applied biosystems

HID SNP Genotyper Plugin

USER GUIDE

v5.2.2

for use with:
Precision ID Ancestry Panel
Precision ID Identity Panel
Precision ID IonCode™ 1–96 Kit in 96 Well PCR Plate

Catalog Numbers A25642, A25643, A33586
Publication Number MAN0010641
Revision D.0

For Research, Forensic, or Paternity Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

Manufacturer: Multiple Life Technologies Corporation manufacturing sites are responsible for manufacturing the products associated with the workflow covered in this guide.

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The information in this guide is subject to change without notice.

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Validation notice: The following Applied Biosystems™ panels have been internally tested but have not been validated under SWGDAM guidelines: Precision ID Ancestry Panel and Precision ID Identity Panel.

Revision history: Pub. No. MAN0010641

Revision	Date	Description
D.0	19 July 2017	• Updated for the 5.5.2 version of the plugin. • Added Appendix B, "Experiments and Results". • Added Appendix C, "Configure the plugin to add Google Maps API key". • Web links for downloading panel files and obtaining help updated.
C.0	17 June 2016	• Product names of Applied Biosystems™ panels and kits for HID applications updated. • For use with Precision ID Library Kit removed. • For use with IonCode™ Barcode Adapters 1–384 Kit added. • Procedures and file names to reflect updated software revised. • Download and install sections streamlined.
B.0	July 2015	• New panel analyses added: Admixture Prediction, Population Likelihoods, 1000 Genomes, and Y Haplogroup Prediction. • Procedures to reflect updated software revised. • Chapters reorganized.
A.0	June 2014	New document.

Contents

1 Plugin overview

Function of the plugin

The HID SNP Genotyper Plugin is a software plugin that runs on the Torrent Server and configured on the Torrent Browser. The plugin analyzes barcoded runs, then finds the genotype at positions specified in the hotspot file. The plugin is based on the Torrent Variant Caller, but unlike the Torrent Variant Caller, it analyzes only hotspot locations, detects only SNPs, and does not detect indels.

The HID SNP Genotyper Plugin currently supports the Precision ID Ancestry Panel and the Precision ID Identity Panel. The plugin produces an HID SNP Genotyper Report that summarizes the data and analysis. The data can be exported for additional analysis.

Plugin features

- Admixture prediction using Ancestry Informative Markers (AIMs) for seven continental populations
- Y haplogroup identification
- Quality checks (QC) for each SNP
- Random Match Probabilities (RMP) calculation using the 1000 Genomes data set

Access Torrent Suite™ Software information

Visit **thermofisher.com/torrent-suite-software-docs.html** to access *Torrent Suite™ Software Help* and current Release Notes.

Torrent Suite™ Software is required for sequence analysis of libraries prepared with Precision ID Library Kits. Once installed, the HID SNP Genotyper Plugin can be used to call genotypes in the genomic regions covered by Precision ID panels.

System requirements

Requirements to run the HID SNP Genotyper Plugin:

- Ion S5™ System—Torrent Suite™ Software 5.2.2
- Ion PGM™ System—Torrent Suite™ Software 5.2.2

Requirements to access the data generated from the HID SNP Genotyper Plugin:

- Google™ Chrome™

  Note: The plugin performs optimally using the Google™ Chrome™ web browser. This application is not recommended for use with other web browsers.

2 General procedures

Install the plugin

1. To install the plugin, sign in to the Torrent Server via the Torrent Browser.
2. Select ⚙ ▼, then click **Plugins**.
3. Click **Install or Upgrade Plugin**.
4. In the **Install or Upgrade Plugin** dialog, click **Select File**, navigate to and select the HID_SNP_Genotyper_v5_2_2 file, then click **Upload and Install**.
5. When the upload completes, refresh the web page to ensure that the upload was successful.
6. In the HID_SNP_Genotyper row, select the checkbox to enable the plugin.
   See *Torrent Suite™ Software Help* for further information.

Download and install hotspots and targets files

Download the BED files

1. Go to **www.ampliseq.com**, then click **Human Identification** under **Pre-designed Panels**.
2. Click on the **Sign in to order** button for the appropriate HID panel, then log in with your Thermo Fisher Scientific account information.
3. Click on the **Download panel files** button, then **Download Now**.
   Panel-specific target and hotspot BED files are downloaded:
   - Precision ID Ancestry Panel
     - PrecisionID_AncestryPanel_targets.bed
     - PrecisionID_AncestryPanel_hotspots.bed
   - Precision ID Identity Panel
     - PrecisionID_IdentityPanel_targets.bed
     - PrecisionID_IdentityPanel_hotspots.bed

When the download is complete, install the targets and hotspots *.bed files.

Install the configuration files in Torrent Suite™ Software

Note: Do not install the *.json file; it is installed in a later setup stage.

1. Log in to the Torrent Server via the Torrent Browser.
2. Select ⚙ ▾, then click **References**.
3. In the left navigation pane, select **Target Regions**, then click **Add Target Regions**.
4. Click **Select File**, then navigate to and select the PrecisionID_AncestryPanel_targets.bed file.
5. From the **Reference** dropdown list, select **hg19 - Homo sapiens hg19**.
6. (*Optional*) Enter a description and notes.
7. Click **Upload Target Regions File**.
8. Refresh the web page to ensure the upload was successful.
9. In the left navigation pane, select **Hotspots**, then click **Add Hotspots**.
10. Click **Select File**, then navigate to and select the PrecisionID_AncestryPanel_hotspots.bed file.
11. From the **Reference** dropdown list, select **hg19 - Homo sapiens hg19**.
12. (*Optional*) Enter a description and notes.
13. Click **Upload Hotspots File**.
14. Refresh the web page to ensure the upload was successful.
15. Repeat steps 2–14 to install the PrecisionID_IdentityPanel_targets.bed and PrecisionID_IdentityPanel_hotspots.bed files.

Run the plugin

Two types of runs are available for the HID SNP Genotyper Plugin:

- **Planned Run** (page 10)—Use this type of run when configuring the plugin through the Planned Run Wizard.
- **Reanalyze Run** (page 15)—Use this type of run to reanalyze the data with different parameters.

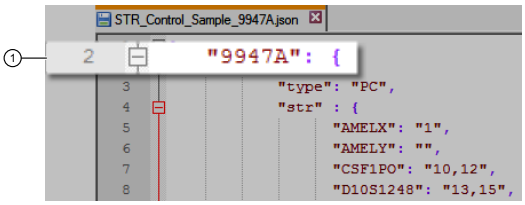
Create a Planned Run

Use this procedure to configure the plugin during Planned Run creation so that the plugin runs automatically after a sequencing run.

1. Log in to the Torrent Server via the Torrent Browser.
2. Select **Plan** ▸ **Templates**.
3. In the navigation pane, click **Human Identification**, then click the appropriate Applied Biosystems Precision ID Ancestry or Identity Panel template from the **Template Name** list
4. In the **Plan** tab, enter and select the following options.

   Note: Sample name information is transferred to the Converge™ Software with the results.

Option	Action
Run Plan Name (required)	Enter a name for the run plan. Do not use spaces or special characters.
Analysis Parameters	Select **Default (Recommended)**
Reference Library	Select **hg19**
Target Regions	Select **PrecisionID_AncestryPanel_targets.bed** or **PrecisionID_IdentityPanel_targets.bed**
Hotspot Regions	Select **PrecisionID_AncestryPanel_hotspots.bed** or **PrecisionID_IdentityPanel_hotspots.bed**
Use same reference & BED filles for all barcodes	Leave the option selected.
Number of barcodes	Select the appropriate number of barcodes.
Sample Tube Label	Leave blank (recommended); information is auto-populated during the Ion Chef™ template preparation run.
Chip ID	Leave blank Information is auto-populated after the Ion Chef™ template preparation run).
Barcode	Select the appropriate information from the drop-down list.
Sample (required)	Enter a name for each sample. Do not use spaces or special characters.
Control Type	Leave blank.

Option	Action
Sample ID	• **If not including a control**—**Sample ID** is optional. • **If including a control**—Enter a **Sample ID** using this naming convention: <Name of your choice>--<`Control Type`>-<`Control Name`> , where – `Control Type` is PC or NTC (positive or negative control) – The `Control Name` is provided in the control profile file.  ① In this example, 9947A is the `Control Name`. **Example positive control name:** Sample1234--PC-9947A **Example negative control name:** Sample5678--NTC-NegCtrl
Sample Description	(*Optional*) If you have already created a case for this sample in the Converge™ Software, enter the Case ID.
Reference	Leave blank.
Bead Loading (%)	Use default (**30**)
Key Signal (1-100)	Use default (**30**)
Usable Sequence (%)	Use default (**30**)

5. Click the **Ion Reporter** tab, confirm the following options, then click **Next**.
   - **Ion Reporter Account—None** (*default*)
   - **Sample Grouping—Self**

6. In the **Application** tab, confirm the following options, then click **Next**.
   - **Application—Human Identification** (*default*)
   - **Target Technique—AmpliSeq DNA** (*default*)

7. In the **Kits** tab, select the appropriate options according to the following table, then click **Next**.

Option	Action
Sample Preparation Kit	Leave blank.
Library Kit Type	• For manual library preparation, select **Ion AmpliSeq 2.0 Library Kit**. IMPORTANT! For HID applications, **Ion AmpliSeq 2.0 Library Kit** is the appropriate selection for use with the Precision ID Library Kit. • For library preparation using the Ion Chef™ System, select **Ion AmpliSeq Kit for Chef DL8**. **Note:** The **Ion AmpliSeq Kit for Chef DL8** option is only available on Torrent Suite™ Software v5.0 and later.
Templating Size	Select **200**.
Template Kit	Select the appropriate template kit. For example: • Ion OneTouch™ 2 System: Select **OneTouch**, then select **Ion PGM Hi-Q OT2 Kit - 200** from the dropdown list. • Ion Chef™ System: Select **IonChef**, then select **Ion Hi-Q Chef Kit** from the dropdown list.
Sequencing Kit	Select the appropriate sequencing kit. For example: • PGM: Select **Ion PGM Hi-Q Sequencing Kit**. • S5: Select **Ion S5 Sequencing Kit**.
Flows	Enter **500**.
Control Sequence	Leave blank.
Chip Type	Select the appropriate chip type.
Barcode Set	Select **IonXpress** or **IonCode**. **Note:** The **IonCode** option is only available on Torrent Suite™ Software v5.0 and later.
Mark as PCR Duplicates	Leave the option deselected.
Base Calibration Mode	Select **Default Calibration**.
Enable Realignment	Leave the option deselected.

8. In the **Plugins** tab, select then configure the HID SNP Genotyper Plugin:
   a. Click **Clear Selections**, select **HID_SNP_Genotyper**, then click the blue **Configure** link.

b. In the **Plugin Configuration** dialog box, select the appropriate options according to the following table.

Option	Action	
	Precision ID Ancestry Panel	**Precision ID Identity Panel**
SNP Panels	Select one or both of these SNP Panels: • **Admixture Prediction – AISNPs** • **Population Likelihoods – AISNPs**	Select one or both of these SNP Panels: • **1000 Genomes – IISNPs** • **Y Haplogroup Prediction – Y SNPs**
Trim Reads	Select **Trim Reads**	Select **Trim Reads**
Minimum allele frequency	Enter the appropriate values **Note:** We recommend the default values.	Enter the appropriate values **Note:** We recommend the default values.
Minimum coverage		
Minimum coverage on either strand		
Maximum strand bias		

c. Click **Save Changes** to close the dialog box, then click **Next**.

9. (*Optional*) In the **Projects** tab, assign a project, then click **Next**.
   - Select an existing project from the list.
     –*or*–
   - Click **Add Project**, then enter one or more project names.

10. In the **Plan** tab, select the appropriate options according to the following table, then click **Plan Run**.

Option	Action	
	Precision ID Ancestry Panel	**Precision ID Identity Panel**
Run Plan Name	Enter a name for the Run Plan.	Enter a name for the Run Plan.
Reference Library	Select **hg19(Homo sapiens hg19)**.	Select **hg19(Homo sapiens hg19)**.
Target Regions	Select **PrecisionID_AncestryPanel_targets.bed**.	Select **PrecisionID_IdentityPanel_targets.bed**.
Hotspot Regions	Select **PrecisionID_AncestryPanel_hotspots.bed**.	Select **PrecisionID_IdentityPanel_hotspots.bed**.
Use same reference & BED files for all barcodes	Select the option.	Select the option.
Number of barcodes	Enter the number of barcodes.	Enter the number of barcodes.
Sample Tube Label	(*Optional*) Enter the Sample Tube Label.	(*Optional*) Enter the Sample Tube Label.
Enter a sample name...	• Enter a unique name for each sample. For replicates, use distinguishing characters (for example, <sample name>_R1). • (*Optional*) Enter an ID and description for each sample.	
Add a note	(*Optional*) Add a note for your laboratory needs.	

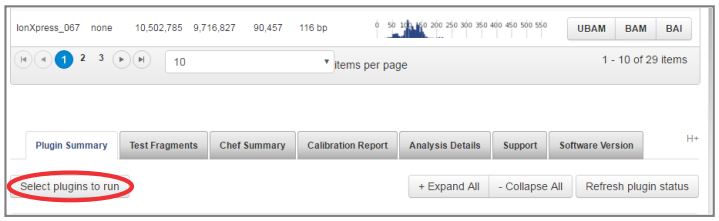
Option	Action	
	Precision ID Ancestry Panel	**Precision ID Identity Panel**
Add LIMS Meta Data	(*Optional*) Add LIMS Meta Data for tracking purposes.	
Monitoring Thresholds	Accept the default settings: • Bead Loading (%): **≤30** • Key Signal (1–100): **≤30** • Usable Sequence (%): **≤30**	Accept the default settings: • Bead Loading (%): **≤30** • Key Signal (1–100): **≤30** • Usable Sequence (%): **≤30**

When the run is complete, go to Chapter 3, "View and interpret results".

Reanalyze

Use this type of run to reanalyze data, for example, if the experiment requires a different BED file or parameters.

1. Log in to the Torrent Server via the Torrent Browser.
2. Select **Data ▸ Completed Runs & Results**, then click a **Report Name** to view the run summary.
3. In the run summary page, click **Plugin Summary** to scroll to the bottom of the page, then click **Select plugins to run**.



4. In the dialog, select **HID_SNP_Genotyper** from the list.
5. In the HID SNP Genotyper Plugin dialog, select the appropriate options according to the following table, then click **Submit**.

Option	Action	
	Precision ID Ancestry Panel	**Precision ID Identity Panel**
SNP Panels	Select one or both of these SNP Panels: • **Admixture Prediction – AISNPs** • **Population Likelihoods – AISNPs**	Select one or both of these SNP Panels: • **1000 Genomes – IISNPs** • **Y Haplogroup Prediction – Y SNPs**
Targeted Regions	Select **PrecisionID_AncestryPanel_targets.bed**.	Select **PrecisionID_IdentityPanel_targets.bed**.
Hotspot Regions	Select **PrecisionID_AncestryPanel_hotspots.bed**.	Select **PrecisionID_IdentityPanel_hotspots.bed**.
Trim Reads	Select **Trim Reads**.	Select **Trim Reads**.
Minimum allele frequency	Enter the appropriate values. **Note:** We recommend the default values.	Enter the appropriate values. **Note:** We recommend the default values.
Minimum coverage		
Minimum coverage on either strand		
Maximum strand bias		

When the run is complete, go to Chapter 3, “View and interpret results“.

