

Recognizing the Promise and Potential Pitfalls of Genomic Medicine Through Routine Rapid Whole Genome Sequencing



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BACKGROUND

Genome sequencing has many advantages over capture-based next-generation sequencing (NGS) methods particularly for critically ill patients in need of a rapid turn-around-time. Clinical utility of such rapid testing has been demonstrated by several large studies; however, these studies must be translated to routine testing for their full potential to be realized. Minimal processing of the isolated DNA for a non-capture based assay, such as genome sequencing (GS), reduces the time from sample receipt to the start of sequencing. GS produces relatively non-biased even sequence coverage across the genome allowing detection of deep-intronic variants, even if not previously reported. The evenness of the depth of coverage provides high specificity for copy number variant (CNV) calling from the cytogenomic to the intra-genic level. In addition to deletions and duplications, this large dataset also allows confident calling of absence of heterozygosity, aneuploidy, mosaic deletions and duplications and uniparental disomy.

RESULTS

DRIED BLOOD SPOT vs WHOLE BLOOD

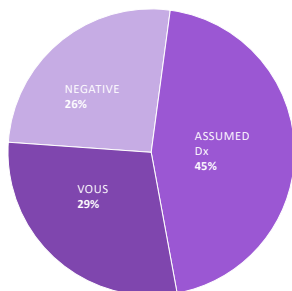
DNA QUANTITY BY SAMPLE TYPE

Sample Type	Average Concentration	Total Yield	Count of Sample
DBS	4.08 ng/ul	244.6 ng	20
WP	37.97 ng/ul	3251.6 ng	20

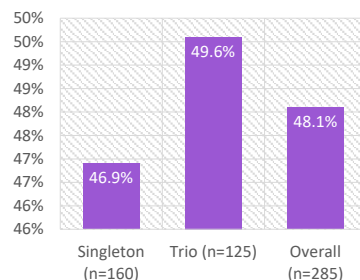
SEQUENCING METRICS BY SAMPLE TYPE

Sample Type	Average of Yield (Mb)	Average of % >=Q30 bases	Average of Coverage	Average of % Mapped Reads	Average of % Duplicate Reads	Average of % Regions >10x	Count of Sample
DBS	146260.13	93.11	40.80	0.99	0.06	90.58	13
WP	146255.73	93.68	40.06	0.99	0.07	90.67	11

Diagnostic Yield of 45% Across a Mixed Cohort 285 Pediatric Cases (74%) 101 Adult cases (26%)



Proband vs Trio Yield from WGS Based on 285 Pediatric Cases



WGS and Beyond from the Dried Blood Spot

WGS Sequence Quality

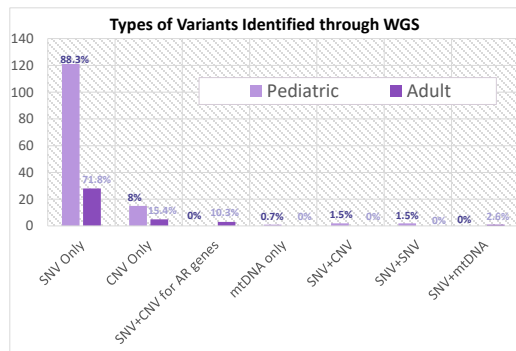
- Coverage of intronic sequences
- Better CNV calling than capture-based assays
- Deep mitochondrial sequencing
- *SMN1* copy number calling

WGS has even more potential

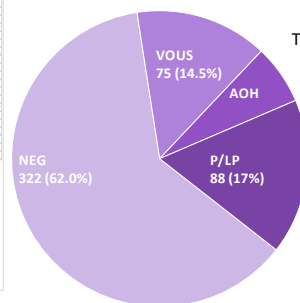
- cCMV from DBS
- Metagenomics – microbiome / virome

DBS Sample

- Amnio acids / Organic acids / Acylcarnitine
- Recommended Universal Newborn Screening + Other targets to exceed state panels



Cytogenomic yield from WGS + CNGnome Based on 485 Cases



Types of Diagnostic Findings (n=88)

PATHOGENIC & LIKELY PATHOGENIC FINDINGS

	Diagnostic	Carrier
Aneuploidy	9 (10.2%)	0
Microdel/dup	48 (9.2%)	3 (0.6%)
Intragenic	13 (2.5%)	12 (2.3%)
UPD	1 (0.2%)	0
Suspected unbalanced translocation	2 (0.4%)	0
Total	73 (14.1%)	15 (2.9%)

mtDNA Coverage



WGS detected a pathogenic m.14658C>T mtDNA variant that was missed by WES due to lower coverage

CONCLUSION

- WGS from DBS greatly reduces the challenges associated with sample collection from an ill newborn.
- Minimal processing steps in WGS result in rapid turn around time
- WGS results in deep coverage of mtDNA sequence
- Increased diagnostic yield due to detection of deep intronic and cytogenomic variants
- Use of DBS also allows for comprehensive biochemical testing (70+ disorders) by the same methods used for newborn screening