Dotmatics
SoftGenetics

NextGENe

Quick Start Guide

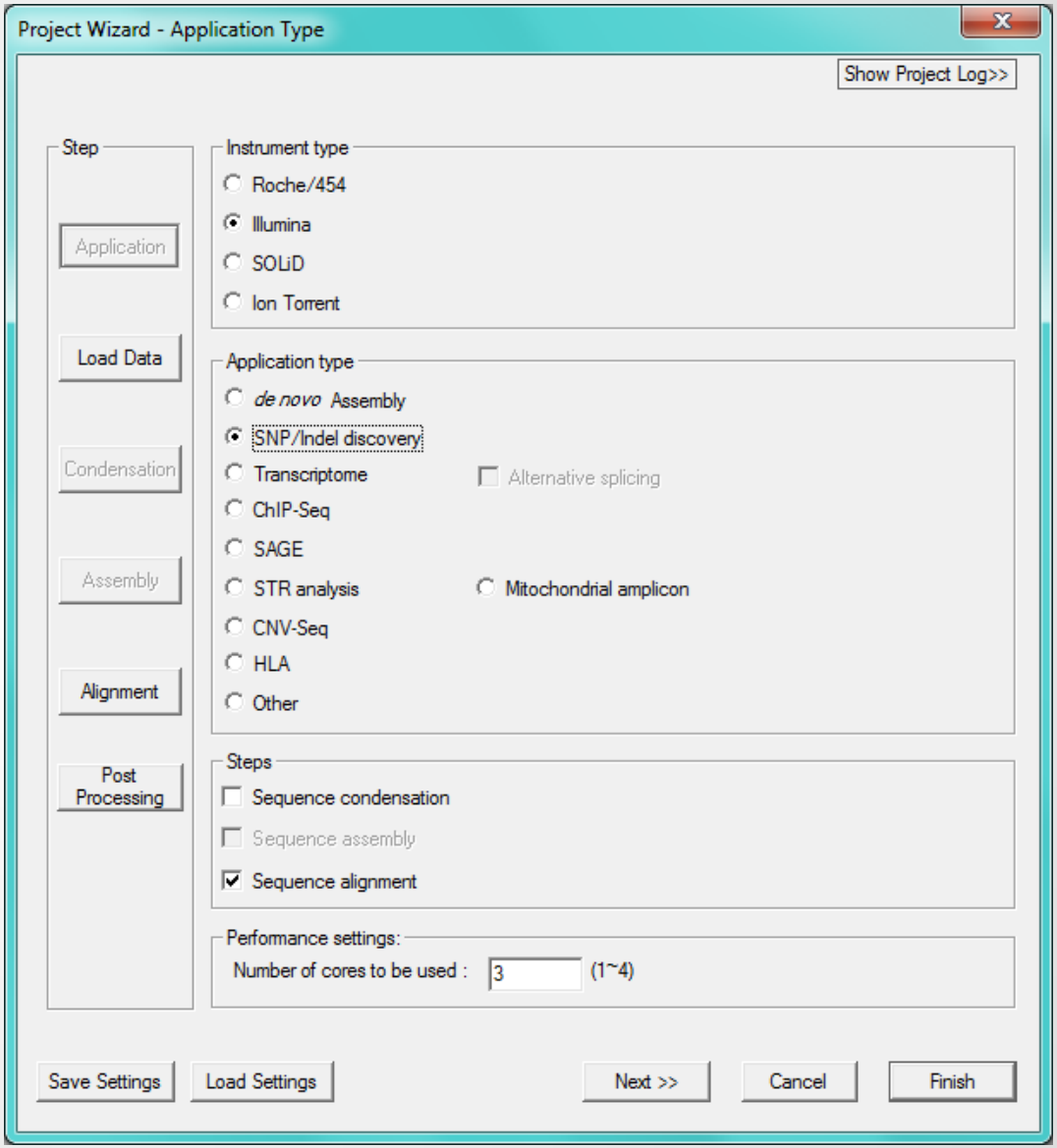
Select Application

1. Open NextGENe Run Wizard
2. Select Instrument Type used to generate data
3. Select Application Type

Steps not necessary for specific Instrument/Application combinations will be grayed out.

Enabled Steps with checkmark will be performed by NextGENe.

