Recombination of exogenous oligonucleotides with chromosomal DNA in yeast

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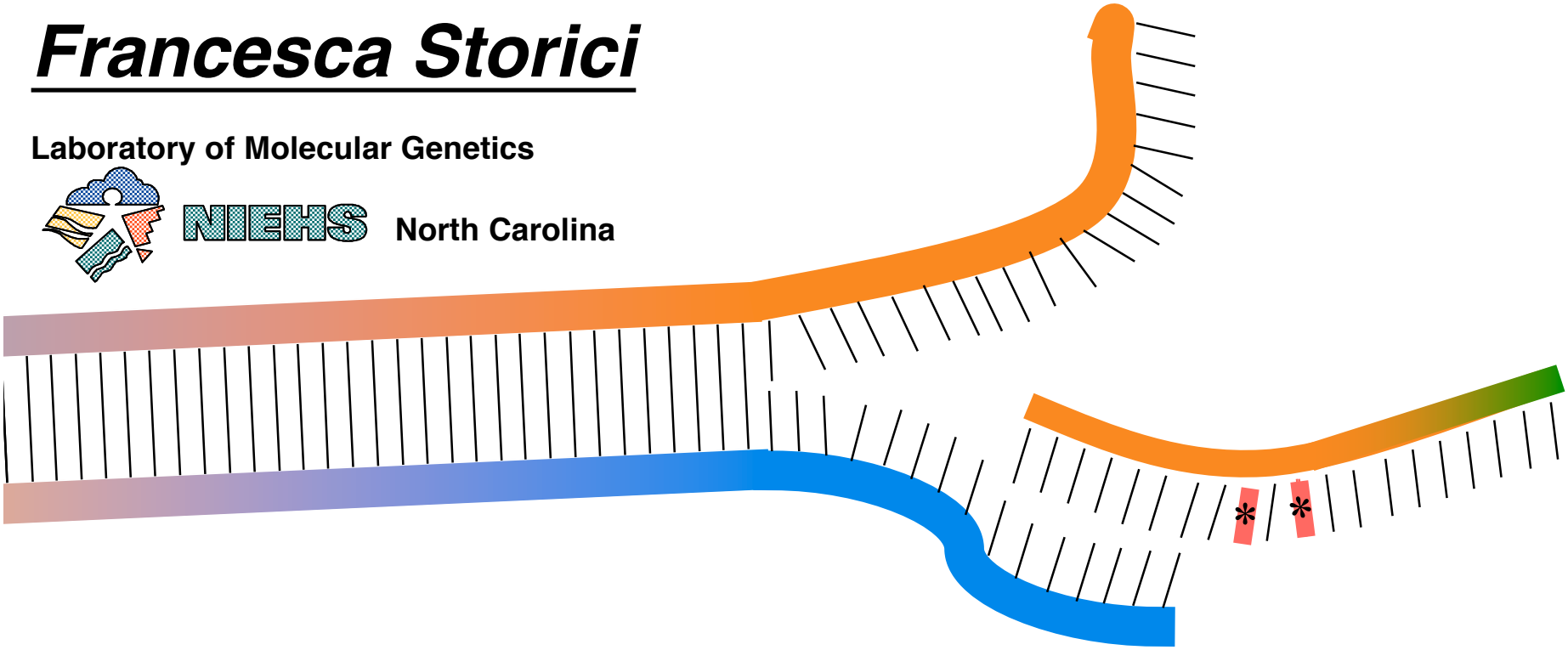


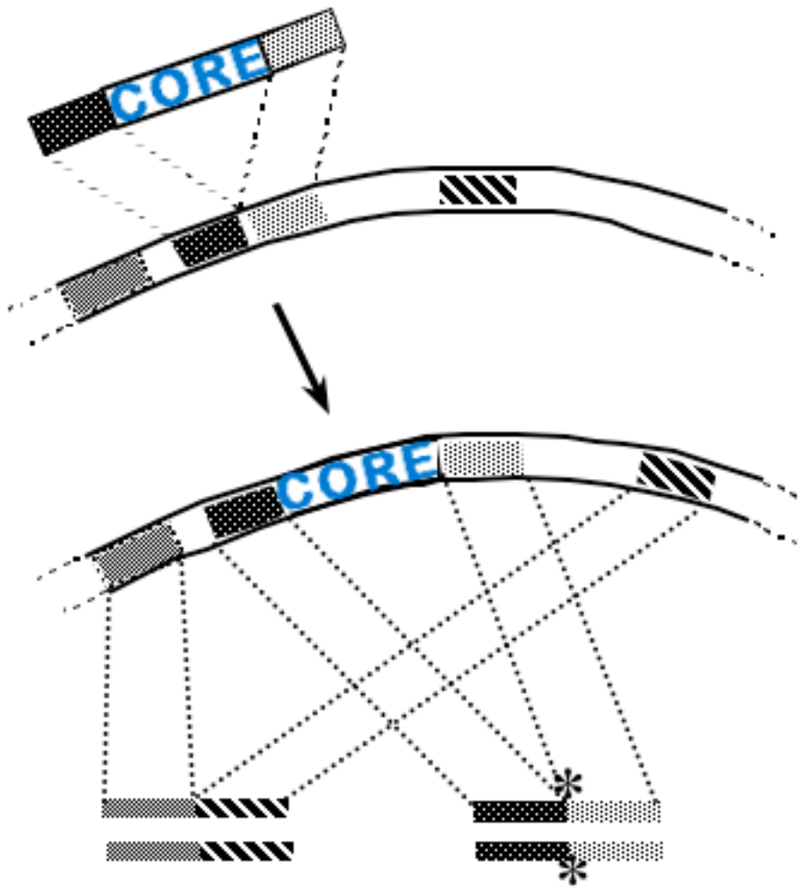
- **The *delitto perfetto* system for *in vivo* targeted mutagenesis using oligonucleotides**
- **Mechanisms of recombination and double strand break repair with oligonucleotides**

In vivo targeted mutagenesis using oligonucleotides (*Delitto perfetto*)

1: INSERT *CORE*

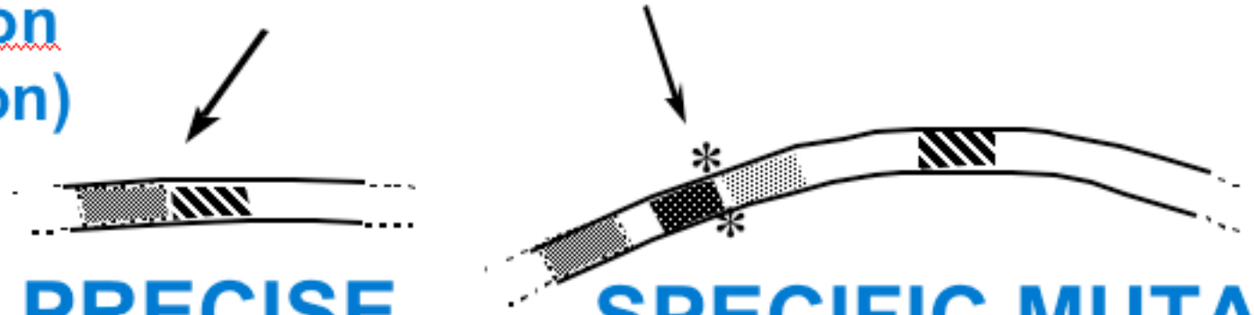
COunter selectable REporter

(e.g., *URA3KI* + *kanMX4*; select for either marker)



