

**Genome-wide  
changes induced by  
long term  
experimental  
warming in  
Mountain Avens**

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# International Tundra Experiment Sites

## Open Top Chambers (OTCs)

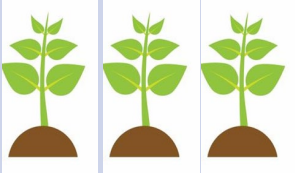
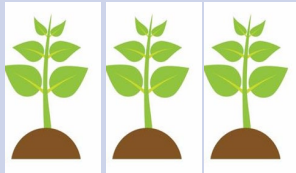








# Background: Past studies

## Changes due to OTCs (warming)

- Increased plant height
- Accelerated phenology
- Increased leaf size
- Increased seed weight
- Increased ovule number per flower
- More seed set overall
- Increased photosynthesis
- Increased green leaf biomass
- Decreased leaf nitrogen
  - (Molau 2001, Welker et al. 2003, Hudson and Henry 2011, Baruah et al. 2017)

Traits	Wild	F1	Later F1
Warming origin			
Control origin			

• Anne Bjorkman (thesis)

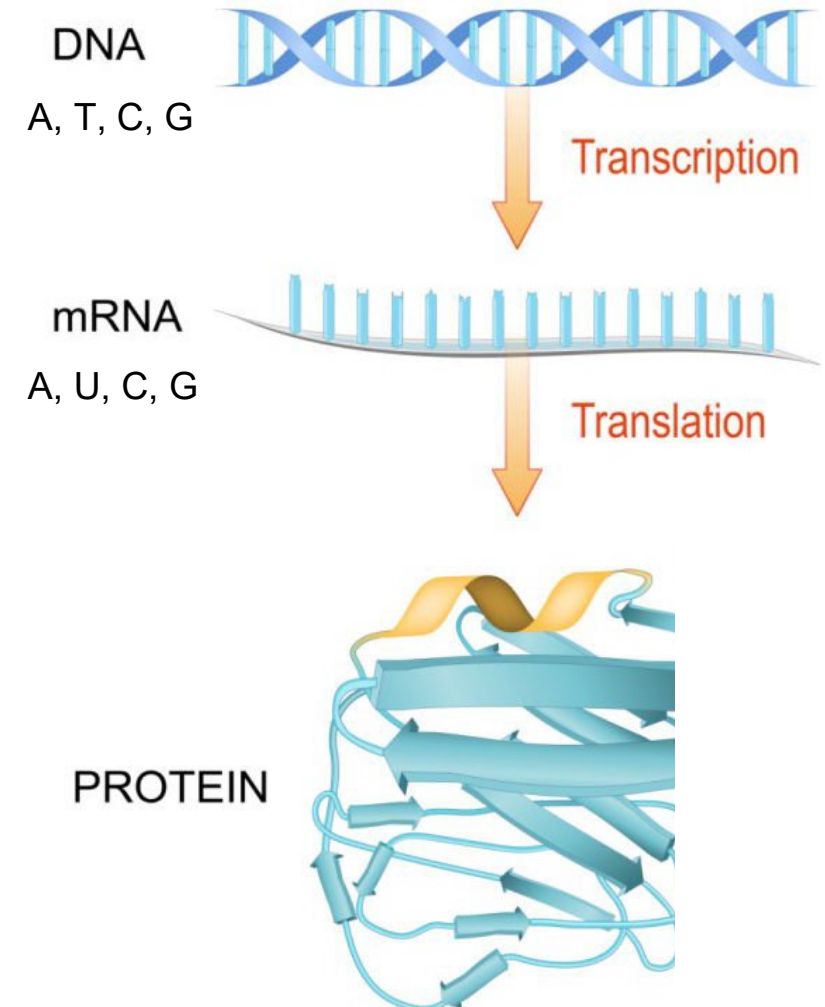
Are these changes passed on to future generations and how?



# Definitions

Central dogma

DNA → RNA → Protein → Phenotype





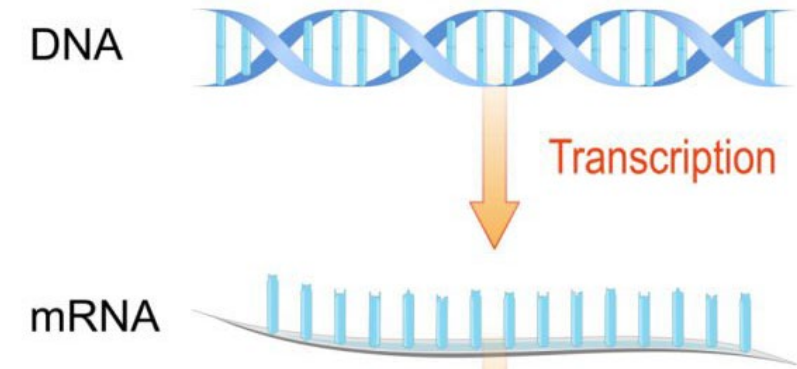
# Definitions

Gene expression

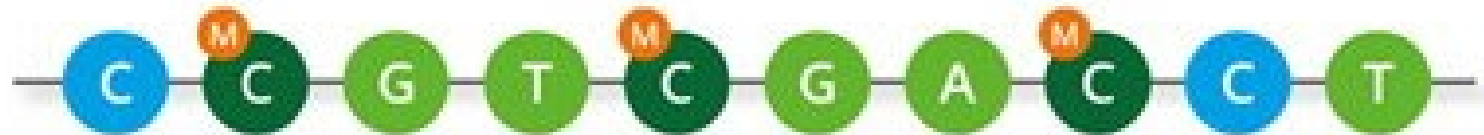
DNA → RNA



Epigenetics



DNA methylation





# Knowledge gap and hypothesis

## Questions

- Are plant responses to warming plastic (no long-term component), epigenetic, or genetic?
- Can warming induced changes be inherited?

## Hypothesis

- There is a combination of plastic and epigenetic responses to warming



# Study species: *Dryas octopetala* and *integrifolia*

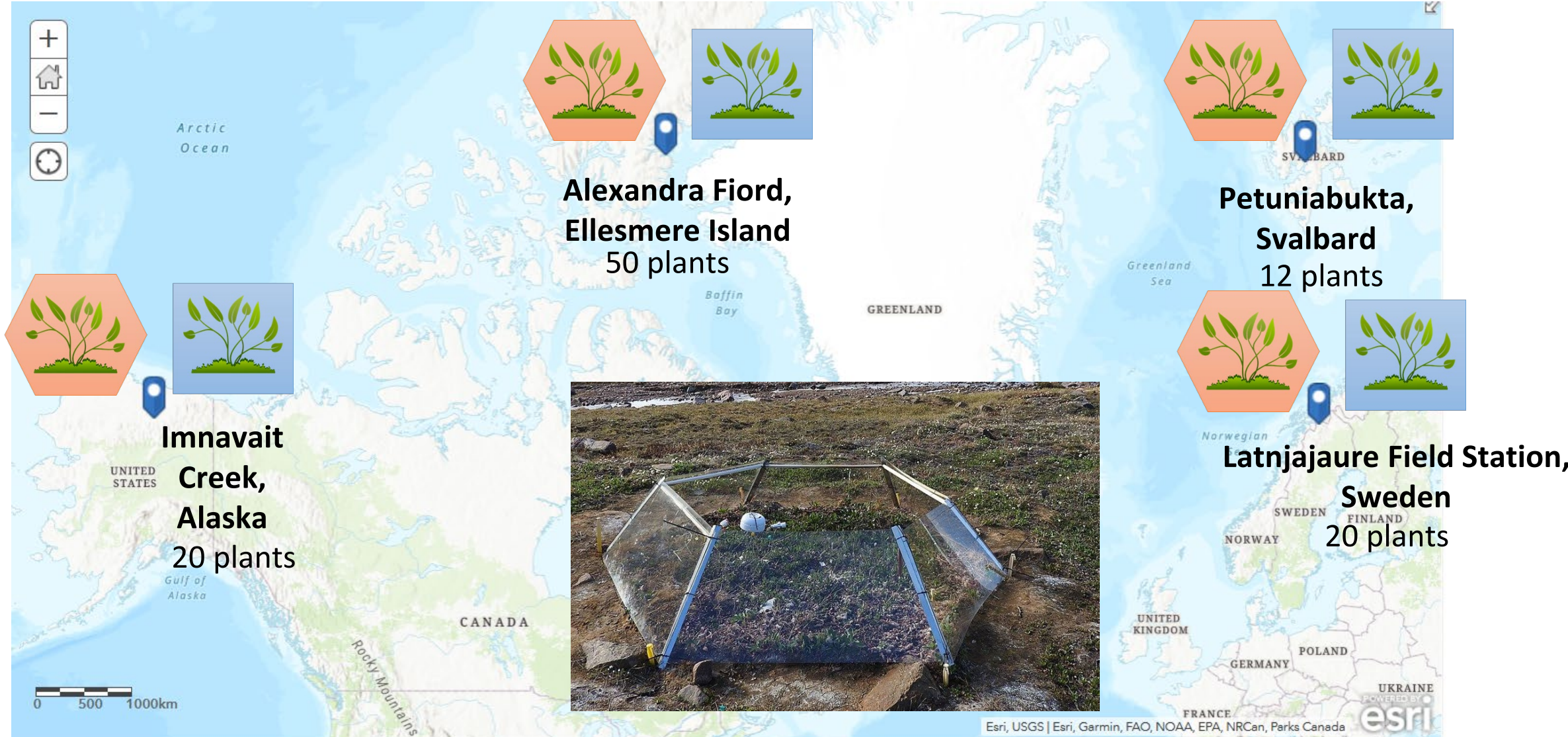
- Reference genome
- $2x = 460$  Mbp
- $2n=18$  chromosomes
- Well studied in Arctic





