POPULATION GENETICS ANALYSIS ON *TRITONIA TETRAQUETRA*

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Master of Science Biological Sciences

By

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Abstract:

Tritonia tetraquetra is a member of gastropoda class. This study was done to investigate the population genetics of *Tritonia* populations. In this study, the whole genome of the organisms from individuals from various populations were sequenced, and then using bioinformatics tools, the mitochondrial DNA was assembled, followed by phylogenetic analysis to look at the populations structure of the organisms. In this study, we found that most *Tritonia* samples from identical or relatively close geographical locations demonstrated similar genetic composition. We hypothesize this to be due to similar environmental and habitat factors between samples.

However, we also suggest that other factors likely influence *Tritonia* genetics given that there were a few *Tritonia* samples from similar locations that showed differences in their phylogenetic relationship (i.e., we found different species of *Tritonia* from similar locations). Since we know that *Tritonia* populations are descendent from a small number of ancestors, the similarities seen between the *Tritonia tetraquetra* and *Tritonia festiva* may be due to the Founder Effect (i.e., establishment of a new population based on a small number of the original population). Future studies may include a greater number of organisms from the same geographic locations as was examined in the current study, as well as consider the possible influence of ocean currents, which may move *Tritonia* larvae from one geographic location to another (thereby potentially facilitating the Founder Effect)

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Background and Introduction:

Mollusca are invertebrates that are a very diverse and they belong to the animal kingdom. Mollusca is recognized with seven different classes: Gastropoda (such as snails and slugs), Bivalvia (such as clams) and Cephalopoda (such as octopuses and squids), Scaphopoda, Monoplacophora, Polyplacophora, Neomeniomorpha and Chaetodermomorpha. (1)

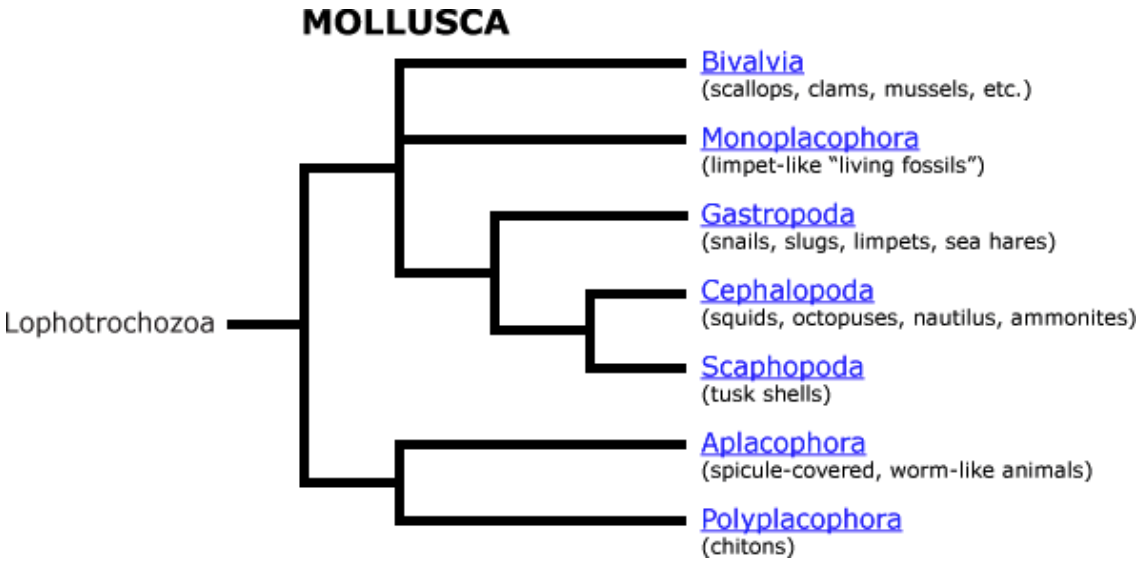

Figure 1: The systematics of Mollusca Phylum (University of California Berkeley)

Gastropoda are a very diverse class of the molluscs phylum. Gastropoda have a wide variety of habitats. They can be living on land like snails or be found in waters and oceans like sea slugs.

(2) Among all molluscs there are many debates over taxonomic relationships of

Gastropoda. Gastropod comes from Latin words gastro which means stomach and pod which stands for foot. According to Thiele's system of classification, Gastropoda are splitted into Opisthobranchia, Pulmonata and Prosobranchia. (3)

The methods of classification such as Theil's were mostly based on morphological aspects which was not very agreeable among people since it is very challenging to be certain about determining synapomorphism of sym-plesiomorphism based on this approach of classification. While the new method of classification of Gastropoda introduced by Bouchet and Rocroi (2017) (4) was mainly based on molecular studies. In Bouchet and Recroi classification, clades were used between classes and superfamilies (basically using clades as taxa) to and with this approach there were six main clades of Gastropoda: Patellogastropoda, Vetigastropoda, Cocculiniformia, Neritimorpha, Caenogastropoda and Heterobranchia. Heterobranchia is further divided into Opisthobranchia, Pulmonata and lower Heterobranchia. some taxonomists grouped Pulmonata and Opisthobranchia together as a clade called Euthyneura. (5,6,7,8,9,10,14,17)

Gastropoda have head, foot and visceral mass which contains organs. the buccal cavity of these organisms contains a radula, which is in the area of the mouth and is for feeding purposes. This is all covered in mantle also known as pallium that typically secretes shell. Gastropoda are known for possessing a shell at least in their larval stage of life. Shells are mostly for protection against predators, mechanical damages and dehydration purposes. Their shells vary from one to other in thickness and shapes.

(11,12) Some members of the Gastropoda lost their shells completely like nudibranchs. (12,13).

Nudibranchia, which translates to “naked gills”. Gills are respiratory organs of nudibranchs. Most nudibranchs are simultaneous hermaphrodites, nudibranchs are mostly known for their beautifully vibrant color patterns, which can be used for camouflage, mating, and a warning signal for predators (Willan and Coleman 1984). Nudibranchs themselves have many suborders which include Aeolidacea, Dendronotacea, Doridacea, Bathydoridoidea and Arminacea. These classifications were mostly based on morphological aspects of individuals and are not certain (15,16,33). In Dendronotacea, due to loss of shells and mantle cavity caused development of secondary gills that their shapes can be feather like structures as seen in *Tritonia* or simple disc shaped as seen in *Melibe* (16,17). In many studies Dendronotida is not supported as monophyletic (18,19,20). Based on RNA sequencing, analysis shows that Cladobranchia contains Dotidae, Tethyidae (including *Melibe*) and Dendonotidae. The dendonotid taxon has a family, Tritoniidae. (18,17). Tritoniidae currently is divided into eight genera: *Tritonia, Tritoniopsis, Tritoniella, Marionia*, *Marianina, Marionopsis, Paratritonia* and *Tochuina*. (21,22) Within *Tritonia* genus there are different species including: *Tritonia affinis, Tritonia bayeri, Tritonia khaleesi, Tritonia festiva*, *Tritonia diomedea* or *tetraquetra* and so on. (Bergh, 1884, Pallas, 1788, Silva, Azevedo & Matthews-Cascon, 2014). *Tritonia tetraquetra* has been used in many of neurobiological and physiological studies and it has given some insights for neurophysiology and evolutionary disciplines. (23,24)

Tritonia also is resistant to toxins, as its food in adult stage of life is sea pens and sea-whips (25), both are known to be toxic. It is not clear whether this resistance is a general adaptation that came from a common ancestor, or the slug is locally adapted to be able to digest and neutralize these toxins due to availability of these materials in its local habitat. There was also a recent study in which the whole mitochondrial genome of *Tritonia tetraquetra* was sequenced and those data addressed the phylogenetic relationship among Gastropoda (26). Genetic makeup of organisms is a dynamic material and is changing over time. (27,28,29) The dynamic nature of genetic material will allow organisms to adapt to environmental changes and this will lead to evolution of organisms. (30)

This study looks at the phylogenetic relationship of two *Tritonia* species (*Tritonia festiva, Tritonia tetraquetra)* using Next Generation Sequencing. to analyze data sequences with up-to- date bioinformatics and phylogenetic tools., the mitochondrial genome of organisms was used, to see whether or not these populations of slugs accumulated enough mutation and genetic changes that can lead to making a new population, or there is enough gene flow among the slugs that made a single population among different geographical locations. The results of this study will be very helpful for many future studies such as chemical ecology (the resistance to toxins is an ancestral trait or a local adaptation). It was hypothesized that individuals from the same geographical location that shared similar environment and ecological factors such as temperature, amount of sunlight exposure, pH of water and climate factors, would show more similar genetic make-up in comparison to same species from another geographical location, this might be due to adaptation or to a founder effect.

In this research, DNA was isolated mostly from buccal mass muscle tissue from different *Tritonia* species that were collected from different areas along the Pacific Coast (a kind gift from Dr. James Murray with the Department of Biological Science at Cal State East Bay). The figures 2 to 4B, below shows the location of samples collection:

Tofino (Latitude 49o13’50.73” N and Longitude 125o56’10.42” W)

Langley (Latitude 48o2’34.81” N and Longitude 122o24’25.01” W)

Squamish (Latitude 47o51’41.40” N and Longitude 122o39’21.24” W) Dash Point (Latitude 47o19’15.08”N and Longitude 122o25’19.80”W)

Buccal tissues were used as a source of DNA for Ion Torrent Next-Generation Sequencing. The mitochondrial DNA of these organisms was assembled and analyzed to reconstruct phylogenetic trees of these organisms, (40, 41, 42) to better understand their genetic makeup, as well as strengthen the phylogenetic relationships between these species.

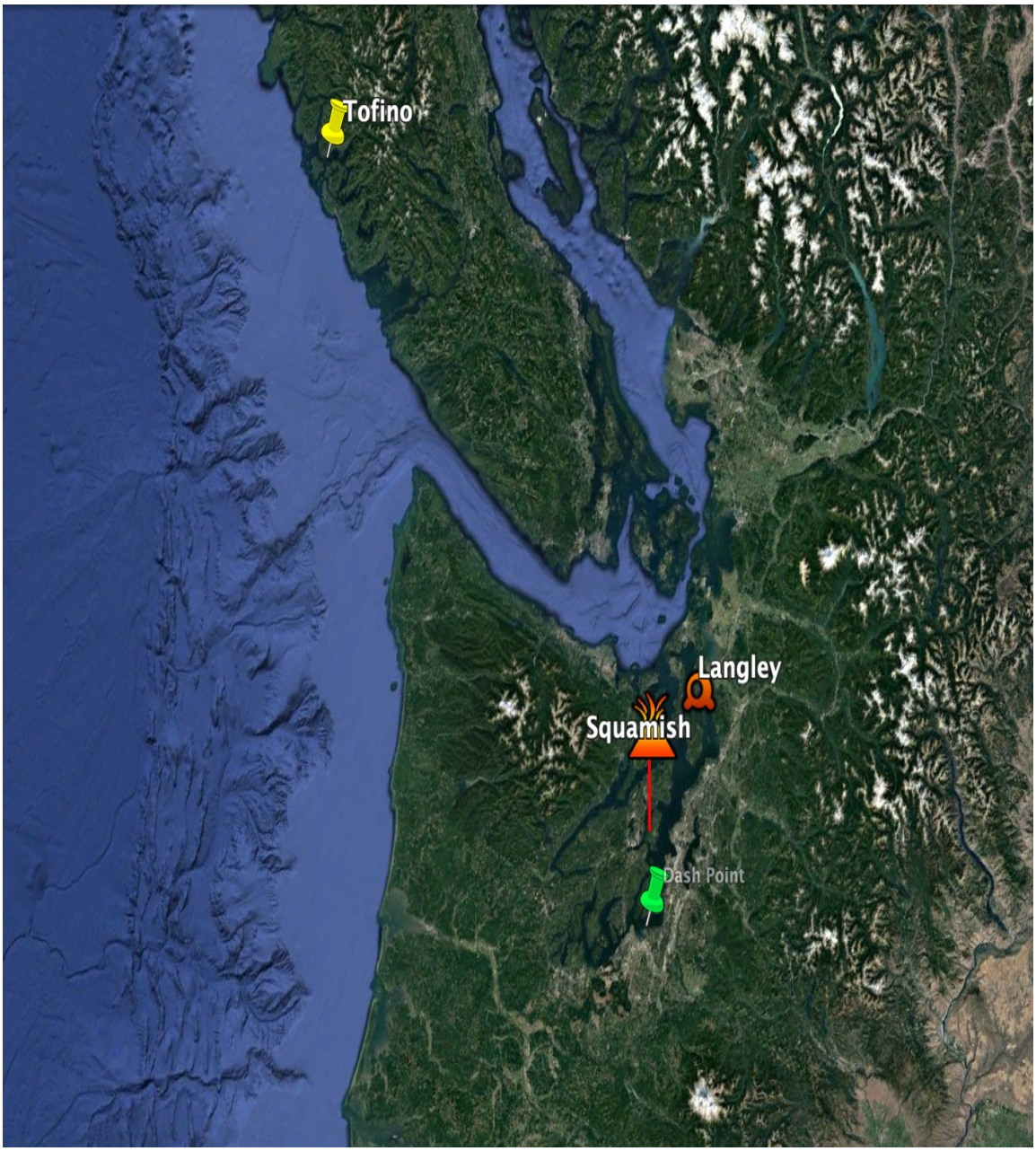

Figure 2- Location that samples were collected from. (the red line shows 30 Km distance)

