CRISPR/Cas9 Mediated Mutagenesis of Moss SUMO System & Development of a Decahistidine(10His)-tagged SUMO Purification Line

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Introduction & Background

SUMOylation

- Small Ubiquitin-related Modifier (SUMO) is a small protein that acts as post translational modifier affecting protein activity, localization, and stability.
- SUMOylation (the process of SUMO attachment) occurs within minutes of expose to environmental stressors, providing resilience ensuring survival under suboptimal conditions.
  - Four main steps of the SUMO cycle include: Activation (E1 - SAE1/2) , Conjugation (E2 – SCE1), Ligation (E3 - SIZI1), and DeSUMOylation by DPS's

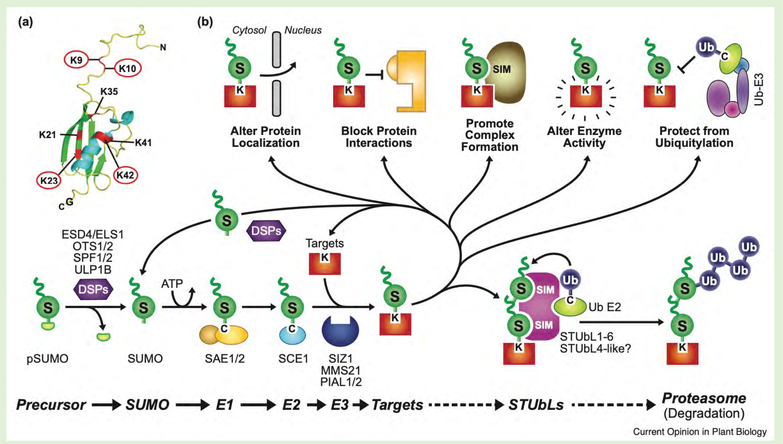
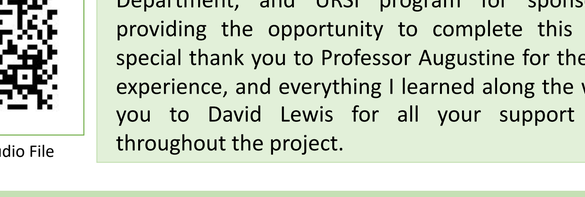


Figure 1. Overview of SUMOylation pathway. (Augustine and Vierstra 2018)

- CRISPR modifies target areas of the genome and can knock out genes of interest in the SUMO pathway to better characterize the system.

The SUMO system in *Physcomitrium patens*

- Highly amenable to genetic and bioanalysis.
- Haploid cells require modification of only one chromosome to create knock outs and express phenotypic effects.



P. Patens life cycle. (Rensing et al. 2020)

Goals

- Genetically interrogate the SUMO system in *Physcomitrium patens* and identify the proteins modified by SUMO.
- Isolation and purification of a 10His-SUMO1a line for identification of SUMOylated proteins.

Selected References

1. Augustine, Robert C., and Richard D. Vierstra. "SUMOylation: Re-Wiring the Plant Nucleus during Stress and Development." Current Opinion in Plant Biology, vol. 45, Oct. 2018, pp. 143–54.
2. Hendriks, Ivo A, and Alfred C. O. Vertegaal. "A High-Yield Double-Purification Proteomics Strategy for the Identification of SUMO Sites." Nature Protocols, vol. 11, no. 9, Sept. 2016, pp. 1630–49..
3. Hendriks, Ivo A., and Alfred C. O. Vertegaal. "Label-Free Identification and Quantification of SUMO Target Proteins." SUMO, edited by Manuel S. Rodriguez, vol. 1475, Springer New York, 2016, pp. 171–93.
4. Peroutka III, Raymond J., et al. "SUMO Fusion Technology for Enhanced Protein Expression and Purification in Prokaryotes and Eukaryotes." Heterologous Gene Expression in E.Coli, edited by Thomas C. Evans, and Ming-Qun Xu, vol. 705, Humana Press, 2011, pp. 15–30.
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6. Rodriguez, Manuel S., editor. SUMO: Methods and Protocols. Springer New York, 2016.

