



School of Chemistry
The Raymond and Beverly Sackler
Faculty of Exact Sciences
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Epigenetic Profiling of of MDS & AML

Epigenetic Classification of MDS and AML using
Peripheral Blood



NanoBioPhotonixLab

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Epigenetics: Beyond The DNA Sequence

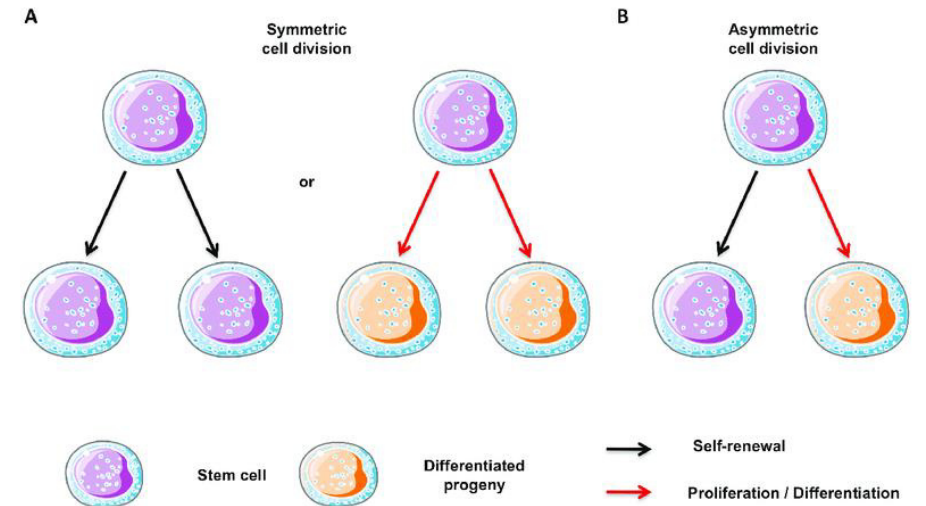
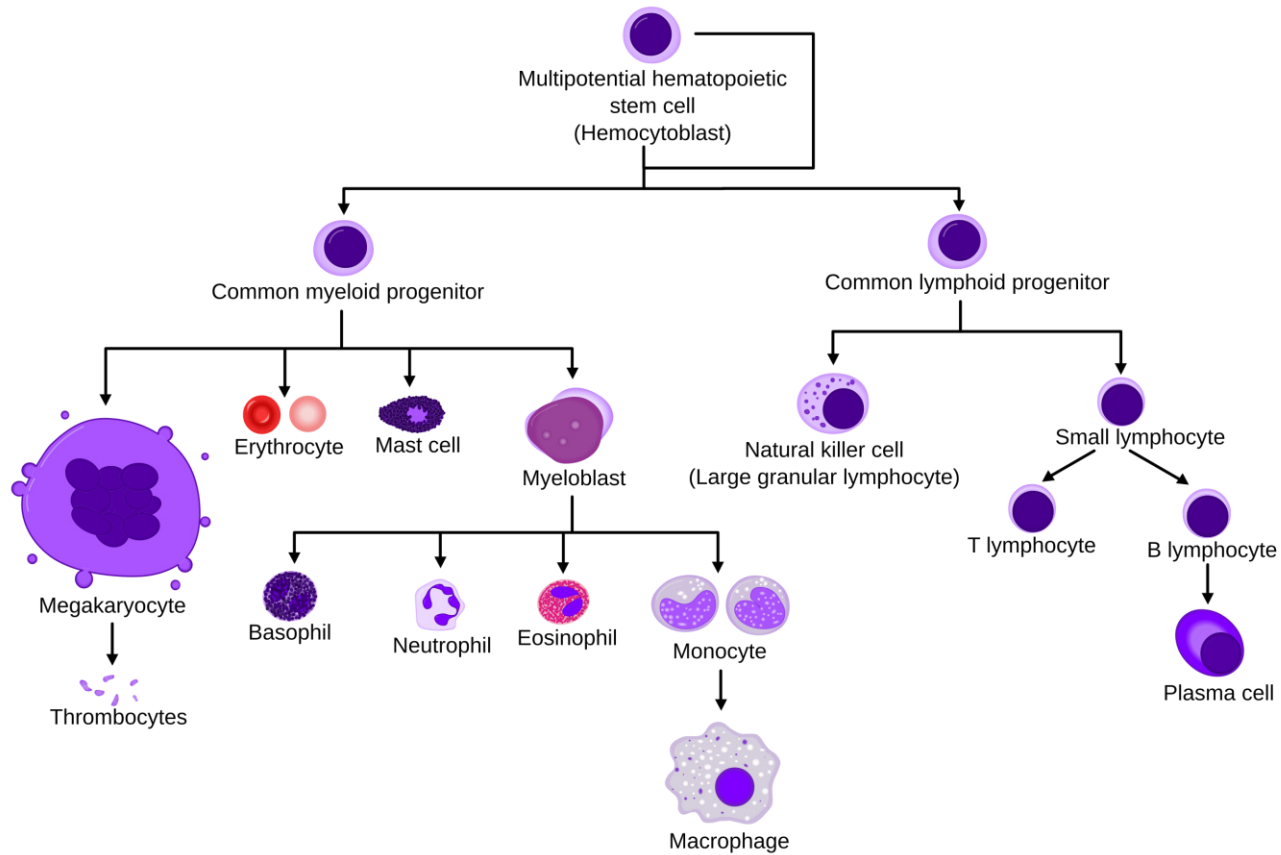


DNA Chemical Modifications Controlling Gene Expression Without Changing the Code

בְּרֵאשִׁית בְּרָא
אֱלֹהִים אֶת הַשָּׁמַיִם
וְאֶת הָאָרֶץ:

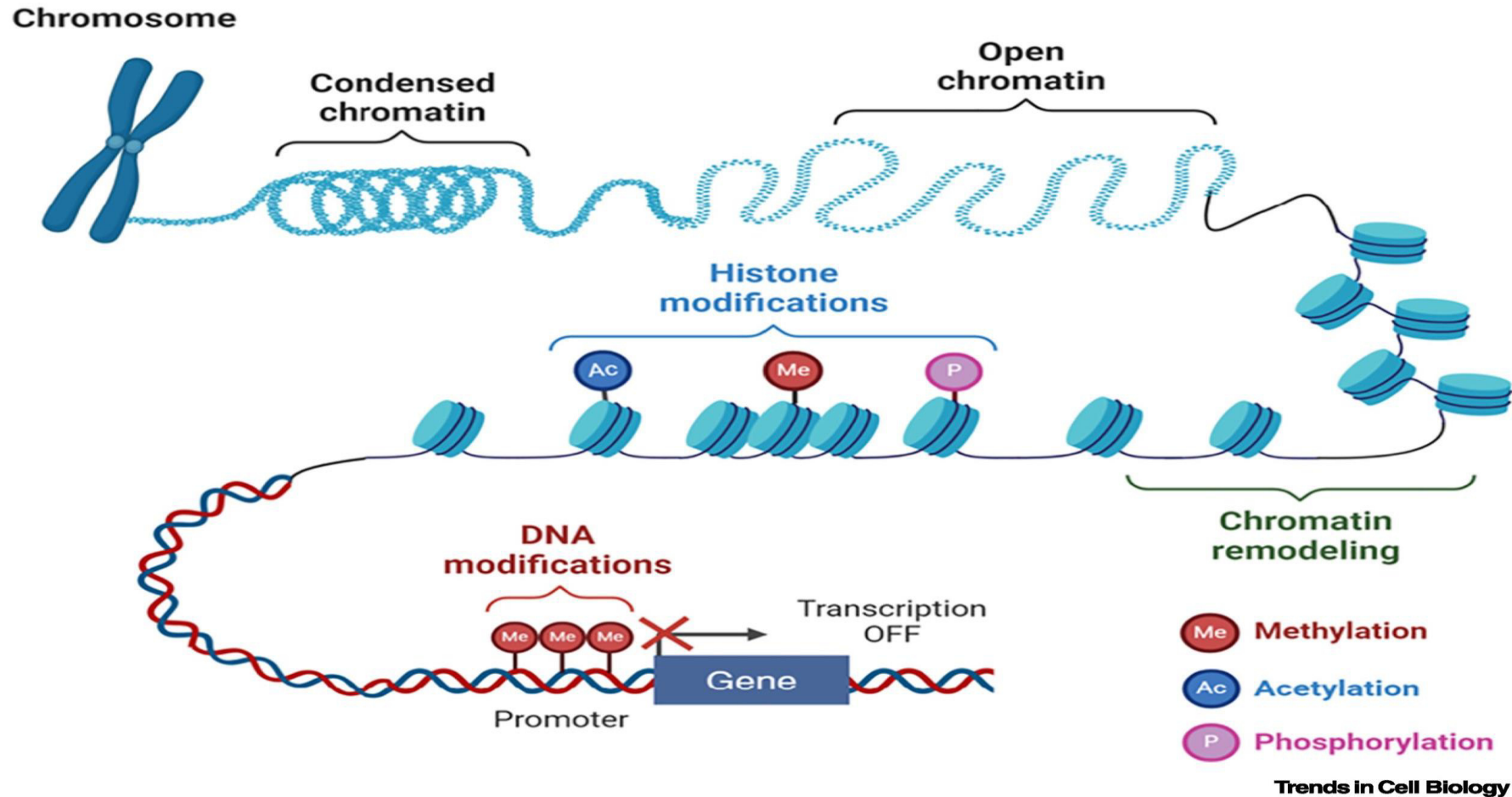
Epigenetic DNA modifications Increase the information content of DNA

Epigenetics in Hematopoiesis

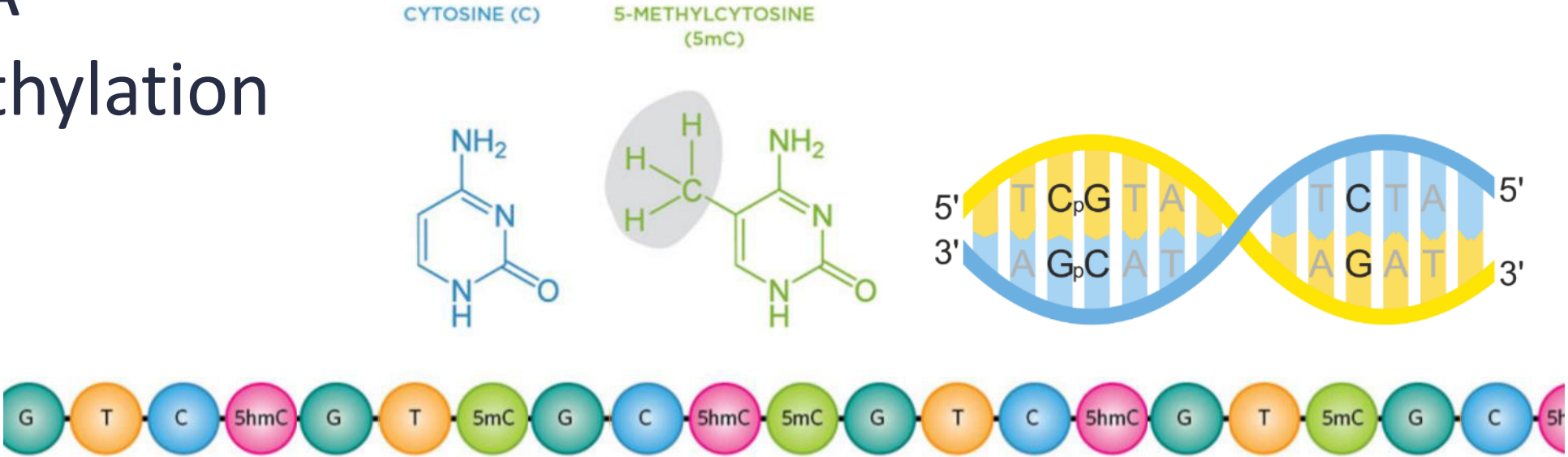


Hematopoiesis regulation : Balancing Self-Renewal and Lineage Differentiation Through Epigenetic Control

