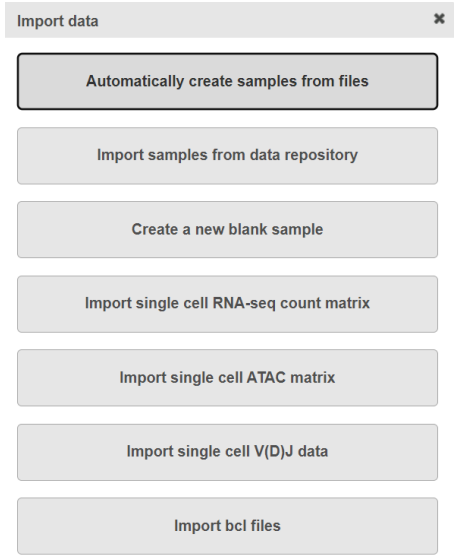
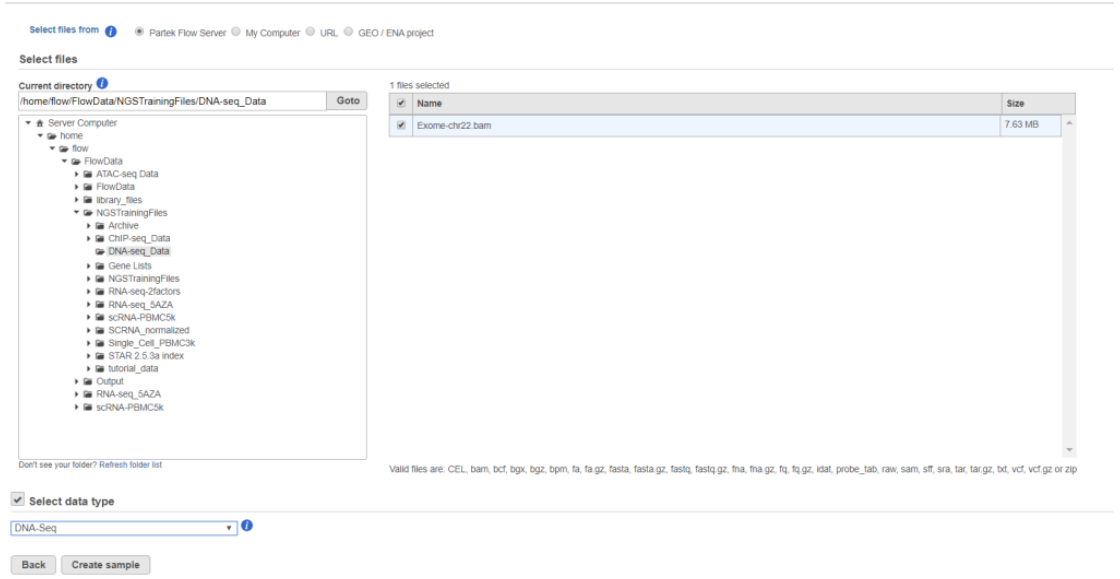
Partek Flow
DNA-Seq Analysis

Eric

Import Data

- Import data by **Automatically create samples from files**
- Browse to **/home/flow/FlowData/DNA-seq**
- Select the available **.bam** file
- Select data type as **DNA-Seq**

