Sample Report

M.Kaller_14_05 - P1170_101

Sample Information

Report Date	2014-11-13
User Contact	phil.ewels@scilifelab.se
Project Name	M.Kaller_14_05 (Swedish Genomes Pilot v3)
User Sample Name	Test Sample #1290
NGI Sample Name	P1170_101
UPPMAX Project ID	b2014832
Sequencing Platform	Illumina
Library Preparation Method	**A:** All samples were sequenced on HiSeq2500 (HiSeq Control Software 2.0.12.0/RTA 1.17.21.3) with a 2x101 setup.The Bcl to Fastq conversion was performed using bcl2Fastq v1.8.3 from the CASAVA software suite. The quality scale used is Sanger / phred33 / Illumina 1.8+.
Sequencing Centre	NGI Stockholm
Reference Genome	gatk_bundle/2.8/b37/human_g1k_v37.fasta
Flow Cells	140815_SN1025_0222_BC4HAPACXX 140815_SN1025_0223_BC4HAPACXX

Library Statistics

Total Reads	908,585,160
Aligned Reads	99.47% - 903,806,933
Duplication Rate	1.9%
Median Insert Size	369 bp
Av. Autosomal Coverage	28.92X

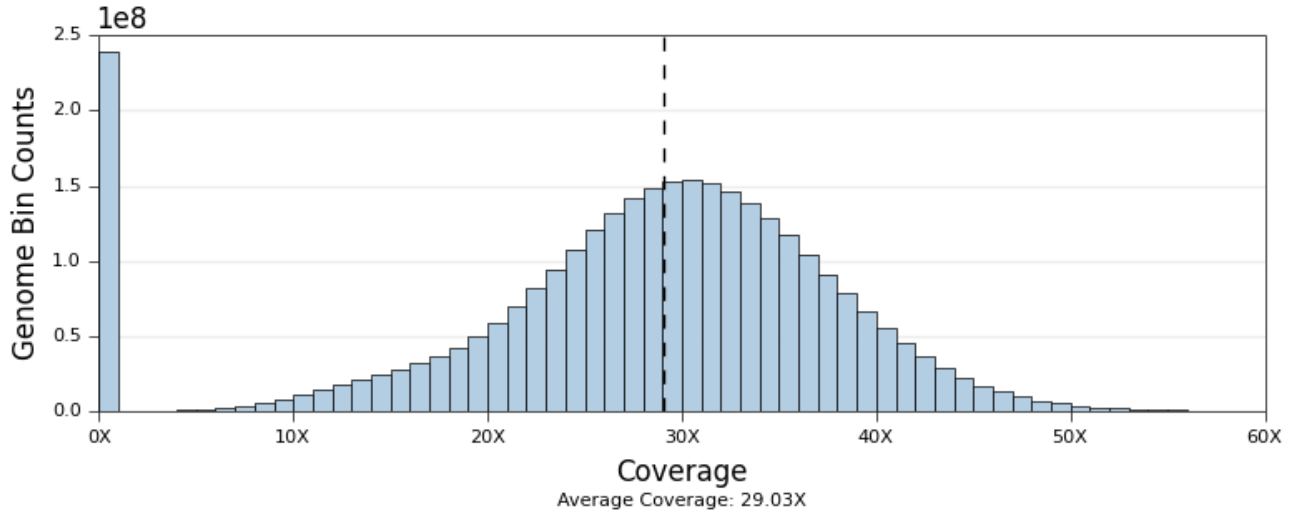
≥ 30X Coverage	51.72% of reference
GC Content	39.87%

See below for more information about coverage and insert size. The `qualimapReport.html` report in your delivery folder contains additional library statistics. Note that the duplication rate above is calculated using Picard Mark Duplicates. Qualimap calculates duplicates differently and the figure in the report will be different to the Picard result..

