

# Introduction to Genotyping

HC70AL  
Spring 2011  
4/7/11

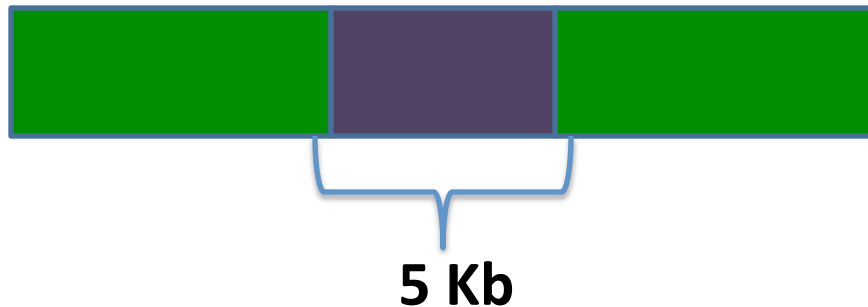
By  
Eden Maloney  
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# What are the two possible alleles present in your plants?

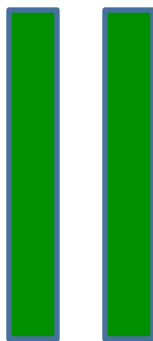
- Wild type allele of the gene



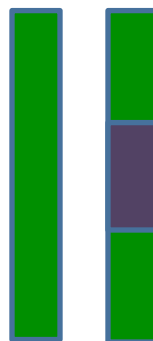
- Gene with T-DNA insert



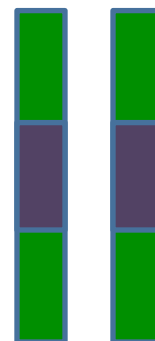
## What are the possible genotypes?



Homozygous Wild Type



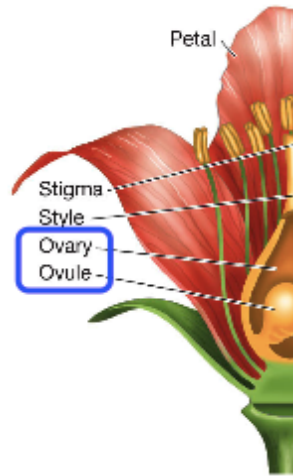
Heterozygous



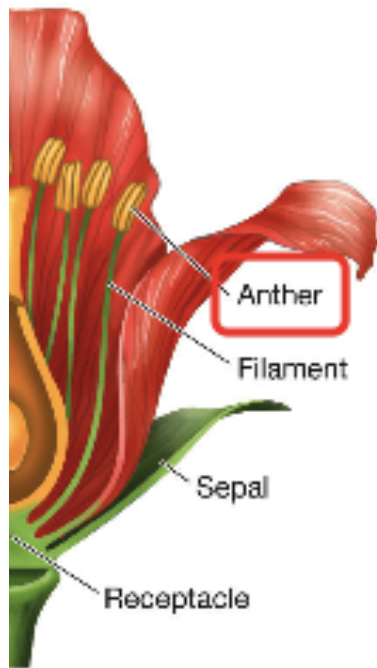
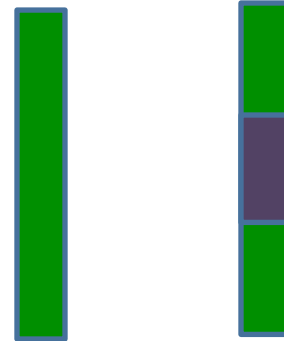
Homozygous for T-DNA

# What is the expected ratio of genotypes?

Remember Arabidopsis is a hermaphrodite!



