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Bridge Capture Permits Cost-Efficient, Rapid, and Sensitive Molecular Precision Diagnostics

Simona Adamusová,*† Anttoni Korkiakoski,*† Nea Laine,* Anna Musku,* Tuula Rantasalo,* Jorma Kim,* Juuso Blomster,*‡ Jukka Laine,*§¶ Tatu Hirvonen,* Juha-Pekka Pursiheimo,* and Manu Tamminen*†

From Genomill Health Inc., Turku; the Departments of Biology,† Cardiology,‡ and Pathology,§ University of Turku, Turku; and TYKS Laboratories, Department of Pathology,¶ Turku University Hospital, Turku, Finland*

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Address correspondence to Simona Adamusová, M.Sc., or Manu Tamminen, Ph.D., Genomill Health, Itäinen Pitkäkatu 4 B, FI-20520 Turku, Finland.
E-mail: sa@genomill.com or manu@genomill.com.

Liquid biopsies quantifying mutations in circulating tumor DNA by targeted next-generation sequencing have been gaining popularity. They are performed by various library preparation methods, each with distinct advantages and limitations. This work introduces Bridge Capture, a novel technology that goes beyond the advantages of market-leading liquid biopsy technologies, eliminating the need to compromise between scalability, cost-efficiency, sensitivity, or panel size. Twenty-four matched contrived colorectal biospecimens mimicking circulating tumor DNA were analyzed by Bridge Capture, Archer LIQUIDPlex, and AmpliSeq CHP version 2 for Illumina to compare variant allele frequency (VAF) detection. Bridge Capture was evaluated for sequencing depth requirement, interlaboratory reproducibility, automatization, and panel scalability. Of all methods, Bridge Capture detected the lowest VAF, and all VAFs strongly correlated with Archer LIQUIDPlex ($R^2 = 0.995$) and AmpliSeq CHPv2 for Illumina ($R^2 = 0.988$). Owing to its unique design, the Bridge Capture is compatible with the commonly used next-generation sequencing platforms and effectively uses sequencing capacity, permitting affordable and sensitive variant detection. The method demonstrated high reproducibility across independent laboratories and between automated and manual workflow. The panel size was increased by 300% and had negligible impact on performance and cross-reactivity of the probes, implying high multiplexing capabilities. Taken together, Bridge Capture is a cost-efficient, simple, rapid, and sensitive cancer diagnostics tool that has a potential to significantly improve the detection of mutations. *(J Mol Diagn 2026, 28: 53–63; https://doi.org/10.1016/j.jmoldx.2025.09.006)*

Tissue biopsies are the primary method for diagnosing and monitoring cancer. However, a new family of techniques, collectively known as liquid biopsies (LBs), is gaining popularity. LBs permit less invasive and simplified diagnostics, and more feasible longitudinal disease monitoring crucial for tracking the disease progression and the effectiveness of treatment.[1]

A key component of LB is circulating tumor DNA (ctDNA), which is a part of cell-free DNA found in blood. ctDNA originates from tumor cells shedding their DNA through apoptosis, necrosis, or active release and reflects the genetic makeup of the tumor.[2,3] A key aspect of ctDNA analysis is quantifying variant allele frequencies (VAFs). A VAF indicates the proportion of a mutation in cell-free DNA. A higher VAF suggests a larger tumor burden, providing insight into the extent of the cancer in the body.[3–6]

Next-generation sequencing (NGS) is a crucial tool for precision cancer diagnostics and is roughly divided into whole-genome sequencing and targeted sequencing. Contrary to the whole-genome sequencing, targeted sequencing focuses only on the regions of interest relevant to the disease.[7] Use of targeted sequencing streamlines sequencing costs through improved sequencing depth use, and decreased data storage and analysis requirements. Importantly, targeting of the sequencing effort permits detecting mutations present at low VAFs.[8]

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J.-P.P. and M.T. contributed equally to this work.

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Targeted sequencing can be achieved through various approaches, such as amplicon-, hybridization-, and molecular inversion probe (MIP)—based NGS library preparation methods. Amplicon-based methods target regions of interest via specifically designed primer pairs by PCR.[9,10] They provide a simple and rapid workflow[11–13] but generally have limited scalability because of primer cross-reactivity and are prone to false variants owing to PCR amplification error rates.[7,14] Hybridization-based methods use chemically modified oligonucleotides to enrich prespecified parts of NGS libraries.[15–17] These methods permit extensive panels and can more efficiently provide information from difficult genomic regions, such as repeat sequences.[8] However, they are generally expensive and time-consuming. MIPs are single-stranded oligonucleotides that hybridize to target genomic region with terminal ends. The resulting gap between the probe ends is filled, and the molecule is circularized.[18] MIPs offer cost efficiency compared with hybridization-based methods, and improved panel size and scalability compared with amplicon-based methods. Their downsides include nonuniform coverage, high probe synthesis cost, and increased noise compared with amplicon-based methods.[19]

To address these shortcomings, Bridge Capture, a novel targeted NGS library preparation technology, was developed to enable sensitive detection of low VAF while remaining rapid, highly multiplexed, and cost-efficient. Bridge Capture consists of a bridge oligomer bound to probes targeting the region of interest (Figure 1). After the probes capture the region of interest, all the gaps between the oligomers are filled and ligated to form a circular molecule. The circular molecule is amplified by rolling circle amplification and is cleaved into monomers by a restriction enzyme. The flowcell binding sequences are attached to the monomers through limited-cycle PCR. This unique design offers several benefits (namely, allowing use of Bridge Capture with commonly available NGS platforms, decreasing the synthesis costs of the panels compared with MIP-based methods, and permitting the use of highly multiplexed panels).

This presents an initial method evaluation of Bridge Capture, a patented NGS library preparation workflow[20] that exceeds the performance of commercially available technologies while providing a workflow that combines the speed, sensitivity, and simplicity of the amplicon- and MIP-based methods with the scalability of the hybrid capture methods.

Key Points

- Bridge Capture, a novel targeted next-generation sequencing method for cancer diagnostics, offers superior sensitivity to commercial methods.
- The method is rapid and uses sequencing depth efficiently; thus, it significantly reduces costs of analysis.
- Bridge Capture is easily adoptable and reproducible in new laboratory settings and readily suited for automation.

