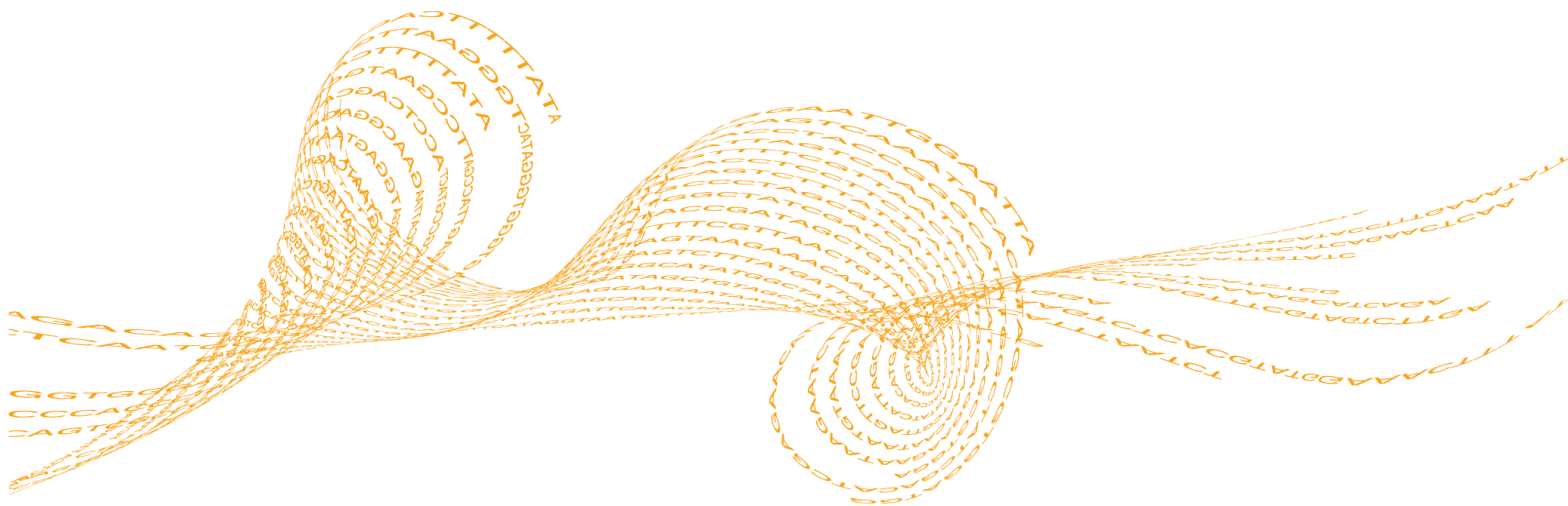


Amplicon DS v1.2

BaseSpace App Guide

For Research Use Only. Not for use in diagnostic procedures.

Introduction	3
Workflow	4
Set Analysis Parameters	5
Analysis Methods	6
Analysis Output	8
Revision History	8
Technical Assistance	19



This document and its contents are proprietary to Illumina, Inc. and its affiliates ("Illumina"), and are intended solely for the contractual use of its customer in connection with the use of the product(s) described herein and for no other purpose. This document and its contents shall not be used or distributed for any other purpose and/or otherwise communicated, disclosed, or reproduced in any way whatsoever without the prior written consent of Illumina. Illumina does not convey any license under its patent, trademark, copyright, or common-law rights nor similar rights of any third parties by this document.

The instructions in this document must be strictly and explicitly followed by qualified and properly trained personnel in order to ensure the proper and safe use of the product(s) described herein. All of the contents of this document must be fully read and understood prior to using such product(s).

FAILURE TO COMPLETELY READ AND EXPLICITLY FOLLOW ALL OF THE INSTRUCTIONS CONTAINED HEREIN MAY RESULT IN DAMAGE TO THE PRODUCT(S), INJURY TO PERSONS, INCLUDING TO USERS OR OTHERS, AND DAMAGE TO OTHER PROPERTY.

