

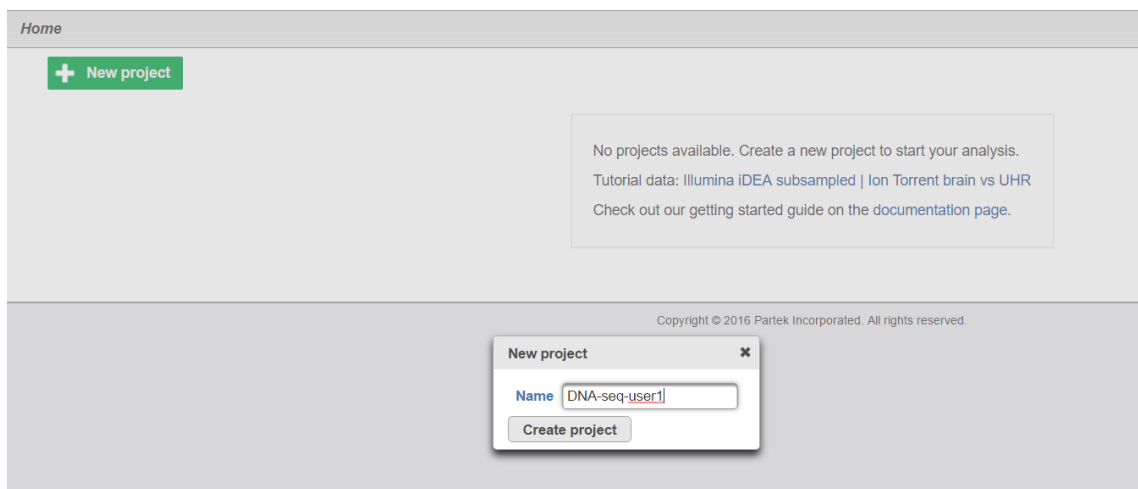
DNA-Seq Analysis in Partek® Flow®

HANDS-ON TRAINING



Login and Project Setup

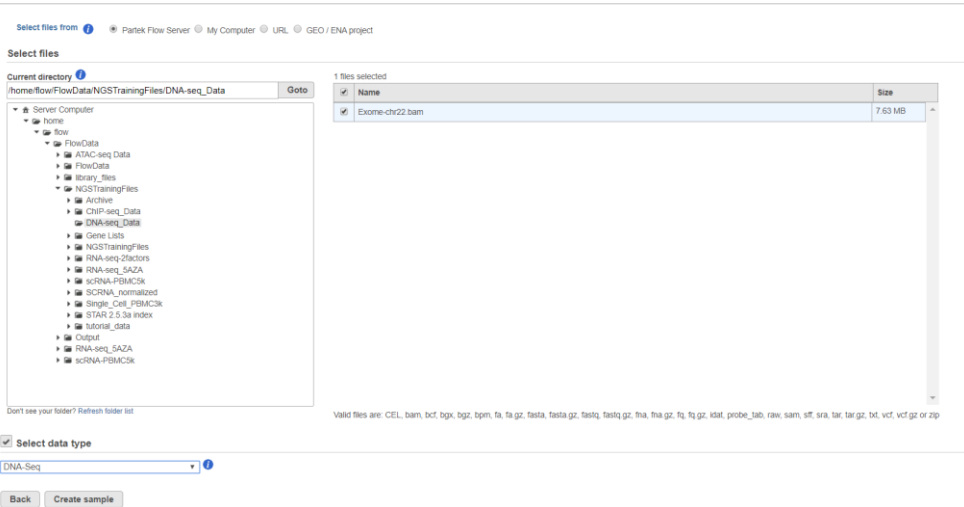
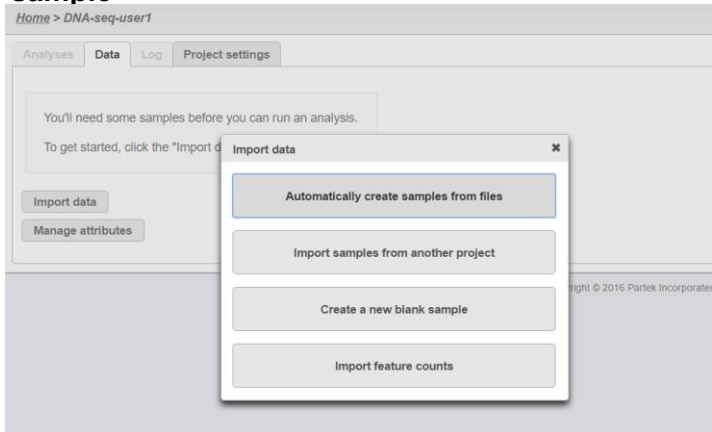
- Log in using the username and password given to you
- This will open to the Partek Flow homepage
- Click **New project** and enter project name: DNA-seq-[username]
- This will create a new project (push **Create project**)



