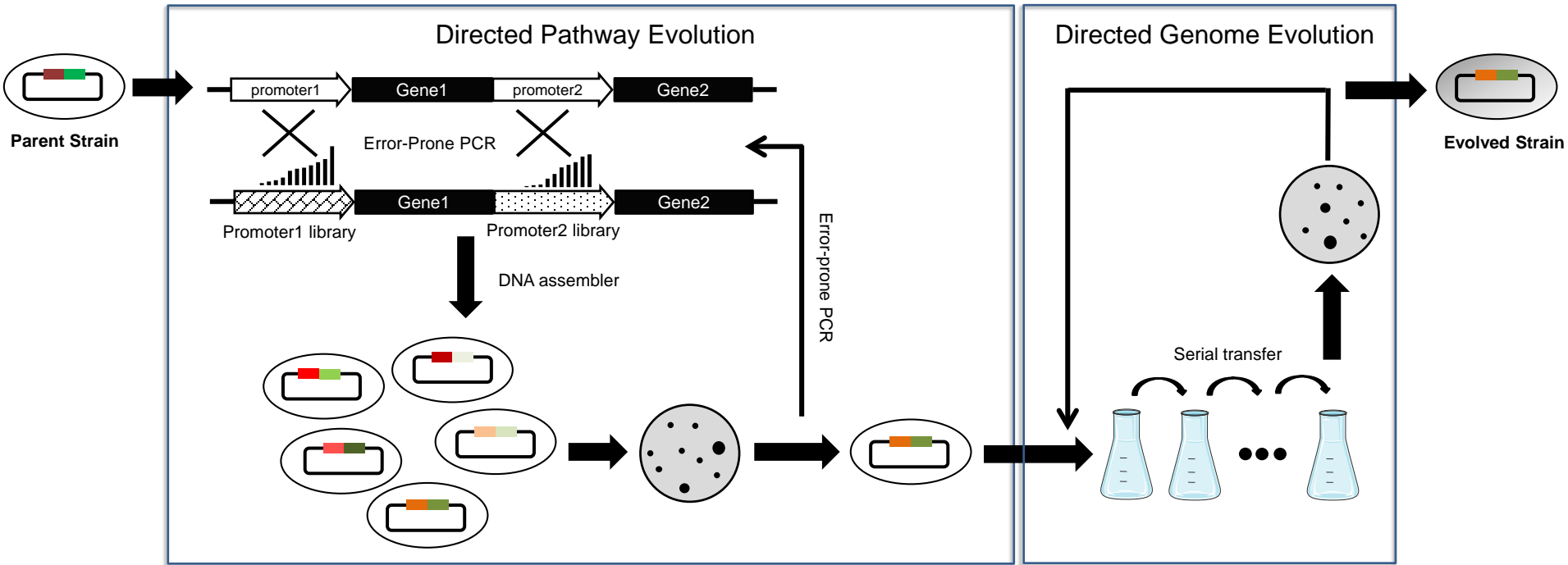


Optimized Xylose Utilizing Pathways are Strain Specific

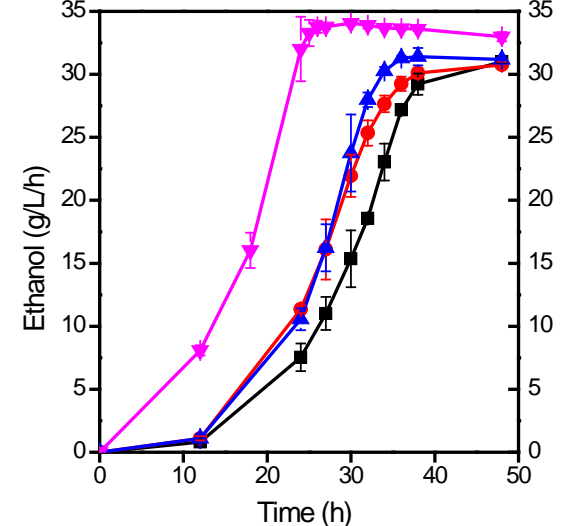
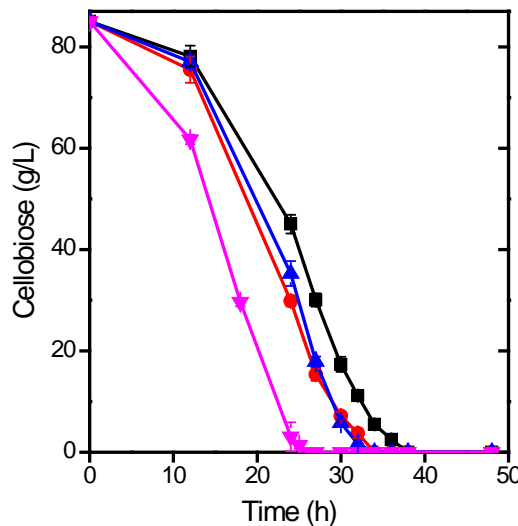
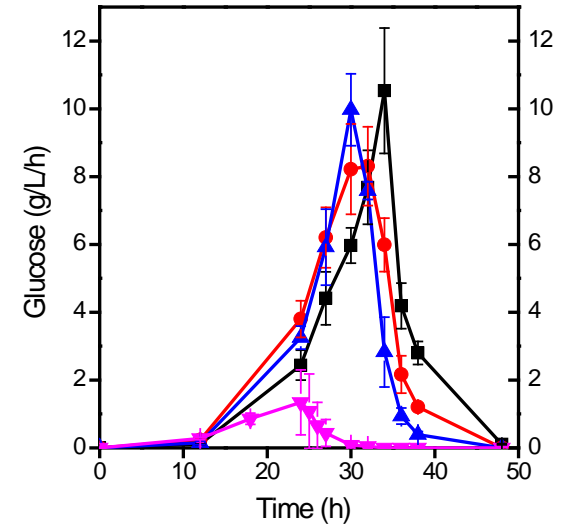
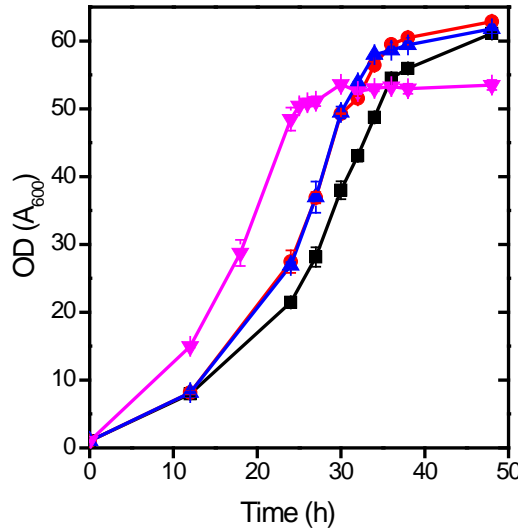
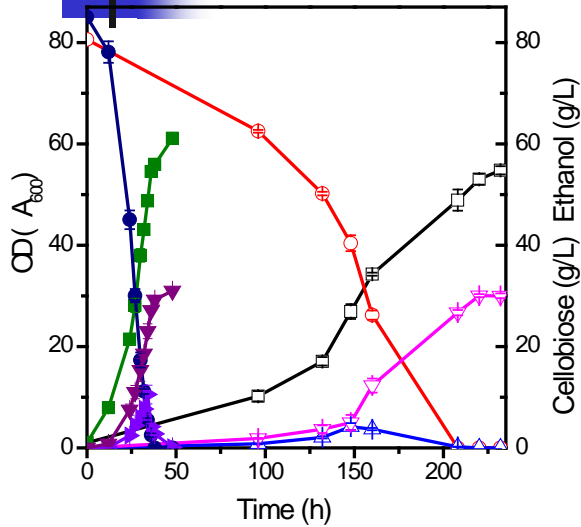


Open symbol: pathway optimized in INVSc1 strain, Solid symbol: pathway optimized in Classic strain, Red circle: cellobiose, Black square: OD (A_{600}), Blue down triangle: ethanol.

Directed Evolution for Strain Development



Directed Evolution for Strain Development



- #9, #91 and #9118 have same final OD, ethanol concentration and glucose accumulation
- A#9118 has lower OD and higher ethanol
- A#9118 has much lower glucose accumulation
- No mutations were found in promoter regions in A#9118

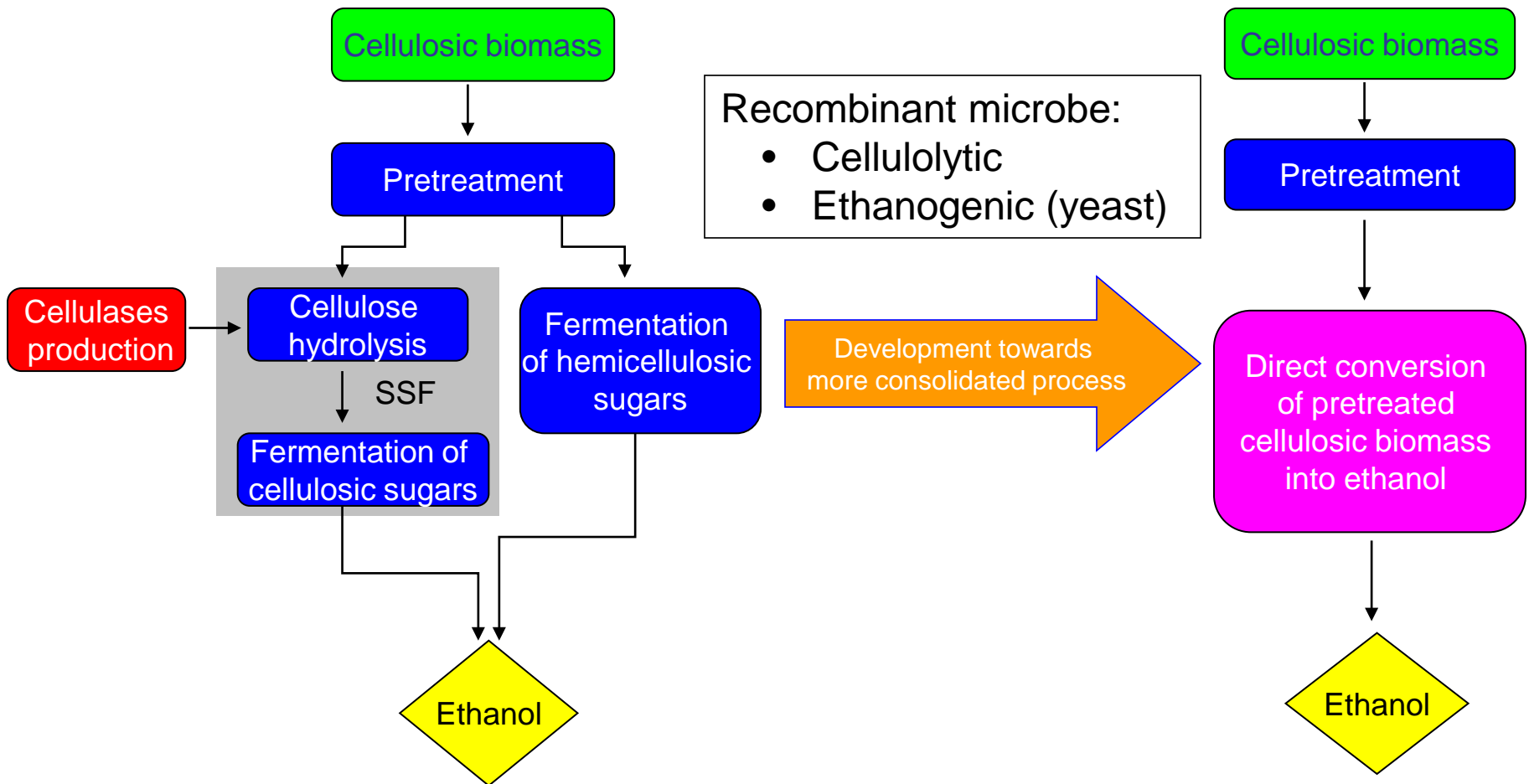
Directed Evolution for Strain Development



Cellobiose fermentation performance of evolved yeast strains #9, #9-1, #9-1-18 and A#9-1-18

