Innovative Control of *Phragmites* and Other Invasive Species: Species Specific Gene Silencing

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Targeting *Phragmites* Success As Invasive Species

- *Ph*
  p

  ion

Managers: Where (Who) are We?

Developing a New Arsenal
Gene Silencing

- What is gene silencing?
  - PTGS- Post Transcriptional Gene Silencing
  - RNAi- RNA interference
- Triggered by <u>double stranded RNA</u>
  - siRNA
  - miRNA
- Natural and common mechanisms in organisms
  - Viral defense
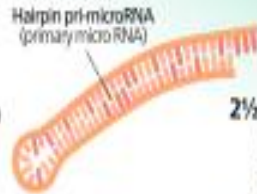 - Gene regulation (development)

siRNA and Viral Defense

the formation and function of micro RNAs

nucleus

Hairpin pri-microRNA (primary micro RNA)
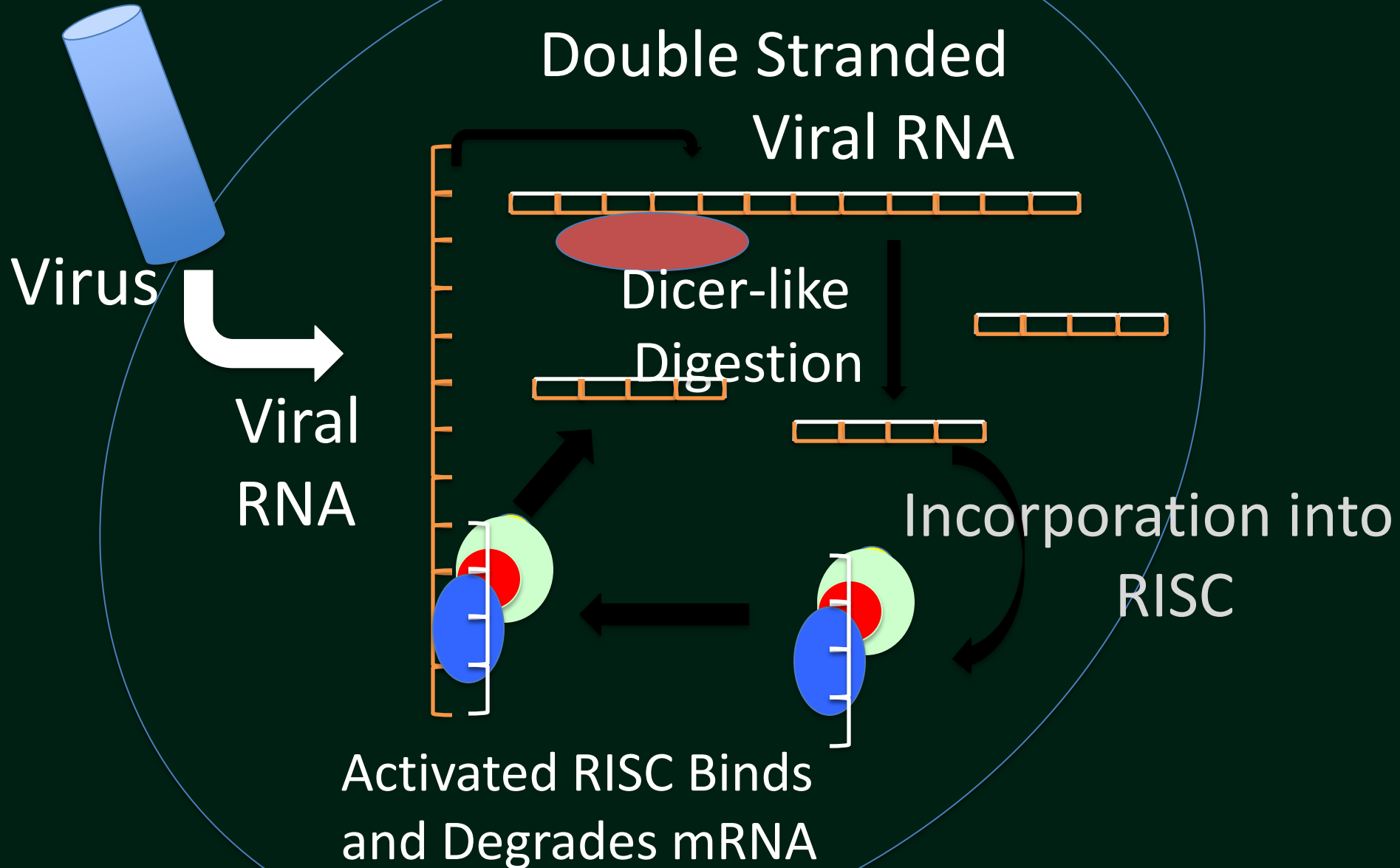
Exportin-5

1 A protein called **exportin-5** transports a hairpin **primary microRNA** (pri-miRNA) out of the nucleus.

Protein

2½ Meanwhile, one of the strands joins a group of proteins, forming an microRNA-protein complex. The other strand, known as a passenger strand is usually discarded. How this all happens is still not very well understood.

2 An enzyme called **dicer** (not shown) trims the pri-microRNA and removes the hairpin loop, leaving a double stranded microRNA duplex molecule.

