Identifying a Translocation

Case 1

Prior information
Using standard cytogenetics tests, an individual was found to have a translocation of the tip of chromosome 4 with the tip of chromosome 22. The breakpoint was narrowed down to around 6 to 7 Mb on chromosome 4 and 50 to 51 Mb on chromosome 22 using MLPA probe sets.

Import the data by pressing the `BAM file` button and then selecting chromosome 4 from the upper dropdown list box and enter the coordinates flanking the approximate position of the breakpoint in the two text boxes to the right of the dropdown list and import the data by selecting the `Analysis` > `Only show reads with secondary alignments` menu option (Figure 1)

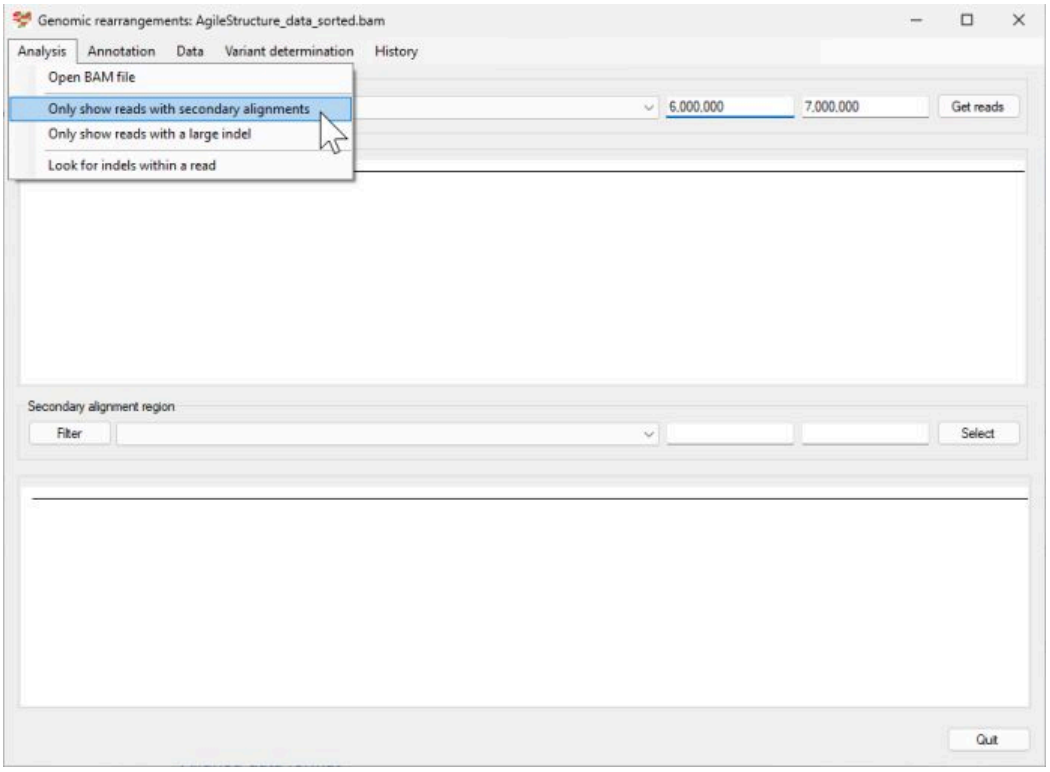

Figure 1

This will populate the upper panel with reads that have unaligned sequences that may span the breakpoint. The lower dropdown list box will now contain a list of regions to which more than two unaligned sequences have been mapped. In the list, there is a position at 50.28 Mb on chromosome 22, which is close to the suggested site of the second breakpoint. Select this region to visualize the secondary alignments (Figure 2).