Materials and Methods

Sample Preparation

Biospecimens were purchased from Indivumed GmbH (Hamburg, Germany). The ethics committee review was waived by the Research Ethics Committee of the well-being services county of Southwest Finland. Total DNA from fresh-frozen colorectal cancer (CRC) tissue samples was isolated using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), and genomic DNA (gDNA) from the whole blood was extracted using NucleoSpin Blood (Macherey-Nagel, Düren, Germany). The extracted DNA was fragmented using Bioruptor Pico (Daigenode, Liege, Belgium). A total of 50 μL of sample was loaded in the 0.1-mL Bioruptor Microtube (Diagenode), and the sample was fragmented using 60 cycles with cycle conditions of 30 seconds ON cycle followed by 30 seconds OFF cycle to mimic size distribution of cell-free DNA. Processed samples were frozen at −80°C, and double-stranded DNA concentration of the sample was measured the following day using Qubit 4 Fluorometer and Qubit dsDNA HS (high sensitivity) Assay Kit (ThermoFisher Scientific, Waltham, MA).

Bridge Capture Library Preparation

To perform a Bridge Capture library preparation, a total of 320 ng of fragmented DNA (approximately 100,000 copies of gDNA) was used as starting material input. Samples underwent overnight target capture using Genomill's proprietary 282-probe panel (Supplemental Table S1). The details of Bridge Capture library preparation are further described in US11486003B2.[20] The Bridge Capture libraries were purified with 0.9× Agencourt Ampure XP beads (Beckman Coulter, Brea, CA). The libraries were quantified by Qubit 4 Fluorometer and Qubit dsDNA HS (high sensitivity) Assay Kit. The sequencing platform used for each experiment is specified in its respective section.

Preparation of Bridge Capture Probe Panels

Bridge oligomer and probes were synthesized by Integrated DNA Technologies (Coralville, IA). Corresponding left and right probes were mixed with the bridge oligomer in a 96-well plate using automatized pipetting robot Opentrons OT-2 (Opentrons, Brooklyn, NY) and afterwards annealed in a C1000 Touch Thermal Cycler (Bio-Rad, Hercules, CA). Each annealed probe pair and bridge were pooled by Opentrons OT-2 to prepare 25 nmol/L panel stock solution for the 282-probe panel and 10 nmol/L panel stock solution for the 887-probe panel. The list of genes and the number of probes per gene in the 282-probe panel and in the 887-probe

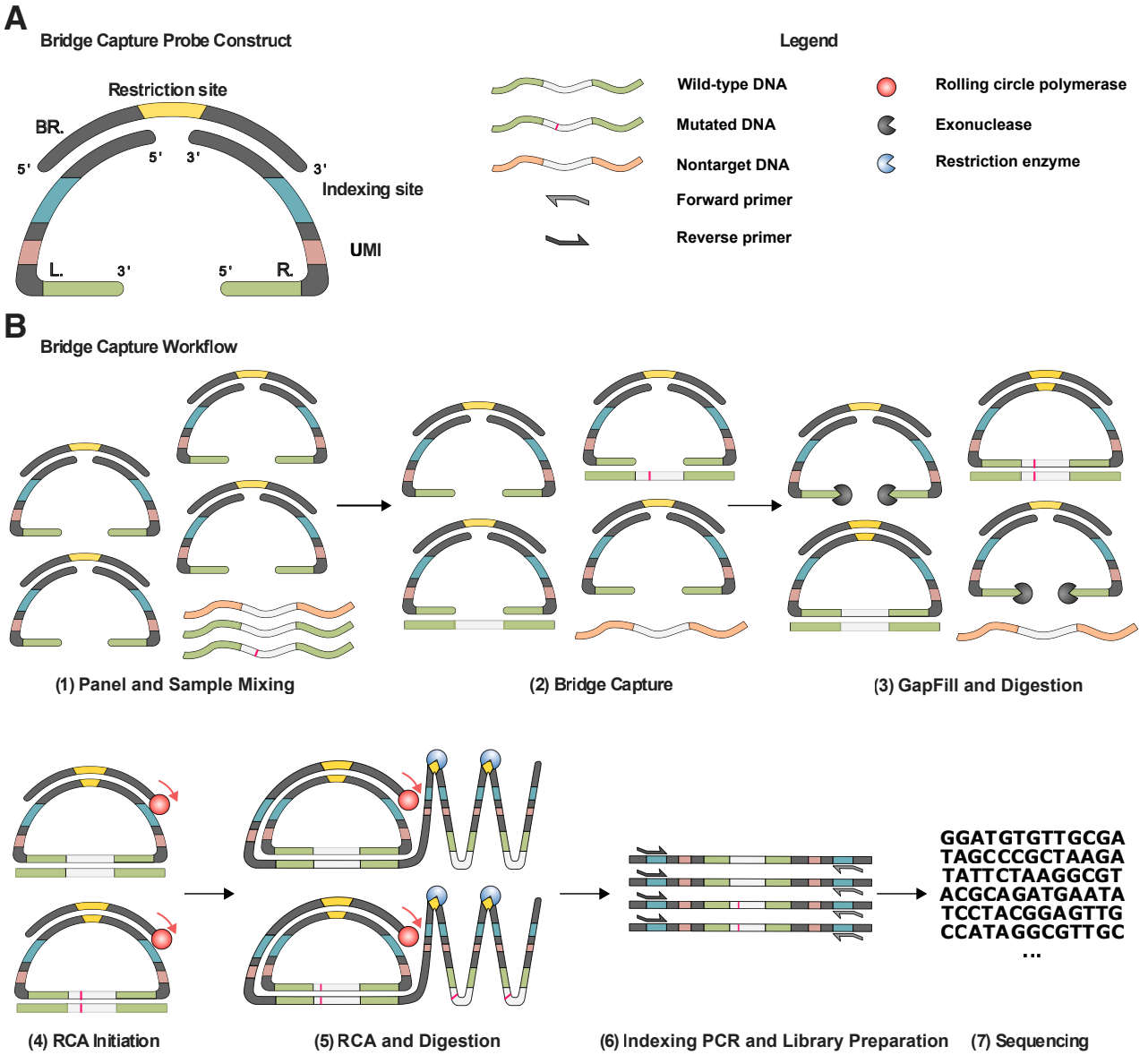

Figure 1 Bridge Capture in summary. **A:** Bridge Capture probe construct consists of a bridge oligomer (BR.) with binding sites to left (L.) and right probe (R.). The bridge contains a restriction recognition site (yellow). The probes contain sequences for binding of dual index primers (blue), unique molecular identifiers (UMIs; pink), and target-specific regions (green). **B:** Bridge Capture workflow. The probe constructs are mixed with a sample containing sequences of interest (step 1), to which the probe constructs hybridize (step 2). DNA polymerase and DNA ligase are used to fill all the gaps in the probe constructs, generating circularized molecules. The constructs that failed to hybridize to target DNA are digested by addition of exonucleases (step 3). Rolling circle amplification (RCA) is initiated from the bridge oligomer of the circularized construct (step 4). A long single-stranded concatemer synthetized by RCA is subsequently cleaved by a restriction enzyme into monomers (step 5). The monomers are indexed with dual indexing primers to generate a library viable for sequencing (step 6). The library is sequenced (step 7).

panel are provided in Supplemental Tables S1 and S2, respectively.