Notes: _____

Data Upload

- Creating a new project automatically opens up the **Data** tab
- To upload your data, click **Import Data > Automatically create samples from files**
- Browse to /home/flow/FlowData/DNA-seq (or equivalent)
- Select the available **.bam** file. Check mark **Select data type** and use **DNA-Seq**. Click **Create sample**




Notes:

Analyses Tab Overview

- Once Partek Flow processes the data, the *Aligned reads* node will appear on the canvas under the Analyses tab

Analyses	Data	Log	Project settings	Notebook	Data Viewer	Attachments
Data	Sample name					
Import	1	Exome-chr22				
Sample attributes	Show data files			Download		
Manage						
Assign values from file						
Add system-wide attribute						

Analyses	Data	Log	Project settings	Notebook	Data Viewer	Attachments
 DNA-Seq						

Notes: _____

Coverage Report Setup

- *Coverage report* provides overview of sequencing coverage across specified regions (e.g. exome or targeted panel)
- Select the **Aligned reads** node and then **Coverage report**
- Set the *Assembly* to **Homo sapiens (human) – hg19_chr22** (or similar, depending on the training setup) and the *Gene/feature annotation* to **refseq** (or similar). The report will, consequently, contain information on coverage on RefSeq genes on the chromosome 22
- Check mark **Generate target enrichment graph** and push **Finish** to start

[Home](#) > [Admin-DNA-Seq](#) > [Coverage report](#)

