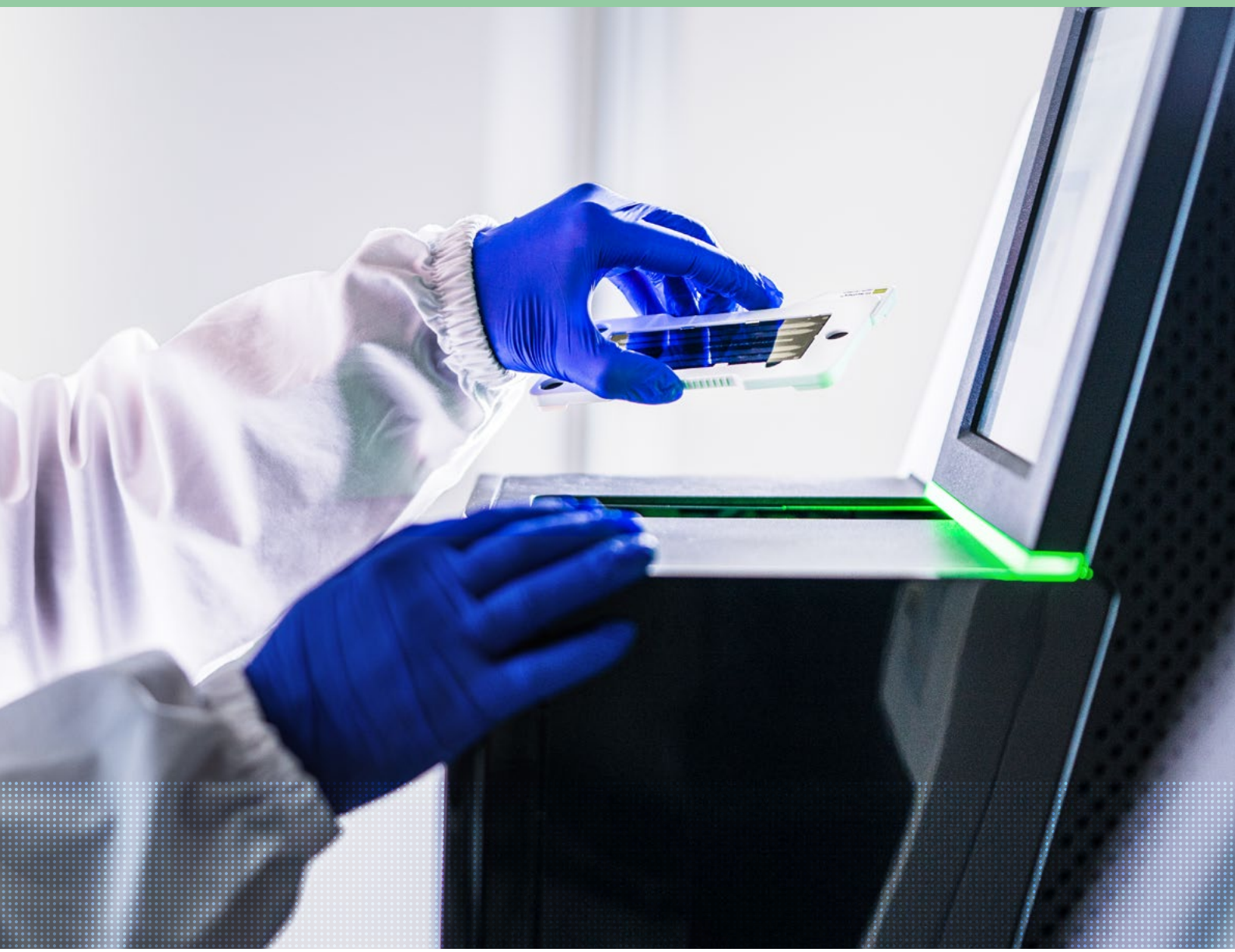
Your solution provider
in the world of genomics

CUSTOM NGS SERVICES
NEXT GENERATION SEQUENCING



Personal Genomics

DNA SEQUENCING

RNA SEQUENCING

METAGENOMICS

SAMPLE PREPARATION

EXTRACTION
Extraction from various matrices (e.g., blood, plasma, FFPE, fresh tissue and saliva) with kits optimized for the requested NGS service.

QUALITY CONTROL
Personal Genomics adopts the most widespread techniques for detemining the quality and quantity of the extracted DNA (NanoDrop, LabChip, Qubit). DNA amplifiability and fragmentation are evaluated for FFPE samples.

SEQUENCING

The sequencing setup depends on the library length and on the choice of the target enrichment kit. Personal Genomics mainly works with Illumina sequencers but, on request, also with other platforms (MinION, PacBio, BGI).

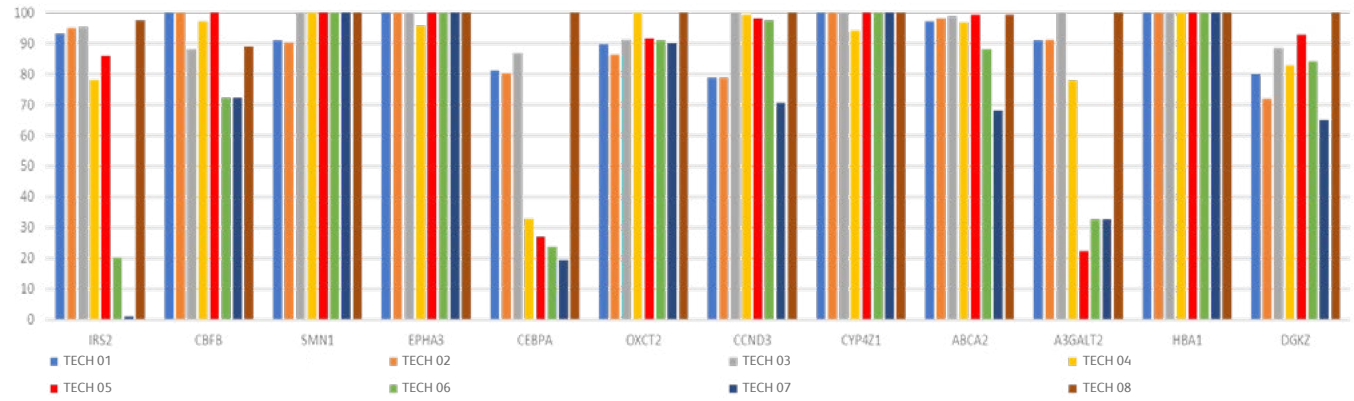
WHOLE EXOME (WES) AND PANELS

Choice of the target enrichment kit

Personal Genomics constantly evaluates the performance of new target enrichment kits. This allows to build a proprietary database that can be queried to assess which could be the best kit for every exome target region.