Technology Comparison and Interlaboratory Reproducibility

To compare Bridge Capture with other commercial technologies, two patient tissue CRC specimens were selected and underwent sample preparation. Specimens 1 and 2 exhibited the following mutations: *APC* p.Q1406*, *KRAS* p.G13D, *PIK3CA* p.E545K, and *APC* p.E1309Dfs*4, *KRAS* p.G12D, *TP53* p.Y126D, respectively. Patient samples were used as is, or they were diluted with fragmented gDNA in various ratios to mimic different levels of VAFs in 10-, 100-, and 1000-fold dilution (Supplemental Table S3). Each sample was tested in three replicates for each technology tested: Bridge Capture, Archer LIQUIDPlex (hereinafter referred to as LIQUIDPlex; Integrated DNA Technologies), and AmpliSeq Cancer HotSpot Panel version 2 for Illumina (hereinafter referred to as AmpliSeq; Illumina, San Diego,

CA). For the Bridge Capture, the 282-probe panel was used (details on panel preparation are provided in *Preparation of Bridge Capture Probe Panels*). The starting material load for Bridge Capture and LIQUIDPlex was 320 ng. To stay within the recommended load range, 100 ng of starting material was used for AmpliSeq. All the samples were processed by each technology at an independent diagnostic service provider. For LIQUIDPlex and AmpliSeq, sequencing was performed using Illumina NextSeq 500, and for Bridge Capture, NextSeq 550 was used. NextSeq 500/550 High Output Kit version 2.5 (300-cycle), 2 × 151-bp paired-end run (Illumina) was used for all three sequencings. Aliquots from the same samples were processed by Bridge Capture at Genomill to evaluate interlaboratory reproducibility. These sequencing libraries were sequenced using the MiSeq - MiSeq Reagent Kit version 3 for a 2 × 300-bp paired-end run (Illumina). Both laboratories processed the samples using a ready-made Bridge Capture kit.

Comparison of Automated and Manual Bridge Capture Workflow

To evaluate the automated Bridge Capture workflow, two CRC specimens and fragmented gDNA were analyzed by automated and manually performed workflows. Detected single-nucleotide variants included *KRAS* p.G12V, *APC* p.R876, and *APC* p.E1408* in one CRC specimen, and *CTNNB1* p.S45P, *KRAS* p.G13D, and *TP53* p.R248W in the other. Four sets of matched contrived samples were prepared in three replicates: undiluted CRC specimens 1 and 2, CRC specimens 1 and 2 diluted 10-fold with fragmented gDNA, and fragmented gDNA ($n = 15$, per set). One of the sets was analyzed following the manual Bridge Capture workflow protocol. The rest of the sets were analyzed in three separate runs by the automated workflow in a 96-well plate using a pipetting robot Opentrons OT-2 and Opentrons Thermocycler GEN1 (Opentrons). For the manual workflow, all incubations were performed in C1000 Touch Thermal Cycler (Bio-Rad). The protocols used for the manual and the automated workflows were identical. The 320 ng of each sample was used and analyzed by the 282-probe panel. The produced libraries were sequenced using NovaSeq 6000 SP2 2 × 150-bp flowcell (Illumina).

Effect of the Hybridization Time on the Bridge Capture Detection Limit

To determine the effect of the hybridization time on the detection limit of Bridge Capture, 320 ng of diluted specimen was incubated with the 282-probe panel under the following incubation times: overnight, 4 hours, 2 hours, 1 hour, and 0.5 hours. The CRC specimen covered single-nucleotide variants: *KRAS* p.G12V, *APC* p.R876*, and *APC* p.E1408*. The specimen was diluted 10-fold with fragmented gDNA, to simulate VAFs 10 times lower than the original. The sample was tested in five replicates at each time point. Produced libraries were sequenced using the MiSeq system, with MiSeq Reagent Kit version 3 for a 2 × 300-bp paired-end run (Illumina).

Panel Scalability

To evaluate the impact of increasing panel size on the evenness of the probe performance, the Bridge Capture analysis was performed with the 282- and the 887-probe panels. A total of 320 ng of fragmented gDNA was used as starting material. In the experiment, 10 technical replicates of fragmented gDNA were analyzed with both panels by Bridge Capture. Obtained libraries were normalized and sequenced using the MiSeq - MiSeq Reagent Kit version 3 for a 2 × 300-bp paired-end run (Illumina).

Data Analysis and Statistical Analysis

Bridge Capture reads were merged using VSEARCH[21] version 2.15.2_linux_x86_64 with the following parameters: –fastq_minovlen 10 –fastq_maxdiffs 15 –fastq_maxee 1 –fastq_allowmergestagger. A proprietary pipeline using unique molecular identifier–based error correction was used to process the Bridge Capture data. Both LIQUIDPlex and AmpliSeq data were processed by the independent diagnostic service provider using ArcherDX's Archer Analysis Unlimited version 6.0.3.1 and Illumina's DNA Amplicon version 2.1.1 data processing pipelines with default parameters (single-nucleotide variant cutoff for LIQUIDPlex, 0.5%; AmpliSeq, 1%). *In silico* subsampling was done without replacement to include 10%, 1%, and 0.1% of the original forward and reverse reads and then processed as mentioned before. For the linear regression analysis of automated and manual workflow, three randomly selected replicas of total nine replicas of automated workflow were paired with the three replicas of manual workflow. For the figures and statistics, Python version 3.11.5 was used (*https://www.python.org/downloads/release/python-3115*). The figures were drawn using the matplotlib version 3.7.2 library (*https://matplotlib.org/3.7.2*), R^2 scores were calculated using the sklearn version 1.2.2 library (*https://scikit-learn.org/stable/whats_new/v1.2.html*), and the scipy version 1.11.1 library (*https://pypi.org/project/scipy/1.11.1*) was used for linear regressions, Spearman correlation, and analysis of variance. Statistical significance for panel performance was established by a permutation test ($n = 1{,}000{,}000$) by calculating if the SD of the 10 replicas of a randomly selected probe in the panel was higher than the SD of 10 randomly selected signals (of any probe and of any replica).