Base mismatch

microRNA duplex

Passenger strand
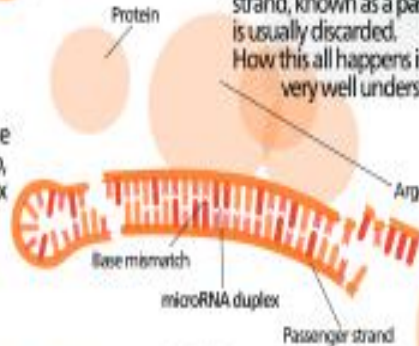
Argonaute proteins

4 In animal cells, the microRNA nucleotides typically don't pair up with the mRNA nucleotides as well. Their base pairing often follows a pattern though.

microRNA

microRNA (abbreviated miRNA) about 22 nucleotides long

microRNA-protein complex

3 In plant cells, the microRNA is usually perfectly complementary to its target mRNA molecule. The microRNA will bond with it, and cause the mRNA to break down.

Endonucleolytic cleavage

Blocked ribosome

Messenger RNA strands (mRNA)
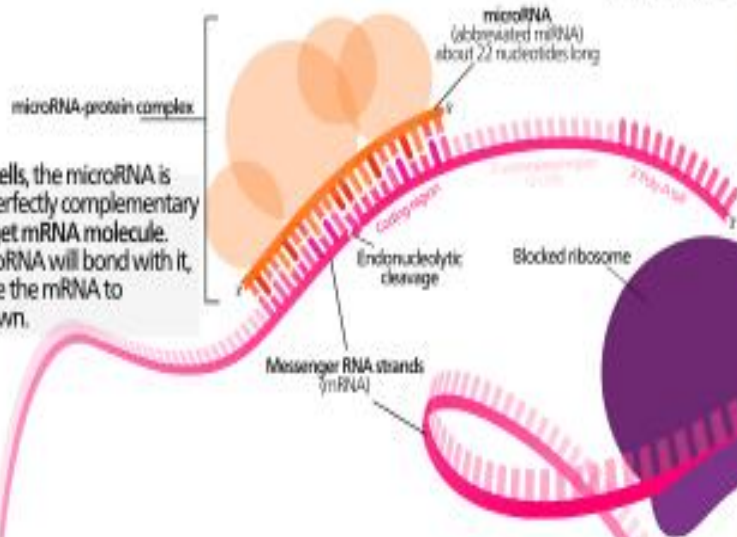
Base mismatches

Nucleotide 1 Has an A across from it

Deadenylation

3' Poly-A tail

Seed Region (Nucleotides 2–8) Perfect base pairing

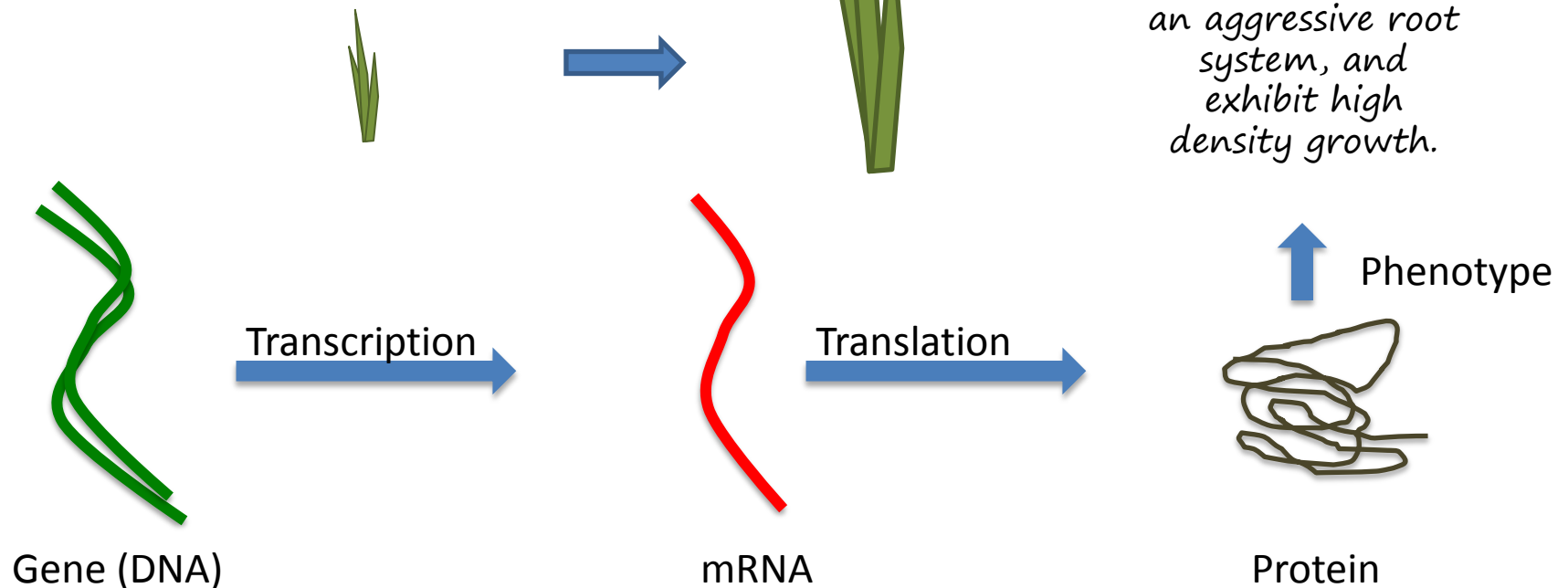**Nucleotide 9** Has an A or U across from it

Nucleotides 13–16 Good base pairing

5 The microRNA-protein complex's presence blocks translation as well as speeding up deadenylation (breakdown of the Poly-A tail), which causes the mRNA to be degraded sooner and translated less.

