Using GFP to study nuclear localization of FLC protein in Arabidopsis plants

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Introduction

- Transport of proteins from the cytoplasm to the nucleus requires their Nuclear Localization Sequence (NLS)
  - Short amino acid sequence
- MADS Transcription Factors are highly conserved proteins that function in the nucleus to regulate gene expression

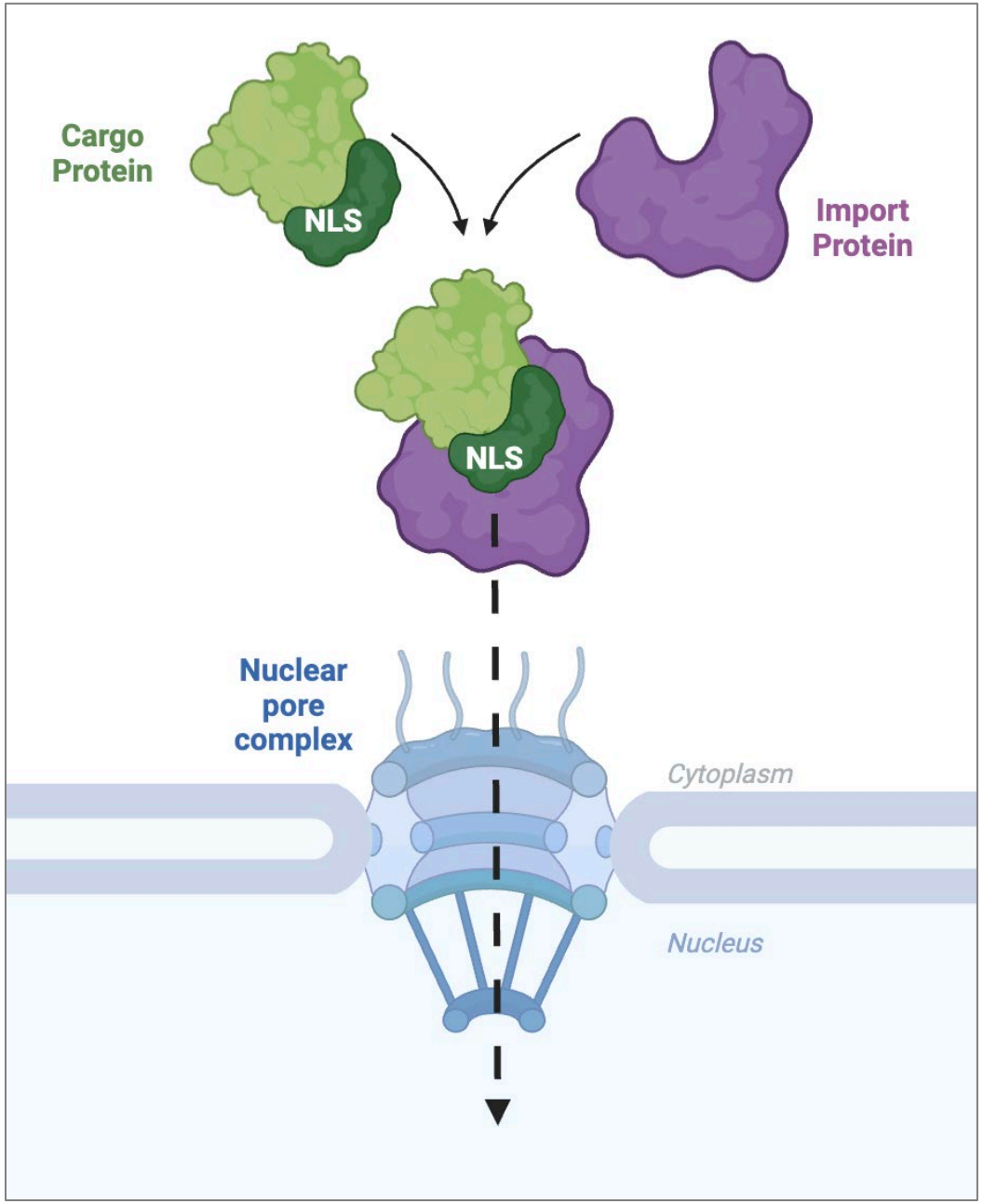


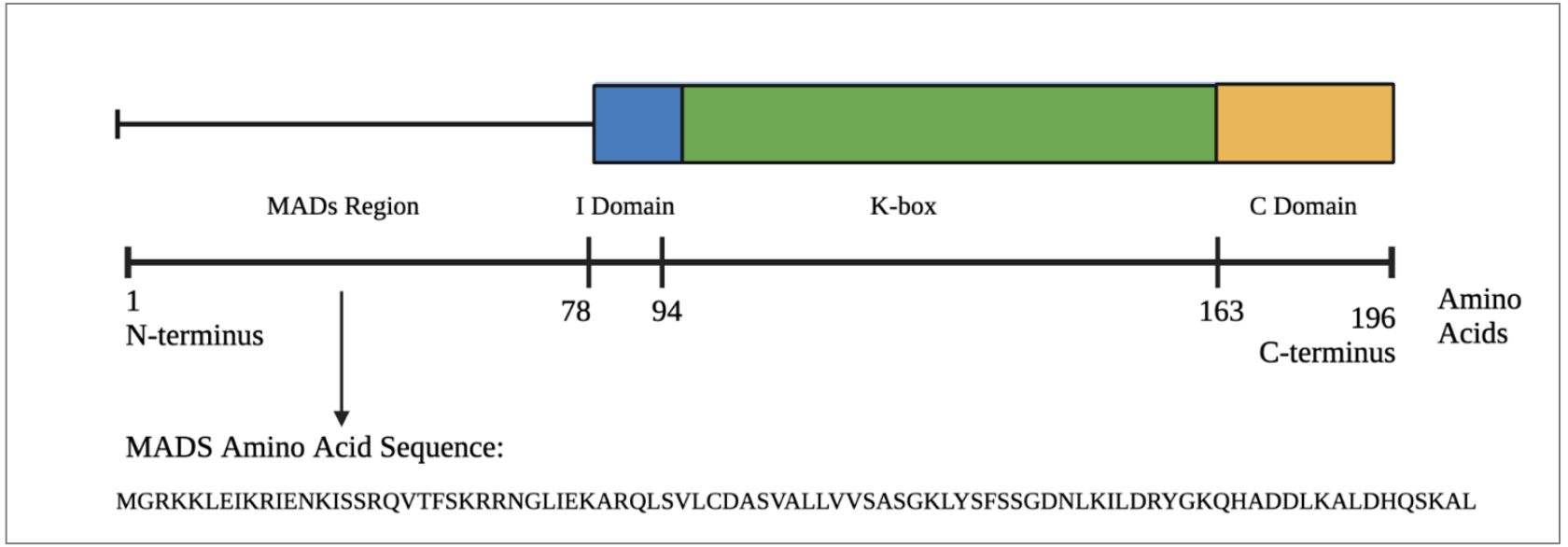
Illustration of nuclear import.

Introduction

- Arabidopsis is a model plant
