



R.C.P.U. NEWSLETTER

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A statewide commitment to the problems of intellectual disabilities

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Next Generation Sequencing- Broadening the Horizon For Genetic Testing

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A Brief History

In 1990, the United States Human Genome Project was formally initiated. This project's goal was to identify the 20,000-25,000 genes in the human genome and sequence each of the 3 billion base pairs that make up our DNA.¹ The Human Genome Project took 13 years and 3 billion dollars to complete. Today, Next Generation Sequencing (NGS), a new and improved technology, allows us to sequence the entire genome in a few weeks for less than \$10,000. This cost-effective technology has opened the doors to entirely new platforms of clinical testing including large disease-targeted gene panels and Whole Exome Sequencing (WES).

Genetics Review

The human genome comprises all of our genetic material and consists of introns and exons. The exome consists of all exons, the portion of DNA that is responsible for protein coding. Current data suggests approximately 1% of the genome consists of exons.² These exons piece together to form the 20,000-25,000 individual genes present in our DNA.² The majority of mutations (genetic changes) that cause disease are located within the exome, therefore, we have the highest chance of finding the mutation(s) responsible for a patient's phenotype by focusing our search on the exome.

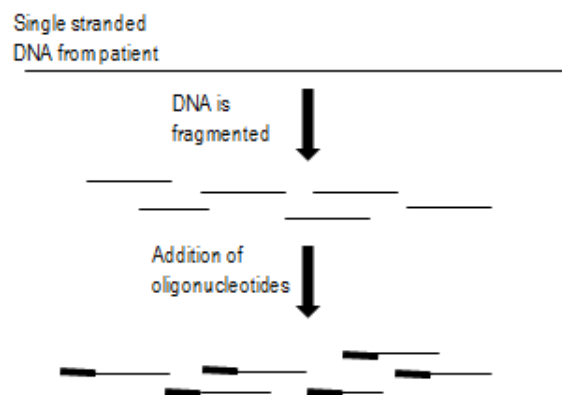
Next Generation Sequencing (NGS) Technology

Sanger sequencing is the traditional technology used for gene sequencing prior to the development of Next Generation technologies. When a gene is sequenced by Sanger sequencing each base pair is read and then sequenced individually which typically allows one gene to be sequenced for each run. In contrast, NGS allows multiple reads of many strands of DNA to occur simultaneously which allows for sequencing and analysis of many genes at once. Each platform for NGS uses a different method for amplification, sequencing, and analysis. Below is a summary of one these methods.

1. Create a Library

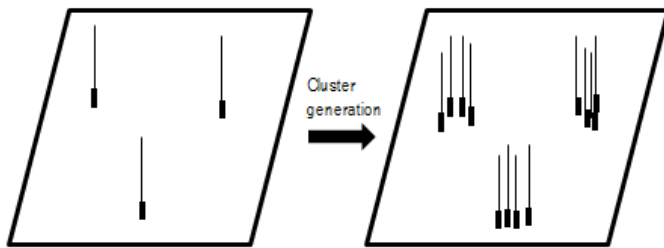
The first step for NGS is to create a library in which the DNA sample from the patient is broken down into shorter, single stranded segments. Pre-made oligonucleotides (single stranded fragments of DNA) are attached to these shorter segments.

Depending on the type of test ordered, gene or exon capturing might be necessary. Small probes "capture" the regions to be studied so that only the exons of the desired genes (or in the case of WES all of the exons) are identified and copied for analysis.



2. Cluster Generation

The approach for cluster generation depends on the platform used. In one method, the previously made library is distributed over a flow sheet and the oligonucleotides attach to a flat chip. These single strands are then replicated to form clusters which results in small groupings of DNA segments that have identical sequences.



mutation that alters the protein. First, variants are processed through a computer filter system that removes the known, benign variants. Humans then analyze the remainder of the variants and use the patient's clinical phenotype to determine which variants could be responsible for the phenotype.

