Development of a Supplemental Newborn Screening Approach that Complements State Public Health Programs for Treatable Conditions

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OVERVIEW

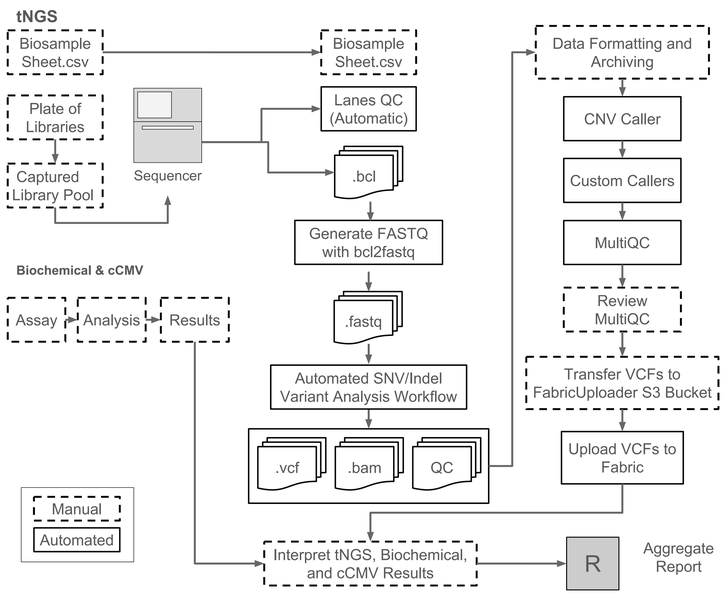
- Newborn screening (NBS) initiated by public health laboratories (PHL) in the US and worldwide has significantly reduced childhood morbidity and mortality for inherited conditions.
- There are several conditions beyond public health screening that may benefit from early identification through NBS but are not yet routinely screened in PHL. To maximize the potential benefit of NBS, we developed an expanded/supplemental NBS service, EliScreen, which integrates molecular and biochemical assays, as well as targeted next generation sequencing (tNGS).
- The Eli Screening Panel tests for 32 conditions and risk factors that are not typically covered in all state PHLs. This includes 6 biochemical assays that are followed with 2nd-tier tNGS, 25 additional stand-alone tNGS screens, and 1 qPCR assay. The full test panel may be used either as a physician ordered service, or the tNGS portion of the panel can be used to complement the needs of PHLs.

OBJECTIVE

To develop a comprehensive, high-throughput, supplemental newborn screening workflow.

NEWBORN SCREENING WORKFLOW

- Variant Knowledge Base - For 31 genes in our targeted panel, we performed manual curation of the variants that included pathogenic (P), likely pathogenic (LP), and pseudodeficiency variants. Common disease variants were pre-classified according to ACMG Guidelines.
- High-Throughput Interpretation - To reduce the turnaround time to interpretation, we developed an automated variant interpretation engine that scores variants taking disease inheritance models into account.



TARGETED NGS PANEL

The Eli panel includes 31 genes with mean coverage of 200x of CDS +/- 25 bp, 98.8% of the target region has >20x coverage.

Disease	Gene	Size (kb)	Homology Problem	Inheritance	% Gene Coverage
Duchenne Muscular Dystrophy	DMD	15.531	FALSE	XLR	100
Pompe	GAA	3.81	FALSE	AR	100
Gaucher[1]	GBA	2.15	TRUE	AR	100
MPS I	IDUA	2.668	FALSE	AR	99
Fabry	GLA	1.633	FALSE	XLR	100
Krabbe	GALC	3.209	FALSE	AR	100
MPS II	IDS	2.291	FALSE	XLR	98
Fructose Intolerance	ALDOB	1.497	FALSE	AR	100
Metachromatic Leukodystrophy	ARSA	1.922	FALSE	AR	100
Maroteaux-Lamy (MPS VI)	ARSB	2.127	FALSE	AR	99
Farber Disease	ASAH1	2.33	FALSE	AR	100
Hypophosphatasia	ALPL	2.114	FALSE	AR	100
Menkes	ATP7A	5.66	FALSE	XLR	100
Wilson	ATP7B	5.437	FALSE	AR	100
Ehlers-Danlos	COL3A1	6.91	FALSE	AD	100
Cystinosis	CTNS	1.954	FALSE	AR	100
G6PD[2]	G6PD	2.423	FALSE	XLD	99
Wolman/ CESD/LAL Deficiency	LIPA	1.751	FALSE	AR	100
Sanfilippo B (MPS IIIB)	NAGLU	2.526	FALSE	AR	89[4]
Sanfilippo A (MPS IIIA)	SGSH	1.936	FALSE	AR	93[4]
Cerebrotendinous Xanthomatosis	CYP27A1	2.037	FALSE	AR	100
Lysinuric Protein Intolerance	SLC7A7	1.977	FALSE	AR	100
Spinal Muscular Atrophy (SMA)[3]	SMN1/SMN2	1.441	TRUE	AR	100
Niemann-Pick Disease A/B	SMPD1	2.19	FALSE	AR	100
Neuronal Ceroid Lipofuscinosis 2 (CLN2)	TPP1	2.35	FALSE	AR	100
Smith-Lemli-Opitz Syndrome	DHCR7	1.771	FALSE	AR	100
Cerebral Creatine Deficiency 2	GAMT	1.245	FALSE	AR	82[4]
Congenital Hearing Loss (Cx26)	GJB2	0.75	FALSE	AD	99
Sly Syndrome (MPS VII)	GUSB	2.554	FALSE	AR	100
α-Mannosidosis I, II	MAN2B1	4.212	FALSE	AR	96
Retinoblastoma	RB1	4.277	FALSE	AD	100

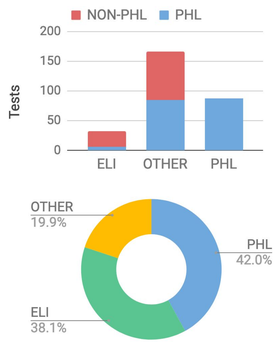
[1] GBA has a high homology pseudogene. We use a validated customized tool to make calls in this gene.

