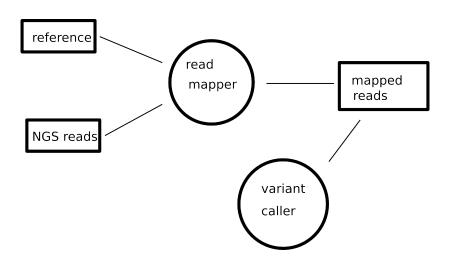
Pipeline overview



Read mapping

• Index reference genome (need to do it only once per reference)

tmap index -f reference.fasta

Read mapping

• Index reference genome (need to do it only once per reference)

```
tmap index -f reference.fasta
```

• Map reads against reference genome

```
tmap map3 -f reference.fasta \
-r reads.fastq \
-i fastq \
-o 2 \
-s mapped_reads.bam
```

BAM file

• Let's see what is inside the bam file samtools view mapped_reads.bam | head -n 20

SFHMR:0	3755:0203	9	4	ż	0	θ	*	ż	0	θ	GATACO	TCGTTG	ACCGA	TGACCC	CGGTTCA	GGCTTC	ACCAC#	AGTGTG	GAACGCG	GTCGTCT	CCGAA	TTAACG	GCGAC	CTAAGGT
TGACGAC	GGACCCAGC	AGTGATG	CTAATCTC	AGCGCTC	CGCTGACCC	CTCAGCA	AAGGGCTTG	GCTCAA	CCCBBBC	ABA;BAB;	BAAA???	?5? <d8< td=""><td>>6:/9</td><td>9/9=09</td><td>???AAAC</td><td>C<@<@B</td><td>A@9@:;</td><td>;;;AA9</td><td>=>96708</td><td><bb;@??< td=""><td>?A6;;4</td><td>1:;A9@<</td><td><<@D@6</td><td>444+5<=</td></bb;@??<></td></d8<>	>6:/9	9/9=09	???AAAC	C<@<@B	A@9@:;	;;;AA9	=>96708	<bb;@??< td=""><td>?A6;;4</td><td>1:;A9@<</td><td><<@D@6</td><td>444+5<=</td></bb;@??<>	?A6;;4	1:;A9@<	<<@D@6	444+5<=
=>?>>AA	??:8@??<8	:@D?4</td <td>::8;</td> <td>١.;;!</td> <td>5/59/==7A</td> <td>:0?000</td> <td>RG:Z:NO</td> <td>ID</td> <td>PG:Z:tm</td> <td>ар</td> <td></td>	::8;	١.;;!	5/59/==7A	:0?000	RG:Z:NO	ID	PG:Z:tm	ар														
SFHMR:0	1788:0254	3	16	gi 385	235550 re	f NC_01	7387.1	2594310	1	131S8M	*	θ	0		CAATGG	CTGGAC	GAGATI	FGAGCC	AAGCCCT	TTGCTGA	GGGGT(AGCGGA	GCGCT	AGATTAC
CATCACT	GCTGGGTCC	GTCGTCA	ACCTTAGO	GTCGCCG	TTAAGTTCG	GAGACGA	CCGCGTTCC	ACACTGTG	GTGAAG	>:BA;AA	A;B@@>@	@@8BBA	?;D <d< td=""><td>?8??7A</td><td>AABCCC81</td><td>DCCBBC</td><td>BGADDE</td><td>BBBBBC</td><td>CCC@BBC</td><td>AAA775=</td><td>=>7+54</td><td>126333</td><td>4661>9</td><td>@9A9)99</td></d<>	?8??7A	AABCCC81	DCCBBC	BGADDE	BBBBBC	CCC@BBC	AAA775=	=>7+54	126333	4661>9	@9A9)99
9>?9?A:	9199;??5A	4B99:C=	CCBC <h=a< td=""><td>AACA?>></td><td>:DCA<aa< td=""><td>RG:Z:N</td><td>OID</td><td>PG:Z:tm</td><td>ар</td><td>MD:Z:8</td><td>NM:1:6</td><td>AS:i</td><td>:8 X</td><td>A:Z:ma</td><td>p3-1</td><td>XS:i</td><td>:-2147</td><td>748364</td><td>7</td><td>XT:i:</td><td>1</td><td></td><td></td><td></td></aa<></td></h=a<>	AACA?>>	:DCA <aa< td=""><td>RG:Z:N</td><td>OID</td><td>PG:Z:tm</td><td>ар</td><td>MD:Z:8</td><td>NM:1:6</td><td>AS:i</td><td>:8 X</td><td>A:Z:ma</td><td>p3-1</td><td>XS:i</td><td>:-2147</td><td>748364</td><td>7</td><td>XT:i:</td><td>1</td><td></td><td></td><td></td></aa<>	RG:Z:N	OID	PG:Z:tm	ар	MD:Z:8	NM:1:6	AS:i	:8 X	A:Z:ma	p3-1	XS:i	:-2147	748364	7	XT:i:	1			
SFHMR:0	0676:0070	9	4	ż	0	θ	*	ż	0	θ	CGCATO	GCTCCG	CCGGC	GACCGA	CGAAGCC	GACGAC	ACTACO	CTGCC	CCCTTCC	GAAAATC	CTGCT	CCACAT	CGCCA	ACACCAC
