

# Analytics Report: LEggert\_UMissouri\_Bass\_20180409-01669

September 04, 2018

## Table of Contents

- 1. Introduction
- 2. Sequencing QA/QC
- 3. Reference Assembly
- 4. Sequence Alignment
- 5. Variant Calling (VCF)
- 6. Tools and Software

541.343.0747



### 1. Introduction

Dear Floragenex Client,

Since 2008, the Floragenex genomics team has proudly provided end to end, full service commercial support for Restriction Site Associated DNA Sequencing (RAD–Seq) marker discovery, genotyping and analysis projects around the world. To date, we have completed over 200 projects in 75 plus species, and have facilitated a wide range of peer–reviewed publications in molecular genetics research.

Please find attached your customized project analysis report. This report was generated using a composite of software tools that have been optimized for bioinformatics analysis of RAD–Seq data. If you have any questions about this report or our analysis procedures, please do not hesitate to contact us. We look forward to helping advance your research.

Best regards,

Rick Nipper, Ph.D. President and CEO Floragenex, Inc.



# 2. Sequencing QA/QC

Section 2.1. FASTQ\_Sequence\_Files\_QAQC\_Report

## Illumina Sequencing FASTQ Quality Control / Quality Assurance Report

Project: LEggert\_UMissouri\_Bass\_20180409-01669

Directory: FASTQ\_Sequence\_Files

### A) Information

Project Name: LEggert\_UMissouri\_Bass\_20180409-01669

Sequence Directory: FASTQ\_Sequence\_Files Analysis Date: 2018-08-31T21:02:23

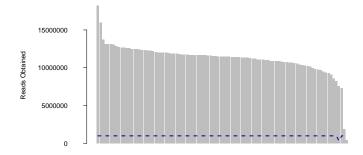
Enzyme: Pstl

Sequencing Chemistry: 1x95bp Number of Samples in Project: 96

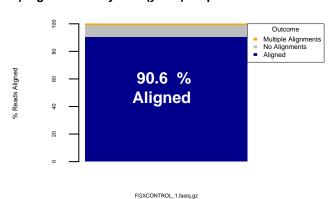
### **B) Statistics**

Total Reads in Project: 1076117933 Mean Reads per Sample: 11209561.8 Median Reads per Sample: 11483651.5 Read Goal per Sample: 1000000 Std. Dev of Reads per Sample: 2063521.9 Number of Samples Meeting Read Goals: 94 Sequencing Coefficient of Variation: 0.18

