AgileStructure user guide

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Installation

WIndows computers

`AgileStructure` can be run simply by downloading AgileStructure.exe, AgileStructure.dll and AgileStructure.runtimeconfig.json files to the same folder and then double clicking on the agileAgileStructure.exe file. The program requires the .NET 6.0 (or later) framework to be install which can be obtained from here: version 6.0 or version 8.0 and selecting the appropriate ***.NET Desktop Runtime *.*.****

Linux, macOS and BSD

Like Windows computers, AgileStructure doesn't require installation (just download the 3 files to the same folder), but does require ***Wine*** to be installed on the computer. A number of short guides covering the installation of ***Wine*** on appropriatlye 20 versions of Linux, BSD and macOS can be found here.
`AgileStructure` also requires the installation of the .NET frameworks usually achieved via ***Winetricks***.

Data requirements

Prior knowledge of the likely location of the breakpoint

`AgileStructure` is designed to identify breakpoints with user assistance rather than scan the whole alignment for possible breakpoints, consequently it is expected that the user will have some prior knowledge as to where the breakpoint is such as a cytogenetics and/or karyotyping report, a list of known disease genes for the patient's condition or a single pathogenic variant in a patient with a recessive disease for whom a second pathogenic variant cannot be found.

Aligned data format

`AgileStructure` is designed to visualise aligned long read data formatted as indexed `BAM` files. It's expected that the index file will have the same name as the `BAM` file with the `*.bai` extension appended to the BAM file's name. For instance, the BAM file:
`CNTNAP2.srt.mm2.bam`
will have an index file named:
`CNTNAP2.srt.mm2.bam.bai`
which will be in the same directory as the `BAM` file.
The `BAM` file must contain the header section, which lists the name and size of each reference sequence in the reference genome.

Preferred long read sequence aligners

Long reads that span a breakpoint will appear to consist of two regions of homology, mapping to different locations in the genome. How these chimeric alignments are reported is aligner-specific. Some aligners, such as Minimap2 (github, paper), treat the two regions as different alignments but will report the secondary alignment as a condensed CIGAR string in the primary alignments tag section, while others report the read as two separate alignments but not directly reference the other alignment's position and CIGAR string. However, for shorter indels, both types of aligners may report them in the CIGAR string (see this section).
`AgileStructure` is only able to analyse reads in which the indel is reported in the primary alignment's CIGAR string or the secondary alignment is reported in the tag section: The reporting of the secondary alignment in the tag section is the most flexible method and will allow more complex breakpoints to be processed. Consequently, it is recommended to align data using an aligner such as minimap2.

Optional data

To aid the analysis, it is possible to view the putative breakpoints with reference to the location of genes and repetitive sequences. This data can be obtained from the USCS genome browser as described here.

Importing alignment data

Data is imported as a pair of files, the pre-aligned BAM file and its index file, by either pressing the `BAM file` button (Figure 1a) or by selecting the `Analysis` > `Open BAM file` menu option (Figure 1b) and selecting the required `BAM` file.

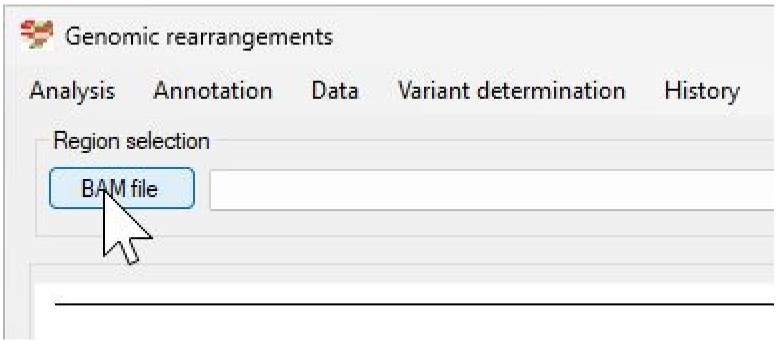


Figure 1a

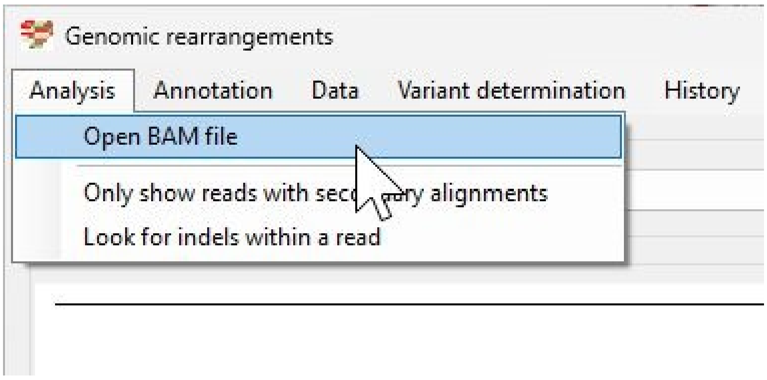


Figure 1b

`AgileStructure` will read the header section of the BAM file and populate the dropdown list box next to the `BAM file` button, with the name of the reference sequences in the BAM file (Figure 2).

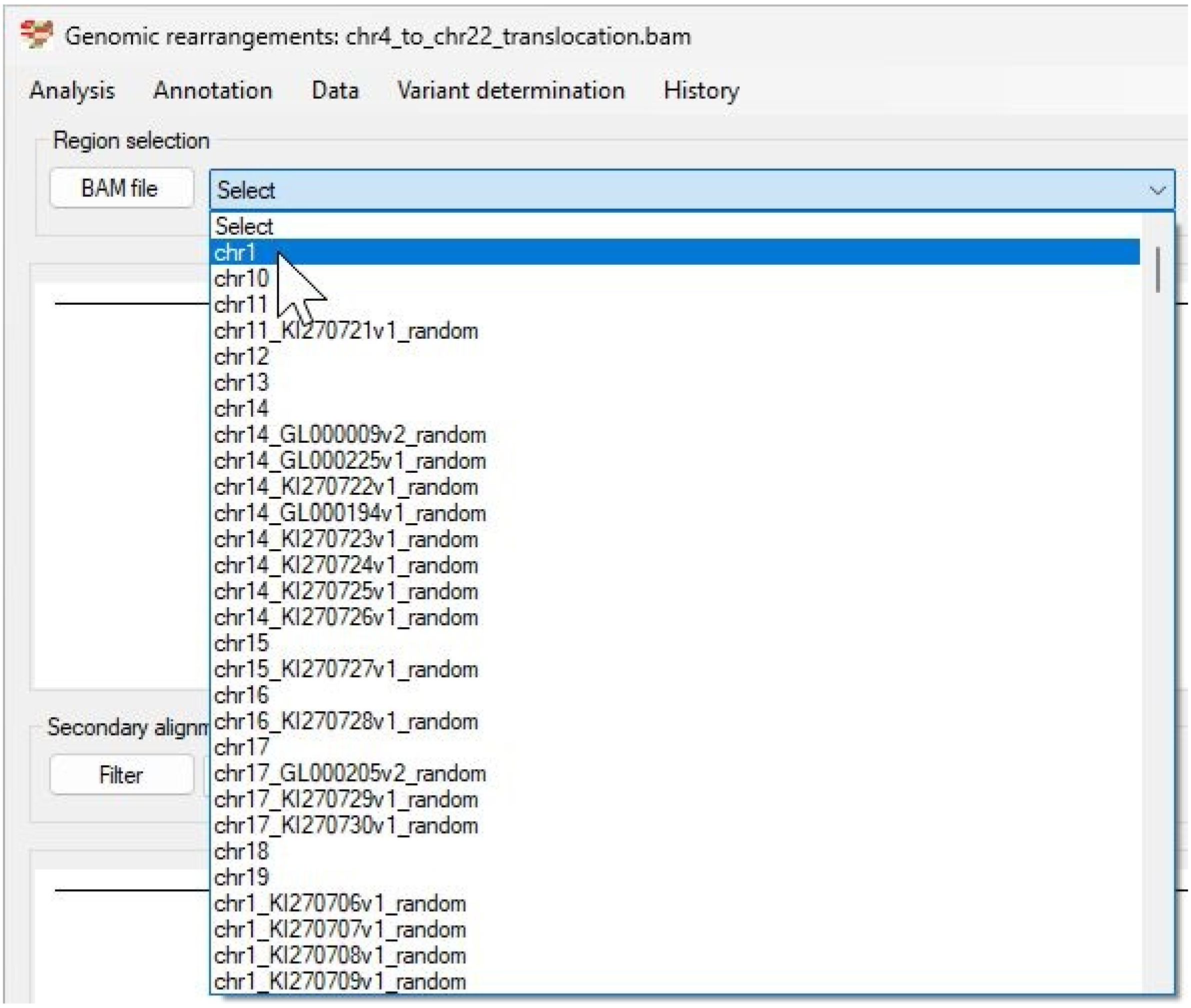


Figure 2

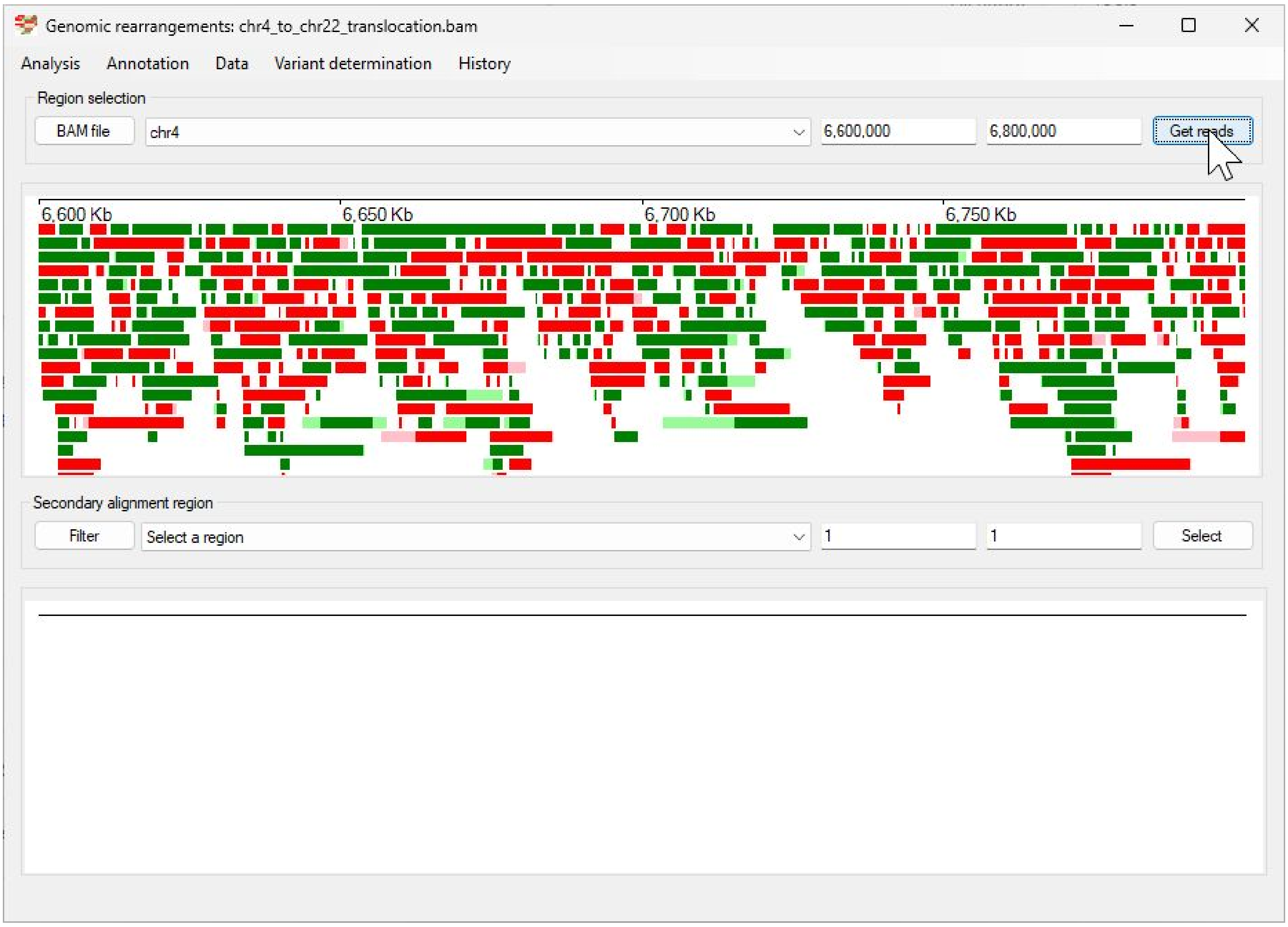
How indexed BAM files are processed

Before indexing a BAM file, reads are ordered with reference to the read's genomic coordinates with the data then compressed with the qzip algorithm to form discrete chunks of compressed data containing reads that start in a small region. For example the first chunk in a file may contain reads with alignments starting on chr 1 between 1 bp and 16,000 bp with the next chunk containing reads whose alignment starts on chr 1 between 16,000 bp and 32,000 bp. The indexing of a file creates a 2nd file (.bam.bai) that lists the start point of each chunk in the BAM file and the start position of the first read in that chunk.
When a program has to find reads mapping to a certain region, it looks in the bam.bai files to find the chunks that contain the start positions of the reads mapped to the region. Once it's found the chunks mapping to the data, it looks up in the index file where that data starts in the BAM file and then reads the data at that point in the BAM file until it comes to the end of the compressed chunk. It repeats the process until it has read all the data for the region of interest. This works fine for short read data since all the reads in a chunk start between two well defined points (the genomic start site of the chunk and the end of the chunk plus the length of the read i.e. 16,000 bp to 32,000 bp plus 150 bp). However, with long read data, a read may be longer than the size of a chunk's region, so a read 20 kb long may start in one chunk while its end may be in the next or the next but one chunk. This causes an issue when you select a small region, as reads that over lap the region may be listed in a chunk much further upstream and so may not be found when reading the BAM file.
Consequently, `AgileStructure` reads the chunk that ends just before the start of the region of interest, but if a read is particularly long it may be missed as it starts even further upstream. Therefore when selecting

regions, initially select a slightly larger (16 kb added to each side) region than required and note if a long read spanning the breakpoint disappears when you shrink the region.