4. Click Next



Load Data

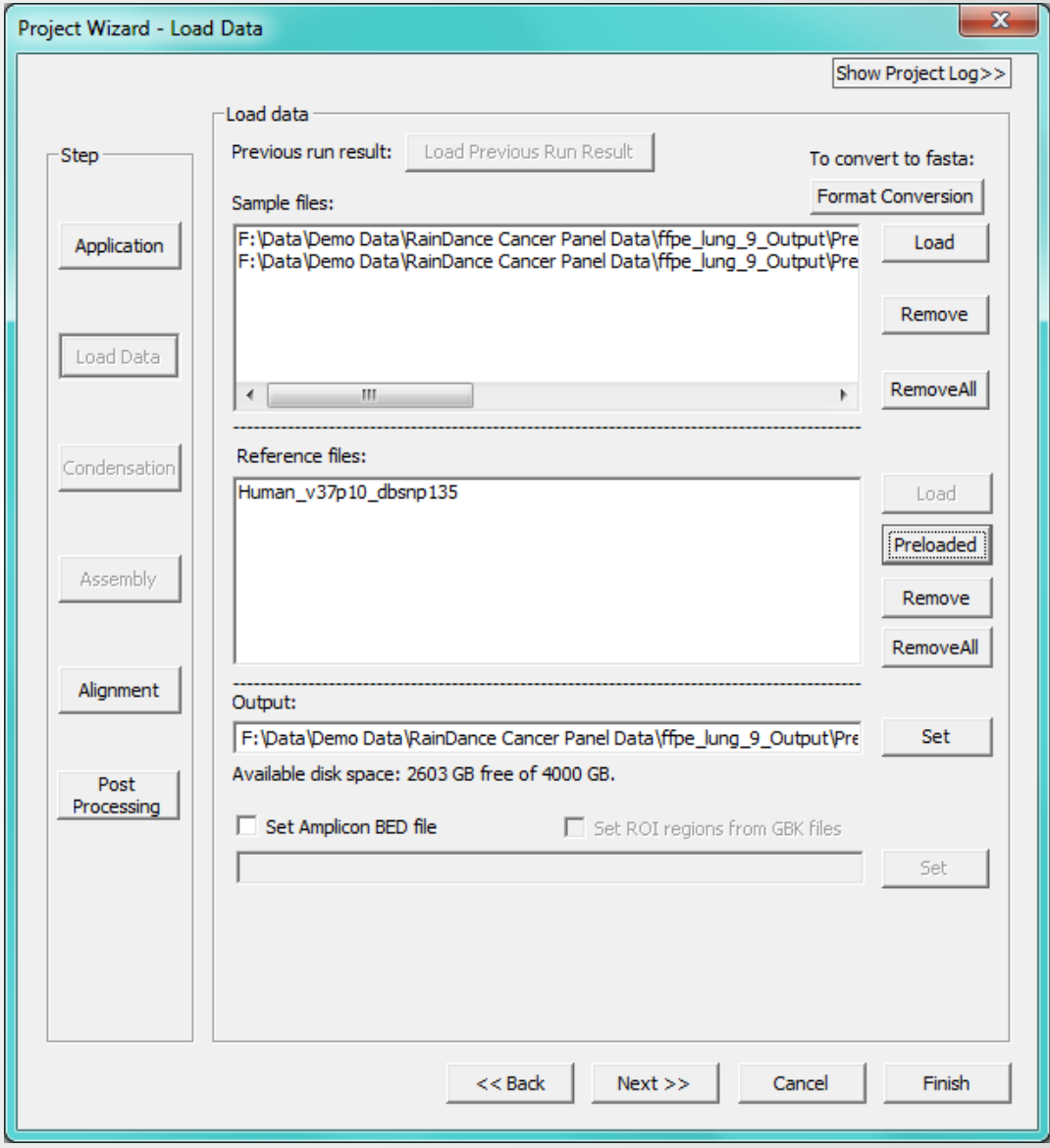
1. Load FASTA or BAM format

If sample is not in FASTA or BAM format, or if low quality calls need to be removed, open Format Conversion Tool (described on next page).

2. Load Reference Files
   1. Load (fasta or GBK format)
   2. Preloaded (large indexed)

3. Select Save Location in Output field

4. Click Next



Load Data – Format Conversion

- Instruments produce several output formats.
- NextGENe input is FASTA.
- Use Format Conversion Tool to convert instrument output to FASTA.

