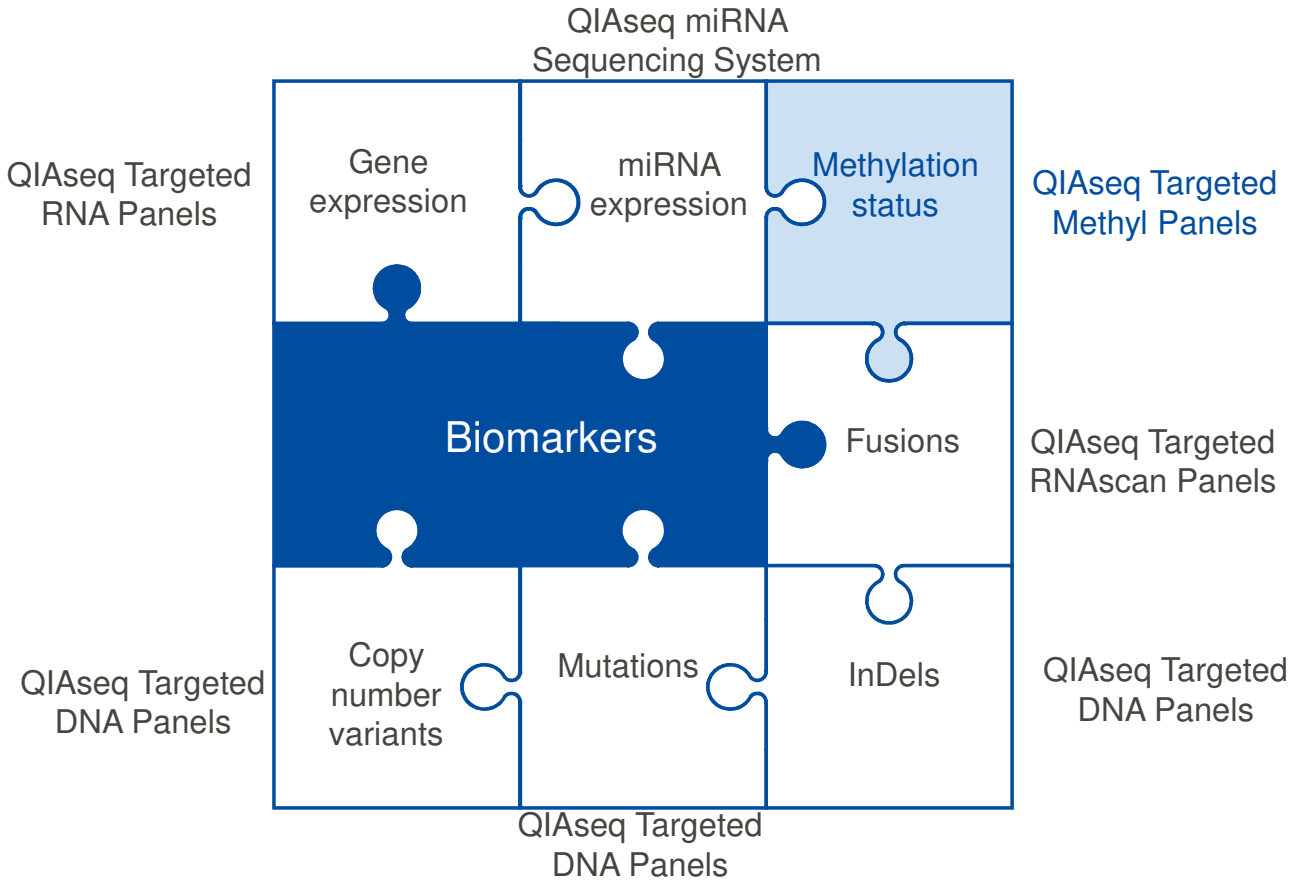
QIAseq Targeted Methyl Panels

A liquid biopsy-compatible solution for detection of methyl markers in NGS

Current QIAseq Sample to Insight solutions



QIAseq Targeted Methyl Panels – an overview

Sample types : FFPE, gDNA and liquid biopsy (circulating cell-free DNA or ccfDNA)