Figure 3- Sample collection location, Tofino

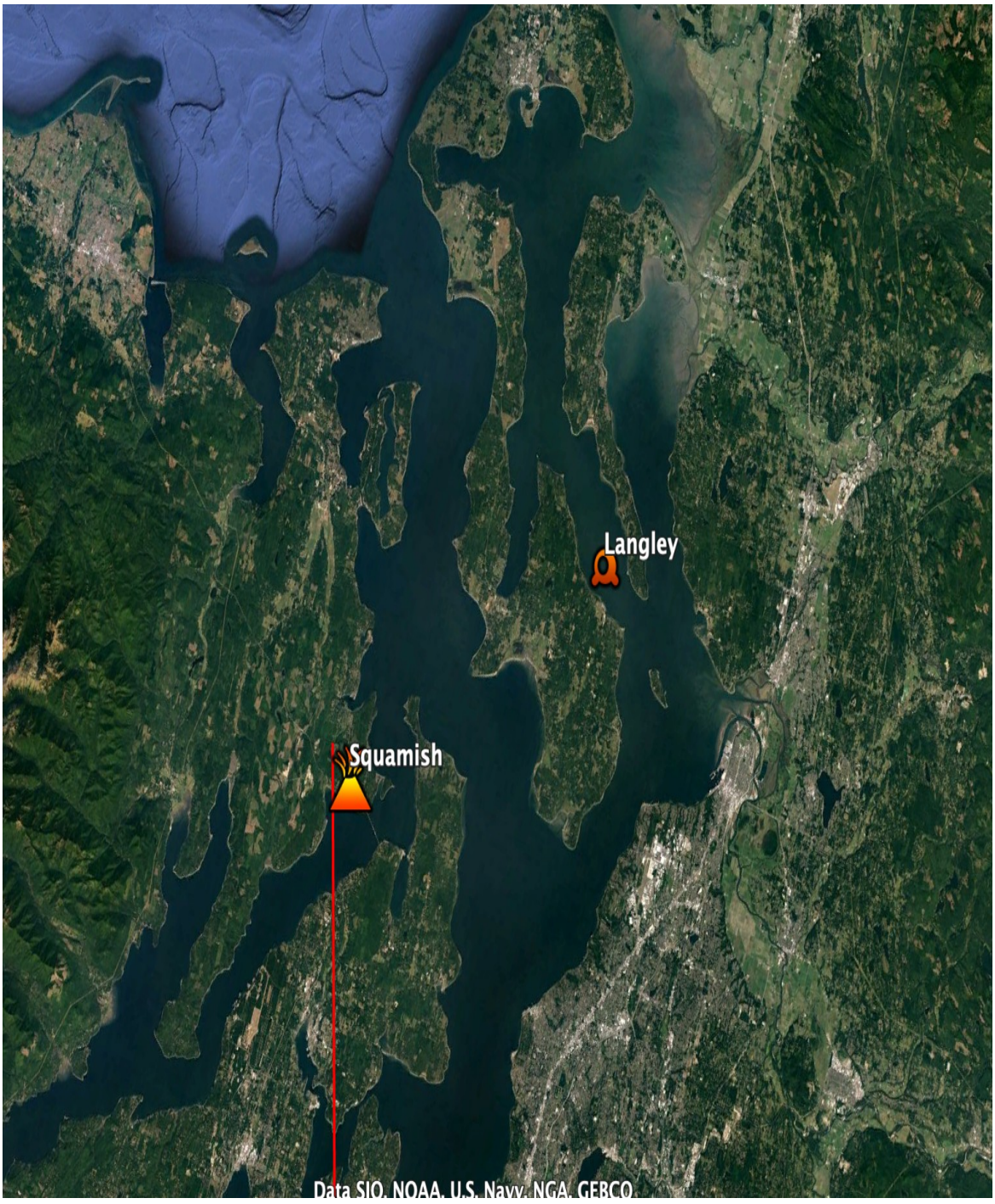

Figure 4 -Sample collection locations, Langley

Fig 4B- Sample collection location, Squamish

Materials and Methods

Frozen (at –80°) or ethanol stored (4°) buccal tissues from *Tritonia* was a kind gift from Dr. James Murray in the Department of Biological Sciences at CSUEB. *Tritonia* samples were from different locations: Dash Point State Park located in Federal Way, Washington, Olympia WA, Squamish WA, Tofino BC Canada, Monterey Bay CA. Total DNA (gDNA and mtDNA) was isolated using the DNeasy Isolation Kit for Tissue and Blood (QIAGEN, Valencia, CA). DNA isolation was accomplished according to manufacturer's instructions. Since some tissues were stored in ethanol, there was a washing step in which samples were washed 2x in sterile ddH_2O. Tissues (50mg) were then treated with Proteinase K at a concentration of 600 mAU/ml treatment overnight at 56°. The eluted DNA (~100 µl) using TE was then stored at –20°until needed. (30)

DNA fragmentation

The procedure for Next-Gene Sequencing begins with utilizing the Ion Torrent PGM Library kit (Thermo-fisher Scientific, Waltham, MA). Stored DNA underwent six rounds of sonication (15 min each round) to fragment DNA into smaller pieces of manageable range, approximately 250 bp in size. The products of sonication were confirmed on a 3% agarose gel in 1xTAE.

End Repair and Adaptor Ligation of Sonicated Samples

Sonicated samples underwent end-repair and bead cleanup, followed by adaptor ligation and bead clean up using standard protocols.

Pippen Prep

The Pippen Prep procedure was then performed to remove adaptors from the DNA sample and narrow the specific size of each DNA sample (~250-300 bp size). The procedure utilizes prepared cassettes and a specialized algorithm to isolate specific DNA fragments (~300 bp fragments). The samples containing the isolated fragments then were cleaned up using Agencourt AMPure beads. (31).

Bioanalyzer:

A bioanalyzer analysis confirmed the size of the library fragments, the efficiency of the adaptor removal step and quantifies the concentration of library obtained. (32)

One Touch and Enrichment:

Ion One Touch 2 system was used to perform template amplification and enrichment according to the manual.

Ion PGM Hi-Q Sequencing: This study employed PGM 318 chips for sequencing following standard protocols. (31)

Bioinformatics tools for analyzing obtained Sequences

For analysis purposes, fastq files were assembled using as a template the mitochondrial genome of *Tritonia* (26). Assembly options were set to the defaults of the DNA Star SeqMan NGen 13 software. Then sequences were edited using Seqman Pro after confirming the coverage of contigs in all areas of mitochondrial genome.

The edited assembled genomes were then saved as a consensus file with FASTA format. (the screenshots of portion of these files are in appendix section) These FASTA files were then aligned using Sequencher. The aligned files were then used for phylogenetic analysis purposes.

Phylogenetics tools:

To understand the relationships among the members of these species, three different methods were applied: Parsimony, Maximum Likelihood and Bayesian analysis.

For performing Parsimony analysis, PAUP (Swofford 2002) was used. Parsimony analysis consisted of a heuristic search with 1000 random stepwise and neighbor joining replicates, TBR branch swapping and equal weighting of all characters. Then Maximum Likelihood analyses were run using RAxML (Stamatakis 2014) with GTRGAMMA

model, consisting of 1000 replicates using default settings, and supported by 1000 replicates of bootstrap value.

Lastly, Bayesian analyses were performed using Metropolis Coupled MCMC methods in MrBayes (Huelsenbeck and Ronquist) software, under GTRGI model with two million generations. Trees with above the average standard deviation of split frequencies of 0.01 were discarded as burn-in, while the rest of the trees were used to calculate posterior probabilities of the clades.

Results

Following are the results of Ion PGM run for each sample (figure are found in appendix section). The chart below shows the read numbers obtained from Ion PGM runs as well as the average length of reads.

Table 1: Sample information on sequence reads: All samples are *Tritonia*, but beside *festiva* the rest are all *tetraquetra*s. These are members of two different species, *festiva and tetraquetra.* There are multiple populations of *tetraquetra* (Dashpoint, Tofino, Squamish and Langley) while there is only one population of *festiva.*

Sample ID	Total read numbers	Read length median(bp)
Mor196 (Tofino)	2,807,362	207
Mor197 (Dashpoint#27)	3,400,350	217
Mor199 (Dashpoint#25)	645,252	223
Mor200 (Dashpoint#23)	4,822,687	218
Mor202 (Festiva)	1,836,446	225
Mor212 (Squamish)	1,488,435	173
Mor213 (Tofino)	2,657,518	188
Mor222 (Festiva)	288,268	240
Mor224 (Langley)	449,067	250

The phylogenetic analysis was performed using three different methods of Parsimony, Bayesian and Maximum Likelihood. (Figure 5 shows Maximum Likelihood Tree, Figure 6 shows Bayesian Tree and Figure 7 shows Parsimony tree).

In all trees below "OZ" is mt genome that was used from NCBI and 2015 publication.

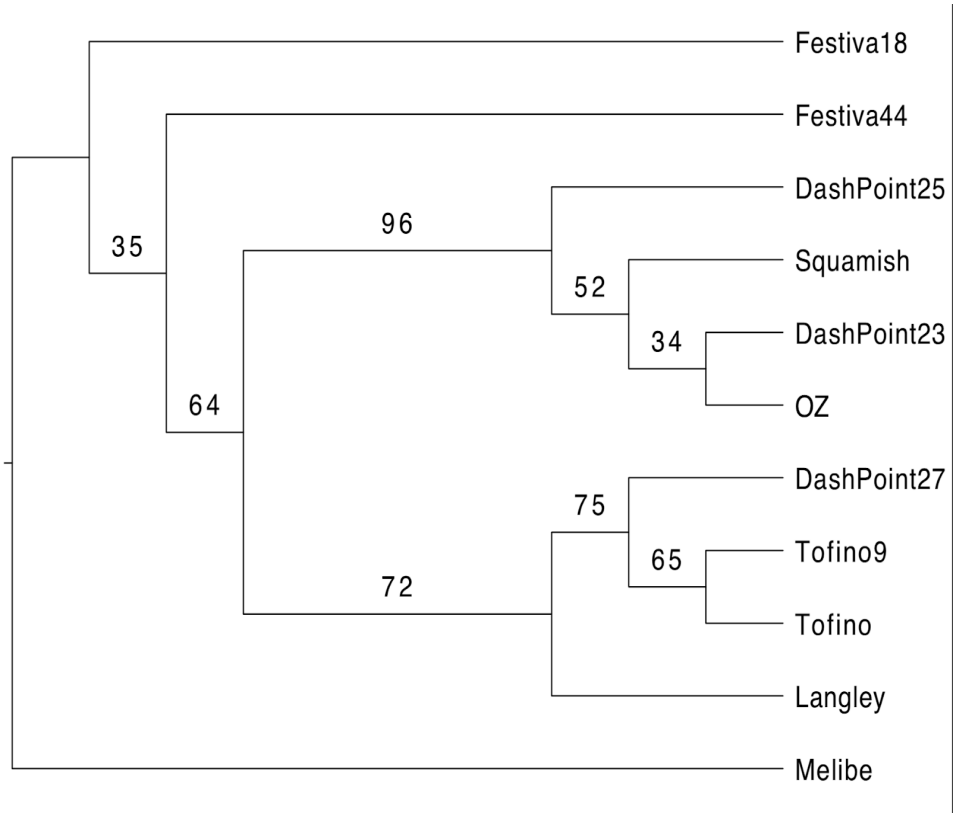

Fig 5A-Maximum Likelihood tree (RAxML)- Proportional tree

(The numbers on the branches show bootstrap values, which is showing out of many times that the analysis was done, how many times the same relationship was found).

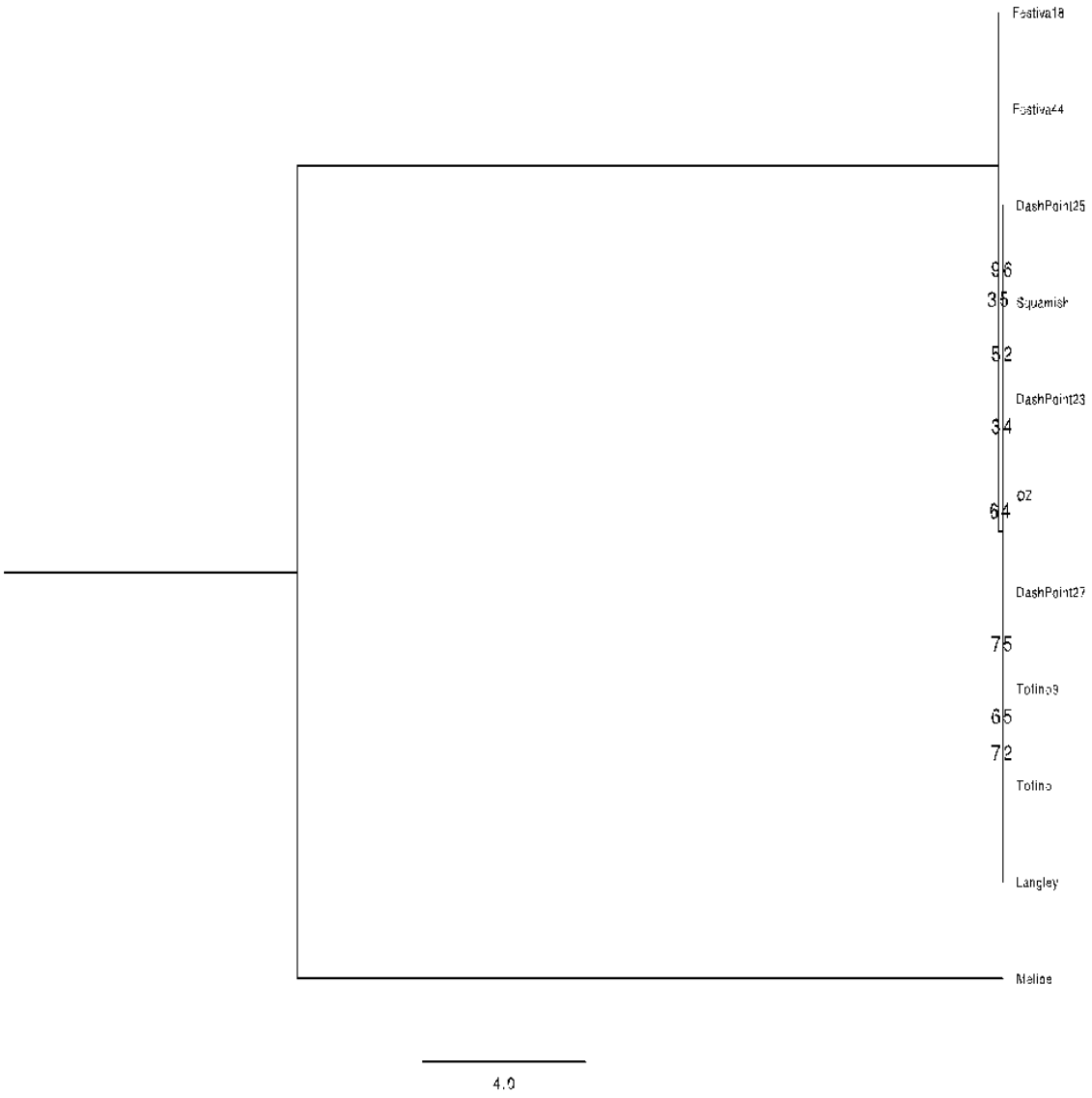

Fig5B- Maximum Likelihood tree-RAxML

The same tree as Fig 3A, but here the length of branches show the actual time of separation from ancestors)