Distribution of sequencing coverage

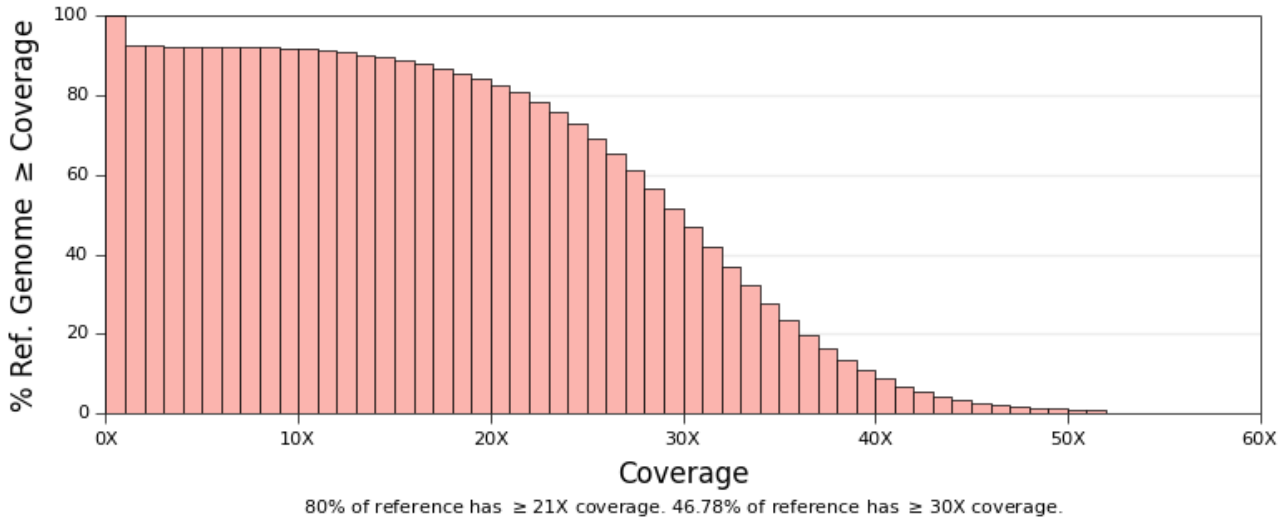
Calculating the coverage of a genome tells you how much information you have about the sequence content of any given position. More coverage means more data and so greater confidence in your results. A specific locus with 30X coverage will have 30 unique reads covering that location. Different positions in the genome may have different coverages due to variations in how well reads map to the underlying sequence. Other factors such as GC bias in library preparation may also have an effect.

To calculate the plot below, we create rolling windows across the genome and count the frequencies of the different coverages observed. This was done using the QualiMap tool and plotted with an NGI script.



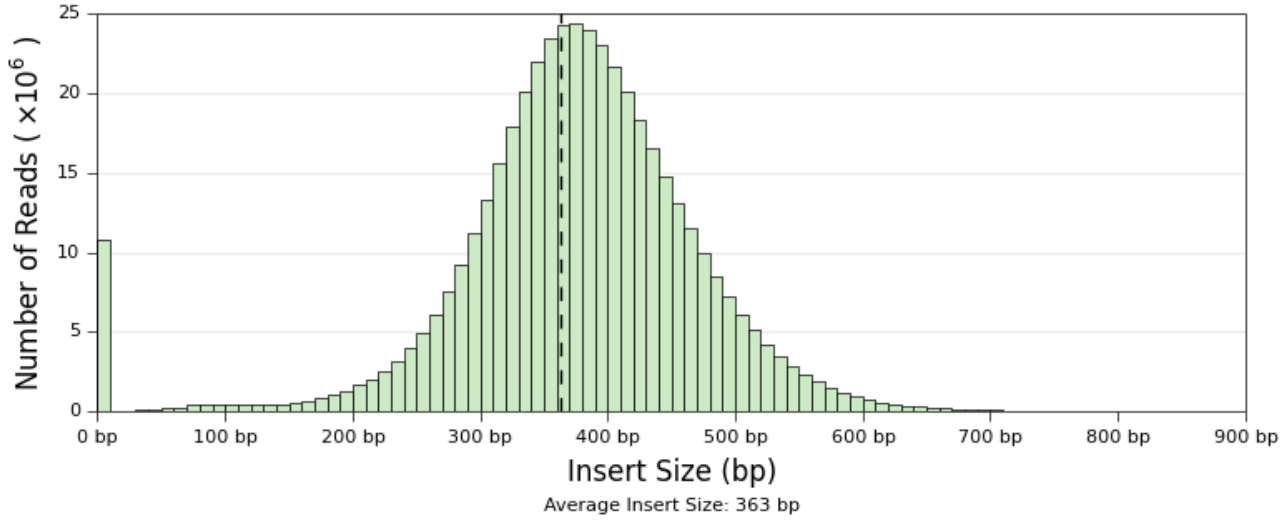
Proportion of library with increasing coverage depths

