# Multi-Cancer Detection Tests: A Look Under the Hood

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# Disclosure Information

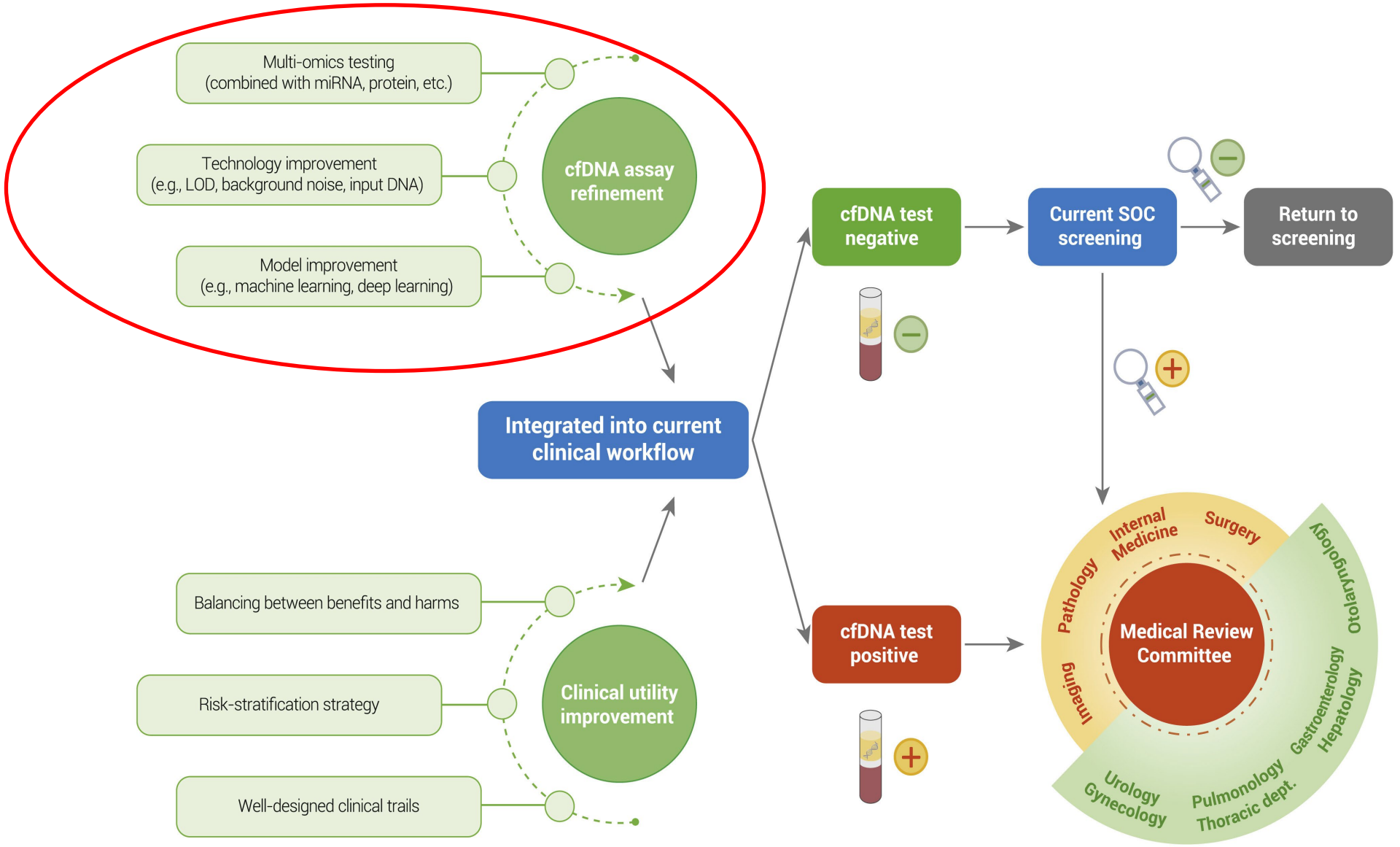
- Freenome — Advisory Board Member
- Guardant Health — Advisory Board Member
- Diacarta — Consultant
- Karius — Consultant
- Research support: — LucidDx Technologies, Cyted Health

# MCD tests

- The current generation of MCD tests are primarily based on cfDNA based biomarkers.