**Female Gametes**



**Male Gametes**

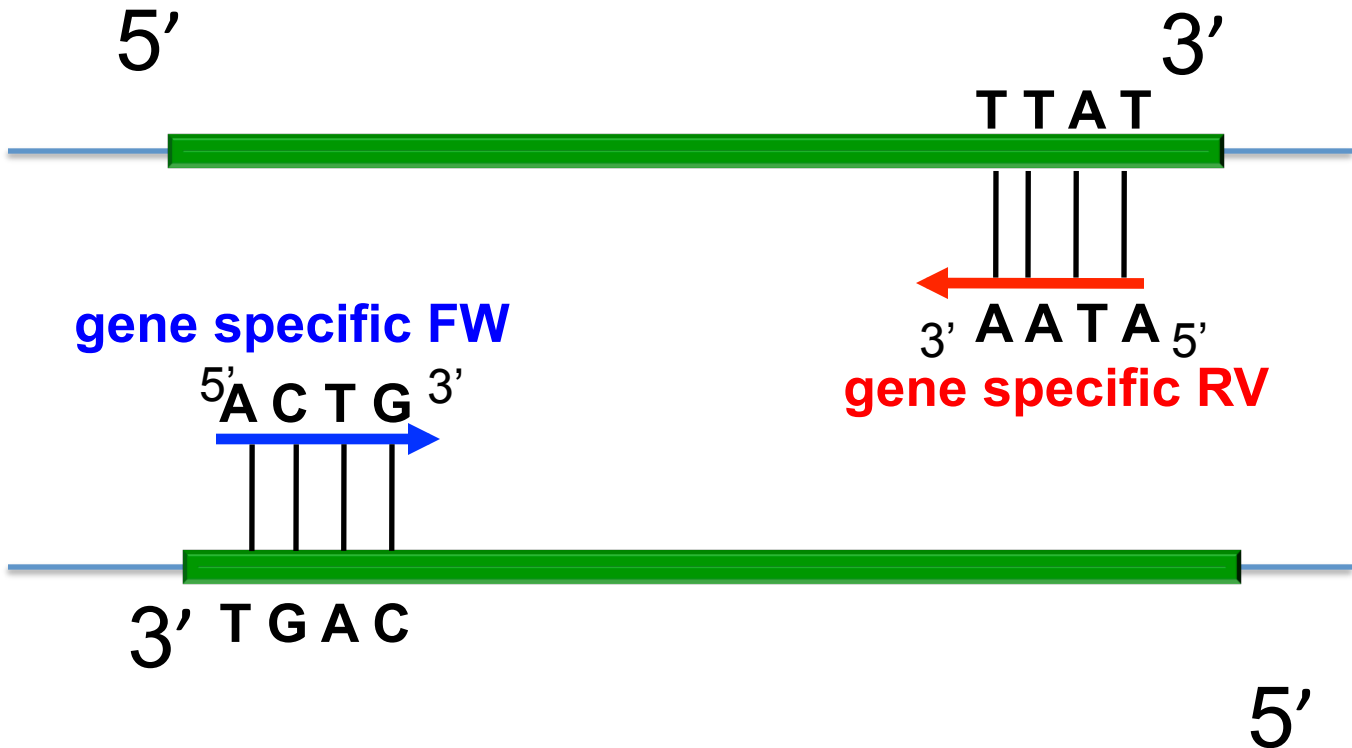


	Wild Type		Hetero	
Wild Type				
Hetero				

Hetero

Homo T-DNA

# Where do Primers Anneal?



## Gene Specific Primers

Forward: 5' ACTG 3'

Reverse: 5' AATA 3'