Another way to assess coverage is to look at the proportion of the reference genome with a certain coverage. The plot below shows what percentage of the reference genome is covered with increasing coverage thresholds. As above, this data was calculated using QualiMap and plotted with an NGI script.



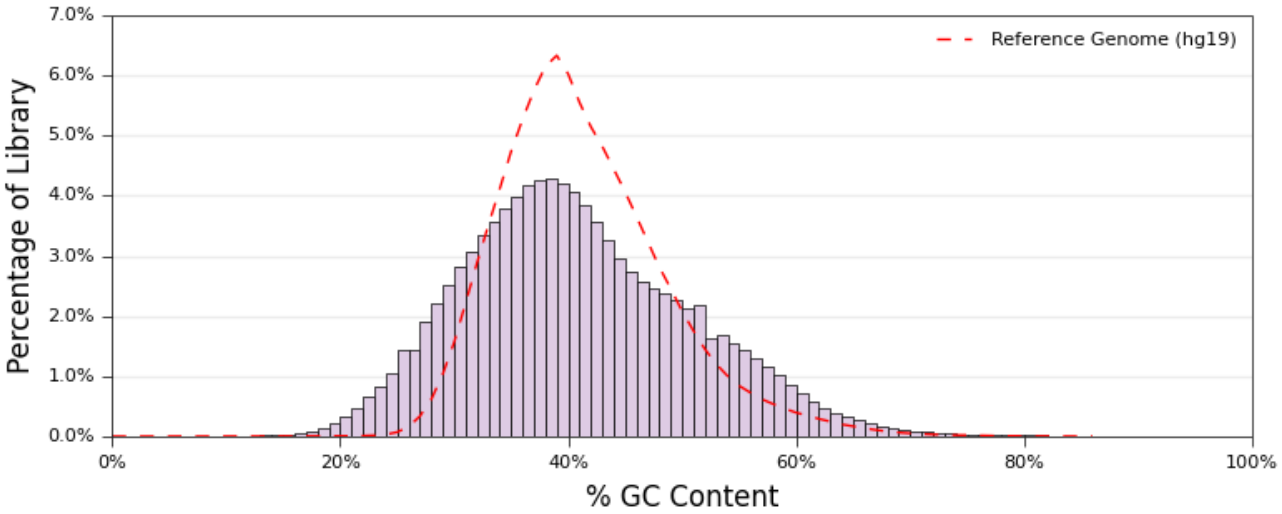
Library fragment insert sizes

By inspecting where each read pair maps to in the reference genome, we can reconstruct the reads that were present in the library and calculate the range of insert sizes. This gives an insight into the quality of the sequencing library. We counted how many reads with each insert size were seen and plotted this in the histogram below. This was done using the QualiMap tool and plotted with an NGI script.



Distribution of reads by GC content

Library preparation and sequencing alignment can be affected by differences in GC content. Here, we plot the proportions of the library reads at each GC content. The red dotted line shows the profile for the reference genome. This data was calculated using QualiMap and plotted with an NGI script.