QR Code of Audio File

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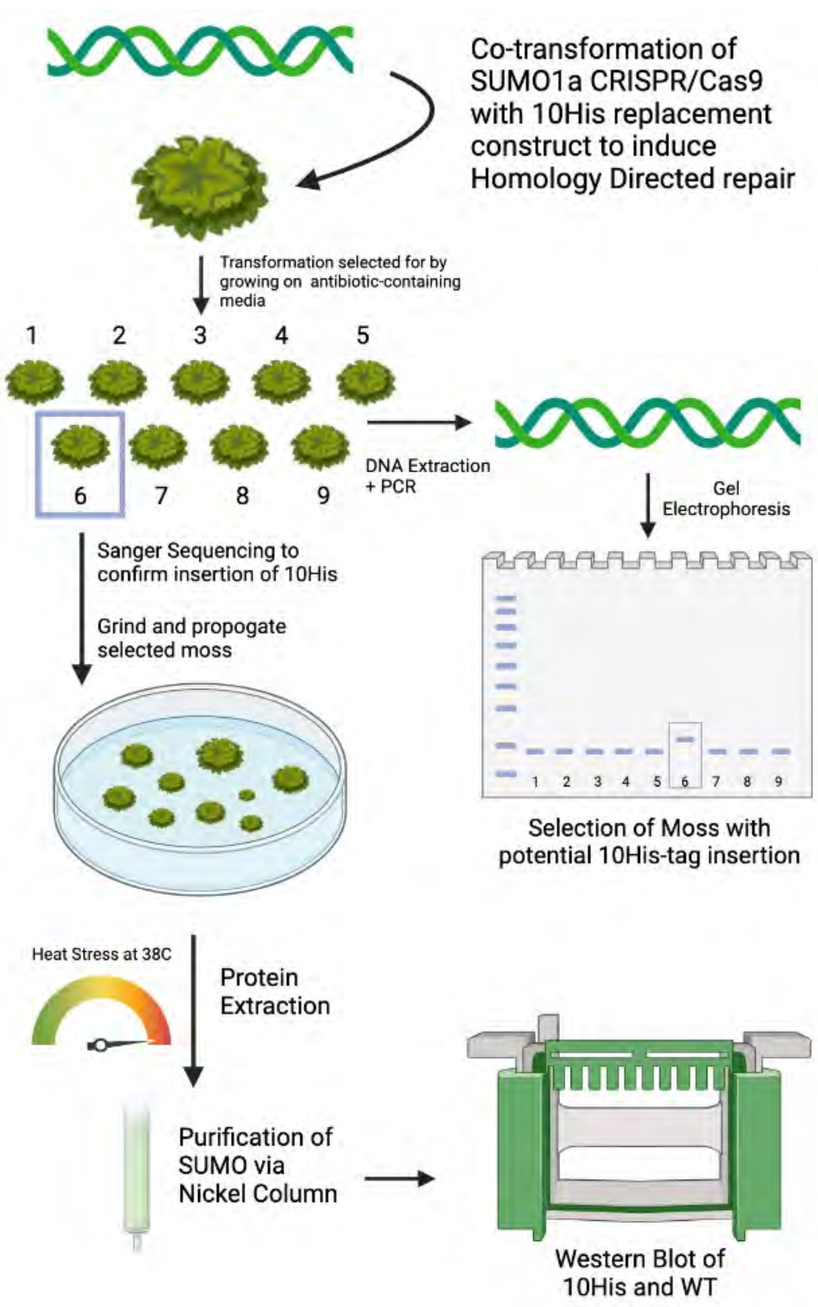
Decahistine (10His)-tag Development