Key Types of Epigenetic mechanisms



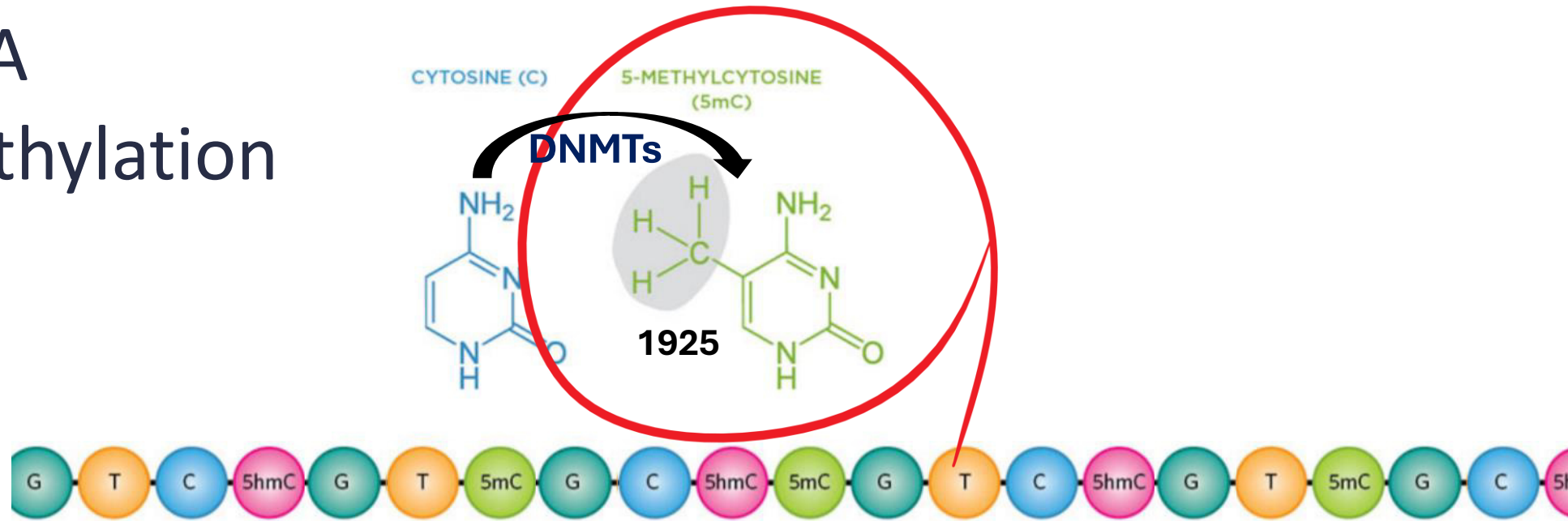
DNA Methylation



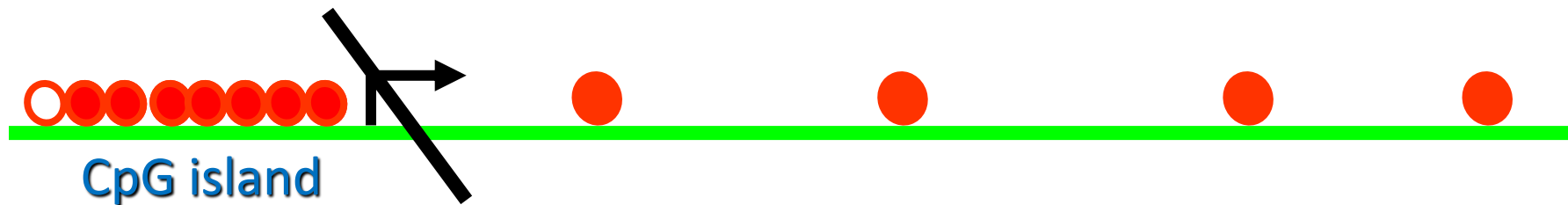
Epigenetic modifications can turn genes on and off



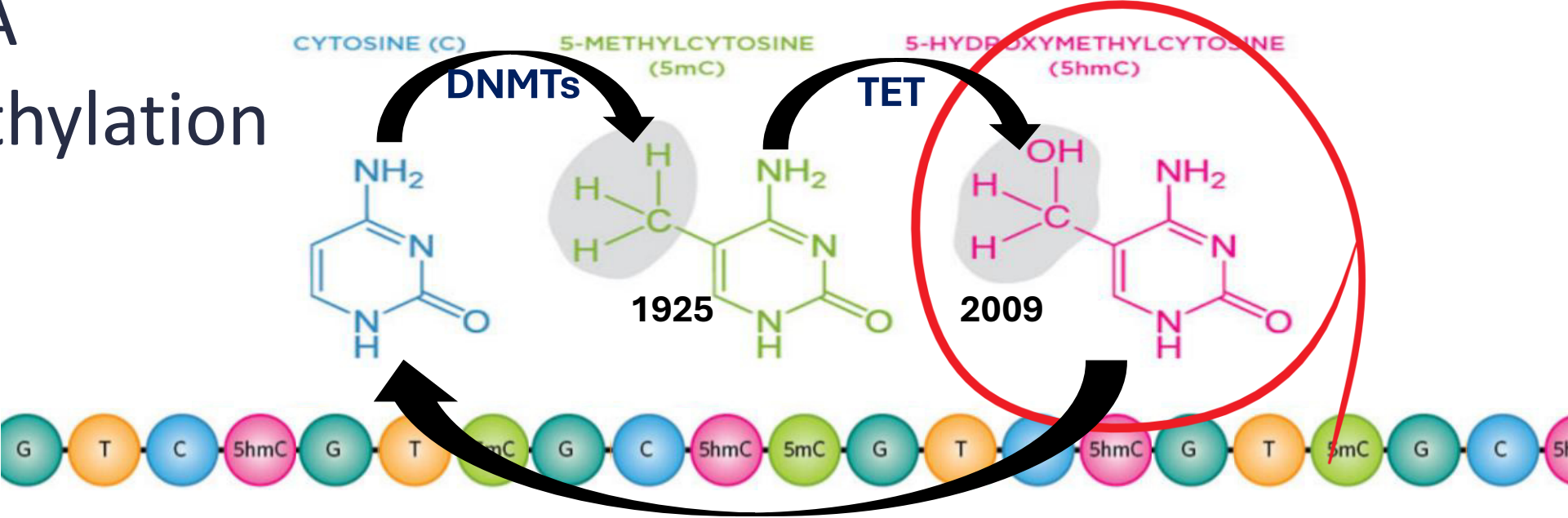
DNA Methylation



Epigenetic modifications can turn genes on and off



DNA Methylation

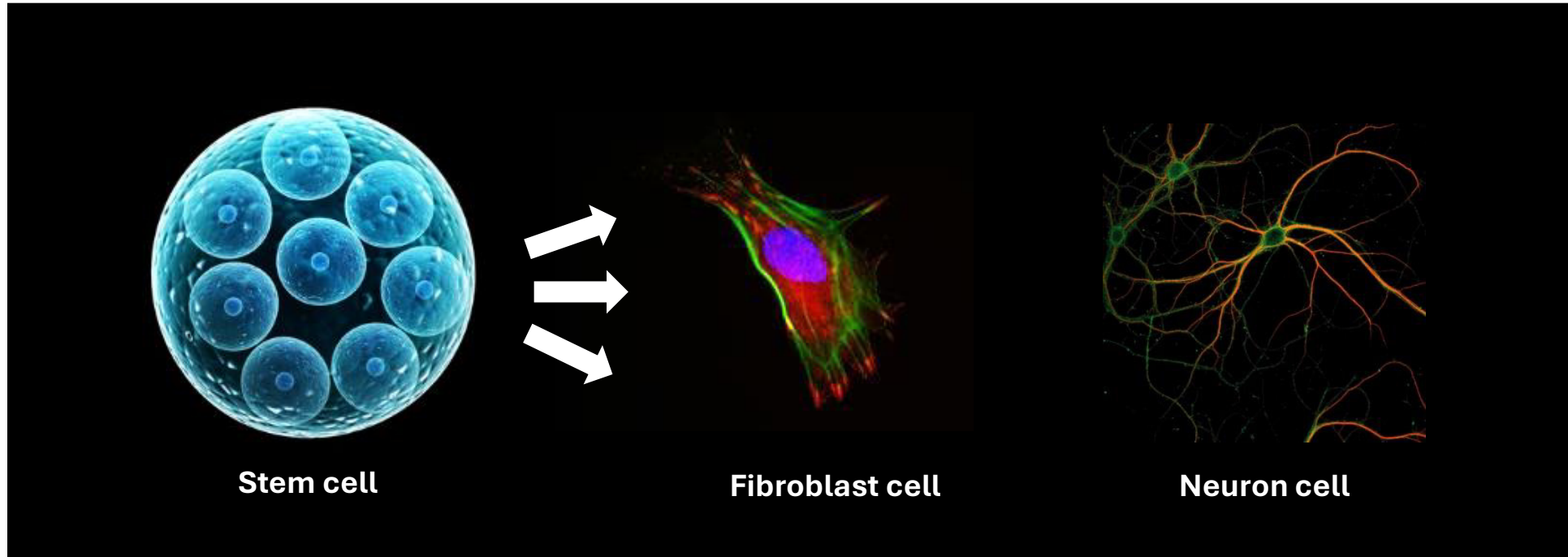


Epigenetic modifications are reversible



Epigenetic DNA modifications

Define cell identity

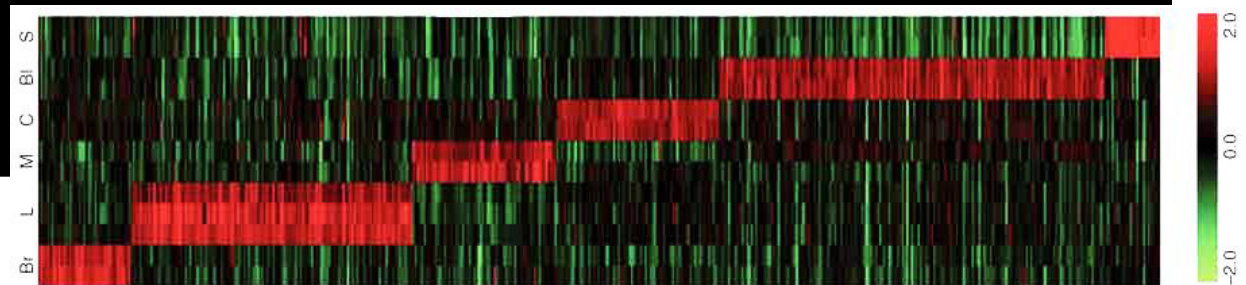


Stem cell

Fibroblast cell

Neuron cell

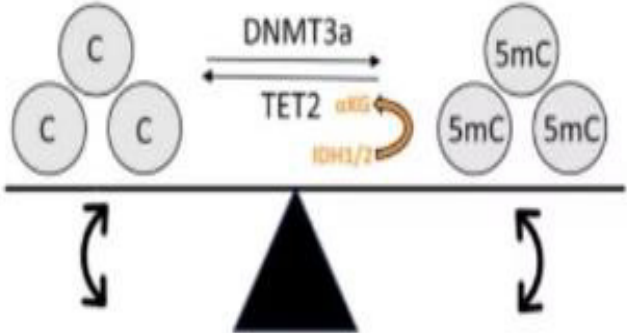
Tissue-specific de novo methylation



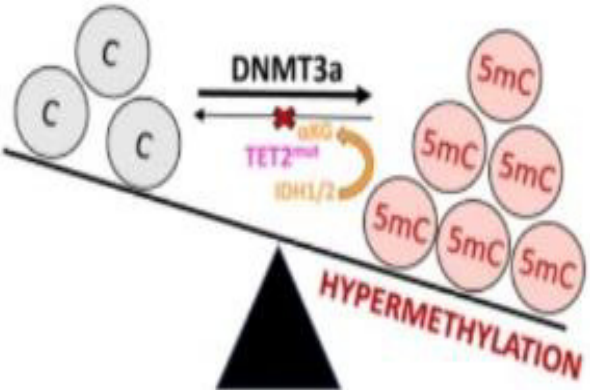
Developmental programming of CpG island methylation profiles in the human genome
Ravid Straussman, Howard Cedar *et.al.* *Nature Structural & Molecular Biology* (2009)