Bayesian Trees-Mr. Bayes:

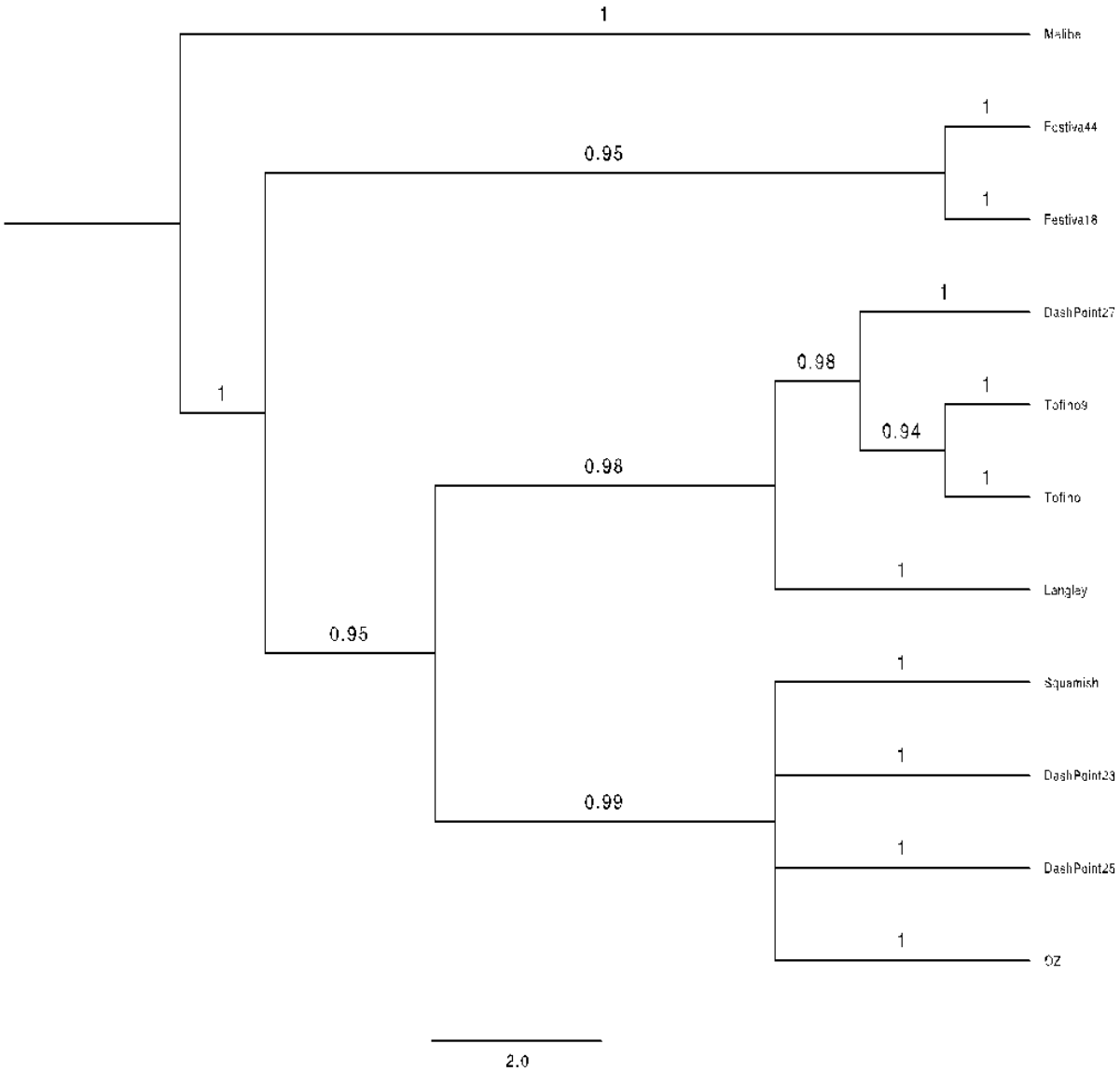

Fig 6A- Bayesian tree- Mr. Bayes- Proportional

The values on the branches show posterior probabilities, which is a rescale of likelihood and posterior probabilities of finding the tree given the model and previous beliefs.

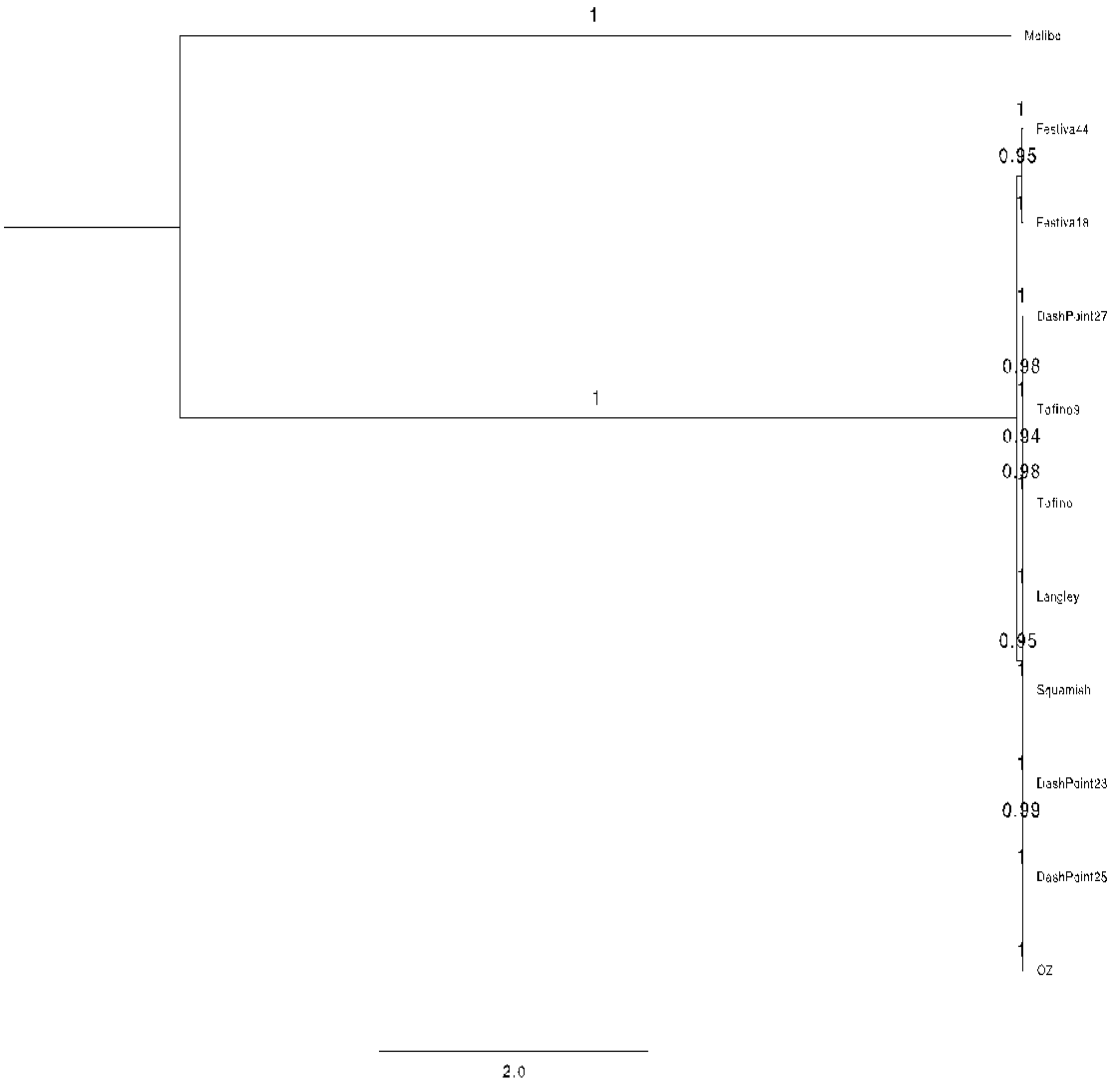

Fig 6B- Bayesian Tree- Mr. Bayes

Same tree as Fig 4A, the branch show the actual time of separation from ancestor.

And lastly is Parsimony tree;

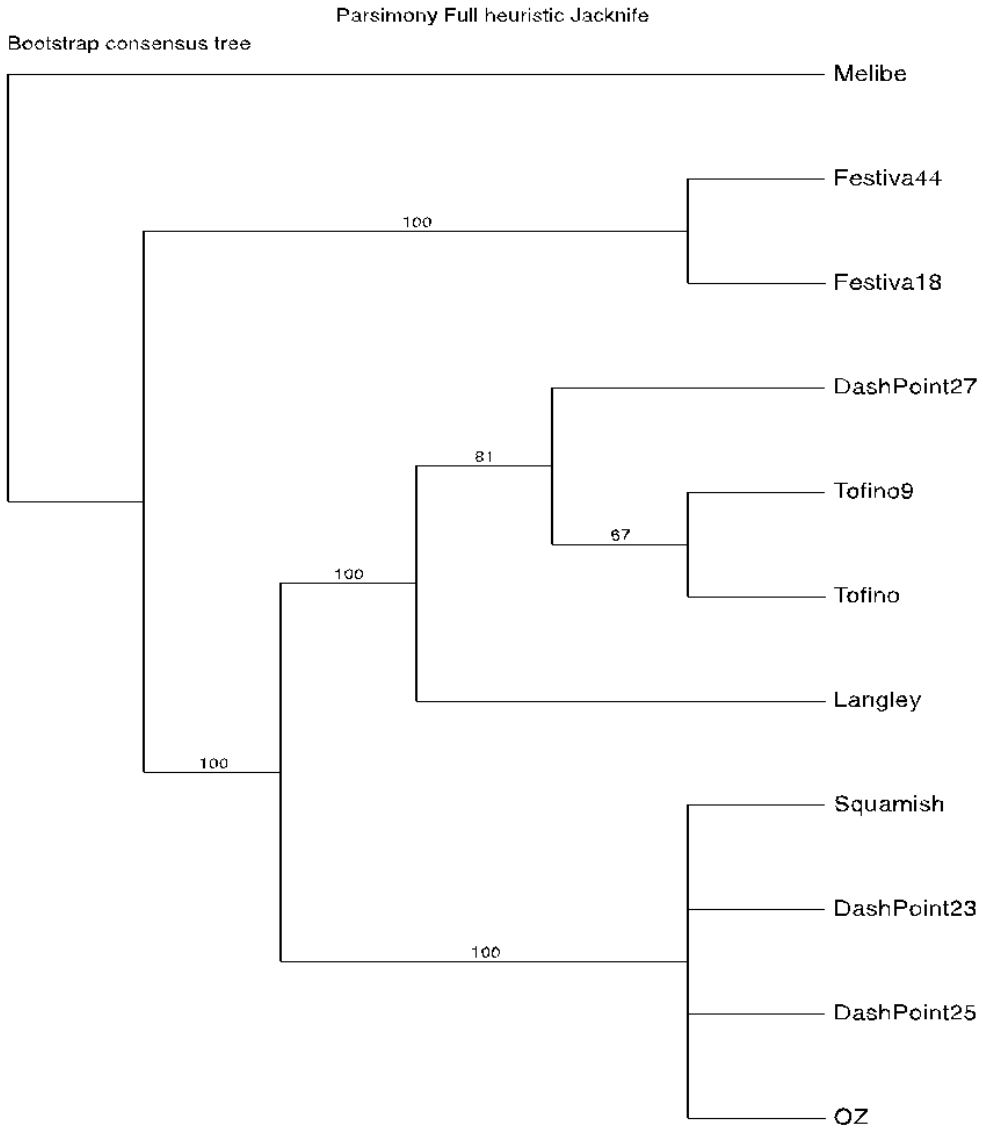

Figure 7-Parsimony tree- the values on the branches how bootstrap values.

During bioinformatics analysis portion of the study, a region of 200 bp of repeated sequences were found in all *Tritonia* mt sequences. After spending some time, I was not able to analyze those data, however having that region during phylogenetic analysis did not change the relationships, so it was decided to continue on with analysis

without that region. In the future, it is attempted to study that region and include that information for possible publication purposes.

Discussion:

Although there were dissimilarities in the mt genomes of *Tritonia tetraquetra* individuals as well as *Tritonia festiva* members, overall the mt genome of *Tritonia tetraquetra* members showed similarities between themselves and were more different than *Tritonia festiva* members which confirmed that *Tritonia festiva* and *tetraquetra* are indeed two different species. This result was confirmed with phylogenetic trees using two different methods of maximum likelihood and Bayesian. (*Tritonia festiva* formed a separate branch on tree from the rest of *tetraquetra* samples in both methods). *Melibe leonina* was used as an outgroup for this study because it was a different family member (from *Tritonia*) , yet it belonged to the same suborder.

