FLUIDIGM | Technical Note

FLDM-00090 Rev 01

Analysis of Advanta Solid Tumor Assay Samples

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An Example Using the SNP/Indel Discovery and CNV Tools in NextGENe Software

To advance our understanding of cancer and make strides toward improved patient care, researchers have a growing need to evaluate somatic mutations, often from limited quantities of available samples. The Advanta™ Solid Tumor NGS Library Prep Assay with the automated Juno™ system produces targeted amplicon-based barcoded libraries for subsequent analysis on Illumina® NGS platforms, optimizing interrogation of high-value, low-variant-frequency SNPs, indels and CNVs within oncology-relevant genes.

This technical note provides steps for analyzing samples prepared with the Fluidigm Advanta Solid Tumor NGS Library Prep Assay using NextGENe® Software by SoftGenetics®, LLC. NextGENe Software can detect low-allele-frequency somatic variants along with copy number variations (CNV).

Figure 1. SNP detection call and the corresponding pile-up coverage information in the NextGENe Viewer

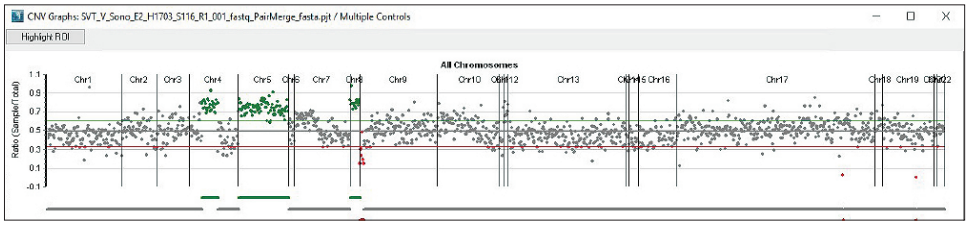


Figure 2. CNV detection call using the NextGENe CNV Tool

SNP and Indel Discovery

Step A: Perform Reference Setup.

Ensure that the desired genome reference (either Human_v37p.13_105 or Human_GRCh38p.7_108) from the SoftGenetics Reference Database is pre-loaded. Refer to the NextGENe User Manual for details of how to import a reference.

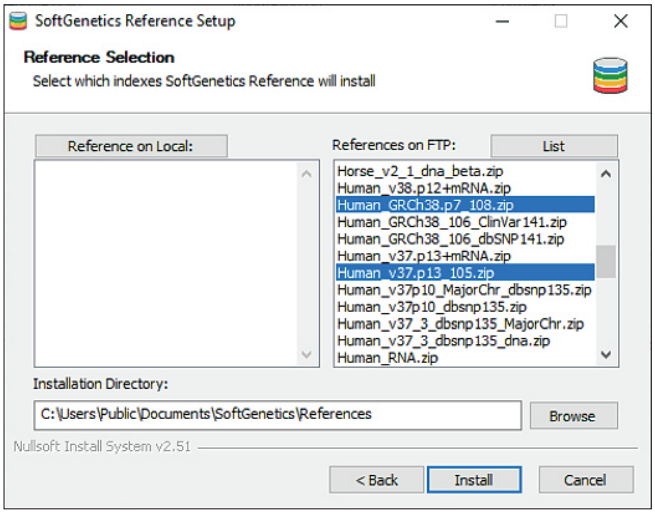


Figure 3. Reference import

Step B: Merge paired-end reads using the Overlap Merger.

Use the Overlap Merger to combine the paired-end R1 and R2 FASTQ reads to generate a FASTA file, *_PairMerged.fasta, of merged reads with higher-quality base calls.

1. Launch NextGENe and close the Project Wizard.
2. Go to the Tools Menu and click Overlap Merger.
3. Either load the previously saved settings file by clicking Load or manually change the settings to those shown in Figure 4.
4. Click Add and select the R1 and R2 FASTQ files of a sample. Note: Up to 96 samples may be processed together and are merged appropriately if sharing the same base sample name.
5. Set the appropriate Output path.
6. Click OK when ready to process. A pop-up window appears when the merging is complete.