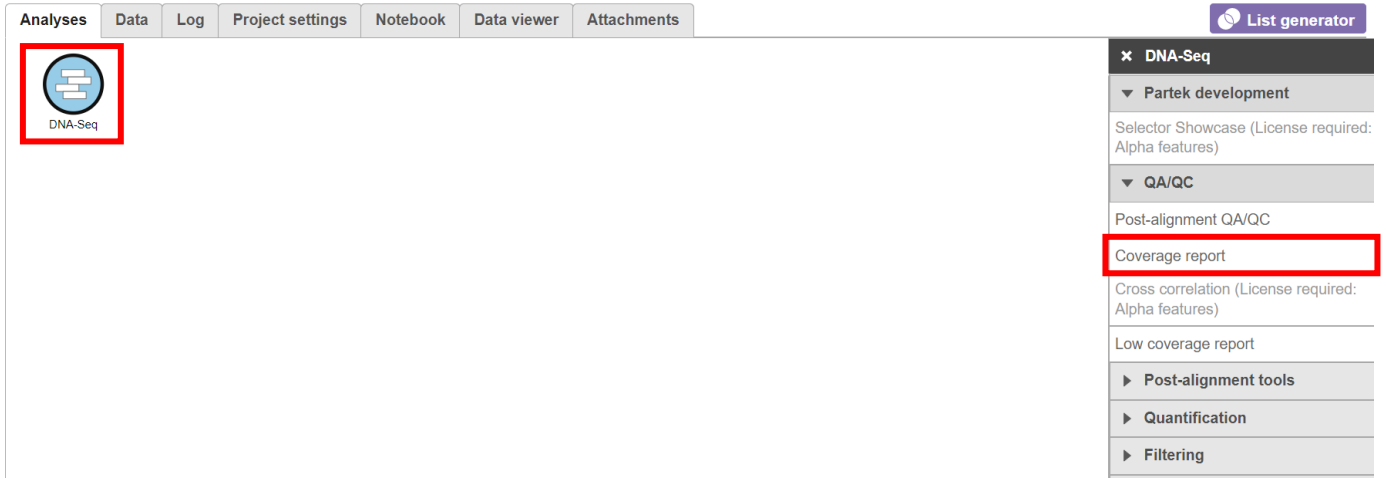




Coverage Report

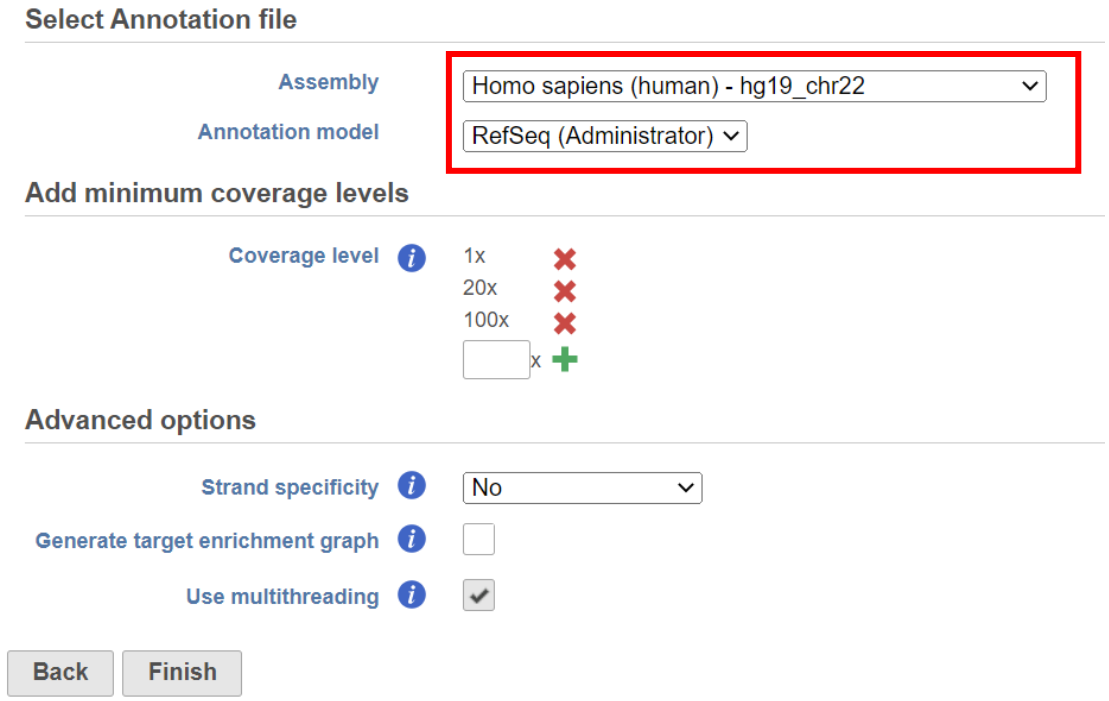
Once the download completes, the sample table will appear in the Data tab.

- Click **Analyses** tab
- Click **DNA-seq** data node
- Click **Coverage report** in the QA/QC section of the task menu