AACCGAC	AACGACGAG	ATTGATG	ACAGCGCT	GCGGCAC	GGGGCGATA	??@@CA	AADC <bb<c< td=""><td>=BAAA=BB</td><td>B??:@B=B</td><td>CBAABBFB</td><td>BB<@@@</td><td>:>=7>:</td><td>C:BCD</td><td>C4CC<b< td=""><td>A: A9999</td><td>779?99</td><td>148==8</td><td>3>;@@@</td><td>:A9@@>></td><td>:>???@D</td><td>:90099</td><td>>>AAAA</td><td>A>??A</td><td>?9>>>@4</td></b<></td></bb<c<>	=BAAA=BB	B??:@B=B	CBAABBFB	BB<@@@	:>=7>:	C:BCD	C4CC <b< td=""><td>A: A9999</td><td>779?99</td><td>148==8</td><td>3>;@@@</td><td>:A9@@>></td><td>:>???@D</td><td>:90099</td><td>>>AAAA</td><td>A>??A</td><td>?9>>>@4</td></b<>	A: A9999	779?99	148==8	3>;@@@	:A9@@>>	:>???@D	:90099	>> AAAA	A>??A	?9>>>@4
B/??@@A	RG:Z:NOI)	PG:Z:tr	ар																				
SFHMR:0	1673:0249	5	4	±	0	θ	*	ż	0	θ	ACCGAC	GAAGCC	GACGA	CACTAC	CGTGCCG	CCTTCC	GAAAAT	CCTGC	TACCACA	TCGCCAG	ACACC/	CAACCG	ACAAC	ACGAGAT
TGATGAC	AGCGCTGCG	GCACGGG	GCGATAAC	CAGCACA	GTTGGCCAA	GTTACTT	C BB:AAB@	?8?@2996	@@:::???	:>>>A>DM	AJ>D>BA	AA7@?7	?CCBB	C;=///	///8BBBI	BCE8AC	D <b7>></b7>	EC<99	9=7:7<=	:AA>>@C	D?>?AE	BAC;<>	:===2	@@>G9A;
=>>??A@	A5;4;7=37	<2>?A;A	RG:Z:NO	ID	PG:Z:tm	ар																		
SFHMR:0	1579:0130	5	4	ż	0	θ	*	ż	0	θ	CTACCA	CATCGC	CAGAC	ACACAC	CACACAC	GAGCTT	- :;	=B576	;=95549	6666666	6/)//	/////5	55/	RG:Z:N
OID	PG:Z:tmap)																						
SFHMR:0	0598:0141	3	16	gi 385	235550 re	f NC_01	7387.1	2603163	1	136S13M	1D2M	*	0		θ	GGTG	TAGCGA	ACGGTT	AAGGCTG	GTTACGC	CAGCG	TAGCGG	AATCG	TATTGTO
CGGGCGC	TCGGTGAAG	TAACTTG	GCCAACTO	TGCTGGT	TATCGCCCC	GTGCCGC	AGCGCTGTC	ATCAATCT	CGTCGTTG	TCGGTTGT	GGTGTCT	GGC	C	>CCBBAI	BBBAA <a:< td=""><td>=C<b=c< td=""><td>BB=B=A</td><td>AAAC=D</td><td>FGD>EB@</td><td>@@@J?BA</td><td>B<bbb:< td=""><td>BAA???</td><td>9????</td><td>'?;@FD=0</td></bbb:<></td></b=c<></td></a:<>	=C <b=c< td=""><td>BB=B=A</td><td>AAAC=D</td><td>FGD>EB@</td><td>@@@J?BA</td><td>B<bbb:< td=""><td>BAA???</td><td>9????</td><td>'?;@FD=0</td></bbb:<></td></b=c<>	BB=B=A	AAAC=D	FGD>EB@	@@@J?BA	B <bbb:< td=""><td>BAA???</td><td>9????</td><td>'?;@FD=0</td></bbb:<>	BAA???	9????	'?;@FD=0
BB <ab<a< td=""><td><b=b<ccce< td=""><td>4>?B9?8</td><td>?@@AAAA6</td><td>ABCD:@A</td><td>FDBABBABB</td><td>BCEC>EI</td><td>CBBBDCC=C</td><td>CBA; A: AA</td><td>A<bbbbba< td=""><td><c< td=""><td>RG:Z:N</td><td>OID</td><td>P</td><td>G:Z:tm</td><td>ар</td><td>MD:Z</td><td>:13^A2</td><td>2</td><td>NM:i:1</td><td>AS:i:</td><td>B XA:</td><td>Z:map3</td><td>-1</td><td>XS:i:-</td></c<></td></bbbbba<></td></b=b<ccce<></td></ab<a<>	<b=b<ccce< td=""><td>4>?B9?8</td><td>?