Hitting the target: An analysis of noncoding alterations as captured by panels and diagnostic exome sequencing at a commercial lab

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Gene panels offer comprehensive targeted analysis of genes of interest and can be useful for diagnosis in clinically-variable or genetically-heterogeneous disorders. Overtime, diagnostic exome sequencing (DES) is more frequently ordered as a 1st or 2nd tier test. We assessed the technical sensitivity of DES for variants in noncoding regions to test the belief that panels offer far better intronic and promoter coverage than DES can.

Using *in silico* analysis, we explored the coverage and sensitivity of DES for detecting VUS, VLP or MUT located in promoter or intronic regions that were reported on cancer and cardiac panels offered by Ambry Genetics. Corresponding nucleotide positions were interrogated in data from 100 randomly-selected DES samples to determine the mean sequence coverage at each position. In total, 1675 noncoding alterations in 108 genes were reported. Most alterations were classified as VUS (1172; 70.0%), and the remaining were split between MUT (217; 13.0%) and VLP (286; 17.0%). In sum, 1207 (72.1%) had mean coverage $\geq$20X on DES, with only 4.0% (20/503) of VLP/MUT alterations with mean coverage <20X. Most noncoding variants with insufficient coverage on DES (428/468; 91.5%) were located in the promoter regions of 3 genes. The remaining 40 alterations without sufficient coverage on DES were located in intronic regions and had a median coverage of 16.6X. Reported alterations in canonical splice sites (+/-1 or +/-2) were more often reported as VLP or MUT than those located 3, 4 or 5 nucleotides from the intron-exon junction; however the percent of loci with $\geq$20X coverage was high (96.9%) for all positions +/-5 of the intron-exon junction. Among 14 variants (13 MUT/ VLP and 1 VUS) reported beyond the 5th intronic nucleotide in panels, 10 had coverage $\geq$20X on DES (71.4%).

In summary, DES has adequate coverage of variants within +/-5, but less sufficient coverage of deep-intronic and promoter region alterations. The vast majority (96.0%) of noncoding MUT/VLP by panels are technically detectable by DES. The number of reported noncoding VUSs on panels greatly outweighs those reported on DES, likely causing the belief that DES has inferior coverage. Intronic alterations beyond +/-2 are mainly uninformative. DES reporting filters to focus on variants at canonical splice sites and established VLP/MUT in deep-intronic regions provide the most informative results while reducing VUSs and lessening the burden of uncertain results for clinicians.

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