ILLUMINA DOES NOT ASSUME ANY LIABILITY ARISING OUT OF THE IMPROPER USE OF THE PRODUCT(S) DESCRIBED HEREIN (INCLUDING PARTS THEREOF OR SOFTWARE).

© 2016 Illumina, Inc. All rights reserved.

Illumina, 24sure, BaseSpace, BeadArray, BlueFish, BlueFuse, BlueGnome, cBot, CSPro, CytoChip, DesignStudio, Epicentre, ForenSeq, Genetic Energy, GenomeStudio, GoldenGate, HiScan, HiSeq, HiSeq X, Infinium, iScan, iSelect, MiSeq, MiSeqDx, MiSeq FGx, NeoPrep, NextBio, Nextera, NextSeq, Powered by Illumina, SureMDA, TruGenome, TruSeq, TruSight, Understand Your Genome, UYG, VeraCode, verifi, VeriSeq, the pumpkin orange color, and the streaming bases design are trademarks of Illumina, Inc. and/or its affiliate(s) in the U.S. and/or other countries. All other names, logos, and other trademarks are the property of their respective owners.

Introduction

The BaseSpace® App, Amplicon DS v1.2, aligns reads against the reference specified in the manifest files using the banded Smith-Waterman algorithm. After alignment, the somatic variant caller performs variant analysis. This workflow is designed specifically for dual-strand targeted resequencing assays. Both Ensembl and RefSeq are available for annotation.

Compatible Libraries

See the BaseSpace support page for a list of library types that are compatible with the Amplicon DS v1.2 App.

Workflow Requirements

- ▶ The minimum read length is 50 bases.
- ▶ No minimum number of reads is required, but use sufficient data for each sample to support appropriate depth of coverage for variant calling.
- ▶ Variants are found in the regions targeted in the manifest.
- ▶ App supports running 1 manifest per analysis.
- ▶ Requires paired-end samples.
- ▶ Requires samples with the same read lengths.

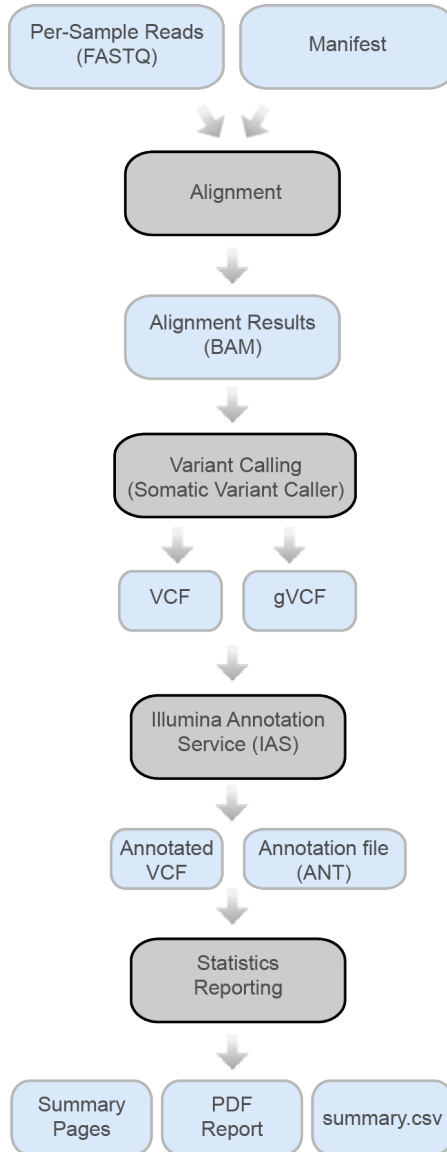
Versions

The following components are used in the Amplicon DS v1.2 App.