The figure below shows an example of WES with the histograms of the percentage of bases covered by at least 20 reads in 12 genes (in silico panels) analysed with 8 different enrichment kits.

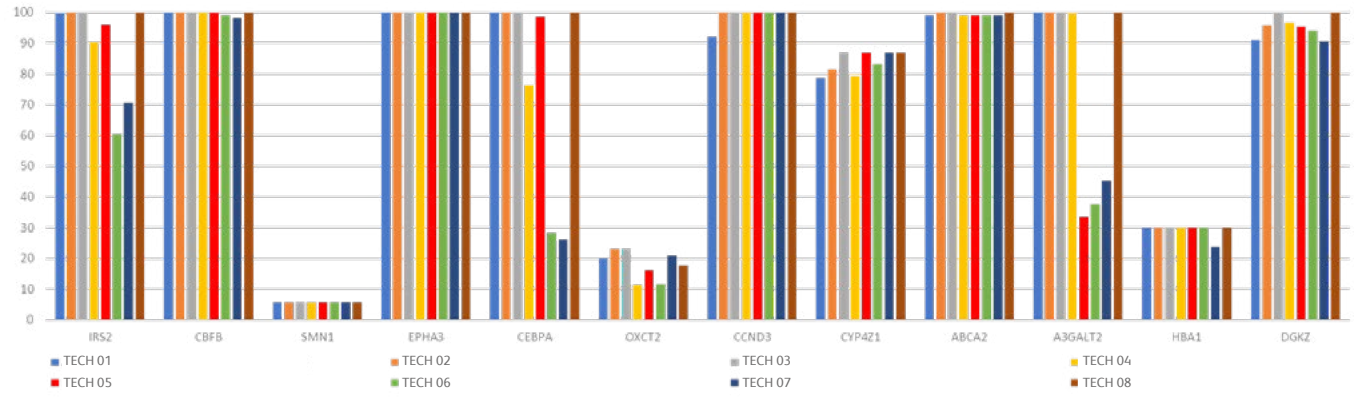
20X



The percentage of bases covered by at least 20 reads varies depending on the enrichment kit; however, a good coverage does not always mean good genotypability of the target region, as this parameter depends on the quality of the alignment.

Some genes are very well covered by all the kits used, however, variants cannot be called due to the low mapping quality. The next figure highlights the percentages of genotypable bases in the same 12 genes and of the same enrichment kits.

PASS

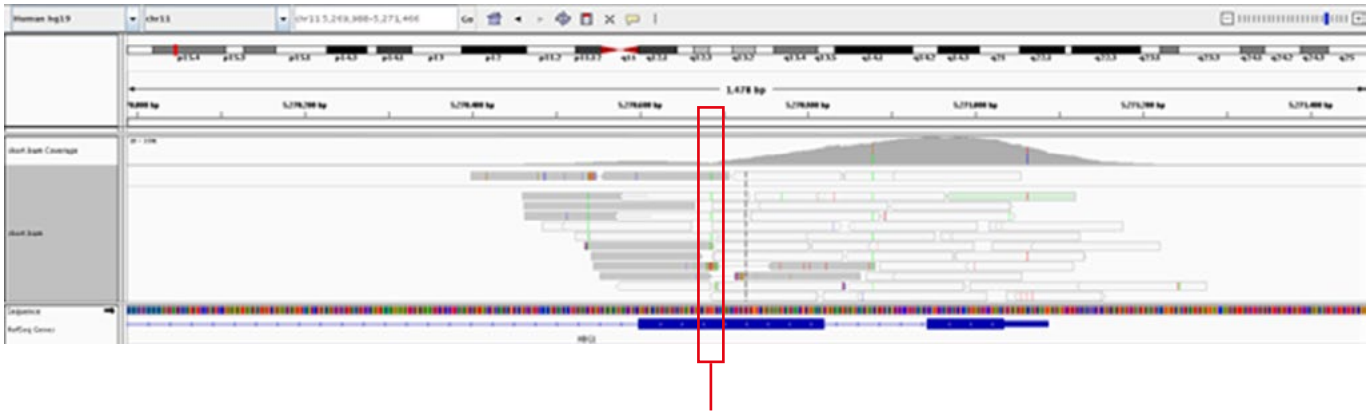


For the above reasons, Personal Genomics deems that coverage is not a sufficient quality parameter, as it is not directly correlated with genotypability.

IMPROVING GENOTYPABILITY

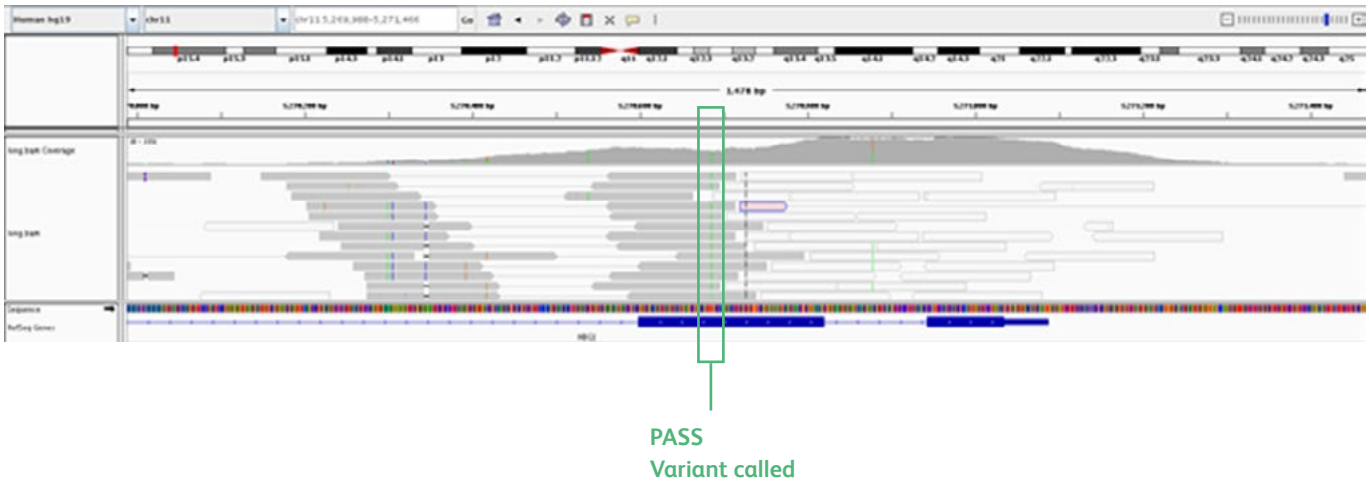
In some regions of the genome (see figure below) a variant cannot be called if there is low mappability, even if the coverage is good.