Phylogenetic results showed consistent relationships among slugs. The results of Bayesian, Maximum likelihood and Parsimony trees showed were very similar relationships (which can be due to chance. The analysis also showed that there were distinct population of slugs in the geographical locations since slugs created separate clades in phylogenetic trees. These differences might be due to low level of gene flow among populations, that lead to forming different population of slugs in these locations. These variations in the mt genome can be a result of adaptive evolution or they can be the result of founder effects that are not adaptive. There are continuous changes in

organism's genetic material and also in population gene pool that may be a result of natural selection and genetic drift. Although gene flow homogenizes the evolutionary forces and decelerates and reduces the differences in the genetic makeup of the population, genetic drift still plays against this force. Genetic drift is due to random mating in the finite sized population, which works erratically and autonomously in various populations. Genetic drift makes the allele frequencies diverge in the absence of gene flow, which causes formation of distinct populations and later on with accumulation enough changes, creating even a new species. In the case of this study there is a probability of low level of gene flow among these slugs which led to create distinct population of slugs. Another possibility is that there are morphotypes (different types of individuals that belong to the same species in a population).

These differences can be due to ecological factors as well, however some of the members *Tritonia tetraquetra* from Langley, Squamish, and Dash Point showed more morphological similarities to populations that were geographically farther from it (Tofino), than to populations of *Tritonia tetraquetra* found in Union or Bellingham WA that are far from Tofino (Murray et al. 2011). These slugs' eggs hatch into planktonic larvae. The larvae can be transported with tidal flows from one location to another and the distance can be variable. (36, 39) There is a chance that a larva of one population was moved from its location to another one and that is one way to explain the similarities and differences that we have in our phylogenetic trees. It is safe to state that initial hypothesis (that close geographical location slugs may show more similar genetic makeup) is inconclusive. Although the numbers of slugs from individual geographical location was

limited, initial hypothesis (that close geographical location slugs may show more similar genetic makeup) was not supported with preliminary phylogenetic analysis: because the individuals that were closer to one another in geographical location did not show closer phylogenetic relationships (measured from similarities in their mitochondrial genetic sequences) and instead formed distant branches from each other separated by groups that were geographically closer. This can be due to the individual slug's diversity in genomic material and due to low number of individuals available from each location during this project. Individuals within a species can also vary in their mitochondrial genome. (40,41) Possibly adding more individuals in future can confirm if the hypothesis is rejected or not. *Tritonia* is a hermaphrodite creature, and creates eggs for reproduction purposes. The eggs will develop into larvae and these larvae can move with tides to suitable habitat, grow and reproduce as an adult organism. Since the larvae stage of *Tritonia* is about 30 days, there is a possibility that these larvae can move with ocean flow and be located in another geographical location. (34,37)

The results of this analysis provided an insight that there are differences between individuals, and each group of slugs has a random selection of different genotypes, which can be due to random larvae movement with ocean tides. Also earlier it was mentioned that *Tritonia* consumes sea pen which is toxic and has resistance to that. The results of this analysis can be very helpful for future studies in chemical ecology field of *Tritonia*, since the resistance of *Tritonia* can be tested for a possibility of local adaptation according to our results. In our analysis it was shown that there are different population

of slugs forming based on geographical location, so maybe the resistance in slugs can be due to available local food for them. (38)

It is attempted in the future, to include more slugs from each location and include them in the study to be able to further analyze the relationships among these slugs from different geographical location, with hopes that the results of this finding can be helpful for future hypothesis testing in future chemical and sensory ecology studies.

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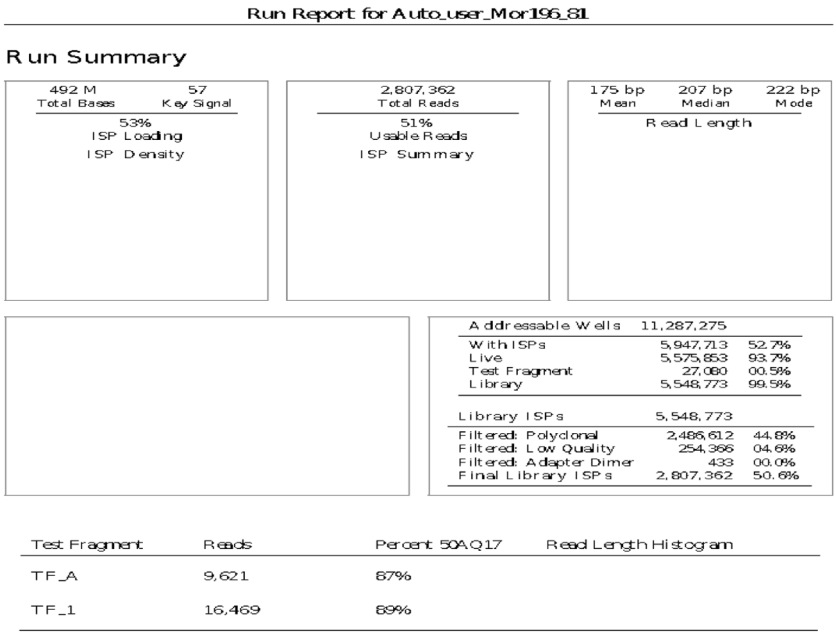
Appendix:



Run Report for Auto_user_Mor196_81

Run Summary

492 M	57
Total Bases	Key Signal

53%
ISP Loading
ISP Density

2,807,362
Total Reads

51%
Usable Reads
ISP Summary

175 bp	207 bp	222 bp
Mean	Median	Mode

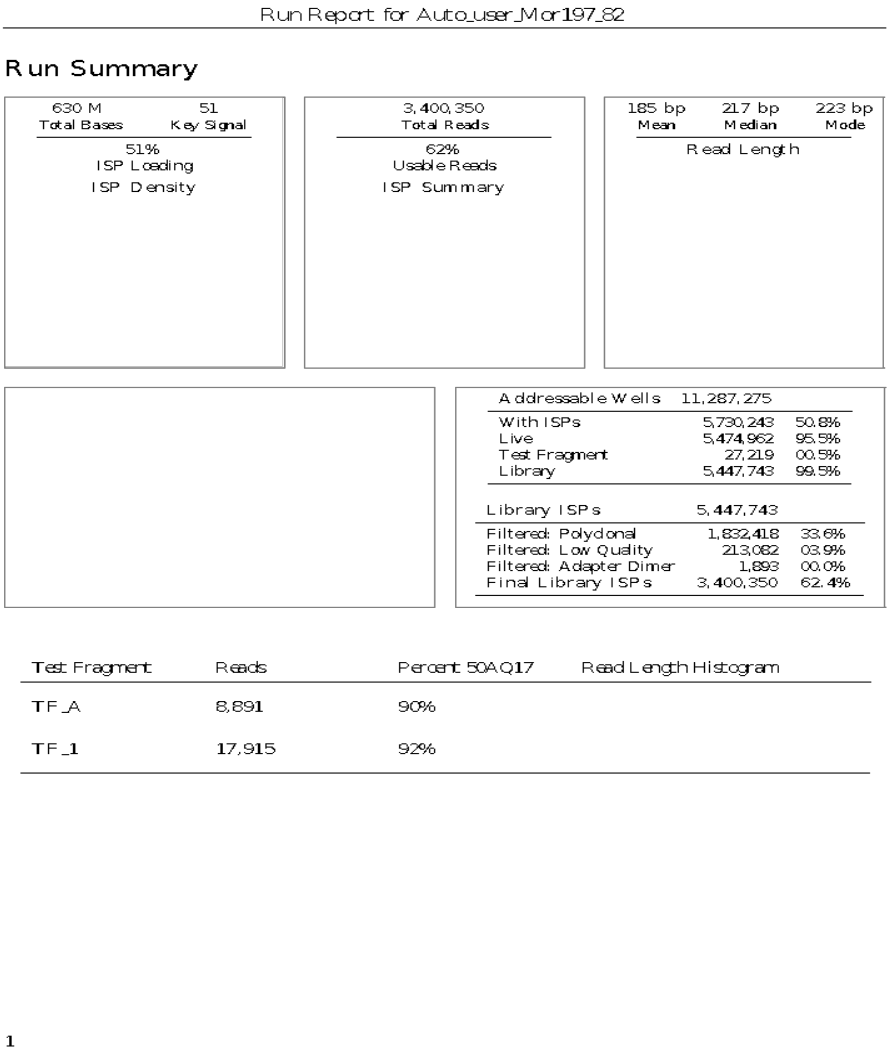
Read Length

Addressable Wells	11,287,275	
With ISPs	5,947,713	52.7%
Live	5,575,853	93.7%
Test Fragment	27,080	00.5%
Library	5,548,773	99.5%
Library ISPs	5,548,773	
Filtered: Polyclonal	2,486,612	44.8%
Filtered: Low Quality	254,366	04.6%
Filtered: Adapter Dimer	433	00.0%
Final Library ISPs	2,807,362	50.6%

Test Fragment	Reads	Percent 50AQ17	Read Length Histogram
TF_A	9,621	87%	
TF_1	16,469	89%	

1

Images 1A- shows the sequencing run report which contains information of library read lengths.



Run Report for Auto_user_Mor197_82

Run Summary

630 M Total Bases | 51 Key Signal

51% ISP Loading

ISP Density

3,400,350 Total Reads

62% Usable Reads

ISP Summary

185 bp Mean | 217 bp Median | 223 bp Mode

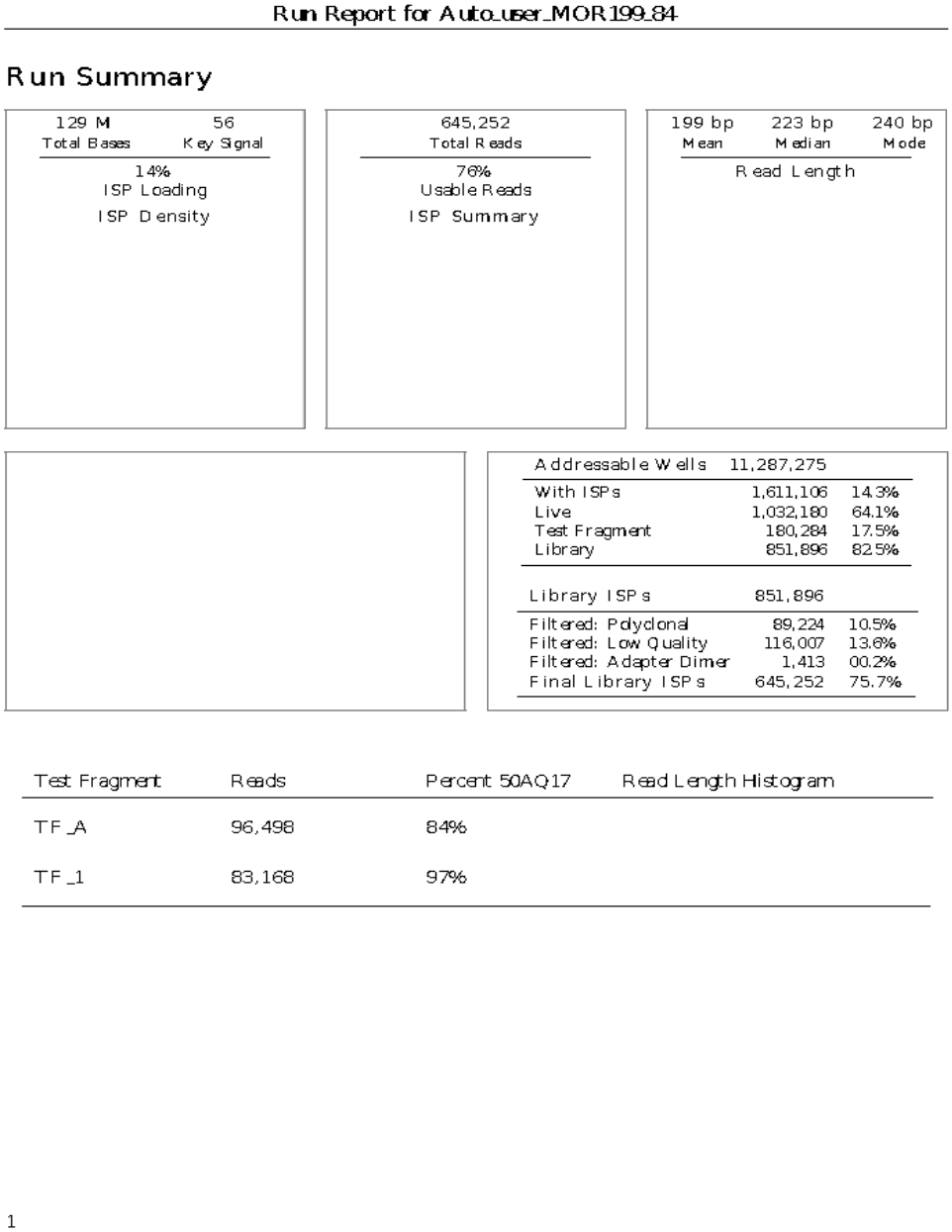
Read Length

Addressable Wells	11,287,275	
With ISPs	5,730,243	50.8%
Live	5,474,962	95.5%
Test Fragment	27,219	00.5%
Library	5,447,743	99.5%
Library ISPs	5,447,743	
Filtered: Polyclonal	1,832,418	33.6%
Filtered: Low Quality	213,082	03.9%
Filtered: Adapter Dimer	1,893	00.0%
Final Library ISPs	3,400,350	62.4%

Test Fragment	Reads	Percent 50AQ17	Read Length Histogram
TF_A	8,891	90%	
TF_1	17,915	92%	

1

Image1B: The image shows the next generation sequencing report and run summary for the shown sample



