Arabidopsis Gene Research Project

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Dynamic Genome

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Plant Roots

- Have different functions:
- providing nutrition for growth
- storing resources for development
- giving and maintaining plant structure

- May take on different appearances for the survival of each plant

- Many components carry out each of these tasks as a system.
- **Meristematic** zone - division and formation
- **Elongation** zone - lengthening
- **Differentiation** zone - specialization

Arabidopsis as a Plant Model

- *Arabidopsis thaliana* is often used as a model organism, esp. for genetic studies
- efficient generation and self-reproduction (Koornneef & Meinke, 2010)
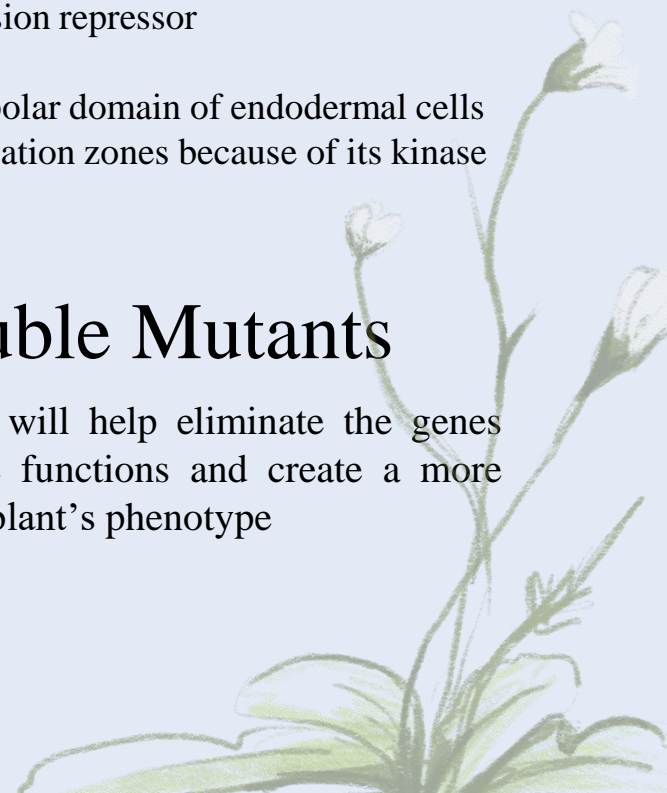- extensive research done globally over time (The Arabidopsis Genome Initiative, 2000).

WFL   - WALLFLOWER

- May provide the positioning of gaps that help with hair growth

- Localized towards the inner polar domain in epidermal cells of the meristematic zones / elongation zones / lateral root (Van Norman, 2021).

KOIN   - KINASE ON THE INSIDE

- Predicted to act as a cell division repressor

- Localized towards the inner polar domain of endodermal cells in the meristematic and elongation zones because of its kinase domain (Van Norman, 2021)

Interest in Double Mutants

- Creating double mutants will help eliminate the genes that may serve the same functions and create a more obvious difference in the plant's phenotype

Experimental Plan

- To "knock-out" genes, mutations are created in various genes related to KOIN and WFL to determine any redundancy in the functionality of these genes. (Van Norman, 2021)
- Two pairs of guide RNAs are chosen (from E-CRISP)
- Primers for the gene work with CRISPR-Cas9 to cleave the genomic sequence and create a large indel.

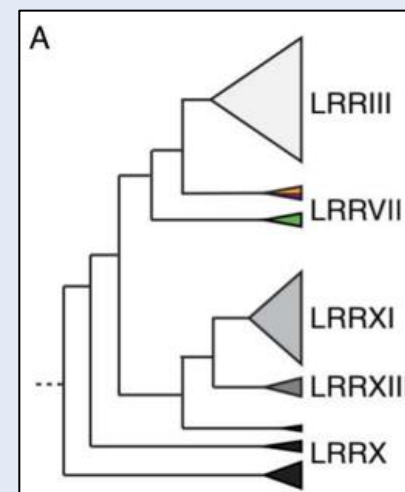- **Assigned Gene: AT3G08680**

gRNA Long Pair: _2_1833 and _0_3055

gRNA Short Pair: _6_3055 and _2_3055

Source: http://www.e-crisp.org/E-CRISP/workdir/Thu_Feb_25_21:25:54_20211614288354/index.html

