

Recombinases and their applications in biotechnology

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Overview

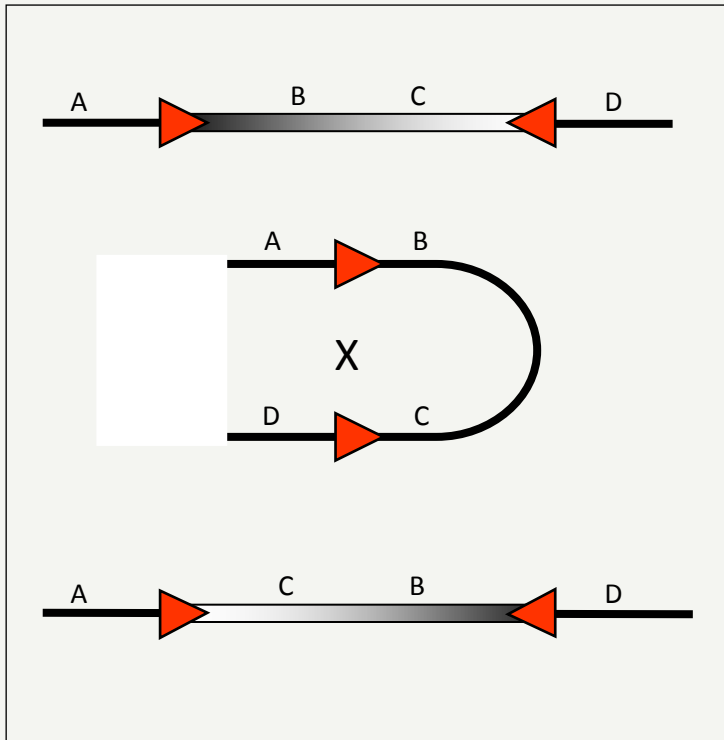
- Recombination
 - Systems
 - Mechanisms
- Previous work
 - Elimination of marker genes
 - Target integration
 - Optimization of recombinase-genes
- Current work
 - Inducible activation of genes
 - Excision of transgenic sequences in germ lines
 - Inducible RNAi and overexpression

Recombination Systems

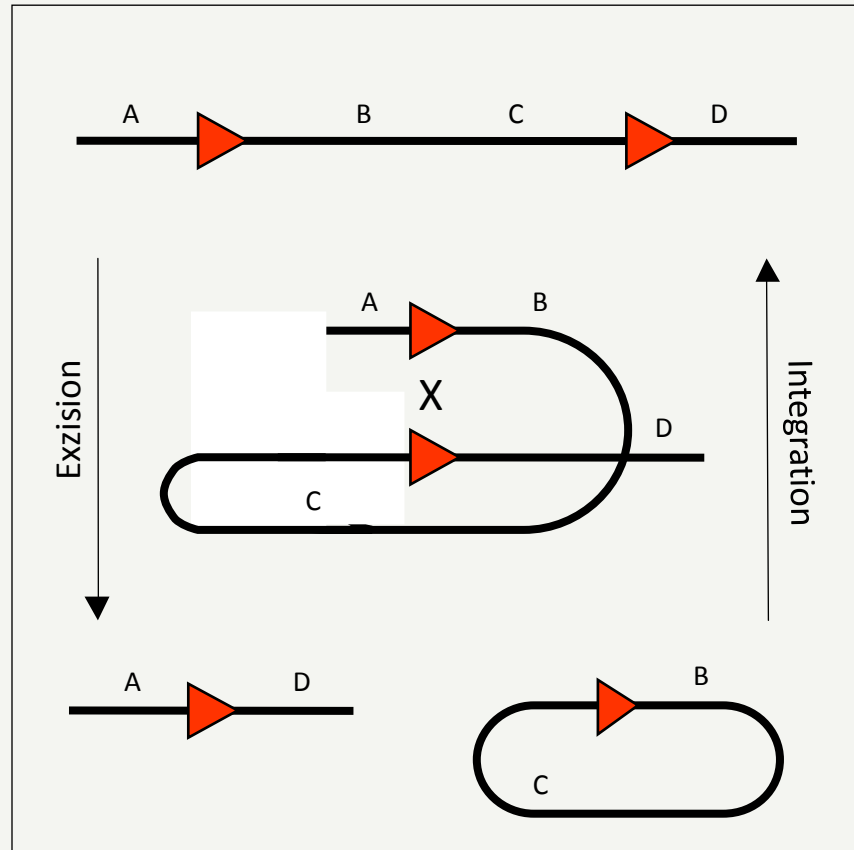
	Origin	Family	Recognition site
Gin/gix	Bacteriophage Mu	Invertase	34 bp, 12bp inverted repeats, 2 bp spacer
R/RS	<i>Zygosaccharomyces rouxii</i>	Integrase	< 59 bp, 12 bp inverted repeats, 7 bp spacer
Cre/loxP	Bacteriophage P1	Integrase	34 bp, 13 bp inverted repeats, 8 bp spacer
FLP/FRT	<i>Saccharomyces cerevisiae</i>	Integrase	34 bp 14 bp inverted repeats, 8 bp spacer

