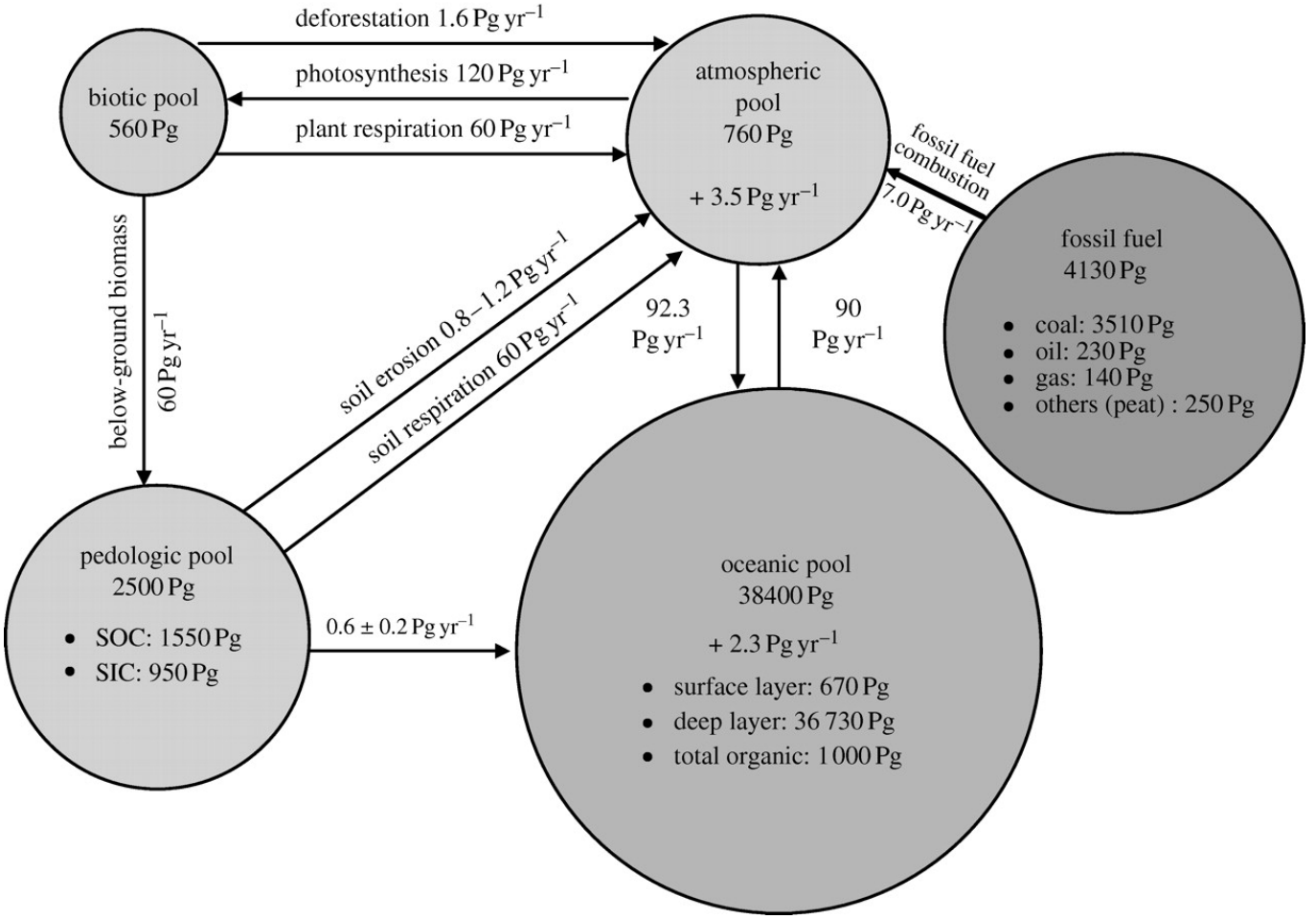
systems and synthetic biology for biorefineries to make sustainable chemicals

Andrew Tolonen

Genoscope

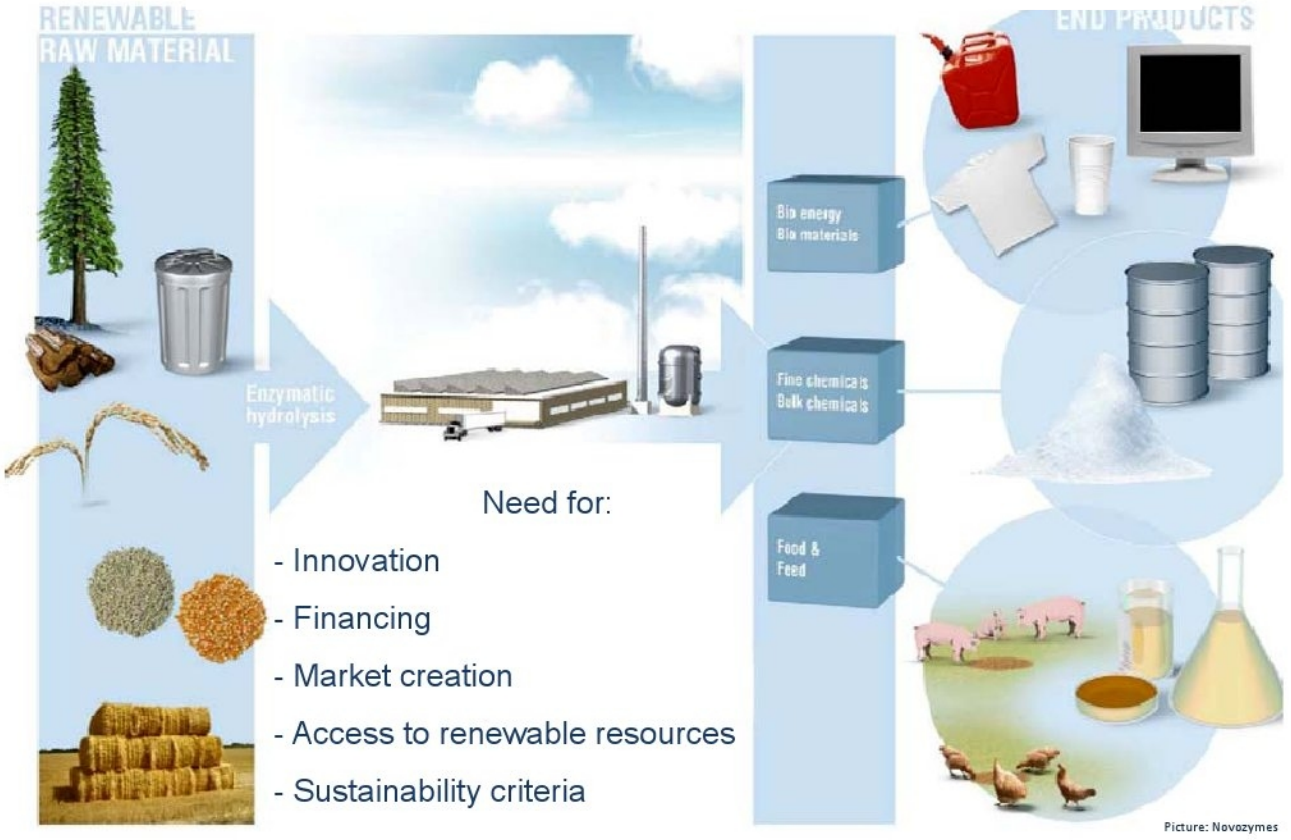
atolonen@gmail.com

a major scientific challenge is to find way to balance the global carbon cycle



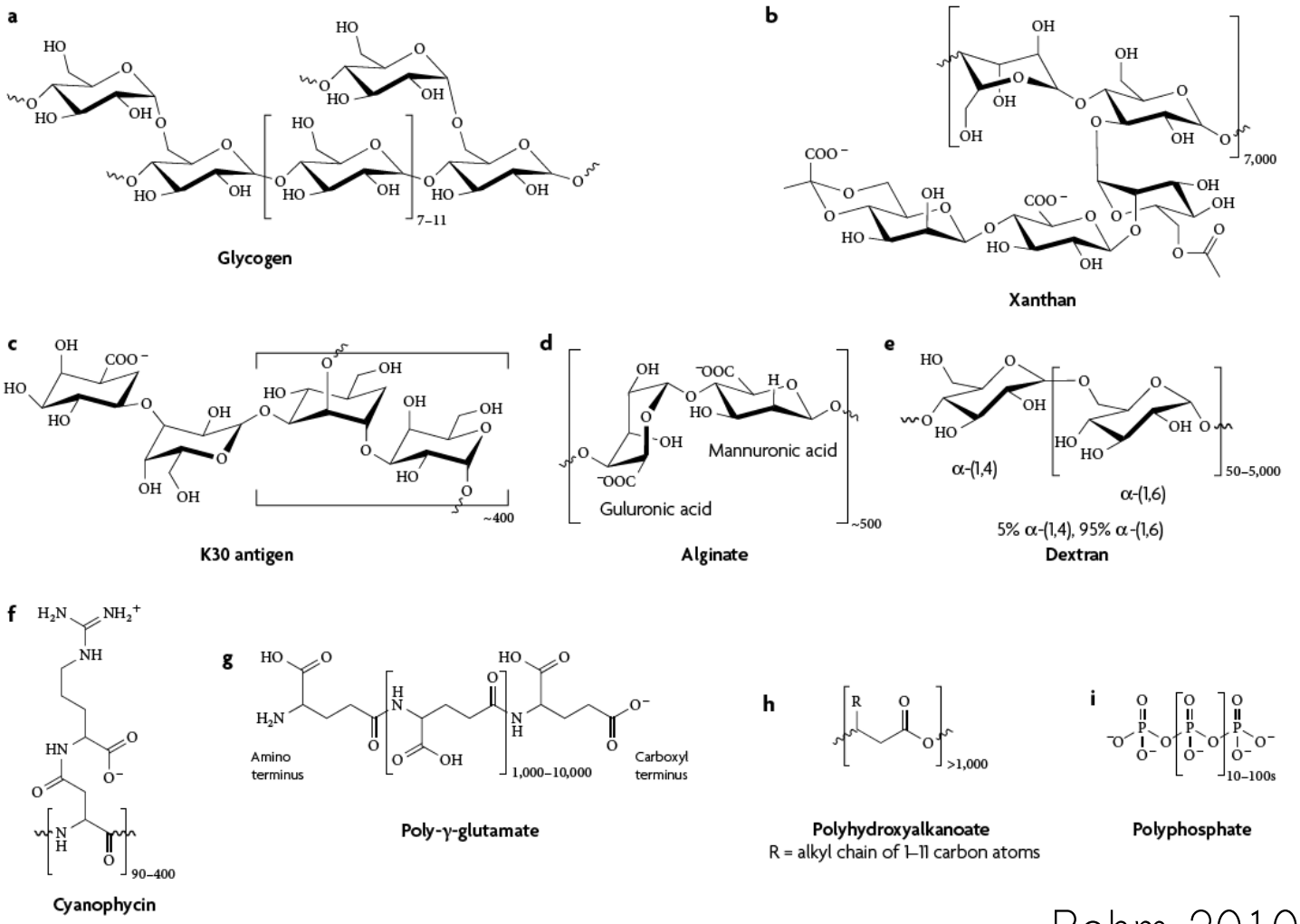
R Phil. Trans. R. Soc. B 2008;363:815-830