Data Availability

All the data supporting the findings of this study are included in the article or Supplemental Data. Because of

ethical and privacy regulations, the raw sequencing data of the human specimens cannot be publicly shared. However, these data are available on request from the corresponding authors.

Results

The hallmark of the novel Bridge Capture method is the use of oligonucleotide probe constructs to target specific genomic regions of interest.[20] The probe construct consists of a bridge oligomer annealed to left and right probes (Figure 1A). The bridge also contains a restriction site and various modifications protecting the bridge from exonuclease activity or unwanted elongation by polymerase. Left and right probes carry binding sites for sequencing platform-specific adapters, unique molecular identifier sequences, and, most importantly, the target-specific binding sites.

In the first step of the Bridge Capture, probe constructs are introduced to a sample containing the targets of interest (step 1 in Figure 1B). A successful capture of the target by the construct results in a gap between the 3′ end of the left and the 5′ end of the right probe with the mutation of interest still located on the targeted DNA strand (step 2 in Figure 1B). Next, the gap is filled by DNA polymerase and ligated by a DNA ligase. The gap between the 5′ end of the left and the 3′ end of the right probe, held together by a bridge oligo, is also filled and ligated. This circularizes the probe construct and captures the mutation(s) of interest. Afterwards, all the noncircularized constructs are digested by exonucleases (step 3 in Figure 1B). Rolling circle amplification is initiated from the 3′ end of bridge of circularized construct (step 4 in Figure 1B). Rolling circle amplification generates multiple copies of the circular construct by generating a long concatemeric single-stranded DNA. After rolling circle amplification, single-stranded DNA concatemer is digested into monomers using a restriction enzyme (the restriction recognition site being provided in the bridge oligomer) (step 5 in Figure 1B). Sequencing platform-specific adapters are introduced to the monomers through limited-cycle indexing PCR (step 6 in Figure 1B). The resulting libraries are purified, pooled, and sequenced on the NGS platform of choice (step 7 in Figure 1B). As of now, the Bridge Capture has been tested on several NGS platforms, including Illumina, Ion Torrent S5, and Element Biosciences AVITI.

Technology Concordance

The performance of Bridge Capture was assessed by comparing it with commercially available cancer diagnostics technologies: LIQUIDPlex and AmpliSeq. The comparison was performed using 24 contrived CRC samples mimicking cell-free DNA and exhibiting different levels of VAFs (Supplemental Table S3).

The VAFs detected by the Bridge Capture were strongly correlated to the VAFs determined by LIQUIDPlex ($R^2 = 0.995$) and AmpliSeq ($R^2 = 0.988$) (Figure 2). LIQUIDPlex did not target any *APC* mutations, and AmpliSeq did not target *APC* p.Q1406*, and therefore these mutations were not included in the comparison. All technologies consistently detected single-nucleotide variants >2% VAF (Table 1). The lowest VAFs identified by Bridge Capture, LIQUIDPlex, and AmpliSeq were 0.08%, 0.58%, and 2.0%, respectively. The only deletion (*APC* p.E1309Dfs*4) was detected by both Bridge Capture and AmpliSeq at VAF of >2%. All technologies exhibited high linear correlation between the sample dilution factor and VAF, implying consistent linear performance across a wide range of VAFs. Average R^2 values of the detected variants were 0.961, 0.999, and 0.995 for Bridge Capture, AmpliSeq, and LIQUIDPlex, respectively. For some variants, the VAF values were higher or lower than expected in serial dilutions. This trend was observed for *KRAS* p.G13D and *PIK3CA* p.E545K detected by LIQUIDPlex; *APC* p.E1309Dfs*4 and *KRAS* p.G12D detected by Bridge Capture and AmpliSeq; and *TP53* p.Y126D detected by all the methods. This variation may be attributed to stochastic variability resulting from the sample mixing and aliquoting.

To determine the sequencing depth required by Bridge Capture for detecting low VAFs, the raw sequencing data (7.5 million reads) were subsampled (Table 1). At 10% subsampling, corresponding to approximately 750,000 reads, results were nearly identical with the nonsubsampled data. Bridge Capture could still detect VAFs of 0.1% for *KRAS* p.G13D and *PIK3CA* p.E545K and 0.5% for *KRAS* p.G12D in 1000× dilution in one of three replicates. At 1% subsampling (approximately 75,000 reads), Bridge Capture could detect VAFs down to 1% for all mutations except *APC* p.E1309Dfs*4.

Interlaboratory Reproducibility of Bridge Capture

The portability and reproducibility of Bridge Capture was assessed by comparing the results of an assay performed by an independent diagnostic service provider (site A) with the results from the Genomill laboratory (site B) (Figure 3A). The VAFs detected between sites A and B were strongly correlated ($R^2 = 0.979$).

Integration of Automated Bridge Capture Workflow

The implementation of automated Bridge Capture workflow was evaluated by comparison to manual workflow using five contrived samples, tested in three replicates with both workflows. The VAFs obtained from the automated and manual workflow were highly correlated ($R^2 = 0.983$, $r = 0.992$) (Figure 3B).

The hands-on time required for processing 15 samples was recorded for both workflows. The basic automated

Table 1 Comparison of VAFs Detected by LIQUIDPlex, AmpliSeq, and Bridge Capture, Including Subsampling Analysis of Bridge Capture

