Performance of a novel NGS-based **Bridge Capture™** technology for mutation profiling in **liquid biopsies** derived from **metastatic colorectal cancer patients**

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Background

Liquid biopsy is a minimally invasive alternative to tissue biopsy and imaging modalities for cancer diagnostics. **Circulating tumor DNA (ctDNA)**, tumor-derived DNA in the bloodstream, can be used to monitor treatment response, detect **minimal residual disease (MRD)**, and identify resistance-associated mutations. This is the first study to evaluate the performance of a novel NGS-based **Bridge Capture™** technology for mutation profiling and MRD detection in ctDNA from patients with metastatic colorectal cancer (mCRC).

Study Design

The performance of **Bridge Capture™** technology was compared to droplet digital PCR (**ddPCR**), **Ion AmpliSeq** Cancer Hotspot Panel v2 (CHPv2), and RT-qPCR based **Idylla** ctKRAS Mutation Assay. Additionally, the analytical performance and scalability of the technology were assessed using synthetic DNA targets.

a

Serial plasma samples from ten patients with *KRAS* mutation in the primary tumor tissue (n = 80)

Bridge Capture™ (n = 80)

282-probe panel targeting hotspots of 84 oncogenes & tumor suppressor genes.

Ion AmpliSeq (n = 10)

207-primer pairs targeting hotspots of 50 oncogenes & tumor suppressor genes.

ddPCR (n = 80)

Single probe per sample targeting 5 *KRAS* mutations in codons 12 and 13.

Idylla (n = 65)

Probes targeting 21 *KRAS* mutations in codons 12, 13, 59, 61, 117, and 146.

b

Synthetic samples with DNA targets for each probe

0.0078–1% VAF

→ **Bridge Capture™**

851-probe panel targeting hotspots of 121 oncogenes & tumor suppressor genes.

Figure 2. Evaluation of Bridge Capture™ using (a) ctDNA extracted from plasma samples of mRCR patients and (b) synthetic DNA targets. ddPCR: droplet digital PCR; NGS: next generation sequencing; RCA: rolling circle amplification; VAF: variant allele frequency

Bridge Capture™

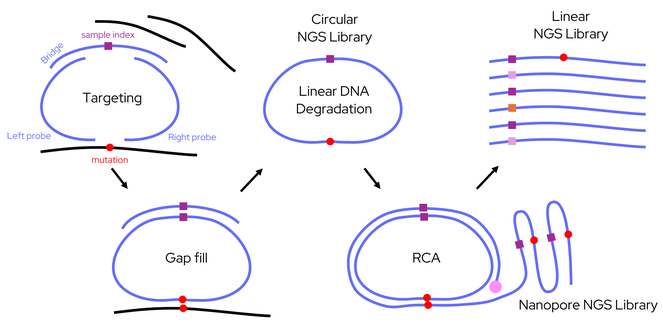

Figure 1. Bridge Capture™ single-tube workflow is simple and rapid. It allows first-step sample indexing, and after only few steps libraries for new generation sequencing platforms can be produced. Red circle depicts a **mutation**, purple, pink, and orange squares depict a **sample index**, and pink circle is a RCA polymerase.

Results

Bridge Capture™ showed **substantial agreement and perfect agreement** with ddPCR (Cohen's Kappa 0.70), and Idylla (Cohen's Kappa 0.79), and with Ion AmpliSeq CHPv2, respectively (Table 1). Bridge Capture™ displayed **very strong correlation** between both ddPCR (ρ = 0.86) (Figure 3a) and Ion AmpliSeq (ρ = 0.74) (Figure 3b). Bridge Capture™ **expanded 851-probe panel** showed very strong correlation until 0.03% (R = 0.99) (Figure 3c). Figure 4 displays serial samples of four mCRC patients with *KRAS* mutations detected by ddPCR and Bridge Capture™, including eight **additional CRC driver mutations** in other genes mirroring the *KRAS* mutations.

Table 1. Concordance between Bridge Capture™ and ddPCR, Idylla, and Ion AmpliSeq CHPv2 in detecting *KRAS* mutations from ctDNA of mCRC patients. Cohen's Kappa measures the agreement between two raters. The range between 0.61–0.8 is regarded as substantial agremeent.