biorefinery: renewable, carbon balanced production of biocommodities



http://www.abakus.be

biorefineries will produce many useful polymers



Rehm 2010

examples of renewable biomaterials

poly-lactic acid

http://www.enviropack.org.uk

chitin

http://www.ecovativedesign.com/

alginate

www.alibaba.com

spider silk

hanopolis.com

biorefineries will also produce renewable fuels

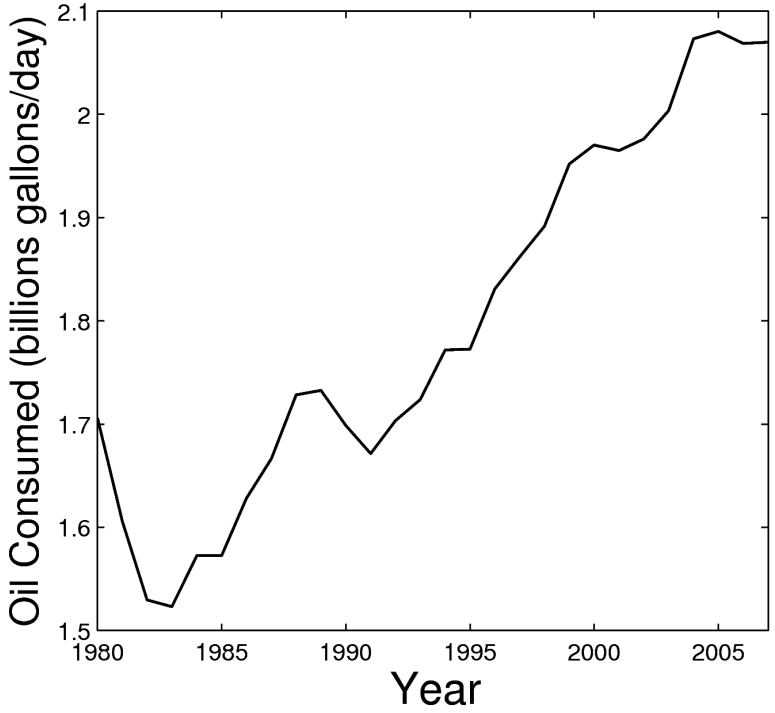
© beboy * www.ClipartOf.com/30312

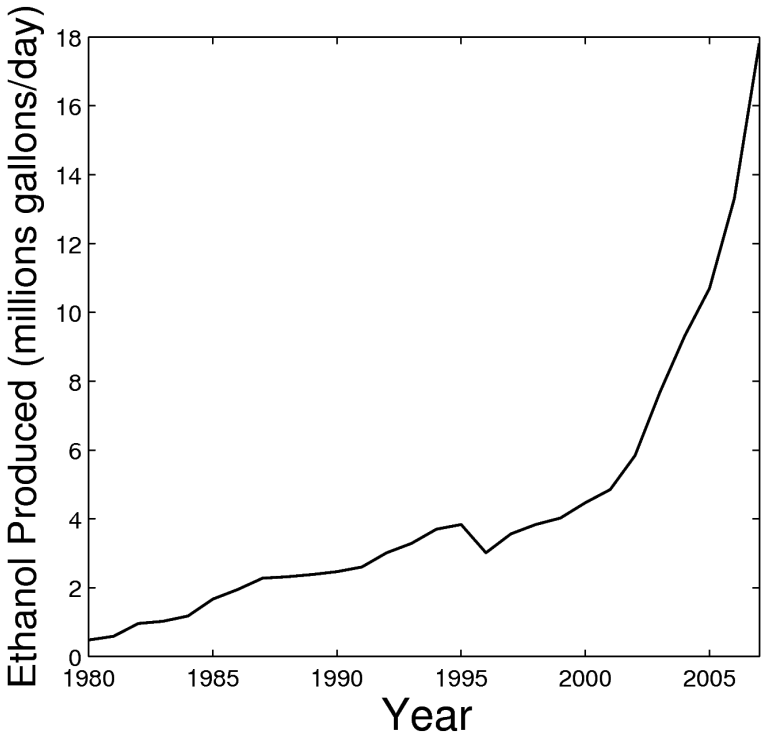
www.qteros.com

http://www.energy-enviro.fi

www.coskata.com

US oil consumption and ethanol production

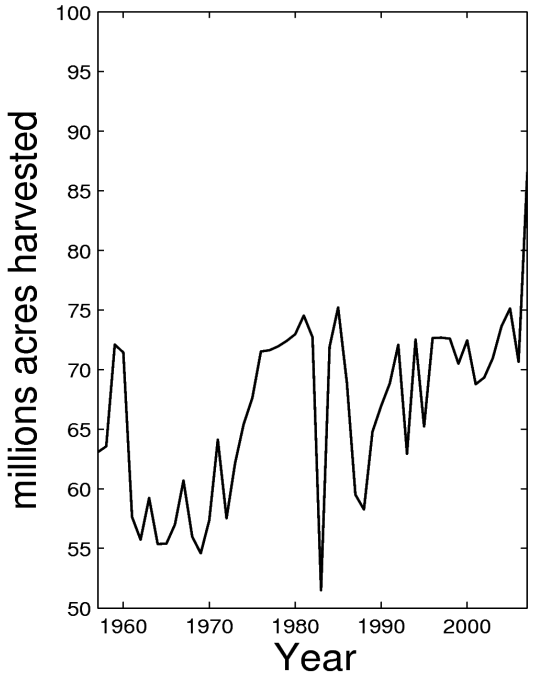


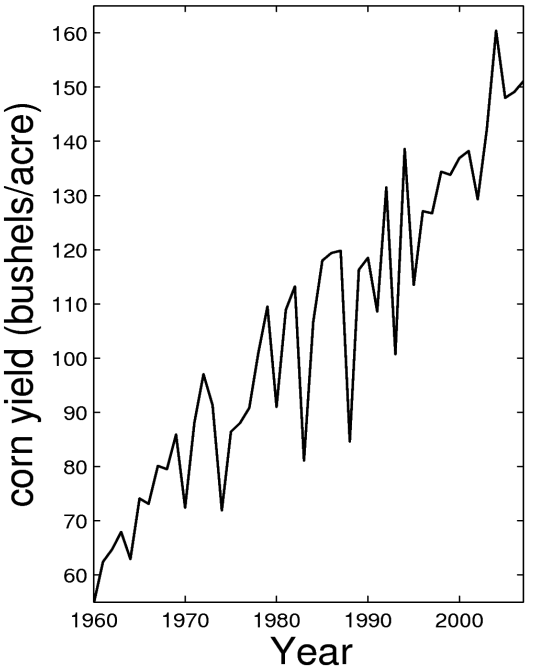


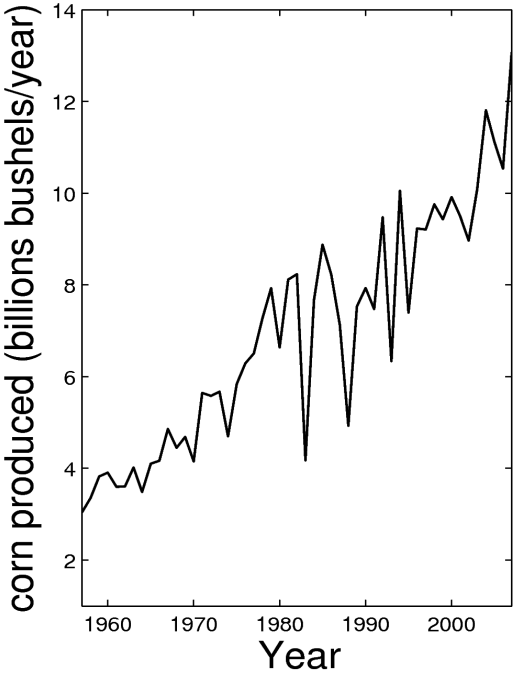
US oil consumption: http://tonto.eia.doe.gov

US ethanol production: http://www.ethanolrfa.org

US corn production

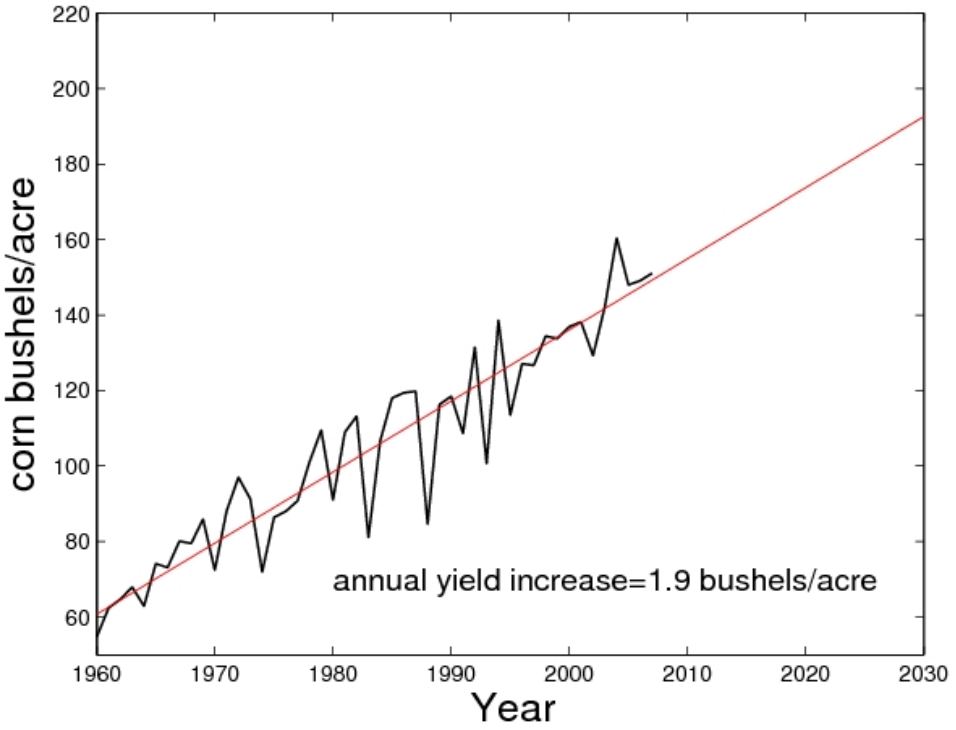






Data from http://www.nass.usda.gov

future corn yields



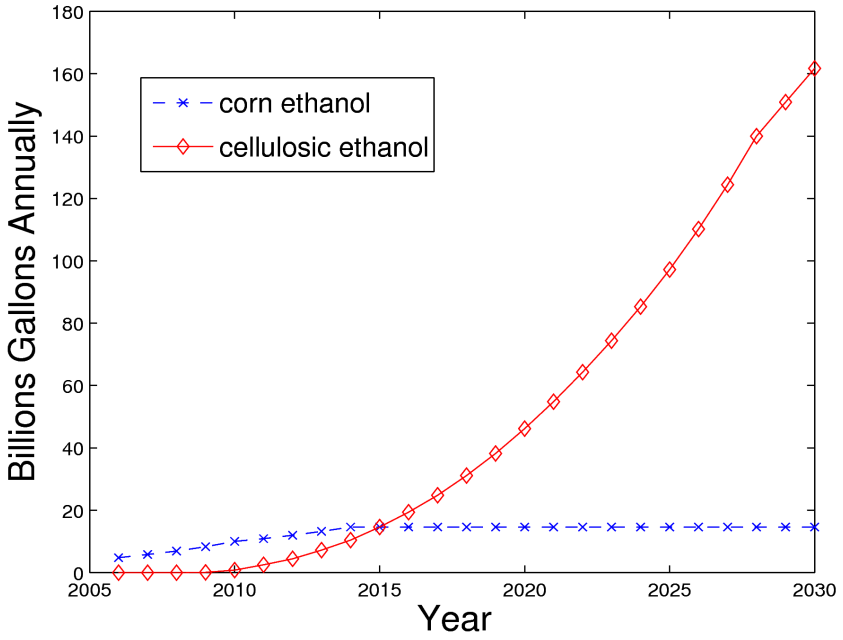
28% improved yields by 2030

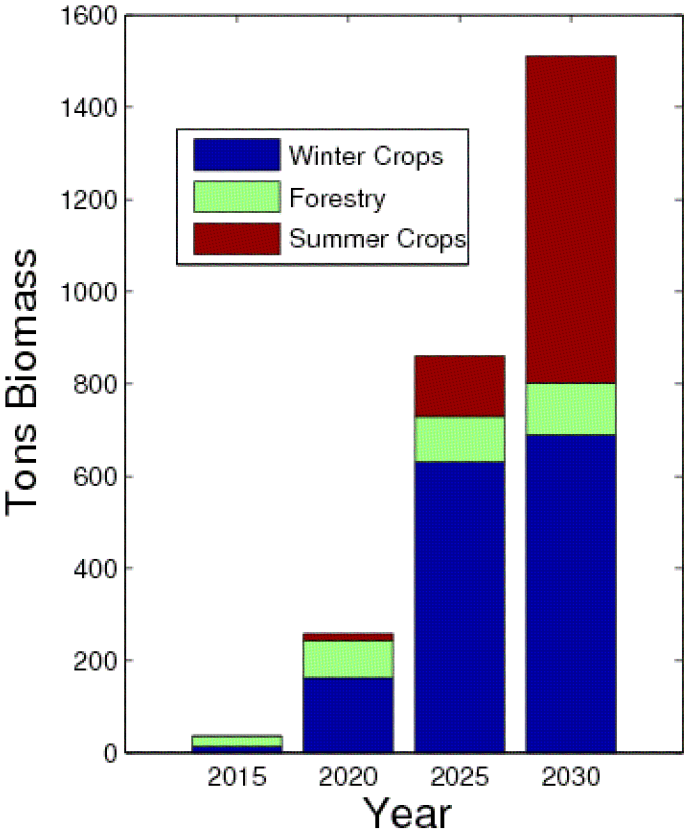
corn data from http://www.nass.usda.gov

we cannot grow enough corn to replace petroleum!

http://www.chicagoboyz.net

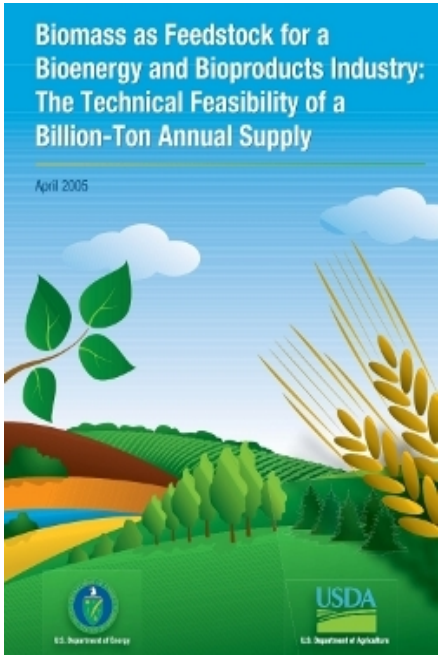
cellulosic biomass can replace corn to make biofuels at larger scale