Select Annotation file

Assembly

Gene/feature annotation

Add minimum coverage levels

Coverage level ⓘ

1x	✖
20x	✖
100x	✖
<input type="text"/> x	+

Advanced options


Strand specificity ⓘ

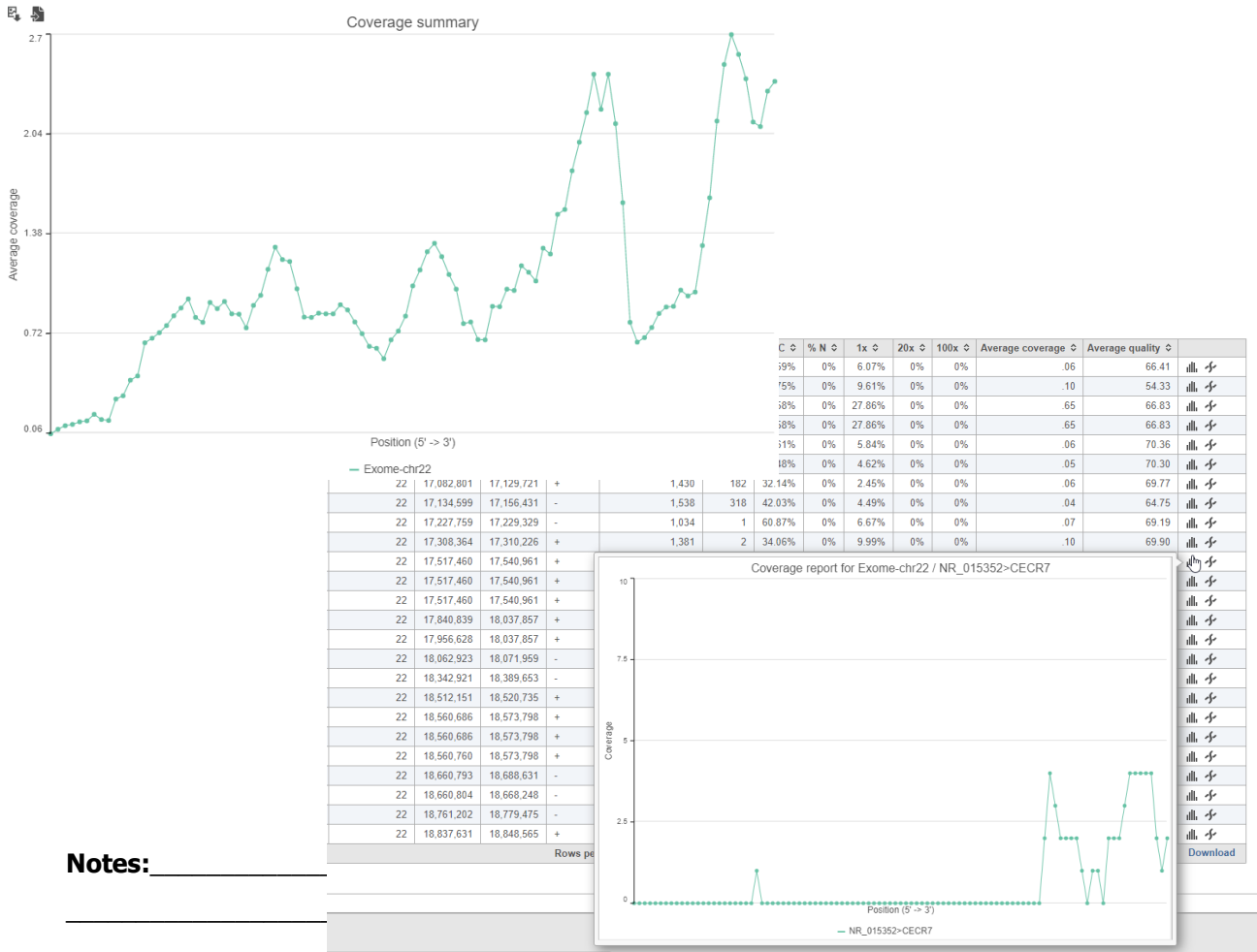
Generate target enrichment graph ⓘ

Use multithreading ⓘ

Notes: _____

Coverage Report Results

- When the task completes open the **Coverage report** task node
- The first page contains an overview of the coverage in the study samples
- To get details per targeted regions select the sample name in the table on the top
- For target enrichment plot, click / mouse over the on the **histogram icon** ()



Deduplication

- Select the **Aligned reads** node and then **Filter alignments** (in the *Post-alignment tools*)
- To get rid of the PCR duplicates check the **Filter duplicates** option
- Choose duplicate as **Same start and same sequence** option
- Select **Filter low mapping quality**
- Push **Finish**
- The filter will create a *Filtered reads* data node

Home > Admin-DNA-Seq > Filter alignments

Filter duplicates [i](#)

Keep duplicates up to [i](#)

Treat the alignment as duplicate if [i](#) Same start position Same start and same sequence

Keep the alignment with [i](#) Highest mapping score Randomly selected

Filter low mapping quality [i](#)

Min mapping quality [i](#)

Filter alignments with mismatches [i](#)

Max mismatched bases [i](#)

Filter by genomic locations [i](#)

Include region overlapping alignments [i](#)

Exclude region overlapping alignments [i](#)

Additional filters

Remove singletons [i](#)

Remove unaligned reads [i](#)

Notes:

Detect Variants Against Reference

- Select the **Filtered reads** data node > **Variant Callers** > **Samtools**
- Choose *Assembly* to **Homo sapiens (human) – hg19_chr22** as reference, click **Finish**
- The result is a *Variants* data node