# Field sampling

- Leaf and seed collection in and out of warming chambers at 4 sites

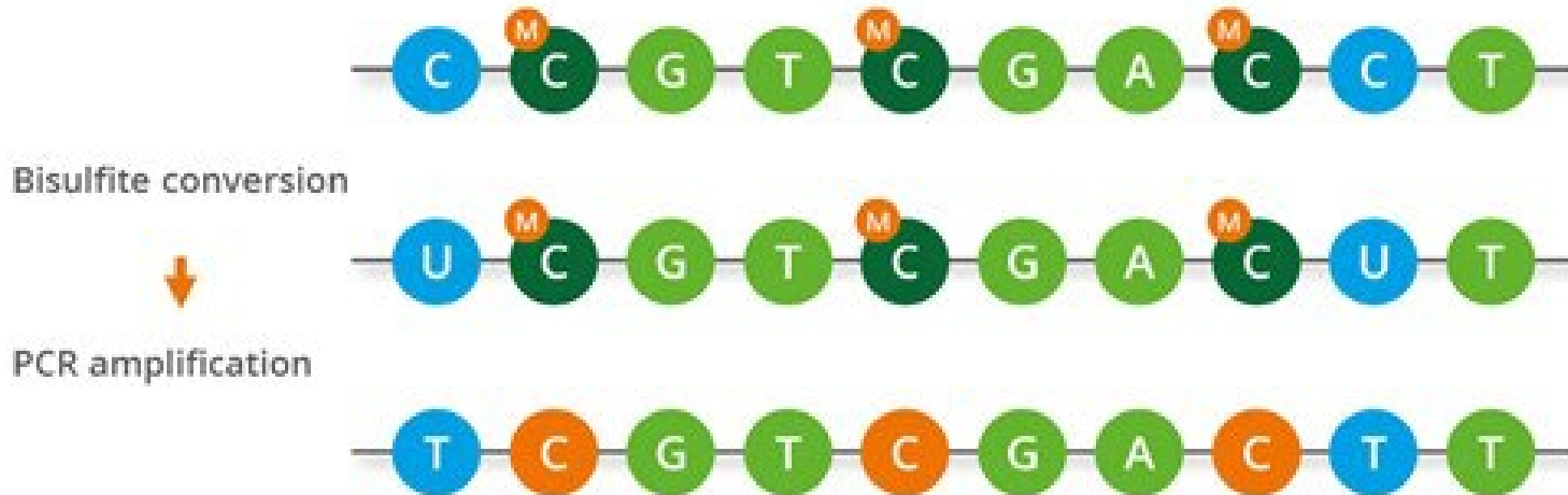






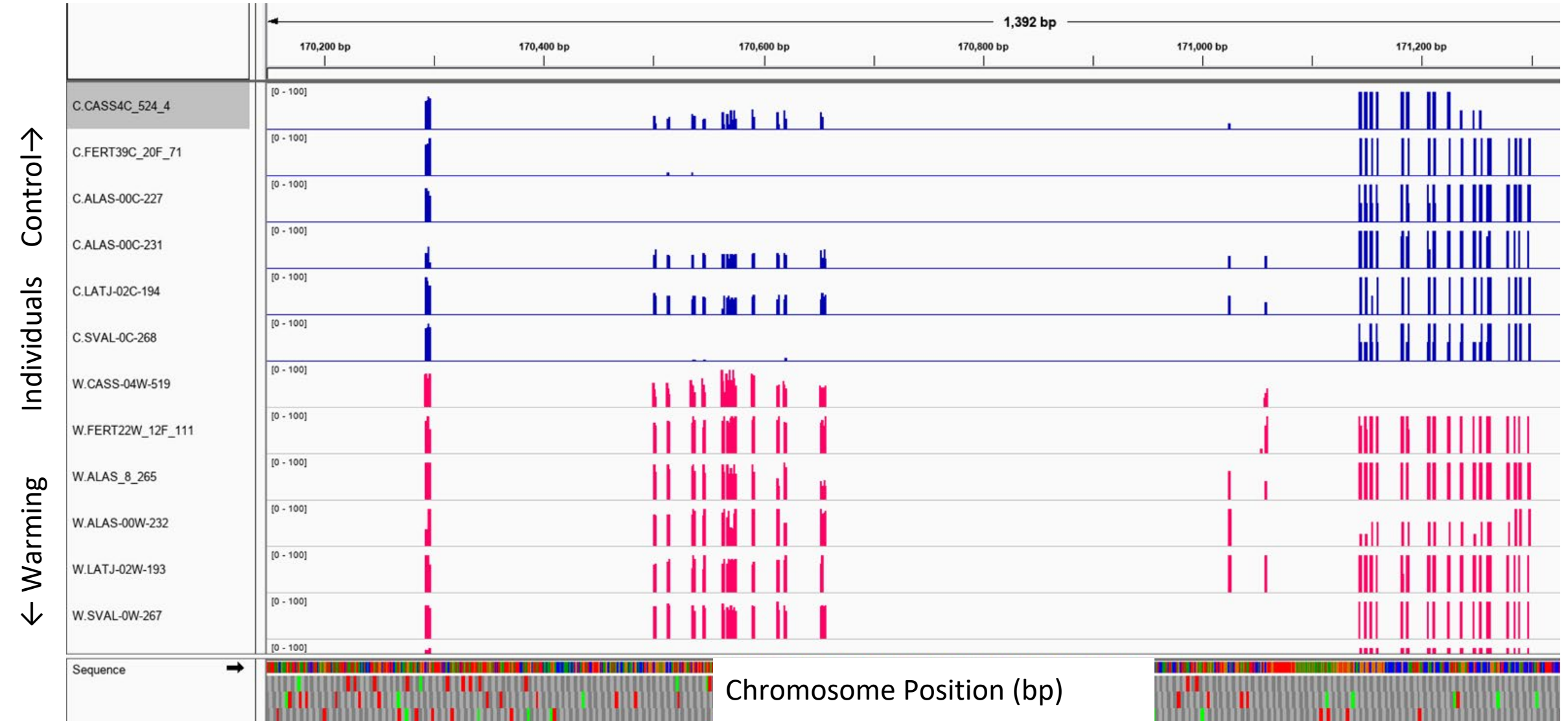
# Whole Genome Bisulphite Sequencing (WGBS)

- Total 102 individuals sequenced at Genome Quebec



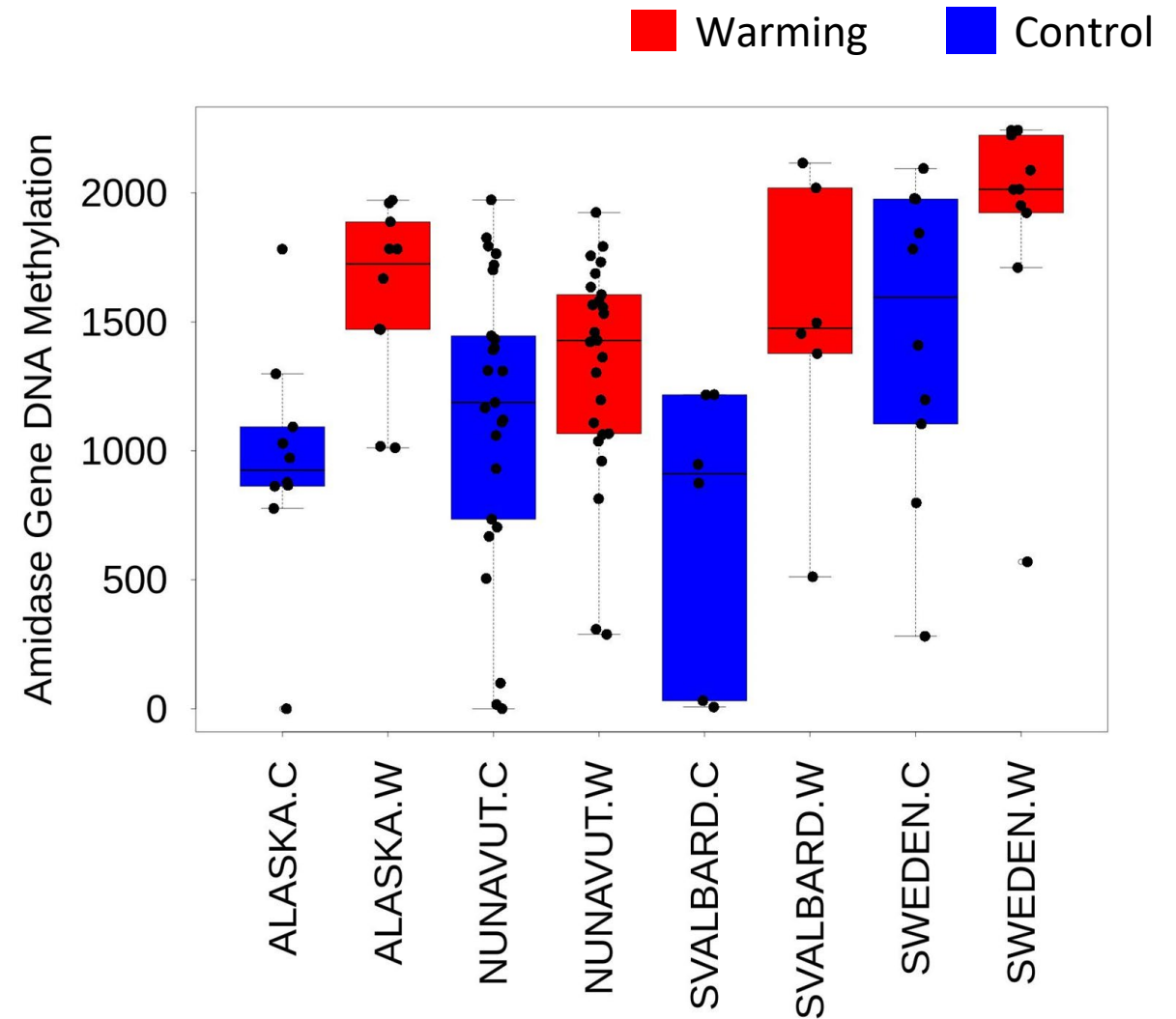
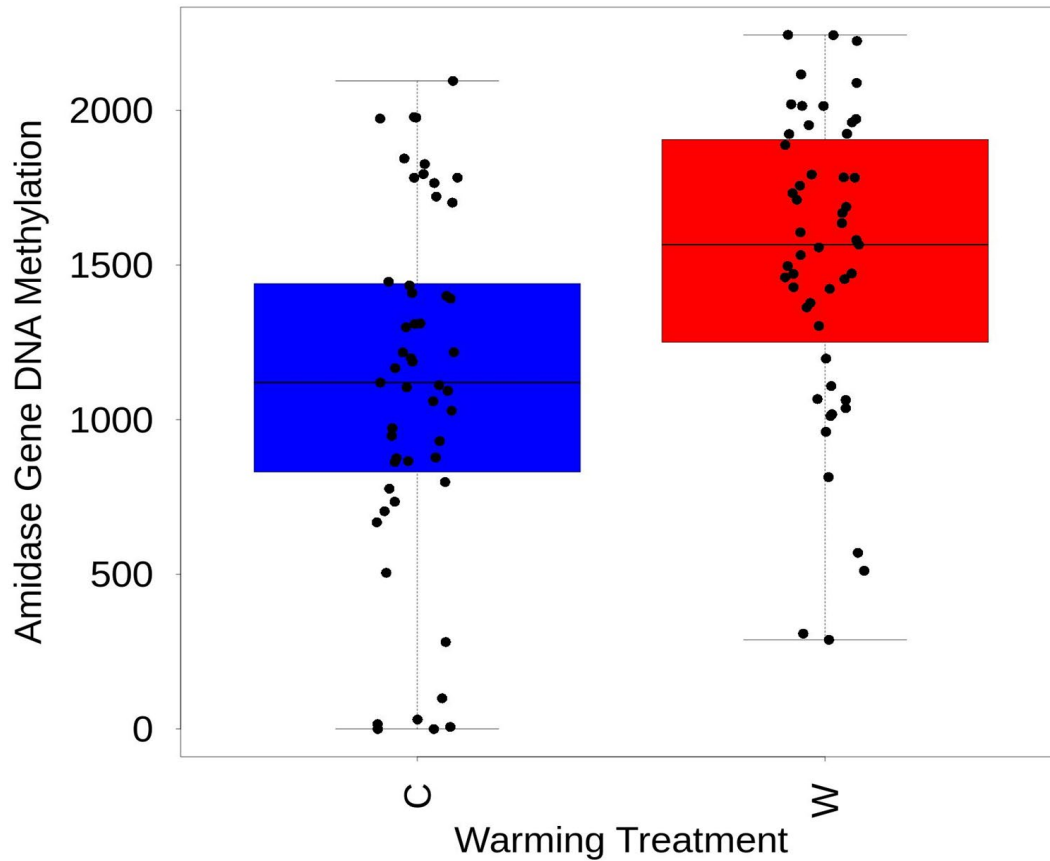