Methods



Results

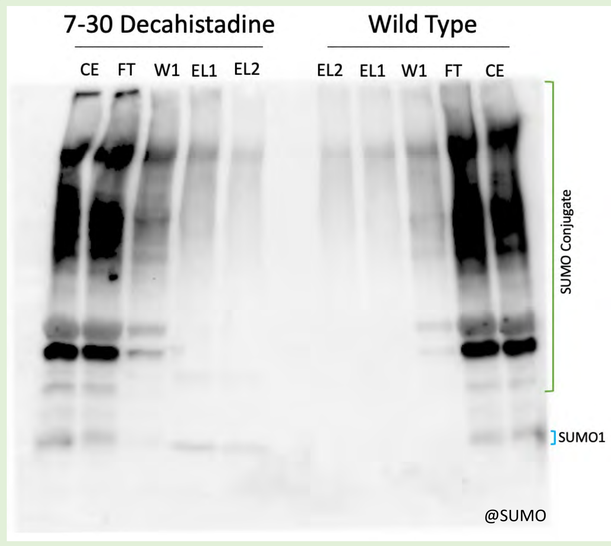


Figure 2. Western Blot of Nickel Purification of SUMO from the10His-SUMO line.

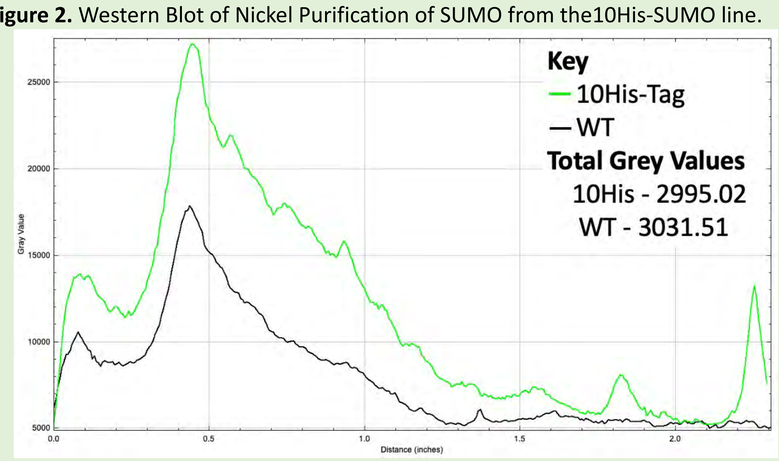


Figure 3. Quantitative comparison of grey values from lanes EL1 of WT vs. 10His tagged moss from the western blot (Figure 2).

Conclusions

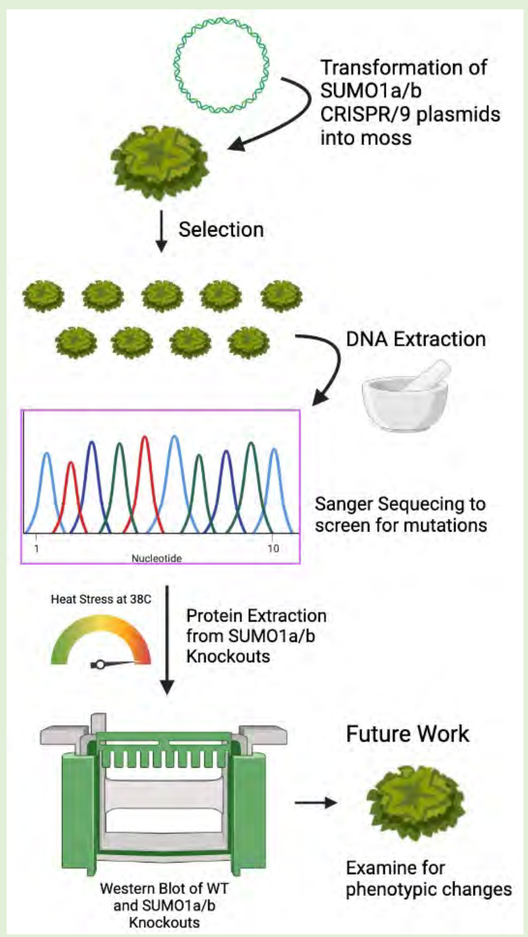
- The 10His-tag enables purification of free SUMO and SUMO1a conjugates thereby enabling their proteomic identification.

