Introduction

Genomill is a pioneering precision diagnostics company with a mission to make molecular diagnostics more sensitive, scalable and accessible. Our product Geno1®, is an NGS technology platform enabling massive improvements in performance, cost, Turn-Around-Time and scalability – leading to a major impact in healthcare. Geno1® consists of three main parts: Geno1® chemistry, Geno1® laboratory workflow and Geno1® data processing. Geno1® workflow is based on the Genomill patented technology. Our current focus within liquid biopsies is MRD & Tx and we continuously monitor potential new verticals.

This document is intended to provide comprehensive information about technical aspects of Geno1®. The questions have been organized into specific categories, including a detailed description of the technology, its compatibility with various sequencing platforms, the types of samples that can be analyzed, a detailed overview of the probes and technology used, and information about UMIs and quality procedures.

List of abbreviations:

cDNA	Complementary DNA
cfDNA	Cell-Free DNA
CNV	Copy Number Variant
CRO	Contract Research Organization
eQMS	Electronic Quality Management System
gDNA	Genomic DNA
Indel	Insertion & Deletion
MAF	Mutant Allelic Fraction
MRD	Minimal/Measurable Residual Disease
NGS	Next-Generation Sequencing
OEM	Original Equipment Manufacturer
QMS	Quality Management System
PCR	Polymerase Chain Reaction
RCA	Rolling Circle Amplification
SNV	Single Nucleotide Variant
SOP	Standard Operating Procedure
ssDNA	Single-Stranded DNA
UMI	Unique Molecular Identifier
VCF	Variant Call Format
Tx	Treatment Selection

> **Note:** You can use references for navigating. For example, "Figure 1" and the number 1 in "Question 1" take you the given figure or question.

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Geno1® in general

Question	Answer
1. What is Geno1®?	Geno1® is a groundbreaking targeted pre-sequencing platform. Geno1® consists of three parts: proprietary chemistry, laboratory workflow and data processing (see Figures 1 and 2 in the Appendix). Geno1® is built on a patented Bridge Capture™ technology. Geno1® incorporates advances in molecular biology, robotics, NGS, and analytics. The result is a rapid, highly sensitive, automated and cost-effective molecular quantification solution for oncology and beyond.

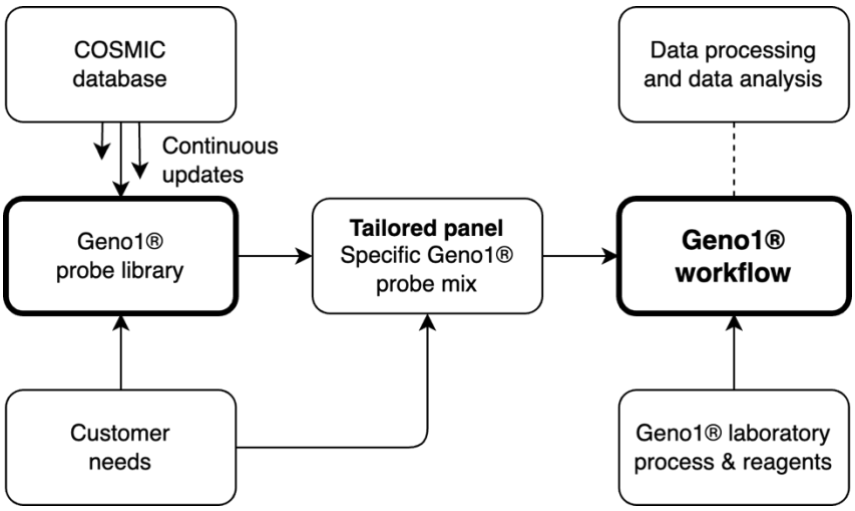


Figure 1 in Appendix

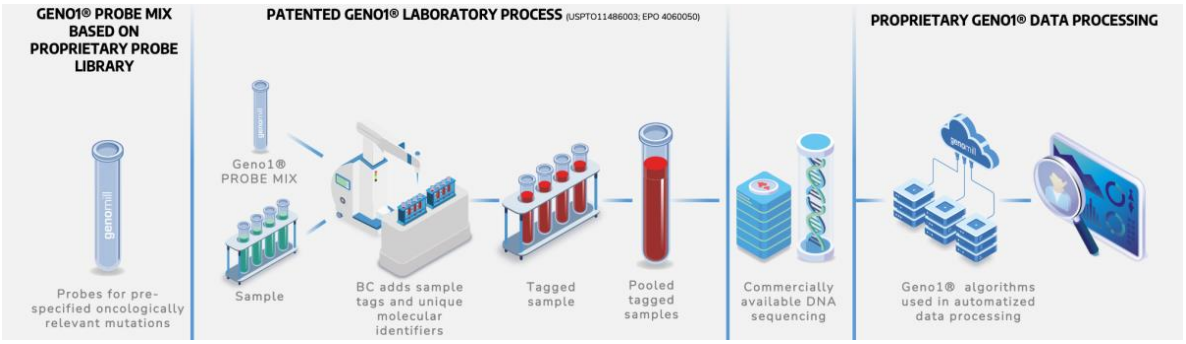


Figure 2 in Appendix

Question	Answer
2. What are typical use cases of Geno1®?	Geno1® is a highly sensitive molecular quantification technique. Its typical oncological use cases involve quantification of mutations (including SNVs, indels and gene fusions) and CNVs (see Question 25). One use case is mutation/CNV detection from tumor DNA. Another use case is mutation/CNV detection from cfDNA extracted from patient's blood plasma, which has applications in Tx and MRD.
3. What is the advantage of Geno1® over other commercially available diagnostic techniques?	Geno1® provides various advantages when compared to different liquid biopsy techniques. We therefore provide three separate answers for this question where Geno1® is compared to amplicon-based techniques (Question 4), hybrid capture-based techniques (Question 5) and molecular inversion probe-based techniques (Question 6). One key benefit Geno1® has over all of these techniques is the capability to pool the samples directly after the targeting step without quantification. This approach ensures that the workload does not increase linearly with the number of samples, offering a distinct advantage in scalability (see also Question 24). To our knowledge, this is unique to Geno1®.
4. Does Geno1® have advantages over amplicon-based techniques?	Yes. Geno1® provides improvements in scalability, sensitivity and workflow simplicity. Geno1® is a simple, UMI-based protocol (see Question 46) with infinite scalability, high evenness, and high sensitivity for rare variants. This results in improved performance as well as a streamlined, highly cost-efficient protocol. Amplicon-based techniques are based on multiplexed PCR which has inherent limitations in multiplexing, sequencing evenness and rare target detection. Amplicon-based workflows can be improved by introducing UMIs but this leads to a more complicated workflow. Geno1® overcomes these limitations.

