L14 Genome Engineering

Instructor: Prof. **K. Solomon Ph.D.**
Assistant Professor
Agricultural & Biological Engineering
Laboratory for Renewable Resources Engineering

Fall 2018

Recall…

Parts assembled by 1 of 4 techniques

Parts are formatted for assembly via PCR

Parts are delivered as DNA via:
- Plasmids/vectors
- Integrated in chromosome

2

This lecture….

- Genome Engineering overview
- Historical approaches
- Site specific approaches

3

Genome engineering permanently alters the host genome

Advantages	Disadvantages
- Stable (no plasmids or selection markers) - Low copy (~1) - Essential for toxic genes	- Difficult/low efficiency

4

Hosts have complex genotypes

DH10B: F⁻ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lacZ*ΔM15 Δ*lacX74 recA1 endA1 ara*D139 Δ(*ara leu*) 7697 *galU galK rpsL nupG* λ⁻

Genotype	Description
F⁻	Missing fertility plasmid; cannot conjugate, DNA less likely to propagate beyond the host
mcrA Δ(mrr-hsdRMS-mcrBC)	Knockout of methylation/restriction system, allows foreign DNA transformation
Φ80lacZΔM15	Φ phage insertion of β-galactosidase subunit; complements with plasmid lacZ for blue/white selection
ΔlacX74	Removes β-galactosidase from genome; allows for blue white selection
recA1	Defective mutant of a recombinase allele; improves plasmid stability
endA1	Defective mutant of an endonuclease; improves plasmid stability
araD139 Δ(ara leu) 7697	Mutant allele of arabinose catabolic enzyme and large scale deletion of other genes in the pathway; blocks arabinose metabolism to allow for stable arabinose induction
galU galK	Transposon mutants that inactivate galactose metabolism
rpsL	Ribosomal subunit mutant; confers resistance to streptromycin
nupG	Mutation for the transport of nucleosides
λ⁻	Lambda phage lysogen is absent

5

Historical approaches

Mutant alleles arise randomly or via mutagenesis

- UV
- Chemical mutagens
- Transposons

If mutant allele is near a natural selection marker, we can move genes to a new host and screen the phenotype

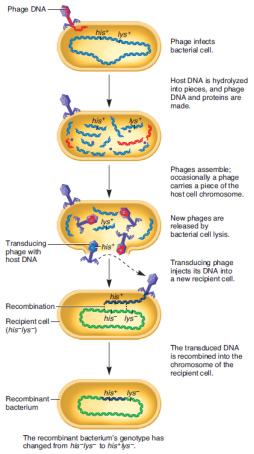
6

P1 Transduction in *E. coli*

- Moves alleles b/n *E. coli* strains
- P1 packages host DNA with low efficiency
  - $<10^{-3}$ % will include ~ 90 kb of host genome including any mutant alleles it contains
- Mutant alleles integrate via homologous recombination
- Low efficiency, **requires** selection marker, can't program

RECOMMENDATION: only good to move mutations

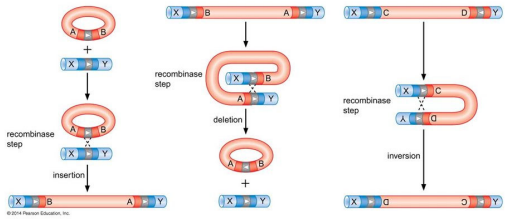
Chegg.com



7

Other historical methods rely on site-specific recombinases

- Site-specific recombinases insert or remove DNA b/n specific sites depending on orientation
  - E.g. FLP/FRT, Cre/LoxP, Int/AttP (Gateway!)



