Tool Development for Transformational Biotechnology Advances

ARPA-E Workshop

Disclaimer

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Introduction

- Jonathan J. Burbaum, PhD MBA
  - Education
    - PhD, Chemistry (Harvard), under Jeremy Knowles
    - Postdoc, Biology (MIT), under Paul Schimmel
    - MBA (UCSD)
  - Professional Background
    - Merck Research Labs (1991)
    - Entrepreneur:
      - Pharmacopeia (1993)
      - ActivX Biosciences (2000)
      - Azure Therapeutics (2004)
      - Gnosys Consulting (2004)
  - Program Director, ARPA-E (since 2010)
    - FOA1 Biofuels Projects (n=6)
    - PETRO Program (n≈10)

Agenda Overview

Thursday, October 6, 2011

8 :30 AM - 9 :00 AM	**Continental Breakfast**
9 :00 AM – 9 :15 AM	**Welcome and Opening Remarks,** Eric Toone, *ARPA-E*
9 :15 AM – 9 :40 AM	**Workshop Overview and Objectives,** Jonathan Burbaum, *ARPA-E*
9 :40 AM – 10 :30 AM	**Technology Overviews**
– 9 :40 AM – 10 :05 AM	**Overview of Plant Biology and Limitations,** June Medford, *Colorado State University*
– 10:05 AM – 10 :30AM	**Overview of Current Plant Transformation Technologies,** Neal Stewart, *University of Tennessee*
10 :30 AM – 10 :45 AM	**Coffee Break**
10 :45 AM – 12 :15 PM	**BREAKOUT SESSIONS Technology Barriers and Opportunities**
12 :15 PM – 1 :00PM	**Working Lunch- Summary of Breakout Sessions by Moderators, Discussion**
1 :00 PM – 2 :45 PM	**EXERCISE: Design the ideal system to rapidly transform and screen plants.**
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3 :30 PM – 4 :30 PM	**Group Discussion/debate on Presented Ideas**
4 :30 PM – 5 :30 PM	**Networking over Refreshments**

Dinner on your own

Friday, October 7, 2011 (Optional)

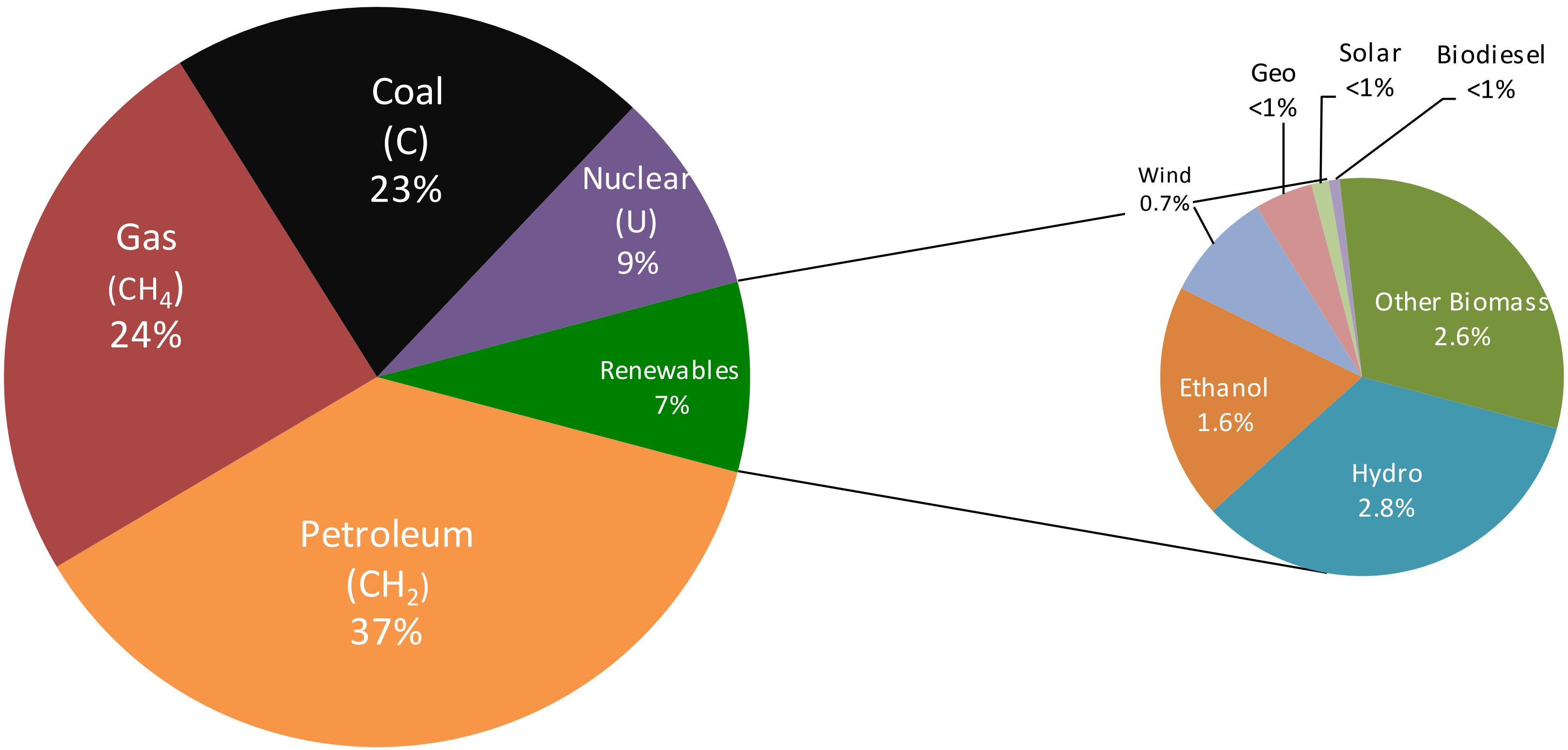
8 :00 AM – 8 :30 AM	**Continental Breakfast**
8 :30 AM – 10 :00 AM	**Preliminary Conclusions, Open Discussion**

- What have we learned?
- What is and is not possible?
- What is “evolutionary” versus “revolutionary”?
- What can we do now?
- How will we tell that we’ve succeeded? (metrics)