- FLC is an important MADS Transcription Factor in Arabidopsis
- Previous research on MADs Nuclear Localization Signals

My Arabidopsis plants starting to flower.

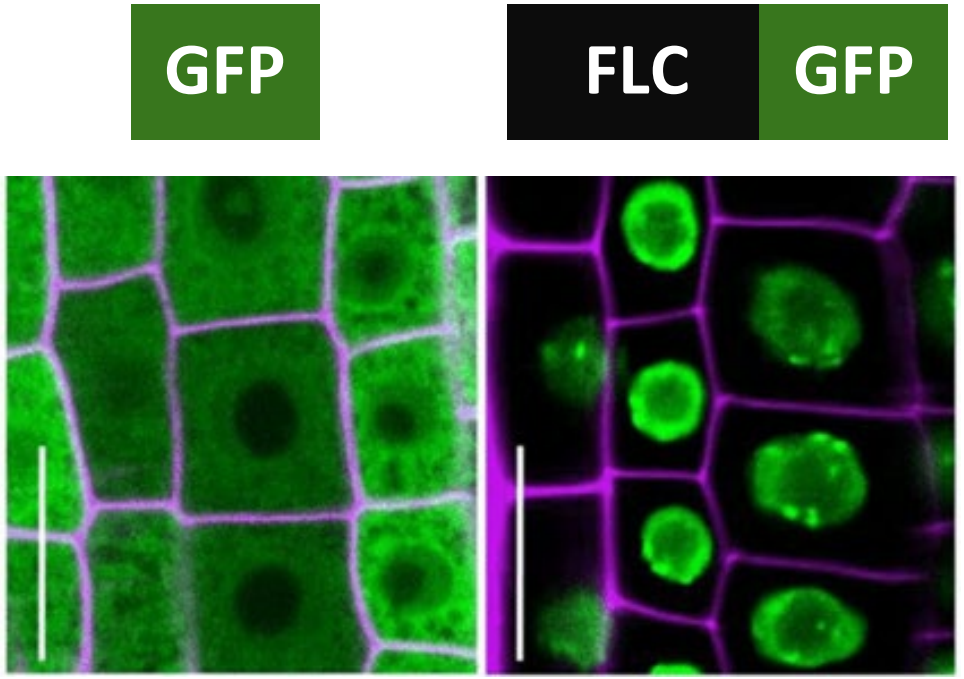
Conserved domains of MADS Transcription Factors



Introduction

Long-term project

- The NLS of the FLC transcription factor has never been characterized
- FLC:GFP fusion proteins will be used to visualize FLC in plant cells
- A mutational analysis approach will be used to try to determine what amino acids are necessary for nuclear localization



