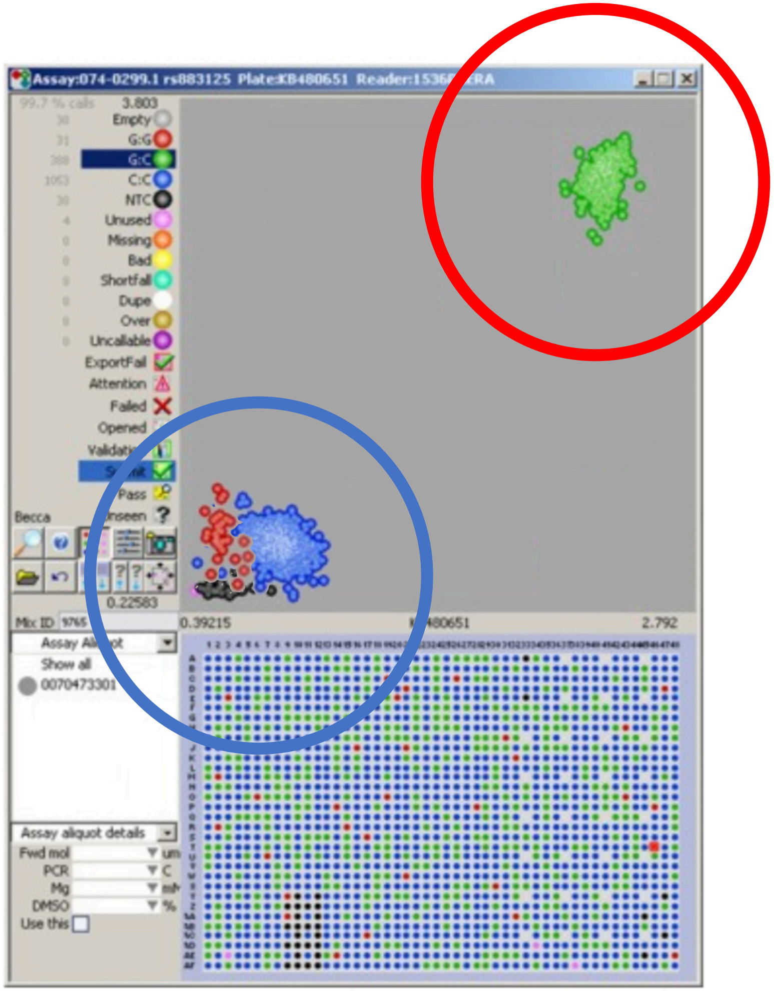
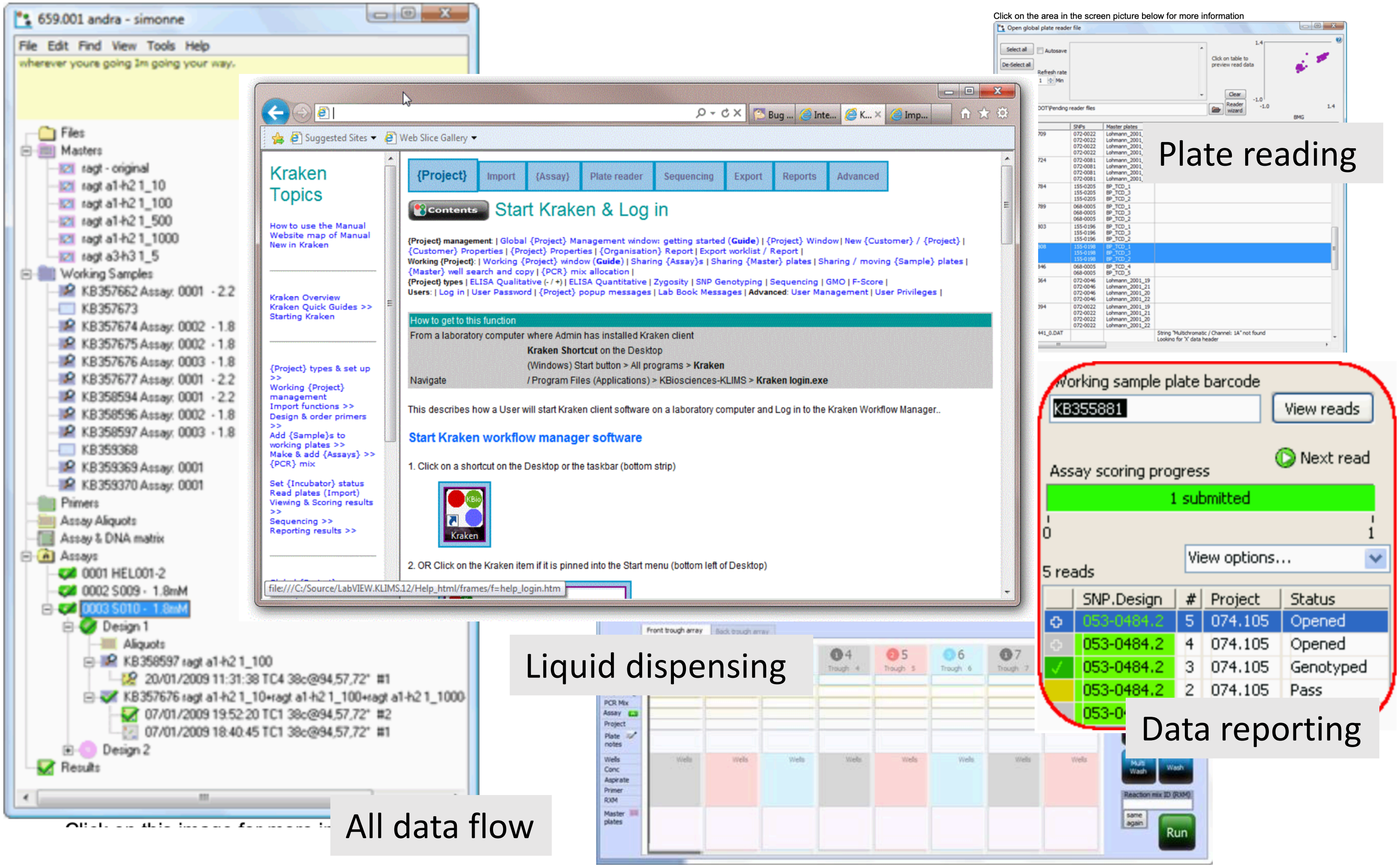
Data Handling: End Point

