Crosslink - polyploid-aware genetic mapping for outbreeders

Synopsis

Builds genetic maps using codominant biallelic markers derived from the F1 progeny of an outcross between two unrelated non-inbred parents, with additional features suitable for allopolyploid species.

Crosslink was written and tested on the Linux operating system, and designed to allow a high degree of automation by the use of scripting. Cross platform deployment is currently supported by the use of virtual machine and docker images. The source code is also freely available.

Crosslink consists of a number of programs, written in C, and helper scripts written in bash and python. The main programs are:

crosslink_group performs grouping, phasing and approximate marker ordering, and also redundant marker removal, missing value imputation and polyploid-related marker-typing error correction

crosslink_map performs final marker ordering and assigns final map positions, and also, for allopolyploids, detects markers which falsely join homeologous linkage groups

crosslink_pos performs a subset of crosslink_map's functionality, assigning final map positions

crosslink_viewer produces a two dimensional colour-coded plot showing an all-versus-all marker comparison indicating phasing, rf, LOD and map distances for one or more linkage groups at a time

crosslink_graphical shows a graphical representation of genotype values along one or more linkage groups

create_map and **sample_map** allow for creation of simulated marker data

File Formats

genotype file

A file containing marker data in a format similar to the loc file format of JoinMap®, but lacking any header information. Each marker occupies one line as follows:

```
<name> <type> <phase> <genotype1> [<genotype2>…]
```

There must be no blank lines or comments. All columns must be separated by a single space. A marker name can be any string, but should not contain any white space characters (space, tab etc.). Marker type must be one of the following three: <lmxll> indicating a biallelic codominant marker heterozygous in the maternal and homozygous in the paternal parent, <nnxnp> a biallelic codominant marker heterozygous in the paternal and homozygous in the maternal parent, or <hkxhk> a biallelic codominant marker heterozygous in both parents. Marker phase must always be specified but can be set arbitrarily to zero where phase is not yet known and will be ignored. For lmxll markers the phase must be either {0-} or {1-}, for nnxnp markers either {-0} or {-1} and for hkxhk markers one of {00}, {01}, {10} or {11} where the first and second digits indicate the maternal and paternal phase respectively. Each marker in the same file must have the same number of genotypes, each of which must consist of exactly two characters. For all marker types missing values must be specified as --, for lmxll markers genotypes must be either lm or ll, for nnxnp either nn or np and for hkxhk one of hh, kk, hk or kh. If the hk genotypes have not been imputed then hk and kh will be treated the same, whereas for imputed genotypes hk is taken to mean allele h was from the maternal parent and kh to mean allele h was from the paternal parent. Example markers:

```
MK-1234 <lmxll> {0-} lm lm ll lm ll -- ll lm
4567.890 <nnxnp> {-1} nn nn -- np -- -- nn np
abcd <hkxhk> {01} hh kk hh hk hk -- hk kh
```

There is no way to specify groupings of markers into linkage groups within a single file. Initially all markers should be together in the same file. When linkage groups are formed each is put into a separate file, and different versions of the map can be organised into their own subdirectory where required.

map file

crosslink_map outputs one map file per linkage group using a four column format giving the marker name and its centimorgan position in the maternal, paternal and combined map. Where a marker is not present or its position cannot be calculated in one of the maps, its position is listed as NA. The first line of the file contains the linkage group name and the number of markers as follows:

```
group <linkage_group_name> ; markers <number_of_markers>
```

Each marker is listed using four whitespace (i.e. tab and space characters) separated columns:

```
<marker_name> <maternal_map_position> <paternal_map_position> <combined_map_position>
```

Example map file:

```
group 000 ; markers 126
PHR10-89849276    0.0000      NA          0.0000
NMH21-89792123    NA          0.0000      0.0000
PHR11-89793667    0.0000      0.0000      0.0000
NMH12-89832883    0.6211      NA          0.7819
```

```
PHR10-89793765    1.2423      NA          1.5637
NMH01-89892406    NA          2.5322      2.1004
PHR21-89835283    NA          3.7823      3.1373
PHR11-89793784    2.4923      3.7823      3.1373
NMH12-89793368    3.1134      NA          3.6021
```

Installation

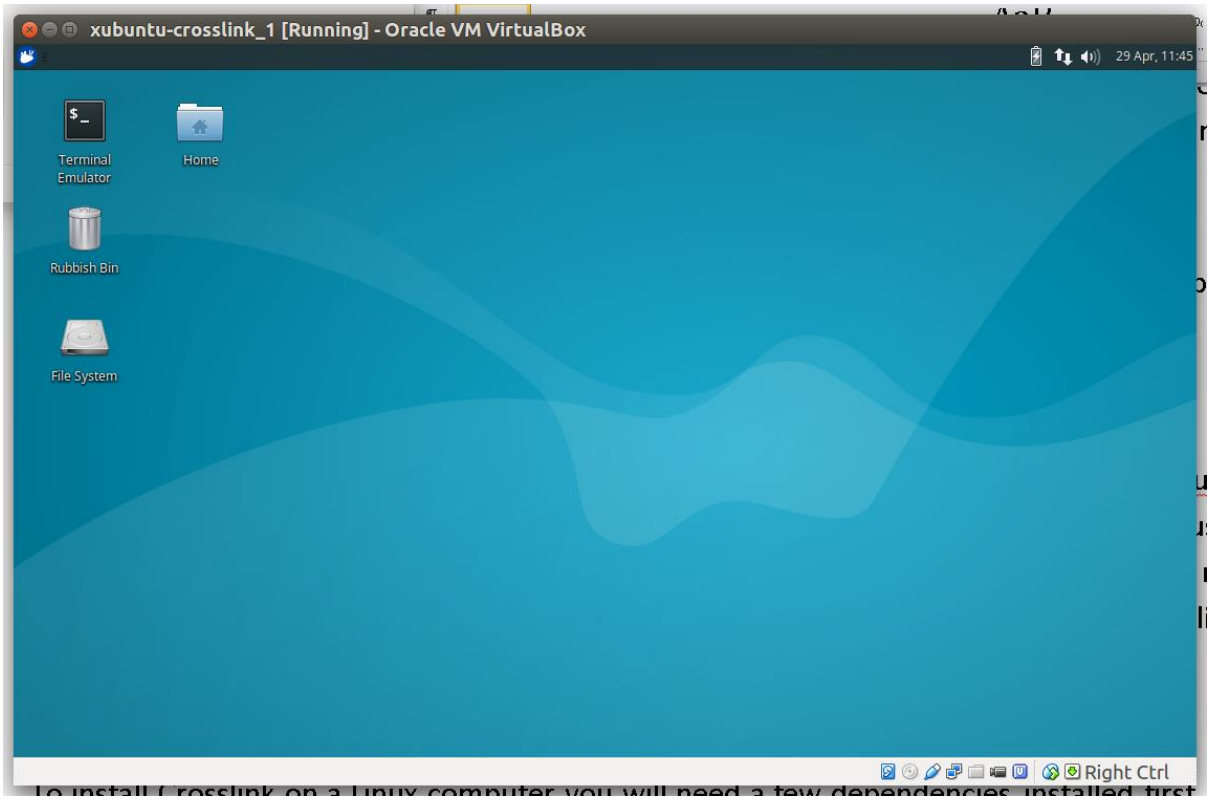
Using the Prebuilt VirtualBox Image

Cross-platform installation of Crosslink is only currently supported through the use of virtual machine and docker images. To use the graphical visualisation tools it is recommended to use the virtual machine rather than docker image, as everything should work without any further setup required. The VirtualBox software is currently available from Oracle for Windows, OS X, Linux and Solaris from: https://www.virtualbox.org and may also be available for your Linux distribution through its built-in package management system, for example on Ubuntu it can usually be installed by using the following command:

```
sudo apt-get install virtualbox
```

Once VirtualBox is installed, download the virtual machine file xubuntu-crosslink.ova (approximately 2GB) from the latest Crosslink github release: https://github.com/eastmallingresearch/crosslink/releases

Launch VirtualBox and import the image using File->Import Appliance, then select the xubuntu-crosslink.ova file you just downloaded. When the Appliance Settings dialog appears adjust the amount of RAM and the number of CPUs you which to allocate to the virtual machine if required, then click Import. Once imported select the virtual machine you have just created and click the green Start arrow. The image contains Xubuntu 16.04, a minimalist version of Ubuntu using the Xfce desktop environment, which should appear like this once loaded:



You may wish to enable copy-pasting between the host and guest operating system, if so Devices -> Shared Clipboard -> BidirectionalCrosslink in the VirtualBox menu system.

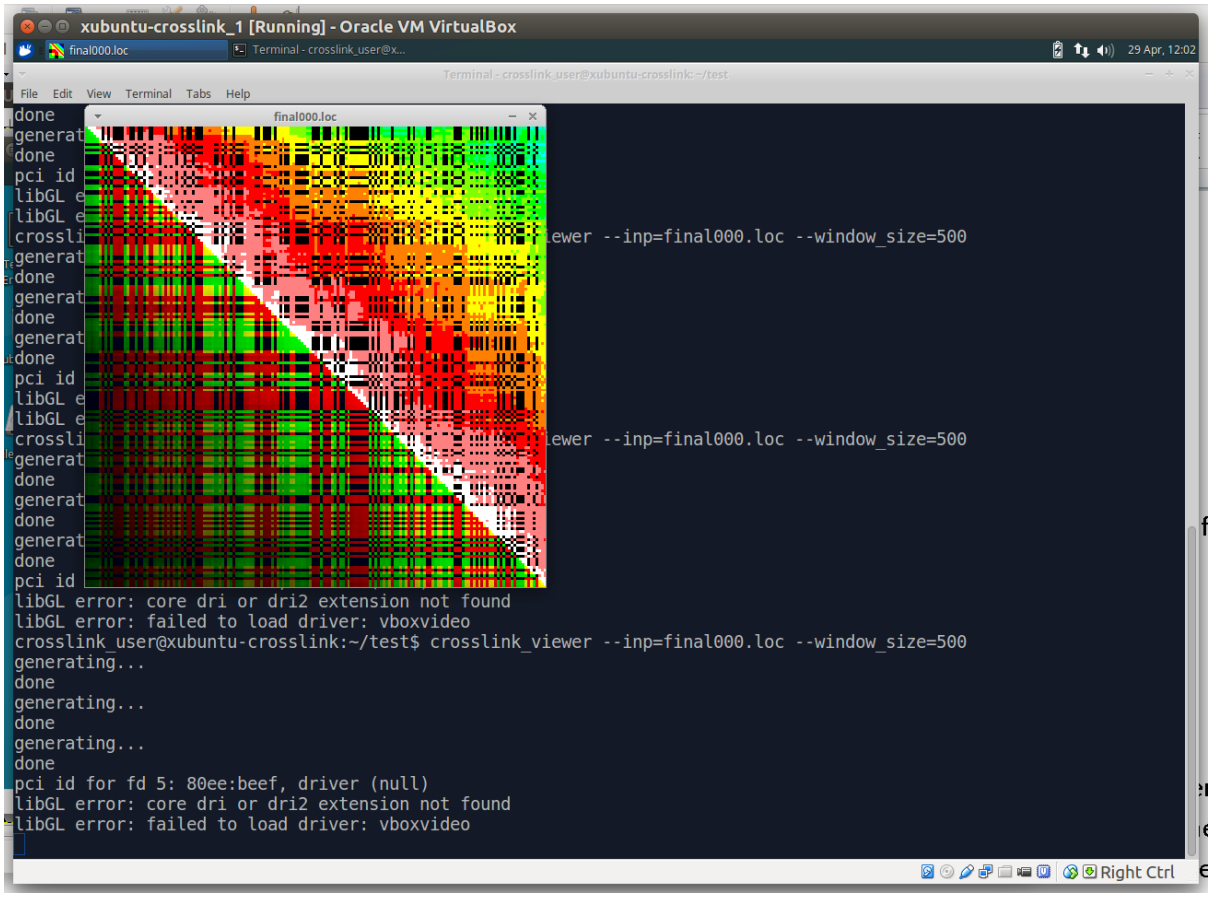
Crosslink is already installed. To test it is working open a terminal by clicking on the black Terminal Emulator icon and run the following commands:

```
mkdir test
cd test
~/crosslink/example_pipelines/simple_simulated.sh
```

After the command finishes type the ‘ls’ command and you should see 28 .loc and .map files called group and final. To test a data visualisation command run the following:

```
crosslink_viewer --inp=final000.loc --window_size=500
```

You should see something like this:



Press ‘q’ or close the graphical window to quit. Type ‘exit’ or close the window to exit the terminal. To shut down the virtual machine click on the mouse icon in the top left and click on the logout button at the bottom right of the menu, then select Shutdown. The password of the crosslink_user account is crosslink, you may want to change this to something more secure using the passwd command.