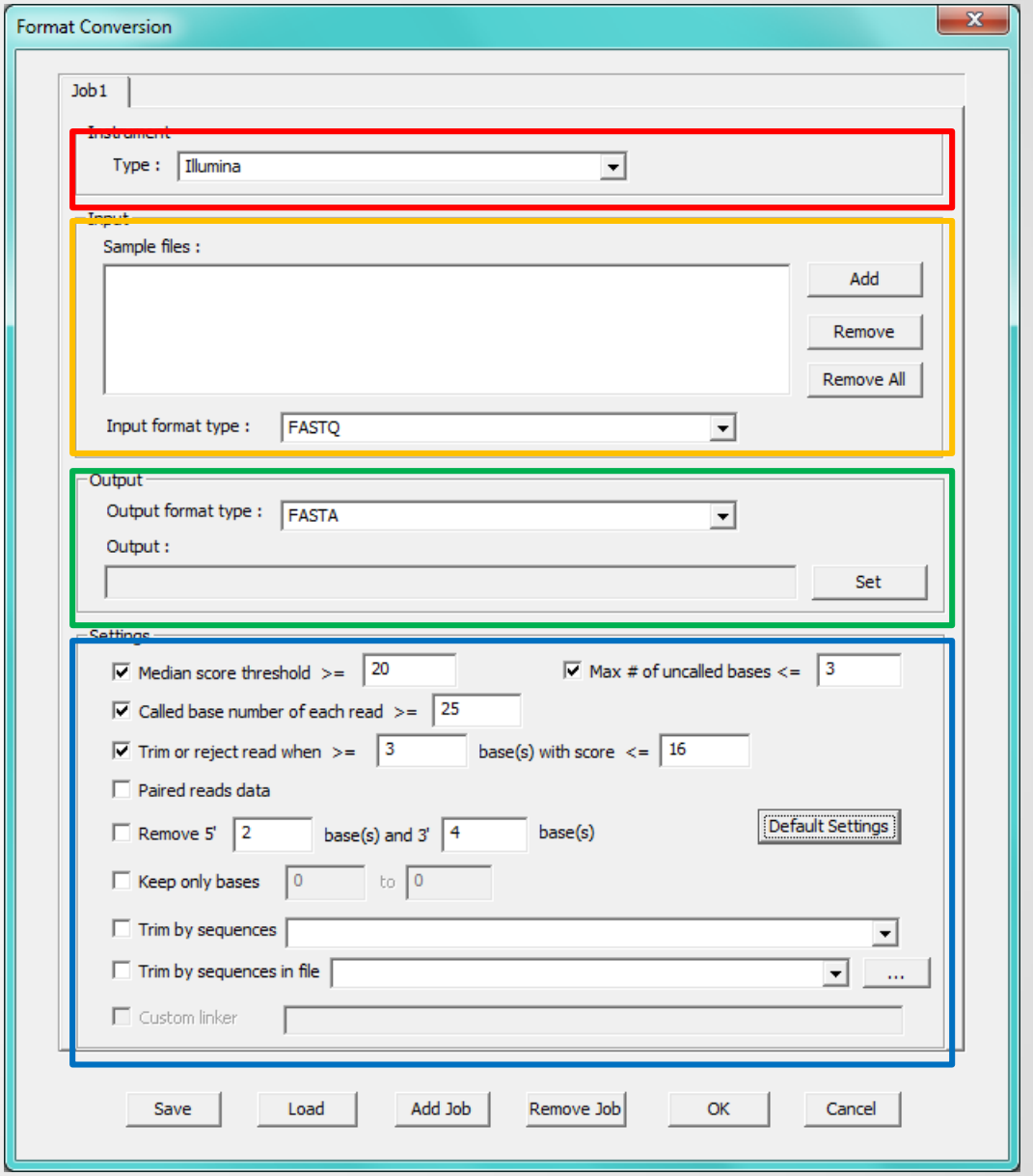
1. Select the Instrument

2. Add sample files to be converted to the Input field

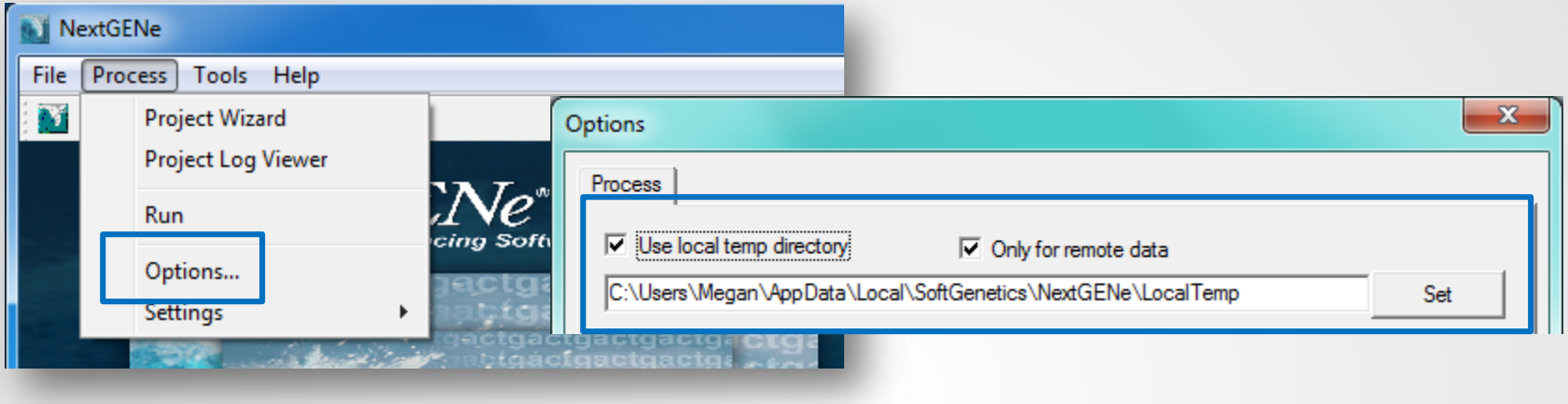
3. Select Output Format and set Output location

4. Select Settings to Clean-up sample file

5. Click OK



Load Data – Analyze Locally to increase speed



When any of the input or output fields contain files that are or will be on a network drive, performance would be improved by processing the project locally.

Within the NextGENe Process Options, choose to use a local temp directory for remote data and specify a local directory with adequate resources.

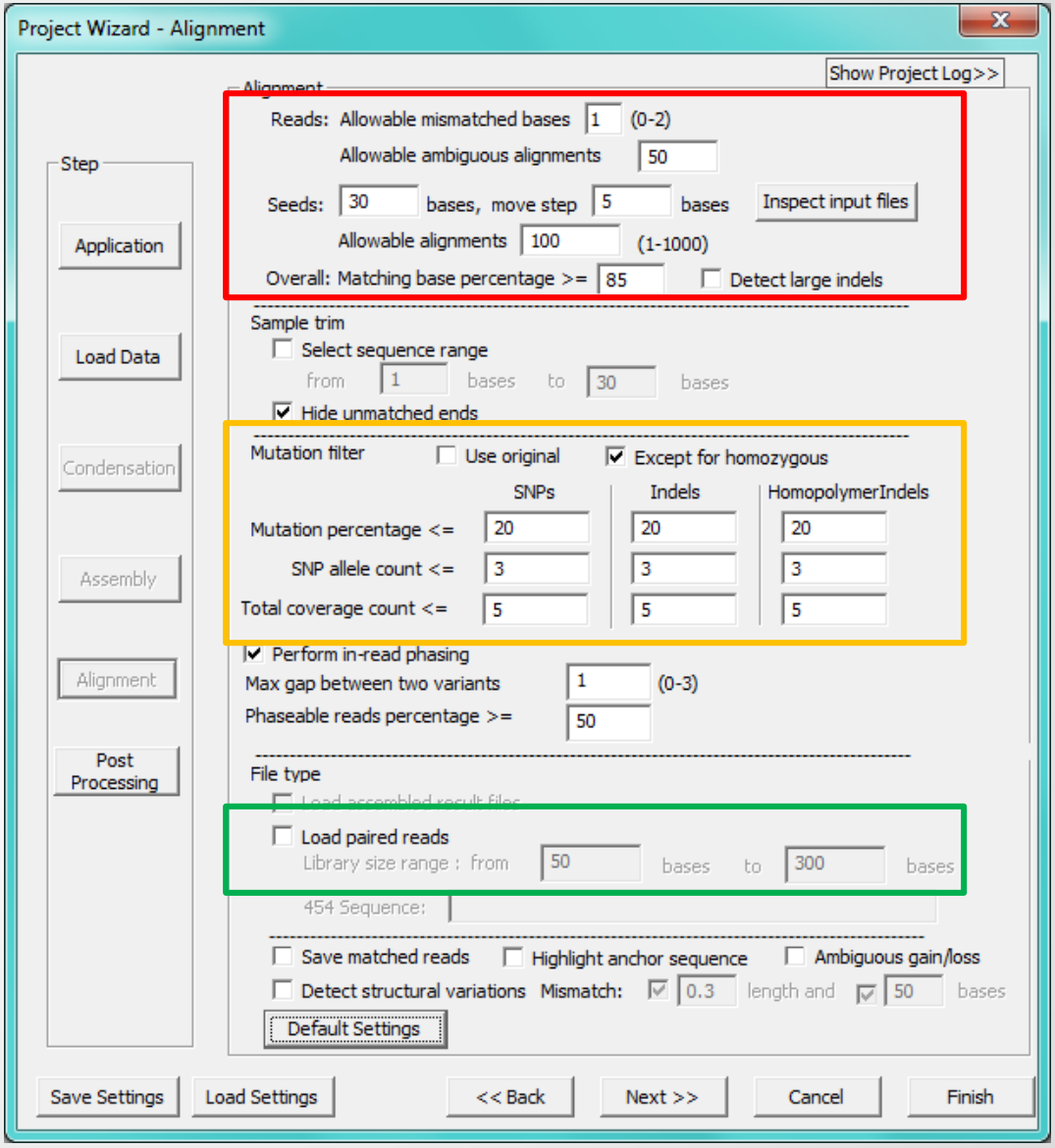
Alignment

1. Set Alignment Specificity – Default value work well for most datasets. Click on the Inspect input files to have NextGENe adjust automatically, or manually adjust.