- Any Mass Testing methodology must match throughput to data handling/scoring
- KRAKEN has been designed as the only dedicated Mass Testing LIMS and has processed Billions of data points on over 350 genomes, on millions of amplicon assays.
- End point allows simplified data scoring reporting as a YES/NO
- Million ++ data points per day is all in house, 1 person per day



KRAKEN LIMS

Laboratory Information Management System
Every aspect of a Mass testing lab “Nose to tail”



Click on the area in the screen picture below for more information

Plate reading

659.001 andra - simonne

wherever youre going Im going your way.

- Files
- Masters
  - ragt - original
  - ragt a1-h2 1_10
  - ragt a1-h2 1_100
  - ragt a1-h2 1_500
  - ragt a1-h2 1_1000
  - ragt a3-h3 1_5
- Working Samples
  - KB357662 Assay 0001 - 2.2
  - KB357673
  - KB357674 Assay 0002 - 1.8
  - KB357675 Assay 0002 - 1.8
  - KB357676 Assay 0003 - 1.8
  - KB357677 Assay 0001 - 2.2
  - KB358594 Assay 0001 - 2.2
  - KB358596 Assay 0002 - 1.8
  - KB358597 Assay 0003 - 1.8
  - KB359368
  - KB359369 Assay 0001
  - KB359370 Assay 0001
- Primers
- Assay Aliquots
- Assay & DNA matrix
- Assays
  - 0001 HEL001-2
  - 0002 S009 - 1.8mM
  - 0003 S010 - 1.8mM
    - Design 1
      - Aliquots
      - KB358597 ragt a1-h2 1_100
        - 20/01/2009 11:31:38 TC4 38c@94.57.72' #1
      - KB357676 ragt a1-h2 1_10+ragt a1-h2 1_100+ragt a1-h2 1_1000
        - 07/01/2009 19:52:20 TC1 38c@94.57.72' #2
        - 07/01/2009 18:40:45 TC1 38c@94.57.72' #1
    - Design 2
- Results

