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Overview

The Local Run Manager Oncology Gene Panel 170 analysis module supports sequencing to analysis for the Oncology Gene Panel 170 IUO/PEO assay. The OGP170 assay generates sequencing libraries for DNA and RNA from formalin-fixed, paraffin-embedded (FFPE) tissue.

The OGP170 analysis module supports run setup, sequencing, and analysis for the prepared DNA and RNA libraries. DNA library output includes variant call files for small variants and copy number variants. RNA library output include fusions and splice variant call files. The OGP170 analysis module supports 3 to 16 libraries per run.

About This Guide

This guide provides instructions for setting up run parameters for sequencing and analysis for the Oncology Gene Panel 170 analysis module. For information about the Local Run Manager dashboard and system settings, see the *NextSeq 550Dx Instrument Reference Guide (document # 1000000009513)*.

Supporting Files

- ▶ See the Oncology Gene Panel 170 Package Insert (document # 1000000042900).
- ▶ The Oncology Gene Panel 170 Module requires a 300 cycle sequencing consumables. See the NextSeq™ 550Dx High Output Reagent Kit v2 (300 cycles) (document # 1000000036666).

Viewing Local Run Manager

To access the Local Run Manager interface, a valid user name and password is required. For more information, see the User Management section in the NextSeq 550Dx Instrument Reference Guide (document # 1000000009513).

The Local Run Manager interface is viewed within NextSeq 550Dx Operating Software (NOS) or through a web browser. The supported web browser is Chromium.



NOTE

If you use an unsupported browser, download the supported browser when prompted on the "Confirm Unsupported Browser" message. Select **here** to download the supported version of Chromium.

View on the Instrument Monitor

To view the Local Run Manager interface on the instrument monitor, select one of the following options:

- ▶ From the NOS Home Screen, select **Local Run Manager**.
Select the X in the upper right corner to return to NOS when done.
- ▶ Select the Minimize NOS icon, open the Chromium web browser that is on the instrument, and enter **http://localhost** in the address bar.
Only admin users can minimize NOS.

View from a Networked Computer

Open a Chromium web browser on a computer with access to the same network as the instrument and connect using the instrument IP address or instrument name. For example, **http://myinstrument**.

Enter Run Information

Set Run Parameters

- 1 Log in to Local Run Manager.
- 2 Select **Create Run**, and select **OGP170**.
- 3 Enter a run name that identifies the run from sequencing through analysis.
Only use alphanumeric characters, underscores, or dashes. Run names cannot begin or end with an underscore or dash.
- 4 **[Optional]** Enter a run description to help identify the run.
Use alphanumeric characters and spaces.

Specify Samples for the Run

Specify samples for the run using one of the options and the directions that follow.

- ▶ **Enter samples manually**—Use the blank table on the Create Run screen.
- ▶ **Import samples**—Navigate to an external file in a comma-separated values (*.csv) format. A template is available for download on the Create Run screen.

After populating the samples table, the sample information can be exported to an external file. You can use the file as a reference when preparing libraries or when you want to use the same sample information for another run.

Enter Samples Manually

Each row in the sample table represents a sample composed of a DNA library, an RNA library, or both. The Oncology Gene Panel 170 analysis module supports 3–16 libraries per run, where the total is the sum of DNA and RNA libraries. Do the following for each sample row.

- 1 Enter a unique sample ID in the Sample ID field.
- 2 **[Optional]** Enter a sample description in the Sample Description field.
Sample descriptions are overwritten if a sample ID is reused in later runs.
- 3 Specify a library (DNA, RNA, or both) for the sample by selecting an index ID from the DNA Index ID or RNA Index ID drop-down lists.
- 4 Specify the tumor type for the sample by selecting a tumor type from the Tumor Type drop-down list.
- 5 If there are exactly three libraries in the run, the three selected libraries must match one of the following sets to ensure that there is enough diversity in the run:
 - ▶ UP01, UP02, and UP03
 - ▶ UP04, UP05, and UP06
 - ▶ UP07, UP08, and UP09
 - ▶ UP10, UP11, and UP12
- 6 **[Optional]** Select **Export Samples to Template** to export sample information to an external file.
The exported sample sheet can be used as a template, or imported when creating new runs.
- 7 Select **Save Run**.