### C) Figure 1: Sequencing Distribution



### D) Figure 2: S. bayanus (yeast) Sequence Control Performance





### E) Individual Sample Sequencing Performance

E) Individual Sample Sequencing Performance									
				NGS	Sequencing Distribution	RAD Clusters	Coverage Distribution	Coverages Low. Quartile: Median:	vs Read Length
Sample Name	NGS Reads	Read Goal	RE	Туре	0 % 100 % of goal	(5x to 1000x)	5x 15x 25x 100		0bp 95bp
GLVR11_1.fastq.gz	18217476	1000000	Pstl	1x95bp		501562		9:20:40	
SPRMO19_1.fastq.gz	15992260	1000000	PstI	1x95bp		459332		8:17:35	
BFC06_1.fastq.gz	13700609	1000000	Pstl	1x95bp		410515		9:18:34	
GRSPB36_1.fastq.gz	13118659	1000000	PstI	1x95bp		394579		9:18:34	
AR21_1.fastq.gz	13117475	1000000	Pstl	1x95bp		443463		8:14:29	
SKIA04_1.fastq.gz	13102064	1000000	PstI	1x95bp		363280		9:18:36	
AT12_1.fastq.gz	13078746	1000000	Pstl	1x95bp		433319		9:18:31	
BC09_1.fastq.gz	12882477	1000000	Pstl	1x95bp		457298		8:16:28	
NOIS08_1.fastq.gz	12754802	1000000	PstI	1x95bp		405543		9:17:32	
BFC02_1.fastq.gz	12683495	1000000	Pstl	1x95bp		419397		9:17:31	
BFORK02_1.fastq.gz	12679586	1000000	PstI	1x95bp		421942		8:15:30	
BFC10_1.fastq.gz	12609609	1000000	Pstl	1x95bp		497601		8:15:24	
GRSPB50_1.fastq.gz	12597697	1000000	PstI	1x95bp		432386		8:16:29	
ER17_1.fastq.gz	12484195	1000000	Pstl	1x95bp		433756		9:17:29	
SKIA09_1.fastq.gz	12473333	1000000	Pstl	1x95bp		416697		9:16:30	
HC44_1.fastq.gz	12452292	1000000	Pstl	1x95bp		417921		8:16:30	
BC14_1.fastq.gz	12372444	1000000	Pstl	1x95bp		419244		9:16:29	
ER20_1.fastq.gz	12350664	1000000	Pstl	1x95bp		449118		9:16:28	
AT02_1.fastq.gz	12344293	1000000	Pstl	1x95bp		433462		9:17:29	
AR18_1.fastq.gz	12287384	1000000	Pstl	1x95bp		418251		8:16:30	
AT09_1.fastq.gz	12281374	1000000	Pstl	1x95bp		403888		9:17:31	
NOIS18_1.fastq.gz	12170181	1000000	PstI	1x95bp		402450		9:17:31	
MI421_1.fastq.gz	12053883	1000000	PstI	1x95bp		388846		10:19:32	
ER33_1.fastq.gz	12023608	1000000	Pstl	1x95bp		407655		9:16:30	
HC43_1.fastq.gz	12021025	1000000	Pstl	1x95bp		402424		9:18:31	
AR16_1.fastq.gz	12002934	1000000	Pstl	1x95bp		403305		8:15:30	
MI425_1.fastq.gz	11988170	1000000	PstI	1x95bp		408991		9:17:30	