Click on this image for more i

All data flow

Kraken Topics

How to use the Manual
Website map of Manual
New in Kraken

Kraken Overview
Kraken Quick Guides >>
Starting Kraken

{Project} types & set up >>
Working {Project} management
Import functions >>
Design & order primers >>
Add {Sample}s to working plates >>
Make & add {Assays} >>
{PCR} mix

Set {Incubator} status
Read plates (Import)
Viewing & Scoring results >>
Sequencing >>
Reporting results >>

Start Kraken & Log in

{Project} management: | Global {Project} Management window: getting started (**Guide**) | {Project} Window | New {Customer} / {Project} | {Customer} Properties | {Project} Properties | {Organisation} Report | Export worklist / Report |
Working {Project}: | Working {Project} window (**Guide**) | Sharing {Assay}s | Sharing {Master} plates | Sharing / moving {Sample} plates | {Master} well search and copy | {PCR} mix allocation |
{Project} types | ELISA Qualitative (- / +) | ELISA Quantitative | Zygosity | SNP Genotyping | Sequencing | GMO | F-Score |
Users: | Log in | User Password | {Project} popup messages | Lab Book Messages | **Advanced**: User Management | User Privileges |

How to get to this function	
From a laboratory computer where Admin has installed Kraken client	
	Kraken Shortcut on the Desktop
	(Windows) Start button > All programs > **Kraken**
Navigate	/ Program Files (Applications) > KBiosciences-KLIMS > **Kraken login.exe**

This describes how a User will start Kraken client software on a laboratory computer and Log in to the Kraken Workflow Manager..

