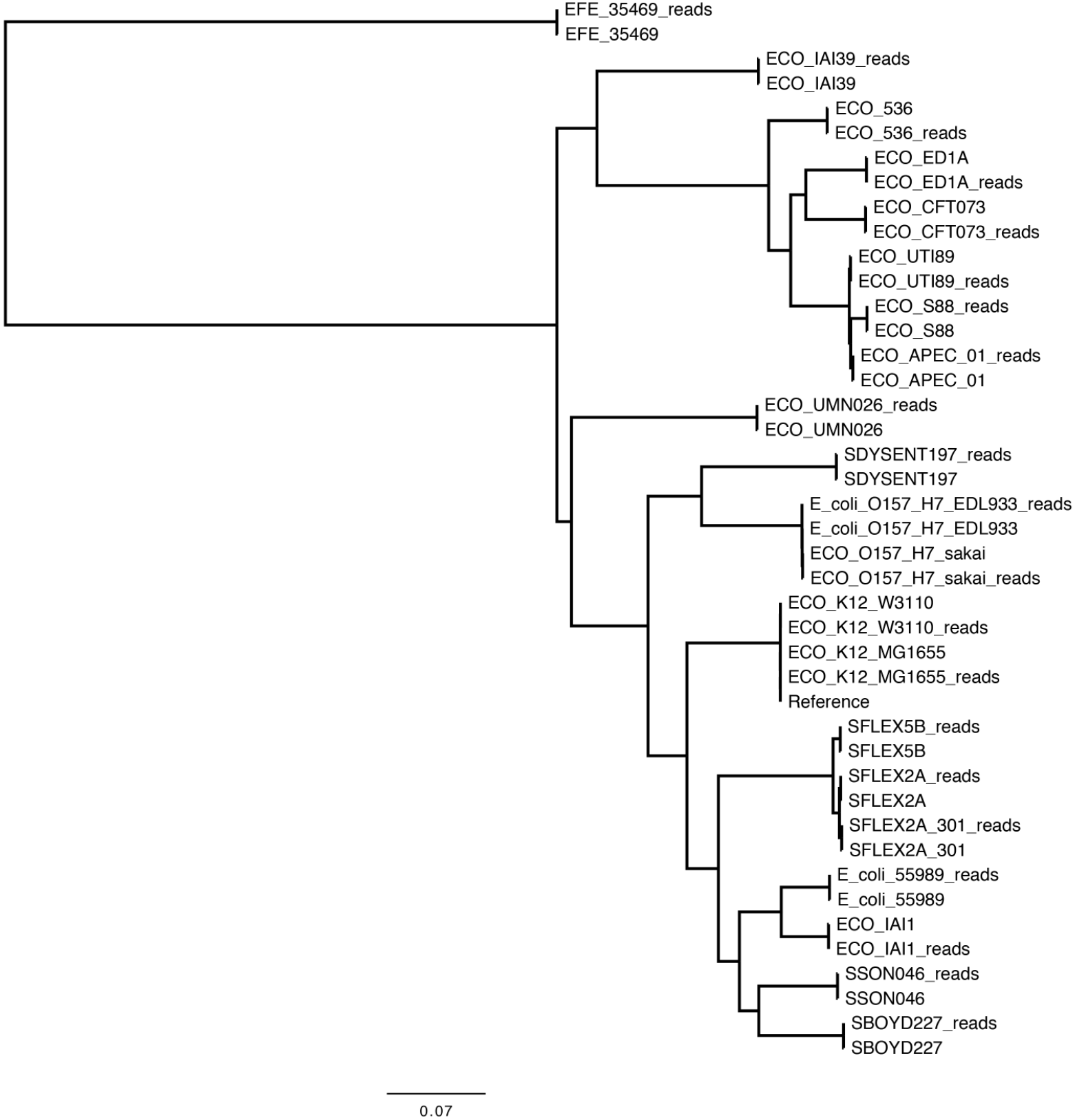
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ECO_CFT073
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ECO_UTI89
ECO_UTI89_reads
ECO_S88_reads
ECO_S88
ECO_APEC_01_reads
ECO_APEC_01
ECO_UMN026_reads
ECO_UMN026
