UTSouthwestern
Simmons Cancer Center

Genetic Testing Technologies

Genetic Testing Technologies for Hereditary Cancer

Different types of genetic variations can affect the function of a gene. Various technologies are used to identify different types of variants, depending on the purpose of the testing. Each has its benefits and limitations, and can impact ordering practices.

Most genetic testing for hereditary cancer predisposition uses at least one of the following techniques/technologies:

- Polymerase Chain Reaction (PCR) with Sanger Sequencing
- Next Generation Sequencing (NGS)
- Paired Germline and Tumor Testing
- RNA Sequencing

PCR and Sanger Sequencing

PCR with Sanger sequencing is considered the 'gold standard' of DNA sequencing.

How it works:

1. PCR uses primers (designed to identify and bind to a specific section of DNA), enzymes, and free nucleotides to replicate a desired section of the patient's DNA exponentially. This creates a "template" for the Sanger sequencing.
2. Sanger sequencing uses dideoxynucleotides tagged with fluorescent probes to bind to the "templates," creating DNA chains of varying lengths. These are then separated by size using gel electrophoresis.
3. Lasers read the DNA chains in order from shortest to longest and determine the sequence. This is aligned with computer programs and compared with reference data to determine if any variations are present in the patient's DNA.

Uses:

- Detect Single Nucleotide Polymorphisms (SNPs) and deletions/insertions <20 bases
- Confirm data generated by other technologies

Limitations:

- Cannot detect deletions/insertions >20 bases
- Allele dropout: one allele is not amplified due to variation in the primer binding site
- Cannot detect low-level mosaicism (<20% allele frequency) (Mosaicism is the presence of more than one cell line in an individual.)
- Cannot determine if variants are on the same allele or separate alleles (cis/trans), which is important for recessive conditions.
- Requires separate reactions for each exon/amplicon. Not scalable for large genes.

Next Generation Sequencing (NGS) or Massively Parallel Sequencing (MPS)

NGS/MPS, a newer and more cost-effective technology than Sanger, can test multiple genes at the same time.

How it works:

1. DNA sample is fragmented (~150 base sections).
2. The fragments are flagged for PCR amplification.
3. Amplified products are applied to a flow cell and generate clusters of identical DNA chains. The different clusters are sequenced simultaneously.
4. Bioinformatics software allows for millions of overlapping sequence fragments to be aligned into a larger fragment to determine the sequence.

Uses:

- Detect SNPs, small insertions/deletions, low-level mosaicism, large Copy Number Variants (CNVs), including deletions/duplications that are ≥1 exon long

Limitations:

- PCR methods are susceptible to allele dropout
- Pseudogenes, areas with very similar DNA sequences, are difficult to align accurately
- Cannot determine if variants are in cis or trans
- Many labs confirm all NGS variants by Sanger

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Paired Germline and Tumor Testing

Paired testing compares germline variants (from saliva, blood, or skin samples) to findings in the somatic tissue (tumor cells).

Uses:

- Determine etiology of tumor development (somatic vs. hereditary)
- Guide therapeutic decision-making FDA approved therapies and/or clinical trials
- Clarify how a variant should be classified (pathogenic, uncertain significance, or benign)

Limitations:

- Requires sufficient tumor sample
- Blood is not an acceptable germline tissue to analyze for hematological malignancies.

RNA Sequencing

Utilizes NGS technology to prepare a library of targeted RNA, sequence, and analyze the data.

Uses:

- May yield higher clarity in classification of variants that could impact RNA splicing.
- Serves as functional analysis of gene expression if RNA levels are different than expected.

Limitations:

- Cannot be performed on a saliva sample.
- May not be covered by insurances at this time.

Additional Genetic Testing Technologies

There are additional methods for evaluating genetic variation for common hereditary conditions. However, these technologies are not suitable for hereditary cancer predisposition testing.

- Restriction Enzyme Digestion
- Allele Specific Oligonucleotides (ASO)
- Fluorescent *in situ* Hybridization (FISH)
- Real Time Quantitative PCR (RT-QPCR)
- Multiplex Ligation-dependent Probe Amplification (MLPA)
- Array-based Comparative Genomic Hybridization (Array CGH or Microarray)
- Multiplex PCR

How Can I Learn More?

Resources

For more information about genetic testing and genetic counseling, visit the UT Southwestern Cancer Genetics for Health Professionals site.

For additional resources on coordinating genetics counseling and testing for patients, or providing genetics services, visit the Genetic Screening and Navigation Toolkit.

To find a genetic counselor near you, visit the National Society of Genetic Counselors Find a Genetic Counselor Tool.

References

Illumina Inc. Methods Guide: Version 5. (2019) Available at: https://www.illumina.com/content/dam/illumina-marketing/documents/gated/methods-guide-770-2014-018.pdf

National Human Genome Research Institute. Educational Resources. Available at: https://www.genome.gov/About-Genomics/Educational-Resources