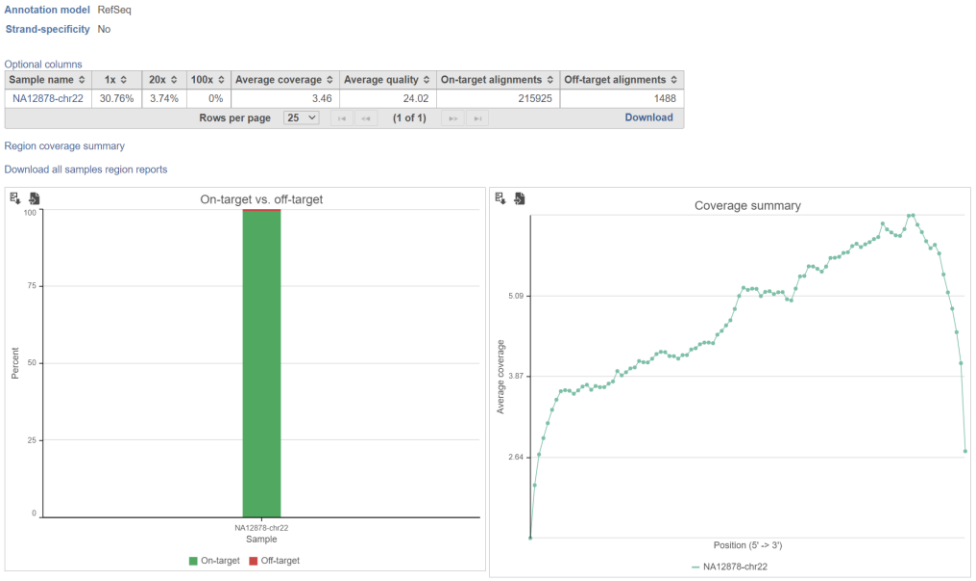
Coverage Report

- Set the Assembly to **Homo sapiens (human) – hg19_chr22** and the Annotation model to **RefSeq**
- Click **Finish** to run



Coverage Report

- Double click the **Coverage Report** node
- Click a sample name for a sample-level table



Annotation model RefSeq

Strand-specificity No

Optional columns

Sample name	1x	20x	100x	Average coverage	Average quality	On-target alignments	Off-target alignments
NA12878-chr22	30.76%	3.74%	0%	3.46	24.02	215925	1488

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Region coverage summary

Download all samples region reports

Region name	Chromosome	Start	Stop	Strand	Total exon length	Reads	% GC	% N	1x	20x	100x	Average coverage	Average quality	
NR_122113>DUXAP8	22	16,150,529	16,193,010	-	2,107	375	44.98%	0.31%	97.06%	14.19%	0%	10.08	25.23	
NM_001136213>POTEH	22	16,256,332	16,287,938	-	2,037	473	47.79%	0.17%	94.31%	1.52%	0%	5.46	24.74	
NR_046571>POTEH-AS1	22	16,274,609	16,277,578	+	1,492	285	45.17%	0.15%	99.73%	0%	0%	9.67	25.85	
NM_001005239>OR11H1	22	16,448,824	16,449,805	-	981	197	43.56%	0.37%	100.00%	2.14%	0%	10.18	25.65	
NM_014406>CCT8L2	22	17,071,648	17,073,701	-	2,053	541	55.70%	0.15%	100.00%	8.57%	0%	13.25	24.12	
NR_001591>TPTEP1	22	17,082,801	17,129,721	+	1,430	271	52.25%	0.23%	96.43%	17.34%	0%	9.81	24.35	
NR_040115>ANKRD62P1-PARP4P3	22	17,134,599	17,156,431	-	1,538	218	38.39%	0.23%	91.55%	0.98%	0%	7.04	26.01	
NM_001318251>XKR3	22	17,264,306	17,302,590	-	1,689	248	38.64%	0.18%	97.63%	0%	0%	6.83	26.11	
NM_175878>XKR3	22	17,264,306	17,302,590	-	1,686	248	38.60%	0.18%	97.63%	0%	0%	6.83	26.11	
NR_003607>HSFY1P1	22	17,308,364	17,310,226	+	1,381	357	37.36%	0.22%	98.19%	4.63%	0%	11.94	26.05	
NM_001037814>GAB4	22	17,442,827	17,489,113	-	2,629	426	56.81%	0.34%	93.65%	2.09%	0%	7.94	23.71	
NR_015352>CECR7	22	17,517,460	17,540,961	+	4,004	678	56.53%	0.17%	97.96%	5.24%	0%	8.72	23.74	
NR_152825>CECR7	22	17,517,460	17,540,961	+	3,942	678	56.44%	0.17%	97.45%	5.33%	0%	8.66	23.73	
NR_152826>CECR7	22	17,517,460	17,540,961	+	3,560	678	58.38%	0.20%	63.96%	4.52%	0%	7.85	23.26	
NM_001289905>IL17RA	22	17,565,849	17,596,585	+	8,506	7	0%	0%	0.01%	0%	0%	0	6.00	
NM_014339>IL17RA	22	17,565,849	17,596,585	+	8,608	7	59.94%	0%	1.07%	0%	0%	0.04	19.04	
NM_001163079>TMEM121B	22	17,597,189	17,602,258	-	3,961	118	69.84%	1.27%	9.67%	0%	0%	0.28	20.06	
NM_031890>TMEM121B	22	17,597,189	17,602,214	-	5,025	118	72.93%	0.30%	28.80%	0%	0%	1.13	19.94	
NR_103793>LINC01664	22	17,602,485	17,612,995	+	1,175	206	54.75%	0.39%	99.49%	0%	0%	8.96	23.29	
NM_017829>HDHD5	22	17,618,410	17,646,178	-	1,726	86	48.18%	0%	4.58%	0%	0%	0.22	27.57	
NM_033070>HDHD5	22	17,618,410	17,640,170	-	1,801	3	81.60%	0%	6.22%	0%	0%	0.09	17.48	
NR_024482>HDHD5-AS1	22	17,640,279	17,646,336	+	340	83	71.83%	1.19%	42.06%	0%	0%	1.97	21.05	
NR_024483>HDHD5-AS1	22	17,640,279	17,646,336	+	626	83	65.04%	0.35%	68.53%	0%	0%	5.94	22.14	
NM_001282225>ADA2	22	17,659,680	17,700,476	-	4,505	53	56.37%	0%	3.88%	0%	0%	0.18	24.77	
NM_001282226>ADA2	22	17,659,680	17,700,476	-	4,472	53	56.69%	0%	3.18%	0%	0%	0.13	23.90	