GFP alone – mainly in cytoplasm of plant cells

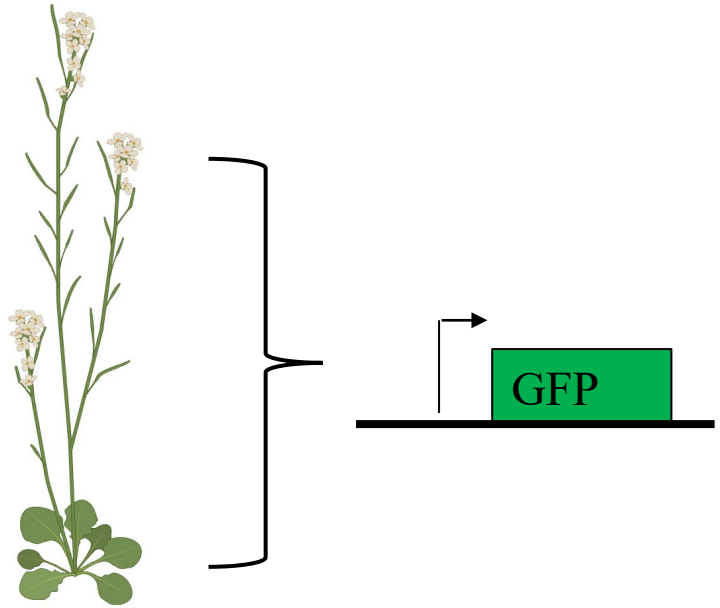
GFP fused to nuclearly localized protein

Example of GFP localization from Massange-Sanchez et al. 2016

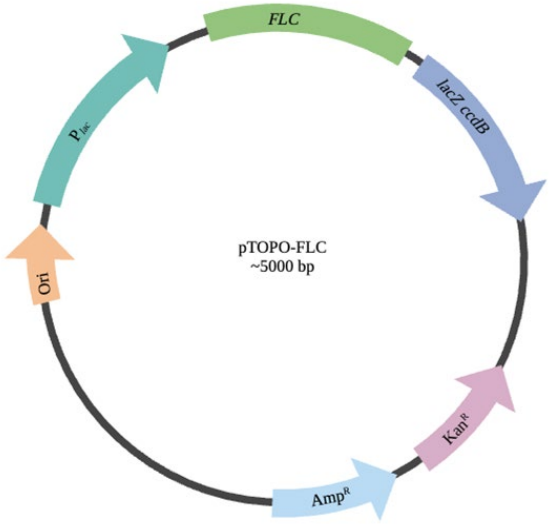
My Objectives

Contribute to the creation of two <u>controls</u> for use in the long-term project to identify the NLS of FLC:

1. Transform Arabidopsis with a plasmid to express GFP alone
2. Prepare *FLC* for the creation of an *FLC:GFP* gene construct by removing an internal restriction site via site-directed mutagenesis