Recombination Mechanism

Inversion

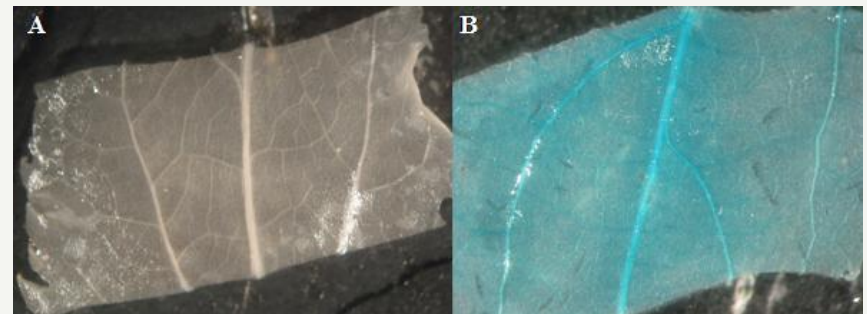
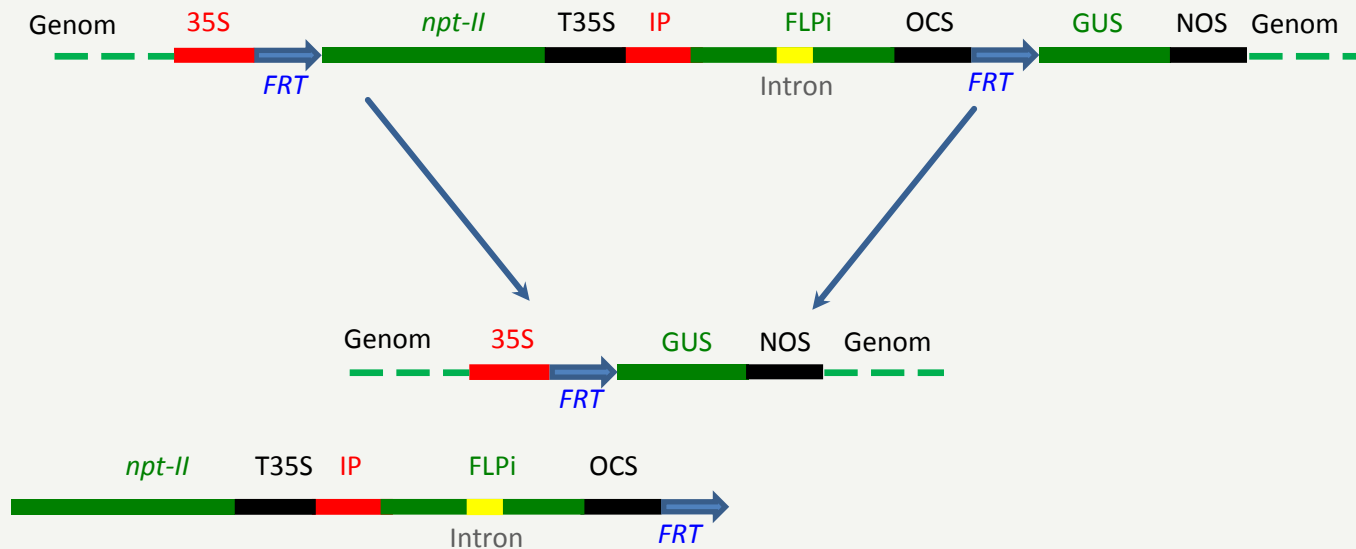