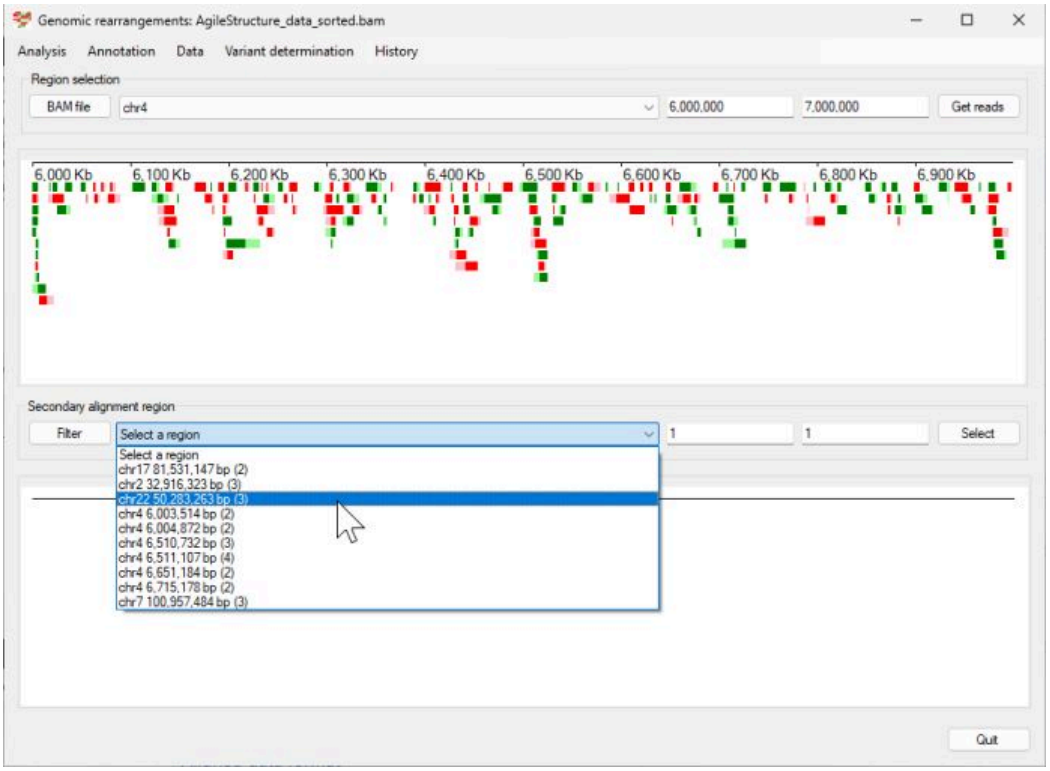

Figure 2

Once displayed, you can select the reads by clicking on them in the lower panel to see where they are located on chromosome 4. The three reads can be seen to map to approximately 6.7 Mb on chromosome 4. Select this region by moving the cursor to about 6.675 Mb, pressing the right mouse button, and moving the mouse to about 6.725 Mb before releasing the button. This will expand the upper panel, showing the reads in greater detail. Since the data has been re-read from the BAM alignment file, it is necessary to select the reads again and the region in the lower dropdown list (Figure 3).