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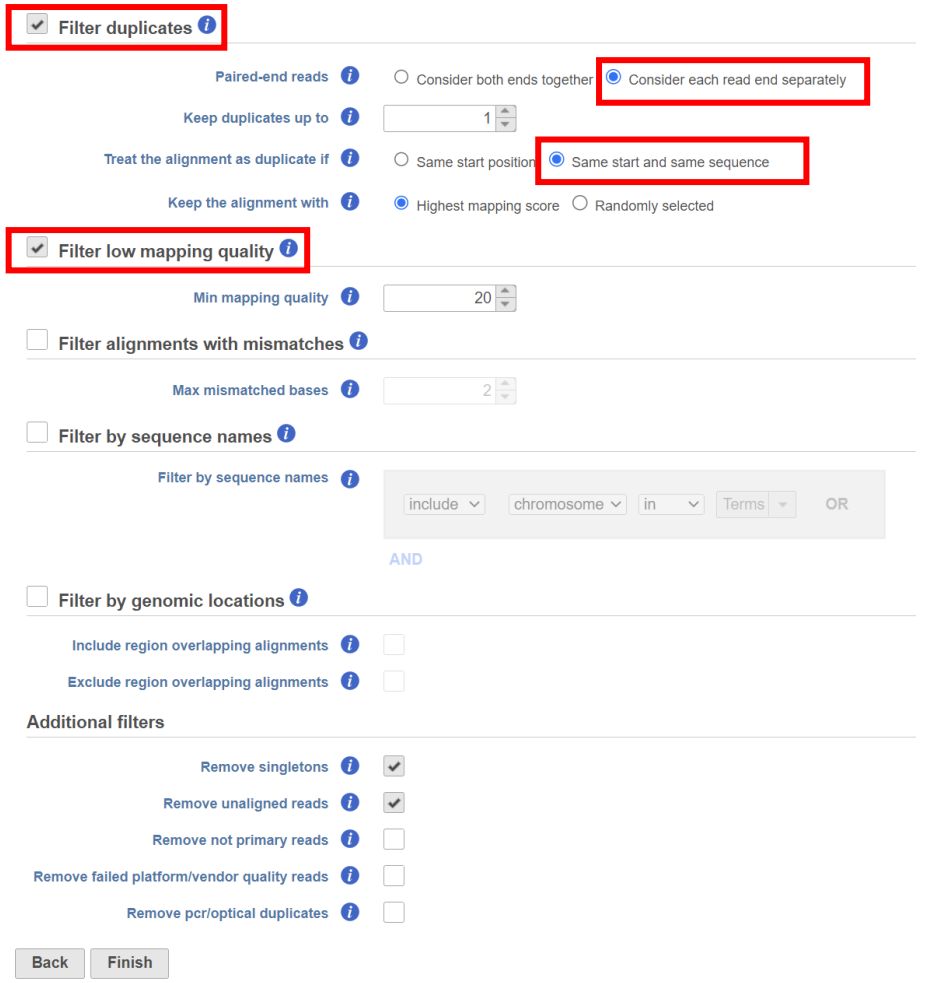
Filter Alignments

- Click the project name to return to the Analyses tab
- Select the **DNA-Seq node**
- Click **Filter alignments** in the Post-alignment tools