10 :00 PM – 2:15 PM	**Coffee and One-on-one Discussions**

Please contact the organizers to schedule one-on-one time with program directors

Energy Sources in the US

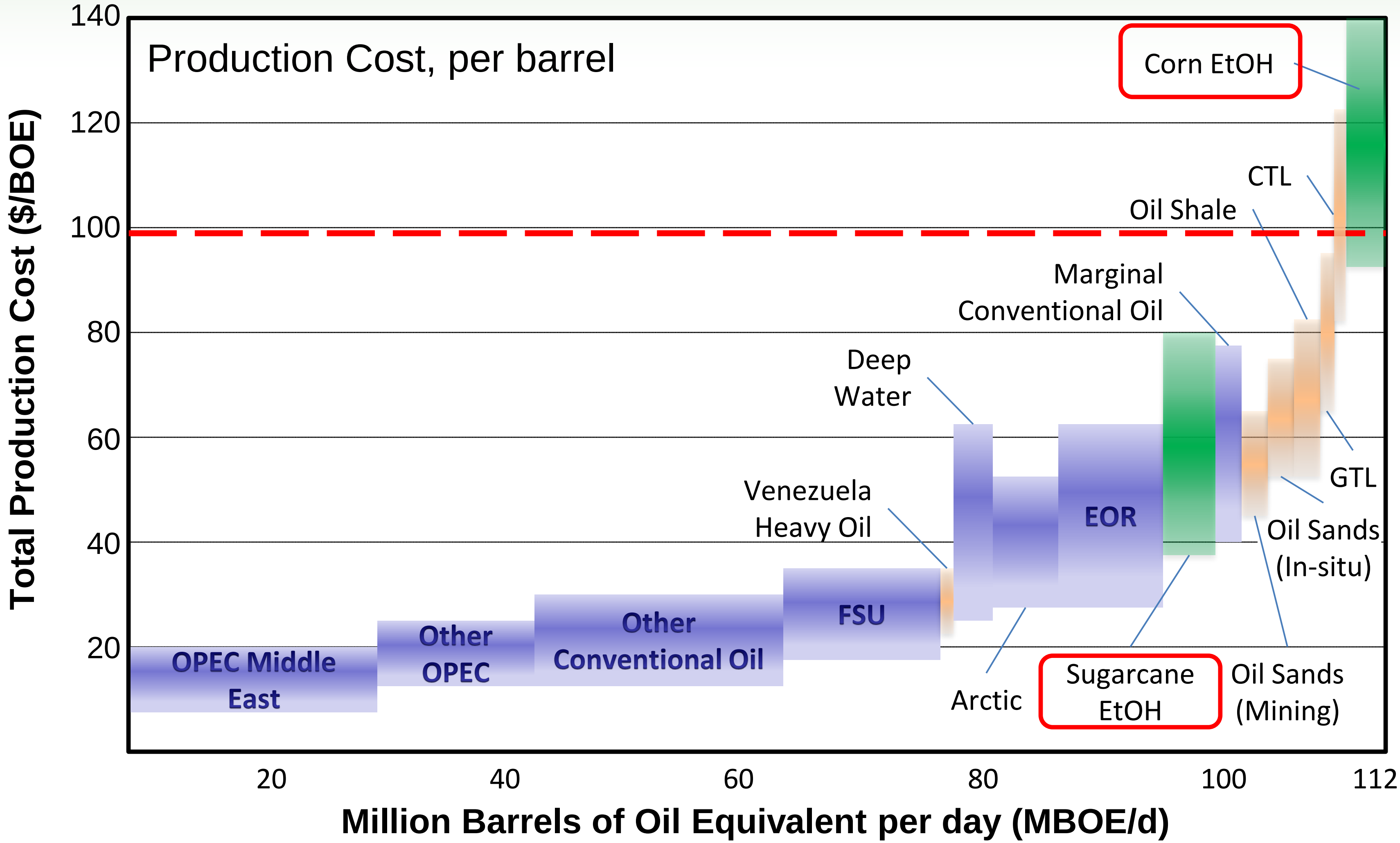


Total Energy Sources

Renewables

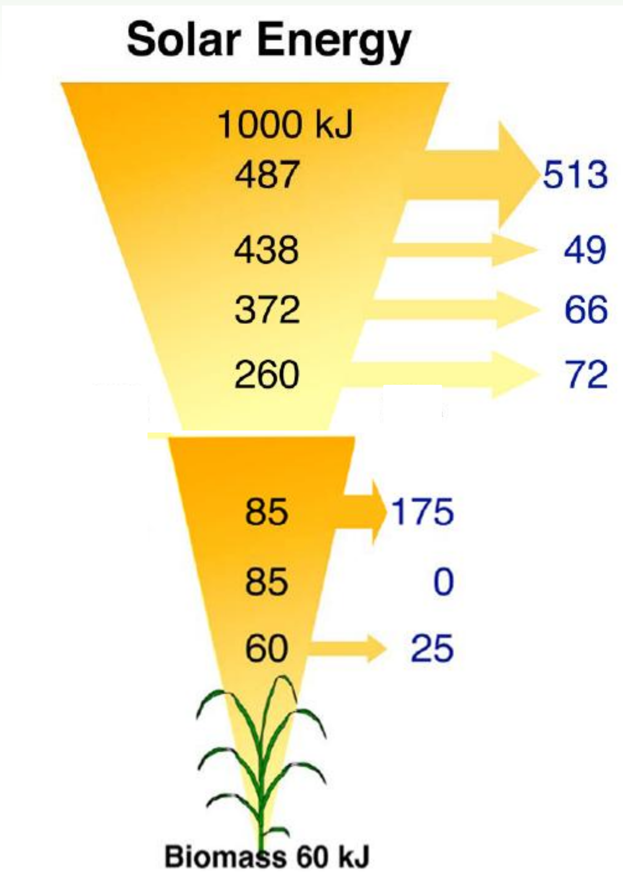
Source: DOE EIA Annual Review, 2009

Biofuels in a Petroleum Context

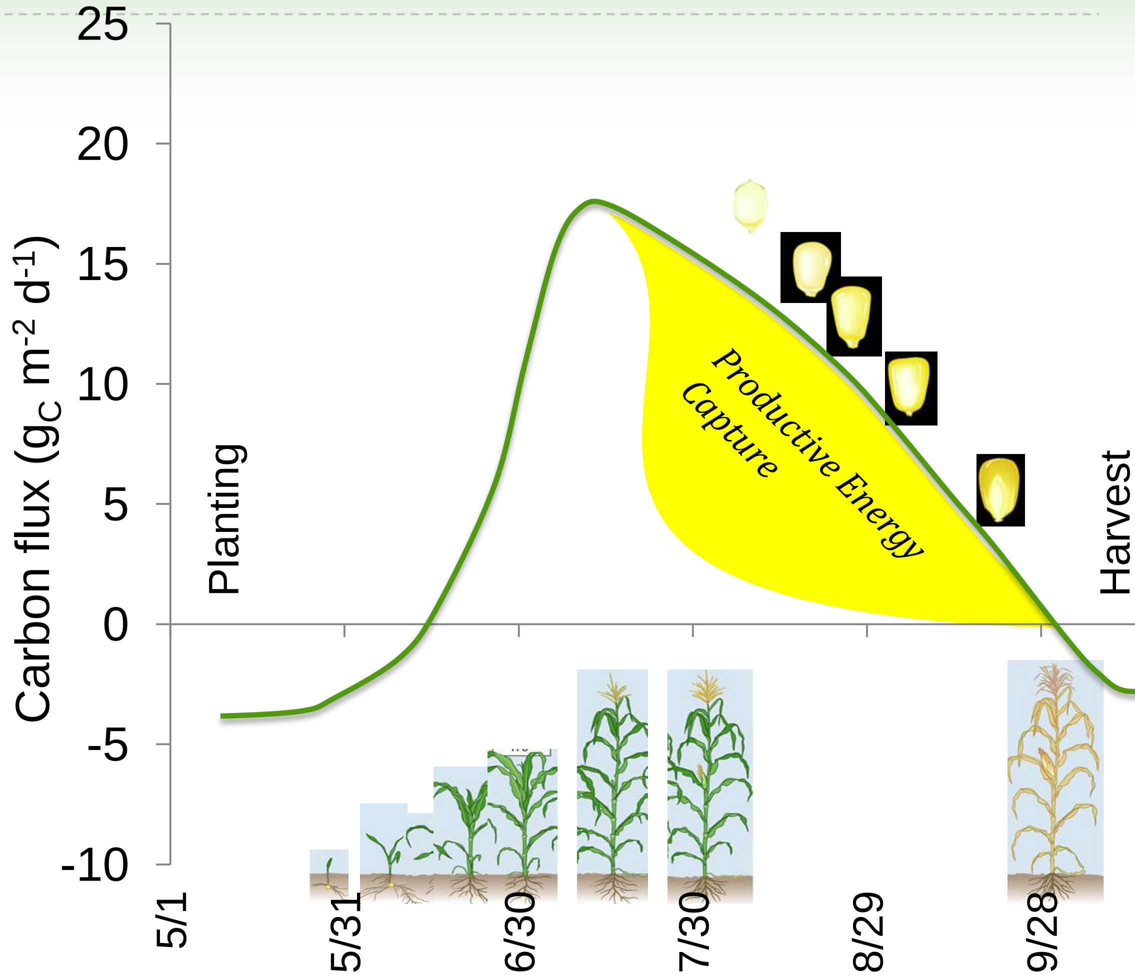


Source: Analysis based on information from IEA, DOE and interviews with super-majors

What Does Corn Do?



Zhu et al. *Current Opinion in Biotechnology* (2008) 19:153-159



Adapted from Verma et al, Ag Forest Meteorology 131, 77-96 (2005) & NDSU "**Corn Growth and Management Quick Guide A-1173" (http://www.ag.ndsu.edu)** , June 1999

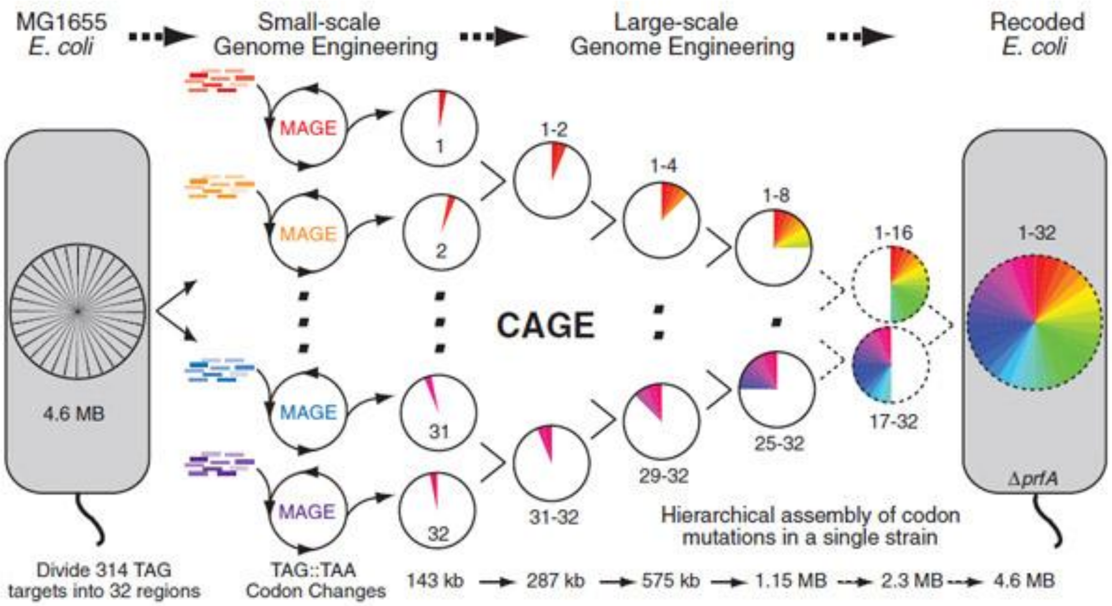
Premise of the Workshop

- Agriculture, as process engineering, is far from optimized,
  - Inefficient energy transduction ($\eta < 6\%$)
  - Wasteful carbon capture and processing (yield < 25%)
  - Sequestration in difficult-to-process forms (e.g., cellulose)
- New tools lead to transformational changes, and
- There are opportunities for significant advances.
  - "Plant Biotechnology" lags other biotechnologies
    - Is it able to progress more rapidly as a consequence??
  - "Genetic Engineering" remains aspirational
    - "Art", at best, for all but a few well-understood organisms…
    - …but progress ≈ data throughput.
  - "Synthetic Biology" is in its infancy
    - "…new biological parts, devices and systems…" [syntheticbiology.org]
    - "… novel artificial biological pathways, organisms or devices…" [Royal Society]
    - "… combines science & engineering [for] novel biological functions & systems…" [SynBERC]
    - "… the engineering of biology…" [European Commission]

vs.