Arabidopsis expressing GFP



FLC in a plasmid

Broader Significance

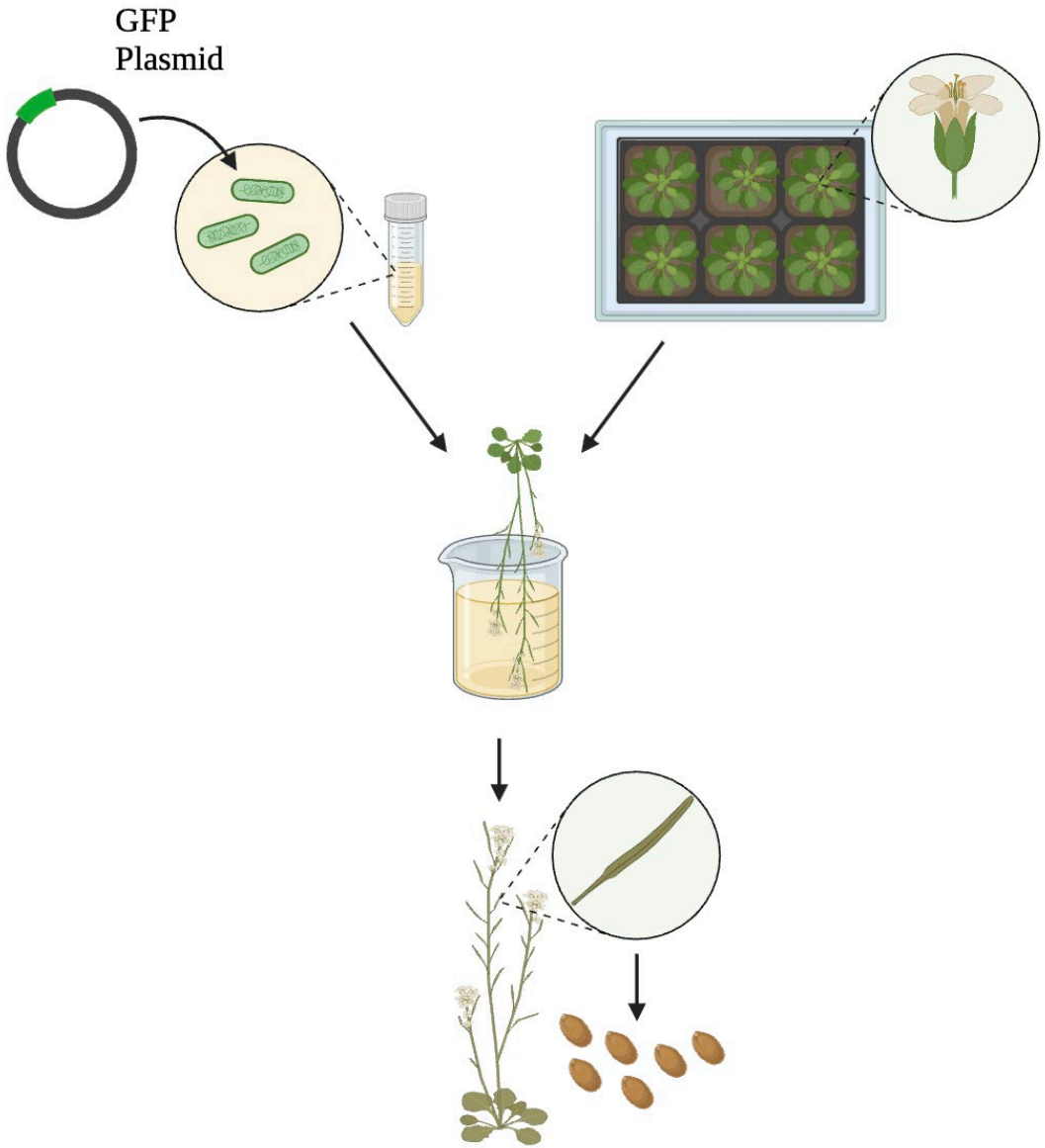
- Determine NLS of FLC (unknown)
- Broaden understanding of nuclear localization in conserved MAD proteins generally
- Add to limited NLS research in plants
- Nuclear localization of proteins is essential to gene function and implicated in disease

Arabidopsis flowering

Objective 1: Methods

Transform Arabidopsis plants with GFP

1. Planted and maintained Arabidopsis until they flowered
2. Used the Agrobacterium floral dip method to transform (genetically modify) Arabidopsis with GFP plasmid
3. Seeds were harvested from transformed plants



Simplified procedure for transforming Arabidopsis

Objective 1: Results - Plant Development

Plants ready for transformation

Sowing

Rosette Formation

Early flowering

Siliques forming

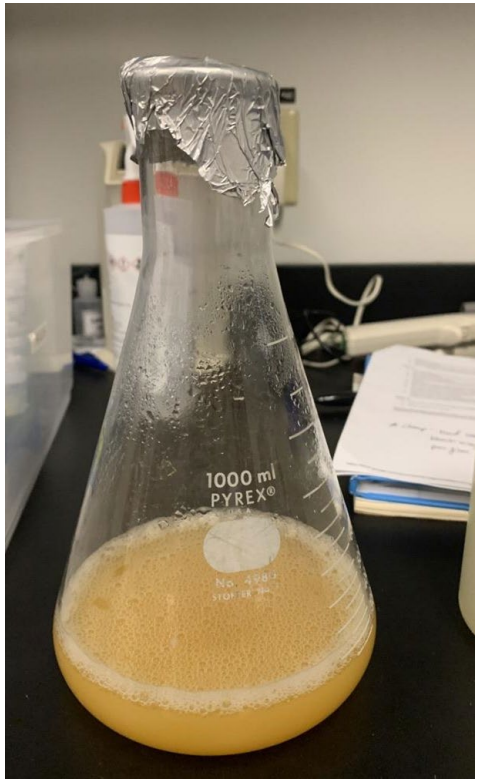
Mature plants

Ready for harvest

Objective 1: Results - Plant Transformation

Agrobacterium floral dip transformation

Trimmed, ready for transformation



Agrobacterium culture

After dipping, let plants rest overnight

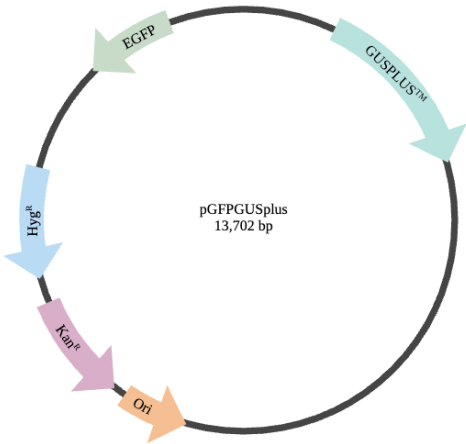
Turn plants upright

Transformed plants successfully producing seed pods

Objective 1: Future Goals

- Transformed seeds have been harvested. The next research student will:
  - Screen seeds for transformants with hygromycin antibiotic selection on plates
  - Confirm GFP expression with fluorescent microscopy
  - Generate a homozygous Arabidopsis GFP control line

Mature Arabidopsis seed pods before harvesting.

