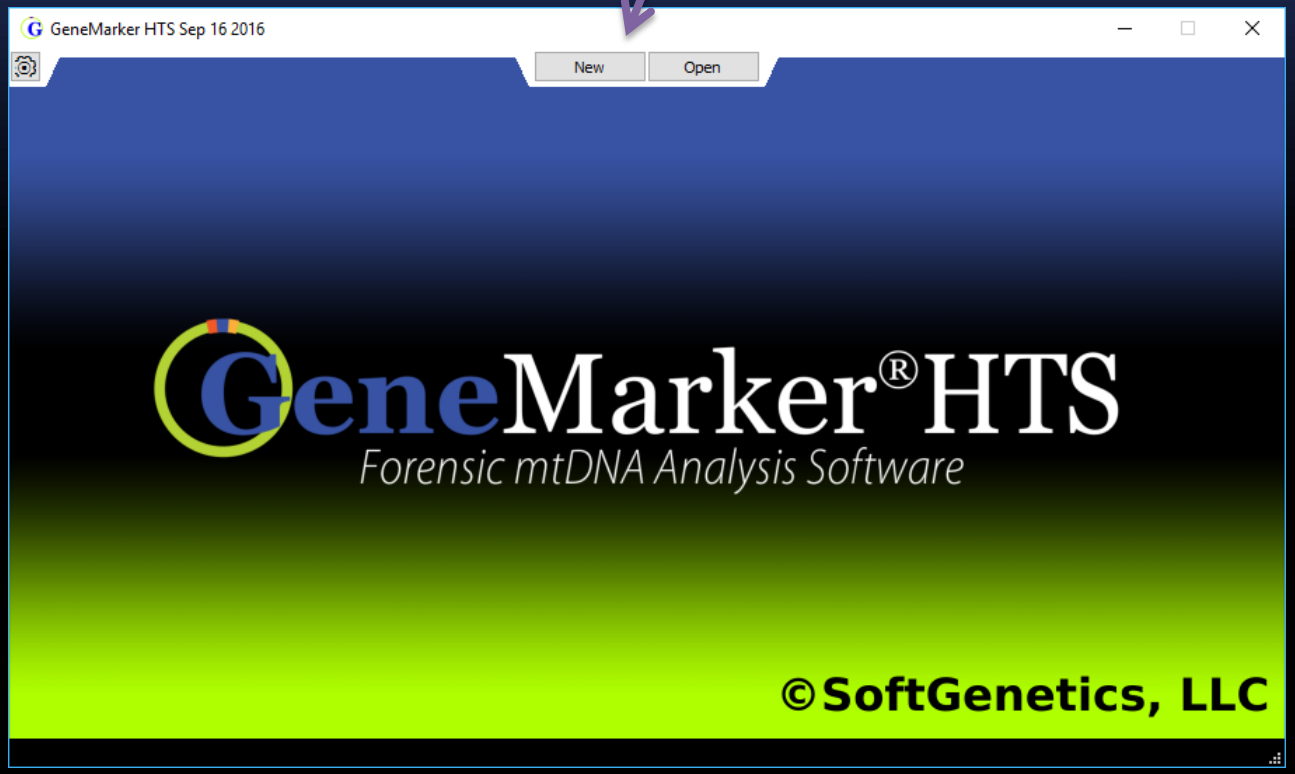
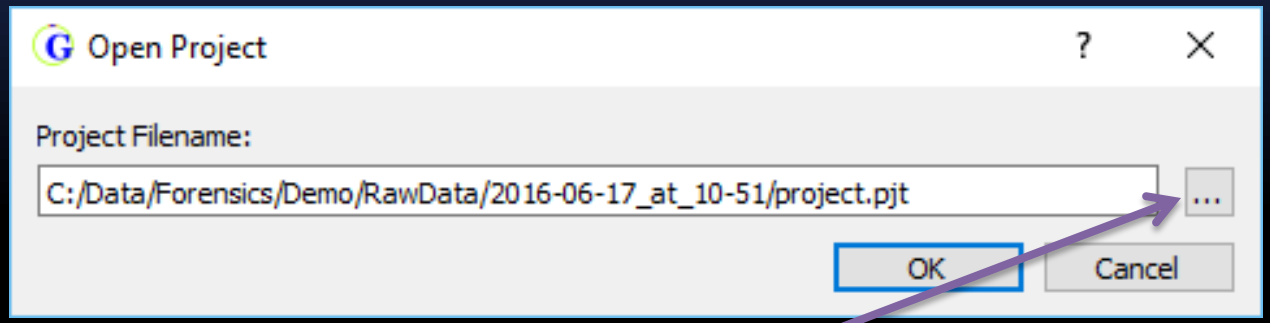
GeneMarker®HTS Software

Quick Start Guide

Upon launching the software, the user will have the option to start a *New* project or *Open* a previously saved project.



Opening a Project

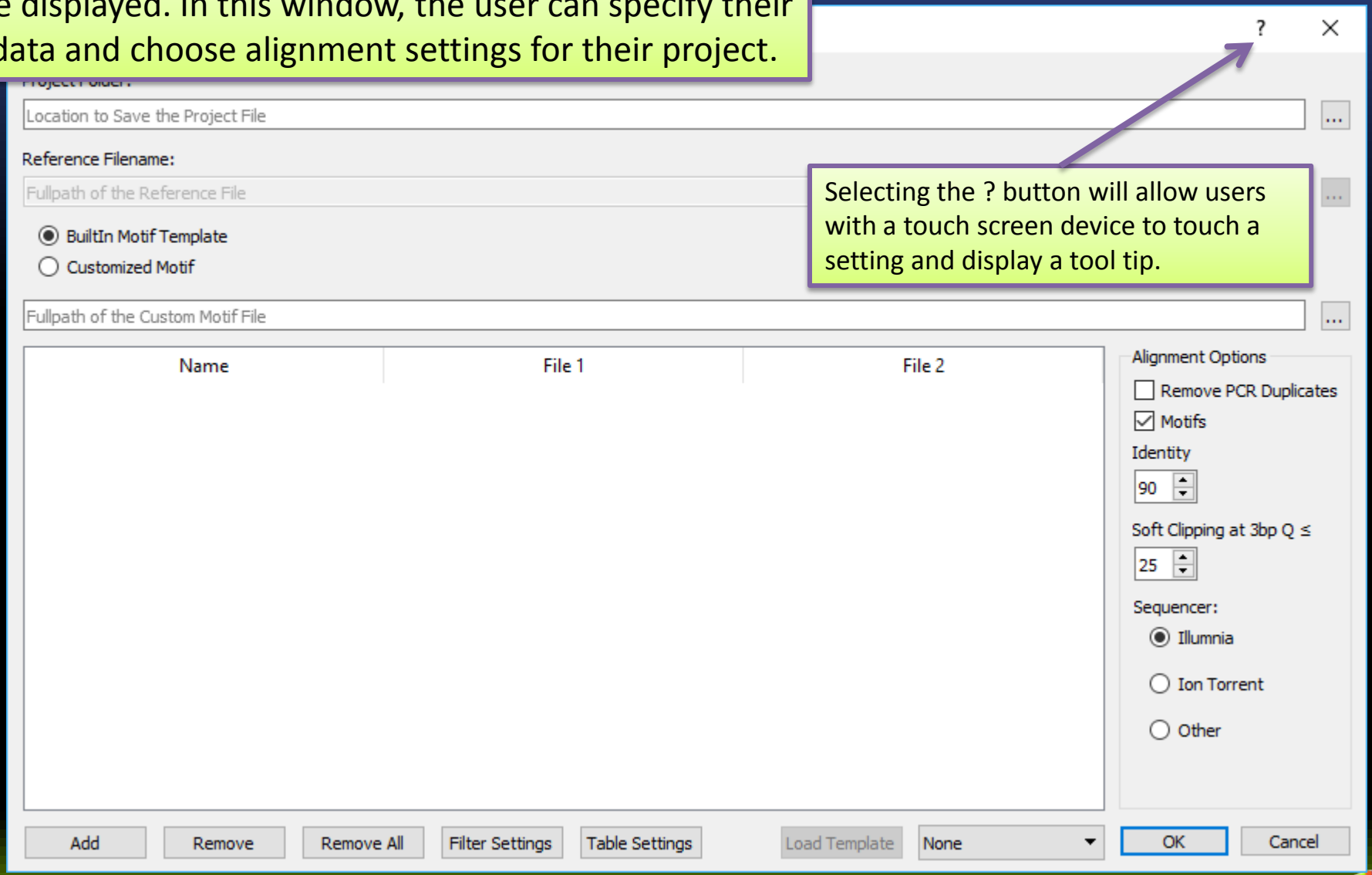


Using the ellipsis button, the user can select a previous project file (project.pjt). After clicking *OK*, the project will be available to open in a *Sample Viewer* window.

Setting up a New Project

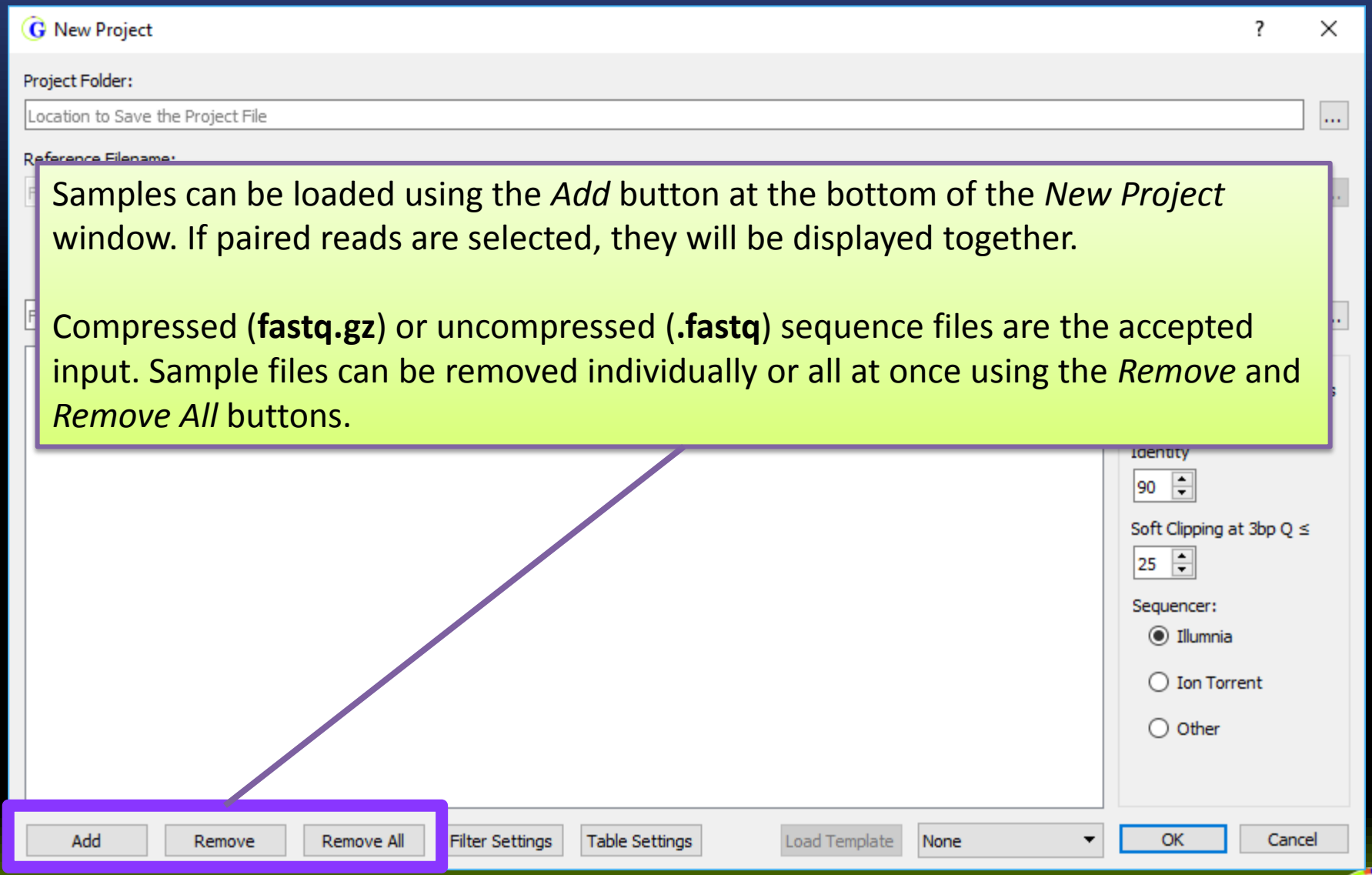
Creating a new project

When *New* is selected, the *New Project* window will be displayed. In this window, the user can specify their data and choose alignment settings for their project.



Selecting the ? button will allow users with a touch screen device to touch a setting and display a tool tip.

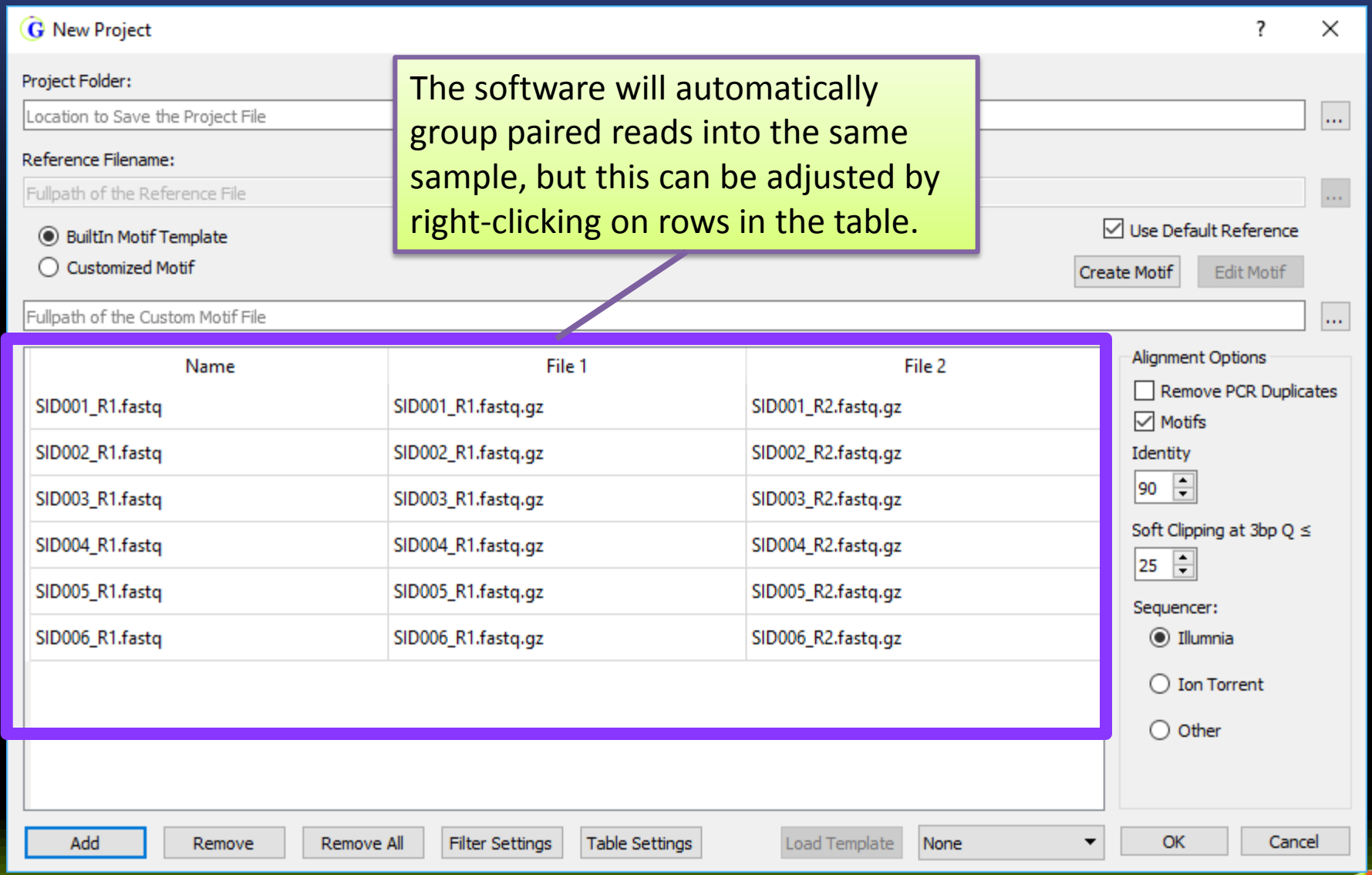
Creating a new project



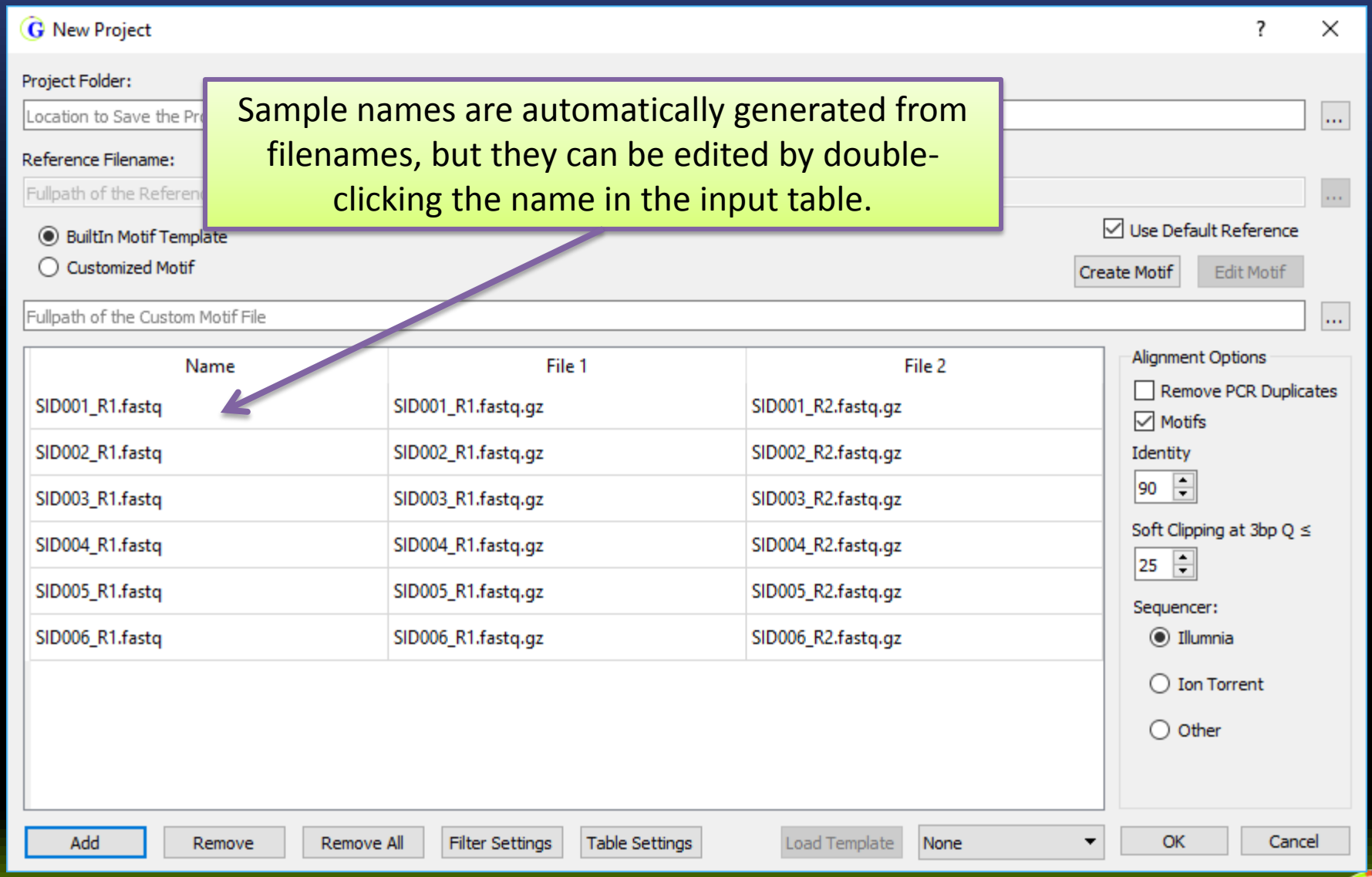
Samples can be loaded using the *Add* button at the bottom of the *New Project* window. If paired reads are selected, they will be displayed together.

Compressed (**fastq.gz**) or uncompressed (**.fastq**) sequence files are the accepted input. Sample files can be removed individually or all at once using the *Remove* and *Remove All* buttons.

Creating a new project



Creating a new project

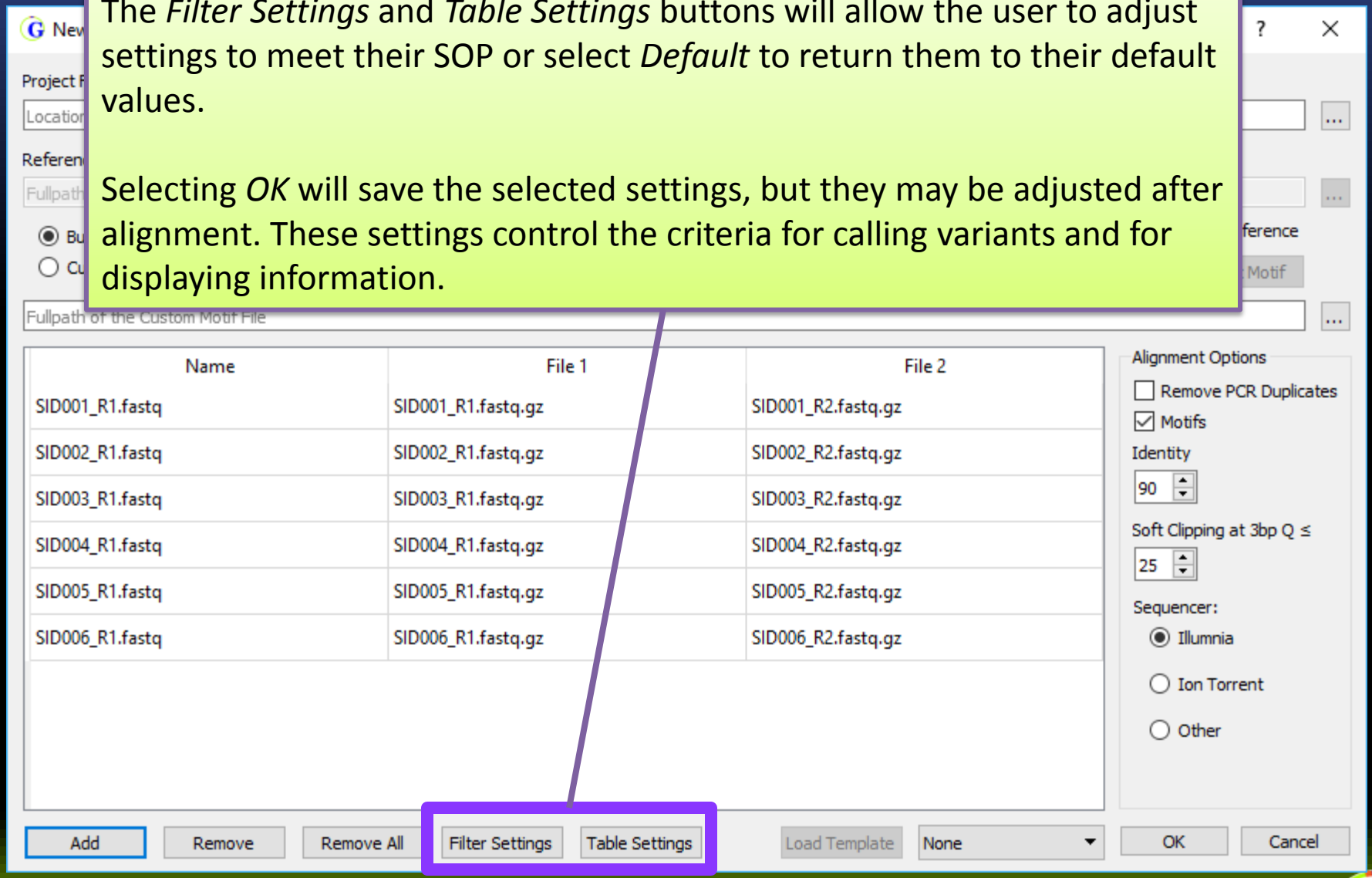


Sample names are automatically generated from filenames, but they can be edited by double-clicking the name in the input table.