STANDARD PROTOCOL (2x75bp)
Insert size: 207 bp



To obtain the best genotypability, Personal Genomics has developed library preparation protocols able of modifying the length of the fragments. By increasing the fragment length to an average of ca. 300-350bp, instead of 150-200bp of standard protocols, sequencing can be performed in 150PE, thus improving mappability of sequences and the subsequent variant calling. Indeed, the variant that was not genotypable with the standard library, reported in the previous example, by using Personal Genomics' modified protocol is correctly called (see figure below).

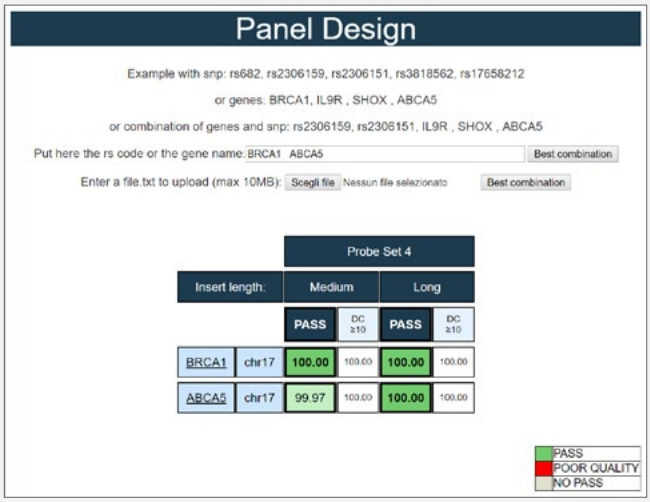
PERSONAL GENOMICS' PROTOCOL (2x150bp)
Insert size: 347 bp



PANEL DESIGN

Information about coverage and genotypability of the different enrichment kits as the mean length of the fragment changes allowed Personal Genomics to develop an application that identifies the best possible configuration to be chosen when sequencing a pool of genes.

Personal Genomics has patented its own library production method.



Software

Personal Genomics has developed a software that allows to identify the best combination of available sequencing technologies, capture kit and fragment length for a panel of genes of interest.

By using the Personal Genomics Panel Design software, it is possible to identify the best configuration to meet the customer requests.

The Personal Genomics R&D unit constantly updates the databases of the Panel Design software with the new enriching kits available on the market.

This information is of utmost importance for diagnostic panel design.

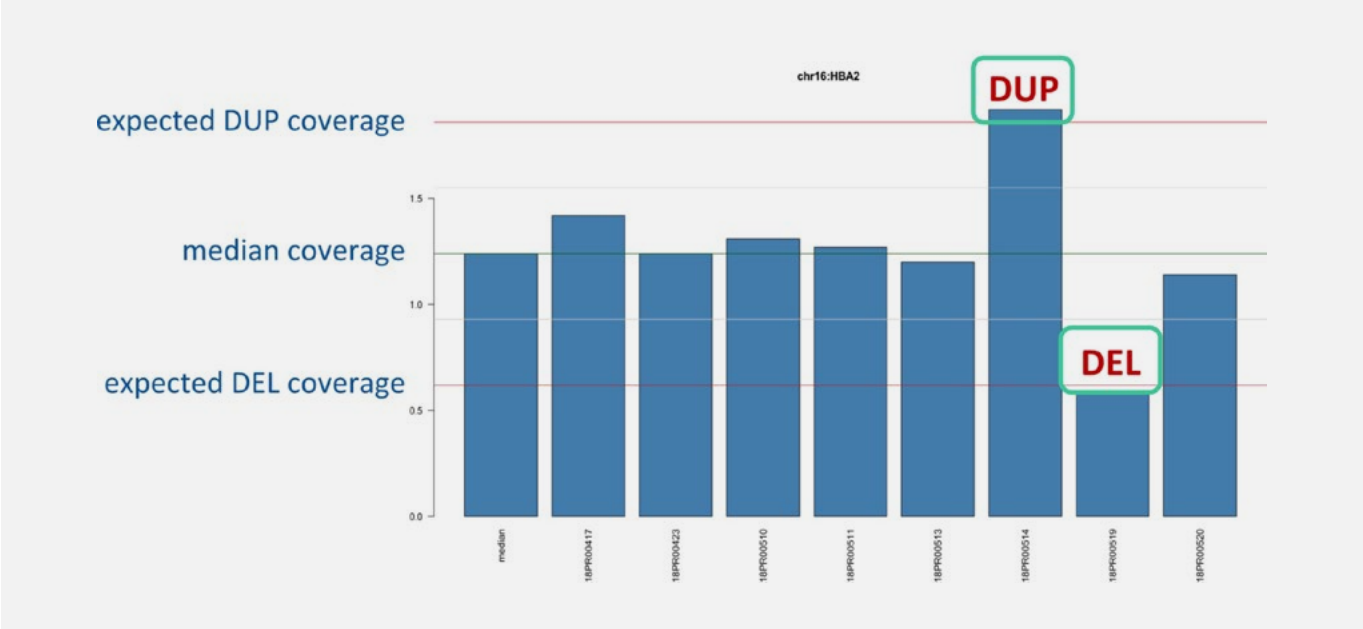
References

- EP3524695 (A1) - METHOD FOR THE PRODUCTION OF KITS FOR THE ENRICHMENT OF GENOMIC REGIONS.
- Barbara Iadarola, Luciano Xumerle, Denise Lavezzari, Marta Paterno, Luca Marcolungo, Cristina Beltrami, Elisabetta Fortunati, Massimo Delledonne. Enhanced targeted resequencing by optimizing the combination of enrichment technology and DNA fragment length. 2019, bioRxiv, DOI: 10.1101/712125.

