A Comprehensive Diagnostic Schema for Fanconi Anemia

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Abstract

Fanconi Anemia (FA) is a multisystem recessive disorder that manifests as anemia and a host of characteristic congenital abnormalities; cancer incidence is also increased. FA patients have functional defects in one of at least 16 proteins associated with DNA repair and thus, lymphocytes from these individuals are uniquely sensitive to DNA cross-linking agents. The current diagnostic test for FA is the chromosome breakage assay, and until recently, positive tests were reflexed to genetic complementation testing followed by single-gene Sanger sequencing; the testing odyssey could routinely take months. Next generation sequencing (NGS) affords an opportunity to streamline this process. We have developed an NGS approach using the Ion Torrent™ PGM platform wherein upon confirmation of breakage positive samples, all exons from 21 FA and FA-associated genes are sequenced. We have validated identification of NGS variants with previously identified Sanger-sequenced variants (including missense, nonsense, insertion, deletion and whole exon deletion mutations) in 22 samples. Our comprehensive in-house annotation pipeline facilitates mutation calls with a high degree of confidence for identification of pathogenic variants. All pathogenic and likely pathogenic variants are confirmed by Sanger sequencing. Additionally, we assess exon-level deletions and duplications using a custom exon-centric microarray. This comprehensive testing scheme, which combines the strengths and experience of both our Cytogenetics Lab (breakage) and Molecular Genetics Lab (sequencing and microarray) provides high quality, rapid, and cost-effective testing for the diagnosis of FA.

Methods

Nineteen FA known-positives and 3 negative controls were sequenced utilizing the Ion Torrent™ PGM and confirmed through Sanger sequencing. All samples were analyzed for large deletions and duplications on an exon-centric custom microarray (OGT), with minimum of 3 probes per exon.

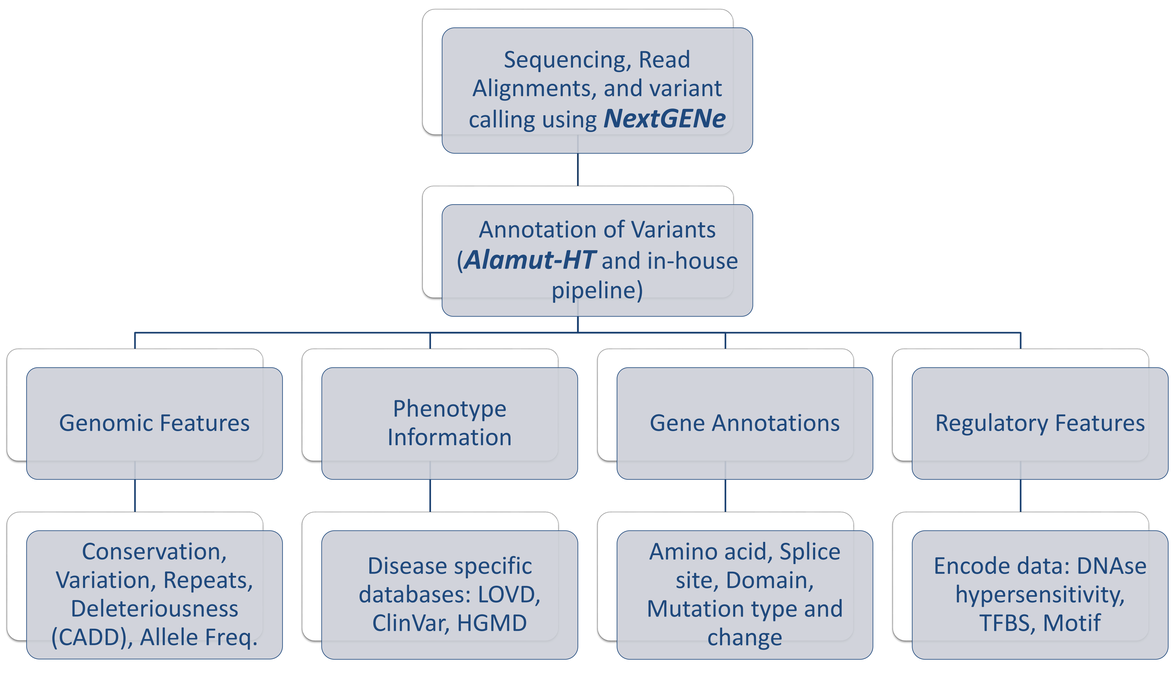
→ *21 genes; 382 total exons sequenced; 985 amplicons; 106.1 kb coding*

21 Genes on FANC Panel

FANCA	FANCE	FANCN/PALB2	FAAP24	FANCM	RAD51
FANCB	FANCF	FANCO/RAD51C	FAAP20	FANCL	FAAP100
FANCC	FANCG	FANCP/SLX4	FANCD2	BLM	RIF1
FANCD1/BRCA2	FANCI	FANCJ/BRIP1	FANCQ*		