Starting material

- 1–100 ng gDNA
- 10–200 ng FFPE DNA
- 10–200 ng ccfDNA

Total workflow time: 7.5–9 h

Total hands-on time. 2.5–4.5 h

Panels for targeted methylation sequencing

- Human Breast Cancer Panel
- Human Colorectal Cancer Panel
- Immuno-Oncology Panel
- Human T-cell Infiltration Panel (coming in 2020)

Compatible with Illumina sequencers

Panel customization: Fully design-novel panel content based on genomic coordinates or CG identifiers

Data analysis options

- GeneGlobe Data Analysis Center
- QIAGEN CLC Genomics Workbench

QIAseq Targeted Methyl Panels powered by unique technologies

UMIs reduce bias

Unique molecular indices (UMIs) are unique index sequences that are ligated on to each bisulfite-treated DNA strand

- UMIs help to overcome bias during PCR and bridge amplification
- Data is now representative of the unique number of molecules in a sample

SPE enhances CpG targeting

Single primer extension (SPE) enables increased targeting and multiplexing capacity

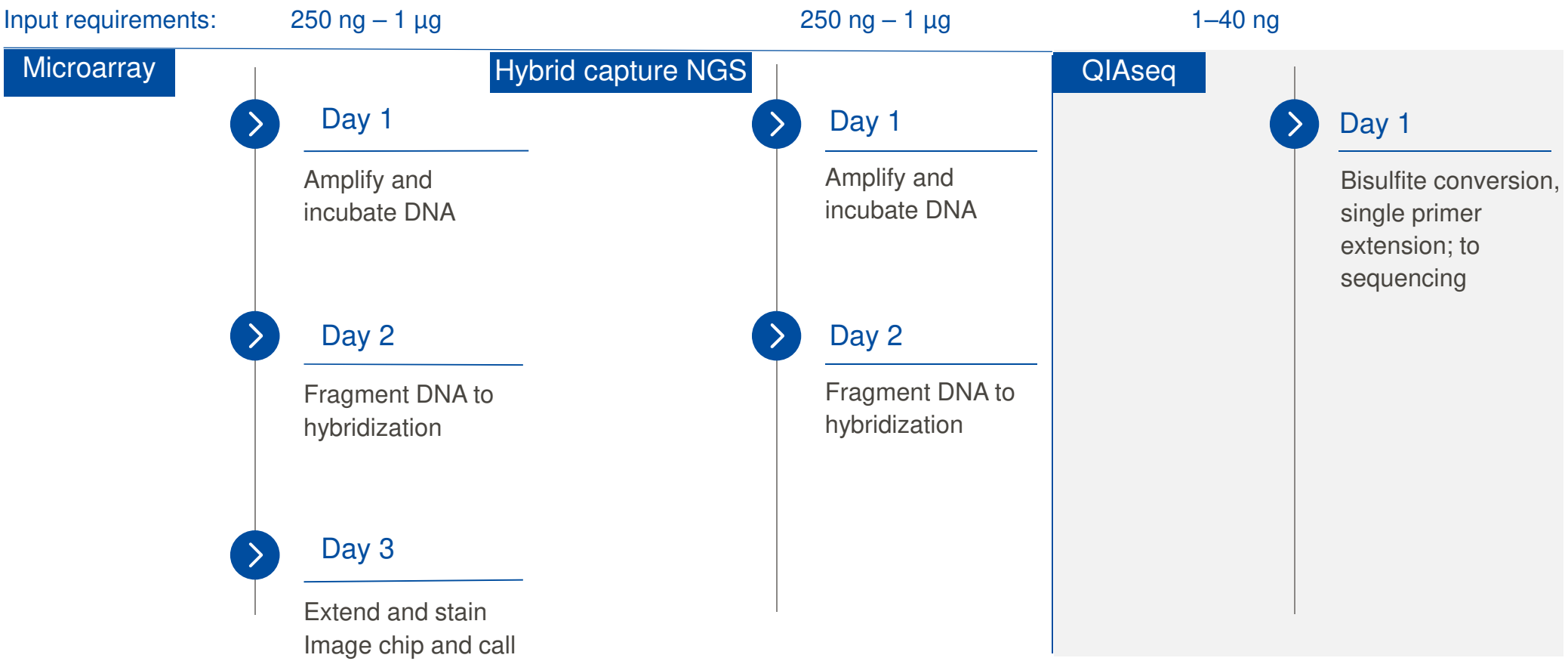
- Targeting only a single region in intronic regions reduces the need for paired primers
- A universal primer is used to create sequencing ready libraries

Liquid biopsy and FFPE compatible

Methylation can be indicative of genomic alterations and identify certain cell and tissue types. The kit is compatible with ccfDNA and ultralow input levels, even from FFPE to help determine these patterns.