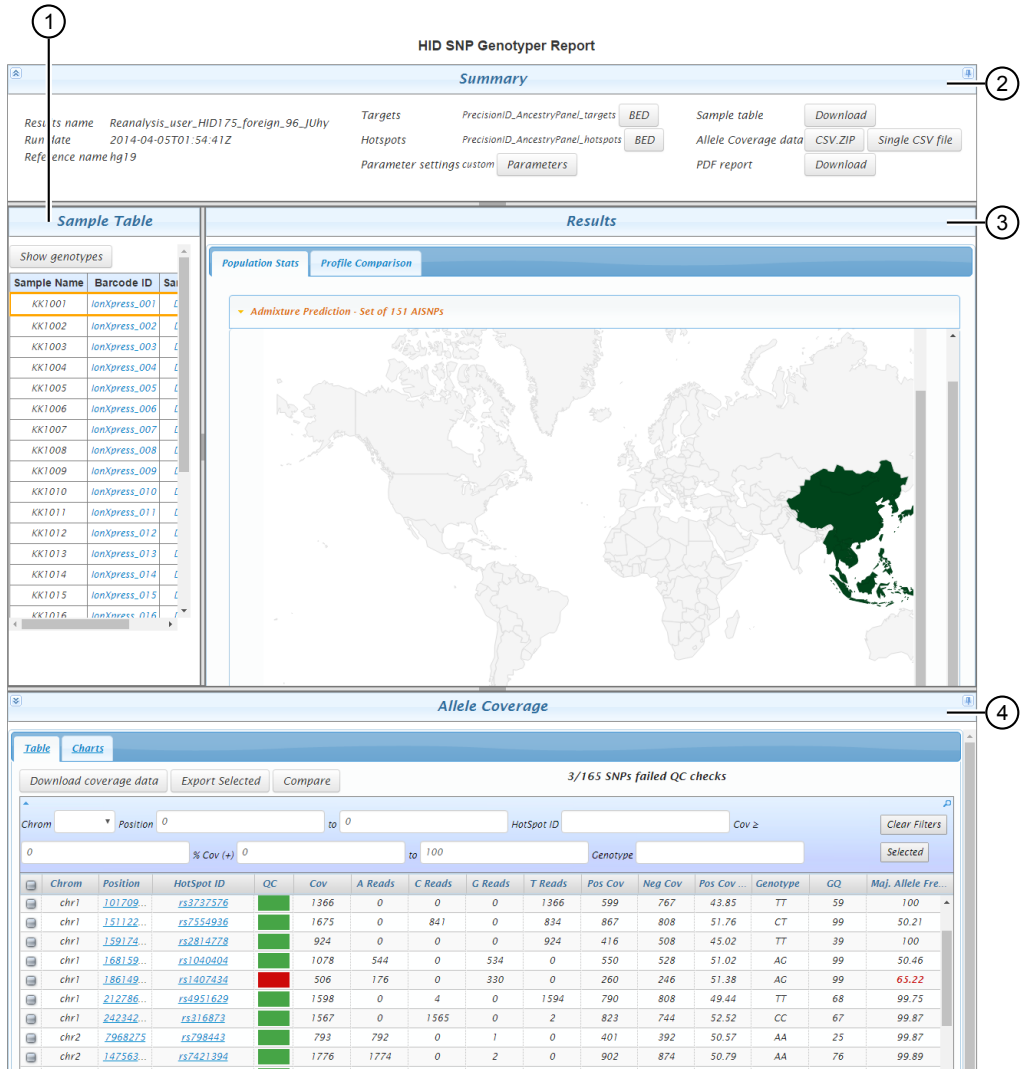
View and interpret results

About the HID SNP Genotyper Report

The HID SNP Genotyper Report is the output of the HID SNP Genotyper Plugin and contains the plugin results.

- To open the report, see "View results" on page 18.
- To interpret the results, follow the procedures for each section of the report.

IMPORTANT! Unless otherwise noted, the HID SNP Genotyper Report features are the same for both the Precision ID Ancestry Panel and the Precision ID Identity Panel.



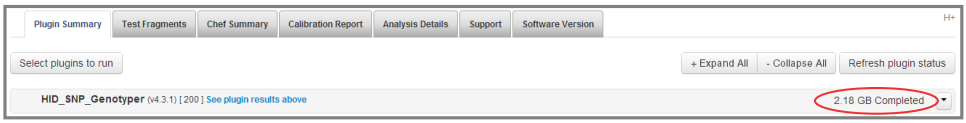
Example HID SNP Genotyper Report (Ancestry Panel results)

① **Sample Table** pane
② **Summary** pane
③ **Results** pane
④ **Allele Coverage** pane

View results

1. Log in to the Torrent Server via the Torrent Browser.
2. Search for the HID SNP Genotyper Report:
   a. Select **Data** ▸ **Completed Runs & Results**.
   b. Use the search features to find the results report of interest.
   c. Click the appropriate blue report link in the **Report Name** column.

3. In the analysis report page, click **Plugin Summary** to scroll to the bottom of the report page.
4. Review the status of the plugin analysis.



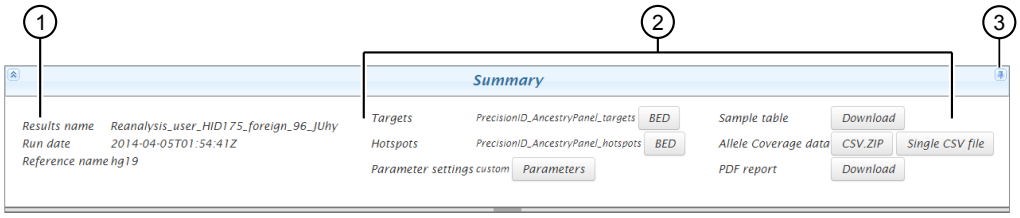
Status	Definition
Completed	Plugin analysis was successful.
Queued	System is processing other actions; your sample has been submitted.
Started	Plugin analysis has started.
Error	Plugin analysis cannot be completed successfully.

5. Open the HID SNP Genotyper Report:
   a. Click **See plugin results above** to scroll to the top of the report page.
   b. Click **HID_SNP_Genotyper.html** to open the HID SNP Genotyper Report.

Summary pane

The **Summary** pane contains run information and links to download the items described in the following table:

Click...	To download...
Targets **BED**	The targets BED file.
Hotspots **BED**	The hotspots BED file.
Parameters	A *.json file that contains the parameters settings.
Sample table **Download**	A *.csv file that contains the sample table. The sample table includes the sample name, barcode, and genotype for each SNP for all samples.
CSV.ZIP	A *.zip file that contains a separate *.csv file for each sample in the run. Each *.csv file contains the allele coverage data for the sample.
Single CSV file	A single *.csv file that contains the allele coverage data for all samples in the run.
PDF report **Download**	A *.pdf file of the HID SNP Genotyper Report (see page 19 for a description of the PDF report).



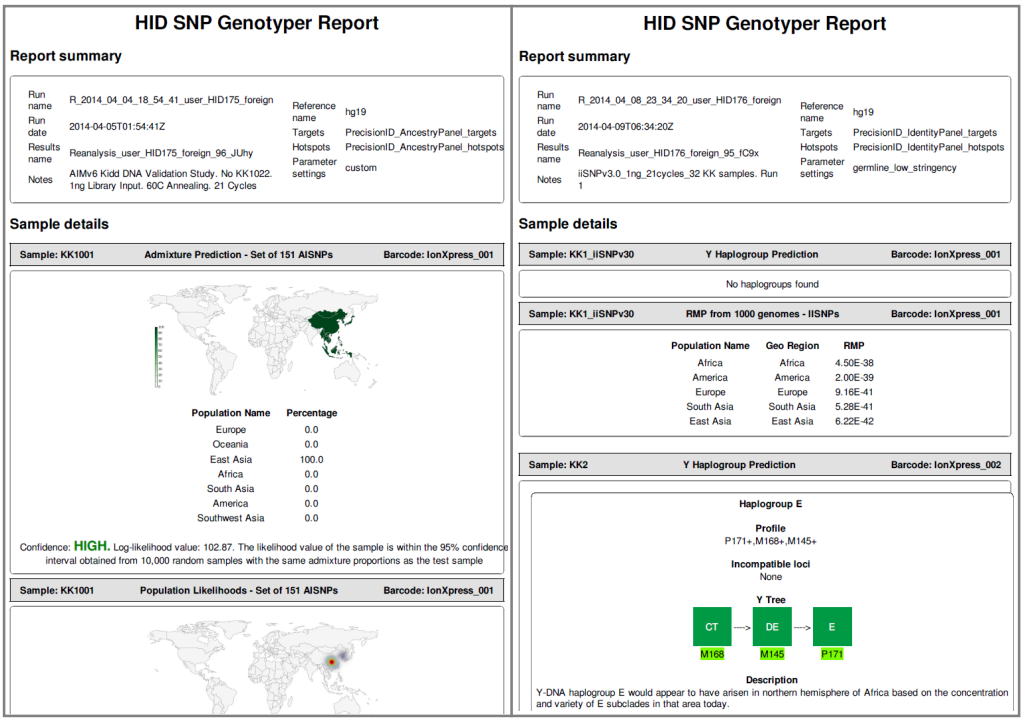
① Run information
② Download links
③ Hide/Show the **Summary** pane

PDF report

The *.pdf report (Example *.pdf reports for the Ancestry and Identity Panels, respectively on page 20) contains the following sections:

- **Report summary**—Provides a brief summary of the run, including the reference name and the BED files that were used.
- **Sample details**—Provides information for each sample in the run, including the sample name, sample barcode, and the analysis name. The section provides additional information for each sample, depending on the analysis used, including:
  - **Admixture Prediction**
  - **Population Likelihoods**
  - **1000 Genomes**
  - **Y Haplogroup Prediction**

Example *.pdf reports for the Ancestry and Identity Panels, respectively



HID SNP Genotyper Report

Report summary

Run name	R_2014_04_04_18_54_41_user_HID175_foreign	Reference name	hg19
Run date	2014-04-05T01:54:41Z	Targets	PrecisionID_AncestryPanel_targets
Results name	Reanalysis_user_HID175_foreign_96_JUhy	Hotspots	PrecisionID_AncestryPanel_hotspots
Notes	AIMv6 Kidd DNA Validation Study. No KK1022. 1ng Library Input. 60C Annealing. 21 Cycles	Parameter settings	custom

Sample details

Sample: KK1001 | Admixture Prediction - Set of 151 AISNPs | Barcode: IonXpress_001

Population Name	Percentage
Europe	0.0
Oceania	0.0
East Asia	100.0
Africa	0.0
South Asia	0.0
America	0.0
Southwest Asia	0.0

Confidence: **HIGH.** Log-likelihood value: 102.87. The likelihood value of the sample is within the 95% confidence interval obtained from 10,000 random samples with the same admixture proportions as the test sample

Sample: KK1001 | Population Likelihoods - Set of 151 AISNPs | Barcode: IonXpress_001

HID SNP Genotyper Report

Report summary

Run name	R_2014_04_08_23_34_20_user_HID176_foreign	Reference name	hg19
Run date	2014-04-09T06:34:20Z	Targets	PrecisionID_IdentityPanel_targets
Results name	Reanalysis_user_HID176_foreign_95_fC9x	Hotspots	PrecisionID_IdentityPanel_hotspots
Notes	iiSNPv3.0_1ng_21cycles_32 KK samples. Run 1	Parameter settings	germline_low_stringency

Sample details

Sample: KK1_iiSNPv30 | Y Haplogroup Prediction | Barcode: IonXpress_001

No haplogroups found

Sample: KK1_iiSNPv30 | RMP from 1000 genomes - IISNPs | Barcode: IonXpress_001

Population Name	Geo Region	RMP
Africa	Africa	4.50E-38
America	America	2.00E-39
Europe	Europe	9.16E-41
South Asia	South Asia	5.28E-41
East Asia	East Asia	6.22E-42

Sample: KK2 | Y Haplogroup Prediction | Barcode: IonXpress_002

Haplogroup E

Profile
P171+,M168+,M145+

Incompatible loci
None

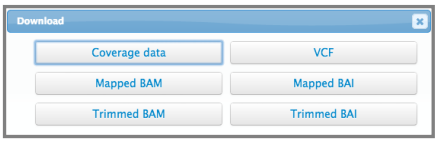
Y Tree
CT → DE → E
M168 M145 P171

Description
Y-DNA haplogroup E would appear to have arisen in northern hemisphere of Africa based on the concentration and variety of E subclades in that area today.

Sample Table pane

The **Sample Table** pane lists all samples in the run. Use the **Sample Table** features as follows:

Click...	To...
Show Genotypes	List the genotypes across all hotspots for a given sample. Information is from the input BED file. The genotype order depends on the sequence of the hotspots in the BED file.
Hide Genotypes	Hide the displayed genotypes.
Barcode ID	Select the sample to view. Information for the selected sample is displayed in the **Sample Table** (Show Genotypes), **Results**, and **Allele Coverage** panes.

Click...	To...
Sample Data **Download**	Open the Variant Caller output for the selected sample. 
(*Click-drag*) Bar between the **Sample Table** and **Results** panes	Resize the **Sample Table** pane.

Allele Coverage pane

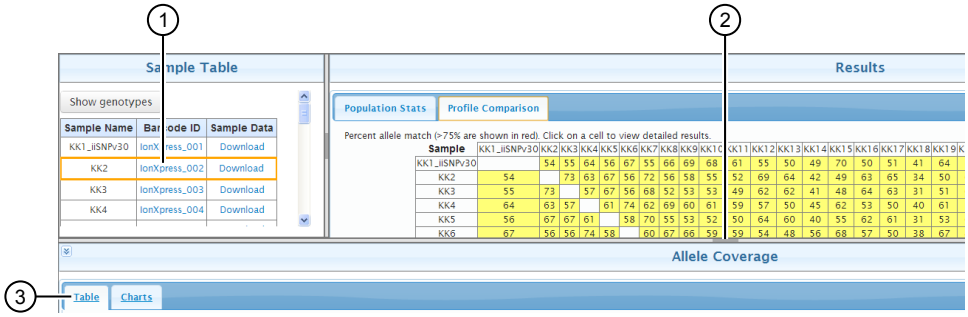
The **Allele Coverage** pane contains the following tabs:

- **Table** (page 21)—Provides allele coverage details.
- **Charts** (page 23)—Displays the read coverage across hotspots.

Review the **Table** tab

The **Table** tab provides the allele coverage details for the selected sample.

1. In the **Sample Table** pane, click the **Barcode ID** for the sample of interest.
2. In the **Allele Coverage** pane, select the **Table** tab.



1. Click the **Barcode ID** for the sample of interest
2. Click-drag to resize the **Allele Coverage** pane
3. Select the **Table** tab

3. (*Optional*) Filter the data to display.

4. Review the **Table** tab using the following features:

Click...	To...
Download coverage data	Download the allele coverage table as a *.csv file.
Export Selected (must first select the header row checkbox)	Export the selected results.
Compare	Compare data from selected SNPs across all samples in the run.
Column header	Sort the column by ascending/descending order.
Position column link	Go to the IGV browser.
HotSpot ID column link	Go to the NCBI web page for SNPs.

Table 1 Data displayed in the **Allele Coverage Table** tab

Column	Description
Chrom	Chromosome. The chromosome location of the SNP.
Position	The position of the SNP on the chromosome.
HotSpot ID	The reference SNP ID number.
QC	Quality checks. See page 22 and page 35.
Cov	Coverage. The number of aligned reads that cover this hotspot.
A Reads	The number of aligned reads that have allele A at this hotspot.
C Reads	The number of aligned reads that have allele C at this hotspot.
G Reads	The number of aligned reads that have allele G at this hotspot.
T Reads	The number of aligned reads that have allele T at this hotspot.
Pos Cov	Positive Coverage. (+) strand coverage.
Neg Cov	Negative Coverage. (–) strand coverage.
Pos Cov %	Positive Coverage percentage. The positive coverage divided by the total coverage. This is a measure of strand bias.
Genotype	Genotype calls at hotspot region.
GQ	Genotype Quality. The Phred-scaled marginal probability of a called genotype. The maximum value is 99. Lower values may be caused by strand bias, low signal strength, or allelic imbalance.
Maj. Allele Freq (%)	Major Allele Frequency percentage. The number of reads for the most frequently appearing allele divided by the total number of reads for all alleles. This value should be about 50% (heterozygote) or 100% (homozygote).