Epigenetic Dysregulation in AML and MDS

WT



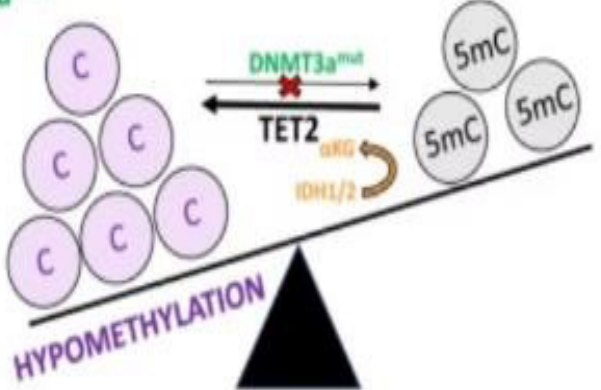
TET2^{mut}



TET2 mut:
~ 20% of both
AML and MDS

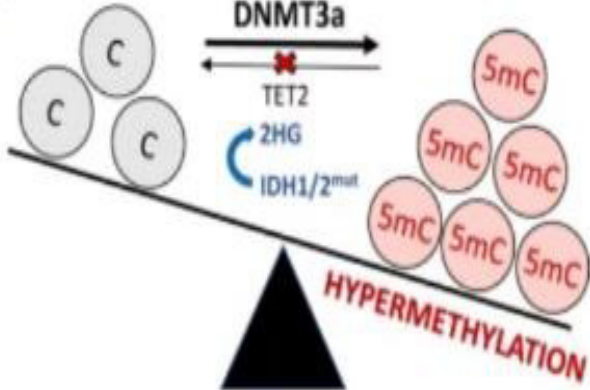
DNMT3a^{mut}

DNMT3A mut:
20-25% of AML cases

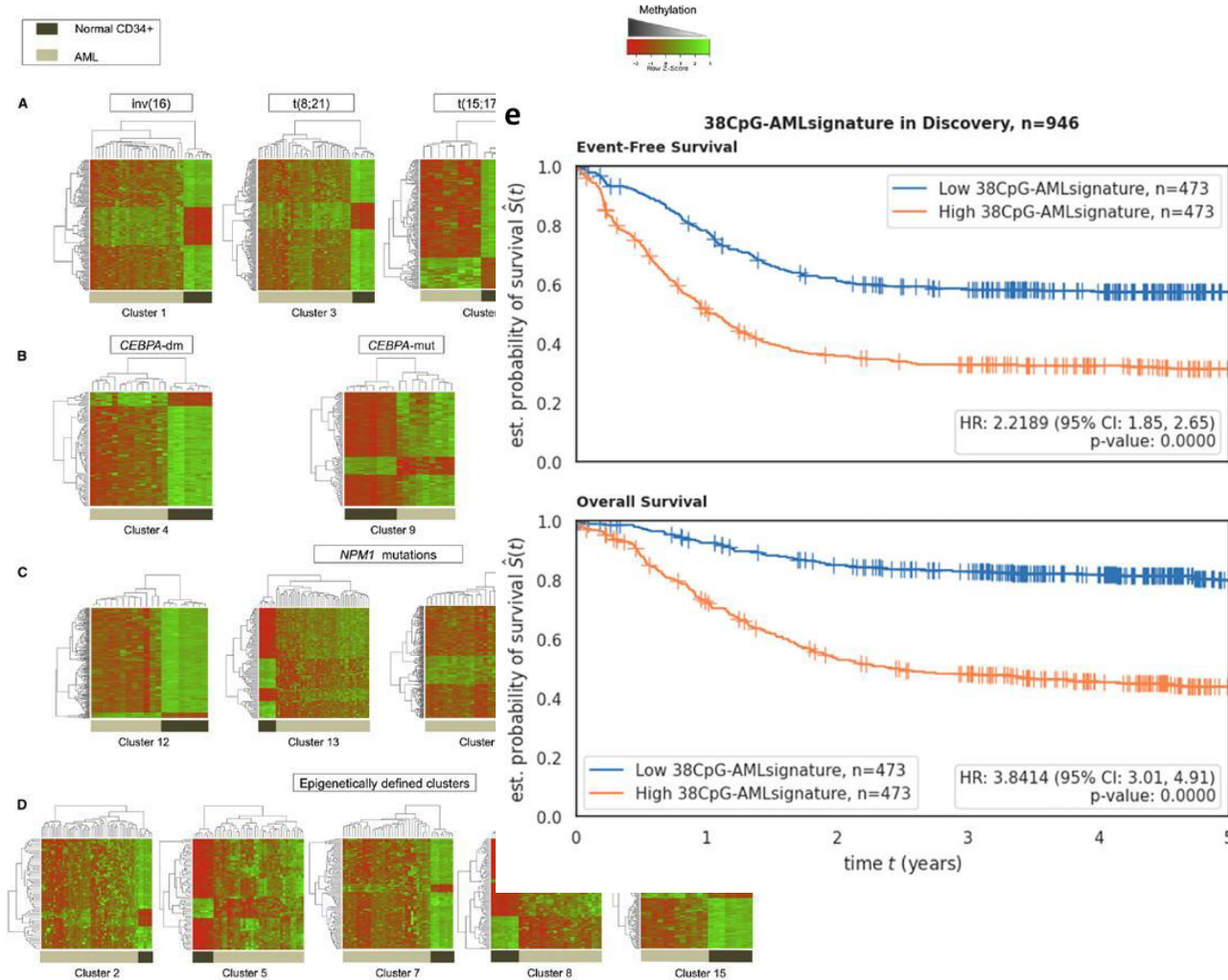


IDH1/2^{mut}

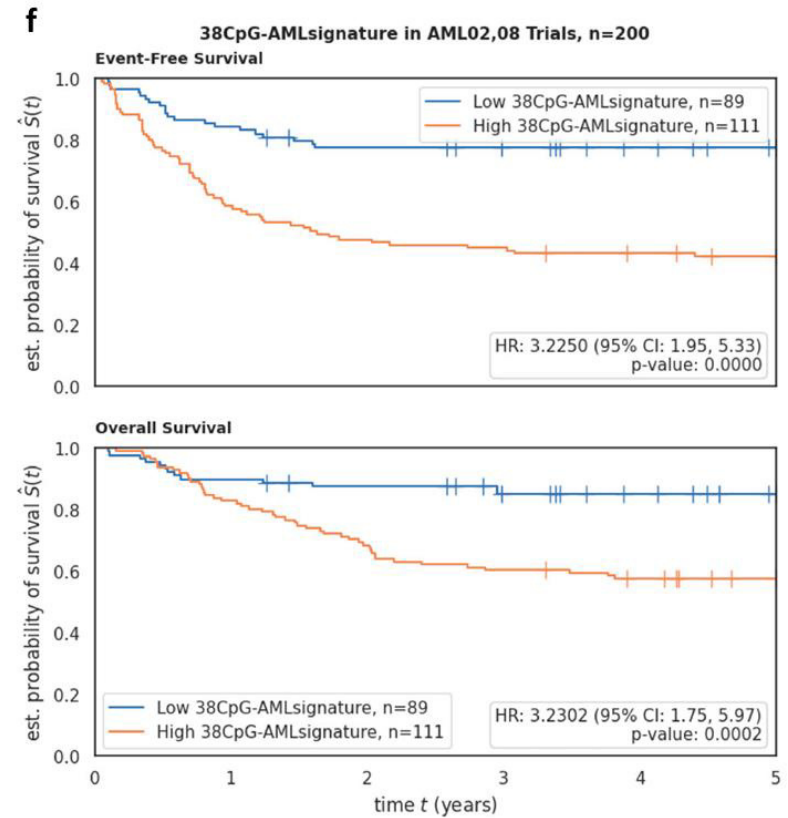
IDH1/2 mut:
20% of AML cases



DNA Methylation in AML and MDS Research

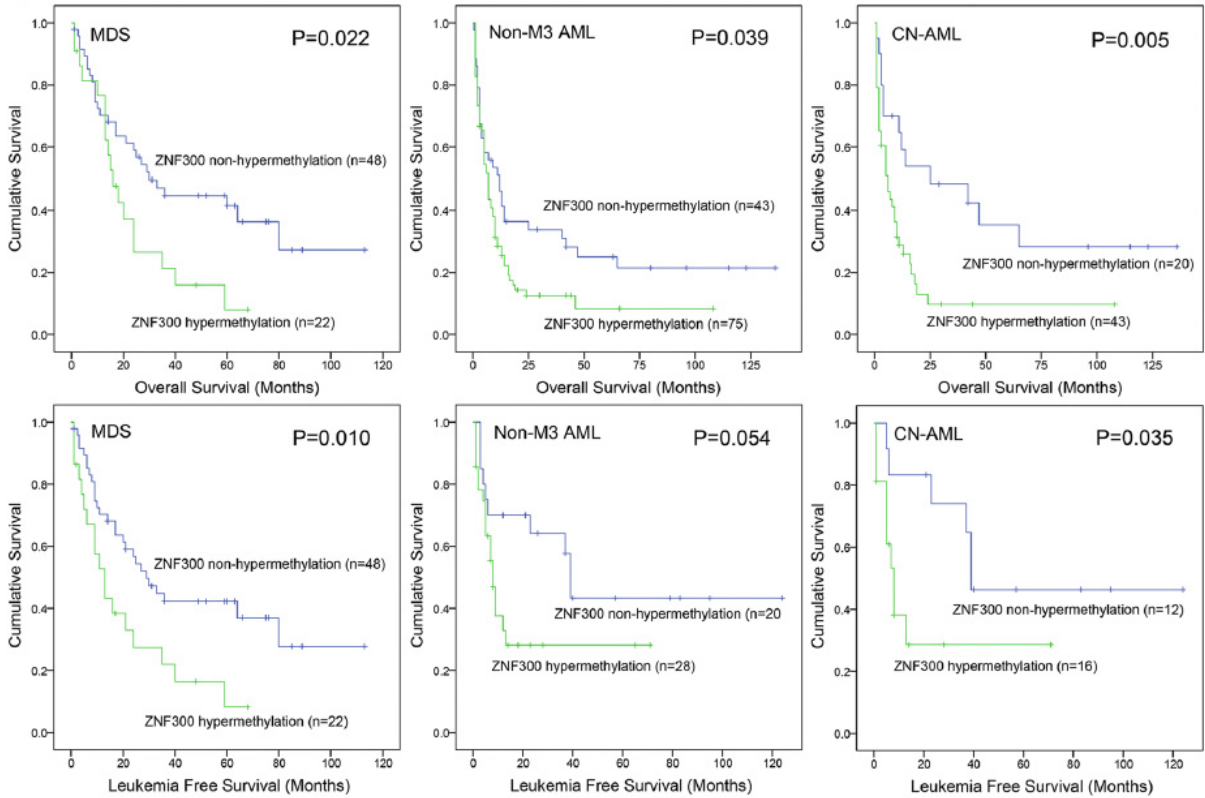
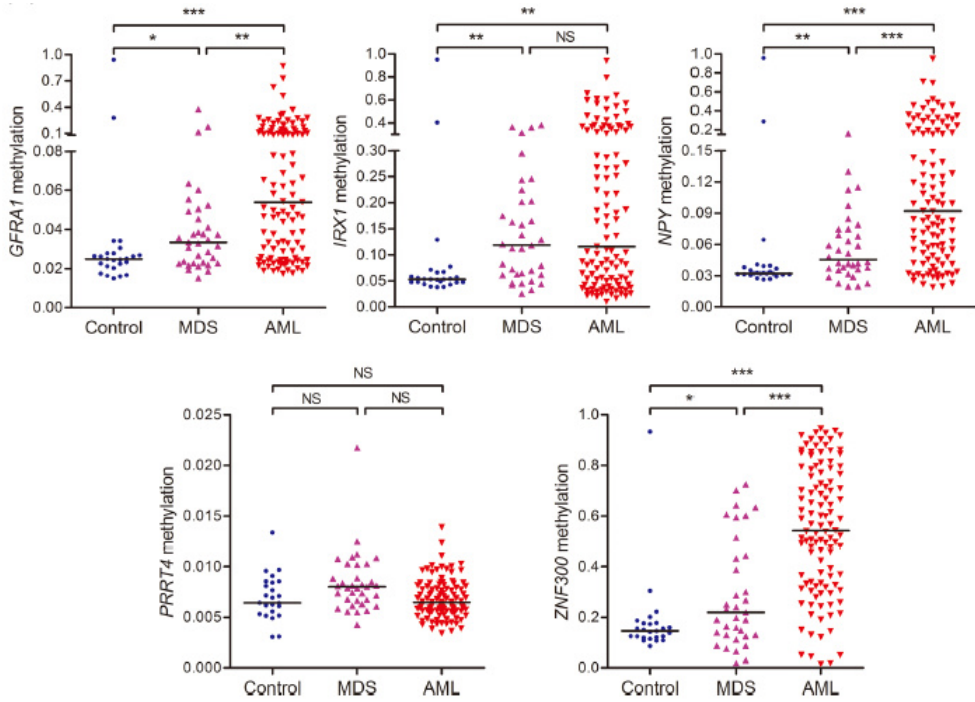


Figueroa et al, Cancer cell 2010



Marchi, F. et al. Nature Communications, 2025

DNA Methylation in AML and MDS Research

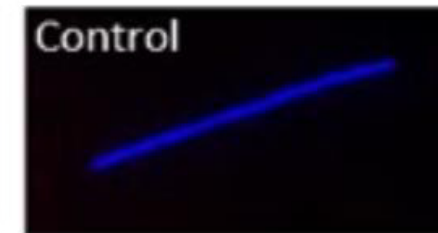
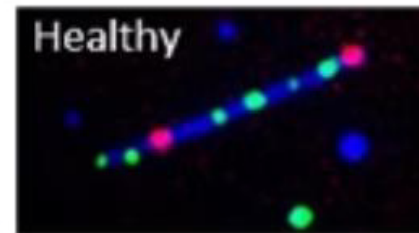
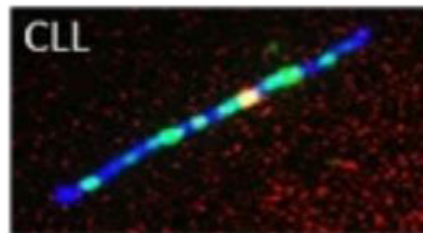
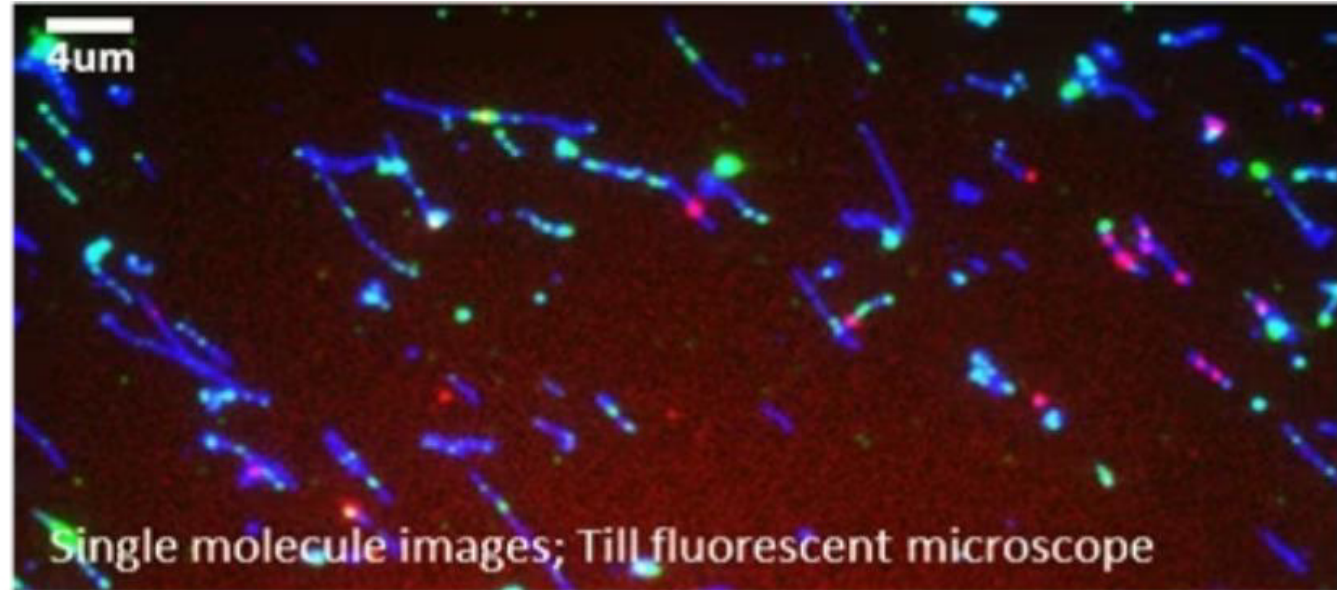