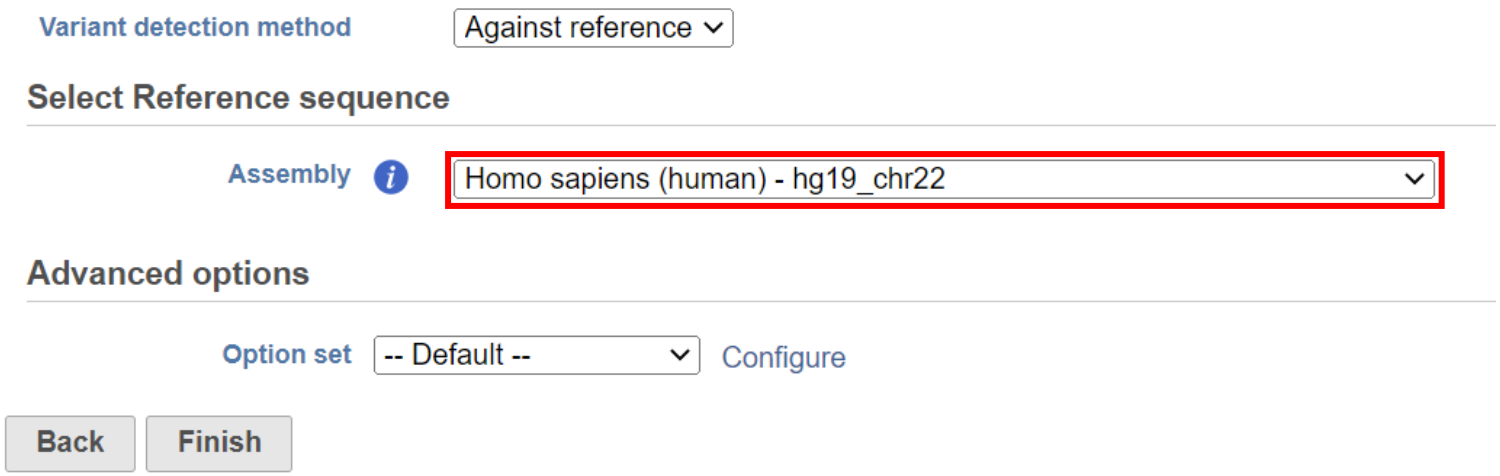
Filter Alignments

- To get rid of the PCR duplicates check the **Filter duplicates** option
- Choose **Consider each read end separately** and duplicate as **Same start and same sequence** option
- Select **Filter low mapping quality**
- Push **Finish**



Detect Variants

- Select the **Filtered reads** data node
- Choose **Samtools** in Variant Callers from the task menu
- Choose Assembly to **Homo sapiens (human) – hg19_chr22** as reference
- Click **Finish**



Inspect Variants

- Select the **Variants** data node
- Choose **View variants** in Variant analysis from the task menu
- Use default settings and click **Finish**
- Double click the **View variants** task node for results

Sample name	Average variant quality	Variants	SNVs	Indels	Ti/Tv ratio	Het/Hom ratio
NA12878-chr22	60.64	1,652	1,552	100	2.12274 (1055/497)	3.39 (1276/376)

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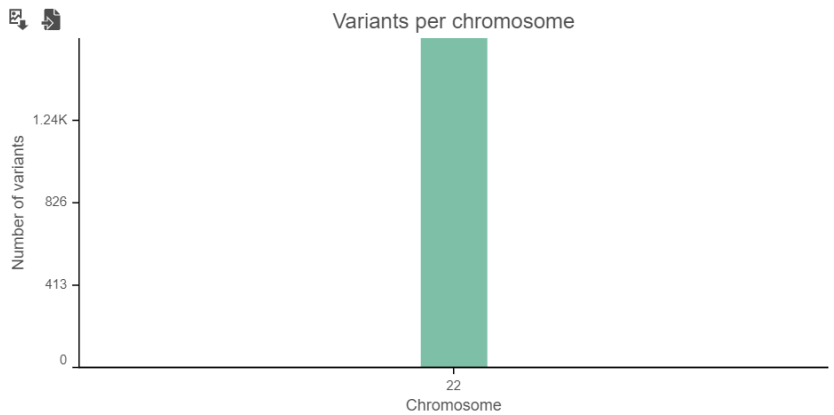
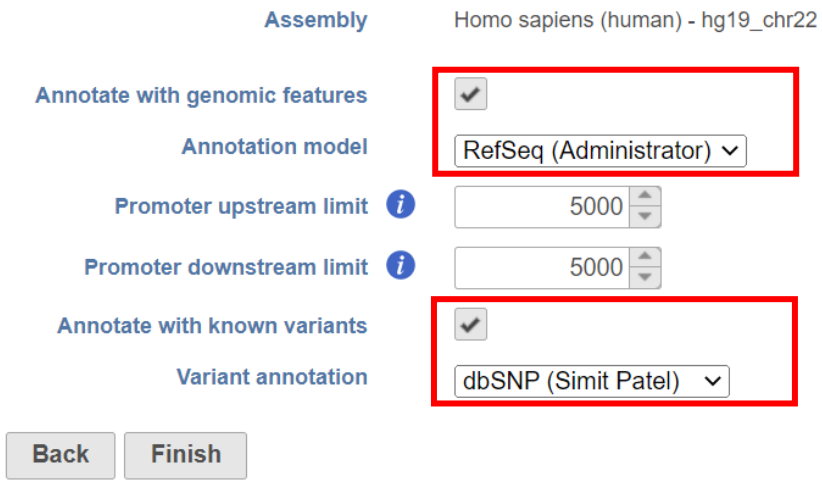


