

Psi (Percent spliced-in) calculation via outrigger psi					
	SE	MXE			
	<div>Isoform1 (inclusion)</div> <div>Isoform2 (exclusion)</div> <div><math display="block">\Psi = \frac{r_{1,2} + r_{2,3}}{r_{1,2} + r_{2,3} + 2r_{1,3}}</math></div>	<div><math display="block">\Psi = \frac{r_{1,2} + r_{2,4}}{r_{1,2} + r_{2,4} + r_{1,3} + r_{3,4}}</math></div>			$\Psi = \frac{\text{inclusion reads}}{\text{inclusion} + \text{exclusion reads}}$
			Notes	Compatible w/ annotation?	
Case 1	Not applicable		Incompatible junctions with sufficient reads	✗	$\Psi = \text{NA}^*$
Case 2			Zero observed reads	✗	$\Psi = \text{NA}$
Case 3			All compatible junctions with insufficient reads	✗	$\Psi = \text{NA}$
Case 4			Only one junction with sufficient reads	✗	$\Psi = \text{NA}$
Case 5			One junction with >10x reads than the other**	✗	$\Psi = \text{NA}$
Case 6			Exclusion: Isoform2 with sufficient reads and Isoform1 with zero reads	✓	$\Psi = 0$
Case 7			Inclusion: Isoform1 with zero reads and Isoform2 with sufficient reads	✓	$\Psi = 1$
Case 8			Sufficient reads on all junctions	✓	$0 < \Psi < 1$
Case 9			Isoform2 with sufficient reads but Isoform1 has one or more junctions with insufficient reads	<div>?</div> <div>a. Total reads <math>\geq r_{\text{threshold}}^{***}</math></div> <div>b. Total reads <math>&lt; r_{\text{threshold}}</math></div>	<div><math>0 &lt; \Psi &lt; 1</math></div> <div><math>\Psi = \text{NA}</math></div>
Case 10			Isoform1 with sufficient reads but Isoform2 has one or more junctions with insufficient reads	<div>?</div> <div>a. Total reads <math>\geq r_{\text{threshold}}</math></div> <div>b. Total reads <math>&lt; r_{\text{threshold}}</math></div>	<div><math>0 &lt; \Psi &lt; 1</math></div> <div><math>\Psi = \text{NA}</math></div>
Case 11	Not applicable		Isoform1 and Isoform2 each have both sufficient and insufficient junctions	<div>?</div> <div>a. Total reads <math>\geq r_{\text{threshold}}</math></div> <div>b. Total reads <math>&lt; r_{\text{threshold}}</math></div>	<div><math>0 &lt; \Psi &lt; 1</math></div> <div><math>\Psi = \text{NA}</math></div>

### Legend

$r_{i,j} \geq r_{\min} \rightarrow$

Sufficient reads on the junction

$r_{i,j} < r_{\min} \rightarrow$

Insufficient reads on the junction

$r_{i,j} \gg r_{\min} \rightarrow$

Much more than sufficient reads

$r_{i,j}$  Reads on junction spanning exon  $i$  to exon  $j$

$r_{\min}$  Minimum number of reads per junction, default 10 and can be user-defined with the flag --min-reads

\*  $\Psi = \text{NA}$  can mean three things:

- Transcript was not expressed
- Insufficient evidence to confidently call exon inclusion or exclusion
- Junctions map to different alternative or flanking exon(s) – considered as distinct events during the indexing step, outrigger index

SE

MXE

Original event

Not applicable, see below

Junctions map to different alternative exon(s)

Same alternative exon(s), different junction(s)

For a SE event, if the junctions map to different alternative exon (small black exon on the top), then the event with smaller exon has a  $\Psi$  value ranging from zero to one, but for the wider exon (on the bottom), which doesn't have matched inclusion reads, this event is called excluded with  $\Psi=0$

$0 < \Psi < 1$

$\Psi = 0$

\*\* The multiplier for how much greater one side junction can be is user-defined with the flag --uneven-coverage-multiplier, here shown with the default value of 10. To deal with 0 reads, a pseudocount of 1 is added to all junctions for this test only:

05

1200

$\pm 1$

16

1201

$\rightarrow 1 \times 10 \not\leq 6$

✓ Passes

050

1200

$\pm 1$

151

1201

$\rightarrow 1 \times 10 \leq 51$

✗ Doesn't pass

\*\*\*  $r_{\text{threshold}}$  Threshold for total junction reads in the event

$$r_{\text{threshold}} = n_{\text{junctions}} \times r_{\min}$$

e.g. for an MXE event (4 junctions) and a minimum of 10 reads per junction:  $\sum_{i,j} r_{i,j} = 4 \times 10 = 40$

$\sum_{i,j} r_{i,j}$

Total Junction reads

$n_{\text{junctions}}$

Number of junctions in splicing event type (e.g. 3 for SE or 4 for MXE)

### Configurable options

Junction read inputs

--bam

--sj-out-tab

--junction-reads-csv

(default: reads from outrigger index)

$r_{\min}$  --min-reads 10 (default)

--uneven-coverage-multiplier 10 (default)