Exzision und Integration



Previous work

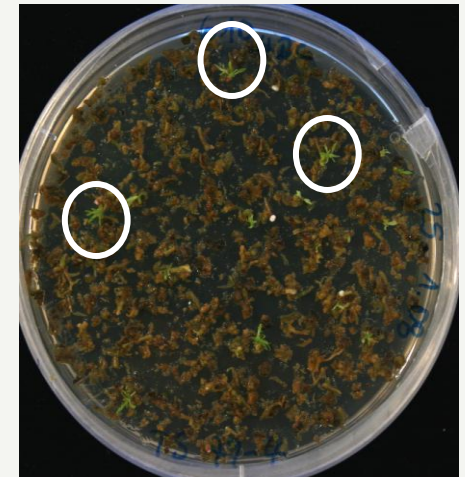
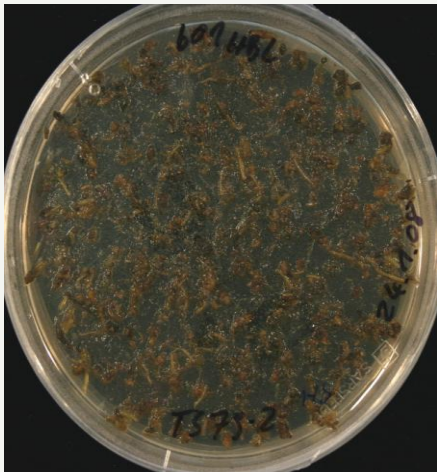
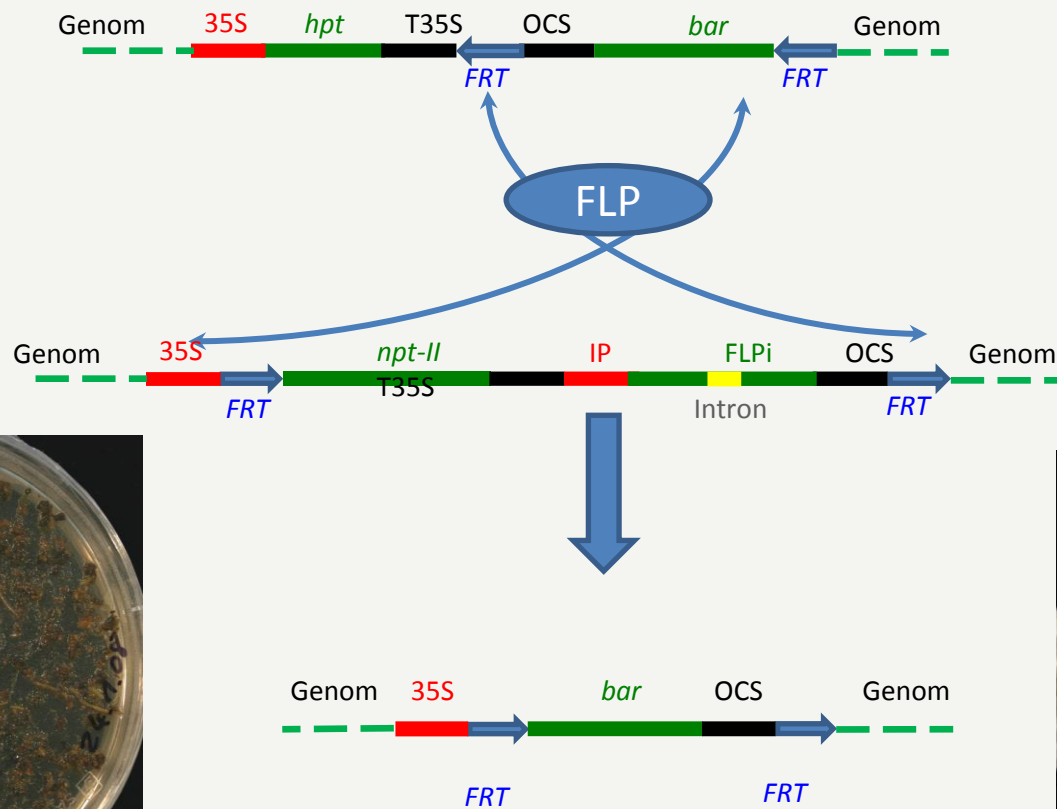
- Excision of marker genes (poplar, wheat, maize)



Poplar leaves before and after excision

Previous work

- Casset exchange/ Site-directed integration of transgenic sequences (poplar, maize)



