DETECT ULTRA-RARE VARIANTS WITH RARESEQ™

HIGHLIGHTS

- **More sensitive sequencing techniques are required to better understand genomic heterogeneity and improve human health through precision medicine**
- **RareSeq is a novel error-corrected NGS method that uses UMIs to surpass the limit of detection possible with standard NGS techniques**
- **RareSeq employs the TruSight® Myeloid Panel from Illumina to detect ultra-rare mutations in genes associated with myeloid malignancies**
- **RareSeq is ideally suited to investigate the prevalence, stability, and origin of rare hematopoietic clones that may be early indicators of disease**

INTRODUCTION

Precision medicine has the potential to dramatically improve human health and transform medical practice as we know it. Until now, medical treatments were designed for the average patient. Consequently, treatment can be successful for some patients but not for others. Precision medicine takes into account an individual's genes, environment, and lifestyle to make more accurate diagnoses and select tailored treatments. Personalized genomics – sequencing and analysis of an individual's genome – is a key component of precision medicine. It is likely that personal genomics will become routine practice of cancer screening in the near future (Denny & Collins, 2021).

Genetic heterogeneity poses a significant challenge to personalized genomics and precision medicine at large. Somatic mutations can arise early or later on in tumor evolution, driving significant phenotypic variation within a single tumor (Burrell et al., 2013). Early identification of the mutations driving clonal expansion may help to inform diagnostic and treatment approaches before tumor progression. Next-generation sequencing (NGS) has rapidly expanded into the clinical setting to assist with diagnosis and treatment selection for many patients.

However, detecting ultra-rare variants is not possible with standard NGS and requires a highly sensitive and targeted sequencing approach. One application where this is particularly important is in studying clonal hematopoiesis – defined as somatic mutations in the blood or bone marrow that may be present in the absence of symptoms. More sensitive sequencing techniques are necessary to detect ultra-rare variants to better understand clonal hematopoiesis and how these mutations may lead to myeloid malignancies.

RareSeq is a novel NGS solution that solves these problems enabling the detection of rare mutations in genes associated with myeloid malignancies (Figure 1). RareSeq leverages error-corrected sequencing to surpass the limit of detection of standard NGS techniques.

MYELOID SEQUENCING PANEL GENE LIST

ABL1	CEBPA	HRAS	MYD88	SF3B1
ASXL1	CSF3R	IDH1	NOTCH1	SMC1A
ATRX	CUX1	IDH2	NPM1	SMC3
BCOR	DNMT3A	IKZF1	NRAS	SRSF2
BCORL1	ETV6/TEL	JAK2	PDGFRA	STAG2
BRAF	EZH2	JAK3	PHF6	TET2
CALR	FBXW7	KDM6A	PTEN	TP53
CBL	FLT3	KIT	PTPN11	U2AF1
CBLB	GATA1	KRAS	RAD21	WT1
CBLC	GATA2	MLL	RUNX1	ZRSR2
CDKN2A	GNAS	MPL	SETBP1	

Figure 1. *RareSeq is a targeted sequencing approach to detect genetic mutations that may lead to myeloid malignancies.*

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RARESEQ WORKFLOW

The RareSeq workflow depicted in Figure 2, begins with extraction of genomic DNA from blood or marrow samples. The gDNA serves as starting material for the steps of library preparation including hybridization, extension-ligation, and amplification. The molecules in the reaction are then tagged with unique molecular indexes (UMIs), which are essential for error correction after sequencing. Sequencing adaptors are added prior to being subjected to a second round of PCR. Sequencing data is processed through our custom bioinformatics pipeline, which clusters the data based on UMI. The custom pipeline distinguishes true variants from errors introduced during the library preparation and sequencing steps to identify ultra-rare variants. Through this, RareSeq enables researchers to detect genetic changes in target genes much earlier than standard NGS.

RARESEQ IS A TARGETED SEQUENCING APPROACH

RareSeq employs the TruSight Myeloid panel from Illumina, which targets 54 genes frequently mutated in myeloid malignancies to provide a comprehensive assessment of disease development and progression in disorders such as:

- Acute myeloid leukemia (AML)
- Myelodysplastic syndrome (MDS)
- Myeloproliferative neoplasms (MPN)
- Chronic myelogenous leukemia (CML)
- Chronic myelomonocytic leukemia (CMML)
- Juvenile myelomonocytic leukemia (JMML)

RareSeq is particularly powerful for investigating mutations that arise in the early stages of these diseases, as in clonal hematopoiesis of indeterminant potential (CHIP) and minimal residual disease (MRD).