Chemoenzymatic Labeling Method

Direct 5hmC and umCpG labeling without bisulfite or sequencing

Simultaneous detection of 2 markers on the same DNA molecule

Sensitive in samples with low 5hmC content such as peripheral blood



Human DNA stained with YOYO-1 intercalator dye (blue). 5hmC residues are labeled with DBCO-Cy5 with DBCO-Cy5 (red), and um-CpG sites are labeled with DBCO-TAMRA (green)

Dual-Color Epigenetic Labeling Approach



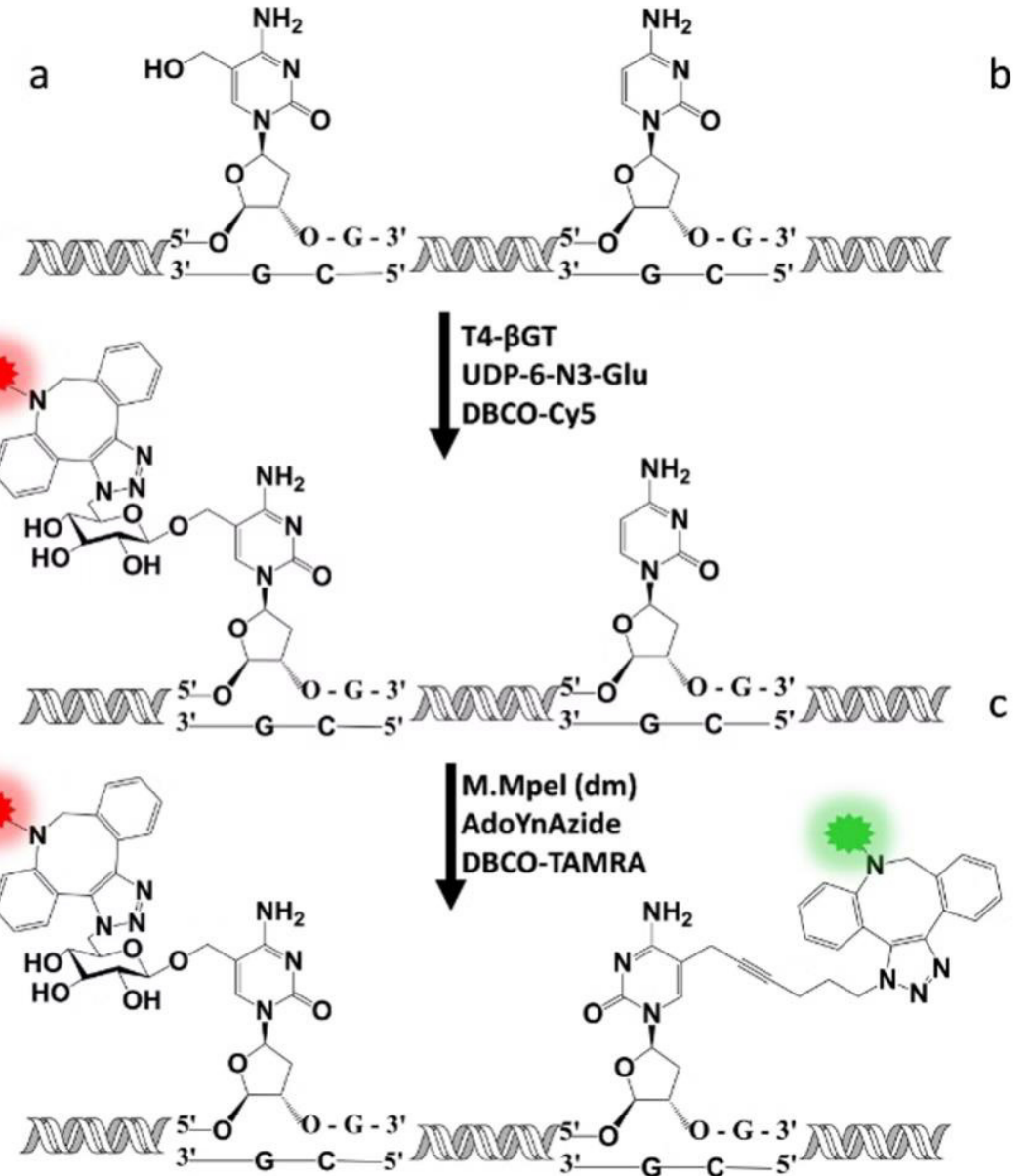
5hmC Labeling

T4-βGT enzyme adds a glucose molecule specifically to 5hmC sites, allowing attachment of a red fluorescent marker (Cy5) through click chemistry.

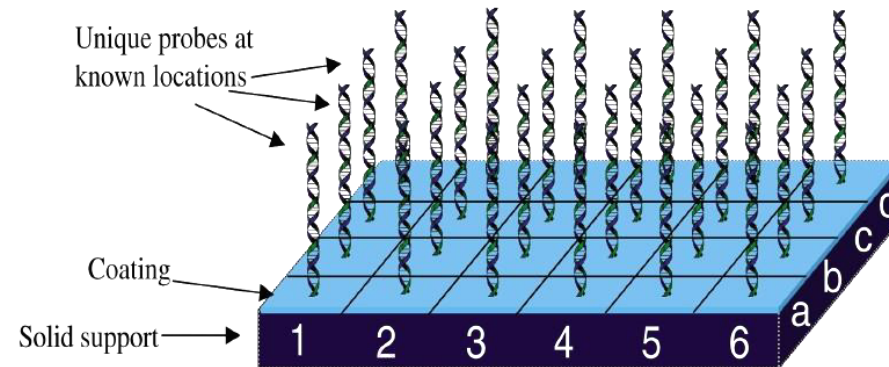
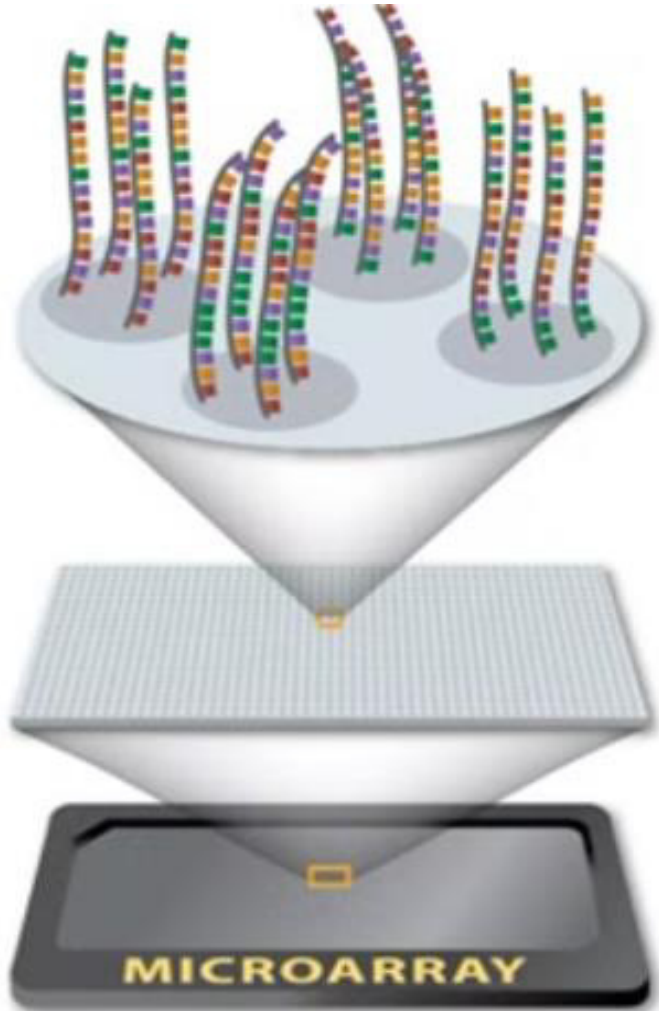


umCpG Labeling

Modified enzyme (M.Mpel) specifically recognizes and labels unmodified cytosines in CpG sequences, followed by attachment of a green fluorescent marker (TAMRA).



DNA Microarray Technology Overview



Structure

Hybridization between fixed DNA sequences and complementary sample DNA

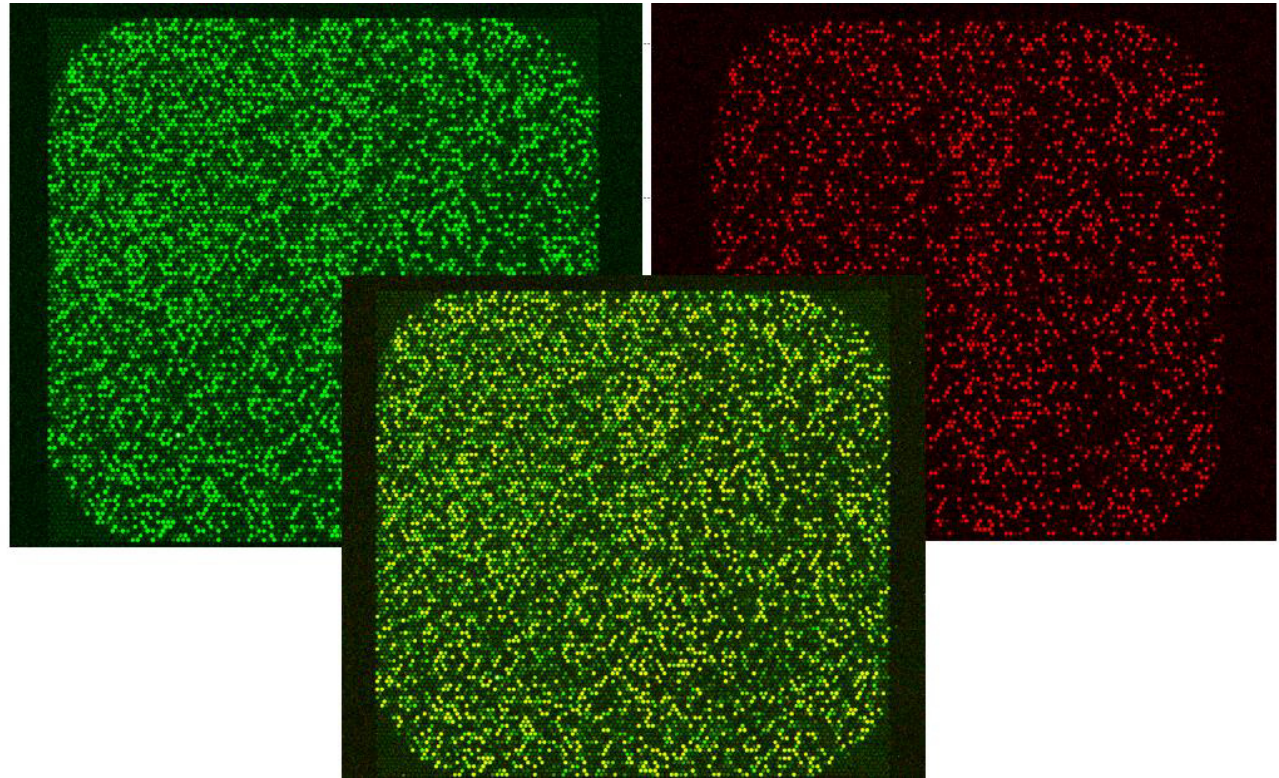
High-throughput analysis through hybridization between immobilized DNA probes and fluorescently labeled target sequences.

Applications

Gene expression and genetic variation assays for cancer diagnostics, classification and treatment guidance.

