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# 1. Introduction

The purpose of this document is to provide an overview of the SNPviewer software from LGC. This software was developed to enable customers to view results from in-house genotyping projects run at LGC and is available as a free download from our website. SNPviewer provides a simple means to graphically view the results as genotyping cluster plots. A Cartesian plot is generated using the FAM and HEX fluorescent values; FAM is plotted on the X-axis and HEX is plotted on the Y axis. Genotyping calls displayed in SNPviewer cannot be edited.

To be able to download SNPviewer you must first create a user account on our webpage. Click [here](#) to set-up an account and [here](#) to download SNPviewer.

## 2. Getting started

1. This section of the guide details how to open the SNPviewer software and how to import a SNPviewer results file. LGC genotyping results files are provided in a ZIP format, and are designed to be viewed with the SNPviewer application.
2. To open SNPviewer, click on the desktop icon.  Alternatively, select 'KBiosciences' from the Start menu of Windows and then click on 'Snpviewer2'.
3. Once SNPviewer opens, you will be prompted to open a genotyping results file. Navigate to the data file (ZIP or CSV format) that you wish to view, and click 'OK'.
4. The data will be imported into SNPviewer.

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### 3. Exploring your genotyping data

This section of the guide explains how to view a genotyping cluster plot, to customise the menu tree, and to search for an assay or plate ID within the project.

Once your data set has opened within SNPviewer, you will see a similar window to that shown in Figure 1. The DNA master plates and assays used in your project will be displayed in the menu tree on the right hand side of the screen.

To view the data for a particular assay or DNA master plate, navigate to the relevant location within the menu tree and click on the plate / assay to display the associated genotyping cluster plot.

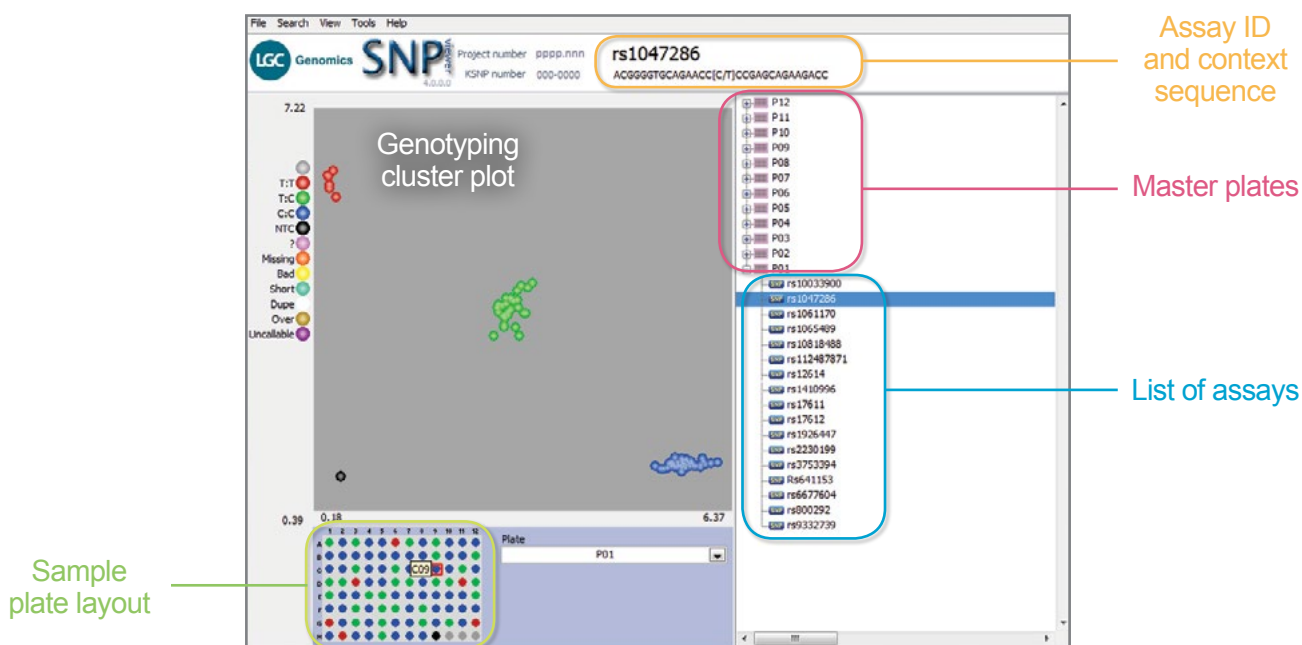
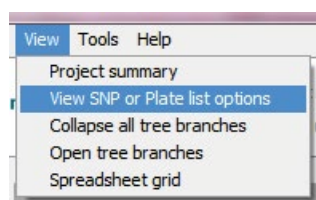
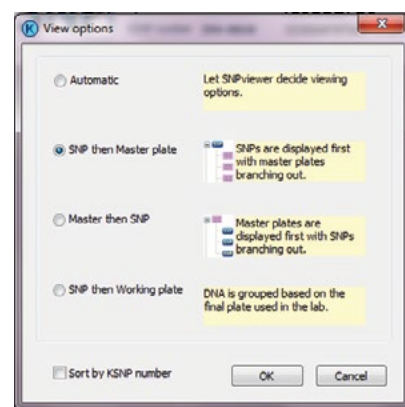