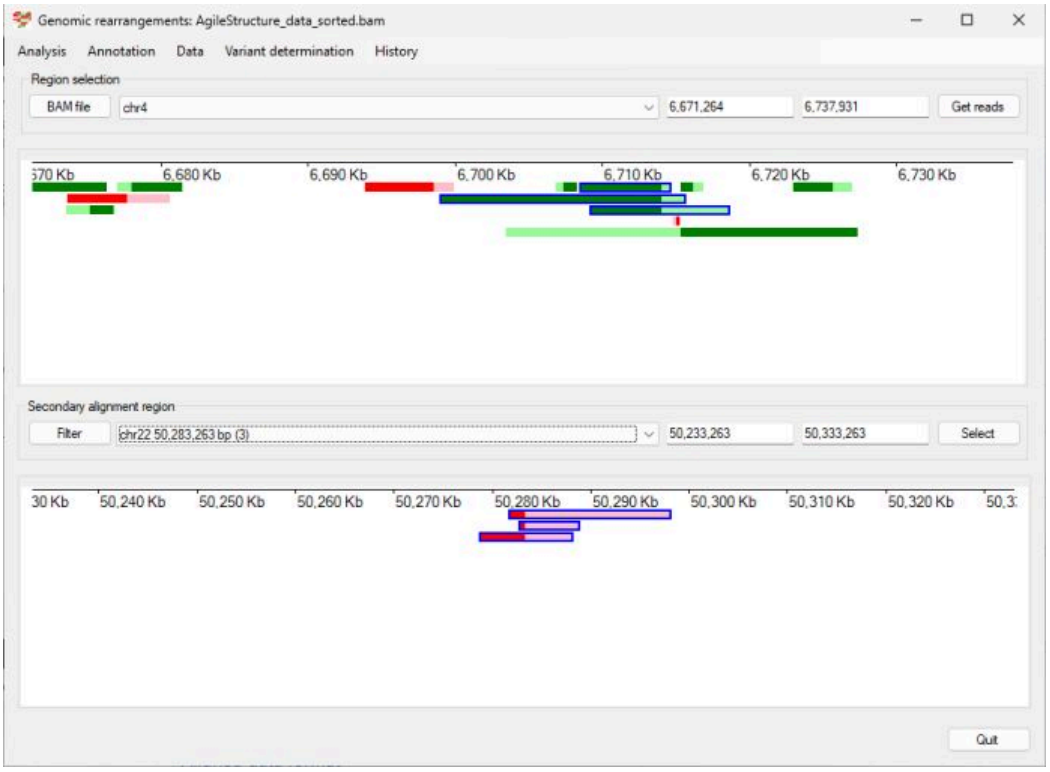

Figure 3

While other reads with secondary alignments have mapped near the breakpoint, it is now evident that they do not span the translocation breakpoint. Selecting the `Variant determination` > `Use soft clip data` > `Translocation` menu option prompts `AgileStructure` to analyse the selected reads and annotate the breakpoint, which it states as: **t(chr4;chr22)g.6,713,999:g.50,283,263** (Figure 4).
Pressing the `Yes` button will save the data to a text file.

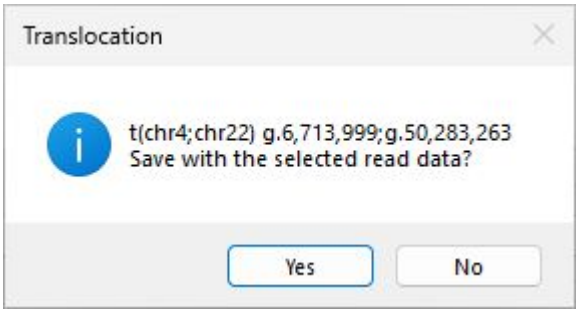

Figure 4

To test the analysis, select the `Variant determination` > `Switch region` menu option (Figure 5).

