A FRAMEWORK FOR EPIGENETIC AND TRANSCRIPTOMIC DATA INTEGRATION TO ANALYSE DROUGHT STRESS

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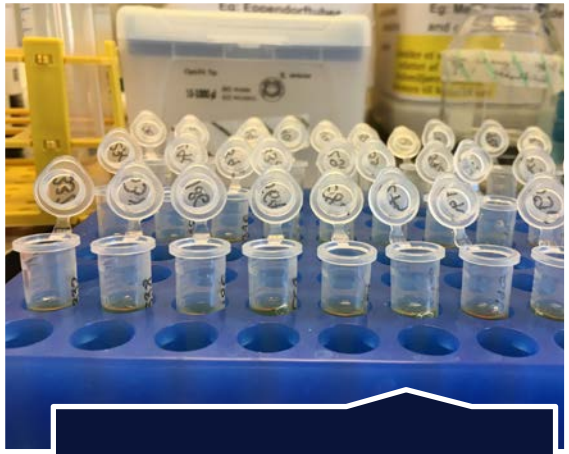
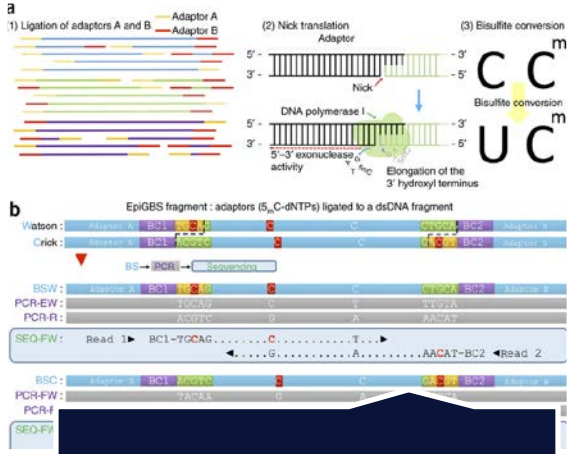
DEPARTMENT OF MOLECULAR BIOLOGY AND GENETICS

PLAN OF THE PRESENTATION

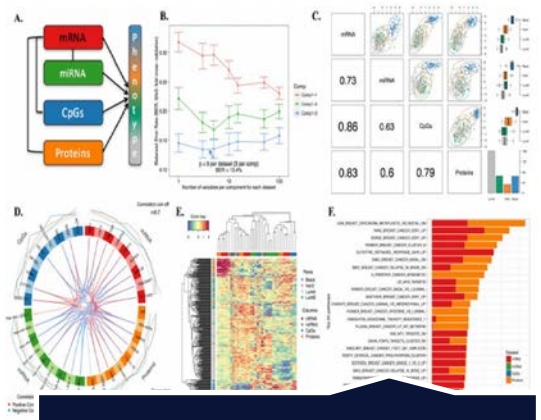
Background



Progress



epiGBS

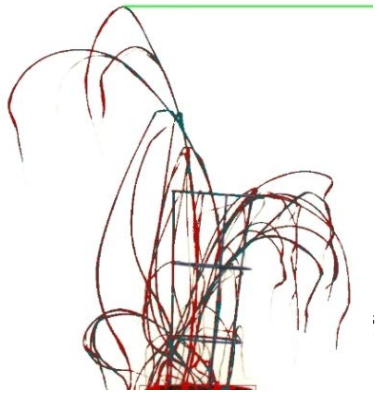


Next 6 months

ABOUT MYSELF...

CV

- Molecular biology
- Drought stress in the perennial biomass crop
- Screening a diverse collection of genotypes for the drought response
- Analysis and integration of the high dimensional data-sets
- Phenomics
- Gene level analysis



SO FAR SO GOOD...

SAMPLING

- Barley – June
  - 7 flag leaves x 600 samples (75 lines; 4 replicates, 2 treatments: Control + Drought)
- Ryegrass – August
  - 15 blades x 1200 samples (262 lines; 2 replicates; 2 treatments: Control + Drought)