@@AAAA6</td><td>ABCD:@A</td><td>FDBABBABB</td><td>BCEC>EI</td><td>CBBBDCC=C</td><td>CBA; A: AA</td><td>A<bbbbba< td=""><td><c< td=""><td>RG:Z:N</td><td>OID</td><td>P</td><td>G:Z:tm</td><td>ар</td><td>MD:Z</td><td>:13^A2</td><td>2</td><td>NM:i:1</td><td>AS:i:</td><td>B XA:</td><td>Z:map3</td><td>-1</td><td>XS:i:-</td></c<></td></bbbbba<></td></b=b<ccce<>	4>?B9?8	?@@AAAA6	ABCD:@A	FDBABBABB	BCEC>EI	CBBBDCC=C	CBA; A: AA	A <bbbbba< td=""><td><c< td=""><td>RG:Z:N</td><td>OID</td><td>P</td><td>G:Z:tm</td><td>ар</td><td>MD:Z</td><td>:13^A2</td><td>2</td><td>NM:i:1</td><td>AS:i:</td><td>B XA:</td><td>Z:map3</td><td>-1</td><td>XS:i:-</td></c<></td></bbbbba<>	<c< td=""><td>RG:Z:N</td><td>OID</td><td>P</td><td>G:Z:tm</td><td>ар</td><td>MD:Z</td><td>:13^A2</td><td>2</td><td>NM:i:1</td><td>AS:i:</td><td>B XA:</td><td>Z:map3</td><td>-1</td><td>XS:i:-</td></c<>	RG:Z:N	OID	P	G:Z:tm	ар	MD:Z	:13^A2	2	NM:i:1	AS:i:	B XA:	Z:map3	-1	XS:i:-
2147483	647	(T:i:1																						
SFHMR:0	0701:0050	9	4	*	0	θ	*	*	0	θ	ACGACO	AGATTG	ATGAC	AGCGCT	GCGGCAC	GGGGCG	ATAACO	CAGCAC	AGTTGGC	CAAGTTA	CTTCA	CGAGCG	CCCGCA	CAATACO
GATTCCG	CTACCGCTG	GCGTAAC	CAGCCTTA	ACCGTCG	CTACACCTT	TGAT	??AAAC?	?@ <a?cac< td=""><td>@AA@BBBB</td><td>CB<bad?3< td=""><td>????@A#</td><td>;A;ABA</td><td>@@BCB</td><td>=B;B;@</td><td><a>9?=@</td><td>;ABA<a< td=""><td>@>>>?7</td><td>7@A9::</td><td>A<aaa=c< td=""><td>BC>C=CB</td><td>BCD?D(</td><td>B?9CC:</td><td>:9E>D0</td><td>:C>B=C>0</td></aaa=c<></td></a<></td></bad?3<></td></a?cac<>	@AA@BBBB	CB <bad?3< td=""><td>????@A#</td><td>;A;ABA</td><td>@@BCB</td><td>=B;B;@</td><td><a>9?=@</td><td>;ABA<a< td=""><td>@>>>?7</td><td>7@A9::</td><td>A<aaa=c< td=""><td>BC>C=CB</td><td>BCD?D(</td><td>B?9CC:</td><td>:9E>D0</td><td>:C>B=C>0</td></aaa=c<></td></a<></td></bad?3<>	????@A#	;A;ABA	@@BCB	=B;B;@	<a>9?=@	;ABA <a< td=""><td>@>>>?7</td><td>7@A9::</td><td>A<aaa=c< td=""><td>BC>C=CB</td><td>BCD?D(</td><td>B?9CC:</td><td>:9E>D0</td><td>:C>B=C>0</td></aaa=c<></td></a<>	@>>>?7	7@A9::	A <aaa=c< td=""><td>BC>C=CB</td><td>BCD?D(</td><td>B?9CC:</td><td>:9E>D0</td><td>:C>B=C>0</td></aaa=c<>	BC>C=CB	BCD?D(B?9CC:	:9E>D0	:C>B=C>0
@???@BA	AABB;@9@@	Agg	RG:Z:NO	ID	PG:Z:tm	ар																		
	2998:0258		16		235550 re				68	109S19M		θ	0						AGGCTGG					
	CGGTGAAGT					TGCCGCA	GCGCTGTCA	TCA	=CCDDD<	B <bbbbba< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>5:BBBB</td><td></td><td></td><td>009>8</td><td>86B:?8</td><td>)AAA9(</td><td>)<8<8A9)</td></bbbbba<>								5:BBBB			009>8	86B:?8)AAA9()<8<8A9)
=::::-?	AAD <d@@aai< td=""><td>>A)<>>6</td><td><@:</td><td>RG:Z:N</td><td>DID</td><td>PG:Z:ti</td><td>map</td><td>MD:Z:13</td><td>A3G1</td><td>NM:i:2</td><td></td><td></td><td></td><td></td><td>XS:i:-</td><td></td><td></td><td></td><td>XT:i:1</td><td></td><td></td><td></td><td></td><td></td></d@@aai<>	>A)<>>6	<@:	RG:Z:N	DID	PG:Z:ti	map	MD:Z:13	A3G1	NM:i:2					XS:i:-				XT:i:1					
