INTRODUCTION TO READ MAPPING

Dhivya Arasappan

(With several slides borrowed from Dr. Jeff Barrick)

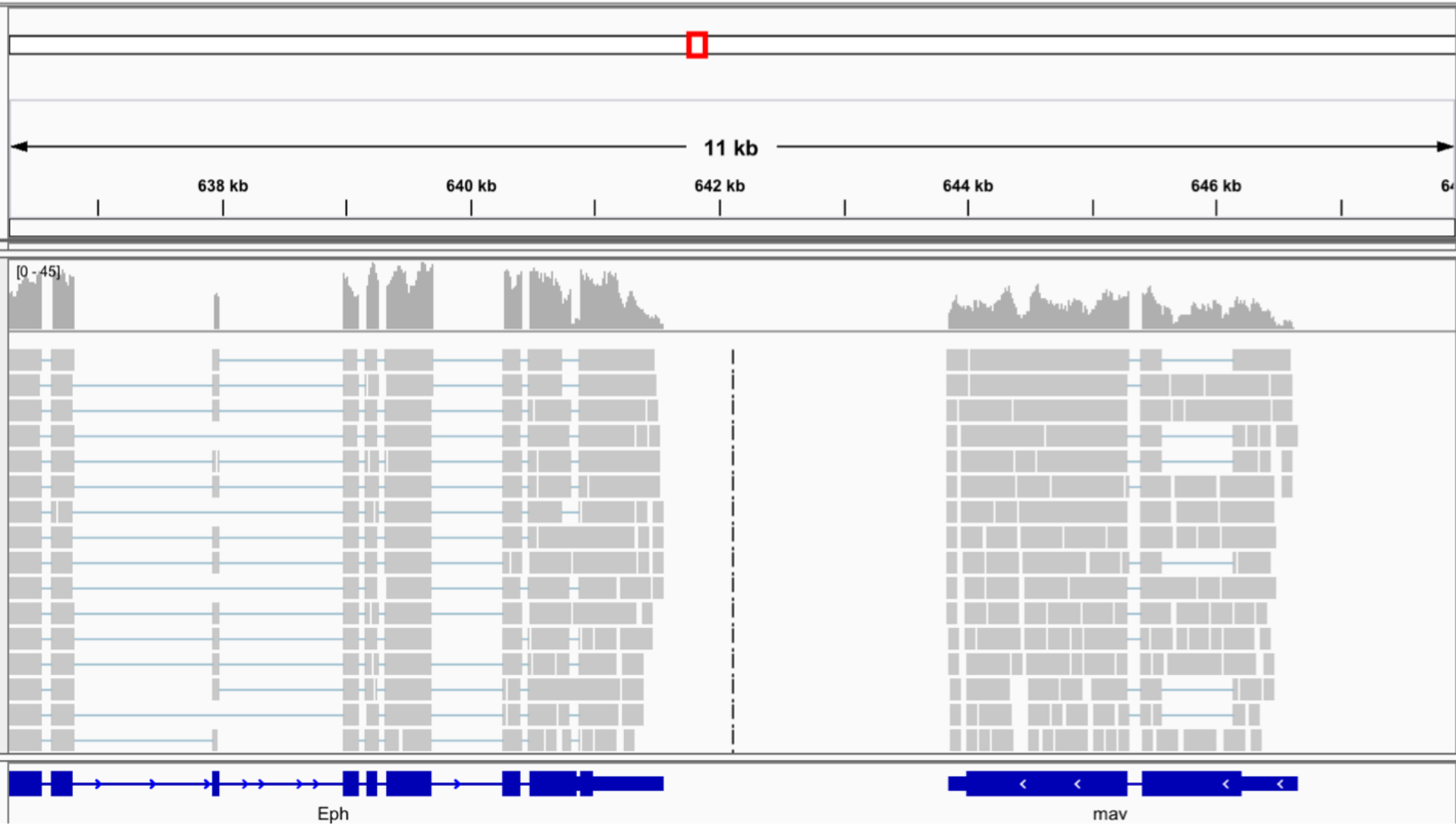
What does an alignment look like?

Ref=TAGATCAGATTCGATACCAGACCATGATCATACGATCCA

Read=AGACCATG

Found at offset 18!
TAGATCAGATTCGATACC**AGACCATG**ATCATACGATCCA

What does an alignment look like?



https://vlsci.github.io/lscc_docs/tutorials/rna_seq_dge_basic/rna_seq_basic_tutorial/

What is an alignment look like?

gatkforums.broadinstitute.org

Why is alignment a difficult problem?

