Screening for NERDs* in *Arabidopsis thaliana*

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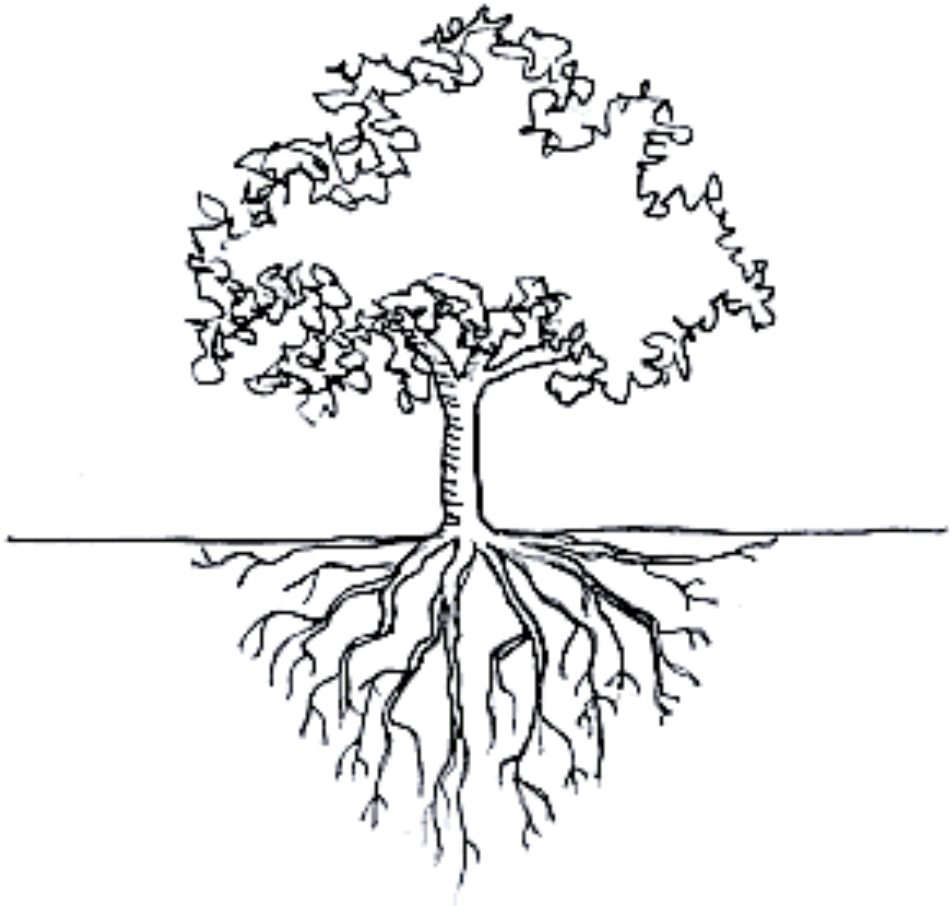
John Fowler Laboratory
Summer 2011

* **N**ew **E**nhancers of **R**oot **D**warfism

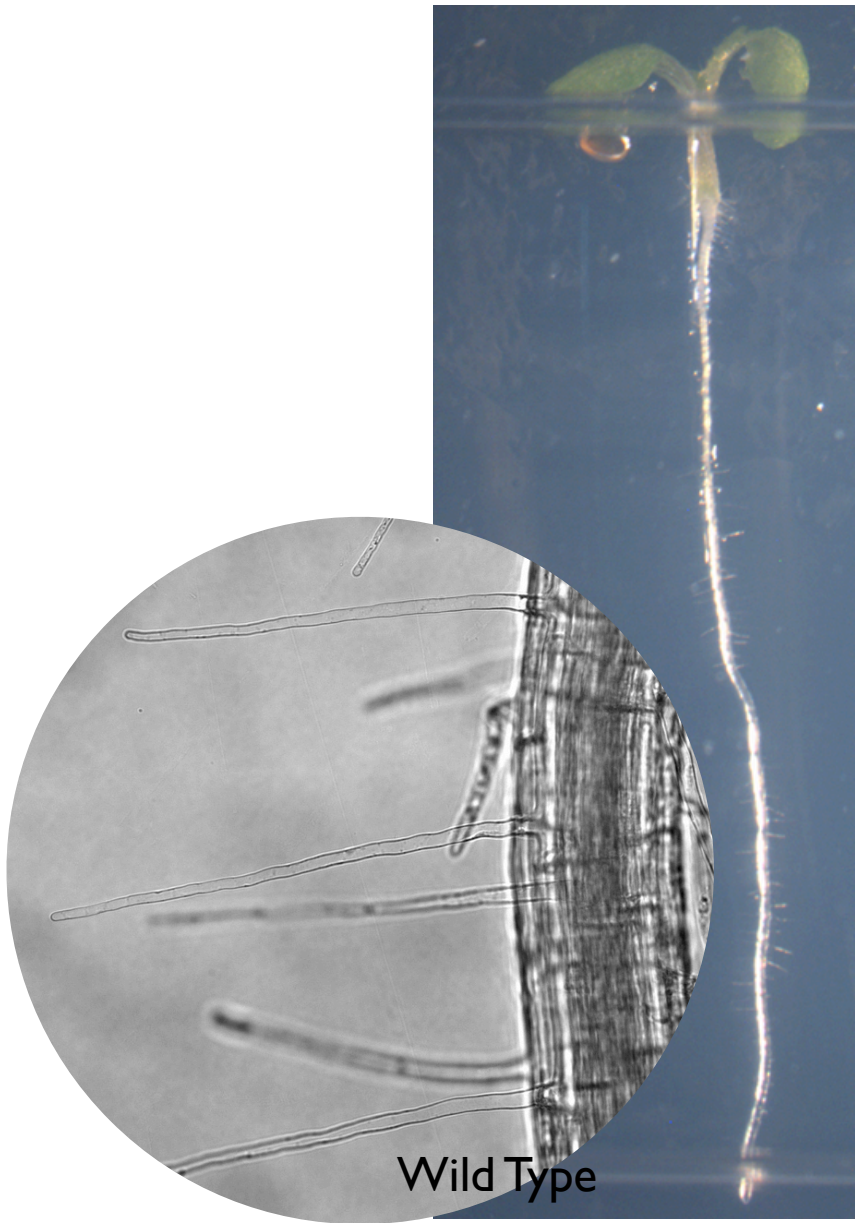
Plant Roots

- Plants obtain the nutrients and water required for survival through root growth



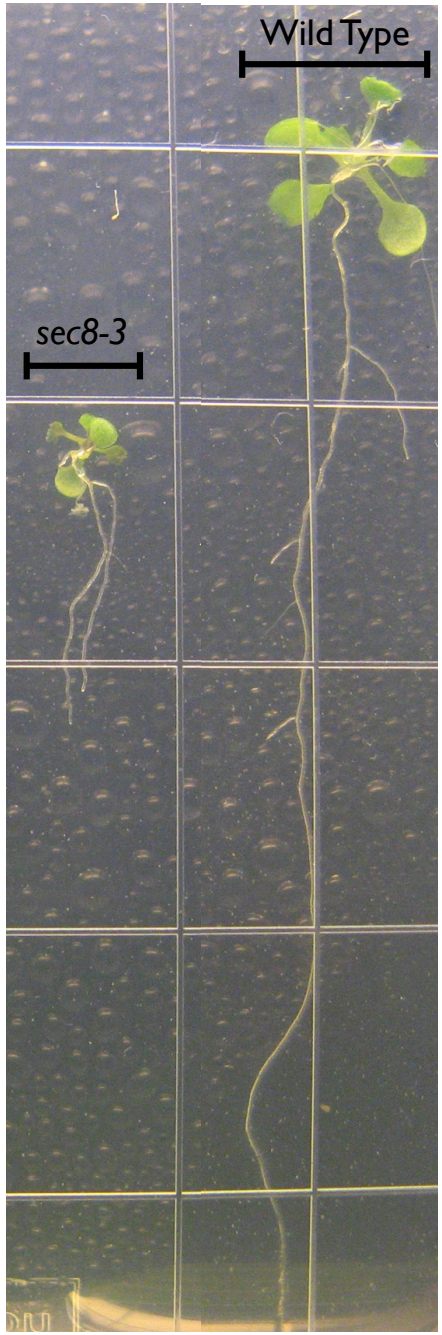
Plant Roots

- Growth through cellular division and cellular elongation
- Root hairs are projections from a single cell
- Targeted exocytosis is important for both of these processes



The Exocyst

- Protein complex involved in exocytosis
- Important for secretion and targeted exocytosis, among other things
- Exocyst mutations result in stunted root and root hair growth
- *sec8-6* is not severe; phenotype is not easily visible



NERD Screen Goal:
identification of exocyst interactors

The NERD Screen

Overview

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- Through EMS exposure, mutations were introduced into many lines homozygous for the *sec8-6* mutation.

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- These lines were manipulated to isolate those new mutations that interacted synergistically with *sec8-6*.

The NERD Screen

Overview

- Through EMS exposure, mutations were introduced into many lines homozygous for the *sec8-6* mutation.
- These lines were manipulated to isolate those new mutations that interacted synergistically with *sec8-6*.
- An exocyst interactor would be identified when a severe mutant phenotype occurred only in *sec8-6* homozygotes.

The NERD Screen

e: *sec8-6* (exocyst mutation) allele, **a**: mutant allele from EMS mutagenesis, **+**: wild type allele

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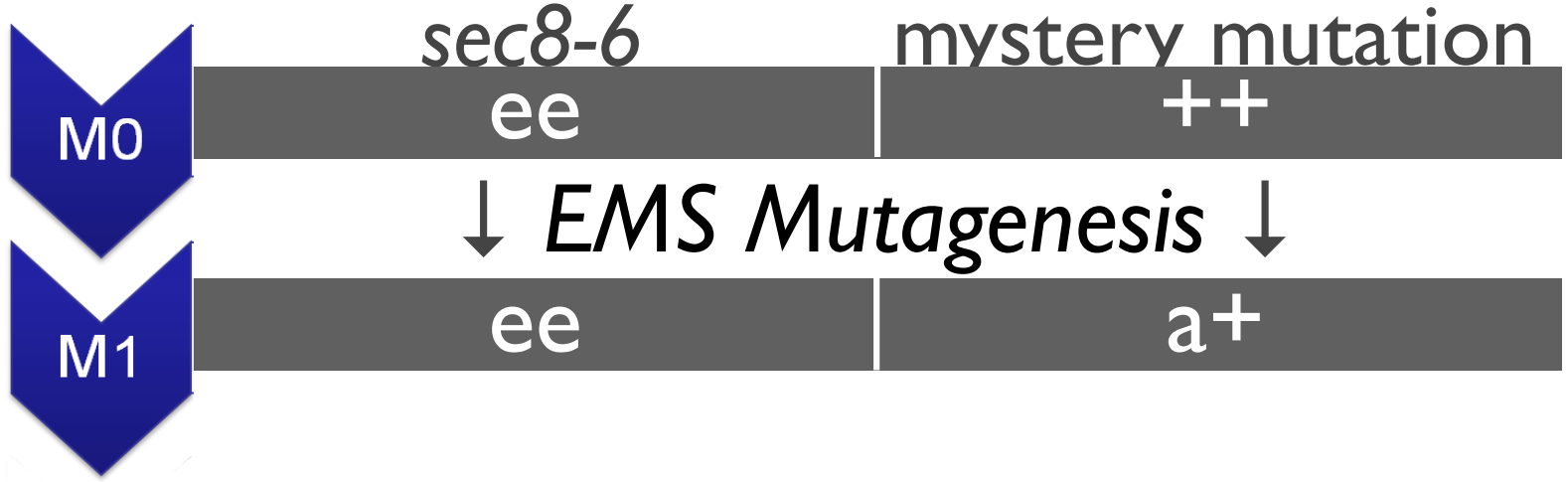