Installing Directly on Linux

To install Crosslink on a Linux computer begin by downloading the latest release of the source code from github at https://github.com/eastmallingresearch/crosslink/releases (choose either Source code (zip) or Source code (tar.gz)) and extract the files somewhere. You may wish to move the folder to a more convenient location such as ~/crosslink. To compile the main Crosslink programs you will need gcc installed. Open a terminal. Type the following to see if you have it already installed:

```
gcc --version
```

If not, on Ubuntu or Debian based systems gcc can usually be installed using:

```
sudo apt-get update
sudo apt-get install gcc
```

If you do not have the ability to install packages on your system ask your system administrator to help you. If you use a different Linux distribution use the appropriate package management tool for your system. Now compile the main Crosslink programs using (assuming the crosslink source code is now in a directory called ~/crosslink):

```
cd ~/crosslink/src
./make.sh
```

If no error messages appear the compilation was probably successful, and the programs crosslink_group, crosslink_map and crosslink_pos should have appear in the bin directory. Test you can run crosslink_group as follows:

```
../bin/crosslink_group --help
```

You should see a help message starting “Crosslink Copyright (C) 2016 NIAB EMR...”. To compile the data visualisation tools you will need SDL2 installed, including the development files. On Ubuntu or Debian based system you should be able to install these using:

```
sudo apt-get install libsdl2-2.0-0 libsdl2-dev
sudo apt-get install libsdl2-image-2.0.0 libsdl2-image-dev
```

Now compile the data visualisation programs (crosslink_viewer and crosslink_graphical) using:

```
./viewer_make.sh
```

Check you can run crosslink_viewer using:

```
../bin/crosslink_viewer --help
```

You should see a similar help message appear. Next, to allow running the programs from any directory on your system add the path to Crosslink's programs to your .bashrc file. Here I will assume that the editor nano will be used (install first using `sudo apt-get install nano` if required). Launch the nano editor using:

```
nano ~/.bashrc
```

Add the following lines to the bottom of the file (again assuming Crosslink files were installed to ~/crosslink):

```
export CROSSLINK_PATH=~/crosslink
export PATH=${CROSSLINK_PATH}/bin:${CROSSLINK_PATH}/scripts:${PATH}
```

Make sure not to omit the final ${PATH} from the second line! Press CTRL-O then ENTER to save and CTRL-X to close the editor. Then close the terminal and open a new one (or log out and then back in again if you are logged in remotely). You should now be able to run Crosslink's programs from any directory:

```
cd ~
mkdir test_crosslink
cd test_crosslink
crosslink_group --help
```

You should see the same help message as before. Now try running one of the example pipelines:

```
${CROSSLINK_PATH}/example_pipelines/simple_simulated.sh
```

The pipeline should run and produce new output files in your current directory, including one called final000.loc. Test you can view this file now using crosslink_viewer:

```
crosslink_viewer --inp=final000.loc
```

which should display a coloured plot similar to the one shown for the virtual machine installation section above. Some of the python helper scripts require one or more of the following python modules: scipy, numpy, matplotlib. Install them using:

```
sudo apt-get install python-scipy python-numpy python-matplotlib
```

Or, if you have pip available you may wish to try installing them only in your user account rather than system-wide, using:

```
pip install --user numpy scipy matplotlib
```

Using the Docker Image

For expert users a Docker image is available called rjvickerstaff/crosslink. This is not the recommended way for non-Linux users to run Crosslink as it is more difficult to setup and use than the VirtualBox method and problems were encountered during testing when trying to produce graphical output. It is included here in case it proves useful for advanced users. To use the image first install Docker on your machine following the installation instructions

for your platform on the https://www.docker.com website. To launch a container use the following:

```
docker run -it -u crosslink_user rjvickerstaff/crosslink:0.2
```

Depending on how Docker is setup on your machine you may need to run this command as root. If running on a Linux host you may be able to get graphical output using:

```
docker run -it -u crosslink_user -v /tmp/.X11-unix:/tmp/.X11-unix\
           -e DISPLAY=$DISPLAY rjvickerstaff/crosslink:0.2
```

The following command, or similar, may also be needed to allow the container permission to create graphical output:

```
xhost +local:
```

The Dockerfile used to create the container can be found at ${CROSSLINK_PATH}/docker/Dockerfile.

Quick Start Guide

Simulated Data Without Polyploid-Related Errors

An example of a simple pipeline using Crosslink to generate and map simulated outcross data is included in the file example_pipelines/simple_simulated.sh, which you may already have run to test your install. If not it can be run using the following:

```
cd ~
mkdir test_crosslink_simple
cd test_crosslink_simple
${CROSSLINK_PATH}/example_pipelines/simple_simulated.sh
```

We will now step through the simple_simulated.sh pipeline one line at a time, by typing (or copy-pasting) the commands into the shell. Begin in a new, empty directory:

```
cd ~
mkdir test_crosslink_simple2
cd test_crosslink_simple2
```

Now invoke the crosslink_group command with the help option to verify that the program runs:

```
crosslink_group --help
```

You should see :

```
Crosslink Copyright (C) 2016 NIAB EMR see included NOTICE file for details
remove redundant markers, form linkage groups, correct marker typing errors,
phase, impute missing values, perform approximate ordering

    --inp=STRING input genotype file (required)… etc
```

Invoking any of the core Crosslink programs (create_map, sample_map, crosslink_group, crosslink_map, crosslink_pos, crosslink_viewer, crosslink_graphical) with the --help option will print a summary of command line options to the terminal and exit. Now create a map specification file with the following command, which can be entered as a single line without the backslash characters (\), or across multiple lines retaining the backslashes:

```
create_map --output-file=sample.spec --numb-lgs=28\
           --map-size=2000 --marker-density=2.0\
```

```
            --prob-both=0.28 --prob-maternal=0.36
```

A new file called sample.spec should have appeared. Take a look at its contents using:

```
head sample.spec
```

It should begin with something similar to:

```
#markers 4000 lgs 28
M00000a98 <nnxnp> {-1}    0 0.465766
M00000029 <lmxll> {0-}    0 2.513004
```

where each line specifies the marker name, type, phase, linkage group and centimorgan map position. The options given to create_map define the number of linkage groups (28), the total map length (2000 centimorgans), the average marker density (2.0 markers per centimorgan), the approximate proportion of markers heterozygous in both parents (0.28) and heterozygous in the maternal parent only (0.36) with the proportion heterozygous in the paternal parent being defined implicitly as 1.0 - 0.28 - 0.36 = 0.36. Use create_map --help for more help with the options. Next we will simulate some genotype data obtained from an F1 cross:

```
sample_map --input-file=sample.spec --output-file=sample.loc\
            --samples=162 --prob-missing=0.007 --prob-error=0.01
```

where we have told sample_map to simulate data from 162 progeny with a missing data rate of 0.007 and a genotyping error rate of 0.01. This program does not generate any allopolyploid-related errors, which are created using the separate create_type_errors.py script. A new file sample.loc should have appeared, which is a genotype file as described in a previous section, and can be viewed using:

```
less -S sample.loc
```

You will notice that all phase values are set to zero and a few genotypes are set to -- indicating they are missing. The markers are also in a random order with respect to their true linkage group and map position. The marker names do not indicate the true marker order in any way, so that it is not possible for a mapping program undergoing testing to inadvertently cheat by alphabetically sorting the markers. For hkxhk markers, all heterozygous genotype calls are given as hk, so that it is unspecified whether the maternally inherited allele was h or k. Press ‘q‘ to quit the less command. Next we will split the markers into a separate file for each linkage group:

```
crosslink_group --inp=sample.loc --outbase=group --min_lod=10.0 --knn=3
```

where we have told the program to split markers based on a linkage LOD threshold of 10, and to impute missing values using the 3 nearest neighbouring markers. The separate linkage group files should have appeared with names starting with group, and be numbered sequentially from 000 to 027. If you wish to rerun the grouping at a different LOD threshold, be aware that crosslink_group does not automatically delete any existing group files, so it is important to first remove all the old files, by a command such as (be sure you are familiar with the rm command before deleting any files from the command prompt in this way as it is easy to delete the wrong files by mistake):

```
rm group???.loc
```

With the present data a LOD of 10 ought to split the markers into the correct 28 groups. View the first linkage group using:

```
less -S group000.loc
```

You will notice the phases are now set and there are no longer any missing values (crosslink_map does not support missing values). The markers have also been approximately ordered, so that you may notice consecutive genotypes have similar patterns. To see how many markers were assigned to each group:

```
wc --lines group???.loc
```

The last step is to produce the final marker order and map positions for each linkage group:

```
for fname in group???.loc
do
    echo ${fname}...
    outname=${fname/group/final}
    crosslink_map --inp=${fname} --out=${outname} --map=${outname/loc/map}
done
```

Here we have told crosslink_map to output the final genotype files with names beginning with final, and to produce associated map files for each one. View the first final genotype file using:

```
less -S final000.loc
```

You will notice that hkxhk markers now have some heterozygous genotypes given as kh as well as hk, indicating that crosslink_map has imputed which allele is likely to be from the maternal parent and which from the paternal (the maternal is the first of the two letters). View the first map file using:

```
less final000.map
```

You should see something like:

```
group group000 ; markers 143
M000001da         0.0000          0.0000          0.0000
M00000700             NA          1.8870          2.2589
M00000055             NA          3.7740          4.5178
M00000542             NA          5.0241          6.0142
M000007ae         8.7471          6.2742          7.5106
M00000f0e        13.2663              NA         10.7209
M00000d96        14.5163              NA         11.6090
etc
```

where the four columns are the marker name followed by its positions on the maternal, paternal and combined maps respectively. lmxll and nnxnp markers are only present on the maternal and paternal maps respectively so the other position is listed as NA. To see the combined length of each linkage group:

```
for x in final???.map
do
    echo ${x} $(tail -n 1 ${x} | cut -f 4)
done
```

You should see something like:

```
final000.map 253.2675
final001.map 234.6211
final002.map 234.7441
final003.map 278.4530
final004.map 265.6859
```

```
final005.map 272.5785
final006.map 268.4784
final007.map 288.5128
final008.map 280.2432
final009.map 256.5174 etc
```

Real Data From A Polyploid Outcross

The file sample_data/rgxha.loc.gz contains a real dataset from the allo-octoploid cultivated strawberry *Fragaria x ananassa* from an outcross between Redgauntlet (maternal parent) and Hapil (paternal parent) cultivars. There are 11587 markers genotyped on 162 progeny using the Affymetrix Axiom® iStraw90K microarray platform. The markers were called as part of a batch of 6x96 samples (including data from other, unrelated plants) using the Affymetrix Power Tools (APT) and SNPolisher pipeline and further filtered to remove extreme segregation distortion. The marker names indicate the classification assigned to each marker by APT: PHR (polymorphic, high resolution) indicates three clusters were detected across all the samples and assigned as the AA, AB and BB genotypes; NMH (no minor homozygote) indicates only two clusters which were assigned as AA and AB genotypes. The two numbers after the three letter code indicate the genotype code assigned by APT to the maternal and paternal parents using the convention 0=AA, 1=AB, 2=BB. For example, PHR12 indicates a PHR marker where Redgauntlet was called as AB and Hapil as BB. The rest of the marker name is the iStraw90K probesetid, for example PHR12-89894503 indicates that the original marker probesetid was AX-89894503.

The script example_pipelines/build_rgxha.sh demonstrates a pipeline which builds a map from the sample data, making use of Crosslink's helper scripts. The helper scripts assist in calling the appropriate Crosslink program (crosslink_group, crosslink_map or crosslink_pos) with the appropriate options when only a subset of their full functionality is required, for example, outputting just a list of the names of redundant markers. Most of the scripts require

a configuration file specifying Crosslink parameters, which are provided in sample_data/rgxha_conf and which can be copied and modified as needed. The pipeline script contains comments explaining each step. To begin open a terminal, create a new directory and change into it:

```
mkdir test_build_rgxha
cd test_build_rgxha
```

To simply run the entire pipeline from start to end, assuming Crosslink has been properly installed, enter the following:

```
${CROSSLINK_PATH}/example_pipelines/build_rgxha.sh
```

Otherwise, to perform each step of the pipeline manually follow all the steps listed below. Begin by making new working copies of the helper script configuration files and genotype data:

```
cp -r ${CROSSLINK_PATH}/sample_data/rgxha_conf ./conf
zcat ${CROSSLINK_PATH}/sample_data/rgxha.loc.gz > all.loc
```

The next step is an initial exploratory grouping, outputting one file per provisional linkage group into a new subdirectory. The helper scripts generally process one genotype file at a time or else deal with all the genotype files within a named subdirectory (specifically all the files with .loc filename extension), these being assumed to represent the linkage groups. In most cases passing the name of a subdirectory to a script will cause it to create the directory if required and remove existing files ending with .loc to prevent old files from getting mixed up with new ones. The following takes the all.loc genotype file and splits it into linkage groups in a new subdirectory called initgrps using a LOD threshold of 7:

```
cl_group.sh   all.loc   initgrps   7
```

This command can be rerun with different LOD values to see how many linkage groups result. If you have graphical output available from your Crosslink install, you can use crosslink_viewer to view the linkage groups. This will be explained in more detail in the next section, but for now we will ensure that we retain all the intermediate results of the pipeline in different subdirectories for later viewing. Polyploidy appears to cause a few markers to be misclassified. Most commonly this is a NMH marker where the classification of the two clusters as homozygote and heterozygote has been reversed, so that an lmxll marker is called as an nnxnp or vice versa. This error is much less common for PHR markers. Crosslink fixes these errors by detecting unexpected linkage between maternal and paternal markers. Where a group of markers are thus found to be linked above a significant LOD threshold but contain a mixture of maternal and paternal markers, the rarer kind of marker is converted to the more common kind (allowing for some markers to be weighted more strongly than others). This is performed using:

```
cl_fixtypes.sh   all.loc   all.loc   conf/fixtypes.000
```

where conf/fixtypes.000 is the configuration file which contains:

```
CL_GROUP_MINLOD=${CL_GROUP_MINLOD:-6.0}
CL_GROUP_MATPATLOD=${CL_GROUP_MATPATLOD:-10.0}
CL_MATPAT_WEIGHTS=${CL_MATPAT_WEIGHTS:-"01P03"}
CL_GROUP_LOGFILE=${CL_GROUP_LOGFILE:-fixtypes.log}
```

The helper script configuration files, written in bash, contain default values which can be overridden in the calling script. For example MYVARIABLE=${MYVARIABLE:-10} indicates that if no value is already set, MYVARIABLE should be set to 10 (*not* -10), otherwise the

existing value is used. In this case CL_GROUP_MINLOD specifies markers should be grouped at a LOD threshold of 6, CL_GROUP_MATPATLOD that anomalous linkage between maternal and paternal markers in the same group should be considered at a LOD threshold of 10, CL_MATPAT_WEIGHTS indicates that by default markers should be weighted as 01 (i.e. 1), but markers with names starting with the letter P should be weighted as 03 (i.e. considered as 3 times more reliable) when deciding the true marker type. For example if two lmxll PHR-type markers and five nnxnp NMR-type markers are found to be linked together, this will count as 2x3=6 votes for lmxll and 5x1=5 for nnxnp and all the markers will be set as lmxll type (because PHR markers are misclassified more rarely than NMHs). CL_GROUP_LOGFILE names the log file that crosslink_group will output containing the names of the markers whose types were switched. cl_fixtypes.sh reads markers in from all.loc, processes them using the configuration provided and then outputs to all.loc again, *overwriting* the existing data. Alternatively a different output filename could have been provided to allow retention of the input data. The linkage groups formed internally are not retained and all markers are outputted into the same output file together. This allows different LOD threshold to be used in different steps of the pipeline. The next step forms linkage groups again, outputting to a new subdirectory. This step is simply to allow the effect of the type error correction to be assessed:

```
cl_group.sh   all.loc   fixgrps   7
```