Question	Answer
5. Does Geno1® have advantages over hybrid capture techniques?	Yes. Geno1® provides improvements in chemistry and workflow costs (see Question 9), as well as improvements in speed, and simplicity. The Geno1® workflow itself is simple and cost-efficient and results in a sequencing library with high target evenness. Since this library is focused on pre-selected loci, even very rare variants can be called with moderate sequencing depth. In contrast, hybrid capture-based techniques are based on sequencing library preparation workflows and extend them by introducing an additional enrichment step where the desired parts of the sequencing libraries are enriched using capture probes. Hybrid capture techniques permit profiling a large number of mutations from sample material but suffer from expensive and complicated laboratory workflows, sequencing unevenness, high sequencing requirement, and complicated data analysis. Geno1® overcomes these limitations and provides a lean, sensitive and cost-efficient alternative to hybrid capture.

Question	Answer
6. Does Geno1® have advantages over Molecular Inversion Probes?	Yes. Geno1® has advantages in performance, scalability and cost-efficiency in comparison to molecular inversion probes. Molecular inversion probes are composed of a lengthy single-stranded DNA molecules including linker region and target-specific segments at both ends. This continuous structure involves high probe synthesis costs as well as significant challenges and limitations on the probe design process. Molecular inversion probes are typically amplified using PCR primers specific to the linker sequence, resulting in uneven target amplification and probe cross-reactivity. In contrast, Geno1® as a Bridge Capture™ technology uses a design featuring short, target-specific probe arms connected by a bridge oligonucleotide (see Question 40). This innovative structure significantly lowers the probe synthesis costs, streamlining the design and testing procedures. The modular nature of Geno1® – with each probe comprising three separate oligonucleotides – facilitates seamless implementation of novel design ideas and modifications on targeting parts, permitting approaches like sample barcoding on targeting, and PCR-free DNA sequencing libraries. Unlike the conventional PCR amplification of molecular inversion probes using primers specific to the linker sequence, Geno1® utilizes linear signal amplification. This approach avoids probe cross-reactivity, ensuring highly uniform and reproducible results.
7. Has Geno1® been compared against other commercially available technologies?	Yes. The performance of Geno1® was compared against ArcherDX LiquidPlex and AmpliSeq for Illumina Cancer Hotspot Panel v2 with comparable results. All the compared technologies were carried out by a global 3rd party CRO highly experienced with NGS. Quote from an expert technician in NGS preparations at the CRO: "*This is by far the easiest workflow I've ever used.*"

Question	Answer
8. Is it sensible to use targeted sequencing since the costs of whole genome sequencing are decreasing?	Yes. Over the past decade, advances in sequencing technologies, competition among sequencing providers, and increased demand and investments have led to a significant decrease in the cost of DNA sequencing. This has enabled, among other things, affordable whole-genome and whole-exome sequencing – applications where the sequencing depth requirement is moderate. Liquid biopsies, on the other hand, are based on detection of rare somatic variants in cell-free DNA, the detection of which requires very deep sequencing. Performing whole-genome or whole-exome sequencing at such sequencing depths will remain unfeasible, irrespective of how much the sequencing costs decrease.
9. What's the estimated cost of Geno1® and how is it determined?	The cost of Geno1® consists of two parts: sample preparation and sequencing. The sample preparation cost is around USD 10 per sample regardless of the panel size with the reagent rates available to us. We expect the cost to come down with further optimizations. The approximated sequencing costs can be seen from Table 1. The low sample preparation cost is based on the simplicity of the protocol, as well as the small amounts of low-cost reagents (see Question 6). The low sequencing cost is based on the efficient targeting of the sequencing effort as well as the faithful representation of the read counts in the sample, provided by the linear signal amplification. Cost of labor is not included in the estimation.

Question	Answer
10. Is Geno1® IP protected? How is the IP field around the technology? How is the situation compared to other technologies in the same field?	Yes. Geno1® is covered by extensive patent portfolio (see Table 2). Please see the full list of obtained and pending patents. Geno1®, being based on proprietary Bridge Capture™ technology, is securely protected. Genomill owns all patents related to Geno1®, providing unparalleled freedom in operations, and eliminating any concerns related to intellectual property issues. Geno1® represents a novel approach and methodology when compared to existing amplicon-based and hybrid capture methods. Therefore, the IP portfolio around Geno1® is highly differentiated from other solutions *e.g.* in MRD applications.
11. What is the turnover and hands-on time of the Geno1® NGS technology platform?	The Geno1® workflow is designed to be fast and efficient, taking less than 5 hours to complete with only 5 minutes of hands-on time required, compared to 7 hours on amplicon-based workflows or at least 8 hours on hybrid capture-based workflows. This streamlined workflow allows for rapid sample processing, saving valuable time and resources (see Figure 3). Once the workflow is complete, the subsequent NGS analysis duration depends on the sequencing technology used.
12. What is the outcome of Geno1® NGS technology platform process?	The outcome of Geno1® is a standard VCF file or easy-to-read tabular file reporting the detected variants and the respective read counts and MAFs. This data can be used by our customers to generate a clinical report for the patients.

Question	Answer
13. How reproducible are the read counts reported by Geno1®?	The reproducibility of the read counts reported by Geno1®, determined by replicated sequencing experiments, and indicated as mean-normalized replicate counts, is typically between 0.5× to 2× of the replicate mean (see Figure 4). The sensitivity is reproducible between replicates and was demonstrated experimentally in validation experimentation done on NovaSeq 6000 (see Question 14).
14. What parameters affect sensitivity?	The main factors that impact the sensitivity of the Geno1® are sample quality and total amount of DNA present. It is worth noting that the sensitivity varies slightly between different probes, which is most likely due to the unique binding characteristics determined by the probe and target sequences. In our sensitivity validation experiment, we observed a sensitivity range of 0.03% to 0.2% MAF, which depends on the specific probe. These MAFs were determined through serial dilutions of fragmented tumor biopsy DNA to fragmented gDNA, which were subsequently sequenced on the NovaSeq 6000. We can optimize the performance characteristics of any probes based on customer preferences.
15. What is the error rate/specificity of the Geno1®? That is, what is the false positive and false negative error rate?	Our clinical testing has not yet been sufficient to establish these numbers. As of 2023-12, we are collecting further sample cohorts to establish numbers for type I (false-positive) and type II errors (false negative).
16. Has the Geno1® workflow been validated by a CRO or similar?	Yes. Geno1® workflow has been validated with two local CROs in early 2023 and with one global-scale CRO / precision diagnostic company in mid-2023.