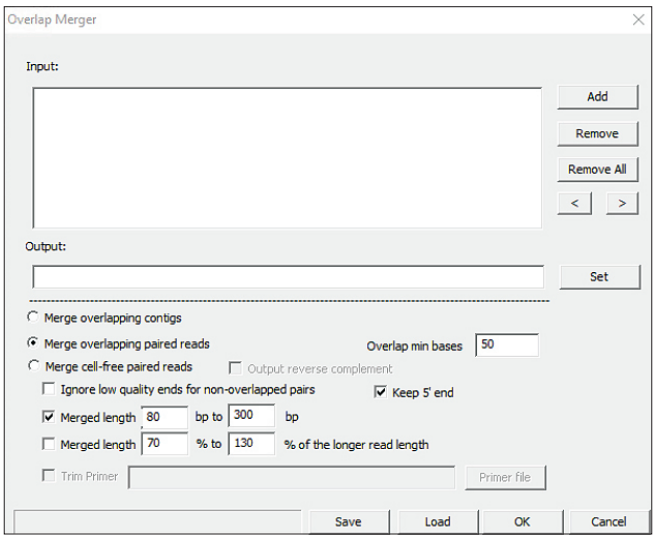


Figure 4. Recommended settings for the Overlap Merger

Step C: Align a sample and generate the Mutation Report.

Samples may be processed individually or as a batch. For batch sample processing, skip to Step E. For a single sample, launch NextGENe and use the Project Wizard to perform the following steps:

1. Load the recommended alignment settings file, Advanta_Solid_Tumor_Alignment_Settings.ini.
2. Click Load Data. In the Sample files field, select the *_PairMerged.fasta file generated in Step B.
3. In the Reference files field, select the desired reference under Preloaded that was imported in Step A.
4. Set the desired output path in the Output field.
5. Ensure that the path to the Amplicon BED file is set appropriately to the Advanta_Solid_Tumor_assays.bed that provides the genomic locations of the targeted regions without the primers.
6. Click Alignment and review the loaded settings. Modify if desired. Recommended: Use very loose parameters for Mutation percentage and SNP allele count for the Mutation filter in this alignment step, outlined in Figure 6, and then use stricter ones in the Mutation Report settings. This still results in the output but prevents the need to re-align if new parameters for curation are desired.
7. Click the Post Processing button to ensure that the Mutation Report is pointed to the correct Settings file location, either Advanta_Solid_Tumor_Mutation_Report_Settings.ini or another desired Mutation Report settings file saved from a previous run, shown in Figure 7.

