Technical and Societal Overview Industrial Biotechnology

Lecture 4
Biofuels and Bioproducts

Bronx Community College - 2017
Chemistry and BioEnergy Technology for Sustainability NSF ATE 1601636

Outline

- Introduction to Industrial Biotechnology and Synthetic Biology
- Common Techniques in Molecular Biology
  - Design Build Loop
- Social and Federal Considerations

Slides and images adapted from:
Industrialization of Biology: A Roadmap to Accelerate the Advanced Manufacturing of Chemicals

Wikipedia, Arizona Chemical – or otherwise noted

Industrial Biotechnology

- Industrial Biotechnology – The process of modifying living organisms to serve human needs, chiefly through genetic engineering
  - Applications typically include:
    - Agriculture (crops, fish, animals engineered to resist pesticides/herbicides, grow larger, with less water/nutrient requirements, etc.)
    - Pharmaceuticals (microbes engineered to overproduce a protein, e.g. insulin, growth hormones, vaccines, monoclonal antibodies, small molecules)
    - Other enzymes (detergents, cellulases, enzymes that enable study of other enzymes, bioprocesses – e.g. TAQ polymerase)

A Brief History of Beer

Beer “receipt”
from Umma brewery
in ancient Iraq (c. 2050 BC)

Brewing in the Middle Ages (16th century brewery shown) shifted from a “at home past time” to artisanal activity (with pubs becoming popular)

The *Alewife* (a female brew master) often ran breweries from ancient to medieval times

Synthetic Biology

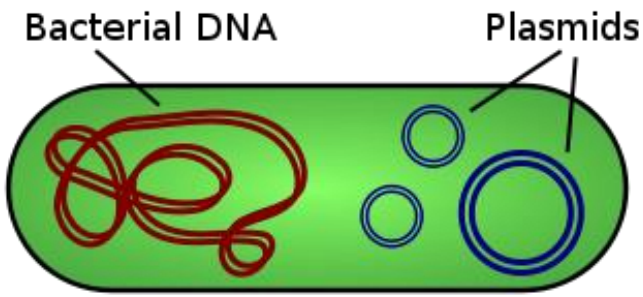
- Desired products are small molecules resulting from *multi-enzyme pathways* (typically one gene per enzyme in a pathway)
- Agricultural and Pharmaceutical Biotechnology has laid important groundwork (gene manipulation, bioprocess scaling)
- Safety of genes “escaping” into natural systems is more easily controlled since microbes are not grown in open fields
- Pathway complexity creates systems level challenges and network analyses are needed to engineer more productive hosts
- Goal is to generate “tunable modules” for the production of biomolecules.
- Some early success stories include artemisinin, lactic acid, 1,3-propanediol, isoprenoids and alcohol-based biofuels

The Design-Build-Test-Learn Loop

- (Design) select host organism, metabolite pathway/enzymes, make a plan for how to build and test what has been selected and tailored
- (Build) synthesize, assemble and transform DNA, use genomic modification tools the create strain variants
- (Test) culture the variants to assess strain performance (e.g. transcriptomics, proteomics, metabolomics, metabolic flux analysis)
- (Learn) evaluate the resulting data to determine the success/failure of the design. Determine how to improve

DNA Vectors: Plasmids

- ***Plasmids*** = small strand of DNA that is physically separate from chromosomal DNA and can replicate independently
- ***Often found in bacteria*** (but can be found in archaea and eukaryotes)
- In nature, plasmids benefit the organism (e.g. confer antibiotic resistance)
- Antibiotics can thus be used as a "selection marker" for cells containing the plasmid of interest



- Large
- gDNA
- Double stranded
- "Life under normal circumstances"

- Small
- Circular
- Double stranded
- used as a *vector* to *clone recombinant DNA* into a host cell

The Seven Steps to Molecular Cloning

in vitro

1. Choose host organism and cloning vector
2. Prepare vector DNA (restriction digest)
3. Prepare DNA to be cloned (isolate -> PCR amplify -> restriction digest for compatibility with vector DNA)
4. Create recombinant DNA (match DNA concentrations from steps 2-3, and *covelently link* with DNA *ligase*)

in vivo

5. Introduce recombinant DNA into host organism (aka transformation)
6. Select organisms containing recombinant DNA (by killing the others!)
7. Screen for clones with desired DNA inserts and biological properties (blue-white screening system, β-gal.)

