### for SICER 2.0

1.bam

2.pair end

3.calculate from bed coverage. Pileup

Sum of all applicable flags. Flags relevant to Bowtie are:

|  |  |
| --- | --- |
| 1 | The read is one of a pair |
| 2 | The alignment is one end of a proper paired-end alignment |
| 4 | The read has no reported alignments |
| 8 | The read is one of a pair and has no reported alignments |
| 16 | The alignment is to the reverse reference strand |
| 32 | The other mate in the paired-end alignment is aligned to the reverse reference strand |
| 64 | The read is mate 1 in a pair |
| 128 | The read is mate 2 in a pair |

Thus, an unpaired read that aligns to the reverse reference strand will have flag 16. A paired-end read that aligns and is the first mate in the pair will have flag 83 (= 64 + 16 + 2 + 1).

Samtools view +

**-f**INT

Only output alignments with all bits set in INT present in the FLAG field. INT can be specified in hex by beginning with `0x' (i.e. /^0x[0-9A-F]+/) or in octal by beginning with `0' (i.e. /^0[0-7]+/) [0].

**-F**INT

Do not output alignments with any bits set in INT present in the FLAG field. INTcan be specified in hex by beginning with `0x' (i.e. /^0x[0-9A-F]+/) or in octal by beginning with `0' (i.e. /^0[0-7]+/) [0].