Targeted genome editing
(part 1: prokaryotes)

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We talked about ways to assemble DNA building blocks

Cloning with restriction enzymes
- -Traditional cloning
- -Biobricks (ecoRI, pstI, xbaI, speI)
- -Golden Gate (type IIS enzymes)

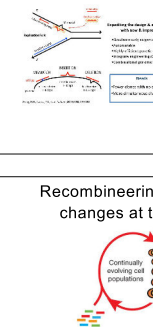
Cloning without restriction enzymes
- -Gateway cloning
- -Circular polymerase extension cloning (CPEC)
- -Ligation-independent cloning
- -SLICE
- -Gibson method (in vitro)
- -Gibson method (in vivo)

Now we will discuss how to use these DNA constructs to modify an organism's genome

Targeted genome editing in prokaryotes

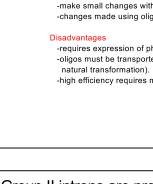
1homologous recombination
2 recombineering (phage lambda recombinases)
3 group II introns
4 Cre-lox
5 genome editing via targetrons and recombinases (GETR)
group II introns + cre-lox (Enyeart et al, 2013 PMID 24002656)
6 Flp-Frt recombination

DNA integration by homologous recombination



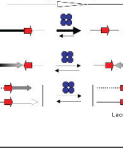
RecBCD are helicases that unwind DNA during repair of double-strand breaks

Gene replacement by homologous recombination

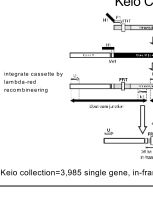


Holliday junctions: 4 stranded recombination intermediates

Good visual description of how Holliday junctions are resolved to recombined linear duplexes:
https://www.youtube.com/watch?v=hpHVryxV4UU



Single gene mutant collection in *Acinetobacter baylyi* ADP1 by homologous recombination

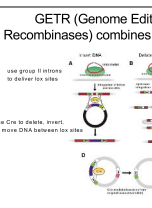


Homologous recombination

Advantages
- -simple to build DNA constructs (flank inserting DNA with 500-1 kb genomic DNA
- -works in many bacteria (RecABCD present in all bacteria)

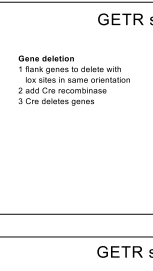
Disadvantages
- -low efficiency <1/1000 cells (need efficient DNA transfer and selectable marker)

Recombineering using phage lambda recombinases



Method
1 introduce ssDNA oligo by electroporation
2 beta-protein coats oligo
3 oligo integrated into genome during replication as Okazaki fragment

Recombineering enables many changes at the same time



Recombineering to replace all 314 TAG stop codons with TAA in *E. coli*

Recombineering

Advantages
- -make small changes with high efficiency (~25%)
- -changes made using oligos (easy and inexpensive)

Disadvantages
- -requires expression of phage proteins
- -oligos must be transported into cell (electroporation or natural transformation).
- -high efficiency requires mismatch repair mutants

Group II introns are programmable transposons

Enyeart et al, 2013 (PMID 24002656)

Group II intron insertion by site-specific recombination

Enyeart et al, 2013 (PMID 24002656)

We use group II introns to knock out genes in Clostridia

Tolonen et al, 2009

Group II introns

Advantages
- -introns insert at ~100% efficiency
- -works in all types of organisms

Disadvantages
- -only useful for targeted insertions (no deletions, rearrangements, point changes)

Cre recombinase enables site-specific recombination between lox sites

Orientation of lox sites (34 bp) determines if DNA is inverted, moved, or deleted

Cre-lox is used in mice for tissue-specific knockouts

Cre-lox enables deletion of genes that are essential in other tissues

Flp-Frt Recombination

Similar to Cre-lox, the Flp recombinase recognizes a 34 bp Frt sequence

Lacroix et al, 2011

Flp-Frt recombination was used to construct the Keio Collection

Keio collection=3,985 single gene, in-frame *E. coli* deletions (4,288 genes in genome).

Keio Collection: Flp-mediated gene excision leaves 28 amino acid scar peptide

Baba et al, 2006

Cre-lox and Flp-Frt recombinase summary

Advantages
- -recombination at ~100% efficiency
- -works in all types of organisms
- -enables tissue-specific knockouts in eukaryotes

Disadvantages
- -requires delivery of genomic recognition sites and recombinase

GETR (Genome Editing via Targetrons and Recombinases) combines group II introns and Cre-lox

First step is to insert lox sites into the group II intron

GETR strategy

Gene deletion
1 flank genes to delete with lox sites in same orientation
2 add Cre recombinase
3 Cre deletes genes

Gene insertion
1 insert lox sites into non-essential gene.
2 flank gene for insertion with lox sites
3 transform gene for insertion into strain on plasmid.
4 transform Cre into strain on second plasmid.
5 Cre recombines gene for insertion and lox sites in genome.

GETR summary

Advantages
- -combines methods that work in many types of organisms.
- -enables insertions, deletions, inversions

Disadvantages
- -requires inserting introns beforehand
- -cannot make small changes (point mutations)