Creating a new project

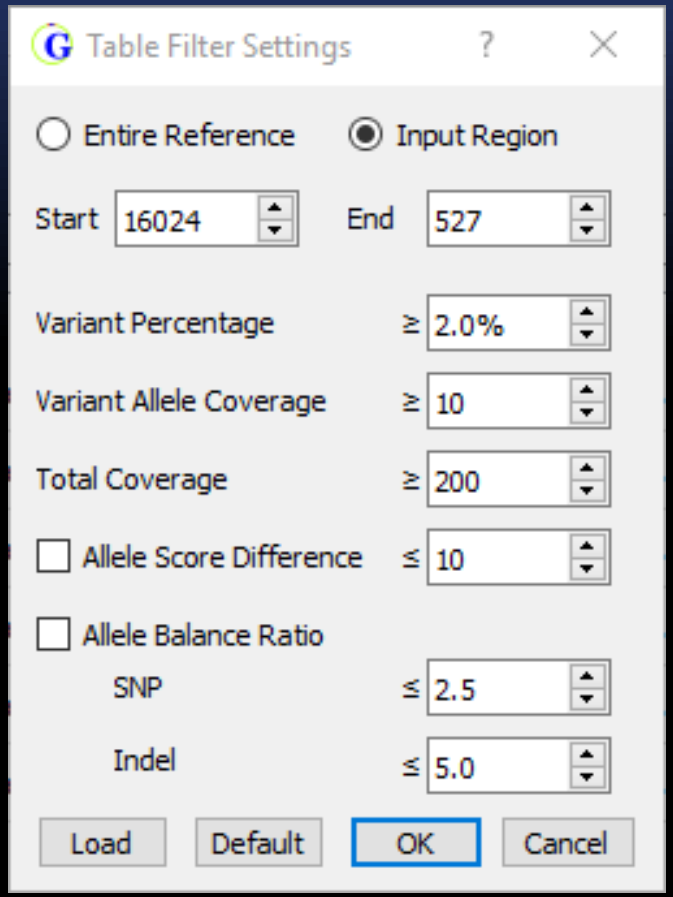
The *Filter Settings* and *Table Settings* buttons will allow the user to adjust settings to meet their SOP or select *Default* to return them to their default values.

Selecting *OK* will save the selected settings, but they may be adjusted after alignment. These settings control the criteria for calling variants and for displaying information.



Filter Settings

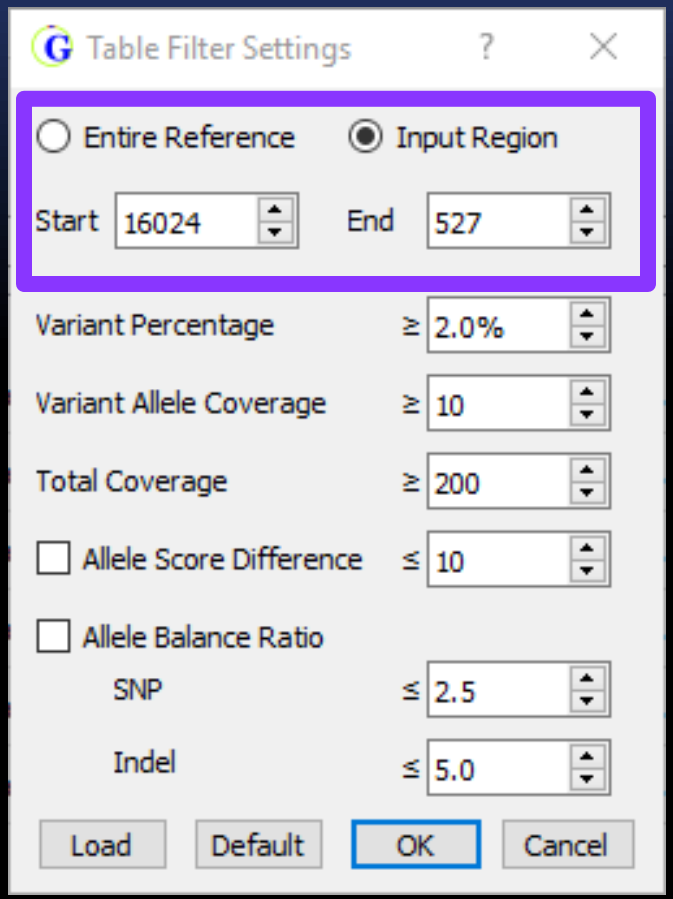
Filter Settings



The *Table Filter Settings* dialog allows for variant calling settings to be adjusted.

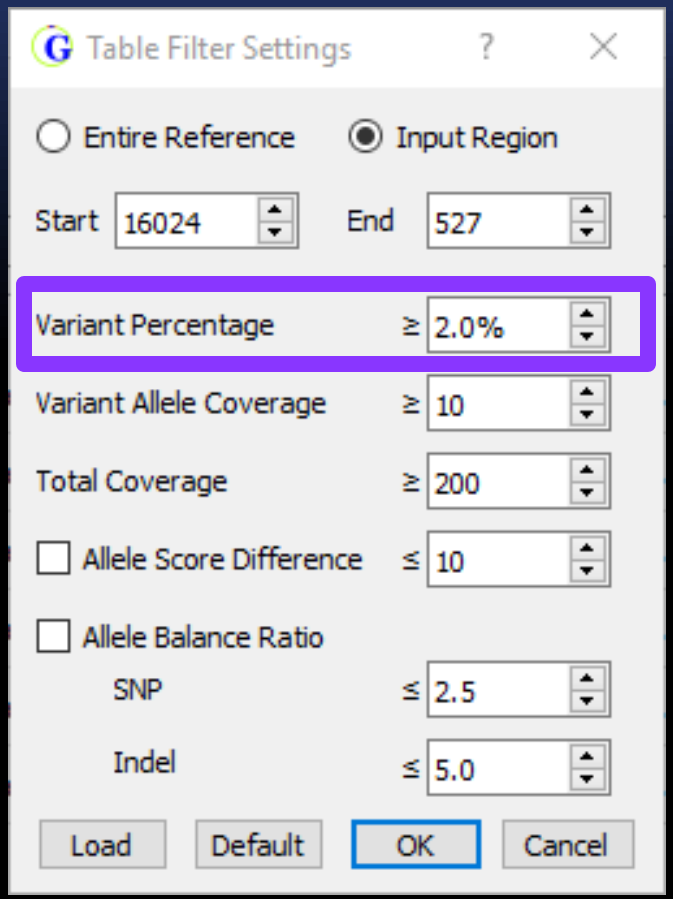
The settings can be returned to their original state by using the *Default* button. A project settings file can be imported using the *Load* button.

Filter Settings



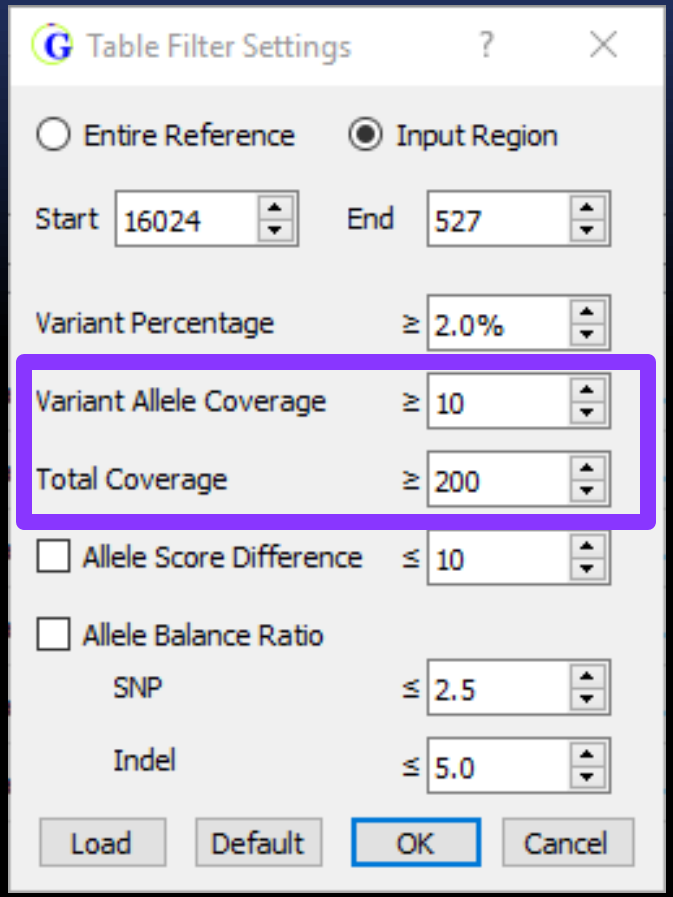
Reference area where variants may be called

Filter Settings

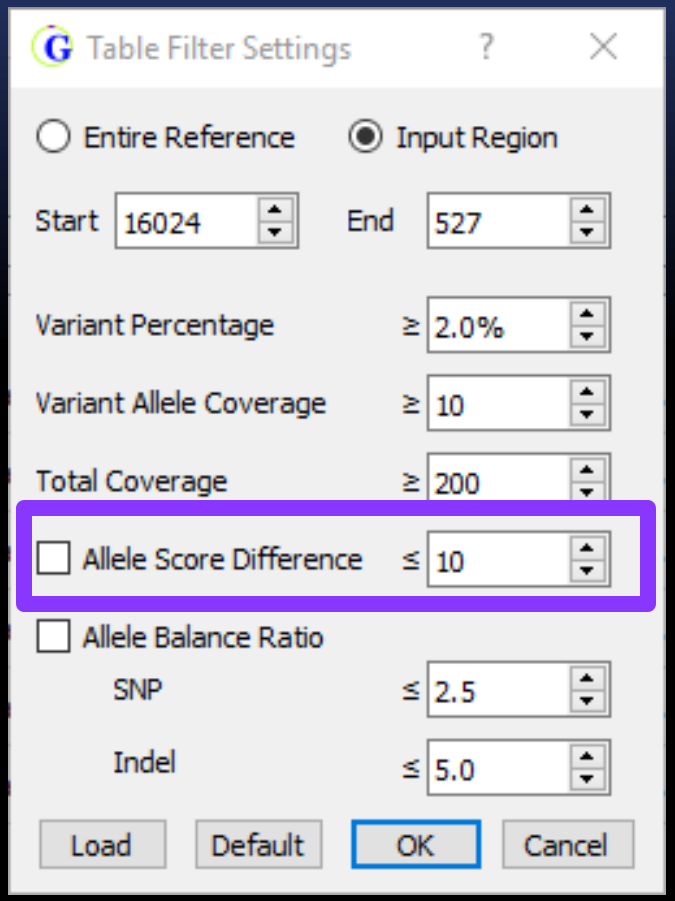


Percent of reads at a position with the variant

Filter Settings



Number of reads with the variant and Total number of reads

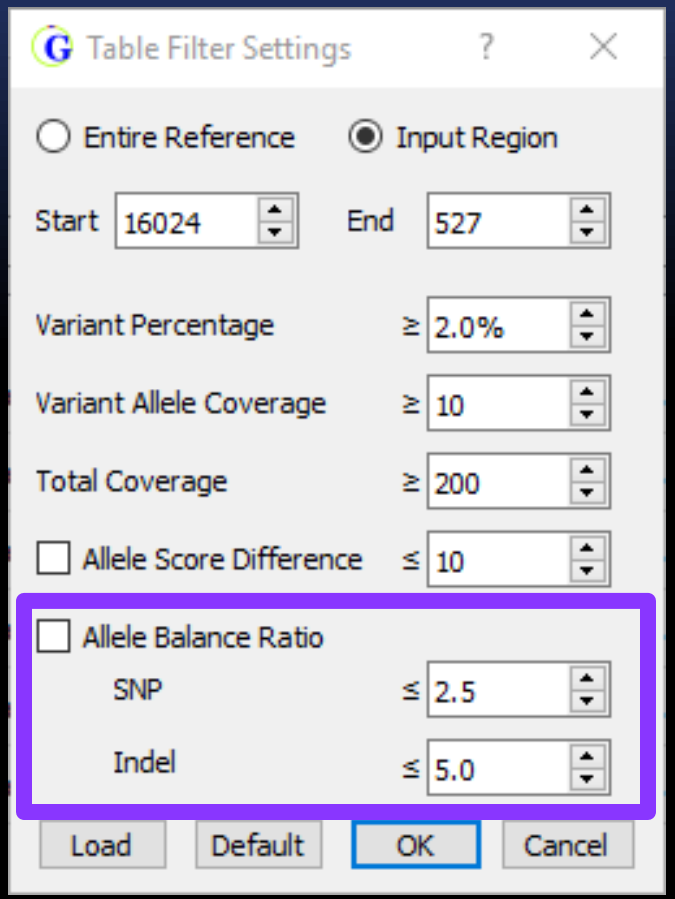


Allele Score Difference

Each allele (A, C, G, T, deletion, and any insertions) has quality scores calculated as the median of the base quality scores from the original data. Deletion base quality scores don't exist, so they are based on the quality scores of surrounding bases.

Each minor allele has its score subtracted from the major allele score and the difference must be less than or equal to the set value.

Both directions are checked individually. If the score difference is higher and the minor allele has at least 5 reads the variant is assumed to be a false positive and is filtered out.



Allele Balance Ratio

The balance ratio is the maximum value of:

- Allele forward % / Total forward %
- Total forward % / Allele forward %
- Allele reverse % / Total reverse%
- Total reverse % / Allele reverse %

This is calculated for each allele. Indels and SNPs have different cutoffs for filtering.

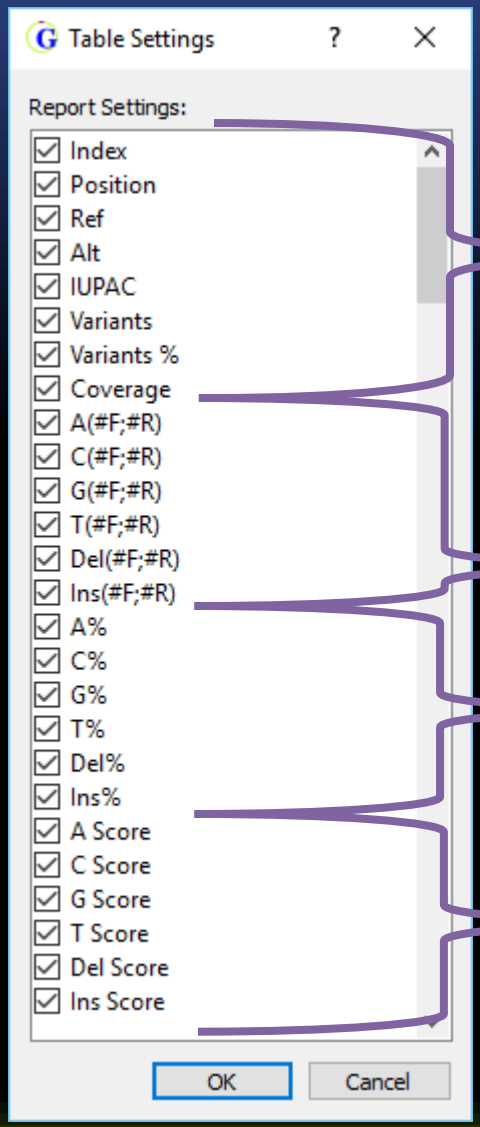
- 2 forward and 79 reverse reads with the allele
- 1758 forward and 6055 reverse reads overall
- Balance is the maximum of:
  - 0.02469/0.22501 = 0.9113
  - 0.22501/0.02469 = **9.1134**
  - 0.97531/0.77499 = 1.2585
  - 0.77499/0.97531 = 0.7946

This allele would be filtered with the default settings because 9.1134 > 2.5

Alleles with frequency >= 85% are not filtered using this setting

Table Settings

Table Settings



- Index (number the variants)
- Position of Variant
- Reference Allele
- Alternative Allele
- IUPAC (ambiguity) formatted variant call
- Standard variant call
- Variant Frequency
- Total Coverage at the Position

Allele Counts (Forward and Reverse)

Allele Percentage

Median Basecall Quality (Forward and Reverse)

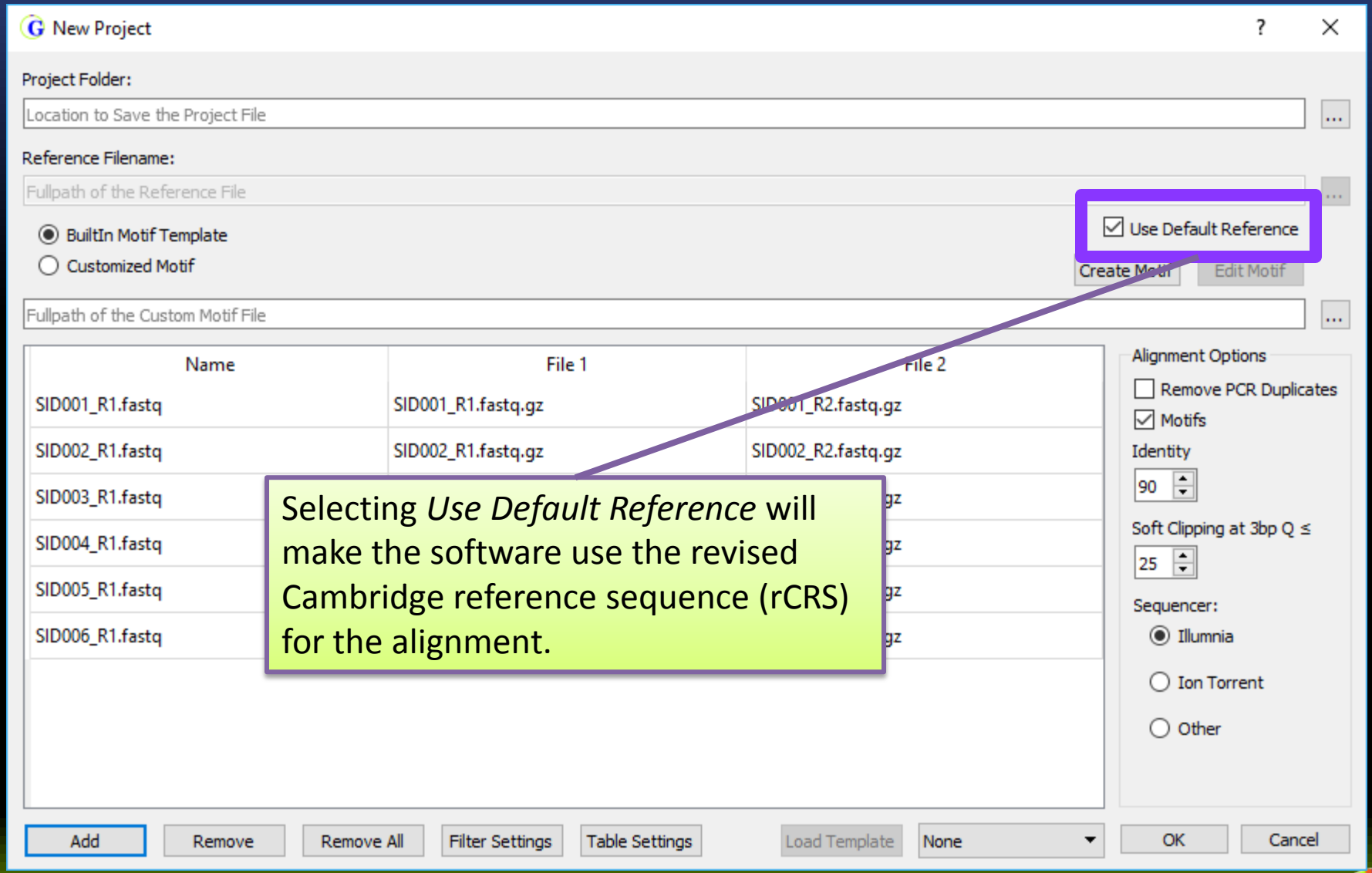
Variant Calls -IUPAC

When there are multiple alleles at a position, ambiguity codes are used when reporting in the “IUPAC” column.