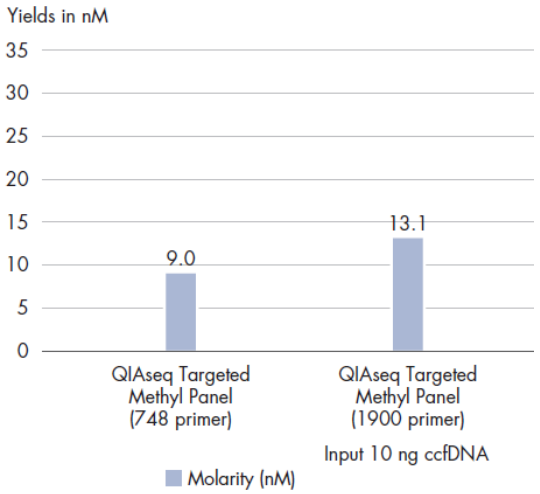
- 10–40 ng of DNA needed for liquid biopsy applications
- Minimum of 1 ng of purified gDNA for cells and tissue for the rarest samples

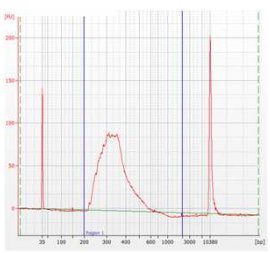
QIASeq Targeted Methyl Panels save you days to months of work

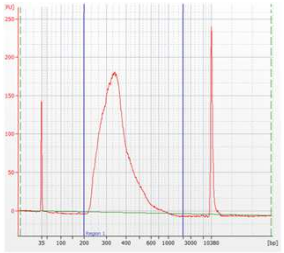


Targeted methylation sequencing from cell-free DNA

Typical library characteristics







QIAseq Targeted Methyl Panel library preparation in combination combination with EpiTect Fast bisulfite conversion allow sensitive applications such as bisulfite sequencing of ccfDNA

High yield by avoiding extended degradation of DNA during bisulfite treatment

Procedure:

ccfDNA was purified using QIAamp chemistry and subsequently bisulfite treated using EpiTect Fast chemistry

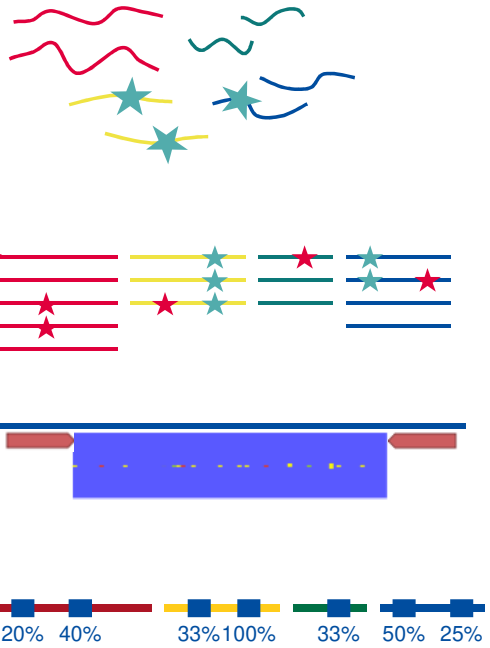
10 ng ccfDNA was processed using 2 different panels

Library amplification with 19 cycles resulted in high library yields and high cytosine coverage

The expected 350 bp library size represents the combination of ccfDNA with typical size of 170 bp and 180 bp adapters

UMI-enabled resolution of true vs false variants improves variant calling accuracy