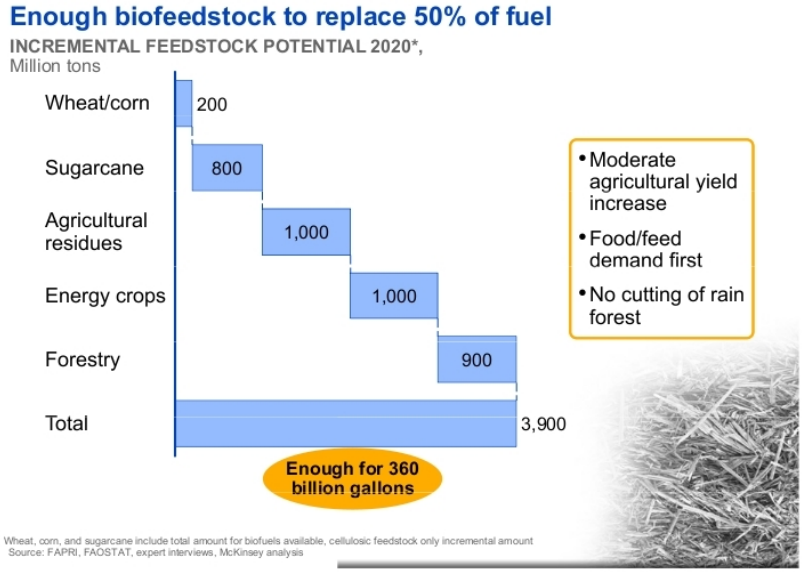


data from http://www.khoslaventures.com

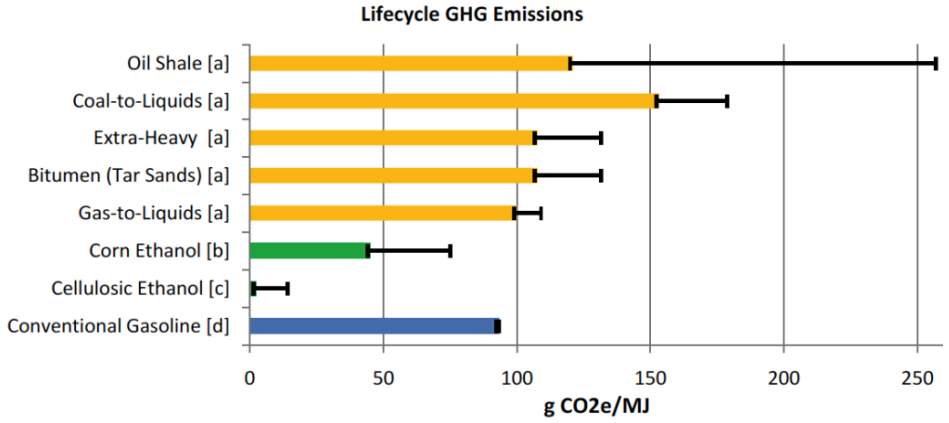
cellulosic feedstocks are abundant, inexpensive and reduce GHG emissions



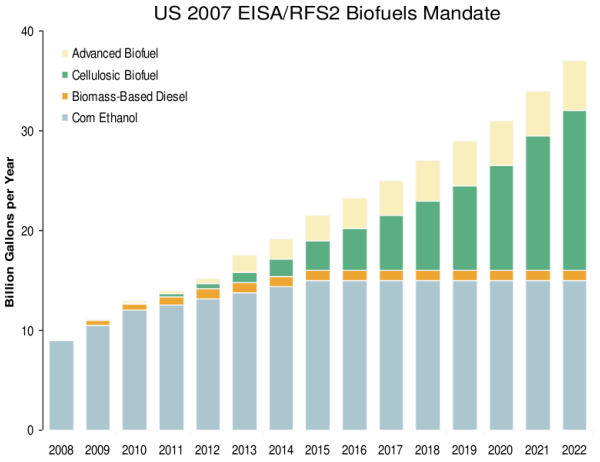
Perlack et al., 2005 DOE/USDA report



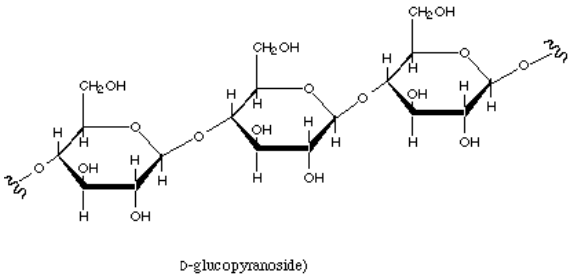
McKinsey and Co.



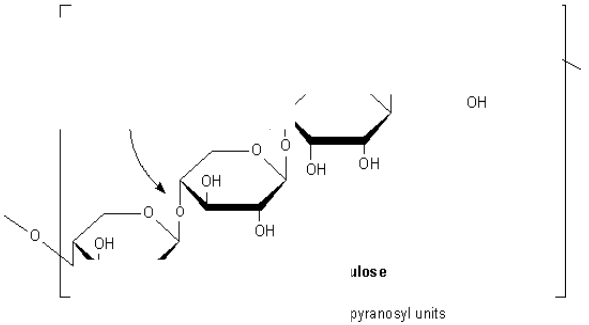
Data from Renewable Fuels Assn



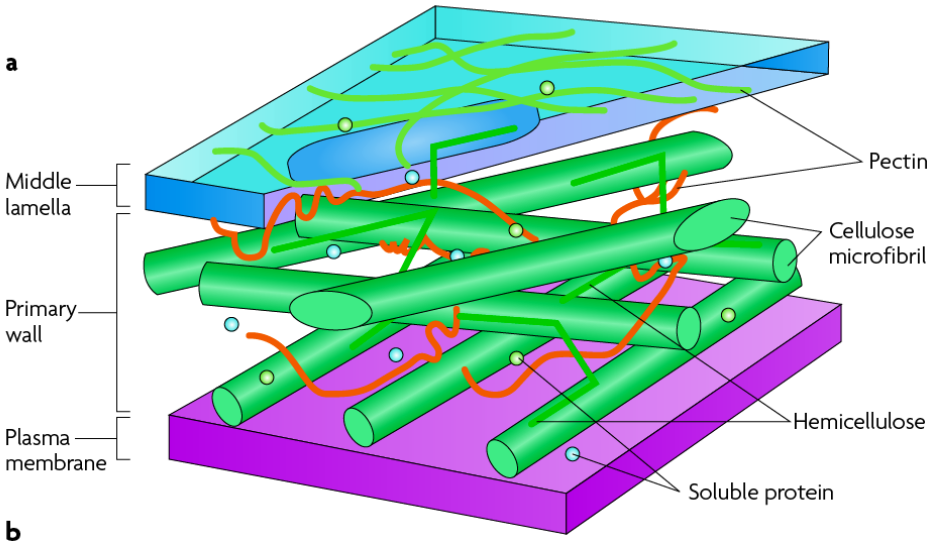
however, plant biomass is tough for microbes to eat



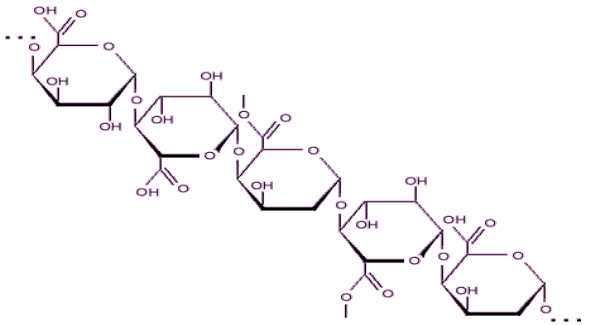
cellulose (1,4-beta-D-glucopyranoside)
35-50% of biomass



hemicellulose (1,4-beta-D-xylopyranoside)
20-30% biomass

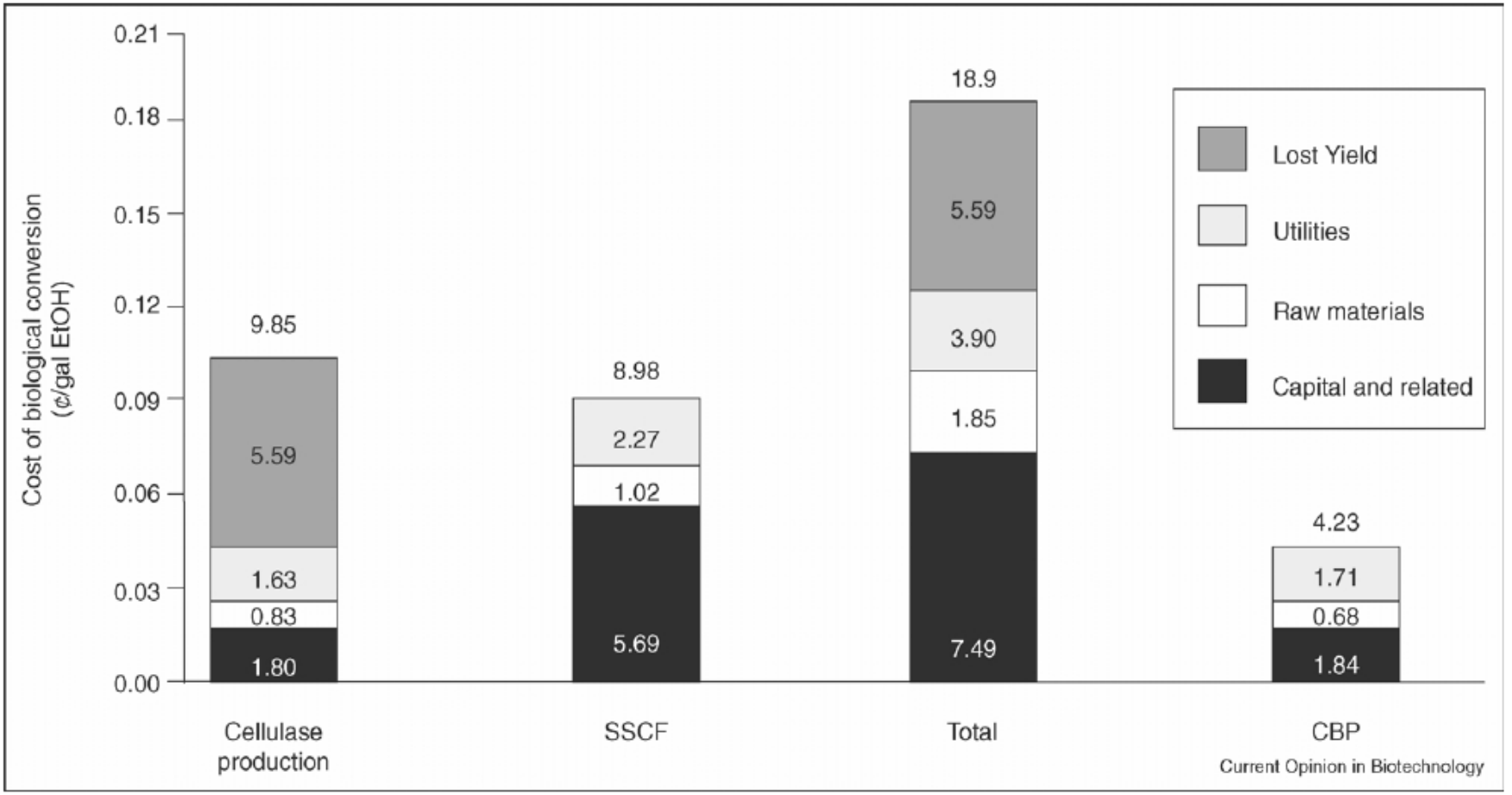


Stricklen et al, 2008



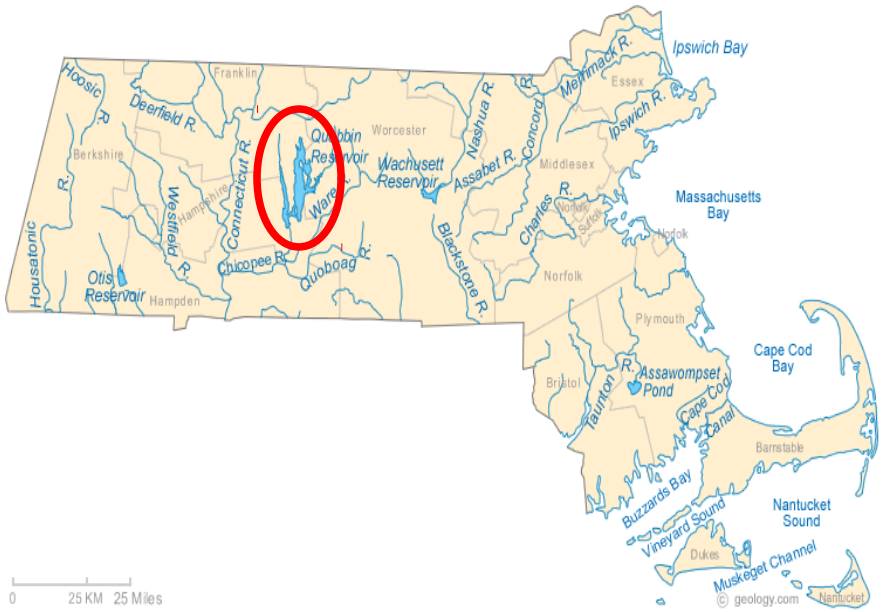
pectin α-(1-4) galacturonic acid ~ 10% biomass

a microbe that can convert biomass to fuel in a single step is ideal



Lynd et al, 2005

Clostridium phytofermentans: a new opportunity for cellulosic ethanol



International Journal of Systematic and Evolutionary Microbiology (2002), **52**, 1155–1160 DOI: 10.1099/ijs.0.02125-0

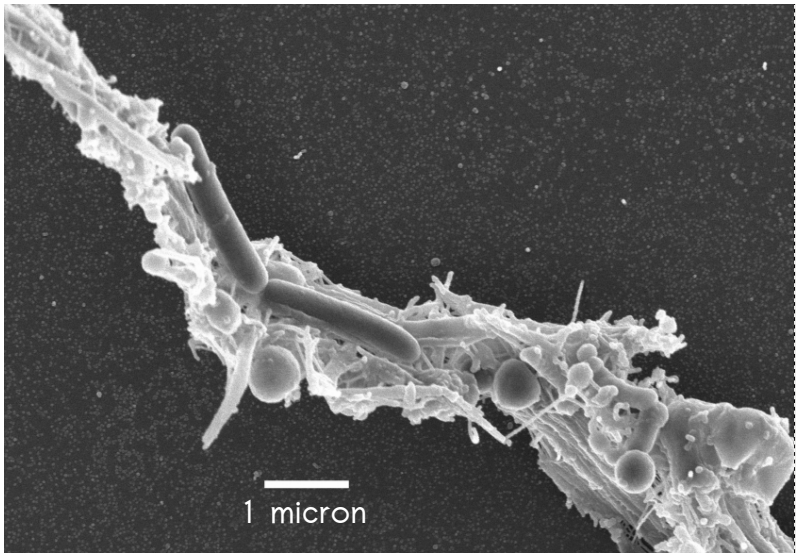
***Clostridium phytofermentans* sp. nov., a cellulolytic mesophile from forest soil**

