# Hitting the Target: An Analysis of Noncoding Alterations as Captured by Panels and Diagnostic Exome Sequencing at a Commercial Lab

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## BACKGROUND

- Multigene panels can be useful for diagnosing patients with clinically-variable or genetically-heterogeneous disorders.
- Diagnostic exome sequencing (DES) is more frequently being ordered as a first or second-tier test[1].
- Most disease-causing alterations are located within coding regions, but non-coding regions (NCRs, i.e. intronic and promoter regions) can also contain disease-causing alterations[2].
- Panels are optimized for coverage of genes-of-interest, presumably increasing detection of NCR alterations.

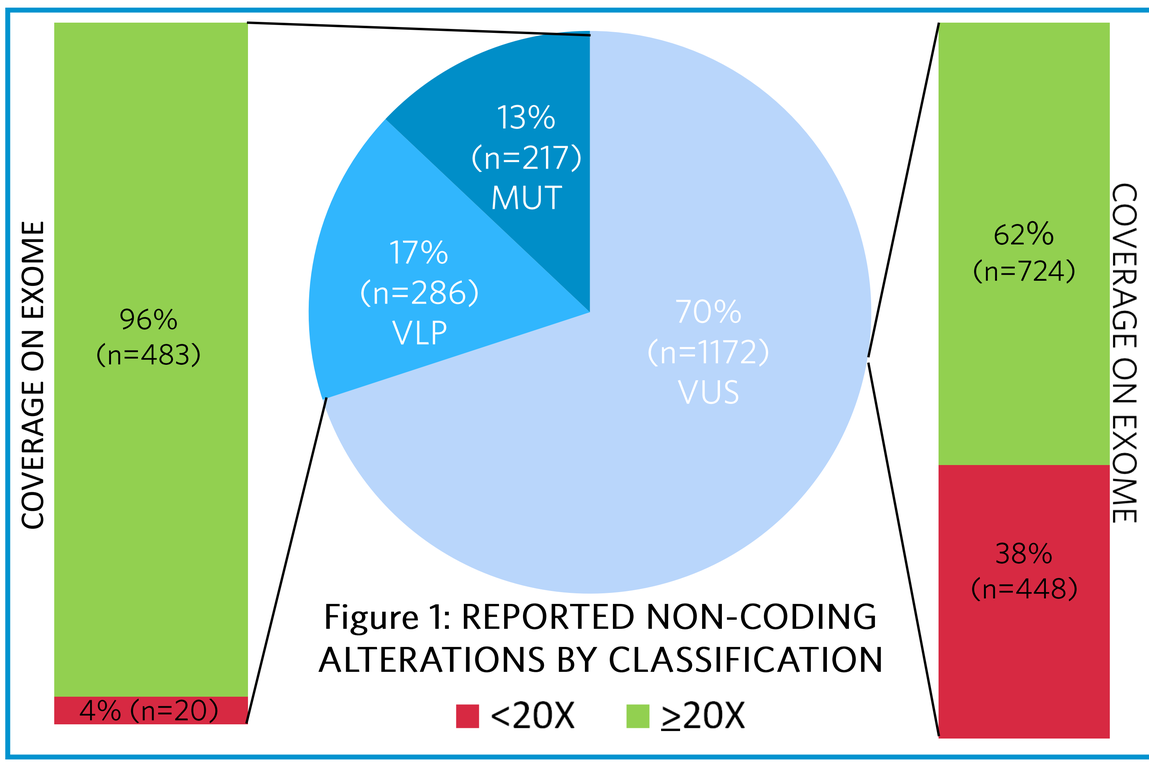



Figure 1: REPORTED NON-CODING ALTERATIONS BY CLASSIFICATION

## AIM

- To assess the technical sensitivity of DES for alterations in NCRs reported on panels.

## METHODS

- Collected all pathogenic mutations (MUT), likely pathogenic variants (VLP), and variants of unknown significance (VUS) in NCRs reported by Ambry Genetics on cancer and cardiac panels over the last 5 years.
- Using *in silico* analysis, corresponding nucleotide positions were interrogated in data from 100 randomly-selected DES samples.
- The mean sequence coverage at each position was determined.

