EMORY GENETICS LABORATORY

One library, many tests: The evolution of Next Generation Sequencing panel testing

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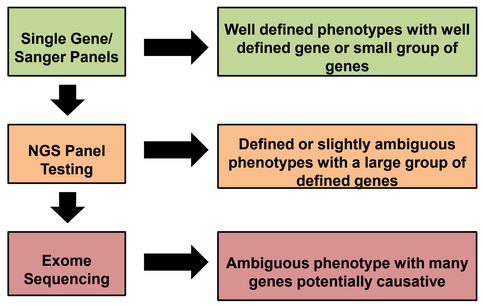
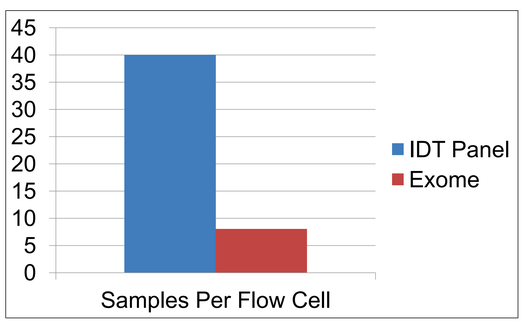
Abstract

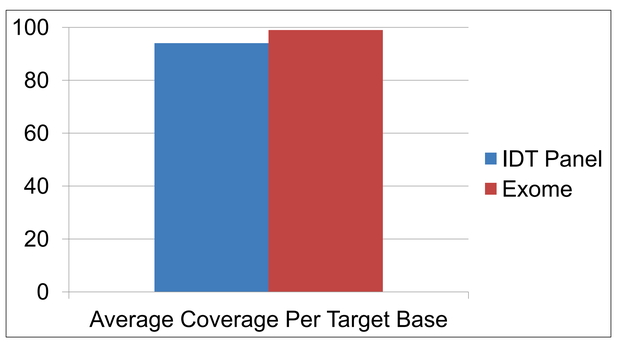
Next Generation Sequencing for clinical diagnostics has been dominated by two assays: gene panels and Whole Exome Sequencing (WES). Traditional panel testing has the benefits of lower costs and higher coverage of the regions of interest with 95% of targets covered at >20X post experiment. However, logistical problems can arise as more libraries and panels are brought into use along with high sample volume leading to increased costs and slower sample processing. While WES has the benefit of capturing many of the regions of interest in one library, simplifying workflow, the library size limits multiplexing and carries a higher sequencing cost, which in turn can quickly raise costs and limit throughput. WES sequencing has the additional issue of incomplete coverage of known disease causing genes with 85% of exons in HGMD listed genes well covered post experiment with standard exome capture kits. This can lead to missed disease causing variants and incomplete coverage of exons targeted for a specific panel. These exons would need to be covered by another method for 100% coverage, creating additional costs and slowing result reporting. In order to combine the ease of WES with the high coverage and low cost for panels, we leveraged IDT Lockdown probes to create a library targeting all exons in HGMD listed genes. To test this methodology, we used 40 samples previously sequenced at Emory Genetics Laboratory using traditional panel testing. Each sample was indexed, hybridized in pools of 10 samples, and sequenced in groups of 2 pools, 20 samples total, on a single flow cell of a HiSeq2500 with 100bp paired end sequencing on Rapid Run Mode. Coverage and run statistics were recorded for each sample for all targets and only variants for the specified panel were returned, limiting incidental findings. HGMD exon coverage increased to 95% well covered post experiment, with each individual panel increased from 95% to 99% coverage with all known variants detected at an average coverage of 75X. The single library approach decreased cost over individual panels by 30% and decreased technologist hands on time by 20%. The creation of the one library allows for simplified workflow, lower costs, rapid sequencing, and the higher coverage necessary for clinical diagnostic Next Generation Sequencing.