# Differentially methylated regions (DMRs)





# DNA methylation



- Bonferroni corrected p-value (MWU) =  $3.95e-14$
- q-value =  $1.54e-25$
- q-values are (per default) Bonferroni adjusted based on MWU-test p-values



# DNA methylation

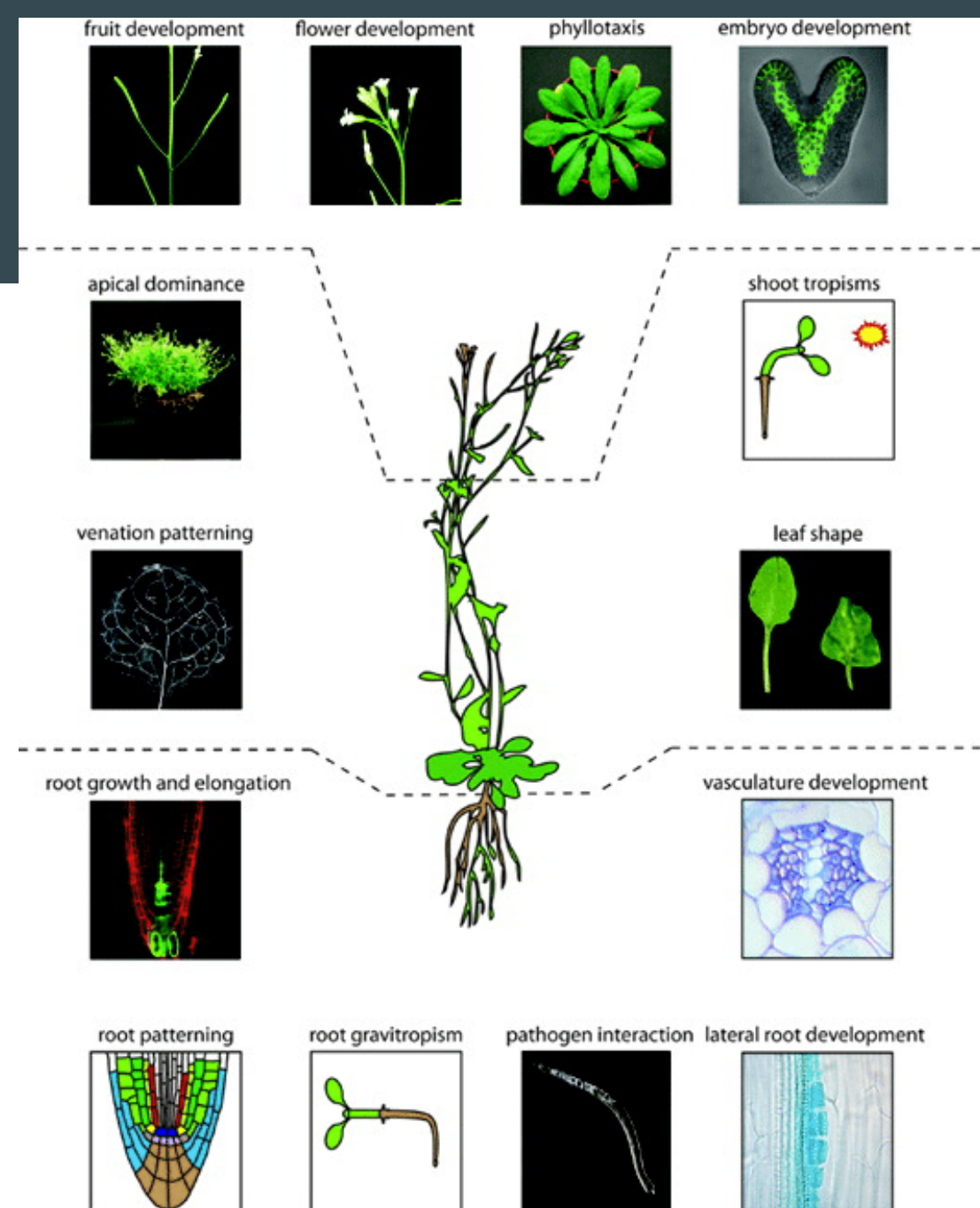
What is this specific region?

- Blast matched an amidase protein in *Prunus persica* (At4g34880)

Some amidase enzymes play a role in auxin biosynthesis

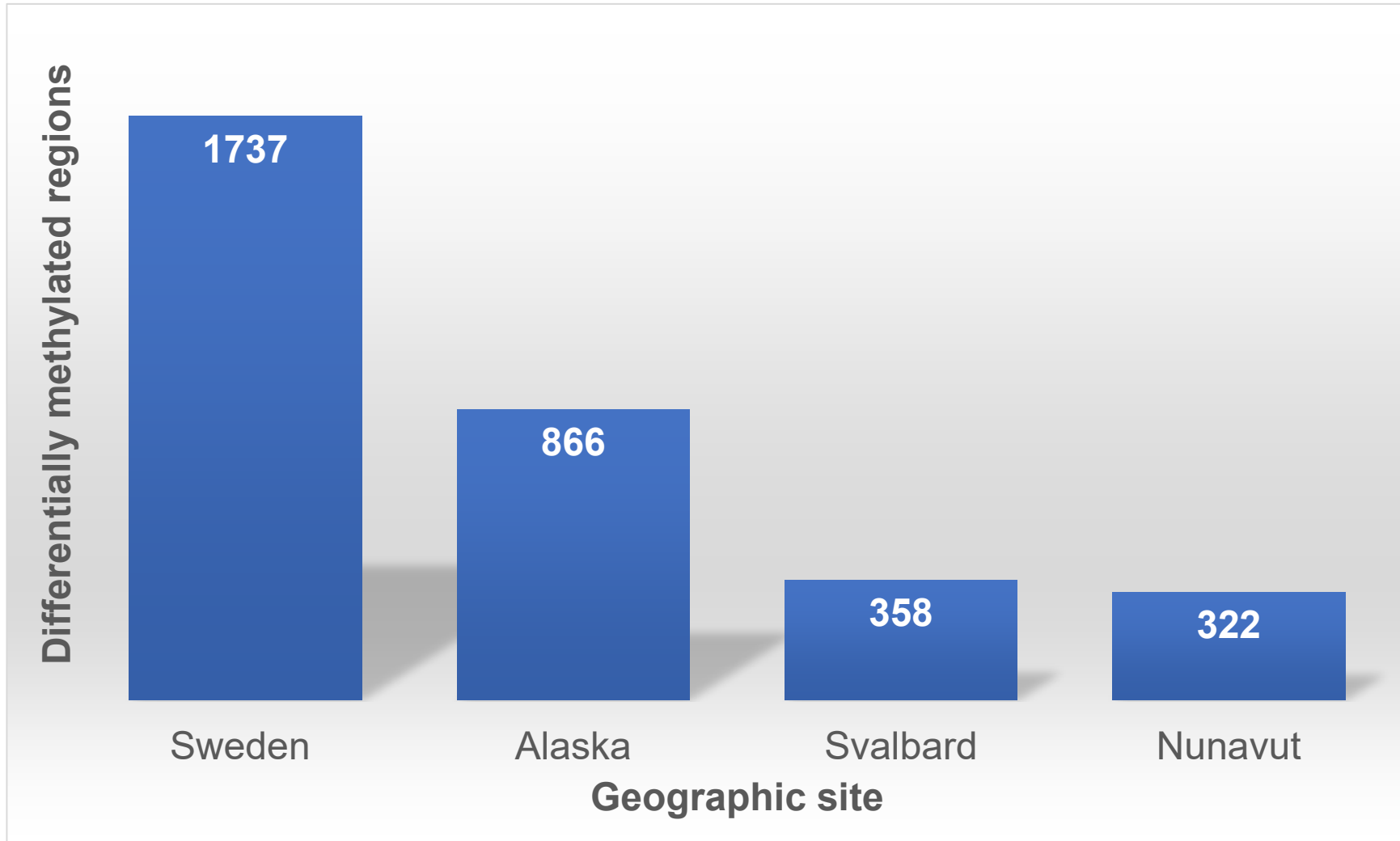
Auxin:

- Controls cell elongation in shoots
- Suppresses lateral buds
- Delays senescence
- Closes stoma for water stress
- Initiates flowering