Variable	Mean sequencing depth	Sample dilution	*APC* p.Q1406*	*KRAS* p.G13D	*PIK3CA* p.E545K	*APC* p.E1309Dfs*4	*KRAS* p.G12D	*TP53* p.Y126D	Total VAF detected (excluding *APC*), *N* (%)	Total VAF detected (including *APC*), *N* (%)
Archer LIQUIDPlex	7.3 Million	No dilution	NA	37.9 ± 0.5	37.4 ± 0.3	NA	81.0 ± 0.5	81.9 ± 0.4	12 (100)	NA
		10×	NA	7.1 ± 0.2	6.7 ± 0.2	NA	6.8 ± 0.1	5.7 ± 0.1	12 (100)	NA
		100×	NA	0.8 ± 0.1	0.8 ± 0.1	NA	0.7 ± 0.1	0.6	10 (83)	NA
		1000×	NA	ND	ND	NA	ND	ND	0 (0)	NA
		R^2	NA	0.992	0.993	NA	0.998	0.997	NA	NA
Illumina AmpliSeq	14.6 Million	No dilution	NA	37.9 ± 0.3	38.0 ± 0.1	75.8 ± 0.3	81.7 ± 0.7	82.1 ± 0.4	12 (100)	NA
		10×	NA	2.6 ± 0.0	3.2 ± 0.1	2.1 ± 0.1	3.0 ± 0.4	3.1 ± 0.1	12 (100)	NA
		100×	NA	ND	ND	ND	ND	ND	0 (0)	NA
		1000×	NA	ND	ND	ND	ND	ND	0 (0)	NA
		R^2	NA	1.0	1.0	1.0	0.998	1.0	NA	NA
Bridge Capture	7.5 Million	No dilution	73.9 ± 2.2	40.0 ± 1.5	36.0 ± 1.1	87.6 ± 0.8	82.8 ± 3.7	79.9 ± 1.6	12 (100)	18 (100)
		10×	6.0 ± 0.7	4.1 ± 1.2	3.2 ± 1.0	3.2 ± 1.3	4.1 ± 1.1	4.9 ± 1.2	12 (100)	18 (100)
		100×	0.5 ± 0.0	1.2 ± 0.2	0.4 ± 0.2	ND	0.5 ± 0.3	1.1 ± 0.3	9 (75)	12 (67)
		1000×	ND	0.2	0.1	ND	0.5	ND	3 (25)	3 (17)
		R^2	0.999	0.974	0.980	0.981	0.875	0.957	NA	NA
Bridge Capture 10% subsampling	0.75 Million	No dilution	73.7 ± 2.6	39.0 ± 0.7	36.1 ± 0.7	86.9 ± 0.3	82.8 ± 3.2	79.7 ± 1.7	12 (100)	18 (100)
		10×	5.9 ± 0.8	4.1 ± 1.3	2.9 ± 0.9	3.2 ± 0.7	3.8 ± 1.2	4.6 ± 0.9	12 (100)	18 (100)
		100×	0.5 ± 0.1	1.2 ± 0.1	0.4 ± 0.1	ND	0.5 ± 0.3	1.2 ± 0.4	9 (75)	12 (67)
		1000×	ND	0.1	0.1	ND	0.6	ND	3 (25)	3 (17)
		R^2	0.997	0.97	0.988	0.994	0.852	0.949	NA	NA
Bridge Capture 1% subsampling	75,000	No dilution	75.2 ± 3.0	39.5 ± 3.1	34.3 ± 3.7	82.4 ± 2.5	86.2 ± 3.0	77.4 ± 1.3	12 (100)	18 (100)
		10×	6.4 ± 0.8	3.9 ± 2.4	2.7 ± 1.3	2.2 ± 0.6	2.9 ± 2.1	4.3 ± 0.4	12 (100)	18 (100)
		100×	0.5 ± 0.3	1.0	0.4 ± 0.2	ND	ND	1.8 ± 0.5	5 (42)	8 (44)
		1000×	ND	ND	ND	ND	0.8	ND	1 (8)	1 (6)
		R^2	0.984	0.922	0.948	0.994	0.723	0.908	NA	NA
Bridge Capture 0.1% subsampling	7500	No dilution	74.1 ± 8.9	32.3 ± 7.5	30.4 ± 2.8	78.6 ± 10.4	91.7 ± 14.4	81.4 ± 10.0	12 (100)	18 (100)
		10×	10.5 ± 5.4	6.7	5.7	ND	ND	4.3 ± 1.6	5 (42)	7 (39)
		100×	3.8	11.6	2.6	ND	ND	5.6	3 (25)	4 (22)
		1000×	ND	ND	ND	ND	ND	ND	0 (0)	0 (0)
		R^2	0.925	0.599	0.971	NA	NA	0.709	NA	NA

The VAFs are shown for each method across sample dilutions (no dilution, 10×, 100×, and 1000×) with R^2 value for each specified gene/variant. The VAFs are reported as means ± SD from three replicates (some variants undetected). For Bridge Capture, the raw reads obtained by sequencing (7.5 million reads) were subsampled by 10% (0.75 million reads), 1% (75,000 reads), and 0.1% (7500 reads). To indicate the number of detected variants in the replicates, the table summarizes the total VAF detected excluding *APC* and including *APC* (if method covered *APC* variants in the panel) for given sample dilution.

NA, not applicable; ND, not detected; VAF, variant allele frequency.

workflow setup took 4 minutes 20 seconds (labware positioning, ice and reagent placement for the first workflow step, and final robot clean-up), and subsequent removal and addition of reagents took 6 minutes 18 seconds. This additional step was obligatory, as the pipetting robot lacks cooling module, and reagents must be added separately during each step of the workflow. However, with a more advanced pipetting robot, this step is redundant. Therefore, the total hands-on time for automated workflow was 10 minutes 38 seconds. For manual workflow, the hands-on time was 35:15 minutes, excluding the bead purification of the ready-made libraries before the sequencing.

The Effect of Hybridization Time on Detection Limit of Bridge Capture

The effect of hybridization time on Bridge Capture performance was tested by detecting VAFs using six different incubation times (overnight, 4 hours, 2 hours, 1.5 hours, 1 hour, and 0.5 hours) in five replicates (Figure 3C). Mutations of interest were successfully identified in all replicates, excluding one replicate in the 0.5-hour incubation time point, where the *APC* p.E1408* was not detected. The SD of the VAFs detected between the five replicates was the lowest for replicates incubated overnight and 4 hours. The SD between replicates increased with shorter incubation time, peaking at the 0.5-hour time point. Only the detection of *KRAS* p.G12V was significantly affected by changes in the incubation time (one-way analysis of variance test, $P < 0.05$).

Panel Scalability

To assess the Bridge Capture scalability, the panel size was increased by >300% from 282 to 887 probes. The effect of the panel size increase was explored by evaluating the change in probe signal intensity and the performance of the matching probes between the two panels. Signal intensity was established for each probe from 10 replicates of fragmented gDNA (Figure 4, A and B) for panels of 282 and 887 probes, by dividing the read count per probe by the total read count of each replicate. Probe signal intensity was varying between the probes, with median intensity of 0.324,