Evolution of Sequencing

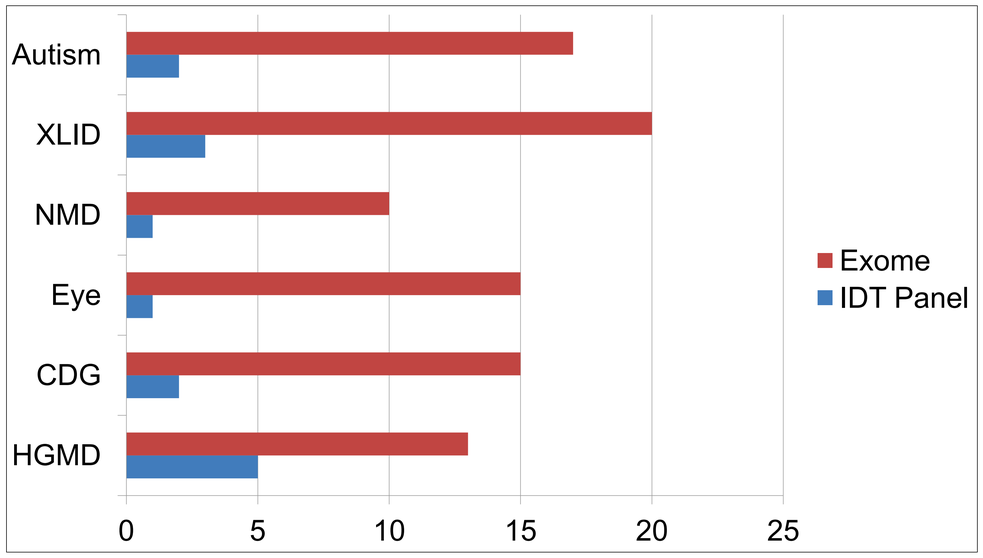


Sequencing Statistics





IDT Lockdown Library Low Coverage vs Exome Low Coverage



This graph gives the percentage of low coverage exons for representative panels in the IDT Lockdown Library compared to the Nimblegen v3 Exome capture. Low coverage is defined as targeted exons with 10bp of flanking sequence with any base or multiple bases less than 15X coverage.

Variant Detection for IDT Lockdown Library

Sample	Gene	Variant	Zygosity	Coverage
Sample 1	DMD	c.1513G>C; p.V505L	Heterozygous	118
	DSC2	c.2686_2687dupGA	Homozygous	81
	TTN	c.32854+4T>C	Heterozygous	75
Sample 2	ANO5	c.364-8delT	Homozygous	87
	COL6A2	c.1671+10A>G	Heterozygous	69
	DYSF	c.428C>A;p.P143Q	Heterozygous	88
Sample 3	AMPD1	c.133C>T;p.Q45*	Heterozygous	53
	COL6A3	c.4399A>G;p.N1467D	Heterozygous	120
	DYSF	c.3196_3197insCGGAGG	Homozygous	49
Sample 4	ACADM	c.1161A>G;p.V387V	Heterozygous	115
	AGA	c.446C>G;p.T149S	Homozygous	101
	ATP2A1	c.*1G>A	Homozygous	44
	EHMT1	c.3541-10_3541-9insTC	Homozygous	60
	FOXE1	c.532_537delGCCGCC;p.A178_A179del	Homozygous	13
	GAA	c.858+7_858+8insAGCGGGC	Homozygous	41
	LPIN1	c.856_858delTCT;p.S286del	Homozygous	100
	ZEB2	c.930C>T;p.Y3210Y	Heterozygous	107
Sample 5	COG1	c.1782G>A;p.E594E	Heterozygous	95
	COG1	c.2024_2026delTCC;p.L675del	Homozygous	88

Conclusions

Sequencing has evolved in the molecular diagnostic laboratory from single gene testing, to Next Generation Sequencing Panels, to Whole Exome Sequencing. As new technologies and methodologies are utilized for diagnostics, more phenotype/genotype relationships can be discovered. Each type of sequencing has its place in the diagnostic world, with Sanger Sequencing best used for well defined phenotypes with well studied gene correlation, panel testing for more defined phenotypes that have multiple possible genetic causes such as Neuromuscular disorders or X-linked Intellectual Disability, and Exome sequencing for ambiguous phenotypes, where any number of genetic disorders could be responsible. The use of a single library to capture many of the disease causing genes listed in HGMD has simplified panel testing for the clinical diagnostic laboratory. This approach has several advantages including a higher throughput, higher coverage, fewer low coverage exons, and lower cost compared to Exome sequencing.

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