Previous work

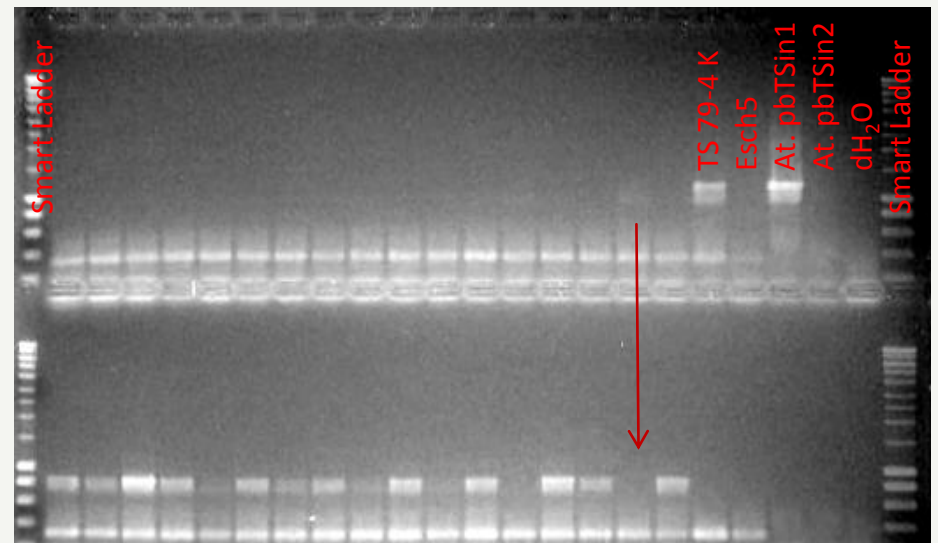
Poplar line	Plant pieces used	Regenerated calli	Regenerations-frequency
TS71-1	42088	45	0,1%
TS71-3	51392	244	0,5%
TS79-1	39663	2946	7,4%
TS79-4	23814	485	2,0%
TS79-5	31058	372	1,2%
TS85-1	39003	412	1,1%
TS85-2	16422	190	1,2%

35S-npt-II

int. control

35S-bar

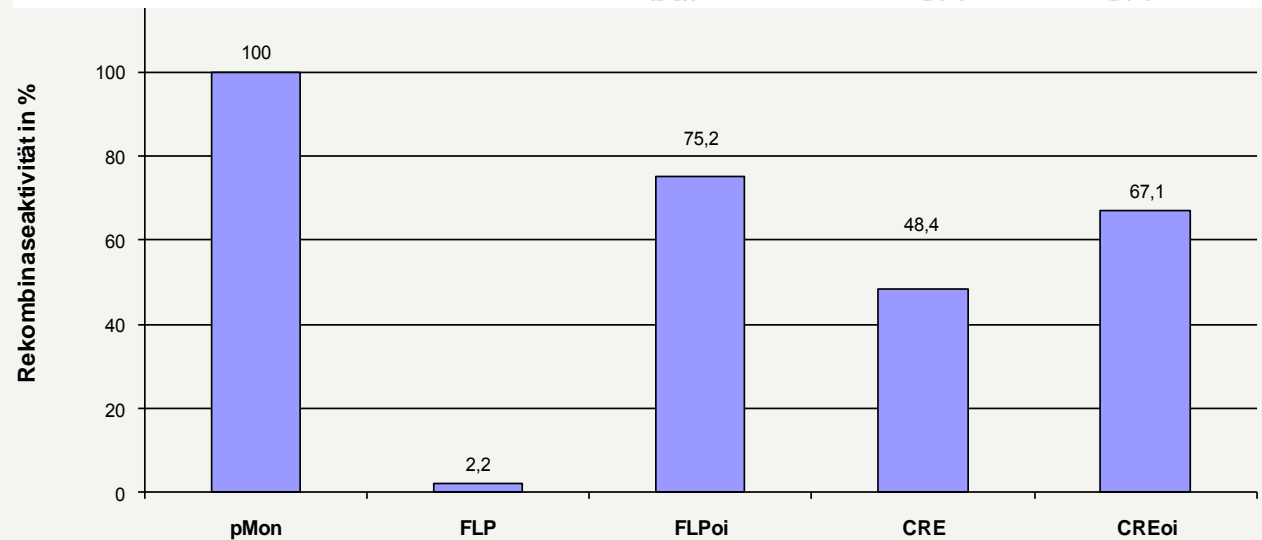
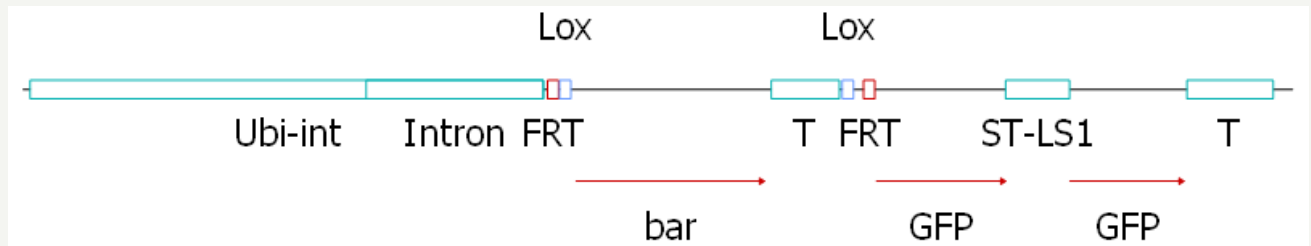
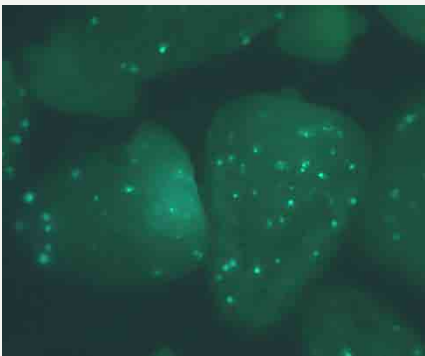
int. control



