

2019年2月15日 臨床ゲノム情報解析ハンズオントレーニング



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VarSeq起動画面の確認





VarSeqを起動した際に、上の図のように表示されていることをご確認ください。下の図のように、ログイン画面や、「Viewer」と表示されている状態では、ソフトウェアを使用できません。

本日使用するデータ





- ✓ Databases
 - 各種データベースのアノテーションファイルが納められている。
- ✓ Exome_trio
 - 遺伝性疾患の解析に用いるサンプルデータが納められている。

✓ Somatic_cancer

- がん体細胞変異の解析に用いるサンプルデータが納められている。

- USBメモリで配布した「VarSeq」フォルダを、PC上の任意の場所(デスクトップなど) にコピーしてください。
- VarSeqフォルダ内に、上記3つのフォルダが入っていることを確認してください。

本日使用するデータ





- VarSeq上より、Tools -> Open Folder -> Annotations Folderをクリックし、 フォルダを開きます。
- 配布した「Databases」フォルダ内のすべてのファイルを、Annotations Folder内 にコピーしてください。

体細胞変異解析ワークフロー



手順1: サンプルデータのインポート

- 解析プロジェクトを作成
- 腫瘍・正常サンプルそれぞれのVCF, BAMファイルをインポート

手順2: アノテーション付加

● 変異データに対して、様々なデータベースを用いたアノテーション付加の実行

手順3: フィルタリング

- 体細胞、生殖細胞系列変異の抽出
- ゲノムブラウザーによる確認

手順4: レポート作成

● 抽出変異情報を用いてレポートの作成



手順1. サンプルデータのインポート

プロジェクトの作成



- O X

	Project Template
	Folder: Project Templates Reset Browse
Coldon Holiy VarSon 2.1.0	
	* C Empty Project
<u>F</u> ile <u>T</u> ools <u>H</u> elp	ACMG Guidelines Gene Panel Template
	ACMG Guidelines Trio Template
	Cancer Gene Panel Starter Template
	Cancer Gene Panel Tutorial
	Exome Trio Template
	Hereditary Gene Panel Starter Template
	Hereditary Gene Panel Starter Template
	TruSight Cancer Panel Japanese Sample
	Tumor-Normal Template
	Empty Project
Counter New Designat	III ►
Create New Project	
	Genome Assembly
Open Existing Project	Homo sapiens (Human), GRCh37 (hg19) (2 2009) 🗸 🗸
New Project 20190116	Duritant
Exome this Analysis	Name: Somatic Cancer
	Folder: C.¥Users¥Ozawa¥GH Data¥Somatic Cancer Browse
	OK Cancel

New Project

- 1. メイン画面の「Create New Project」をクリック
- 2. 任意のプロジェクト名を入力し、またProject Templateに「Empty Project」、Genome Assemblyが 「Homo sapiens (Human), GRCh37 (hg19) (2 2009)」となっていることを確認したら「OK」をクリック

腫瘍・正常サンプルファイルのインポート



		V Impore Varianco Vilzara		
		Import Variant S	Sources	
Somatic Cancer - Golden Helix VarSeq 2.1.0 File View Tools Help Table Import Export Import ? Table Import Get started by importing some data	Samples: 0 Variants: 0	 ① Define Input ② Scan Input ③ Change Options ④ Review Select one or more variant files to import. 	Append Records	Add Files Remove Add Folder Remove All
		Help	< Back Next >	Cancel

Import Variante Wizard

- 3. 次の画面で、「Import Variants」をクリック
- 4. Import Variant Sources画面で「Add Files」をクリックし、Somatic_cancerフォルダ内の「N990005.vcf.gz」 と「T990005.vcf.gz」を選択
- 5. Import Variant Sources画面に両ファイルが表示されたら、「Next」をクリック

腫瘍・正常サンプルファイルのインポート





① Define Input	Add sample fields: From Text File Associate BAM File								
② Scan Input	Original Sam	oles 📝 le Source File I	Samples	Normal Sample	Tumo				
③ Change Options	1 N990005	N990005	N990005		Norma				
(4) Review	2 T990005	T990005	T990005	N990005 -	Tumor				

- 6. Sample Relationshipsで、「Tumor/Normal Samples」を選択し、Nextクリック
- 7. サンプル情報の入力画面で、「T990005」のNormal Sampleフィールドに「N99005」、Tumorフィールドに 「Tumor」を選択
- 8. Add sample fieldsの「Associate BAM File」をクリック





У Select BAM Files	to be Associated with Each Sample
Select each sample	's corresponding BAM file by selecting it from the drop down menu.
C:/	/VarSeq/Somatic_cancer Browse Found 2 BAM files 💼
Sample Name	File Name
N990005	C:/
T990005	C:/
📃 Make Paths Rel	ative to Project
	OK Cancel Help

- 9. 各サンプルのFile Nameフィールドのドロップメニューより、各サンプル名のBAMファイルを選択し、 「OK」をクリック
 - *ドロップメニューにBAMファイルが表示されない場合は、上部の「Browse」よりSomatic_cancer フォルダを選択する





🤣 Import Variants Wizard							
Import Variant S	Sources						
① Define Input	Add sample fields: Fro	om Text File Assoc	ciate BAM File				
② Scan Input	Original Samples	🔽 le Source File I	Samples	Normal Sample	Tumor		BAM Path
③ Change Options	1 N990005	N990005	N990005	-	Normal	C:/	/VarSeq/Somatic_cancer/N990005.bam
(4) Review	2 T990005	T990005	T990005	N990005 -	Tumor	C:/	/VarSeq/Somatic_cancer/T990005.bam
Select the samples of interest and appropriately adjust their attributes							
Advanced Options	Change the sample name	s: Reset Se	t to File Name Set	to File Name_Sample			
Help							A Back Next > Cancel Cancel

10. 各サンプルのBAM Pathフィールドに、先の画面で指定したBAMファイルへのパスが正しく表示されている ことを確認し、「Next」をクリック 腫瘍・正常サンプルファイルのインポート



🤣 Import Variants Wizar	d	
Import Variant	Sources	
 Define Input Scan Input Change Options Review The new source file will be saved to the project data folder. (Advanced) You may also choose to left align, or split variants into allelic primitives.	Summary: • Total size: 336K, 2 Files • 14 fields, 2 tumor/normal samples(1 tumor). • Assembly GRCh_37_g1k,Chromosome,Homo sap Assembly of Input Files: Homo sapiens (Human), GRCh37 (hg 19) (2 2009) Matches project, no liftover performed. Specify Genomic Regions to Import Matches project, no liftover performed. Select files: Select an Annotation Source Select Track 20 + +/- BP © Exons Only Full Transcript Select filters to reduce the number of variants imported. If no filters are selected then all of the variants will be imported. PASS Select All Clear Selection	piens
Advanced Options		
Help		Kerken State Cancel Cancel

11. Assembly of Input Filesに、「Homo sapiens (Human), GRCh37 (hg19) (2 2009)」と表示されていることを確認し、「Finished」をクリック





*Somatic Cancer - Golden Helix VarSeq 2.1.0										
File View Tools Help										
🔚 🕋 🗔 Add 🖉 Export 🙀 Plot 🚫 Co	nnect 🔰 Tumor (Tumor (T990005) → ▶ # ?							Samples: 2 Variants: 11,076	
🝸 Filter Variants 🗙 🕂	Variants: 11,0	T Variants: 11,076 x +								
Variants 👻	Variants 👻 🖽	© 2 D		Filter Variants: T99	0005 - 🗗				Variants: 11,076	
V nº Filter Variants 🔧 11,076		Variant Info		г	umor (T000005)		Ν	ormal (N000005)		
	Chr:Pos	Bef/Alt	Identifier	Bead Depths (DP)	I/1 Genotynes (GT)	AF	Read Depths (DP)	I/1 Genotypes (GT)	AF	
	3:238566	G/C	rs2271500	?	./.	?	5	1/1	1	
	3:350861	A/G	rs12639486	5	1/1	1	8	1/1	1	
	3:361508	C/T	rs2272522	70	1/1	1	54	1/1	0.981481	
	3:391100	A/G	rs13060847	177	1/1	0.988701	120	1/1	1	
	3:405202	A/T	rs2387180	19	1/1	1	5	1/1	1	
	3:423983	C/T	?	?	./.	?	26	0/1	0.153846	
	3:439963	A/G	rs6442827	181	1/1	1	96	1/1	1	
	3:440028	T/C	rs6771714	230	1/1	1	134	1/1	1	
	3:440088	T/A	rs6771803	185	1/1	1	141	1/1	1	
	3:448063	A/G	rs3956164	2	1/1	1	?	./.	?	
	3:449832	A/G	rs4328791	4	1/1	1	?	./.	?	
	3:450391	T/C	rs3856876	3	1/1	1	?	./.	?	
	3:884279	G/A	rs4345060	2	1/1	1	?	./.	?	
	3:886106	T/G	rs1403909	2	1/1	1	?	./.	?	
	3:886304	C/A	rs1403910	3	1/1	1	?	./.	?	
	3:1339681	GTTTTT/-	?	60	0/1	0.293103	47	0/1	0.288889	
	3:1418753	G/A	rs17038365	247	0/1	0.40081	177	0/1	0.443182	
	3:1424/18	G/A	rs2291101	250	0/1	0.4/3896	213	0/1	0.43128	
	3:1424/45	C/ T	rs4684146	226	0/1	0.451327	1/6	0/1	0.4375	
	3:1424850	1/G	rs2291100	182	0/1	0.5	99	0/1	0.585859	
?	3:103/940	G/A	rs900244	9	0/1	0.3/5	8	0/1	0.5	
	3.1//1/49	A/C	150442004				18	1/1		

12. Tumor (T990005)とNormal (N990005)両サンプルの変異データがインポートされ、 プロジェクト画面に表示される



手順2. アノテーション付加



V *Somatic Cancer - Golden Helix VarSeq 2.1.0									
File View	Tools Help								
8	🔂 Add 🕽 🛃 Export 🛃 🚧 Plot	Connect							
Filter	🗔 Variant Annotation								
Variants 👻	Secondary Tables	Varia							
🔽 🖬 Filter	Computed Data	11,076							

Select Data Source		
Select tracks to use as annotation sourc	es against the imported variant set.	
 Select Data Source Select tracks to use as annotation source Locations Browse Url Warehouse Warehouse Local User Annotations Assessment Catalogs Public Annotations Secure Annotations Secure Annotations Example Samples 	es against the imported variant set. User Annotations Filter: * (Any type Homo sapiens (Human), GRCh37 (hg 19) (Name Name Name Nextera Rapid Capture Exome Targeted Regions 2013-03-07, Illu NHLBI ESP6500SI-V2-SSA137 Exomes Variant Frequencies 0.0.3 OMIM Genes 2019-01-01, GHI OMIM Genes with Details 2017-01-01, GHI OMIM Phenotype Ontology 2017-06-15 OMIM Phenotypes 2019-01-01, GHI OMIM Phenotypes with Details 2017-01-01, GHI OMIM Variants 2019-01-01, GHI OMIM Variants 2019-01-01, GHI TruSight Cancer Amplicon Design 2013-02-05, Illumina Information Showing (29/58), 1 selected RefSeq Genes 105 Interim v1, NCBI (Edit) Description	Current Type Type Interval Interval Interval Interval Interval Interval Variant Gene Variant Interval Variant Current
	Description This track contains RefSeq Gene transcripts annotated by the NCBI Homo sa Annotation Release 105 Interim v1.	apiens 👻
Convert Download	Select Cancel	Help

- 1. プロジェクト画面の「Add」をクリックし、メニューより「Variant Annotation」を選択してクリック
- 2. Select Data Source画面において、画面左側のLocationに「User Annotations」を選択し、続く画面右側 のデータベースリストより「RefSeq Genes 105 Interim v1, NCBI」にチェックを入れ、「Select」をクリック