** Coming soon in updated panel design*

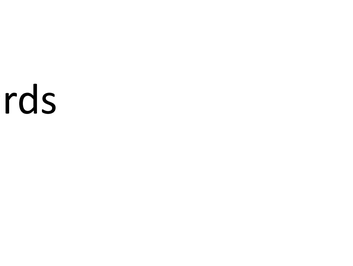
Variant Calling, Annotation and filtering



Sample ID	Total Aligned Reads	Average base Coverage	Target Base Coverage at 100X	Known Mutations	Mutations Detected by Ion Torrent
Sample1	521,719	503.3	98.08%	c.3240-2A>G, c.1683_1689del7*	c.3240-2A>G
Sample2	619,621	613.5	98.18%	None (Control Sample)	None (Control Sample)
Sample3	452,701	412.9	82.51%	c.2T>C*, c.1115_1118delTTGG	c.1115_1118delTTGG
Sample4	642,627	536.5	87.12%	None (Control Sample)	None (Control Sample)
Sample5	375,327	315.1	80.40%	c.484_485delCT (Homozygous)	c.484_485delCT (Homozygous)
Sample6	678,712	455.4	87.40%	None (Control Sample)	None (Control Sample)
Sample7	690,127	604.2	90.13%	c.1163+2T>A (Homozygous)	c.1163+2T>A (Homozygous)
Sample8	671,976	671.3	96.58%	c.67delG, c.1642C>T	c.67delG, c.1642C>T
Sample9	578,762	568.9	97.78%	c.3391A>G, deletion exons 4-8	c.3391A>G, deletion exons 4-8
Sample10	503,019	491.0	97.40%	c.1115_1118delTTGG, c.893+1G>T	c.1115_1118delTTGG, c.893+1G>T
Sample11	606,523	549.2	89.51%	c.37C>T, c.456+4A>T	c.37C>T, c.456+4A>T
Sample12	489,444	393.4	83.45%	c.2738A>C, c.1A>C*	c.2738A>C
Sample13	495,360	452.3	96.37%	c.1115_1118delTTGG, c.3349A>G	c.1115_1118delTTGG, c.3349A>G
Sample14	562,228	498.9	96.60%	c.3788_3790del, c.827-1G>T	c.3788_3790del, c.827-1G>T
Sample17	480,131	449.6	96.70%	c.4288_4289insCGAC	c.4288_4289insCGAC
Sample18	355,804	317.9	91.64%	c.1771C>T	c.1771C>T
Sample19	549,640	511.9	96.89%	c.2851C>T	c.2851C>T
Sample20	578,382	560.5	96.83%	c.2066delG	c.2066delG
Sample21	737,722	748.1	98.28%	c.916_917delAC	c.916_917delAC
Sample22	651,051	653.3	97.87%	c.1475T>C (Homozygous)	c.1475T>C (Homozygous)
Sample23	647,823	653.4	97.82%	c.2730_2731delCT	c.2730_2731delCT
Sample24	599,463	588.8	96.96%	deletion exons 30-43	deletion exons 30-43

Table1: Validation of blinded, positive FA samples and controls using Ion Torrent™ PGM and Sanger sequencing
***missed by design, see Summary**

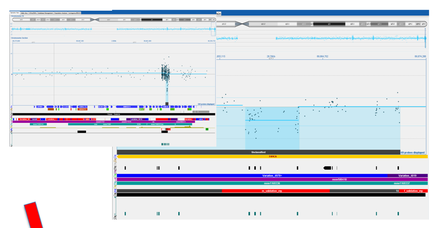
Work Flow



1 Peripheral Blood → Positive Chromosomal Breakage Study (TAT 5-10d)

2 Next-Gen Sequencing of Targeted Gene Panel using Ion Torrent™ PGM → Data Analysis Pipeline and Variant Annotation → Sanger Sequencing Confirmation of Variants

\+ Large Deletion and Duplication By Exon Centric Array



del exon 11-14

Comprehensive Report (TAT 4-6 weeks)

Summary

1. Our protocol uses NGS, data analysis pipeline, and exon-centric microarray, to identify DNA-level mutations, indels and CNVs (large del/dups), which provides an accurate, rapid, and cost-effective solution to FA diagnosis.
   - Comprehensive testing for FA is useful for diagnosis in affected persons, carrier testing in potential donors for transplantation, and prenatal diagnostics.
2. In our validation series, 25/28 variants were correctly identified utilizing NGS and our data analysis pipeline. The missed variants (2/28) were located in regions not covered by the sequencing probes, ie. missed by design.
   - Sanger sequencing of the uncovered regions provides 100% coverage for genes in the panel.

References:
Kottemann MC1, Smogorzewska A. (2013) Fanconi anaemia and the repair of Watson and Crick DNA crosslinks. *Nature*. 2013 493(7432):356-63.