Home > Admin-DNA-Seq > Samtools

Variant detection method

Select Reference sequence

Assembly

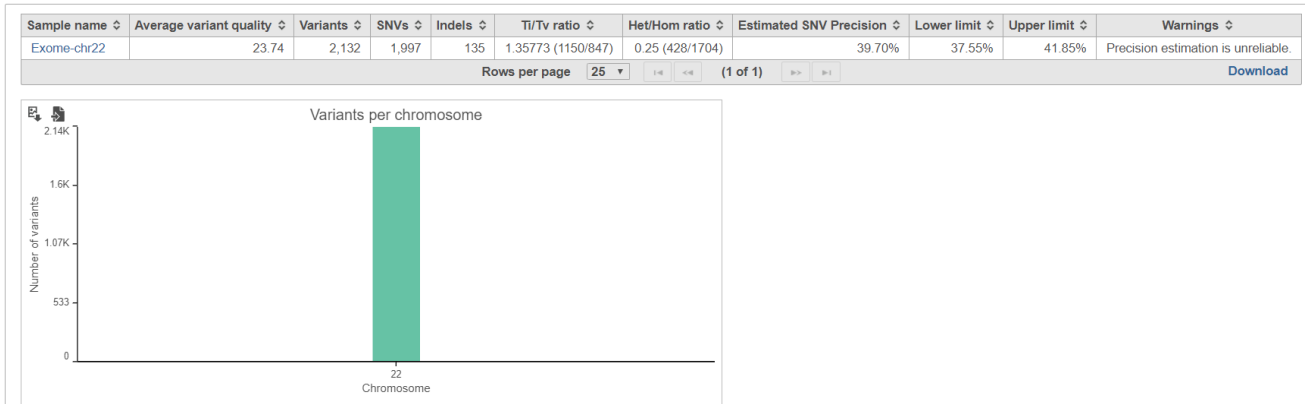
Advanced options

Option set [Configure](#)

Notes: _____

Inspecting Variants

- Select the **Variants** data node > **Variant analysis** > **View variants**
- Use default settings and click **Finish**
- To inspect the result open the **View variants** task node
- Ti/Tv ratio: ratio of transitions vs. transversions in SNPs
 - Transition: mutation within the same type of nucleotide (C->T; A->G)
 - Transversion: mutation from pyrimidine to purine or vice versa



Task details

Notes: _____

Annotating Variants

- Select the **Variants** data node > **Variant analysis** > **Annotate variants**
- Select **Annotate with genomic features** and point to **refseq**
- Select **Annotate with known variants** and point to **dbSNP**
- Click **Finish** to start. The output is *Annotated variants* node

