Variant calling algorithm

decision making based on
binomial distribution

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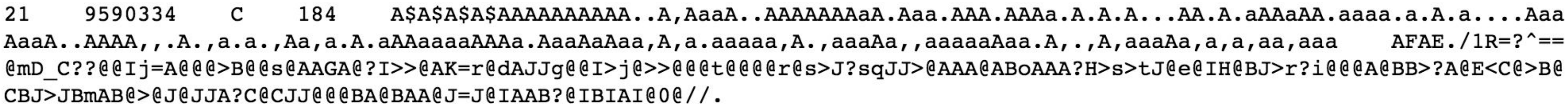
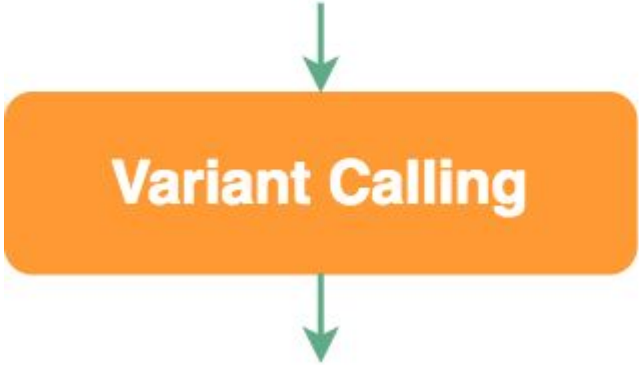
Variant Calling Algorithm

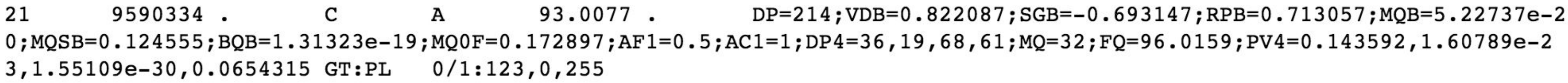


```
21    9590334    C    184    A$A$A$A$AAAAAAAAAA..A,AaaA..AAAAAAAaA.Aaa.AAA.AAAa.A.A.A...AA.A.aAAaAA.aaaa.a.A.a....Aaa
AaaA..AAAA,,.A.,a.a.,Aa,a.A.aAAaaaaAAAa.AaaAaAaa,A,a.aaaaa,A.,aaaAa,,aaaaaAaa.A,.,A,aaaAa,a,a,aa,aaa    AFAE./1R=?^==
@mD_C??@@Ij=A@@@>B@@s@AAGA@?I>>@AK=r@dAJJg@@I>j@>>@@@t@@@@r@s>J?sqJJ>@AAA@ABoAAA?H>s>tJ@e@IH@BJ>r?i@@@A@BB>?A@E<C@>B@
CBJ>JBmAB@>@J@JJA?C@CJJ@@@BA@BAA@J=J@IAAB?@IBIAI@0@//.
```

PILEUP FILE



VCF FILE



```
21       9590334 .        C       A       93.0077 .       DP=214;VDB=0.822087;SGB=-0.693147;RPB=0.713057;MQB=5.22737e-2
0;MQSB=0.124555;BQB=1.31323e-19;MQ0F=0.172897;AF1=0.5;AC1=1;DP4=36,19,68,61;MQ=32;FQ=96.0159;PV4=0.143592,1.60789e-2
3,1.55109e-30,0.0654315 GT:PL   0/1:123,0,255
```