## RESULTS

- In total, 1675 NCR alterations were reported among 108 genes.
- Majority of NCR alteration classifications were VUS, with similar numbers of MUT and VLP reported (Figure 1)
- In total, 1207 (72.1%) NCR alterations had mean coverage ≥20X on DES
  - Only 4.0% (20/503) of MUT/VLP had mean coverage <20X on DES
- Promoter regions of 3 genes accounted for 91.5% (428/468) of alterations with insufficient coverage on DES
  - *PTEN* (293/428); *MSH2* (96/428); *MLH1* (39/428)
  - Only 2 promoter alterations were reported as MUT & had DES mean coverage of 52 and 111, respectively
  - All other reported promoter alterations were VUS
- 96.9% of alterations +/-5 of the intron-exon junction had coverage ≥20X (Figure 2)
- Reported alterations in canonical splice sites (+/-1 or +/-2) were more often reported as MUT or VLP than those located 3, 4 or 5 nucleotides from the intron-exon junction (Figure 3)
  - Only 7.7% of alterations +/-2 are VUS, whereas 93.4% are VUS in positions 3,4 and 5
- 71.4% (10/14) of alterations reported beyond the 5th intronic nucleotide had coverage ≥20X on DES
  - Of these 14 reported alterations, 13 were MUT/VLP and one was a VUS

Figure 2: COVERAGE OF NCR ALTERATIONS BY LOCATION

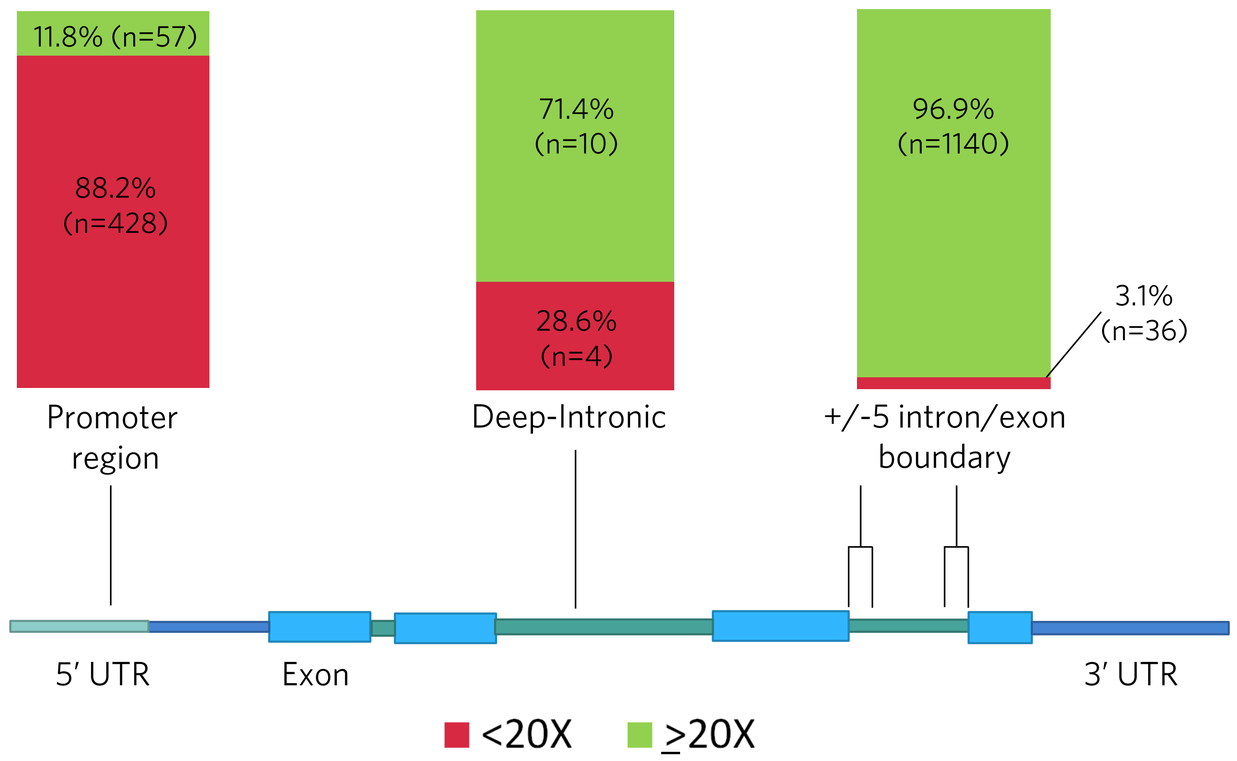
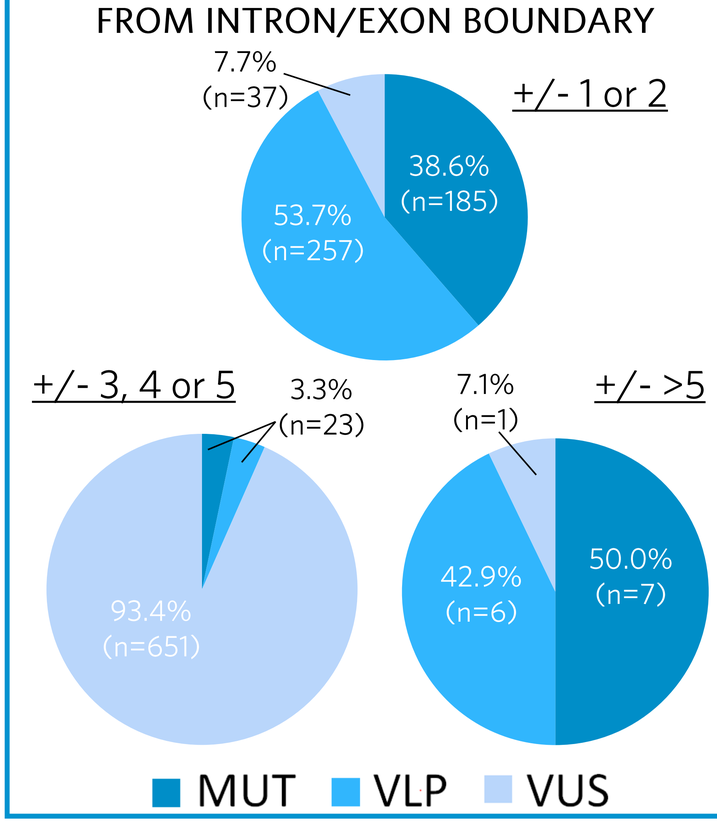



