Sensitive detection of molecular biomarkers in custom gene panels in support of clinical trials: sequencing under a quality system

Chris Friedrich, Andrew Barry, Alyssa MacBeth, Cassandra Crawford, Betty Woolf, Amaro Taylor-Weiner, Kristian Cibulskis
Tracey Honan, Jon Thompson, Andrew Zimmer, Jim Meldrim, Niall Lennon, Sheila Dodge, Stacey Gabriel
Genomics Platform, Broad Institute, 320 Charles St. Cambridge, MA

Introduction

The application of next generation sequencing in a clinical laboratory setting is a new endeavor. As part of the Broad Institute's efforts to facilitate translational genomics, the Clinical Research Sequencing Platform (CRSP) was created in October 2013. Within CRSP, we have developed a customizable offering aimed at sequencing clinical trials at scale, rapidly, and with high analytical sensitivity. Furthermore, we have designed a process for rapid validation and implementation, and created a new LIMS system for our clinical laboratory with many new features designed to support clinical testing in a high quality and compliant way.

Here we describe the lifecycle of a custom content clinical trial project including experiment design, assay selection, validation, and process performance of 1,000 samples in a hematological custom target panel. In addition, we describe the creation and implementation of a new LIMS system to support complex NGS workflows and associated activities in a clinical lab setting. These recent developments enable quality tracking and scalability of all of our clinical offerings.

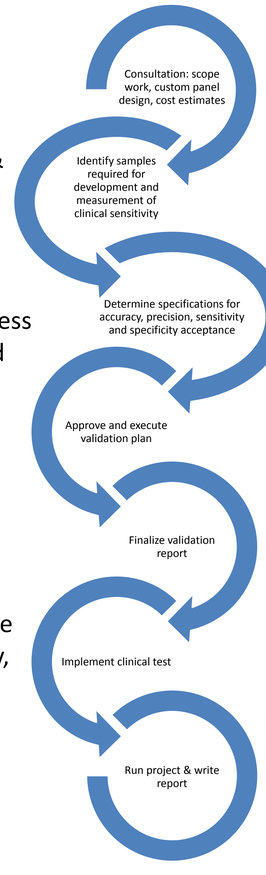
Clinical Custom Panel Life Cycle

CRSP Experience:

Consultation on experimental design & acquisition of samples

Clear definition of acceptance and success criteria with required power to detect

Qualification of sample integrity: tumor purity, process control, contamination



Customer Deliverable:

Draft validation plan

Panel acceptance report of interval read coverage

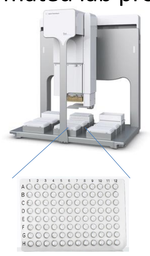
Validation report

Project report summary, BAMs, VCFs, MAFs

Informatics Capabilities: Broad Mercury LIMS

Mercury, a Broad developed LIMS system, integrates with laboratory equipment, as well as downstream analytical functions to provide end-to-end process tracking and an audit trail suitable for clinical use. Actual workflow, reagents and decision points are captured, and data is secure and centralized for analysis and custom reporting.

1) Automated lab processes



2) In-process information capture

-positive LIMS tracking of samples
-reagent lot and expiration date
-Lab user ID
-Machine name
-time stamp
-QC check points

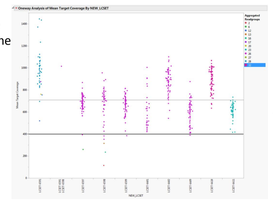
3) Database

-external sample ID
-process QC metrics
-chain of custody record

-secure
-audit trail

4) Reports

-sample performance
-verification of machine performance
-batch effects
-process monitoring
-sample qualification measures
-trends



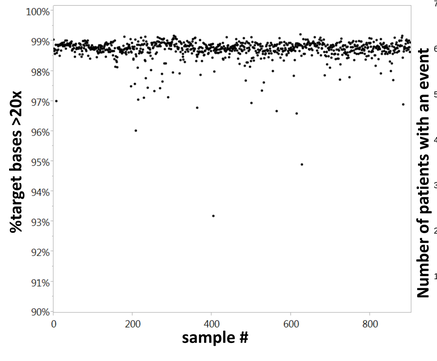
Example: 3.3Mb Custom Design Project

A 1,000 sample clinical research hematology project executed in December 2014 resulted in an average mean target coverage of 694x and 98.6% of targets covered at >20x. Additionally, a measurement of tumor contamination in normal was provided to address cancer type specific concerns and 15 samples were flagged (>0.5%) for risk of reduced sensitivity.