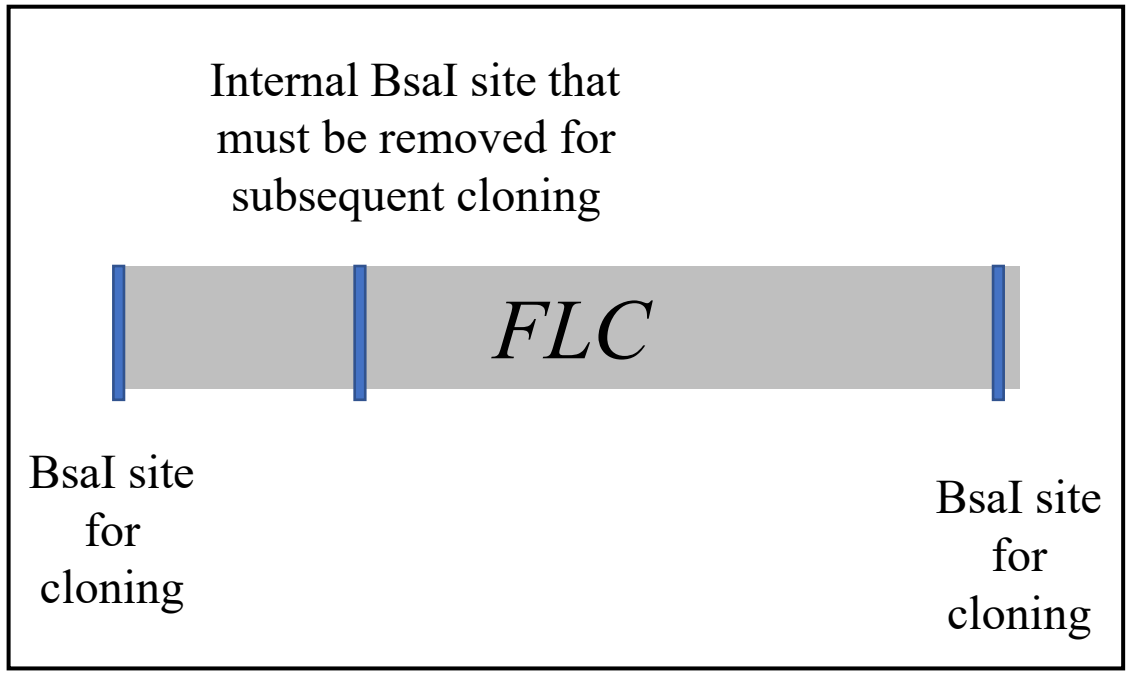


GFP in a plasmid

Methods: Research Objective 2

Remove internal BsaI restriction site from *FLC* for subsequent cloning to make FLC:GFP fusion

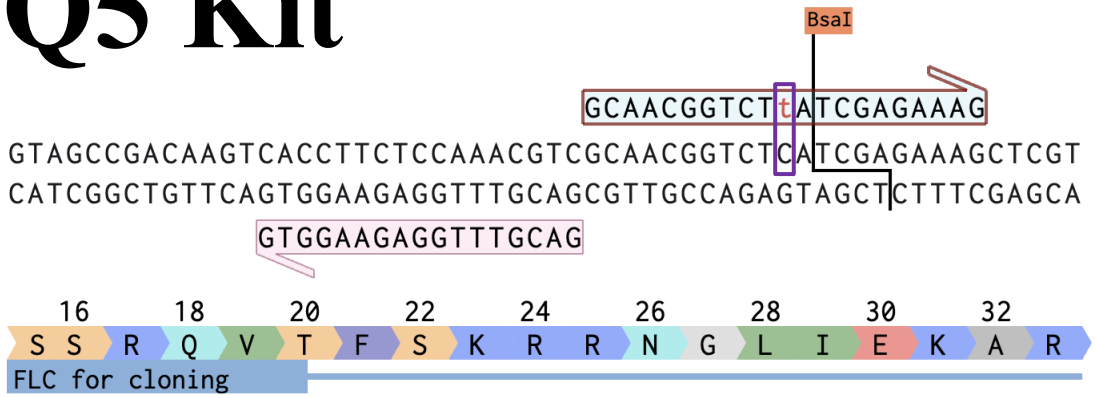
1. Determine suitable mutation that does not change the amino acid sequence
2. Design primers for site-directed mutagenesis
    a. New England Biolabs **Q5 kit**
    b. BIOL 421 **QuikChange II** kit