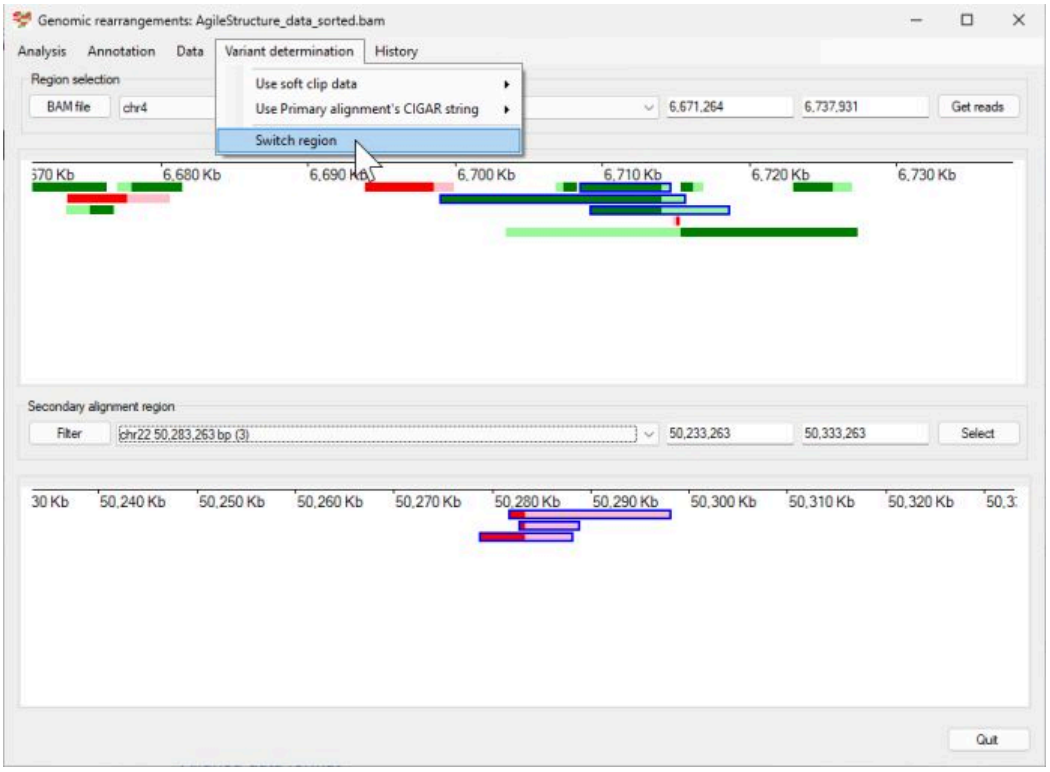

Figure 5

The coordinates used in the lower panel will now define the region viewed in the upper panel. Since data is re-read from the BAM alignment file, it is necessary to select the region in the lower panel and any reads that span the translocation (Figure 6), before selecting the `Variant determination` > `Use soft clip data` > `Translocation` menu option (Figure 7).

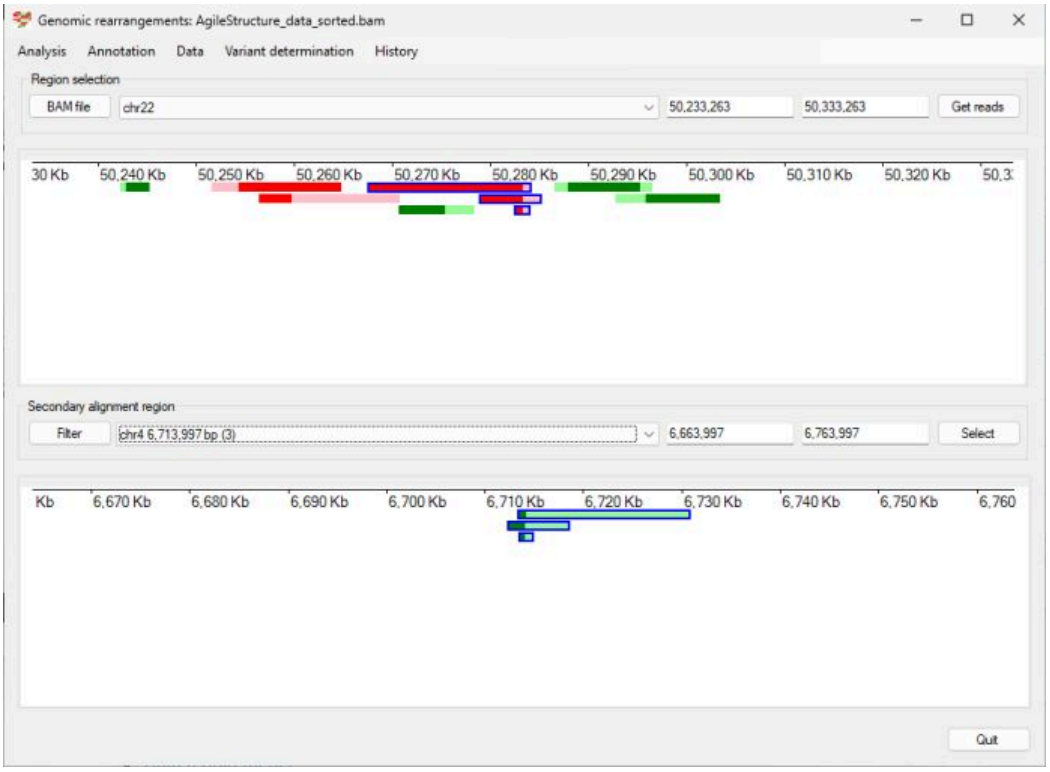

Figure 6

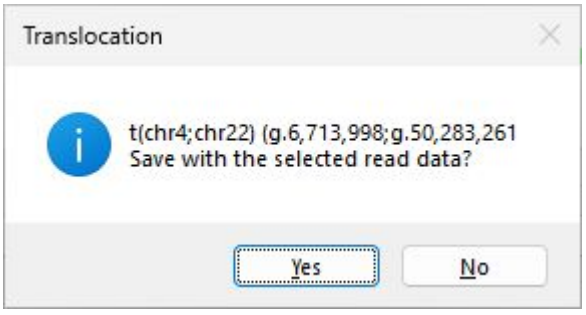

Figure 7

The breakpoint identified in the 2nd analysis was
t(chr4;chr22)(g.6,713,998:g.50,283,262)
This is derived from a different set of reads and so could be considered an independent test. While the base pair position of the two breakpoints are slightly different, it should be close enough to design a diagnostic PCR test.