Examples of Recent Advances in Science

- Large scale genome wide modifications in microbes using techniques such as Multiplex Automated Genome Engineering (MAGE).



- Conjugative Assembly Genome Engineering (CAGE) converts all TAG codons in E. coli to TAA [Isaacs et al., *Science* **333**, 348 (2011)

Examples of Recent Advances in Science

- Endophytes
  - Live inside all plants
  - Confer beneficial phenotypes, e.g.,
    - drought tolerance
    - nitrogen fixation
    - protection against pathogens
  - Can potentially be used as gene delivery systems

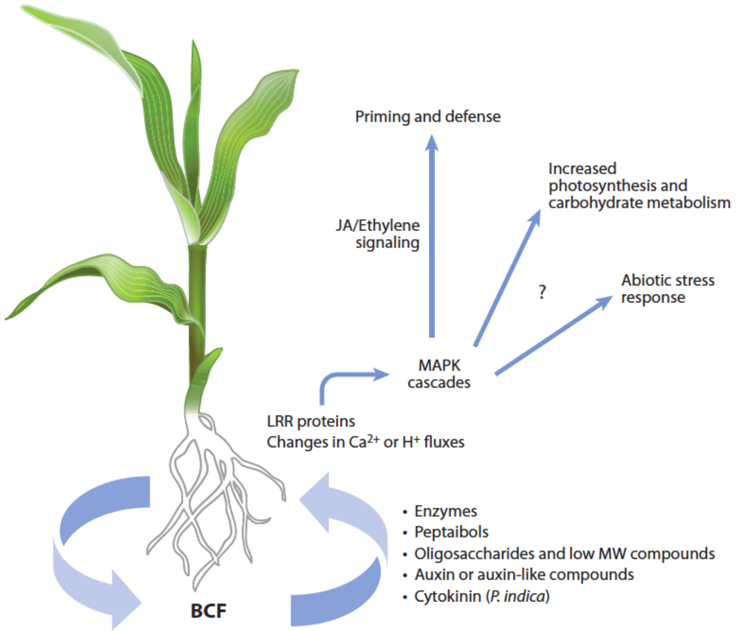
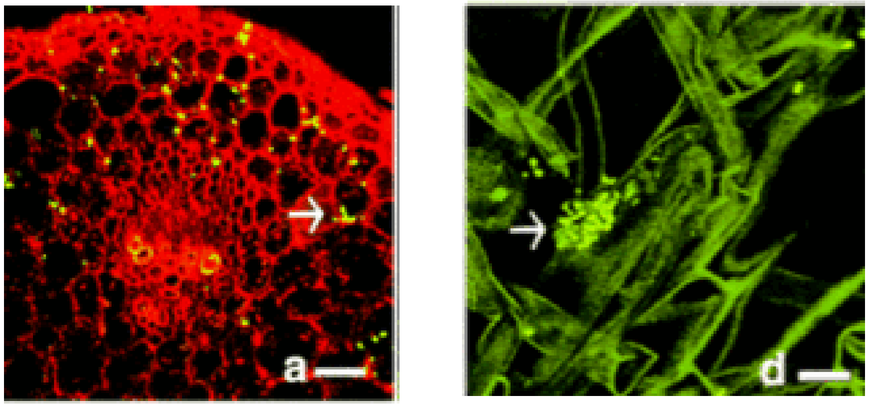


Figure 1

Biocontrol fungi (BCF) grow to interact with the roots. By forming this interaction, the BCF and the plant exchange signals. BCF releases elicitors into the zone of chemical communication (both outside and inside

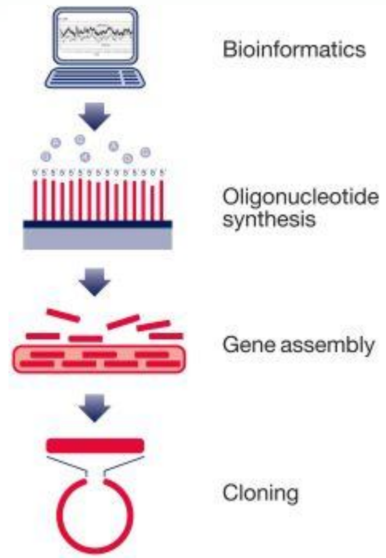
Shoresh et al., *Annu. Rev. Phytopathol.*, **48**, 21 (2010)



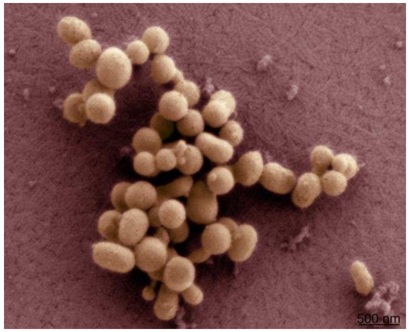
K. pneumoniae in maize cells. (Chelius & Triplett, 2000).

Examples of Recent Advances in Science

- Large scale gene synthesis capabilities now allow the routine production of plasmids containing entire gene pathways.



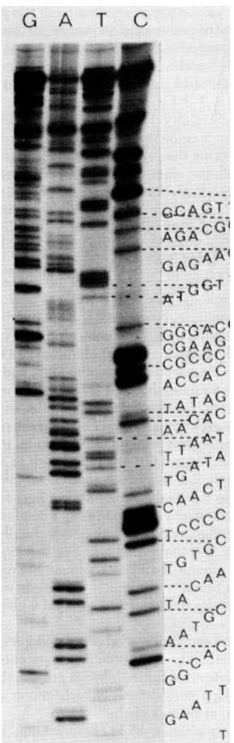
- This technology can be scaled up to synthesize entire genomes *de novo* and incorporated into microbial cells.