- 100's of millions of reads
- Billions of bases to search through
- Approximate matching
- Looking for a tiny pattern (~100-120 bp read) in a large, often redundant sequence.

Basic steps of mapping reads

- Pre-mapping QC
- Build a reference sequence index.
- Map sequencing reads to the reference index.
- Convert results to SAM/BAM format and obtain mapping statistics.
- Post-mapping analysis.

What will your reads look like?

FASTQ FORMAT

```
@HWI-EAS216_91209:1:2:454:192#0/1
GTTGATGAATTTCTCCAGCGCGAATTTGTGGGCT
+HWI-EAS216_91209:1:2:454:192#0/1
B@BBBBBB@BBBBAAAA>@AABA?BBBAAB??>A?
```

Line 1: @read name

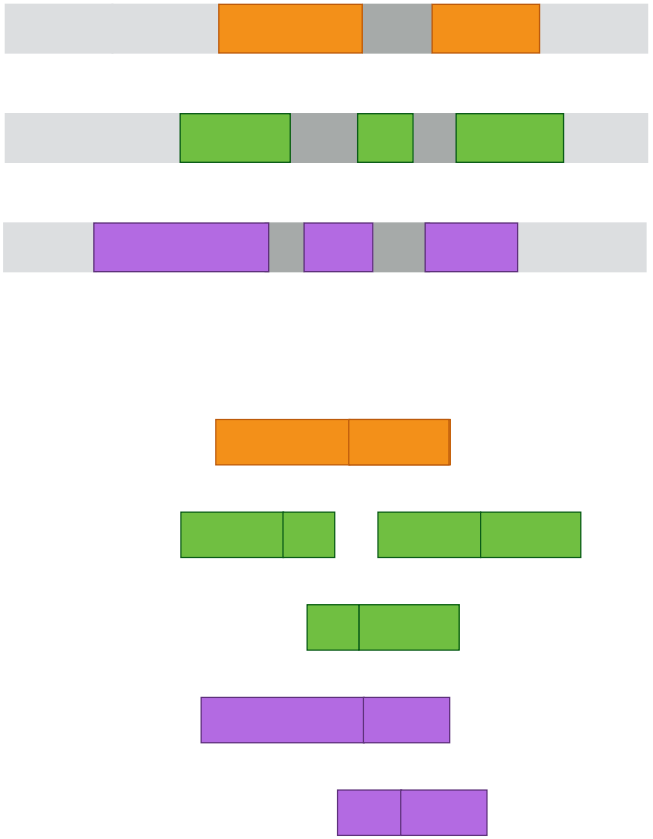
Line 2: called base sequence

Line 3: +read name (optional after +)

Line 4: base quality scores

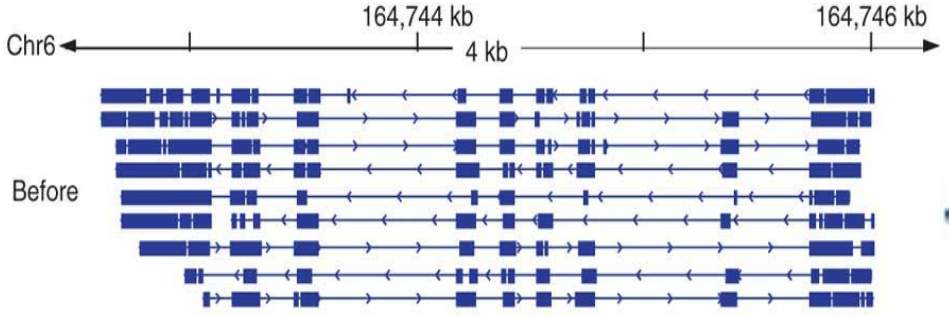
What does the reference look like?

- **Genome:** All the DNA of an individual, organized by chromosome, containing non-coding and coding regions.
- **Transcriptome**: All the gene isoforms. No non-coding sequences.



What to know about your reference before mapping?

- Mapping to genome vs transcriptome?



- Is your reference the right version?
- Does your annotation match your reference?

What will your reference look like?

- FASTA Format

  >gi|254160123|ref|NC_012967.1| Escherichia coli B str. REL606
  agcttttcattctgactgcaacgggcaatatgtctctgtgtggattaaaaaaagagtgtc
  tgatagcagcttctgaactggttacctgccgtgagtaaattaaaattttattgacttagg

tcactaaatactttaaccaatataggcatagcgcacagacagataaaaattacagagtac

acaacatccatgaaacgcattagcaccaccattaccaccaccatcaccattaccacaggt

  ....