Quality checks (QC)

The **Table** tab displays the quality checks. If any of the QC metrics fail:

- The **QC** column cell displays as red.
- The individual metrics that failed display as red text.

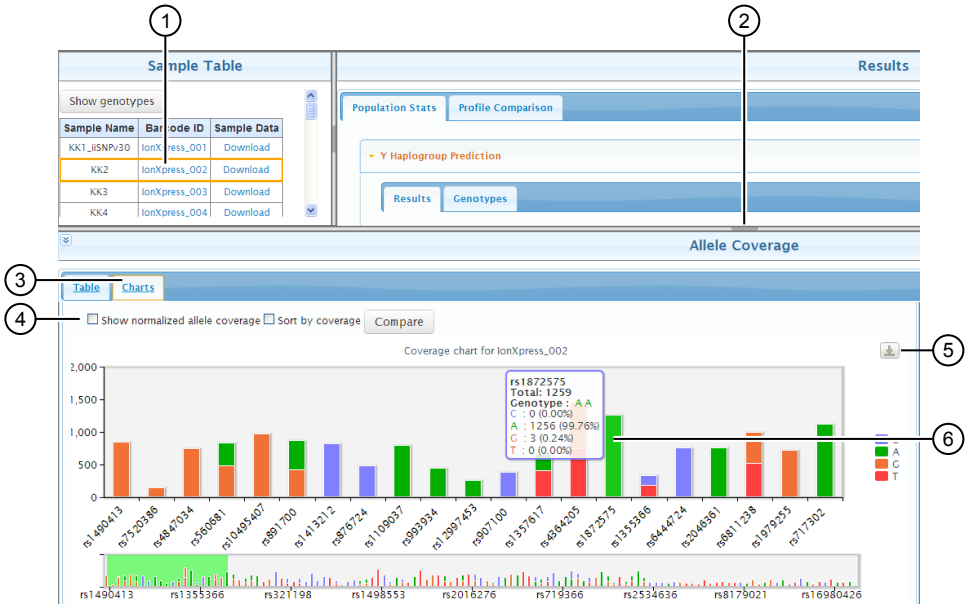
For further information, see "Quality checks (QC)" on page 35.

Review the **Charts** tab

The **Charts** tab displays the read coverage across hotspots for the selected sample.

1. In the **Sample Table** pane, click the **Barcode ID** for the sample of interest.
2. In the **Allele Coverage** pane, select the **Charts** tab.
3. Review the **Charts** tab using the following features:

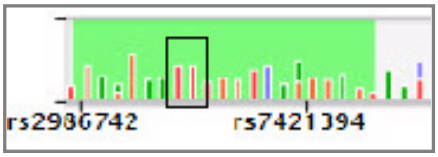
Click...	To...
Show normalized allele coverage	Display normalized allele coverages at each SNP with regards to its total coverage.
Sort by coverage	Sort the entire panel by increasing coverage. **Note:** Default sorting is based on the sequence of hotspots in the BED file.
Compare	Compare data from selected SNPs across all samples in the run. See step step 4 for detailed instructions.
(*Hover over*) SNP details	View the details for the SNP.
Export icon	Save the Coverage Plot in various formats.



1. Click the **Barcode ID** for the sample of interest
2. Click-drag to resize the **Allele Coverage** pane
3. Select the **Charts** tab
4. Select options for chart display
5. Click icon to export files
6. Hover over data points to display SNP details

4. Compare data from selected SNPs across all samples in the run:
   a. Click-drag on the bottom of the chart to select a region.
   The region highlights in green.

b. Click-drag around specific SNPs in the highlighted region.



c. Click **Compare** to show the comparison results for two selected SNPs.

Results pane – Ancestry Panel

The **Results** pane for the Ancestry Panel contains the following tabs:

- **Population Stats** (page 25 and page 27)—Displays the population statistics for the selected sample. The population statistics vary and depend on the analysis used by the HID SNP Genotyper Plugin: Admixture Prediction or Population Likelihoods (see "Available analyses").
- **Profile Comparison** (page 28)—Displays a pair-wise profile comparison for all samples in the run.

Available analyses

For the Ancestry Panel, configure the plugin to use one or both of the following analyses:

- **Admixture Prediction**—Use the Admixture Prediction analysis to find the most likely combination of specific root populations that best explains the genotypes observed in the sample of interest. This analysis provides an estimate of the overall ancestral composition of the sample.

  For example, use the Admixture Prediction analysis to determine if a sample is predominantly of African origin or admixed between more than one population.
- **Population Likelihood**—Use the Population Likelihood analysis to calculate the likelihood of the sample genotypes for a particular population based on its allele/genotype frequencies. This analysis helps further resolve the results obtained from the Ancestry Prediction analysis.

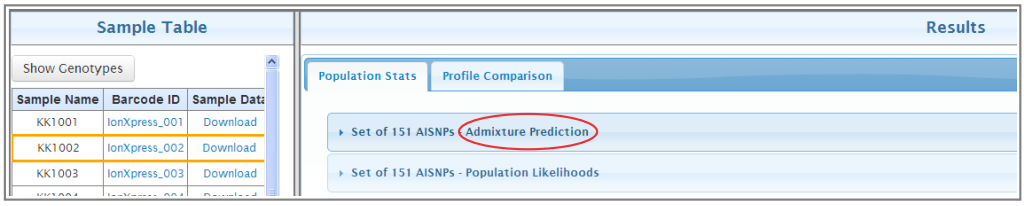
  For example, for an African sample, use the Population Likelihood analysis to find the sub-population (such as Ibo, Yoruba, Hausa) to which a sample is most likely to belong.

  Note: This level of resolution can be beyond the design of the SNPs in the panel.

View the Population Stats ► Admixture Prediction

For the Admixture Prediction analysis, use the **Population Stats** tab features:

1. In the **Sample Table** pane, click the **Barcode ID** for the sample of interest.
2. In the **Results** pane, click **Admixture Prediction** to display the following subtabs:
   - **Map**—see page 25
   - **Results**—see page 25
   - **Genotypes**—see page 26



Review the Admixture Prediction ► Map subtab

The **Map** subtab displays a heat map overlaid on a world map. It provides a quick way to view the most likely geographical origin of the selected sample.

The darkness of the color indicates the proportion of the corresponding population in the sample. That is, the darker the color, the greater the proportion of the corresponding population in the sample.

1. Select the **Map** subtab.

   Note: If the Torrent Server cannot connect to the internet, the displayed heat map is a static version.

2. Scroll to view the region of interest, if needed.
3. Hover over the region to display the proportion value of the corresponding population in the sample.

Review the Admixture Prediction ► Results subtab

The **Results** subtab displays the proportions for all populations.

Note: Populations with a proportion of 0% are not visible in the plot.

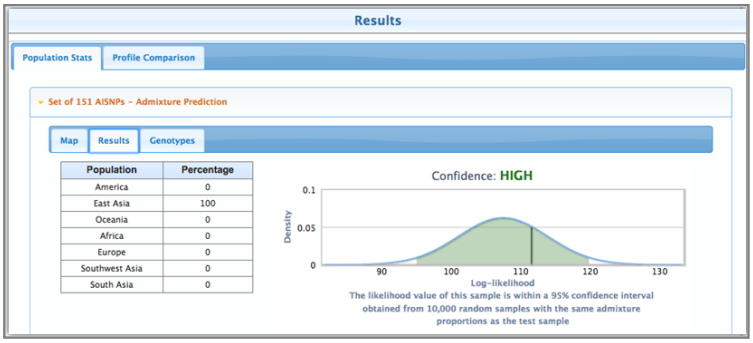
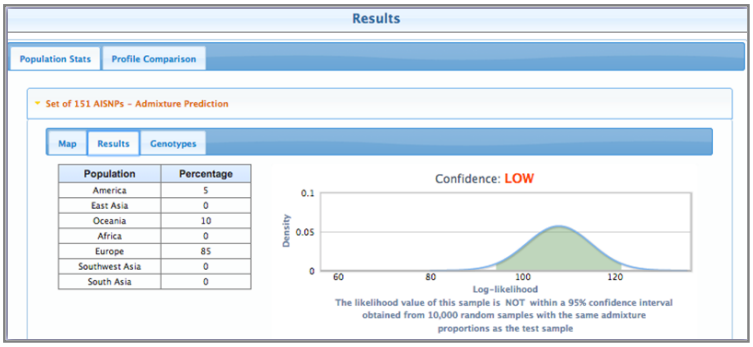
1. Select the **Results** subtab.
2. Review the table at left, which lists the most likely geographical origin of the selected sample.
3. Review the plot at right, which shows the distribution of log-likelihood values and a level of confidence for the calculated results.

   The confidence value answers the question: *Given a random population that has the same ancestral makeup as that of the test sample, what is the chance of observing the test sample?*

To calculate the confidence value, the plugin will:

1. Simulate 10,000 random samples that have the same predicted admixture proportions as the test sample.
2. Calculate the likelihood value for each simulated sample and the test sample.
3. Generate a distribution of log-likelihood values from the simulated samples, then compare the log-likelihood of the test sample to the distribution.

- The green region represents the 95% confidence interval. The 95% confidence interval means that the tested sample is similar to a sample obtained from 10,000 randomly generated samples.
- The black line indicates the log-likelihood of the tested sample. If the log-likelihood (black line) falls inside the green region, confidence is high. If the log-likelihood falls outside the green region, confidence is low.

Example	Description
	Confidence is high.
	For degraded samples, the confidence value decreases with the level of degradation. In this example, the sample is so degraded that there are valid genotypes for only 50% of the loci. As expected, the confidence is low.

Review the **Admixture Prediction ► Genotypes** subtab

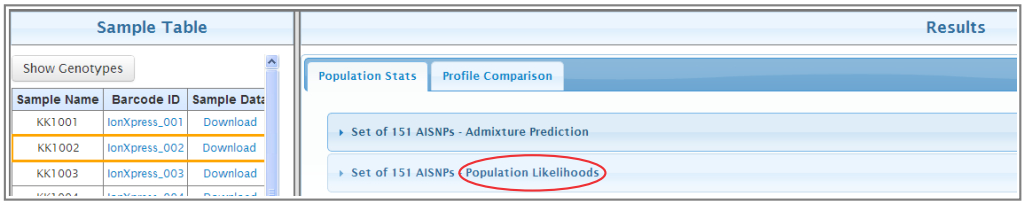
The **Genotypes** subtab lists the genotypes of the SNPs that were used for analysis.

1. Select the **Genotypes** subtab.
2. Scroll to view the entire table, if needed.

View the Populations Stats ► Population Likelihoods

For the Population Likelihoods analysis, use the **Population Stats** tab features:

1. In the **Sample Table** pane, click the **Barcode ID** for the sample of interest.
2. In the **Results** pane, click **Population Likelihoods** to display the following subtabs:
   - **Map**—see page 27
   - **Results**—see page 27
   - **Genotypes**—see page 28



Review the Population Likelihoods ► Map subtab

The **Map** subtab displays a heat map overlaid on a world map. It provides a quick way to view the most likely geographical origin of the selected sample.

The intensity of the color indicates the proportion of the corresponding population in the sample. That is, the darker the color, the greater the proportion of the corresponding population in the sample.

1. Select the **Map** subtab.

   Note: If the Torrent Server cannot connect to the internet, the displayed heat map is a static version.

2. Scroll to zoom in and click-drag to pan around the map, if needed.
3. Click **Show/hide populations**, then hover over the pins to view the names of each population.

   Note: Location information for the following populations is not available, so the map does not display these populations: Europeans-HapMap, Han-HapMap, Yoruba-HapMap, Japanese-HapMap, Sephardic Jews, Ashkenazi Jews. However, you can view likelihood values for these populations in the **Results** subtab (on page 27).

Review the Population Likelihoods ► Results subtab

The **Results** subtab displays the likelihood values for the sample; that is, how likely it is that the sample comes from each population used in this analysis.

1. Select the **Results** subtab.
2. Review the table, which lists all populations in the analysis, their geographical regions, and the likelihood values.

Review the **Population Likelihoods** ▶ **Genotypes** subtab

The **Genotypes** subtab lists the genotypes of the SNPs that were used for analysis.

1. Select the **Genotypes** subtab.
2. Scroll to view the entire table, if needed.

Review the **Profile Comparison** tab

The **Profile Comparison** tab displays a pair-wise profile comparison for all samples in the run. It shows the percentage of alleles that are common between any two samples in the current run. SNPs that have missing calls or No Calls (N) in both the samples are ignored in the calculation.

1. In the Results pane, select the **Profile Comparison** tab, then review the results.
   - Red cells indicate an allele match >75%.
   - Yellow cells indicate an allele match ≤75%.
2. Click a cell to display:
   - SNPs that are discordant between the two samples.
   - SNPs that were ignored in the calculation.

Results pane – Identity Panel

The **Results** pane for the Identity Panel contains the following tabs:

- **Population Stats** (page 29 and page 31)—Displays the population statistics for the selected sample. The population statistics vary and depend on the analysis used by the HID SNP Genotyper Plugin: Y Haplogroup Prediction or 1000 Genomes (see "Available analyses" on page 28).
- **Profile Comparison** (page 28)—Displays a pair-wise profile comparison for all samples in the run.

Available analyses

For the Identity Panel, configure the plugin to use one or both of the following analyses:

- **Y Haplogroup Prediction**—Use this analysis to predict the Y lineage of the sample.
- **1000 Genomes**—Use this analysis to find the Random Match Probability (RMP) for each of the five global populations from the 1000 Genomes data set.

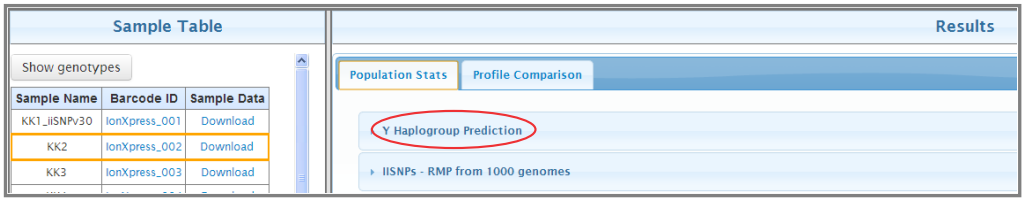
Select analyses

Select the analyses before running the plugin (see "Create a Planned Run" on page 10).

View the Population Stats ► Y Haplogroup Prediction

For the Y Haplogroup Prediction analysis, use the **Population Stats** tab features as follows:

1. In the **Sample Table** pane, click the **Barcode ID** for the sample of interest.
2. In the **Results** pane, click **Y Haplogroup Prediction** to display the following subtabs:
   - **Results**—see page 29
   - **Genotypes**—see page 31



Review the Y Haplogroup Prediction ► Results subtab

The **Results** subtab displays information about the identified haplogroup of a sample.