Selecting the region to view

Select the required chromosome (reference sequence) from the upper dropdown list box and enter the region's coordinates in the two text boxes to the right of this drop down list box and press the `Get reads` button (Figure 3). The coordinates are checked to make sure they are not greater than the chromosomes length as reported in the `BAM` file. If no chromosome has been selected these values will be limited to '1'.



Genomic rearrangements: chr4_to_chr22_translocation.bam

Region selection: chr4 6,600,000 6,800,000

Secondary alignment region: Select a region 1 1

Figure 3

The positions of reads mapping to the region are depicted as green rectangles (aligned to the forward strand) and red rectangles (aligned to the reverse strand), scaled according to the length of the read. Soft-clipped sequences are represented as pale green or pale red extensions adjacent to the darker green/red aligned data. The size of these pale rectangles is proportional to the length of the unaligned sequence, and their placement merely indicates whether they are on the 5′ or 3′ end of the aligned sequence.
It is important to note that in the default view, reads are represented as a solid box spanning the length of the alignment, if a read has a large deletion this will not be shown, however they can be visualised by selecting the `Analysis` > `Look for indels within a read` menu option (see section Identifying Indels using the primary alignments CIGAR string).
`AgileStructure` does not have an upper limit on the size of the region or number of reads it will process and will attempt to read the requested data until it has processed the region or the computer runs out of

memory. While there is no upper limit, you should try to limit the amount of data it reads because reading the underlying BAM file can be a slow process due to its size.

Hiding reads without a soft-clipped segment

When adding reads to the display, they are stacked to use as little space as possible. However, for alignments with a high read depth, the stacks may be too tall to fit in the image. Since reads that have a soft-clipped region are more important in breakpoint detection, it is possible to hide those without an unaligned fragment by selecting the `Analysis` > `Only show reads with secondary alignments` menu option (Figure 4).

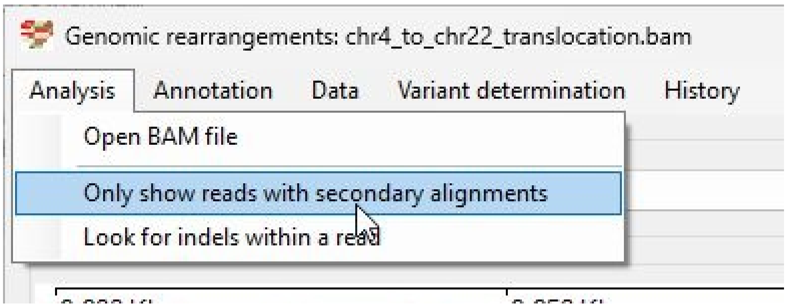

Genomic rearrangements: chr4_to_chr22_translocation.bam
Analysis
Annotation
Data
Variant determination
History
Open BAM file
Only show reads with secondary alignments
Look for indels within a read

Figure 4

The filtered image will contain fewer reads, making those at the breakpoint more apparent.

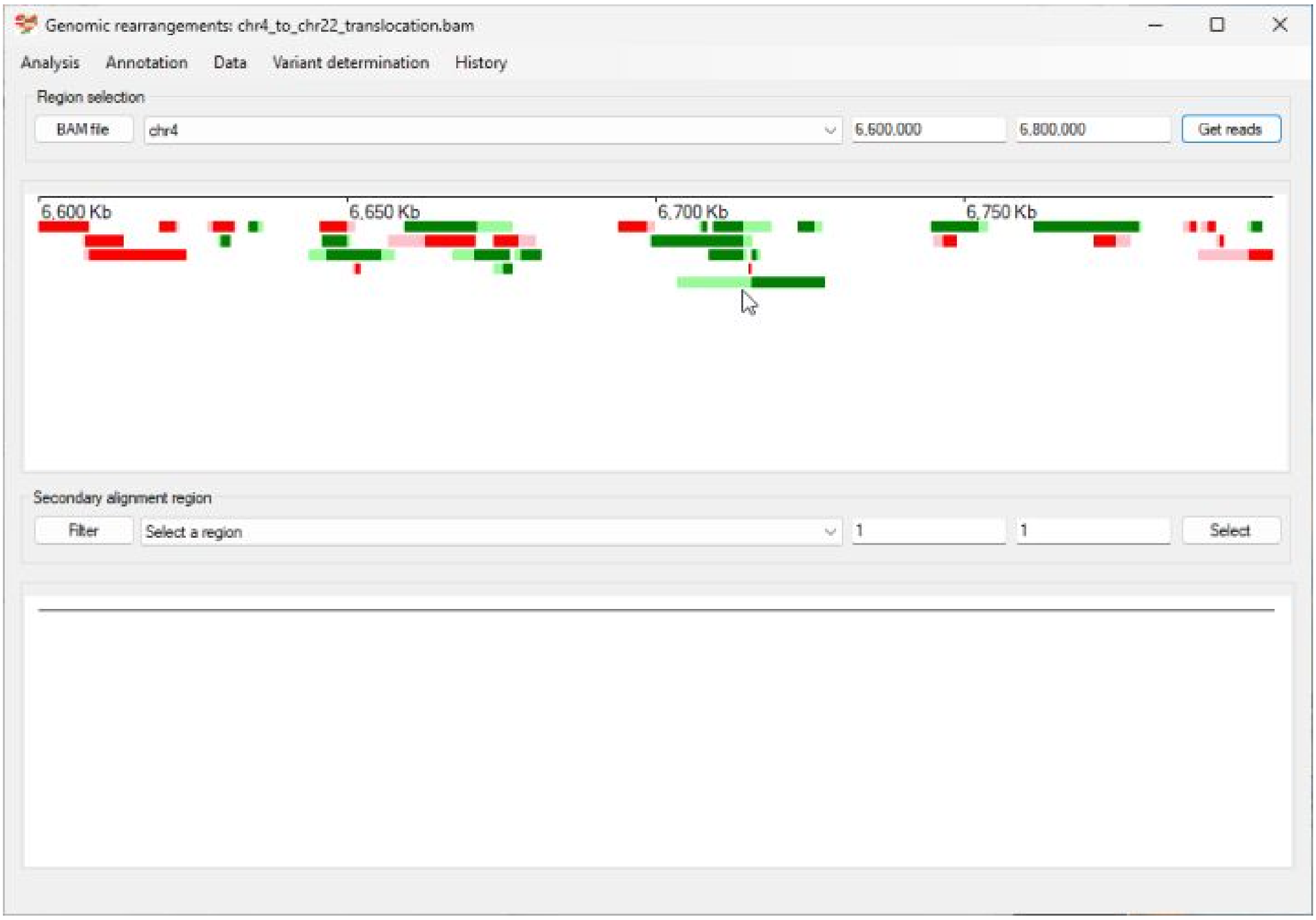
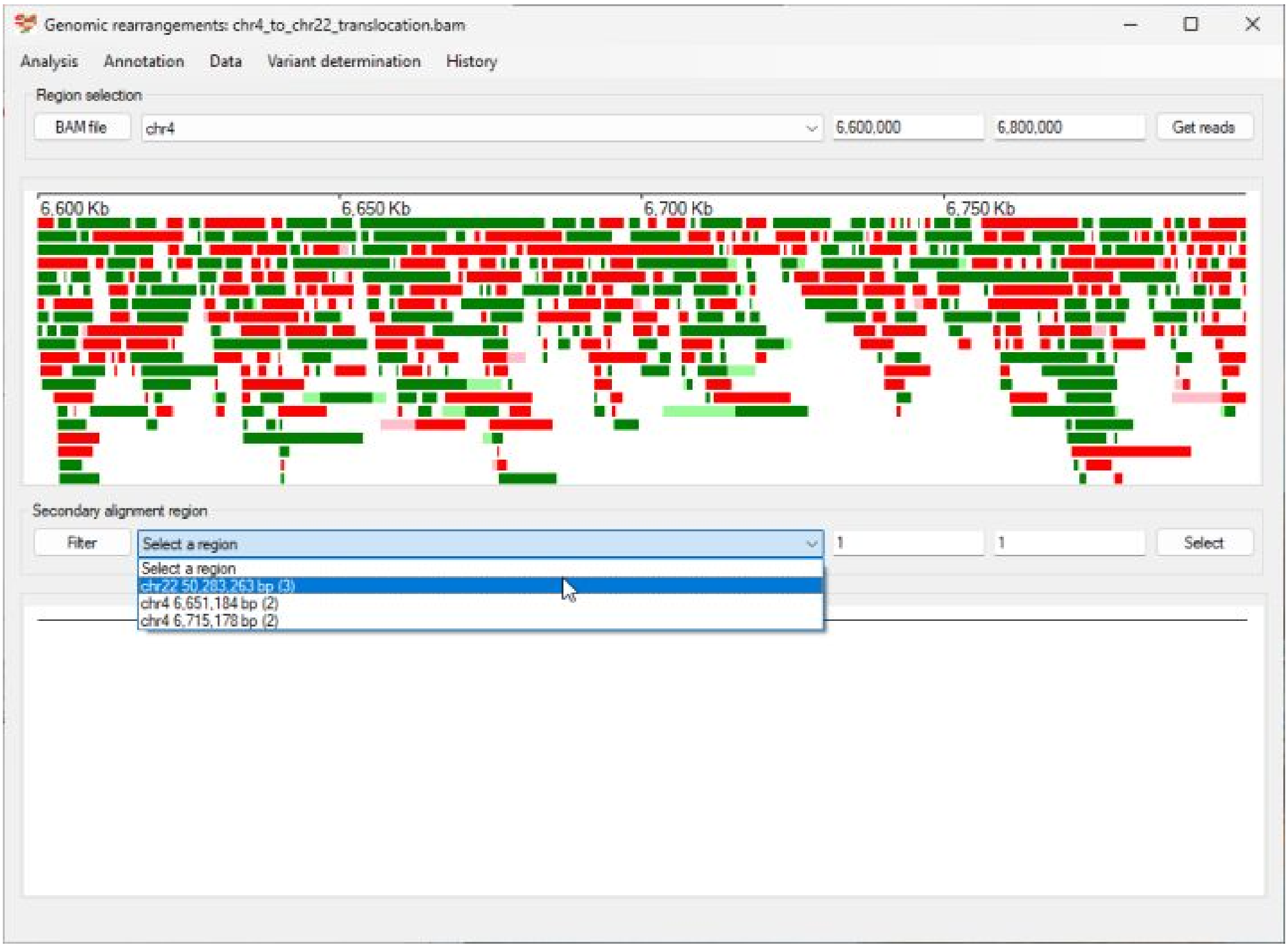


Figure 5: When reads with no secondary alignment are removed the breakpoint is more apparent: see mouse cursor in Figure 5 and compare to same region in Figure 3

Looking for putative breakpoints in the selected region.

It may be possible to simply identify the breakpoint at this point, especially for large homozygous deletions, but in many situations, particularly for heterozygous breakpoints, they may not stand out. Consequently, AgileStructure scans the displayed reads, looking for 250 bp regions in which multiple read alignments prematurely terminate and the remaining soft clipped sequences all map to the same secondary location. These regions are then noted and entered into the lower drop-down list box (Figure 6).


Genomic rearrangements: chr4_to_chr22_translocation.bam

Region selection

BAM file: chr4, 6,600,000, 6,800,000

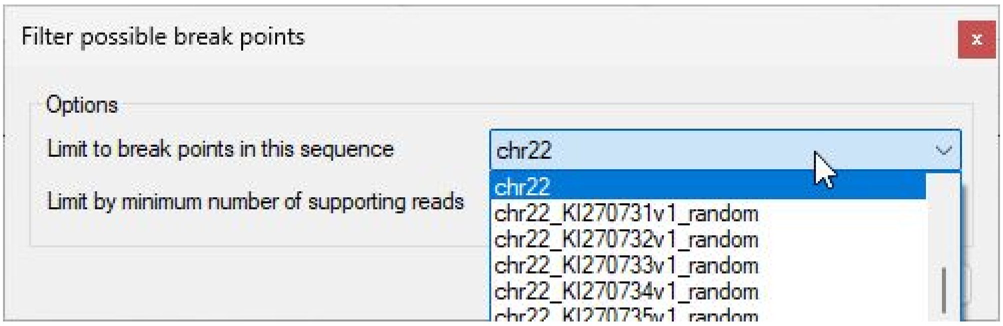
Secondary alignment region

Select a region
chr22 50,283,263 bp (3)
chr4 6,651,184 bp (2)
chr4 6,715,178 bp (2)

1, 1

Figure 6

For extended regions and/or alignments with a high read depth, this list may contain a large number of entries. To filter these regions, press the Filter button to the left of the lower drop-down list box. This will open the Filter possible breakpoints window (Figure 7a and 7b). The upper drop-down list box allows the breakpoints to be filtered by the chromosome that the secondary alignments are mapped to (Figure 7a), while the lower number select box will filter the results by the number of reads linked to each putative breakpoint.