Department of Microbiology, University of Massachusetts, Amherst, MA 01003, USA

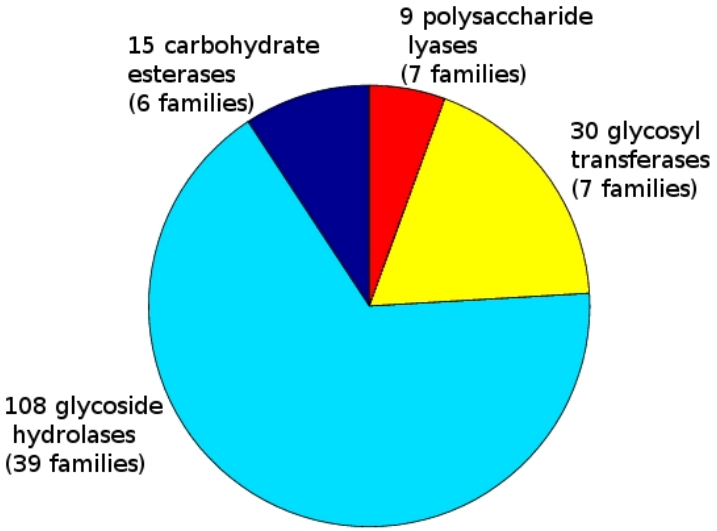
Thomas A. Warnick, Barbara A. Methé and Susan B. Leschine

Author for correspondence: Susan B. Leschine. Tel: +1 413 545 0673. Fax: +1 413 545 1578. e-mail: suel@microbio.umass.edu

Clostridium phytofermentans (Cphy) ferments cellulosic biomass to ethanol and hydrogen

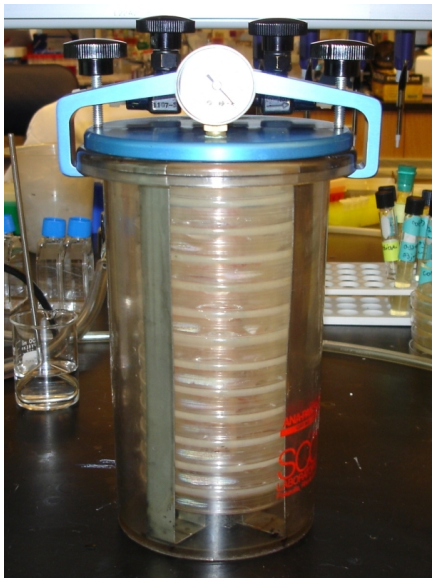


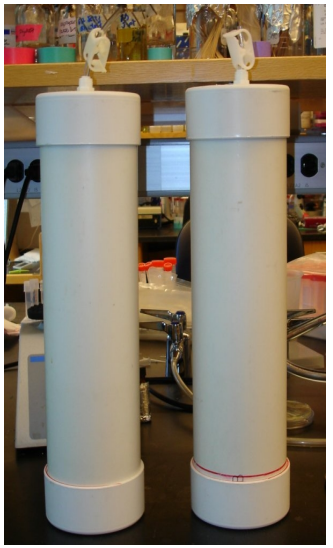
Cphy genome has 161 carbohydratases (CAZy)



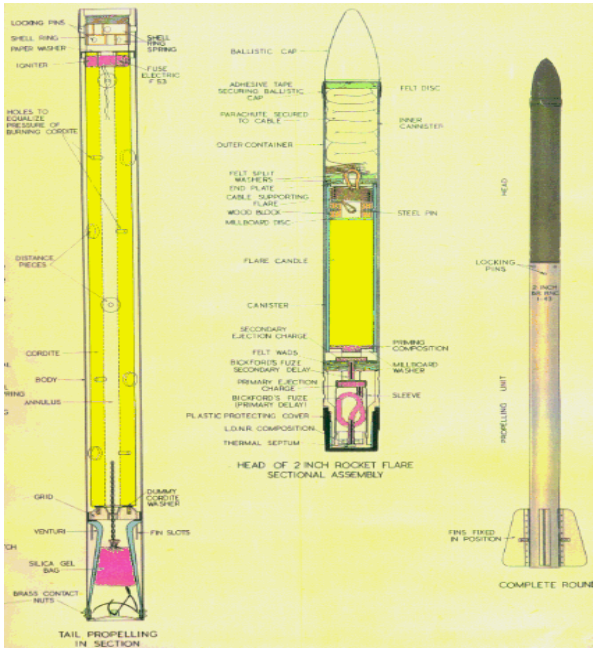
Cphy is an obligate anaerobe

"la vie sans l'air" -Louis Pasteur 1862





Chaim Weizmann: clostridia, cordite, and the founding of Israel



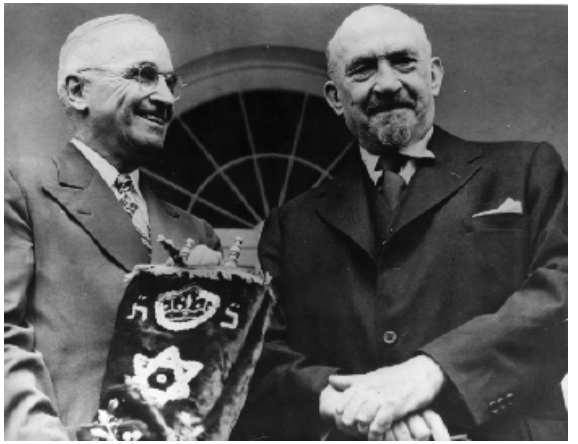
http://www.cyber-heritage.co.uk

http://www.lesjones.com

http://www.wereldoorlog1418.nl

"There is only one thing I want: A national home for my people." -Chaim Weizmann

● Clostridial fermentation plant http://www.world-atlas.us



http://www.trumanlibrary.org

http://www.lonklab.ac.uk

my research focus: new technologies

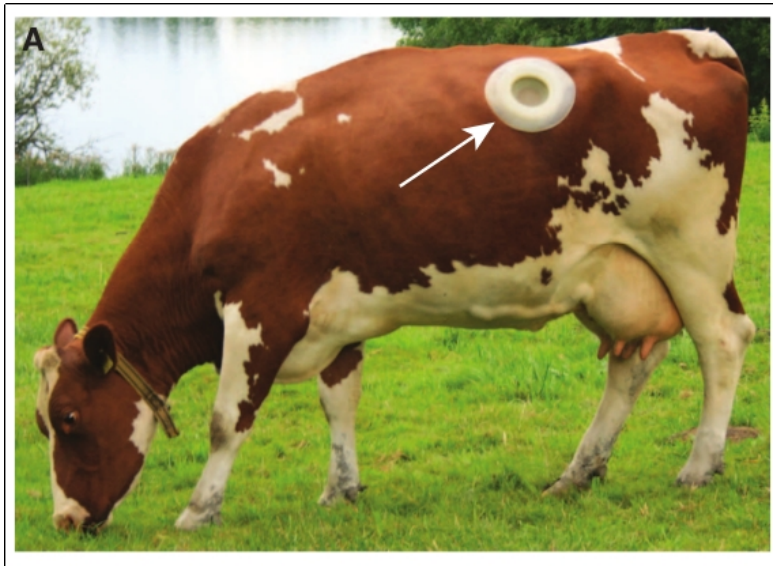
1. Systems biology: identify key enzymes/pathways
2. Synthetic biology: genome engineering