2: REPLACE CORE WITH OLIGOS

(*URA3KI* counterselection and *kanMX4* confirmation)



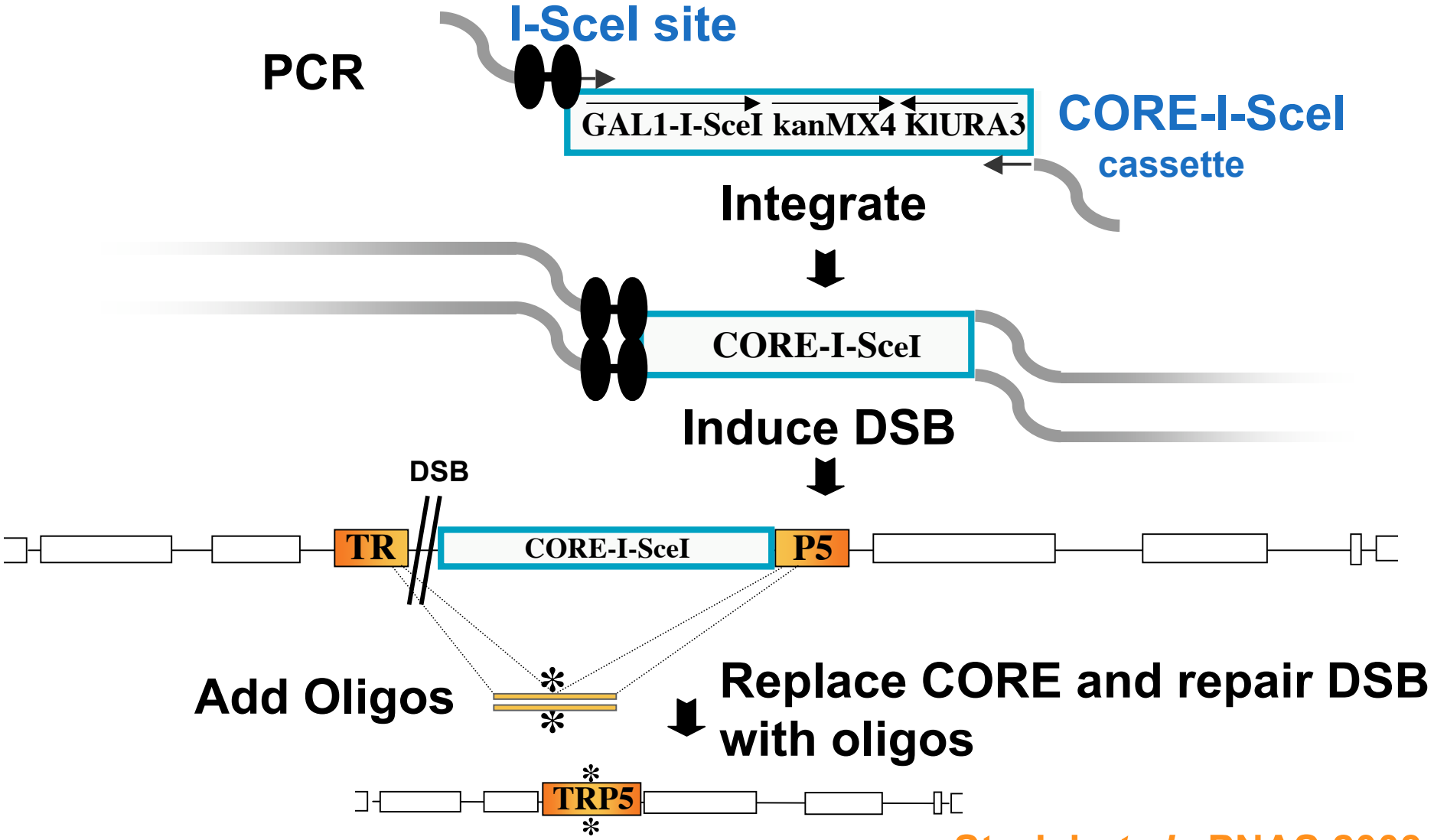
Frequency: 10^{-5} -10^{-4}

PRECISE DELETION

SPECIFIC MUTATION

Storici *et al.*, Nat. Biotech. 2001

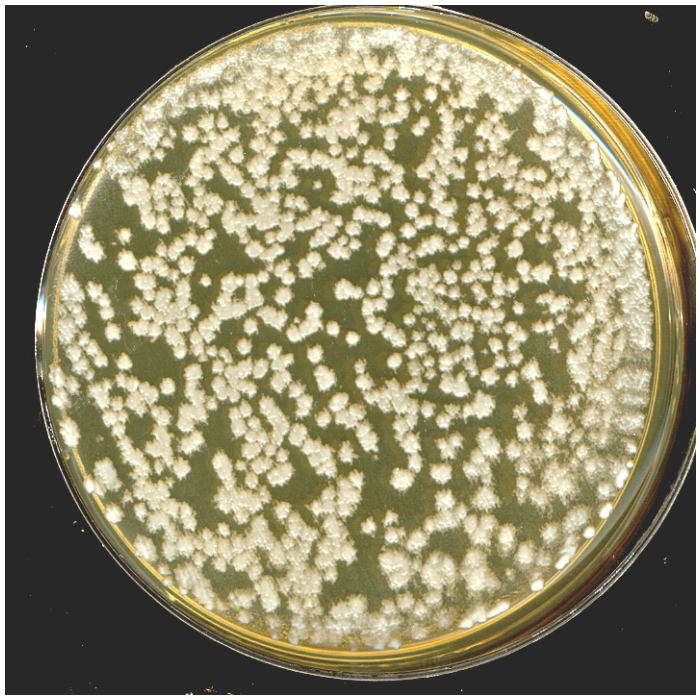
Induction of a double-strand break (DSB) stimulates oligo targeting more than 1000 fold



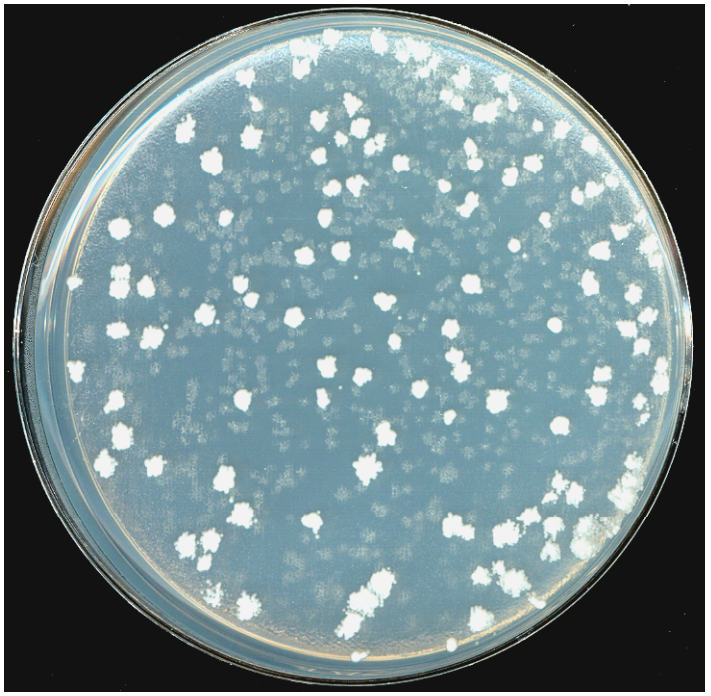
Storici *et al.*, PNAS 2003

Targeting is highly efficient -- up to 15%!