Assembly Homo sapiens (human) - hg19_chr22

Annotate with genomic features

Gene/feature annotation

Promoter upstream limit *i*

Promoter downstream limit *i*

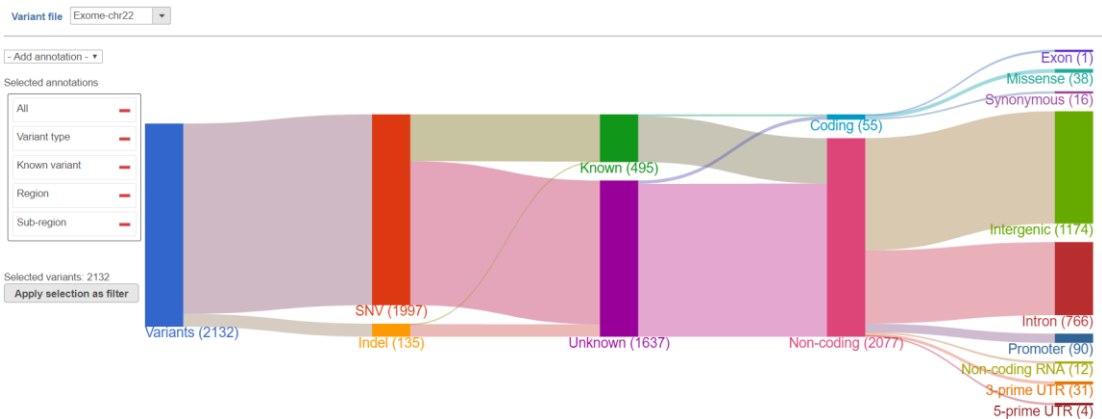
Annotate with known variants

Variant annotation

Notes: _____

Variants Report

- Select the **Annotated variants** data node, choose **Task report** on the menu
- Sankey plot presenting different classes of variant based on the annotation
 - Each vertical block is one type of annotation
 - Annotation can be added or removed
 - Click on any colored section to apply filter
 - Click on the any blank to remove the filter
- The record in the bottom table will reflect the filter applied



Optional columns

	View	Chr	Position	ID	Ref	Alts	VarQual	Type	Gene symbol	Transcript	Strand	Gene section	Nt change	AA change	Genotype
		Search...	Search...	Search	Search	Search	Search...	Search...	Search...	Search...	Search...	Search...	Search...	Search...	Search...
1	✂	22	16053317	.	G	C	14.57	CC
2	✂	22	16058812	.	G	C	14.57	CC
3	✂	22	16058891	.	C	T	6.51	TT
4	✂	22	16062817	.	G	A	14.57	AA
5	✂	22	16062906	.	A	T	9.00	TT
6	✂	22	16063044	.	G	T	5.76	TT
7	✂	22	16063077	.	C	G	5.76	GG
8	✂	22	16064159	.	C	A	5.76	AA
9	✂	22	16064166	.	T	A	24.43	AA
10	✂	22	16069573	.	T	C	5.76	CC
11	✂	22	16069589	.	C	T	5.76	TT
12	✂	22	16077491	.	T	C	5.76	CC

Notes:

Filtering Based on Annotation

- Select the **Annotated variants** data node > **Variant analysis** > **Filter variants**
- Set *Minimum read depth* to **20**. The filter shows variants covered at least 20 × .
- Set *Minimum alternate calls* to **10**. The filter shows variants with at least 10 supporting reads.
- Push **Finish**. The output is a *Variants* data node

Annotation

- Include region overlapping variants f
- Exclude region overlapping variants f

Samples

- Filter by samples

Variant Type

- Indels** f Indels only Non-indels All
- Zygoty** f Heterozygous only Homozygous only All
- Mutation type** f Synonymous Missense Nonsense Exonic indels
- Feature section** f Splice-5 Splice-3 Non-coding RNA 5-prime UTR 3-prime UTR
- Intron Promoter Intergenic

Variant Novelty

- Known variants** f Known only Novel only All

Quality

- Minimum read depth** f
- Minimum high-quality alternate calls** f
- Minimum mean-square mapping quality** f
- Minimum variant quality** f

Notes:

Inspecting Variants

- Select the **Variants** data node > **Variant analysis** > **View variants**
- Use default settings and click **Finish**
- To inspect the result open the **View variants** task node
- To get detailed output click on the **sample name**



Task details

Notes: _____

Filtering the Results Table

- To select missense variants only, select the **Missense** column in the *Variant occurrences by type* chart
- When the table gets refreshed, all the entries in the *Type* column are set to *Missense*

Sample Exome-chr22 Variants 65 Show one overlap per variant Apply precedence rules Show all overlaps

Optional columns

View	Chr	Position	ID	Ref	Alts	VarQual	Type	Gene symbol	Transcript	Strand	Gene section	Nt change	AA change	Genotype
1	22	29091127	.	C	T	98.00	Missense	CHEK2	NM_001005735	-	Exon 13	c.1492G>A	V498I	CT
2	22	29091142	.	C	T	221.00	Missense	CHEK2	NM_001005735	-	Exon 13	c.1477G>A	E493K	CT
3	22	29091157	rs587778194	A	G	221.00	Missense	CHEK2	NM_001005735	-	Exon 13	c.1462T>C	Y488H	AG
4	22	29091740	rs200649225	C	T	132.00	Missense	CHEK2	NM_001005735	-	Exon 12	c.1346G>A	R449H	CT
5	22	29091788	rs200928781	T	C	197.00	Missense	CHEK2	NM_001005735	-	Exon 12	c.1298A>G	Y433C	TC
6	22	29091816	rs375130261	T	C	14.85	Missense	CHEK2	NM_001005735	-	Exon 12	c.1270A>G	M424V	TC




