We are uniquely positioned to fully integrate genomics into the clinical setting at Rady Children's.
We have a vision to precisely diagnose and treat as many kids as possible with the use of WGS and provide the evidence that genomics can transform the practice of medicine.

**THE FUTURE OF MEDICINE IS HERE AND NOW.**

For more information, please contact:

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CLUSTER GENERATION AND SEQUENCING
The process begins by taking a blood sample and isolating genomic DNA from it. The DNA contains the 4 letters (ATCG) that make up our genetic code, and we hope that within those 3 billion letters, we are able to find the letter changes that are responsible for causing the disease in our patient.

DNA ISOLATED FROM BLOOD AND PREPARED FOR SEQUENCING
The process begins by obtaining blood from the affected child in the NICU/PICU, and in some cases, the parents. Often these kids are undergoing numerous tests and are acutely ill, so we try to use as little blood as possible for our testing, as little as 0.5 mL of blood which equals a spot the size of a dime.

BLOOD SAMPLES DELIVERED TO CLINICAL GENOME CENTER LABORATORY
The test is ordered and the blood sample is delivered from the hospital to the Clinical Genome Center at the Institute. We confirm the patient, date of birth and that the test has been ordered properly, and prepare the sample for the process of whole genome sequencing.

LIBRARY PREPARATION
Once the DNA is extracted, we measure the quality and quantity of the DNA. The isolation and quantification takes about 1.5 hours. With high stakes involved, including acutely ill children and proper stewardship of the time and cost to run the Whole Genome Sequencing (WGS), we have multiple quality checks in place to ensure a successful whole genome sequencing run on the first try. Every hour counts every time!

CLUSTER GENERATION AND SEQUENCING
Now the samples are ready to be loaded onto our Illumina instruments and undergo the process of cluster generation and sequencing. In this step, our fragments of DNA are bound to a flow cell. A flow cell is where the sequencing reactions occur and contains millions of oligos (synthesized fragments of bases or letters). Each fragment of DNA that is bound to the flow cell is copied thousands of times, so we have multiple data points for each position in the genome. On the instrument, the fragments now undergo a process called “sequencing by synthesis.” This is a proprietary process from Illumina which involves incorporating DNA letters (ATCG) to our DNA strands on the flow cell. As bands with T’s and C’s with Gs. When the letters bind to our fragments, a signal is generated that lets the machine know what letter has matched. These reactions occur over millions of fragments, all within the tiny area of a flow cell.

BIOINFORMATICS - ALIGNMENT AND CALLING VARIANT (LETTER CHANGES) IN THE GENOME
We must now take the fragments of sequenced DNA and try to line up to the reference genome. The reference genome is a standard human genome used by laboratories to find letter change differences between the patient and the reference. On average, an individual has over 5 million letter changes different than the reference. We must now try to parse through these changes to see if one or more of them are causing disease.

Thanks to cutting edge technology we are able to align and call variants in 3 hours. To process this large amount of data that fast, is unbelievable. Storing this data is also a huge task and we work with the SD computing center to ensure we can store 1000s of genomes of very large size.

For the machines called the HiSeq2500, this process takes 26 hours, while on the 4000’s it takes 3.5 days. The reason for the difference in time is that the 2500S can process only one genome at a time, while the 4000S can process up to 12 genomes at a time. Because it is so much information to process, more genomes take a longer time to run on the machines.

We now take our DNA and perform what is called library preparation. This process involves chopping up the DNA into small pieces using an instrument called the Covaris. This machine is used to ensure that our pieces are cut in equal sizes. We then apply chemicals, including phosphate and hydroxyl, to cause reactions at the end of the DNA to tag our fragments. This will be important later in the process.

Literary preparation takes 8 hours and is performed manually by certified technologists. As we grow, we will add the use of robotics in this step.

Once the DNA is extracted, we measure the quality and quantity of the DNA. The isolation and quantification takes about 1.5 hours. With high stakes involved, including acutely ill children and proper stewardship of the time and cost to run the Whole Genome Sequencing (WGS), we have multiple quality checks in place to ensure a successful whole genome sequencing run on the first try. Every hour counts every time!

The results are negative. Next-Gen Sequencing comparison sequencing. It is the standard of care for identifying variants in 3 hours. To process this large amount of data that fast, is unbelievable. Storing this data is also a huge task and we work with the SD computing center to ensure we can store 1000s of genomes of very large size.

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We now are left with millions of fragments of sequenced DNA. The next step in our journey is bioinformatics.

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VARIANT INTERPRETATION
With the files generated from the bioinformatics platform, we narrow down the large number of variants to try and make our diagnosis. We do this by asking a series of questions such as, is the change predicted to effect downstream protein function? Is the change common in the population using publicly available databases? If it is quite common in the population, then the variant probably does not cause disease or a lot of people would have that condition. Does the variant make sense from a family structure perspective (one variant inherited from mom and one from dad for a recessive condition - requires 2 variants in one gene?) is the variant de novo, meaning only found in the child and not in the parents?

We also rely on the expertise of our physicians to provide thorough clinical information of the symptoms the child has to help us narrow our search. This helps us hone our search to genes already identified through previous bench research as having involvement with child’s condition. Once we overlay the genetic analysis with the clinical information, we end up with a small number of variants to manually inspect.

For a variant of interest, we do a thorough review of the literature to see if this variant has been seen in other children with similar phenotype or characteristics, behaviors and symptoms. Is there evidence showing this variant affects the function of the gene? Does this variant get passed to members of the family who also have the disease? We take this all into account to determine if the variant is causing disease.

This process can take as little as 30 minutes to hours as we dig through the genome to try and find a diagnosis.

CONFIRMATION AND REPORTING
In the process of analyzing genomes, multiple carriers can occur that lead us to different paths. They are:

1. We find variants that explain the phenotype in the child in a critically ill situation. If the child is in critical condition and the genetic diagnosis can lead to a potentially lifesaving treatment, we will use this information to provide guidance to the medical team.

2. We find variants that explain the phenotype in the child, but the condition of the patient is stable. We perform Sanger sequencing and report the results to the medical team once the confirmation has been completed. This often can lead to providing answers to the family, allowing the family to know what to expect, inform decisions on therapeutic interventions and plan for the future. Providing an answer, even if we cannot treat the disease, gives closure to families as they do not need to search for the diagnosis, treat using trial and error and help them move forward in the process of taking care of their child.

3. The testing reveals newly discovered alleles that may explain the phenotype. We pursue additional testing and partner with our research colleagues to perform additional research using animal models, examining if the variant of interest is causing disease. We may also find new disease causing genes that have not been described before.

4. The results are negative. We have not identified clear variants or interesting new candidates. We file the case and data to make sure we look at the genome again in the future as the technology improves and our ability to interpret the genome gets better.