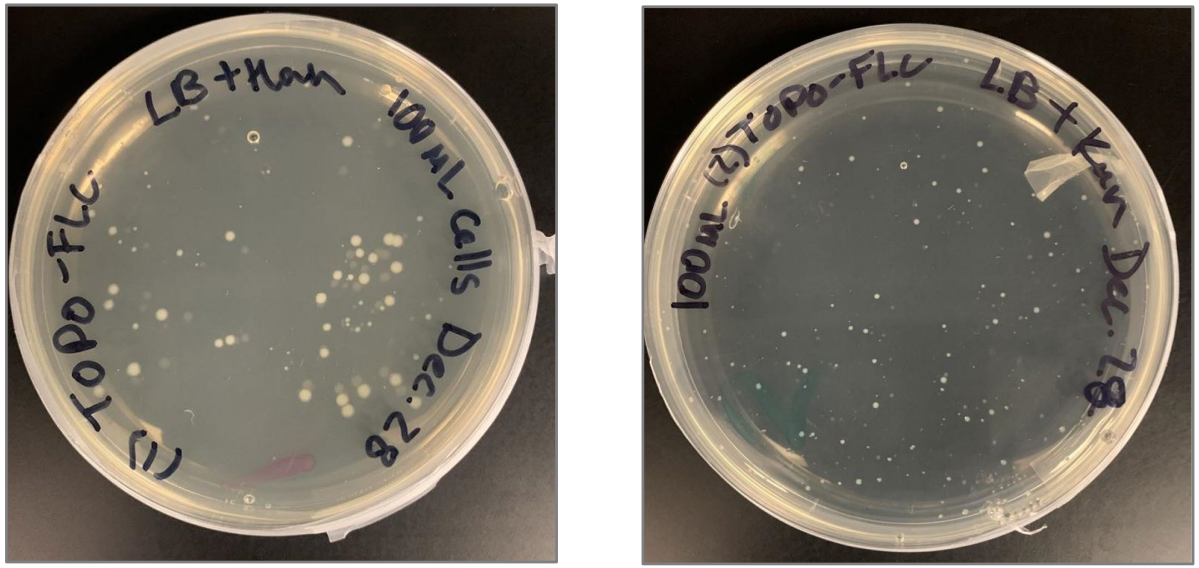
FLC gene with three BsaI restriction enzyme cut sites.

Objective 2: Results - Q5 Kit

- Primers designed to create mutation (97 T>C)
- Template DNA: pTOPO-FLC plasmid (Wiseman, 2021)
- Site-directed mutagenesis
- Transformation of *E. coli*
  - Overnight cultures of large well isolated colonies
  - Plasmid minipreps



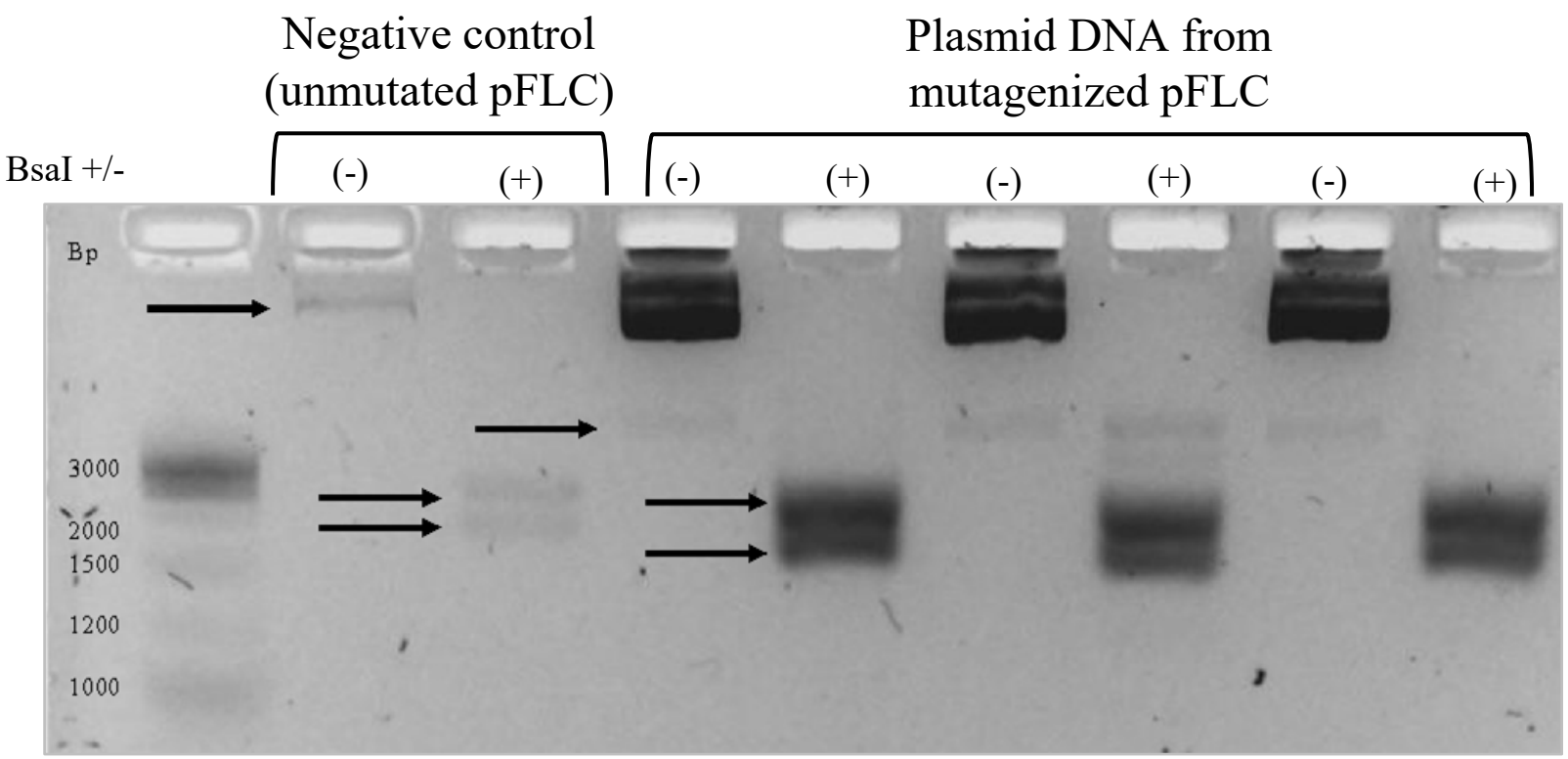
FLC gene showing internal BsaI restriction site and Q5 compatible mutagenic primers.