Ryegrass on 26th of August 2017

RYEGRASS SAMPLING

Collective effort with DLF staff

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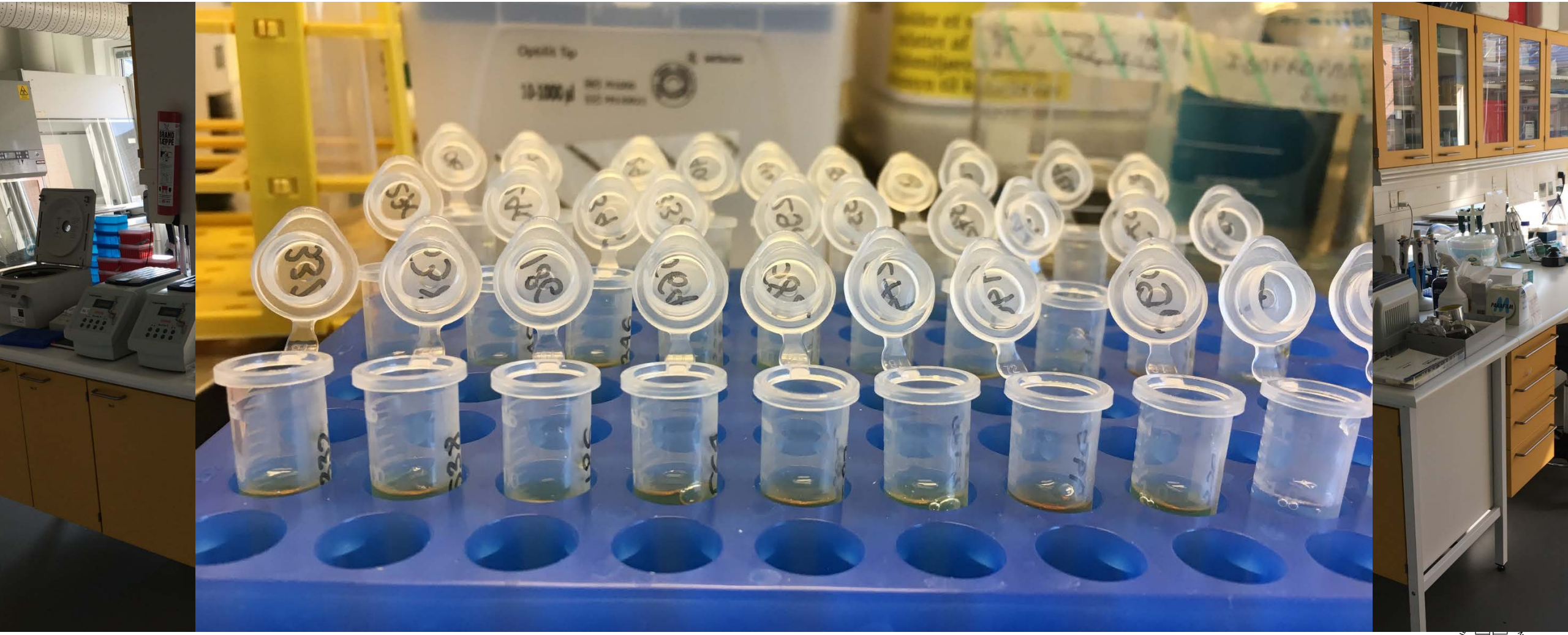
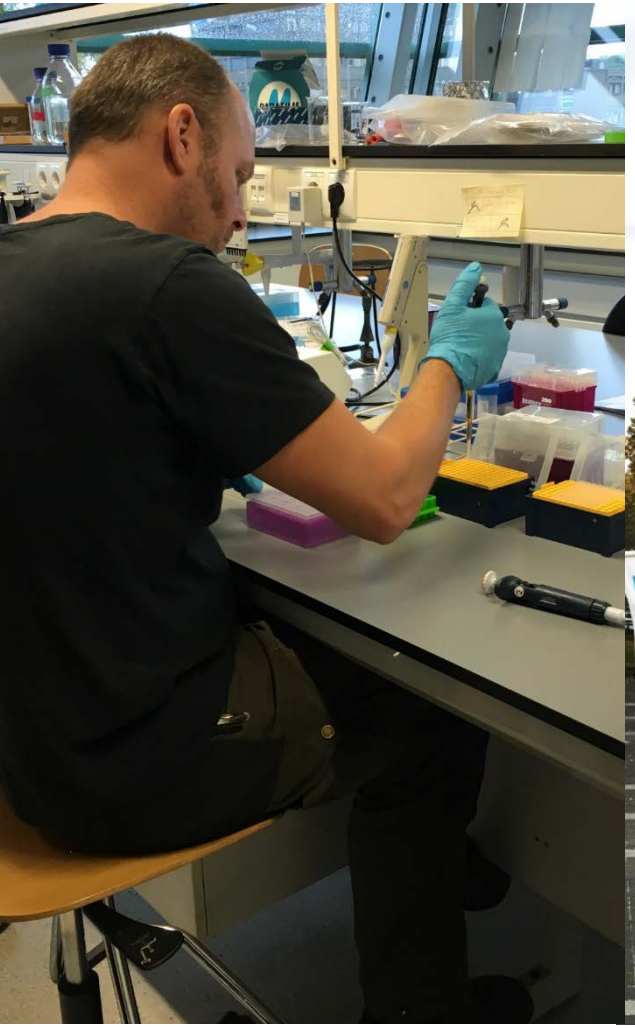
DNA AND RNA ISOLATION



RADBOUD UNIVERSITY VISIT



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POSTDOC

epiGBS library preparation with
N. Wagemaker, Nijmegen

EPIGENETIC CONTROL BY DNA METHYLATION AT CYTOSINES...