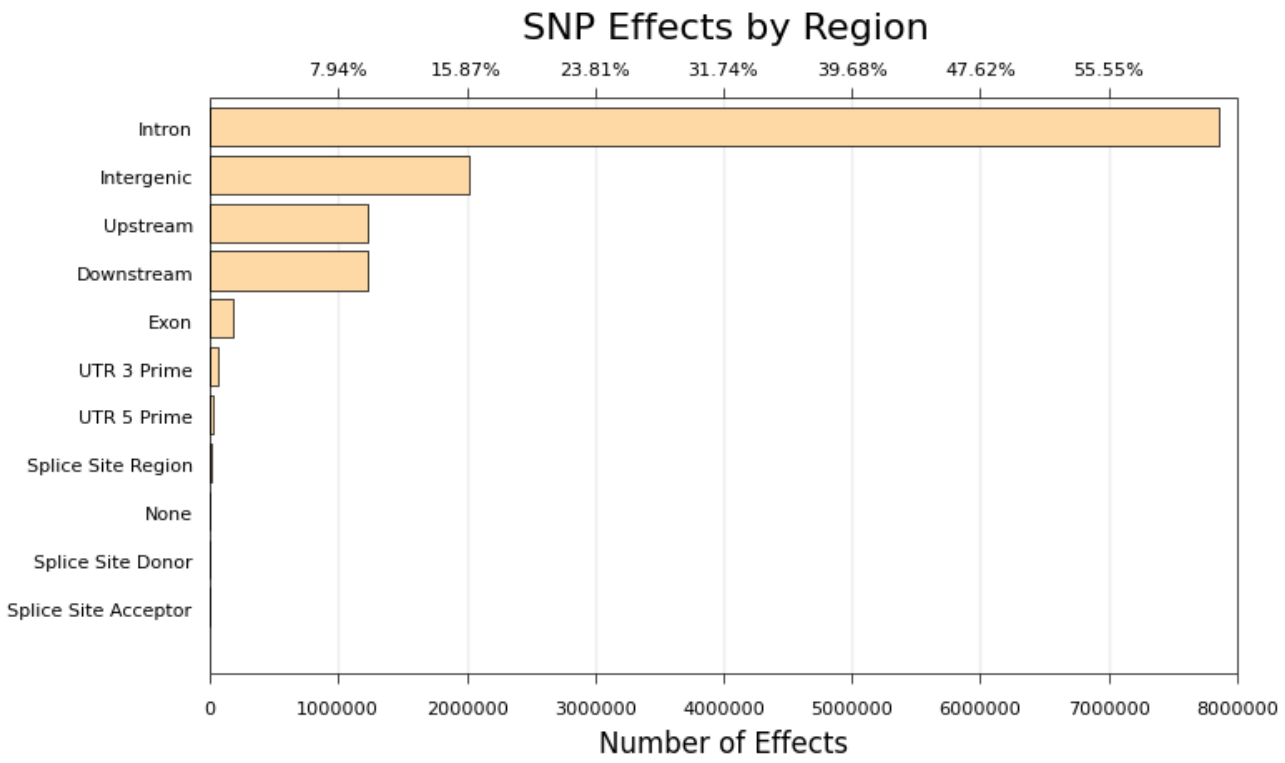


Variants

Change Rate	1 change per 774 bp
Total SNPs	4,004,647
Homotypic SNPs	1,491,592
Heterotypic SNPs	2,513,055
Ts/Tv Ratio	1.9895
Synonymous SNPs	35,078
Non-Synonymous SNPs	30,232
Stop Gained / Lost	273 / 58
Missense SNPs	46.2% - 30,366
Nonsense SNPs	0.4% - 273
Silent SNPs	53.4% - 35,078

Different effects can be attributed to each SNP depending on where it occurs. Here we have used the snpEff tool to categorise and count different effects. The results were plotted with an NGI script.

See the `snpEff_summary.html` report in your delivery folder for more details.


SNP Effects by Region
7.94%
15.87%
23.81%
31.74%
39.68%
47.62%
55.55%
Intron
Intergenic
Upstream
Downstream
Exon
UTR 3 Prime
UTR 5 Prime
Splice Site Region
None
Splice Site Donor
Splice Site Acceptor
0
1000000
2000000
3000000
4000000
5000000
6000000
7000000
8000000
Number of Effects