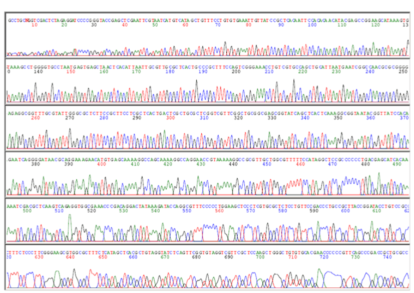
M. mycoides, JCVI-syn1.0, Venter Institute

Progress of Biotechnology

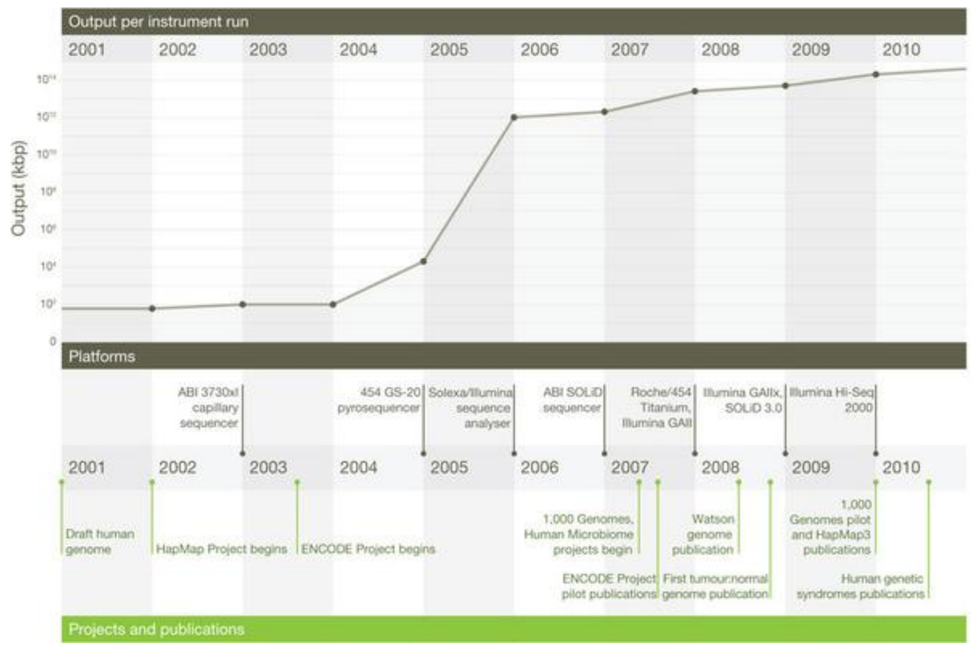
Year	Strain	Plasmid	Genotype (DNA genes only)	Efficiency (cfu/ug)	Efficiency (%)
1972	C600	R6	-	5 x 10^4	0.00001
1985	DH1	pBR322	endA1 recA1 gyrA96 relA1 hsdR17(r_K^- m_K^+)	5 x 10^6	0.001
1993	XL-10	pUC18	endA1 recA1 gyrA96 relA1 Δ(mcrA)183 Δ(mcrCB-hsdSMR-mrr)173	5 x 10^9	1



1977



1998



2001

2010

Examples of Recent Advances in Technology

- Image analysis systems can follow plants from seedling to maturity

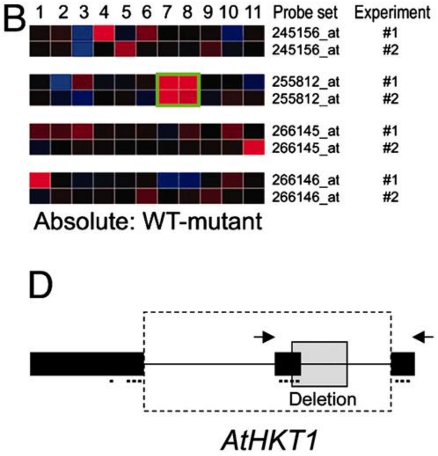
- High-throughput phenotype analysis:
  - Growth rate
  - Developmental abnormalities
  - Responses to stressors
  - Chlorophyll content

Phenofab

Examples of Recent Advances in Technology

- Following the development of DNA based genechips for genomic analysis, microarrays have been developed to quantify protein expression, carbohydrates, and levels of small molecules.

One application of genechips has been to allow rapid mapping of genetic modifications in plants. Genechip analysis of an Arabidopsis mutant that overaccumulated sodium identified a deleted region in the genome (highlighted in red, panel B) corresponding to the *AtHKT1* transporter.

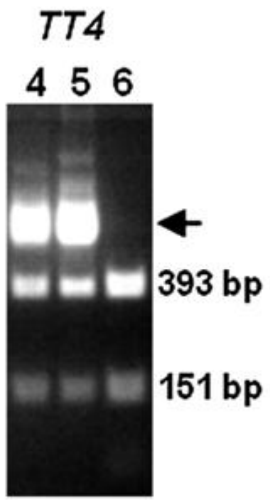


Gong et al, PNAS (2004)

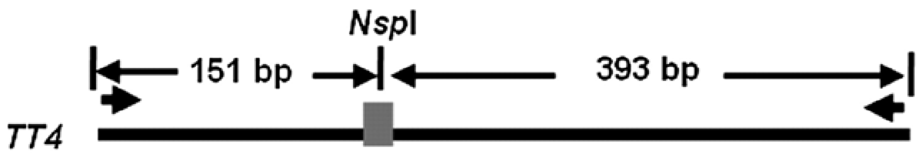
Examples of Recent Advances in Technology

- Targeted genome engineering to allow precise integration of a transgene or disruption of native genes.

- Enzymes (zinc-finger, meganucleases, TALENs) can be engineered to recognize specific gene sequences (> 20 bp) in a plant genome.



A zinc finger nuclease was engineered to recognize an 18 bp sequence in Arabidopsis containing a NspI restriction site.