Figure 1: SNPviewer window. In the tree on the right hand side, each assay and DNA master plate is listed. Cluster plots are displayed by clicking on the relevant plate in the menu tree. Blue data points are homozygous for the allele reported by FAM, green data points are heterozygous and red data points are homozygous for the allele reported by HEX. The black data points represent the no template controls (NTC). The DNA sample plate layout is shown below the cluster plot.

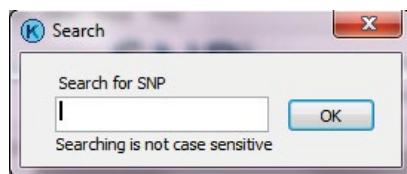


You can change the order within which the data tree is displayed. To do this, click on 'View' in the top menu bar and select 'View SNP or Plate list options'.

A dialogue box will open with the four available options for viewing data. Select your preferred option and click 'OK'.



To view the raw data values for a particular DNA sample on a cluster plot, place the cursor over the relevant well on the DNA plate layout or the relevant data point on the cluster plot. The raw data will be displayed in the yellow dialogue box (Figure 2).



To search for an assay ID or DNA plate within your project, click on 'Search' in the top menu bar. Type in the assay or plate name that you wish to search for (search is not case sensitive), and click 'OK'. The first instance of the search term will be selected within your project.

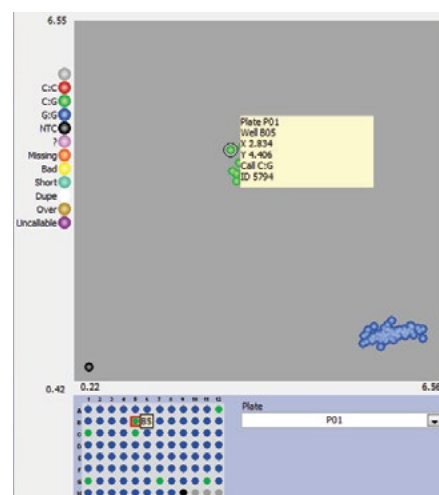


Figure 2: Viewing raw data on the SNPviewer cluster plot. The raw data (X and Y signal) for well B5 is displayed in the yellow dialogue box, in addition to the genotyping call and the sample ID.

## 4. Interpreting the genotyping calls

Each well on the plate is colour coded to indicate the genotyping call that has been assigned to the DNA sample. Table 1 provides a key to the colours used on the genotyping cluster plot.

Type	Title	Details
	Empty	Wells that have deliberately not been filled and are to be ignored in the analysis.
	1:1	Samples genotyped as homozygous for Allele 1 (reported by FAM on the X-axis). The genotype specific to the assay is displayed on the legend e.g. T:T
	1:2	Samples genotyped as heterozygous (both FAM and HEX signal generated). The genotype specific to the assay is displayed on the legend e.g. T:C
	2:2	Samples genotyped as homozygous for Allele 2 (reported by HEX on the Y-axis). The genotype specific to the assay is displayed on the legend e.g. C:C
	NTC	No template controls. These are wells that do not contain any template DNA and will therefore not generate any fluorescent signal. They will be positioned in the bottom left corner of the cluster plot.
	Unused	Samples that have not been assigned a genotyping call because they did not generate consistent signal or failed to amplify. These will be identified with '?' in the .csv results file.
	Missing	Wells that were expected to contain DNA samples but that have been manually flagged as missing samples.
	Bad	Samples that consistently provide unreliable genotyping results across several assays. This indicates an inherent problem with the DNA.

Table 1: Key to the colours used in the SNPviewer cluster plot and plate map.

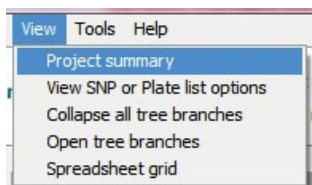
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## 5. View a project summary

The 'Project summary' option within SNPviewer can be used to display an overview of the project being viewed in SNPviewer including percentage call rate and total number of data points.



