Analysis of Advanta RNA Fusions Assay Samples

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An Example Using the Structural Variation Feature of NextGENe Software

RNA fusion transcripts are an important class of somatic alterations, resulting in chimeric proteins that can contribute to oncogene activation or tumor suppressor inactivation. The Advanta™ RNA Fusions NGS Library Prep Assay with the automated Juno™ system produces targeted amplicon-based libraries for subsequent analysis on Illumina® NGS platforms and optimized interrogation of high-value gene fusion pairs.

This technical note provides steps to analyze samples processed with the Advanta RNA Fusions NGS Library Prep Assay. Using NextGENe® Software by SoftGenetics®, LLC., generate a Structural Variation Report to identify reads spanning a fusion breakpoint and split them appropriately to align to multiple genomic and transcriptomic references.

Figure 1. The Structural Variation Report output of a sample prepared with the Advanta RNA Fusions NGS Library Prep Assay in the NextGENe Viewer, displaying identified fusion events. The dual viewer allows side-by-side comparison of both sides of the fusion breakpoint.

Step A: Perform Reference Setup.

Import the reference file containing both genomic and transcriptomic sequences. Two reference files titled Human_v37.p13+mRNA and Human_v38.p13+mRNA, corresponding to hg19 and hg38, respectively, are available from the SoftGenetics Reference Database. Refer to the NextGENe User Manual (NextGENe-2.4.2-UG001) for details of how to import a reference.
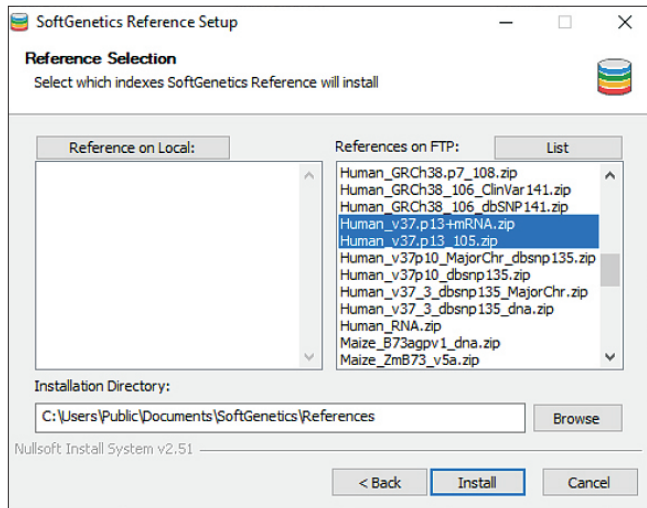

Figure 2. Reference import containing both DNA and RNA reference sequences

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

Use the Overlap Merger tool to combine the paired-end R1 and R2 FASTQ reads to generate a FASTA file of merged reads with the file extension *_PairMerged.fasta. To do this, make a consensus call for each overlapping base from both reads while assessing individual base quality, resulting in a merged read 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 a previously saved Settings file by clicking Load or manually change the settings to those shown shown in Figure 3.

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.

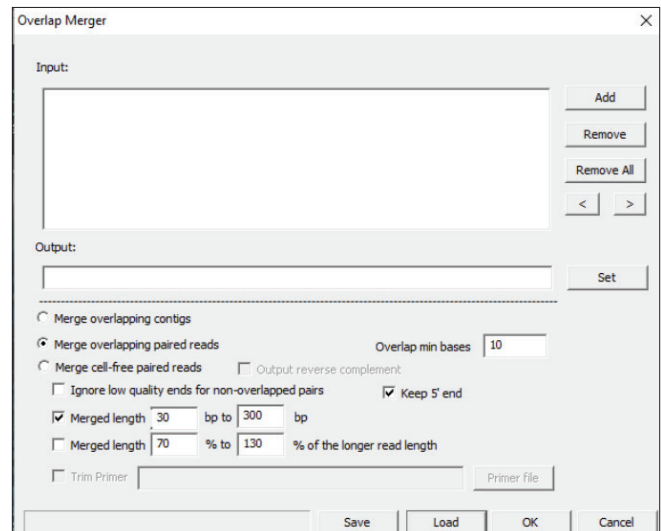6 Click OK when ready to process. A pop-up window appears when merging is complete.

Figure 3. Recommended settings for the Overlap Merger

Step C: Align a sample and generate the Structural Variation Report.

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

1 Load the recommended alignment Settings file, Advanta_RNA_Fusion_Alignment Settings.ini.

2 Click Load Data. In the Sample files field, select the *_PairMerged.fasta file corresponding to the sample to be analyzed that was generated in Step B.