Insertion **Deletion (reversal of insertion)** **Inversion**

8

Site-specific recombination

- Can clone genes between sites in a plasmid and induce recombinase to modify genome **BUT**
  - **Can't specify locus of insertion** (rely on native sites within genome)
  - Copy # uncertain if multiple sites in genome
  - Can't use for multiple integration events

RECOMMENDATION: efficient to quickly integrate DNA but cannot program locus or use repeatedly

9

Transposons

- Transposons or 'jumping genes' cut out genes of interest between an inverted repeat with a transposase and insert them in the genome
  - Locus is random
  - But multiple integrations possible

10

Programmable Genome Engineering

- Can target to specific loci
- Have higher efficiency
- Can delete genes, insert new genes and/or mutate alleles

Locus programmed by:

1. **Sequence homology**: can induce integration via recombination b/n donor DNA and genome (>50 bp)

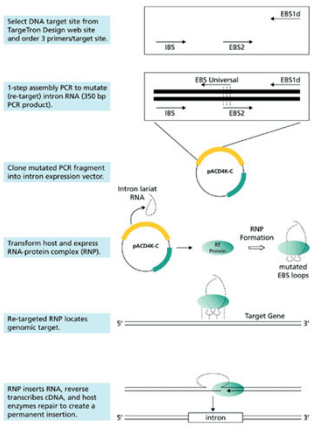
2. **Targeted dsDNA breaks**
   - DNA damage is lethal
   - Breaks induce repair pathways that incorporate donor DNA
   - Efficiency is higher when donor and target are homologous

11

Targetrons/ Group II Introns

- Targeted to specific loci via site-specific mutations in 3 regions of intron structure
- Intron contains:
  - Endonuclease
  - Intron targeting sequences
  - Reverse transcriptase to integrate intron & repair break with into indsertion
- Can insert DNA with RT deficient intron and homologous donor DNA in plasmid

Fairly specific but moderate efficiency and difficult to program

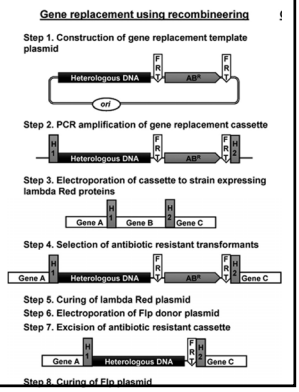


12

Recombineering (Recombinase engineering)

- Uses viral/phage homologous recombinases to integrate with high efficiency (λ RED)
  - Can use linear (PCR) donor DNA w/ homology directly
- Targeting primarily via sequence homology
  - Can improve efficiency w/ dsDNA break (e.g. homing endonucleases/recognition sites, plasmid delivery of DNA, use of selection markers)
- Selection marker flanked by site-specific recombinase sites to facilitate later removal and reuse

High efficiency, easy to program but only in prokaryotes with upper bound of 10-20 kb insertions



Pyne et al, AEM 2015.

Programmable Nucleases

dsDNA breaks can be repaired with homologous DNA thereby inserting new sequences

dsDNA breaks made by:

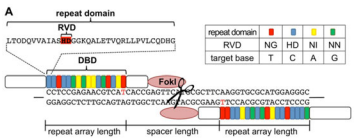
1. TALENs (Transcription-Activator Like Effector Nucleases)
2. Zinc Finger Nucleases
3. CRISPR/Cas9

These approaches work better in eukaryotes and can be adapted for prokaryotes

14

TALENs & ZFNs

- are synthetic proteins with DNA-binding domain and endonuclease domain
- Design rules for target nt/AA sequence
- Can target sequence of desired length (up to 18 bp)
- Need to custom build nuclease for each targeted mutation
- Included repetitive sequences difficult to assemble



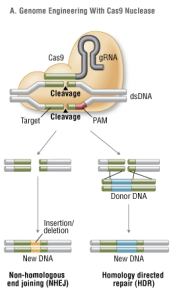
Naitou et al, Biology Open, 2015.

15

CRISPR/Cas9

- Programmed by guide RNA that consists of 20 nts + a 3 bp protospacer adjacent motif (PAM)
- gRNA easily swapped out with an oligonucleotide
- Cas9 endonuclease will cleave ween DNA target + guide RNA recognized
- Growing family of endonucleases with distinct PAMs

Works in all domains of life with high efficiency but limited by PAM restriction



neb.com

16

Next time

- Genome Scale Engineering

17