Question	Answer
17. Can Geno1® workflow be automated?	Yes. The Geno1® workflow is highly versatile and can easily be adapted to automated and scalable environments. Recent demonstrations of the workflow were performed with the Opentrons OT-2, a popular automation platform for life sciences, where the workflow was completed in a single run. Geno1® workflow can be fully automated depending on the chosen automation platform and configuration, encompassing both workflow and library preparation for NGS analysis in the same run. This allows for even greater efficiency and precision in processing large numbers of samples. In addition, the hands-on load time for the automation workflow is brief, taking only approximately 5 minutes to complete. The automation was piloted with 1 to 23 samples. In this pilot test, the run duration was about 7 hours for one sample and 9 hours for 23 samples. This pilot test did not use sample pooling (see Question 24), the incubation times were unoptimized, the robot prepared the master mixes as a part of the protocol, and the robot was using single-channel pipettes for reaction aliquoting. Therefore, we can safely estimate the reaction durations to drop by at least 2 hours with optimizations. This means a duration of 5 hours for one sample and 7 hours for 23 samples. We are currently optimizing the robotic protocol for improved workflow duration.
18. Is any special equipment needed to successfully complete the Geno1® workflow?	No. The Geno1® workflow can be completed using standard molecular laboratory equipment such as pipettes, vortexes, spinners, and PCR instrument. No specialized or uncommon equipment is necessary to successfully carry out the protocol.

Sequencing platforms

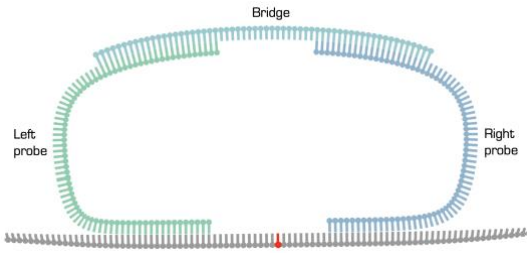
Question	Answer
19. Is Illumina the only sequencing platform that can be used with Geno1®?	No. Geno1® NGS technology platform is compatible with all NGS platforms on the market. In addition to Illumina, Geno1® workflow has been successfully completed with Ion Torrent and Oxford Nanopore NGS platforms.
20. Is the Geno1® NGS technology platform compatible to both short read and long read sequencing technologies?	Yes. The Geno1® NGS technology platform is designed to produce sequencing libraries that are compatible with any short-read NGS platform. However, the workflow can be halted after the linear amplification stage. At this point, the concatemeric ssDNA molecule, which contains both the probe and target sequences, is obtained. This elongated DNA molecule can be utilized in long read sequencing technology preparation kits, such as those provided by Oxford Nanopore. This alternate workflow has been demonstrated with Geno1® using Oxford Nanopore (see Question 19).

Sample types

Question	Answer
21. Is Geno1® compatible with different sample types and materials?	Yes. Geno1® can analyze variety of the different sample materials. Routinely tested sample types are cfDNA, gDNA, and cDNA. The technology has demonstrated promising results on direct targeting of RNA, as well as capturing unpurified DNA from urine and saliva.
22. Can Geno1® be used with samples having very low amounts of target DNA?	Yes. The smallest amount of patient cfDNA tested thus far has been approximately 2000 genomic copies (5 ng). The detection of low allelic fractions benefits from a larger amount of sample used in the workflow.
23. Do samples need to be diluted or amplified prior to analysis? What pre-processing steps must be done to the samples?	No. After the cfDNA is extracted from the sample, purified material can be used straight for workflow. Sample doesn't need to be diluted, as it is unlikely that amount of cfDNA in sample would saturate the number of probes used in the reaction.
24. Can samples be pooled in Geno1® workflow?	Yes. Geno1® workflow permits sample pooling after the initial targeting step without the need for normalization. This is specific to Geno1® unlike other commercially available technologies where pre-indexed amplified libraries are pooled before sequencing. This approach ensures that as the number of samples processed in parallel increases, the associated increase in workflow duration, cost, and complexity does not follow a linear trend, offering a distinct advantage in efficiency and scalability, a feature unique to Geno1®. Our pooling approach is currently protected within our patents and undergoing further improvements.

Question	Answer
25. Can Geno1® detect different mutation types?	Yes. Geno1® has been demonstrated to reliably detect SNVs, indels, and gene fusions. We also have preliminary results of detecting CNVs. Geno1® can detect mutations associated with any cancer type, given the right panel design.

Probes in detail

Question	Answer
26. What is a probe?  Figure 5 in Appendix	A probe is a DNA oligomer that binds to complementary DNA sequence. Geno1® uses probe pairs which are bound together by a third, non-target-binding oligomer. See image on the left (probes in green and blue) and Figure 5 in the Appendix for more detail.
27. What is a probe library?	The probe library contains all the probes Genomill has developed.
28. What is a panel?	A panel is a subset of the probe library. In context of cancer diagnostics, a panel is a predefined set of probes to target specific regions of the genome to detect genetic mutations, variations or other alterations associated with a particular cancer type.
29. What is a probe mix?	Probe mix is a solution containing specific probes, or a physical embodiment of a panel it represents. Probe mix is an essential part of the Geno1® chemistry.
30. Are there predetermined panels?	Yes. Genomill has developed a panel with 887 probes which targets 123 genes containing 24,236 mutations, from which 1,870 are oncologically relevant mutations (see Figure 6). The panel design is based on the COSMIC database, CMC v.99 (https://cancer.sanger.ac.uk/cosmic). Genomill's panel is fully modular, since panel can be split into multiple smaller panels and from these the desired subpanels can be combined back into one bigger panel, still retaining their probe performance.

Question	Answer
31. Can new probes be introduced into an existing panel?	Yes. New probes can be introduced into an existing panel when required. Experimental panel validation is mandatory after introduction of new content.
32. What information is needed to create a probe or panel?	Probe design requires detailed information of target DNA sequence or genomic coordinates of the mutation.
33. How many targets/probes can be in one panel?	There is no predefined upper limit for the targets (number of probes) in a panel. So far, our largest panel has contained 887 probes and it worked as expected.
34. How long is the region between the probe arms (the gap region)? How long are the probe sequences hybridizing to the target? How long is the combined footprint of the probe?	The standard gap length in Bridge Capture™ is approximately 50 base pairs. This length was chosen to strike a balance between the small size of cfDNA fragments and the number of mutations that can be captured by a single probe pair when the gap length is increased. The longest gap length tested so far is 110 base pairs, while the shortest gap tested was a nick between the probes, which was used to quantify specific molecules. Probe arms are 20 base pairs long on average. This results in a typical combined footprint of 90 bp for left and right probes together with the gap region.
35. Does the panel need optimizations? How is the panel evenness?	Not necessarily. With our in-house probe designer, the probes typically work with good evenness (see Figure 7). We have shown that panel evenness can be further improved by adjusting individual probe concentrations.
36. Does the probe design take long? How long does the probe design take?	No. Genomill has developed an efficient and highly scalable probe design method. It is based on a streamlined proprietary in-house algorithm able to design functional probes in minutes. Genomill's probe production pipeline involves also rigorous experimental testing, ensuring the highest possible quality of each probe used.