1. Select the **Results** subtab.
2. Scroll or expand to view the entire pane, if needed.
   The **Results** subtab displays the following information:

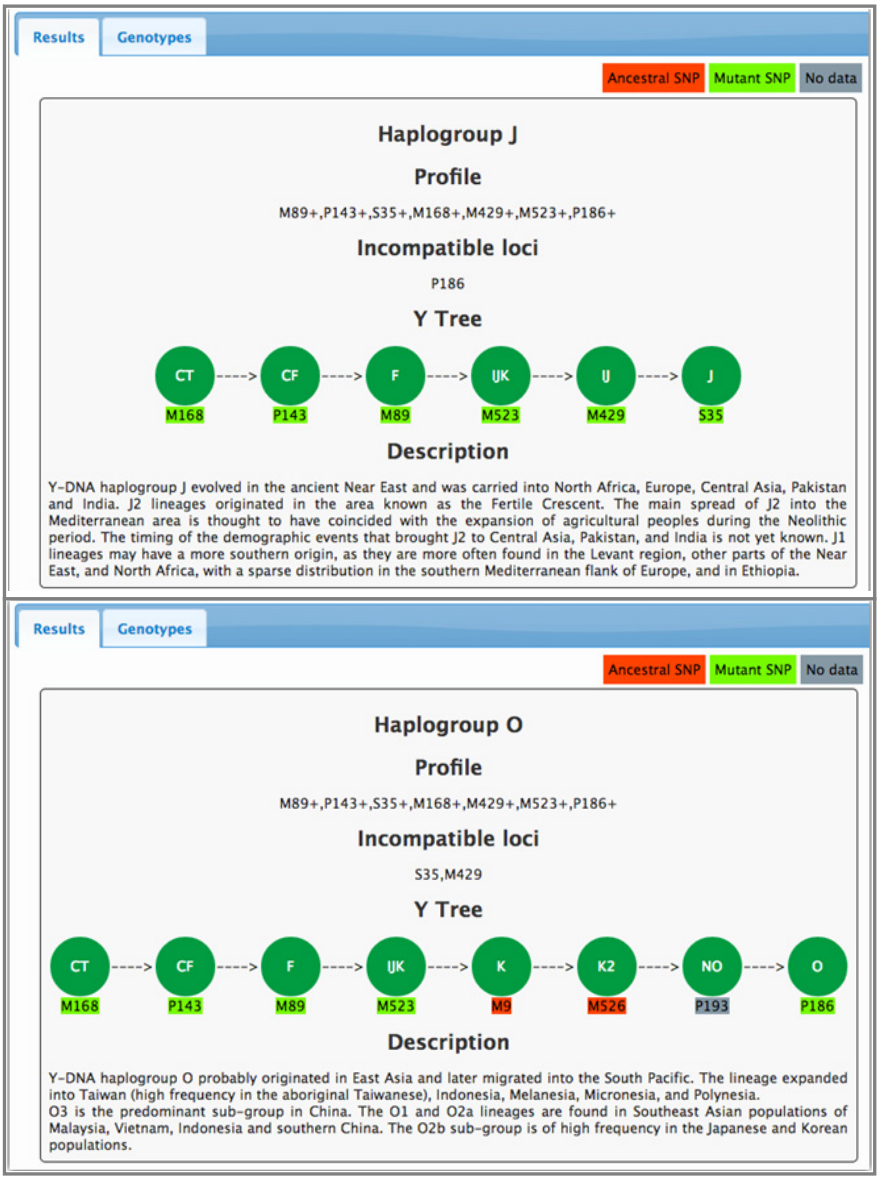
Item	Description
Haplogroup	The identified haplogroup.
Profile	The genotypes of the SNPs, provided in the Family Tree DNA (FTDNA) format. Only loci that are in the derived state are represented. Use the profile information to search against third-party tools, such as **http://ytree.morleydna.com**.
Incompatible loci	Loci that are present in the derived state but are not compatible with the identified lineage.
Y Tree	The Y tree with clades that have been typed for this lineage. Loci that define each clade are color-coded: • Red—loci in the ancestral state (**Ancestral SNP**) • Green—loci in the derived state (**Mutant SNP**) • Grey—loci that do not have a valid genotype (**No data**)
Description	A short description of the haplogroup, including details about its origin and geographical distribution.

Simulated profile example 1

This example shows results from a simulated profile that can be assigned to both haplogroups J and O.

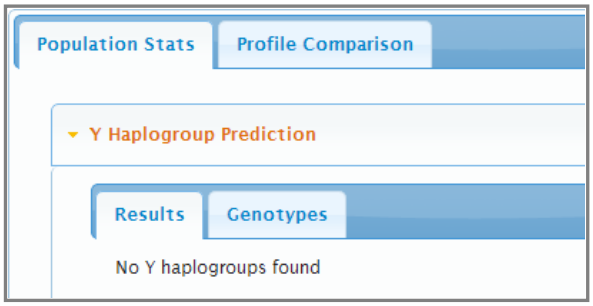
The sample has both S35 (defines haplogroup J) and P186 (defines haplogroup O) in the derived state. When considering the branch (lineage) leading up to clade J, locus P186 is absent and is therefore incompatible.

Similarly, when considering the branch (lineage) leading up to O, both S35 and M429 are absent. In the O branch, loci M9 and M526 are in the ancestral state and there is no data present for P193. These results indicate a genotyping issue and require a more detailed review of the data.



Simulated profile example 2

This example shows results from a simulated profile where no haplogroups were found. These results indicate a female sample.



Review the Y Haplogroup Prediction ► Genotypes subtab

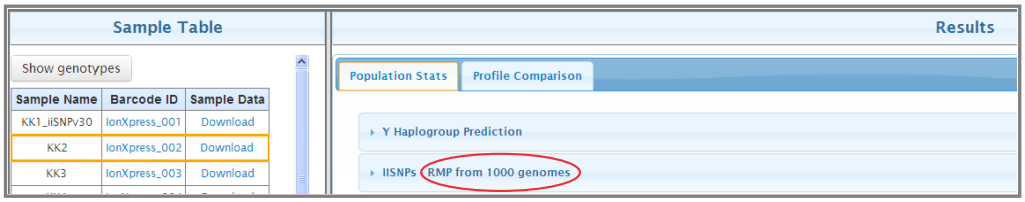
The **Genotypes** subtab lists the genotypes of the SNPs that were used for analysis.

1. Select the **Genotypes** subtab.
2. Scroll to view the entire table, if needed.

View the Population Stats ► 1000 Genomes

For the 1000 Genomes analysis, use the **Population Stats** tab features:

1. In the **Sample Table** pane, click the **Barcode ID** for the sample of interest.
2. In the **Results** pane, click **1000 Genomes** to display the following subtabs:
   - **Results**—see page 31
   - **Genotypes**—see page 32



Review the 1000 Genomes ► Results subtab

The **Results** subtab displays the Random Match Probabilities (RMP) results.

1. Select the **Results** subtab.
2. Review the table, which lists all populations in the analysis, their geographical regions, the RMP values, and the overall RMP range (minimum to maximum).

Review the 1000 Genomes ► Genotypes subtab

The **Genotypes** subtab lists the genotypes of the SNPs that were used for analysis.

1. Select the **Genotypes** subtab.
2. Scroll to view the entire table, if needed.

Review the Profile Comparison tab

The **Profile Comparison** tab displays a pair-wise profile comparison for all samples in the run. It shows the percentage of alleles that are common between any two samples in the current run. SNPs that have missing calls or No Calls (N) in both the samples are ignored in the calculation.

1. In the Results pane, select the **Profile Comparison** tab, then review the results.
   - Red cells indicate an allele match >75%.
   - Yellow cells indicate an allele match ≤75%.
2. Click a cell to display:
   - SNPs that are discordant between the two samples.
   - SNPs that were ignored in the calculation.

Calculations

Admixture Prediction

The Admixture Prediction method finds the ancestral composition of a sample. From a set of "root" populations, this method finds the most likely combination of these populations that best explains the genotypes observed in the sample of interest.

As an analogy: If there are three known colors — red, green, and blue — and an unknown color, the algorithm tries to find the best way to mix red, green, and blue to obtain the same color as that of the unknown.

To determine how reliable the results are, the HID SNP Genoytper Plugin provides a level of confidence for the calculated results. The confidence value answers the question: *Given a random population that has the same ancestral makeup as that of the test sample, what is the chance of observing the test sample?*

In general, confidence values are lower for degraded samples and for samples that cannot be satisfactorily expressed as an admixture of the root populations.

Data set

Currently, the HID SNP Genotyper Plugin considers seven root populations:

- Africa
- America
- Southwest Europe (Middle East)
- Europe
- Oceania
- East Asia
- South Asia

151 Ancestry Informative Markers (AIMs) are used to calculate the admixture proportions of these populations based on their genotype frequencies. The 151 SNPs are a subset of Kidd's 55 AIMs (Kidd et al. 2014) and Seldin's 128 AIMs (Kosey et al., 2009).

Download the list of loci and genotype frequency data from **https://s3-us-west-1.amazonaws.com/hid-ngs-public/ai151_regions.zip**.

Note: The AIM SNPs described above are a combined subset of Kidd and Seldin SNPs when compared with previous versions of the HID SNP Genotyper Plugin (v4.3 and earlier). The results obtained from the 151 SNPs are more robust and reliable.

Population Likelihoods

The Population Likelihoods method calculates the ancestral population likelihood as the product of genotype frequencies for each locus:

$$L_P = \prod_N f_P(g,i)$$

where,

- L_p is the likelihood of ancestral population P
- f_p (g, i) is the frequency of genotype g at locus i
- N is the number of loci

Data set

The HID SNP Genotyper Plugin calculates the likelihood for 65 populations based on the genotype frequencies of 151 SNPs.

Download the complete list of populations and frequency data from **https://s3-us-west-1.amazonaws.com/hid-ngs-public/ai151.zip**. These data are also available from the **AL**lele **FRE**quency **D**atabase at **http://alfred.med.yale.edu**.

1000 Genomes (Random Match Probability)

The HID SNP Genotyper Plugin calculates the Random Match Probability (RMP) as the product of genotype frequencies for each locus.

Data set

The HID SNP Genotyper Plugin calculates the RMP for the following five populations:

- Africa
- Europe
- America
- East Asia
- South Asia

The calculation is based on:

- 85 unlinked identity SNPs of the 90 SNPs in the panel.
- The genotype frequencies obtained from the 1000 Genomes data set. Download the genotype frequency data from **https://s3-us-west-1.amazonaws.com/hid-ngs-public/ii85_1000g.zip**.

Y Haplogroup Prediction

The HID SNP Genotyper Plugin identifies Y haplogroups based on the 2014 ISOGG Y Tree at **http://isogg.org/tree/index14.html**. In addition, the HID SNP Genotyper Plugin provides a short description of the identified haplogroup below the Y Tree (see "View the Population Stats ► Y Haplogroup Prediction" on page 29).

The prediction algorithm finds the longest branch (lineage) in the tree, then reports all possible lineages. The plugin flags loci that can be incompatible (not in the derived state) with a particular lineage and loci that have no genotypes.

To successfully identify a haplogroup, at least 30% of the Y SNPs should have a valid genotype.

Data set

The HID SNP Genotyper Plugin identifies haplogroups based on the 34 Y SNPs in the Precision ID Identity Panel.

Quality checks (QC)

In the **Allele Coverage** pane, the plugin applies locus-level quality checks to flag possible bad data. The following table lists the checks that the plugin performs and the conditions that raise an exception.

Quality check	Condition that raises exception
Coverage	Total coverage at locus is less than twice the standard deviation compared to the mean. The genotyper calculates the mean and standard deviation independently for autosomal and Y SNPs. This indicates drop-out.
Percent positive coverage	The ratio of coverage from (+) strand to (–) strand is <30% or >70%. This indicates strand imbalance. 50% is perfect strand balance.
Major allele frequency	The ratio of coverage of major allele to total coverage is: • <95% for homozygotes • <35% or >65% for heterozygotes This indicates allele imbalance.
Genotype	The genotype is not valid. Occasionally, a No Call (NN) may be observed even if all of the above metrics are good. This is because the variant caller performs sophisticated data analysis and looks at many other metrics. The exact reason for failure can be seen in the VCF file. Similarly, a genotype can be valid even if one or more of the above metrics fail. A flag does not necessarily mean an incorrect result; the flag only indicates an unexpected deviation from the norm and requires a more detailed review of the data.

QC for female samples

The genotyper calculates the mean and standard deviation independently for autosomal and Y SNPs. If the mean coverage for Y SNPs is <20, it is considered a female sample. The genotyper does not perform quality checks for the Y SNPs, and the QC column displays as gray. Because Y SNPs have no coverage for females, they are not flagged.

B

Experiments and results

This appendix describes the results of the developmental experiments performed using the Precision ID Identity Panel and the Precision ID Ancestry Panel for use with forensic samples on the Ion PGM™ System.

About Ion AmpliSeq™ technology

Ion AmpliSeq™ technology enables complex PCR multiplexing while minimizing primer-dimer formation. Both the Precision ID Identity Panel and the Precision ID Ancestry Panel contain primers for PCR amplification using Ion AmpliSeq™ technology, and use a recommended sample input of 1 ng of genomic DNA. We chose conditions that produce optimum PCR product yield and that meet reproducible performance standards.

Precision ID panel design

Precision ID Identity Panel

The Precision ID Identity Panel is made up of 124 markers; 90 autosomal markers and 34 upper Y-clade markers. The autosomal SNPs are from Ken Kidd's 45- unlinked set (Pakstis, et al. 2010) and from the SNPforID set (Kosey et al., 2009). The average size for autosomal markers is 132 bp. The average size for upper Y-clade markers is 141 bp (Karafet et al. 2008). Amplicon size distributions are shown in Figure 1. The chromosomal location of the SNPs in the panel is shown in Figure 2.

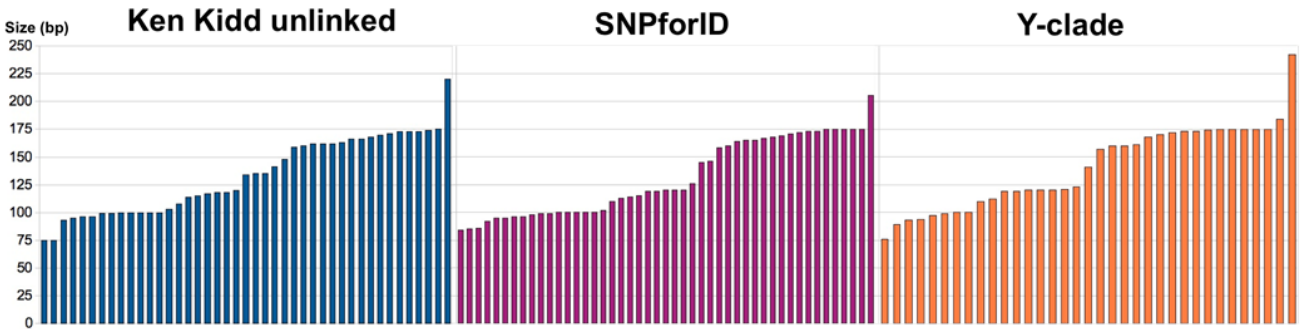


Figure 1 Amplicon size distributions of SNP subsets in the Precision ID Identity Panel.