BFC49_1.fastq.gz	11899052	1000000	Pstl	1x95bp		414379		9:17:29	
BC01_1.fastq.gz	11868958	1000000	Pstl	1x95bp		402893		9:17:30	
STOCK06_1.fastq.gz	11841730	1000000	PstI	1x95bp		393467		8:15:28	
GRSPB41_1.fastq.gz	11832689	1000000	PstI	1x95bp		398954		8:16:30	
SPVW07_1.fastq.gz	11814887	1000000	PstI	1x95bp		371497		8:16:31	
SPVW05_1.fastq.gz	11760746	1000000	PstI	1x95bp		391294		8:15:30	
AT08_1.fastq.gz	11731028	1000000	Pstl	1x95bp		428865		8:15:28	
GRSPB51_1.fastq.gz	11730184	1000000	PstI	1x95bp		408032		8:15:29	
BC07_1.fastq.gz	11693232	1000000	Pstl	1x95bp		429869		8:15:27	
AT05_1.fastq.gz	11686746	1000000	Pstl	1x95bp		410440		8:16:28	
GRSPB39_1.fastq.gz	11674383	1000000	Pstl	1x95bp		379090		8:14:29	
BC08_1.fastq.gz	11671727	1000000	Pstl	1x95bp		437159		8:14:26	
GRSPB03_1.fastq.gz	11665906	1000000	Pstl	1x95bp		404952		8:16:29	
NOIS07_1.fastq.gz	11653095	1000000	Pstl	1x95bp		389238		9:17:31	
STOCK03_1.fastq.gz	11648079	1000000	Pstl	1x95bp		360428		9:17:33	
MI420_1.fastq.gz	11619901	1000000	Pstl	1x95bp		404812		9:17:29	
AR29_1.fastq.gz	11592215	1000000	Pstl	1x95bp		421544		8:14:27	
GRSPB35_1.fastq.gz	11547795	1000000	PstI	1x95bp		401880		8:15:29	
GRSPB52_1.fastq.gz	11546152	1000000	Pstl	1x95bp		423773		8:16:28	
BFORK49_1.fastq.gz	11486097	1000000	PstI	1x95bp		417428		8:15:28	
SPVW12_1.fastq.gz	11484081	1000000	PstI	1x95bp		386707		8:16:30	
TBLR02_1.fastq.gz	11483222	1000000	PstI	1x95bp		427529		8:15:27	
SPRMO11_1.fastq.gz	11450594	1000000	PstI	1x95bp		414262		7:13:26	
ER35_1.fastq.gz	11438869	1000000	Pstl	1x95bp		419289		8:15:27	
MI422_1.fastq.gz	11384414	1000000	PstI	1x95bp		375151		9:17:31	
GRSPB37_1.fastq.gz	11380653	1000000	PstI	1x95bp		426393		8:15:27	
BP07_1.fastq.gz	11376973	1000000	Pstl	1x95bp		412819		8:15:27	
SPVW02_1.fastq.gz	11370210	1000000	Pstl	1x95bp		393795		9:16:29	
GLVR4_1.fastq.gz	11352894	1000000	Pstl	1x95bp		409009		8:14:27	
SPRMO49_1.fastq.gz	11333848	1000000	PstI	1x95bp		421525		8:15:27	
BFORK24_1.fastq.gz	11322723	1000000	Pstl	1x95bp		421245		8:14:26	
MI419_1.fastq.gz	11295066	1000000	Pstl	1x95bp		413970		9:16:28	
BP17_1.fastq.gz	11188566	1000000	PstI	1x95bp		422105		8:15:27	
. 5				•		722100	, L	3.13.27	



