Supporting Neoantigen Identification for Personalized Cancer Vaccines Through Analytical Validation of an Augmented Content Enhanced (ACE) Transcriptome

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Jennifer Yen, Sean Michael Boyle, Ravi Alla, Jason Harris, Martina Lefterova, Richard Chen
Personalis, Inc. 1330 O'Brien Drive, Menlo Park, CA 94025

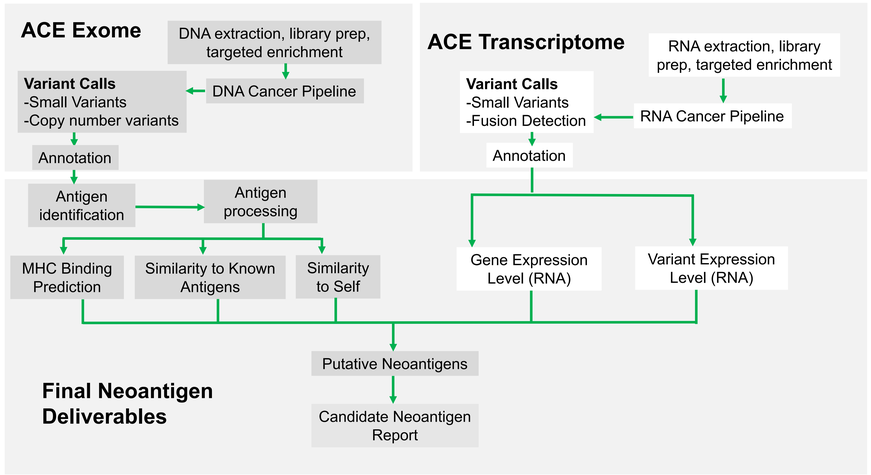
Contact:
jennifer.yen@personalis.com

Background

Neoantigen identification, or the detection of tumor-specific variants, is a critical step in personalized cancer vaccine development. Due to the robust clinical standards required for producing cancer vaccines, there is a strong demand for well validated neoantigen detection platforms. The ACE Exome and Transcriptome platforms harness a unique, augmented enrichment approach specifically designed to increase sensitivity for neoantigen detection in low complexity, difficult to sequence regions. Here we describe the analytical validation of the ACE Transcriptome platform for the detection of tumor expressed DNA-based neoantigens.

Neogantigen Detection Pipeline

Our Neoantigen Workflow is dependent on the ACE Exome and ACE Transcriptome platforms, comprising of both DNA and RNA Cancer Pipelines.



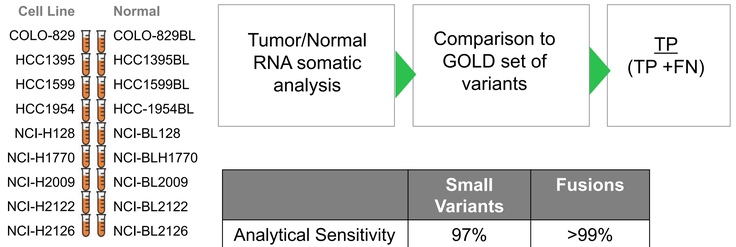
Methods

- To validate the Transcriptome platform, we curated a reference 'GOLD' set of DNA variants that had been either orthogonally validated, corroborated by two other sources (CCLE and COSMIC), or detected in the COSMIC Cell Line Project for a total of 875 SNvs and 19 InDels.
- The validation set comprised of
  - 10 cancer cell lines and their corresponding matched normals for small variants in the RNA
  - 10 cancer cell lines for known RNA fusions
- Indexed genomic libraries were pooled and enriched using ACE enrichment technology, followed by paired-end Illumina sequencing.

Results

Analytical Sensitivity – Small Variants

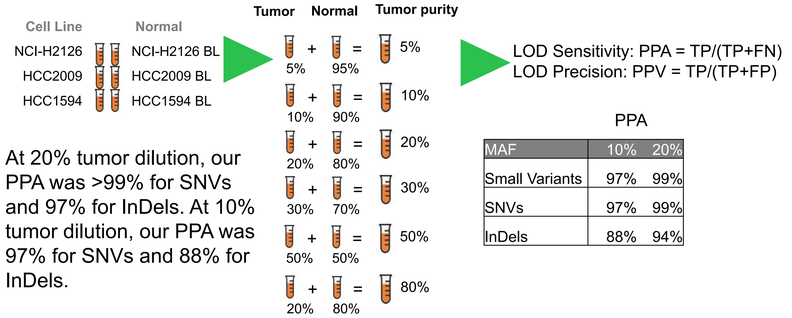
Sensitivity of SNV and indel calling in RNA was determined by comparing variants called in the 10 cell lines with known variants in the GOLD reference set.



	Small Variants	Fusions
Analytical Sensitivity	97%	>99%

Limits of Detection – Small Variants

To determine the limit of detection for SNVs and InDels, the RNA from three cell lines were diluted with increasing fractions of their matched normal RNA. We calculated the Positive Percent Agreement (PPA) as a measure of sensitivity, and Positive Predictive Value as a measure of precision for each dilution set.



At 20% tumor dilution, our PPA was >99% for SNVs and 97% for InDels. At 10% tumor dilution, our PPA was 97% for SNVs and 88% for InDels.

PPA

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

Analytical Sensitivity – Fusions

We confirmed the detection of all 16 RNA fusion events across the 10 cell lines in our Gold Set, demonstrating an analytical sensitivity for fusions as >99%.

Precision

To obtain a high level of precision for variants called in the RNA, we suggest selecting RNA variants that intersect with the DNA variants, for which we calculated a precision (PPV) of >99%.

Conclusion

Our validation demonstrates that the ACE Transcriptome is a highly sensitive and precise assay for small variant and fusion calling, which is a requisite step for neoantigen detection and personalized cancer vaccine development.

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