

PRACTICAL CONSIDERATIONS FOR CRISPR-CAS9

Genome editing in plant... less scary than it looks

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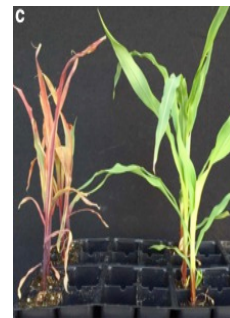
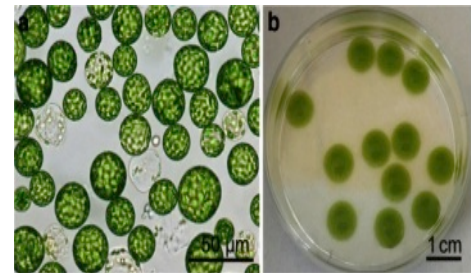
New breeding technologies in the plant sci

7th and 8th July 2017

- 09:00 - 09:20 *Designing guide RNAs*
- Advice and questions
- 09:20 - 09:40 *designing your construct*
- Advice, questions and discussion
- **09:40 - 10:00 *Selecting Transformant***
- **Advice, questions and discussion**

Transformation

- Tissue culture then regeneration – potato, tomato...
- Floral dip transformation – Arabidopsis
- Protoplast transformation followed by regeneration
- Biolistic delivery of pre-assembled Cas9–gRNA targeting Acetolactate Synthase (ALS) gene ribonucleoproteins into maize embryo cells and regeneration of plants with both mutated and edited alleles. Svitashhev et al 2016
Nat. Comm.



Selectable marker: FASTred / FASTgreen

the plant journal



The Plant Journal (2010) 61, 519–528

doi: 10.1111/j.1365-3113X.2009.04060.x

TECHNICAL ADVANCE

A rapid and non-destructive screenable marker, FAST, for identifying transformed seeds of *Arabidopsis thaliana*

Takashi L. Shimada, Tomoo Shimada and Ikuko Hara-Nishimura*

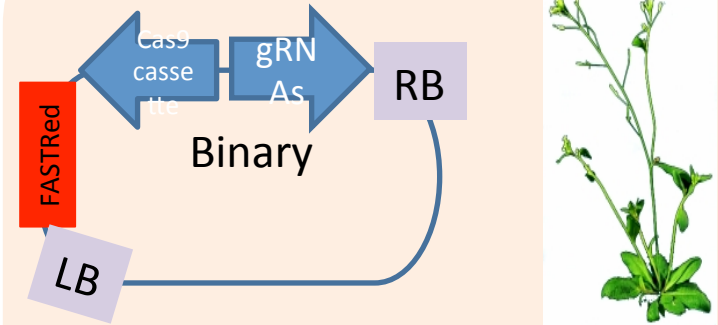
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The FAST marker harbors a fusion gene encoding either GFP or RFP with an oil body membrane protein that is prominent

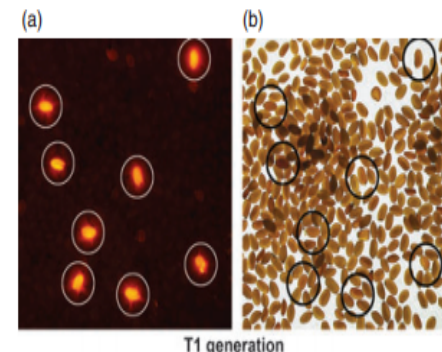
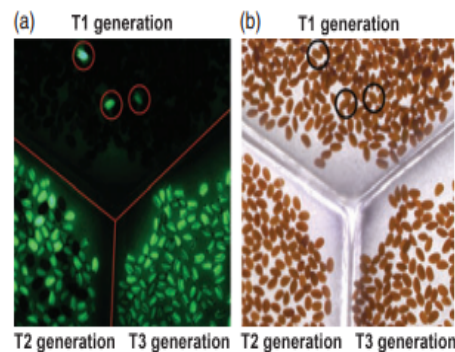
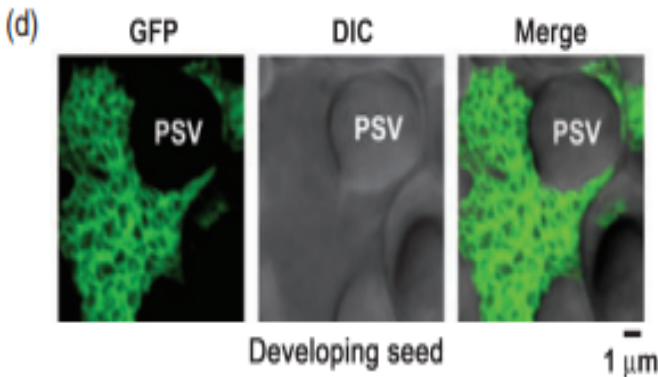
Floral dip transformation