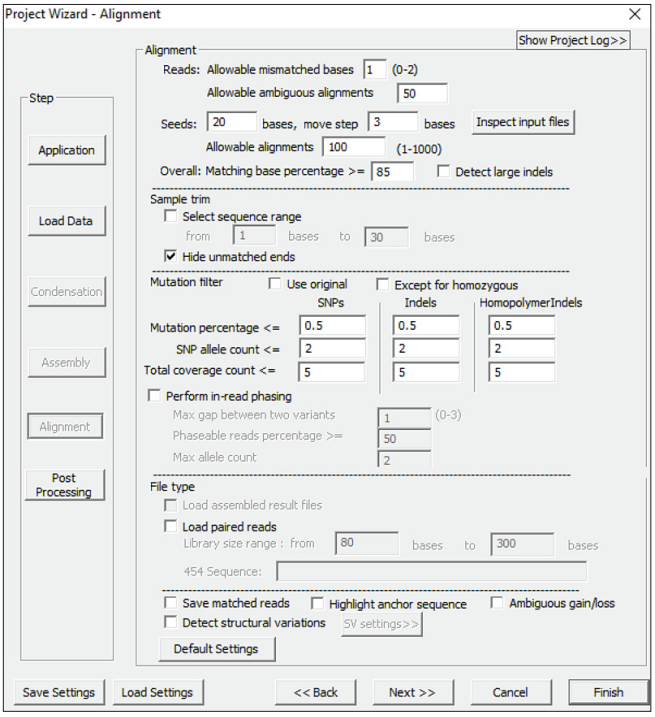


Figure 6. Recommended settings for the Alignment and Mutation filter settings

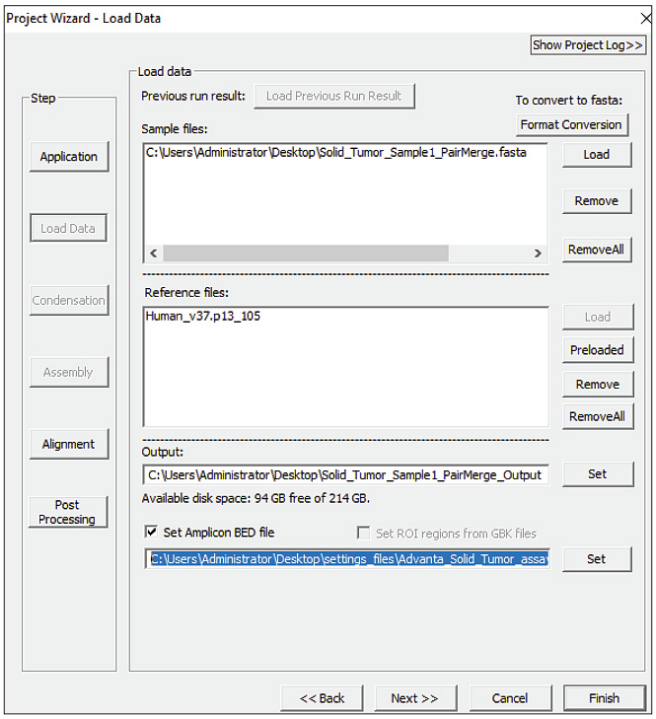


Figure 5. Project Wizard single-sample data loading step

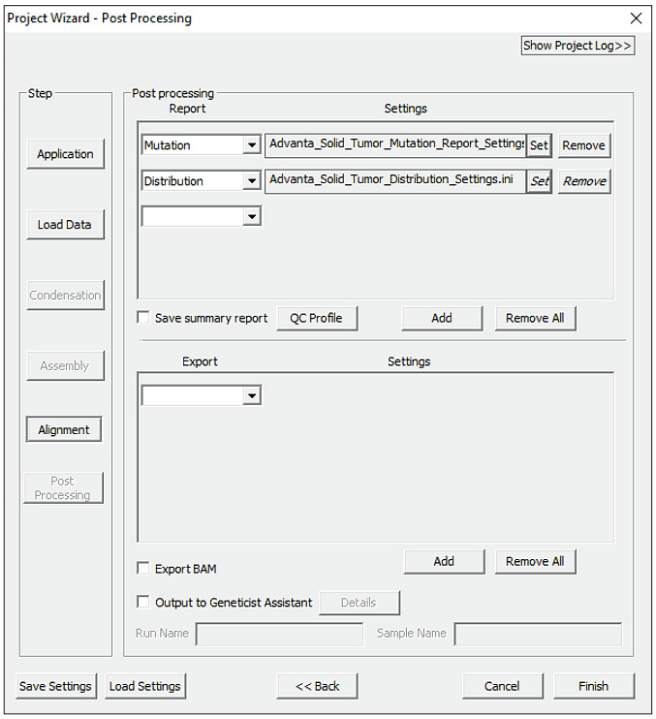


Figure 7. Project Wizard single-sample report settings

8 Ensure that the Distribution Report is pointed to the correct Settings file location, either Advanta_Solid_Tumor_Distribution_Settings.ini or another desired Distribution Report settings file saved from a previous run.

9 Click Finish to close the Project Wizard.

10 In the Projects window, click Run to process the sample.

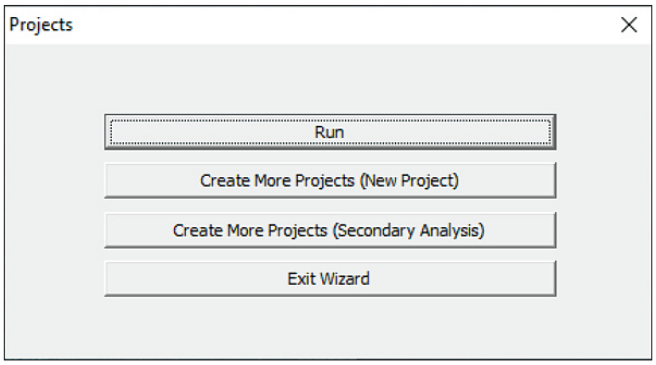


Figure 8. Final window to launch processing

Step D: View output.

After completion, the NextGENe Viewer automatically opens, displaying the pile-ups as shown in Figure 1 and the Mutation Report (not shown).

When the user clicks the SNP/indel events listed in the report, the viewer automatically changes to the genomic location of the event.

Step E: Perform multiple sample processing.

Many samples can be processed as a batch after merging is completed in Step B.

Refer to the NextGENe User Manual (NextGENe-2.4.2-UG001) for details to set up AutoRun and to create a batch job using the Fluidigm Advanta Solid Tumor template or another settings file that generates the Mutation Report and the Distribution Coverage Report. Use of the Fluidigm Advanta Solid Tumor template automatically applies parameters used to conduct the internal analytical validation.