### E) Individual Sample Sequencing Performance

E) Individual Sample S	Sequencing F	erforman	ce	NGS	Sequencing Distribution	RAD Clusters	Coverage Distribution	Coverages Low. Quartile:	Median Phred QScore vs Read Length
Sample Name	NGS Reads	Read Goal	RE	Туре	0 % 100 % of goal	(5x to 1000x)	5x 15x 25x 100x	250x Median: Upp. Quartile	0bp 95bp
SPVW14_1.fastq.gz	11187685	1000000	PstI	1x95bp		399425		8:15:28	
ER30_1.fastq.gz	11102055	1000000	Pstl	1x95bp		382156		8:16:29	
CANEY16_1.fastq.gz	11084736	1000000	PstI	1x95bp		397863		8:15:28	
SC06_1.fastq.gz	11038455	1000000	Pstl	1x95bp		416619		9:16:27	
BP01_1.fastq.gz	11015654	1000000	PstI	1x95bp		341451		8:15:30	
TBLR03_1.fastq.gz	10975208	1000000	PstI	1x95bp		403458		8:14:27	
HC41_1.fastq.gz	10900200	1000000	Pstl	1x95bp		405850		8:15:27	
BP10_1.fastq.gz	10899720	1000000	PstI	1x95bp		427223		8:15:26	
TBLR04_1.fastq.gz	10869552	1000000	PstI	1x95bp		406214		8:14:26	
CANEY15_1.fastq.gz	10849620	1000000	PstI	1x95bp		396071		8:15:27	
AR19_1.fastq.gz	10818029	1000000	Pstl	1x95bp		404294		8:15:27	
ER44_1.fastq.gz	10757734	1000000	Pstl	1x95bp		391406		9:16:27	
GRSPB02_1.fastq.gz	10709036	1000000	PstI	1x95bp		398950		8:14:26	
BP02_1.fastq.gz	10691523	1000000	PstI	1x95bp		404749		8:15:26	
NOIS12_1.fastq.gz	10655620	1000000	PstI	1x95bp		393102		8:13:26	
BC12_1.fastq.gz	10633483	1000000	Pstl	1x95bp		391639		8:15:26	
HC40_1.fastq.gz	10571344	1000000	PstI	1x95bp		412767		8:14:25	
STOCK04_1.fastq.gz	10384320	1000000	PstI	1x95bp		316431		8:16:32	
AR30_1.fastq.gz	10321742	1000000	Pstl	1x95bp		382304		8:14:26	
SKIA05_1.fastq.gz	10249968	1000000	PstI	1x95bp		360100		8:15:29	
GRSPB69_1.fastq.gz	10210701	1000000	PstI	1x95bp		388053		8:15:26	
MI423_1.fastq.gz	10135162	1000000	PstI	1x95bp		380758		8:15:26	
HC38_1.fastq.gz	9961349	1000000	PstI	1x95bp		402480		7:12:23	
BFORK23_1.fastq.gz	9836489	1000000	PstI	1x95bp		388978		8:14:24	
SKIA06_1.fastq.gz	9764384	1000000	PstI	1x95bp		357052		8:15:27	
SPVW11_1.fastq.gz	9659111	1000000	PstI	1x95bp		357142		8:14:26	
SC37_1.fastq.gz	9483195	1000000	Pstl	1x95bp		380912		8:14:25	
ER05_1.fastq.gz	9384025	1000000	Pstl	1x95bp		230408		11:22:39	
HC42_1.fastq.gz	9279559	1000000	PstI	1x95bp		355806		8:15:26	
TBLR01_1.fastq.gz	9098318	1000000	PstI	1x95bp		378475		7:13:23	
SKIA03_1.fastq.gz	8559541	1000000	PstI	1x95bp		331928		8:14:25	
STOCK05_1.fastq.gz	8204441	1000000	PstI	1x95bp		328770		8:14:24	
FGXCONTROL_1.fastq.gz	7589235	330000	PstI	1x95bp		37528		5:6:9	
BFORK30_1.fastq.gz	7291023	1000000	Pstl	1x95bp		241816		8:13:24	
GRSPB34_1.fastq.gz	1873661	1000000	PstI	1x95bp		23426		10:29:75	
BFORK32_1.fastq.gz	404605	1000000	PstI	1x95bp		8122		5:6:7	