2. Set Mutation Detection Sensitivity – mutation percentage and total depth of coverage for each base position.

3. For sample files with only single end reads, keep the Load paired reads deselected. For paired sample files, select this option and adjust range accordingly.

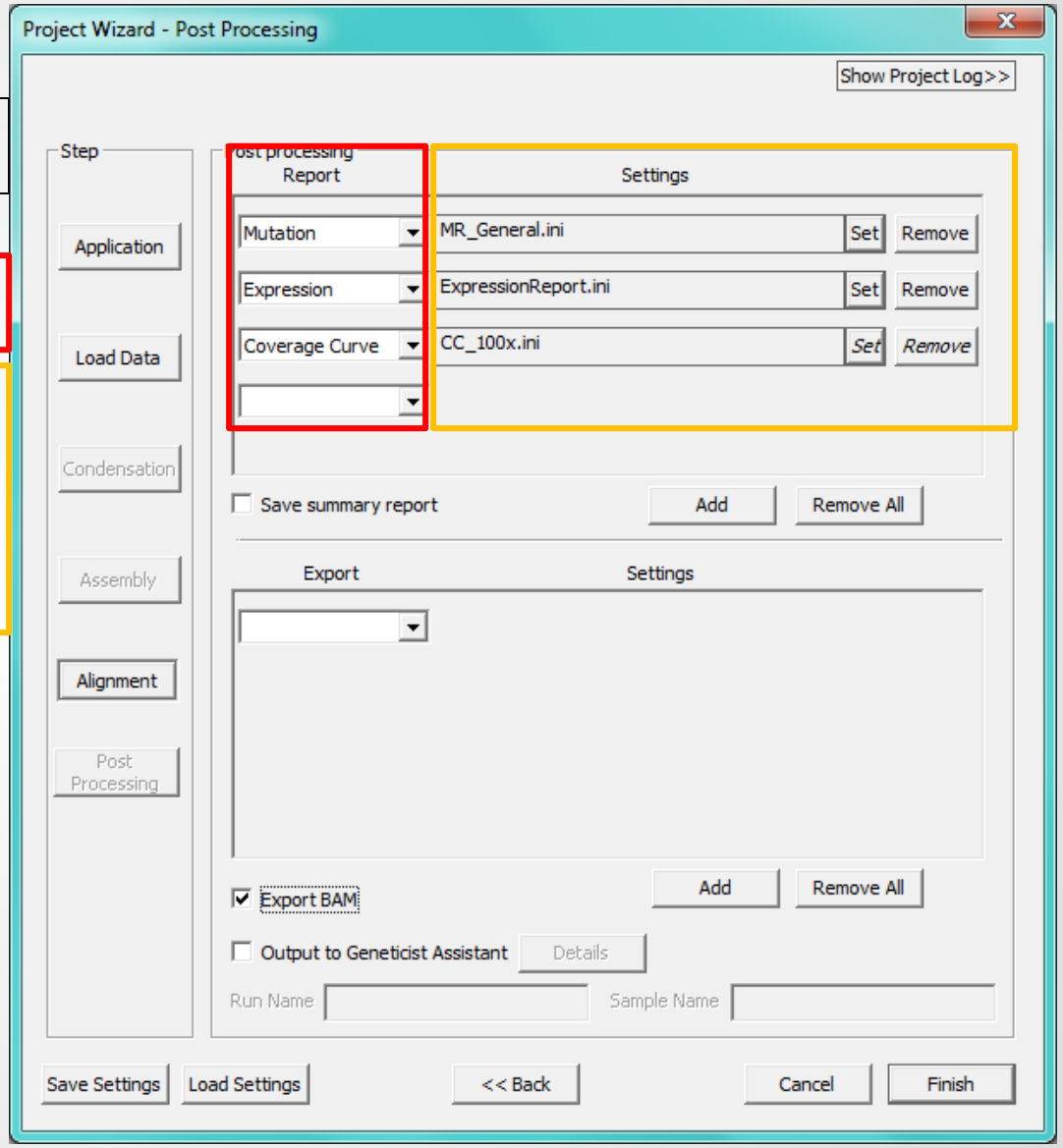
4. Click Next.



Post-Processing Reports

- Set the reports that you want to be automatically generated in each project.

1. Choose the report.
2. Choose a configuration file that you previously saved from the NextGENe Viewer report's Settings dialog.
3. Click Finish to Run NextGENe.



Project Analysis – Run Log

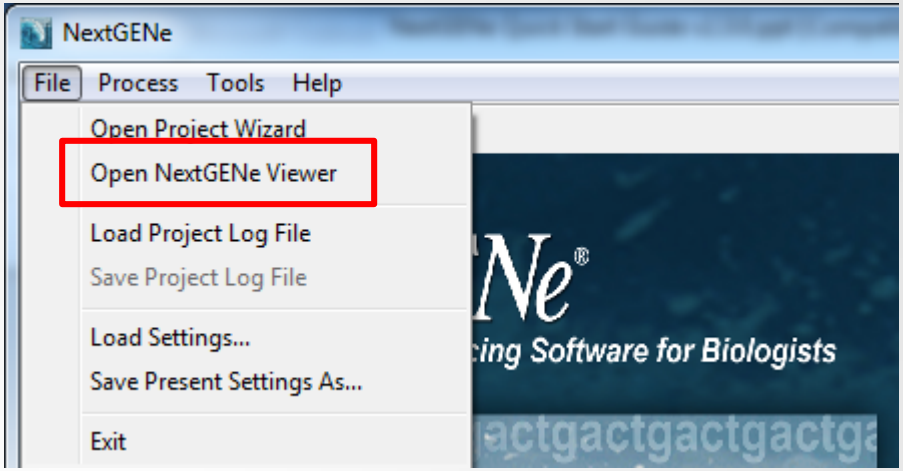
1. Statistics are calculated
2. Log denotes project analysis completion

