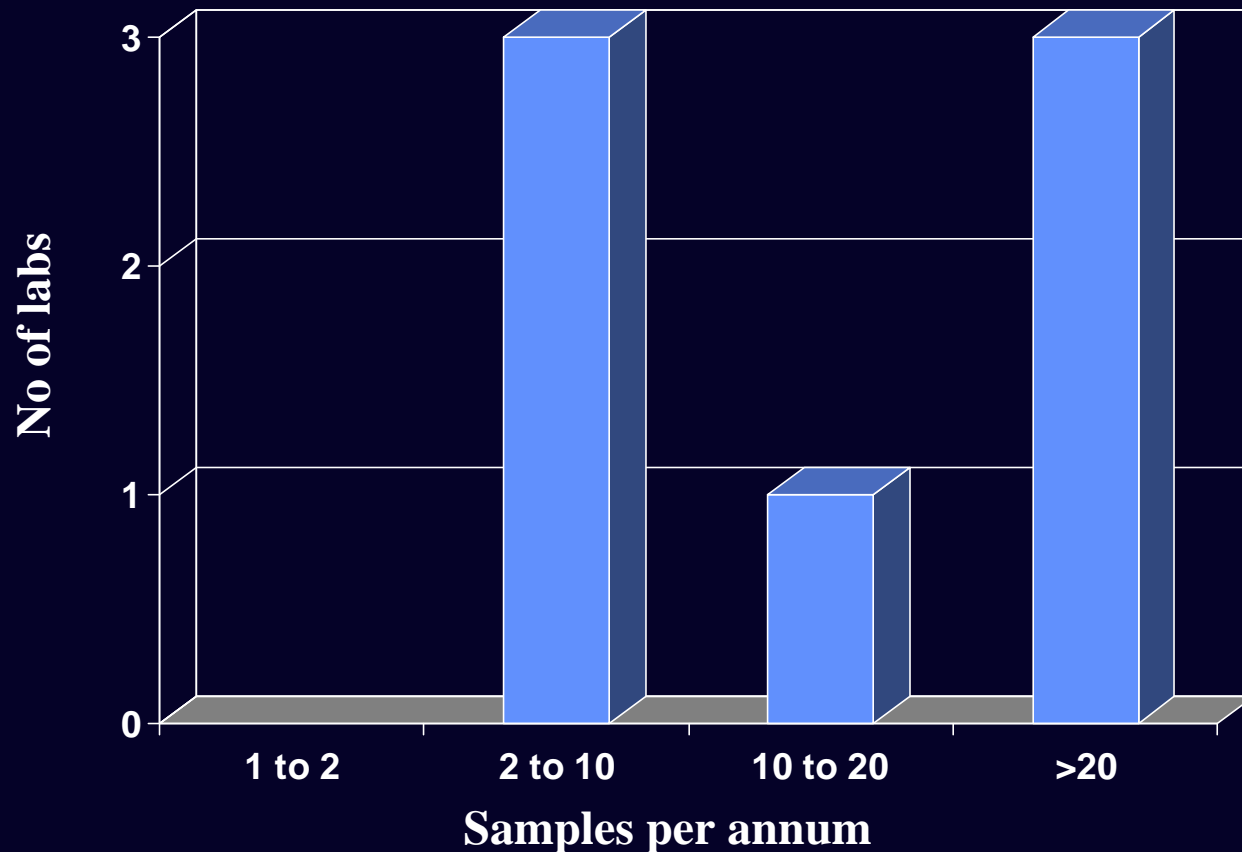


RNA analysis survey results

Labs performing splicing analysis

- **7 surveys returned**
 - **Exeter**
 - **Guy's**
 - **Bart's/London**
 - **Birmingham**
 - **Cardiff**
 - **Belfast**
 - **Salisbury**

How many samples have you analysed in the past year for possible splicing abnormalities on the basis of finding a DNA variant of unknown significance?



What genes have you analysed for splicing mutations? Roughly how many patients have you tested for each gene in the past year?

- **Splicing analysis initiated by finding of UV**
- **Upfront screen for some genes (eg collagen VI)**
- **Secondary screen of no mutations detected by full sequencing or MLPA (DMD)**

- **DMD (2), X-linked Alports (2), Collagen VI (20)**
- **RB (64)**
- **NF1 (50)**
- **RET, HNF1A, HNF4A, HNF1B, ABCC8 (1-3 of each)**
- **BRCA (10), FAP (4), E-Cadherin (2), PTCH (4) MLH1/MSH2 (3), SDHB/SDHD (4), ALMS1 (1), VHL (3)**
- **APC (8), LDLR (15)**
- **BRCA1 (8), BRCA2 (8), hMLH1 (3), hMSH2(3)**

Does your lab routinely perform RT-PCR analysis for other purposes, eg detection of fusion genes in leukaemia?

- **No (4), Yes (3)**

How do you process the blood sample to remove red cells?

- **RCL (6; one with Paxgene)**

What method do you use for RNA extraction?

- **Qiagen (4), Trizol/Trireagent (2), Paxgene (2), PureGene (1)**

What quantity of RNA (μg or ng) do you use in each RT reaction?

- **OD not measured (6)** (1 estimates on gel; 1 extracts from fixed number of cells).
- **2.5 μg (1)**

What cDNA synthesis method do you use?

- **Kit (6); Invitrogen (5: SS, SSII, SSIII, Thermoscript; 1: Qiagen)**
- **Homebrew (1): SSIII**

What RT primers do you use?

- **Hexamers (3); oligo dT (1); gene specific (2)**

How do you test if the cDNA is of adequate quality?

- **Amplifying the gene of interest (6) (plus sequence confirmation)**

If you design your own primers, do you confirm that they are cDNA specific?

- **Design to be DNA specific (3); test on DNA (3)**

How many normal controls do you routinely analyse for comparison with the test sample?

- **Zero (2); 2+ (3); 4+ (2)**
- **Difficult to get normal controls**
- **What is normal?**

What are the main problems you have encountered when performing RT-PCR analysis to determine the effects of sequence variants on splicing?

- **Studying intronic variants when no aberrant product is observed: it is not always possible to confirm loss of expression of the mutant allele**
- **The identification of previously unreported isoforms / multiple splice variants**
- **Interpretation of splicing patterns when more than one band present in normal controls and band intensities vary**
- **Nonsense-mediated decay**
- **Not always able to confirm sequence changes (?splice variants) in gDNA**
- **Getting RNA of sufficient quality and quantity (particularly for 5' ends of genes)**

What are the main problems you have encountered when performing RT-PCR analysis to determine the effects of sequence variants on splicing?

- **Quality of RNA hard to check**
- **Tissue-specific expression expression – eg the HNF1A and HNF1B genes are only expressed in tissues that are not available for study. Development of techniques to harness ectopic or illegitimate transcripts in lymphoblastoid cell lines. The low levels of ectopic transcripts present in such lines can make the analysis difficult**
- **RNA from paraffin sections**

Have you used any other laboratory (not bioinformatic) methods to analyse the effects of mutations, eg minigene analysis, functional analysis, expression analysis? If yes please give details

- **One gastric cancer (E-cadherin) analysed in research lab**
- **Expression analysis to evaluate the effect of splicing mutations on the expression profiles of alternatively processed transcripts**
- **Investigation of the effects of nonsense-mediated decay on transcripts harbouring stop codons that can be generated by splicing errors**
- **BRCA minigene analysis (research project)**