

Automatic NGS HLA Data Analysis Using NGSignition and NGSserver

Next-generation sequencing (NGS) allows for HLA typing of large numbers of samples within a single sequencing run. Currently, laboratories have to wait for the sequencer to be finished to manually start data analysis in NGSengine, a software package for high-resolution identification of HLA alleles.

Here we present NGSignition and NGSserver as new software applications that together enable an automatic start of data analysis as soon as the NGS run has completed.

Materials & Methods

NGSserver software was developed as an independent application that runs as a Windows service in the background without a Graphical User Interface (GUI). After a sequencing run, the sequencing machine can place the resulting data on a certain server location. This location was monitored by NGSserver for the creation of folders with NGS data. When new data was identified, NGSserver automatically started HLA typing which was performed in the same way as done by NGSengine.

NGSserver was developed to use the Windows file system API to receive signals on each change to the file system within the monitoring folder for new sequencing files. Analysis started when no more changes were detected in the data within an adjustable time limit. For communication with NGSserver, a RESTful API was presented that enabled starting and stopping of new analysis jobs.

To facilitate user interaction, a Windows desktop application called NGSignition that acts as a GUI client was built. It communicates with NGSserver, which can be on any network location, provided with the correct user credentials. NGSignition was used to start or stop monitoring, set the monitoring location, and modify the settings of NGSserver including the project location and the analysis settings. All software was developed using the .NET framework, with external libraries for tasks such as HTTP processing.

Results

NGSignition and NGSserver were successfully implemented in laboratory test sites in Utrecht (The Netherlands) and Freiburg (Germany). NGSignition (Fig 1) served as an interface for NGSserver and was used to set the data analysis preferences such as analysis region, thresholds, and quality trimming (Fig 2).

After the sequencing run was finished, NGSserver created a project based on the run, and automatically started the analysis of the data, using the same algorithms as NGSengine. After NGSserver analysis was completed, data was opened in NGSengine for manual inspection (Fig 3). Data analysis was performed in the same way as data analysis in NGSengine, producing the same quality metrics, statistics and typing results.

Conclusion

Combining NGSignition and NGSserver significantly streamlines laboratory workflows and reduces manual actions. This allows laboratories for example to start the sequencer on Friday afternoon and have all the analysis done over the weekend. Analysis is performed in the same way as done with NGSengine, producing the same reliable data quality and typing results.

Figure 1. NGSignition software, showing which folder is being monitored and which projects are currently being analyzed or have finished recently.