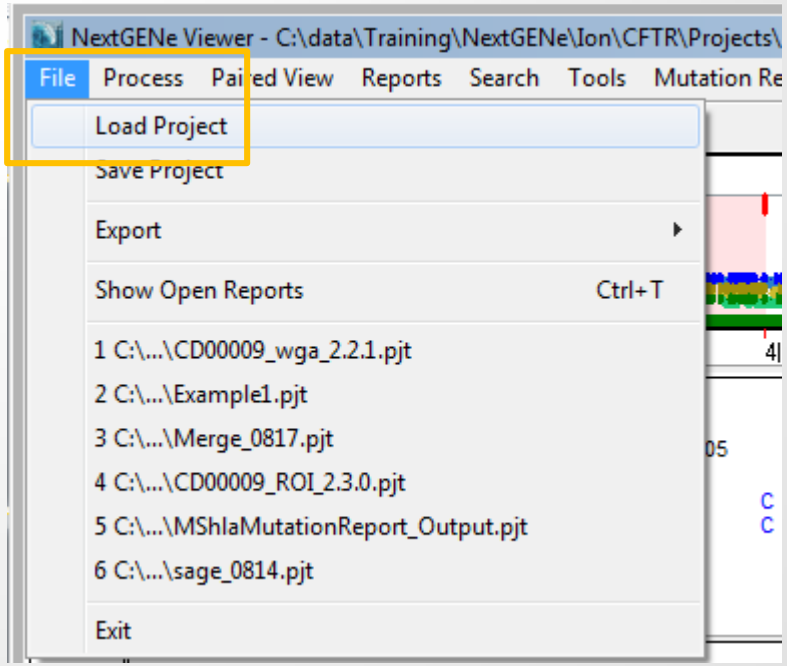
View Projects in NextGENe Viewer

Projects can be reviewed in the NextGENe Viewer Application.

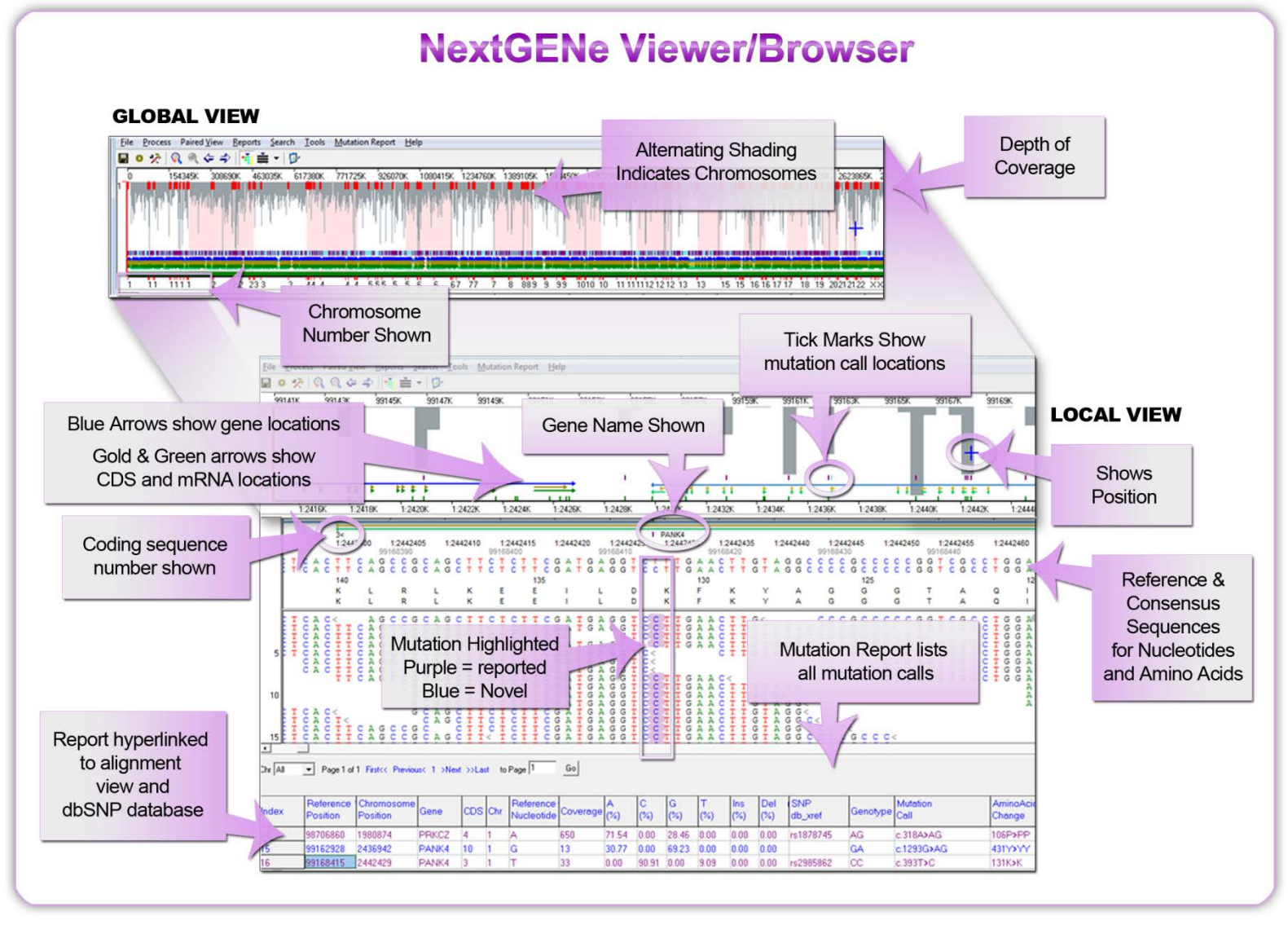
1. Click on Open NextGENe Viewer from NextGENe toolbar.