# Geographic sites differential methylation between warmed and control



## ACROSS ALL SITES

385 DMRs

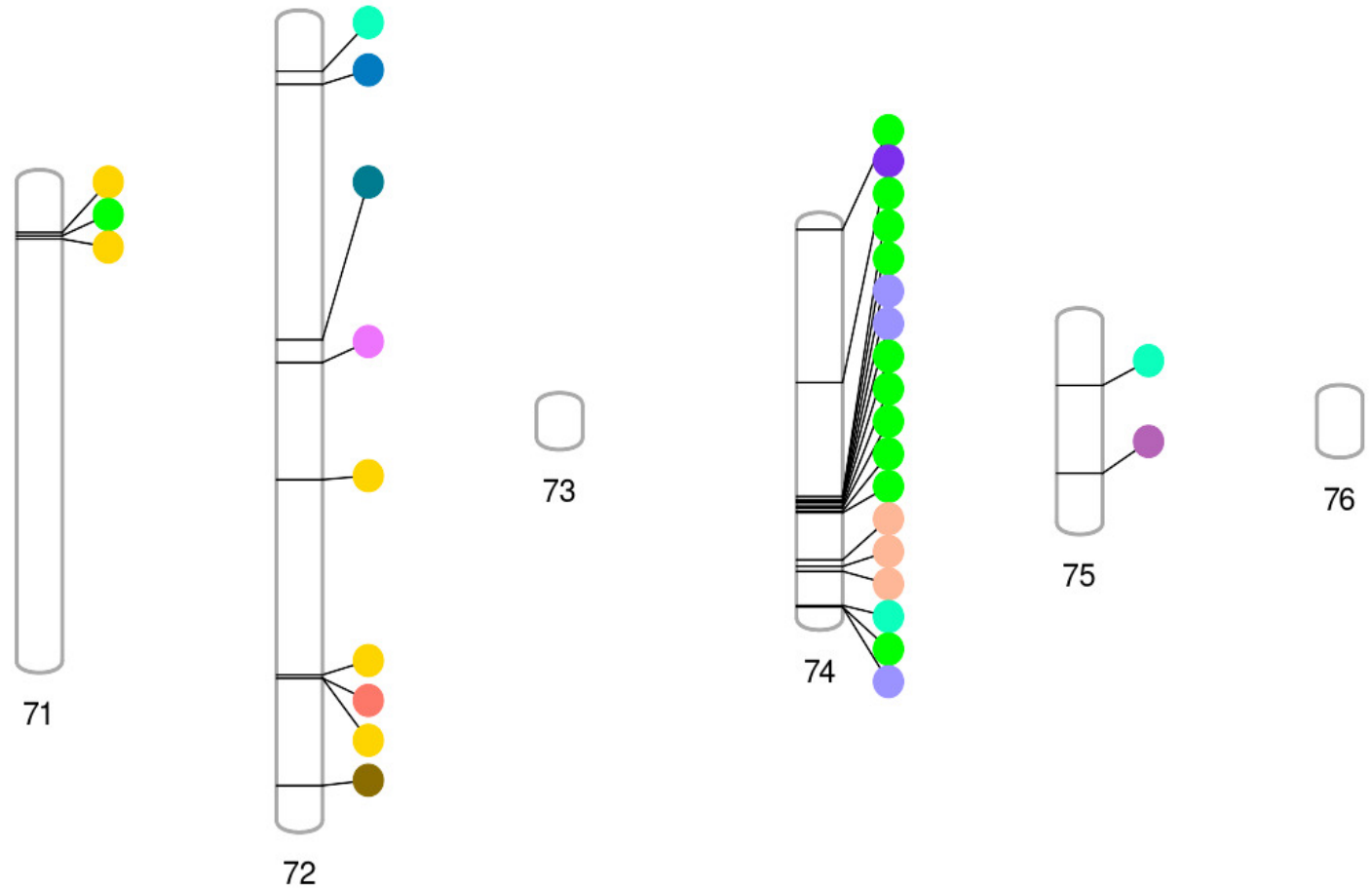
N=102

51 control and 51 warming



# Differentially methylated defense genes on chromosome 7

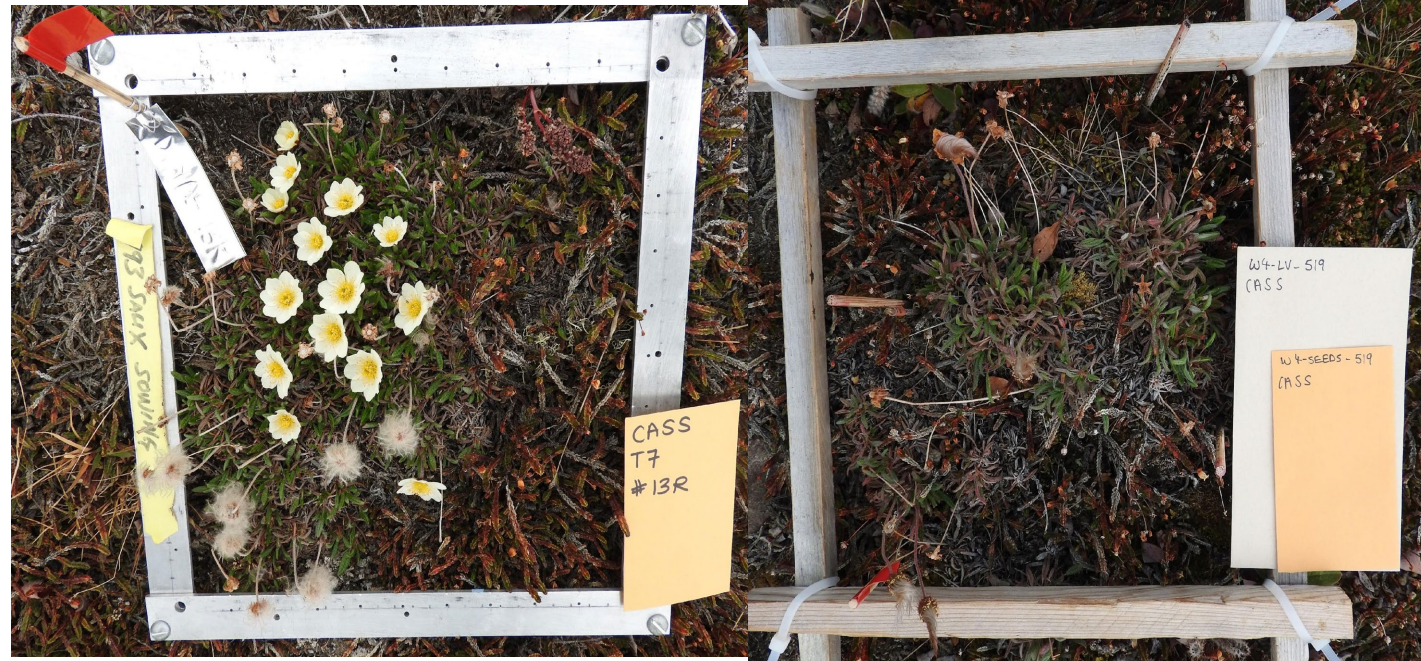
- GO:polysaccharide
- GO:defense
- GO:protein
- GO:DNA





# Phenology DMRs

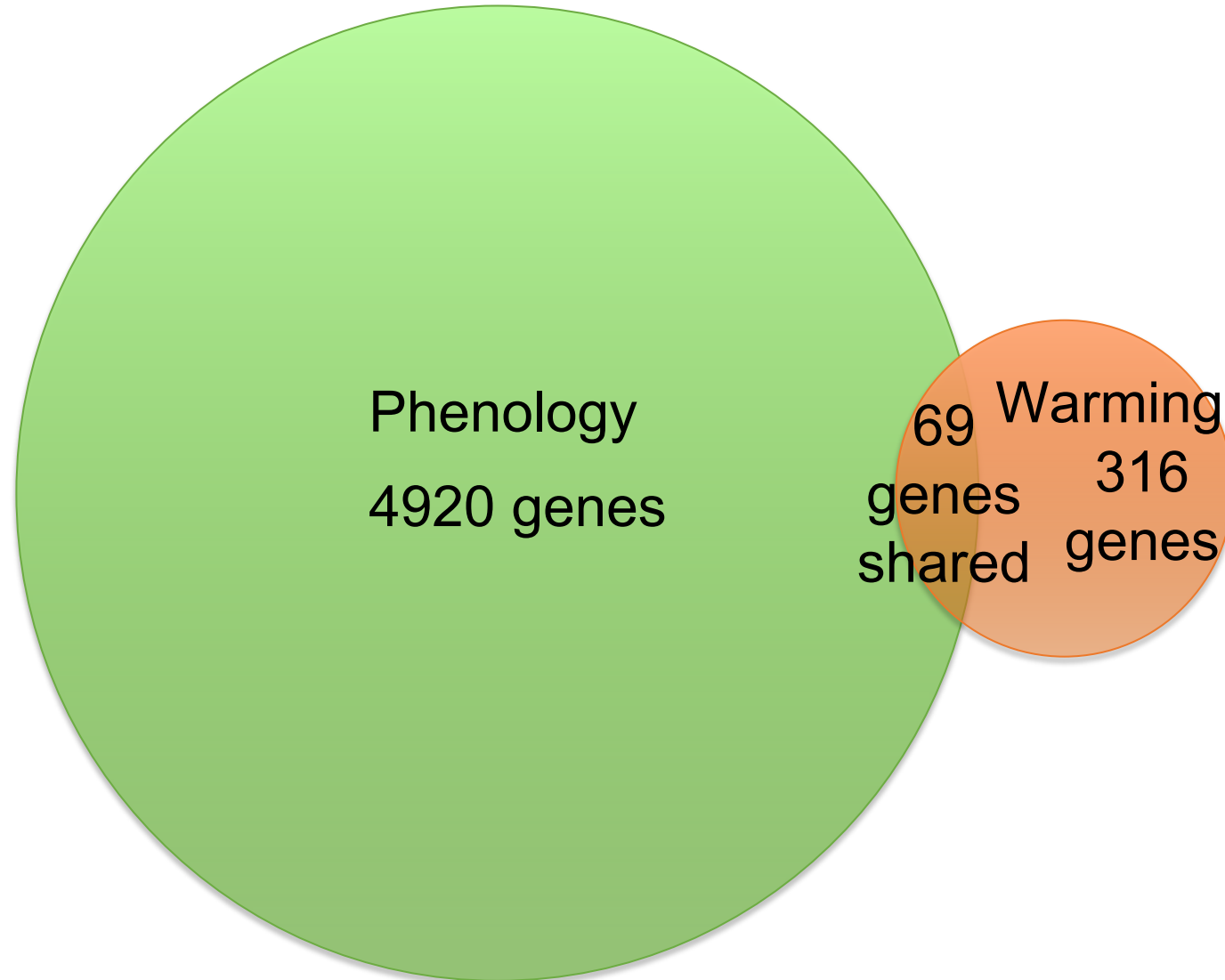
- Does differential methylation occur just due to phenology stage of sampling?
- Sampled 10 individuals from Alexandra Fiord at mature flower and senescence (5W and 5C)