Filter possible break points

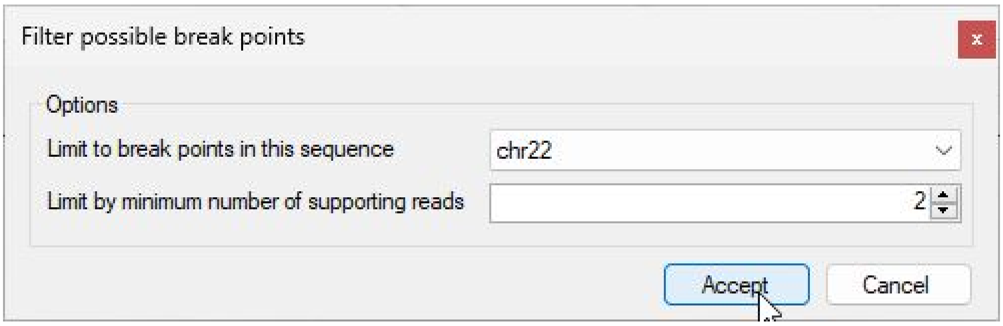
Options

Limit to break points in this sequence: chr22

Limit by minimum number of supporting reads

chr22
chr22_KI270731v1_random
chr22_KI270732v1_random
chr22_KI270733v1_random
chr22_KI270734v1_random
chr22_KI270735v1_random

Figure 7a



Filter possible break points

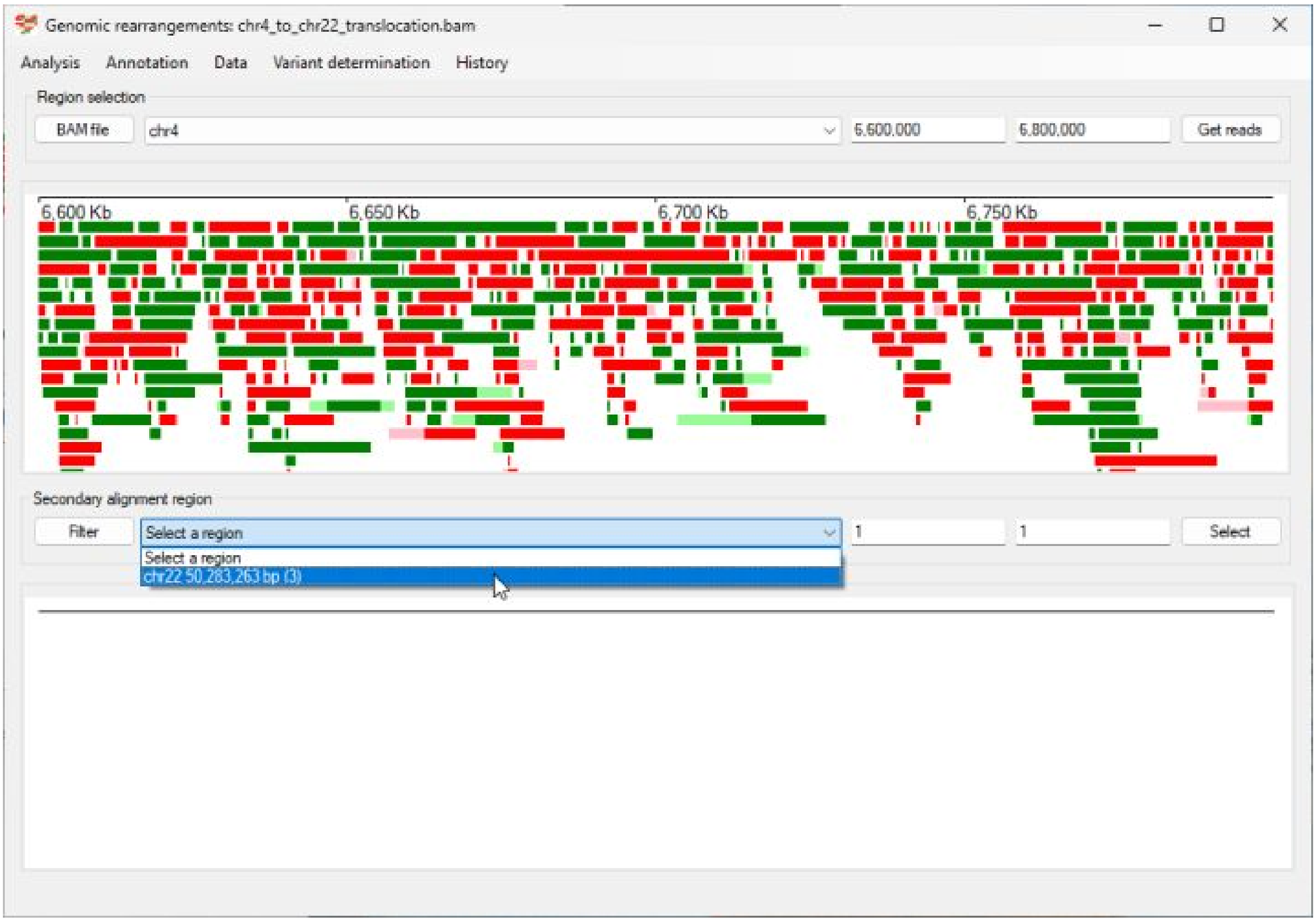
Options

Limit to break points in this sequence: chr22

Limit by minimum number of supporting reads: 2

Figure 7b

Pressing the `Accept` button will remove all breakpoints that do not match the criteria (Figure 8), while pressing the `Cancel` will remove all filtering.



Genomic rearrangements: chr4_to_chr22_translocation.bam

Region selection: chr4, 6,600,000 – 6,800,000

Secondary alignment region: Select a region, 1, 1

Select a region
chr22 50,283,263 bp (3)

Figure 8

Selecting a breakpoint from this list will cause the reads with soft-clipped sequences mapped to the second breakpoint's flanking regions to be displayed in the lower panel (Figure 9). As before, reads are represented in green or red for those mapping to the forward and reverse strands, with aligned sequences darker than the unaligned soft-clipped sequences. It is important to note that only reads that are present in the upper image are shown in the lower image, and that sequences that were aligned to the reference sequence in the upper image will be unaligned, soft-clipped sequences in the lower image

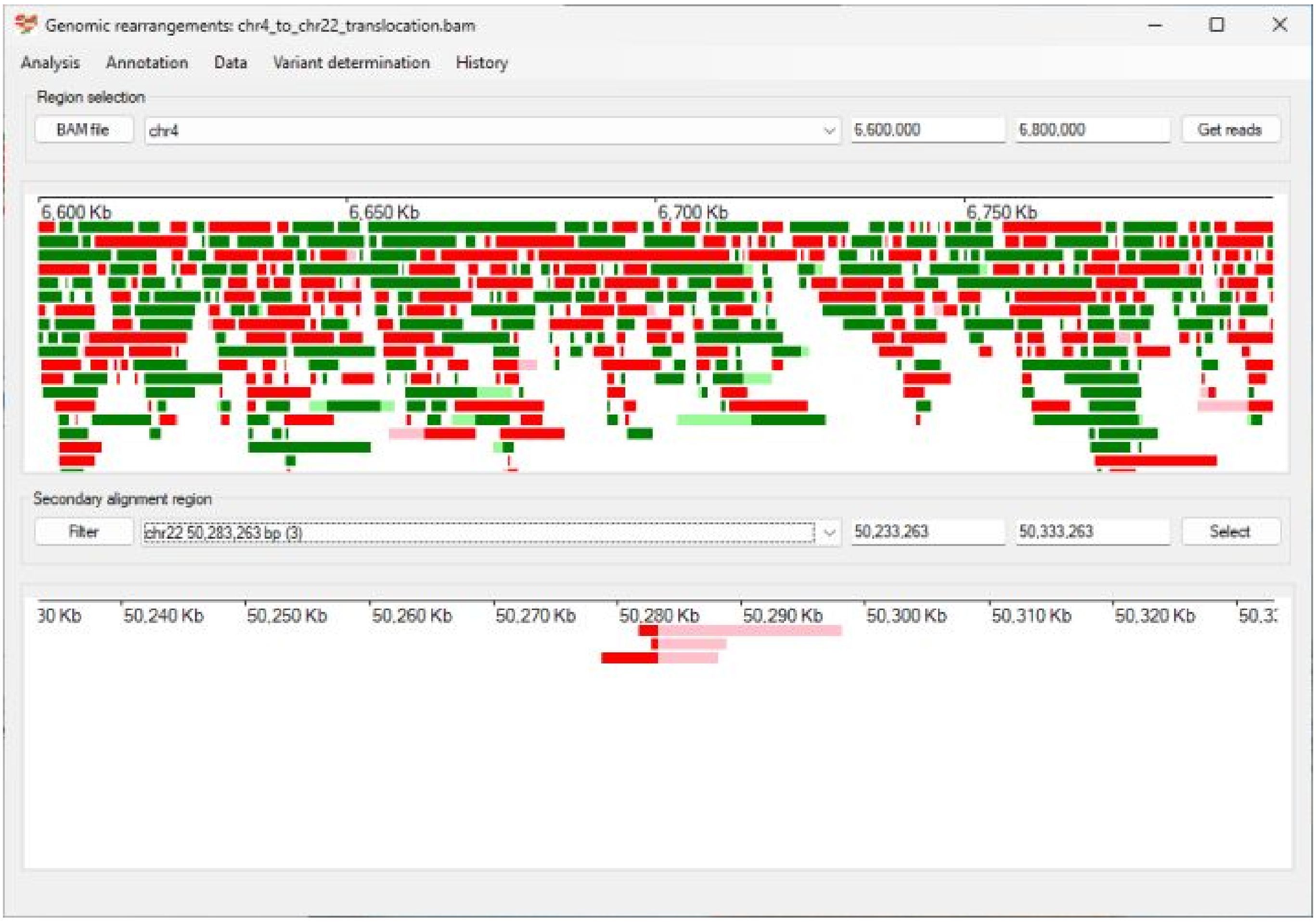


Figure 9

Viewing read alignment information

Selecting the `Data` > `View read data` (Figure 10a) will cause a resizable window to appear that consists solely of a text area. If the mouse cursor is held over a read, its underlying data will be written to the text area (Figure 10b). For a sequence to be shown, the cursor has to hover over the read for a little while. This makes it possible to select a read, then quickly move the cursor to the new window, and copy the data to paste into a document.

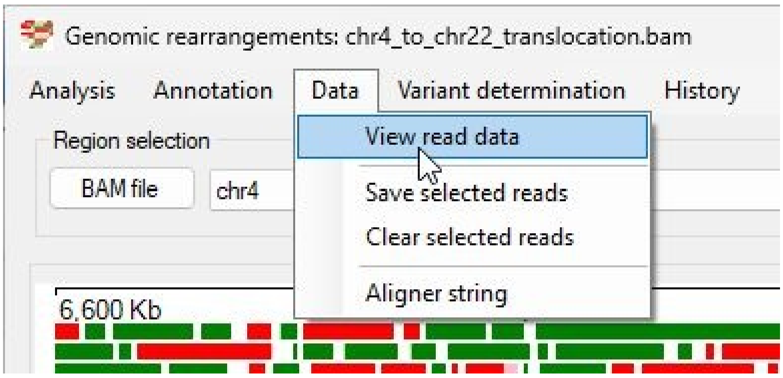
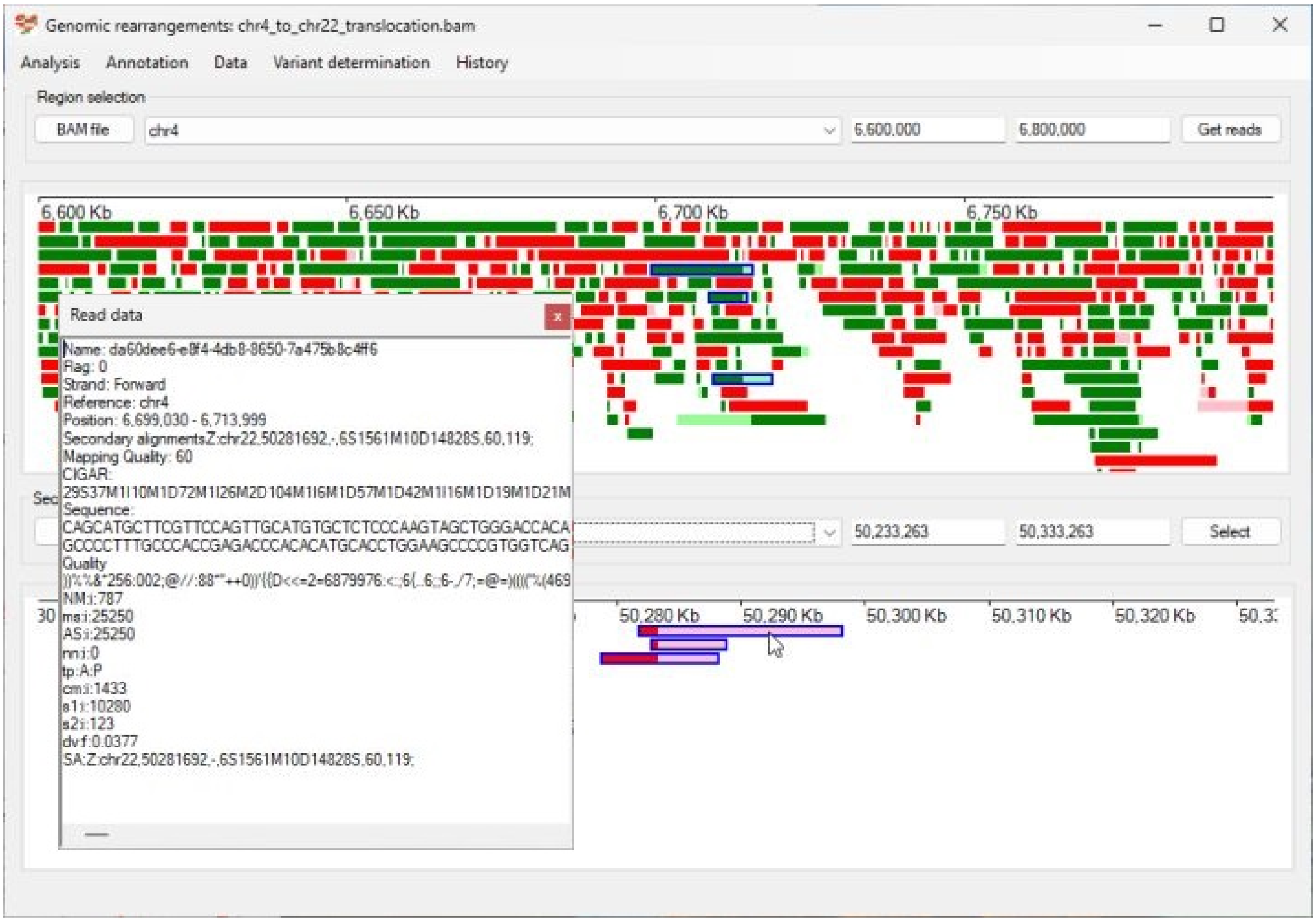


Figure 10a



```
Name: da60dee6-e8f4-4db8-8650-7a475b8c4ff6
Flag: 0
Strand: Forward
Reference: chr4
Position: 6,699,030 - 6,713,999
Secondary alignmentsZ:chr22,50281692,-,6S1561M10D14828S,60,119;
Mapping Quality: 60
CIGAR:
29S37M1I10M1D72M1I26M2D104M1I6M1D57M1D42M1I16M1D19M1D21M
Sequence:
CAGCATGCTTCGTTCCAGTTGCATGTGCTCTCCCAAGTAGCTGGGACCACA
GCCCCTTTGCCCACCGAGACCCACACATGCACCTGGAAGCCCCGTGGTCAG
Quality
))%%&"256:002;@//:88""++0))'{{D<<=2=6879976:<:;6(..6;;6-./7;=@=))))("%(469
NM:i:787
ms:i:25250
AS:i:25250
nn:i:0
tp:A:P
cm:i:1433
s1:i:10280
s2:i:123
dv:f:0.0377
SA:Z:chr22,50281692,-,6S1561M10D14828S,60,119;
```

Figure 10b

Since the read, quality score string, and CIGAR string can be several thousand characters long, the text area doesn't word wrap text (unless it's very long), and so if you want to read the end of a CIGAR string, you must use the horizontal scroll bar. In addition to the sequence and quality string, this information contains the primary and secondary alignment locations, as well as all the tags added by the aligner. The format of the tag data can be aligner-specific, with the aligner's documentation providing a full description of each tag's meaning.