Selection is NOT needed



YPD



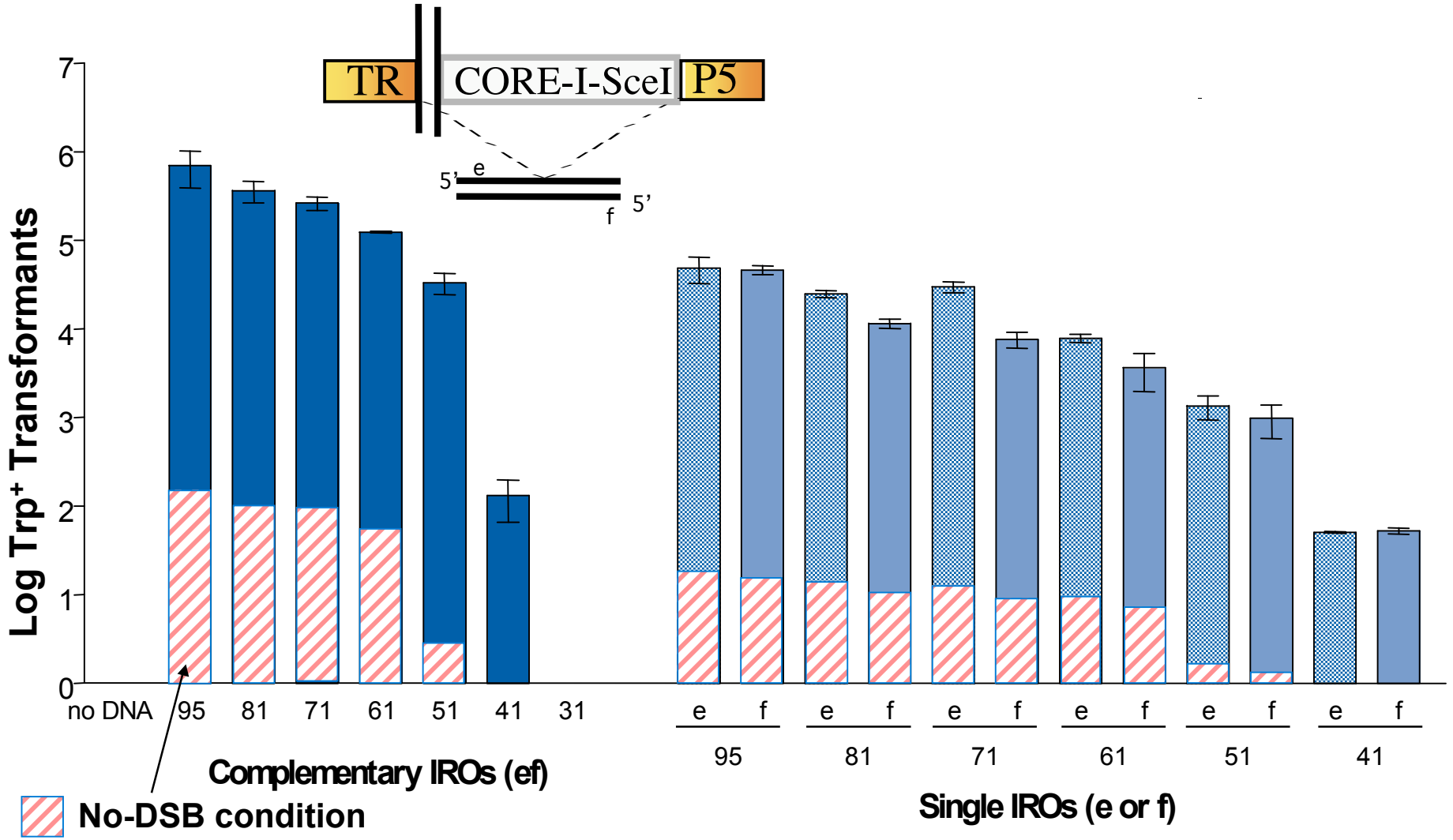
Trp$^-$

Plate to YPD

Replica plate

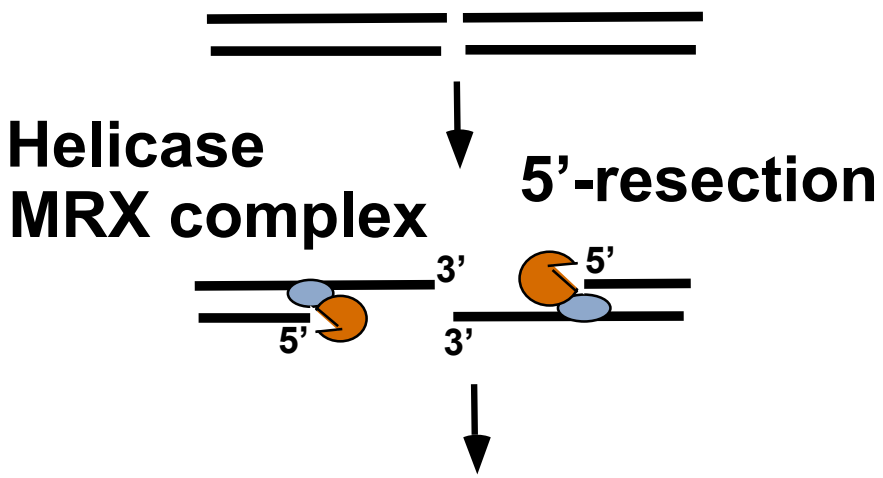
Targeting to DSB is efficient with oligos >50 nucleotide

AND with single-stranded oligos



What is the mechanism of oligonucleotide targeting to a DSB?