Question	Answer
37. Do you manufacture the probes?	No. Genomill obtains probe oligomers from well-established commercial providers. Currently, Genomill uses IDT as a commercial provider.

Geno1® technology in detail

Question	Answer
38. How does Geno1® technology work?	Geno1® technology is illustrated in Figure 8 in the Appendix (also presented below). Successful targeting of a specific sequence requires simultaneous binding of two probes, which are held together by a bridge oligo. Both probes must bind to their respective target sequences for the Bridge Capture™ to be successful. To ensure accurate identification of unique binding events, UMIs (see Question 45) are included in the probes. Once the probes have found their intended targets, the gap between probes is copied from the target sequence and ligated to form gap-filled circular probe construct. This circular construct is linearly amplified by RCA to generate multiple copies of the circular probe. After linear amplification, sequencing platform-specific adapters are introduced through a limited-cycle indexing PCR. To ensure optimal quality and purity, the resulting libraries are purified with bead purification protocols. This step helps to remove any impurities and contaminants that may affect the accuracy of the sequencing data. This process is collectively known as Bridge Capture™ (see Question 39).

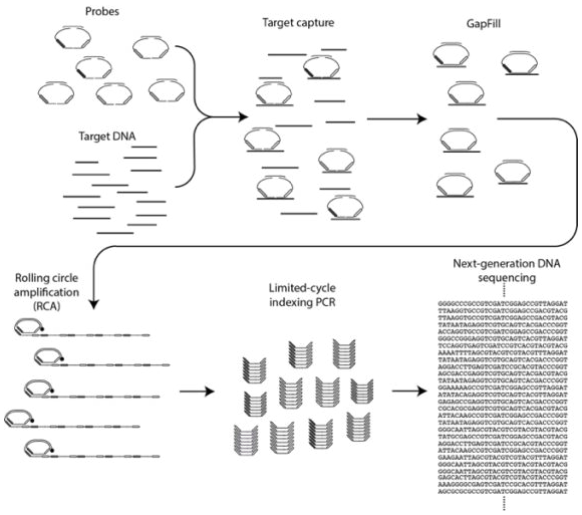
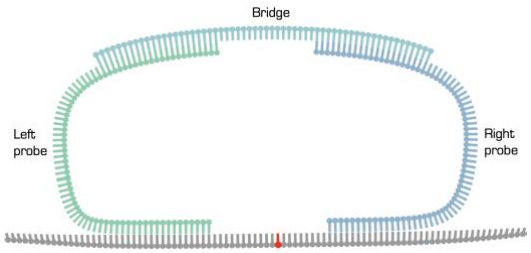


Figure 8 in Appendix

Question	Answer
39. What the bridge and Bridge Capture™ in the Geno1® technology means? What are the benefits of using the Bridge Capture™?  Figure 5 in Appendix	Bridge Capture™ is a patented laboratory process workflow (USPTO 11486003, EPO 4060050). The bridge structure connects the two target-specific probes, enabling the capture of the target region and its subsequent linear amplification. This innovative design allows the targeted region to be copied to the probe gap. This permits capture of relatively long target regions while keeping the cost of probe synthesis down by using shorter individual oligos. See figure to the left and Figure 5 in the Appendix for more detail. Bridge is the turquoise structure on top.
40. How does the targeting phase work?	The probe-bridge constructs are introduced into a hybridization reaction with the sample material. The number of probe-bridge constructs is significantly higher than the sample material to improve the kinetics of the hybridization.
41. How does the GapFill work?	In the Geno1® workflow, the process of circularizing the probe involves several steps. First, a polymerase is introduced to fill the gap between the two probes. The polymerase uses the target sequence as a template to fill in the gap between the probes. As the polymerase extends the sequence, it eventually meets the other probe, and a ligase joins the two probes together. Furthermore, during the polymerase and ligase reaction, a gap that exists between the probes in the bridge area is also filled, thus ligating the probes together from this end as well. This leads to the formation of a full circular molecule with the bridge still hybridized to it. The bridge sequence is an essential part of the probe design, as it helps to hold the two probes together and maintain the circularized structure of the molecule. The resulting circularized probe is now ready for amplification through RCA.

Question	Answer
42. How does the RCA work?	RCA is a nucleic acid amplification technique that involves the circularization of an ssDNA template, followed by repeated extension of a DNA polymerase around the circularized molecule, resulting in the formation of long, branched DNA molecules. In Geno1® process the RCA is primed from the bridge oligo, which is extended by Phi29 DNA polymerase. As the Phi29 polymerase progresses around the circularized template, it displaces the previously synthesized strand, resulting in the formation of a long, concatemeric DNA molecule. Phi29 is highly processive, and extension results in the formation of many copies of the original circular template, leading to efficient linear amplification of the target sequence.
43. How does the limited-cycle indexing PCR work?	Limited-cycle indexing PCR uses primers with overhangs to introduce the required adapters for the sequencing platform of choice. The PCR is done in limited cycles to minimize the exponential amplification as the main amplification is done in RCA.
44. What is the base pair size of the libraries created by Geno1® workflow?	The base pair size of the libraries varies depending on the probe design. Usually, the length is approximately 280 base pairs.

UMIs

Question	Answer
45. What is a UMI?	UMIs are sequences associated with each read that permit identifying the original molecule from which amplification took off. UMIs permit accurate target quantification as well as error correction. UMIs are introduced by incorporating random nucleotides into predefined regions of the probes. When the probes are sequenced, the UMI sequences are also read. The UMIs are designed to be long enough to create sufficient variation, so that each probe hybridization event to the target material will have a high probability of having its own unique UMI. This high level of variation is crucial for accurate quantification, as it ensures that each individual molecule is accurately identified and counted. This permits even rare variants to be quantified with high accuracy.
46. Does Geno1® use UMIs?	Yes. Geno1® is designed to be compatible with UMIs, which can be particularly useful for ultrasensitive quantitative applications, such as detecting low allele fractions from cfDNA. However, UMIs may not be necessary for the applications where simple presence or absence quantification is required.
47. How are the UMIs implemented in the data processing?	The UMI sequences are used in the Geno1® data processing in error correction. Error correction assumes that a single UMI should be associated with a single sequence, and any deviation from this is a result of an amplification or sequencing error. Taken together with the read count, the UMI sequences permit very accurate quantification of molecular counts with a high signal-to-noise ratio.

Quality

Note: Quality-related documents are given a unique document ID, which includes a type code so the document class can be easily distinguished at a glance. The type code itself includes a two-figure number (denoted "xx") unique to that document. Some of the type codes are Qxx (quality manual, policies), Sxx (standard operating procedures or SOPs), Fxx (forms) and Rxx (records).

