LARIAT BIOSCIENCES

100 Cummings Center, Suite 451C
Beverly, MA 01915
603-244-9657
www.lariatbio.com

Toward Ultra-Rare Variant Detection by Allele-Specific Enrichment with Error-Corrected Sequencing

Application to Lung Cancer Diagnostics

Jonathan Larson, Alexandra Damiano, Madeleine Harlan, Dona Hevroni, Lisa Squatrito, Edward Townsend, and Makswell Vittands

INTRODUCTION

Many cancers advance into late stages before the telltale symptoms arise, resulting in unnecessarily poor prognosis. Cell free DNA (cfDNA) in the bloodstream is now recognized as a reservoir of biomarkers that promises non-invasive, pre-symptomatic screening to catch cancer early. Very encouraging recent approaches have been reported that use multiple biomarkers or epigenetic patterns to uncover cancer from a simple blood draw at early stages, firmly establishing the connection between cancer and cell-free tumor DNA. However, the results to-date have exhibited substantial false positive rates, and furthermore the types of cancer were not identified. Such ambiguity will confuse the oncologist and frighten the patient: "there may be a malignant tumor somewhere." In such cases, the next step for clinical action is likely to be worried watchful waiting, because the battery of tests otherwise required to search for and to confirm the tumor could be prohibitively expensive, invasive, and potentially never-ending. Since watchful waiting with judicious use of invasive screening is already the standard of care for many high-risk groups, it is somewhat doubtful that today's liquid biopsies will benefit those patients who otherwise would be most likely to opt-in for screening.

Lariat Biosciences is developing a completely novel liquid biopsy assay to overcome these last hurdles of certainty, location, and clinical actionability. Focusing first on lung cancer—but with an approach that allows the panel to expand—Lariat technology promises pre-symptomatic detection of lung cancer at the highest level of sensitivity for common forms of the disease.

TECHNICAL APPROACH

The existing liquid biopsies for early stage screening monitor the widespread effects of the deteriorating genomes of malignant cells (changing epigenetic patterns, etc.). In contrast, Lariat technology detects the original mutations that drive oncogenesis. Of course, driver mutations are the earliest signs of disease: the first opportunity for diagnosis. In addition, the different driver mutations are often non-overlapping, revealing the genetic subtype of the disease. Subtyping the cancer can pinpoint the afflicted organ and also guide the selection of treatment.

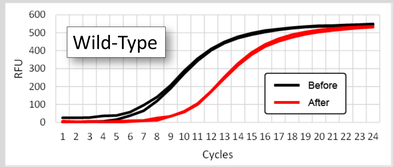
Genotyping these rare genetic variants in the bloodstream is a Herculean hunt for the needle-in-the-haystack (typically 1 mutant amidst 10^{13} wild-type DNA fragments). Yet, even if all of the background DNA is removed via multiplexed, targeted, and error-corrected enrichment, the mutant-to-wild type ratio is still near 1: 10^6 for cancer at the earliest stages. Assuming a minimum of 10 mutant molecules for diagnosis, and with a small panel of 10 targets, the sequencing burden substantially exceeds a typical NGS run. Hence, the brute-force combination of existing enrichment technologies and sequencing is prohibitively expensive.

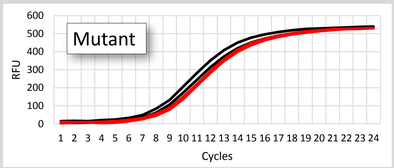
Lariat technology reduces assay costs with a novel allele-specific enrichment assay that accomplishes all of the following simultaneously:

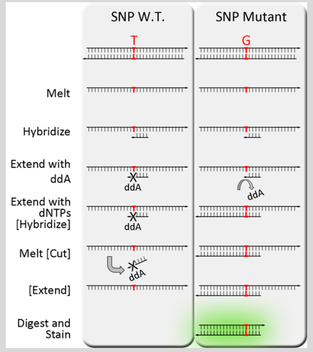
1) selectively depletes wild-type DNA by >95%,
2) reduces the genomic background by >10^6×, and
3) maintains fidelity by error-free enrichment and error-corrected sequencing.