3 In the Reference files field, select the Human_v[37/38]+mRNA reference under Preloaded that was imported in Step A.

4 Set the desired output path in the Output field.

5 Click Alignment and review the loaded settings. Modify if desired.

6 Ensure that the path under Post processing for the Structural Variation Report is pointed to the correct Settings file location, either Advanta_RNA_Fusion_SV_Report_Settings.ini or another user-defined Structural Variation Report settings file saved from a previous run.

7 Click Finish to close the Project Wizard.

8 In the subsequent pop-up window, click Run to process the sample.
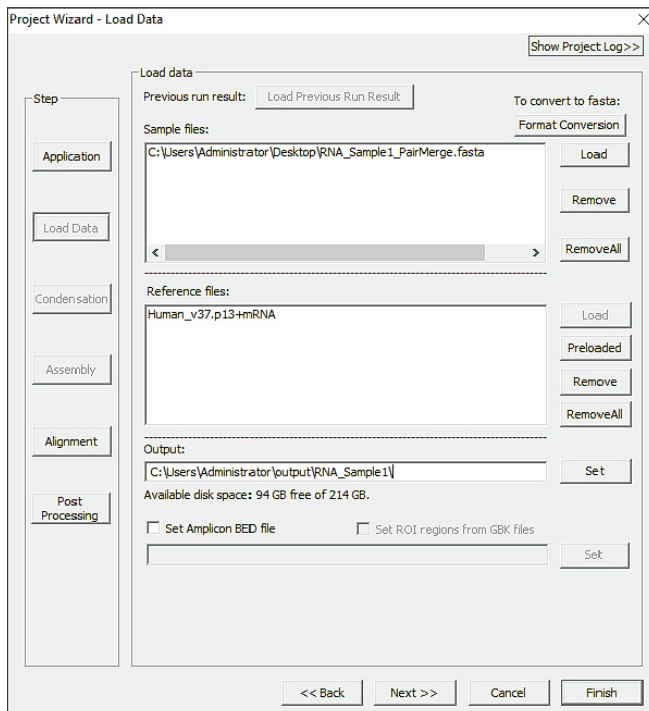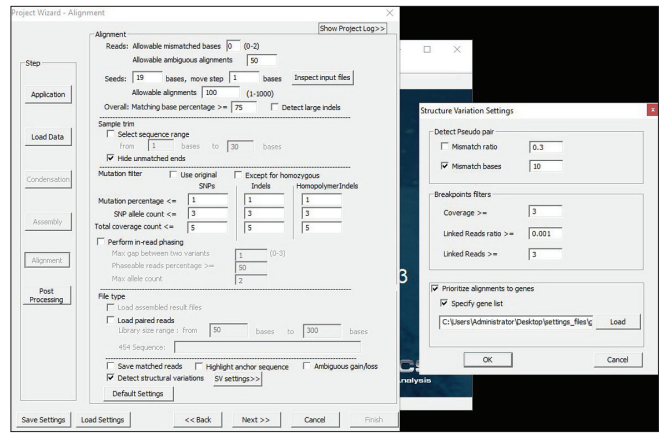

Figure 5. Project Wizard single-sample alignment settings
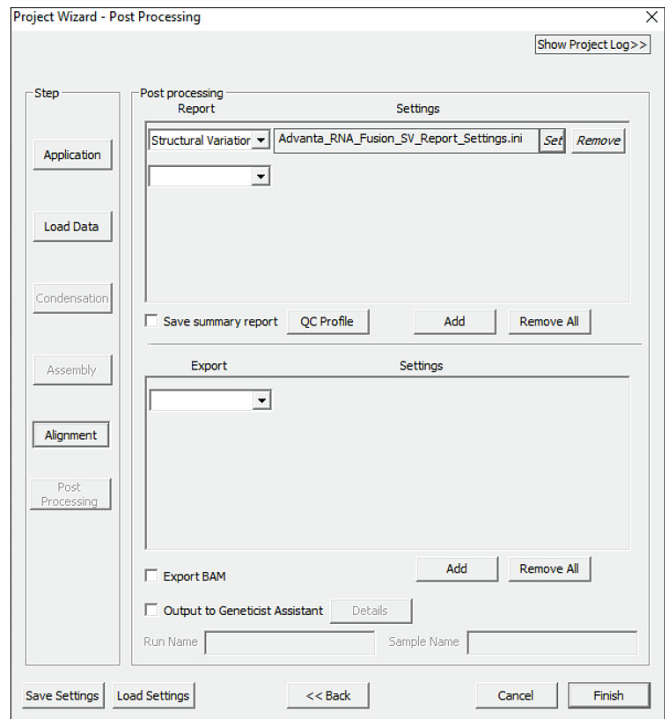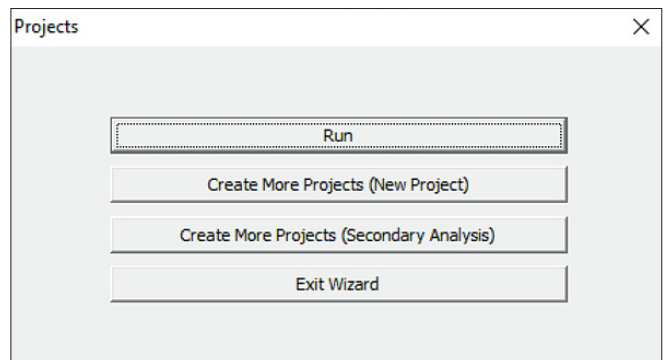