RefSeq Genesアノテーションの付加

			RefSeq Genes	105 Interim v1, NCBI		
Gene Names	Sequence Ontology (Combined)	Effect (Combined)	Nof4PredictedSplicingDisruptedCombined	Predicted Splicing Disrupted (Combined)	Transcript Name (Clinically Relevant)	HGVS c. (Clinically Relevant)
RAI14	missense_variant	Missense	?	?	NM_001145525.1	NM_001145525.1:c.497G>T
?	intergenic_variant	Other	?	?	?	?
TTC23L	missense_variant	Missense	0 of 4 Predicted Splicing Disrupted	?	NM_001317949.1	NM_001317949.1:c.92A>G
BRIX1	splice_region_variant	Other	0 of 4 Predicted Splicing Disrupted	?	NM_018321.3	NM_018321.3:c.793-3T>A
BRIX1	splice_acceptor_variant	LoF	3 of 4 Predicted Splicing Disrupted	GeneSplicer,MaxEntScan,NNSplice	NM_018321.3	NM_018321.3:c.793-2dupA
AGXT2	splice_donor_variant	LoF	2 of 4 Predicted Splicing Disrupted	MaxEntScan,PWM	NM_031900.3	NM_031900.3:c.*46G>T
AGXT2	synonymous_variant	Other	?	?	NM_031900.3	NM_031900.3:c.1305T>C
AGXT2	missense_variant	Missense	?	?	NM_031900.3	NM_031900.3:c.635C>T
AGXT2	missense_variant	Missense	?	?	NM_031900.3	NM_031900.3:c.418G>A
AGXT2	missense_variant	Missense	?	?	NM_031900.3	NM_031900.3:c.305G>A
SPEF2	missense_variant	Missense	?	?	NM_024867.3	NM_024867.3:c.211A>C
SPEF2	synonymous_variant	Other	0 of 4 Predicted Splicing Disrupted	?	NM_024867.3	NM_024867.3:c.579T>C
SPEF2	synonymous_variant	Other	?	?	NM_024867.3	NM_024867.3:c.861C>T
SPEF2	missense_variant	Missense	?	?	NM_024867.3	NM_024867.3:c.1498G>A
SPEF2	synonymous_variant	Other	0 of 4 Predicted Splicing Disrupted	?	NM_024867.3	NM_024867.3:c.2142T>C
SPEF2	missense_variant	Missense	?	?	NM_024867.3	NM_024867.3:c.2711C>T
SPEF2	missense_variant	Missense	?	?	NM_024867.3	NM_024867.3:c.2800G>C
SPEF2	intron_variant	Other	?	?	NM_024867.3	NM_024867.3:c.2839+630C>T
SPEF2	intron_variant	Other	?	?	NM_024867.3	NM_024867.3:c.2839+815C>T
SPEF2	intron_variant	Other	?	?	NM_024867.3	NM_024867.3:c.2839+1107G***
SPEF2	intron_variant	Other	?	?	NM_024867.3	NM_024867.3:c.2839+1163A
SPEF2	intron_variant	Other	?	?	NM_024867.3	NM_024867.3:c.2839+1505C+++
SPEF2	intron_variant	Other	0 of 4 Predicted Splicing Disrupted	?	NM_024867.3	NM_024867.3:c.2914+19T>G
SPEF2	intron_variant	Other	0 of 4 Predicted Splicing Disrupted	?	NM_024867.3	NM_024867.3:c.3331-11T>C
CAPSL	missense_variant	Missense	?	?	NM_144647.3	NM_144647.3:c.254G>A
CAPSL	intron_variant	Other	0 of 4 Predicted Splicing Disrupted	?	NM_144647.3	NM_144647.3:c.137+17A>G
UGT3A1	3_prime_UTR_variant	Other	?	?	NM_152404.3	NM_152404.3:c.*607T>C
UGT3A1	splice_region_variant	Other	0 of 4 Predicted Splicing Disrupted	?	NM_152404.3	NM_152404.3:c.1296-8G>A

3. アノテーション付加が完了すると、変異データテーブルにRefSeq Genesデータベースのアノテーション列が 追加される

RefSeq Genesアノテーションの付加



/ 🛅 Variants: 11,1)76 × +								
Variants 🔻 🖬		L 📴 🔲 🗹	Filter Variant	ts: T990005 ~ 					Variants: 11,076
Variar	it Info	Tumor (T99	0005)	Normal (N99	10005)	RefSeq Gen	es 105 Interim v1, NCBI	▲ 5:35033605 - G/A (1bp sub)	□ ×
Chr:Pos	Ref/Alt	Read Depths (DP)	AF	Read Depths (DP)	AF	Gene Names	Sequence Ontology (Combined)	Sequence Ontology (Combined): missense_v	ariant
5:34811154	G/T	163	0.429448	107	0.542056	RAI14	missense_variant	rs180749	
5:34838930	-/G	?	?	5	1	?	intergenic_variant		
5:34840841	A/G	6	0.5	?	?	TTC23L	missense_variant	5:35033605 - G/A (1bp sub)	
5:34925328	T/A	21	0.428571	?	?	BRIX1	splice_region_variant	Variant Info	
5:34925329	-/A	?	?	17	0.529412	BRIX1	splice_acceptor_variant	Chr.Ros E:25022805	
5:34998778	C/A	36	0.361111	32	0.46875	AGXT2	splice_donor_variant	Cill.P05 5.35033605	
5:35010138	A/G	120	0.991667	68	0.985294	AGXT2	svnonvmous variant	Ref/Alt G/A	
5:35033605	G/A	117	1	104	0.990385	AGXT2	missense_variant	Identifier rs180749	
5:35037115	C/T	118	0.589744	91	0.637363	AGXT2	missense_variant	Show 3 hidden fields	
5:35039486	C/T	86	0.360465	54	0.444444	AGXT2	missense_variant	Show 5 hidden heids	
5:35641582	A/C	217	0.56682	132	0.522727	SPEF2	missense_variant	Sample Fields	
5:35644621	T/C	70	0.442857	38	0.368421	SPEF2	synonymous_variant	Sample Normal (N990005) Tur	nor (T990005)
5:35654711	C/T	201	0.49	133	0.398496	SPEF2	synonymous_variant	Read Denths (DP) 104 117	, E
5:35670303	G/A	237	0.481013	167	0.463855	SPEF2	missense_variant		
5:35700598	T/C	243	0.433884	143	0.507042	SPEF2	synonymous_variant	AF 0.990385 1	
5:35709095	C/T	132	0.992424	84	1	SPEF2	missense_variant	Show 2 hidden fields	
5:35709184	G/C	111	1	59	1	SPEF2	missense_variant		
5:35709853	C/T	3	1	?	?	SPEF2	intron_variant	RefSeq Genes 105 Interim v1, NCBI	
5:35710038	C/T	2	1	2	1	SPEF2	intron_variant	Gene Names	AGXT2
5:35710330	G/A	3	1	?	?	SPEF2	intron_variant	Sequence Ontology (Combined)	missense_variant
5:35710386	A/G	3	1	?	?	SPEF2	intron_variant	Effect (Combined)	Missonso
5:35710728	C/T	3	1	?	?	SPEF2	intron_variant		missense
5:35713007	T/G	81	1	57	1	SPEF2	intron_variant	N of 4 Predicted Splicing Disrupted (Combined)	?
5:35753715	T/C	248	0.995968	244	1	SPEF2	intron_variant	Displicited Collicing Disputed (Combined)	0
5:35910529	C/T	173	1	122	1	CAPSL	missense_variant	Predicted Splicing Disrupted (Combined)	?
5:35921069	T/C	250	1	249	1	CAPSL	intron_variant	Transcript Name (Clinically Relevant)	NM_031900.3
5:35953697	A/G	2	1	3	1	UGT3A1	3_prime_UTR_variant	HGVS c. (Clinically Relevant)	NM_031900.3:c.635C>T
5:35954588	C/T	103	0.990196	65	0.984615	UGT3A1	splice_region_variant	HGVS p. (Clinically Relevant)	NP 114106.1:p.Thr212lle
5:35960841	ſ/C	5	0.6	?	?	UGT3A1	intron_variant		
5:35962984	A/G	3	1	3	1	UG13A1	missense_variant	Show 10 hidden fields	
5:361//269	C/A	130	0.45/364	63	0.4/619	SKP2	intron variant		

4. 変異テーブル上部の「Hide/Show details window」をクリックすると、テーブル右側に詳細データの表示 スペースが現れ、テーブル上で選択した変異に付加されたアノテーション情報を確認できる RefSeq Genesアノテーションの付加



				(0. 100000 ·]						v	andres (1,01	
Variant	Tumor (T99	0005)	Normal (N9	30005)	R	RefSeq Genes 105 Interim v1, NCBI		ibined)				
Ret/Alt F	Read Depths (DP)	AF	Read Depths (DP)	AF	Gene Names	Sequence Ontology (Combined)	Effect (Combined)	The highest p	The highest priority of the effect annotations found among the interactions			
G/1	163	0.429448	107	0.542056	RAI14	missense_variant	Missense	Tupo: Cotoro	Turner Onterenting			
-/G	?	?	5	1	?	intergenic_variant	Other	Type: Catego	rical			
A/G	6	0.5	2	1	TTC23L	missense_variant	Missense	Field: Effect	(Combined)			
1/A	21	0.4285/1	(BRIXI	splice_region_variant	Other	Symbol: Eff	Symbol: EffectCombined			
-/A	?	2	1/	0.529412	BRIXI	splice_acceptor_variant	LOF	DesiThe hi				
C/A	36	0.361111	32	0.468/5	AGX12	splice_donor_variant	LOF	transcript in	gnest phonty of the effect annotations for iteractions. The likely effect that the varia	und among nt will have i	ine variant	
A/G	120	0.99166/	68	0.985294	AGX12	synonymous_variant	Other	transcript's	product. The ontologies that correspond	to each effe	ct category	
G/A	11/	1	104	0.990385	AGX12	missense_variant	Missense	can be four	id at the bottom of this page in the docun	nentation fo	the effect	
C/T	118	0.589744	91	0.637363	AGXT2	missense_variant	Missense	category.				
C/T	86	0.360465	54	0.444444	AGXT2	missense_variant	Missense	Category	Counts (11,076 Records)			
A/C	217	0.56682	132	0.522727	SPEF2	missense_variant	Missense	Catagoria		Count	Deserved	
T/C	70	0.442857	38	0.368421	SPEF2	synonymous_variant	Other	Category		Count	Percent	
C/T	201	0.49	133	0.398496	SPEF2	synonymous_variant	Other	Other	86.43%	9573	86.43%	
G/A	237	0.481013	167	0.463855	SPEF2	missense_variant	Missense					
T/C	243	0.433884	143	0.507042	SPEF2	synonymous_variant	Other	Missense	13.139	1454	13.139	
C/T	132	0.992424	84	1	SPEF2	missense_variant	Missense	LoF	0	49	0.44%	
G/C	111	1	59	1	SPEF2	missense_variant	Missense					
C/T	3	1	?	?	SPEF2	intron_variant	Other	Total	0.00% 17.3% 34.8% 51.9% 69.1% 88.4%	11076	100.0%	
C/T	2	1	2	1	SPEF2	intron_variant	Other					
G/A	3	1	?	?	SPEF2	intron_variant	Other	Categorie	s of Effect (Combined)			
A/G	3	1	?	?	SPEF2	intron_variant	Other	Other	The variant is likely to have a low or unl	known effec	t on the	
C/T	3	1	?	?	SPEF2	intron_variant	Other		transcript's functional product. These c	hanges do i	not change	
T/G	81	1	57	1	SPEF2	intron_variant	Other		the amino acid sequence of the protein	1. The ontold	gies	
T/C	248	0.995968	244	1	SPEF2	intron_variant	Other		stop_retained_variant, splice_region_v	ariant,		
C/T	173	1	122	1	CAPSL	missense_variant	Missense		3_prime_UTR_variant, 5_prime_UTR_	variant, intro	on_variant,	
T/C	250	1	249	1	CAPSL	intron_variant	Other		non_coding_exon_variant, intergenic_v	/ariant, unkr	iown.	
A/G	2	1	3	1	UGT3A1	3_prime_UTR_variant	Other	Missense	The variant will cause at least one amin	no acid to ch	nange or	
C/T	103	0.990196	65	0.984615	UGT3A1	splice_region_variant	Other		cause a premature start codon in the U	ITR5. The o	ntologies	
T/C	5	0.6	?	?	UGT3A1	intron_variant	Other		disruptive inframe insertion, inframe	deletion.	eletion,	
A/G	3	1	3	1	UGT3A1	missense_variant	Missense	-	inframe_insertion,			
C/A	130	0.457364	63	0.47619	SKP2	intron_variant	Other		5_prime_UTR_premature_start_codor	n_gain_varia	ant,	
T/A	115	0.443478	79	0.481013	NADK2	intron_variant	Other		missense_variant.			
T/C	6	0.333333	12	0.583333	NADK2	intron_variant	Other	LoF	Loss of Function. The variant is likely to	cause the	ranscript's	
G/A	?	?	8	0.5	NADK2	intron_variant	Other		category are: transcript ablation exon	loss variar	nt.	
G/A	6	0.666667	?	?	NADK2	intron_variant	Other		stop_lost, stop_gained, initiator_codor	variant,		
T/C	6	0.666667	?	?	NADK2	5_prime_UTR_variant	Other		frameshift_variant, splice_acceptor_va	riant,		
T/G	4	1	2	2	RANBP3I	missense variant	Missense		splice_donor_variant.			