Save a generation

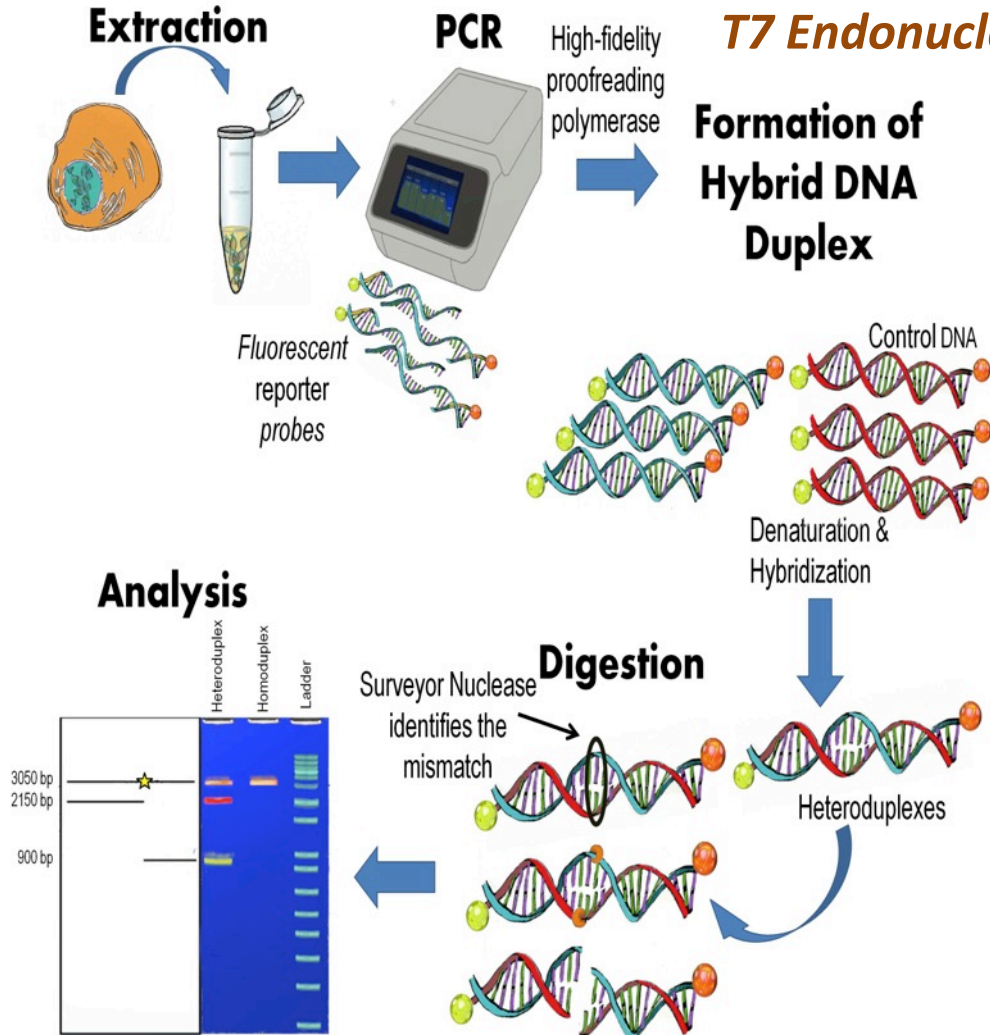
T1 plants selected for presence of Fluor