Vectors <u>must</u> contain DNA segments for:

- ***Replication Origin*** = necessary for replication of recombinant DNA in host

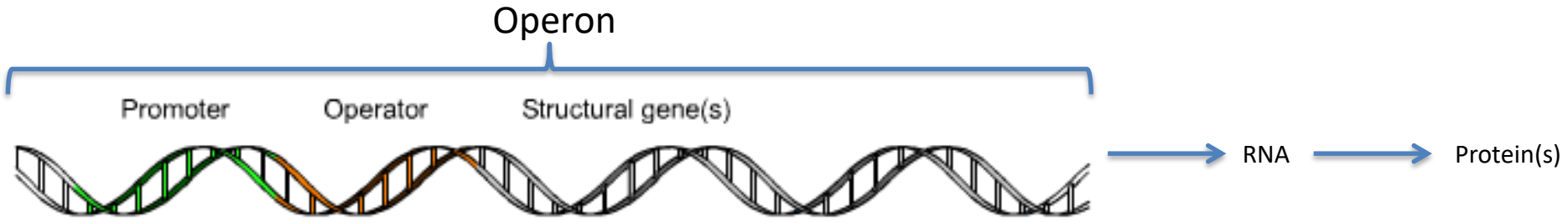
- ***Restriction Endonuclease Recognition Sites*** = sites where foreign DNA can be introduced

- ***Selection Markers*** = (e.g. antibiotic resistance genes) used to enable survival of transformed cells

- ***Genetic Tag*** = used to screen cells containing foreign DNA

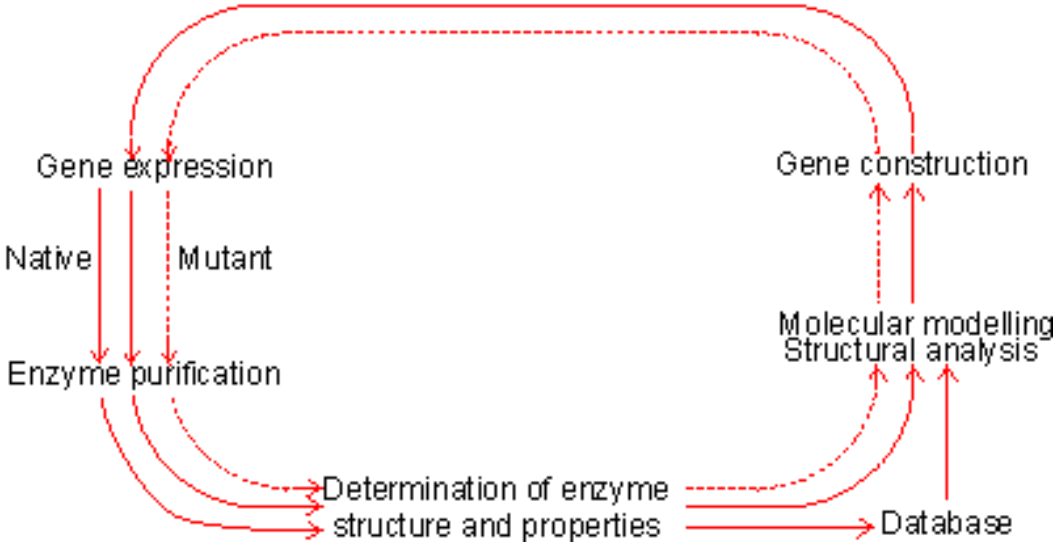
Biological Switches: Operons

- Biological Circuits: Switches that allow protein modules to be turned on or off
- *Lactose (lac) operon* = First discovered natural gene circuit
  - *E.coli* prefers glucose before lactose metabolism
  - When lactose is present the β-galactosidase enzyme is produced that converts lactose into glucose
  - "Exogenous" genes can be placed on a plasmid under the control of a lac promoter
  - Initially, cells are grown in media without lactose so the desired genes are not expressed
  - Once cells reach exponential growth, they are induced with isopropyl-β-D-thiogalactopyranoside (**IPTG**), a non-hydrolyzable lactose mimic that therefore keeps the biological circuit in the on position



