PretextView - Paired REad TEXTure Viewer

Basic Operation

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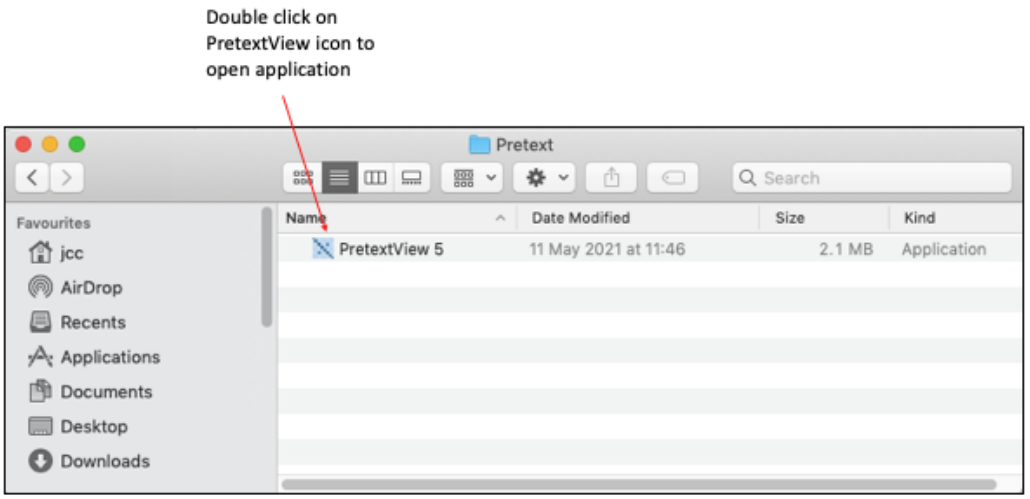
1) Introduction

PretextView is a desktop application for viewing and editing HiC contact maps made using the **PretextMap** application - information for which can be found in the following location https://github.com/wtsi-hpag/PretextMap.

The latest version of **PretextView** can be downloaded from the following location:
https://github.com/wtsi-hpag/PretextView/releases
This tutorial was written for PretextView version 0.2.5

2) Opening up the PretextView interface

In order to open the main application window navigate to where you have downloaded the **PretextView** application and double click on the **PretextView** icon.

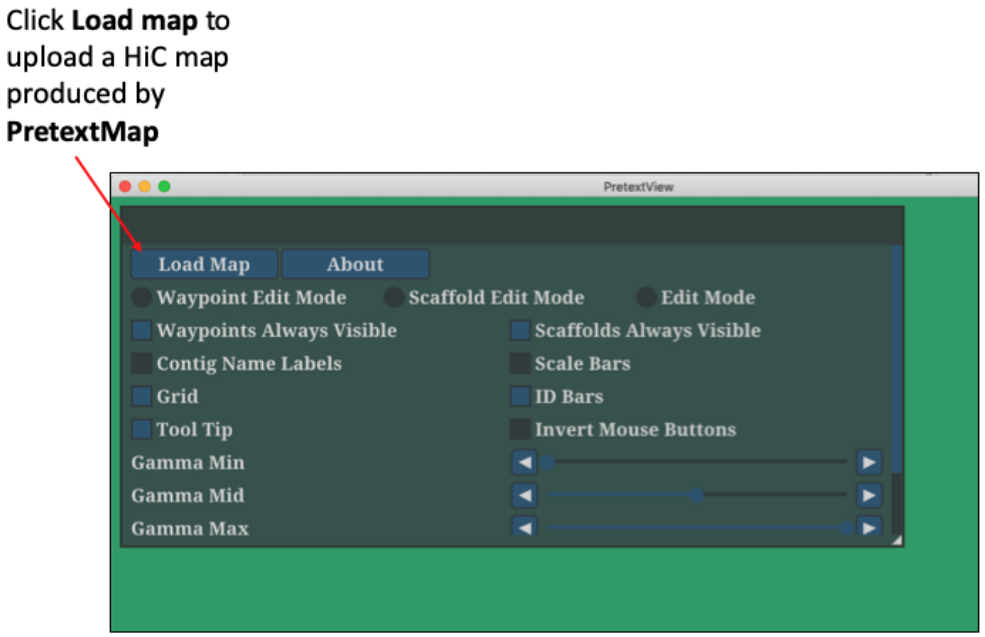


Alternatively the application can be launched via command line in linux:

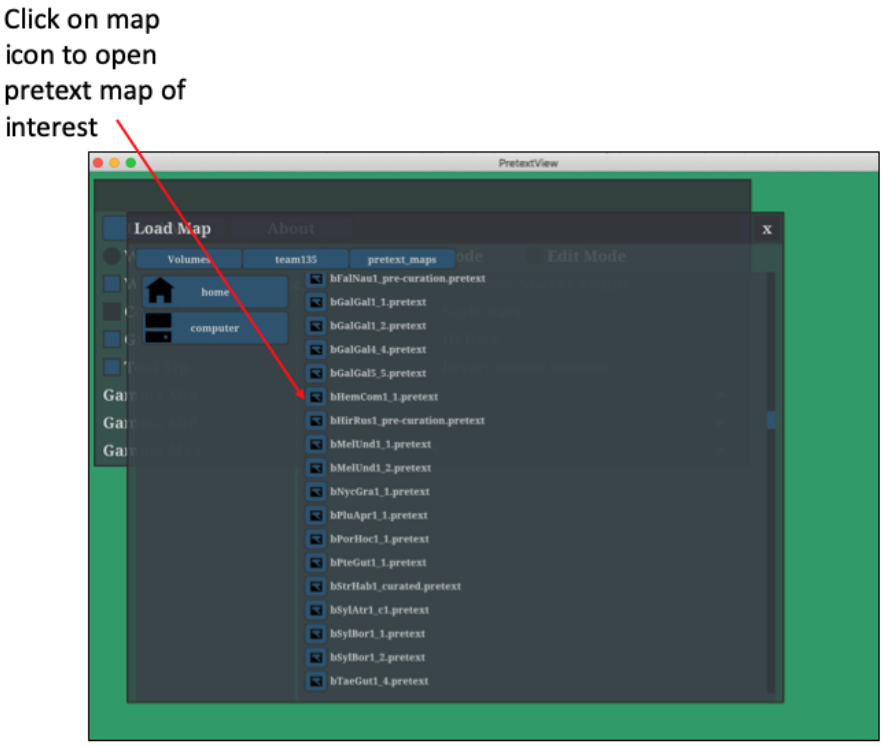
- ➢ Navigate to the **PretextView** application directory
- ➢ Run: ./PretextView

Launching the **PretextView** application opens up two windows, the main **PretextView screen** (green) where the HiC map will be displayed, and the **PretextView** main menu.