T2 seeds selected for absence of Fluor



1. Molecular Biology

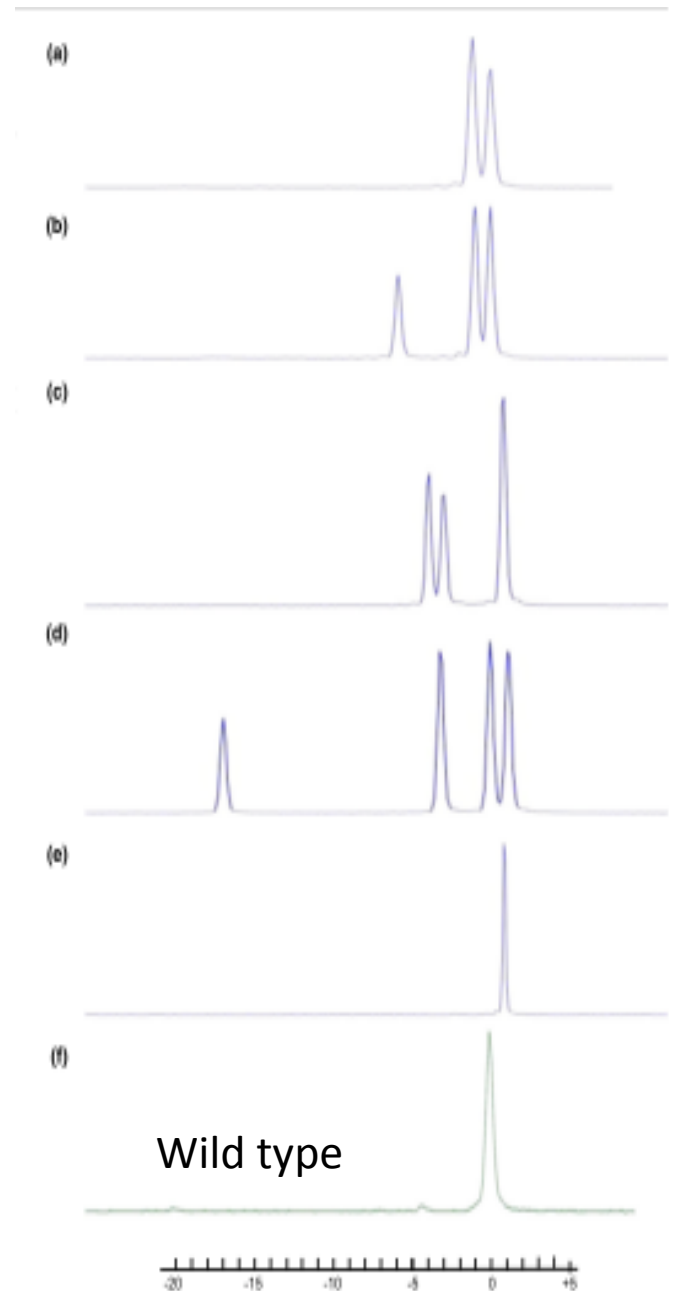
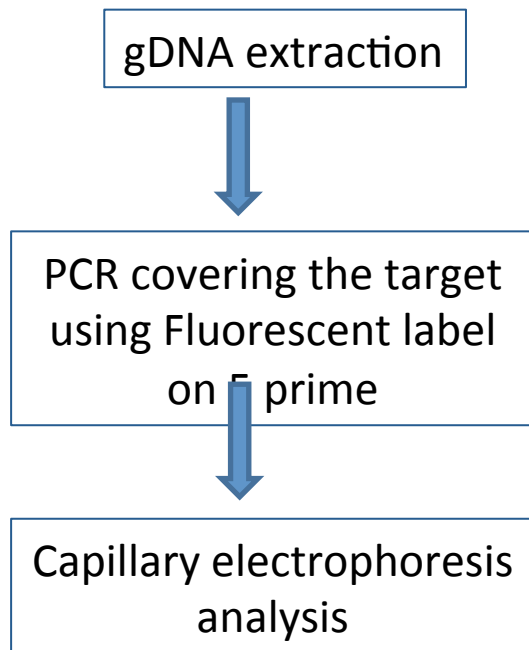
Surveyor



- It did not work reliably in our hands.
- It is expensive

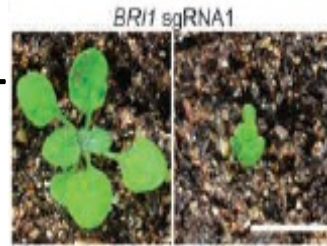
1. Molecular Biology

High-resolution fragment analysis (HRFA) for screening of mutations



2. Phenotype

- Mutant could be selected with regards to their size: DELLA, Bri1



Feng et al 2013 Cell research

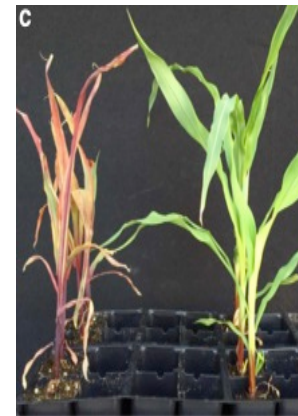
- Mutant could become more resistance to pathogen or herbicide
- Mutant could harbour different colour, resistant to abiotic stress...



Cermak et al. Genome biology 2015

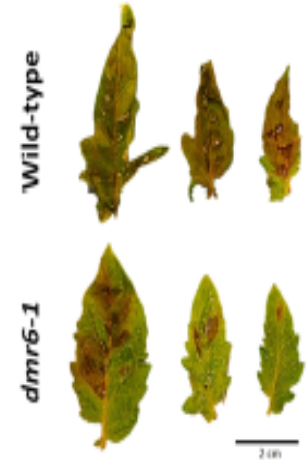


Tomlinson et al in preparation



Svitashev et al 2016 Nat. Comm

Phytophthora capsici LT1534 (10 DAI)



Daniela Paula de Toledo

Thomazella et al 2016 BioRxiv