Binomial distribution

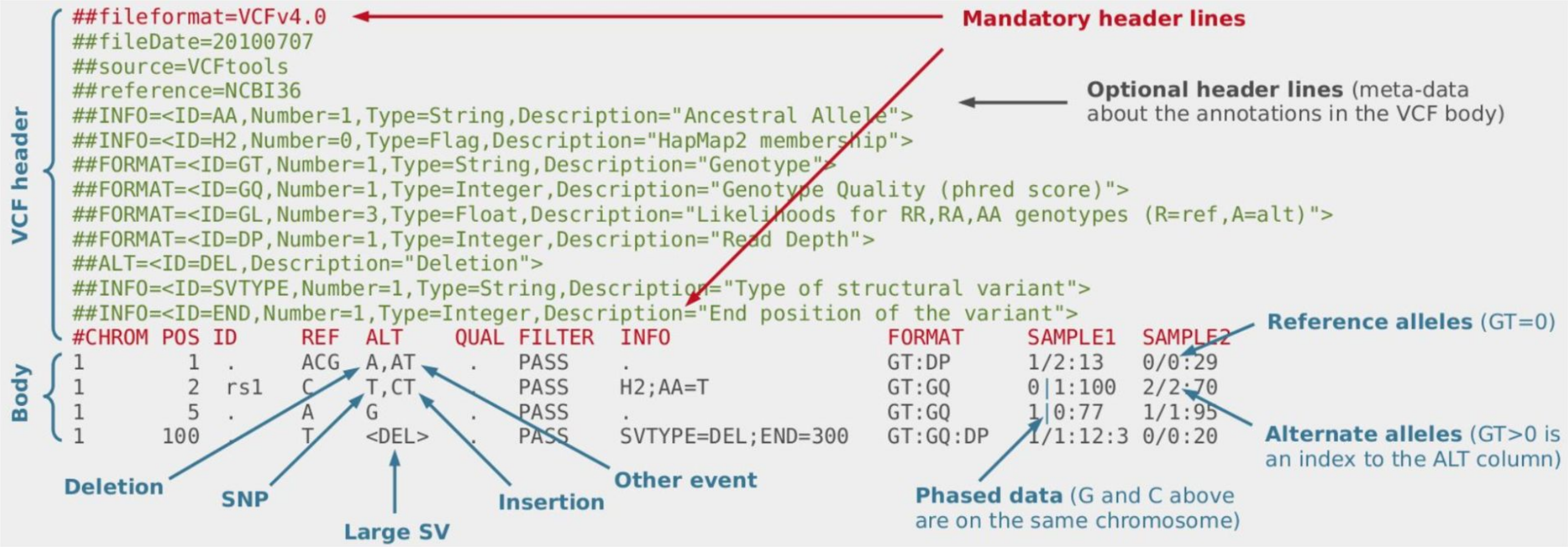
number of trials - total number of sequences with bases mapped to current position

$$f(k;n,p) = \Pr(X = k) = \binom{n}{k} p^k (1-p)^{n-k}$$

probability of success in a single trial

- calculate probabilities of each possible genotype
- choose the most probable one

Results ⟶ VCF format



VCF header

```
##fileformat=VCFv4.0
##fileDate=20100707
##source=VCFtools
##reference=NCBI36
##INFO=<ID=AA,Number=1,Type=String,Description="Ancestral Allele">
##INFO=<ID=H2,Number=0,Type=Flag,Description="HapMap2 membership">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality (phred score)">
##FORMAT=<ID=GL,Number=3,Type=Float,Description="Likelihoods for RR,RA,AA genotypes (R=ref,A=alt)">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##ALT=<ID=DEL,Description="Deletion">
##INFO=<ID=SVTYPE,Number=1,Type=String,Description="Type of structural variant">
##INFO=<ID=END,Number=1,Type=Integer,Description="End position of the variant">
#CHROM POS ID      REF  ALT     QUAL FILTER  INFO                 FORMAT     SAMPLE1    SAMPLE2
1        1 .       ACG  A,AT    .    PASS    .                    GT:DP      1/2:13     0/0:29
1        2 rs1     C    T,CT    .    PASS    H2;AA=T              GT:GQ      0|1:100    2/2:70
1        5 .       A    G       .    PASS    .                    GT:GQ      1|0:77     1/1:95
1      100 .       T    <DEL>   .    PASS    SVTYPE=DEL;END=300   GT:GQ:DP   1/1:12:3   0/0:20
```