# Phenology

## Differentially Methylated Regions

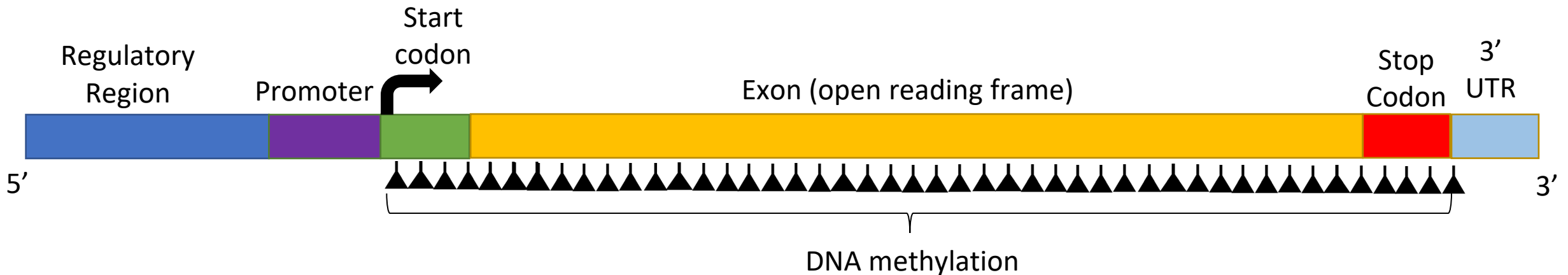






# Location of methylation: senescence protein

- Open reading frame is conserved between species and is methylated

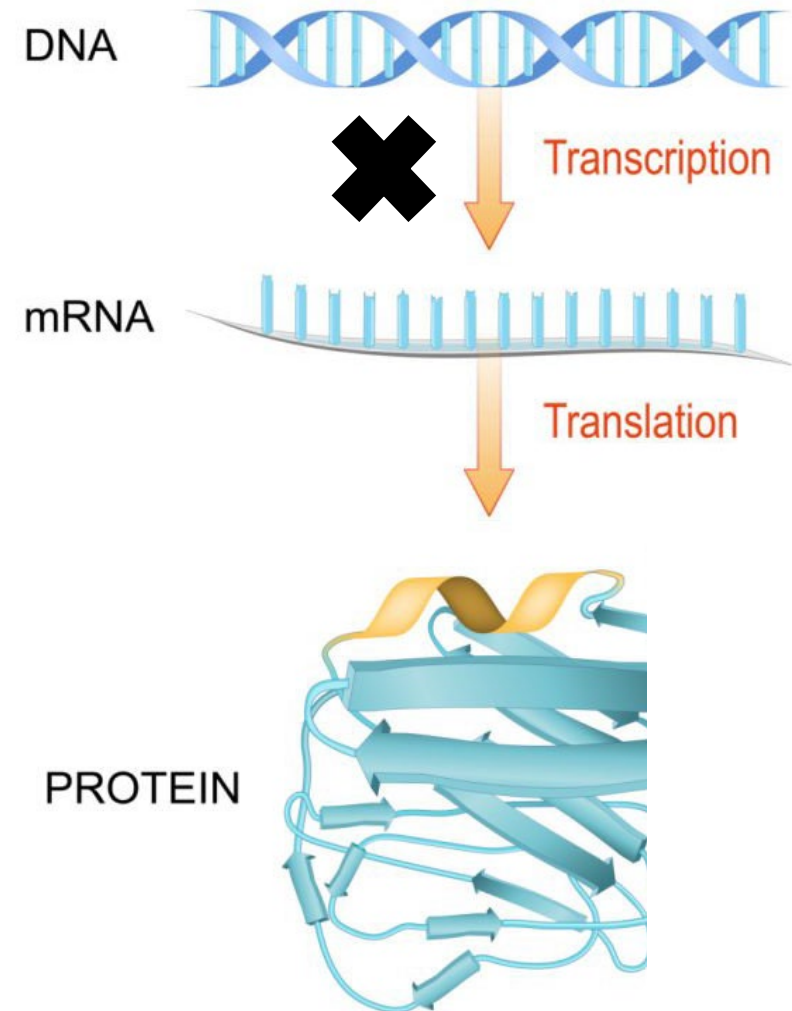




# What about changes in gene expression?

## RNAseq

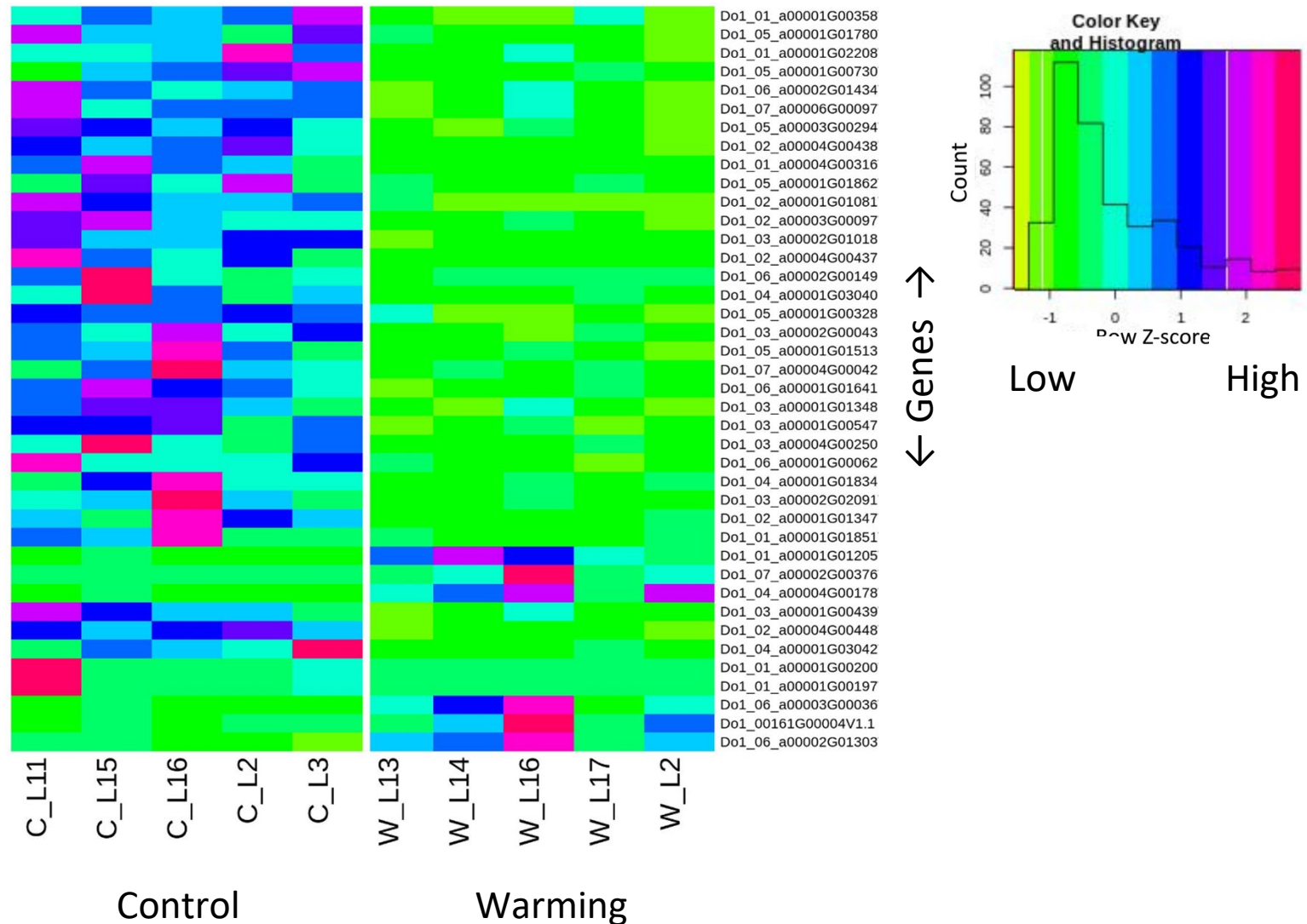
- We sequenced RNA to determine how gene expression is affected by the DNA methylation
- Expect methylation to prevent transcription











# Sweden RNAseq

- Control plants show higher expression than warming individuals across several genes
- Other sites show varying patterns but clear expression differences





# Common Garden: Will the differentially methylated regions be inherited?

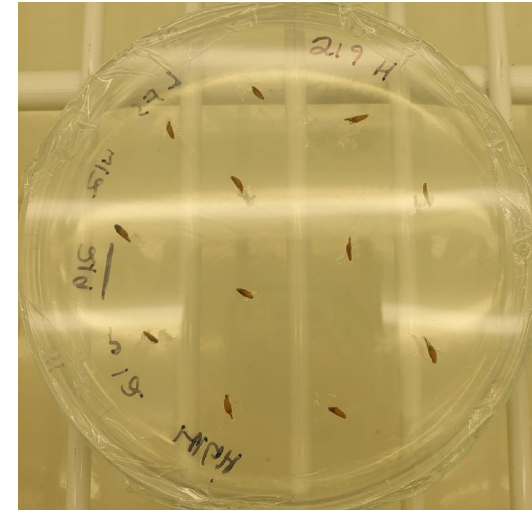
Site	Parental Warming	Parental Control
Imnavait Creek, Alaska		
Alexandra Fiord		
Latnjajaure, Sweden		

- Lots of plants died before sampling
- Started with 2000 seeds in each chamber
- Down to 18 sequenced

