Accurately identifying expressed somatic variants for neoantigen detection and immuno-oncology

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Introduction

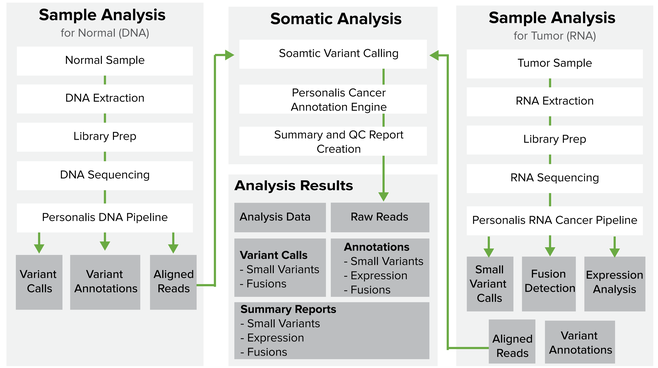
Accurate detection of somatic variants is a staple of both research and clinical cancer analysis, with applications ranging from detecting new common driver mutations in large patient cohorts to selecting therapeutic small molecule treatment courses for an individual patient. Recent research into neoantigens and immunotherapy has shown great promise as a precision therapeutic, and somatic variant detection by next-generation sequencing represents an ideal method of identifying candidate neoantigens. Somatic variant detection typically involves assaying the DNA for changes in gene sequences without assessing whether those variants are actually expressed in RNA. However, the expression of small variants is key because only expressed peptides will be displayed as neoantigens on the cell surface.

We have designed a highly accurate expression-based somatic variant detection pipeline utilizing extensive discovery and filtering methods to overcome the challenges inherent in RNA somatic variant calling. We validated our pipeline using a combination of well-characterized cell lines, commercially available reference standards, and real world FFPE patient samples.

Detecting Somatic Variants In RNA

Cancer RNA Workflow and Data Deliverables

The diagram below shows our process from receipt of the sample to data delivery

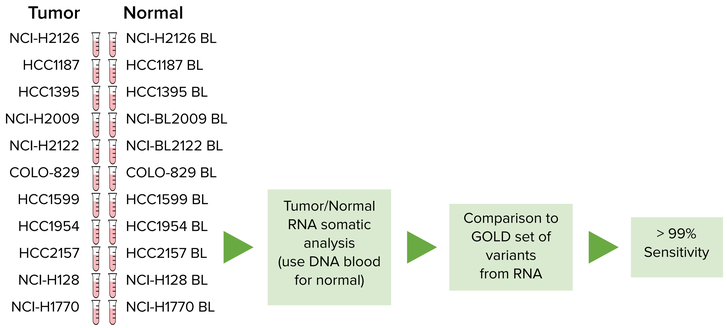


From a technical standpoint, detection of somatic variants in the RNA represents additional challenges above and beyond those of somatic detection in DNA. The widely varying expression levels of cancer genes, alternative splicing, and RNA editing are all features that make somatic variant calling in RNA uniquely challenging. However, accurately detecting variants directly from expressed transcripts is beneficial to neoantigen prediction, and therefore we sought to create and validate a method for somatic variant calling in RNA.

Validating Somatic Variant Detection In RNA

Analytical Sensitivity

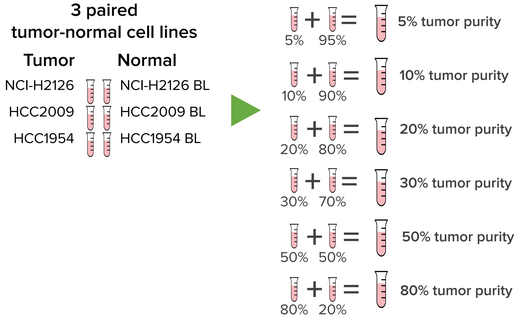
We have designed a highly accurate expression-based somatic variant detection pipeline utilizing extensive discovery and filtering methods to overcome the challenges inherent in RNA somatic variant calling. We validated our pipeline using a combination of well-characterized cell lines, commercially available reference standards, and real world FFPE patient samples. To our knowledge, this is the most extensive validation of its kind to date.



Specificity

RNA somatic variants were intersected with our DNA somatic variant set for each sample, allowing for highly specific detection of expressed small variants. DNA specificity (PPV) = >99%

Limits Of Detection



Independently, RNA from three tumor cell lines were diluted with RNA from their paired normal cell lines. Dilutions ranged from 5% to 80% tumor purity. Somatic variants detected in RNA were filtered for somatic detection in DNA. Positive predictive value (PPV) was calculated using expected MAF variant frequencies from pure samples.

Fusion Validation Results

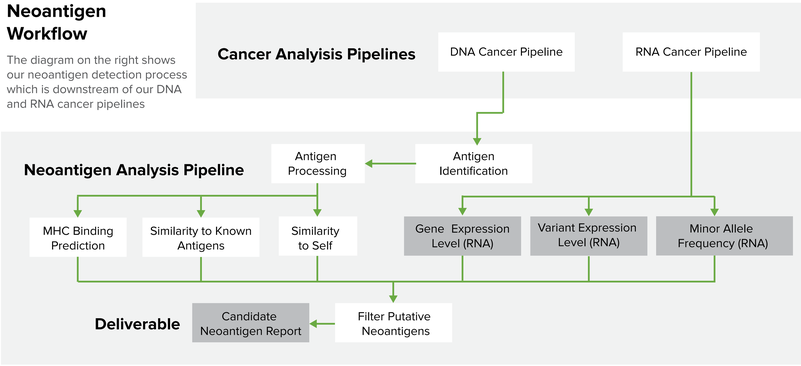
In total, sixteen well described, previously known fusions were verified by RNA sequencing using the ACE Extended Cancer Panel.

Sensitivity	Specificity
> 99%	> 99% (DNA Intersection)

Detecting Neoantigens

Neoantigen Workflow

The diagram on the right shows our neoantigen detection process which is downstream of our DNA and RNA cancer pipelines



We are currently developing a neoantigen detection pipeline, which builds upon our existing comprehensive ACE platform and highly accurate DNA and RNA cancer analysis pipelines. Small variants will be classified as neoantigens through analysis of many important features, including MHC presentation prediction, similarity to known antigens, similarity to self, gene expression levels, variant expression level, and variant allele frequency.

Conclusion

We have developed a highly accurate RNA based somatic variant detection pipeline, which we have validated using previously characterized variants from paired tumor-normal cell lines. We are further using our RNA somatic detection pipeline as a major component in a neoantigen pipeline.

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