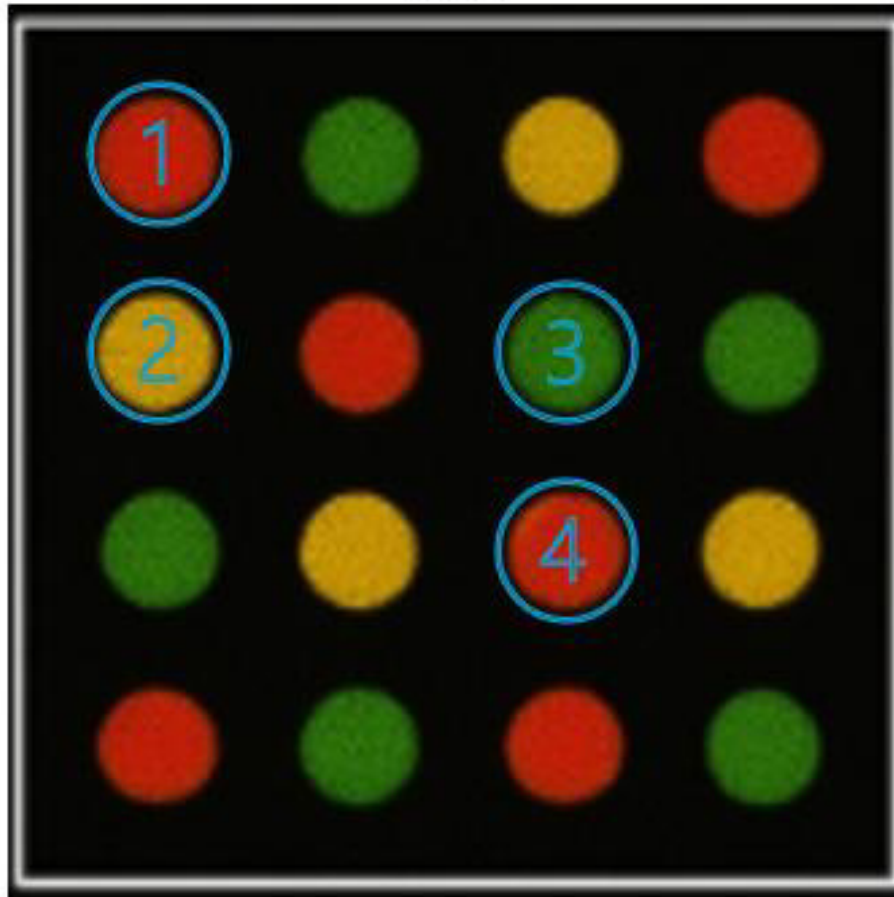
The Best of Both Worlds

- Dual epigenetic marking with established lab chemistry
- Microarray hybridization in clinical use
- Precise genomic mapping of both modifications

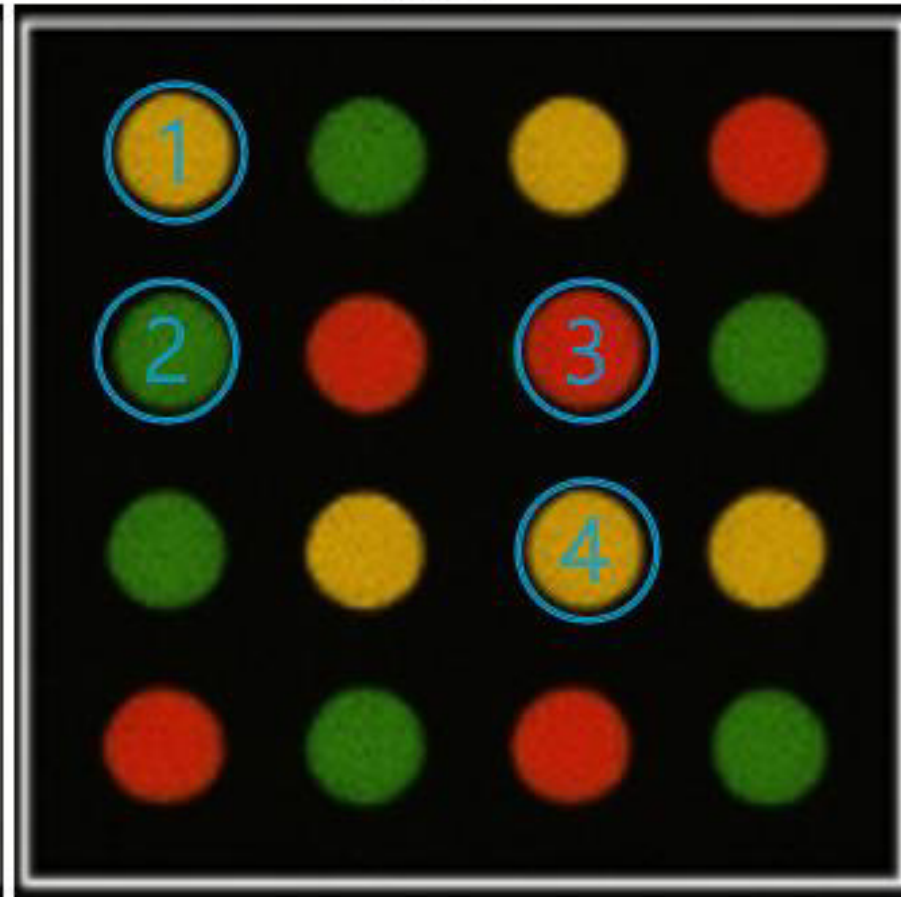


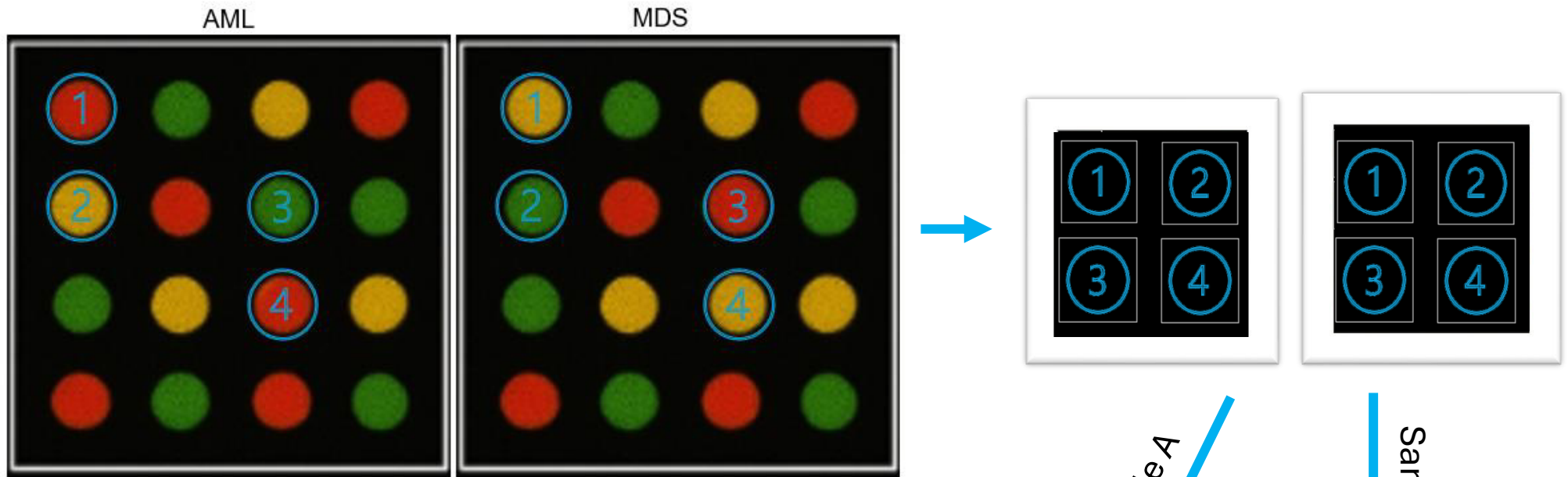
Principle of Targeted Array Design (illustration)

AML



MDS





Main Research Goal:

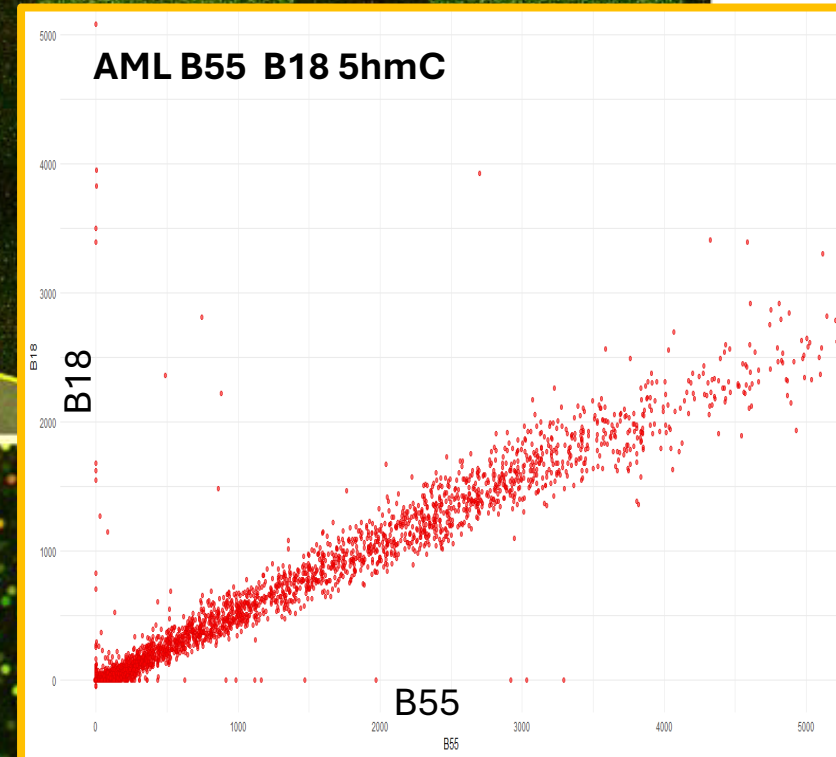
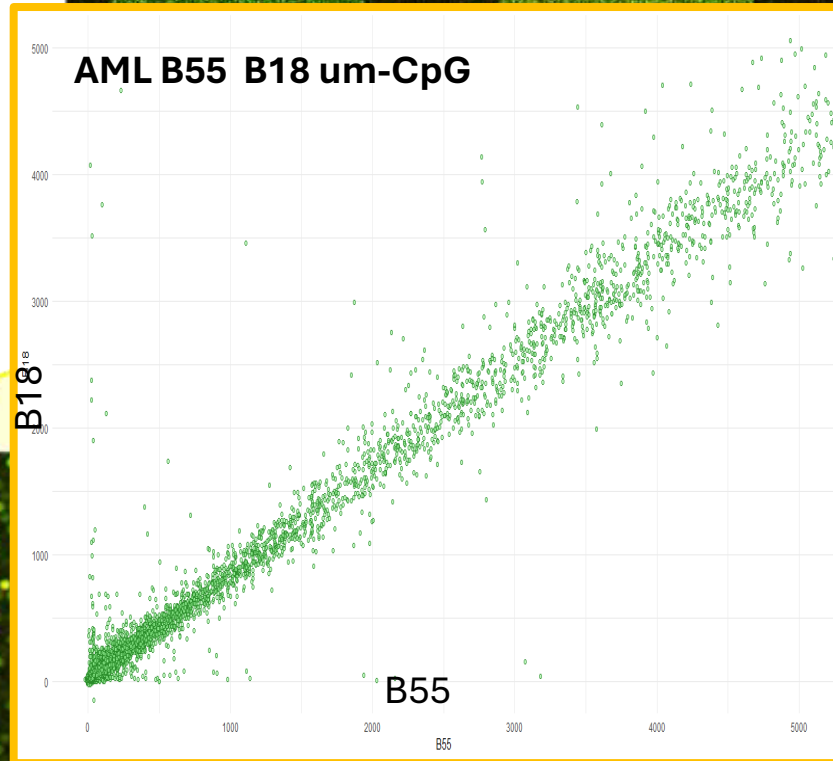
Develop an accurate and cost
affective tool for AML and MDS
Detection by Epigenetic markers