Question	Answer
48. Have you implemented a quality system solution? What kind?	Yes. Our quality management system is completely electronic (eQMS). The QMS will be maintained mainly in Confluence. The software used in QMS has been validated.
49. Is your quality management system ISO certified?	It will be. We are working to have a QMS that complies with at least ISO 13485 (quality management systems of medical devices), ISO 14971 (risk management of medical devices) and FDA 21 CFR Part 11 (electronic records and electronic signatures).
50. Do you have a Quality Manual?	Yes. Document ID: Q01 Quality manual.
51. Do you have a Quality Policy?	Yes. Document ID: Q02 Quality policy and quality objectives.
52. Do you have a system for document control?	Yes. Quality documentation is controlled according to S01 Documentation management.
53. How do you ensure the quality of your products?	We will evaluate our subcontractors – such as OEMs who will supply the reagents and probes for our products – according to S13 Supplier evaluation. Kit-level quality control is not mandatory for research use only products. Despite this, as of 2023-12, we are discussing about the possibility of having some level of quality control for probe mix.

Question	Answer
54. Do you document and record company training activities?	Yes. Training is given and documented according to SOP6 Training of personnel. Confirmation on the received training is recorded using electronic signatures in our eQMS. Training records of individual employees are maintained in Confluence.
55. Is there a training program for new and current staff?	Yes. New staff undergo orientation training according to SOP6 Training of personnel. The orientation is recorded using FOP2 Orientation form. Current staff undergoes training whenever needed as new SOPs and policies are established. This training is recorded as described above.
56. Are CVs and job descriptions for current staff in place and maintained?	Yes. Genomill's Confluence site includes space for each individual employee. Employees maintain their CVs and job description in these pages.
57. How often do you back up the data?	eQMS is backed up once a month. Export is done in two formats: PDF, which is human readable, and CSV, which can be imported to Confluence. In addition, each individual quality document is backed up with each approved version.
58. How long do you store data?	We are planning to store the data for 10 years after an appropriate date, such as since publication of the document, retiring the product, etc. Data retention periods for different types of quality documentation is described in SOP1 Documentation management.

Appendix

Geno1® workflow

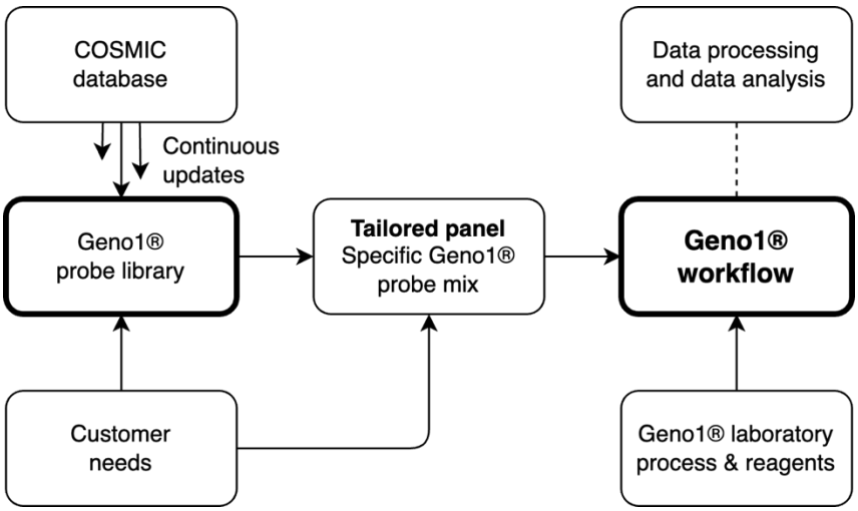


Figure 1 Complete Geno1® workflow comprises probe mix, process and reagents, and Geno1® data processing.

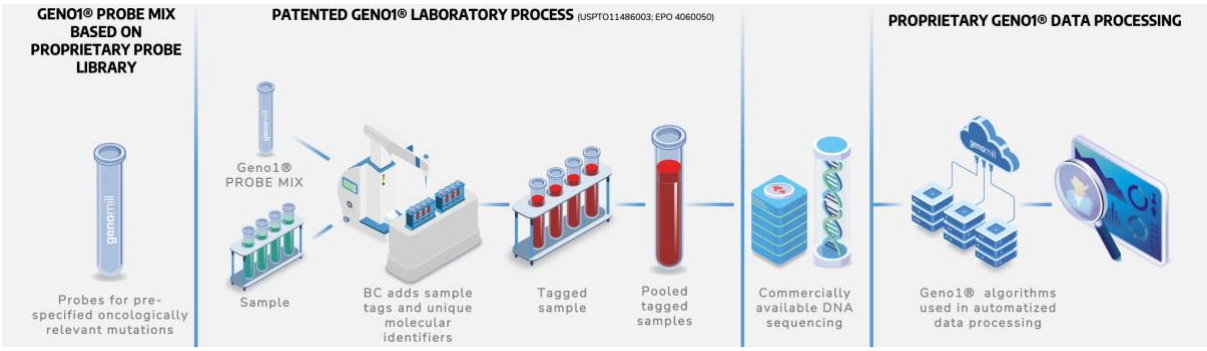


Figure 2 Illustration of Geno1® workflow.

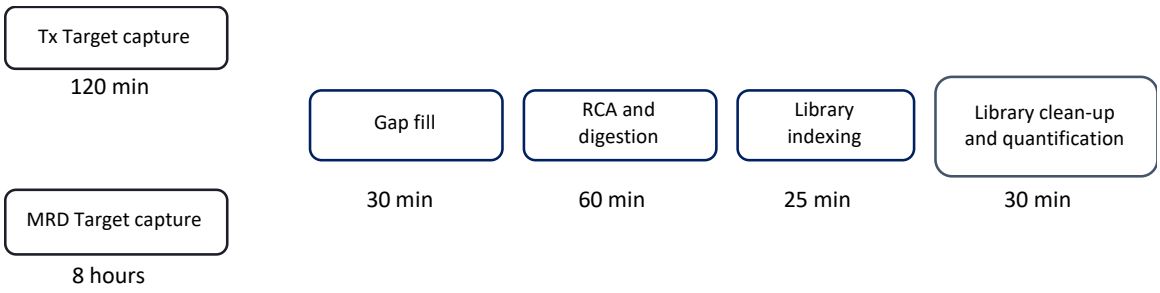


Figure 3 Duration breakdown of Geno1® Tx and MRD laboratory workflows.

