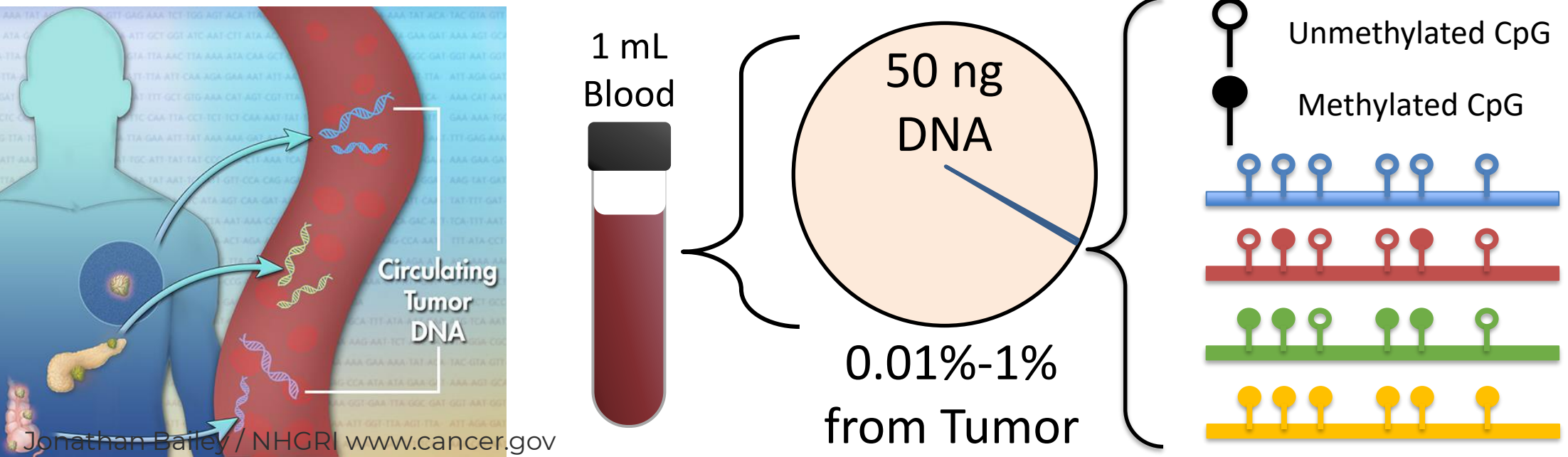


Targeted Methylation Profiling for Early Detection of Small Nodule Non-Small Cell Lung Cancer from Low-Volume Plasma