2. Click on File menu from the NextGENe Viewer toolbar and select Load Project.

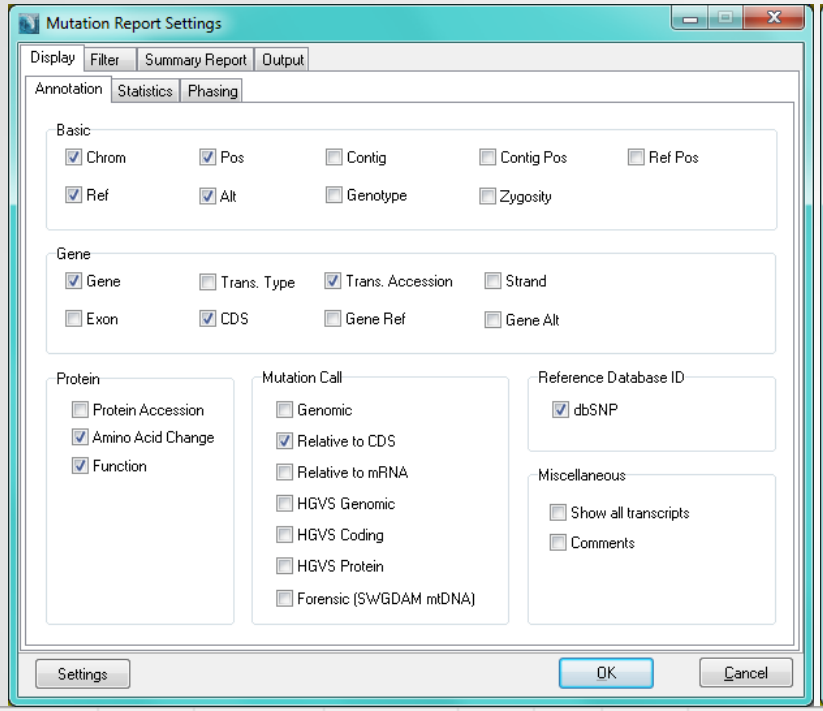


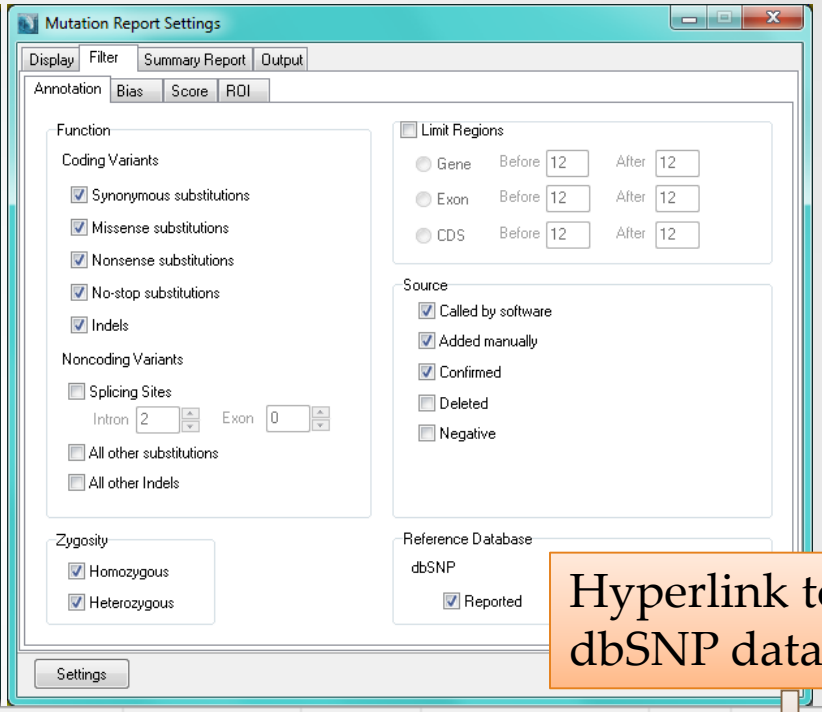


NextGENe Viewer



NextGENe Viewer – Mutation Report





Hyperlink to NCBI dbSNP database

Index	Chrom	Pos	Coverage	Ref	Alt	Alt%	Mutation Call: Relative To CDS	Function	Gene	Trans Accession	CDS	dbSNP	Amino Acid Change
14	13	32915005	1564	G	C	97.89	c.6513G>CG	Synonymous	BRCA2	NM_000059.3	10	rs206076	p.V2171VV
15	13	32929007	2296	G	C	1.96	c.7017G>CG	Missense	BRCA2	NM_000059.3	13	rs45574331	p.K2339NK
16	13	32929232	819	A	G	14.29	c.7242A>AG	Synonymous	BRCA2	NM_000059.3	13	rs1799955	p.S2414SS
17	13	32929331	1410	T	A	23.76	c.7341T>AT	Missense	BRCA2	NM_000059.3	13	rs4986858	p.N2447KN
18	13	32929387	1962	T	C	97.66	c.7397T>CT	Missense	BRCA2	NM_000059.3	13	rs169547	p.V2466AV
19	13	32971071	1551	C	T	2.13	c.9538C>CT	Missense	BRCA2	NM_000059.3	25		p.L3180LF
20	13	32972626	1524	A	T	1.64	c.9976A>AT	Nonsense	BRCA2	NM_000059.3	26	rs11571833	p.K3326KX
21	13	32972884	896	A	G	2.90	c.10234A>AG	Missense	BRCA2	NM_000059.3	26	rs1801426	p.I3412IV