Zhang et al. PNAS (2010)

An ARPA-E Project has four main attributes

IMPACT

If successful, project could have:

- High impact on ARPA-E mission areas
- Large commercial application

BREAKTHROUGH TECHNOLOGY

Technologies that:

- Do not exist in today's energy market
- Are not just incremental improvements; could make today's technologies obsolete

ADDITIONALITY

- Difficult to move forward without ARPA-E funding
- But able to attract cost share and follow-on funding
- Not already being researched or funded by others

PEOPLE

- Best-in-class people
- Teams with both scientists and engineers
- Brings new people, talent and skill sets to energy R&D

ARPA-E's Funding Rubric

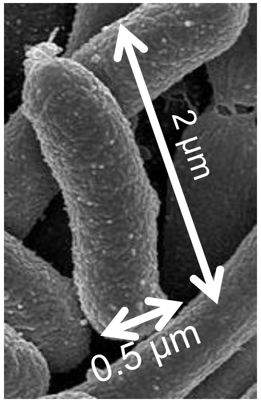
- arpa·e
  - Funds the development of **disruptive new technologies rather than new scientific knowledge**
  - Focuses on **high-risk, high-reward projects with significant commercial potential**
  - Chooses projects that that are **generally unable to attract private sector financing** because of the significant risks involved

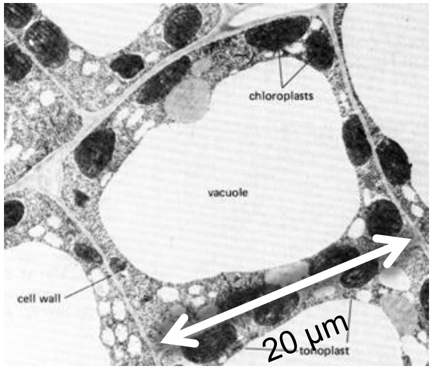
Agriculture provides a low cost production platform at scale

- Fuels = Commodity: Cost is the major determinant
- Low capital costs for large area
- Low operational costs: Can use a variety of inputs

But genetic engineering of plants is *much* more difficult

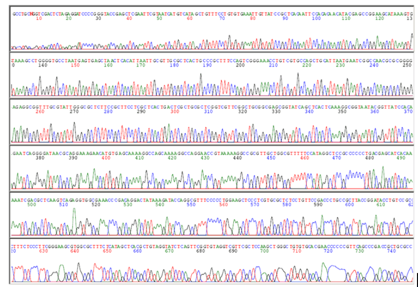
E. Coli	Agricultural Crops
• Single type of cell	• Differentiated cells
• One compartment	• Multiple compartments/organelles
• Single membrane	• Multiple membranes/cell walls
• Single genome	• Multiple genomes
• Reproduce every 20’	• Reproduce annually
• Haploid	• Diploid/polyploid
• Heterotrophic (fixed food)	• Autotrophic (diurnal, CO2)

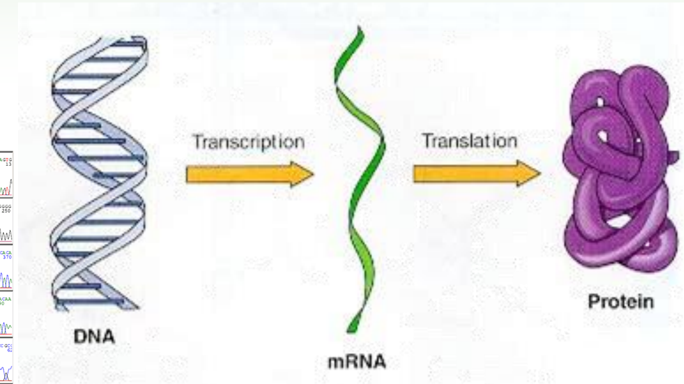


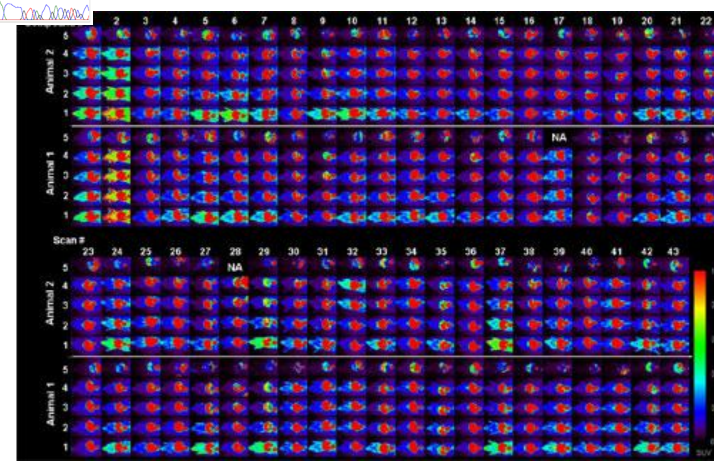


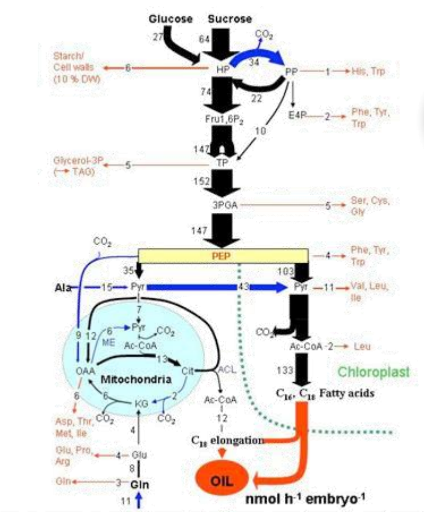
What has biology + engineering done already?

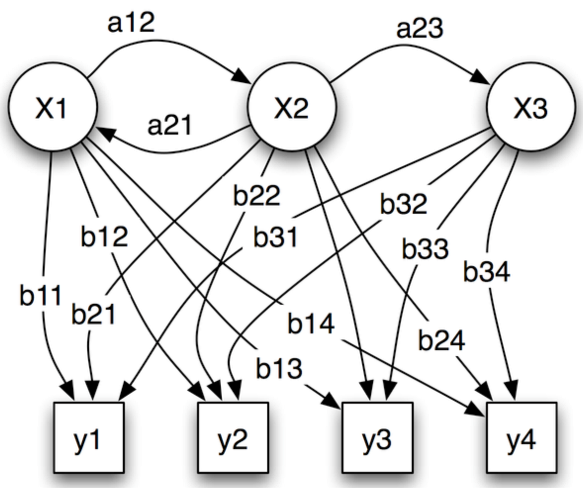
- DNA Technologies
  - Synthesis
  - Analysis
- ‘High-throughput’ analysis
  - More data/time
  - Fewer human errors
- Computational Algorithms