# 3. Reference Assembly

Section 3.1. AR21\_denovo\_clusters\_SR\_denovo\_Clustering\_Report

### RAD-Seq Single End Clustering Report

Project: LEggert\_UMissouri\_Bass\_20180409-01669 FASTA Assembly: AR21\_denovo\_clusters.fasta

### A) Information

Project: LEggert\_UMissouri\_Bass\_20180409-01669

Date: 2018-08-31T18:15:45

FASTQ file used for Clustering: AR21\_1.fastq.gz FASTA genome directory: Genome\_Assemblies FASTA genome name: AR21\_denovo\_clusters.fasta

Clustering Options:

Min. Cluster Depth: 10 x Max. Cluster Depth: 1000 x 5' Trimming: 0 bp 3' Trimming 0 bp Max. Variants / Cluster: 3 Max. Haplotypes / Cluster: 2

#### **B) Statistics**

Total Reads in Sample AR21\_1.fastq.gz: 13117475 Reads Passing FASTQ Quality Filters: 12859536 Reads Failing FASTQ Quality Filters: 257939 Reads in Provisional Clusters: 8491818 Total Contigs in Provisonal Clusters: 288336 Contigs Extracted from Provisonal Clusters: 48248

Total Contigs in Final Assembly: 240085

Contig Length: 96 bp

Total Cluster Length: 23048160 bp

### C) Figure 1. Assembly Metrics

Sample NGS Reads

13<sub>-</sub>1 M

Sample NGS Reads in Provisional Assembly

**Contigs in Provisional Clusters** 

288.3 K

**Contigs Removed** from Provisional Clusters

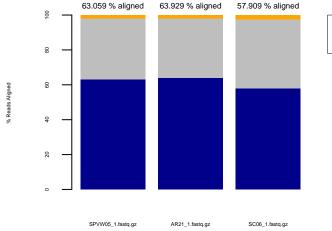
8.5 M (64.7 %) 48.2 K (16.7 %) 23.0 Mb

**Contigs Retained** in Final Clusters

240.1 K (83.3 %)

Approx. Assembly Length

### D) Figure 2. Test Alignment Performance of Clusters: AR21\_denovo\_clusters.fasta



Multiple AlignmentsNo AlignmentsAligned





# 4. Sequence Alignment

Section 4.1. BAM\_mpileups\_Alignment\_Report

## Alignment Summary: BAM\_mpileups

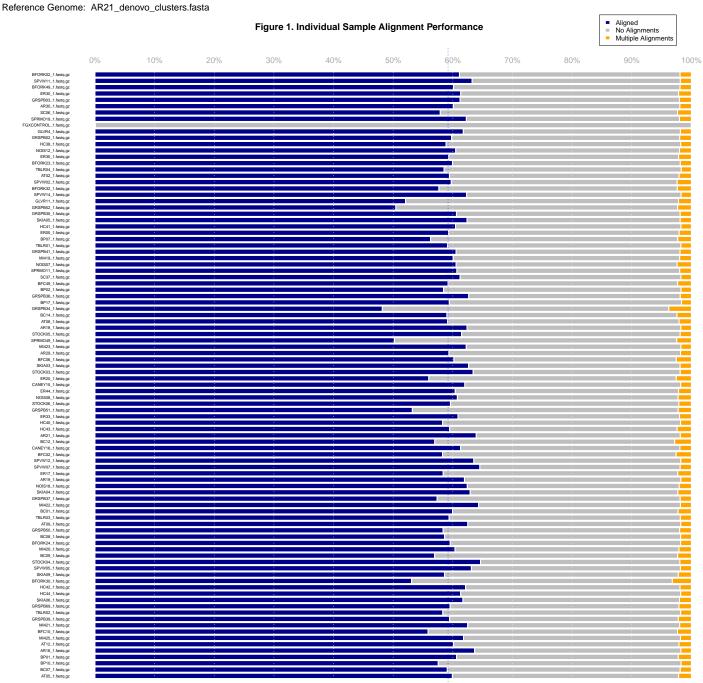
### A) Information and Statistics

Project Name: LEggert\_UMissouri\_Bass\_20180409-01669

Analysis Date: 2018-09-01T19:15:59 Sequence Directory: FASTQ\_Sequence\_Files BAM Pileup Directory: BAM\_mpileups

0%

Avg % Reads Aligning to Reference: 59.2 Avg % Reads Failing to Align: 38.8 Avg % Reads with Multiple Alignments: 2.0



30%

40%

50%

60%



100%



# 5. Variant Calling (VCF)

Section 5.1. AR21\_Aligned\_Genotypes\_VCF\_Report

## VCF File Summary: AR21\_Aligned\_Genotypes\_relaxed.vcf

### A) Information

VCF File: AR21\_Aligned\_Genotypes\_relaxed.vcf

Date: 2018-09-02T03:39:11

Project: LEggert\_UMissouri\_Bass\_20180409-01669 Reference Genome: AR21\_denovo\_clusters.fasta

BAM / mpileup Folder: BAM\_mpileups

VCF Options:

Min. % Population Genotyped: 75
Min. Individual Sequencing Depth: 6 x
Min. Individual Genotype Quality: 10
Min. FASTQ Quality Score: 20
MAF to be Screened: 0.2
Min. Distance Free of Other Variants: 50
Genome Landscape Flanking SNP: 50

### **B) Variant Statistics**

Number of Samples Screened: 95

Total Candidate Variants Detected: 357123

Candidate Variants Filtered (due to missing or low quality data): 77144

Candidate Variants with Low Probability: 2066 Candidate Variants Passing All Filters: 277913

Average Number of Polymorphisms within 200bp of Each Variant: 3 Sequencing Coverage Across Population for Each Variant: 3249.0 x