SFHMR:0	1284:0029	9	4	*	0	0	*	ż	0	θ	CCCCCT	GGTTCC	ATCTG	GGGCGA	GTCCGGT	CCGTCG	GCAAGA	ACACAC	CTGCTGA	CACGCGG	CAGGC	ATCCTA	TGCCCA	ACGGTTC
TTCCCGG	GAATCGCAG	ũ	(33333-	3/<8::;	;8,868696	63'-(2(6	89:;///6	::8<8888	87><656/	2663//(//><>4	BA8<:	46/665	6*57*>>	75////	/)/ RO	5:Z:NO	ID	PG:Z:	tmap			
SFHMR:0	3138:0298	9	4	*	0	θ	*	*	0	θ	GGTCTC	GGCAAG	ACACA	CCTGCT	ACACGCG	GCAGGC	AACTAT	TGCCCA	ACGGTTG	TTCCCGG	GAATG	AGGTCA	AATAT	TCTCCAC
	TTCACCAAC									E>EDBBB			??ACB	B?@@B=I	BBCAFD@	CCBCBB	C <b=??< td=""><td>?9?<@@</td><td>;BB:BB:</td><td>B=ECAAC</td><td>=C>=>!</td><td>>>>>?</td><td>>=8>B9</td><td>?@@;A3;</td></b=??<>	?9?<@@	;BB:BB:	B=ECAAC	=C>=>!	>>>>?	>=8>B9	?@@;A3;
3;96171	11-2BB	<c<bbcd< td=""><td>MCC@BEDD</td><td></td><td>A<a<bbaab< td=""><td>C=AAA9A</td><td>A???AB??9</td><td>0</td><td>RG:Z:NO</td><td>ID</td><td>PG:Z:t</td><td>map</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></a<bbaab<></td></c<bbcd<>	MCC@BEDD		A <a<bbaab< td=""><td>C=AAA9A</td><td>A???AB??9</td><td>0</td><td>RG:Z:NO</td><td>ID</td><td>PG:Z:t</td><td>map</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></a<bbaab<>	C=AAA9A	A???AB??9	0	RG:Z:NO	ID	PG:Z:t	map												
	2085:0198		4	*	0	θ	*	*	0	θ					CGCATTC									
	GAGGGTATT								>=???9@	@??776:2	:::6=6<	<;7659	;;2D@	@@@BB?	00:0;;;	A?>?@>	>>??86	661/4-	98888:>	>A@=;@7	E=A;::	IA?9>7>	=4????	'5A959=0
	@@:AB;B;CI								PG:Z:tm															
	0470:0044		0		235550 re					18M123S		θ	0						GGAGACC					
	CTCGCGGTC																						9>A??	'BBF <aab< td=""></aab<>
	BBB <abbbbi< td=""><td></td><td>?>?B=B=8</td><td></td><td></td><td></td><td></td><td>PG:Z:tm</td><td>ар</td><td>MD:Z:1T</td><td></td><td></td><td></td><td></td><td>XA:Z:m</td><td></td><td></td><td></td><td>1474836</td><td></td><td></td><td>i:1</td><td></td><td></td></abbbbi<>		?>?B=B=8					PG:Z:tm	ар	MD:Z:1T					XA:Z:m				1474836			i:1		
	3248:0165		4	*	0	0	*	*	0	θ					GTATCGA									
	AACACCAAT														@@AAABB	?BB@@:	B9::=E	BBDE=E	CCCBBB<	CBBCDA@	<<<27	1:AAA<	:CAAA<	CCACBBC
	C7AAB <a;></a;>										RG:Z:N			G:Z:tm										
	2004:0135		16		235550 re					130S13M		θ	0						TATTCGG					
	TGTTGGCGT														B@;::3?									
99===>7	51@?AA?7>:	>C>@:C?	CAA>AB:E	A=<<73:	2?>>/=???	;@B <ccc< td=""><td>C=C</td><td>RG:Z:NO</td><td>ID</td><td>PG:Z:tm</td><td>ар</td><td>MD:Z</td><td>:13 N</td><td>M:i:0</td><td>AS:1:1</td><td>3 XA:7</td><td>:mup3-</td><td>-1⊴ ∃</td><td>X9:i:-</td><td>2147483</td><td>547</td><td>≣ χ</td><td>T:in</td><td>00</td></ccc<>	C=C	RG:Z:NO	ID	PG:Z:tm	ар	MD:Z	:13 N	M:i:0	AS:1:1	3 XA:7	:mup3-	-1⊴ ∃	X9:i:-	2147483	547	≣ χ	T:in	00