CNV DETECTION FROM PANELS

Personal Genomics has developed panels that allow to call CNVs on target regions by using molecular probes for quantitative assay of the genomic DNA.

The figure shows the results obtained for HBA2 gene of 8 subjects. The observed duplication and deletion have been validated with MLPA.



WHOLE GENOME (WGS)

Personal Genomics uses PCR-free libraries to achieve the highest coverage. Genomic libraries produced with PCR-based protocols show a more irregular or absent coverage.

An example of these limitations is provided by GC-rich regions, such as the CGG-repeat of FMR1 gene:

Several types of variants can be identified through Whole Genome Sequencing:

- SNVs and INDELs
- Structural variants (e.g., CNV, inversions, translocations)
- Trinucleotide Repeat Expansions (e.g., FMR1, HTT)

BIOINFORMATIC ANALYSIS

	Bioinformatic analysis level I	Bioinformatic analysis level II	Bioinformatic analysis level III
Demultiplexing			
Production of FastQ files			
Alignment			
Variant calling			
Variant annotation			
Prioritisation			

ALIGNMENT AND VARIANT CALLING

- Alignment on reference genome (hg19 or hg38) and generation of BAM files;
- Variant calling using BWA-mem/GATK4 pipeline and generation of VCF files + file of genotypable regions.

The standard VCF file reports information about the variants called in one sample and implies that no information is reported for a specific position if it corresponds to an homozygous reference allele. This assumption is false because a specific variant may not be present in the VCF file due to the lack of coverage in the panel or due to low mappability. For this reason, Personal Genomics provides also the file reporting the genotypable regions.
As an alternative to the standard pipeline (BWA-mem/GATK4), a gVCF file can be produced by using the iSAAC- align/Strelka pipeline.

COVERAGE ANALYSIS AND GENOTYPABILITY

Personal Genomics does NOT work with theoretical coverage but the effective one, thus excluding:

- Off target regions
- Padded Region
- Duplicates

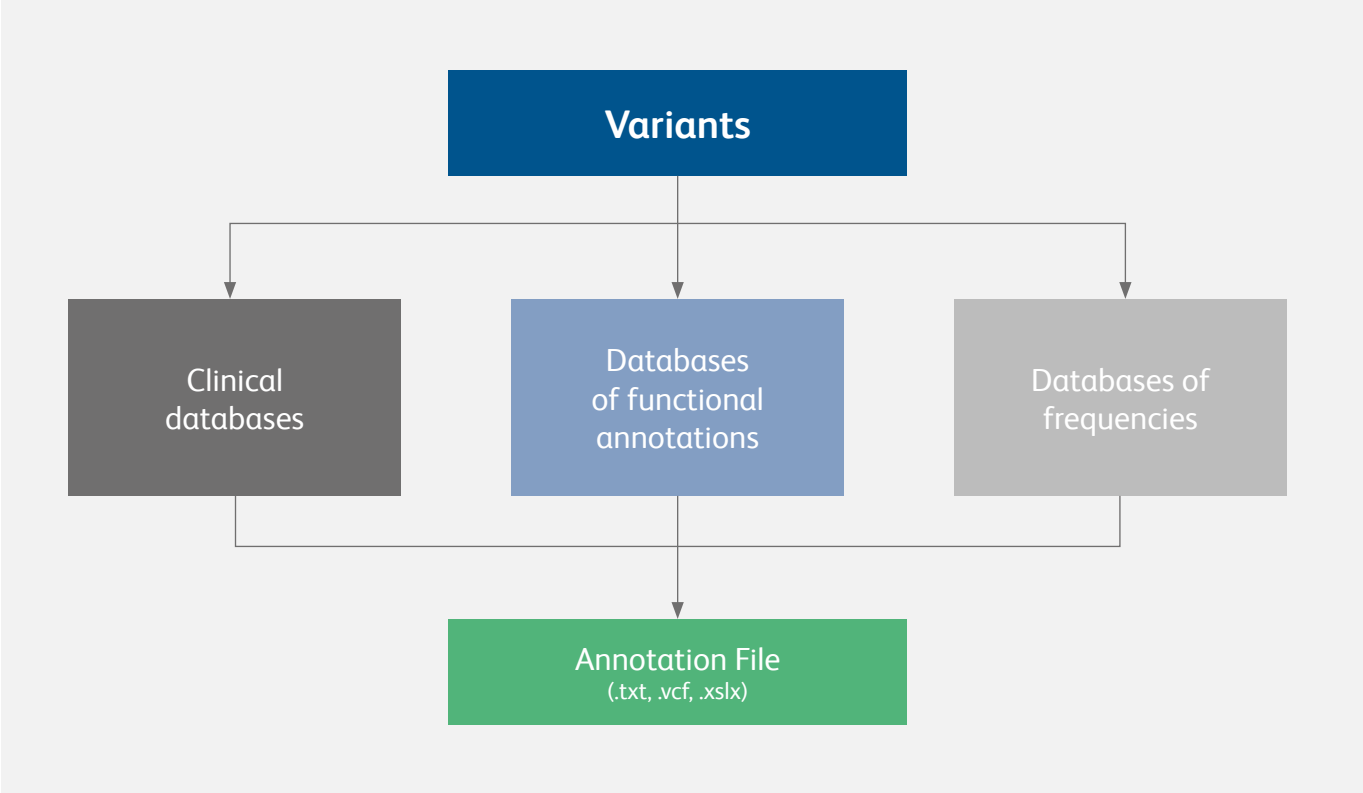
Personal Genomics has developed a pipeline to provide detailed coverage analysis and genotypability for all RefSeq protein-coding regions in case of WES, and of the target regions in case of panels.