Start Kraken workflow manager software

1. Click on a shortcut on the Desktop or the taskbar (bottom strip)

2. OR Click on the Kraken item if it is pinned into the Start menu (bottom left of Desktop)

file:///C:/Source/LabVIEW.KLIMS.12/Help_html/frames/f=help_login.htm

Liquid dispensing

Working sample plate barcode: KB355881

Assay scoring progress

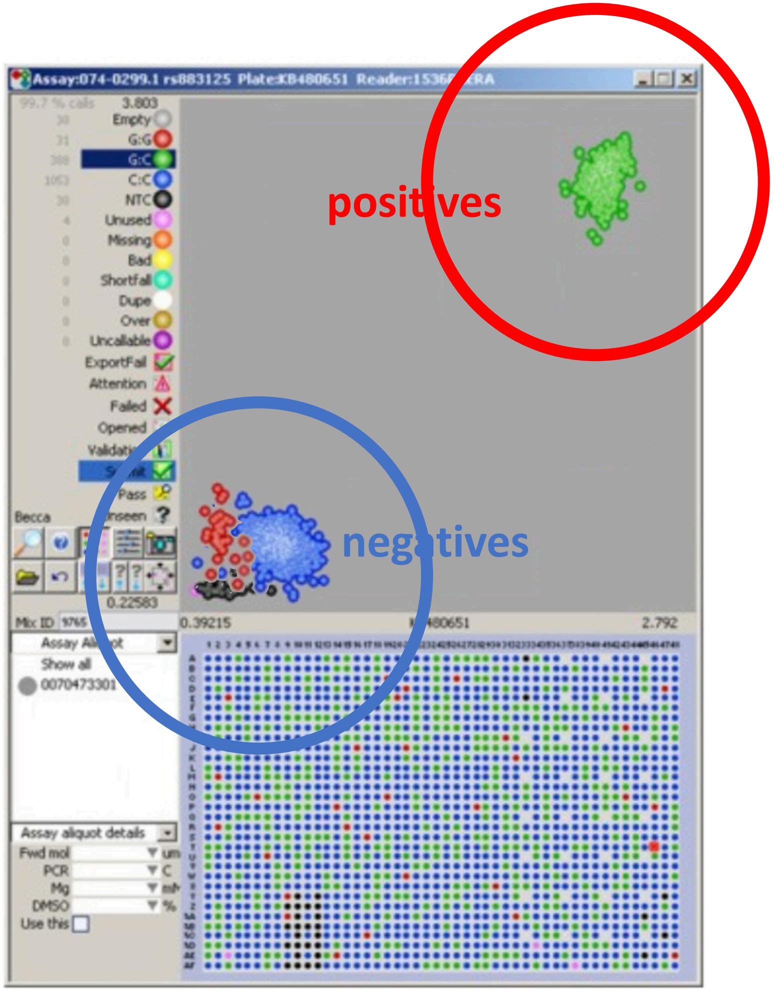
1 submitted

5 reads

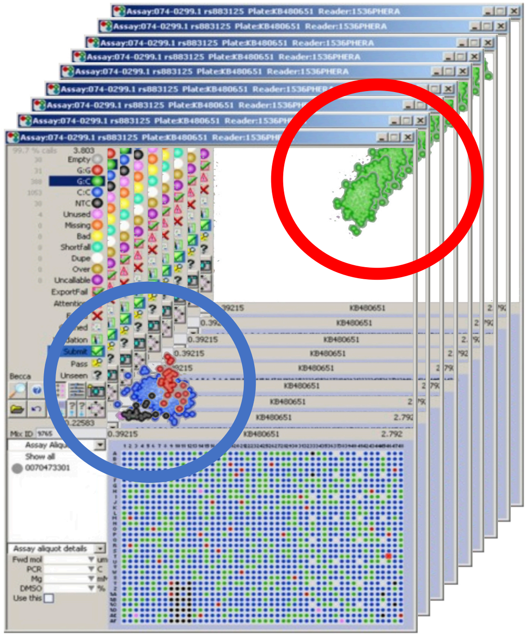
	SNP.Design	#	Project	Status
✚	053-0484.2	5	074.105	Opened
✚	053-0484.2	4	074.105	Opened
✓	053-0484.2	3	074.105	Genotyped
🟨	053-0484.2	2	074.105	Pass
	053-0			

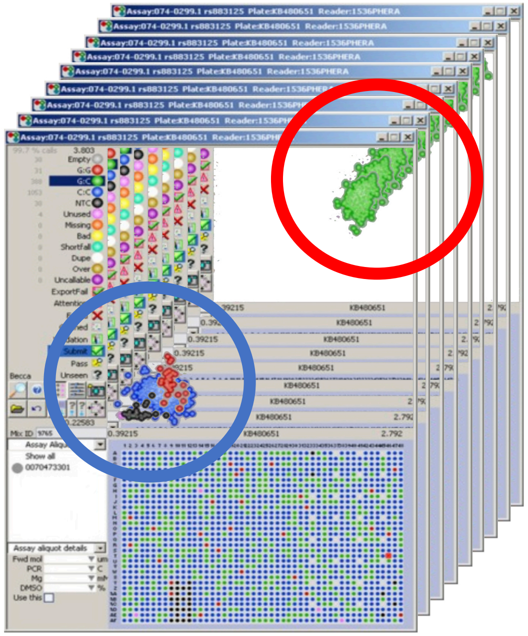
Data reporting

Data calling: End Point



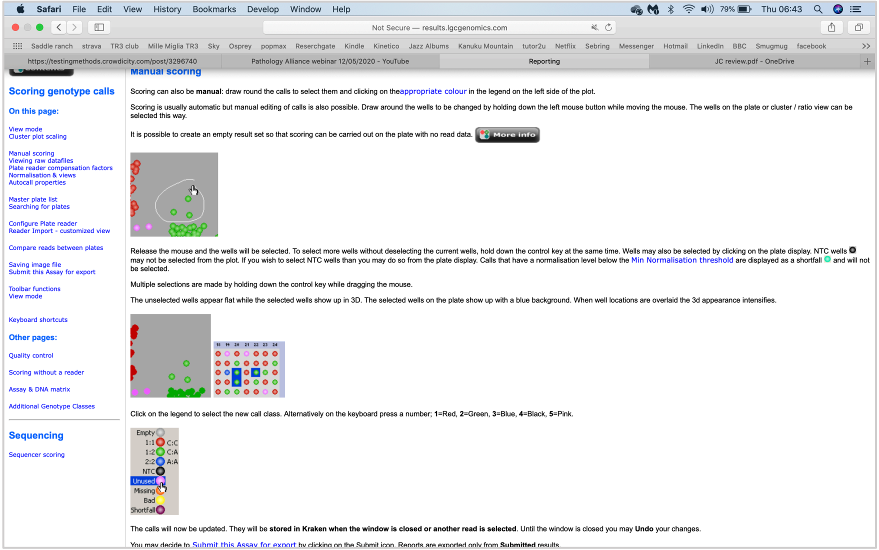
Data is automatically or manually scored with definable levels of user security