Figure 6. Project Wizard single-sample report settings

Figure 7. Final window to launch processing

Figure 4. Single-sample data loading step in the Project Wizard

Step D: View output.

1 After completion, the NextGENe Viewer automatically opens. To view the detected RNA fusion events, go to the Reports menu and select Structural Variation Report.

2 After reviewing/modifying the Structural Variation Report settings, a secondary dual viewer opens as shown in Figure 1.

3 Selecting different lines in the report on the bottom changes the dual viewer to the selected RNA fusion event.

4 Review the dual alignment pile-ups to ensure high confidence in the RNA fusion detection call.
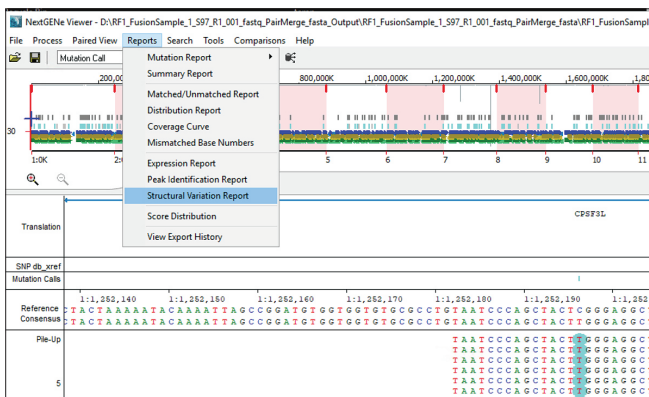
Figure 8. Opening the Structural Variation Report from the NextGENe Viewer

Step E: Perform multiple sample processing.

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

Refer to the NextGENe User Manual for details to set up AutoRun and to create a batch job using the Fluidigm Advanta RNA Fusions template provided by Fluidigm or another user-defined setting file to generate the Structural Variation Report. Use of the Fluidigm Advanta RNA Fusions template automatically applies parameters used to conduct the internal analytical validation.

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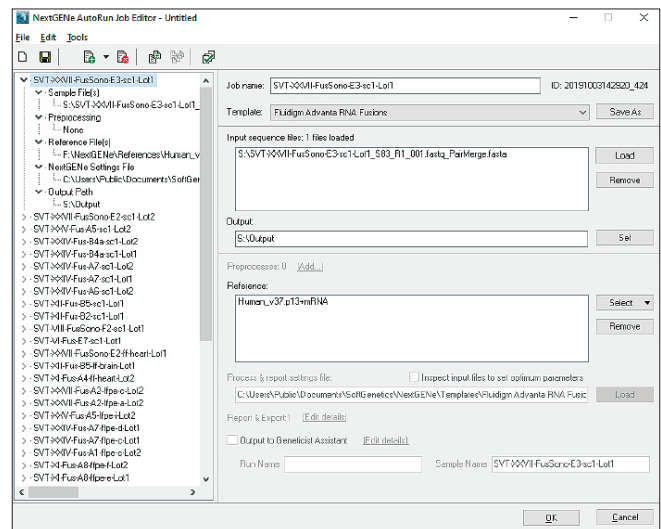

Figure 9. The Job Editor is used for batch processing of samples in the AutoRun feature.

For more information

The Advanta RNA Fusions NGS Library Prep Assay:
Contact **tech.support@fluidigm.com**.

Receive a 30-day free trial of the NextGENe software:
Contact **tech_support@softgenetics.com**.

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