CHROM	gene/region	gene/region lenght	avg_cov	%1X	%5X	%10X	%20X	%30X	%PASS	%PASS DP10
all	all	33642212	84.46	99.88	99.81	99.78	99.68	99.16	97	96.98
chr1	A3GALT2	1023	86.56	100	100	100	100	100	100	100
chr1	AADACL3	1057	95.63	100	100	100	100	100	100	100
chr1	AADACL4	1224	97.74	100	100	100	100	100	100	100
chr1	ABCA4	6822	70.89	100	100	100	100	100	100	100
chr1	ABCB10	2217	76.48	100	100	100	100	100	100	100
chr1	ABCD3	2007	63.68	100	100	100	100	100	100	100
chr1	ABL2	3702	98.19	100	100	100	100	100	100	100
chr1	ACADM	1377	72.38	100	100	100	100	100	100	100
chr1	ACAP3	2505	72.64	100	100	100	100	98.12	100	100
...	...	...	...	...	...	...	...	...	...	...
chr1	A3GALT2::33306765	688	93.39	100	100	100	100	100	100	100
chr1	A3GALT2::33312051	138	79.22	100	100	100	100	100	100	100
chr1	A3GALT2::33312500	90	68.11	100	100	100	100	100	100	100
chr1	A3GALT2::33312806	84	71.56	100	100	100	100	100	100	100
chr1	A3GALT2::33321075	23	53.57	100	100	100	100	100	100	100
chr1	AADACL3::12716340	4	68.5	100	100	100	100	100	100	100
chr1	AADACL3::12719477	214	80.39	100	100	100	100	100	100	100
chr1	AADACL3::12720882	64	51.48	100	100	100	100	100	100	100
chr1	AADACL3::12725221	775	103.62	100	100	100	100	100	100	100
chr1	AADACL4::12644546	168	82.2	100	100	100	100	100	100	100

The table reports the chromosome; the name of the gene or the region; the length of the region; the average coverage after filtering and duplicates removal; the percentage of covered bases; the percentage of genotypable bases.

VARIANT ANNOTATION

The identified variants are annotated based on the predicted effect on the protein and on annotations stored in public databases.



Annotation with public databases

- Clinical (OMIM, ClinVar, MedGen, CIViC, ICGC Simple Somatic Mutations);
- Frequency in the population (1000 Genomes project, NHLBI ESP6500, ExAC, gnomAD, PGVD);
- Functional annotations (RefSeq, GWAS catalog, GTEx, ACMG IF, ExAC LoF, dbNFSP, ENCODE).

Annotations with commercial databases

- HGMD Professional: contains more than 250,000 known causative variants. The database is manually curated by expert geneticists and is constantly updated based on literature review. Personal Genomics uses the locally installed version of HGMD Professional, allowing to perform the variant annotation process before the prioritisation.

PRIORITISATION

Single sample bioinformatic analysis

The annotated variants are prioritised, i.e., they are filtered using specific parameters and reported as:

- Clinically significant (as reported by the clinical databases);
- Variants not reported in clinical databases but with predicted clinical significance;
- Frequency in the population.
- Frequency in Personal Genomics Variant Database (PGVD), which stores additional clinical and phenotypical data regarding the variants, including rare ones.

(Familial) Trio bioinformatic analysis

The annotated variants are prioritised, i.e., they are filtered using specific parameters and reported as:

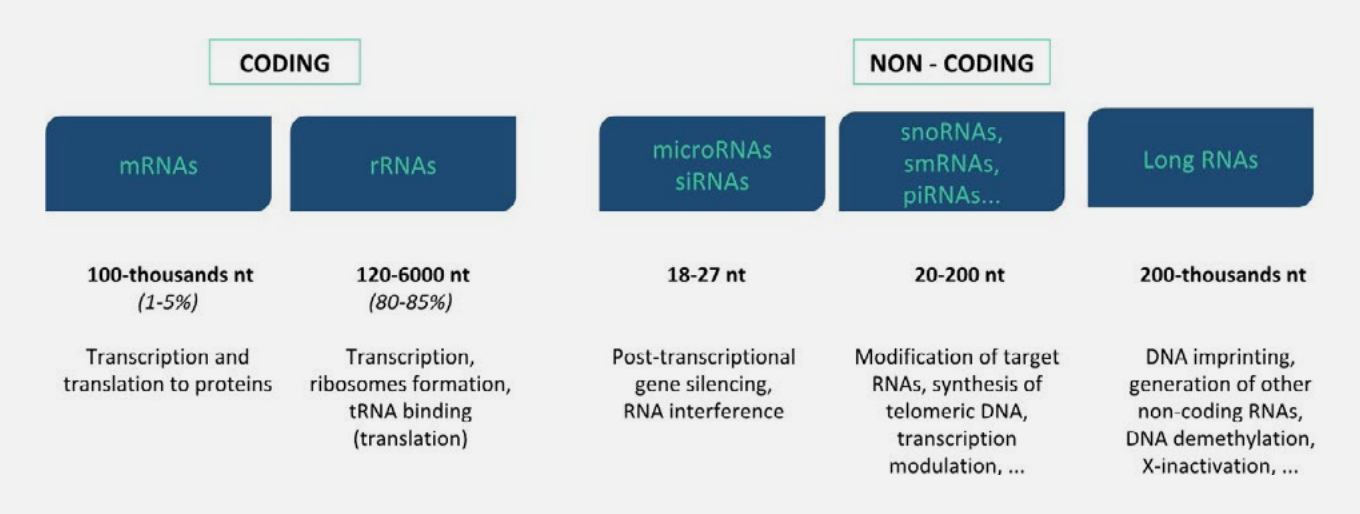
- Clinically significant (as reported by the clinical databases);
- Variants not reported in clinical databases but with predicted clinical significance;
- Frequency in the population.
- Frequency in Personal Genomics Variant Database (PGVD), which stores additional clinical and phenotypical data regarding the variants, including rare ones.
- Reconstruction of the variant's segregation in the family (de-novo variants, compound heterozygous, homozygous recessive).