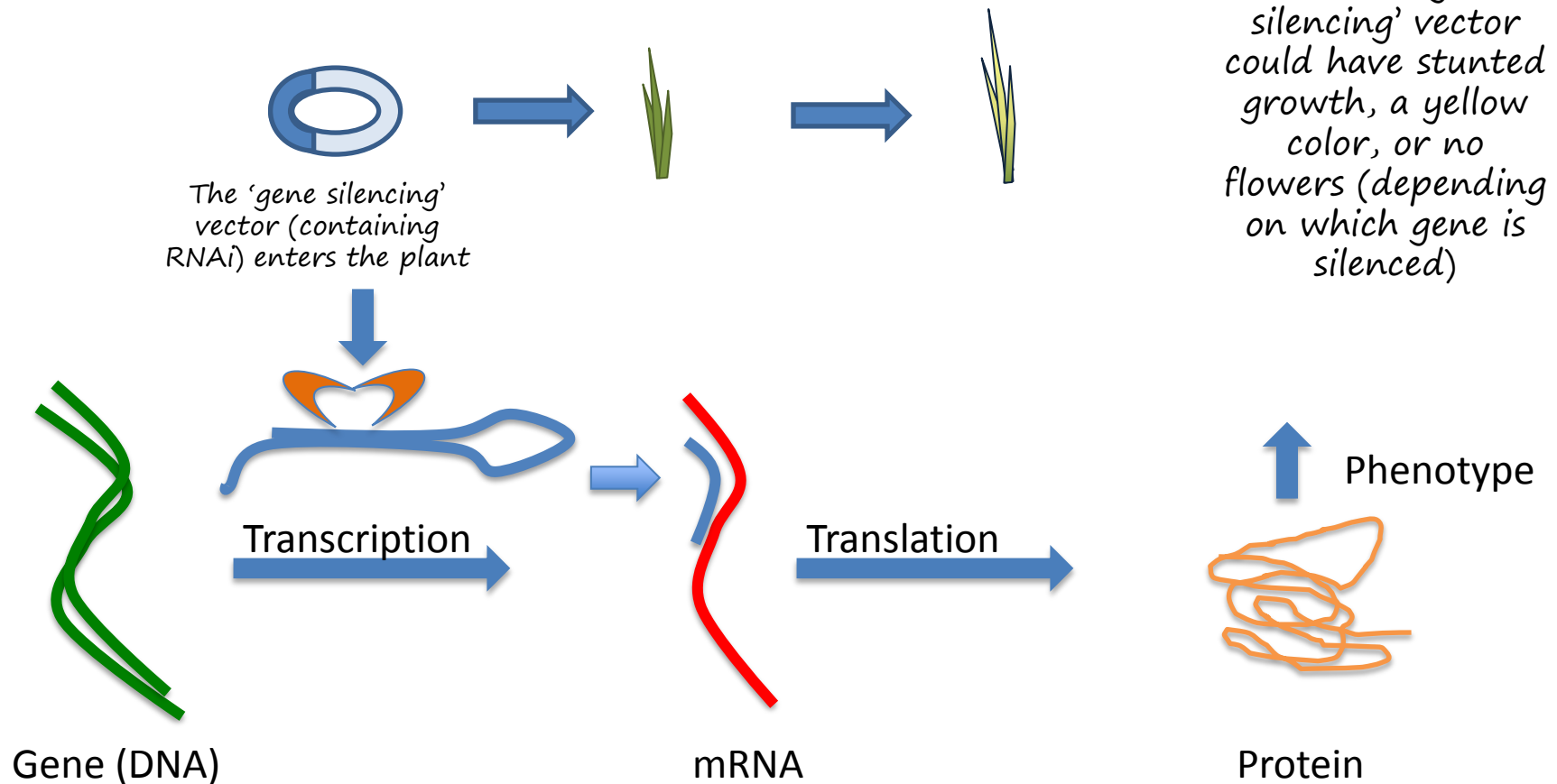# What is Gene Silencing?

Plant processes, such as photosynthesis, are controlled by genetic material within the plant cells

Healthy *Phragmites* plants are tall and robust, with abundant seeds, an aggressive root system, and exhibit high density growth.

Phenotype

Gene (DNA) → Transcription → mRNA → Translation → Protein

What is Gene Silencing?

Gene Silencing inhibits these intracellular processes resulting in muted trait expression

Plants infected with the 'gene silencing' vector could have stunted growth, a yellow color, or no flowers (depending on which gene is silenced)

The 'gene silencing' vector (containing RNAi) enters the plant

Transcription

Translation

Phenotype

Gene (DNA)

mRNA

Protein

# Goal: Establish Gene Silencing for Control of Invasive Species

- Develop robust mode for the **transient** generation of dsRNA in *Phragmites*
- Develop efficient means of application to *Phragmites*
- Target genes involved in:
  - photosynthesis (biomass production)
  - flower development (sexual reproduction)
  - root development (asexual reproduction)
- Demonstrate reliable and effective gene silencing in *Phragmites*
- Test for reduction in competitive dominance of *Phragmites*