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Case 2

This patient was first described in:

> Hu L, Liang F, Cheng D, Zhang Z, Yu G, Zha J, Wang Y, Xia Q, Yuan D, Tan Y, Wang D, Liang Y, Lin G. Location of Balanced Chromosome-Translocation Breakpoints by Long-Read Sequencing on the Oxford Nanopore Platform. Front Genet. 2020 Jan 14;10:1313. doi: 10.3389/fgene.2019.01313. PMID: 32010185; PMCID: PMC6972507.

Prior information
Using standard cytogenetics tests, an individual was found to have a translocation between chromosome 6 and chromosome 8. The breakpoint was narrowed down to around 113 to 114 Mb on chromosome 8 and 167 to 168 Mb on chromosome 6.

The read data is available from the NCBI SRA page as sample SRR9982132. For this guide, the data was aligned to the hg19 human reference sequence using minimap2.

Import the data by pressing the `BAM file` button and selecting the appropriate BAM file. Then, select chromosome 8 from the upper dropdown list box and enter the coordinates for the approximate position of the breakpoint in the two text boxes to the right of the dropdown list. Import the data by selecting the `Get reads` button (the data on the SRA website only contains data for the region flanking the breakpoint on chromosome 8) (Figure 1)

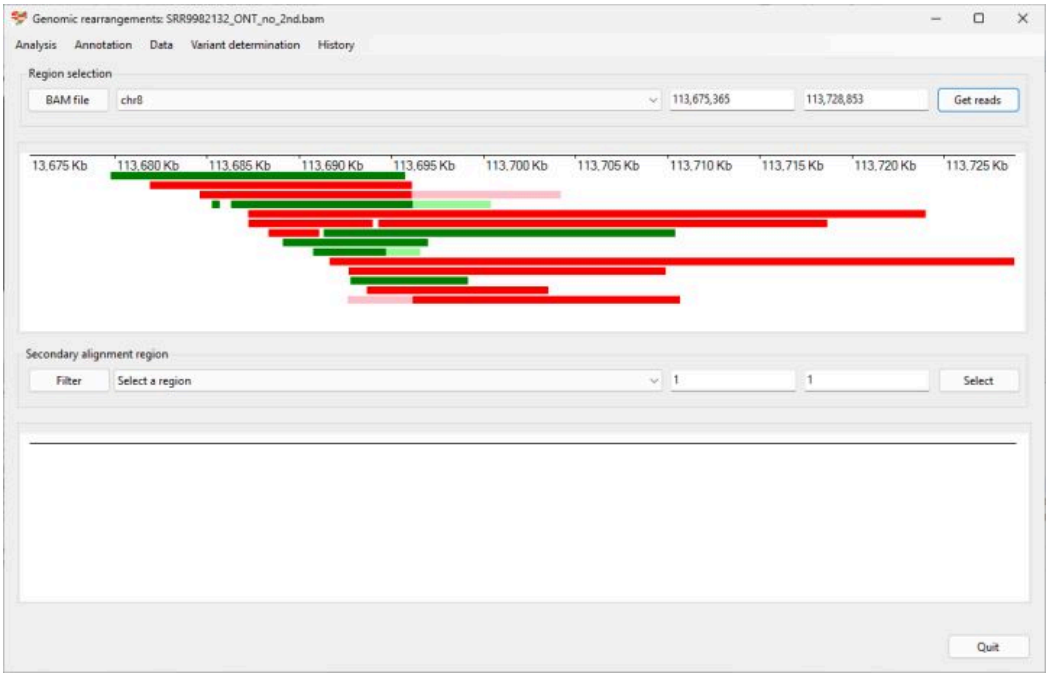

Figure 1

This will populate the upper panel with reads that have unaligned sequences that may span the breakpoint. The lower dropdown list box will now contain a region where more than two unaligned sequences have been mapped. This region is at 167.28 Mb on chromosome 6, which is close to the suggested site of the second breakpoint. Select the chromosome 6 region to visualise the secondary alignments of the reads on chromosome 8 (Figure 2).