Bioinformatic analysis of tumour samples

Personal Genomics provides the variant prioritisation service for tumour samples, which allows to identify:

- Somatic variants that are either pathogenic or likely pathogenics in clinical oncology databases;
- Frequency of the somatic variant in the tumour tissue;
- Variants not reported in clinical databases but with predicted clinical significance.

Personal Genomics uses Next Generation Sequencing protocols that allow to analyse total RNA (WTS), but also to specifically analyse protein-coding RNAs (RNA-seq) or microRNAs (miRNA-seq).



SAMPLE PREPARATION

EXTRACTION

Extraction from various matrices (e.g., blood, plasma, FFPE, fresh tissue and saliva) with kits optimized for the requested NGS service.

QUALITY CONTROL

Personal Genomics adopts the most widespread techniques for determining the quality and the quantity of the extracted RNA (NanoDrop, LabChip, Qubit).

SEQUENCING

The library preparation protocol and the sequencing configuration closely depend on the aim of the project.

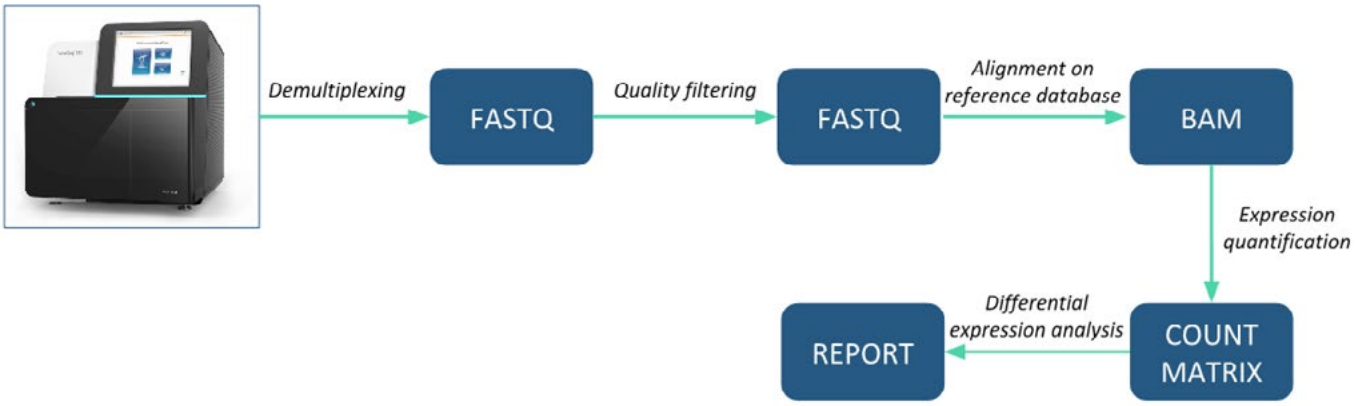
	RNA-seq	WTS	miRNA-seq
Type of RNA isolated	Mainly protein-coding (with poly-A tail)	protein-coding and non-coding	microRNA
Selection method	Selection of poly-A	rRNA depletion	Selection of mature miRNA (3' OH, 5' P)
Library type	75SE / 75PE / 150PE	75SE / 75PE / 150PE	75SE

BIOINFORMATIC ANALYSIS *

	Bioinformatic analysis level I	Bioinformatic analysis level II	Bioinformatic analysis level III
Demultiplexing			
Production of FastQ files			
Alignment on reference			
Analysis of the expression of annotated genes			
Analysis of the expression of annotated isoforms			

*** Personal Genomics, on request, develops custom pipelines that may include different and/or additional analyses with respect to those listed in the above table.**

STANDARD DIFFERENTIAL EXPRESSION ANALYSIS



EXAMPLES OF CUSTOM ANALYSES

- Variant calling (germinal/somatic)
- T-cell receptor / B-cell receptor analysis;
- HLA typing;
- scRNA-seq analysis.

Personal Genomics uses Next Generation Sequencing protocols for the analysis of microbial communities through sequencing of a target gene (e.g., 16S or ITS), or the whole genome or transcriptome (Single-species genome sequencing or Shotgun sequencing of the microbial community).

SAMPLE PREPARATION

EXTRACTION
DNA extraction from various biological specimens with optimised kits for the requested NGS analysis.

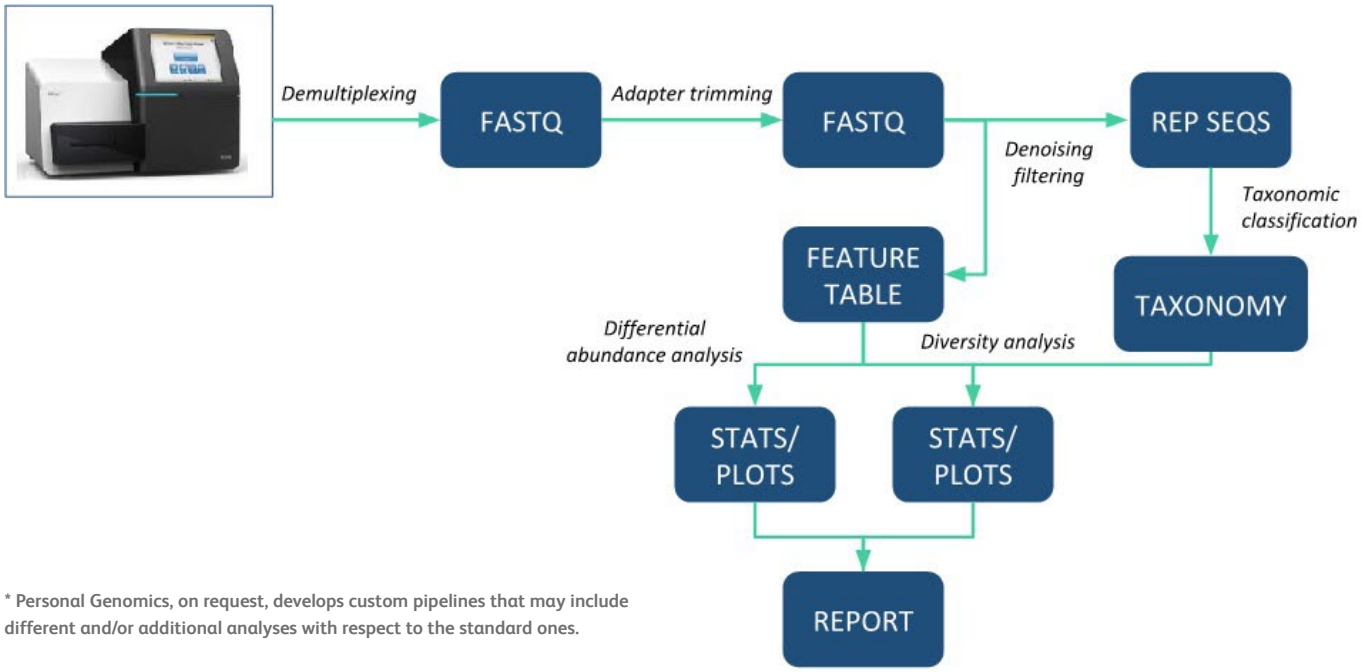
QUALITY CONTROL
Personal Genomics adopts the most widespread techniques for determining the quality and quantity of the extracted DNA (NanoDrop, LabChip, Qubit).