Introduction

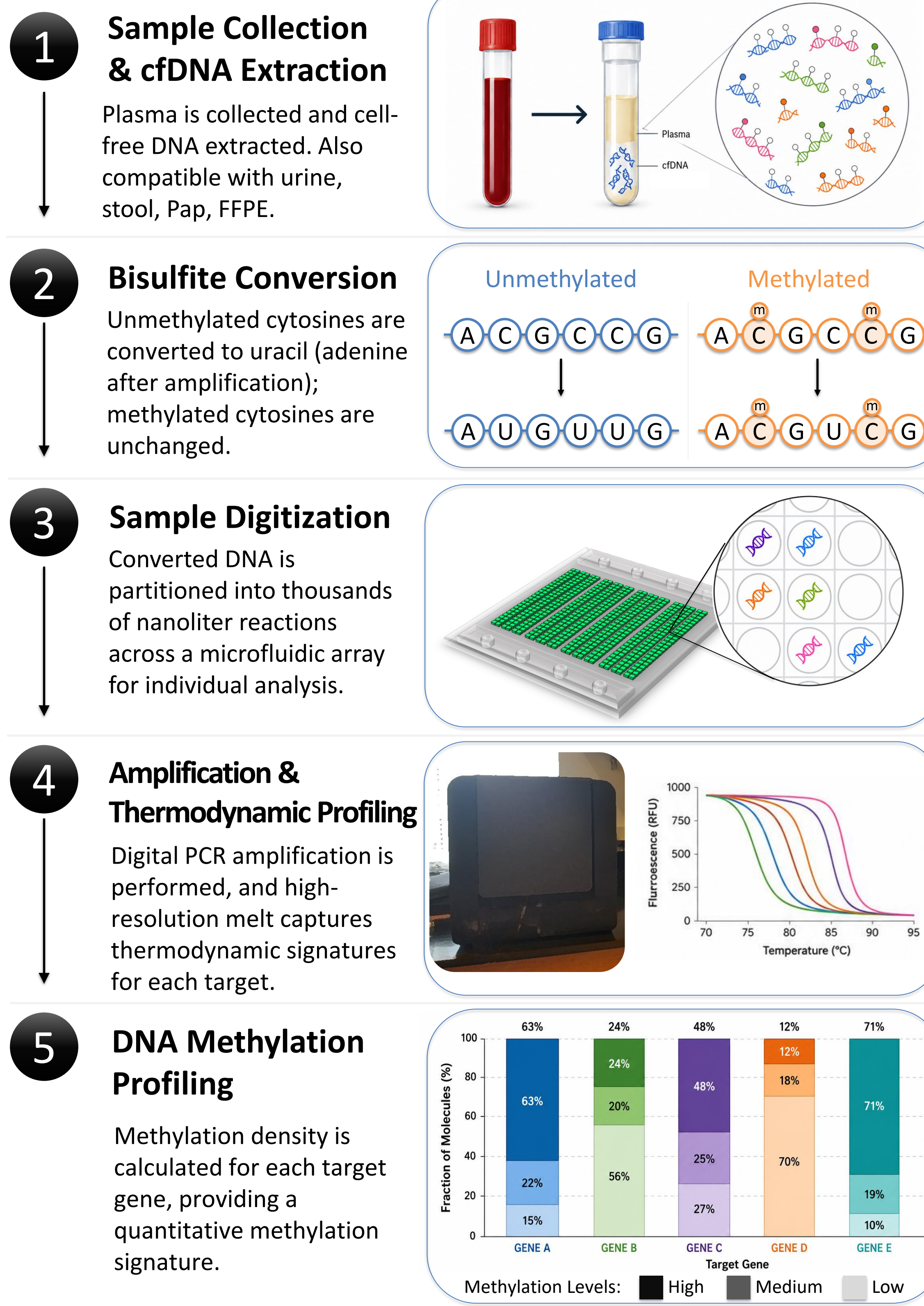
DNA methylation is a promising biomarker for early cancer detection and prognosis. Detection of cell-free DNA methylation biomarkers within non-invasive fluid, or liquid biopsies, is a promising avenue for routine analysis. However, tumor-derived DNA may represent less than 0.1% of DNA in a sample. Existing DNA methylation workflows are not well suited for detection of heterogeneous methylation patterns in samples with limited DNA quantities or high levels of background DNA.



Objectives

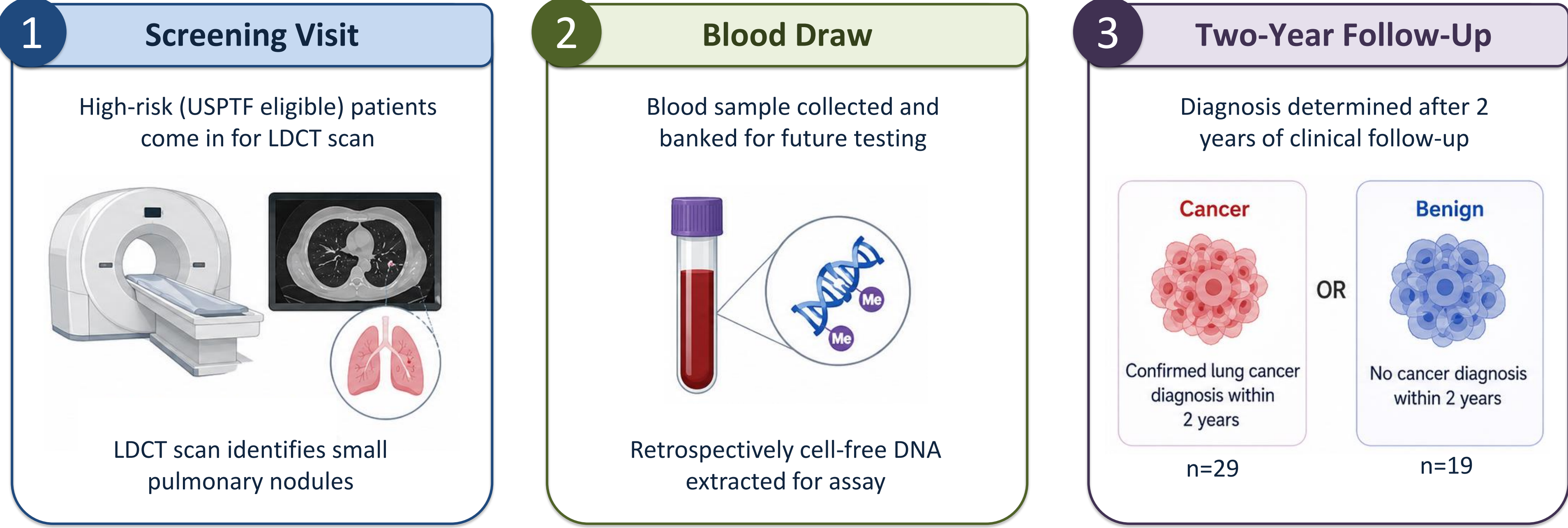
- ✓ To detect and discriminate circulating tumor DNA and rare subclonal epiallelic variants based on methylation density
- ✓ To create a simple, low-cost, and highly sensitive platform for the assessment of intermolecular heterogeneity on a per-molecule basis