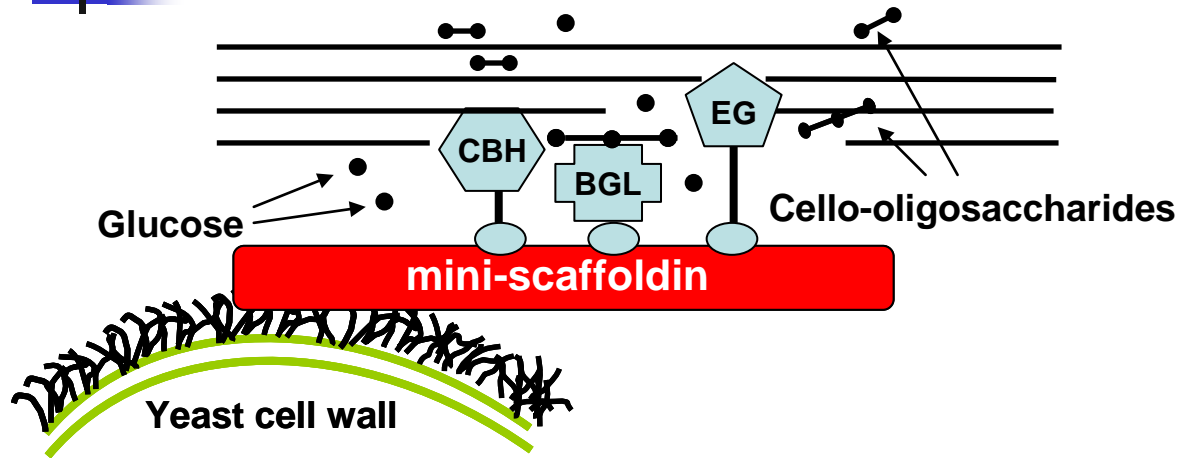
	WT	#9	#9-1	#9-1-18	A#9-1-18
Cellobiose consumption (g cellobiose/L/h)	0.388	2.24	2.5	2.5	3.27
Ethanol productivity (g ethanol/L/h)	0.137	0.77	0.81	0.89	1.30
Yield (g ethanol/g cellobiose)	0.373	0.36	0.36	0.37	0.40

Consolidated Bioprocessing (CBP)



- Consolidated bioprocessing (CBP): save ~10-20 cents/gallon of ethanol

Direct Conversion of Cellulose to Ethanol by Engineered Mini-cellulosomes



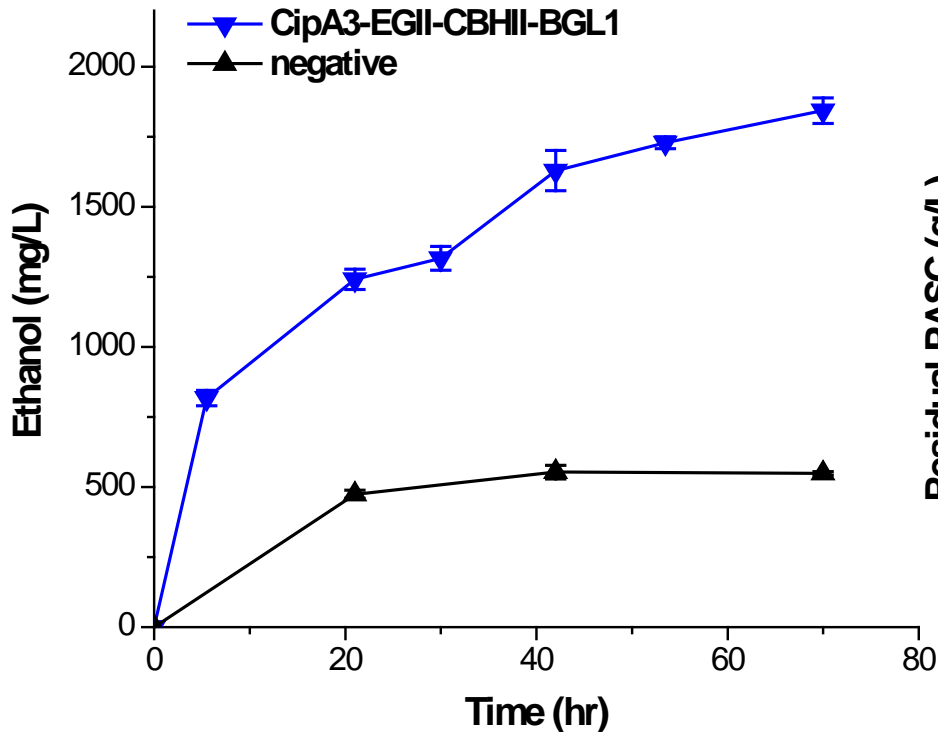
- EG: Endoglucanase
- CBH: Exoglucanase
- BG: β -glucosidase

- Yeast surface display of functional minicellulosomes
 - Functional display of a mini-scaffoldin
 - Successful assembly of minicellulosomes through cohesin-dockerin interaction
 - Synergistic hydrolysis of cellulose
 - Direct fermentation of hydrolysate (glucose) to ethanol

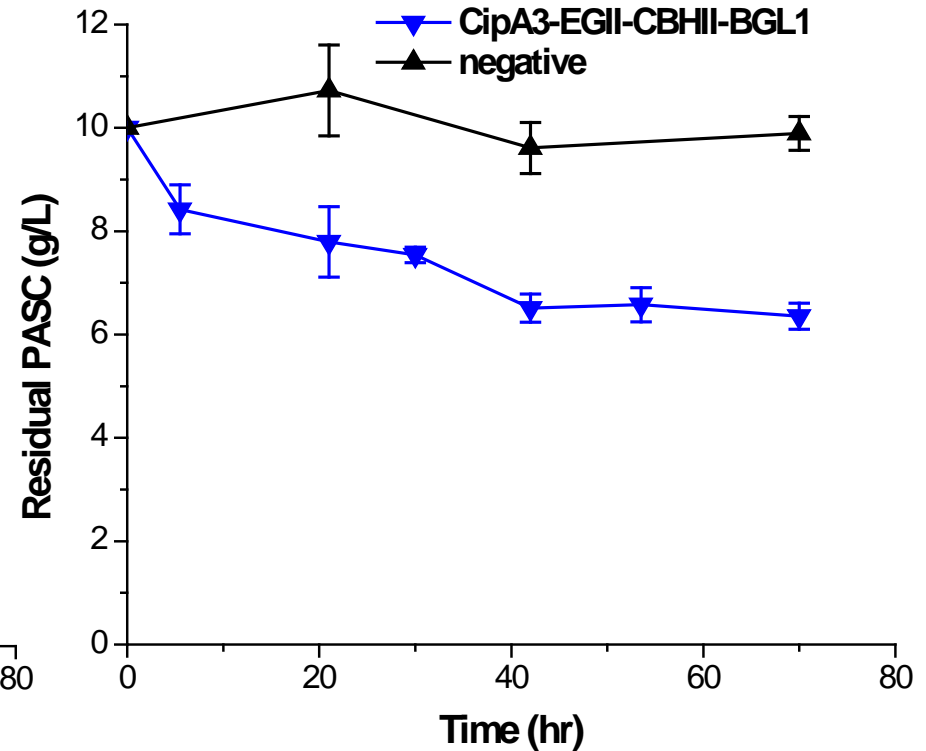
Direct Ethanol Production from PASC



Ethanol production



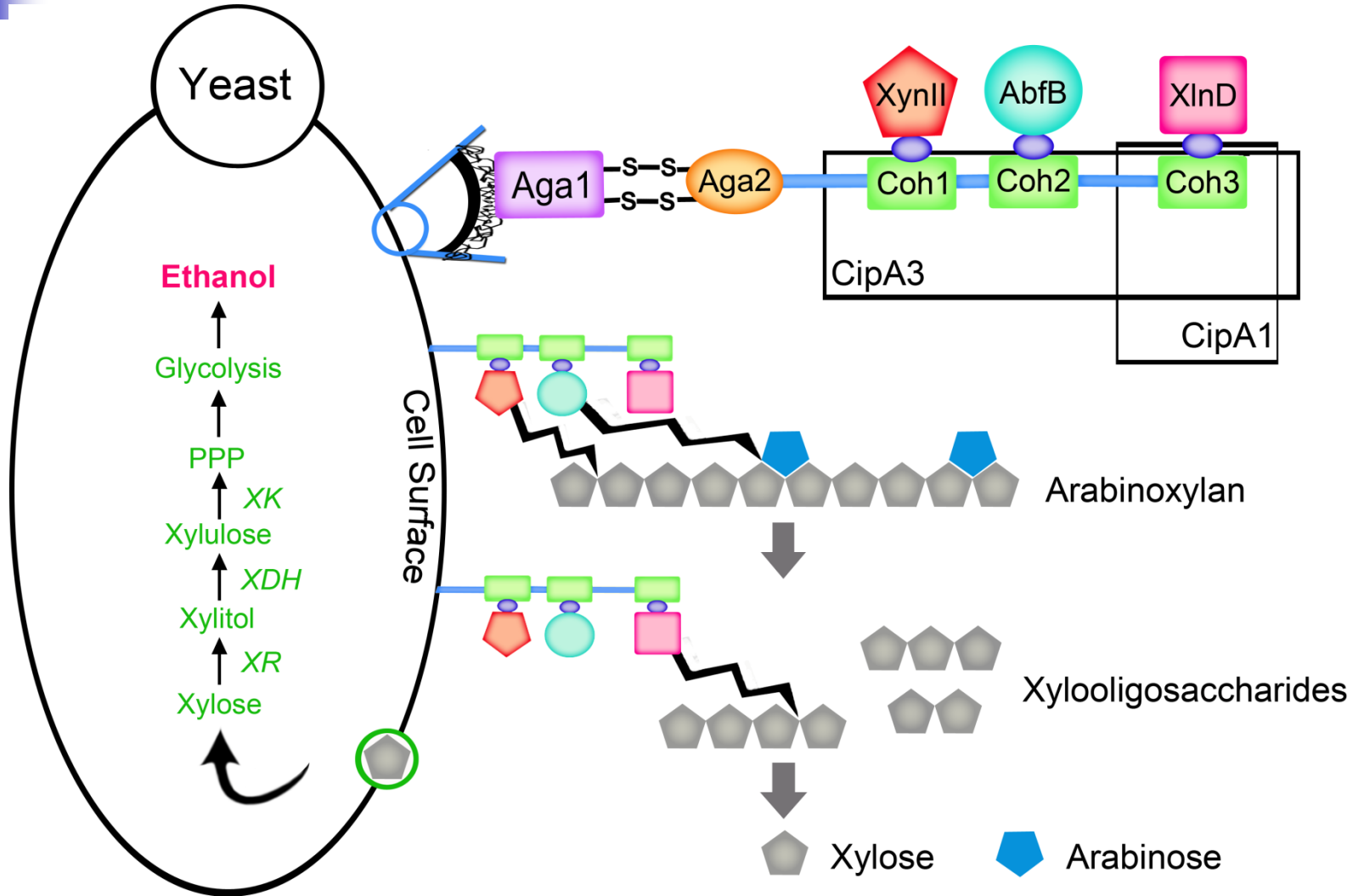
Residual PASC



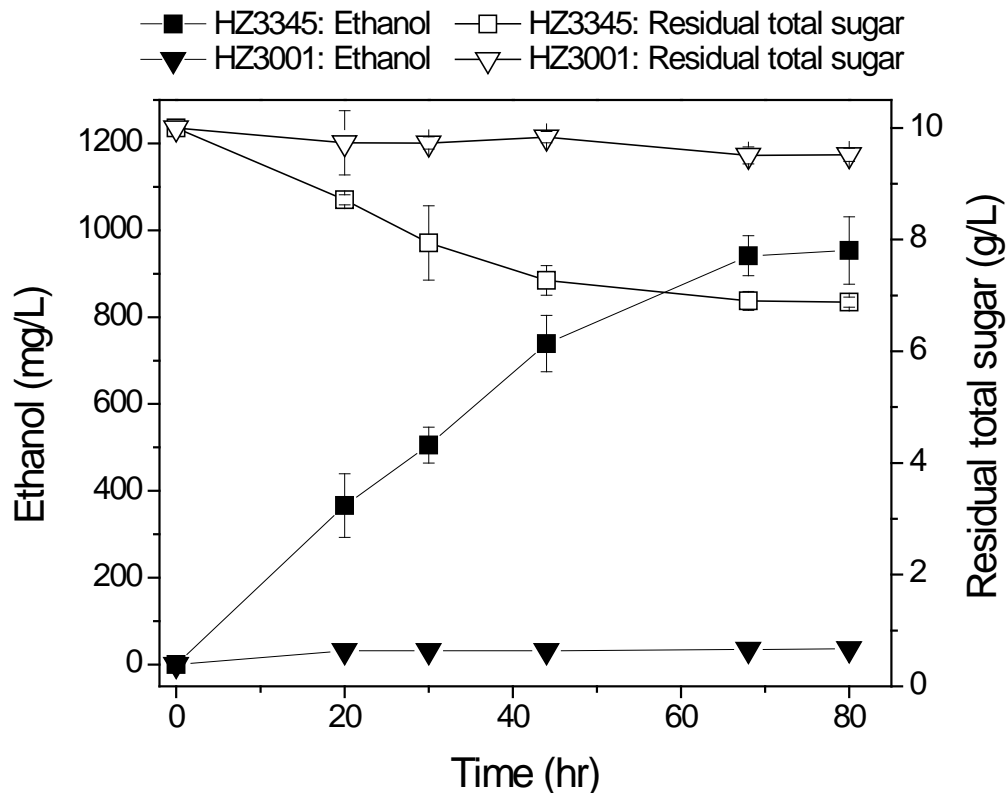
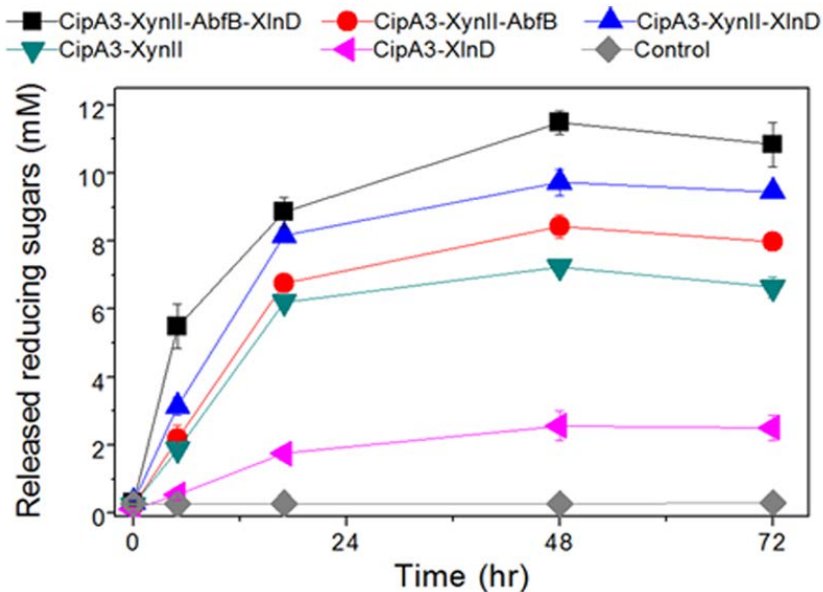
Yield: 0.31 grams of ethanol per gram of PASC