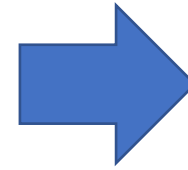


# Seedling growth

Parent plant August 2018



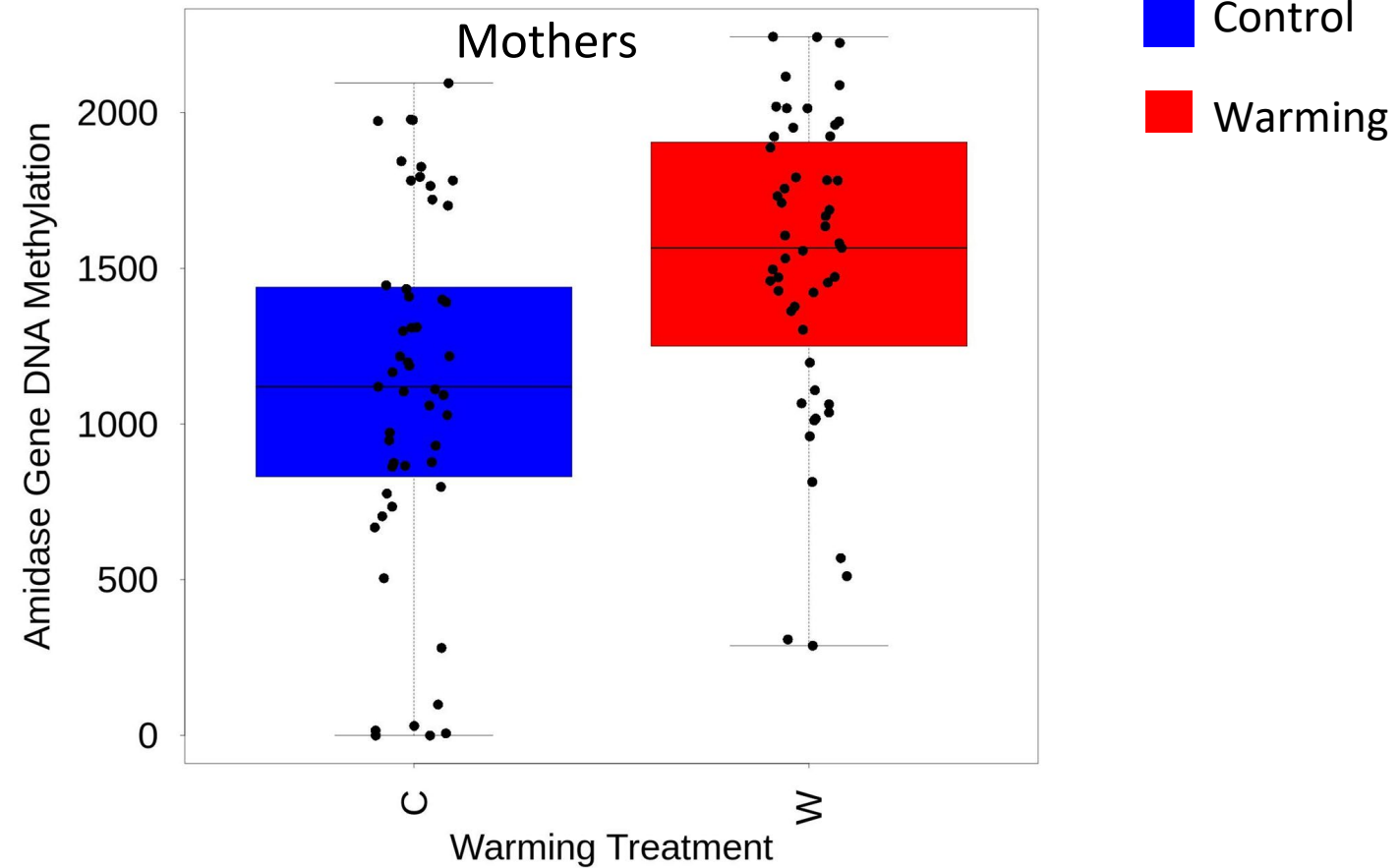
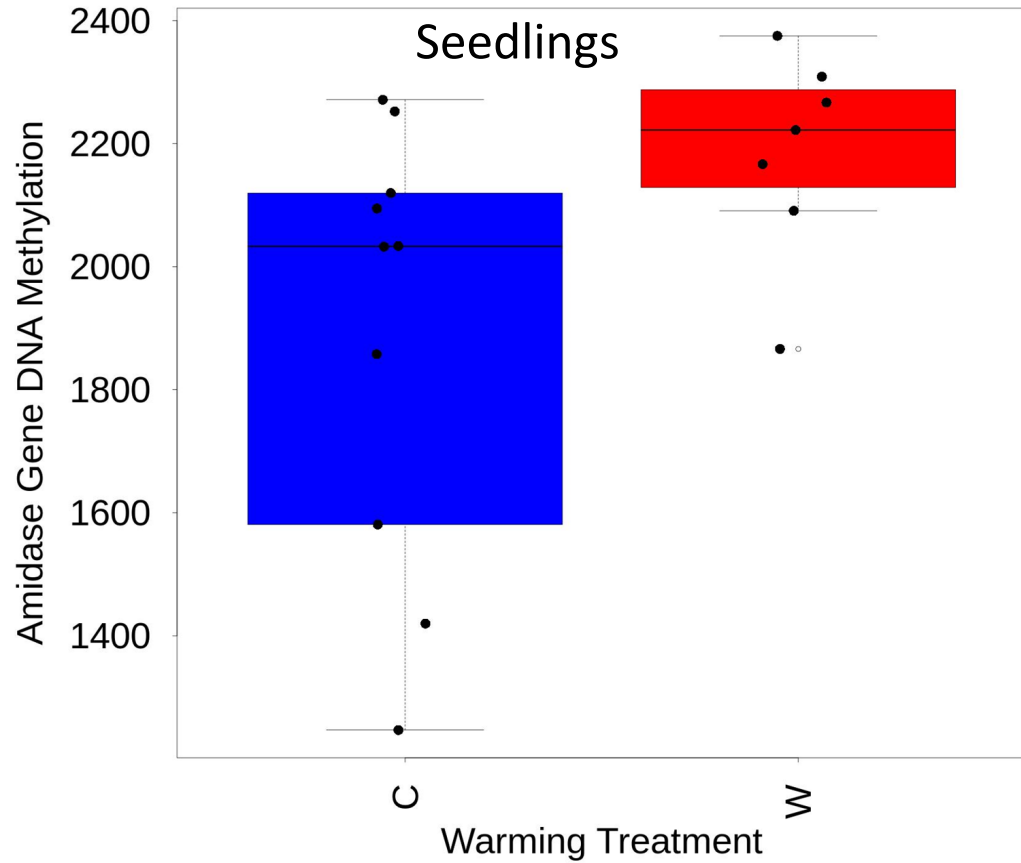
Photos by Huba Arif, Mario Rudner and Johan Martinelli



Leaves Aug 18<sup>th</sup> 2021



# Inheritance of Warming DMRs (Amidase)





# Overall Results Summary

## WARMING METHYLATION

385 differentially methylated regions in response to the warming chambers

Variation in responses with more differential methylation in low Arctic sites



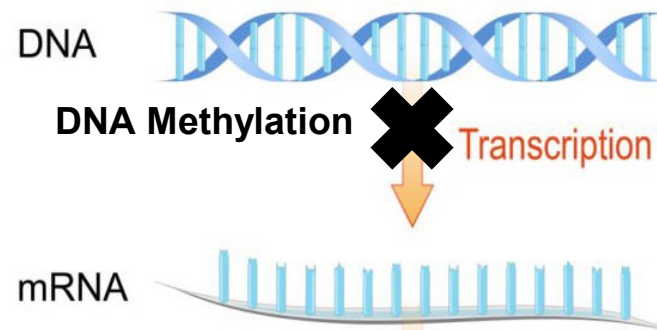
## PHENOLOGY

4920 differentially methylated regions between mature flower and senescence



## WARMING GENE EXPRESSION

Many genes are expressed differentially between warming and control



<https://www.istockphoto.com/vector/dna-rna-mrna-and-protein-synthesis-difference-between-transcription-and-translation-gm1323350905-409029650>

## INHERITANCE

Changes in DNA methylation and gene expression are inherited



Photo by Huba Arif



# Ecological significance

- Many biotic defense genes appear important responses to warming
- Geographic sites have variation in genomic responses to warming chambers
  - Stronger response in Low Arctic sites
- Some environmentally induced changes are seen in the next generation in a new environment
- Thousands of genomic regions involved in phenology changes
- Long list of genes to investigate for specific functions



(Lizzie Harper)







# Acknowledgements

Thanks to the Rieseberg Lab, field collectors and my supervisory committee!

**Supervisory committee:** Loren Rieseberg, Marco Todesco, Greg Henry, Quentin Cronk

**Rieseberg lab members who have helped a lot:**

- Marie Sandler
- Marco Todesco
- Huba Arif
- Natalia Bercovich
- Dylan Moxley
- Winnie Cheung

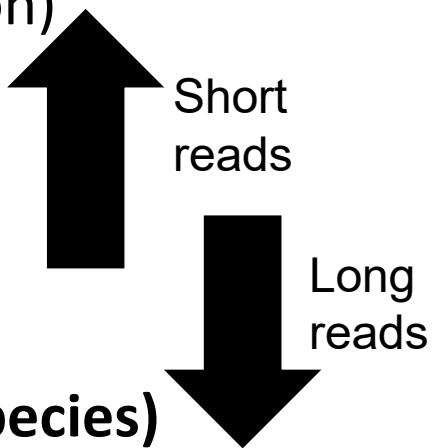
**Field collections:** Sofie Agger, Petr Macek, Jeremy May, Mats Björkman, Robert Björk, Kari Klanderud

# Increased sequencing of non-model species



Photo credit: <https://sangerinstitute.blog/2018/11/01/sequencing-all-life-on-earth-facts-and-figures/>