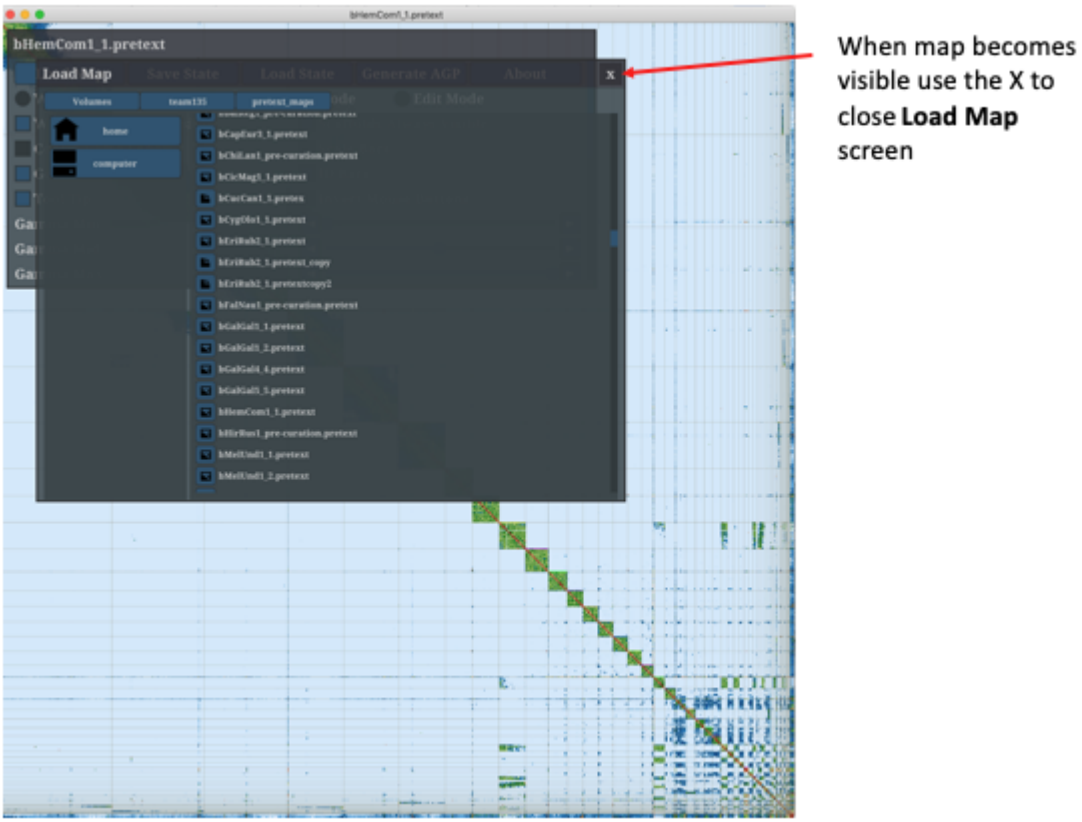
To display a HiC map that has been produced using **PretextMap** click on the **Load Map** button on the menu.



After you click the **Load Map** button another window pops up that allows you to navigate to your pretext HiC contact map. Clicking on the map icon opens the map.



When the map has opened in the viewer the **Load Map** screen can then be closed using the **X.**



3) Customising the PretextView interface

With the map open it is now possible to customise the **PretextView** interface using the options available from the menu. A number of these options will be turned on as default but others are selectable depending on your preference. All of these options can be switched off or on at any point and the map will update accordingly.

Once the map is loaded up the main menu is opened by the middle mouse click anywhere on the map, or by left clicking and then pressing ‘u’ on the keyboard. In order to view the full PretextView menu drag out the window from the bottom right corner.

As a starting point for curation the following set up is recommended:



Highlighted in the red box on the above menu are the “Gamma’ Sliders’. These allow the user to adjust the Gamma signal on the map which can be useful when trying to determine relationships between scaffolds where for whatever reason the HiC signal strength is weak. Typically the **Default Gamma** setting is used.

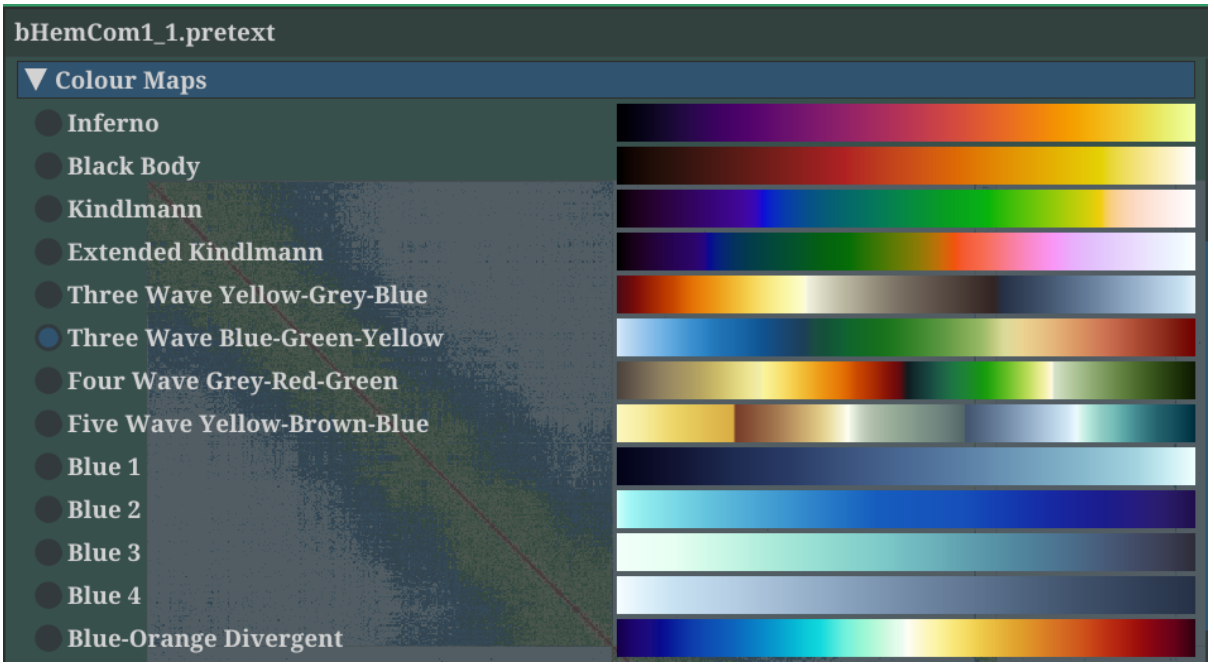
A full explanation of the function of all the selectable options is found in the table below:

Menu Option	Function
Contig Names Labels	Displays the contig names on both axis as taken from the fasta file used to create the Pretext map
Edit Mode	Allows the user to directly edit the map using the mouse functions
Grid	Displays the map with a grid overlaid in order to see contig separation
ID Bars	Displays a uniquely coloured bar across the top of each individual scaffold in order to help track edits
Invert Mouse Buttons	Swaps the order of the mouse buttons used for Edit Mode (This would revert the mouse to normal mouse button assignment)
Scaffolds Always Visible	When used in conjunction with scaffold Edit mode. Allows the user to temporarily remove scaffold painting
Scaffold Edit Mode	Allows scaffold painting to facilitate the ‘Generate AGP’ option
Scale Bars	Displays a scale auto adjusting scale bar across the top of the map
Tool Tip	Displays the mouse position with a menu showing scaffold coordinates
Waypoints Always Visible	Displays waypoints that have been added to the map. These are numbered chronologically.
Waypoint Edit Mode	Allows users to add waypoints directly on to the map.
MetaData Tags	This editor allows the user to define the Meta data tags they require. Six tags are set as default when the application is installed. These are Haplotigs, Unloc(alised) and Sex Chromosome assignments.
MetaData Tag Mode	Allows users to add selected Meta Data tags directly onto the map.
MetaData Tags always visible	Displays Meta Data tags that have been added to the map
Sort by MetaData tags	Allows users to group scaffolds by Meta Data tag type. All groups are then relocated to the right hand corner of the map.

The dropdown menus on the lower part of the menu provide further options for map customization.

➢ Colour Maps

Clicking on the arrow reveals a dropdown menu with a number of different colour options for visualising the mapped data.



The preferred colour choices for curation are typically:

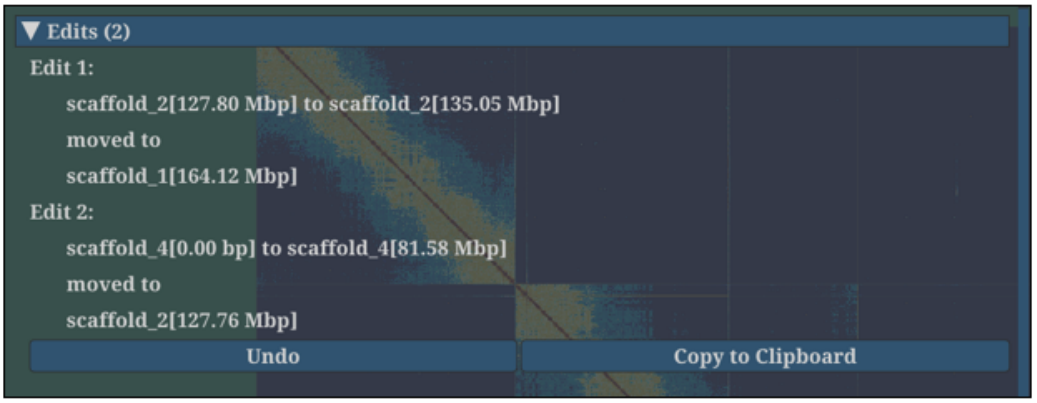
- **Three Wave Blue-Green_Yellow**
- **Blue-Orange Divergent**
- **Orange 1**

Other colour schemes may be used but the 3 options listed above have been found to be most useful for HiC contact interpretation.

Note: With the map open using **‘cmd up/down’ (mac) ‘ctrl up/down’ (windows)** it is possible to cycle through the different colour schemes without going into the main menu. This is very useful as you can scroll through the colours easily when editing the map which can help in interpretation of ambiguous HiC signal.

➢ Edits

The **Edits** menu bar lists the number of edits that have been in the assembly. Clicking on the **Edits** arrow reveals a dropdown menu that provides information about the edits that have been made in the map. From here this list can also be copied to clipboard if required:



Using the '**Undo**' button it is possible to revert any edits that have been made in the map. These are undone in reverse order, there is no option to choose specific edits. When an edit is undone in the menu the map will automatically update accordingly.

➢ Waypoints

Clicking on the arrow reveals a dropdown which lists the **Waypoints** that have been placed on the map:



Selected **Waypoints** can be removed from the map using this menu.

➢ Input Sequences

Clicking on the arrow reveals a dropdown which lists all of the scaffolds in order in the assembly . The number in brackets indicates the number of pieces that the scaffold is in. This will be (1) for all unedited scaffolds. Where breaking has occurred this is represented in this number as is seen for scaffold 2 below:



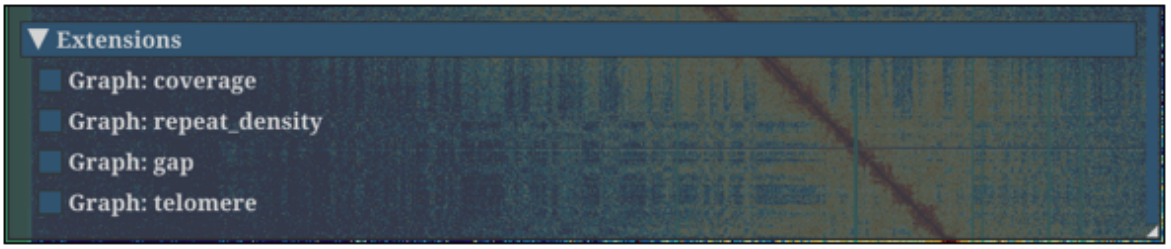
Selecting a scaffold allows the user to revert breaks they have made in particular scaffolds.