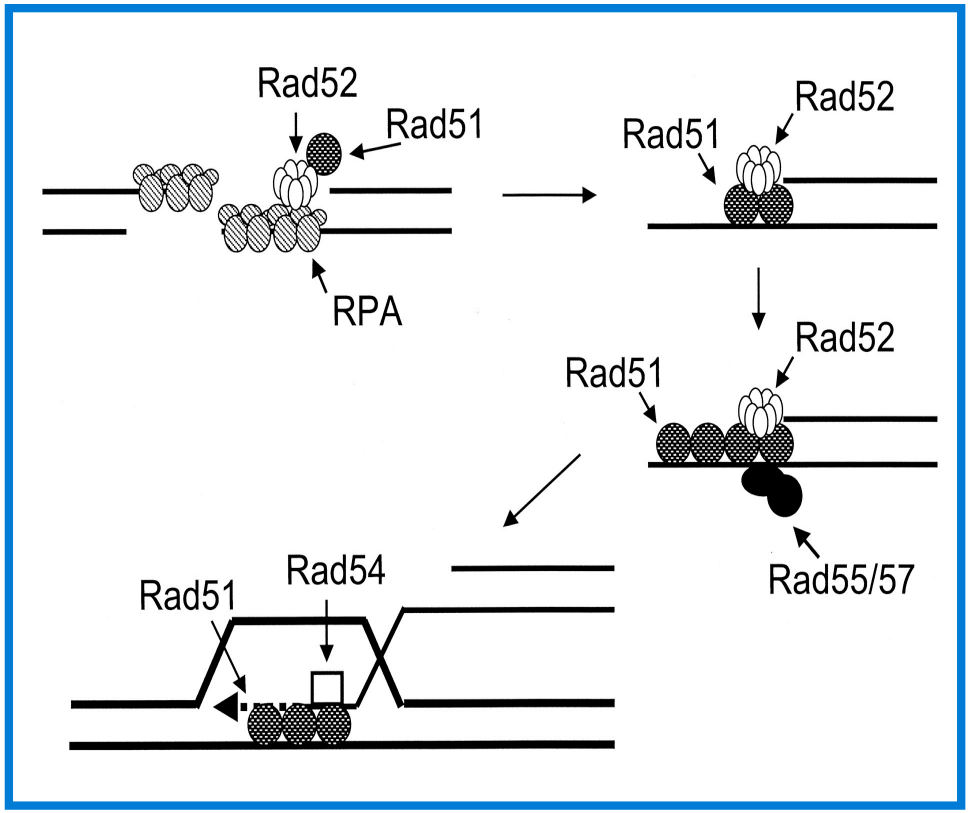
Homology dependent DSB repair in yeast (initial steps)



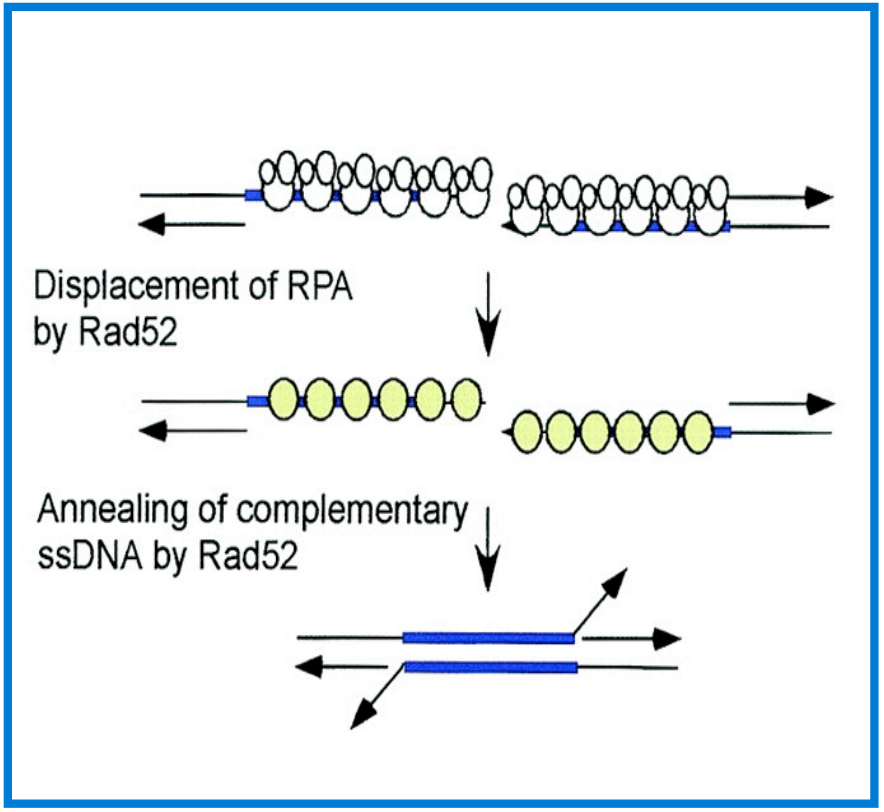
Strand invasion or
Single strand annealing

Homology dependent DSB repair

Strand invasion

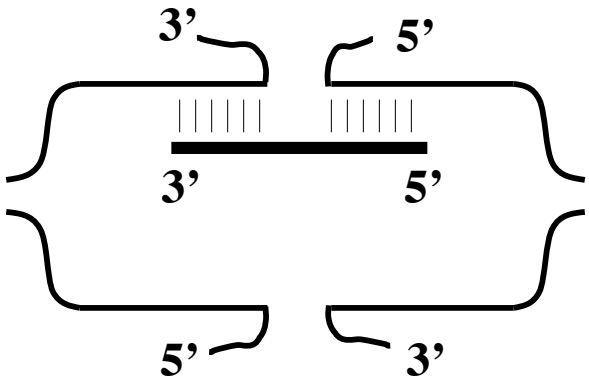


Single strand annealing (SSA)

