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Abstract Topic:- Clinical Genetics

Abstract Title:- Whole exome with enhanced coverage (ExomeMAX) of Disease causing Genes

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Aims:-To create a comprehensive design of whole exome panel, with layered tiling and coverage to improve variant calling of both SNVs and CNVs. Validation and evaluation of this custom designed panel for clinical diagnostic testing.

Methods:- Panel design: The genomic coordinates were obtained from all primary and alternative transcripts from multiple gene models; MANE(v.0), GENCODE(v41), RefSeq, APPRIS databases. Mitochondrial genome was also added. In addition all reported pathogenic (coding, non-coding, promoters) variants in disease databases [HGMD (2021.3), Clinvar (17Oct 2021)] were included in the design. The final design was a comprehensive panel of 47 Mb including additional probes for ~700 genes to improve CNV calling.

Validation: National Institute of Standards and Technology (NIST) reference sample NA12878 was evaluated for determining the sensitivity and specificity. A total of 45 samples (Males-22; Females-23) were processed end to end. They were analysed, interpreted and concordance estimated based on prioritised disease causal variants. The coverage of the reported variants in ClinVar and HGMD were also evaluated.

Results:- The average coverage of the enriched whole exome panel- ExomeMAX at 1x, 10x and 20x based on ~12Gb data was 99.6%, 98.8% and 97.8%. The sensitivity percentages, as estimated for this panel were ≥ 99% and ≥ 97% with respect to SNVs and Indels, respectively for the NIST reference (NA12878), which was comparable to the expected numbers.

The variants detected in all 45 samples were concordant. Twenty seven were SNVs and Indels [Missense (37%), Inframe (4%), Promoter/UTR variants (7%), Splice/splice proximal (11%), nonsense(15%), frameshift (26%)]; Five were mitochondrial variants (MT-ND1: c.154G>A (p.Ala52Thr), Heteroplasmic; MT-TL1: n.14A>G, (Exonic/Non-coding), Heteroplasmic; MT-CO2: c.274G>A (p.Asp92Asn), Homoplasmic; MT-ND5: c.706G>A (p.Ala236Thr), Heteroplasmic; MT-ND5: c.706G>A (p.Ala236Thr), Heteroplasmic) ; thirteen were CNVs previously confirmed by orthogonal.

Analysis of a prospective cases: We have processed ~1000 samples for clinical diagnostics including carrier testing. The diagnostic yield was 37% including CNV in ~11%. For a subgroup of reported variants on ExomeMAX we evaluated the variant position coverages in other commercial exomes. We observed that the ExomeMAX provide a consistent higher coverage of the variants over other available commercial exome panels. On further evaluation, the low and uncovered regions in the other commercial panels mapped to ~61 clinically relevant genes.

Conclusions:- Whole exome sequencing (WES) is being successfully used in the detection of disease-causing variants in genetic diagnostics or inherited diseases. It is an efficient, cost effect and comprehensive method

to evaluate genetically heterogeneous diseases. Based on known literature on inherited diseases most of the known pathogenic variants (~80%) are in the coding exons. Though there are several commercial whole exome panels available in the market, one of the well appreciated deficiency is the uneven coverage distribution and/or no coverage of certain targets due to either probe design or inherent genomic composition (GC rich/repeat regions) which hampers accurate variant calling. In an effort to overcome the inadequacy of the commercial WES panels we designed a comprehensive whole exome based on the experience of clinical diagnostic testing of several hundred exomes.

Here, we demonstrate comprehensive validation and its effective use in diagnostic testing for inherited genetic disorders. The technical performance of this pipeline is very similar to the routinely used gold-standard-targeted NGS-based approaches. Furthermore, this study shows that the Custom Enriched Whole Exome Design (ExomeMAX) efficiently detects novel disease-causing variants, known pathogenic coding/non coding variants including comprehensive evaluation of copy number variants and mitochondrial genome.

Keywords:- Genetic testing, whole exome sequencing, diagnostic yield, non coding variants, gene coverage