- Milestones that have led to MCD tests:
  - Technical advances over prior barriers:
    - low recovery of cfDNA molecules
    - technical error suppression
    - DNA sequence analysis methods development

# Overview of MCD assay development



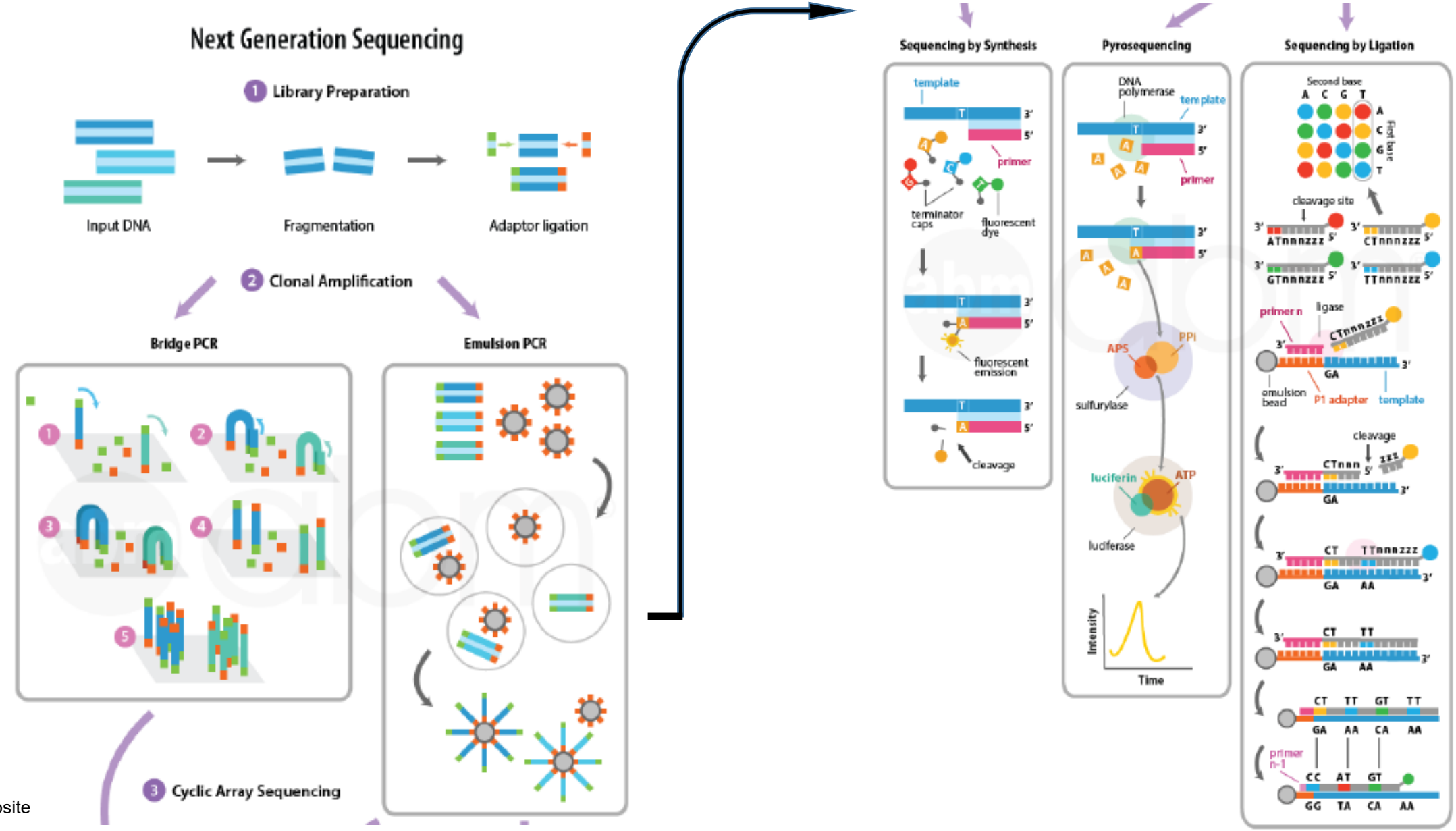
# Overview:  MCD test technology

- Biomarker assays based on panels of:
  - proteins
  - cfDNA: mutations (aka SNVs and CNAs), methylated DNA, chromatin fragments
  - cfDNA + proteins