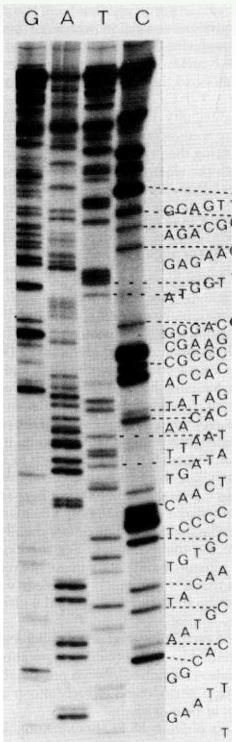


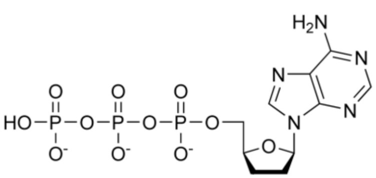






Example 1: Automated DNA Sequencing





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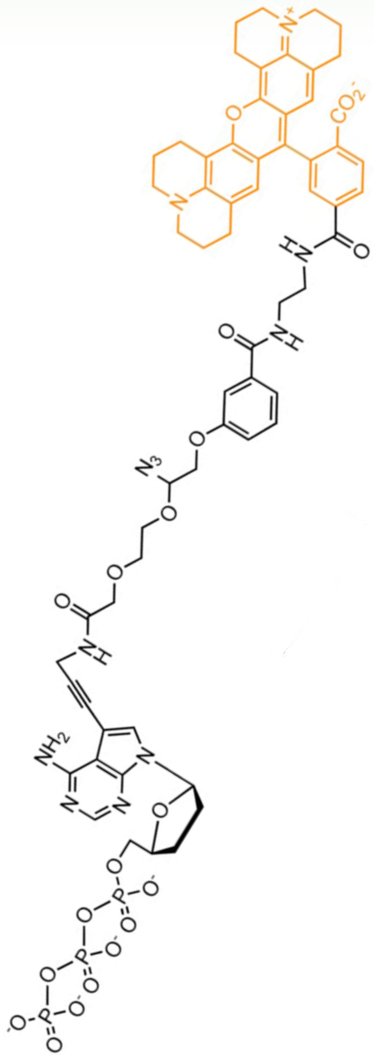

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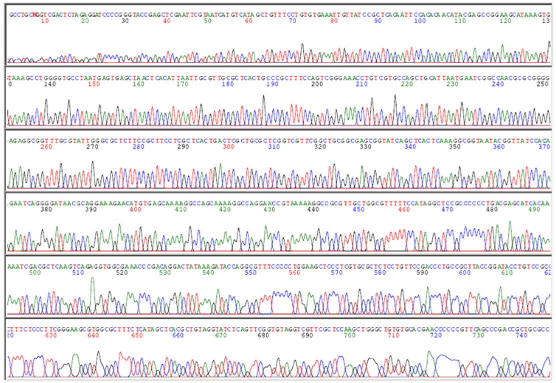

Example 2: PCR

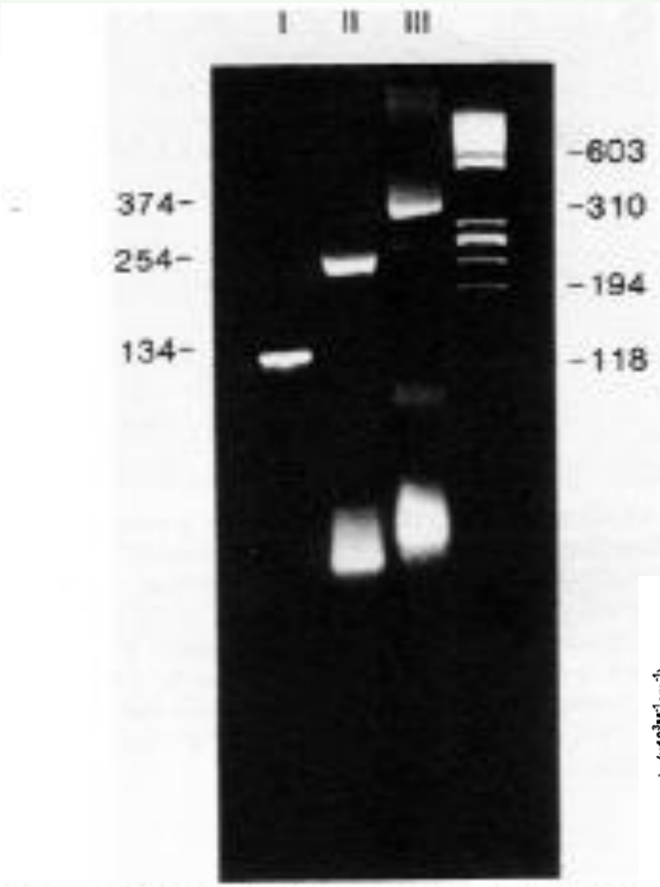

Figure 5. PCR construction of a 374-bp DNA frag...

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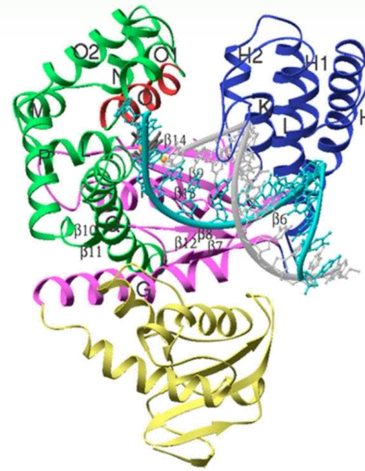

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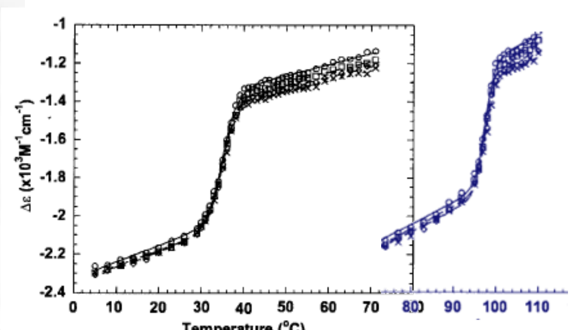
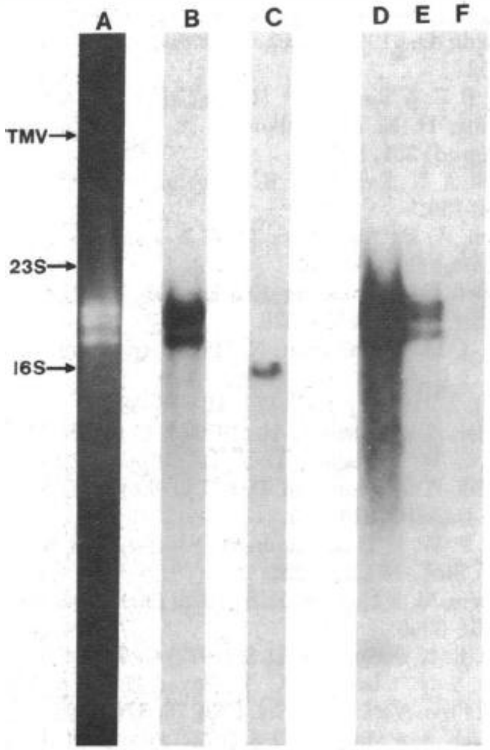