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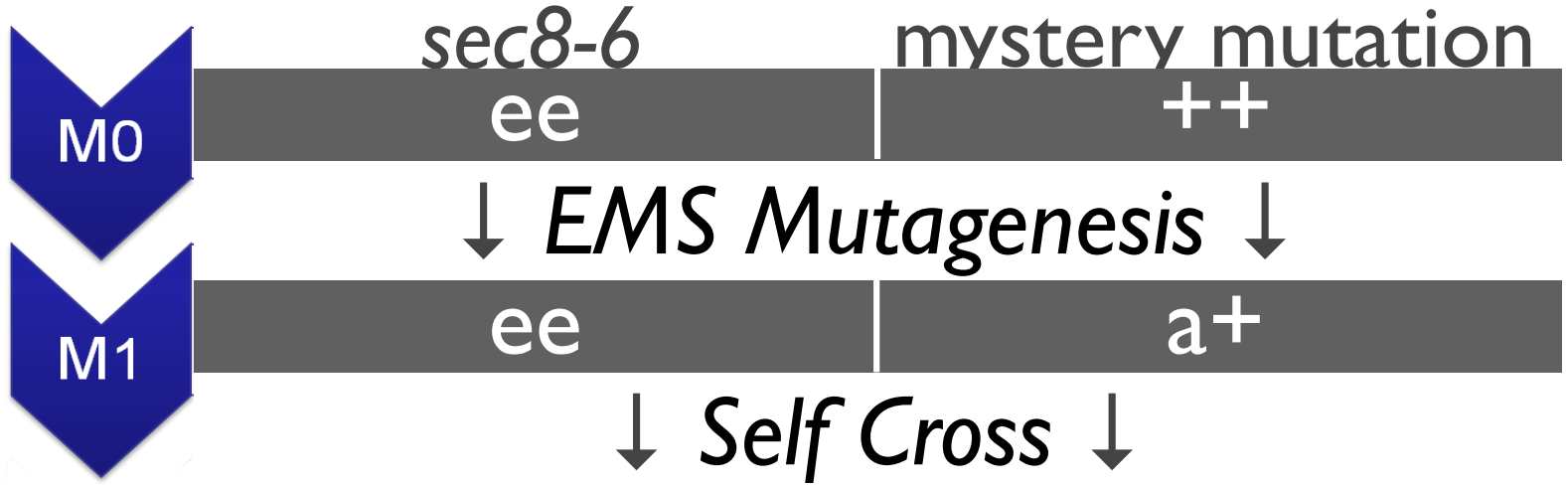

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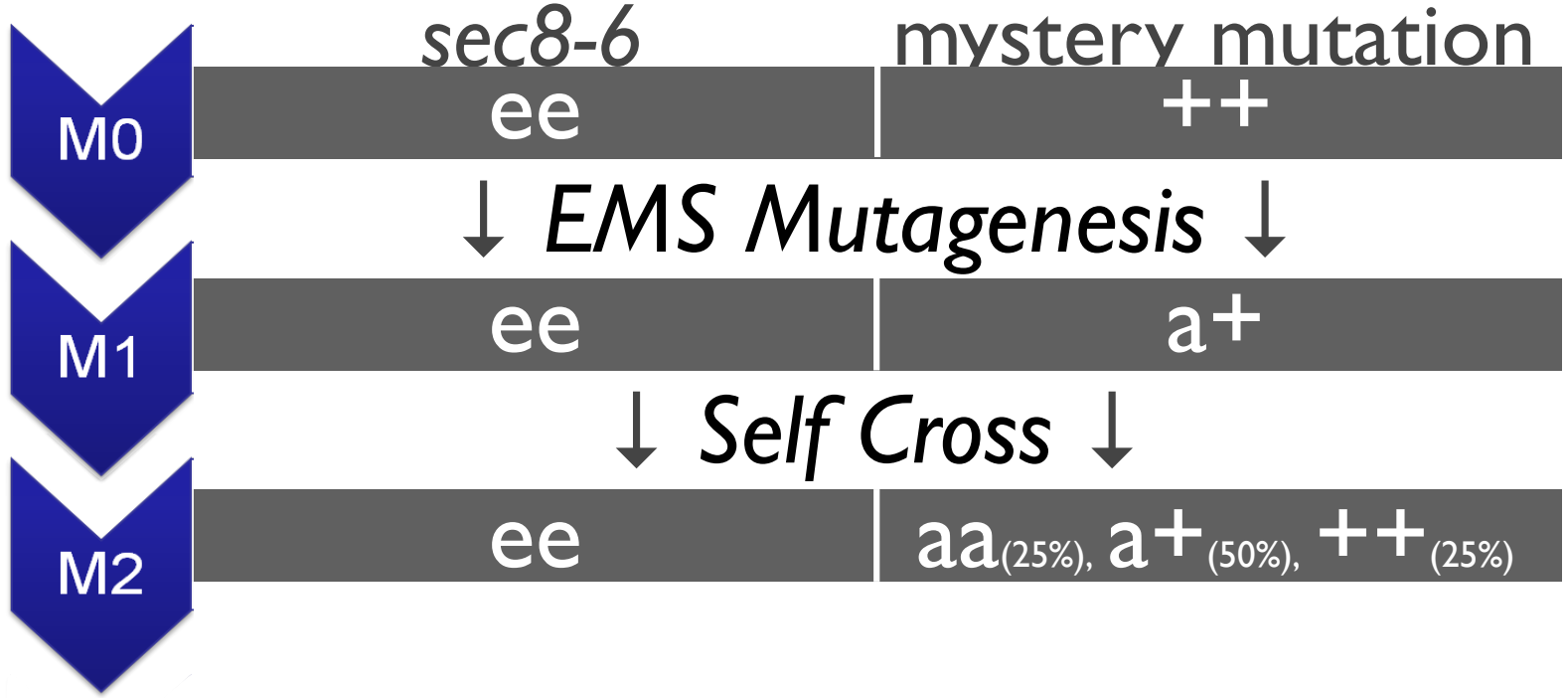

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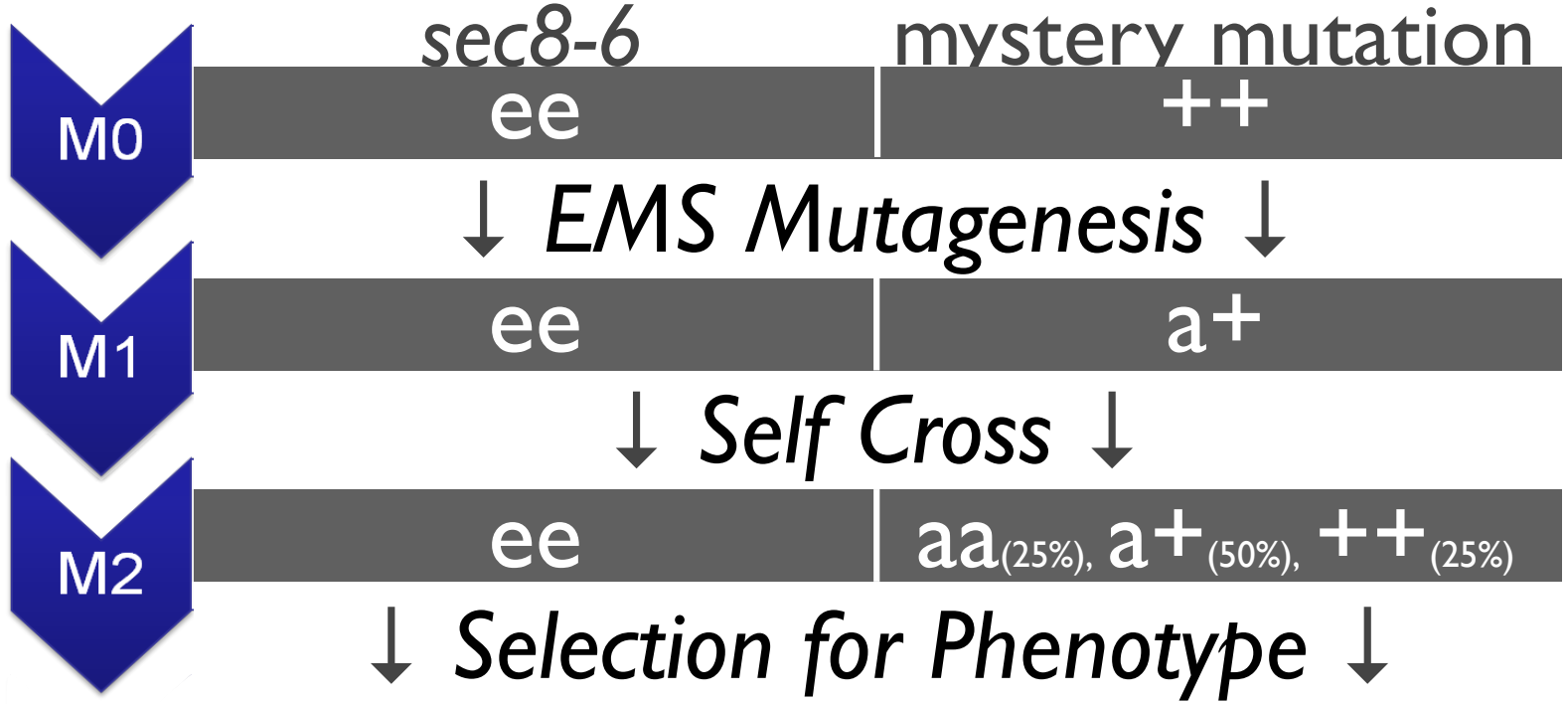