AMPLICON SEQUENCING (16S OR ITS)

The sequencing of the 16S rRNA gene and of the Internal Transcribed Spacer (ITS) are the most common applications of amplicon sequencing in metagenomics.

This type of sequencing allows to identify and compare the bacterial or fungal species present in a sample.

BIOINFORMATIC ANALYSIS *



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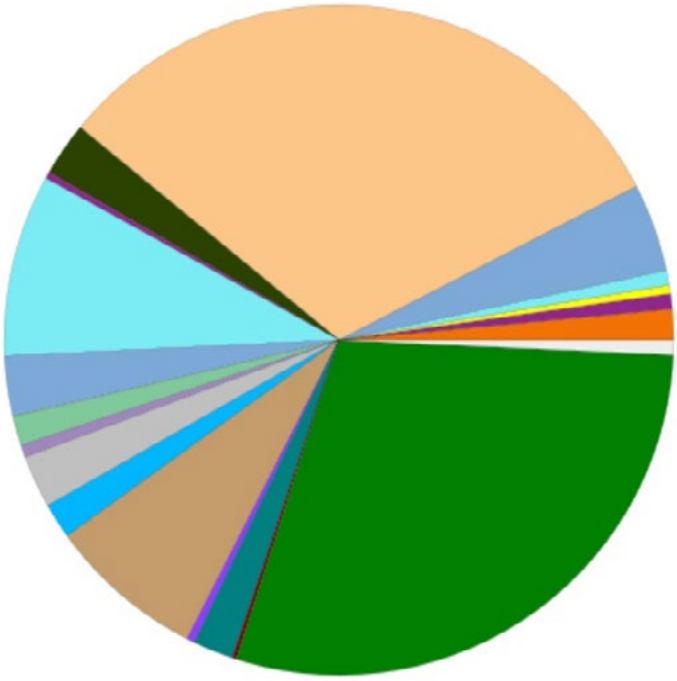
EXAMPLE OF TAXONOMIC CLASSIFICATION



k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Capnodiales;f__Cladosporiaceae;g__Cladosporium;s__Cladosporium_sp
k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Dothideales;f__Aureobasidiaceae;g__Aureobasidium;s__Aureobasidium_pullulans
k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Pleosporales;f__Didymellaceae;g__Leptosphaerulina;s__Leptosphaerulina_sp
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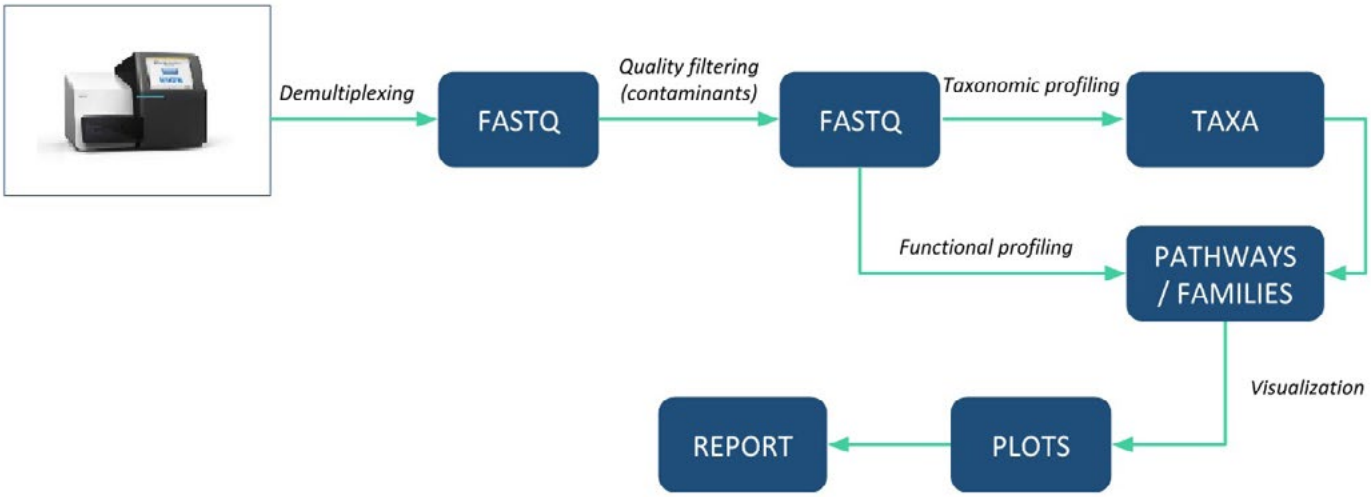
SHOTGUN SEQUENCING OF THE MICROBIAL COMMUNITY

Shotgun sequencing of the microbial community allows to analyse the entire genome or transcriptome of the entire community of microorganisms (metagenome/ metatranscriptome) in a sample.