Initial Platform Development

 Agilent Commercial CGH Arrays, 8 samples, 60K probes

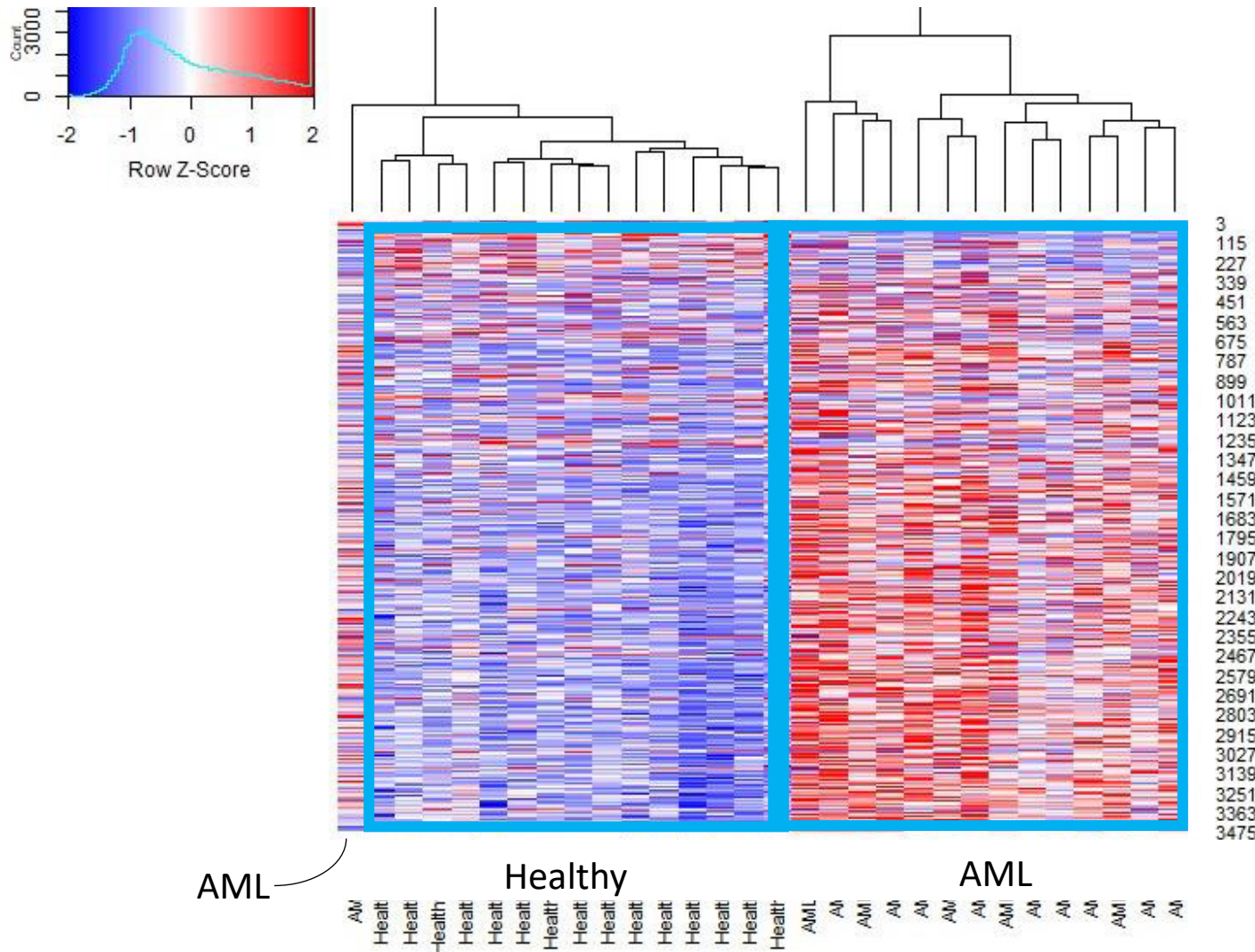
Hybridization Specificity Validation



Consistent patterns across samples indicate specific binding



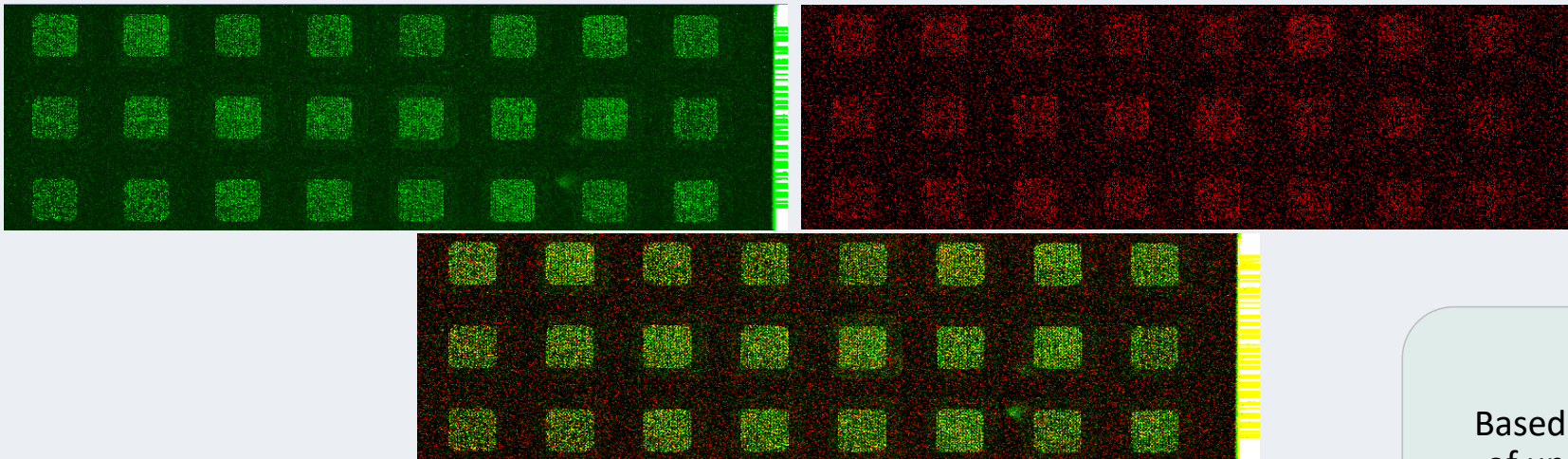
Proof Of Concept: AML & Control samples



- 15 AML and 15 control samples on 60k genomic loci microarray
- 3476 differentially Hydroxymethylated genomic locations
- Heat map showing top 340 genomic loci
- 93% sensitivity and 100% specificity for AML detection

Custom AML microarray design

AML microarray, 24 samples, 13K probes

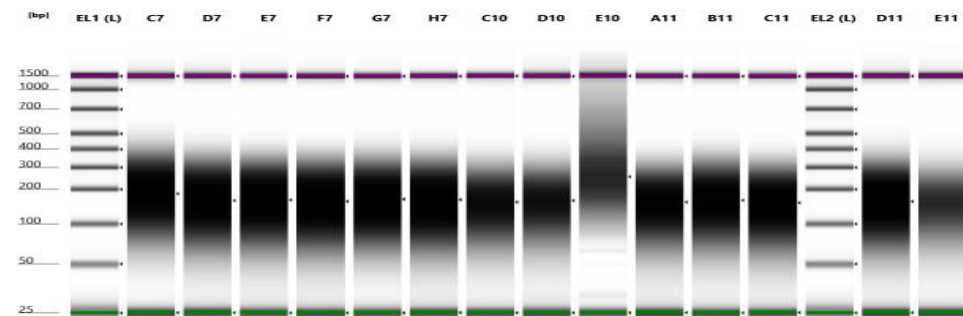
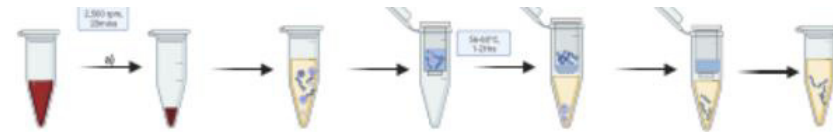


Based on Literature review
of unique 5mc and 5hmC
patterns in AML

Methods & intermediate Results:

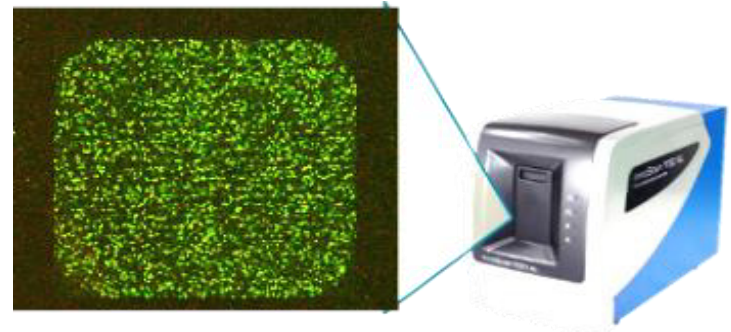
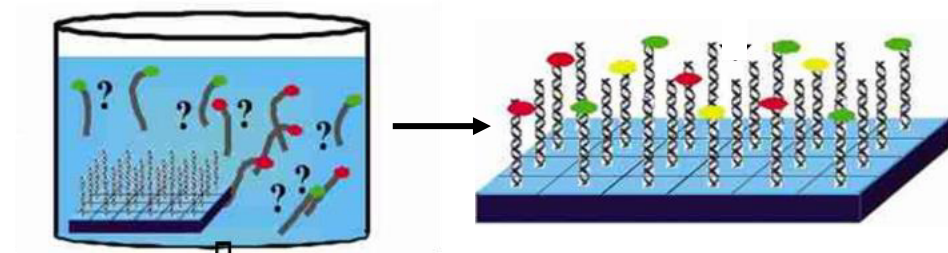
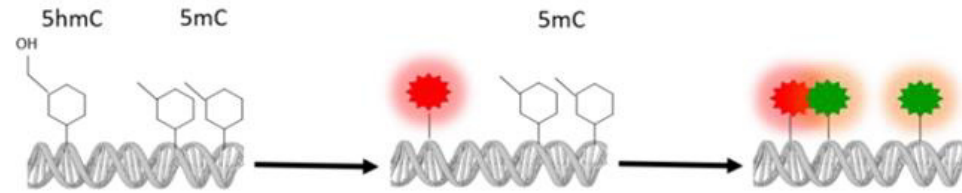
Sample Collection and Processing

- 1** Samples Collection
Three European clinical sites and TSMC
- 2** Processing Timeline
Samples are processed within 7-10 days of collection
- 3** DNA Extraction & Fragmentation
Genomic DNA is extracted and fragmented
fragmented with Covaris Sonication system



Microarray Workflow

- 1 — Epigenetic Markers Labeling
- 2 — Loading and Hybridization
- 3 — Scanning and Analyzing



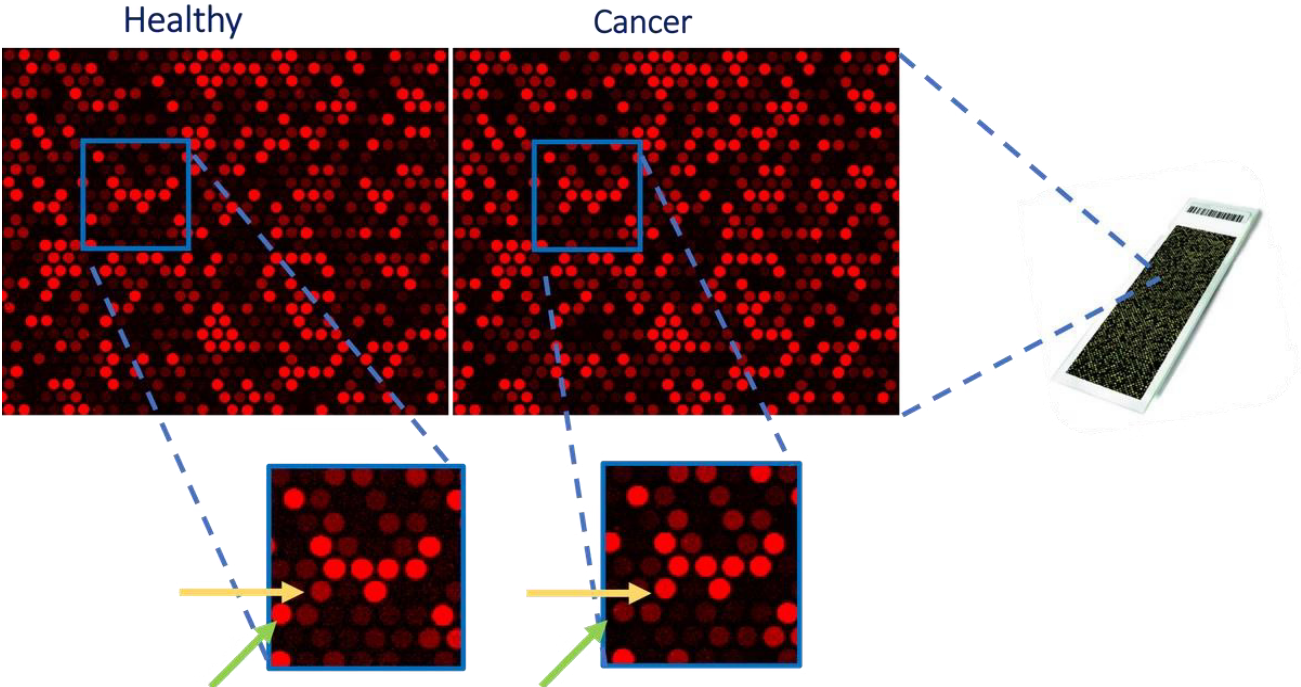
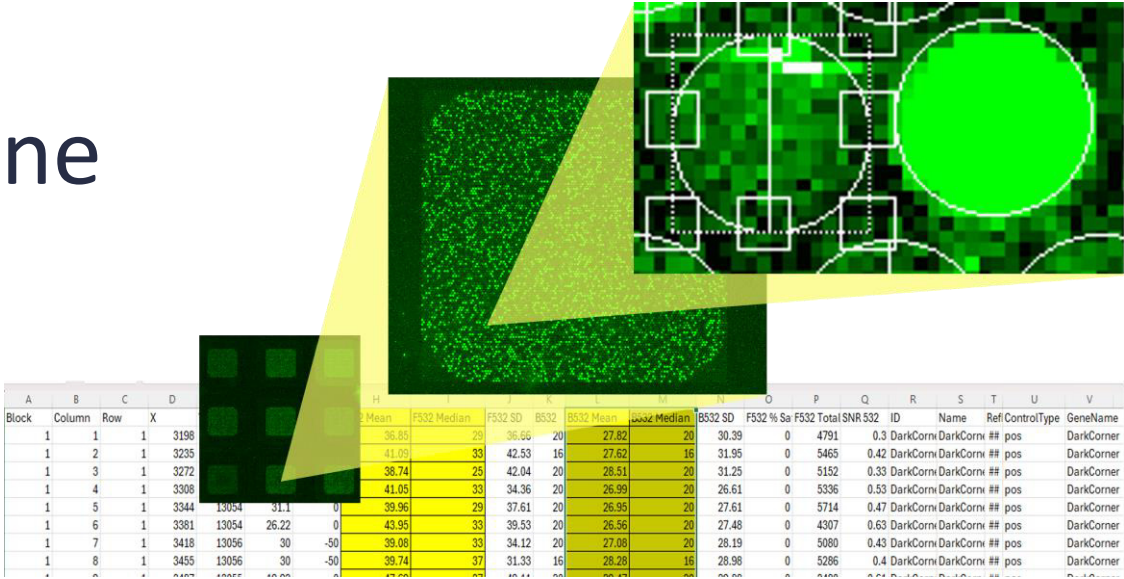
Data Analysis Pipeline