Selecting reads linked to a breakpoint

When the upper image contains a large number of reads, it may not be possible to identify the reads associated with the selected breakpoint. However, clicking on a read in either image will cause it to be selected and outlined with a blue border. Clicking on all the reads linked to a breakpoint in the lower image will help to identify the location of the breakpoint in the upper image (Figure 11).

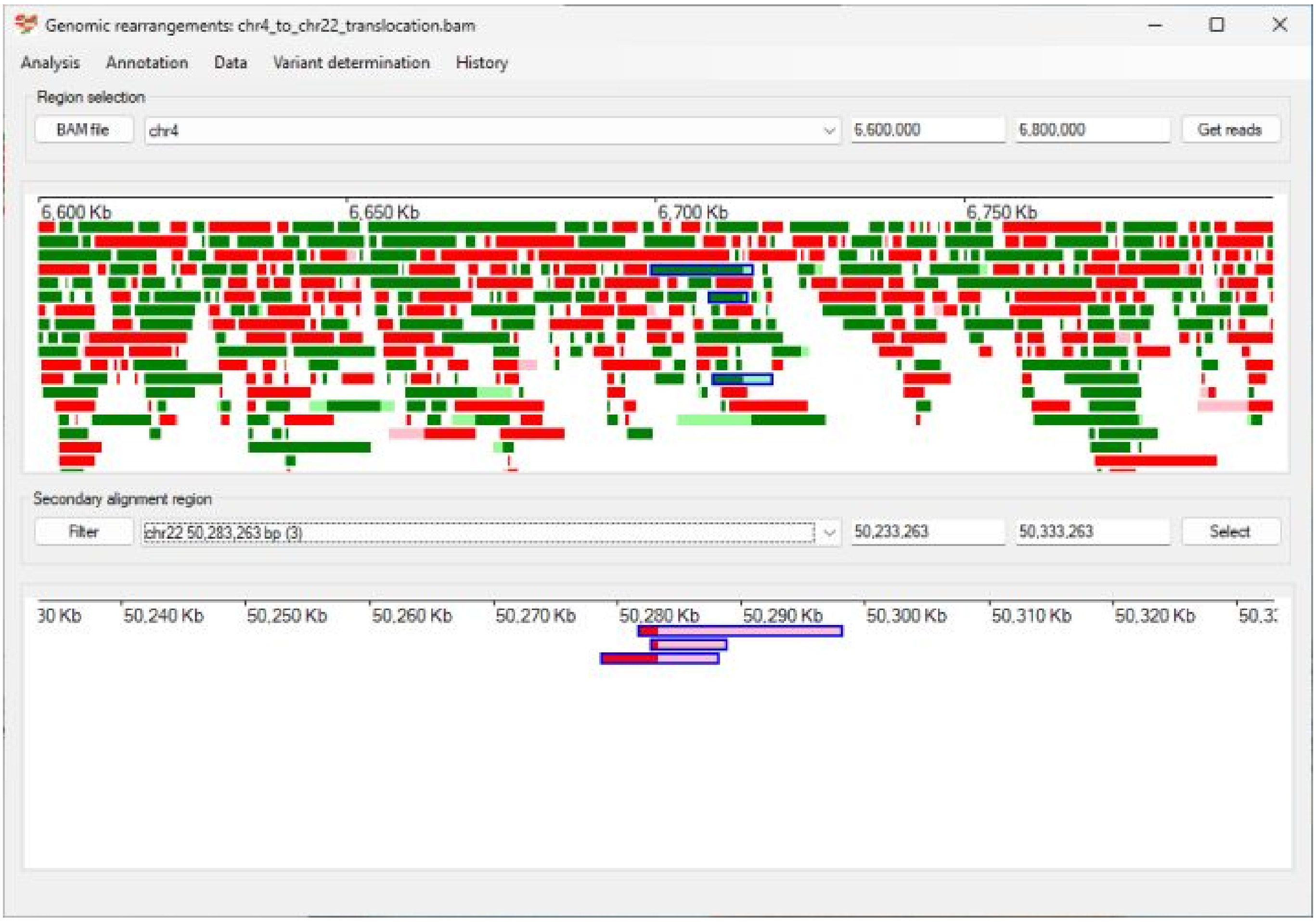


Figure 11

If you click on a selected read, it will be deselected, while selecting the `Data` > `Clear selected reads` option will deselect all selected reads (Figure 12). Finally, if new data is imported from the BAM file (i.e. the `Get reads` button is pressed) the selection will be cleared.

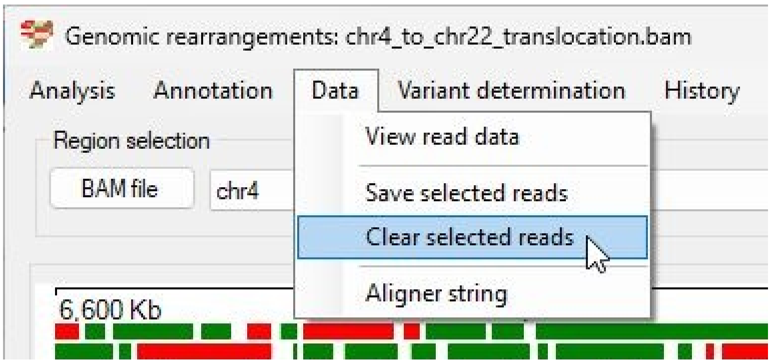


Figure 12

Saving alignment information for selected reads

Rather than manually saving the data for a series of read alignments, it's possible to save the data of selected reads to a text file using the `Data` > `Save selected reads` (Figure 13).

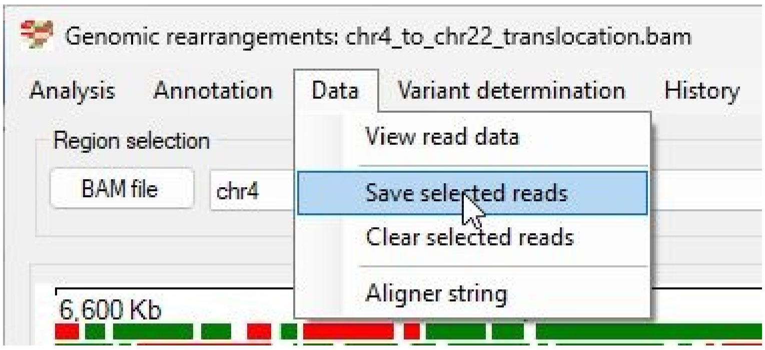


Figure 13

Annotating breakpoints using soft-clipped data

Once the reads spanning a breakpoint have been selected, it is possible to get `AgileStructure` to attempt to identify the type of variant: deletion, duplication, insertion, inversion or translocation. To identify what type of mutation the breakpoint represents, select the `Variant determination` > `Use soft-clip data` > `Variant type` menu option (Figure 14a). `AgileStructure` will then scan the orientation of the primary and secondary alignments of the selected reads to determine what type of mutation it is. This is reported in a message box with the possible answers of “Deletion”, “Insertion”, “Inversion”, “Duplication” or “Translocation” as well as messages indicating any error processing the data or user data selection issues (Figure 14b).
For this feature to work a region must be selected in the lower panel.

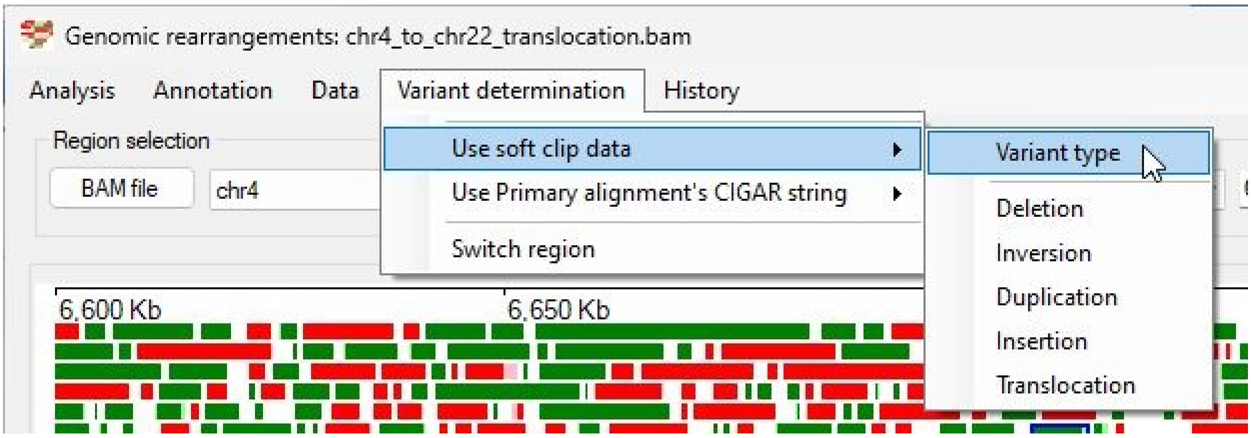
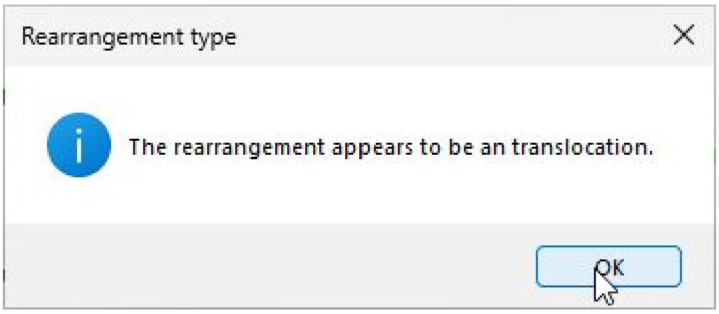


Figure 14a



The rearrangement appears to be an translocation.

Figure 14b

Once the variant type is determined, it is then possible to annotate the breakpoint by selecting the appropriate option. The links below show examples of analysing each type of mutation.

Deletion

A worked example is here.

Duplication

A worked example is here.

Insertion

A worked example is here.

Inversion

A worked example is here.

Translocation

A worked example is here.

Identifying Indels using the primary alignments CIGAR string

`AgileStructure` is primarily designed to identify chromosomal breakpoints by looking for sets of reads whose alignment is broken in two, such that their primary alignment aligns at one location and their secondary alignments are all located to a more distant common region possibly on a different chromosome. However, it is also able to identify insertions and deletions that do not cause the alignment to be fragmented, but whose presence is noted in the primary alignment's CIGAR string.
Selecting the `Analysis` > `Look for indels within a read` menu option (Figure 15) causes the reads to be redrawn with deletions shown as a horizontal black line linking two blocks of aligned sequences while an insertion is shown as a vertical line projecting above and below the aligned sequence. Since ONT data contains numerous short indels, only insertions/deletions longer than 10 bp are shown.