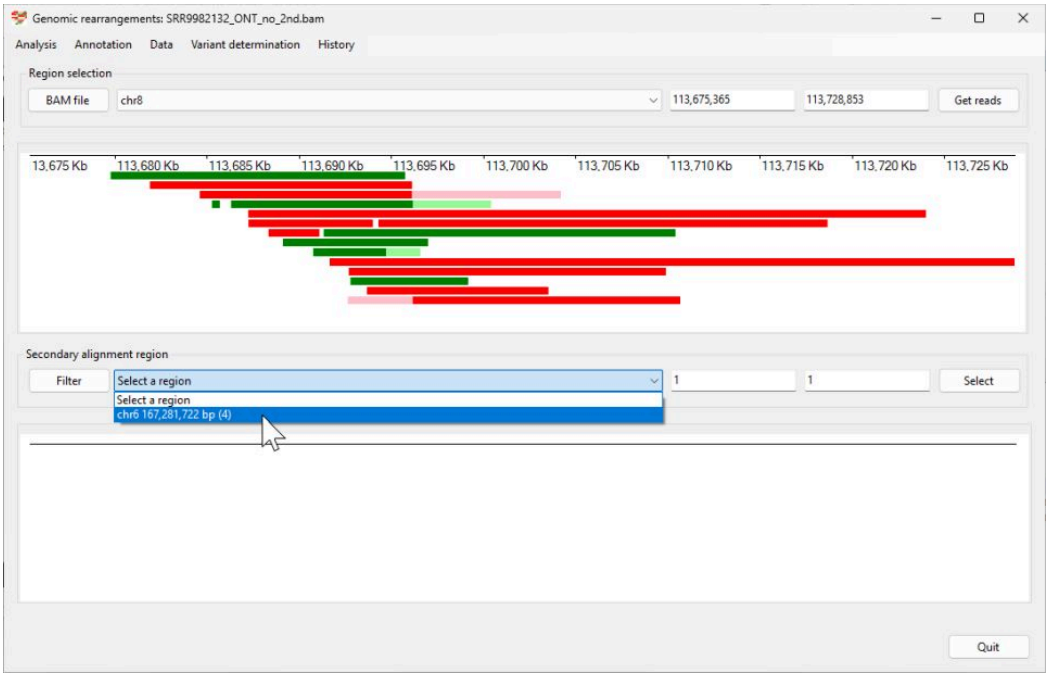

Figure 2

Once displayed, you can select the reads in the lower panel by clicking on them with the mouse to see where they are located on chromosome 8. The four reads can be seen to be mapped to approximately 113.7 Mb on chromosome 8 (Figure 3).