Software	Version
Amplicon DS (BaseSpace Workflow)	1.2
Isis (Analysis Software)	2.5.40.16
SAMtools	0.1.19-isis-1.0.3
Somatic Variant Caller	3.5.2.1
IAS (Illumina Annotation Service)	v3
Annotation Dataset	72.5

Workflow

Figure 1 Amplicon DS v1.2 App Workflow



Set Analysis Parameters

- 1 In BaseSpace, click the **Apps** tab.
- 2 Click **Amplicon DS**.
- 3 From the drop-down list, select **version 1.2**, and then click **Launch** to open the app.
- 4 In the **Analysis Name** field on the input form, enter the analysis name. By default, the analysis name includes the app name, followed by the date and time that the analysis session starts.
- 5 From the **Save Results To** field, select the project that stores the app results.
- 6 From the **Targeted Amplicons** field, select a panel of targeted amplicons representative of the selected samples.
- 7 If you selected **Custom Manifest** in the **Targeted Amplicons** drop-down list, upload a custom manifest and select the manifest file from the **Custom Pool A/B Manifest File** field. Upload a custom manifest as follows.
 - a Navigate to your project in BaseSpace.
 - b Click **Import**.
 - c Follow the instructions to add the Custom Amplicon manifest file (*.txt) to the project.



NOTE

By default, the Amplicon DS v1.2 App uses the Smith-Waterman algorithm for alignment and the Somatic variant caller.

- 8 From the **Annotation** field, select a preferred gene and transcript annotation reference database.
- 9 From the **Sample Pairs** field, select the samples for a pooled sample pair. Select the samples as follows.
 - a Click **Select Pairs** to open the Select Pairs window.
 - b [Optional] Search for the samples you want to analyze.
 - c Select a sample from the search results, or click **Select All** to select all samples, and then drag and drop the samples to the appropriate location (Pool A Samples or Pool B Samples).
 - d Click **Confirm**.



Note

Select matching pooled sample pairs.

- 10 Click **Continue**.

The Amplicon DS v1.2 App begins analysis of the sample.
When analysis is complete, the status of the app session is updated automatically and an email is sent to notify you.

Analysis Methods

The Amplicon DS v1.2 workflow evaluates short regions of amplified DNA, or amplicons, for variants. Focused sequencing of amplicons enables high coverage of particular regions across many samples. Amplicon DS samples are generated using a mirrored, dual strand amplicon assay.

Alignment

During the alignment step, the banded Smith-Waterman algorithm aligns clusters from each sample against amplicon sequences specified in the manifest file.

The banded Smith-Waterman algorithm performs local sequence alignments to determine similar regions between 2 sequences. Instead of comparing the total sequence, the Smith-Waterman algorithm compares segments of all possible lengths. Local alignments are useful for dissimilar sequences that are suspected to contain regions of similarity within the larger sequence. This process allows alignment across small amplicon targets, often less than 10 bp.

Each paired-end read is evaluated in terms of its alignment to the relevant probe sequences for that read.

- ▶ Read 1 is evaluated against the reverse complement of the Downstream Locus-Specific Oligos (DLSO).
- ▶ Read 2 is evaluated against the Upstream Locus-Specific Oligos (ULSO).
- ▶ If the start of a read matches a probe sequence with no more than 1 mismatch, the full length of the read is aligned against the amplicon target for that sequence.

Alignments that include more than 3 indels are filtered from alignment results. Filtered alignments are written in alignment files as unaligned and are not used in variant calling.

Variant Calling

Variant calling is performed with the Somatic Variant Caller.

Variant Calling

Developed by Illumina, the somatic variant caller identifies variants present at low frequency in the DNA sample.

The somatic variant caller identifies SNPs in 3 steps:

- ▶ Considers each position in the reference genome separately
- ▶ Counts bases at the given position for aligned reads that overlap the position
- ▶ Computes a variant score that measures the quality of the call using Poisson model.

Variants are first called for each pool separately. Then, variants from each pool are compared and combined into a single output file. If a variant meets the following criteria, the variant is marked as PASS in the variant call (VCF) file:

- ▶ The variant is present in both pools
- ▶ Has a cumulative depth of 1000 or an average depth of 500x per pool
- ▶ Has a variant frequency of $\geq 3\%$ as reported in the merged VCF file

Illumina Annotation Service (IAS)

Annotation with IAS populates several values in the VCF file, including dbSNP ID (in the ID column), and some values in the INFO column. More detailed and extensive

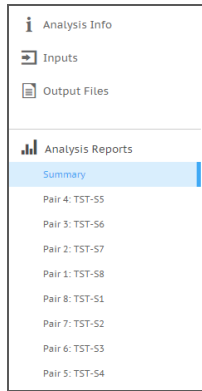
annotations are stored in a binary ANT file. This binary file can be imported into VariantStudio. For more information, see www.illumina.com/informatics/research/biological-data-interpretation/variantstudio.html.

