Circulating cell-free DNA detection on the GenapSys sequencer for organ transplant applications

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Abstract

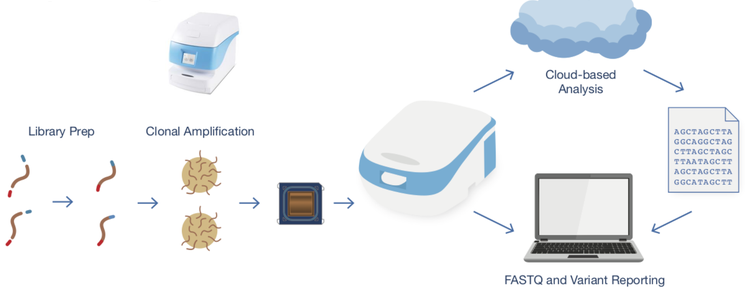
Circulating cell-free DNA (cfDNA) can be used as a biomarker for transplant rejection in organ transplant patients. A cfDNA-based assay offers several advantages over traditional invasive tissue biopsy approaches, including lower cost, reduced complications, and reduced interpretation bias. Next-Generation Sequencing (NGS) technology has enabled the accurate determination of the fraction of donor derived cfDNA in a transplant recipient's blood sample, which can be correlated with the potential for organ rejection.

GenapSys™ has developed a novel, portable, and inexpensive NGS technology that uses electronic detection to sequence DNA. Implementation of a cfDNA assay for organ transplant rejection on this platform could lead the way to enabling low cost and accessible point-of-care testing for transplant patients in the future.

We evaluated a comprehensive NGS-based cfDNA assay for organ transplants on the GenapSys sequencing platform and developed bioinformatics algorithms for determination of donor-derived cfDNA fraction. Amplicon libraries that target >200 single-nucleotide polymorphisms were generated from samples which contained donor-derived DNA at a range of different fraction levels, including 0% (control), <0.5% and >1%. Libraries were sequenced on the GenapSys sequencing system and sequencing data was analyzed using a custom bioinformatics approach. Briefly, adapter sequences were removed using cutadapt and trimmed reads were aligned to the hg38 reference genome using BWA-MEM. The allele frequencies of heterozygous and homozygous SNPs were calculated using BCFtools mpileup and calibrated with reference to control samples.

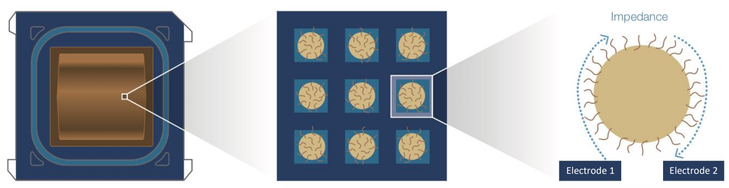
GenapSys Sequencing Workflow

Sequencing Workflow Overview



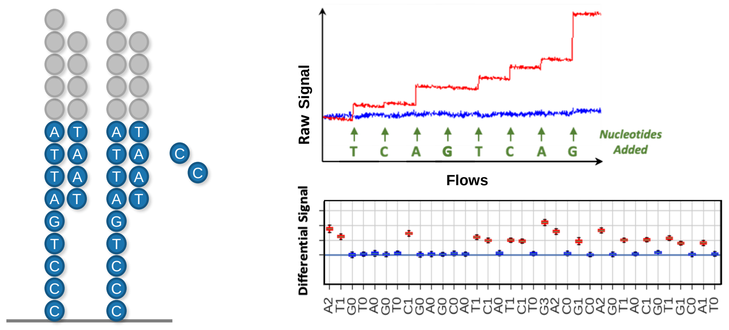
Amplicons generated from cfDNA were converted into a sequencing library via ligation of adapters using industry standard methods. Individual library molecules are clonally amplified onto beads and loaded into the sequencing chip. Automated sequencing is carried out on the GenapSys Sequencer which utilizes cloud-based analysis methods that ultimately deliver FASTQ and variant reporting files.

GenapSys Sequencing Chip



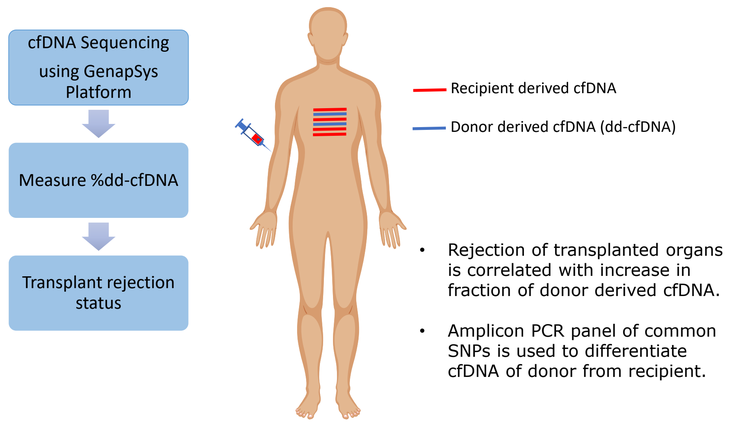
The CMOS sequencing chip contains millions of individual sensors, which are each loaded with a clonally amplified bead. The electrodes in each sensor are capable of measuring minute changes in impedance when nucleotides are incorporated opposite the bead-bound templates.