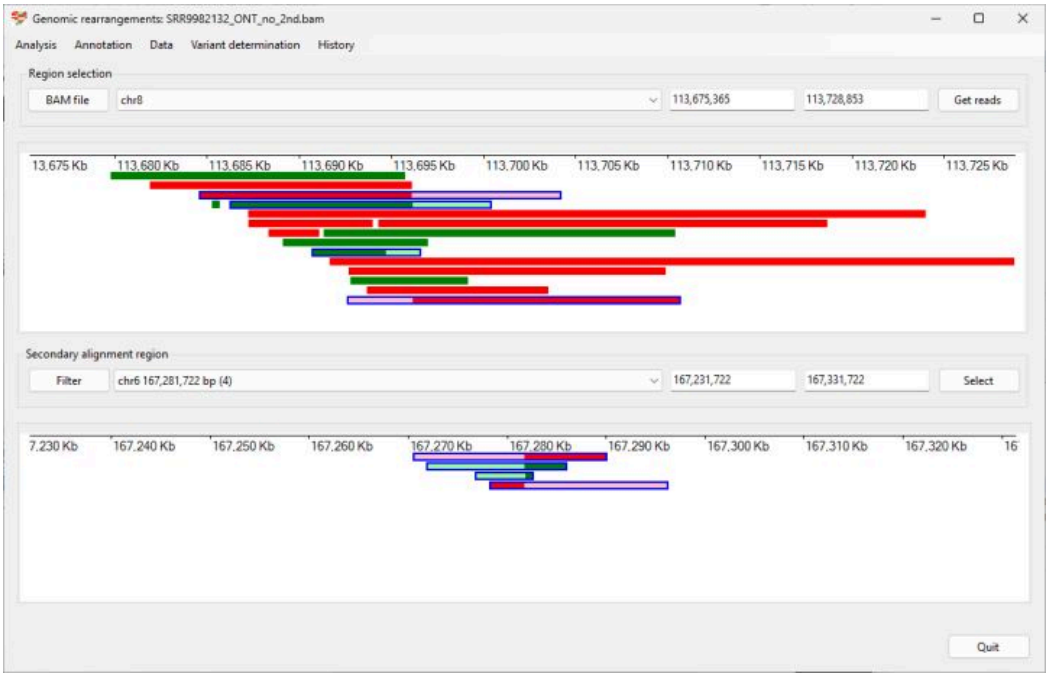

Figure 3

Selecting the `Variant determination` > `Use soft clip data` > `Translocation` menu option prompts `AgileStructure` to analyse the selected reads and annotate the breakpoint as: **t(chr6;chr8) (g.167,281,719;g.113,696,098)** (Figure 4).
Pressing the `Yes` button will save the data to a text file.

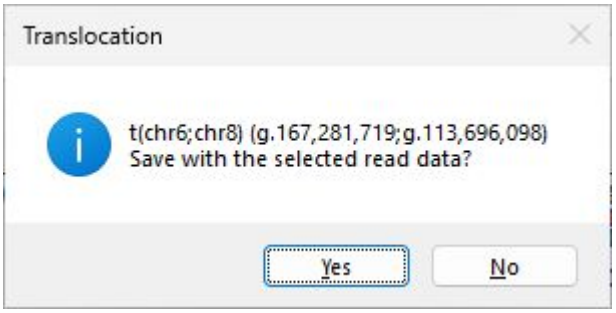

Figure 4

To test the analysis, select the `Variant determination` > `Switch region` menu option (Figure 5).

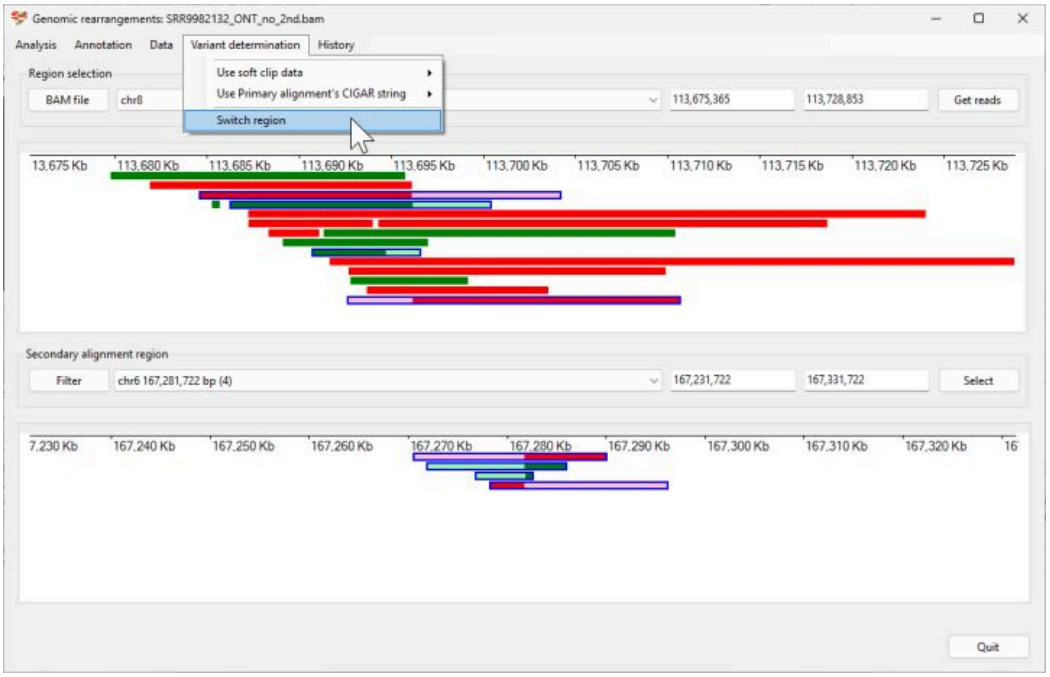

Figure 5

The coordinates used in the lower panel will now define the region viewed in the upper panel. Since data is re-read from the BAM alignment file, it is necessary to select the region in the lower panel and any reads that span the translocation (Figure 6) before selecting the `Variant determination` > `Use soft clip data` > `Translocation` menu option (Figure 7).