Import Samples

- 1 Select **Import Samples** and browse to the location of the sample information file. There are two supported workflows for sample import.
 - ▶ Select **Download Template** on the Create Run screen to make a new plate layout. The template file contains the column headings required for import. Enter sample information in each column for the samples in the run. Delete example information in unused cells, and then save the file.
 - ▶ Use a file of sample information that was exported from the Oncology Gene Panel 170 module using the Export Samples to Template feature.
- 2 Review the information that was imported on the Create Run Screen. Make sure that sample information is correct. Incorrect information can impact results.
- 3 **[Optional]** Select **Export Samples to Template** to export sample information to an external file.
- 4 Select **Save Run**.

Edit a Run

For instructions on editing the information in your run before sequencing, see *NextSeq 550Dx Instrument Reference Guide (document # 1000000009513)*.

Requeue an Analysis

For instructions on editing the information in your run prior to requeueing, see *NextSeq 550Dx Instrument Reference Guide (document # 1000000009513)*.

Analysis Methods

The Oncology Gene Panel 170 analysis module evaluates DNA and RNA libraries targeting variants of cancer-related genes in the panel.

DNA Analysis Methods

Inputs Validation

Validates the existence and format of required analysis inputs.

Run QC

The Run Metrics report provides suggested values to determine if run quality results are within an acceptable range using InterOp files from the sequencing run folder. For Read 1 and Read 2, the report provides the average percentage of bases \geq Q30, which is a quality score (Q-score) measurement. A Q-score is a prediction of the probability of an incorrect base call.

FASTQ Generation

BCL files are demultiplexed and the software generates intermediate analysis files in the FASTQ format. FASTQ files use a text format to represent sequences. They contain reads for each sample and the associated quality scores. Any controls used for the run and clusters that did not pass filter are excluded. Each FASTQ file contains reads for only one sample, and the name of that sample is included in the FASTQ file name.

Alignment

The alignment step uses the BWA-MEM alignment algorithm to align DNA sequences in FASTQ files to the hg19 genome. This alignment step generates BAM files (*.bam) and BAM index files (*.bam.bai) which are saved to the alignment folder.

Indel Realignment

Reads near detected indels are realigned to remove alignment artifacts. The realignment identifies likely indels observed across all reads and realigns reads against those targets. This step is necessary because reads with indels at the end are sometimes aligned incorrectly during the first alignment. The realigned BAM and BAI files are saved to the alignment folder.

Small Variant Calling

Pisces performs somatic variant calling to identify variants at low frequency in the DNA sample.

For more information, see github.com/Illumina/Pisces/wiki.

Variant Filtering

The DNA Variant Filter software performs post-processing on the small variant calling VCFs, with the intended result of removing false positive variant calls due to systematic noise. The inputs and outputs are gVCF files.

Annotation

The Illumina Annotation Engine performs annotation of small variants. The inputs and outputs are gVCF files.

Copy Number Variants Calling

The CRAFT copy number variant caller performs amplification, reference, and deletion calling for target amplification genes within the assay. This calling is based on the calculated fold change value. Fold change is defined as the median normalized bin count value for a target gene over the median bin count value of the entire panel. The inputs are in BAM format and the outputs are VCF files. Deletion is marked with the LowValidation filter in the VCF file because they have only been tested with *in silico* data sets.

Complex Variants Alignment

The BWA aligner performs alignment of DNA reads to a custom reference genome targeting 29 EGFR long deletions and multinucleotide variants. The inputs are adapter-trimmed FASTQ files. This alignment step generates BAM files (*.bam) and BAM index files (*.bam.bai) which are saved to the alignment folder.

Complex Variant Calling

The complex variant caller detects 29 targeted EGFR long deletions and multinucleotide variants based on complex variant alignment results. The inputs are complex variant BAM files and the gVCF file from the variant filtering step and the output is a complex variant VCF file.

RNA Analysis Methods

Inputs Validation

Validates the existence and format of required analysis inputs.