Next the pipeline identifies which markers are redundant, containing only genotype calls all exactly the same as another marker(s). Missing values are not counted as being new information:

```
cl_findredun.sh   all.loc   all.redun   conf/findredun.000
```

Input is read from all.loc (which is not modified) and a list of redundant markers output to all.redun in a two column format. Column one gives the name of the redundant marker, column two gives the name of the non-redundant marker that represents it. conf/findredun.000 is the configuration file:

```
CL_GROUP_MINLOD=${CL_GROUP_MINLOD:-6.0}
CL_GROUP_REDUNLOD=${CL_GROUP_REDUNLOD:-20.0}
```

where a CL_GROUP_MINLOD of 6.0 indicates that linkage groups be formed above a LOD threshold of 6, and a CL_GROUP_REDUNLOD of 20 indicates that markers linked above this LOD are explicitly checked to see if one can be flagged as redundant with respect to the other. Only the marker list is output, the grouping is not retained. The next step is to impute missing genotype calls:

```
cl_knnimpute.sh   all.loc   all.loc   conf/knnimpute.000
```

The configuration file contains:

```
CL_GROUP_MINLOD=${CL_GROUP_MINLOD:-6.0}
CL_GROUP_KNN=${CL_GROUP_KNN:-3}
```

Genotypes are read in from all.loc and once imputed are output to all.loc overwriting the original data. A CL_GROUP_MINLOD of 6 indicates that linkage groups should be formed down to a LOD threshold of 6, a CL_GROUP_KNN of 3 indicates that each missing genotype is imputed from the mean value of the 3 most similar markers in the same linkage group which do not lack the corresponding genotype. The imputed data are output into a single file, the grouping is not retained. Next just the redundant markers (listed in all.redun) are excluded from the fully-imputed genotypes (all.loc) and saved as a new file (all.uniq). The redundant markers will be used again later and are therefore not overwritten:

```
cl_extract.sh   all.loc   all.redun   all.uniq
```

Next linkage groups are formed from the unique markers and put in a new subdirectory called uniqgrps:

```
cl_group.sh   all.uniq   uniqgrps   7
```

Because polyploid related errors are causing some markers to be linked strongly to two different (but homeologous) linkage groups, the data do not split into the expected 28 linkage groups. This causes crosslink_group to give up phasing the affected groups, as phasing information will not propagate to all the markers in those groups, therefore we use an additional step to force phasing to complete down to a LOD threshold of zero for each linkage group individually. The phasing between unlinked markers will be meaningless, but at least all genuinely linked markers should have a usable phase. Here we retain the data before and after forced phasing (which just runs crosslink_group with a LOD threshold of zero) for later visualisation if desired:

```
cl_phase.sh   uniqgrps   phasegrps
```

Next, we try to automatically detect all the markers which are incorrectly joining two linkage groups. This step *must* be run on fully phased markers:

```
cl_detect_crosslg.sh phasegrps crosslg_markers conf/detectcrosslg.000
```

The configuration file contains:

```
CL_PARALLEL_JOBS=${CL_PARALLEL_JOBS:-1}
CL_MAP_RANDOMISE=${CL_MAP_RANDOMISE:-0}
```

```
CL_GA_SKIPORDER1=${CL_GA_SKIPORDER1:-1}
CL_MAP_CYCLES=${CL_MAP_CYCLES:-5}
CL_GA_ITERS=${CL_GA_ITERS:-150000}
CL_GA_OPTIMISEMETH=${CL_GA_OPTIMISEMETH:-0}
CL_GA_USEMST=${CL_GA_USEMST:-5}
CL_GA_MINLOD=${CL_GA_MINLOD:-3.0}
CL_GA_MSTNONHK=${CL_GA_MSTNONHK:-0}
CL_GA_PROBHOP=${CL_GA_PROBHOP:-0.3333}
CL_GA_MAXHOP=${CL_GA_MAXHOP:-1.0}
CL_GA_PROBMOVE=${CL_GA_PROBMOVE:-0.3333}
CL_GA_MAXMOVESEG=${CL_GA_MAXMOVESEG:-1.0}
CL_GA_MAXMOVEDIST=${CL_GA_MAXMOVEDIST:-1.0}
CL_GA_PROBINV=${CL_GA_PROBINV:-0.5}
CL_GA_MAXSEG=${CL_GA_MAXSEG:-1.0}
CL_GIBBS_SAMPLES=${CL_GIBBS_SAMPLES:-200}
CL_GIBBS_BURNIN=${CL_GIBBS_BURNIN:-5}
CL_GIBBS_PERIOD=${CL_GIBBS_PERIOD:-1}
CL_GIBBS_PROBSEQUEN=${CL_GIBBS_PROBSEQUEN:-0.0}
CL_GIBBS_PROBUNIDIR=${CL_GIBBS_PROBUNIDIR:-1.0}
CL_GIBBS_MINPROB1=${CL_GIBBS_MINPROB1:-0.1}
CL_GIBBS_MINPROB2=${CL_GIBBS_MINPROB2:-0.0}
CL_GIBBS_TWOPT1=${CL_GIBBS_TWOPT1:-1.0}
CL_GIBBS_TWOPT2=${CL_GIBBS_TWOPT2:-1.0}
CL_HOMEO_MINCOUNT=${CL_HOMEO_MINCOUNT:-2}
CL_HOMEO_MINLOD=${CL_HOMEO_MINLOD:-1}
CL_HOMEO_MAXLOD=${CL_HOMEO_MAXLOD:-25}
```

This step uses crosslink_map internally to impute the missing information in the hkxhk markers before detecting those joining two linkage groups. CL_PARALLEL_JOBS=1 indicates to only process one linkage group at a time. Increase the value to utilise more than one CPU core. The many options relating to marker ordering and hk genotype imputation will be described in detail in a later section. The three final options relate directly to detection of cross linkage group markers. The problem appears to be the result of a few hkxhk markers

whose maternal information comes from one linkage group while the paternal information comes from one of the homeologous linkage groups. That is to say that the marker's probes seem to be hybridising to two or more homeologous loci, one of which is heterozygous in the maternal genome and another in the paternal genome. These cases are detected by comparing pairs of hkxhk markers, where we expect the markers to be truly linked according to one parent but unlinked according to the other. For a pair of hkxhk markers if one parent indicates a LOD below CL_HOMEO_MINLOD and the other a LOD above CL_HOMEO_MAXLOD then both markers are given one point. Markers accumulate points for each qualifying hkxhk marker pair they are involved in. Those that receive a total number of points greater than or equal to CL_HOMEO_MINCOUNT are flagged as being cross linkage group markers, and listed in the output file (here we have called it crosslg_markers). The next three steps remove the flagged markers then regroup and rephase the data:

```
cl_removemarkers.sh   all.uniq   filt.uniq   crosslg_markers
cl_group.sh   filt.uniq   filtgrps   7
cl_phase.sh   filtgrps   filtgrps
```

There are still 27 rather than the expected 28 linkage groups. If you have graphical output available, crosslink_viewer can be used to view each linkage group in turn (explained in detail in the next section), which should make it fairly obvious which group needs to be split. There should be a single hkxhk marker holding the two true linkage groups together. Zoom in using the cursor keys and plus/minus keys to find the marker. Clicking on the marker should print its name to the terminal. We can then manually add it to the list of cross linkage group markers and repeat the filtering and regrouping steps. If you don't have crosslink_viewer available just follow these steps below anyway for now:

```
echo 'PHR11-89834490' >> crosslg_markers
cl_removemarkers.sh   filt.uniq   filt.uniq   crosslg_markers
cl_group.sh   filt.uniq   filtgrps   7
```

```
cl_phase.sh   filtgrps   filtgrps
```

There should now be 28 linkage groups. To produce the final map ordering use the following:

```
cl_order_hkimpute.sh   filtgrps   finalgrps   conf/orderhkimpute.000
```

which will output the final markers orderings with imputed hk genotypes into the new subdirectory finalgrps. The configuration file contains:

```
CL_PARALLEL_JOBS=${CL_PARALLEL_JOBS:-1}
CL_MAP_RANDOMISE=${CL_MAP_RANDOMISE:-0}
CL_GA_SKIPORDER1=${CL_GA_SKIPORDER1:-1}
CL_MAP_CYCLES=${CL_MAP_CYCLES:-5}
CL_GA_ITERS=${CL_GA_ITERS:-150000}
CL_GA_OPTIMISEMETH=${CL_GA_OPTIMISEMETH:-0}
CL_GA_USEMST=${CL_GA_USEMST:-5}
CL_GA_MINLOD=${CL_GA_MINLOD:-3.0}
CL_GA_MSTNONHK=${CL_GA_MSTNONHK:-0}
CL_GA_PROBHOP=${CL_GA_PROBHOP:-0.3333}
CL_GA_MAXHOP=${CL_GA_MAXHOP:-1.0}
CL_GA_PROBMOVE=${CL_GA_PROBMOVE:-0.3333}
CL_GA_MAXMOVESEG=${CL_GA_MAXMOVESEG:-1.0}
CL_GA_MAXMOVEDIST=${CL_GA_MAXMOVEDIST:-1.0}
CL_GA_PROBINV=${CL_GA_PROBINV:-0.5}
CL_GA_MAXSEG=${CL_GA_MAXSEG:-1.0}
CL_GIBBS_SAMPLES=${CL_GIBBS_SAMPLES:-200}
CL_GIBBS_BURNIN=${CL_GIBBS_BURNIN:-5}
CL_GIBBS_PERIOD=${CL_GIBBS_PERIOD:-1}
CL_GIBBS_PROBSEQUEN=${CL_GIBBS_PROBSEQUEN:-0.0}
CL_GIBBS_PROBUNIDIR=${CL_GIBBS_PROBUNIDIR:-1.0}
CL_GIBBS_MINPROB1=${CL_GIBBS_MINPROB1:-0.1}
```

```
CL_GIBBS_MINPROB2=${CL_GIBBS_MINPROB2:-0.0}
CL_GIBBS_TWOPT1=${CL_GIBBS_TWOPT1:-1.0}
CL_GIBBS_TWOPT2=${CL_GIBBS_TWOPT2:-1.0}
```

Again CL_PARALLEL_JOBS controls how many linkage groups will be processed in parallel using multiple CPU cores if available. CL_MAP_CYCLES=5 means to run 5 cycles of marker ordering and hk imputation. CL_MAP_RANDOMISE=0 and CL_GA_SKIPORDER1=1 means to skip the very first round of marker ordering, retaining the approximate ordering of the input data produced previously by crosslink_group and proceed straight to hk imputation. For subsequent rounds marker ordering is performed as normal. CL_MAP_RANDOMISE=1 would cause the program to ignore the order of the markers in the input file and start the first round of ordering from a random initial order (this option cannot be used with CL_GA_SKIPORDER1=1, otherwise randomly ordered markers would be passed to the hk imputation step). The remaining options will be explained in detail in the reference section of this document. In summary, this step produces a final marker ordering for each linkage group and also imputes the likely parental origin of the h and k allele for all hk genotypes. The output genotype files contain the markers in map order with hk genotypes listed explicitly as either hk or kh. The final three commands produce the final map positions of the markers, and produce a redundant version of the genotype and map file for each linkage group where the redundant markers have been reinserted at the position of their representative marker:

```
cl_mappos.sh   finalgrps   finalgrps
cl_reinsert_loc.sh finalgrps all.loc all.redun finalredun conf/reinsert.000
cl_reinsert_map.sh   finalgrps   all.redun   finalredun
```

The configuration file contains:

```
CL_GIBBS_SAMPLES=${CL_GIBBS_SAMPLES:-200}
```

```
CL_GIBBS_BURNIN=${CL_GIBBS_BURNIN:-5}
CL_GIBBS_PERIOD=${CL_GIBBS_PERIOD:-1}
CL_GIBBS_PROBSEQUEN=${CL_GIBBS_PROBSEQUEN:-0.0}
CL_GIBBS_PROBUNIDIR=${CL_GIBBS_PROBUNIDIR:-1.0}
CL_GIBBS_MINPROB1=${CL_GIBBS_MINPROB1:-0.1}
CL_GIBBS_MINPROB2=${CL_GIBBS_MINPROB2:-0.0}
CL_GIBBS_TWOPT1=${CL_GIBBS_TWOPT1:-1.0}
CL_GIBBS_TWOPT2=${CL_GIBBS_TWOPT2:-1.0}
```

and controls the process of imputing the hk genotypes of the reinserted markers. The version of the map containing only unique markers is now in the finalgrps subdirectory, with the redundant version in finalredun.

Data Visualisation

crosslink_viewer is the main visualisation tool, which creates coloured plots showing linkage LOD and map distances between all the markers. To use it you must have either successfully compiled Crosslink's data visualisation programs using the viewer_make.sh script during the installation procedure, or be using the Crosslink virtual machine inside VirtualBox or have set up graphical output from the Crosslink docker image. crosslink_viewer can display a single linkage group, a subset of a linkage group or multiple linkage groups at once. It can be set to begin centred on a named marker of interest, and clicking on the plot will output information about the markers to the terminal. The plot itself conveys information only through the colour and brightness of the pixels. For this reason it makes full use of the red, green and blue colour channels. An alternative colour scheme is available by editing the src/viewer_make.sh script and uncommenting (i.e. deleting the initial # from) the line AUX="-DALTCOLSCHEME", changing into the src directory and running the script as ./viewer_make.sh to recompile. This scheme may be more useful for people with red-green colour blindness. The file src/crosslink_viewer.h can also be edited to change the definition of the function setpixelrgb by simply rearranging the position of the variables r, g and b. The descriptions below assume the default colour scheme.