Develop robust mode for generation of dsRNA in *Phragmites*

- Viral Induced Gene Silencing-VIGS

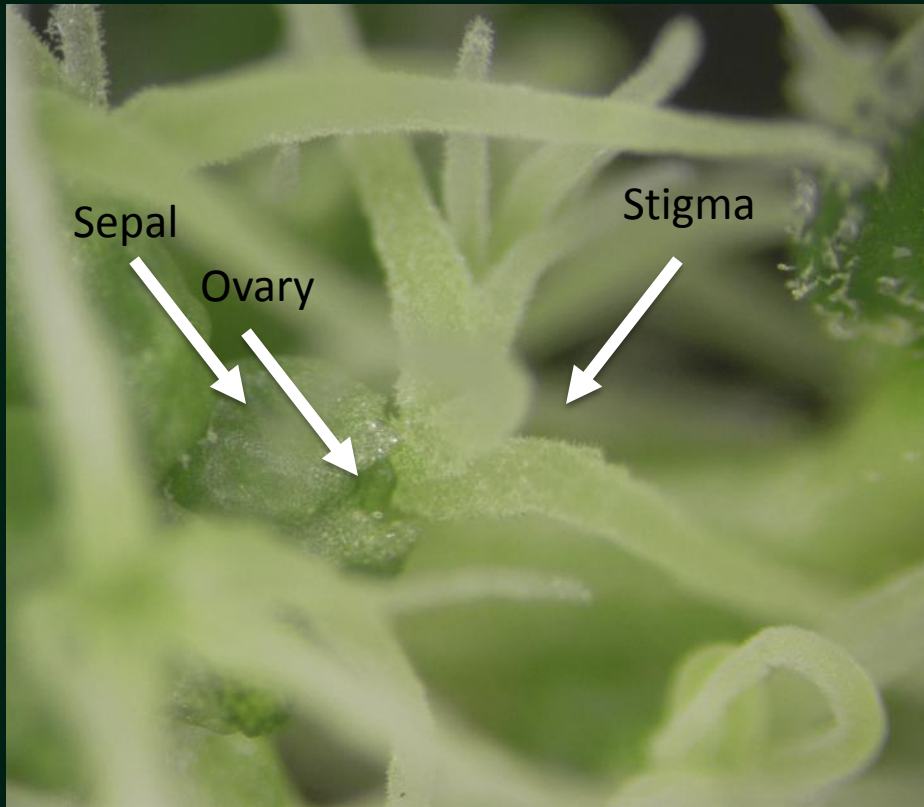- Hairpin generating vectors

- Artificial microRNA- amiR

VIGS- Viral Induced Gene Silencing

pWSRi is a Beet curly top virus (BCTV) based vector system (Golenberg et al 2009)
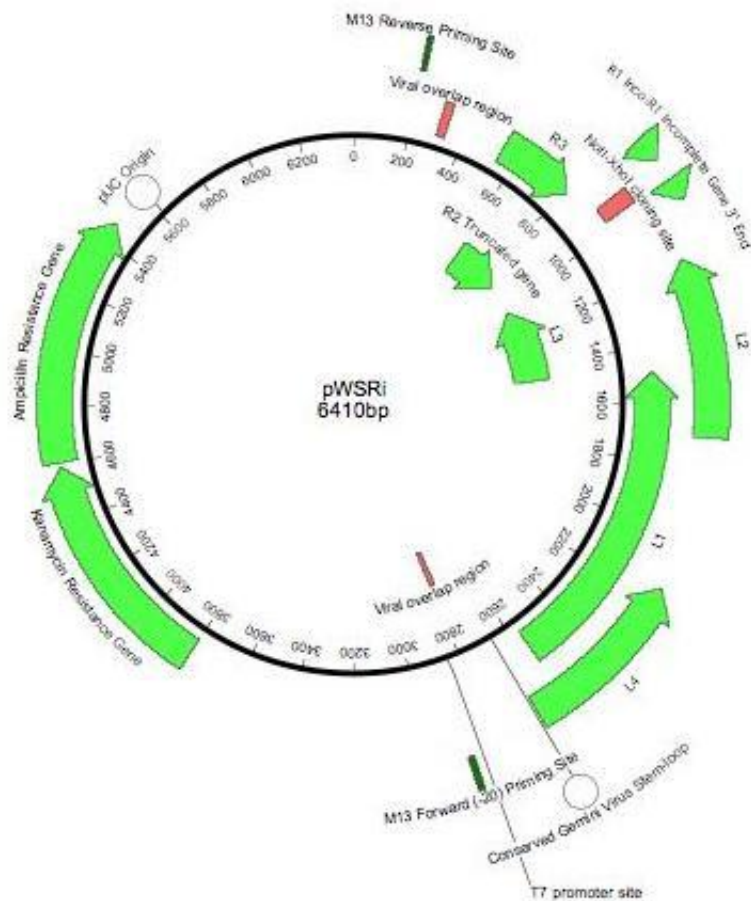
Examples of Gene Silencing
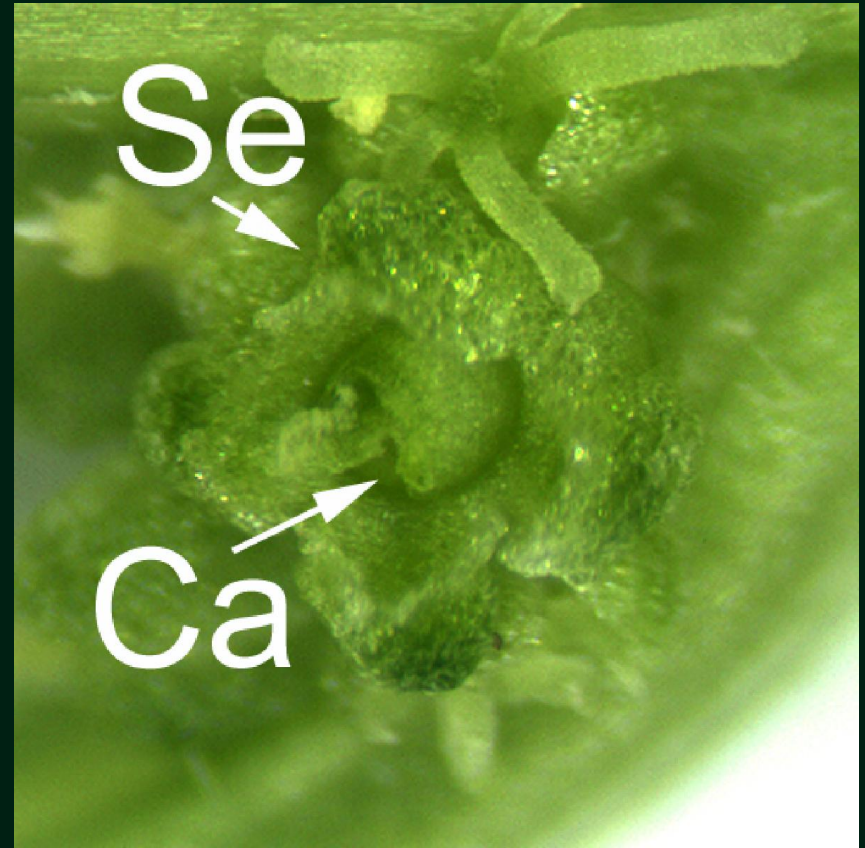# Sterilizing a Flower