Targeted Next-Generation Sequencing (NGS) Panels

NGS has led to the development of large multi-gene panels for specific indications or diseases. Targeted NGS panels allow patients with a defined phenotype that is associated with many candidate genes to undergo one targeted test to attempt to find the specific mutation responsible for their phenotype. Many of these panels offer sequencing of the genes associated with one disease or sequencing for diseases with similar phenotypes. For example, there are at least 7 genes responsible for Noonan syndrome and the Noonan syndrome phenotype overlaps with related disorders such as Costello Syndrome, Cardio-Facio-Cutaneous Syndrome, and LEOPARD Syndrome.³ Using NGS Panels we can sequence all of the known genes responsible for these conditions at the same time and for a much lower cost than sequencing each individual gene in a stepwise fashion. These panels can help find the causative mutation for a condition at a lower cost and with less stress for the family since only one sample and blood draw is needed.

Several laboratories are offering NGS Panels for many conditions including hearing loss, epilepsy, Noonan Syndrome and related disorders, cardiomyopathies, arrhythmias, heterotaxy, aortopathies, Retinitis Pigmentosa, Bardet-Biedel, Cone-Rod Dystrophy, Neurofibromatosis, Usher Syndrome, Waardenburg Syndrome, Senior-Loken Syndrome, Septo-optic Dysplasia, ciliopathies, mitochondrial conditions, Marfan and aneurysm related disorders, hereditary cancer conditions, autism spectrum disorders, congenital disorders of glycosylation, X-linked intellectual disability, neuromuscular disorders, short stature, microcephaly, and brain anomalies.^{4,5,6,7,9,10} These panels can contain anywhere between a few genes to over a hundred.

Whole Exome Sequencing

WES through NGS has led to a paradigm shift in the field of clinical genetic testing.

Who is a good candidate for WES?

1. A child with an unknown phenotype that strongly suggests an underlying genetic condition
2. A child with a known phenotype with negative testing for the most common and/or known genes responsible for the phenotype

What type of results can/will we receive?

The amount of information generated by WES is vast and complex. The amount of information released in a final report varies between laboratories. Typically, all labs report pathogenic mutations within genes that could account for the patient's phenotype. You might also receive variants of uncertain significance (VUS) within candidate genes that fit the phenotype. VUSs are not definitely causative of protein alteration. Over time, these variants may be reclassified as disease causing or benign.

In addition, some labs report actionable, disease-causing mutations that are identified and not related to the patient's current clinical picture (for instance a mutation in *FBN1* causing Marfan) or actionable pharmacogenetic mutations (that affect the way a patient processes medications). Labs may also report mutations that indicate carrier status for an autosomal recessive condition although many labs give patients the opportunity to opt out of this type of result.

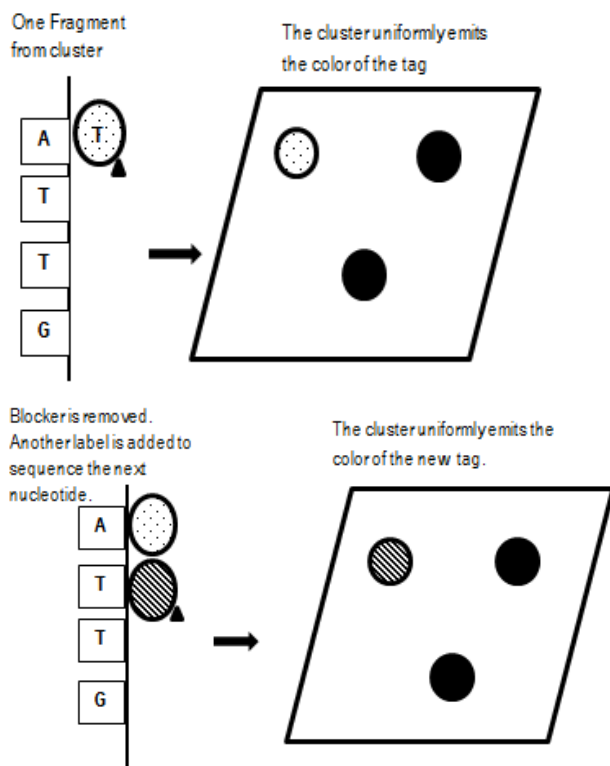
Some companies offer extended reports that include non-actionable pathogenic mutations within genes that are not associated

3. Sequencing

The next step is sequencing of the clusters. Fluorescent labels attached to nucleotides are distributed over the flow sheet. Each label is colored based on the nucleotide it is attached to. These labels will attach to the DNA strands in each cluster so that strands with an exposed Adenine (A) nucleotide will have a correlating label. In the diagram below, we used different patterns to indicate different colors of the labels. The labels attach to each fragment in the cluster so that each cluster emits one uniform color (as shown on the chip to the right of the fragment in the diagram below). The labels also have a blocker (black triangle) that prevents additional labels from attaching.