Figure 3: CLASSIFICATION BY DISTANCE FROM INTRON/EXON BOUNDARY




## DISCUSSION

- DES has adequate coverage of alterations within +/-5, but less sufficient coverage of deep-intronic and promoter region alterations.
- Most reported promoter sequencing alterations were in *PTEN*, and the clinical importance of *PTEN* promoter alterations is moving towards VLB (likely benign variants)[3].
- 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.
- DES reporting focuses on alterations at canonical splice sites and established MUT/VLP in deep-intronic regions.
  - Panel reporting criteria includes +/-5 nucleotides from the intron-exon boarder
  - Previously unreported intronic alterations beyond +/-2 are at most VUSs without further studies
  - Most deep-intronic alterations reported on panels were MUT/VLP and the majority of these had good coverage on DES, suggesting platforms for both tests are optimized to capture these known pathogenic alterations.

## TAKE-HOME POINTS

- Panels detect more alterations in the promoter region and deep-intronic regions compared to DES.
- DES had adequate coverage of 96% of NCR alterations reported as VLP/MUT on cancer and cardio panel testing.
- DES focusing on alterations at canonical splice sites and established MUT/VLPs in other NCR regions may provide the most informative results while reducing VUSs and lessening the burden of uncertain results for clinicians.

## REFERENCES

1. Xue, Y, et al (2015) Genet Med
2. Scacheri, CA & Scacheri P.C. (2015) *Current Opinion in Pediatrics*, *27*(6), 659–664.
3. Pilarski, R et al. (2016, October) *The PTEN promoter: to be or not be included on multigene panel tests?* Poster presented at the annual meeting of the ASHG, Vancouver, BC

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