Previous work

Codon optimization of recombinase-genes

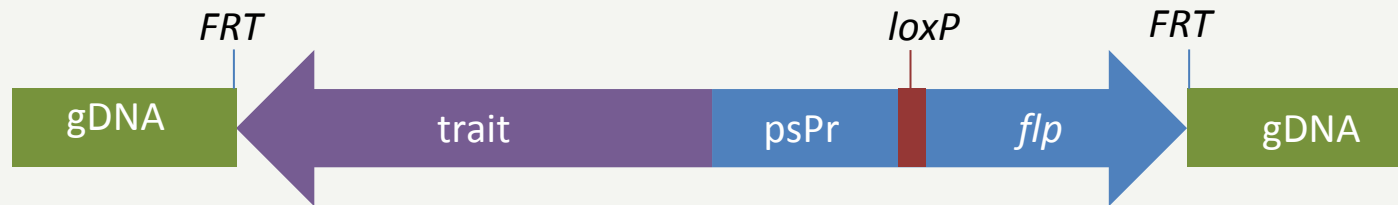
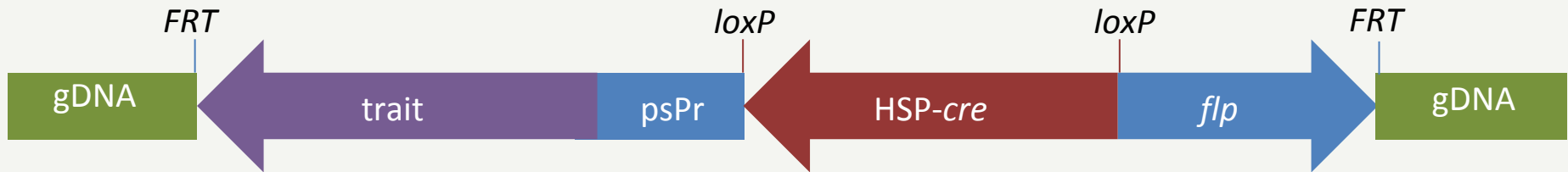
Transient co-transformation of a constitutively expressed recombinase and a marker plasmid



