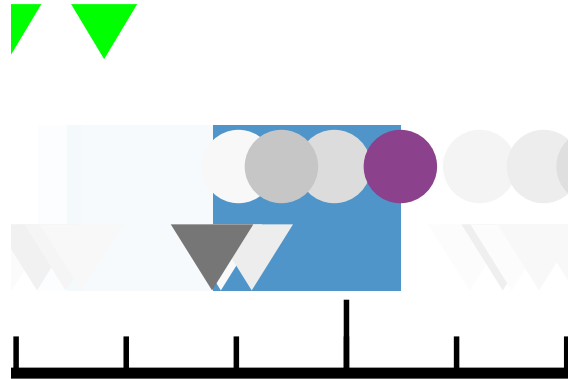
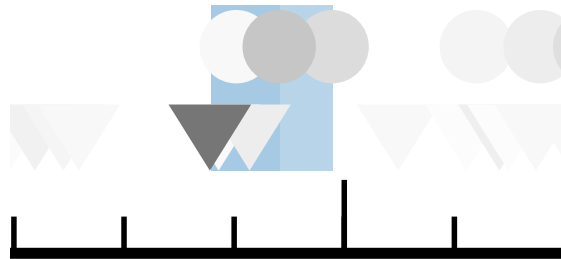


Example of predicting a mutation effect with SpliceScan II



(a) SpliceScan II predicting normal non-canonical exon in BMPR2 gene.



(b) An example of the successfully predicted effect of mutation IVS2+2delC causing familial pulmonary arterial hypertension [1], a single nucleotide deletion which disrupts a strong non-canonical 5'GC SS (shown as purple circle in subfigure 1(a)) and causing activation of two cryptic alternatively committed canonical 5' SSs located -60 and -108 nucleotides upstream of the original SS. Here we predict not only the effect of mutation, but the two alternatively used aberrant exonic isoforms.

Figure 1: Example of predicting an effect of IVS2+2delC mutation on splicing as annotated in DBASS5 [2] database.

The following sequence annotated in DBASS5 [2] for IVS2+2delC mutation have been used for the prediction results shown in Figure 1.

```
ttcggataag acaaagattt tatataatat tttgaaaaca ttaaataatt tgtcattcct ttatttcctt tatttttagCT
TCGCAGAATC AAGAACGGCT ATGTGCGTTT AAAGATCCGT ATCAGCAAGA CCTTGGGATA G/GTGAGAGTA GAATCTCTCA
TGAAAATGGG ACAATATTAT GCTCGAAAG/ GTAGCACCTG CTATGGCCTT TGGGAGAAAAT CAAAAGGGGA CATAAATCTT
GTAAAACAAG g(c)aagtga tacttttcctt acctgaaatg actgtgtttt atacaattga tattttatcta aaaaggacat
gggagtatgt taaaatcctg ttcagaaaaa cagtgaattt aaaagtgtat atataaagcc aggtgtgggtg gctcatgcct
gtaattccag cacttttcga ggctgagggtg ggcggatcac ttgaggccag gagtttgaga ccagcctggg taataacatg
gtgaaacccc gt
```