Wildtype Spinach Female Flower

Sterile Silenced Female Flower

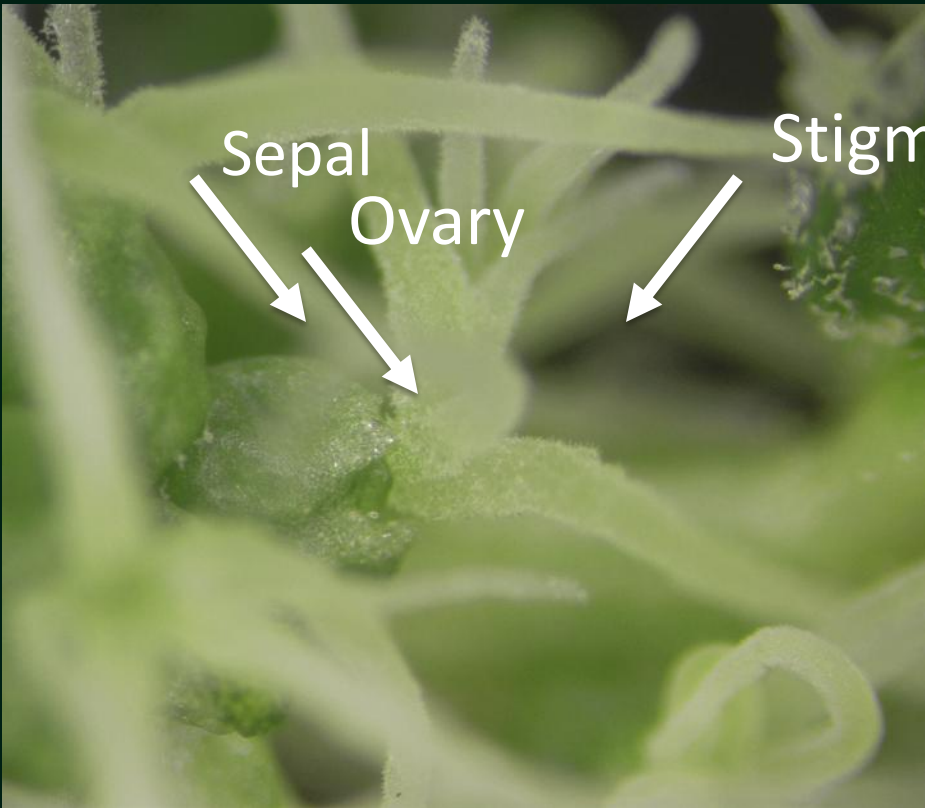# Examples of Gene Silencing Changing Flower Gender

Wildtype Spinach Male Flower

Feminized Spinach Male Flower

Examples of Gene Silencing Changing Flower Gender

Wildtype Spinach Female Flower

Masculinized Female Flower

Examples of Gene Silencing
Changing Flower Gender
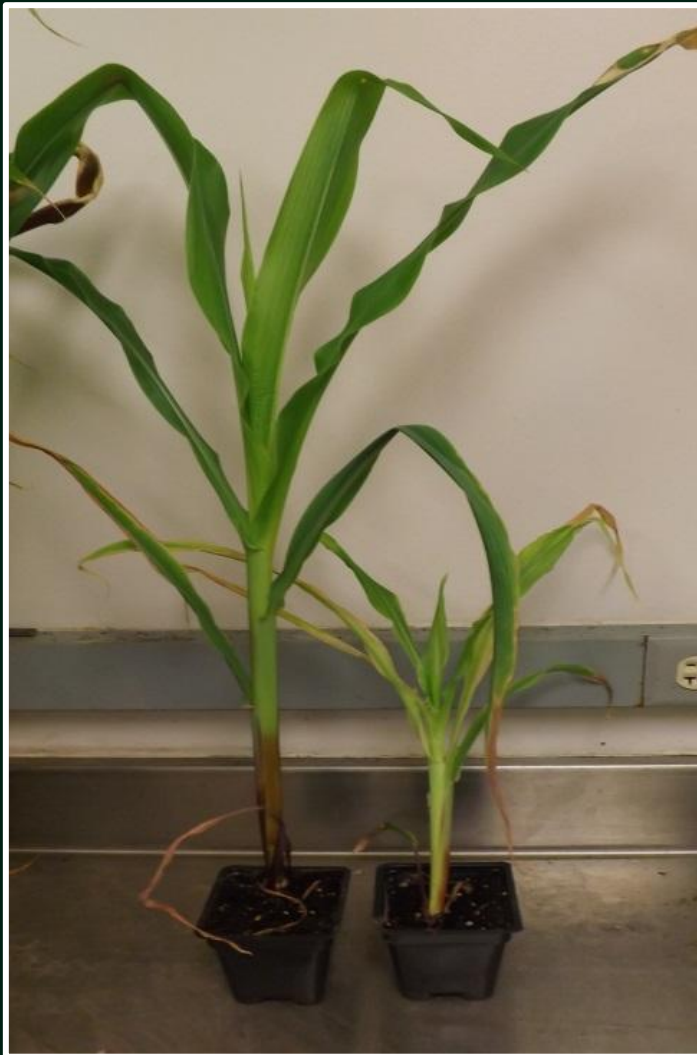