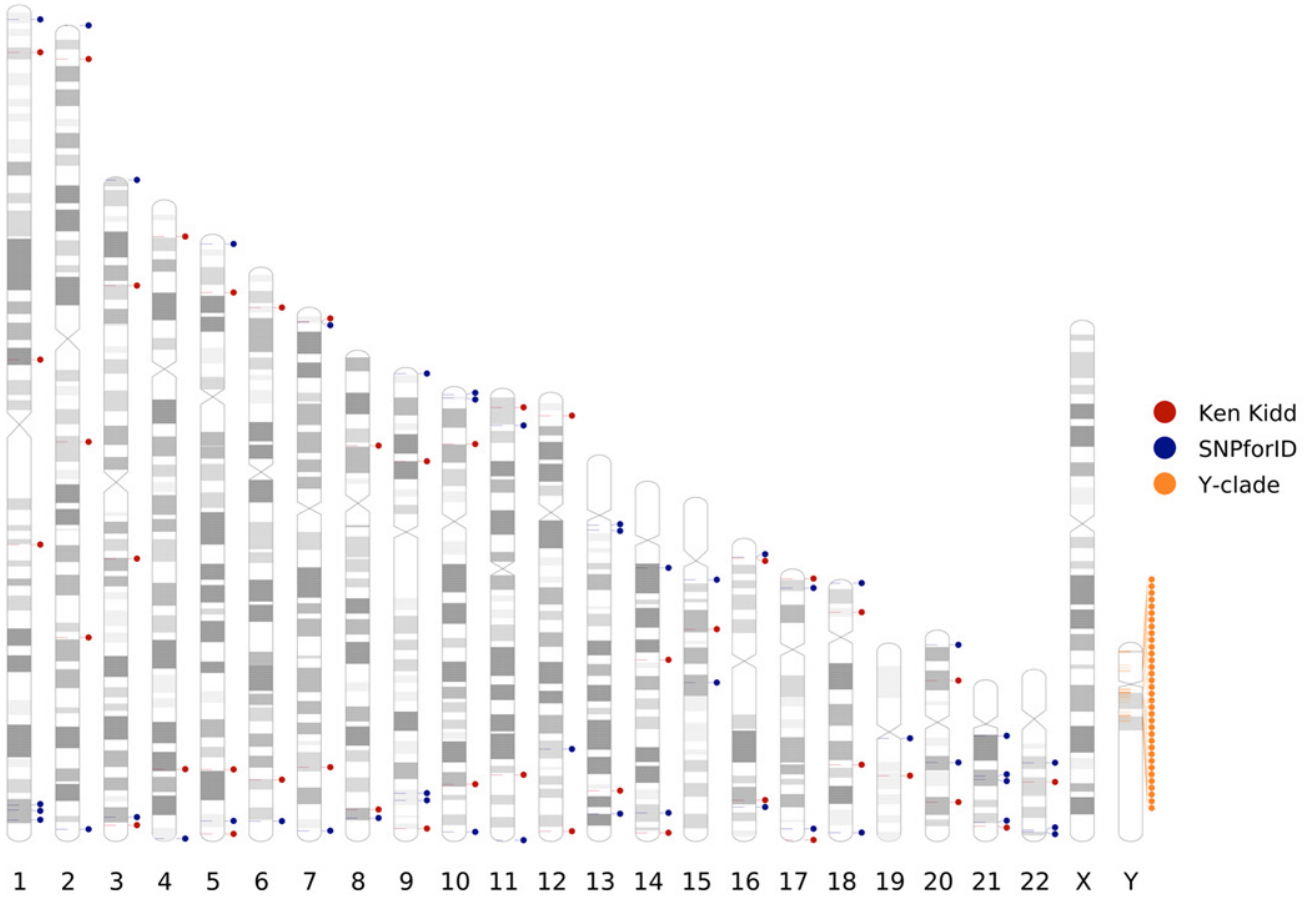


Figure 2 Chromosomal location of identity SNPs in the Precision ID Identity Panel.

Precision ID Ancestry Panel

The Precision ID Ancestry Panel is made up of 165 autosomal markers; 55 from Ken Kidd's AIM set (Kidd et al. 2014) and 123 Seldin markers (Nassir et al. 2009). The average size of the Ken Kidd markers is 130 bp. The average size of the Seldin markers is 122 bp. Amplicon size distributions are shown in Figure 3. The chromosomal location of the SNPs in the Precision ID Ancestry Panel is shown in Figure 4.

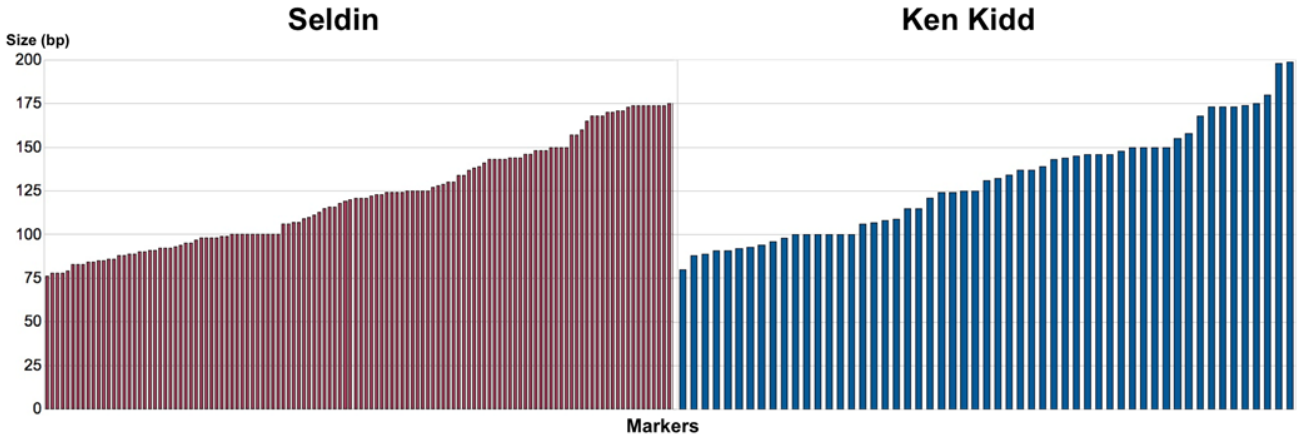


Figure 3 Amplicon size distributions of SNP subsets in the Precision ID Ancestry Panel.

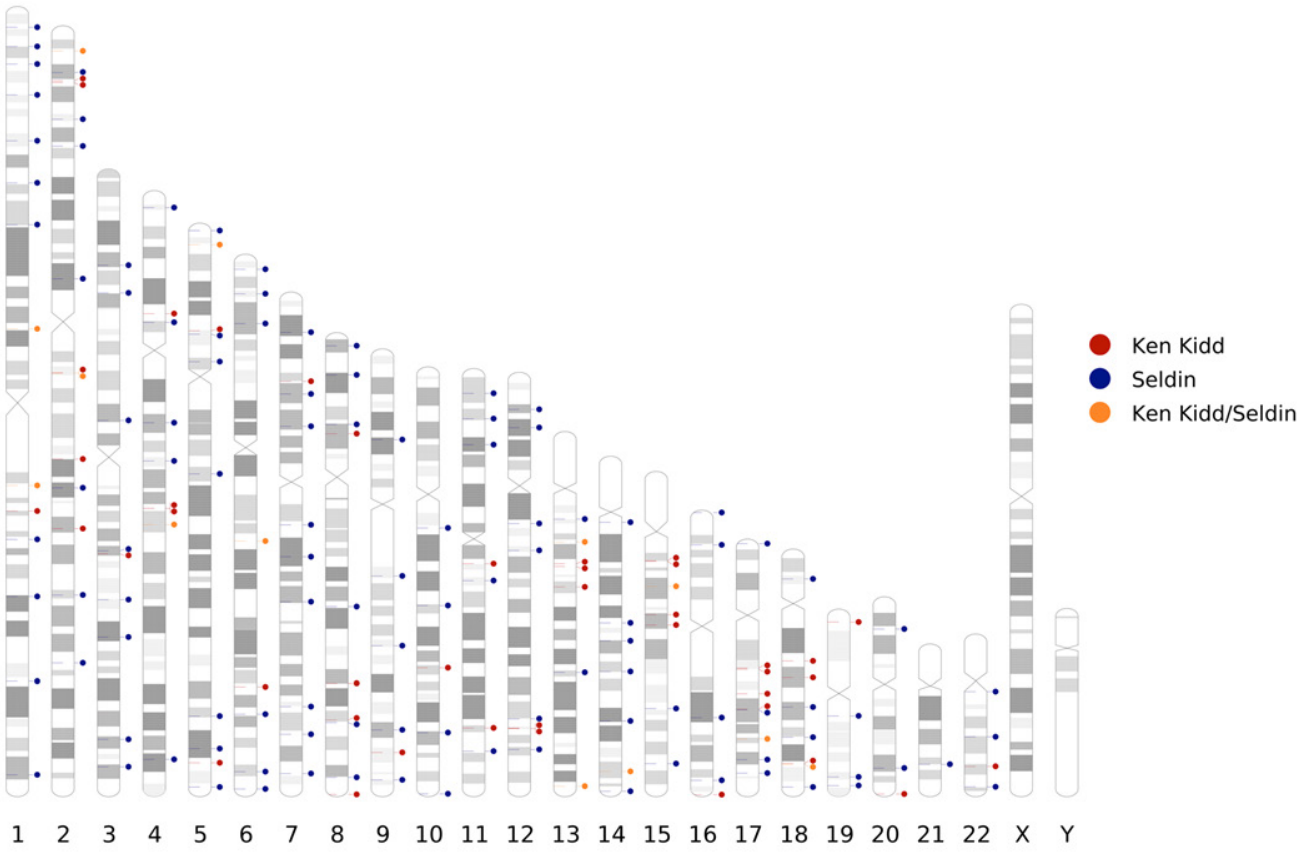


Figure 4 Chromosomal location of identity SNPs in the Precision ID Ancestry Panel.

Reference DNA samples

Precision ID Identity Panel

Thirty identity DNA samples and corresponding TaqMan® genotype data for 46 Ken Kidd SNPs were provided by Dr. Ken Kidd (Pakstis et al. 2010). These samples were used in the development of the Precision ID Identity Panel.

The input genomic DNA per PCR reaction ranged from 180 pg to 1 ng. The resulting 30 library samples were quantified by qPCR using the Ion Library TaqMan® Quantitation Kit. Library samples were diluted and pooled before template preparation and sequenced on a single Ion 318™ Chip v2.

Precision ID Ancestry Panel

A total of 24 ancestral DNA samples and corresponding TaqMan® genotype data for 55 Ken Kidd SNPs were provided by Dr. Ken Kidd (Yale University). These samples were used in the development of the Precision ID Ancestry Panel.

The input genomic DNA per PCR reaction was 1 ng. Using the same workflow as in testing of the identity samples, the resulting ancestral library samples were quantified by qPCR, diluted, pooled, and sequenced on a single Ion 318™ Chip v2.

Performance metrics and panel evaluation

For the development of the Precision ID panels, a metric system was developed to assess marker selections and panel robustness.

The metrics used in evaluating marker selections and overall panel performance are:

- Amplicon Strand Balance (Figures 5 and 6 on page 41)
- Amplicon Heterozygosity Balance (Figure 7 on page 41 and Figure 8 on page 42)
- Amplicon Coverage (Figures 9 and 10 on page 42)

These metrics were evaluated using data sets generated from the 30 samples provided by Ken Kidd.

Amplicon Strand Balance is defined as the ratio of the minimum coverage strand counts to the maximum coverage strand counts. The average Amplicon Strand Balance should be 50±20% and have a strand ratio of >0.45 (see Figure 11 on page 43). Target average Amplicon Heterozygosity Balance is 50±15%. The average Amplicon Coverage across all markers should be within 2 standard deviations from the mean (see Figure 12 on page 43).

Sequence coverage for both forward and reverse strands was checked. To minimize heterozygosity bias, any amplicon where sequencing of one strand was favored over the other strand was removed from the panel. Minimizing heterozygosity bias is essential for ensuring equal representation of alleles, and eliminating false allele calls.

Variability in Amplicon Coverage across the panel was minimized by removing markers with lower sequencing performance as compared to other markers.

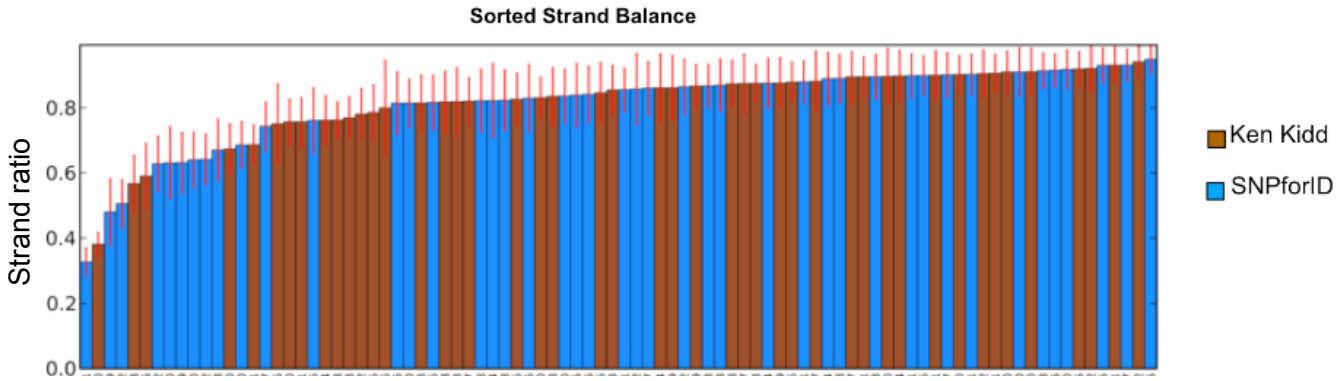


Figure 5 Amplicon Strand Balance for the autosomal SNPs in the Precision ID Identity Panel across all markers. Target average Amplicon Strand Balance is 50±20%. 95% of all markers were between 50–90%.

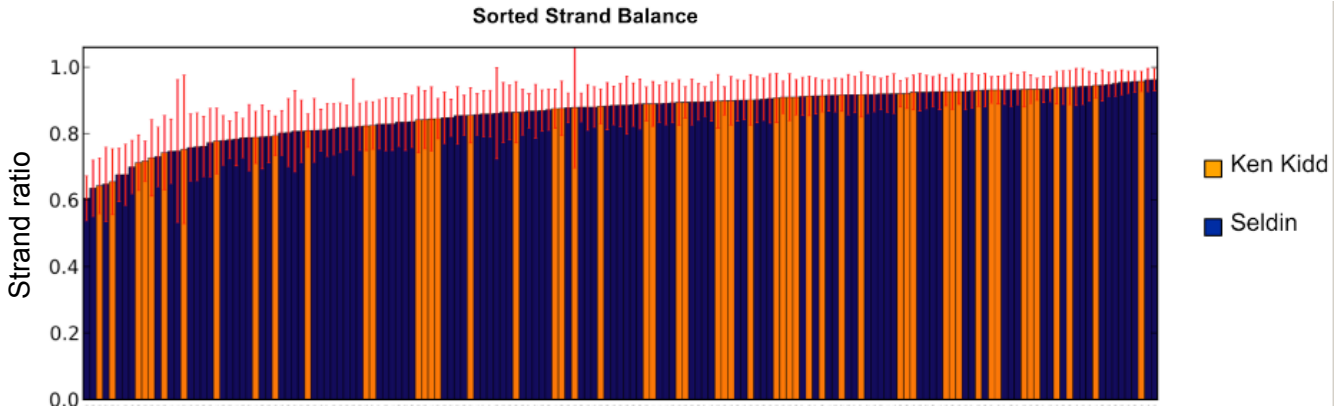


Figure 6 Amplicon Strand Balance for the Precision ID Ancestry Panel across all markers. Target average Amplicon Strand Balance is 50±20%. All markers were between 60–90%.