Code	Alleles
M	A, C
R	A, G
W	A, T
S	C, G
Y	C, T
K	G, T
V	A, C, G
H	A, C, T
D	A, G, T
B	C, G, T
N	A, C, G, T

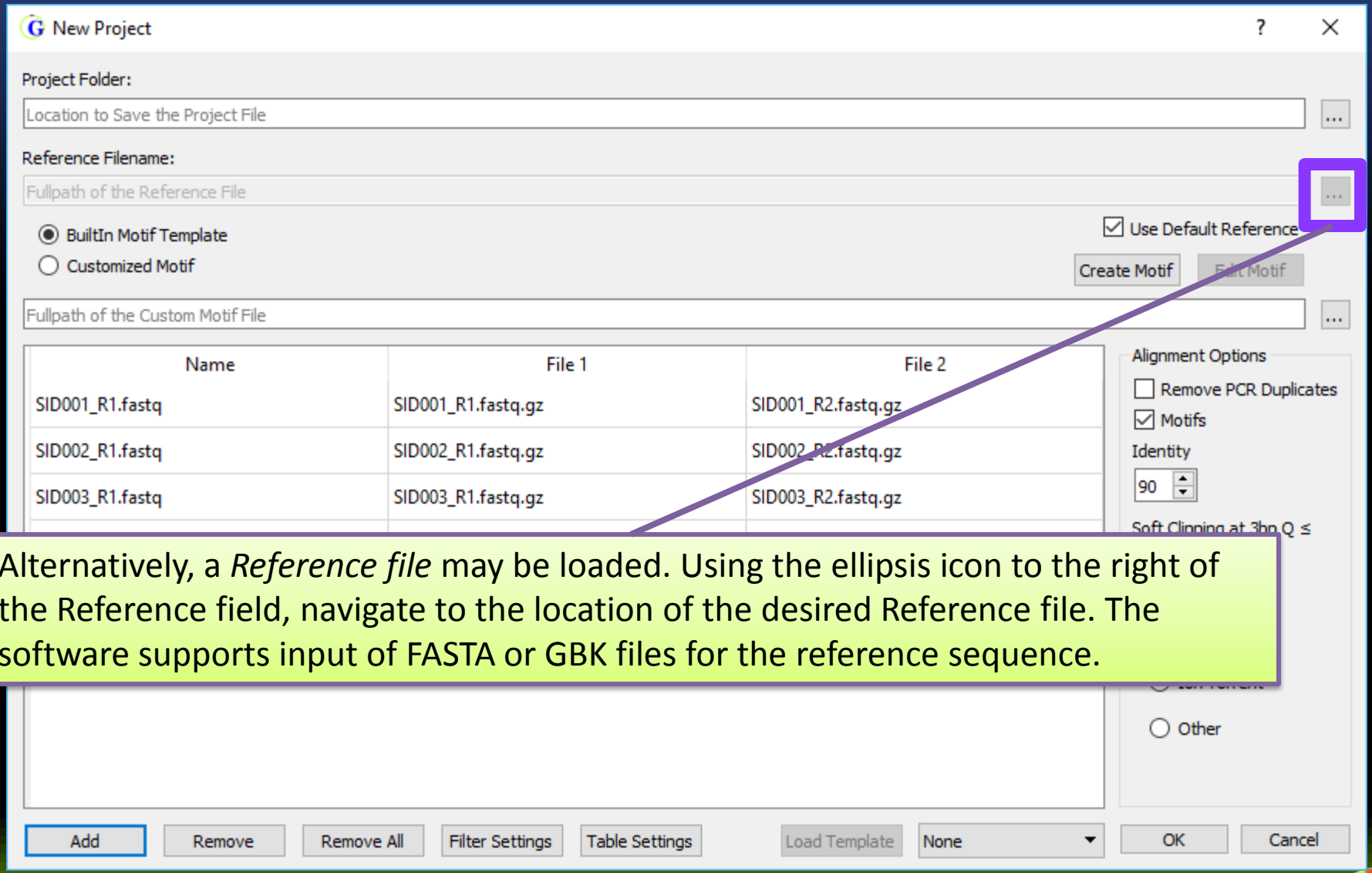
Mixtures of a deletion and other alleles use a lowercase letter.

Creating a new project



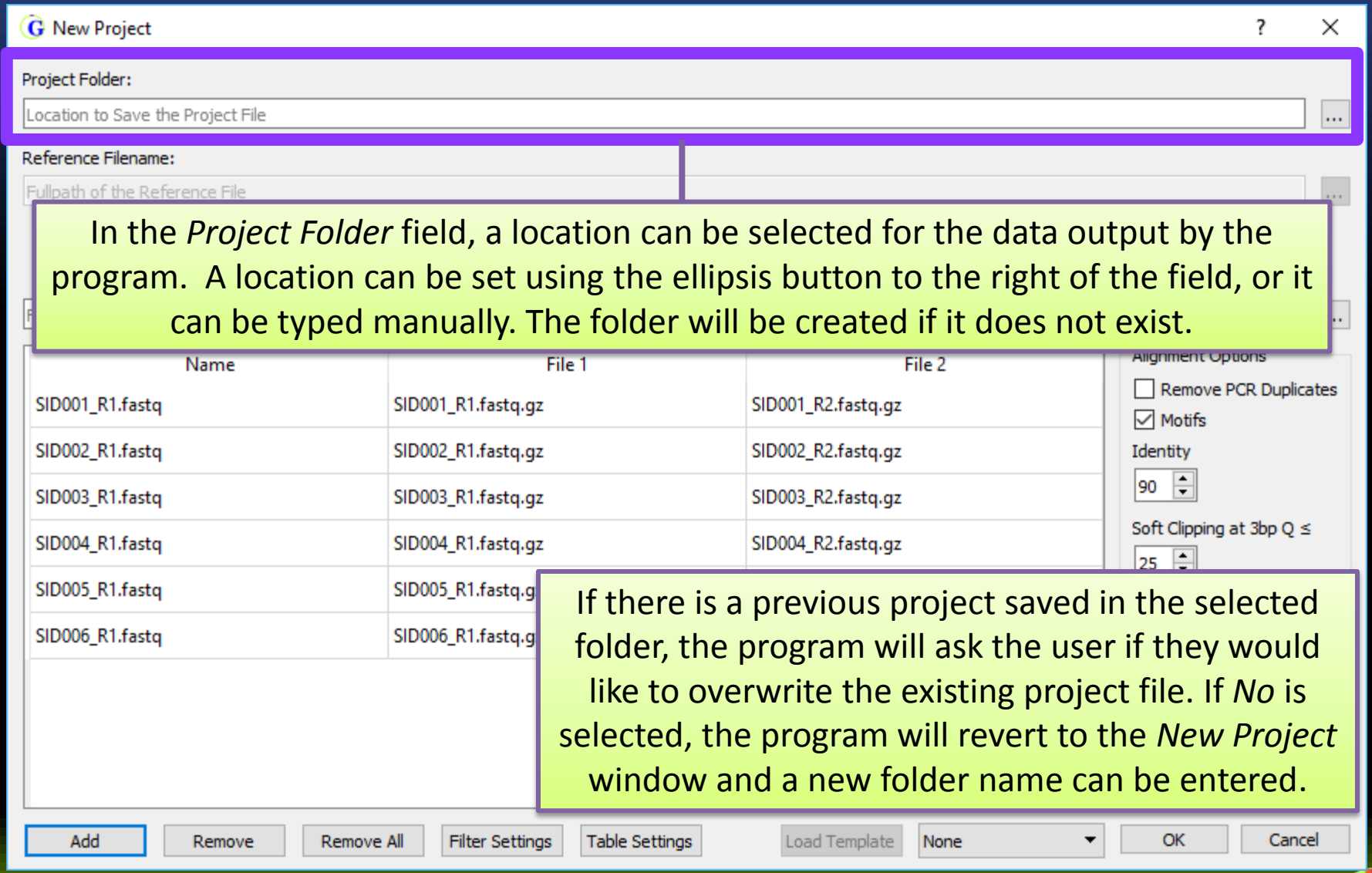
Selecting *Use Default Reference* will make the software use the revised Cambridge reference sequence (rCRS) for the alignment.

Creating a new project



Alternatively, a *Reference file* may be loaded. Using the ellipsis icon to the right of the Reference field, navigate to the location of the desired Reference file. The software supports input of FASTA or GBK files for the reference sequence.

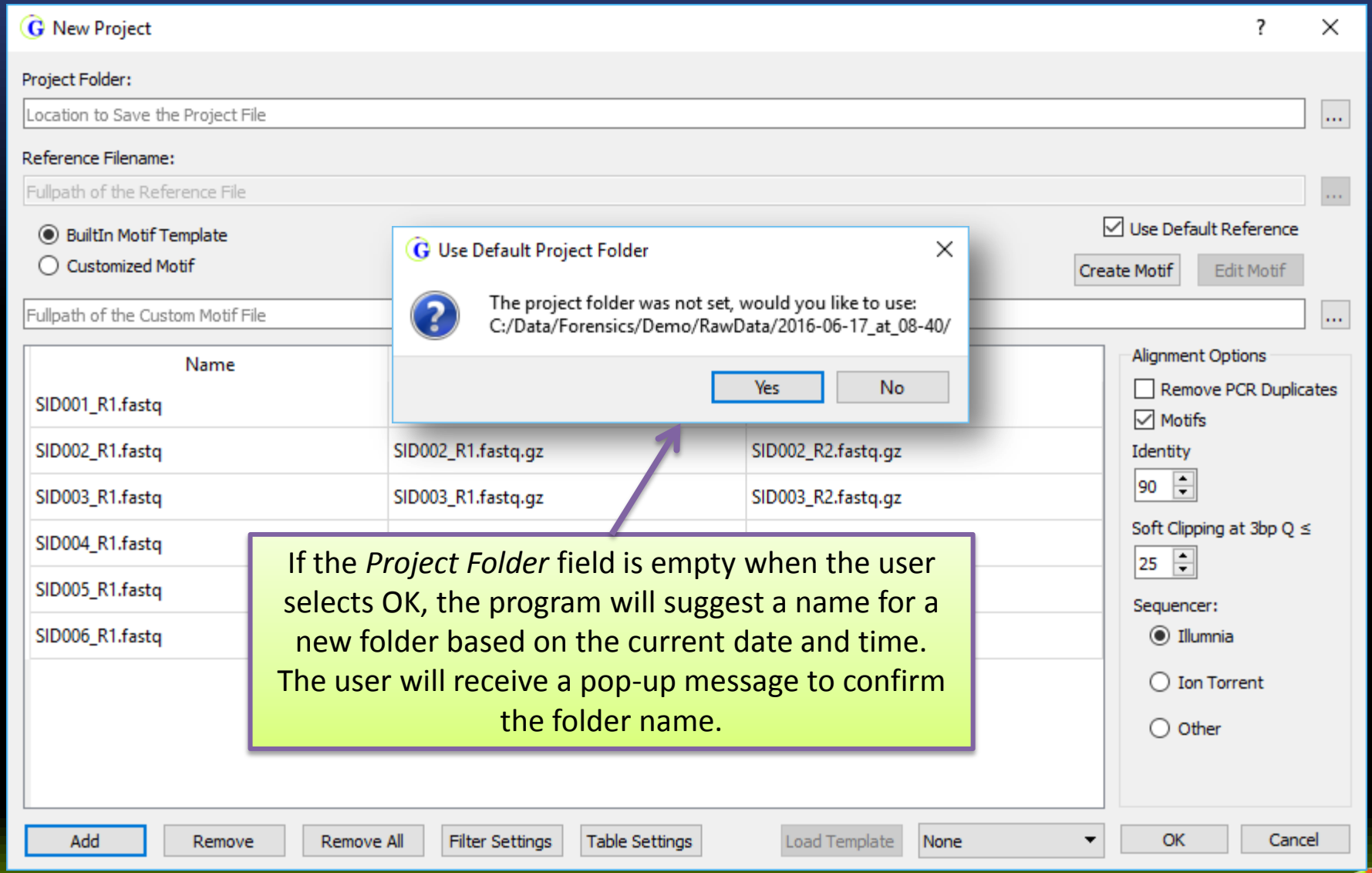
Creating a new project



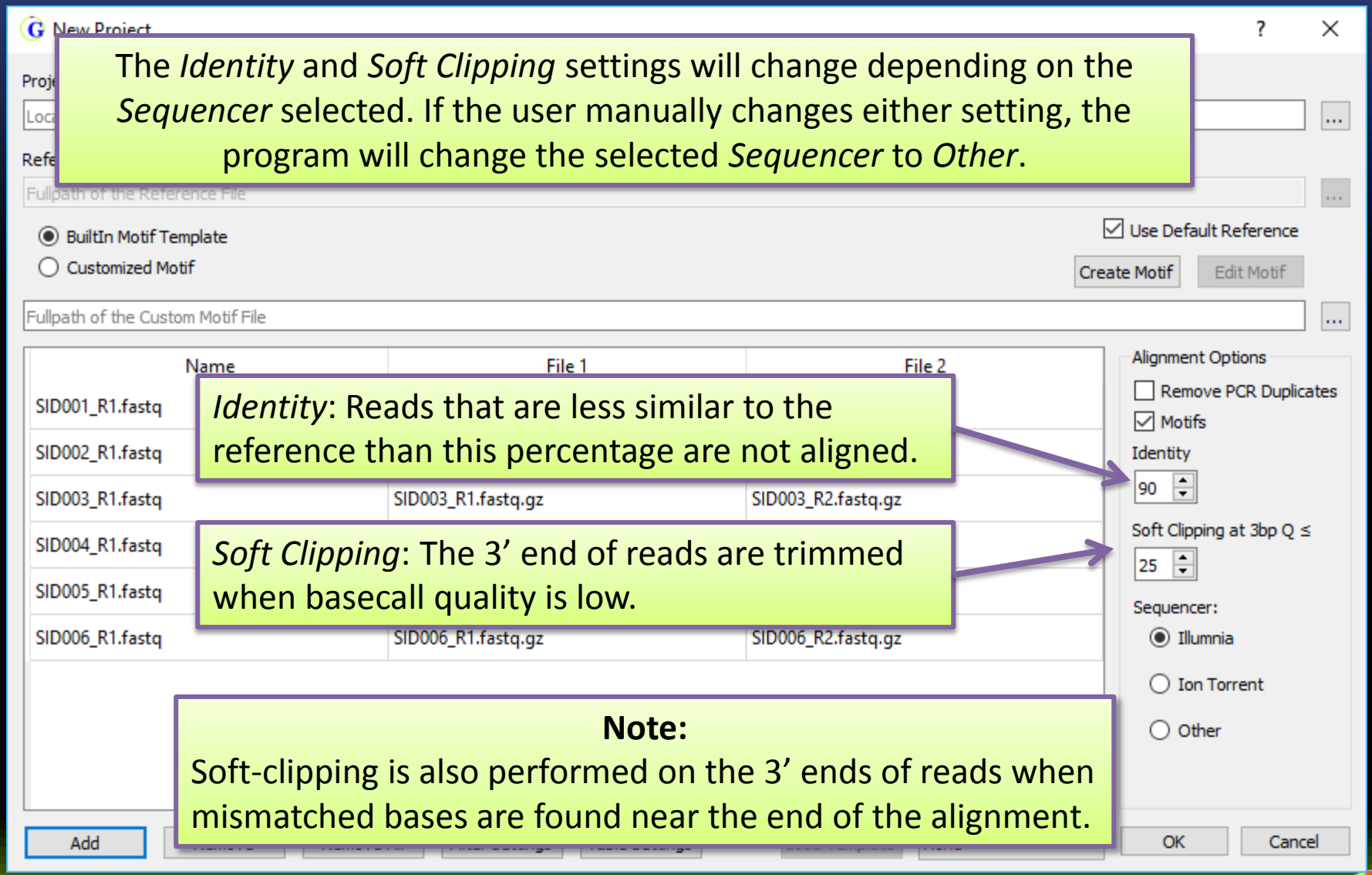
In the *Project Folder* field, a location can be selected for the data output by the program. A location can be set using the ellipsis button to the right of the field, or it can be typed manually. The folder will be created if it does not exist.

If there is a previous project saved in the selected folder, the program will ask the user if they would like to overwrite the existing project file. If *No* is selected, the program will revert to the *New Project* window and a new folder name can be entered.

Creating a new project

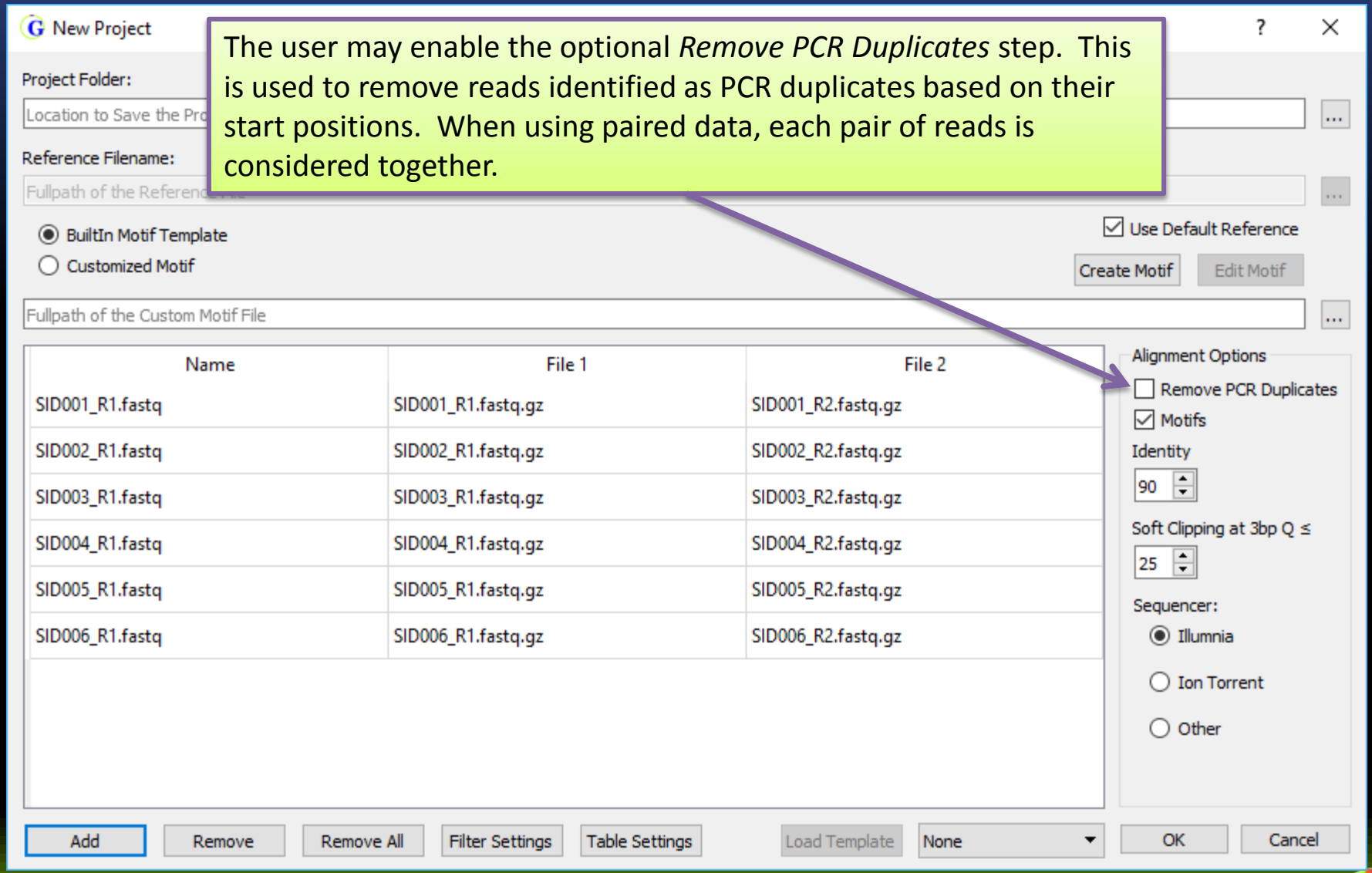


If the *Project Folder* field is empty when the user selects OK, the program will suggest a name for a new folder based on the current date and time. The user will receive a pop-up message to confirm the folder name.


New Project
The *Identity* and *Soft Clipping* settings will change depending on the *Sequencer* selected. If the user manually changes either setting, the program will change the selected *Sequencer* to *Other*.
Fullpath of the Reference File
BuiltIn Motif Template
Customized Motif
Use Default Reference
Create Motif
Edit Motif
Fullpath of the Custom Motif File
Name
File 1
File 2
SID001_R1.fastq
SID002_R1.fastq
SID003_R1.fastq
SID003_R1.fastq.gz
SID003_R2.fastq.gz
SID004_R1.fastq
SID005_R1.fastq
SID006_R1.fastq
SID006_R1.fastq.gz
SID006_R2.fastq.gz
Identity: Reads that are less similar to the reference than this percentage are not aligned.
Soft Clipping: The 3' end of reads are trimmed when basecall quality is low.
Alignment Options
Remove PCR Duplicates
Motifs
Identity
90
Soft Clipping at 3bp Q ≤
25
Sequencer:
Illumnia
Ion Torrent
Other
Note:
Soft-clipping is also performed on the 3' ends of reads when mismatched bases are found near the end of the alignment.
Add
OK
Cancel

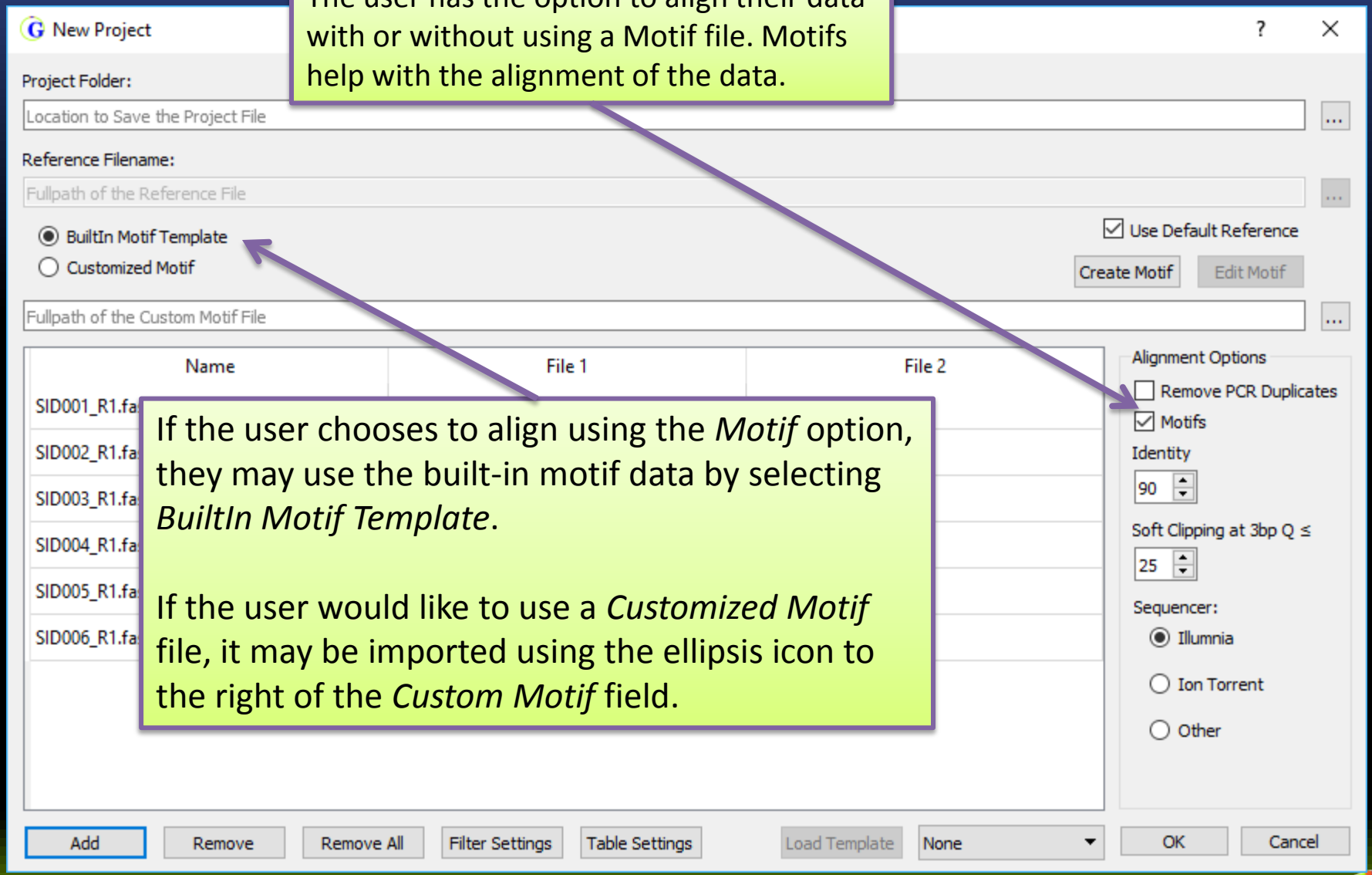
Setting up your project

The user may enable the optional *Remove PCR Duplicates* step. This is used to remove reads identified as PCR duplicates based on their start positions. When using paired data, each pair of reads is considered together.



