Analytical Validation of Comprehensive Assays for Genomic Profiling of Cancer from DNA and RNA

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Introduction

Genomic assays are increasingly used in oncology to guide clinical management and assess tumor responsiveness to novel therapeutics. However, the number of cancer genes with clinical relevance continuously expands, necessitating broader mutational profiling of tumors. Here we present the analytical validation of the ACE Cancer Portfolio, which includes an extended DNA and RNA cancer gene panel and an augmented exome and transcriptome for more complete genomic characterization.

Methods

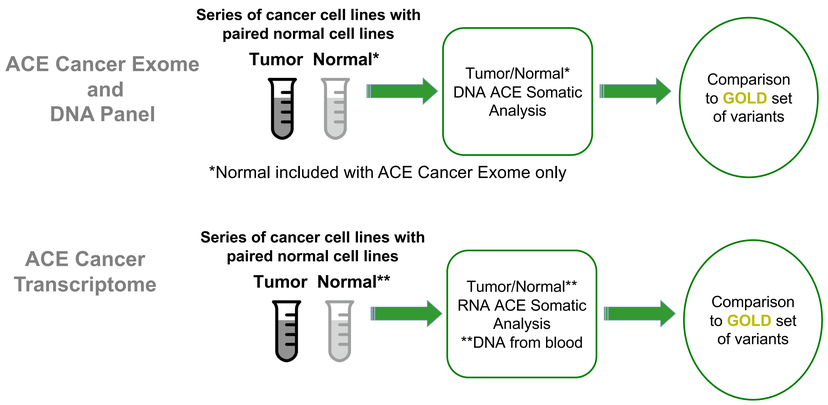
Cancer Reference Standards

Since commercially available standards are often of limited utility, we aimed to create a cancer standard reference set by procuring a series of well characterized cancer cell lines for analyses. A Gold Set of variants (SNVs and indels) was established representing a set of COSMIC verified events also determined by two independent sources.

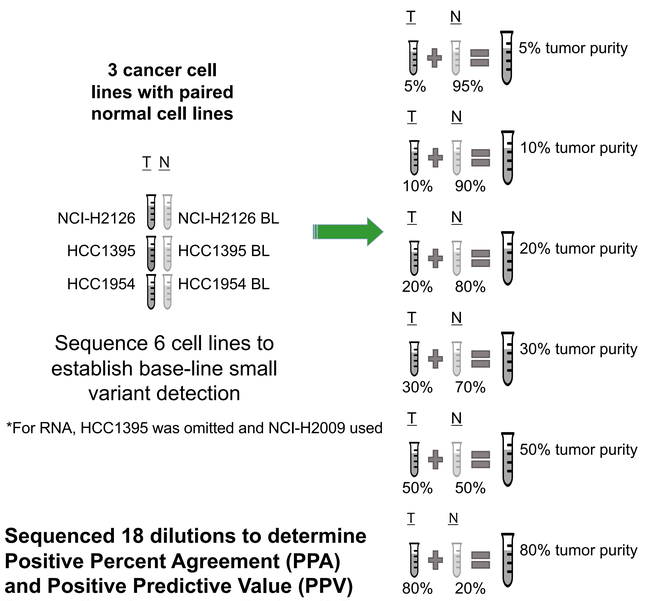
Assays Evaluated

Validation was performed on our targeted cancer gene panel for DNA and RNA containing >1400 cancer and pharmacogenomic genes. Likewise, analysis was also performed on our augmented exome and transcriptome assay containing >20,000 genes, 8,000 of which are supplemented for more uniform coverage. The assays were validated for use in different clinical specimen types, including formalin fixed paraffin embedded (FFPE), fresh frozen (FF), and blood.

Small Variant Analytical Sensitivity (DNA and RNA)



ACE Cancer Exome and Transcriptome Small Variant Limit of Detection (DNA and RNA*)



Sequenced 18 dilutions to determine Positive Percent Agreement (PPA) and Positive Predictive Value (PPV) for small variants at particular allele frequencies. A similar approach was utilized for the ACE DNA Cancer Panel.

ACE DNA Cancer Panel Copy Number Alterations (CNA)

Nineteen Cell Lines were selected for CNA analysis and a similar limit of detection approach used as outlined above.

ACE RNA Cancer Panel and ACE Cancer Transcriptome Copy Number Alterations (CNA)

Well characterized cancer cell lines (15 for panel; 10 for WTS) were chosen for fusion analytical validity including events such as ALK, BCR-ABL1, and ROS1 fusions.

Results

ACE DNA and RNA Cancer Panel – Tumor Only Median depth ≥ 500x

Sensitivity	**Base Substitutions**	**(AF ≥5%) >99%**
	Indels	**(AF ≥10%) >99%**
	Copy Number Alterations	**96% for tumor content ≥ 30%**
	Gene Fusions	**>95%**
Specificity	**Base Substitutions**	**(AF ≥5%) >99%**
	Indels	**(AF ≥10%) >99%**

ACE Cancer Exome – Tumor/ Normal Median depth ≥ 200x (28G Tumor / 10G Normal)

	SNVs	Indels
Analytical Sensitivity	98%	95%

	Sensitivity (PPA)		Specificity (PPV)	
MAF	10%	20%	10%	20%
Small Variants	97%	99%	98%	99%
SNVs	97%	99%	98%	99%
InDels	87%	94%	97%	97%

ACE Cancer Transcriptome 50 Million PE

	Small Variants	Fusions
Analytical Sensitivity	97%	100%

MAF	10%	20%
Small Variants	97%	99%
SNVs	97%	99%
InDels	88%	94%

Conclusion

We have developed and validated comprehensive cancer NGS assays with uniform and deep coverage ensuring high sensitivity and specificity for all variant types. This platform encompasses assays for use as a versatile diagnostic to test a core set of clinically actionable genes, but also solutions to facilitate discovery of novel therapeutic targets.

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