5. 変異テーブル上のフィールドのヘッダーをクリックすると、現在表示されている変異データから選択フィールドの項目を集計したグラフが、詳細データの表示スペースに表示される

その他アノテーションの付加



選択データベースリスト

- 1kG Phase3 Variant Frequencies 5a
- CIViC
- ClinVar
- ClinVar Assessments
- COSMIC Mutations 87
- dbNSFP Functional Predictions 3.0
- HGVD1210-V2_30

- 1. 再びプロジェクト画面の「Add」をクリックし、メニューより「Variant Annotation」を選択してクリック
- 2. Select Data Source画面において、上記データベース名にすべてチェックを入れ、「Select」をクリック

その他アノテーションの付加



	Mariants: 11,076 × +							
Var	riants 💌 📠	<u>ତ</u> ୧ ୮) 📑 🔳 🔽 Filter Variant	s: T990005 ~ 🗗				
				ClinVar 20	9-01-01, NCBI			
	Ref/Alt	Variant ID	Classification	Clinical Significance	Aggregate of Interpretations from Submissions	Review Status		
	C/G	350806	Benign	Benign	Benign (1)	(1 Stars) Criteria Provided, Single Sub…		
	G/C	350810	Uncertain Significance	Uncertain Significance	Uncertain significance (1)	(1 Stars) Criteria Provided, Single Sub…		
	T/C	94099	Benign	Benign	Benign (5)	(2 Stars) Criteria Provided, Multiple Su····		
	A/G	25382	Benign	Benign	Benign (7)	(2 Stars) Criteria Provided, Multiple Su····		
	A/G	94101	Benign	Benign	Benign (5)	(2 Stars) Criteria Provided, Multiple Su····		
	T/C	94102	Benign	Benign	Benign (4)	(2 Stars) Criteria Provided, Multiple Su····		
	A/-	350820	Benign	Benign	Benign (1)	(1 Stars) Criteria Provided, Single Sub…		
	T/C	350827	Benign	Benign	Benign (1)	(1 Stars) Criteria Provided, Single Sub…		
	?	?	?	?	?	?		
	?	?	?	?	?	?		
	?	?	?	?	?	?		
	?	?	?	?	?	?		
	?	?	?	?	?	?		
	?	?	?	?	?	?		
	?	?	?	?	?	?		
	?	?	?	?	?	?		
	C/T	216097	Pathogenic	Pathogenic	Pathogenic (3)	(2 Stars) Criteria Provided, Multiple Su····		
	A/G	14673	Other	Risk Factor	risk factor (2)	(0 Stars) No Assertion Criteria Provided		
	?	?	?	?	?	?		
	?	?	?	?	?	?		

3. 変異データテーブルに、選択した全データベースのアノテーション列が追加される

4. アノテーションフィールドの表示・非表示や順序を変更する場合は、変異テーブル上部の「Hide/Show columns and column groups」をクリックして実行する



手順3. フィルタリング

フィルターコンテナの作成



Variant Info		Tumor (T9	9000	5)	Normal (N990005)		1
Chr:Pos	Ref/Alt	Read Depths (DP)	A	Sort A	scending		AF
3:238566	G/C	?	Z+	001070	i.		1
3:350861	A/G	5	Ā	Sort D	escending		1
3:361508	C/T	70		Hide			1481
3:391100	A/G	177	Х	Delete			1
3:405202	A/T	19	A •	Diat for	Current Comp		1
3:423983	C/T	?	202	PIOC TO	Current Samp	le	3846
3:439963	A/G	181	\sim	Plot for	r All Samples		1
3:440028	T/C	230	P	Query	Column Values		1
3:440088	T/A	185	Y	Add to	Filter Chain		1
3:448063	A/G	2	-				?
3:449832	A/G	4		Renam	e		?

🝸 Filter Variants 🗙 🕂	
Variants 👻	
🗸 🖬 Filter Variants	🔦 11,076
Read Depths (DP) (Current) >= 50	× -
50	kilu
	+
Less than 50	5,392
Equal to 50	40
Greater than 50	4,470
Missing	1,174
	4,510

- 1. 変異テーブル上で任意のフィールドのヘッダー(この例では「Tumor (T990005)」の「Read Depths (DP)」) を右クリックし、メニューより「Add to Filter Chain」を選択してクリック
- 2. 画面左側のFilter Variantsに、選択したフィールドのフィルターコンテナが表示されるので、任意の検索条件を 指定する
- 3. コンテナ内の右側に表示される各数字は、指定された条件で抽出される変異数を表し、この数字をクリックすると、変異テーブルに表示される変異データ数も変更される



🔽 🖬 Filter Variants	🔧 11,076
Read Depths (DP) (Current) >= 50	× -
50	iiiii
	+
Less than 50	5,392
Equal to 50	40
Greater than 50	4,470
Missing	1,174
	4,510
✓ AF (Current) >= 0.05	× -
0.05	ith
	+
Less than 0.05	8
Equal to 0.05	0
Greater than 0.05	4,501
Missing	1
	4,501
AF (Normal) < 0.01 OR missing	× -
0.01	idu
	+
Less than 0.01	1
Equal to 0.01	0
Greater than 0.01	4,397
Missing	103
	104

- Tumor (T990005) $\mathcal{O}[\text{Read Depths (DP)}] \ge 50$
- Tumor (T990005)の「AF」≧ 0.05
- Normal (N990005)の「AF」 < 0.01 or Missing

 腫瘍サンプルにおける体細胞変異抽出のため、T990005(Currentサンプル)の「Read Depths (DP)」と 「AF」、さらにN990005(Normalサンプル)の「AF」の3フィールドのコンテナをつくり、上記のとおり検索条件 を指定する



Filter Variants	2	11,076
Read Depths (DP) (Current) >= 50	3	
		4,510
✓ AF (Current) >= 0.05	4	
		4,501
AF (Normal) < 0.01 OR missing	4	
		104
Allele Frequencies < 0.01 OR missing	٩	-
0.01		<u>ith</u>
		+
Less than 0.01		3
Equal to 0.01		0
Greater than 0.01		15
Missing		86
		89
☑ Alt_allele_freq < 0.01 OR missing	٩	-
0.01		i.
		+
Less than 0.01		3
Equal to 0.01		0
Greater than 0.01		23
Missing		63
		66

- 1kG Phase3 Variant Frequencies 5aの 「Allele Frequencies」 < 0.01 or Missing
- HGVD1210-V2_30の「Alt_allele_freq」< 0.01 or Missing

2. 人種特異的なSNPの除去のため、1kG Phase3 - Variant Frequencies 5aの「Allele Frequencies」、 さらにHGVD1210-V2_30の「Alt_allele_freq」の2フィールドのコンテナをつくり、上記のとおり検索条件を指 定する



🔽 🖬 Filter Variants	4	11,076
Read Depths (DP) (Current) >= 50	٩	
		4,510
✓ AF (Current) >= 0.05	3	
	l	4,501
AF (Normal) < 0.01 OR missing	3	
		104
Allele Frequencies < 0.01 OR missing	3	
		89
Alt_allele_freq < 0.01 OR missing	3	
		66
In COSMIC? is true	3	-
True		7
False		59
Missing		0
		7
FATHMM Prediction is PATHOGENIC	3	-
NEUTRAL		3
PATHOGENIC		4
Missing		0
		4

- Summary of COSMIC Mutationsの「In COSMIC?」 is TRUE
- COSMIC Mutationsの「FATHMM Prediction」 is
 PATHOGENIC

 データベースに登録されている生体に有害な変異の抽出のため、Summary of COSMIC Mutations の「In COSMIC?」、COSMIC Mutationsの「FATHMM Prediction」の2フィールドのコンテナをつくり、 上記のとおり検索条件を指定する



📝 🖬 Filter Variants		⊸ 1	1,076
Read Depths (DP)	(Current) >= 50	4	
		4.	510
AF (Current) >= 0.0	5	3	
		4,	501
AF (Normal) < 0.01	OR missing	3	
			104
Allele Frequencies	< 0.01 OR missing	2	
			89
Alt_allele_freq < 0.	01 OR missing	3	
			66
In COSMIC? is true)	3	
			7
FATHMM Prediction	is PATHOGENIC	3	
			4
	Enabled		
	Inverted		
	AND		
	OR		3
		-1-	
E C	Lock		
	Add Filter		
	Add Filter Container		

👽 🗗 Filter Variants	× 11,076
Read Depths (DP) (Current) >= 50	* □
	4,510
✓ AF (Current) >= 0.05	* □
	4,501
AF (Normal) < 0.01 OR missing	* □
	104
Allele Frequencies < 0.01 OR missing	₹ □
	89
Alt_allele_freq < 0.01 OR missing	* □
	66
In COSMIC? is true	* □
	7
FATHMM Prediction is PATHOGENIC	* □
	4
Somatic	4 -
	2

🔽 📭 Filter Variants	× 11,076
Somatic	r –
Read Depths (DP) (Current) >= 50	∢ □
	4,510
✓ AF (Current) >= 0.05	∢ □
	4,501
AF (Normal) < 0.01 OR missing	▲ □
	104
☑ Allele Frequencies < 0.01 OR missing	₹ □
	89
▼ Alt_allele_freq < 0.01 OR missing	₹ □
	66
In COSMIC? is true	₹ □
	7
FATHMM Prediction is PATHOGENIC	₹ □
	4
	4

- 4. ワークフロー下側の空きスペース上で右クリックし、メニューから「Add Filter Container」選択してクリック
- 5. 新たなコンテナが作成されるので、コンテナ名をダブルクリックし、「Somatic」に変更
- 6. Somatic以外のコンテナをすべて選択し、Somaticコンテナ内にドラッグ&ドロップ





- 1. ワークフロー最上段の「Filter Variants」の「Show Filter Configuration」をクリックし、検索条件に「OR」を指定
- 2. Somaticワークフロー右側の空きスペースで右クリックし、メニューから「Add Filter Container」選択してクリック
- 3. 新たなコンテナが作成されるので、コンテナ名をダブルクリックし、「Germline」に変更