Run Report for Auto_user_MOR199_84

Run Summary

129 M Total Bases | 56 Key Signal
14% ISP Loading
ISP Density

645,252 Total Reads
76% Usable Reads
ISP Summary

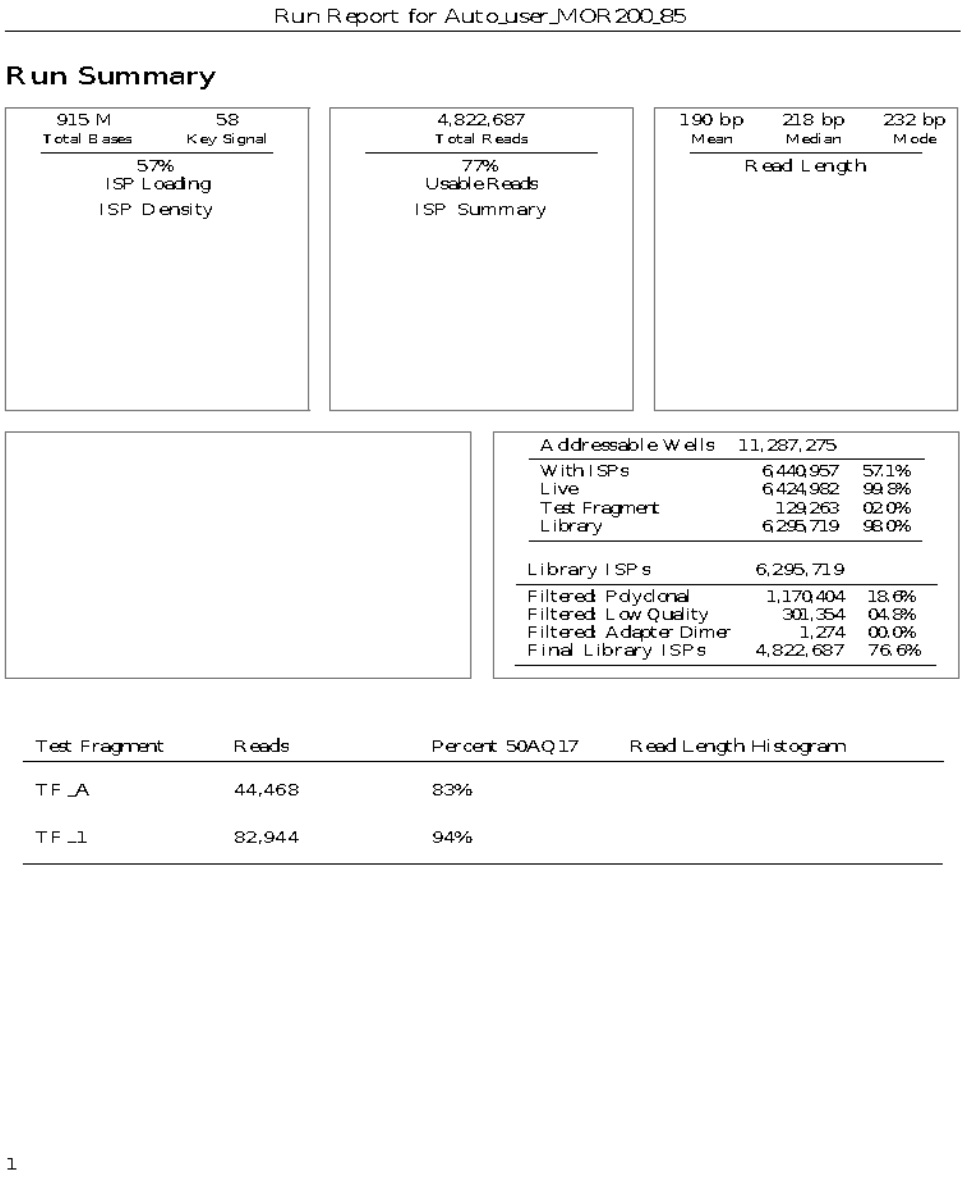
199 bp Mean | 223 bp Median | 240 bp Mode
Read Length

Addressable Wells	11,287,275	
With ISPs	1,611,106	14.3%
Live	1,032,180	64.1%
Test Fragment	180,284	17.5%
Library	851,896	82.5%
Library ISPs	851,896	
Filtered: Polyclonal	89,224	10.5%
Filtered: Low Quality	116,007	13.6%
Filtered: Adapter Dimer	1,413	00.2%
Final Library ISPs	645,252	75.7%

Test Fragment	Reads	Percent 50AQ17	Read Length Histogram
TF_A	96,498	84%	
TF_1	83,168	97%	

1

Image 1C: The image shows the next generation sequencing report and run summary for the shown sample



Run Report for Auto_user_MOR200_85

Run Summary

915 M Total Bases | 58 Key Signal

57% ISP Loading

ISP Density

4,822,687 Total Reads

77% Usable Reads

ISP Summary

190 bp Mean | 218 bp Median | 232 bp Mode

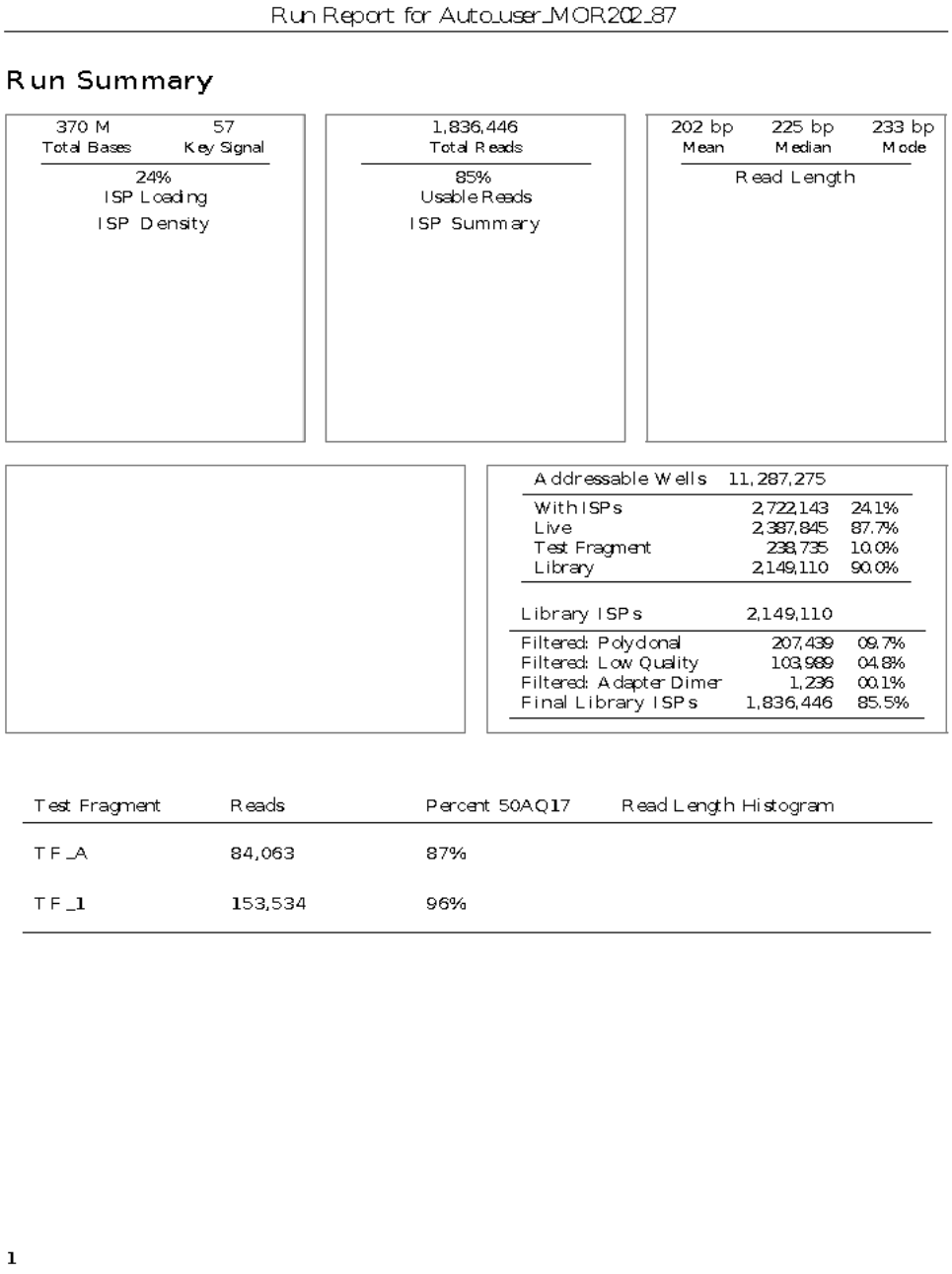
Read Length

Addressable Wells	11,287,275	
With ISPs	6,440,957	57.1%
Live	6,424,982	99.8%
Test Fragment	129,263	02.0%
Library	6,295,719	98.0%
Library ISPs	6,295,719	
Filtered: Polyclonal	1,170,404	18.6%
Filtered: Low Quality	301,354	04.8%
Filtered: Adapter Dimer	1,274	00.0%
Final Library ISPs	4,822,687	76.6%

Test Fragment	Reads	Percent 50AQ17	Read Length Histogram
TF_A	44,468	83%	
TF_1	82,944	94%	

1

Image 1D: The image shows the next generation sequencing report and run summary for the shown sample



Run Report for Auto_user_MOR202_87

Run Summary

370 M
Total Bases
57
Key Signal
24%
ISP Loading
ISP Density

1,836,446
Total Reads
85%
Usable Reads
ISP Summary

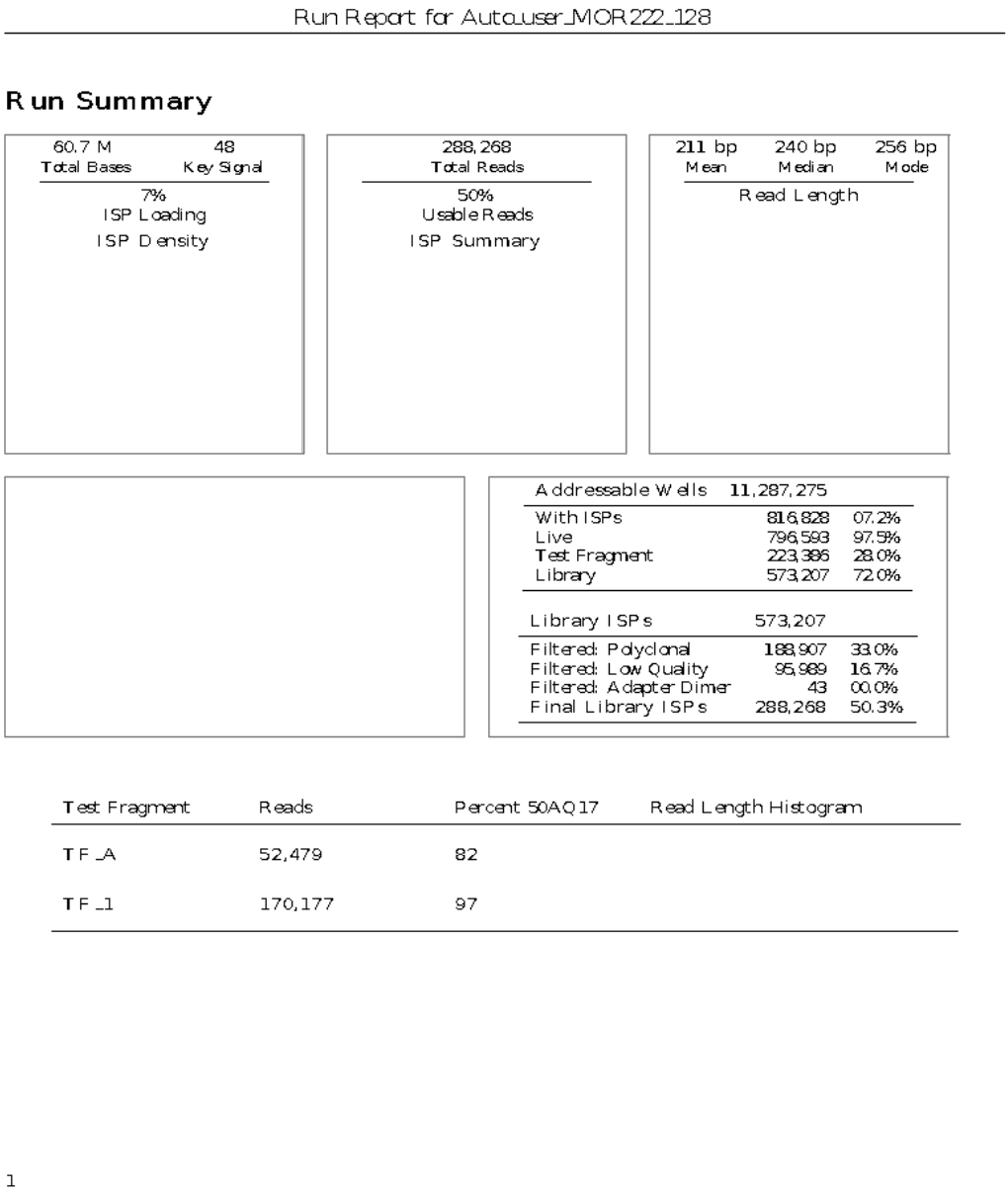
202 bp
Mean
225 bp
Median
233 bp
Mode
Read Length

Addressable Wells	11,287,275	
With ISPs	2,722,143	24.1%
Live	2,387,845	87.7%
Test Fragment	238,735	10.0%
Library	2,149,110	90.0%
Library ISPs	2,149,110	
Filtered: Polyclonal	207,439	09.7%
Filtered: Low Quality	103,989	04.8%
Filtered: Adapter Dimer	1,236	00.1%
Final Library ISPs	1,836,446	85.5%

Test Fragment	Reads	Percent 50AQ17	Read Length Histogram
TF_A	84,063	87%	
TF_1	153,534	96%	

