Predicting and assessing SAUR gene function through phylogenetics and CRISPR-Cas9 genome editing

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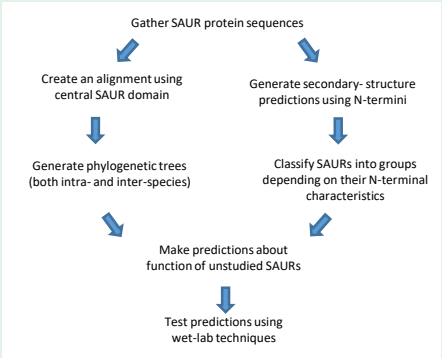
Highlights

- Analyzing phylogenetic trees allows us to predict the functions of unstudied *SAUR* genes based on their evolutionary relationship to *SAUR*s that are studied
- Thirty-six new *SAUR* genes have been discovered through genome mining
- N-terminal analysis reveals *SAUR* lineages with potentially conserved functions, based on their predicted secondary structure
- Successfully knocked out two *saurs* in *Arabidopsis thaliana* in a continuing effort to observe a phenotype

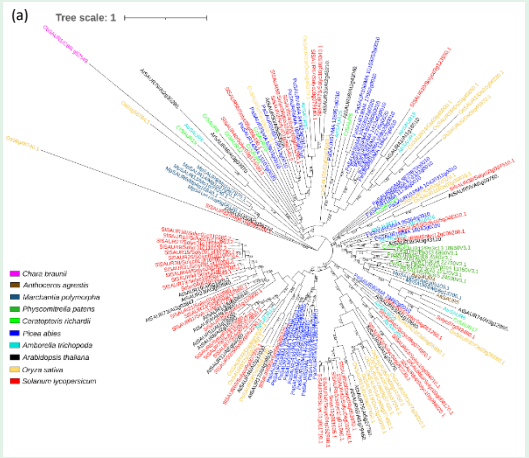
Rationale

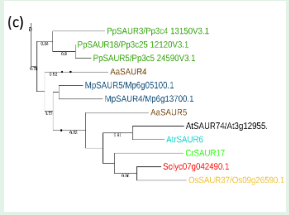


The phylogenetic and N-terminal analysis portion of the project generates hypotheses about *SAUR* function. The CRISPR-Cas9 portion tests those hypotheses by knocking out genes to look for a phenotype.

We designed a CRISPR-Cas9 multiplex construct targeting four *SAUR* genes, *saur9, saur10, saur16,* and *saur50* in *Arabidopsis thaliana*. We introduced it in a background already missing 19 *saurs* since the high multiplicity and functional redundancy of the family often makes it difficult to observe a phenotype in a one- or two-mer knockout

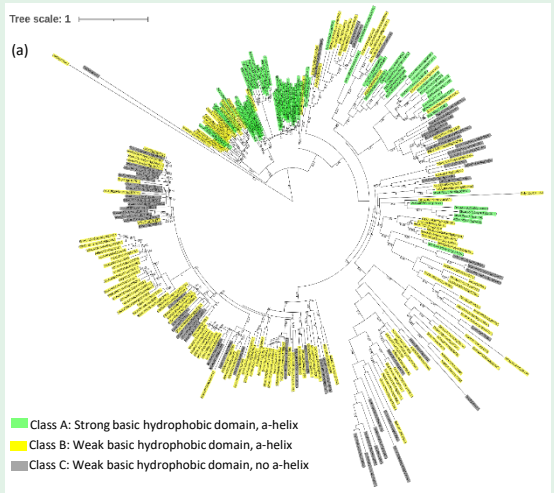
Phylogeny across ten species

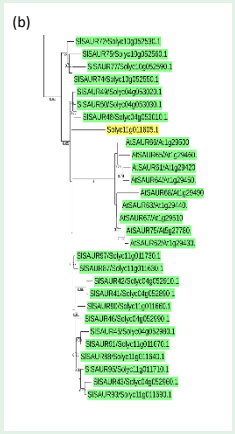




Phylogenetic tree including clade representatives of all ten species studied. (a). Support values are shown only for branches with greater than 0.7 confidence **(b).** (excerpt from (a), enlarged and de-circularized for ease of viewing) This clade only includes members from *Solanum lycopersicum* (Sl), suggesting a more recently evolved SAUR lineage **(c).** This clade includes members from every species studied except *Picea abies,* and *Chara braunii,* suggesting a more ancient SAUR lineage

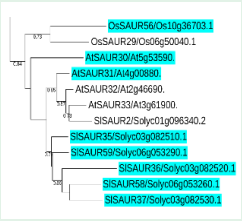
Angiosperm phylogeny colored by N-terminal class





N-terminal analysis of rice, tomato, and *Arabidopsis*. (a): A phylogeny of all 271 SAURs from rice (Os), tomato (Sl), and *Arabidopsis* (At), color coded by N-terminal classification. **(b):** An excerpt from subfigure A depicting a group of SAURs with a well-conserved strong basic hydrophobic domain, indicating the potential for a shared localization and/or function.

Histidine-rich N-termini



An excerpt from the At Os Sl tree displaying all SAURs that have a histidine-rich N-terminus (blue highlight).

Why it matters: There is some evidence that these are expressed in phloem companion cells (AtSAUR31 and SlSAUR59 specifically). From this, we can infer that their function in phloem companion cells is likely to be evolutionarily conserved.

CRISPR-Cas9 *saur* knockouts

Two of the four targeted *saur* genes were successfully knocked out: *saur9* and *saur16* (mutations diagrammed below)

Mutants were identified via PCR and CAPS-marker testing and confirmed through gene sequencing. Though performing a heat shock treatment increased the mutation rate to some extent, mutants still did not show up with the expected frequency, as we have not found mutants for either *saur10* or *saur50.*

Future Directions

Taken together, phylogenetic results can be used to:

- Get a broader understanding of how *SAURs* arrived at their current form
- Predict structural attributes and functioning of unstudied *SAUR* genes
- Inform future decision-making about genes to target with CRISPR-Cas9

The CRISPR-Cas9 genome editing will continue through:

- Refined screening for *saur10* and *saur50* mutants
- Functionality experiments on the 21-mer mutant
- Targeting new genes with CRISPR-cas9 based on phylogenetic results and expression data

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