Reproducibility

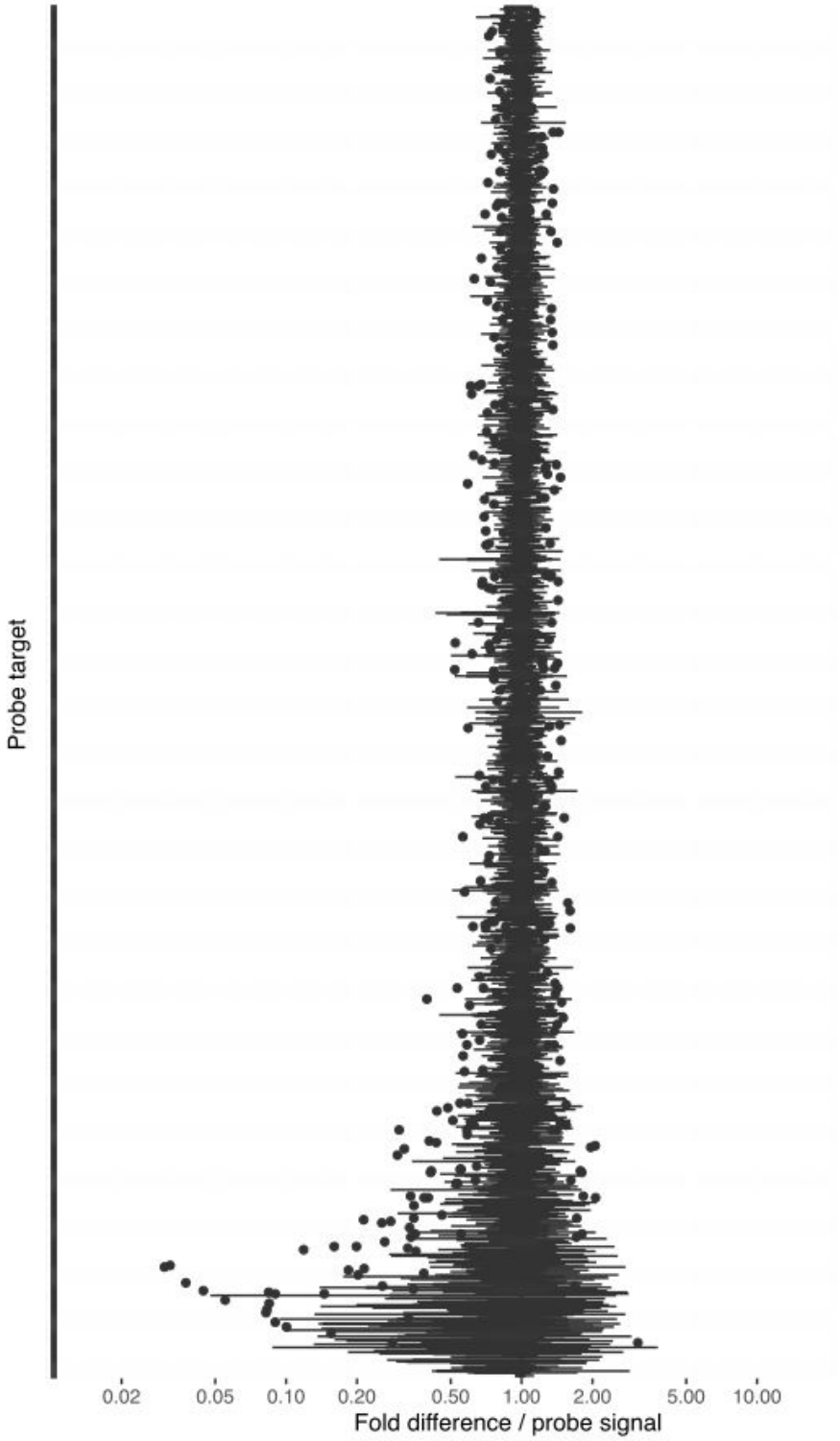


Figure 4 Reproducibility of a panel consisting of 887 probes over 10 sample replicates. The between-replicate differences are typically < 2-fold from the replicate mean, as shown by thin boxplots of replicate values calculated by dividing the replicate values of each probe target by the mean of all replicates.

Probe construct

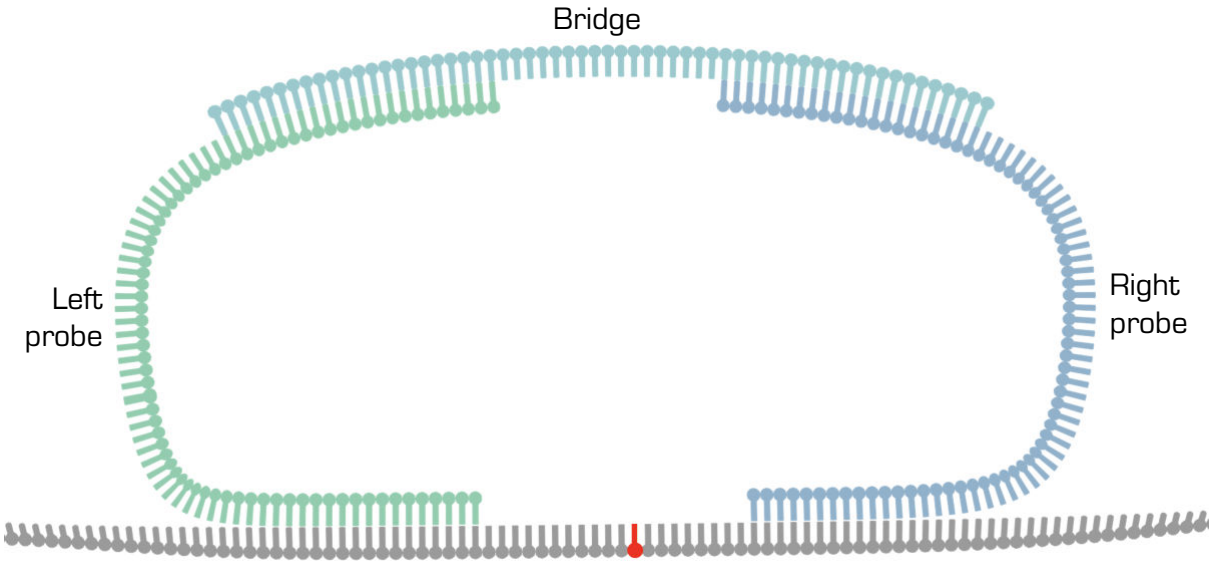


Figure 5 Probe construct. Left (green) and right (blue) target-specific probes are linked by a bridge-oligo (turquoise) forming a probe construct. The probe construct binds specifically to the target (gray) around the mutation of interest (red).