SDYSENT197_reads
SDYSENT197
E_coli_O157_H7_EDL933_reads
E_coli_O157_H7_EDL933
ECO_O157_H7_sakai
ECO_O157_H7_sakai_reads
ECO_K12_W3110
ECO_K12_W3110_reads
ECO_K12_MG1655
ECO_K12_MG1655_reads
Reference
SFLEX5B_reads
SFLEX5B
SFLEX2A_reads
SFLEX2A
SFLEX2A_301_reads
SFLEX2A_301
E_coli_55989_reads
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SBOYD227
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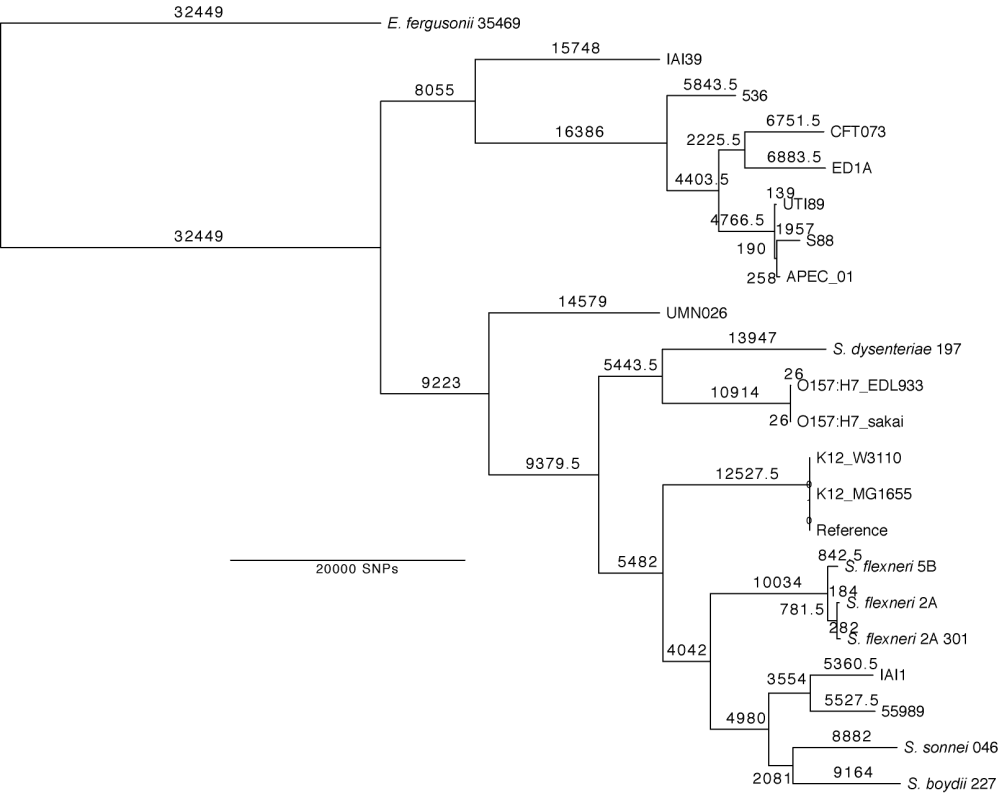
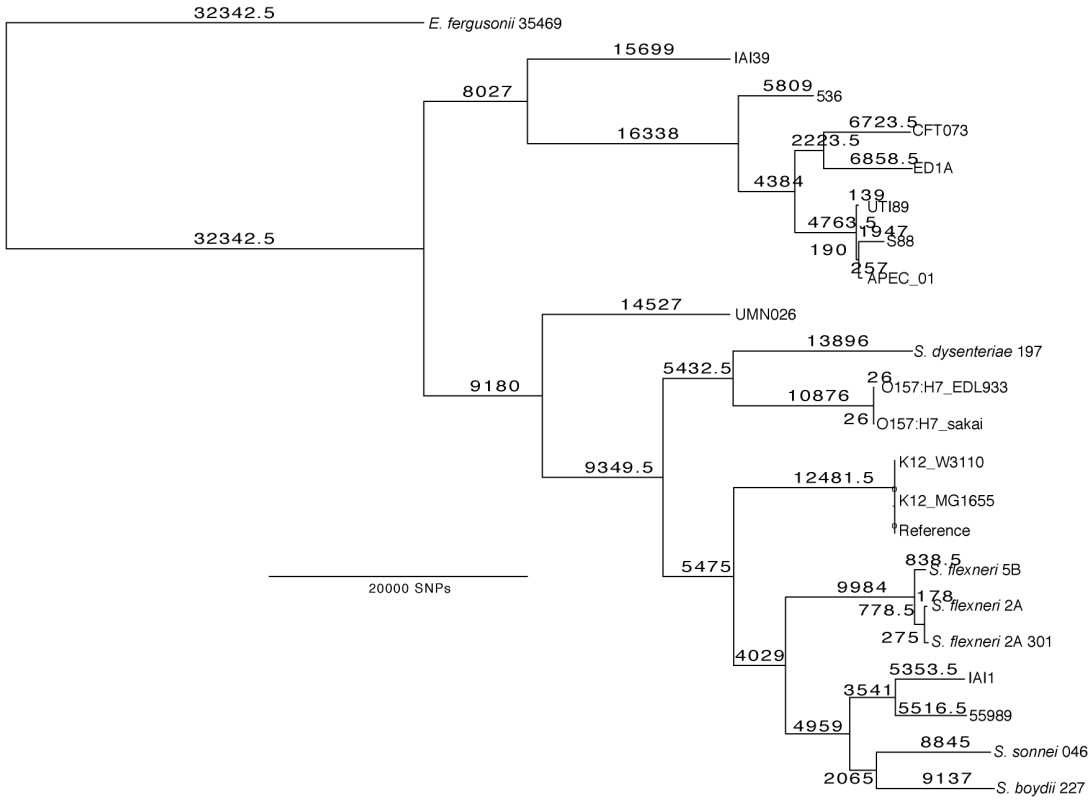
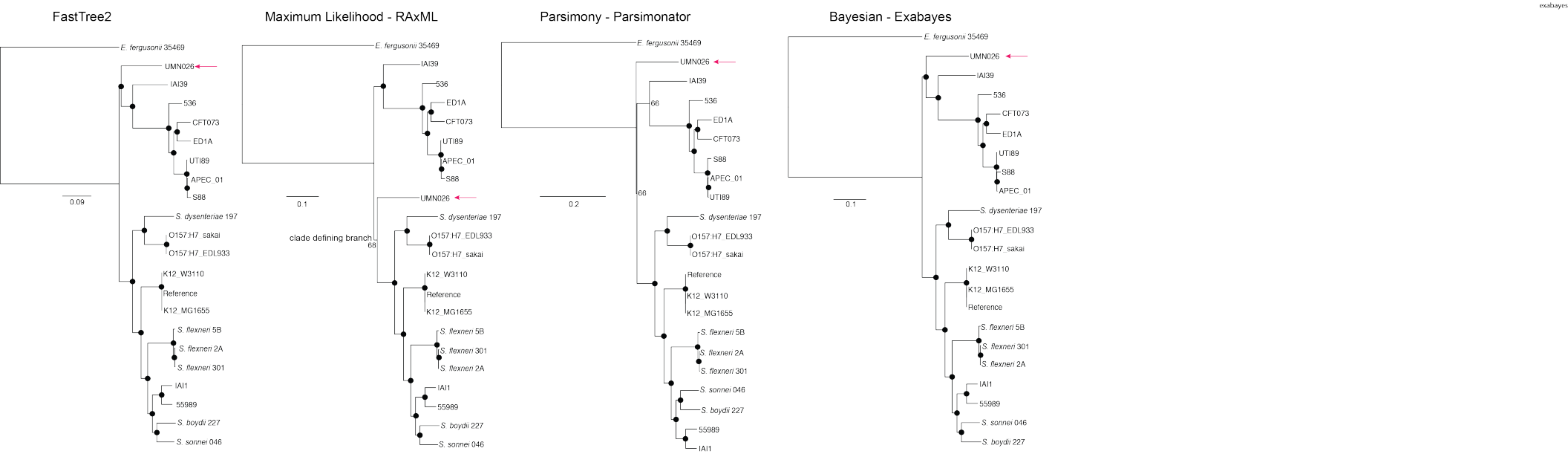
All positions