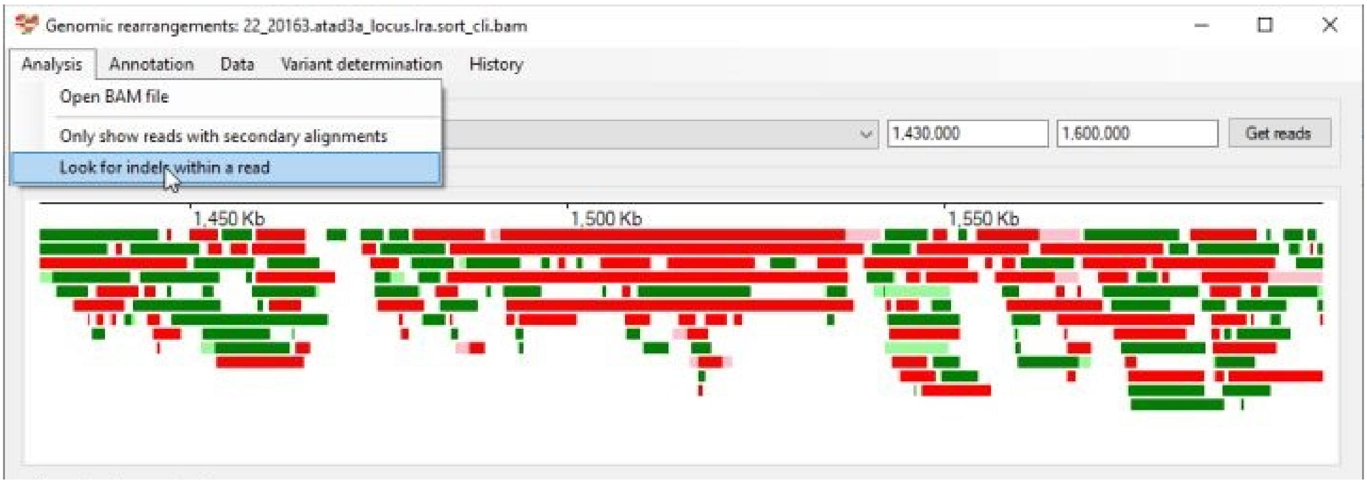

Genomic rearrangements: 22_20163.atad3a_locus.lra.sort_cli.bam
Analysis
Annotation
Data
Variant determination
History
Open BAM file
Only show reads with secondary alignments
Look for indels within a read
1,430,000
1,600,000
Get reads
1,450 Kb
1,500 Kb
1,550 Kb

Figure 15

When redrawn using the CIGAR string to identify insertions and deletions, their presence becomes apparent. For example in Figure 16 the large 39 kb deletion spanning 1,495,000 bp to 1,534,000 bp of chromosome 1 and the insertion at 1,586,000 bp (above the cursor) are easily identified, as a series of broken read alignments linked by a pale grey line..

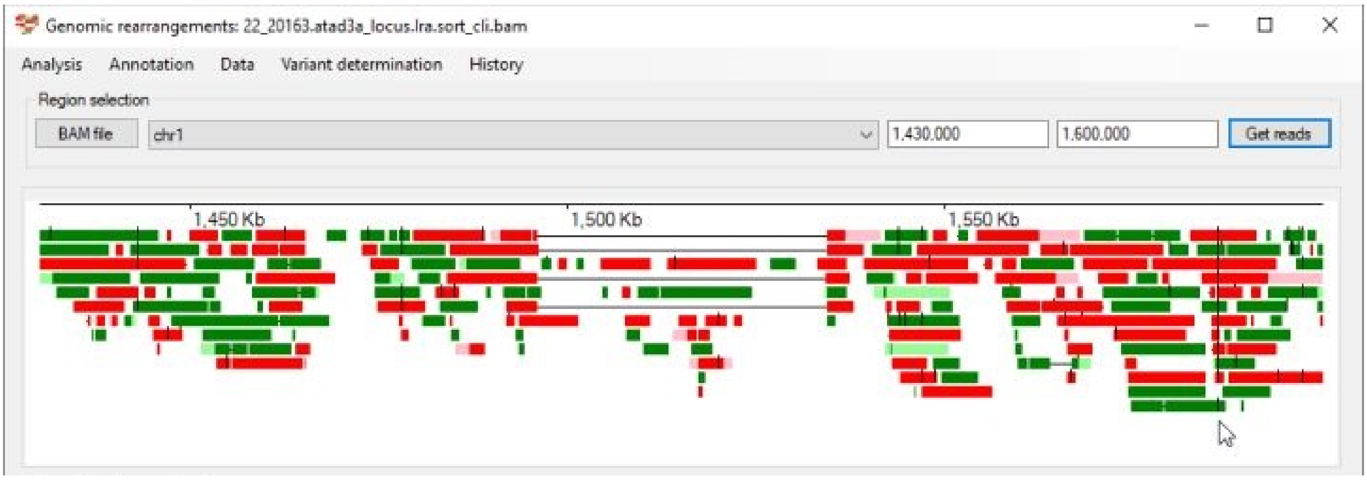


Figure 16

Important note

Since ONT data is very noisy, the exact point of the breakpoint may appear to vary by a number of base pairs between different reads, while artefactual indels may also be present in the reads. Consequently, `AgileStructure` scans the beginning and ends of the indels, sorts them by position, and then reports the median values for the reported variant. Using the median value rather than the average reduces the chance an artifactual indel unduly influencing the annotation, but it is important to ensure that the individual indels are checked to make sure a 2nd, possibly artifactual, indel is not somehow disrupting the annotation.

Identifying insertions using the primary alignments CIGAR string

To annotate an insertion, select the reads containing the variant of interest, then navigate to `Variant determination` > `Use primary alignment's CIGAR string` > `Insertion` menu option (see Figure 17a). A message box will appear, listing any insertions larger than 10 bp along with the names of the selected reads and the variant's annotations. ***Note:*** Reads with multiple insertions may need to be deselected for accurate analysis.

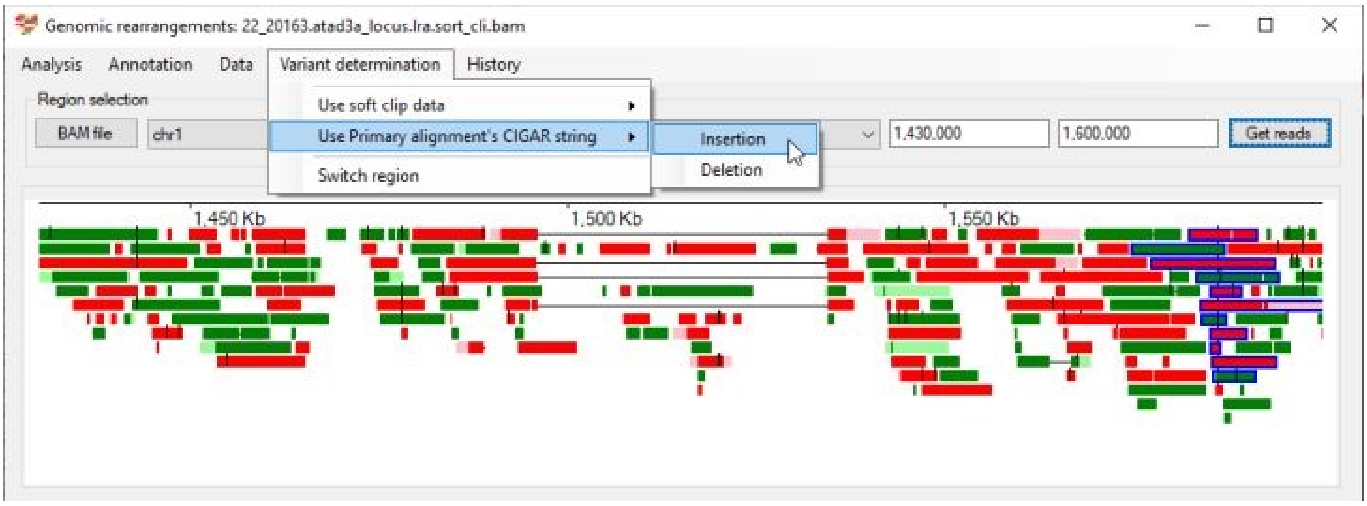
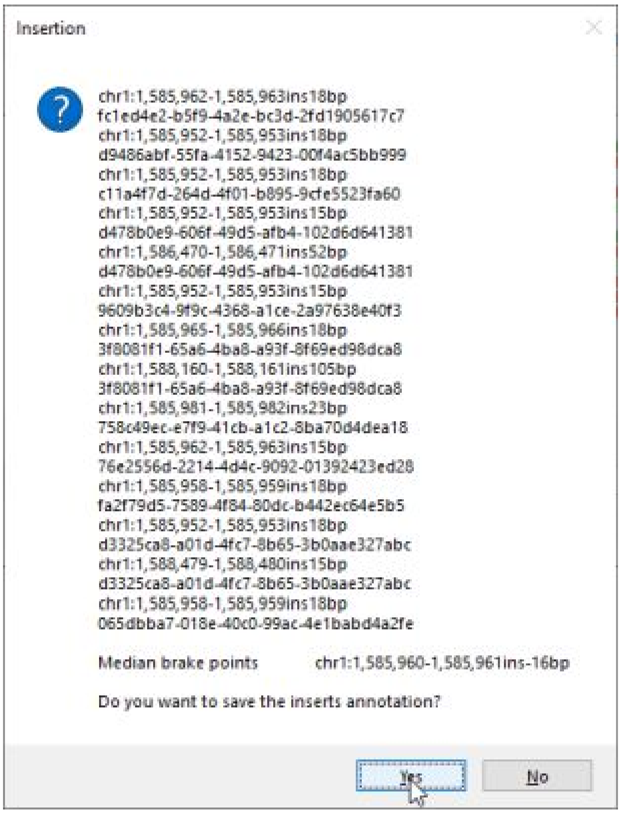


Figure 17



```
chr1:1,585,962-1,585,963ins18bp
fc1ed4e2-b5f9-4a2e-bc3d-2fd1905617c7
chr1:1,585,952-1,585,953ins18bp
d9486abf-55fa-4152-9423-00f4ac5bb999
chr1:1,585,952-1,585,953ins18bp
c11a4f7d-264d-4f01-b895-9cfe5523fa60
chr1:1,585,952-1,585,953ins15bp
d478b0e9-606f-49d5-afb4-102d6d641381
chr1:1,586,470-1,586,471ins52bp
d478b0e9-606f-49d5-afb4-102d6d641381
chr1:1,585,952-1,585,953ins15bp
9609b3c4-9f9c-4368-a1ce-2a97638e40f3
chr1:1,585,965-1,585,966ins18bp
3f8081f1-65a6-4ba8-a93f-8f69ed98dca8
chr1:1,588,160-1,588,161ins105bp
3f8081f1-65a6-4ba8-a93f-8f69ed98dca8
chr1:1,585,981-1,585,982ins23bp
758c49ec-e7f9-41cb-a1c2-8ba70d4dea18
chr1:1,585,962-1,585,963ins15bp
76e2556d-2214-4d4c-9092-01392423ed28
chr1:1,585,958-1,585,959ins18bp
fa2f79d5-7589-4f84-80dc-b442ec64e5b5
chr1:1,585,952-1,585,953ins18bp
d3325ca8-a01d-4fc7-8b65-3b0aae327abc
chr1:1,588,479-1,588,480ins15bp
d3325ca8-a01d-4fc7-8b65-3b0aae327abc
chr1:1,585,958-1,585,959ins18bp
065dbba7-018e-40c0-99ac-4e1babd4a2fe

Median brake points        chr1:1,585,960-1,585,961ins-16bp

Do you want to save the inserts annotation?
```

Figure 17b: The order of the reads in the message box is determined by chromosomal position, which may differ from the displayed order of the reads.

Identifying deletions using the primary alignments CIGAR string

To annotate a deletion, select the reads containing the variant of interest and then select the `Variant determination` > `Use primary alignment's CIGAR string` > `Deletion` menu option (Figure 18a). This will open a message box, listing the deletions over 10 bp followed by the names of the reads and finally, the variant's annotation (Figure 18b).

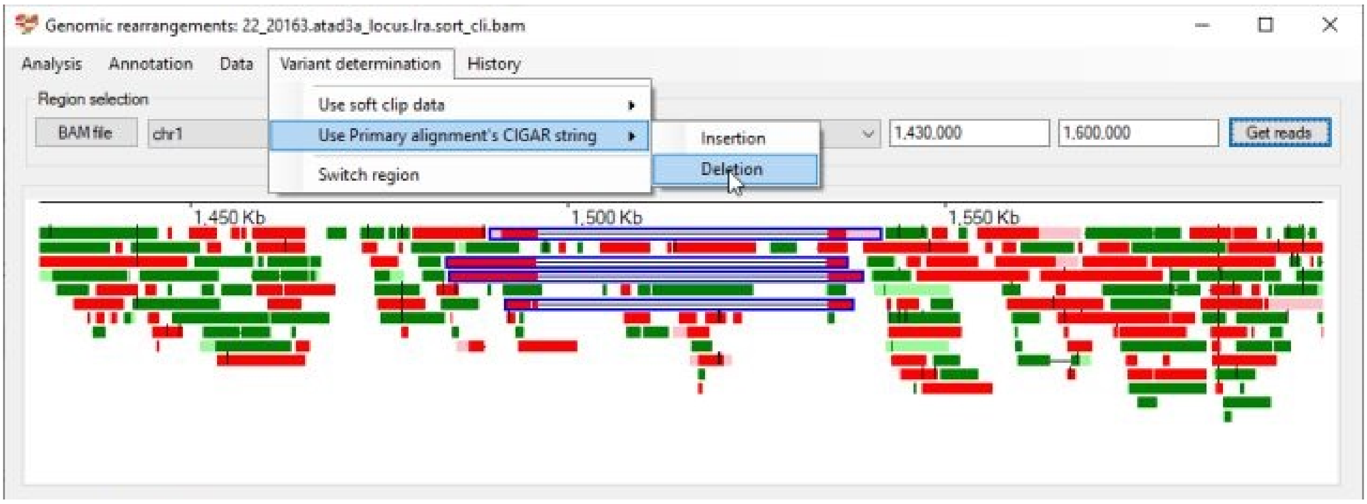
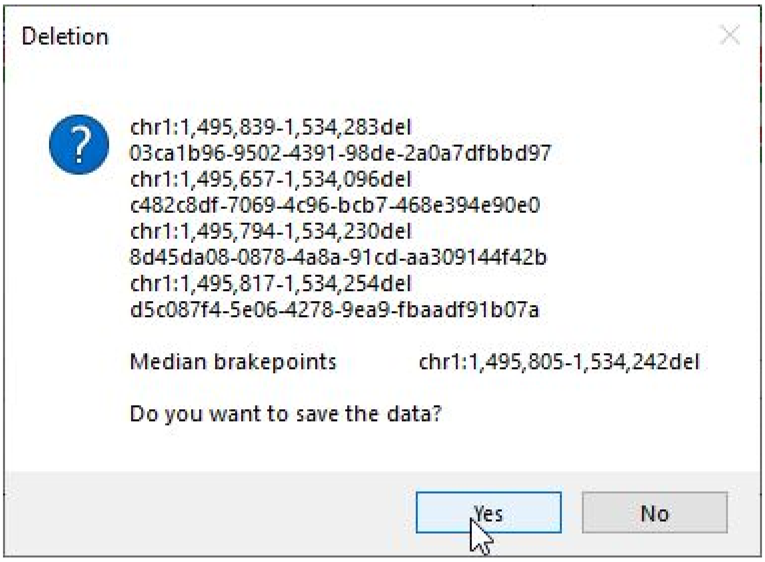


Figure 18



chr1:1,495,839-1,534,283del
03ca1b96-9502-4391-98de-2a0a7dfbbd97
chr1:1,495,657-1,534,096del
c482c8df-7069-4c96-bcb7-468e394e90e0
chr1:1,495,794-1,534,230del
8d45da08-0878-4a8a-91cd-aa309144f42b
chr1:1,495,817-1,534,254del
d5c087f4-5e06-4278-9ea9-fbaadf91b07a

Median brakepoints chr1:1,495,805-1,534,242del

Do you want to save the data?

