A dual sgRNA approach for functional genomics in Arabidopsis thaliana

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Outline

- Workflow for CRISPR/Cas9 in *Arabidopsis thaliana*
- Dual sgRNA approach for functional genomics
- PEAPOD signalling pathway in *tomato*
- Targeting *cis*-regulatory elements for crop improvement
- CRISPR in *maize* and the transformation bottleneck
Arabidopsis thaliana

Workhorse for plant biology

- Atypical floral dip transformation method
- Egg cell transformed
- Chimerism
  - T1 not chimeric for T-DNA
  - Chimeric for CRISPR/Cas9 mutations

(Mao et al., 2016)
High gene editing efficiency in T1 somatic tissue

- Vectors based on Puchta lab (Fauser et al., 2014)
- Analysis of T1 plants using TIDE (Brinkman et al., 2014)
Arabidopsis thaliana

High gene editing efficiency in T1 somatic tissue

- All sgRNAs tested resulted in T1 plants with very high editing levels
- Variation between sgRNAs, but also between T1 plants
- TIDE allows studying indel spectra

Pauwels et al., 2017 BioRxiv
Arabidopsis thaliana

Inheritance of mutations to T2

- 3 lines selected with
  a) High editing levels in T1
  b) 1:3 segregation (1 T-DNA locus)

- Look for Cas9 null-segregants

Pauwels et al., 2017 BioRxiv
*Arabidopsis thaliana*

**Example for FRS7 + FRS12**

- Cloning of 2 sgRNAs
- Homoallelic double mutant in T2

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**Arabidopsis thaliana**

Isolation of a new *grxs17* CRISPR allele

- Homoallelic mutant
- NMD
- No phenocopy of known KO lines!
- Truncated protein?

“know your gene”
- Alternative start site
- Alternative first exon
- Alternative splicing
- etc.

Pauwels et al., 2017 BioRxiv
**Arabidopsis thaliana**

A dual sgRNA approach for gene deletions

- Target protein domain with 2 sgRNAs
- Efficient deletion in T₁
- Inheritance
- Cas9 free deletion mutants in T₂
- Often ‘perfect cut’

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**References**

Pauwels et al., 2017 BioRxiv
**Arabidopsis thaliana**

**Workflow**

- Comparable with generating OE line
- Bi-allelic mutant in T2
  - No visual phenotype
  - No positive selection

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Pauwels et al., 2017 BioRxiv
Arabidopsis thaliana

PEAOPOD leaf development pathway

- KD of PPD transcriptional regulators
- Larger, dome-shaped leaves
- Increased meristemoid divisions

Gonzalez, Pauwels, Baekelandt et al. 2016 TPC
WO2016005449
**Arabidopsis thaliana**

**PPD pathway controls also seed size**

*Glycine max* PPD amiRNA  
*Naito et al. 2016*

*Vigna mungo* ppd mutant  
*Naito et al. 2017*
**Arabidopsis thaliana**

*PEAOPOD leaf development pathway*

- KIX8 and KIX9 are PPD interacting proteins
- Double kix8 kix9 mutant mimics ppd phenotype

Gonzalez, Pauwels, Baekelandt *et al.* 2016 TPC
WO2016005449
**Solanum lycopersicum**

Conservation of *KIX8* and *KIX9* function in tomato

- Same vectors as Arabidopsis
- Here: 1 sgRNA for each target
- Mimics *kix8 kix9* phenotype from Arabidopsis already in T1

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*WT MicroTom*  

*kix8 kix9* CRISPR/Cas9 mutants (T1)  

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Swinnen *et al.*, unpublished
**Solanum lycopersicum**

Conservation of \textit{KIX8} and \textit{KIX9} function in tomato

WT MicroTom

\textit{kix8 kix9} CRISPR/Cas9

Swinnen \textit{et al.}, unpublished
Solanum lycopersicum

Targeting cis-regulatory elements for crop improvement

- Crop domestication traits are often caused by mutations in cis-regulatory elements.
- Subtle alterations in expression are favored to avoid pleiotropic effects.
- Regulatory networks (affecting domestication traits) are beginning to be unraveled.
- Genome editing holds promise for engineering of cis-regulatory elements.

Swinnen et al., 2016 TIPS
Solanum lycopersicum

Boosting the nutritional value of tomato

- Altering steroidal glycoalkaloid composition
- Identification of AP2-ERF that targets GAME4 during ripening
- CRISPR KO leads to increased GAME4 in breaker fruit
- TF controls also other genes/processes
- Targeting of cis-regulatory element in GAME4 promoter

Swinnen et al., unpublished
Zea mays

B104 transformation pipeline

- 3 cobs $\rightarrow$ 600 embryos $\rightarrow$ 15 shoots on average
- 7 months (to T1 seed)
- Very reproducible = unique
- CRISPR/Cas9 = extremely efficient: > 20 constructs

=> External service

Coussens et al. 2012
Maize Transformation Service
**Zea mays**

**Example 1 sgRNA**

- Initially: pBUN411 (Qi-Jun Chen's lab)
- Now: standard MS Gateway pDEST
- Extra Cas9 module
- Bi-allelic mutations in T0

Vanderhaeghen R *et al.*, unpublished
**Zea mays**

*Transformation = bottleneck: current research*

A) Delivery: proteolistics

B) Plant regeneration

- QuickCorn (Dupont Pioneer)
  - OE of *Odp2 + Wus2*

- Developing other *morphogenic regulators*
  - OE of TF1

Vanderhaeghen R *et al.*, unpublished
**Zea mays**

Transformation = bottleneck

- Standard MS Gateway pDEST
- Cas9 module
- 2 sgRNAs
- Morphogenic regulator module

Vanderhaeghen R et al., unpublished
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Crop Genome Engineering