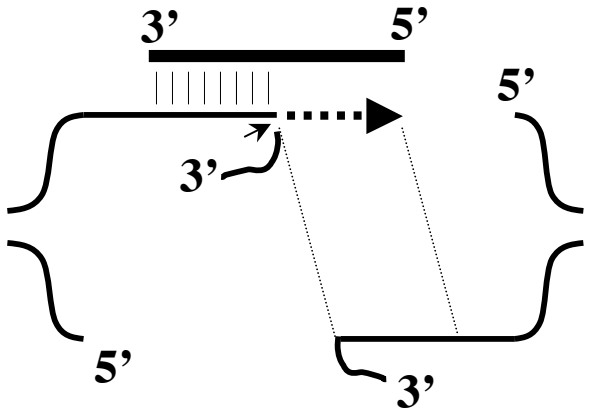


From: Symington, Microb. and Mol. Biol. Rev., 2002

DSB repair with an oligonucleotide: possible intermediates



bridge

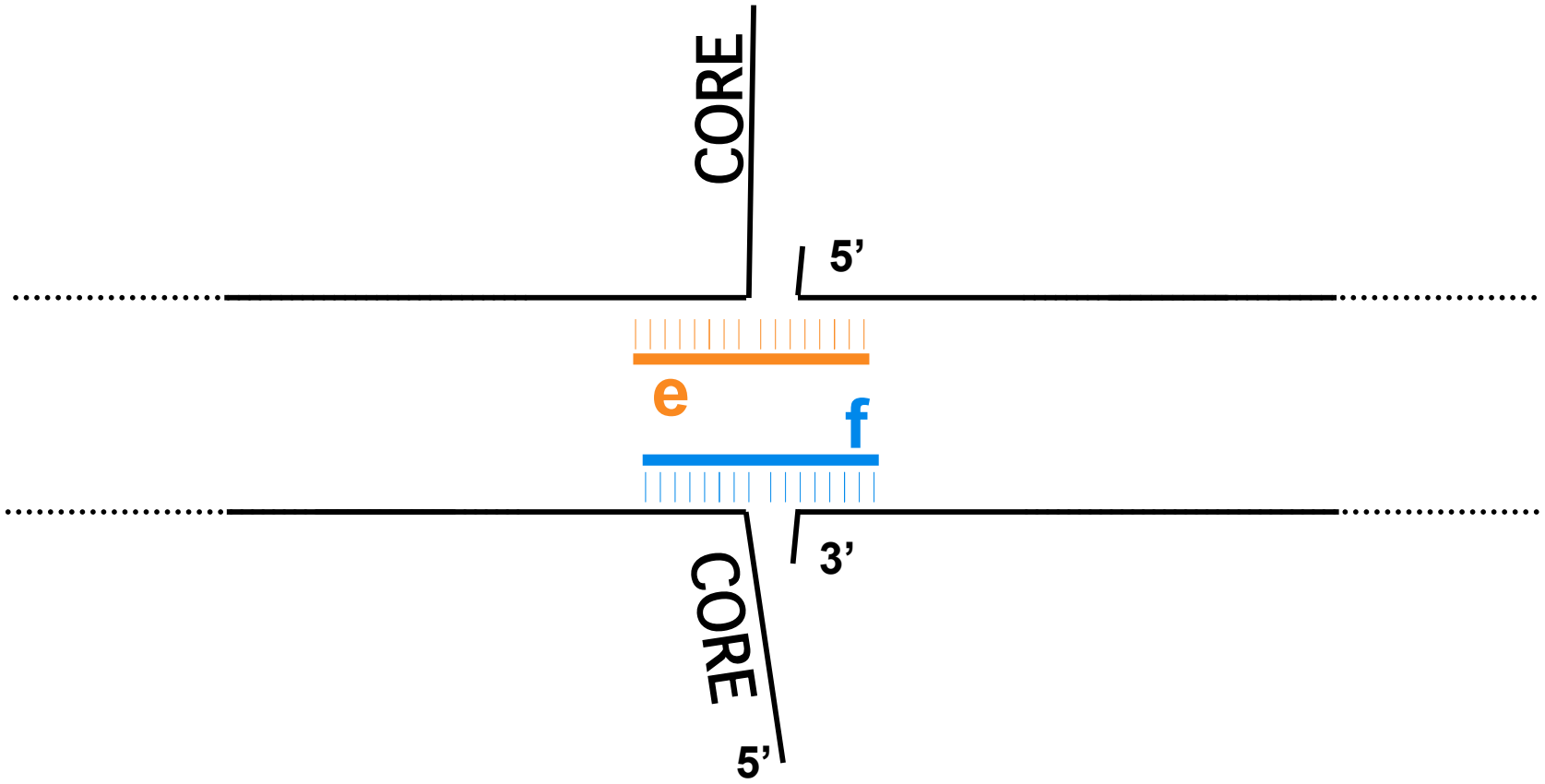


template

Focus of this study

- Understand mechanisms of DSB-mediated oligonucleotide targeting in yeast
- Dissect DSB repair pathways using oligo-mediated targeting

Defined substrates for dissecting DSB repair



Effect of null mutations in DSB repair on targeting of ss-oligos to a DSB

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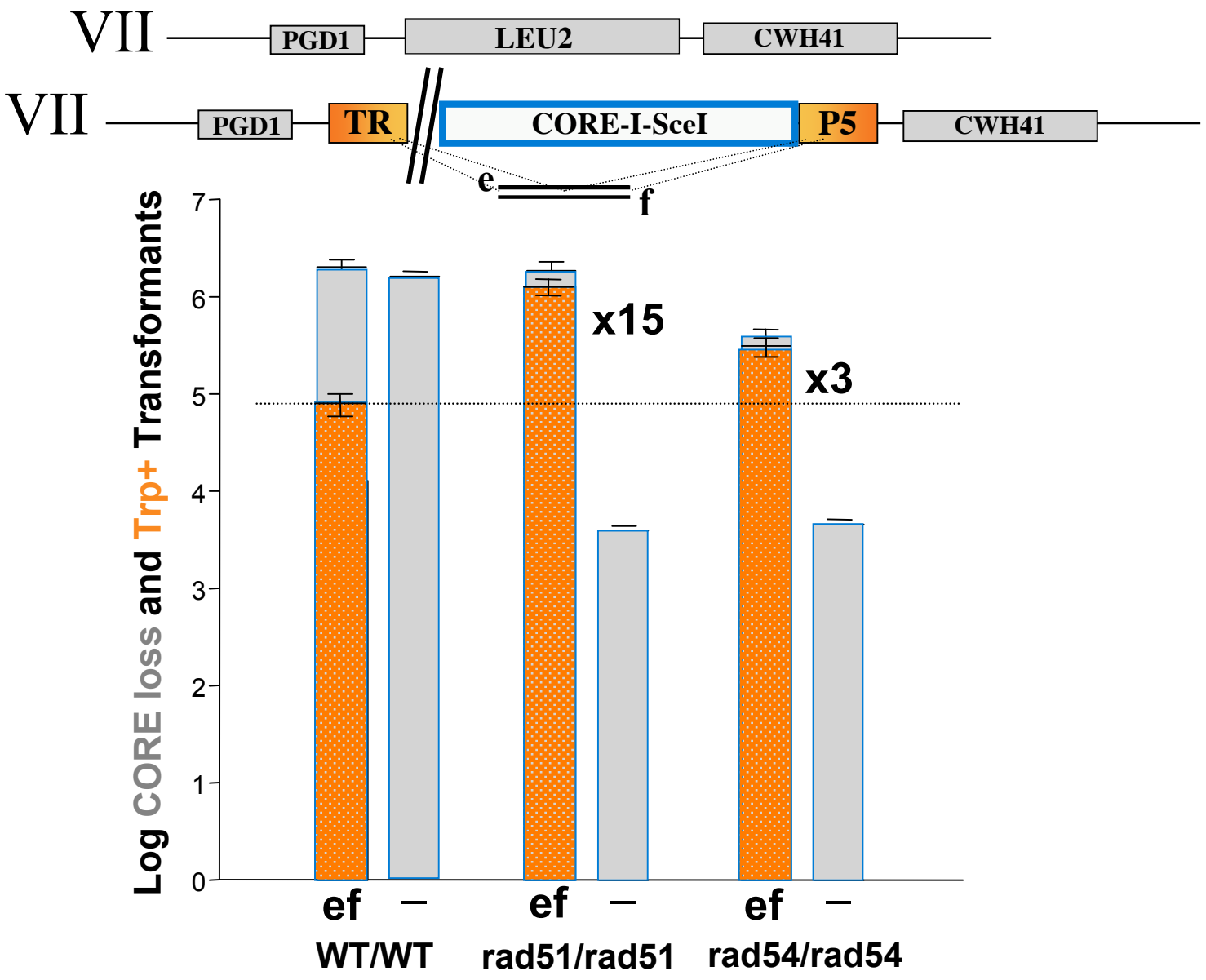
Genotype	Oligo e / f
WT	x 1 (0.8%)
MRX *(mre11/rad50/xrs2)*	x 0.4
rad52	x 0.005
rad59	x 0.05
rad51	x 3.0
rad54	x 2.0
rad55	x 3.4
ku70	x 1.1
lig4	x 1.3