new generation biorefineries

fuels

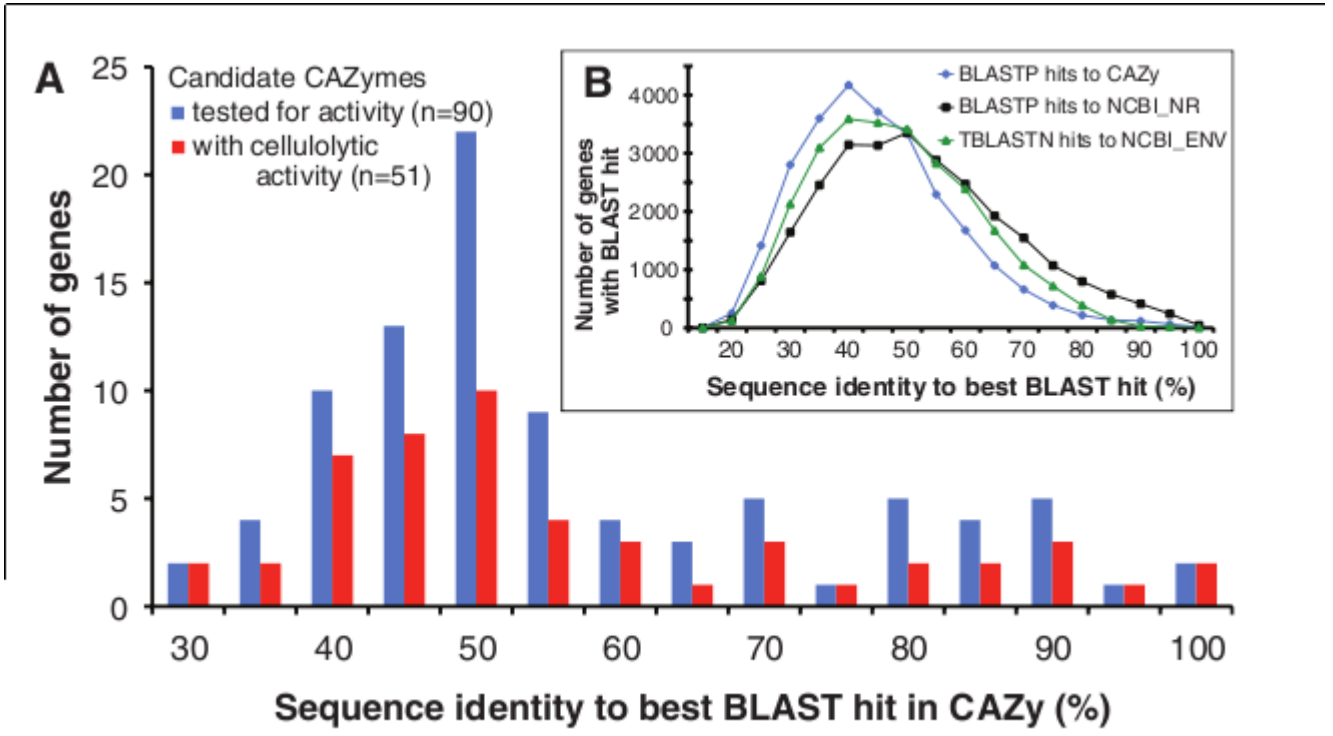
biocommodities

metagenomics for new enzymes from the cow rumen

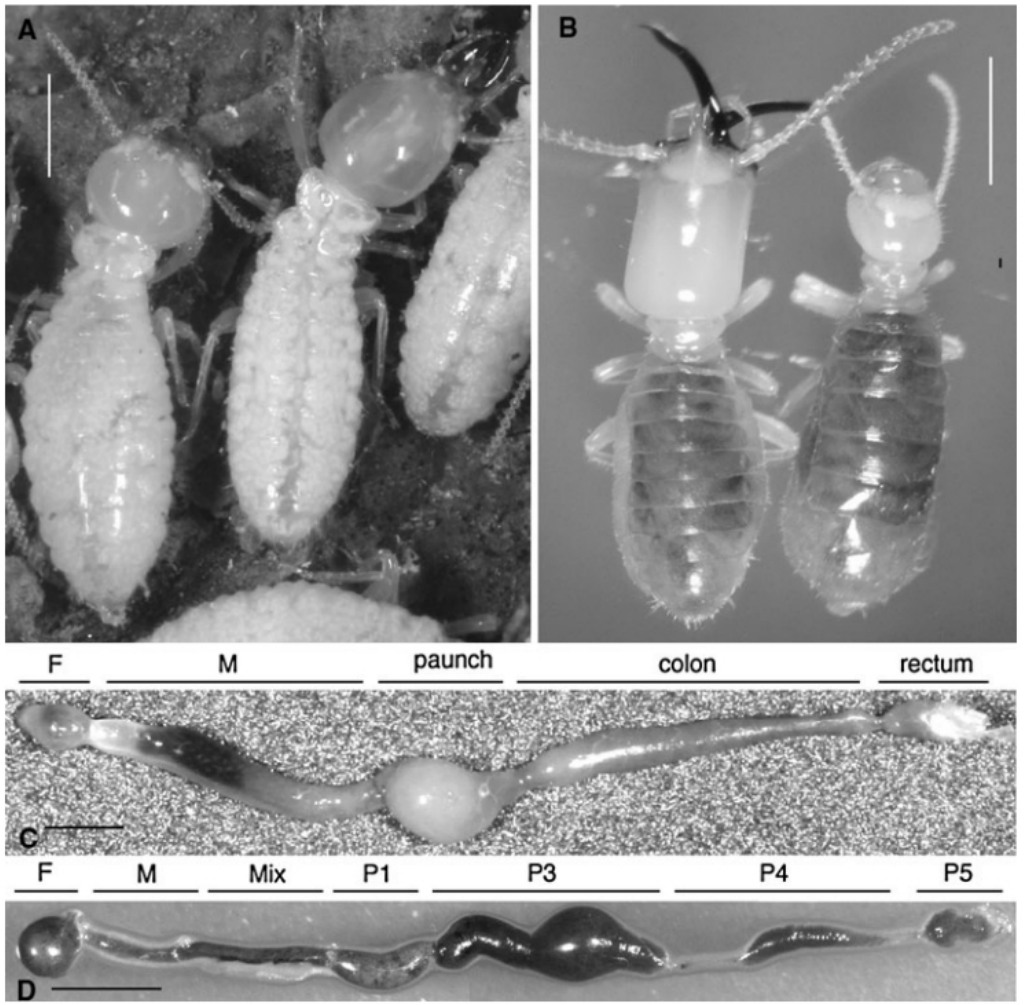


Hess et al, 2011

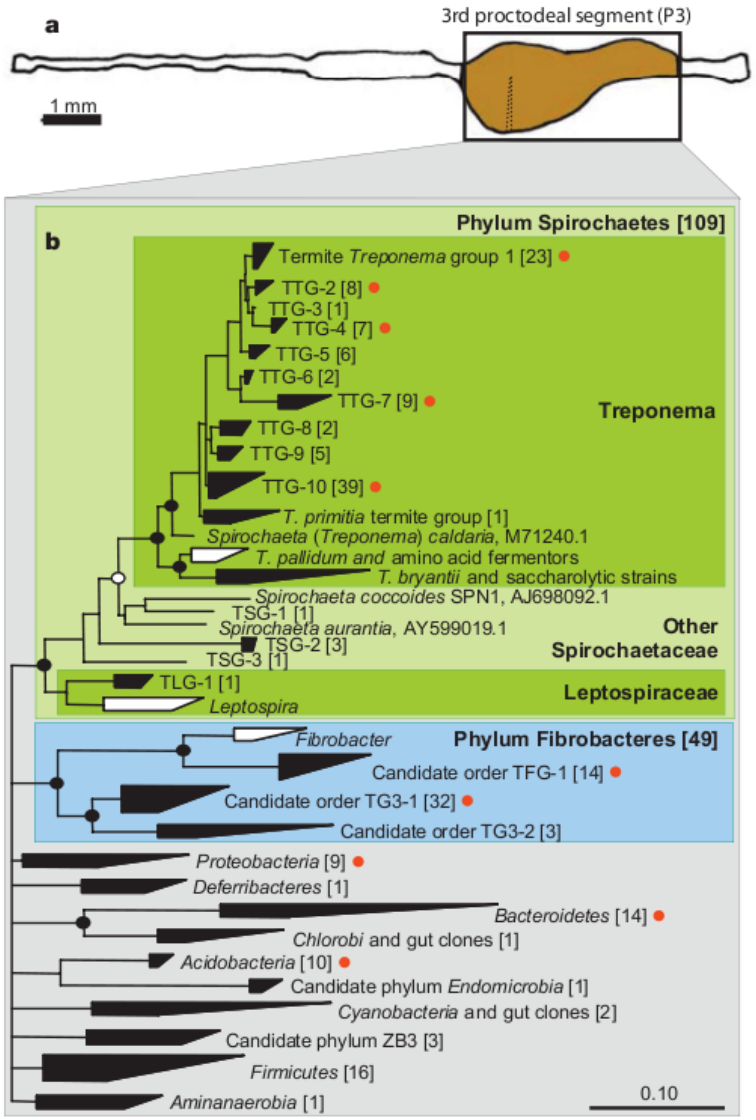
Identified 27,755 putative carbohydrate-active genes



...and also metagenomics of the termite gut

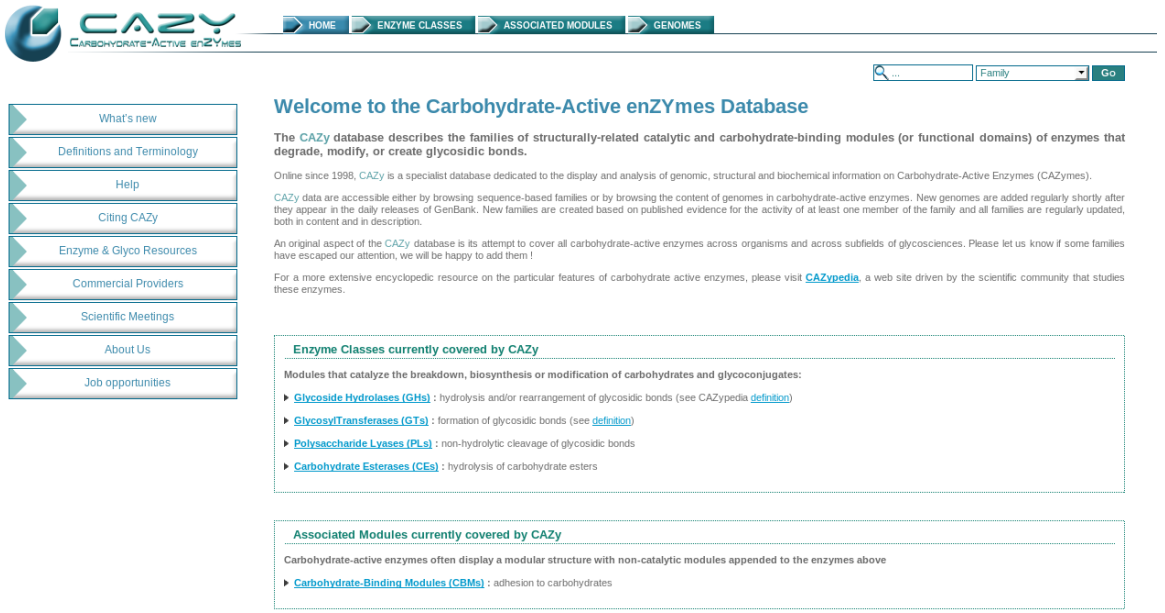


Hongoh 2011

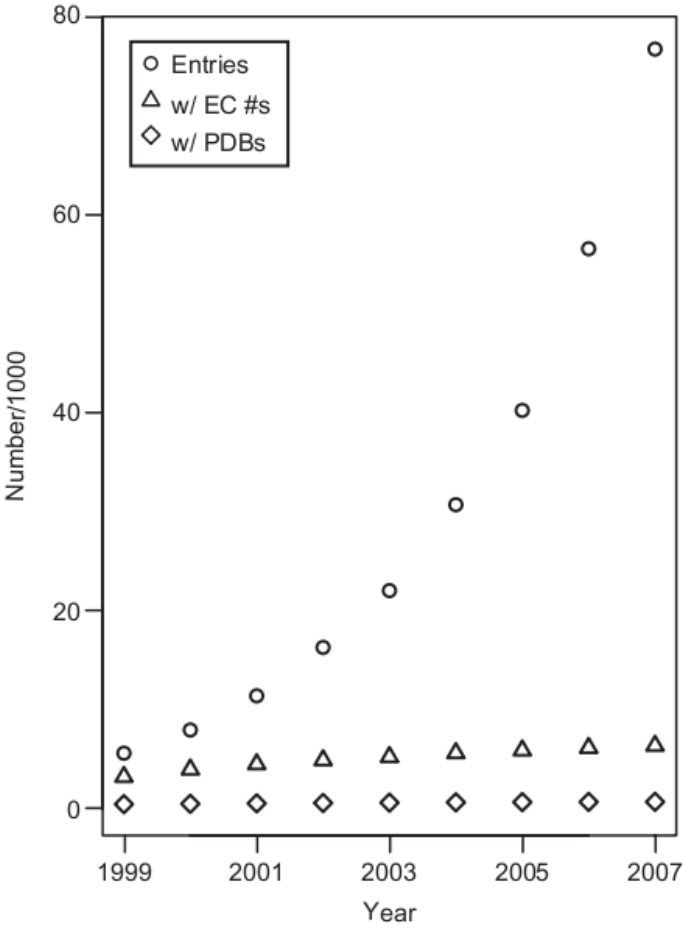


Warnecke et al, 2007

many putative cellulolytic enzymes sequenced, but what are their substrates?

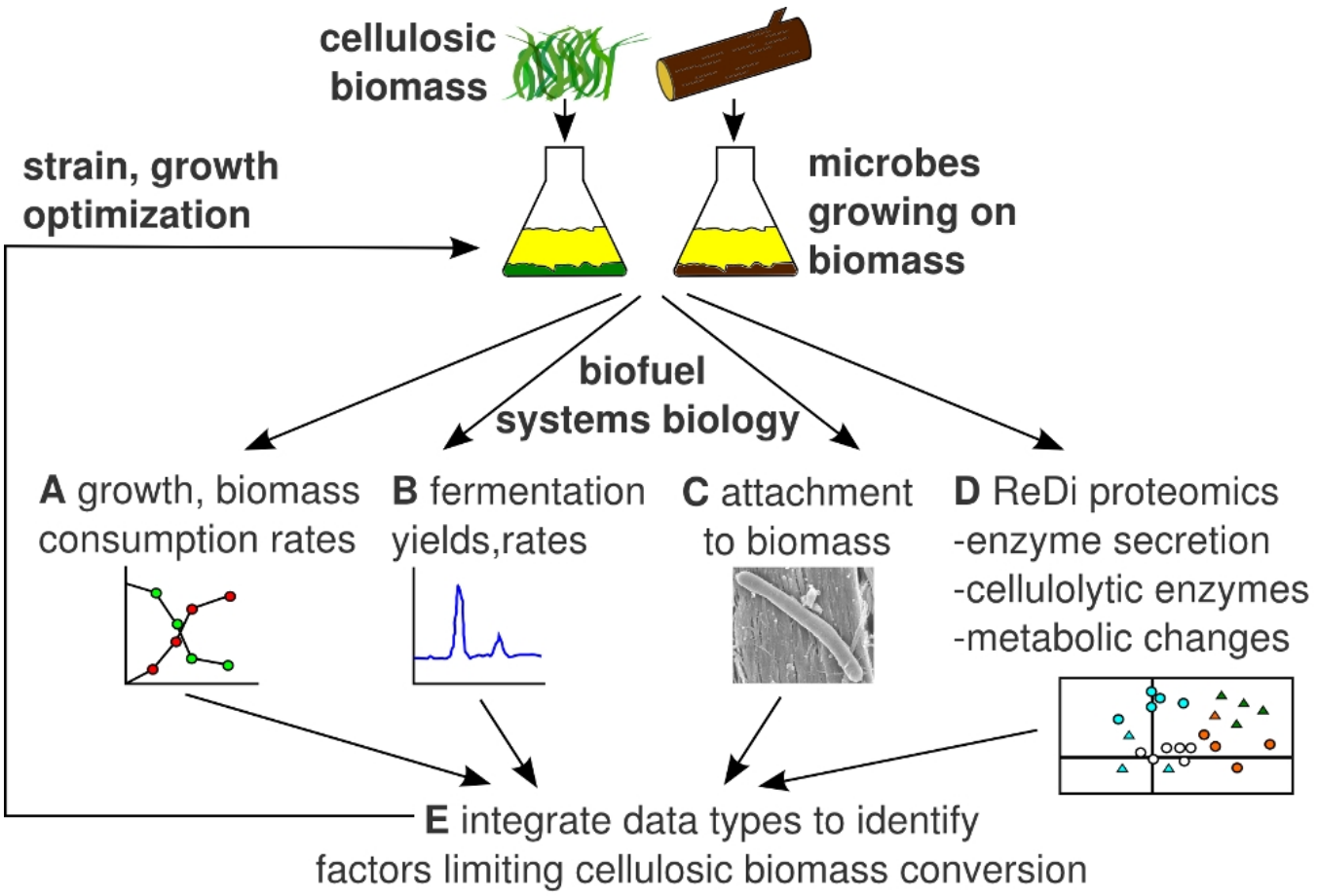


www.cazy.org



Cantarel et al, 2009

systems biology of cellulosic bioconversion



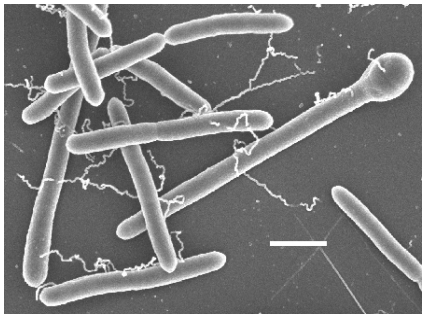
Tolonen et al, 2011

how proteins are identified by mass spectrometry

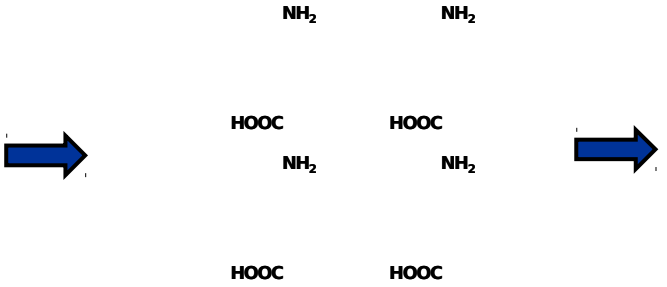
Sample Preparation

Data Acquisition

Data Analysis



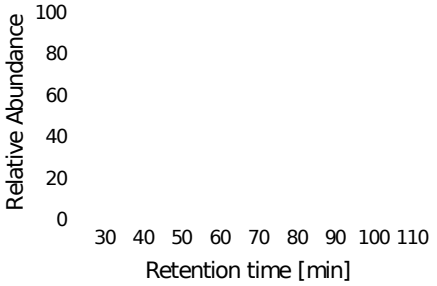
Cphy culture



protein lysate by french press

proteins trypsin peptides

LC-MS/MS



peparation of Peptides

Survey MS

MS Spectrum

MS/MS

MS/MS Spectra

MS/MS

Search Algorithm (SEQUEST)