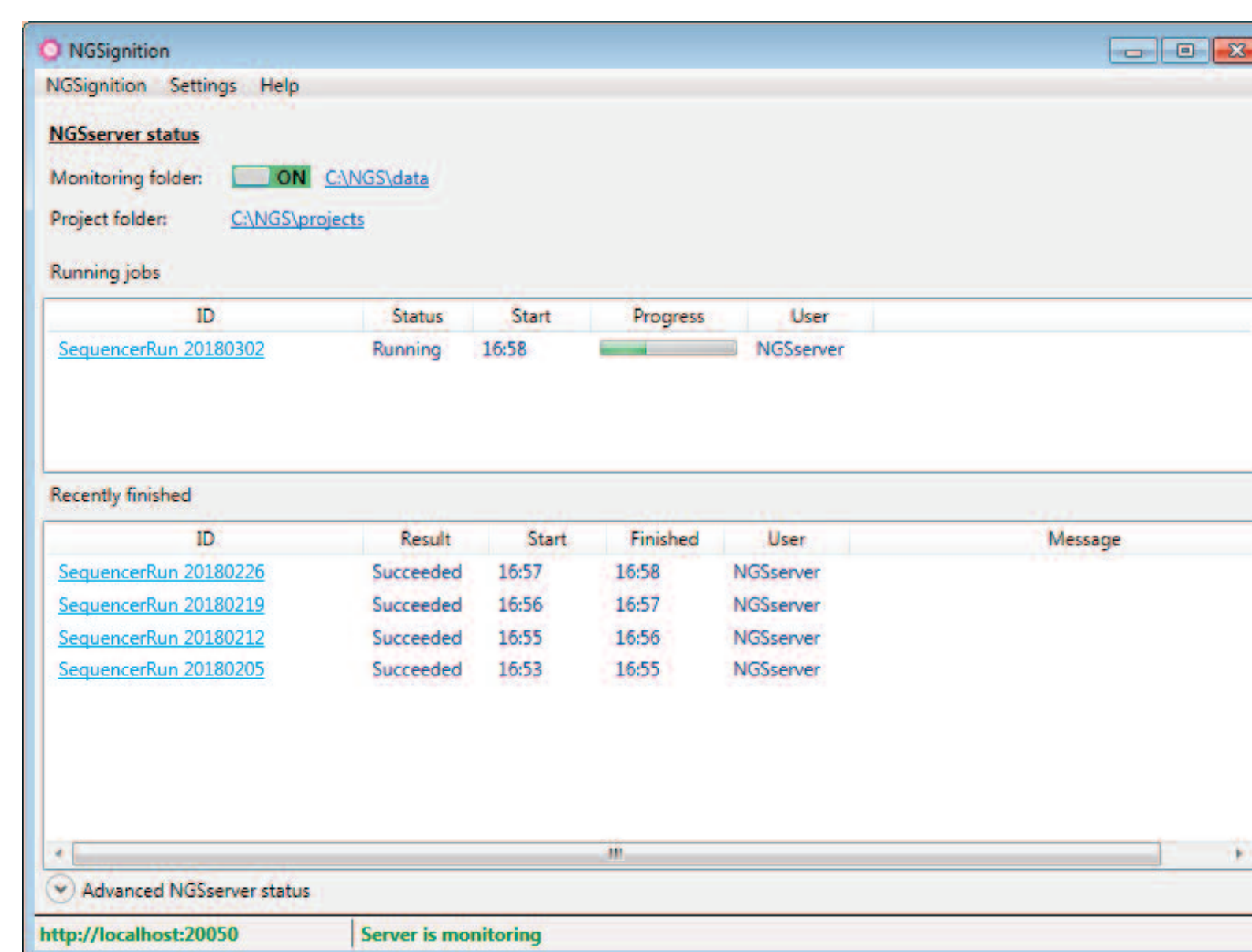


Figure 2. NGSignition locus settings, allowing the user to select the preferred analysis settings.

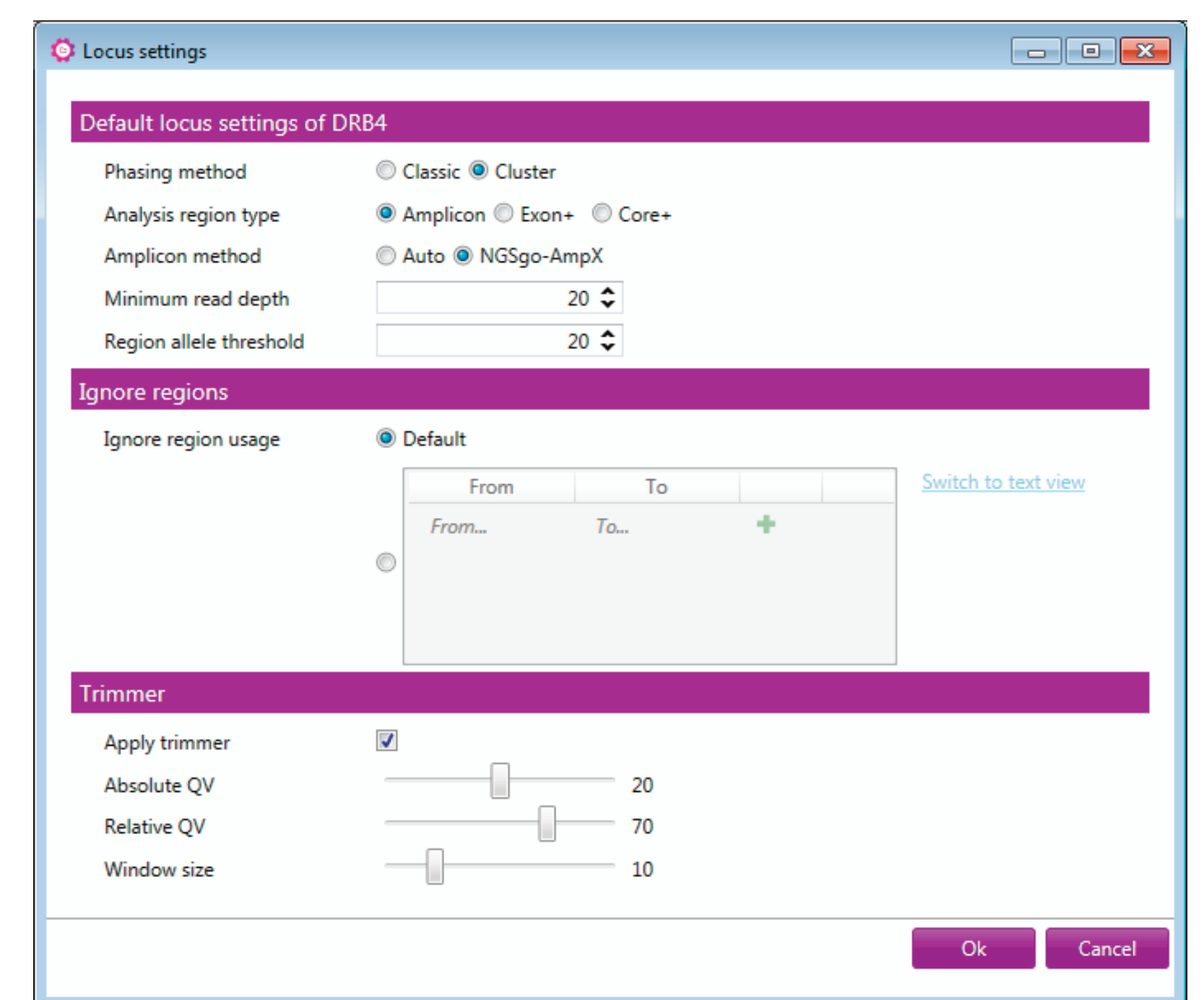


Figure 3. Results can be opened and reviewed in NGSengine. This figure shows the alignment of an HLA-A locus.