Figure 18b: The reads in the message box are ordered by chromosomal position, which may differ from their display order.

Navigating the read data

Changing the region by typing the coordinates

As noted earlier, `AgileStructure` presents the primary and secondary alignments in separate panels. At the top of each panel, there are text fields for adjusting the start and end points of the data displayed. Retrieval of primary read data from the BAM file can be time-consuming, so updates to the primary alignment region occur only upon clicking the `Get reads`. In contrast, modifications to the secondary alignment coordinates are reflected immediately.

Moving the region to the left and right with the left and right arrow keys

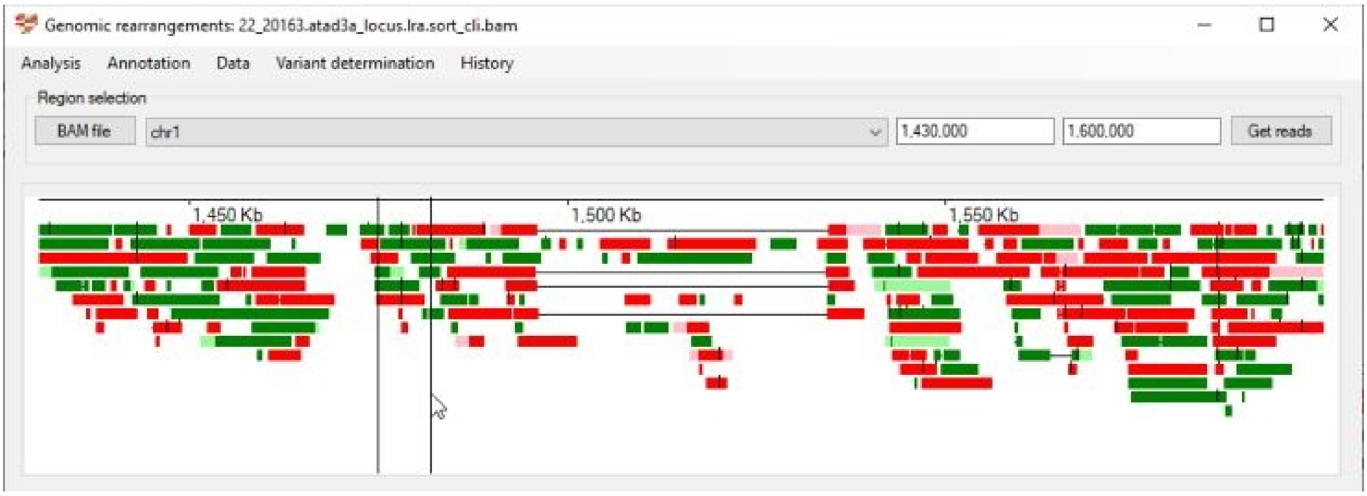
Instead of manually entering new locations into the text areas, you can easily shift the region to the left or right. Simply click on one of the text areas to activate it (i.e., you could edit the value by typing), and then press the `Ctrl` + `left arrow` or `Ctrl` + `right arrow` keys. This will move the region in the desired direction to an adjacent, non-overlapping region of the same length as the original.

Changing the width of the region with the Up and Down arrow keys

In a similar manner to moving the region to the left or right, you can double or half the width of the region. Simply activate a text area and then press the `Ctrl` + `Up arrow` or `Ctrl` + `Down arrow` keys. Even though the size of the region changes, it remains centered on the same point in the reference sequence.

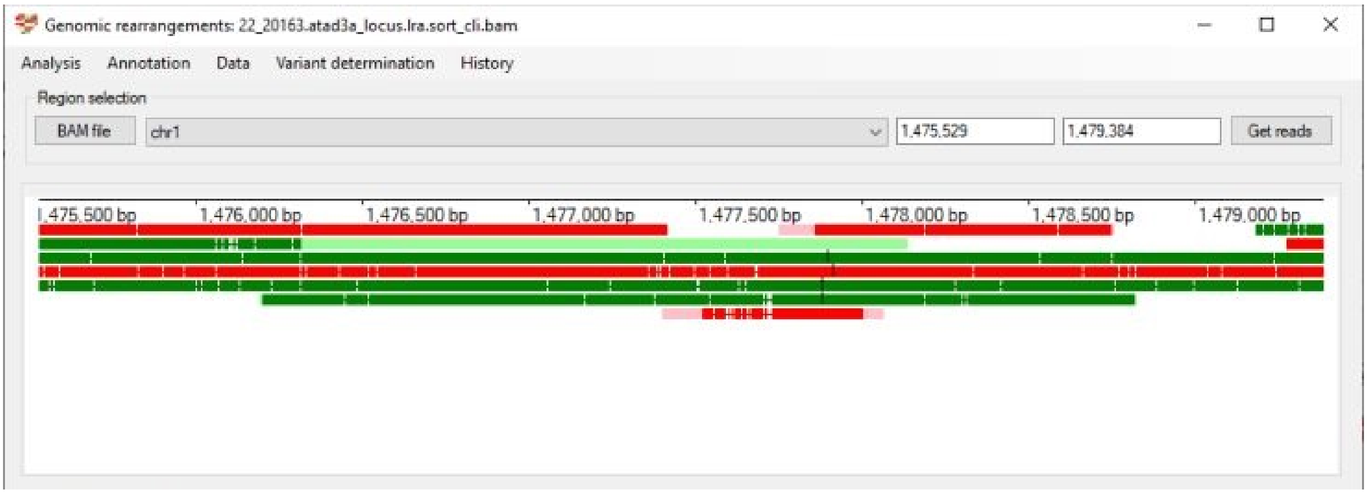
Changing the region by selecting a region with the mouse

Using the mouse, a sub-region can be selected within either panel by clicking at the desired start point and dragging the cursor to the end point with the right mouse button held down (refer to Figure 19a). Upon releasing the button, the display updates to show the selected area (see Figure 19b). This feature enables detailed examination of specific regions. For example, Figure 19a shows four reads with a seemingly common insertion. However, closer inspection in Figure 19b reveals discrepancies in their locations, indicating they may not be authentic. To further investigate, selecting `Variant determination` > `Use primary alignment's CIGAR string` > `Insertion` menu option, reveals that all reads share a 134 to 135 bp insertion. This uniformity suggests that the insertion's position might be inaccurately represented, potentially due to sequencing errors or alignment issues with low complexity sequences (illustrated in Figure 19c)..



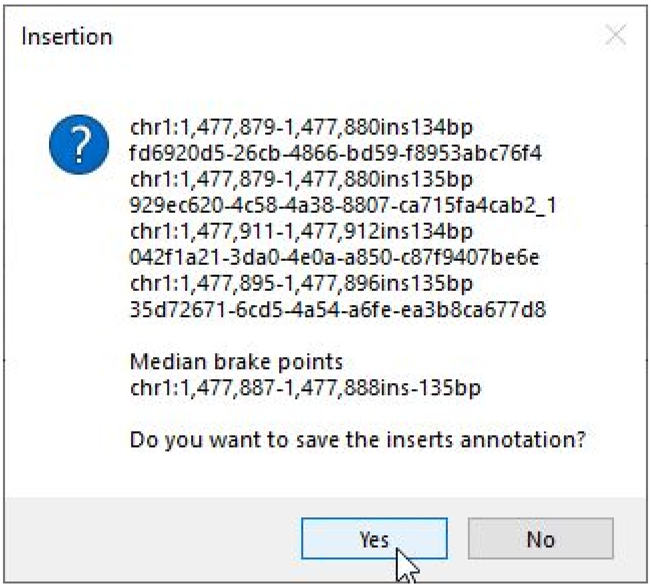
chr1 1,430,000 1,600,000

Figure 19a:



chr1 1,475,529 1,479,384

Figure 19b



chr1:1,477,879-1,477,880ins134bp
fd6920d5-26cb-4866-bd59-f8953abc76f4
chr1:1,477,879-1,477,880ins135bp
929ec620-4c58-4a38-8807-ca715fa4cab2_1
chr1:1,477,911-1,477,912ins134bp
042f1a21-3da0-4e0a-a850-c87f9407be6e
chr1:1,477,895-1,477,896ins135bp
35d72671-6cd5-4a54-a6fe-ea3b8ca677d8

Median brake points
chr1:1,477,887-1,477,888ins-135bp

Do you want to save the inserts annotation?

Figure 19c

Changing the regions using the History menu options

Whenever adjustments are made to the primary or secondary display coordinates, the previous positions are automatically stored. This feature enables users to revisit earlier views by choosing the saved coordinates from the `History` dropdown menu, under `Primary alignments` or `Secondary alignments` (as shown in Figure 20).

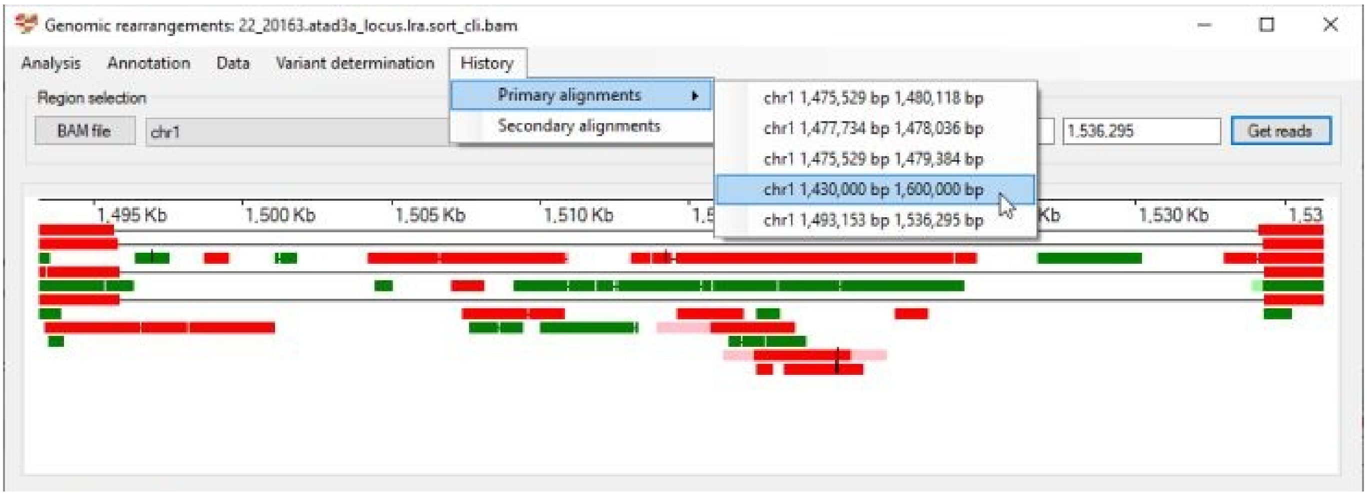

Genomic rearrangements: 22_20163.atad3a_locus.lra.sort_cli.bam
Analysis
Annotation
Data
Variant determination
History
Primary alignments
Secondary alignments
chr1 1,475,529 bp 1,480,118 bp
chr1 1,477,734 bp 1,478,036 bp
chr1 1,475,529 bp 1,479,384 bp
chr1 1,430,000 bp 1,600,000 bp
chr1 1,493,153 bp 1,536,295 bp
Region selection
BAM file
chr1
1,536,295
Get reads
1,495 Kb
1,500 Kb
1,505 Kb
1,510 Kb
1,530 Kb

Figure 20

Selecting an area that contains a specific gene

The Displaying gene positions section explains how to import gene locations, once imported, it is possible to navigate to a region that contains a specific gene by selecting the `Annotation` > `Gene coordinates` menu option (Figure 21a).