FASTQ Generation

BCL files are demultiplexed and the software generates intermediate analysis files in the FASTQ format. FASTQ files use a text format to represent sequences. They contain reads for each sample and the associated quality scores. Any controls used for the run and clusters that did not pass filter are excluded. Each FASTQ file contains reads for only one sample, and the name of that sample is included in the FASTQ file name.

Downsampling

The Oncology Gene Panel 170 analysis pipeline downsamples each sample to 300 million RNA reads. This number represents the total number of single reads (ie, R1 + R2, from all lanes). When using the recommended sequencing configurations or plexing, the samples can have fewer reads than the down-sampling limit. In these cases, the FASTQ files are left as-is.

Adapter Trimming

Adapter trimming is performed on the downsampled FASTQ files corresponding to each RNA library. Reads are then trimmed to 76 base pairs for further processing.

Alignment

The STAR Aligner aligns RNA reads to the human hg19 genome. The alignment step identifies candidates for single-end realignment and reruns the candidates through STAR. The two STAR outputs are merged into a single BAM file per library. The inputs are trimmed FASTQ files and the outputs are BAM files and SJ.out.tab files containing potential splice junctions.

Fusion Calling

Manta performs the fusion calling. The inputs are BAM files and the outputs are VCF files.

For more information on Manta, see [biorxiv.org/content/biorxiv/suppl/2015/08/10/024232.DC1/024232-1.pdf](https://www.biorxiv.org/content/biorxiv/suppl/2015/08/10/024232.DC1/024232-1.pdf).

RNA Fusion Filtering

The RNAFusionFilter performs fusion filtering. The inputs are BAM and VCF files, and the outputs are CSV files.

Fusion Merging

Fusions identified through the fusion calling and RNA fusion filtering are combined with the intergenic calls made during splice variant calling. Also, each precise fusion event from the RNA fusion filter is recalculated for read count support. The inputs are intergenic splice variant TSV files, fusion filter CSV files, and BAM files. The outputs are CSV files.

Splice Variant Calling

Splice variant calling is performed using the RNA Splice Variant Calling software. The inputs are BAM files and SJ.out.tab files from STAR. The outputs are VCF files, which are the final output, and TSV file containing intergenic variants, which are used in fusion merging.

Annotation

The Illumina Annotation Engine performs annotation of splice variants. The inputs and outputs are VCF files.

Report Generation

The Oncology Gene Panel 170 analysis module generates an HRR report for each sample associated to a DNA library with tumor type *Carcinoma of Bladder* as input.

Analysis Output

When the analysis run is completed, the Local Run Manager App generates an analysis output folder in the user specified location.

To view analysis output:

- 1 Navigate to the directory that contains the analysis output folder.
- 2 Open the folder to view output files.

Folders

This section describes the content of each output folder generated during analysis.

- ▶ **Module_Logs**—Contains logs for module and auxiliary software
- ▶ **Logs_Intermediates**
 - ▶ **FASTQs**—Contains original sample FASTQ files
 - ▶ **Logs**
 - ▶ AnalysisLog-YYYYMMDD-HHMMSS .txt
 - ▶ **FASTQ_Logs**—Contains the following:
 - ▶ Sample-specific FASTQ logs
 - ▶ Intermediate SampleSheet.csv
 - ▶ **DNA**—Contains component logs.
 - ▶ **RNA**—Contains component logs.
 - ▶ **Reporting**—Contains logs for metrics output reporting and, when eligible samples are present in the run, logs for HRR reporting.
- ▶ **DNA_IntermediateFiles**
 - ▶ Alignment subfolder containing all library-specific BAM and BAI files
 - ▶ Annotation subfolder containing small and complex variants annotation files
- ▶ **RNA_IntermediateFiles**
 - ▶ Alignment subfolder containing all library-specific BAM and BAI files
 - ▶ Annotation subfolder containing library-specific variant annotation files
 - ▶ VariantCalling subfolder containing the fusion calling and splice variants calling outputs for each library
- ▶ **{Sample_ID}** (per sample)
 - ▶ {Library_ID}_Complex.vcf (when sample is associated with a DNA library)
 - ▶ {Library_ID}_SmallVariants.genome.vcf (when sample is associated with a DNA library)
 - ▶ {Library_ID}_CopyNumberVariants.vcf (when sample is associated with a DNA library)
 - ▶ {Library_ID}_HighConfidenceVariants.vcf (when sample is associated with an RNA library)
- ▶ **Reports**
 - ▶ {Sample_ID}_OGP170_2.0.0_HRR_Report.json (when sample is associated with a DNA library with *Carcinoma of bladder* tumor type)

