Low DNA Input Technologies Enable Rare Tumor Cell Sequencing

Dr. Nicholas Navin at the MD Anderson Cancer Center uses the Nextera® Rapid Capture Exome kit to analyze heterogeneity in tumor subpopulations.

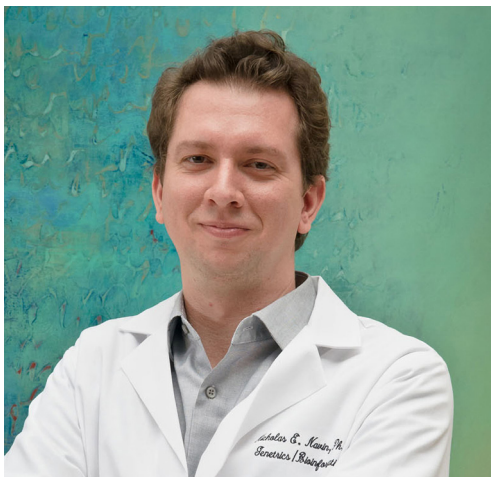
Next-generation sequencing (NGS) has emerged as a valuable tool in cancer research, enabling the detection of somatic mutations and gene expression changes in tumor DNA at high resolution and accuracy. Yet, there are challenges associated with sequencing DNA from human tumors. Only limited sample quantities are usually available and obtaining additional material is often not possible. Because tumor biopsies are prepared and preserved for later analysis as formalin-fixed, paraffin-embedded (FFPE) samples, the resulting DNA degradation can also make NGS library preparation difficult.

Dr. Nicholas Navin, an Assistant Professor at the MD Anderson Cancer Center in the Department of Genetics with a joint appointment in the Department of Bioinformatics, is no stranger to these challenges. Dr. Navin's laboratory routinely performs single-cell sequencing on DNA from extremely limited quantities of scarce and rare tumor sources to study the genetic complexity, evolution, and diversity in human cancer. The Illumina Nextera Rapid Capture Exome (NRCE) sample preparation kit has become an essential tool in his laboratory, enabling Dr. Navin to isolate low quantities of DNA (< 50 ng) and generate high-quality libraries that can be multiplexed and sequenced in just a day and a half. With the mutational data, Dr. Navin seeks to decipher the selective pressures that drive tumors to evolve and diversify.

Q: What are the goals of your research?

NN: My goal is to understand genome evolution in cancers. Our lab is interested in how genomes evolve hundreds and thousands of somatic mutations, very complex mutations, starting from just a single normal cell in a somatic tissue. This intra-tumor heterogeneity has impeded basic research and clinical diagnosis because tools to resolve it are lacking. To enable our studies, we've developed single-cell and low-input sequencing methods that use next-generation sequencing technologies to obtain mutational profiles of rare tumor subpopulations. In a breast cancer study[1], we profiled hundreds of single cells and discovered that the tumors grew by punctuated clonal expansions, in which hundreds of genomic rearrangements were acquired in short bursts of evolution.

> "Nextera Rapid Capture Exome is enabling researchers to examine the scarce clinical samples that just weren't possible to sequence before."



Nicholas Navin, Ph. D. is an Assistant Professor in the Department of Genetics at the University of Texas MD Anderson Cancer Center in Houston, TX where he uses the Nextera Rapid Capture Exome kit to study genome evolution in human cancers.

Q: What features impacted your decision to select the Nextera Rapid Capture Exome Kit?

NN: The features I like most about the kit are its ability to accommodate low DNA input, and its high exome coverage, sample multiplexing, and short protocol time. NRCE kits are also well-integrated with the MiSeq® and the HiSeq® systems, enabling us to easily sequence libraries without needing to use any special sequencing primers. And the software is set up for multiplexing. Everything is very plug-and-play, and streamlined.

Q: How does the ability of the NRCE kit to accommodate low-input DNA enable your research?

NN: We isolate small, minor tumor subpopulations such as cancer stem cells. Generally, we only have nanogram amounts of DNA and we're working from human tumor samples where the supply is limited. The NRCE kit has enabled us to sequence these samples. While the kit specification is around 50 ng, we've started with as little as 1 ng. By increasing the PCR cycles, we can generate good libraries that we can multiplex, exome capture, and obtain high-quality data. Nextera Rapid Capture Exome is enabling researchers to examine the scarce clinical samples that just weren't possible to sequence before.