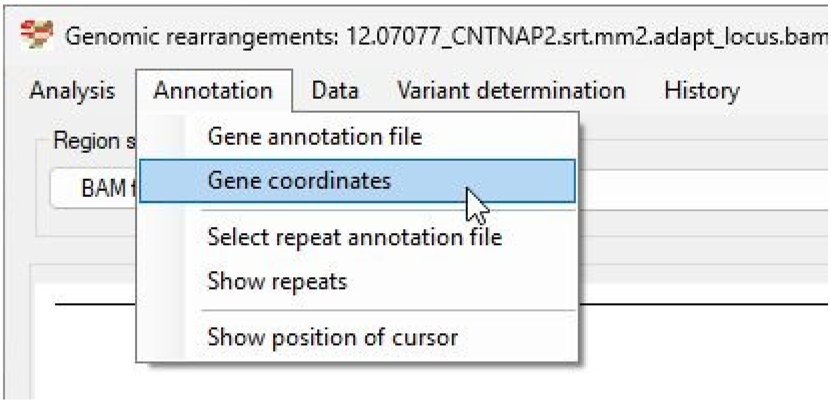
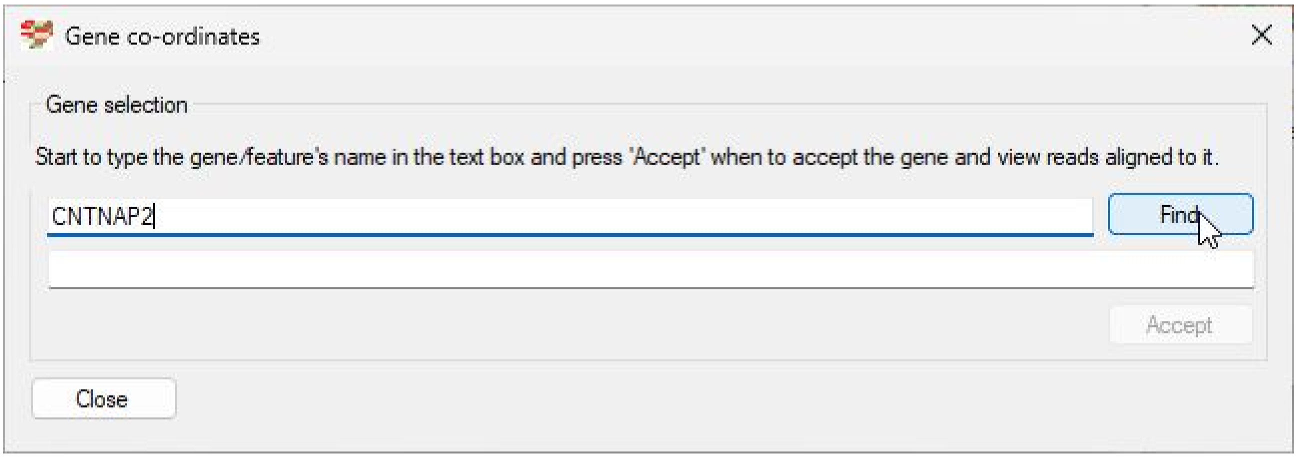


Figure 21a

Activating this option will launch the `Gene coordinates` window, which features two text fields. Enter the gene symbol of interest into the top text field (refer to Figure 21b).



Gene co-ordinates

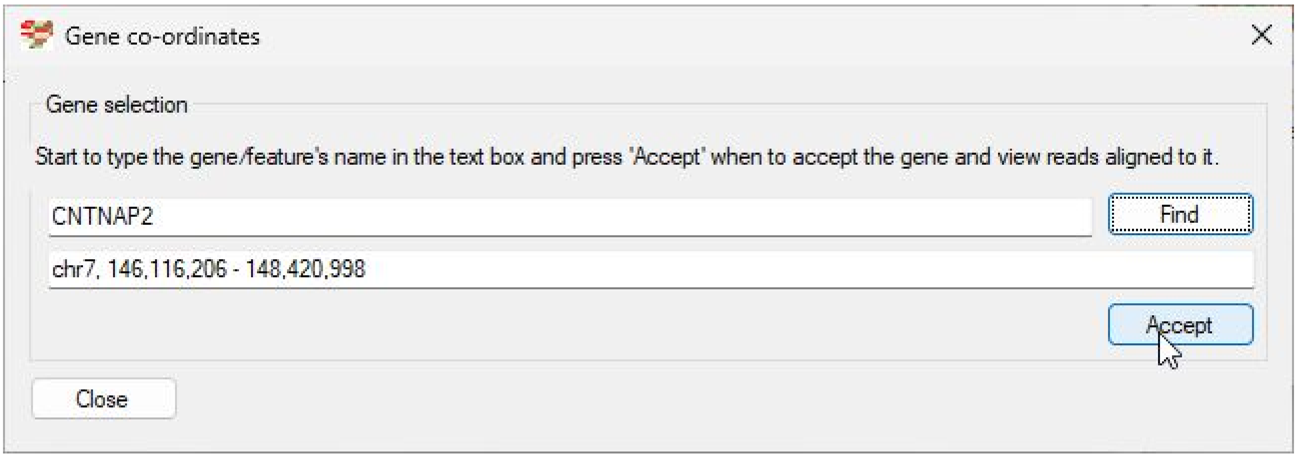
Gene selection

Start to type the gene/feature's name in the text box and press 'Accept' when to accept the gene and view reads aligned to it.

CNTNAP2

Figure 21b

Click the `Find` button and if the gene symbol is present in the imported gene coordinate data, its coordinates will be displayed in the lower text field (Figure 21c).



Gene co-ordinates

Gene selection

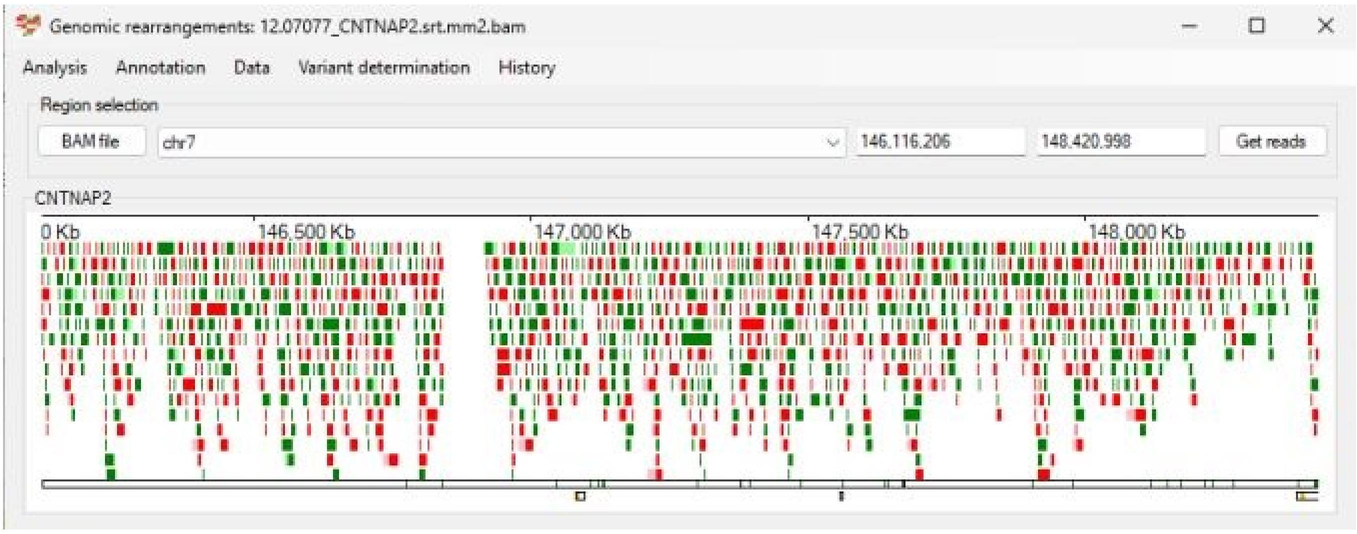
Start to type the gene/feature's name in the text box and press 'Accept' when to accept the gene and view reads aligned to it.

CNTNAP2

chr7, 146,116,206 - 148,420,998

Figure 21c

Pressing `Accept` will reset the coordinates in `AgileStructure` main window. Pressing the `Get reads` button will update the Primary alignment window (Figure 21d).



Genomic rearrangements: 12.07077_CNTNAP2.srt.mm2.bam

Region selection

chr7 146,116,206 148,420,998

Figure 21d

Viewing data with reference to genomic features

It is possible to visualise the read data in relation to the genes and repeats around the breakpoint. The chromosomal coordinates of the genes and repeats can be downloaded from the UCSC Genome Browser as outlined in this guide.

Displaying gene positions

Gene coordinate data can be imported by selecting the `Annotation` > `Gene annotation file` menu option (Figure 22).

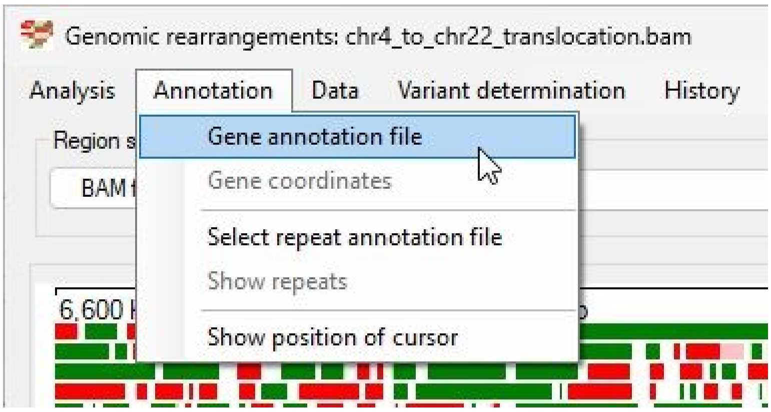


Figure 22

Genes are displayed as black rectangles, with their exons depicted by green (gene on forward strand) or yellow (gene on reverse strand) positioned at the bottom of the displays (Figure 23).

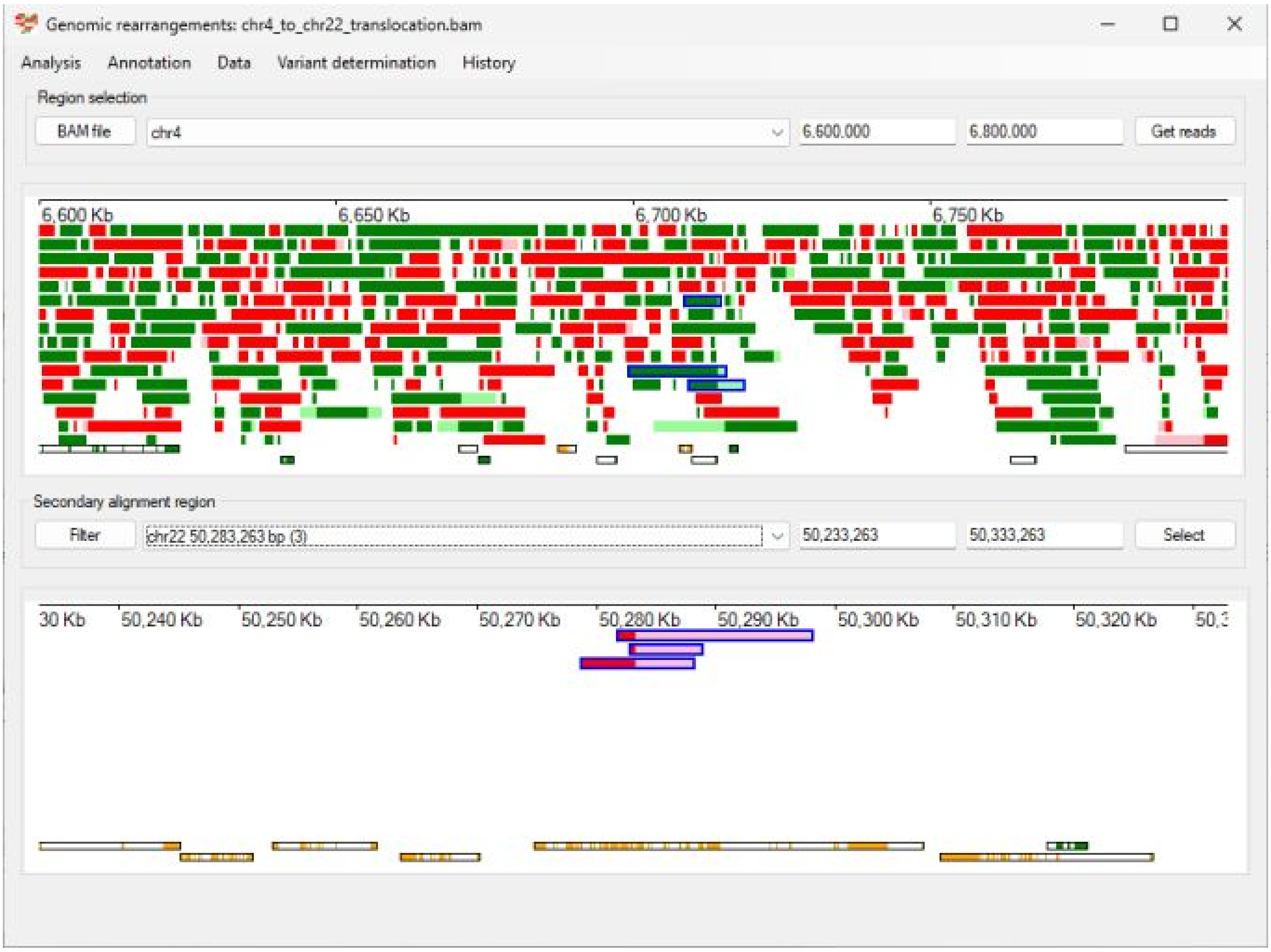
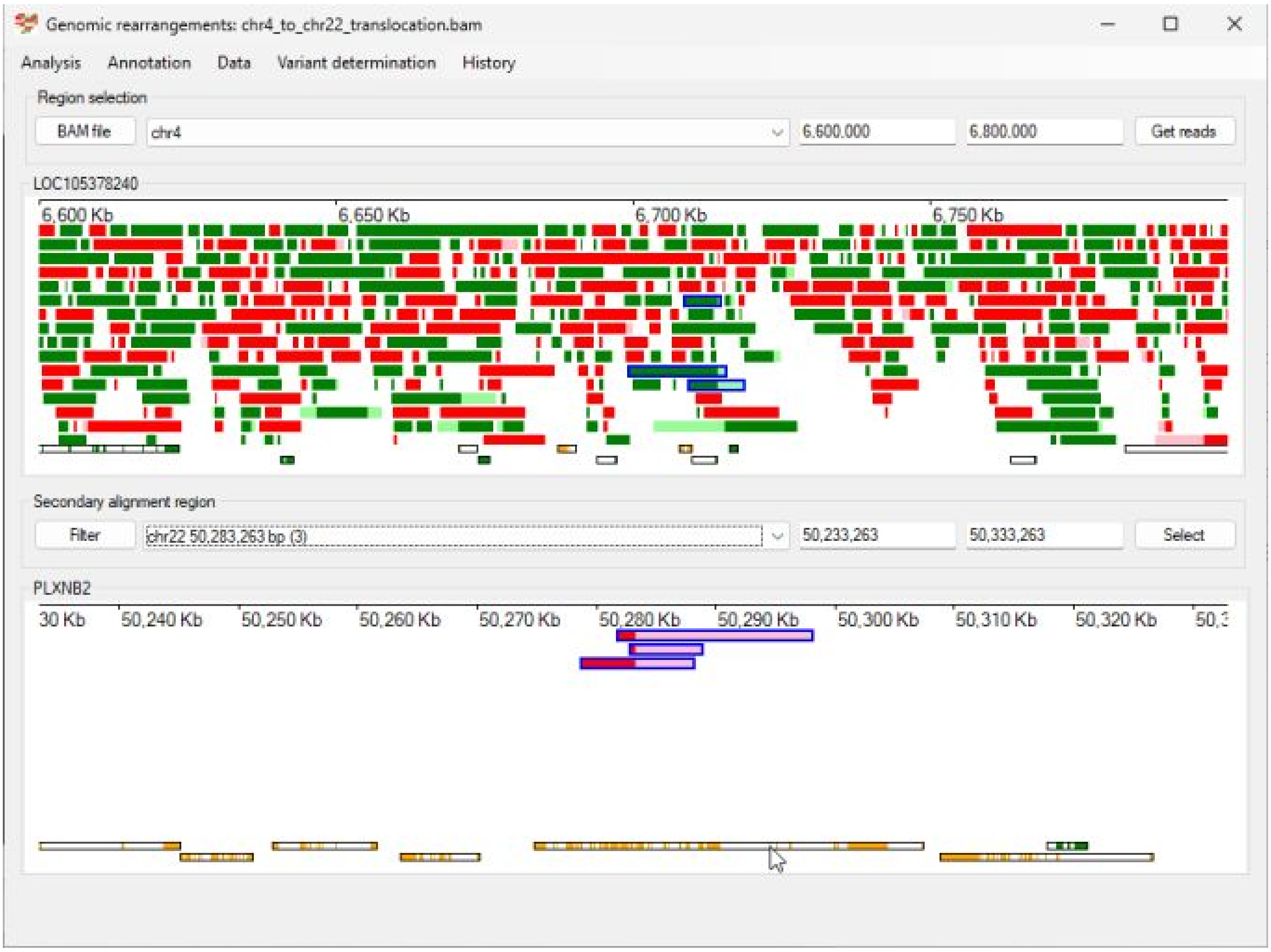