Rows per page 25 (1 of 1) [Download](#)

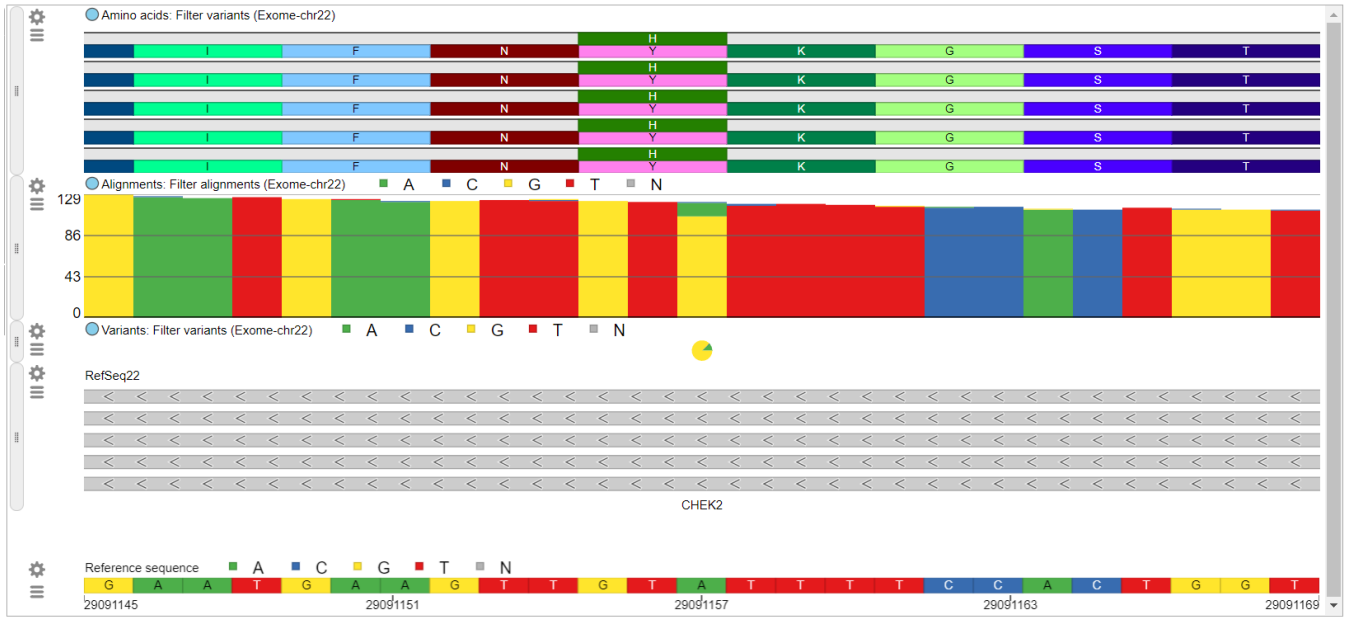
Operators such as >= or <= can be used in the numeric columns' filters.

Task details

Notes:

Exploring the Browser View

- Click on  to browse in chromosome view
- Click on Select tracks to add or remove data tracks or annotation tracks
- On each track, click on  button to configure the track
- Click on  button to hide or pin track
- Drag and drop to change the order of the tracks



Notes: _____

Further Training

Self-learning

- Check out <http://www.partek.com/resources-partek-flow> for resources
- Recorded webinars available on Partek Incorporated's YouTube page

Regional Technical Support

- Email: support@partek.com
- Phone: +1-314-878-2329

Notes: _____
