What constitutes an ‘alternative TSS’?

Case 1

First exon shows novel splice structure (likely real)

This includes:

- any
- any

Case 2

First exon is significant variation on existing exon (but may also be artifact)

Condition: x>100 bp

- any
- any

- any
- any

• Genes with a single TSS: by default, longest form included for multi-exon transcripts only
• Case 2 events account for ~40% of the cases (genes with a single TSS not included)
• Event “signature” is the in-boundary of the first exon (see below). Therefore, different events may lead to the same transcription start site in genomic coordinates:

Event 1001
Event 1002

Example 1: Alternative TSS at Uqcrcl
What constitutes a ‘skipped’ (cassette) exon?

(X)SKIP

- 'on'
- 'off'

(X)MSKIP

- 'on'
- 'off'

- Transfrags must belong to the same gene, have the same orientation
- Both transfrags must contain ≥3 exons
- X – exact boundary match for SKIP, approximate (simple exon overlap) for XSKIP
- * - event “pattern” or “signature” (column 7)

Example 2: ‘Skipped’ exons at Sp140
What constitutes a ‘retained intron’?

What if the event involves a terminal exon?

1. Long (‘on’) exon? No – APA!
2. Short (‘off’) exon? Yes - IR!

What constitutes an ‘alternative exon end’?

Both ends
Relationship to a reference gene (column 19)

<table>
<thead>
<tr>
<th>5’</th>
<th>~2 (overlap)</th>
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<tbody>
<tr>
<td></td>
<td>~2 (overlap)</td>
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<tr>
<td>3’</td>
<td>&gt;2 (intronic)</td>
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<tr>
<td></td>
<td>&lt;1 (out of range)</td>
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