The Protein Engineering Cycle

1. Isolate and characterize desired enzyme
2. Analyze info against a known database
3. Hypothesize which amino acid mutations will allow for higher activity/ease of purification
4. Construct new enzyme via site-direct mutagenesis
5. Isolate and characterize mutants
6. Repeat until desired result is obtained



http://www1.lsbu.ac.uk/water/enztech/engineering.html

Other Common Terms Encountered in Molecular Biology

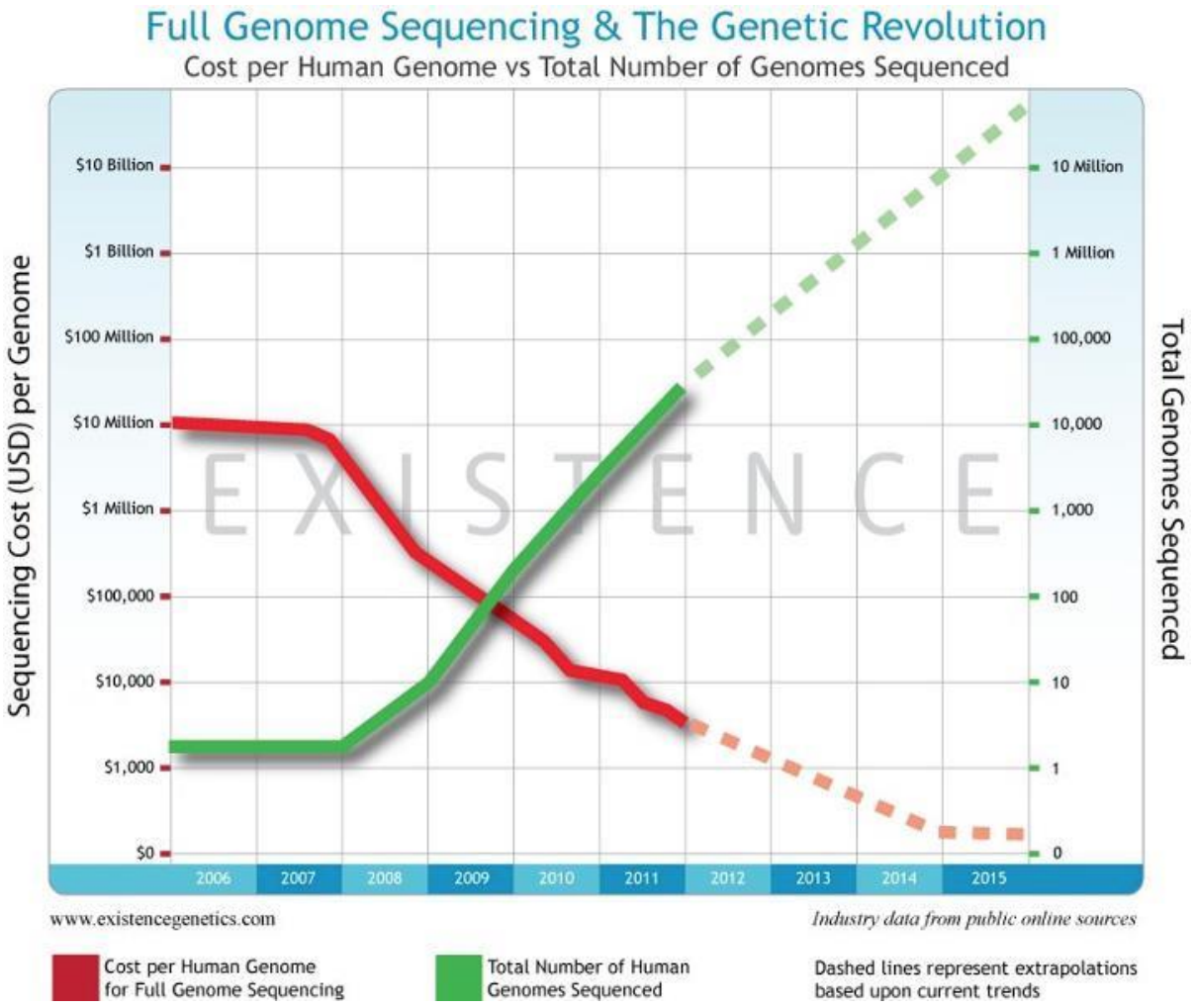
- ***DNA Construct*** = an artificially constructed DNA segment that has been sub-cloned into a vector
- ***DNA Library*** = total population of clones obtained from a cloning experiment
- ***DNA Sequencing*** = a process used to determine the exact order of nucleotide bases in a DNA molecule. Confirms the success of a DNA construct
- ***Genetically Modified Organisms (GMOs)*** = organisms containing a set of cloned genes
- ***Gene Therapy*** = supplying a functional gene to cells lacking that function (with aim to correct a disease or genetic disorder)