BIOINFORMATIC ANALYSIS *

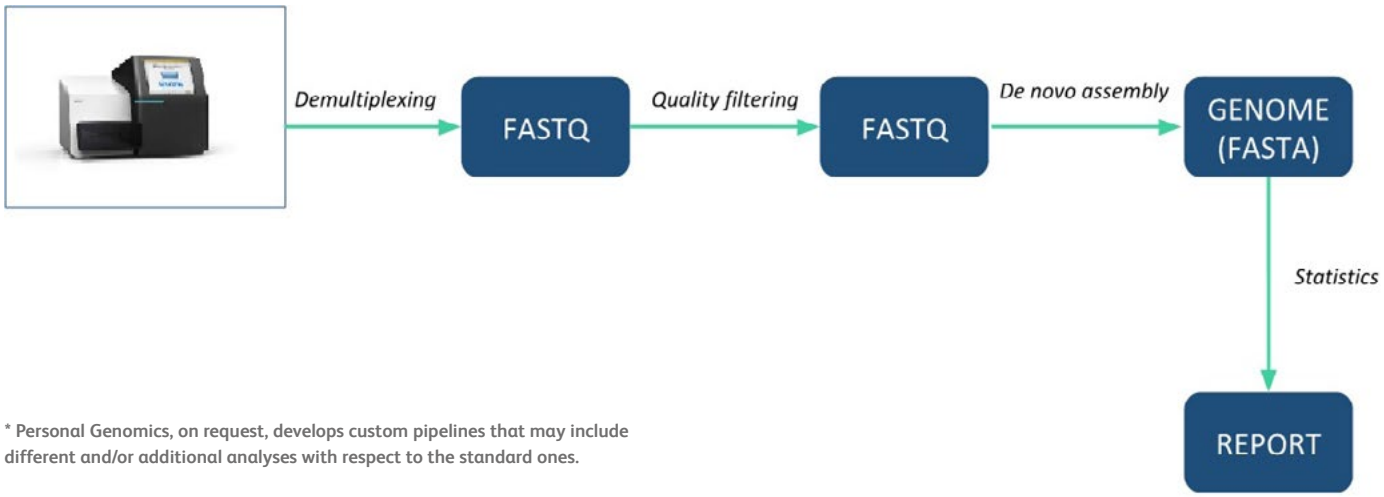


* Personal Genomics, on request, develops custom pipelines that may include different and/or additional analyses with respect to the standard ones.

MICROBIAL GENOME SEQUENCING AND ASSEMBLY

Shotgun sequencing of the whole microbial genome and de novo assembly.

BIOINFORMATIC ANALYSIS *



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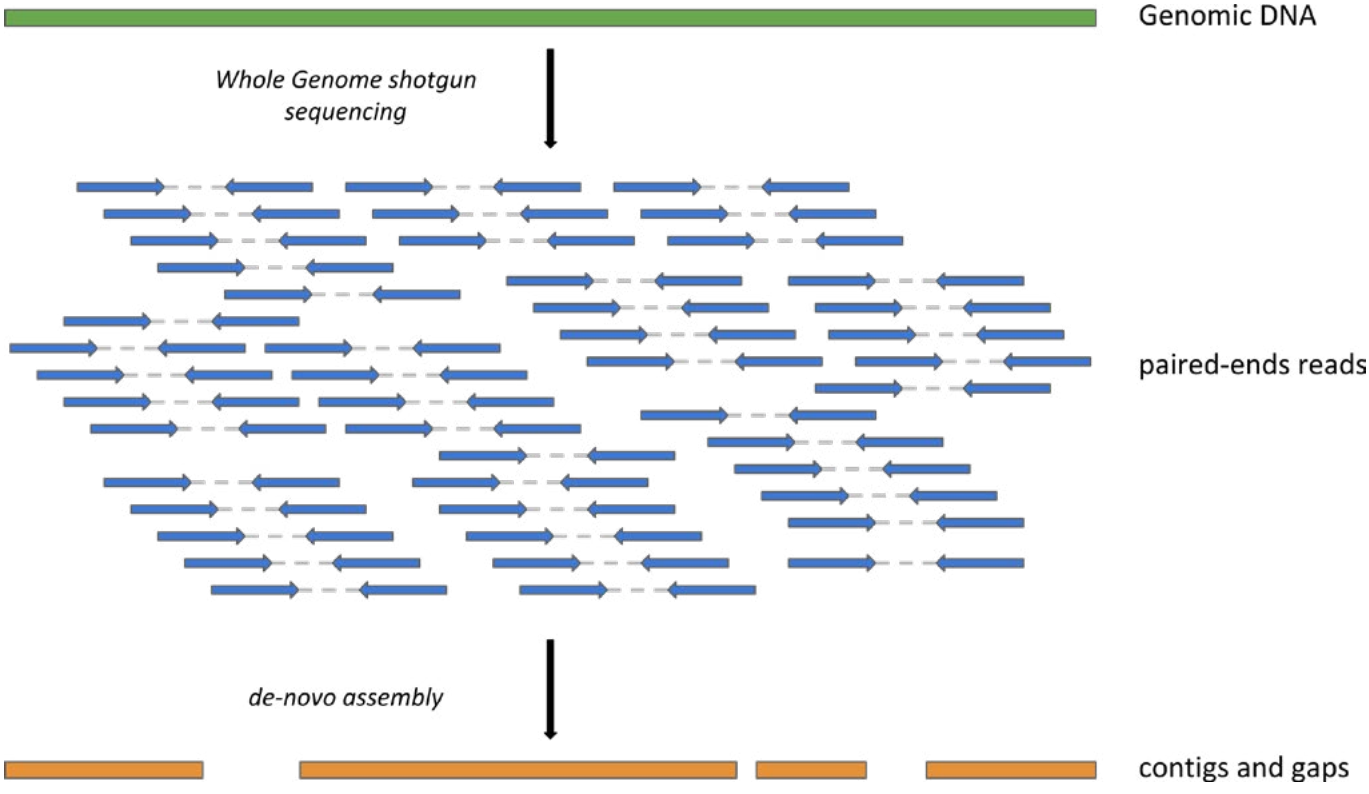

Your solution provider in the world of genomics

Personal Genomics

Personal Genomics Srl
Via Roveggia, 43b
37136 Verona (VR) Italy
t +39 045 8202351
info@personalgenomics.it
www.personalgenomics.it

customercare@personalgenomics.it

Biotechnology Division

DIATHEVA
Bringing Research to application

Personal Genomics

CRYOLAB

SOLGROUP
a breath of life