Body

Mandatory header lines

Optional header lines (meta-data about the annotations in the VCF body)

Reference alleles (GT=0)

Alternate alleles (GT>0 is an index to the ALT column)

Deletion

SNP

Large SV

Insertion

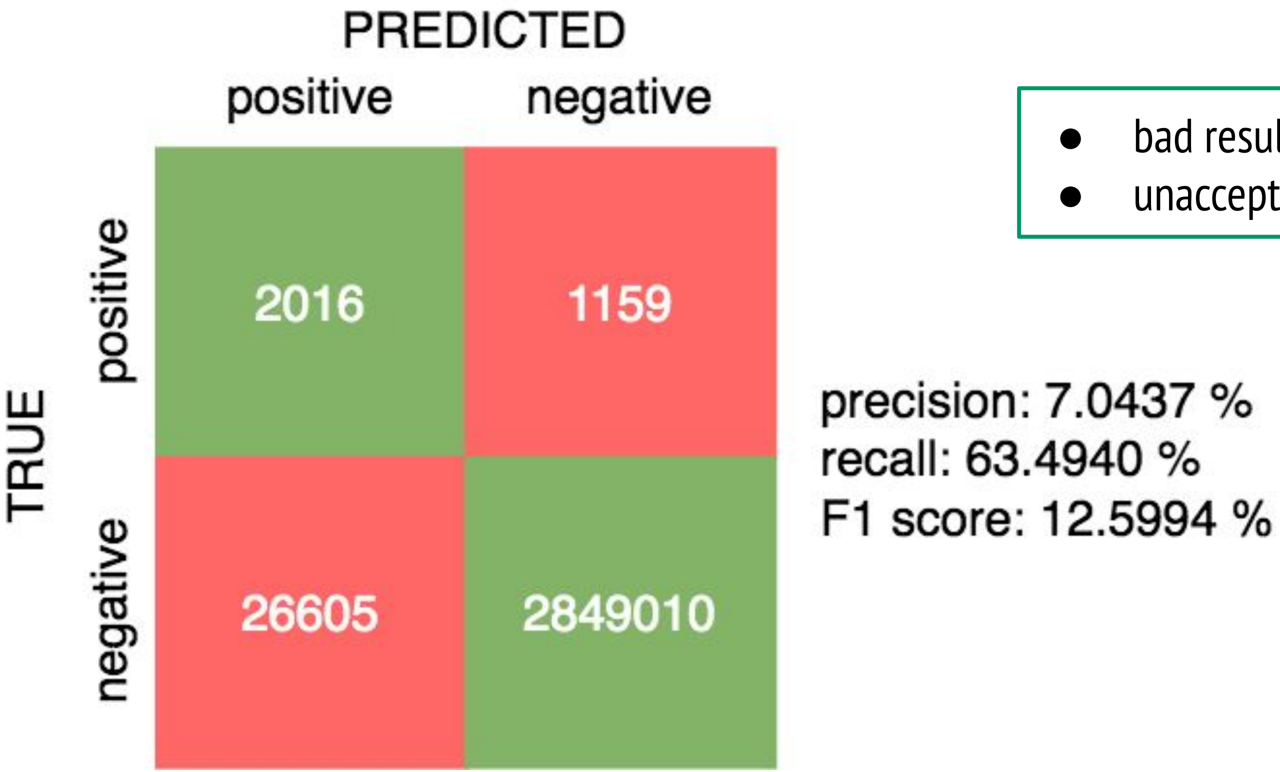
Other event

Phased data (G and C above are on the same chromosome)