Targeting the Gene and its Homologs

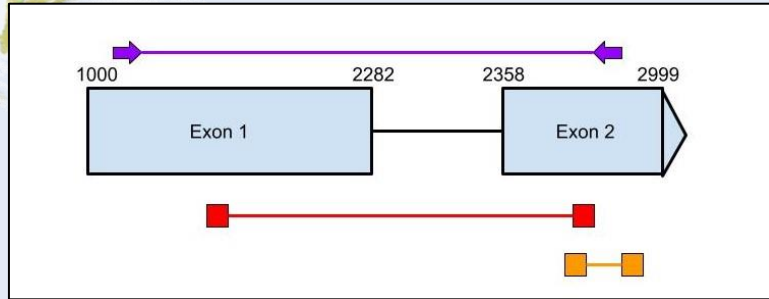

A. thaliana Phylogenetic Tree (Van Norman, 2021)
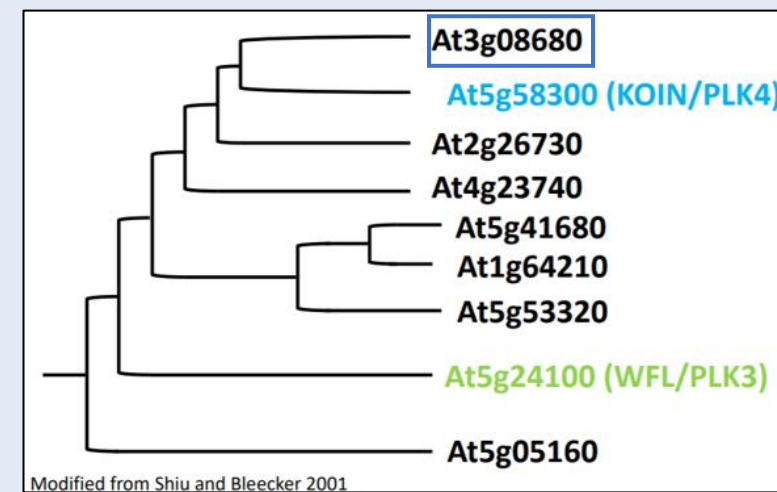
Genes Related to WFL and KOIN (Van Norman, 2021)

- Using gRNAs with several hits can help eliminate genes that provide the same function as the assigned gene
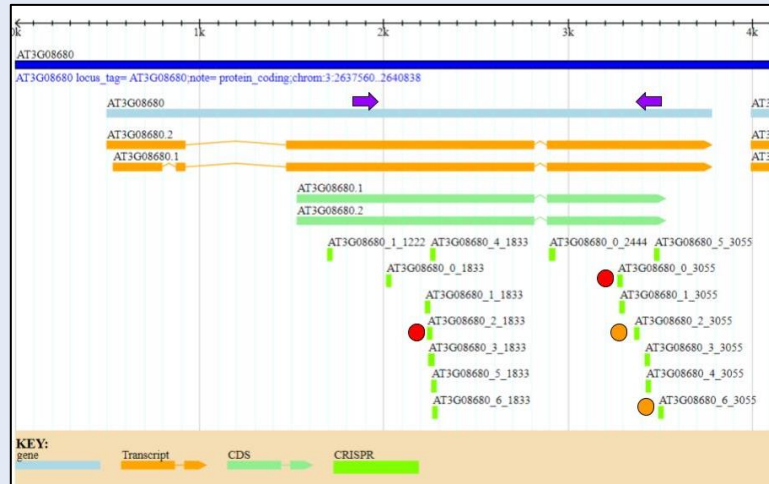
Image Source for gRNA pairs: http://www.e-crisp.org/E-CRISP/workdir/Thu_Feb_25_21:25:54_20211614288354/index.html
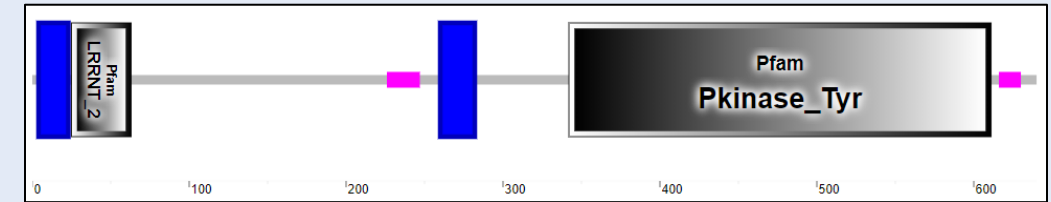
Diagram of Gene **AT3G08680**

— = Primer
— = gRNA Long Pair
— = gRNA Short Pair

* Primer does not cover entirety of the short gRNA pair

Source: http://www.e-crisp.org/E-CRISP/workdir/Thu_Feb_25_21:25:54_20211614288354/index.html