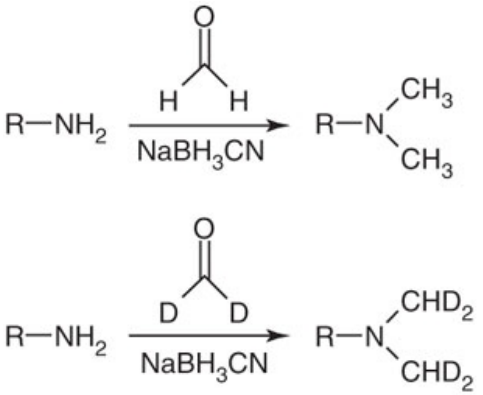
Protein Sequence Database

Peptide Identifications

Protein Identifications

quantify protein differences between treatments by reduction dimethylation (ReDi)

label amines with methyl isotopes

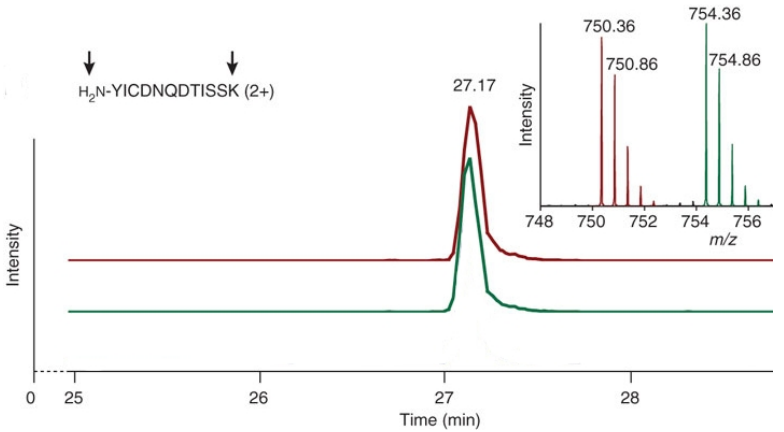


glucose

cellulose

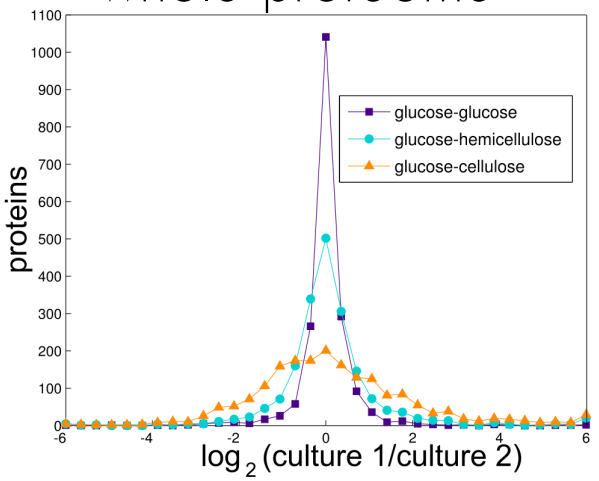
Mix 1:1

quantify expression changes using MS1 peak area ratios

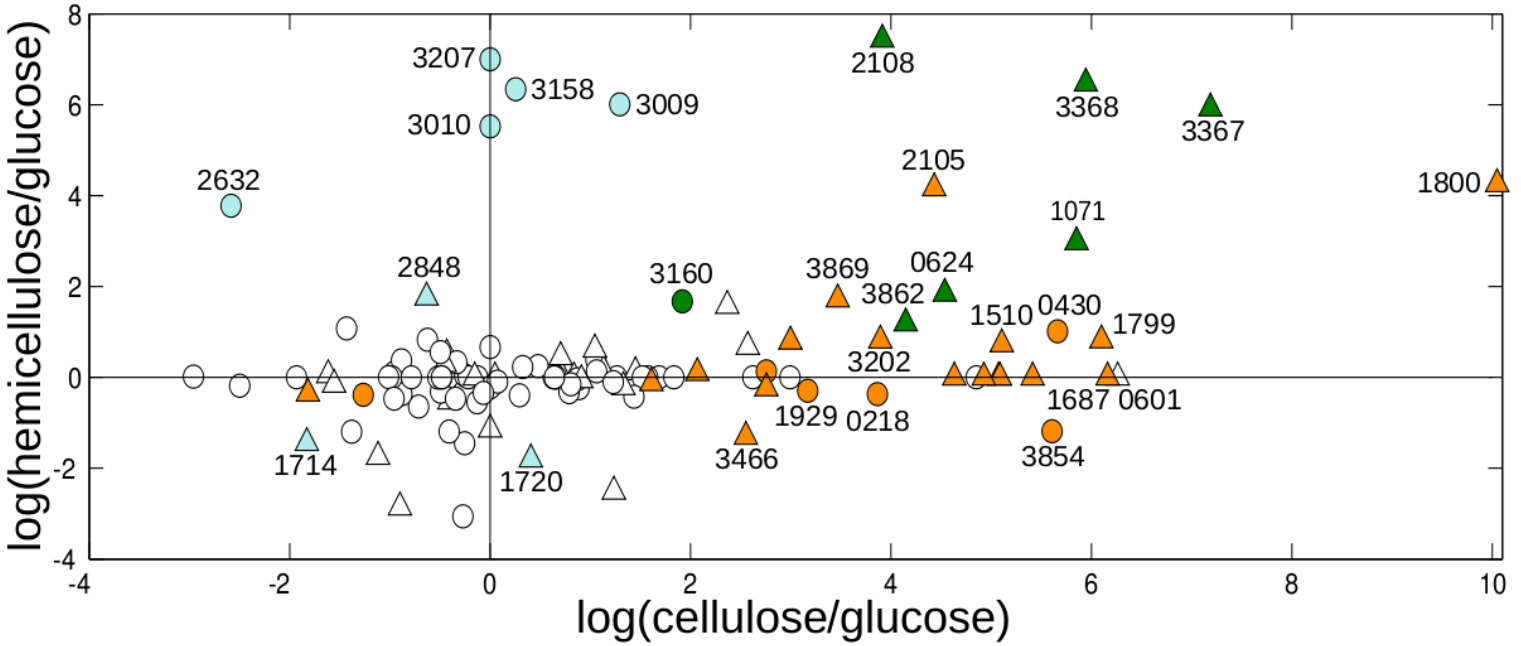


Boersema et al, 2009

whole proteome



distinct CAZy repond to hemicellulose and cellulose

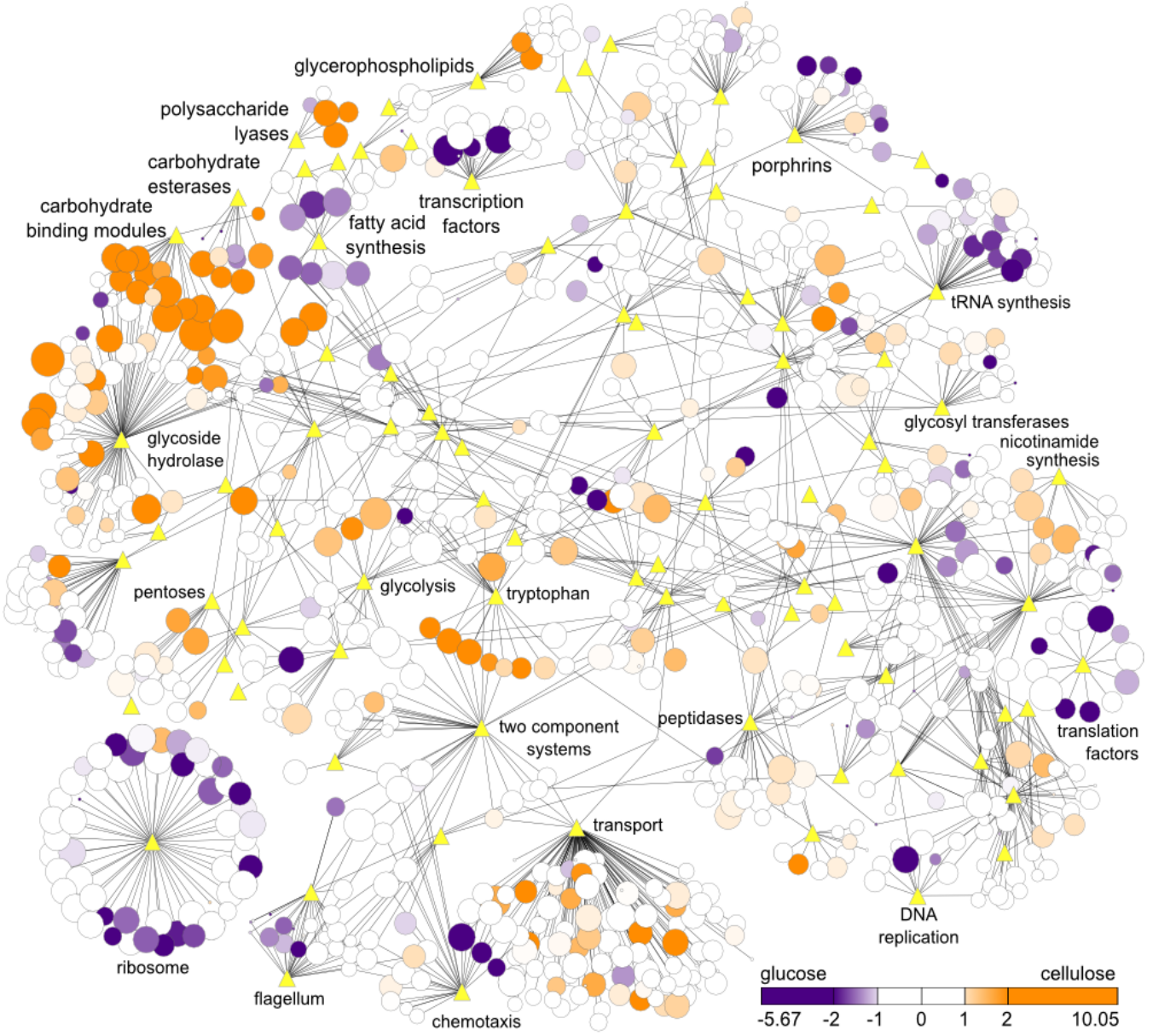


Tolonen et al, 2011

significant differential expression
cellulose=orange, hemicellulose=cyan, both=green

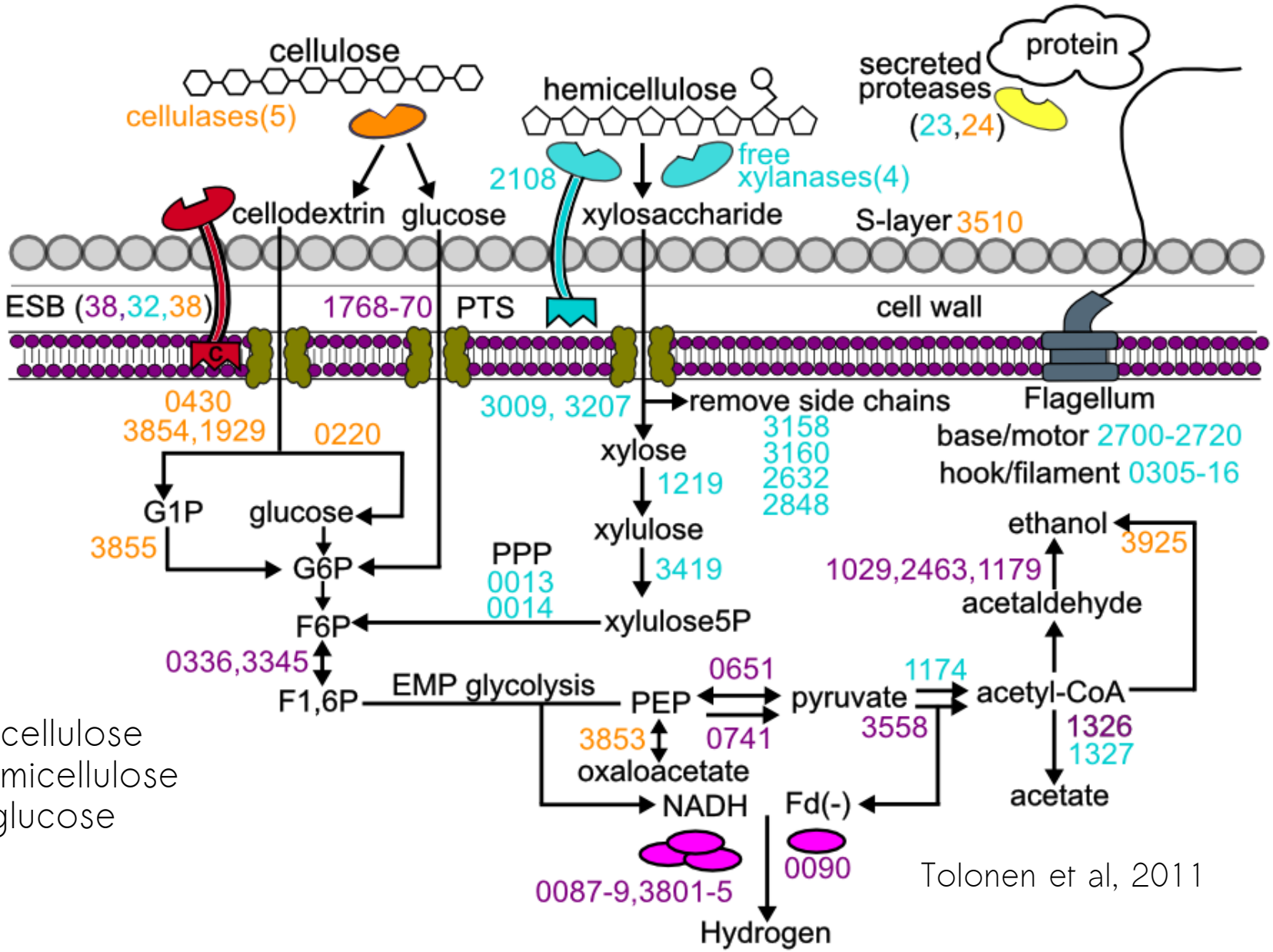
localization
secreted=triangle, intracellular=circle