Generated files include VCF format files, which can be compiled and manipulated as other VCF files, and NextGENe formatted text files, which can be loaded into a spreadsheet program like Excel® as tables for review.

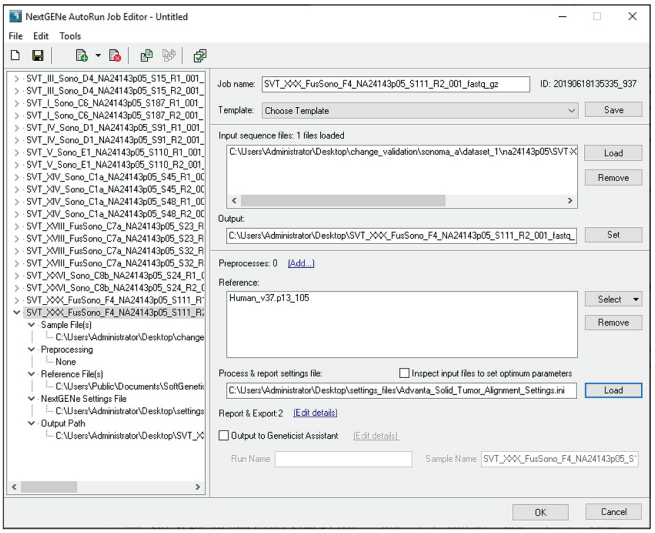


Figure 9. The Job Editor is used for batch processing of samples in the AutoRun feature.

CNV Detection

Step A: Generate project files for samples.

Execute the methods listed under the previous section, SNP and Indel Discovery, for all samples that the CNV analysis is to be performed on.

Step B: Generate project files for controls.

Execute the methods listed under the previous section, SNP and Indel Discovery, for a set of control samples that are known to not have CNVs. For this reference, a set of 12 Genome in a Bottle (GIAB) samples that were sequenced using the same methods as the samples to be analyzed for CNV is used as a control set.

Step C: Set CNV Report parameters.

1. Launch NextGENe and close the Project Wizard.
2. Go to the File menu and click Open NextGENe Viewer.
3. After the NextGENe Viewer has opened, go to the Comparisons menu and click CNV Tool.
4. In the CNV Tool settings window, click Load Settings and load the Advanta_Solid_Tumor_CNV_Settings.ini file or a different settings file from a previous run.

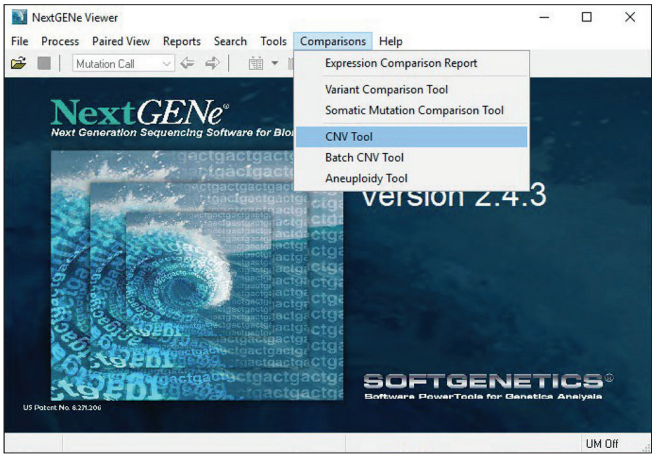


Figure 10. Open the CNV Tool from the NextGENe Viewer.

