

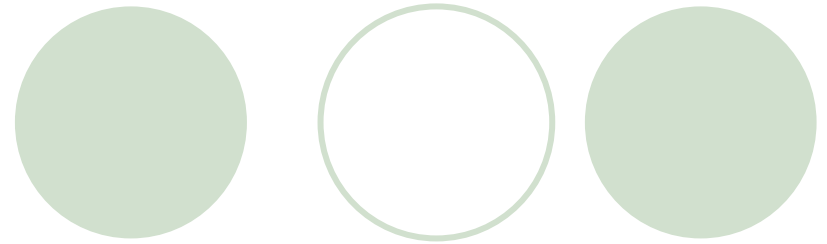
RNA work on UVs - Our Experience So Far

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03/03/08

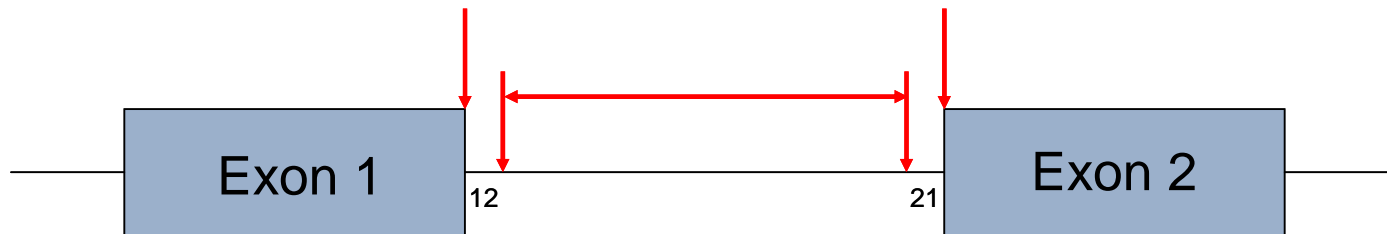
Protocol

- Sample- 5-10ml EDTA
- WCC- 10×10^6 cells extracted
- RNA extraction- Stage 1- Qiagen RNeasy mini kit
- Stage 2 - QIAcube
- cDNA preparation- Invitrogen Superscript III
- PCR- design exonic primers for exons near to UV
- Resolve products- agarose gel
- Clean up products- QIAquick gel extraction kit
- Sequence

When we test



- Exonic UV near intron/exon junctions
 - Intronic UV
-
- Fruitfly suggests effect
 - Literature suggests effect
 - UV potentially effects branch site



Testing

- Dabbling since 2002, increase in number of cases recently
- Diseases;

Disease	Total	Confirmed splice variant	Apparently normal	Inconclusive	Ongoing
BRCA	11	3	7		1
FAP	4	3	1		
GACA	2	1	1		
GS	8	5	1	1	1
HNPCC	9	7		1	1
PP	9	3	3		3
SOTOS	2				2
VHL	3	1	2		
Total	48	23	15	2	8
Percent		48%	31%	4%	17%

CMT	4	To confirm deletions found on MLPA
Alstrom	1	To look for multiple exon deletion in a consanguous family
INAD	1	To look for multiple exon deletion in a consanguous family

- BRCA training project including samples tested due to ESE Finder



Mutations

Disease	Gene	Confirmed splice variant	Apparently normal	Inconclusive	Ongoing
BRCA	BRCA1	c.4476+6T>C c.5272-26A>G c.5396+48ins12	c.4931A>G		
	BRCA2		c.296-7T>A c.296-7delT c.8544A>G c.8860+132dupC		c.275A>G
FAP	APC	c.1957A>G c.1312+3A>G c.1312+3A>G	c.1313-18G>A		
GACA		c.832G>A	c.1565+2dupT		
GS	PTCH	c.572G>C	c.1335+17T>C	c.3537+5G>T *1	c.383-33G>C
		c.642+3A>G c.734+3_6del4 c.3438-1_3469dup33 c.3537G>T			
HNPCC	hMLH1	3x c.306G>T c.380G>A c.677G>A		c.544A>G *2	c.116+5G>C
	hMSH2	c.1759G>C c.2211-2A>T *3			
PP	SDHB	c.72+1G>T c.286G>A	c.424-19_-14dup6		c.300T>C 2x c.423+20T>A
SOTOS	NSD				2x c.6259-25T>G
VHL	VHL	c.554-1insAA	c.555_556dup3 c.676+8C>T		

- 1 Poor sample quality
- 2 Presence of faint spurious products, unable to sequence them
- 3 Confirmed in Newcastle