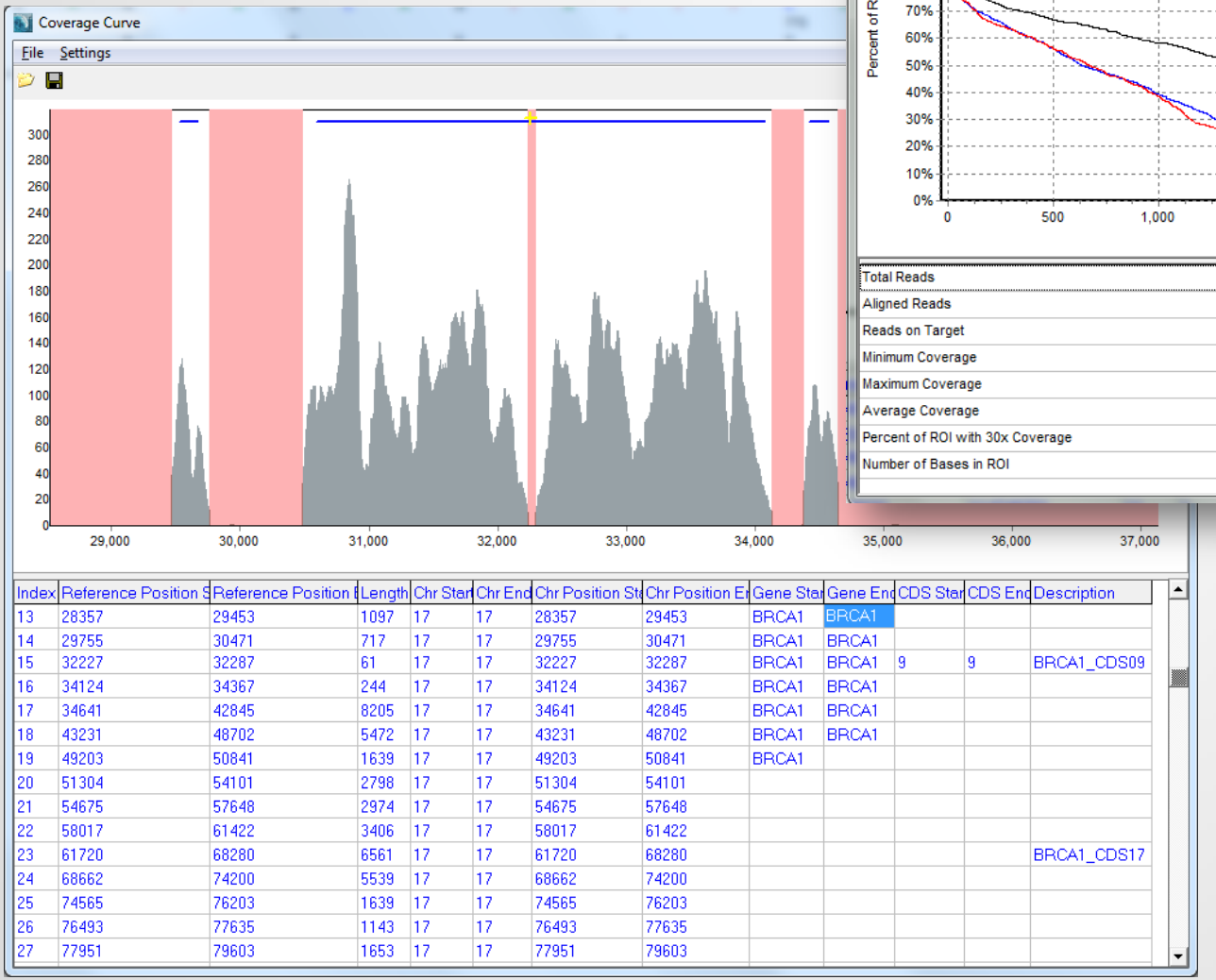
NextGENe Viewer – Variant Comparison Tool

Compare mutations detected in two or more samples aligned to the same reference.

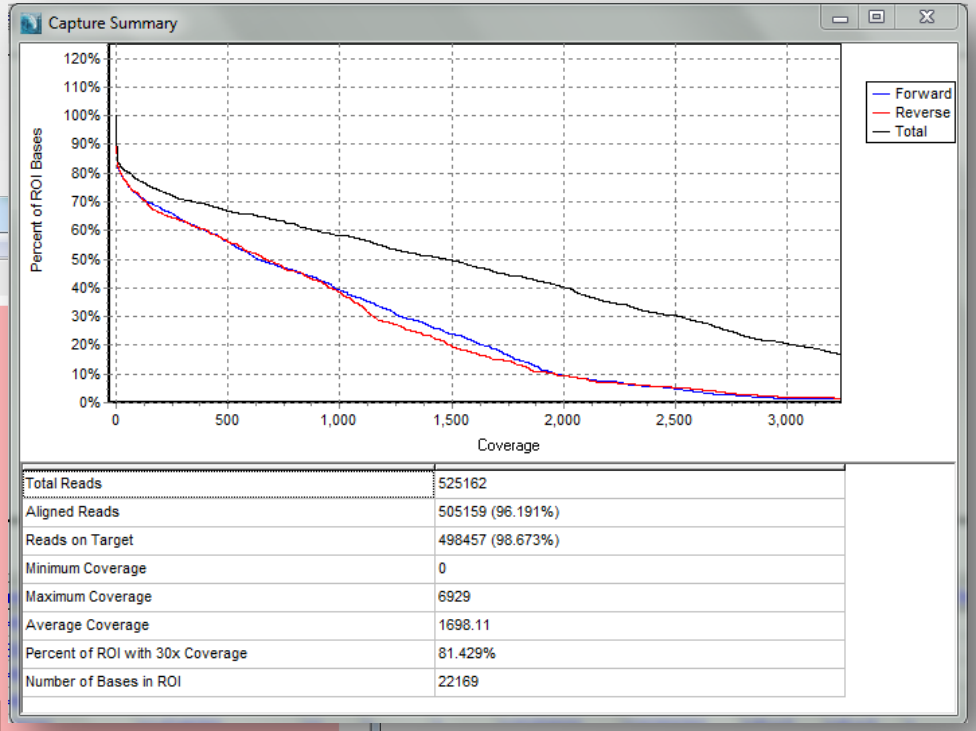
Use the Rare Disease tool to filter by relationship and phenotype.