BAM file format The header

Let's look at the bam header

samtools view -H mapped_reads.bam

BAM file header modifying

• Let's change the header so that it includes our sample name!

```
samtools view -H mapped_reads.bam \
| sed 's/SM:NOSM/SM:Sample1/' \
| samtools reheader - mapped_reads.bam \
> mapped_reads.reheaded.bam
```

Variant calling

Variant callers need sorted bam file

```
samtools sort mapped_reads.reheaded.bam \
mapped_reads.reheaded.sorted
```

Variant calling

```
samtools mpileup -uf reference.fasta \
mapped_reads.reheaded.sorted.bam \
| bcftools call -cv \
| vcfutils.pl varFilter > variants.samtools.vcf
```

Variant call format (VCF)

```
##fileformat=VCEv4 2
##FTLTER=<TD=PASS_Description="All filters_passed">
##samtoolsVersion=1.0-11-geeb4b22+htslib-1.0-2-g1903fd4
##samtoolsCommand=samtools mpileup -uf reference.fasta mapped reads.sorted.bam
##reference=file://reference.fasta
##contig=<ID=gi|385235550|ref|NC 017387.1|.length=4138388>
##ALT=<TD=X.Description="Represents allele(s) other than observed.">
##INFO=<ID=INDEL.Number=0.Type=Flag.Description="Indicates that the variant is an INDEL.">
##INFO=<ID=IDV,Number=1,Type=Integer,Description="Maximum number of reads supporting an indel">
##INFO=<ID=IMF.Number=1.Type=Float.Description="Maximum fraction of reads supporting an indel">
##INFO=<ID=DP.Number=1.Type=Integer.Description="Raw read depth">
##INFO=<ID=VDB,Number=1,Type=Float,Description="Variant Distance Bias for filtering splice-site artefacts in RNA-seq data (biager is better)". Version=3>
##INFO=<ID=RPB.Number=1.Type=Float,Description="Mann-Whitney U test of Read Position Bias (bigger is better)">
##INFO=<ID=MQB,Number=1,Type=Float,Description="Mann-Whitney U test of Mapping Quality Bias (bigger is better)">
##INFO=<ID=BOB.Number=1.Type=Float.Description="Mann-Whitney U test of Base Quality Bias (bigger is better)">
##INFO=<ID=MOSB,Number=1,Type=Float,Description="Mann-Whitney U test of Mapping Quality vs Strand Bias (bigger is better)">
##INFO=<ID=SGB.Number=1.Type=Float.Description="Segregation based metric.">
##INFO=<ID=MOOF.Number=1.Type=Float.Description="Fraction of MOO reads (smaller is better)">
##FORMAT=<ID=PL.Number=G.Type=Integer.Description="List of Phred-scaled genotype likelihoods">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##INFO=<ID=AF1.Number=1.Type=Float.Description="Max-likelihood estimate of the first ALT allele frequency (assuming HWE)">
##INFO=<ID=AF2.Number=1.Type=Float.Description="Max-likelihood estimate of the first and second group ALT allele frequency (assuming HWE)">
##INFO=<ID=AC1, Number=1, Type=Float, Description="Max-likelihood estimate of the first ALT allele count (no HWE assumption)">