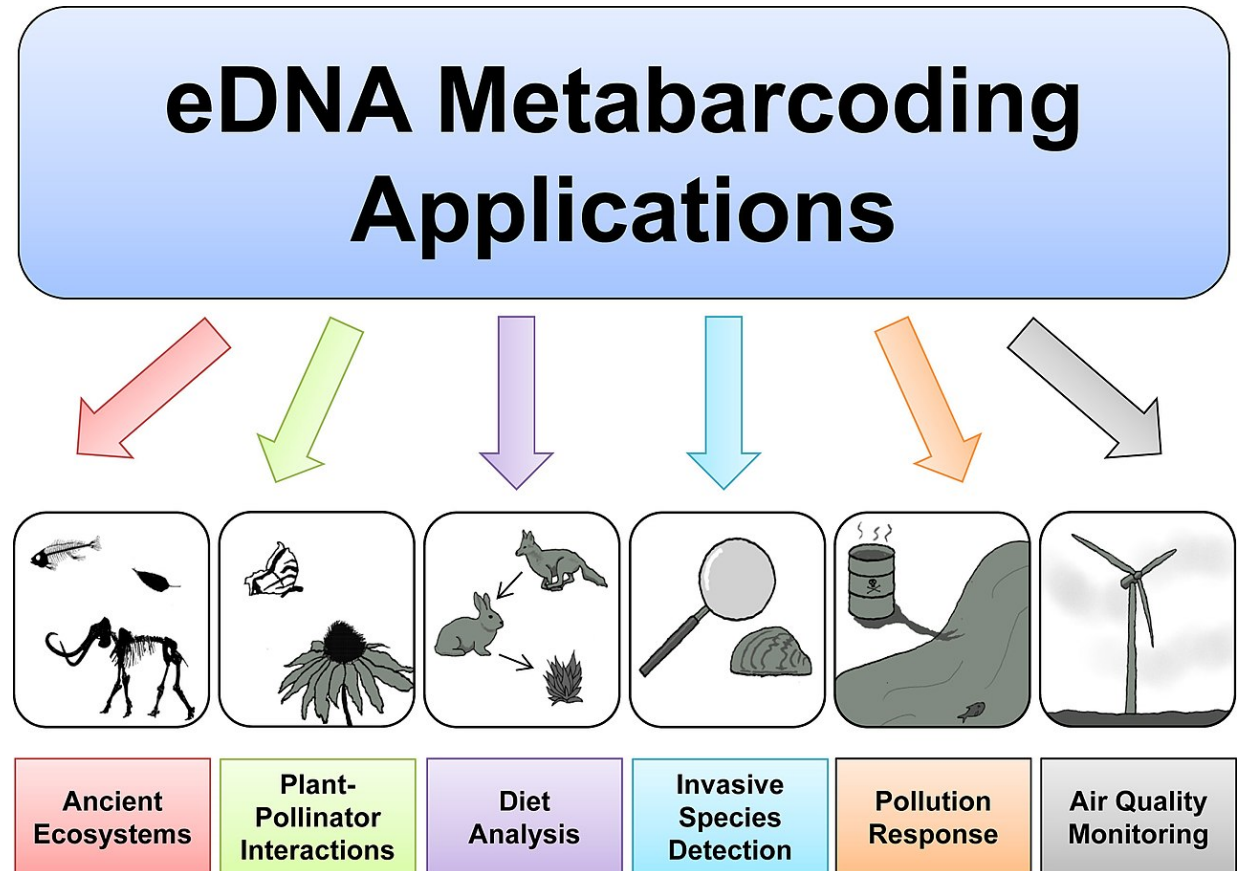
# Genomics vs genetics

- What is the difference between genetics and genomics?
  - Types of studies
    - Barcoding (eDNA, Species identification)
    - **Population genetics (biogeography)**
    - Gene editing (CRISPR)
    - **Gene expression studies (RNA)**
    - **Epigenetics (DNA methylation)**
    - **Comparative genomics (comparing species)**
- 

What types of ecological questions can these techniques address?

# Barcoding (eDNA, Species identification)

- Species diversity changes with experimental manipulations (soil, water)

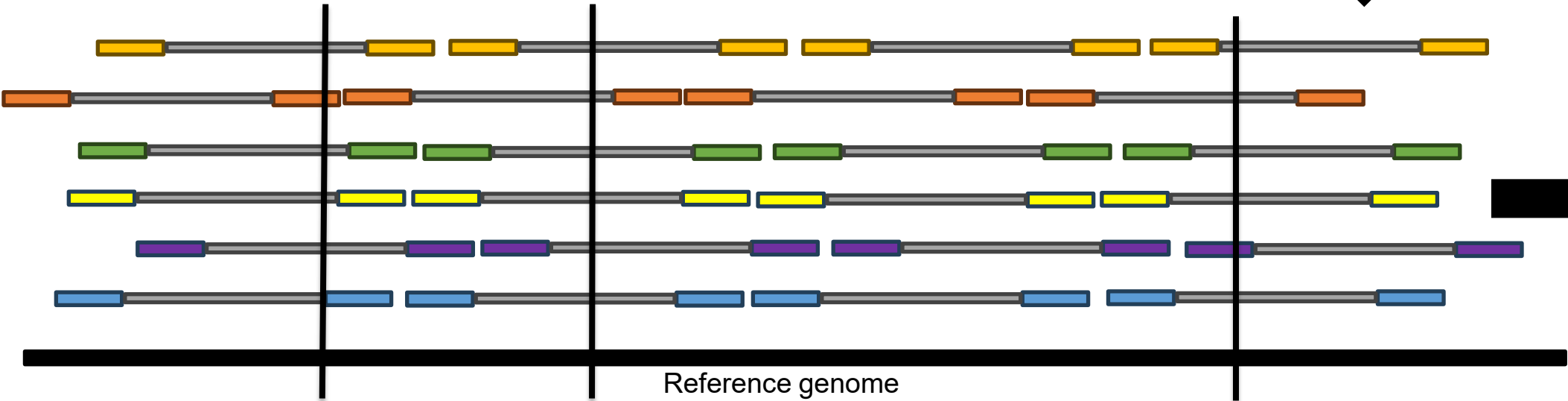
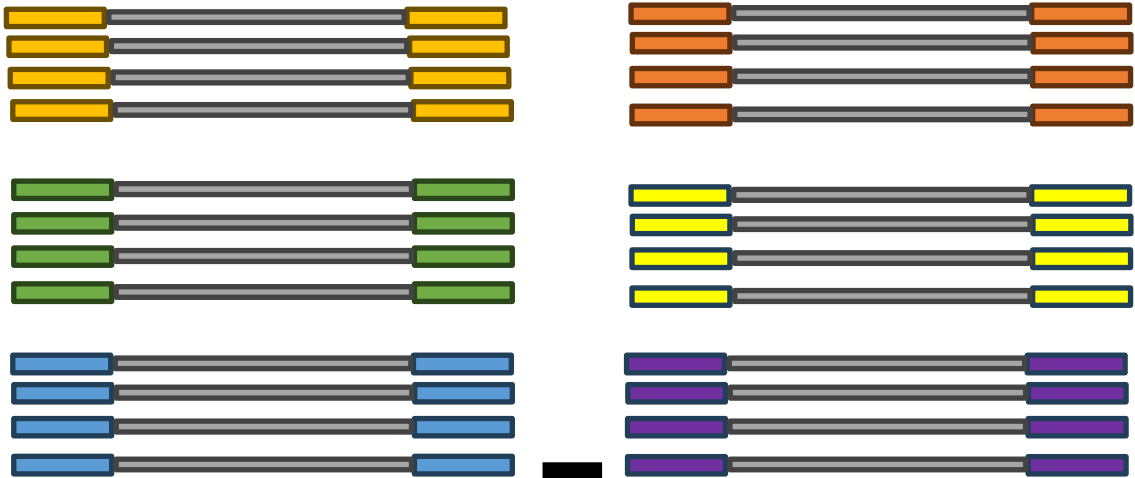


# Population genetics (biogeography)

- Biogeographic history of species
- Barriers to gene flow
- Recombination changes

# How do we detect differences between individuals?

DNA samples  
Each individual is a different  
colour to visualize



C T T  
C T T  
C T T  
T C T  
C C T  
T C T  
C C T

# Gene expression studies (RNA)

- Phenology
- Winter expression

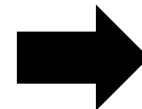
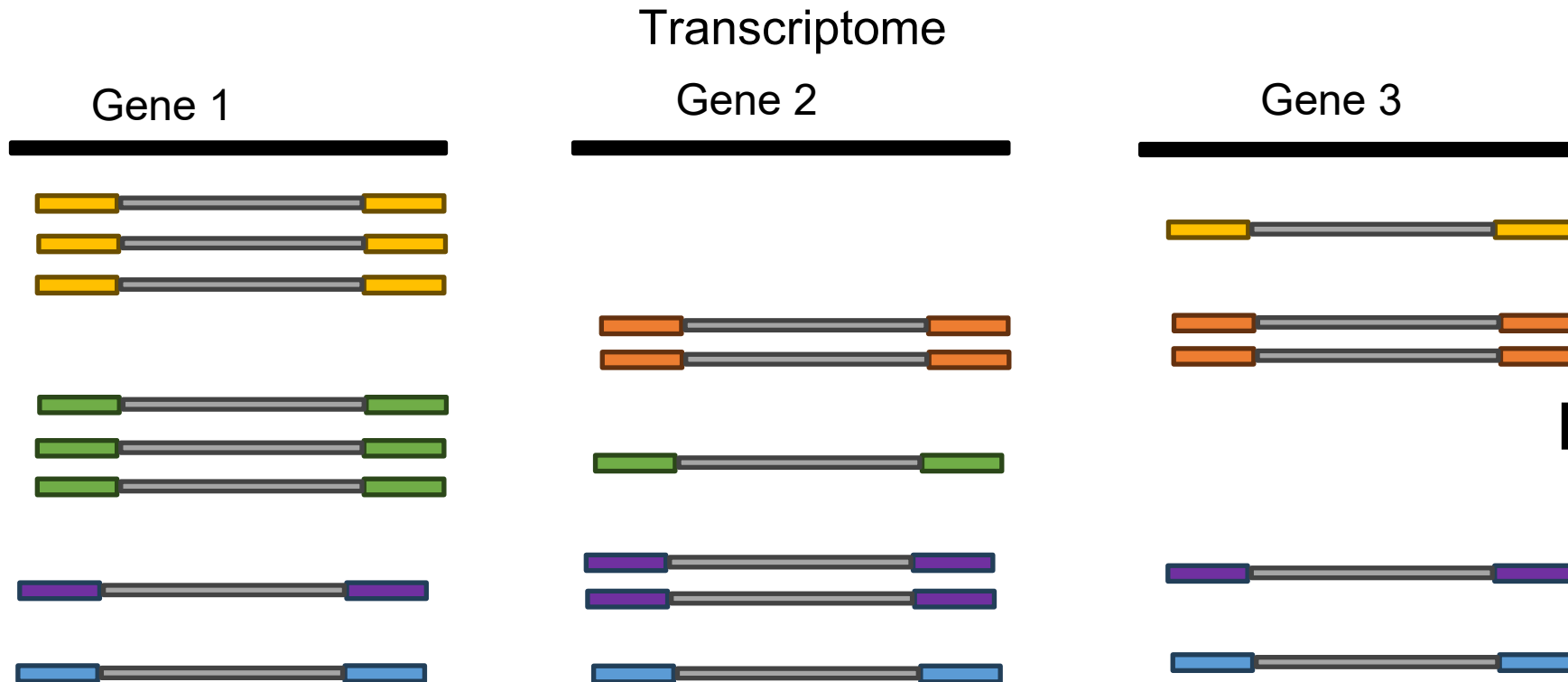




