

NextGENe

Quick Start Guide



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Load Data	Project Wizard - Load Data	×
	Show P	Project Log>>
1. Load FASTA or BAM format	Step Previous run result: Load Previous Run Result To convert	t to fasta:
	Application F:\Data\Demo Data\RainDance Cancer Panel Data\ffpe_lung_9_Output\Pre F:\Data\Demo Data\RainDance Cancer Panel Data\ffpe_lung_9_Output\Pre	Load
If sample is not in FASTA or BAM format, or if low quality calls need to	Load Data	Remove
be removed, open Format Conversion Tool (described on next	Condensation	RemoveAll
page).	Human_V3/p10_doshp135	Preloaded
2. Load Reference Files	Assembly	Remove
1. Load (fasta or GBK format)	Alignment	RemoveAll
2. I Teloaded (large indexed)	F:\Data\Demo Data\RainDance Cancer Panel Data\ffpe_lung_9_Output\Pre	Set
3. Select Save Location in Output	Post Processing Processing Set Amplicon BED file Set ROI regions from GBK files	Cat
neid		
4. Click Next		
	<< Back Next >> Cancel	Finish



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

Instrument			
Type: Illumina		•	
Comple files :			
Sample files :			Add
			Remove
			Remove All
I I I I I I I I I I I I I I I I I I I	EASTO		
input format type		<u> </u>	
Output Output format typ	e: FASTA		
Output :			
			Set
Settings			
Median score	threshold >= 20	Max # of uncalled base	s <= 3
Called base n	umber of each read >= 25		
Trim or reject	read when >= 3 bas	se(s) with score $\langle = 16 \rangle$	
Paired reads		— hara(a)	Default Settings
E Remove 5	2 Dase(s) and 3	Dase(s)	
Keep only bas	es 0 to 0		
Trim by seque	nces		-
	nces in file		▼
Trim by seque			

Load Data – Analyze Locally to increase speed

	NextGENe		
File	Process Tools Help		
	Project Wizard	Options	×
	Project Log Viewer	Process	
	Run	Ne^*	
	Options	Image Soft Image Use local temp directory Image Only for remote data	
	Settings +	C:\Users\Megan\AppData\Local\SoftGenetics\NextGENe\LocalTemp	Set
	and the second second	gactgactgactgactgactgactgactgactgactgact	

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

- 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.

Project Wizard - Alignn	nent X
	Alignment Show Project Log>>
	Reads: Allowable mismatched bases 1 (0-2)
Step	Allowable ambiguous alignments 50
Application	Seeds: 30 bases, move step 5 bases Inspect input files
Application	Overall: Matching base percentage >= 85
Load Data	Sample trim Select sequence range from 1 bases to 30 bases V Hide unmatched ends
Condensation	Mutation filter Use original 🔽 Except for homozygous
	SNPs Indels HomopolymerIndels Mutation percentage <= 20 20 20
Assembly	SNP allele count <= 3 3 3
	Total coverage count <= 5 5 5
Alignment	Max gap between two variants 1 (0-3) Phaseable reads percentage >= 50
Post Processing	File type
	Library size range ; from 50 bases to 300 bases
	454 Sequence:
	Save matched reads Highlight anchor sequence Ambiguous gain/loss Detect structural variations Mismatch: 0.3 length and 50 bases Default Settings Image: Setting set
Save Settings	ad Settings << Back Next >> Cancel Finish



Project Analysis – Run Log

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

	Sector actgactgactgactgactgactgactgactgactgactg	SOFTGENETICS® Software PowerTools for Genetics Analysis
	[Alignment Statistics Information] Matched Reads Count: 248806 Unmatched Reads Count: 138368 Short Reads Count: 0 Number of Matched Bases: 19856355 Number of Unmatched Bases That are Recorded as Mutations: Mismatches: 4701 Deletions: 9417 Insertions: 35 Number of Unmatched Bases That are NOT Recorded as Mutations: Mismatches: 30402 Deletions: 35812 Deletions: 35812	
L	Inservors: 31241 Average Read Length: 80 Average Coverage: 856 Reference Length: 188703 Number of Coverage Reace: 23273	E
q	[Monday, August 20, 2012, 08:54:23] Processing Complete.	₹

View Projects in NextGENe Viewer

Projects can be reviewed in the NextGENe Viewer Application.