The visualisation programs can work your computer's graphical system quite hard, especially for large datasets, and in my experience are more likely to cause crashes or other problem than the rest of Crosslink. For this reason make sure you have saved any other work you are doing before running them.

crosslink_viewer works by loading the marker data, interpreting it as either unphased, phased or imputed depending on the --datatype option, then rendering three versions of the plot: maternal, paternal and combined information. The plots are fully rendered in memory before being displayed on screen, which can cause a considerable delay before anything appears on screen for large datasets. An all-versus-all marker comparison is performed, meaning that for n markers on the order of n^2 memory and time will be required. The plots are created at full resolution, using the graphics card for scaling, panning and zooming. For this reason you may wish to limit the number of markers you load at once, at least to begin with, or use a computer with more video memory.

To illustrate data visualisation we will go through most of the steps of the example_pipelines/build_rgxha.sh pipeline again, but using only one of the linkage group files as an example. Recall that the first step of the Redgauntlet x Hapil example pipeline formed linkage groups using a simple LOD threshold, and that 21 instead of the expected 28 linkage groups were formed. This was caused by some hkxhk markers which had strong linkage to two different linkage groups, resulting in two or more true linkage groups remaining joined together. The example file we will use here is sample_data/000.loc.gz which was created by running cl_group.sh on the sample dataset sample_data/rgxha.loc.gz with a LOD threshold of 7. The file 000.loc grouped correctly at the first try. After running through all three of the quick start guide sections you will have the knowledge to visualise the groups which did not group correctly to begin with and follow them through the steps of the Real Data example pipeline example_pipelines/build_rgxha.sh. We will begin by

processing 000.loc through all the necessary steps. Starting from a clean working directory as follows:

```
cd ~
mkdir test_crosslink_vis
cd test_crosslink_vis
```

Then run the data through the required pipeline steps (note that because the grouping is already correct we do not need to run cl_detect_crosslg.sh or cl_removemarkers.sh at any point) by entering the following commands:

```
cp -r ${CROSSLINK_PATH}/sample_data/rgxha_conf ./conf
zcat ${CROSSLINK_PATH}/sample_data/000.loc.gz > 000.loc
cl_fixtypes.sh   000.loc   000.fix   conf/fixtypes.000
cl_findredun.sh   000.fix   000.redun   conf/findredun.000
cl_knnimpute.sh   000.fix   000.imp   conf/knnimpute.000
cl_extract.sh   000.imp   000.redun   000.uniq
mkdir -p 000.dir
cp 000.uniq 000.dir/000.loc
cl_order_hkimpute.sh   000.dir   000.final   conf/orderhkimpute.000
cl_mappos.sh   000.final   000.final
cl_reinsert_loc.sh     000.final     000.imp     000.redun     000.finalredun
conf/reinsert.000
cl_reinsert_map.sh   000.final   000.redun   000.finalredun
```

Or run the same commands using the provided script:

```
${CROSSLINK_PATH}/example_pipelines/build_one_group.sh
```

The initial file 000.loc contains the first linkage group output by crosslink_group when run on the sample dataset rgxha.loc. The markers are already phased and approximately ordered. To view only the first 100 markers of this linkage group use the following:

```
crosslink_viewer --inp=000.loc --datatype=phased --total=100
```

This plot should display at the default window size of 1000x1000 pixels. If the window is too large or small for your monitor press ‘q’ to quit and restart using the --window_size option to adjust the window size, for example to use an 800x800 pixel window:

```
crosslink_viewer --inp=000.loc --datatype=phased --total=100 --window_size=800
```

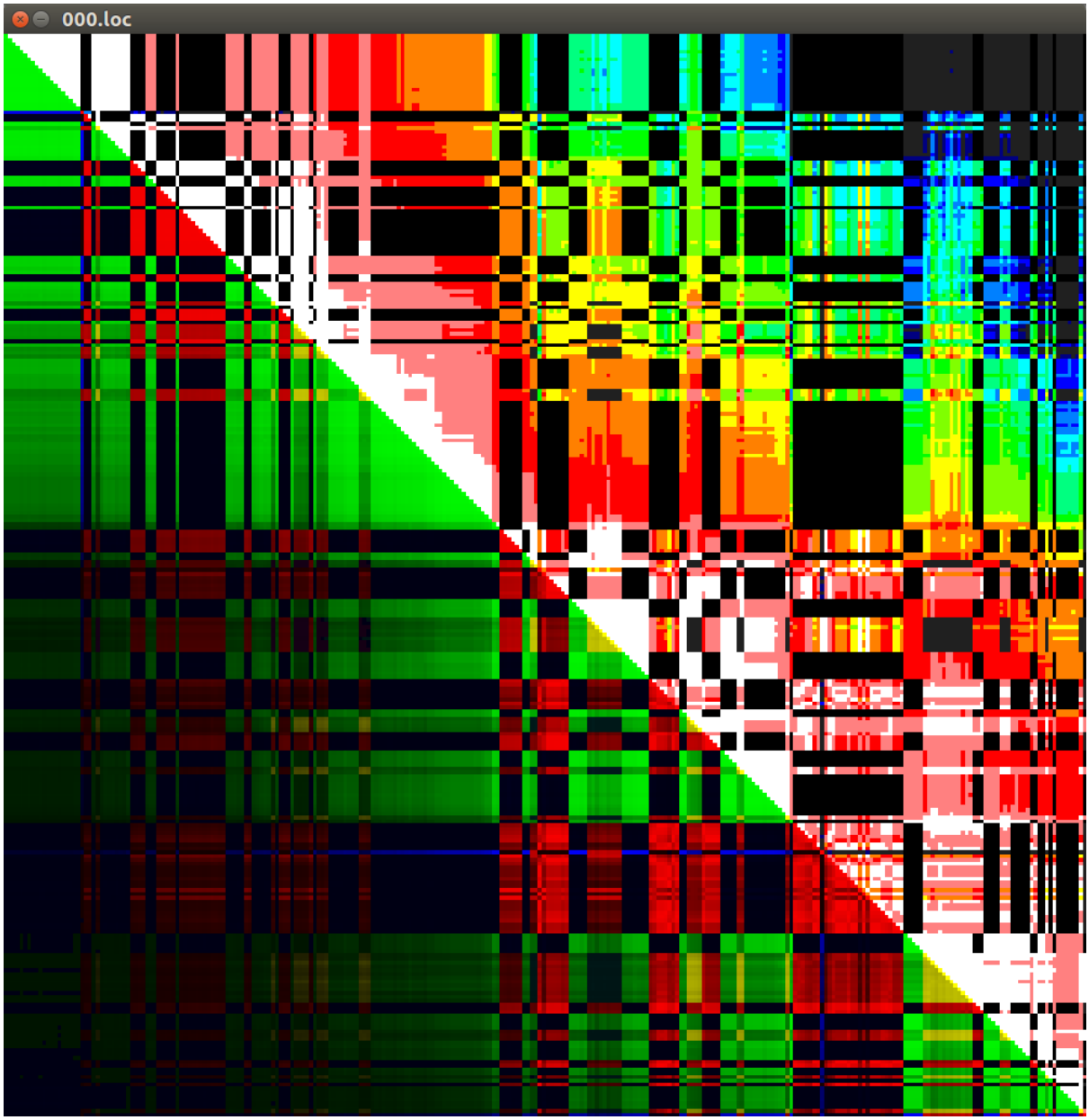
The cursor keys pan the plot, plus and minus zoom in and out, and ENTER re-centres the plot. The combined plot is shown first, press ‘m’ or ‘p’ to switch to the maternal or paternal plots and ‘c’ to return to the combined view. To view the next 100 markers in the linkage group we would use:

```
crosslink_viewer --inp=000.loc --datatype=phased --skip=100 --total=100
```

To view all the markers at once:

```
crosslink_viewer --inp=000.loc --datatype=phased
```

which should look like this:



The markers are represented as coloured squares along the main diagonal with the first at the top left and the last at bottom right. Maternal (lmxll) markers are red, paternal (nnxnp) green and shared (hkxhk) yellow. These squares are always shown at full brightness. Click on a marker, making sure to click only on one of the squares along the main diagonal (you may need to zoom in to be sure you have clicked on the marker you intend) and information about the marker will appear in the terminal, for example:

```
Xaxis: (0)NMH01-89891327 nnxnp {-0} Yaxis: (0)NMH01-89891327 nnxnp {-0}\
    Comp: pat:N=162 rf=0.00000 lod=48.77
```

This tells us the linkage group (0), the marker name (NMH01-89891327), type (nnxnp) and phase ({-0}). In this case we get the same information twice, because, as we clicked on the main diagonal, both the row and column we clicked on correspond to the same marker. We therefore get a comparison of the marker with itself, telling us the paternal information (pat) has N=162 non missing genotype values with an rf of zero (of course) and a LOD of 48.77. There is no maternal information as this is an nnxnp marker. Now click away from the main diagonal. We get information comparing the markers corresponding to the row and column that we clicked on, for example:

```
Xaxis: (0)NMH01-89891327 nnxnp {-0}...
Yaxis: (0)PHR11-89818133 hkxhk {00}...
Comp: pat:N=76 rf=0.03947 lod=17.39
```

This indicates that there were N=76 genotypes available for the comparison, (before hk genotypes have been imputed they are not available and must be treated as missing), showing an rf of 0.03947 corresponding to a LOD of 17.39. Below the main diagonal the square colour shows information about the rf, LOD and phasing between the two markers. In general, to find the square showing the rf/LOD/phasing information between two markers, find the first (higher) of the two markers and move down its column until you reach the row corresponding to the second of the two markers. This square's red channel shows the maternal information and the green channel the paternal. The LOD is represented by the brightness, so a brighter square means a larger LOD. Squares corresponding to a pair of lmxll markers therefore indicate the LOD between them as the red brightness, squares corresponding to a pair of nnxnp markers as the green brightness and squares corresponding to a pair of hkxhk markers as the red and green brightness, resulting in a yellow square. Comparing an lmxll with an hkxhk allows only the maternal information of the hkxhk to be used, therefore the square will be red, likewise an nnxnp and an hkxhk

comparison can only use the paternal information and is represented as a green square. Comparing an lmxll with an nnxnp marker does not normally make sense, as the markers should not show any linkage. However, due to the polyploid nature of the strawberry, some markers are misclassified as lmxll when they are actually nnxnp and vice versa. To visualise this phenomenon crosslink_viewer *does* perform a linkage analysis between the maternal information of all lmxll and the paternal information of all nnxnp markers. These squares show the linkage LOD with the blue brightness, making it easier to pick out these likely errors. Blue is also used to indicate likely phasing errors (for comparisons other than lmxll versus nnxnp). When treating the data as phased by passing option --datatype=phased, if the rf value between two markers is in disagreement with their declared phases, this may indicate a phasing error. For example, if two linked lmxll markers have been assigned different phases (i.e. one is {0-} and the other is {1-}) then we expect the rf to be greater than 0.5, conversely if they have the same phase we expect the rf to be less than 0.5. If the rf is found to be in agreement with the phase of the two markers then the usual colour channel is used, whereas if a disagreement is found the colour channel is switched to blue. Therefore an lmxll to lmxll square would be blue instead of red and an nnxnp to nnxnp blue rather than green. For an hkxhk to hkxhk comparison there are two independent phases. If both checks fail the square changes from yellow to blue, if only the maternal phase check fails the red channel changes to blue but the green channel stays green resulting in a cyan colour, if only the paternal check fails the green channel changes to blue resulting in a purple colour.

If you zoom in a little and pan along the main diagonal you should notice that three of the markers are showing bright blue squares against some of the other markers. Click on some of the blue squares below the main diagonal and see if you can work out what is going on from the rf, LOD and marker type information displayed to the terminal. Three lmxll markers are showing a strong linkage to many of the nnxnp markers, causing the corresponding squares to appear bright blue, but lack any strong linkage to other lmxll markers, causing the corresponding squares to appear almost black, all of which indicates they are likely

misclassified nnxnp markers. There are no other bright blue squares, suggesting that the remaining markers do not have any obvious phasing errors.

Summary of crosslink_viewer colour scheme (if compiled using default colour mode) when viewing phased data, for the bottom left half of the plot which shows rf/LOD/phasing information. This applies to combined mode (press 'c' to switch to this mode). Increasing brightness indicates increasing linkage LOD.

Marker 1	Marker 2	Correct Phasing	Incorrect Phasing
lmxll	lmxll	red	blue
nnxnp	nnxnp	green	blue
hkxhk	hkxhk	both correct => yellow	**maternal error => cyan** **paternal error => purple** **both in error => blue**
lmxll	hkxhk	red	blue
nnxnp	hkxhk	green	blue
If *one* of the two markers has been incorrectly typed:			
lmxll	nnxnp	blue	blue

If we are viewing fully hk imputed data using the --datatype=imputed option the colour scheme is the same as the above. If we are viewing unphased data using the --datatype=unphased option then the colour scheme is interpreted slightly differently. Because there are no declared phases to be check now the blue channel is simply used to represent where the rf value is greater than 0.5 indicating a likely repulsion phase. So in the table above we simply change the meaning of "correct phasing" to "coupling phase" and "incorrect phasing" to "repulsion phase". So for an hkxhk to hkxhk comparison a yellow square would indicate coupling phase in both maternal and paternal genomes for example. If

viewing in maternal or paternal mode (by pressing ‘m’ or ‘p’) then only information about the respective parent will be shown so, for example, in maternal mode an hkxhk to hkxhk square will appear as red, showing only information about the maternal part of the genotypes.