Targeting of oligonucleotides to a DSB:

- Homology dependent repair
- Only Rad52 is essential
- Independent of Rad51, Rad54 and Rad55
- Increased 2-3 X in *rad51*, *rad54*, *rad55* mutants

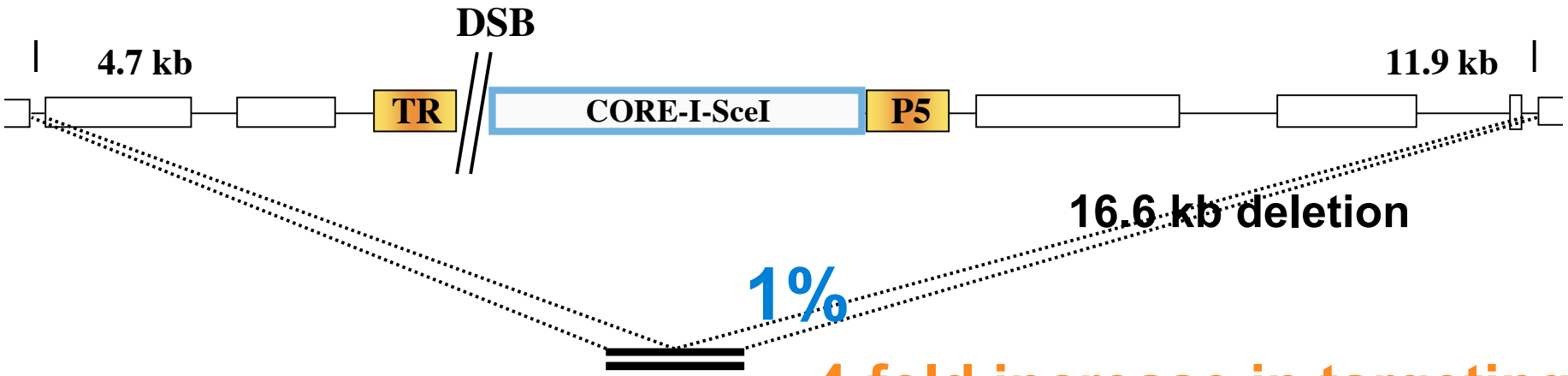
Major pathway: Rad52-dependent SSA

rad51 and *rad54* stimulate oligonucleotide targeting



Can oligos target sites distant from the DSB?

Does the targeting require Rad51 function?



4-fold increase in targeting in the absence of Rad51

Oligos can effciently target sites distant (up to 12 kb) from the DSB by a SSA mechanism

A large region around a DSB is activated for recombination

Is targeting stimulated when oligos are homologous to only one side of a break?