Annotation through IAS is available for alignments against the human reference genome: UCSC build hg19 / Ensembl build GRCh37 / NCBI build 37.2.

Analysis Output

To view the results, click the **Projects** tab, then the project name, and then the analysis.

Figure 2 Output Navigation Bar



After analysis is complete, access the output through the left navigation bar.

- ▶ **Analysis Info**—Information about the analysis session, including log files.
- ▶ **Inputs**—Lists the samples and settings specified for the analysis session.
- ▶ **Output Files**—Output files for the sample.
- ▶ **Summary Analysis Reports**—Analysis metrics for the aggregate results, displayed when multiple samples are analyzed.
- ▶ **Sample Analysis Reports**—Analysis reports for a single sample pair. One entry for each sample pair that was analyzed, with the entries displayed in reverse chronological order of analysis.

Analysis Info

The Analysis Info page displays the analysis settings and execution details.

Row Heading	Definition
Name	Name of the analysis session.
Application	App that generated this analysis.
Date Started	Date and time the analysis session started.
Date Completed	Date and time the analysis session completed.
Duration	Duration of the analysis.
Session Type	Number of nodes used.
Status	Status of the analysis session. The status shows either Running or Complete.

Log Files

File Name	Description
AmpliconRunStatistics.xml	Provides amplicon-related information.
CompletedJobInfo.xml	Contains information about the completed analysis session.
Logging.zip	Contains all detailed log files for each step of the workflow.
output-{timestamp}.log	Shows the raw console output from the app.
SampleSheet.csv	Sample sheet.
SampleSheetUsed.csv	A copy of the sample sheet, generated at the beginning of the workflow.
[Manifest name].txt	Manifest files used in the analysis.
VariantCallingLogs.zip	Zip file containing the intermediate files used to produce the final variant calls.
WorkflowError.txt	Workflow standard error output (contains error messages created when running the workflow).
WorkflowLog.txt	Workflow standard output (contains details about workflow steps, command line calls with parameters, timing, and progress).

Inputs

The Inputs page lists the samples and settings specified for the analysis session.

Output Files

The Output Files page provides access to the output files for each sample pair analysis.

- ▶ **BAM Files**—Aligned sequences and quality scores in the BAM (*.bam) file format.
- ▶ **VCF Files**—Variant calls in the VCF (*.vcf) file format.
- ▶ **Genome VCF Files**—Variants, references, and no calls for all sites in the genome in the genome VCF (gVCF) file format.
- ▶ **Annotation File**—Detailed annotations in a binary file format.
- ▶ **Summary File**—Statistics for each sample.

BAM File Format

A BAM file (*.bam) is the compressed binary version of a SAM file that is used to represent aligned sequences up to 128 Mb. SAM and BAM formats are described in detail at <https://samtools.github.io/hts-specs/SAMv1.pdf>.

BAM files use the file naming format of SampleName_S#.bam, where # is the sample number determined by the order that samples are listed for the run.

BAM files contain a header section and an alignments section:

- ▶ **Header**—Contains information about the entire file, such as sample name, sample length, and alignment method. Alignments in the alignments section are associated with specific information in the header section.
- ▶ **Alignments**—Contains read name, read sequence, read quality, alignment information, and custom tags. The read name includes the chromosome, start coordinate, alignment quality, and the match descriptor string.

The alignments section includes the following information for each or read pair:

- ▶ **RG:** Read group, which indicates the number of reads for a specific sample.
- ▶ **BC:** Barcode tag, which indicates the demultiplexed sample ID associated with the read.
- ▶ **SM:** Single-end alignment quality.
- ▶ **AS:** Paired-end alignment quality.
- ▶ **NM:** Edit distance tag, which records the Levenshtein distance between the read and the reference.
- ▶ **XN:** Amplicon name tag, which records the amplicon tile ID associated with the read.