interaction map of proteome expression changes



Tolonen et al, 2011

deconstruction and fermentation model

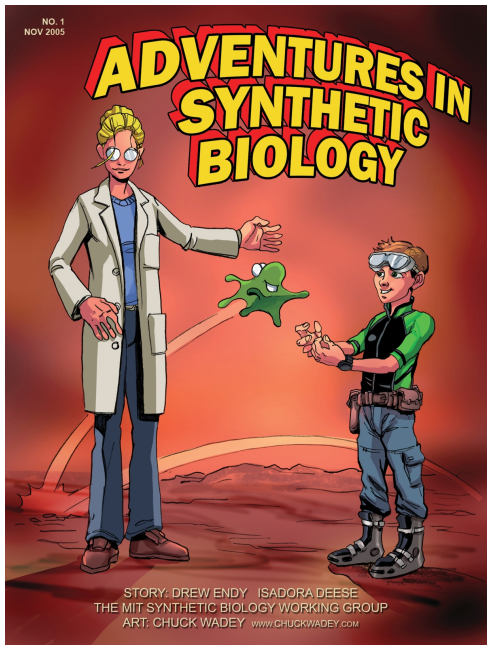


orange: cellulose
cyan: hemicellulose
purple: glucose

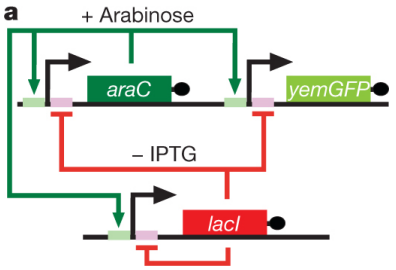
Tolonen et al, 2011

Synthetic biology: once we know the enzymes, we can engineer the microbes

de novo construct biocatalysts from model microbes

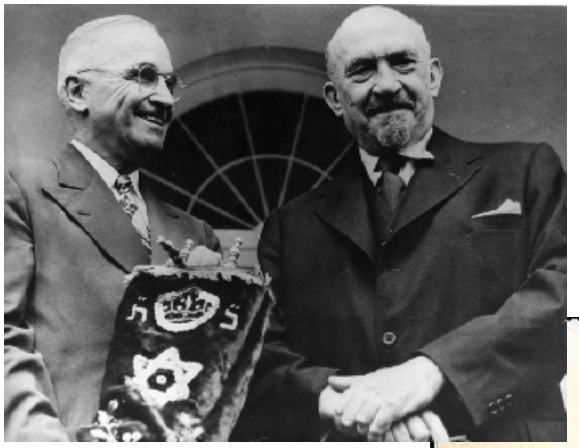


nature.com

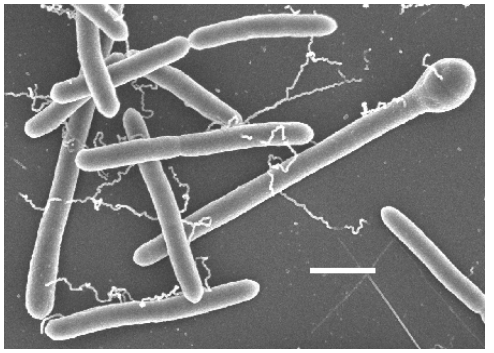


http://ucsdnews.ucsd.ed

study, modify natural isolates

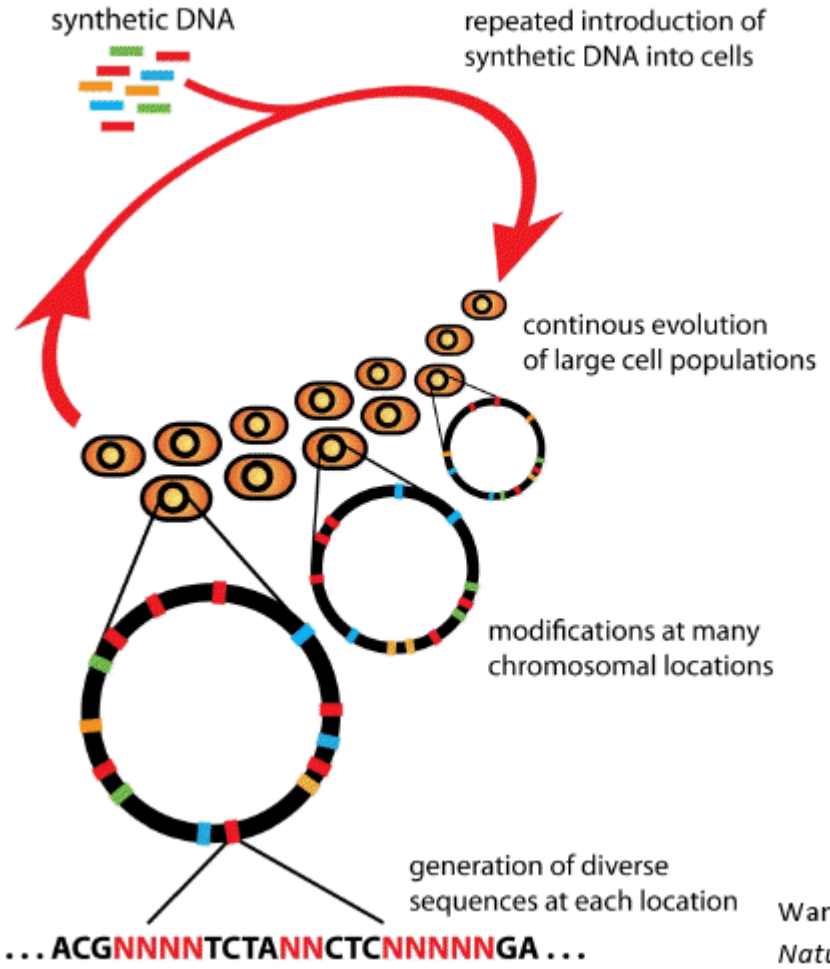


www.trumanlibrary.org



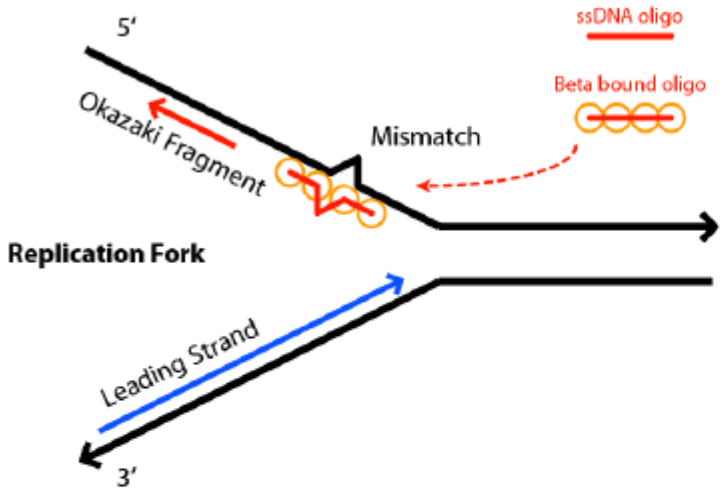
http://www.lesjones.com

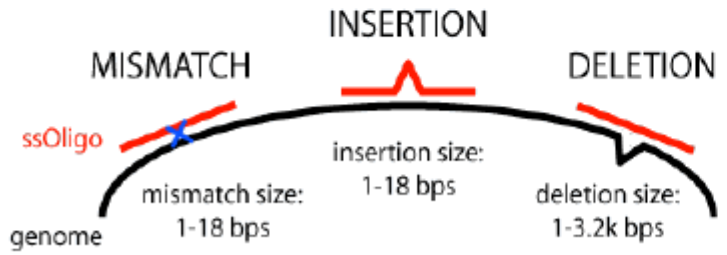
MAGE is a method to make many small changes to the *E .coli* genome



Wang, H.H., Isaacs, F.J., et al.
Nature (2009) ***460,*** 894-898

How MAGE works





Wang, H.H., Isaacs, F.J., et al. *Nature* (2009) ***460,*** 894-898

Expediting the design & evolution of organisms with new & improved properties

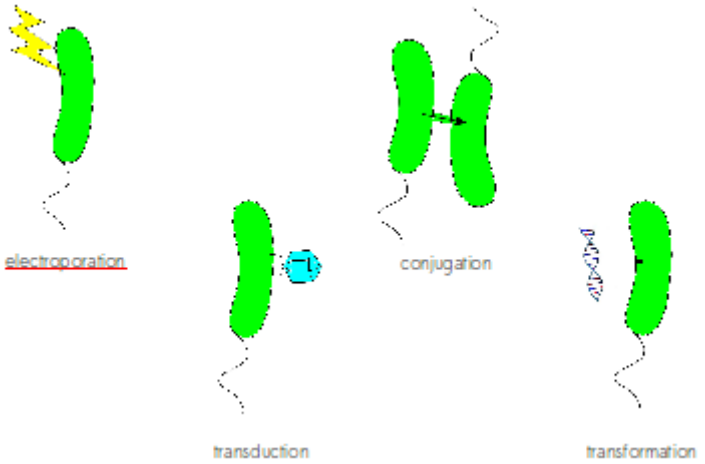
- Simultaneously targets many genetic locations
- Automatable
- Highly efficient genetic changes (>30%)
- Integrate engineering & evolution
- Combinatorial genomic diversity

Needs

- Fewer clones with no changes
- More simultaneous changes

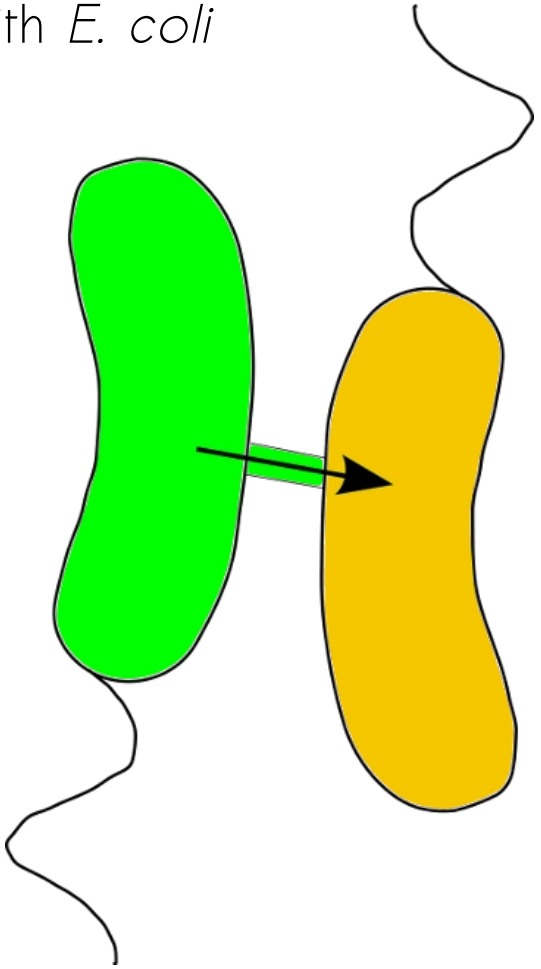
What about engineering novel bacteria? (ie *Clostridium phytofermentans*)