NextGENe Viewer – Coverage Curve

Identify regions below a user-defined threshold of coverage

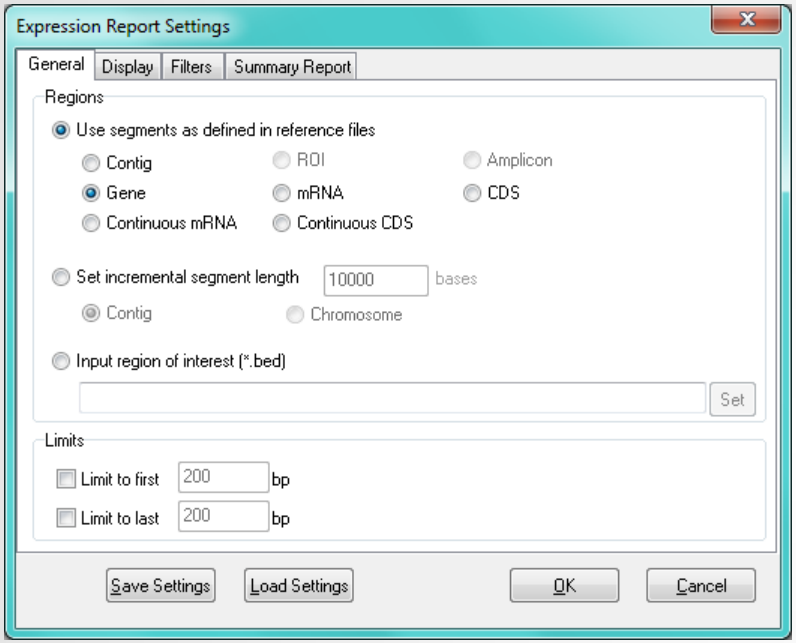


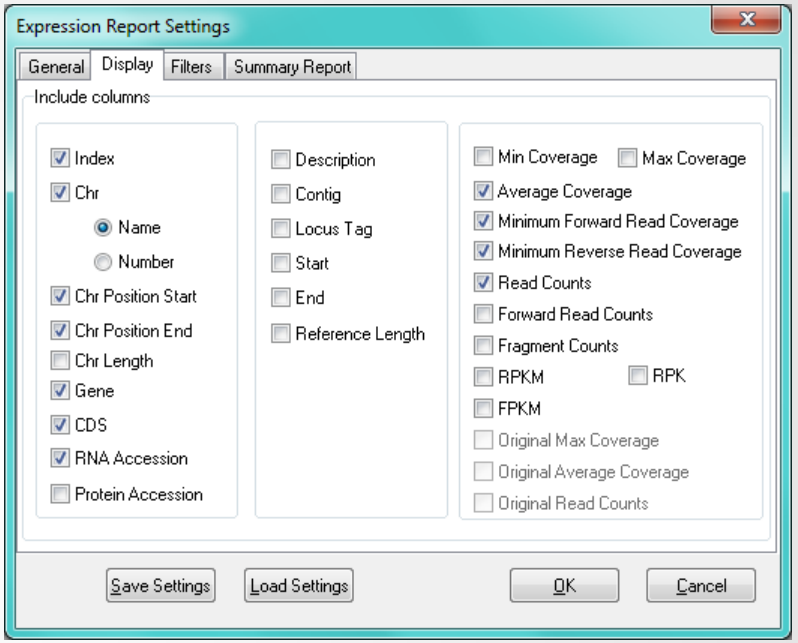
Index	Reference Position S	Reference Position E	Length	Chr Start	Chr End	Chr Position St	Chr Position En	Gene Start	Gene End	CDS Start	CDS End	Description
13	28357	29453	1097	17	17	28357	29453	BRCA1	BRCA1			
14	29755	30471	717	17	17	29755	30471	BRCA1	BRCA1			
15	32227	32287	61	17	17	32227	32287	BRCA1	BRCA1	9	9	BRCA1_CDS09
16	34124	34367	244	17	17	34124	34367	BRCA1	BRCA1			
17	34641	42845	8205	17	17	34641	42845	BRCA1	BRCA1			
18	43231	48702	5472	17	17	43231	48702	BRCA1	BRCA1			
19	49203	50841	1639	17	17	49203	50841	BRCA1				
20	51304	54101	2798	17	17	51304	54101					
21	54675	57648	2974	17	17	54675	57648					
22	58017	61422	3406	17	17	58017	61422					
23	61720	68280	6561	17	17	61720	68280					BRCA1_CDS17
24	68662	74200	5539	17	17	68662	74200					
25	74565	76203	1639	17	17	74565	76203					
26	76493	77635	1143	17	17	76493	77635					
27	77951	79603	1653	17	17	77951	79603					



Total Reads	525162
Aligned Reads	505159 (96.191%)
Reads on Target	498457 (98.673%)
Minimum Coverage	0
Maximum Coverage	6929
Average Coverage	1698.11
Percent of ROI with 30x Coverage	81.429%
Number of Bases in ROI	22169

NextGENe Viewer – Expression Report





Index	Chr	Chr Position Start	Chr Position End	Gene	CDS	RNA Accession	Min Coverage	Minimum Forward R	Average Coverage	Read Counts	RPKM
1	chr13	32890598	32890664	BRCA2; +	1	NM_000059.3	403	13	805.10	1073	38469.2122
2	chr13	32893214	32893462	BRCA2; +	2	NM_000059.3	609	230	1824.30	6590	63573.2539
3	chr13	32899213	32899321	BRCA2; +	3	NM_000059.3	417	28	546.15	903	19899.8419
4	chr13	32900238	32900287	BRCA2; +	4	NM_000059.3	514	456	605.80	532	25558.1845
5	chr13	32900379	32900419	BRCA2; +	5	NM_000059.3	342	52	453.39	256	14998.3845
6	chr13	32900636	32900750	BRCA2; +	6	NM_000059.3	975	582	1715.73	3355	70078.2192
7	chr13	32903580	32903629	BRCA2; +	7	NM_000059.3	705	479	1147.82	1144	54959.7050
8	chr13	32905056	32905167	BRCA2; +	8	NM_000059.3	340	17	827.46	1368	29339.7526