- ▶ {Sample_ID}_OGP170_2.0.0_HRR_Report.pdf (when sample is associated with a DNA library with *Carcinoma of bladder* tumor type)
- ▶ **MetricsOutput.tsv**—See *Run Metrics Report* on page 1
- ▶ **SampleSheet.csv**—See *SampleSheet.csv* on page 1
- ▶ **Summary.tsv**—See *Summary TSV* on page 1

Files

This section describes the summary output files generated during analysis.

Metrics Output

File name: MetricsOutput.tsv

This report contains sequencing run metrics, DNA library QC metrics for small variant and copy number variant calling, RNA library QC metrics, and expanded metrics for DNA and RNA libraries.

Summary.tsv

File name: Summary.tsv

A tab-delimited summary report is generated for each analysis showing the analysis status for each sample analyzed. The Summary column in the output displays Analysis Completed when the sample completes analysis. When there is an error, the Summary column displays the analysis step(s) that failed or were skipped.

SampleSheet.csv

File name: SampleSheet.csv

The Oncology Gene Panel 170 module-specific sample sheet is a comma-delimited file that stores the information to set up and analyze a sequencing experiment. The file includes a list of samples and the index sequences, as well as the workflow to be employed for each sample in the analysis. For information on populating the sample sheet, see *Workflow Requirements* on page 1.

DNA Outputs

Files

This section describes the output files generated during a DNA sample analysis.

Small Variants Genome VCF

File name: {Library_ID}_SmallVariants.genome.vcf

This file contains small variant calls for each target coordinate in the analysis DNA panel.

The following variant call filters are applied during the variant call and filter step.

Filter Name	Description
Low Variant Quality	Filtered variant quality score is < 20 (applies to reference and variant allele calls)
Low Depth	Coverage is < 100 for variant calls and < 250 for reference calls
Strand Bias	Strand bias score is \geq to 0.5 (applies to variant allele calls only)

Filter Name	Description
Low Variant Frequency	Frequency is < 0.026 (applies to variant allele calls only)
Indel Reference Repeat	Repeat is ≥ 8 (applies to insertion and deletion variant calls only)
Adjusted Quality	Baseline quality score is < 10 (applies to variant allele calls only)
Low Variant Support	Variants with supporting reads < 7 (applies to variant allele calls only)

If available, variant calls are annotated using the Illumina Annotation Engine with the following information:

- ▶ HGNC Gene
- ▶ Transcript (using the canonical Ensembl transcript)
- ▶ Exon (using the canonical Ensembl transcript)
- ▶ Consequence (using the canonical Ensembl transcript)
- ▶ HGVS Coding Sequence Name (using the canonical Ensembl transcript)
- ▶ HGVS Protein Sequence Name (using the canonical Ensembl transcript)
- ▶ COSMIC ID

Copy Number Variants VCF

File name: {Library_ID}_CopyNumberVariants.vcf

Contains CNV calls for DNA libraries of the amplification genes targeted by the analysis panel. The CNV call indicates fold change results for each gene classified as reference, deletion, or amplification. The value in the QUAL column of the VCF is a Phred transformation of the p-value where $Q = -10 \times \log_{10}(p\text{-value})$. The p-value is derived from the t-test between the fold change of the gene against rest of the genome. Higher Q-scores indicate higher confidence in the CNV call.

In the VCF notation, <DUP> indicates the detected fold change (FC) is greater than a predefined amplification cutoff. indicates the detected fold change (FC) is less than a predefined deletion cutoff for that gene. This cutoff can vary from gene to gene.

 calls have only been validated with in silico data sets. As a result, all calls have LowValidation filter in the VCF.

Each copy number variant is reported in the form of fold change on normalized read depth in a testing sample relative to the normalized read depth in diploid genomes. Given tumor purity, one can infer the ploidy of a gene in the sample from the reported fold change.