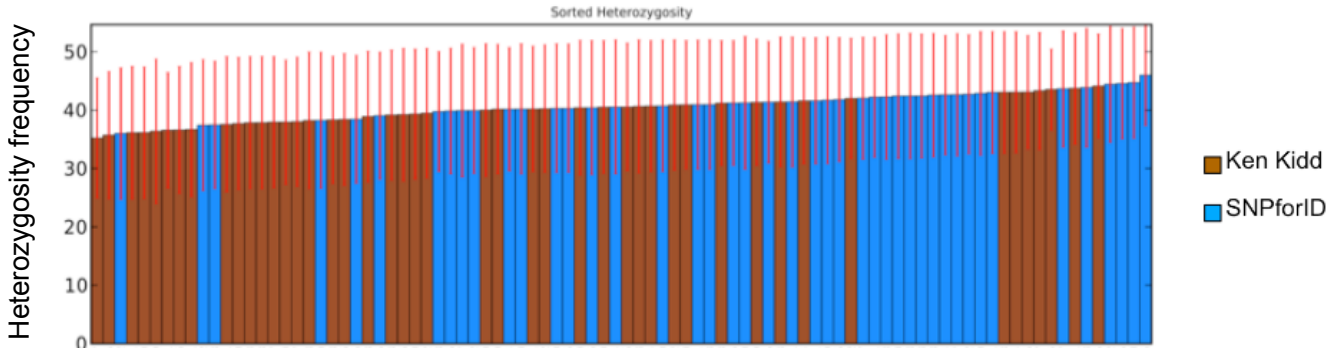


Figure 7 Average Amplicon Heterozygosity balance of autosomal SNPs in the Precision ID Identity Panel across all markers. Target average heterozygosity is 50±15%. The average heterozygosity across all markers was 35–45%.

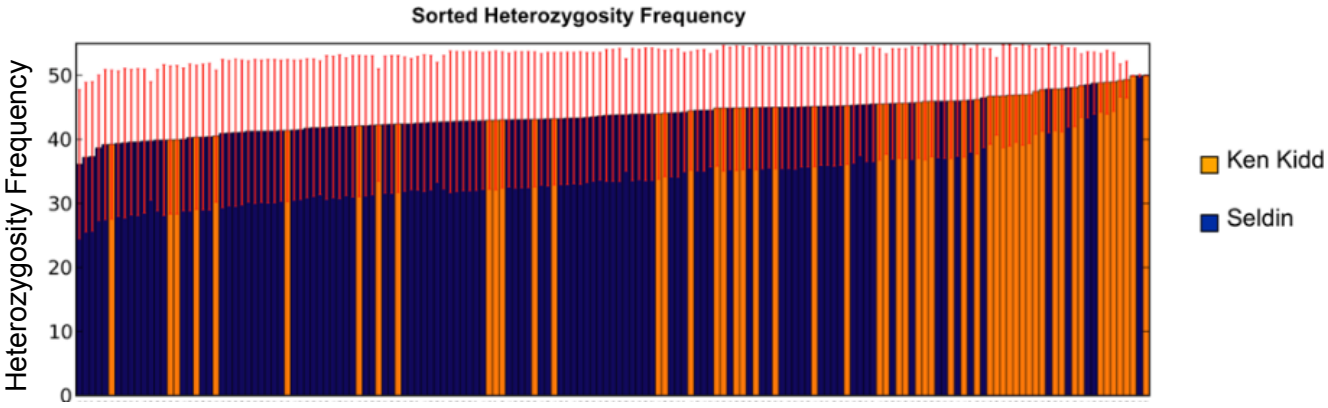


Figure 8 Average Amplicon Heterozygosity balance for the Precision ID Ancestry Panel across all markers. Target average heterozygosity is 50±15%. The average heterozygosity across all markers was 37–50%.

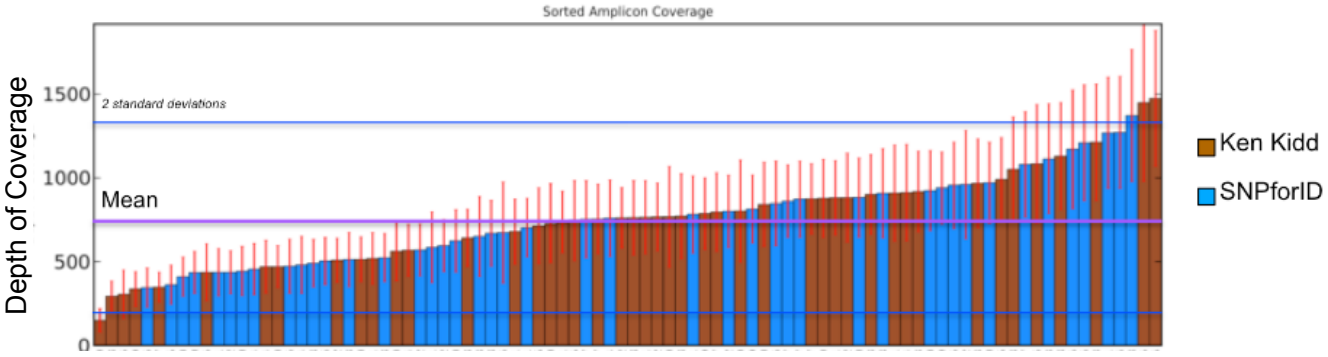


Figure 9 Amplicon Coverage for autosomal SNPs in the Precision ID Identity Panel across all markers. 96% of all markers were within 2 standard deviations of the mean.

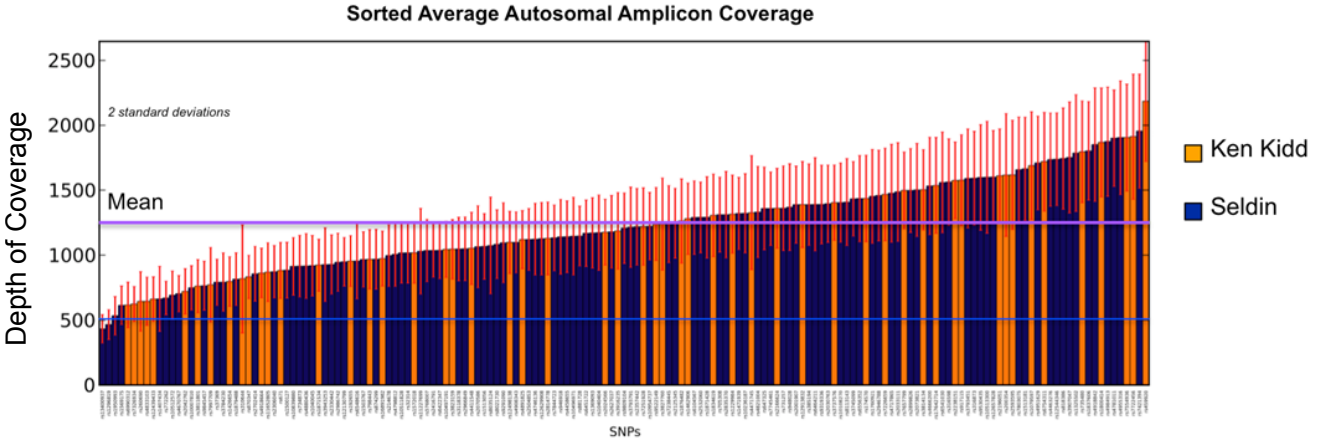


Figure 10 Amplicon Coverage for the Precision ID Ancestry Panel across all markers. 98% of all markers were within 2 standard deviations of the mean.

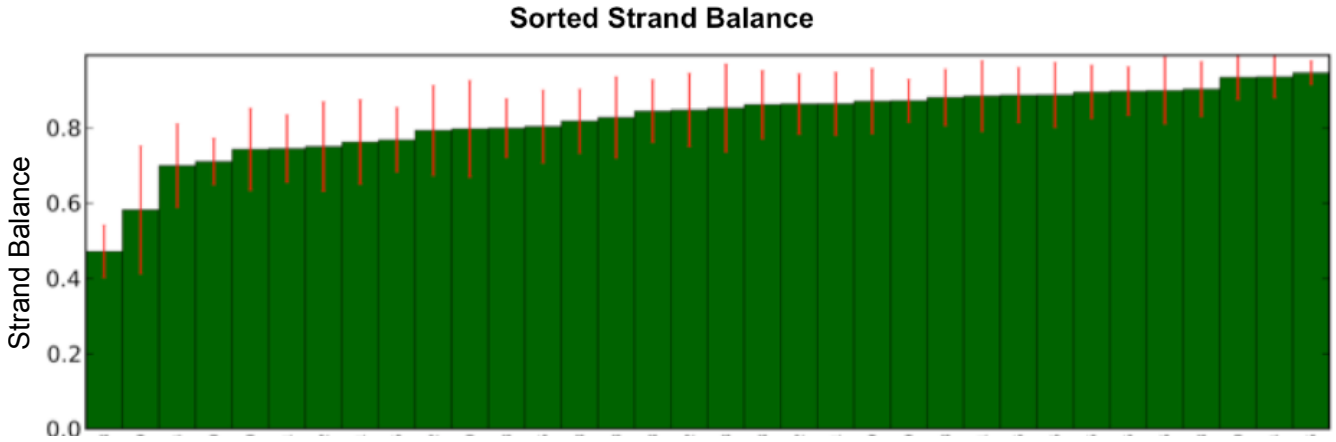


Figure 11 Amplicon Strand Balance for Y-Clade Markers in the Precision ID Identity Panel. 95% of the markers have an average amplicon strand balance between 70-90%.

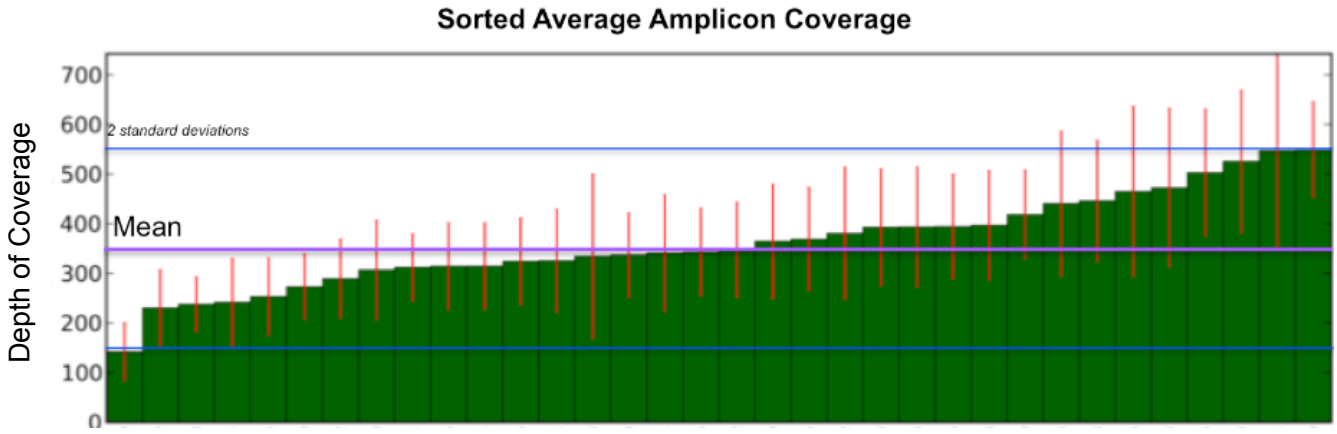


Figure 12 Average Amplicon Coverage for Y-Clade Markers in the Precision ID Identity Panel. 97% of the markers have an average amplicon coverage within 2 standard deviations of the mean.

Concordance results

The HID SNP Genotyper Plugin was used to analyze sequencing data for both the Precision ID Identity Panel and the Precision ID Ancestry Panel. Concordance results from genotype calls made using the plugin analysis output were compared to the reference TaqMan® genotype data provided by Ken Kidd. Concordance data were analyzed in the following subgroups:

- Genotype disparity (both alleles in the result disagree with the reference)
- Heterozygote disparity (data appear to have a dropout/loss of one allele)
- Homozygote disparity (data appear to have an additional allele)
- Concordant (both alleles match the reference)

A 10% minor allele frequency threshold was applied to genotype calls made using the plugin.

Precision ID Identity Panel

Genotype data for 32 individuals was analyzed for concordance (see Figure 13). Library samples were prepared using 180 pg to 1 ng of input gDNA per PCR reaction, and were sequenced on a single Ion 318™ Chip v2. TaqMan® genotype data for the 32 individuals was provided for 46 Ken Kidd SNPs. Two homozygote disparities were observed. Genotype data between calls made using the plugin and the TaqMan® data were 99.99% concordant.

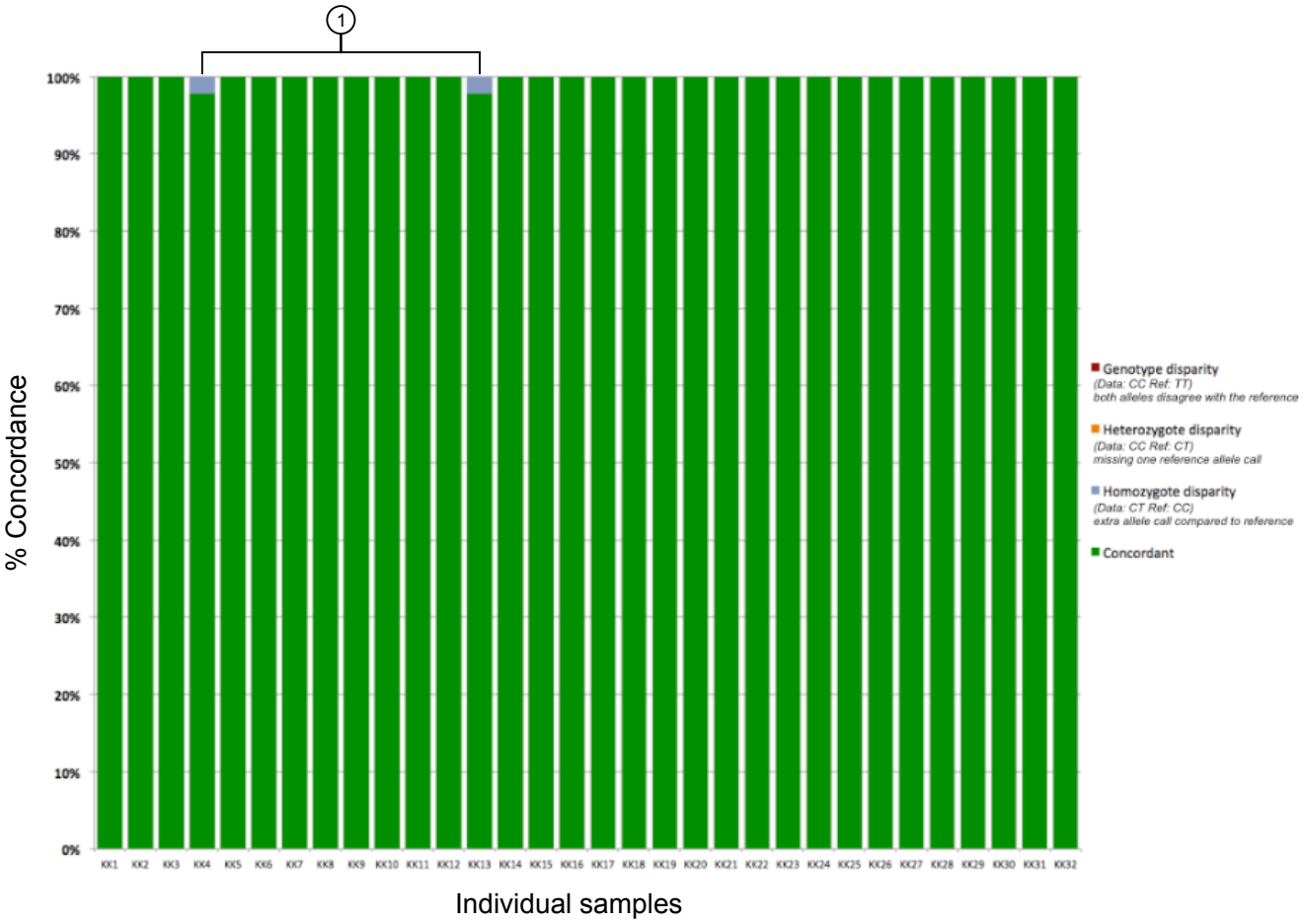


Figure 13 Precision ID Identity Panel concordance results

① Homozygote disparity

Precision ID Ancestry Panel

Genotype data for 24 individuals was analyzed for concordance (see Figure 14). Library samples were prepared using 1 ng of input gDNA per PCR reaction, and were sequenced on a single Ion 318™ Chip v2. TaqMan® genotype data for the 24 individuals were provided for 55 Ken Kidd SNPs. Two homozygote disparities and one heterozygote disparity were observed. Genotype data between calls made using the plugin and the TaqMan® data were 99.77% concordant.