Setting up your project

The user has the option to align their data with or without using a Motif file. Motifs help with the alignment of the data.



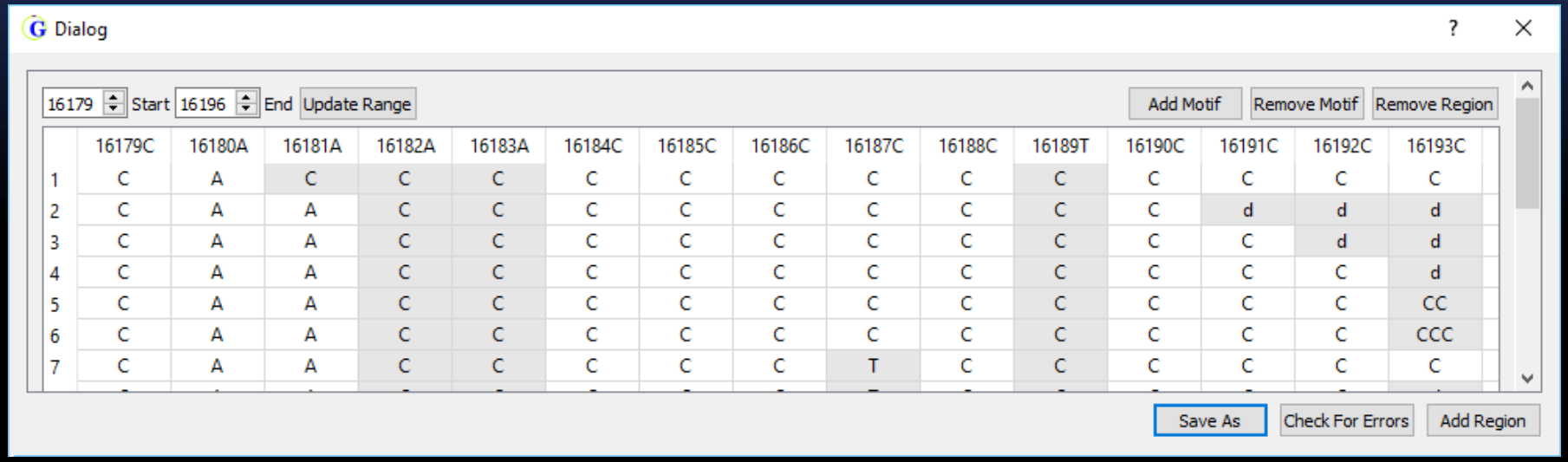
If the user chooses to align using the *Motif* option, they may use the built-in motif data by selecting *BuiltIn Motif Template*.

If the user would like to use a *Customized Motif* file, it may be imported using the ellipsis icon to the right of the *Custom Motif* field.

Motifs

Setting up your project

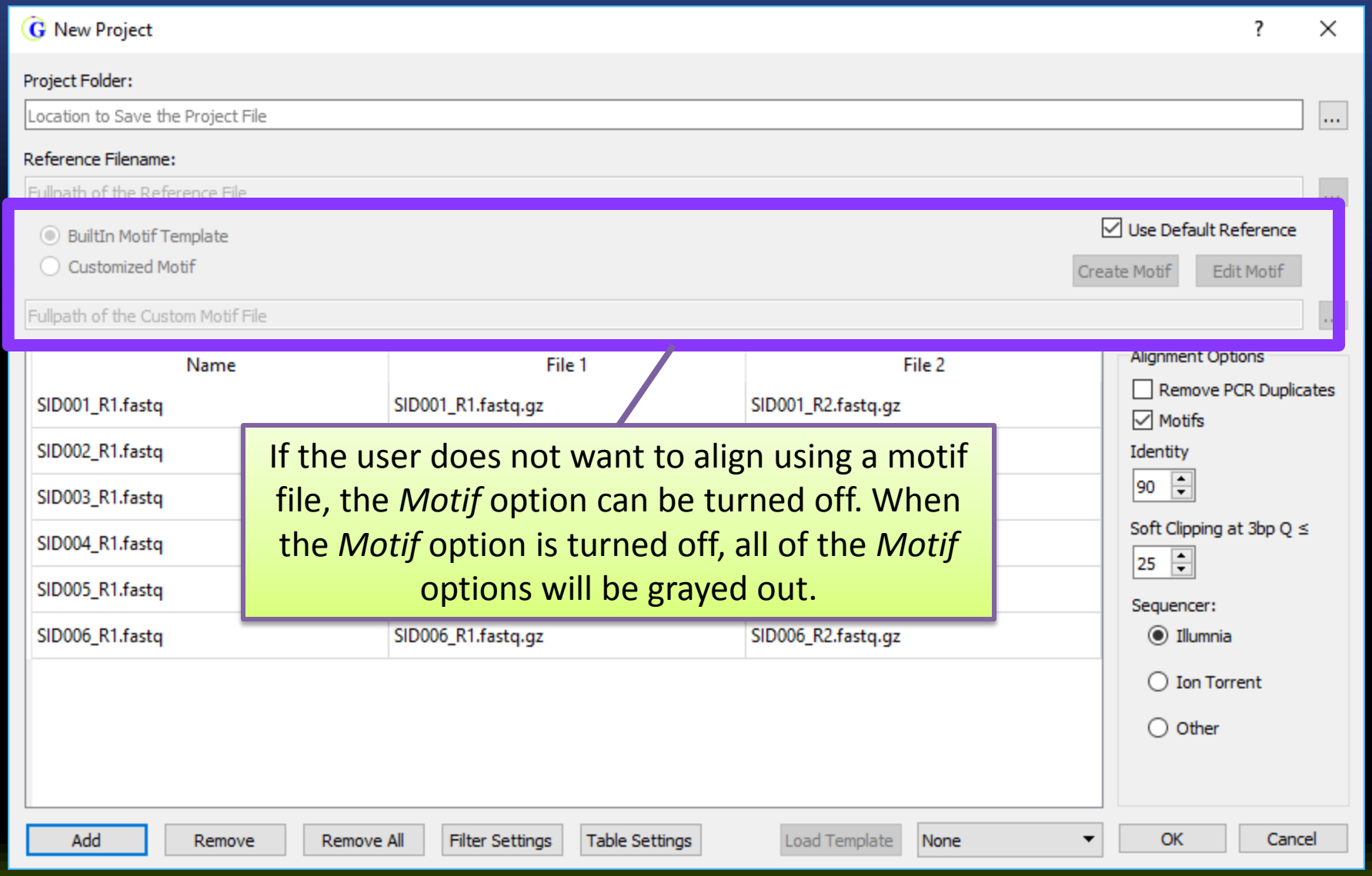
The *Edit Motif* icon opens the custom motif in the *Motif Editor* window.
The *Create Motif* icon will open the built-in motif file.



	16179C	16180A	16181A	16182A	16183A	16184C	16185C	16186C	16187C	16188C	16189T	16190C	16191C	16192C	16193C
1	C	A	C	C	C	C	C	C	C	C	C	C	C	C	C
2	C	A	A	C	C	C	C	C	C	C	C	C	d	d	d
3	C	A	A	C	C	C	C	C	C	C	C	C	C	d	d
4	C	A	A	C	C	C	C	C	C	C	C	C	C	C	d
5	C	A	A	C	C	C	C	C	C	C	C	C	C	C	CC
6	C	A	A	C	C	C	C	C	C	C	C	C	C	C	CCC
7	C	A	A	C	C	C	C	C	T	C	C	C	C	C	C

The *Motif Editor* window makes it easy to add new motifs or edit existing motifs. It is also possible to add new motif regions. The *Check for Errors* button will ensure that all motifs in the file are valid. After editing the user can save the motifs to a new file using the *Save As* button.

Setting up your project



If the user does not want to align using a motif file, the *Motif* option can be turned off. When the *Motif* option is turned off, all of the *Motif* options will be grayed out.

Using Motifs

Alignment traditionally is focused on minimizing the number of differences between the read and the reference. This isn't optimal for forensic analysis since there is an established convention for the positioning of many common variants.

	16180					16185					16190					16195	
Reference	A	A	A	A	C	C	C	C	C	T	C	C	C	C	A	T	G
Optimal Alignment	A	A	-	-	C	C	C	C	C	T	C	C	C	CC	A	T	G
Forensic Alignment	A	A	C	C	C	C	C	T	C	C	C	C	C	-	A	T	G

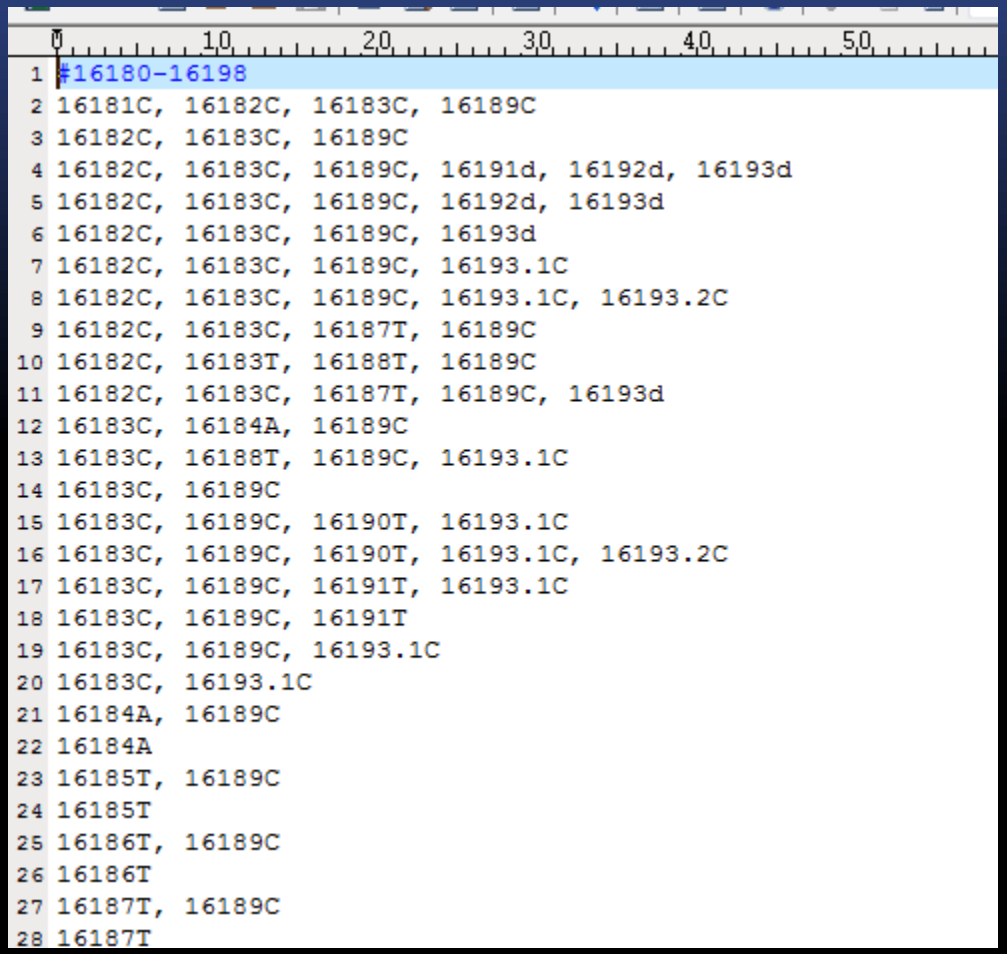
```
#16179-16196
16181C, 16182C, 16183C, 16189C
16182C, 16183C, 16189C, 16191d, 16192d, 16193d
16182C, 16183C, 16189C, 16192d, 16193d
16182C, 16183C, 16189C, 16193d
16182C, 16183C, 16189C, 16193.1C
16182C, 16183C, 16189C, 16193.1C, 16193.2C
16182C, 16183C, 16187T, 16189C
16182C, 16183C, 16187T, 16189C, 16193d
16182C, 16183C, 16189C
```

Each motif is a list of variant calls. These variant calls are translated into an expected sequence.

When using "motif alignment", the alignments of reads crossing motif regions (specified by the "#" line) with an expected sequence will be adjusted to match the list of variants.

Reads starting or ending in motif regions are soft-clipped.

Motif File Format



```
#16180-16198
16181C, 16182C, 16183C, 16189C
16182C, 16183C, 16189C
16182C, 16183C, 16189C, 16191d, 16192d, 16193d
16182C, 16183C, 16189C, 16192d, 16193d
16182C, 16183C, 16189C, 16193d
16182C, 16183C, 16189C, 16193.1C
16182C, 16183C, 16189C, 16193.1C, 16193.2C
16182C, 16183C, 16187T, 16189C
16182C, 16183T, 16188T, 16189C
16182C, 16183C, 16187T, 16189C, 16193d
16183C, 16184A, 16189C
16183C, 16188T, 16189C, 16193.1C
16183C, 16189C
16183C, 16189C, 16190T, 16193.1C
16183C, 16189C, 16190T, 16193.1C, 16193.2C
16183C, 16189C, 16191T, 16193.1C
16183C, 16189C, 16191T
16183C, 16189C, 16193.1C
16183C, 16193.1C
16184A, 16189C
16184A
16185T, 16189C
16185T
16186T, 16189C
16186T
16187T, 16189C
16187T
```

It's possible to edit motif files manually, but the built-in editor is recommended.

Lines starting with "#" define the region of motifs listed below it.

#start-end (inclusive)

Mutations are listed with standard forensic nomenclature:

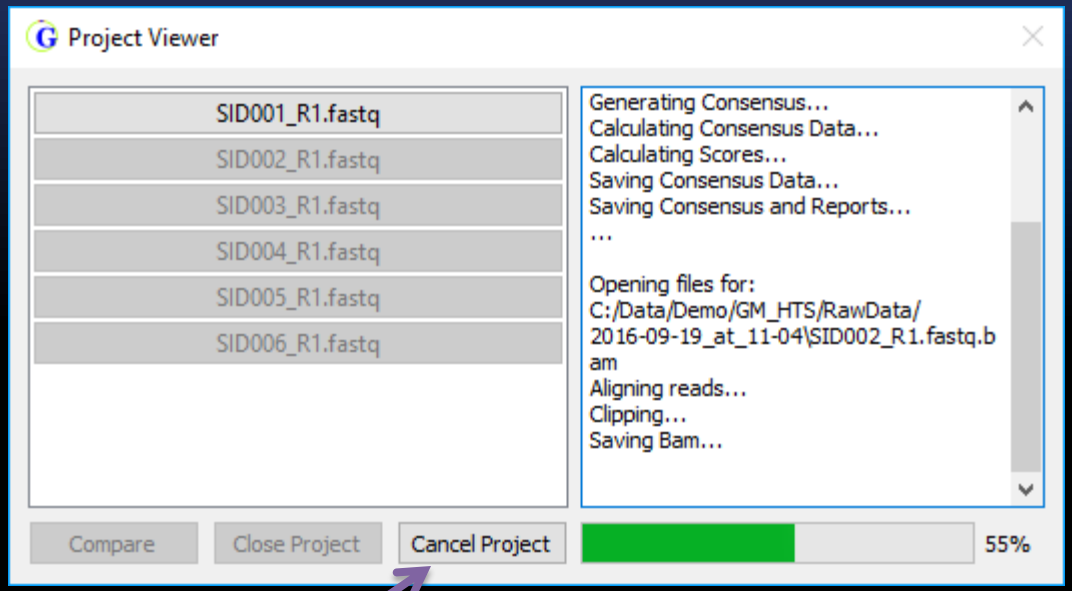
- Deletion: 16191d
- Insertion: 16193.1C
- SNP: 16184A

<u>Important:</u>

- Spacing of text is not important, but commas are.
- Regions cannot overlap

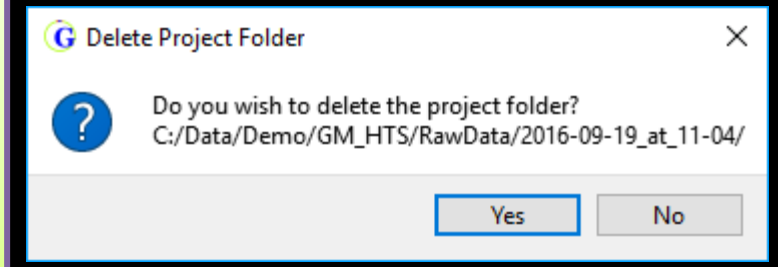
Sample Processing

After the all the desired settings are chosen, selecting *OK* will being alignment.



When a sample is finished it is possible to click on the button in the list of projects to open it- even before all samples finish processing

Projects can be canceled using the *Cancel Project* button. The *Project Viewer* will be closed after the next alignment finishes.

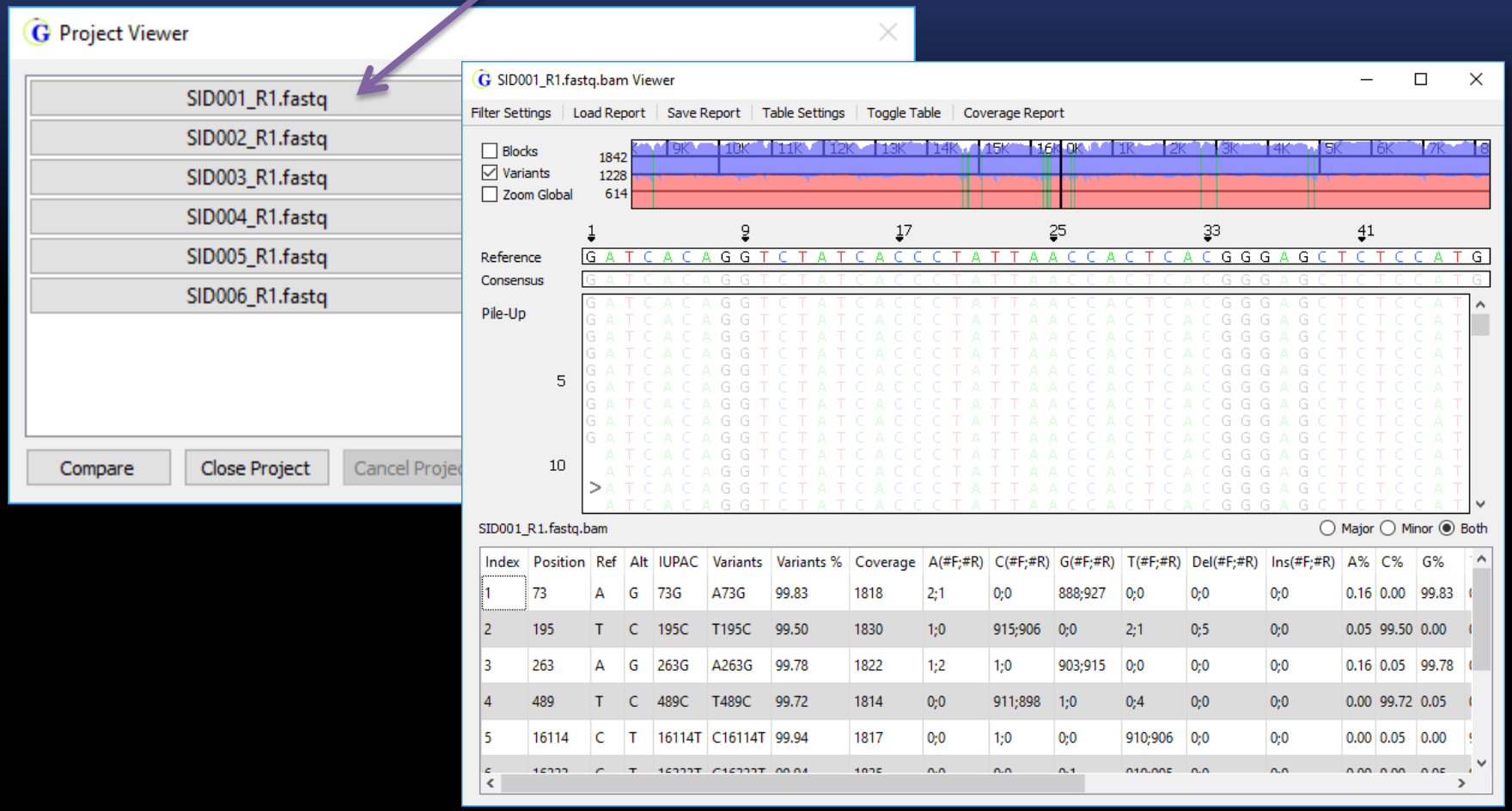


If the project is cancelled, the program will ask the user if they would like to delete the project folder that was created.

Viewing the Results

Viewing the Results

Clicking on a sample name will open it in a *Viewer* window.