Five separate technologies, described as follows, were developed that are tailored specifically for the tremendous challenge of genotyping ultra-rare genetic variants in the bloodstream.

SNP Enrichment

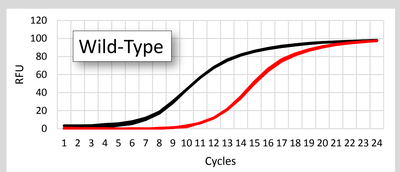


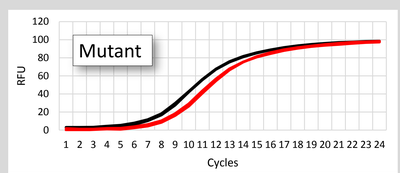


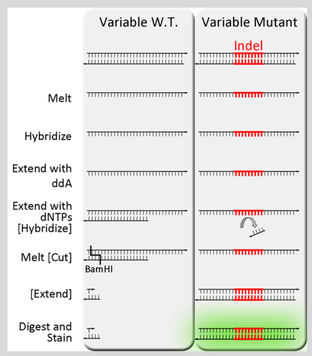


Selective depletion of EGFR L858R wild-type DNA by a chain of transformations, converting a difference in a pair of bases sequentially into differences in (1) primer extensibility, (2) melting temperature of hybridized complex, and (3) susceptibility to exonuclease digestion. The work flow is step-by-step compatible with the indel assay, below (steps specific to the indel assay are in [square brackets] above). Also demonstrated with BRAF and KRAS mutations (not shown).

Indel Enrichment

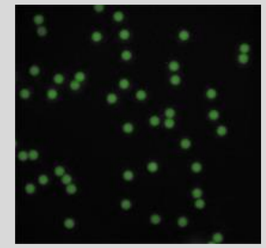


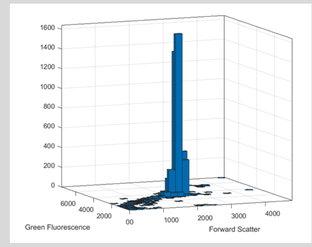




Selective depletion of EGFR exon 19 wild-type DNA by a chain of transformations, converting a small deletion mutation sequentially into differences in (1) hybridization, and (2) susceptibility to endonuclease digestion. The work flow is step-by-step compatible with the SNP assay, above (steps specific to the indel assay are in [square brackets]).

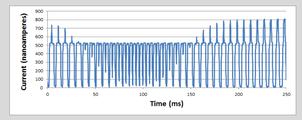
microGel Solid Support

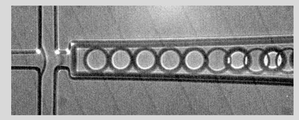


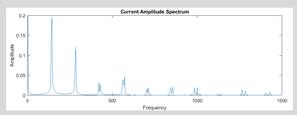


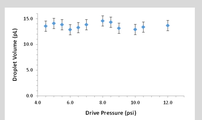
Efficient allelic enrichment was accomplished using µgels as the solid support. Success required excellent uniformity in µgel size and porosity.

Bridge-Mode Droplet µFluidics



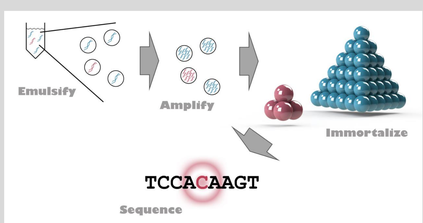






µgels are synthesized from µdroplets. DropTini™ is an inexpensive, high-throughput, and stable droplet generator featuring electronic droplet detection. Invented at Lariat for rare variant detection. ***Licensing available.***

Error-Corrected Sequencing



Work-in-progress, with something new coming soon. Our original strategy, shown above, ultimately proved incompatible with the allelic enrichment that we have developed. ***Partners welcome in development.***