Delivery of foreign DNA into bacteria

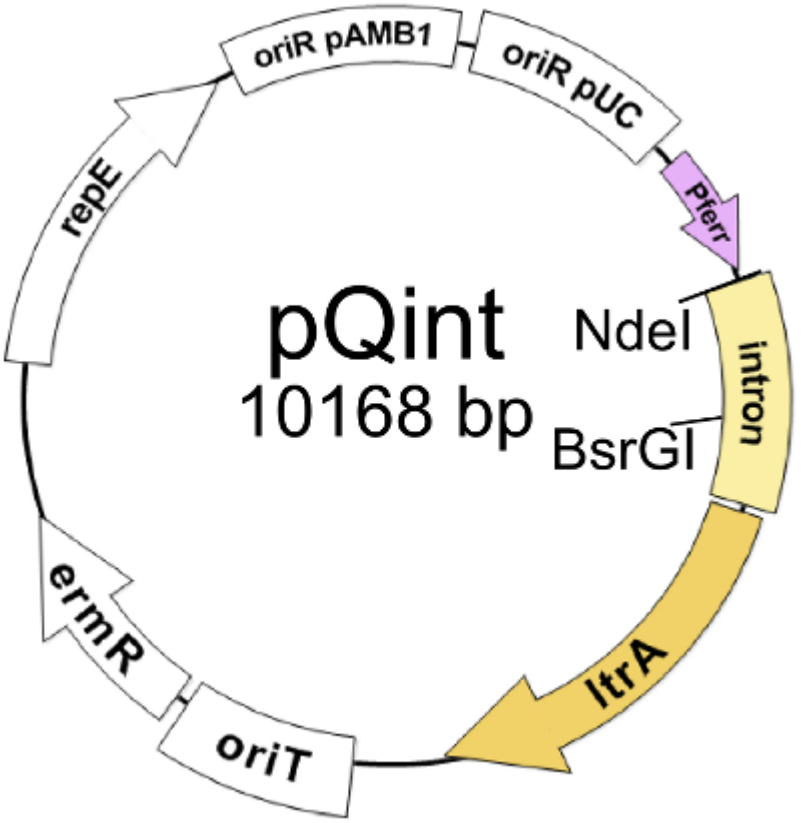


Plasmid DNA transfer to bacteria

DNA delivery by conjugation with *E. coli*



Plasmid for targeted chromosomal insertions

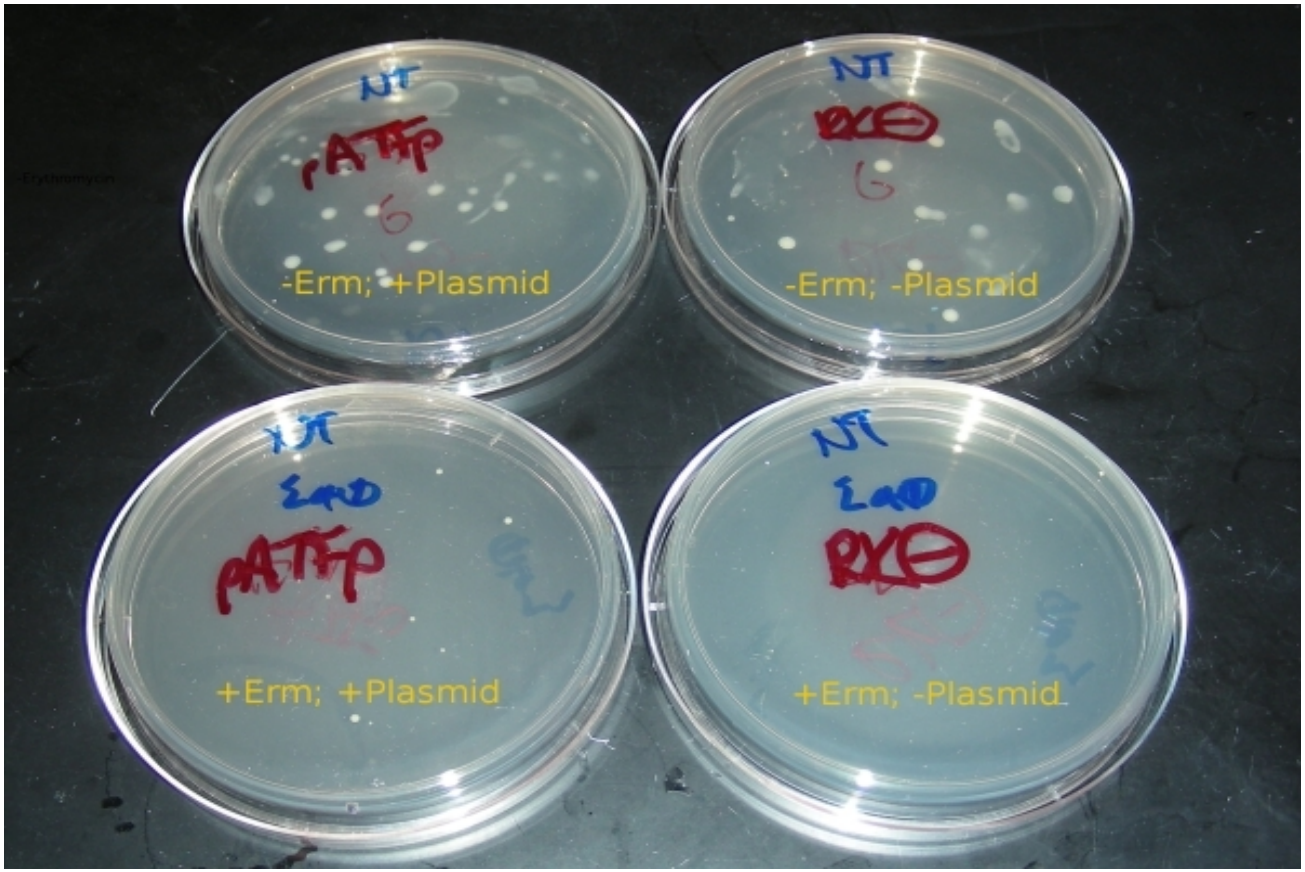


Tolonen et al, 2009

we need antibiotics to determine which cells got the plasmid DNA

10^{-6}x dilution

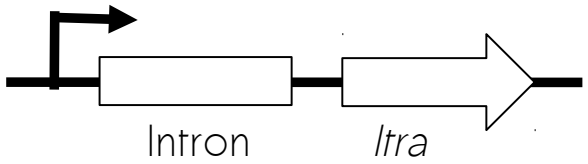
1x dilution

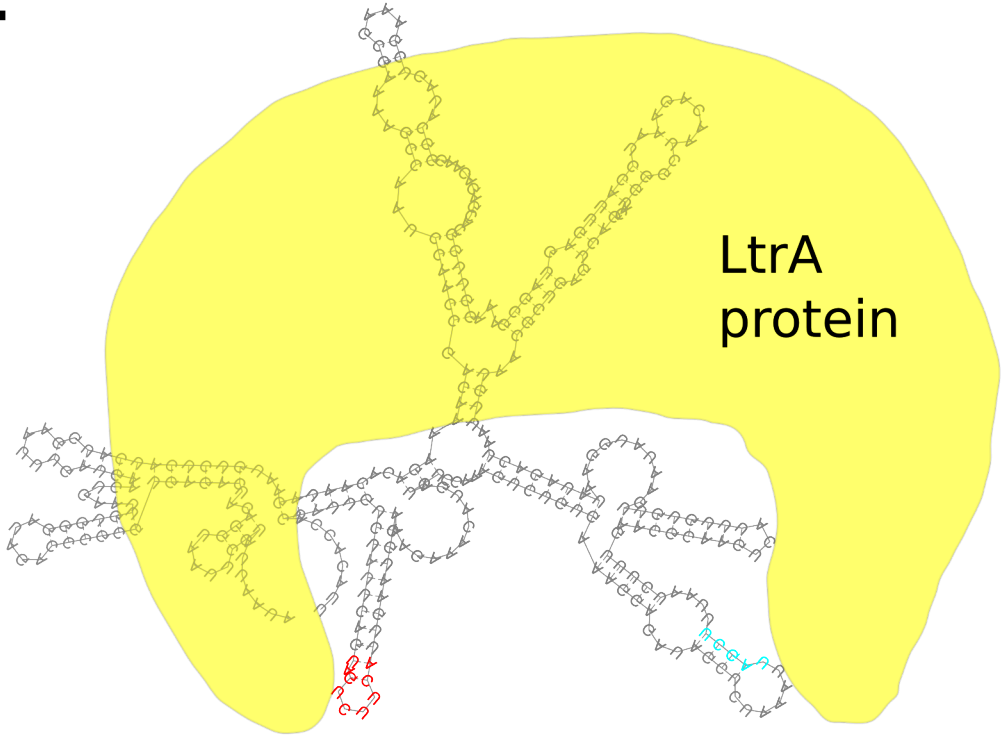


Now we have DNA in the cell. How do we get it into the genome?

Homologous recombination

group II introns are more efficient than recombination





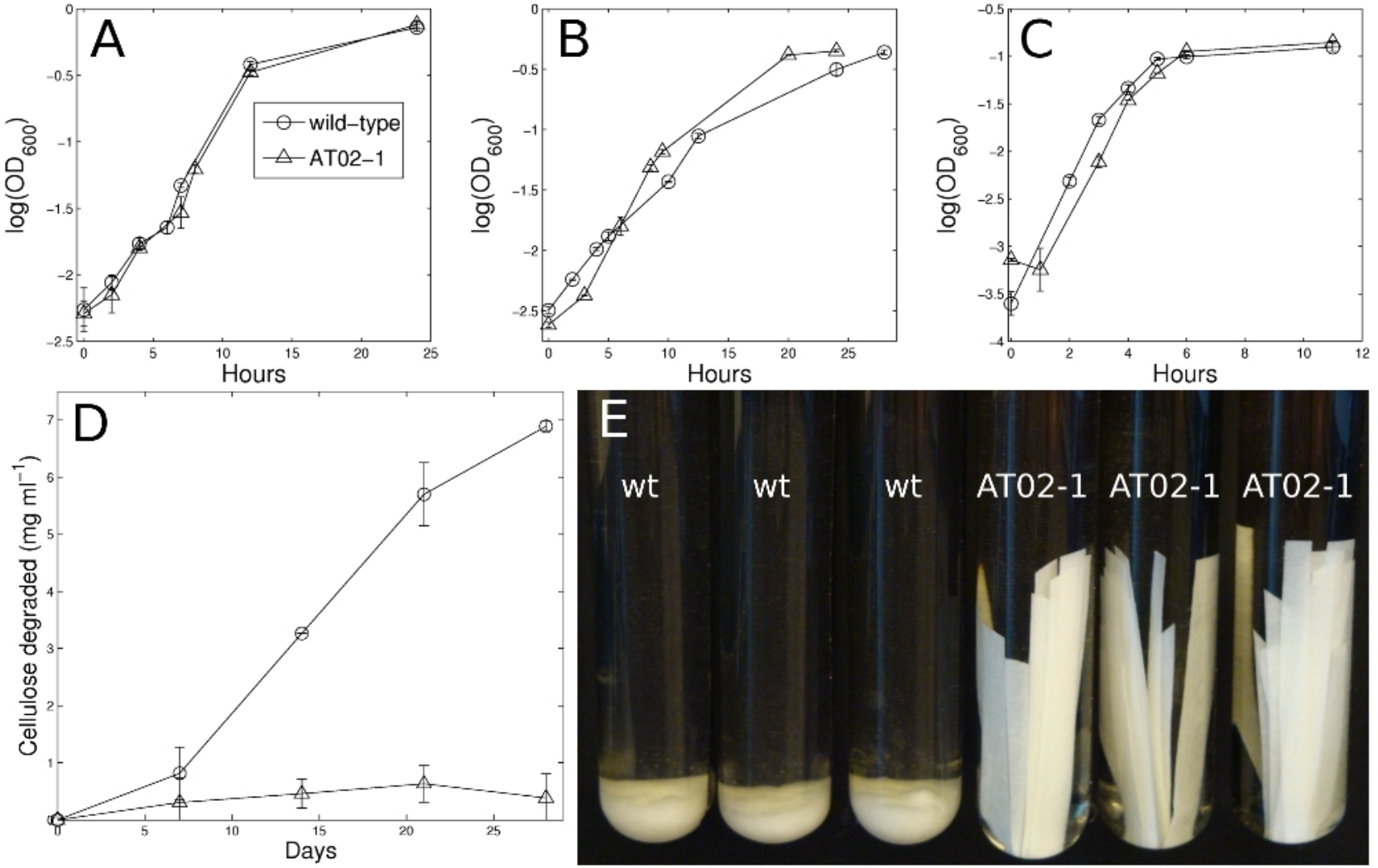
5'atgaaaaagataataagtcttttattagtgataacaCttctgatatccatggcaccatcgaaagctgacgcagcg3'
3'tacttttttctattattcagaaaataatcactattgtGaagactataggtaccgtggtagctttcgactgcgtcgc5'

LtrA EBS1 ESB2 LtrA

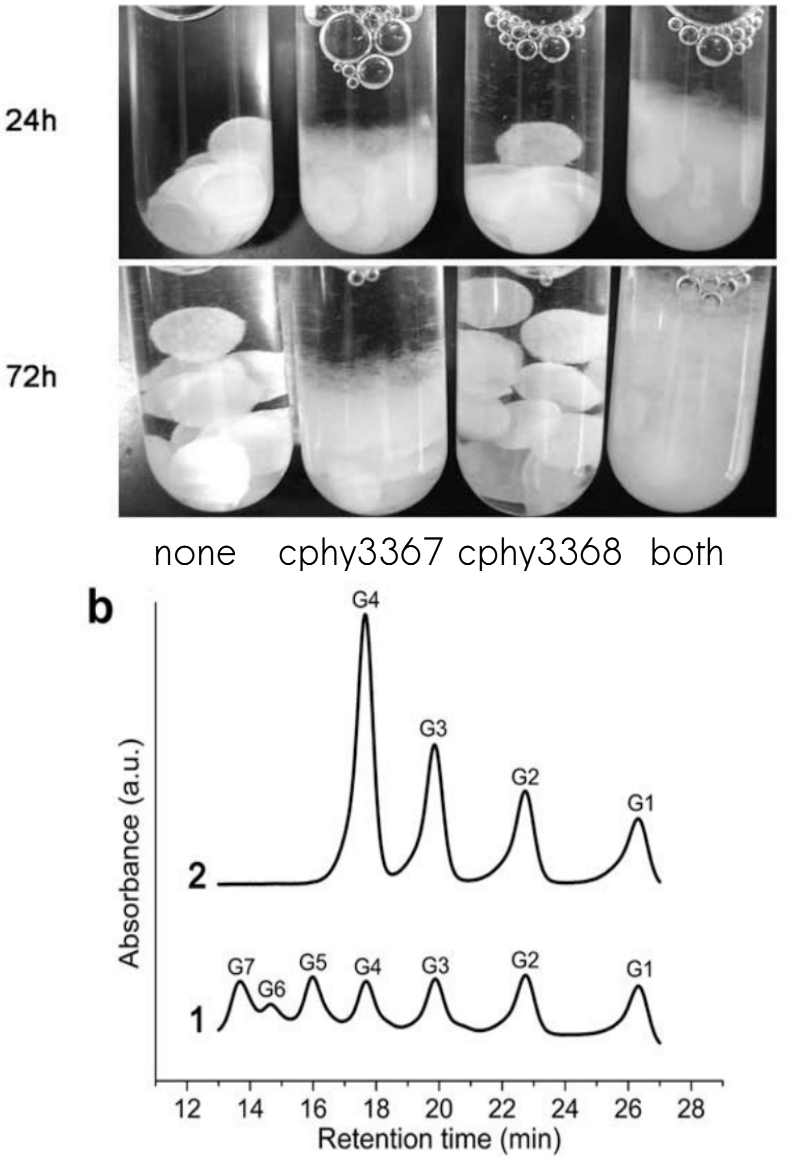
Insert site

a single cellulase is required for cellulose degradation

glucose cellobiose hemicellulose



Cphy3367: a "Rambo" cellulase



images from Zhang et al., 2010 (except Rambo)

Now we can efficiently modify the *Cphy* genome, our future directions are to use systems biology to guide genome engineering