If we input the normal and mutant sequences in FASTA format, as shown in Figure 2, to the SpliceScan II we get the prediction results identical to the shown in Figure 1. For this run SpliceScan II option [Show only non-overlapping exons predicted] should be unchecked and [Show exons with non-canonical GC donor] should be checked.

```
>Normal:IVS2+2delC
GCAATTTCCAGAATTATTCAGCCTTCTAGGTTTGTGTTTGAAGACTTGGTAATTTGAACATGGACATTTT
CTTTGATGATATTTTTATTGGACACCAAAATTATTTAATAGAAGCAAGAGTGGTGAGAATAATTTGAG
TACGAGGGGATTGCTTTTGGGAAATGAGAATTTTAAAAATTCCTTCTTGTGTTGGAGTTTACAAATGAATT
CATGAACAGAAGAACGTCATTGAATGTAATAATTAGAAATTTATGTAAGATTTTCAGTTCAAATAATTTAG
TAGGGAAATTTATGAAGTCATTCCGATAAGACAAAGATTTTATATAATATTTTGAAAACATTAATAAATT
TGTCATTCCTTTATTTCTTTATTTTAGCTTCGCAGAATCAAGAACGGCTATGTGCGTTTAAAGATCCGT
ATCAGCAAGACCTTGGGATAGGTGAGAGTGAATCTCTCATGAAAATGGGACAATATTATGCTCGAAAGG
TAGCACCTGCTATGGCCTTTGGGAGAAATCAAAAAGGGGACATAAATCTTGTAACAAGCAAGTATGATAC
TTTCTTACCTGAAATGACTGTGTTTATACAATTGATATTTATCTAAAAAGGACATGGGAGTATGTAA
AATCCTGTTGAGAAAAACAGTGAATTTAAAAAGTGTATATATAAAGCCAGGTGTGGTGGCTCATGCCTGTA
ATTCCAGCACTTTTCGAGGCTGAGGTGGGCGGATCACTTGAGGCCAGGAGTTTGAGACCAGCCTGGGTAA
TAACATGGTGAACCCCGT
>Mutant:IVS2+2delC
GCAATTTCCAGAATTATTCAGCCTTCTAGGTTTGTGTTTGAAGACTTGGTAATTTGAACATGGACATTTT
CTTTGATGATATTTTTATTGGACACCAAAATTATTTAATAGAAGCAAGAGTGGTGAGAATAATTTGAG
TACGAGGGGATTGCTTTTGGGAAATGAGAATTTTAAAAATTCCTTCTTGTGTTGGAGTTTACAAATGAATT
CATGAACAGAAGAACGTCATTGAATGTAATAATTAGAAATTTATGTAAGATTTTCAGTTCAAATAATTTAG
TAGGGAAATTTATGAAGTCATTCCGATAAGACAAAGATTTTATATAATATTTTGAAAACATTAATAAATT
TGTCATTCCTTTATTTCTTTATTTTAGCTTCGCAGAATCAAGAACGGCTATGTGCGTTTAAAGATCCGT
ATCAGCAAGACCTTGGGATAGGTGAGAGTGAATCTCTCATGAAAATGGGACAATATTATGCTCGAAAGG
TAGCACCTGCTATGGCCTTTGGGAGAAATCAAAAAGGGGACATAAATCTTGTAACAAGCAAGTATGATAC
TTTCTTACCTGAAATGACTGTGTTTATACAATTGATATTTATCTAAAAAGGACATGGGAGTATGTAA
AATCCTGTTGAGAAAAACAGTGAATTTAAAAAGTGTATATATAAAGCCAGGTGTGGTGGCTCATGCCTGTA
ATTCCAGCACTTTTCGAGGCTGAGGTGGGCGGATCACTTGAGGCCAGGAGTTTGAGACCAGCCTGGGTAA
TAACATGGTGAACCCCGT
```

Figure 2: Normal and mutant allele fragments in FASTA format corresponding to IVS2+2delC mutation as annotated in DBASS5 [2] database.

References

- [1] Cogan J, Pauciulo M, Batchman A, Prince M, Robbins I, Hedges L, Stanton K, Wheeler L, Phillips LJ JA 3rd, Nichols WC Am J Respir Crit Care Med: **High frequency of BMPR2 exonic deletions/duplications in familial pulmonary arterial hypertension.** *Am. J. Respir. Crit. Care Med.* 2006, **174**:590–598.
- [2] Buratti E, Chivers M, Královičová J, Romano M, Baralle M, Krainer A, Vořechovský I: **Aberrant 5' splice sites in human disease genes: mutation pattern, nucleotide structure and comparison of computational tools that predict their utilization.** *Nucleic Acids Res.* 2007, **35**(13):4250–4263.