➢ Extensions

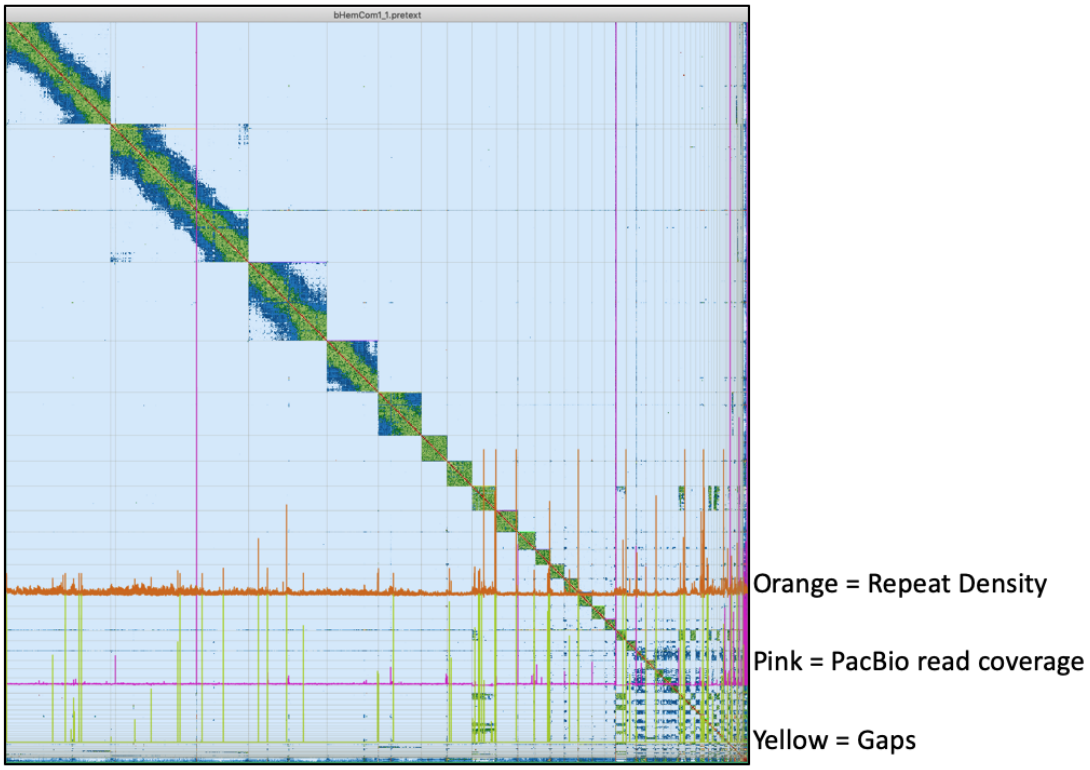
Clicking on the arrow reveals a dropdown menu which lists all available data sets

that can be embedded into the pretext map. These extensions are only available where the bedgraph formatted data has been converted using the **PretextGraph** application, instructions on how to use this can be found here:

https://github.com/wtsi-hpag/PretextGraph

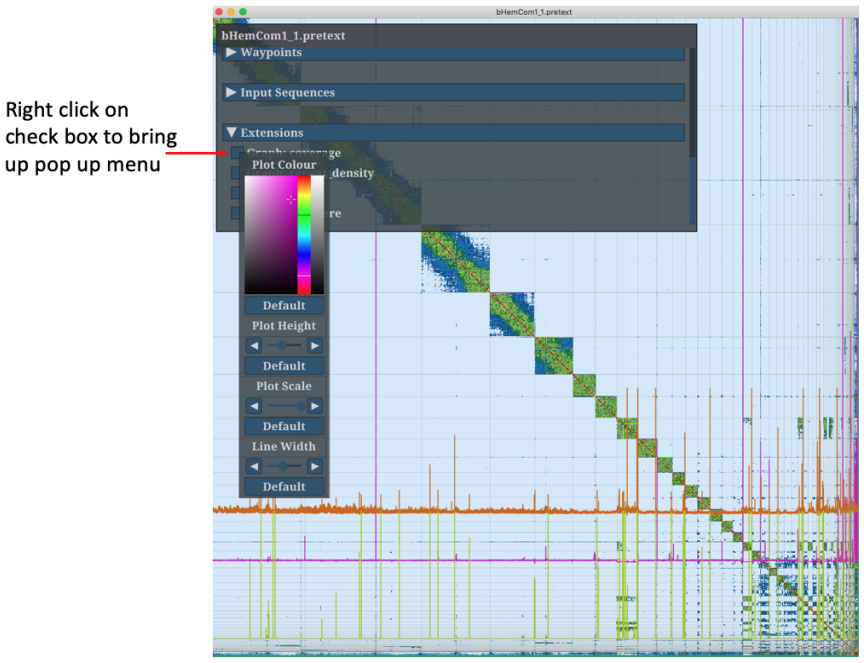


Selected extensions appear as graphs on the pretext map.



PretextView with extension graphs displayed

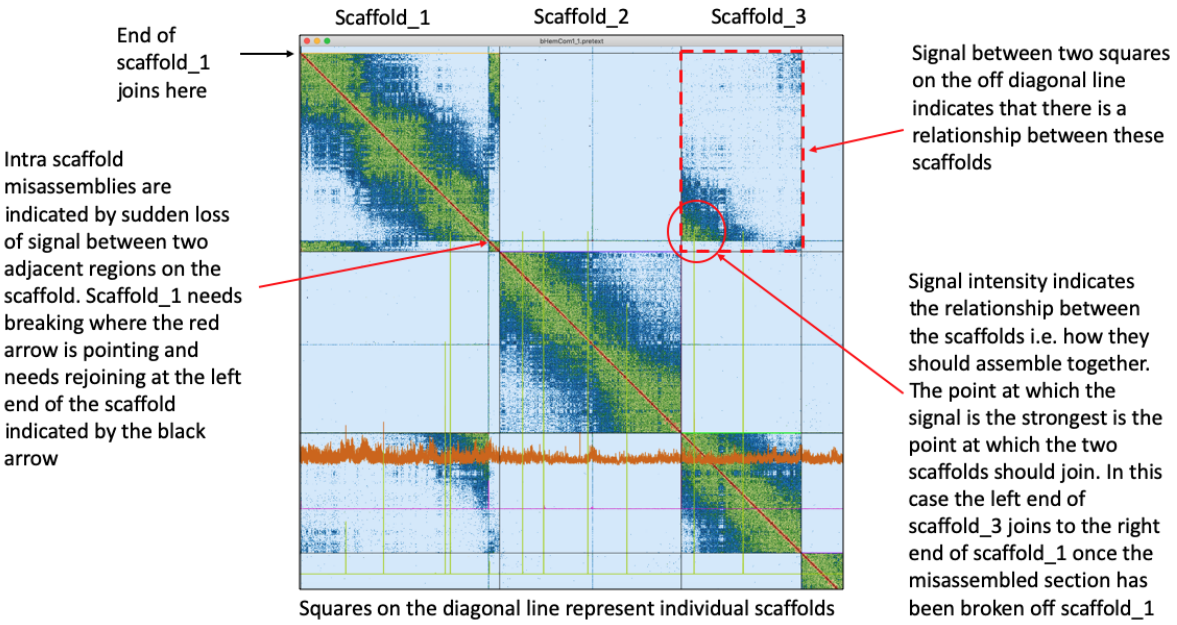
In the default setting the graphs are all displayed in the same colour on the map view. This can be changed by right clicking on the check box for each graph. A pop up menu will appear and a custom colour can be selected. Also in this menu there are sliders that allow the user to customise the height, scale and line width of the graphs as they are displayed on the map.



The displayed graphs are not static, they adjust accordingly in order that they are still visible to the user as they navigate around the map, this includes when the zoom function is used.