1 Signal Quantification

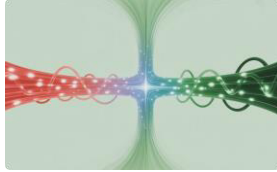
2 Normalization

3 Differential Analysis

4 Classification



Analysis Methodology



5mC & 5hmC Data Integration

Combined 5hmC and 5mC signals of each probe using a projection method, to create a single score that reflects both



Model Training & Validation

Top-ranked probe sets (50–400) trained with several classifiers; performance tested by leave-one-out cross-validation



Feature Ranking

Compared ~13,000 probes between groups and ranked them by statistical separation (Welch's t-test)



Independent Test

Applied chosen model to a small separate test set

13k Array Results: Multi-Center Study



198 participants
71 AML, 55 MDS, 72 controls

Train Model
60 AML, 50 MDS,
60 controls

Test
11 AML, 5 MDS,
12 controls



Patient Characteristics

Train samples:	AML n=60	MDS n=50	Controls n=60	P value
Median Age (IQR)	66.5 (52.8-74.0)	73.5 (68.3-77.0)	68.5 (56.5-73.3)	AML vs control* 0.238
Sex, n (%)				0.936
Male	31 (51.7)	25 (50.0)	29 (48.3)	
Female	29 (48.3)	25 (50.0)	31 (51.7)	
Risk Stratification	13 (22.0%) favorable	85% Low-risk		
	22 (37.3%) intermediate			
	24 (40.7%) adverse			

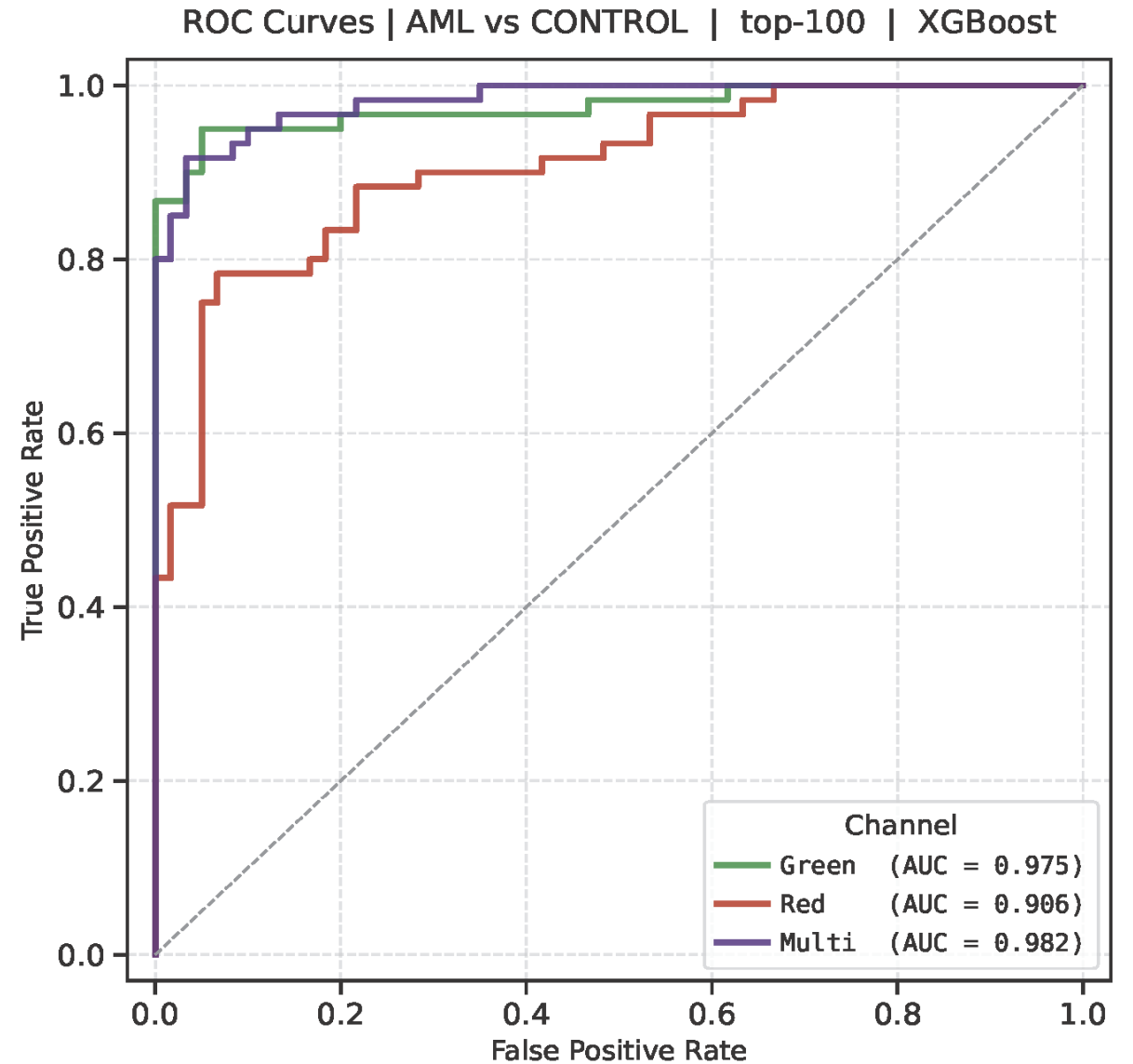
AML & Control:

Configuration	Classifier	Top K	ROC_AUC
multi polar cartesian	XGBOOST	100	0.982
single grn	XGBOOST	100	0.975
single red	XGBOOST	100	0.905

Optimal Threshold	Sensitivity % (TP/Total)	Specificity % (TN/Total)
0.63	91.7% (55/60)	96.7% (58/60)

Test:

Sensitivity % (TP/Total)	Specificity % (TN/Total)
81.8% (9/11)	91.7% (11/12)



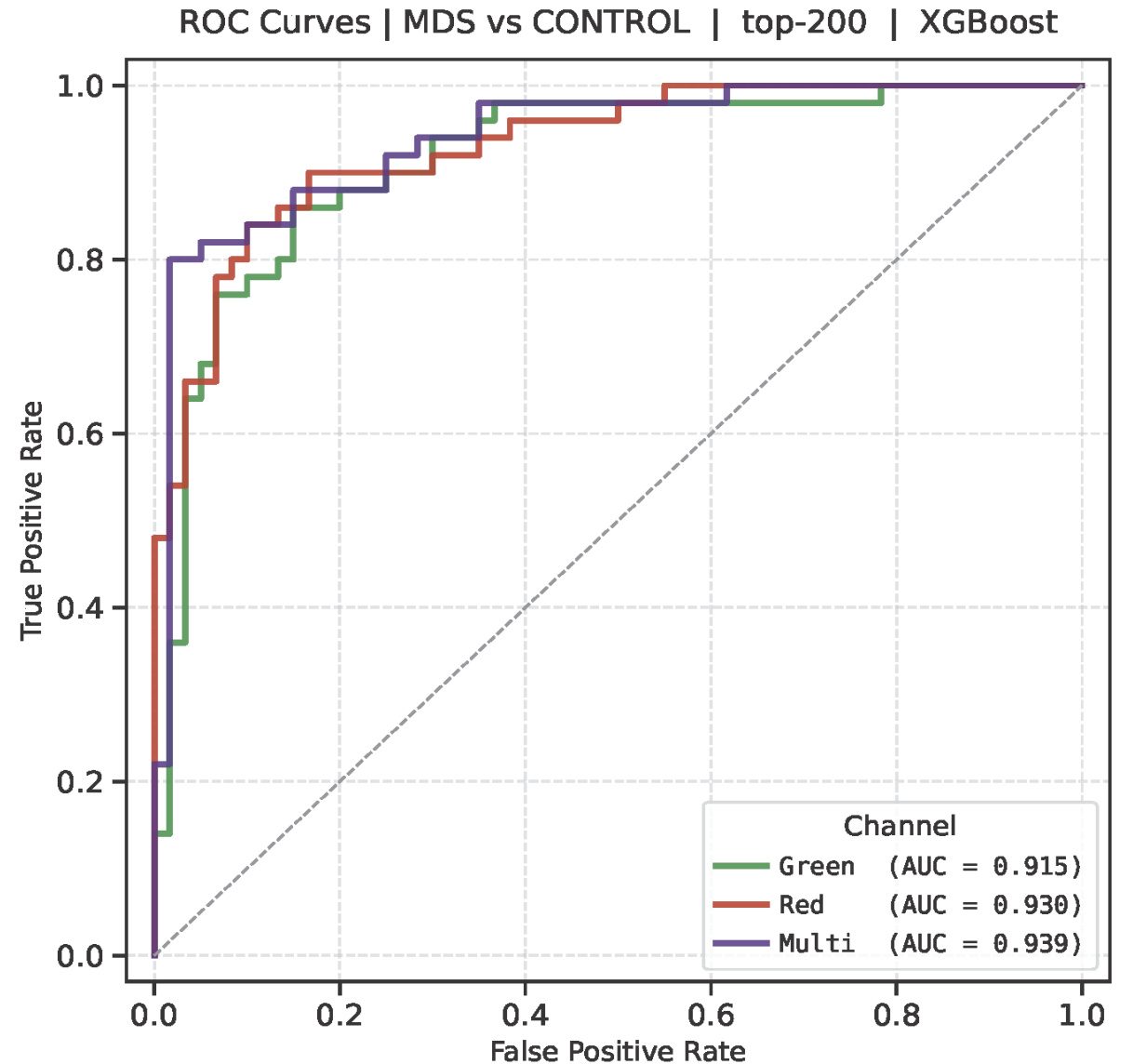
MDS & Control:

Configuration	Classifier	Top K	ROC_AUC
multi polar cartesian	XGBOOST	200	0.939
single grn	XGBOOST	200	0.915
single red	XGBOOST	200	0.930

Optimal Threshold	Sensitivity % (TP/Total)	Specificity % (TN/Total)
0.64	80% (40/50)	95% (57/60)

Test:

Sensitivity % (TP/Total)	Specificity % (TN/Total)
100% (5/5)	100% (12/12)



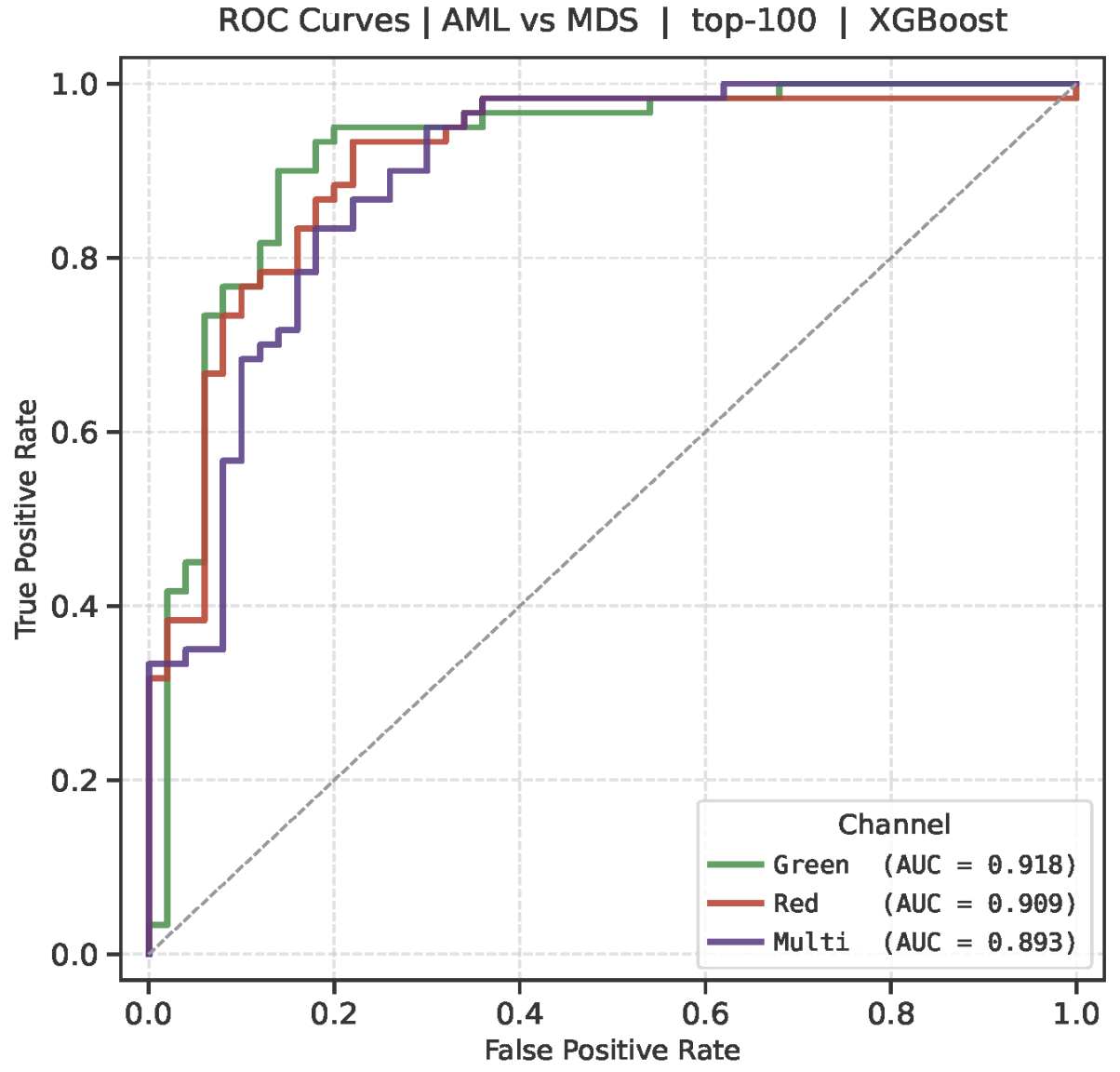
AML & MDS:

Configuration	Classifier	Top K	ROC_AUC
multi polar cartesian	XGBOOST	400	0.922
single grn	XGBOOST	400	0.942
single red	XGBOOST	400	0.926

Optimal Threshold	AML Sensitivity %	MDS sensitivity %
0.59	86.7% (52/60)	86% (43/50)

Test (top K = 100):

AML Sensitivity %	MDS sensitivity %
90.9% (10/11)	100% (5/5)



Future Plans



Validation on final Microarray

Expand all cohorts and validate on the new targeted microarray.