Conventional methodology



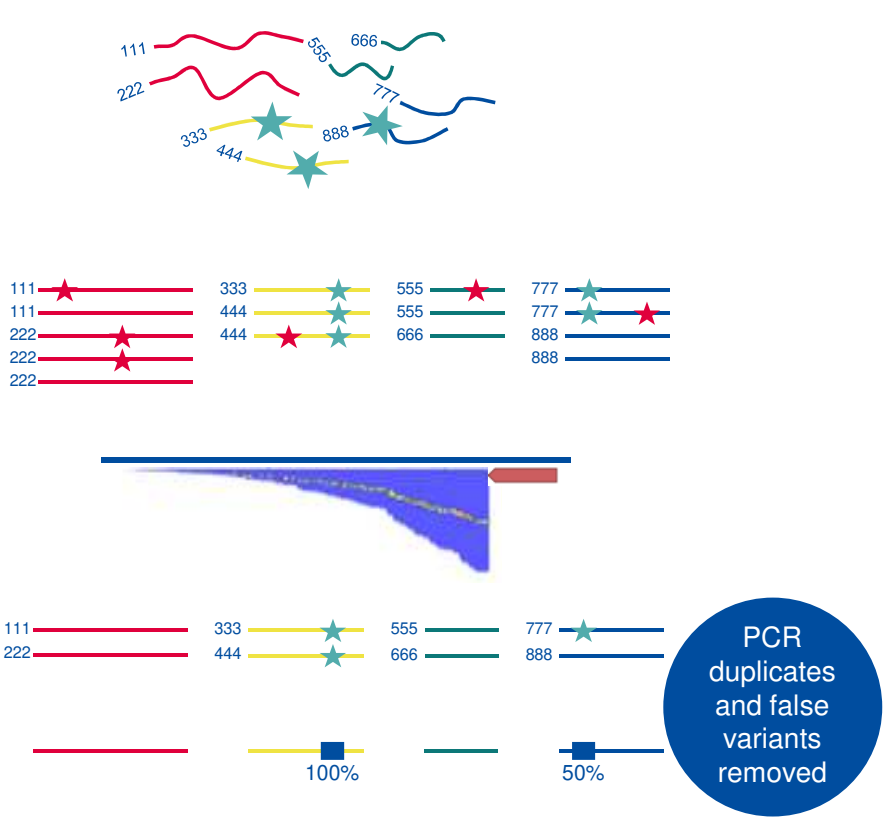
Library amplification

Map to reference

Collapse UMI

Variant calling

QIAseq approach



Single primer extension (SPE) enhances uniformity and sensitivity for degraded samples

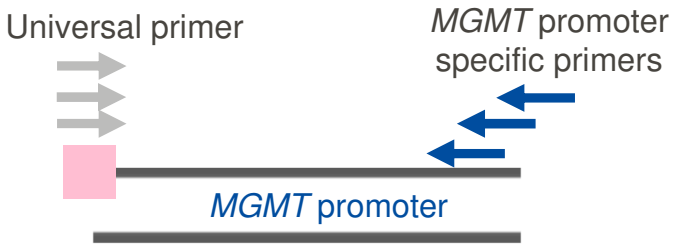
2-primer amplicon design

Two primers are required to define a targeted region

Outcomes:

- Increased risk of primer dimers and dropouts
- Lower uniformity
- Multiple pools of primers might be required
- Low library complexity
- Increasing panel content might require primer redesign