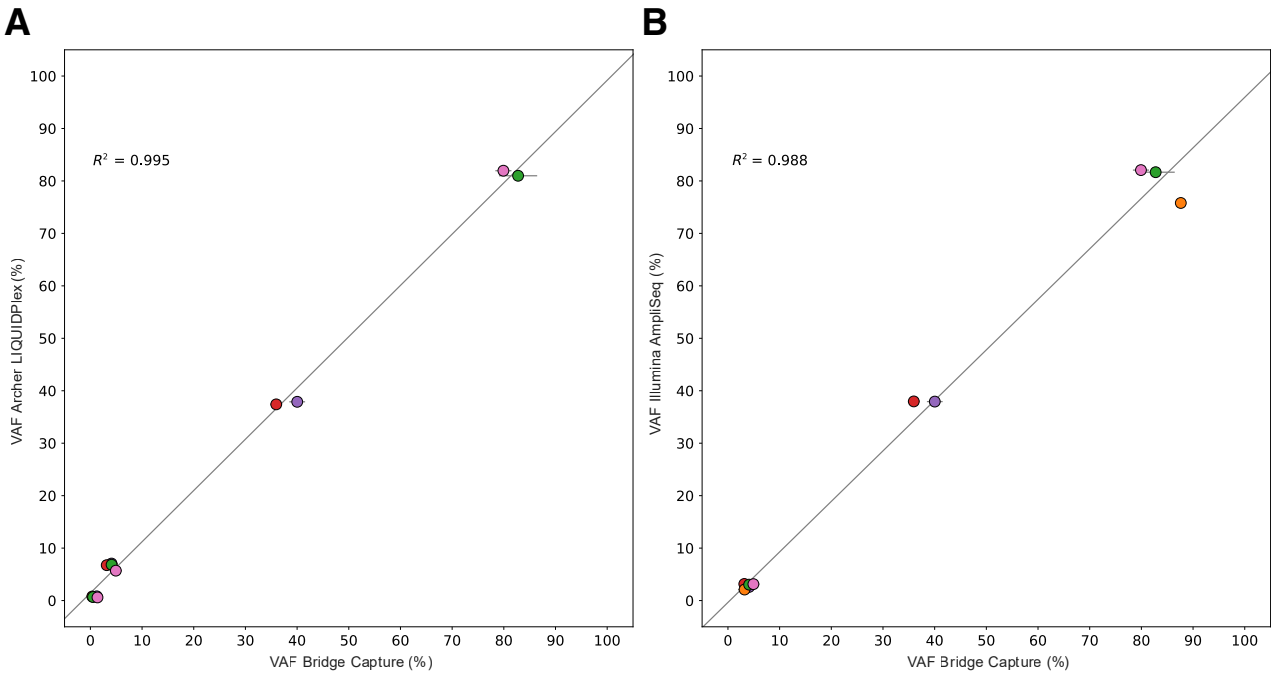

Figure 2 Concordance between variant allele frequencies (VAFs) detected by Bridge Capture and commercially available technologies. **A:** Concordance between VAFs detected by Archer LIQUIDPlex assay and Bridge Capture. **B:** Concordance between VAFs detected by AmpliSeq Cancer HotSpot Panel version 2 for Illumina and Bridge Capture. The mutations are indicated as follows: *APC* p.Q1406* (blue), *KRAS* p.G13D (purple), *PIK3CA* p.E545K (red), *APC* p.E1309Dfs*4 (orange), *KRAS* p.G12D (green), and *TP53* p.Y126D (pink).

compared with theoretical intensity of 0.355, assuming a uniform performance for the 282-probe panel. In comparison, for the 887-probe panel, the median intensity was 0.087, compared with the theoretical intensity of 0.087. Signal intensity per probe between replicates was notably consistent, with a mean SD of 0.0267 for the 282-probe panel ($P < 0.001$) and 0.0091 for the 887-probe panel ($P < 0.001$).

The probes present in both panels performed highly similarly as indicated by Spearman rank correlation ($\varrho = 0.904$), demonstrating that panel size expansion had negligible impact on performance and cross-reactivity of individual probes (Figure 4C).

A relationship between probe signal intensity and the respective GC content of the target region (defined as 300 nucleotides around the probe binding site) was observed (Figure 4D). For both panels, high GC content affected probe performance, evidenced by a dip in signal intensity starting from 55% to 59% with a noticeable decline from 60% onwards. Similarly, low GC content (25% to 29%) negatively affected probe performance, but to a lesser extent.

The 282-probe panel covered 64 cancer types and associated driver genes obtained from intOgen-framework version 2023.05.31[22] and their respective mutations of one and two significance, which are based on the Catalogue of Somatic Mutations in Cancer database, CMC version 99.[23] Most cancer type mutations were covered by the 282-probe panel (Supplemental Figure S1). The expanded 887-probe panel covered nearly 71 driver genes, with most of the cancer types (65/71) covered by at least 90%. This panel included probes for the most common cancer types, such as colorectal adenocarcinoma, breast carcinoma, non–small-cell lung cancer, prostate adenocarcinoma, and ovarian cancer.

Discussion

This study demonstrates that Bridge Capture surpasses the performance of commercially available leading technologies for cancer diagnostics, such as LIQUIDPlex and AmpliSeq. For example, Bridge Capture identified certain mutations that were below the cutoff limit of LIQUIDPlex (>0.5%) and AmpliSeq (>1%). It was demonstrated that Bridge Capture is easily adoptable in a new laboratory setting, shown by the high correlation between two independent laboratories. This was achieved despite the use of different sequencing platforms, over ninefold difference in sequencing depth, and operators having varying levels of experience.

Various library preparation methods are available for NGS-based LB analysis, each with their advantages and limitations. For instance, amplicon-based technologies, such as AmpliSeq and TruSeq Amplicon, are simple and rapid in terms of their workflow but provide limited