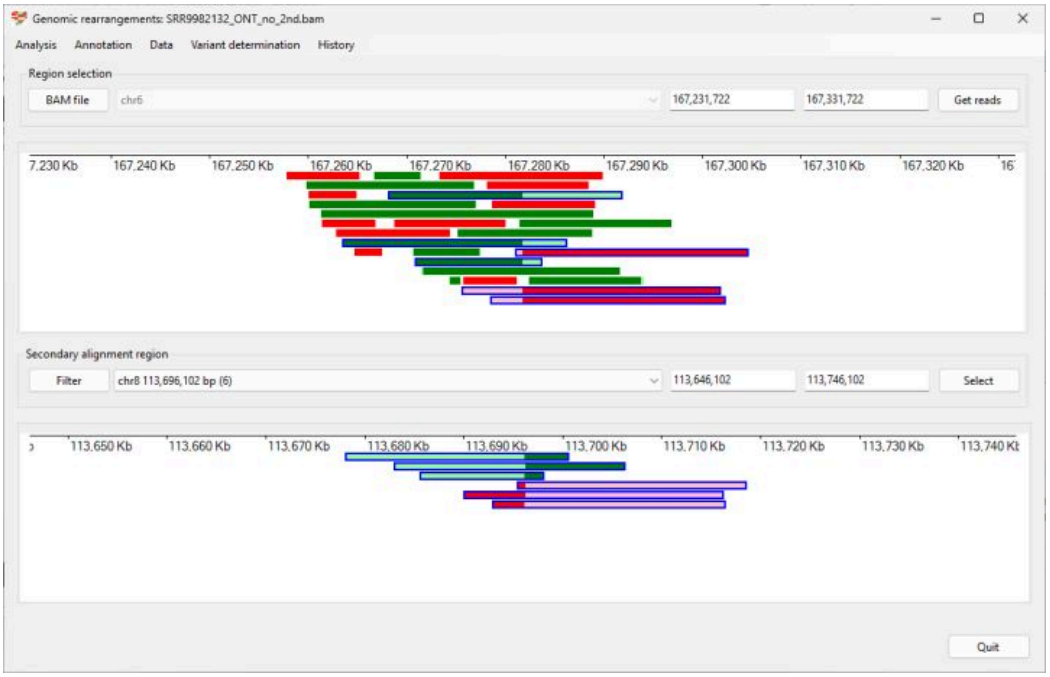

Figure 6

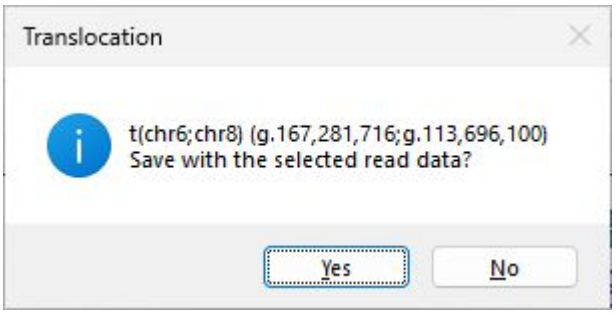

Figure 7

The breakpoint identified in the 2nd analysis:
t(chr6;chr8)(g.167,281,716;g.113,696,100)
is derived from a different set of reads and could be considered an independent test. While the base pair positions of the two breakpoints are slightly different from each other (Table 1) and the published position (less than 3 bp), it should be close enough to design a diagnostic PCR test.

Origin	Variant
Published variant	t(chr6;chr8) (g.167,281,717:g.113,696,089)
Using primary alignments on chr8	t(chr6;chr8) (g.167,281,719;g.113,696,098)
Using primary alignments on chr6	t(chr6;chr8) (g.167,281,716;g.113,696,100)

Table 1

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