**A.** Junction detection: GT/AG sites in reference genes

Known and novel junctions  Ignored junction

TTTTCCTTCT**GT**ATCTAA**AG**ATTGTTA**GT**TTCCCA**AG**TACAACATTA**GT**TAAAATAACG**AG**TGAATCTATTAGAATTATG**GT**CGATAACATA**AG**TGAAACAT**GT**AAT**AG**CTGC

**B.** Junction quantification: Count reads supporting splicing or intron retention

Non-split read supporting intron retention  Uninformative read

Split reads supporting splicing
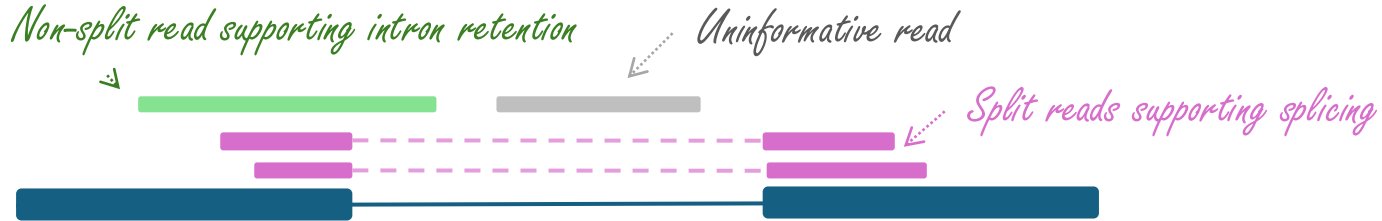
**C.** Dataset of all junctions and samples

| junction | sample | condition | support | count |
|----------|--------|-----------|---------|-------|
| JX001 | A | WT | Split | 16 |
| JX001 | A | WT | Non-split | 34 |
| JX001 | B | Ctrl | Split | 24 |
| JX001 | B | Ctrl | Non-split | 40 |
| JX001 | C | WT | Split | 10 |
| JX001 | C | WT | Non-split | 32 |
| JX001 | D | Ctrl | Split | 24 |
| JX001 | D | Ctrl | Non-split | 45 |
| JX002 | A | WT | Split | 20 |
| ... | ... | ... | ... | ... |
| JX999 | ... | ... | ... | ... |

**D.** GLM analysis: Fictional examples of outcome

DIFF EXPR W/O DIFF SPLICE    DIFF SPLICE W/O DIFF EXPR    DIFF EXPR & DIFF SPLICE

LOG2(COUNT)

CTRL  WT  WT  CTRL  CTRL  WT

SPLICE  INTRON  SPLICE  INTRON  SPLICE  INTRON

READS SUPPORTING