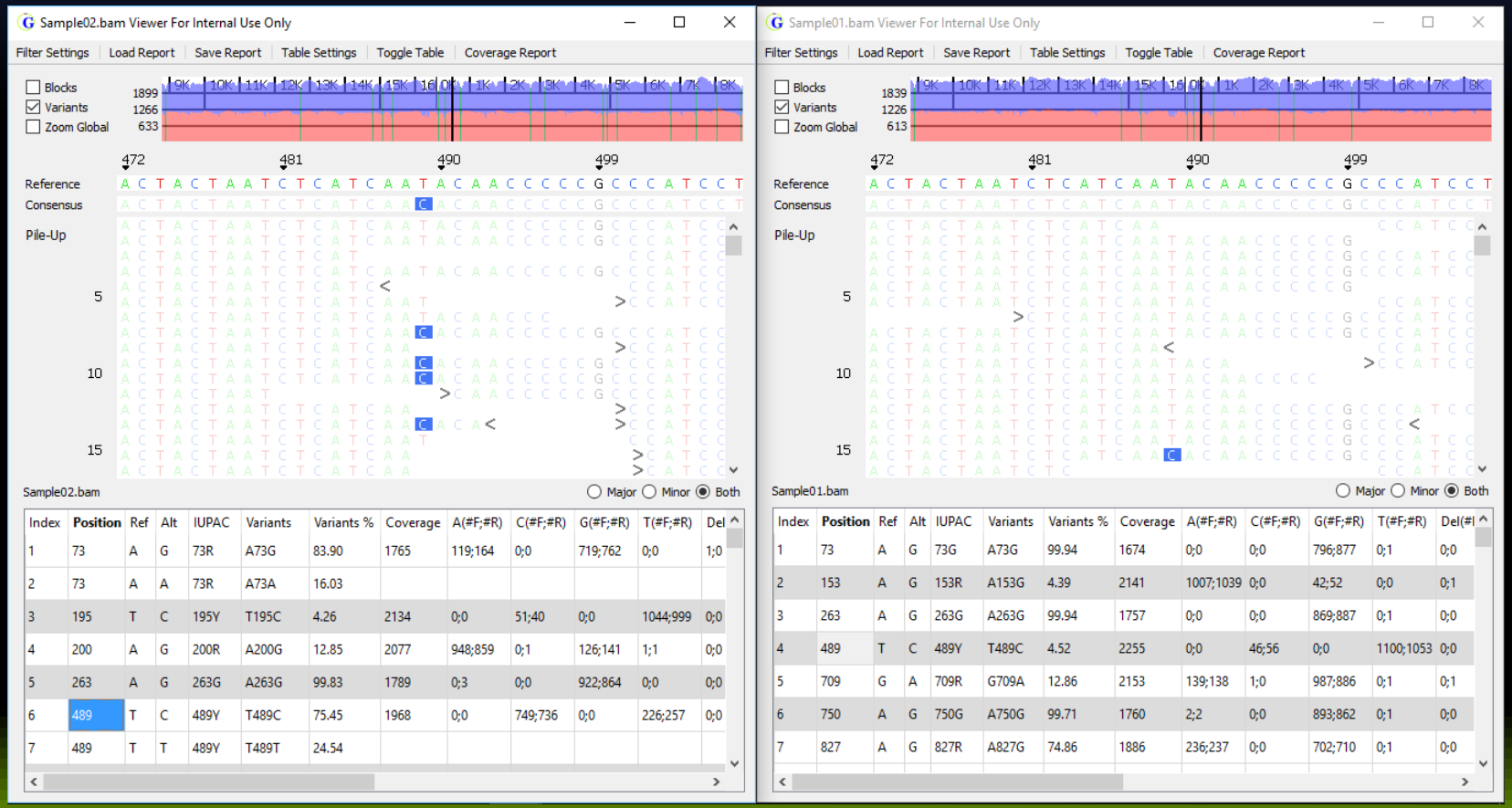


Index	Position	Ref	Alt	IUPAC	Variants	Variants %	Coverage	A(#F;#R)	C(#F;#R)	G(#F;#R)	T(#F;#R)	Del(#F;#R)	Ins(#F;#R)	A%	C%	G%
1	73	A	G	73G	A73G	99.83	1818	2;1	0;0	888;927	0;0	0;0	0;0	0.16	0.00	99.83
2	195	T	C	195C	T195C	99.50	1830	1;0	915;906	0;0	2;1	0;5	0;0	0.05	99.50	0.00
3	263	A	G	263G	A263G	99.78	1822	1;2	1;0	903;915	0;0	0;0	0;0	0.16	0.05	99.78
4	489	T	C	489C	T489C	99.72	1814	0;0	911;898	1;0	0;4	0;0	0;0	0.00	99.72	0.05
5	16114	C	T	16114T	C16114T	99.94	1817	0;0	1;0	0;0	910;906	0;0	0;0	0.00	0.05	0.00

Viewing the Results

Multiple samples from the sample project can be opened at the same time. The *Viewer* windows will zoom and scroll horizontally in unison but they will not scroll vertically in unison. Any changes made to the *Filter Settings* will be applied to all *Viewers* of the same project.

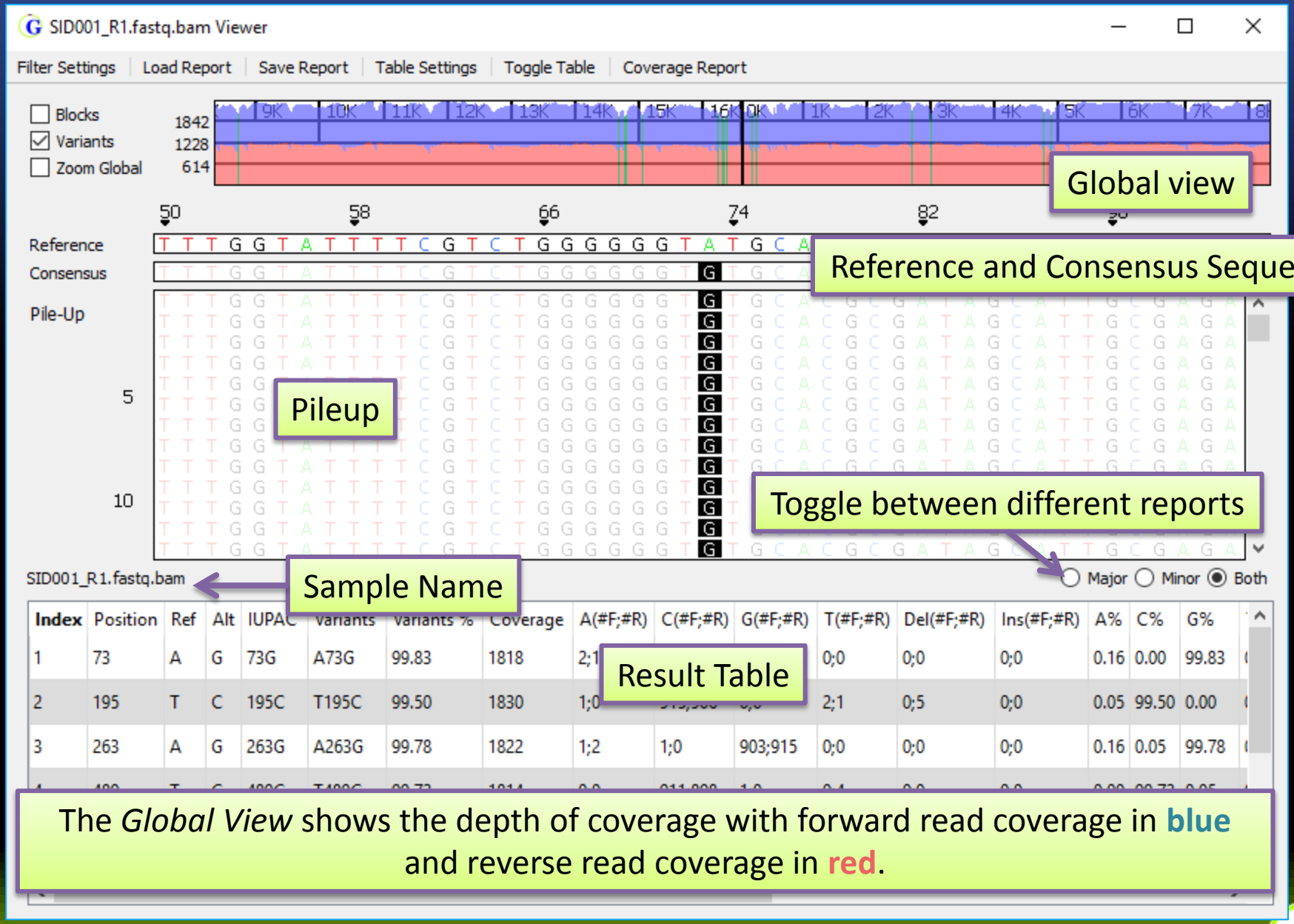
If there are two separate projects open at the same time, the *Viewers* between projects are not linked.



Index	Position	Ref	Alt	IUPAC	Variants	Variants %	Coverage	A(#F;#R)	C(#F;#R)	G(#F;#R)	T(#F;#R)	Del
1	73	A	G	73R	A73G	83.90	1765	119;164	0;0	719;762	0;0	1;0
2	73	A	A	73R	A73A	16.03						
3	195	T	C	195Y	T195C	4.26	2134	0;0	51;40	0;0	1044;999	0;0
4	200	A	G	200R	A200G	12.85	2077	948;859	0;1	126;141	1;1	0;0
5	263	A	G	263G	A263G	99.83	1789	0;3	0;0	922;864	0;0	0;0
6	489	T	C	489Y	T489C	75.45	1968	0;0	749;736	0;0	226;257	0;0
7	489	T	T	489Y	T489T	24.54						

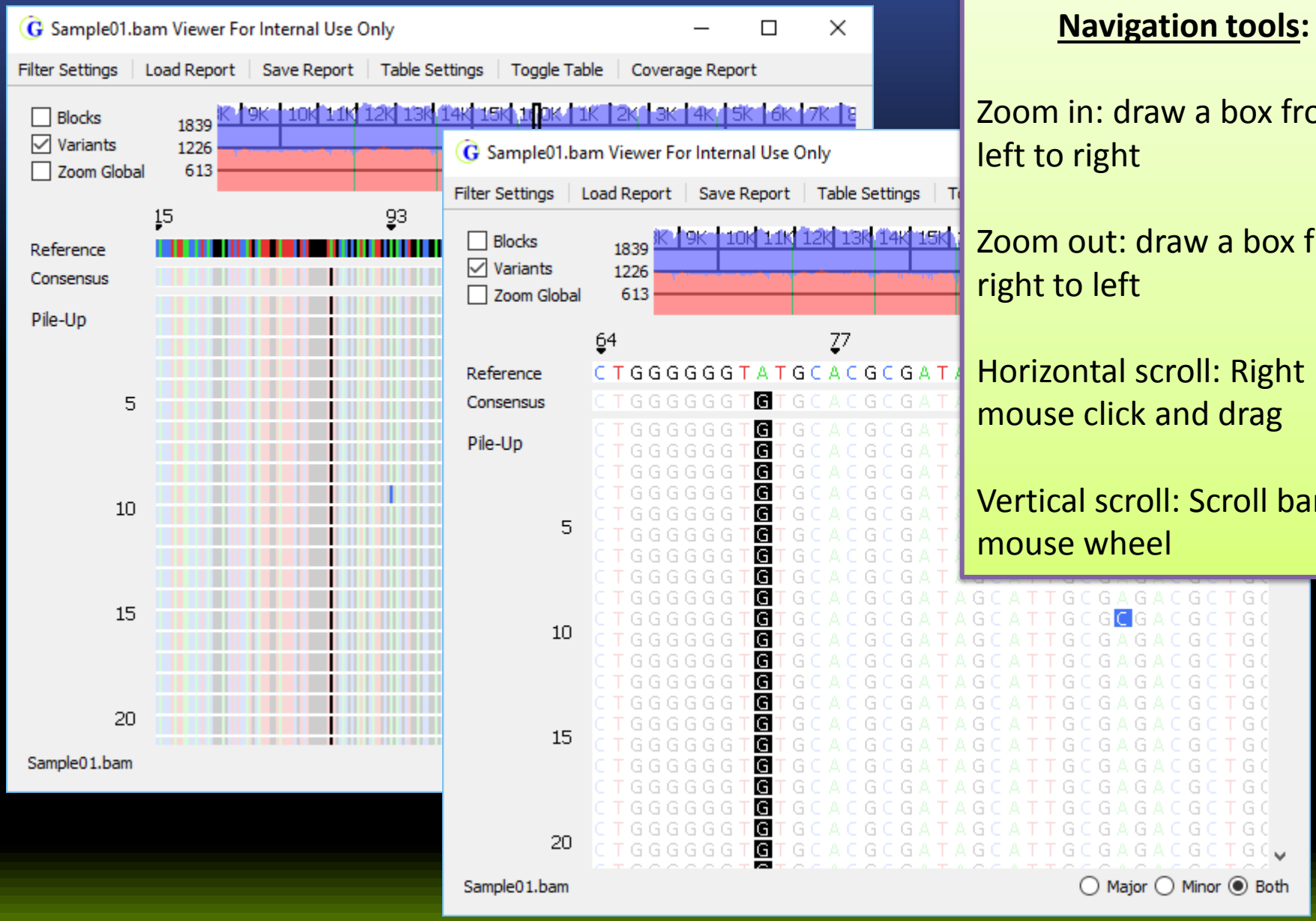
Index	Position	Ref	Alt	IUPAC	Variants	Variants %	Coverage	A(#F;#R)	C(#F;#R)	G(#F;#R)	T(#F;#R)	Del(#I
1	73	A	G	73G	A73G	99.94	1674	0;0	0;0	796;877	0;1	0;0
2	153	A	G	153R	A153G	4.39	2141	1007;1039	0;0	42;52	0;0	0;1
3	263	A	G	263G	A263G	99.94	1757	0;0	0;0	869;887	0;1	0;0
4	489	T	C	489Y	T489C	4.52	2255	0;0	46;56	0;0	1100;1053	0;0
5	709	G	A	709R	G709A	12.86	2153	139;138	1;0	987;886	0;1	0;1
6	750	A	G	750G	A750G	99.71	1760	2;2	0;0	893;862	0;1	0;0
7	827	A	G	827R	A827G	74.86	1886	236;237	0;0	702;710	0;1	0;0

Viewing the Results



The *Global View* shows the depth of coverage with forward read coverage in **blue** and reverse read coverage in **red**.

Viewing the Results



Navigation tools:

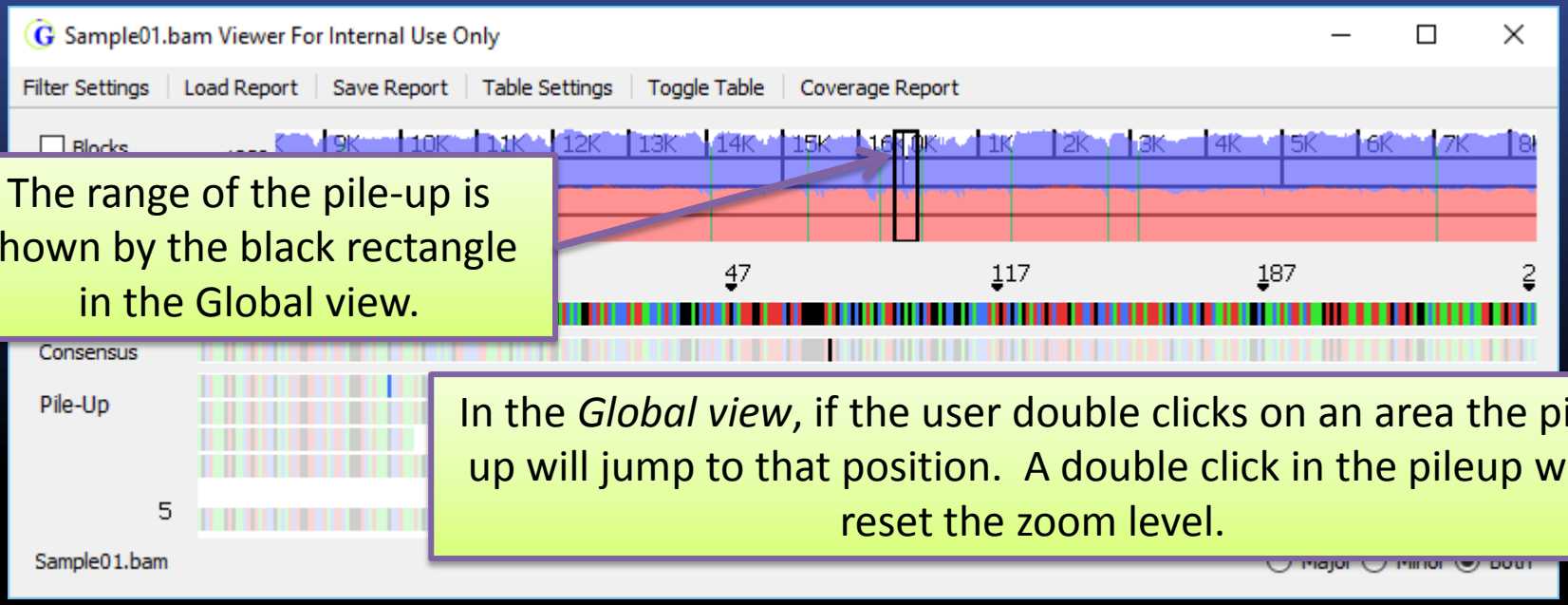
Zoom in: draw a box from left to right

Zoom out: draw a box from right to left

Horizontal scroll: Right mouse click and drag

Vertical scroll: Scroll bar or mouse wheel

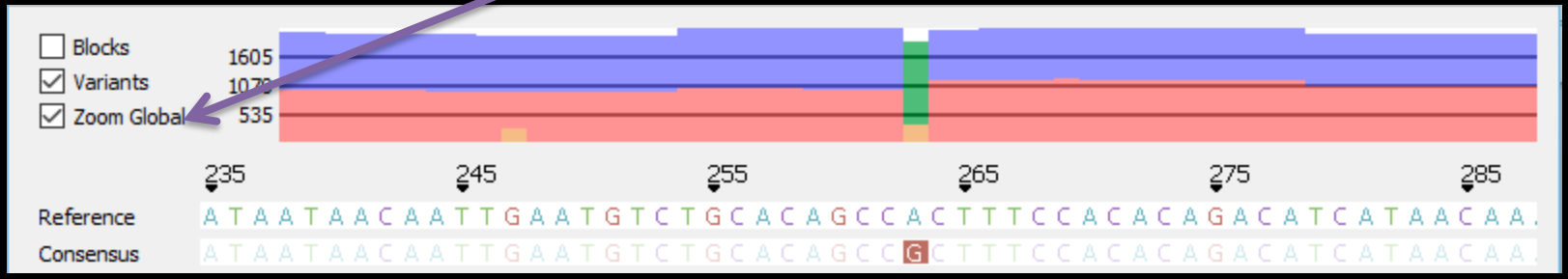
Viewing the Results



The range of the pile-up is shown by the black rectangle in the Global view.

In the *Global view*, if the user double clicks on an area the pile up will jump to that position. A double click in the pileup will reset the zoom level.

Zoom Global will zoom the global view to only the pileup region

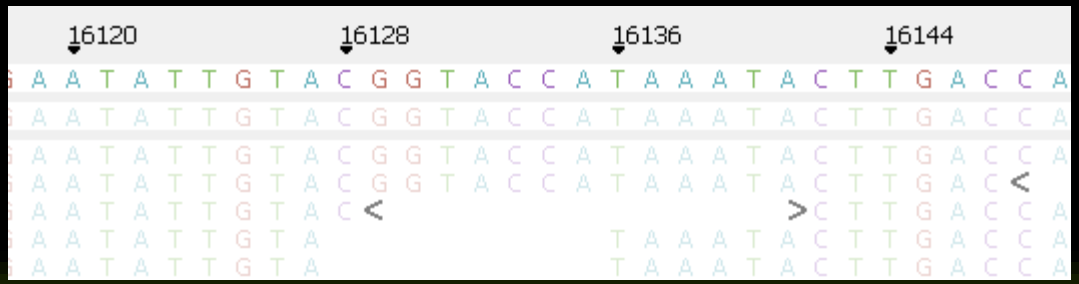


The **teal lines** in the *Global View* represent the location of variants. The **orange lines** represent the percent of reads that didn't match the consensus sequence at that position.

Viewing the Results

Pink shading in the global view alerts the user to areas of coverage below the total coverage value set in the *Table Filter Settings* window.
The consensus sequence is "N" in these regions.

Greater-than and less-than symbols point to the 5' end of the read:
">" for forward reads
"<" for reverse reads