Data

- Pileup file generated with samtools pileup tool from bam and reference file
    - bam file created from the exome portions of chromosomes 21, 22, Y and MT
    - fasta file used in 1000 genomes phase 3 project
- Test file for the purpose of comparing results generated with bcftools call tool

p = 0.9

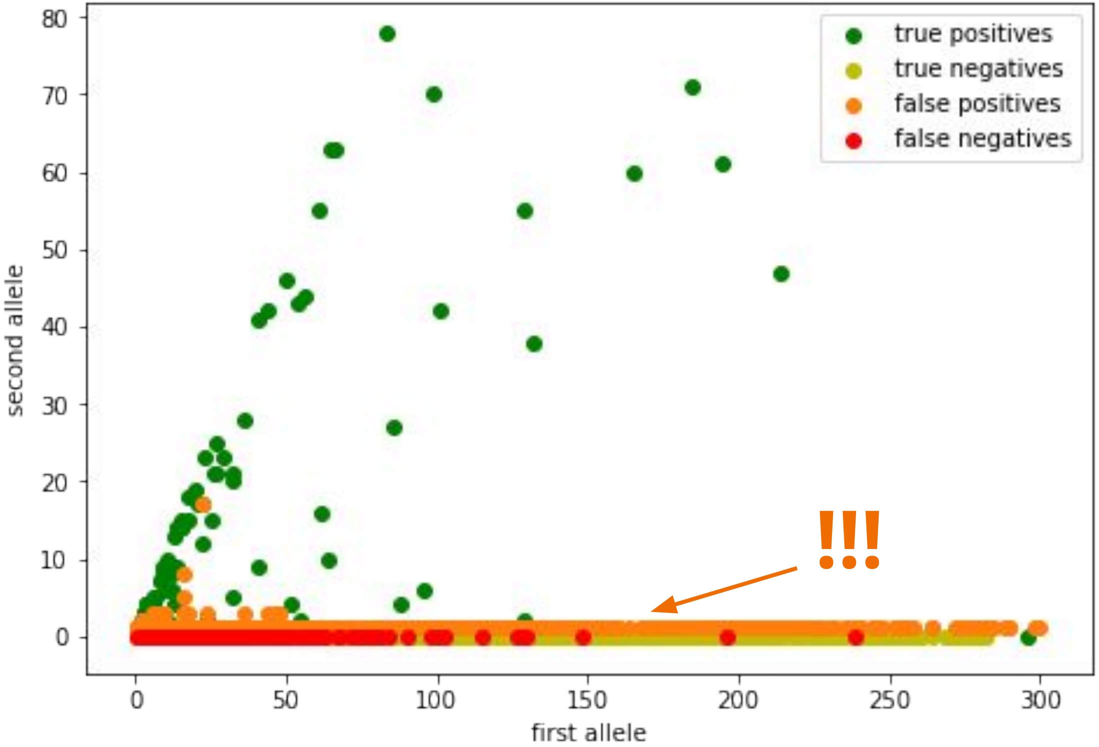


		PREDICTED	
		positive	negative
TRUE	positive	2016	1159
	negative	26605	2849010

precision: 7.0437 %
recall: 63.4940 %
F1 score: 12.5994 %

- bad results
- unacceptably low precision

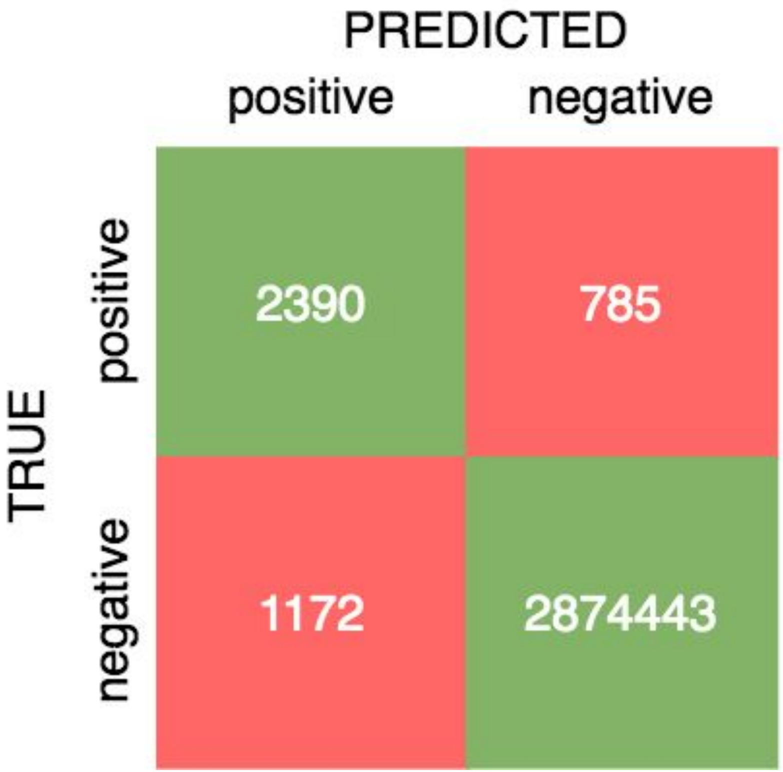
p = 0.9



true positives
true negatives
false positives
false negatives
second allele
first allele
0
10
20
30
40
50
60
70
80
0
50
100
150
200
250
300

!!!