The bottom left half of the plot therefore shows information about rf, LOD and phasing, and is useful for detecting grouping, phasing and marker typing errors. However the implied map distance between two markers is not linearly related to the LOD, making subtle problems with marker ordering difficult to spot. Above the diagonal a colour scheme is used to represent the centimorgan distance between the markers which is designed to make problems with the marker ordering easier to see. The rf value is converted into a centimorgan distance using the Haldane mapping function and displayed on a heatmap scale where white represents the shortest distances and grey the longest, with black indicating no information (between lmxll and nnxnp markers).

Colour scheme used in the top right half of the crosslink_viewer plot, which displays map distance information, calculated using the Haldane mapping function.

Colour	Distance Range (cM)
white	< 10
pink	10 - 20
red	20 - 30
orange	30 - 40
yellow	40 - 50
yellow-green	50 - 60
green	60 - 70
green-cyan	70 - 80
cyan	80 - 90
cyan-blue	90 - 100

blue	100 - 110
dark blue	110 - 120
grey	> 120
black	no information

000.fix contains the same markers after typing errors were fixed using cl_fixtype.sh, view it now using:

```
crosslink_viewer --inp=000.fix --datatype=phased
```

which should look the same as the previous plot except that the erroneously typed markers have now been recoded as the correct type. To check this view the uncorrected plot, click on an affected marker to get its name, then view the corrected file using the above command but add the --marker option to specify that the affected marker should appear in the centre of the view, e.g.:

```
crosslink_viewer --inp=000.fix --datatype=phased --marker=NMH10-89914030
```

The next steps identified redundant markers (cl_findredun.sh), imputed missing genotype calls (cl_knnimpute.sh) and extracted the imputed versions of the non-redundant markers (cl_extract.sh) producing 000.uniq which contains 126 instead of 284 markers, and contains no missing calls. View it using:

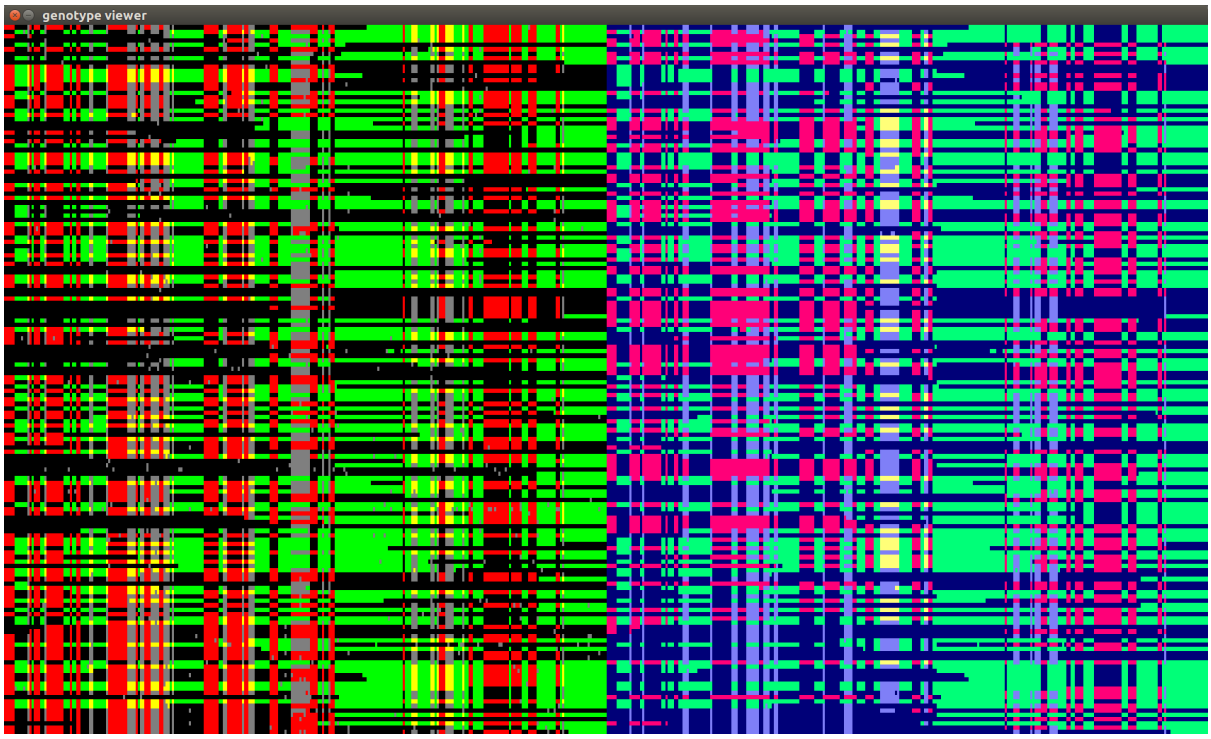
```
crosslink_viewer --inp=000.uniq --datatype=phased
```

To compare the data before and after missing calls have been imputed we can use the crosslink_graphical command, which displays a simple colour coded representation of the genotype values. The following command will open 000.fix and 000.imp (the data before and

after missing value imputation) side-by-side in a window 1700x1000 pixels. You may wish to adjust the size to fit your monitor:

```
crosslink_graphical --inp="000.fix 000.imp" --datatype=phased\
                    --sizex=1700 --sizey=1000
```

which should look like this (this figure shows the default combined-phased mode):



Each row represents a progeny and each column a marker. The slight blue colouration on the right indicates that these columns are the data from the second genotype file (000.imp). Use ‘m’, ‘p’ and ‘c’ to change between maternal, paternal and combined views, and ‘z’ to which between phased and unphased views. In *unphased* view mode a red or green colour represents an m or p allele for an lmxll or nnxnp marker respectively, whereas a black or dark blue rectangle represents the complementary allele (l or n); and for hkxhk markers yellow, red, green and black/dark blue represent the genotypes kk, kh, hk and hh respectively. For data lacking imputed hk information, as in the present example, grey is used to represent hk/kh genotypes. In *phased* mode red and green represent a phase 0 allele m or p or a phase 1 allele l or n. For hkxhk markers the red and green channels

represent the maternal and paternal information, so the red channel is activated if there is a maternal phase 0 k or phase 1 h, and the green channel activated if there is a paternal phase 0 k or phase 1 h. If red and green are active a yellow colour results, if neither are active a black / dark blue colours results. Grey also represents any missing genotype calls.

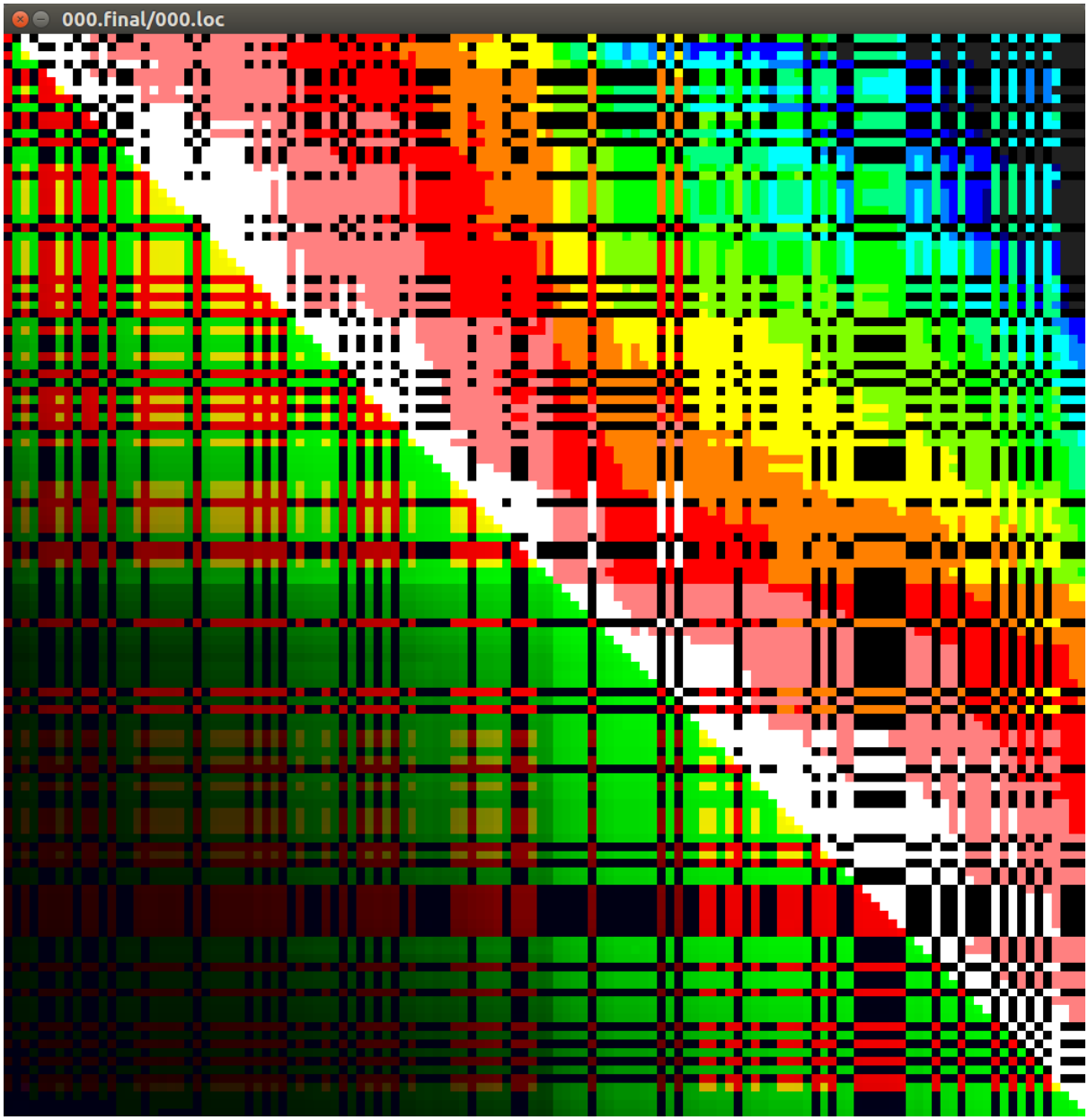
As with crosslink_viewer plus/minus perform zooming, cursor keys pan and ENTER recentres the view. Shift combined with a cursor key can be used to shrink or grow the plot in one direction only. You should be able to see a few grey squares in the left plot (000.fix), these represent the missing values which have all been imputed in the right plot. Identifying markers/progeny by clicking on them is not yet implemented in this program.

As a quirk of the way that the helper script pipeline works by calling crosslink_group internally, the process of imputing missing values actually also repeats the process of phasing and approximate ordering. (The advantage of breaking the crosslink_group functionality down into separate steps is that the full dataset can be used to impute missing values at the same time as identifying redundant markers in the non-imputed data, so that markers with less missing data are retained preferentially). You will therefore be able to see in the above figure that the maternal information in 000.fix is phased using the opposite convention to those in 000.imp (i.e. phase 0 in 000.fix is called phase 1 in 000.imp), whereas the paternal markers have been assigned the same phasing convention in both files (you may have experienced a different outcome when you ran the pipeline, as a small degree of randomness can arise in imputing missing values). The markers are also in a slightly different order. The get around this problem press ‘m’ to switch to maternal-only view, then press ‘z’ to switch to unphased view. Now both datasets should look almost the same, except for the slight difference in ordering.

The final version of the non-redundant markers was produced using cl_order_hkimpute.sh to refine the map order and impute the hk genotype missing information. View this version of the file using:

```
crosslink_viewer --inp=000.final/000.loc --datatype=imputed
```

which should look like this:

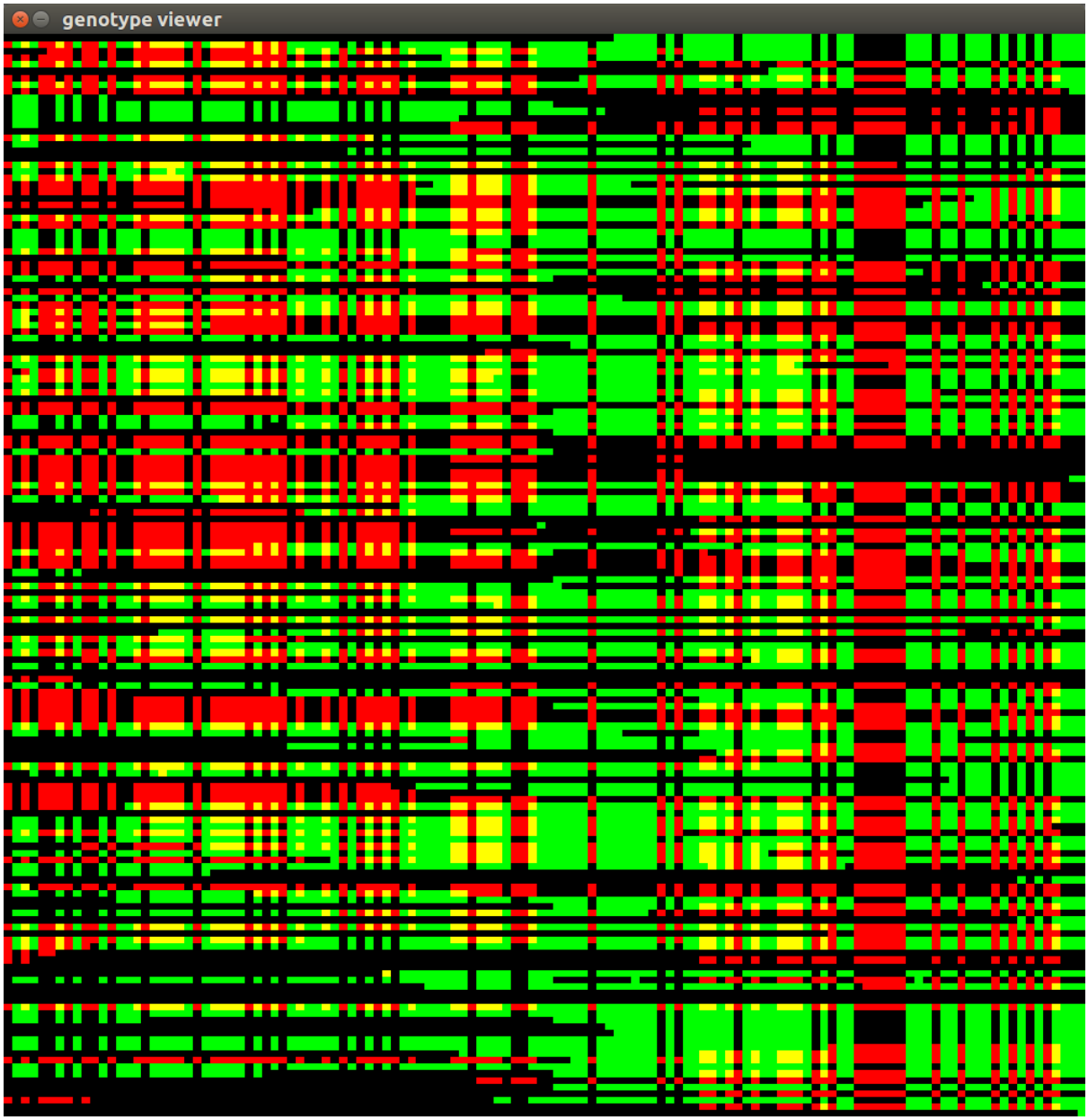


To check the ordering switch to maternal-only view by pressing ‘m’ and check that the map distances increase monotonically away from the main diagonal, then repeat this process for the paternal map by pressing ‘p’.