To view a project summary, click on 'View' in the top menu bar. Select 'Project summary'.

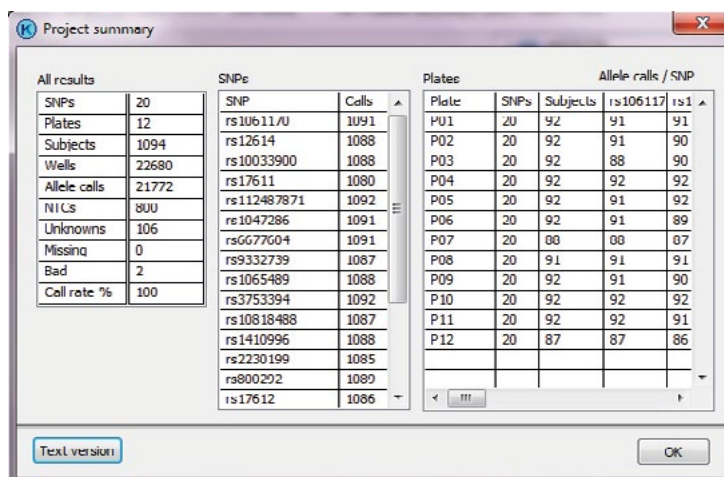


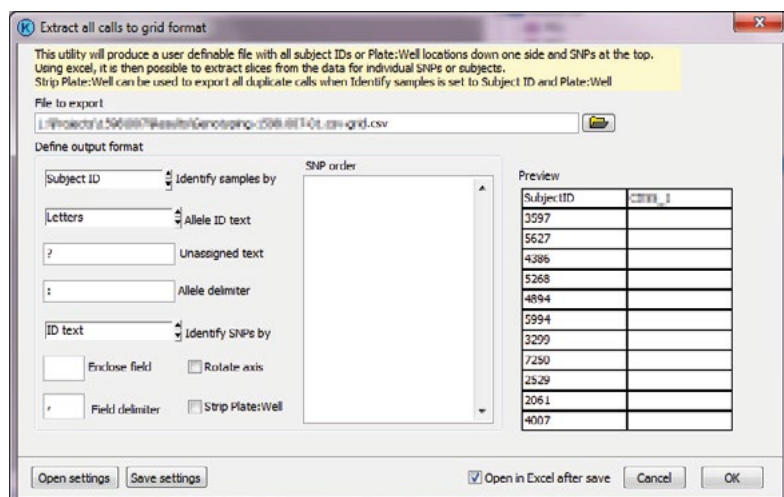
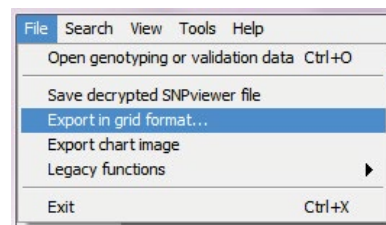
Figure 3. A 'Project summary' window for a genotyping project viewed in SNPviewer. This can be exported as a text file by clicking on 'Text version'.

A dialogue window will open containing a summary of the genotyping project that is currently being viewed within SNPviewer (Figure 3). This will detail a range of parameters including the total number of assays and plates within your project, the number samples designated as 'bad' and the percentage call rate.

## 6. Export of data

This section of the guide provides details on how to customise genotyping data for export to a CSV file.

Data can be exported from SNPviewer in a grid format. Click on 'File' in the top menu bar and select 'Export in grid format...'



This will open a dialogue window within which the user can define preferences for data export (Figure 4).

Figure 4. Dialogue box for customisation of data export from SNPviewer

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Customise the options for the data export to suit your preferences.

- Identify samples by:
  - Subject ID – the unique sample name supplied by the customer
  - Plate:Well – reference to the DNA master plate and well location
  - Subject ID Plate:Well – unique sample name, plus DNA master plate and well location
- Allele ID text
  - Letters – allele calls will be identified with two letters e.g. A:A, A:C, C:C
  - Letter single – allele calls will be identified with one letter, hence IUPAC codes will be used for the heterozygous calls e.g. A, M, C.
- Unassigned text – this text will be used when an allele call has not been assigned to a sample. The default text for this is a question mark ‘?’.
- Allele delimiter – this text will be used between the two bases of an allele call. The default text for this is a semicolon ‘:’ e.g. A:A. You can change this to a different delimiter if required e.g. A-A, A.A.
- Identify SNPs by:
  - ID text – the unique assay name supplied by the customer
  - KB SNP numbers – the assay ID number assigned by LGC
- Enclose field – this gives the option to enclose the allele calls with text such as inverted commas e.g. ‘A:C’.
- Field delimiter – this is used to separate each allele call. The default delimiter is a comma.