Manual Auto calling data

KRAKEN Manual calling data



Scoring genotype calls

On this page:

View mode
Cluster plot scaling

Manual scoring
Viewing raw datafiles
Plate reader compensation factors
Normalisation & views
Autocall properties

Master plate list
Searching for plates

Configure Plate reader
Reader Import - customized view

Compare reads between plates

Saving image file
Submit this Assay for export

Toolbar functions
View mode

Keyboard shortcuts

Other pages:

Quality control

Scoring without a reader

Assay & DNA matrix

Additional Genotype Classes

Sequencing

Sequencer scoring

Manual scoring

Scoring can also be **manual**: draw round the calls to select them and clicking on theappropriate colour in the legend on the left side of the plot.

Scoring is usually automatic but manual editing of calls is also possible. Draw around the wells to be changed by holding down the left mouse button while moving the mouse. The wells on the plate or cluster / ratio view can be selected this way.

It is possible to create an empty result set so that scoring can be carried out on the plate with no read data.

Release the mouse and the wells will be selected. To select more wells without deselecting the current wells, hold down the control key at the same time. Wells may also be selected by clicking on the plate display. NTC wells ● may not be selected from the plot. If you wish to select NTC wells than you may do so from the plate display. Calls that have a normalisation level below the Min Normalisation threshold are displayed as a shortfall ● and will not be selected.

Multiple selections are made by holding down the control key while dragging the mouse.

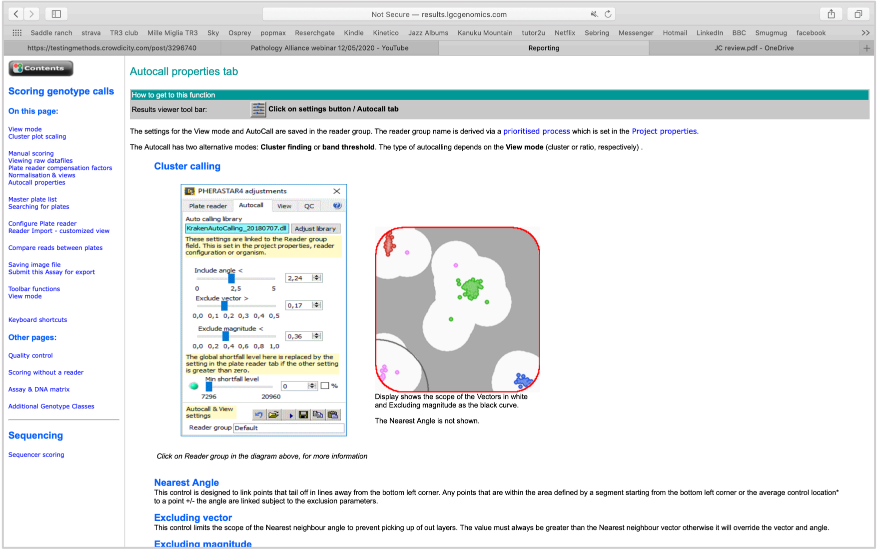
The unselected wells appear flat while the selected wells show up in 3D. The selected wells on the plate show up with a blue background. When well locations are overlaid the 3d appearance intensifies.

Click on the legend to select the new call class. Alternatively on the keyboard press a number; **1**=Red, **2**=Green, **3**=Blue, **4**=Black, **5**=Pink.

The calls will now be updated. They will be **stored in Kraken when the window is closed or another read is selected**. Until the window is closed you may **Undo** your changes.

You may decide to Submit this Assay for export by clicking on the Submit icon. Reports are exported only from **Submitted** results.

KRAKEN Auto calling data



Scoring genotype calls

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Autocall properties tab

How to get to this function
Results viewer tool bar: **Click on settings button / Autocall tab**

The settings for the View mode and AutoCall are saved in the reader group. The reader group name is derived via a prioritised process which is set in the Project properties.