		Bridge Capture™ +	Bridge Capture™ −	Cohen's Kappa
ddPCR (n = 80)	+	35	4	0.70
	−	8	33	
Idylla (n = 58)*	+	31	4	0.79
	−	2	21	
Ion AmpliSeq (n = 10)	+	7	0	1.0
	−	0	3	

*7 samples failed (1 positive and 6 negative for Bridge Capture™).

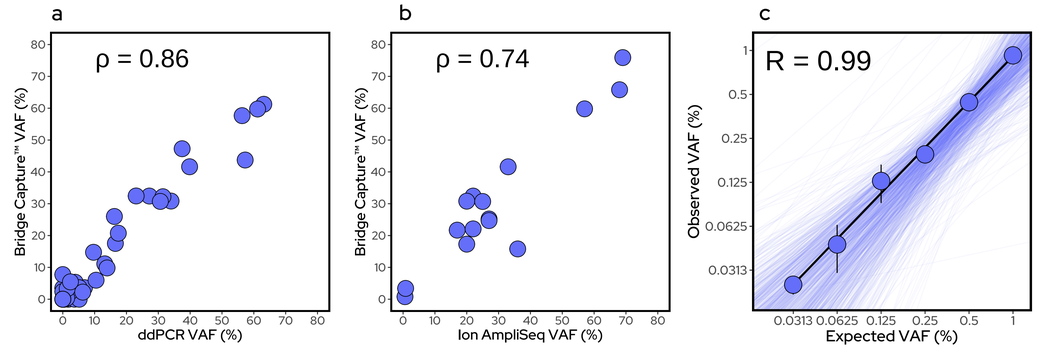

Figure 3. Correlation of ctDNA VAF values from mCRC patients between Bridge Capture™ and other methods. (a) Comparison with ddPCR for *KRAS* mutations across 80 patient samples (ρ = 0.86). (b) Comparison with Ion AmpliSeq CHPv2 across 10 patient samples (ρ = 0.74). (c) Dilutions from 0.0313–1% displayed strong linear correlation between the observed and expected total specific VAF values (Pearson R = 0.99). Violet circles are mean values of 5 replicates and SD is displayed as error bars. Individual linear regressions of each probe are displayed as violet lines.

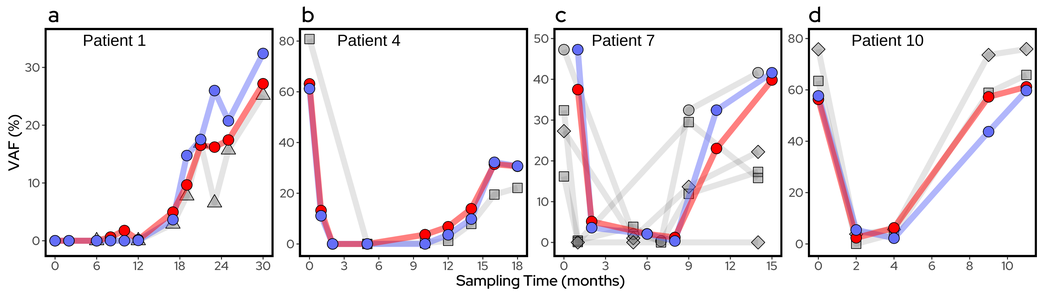

Figure 4. Serial samples of four mCRC patients with *KRAS* (circle) mutations detected by **Bridge Capture™ (violet)** and **ddPCR (red)**. Bridge Capture™ reported additional oncogenic mutations (gray) in *TP53* (square), *APC* (diamond), and *PIK3CA* genes (triangle).

Conclusions

Bridge Capture™ is simple, rapid and sensitive targeted sequencing technology **compatible with all new generation sequencing platforms**. The technology's unique features enable **first-step sample indexing** which simplifies even further the already few-step **single-tube** sample processing enabling increased cost benefits.

Bridge Capture™ improved mutation profiling and MRD detection in mCRC ctDNA samples by reporting the **broader mutational landscape without sacrificing sensitivity**. It displayed substantial agreement with other technologies and the observed scalability enables even more comprehensive view of the mutational landscape **without the need for extremely deep sequencing.**

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