SPE amplicon design



Only one primer is required to define a targeted region

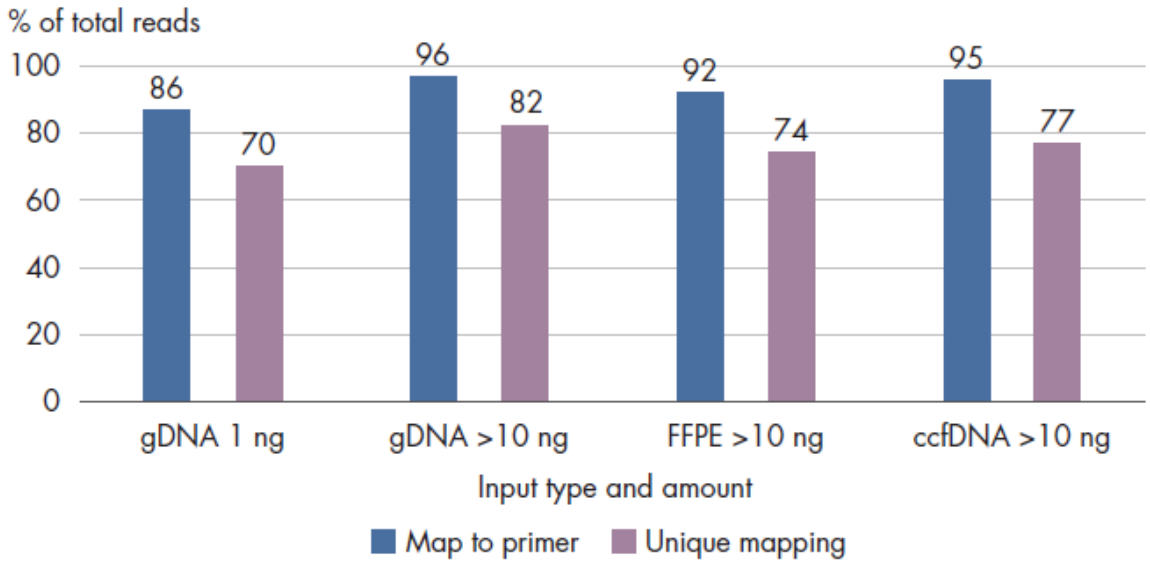
Outcomes:

- Lower risk of primer dimers and dropouts
- Higher uniformity
- Only a single pool of primers required
- Higher library complexity
- Increasing panel content does not require primer redesign

Universal primer (→) binds to universal sequences introduced through UMI-containing library adapters (▭).