Current work

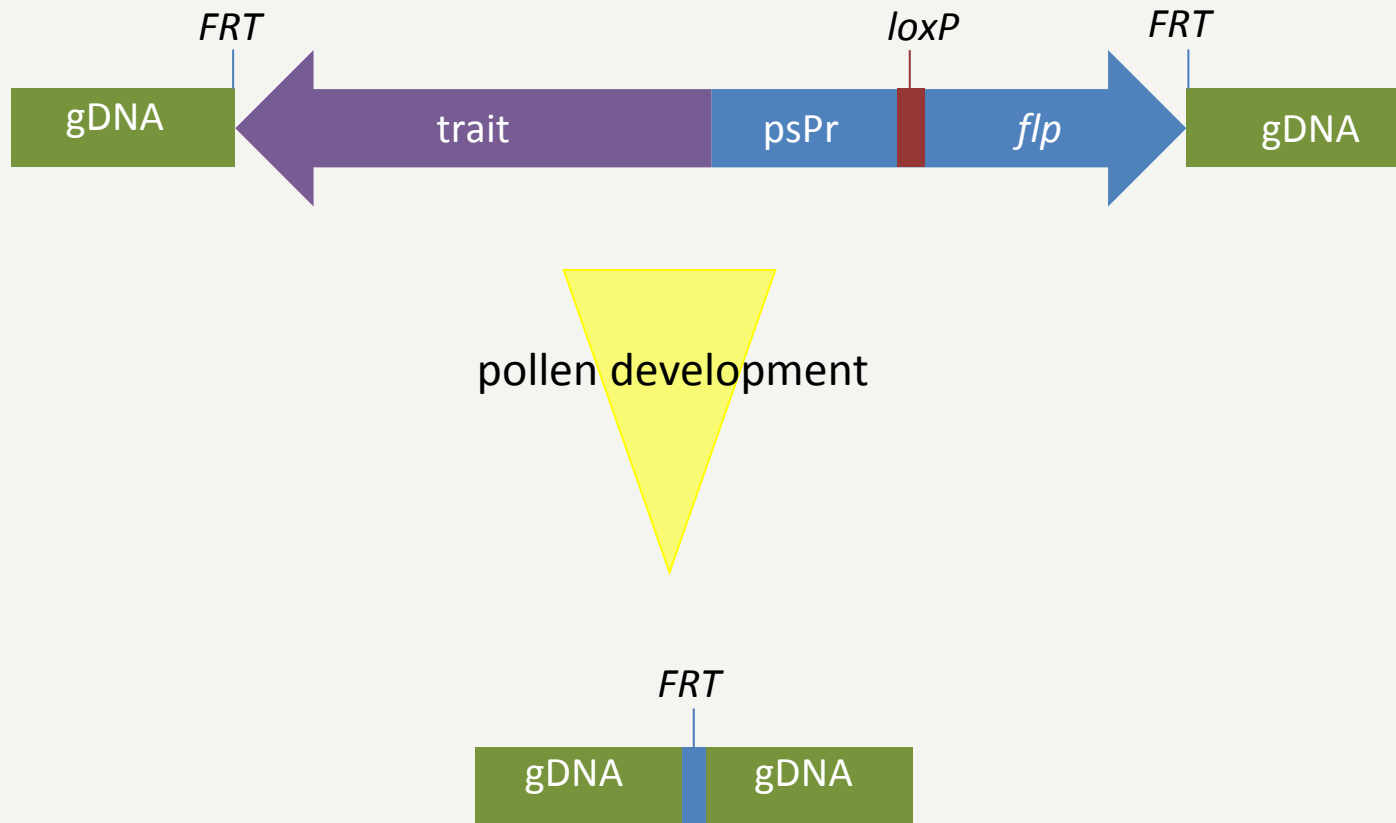
- Inducible gene activation (maize, arabidopsis, poplar)
- Excision of transgenic sequences in germ lines (maize, arabidopsis, poplar)
- Inducible RNAi and overexpression (maize)