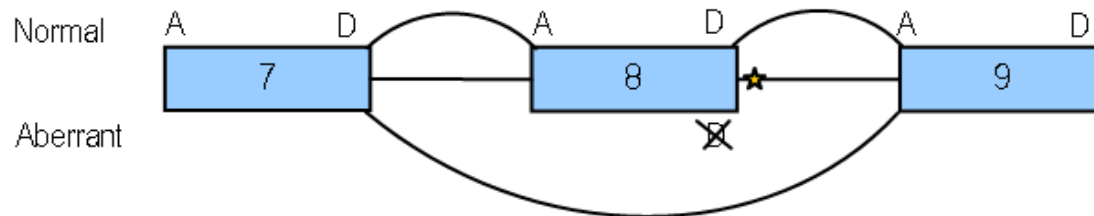
Example 1

- BRCA2 c.681+2dupT
- Fruitfly predicted the splice donor was abolished

Normal; **CTACTGCTGTAAGTAAA** c.909+2dupT; **CTACTGCTGTTAAGTAA**

Start	End	Score	Exon	Intron	Start	End	Score	Exon	Intron
18	32	0.93	tactgctg	taagtaa					

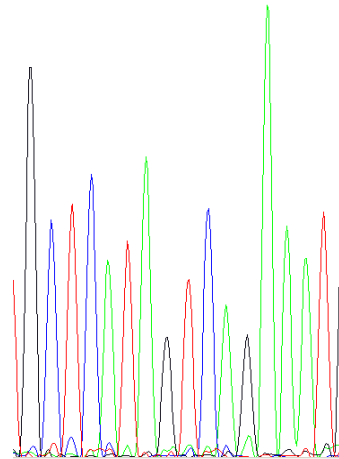
- Predicted effect on splicing



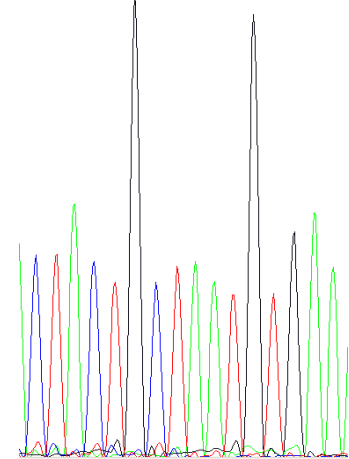
Results

● r.860_909del

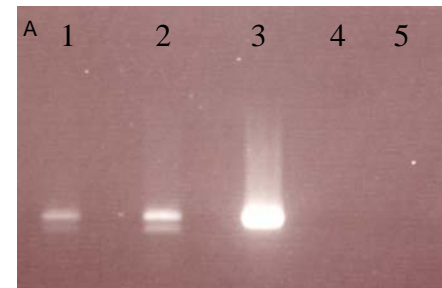
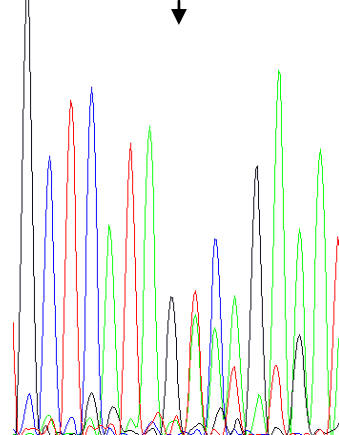
B GCTCATAGTCAGAAAT
Ex 7 Ex 8



C CTACTGCTAATGTGAA
Ex 8 Ex 9



D GCTCATAGTCAGAAAT Ex 8
Ex 7 AATGTGAA Ex 9



BRCA2 gene screen (trainee project)

- 2 male patients
- Strong FH of male breast cancer
- No pathogenic mutations detected in BRCA1/BRCA2 by sequencing of coding regions or by MLPA
- Designed primers for overlapping fragments
- all products less than 1kb



Isoforms

● No clear pathogenic variants identified

Full transcript; r.=



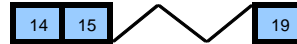
Deletion of exon 12; r. 7070_7165del **KNOWN ISOFORM** Inframe



Deletion of exons 15, 16 and 18; r. 7664_8033del 8205_8559del NOT PREVIOUSLY IDENTIFIED Out of frame



Deletion of exons 16, 17 and 18; r. 7845_8559del NOT PREVIOUSLY IDENTIFIED Inframe



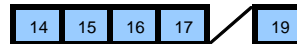
Deletion of exons 16 and 18; r.7846_8033del8205_8559del NOT PREVIOUSLY IDENTIFIED Out of frame



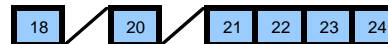
Deletion of exons 17 and 18; r.8034_8559del NOT PREVIOUSLY IDENTIFIED Out of frame



Deletion of exon 18; r.8205_8559del **KNOWN ISOFORM** Out of frame



Deletion of exon 19; r.8560_8715del **THOUGHT TO BE DELETERIOUS** Inframe



Insertion of 64bp from intron 20; r.8860_8861ins8861-1327_8861-1263 **KNOWN ISOFORM** Out of frame



Insertion of 64bp from intron 20 and deletion of exon 22; r.8860_8861ins8861-1327_8861-1263 8983_9181del **KNOWN ISOFORM** Out of frame



Deletion of exon 22; r.del8983_9181 NOT PREVIOUSLY IDENTIFIED Inframe



Normal Controls