- Emerging markers:
  - exosomal RNA and DNA
  - circulating microbial DNA
  - autoantibodies

- Most MCD tests depend on AI informed patterns of specific cancers

# NGS Technology Overview




Illumina website

# Genomic DNA sequence alterations

The array of genomic alterations that can be assessed by NGS has expanded over the last 5-10 years due to increased sequence depths and advances in sequence analysis

Type of genomic alterations that can be included in this type of analysis depends on whether the assay is a targeted mutation panel, whole exome or whole genome assay

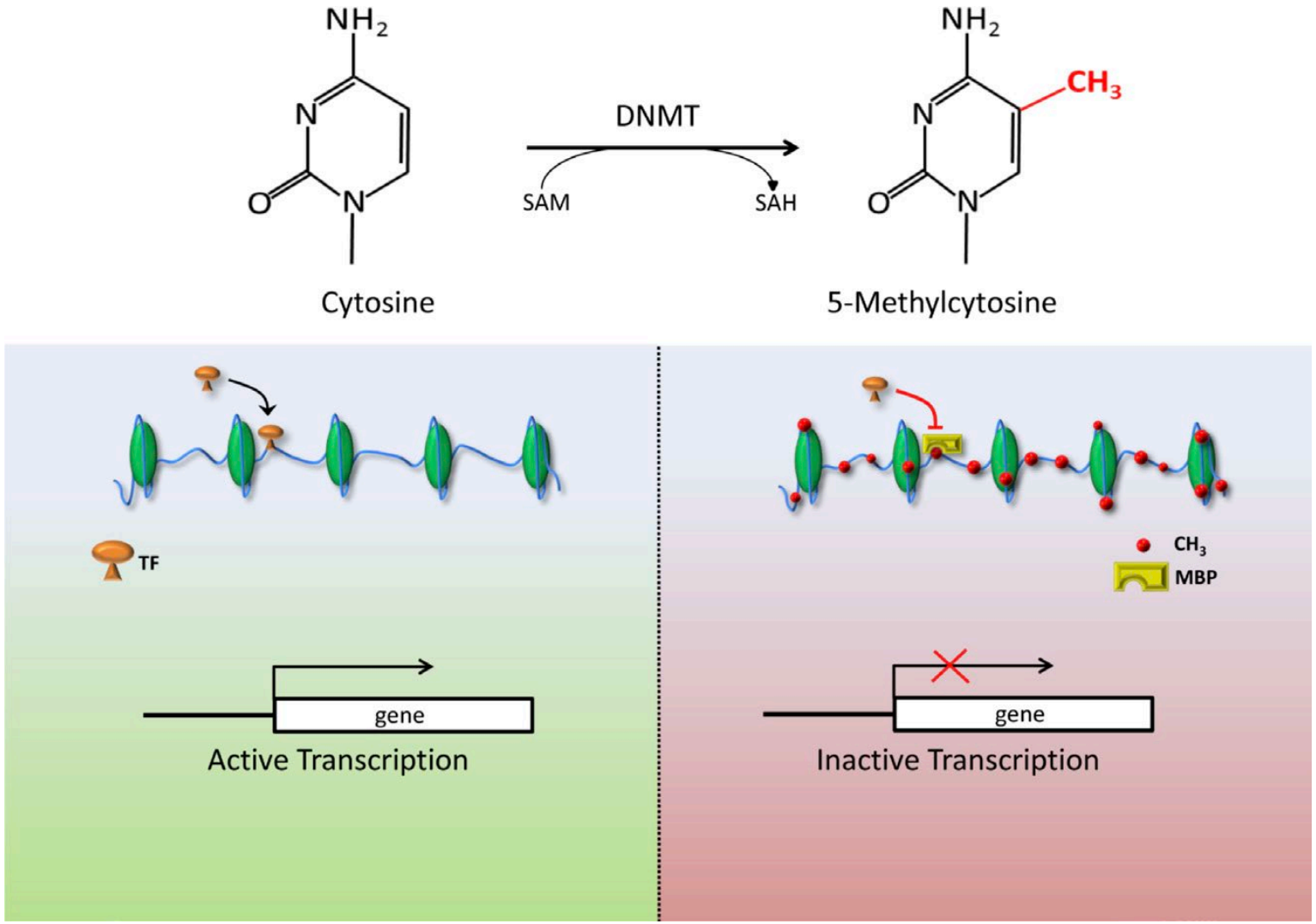
Types of alterations that can be assessed include:

- Nucleotide sequence variants (SNV): aka mutations
- Copy number alterations (CNA):  aka LOH, copy number gain and loss
- Fusion genes
- Gene deletions

# Methylomics

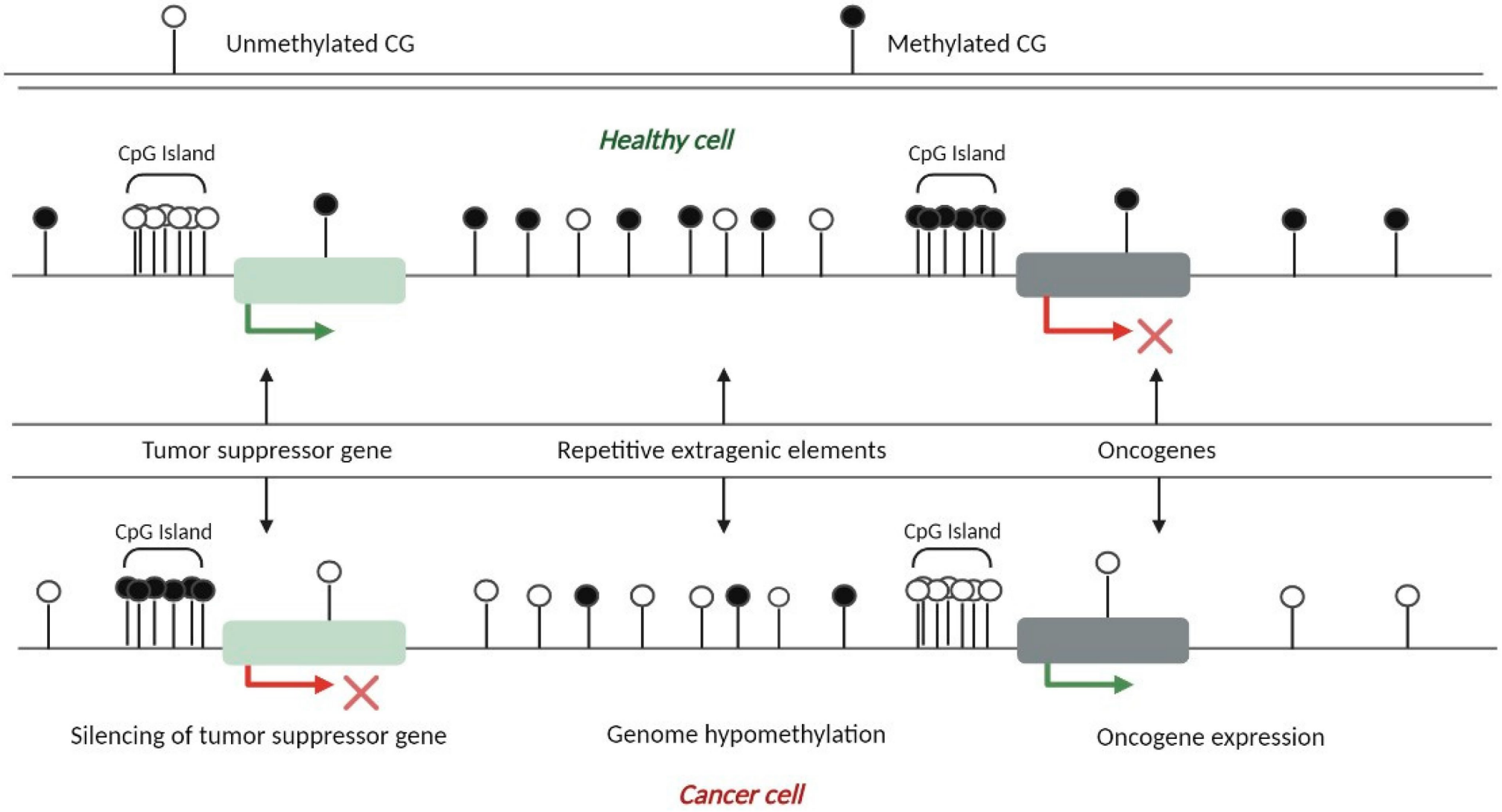
- Normal cell DNA has an established DNA methylation state that is
  - Unique to each tissue type
  - Reflects the gene expression pattern of the tissue
  - Reflects the stem cell of origin of the tissue.
- The methylation pattern is altered in cancers and premalignant lesions
- DNA methylation can also be altered by
  - Aging,
  - Exposure to certain dietary factors and medications, and
  - Benign disease states