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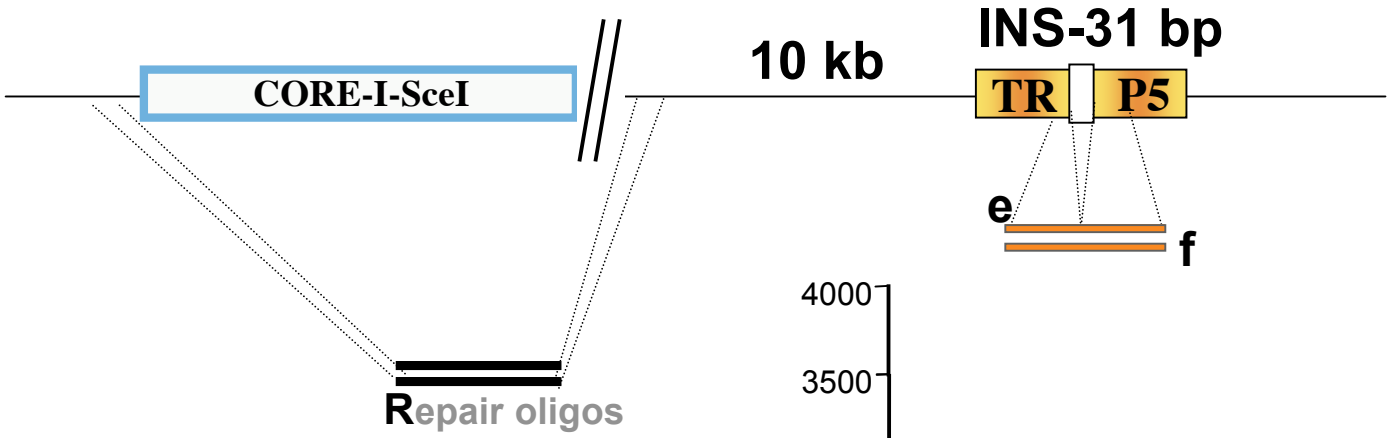
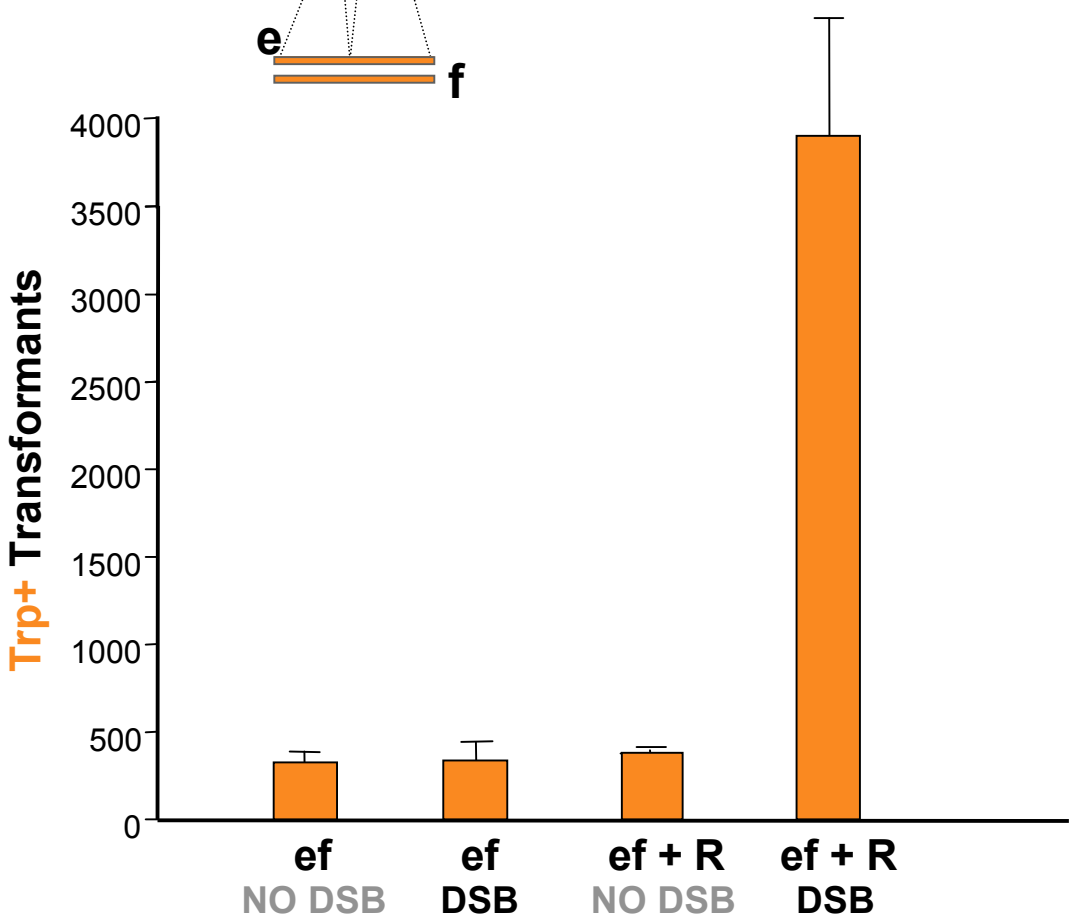
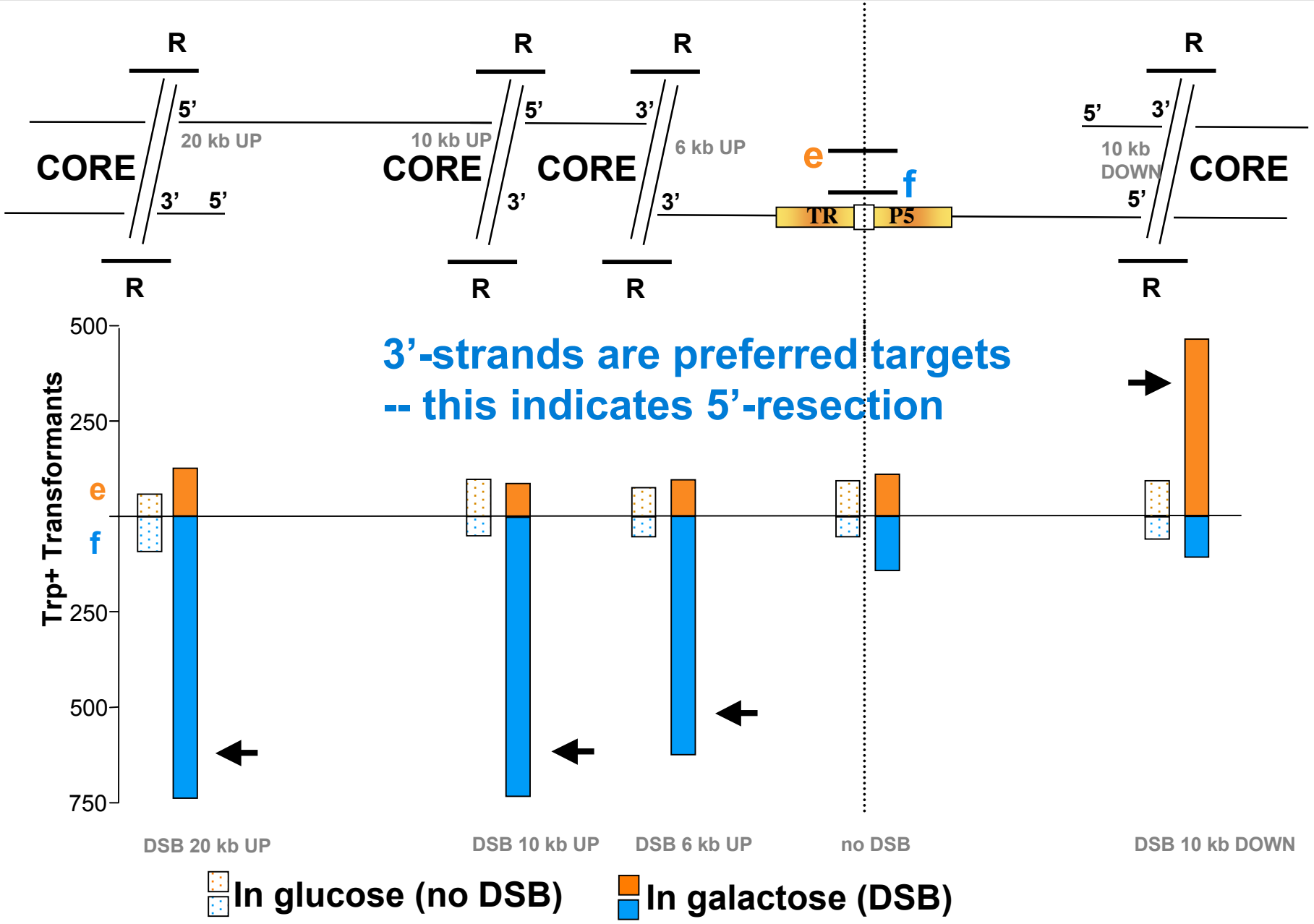


A DSB stimulates targeting of oligonucleotides homologous to only one side of the break

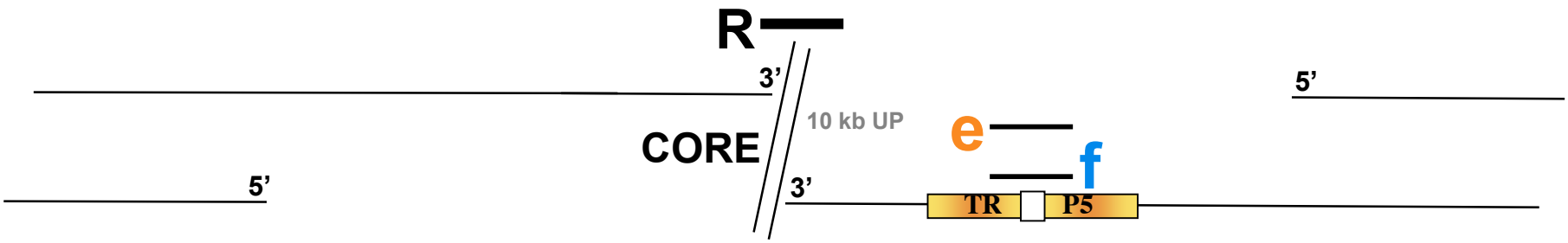
Formation of viable transformants requires DSB repair

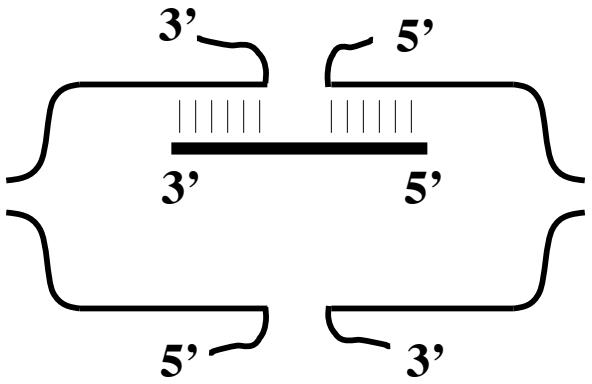


Is there strand bias for oligos targeting to one side of the break?