Figure 23

Clicking on a gene will cause is name to be displayed to the top left of the appropriate display. For instance, in Figure 24, the genes near the breakpoint (Primary alignment display: LOC105378240 and secondary alignment display: PLXNB2) have been selected.



Genomic rearrangements: chr4_to_chr22_translocation.bam

Region selection

chr4 6,600,000 6,800,000

LOC105378240

Secondary alignment region

chr22 50,283,263 bp (3) 50,233,263 50,333,263

PLXNB2

Figure 24

Displaying repeat positions

Repeat coordinates are imported by selecting the `Annotation` > `Select repeat annotation file` option (Figure 25). Unlike the gene positions, repeats are only drawn when the `Annotation` > `Show repeats` option is selected (Figure 25). This is due to the large number of repeats, which require substantial memory to store and can be slow to render across extensive regions. Consequently, `AgileStructure` will only retain the repeat file's filename and reloading the required data from the file each time the repeats need to be drawn..

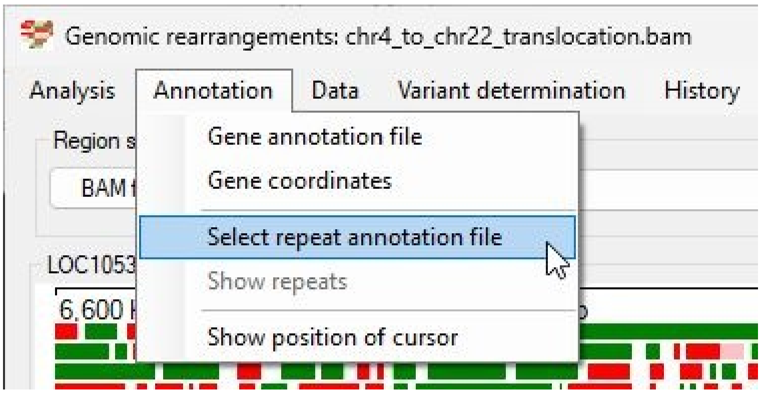


Figure 25

The repeats are drawn as black rectangles filled in pale blue (forward strand) or pale yellow (reverse strand) across a single row at the very bottom of the displays. As with genes, clicking on a repeat will cause its name, class, and family to be displayed at the top left of the display. For example in Figure 26, the repeats close to the breakpoint (Primary alignment: AluSz, SINE, Alu and Secondary alignment: (CCCACC)n, Simple repeat, Simple repeat) have been selected.

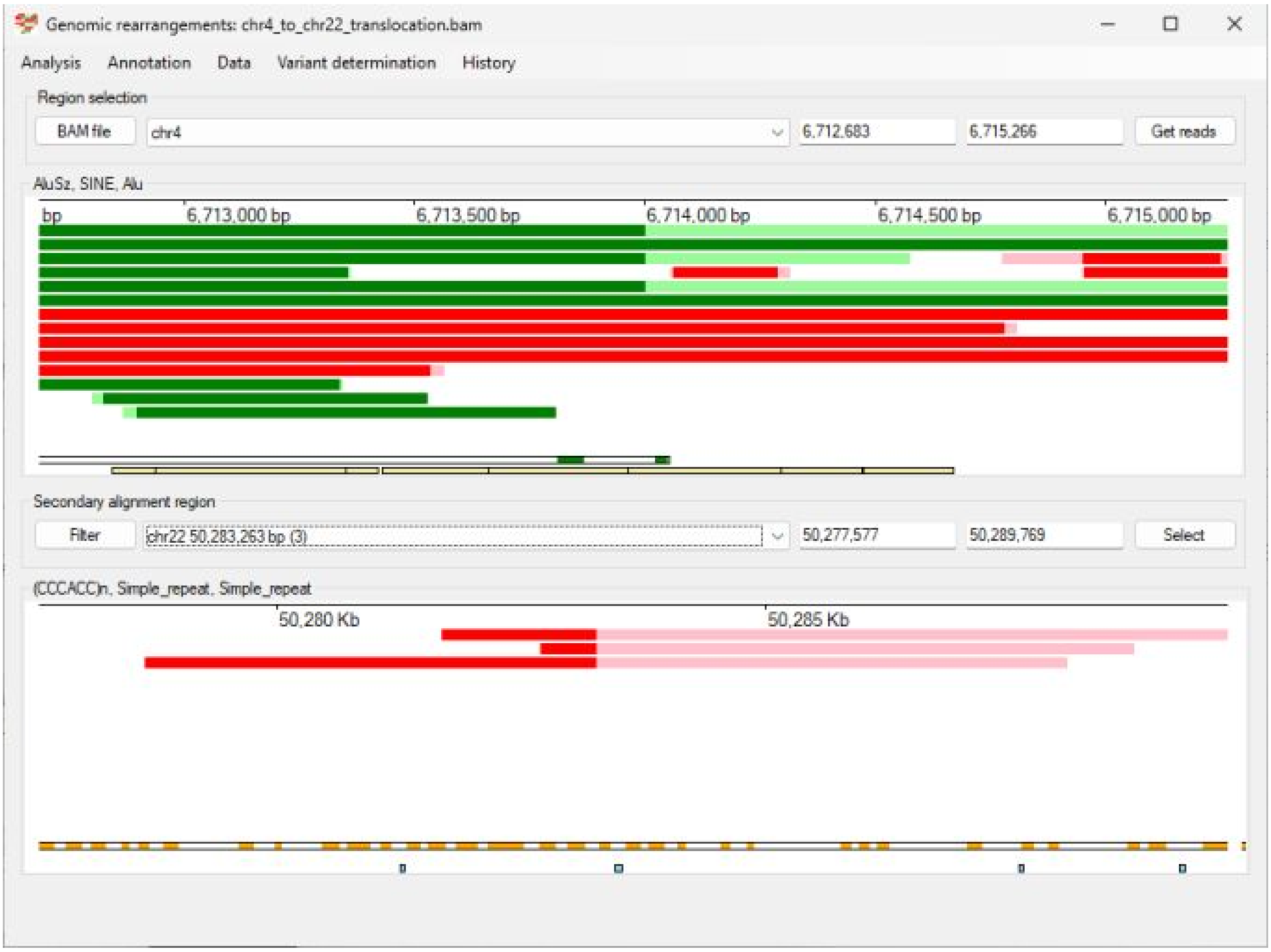


Figure 26

Miscellaneous functions

Cursor location

The `Annotation` > `Show position of cursor` menu option reveals the genomic coordinates of cursor's position (Figure 27b).It's important to note that this representation is approximate. For instance, a 1 Mb region depicted on an 860-pixel-wide image results in approximately 1,162.8 bps per pixel. Therefore, this feature should be used as a general guide to understand the region rather than a precise tool.

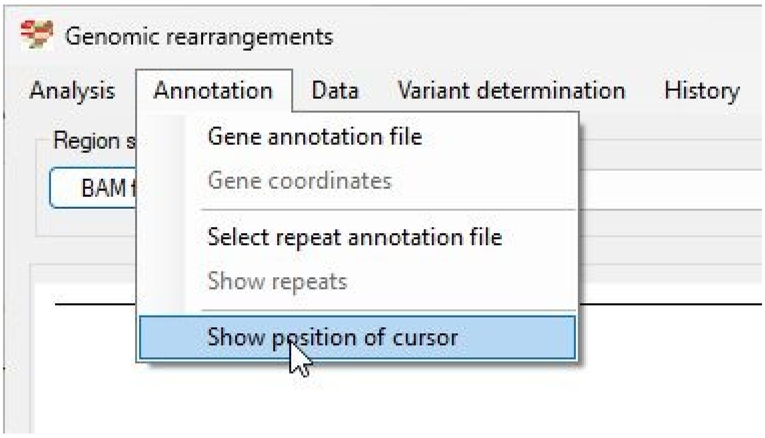


Figure 27a

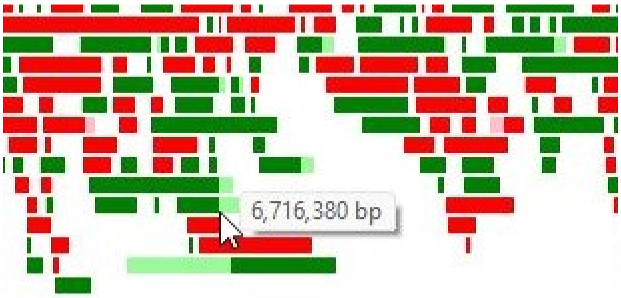


Figure 27b

Aligner string

Typically, the aligner used to map reads to the reference genome includes the command line arguments in the BAM file's header section. This information can be accessed by selecting the `Data` > `Aligner string` menu option (see Figures 28a and 28b). This feature is particularly useful when, for instance, you need to verify which reference genome was used for the alignment.

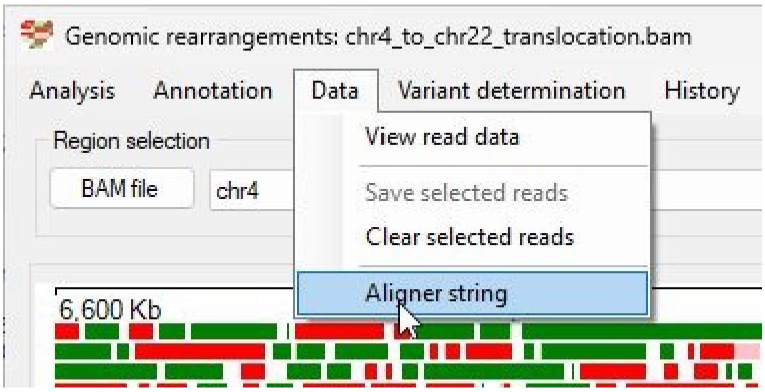
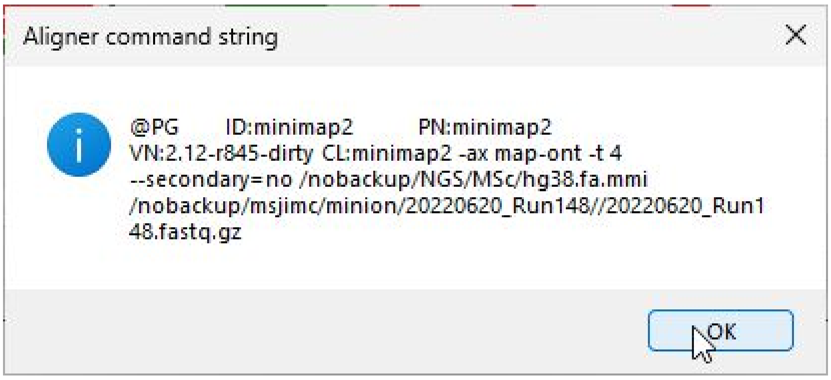


Figure 28a



```
@PG	ID:minimap2	PN:minimap2
VN:2.12-r845-dirty CL:minimap2 -ax map-ont -t 4
--secondary=no /nobackup/NGS/MSc/hg38.fa.mmi
/nobackup/msjimc/minion/20220620_Run148//20220620_Run1
48.fastq.gz
```

Figure 28b: The command string identifies the data in the '20220620_Run148.fastq.gz' file was aligned to the 'hg38.fa.mmi' minimap2 index (human genome : hg38).