# What about changes in gene expression? RNAseq

- Sequenced long non-coding RNA and mRNA
- Ribosomal RNA depletion protocol

- After sequencing, mapped reads to transcriptome using RSEM



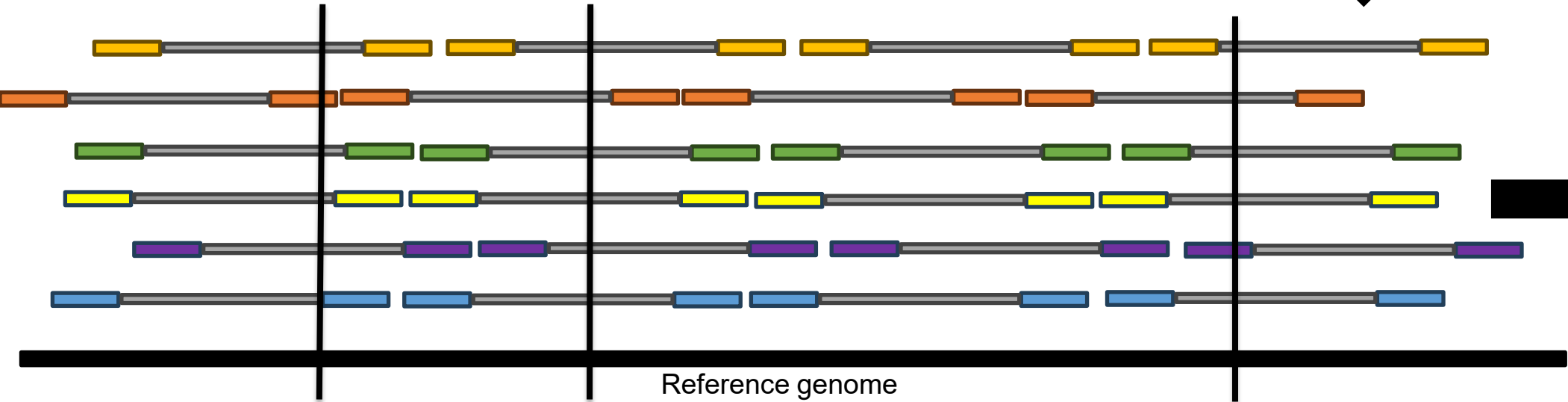
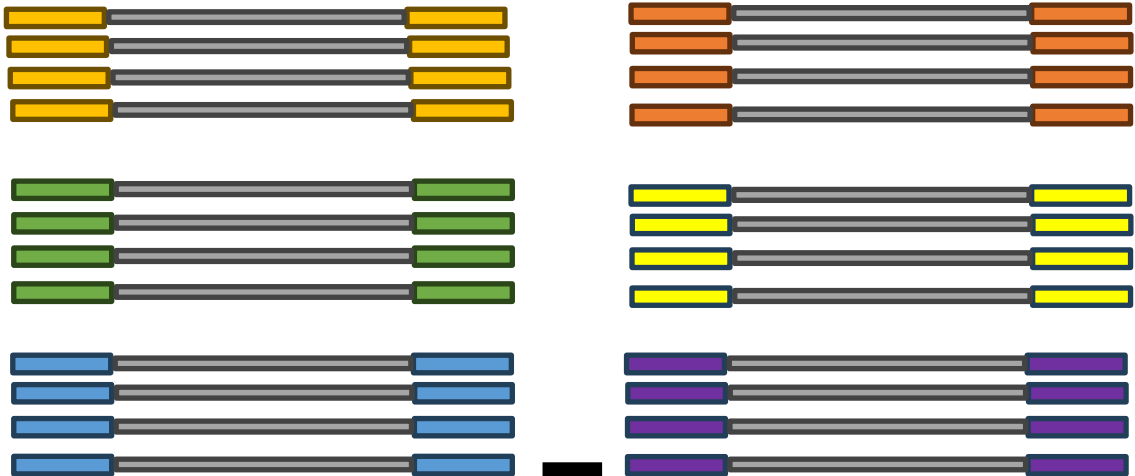
Gene 1	Gene 2	Gene 3
3	0	1
0	2	2
3	1	0
1	2	1
1	1	1

# Epigenetics (DNA methylation)

- Common gardens

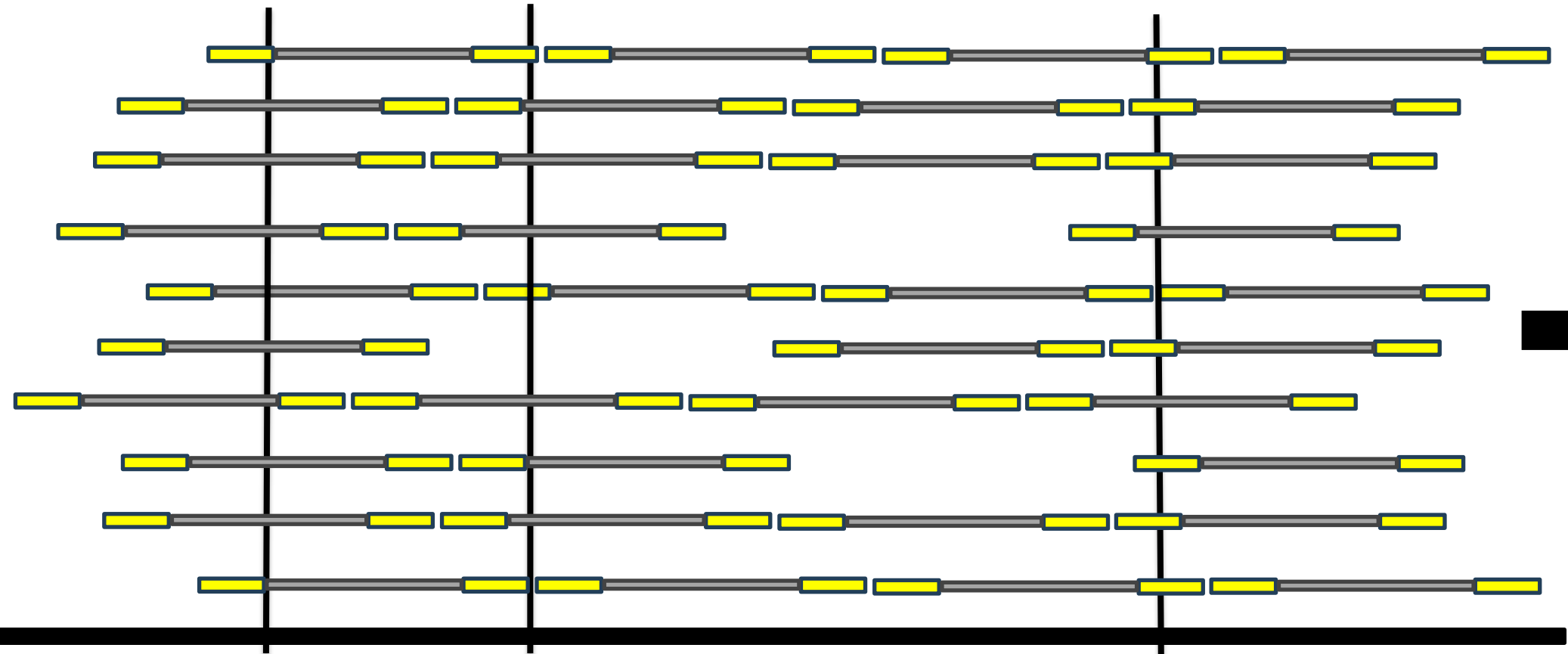
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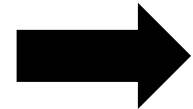


C	T	T
C	T	T
C	T	T
T	C	T
C	C	T
T	C	T
C	C	T

# Coverage (30x) and sequencing many cells



Reference genome



T	T	C
T	C	C
T	C	C
T	C	C
T	T	C
T	<b>N</b>	C
C	C	C
T	C	C
T	T	C
C	T	C
T	C	C
<b>20</b>	<b>66</b>	<b>100%</b>

# Comparative genomics (comparing species)

- High and low latitude species responses

# Making a reference genome

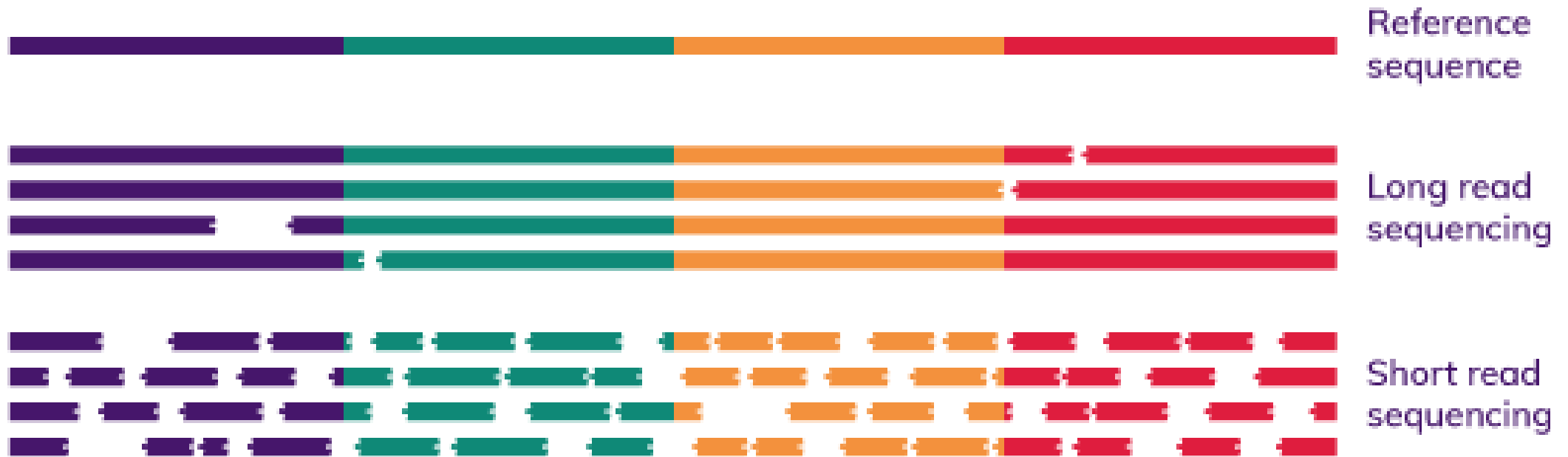


Image from:

<https://www.phgfoundation.org/briefing/clinical-long-read-sequencing>