62% of theoretical yield

Direct Conversion of Xylan to Ethanol by Engineered Hemicellulosomes

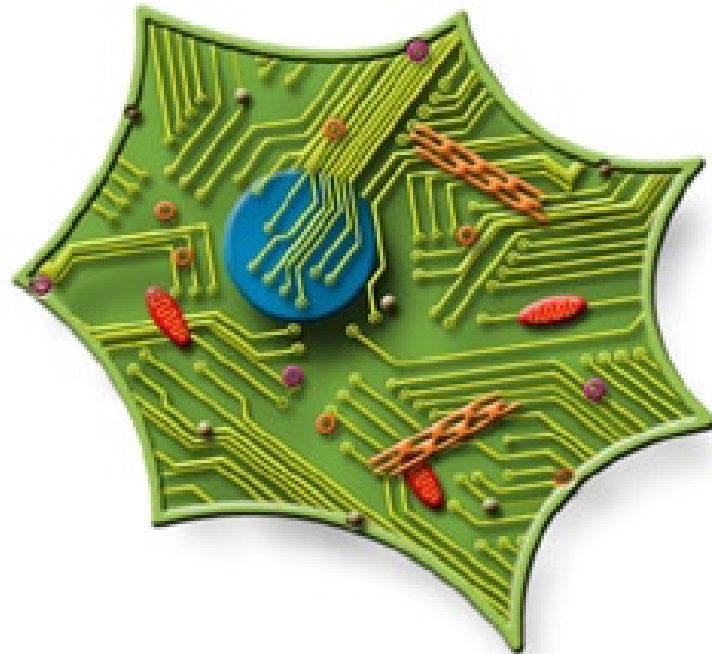


Direct Conversion of Xylan to Ethanol by Engineered Hemicellulosomes



Yield: 0.31 grams of ethanol per gram of birchwood xylan

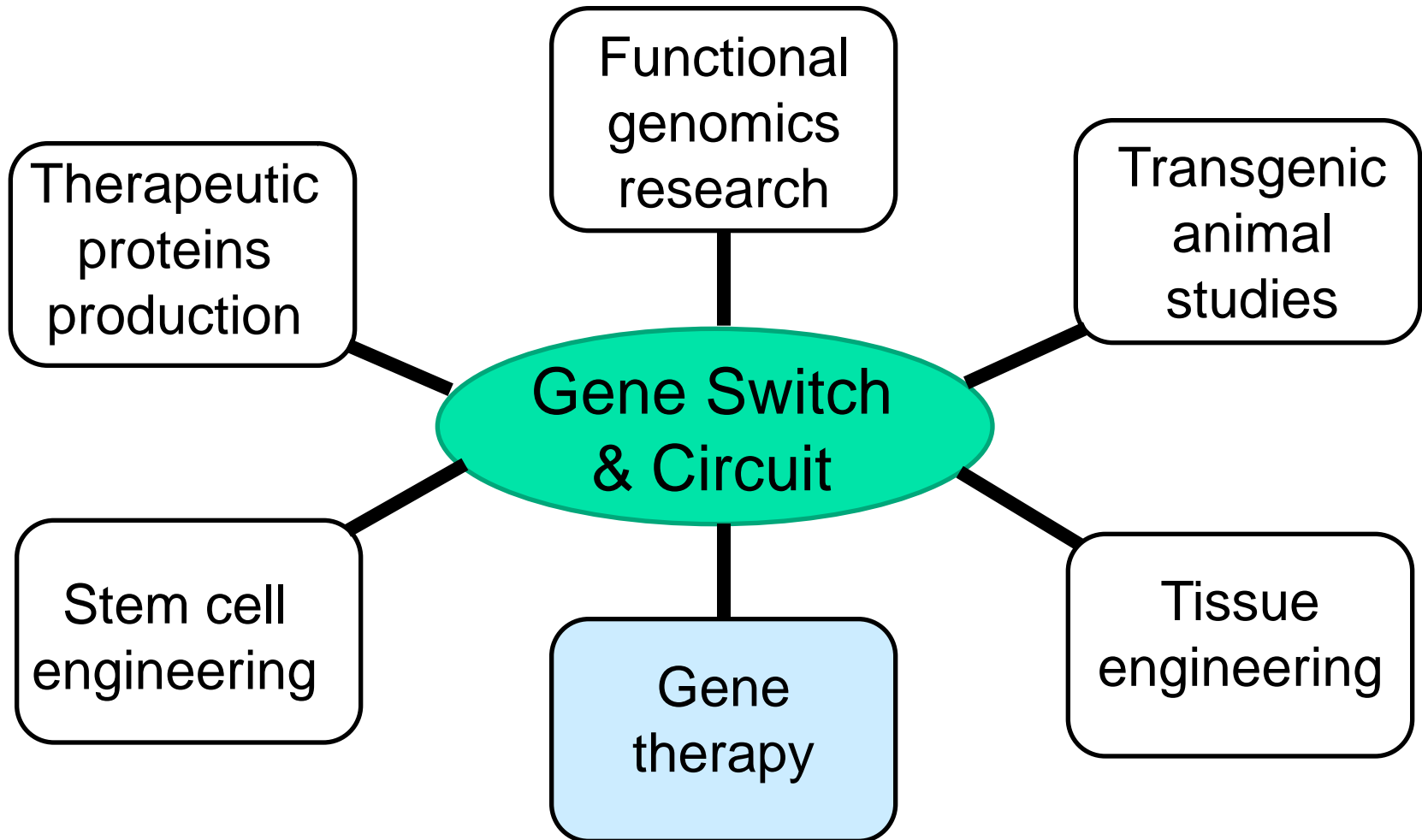
Engineering Gene Switches



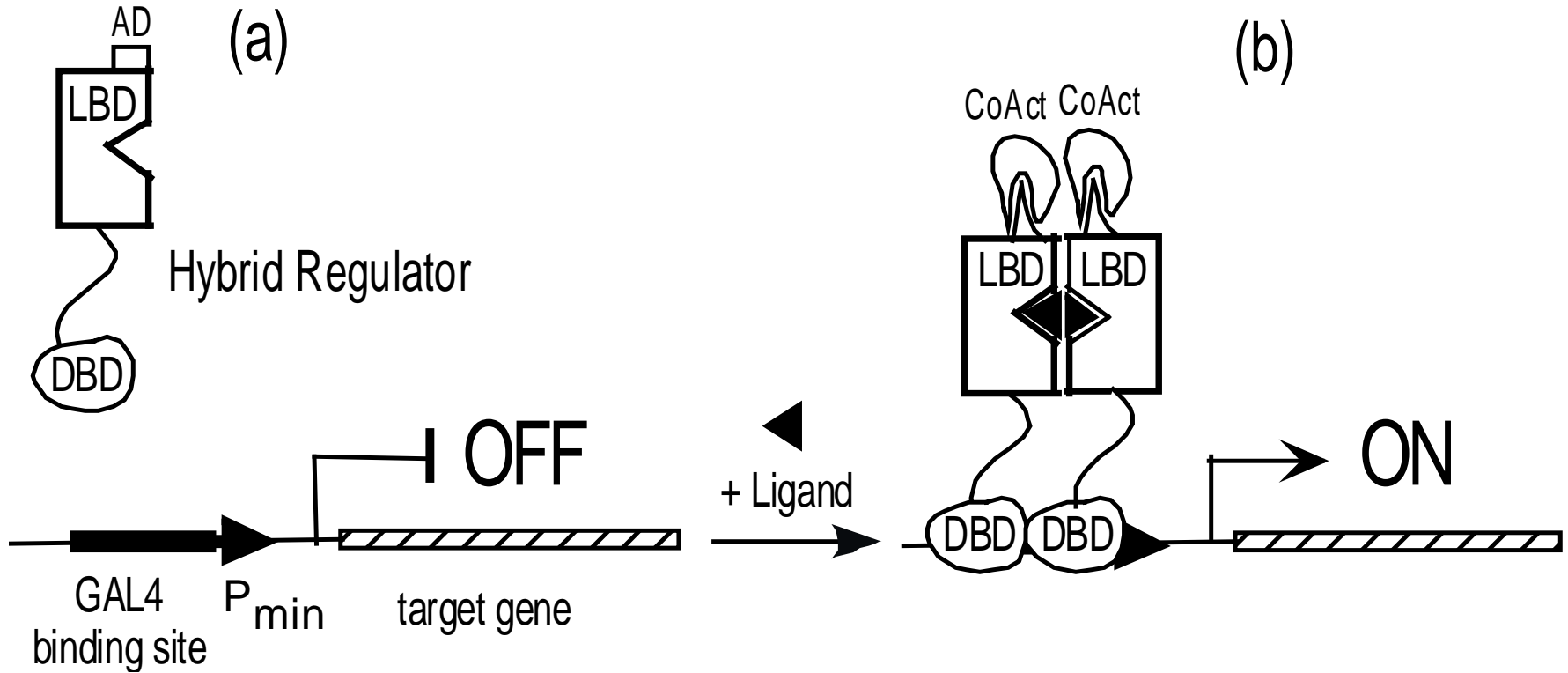
Motivation



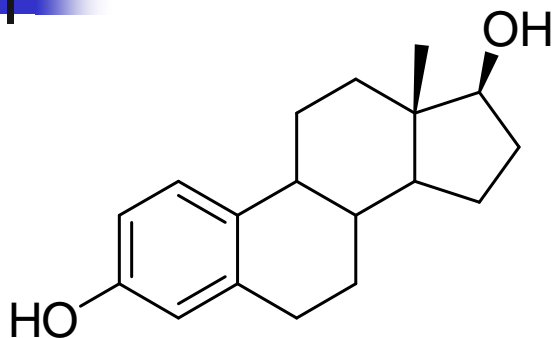
Precisely regulatable gene expression system (Gene Switch) in mammalian cells plays an important role in diverse fields



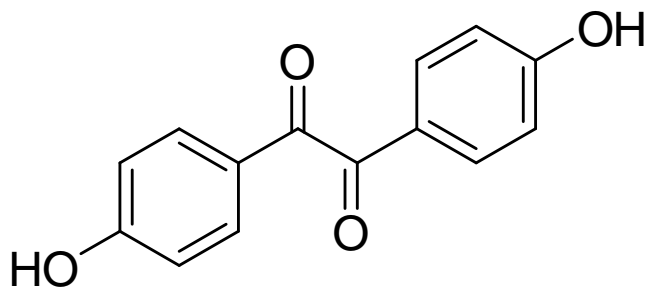
Engineering hER-based Gene Switches



Choice of Ligand for a Gene Switch

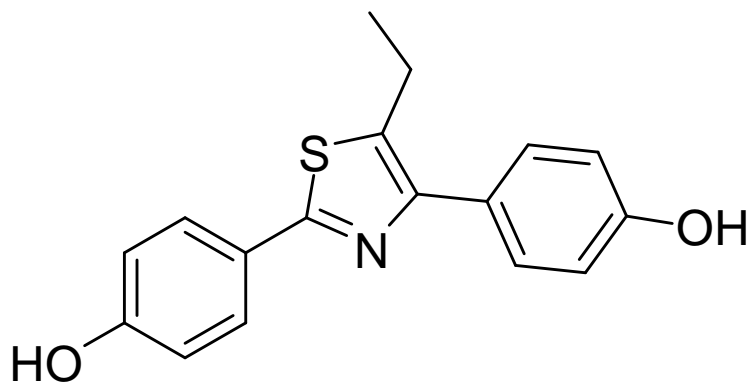


17 β -Estradiol (E2)
Natural ligand for wt hER α



4,4'-dihydroxybenzil (DHB)