- Using complex reference sequence names is a common problem during analysis. Might rename:

  >REL606
  agcttttcattctgactgcaacgggcaatatgtctctgtgtggattaaaaaaagagtgtc
  tgatagcagcttctgaactggttacctgccgtgagtaaattaaaattttattgacttagg

What will your annotation look like?

- GFF3 Format
  - seqname - The name of the sequence.
  - source - The program that generated this feature.
  - feature – Examples: "CDS", "start_codon", "stop_codon", and "exon".
  - start - The starting position of the feature in the sequence.
  - end - The ending position of the feature (inclusive).
  - score - A score between 0 and 1000.
  - strand - Valid entries include '+', '-', or '.' (for don't know/don't care).
  - Frame – reading frame
  - group – ID and other information about the entry

  Example:

  Rel606 refseq cds 1450 1540 500 + . Gene_id=« test_gene »

- Make sure the GFF3 file matches your reference fasta file.

Where to get your references?

- Ensembl ftp
- UCSC
- Gencode
- Organism specific databases/websites.

First Step : Reference Indexing

- **Indexing**: Think of the index of the book.
- Break reference into substrings of K length (Kmers) and index all locations of the Kmer.

Reference: TTACTTTACG

ACG	6
ACT	3
CTT	4
TAC	2,7
TTA	1,6
TTT	5

Read:CTTAAC

Find the prefix and extend the alignment

Indexing

- Many different ways to represent reference indexes

hash table

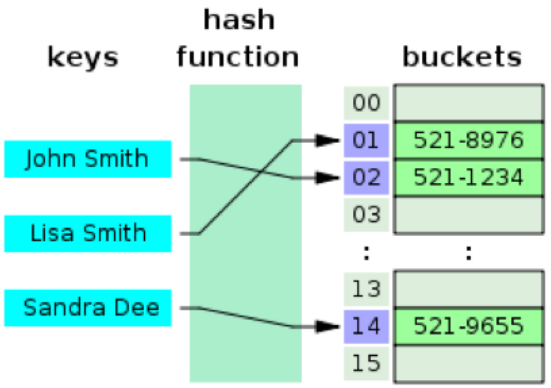


image from wikipedia

suffix array

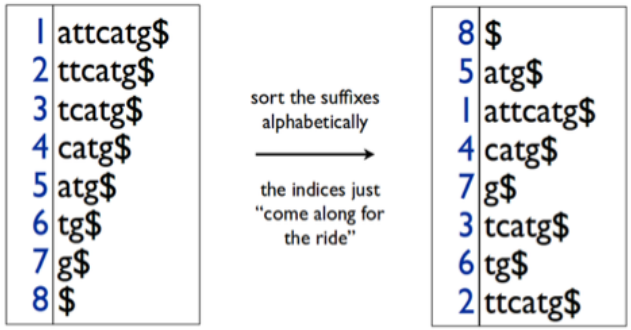


image from discuss.codechef.com

suffix tree

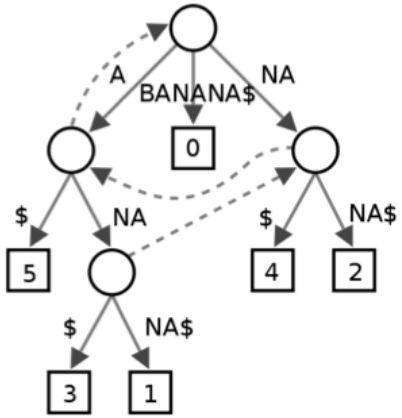


image from wikipedia

Types of Mappers

Class	Category	Package	Notes	Uses
Read mapping				
Unspliced aligners[a]	Seed methods	Short-read mapping package (SHRiMP)[41]	Smith-Waterman extension	Aligning reads to a reference transcriptome
		Stampy[39]	Probabilistic model	
	Burrows-Wheeler transform methods	Bowtie[43]		
		BWA[44]	Incorporates quality scores	
Spliced aligners	Exon-first methods	MapSplice[52]	Works with multiple unspliced aligners	Aligning reads to a reference genome. Allows for the identification of novel splice junctions
		SpliceMap[50]		
		TopHat[51]	Uses Bowtie alignments	
	Seed-extend methods	GSNAP[53]	Can use SNP databases	
		QPALMA[54]	Smith-Waterman for large gaps	