A machine reads the entire sheet and marks the color of each individual cluster.

The blocker is removed from each label so that a new label can attach. The process is repeated so that each cluster has a unique sequence based on the colors expressed after each round.



4. Analysis

A computer uses a reference sequence to align all the sequences generated from each cluster. The program then identifies variants that differ from the reference sequence and can be considered personal mutations.

5. Interpretation of Results

Each variant is analyzed to determine if it is a known, benign variant, a variant of uncertain significance (VUS), or a pathogenic

with the clinical phenotype as well as pathogenic mutations within genes that do not have an associated phenotype.

What is the likelihood we will receive an answer?

WES is not perfect. Labs have reported approximately a 30% success rate for all WES cases to date.¹² It is important that the details of the patient's clinical picture are provided to the laboratory so they can use that information in the analysis of variants and provide appropriate interpretation.

Ethical and Legal Dilemmas of Whole Exome Sequencing

WES leads to ethical and legal dilemmas that were not previously encountered when sequencing single genes. As mentioned, the amount of information provided by WES is vast, which opens the question of how much information is too much information. Some worry that patients will have a difficult time understanding basic genetic concepts, and will not be able to make an informed, educated decision to participate in WES. Others feel the general population can handle this information and have the right to all aspects of their personal genetic information. (Personal Observation)

With this said, defining "all aspects of personal genetics" can be difficult. Patients might not understand all of the possible results they might receive and the implications of these results so they might not know whether or not they should consent to receiving a specific type of result. One large debate is whether or not to provide pediatric patients with information pertaining to adult-onset conditions with no treatment or prevention. Many feel that if this information is found, the patient has the right to know even though common practice typically is to wait until 18 years of age to test for such conditions. If a provider or laboratory decides to wait until the patient turns 18 to disclose the results, who is responsible for following-up with the patient?

Even follow-up of VUSs found can be complicated. At this point, there is no consensus as to who should follow-up on changes of classifications of VUSs and how often the literature and databases should be re-assessed. Depending on the practice, physicians could be faced with checking on hundreds of VUSs each year for their patients who had undergone WES.

Future Directions

We are just beginning to tap into the potential of clinical tests made possible through NGS. As our knowledge of the exome expands, our ability to provide answers for patients who have been in the dark without a known diagnosis will increase.

Whole genome sequencing is also on the verge of becoming clinically available for physicians. Opening the entire genome to interpretation and analysis may allow us to solve some of the mysteries of the function of the non-coding regions of the genome.

NGS has decreased some of the previous limitations we've faced when offering genetic testing. NGS has broadened the horizon of genetic testing and we are just now on the brink of understanding the impact of this new technology.

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About the RCPU

The Raymond C. Philips Research and Education Unit began in 1978 when the legislature established section 393.20, F.S., of what is now known as the "prevention" legislation. It is named after Raymond C. Philips, who was the Superintendent of Gainesville's Tacachale (formerly Sunland) Center for 38 years, and was an acknowledged state and national leader in services for mentally retarded persons. The Unit is located on the Tacachale campus and is funded through a contract with the Department of Children and Families and the Department of Health.

The purpose of the R.C.P.U. is to treat, prevent, and/or ameliorate intellectual disabilities through medical evaluations, education and research. The unit provides direct evaluations and counseling to families and promotes service, education, and prevention projects.

Some of the conditions currently under study at the RCPU involve Angelman, Velo-Cardio-Facial, Prader-Willi, Fragile X, Williams and Smith-Lemli-Opitz syndromes.

The R.C. Philips Unit is a resource for all Floridians interested in the diagnosis, treatment and prevention of intellectual disabilities. Staff members are available for consultation and for educational programs for health professionals and for the community at large.

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