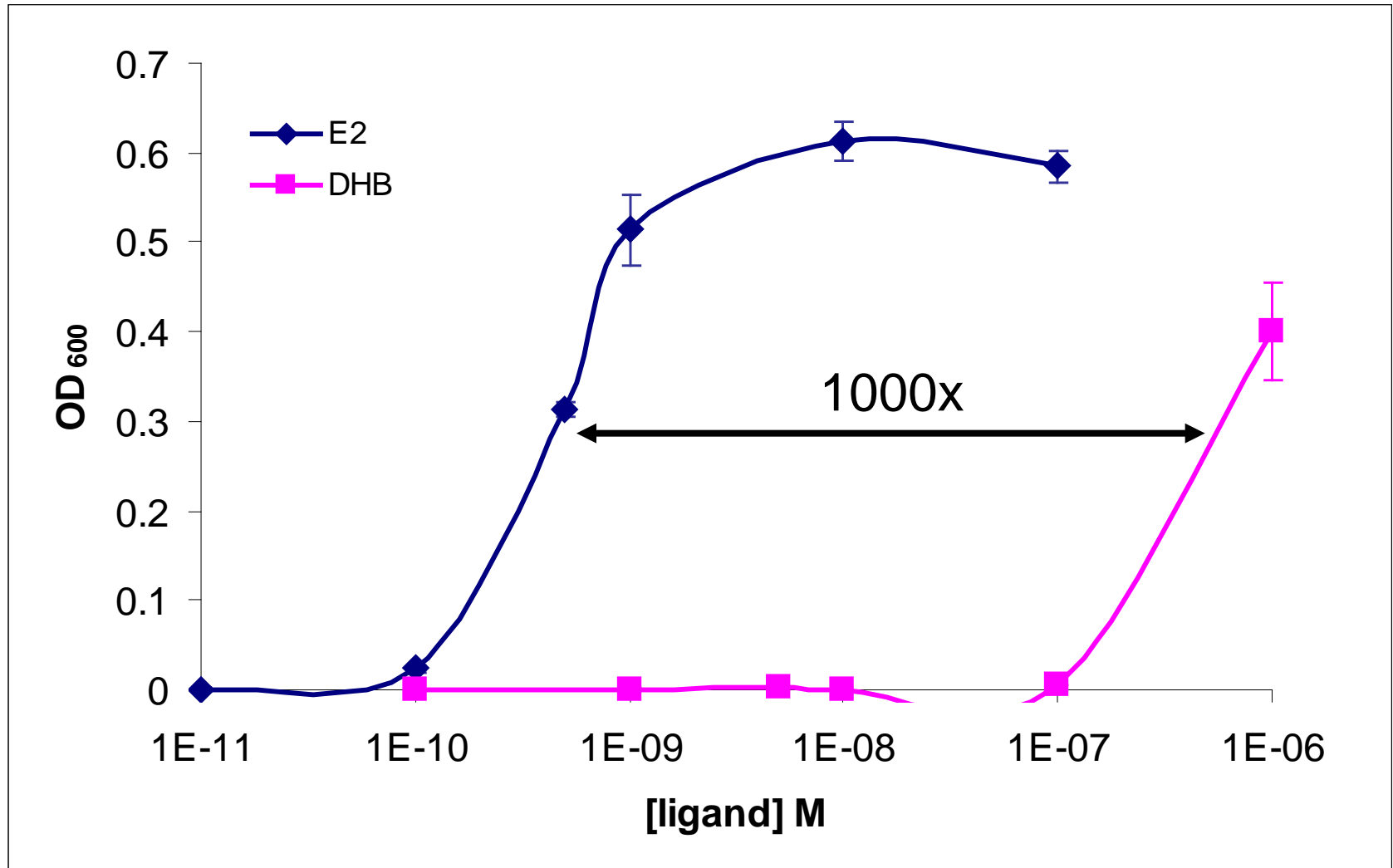
- Poor synthetic agonist of wt-ER α
- Non-toxic to mammalian cells



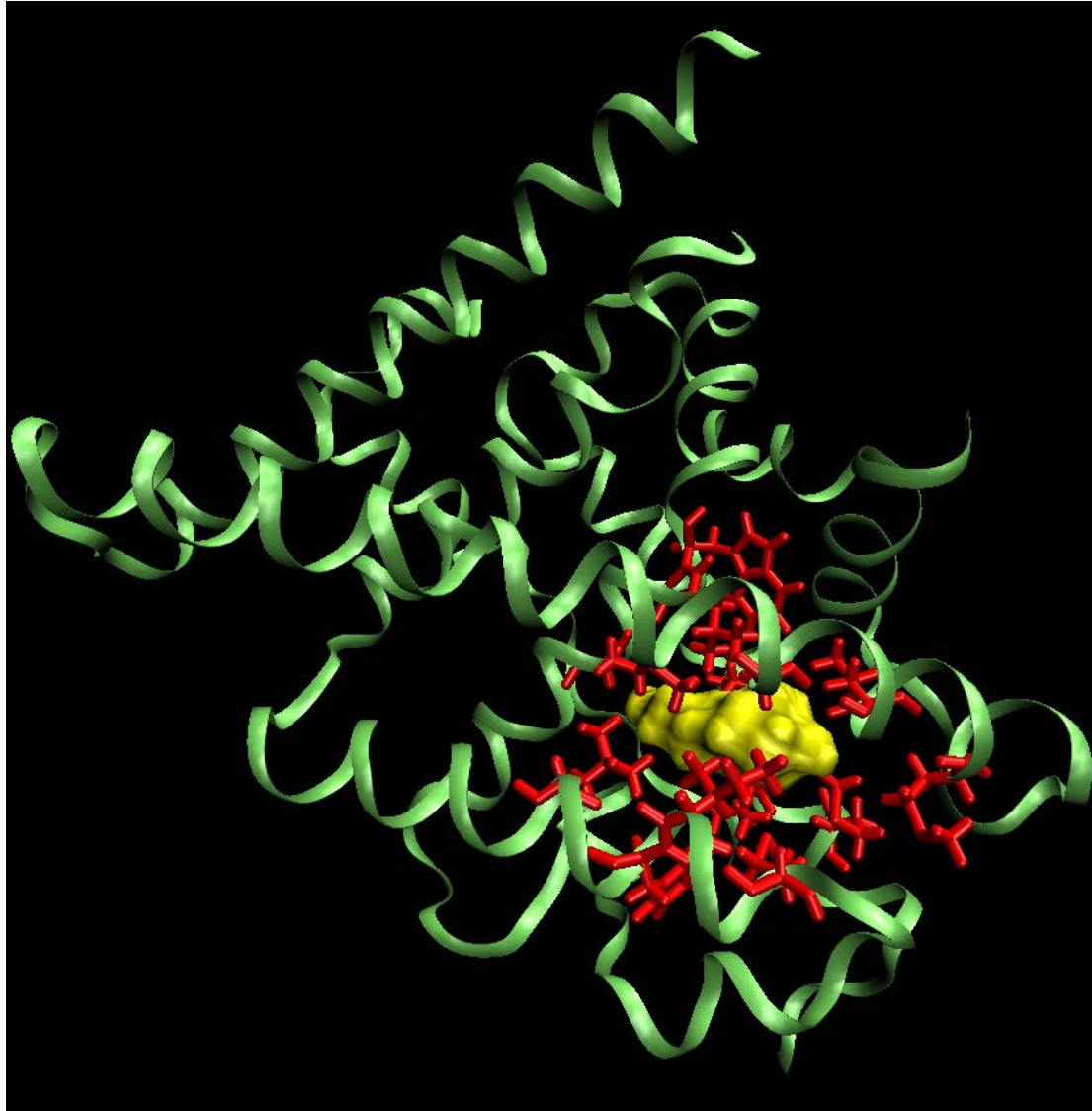
2,4-di(4-hydroxy-phenyl)-5-ethyl-thiazole (DHET, L9)

- Poor synthetic agonist of wt-ER α
- Low toxic to mammalian cells

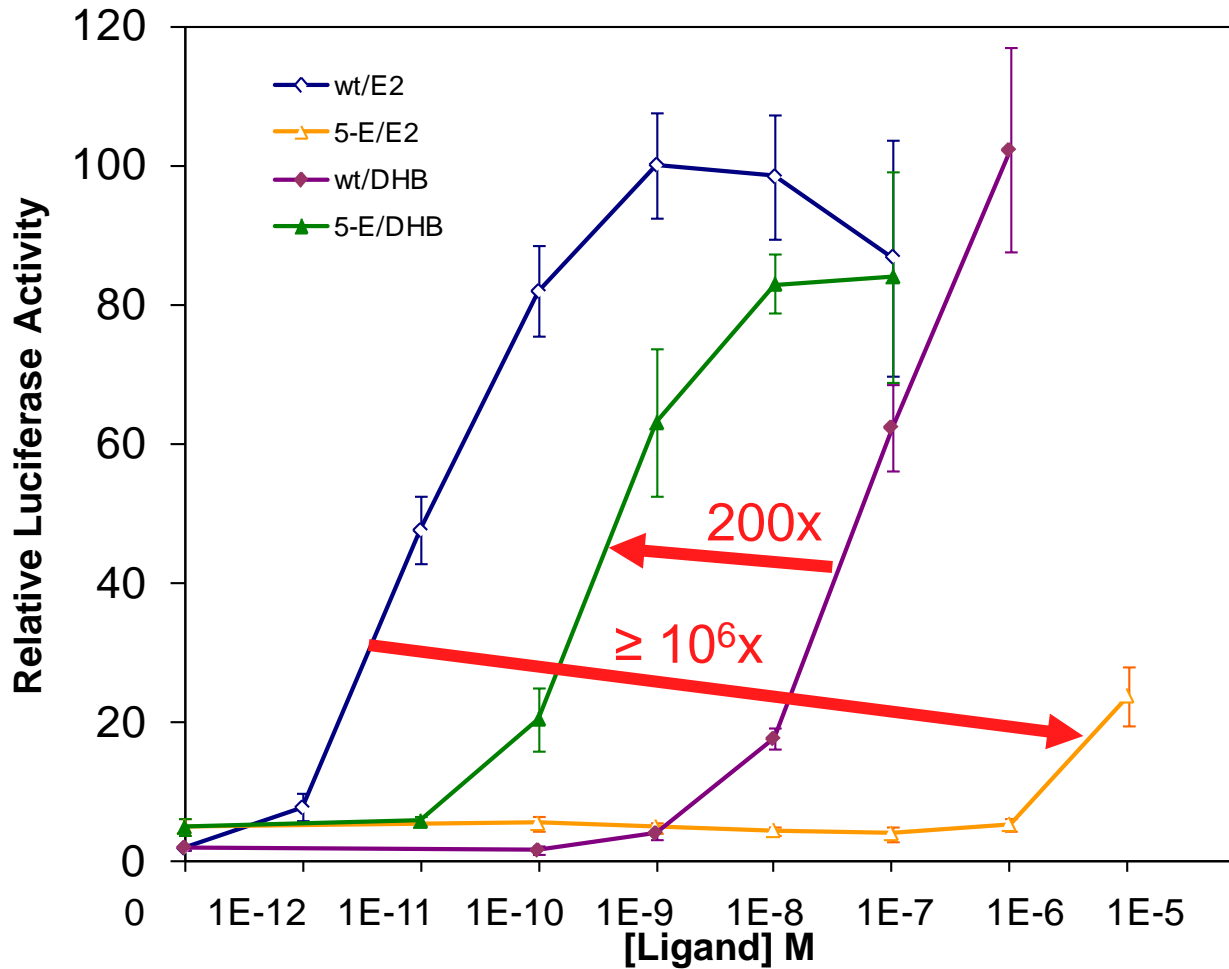
Dose Response of wt-ER to 17β -Estradiol and DHB



