

What does secondary (and some other fields) mean in flagstat output?



This is my samtools flagstat output for the alignment of a paired-end sample using HISAT (filtered with MQ<1):

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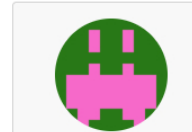
```
45084184 + 0 in total (QC-passed reads + QC-failed reads)
4717987 + 0 secondary
0 + 0 supplementary
0 + 0 duplicates
45084184 + 0 mapped (100.00%:-nan%)
40366197 + 0 paired in sequencing
20273012 + 0 read1
20093185 + 0 read2
39146254 + 0 properly paired (96.98%:-nan%)
39644363 + 0 with itself and mate mapped
721834 + 0 singletons (1.79%:-nan%)
260722 + 0 with mate mapped to a different chr
235361 + 0 with mate mapped to a different chr (mapQ>=5)
(END)
```

There's quite a few questions about flagstat on Biostars, but I haven't seen the secondary, supplementary or duplicates flag before. Assuming that this is HISAT specific, are is secondary the number of mapped reads that get mapped using the second (non-global) HISAT index? What would supplementary mean? And are duplicates the amount of reads that map non-uniquely?

[flagstat](#)
[samtools](#) • 16k views

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updated 2.5 years ago by [Ram](#) ⚡ 45k • written 9.9 years ago by [Niek De Klein](#) ★ 2.6k



9.9 years ago
[Niek De Klein](#) ★ 2.6k



These are defined more formally in the SAM specification, though perhaps the wording there isn't great.

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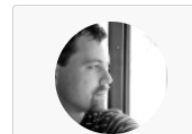
Secondary: One of the many places a multimapper can align. Note that multimappers will have one primary and 0 or more such secondary alignments.

Supplementary: For chimeric/fusion/non-linear alignments, this is the location of one part of the alignment. For reads that align in a chimeric fashion, one segment will be designated as primary and the remainder supplementary.

Duplicate: If you've marked possible PCR duplicates, then this will be set. The definition of a duplicate is somewhat dependent on the tool used (N.B., aligners don't typically set this flag, it's down by picard's markDuplicates command or similar).

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[WouterDeCoster](#) ⚡ 48k • written
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Hello, I'm mapping with three
 different programs: TopHat,
 HISAT2, and STAR. I'm using
 default values for all but STAR (I
 have short read...

["Secondary", "Supplementary",
 "Duplicates" and "Paired in
 sequencing" in samtools flagstat](#) •

updated 2.5 years ago by [Ram](#)
 ⚡ 45k • written 3.2 years ago by
[pasha64t](#) • 0

I wonder if someone please
 explain what secondary,
 supplementary, duplicates and
 paired in sequencing mean in
 samtools flagstat Example: ...

[How to extract uniquely aligned
 paired end reads obtained from
 freebayes using different
 parameter combinations of
 samtools?](#) •

4.9 years ago by
[nadiabeg.comsats](#) ▲ 10

I have 10x reads and I need
 uniquely aligned reads for SNP
 calling. I am using samtools to
 extract uniquely aligned reads
 but I am not sat...

[Extracting supplementary reads
 from bam file](#) •

updated 9 months ago by
[cmdcolin](#) ★ 4.2k • written 9
 months ago by [g1ang](#) ▲ 20

Hi everyone, bioinformatics
 noob here :) I'm trying to extract
 supplementary reads (Illumina
 paired-end 150bp) from a series
 of bam files...

[Separating unmapped reads
 from bam files is not working
 using samtools -f 4](#) •

updated 5.0 years ago by
[GenoMax](#) ⚡ 151k • written 5.0

[Login](#) before adding your answer.

years ago by [sorrymouse](#) ▲ 120

I mapped reads using bwa mem and created a sorted bam file. An example flagstat is as follows: 12446425 + 0 in total (QC-passed rea...

[BWA MEM Definition of a Primary Alignment](#) •

updated 4.0 years ago by [d-cameron](#) ★ 2.9k • written 6.0 years ago by

[QVINTVS_FABIVS_MAXIMVS](#) ★ 2.6k

Quick question: When bwa mem splits a read into a primary and a secondary/supplementary alignment, is the primary always the "left-most..."

[Interpreting mapping contaminants](#) •

updated 6.5 years ago by [h.mon](#) ⚡ 35k • written 6.5 years ago by [cecilio11](#) ▲ 120

Dear Biostars, I am posting this question in this forum because I found this wonderful site is populated mostly by very kind and helpful...

[Different mapping results between HISAT and TopHat](#) •

5.0 years ago by [conchetta](#) ▲ 10

Hi all! I am performing a genome-guided transcriptome assembly with Stringtie. Before the transcriptome assembly, I have mapped my pa...

[Difference between chimeric alignments and multiple mapping](#) •

updated 8.0 years ago by [Devon Ryan](#) ⚡ 105k • written 8.0 years ago by [Vanilla](#) ▲ 110

Hi all: I recently got quite confused with two SAM flags got from BWA alignment, which is "***supplementary alignment***" from chimeric al...

[Total number of trimmed reads less than number of mapped reads](#) •

4.3 years ago by [kspata](#) ▲ 90

Hi All, I have forward and reverse trimmed reads. The total number of forward and reverse trimmed reads calculated using fastqc was 9,83...

[How to interpret flagstat output](#) •

updated 3.0 years ago by [lstvan](#)

Albert ⚡ 102k • written 3.0 years ago by [deniselavezzari](#) • 0

Hi, I have an issue similar to some published. FastQC gives me 2,941,170 Total sequence, whereas the flagstat outputs these: ``` 351046...