Wildtype Spinach Male Flower

Hermaphroditized Spinach Male Flower

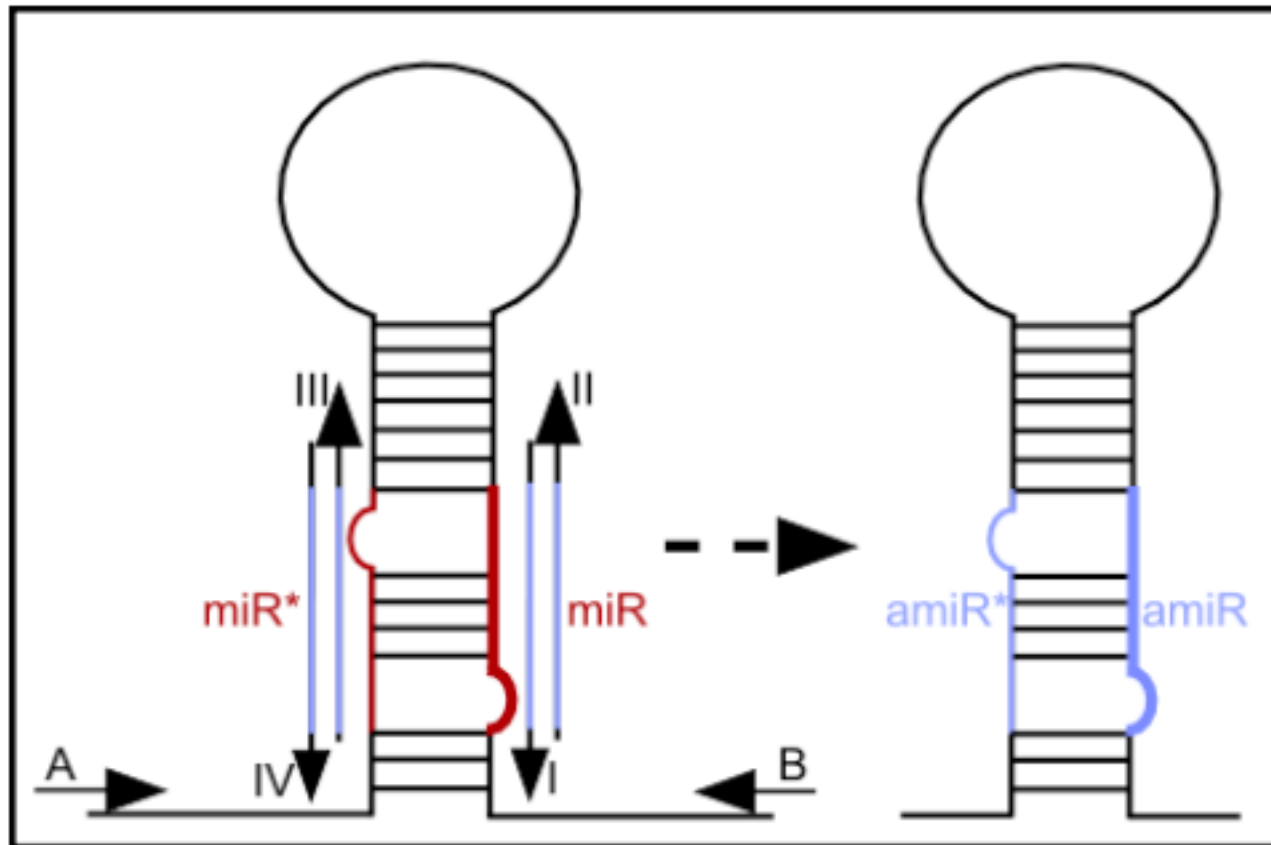# *pWSRiMSV:ZmPDS*
Agrobacterium infected

pIPKb007:ZmPDS
Agrobacterium Infected

Silenced Photosynthetic abilities in model grass species

amiR

What's the advantage of switching to Artificial micro RNA (amiR)?