	11,070
Z Germline	3 √
📝 Read Depths (DP) (Normal) >= 50	s -
50	kth
	+
Less than 50	4,728
Equal to 50	40
Greater than 50	4,027
Missing	2,281
	4,067
✓ AF (Normal) >= 0.05	× -
0.05	http
	+
Less than 0.05	6
Equal to 0.05	0
Greater than 0.05	4,058
Missing	3
	4,058

- Normal (N990005) $\mathcal{O}[\text{Read Depths (DP)}] \ge 50$
- Normal (N990005)の「AF」 ≧ 0.05

4. 正常サンプルにおける生殖細胞変異抽出のため、N990005(Normalサンプル)の「Read Depths (DP)」 と「AF」の2フィールドのコンテナをつくり、上記のとおり検索条件を指定する



	3	11,076
🗹 Germline	4	1
Read Depths (DP) (Normal) >= 50	3	
	4	,067
☑ AF (Normal) >= 0.05	3	
	4	,058
Allele Frequencies < 0.01 OR missing	3	-
0.01		lub
		+
Less than 0.01		116
Equal to 0.01		0
Greater than 0.01	3	,677
Missing		265
		381
Alt_allele_freq < 0.01 OR missing	3	-
0.01		lub
		+
Less than 0.01		45
Equal to 0.01		0
Greater than 0.01		141
Missing		195
		240

- 1kG Phase3 Variant Frequencies 5aの 「Allele Frequencies」 < 0.01 or Missing
- HGVD1210-V2_30の「Alt_allele_freq」< 0.01 or Missing

5. 人種特異的なSNPの除去のため、1kG Phase3 - Variant Frequencies 5aの「Allele Frequencies」、 さらにHGVD1210-V2_30の「Alt_allele_freq」の2フィールドのコンテナをつくり、上記のとおり検索条件を指 定する



	2	11,076
Germline	٩	۱.
Read Depths (DP) (Normal) >= 50	3	
	4	.067
✓ AF (Normal) >= 0.05	3	
	4	.058
Allele Frequencies < 0.01 OR missing	3	
		381
Alt_allele_freq < 0.01 OR missing	3	
		240
Effect (Combined) is (LoF, Missense)	з,	-
LoF		8
Missense		61
Other		171
Missing		0
_		69
Classification is (Likely Pathogenic, Pa	3	-
Benign		0
Conflicting		2
Likely Benign		2
Likely Pathogenic		0
Other		0
Pathogenic		1
Uncertain Significance		0
Missing		64
		1

- RefSeq Geneの「Effect (Combined)」 is LoF, Missense
- ClinVarの「Classification」 is Likely Pathogenic, Pathogenic

6. データベースに登録されている生体に有害な変異の抽出のため、RefSeq Geneの「Effect (Combined)」、 ClinVarの「Classification」の2フィールドのコンテナをつくり、上記のとおり検索条件を指定する

ワークフロー解析結果の確認



🛛 🍸 Filter Variants 🗙 🕂				🔟 Variants: 5 🕽	< +					
Variants 👻			Va	ariants	• E		i 🗆 🗐	🔽 Filter Variants: TS	90005 🕶 💼	
Filter Variants		A 11,076		Variant	t Info	Tumor (T99	0005)	Normal (N99	0005)	
Somatic 🔹 I	Germline	× 1		Ĉhr:Pos	Ref/Alt	Read Depths (DP)	AF	Read Depths (DP)	AF	Gene Names
🕼 Read Depths (DP) (Current) >= 50 🔌 🗖	Read Depths (DP) (Normal) >= 50	▲ □		3:178936082	G/A	177	0.107345	?	?	PIK3CA
4,510		4,067		4:66280142	G/A	243	0.127572	?	?	EPHA5
Ø AF (Current) ≥= 0.05	✓ AF (Normal) >= 0.05	•		4:147560457	T/C	63	0.126984	?	?	POU4F2
4501		4 058		5:131973850	C/T	95	0.568421	81	0.592593	RAD50
				5:137519659	T/C	246	0.150407	?	?	KIF20A
AF (Normal) < 0.01 OR missing 🐴 🗖	Alt_allele_freq < 0.01 OR missing	× □	11							
104		1,357								
🕼 Alt_allele_freq < 0.01 OR missing 🔌 🗖	Allele Frequencies < 0.01 OR missing	1								
80		240								
Allele Frequencies < 0.01 OR missi 🔧 🗖	Effect (Combined) is (LoF, Missense)	* -								
66		69								
	Classification in (Likely Pothagania P									
	Classification is (Likely Patriogenic, P	· · ·								
·										
FATHMM Prediction is PATHOGENIC 🔧 🛛										
4										
4		1								
		5	•							

1. Somaticワークフローでは4つ、Germlineワークフローでは1つの変異が検出され、合計5種類の変異が テーブルに表示される

ワークフロー解析結果の確認



/ 🔲 Variants:	5	X	+	
---------------	---	---	---	--

Variants	•	।	0, 🗔 🛛	📕 📝 Filter Varia	nts: T990005							Variant	ts: 5
Variant	[nfo	Tumor (T99	0005)	Normal (N99	0005)	R	efSeq Genes 105 Interim v1,	, NCBI		3:178936082 -	G/A (1bp sub)		×
Ćhr:Pos	Ref/Alt	Read Depths (DP)	AF	Read Depths (DP)	AF	Gene Names	Sequence Ontology (Combined)	Effect (Co	ombined)	AF: 0.107345			
3:178936082	G/A	177	0.107345	?	?	PIK3CA	missense_variant		Missense	<u>rs121913273</u>			
4:66280142	G/A	243	0.127572	?	?	EPHA5	missense_variant		Missense	COSMIC Muta	ations 87, GHI		-
4:14/50045/	1/C	03	0.120984	۲ 01	0 502502	P004F2	synonymous_variant		Other		1		
5:137519659	T/C	246	0.150407	?	?	KIF20A	missense variant		Missense	Ref/Alt	G/A		
	-7-									Mutation ID	COSM760, COSM125369		
										Mutation CDS	c.1624G>A, c.1624G>A		
										Mutation AA	p.E542K, p.E542K		
										Ensembl Transcript ID	?, ENST00000263967		
										RefSeq Transcript ID	NM_006218.1, NM_006218.3		
										CDS Length	3207, 3207		Ξ
										Gene Name	PIK3CA		
										HGNC ID	?		
										Pubmed ID	27149842, 17947469, 27135926, 22357840, 19671852, 28481359, 26184520, 27901576, 16353168, 21533174,	[more]	
										Study ID	?		
										Sample Coun	t 535		
										Oncotree Tissue Type	Breast (BREAST), Bowel (BOWEL), Bladder/Urinary Trac (BLADDER), Lung (LUNG), Esophagus/Stomach ([more		
										Oncotree Tissue Type Counts	183, 99, 57, 35, 30, 23, 17, 17, 15, 10, 9, 9, 6, 5, 5, 5, 3, 3,	2, 1, 1	
										Primary Site	Breast, Large intestine, Urinary tract, Lung, Upper aerodi tract, Oesophagus, Cervix, [more]	gestive	
										Primary Site Counts	183, 98, 55, 35, 21, 18, 17, 17, 12, 11, 10, 9, 9, 6, 5, 5, 5, 4 2, 2, 1, 1, 1	, 3, 3, 2,	
										Site Subtype	Bladder, Colon, Brain, Caecum, Mouth, Anus, Head neck Upper urinary tract, Pharynx, [more]	Bile duct,	
										Site Subtype Counts	49, 44, 10, 9, 9, 7, 7, 7, 5, 5, 5, 3, 2, 2, 2, 1, 1, 1, 1, 1, 1, 1, 1,	1, 1, 1	
										Primary Histology	Carcinoma, Glioma, Germ cell tumour, Malignant melano Liposarcoma, Thymic carcinoma, Other, Rhabdomyosar Sarcoma, Haemangioblastoma	ma, oma,	









- 1. プロジェクト画面の「Plot」をクリックし、メニューより「Plot BAM for Current Sample」を選択してクリック
- ゲノムブラウザーが起動し、CurrentサンプルのRead Alignmentデータが表示されたら、同じくPlot -> Plot BAM for Current Normal」を選択してクリック





	το Τι	mor (T99	00005)	Normal (N9	90005)		Re	efSeq Genes 105	Interim v1, M	VCBI			
Ćhr:Pos F	lef/Alt	DP	AF	DP	AF	GeneNa…	SequenceOntologyCombined	EffectCombined	Nof4PredictedS	SplicingDisrupted	ICo··· F	redicted	Splicing
3:178936082	G/A	177	0.107345	?	?	PIK3CA	missense_variant	Missense			?		
4:66280142	G/A	243	0.127572	?	?	EPHA5	missense_variant	Missense			?		
5:131973850	C/T	95	0.568421	81	0.592593	RAD50	stop_gained	LoF			?		
5:137519659	T/C	246	0.150407	?	?	KIF20A	missense_variant	Missense	0 of 4 Predict	ed Splicing Disru	upted		
*GenomeBrows	e × +					70.000.001							
Plot 🖸 💭		3		▼ 3: 1	/8,936,072 - 1	78,936,091							
o sapiens (Humar	n), GRCh37 (he	19) (2 2009)	ř.		•	© -	r.					U	
			-		8					Console			1
3.178	36.073 3-179	036.075 3-	178 036 077	3: 178 036 070 3:	178 036 081	3-178.036.08	3 3 178 036 085 3 178 036 087	3-179.036.090 3-17	8 936 091	🔲 History		C	opy (
 I 1/0,3 	1 1	,000,070 0.	170,000,077	1 1	1, 3, 300,001	0. 170,000,000		1 1 1 1	0,000,001	Chr3: 178,936	6,082		
						7, 7, 7, 7 T				Matchos / M	iemato	hos / Do	lations
Current Normal	Read Alignme	ent						Somat	ic_cancer 💼 🦳	Matches / M	Ismatt	nes / De	letions
Coverage 듣 250년										Type	Base	Count %	of Me tal Qu
0 150										(match)	G	153 80	0 36
50 50		TC	TC	TC	TC	A	AATCA	СТ	GA	(match)		100 44	.0 30.
œ °° ¶									U , •	(mismatch)	A	19 11	.0 34.
Pile-up												172 10	0 36.
										Total			
100	-46		-	e				1	*	Total			
100	-ie			4. A.		1		<u> </u>	* 5	Total 5 alignments i	filtered o	out by qua	ality set
100 60 20				к		-		n n n	*	Total 5 alignments i	filtered o	out by qua	ality sett
100 60 20		9. 17	i i	6 8				- 7 1 1. - 1. - 1.		5 alignments i	filtered o	out by qua	ality sett
100 60 20 20 Current Sample	Read Alignm	ent	4 4	к. д.		4444		Somat	ic_cancer 💼	5 alignments i Chr3 betweer 178,936,083	filtered o	out by qua	ality sett d
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Current Sample	Read Alignm	ent C	T		т			Somat	c_cancer	Total 5 alignments I Chr3 betweer 178,936,083 Insertions Base(s)	filtered of n 178,93 Count	out by que 6,082 an % of	d Mean
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- 3. 変異テーブルの任意の変異データをクリックすると、ゲノムブラウザーの該当位置に自動的に移動する
- 4. 各サンプルのCoverageグラフの任意の位置をクリックすると、リード数の集計データなどが表示される

ゲノムブラウザーの表示





- 5. ゲノムブラウザーの「Plot」をクリック
- 6. Select Data Source画面において、データベースリストより「ClinVar」と「COSMIC Mutation」に チェックを入れ、「Plot & Close」をクリック

ゲノムブラウザーの表示





- 7. ゲノムブラウザーに、データベースのアノテーションがプロットされる
- 8. プロットされた任意のアノテーションをクリックすると、アノテーションの詳細が表示される