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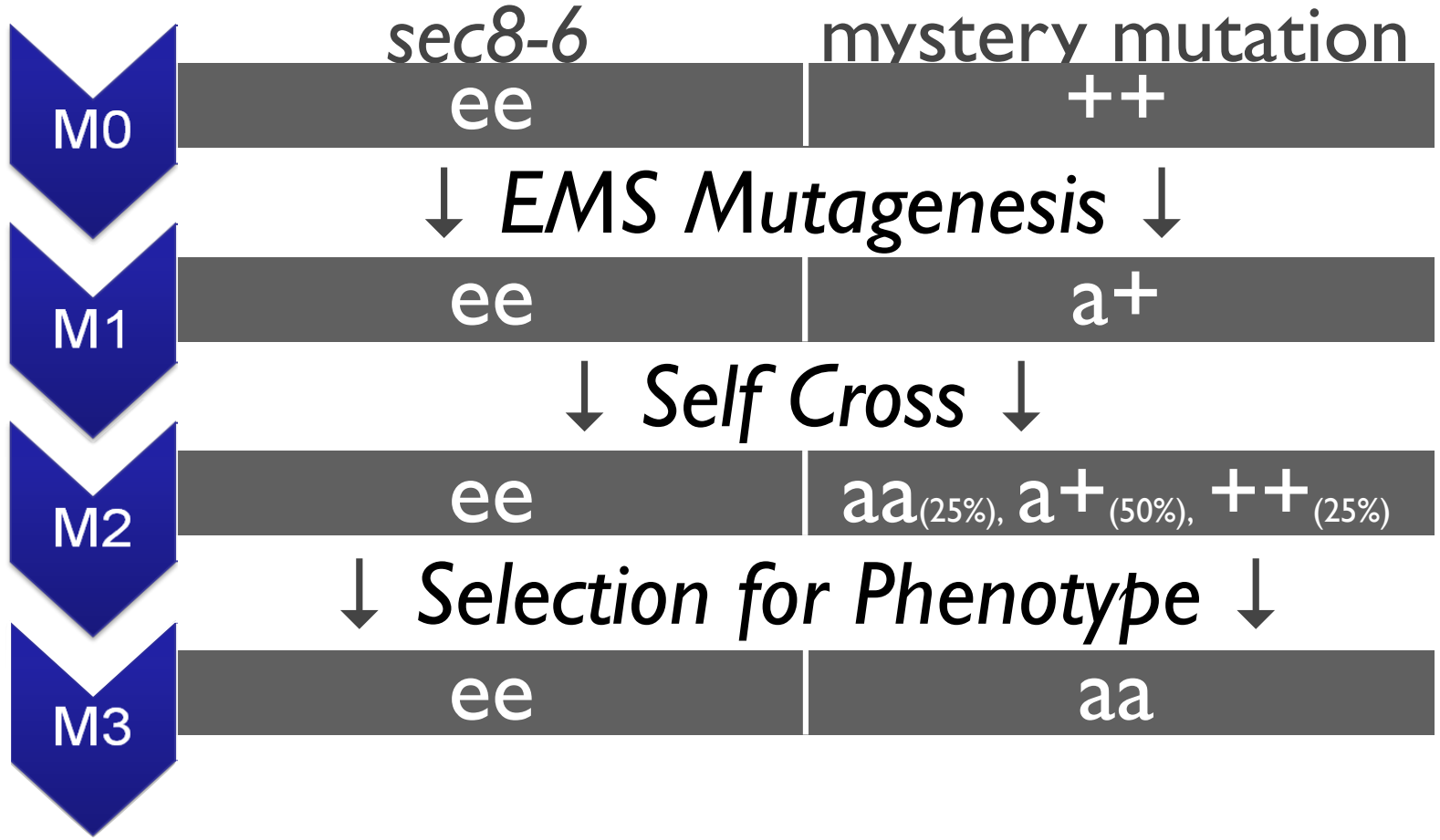

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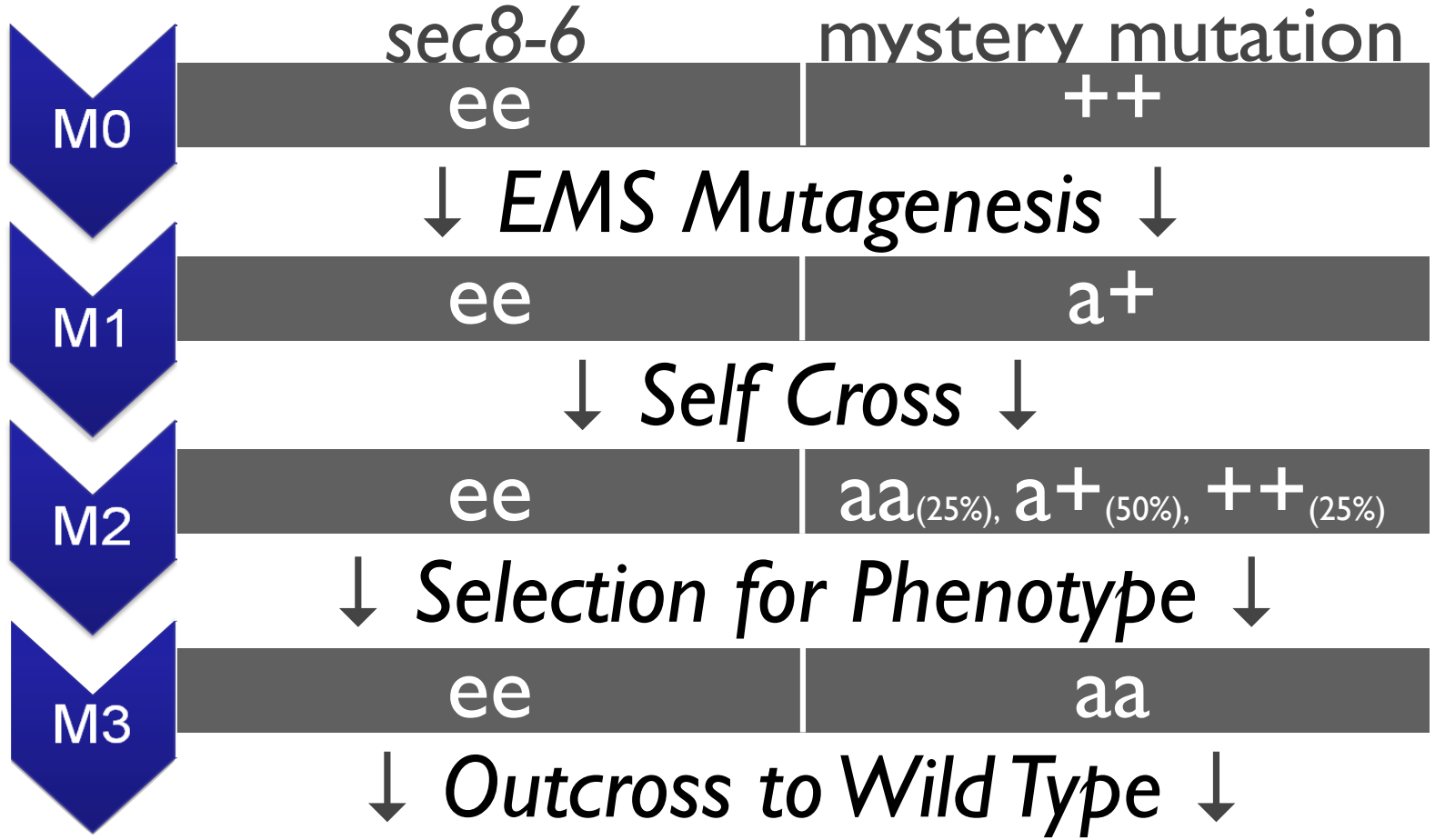

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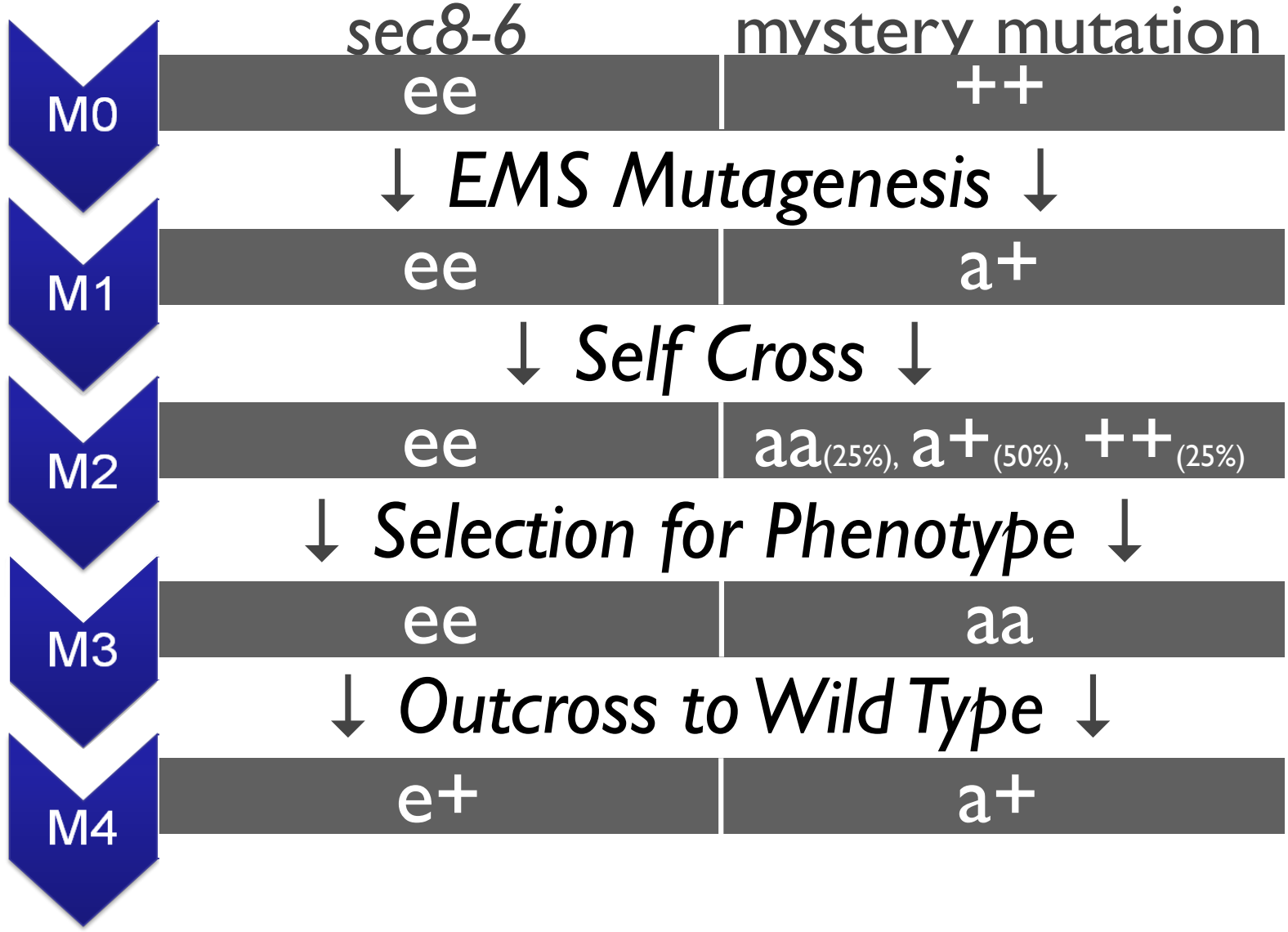