Technical Breakthroughs

- Technology that can compose, read, write and “de-bug” DNA
- Leads to more sophisticated engineering projects which require precise control over dozens of genes
- Structures and composite nanomaterials (e.g. mining drug candidates from human microbiome, pesticides from environmental samples, production of metal nanoparticles for electronic and medical devices)

Advances in Genomics

- Lower genome sequencing costs (now <$1000/genome)
- 300,000 different organisms sequenced
- An enormous natural “parts catalogue” of DNA units from which pathways can be discovered or created



Organism as a Molecular Assembly Line

- Consolidated bioprocessing and automated product assembly in ***same*** organism
- e.g. nitrogen fixation and cellulase enzymes and biosynthetic genes/proteins for e.g. terpenes production from biomass

Biological Bottle Necks

- Composing and/or selecting a sequence of DNA to use is slower than our ability to read and write DNA
- Most valuable functions require many genes, and complex regulatory control over how much, when and where they are turned on
- Synthetic biologists create tools to solve these problems (e.g. genetic circuits, precision gene regulatory parts, and computer aided design to systematically recode multi-gene systems)

State of the Art

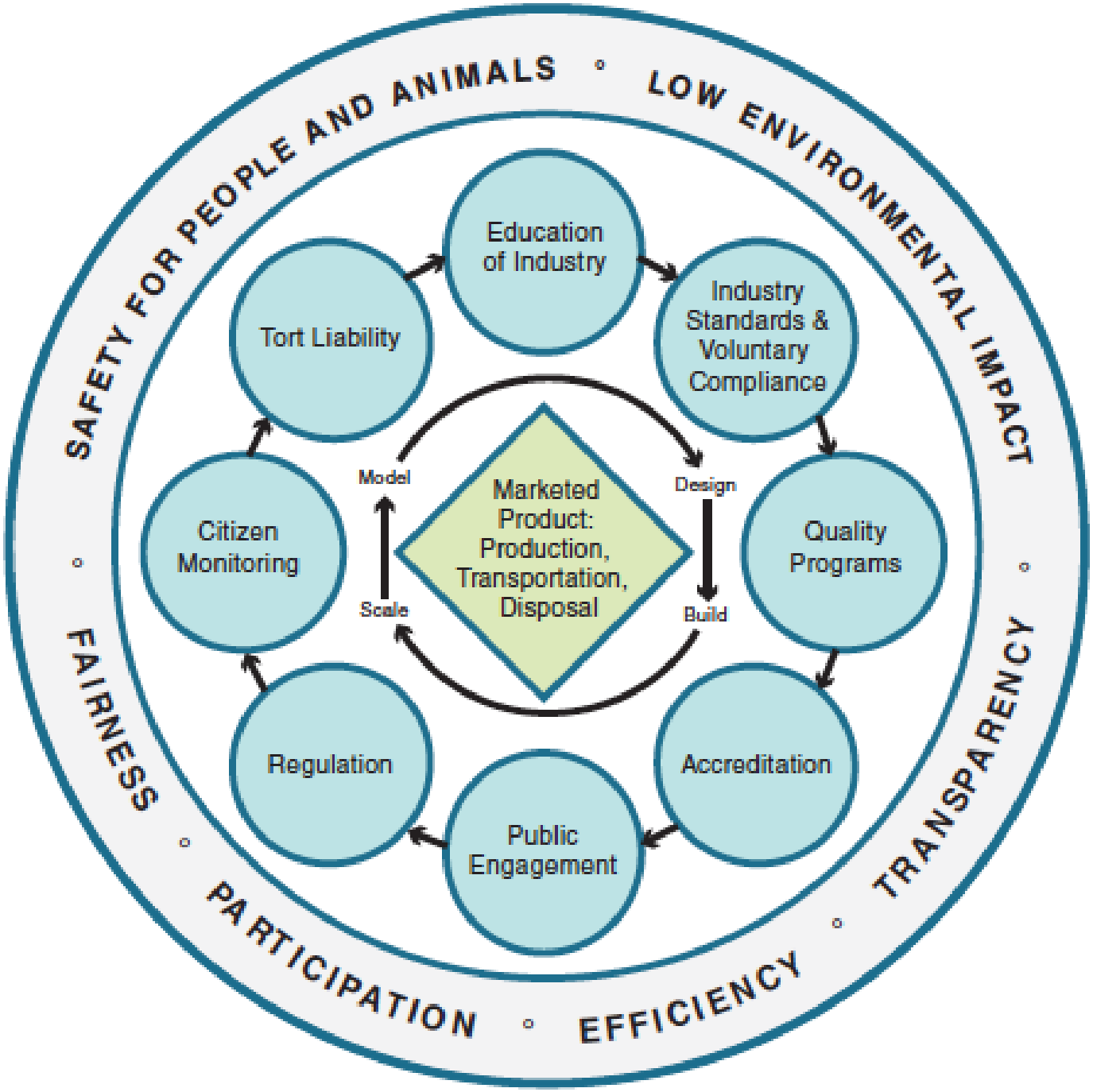
- Targetrons, TALENS, MAGE and CRISPR/Cas9 are technologies that allow “top-down editing” of existing genomes
- They introduce incremental changes to an otherwise natural genome
- Site-specific insertion, mutation or deletion of genes