手順4. レポート作成

OMIMアノテーションの付加



Select Data Source		
Select tracks to use as annotation source	s against the imported variant set.	
 Select Data Source Select tracks to use as annotation source Locations Browse Url Warehouse Warehouse Local Local User Annotations Assessment Catalogs Public Annotations Secure Annotations Example Samples 	s against the imported variant set. User Annotations Filter: * (Any type) Homo sapiens (Human), GRCh37 (hg 19) (2 Name ICGC Simple Somatic Mutations 27, GHI Nextera Rapid Capture Exome Targeted Regions 2013-03-07, Illumina NHLBI ESP6500SI-V2-SSA137 Exomes Variant Frequencies 0.0.30, GHI OMIM Genes 2019-01-01, GHI OMIM Phenotype Ontology 2017-06-15 OMIM Phenotypes 2019-01-01, GHI OMIM Phenotypes 2019-01-01, GHI OMIM Variants 2019-01-01, GHI Filt, RefSeq Genes 105 Interim V1, NCBI SIFT and PolyPhen2 Missense Predictions, GHI TruSight Cancer Amplicon Design 2013-02-05, Illumina Immetide OMIM Variants 2019-01-01, GHI (Edit) Description	C Current Type Variant Interval Variant Interval Variant Interval Variant Interval MB) Clear
	OMIM is a comprehensive, authoritative compendium of human genes and genetic phenotypes that is freely available and updated daily. OMIM is authored and edited at McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, under the direction of Dr. Ada Hamosh. Its official home is omim.org.	the 🗸
Convert Download	Select Cancel	Help

- 選択データベースリスト
- OMIM Genes
- OMIM Phenotypes
- OMIM Variants

- 1. 再びプロジェクト画面の「Add」をクリックし、メニューより「Variant Annotation」を選択してクリック
- 2. Select Data Source画面において、上記3つのOMIMデータベース名にすべてチェックを入れ、「Select」を クリック



/	Variants: 5	× +							
V	ariants	• 🗗 💿	옷 다 🗔	Filter	Variants: T99000	5 ▼ m î			
						C	MIM Genes 2019-01-01, GHI		
	Gene Name	Cytogenetic Location	OMIM ID	Entrez Gene ID	PubMed ID	HasPubMedID	Title	Alternative Title(s)	Description
	PIK3CA	3q26.3	171834	5290	16432179,…	True	PHOSPHATIDYLINOSITOL 3-···	PHOSPHATIDYLINOSITOL 3-KI····	?
	EPHA5	4q13	600004	2044	7898931,1…	True	EPHRIN RECEPTOR EphA5; E····	HEK7, EPH HOMOLOGY KINASE ···	Receptor protein tyro…
	POU4F2	4q31.2	113725	5458	8995448,1…	True	POU DOMAIN, CLASS 4, TRA···	POU-DOMAIN TRANSCRIPTION ···	POU4F2 is a member \cdots
	RAD50	5q31	604040	10111	19487811,…	True	RAD50, S. CEREVISIAE, HOM…	?	?
	KIF20A	5q31	605664	10112	10233894,…	True	KINESIN FAMILY MEMBER 20	RAB6-INTERACTING PROTEIN ···	Kinesin-like proteins, …

3. 変異データテーブルに、選択した3種類のOMIMデータベースのアノテーション列が追加される

変異セットの作成



		/ 🔲 Varia	nts:4 🗙 🕂										
		Variants 👻	• •	오 🗆	🗔 🗆	🔽 F	lter Variant	s: T990005 T 					
		Va	riant Info		Create Va	ariant Se	t No	rmal (N99000	(5)				
		2	hr:Pos Ref/	Alt Read D	epths (DP)		AF Read D	epths (DP)	AF				
		3:1789	36082 G	/A	177	0.1073	45	?	?				
											2		
	🗸 Create	e Variant Set				VC	reate varia	int Set			_		
	Name:	Primary Findir	ıg			Nam	ie: Inciden	tal Finding					
	Initials:	PF				Initia	als: IF						
	Color: (#FF696B			Colo	ir: 🔁	#387	4E5				
	v Samp	le Specific	ОК	Canc	el		Sample Spec	oific OI	K [Cancel			
∕ IIII Variants: 5 🗙 Variants	+				Filter Varian	- nts: T9900	05-						
Variant In	fo		umor (T00)	005)	Norm	al (NOO	0005)		Rof		105 Interir	m v1 NCBI	
Ĉhr:Pos F	Ref/Alt P	F IF Read	Depths (DP)	AF	Read Dept	hs (DP)	AF	Gene Names	Sequence	ce Ontology	(Combined)	Effect (Combined)
3:178936082	G/A		177	0.107345		?	?	PIK3CA		misse	ense_variant	Missens	e
4:66280142	G/A		243	0.127572		?	?	EPHA5		misse	ense_variant	Missens	a
4:147560457	T/C		63	0.126984		?	?	POU4F2		synonym	nous_variant	Othe	r
5:131973850	C/T		95	0.568421		81	0.592593	RAD50			stop_gained	Lol	F
5:137519659	T/C		246	0.150407		?	?	KIF20A		misse	ense_variant	Missense	a

- 変異テーブル上部の「Manage Variant sets」をクリックし、メニューより「Create Variant Set」を選択してクリック 1.
- Primary FindingとIncidental Findingの2セットを作成すると、変異テーブルにフラグが表示される 2.



/ 🔲 Variants: 5 🔉	<u>ر</u> +								
Variants				0, 🗔			Filter Variants: T990)05 ~ 	
Variant I	nfo	Flag…	Turr	nor (T990	0005)		Normal (N99	0005)	
Ĉhr:Pos	Ref/Alt	PF IF	Read Dep	oths (DP)		AF	Read Depths (DP)	AF	Gene Names
3:178936082	G/A			177	0.107	345	?	?	PIK3CA
4:66280142	G/A			243	0.127	572	?	?	EPHA5
4:147560457	T/C			63	0.126	984	?	?	POU4F2
5:131973850	C/T			95	0.568	421	81	0.592593	RAD50
5:137519659	T/C			246	0.150	407	?	?	KIF20A
Variants: 5 🗴	د\ +	Assessn	nent Catal	og I			Filter Variants: T990	105 -	
variants		Filtor		- J					
Variant Ir	nfc T	Filter	_	1	0005)		Normal (N99	0005)	
Ćhr:Pos	Re 🙆	Genome	Browse			AF	Read Depths (DP)	AF	Gene Names
3:178936082	85	Log			0.107	345	?	?	PIK3CA
4:66280142		Note			0.127	572	?	?	EPHA5
4:147560457		Report			0.126	984	?	?	POU4F2
5:131973850		Table	-		0.568	421	81	0.592593	RAD50
5:137519659		TODIE .			0.150	407	?	?	KIF20A
	0	web Bro	owser						
	$\mathbf{\vee}$	VS Clini	cal						

- 1. 変異テーブル上で各フラグをクリックし、任意の変異をPrimary FindingとIncidental Findingに指定 (この例ではPIK3CA遺伝子の変異をPF、RAD50遺伝子の変異をIFに指定)
- 2. 「Open a new tab」をクリックし、メニューより「Report」を選択してクリック





- 3. 「Create a New Report Template」をクリック
- 4. レポートテンプレートの選択で、「Based On:」に「Cancer Gene Panel Template」を選択し、 「Name:」に任意の名前(この例ではCancer Gene Panel)を入力し、「OK」をクリック



ancer Gene Pane	+ O	
		🕸 Configure Report Template
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comment:	Mutations with an establish somatic link detected.	C
nterpretation Sur	mmary:	
		C
lecommendation	s:	
		C

- 5. レポートの情報入力画面で、必要に応じてサンプル情報やコメントなどを入力
- 6. 最上段右側の「Configure and reload this report template」をクリックし、メニューより「Configure Report Template」を選択してクリック



et Report Parame	ters	
- Lab Information	ма кыл х	_
Name:	Insert Name>	_
Address:	Insert Address>	
City:	Insert City>	
State:	Insert State>	
Zip Code:	Jnsert Zip>	
Phone Number:	Insert Phone>	
Fax Number:	Insert Fax>	
Logo File: L	sing previously saved image 🔛 Select Image	
Test Information -		
Test	(Incert Tevt)	_
Indication	Zhoort Touth	_
Background:	B 1 <u>0</u> Ø	_
Method:	B I <u>U</u> Ø	
Limitations:		
Emiliarions.	D 1 <u>D</u> (*	_
Reportable Gene:	APC, CASP10, CDH1, CHEK2, ERBB2, FGFR2, IRF1, KLF6, KRAS, MSH3, MUTYH, PIK3CA	
		i
		Cancel

7. Somatic_cancerフォルダ内の「Gene_list.txt」の遺伝子名リストをコピーし、Set Report Parametersの 「Reportable Genes」にペーストして「OK」をクリック



ancer Gene Pane	el 🔹 + 📀	
rimary Findings		
Primary Find	ing	•
Variant:	3:178936082 G/A (<i>PIK3CA</i>)	<u>(</u>)
Classification:	Pathogenic	• (i)
Interpretation:	BIUØ	
	This is a Missense Variant located in the PIK3CA gene.	^ (i)
	CLOVE Syndrome	
	In a 11-wax-ald airl and an unrelated 1-wax-ald how with CLOVE sundrame (619019). Kurak at al. (9019) identified compation	Ŧ
cidental Finding	2	
cidental Finding	s ndine	•
cidental Finding	is nding	T
cidental Finding Incidental Fir Variant:	is Inding 5:131973850 C/T (<i>RAD50</i>)	•
cidental Finding Incidental Fir Variant: Interpretation:	s nding 5:131973850 C/T (<i>RAD50</i>) B <i>I</i> U Ø	•
cidental Finding Incidental Fir Variant: Interpretation:	s nding 5:131973850 C/T (<i>RADs0</i>) B I U @ [This is a Stop Gained located in the RAD50 gene.	• •
cidental Finding Incidental Fir Variant: Interpretation:	s nding 5:131973850 C/T (<i>RAD50</i>) B <i>I</i> <u>U</u> <i>©</i> [This is a Stop Gained located in the RAD50 gene. This gene has been observed to exhibit ? inheritance pattern.	• • •
cidental Finding Incidental Fir Variant: Interpretation:	s nding 5:131973850 C/T (<i>RAD50</i>) B <i>I</i> <u>U</u> <i>©</i> [This is a Stop Gained located in the RAD50 gene. This gene has been observed to exhibit ? inheritance pattern. This gene has been observed to exhibit ? inheritance pattern.	• • •
cidental Finding Incidental Fir Variant: Interpretation: eport Signoff	s nding 5:131973850 C/T (<i>RAD50</i>) B <i>I</i> <u>U</u> <i>©</i> [This is a Stop Gained located in the RAD50 gene. This gene has been observed to exhibit ? inheritance pattern. It has been observed to exhibit ? inheritance pattern.	• • •
cidental Finding Incidental Fir Variant: Interpretation: eport Signoff /erify: 🔲 Repol	s nding 5:131973850 C/T (<i>RAD50</i>) B <i>I</i> <u>U</u> ∅ [This is a Stop Gained located in the RAD50 gene. This gene has been observed to exhibit ? inheritance pattern. It has been accessiated with Niimegen breakage aundkomea-like disorder It has not been signed off.	• • • •

- 8. Reportタブに戻り、「Primary Findings」と「Incidental Findings」のそれぞれの「Select a Variant set」に、 フラグ付けした変異セットを選択
- 9. 最上段の「Create the Report」をクリック





Filge	, Pr Fa	none: ax:		Provider Infor Physician Institution Case Id	mation	
Patient Inf Name Gender Date of Birth Id	ormation T99000 Male 1/20/20 1234)5)19	Sample Information Sample Site Sample Type Collection Method Panel Coverage	n G F	Avg. Read Depth Collection Date Receipt Date Report Date	1/20/2019 1/20/2019 1/20/2019
Results						
Affected G	enes CASP10 (0) PIK3CA (1) ndings Zygosity	establish somatic link detected.	RBB2 (0) FGFR2 (0)	IRF1 (0)	LF6 (0) (0) Exon	MSH3 (0)
Gene	Zygosity	Variant	000000 0:0 0005 401		Exon I	Pathogenicity
PIK3CA	f	NNI_006218.3.C.1624G>A(NP_)	มมช่วมษ.2:p.Gluอ42LyS)		10 1	Patriogenic