Figure :
Garber et al, Nature Methods, 2011

Unspliced Mapping

Class	Category	Package	Notes
Read mapping			
Unspliced aligners[a]	Seed methods	Short-read mapping package (SHRiMP)[41]	Smith-Waterman extension
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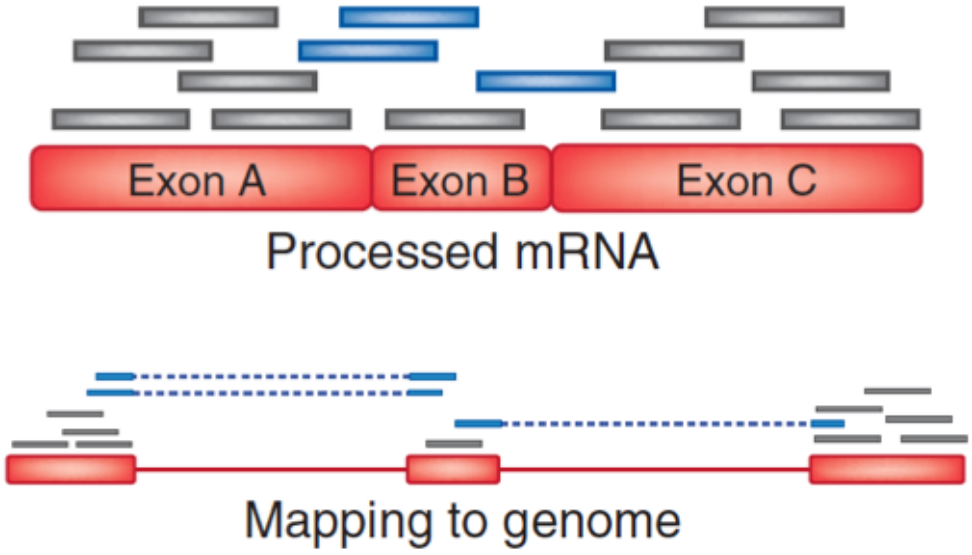
		BWA[44]	Incorporates quality scores

Figure :
Garber et al, Nature Methods, 2011

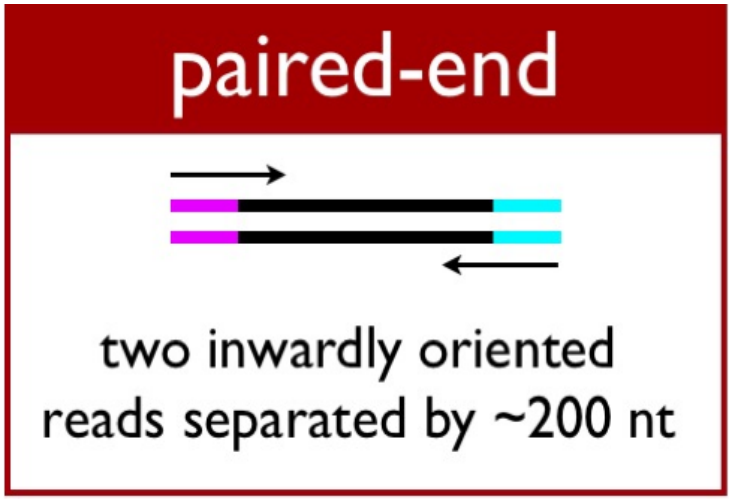
Spliced mapping

- Needed for quantifying and identifying splice variants from RNA Seq data.
- Tools:
  - HISAT2
  - STAR
  - Tophat
  - SpliceMap
  - MapSplice
  - RUM



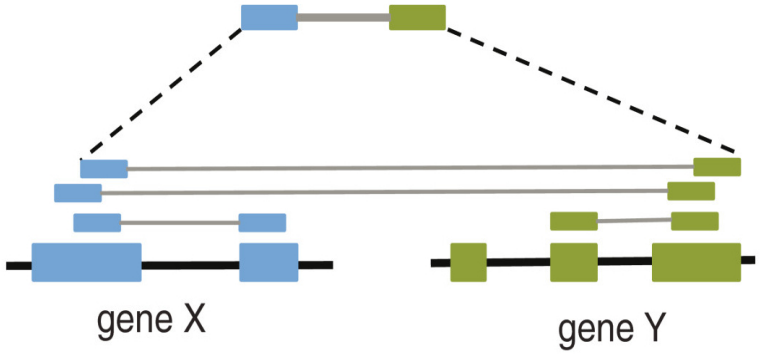
Trapnell, C. & Salzberg, S. L. How to map billions of short reads onto genomes. *Nature Biotech.* **27**, 455–457 (2009).

Paired end mapping



- Pairs map with expected insert size.
- One part of the pair, after mapping, is the anchor for the next read's mapping.