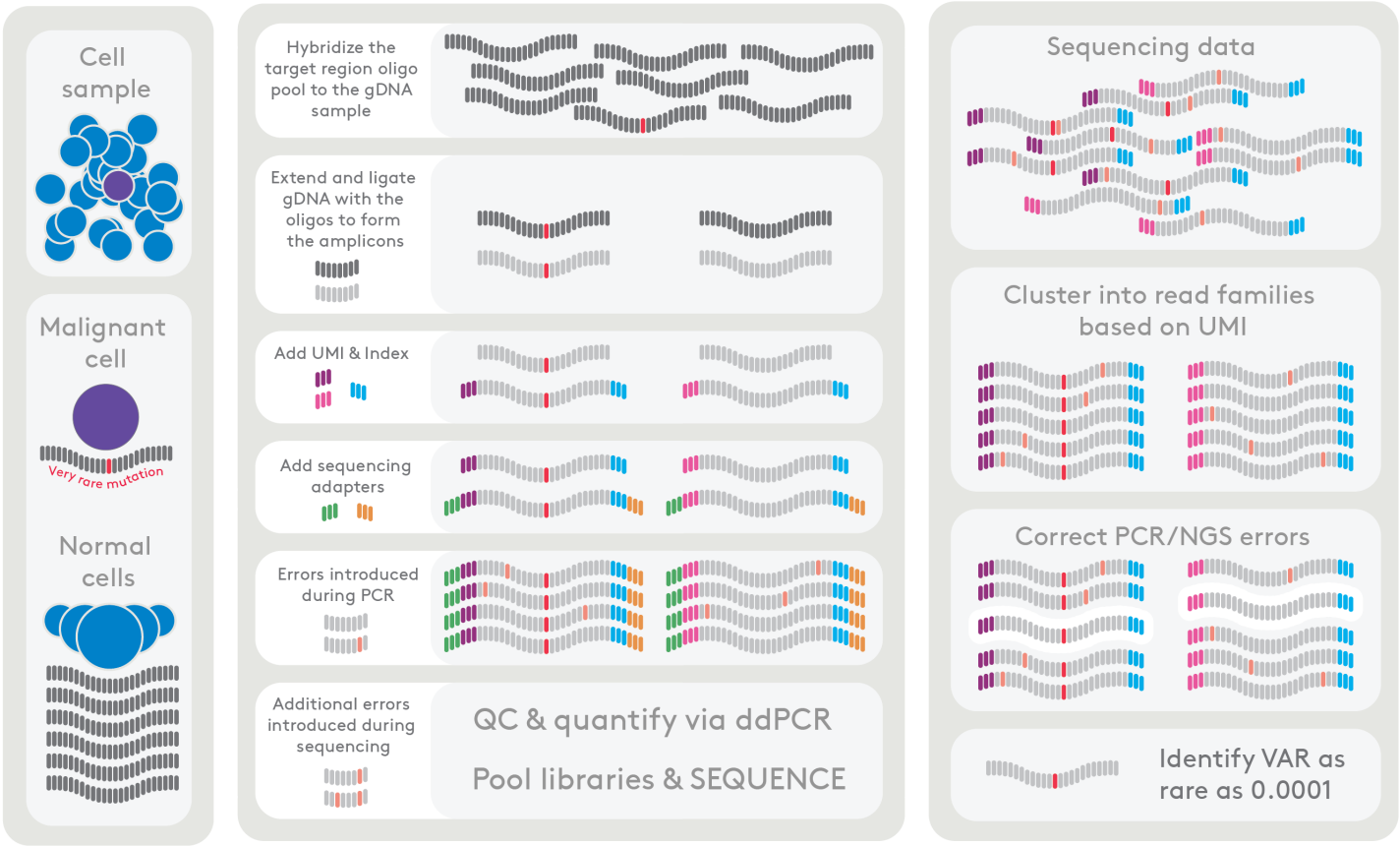

Figure 2. *Left column: The RareSeq workflow begins with extraction of genomic DNA from cells in a sample and subsequent QC. Middle column: The gDNA is then used as the starting material for the library preparation and sequencing steps. Right column: Using bioinformatics software, the sequencing data is clustered into read families based on UMI to quantify read counts and identify true variants.*

RARESEQ IS DESIGNED TO ENHANCE READ SENSITIVITY TO DETECT ULTRA-RARE VARIANTS

Routine techniques lack the sensitivity required to detect small genomic alterations. Studying early incidences of clonal hematopoiesis requires a limit of detection not available with traditional NGS techniques. To address this challenge, researchers at Washington University Medical School, St. Louis developed RareSeq, an error-corrected sequencing approach that dramatically increases sensitivity by one hundred times greater than standard NGS (Crowgey et al., 2020). RareSeq is ideally suited for investigating clonal hematopoiesis and been used in a number of research applications to better understand disease progression.