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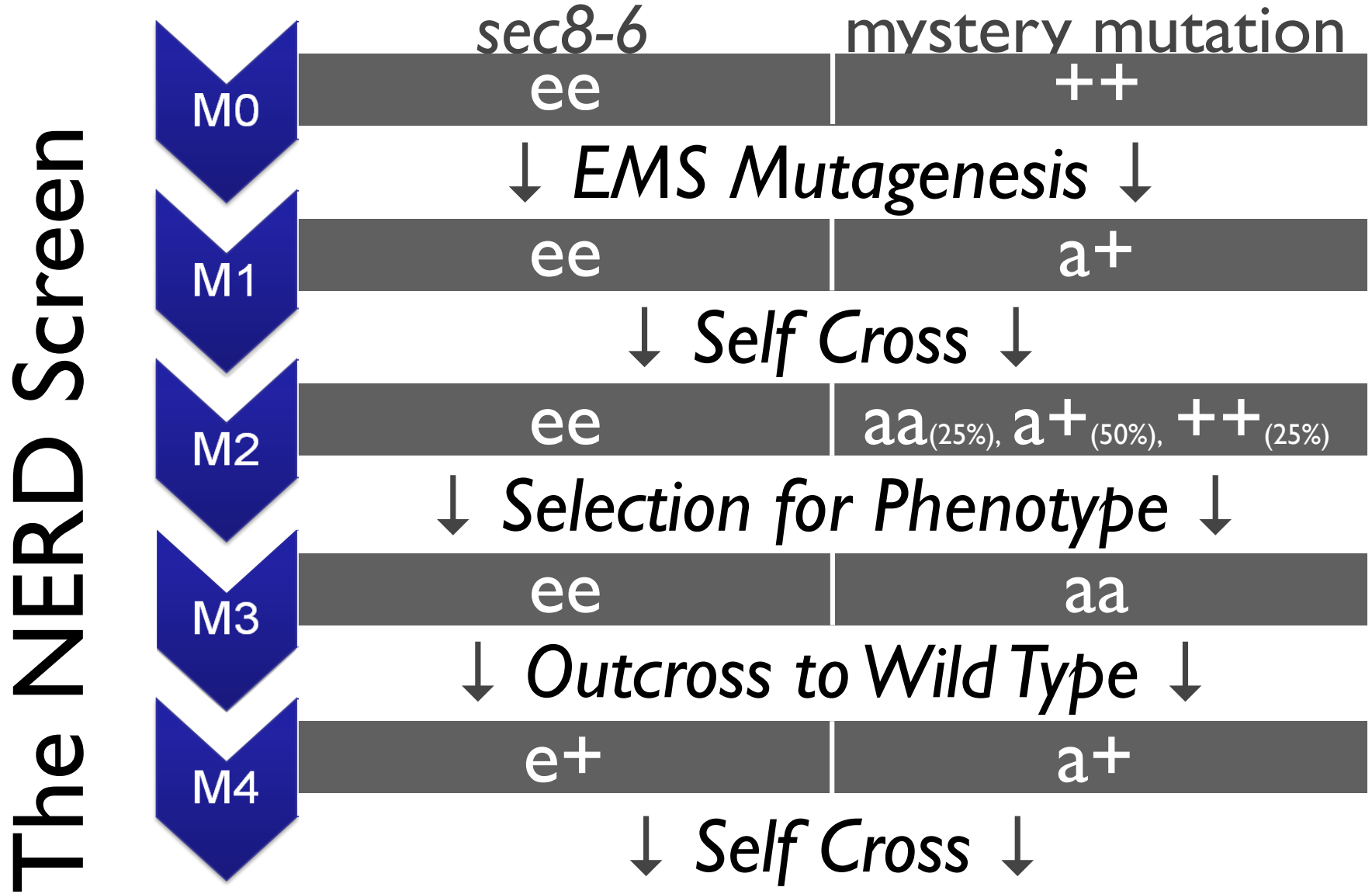

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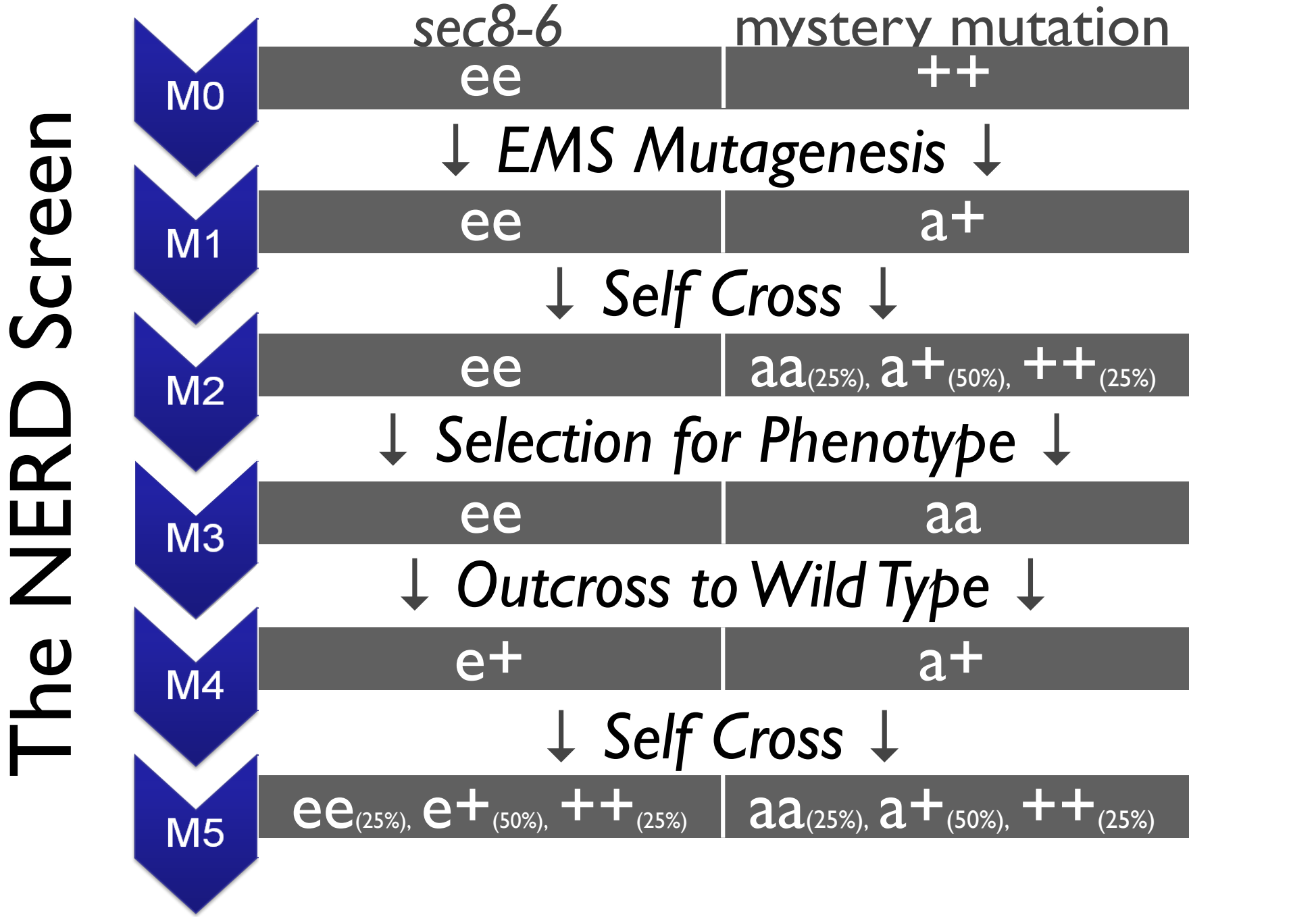

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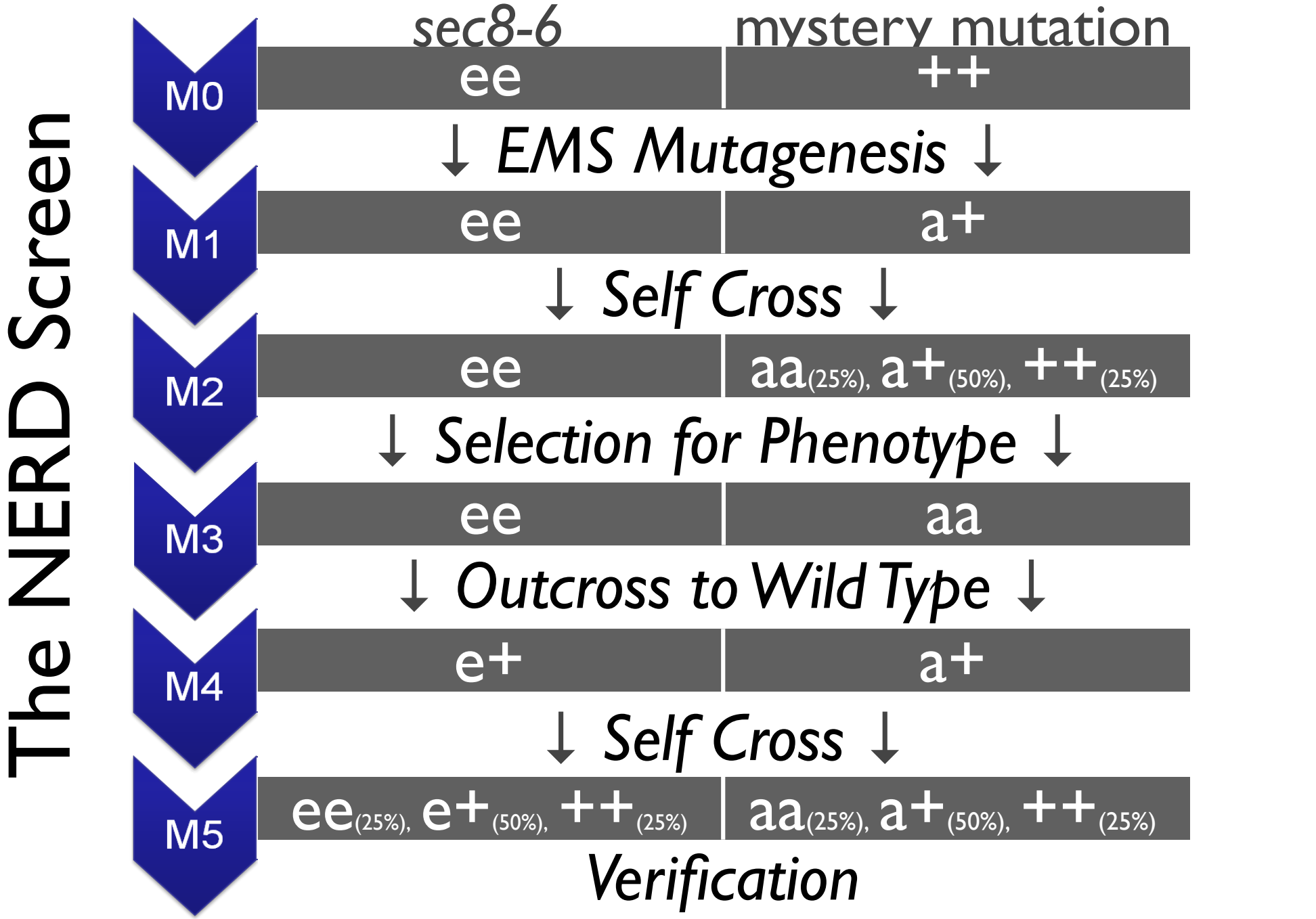