The Autocall has two alternative modes: **Cluster finding** or **band threshold**. The type of autocalling depends on the **View mode** (cluster or ratio, respectively) .

Cluster calling

Auto calling library: KrakenAutoCalling_20180707.dll

These settings are linked to the Reader group field. This is set in the project properties, reader configuration or organism.

Include angle < 2,24

Exclude vector > 0,17

Exclude magnitude < 0,36

The global shortfall level here is replaced by the setting in the plate reader tab if the other setting is greater than zero.

Min shortfall level 0 ☐ %

Autocall & View settings

Reader group Default

Display shows the scope of the Vectors in white and Excluding magnitude as the black curve.

The Nearest Angle is not shown.

Click on Reader group in the diagram above, for more information

Nearest Angle

This control is designed to link points that tail off in lines away from the bottom left corner. Any points that are within the area defined by a segment starting from the bottom left corner or the average control location* to a point +/- the angle are linked subject to the exclusion parameters.

Excluding vector

This control limits the scope of the Nearest neighbour angle to prevent picking up of out layers. The value must always be greater than the Nearest neighbour vector otherwise it will override the vector and angle.

Excluding magnitude

KRAKEN has been and is still used to call Millions of data points, capable of Auto calling 1.5 million or 10,000,000++ Data points per day.
Its with out doubt the most significant part of any mass testing program.

KRAKEN LIMS Laboratory Information Management System

Troubleshooting
FAQ

Kraken Overview
Kraken Quick Guides >>
Starting Kraken

Project types & set up >>
Working Project management
Import functions >>
Design & order primers >>
Add Samples to working plates >>
Make & add {Assays} >>
PCR mix

Set Incubator status
Read plates (Import)
Viewing & Scoring results >>
Sequencing >>
Reporting results >>

Global Project management
Event log
Searching >>
Definitions
Lab book
Managing users

Advanced >>
Installing server & clients
Database tables

Troubleshooting
FAQ

Kraken

Multiple users log on to password protected Kraken. Each User has their own privileges. User names are recorded in logs, as users interact with Kraken.

Kraken has a single database and a shared fileserver area, allowing users to share data in real time.

Kraken data is subdivided by Customer. All entities (other than Kraken users) belong to a Customer or are first created for a Customer.

Customer

Customers are added to Kraken. A Customer holds source information for Assays, oligonucleotide Primers, DNA Sample tracking, default QC criteria, organism, notes and contact details. A Customer may also be subdivided by Organisation.

Each Customer has one or more Projects associated with it.

Customer

Customers are added to Kraken. A Customer holds source information for Assays, oligonucleotide Primers, DNA Sample tracking, default QC criteria, organism, notes and contact details. A Customer may also be subdivided by Organisation.

Each Customer has one or more Projects associated with it.

{Customers} contain Projects

Project

Every Project is assigned to a Customer. Each Project has Master plates containing Samples provided for the Customer and Assays used on working Sample plates. It links to Assays and Primers from any Project as needed.

Projects are displayed as a navigation tree, the Project tree. From the Project tree, Assays from this or other Customers may be viewed and imported into the Project (to assist in locating the Assay Aliquot physical tubes). The Project tree containsits own working Sample plates, which may be dispensed from dilutions of the Master DNA (sisters) and daughter plates which are stamped from sisters. Assay results are linked to the Project so that experimental set up and data analysis is performed from the Project tree.

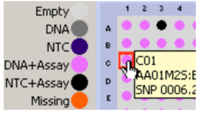
SNPs
- 0001 GHGln90
- 0002 GHArg51Gln
- 0003 GHLeu72Met
- 0004 BDNFC270T

Each Project is a self-contained information set defining everything from incoming Master sample information, to the results and the final report.

Notes, Files, DNA samples, Working Sample copies, Raw data

Items stored with each Project are: Master plates containing DNA with their storage location. To each Project, Master plates may be, replicated, subsets copied and Assays added virtually, modeling the experimental workflow in the laboratory. Location tracking information is stored in real time so that you can see where a Sample plate is at any given moment. Files on the computer system, which are destinations for Reports, are make available in the Project tree.