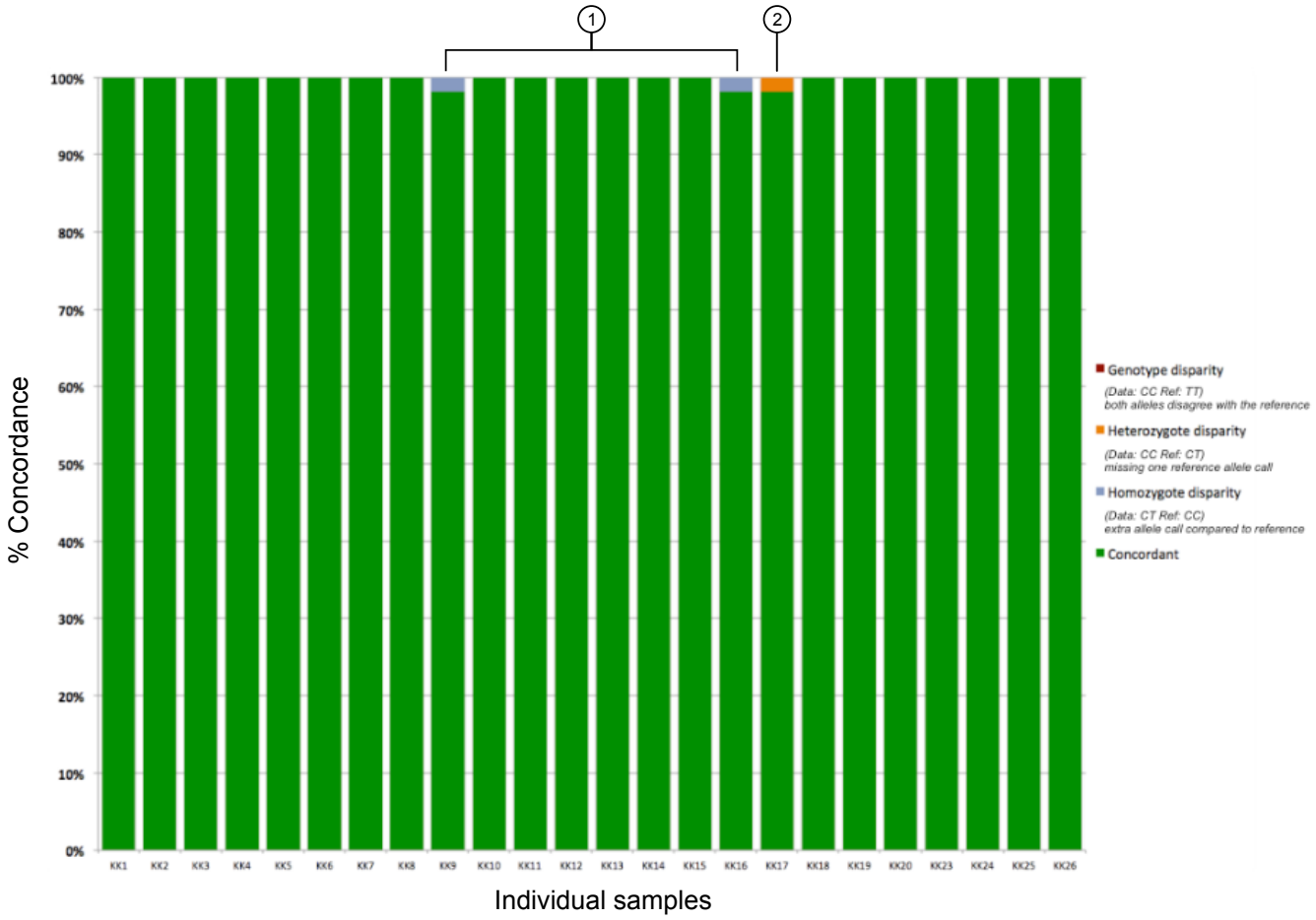


Figure 14 Precision ID Ancestry Panel concordance results

① Homozygote disparity

② Heterozygote disparity

Effect of degradation on the probability of identity

Using data from the 1000 Genomes database, it was determined that 85 of 90 autosomal identity SNPs were unlinked. The unlinked SNP data set was degraded in silico to determine the effect of degradation on the probability of identity. Data for four populations from the 1000 Genomes database (East Asian, Ad Mixed American, African, and European) along with values for a CE-based 21-plex STR assay were plotted to detect changes in probability of identity with decreasing amplicon size (see Figure 15). Based on these data, the identity SNP panel has lower combined match probabilities than the standard CE-based STR assay at all levels of degradation.

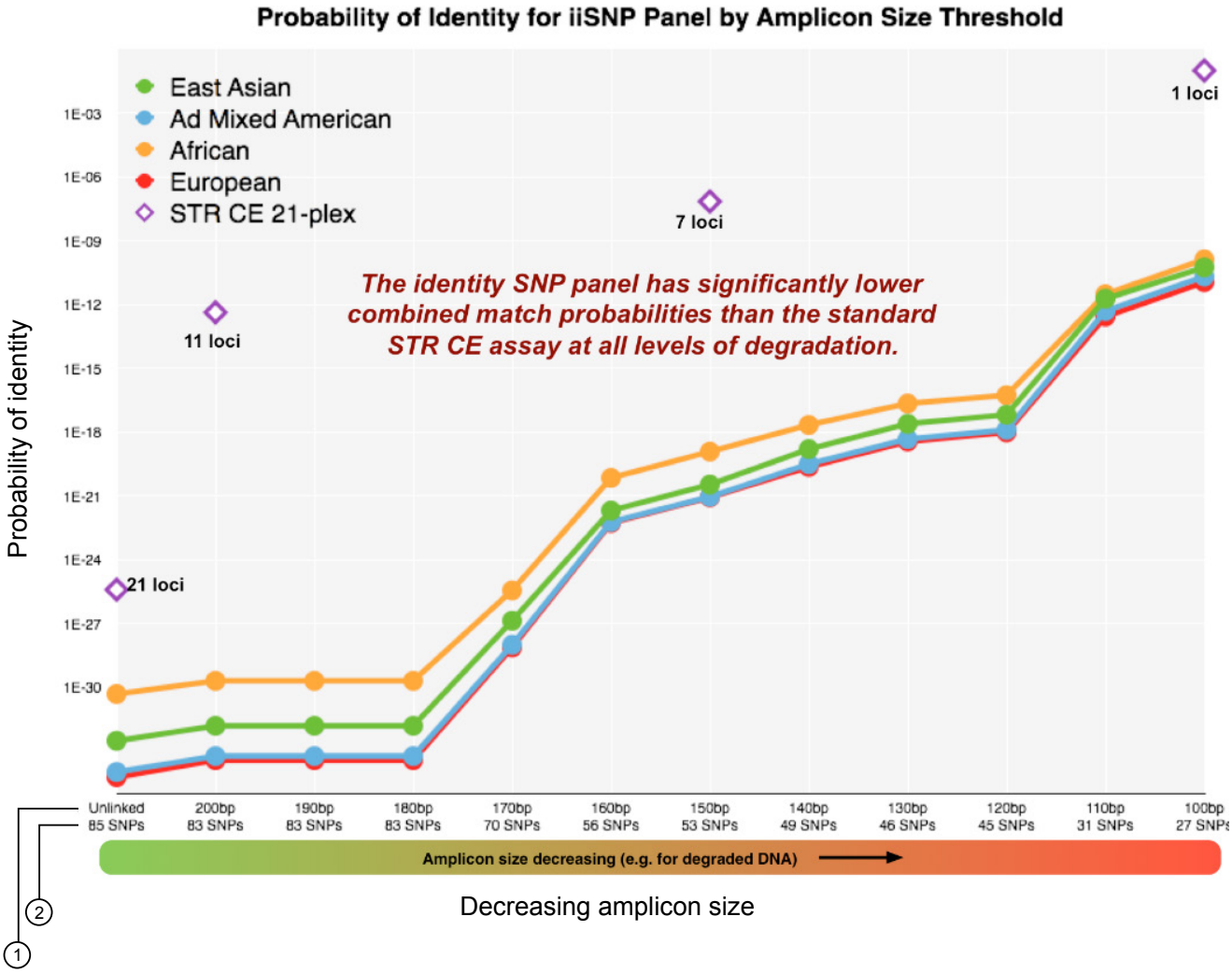


Figure 15 Effect of degradation on probability of identity for the Precision ID Identity Panel using probability of identity calculated from the 1000 Genomes database.

① Amplicon size

② Number of intact SNPs

Panel throughput

Using 80% chip loading and 60% usable reads, the minimum amount of coverage and, therefore, the number of individuals per Ion chip type can be determined for each SNP panel.

Precision ID Identity Panel

To obtain a minimum of 300X coverage for 97% of the autosomal SNPs, an average coverage depth of 738X is required. To obtain a minimum of 150X coverage for 97% of the Y-clade SNPs, an average coverage of 236X is required. Using the condition of 80% chip loading and 60% usable reads, this translates to the following run recommendations by chip type:

- Ion 314™ Chip v2: 8 individuals
- Ion 316™ Chip v2: 38 individuals
- Ion 318™ Chip v2: 77 individuals

Precision ID Ancestry Panel

To obtain a minimum of 300X coverage for 97% of the autosomal SNPs, an average coverage depth of 594X is required. Using the condition of 80% chip loading and 60% usable reads, this translates to the following run recommendations by chip type:

- Ion 314™ Chip v2: 6 individuals
- Ion 316™ Chip v2: 30 individuals
- Ion 318™ Chip v2: 59 individuals

C

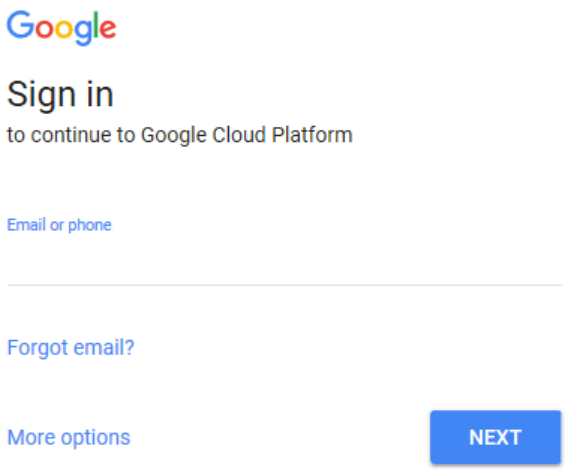
Configure the HID SNP Genotyper Plugin to add a Google™ Maps API key

The HID SNP Genotyper Plugin needs to be configured to add a Google™ Maps API key to access the Google™ Maps feature in population likelihood and admixture results. If the key is not added, maps may not be displayed on the result pages.

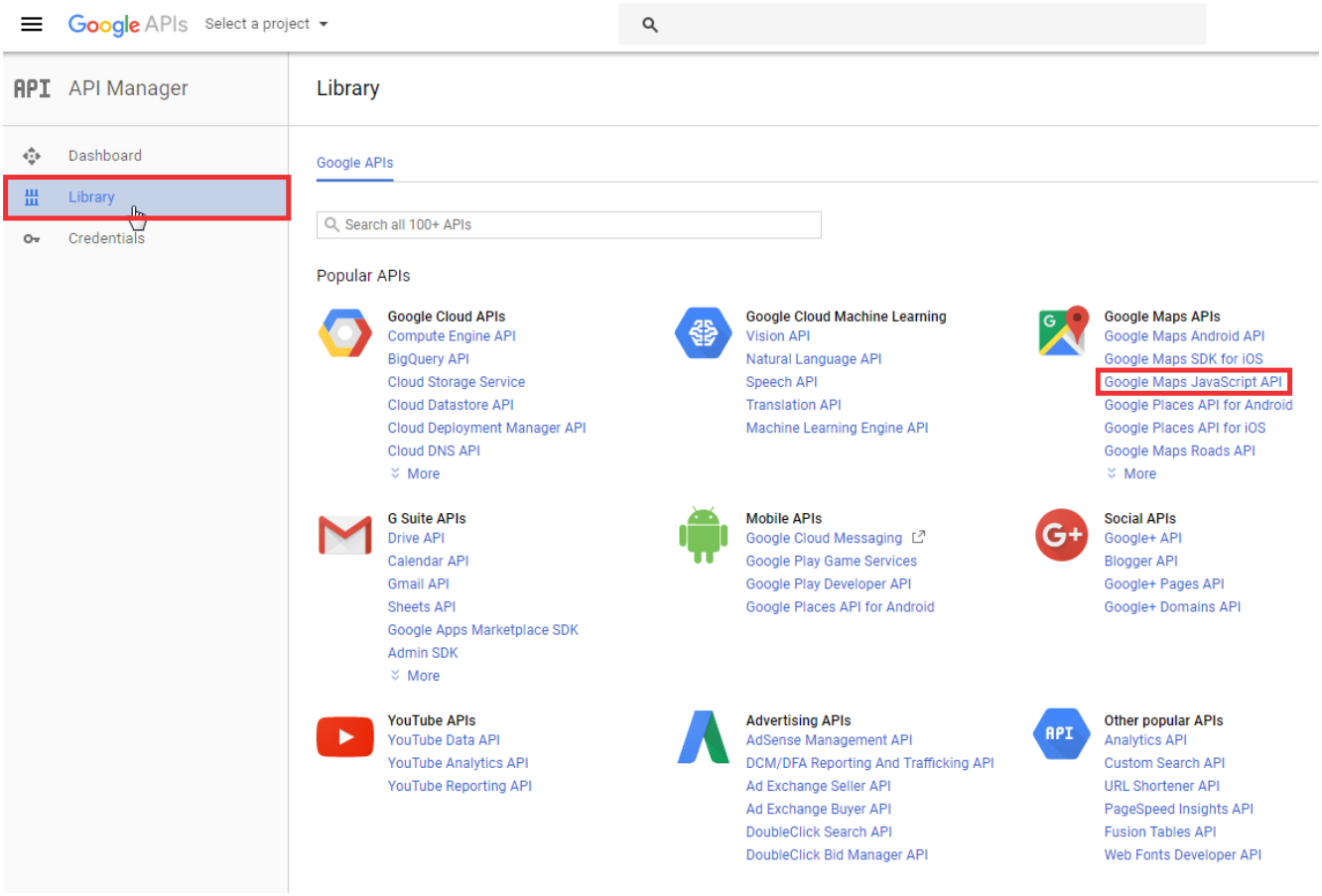
Use the following step-by-step procedure first to generate the key, then use the key to configure the plugin.