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



N 10	lextGENe V	iewe	r - C:\data	a\Training	NextGEN	e\Ion\C	FTR\Projects\			
File	Process	Pai	ed View	Reports	Search	Tools	Mutation Re			
	Load Proj	ect								
	Save Proje	ect								
	Export						• •			
	Show Ope	Ctrl+	-T Nati							
	1 C:\\CE	0000	09_wga_2.	2.1.pjt			4			
	2 C:\\Ex	amp	le1.pjt							
	3 C:\\M	erge	_0817.pjt				05			
	4 C:\\CE	0000	09_ROI_2.3	3.0.pjt						
	5 C:\\MShlaMutationReport_Output.pjt									
	6 C:\\sa	ge_0	814.pjt							
	Exit									

NextGENe Viewer



NextGENe Viewer – Mutation Report

M 🛐	utation Report Sett	ings						Mutation	n Report Settings						
Displ	ay Filter Summar	y Report Output						Display Fil	ter Summary Report Outp	out					
Ann	otation Statistics F	Phasing						Annotation	Bias Score ROI						
E	Basic Drom	Pos	Contia	Con	tia Pos	Bef		-Functio	n Marianta		Limit Regions	40 40-			
	Ref	■ 1 03	Genotupe	Zua	ooitu	- Herr	0.5		nonumous substitutions		Giene Berore	12 After	(12)		
	i no	M AK	achogec	2yg	USILY				issense substitutions		CDS Before	12 After	12		
	Gene			_				🔽 N	onsense substitutions		CD3 Beloic	12 Altoi	12		
	📝 Gene	Trans. Type	V Trans. Accession	i 📃 Stra	and			🔽 N	o-stop substitutions		Source				
	Exon Exon	CDS	🔲 Gene Ref	🗖 Ge	ne Alt			🔽 In	dels		Added manually	-			
-F	Protein	Mutation	n Call		Reference	e Database II		Nonco	ding Variants		Confirmed				
	Protein Accessio	on 🗖 Ge	enomic		🔽 db9	5NP		Sp Sp	ilicing Sites tron 2 🛋 Exon 0		Deleted				
	V Amino Acid Char	nge 🛛 📝 Re	elative to CDS						other substitutions	Y	Negative				
	Function	Re	elative to mRNA		Miscellar	neous		AI	other Indels						
		П НС	iVS Genomic SVS Coding		Sho	ow all transcrip	ts				Deference Detetere				
		E HG	GVS Protein		Con	nments		Zygosity	mozvaous		dbSNP				
		E Foi	rensic (SWGDAM mtDI	NA)					eterozygous		📝 Reported	Hy	perli	ink to N	CBI
												dhS	NIP	databas	
	Settings					<u>Ω</u> K	<u>C</u> ancel	Settings				ubc	N N I		
dex	Chrom	Pos	Coverage	e Ref	Alt	Alt%	Mutation (Relative T	all: o CDS	Function	Gene	Trans Ac	cession	CDS	dbi NP	Amino Aci Change
	13	32915005	1564	G	С	97.89	c.6513G>C	G	Synonymous	BRCA2	NM_00005	9.3	10	rs206076	p.V2171VV
	13	32929007	2296	G	С	1.96	c.7017G>C	G	Missense	BRCA2	NM_00005	9.3	13	rs45574331	p.K2339NK
	13	32929232	819	A	G	14.29	c.7242A>A	G	Synonymous	BRCA2	NM_00005	9.3	13	rs1799955	p.S2414SS
	13	32929331	1410	Т	A	23.76	c.7341T>A	Г	Missense	BRCA2	NM_00005	9.3	13	rs4986858	p.N2447KN
	13	32929387	1962	Т	С	97.66	c.7397T>C	Г	Missense	BRCA2	NM 00005	9.3	13	rs169547	p.V2466AV
	13	32971071	1551	С	Т	2.13	c.9538C>C	Г	Missense	BRCA2	NM_00005	9.3	25		p.L3180LF
	13	32972626	1524	Δ	т	1.64	c.9976A>A	Г	Nonsense	BBCA2	NM 00005	9.3	26	rs11571833	p.K3326KX
	1.0	00010000	1000	<u> </u>		1.100			NONDONDO	DI YOU ID	14141_00000	· • · • ·			

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.





Identify regions below a user-defined threshold of coverage





36.000

37.000

Index	Reference Position 9	Reference Position I	Length	Chr Star	Chr End	Chr Position Ste	Chr Position Er	Gene Sta	Gene End	CDS Star	CDS End	Descriptio	'n
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_C	DS09
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_C	DS17
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						

NextGENe Viewer – Expression Report

Expression Report Settings	Expression Report Settings
Contig ROI Amplicon Gene MRNA CDS Continuous mRNA Continuous CDS Set incremental segment length 10000 bases Contig Chromosome Input region of interest (*.bed) Limits Limit to first 200 bp Limit to last 200 bp	Image: IndexDescriptionMin CoverageMax CoverageImage: IndexContigImage: Image: Image
Save Settings Load Settings DK Cancel	Save Settings

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