Mullis, 1986: 25 addition steps of Klenow DNA Polymerase

Example 3: Whole genome expression analysis



Alwine et al., 1977

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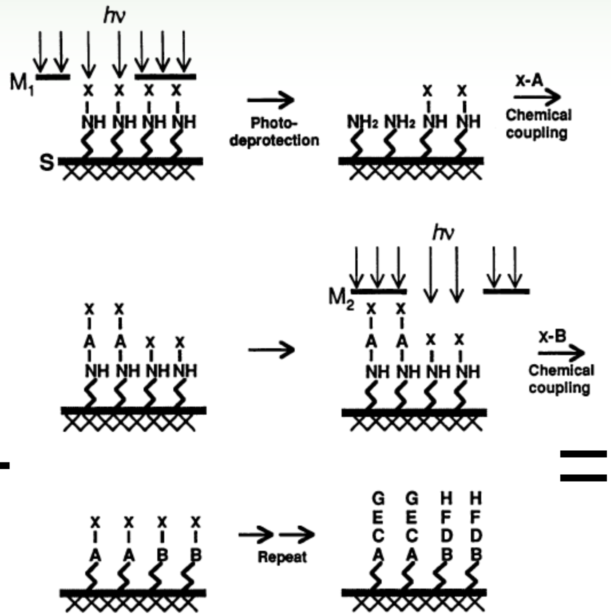

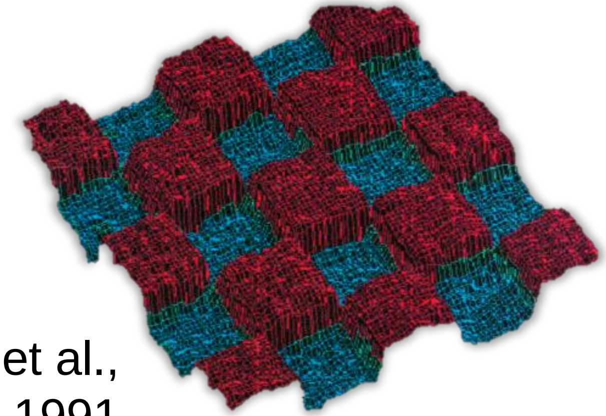

Fodor et al.,
1991

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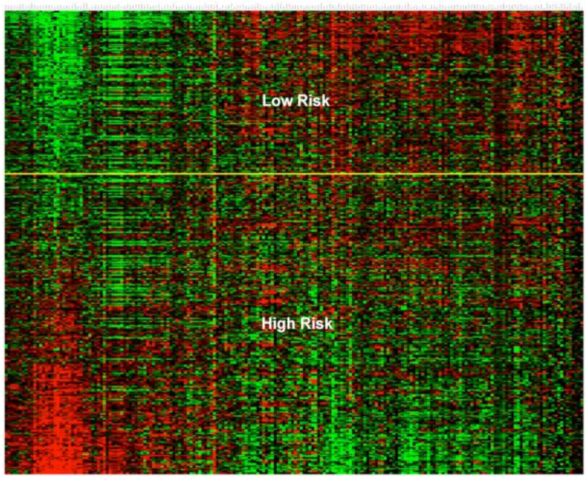

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Afternoon Breakout Sessions

Last	First	Group	Session Location
Chesnut	Jon	Lifetech	10th Floor (Edwin)
Citovsky	Vitaly	University of NY, Stony Brook	10th Floor (Edwin)
Daniell	Henry	University of Central Florida	10th Floor (Edwin)
Davis	Mark	NREL	6th Floor (Hamilton)
Doty	Sharon	University of Washington	10th Floor (Edwin)
Ferguson	Bruce	Edenspace	10th Floor (Edwin)
Flavell	Dick	Ceres	6th Floor (Hamilton)
Guiltinan	Mark	Penn State	10th Floor (James)
Guo	Lining	Metabolon	10th Floor (James)
Hanson	David	University of New Mexico	10th Floor (Edwin)
Haseloff	Jim	University of Cambridge	10th Floor (James)
Jackson	Alicia	DARPA	10th Floor (James)
Jessen	Dave	Chromatin	10th Floor (Edwin)
Kelly	Jason	Ginkgo Bioworks	6th Floor (Hamilton)
Kirk	Greg	KLA Tencor	6th Floor (Hamilton)
Lessard	Philip	Agrivida	10th Floor (James)
Li	Zhonshen	DuPont	6th Floor (Hamilton)
Maliga	Pal	Rutgers University	6th Floor (Hamilton)

Last	First	Group	Session Location
McElroy	David	Calysta	6th Floor (Hamilton)
McKee	Adrienne	Solazyme	10th Floor (James)
Mclean	Gail	DOE BES	10th Floor (James)
Medford	June	Colorado State	10th Floor (Edwin)
Merryman	Chuck	Venter Institute	10th Floor (James)
Modlin	Doug	LLNL	6th Floor (Hamilton)
Okamura	Jack	USDA ARS	10th Floor (Edwin)
Poon	Yan	Sapphire	10th Floor (James)
Schroth	Gary	Illumina	10th Floor (James)
Singh	Anup	JBEI	10th Floor (Edwin)
Snell	Kristi	Metabolix	6th Floor (Hamilton)
Stewart	Neal	University of TN	10th Floor (Edwin)
Streatfield	Stephen	Fraunhofer	10th Floor (James)
Thomson	Jim	USDA-ARS	6th Floor (Hamilton)
Wang	Zeng-Yu	Noble Foundation	6th Floor (Hamilton)
Welch	Mark	DNA 2.0	6th Floor (Hamilton)
Zhang	Zhanyuan	University of MO	10th Floor (Edwin)
Zhang	Feng	Cellectis plant sciences	10th Floor (James)