Once you have set up the export parameters to your preferences, you can save these settings for future exports by clicking on the ‘Save Settings’ button.

Click ‘OK’ to save your data export file. If ‘Open in Excel after save’ is ticked, your exported data will automatically open in Excel. The exported grid file will look similar to the data shown in Figure 5.

The table shows a grid of genotyping data. The columns are labeled 'DNA Assay' and contain assay IDs: rs1234, rs5678, rs9101, rs1213, rs1415, rs1617, rs1819, rs2021, rs2223, rs2425, rs2627, rs2829. The rows are labeled 'Sample1' through 'Sample19'. Each cell contains a genotype call, such as 'T:T', 'C:C', 'T:C', 'T:T', 'T:A', 'C:C', 'G:G', 'G:G', 'T:G', 'C:T', 'G:A', 'T:C', 'C:C', 'T:C', 'T:C', 'T:T', 'T:T', 'T:C', 'G:A', 'G:G', 'T:G', 'C:T', 'G:A', 'T:C', 'C:C', 'T:C', 'T:C', 'T:T', 'T:T', 'T:T', 'C:C', 'G:G', 'G:G', 'G:G', 'C:C', 'G:A', 'C:C', 'C:T', 'C:C', 'T:C', 'T:C', 'T:A', 'T:C', 'G:A', 'C:G', 'G:G', 'C:T', 'G:A', 'T:C', 'T:T', 'T:C', 'T:C', 'T:C', 'T:T', 'T:C', 'A:A', 'G:G', 'G:G', 'C:C', 'G:A', 'T:T', 'C:C', 'C:C', 'C:C', 'C:C', 'T:T', 'T:C', 'G:G', 'G:G', 'G:G', 'C:C', 'G:A', 'C:C', 'C:C', 'T:C', 'T:T', 'T:C', 'T:T', 'C:C', 'G:G', 'G:G', 'T:G', 'C:T', 'G:A', 'T:C', 'C:T', 'C:C', 'T:C', 'C:C', 'G:A', 'G:G', 'G:G', 'C:C', 'G:G', 'T:C', 'T:T', 'T:C', 'G:G', 'G:G', 'G:G', 'C:C', 'G:A', 'T:T'.

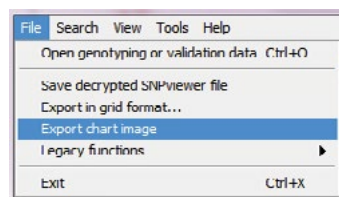
Figure 5. Grid file of genotyping data exported from SNPviewer and viewed in Excel.

Genotyping calls in grid layout

## 7. Export of cluster plot

Genotyping cluster plots can be exported from SNPviewer as bitmap (BMP) image files.

To export an image file containing a cluster plot from SNPviewer, click on 'File' in the top menu bar and select 'Export chart image'.



You will then be prompted to name and save the image file. The image will be exported as a bitmap file, as illustrated in Figure 6.

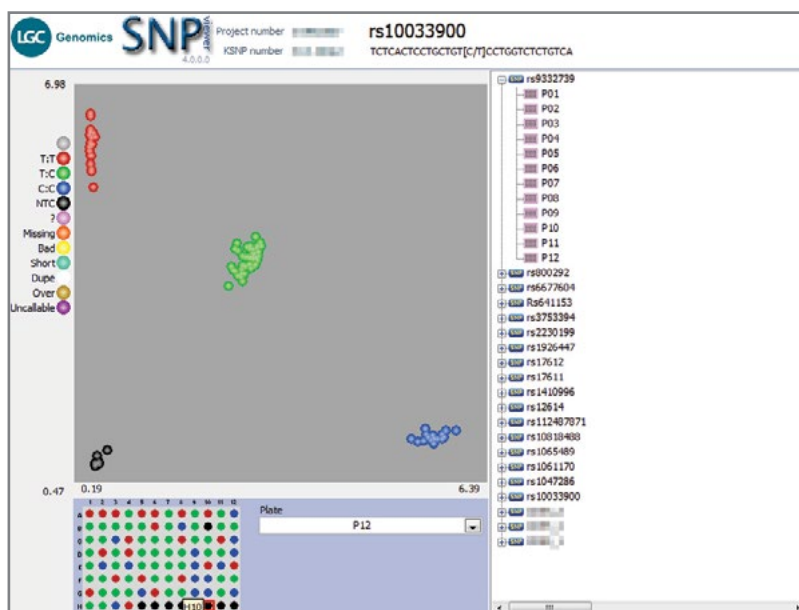


Figure 6. Cluster plot image file exported from SNPviewer.

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