High performance from tissue and liquid biopsy: 1–10 ng of input range

QIAseq Targeted Methyl Panel: High mapping efficiency even at 1 ng of input

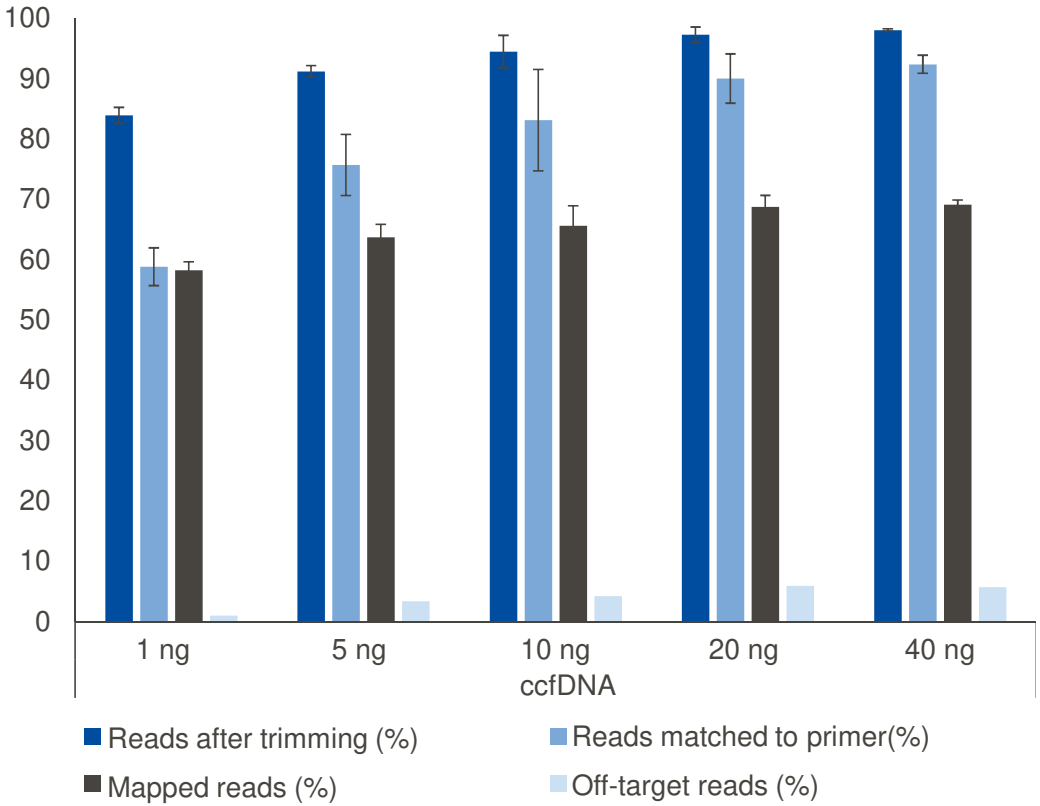


- gDNA was run at both 1 and 10 ng
- FFPE was run at 10 ng
- ccfDNA was run at 10 ng

High mapping on primer and unique reads even from 10 ng inputs

High mapping of sequencing reads even from ultra low input ccfDNA

Percentage of total reads

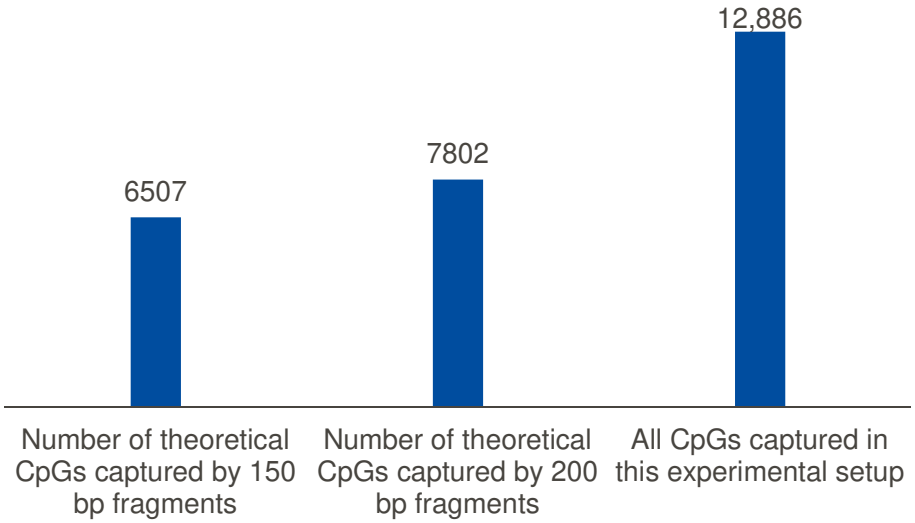


- Input: 1,5,10,20 and 40 ng of on stabilized ccfDNA purified with QIAamp chemistry
- Samples were processed using EpiTect Fast and a custom panel consisting of 1791 primers
- Off-target effects are minimal with SPE-based chemistry

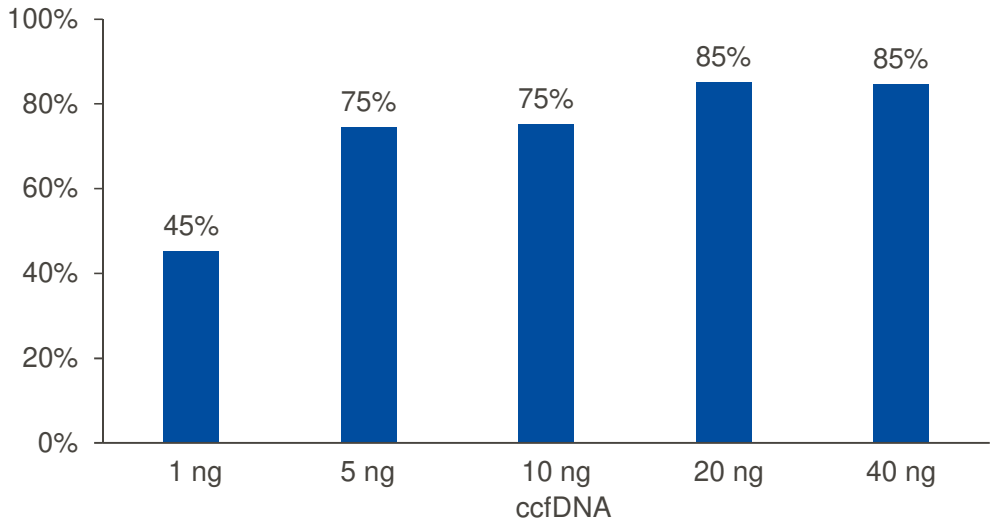
Coverage of expected CpGs is highest between 20–40 ng of ccfDNA input