[2] G6PD common mutations in African, Mediterranean and Asian are covered only.

[3] SMN1 exon 7 deletion: The SMN1 gene is identical to the SMN2 gene with the exception of exon 8 (typically referred to as exon 7). This assay unambiguously detects SMN1 exon 8 copy number and sequence variant by force calling on SMN1. Sequence variants outside of exon 8 will also be detected, but this assay cannot determine whether the variant is located in SMN1 or SMN2.

[4] NAGLU, SGSH, and GAMT have lower coverage (<20x) in exon 1 due to sequences with high GC content.

- The variant calling performance of the tNGS test was evaluated against previously characterized samples. Variants were called independently for each sample. Both SNV/Indel and CNV variants were concordant with expected calls and thus sensitivity of the test is 100% (n=62) and 100% (n=8) respectively.



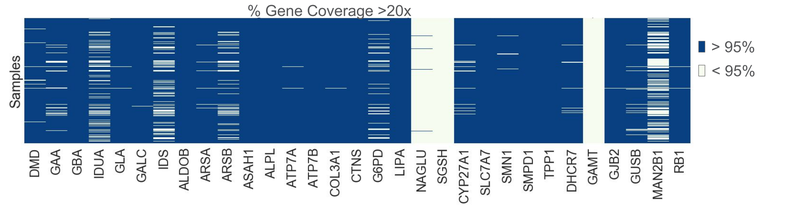
- The Eli Newborn Screening Panel is supplemental to testing performed by PHL. Newborn Screening products offered by other laboratories often sell the same tests that are offered by PHL.
- Though Eli offers fewer tests than other screening laboratories' Newborn Screening products, it detects a higher percentage of positive cases.*

*estimated based on disease incidence.

- Our NGS workflow takes on average 7 days from sample receipt to report generation and fits within our promised short turnaround time.

COVERAGE, VUS MODEL, & CNV

Panel performance of 888 samples based on % gene coverage >20x shows that the majority of the genes in the panel had >95% gene coverage.

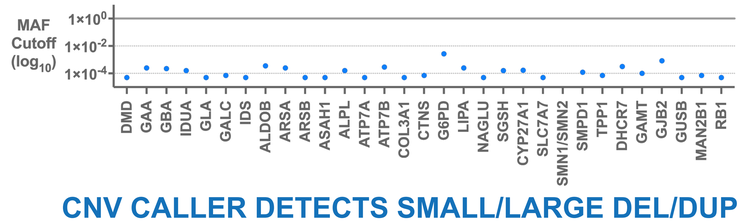


VUS CUTOFF MODEL

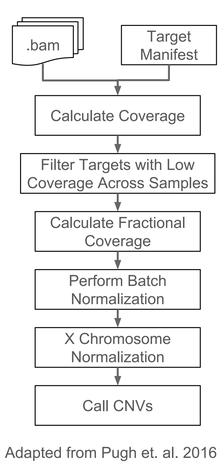
To reduce VUS burden, we applied a VUS cutoff model. For each gene, we calculate MAF using the statistical framework applied in Whiffin et al. 2017.

$$MAF = \frac{c\sqrt{gv}}{\sqrt{p}}$$

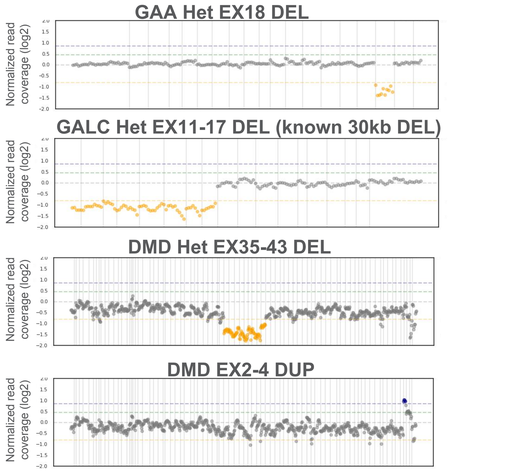
c = max. allelic contribution (proportion of cases attributable to gene vs. those attributable to individual variant)
g = max. genetic contribution (proportion of all cases due to gene)
v = prevalence; and ***p*** is the penetrance



CNV CALLER DETECTS SMALL/LARGE DEL/DUP



Adapted from Pugh et. al. 2016



CONCLUSIONS

Our comprehensive supplemental newborn screening panel addresses several technical challenges associated with NGS. The panel is complementary to tests offered by PHL and has a higher detection rate of percent positive cases than other Newborn Screening products. Our workflow includes a VUS cutoff model to reduce VUS interpretation and a CNV caller capable of detecting small and large DEL/DUP events. Finally, the automated workflow is scalable to handle short TAT for reporting.

REFERENCES

1. Whiffin, N. et al. Using high-resolution variant frequencies to empower clinical genome interpretation. Genet. Med. 19, 1151–1158 (2017).
2. Pugh, T. J. et al. VisCap: inference and visualization of germ-line copy-number variants from targeted clinical sequencing data. Genet. Med. 18, 712–719 (2016).