Stony Brook University
The Graduate School

Doctoral Defense Announcement

Abstract

ACME: an Affinity-based Cas9 Mediated Enrichment method for targeted nanopore sequencing to identify genetic variation in cancer

By

Shruti Iyer

Structural variations (SV) tend to be recurrent and contribute to cancer. Next-generation sequencing (NGS) is mostly blind to many SVs, with false positive and false negative rates >50% in SV detection. Long-read sequencing (LRS) generates read lengths of tens of thousands of bases, helping identify thousands of genomic features pertinent to cancer previously missed by NGS. However, whole genome LRS does not generate adequate coverage of alleles in heterogeneous samples like tumors, requiring additional sequencing to generate adequate coverage, increasing cost.

Targeted sequencing improves accuracy and coverage by providing depth necessary to detect rare alleles in a heterogenous population of cells. However, a lack of efficient LRS compatible targeting techniques made it difficult to study specific regions of interest on these platforms, limiting their wide adoption. To address this gap, we developed an Affinity-based Cas9 Mediated Enrichment (ACME) approach to better identify and study large genetic variation in cancer using long reads. ACME is a targeted LRS approach that helps reduce background non-target reads, increasing coverage and size of target regions that are captured. The main advantage ACME offers over other amplification-free long-read targeting approaches is the ability to capture contiguous reads, up to 100kb in size, that span the whole target from start to end. This reduces mapping errors and aids in SV detection even with lower target coverage for large gene targets. As proof of concept, we showed that ACME successfully detects all SVs within our targets previously inferred by whole genome LRS.

Since its development we further optimized ACME for different applications. We used ACME to target gene promoter regions in acute myeloid leukemia (AML) to determine their methylation pattern across different AML cell lines, helping confirm gene pathway dependencies. We also used ACME on organoid models of pancreatic ductal adenocarcinoma (PDAC) to identify variants of interest that were undetected by short-read exome and whole genome sequencing. Given its ease in design and flexibility, ACME has applications that can extend to not only different cancer types, but also other diseases and biological samples, making it a versatile, cost-effective approach that could help answer longstanding biological questions.

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Place: Bush Auditorium, Cold Spring Harbor Laboratory