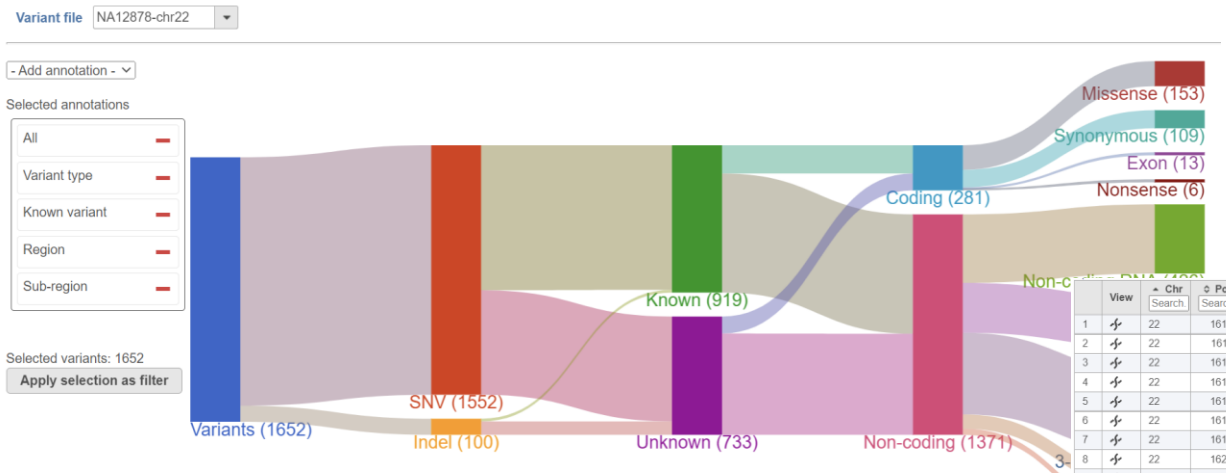
Annotate Variants

- Click the project name to return to the Analyses tab
- Select the **Variants** data node
- Choose **Annotate variants** in Variant analysis
- Select **Annotate with genomic features** and point to **RefSeq**
- Select **Annotate with known variants** and point to **dbSNP**
- Click **Finish** to start