View the same data in crosslink_graphical using:

```
crosslink_graphical --inp=000.final/000.loc --datatype=imputed
```

which should look like this:



Switch between combined, maternal and paternal views (by pressing ‘c’, ‘m’ and ‘p’) to check that the ordering and hk imputation seem correct (there will be a few remaining errors as Crosslink does not currently support error correction). Finally the redundant markers were reinserted into the ordering producing the redundant version of the final ordering, viewable using:

```
crosslink_viewer --inp=000.finalredun/000.loc --datatype=imputed
```

As well as viewing single files, crosslink_viewer can display multiple genotype files at the same time. To view all the linkage groups from the build_rgxha.sh pipeline at the same time (be aware this will use a lot more video memory than the previous single linkage group examples) we would use a command like this executed in the directory where you ran the full build_rgxha.sh pipeline:

```
crosslink_viewer --inp="$(echo initgrps/*.loc)" --datatype=phased
```

To view each linkage group one at a time:

```
for x in initgrps/*.loc
do
      crosslink_viewer --inp=${x} --datatype=phased || break
done
```

In this example pressing 'q' quits the current linkage group and moves on to the next one whereas pressing 'escape' breaks out of the for loop without viewing anymore. This works because crosslink_viewer returns an exit status (accessible in bash as the $? variable) of 0 if 'q' is pressed, but a status of 100 if 'escape' is pressed. If you have a large batch of file to view and want to automatically process each one differently depending on a decision made by a user viewing the files, crosslink_viewer also exits when any of the number keys 0-9 are pressed with an exit status of ten plus the number of the key pressed.

Export To JoinMap® Compatible Files

Given a subdirectory containing the final genotype (*.loc) and map (*.map) files, the following commands will export the data into JoinMap compatible loc and map files:

```
cl_loc2joinmap.sh   final_lgs    jm.loc
cl_map2joinmap.sh   final_lgs    jm.map
```

Commands and Options

crosslink_group

```
    --inp=STRING input genotype file (required)
    --outbase=STRING basename for output genotype files (optional)
    --mapbase=STRING basename for output map files (optional)
    --redun=STRING filename for outputting marker redundancy information (optional)
    --log=STRING filename for outputting logging information (optional)
    --seed=UNSIGNED random number generator seed, 0=use system time (default: 1)
    --map_func=UNSIGNED mapping func, 1=Haldane,2=Kosambi (default: 1)
    --randomise_order=UNSIGNED start from a random initial marker ordering
      (default: 0)
    --bitstrings=UNSIGNED use bitstring data representation internally (default: 1)
    --matpat_lod=FLOAT minimum LOD used to identify spurious linkage between
      maternal and paternal markers, 0.0=disable (default: 0.0)
    --matpat_weights=STRING conditional weightings to give markers when correcting
      marker typing errors
      eg 01P03L05 gives default weight of 1 but 3 to markers starting with P and 5
      for those starting with L (default: 01)
    --min_lod=FLOAT minimum linkage LOD to use when forming linkage groups
      (default: 3.0)
    --em_tol=FLOAT for 2 point rf calculations, convergence tolerance for EM
      algorithm (default: 1e-5)
    --em_maxit=UNSIGNED for 2 point rf calculations, max EM iterations (default:
      100)
    --knn=UNSIGNED how many nearest neighbours to use for kNN missing data
      imputation, 0=disable imputation (default: 0)
    --ignore_cxr=UNSIGNED 1=ignore cxr and rxc linkage during grouping (default: 0)
    --redundancy_lod=FLOAT minimum linkage LOD to use when identifying redundant
      markers, 0.0=disable (default: 0.0)
```

This program, implemented in C, performs the first part of the mapping pipeline, consisting of: Two-point rf and LOD Calculation, Form Linkage Groups, Fix Marker-Typing Errors, Phasing, Impute Missing Values, Approximate Marker Ordering and Combined Map Positions.

Data is read from the genotype file named by required option --inp. Option --seed is used to seed the integer pseudo-random number generator using the C function call srand. The floating point pseudo-random number generator is then seeded using srand48(rand()). A seed value of zero causes the microsecond resolution system time to be used instead via a gettimeofday function call, giving different random values for each invocation. The default seed is 1, causing the same behaviour each invocation, provided the genotype data is identical and no other options are changed. Option --bitstrings=1 causes the genotype calls to be encoded in memory using a compact bitstring representation which may execute faster on some machines (enabled by default, using --bitstrings=0 to disable). Option --randomise_order=1 causes the order of the genotypes to be randomised as soon as they are loaded (disabled by default). This may occasionally leading to a better approximate ordering of the markers for some datasets. Note this will be the same “random” ordering every time if the --seed option is left at the default. If provided logging messages are output for the file named by option --log, otherwise no log messages are generated.

(1) Two-point rf and LOD Calculation

This step estimates rf and linkage LOD between all pairs of markers using the so-called two-point rf method, by treating all hk genotypes of hkxhk markers as missing. Estimating rf values between pairs of hkxhk markers involves using an iterative expectation-maximisation algorithm. Options --em_tol and --em_maxit control the tolerance and maximum iterations respectively. Any LOD found to be less than the value of option --min_lod is ignored and not

stored in memory. hkxhk to hkxhk rf/LOD estimation sometimes fails to provide complete phasing information as cxr and rxc phases cannot be distinguished, but given sufficient marker density, phasing can likely be worked out indirectly via other markers. An option, --ignore_cxr=1, is provided which causes cxr and rxc hkxhk to hkxhk linkage to be ignored. In a dense map nearby markers will often have identical genotype calls, or differ only in the pattern of missing calls. An option, --redundancy_lod, specifies a LOD threshold above which two markers are checked to see if one can be deleted without losing any information, which can speed up rf/LOD calculations for some datasets. A list of redundant markers will be output to the file named by option --redun if set, this file will have two columns contains the names of redundant markers followed by the names of the remaining non-redundant markers representing them. To deal with lmxll markers incorrectly classified as nnxnp and vice versa an option, --matpat_lod, is provided. If enabled, rf and LOD values are calculated between the maternal information of lmxll markers and the paternal information of nnxnp markers, and linkage is retained if the LOD meets the specified threshold value (which should be greater than --min_lod), and will be used in steps (2) and (3) below.

(2) Form Linkage Groups

This step forms linkage groups consisting of all markers connected (directly or indirectly) by edges from step (1) (i.e. via LOD values above --min_lod and, if linkage between lmxll and nnxnp markers was enabled, above --matpat_lod between these markers). If the --ignore_cxr option is disabled then cxr and rxc linkage between pairs of hkxhk markers contributes to linkage group formation even though it will not directly provide phasing information. Markers are split into their respective linkage groups which are processed separately for the remaining steps.

(3) Fix Marker-Typing Errors

The --matpat_lod option when set greater than zero activates an error correction method to resolve lmxll markers incorrectly typed as nnxnp and vice versa, the --matpat_weights option allows markers to receive a different reliability weighting based on the first letter of the marker name. Using the marker linkages identified during step (1), but excluding all linkage involving hkxhk markers, markers are formed into subgroups. For a well-covered linkage group with no marker typing errors one subgroup of maternal markers and one of paternal would be expected. The correct marker type of each subgroup is assumed to be that of the most common type it contains, after taking account of the weighting system. The option --matpat_weights must be set to a string starting with two digits defining the default weight followed by any number of weight categories consisting of a single character matching the first character of the marker's name followed by two digits. For example to set the default weight to 10, and a category for markers starting with X with a weight of 20 and one for markers starting with Y with a weight of 95 the option would be --matpat_weights=10X20Y95. Weights are therefore limited to integers in the range 0 to 99 inclusive. In the event of a tied score markers are arbitrarily set to maternal type. If option --matpat_weights is omitted all markers have a weight of 1.

(4) Phasing

lmxll and nnxnp markers require one phase each whereas hkxhk markers require a maternal and paternal phase. The phases calculated here are purely relative, so that the same marker phase value could be assigned as 0 or 1, but should always be assigned the same phase relative to the other markers in its linkage group. Phasing can be estimated between any pair of markers that show linkage (except for cxr and rxc linkage between hkxhk markers) based on the rf value being greater than or less than 0.5, but is more reliable between markers with

a high LOD value. Maternal and paternal phasing is performed separately, so that the same algorithm is run twice for each linkage group, hkxhk markers taking part in both runs.

For each of the two phasing passes (maternal and paternal), markers are joined together into a minimum spanning tree (MST) using the relevant linkages identified in step (1) in descending order of LOD, such that the LOD of the weakest edge in the tree is minimised. If cxr and rxc linkage was allowed (--ignore_cxr=0) these linkages are given lower priority than any other linkage, because they provide only partial phasing information. A warning is written to the logfile (if option --log is being used) if the MST required the using any cxr/rxc linkage. Phasing is aborted with a warning if a single tree was not produced after all edges were used.

An arbitrary starting point marker is assigned to phase 0 and phasing is propagated throughout the tree using a depth-first search, propagating the same phase value across linkages where rf is less than 0.5 and switching to the other phase value where rf is greater than 0.5. If cxr/rxc edges are encountered the phase information is incomplete and is simply guessed as always being cxr (i.e. coupling in the maternal and repulsion in the paternal), which will be incorrect on average 50% of the time. If cxr/rxc linkage is required to correctly form linkage groups at a stringent LOD threshold but causes incorrect phasing, crosslink_group can be run a second time on each linkage group separately to rephase them excluding cxr/rxc linkage with a lower LOD threshold (helper script cl_phase.sh assists this process).

(5) Impute Missing Values

Missing genotype calls are imputed using the k nearest neighbour algorithm, whereby missing calls are set to the mean value of the k most similar markers which do not lack the call. Set the value of k using option --knn. By default --knn=0 disabling missing data imputation. Small odd values, such as 1 or 3 are likely to give better results, but performance will obviously depend on marker density, genotyping errors and the proportion of missing data. Only markers connected by edges from step (1) are considered as potential neighbours. hk genotype calls are treated as missing but are not imputed here. Similarity is defined using a lower bound of the rf rather than the maximum likelihood rf to guard against overestimation between marker pairs with many missing values. Specifically the rf is estimated assuming missing values differ with a probability of 0.5 (mimicking unlinked markers). If no neighbours are found or there is a tie the value will be imputed to a random value and a warning written to the log file. With polyploid genomes often the loss of whole chromosomes can be tolerated, meaning some progeny could be aneuploid. If so all the marker calls for one chromosome could be missing, and would be filled with random values, which should be obvious from the log file or from examining the linkage group using crosslink_graphical. In such a case the linkage group should have the effected progeny removed and then be reprocessed.

(6) Approximate Marker Ordering

The markers are approximately ordered using a modified version of the MST method employed by MSTMap. This involves building a map-distance minimising spanning-tree, extracting a longest path through the tree as the map backbone, then collapsing any remaining side branches onto the backbone at their point of attachment. This overall procedure is performed separately for maternal and paternal maps, which will then be merged in the next step. The MST is built using Kruskal's algorithm using the linkages from step (1) sorted into ascending order by inter-marker map distance (option --map_func

selects either the Haldane or Kosambi function). As hk genotypes of hkxhk markers must be treated as missing at this stage in the mapping process, a simple implementation of the MSTMap's approach would cause the backbone to form along a path where missing hk values are masking the largest possible number of recombination events, and therefore likely distort the true ordering. To counter this Kruskal's algorithm is run with the edge list sorted so that linkage involving hkxhk markers appear below those involving only lmxll and nnxnp markers, as the latter edges cannot mask recombination events to the same extent. Since the linkage groups were formed using both maternal and paternal markers there is no guarantee that the separate maternal and paternal MST will span the full linkage group. If this is the case the approximate ordering is abandoned (crosslink_map can still be used to produce a final ordering). Once the MST is built a longest path is extracted using two depth-first searches. The first search starts at an arbitrary marker and finds the most distant marker from the starting point. The second search starts at this marker and finds the marker most distant from it in turn. The map backbone path is extracted by backtracking from this second marker to the first, and is traversed to assigned map positions to backbone markers. Finally, a depth-first search from each backbone marker of any side branch markers assigns them the same position as the associated backbone marker.