Variant Transition / Transversion Ratio: 1.43

Transitions: 167236 A->G: 61214 C->T: 106022 Transversions: 116934

> G->T: 41133 A->C: 21860 A->T: 27627 C->G: 26314

### C) Genotyping Statistics

Total Genotypes in VCF File: 26598005

Genotypes Scored: 25824119 Genotypes Missing: 773886

Average Individual Genotype Quality: 69.6 (Phred-scaled) Average Individual Sequencing Coverage: 34.2 x

Homozygous Genotypes: 23202008 Heterozygous Genotypes: 2622111

#### D) Genotyping Design Statistics (if applicable)

Variants Screened: 0

Variants Passing Design Criteria: 0 Variants Failing Design Criteria: 0

Failed due to flanking polymorphism: 0 Failed due to lack of flanking sequence: 0

Figure 1. Variants Passing Filters

277.9 K

#### Filtering Criteria:

Minimum Sequencing Depth per Sample: 6 x Minimum Phred Scaled Genotype Quality Per Sample: 10 Minimum Percent of Population Genotyped: 75 %

Figure 2. Histogram Plotting Number of Variations Over 200bp Genomic Interval

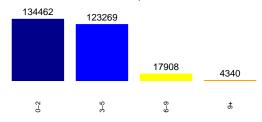


Figure 3. Variant Transition / Transversion Rates

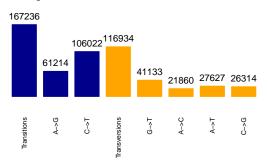
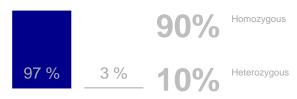


Figure 4.
Percentage of Missing
Genotype Data

Figure 5. Percent Homozygous & Heterozygous Genotypes



Quality Genotype Missing Genotype

Figure 6. Individual Sequencing Coverage and Genotype Quality

34.2x

Average Sequencing Coverage

69.6





## VCF File Summary: AR21\_Aligned\_Genotypes\_standard.vcf

### A) Information

VCF File: AR21\_Aligned\_Genotypes\_standard.vcf

Date: 2018-09-02T03:41:20

Project: LEggert\_UMissouri\_Bass\_20180409-01669 Reference Genome: AR21\_denovo\_clusters.fasta

BAM / mpileup Folder: BAM\_mpileups

VCF Options:

Min. % Population Genotyped: 82.5 Min. Individual Sequencing Depth: 10 x Min. Individual Genotype Quality: 13 Min. FASTQ Quality Score: 20 MAF to be Screened: 0.2 Min. Distance Free of Other Variants: 50 Genome Landscape Flanking SNP: 50

### **B) Variant Statistics**

Number of Samples Screened: 95

Total Candidate Variants Detected: 357124

Candidate Variants Filtered (due to missing or low quality data): 136885

Candidate Variants with Low Probability: 1167 Candidate Variants Passing All Filters: 219071

Average Number of Polymorphisms within 200bp of Each Variant: 2.9 Sequencing Coverage Across Population for Each Variant: 3705.0 x

Variant Transition / Transversion Ratio: 1.43

Transitions: 131051 A->G: 46085 C->T: 84966 Transversions: 91795

> G->T: 32218 A->C: 16974 A->T: 21447 C->G: 21156

### C) Genotyping Statistics

Total Genotypes in VCF File: 20922610

Genotypes Scored: 20403313 Genotypes Missing: 519297

Average Individual Genotype Quality: 79.9 (Phred-scaled)

Average Individual Sequencing Coverage: 39 x

Homozygous Genotypes: 18358647 Heterozygous Genotypes: 2044666

#### D) Genotyping Design Statistics (if applicable)

Variants Screened: 0

Variants Passing Design Criteria: 0 Variants Failing Design Criteria: 0

Failed due to flanking polymorphism: 0
Failed due to lack of flanking sequence: 0

Figure 1. Variants Passing Filters

219.1 K

#### Filtering Criteria:

Minimum Sequencing Depth per Sample: 10 x Minimum Phred Scaled Genotype Quality Per Sample: 13 Minimum Percent of Population Genotyped: 82.5 %