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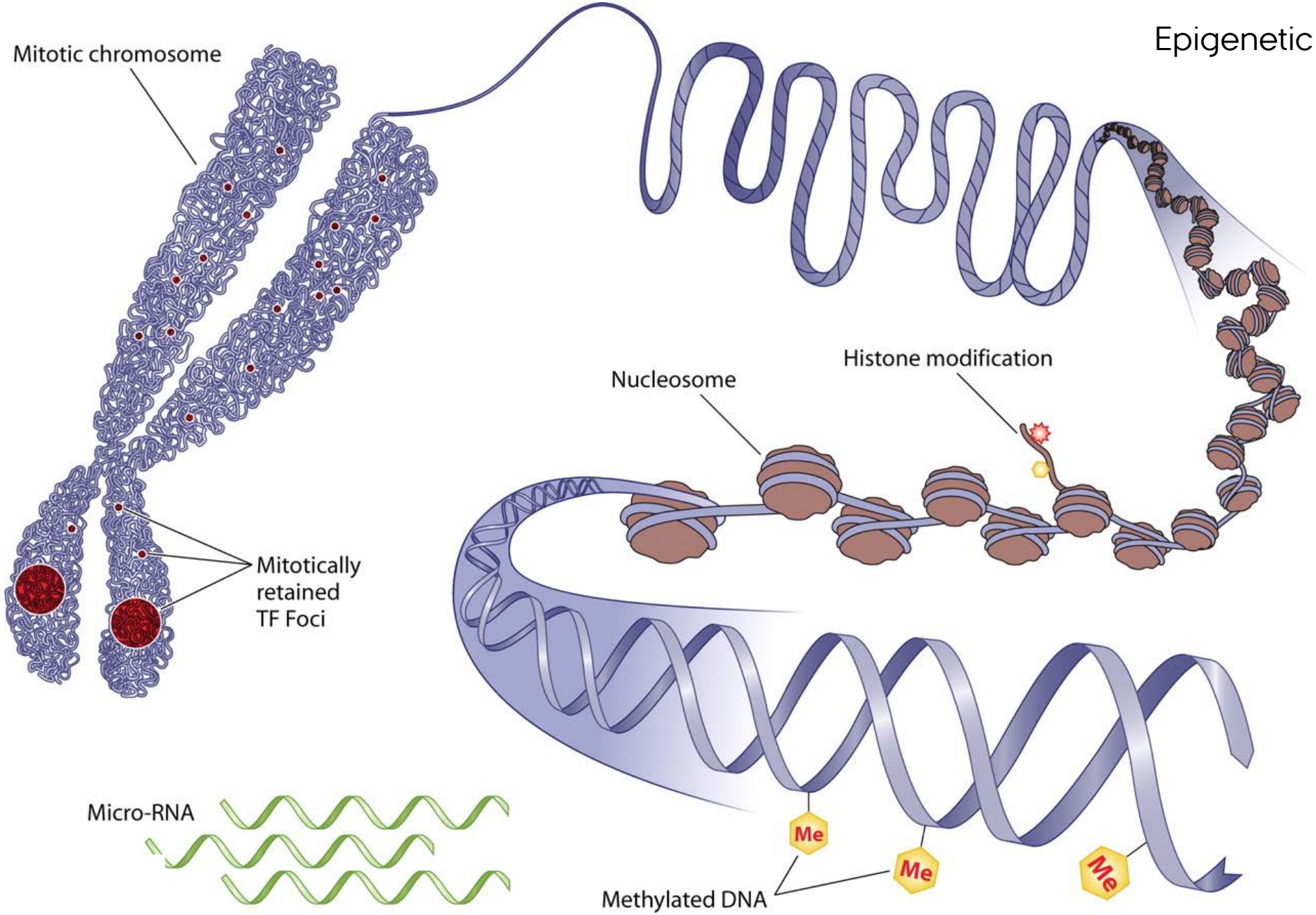
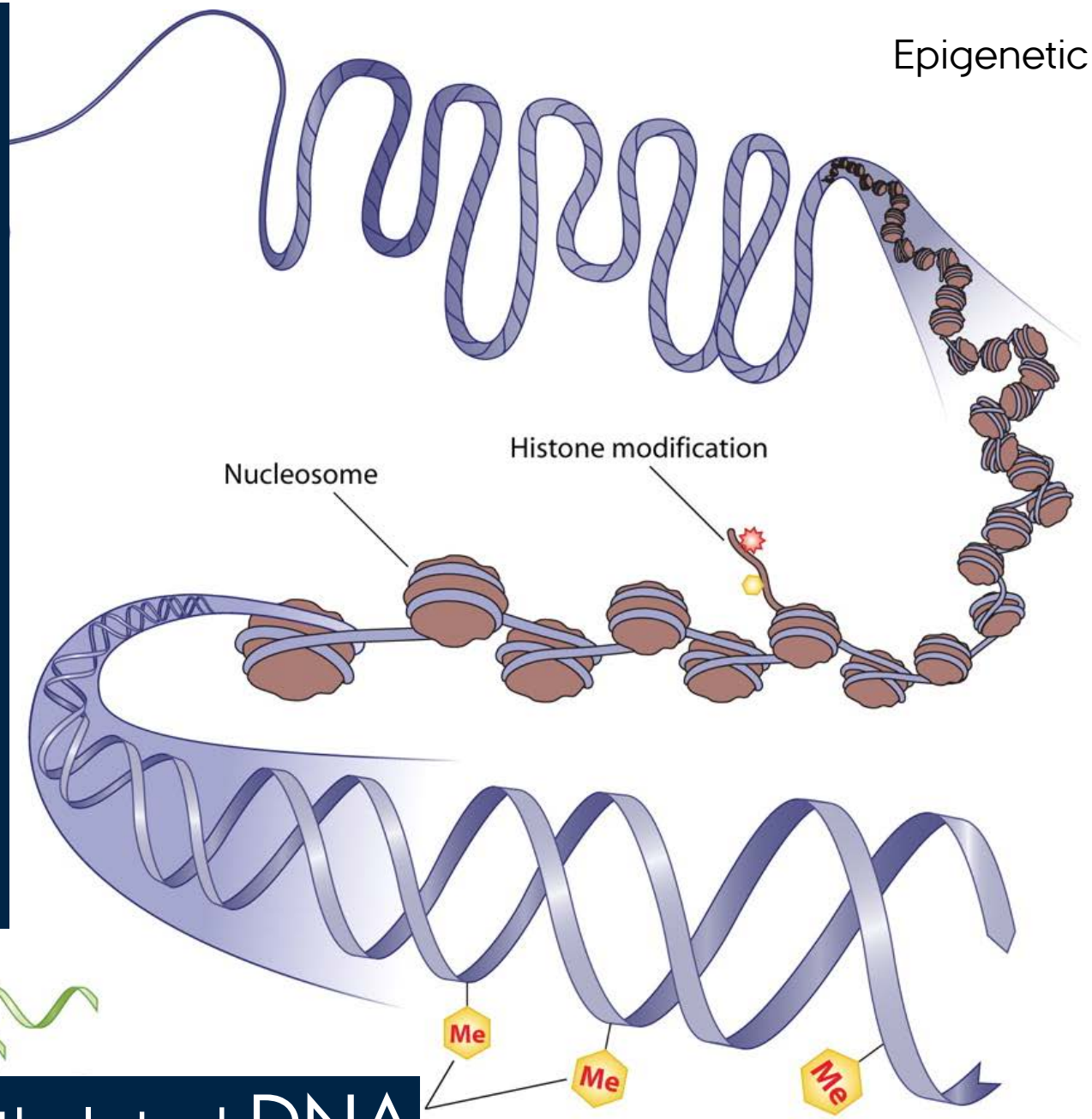
Epigenetic modifications