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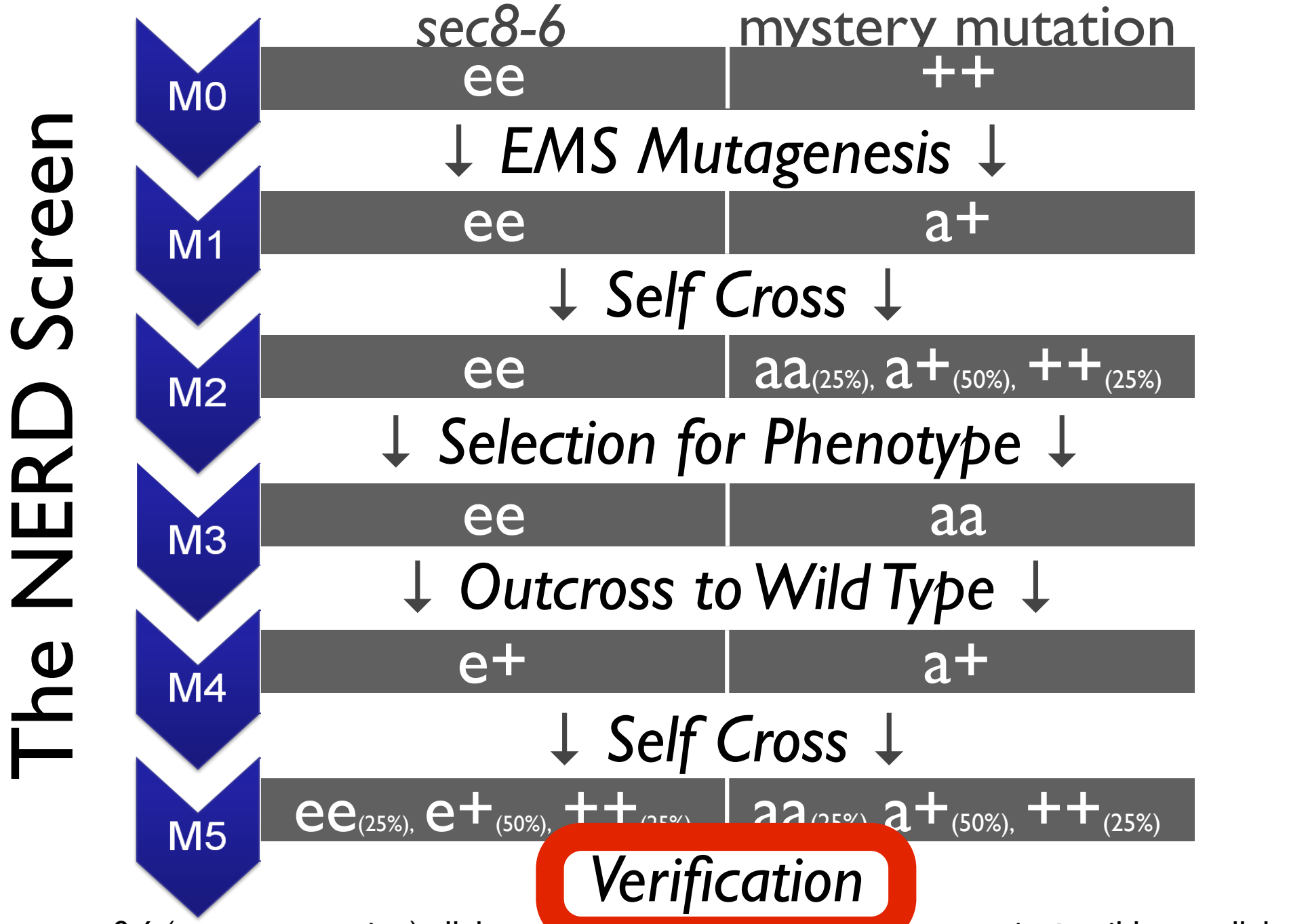
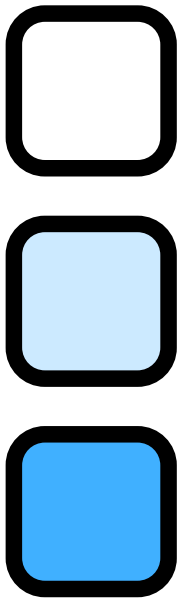


e: *sec8-6* (exocyst mutation) allele, **a**: [illegible] utagenesis, **+**: wild type allele

M5 NERD Verification

M4 Pollen Parent

M4 Female Parent	++	e+	+a	ea
++	++ ++	e+ ++	++ a+	e+ a+
e+	e+ ++	ee ++	e+ a+	ee a+
+a	++ a+	e+ a+	++ aa	e+ aa
ea	e+ a+	ee a+	e+ aa	ee aa



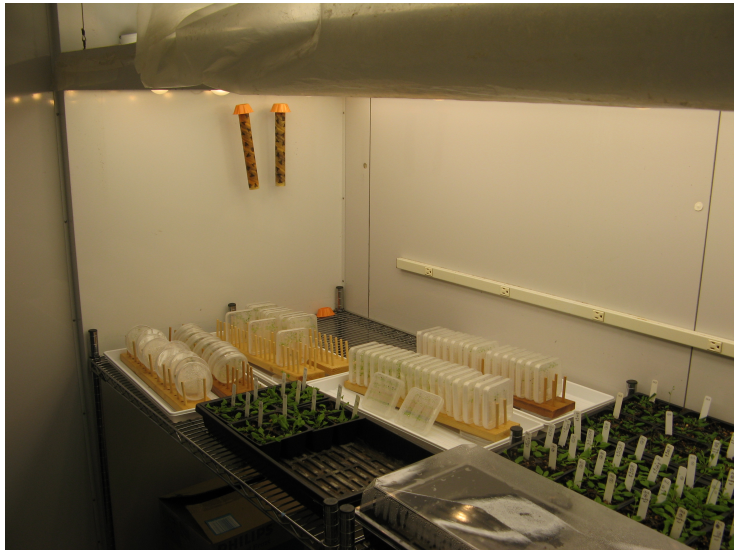
No Phenotype (3/4)

Mutant Phenotype (3/16)

Severe Mutant Phenotype (1/16)

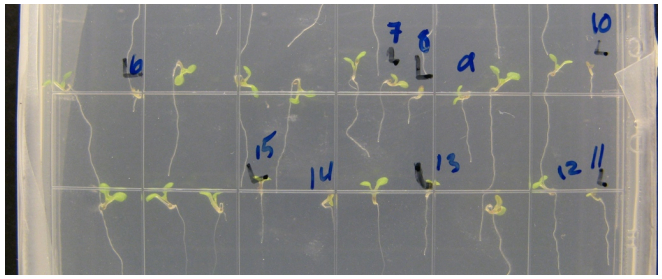
e: *sec8-6* (exocyst mutation) allele, **a**: mutant allele from EMS mutagenesis, **+**: wild type allele

Techniques/Process



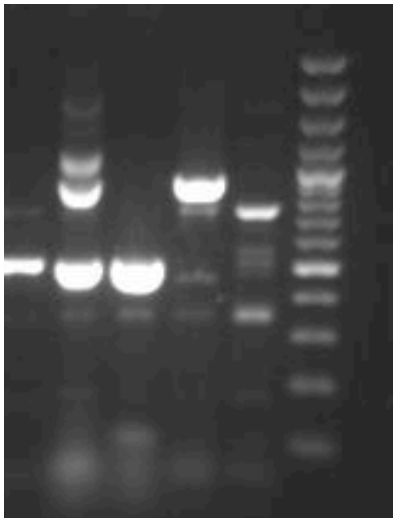
- Plates were poured and planted under a laminar flow hood
- Plants were grown in a climate controlled growth chamber

Techniques/Process



- Phenotypic observations were made with the aid of a dissecting microscope

Techniques/Process



- Genotypic observations were made through DNA extraction, PCR amplification, and gel electrophoresis

Lines Tested

1		12	
2		13	
3		14	
4		15	
5		16	
6		17	
7		18	
8		19	
9		20	
10		21	
11			

Lines Tested

1	appears to be 1/4	12	1/16 category not valid
2	genotyping negative	13	appears to be 1/4
3	genotyping negative	14	appears to be 1/4
4	too many phenotypes, dead	15	*sec8-6* not present
5	genotyping negative	16	*sec8-6* not present
6	genotyping negative	17	*sec8-6* not present
7	chlorosis	18	*sec8-6* not present
8	genotyping negative	19	*sec8-6* not present
9	putative NERD	20	*sec8-6* not present
10	putative NERD	21	*sec8-6* not present
11	no 1/16 category		

Lines Tested

1	appears to be 1/4	12	1/16 category not valid
2	genotyping negative	13	appears to be 1/4
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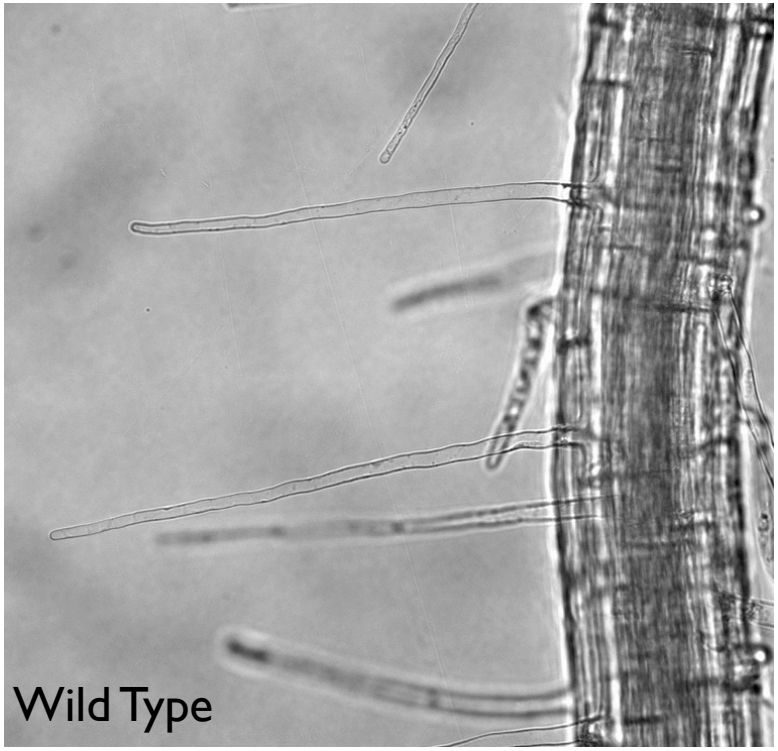
11	no 1/16 category		