- Overall high CpG coverage from 5–40 ng
- Expected CpG coverage from coordinate designs often underreports the total number of CpGs seen in sequencing data
- 1791 primers cover 12,886 CpG regions
- High coverage was obtained even from 5 ng based on 150 bp read length

Number of CpGs captured by the MCHS-0017 Panel

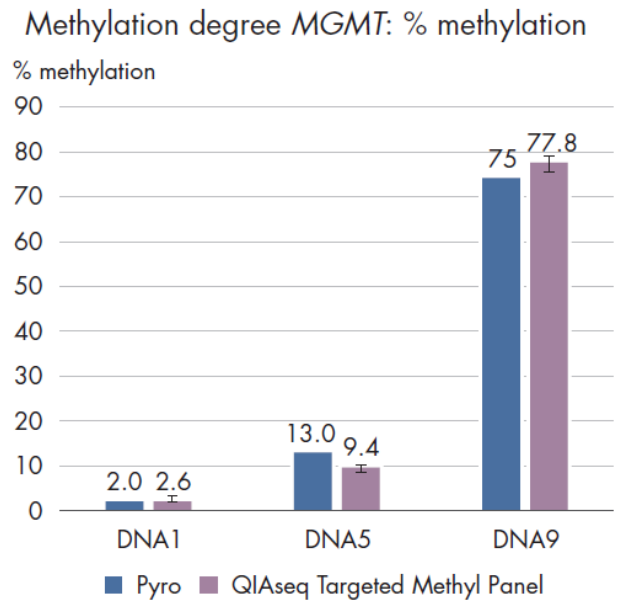


Percentage of total reads



Much lower input amounts with high correlation to established methods

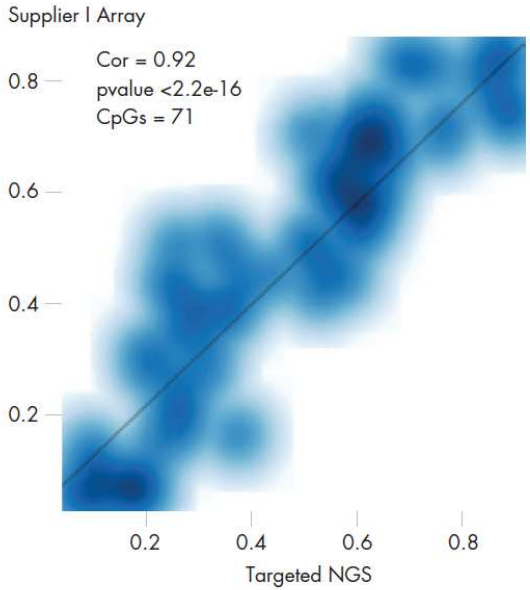
High correlation to Pyromark assays



Input: 40 ng FFPE DNA

Primers: 93 primers covering 566 CpG sites; 7 CPG sites on the *MGMT* gene, previously validated with pyrosequencing, were compared to the targeted methyl result

High correlation (92%) to EPIC array despite 1/5th the input



Input: 40 ng gDNA from hepatocytes

Primers: 102 primers covering 71 CpGs

QIASeq Targeted Methyl Panels – summary

Liquid biopsy-capable panels with a single day-to-sequencer workflow from a few nanograms of input

Can’t get 40 ng of input

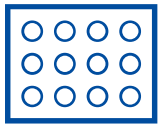
You can go from as low as 1–10 ng of input to your bisulfite reaction

Pressed for time

With less than 7 h for library prep and another 1.5 h for bisulfite conversion, you can get to sequencer in a day

Never analyzed methylation data

Cloud-based and local solutions on Geneglobe.com and through the QIAGEN CLC Workbench



Need to customize the panel content

Custom design 1000s of targets from a single pool

Need to work with FFPE and ccfDNA

Compatible with fresh tissue, FFPE and ccfDNA

Thank you for your attention

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