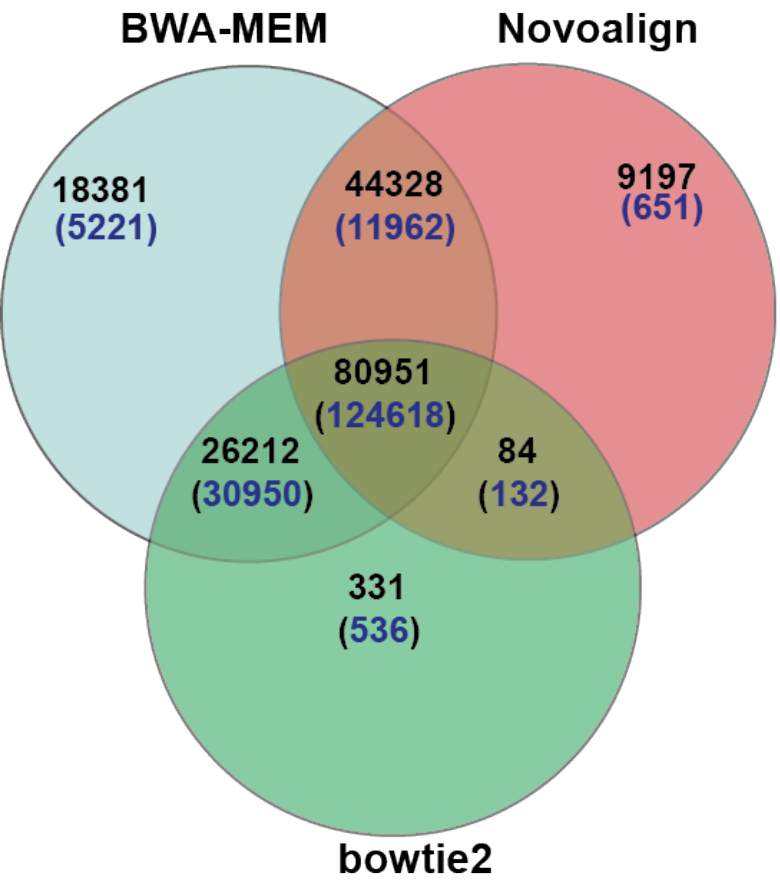
Polymorphisms only





● Boostrap or Posterior probabilities = 100

A.


BWA-MEM
Novoalign
bowtie2
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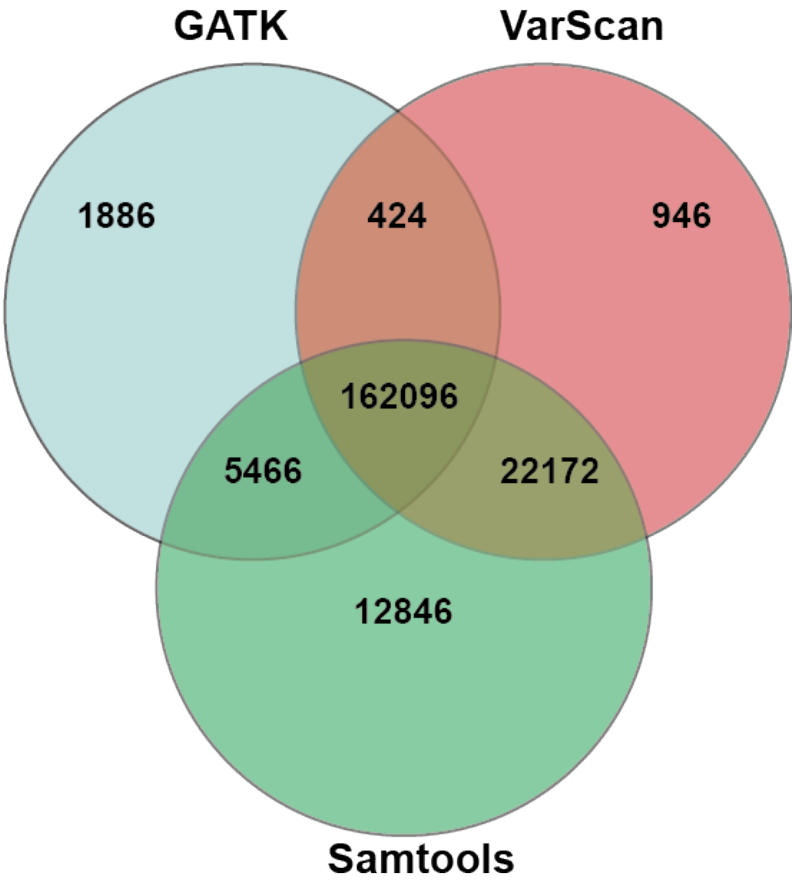
(5221)
44328
(11962)
9197
(651)
80951
(124618)
26212
(30950)
84
(132)
331
(536)