Family 9

Phenotype S



No Root Hairs, Squiggly Roots

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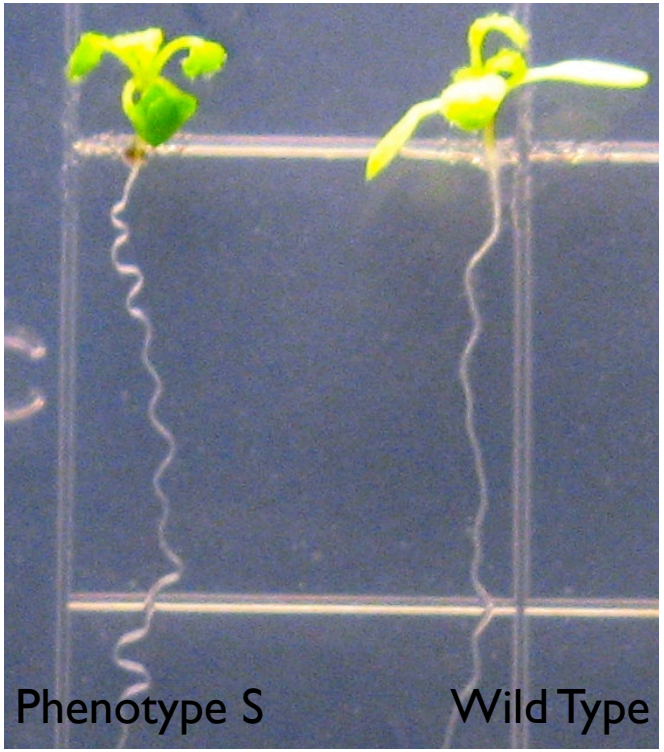

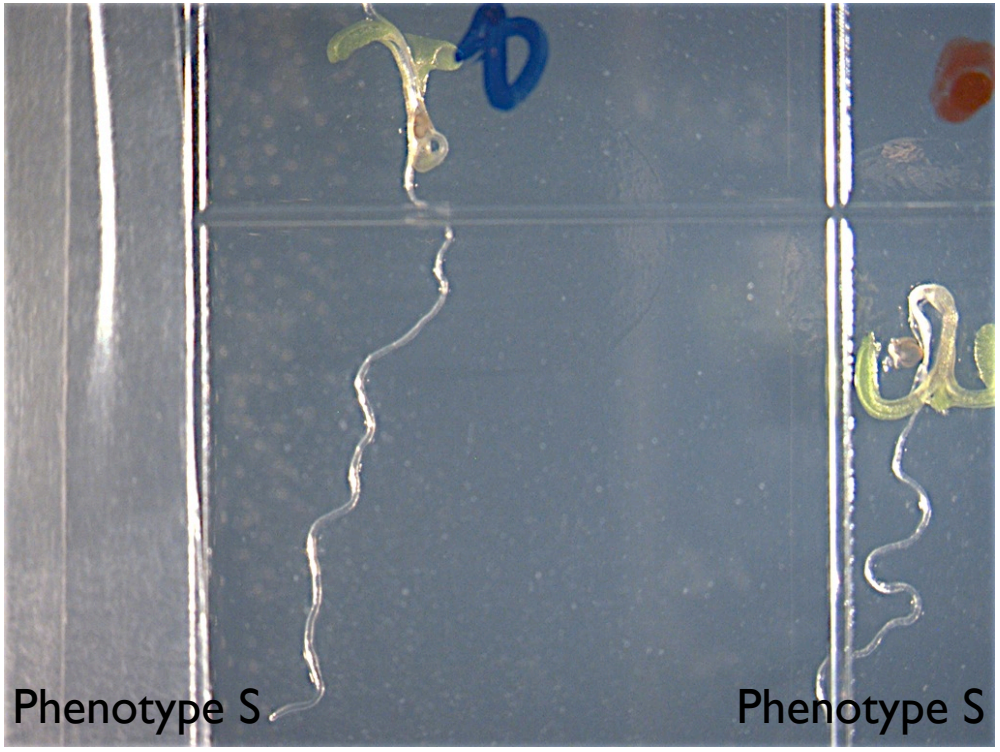

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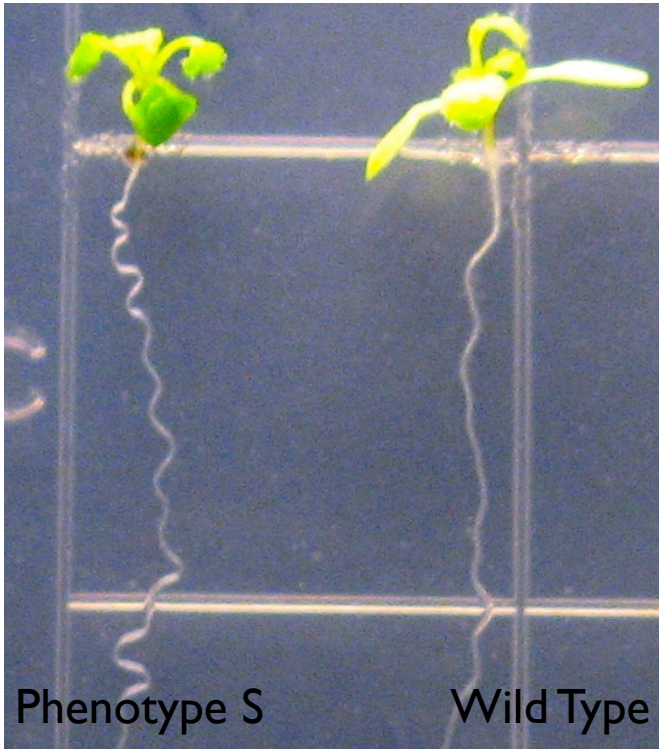
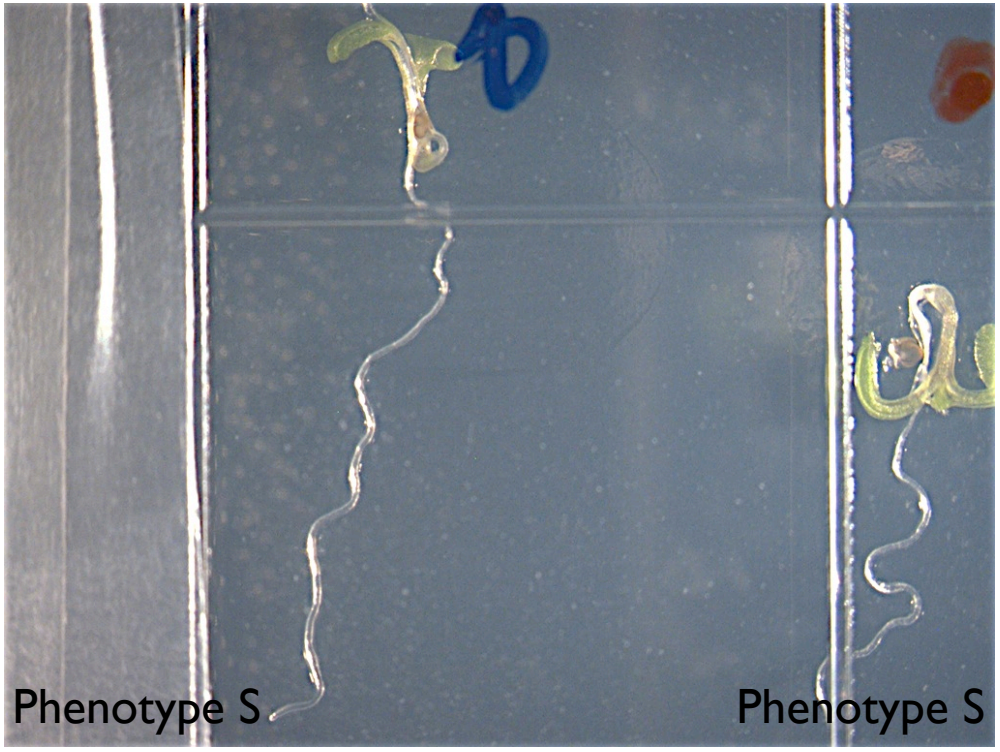




No Root Hairs, Squiggly Roots

Ratio Observed: 56/332 or **~2.7/16**

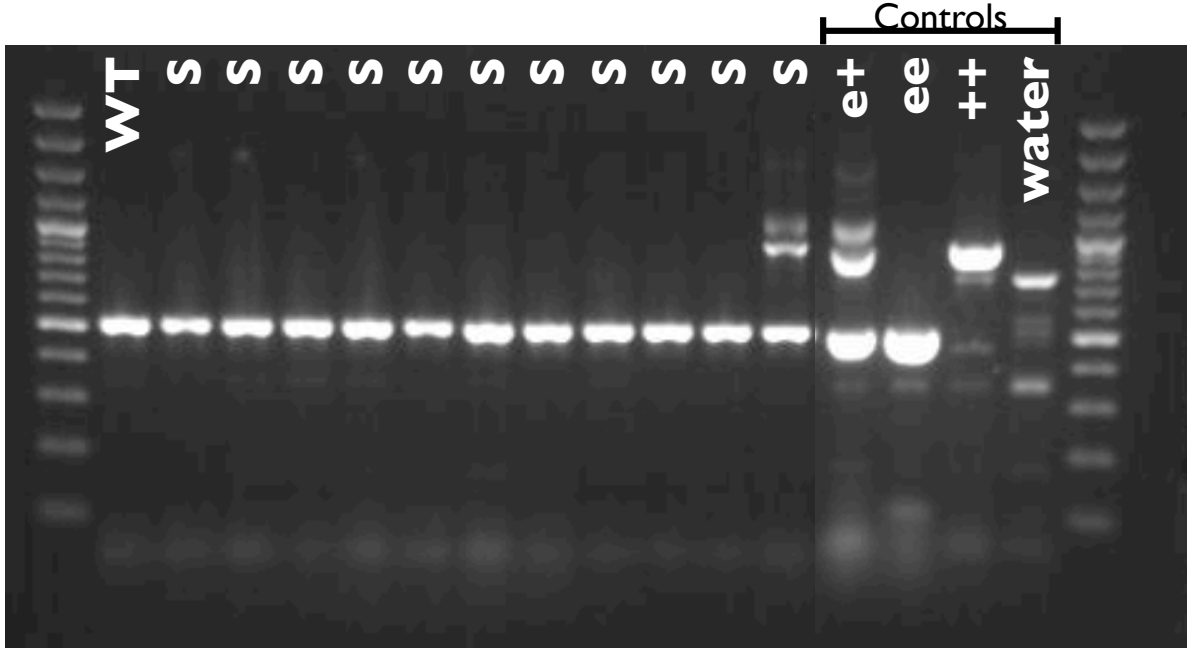
Phenotype	Total	Family 9 Genotypes					
		ee		e+		++	
		#	%	#	%	#	%
Negative Result			25%		50%		25%
WT	9	1	11%	5	56%	3	33%
S	20	18	90%	2	10%	0	0%