PAIRED END READ MAPPING IS VERY HELPFUL IN RNA SEQ!!



http://genomebiology.com/content/12/1/R6

Mapping Quality

- Mapping quality is the probability that a read is aligned to the wrong place.

$$p = 10^{**} (-q/10)$$

- BWA mapping quality calculated by considering:
  - Repeat structure of reference
  - Read alignment quality (mismatches etc)
  - Number of mappings
  - BWA will assign a mapping quality of 0 to reads that mapped equally well to multiple places

SAM file format

- Alignment results generated in Sequence Alignment/Map format
- Tab delimited, with fixed columns followed by user-extendable key:data values.
- Most mappers also output unmapped reads in SAM file.
- SAMTOOLS – toolkit to manipulate, parse SAM files.

SAM File Format

SAM fixed fields:

http://samtools.sourceforge.net/

Col	Field	Type	Regexp/Range	Brief description
1	QNAME	String	`[!-?A-~]{1,255}`	Query template NAME
2	FLAG	Int	$[0,2^{16}-1]$	bitwise FLAG
3	RNAME	String	`\*|[!-()+-<>-~][!-~]*`	Reference sequence NAME
4	POS	Int	$[0,2^{29}-1]$	1-based leftmost mapping POSition
5	MAPQ	Int	$[0,2^{8}-1]$	MAPping Quality
6	CIGAR	String	`\*|([0-9]+[MIDNSHPX=])+`	CIGAR string
7	RNEXT	String	`\*|=|[!-()+-<>-~][!-~]*`	Ref. name of the mate/next segment
8	PNEXT	Int	$[0,2^{29}-1]$	Position of the mate/next segment
9	TLEN	Int	$[-2^{29}+1,2^{29}-1]$	observed Template LENgth
10	SEQ	String	`\*|[A-Za-z=.]+`	segment SEQuence
11	QUAL	String	`[!-~]+`	ASCII of Phred-scaled base QUALity+33

SRR030257.264529 99 NC_012967 1521 29 34M2S = 1564
79 CTGGCCATTATCTCGGTGGTAGGACATGGCATGCCC
AAAAAA;AA;AAAAAA??A%.;?&'3735',()0*,
XT:A:M NM:i:3 SM:i:29 AM:i:29 XM:i:3 XO:i:0 XG:i:0 MD:Z:23T0G4T4

CIGAR score

```
Ref   CTGGCCATTATCTC--GGTGGTAGGACATGGCATGCCC!
Read        aaATGTCGCGGTG.TAGGAggatcc!
```

2S5M2I4M1D4M6S

	Op	BAM	Description
	M	0	alignment match (can be a sequence match or mismatch)
	I	1	insertion to the reference
	D	2	deletion from the reference
*	N	3	skipped region from the reference
	S	4	soft clipping (clipped sequences present in SEQ)
*	H	5	hard clipping (clipped sequences NOT present in SEQ)
*	P	6	padding (silent deletion from padded reference)
*	=	7	sequence match
*	X	8	sequence mismatch

*Rarer / newer

CIGAR = "Concise Idiosyncratic Gapped Alignment Report"

BAM format

- SAM files are converted to BAM format through SAMTOOLS command:
    - samtools view –b –S samfile > bamfile
- BAM file is binary format.
- BAM file is compressed.
- BAM files are usually what you need for post mapping analysis and visualization.

TAKEAWAYS

- Unspliced mapper-
  - Most suited for mapping to transcriptome
  - Example: BWA
- Spliced mapper-
  - Most suited for mapping to genome
  - Example: Hisat2, Star
- Mapping output
  - SAM File: tab-delimited file
  - Filter SAM file, Assess mapping stats

SAM FILE FLAGS EXPLAINED

QNAME SRR035022.2621862
FLAG 163

The QNAME is the query name. For the FLAG of 163 we transform this into a binary string: 10100011. So accordingly to the flag table:

Flag	Description
0x0001	the read is paired in sequencing, no matter whether it is mapped in a pair
0x0002	the read is mapped in a proper pair (depends on the protocol, normally inferred during alignment) [1]
0x0004	the query sequence itself is unmapped
0x0008	the mate is unmapped [1]
0x0010	strand of the query (0 for forward; 1 for reverse strand)
0x0020	strand of the mate [1]
0x0040	the read is the first read in a pair [1,2]
0x0080	the read is the second read in a pair [1,2]
0x0100	the alignment is not primary (a read having split hits may have multiple primary alignment records)
0x0200	the read fails platform/vendor quality checks
0x0400	the read is either a PCR duplicate or an optical duplicate

1 the read is paired in sequencing, no matter whether it is mapped in a pair
1 the read is mapped in a proper pair
0 not unmapped

0 mate is not unmapped
0 forward strand
1 mate strand is negative
0 the read is not the first read in a pair
1 the read is the second read in a pair