4) The Graphical view

The Pretext graphical view represents each separate scaffold in an assembly as a distinct square on the contact map. Interactions between squares on the off diagonal indicate a relationship between scaffolds - i.e that they are part of the same chromosome. The stronger the relationship is between the scaffolds, the more intense the off diagonal signal is. The HiC map also indicates where there are scaffolds that have been incorrectly assembled - either intra - or inter chromosomally.



5) Map Navigation

A three button mouse is recommended

Pan - Hold down right mouse button and drag from left to right
Zoom - Use scroll to zoom in and out of map

6) PretextView Edit Mode

PretextView Edit mode is a feature that allows the user to to make edits directly within the pretext map. This includes moving scaffolds around the map, reorienting scaffolds and making breaks and joins within scaffolds.

There are two ways to activate **Edit mode** in the map:

- ➢ **Left mouse click on map and then press the ‘e’ key on the keyboard**
- ➢ **Middle click on the mouse or press ‘u’ and then select Edit Mode from the main menu**

Once **Edit mode** is switched on crosshairs appear on the map as a green cross. The user can navigate around the map with the crosshairs, the expandable region between the crosshairs denotes the area to be edited which needs to be defined before editing occurs.

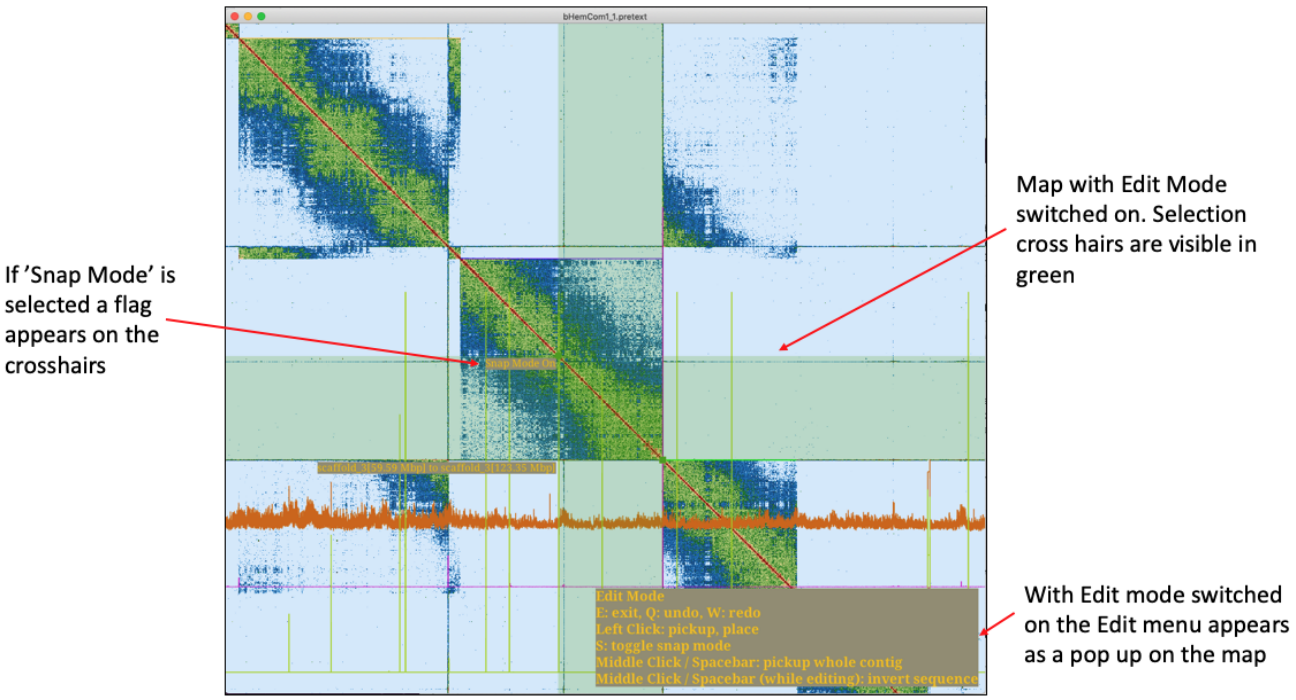
Crosshair navigation:

Pan - Move left and right with mouse over map
Zoom - Move mouse up and down

In addition to the crosshairs an **Edit Mode menu** also pops up on the bottom right hand side of the map. This lists all of the editing functions and their usage:

Edit Mode menu:

Mouse left click - selects the region defined by the crosshairs and also then places that region at the desired location.
Mouse middle click/Spacebar - selects the whole scaffold.
Mouse middle click/Spacebar (after selection with Edit mode) - inverts the selection region or whole scaffold.
Keyboard ‘q’ - undoes the last edit
Keyboard ‘w’ - redoes the last edit
Keyboard ‘s’ - switches on **‘Snap Mode’,** this mode prevents the user from joining the selected region/scaffold internally within another scaffold (unless that scaffold has been previously broken). With Snap Mode selected it is only possible to place selected regions adjacent to other scaffolds. When **‘Snap Mode’** is selected a banner pops up on the crosshairs to alert the user.
Keyboard ‘e’ - Exits **Edit Mode**



7) PretextView Waypoint Edit mode

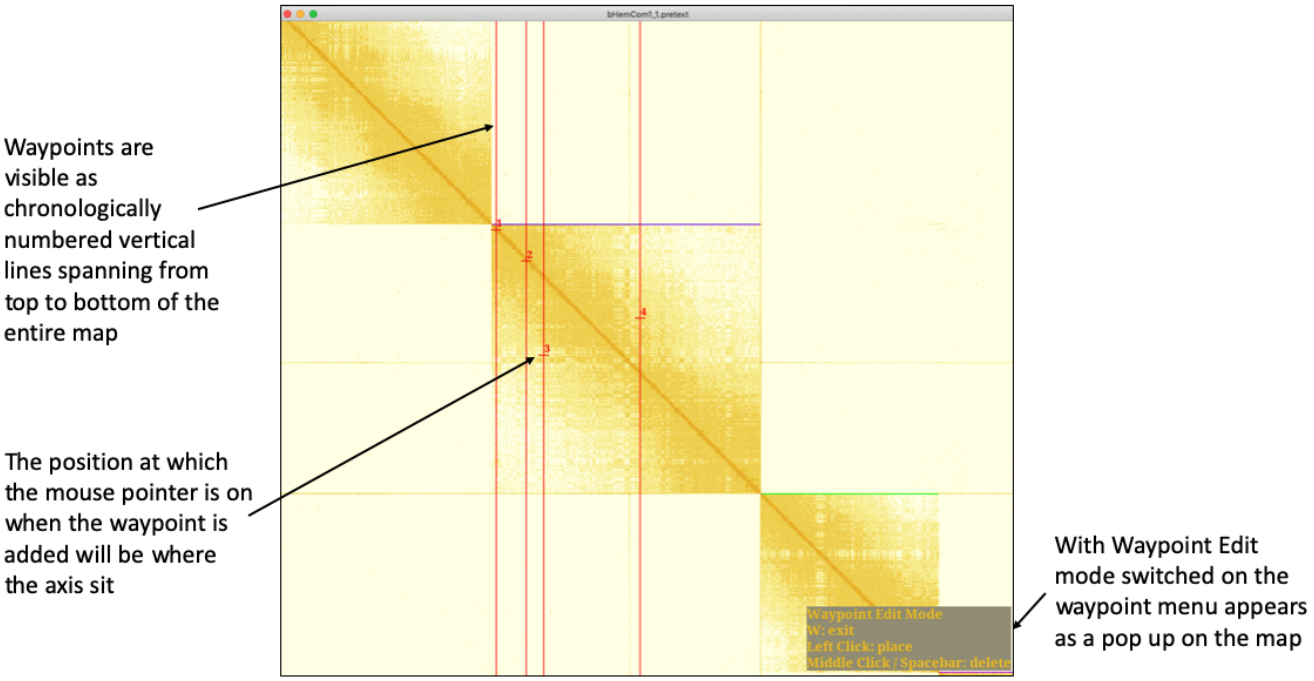
PretextView Waypoint Edit mode allows the user to place numbered waypoints on the map.