Materials and Methods



Results

Clinical Proof-of-Concept in Non-small cell Lung Cancer

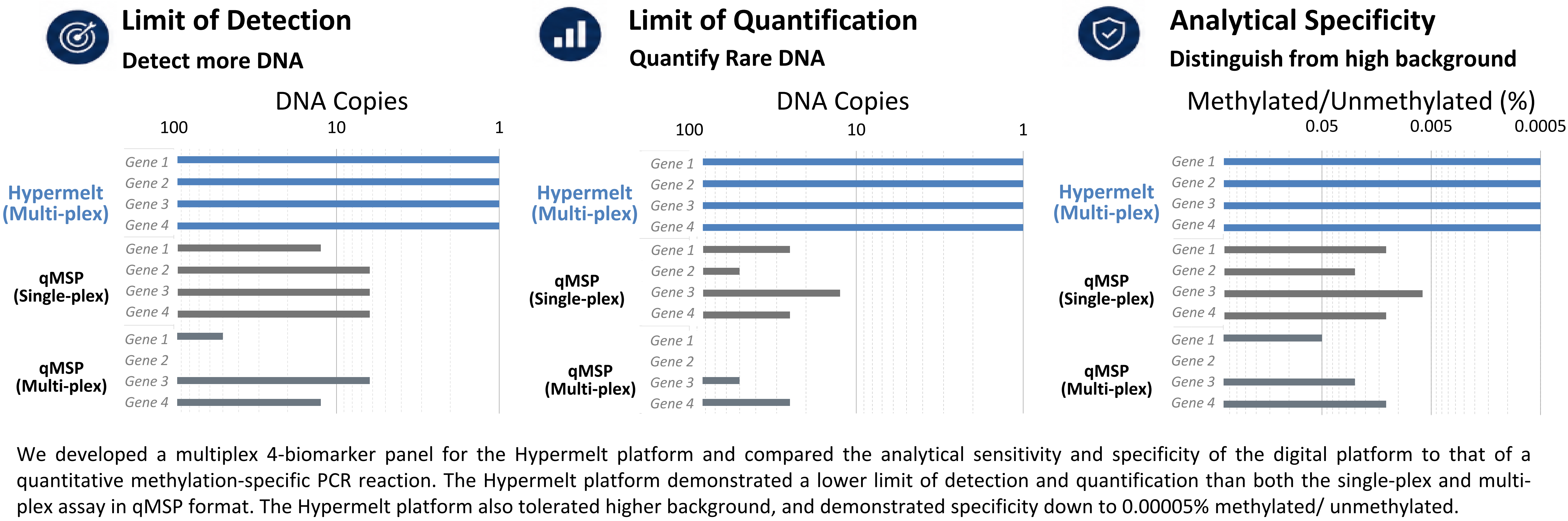


We developed a DNA methylation biomarker panel for early-stage NSCLC. We tested clinical feasibility by evaluating cell-free DNA in plasma from patients in a high-risk cohort who were undergoing routine screening via LDCT scans. Blood was collected at the time of an initial LDCT scan. After 2 years of follow-up, patients with small nodules were either diagnosed with NSCLC or nodules were determined to be benign.