##INFO=<ID=MO.Number=1.Type=Integer.Description="Root-mean-square mapping quality of covering reads">
##INFO=<ID=F0.Number=1.Type=Float.Description="Phred probability of all samples being the same">
##INFO=<ID=PV4.Number=4.Type=Float.Description="P-values for strand bias, base0 bias, map0 bias and tail distance bias">
##INFO=<ID=G3.Number=3.Type=Float.Description="ML estimate of genotype frequencies">
##INFO=<ID=HWE.Number=1.Type=Float.Description="Chi^2 based HWE test P-value based on G3">
##INFO=<ID=DP4.Number=4.Type=Integer.Description="Number of high-quality ref-forward , ref-reverse, alt-forward and alt-reverse bases">
##bcftools_callVersion=1.0-26-g53fcd5c+htslib-1.0-6-g546bfcb
##bcftools callCommand=call -cv
#CHROM POS ID REF ALT QUAL FILTER INFO FORMAT NOSM
gi|385235550|ref|NC 017387.1| 5461 . T G 20.8005 . DP=4:VDB=0.14:SGB=-0.453602:MO0F=0:AF1=1:AC1=2:DP4=0.0.0.2:MO=60:FO=-32.988 GT:PL 1/1:52.6.0
gt 385235550 ref NC 017387.1 14453 . A C 20.8005 . DP=2; VDB=0.02; SGB=-0.453602; MO0F=0; AF1=1; AC1=2; DP4=0.0, 0, 2; MO=47; FO=-32.988 GT:PL 1/1:52,6,0
01/385235550 refixC 017387.1 51777 . TAA T 14.1422 . INDEL:IDV=3:IMF=0.75:DP=4:VDB=0.0381671:SGB=-0.556411:MO0F=0:AF1=1:AC1=2:DP4=0.0.0.4:MO=25:FO=-46.5
1:54.12.0
gi|385235550|ref|NC_017387.1| 69668 . G C 17.8363 . DP=4;VDB=0.02;SGB=-0.453602;MQ0F=0;AF1=1;AC1=2;DP4=0,0,0,2;MQ=60;FQ=-32.988 GT:PL 1/1:49,6,0
gi 385235559 ref NC 017387.1 108655 . G C 3.01703 . DP=4:VDB=0.1:5GB=-0.453602:RPB=0.5:MOB=0.75:BOB=0.5:MO0F=0.25:AF1=0.500542:AC1=1:DP4=0.2.0.2:MO=45:F1
=1.0.352957.1.1 GT:PL 0/1:30.0.25
```

Summary of used commands

Reference genome indexing

```
tmap index -f reference.fasta
```

• Read mapping against reference genome

```
tmap map3 -f reference.fasta \
-r reads.fastq \
-i fastq \
-o 2 \
-s mapped_reads.bam
```

Bam header modifying

```
samtools view -H mapped_reads.bam \
| sed 's/SM:NOSM/SM:Sample1/' \
| samtools reheader - mapped_reads.bam \
> mapped reads.reheaded.bam
```

Mapped read sorting
 samtools sort mapped_reads.reheaded.bam \
mapped_reads.reheaded.sorted

Variant calling

```
samtools mpileup -uf reference.fasta \
mapped_reads.reheaded.sorted.bam \
| bcftools call -cv \
| vcfutils.pl varFilter > variants.samtools.vcf
```

Let's build pipeline!

```
tmap map3 -f reference.fasta \
-r reads.fastq \
-i fastq \
-o 2 \
-s mapped reads.bam
samtools view -H mapped reads.bam \
| sed 's/SM:NOSM/SM:Sample1/' \
  samtools reheader - mapped reads.bam \
> mapped reads.reheaded.bam
samtools sort mapped_reads.reheaded.bam \
mapped_reads.reheaded.sorted
samtools mpileup -uf reference.fasta \
mapped_reads.reheaded.sorted.bam \
| bcftools call -cv \
| vcfutils.pl varFilter > variants.samtools.vcf
```