There are two ways to activate **Waypoint Edit Mode** in the map:

- **Left mouse click on map and then press the 'w' key on the keyboard**
- **Middle click on the mouse or press 'u' and then select Waypoint Edit Mode from the main menu**

With **Waypoint Edit Mode** selected a menu appears in the bottom right hand side of the map, this lists all of the waypoint functions and their usage:

Mouse left click - places a waypoint
Mouse middle click/Spacebar - deletes a waypoint
Keyboard 'w' - exits Waypoint Edit Mode



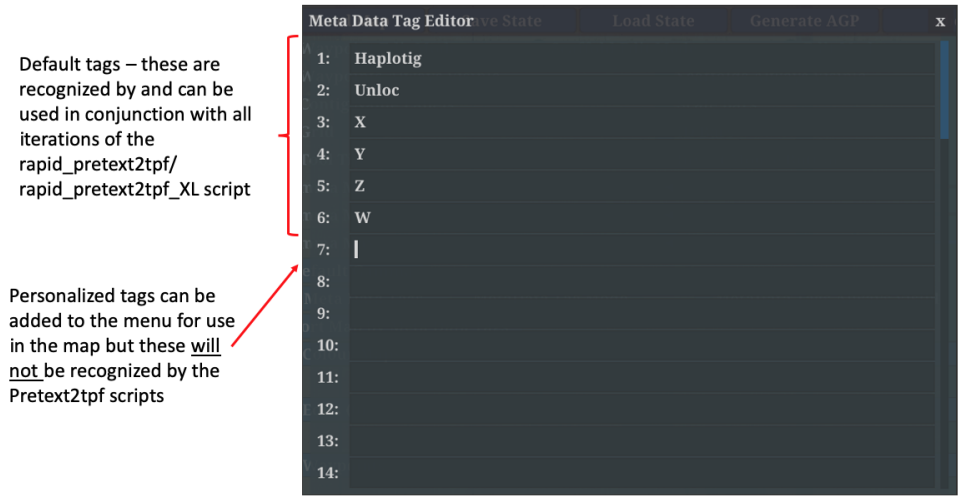
8) PretextView MetaData Tag Mode

PretextView MetaData Tag Mode allows the user to add predefined tags to the scaffolds in the map. This function can be used as a stand alone function in order for the user to mark up sections of the map for reference purposes. It is most powerful however when used in conjunction with the rapid curation pipeline (**rapid_pretext2tpf/rapid_pretext2tpf_XL scripts)** where the script will take the MetaData tag information and use it in production of the resulting tpf.

For further explanation of how the **MetaData Tag Mode** function works as part of the rapid pipeline lease refer to:

https://gitlab.com/wtsi-grit/rapid-curation/-/blob/main/README_curation.md

Clicking the MetaData tags button brings up the **Meta Data tag Editor Menu**:



There are two ways to activate Meta Data Tag mode in the map:

- ➢ **Left mouse click on map and then press the 'M' key on the keyboard**
- ➢ **Middle click on the mouse or press 'u' and then select MetaData Tag Mode from the main menu**

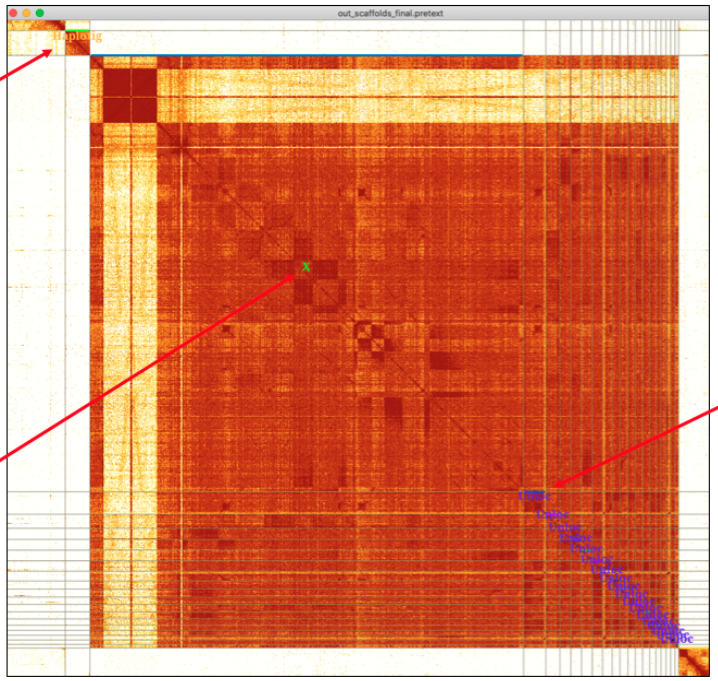
With **MetaData Tag Mode** selected a menu appears in the bottom right hand side of the map, this lists all of the editing functions and their usage:

Keyboard 'm' - exits Meta Data Tag Mode
Mouse left click - places a tag
Mouse middle click - removes a tag (a tag can only be removed if the 'active tag' is set to the same type of tag as the tag to be removed).
Shift-D - deletes all tags
Keyboard up and down arrow keys - selects the active tag type
Active tag - shows the selected tag type that will be placed