Zaidi et al. (2010) Molecular and Cellular Biology

Dynamical regulation of the DNA methylation, shaping the phenotype, and determining the physiological resilience of analysed plants in response to drought stress

Epigenetic modifications

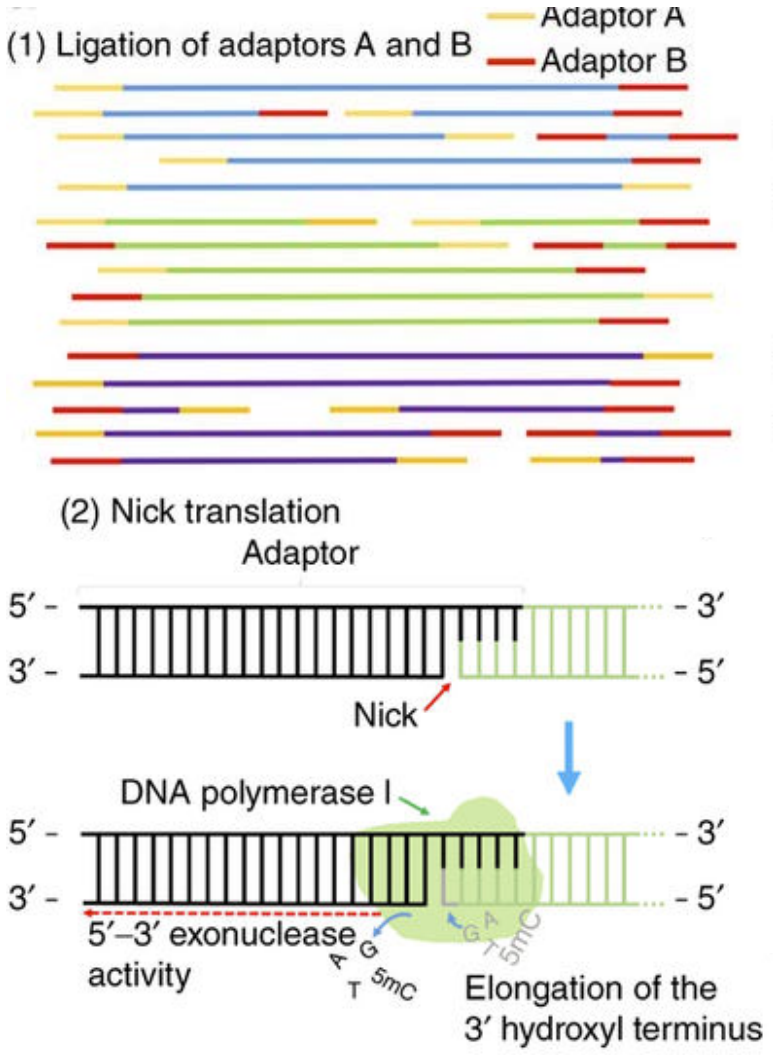


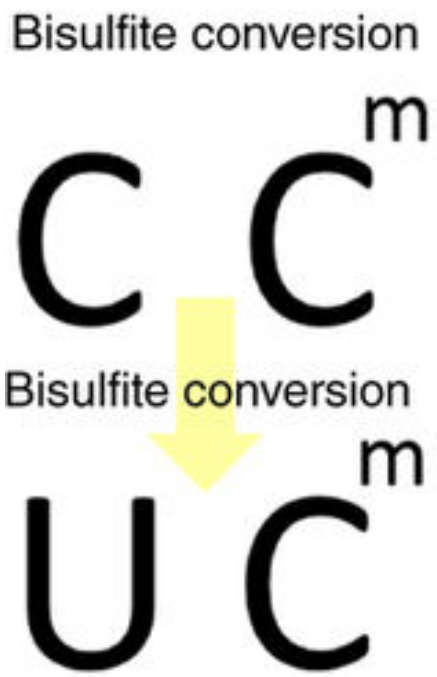
Methylated DNA