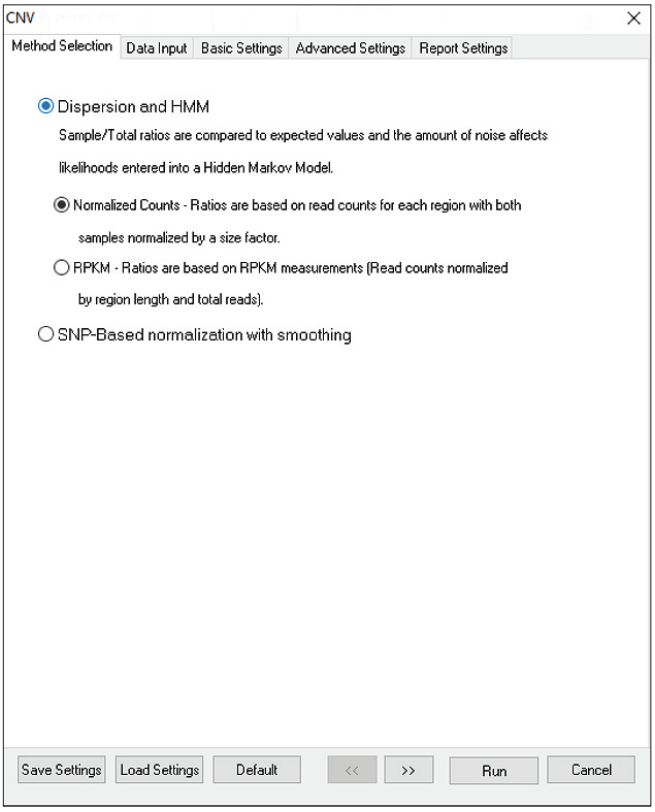


Figure 11. CNV Tool settings window

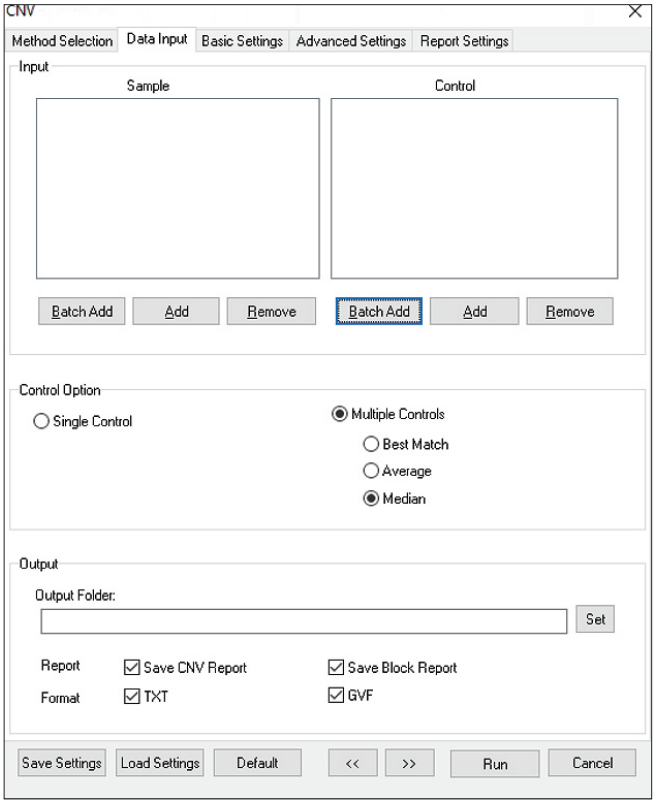


Figure 12. Data Input for CNV Tool

5 Click the Data Input tab and under the Control field, select Batch Add.

6 Navigate to the folder that contains the project files generated in Step B and click OK. (The software recursively searches that folder for all project files.)

7 Under the Sample field, select Batch Add, navigate to the folder that contains the project files generated in Step A, and click OK. (The software recursively searches for all project files in the selected folder. Up to 48 samples may be processed at one time for CNVs.)

8 Set the Output Folder path as desired.

9 Click the Basic Settings tab and check that the Input region of interest path is set to the correct location of Advanta_Solid_Tumor_assays.bed, as in Figure 13.