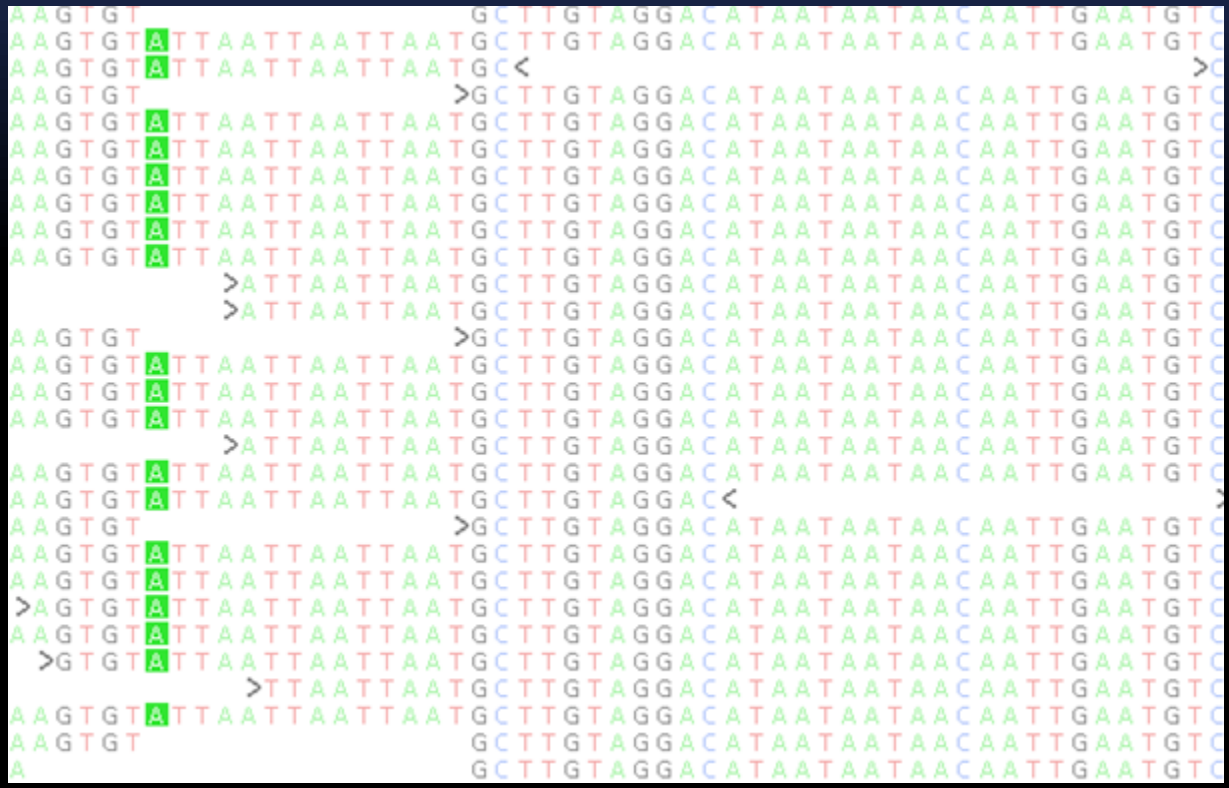


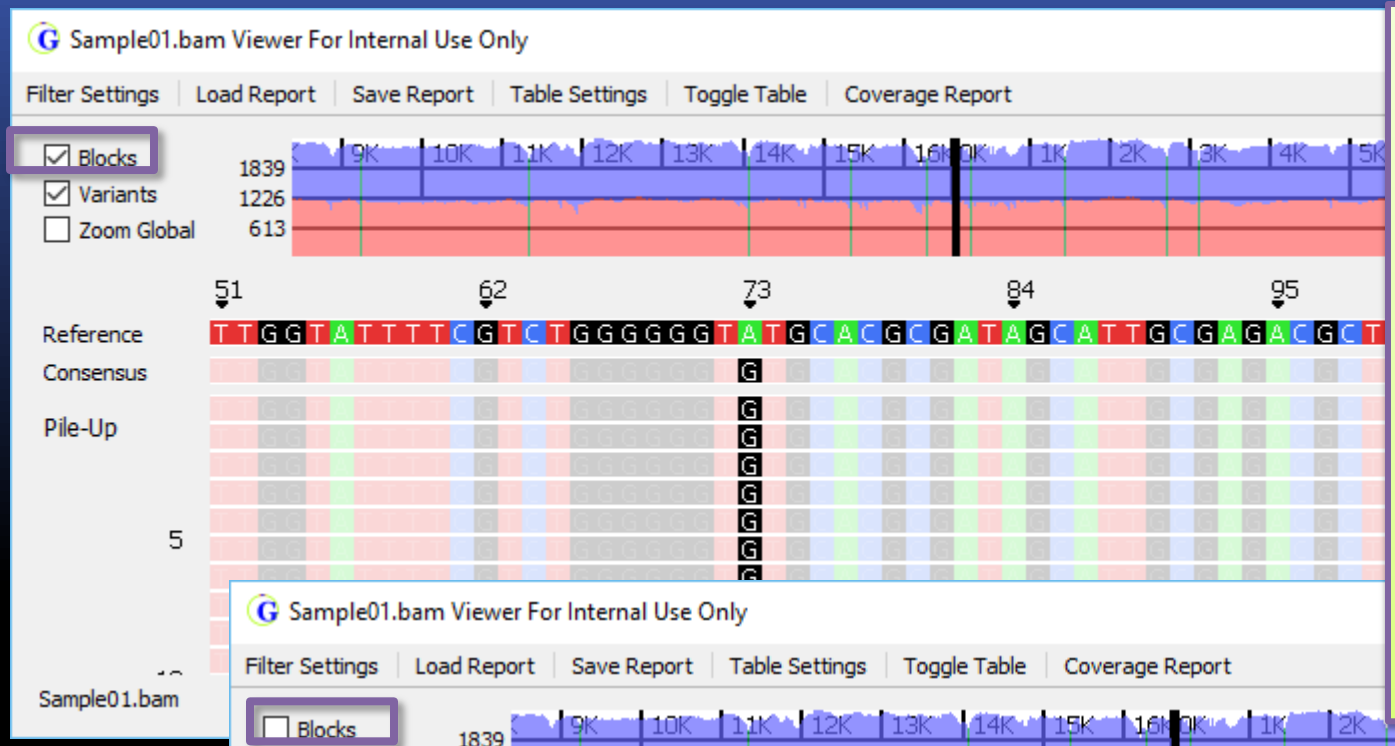
Viewing the Results

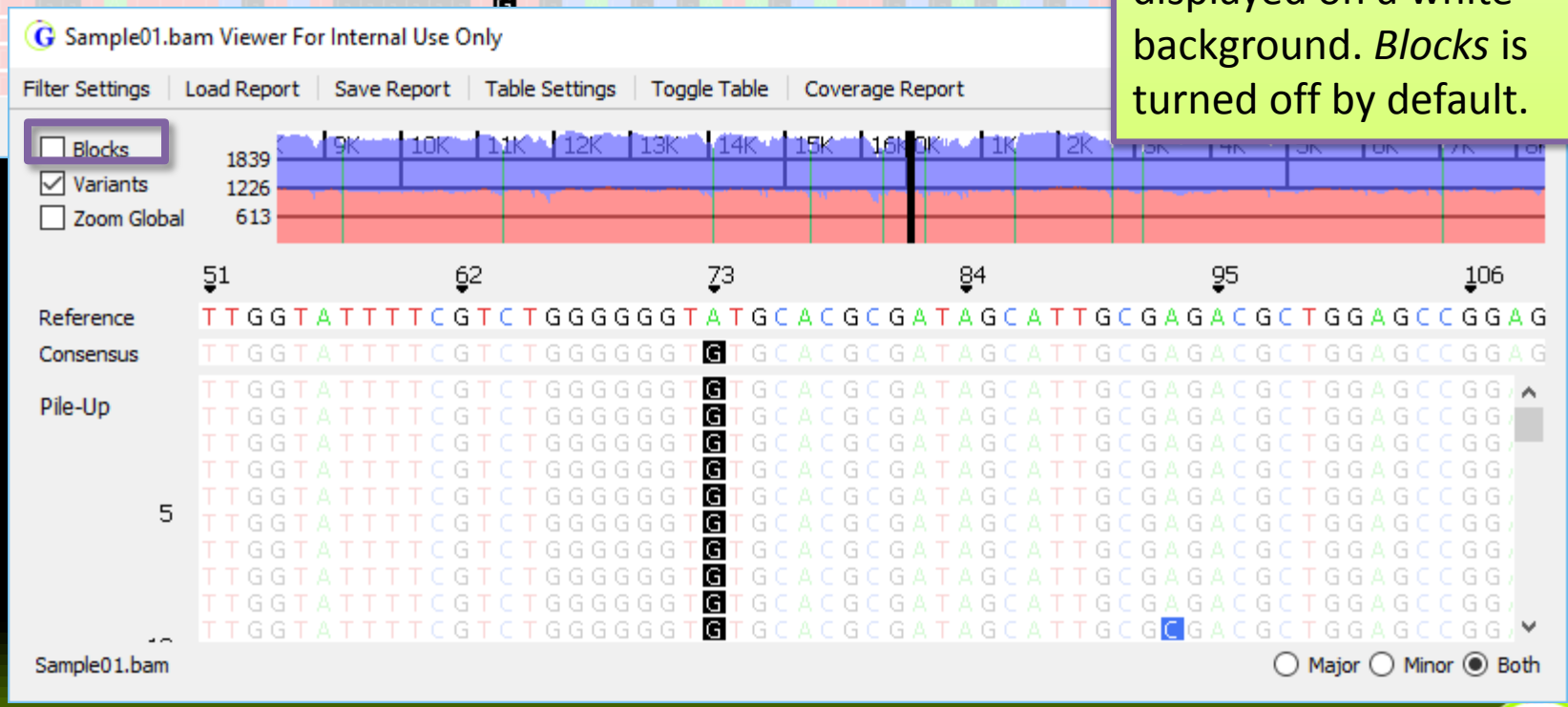
Default Color Key:

White area/space: in between reads

Deletion: Burnt Orange
Insertion: Purple
A: Green
C: Blue
G: Black
T: Red
N: Gray

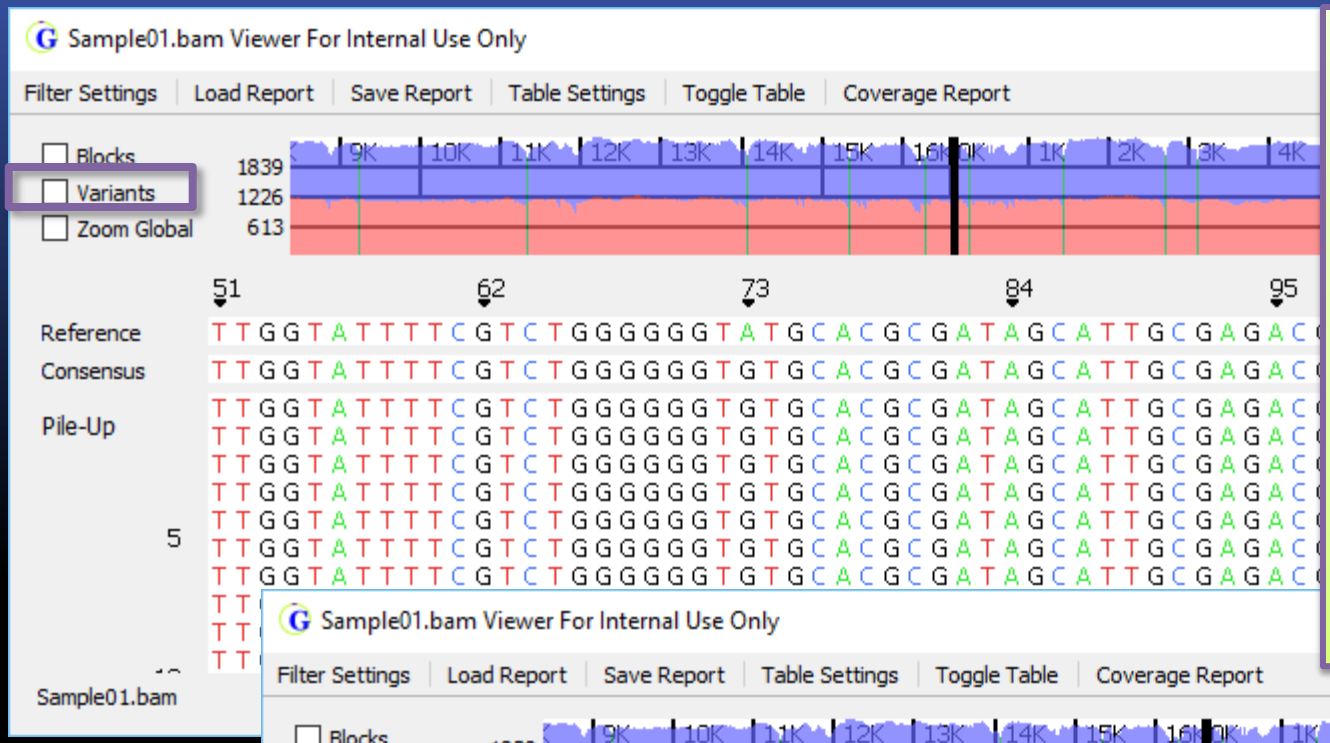


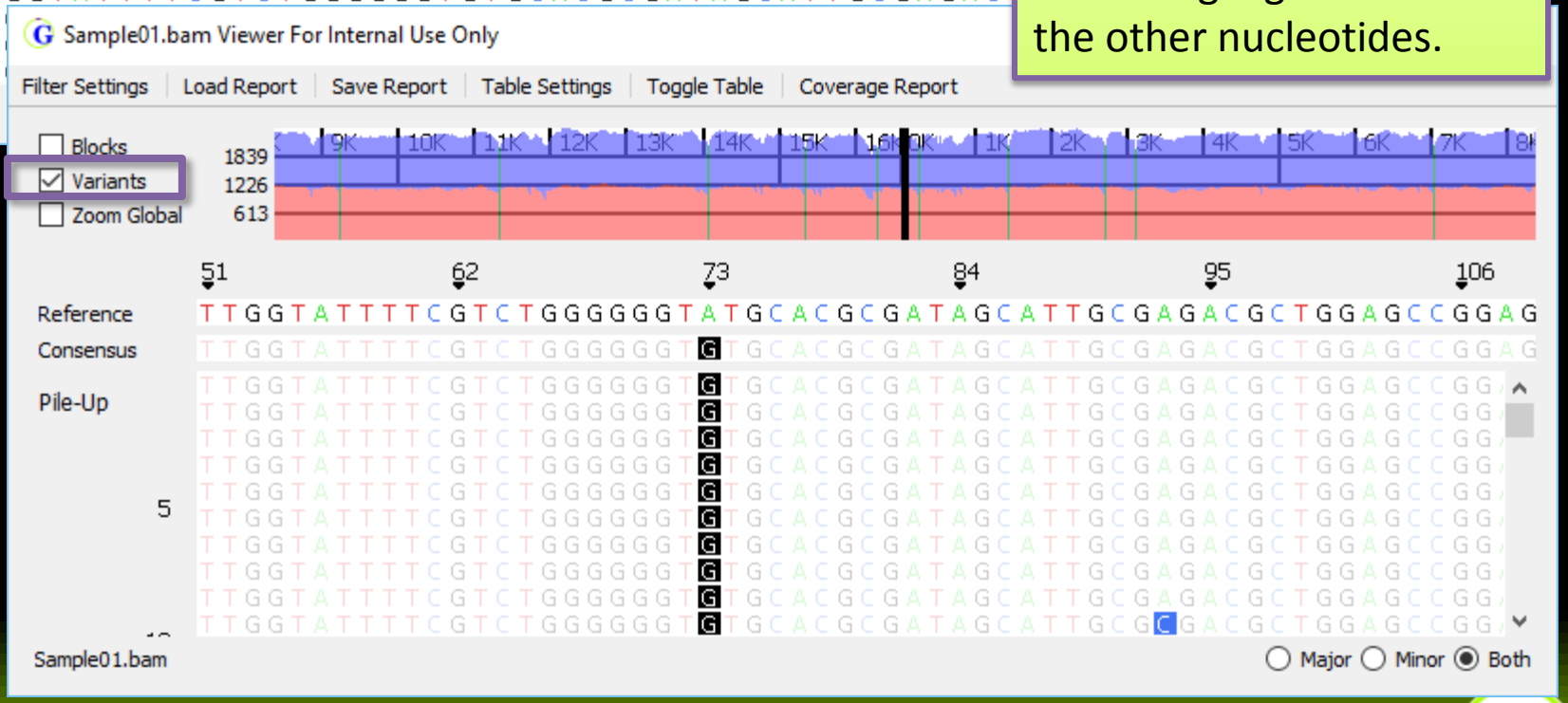




When *Blocks* is selected, the background becomes colored and the font is white.

When *Blocks* is not selected, the text will be colored and displayed on a white background. *Blocks* is turned off by default.



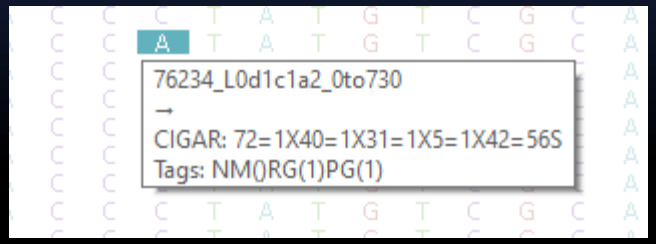


When *Variants* is selected, the variants will be shown in block format and non-variants are dimmed. *Variants* is selected by default.

When *Variants* is not selected, the variants will not be highlighted from the other nucleotides.

Viewing the Results

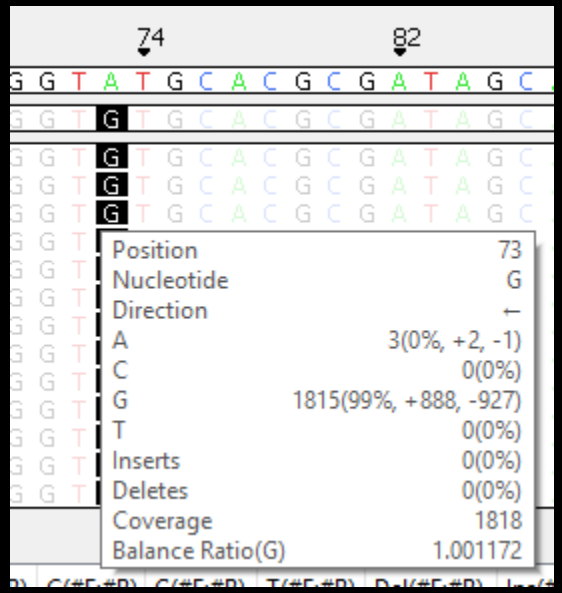
To show information for a specific read:
- Hold SHIFT and center mouse wheel click
- Hold CTRL + SHIFT and left-click with mouse



Keyboard Shortcuts:

- Ctrl + f: The pile-up will jump to center on the next entry in the table.
- Ctrl + b: The pile-up will jump to center on the previous entry in the table.

To show information for a specific variant:
- Center mouse wheel click
- Hold CTRL and left-click with mouse

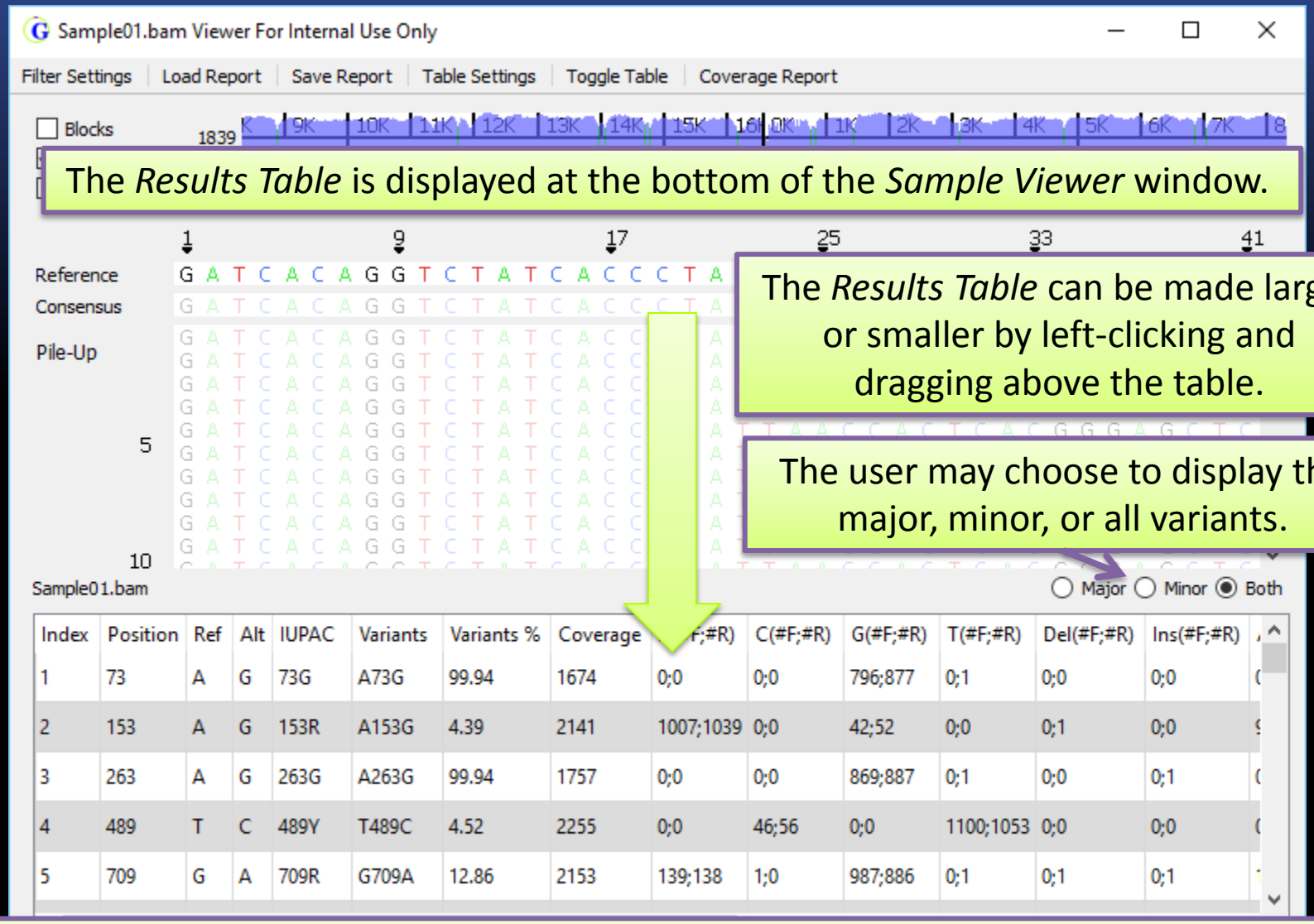


Viewing the Results

The user may right-click at any position in the *Pile-Up* and choose *Go to closest position in table* and the *Results Table* will highlight the closest position.

There is also the option to copy the sequence from any read.

Viewing the Results



Allele Calls

Major alleles are the highest frequency allele at that position
Minor alleles are any other alleles that have frequency > the minimum threshold

AUT0500037(0.8)_AUT0500009(0.15)_AUT0500182(0.05)_100k_250bp.bam — Major / Minor / Both

Index	Position	Ref	Alt	Variants	Variants %	A%	C%	G%	T%	Del%	Ins%
1	73	A	G	A73G	83.71	16.17	0.04	83.71	0.01	0.04	0.00
2	73	A	A	A73A	16.17						
3	94	G	A	G94A	4.70	4.70	0.02	95.24	0.01	0.00	0.00
4	189	A	G	A189G	14.50	85.44	0.01	14.50	0.02	0.00	0.00
5	195	T	C	T195C	79.13	0.00	79.13	0.02	20.81	0.01	0.01

AUT0500037(0.8)_AUT0500009(0.15)_AUT0500182(0.05)_100k_250bp.bam — Major / Minor / Both

Index	Position	Ref	Alt	Variants	Variants %	A%	C%	G%	T%	Del%	Ins%
1	73	A	G	A73G	83.71	16.17	0.04	83.71	0.01	0.04	0.00
2	195	T	C	T195C	79.13	0.00	79.13	0.02	20.81	0.01	0.01
3	263	A	G	A263G	99.87	0.02	0.04	99.87	0.02	0.02	0.00
4	315	C	insC	C315insC	99.80	0.01	99.95	0.01	0.00	0.00	99.80
5	16126	T	C	T16126C	78.44	0.01	78.44	0.01	21.50	0.00	0.01

AUT0500037(0.8)_AUT0500009(0.15)_AUT0500182(0.05)_100k_250bp.bam — Major / Minor / Both

Index	Position	Ref	Alt	Variants	Variants %	A%	C%	G%	T%	Del%	Ins%
1	73	A	A	A73A	16.17	16.17	0.04	83.71	0.01	0.04	0.00
2	94	G	A	G94A	4.70	4.70	0.02	95.24	0.01	0.00	0.00
3	189	A	G	A189G	14.50	85.44	0.01	14.50	0.02	0.00	0.00
4	195	T	T	T195T	20.81	0.00	79.13	0.02	20.81	0.01	0.01
5	309	C	insC	C309insC	4.75	0.00	99.89	0.01	0.02	0.05	4.75

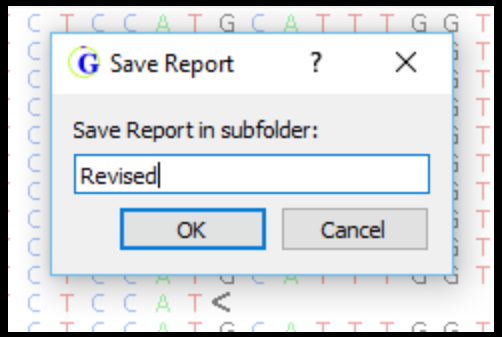
Reference alleles may be reported as minor alleles when their frequency fits the criteria because they may represent a secondary mutation back from the major allele.