Given tumor purity X%, for a reported fold change Y, the copy number n can be calculated by: $n = [(200 * Y) - 2 * (100 - X)] / X$

For example, in a testing sample of tumor purity at 30%, MET with fold change of 2.2x indicates that 10 copies of MET DNA are observed.

Complex Variant VCF

File name: {Library_ID}_Complex.vcf

The VCF file contains multinucleotide variants that meet all of the following criteria

- ▶ Quality score value ≥ 20
- ▶ Variant support value ≥ 6
- ▶ Variant frequency value ≥ 0.0200
- ▶ Total Depth value ≥ 100

Each variant included in the VCF File will contain the following information:

- ▶ Gene's chromosome for CHROM column
- ▶ Start position of the reference call in POS column
- ▶ "." in ID column
- ▶ The reference base(s) in REF column
- ▶ Alternate base(s) in ALT column
- ▶ The quality score in QUAL column
- ▶ "PASS" in FILTER column.
- ▶ Total depth in VALUE column
- ▶ "GT:GQ:AD:DP:VF:GQX" in FORMAT column, where
 - ▶ GT is genotype value
 - ▶ GQ is genotype quality
 - ▶ AD is allele depth
 - ▶ DP is total depth
 - ▶ VF is variant frequency
 - ▶ GQX is more conservative measure of the genotype quality, taken as the minimum of the GQ value and the QUAL column.
 - ▶ {Library ID}, with column header "{Library ID}", and the values for GT, GQ, AD, DP, VF, GQX in the format: GT:GQ:AD:DP:VF:GQX.

If available, variant calls are annotated using the Illumina Annotation Engine with the following information:

- ▶ HGNC Gene
- ▶ Transcript (using the canonical Ensembl transcript)
- ▶ Exon (using the canonical Ensembl transcript)
- ▶ Consequence (using the canonical Ensembl transcript)
- ▶ HGVS Coding Sequencing Name (using the canonical Ensembl transcript)
- ▶ HGVS Protein Sequence Name (using the canonical Ensembl transcript)
- ▶ COSMIC ID

RNA Outputs

Files

This section describes the top-level output files generated during an RNA sample analysis.

Fusions CSV

File name: {Library_ID}_Fusions.csv

This file contains all candidate fusions identified by the RNA analysis pipeline. Candidate fusions from the splice variant caller are listed in this output with relevant supporting information but are not considered high confidence. Two key output columns in the file describe the candidate fusions: Filter and KeepFusion.

The following table describes the output found in the Filter columns. The output is either a confidence filter or information only as indicated. If none of these filters are triggered, the Filter column displays PASS.

Filter	Description
Imprecise	A low-resolution candidate, not an assembled fusion call. (Confidence filter)
RepeatOverlap	The fusion is tagged as overlapping with a repeat region. Only used as a confidence filter for nonuniquely mapping fusion candidates, otherwise information only. (Confidence filter)
WeakBreakend	The read/alignment evidence on one side of the fusion is weak. Usually this filter indicates that the reads only overlap the fusion by a few base pairs. Alternatively, it can indicate too much homology (no unique sequence). (Confidence filter)
Homology	The fusion contig is a substring of another fusion contig. (Confidence filter)
DuplicateContig	The two contigs of the fusion are the same contig. (Confidence filter)
ContigIntragenic	The realignment of half-contigs produces alignments that map to the same gene on both sides (or within 1 kb if unannotated). (Confidence filter)
LowQ	Fusion supporting reads (unique) < 5 (+ 1 for every 10 million reads over 16 million reads). (Confidence filter)
LowDupReads	Fusion supporting reads (duplicate) < 5. (Confidence filter)
NonExonic	Fusion breakpoint does not fall within an exon. (Information only)
LocalContigAlign	Contig realignment found a nonfusion alignment for this contig. (Information only)
LowFusionRatio	Few strong evidence reads compared to wild type reads. (Information only)
NoReferenceReads	No reads on either side of the presumed breakpoint are marked as reference (structurally normal) reads. (Information only)

The KeepFusion column of the output has a value of True when the RNAFusionFilter score is ≥ 0.45 and none of the confidence filters are triggered.

