

# A Pathway to the Exploitation of Epigenetic Variation in UK, US and International Breeding Programmes

**Project Leads:** Anthony Hall, Earlham Institute, GBR & Eduard Akhunov, Kansas State University, USA

## Project Description

1. Develop a platform to analyze the methylation state of agronomically important wheat genes. Within this project we aim to characterize the methylation state across the entire regulatory and coding region of a subset of gene over a large number of cultivars. Through our collaboration with CIMMYT, the International Wheat yield Partnership (IWYP), NIFA-IWYP and national genetic improvement networks we have identified a set of genes controlling adaptive traits relevant to national and International breeding programs. We will utilize the targeted capture and bisulfite treatment and sequencing approach we have developed, Methyl-seq5 and would aim to sequence across 500 genes and their homoeologous copies.

**Goal:** Design, build and test capture probe set for 500 agronomically important genes and automate experimental protocol for methyl-seq

2. Survey variation in epigenetic state across UK, US and international elite cultivars. The aim will be to use the Methyl-Seq approach to investigate the methylation state of agronomically important genes across 96 US, 96 UK and 96 CIMMYT cultivars that have already been extensively phenotyped for adaptive agronomic traits (development, phenology, disease resistance, drought and heat tolerance, and yield) in the US and UK IWYP projects. Cultivars will be selected in consultation with breeders and on the amount of pre-existing genotype and pedigree data for a line. Cultivars will be specifically chosen to allow us to test epi-allele stability and inheritance, while providing a broad range of phenotypes. For example, for the UK cultivars, highly related lines would be selected with common parents but also winter and spring cultivars and cultivars with different qualities. For the CIMMYT cultivars we would select synthetic and non-synthetic material adapted to different mega environments.

**Goal:** Methylation state of 500 genes across 288 elite cultivars displayed in a searchable database, searchable by cultivar or gene.

3. Uncovering inheritance and stability of epi-allele and their role in adaptation. By choosing lines with well understood pedigrees it should be possible to follow epi-alleles through breeder's crosses and establish inheritance and stability. It will allow us to address important questions that breeders are asking: Does epi-allele simply follow haplotypes? Is methylation gained and lost from a haplotype block? Have existing breeding programs already been selecting epi-alleles? By carefully selecting cultivars with well understood pedigrees we should be able to address these questions. In addition, by choosing cultivars adapted to different mega-environments we should be able to identify whether methylation is playing a role in adaptation. In addition to helping us understand basic rules of inheritance, stability and adaptation of epi-alleles, this work should identify valuable epi-alleles that may be introduced into new cultivars or be used by researchers to confirm the role of an epi-allele in controlling differential gene-expression and phenotypic traits.

**Goal:** Understand inheritance, stability and role in adaptation of epi-alleles in wheat

4. Develop an epi-allele marker system and test the role of epi-alleles in controlling gene-expression and phenotypic variation. We already have evidence that differential methylation associates with altered gene-expression and ion-content from our work in the Watkins collection. In aim 3 we should identify epi-allele variation for some of our genes and be in a position to assess their role in controlling phenotypic variation. To test these hypotheses, we will develop a high throughput assay with LGC to track epi-alleles and use existing genetic resources to correlate a specific epi-allele with changes in gene expression and phenotype.

**Goal.** The development of an enabling technology to track epi-alleles through crosses and over generations in collaboration with LGC. Use existing genetic resources as a proof of concept to demonstrate epi-allele effects on agricultural traits.