B.


GATK
VarScan
Samtools
1886
424
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162096
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22172
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Supplemental Table 1. An overview of NASP functionality

Language:	·Python ·GO
Job Management Systems:	·Torque ·Slurm ·Sun/Oracle Grid Engine ·Single Node (not recommended)
Inputs:	·FASTA ·BAM ·SAM ·VCF ·FASTQ ·FASTQ.gz
Outputs:	·master.tsv ·master_masked.tsv (optional) ·missingdata.tsv ·bestsnps.tsv ·missingdata.vcf ·bestsnps.vcf ·general_statistics.tsv ·sample_statistics.tsv ·bestsnps.fasta ·missingdata.fasta
Aligners:	·BWA-MEM ·Novoalign ·bowtie2 ·SNAP
SNP Callers:	·GATK ·Samtools ·SoLSNP ·VarScan

Supplemental Table 2. Accession information for *E. coli* genomes tested in this study

Species	Genome	Accession
E. coli	536	NC_008253
E. coli	APEC_01	NC_008563
E. coli	CFT073	NC_004431
E. coli	ED1A	NC_011745
E. coli	IAI1	NC_011741
E. coli	IAI39	NC_011750
E. coli	K12_MG1655	NC_000913
E. coli	K12_W3110	AC_000091
E. coli	55989	NC_011748
E. coli	O157:H7_EDL933	NZ_CP008957
E. coli	O157:H7_sakai	NC_002695
E. coli	S88	NC_011742
E. coli	UMN026	NC_011751
E. coli	UTI89	NC_007946
E. fergusonii	35469	NC_011740
S. boydii	227	NC_007613
S. dystenteriae	197	NC_007606
S. flexneri	301	NC_004337
S. flexneri	2457T	NC_004741
S. flexneri	8401	NC_008258
S. sonnei	SSON046	NC_007384

Supplemental Table 3. *Yersinia pestis* genome accession information

Genome	Accession
Yersinia pestis A1122	CP002956
Yersinia pestis biovar Orientalis str AS200901156	AGJU01000001
Yersinia pestis biovar Orientalis str AS200901434	AGJZ01000001
Yersinia pestis biovar Orientalis str AS200901509	AGKA01000001
Yersinia pestis biovar Orientalis str AS200901539	AGJT01000001
Yersinia pestis biovar Orientalis str AS200902147	AGJY01000001
Yersinia pestis biovar Orientalis str BA200901703	AGJV01000001
Yersinia pestis biovar Orientalis str BA200901799	AGJS01000001
Yersinia pestis biovar Orientalis str BA200901990	AGJW01000001
Yersinia pestis biovar Orientalis str BA200902009	AGJX01000001
Yersinia pestis CA88-4125	NC_009595
Yersinia pestis El Dorado	CP009785
Yersinia pestis Java9	NZ_CP009996
Yersinia pestis Shasta	CP009723
Yersinia pestis EBD10 O58	ATWT01000001