10 Modify any additional parameters as desired and click Run.

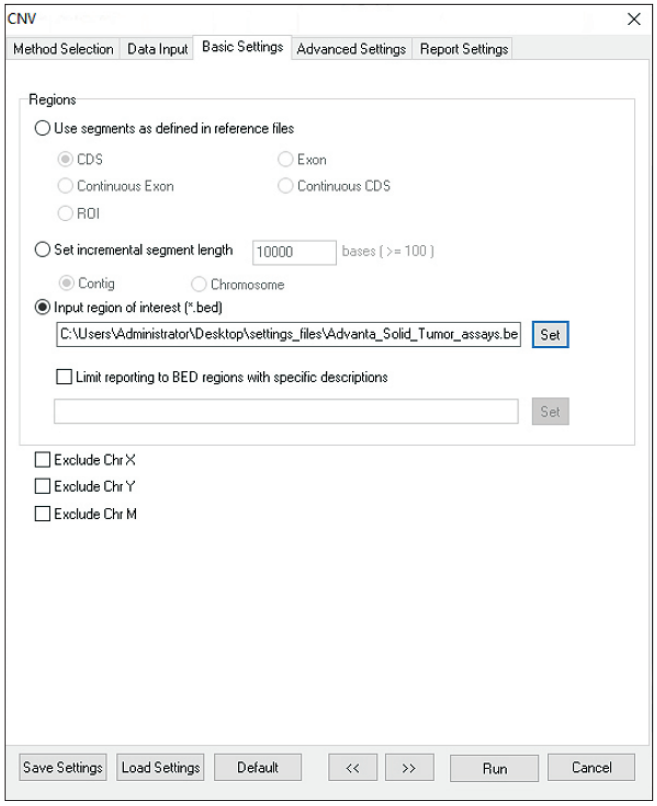
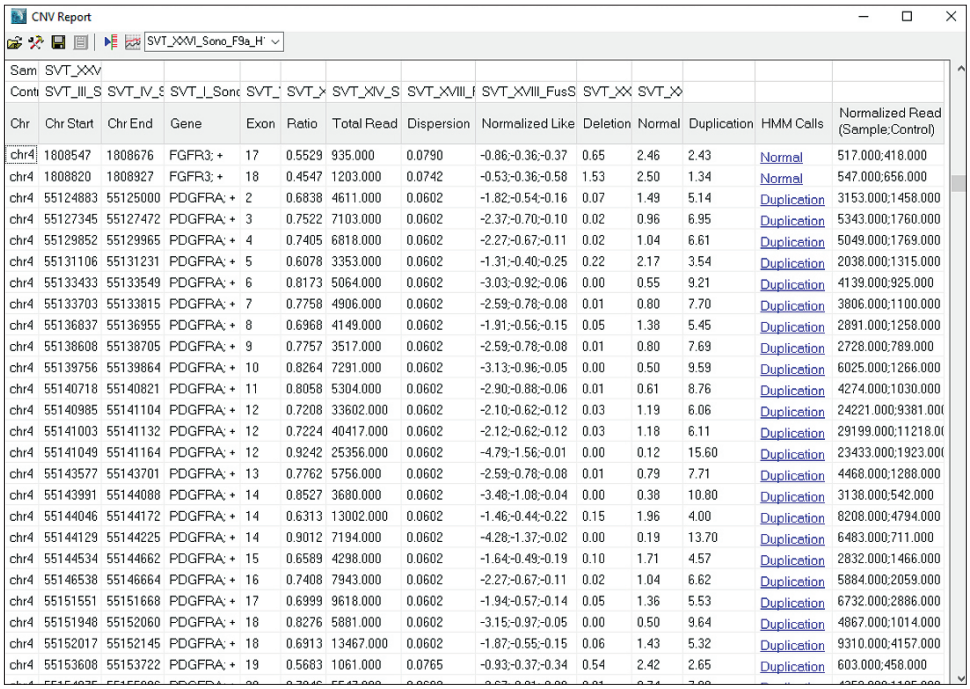


Figure 13. Basic Settings for CNV Tool

Step D: View CNV Report and plots.

After the CNV Tool has finished running, the CNV Report for the first sample is displayed.

Generated files include GVF and NextGENe formatted text files, which may be loaded into a spreadsheet program like Excel as tables for review.