Variants Report

- Double click the **Annotated variants** task node for reports



	View	Chr	Position	ID	Ref	Alts	VarQual	Type	Gene ID	Transcript ID	Strand	Gene section	Nt change	AA change	Genotype
1		22	16150589	rs2844966	C	A	3.22	Non-coding RNA	DUXAP8	NR_122113	-	Exon 8		None	AA
2		22	16151138	rs11702921	C	T	36.37	Non-coding RNA	DUXAP8	NR_122113	-	Exon 8		None	CT
3		22	16151692	rs2019757	T	C	13.30	Non-coding RNA	DUXAP8	NR_122113	-	Exon 8		None	TC
4		22	16151782	rs7285270	C	T	11.61	Non-coding RNA	DUXAP8	NR_122113	-	Exon 8		None	CT
5		22	16186885	.	A	G	62.00	Non-coding RNA	DUXAP8	NR_122113	-	Exon 6		None	AG
6		22	16186937	.	T	C	6.00	Non-coding RNA	DUXAP8	NR_122113	-	Exon 6		None	TC
7		22	16186939	.	C	G	29.42	Non-coding RNA	DUXAP8	NR_122113	-	Exon 6		None	GG
8		22	16269934	rs2845206	A	G	72.00	Missense	POTEH	NM_001136213	-	Exon 7	c.1247T>C	L416S	GG
9		22	16276569	.	A	G	18.01	Intron	POTEH	NM_001136213	-	Intron 5	c.1166+1179T>C	None	AG
10		22	16276820	rs376561434	G	GT	29.39	Intron indel	POTEH	NM_001136213	-	Intron 5	c.1166+928C>C	None	REF/ALT
11		22	16276889	rs7292440	A	G	49.32	Intron	POTEH	NM_001136213	-	Intron 5	c.1166+859T>C	None	AG
12		22	16277059	rs9680617	C	T	11.16	Intron	POTEH	NM_001136213	-	Intron 5	c.1166+689G>A	None	CT
13		22	16282259	rs3931625	C	T	26.32	Synonymous	POTEH	NM_001136213	-	Exon 3	c.807G>A	None	CT
14		22	16449491	rs202124975	T	G	13.44	Missense	OR11H1	NM_001005239	-	Exon 1	c.314A>C	K105T	TG
15		22	17072483	rs2236639	A	G	103.00	Missense	CCT8L2	NM_014406	-	Exon 1	c.958T>C	W320R	GG
16		22	17073066	rs5747988	A	G	95.00	Synonymous	CCT8L2	NM_014406	-	Exon 1	c.375T>C	None	GG
17		22	17092597	rs537791337	G	A	30.40	Non-coding RNA	TPTEP1	NR_001591	+	Exon 2		None	GA
18		22	17092673	.	A	G	15.85	Non-coding RNA	TPTEP1	NR_001591	+	Exon 2		None	AG
19		22	17092688	rs5748118	C	T	118.00	Non-coding RNA	TPTEP1	NR_001591	+	Exon 2		None	TT
20		22	17092702	.	T	C	28.30	Non-coding RNA	TPTEP1	NR_001591	+	Exon 2		None	TC
21		22	17092720	.	C	G	16.09	Non-coding RNA	TPTEP1	NR_001591	+	Exon 2		None	CG
22		22	17092759	.	A	G	52.00	Non-coding RNA	TPTEP1	NR_001591	+	Exon 2		None	AG
23		22	17156280	rs6518594	A	G	185.00	Non-coding RNA	ANKRD62P1-PARP4P3	NR_040115	-	Exon 1		None	GG
24		22	17309881	rs16981741	A	G	199.00	Non-coding RNA	HSFY1P1	NR_003607	+	Exon 2		None	GG
25		22	17443238	rs1024731	T	A	157.00	3-prime UTR	GAB4	NM_001037814	-	Exon 10	c.385A>T	None	AA

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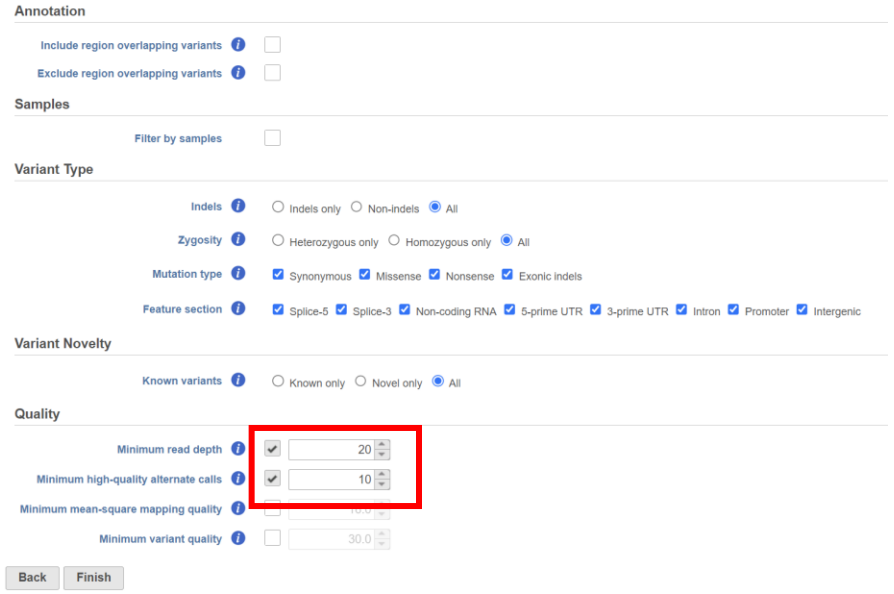
Filter Variants

- Click the project name to return to the Analyses tab
- Select the **Annotated variants** data node
- Choose **Filter variants** in Variant analysis
- Set Minimum read depth to **20**
  - The filter shows variants covered at least 20x
- Set Minimum alternate calls to **10**
  - The filter shows variants with at least 10 supporting reads
- Push **Finish**