1

Image 1E: The image shows the next generation sequencing report and run summary for the shown sample



Run Report for Auto_user_MOR222_128

Run Summary

60.7 M Total Bases | 48 Key Signal

7% ISP Loading

ISP Density

288,268 Total Reads

50% Usable Reads

ISP Summary

211 bp Mean | 240 bp Median | 256 bp Mode

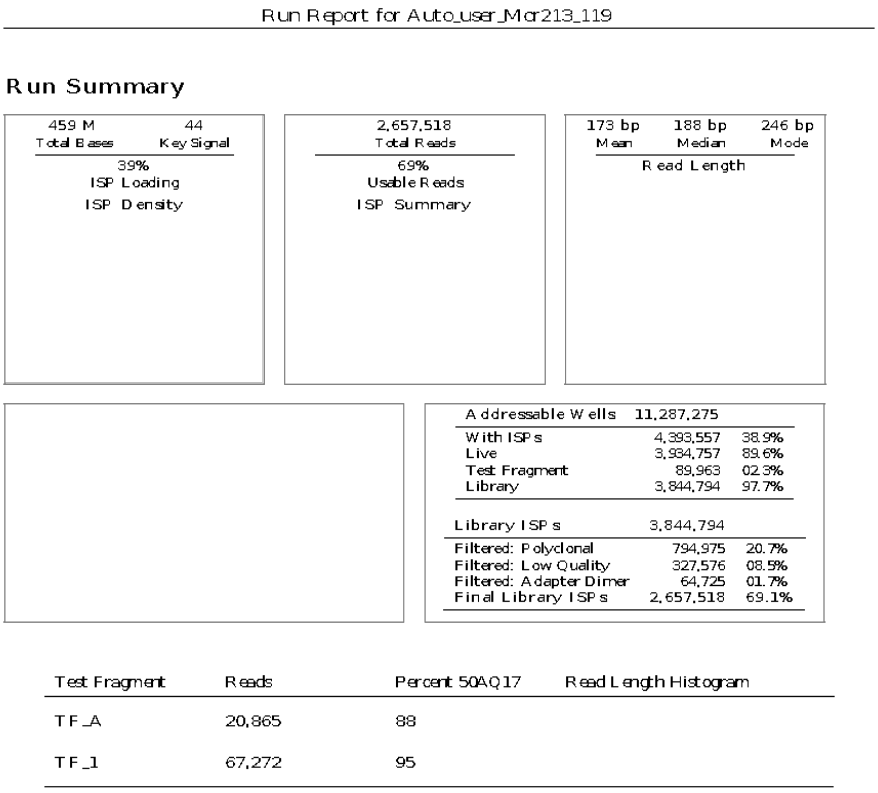
Read Length

Addressable Wells	11,287,275	
With ISPs	816,828	07.2%
Live	796,593	97.5%
Test Fragment	223,386	28.0%
Library	573,207	72.0%
Library ISPs	573,207	
Filtered: Polyclonal	188,907	33.0%
Filtered: Low Quality	95,989	16.7%
Filtered: Adapter Dimer	43	00.0%
Final Library ISPs	288,268	50.3%

Test Fragment	Reads	Percent 50AQ17	Read Length Histogram
TF_A	52,479	82	
TF_1	170,177	97	

1

Image 1F: The image shows the next generation sequencing report and run summary for the shown sample



Run Report for Auto_user_Mcr213_119

Run Summary

459 M Total Bases | 44 Key Signal

39% ISP Loading

ISP Density

2,657,518 Total Reads

69% Usable Reads

ISP Summary

173 bp Mean | 188 bp Median | 246 bp Mode

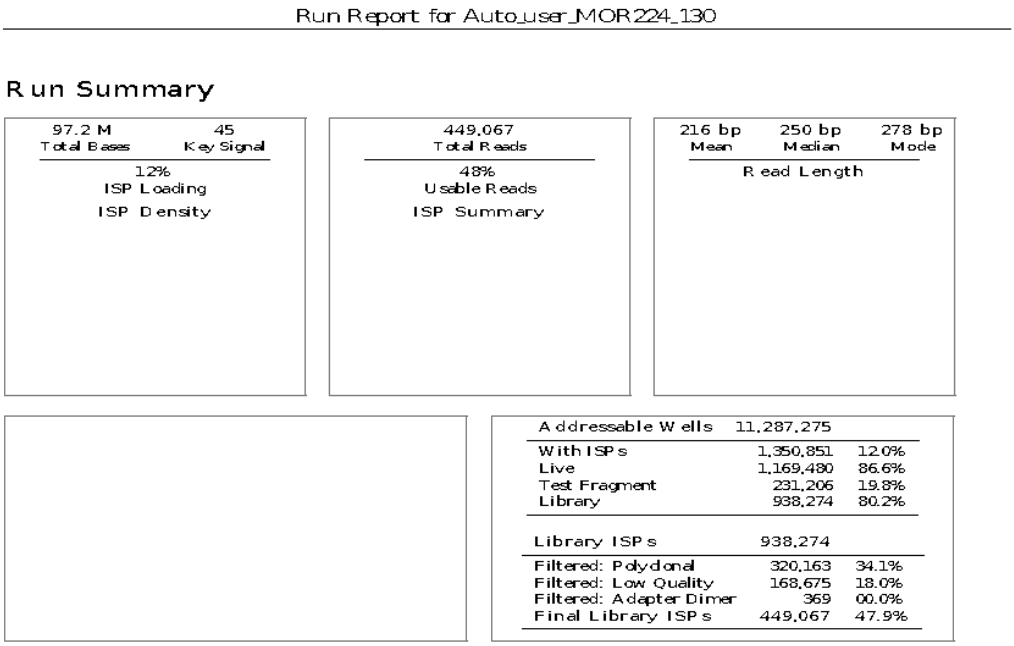
Read Length

Addressable Wells	11,287,275	
With ISPs	4,393,557	38.9%
Live	3,934,757	89.6%
Test Fragment	89,963	02.3%
Library	3,844,794	97.7%
Library ISPs	3,844,794	
Filtered: Polyclonal	794,975	20.7%
Filtered: Low Quality	327,576	08.5%
Filtered: Adapter Dimer	64,725	01.7%
Final Library ISPs	2,657,518	69.1%

Test Fragment	Reads	Percent 50AQ17	Read Length Histogram
TF_A	20,865	88	
TF_1	67,272	95	

1

Image 1G: The image shows the next generation sequencing report and run summary for the shown sample



Run Report for Auto_user_MOR224_130

Run Summary

97.2 M Total Bases | 45 Key Signal

12% ISP Loading

ISP Density

449,067 Total Reads

48% Usable Reads

ISP Summary

216 bp Mean | 250 bp Median | 278 bp Mode

Read Length

Addressable Wells	11,287,275	
With ISPs	1,350,851	12.0%
Live	1,169,480	86.6%
Test Fragment	231,206	19.8%
Library	938,274	80.2%
Library ISPs	938,274	
Filtered: Polyclonal	320,163	34.1%
Filtered: Low Quality	168,675	18.0%
Filtered: Adapter Dimer	369	00.0%
Final Library ISPs	449,067	47.9%

Test Fragment	Reads	Percent 50AQ17	Read Length Histogram
TF_A	51,673	85	
TF_1	178,879	97	

1

Image 1H: The image shows the next generation sequencing report and run summary for the shown sample