See the headers in the output for more information about each column.



NOTE

When using Microsoft Excel to view this report, genes that are convertible to dates (such as MARCH1) automatically convert to dd-mm format (1-Mar) by Excel.

Splice Variants VCF

File name: {Library_ID}_SpliceVariants.vcf

Contains all candidate splice variants targeted by the Analysis panel identified by the RNA analysis pipeline.

The following filters can be applied for each variant call.

Filter Name	Description
LowQ	Splice Variant Score is < the Passing Quality Score threshold value of 1.
PASS	Splice Variant Score is \geq to the Passing Quality Score threshold value of 1.

Each splice variant is annotated using the Illumina Annotation Engine and the following information is captured in the VCF if available:

- ▶ HGNC Gene
- ▶ Transcript
- ▶ Exons
- ▶ Introns
- ▶ Canonical

► Consequence

See the headers in the output for more information about each column.

High Confidence Variants

File name: {Library_ID}_HighConfidenceVariants.csv

Contains the high confidence fusion and splice variant calls.

High confidence fusions are fusions targeted with passing filter (KeepFusions column = True in the {Sample_ID}_Fusions.csv). There are a total of 55 genes targeted for fusion calls. The fusion is reported if at least one gene of the gene pair is in the targeted gene list.

Each reported gene fusion has the following information: breakpoint 1, breakpoint 2, gene 1 reference reads, gene 2 reference reads, score.

- The order of gene1/gene2 fusion reported in the Gene Fusion column does not indicate directionality of the fusion. For any fusion of interest, search for the gene pair in both orientations.
- Semicolons denote fusions with overlapping genes. For example, Gene1;Gene2/Gene 3 indicates possible fusion event Gene1/Gene3 and Gene2/Gene3.
- The fusion variants are sorted, in ascending order, by Gene 1, then by Gene 2, then by Gene 1 Breakpoint, and then by Gene 2 Breakpoint.

High confidence splice variant calls are splice variants targeted by the panel (total of three genes) with a passing filter.

- Each reported splice variant has the following information: Affected Exon(s), Transcript, Breakpoint Start, Breakpoint End, Splice Supporting Reads, Reference Reads, and score.
- The splice variants are sorted by, in ascending order, Gene, then by Transcript, then by Breakpoint Start, and then by Breakpoint End.

View Analysis Results

- 1 From the Local Run Manager dashboard, select the run name.
- 2 From the Run Overview tab, review the sequencing run metrics.
- 3 To change the analysis data file location for future requeues of the selected run, select **Edit**, and edit the output run folder file path.
The file path leading up to the output run folder name is editable. The output run folder name cannot be changed.
- 4 **[Optional]** Select **Copy to Clipboard** for access to the output run folder.
- 5 Select the Sequencing Information tab to review run parameters and consumables information.
- 6 Select the Samples & Results tab to view the analysis report.
 - If analysis was repeated, expand the Select Analysis drop-down list and select the appropriate analysis.
- 7 **[Optional]** Select **Copy to Clipboard** to copy the Analysis folder file path.

For more information on the Run Overview and Sequencing Information tabs, and how to requeue analysis, see *NextSeq 550Dx Instrument Reference Guide (document # 1000000009513)*.

Module Settings

To view more information about the Local Run Manager Module, select to the **Module Settings** from the Tools drop-down menu. The module settings menu is only accessible to admin users or users with admin-specified access permission.

The Module settings screen displays the following information:

- ▶ Module Name
- ▶ Module Version
- ▶ RTA Version used for primary analysis
- ▶ Last date modified on
- ▶ Regulatory Label
- ▶ Sequencing Run Settings

Technical Assistance

For technical assistance, contact Illumina Technical Support.

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

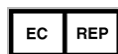
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France	+33 805102193	+33 170770446
Germany	+49 8001014940	+49 8938035677
Hong Kong	800960230	
Ireland	+353 1800936608	+353 016950506
Italy	+39 800985513	+39 236003759
Japan	0800.111.5011	
Netherlands	+31 8000222493	+31 207132960
New Zealand	0800.451.650	
Norway	+47 800 16836	+47 21939693
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Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.



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