Major alleles matching the reference are not reported
Insertions are reported as *Major* alleles if they are above 50% and *Minor* if they are below

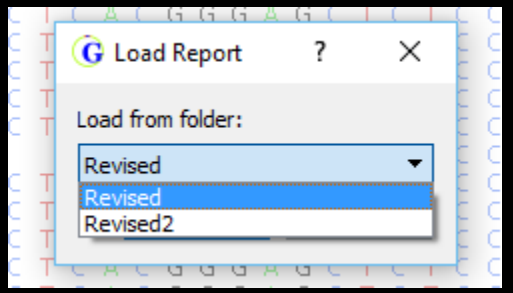
Adding and Removing Variants

Variants may be manually added (right-click in the pileup) or removed (right-click in the table). Added variants are **shaded green** and removed variants are **shaded red.** Either kind of edit can include comments

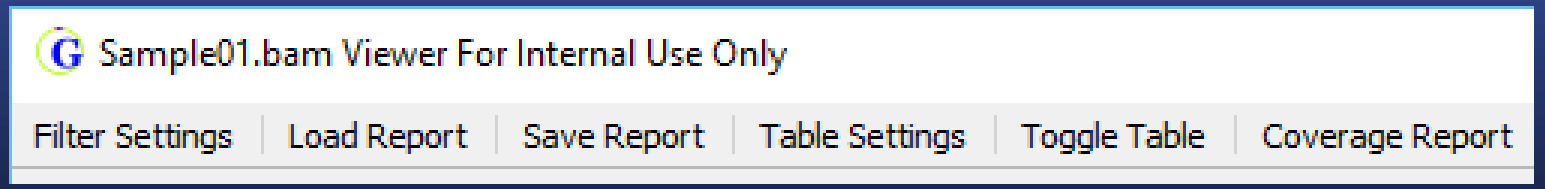
Index	Position	Ref	Alt	Variants	Variants %	Coverage	A%	C%	G%	T%	Del%	Ins%
1	73	A	G	A73G	99.83	1805	0.11	0.00	99.83	0.05	0.00	0.00
2	114	C	A	C114A	12.19	2001	12.19	87.75	0.00	0.04	0.00	0.04
3	143	G	C	G143C	0.04	2240	0.00	0.04	99.95	0.00	0.00	0.00
4	146	T	C	T146C	12.01	2206	0.00	12.01	0.00	87.98	0.00	0.00
5	152	T	C	T152C	16.98	2220	0.00	16.98	0.00	83.01	0.00	0.00
6	195	T	C	T195C	16.75	2172	0.00	16.75	0.00	83.24	0.00	0.00



The report can be saved as a new report after changes to settings or manual edits. The original project is opened by default, but all previously saved reports are maintained in subfolders with changelogs.



Viewing the Results



The ***Filter Settings*** button will open the *Table Filter Settings* window that was available in the *New Project* window.

The ***Load Report*** button will allow the user to change to a different saved report.

The ***Save Report*** button will allow the user to save the report using the current filter settings and edited variants.

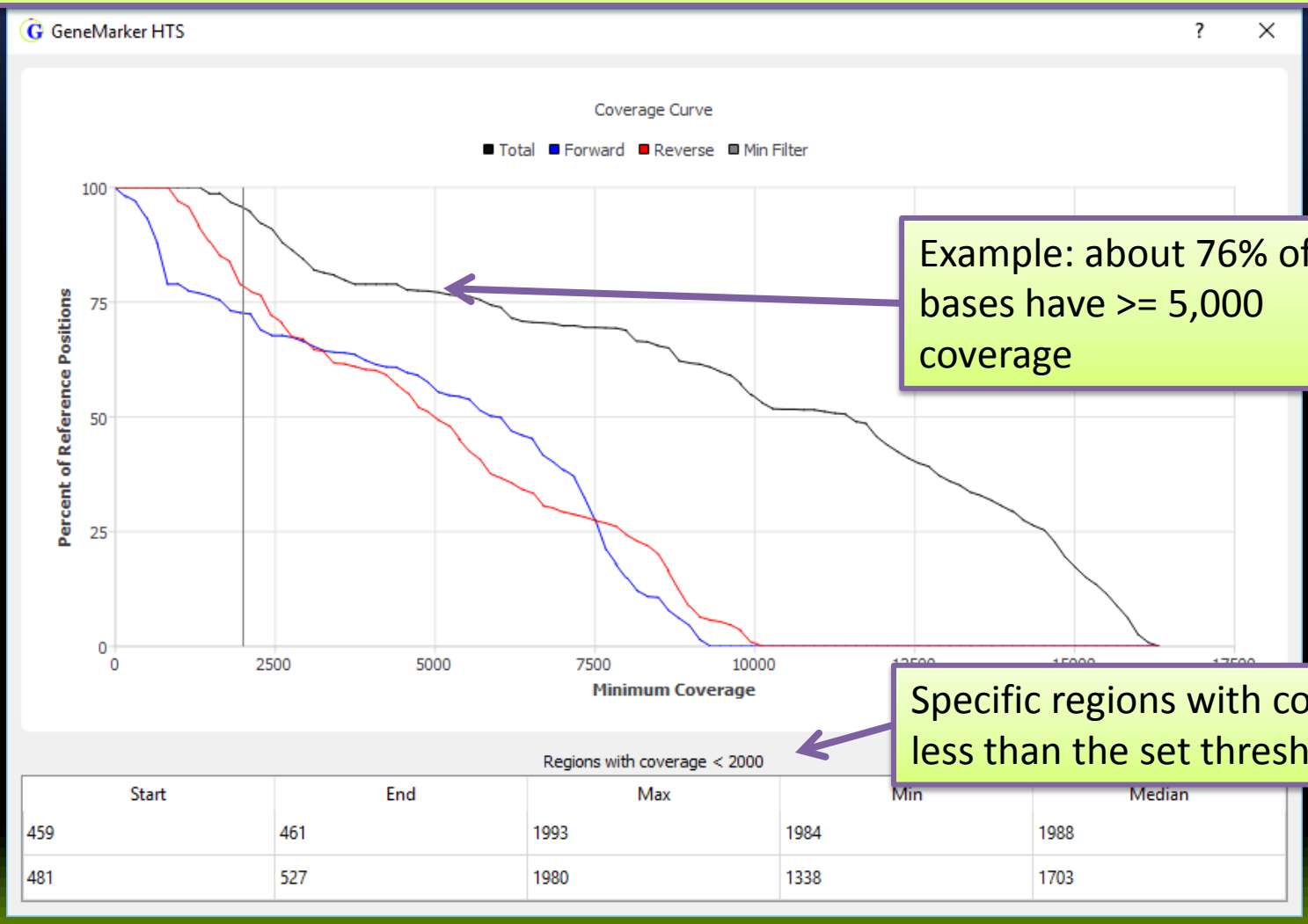
The ***Table Settings*** button will open the *Table Settings* window. Here the user can choose what information is displayed in the table.

The ***Toggle Table*** button will allow the user to display or hide the table below the pile-up.

The ***Coverage Report*** button will open the coverage report.

Coverage Report

The *Coverage Report* gives a quick overview of the coverage depth, specifically the fraction of all bases (entire reference, or range specified in filter settings) that have >= some level of coverage (specified in filter settings)

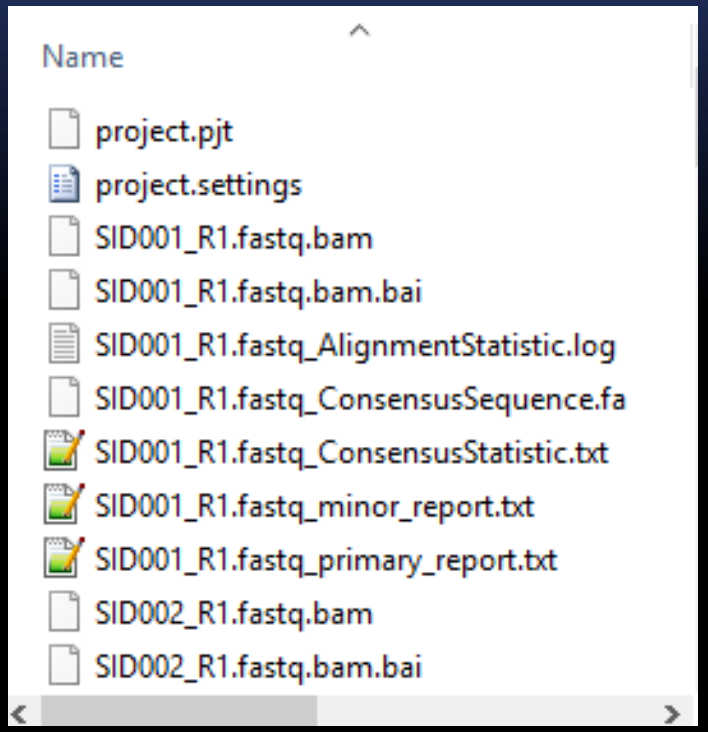


Start	End	Max	Min	Median
459	461	1993	1984	1988
481	527	1980	1338	1703

Output Files

The program will output the following pieces of information for each sample in the project:

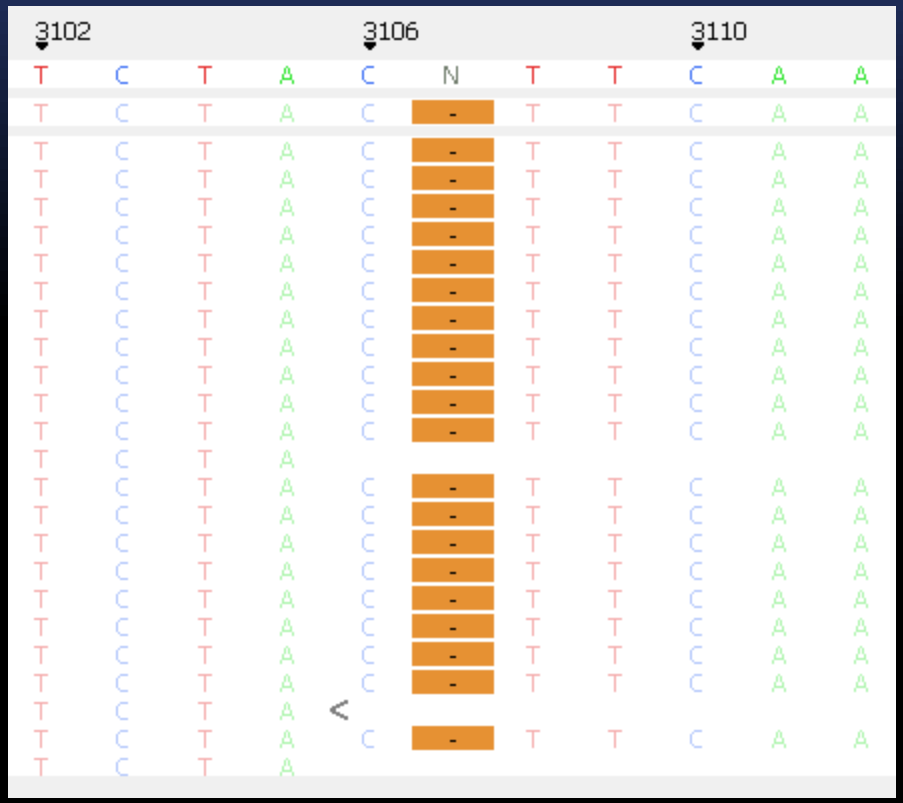
- **BAM/BAI files**: Alignment results
- **Alignment Statistic Log**: Summary of alignment results
- **Consensus Sequence**: Consensus sequence with primary alleles in FASTA format
- **Consensus Statistics**: Report listing the number of copies of each allele at each position in the sample.
- **Primary Allele Report**: Variant report for primary alleles
- **Minor Allele Report**: Variant report for minor alleles



Project file and **Project Settings** file: Used by software to track settings and data

N bases in the Reference Sequence

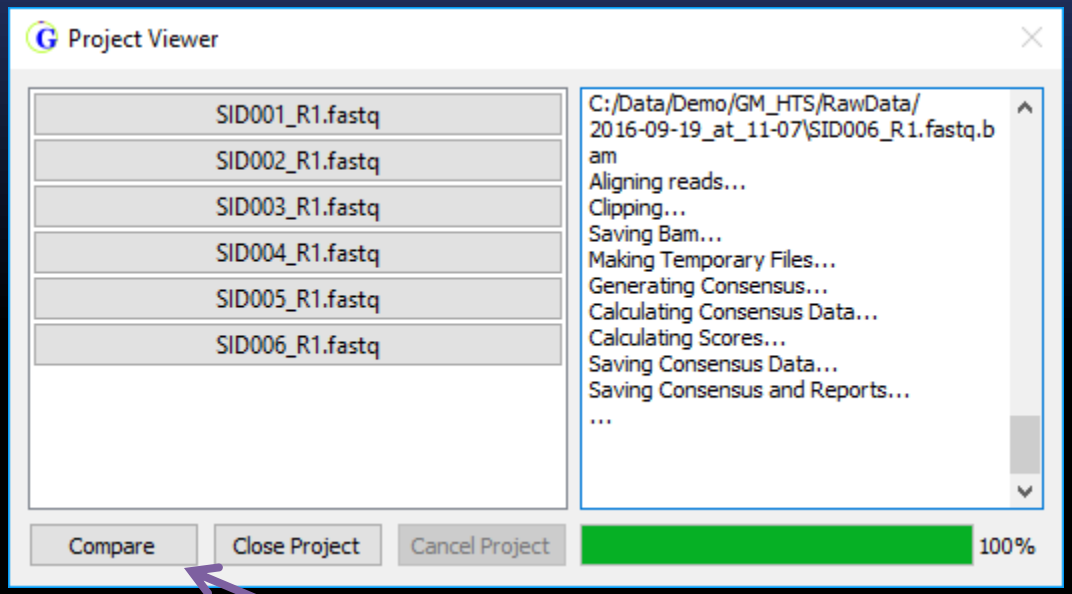
Positions with “N” in the reference sequence will not have variant calls in the report.



Position 3107 in the rCRS has an “N” to maintain position numbering.
Bases aligned at this position are ignored.

Comparison Viewer

Comparison Tool

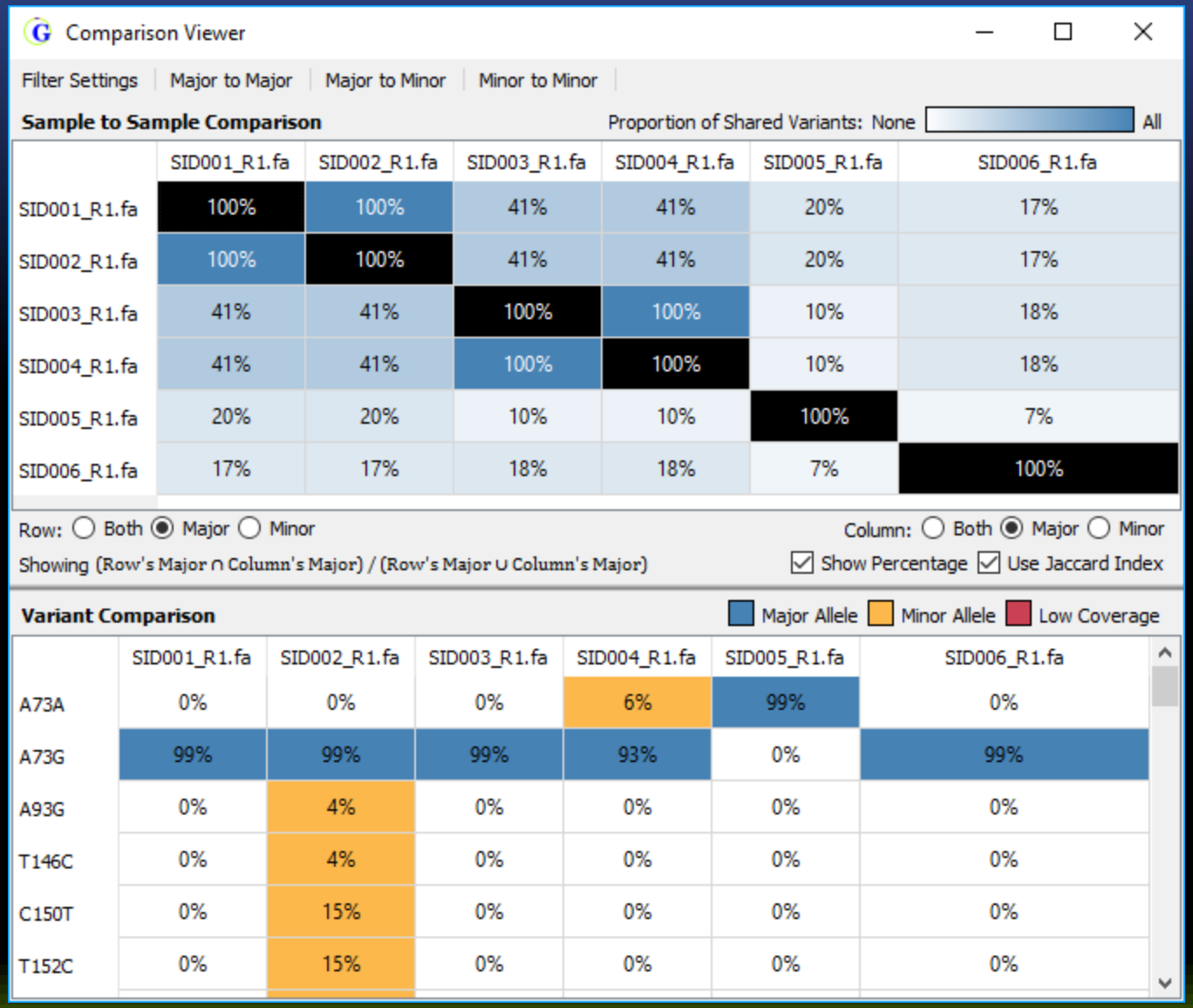


All of the samples within a project can be opened in a *Comparison Viewer* using the “Compare” button

Comparison Tool

Sample to Sample

Variant Comparison

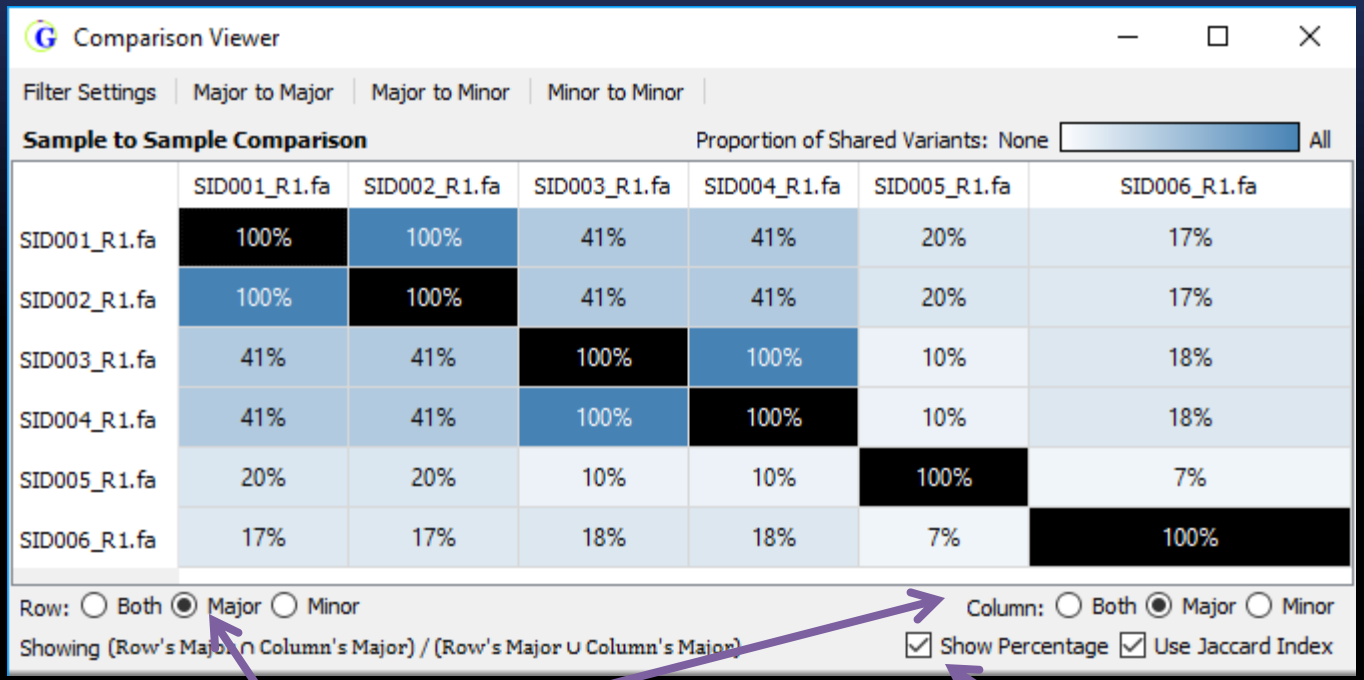


	SID001_R1.fa	SID002_R1.fa	SID003_R1.fa	SID004_R1.fa	SID005_R1.fa	SID006_R1.fa
SID001_R1.fa	100%	100%	41%	41%	20%	17%
SID002_R1.fa	100%	100%	41%	41%	20%	17%
SID003_R1.fa	41%	41%	100%	100%	10%	18%
SID004_R1.fa	41%	41%	100%	100%	10%	18%
SID005_R1.fa	20%	20%	10%	10%	100%	7%
SID006_R1.fa	17%	17%	18%	18%	7%	100%

	SID001_R1.fa	SID002_R1.fa	SID003_R1.fa	SID004_R1.fa	SID005_R1.fa	SID006_R1.fa
A73A	0%	0%	0%	6%	99%	0%
A73G	99%	99%	99%	93%	0%	99%
A93G	0%	4%	0%	0%	0%	0%
T146C	0%	4%	0%	0%	0%	0%
C150T	0%	15%	0%	0%	0%	0%
T152C	0%	15%	0%	0%	0%	0%

Comparison Tool

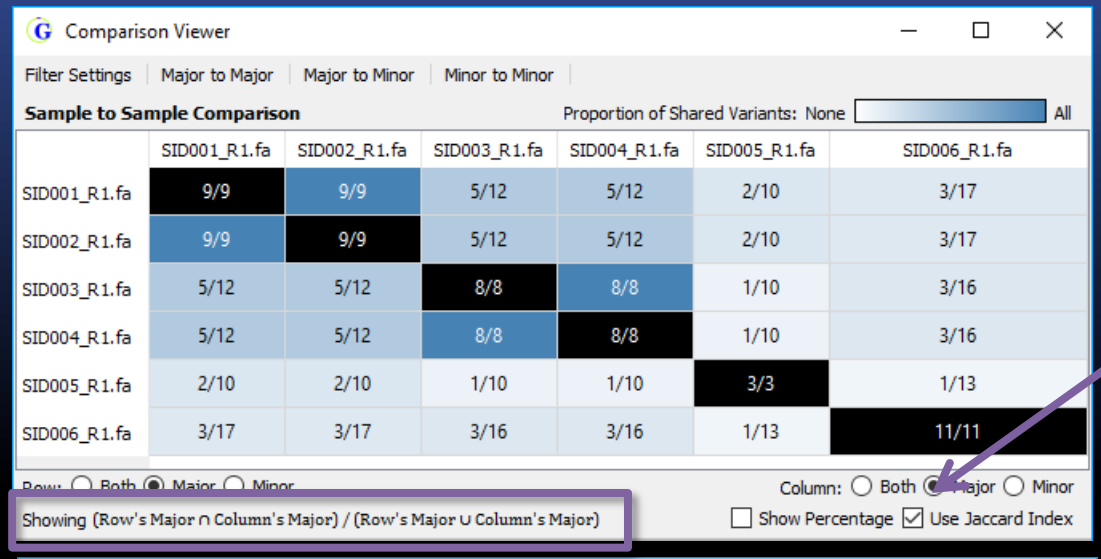
The *Sample to Sample Comparison* table (top half) shows a similarity table for all samples in the project.