BAM index files (*.bam.bai) provide an index of the corresponding BAM file.

VCF File Format

VCF is a widely used file format developed by the genomics scientific community that contains information about variants found at specific positions in a reference genome.

VCF files use the file naming format SampleName_S#.vcf, where # is the sample number determined by the order that samples are listed for the run.

VCF File Header—Includes the VCF file format version and the variant caller version. The header lists the annotations used in the remainder of the file. If MARS is listed, the Illumina internal annotation algorithm annotated the VCF file. The VCF header includes the reference genome file and BAM file. The last line in the header contains the column headings for the data lines.

VCF File Data Lines—Each data line contains information about a single variant.

VCF Annotations

The VCF files for Amplicon DS v1.2 can have the following annotations in the FILTER, FORMAT, and INFO fields.

Table 1 VCF FILTER Entries

Entry	Description
LowGQ	The genotyping quality (GQ) is below a cutoff.
LowVariantFreq	The variant frequency is less than the given threshold.
PB	The prevalence of the variant is significantly biased between the 2 forward and reverse probe pools.
R8	For an indel, the number of adjacent repeats (1-base or 2-base) in the reference is greater than 8.
SB	The strand bias is more than the given threshold.
LowDP	Applied to sites with depth of coverage that is below a cutoff.

Table 2 VCF FORMAT Entries

Entry	Description
AD	Allelic depths for the ref and alt alleles in the order listed. For indels, this value includes only the reads that confidently support each allele (posterior probability 0.999 or higher that read contains indicated allele vs all other intersecting indel alleles).
GQ	Genotype Quality.
GQX	Minimum of {Genotype quality assuming variant position, Genotype quality assuming nonvariant position}.
GT	Genotype.
NL	Noise level, as a Q-score.
PB	Probe pool bias.
SB	Strand bias.
VF	Variant frequency in the sample.

Table 3 VCF INFO Entries

Entry	Description
AA	The inferred allele ancestral to the chimpanzee/human lineage.
AF1000G	The allele frequency from all populations of 1000 genomes data.
clinvar	Clinical significance from the ClinVar database (www.ncbi.nlm.nih.gov/clinvar/).
cosmic	The numeric identifier for the variant in the Catalogue of Somatic Mutations in Cancer (COSMIC) database (cancer.sanger.ac.uk/cosmic).
CSQR	Regulatory consequence as predicted by Variant Effect Predictor (www.ensembl.org/info/docs/tools/vep/index.html) version 72. A comma-separated list for each affected regulatory region (including transcription factor binding sites) is provided using the following delimited format: RegulatoryID Consequence. The annotations provided in this field come from the Ensembl database of regulatory features even if RefSeq was selected as the annotation source. Many of the RegulatoryIDs begin with ENSR. The consequences are indicated using valid Sequence Ontology (SO) terms (www.ensembl.org/info/genome/variation/predicted_data.html#consequences) and typically are either regulatory_region_variant or TF_binding_site_variant.

Entry	Description
CSQT	Transcript consequence as predicted by Variant Effect Predictor (www.ensembl.org/info/docs/tools/vep/index.html) version 72. Only canonical transcripts are included in the VCF file to maintain readability. The ANT file contains consequences for all affected transcripts. This binary file can be loaded into VariantStudio for viewing. See www.illumina.com/informatics/research/biological-data-interpretation/variantstudio.html .
DP	The depth (number of base calls aligned to a position and used in variant calling). In regions of high coverage, GATK down-samples the available reads.
EVS	Allele frequency, sample count, and coverage taken from the Exome Variant Server (EVS). Format: AlleleFreqEVS EVSCoverage EVSSamples.
EXON	A comma-separated list of exon regions read from RefGene.
FC	Functional consequence.
GI	A comma-separated list of gene IDs read from RefGene.
GMAF	Global minor allele frequency (GMAF); technically, the frequency of the second most frequent allele. Format: GlobalMinorAllele AlleleFreqGlobalMinor.
phastCons	Denotes if the variant is an identical or similar sequence that occurs between species and maintained between species throughout evolution.
TI	A comma-separated list of transcript IDs read from RefGene.