p = 0.8

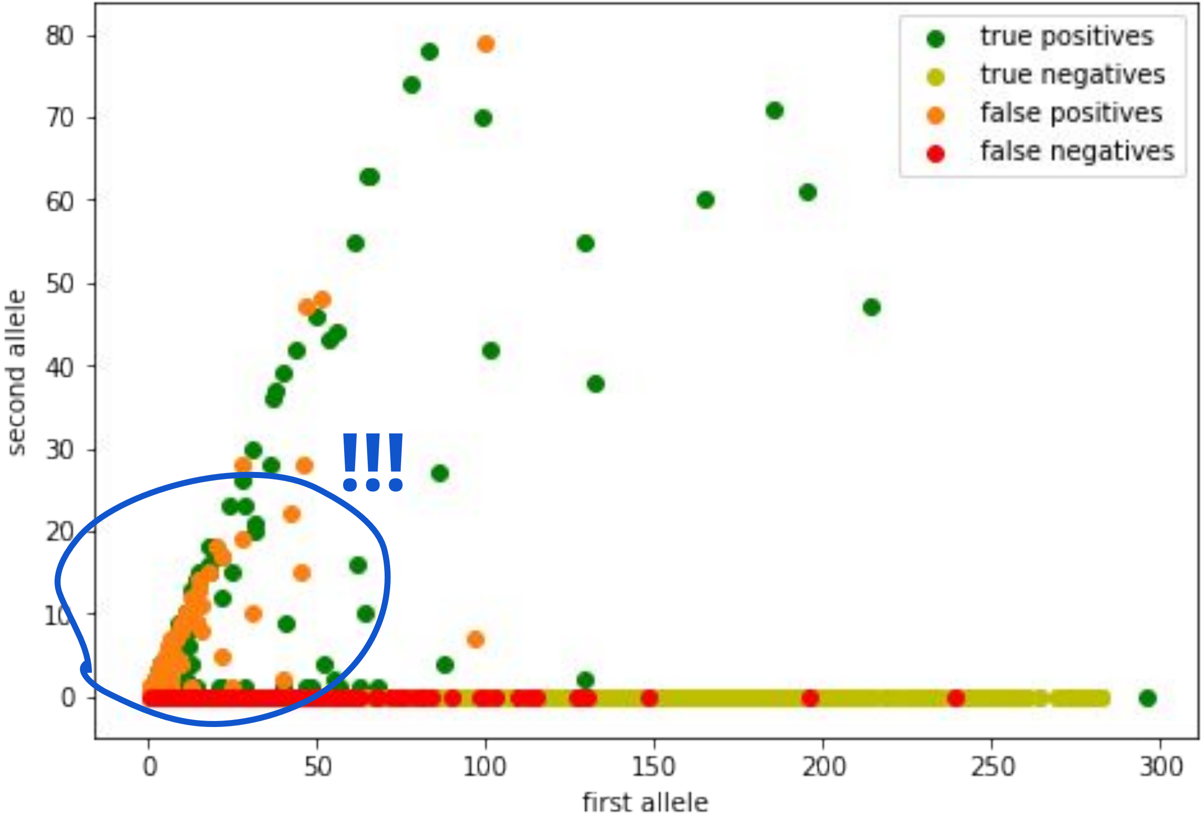


		PREDICTED	
		positive	negative
TRUE	positive	2390	785
	negative	1172	2874443

- much better
- number of good predictions increased, number of bad predictions decreased

precision: 67.0971 %
recall: 75.2756 %
F1 score: 70.9515 %
F2 score: 73.4842 %

p = 0.8



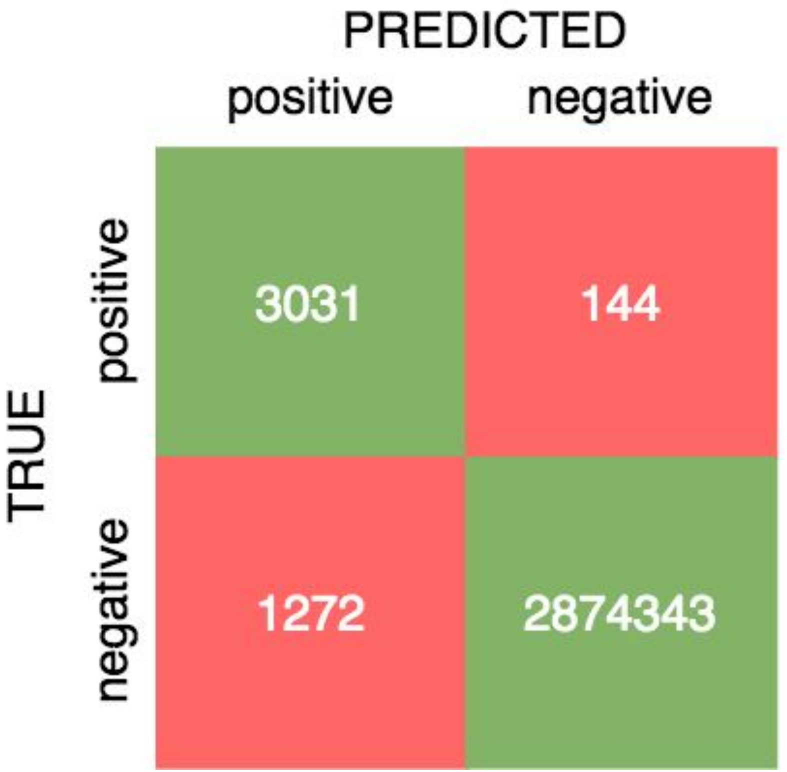
true positives
true negatives
false positives
false negatives
second allele
first allele
0
10
20
30
40
50
60
70
80
0
50
100
150
200
250
300
!!!

Modified p

- overlapping regions - existing information not enough
- Indels appear less frequently!

$$p = \begin{cases} 0.8, & \text{for SNVs and matches} \\ 0.6, & \text{for indels} \end{cases}$$