Current work



Recombination to activate the construct

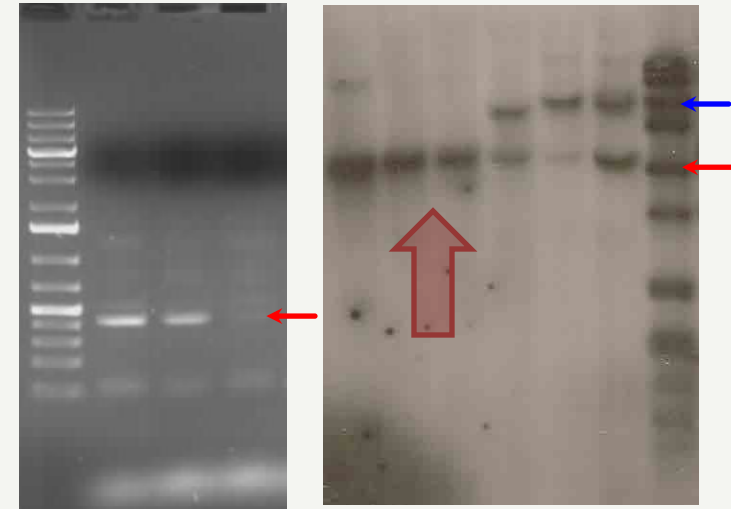
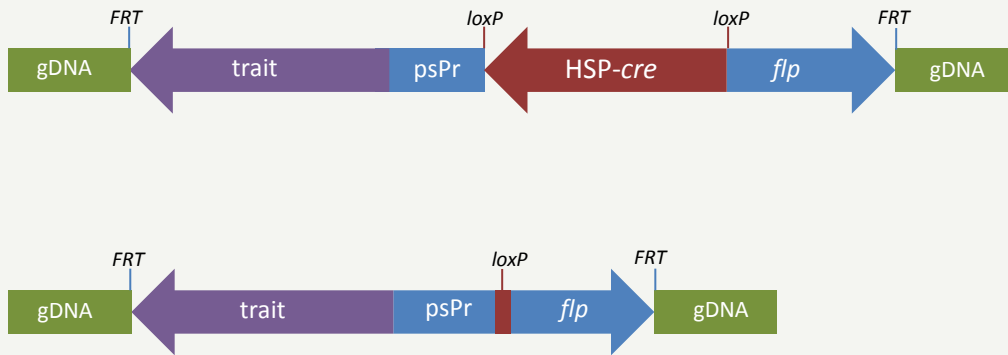
Current work



Final recombination excises coding sequences from the genome

Current work

Activation through heat shock

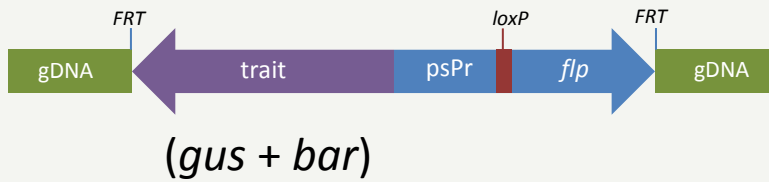


- Activation shown via PCR
- Full activation validated via Southern Blot

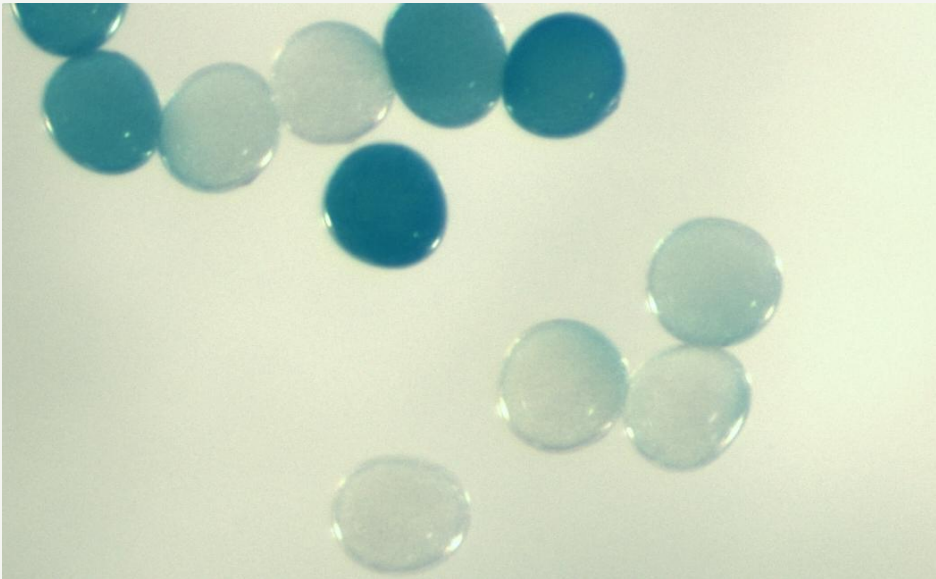
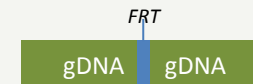
Current work

