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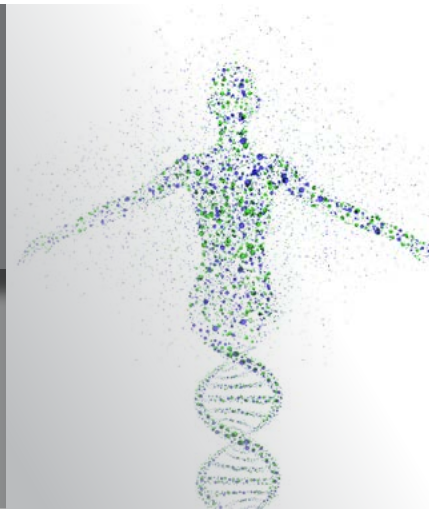
**PERSONALISED MOLECULAR DIAGNOSTICS > FOR SOMATIC DISEASE > FOR YOU**

# Targeted Sequencing of DNA in Cancer



The Human Genome Project was completed in 2003 at a cost of approximately US $3 billion. In the last decade there have been rapid advancements in technology with a marked reduction in the costs of genomic sequencing and a reduced sequencing time.

The initial sequencing was performed using Sanger sequencing and whilst this methodology is still in use today, it is being supplanted by massive parallel sequencing (MPS) or next generation sequencing (NGS).

## Massive Parallel Sequencing (MPS) or Next Generation Sequencing (NGS)

There are a number of different platforms available including instrumentation from Thermo Fisher/Life Technologies, Illumina and Pacific Biosciences. A number of other groups such as Complete Genomics (BGI Dx) offer a service in sequencing. Massive parallel sequencing uses a number of different technologies, but essentially DNA or RNA is fragmented, barcoded, adapters are attached and each base is then interrogated multiple times. The majority of the current instruments use short reads (<500base pairs (bps)) and then assemble the genome from the various pieces. Longer read instruments such as PacBio or Roche 454 can read thousands of base pairs.

Various measures of quality are quoted for MPS and one is coverage. This is the number of times a base has been interrogated at the position and is usually reported as NumberX. For example if the coverage at a particular base is 500X then that site has been read 500 times. For discovery projects 30x is often used as this reduces costs and will identify common mutations. For germline mutations (inherited) a minimum of 120X is usually required as there are only three percentages at each base (0%, 100% and 50%) for homozygous and heterozygous cases. However, the higher the coverage, the more reliable the results. For somatic mutations, where mutations in cancers may occur in a small percent of tumour cells, a minimum coverage of ~500X is required. For high sensitivity and minimal DNA amounts a minimum coverage of ~25,000 - 50,000X may be required. It should also be noted that results may be quoted as an average cover or a percentage of targets having more than 20X coverage. Care should be taken when interpreting results in these circumstances as some exons or bases of interest will fall below the minimum threshold for accurate detection of variants.

## MPS - in our laboratory

In our laboratory we use the ThermoFisher/ Life Technologies Ion Torrent system and aim for a minimum coverage of ~500X for inherited diseases and a minimum coverage of ~500X for the worst performing site for somatic mutations. For somatic testing the average coverage is usually ~2000X. Using validated control samples we can reliable detect mutations in formalin fixed tissue samples (FFPE) at 1-2%.

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## Samples

A large number of assays developed for genomic testing require the use of fresh/ frozen tissue. This enables the isolation of good quality long DNA or RNA.

**For tumour tissue this poses a number of problems:**

- The current pathology work-flow does not easily accommodate the collection of fresh tissue as transit times vary depending on the location of the laboratory and the collection site.
- Histological diagnosis is the current standard of care and collection of fresh tissue may compromise the diagnostic sample.
- Fresh tissue is usually homogenised for analysis and it is not possible to distinguish normal from tumour or in-situ disease or to ascertain the percentage of tumour in the sample.
- Some solutions have been developed such as RNALater that preserves DNA and RNA for transport, but the issues of determining tumour amount remain.
- Alternate preservatives are available instead of formalin, but again there are problems with cost, logistics (some require processing at 4° C) and workflow.

**Genomic analysis from formalin fixed tissue poses significant challenges:**

- Fragmentation of DNA and RNA.
- Crosslinking of DNA to proteins.
- Damaged bases are present in DNA extracted from FFPE material and are estimated at approximately 1 per 1000 bases.
- The majority of the changes are transitional C:G>T:A changes resulting from cytosine deamination to uracil.

## Target enrichment

A number of different methods are used for enrichment of target DNA or RNA. Targeted sequencing enriches a specific subset of genes or specific gene portions. With targeted enrichment, testing does not usually enable the complete sequencing of every base of each one of the genes. The two main types of enrichment are amplicon based and oligonucleotide capture.

### Amplicon based enrichment

Our targeted sequencing assays use amplicon based enrichment:

- Amplicon based enrichment uses the polymerase chain reaction (PCR) to make multiple copies of portions of DNA (or convert RNA into cDNA and then copy).
- The amplicons are then sequenced.
- The areas sequenced are determined by the design of the primers to make the amplicons.
- This methodology is very good for exons but is less useful for intronic regions and repetitive regions in genes.
- However, it uses significantly less starting material than other capture methods.
- It is a more rapid methodology.

### Oligonucleotide capture

In comparison oligonucleotide capture uses either in-solution or fixed complementary DNA or RNA to hybridise to the regions of interest.

- These portions of DNA are then captured and the DNA is then amplified and sequenced.
- Issues with this methodology are that there is often off-target capture so DNA from other regions is extracted and then sequenced and that not all regions are captured uniformly.

## Targeted Panels

THE ASSAYS WE USE HAVE BEEN OPTIMIZED FOR THE USE OF FFPE SAMPLES USING MINIMAL MATERIAL. THE ASSAYS USE THE LOWEST INPUT MATERIAL (<10 ng DNA) ENABLING THE USE OF BIOPSY SAMPLES, FNA SAMPLES AND CELL BLOCKS

**TSP19**

- 19 genes associated with acute myeloid leukaemia and myelodysplastic syndromes.

**TSP22**

- 22 genes associated with colorectal and lung cancer.

**RNA Fusion Lung cancer panel**

- Over 70 fusion transcripts associated with lung cancer.

**TSP50**

- 50 genes associated with actionable targets in cancer.

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