Inspect Variants

- Select the **Variants** data node
- Choose **View variants** in Variant analysis from the task menu
- Use default settings and click **Finish**
- Double click the **View variants** task node for results

Sample name	Average variant quality	Variants	SNVs	Indels	Known variants	% known variants	Ti/Tv ratio	Het/Hom ratio
NA12878-chr22	83.20	272	263	9	80	29.41%	2.05814 (177/86)	23.73 (261/11)

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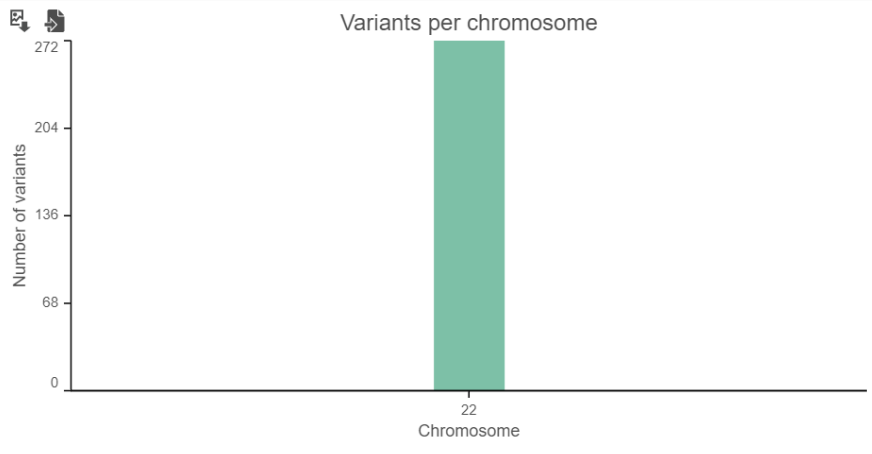
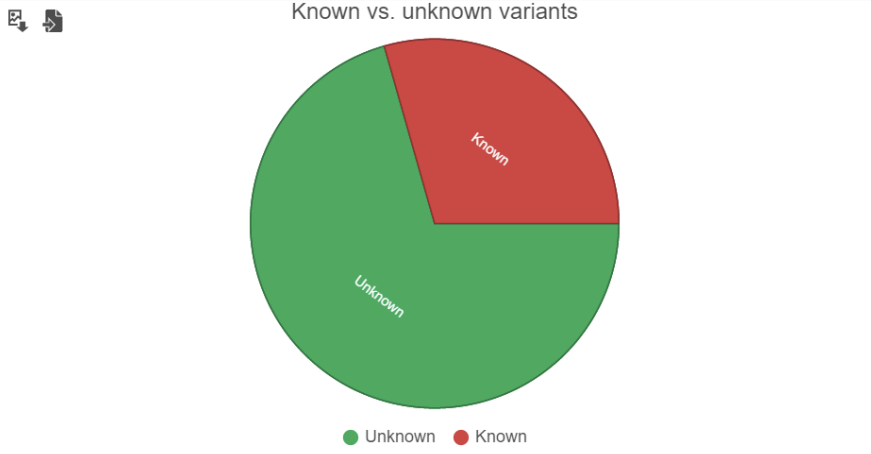
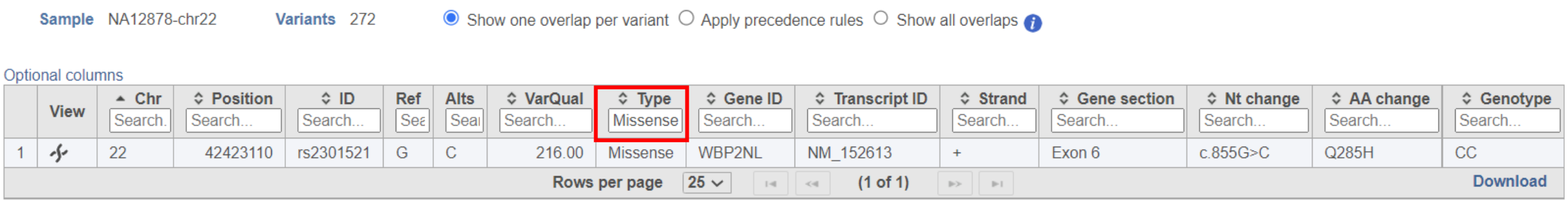




Filter Results Table

- Click a sample name for a sample-level table
- To select missense variants only, type **Missense** under Type



Exploring Browser View

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

DNA-Seq Pipeline