Patient characteristics

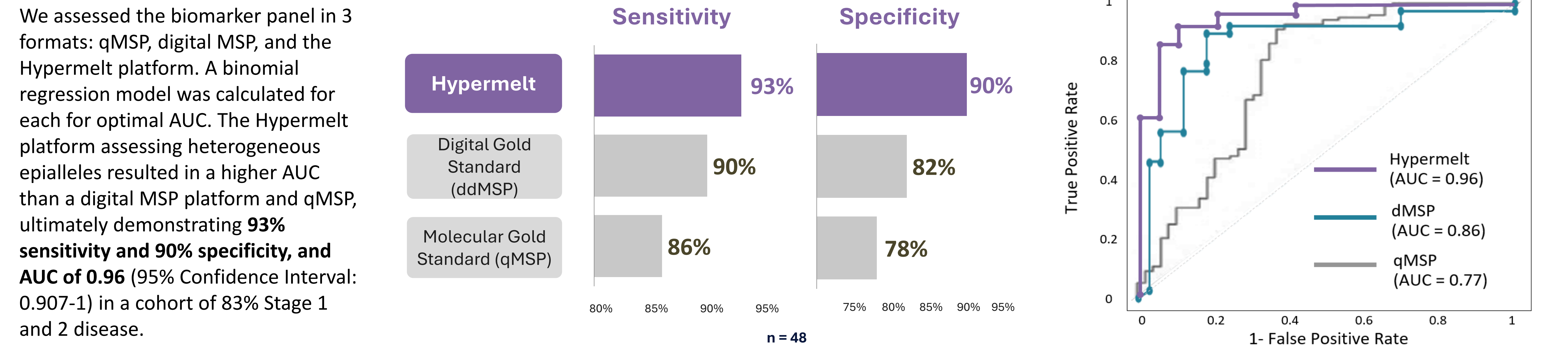
	Cancer (n = 29)	Control (n = 19)
Age at surgery (years; IQR)	66 (14)	61 (9)
Gender		
Male (%)	16 (55%)	11 (58%)
Female (%)	13 (45%)	8 (42%)
Smoking status		
Current (%)	14 (48%)	5 (26%)
Former (%)	15 (52%)	11 (58%)
Never (%)		3 (16%)
Stage		
I (%)	22 (76%)	N/A
II (%)	2 (7%)	
III (%)	4 (14%)	
IV (%)	1 (3%)	
Histology		
Adenocarcinoma	15 (52%)	N/A
Squamous cell	13 (45%)	
Large cell	1 (3%)	
Pack-year (IQR)	44 (35)	33 (37)

Assay Performance vs. qMSP



We developed a multiplex 4-biomarker panel for the Hypermelt platform and compared the analytical sensitivity and specificity of the digital platform to that of a quantitative methylation-specific PCR reaction. The Hypermelt platform demonstrated a lower limit of detection and quantification than both the single-plex and multi-plex assay in qMSP format. The Hypermelt platform also tolerated higher background, and demonstrated specificity down to 0.00005% methylated/ unmethylated.

Preliminary Performance Across Platforms



AI Disclaimer: AI was NOT utilized for generation of any text or data in this work. AI was utilized to generate cartoon images in the workflow description.

Seeking Collaborators

Do you want to help reduce the mortality rate of lung cancer? Please reach out to discuss possible collaborations. (Screening partners, biobank partners, distribution partners)
Emails: cmokeefe@jhmi.edu, thwang@jhu.edu, chrissy@hypermelt.com

Conclusion

- ✓ Achieves high-sensitivity targeted methylation profiling
- ✓ Quantifies epiallelic heterogeneity on a per-molecule basis
- ✓ Outperforms qMSP in sensitivity, quantification, and specificity
- ✓ Detects single methylated epialleles in a background of 2 million unmethylated molecules
- ✓ Shows feasibility with complex samples for detection of NSCLC biomarkers

Acknowledgements

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