(7) Combined Map Positions

A combined map is produced using the maternal and paternal maps created in the previous step, following the method used by JoinMap®. In order to produce a useful combined map there must be at least two hkxhk markers (preferably widely spaced) otherwise it is not possible to work out the relative orientations of the two maps. A warning is written to the log file if any linkage group has less than 2 hkxhk markers. Otherwise, the covariance between the maternal and paternal positions of all hkxhk markers is found, and the paternal map positions are inverted if the covariance is negative. hkxhk marker combined positions are set

to the average of their maternal and paternal map positions. lmxll and nnxnp marker combined positions are interpolated between the nearest two flanking hkxhk markers, or extrapolated from the nearest hkxhk if they are not flanked by two. Finally, the markers are sorted into the order defined by their combined map position.

This approximate ordering defines the marker order in the output genotype and map files. For each linkage group the genotypes are written to a file named as the option --outbase followed by the linkage group number with a .loc extension. The corresponding maps are output to files names as the --mapbase option with a .map extension. These files are not written unless the --outbase and --mapbase options are explicitly set.

crosslink_map

```
--inp=STRING input genotype file (required)
--out=STRING output genotype file (optional)
--log=STRING output log file (optional)
--map=STRING output map file (optional)
--seed=UNSIGNED random number generator seed, 0=use system time
  (default: 1)
--map_func=UNSIGNED mapping func, 1=Haldane,2=Kosambi (default: 1)
--randomise_order=UNSIGNED start from a random initial marker ordering
  (default: 0)
--bitstrings=UNSIGNED use bitstring data representation internally
  (default: 1)
--ga_gibbs_cycles=UNSIGNED number of GA-Gibbs cycles (default: 5)
--ga_iters=UNSIGNED number of GA iterations per GA-Gibbs cycle (default:
  100000)
```

```
--ga_use_mst=UNSIGNED how many GA-Gibbs cycles to perform initial MST
  ordering before the GA (0=none,N=up to and including the Nth cycle)
  (default: 999)
--ga_minlod=FLOAT min LOD for MST construction and global order
  optimisation scoring (default: 3.0)
--ga_mst_nonhk=UNSIGNED prioritise non-hk linkage when building the MST
  (default: 0)
--ga_optimise_meth=UNSIGNED 0=optimse map total recombination events,
  1=optimise total map distance, 2=optimise a global measure of map
  quality (sets --ga_skip_order1=1 --randomise_order=0) (default: 0)
--ga_prob_hop=FLOAT probability a mutation moves a single marker
  (default: 0.333)
--ga_max_hop=FLOAT max distance a single marker can move as proportion
  of whole linkage group (default: 0.1)
--ga_prob_move=FLOAT probability a mutation moves a block of multiple
  markers (default: 0.333)
--ga_max_mvseg=FLOAT max number of markers in the block as proportion of
  whole linkage group (default: 0.1)
--ga_max_mvdist=FLOAT max distance the block of markers can move as
  proportion of whole linkage group (default: 0.1)
--ga_prob_inv=FLOAT probability the block of markers also inverts as
  well as moves (default: 0.5)
--ga_max_seg=FLOAT for in-place inversion mutations, max number of
  markers to be inverted as proportion of whole linkage group (default:
  0.1)
--ga_cache=UNSIGNED 1=use cache of rf values in GA (default: 1)
--ga_em_tol=FLOAT for 2 point rf calculations, convergence tolerance for
  EM algorithm (default: 1e-5)
--ga_em_maxit=UNSIGNED for 2 point rf calculations, max EM iterations
  (default: 100)
--ga_skip_order1=UNSIGNED 1=skip first GA ordering, go straight to Gibbs
  using the marker order from the input file (default: 0)
--ga_report=UNSIGNED GA log reporting period, 0=disabled (default: 0)
```

```
--gibbs_samples=UNSIGNED number of Gibbs samples to collect per GA-Gibbs
  cycle (default: 300)
--gibbs_burnin=UNSIGNED Gibbs burn in cycles (default: 10)
--gibbs_period=UNSIGNED Gibbs cycles per sample (default: 1)
--gibbs_prob_sequential=FLOAT probability Gibbs cycle uses sequential
  mode (default: 0.0)
--gibbs_prob_unidir=FLOAT probability Gibbs cycle uses unidirectional
  mode (default: 1.0)
--gibbs_min_prob_1=FLOAT minimum permitted probability of a state
  transition at the start of burn in period (default: 0.1)
--gibbs_min_prob_2=FLOAT minimum permitted probability of a state
  transition by the end of burn in period (default: 0.0)
--gibbs_twopt_1=FLOAT weighting given to two point rf at start of burn
  in period (default: 1.0)
--gibbs_twopt_2=FLOAT weighting given to two point rf by the end of burn
  in period (default: 1.0)
--gibbs_report=UNSIGNED Gibbs log reporting period, 0=disabled (default:
  0)
--homeo_minlod=FLOAT detect cross homeolog markers, minlod (default:
  1.0)
--homeo_maxlod=FLOAT detect cross homeolog markers, maxlod (default:
  16.0)
--homeo_mincount=UNSIGNED report as possible cross homeolog if
  implicated more than this many time, 0 to disable (default: 0)
```

This program, also implemented in C, performs the second part of the mapping pipeline, which produces the final map ordering, imputes the missing information for hk genotypes and calculates final map positions. It also has a method to detect cross-linkage-group hkxhk markers which have strong linkage to more than one true linkage group. It loads only one linkage group per invocation, from the genotype file specified by required option --inp. The final hk-imputed genotypes in their final ordering are output to a file specified by option --out,

with final map positions going into the file given by option --map and with optional logging information going to a file specified by --log. The --seed , --randomise_order , --bitstrings and --map_func options have the same function as for crosslink_group (see above). On multicore systems multiple linkage groups can to processed in parallel (helper script cl_order_hkimpute.sh can be used to facilitate this).

The two main steps are (1) ordering the markers using a genetic algorithm (GA) and (2) imputing the missing hk information using a Gibbs sampler. These steps are alternated the specified number of times. Option --ga_gibbs_cycles controls the number of cycles of GA+Gibbs that are run, --ga_iters controls how many iterations of the GA are run each cycle, and --gibbs_burnin, --gibbs_samples and --gibbs_period control the iterations of the Gibbs sampler (see below). The --ga_skip_order1 option allows the first round of GA ordering to be skipped, so that the approximate ordering output from crosslink_group will be used directly by step (2) before any further ordering changes are made. If not skipped, the first round of ordering will use two-point rf values (treating hk genotypes as missing). All subsequent ordering rounds use multipoint rf values from the imputed hk information. Final map positions are calculated in the same way as crosslink_group following the method of JoinMap, by averaging the maternal and paternal positions of the shared hkxhk markers, and interpolating/extrapolating the positions of lmxll and nnxnp markers based on the nearest hkxhk marker(s). Detection of cross-linkage-group hkxhk markers takes place before the final cycle of marker ordering.

(1) Improve Marker Ordering With A Genetic Algorithm

The genetic algorithm works by scoring the optimality of the current marker ordering, making a random change to the ordering, then reassessing the score. If the score improves or stays

the same the new ordering is accepted, otherwise the change is rejected. The idea behind accepting neutral changes is that they may allow the ordering to escape from local optima.

For each of the cycles specified by option --ga_gibbs_cycles (minus the first cycle if --ga_skip_order1 is enabled) the GA runs for --ga_iters iterations, trying one mutation per iteration. There are three available scoring methods, all of which take account of both maternal and paternal information simultaneously: --ga_optimise_meth=0 minimises the sum of recombination events in maternal and paternal maps, --ga_optimise_meth=1 minimises the combined map lengths of the maternal and paternal maps, quantised to 100ths of a centimorgan, and --ga_optimise_meth=1 maximises a metric which rewards small recombination fractions between markers which are also nearby in the current ordering. The third method provides more of a "global" picture of the ordering optimality by comparing all against all markers (with a LOD threshold) instead of just adjacent ones, but is more computationally intensive for this reason. For options 0 and 1, each time a pair of markers are compared the calculated recombination count or map distance is cached (disabled using --ga_cache=0) so that next time those two markers are compared the value will not need to be recalculated. For the global scoring method, at the start of each GA round a list of rf and LOD values are calculated by comparing all markers against all others, those with LOD values lower than option --ga_minlod are dropped, the otherwise the rf values are converted into map distances. To find the overall score for a map ordering each map distance stored in the list is given a score which disproportionately rewards short map distances which are also nearby in the ordering. Longer map distances and/or larger separations in the ordering reduce the score. The best score is obtained by having as many short map distances correspond to nearby ordering positions as possible. This will penalise any strongly linked markers which are widely separated by the ordering even where all immediately adjacent pairs have low recombination counts. The overall score for the ordering is the sum of all the individual scores.

Option --ga_use_mst, when set to greater than zero, causes the markers to be pre-ordered using the same MST method as use by crosslink_group at the start of GA cycles. This process is not supported for the first GA cycle (as it is assumed that the ordering given by crosslink_group would be available already). It is possible to have all subsequent cycles approximately ordered, or only up to a given cycle number. For example, numbering the GA cycles starting from 0, setting --ga_use_mst=3 would cause cycles numbers 1, 2 and 3 to use approximate ordering, but subsequent cycles to not use it. The specified number of GA iterations are always performed regardless of whether pre-ordering is used. Option --ga_mst_nonhk=1 causes the pre-ordering to prioritise linkages not involving hkxhk markers, whereas --ga_mst_nonhk=0 (the default) pre-orders making full use of hkxhk markers at the same priority as the other marker types.

If the first round of ordering is not skipped then rf values are calculated using the same two-point methods as crosslink_group, with the EM algorithm tolerance and maximum iterations defined by parameters --ga_em_tol and --ga_em_maxit respectively.

Three types of mutation operator are provided: single marker hop, segment move and segment inversion, chosen with probabilities controlled by options --ga_prob_hop and --ga_prob_move (with the probability of inversion being the remainder after subtraction from 1.0). A single marker hop moves a single marker a random distance up to a proportion of the total marker count controlled by option --ga_max_hop. A segment move moves a randomly chosen contiguous block of markers with a size from 1 up to a proportion of the total marker count controlled by option --ga_max_mvseg, a random distance up to a proportionate maximum controlled by option --ga_max_mvdist, and inverts the block with a probability --ga_prob_inv. A segment inversion inverts a randomly chosen block of markers in-place with a size of 2 up to a proportionate maximum controlled by option --ga_max_seg.

If logging is enabled by option --log, the option --ga_report controls how often the current ordering score is written to the log file, --ga_report=0 disables reporting the ordering score, whereas values greater than 0 report the score every nth iteration.

At the start of the final cycle of marker ordering if option --homeo_mincount is greater than zero then detection of cross-linkage-group hkxhk markers takes place.

In our allo-polyploid strawberry data we have encountered a few hkxhk markers whose maternal information comes from one linkage group while the paternal information comes from one of the homeologous linkage groups. These cases are detected by comparing pairs of hkxhk markers, where we expect the markers to be linked according to one parent but unlinked according to the other. For a pair of hkxhk markers if one parent indicates a LOD below --homeo_minlod and the other a LOD above --homeo_maxlod then both markers are given one point. Markers accumulate points for each qualifying hkxhk marker pair they are involved in. Those that receive a total number of points greater than or equal to --homeo_mincount are flagged as being cross linkage group markers, and reported in the log file (provided logging is enabled).

(2) Impute hk Information By Gibbs Sampler

For each hkxhk marker genotype called as hk it is unknown whether the h allele came from the maternal and the k allele from the paternal parent or vice versa. Without this information the rf values cannot be known as accurately, as all hk genotypes must be treated as missing. Here using the convention of listing the maternal allele first, the two possible imputed forms of the genotype call will be written as HK and KH, using uppercase to indicate they have been imputed (note lowercase is still used in the actual genotype files even for imputed markers). The marker is present in both the maternal and paternal maps, therefore both need to be considered simultaneously. The most likely choice between HK or KH

depends on the current marker ordering, whether either imply a recombination before and/or after the marker and the current recombination fractions between the previous and next markers in the maternal and paternal maps. The problem then is to find the most probable set of choices for all the hk genotypes considered jointly. A Gibbs sampler can address this problem by initially randomising all hk genotypes, and then resampling each individually in a random order many times. The final state of each is then set to the most common during resampling, discarding an initial burn-in period. Resampling an hk genotype entails assuming its true state is unknown but that all other states are known and correct, calculating the probabilities of HK and KH given the neighbouring marker states, and assigning the state to either HK or KH with the respective probabilities.

Three implementations are provided all based on the basic Gibbs sampler algorithm: (A) a Gibbs sampler which resamples hk genotypes in a completely random order, (B) a Gibbs sampler which resamples states sequentially along the current map order one progeny at a time, treating the progeny in a random order, and in either forward or reverse map order at random (C), a simplified heuristic which resamples sequentially per progeny in the same manner as method B but only takes account of the preceding marker states as it progresses.

Each Gibbs cycle begins by randomising all hk genotype states. Then, during a burn-in period, the states are resampled a given number of times (controlled by option --gibbs_burnin) using one or more of methods A-C. A counter associated with each hk genotype is then increment if the state is currently HK or decremented if it is KH. Then the states are resampled again a given number of times (controlled by option --gibbs_period) and the counters updated again. This is repeated until the required number of samples (controlled by option --gibbs_samples) have been obtained. For example if --gibbs_burnin is 20, --gibbs_period is 1 and --gibbs_samples is 300 then every state will be resampled 20 times during the burn-in before the first counter update, then exactly once between all

subsequent counter updates until each counter has been updated a total of 300 times. Therefore each Gibbs iteration consists of resampling each hk state exactly once.

Each iteration the method of resampling is chosen from the three available methods (controlled by options --gibbs_sequential, the probability of using method B, and --gibbs_unidir, the probability of method C, the probability of A being the retainder after subtraction from 1.0), such that any one method can be used exclusively or a random mixture of some or all.