Mutations

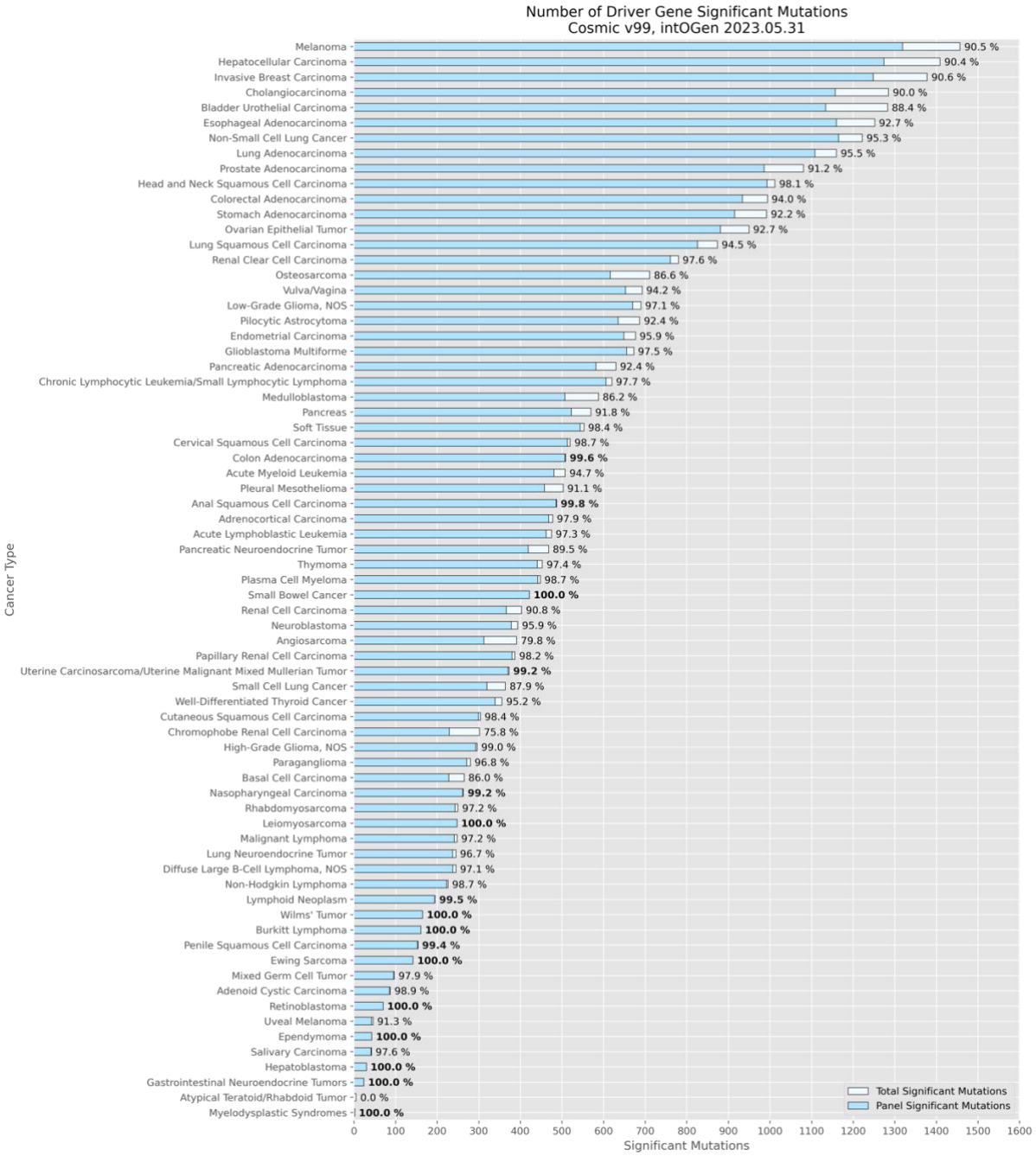


Figure 6 Cancer types and the driver genes associated with them and their significant mutations. Data of different cancer types and their associated driver genes are from intOgen-framework release 2023-05-31 (https://www.intogen.org). Significant mutations are mutations from these driver genes that have mutation significance 1 or 2 in COSMIC database, CMC v.99 (https://cancer.sanger.ac.uk/cosmic). The percentage is a ratio of number of significant mutations that the panel targets and total amount of significant mutations for that specific cancer type.

Panel evenness

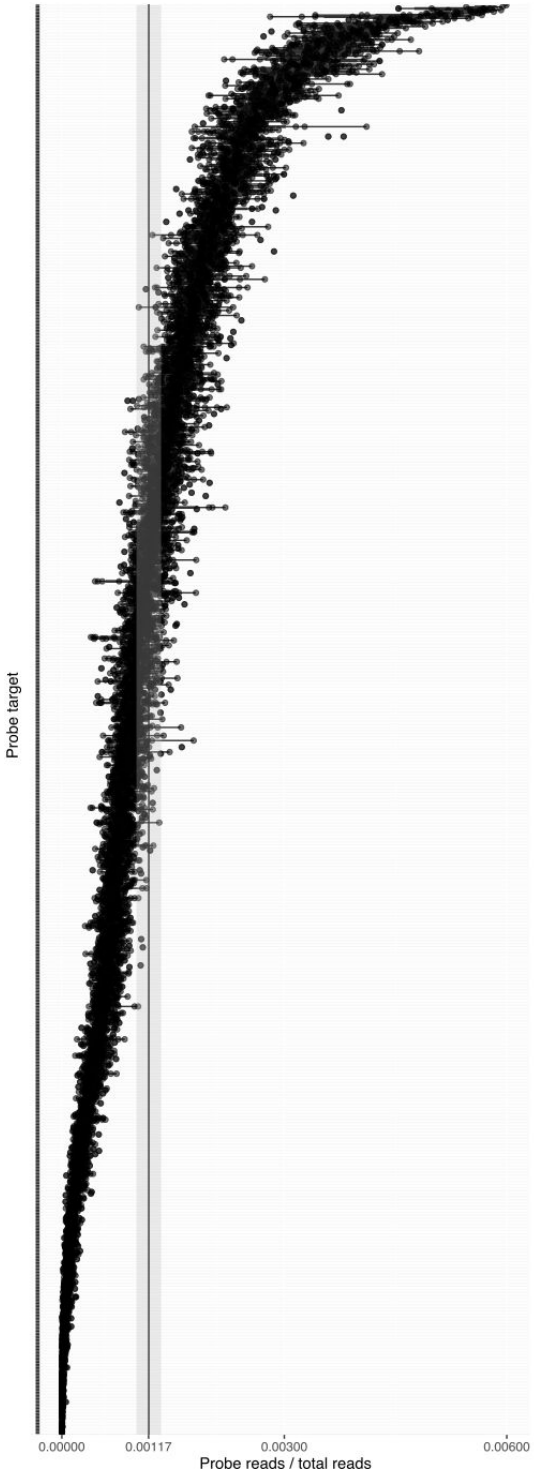


Figure 7 Panel evenness depicted as a series of boxplots of individual probe targets from 10 replicate Geno1® workflows. The thin boxplots of replicate values were calculated by dividing the replicate values of each probe target by the read sum of corresponding replicate workflow's all reads, representing the probe target's proportional signal in the whole panel across the replicates. The mean proportional probe signal is 0.00117 ± 0.00017 depicted with a vertical line, with the 1× standard deviation of depicted as a grey area around the vertical line. The total reads per sample were 830,000 ± 71,000.