- Natural Micro RNA
  - Express genes in normal genomes
  - Used to regulate gene expression in development
  - Utilizes silencing machinery in the cell
- Artificial Micro RNA
  - Uses very small sequences for silencing
  - Gives the ability to design targets that are very specific
    - Can be species specific
    - Maybe even at the genotype level
  (i.e. native vs. invasive *Phragmites*)

pEARLEYGATE100:ZmPDS_IR-1

amiR Trials in Sunflower

pEARLEYGATE100:HaKOA1miR2

GS Trials in *Phragmites*

Construct Development

Species	Gene	System/Function	Name
Helianthus annua (Sunflower)	*rbcS*	Photosynthesis	**HarbcSmiR**
Helianthus annua (Sunflower)	*ent-kaurenoic acid oxidase (KAO1 gene), dwarf2 mutant*	Gibberellin Production	**HaKOAmiR1**
Helianthus annua (Sunflower)	*ent-kaurenoic acid oxidase (KAO1 gene), dwarf2 mutant*	Gibberellin Production	**HaKOAmiR2**
Helianthus annua (Sunflower)	*ent-kaurenoic acid oxidase (KAO1 gene), dwarf2 mutant*	Gibberellin Production	**HaKOAmiR3**
Zea mays (Maize)	*rbcS*	Photosynthesis	**ZmrbcSmiR1**
Zea mays (Maize)	*rbcS*	Photosynthesis	**ZmrbcSmiR2**
Zea mays (Maize)	*GA 3-oxidase 1 (D1)*	Gibberellin Production	**ZmGA3OxmiR1**
Zea mays (Maize)	*phytoene desaturase (pds1)*	Photosynthesis	**ZmPDStasiR**
Zea mays (Maize)	*phytoene desaturase (pds1)*	Photosynthesis	**ZmPDSmiR1**
Zea mays (Maize)	*phytoene desaturase (pds1)*	Photosynthesis	**ZmPDSmiR2**
Zea mays (Maize)	*phytoene desaturase (pds1)*	Photosynthesis	**ZmPDSmiR3**
Zea mays (Maize)	*phytoene desaturase (pds1)*	Photosynthesis	**ZmPDSmiR4**
Zea mays (Maize)	*magnesium chelatase subunit I precursor (ChlI), oil yellow*	Photosynthesis	**ZmYO1ImiR1**
Zea mays (Maize)	*magnesium chelatase subunit I precursor (ChlI), oil yellow*	Photosynthesis	**ZmYO1ImiR2**
Phragmites australis	*phytoene desaturase –derived from transcriptome*	Photosynthesis	**PaPDSmiR**
Phragmites australis	*phytoene desaturase –derived from transcriptome*	Photosynthesis	**PaPDSmiR**
Phragmites australis	*phytoene desaturase –derived from transcriptome*	Photosynthesis	**PaPDStasiR**
Phragmites australis	*GA 3-oxidase 1- derived from transcriptome*	Gibberellin Production	**PaGA3OxmiR1**
Phragmites australis	*GA 3-oxidase 1- derived from transcriptome*	Gibberellin Production	**PaGA3OxmiR2**

How do we transfer from model species to *Phragmites*?

Building *Phragmites* Data Base

- Identify genetic code for particular traits in *Phragmites*
  - *Phragmites* transcriptome sequenced
    - Generated 4 RNAseq libraries: Inflorescence, Leaf, Root, Ramet meristem
    - Pair-end sequences for each library
    - Sequences each direction per library: Inflorescence and leaf 35 million each , Root and meristem 25 million each
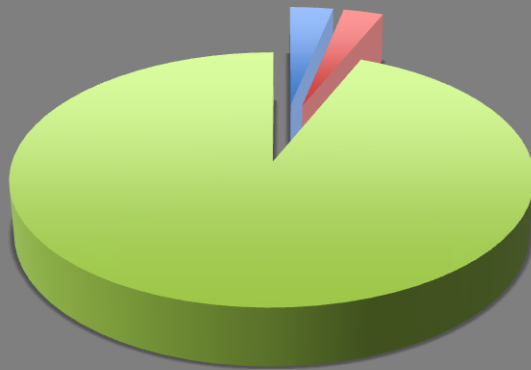 - Target traits Identified

Phragmites Transcriptome

Tissue	Transcriptome Length	Proportion of total
Inflorescence	32826103	0.262
Leaf	146724	0.001
Root	151693	0.001
Shoot tip	22670484	0.181
Shared	69294171	0.554