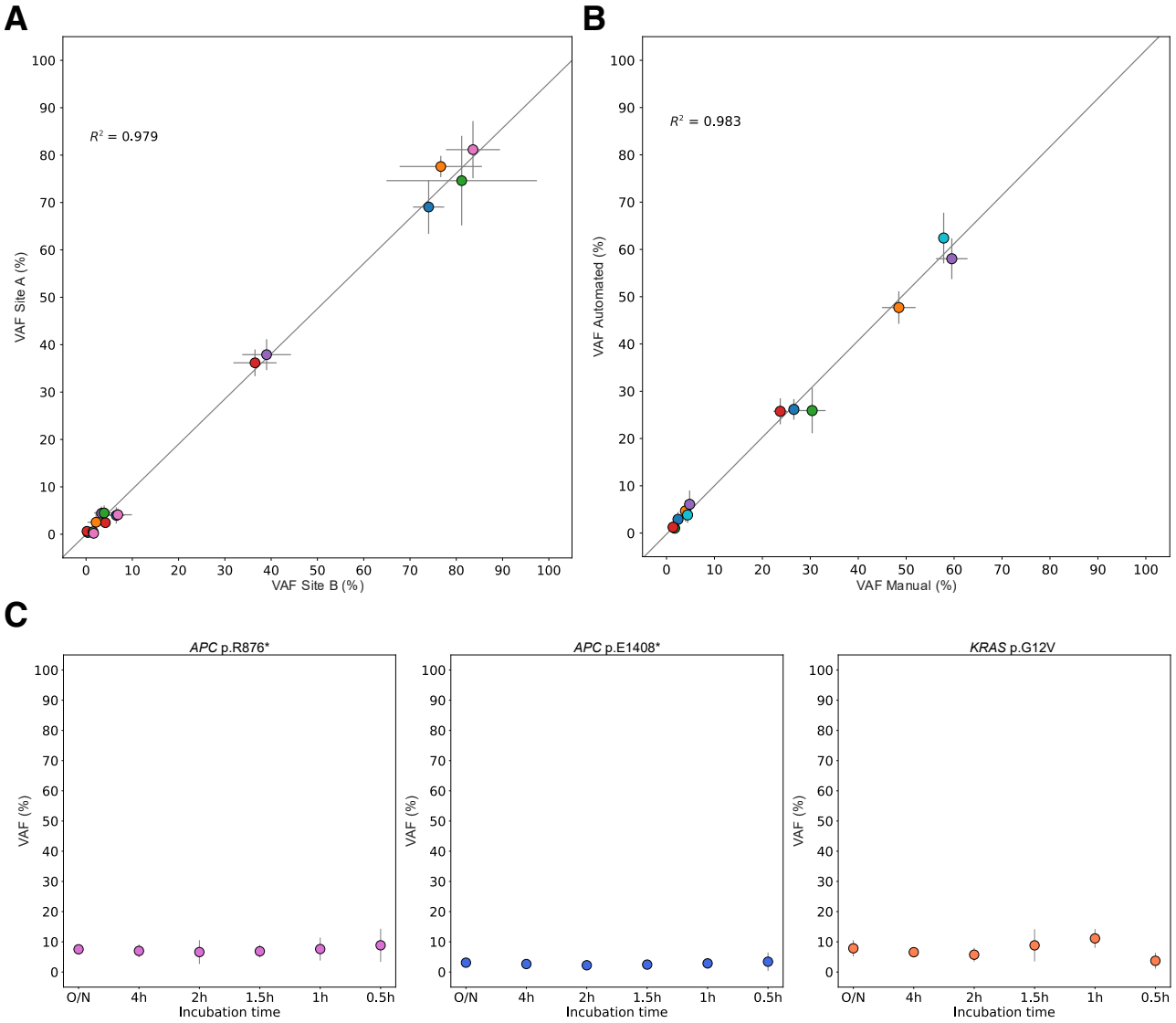

Figure 3 The performance of Bridge Capture. **A:** The interlaboratory reproducibility testing of Bridge Capture between two independent laboratories (site A and site B) shows a high correlation ($R^2 = 0.979$). The mutations are indicated as follows: *APC* p.Q1406* (blue), *KRAS* p.G13D (purple), *PIK3CA* p.E545K (red), *APC* p.E1309Dfs*4 (orange), *KRAS* p.G12D (green), and *TP53* p.Y126D (pink). **B:** The reproducibility of Bridge Capture between automated and manual workflow ($R^2 = 0.983$, $r = 0.992$). The mutations are indicated as follows: *APC* p.R876* (purple), *APC* p.E1408* (red), *CTNNB1* p.S45P (blue), *KRAS* p.G12V (cyan), *KRAS* p.G13D (orange), and *TP53* p.R248W (green). **C:** Hybridization time had a significant effect on *KRAS* p.G12V (one-way analysis of variance, $P < 0.05$) and did not have a significant effect on the other mutations. The points are represented as the means ± SD of the replicas (**A**–**C**). O/N, overnight; VAF, variant allele frequency.

scalability to a larger number of targets.[11–13] Hybrid capture methods, such as AVENIO and FoundationACT, provide scalability to a large number of target genes but are slow, cumbersome, and expensive.[16,17,24,25] MIP-based workflows are rapid and simple but have high probe synthesis costs as well as poor uniformity.[19,26] The results presented in this research provide initial evidence that Bridge Capture–based sequencing has the potential to combine the advantages of the aforementioned methods. The sequencing depth required by Bridge Capture is low, with approximately 750,000 reads sufficient for detecting mutations of 0.1% VAF (282-probe panel). The low sequencing depth requirement permits pooling dozens of samples on benchtop devices, such as Illumina MiniSeq, and thousands of samples on production-scale sequencers, such as NovaSeq X. This could be leveraged in small- and medium-scale facilities where sample volumes are insufficient to fill production-scale sequencer runs, or in larger, centralized facilities to enable higher throughput.

The panel expansion from 282 to 887 probes was demonstrated. Despite the panel coverage increasing by >300%, the panel uniformity remains unchanged, implying that the Bridge Capture panels can be scaled without any upper limit. However, similar to other available methods, the performance between probes can be variable, which can be mitigated by probe design. The results presented in this