Interpretation Summary

Recommendations

10. フラグ付けした変異セットのOMIMアノテーションの情報をまとめたレポートが作成される

単一遺伝子病解析ワークフロー



手順1: サンプルデータのインポート

- 解析プロジェクトを作成
- 患者、父親、母親サンプルのVCFファイルをインポート

手順2: アノテーション付加

● 変異データに対して、様々なデータベースを用いたアノテーション付加の実行

手順3: フィルタリング

- 遺伝形式に基づいた変異の抽出
- 表現型関連変異のスコアリング



手順1. サンプルデータのインポート

プロジェクトの作成



- 0 X

	Project Template	
	Folder: Project Templates	Reset Browse
😵 Golden Helix VarSeq 2.1.0	* Q E	Empty Project
<u>File Iools Help</u>	ACMG Guidelines Gene Panel Template ACMG Guidelines Trio Template Cancer Gene Panel Starter Template Cancer Gene Panel Tutorial Exome Trio Template Hereditary Gene Panel Starter Template	Start from a blank slate
VOrseq	TruSight Cancer Panel Starter Template TruSight Cancer Panel Japanese Sample Tumor-Normal Template Empty Project	
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Open Existing Project	Genome Assembly Homo sapiens (Human), GRCh37 (hg19) (2 2009)	▼
New Project 20190116	Project	
Exome Trio Analysis	Name: Exome Trio	
	Folder: C:¥Users¥Ozawa¥GH_Data¥Exome Trio	Browse
		<u>O</u> K <u>C</u> ancel

New Project

- 1. メイン画面の「Create New Project」をクリック
- 2. 任意のプロジェクト名を入力し、またProject Templateに「Empty Project」、Genome Assemblyが 「Homo sapiens (Human), GRCh37 (hg19) (2 2009)」となっていることを確認したら「OK」をクリック





- 3. 次の画面で、「Import Variants」をクリック
- 4. Import Variant Sources画面で「Add Files」をクリックし、Exome_trioフォルダ内の「F12891.vcf.gz」「M12892.vcf.gz」「P12878.vcf.gz」を選択
- 5. Import Variant Sources画面に両ファイルが表示されたら、「Next」をクリック





① Define Input		idd sample fields: Fi	rom Text File Ass	ociate BAM File			
② Scan Input		Original Samples	🔽 le Source File I	Samples	Mother	Father	Affected
③ Change Options	1	F12891	F12891	F12891	-	•	Unaffecte
(4) Review	2	M12892	M12892	M12892	- -		Unaffecte
Select the samples of interest and appropriately	3	P12878	P12878	P12878	M12892 🗸	F12891 -	Affected

- 6. Sample Relationshipsで、「Family Samples」を選択し、Nextをクリック
- 7. サンプル情報の入力画面で、「P12878」のMotherフィールドに「M12892」、Fatherフィールドに「F12891」、 Tumorフィールドに「Affected」を選択してNextをクリック



V Import Variants Wizard	d	
Import Variant	Sources	
 Define Input Scan Input Change Options Review The new source file will be saved to the project data folder. (Advanced) You may also choose to left align, or split variants into allelic primitives.	Summary: • Total size: 463K , 3 Files • 16 fields, 3 related samples(1 affected). • Assembly GRCh_37_g1k,Chromosome,Homo sapiens Assembly of Input Files: Homo sapiens (Human), GRCh87 (hg19) (2 2009) Matches project, no liftover performed. Specify Genomic Regions to Import © Import Regions Defined by Annotation File Select an Annotation Source Select Track 20 ÷ +/- BP © Exons Only © Full Transcript Select filters to reduce the number of variants imported. If no filters are selected then all of the variants will be imported. PASS Select All Clear Selection	
Advanced Options		K Back Einished Qancel

8. Assembly of Input Filesに、「Homo sapiens (Human), GRCh37 (hg19) (2 2009)」と表示されていることを確認し、「Finished」をクリック



📎 *Exome Trio - Golden Helix Vars	Seq 2.1.0								
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🔚 🕋 🗔 Add 🕻 🛃 Export	[<u>/_</u> Plot_ (👽 Connect 🛛 🔌	Proband (P12878	◎ • ▶ 🛱 ?					Samples: 3 Variants: 13,086
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Variants 💌		Variants 👻 🛄		, 🗔 🔲 📝 Filter	Variants: P12878 ▼)			Variants: 13,086
Filter Variants	A 13,086	Varian	t Info	Proband (P	12878)	Mother (M	12892)	Father (F1	2891)
		Chr:Pos	Ref/Alt	Variant Allele Freq	Read Depths (DP)	Variant Allele Freq	Read Depths (DP)	Variant Allele Freq	Read Depths (DP)
		6:266473	C/T	0.75	12	?	?	?	?
		6:267575	C/G	0.833333	12	?	?	?	?
		6:284016	-/A	0.833333	12	?	?	?	?
		6:286281	G/A	0.307692	13	?	?	?	?
		6:286288	G/T	0.363636	12	?	?	?	?
		6:287725	A/G	0.6	10	?	?	?	?
		6:288336	A/G	0.818182	11	?	?	?	?
		6:292695	C/-	?	?	0.666667	12	?	?
		6:294361	-/A	1	11	?	?	?	?
		6:301859	C/A	1	11	?	?	?	?
		6:302368	A/G	1	10	?	?	?	?
		6:302913	C/T	0.166667	18	?	?	?	?
		6:302973	C/G	0.230769	13	?	?	?	?
		6:304890	I/A	0.411/65	1/	0.545455	55	?	?
		6:304900	TA/-	1	14	0.948276	58	?	?
		6:305095	C/1	?	?	0.35/143	14	0.6	10
		6:308331	G/A	0.333333	12	?	?	?	?
		6:309718	Aſ/-	0.916667	12	?	?	?	?
		6:311548	C/T	?	?	0.272727	11	?	?
	?	6:311680	-/A	?	?	0.886792	58	2	2 -

9. Proband (P12878)、Mother (M12892)、Father (F12891) の変異データがインポート され、プロジェクト画面に表示される



手順2. アノテーション付加



♥ *Somatic Cancer - Golden Helix VarSeq 2.1.0									
File View Tools Help									
		🕞 Add 🛃 🛃 Export 🛛 🗶 Plot 🗸 🚫 Connect							
/ 🝸	Filter		Variant Annotation						
Varian	its 🔻		Secondary Tables	Varia					
🔽 🖬	Filter	Σ.	Computed Data	11,076					

選択データベースリスト

- RefSeq Genes
- 1kG Phase3 Variant Frequencies 5a
- ClinVar
- ClinVar Assessments
- dbNSFP Functional Predictions 3.0
- HGVD1210-V2_30

Select Data Source		
Select tracks to use as annotation source	es against the imported variant set.	
 Select Data Source Select tracks to use as annotation source Locations Browse Url Warehouse Warehouse Local Local Secure Annotations Secure Annotations Example Samples 	es against the imported variant set. User Annotations Filter: Name Name Name Name ONIM Genes Rapid Capture Exome Targeted Regions 2013-03-07, Illu NHLBI ESP6500SI-V2-SSA137 Exomes Variant Frequencies 0.0.3 OMIM Genes 2019-01-01, GHI OMIM Genes with Details 2017-01-01, GHI OMIM Phenotype Ontology 2017-06-15 OMIM Phenotypes 2019-01-01, GHI OMIM Phenotypes with Details 2017-01-01, GHI OMIM Phenotypes with Details 2017-01-01, GHI MIM Phenotypes with Details 2017-01-01, GHI MIM Phenotypes 105 Interim v1, NCBI MIM SIFT and PolyPhen2 Missense Predictions, GHI TruSight Cancer Amplicon Design 2013-02-05, Illumina	Current Type Type Variant Interval Interval Interval Interval Variant Gene Variant Interval
	Information showing (29/58), 1 selected (RefSeq Genes 105 Interim v1, NCBI (Edit) Description This track contains RefSeq Gene transcripts annotated by the NCBI Homo say Annotation Release 105 Interim v1.	6.0 MB) Clear
Convert Download	Select Cancel	Help

- 1. プロジェクト画面の「Add」をクリックし、メニューより「Variant Annotation」を選択してクリック
- 2. Select Data Source画面において、上記データベース名にすべてチェックを入れ、「Select」をクリック



アノテーションの付加

RefSeq Genes 105 Interim v1. NCBI									
Gene Names	Sequence Ontology (Combined)	Effect (Combined)	Nof4PredictedSplicingDisruptedCombined	Predicted Splicing Disrupted (Combined)	Transcript Name (Clinically Relevant)	HGVS c. (Clinically Relevant)			
RAI14	missense_variant	Missense	?	?	NM_001145525.1	NM_001145525.1:c.497G>T			
?	intergenic_variant	Other	?	?	?	?			
TTC23L	missense_variant	Missense	0 of 4 Predicted Splicing Disrupted	?	NM_001317949.1	NM_001317949.1:c.92A>G			
BRIX1	splice_region_variant	Other	0 of 4 Predicted Splicing Disrupted	?	NM_018321.3	NM_018321.3:c.793-3T>A			
BRIX1	splice_acceptor_variant	LoF	3 of 4 Predicted Splicing Disrupted	GeneSplicer,MaxEntScan,NNSplice	NM_018321.3	NM_018321.3:c.793-2dupA			
AGXT2	splice_donor_variant	LoF	2 of 4 Predicted Splicing Disrupted	MaxEntScan,PWM	NM_031900.3	NM_031900.3:c.*46G>T			
AGXT2	synonymous_variant	Other	?	?	NM_031900.3	NM_031900.3:c.1305T>C			
AGXT2	missense_variant	Missense	?	?	NM_031900.3	NM_031900.3:c.635C>T			
AGXT2	missense_variant	Missense	?	?	NM_031900.3	NM_031900.3:c.418G>A			
AGXT2	missense_variant	Missense	?	?	NM_031900.3	NM_031900.3:c.305G>A			
SPEF2	missense_variant	Missense	?	?	NM_024867.3	NM_024867.3:c.211A>C			
SPEF2	synonymous_variant	Other	0 of 4 Predicted Splicing Disrupted	?	NM_024867.3	NM_024867.3:c.579T>C			
SPEF2	synonymous_variant	Other	?	?	NM_024867.3	NM_024867.3:c.861C>T			
SPEF2	missense_variant	Missense	?	?	NM_024867.3	NM_024867.3:c.1498G>A			
SPEF2	synonymous_variant	Other	0 of 4 Predicted Splicing Disrupted	?	NM_024867.3	NM_024867.3:c.2142T>C			
SPEF2	missense_variant	Missense	?	?	NM_024867.3	NM_024867.3:c.2711C>T			
SPEF2	missense_variant	Missense	?	?	NM_024867.3	NM_024867.3:c.2800G>C			
SPEF2	intron_variant	Other	?	?	NM_024867.3	NM_024867.3:c.2839+630C>T			
SPEF2	intron_variant	Other	?	?	NM_024867.3	NM_024867.3:c.2839+815C>T			
SPEF2	intron_variant	Other	?	?	NM_024867.3	NM_024867.3:c.2839+1107G····			
SPEF2	intron_variant	Other	?	?	NM_024867.3	NM_024867.3:c.2839+1163A			
SPEF2	intron_variant	Other	?	?	NM_024867.3	NM_024867.3:c.2839+1505C····			
SPEF2	intron_variant	Other	0 of 4 Predicted Splicing Disrupted	?	NM_024867.3	NM_024867.3:c.2914+19T>G			
SPEF2	intron_variant	Other	0 of 4 Predicted Splicing Disrupted	?	NM_024867.3	NM_024867.3:c.3331-11T>C			
CAPSL	missense_variant	Missense	?	?	NM_144647.3	NM_144647.3:c.254G>A			
CAPSL	intron_variant	Other	0 of 4 Predicted Splicing Disrupted	?	NM_144647.3	NM_144647.3:c.137+17A>G			
UGT3A1	3_prime_UTR_variant	Other	?	?	NM_152404.3	NM_152404.3:c.*607T>C			
UGT3A1	splice_region_variant	Other	0 of 4 Predicted Splicing Disrupted	?	NM_152404.3	NM_152404.3:c.1296-8G>A			