Future Work

- Creation of a line where both SUMO1a and SUMO1b are 10His tagged.

CRISPR/Cas9 Mediated Mutagenesis

Methods

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WT      1  MSGVEDGSKMANNQTNTQDQEEKKPLDGAGQHINLKVKGQDGGEVFFRIKSTATLRKLMN
10-1    1  MSGVEDGSKMANNQTNTQDQEEKKPLDGAGQHINLKVKGQDGGEVFFRIKSTATLRKLMN
10-2    1  MSGVEDGSKMANNQTNTQDQEEKKPLDGAGQHINLKVKGQDGGEVFFRIKSTATLRKLMN
10-4    1  MSGVEDGSKMANNQTNTQDQEEKKPLDGAGQHINLKVKGQDGGEVFFRIKSTATLRKLMN
10-7    1  MSGVEDGSKMANNQTNTQDQEEKKPLDGAGQHINLKVKGQDGGEVFFRIKSTATLRKLMN
10-11   1  MSGVEDGSKMANNQTNTQDQEEKKPLDGAGQHINLKVKGQDGGEVFFRIKSTATLRKLMN
10-12   1  MSGVEDGSKMANNQTNTQDQEEKKPLDGAGQHINLKVKGQDGGEVFFRIKSTATLRKLMN

WT     61  AYCDRQSVDPSSIAFLFDGRRLRADQTPAELEMEDGDEIDAMLHQTGGNAC---
10-1   61  AYCDRQSVDPSSIAFLFDGRRLRADQTGGGA*NGRWR*DRCHASSNWWKCLL--
10-2   61  AYCDRQSVDPSSIAFLFDGRRLRADQTLRRWSLKWKMEMRSMPCFIKLVEMLA-
10-4   61  AYCDRQSVDPSSIAFLFDGRRLRADQTYLRRWSLKWKMEMRSMPCFIKLVEMLA
10-7   61  AYCDRQSVDPSSIAFLFDGRRLRADLGA*NGRWR*DRCHASSNWWKCLL-----
10-11  61  AYCDRQSVDPSSIAFLFDGRRLRADHTCGGGA*NGRWR*DRCHASSNWWKCLL-
10-12  61  AYCDRQSVDPSSIAFLFDGRRLRADQTCGGGA*NGRWR*DRCHASSNWWKCLL-
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Figure 4. Amino acid sequence alignment of predicted sequences of wild type and selected SUMO1b mutants. DiGlycine (highlighted by the red box) residues are required for SUMOylation.

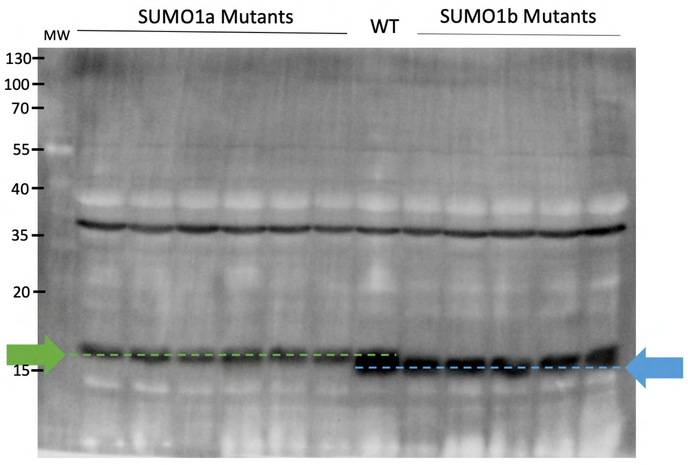

Figure 5. Western Blot of SUMO protein extracted from moss with SUMO1a and SUMO1b genes knockouts.

Conclusions

- SUMO1a and SUMO1b genes encode for distinct variants of the SUMO protein.

Future Work

- Analysis of SUMO1a/b knockouts on the phenotype.
- Further exploration of SUMO variants.