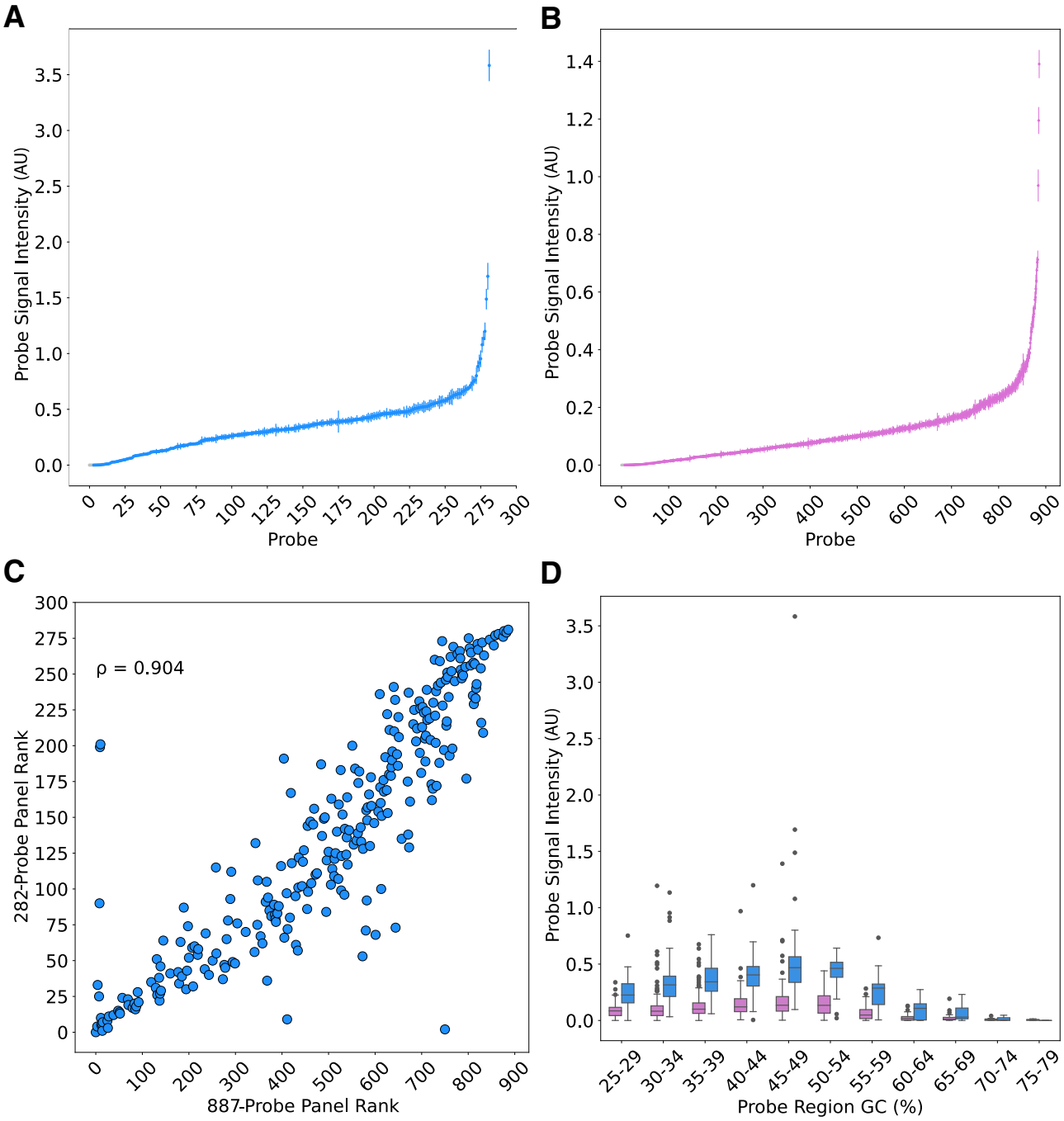

Figure 4 **A** and **B:** Probe signal intensity of the 282-probe panel (**A**) and the 887-probe panel (**B**). Mean probe signal intensity of 10 replicates (*y* axis) was calculated by dividing the read count per probe by the total read count of the replicate. The SD between the replicates is displayed as error bar. **C:** Spearman rank correlation ($\varrho = 0.904$) depicting the performance relationship of the probes shared between the 282- and the 887-probe panels. **D:** The relationship between probe signal intensity and GC content of the probe target region (300 nucleotides around the probe binding site) of the 282-probe panel (blue) and the 887-probe panel (pink). AU, arbitrary unit.

study indicate that the probe performance is related to the high or low GC content of the probe target region. This performance effect could result from the indexing PCR,[27–30] because PCR has been shown to deplete loci with GC content of >65%.[29,30] Another potential source for the probe performance variation could be the sequencing by synthesis on Illumina platforms, which is also negatively impacted by high GC content.[28,29,31] Outlier above-average performance could partly be explained by the binding site overrepresentation by pseudogenes, for instance the probe targeting *NF1* gene, a known homologous pseudogene in the human genome.[32,33]

Although this study was limited to samples mimicking ctDNA that does not exhibit the full complexity of plasma-derived ctDNA, the results demonstrate potential for use of Bridge Capture in LB. This has been further supported by a follow-up study evaluating the performance of Bridge Capture on 80 ctDNA samples from patients with metastatic CRC.[34] In addition, the future studies should evaluate performance of Bridge Capture on other alterations, such as copy number variations and fusions. The ongoing development efforts aim to further enhance sensitivity of Bridge Capture by refining the probe design and optimizing the workflow. Bridge Capture provides a scalable but highly targeted diagnostic approach, as with the 887-probe panel and low sequencing depth requirements, it addresses the most common cancer types, such as colorectal adenocarcinoma, breast carcinoma, non−small-cell lung cancer, and prostate adenocarcinoma.

In conclusion, Bridge Capture is an easy-to-use, cost-efficient, scalable, streamlined, and platform-agnostic NGS library preparation method that combines the speed and simplicity of the amplicon- and MIP-based methods while maintaining the performance with the scalability and sensitivity of the hybrid capture methods. Hence, Bridge Capture is a novel cancer diagnostics tool that significantly improves the detection of mutations.

Declaration of Generative AI and AI−Assisted Technologies in the Writing Process

During the preparation of this work, the authors used ChatGPT 4.0 (OpenAI, San Francisco, CA) to refine the language and improve readability in certain parts of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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Author Contributions

S.A., A.K., N.L., T.H., J.-P.P., and M.T. conceptualized the study; A.K., A.M., T.R., and J.K. curated data; A.K. and N. L. analyzed data; J.-P.P. and M.T. acquired funding; S.A., A.K., N.L., A.M., and T.R. performed investigations; S.A., A.K., N.L., T.H., J.-P.P., and M.T. developed methods; J.-P.P. and M.T. administered the project; A.K., N.L., and M. T. obtained software; J.-P.P. and M.T. supervised the study; S.A., A.K., and N.L. visualized the data; S.A., A.K., J.-P.P., and M.T. wrote the manuscript; N.L., J.K., J.B., J.L., and T. H. reviewed and edited the manuscript; and M.T. is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Disclosure Statement

M.T. is a Chief Executive Officer of Genomill Health Inc. J.-P.P. is a Chief Technology Officer of Genomill Health Inc. J.K. is a medical advisor at Genomill. J.B., J.L., and M. T. hold equity in Genomill. S.A., A.K., N.L., A.M., T.R., J. K., J.B., J.L., T.H., J.-P.P., and M.T. are entitled to stock options in Genomill. S.A., A.K., N.L., A.M., T.H., and J.-P. P. are currently employed at Genomill. The research detailed in this article is related to Genomill patents EP-3673081, JP-7074978, EP-4060049, US-11486003, ZL-201880055271.3, HK-40017021, JP-7651497, and TWI-864378. Genomill is in the process of filing patents related to this work, and A.K., T.H., J.-P.P., and M.T. are named inventors on these patent applications. J.B. has received honoraria from Novo Nordisk and Boehringer Ingelheim. In preparing this manuscript, every effort was made to ensure that the research was conducted and presented objectively. The study's design, data collection, analysis, interpretation, and the writing of the manuscript were conducted independently of Genomill's commercial interests. The authors affirm that the information provided here is accurate and complete to the best of their knowledge.

Supplemental Data

Supplemental material for this article can be found at *https://doi.org/10.1016/j.jmoldx.2025.09.006*.

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