Epigenetic Influences on Plant Responses to the Environment

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INTRODUCTION AND BACKGROUND

I am addressing the genetic basis of phenotype changes with environment (phenotypic plasticity) in *Arabidopsis thaliana. A. thaliana* is an annual plant, i.e. that grows and reproduces all within a year. Seasonal cues, such as photoperiod and temperature, tell the plant when it's time to germinate and flower. **For *A. thaliana*, the response to those cues is variable**. Plants flower in the spring in response to long days. When their seeds germinate varies among populations. Most germinate the following fall, while others spend the winter as a seed. The cold temperatures of winter experienced by overwintering seeds acts as a warning sign to hurry up and flower the following spring (Figure 1). I am examining the sequences differences among three genetic inbred strains (lines) with dramatically different sensitivities to seasonal cues and specifically the methylation patterns of their cytosines and adenines

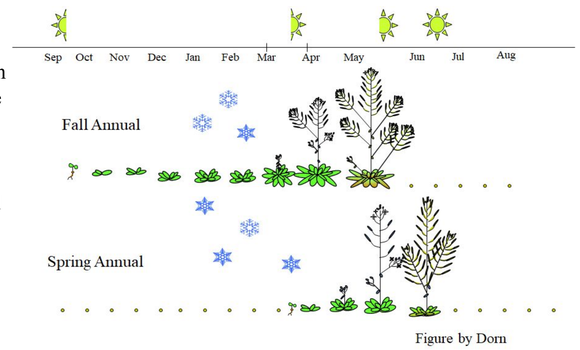

Figure 1. *Arabidopsis thaliana* life cycle

I used a set of recombinant inbred lines of *Arabidopsis thaliana.* Recombinant inbred lines (RIL) are the result of crossing two genetically uniform strains (Cal-0 & Tac), allowing recombination to occur in the resulting hybrid then self-fertilizing each recombinant offspring to make inbred lines where all lines have fixed and widely variable recombination events. Just like humans, recombination is a way for a plant to generate a new combination of genes that can affect the expression of the protein product of those genes and thus their phenotypes. To determine how recombination and methylation may have influenced gene expression and sensitivity to the environment we are sequencing 3 RILs with dramatically different phenotypic responses to cold treatment of seeds as well as gene expression responses to environment (Figure 2) along with one parental strain. The goal of this experiment is to find the rearrangements that caused phenotypic changes among the three RILs and ask if commonly methylated adenines and cytosines were important in the subsequent gene expression differences.

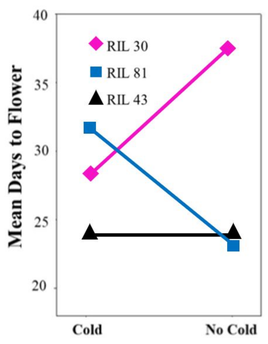

Figure 2: Reaction norm graph showing the degree and direction of phenotypic plasticity to the cold treatment (4C for 11 days) of seeds. Three kinds of plasticity to the seed treatment are shown, the sensitive negative (C-NC) line, RIL 30, sensitive positive, RIL81 and insensitive, RIL 43.

Sequencing Data Analysis

We used EPI2ME software (Metrichor) to separate all of the reads by barcode (i.e. genotype; see Figure 4). To determine how much of each chromosome we have sequenced so far, we used the TAIR 10.0 reference genome to align pooled and barcoded sequences against the chloroplast and mitochondrial genomes as well as the 5 chromosomes (Table 1A). We did the same for un-pooled sequence data, to assess how much of each chromosome was sequenced for each strain Table 1B). We used the program UGene to visualize our reads aligned to the TAIR 10.0 reference sequence (Figure 6).

Still to do:

We can use the minION flow cell for 72 more hours to collect more sequence information.

- We will continue to analyze the data for methylation patterns
- To quantify the gene expression we will perform qPCR assays on these 4 strains plus other less extreme RILs

Nanopore Sequencing

The Oxford Nanopore Technologies (ONT) minION is a portable device that plugs into a computer or phone via a USB port. It uses an ionic current that passes through each nanopore on the flow cell membrane and measures the changes in that current as DNA base pairs pass through it (Figure 3). These measurable changes in the ionic current can be used to determine what biological molecule it is, that is, what base pairs are flowing through. It sequences DNA in real time, so base calling (nucleotide identification) occurs at the same time.