Genome VCF Files

Genome VCF (gVCF) files are VCF v4.1 files that follow a set of conventions for representing all sites within the genome in a reasonably compact format. The gVCF files include all sites within the region of interest in a single file for each sample.

The gVCF file shows no-calls at positions with low coverage, or where a low-frequency variant (< 3%) occurs often enough (> 1%) that the position cannot be called to the reference. A genotype (GT) tag of ./ indicates a no-call.

For more information, see sites.google.com/site/gvcftools/home/about-gvcf.

Annotation File

The Illumina Annotation Service (IAS) generates a binary annotation file (*.ant), which contains consequences for all affected transcripts. The annotations are more detailed than the annotations in the VCF file. You can view this binary file in VariantStudio. For more information, see www.illumina.com/informatics/research/biological-data-interpretation/variantstudio.html.

Summary File

The Amplicon DS v1.2 App produces an Amplicon DS Summary Report for each sample and the aggregate results in a comma-separated values (CSV) format: *.summary.csv.

These files are located in the results folder for each sample.

Statistic	Definition
Sample ID	IDs of samples reported in the file.
Sample Name	Names of samples reported in the file.
Run Folder	Run folders for samples reported in the file.
Manifest	The manifest file used for analysis. This file specifies the targeted regions for the aligner and variant caller.
Reference genome	Reference genome selected.
Number of amplicons	The number of amplicon regions sequenced.
Total length of amplicons	The total length of the sequenced bases in the target region.
Total PF reads	The number of reads passing filter for the sample.
Total aligned reads	The total number of reads passing filter present in the data set that aligned to the reference genome. Numbers are calculated per read, and over both reads.
Percent aligned reads	The percentage of reads passing filter that aligned to the reference genome. Numbers are calculated per read, and over both reads.
Total PF bases	The number of bases passing filter for the sample.
Total aligned bases	The total number of bases present in the data set that aligned to the reference genome. Numbers are calculated per read, and over both reads.
Percent aligned bases	The percentage of bases that aligned to the reference genome. Numbers are calculated per read, and over both reads.
Percent Q30	The percentage of bases with a quality score of 30 or higher. Numbers are calculated per read.
Mismatch rate	The average percentage of mismatches across both reads 1 and 2 over all cycles. Numbers are calculated per read.
Amplicon mean coverage	The total number of aligned bases to the targeted region divided by the targeted region size.
Uniformity of Coverage	The percentage of amplicon regions with coverage values of less than the low coverage threshold ($0.2 * \text{amplicon mean coverage}$).
SNVs, Insertions, Deletions	Total number of variants present in the data set that pass the quality filters.
SNVs, Insertions, Deletions (Percent found in dbSNP)	$100 * (\text{Number of variants in dbSNP} / \text{Number of variants})$.

Statistic	Definition
SNV Ts/Tv ratio	The number of Transition SNVs that pass the quality filters divided by the number of Transversion SNVs that pass the quality filters. Transitions are interchanges of purines (A, G) or of pyrimidines (C, T). Transversions are interchanges of purine and pyrimidine bases (for example, A to T).
SNVs, Insertions, Deletions Het/Hom ratio	Number of heterozygous variants/Number of homozygous variants.

Sample Analysis Reports

The Amplicon DS v1.2 App provides an overview of statistics per sample pair separated by pools on the Analysis Reports pairwise pages. To download statistics in the AmpliconDS Sequencing Report, click **PDF Summary Report**.

Amplicon Summary

Table 4 Amplicon Summary Table

Statistic	Definition
Number of Amplicons	The number of amplicon regions that were sequenced.
Total Length of Amplicon	The total length of the sequenced amplicon regions in base pairs.

Table 5 Read Level Statistics Table

Statistic	Definition
Total Aligned Reads	The total number of reads passing filter present in the data set that aligned to the reference genome.
Percent Aligned Reads	The percentage of reads passing filter that aligned to the reference genome.