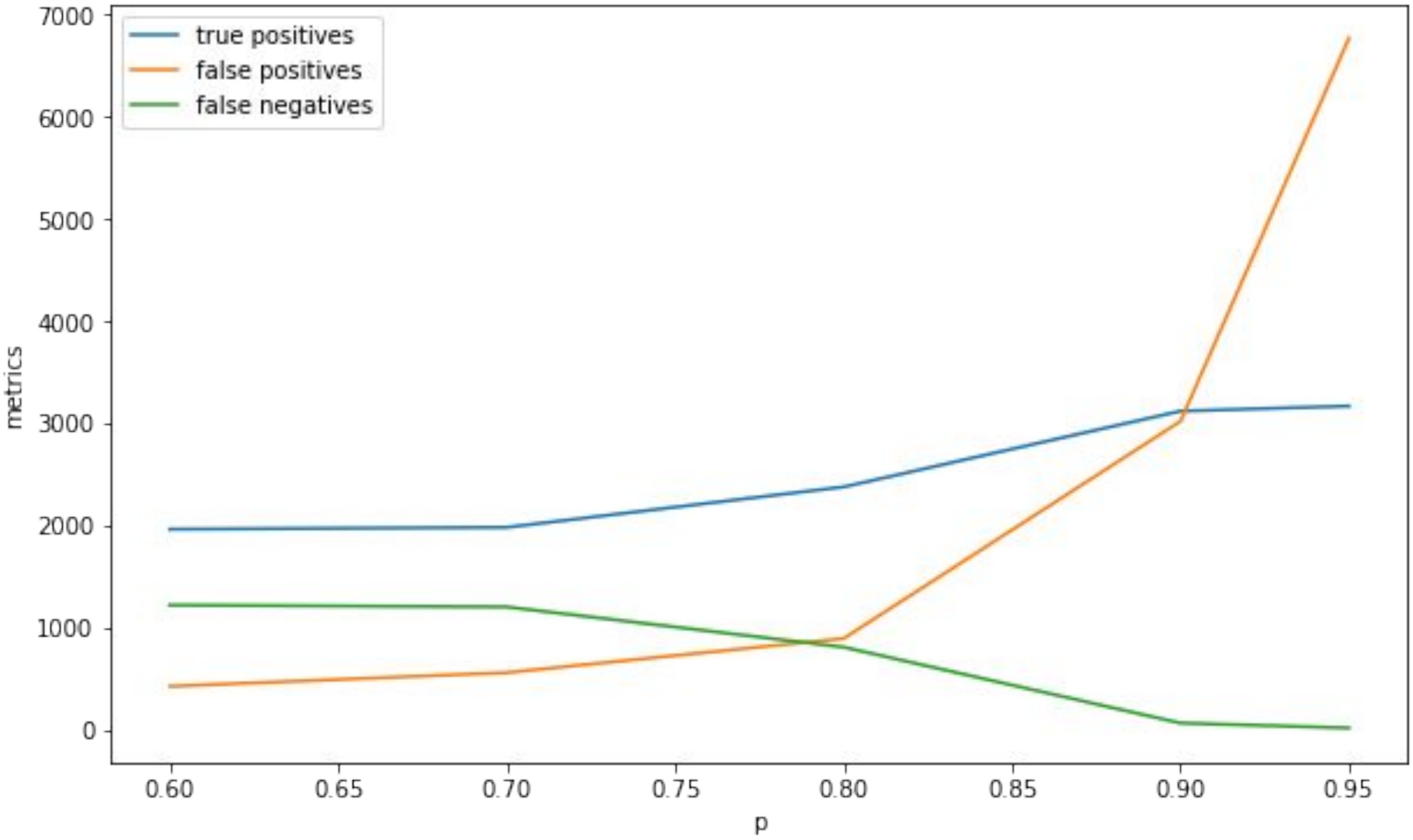
Modified p

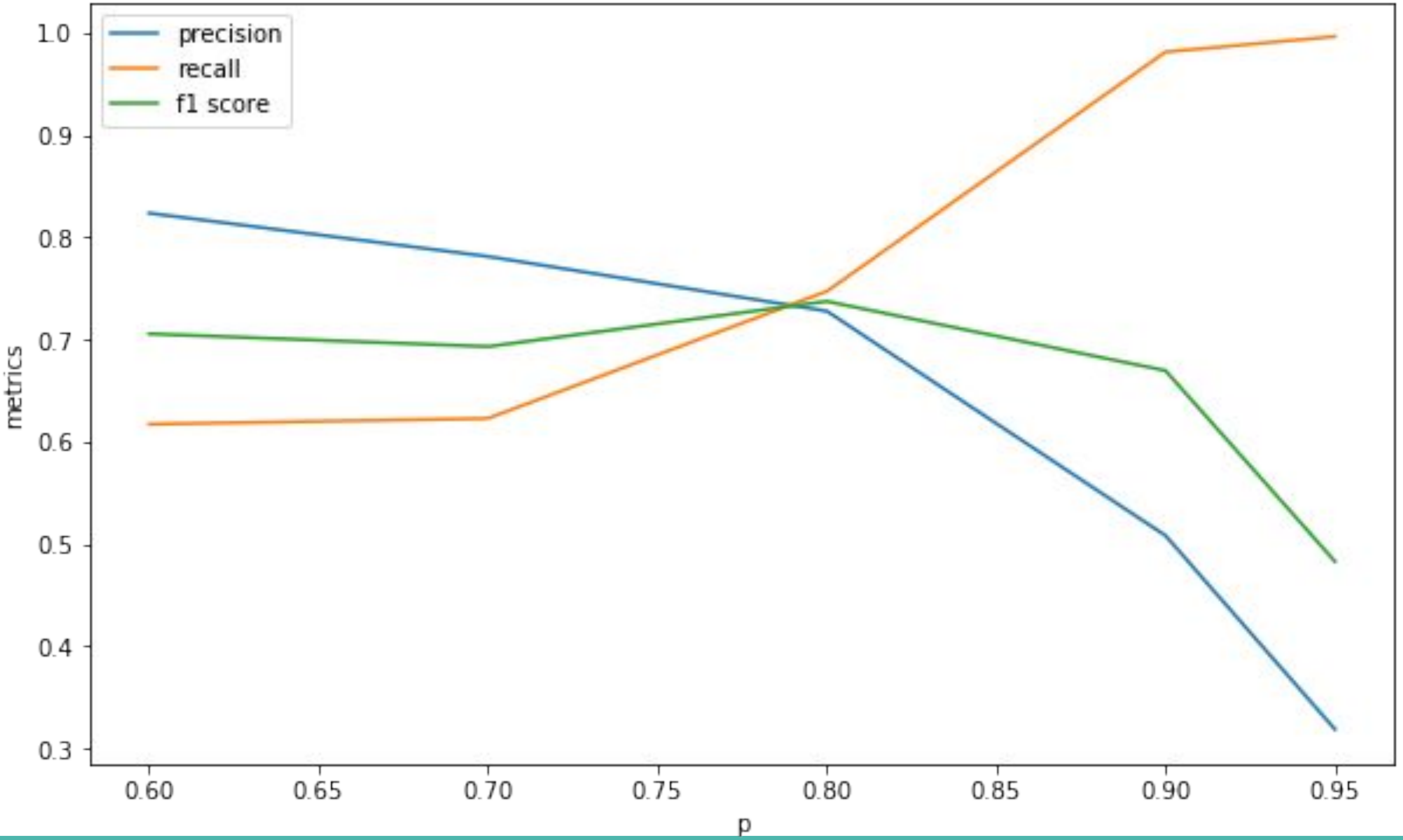


		PREDICTED	
		positive	negative
TRUE	positive	3031	144
	negative	1272	2874343

- Almost 96% of all mutations found!!!
- Low coverage reads - could probably improve with filtering

precision: 70.4392 %
recall: 95.4646 %
F1 score: 81.0645 %
F2 score: 84.3076 %


true positives
false positives
false negatives
metrics
p
0
1000
2000
3000
4000
5000
6000
7000
0.60
0.65
0.70
0.75
0.80
0.85
0.90
0.95


precision
recall
f1 score
metrics
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.60
0.65
0.70
0.75
0.80
0.85
0.90
0.95
p