Using Meta Data Tag mode scaffolds have been marked up where required

When the 'Haplotig' tag is used in conjunction with rapid_pretext2tpf/ rapid_pretext2tpf_XL the script will dump all 'Haplotig' assigned scaffolds into a separate text file

Using the Sex Chromosome tag only one of the scaffolds within what has been grouped as the chromosomal entity requires tagging in order for rapid_pretext2tpf/ rapid_pretext2tpf_XL to correctly label the chromosome in the resulting tpf



When the 'Unloc' tag is used is conjunction with rapid_pretext2tpf/ rapid_pretext2tpf_XL the 'Unloc' scaffolds need to be grouped adjacent to the chromosome to which they localize to and painted (see section 9) as part of the primary chromosome

9) PretextView Scaffold Edit mode

PretextView Scaffold Edit Mode allows the user to 'paint' the scaffolds in the map. The map state with the 'painted' objects defined in scaffolding mode can then be outputted in AGP (Accessioned Golden Path - for an explanation of AGP files please refer to AGP Specification v2.1) format via the main menu using the **Generate AGP** function. Any unpainted sequences are output as singletons.

For a full explanation of how the **Scaffold Edit Mode** and **Generate AGP** functions should be incorporated as part of the Rapid pipeline please refer to:

https://gitlab.com/wtsi-grit/rapid-curation/-/blob/main/README_curation.md

There are two ways to activate **Scaffold Edit mode** in the map:

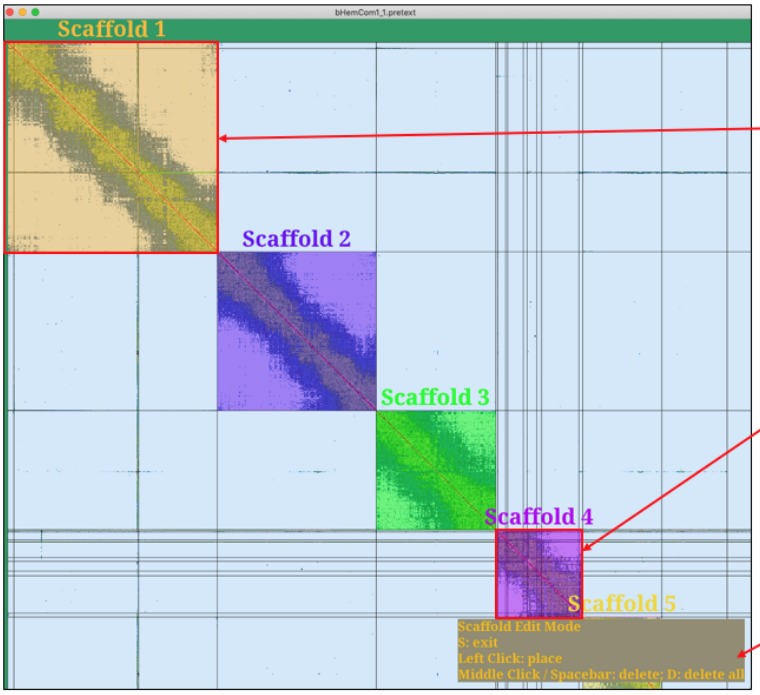
- **Left mouse click on map and then press the 's' key on the keyboard**
- **Middle click on the mouse or press 'u' and then select Scaffold Edit Mode from the main menu**

With **Scaffold Edit mode** selected a menu appears in the bottom right hand side of the map, this lists all of the editing functions and their usage:

Mouse left click - paints a scaffold

Mouse middle click/Spacebar - deletes the 'painting'
Mouse left click hold down and drag - paints adjacent scaffolds naming them as part of the same scaffold
Mouse left click hold down and scroll - enables zoom while painting
Keyboard 'd' - deletes all painting
Keyboard 's' - exits Scaffold Edit Mode

Using Scaffold Edit Mode the scaffolds are now 'painted'. The 'painting' denotes how the scaffolds are represented in the AGP when using the 'Generate AGP' function in the main menu



Painted scaffolds constitute entire, ordered and orientated chromosomal units that pre-curation existed as separate scaffolds. As a result of 'painting' these related scaffolds are named collectively in the output AGP as Scaffolds 1 and 4

With Scaffold Edit mode switched on the edit menu appears as a pop up on the map

After painting the map state is now ready to be output in AGP format. **See section 10) for details of how to proceed.**

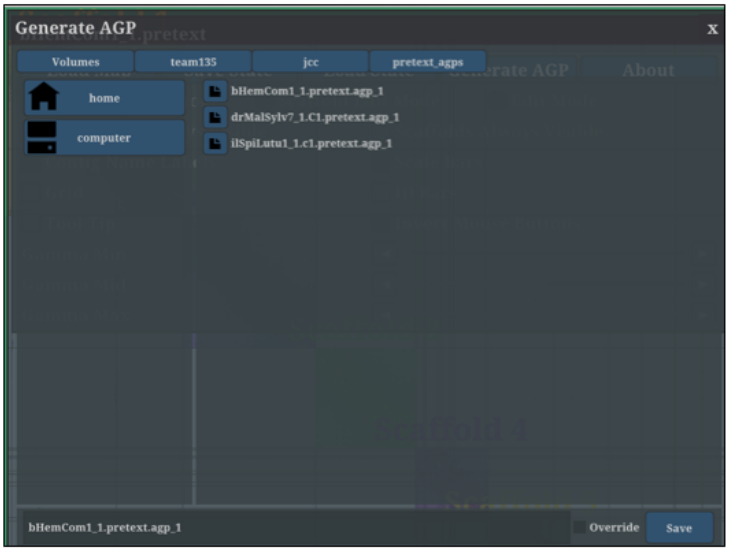
10) PretextView Generate AGP function

The **PretextView Generate AGP** function allows the user to output an AGP directly from the pretext map. This function can be used to generate an AGP of the pre-curated map, or in conjunction with **'Scaffold Edit Mode'** (see section 9) in order to create an AGP of the curated map. If used with **'Scaffold Edit Mode'** all 'painting' should be carefully checked in the map before AGP generation in order that the output AGP is an accurate reflection of the curated assembly.

To navigate to the **Generate AGP** function in the map:

- **Middle click on the mouse or press 'u' and then select Generate AGP from the main menu**

Selecting the Generate AGP function from the main menu brings up a new window that allows the user to save the AGP file, or override a previous file.