3. アノテーション付加が完了すると、変異データテーブルに各データベースのアノテーション列が追加される



手順3. フィルタリング

基本ワークフローの作成



🗹 🖆 Filter Variants	🔦 13,086
Read Depths (DP) (Current) >= 30	- به
30	kth
	+
Less than 30	3,114
Equal to 30	79
Greater than 30	3,881
Missing	6,012
	3,960
Variant Allele Freq (Current) >= 0.2	× -
0.2	http
	+
Less than 0.2	141
Equal to 0.2	0
Greater than 0.2	3,814
Missing	27
	3,814

- Proband (P12878) \mathcal{O} [Read Depths (DP)] \geq 30
- Proband (P12878) $\mathcal{O}[Variant Allele Freq] \ge 0.2$

1. Proband (P12878サンプル)の「Read Depths (DP)」と「Variant Allele Freq」の2フィールドのコンテナを つくり、上記のとおり検索条件を指定する 基本ワークフローの作成



🔽 🖆 Filter Variants	3	13,086
Read Depths (DP) (Current) >= 30	٩	
		3,960
Variant Allele Freq (Current) >= 0.2	٩	
		3,814
Allele Frequencies < 0.01 OR missing	٩	
0.01		idu
		+
Less than 0.01		76
Equal to 0.01		0
Greater than 0.01		3,249
Missing		490
		566
☑ Alt_allele_freq < 0.01 OR missing	3	. –
0.01		idu
		+
Less than 0.01		24
Equal to 0.01		0
Greater than 0.01		97
Missing		445
		469

- 1kG Phase3 Variant Frequencies 5aの 「Allele Frequencies」 < 0.01 or Missing
- HGVD1210-V2_30の「Alt_allele_freq」< 0.01 or Missing

2. 人種特異的なSNPの除去のため、1kG Phase3 - Variant Frequencies 5aの「Allele Frequencies」、 さらにHGVD1210-V2_30の「Alt_allele_freq」の2フィールドのコンテナをつくり、上記のとおり検索条件を指 定する



🗹 🖬 Filter Variants	٩	1	3,086
Read Depths (DP) (Current) >= 30	J	6	
		3,	960
Variant Allele Freq (Current) >= 0.2	3	6	
		3,	814
Allele Frequencies < 0.01 OR missing	J	6	
			566
☑ Alt_allele_freq < 0.01 OR missing	J	6	
			469
Effect (Combined) is (LoF, Missense)	J	6	-
LoF			8
Missense			126
Other			335
Missing			0
			134

• RefSeq Geneの「Effect (Combined)」 is LoF, Missense

3. 生体に有害な変異の抽出のため、RefSeq Geneの「Effect (Combined)」のフィールドのコンテナをつくり、 上記のとおり検索条件を指定する

解析アルゴリズムの実行



父 *Exome Trio - Golden Helix VarSeq 2.1.0									
<u>File View Tools H</u> elp									
		Add 🛃 🛃 Export 🛔 📈 Plot	. 🔍 o	onnect					
Filter	□,	Variant Annotation							
Variants 👻		Secondary Tables		Variant					
🔽 🖬 Filter	$\Sigma_{\mathbf{r}}$	Computed Data	13,086						
			-						
		_							
		•							



- 1. プロジェクト画面の「Add」をクリックし、メニューより「Computed Data」を選択してクリック
- 2. Select an Algorithm画面において、任意の解析アルゴリズムを選択して、「OK」をクリック
- 3. 解析アルゴリズムの種類によっては、計算時のパラメータなどを指定する
- 4. 多くの解析アルゴリズムでは、計算が終了すると、変異テーブルに計算結果のアノテーションが付加され、 フィルタリングに使用できるようになる





Variant Info		Pro	band (P12878)		Mother (M12892)			Father (F12891)		
Chr:Pos	Ref/Alt	Variant Allele Freq	Read Depths (DP)	Zygosity	Variant Allele Freq	Read Depths (DP)	Zygosity	Variant Allele Freq	Read Depths (DP)	Zygosity
6:564567	C/G	0.393443	122	Heterozygous	?	?	?	0.496552	145	Heterozygous
6:7405508	G/A	0.457143	70	Heterozygous	0.514925	134	Heterozygous	?	?	?
6:8428249	A/C	0.505155	194	Heterozygous	?	?	?	?	?	?
6:10626704	T/G	0.409836	122	Heterozygous	0.46	150	Heterozygous	?	?	?
6:10709632	G/A	0.552941	85	Heterozygous	?	?	?	0.514563	103	Heterozygous
6:12124988	T/C	0.433862	189	Heterozygous	?	?	?	?	?	?
6:12290906	G/T	0.336538	104	Heterozygous	0.369295	241	Heterozygous	0.38806	67	Heterozygous
6:15501276	C/G	0.417722	79	Heterozygous	?	?	?	?	?	?
6:17794528	A/C	0.381818	112	Heterozygous	?	?	?	?	?	?
6:18208415	G/A	0.416667	110	Heterozygous	?	?	?	0.481481	135	Heterozygous
6:24850081	A/C	0.603239	250	Heterozygous	?	?	?	0.58871	250	Heterozygous
6:26056427	T/G	0.462963	162	Heterozygous	?	?	?	0.443787	169	Heterozygous
6:26108282	C/A	0.245902	62	Heterozygous	?	80	Heterozygous	?	?	?
6:26234929	T/G	0.42029	138	Heterozygous	0.513043	115	Heterozygous	0.470588	153	Heterozygous
6:26506950	T/G	0.389558	249	Heterozygous	0.512295	245	Heterozygous	0.436214	243	Heterozygous
6:27420048	G/C	0.44697	132	Heterozygous	0.446541	160	Heterozygous	?	?	?

- 1. Select an Algorithm画面より「Genotype Zygosity」を選択して「OK」をクリック
- 2. 変異テーブルに、各サンプルの各変異ごとに接合体情報のフィールドが追加される

メンデル遺伝情報の付加







Variant Info			Proband (P12	878)		Mother (M12892)				Father (F12891)			
Chr:Pos	Ref/Alt	Variant Allele Freq	Read Depths (DP)	Zygosity	Mendel Error	Variant Allele Freq	Read Depths (DP)	Zygosity	Mendel Error	Variant Allele Freq	Read Depths (DP)	Zygosity	Mendel Error
6:564567	C/G	0.393443	122	Heterozygous	Transmitted	?	?	?	?	0.496552	145	Heterozygous	?
6:7405508	G/A	0.457143	70	Heterozygous	Transmitted	0.514925	134	Heterozygous	?	?	?	?	?
6:8428249	A/C	0.505155	194	Heterozygous	de Novo Allele	?	?	?	?	?	?	?	?
6:10626704	T/G	0.409836	122	Heterozygous	Transmitted	0.46	150	Heterozygous	?	?	?	?	?
6:10709632	G/A	0.552941	85	Heterozygous	Transmitted	?	?	?	?	0.514563	103	Heterozygous	?
6:12124988	T/C	0.433862	189	Heterozygous	de Novo Allele	?	?	?	?	?	?	?	?
6:12290906	G/T	0.336538	104	Heterozygous	Transmitted	0.369295	241	Heterozygous	?	0.38806	67	Heterozygous	?
6:15501276	C/G	0.417722	79	Heterozygous	de Novo Allele	?	?	?	?	?	?	?	?
6:17794528	A/C	0.381818	112	Heterozygous	de Novo Allele	?	?	?	?	?	?	?	?
6:18208415	G/A	0.416667	110	Heterozygous	Transmitted	?	?	?	?	0.481481	135	Heterozygous	?
6:24850081	A/C	0.603239	250	Heterozygous	Transmitted	?	?	?	?	0.58871	250	Heterozygous	?
6:26056427	T/G	0.462963	162	Heterozygous	Transmitted	?	?	?	?	0.443787	169	Heterozygous	?
6:26108282	C/A	0.245902	62	Heterozygous	Transmitted	?	80	Heterozygous	?	?	?	?	?
6:26234929	T/G	0.42029	138	Heterozygous	Transmitted	0.513043	115	Heterozygous	?	0.470588	153	Heterozygous	?
6:26506950	T/G	0.389558	249	Heterozygous	Transmitted	0.512295	245	Heterozygous	?	0.436214	243	Heterozygous	?
6:27420048	G/C	0.44697	132	Heterozygous	Transmitted	0.446541	160	Heterozygous	?	?	?	?	?

- 1. Select an Algorithm画面より「Mendel Error」を選択して「OK」をクリック
- 2. 変異テーブルのProbandサンプルにおいて、各変異ごとのメンデル遺伝情報のフィールドが追加される

複合ヘテロ接合体の検出





- 1. Select an Algorithm画面より「Mendel Error」を選択して「OK」をクリック
- 2. Select a Source and Field画面において、変異テーブル上の遺伝子名のフィールド(この例では RefSeq GenesのGene Namesフィールド)を選択して、「OK」をクリック

複合ヘテロ接合体の検出



burces	Fields				
Exome Trio	Filter *		Only Allowed Types	-	
ClinVar 2019-01-01, NCBI	Name	Туре	Doc		
dbNSFP Functional Prediction Voting	Ref/Alt	String	Reference and Alternate all… _■		
dbNSFP Functional Predictions 3.0, GHI	Identifier	String Array	Known identifier (often dbS…		VarSeq 2
1kG Phase3 - Variant Frequencies 5a with	Flags	Categorical Array	All boolean flags defined in t…		Advanced Parameters
HGVD1210-V2_30	Read Depth (DP)	Int	Total read depth; only low c···		Allow de Novo het mutations to be considered
RefSeq Genes 105 Interim v1, NCBI	Allele Counts	Int Array	Counts of each alternate all…		Allow duos (one missing parent)
Aux Fields RefSeq Genes 105 Interim v1,	Allele Frequencies	Float Array	The Allele Counts divided b…		
Genotype Zygosity	# Alleles	Int	Total number of observed a…		
Mendel Error	# Het	Int	Count of the number of het		
G Phase3 - Variant Frequencies 5a wit atching variants of 1kG Phase3 - Variant Frequenci	h Genotype Counts	, GHI nts, GHI			

- 2つ目のSelect a Source and Field画面において、変異テーブル上のアレル頻度データのフィールド (この例では1kG Phase3のAllele Frequenciesフィールド)を選択して「OK」または「Skip」をクリック *本トレーニングでは「Skip」をクリック
- 4. Advanced Parametersで、de Novoのヘテロ接合性変異を含めるか、また片親だけのサンプルデータしか存在しない場合も計算を行うかどうかを指定し、「OK」をクリック

複合ヘテロ接合体の検出



変異テーブル

Gene	Compound Het	Variants for…	Compound Het Genes for Proband (P12878)					
Gene Names	Compound Het?	Inherited From	Has Compound Het?	Inherited from Father	Inherited from Mother	Inherited Total	Hets In Both Parents	
EXOC2	False	NA	False	1	0	1	0	
RIOK1	False	NA	False	0	1	1	0	
SLC35B3	False	NA	False	0	0	0	0	
GCNT2	False	NA	False	0	1	1	0	
PAK1IP1	False	NA	False	1	0	1	0	
HIVEP1	False	NA	False	0	0	0	0	
EDN1	False	NA	False	0	0	0	1	
JARID2	False	NA	False	0	0	0	0	
KIF13A	False	NA	False	0	0	0	0	
KDM1B	False	NA	False	1	0	1	0	
FAM65B	False	NA	False	1	0	1	0	
HIST1H1C	False	NA	False	1	0	1	0	
HIST1H1T	False	NA	False	0	1	1	0	
HIST1H1D	False	NA	False	0	0	0	1	