Zaidi et al. (2010) Molecular and Cellular Biology

BISULFITE SEQUENCING METHOD DESIGN

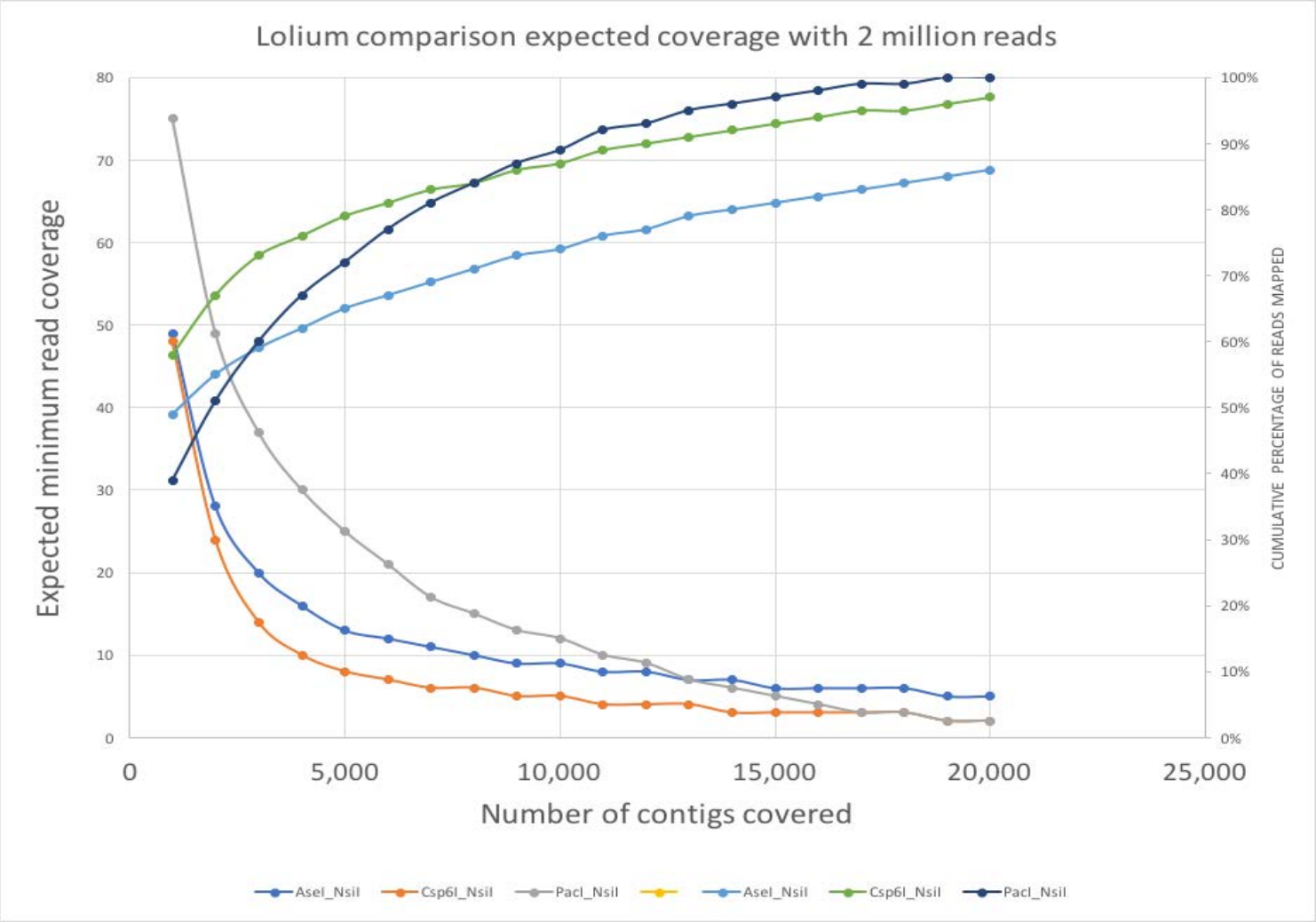
- DNA digestion with selected restriction enzymes
- Adaptor ligation
- Purification and size selection
- Nick translation
- Bisulfite treatment
- PCR amplification to yield a sequencing library