Table 6 Base Level Statistics Table

Statistic	Definition
Total Aligned Bases	The total number of bases present in the data set that aligned to the reference genome.
Percent Aligned Bases	The percentage of bases that aligned to the reference genome.
Percent Q30	The percentage of bases with a quality score of 30 or higher.
Mismatch Rate	The average percentage of mismatches across both reads 1 and 2 over all cycles.

Small Variants Summary

This table provides metrics about the number of SNVs, deletions, and insertions. Data are first analyzed for each individual pool (Pool A and Pool B). The data are then reconciled for the 2 pools and a consensus call is made for all the called variants.

Table 7 Small Variants Summary Table

Statistic	Definition
Total Passing	The total number of variants present in the data set that passed the variant quality filters.
Percent Found in dbSNP	100*(Number of variants in dbSNP/Number of variants). The SNVs that were found in the dbSNP are annotated accordingly.
Het/Hom Ratio	Number of heterozygous variants/Number of homozygous variants.
Ts/Tv Ratio	Transition rate of SNVs that pass the quality filters divided by transversion rate of SNVs that pass the quality filters. Transitions are interchanges of purines (A, G) or of pyrimidines (C, T). Transversions are interchanges between purine and pyrimidine bases (for example, A to T).

Coverage Summary

Table 8 Coverage Summary Table

Statistic	Definition
Amplicon Mean Coverage	The mean coverage across all sequenced amplicons.
Uniformity of Coverage	The percentage of amplicon regions with coverage values greater than the low coverage threshold, where the low coverage threshold is defined as (0.2 * Amplicon Mean coverage).

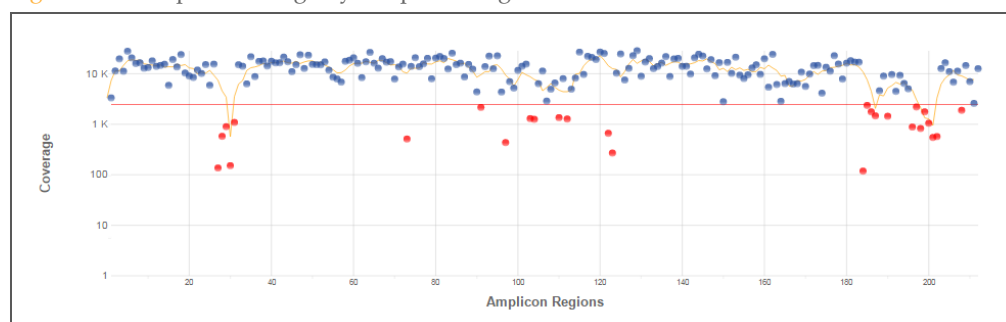
Coverage by Amplicon Region Plot

The Coverage by Amplicon Region plot shows the number of bases plotted against the amplicon region. It has the following features:

- ▶ Amplicon regions with coverage values less than the low coverage threshold (0.2 * amplicon mean coverage) are highlighted in red.
- ▶ Amplicon regions with coverage values greater than the low coverage threshold (0.2 * amplicon mean coverage) are highlighted in blue.
- ▶ The horizontal red line marks the low coverage threshold.
- ▶ The orange line marks the moving average of all coverage values.

Plots detail Amplicon Region overall and Amplicon Region by pool.

Figure 3 Example Coverage by Amplicon Region Plot



Coverage values for each amplicon are detailed in the downloadable Export (CSV) file.

Summary Analysis Report

The Amplicon DS v1.2 App provides an Aggregate Summary for all samples. Statistics are plotted against samples with tables providing additional metrics.

Manifest Information

The manifest is the list of targeted regions analyzed. The Manifest Information is a fixed parameter.

Amplicon Summary

Table 9 Read Level Statistics Table

Statistic	Definition
Total Aligned Reads (R1/R2)	The total number of reads passing filter present in the data set that aligned to the reference genome. Numbers are calculated per read.
Percent Aligned Reads (R1/R2)	The percentage of reads passing filter that aligned to the reference genome.
Overall Aligned Reads	The percentage of reads passing filter that aligned to the reference genome across both reads (R1 and R2). The value is the average of the individual Percent Aligned Reads values.