Directed Evolution Strategy – Sequential Saturation Mutagenesis



Dose Response in Mammalian Cells

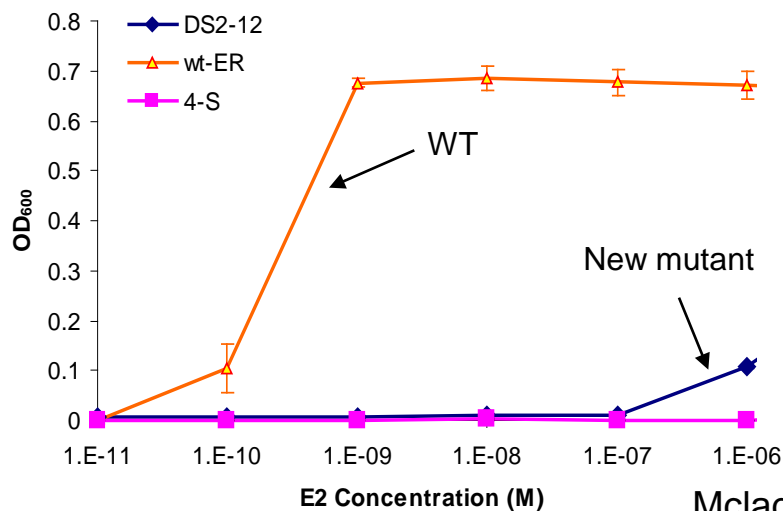
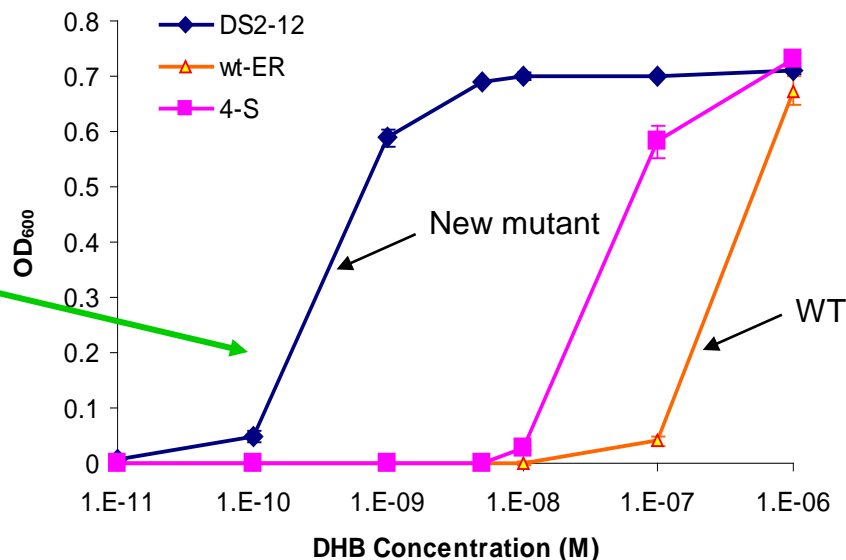


Net selectivity enhancement of $>10^8$ -fold

Complete Reversal of Ligand Selectivity

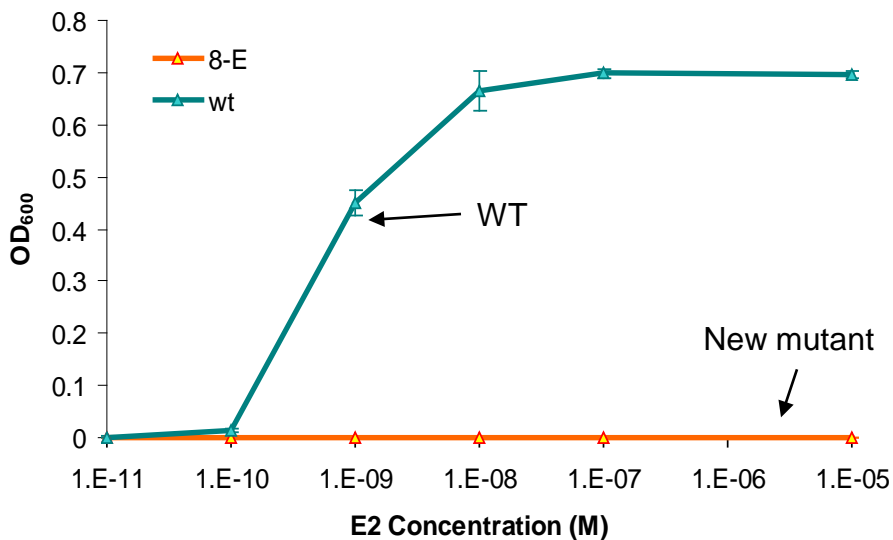
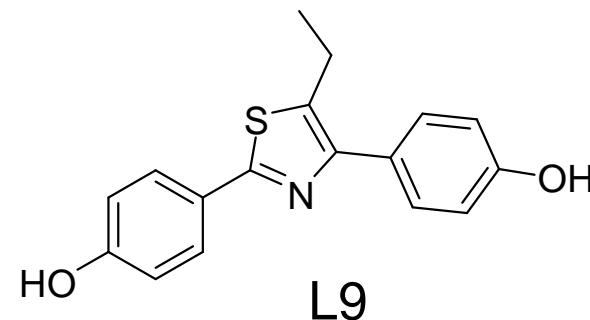
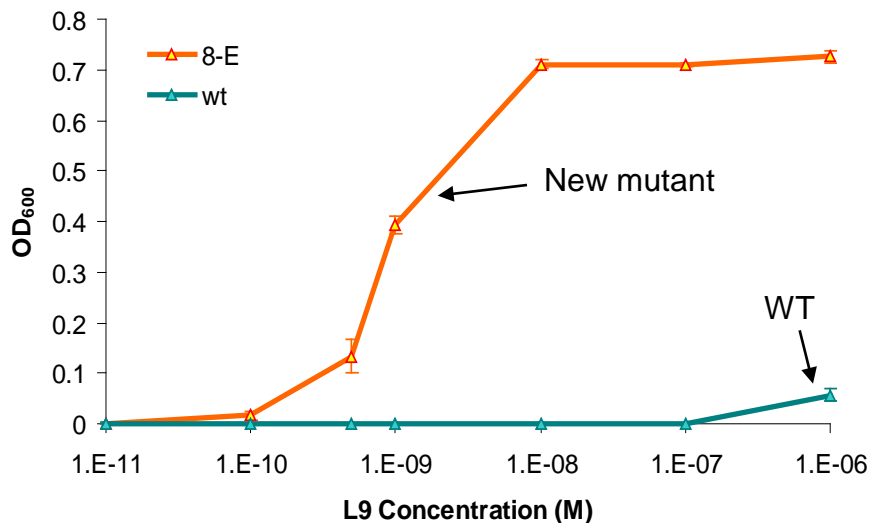


1.8-3.3 molecules



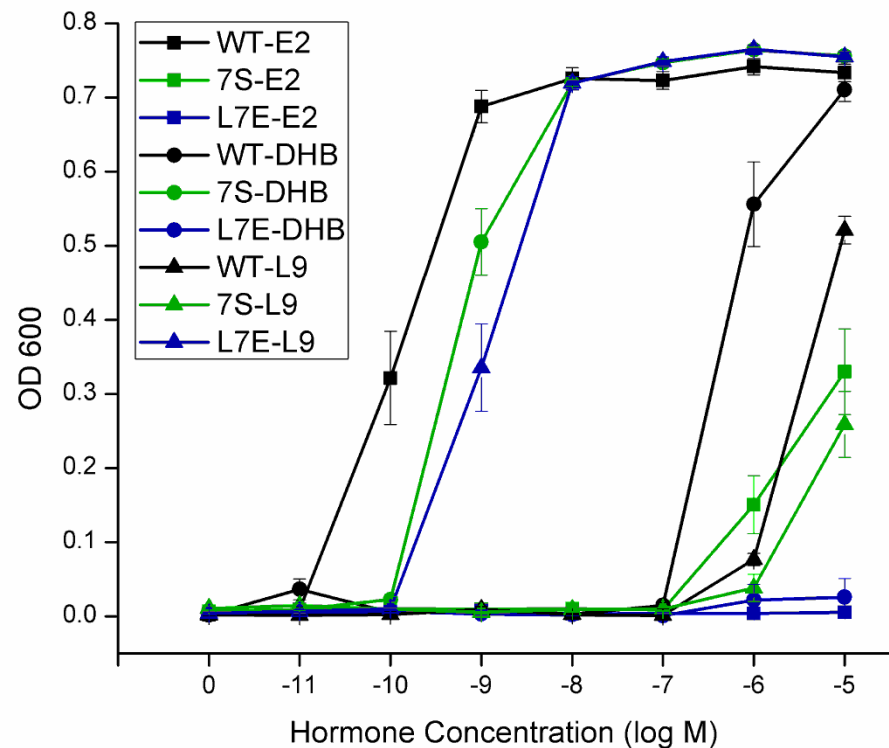
>10¹⁰-fold improvement!

Complete Reversal of Ligand Selectivity



>10¹⁰-fold improvement!

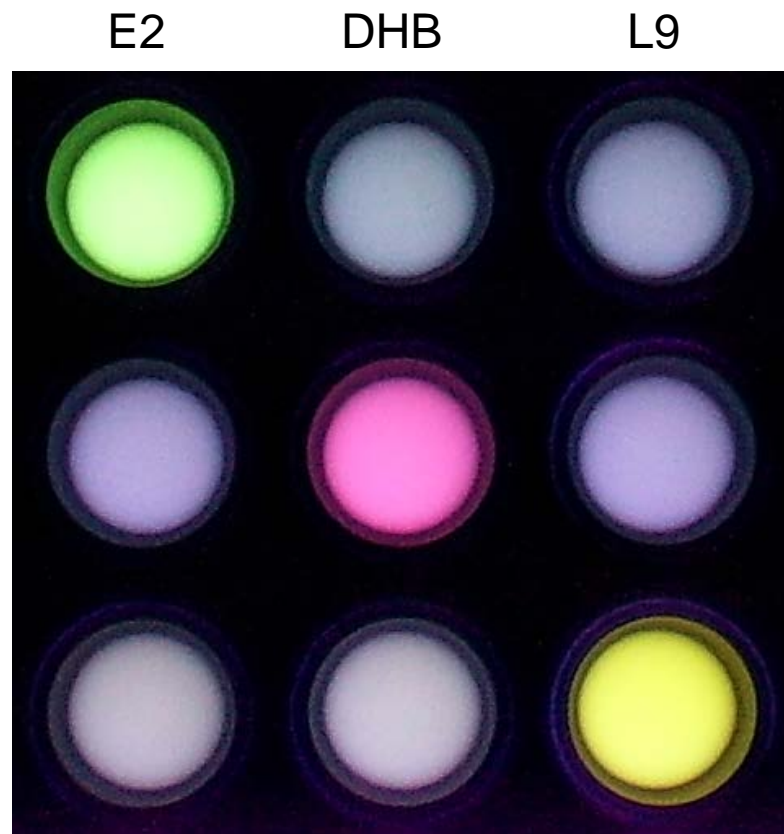
Three Orthogonal Ligand-Receptor Pairs



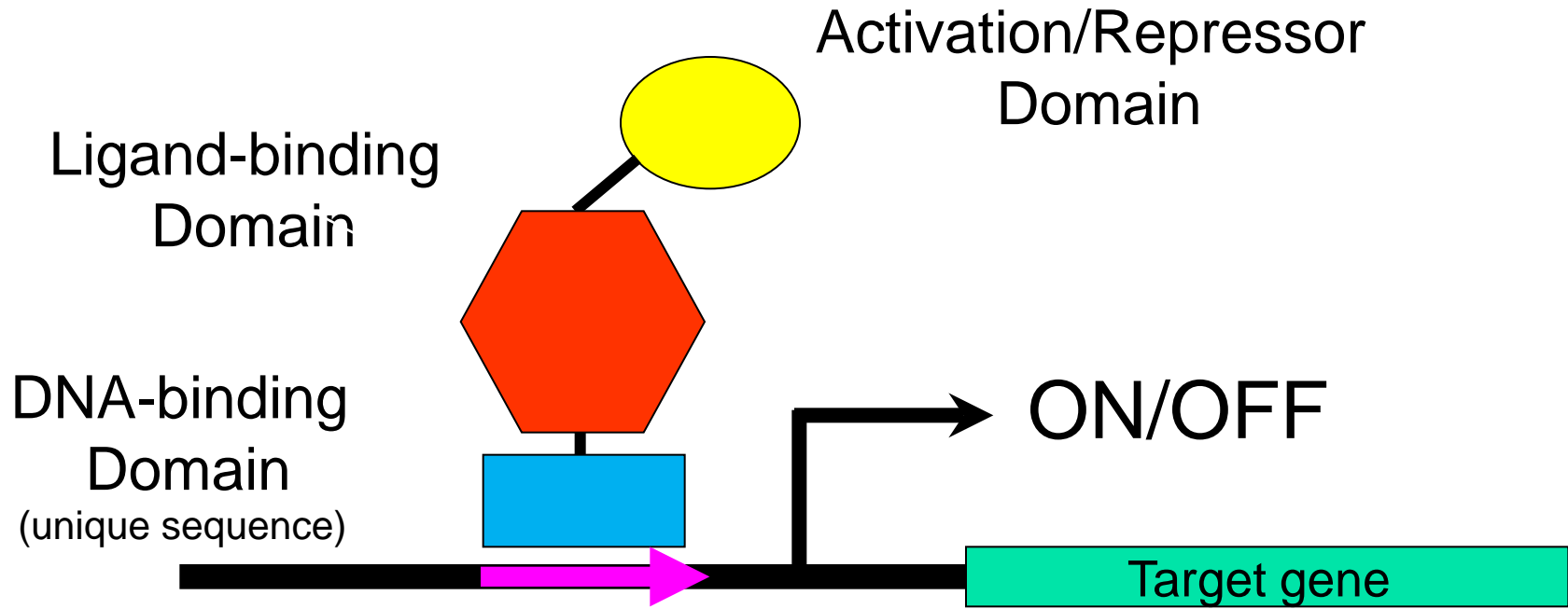
WT
(GFP)

7S
(mCherry)

L7E
(YFP)



Ligand-Dependent Gene Switches



- Regulate exogenous gene expression
- Regulate endogenous gene expression
- Regulate enzyme activity

Regulation of Endogenous Gene Expression



- Motivation

- It can address diseases caused by gene regulation.
- It has certain therapeutic advantage.