Supplemental Table 4. SNP locations in the Colorado 92 genome

SNP location	SNP type
gi|16120353|ref|NC_003143.1|::74539	Bench validated SNP
gi|16120353|ref|NC_003143.1|::432785	Bench validated SNP
gi|16120353|ref|NC_003143.1|::471201	Bench validated SNP
gi|16120353|ref|NC_003143.1|::681456	Bench validated SNP
gi|16120353|ref|NC_003143.1|::877258	Bench validated SNP
gi|16120353|ref|NC_003143.1|::904485	Bench validated SNP
gi|16120353|ref|NC_003143.1|::1017647	Bench validated SNP
gi|16120353|ref|NC_003143.1|::1178178	Bench validated SNP
gi|16120353|ref|NC_003143.1|::1306718	Bench validated SNP
gi|16120353|ref|NC_003143.1|::1391153	Bench validated SNP
gi|16120353|ref|NC_003143.1|::1558606	Bench validated SNP
gi|16120353|ref|NC_003143.1|::1564799	Bench validated SNP
gi|16120353|ref|NC_003143.1|::1971625	Bench validated SNP
gi|16120353|ref|NC_003143.1|::2022335	Bench validated SNP
gi|16120353|ref|NC_003143.1|::2300659	Bench validated SNP
gi|16120353|ref|NC_003143.1|::2619611	Bench validated SNP
gi|16120353|ref|NC_003143.1|::2950954	Bench validated SNP
gi|16120353|ref|NC_003143.1|::2968425	Bench validated SNP
gi|16120353|ref|NC_003143.1|::3145523	Bench validated SNP
gi|16120353|ref|NC_003143.1|::3190399	Bench validated SNP
gi|16120353|ref|NC_003143.1|::3244204	Bench validated SNP
gi|16120353|ref|NC_003143.1|::3394022	Bench validated SNP
gi|16120353|ref|NC_003143.1|::3406528	Bench validated SNP
gi|16120353|ref|NC_003143.1|::3739401	Bench validated SNP
gi|16120353|ref|NC_003143.1|::3789780	Bench validated SNP
gi|16120353|ref|NC_003143.1|::4579183	Bench validated SNP
gi|16120353|ref|NC_003143.1|::82	Colorado 92 error
gi|16120353|ref|NC_003143.1|::130	Colorado 92 error
gi|16120353|ref|NC_003143.1|::150946	Colorado 92 error
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gi|16120353|ref|NC_003143.1|::1939828	Colorado 92 error
gi|16120353|ref|NC_003143.1|::1939841	Colorado 92 error
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gi|16120353|ref|NC_003143.1|::3608932	Colorado 92 error
gi|16120353|ref|NC_003143.1|::3647867	Colorado 92 error
gi|16120353|ref|NC_003143.1|::3655609	Colorado 92 error
gi|16120353|ref|NC_003143.1|::4624135	Colorado 92 error
gi|16120353|ref|NC_003143.1|::3886839	Colorado 92 error

Supplemental Table 8. *E. coli* genome accession information

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Supplemental Data File 1

--E. coli dataset--

-NASP (assemblies)

Interactive, default values

-NASP (reads)

Interactive mode
Min depth = 3x
Min proportion = 0.9
Aligner = BWA-MEM
SNP caller = GATK

-ParSNP

```
parsnp -c -d genomes/ -r genomes/ECO_K12_MG1655.fasta -o parsnp_out -p 8
```

-ISG (assemblies)

```
java -jar ISGPipeline-0.16.10-3.jar -S ISGPipelineQScript.scala -I touchon_isg_in -O ISG_touchon -R ECO_K12_MG1655.fasta -mummer /packages/MUMmer/3.23/ -nt 8 -run
```

-ISG (reads)

```
java -jar ISGPipeline.jar -S ISGPipelineQScript.scala -I art_reads -O isg_reads_out/ -R ECO_K12_MG1655.fasta -gatk GenomeAnalysisTK.jar -bwa bwa -mummer /packages/MUMmer/3.23/ --minaf 0.9 --mindp 3 --usebwamem -nt 8 -run
```