WT: wild type phenotype

S: squiggly roots/no root hairs phenotype

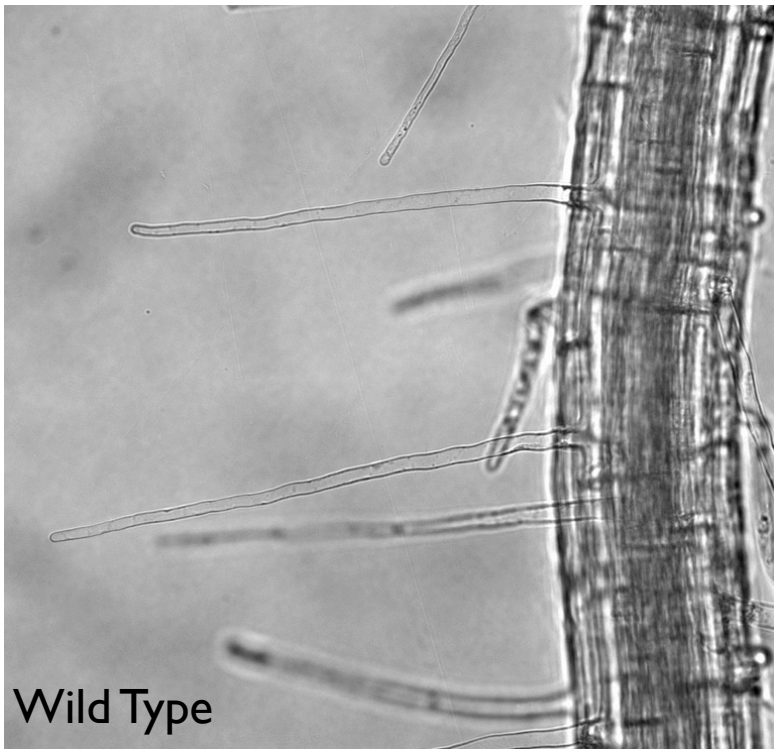
e: *sec8-6* mutation allele

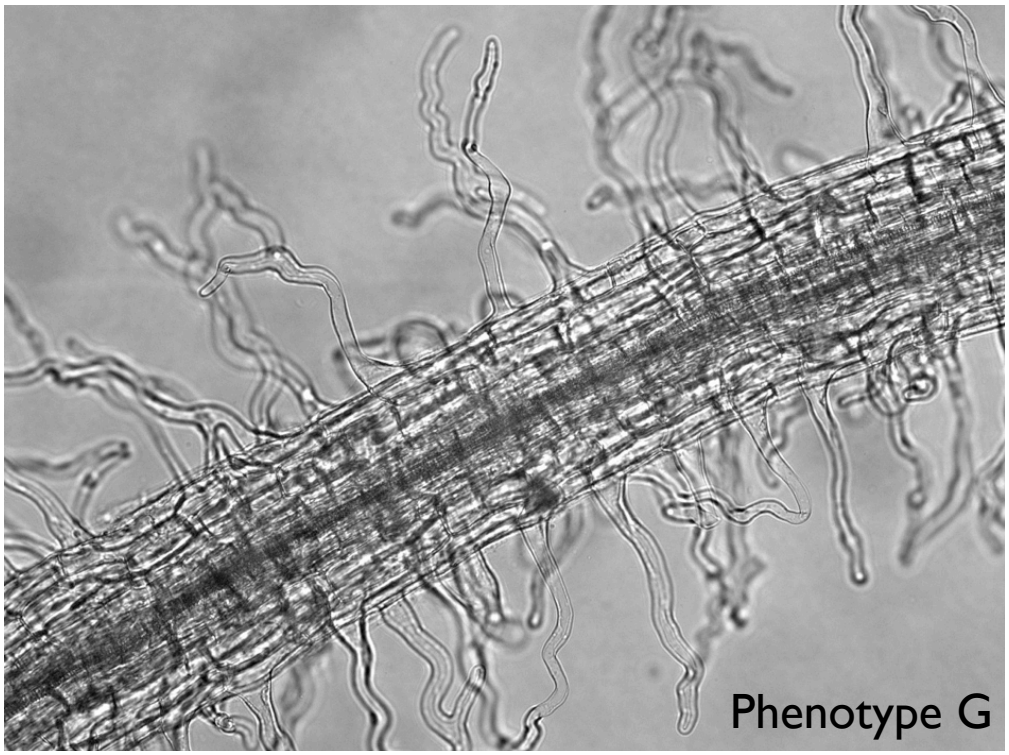
+: wild type allele



Family 10

Phenotype G

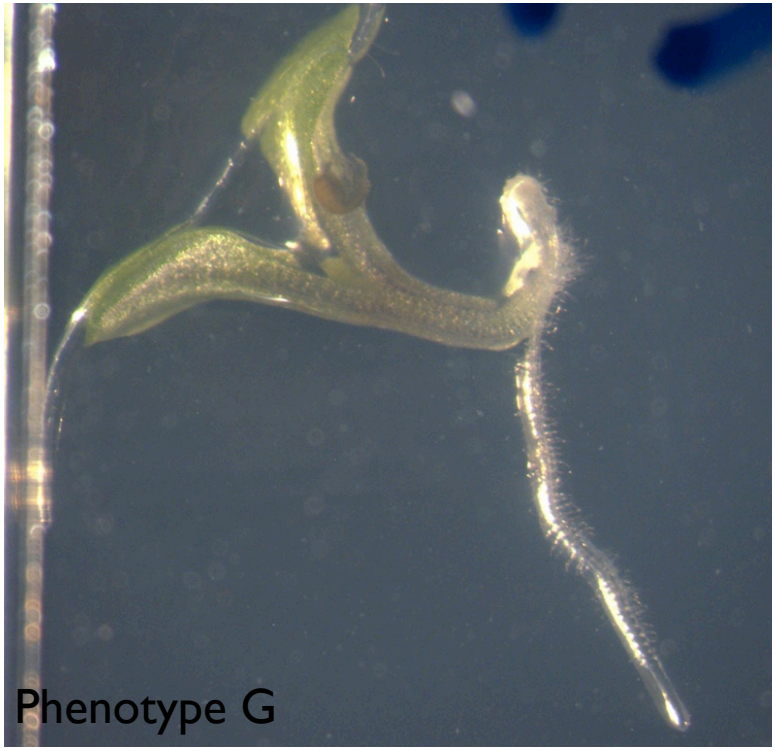


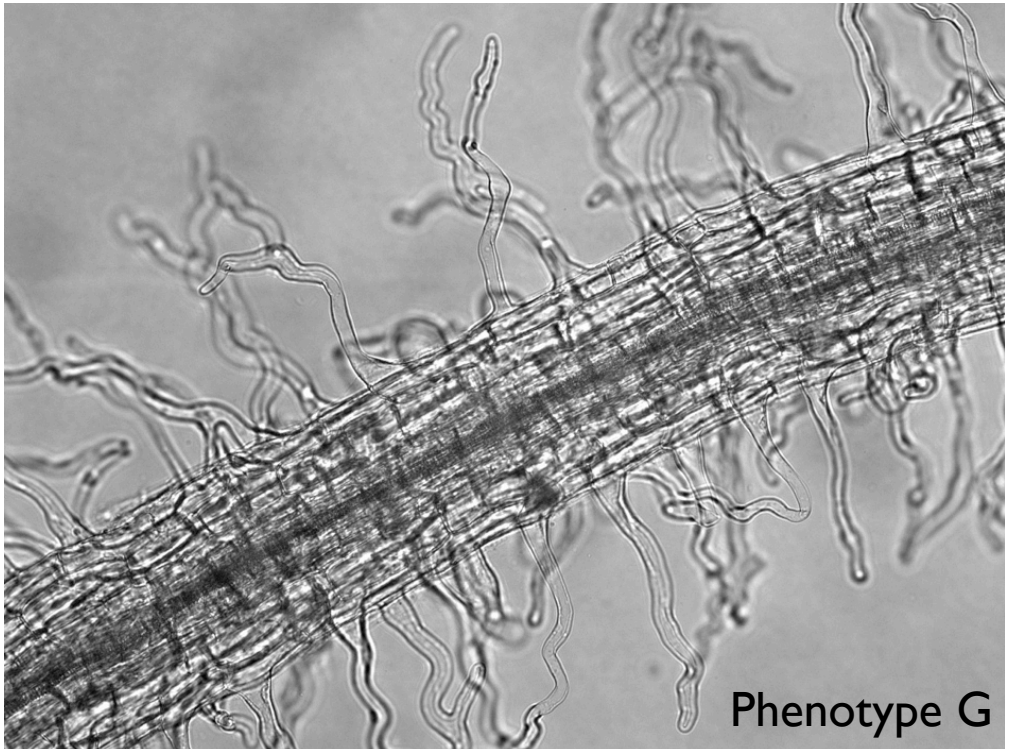


Short, Dense Root Hairs on a Shorter Root

Family 10

Phenotype G





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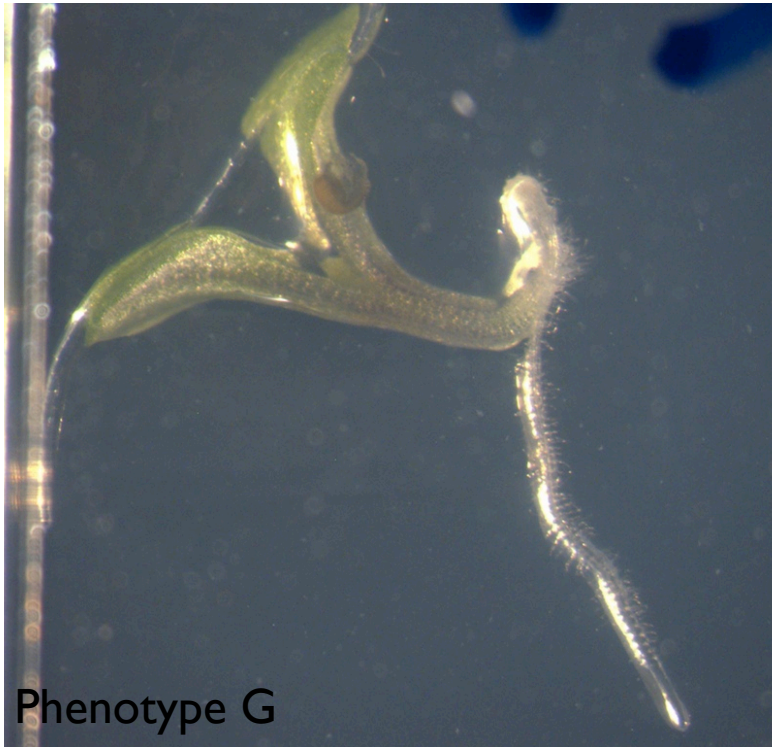
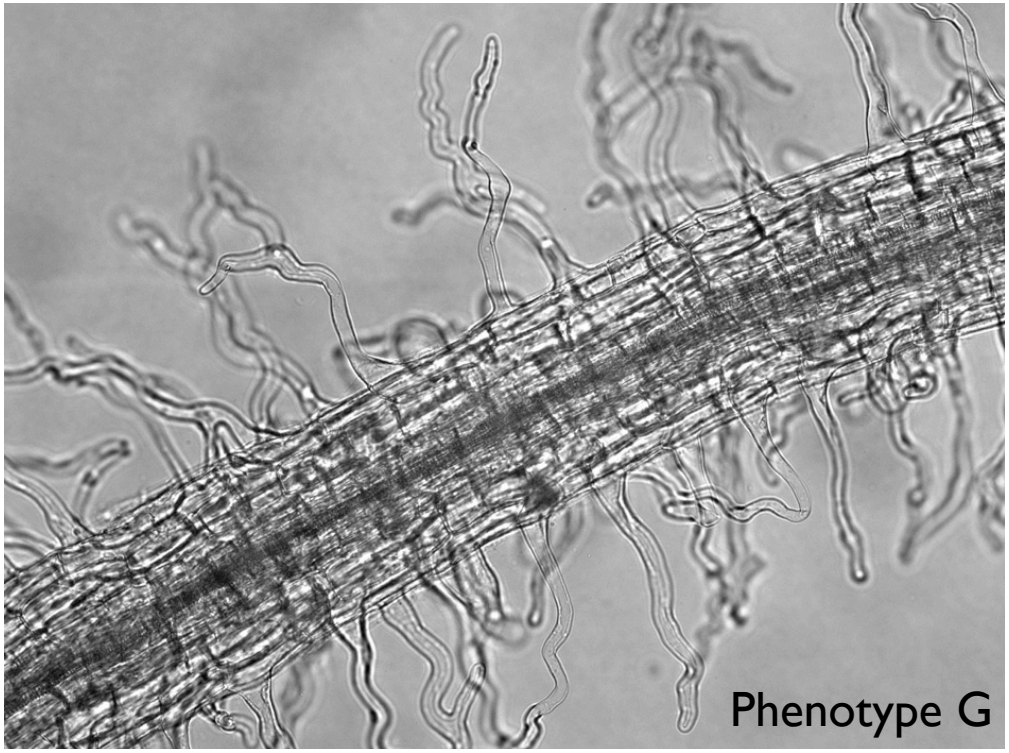




Short, Dense Root Hairs on a Shorter Root

Ratio Observed: 50/299 or **~2.7/16**

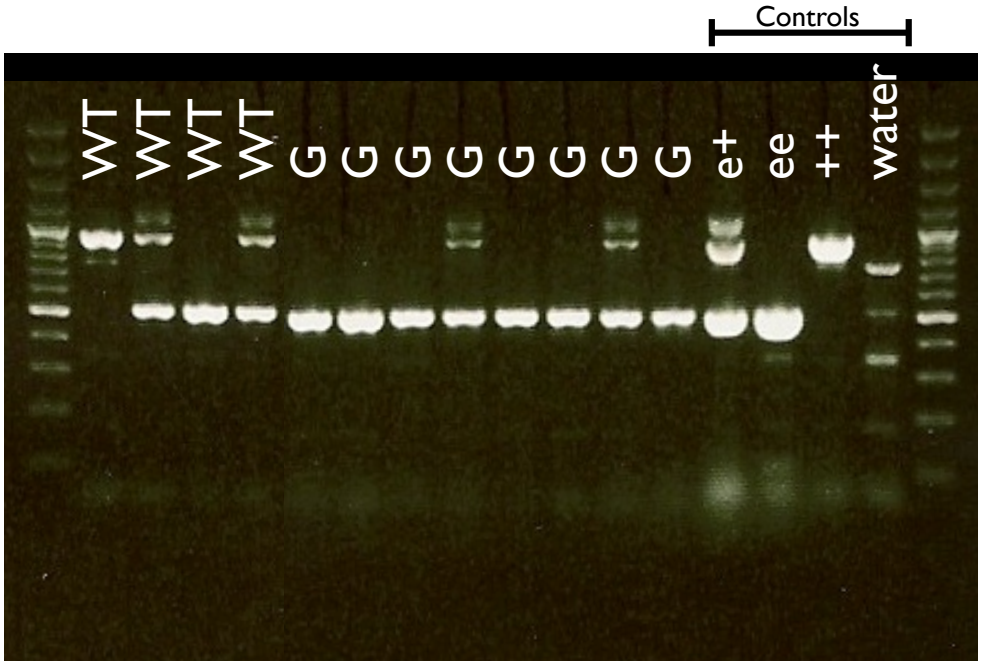
Phenotype	Total	Family 10 Genotypes					
		ee		e+		++	
		#	%	#	%	#	%
Negative Result			25%		50%		25%
WT	13	1	8%	8	62%	4	31%
G	15	13	87%	2	13%	0	0%

WT: wild type phenotype

G: short roots/root hairs phenotype

e: *sec8-6* mutation allele

+: wild type allele



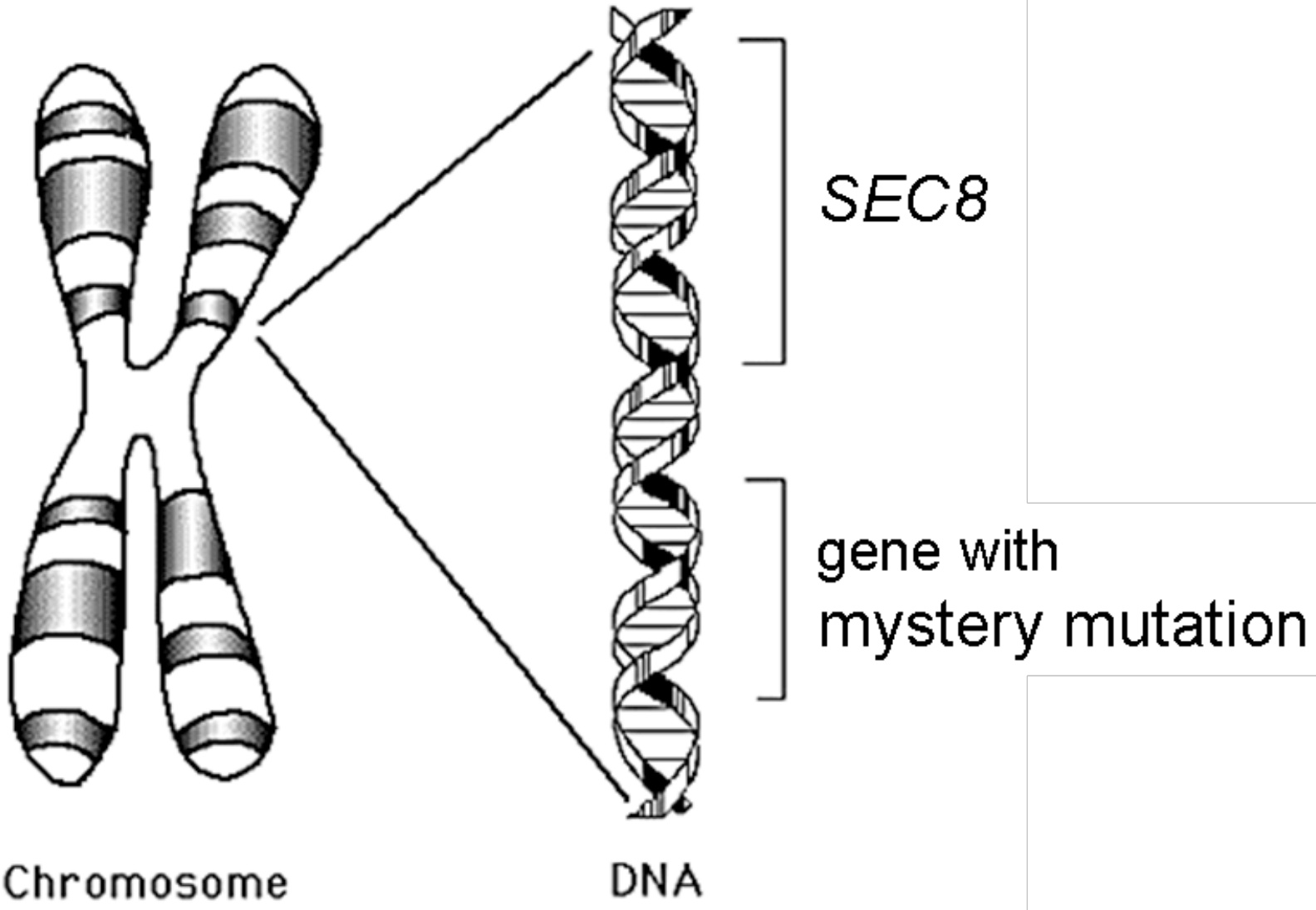
Preliminary Hypothesis A

Phenotype S & Phenotype G

- *sec8-6* and the mutations that cause phenotypes S and G may be linked genes.
- Linked genes are located nearby each other on the same chromosome, increasing their chances of being inherited together.

Preliminary Hypothesis A

Phenotype S & Phenotype G



Preliminary Hypothesis A

Phenotype S & Phenotype G