# DNA methylation in health and disease




Zhong, 2016

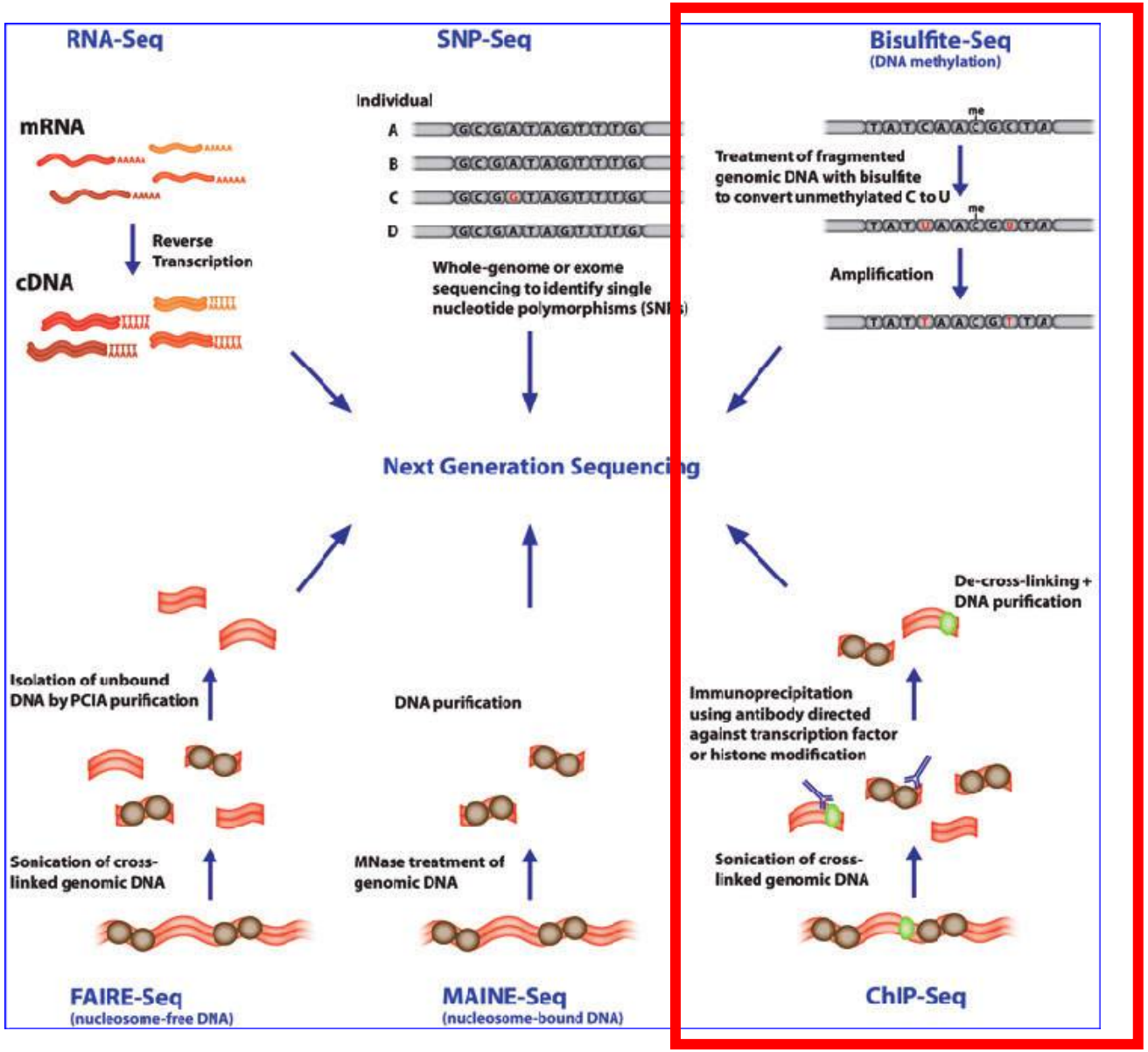
# DNA methylation alterations in cancer




Heredia-Mendez, 2023

# NGS methods for mDNA assessment

NGS based methods



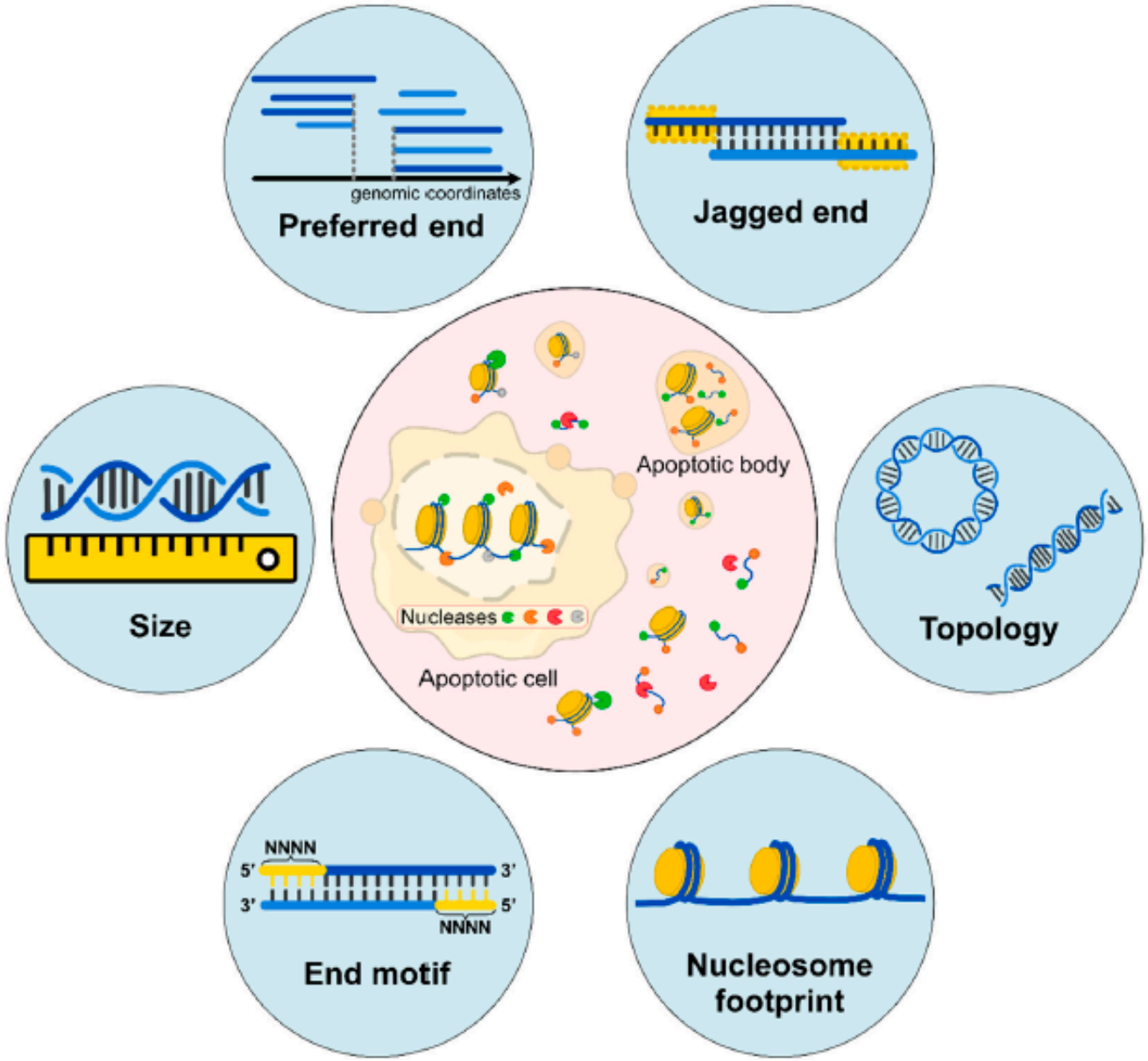
# Fragmentomics

- ‘Fragmentomics’:
  - utilizes patterns in healthy cell-free DNA to identify non-conforming molecules as potentially tumor-derived,
  - No need for knowledge of mutations commonly present in cancer cells

- Fragment Lengths:
  - Tumor DNA fragments are typically 3-6bp shorter than normal DNA
  - Tumor DNA fragments are more variable in length.