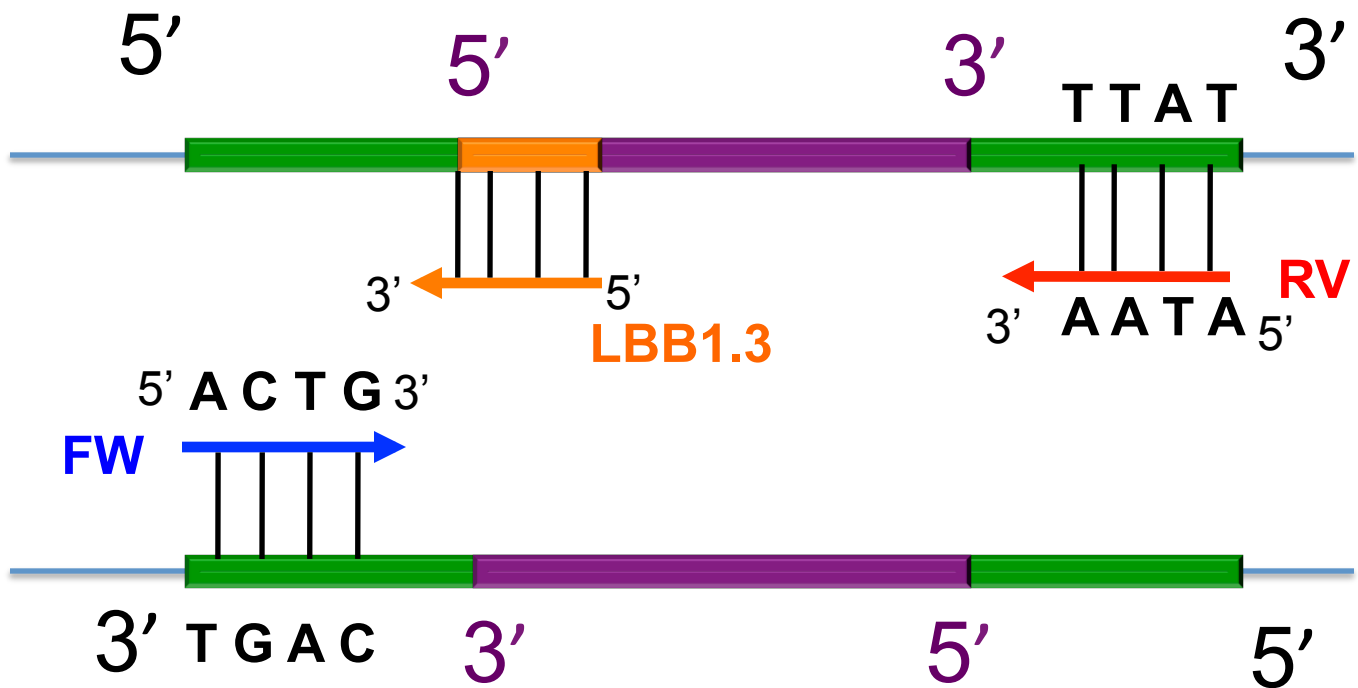
Reverse Complement

## In Sequence Look For

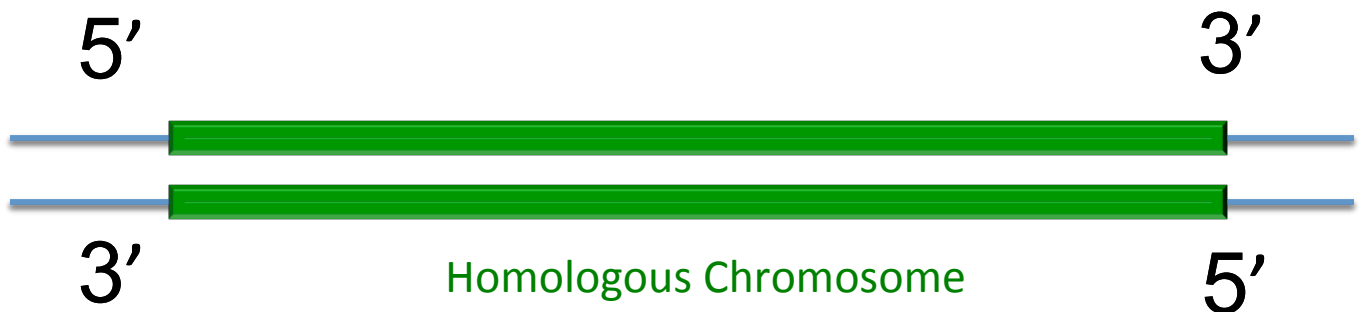
Forward: 5' TGAC 3'

Reverse: 5' TTAT 3'

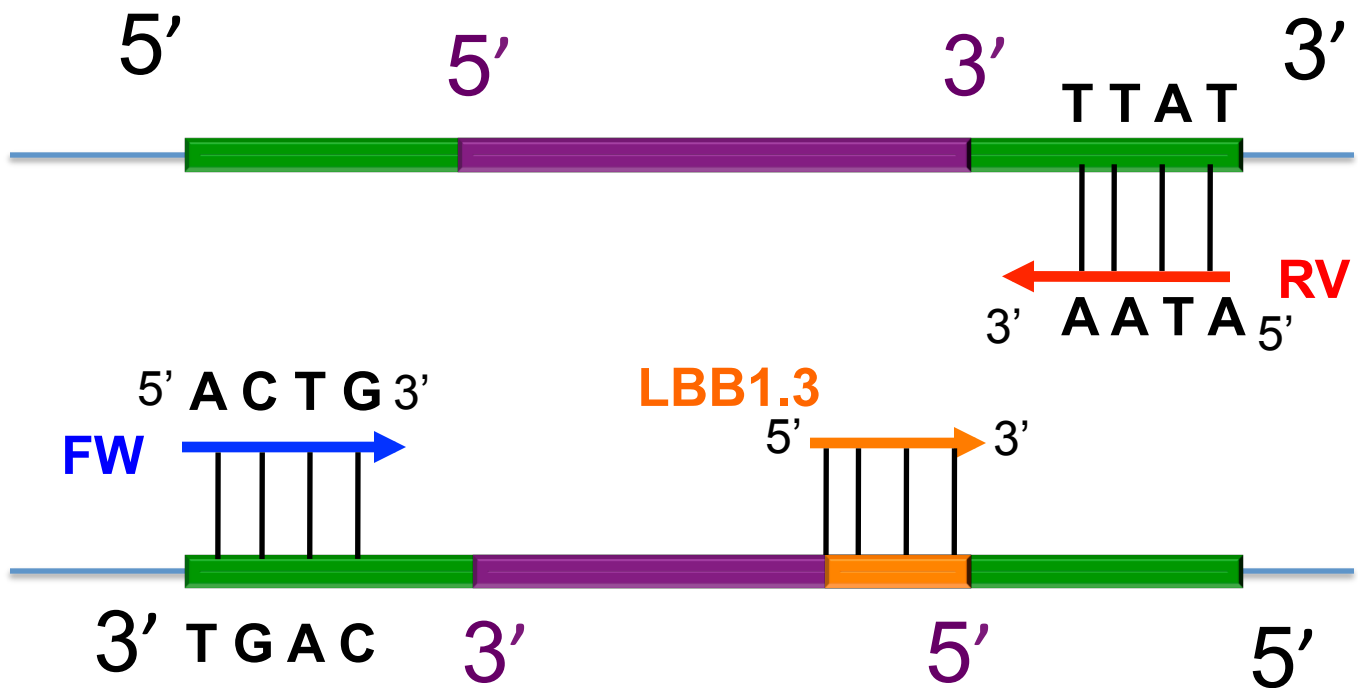
# What is the orientation of a T-DNA insert relative to the gene of interest?