Case Study #1

Previous studies were able to identify hematopoietic clones in only 10% of 70-year olds and rarely in younger individuals, due to the limited sensitivity offered by standard NGS techniques. By employing RareSeq, researchers were able to detect hematopoietic clones in 95% of 50-60-year olds studied (Young et al., 2016). The results demonstrate how RareSeq is superior to standard NGS for accurate detection of clonal mutations at a much lower limit of detection (0.0003 VAF) and in much younger individuals. Figure 3 shows the mutations detected in 19 of the individuals using RareSeq.

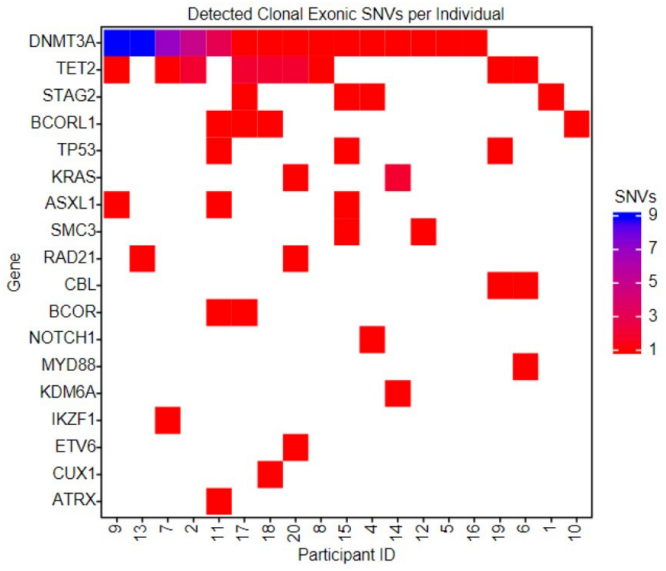

Figure 3. *Heat map showing the single nucleotide variants (SNVs) detected in each gene in 19 individuals. DNMT3A was the most frequently mutated gene both within a single individual (blue squares) and across all study participants.*

Case Study #2

Routine karyotype, fluorescence in situ hybridization (FISH), and NGS assays are typically used to assess the diverse genomic landscape of pediatric leukemias, but lack the sensitivity required for minimal residual disease monitoring (MRD). Researchers employed RareSeq to enable comprehensive detection of leukemic mutations relevant for diagnosis and minimal residual disease monitoring (Crowgey et al., 2020). Primary bone marrow samples from leukemia patients were analyzed, revealing multiple novel gene fusions. Further analyses enabled the characterization of exon duplications, exon deletions, and introns. The results of this study demonstrate how RareSeq can be employed for highly sensitive and comprehensive detection of various clonal leukemic mutations, which can be tracked from diagnosis to relapse.

Case Study #3

Wong and colleagues (2020) noted that nearly every healthy individual younger than 50 years old harbors rare hematopoietic clones below the detection limit of standard NGS. This poses a significant problem for hematopoietic stem cell transplants where blood and marrow are taken from donors and engrafted into recipients. The researchers wanted to investigate how often hematopoietic clones with pathogenic mutations are transferred to recipients, and what happened to these clones over time. With RareSeq, they found that the donor cohort, 44% harbored at least one somatic mutation, and those mutations were passed onto bone marrow donors (Wong et al., 2020). Recipients also exhibited clonal expansion within the first 100 days after transplantation. The results demonstrate how RareSeq can be used to identify ultra-rare hematopoietic clones and monitor clonal expansion, which may lead to disease progression.

Taken together, the data in these studies demonstrate the power of RareSeq to detect ultra-rare mutations that may lead to myeloid malignancy. RareSeq is a unique error-corrected sequencing approach for enhanced read sensitivity that is ideally suited for research aimed to investigate clonal hematopoiesis, clonal evolution, and disease progression.

SUMMARY

As the need for more accurate and sensitive sequencing approaches becomes clear, scientists are turning to UMI-based approaches because of their potential to advance precision medicine and personalized genomics. RareSeq is one approach that employs UMIs to surpass the limit of detection of standard NGS to detect single mutations in genes associated with myeloid malignancies. In this report, we described the key features of RareSeq – namely to investigate the prevalence, stability, and origin of rare genetic mutations in blood samples – and demonstrated how these aid research aimed to understand how genetic heterogeneity drives tumor evolution and disease (Young et al., 2016; Crowgey et al., 2020; Wong et al., 2020).

In conclusion, RareSeq has proven to be a key solution in NGS research and addresses the need for a highly sensitive sequencing approach. It is likely that these UMI-based sequencing techniques will continue to expand into the NGS space, as the focus on precision medicine continues to grow.

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