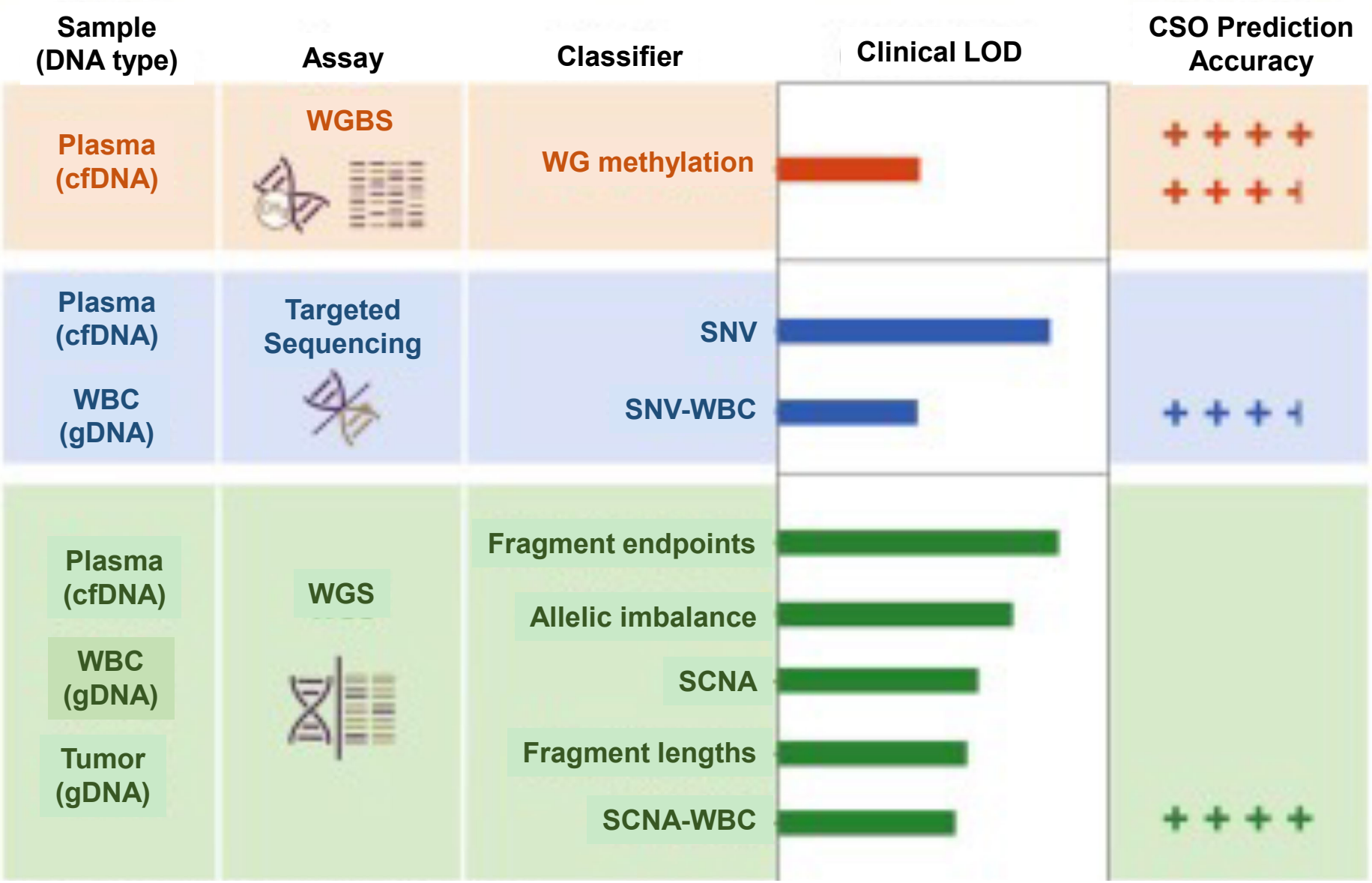
- The Fragment Pattern:
  - can also be used to infer transcriptionally active and inactive regions based on predicted nucleosome position
  - in vivo nucleosome footprints have been used to describe cell-of-origin