- Objective

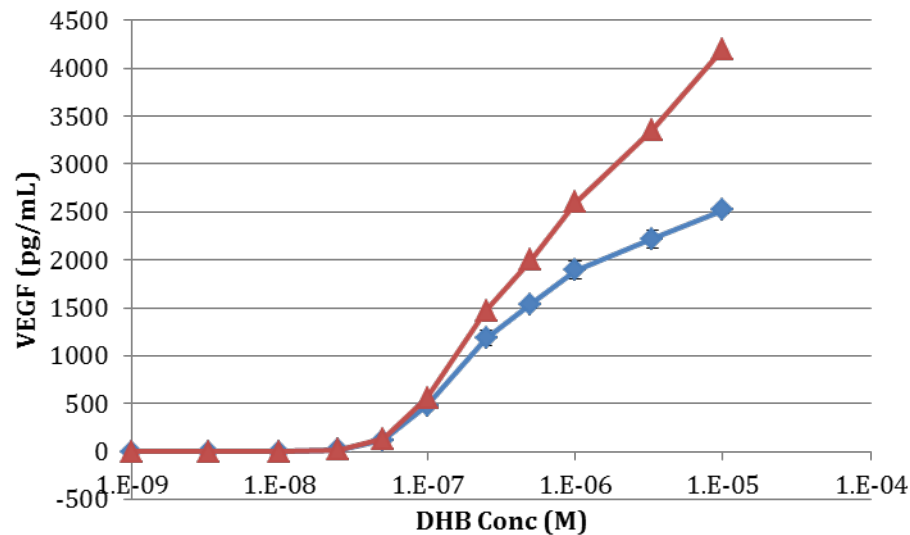
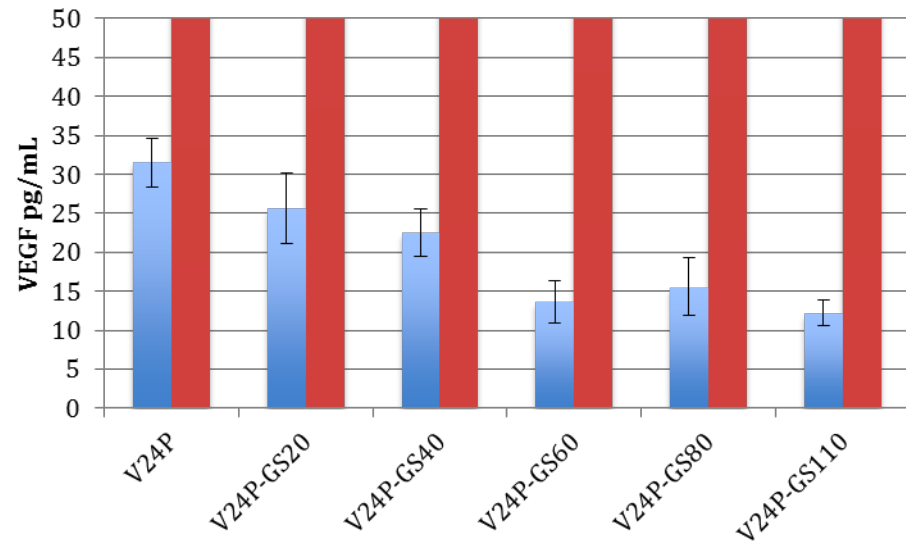
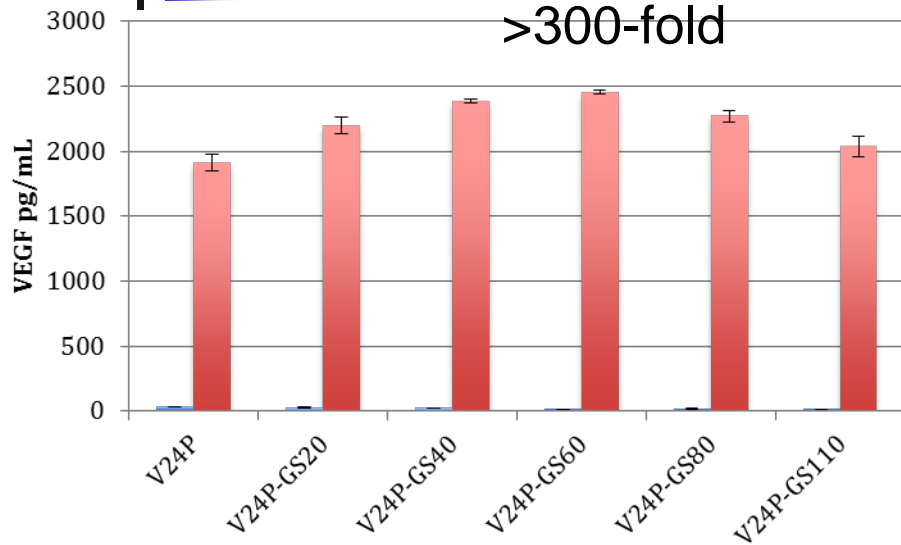
- To achieve ligand responsive induction of an endogenous gene, vascular endothelial growth factor (VEGF) gene.

Exogenous VEGF Endogenous VEGF

Human *VEGF* gene is a chromosomally embedded gene and has splice variants whose correct stoichiometric expression is important for proper angiogenesis.



Regulation of VEGF



Engineering Gene Scissors



Targeted
genome
engineering

Sickle Cell Disease (SCD)

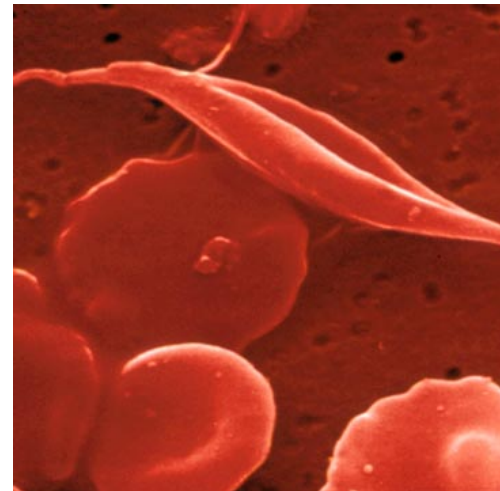


- An inherited autosome recessive disease
- ~120,000 infants are born with SCD every year world wide
- Mutation of β -globin (HBB) gene
- No widely available cure