Diagram of Protein Domains

Source: http://smart.embl-heidelberg.de/smart/show_motifs.pl

— = Transmembrane region

- Total Length: 640 aa
- Kinase Domain Length: 342 to 611 aa
- Leucine Rich Repeat Domain: 25 to 63 aa

Guide RNAs

Long Pair: _2_1833 and _0_3055, Targets off-target sites

Short Pair: _6_3055 and _2_3055, Targets on-target sites

Source: http://www.e-crisp.org/E-CRISP/workdir/Thu_Feb_25_21:25:54_20211614288354/index.html

PCR Primers to Verify gRNA Targeted Sites

Primer pair 1	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	ACTCCGTTTGCCTGGATCTG	Plus	20	1212	1231	60.04	55.00	4.00	1.00
Reverse primer	TCTCACCACTGATTGCACCC	Minus	20	2756	2737	59.96	55.00	4.00	2.00
Product length	1545								

Source: https://www.ncbi.nlm.nih.gov/tools/primer-blast/primertool.cgi?ctg_time=1614899807&job_key=KyH0ZvwQ8bjWhvSD-ePQsYP4wYOu69qerw
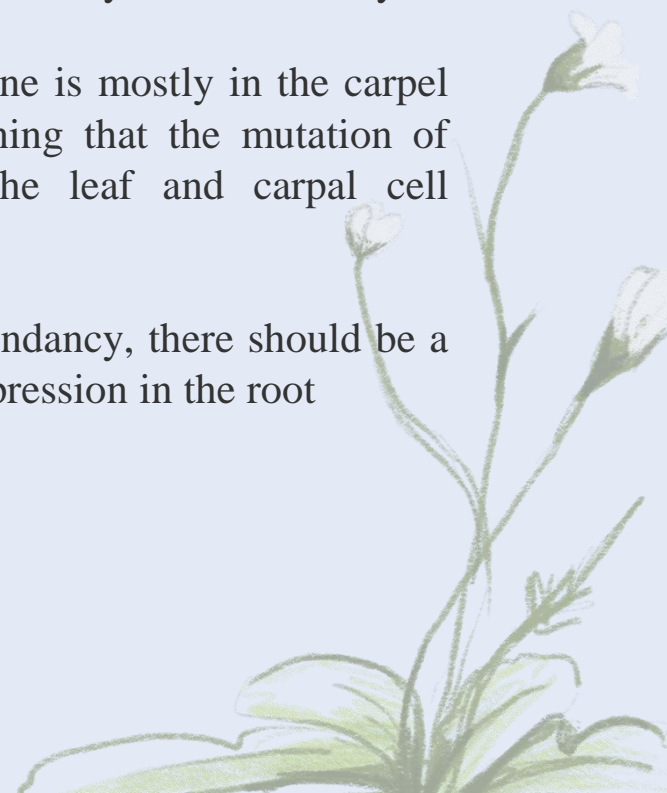
Predicted Efficacy of gRNA in Mutagenesis

▪ Mutated copy of gene replaces the wild type copy

- Using the gRNAs, the genomic sequence can be cleaved, referencing to a protospacer adjacent motif (PAM) sequence NGG (Van Norman, 2021)
- Large deletion of nucleotides result in a shifted frame for translation and cause different proteins to be coded for than the original

▪ _0_3055 in long gRNA pair has four hits, which may also "knock-out" genes that are homologs of AT3G08680

▪ The primer chosen does not cover the span of the short gRNA pair, so the deletion between the two target sites may be incomplete

Predicted Consequence of Mutation on Phenotype

▪ Gene **AT3G08680** functions primarily in protein serine and threonine kinase activity, kinase activity, and ATP binding

▪ Larger change in phenotype of *A. thaliana* if the gene and KOIN have redundancy in functionality

▪ The expression of this gene is mostly in the carpel and cauline leaves, meaning that the mutation of this gene can impact the leaf and carpal cell structures

▪ If there is functional redundancy, there should be a change of cell division repression in the root

References

Arabidopsis Genome Initiative. (2000 Dec 14) Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. *Nature*. doi: 10.1038/35048692.

Koornneef, M., & Meinke, D. (2010). The development of Arabidopsis as a model plant. *The Plant Journal, 61*, 909-921. doi: 10.1111/j.1365-313X.2009.04086.x

Van Norman, J. (2021). *Introductory Presentation* [PowerPoint slides]. College of Natural and Agricultural Sciences, University of California, Riverside. https://ilearn.ucr.edu/bbcswebdav/pid-4223925-dt-content-rid-44556217_1/courses/BIOL_020_004_21W/BIOL20_RootIntro_WFL%2CKOIN_11Feb2021small.pdf