Kanamycin (antibiotic) selection plates with *E. coli* colonies

Objective 2: Results - Q5 kit

BsaI Digestion to confirm successful mutagenesis

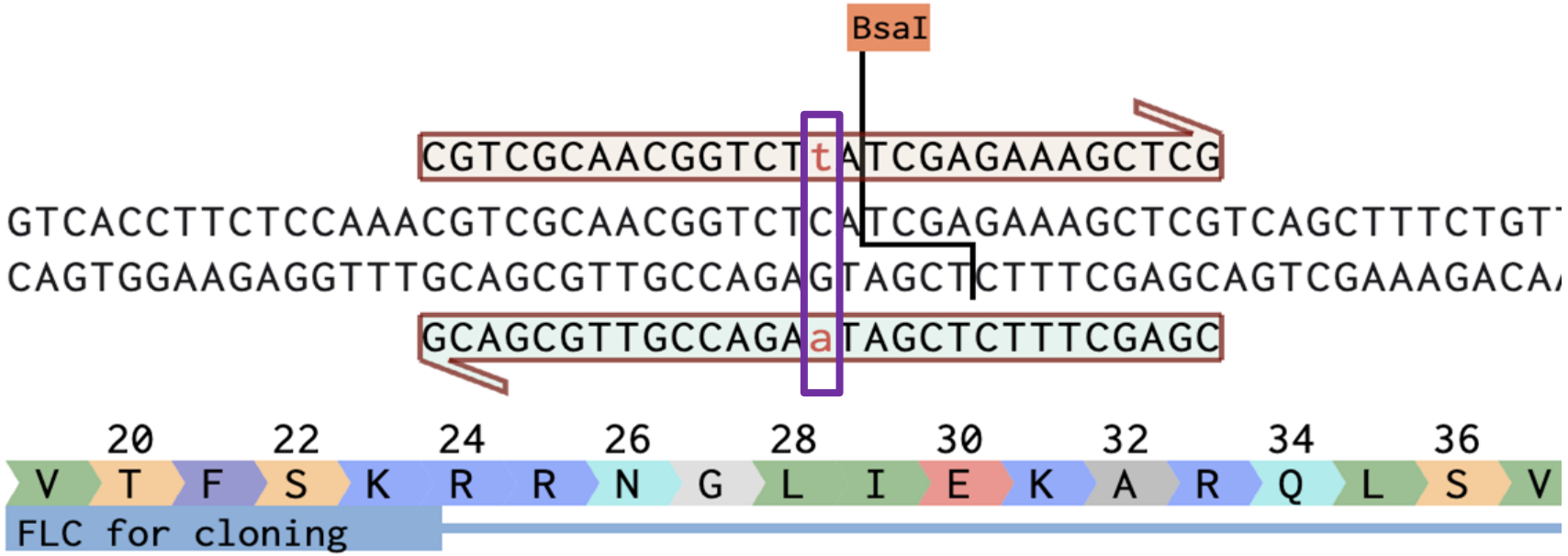


Unsuccessful:
Two bands, 100 bp and 500 bp

Success:
One 600 bp band

Objective 2: Results - QuikChange

- New primers designed
- Transformation unsuccessful (no colonies on any plates), needs to be repeated