Any item contained on a plate or in a multi-well box may be reviewed on a picture of that plate. Each spot in a picture is fully interactive, so that you can drill for information about a single well (shown in the image, right). .



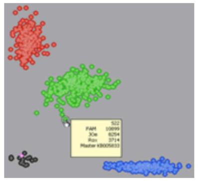
Many items are stored with a Project but only a subset of the scored plate read data is required for the final report and stored as Results.

Result

Results are automatically collated from plate readers into the correct Project based on the barcode (or name) assigned to each Sample plate. This feature allows multiple Projects to be bundled in the same experimental batch, because each set of results is immediately associated with its own Project/DNA/Assay.

After data has been read it may be automatically called, and made available for display within its Project, with its identifiers for Assays and DNA Samples already assigned.

When reviewing data, spots on a cluster plot interact with a plate diagram and normalisation data. Calls may be automatic, but are also assigned manually, using these interfaces. Each spot may be drilled for information (shown in the image, right).

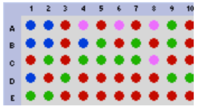


Automatic reports generated from Result set

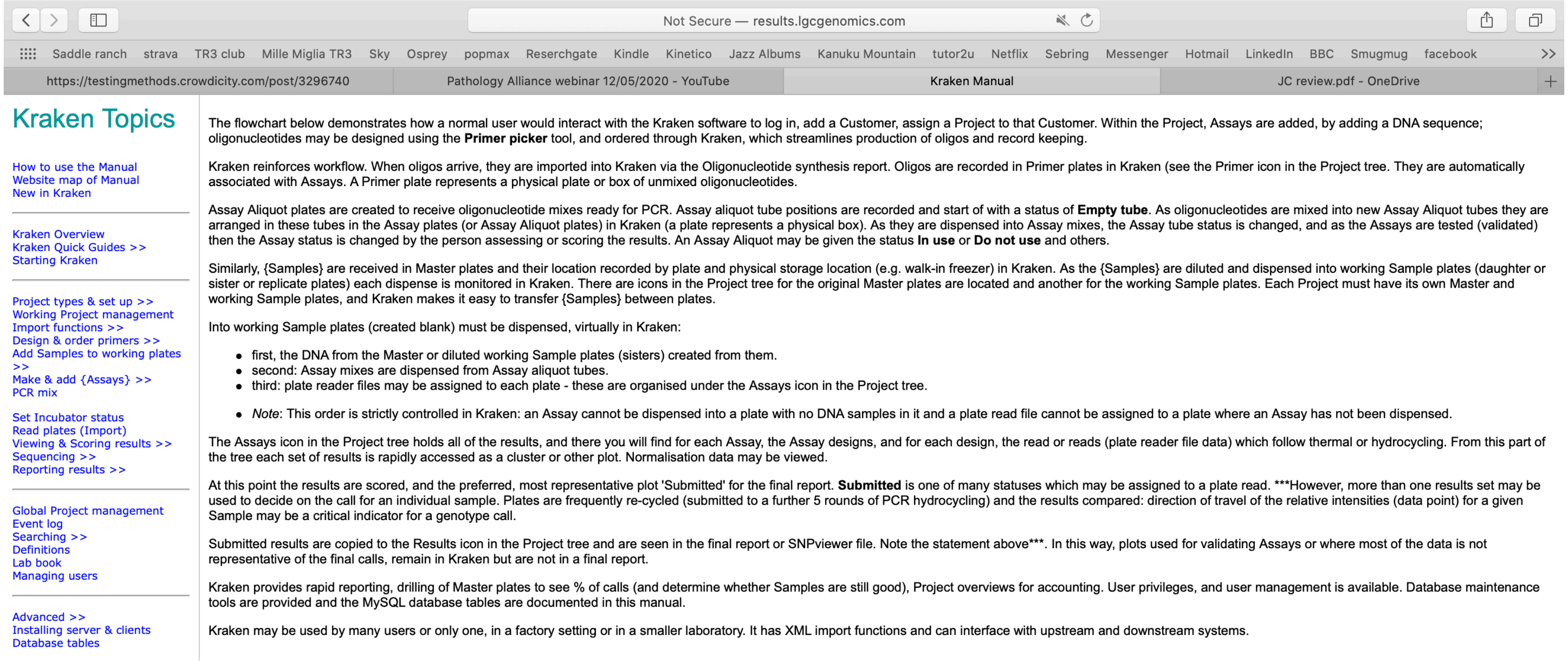
Report

Reports may be generated for general use. Results may be exported for use in the free SNPviewer tool. The SNPviewer can then export subsets of the data to other systems.