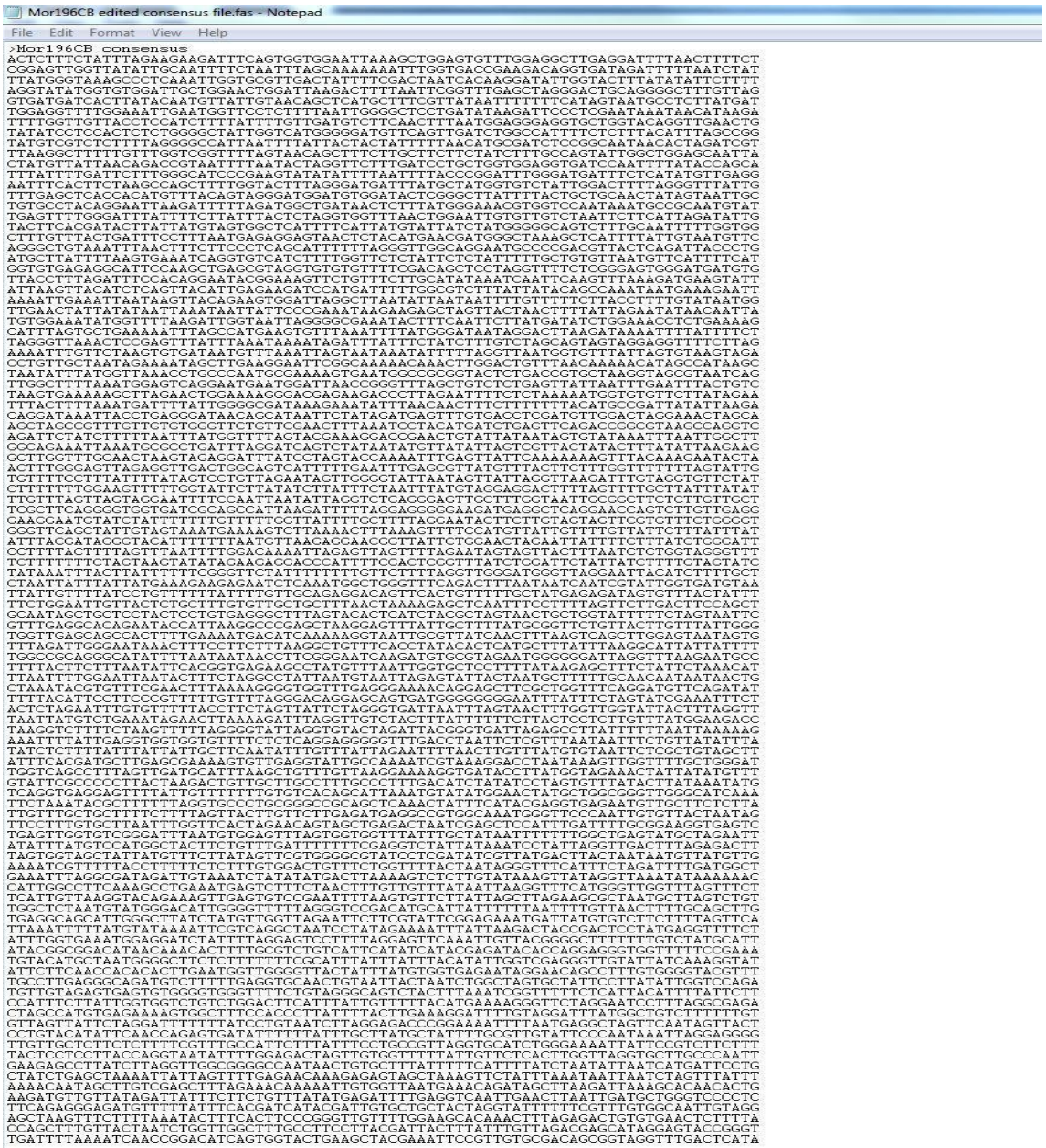


Image 2A-Portion of Mor196 consensus file

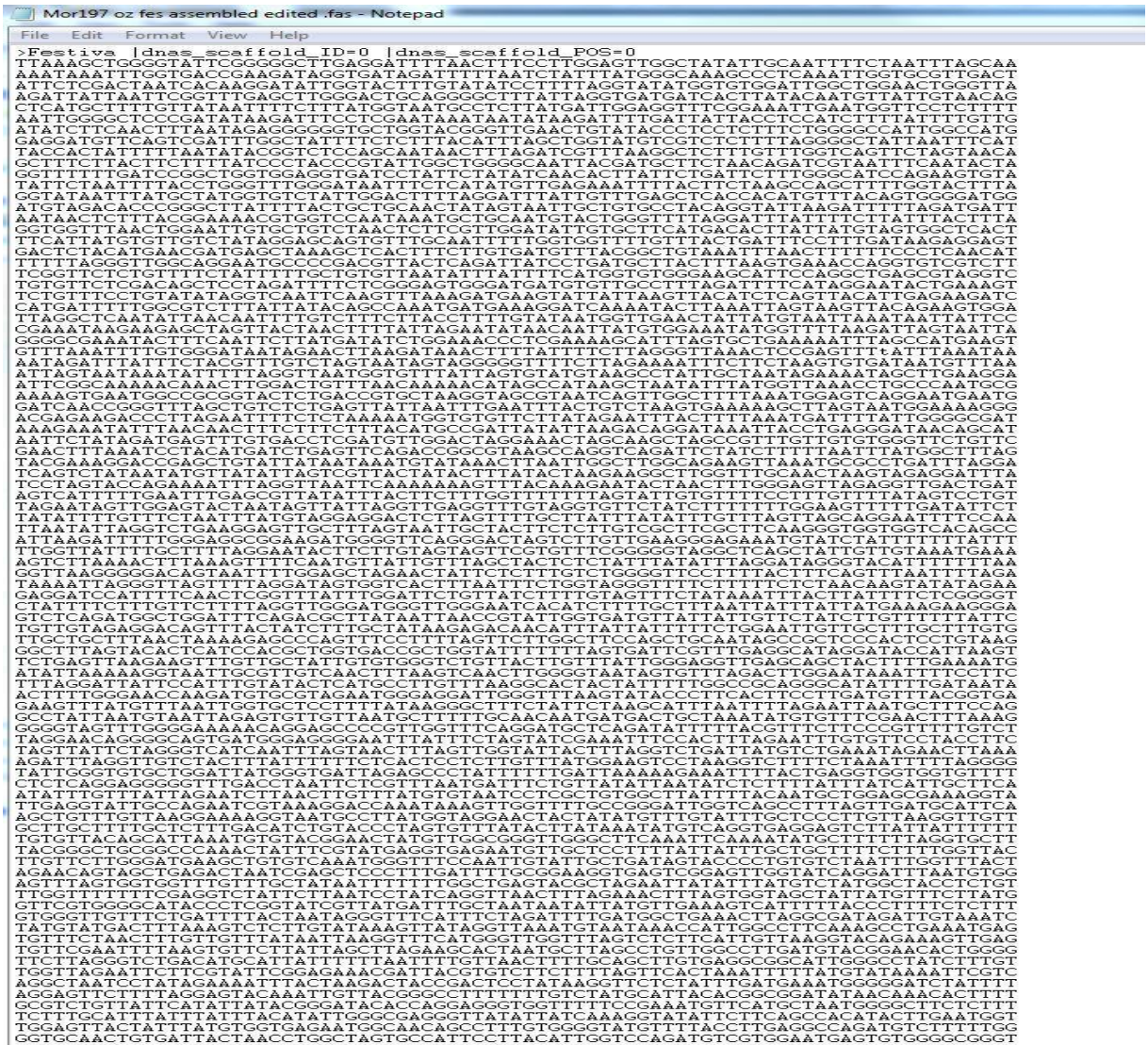


Image 2B-Portion of Mor197 consensus file

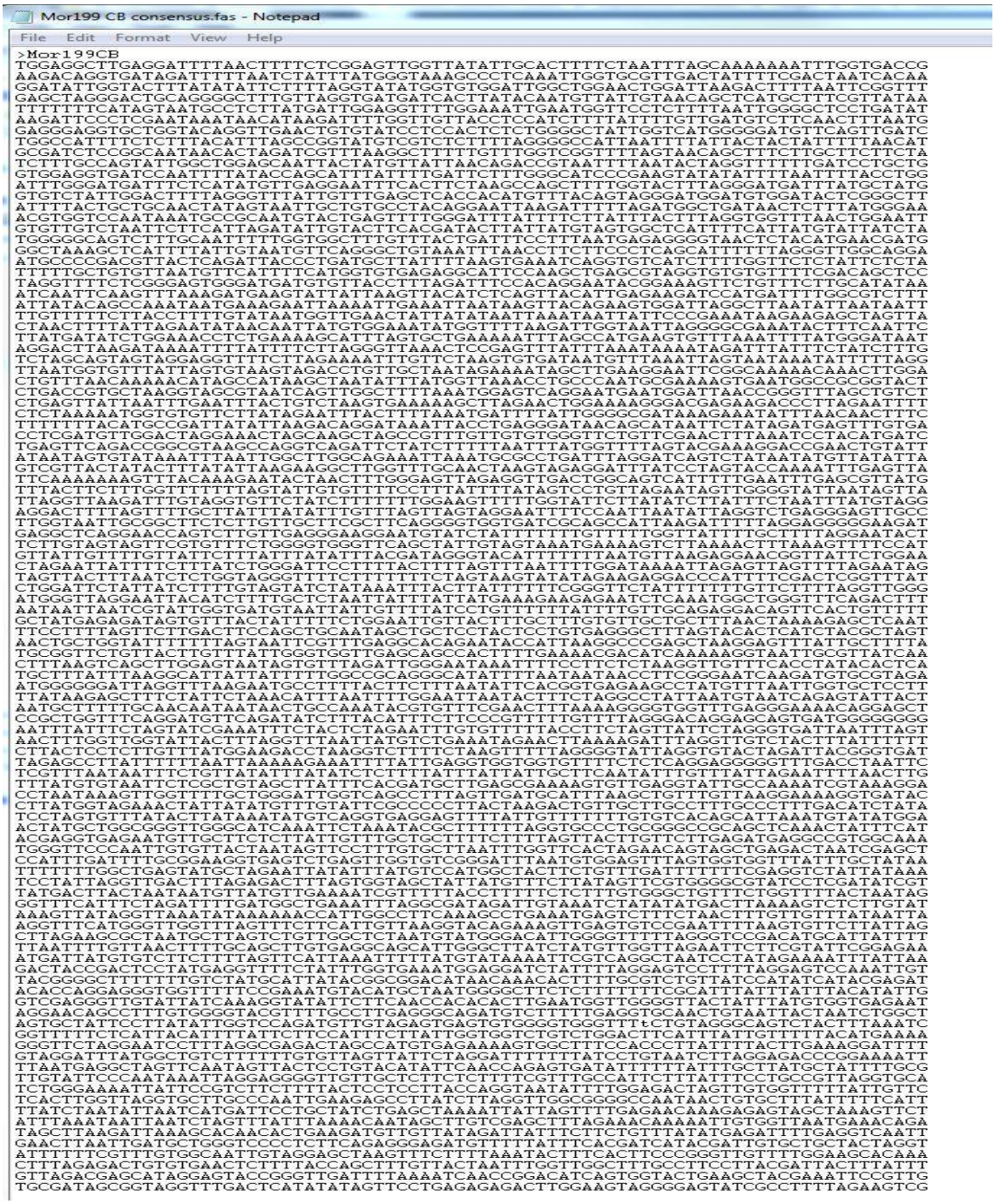


Image 2C: Portion of Mor199 Consensus file

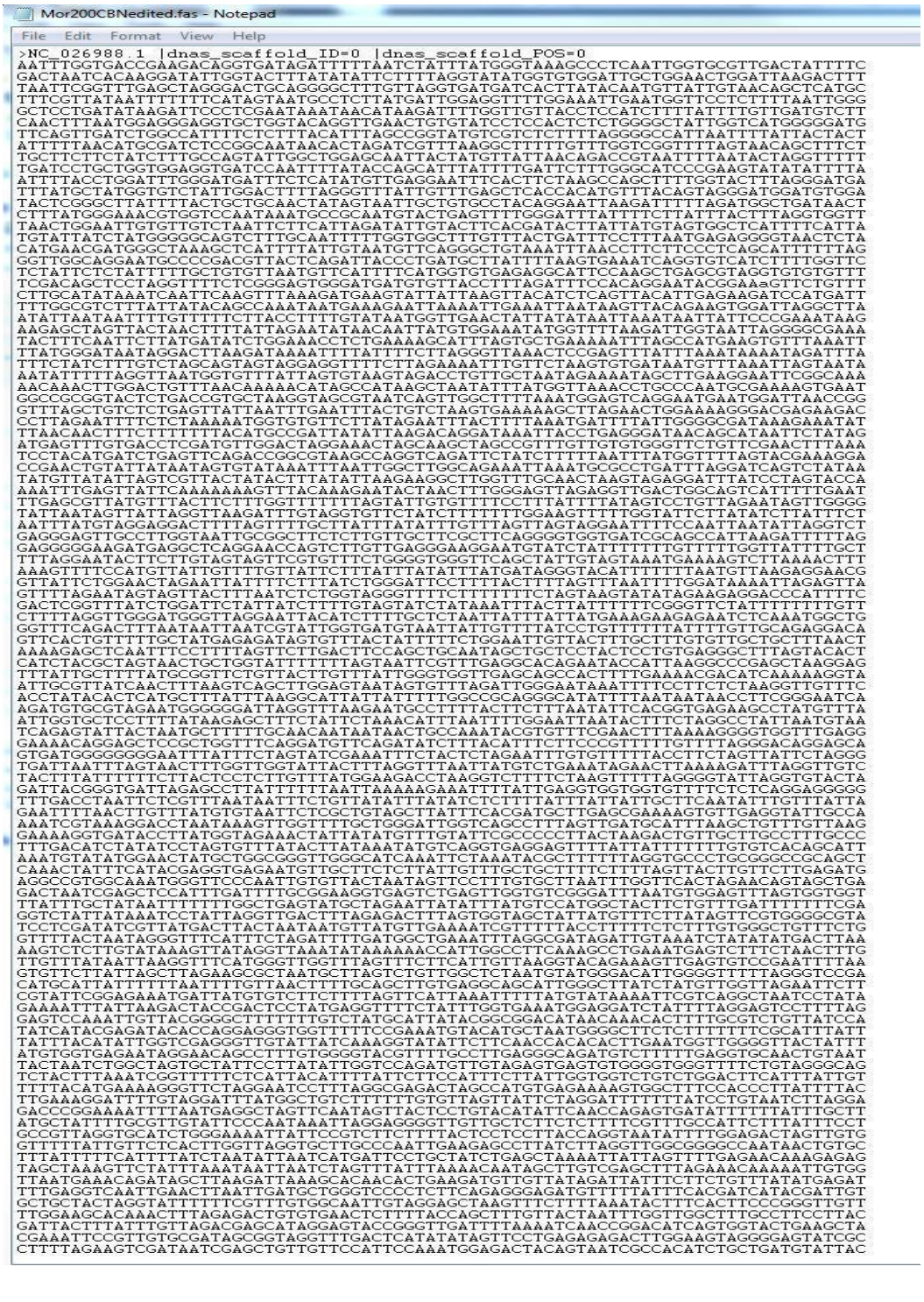


Image 2D- Portion of Mor200 consensus file

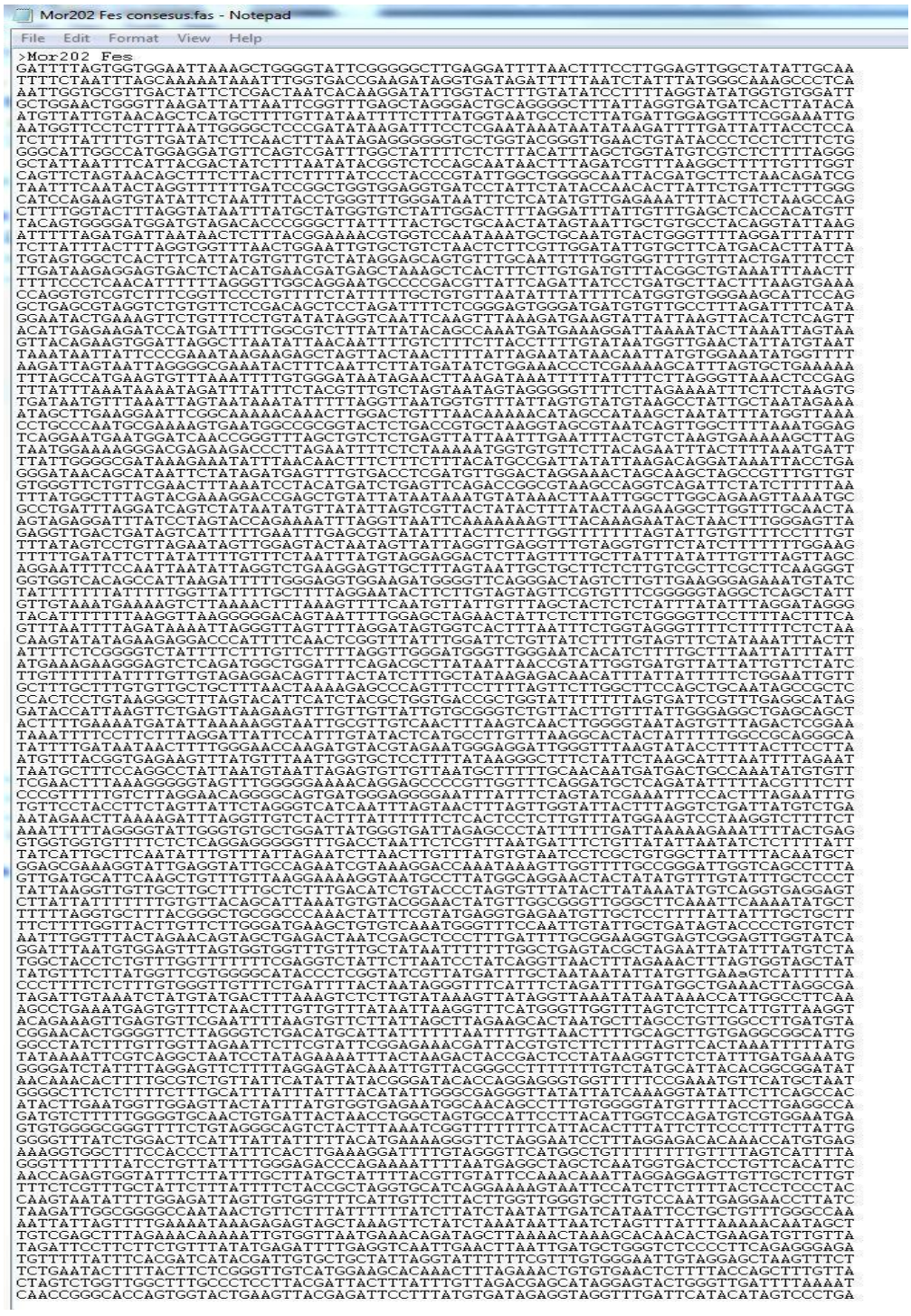