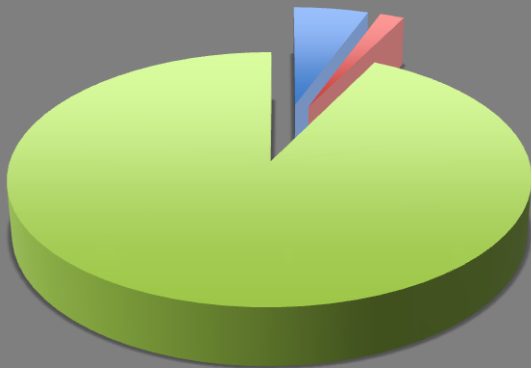# *Phragmites* Transcriptome Transcript Distribution by Tissue
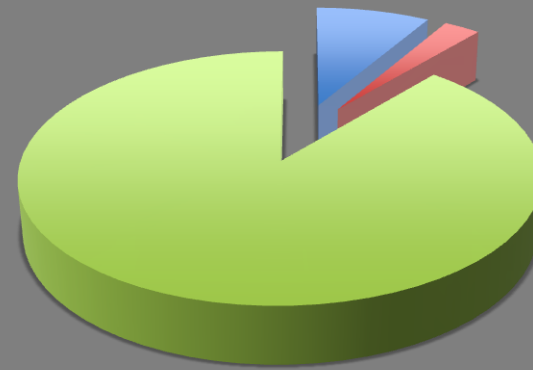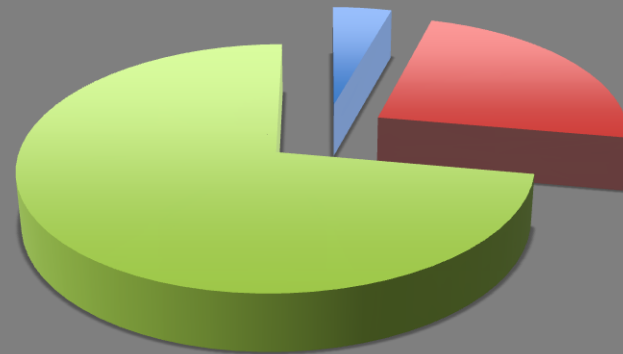
Transcriptome Analysis
Phragmites Differential Expression
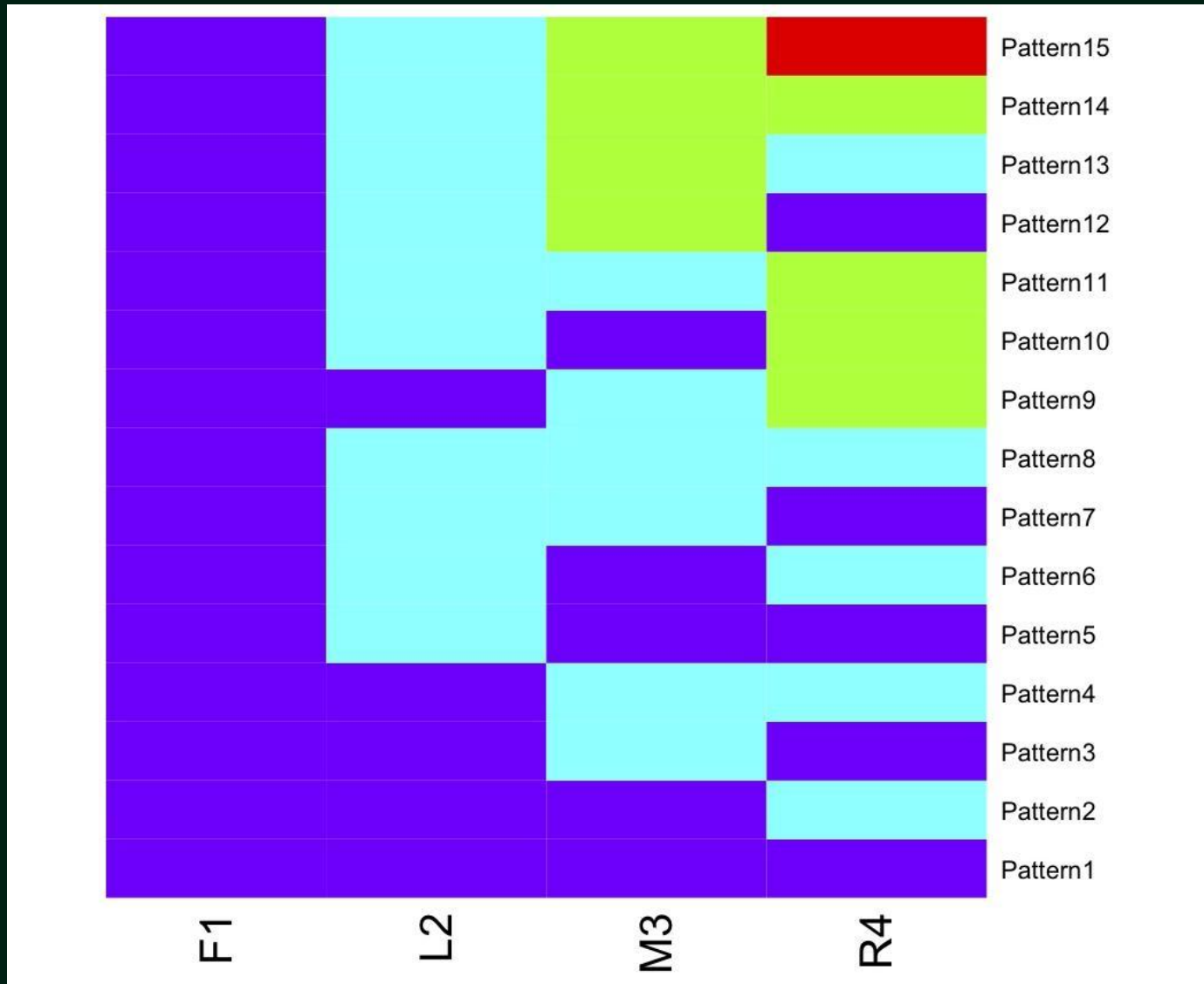
Transcriptome Analysis
Phragmites Differential Expression

	0	1	2	3	4
#	184	135597	608	639	31155
5	6	7	8	9	10
17574	586	350	48574	455	317
11	12	13	14	15	
136	105	307	9983	330	

Pattern 1- All the same   Pattern 4- (FL)(MR)   Pattern 5-   L(FMR)

Pattern 8- F(LMR)   Pattern 14- (F)(L)(MR)

Why *Phragmites* Transcriptome is Important

- Once gene sequences are identified, specific traits can be targeted for silencing

- Differential expression patterns among strains of *Phragmites* (native vs. invasive) may identify specific targets

- Generates broad database for scientific community

Why is Gene Silencing Important?

- The Holy Grail- This will show first real proof of gene silencing as a viable management/control option

- If silencing vector is effective in mixed plant compositions, widespread application in the field could be an option

- If invasive *Phragmites* is affected and native is not, that makes widespread application more ecologically viable

Caveats and Concerns

- Penetrance and expressivity of miRs in *Phragmites*
- Persistence of silencing temporally
- Modes of applications
  - Mechanistically how to scale up
  - Environmentally are there unintended effects
- Development of management plans that includes guidelines for applications and reintroduction of native species

This could be BIG

If gene silencing is effective in a field setting and proves to be species specific, it will represent a <u>huge breakthrough</u> in invasive species management

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