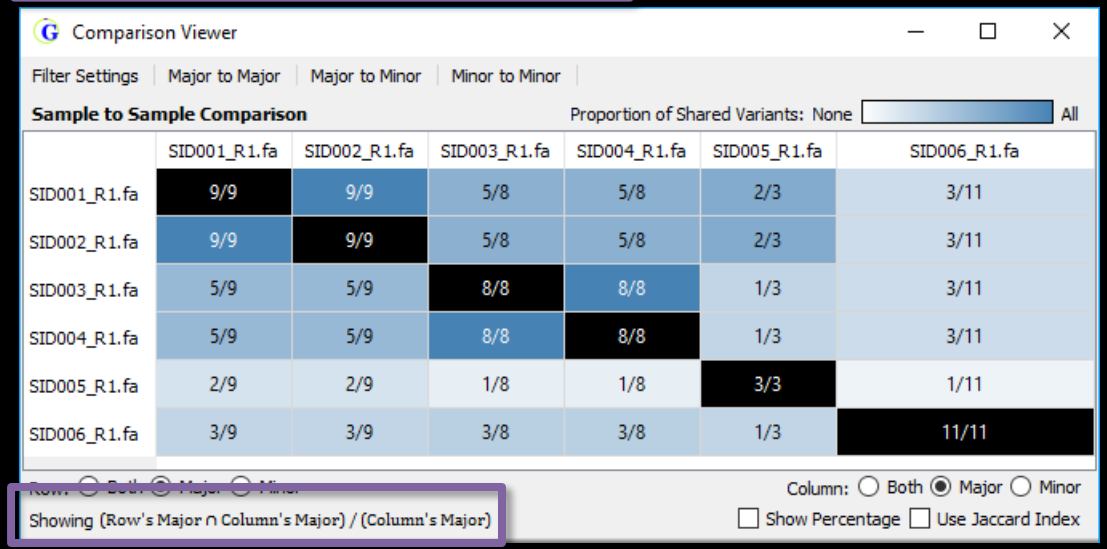


Comparisons can be made between major alleles, minor alleles, or both on each axis (row and column)

It is possible to show the absolute number of variants in common, or the percentage.

Comparison Tool





The formula used to calculate similarity is shown. It is possible to use:

- A Jaccard Index measurement (Row AND Column / Row OR Column)
- Simple measurement (Row AND Column / Column)

Diagonal cells (comparing a sample to itself) are colored black, while others are based on a color gradient from 0 to 100%

Comparison Tool

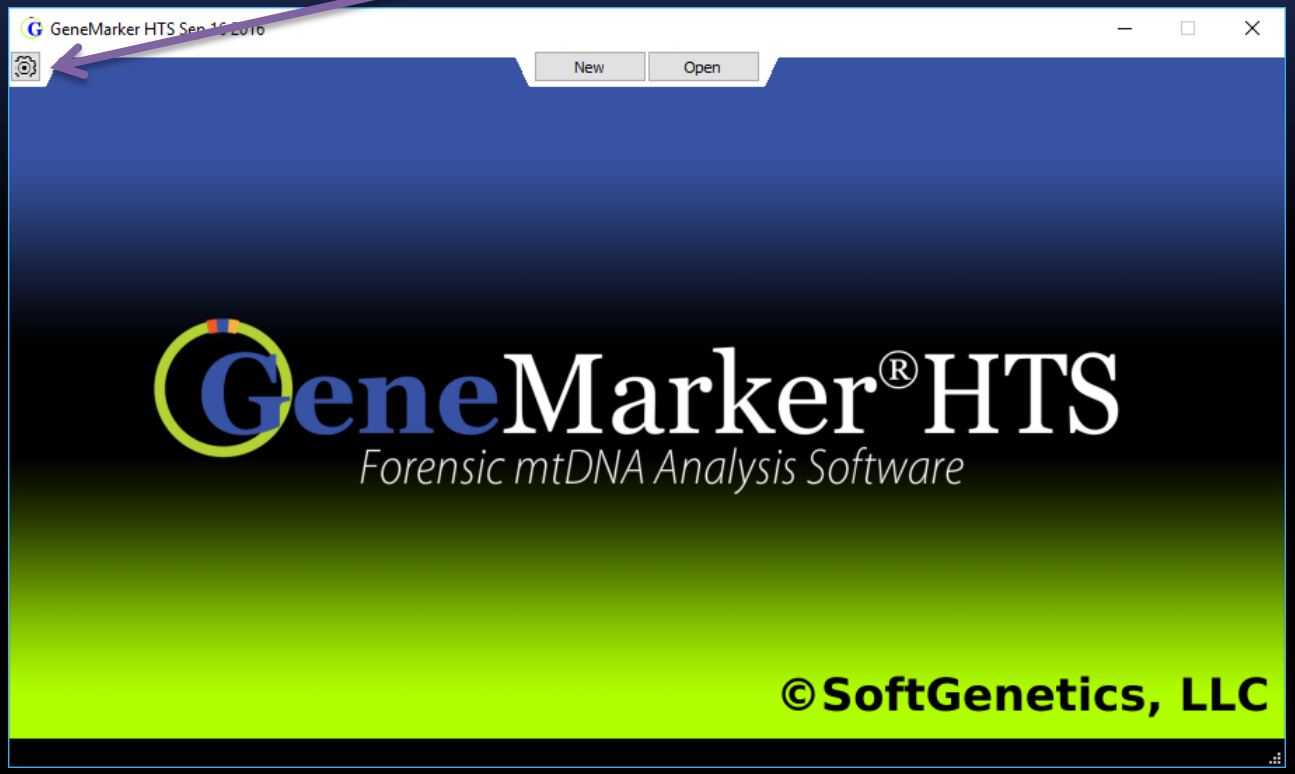
The *Variant Comparison* table (bottom half) simply shows the allele frequency of all variants called in at lease one sample. Cells in the table are colored according to whether or not the variant was a major allele or minor allele in that sample, or if the total coverage was below the set threshold.

Variant Comparison — Major Allele | Minor Allele | Low Coverage

	SID001_R1.fa	SID002_R1.fa	SID003_R1.fa	SID004_R1.fa	SID005_R1.fa	SID006_R1.fa
A73A	0%	0%	0%	6%	99%	0%
A73G	99%	99%	99%	93%	0%	99%
A93G	0%	4%	0%	0%	0%	0%
T146C	0%	4%	0%	0%	0%	0%
C150T	0%	15%	0%	0%	0%	0%
T152C	0%	15%	0%	0%	0%	0%

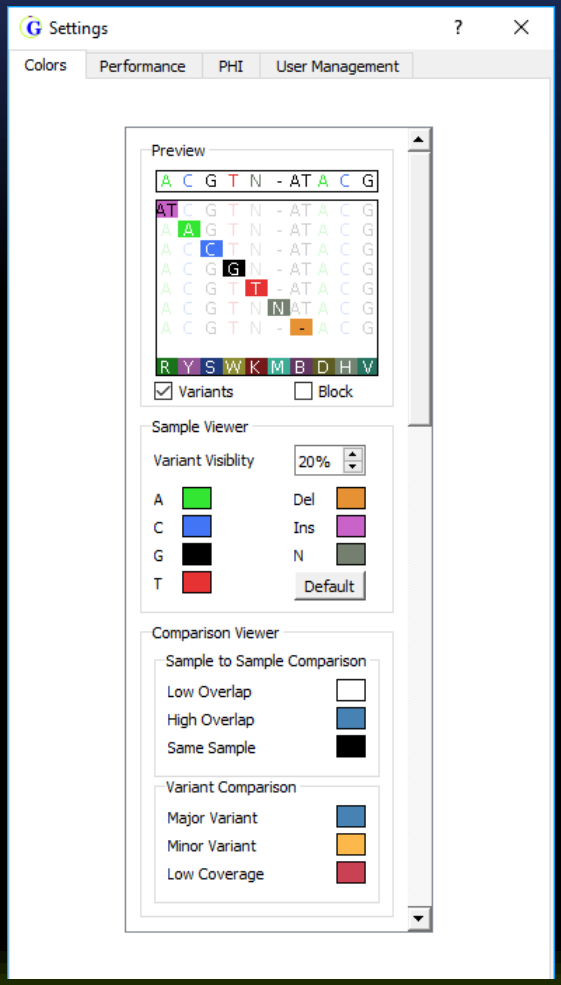
Program Settings

A settings button in the upper-left of the window allows for some customization and control of user management



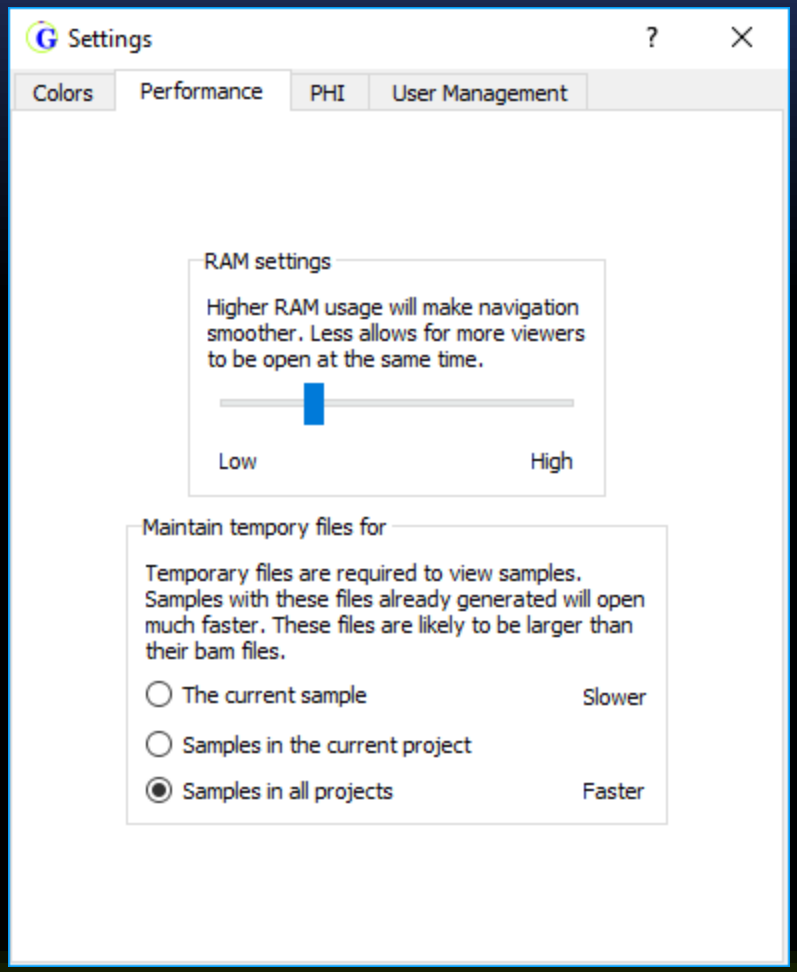
Program Settings - Colors

Colors used for highlighting in the pileup and in the comparison tool can be edited.



Program Settings - Performance

These settings can adjust the RAM and disk space usage of the software to optimize resource usage



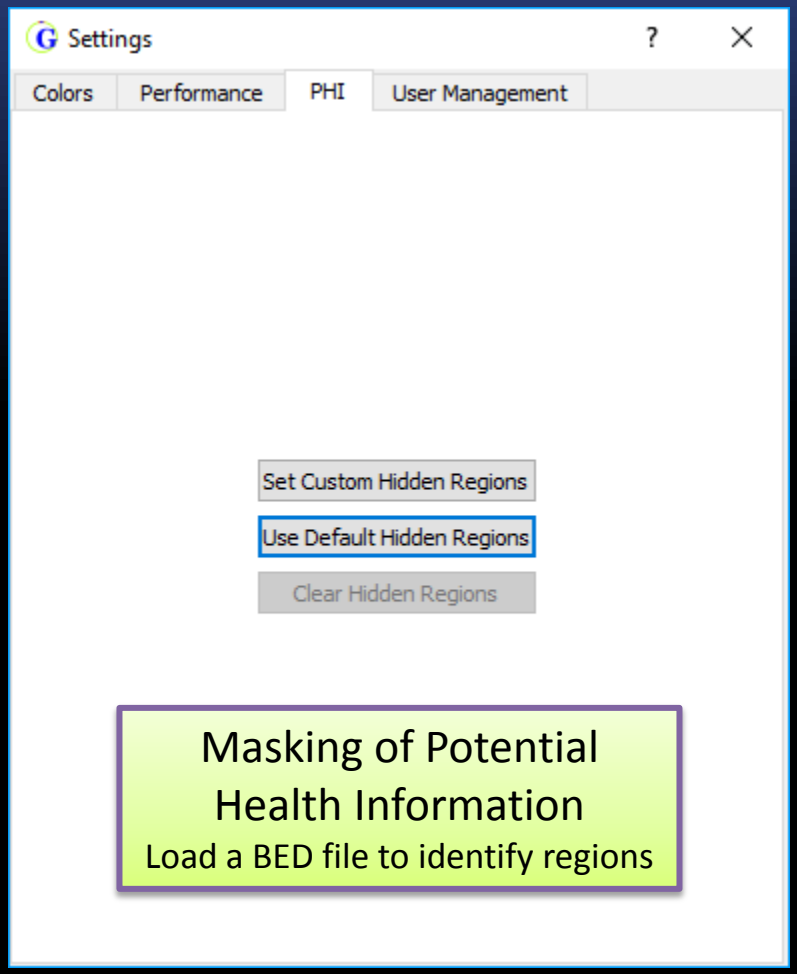
Ram Settings

Setting this value higher will allow the program to use more RAM while viewing projects. This will enhance the speed of moving around in the pileup, but setting it lower will allow more projects to be opened at once.

Temporary Files

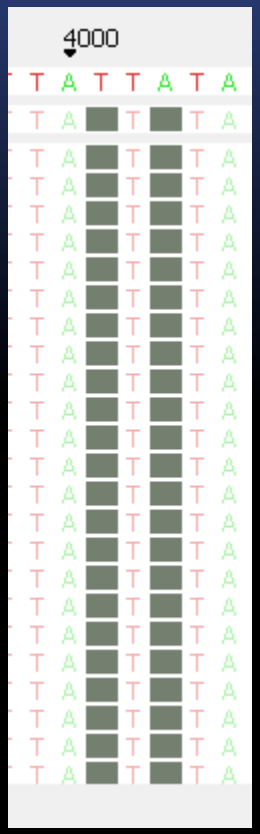
The temp files must be generated before opening a project. The files can be kept in order to save time whenever a sample is opened later on. This setting can allow them to be kept on-disk always, only for the currently opened project, or only for the currently opened sample. These files will take up extra disk space when they are kept.

Program Settings – Personal Health Information



Masking of Potential Health Information

Load a BED file to identify regions

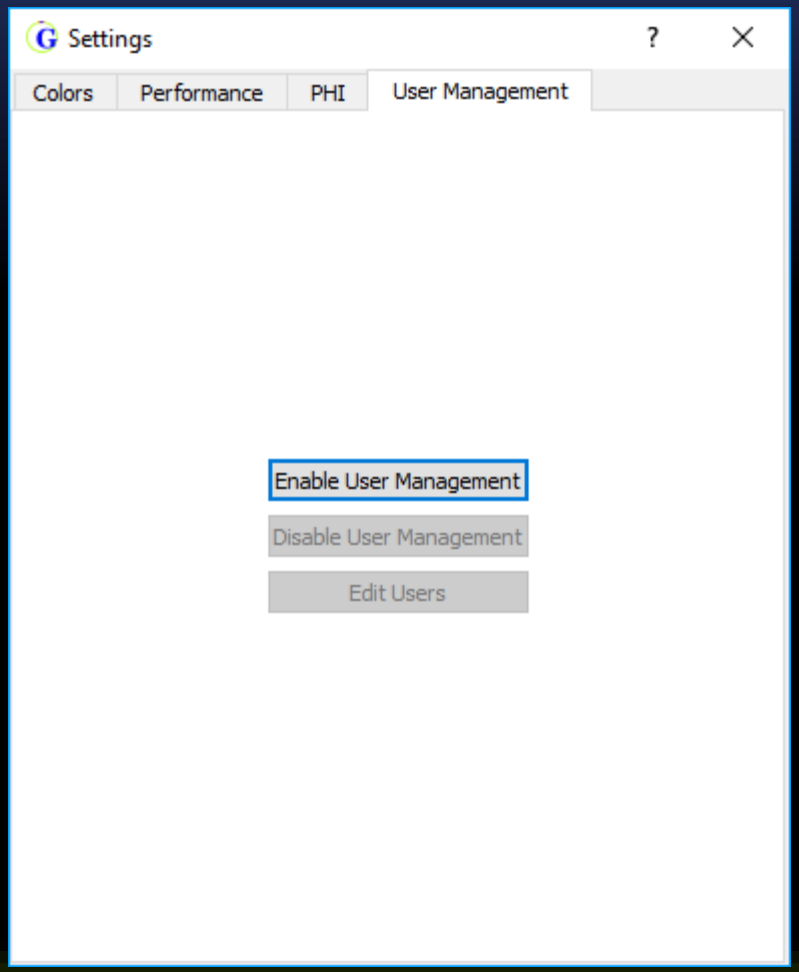


- Bases in the pileup are hidden
- Sequence cannot be copied for reads crossing these regions
- The CIGAR string is hidden from ctrl-shift-click
- Allele counts are hidden from all reports
- No variants are called

Default regions are available as marked in MitoMap

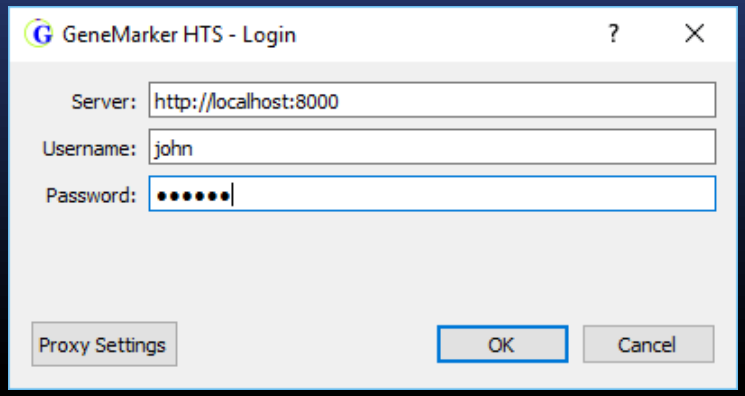
Program Settings – User Management

The software has User Management functionality which can be enabled or disabled by the administrator account of the current computer

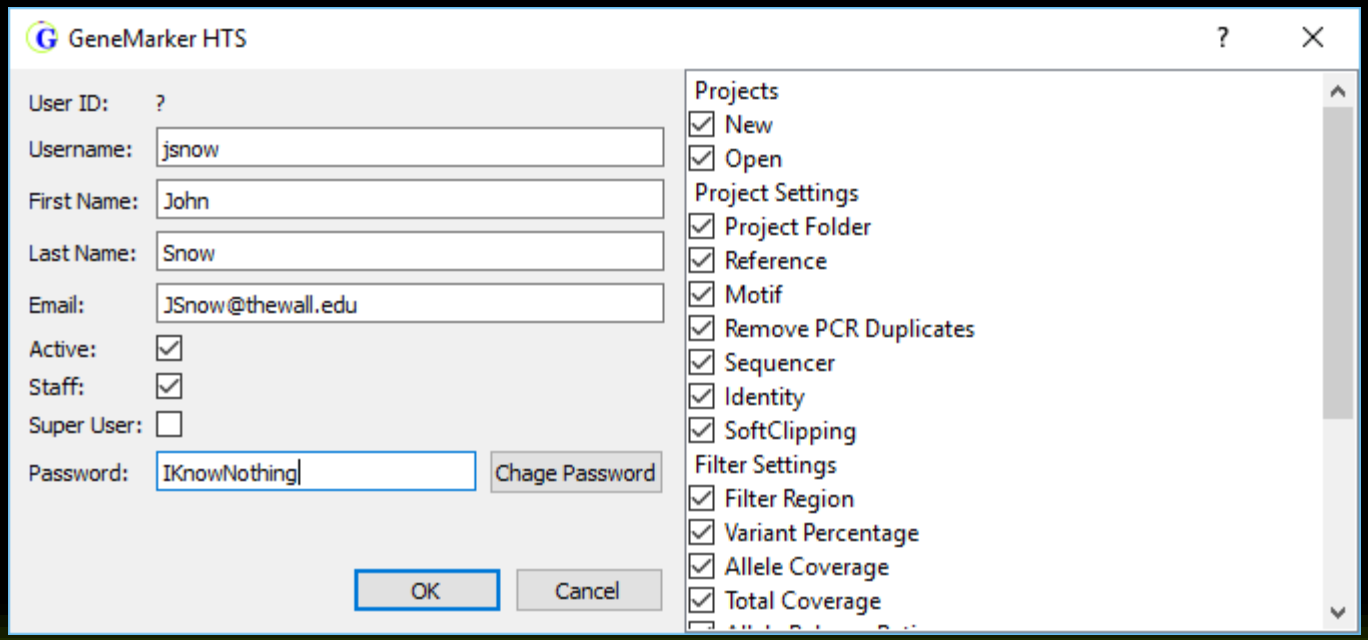


User Management

A windows administrator can enable or disable user management.



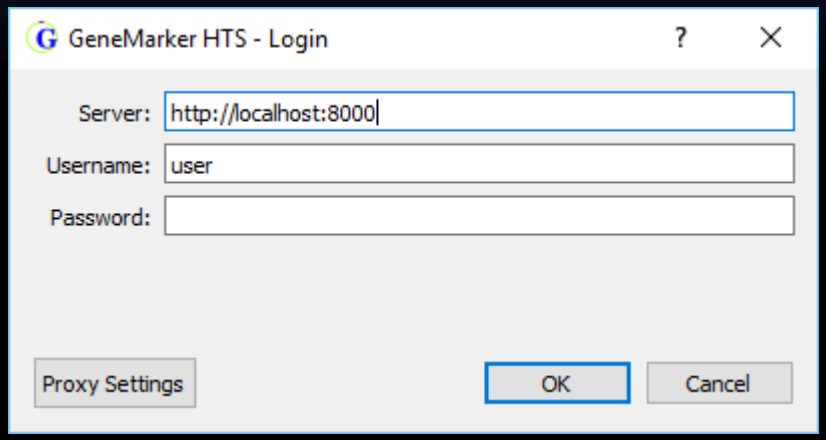
User management is made possible by the SoftGenetics server, which may run on a different computer.



Super users can create new users with limited permissions for almost any feature in the software

User Management

When user management is enabled, the software prompts the user for a login at startup.



Please contact tech_support@softgenetics.com if further assistance is needed.

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