# Fragmentomics: Features of fragmented DNA




Ding, 2022

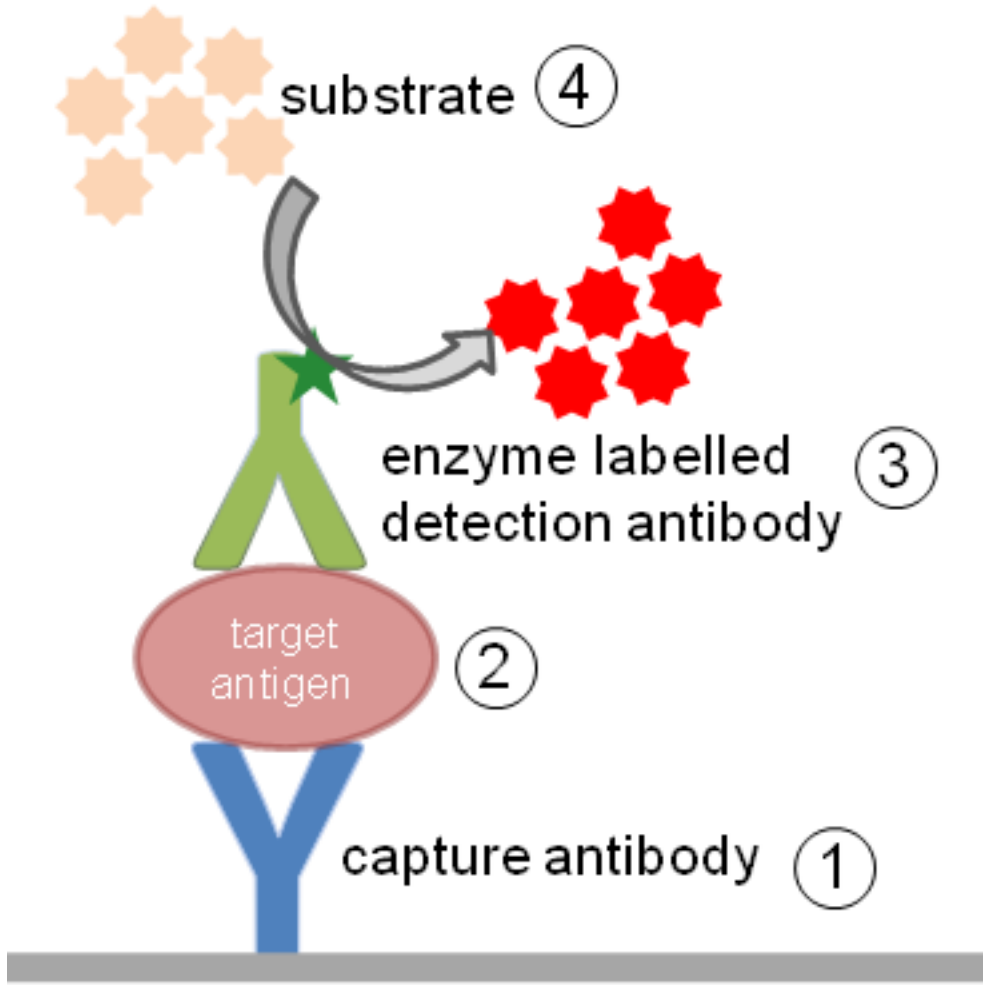
# cfDNA Features and Assays



# Protein based biomarkers

- Panels of protein based biomarkers have been included in some MCD tests.
- The proteins are typically detected using standard antibody-based assays—ELISA, RPA array
- These assays are tedious to develop and validate and are limited by the performance of the antibodies.
- They have the potential to increase sensitivity of the MCD test for early cancers.

# Antibody based detection assay



# Analysis of Biomarkers for use in MCD Tests

- There are millions of data points generated by the cfDNA analysis and protein analysis
- The datasets are assessed using AI based methods using sample sets of representative tumors to develop a specific biomarker signature.
- Key points
  - The specificity of the cancer signature appears to be mainly based on the mDNA data
  - The details of the MCD test methods and assay output are not available in the public domain.

# Conclusions

- Blood based cfDNA
- Blood based protein assays
- Multi-cancer detection assays
  - Technical performance of the assays is promising.
  - With current bioanalytes, sensitivity for earliest stage cancers variable and low compared to advanced cancers