Reports and exported data are sent to Files available both from Kraken's Project tree and from a general computer navigation system (such as an Explorer window).



KRAKEN LIMS Laboratory Information Management System



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How to use the Manual
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Kraken Overview
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Set Incubator status
Read plates (Import)
Viewing & Scoring results >>
Sequencing >>
Reporting results >>

Global Project management
Event log
Searching >>
Definitions
Lab book
Managing users

Advanced >>
Installing server & clients
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The flowchart below demonstrates how a normal user would interact with the Kraken software to log in, add a Customer, assign a Project to that Customer. Within the Project, Assays are added, by adding a DNA sequence; oligonucleotides may be designed using the **Primer picker** tool, and ordered through Kraken, which streamlines production of oligos and record keeping.

Kraken reinforces workflow. When oligos arrive, they are imported into Kraken via the Oligonucleotide synthesis report. Oligos are recorded in Primer plates in Kraken (see the Primer icon in the Project tree. They are automatically associated with Assays. A Primer plate represents a physical plate or box of unmixed oligonucleotides.

Assay Aliquot plates are created to receive oligonucleotide mixes ready for PCR. Assay aliquot tube positions are recorded and start of with a status of **Empty tube**. As oligonucleotides are mixed into new Assay Aliquot tubes they are arranged in these tubes in the Assay plates (or Assay Aliquot plates) in Kraken (a plate represents a physical box). As they are dispensed into Assay mixes, the Assay tube status is changed, and as the Assays are tested (validated) then the Assay status is changed by the person assessing or scoring the results. An Assay Aliquot may be given the status **In use** or **Do not use** and others.

Similarly, {Samples} are received in Master plates and their location recorded by plate and physical storage location (e.g. walk-in freezer) in Kraken. As the {Samples} are diluted and dispensed into working Sample plates (daughter or sister or replicate plates) each dispense is monitored in Kraken. There are icons in the Project tree for the original Master plates are located and another for the working Sample plates. Each Project must have its own Master and working Sample plates, and Kraken makes it easy to transfer {Samples} between plates.

Into working Sample plates (created blank) must be dispensed, virtually in Kraken:

- first, the DNA from the Master or diluted working Sample plates (sisters) created from them.
- second: Assay mixes are dispensed from Assay aliquot tubes.
- third: plate reader files may be assigned to each plate - these are organised under the Assays icon in the Project tree.

- *Note*: This order is strictly controlled in Kraken: an Assay cannot be dispensed into a plate with no DNA samples in it and a plate read file cannot be assigned to a plate where an Assay has not been dispensed.

The Assays icon in the Project tree holds all of the results, and there you will find for each Assay, the Assay designs, and for each design, the read or reads (plate reader file data) which follow thermal or hydrocycling. From this part of the tree each set of results is rapidly accessed as a cluster or other plot. Normalisation data may be viewed.

At this point the results are scored, and the preferred, most representative plot 'Submitted' for the final report. **Submitted** is one of many statuses which may be assigned to a plate read. ***However, more than one results set may be used to decide on the call for an individual sample. Plates are frequently re-cycled (submitted to a further 5 rounds of PCR hydrocycling) and the results compared: direction of travel of the relative intensities (data point) for a given Sample may be a critical indicator for a genotype call.

Submitted results are copied to the Results icon in the Project tree and are seen in the final report or SNPviewer file. Note the statement above***. In this way, plots used for validating Assays or where most of the data is not representative of the final calls, remain in Kraken but are not in a final report.

Kraken provides rapid reporting, drilling of Master plates to see % of calls (and determine whether Samples are still good), Project overviews for accounting. User privileges, and user management is available. Database maintenance tools are provided and the MySQL database tables are documented in this manual.

Kraken may be used by many users or only one, in a factory setting or in a smaller laboratory. It has XML import functions and can interface with upstream and downstream systems.

KRAKEN LIMS Laboratory Information Management System

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