Geno1® workflow in detail

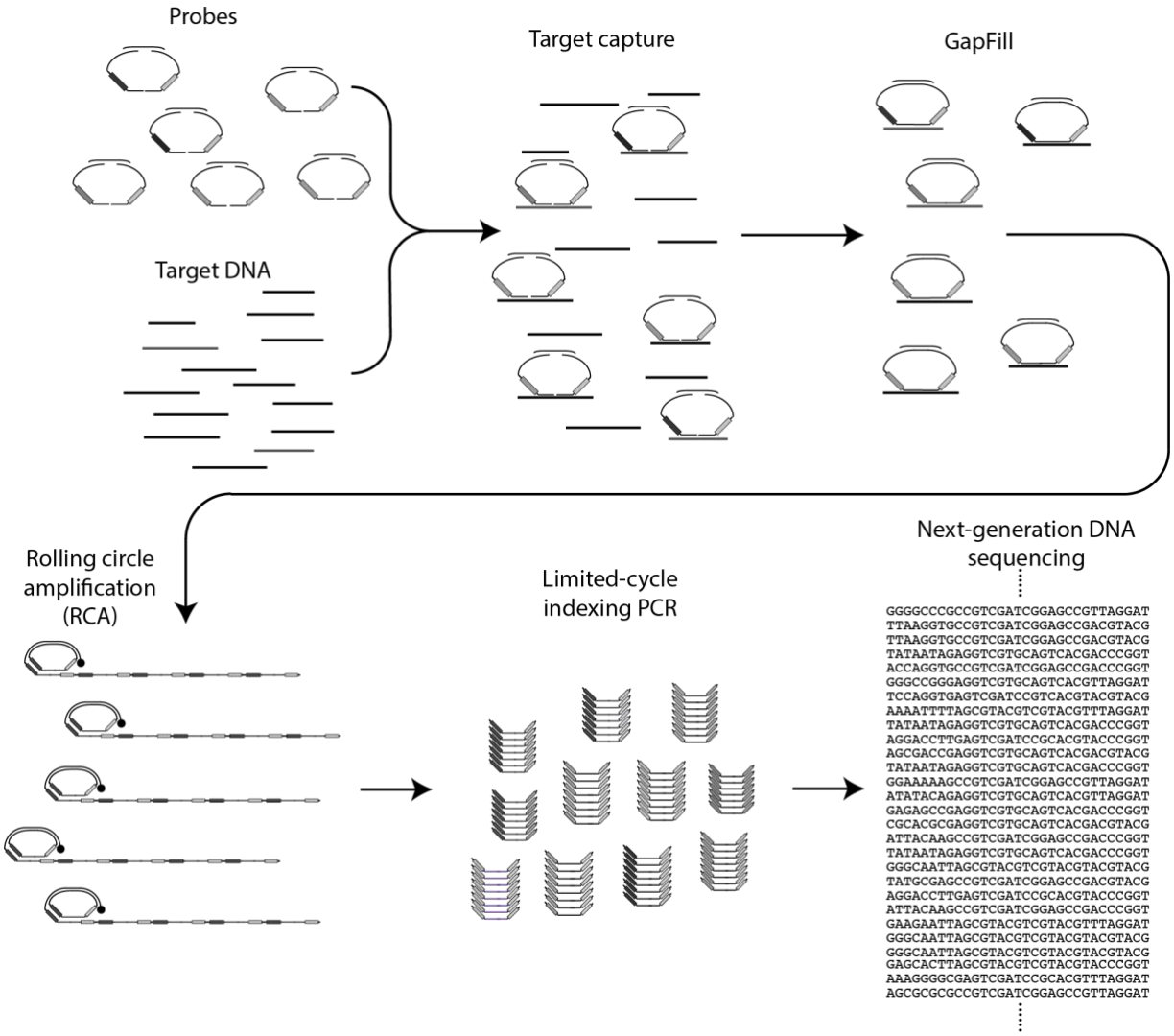


Figure 8 Geno1® workflow depicted in step-by-step manner. Probes hybridize to their targets and the gap is filled and the circular structure is formed by ligation. RCA is primed from the bridge and the adapter sequences are introduced in a limited-cycle indexing PCR.

Sequencing cost estimations

Table 1 Geno1® multiplexing cost on Illumina sequencing platforms using a panel of 282 probes. M = million, B = billion (thousand million).

	Benchtop sequencers			Production-scale sequencers			
Platform	iSeq 100	MiniSeq	MiSeq	NextSeq 550 (High output 75 cycle kit)	NextSeq 1000 & 2000 (P3)	NovaSeq 6000 (S4)	NovaSeq X (10B)
Reads per run	4 M	25 M	25 M	0.4 B	1.2 B	20 B	20 B
Cost per run	€500	€1,000	€2,000	€1,800	€6,000	€12,000	€7,100
Samples per run (Geno1® Tx – 8M reads)	0.5	3	3	50	150	2500	2500
Samples per run (Geno1® Tx – 800k reads)	5	31	31	500	1,500	25,000	25,000
Cost per sample (Geno1® Tx – 8M reads)	€1,000	€330	€640	€36	€40	€4	€3
Cost per sample (Geno1® Tx – 800k reads)	€100	€33	€64	€4	€4	€0.48	€0.28

Genomill patents

Table 2 List of granted and pending patents of Genomill.

NAME	TOPIC	STATUS	EXPANSION TO	PATENT NR.
Geno001	Foundational patent; standard protocol	**EU patent granted** **Japan patent granted** **China patent granted**	US, Canada, China, Hongkong, Japan	EP-3673081 JP-7074978 ZL-201880055271.3
Geno002	Dirty samples	**EU patent granted** **US patent granted**	US, Canada, China, Hongkong, Japan, South Korea	EP-4060049 US-11898202
Geno003	Improved workflow 1 (current standard)	**US patent granted** **EU patent granted**	Canada, China, Hongkong, Japan, South Korea	US-11486003 EP-4060050
Geno004	Improved workflow 2	US patent filed; EU examination in rebuttal stage	EU, Canada, China, Hongkong, Japan, South Korea	
Geno005	Improved sample indexing and barcodes	**US patent in grant phase**	EU, Canada, China, Hongkong, Japan, South Korea	USPTO # pending
Geno006	Rare allelic boosting	US patent filed	EU, Canada, China, Hongkong, Japan, South Korea	
Geno007	Compatibility with circular sequencing platforms.	US patent filed	EU, Canada, China, Hongkong, Japan, South Korea	