Chr	Chr Start	Chr End	Gene	Exon	Ratio	Total Read	Dispersion	Normalized Like	Deletion	Normal	Duplication	HMM Calls	Normalized Read (Sample:Control)
chr4	1808547	1808676	FGFR3; +	17	0.5529	935.000	0.0790	-0.86;-0.36;-0.37	0.65	2.46	2.43	Normal	517.000;418.000
chr4	1808820	1808927	FGFR3; +	18	0.4547	1203.000	0.0742	-0.53;-0.36;-0.58	1.53	2.50	1.34	Normal	547.000;656.000
chr4	55124883	55125000	PDGFRA; +	2	0.6838	4611.000	0.0602	-1.82;-0.54;-0.16	0.07	1.49	5.14	Duplication	3153.000;1458.000
chr4	55127345	55127472	PDGFRA; +	3	0.7522	7103.000	0.0602	-2.37;-0.70;-0.10	0.02	0.96	6.95	Duplication	5343.000;1760.000
chr4	55129852	55129965	PDGFRA; +	4	0.7405	6818.000	0.0602	-2.27;-0.67;-0.11	0.02	1.04	6.61	Duplication	5049.000;1769.000
chr4	55131106	55131231	PDGFRA; +	5	0.6078	3353.000	0.0602	-1.31;-0.40;-0.25	0.22	2.17	3.54	Duplication	2038.000;1315.000
chr4	55133433	55133549	PDGFRA; +	6	0.8173	5064.000	0.0602	-3.03;-0.92;-0.06	0.00	0.55	9.21	Duplication	4139.000;925.000
chr4	55133703	55133815	PDGFRA; +	7	0.7758	4906.000	0.0602	-2.59;-0.78;-0.08	0.01	0.80	7.70	Duplication	3806.000;1100.000
chr4	55136837	55136955	PDGFRA; +	8	0.6968	4149.000	0.0602	-1.91;-0.56;-0.15	0.05	1.38	5.45	Duplication	2891.000;1258.000
chr4	55138608	55138705	PDGFRA; +	9	0.7757	3517.000	0.0602	-2.59;-0.78;-0.08	0.01	0.80	7.69	Duplication	2728.000;789.000
chr4	55139756	55139864	PDGFRA; +	10	0.8264	7291.000	0.0602	-3.13;-0.96;-0.05	0.00	0.50	9.59	Duplication	6025.000;1266.000
chr4	55140718	55140821	PDGFRA; +	11	0.8058	5304.000	0.0602	-2.90;-0.88;-0.06	0.01	0.61	8.76	Duplication	4274.000;1030.000
chr4	55140985	55141104	PDGFRA; +	12	0.7208	33602.000	0.0602	-2.10;-0.62;-0.12	0.03	1.19	6.06	Duplication	24221.000;9381.00(
chr4	55141003	55141132	PDGFRA; +	12	0.7224	40417.000	0.0602	-2.12;-0.62;-0.12	0.03	1.18	6.11	Duplication	29199.000;11218.0(
chr4	55141049	55141164	PDGFRA; +	12	0.9242	25356.000	0.0602	-4.79;-1.56;-0.01	0.00	0.12	15.60	Duplication	23433.000;1923.00(
chr4	55143577	55143701	PDGFRA; +	13	0.7762	5756.000	0.0602	-2.59;-0.78;-0.08	0.01	0.79	7.71	Duplication	4468.000;1288.000
chr4	55143991	55144088	PDGFRA; +	14	0.8527	3680.000	0.0602	-3.48;-1.08;-0.04	0.00	0.38	10.80	Duplication	3138.000;542.000
chr4	55144046	55144172	PDGFRA; +	14	0.6313	13002.000	0.0602	-1.46;-0.44;-0.22	0.15	1.96	4.00	Duplication	8208.000;4794.000
chr4	55144129	55144225	PDGFRA; +	14	0.9012	7194.000	0.0602	-4.28;-1.37;-0.02	0.00	0.19	13.70	Duplication	6483.000;711.000
chr4	55144534	55144662	PDGFRA; +	15	0.6589	4298.000	0.0602	-1.64;-0.49;-0.19	0.10	1.71	4.57	Duplication	2832.000;1466.000
chr4	55146538	55146664	PDGFRA; +	16	0.7408	7943.000	0.0602	-2.27;-0.67;-0.11	0.02	1.04	6.62	Duplication	5884.000;2059.000
chr4	55151551	55151668	PDGFRA; +	17	0.6999	9618.000	0.0602	-1.94;-0.57;-0.14	0.05	1.36	5.53	Duplication	6732.000;2886.000
chr4	55151948	55152060	PDGFRA; +	18	0.8276	5881.000	0.0602	-3.15;-0.97;-0.05	0.00	0.50	9.64	Duplication	4867.000;1014.000
chr4	55152017	55152145	PDGFRA; +	18	0.6913	13467.000	0.0602	-1.87;-0.55;-0.15	0.06	1.43	5.32	Duplication	9310.000;4157.000
chr4	55153608	55153722	PDGFRA; +	19	0.5683	1061.000	0.0765	-0.93;-0.37;-0.34	0.54	2.42	2.65	Duplication	603.000;458.000

Figure 14: An example of a CNV report. Select a different sample by clicking the drop-down menu on the toolbar. To view the CNV plots, click the CNV Graphs button next to the drop-down menu, including one similar to Figure 2.

For more information

The Advanta Solid Tumor NGS Library Prep Assay:
Contact **tech.support@fluidigm.com**.

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