-REALPHY

```
REALPHY_v112 genomes/ realphy_single/ -readLength 100 -polyThreshold 0.7 -perBaseCov 5 -ref ECO_K12_MG1655 -treeBuilder 2
```

-REALPHY (failed attempt using multiple references)

```
REALPHY_v112 genomes/ realphy_single/ -readLength 100 -polyThreshold 0.7 -perBaseCov 5 -treeBuilder 2
```

-kSNP3 (assemblies)

```
kSNP3 -in ksnp_paths.txt -outdir ksnp_results -annotate annotate_list -CPU 8 -ML -k 23 -core -min_frac 0.9
```

-SPANDx (reads)

(v2.7) SPANDx.sh -r ECO_K12_MG1655 -m yes -p PE
(v3.1) SPANDx.sh -m yes -r ECO_K12_MG1655 -t Illumina -p PE -z yes

-MUGSY (assemblies)

```
mugsy -p touchon --directory . -duplications 1 *.fasta
```

-CFSAN (reads)

```
run_snp_pipeline.sh -s cfsan_in/ genomes/ECO_K12_MG1655.fasta
```

-lyve-SET

```
launch_set.pl ecoli_set --numcpus 8 min_alt_frac 0.9 --min_coverage 3
```

--YP dataset--

-NASP (mixed)
Interactive mode
Min depth = 3x
Min proportion = 0.9
Aligner = BWA-MEM
SNP caller = GATK

-NASP (assemblies)
Interactive mode
Default values

-ISG(assemblies)

```
java -jar ISGPipeline.jar -S ISGPipelineQScript.scala -I ISG_in_assemblies/ -O ISG_out_assemblies -mummer /packages/mummer/3.23/ -R YP_CO92_all.fasta -nt 8 -run
```

-ISG (mixed)

```
java -jar ISGPipeline.jar -S ISGPipelineQScript.scala -I ISG_in/ -O ISG_out -mummer /packages/mummer/3.23/ -R YP_CO92_all.fasta -nt 8 -run --minaf 0.9 --mindp 3 -bwa bwa --usebwamem -gatk GenomeAnalysisTK.jar
```

-REALPHY

```
REALPHY_v112 all_good_assemblies realphy_YP_out/ -clean -readLength 100 -polyThreshold 0.70 -ref YP_CO92_all
```

-kSNP

```
kSNP3 -k 19 -in fasta_list -annotate annotated_list -core -ML -CPU 8 -outdir ksnp_results/
```

-SPANDx

(v2.7) SPANDx.sh -r YP_CO92_all -m yes -p PE
(v3.1) SPANDx.sh -r YP_CO92_all -m yes -p PE -t Illumina -z yes

-CFSAN

-lyve-SET

--ART sequence simulation--

```
art_illumina -i genome.fasta -l 100 -f 20 -p -ss HS25 -m 300 -s 50 -o genome
```

--Commands for phylogenetic comparisons--

--RAxML--

-GTRGAMMA model for monomorphic/polymorphic sites

```
raxmlHPC-SSE3 -f d -p 12345 -s GTRGAMMA -s alignment.fasta -n out
```

-ASC model with ascertainment bias correction

```
raxmlHPC-SSE3 -f d -p 12345 -s ASC_GTRGAMMA -s alignment.fasta -n out --asc-corr=lewis --no-bfgs
```

-including bootstraps

```
raxmlHPC-PTHREADS-SSE3 -p 12345 -s alignment.fasta -f a -# 100 -T 8 -n raxboots -m GTRGAMMA -x 12345
```

--Parsimonator--

```
parsimonator-SSE3 -p 12345 -s bestsnps.phy -n test
```

--ExaBayes

```
yggdrasil -f bestsnps.phy -m DNA -n test -s 22552 -T 16 -M 0 -c config.nex
```

-contents of config.nex

```
#NEXUS

begin run;
 numruns 2
 numgen 1e8
 numCoupledChains 2
end;
```

--FastTree2--

```
FastTree -nt -noboot in.fasta
```