Clinical Correlation

Correlate epigenetic signatures with patients' clinical data, including cytogenetic characteristics, characteristics, risk stratification, treatment responses, and survival outcomes.



Pathway Analysis

Conduct bioinformatic analysis to identify biological pathways associated with differentially methylated regions, using established tools for pathway enrichment analysis.

THANKS!



NanoBioPhotonixLab



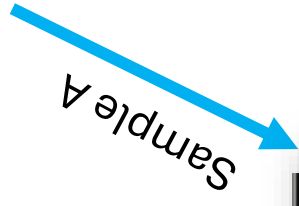
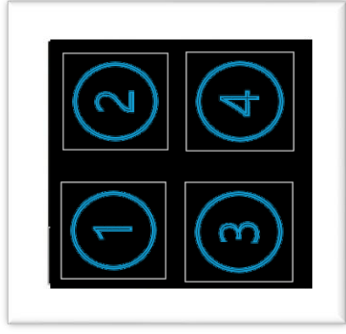
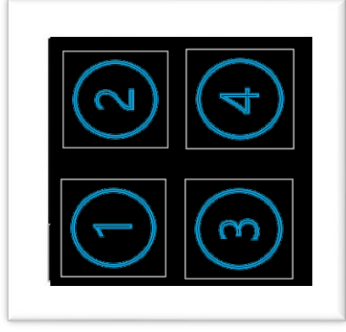
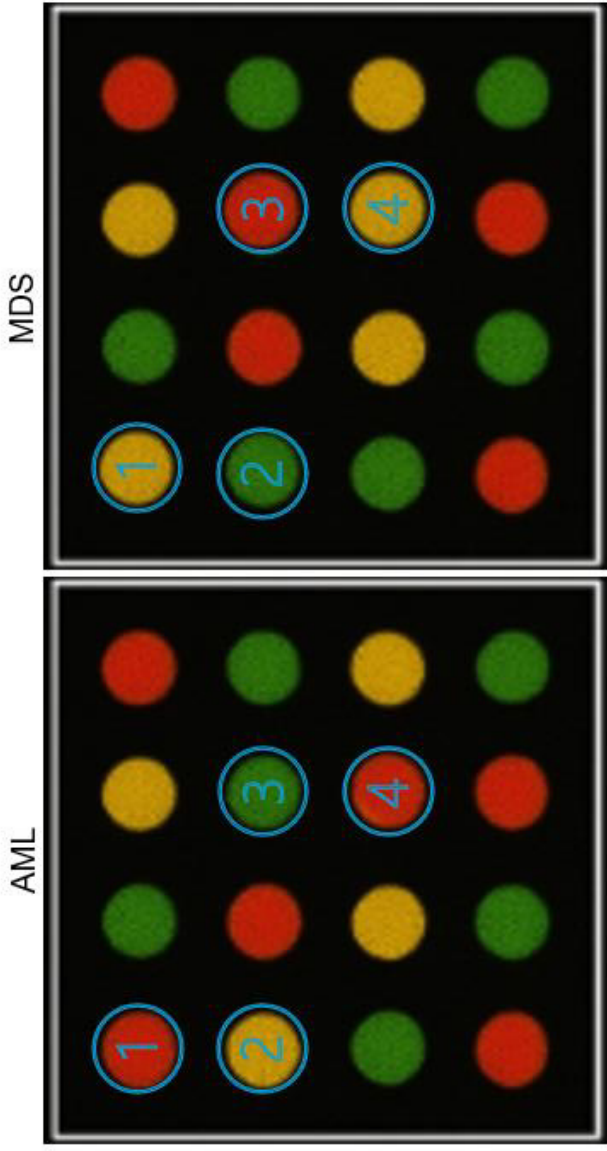
**THE
END**

Patient characteristics

Characteristic	AML (n=60)	MDS (n=50)	Controls (n=60)	p-value*
Age, median (IQR) years	66.5 (52.8-74.0)	73.5 (68.3-77.0)	68.5 (56.5-73.3)	0.238 (AML vs Control)
Sex, n (%)				0.936
Male	31 (51.7)	25 (50.0)	29 (48.3)	
Female	29 (48.3)	25 (50.0)	31 (51.7)	

Thanks! NanoBioPhotonixLab



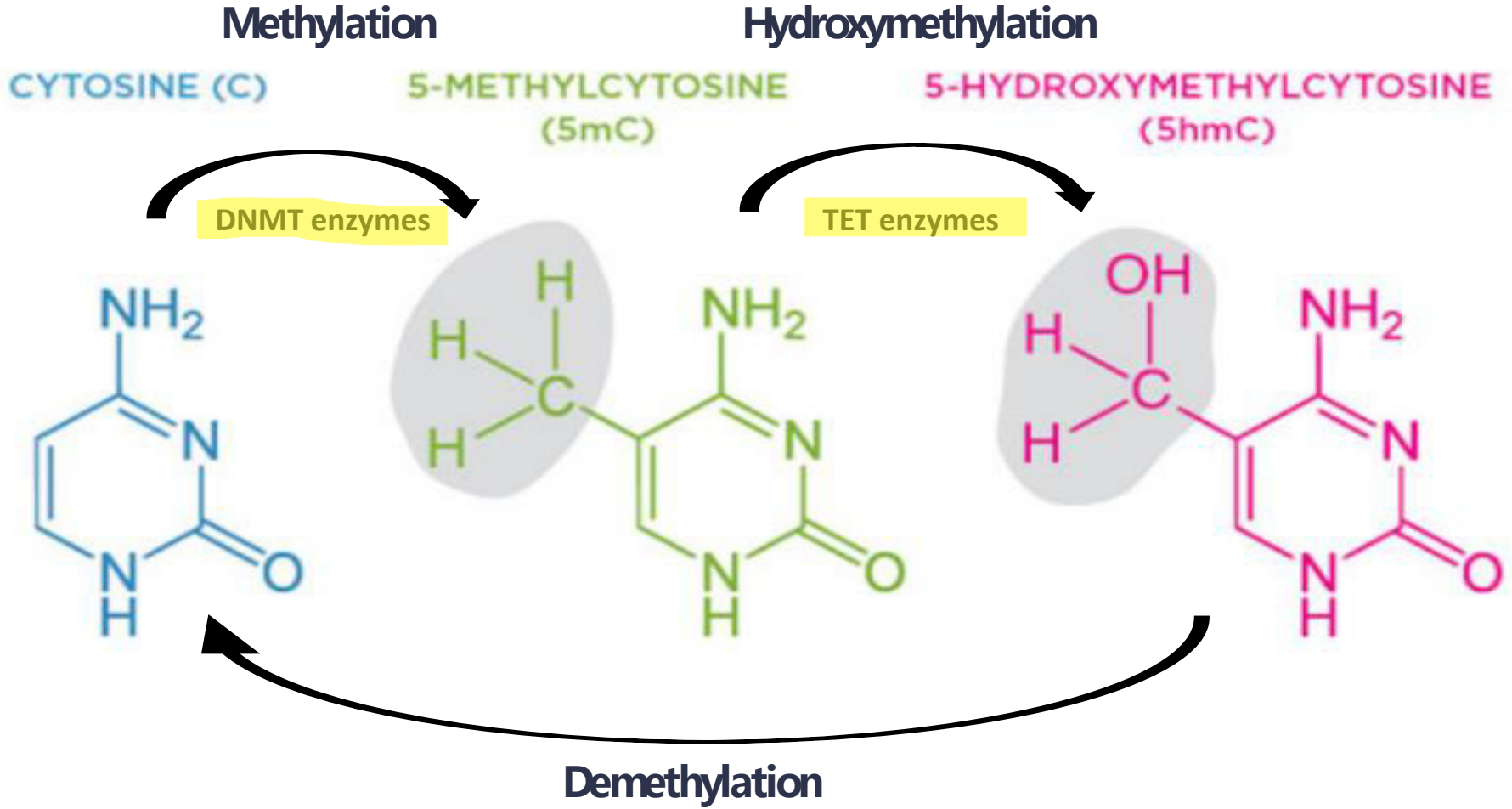


AML

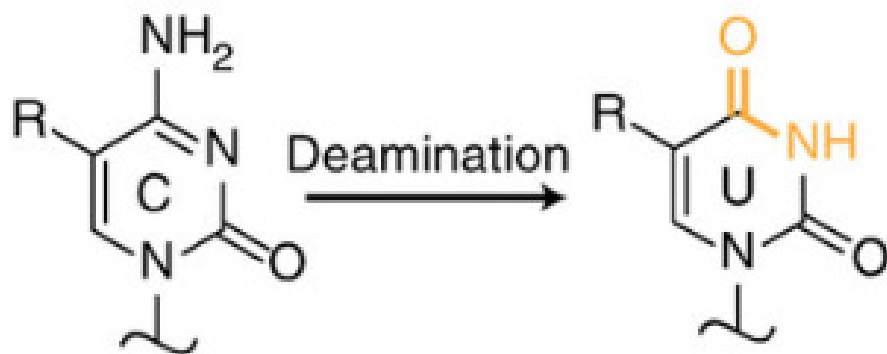


MDS

DNA Methylation is a Dynamic process



Current Methods for Detecting DNA Methylation



Base	BS-seq	oxBS-seq	TAB-seq	ACE-seq
C	●	●	●	●
5mC	○	○	●	●
5hmC	○	●	○	○

Schutsky et al., Nature Biotechnology, 2018

Bisulfite Sequencing

5mC + 5hmC

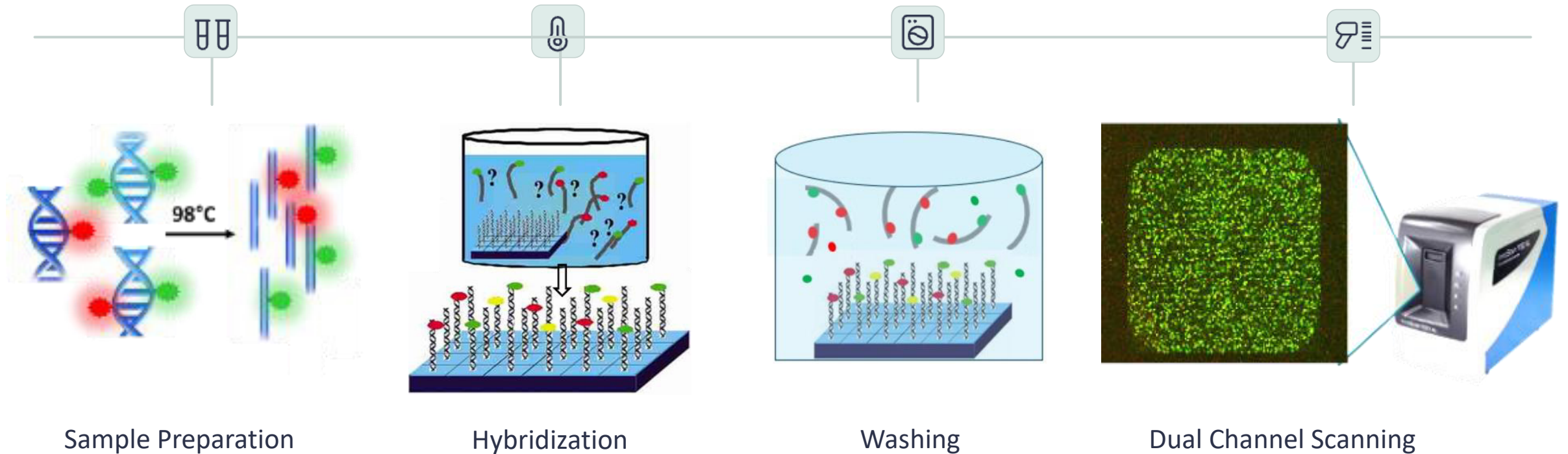
OxBS-seq

5mC

TAB-seq

5hmC

Microarray Hybridization Process



⚠ **Specific hybridization is critical** for accurate results while Non-specific binding creates false signals. Key factors determining specificity: Hybridization buffer and temperature

Technical Optimization for 24-Sample Arrays



Sample Concentration

Modified post-labeling cleanup protocol using specialized specialized magnetic beads kit for low-volume elution

Loading Technique

Precise 10-11 μL droplet placement in gasket wells with wells with careful attention to surface tension

Buffer Modification

Addition of wetting agents with optimized surfactant surfactant concentrations for sample spreading

Hybridization Protocol

Initial 10-minute incubation at 80°C followed by by overnight at 46°C