Selecting 'Generate AGP' from the main menu brings up a new window that allows the user to save/override an AGP of the current map state

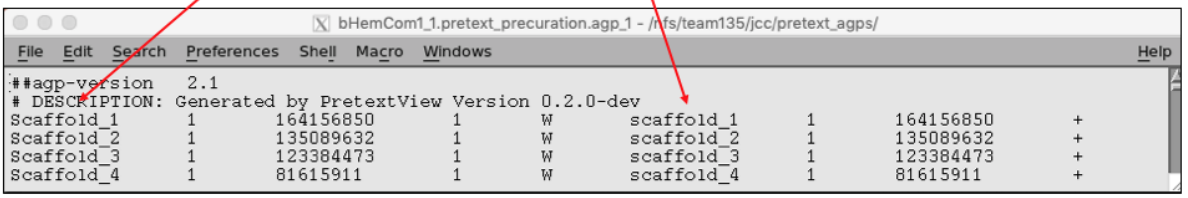
The AGP file can then be opened with the relevant text editing program e.g NEdit.

Note that object/part sizes will only be accurate up to the size of an individual map texel, and that any input sequences smaller than an individual texel will not be output.

AGP output examples

Pre-curated:

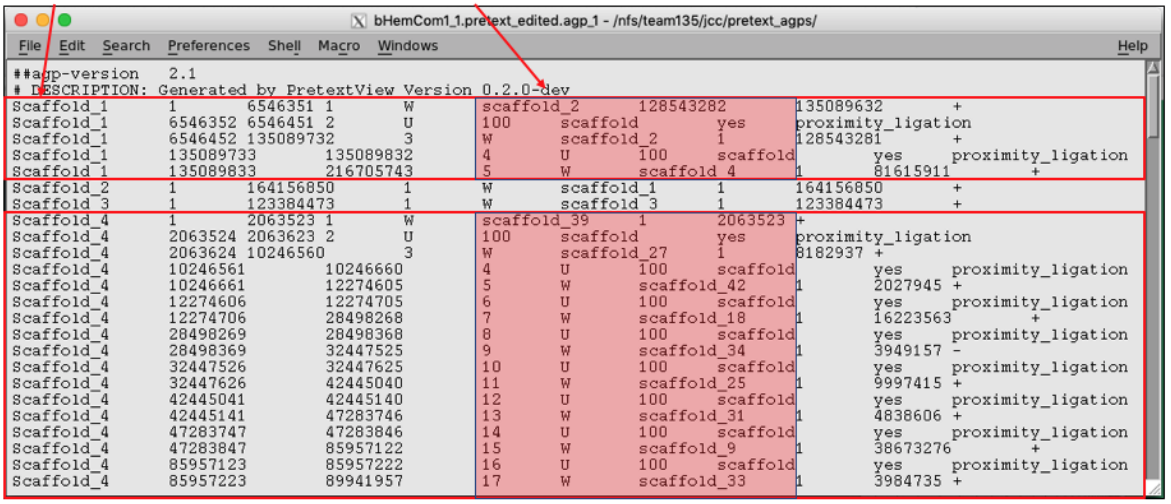
AGP scaffolds reflect exactly the unedited original scaffolds



Example extract AGP from a pre curated assembly

Curated:

AGP scaffolds reflect the edited map – where some newly assembled chromosomal units now comprise several original scaffolds that have been grouped by the 'painting' process e.g. AGP scaffold 1 comprises original scaffolds_2 and 4



Example extract AGP from a curated assembly

11) PretextView Save Functions

The **pretextView** map is automatically saved ($XDG_CONFIG_DIR or ~/.config on Unix, and the %APPDATA% folder on Windows) while the application is open. If the application is exited correctly the edited map will be loaded up the next time the same map is opened in the viewer. If the application crashes it is likely that all edits may be lost.

There is also an option within the **PretextView** main menu to manually save a map state. This provides additional security if the application were to unexpectedly close but is also useful for sharing an edited pretext map between users. **Save State** files are viewed in the map using the **Load State** Function.

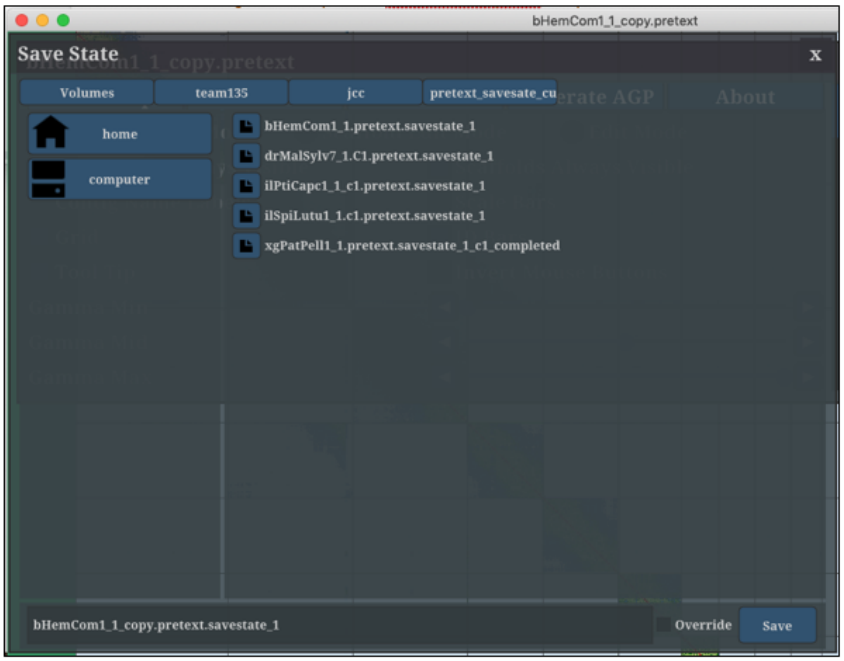
11a) Directions for using SaveState/Load State functions

First make a copy of the original pretext file and rename it e.g. edited.pretext - this is because when you use the **Save State** function this overwrites the original map.

Make edits in the copied version of the file and then navigate to the **Save State** function:

➢ **Middle click on the mouse or press ‘u’ and then select Save State from the main menu**

Selecting the **Save State** function from the main menu brings up a new window that allows the user to save the edited map in a chosen location. There is also an override option which if selected overrides the previous iteration of the saved map.



Selecting ‘Save State’ from the main menu brings up a new window that allows the user to save/override the current map state

In order for the **Load State** function to work when the user wants to view the edited map the **Save State** file needs the same file name as the pretext map that has been edited plus .savestate.

For example:

For map - **bHemCom1_1.edited.pretext**

Save State file should be - **bHemCom1_1.edited.pretext.savestate_1**

To open the **Save State** version of the map the map of the same name must first be loaded into the **PretextView** application. When this has loaded, **Load State** needs to be selected from the main menu, this will bring up a new window from which the user can then navigate to the relevant **Save State** file. If the **Save State** file has loaded correctly the map will instantly update in the viewer to the saved version.

Load state will only open on an unedited map

Note: There is no forward or backward compatibility with save-states from previous PretextView release versions.