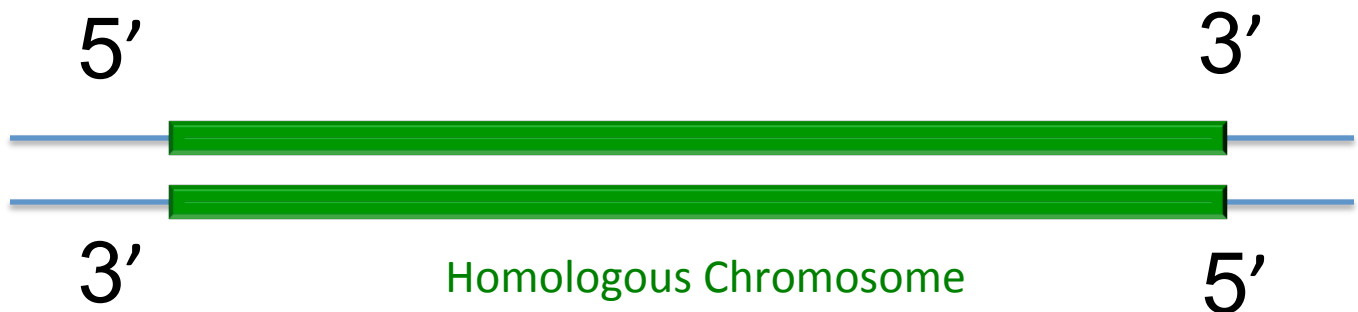
What primer does the LBB1.3 primer form a PCR Product with?



# What is the orientation of a T-DNA insert relative to the gene of interest?



What primer does the LBB1.3 primer form a PCR Product with?



# How do you set up a PCR reaction for genotyping?

**MASTER MIX A**



**MASTER MIX B**



**MASTER MIX C**



**Gene Specific FW +  
LBb1.3**

**Gene Specific RV +  
LBb1.3**

**Gene Specific FW +  
Gene Specific RV**

**Do you know what direction the T-DNA  
inserted into your gene of interest?**

# How do we visualize PCR products?

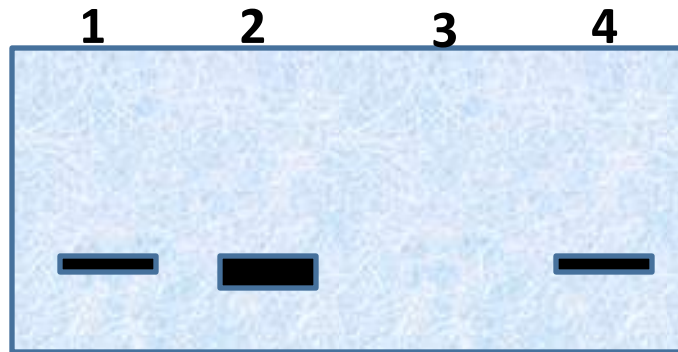
- What do different sizes of PCR products represent?
- How many different alleles are in a diploid organism?
- How many bands would you expect to see in a homozygous plant?
- How many bands would you expect to see in a heterozygous plant?



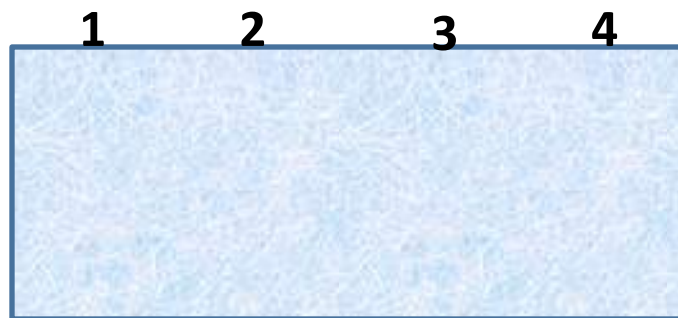
# How do we interpret the gel results?

PCR products  
from plant #:

Rxn A  
F + LBb1.3



Rxn B  
R + LBb1.3



Rxn C  
F + R