Table 10 Base Level Statistics Table

Statistic	Definition
Total Aligned Bases (R1/R2)	The total number of bases present in the data set that aligned to the reference genome. Numbers are calculated per read.
Overall Total Aligned Bases	The total number of bases present in the data set that aligned to the reference genome across both reads (R1 and R2). The value is the average of the individual Total Aligned Bases values.
Percent Aligned Bases (R1/R2)	The percentage of bases that aligned to the reference genome. Numbers are calculated per read.
Overall Percent Aligned Bases	The percentage of bases that aligned to the reference genome across both reads (R1 and R2). The value is the average of the individual Percent Aligned Bases values.
Percent Q30 (R1/R2)	The percentage of bases with a quality score of 30 or higher.
Mismatch Rate (R1/R2)	The average percentage of mismatches to the reference genome across both reads 1 and 2 over all cycles.

Small Variants Summary

Table 11 SNVs Table

Statistic	Definition
SNVs	The total number of SNVs present in the data set that passed the variant quality filters.
Percent Found in dbSNP	100*(Number of SNVs in dbSNP/Number of SNVs). The SNVs found in the dbSNP are annotated accordingly.
SNV Ts/Tv Ratio	Transition rate of SNVs that pass the quality filters divided by transversion rate of SNVs that pass the quality filters. Transitions are interchanges of purines (A, G) or of pyrimidines (C, T). Transversions are interchanges between purine and pyrimidine bases (for example, A to T).
SNV Het/Hom Ratio	Number of heterozygous variants/Number of homozygous variants.

Table 12 Insertions Table

Statistic	Definition
Insertions	The total number of insertions present in the data set that passed the variant quality filters.
Percent Found in dbSNP	100*(Number of SNVs in dbSNP/Number of SNVs). The SNVs that were found in the dbSNP are annotated accordingly.
Insertion Het/Hom Ratio	Number of heterozygous variants/Number of homozygous variants.

Table 13 Deletions Table

Statistic	Definition
Deletions	The total number of deletions present in the data set that passed the variant quality filters.
Percent Found in dbSNP	100*(Number of SNVs in dbSNP/Number of SNVs). The SNVs that were found in the dbSNP are annotated accordingly.
Deletions Het/Hom Ratio	Number of heterozygous variants/Number of homozygous variants.

Coverage Summary

Table 14 Coverage Summary Table

Statistic	Definition
Amplicon Mean Coverage Depth	The total number of aligned reads to the targeted region divided by the targeted region size.

Statistic	Definition
Uniformity of Coverage	The percentage of amplicon regions with coverage values greater than the low coverage threshold, where the low coverage threshold is defined as $(0.2 * \text{Amplicon Mean coverage})$.

Revision History

Document	Date	Description of Change
Document # 15066594 v03	January 2016	Reorganized topics, updated writing style.
Document # 15066594 v02	October 2015	Updated the product name to TruSight Tumor 26.
Document # 15066594 v01	September 2015	Supports Amplicon DS v1.2.

Notes

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 15 Illumina General Contact Information

Website	www.illumina.com
Email	techsupport@illumina.com

Table 16 Illumina Customer Support Telephone Numbers

Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Japan	0800.111.5011
Australia	1.800.775.688	Netherlands	0800.0223859
Austria	0800.296575	New Zealand	0800.451.650
Belgium	0800.81102	Norway	800.16836
China	400.635.9898	Singapore	1.800.579.2745
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	Taiwan	00806651752
Hong Kong	800960230	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000
Italy	800.874909		

Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download in PDF from the Illumina website. Go to support.illumina.com, select a product, then select **Documentation & Literature**.



Illumina

5200 Illumina Way

San Diego, California 92122 U.S.A.

+1.800.809.ILMN (4566)

+1.858.202.4566 (outside North America)

techsupport@illumina.com

www.illumina.com