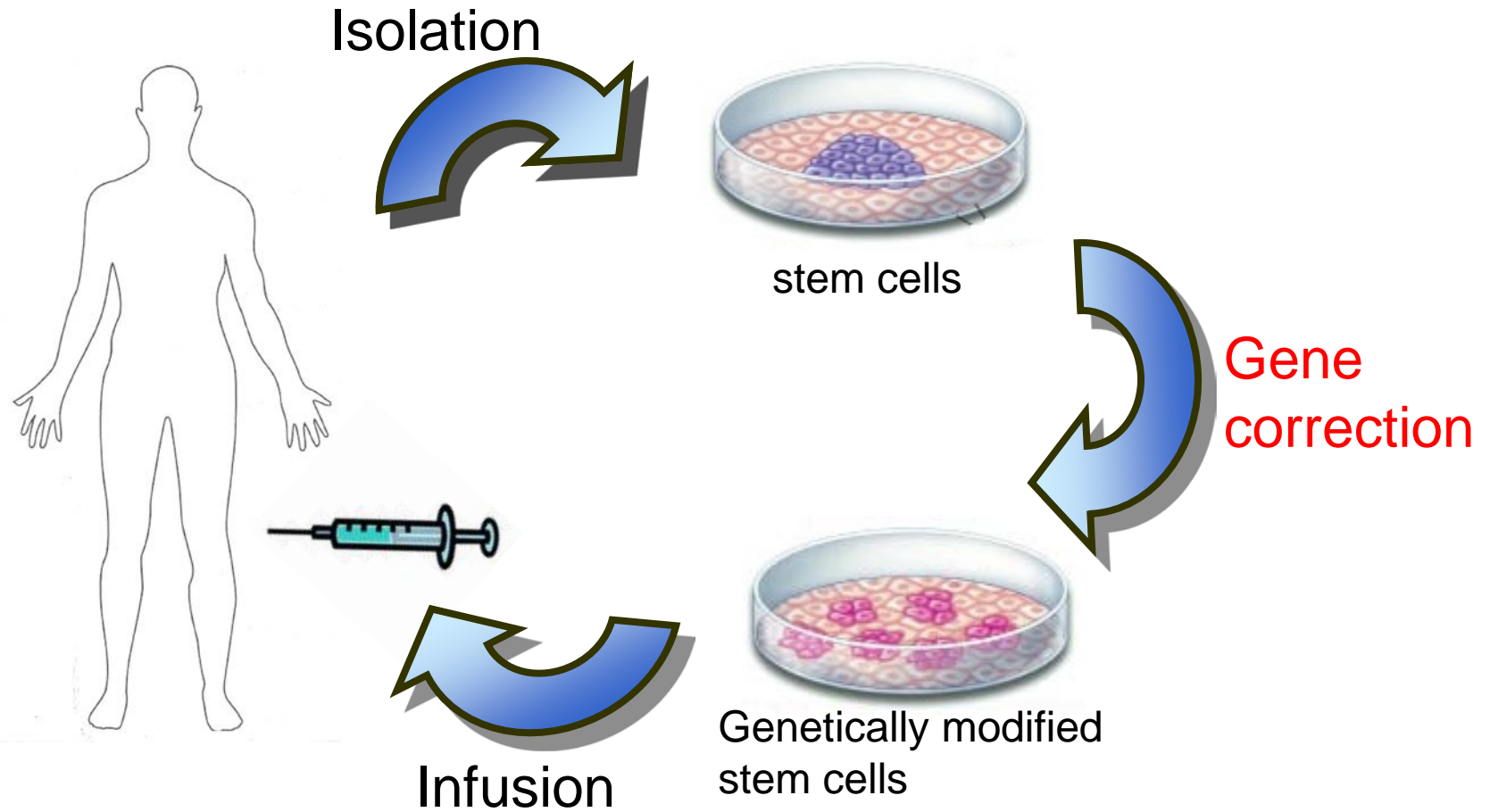
5' - ACCATGGTGCATCTGACTCCTGAGGAGAAGTCTGCCGTTACT
M V H L T P E E K S A V T



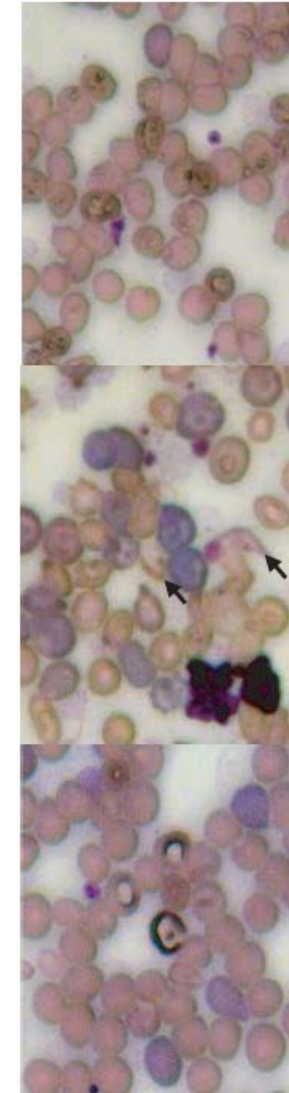
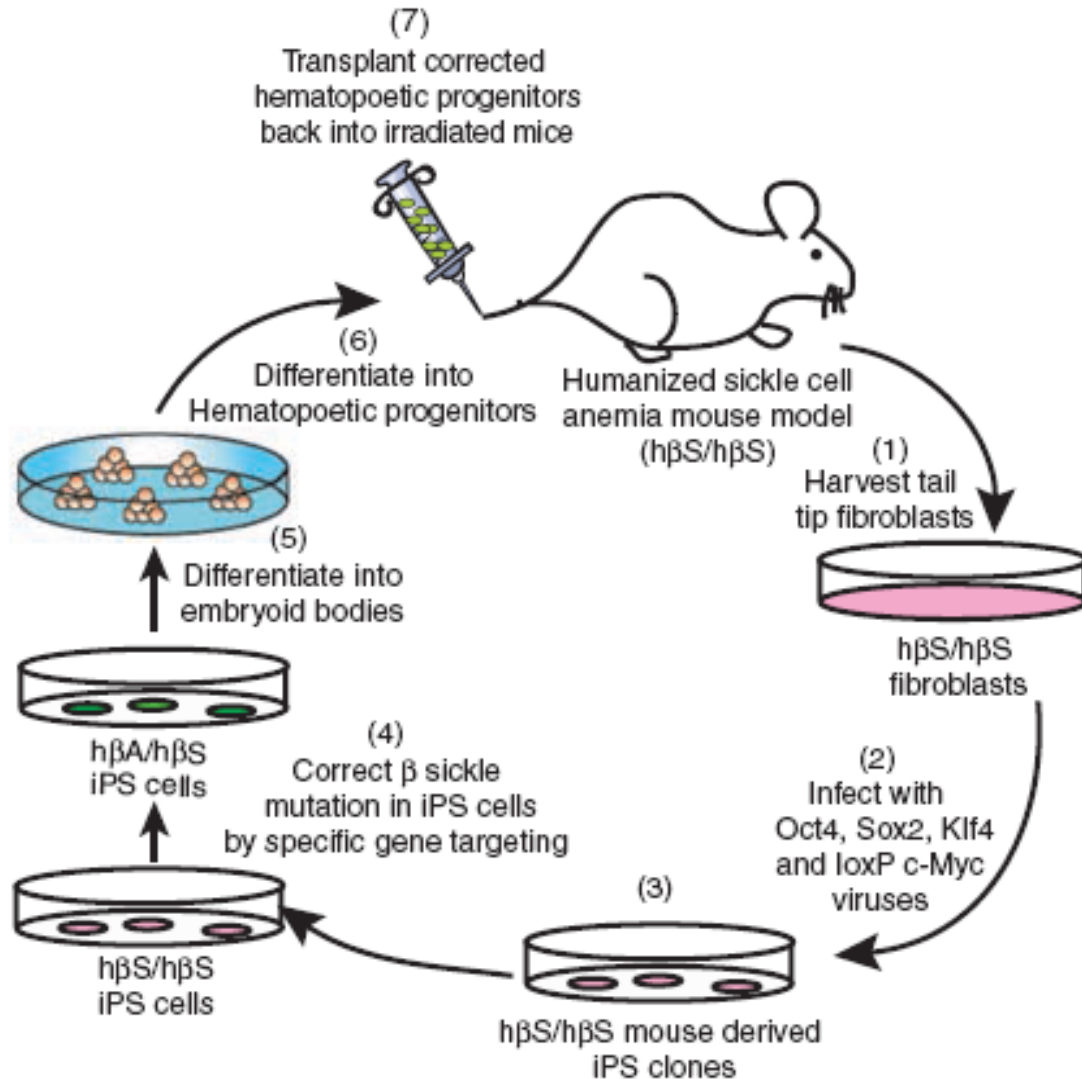
5' - ACCATGGTGCATCTGACTCCTG**T**GGAGAAGTCTGCCGTTACT
M V H L T P **V** E K S A V T



Ex vivo Gene Therapy



iPSC Based Gene Therapy

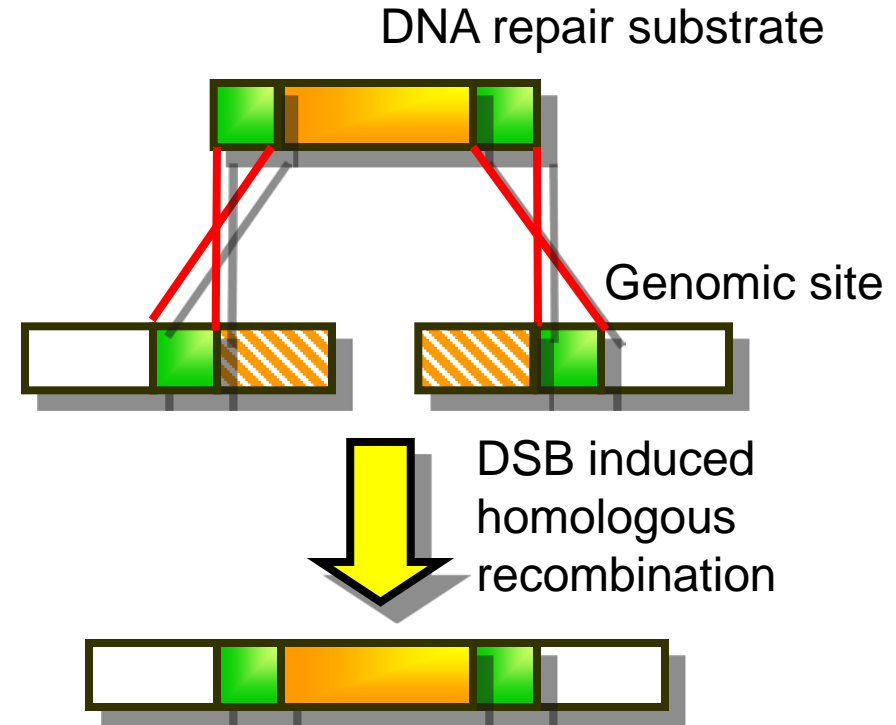
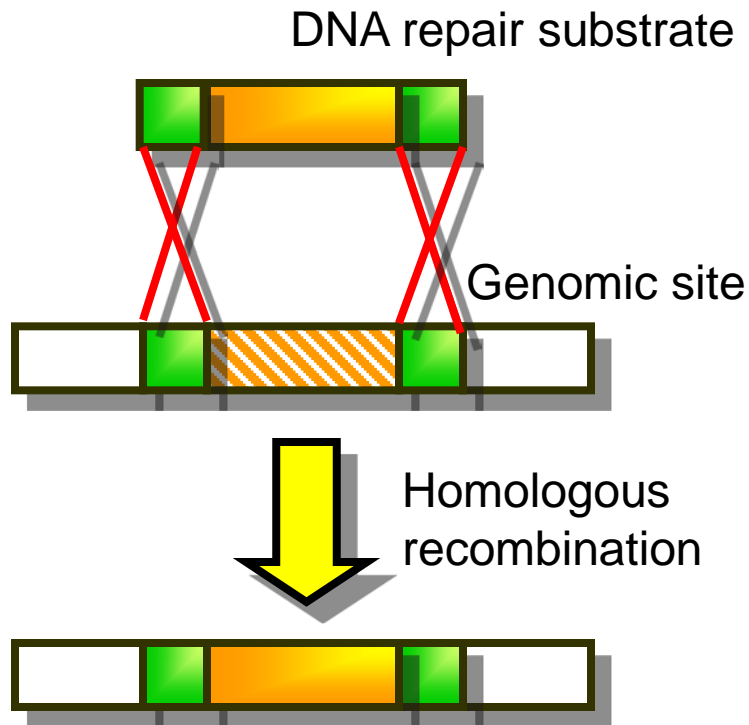


Control

Untreated

Treated

Gene Targeting



Recombination rate:
 $10^{-6} \sim 10^{-9}$ events/cell/generation

Double strand break:
Increase >1000 fold

TAL Effector Nucleases (TALENs)



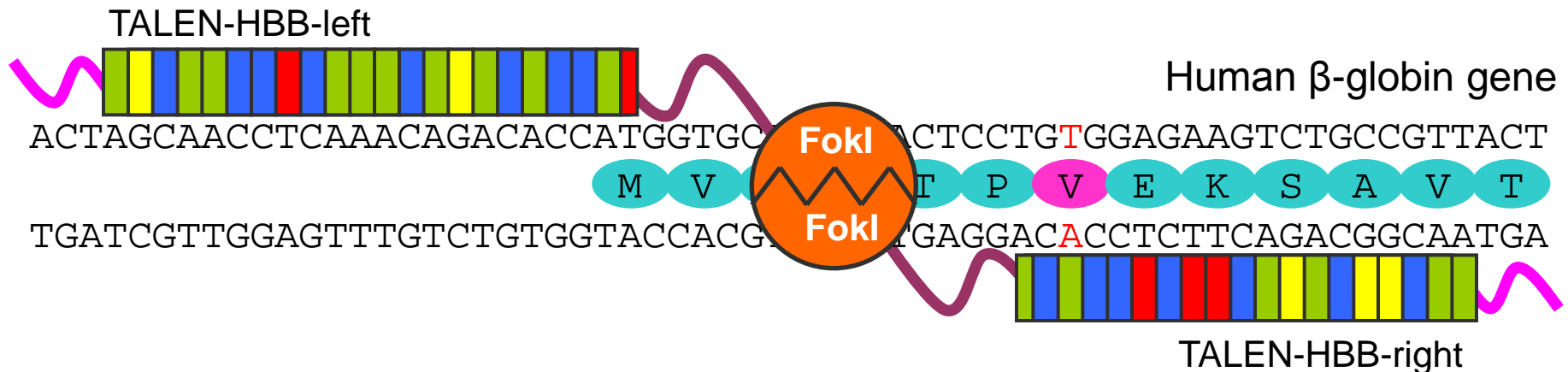
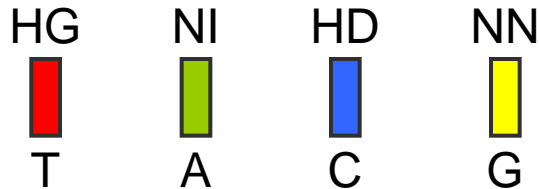
- TAL effectors were found in *Xanthomonas* plant pathogens and acted as transcriptional activator of plant genes



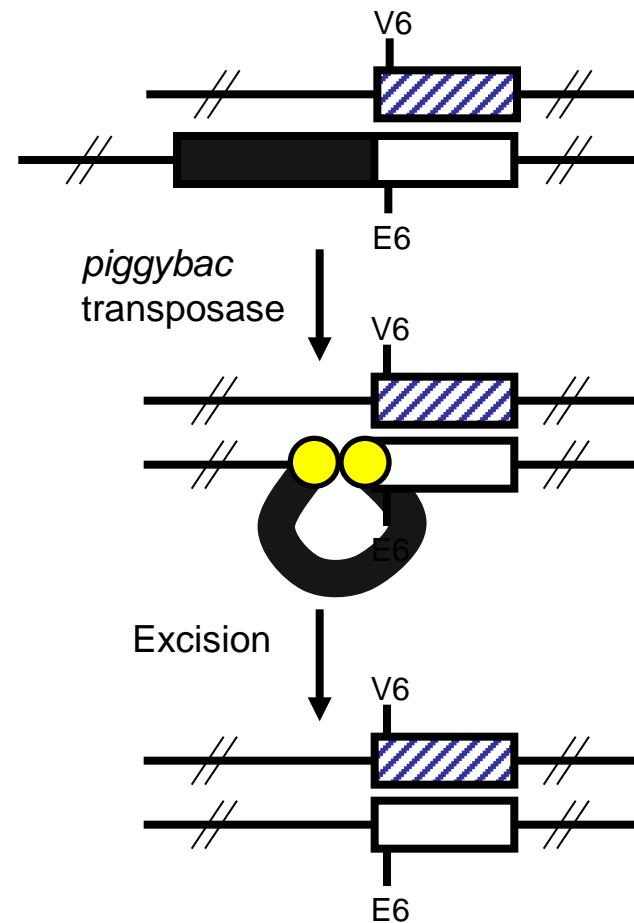
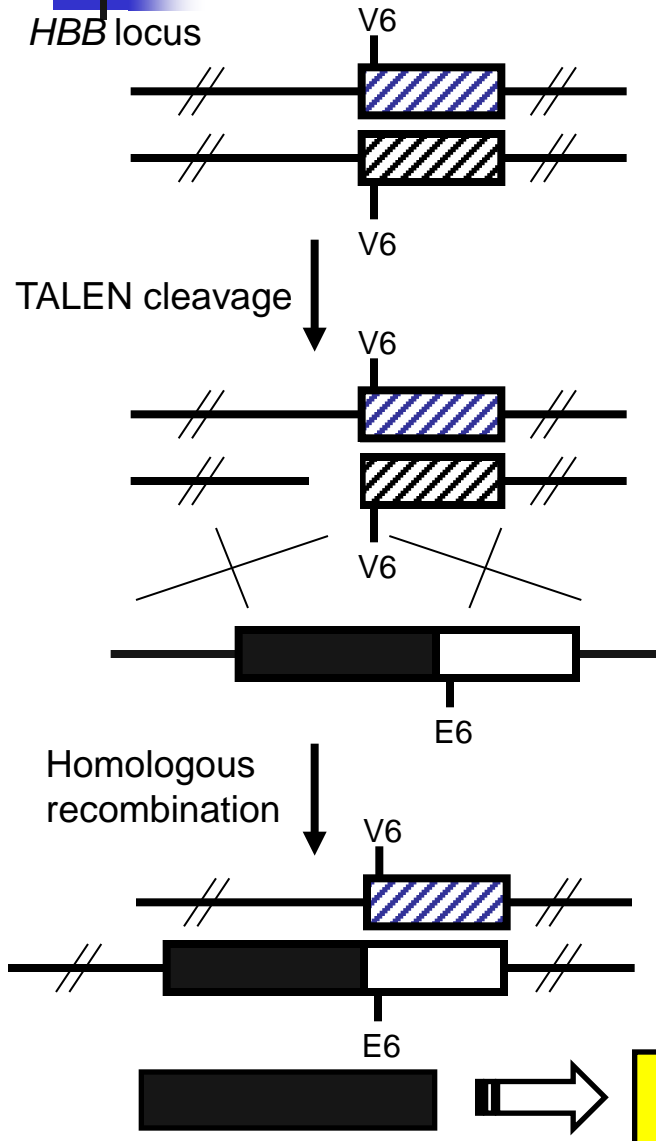
L T P E Q V V A I A S **12 13** H D G G K Q A L E T V Q R L L P V L C Q A H G



