Analytical Validation and Performance of a 7-gene Next-generation Sequencing Panel in Uveal Melanoma

Katherina Maria Alsina
Castle Biosciences Inc
Lauren M Sholl
Castle Biosciences Inc
Kyle R Covington
Castle Biosciences Inc
Suzette M Arnal
Castle Biosciences Inc
Michael M Durante
University of Miami Bascom Palmer Eye Institute
Christina L Decatur
University of Miami Bascom Palmer Eye Institute
John F Stone
Castle Biosciences Inc
Kristen M Oelschlager
Castle Biosciences Inc
J William Harbour
University of Miami Bascom Palmer Eye Institute
Federico A Monzon
Castle Biosciences Inc
Robert W Cook
Castle Biosciences Inc
Sherri Borman (sborman@castlebiosciences.com)
Castle Biosciences, Inc., Friendswood, TX, USA

Research

Keywords: gene expression profiling (GEP), next generation sequencing (NGS), formalin-fixed paraffin embedded (FFPE)

Posted Date: October 9th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-86927/v1

Version of Record: A version of this preprint was published at Ocular Oncology and Pathology on August 4th, 2021. See the published version at https://doi.org/10.1159/000518829.

Abstract

Background: A 15-gene expression profiling (GEP) test is widely used for prognostication of metastatic risk in uveal melanoma (UM) patients. Because the amount of tumor tissue that can be safely obtained by biopsy from UM is limited, it is critical to obtain as much individualized genomic information as possible from each biopsy sample. Mutational profiling of UM tumors using next generation sequencing (NGS) in combination with GEP allows for analysis of both DNA and RNA from a single tumor sample, offers additional prognostic value, and can potentially inform therapy selection. This study evaluated the analytical performance of a targeted custom NGS panel for mutational profiling of the seven genes known to be commonly mutated in primary UM.

Methods: 105 primary UM samples were analyzed, including 37 formalin-fixed paraffin embedded (FFPE) specimens and 68 fine needle aspiration biopsy (FNAB) specimens obtained with a 25- or 27-gauge needle. Sequencing was performed on the Ion GeneStudio S5 platform to an average read depth of greater than 500X per region of interest in a clinical laboratory accredited by the College of American Pathologists (CAP) and certified under the Clinical Laboratory Improvement Amendments (CLIA).

Results: The 7-gene panel assay achieved a positive percent agreement (PPA) of 100% for detection of both single nucleotide variants (SNVs) and insertions/deletions (INDELs), with a technical positive predictive value (TPPV) of 99.4% and 100%, respectively. Intra-assay and inter-assay concordance studies confirmed the reproducibility and repeatability of the assay. The limit of detection was determined to be 5% variant allele frequency (VAF) for both SNVs and INDELs, with a minimum DNA input requirement of 1.5ng for FNAB and 5ng for FFPE samples.

Conclusions: The 7-gene panel is a robust, highly accurate NGS test that can be successfully performed, along with GEP, from a single small gauge needle biopsy sample.

Full Text

This preprint is available for download as a PDF.

Tables

Due to technical limitations, table 1-7 is only available as a download in the Supplemental Files section.

Figures

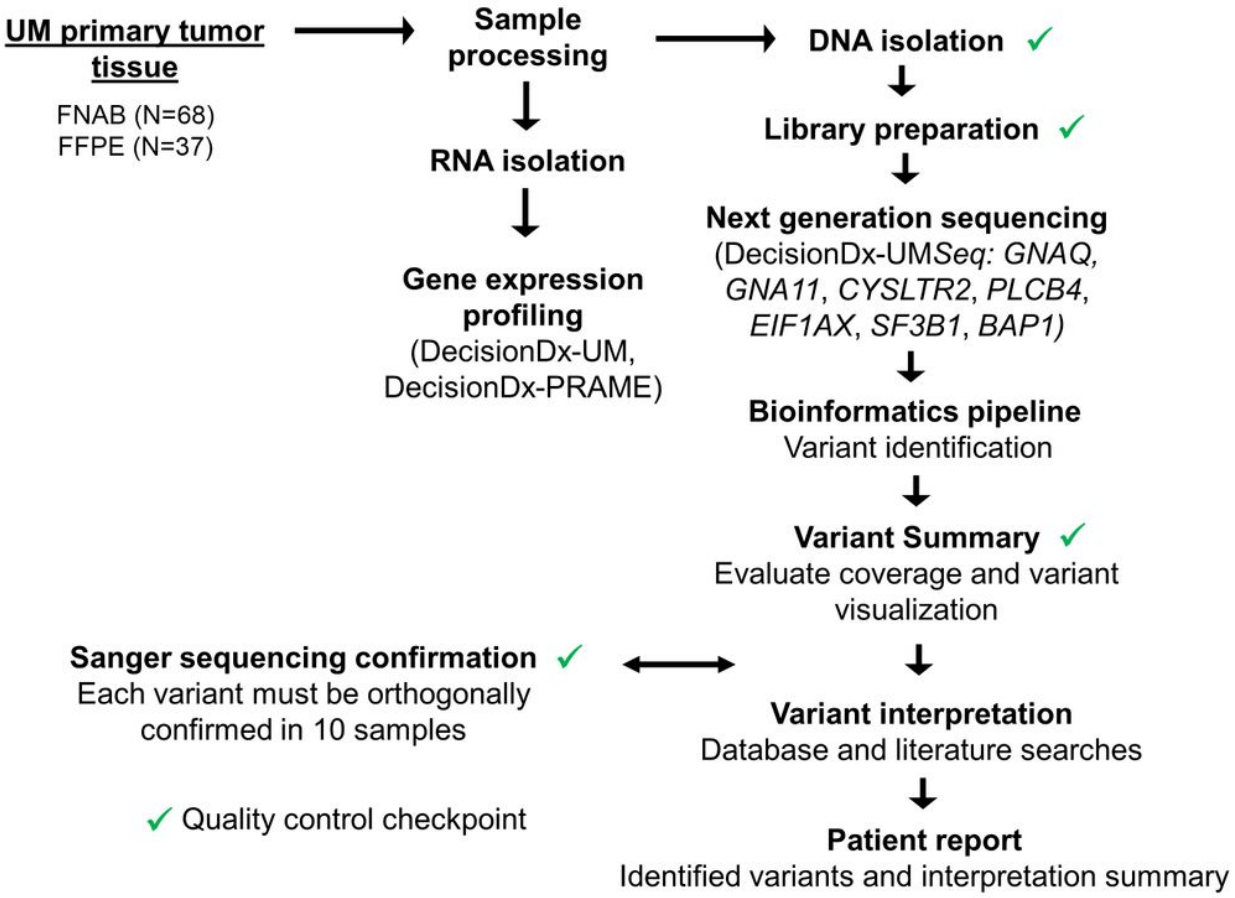

Figure 1

Clinical workflow for the 7-gene panel. Primary tumor tissue (FFPE or FNAB) from a single biopsy is processed upon arrival at Castle Biosciences' clinical lab, with one aliquot used for RNA isolation and gene expression profiling, and the other reserved for DNA isolation and sequencing. Targeted regions of the genome are amplified for sequencing on the Thermofisher Ion S5, after which variants are called using the Ion Reporter bioinformatics pipeline. Variants that meet minimum quality and coverage criteria and that are within reportable range are reported along with their potential clinical relevance according to the 4-tiered system for classification of somatic variants established by joint AMP, ASCO and CAP guidelines [21].

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TablesUMSeqAVv7.pdf
- Fig2.jpg