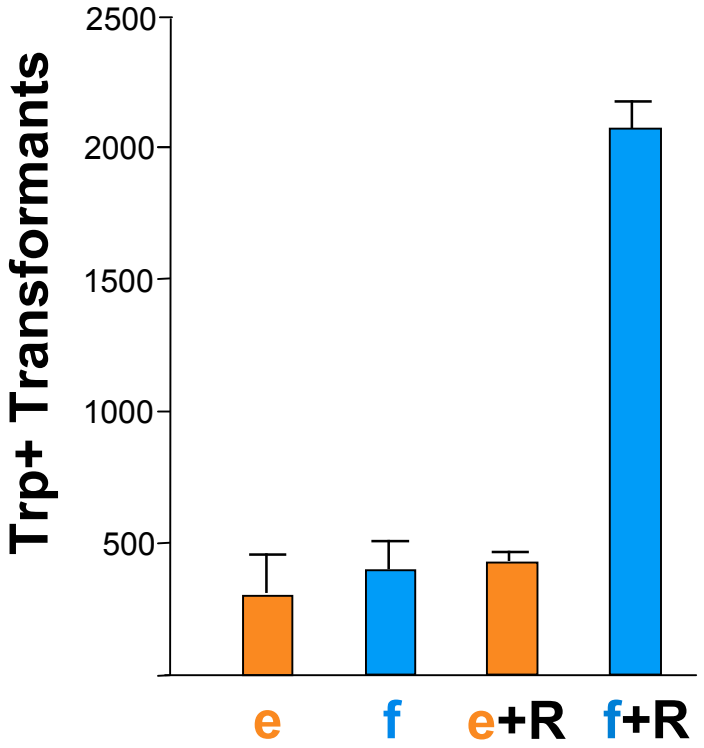


Repair of a DSB with a ss-oligo supports ‘template’ model





bridge



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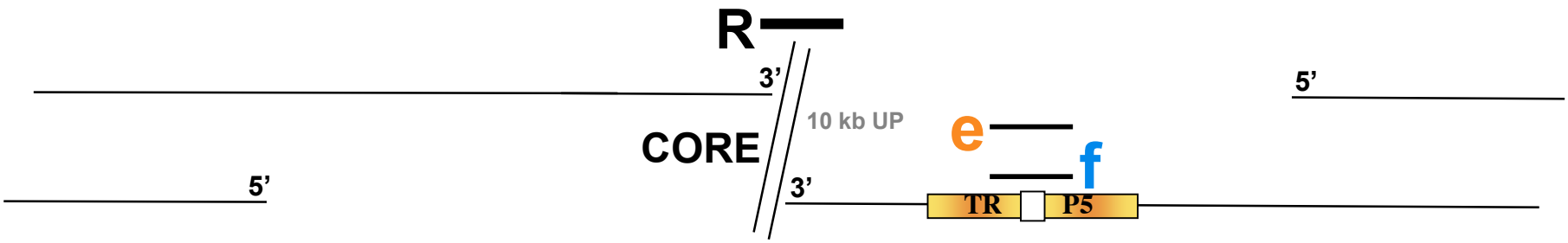

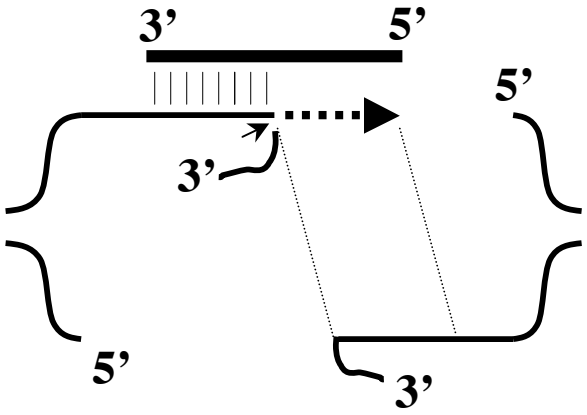

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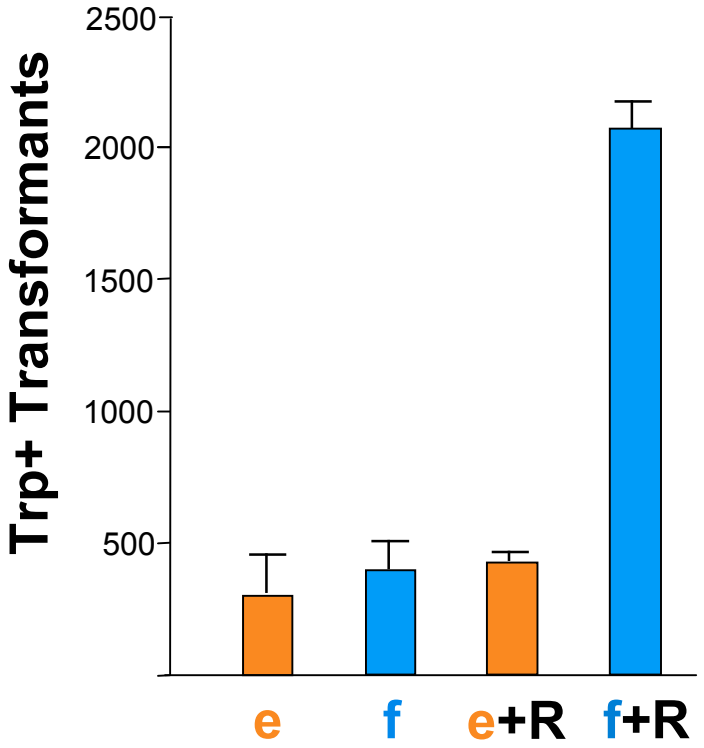

Conclusions

Delitto perfetto in vivo **mutagenesis using oligonucleotides**

- **very efficient**
- **high throughput**

Mechanisms of recombination and double strand break repair with oligonucleotides

- **via single-strand annealing (SSA) with resected ends of a DSB**
- **also distant sites (up to 12 kb) from a DSB are targeted via SSA**
- **primarily through a "template" mechanism**
- **only *RAD52* is essential**
- **we confirm the *RAD52* capacity to perform SSA *in vivo***
- **Rad51, Rad54 and Rad55 suppress oligonucleotide targeting**

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