Electronic Sequencing by Synthesis



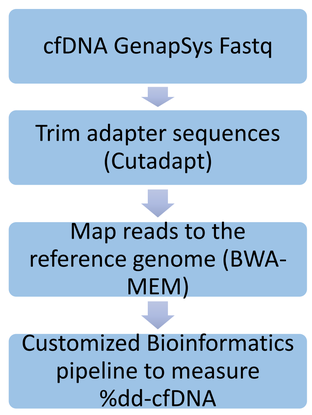
Nucleotides are injected one base at a time. When a nucleotide is incorporated, the measured impedance value of that sensor will jump, creating a graph that resembles a staircase. The magnitude of the differential signal correlates with the number of incorporated nucleotides. Due to the steady state nature of the impedance metric, the measured values do not significantly change over time. Thus, if desired, multiple measurements can be taken to improve precision.

cfDNA Sequencing

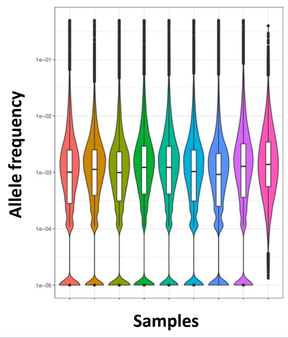


- Rejection of transplanted organs is correlated with increase in fraction of donor derived cfDNA.
- Amplicon PCR panel of common SNPs is used to differentiate cfDNA of donor from recipient.

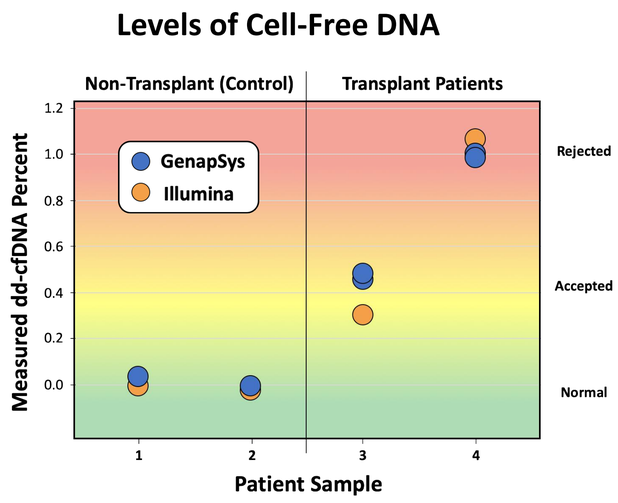
Analysis Workflow

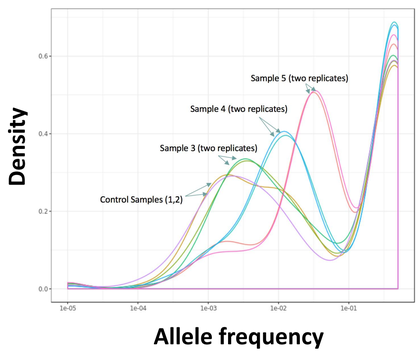


The allele frequencies of SNPs were calculated using BCFtools mpileup v1.9. The samples were also sequenced using Illumina MiSeq and were analyzed with the same Bioinformatics pipeline.



Results





As a demonstration of the technology applied to human samples, we carried out sequencing of Cell-Free DNA from multiple samples with technical replicates. Here we show results for controls and human samples. We were able to reproducibly identify the expected donor derived levels and successfully discriminate between the level of active and non-active rejection and recapitulate the standard pipeline which uses Illumina data.

Summary

We demonstrated successful implementation of the cfDNA assay on the GenapSys Sequencing Platform and the ability to accurately detect donor-derived cfDNA fractions at expected values, including allele-frequencies less than 0.5%. Additionally, sequencing replicates for a given library correlated well, indicating robust and reproducible performance.

The GenapSys Sequencing Platform offers:

- Low price per run and low price per sample
- 1.2 to 2 Gb of highly accurate DNA sequence per run (85% of bases >Q30)
- Highly accurate sequencing data yields high confidence rare variant calling
- Excellent concordance for variant calling relative to established sequencing technologies