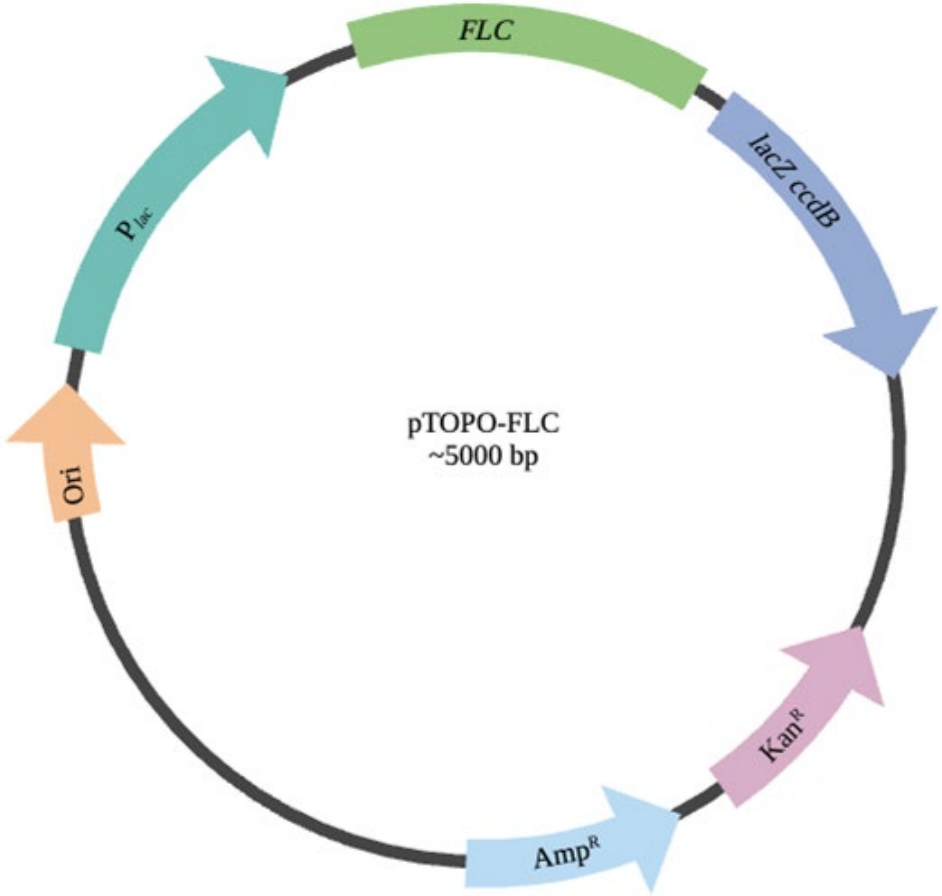


FLC gene showing internal BsaI restriction site and QuikChange II mutagenic primers.

Objective 2: Future Goals

Site-Directed Mutagenesis

- Repeat transformation of QuikChange II mutagenesis plasmid into E.coli
- Troubleshoot as necessary
  - PCR conditions?
  - Procedure optimization



pTOPO-FLC plasmid.

Conclusions

- The creation of two Arabidopsis control lines (GFP and FLC:GFP) is essential for later projects that will use targeted mutations to identify the NLS of FLC
- My work has contributed to this goal by
  - Transforming Arabidopsis with a construct to express GFP (seed is ready for screening)
  - Establishing a protocol for Agrobacterium mediated transformation (not previously done at MacEwan)
  - Designing primers for two different approaches to site-directed mutagenesis to prepare FLC for cloning of FLC:GFP

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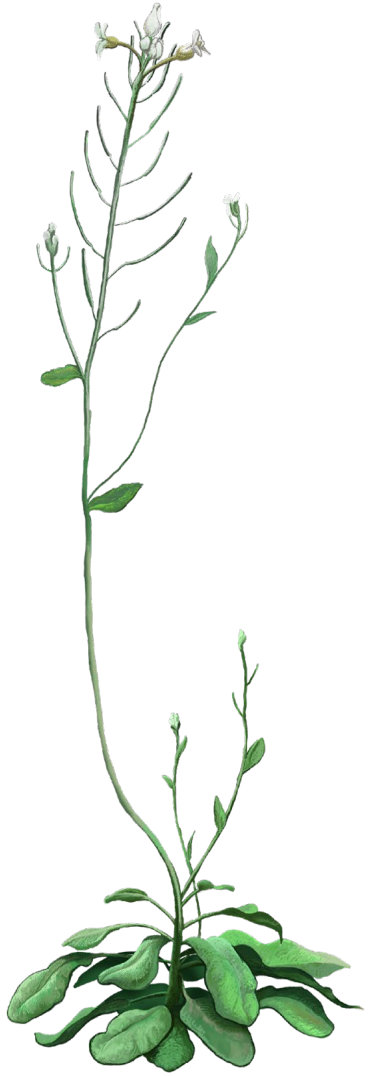
Left: Myself (Sam) Middle: Dr. Melissa Hills Right: Lauren Tkalcic



Questions?

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Image sources

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