van Gurp et al. (2016) Nature Methods

SELECTION OF THE RESTRICTION ENZYMES



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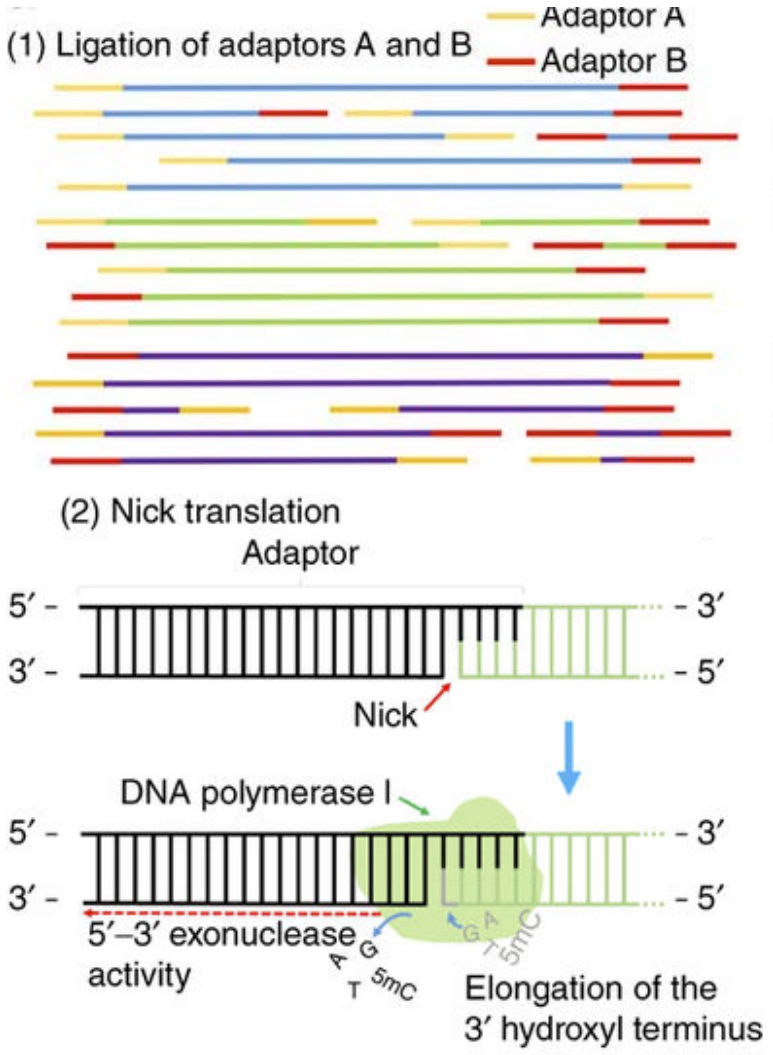