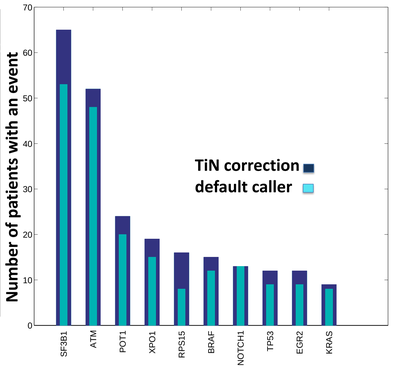
Analytical tools

1) Co-cleaned variant detection through **MuTect** (SNVs) & **Indelocator**
2) CNV detection through **ReCapSeg**
3) **ABSOLUTE** measurement of tumor purity
4) **DeTiN** tumor in normal measures and correction
5) **ConTest** verification of sample purity (%contamination)

Coverage across targets



deTiN rescues mutations in driver genes



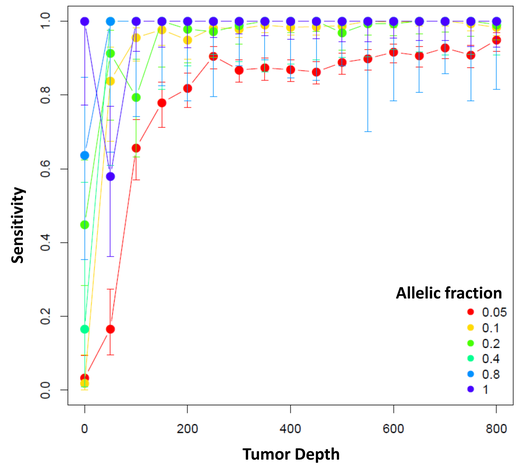
Clinical Test Validation Results

Analytical sensitivity: assess ability to detect SNVs and InDels
Clinical accuracy: measure ability to detect known variants within truth data
Analytical specificity: measure of false positive rate for detection of SNVs and InDels
Precision: reproducibility of variant detection between assay technical replicates

Validation test results are compared to acceptance criteria

Sample Set	Test	Specification	400x MTC	700x MTC	Result
Hematological Cell Line Pools	Analytical Sensitivity for SNVs	≥95%	97.8%	96.6%	PASS
	Analytical Sensitivity for InDels	≥80%	84.7%	88.3%	PASS
HapMap Mixed Pools	Analytical Sensitivity for SNVs	≥95%	98.8%	98.8%	PASS
	Analytical Sensitivity for InDels	≥80%	92.3%	96.9%	PASS
Multiple Myeloma Samples	Clinical Sensitivity for SNVs	≥90%	91.2%	93.2%	PASS
	Clinical Sensitivity for InDels	≥80%	80.0%	80.0%	PASS
NA12878 Replicates	False Positive Rate for SNVs	≤ 1 FP/mB	0.27	0.16	PASS
	False Positive Rate for InDels	≤ 1 FP/mB	0.12	0.16	PASS
HapMap Mixed Pools	Precision for SNVs	≥90%	92.3%	94.1%	PASS
	Precision for Indels	≥90%	95.2%	96.7%	PASS

Analytical sensitivity by varying read depth and allelic fraction



Summary

The Broad CLIA-CAP certified clinical laboratory offers customization, scalability and expertise in small panel capture designs. Analytical and clinical sensitivity is high at low allelic fractions. The Broad leverages its extensive knowledge base in NGS library preparation and analytical tools to provide a best in class product, while taking into account considerations that are specific to the experimental design, and has tools that can qualify data quality for high sensitivity and specificity in calling variants. The Clinical Research Sequencing Platform also offers germline and somatic technical exome products.