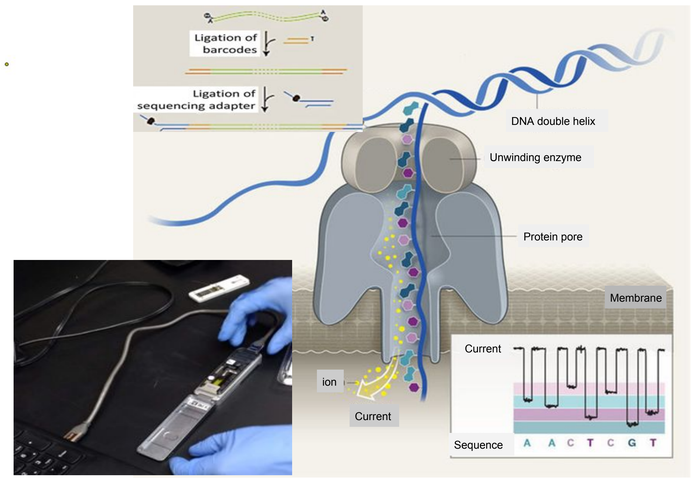

Figure 3. The ONT minION flow cell and illustration of how the minION flow cell operates. *Nature* 2017, GTEx Consortium.

Re-Sequencing Workflow

- We used the native barcoding kit from ONT (NBD104) to attach short 24 base pair nucleotide sequences to uniquely identify the DNA from 3 RILs (RIL30, RIL43, RIL 81; see Figure 2) and one parental strain (Cal-0)
- All 4 samples were pooled and sequencing adapters ligated to both ends
- Loaded on a minION flow cell that ran for 22+ hours (Figures 4 and 5)

ID	Read Count	% Total Reads
Cal	9,131	11.5%
RIL30	16,044	20.2%
RIL43	8,407	10.6%
RIL80	6,862	8.6%
None	38,894	49%
	TOTAL READS =79,342	

Figure 4 The number of chunks of DNA (i.e. reads) that were sequenced by the minION for the 4 strains.

A.

Sequence ID	% Alignments
Chloroplast	17.6%
Chr1	16.5%
Chr2	13%
Chr3	14%
Chr4	10.5%
Chr5	15%
Mito	4%

B.

	Cal-0	RIL30	RIL43	RIL81
Chr1	16.6%	16.5%	17.0%	16.5%
Chr2	13.6%	12.2%	12.0%	14.6%
Chr3	14.2%	13.5%	13.9%	14.1%
Chr4	10.1%	10.4%	10.8%	10.9%
Chr5	15.5%	14.8%	15.1%	15.1%
Total Reads	**8,994**	**15,722**	**8,310**	**6,495**

Table 1. A. The percentage of reads for the pooled sequences found on each chromosome, along with chloroplast and mitochondrial DNA. **B.** The percentage of reads per RIL found on each chromosome.

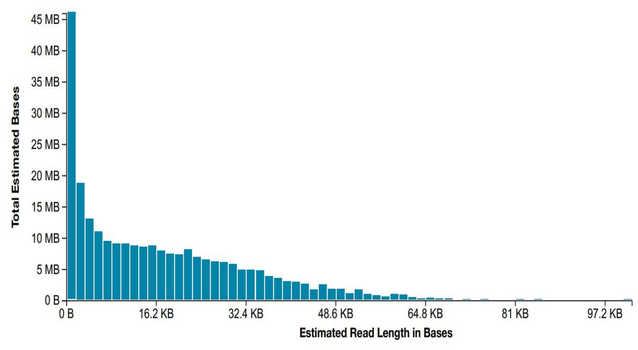

Figure 5. The distribution of the lengths of each read sequenced in the 22 hr. run.

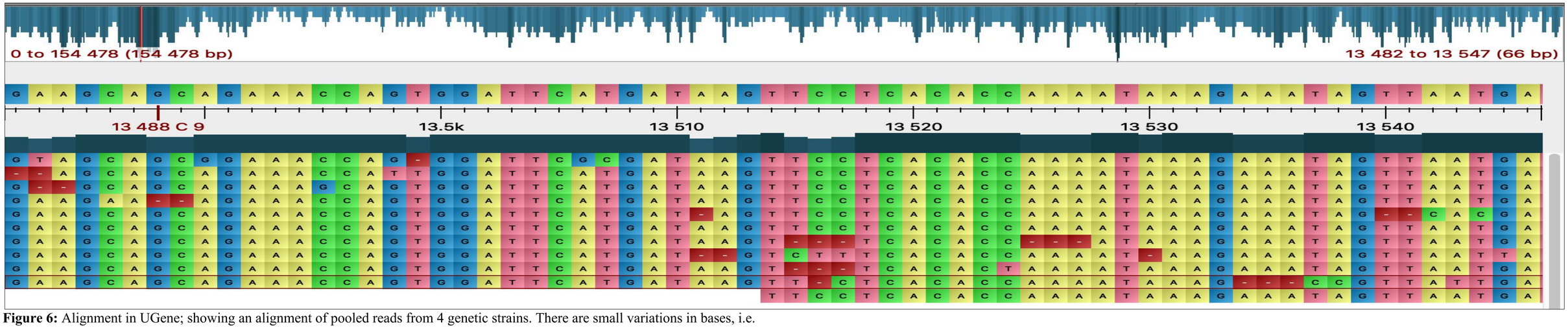

Figure 6: Alignment in UGene; showing an alignment of pooled reads from 4 genetic strains. There are small variations in bases, i.e. possible single base pair polymorphisms.

Acknowledgments

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