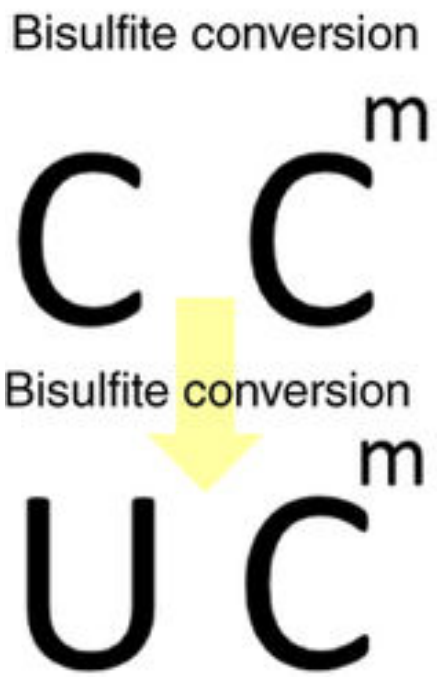

van Gurp et al. (2016) Nature Methods

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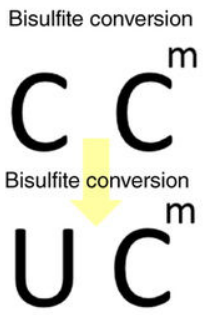
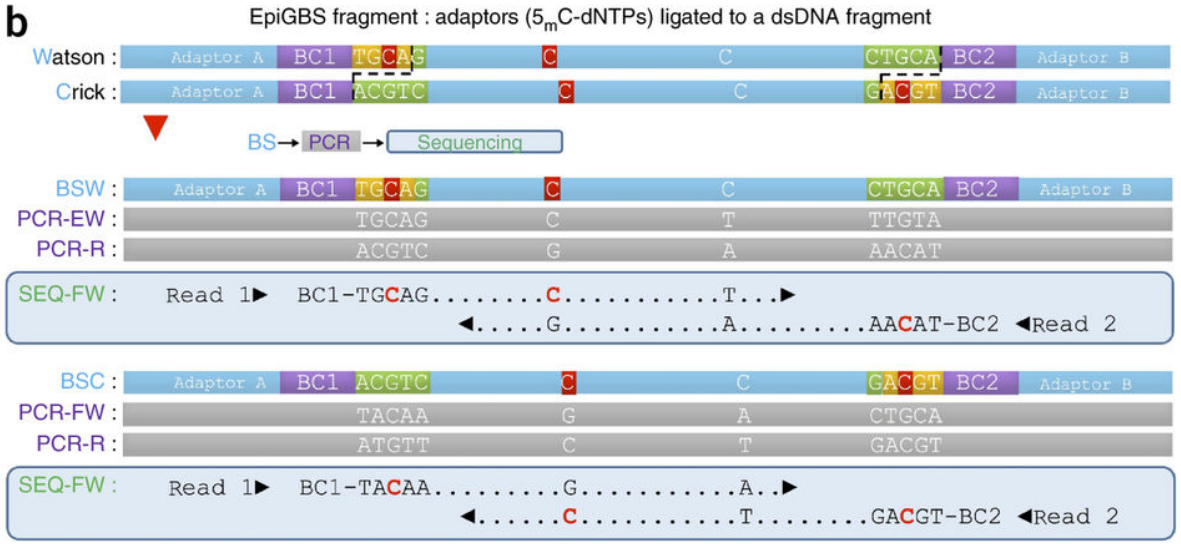
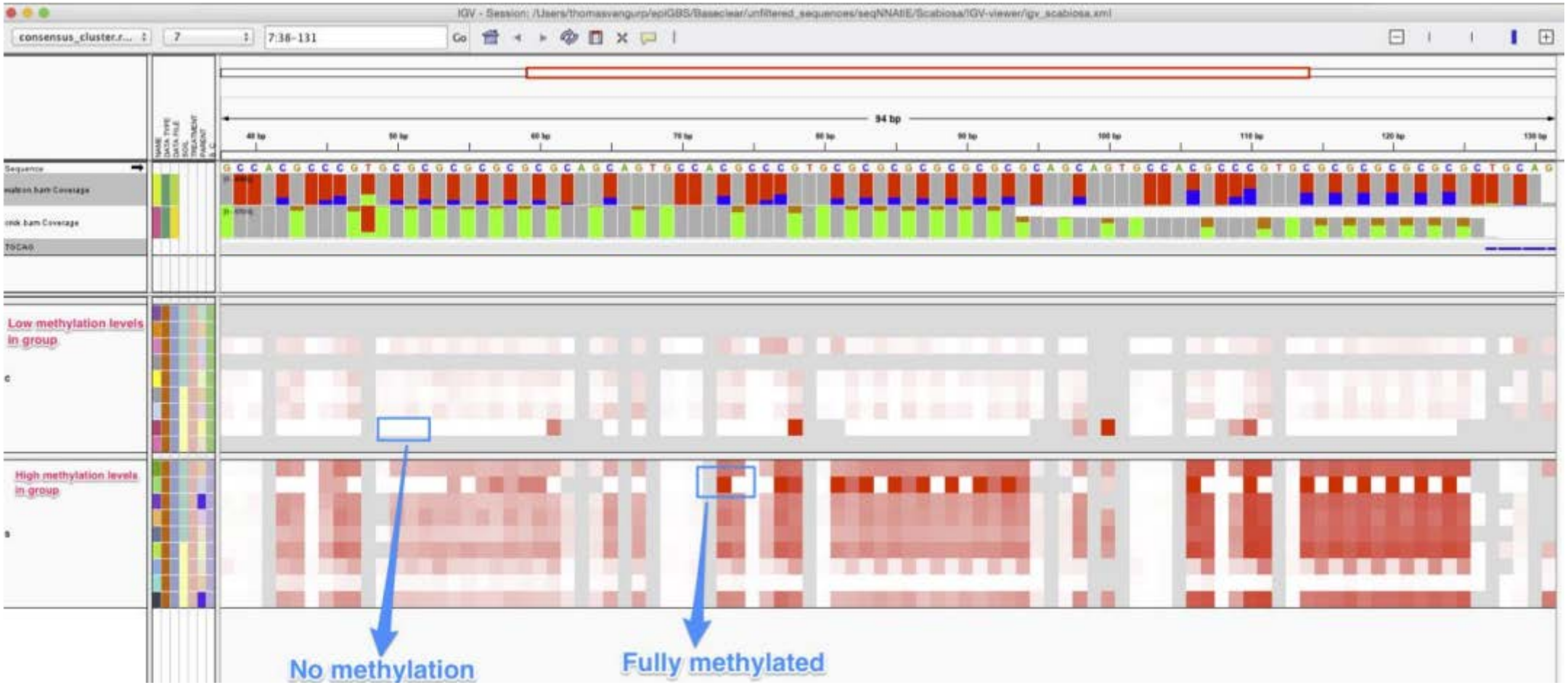
Unmethylated cytosines are bisulfite converted to uracil, whereas methylated cytosines remain intact