Tissue specific recombination

- Analysis of GUS-activity in pollen



- Cross-breeding into maize
- GUS-Test from progeny

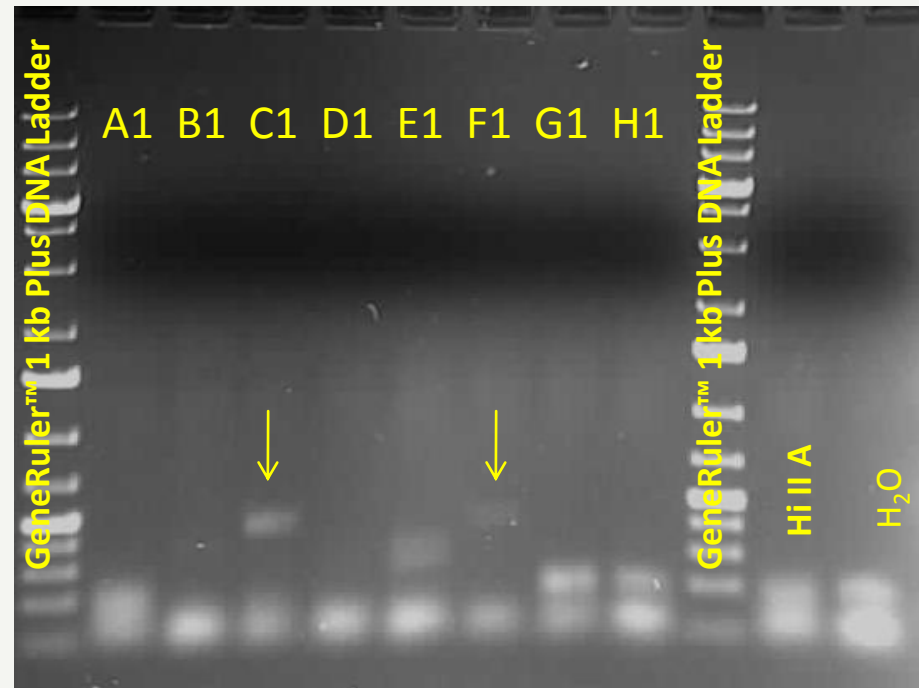


Current work

Tissue specific recombination



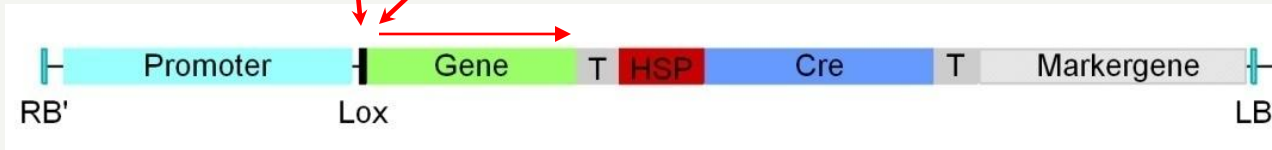
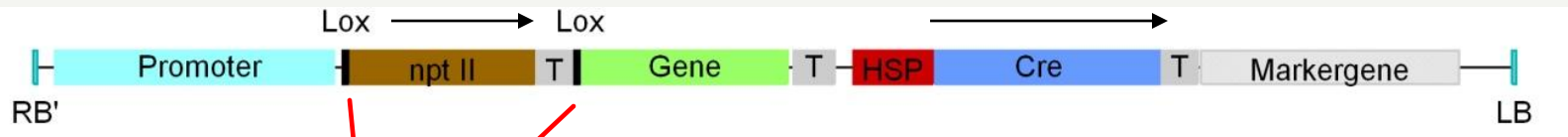
- 5 lines show significantly different segregation events
- First PCR results indicate the final recombination



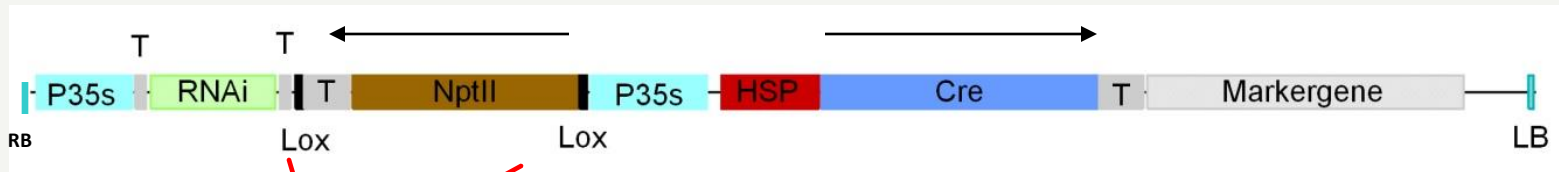
Current work

Inducible expression systems

Inducible overexpression



Inducible RNAi



Summary

- Transformation of inactivated constructs
- Inducible effects: activation, conversions, removal
- Sophisticated vectors for applications