- In this scenario, the mutated genes that cause phenotype S and G may not be exocyst interactors.

Preliminary Hypothesis B

Phenotype S & Phenotype G

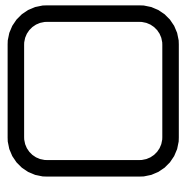
- The mystery mutation may act as a dominant allele.
- Phenotypic expression may occur with only one copy the allele present.
- The mutation would be expressed if a plant were homozygous for *sec8-6* and had one or two copies of the mystery mutation allele.

Preliminary Hypothesis B

Phenotype S & Phenotype G

M4 Pollen Parent

M4 Seed Parent	++	e+	+a	ea
++	++ ++	e+ ++	++ a+	e+ a+
e+	e+ ++	ee ++	e+ a+	ee a+
+a	++ a+	e+ a+	++ aa	e+ aa
ea	e+ a+	ee a+	e+ aa	ee aa



No Phenotype (13/16)

Mutant Phenotype (3/16)

e: *sec8-6* (exocyst mutation) allele, **a**: mutant allele from EMS mutagenesis, **+**: wild type allele

Preliminary Hypothesis B

Phenotype S & Phenotype G

- The phenotypic expression ratio would be 3/16, which is similar to observations.
- In this scenario, the mutated genes that cause phenotype S and G may be exocyst interactors.

Conclusion

- Families 9 and 10 are putative NERDs.
- More study is required to confirm and investigate this finding.

Acknowledgement:

- John Fowler Laboratory
- Dr. Rex Cole
- Anne Kettler
- Renee Cool

OSU
Oregon State
UNIVERSITY

saturday academy

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