Figure 2. Histogram Plotting Number of Variations Over 200bp Genomic Interval

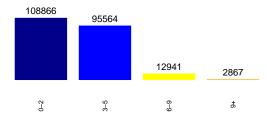


Figure 3. Variant Transition / Transversion Rates

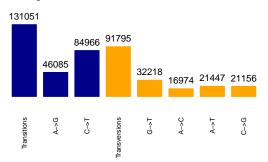


Figure 4.
Percentage of Missing
Genotype Data

Figure 5. Percent Homozygous & Heterozygous Genotypes



Quality Genotype Missing Genotype

Figure 6. Individual Sequencing Coverage and Genotype Quality

39x

79.9

Average Sequencing Coverage

Average Genotype Quality



## VCF File Summary: AR21\_Aligned\_Genotypes\_stringent.vcf

### A) Information

VCF File: AR21\_Aligned\_Genotypes\_stringent.vcf

Date: 2018-09-02T03:43:11

Project: LEggert\_UMissouri\_Bass\_20180409-01669 Reference Genome: AR21\_denovo\_clusters.fasta

BAM / mpileup Folder: BAM\_mpileups

VCF Options:

Min. % Population Genotyped: 90
Min. Individual Sequencing Depth: 15 x
Min. Individual Genotype Quality: 20
Min. FASTQ Quality Score: 20
MAF to be Screened: 0.2
Min. Distance Free of Other Variants: 50
Genome Landscape Flanking SNP: 50

### **B) Variant Statistics**

Number of Samples Screened: 95

Total Candidate Variants Detected: 357124

Candidate Variants Filtered (due to missing or low quality data): 229694

Candidate Variants with Low Probability: 406 Candidate Variants Passing All Filters: 127023

Average Number of Polymorphisms within 200bp of Each Variant: 2.8 Sequencing Coverage Across Population for Each Variant: 4674.0 x

Variant Transition / Transversion Ratio: 1.45

Transitions: 76063 A->G: 25295 C->T: 50768 Transversions: 52544

> G->T: 18522 A->C: 9550 A->T: 11868 C->G: 12604

### C) Genotyping Statistics

Total Genotypes in VCF File: 12105755

Genotypes Scored: 11873918 Genotypes Missing: 231837

Average Individual Genotype Quality: 102.1 (Phred-scaled)

Average Individual Sequencing Coverage: 49.2 x

Homozygous Genotypes: 10699755 Heterozygous Genotypes: 1174163

#### D) Genotyping Design Statistics (if applicable)

Variants Screened: 0

Variants Passing Design Criteria: 0 Variants Failing Design Criteria: 0

Failed due to flanking polymorphism: 0 Failed due to lack of flanking sequence: 0

Figure 1. Variants Passing Filters

127.0 K

#### Filtering Criteria:

Minimum Sequencing Depth per Sample: 15 x Minimum Phred Scaled Genotype Quality Per Sample: 20 Minimum Percent of Population Genotyped: 90 %

Figure 2. Histogram Plotting Number of Variations Over 200bp Genomic Interval

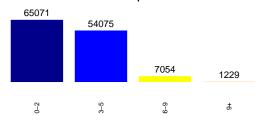


Figure 3. Variant Transition / Transversion Rates

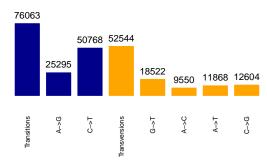
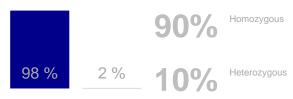


Figure 4.
Percentage of Missing
Genotype Data

Figure 5. Percent Homozygous & Heterozygous Genotypes



Quality Genotype Missing Genotype

Figure 6. Individual Sequencing Coverage and Genotype Quality

49.2x

TUZ.

Average Sequencing Coverage

Average Genotype Quality





## 6. Tools and Software

BOWTIE Version: bowtie-1.1.1

BWA Version: bwa-0.6.1

SAMTOOLS Version: samtools-0.1.16

VELVET Version: velvet\_1.2.10