[High number of secondary alignments with HISAT2](#) • updated 7.2 years ago by [Biostar](#) ⚡ 20 • written 7.3 years ago by [JJ](#) ▲ 760

Hi all, So I am working with a public dataset and I am a bit worried about the high number of secondary alignment I get. Here is the sam...

[The meaning of uniquely mapped](#) •

MISSING

[Can anyone suggest resources for troubleshooting why my alignment using Bowtie1.1.1 is incorrect?](#) •

4.1 years ago by [nessj](#) • 0

My bowtie command : bowtie GCF_000001215.4_Release_6_plus_ISO1_MT_genomic-q SRR8191524.fastq -v 2 -m 1 -3 1 -S 2> ./SRR8191524.out...

[Different alignments rates with bwa mem \(0%\) and bowtie2 \(82%\)](#) •

8.3 years ago by [James Ashmore](#) ★ 3.5k

I have ChIP-seq data which has been trimmed using trim galore (all default settings). When I align these trimmed reads using bwa mem (all d...

[Samtools rmdup and Piccard Markduplicates](#) •

updated 7.8 years ago by [lakhujanivijay](#) ⚡ 5.9k • written 7.8 years ago by [Prakash](#) ★ 2.2k

Hello Bio Stars, I have doubt regarding duplicate removal from BAM file. I used two tools ***samtools rmdup*** and **Piccard MarkDuplic...

[Can't convert paired end BAM to bed using bedtools](#) •

updated 2.3 years ago by [GenoMax](#) ⚡ 151k • written 2.3 years ago by [oksana03fel](#) • 0

Hello, I got .bam files from my pipeline and i merged them with

samtools merge ALL.bam *.bam
and I got this % of mapping
3825773 + ...

[PCR duplicates in RNASeq](#) •
updated 2.1 years ago by [Ram](#)
🚧 45k • written 7.8 years ago by
[Prakash](#) ★ 2.2k

Hello Bio stars, I have small
query regarding identification
and removal of PCR duplicates
from RNASeq data. The
Tophat2 alignment stats c...

[Variant calling and alignment
stats](#) •

5.0 years ago by
[nadiabeg.comsats](#) ▲ 10

Hi. I am using samtools
flagstats to see the statistics of
my alignment file. It looks
something like this: I have 10x
genomics reads. ...

[different result using minimap2
and pbmm2](#) •
updated 2.6 years ago by
[gconcepcion](#) ▲ 410 • written 3.2
years ago by [pingu77](#) ▲ 40

Hi all! I am analysing CSS
Pacbio data and each sample
came from different run, in
particular I have three files for
each sample. I teste...

[Variation in mapping percentage
with genome](#) •

updated 24 months ago by [Ram](#)
🚧 45k • written 3.4 years ago by
[onkar](#) ▲ 10

I have a few resequencing data
(Illumina DNA Seq) for various
cultivars/varieties of same plant.
I had mapped the reads with a
published c...

[Bowtie2 and BWA-MEM giving
very different results in
metagenomic data](#) •

5.5 years ago by [Antonio
Camargo](#) ▲ 160

I've assembled a metagenome
using MEGAHIT and begun
testing different mapping
options to perform the binning of
the contigs. However, I've ...

[Cannot align reads to plasmid](#) •
updated 7.3 years ago by [h.mon](#)
🚧 35k • written 7.3 years ago by
[David](#) ▲ 240

Hi, I have sequenced a bacterial
genome for which i have a
reference genome (98%
similarity). I have used bwa to

map reads to the refe...

[Samtools: How can I extract properly-paired QC-passed reads instead of extracting only properly-paired?](#) •

8.0 years ago by [bioinfo8](#) ▲ 230

Hi, Here is the 'flagstat' output of my bam file: 37750740 + 352032 in total (QC-passed reads + QC-failed reads) 0 + 0 secon...

[Samtools flagstat confusing result of a merged bam file](#) •

MISSING

[How does Base Call casing affect BWA](#) •

4.8 years ago by [oconnwald](#) ▲ 20

I was wondering how the casing of base calls affected BWA MEM performance. I ran three sets of data to see if there was any difference a...

[tophat --max-multihits impacts 'mapped in a proper pair'](#) •

updated 8.6 years ago by [John](#) ⚡ 13k • written 8.6 years ago by [Carlo Yague](#) ⚡ 9.0k

Hi everyone, I have a RNA-seq library (paired-end) with a large amount of multimappers because it comes from a total RNA extract with pa...

[samtools flagstat interpretation](#) •

updated 5.7 years ago by [ATpoint](#) ⚡ 88k • written 5.7 years ago by [asmaaaljuhani](#) • 0

How can I interpret the following results? and is there a specific percentage that we accept the allignment? samtools flagstat aln-p...

[Different flagstat number after using MergeBamAlignment](#) •

13 months ago by [ThomasLam](#) • 0

Hi everyone, I'm utilizing the GATK Best Practices for analyzing mitochondrial NGS data. I utilized FastqToSam to generate a ualign BAM fi...

[Samtools Flagstat Comments](#) •

updated 3.9 years ago by [GenoMax](#) ⚡ 151k • written 3.9 years ago by [santos48](#) ▲ 40

Hi, I am trying to solve [total alignments :0 results of

featureCounts]]]] this problem.
Now I checked my bam files
with that command `sam...

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