Image 2E- Portion of Mor202 consensus file

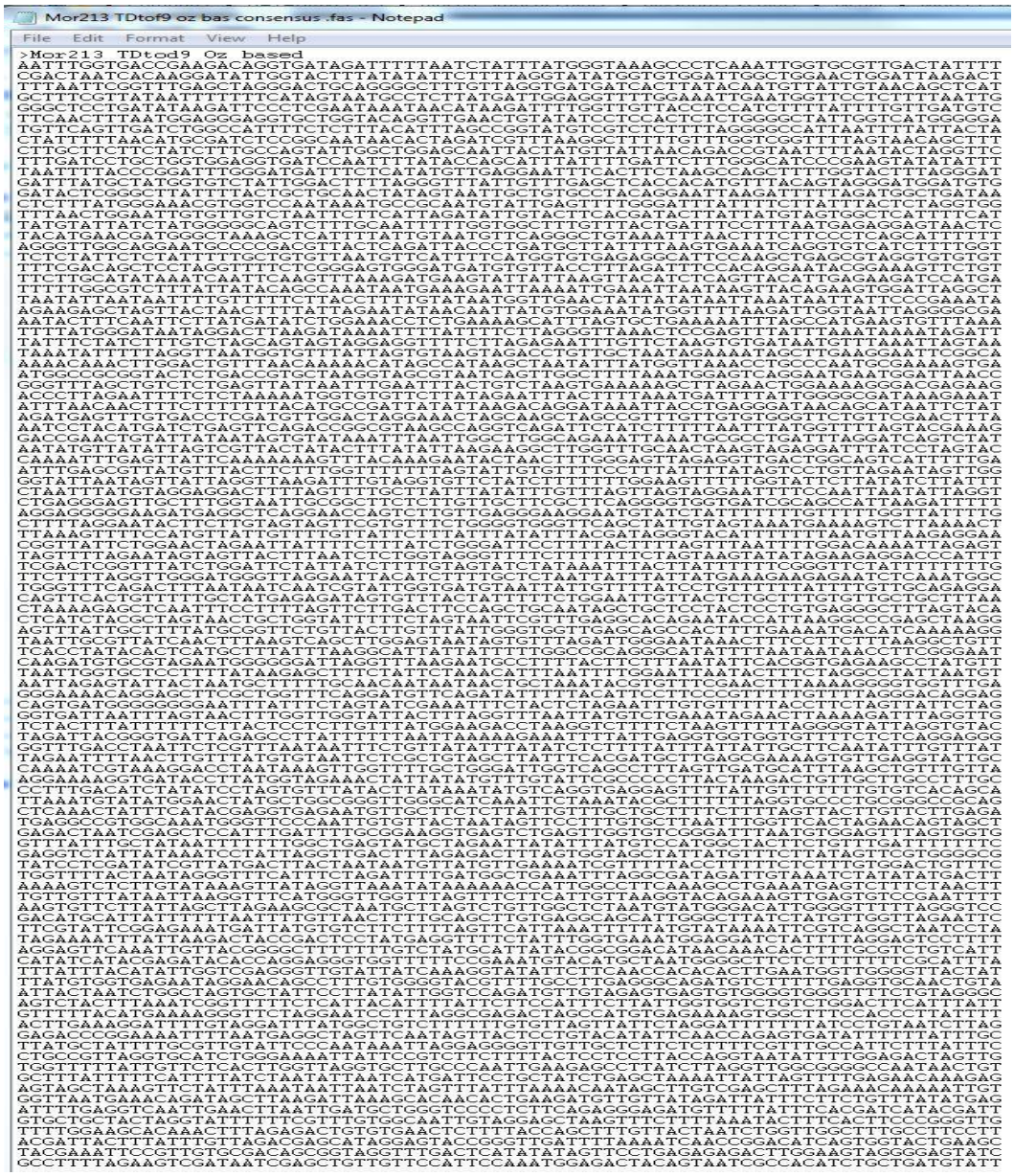


Image 2F: Portion of Mor213 Consensus file

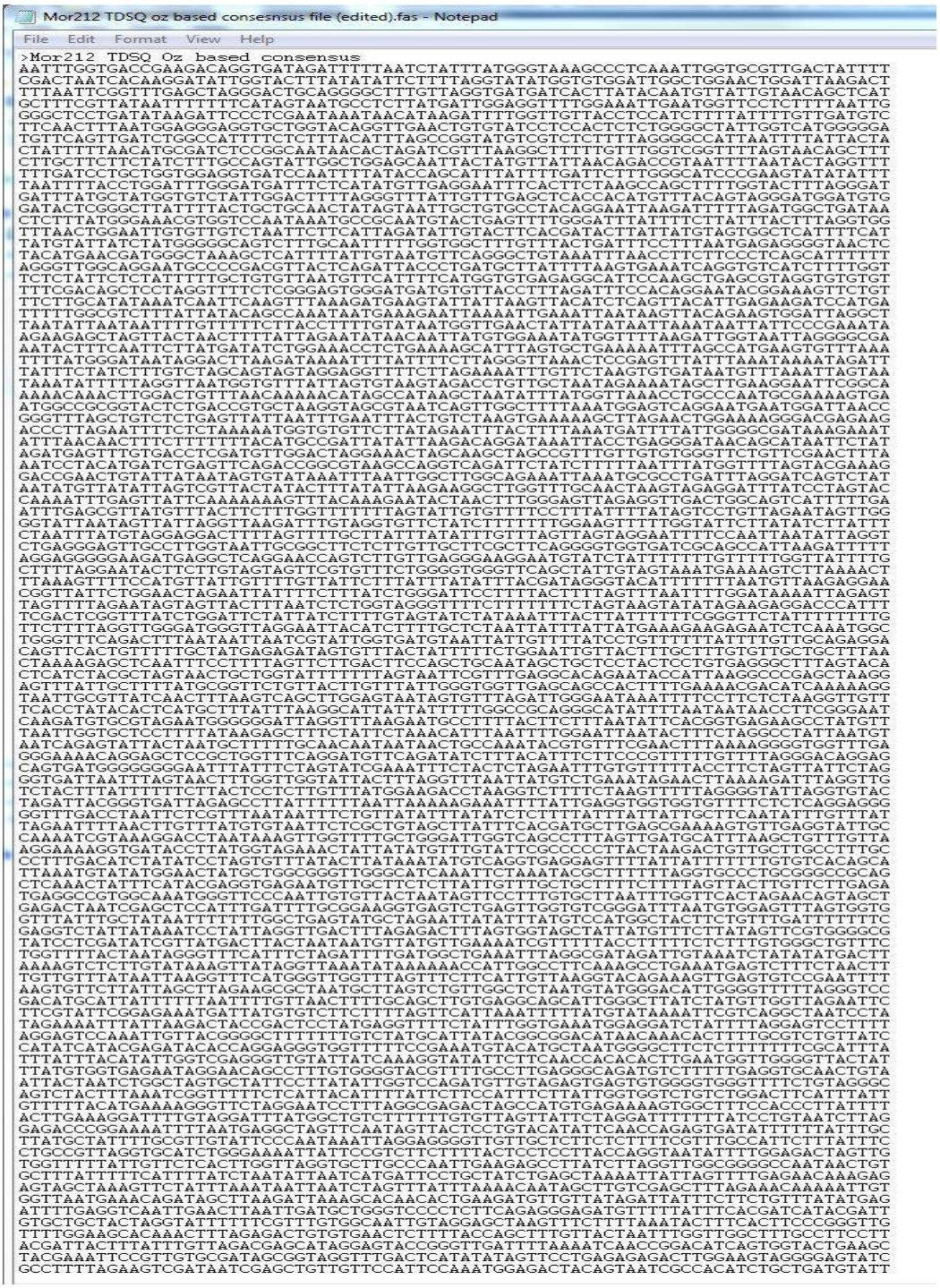


Image 2G: Portion of Mor212 Consensus file

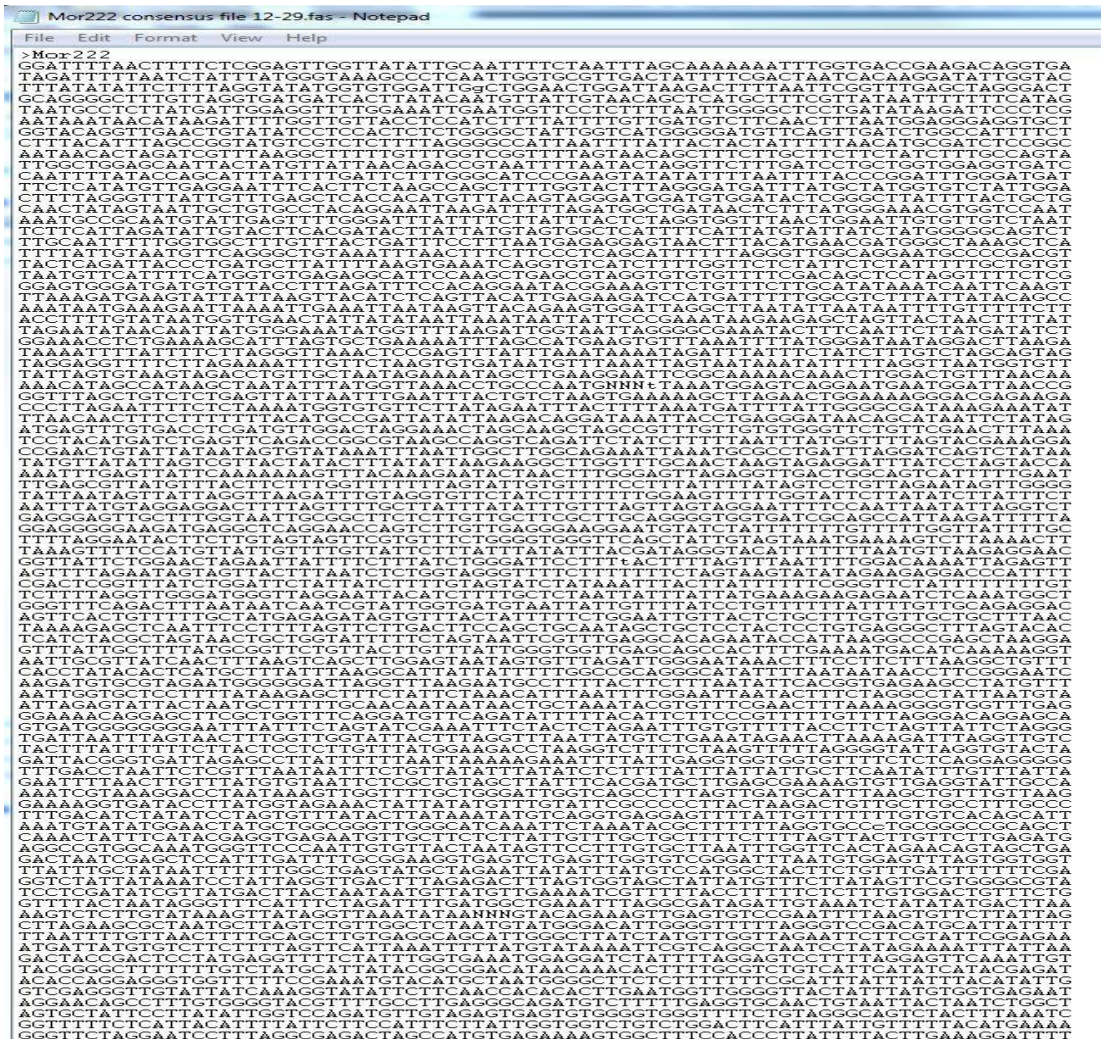


Image 2I: Portion of Mor222 Consensus file

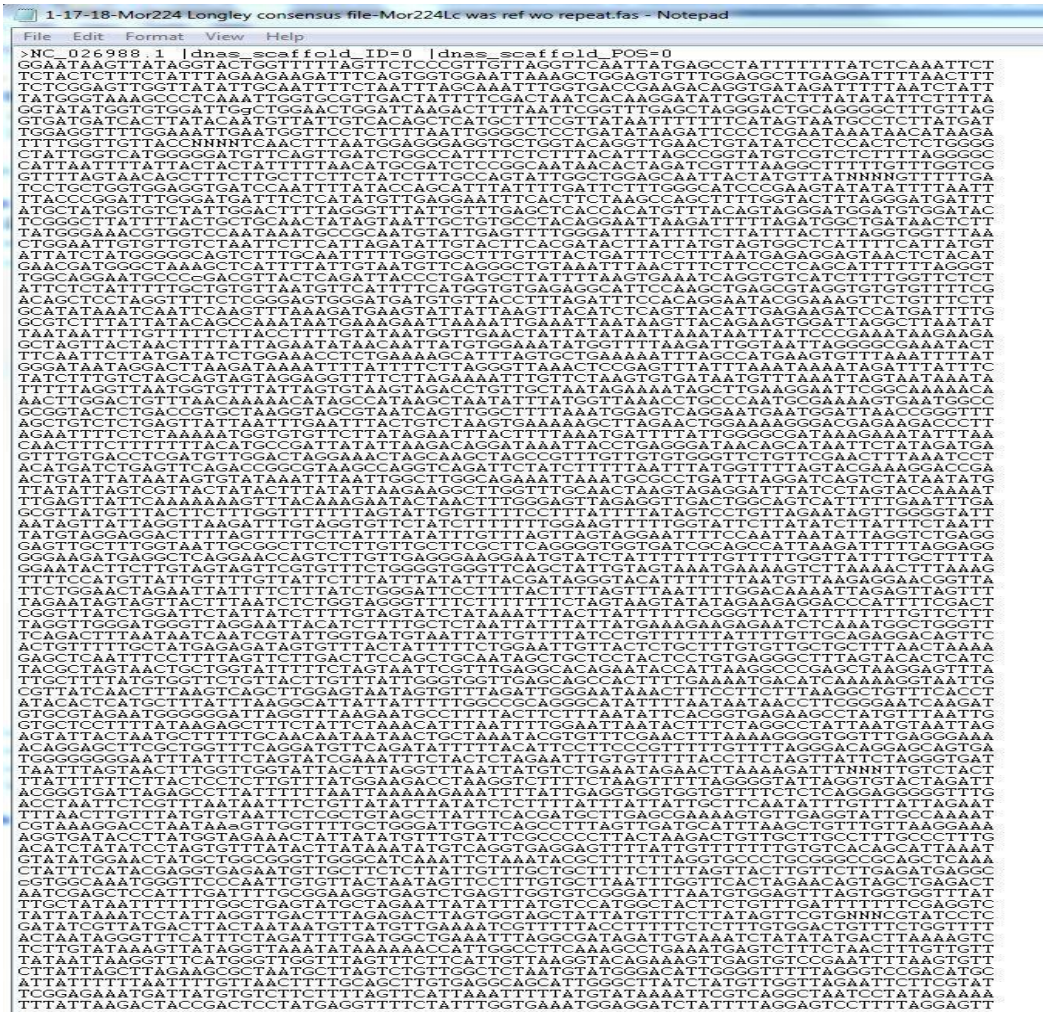


Image 2J: Portion of Mor224 Consensus file