van Gurp et al. (2016) Nature Methods

VISUALISATION OF DNA METHYLATION

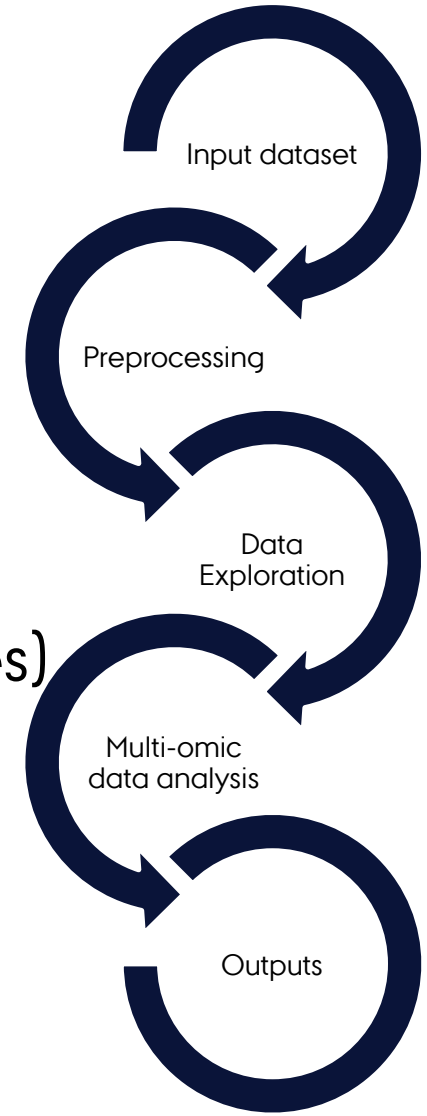


NEXT FEW MONTHS...

WHAT IS AHEAD

- By the end of the year...
  - RNAseq (barley)
  - DNA and RNA isolation (ryegrass)

- From January on...
  - epiGBS librararies preparation and sequencing (both species)
  - Data analysis



SUMMING UP...

DONE

- ✓ Pilot experiments to select restriction enzymes
- ✓ Barley and ryegrass sampled and processed
- ✓ Barley
  - ✓ DNA isolation (600 samples)
  - ✓ RNA isolation (150 samples)
  - ✓ epiGBS library (150 samples: 1 replication, both treatments) sent for sequencing

DONE & DONE

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- ❑ Plenty of fun ahead...

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