- Genome scale design tools have also emerged which allow user to control flux through a metabolic pathway

Organismal “Chassis”

- The “chassis” is the host organism
- More productive organisms must be “domesticated” into the lab
- Metabolic pathways must be introduced into a good chassis (heat tolerant, salt tolerant, fast doubling time, etc)

Societal Aspects of Biotech

- Inform the public about the nature of industrial biotech/synthetic biology
- Societal Benefits (job creation, reduced dependence on foreign oil, cleaner environment)
- Ensure that public concerns are heard by Corporations and Law Makers
- Biological and chemical routes must be considered equals by a customer


SAFETY FOR PEOPLE AND ANIMALS
LOW ENVIRONMENTAL IMPACT
TRANSPARENCY
EFFICIENCY
PARTICIPATION
FAIRNESS
Education of Industry
Industry Standards & Voluntary Compliance
Quality Programs
Accreditation
Public Engagement
Regulation
Citizen Monitoring
Tort Liability
Model
Design
Build
Scale
Marketed Product: Production, Transportation, Disposal

Global Governmental Standards

- Regimes must be harmonized across national boundaries, enabling rapid, safe and global access to new technologies and products, for example:
  - Read/write accuracy for DNA
  - DNA “part” performance specifications
  - Data and machine standards across “-omics” technologies (e.g. for genomics, proteomics, metabolomics)
  - Organism performance (rate, titer, yield)
  - These will SPEED the SAFE commercialization of new host organisms, new metabolic pathways, and new chemical products