Q: What low input DNA methods did you previously rely upon, and how do they compare to the NRCE kit?

NN: Before NCRE, we were never very successful constructing libraries with DNA amounts below 100 ng. We would use low-input, high-ligation efficiency methods, but the library quality always suffered. We had many PCR duplicates due to the increase in number of the PCR cycles needed.

Q: How does the shortened protocol of the NRCE kit impact your experiments?

NN: The overall timeframe is very nice. Other capture systems require three days of work just to perform the exome capture. With NRCE, you can go from DNA sample to multiplexed libraries and exome capture all within one day. If you want, you can start the sequencing run that night or the next day.

Q: What advantage does multiplexing provide for you?

NN: Multiplexing is another great feature we like about the NRCE kit. Illumina was smart to release a kit that only covers 37 Mb, which are the exonic regions. This is great for us because we can multiplex 10 or 12 samples and still get 30–50× coverage on each sample using the HiSeq 2000 system, which is what we need for variant calling. If you want to get the 3' and 5' UTRs in the other regions, Illumina also offers a larger capture platform, which covers 60 Mb.

> "Tools like Nextera Rapid Capture Exome are allowing researchers to get a whole new view of tumors and their genetic diversity and complexity."

Q: How did you test the NRCE kit?

NN: We have a MiSeq system in the lab so we quickly evaluated the NRCE kit. We used control DNA from a breast cancer cell line that we knew well. We made the libraries and performed the exome capture in one day. We then ran the MiSeq system overnight. We had sequence data to look at the next day.

Q: How do you measure the assay's performance?

NN: To measure how well the systems are working, we look at a number of metrics including percent on-target reads, percent off-target reads, coverage uniformity, GC bias, and allelic dropout. We obtain high exome coverage in our data analysis when we look at how many of the reads actually cover the exonic regions. It usually is over 90%, which is high for these targeted capture platforms.

Q: What other applications benefit from the NRCE kit?

NN: NRCE is great for FFPE samples that are often seen in the clinic. It's challenging to isolate DNA from a small block of tissue. Typically, a few hundred nanograms at most can be isolated, and the sample is often fragmented. NRCE requires just 50 ng of DNA and produces high-quality results. Another application we perform is laser capture microdissection (LCM) where we cut out small populations within a tumor. DNA isolation yields nanograms of DNA, which is compatible with the NRCE kit. Finally, we perform a lot of flow sorting to isolate subpopulations in tumors, such as cancer stem cells. Because these are extremely rare cells in the tumor, we're left with only nanograms of DNA, and it's unlikely we'll get more tissue. It's not possible to analyze these types of samples with capture kits that require 1–2 µg DNA for library construction. NRCE makes it possible to generate these libraries, do the sequencing, and actually obtain high-quality mutational data on scarce samples.

Q: What advice do you have for other researchers considering the NRCE kit?

NN: For researchers who have already purchased the NRCE kit, my advice is to consider the insert size distribution of their libraries before exome capture. This is critical in how well they're going to efficiently hybridize the probes. For those thinking about which low-input DNA capture method to use, my advice is to consider the timeframe, the multiplexing, the efficiency, and data metrics like the percent of reads on-target.

Q: How does the NRCE kit enable major changes in cancer research?

NN: There are many cancer genomes being sequenced at genome centers, but they're mainly performed on major cancer cell populations, and they're usually done at 30–50× coverage. Most of these studies have not examined minor populations in tumors. Tools like NRCE allow researchers to start investigating those minor populations—cancer stem cells, circulating tumor cells, and various populations within the tumor that behave differently or have different organization. NRCE allows scientists to geographically sample within the tumor and isolate small amounts of DNA to look at spatial heterogeneity, which is a hot topic right now in cancer research. Tools like NRCE are allowing researchers to get a whole new view of tumors and their genetic diversity and complexity.

Learn more about the Nextera Rapid Capture Exome kit at www.illumina.com/products/nextera-rapid-capture-exome-kits.ilmn.

1. Navin N, Kendall J, Troge J, Andrews P, Rodgers L, et al. (2011) Tumour evolution inferred by single-cell sequencing. Nature 472(7341):90–4.

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