The initial burn-in period is used to allow the states to converge from their initial random values towards a more optimal part of the state space. Some additional options are provided to fine tune the resampling process during burn-in. Firstly, to prevent the state getting stuck prematurely in a suboptimal part of state space, options --gibbs_min_prob_1 and --gibbs_min_prob_2 control the minimum probability that a resampled hk state will change to the other value. For the very first iteration of burn-in the minimum probability is --gibbs_min_prob_1, for the last burn-in iteration it is --gibbs_min_prob_2, and for intermediate iterations the probability changes linearly from the former to the latter. After burn-in, during normal iterations, the minimum probability remains at --gibbs_min_prob_2. At the start of burn-in, multipoint rf values are available immediately, but are initially determined from the randomly chosen hk states, consequently the two-point rf values may be more accurate until some point through the burn-in period. For this reason, when resampling hk states a weighted average of the multipoint and two-point rf values is used to calculate the state probabilities. Two control parameters are provided, --gibbs_twopt_1 and --gibbs_twopt_2, the weighting given to two-point values being changed linearly from the first to the second over the course of the burn-in period and remaining at the second value during normal iterations.

If logging is enabled by option --log, the option --gibbs_report controls how often the current total number of recombination events is written to the log file, --gibbs_report=0 disables reporting, whereas values greater than 0 report the count every nth iteration.

(3) Calculate Final Map Positions

This step follows the same procedure as the final step of the crosslink_group program.

crosslink_pos

```
--inp=STRING name of input genotype file (required)
--out=STRING name of output map file (optional)
--seed=UNSIGNED random number generator seed, 0=use system time (default: 1)
--map_func=UNSIGNED mapping func, 1=Haldane,2=Kosambi (default: 1)
--bitstrings=UNSIGNED use bitstring data representation internally (default: 1)
```

This program loads genotype data from the file specified by option --inp and, treating the data as fully hk-imputed genotypes, calculates the final map positions. This is a convenience function in that it only implements a subset of the functionality of crosslink_map, and the options it shares have the same meaning.

crosslink_viewer

```
--inp=STRING input genotype file(s) (required)
--window_size=UNSIGNED window size (pixels) (default: 1000)
--datatype=STRING state of the genotype data: imputed, phased, unphased (default:
   imputed)
--bitstrings=UNSIGNED 1=use bitstring representation of the data internally
   (default: 1)
--hardware=UNSIGNED 1=use hardware graphical acceleration when available
   (default: 0)
--skip=UNSIGNED how many markers to skip at the start of the genotype file
   (default: 0)
```

```
    --total=UNSIGNED how many markers to load in total, 0=load all (default: 0)
    --marker=STRING named marker to centre intial view on (optional)
```

This program allows visualisation of the pattern of LOD, phasing and map distances between markers on one or more linkage groups. Input is read from a list of one or more genotype files specified with option --inp. To view more than one linkage group at once surround the space-separated file list with double or single quotes. The markers are treated as unphased, phased or fully hk-imputed according to whether option --datatype is set to unphased, phased or imputed respectively. --window_size specified the width and height of the plot in pixels (excluding an window decoration). --bitstrings has the same meaning as for crosslink_group. --hardware=1 activates hardware accelerated rendering is available. --skip=N allows the first N markers to be dropped and --total=M means to retain only the next M markers in total (useful for viewing part of a large dataset). --marker allows the initial view to be centred on a particular named marker. See the Quickstart Guide section for a explanation of the coloured plot produced by this program.

The control keys to control the plot are:

```
CURSOR KEYS  = pan
+/-          = zoom
ENTER        = recentre
m,p,c        = maternal,paternal,combined mode
q,escape,0-9 = quit with various bash exit codes ($?)
```

crosslink_graphical

```
    --inp=STRING input genotype file(s) (required)
    --sizex=UNSIGNED window width (pixels) (default: 1000)
    --sizey=UNSIGNED window height (pixels) (default: 1000)
    --datatype=STRING state of the genotype data: imputed, phased, unphased
      (default: imputed)
    --bitstrings=UNSIGNED 1=use bitstring representation of the data
      internally (default: 1)
    --hardware=UNSIGNED 1=use hardware graphical acceleration when available
      (default: 0)
    --skip=UNSIGNED how many markers to skip at the start of the genotype
      file (default: 0)
    --total=UNSIGNED how many markers to load in total, 0=load all (default:
      0)
```

This program displays the pattern of genotypes along one or more linkage groups. Options are the same as for crosslink_viewer except: option --sizex controls only the width and --sizey only the height of the plot in pixels. The control keys are:

```
CURSOR KEYS         = pan
SHIFT+CURSOR KEYS   = reshape
+/-                 = zoom
ENTER               = recentre
m,p,c,z             = maternal,paternal,combined,phased/unphased mode
q,escape,0-9        = quit with various bash exit codes ($?)
```

See the Quickstart Guide section for an explanation of the coloured plot produced.

create_map

```
    --output-file=STRING output map specification file (required)
    --random-seed=UNSIGNED random number generator seed (0=use system time)
      (default: 0)
```

```
--numb-lgs=UNSIGNED divide map into this number of equally sized linkage
  groups (default: 10)
--map-size=FLOAT total map size in centimorgans (default: 100.0)
--marker-density=FLOAT average markers per centimorgan (default: 1.0)
--prob-both=FLOAT probability marker is heterozygous in both parents
  (default: 0.3333)
--prob-maternal=FLOAT probability marker is heterozygous only in
  maternal parent (default: 0.333)
```

This program outputs a map specification file to the file specified by option --output-file. --random-seed has the same meaning as the --seed option of crosslink_group, except the default value is 0 (meaning to use microsecond resolution system time). --numb-lgs determines the number of linkage groups, all of equal size. --map-size determines the total map length in centimorgans. --marker-density is the average number of markers per centimorgan. --prob-maternal is probability that a marker is a maternal (lmxll) marker, --prob-both is the probability of a shared (hkxhk) marker, the probability of a paternal (nnxnp) marker is the remainder after subtraction from 1.0. The map specification file is used by sample_map to create simulated outcross genotype data.

sample_map

```
--input-file=STRING input map specification file (required)
--output-file=STRING output genotype file including any errors
  (required)
--orig-dir=STRING output grouped genotype files without any errors
  (optional)
--random-seed=INTEGER random number generator seed, 0=use system time
  (default: 0)
--samples=INTEGER number of offspring to simulate (default: 200)
--prob-missing=FLOAT probability a genotype call is missing (default:
  0.0)
--prob-error=FLOAT probability a genotype call is incorrect (default:
  0.0)
--map-function=INTEGER 1=Haldane, 2=Kosambi (default: 1)
```

This program takes a map specification file (--input-file) and produces an output file (--output-file) containing simulated outcross genotype data containing optional errors, plus an option set of files in a subdirectory (--orig-dir) containing the same markers correctly grouped and ordered without an errors. --random-seed has the same meaning as for create_map. --samples determines the number of progeny to simulated from the cross. --map-function determines which mapping function to assume (Haldane or Kosambi) when calculating the probability of recombination from map distances. --prob-missing determines the probability that a genotype call will be set to missing and --prob-error that a call will be set to an incorrect value. This program does not simulate any allo-polyploidy related errors (see create_type_errors.py in the helper script section below for this functionality).

Helper Scripts

It was originally intended that the two programs crosslink_group and crosslink_map would each have to be run only once on the data to produce a final map. Later it became clear that more flexibility was advantageous. Ideally all the functionality would be broken out into separate functions accessible via a Python or R interface and available to run in any order. The helper scripts described in this section provide a similar functionality, but work by simply calling the two main programs internally and ignoring any of the output they don't need. Other scripts also automate common tasks such as renaming linkage groups to match an existing reference map. See the example pipeline dealing with real data in the QuickStart section for examples of how to use some of the helper scripts and for a description of the configuration files used by some of them. Example configuration files are also provided in the directory ${CROSSLINK_PATH}/sample_data/rgxha_conf. In general if a directory name is required as an input then all files with a .loc extension in the directory will be treated as linkage groups. If a directory name is required for output then generally .loc genotype files or .map files will be output to that directory. The last option for many of the scripts is

configuration file containing the parameters to be passed to crosslink_group and/or crosslink_map.

cl_adjustlgs.sh <input-dir> <vsref-file> <output-dir>

Renames and reorients linkage groups to match a reference map. All .loc files in the input directory will be processed. The vsref file is a file created by cl_match2ref.sh and specifies the new name to be given to each linkage groups and whether it should be reoriented. The new .loc files will be written to the output directory, which is created if required.

cl_break.sh <input-file> <marker-name> <output-dir> <conf-file>

Break the linkage group in the input genotype file into two parts after the named marker, and output the new linkage groups into the output directory.

cl_detect_crosslg.sh <input-dir> <output-file> <conf-file>

Detect cross-linkage-group hkxhk markers in all .loc files in the input directory, output a list of their names to the output file. Reads configuration options from conf-file.

cl_extract.sh <input-file> <redun-file> <output-file>

Extracts from the input genotype file all markers not listed as redundant in the redun-file (created by crosslink_group or cl_findredun.sh) and output them to a new output genotype file.

cl_findredun.sh <input-file> <output-file> <conf-file>

Run crosslink_group on the input genotype file with the parameters defined in the conf-file, output a list of redundant markers to the output file.

cl_fixtypes.sh <input-file> <output-file> <conf-file>

Fix marker typing errors in input genotype file using parameters from conf-file, output corrected genotypes to output file. Can safely overwrite the original if output filename set same as input.

cl_group.sh <input-file> <output-dir> <min-lod>

Form markers from input genotype file into linkage groups based on the specified LOD threshold, output to new linkage group files in the output directory, which is created if required. Any existing .loc or .map files in the output directory are **deleted** first to avoid retaining any stale files from a previous grouping attempt.

cl_hkimpute.sh <input-file> <output-file> <conf-file>

Impute hk genotypes in the input genotype file without changing marker order and output to a new genotype file.

cl_knnimpute.sh <input-file> <output-file> <conf-file>

Impute missing genotype calls using k nearest neighbour method for markers in input genotype file, output to a new genotype file. Note: marker order may not be preserved.

cl_loc2joinmap.sh <input-dir> <output-file>

Convert all the .loc genotype files from the input directory into a JoinMap compatible .loc file.

cl_map2joinmap.sh <input-dir> <output-file>

Convert all the .map files from the input directory into a JoinMap compatible .map file.

cl_mappos.sh <input-dir> <output-dir>

For each .loc file in the input directory create a .map file containing the map positions in the output directory (which can be the same as the input directory). Does not alter marker ordering.

cl_match2ref.sh <input-dir> <refmap-file> <ps2snp-file>

This script is specific to the type of markers used in the Redgauntlet x Hapil dataset. Match all the per-linkage group .map files in the input directory to linkage groups in the reference map described in the refmap file. ps2snp-file is a file matching probesetids to snpids for the microarray used to genotype the samples.

cl_merge.sh <input-dir> <merge-file> <output-dir> <conf-file>

Each line of the merge file contains a space separated list of linkage group names (i.e. filenames from the input directory with the .loc extension removed) that will be merged and placed in the output directory. The extension of the original .loc files are changed to .old and the name of the new file is formed by joining the names of the original files with underscore characters (_). The new linkage group is then rephased, ordered and hk-imputed.

cl_modifymarkers.sh <input-file> <output-file> <mat2pat-file> <pat2mat-file>

For markers named in file mat2pat-file manually change the marker type from lmxll to nnxnp and for those named in file pat2mat-file change type from nnxnp to lmxll. Markers are read from the input genotype file and written to the output file. Markers names must appear one per line in the mat2pat and pat2mat files.

cl_order_hkimpute.sh <input-dir> <output-dir> <conf-file>

Produce final marker ordering and impute missing hk information for each .loc genotype file in the input directory, writing output to the output directory, which can be the same as the input directory. By setting parameter CL_PARALLEL_JOBS in the conf-file to greater than 1, multiple linkage groups can be processed in parallel on multicore systems.

cl_phase.sh <input-dir> <output-dir>

Force phasing to complete for each .loc genotype file in the input directory, write output to output directory which can be the same as the input directory.

cl_refine_order.sh <input-dir> <output-dir> <trials> <parallel-jobs> <conf-file> [<conf-file>...]

For each .loc genotype file in the input directory, run map ordering and hk-imputation 'trials' times for each of the list of one or more configuration files and retain only the best map obtained, writing it to the output directory, which can be the same as the input directory. Process 'parallel-jobs' linkage groups in parallel. If the configuration option CL_IGNORE_PREVIOUS is set to 1 in the first configuration file then the best *new* ordering is retained regardless of whether it is worse than the original, otherwise if

CL_IGNORE_PREVIOUS=0, the original ordering is retained unless a better one is found. Orderings are scored based on the combined lengths of the maternal and paternal maps.

cl_reinsert_loc.sh <input-dir> <all-markers> <redun-file> <output-dir> <conf-file>

For each .loc genotype file in the input directory, reinsert redundant markers from all-markers file using redundancy information from redun-file, and write output to output directory, which can be the same as the input directory. Reinserted markers are rephased and hk-imputed but the ordering of the original markers is not changed.

cl_reinsert_map.sh <input-dir> <redun-file> <output-dir>

For each .map file in the input directory, reinsert redundant markers into the map at the same position as their representative non-redundant marker, without reordering the map, write output to output directory, which can be the same as the input directory.

cl_removemarkers.sh <input-file> <output-file> <bad-markers-file>

Filter out markers names one-per-line in the bad markers file from the input genotype file and write to the output file which can be the same as the input file.

cl_subgroup.sh <input-file> <min-lod> <output-dir> <conf-file>

Regroup markers in the input genotype file at the specified LOD threshold, write new linkage group(s) to the output directory. Each new linkage group is rephased, ordered and hk-imputed.

Glossary

lmxll - a marker which is heterozygous in the maternal parent but homozygous in the paternal

nnxnp - a marker which is heterozygous in the paternal parent but homozygous in the maternal

hkxhk - a marker heterozygous in both parents

rf - recombination fraction, the proportion of progeny showing recombination between two markers

LOD - the (linkage) logarithm of odds, a measure of the statistical significance of the linkage between two markers, specifically the base-10 logarithm of the ratio of probabilities that the markers are truly linked versus unlinked

GA - genetic algorithm

MST - minimum spanning tree