Generate a Google™ Maps API key

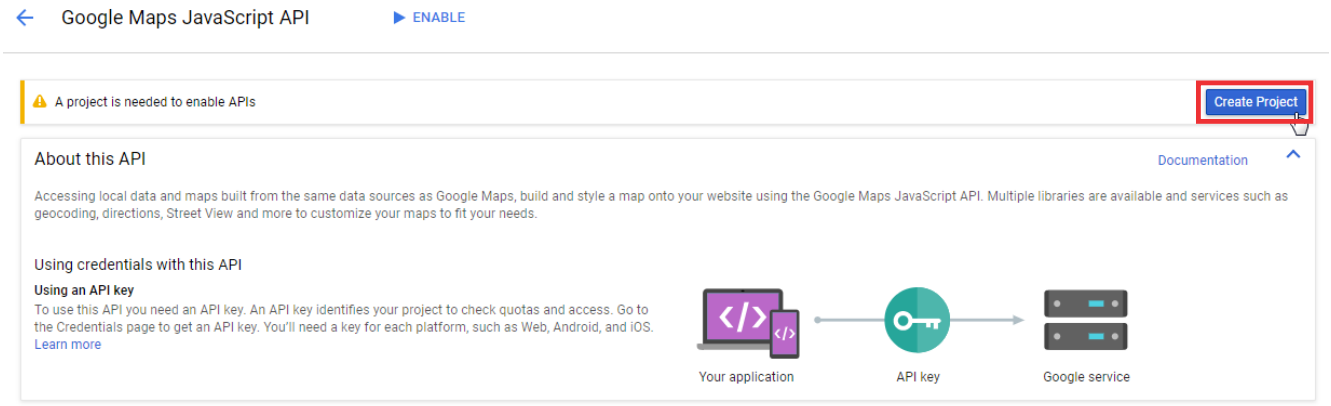
1. Go to **https://console.developers.google.com/**. Sign in using your Google™ account log in information.



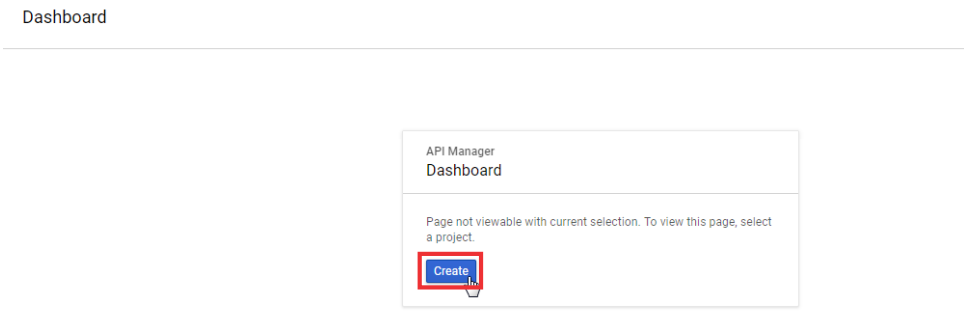
2. After signing in, click the **Library** link at the upper left corner of the screen, then click **Google Maps JavaScript API**.



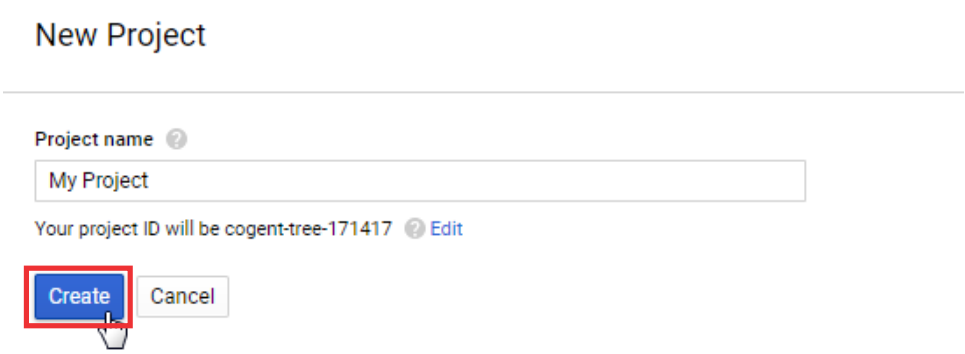
3. If you are configuring the Google™ Maps Javascript API for first time, click **Create Project**.



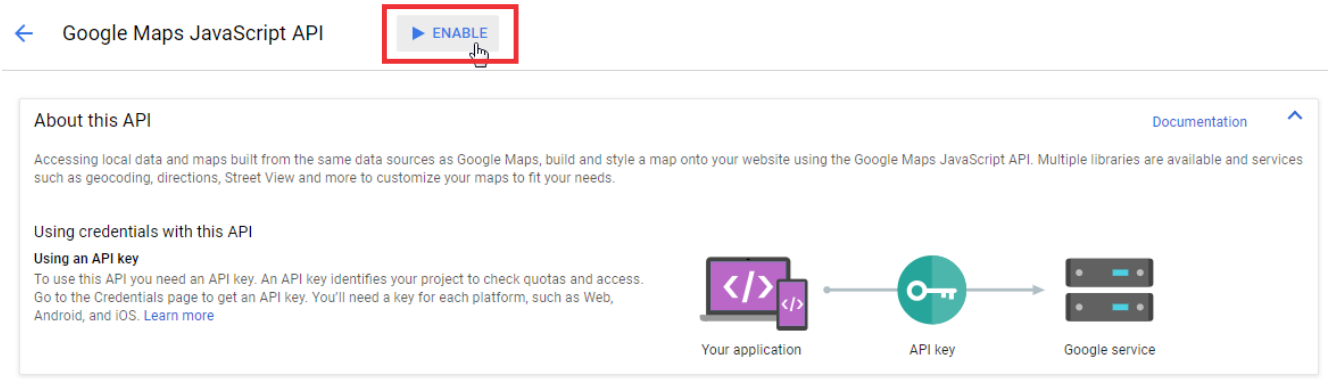
4. On the **Dashboard** screen, click **Create**.



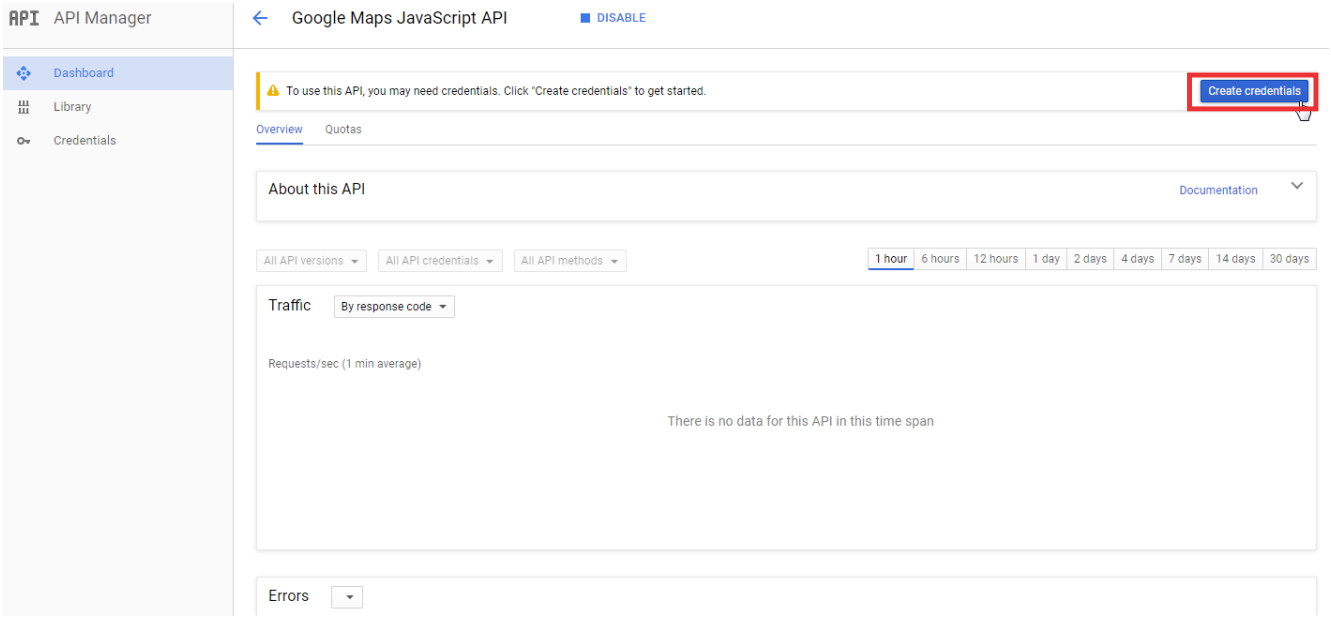
5. Enter a project name, then click **Create**.



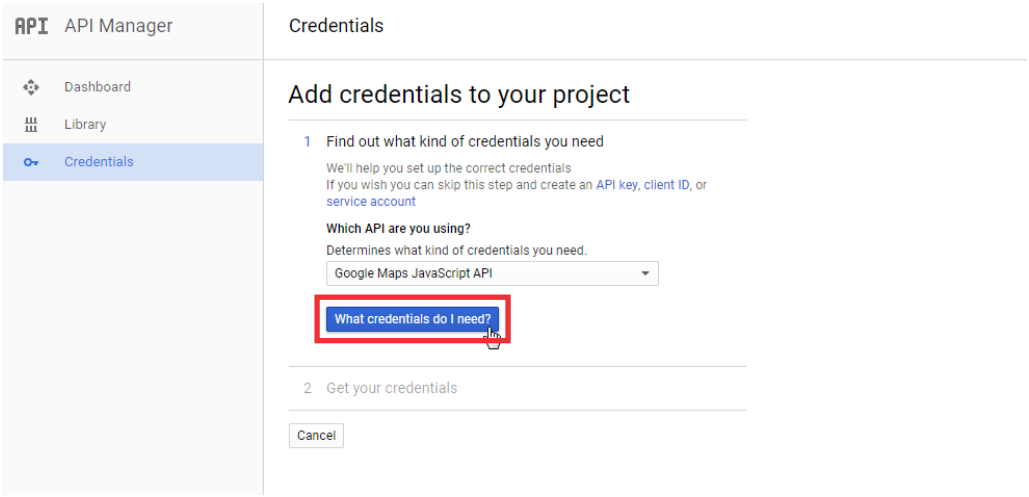
6. Click **Enable** to initiate key generation.



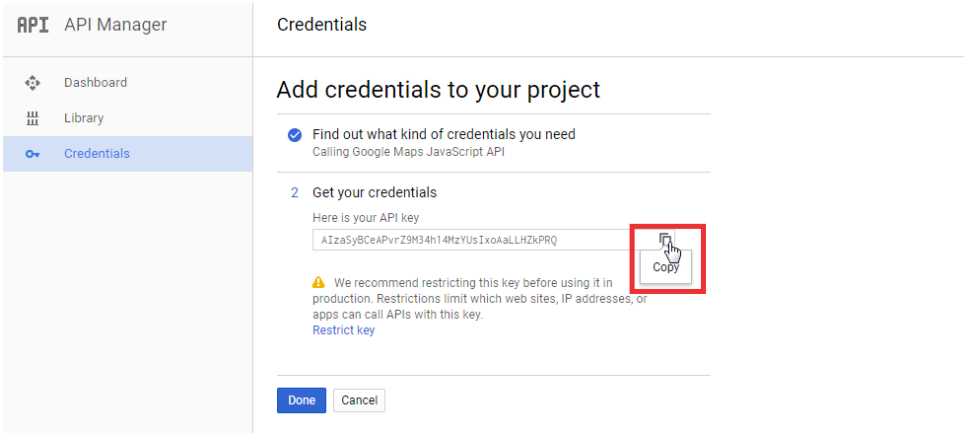
7. On the next page, click **Create credentials**.



8. Click **What credentials do I need?** to generate a key.

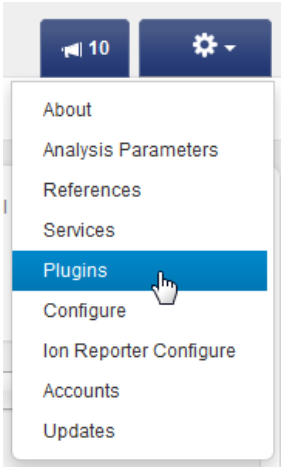


9. Click the **Copy** icon to the right of the field where the key appears.

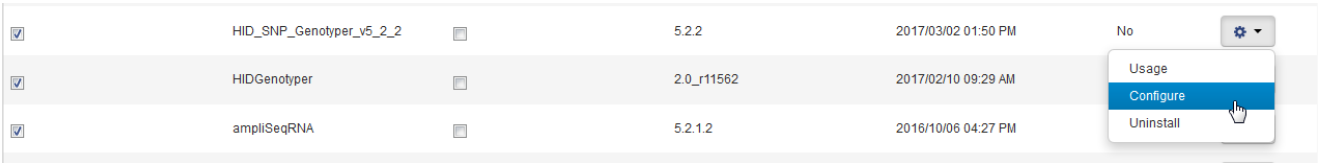


Configure the plugin with the API key

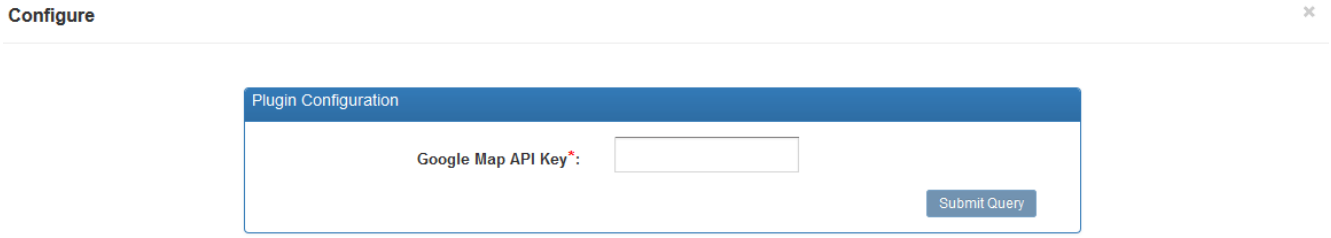
1. Sign in to the Torrent Server via the Torrent Browser.
2. Go to the Plugins page by clicking ⚙ ▼, then **Plugins**.



3. Locate the HID_SNP Genotyper_v5_2_2 plugin, then click **Configure** under the ⚙ ▼ menu for the plugin.



4. Paste the API key you generated into the **Google Map API Key** field, then click **Submit Query**.



Close the dialog. You should see that Google™ Maps is displayed for all the previously generated results, as well as for future run results.

Note: Certain usage restrictions for the key may exist, and you may need to regenerate it. In that case, repeat the steps to generate a new API key, then reconfigure the plugin.

D Kits and reagents

Precision ID SNP panels

The Precision ID Library Kit supports the following Precision ID SNP panels:

Item	Cat. No.	Amount	Average amplicon size[1]	No. of primer pairs	Storage
Precision ID Ancestry Panel[2]	A25642	1 tube	127 bp	165	–30°C to –10°C
Precision ID Identity Panel[3]	A25643	1 tube	138 bp	124	

[1] Libraries have an additional ~80 bp due to barcode adapters.

[2] Also available bundled with the Precision ID Library Kit as Cat. No. A26807.

[3] Also available bundled with the Precision ID Library Kit as Cat. No. A26808.

Precision ID IonCode™ 1–96 Kit in 96 Well PCR Plate

The Precision ID IonCode™ 1–96 Kit in 96 Well PCR Plate (Cat. No. A33586) contains a set of 96 unique barcode adapters in a 96-well plate format for use in manual library preparation. When used in combination with the Precision ID Library Kit, this kit enables pooling of up to 96 libraries for multiplex sequence analysis.

Component	Quantity	No. of reactions	Storage
Precision ID IonCode™ 1–96 Kit in 96 Well PCR Plate	1 × 96-well plate (20 µL/well)	960 (10 reactions per barcode)	–30°C to –10°C

Documentation and support

Related documentation

Document	Publication number
Precision ID Panels with Ion PGM™ System Application Guide	MAN0015830
Precision ID Panels with Ion S5™ System Application Guide	MAN0015831
Torrent Suite™ Software Help	See **thermofisher.com/torrent-suite-software-docs.html**
IonCode™ Barcode Adapters 1–384 Kit Product Information Sheet	MAN0014640

Customer and technical support

For support:

- **In North America**—Send an email to **HIDTechSupport@thermofisher.com**, or call **888-821-4443 option 1**.
- **Outside North America**—Contact your local support office.
- For latest services and support information for all locations, go to **thermofisher.com/support**.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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