hypervariable, confers DNA target specificity



HBB Gene Correction in SCD iPSCs



4.2%



Bsu36I Digestion



5' - ACCATGGTGCATCTGACTCCTG**T**GGAGAAGTCTGCCGTTACT



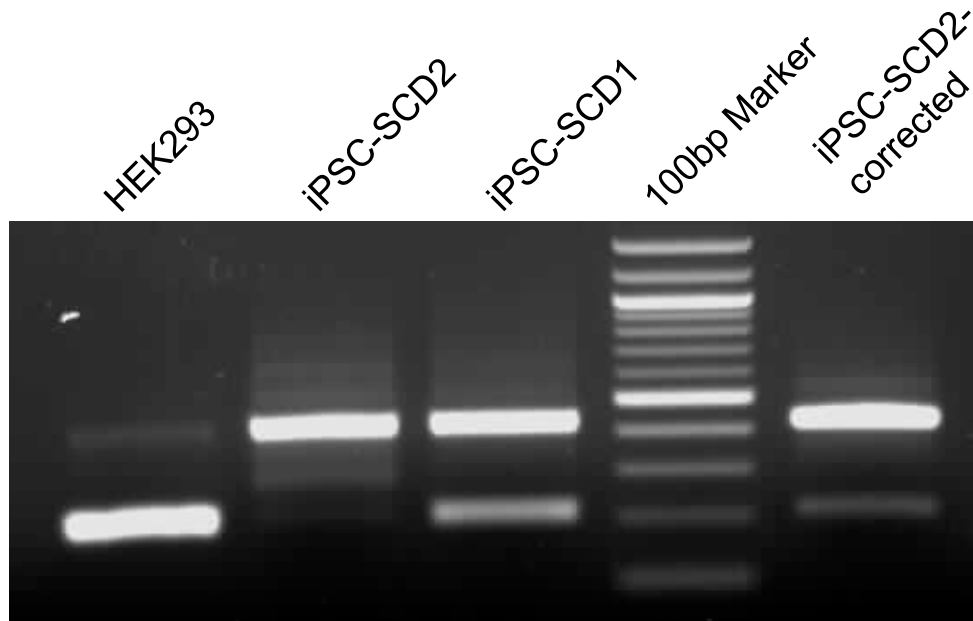
Sickle allele

Bsu36I

5' - ACCATGGTGCATCTGACTCCTG**A**GGAGAAGTCTGCCGTTACT



WT allele



Seamless HBB Gene Correction



5' - ACCATGGTGCATCTGACTCCTG**T**GGAGAAGTCTGCCGTTACT

M V H L T P **V** E K S A V T

Sickle allele

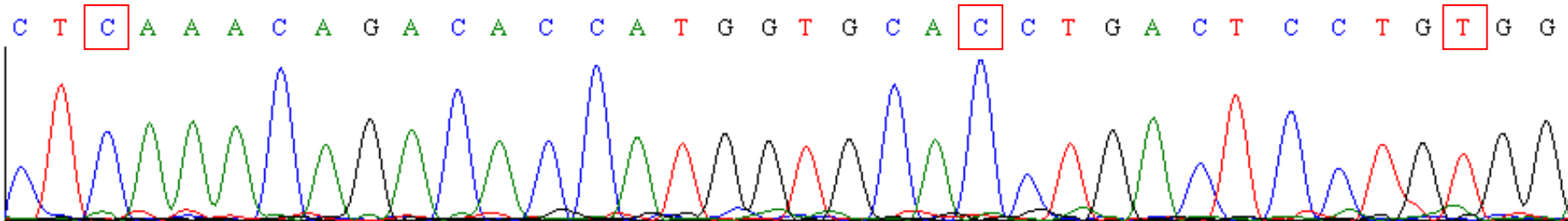
Bsu36I

5' - ACCATGGTGCATCTGACTCCTG**A**GGAGAAGTCTGCCGTTACT

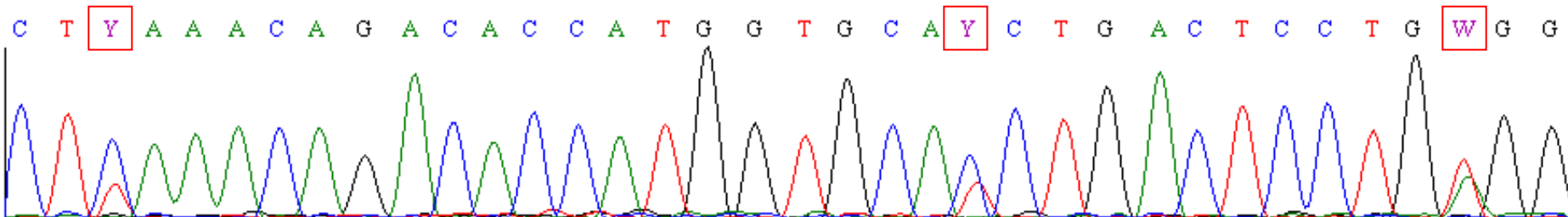
M V H L T P **E** E K S A V T

WT allele

iPSC-SCD2



iPSC-SCD2-corrected clone #22



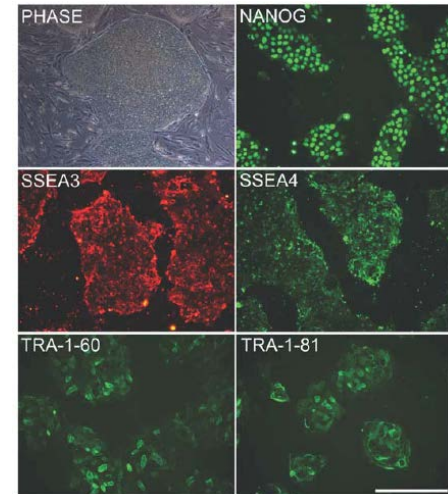
Pluripotency Test



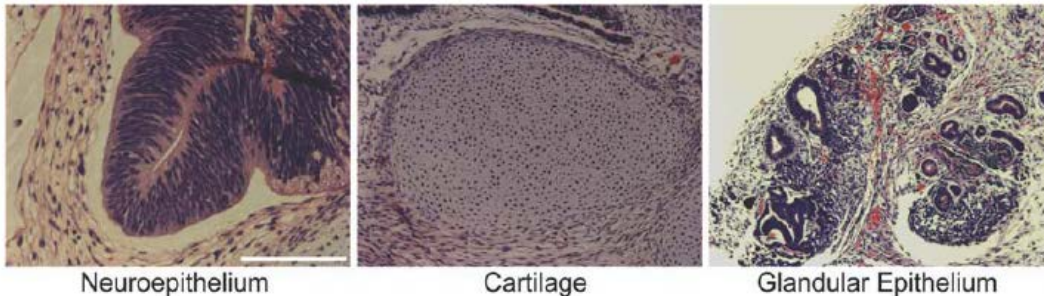
1. Karyotyping



2. Immunostaining



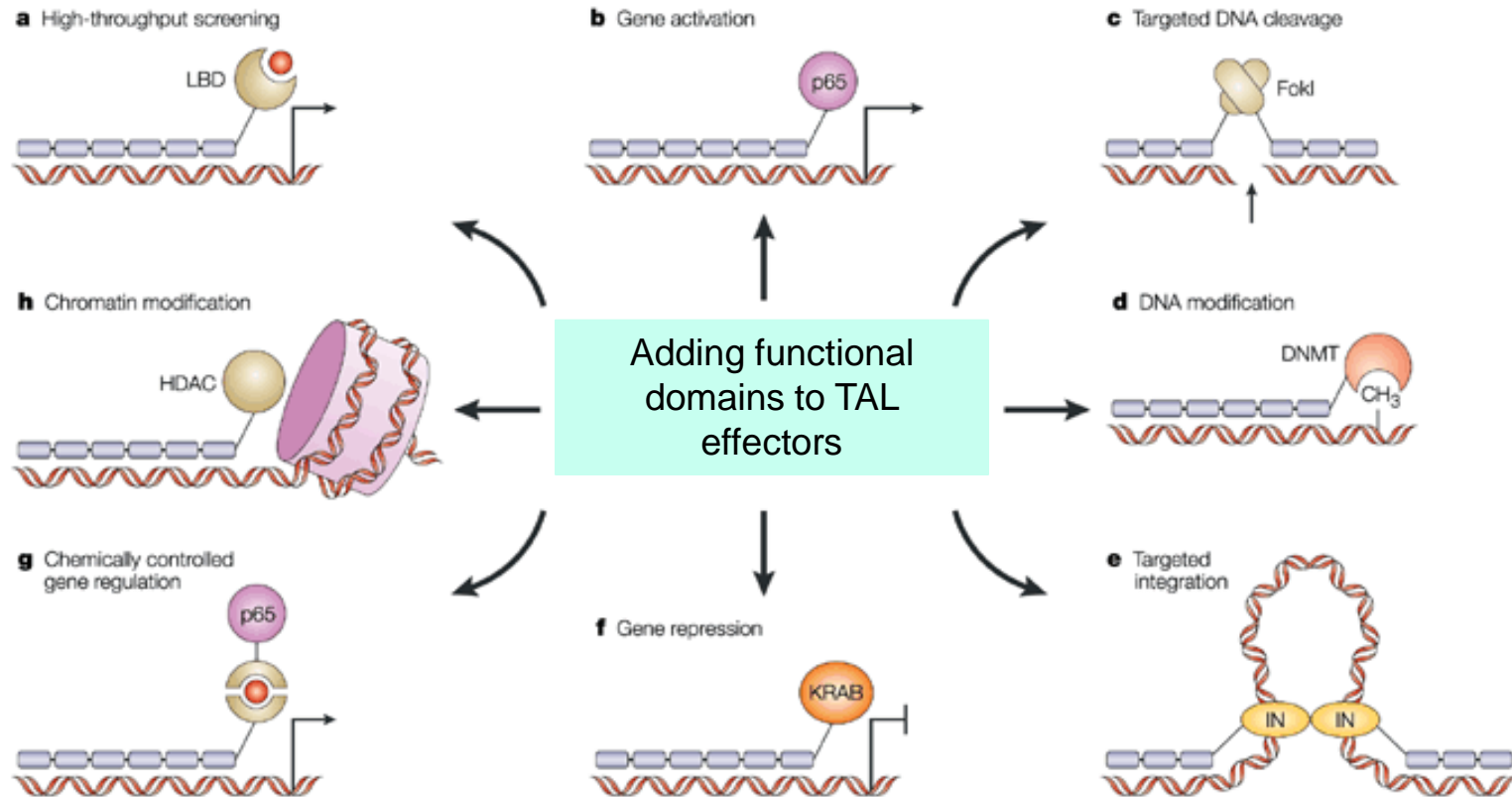
3. Teratoma formation (in progress)



4. Cell differentiation (in progress)

Hematopoietic progenitor cells

Establishing a TAL Effector Based Genome Engineering Platform



- New TAL effector nuclease (TALEN) architectures
- New methods for high throughput synthesis of TAL effector DBDs
- New applications for genome-scale analysis and engineering
- Plant and mammalian systems

Sun et al. *Mol Biosyst* (2012)

Sun et al. *Biotech J.* (2012)



Summary



- Developed a DNA assembler method for constructing large DNA molecules such as pathways, plasmids, and genomes.
- Developed a DNA assembler based synthetic biology method for discovering, characterizing, and engineering cryptic biosynthetic pathways from sequenced microbial genomes and metagenomes.
- Developed a DNA assembler based synthetic biology method (COMPACTER) for optimizing the metabolic flux in a heterologous pathway.
- Engineered a yeast strain capable of simultaneously and efficiently utilizing C5/C6 sugars
- Engineered yeast strains for consolidated bioprocessing of cellulose and xylan respectively.
- Developed new tools for orthogonal control of gene expression and targeted genome editing in mammalian cells.

The Zhao Group



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Dan Coursolle, Hua Huang

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