ワークフロー

🔽 📭 Filter Variants	🔦 13,086
Read Depths (DP) (Current) >= 30	∢ □
	3,960
Variant Allele Freq (Current) >= 0.2	₹ □
	3,814
☑ Allele Frequencies < 0.01 OR missing	۹ ۵
	566
☑ Alt_allele_freq < 0.01 OR missing	۹ 🗆
	469
Effect (Combined) is (LoF, Missense)	₹ □
	134
Compound Het? (Current)	r –
True	3
False	131
Missing	0
	134

Variant Gene Info		Compound Het	Genes for Proband	(P12878)	
Gene Names	Has Compound Het?	Inherited from Father	Inherited from Mother	Inherited Total	Hets In Both Parents
AARS2	False	0	0	0	0
ABCC10	False	0	0	0	0
ABCF1	False	0	0	0	0
ABHD16A	False	0	0	0	0
ABRACL	False	0	0	0	0
ABT1	False	0	0	0	0
ACAT2	False	1	0	1	0
ACOT13	False	0	0	0	0
ADAT2	False	0	0	0	0
ADGB	False	0	0	0	0
ADGRB3	False	1	0	1	0
ADGRF1	False	0	0	0	0
ADGRF2	False	1	0	1	0
	Variant Gene Info Gene Names AARS2 ABCC10 ABCC10 ABCF1 ABHD16A ABRACL ABT1 ACAT2 ACAT2 ACOT13 ADAT2 ADGB ADGRB3 ADGRF1 ADGRF2	Variant Gene InfoGene NamesHas Compound Het?AARS2FalseABCC10FalseABCF1FalseABHD16AFalseABRACLFalseABRACLFalseABRACLFalseABRACLFalseABRACLFalseABRACLFalseACAT2FalseACOT13FalseADAT2FalseADGRBFalseADGRF1False	Variant Gene InfoCompound HettGene NamesHas Compound Het?Inherited from FatherAARS2False0ABCC10False0ABCF1False0ABHD16AFalse0ABRACLFalse0ABRAC1False0ABRAC2False0ABRAC3False0ABRAC4False0ACAT2False0ADAT2False0ADGRB3False0ADGRF1False0ADGRF2False0	Variant Gene InfoCompound Het Compound Het Genes for Proband of Inherited from FatherInherited from MotherAARS2FalseInherited from FatherInherited from MotherABRC10False00ABCF1False00ABHD16AFalse00ABRACLFalse00ABRACLFalse00ABRACLFalse00ABRACLFalse00ABRACLFalse00ABRACLFalse00ACAT2False00ACAT3False00ADGRBFalse00ADGRB1False00ADGRF2False00ADGRF2False00	Variant Gene InfoCompound Het Genes for Proband (P12878)Gene NamesHas Compound Het?Inherited from FatherInherited from MotherInherited TotalAARS2False000ABCC10False000ABCF1False000ABHD16AFalse000ABRACLFalse000ABRAC1False000ABRAC2False000ABT1False000ACAT2False000ADAT2False000ADGBFalse000ADGR51False000ADGR52False000

5. ワークフローに自動的にCompound Het?のフィルターコンテナが作成される

6. 同時に変異テーブルへアノテーション付けされ、別タブで情報が付加された遺伝子テーブルも作成される

トリオ解析ワークフローの作成



🔽 🖬 Filter Variants	A 13,086
Read Depths (DP) (Current) >= 30	∢ □
	3,960
Variant Allele Freq (Current) >= 0.2	۹ ۵
	3,814
☑ Allele Frequencies < 0.01 OR missing	۹ ۵
	566
☑ Alt_allele_freq < 0.01 OR missing	× 🗆
	469
Effect (Combined) is (LoF, Missense)	× 🗆
	134
Compound Het? (Current) is true	* -
True	3
False	131
Missing	0
	8
Enabled	
Inverted	
AND AND	
OR	
Lock	

Add Filter

Add Filter Container

🗹 🖬 Filter Variants	з,	13,086					
Read Depths (DP) (Current) >= 30	٩	. 🗆					
		3,960					
Variant Allele Freq (Current) >= 0.2	٩	. 🗆					
		3,814					
Allele Frequencies < 0.01 OR missing	٩	. 🗆					
		566					
☑ Alt_allele_freq < 0.01 OR missing	٩	. 🗆					
		469					
Effect (Combined) is (LoF, Missense)	٩						
		134					
Compound Het? (Current) is true	٩)					
True		3					
False		131					
Missing		0					
		3					
Trio Analysis	1						
AND Results satisfy criteria of all contained filters							
OR Results satisfy criteria of any contained	l filte	r					
		2					

🗹 🖬 Filter Variants	3	1	3,086
Read Depths (DP) (Current) >= 30	3	6	
		3,	960
Variant Allele Freq (Current) >= 0.2	3	6	
		3,	814
Allele Frequencies < 0.01 OR missing	3	6	
			566
Alt_allele_freq < 0.01 OR missing	a	6	
			469
Ffect (Combined) is (LoF, Missense)	a	6	
			134
🔽 Trio Analysis		۴	-
 AND Results satisfy criteria of all contain OR Results satisfy criteria of any contain 	ned filter ined filte	rs er	
🕼 Compound Het? (Current) is tri 🔌 🛽			
True 3			
False 131			
Missing 0			
3			
			3

- 1. ワークフロー下側の空きスペース上で右クリックし、メニューから「Add Filter Container」選択してクリック
- 2. 新たなコンテナが作成されるので、コンテナ名をダブルクリックして「Trio Analysis」に変更し、さらに「Show Filter Configuration」をクリックし、検索条件に「OR」を指定
- 3. Compound Het?コンテナを選択し、Trio Analysisコンテナ内にドラッグ&ドロップ

トリオ解析ワークフローの作成



🗹 🖬 Filter Variants						
Read Depths (DP) (Current) >= 30						
Variant Allele Freq (Current) >= 0.2						
Allele Frequencies < 0.01 OR missing						
☑ Alt_allele_freq < 0.01 OR missing						
	469					
Effect (Combined) is (LoF, Missense)						
🕼 Trio Analysis						
Compound Het? 🔦 I	ч I					
True 3 MIE	1					
False 131 Transmitted	96					
Missing 0 Untransmitted	0					
de Novo Allele	37					
Missing	0					
3	37					
	40					

• Proband (P12878)の「Mendel Error」 is de Novo Allele

4. Proband (P12878サンプル)の「Mendel Error」のフィールドのコンテナをつくり、上記のとおり検索条件を 指定する トリオ解析ワークフローの作成





- Proband (P12878)の「Zygosity」 is Homozygous Variant
- Mother (M12892)の「Zygosity」 is Heterozygous
- Father (F12891)の「Zygosity」 is Heterozygous

- 5. Trio Analysisコンテナ内の空きスペース上で右クリックし、メニューから「Add Filter Container」選択して クリック
- 6. 新たなコンテナが作成されるので、コンテナ名をダブルクリックして「Recessive Homozygous」に変更
- 7. Proband (P12878サンプル)、Mother (M12892サンプル)、Father (F12891サンプル)それぞれの 「Zygosity」のフィールドのコンテナをつくり、上記のとおり検索条件を指定する

遺伝子ランキングの計算





- 1. Select an Algorithm画面より「Variant PhoRank Gene Ranking」を選択して「OK」をクリック
- 2. Select a Source and Field画面において、変異テーブル上の遺伝子名のフィールド(この例では RefSeq GenesのGene Namesフィールド)を選択して、「OK」をクリック

遺伝子ランキングの計算



V PhoRank			
Phenotype Group Name: Muscular Dystrophy	Muscular	Dystrophy Pl	noRank
Phenotype Terms:	Gene Rank	Gene Score	Path
	0.425273	0.00136835	SLC35B3 / G···
late-onset muscular dystrophy	0.221142	0.000465594	HIVEP1 / GO···
congenital muscular dystrophy muscular dystrophy	0.379101	0.000974205	JARID2 / GO···
scapulohumeral muscular dystrophy	0.281896	0.000590947	KIF13A / GO
limb-girdle muscular dystrophy	0.377886	0.000968939	DHX16 / GO:
	0.247874	0.000517044	C6orf47 / GO···
	0.215067	0.000437204	LY6G6F / GO···
	0.247874	0.000517044	DXO / GO:00…
	0.336574	0.000794162	EGFL8 / GO:···
	0.221142	0.000465594	PBX2 / GO:0
	0.221142	0.000465594	PBX2 / GO:0
	0.36695	0.000910578	HLA-DQB2 / ···
	0.550425	0.00761623	SYNGAP1 / ···
OK Cancel			

- 3. PhoRank画面において、Phenotype Terms:に任意のHPO Term(この例ではmuscular dystrophy) を入力、あるいはTermの候補リストから選択して、「OK」をクリック
- 4. 変異あるいは遺伝子テーブルに、遺伝子ごとの入力HPO Termとの関連の強さを数値化したフィールドが 追加される



ワークフロー解析結果の確認

Tilter Variants X +						/ Variants: 40 🗙 🕅 Variant Genes: 902 🗙 🕂						
Variants 💌					V	ariants			Tric	Analysis: P12878	∼ m î	
🔽 🖬 Filter Variants 🔹 13,086					Variant Info							
Read Depths (DP) (Current)) >= 30			* □		Chr:Pos	Bef/Alt	Variant Allele Freg	Read Denths (DP)	Zvensity	Mendel Error	
				3,960		6:8428249	A/C	0.505155	194	Heterozvaous	de Novo Allele	
Voriant Allela Frag (Ourrant)						6:12124988	T/C	0.433862	189	Heterozygous	de Novo Allele	
Variant Anele Freq (Current)	~= 0.2					6:15501276	C/G	0.417722	79	Heterozygous	de Novo Allele	
				3,814		6:17794528	A/C	0.381818	112	Heterozygous	de Novo Allele	
Allele Frequencies < 0.01 O	R missing			× 🗆		6:30622603	G/T	0.449275	69	Heterozygous	de Novo Allele	
				566		6:31626986	C/G	0.244444	91	Heterozygous	de Novo Allele	
Alt allele free < 0.01 OP m	iccina			• •		6:31675842	A/G	0.39375	160	Heterozygous	de Novo Allele	
M All_allele_lieq < 0.01 OK III	issing					6:31938841	T/C	0.4375	112	Heterozygous	de Novo Allele	
				409		6:32135202	A/G	0.528302	54	Heterozygous	de Novo Allele	
Effect (Combined) is (LoF, M	lissense)			× 🗆		6:32155057	T/G	0.438596	57	Heterozygous	de Novo Allele	
				134		6:32156189	G/C	0.470588	34	Heterozygous	de Novo Allele	
				<u> </u>		6:32725625	T/C	0.321429	57	Heterozygous	de Novo Allele	
						6:33410691	T/C	0.343137	102	Heterozygous	de Novo Allele	
Compound Het?	Mendel Error (Current) 🔌		Recessive Homozygous	A 1		6:34985432	G/C	0.210526	38	Heterozygous	de Novo Allele	
True 3	MIE	1	🛛 🕅 Zygosity (Current) is Homozygous Vari	٤ ٩ 🛛 🗎		6:35782521	A/G	0.523077	65	Heterozygous	de Novo Allele	
False 131	Transmitted	96		1		6:41533574	C/A	0.6	146	Heterozygous	de Novo Allele	
Missing	Untransmitted	0	Zvaosity (Mother) is Heterozvaous	* -		6:43267651	A/G	0.5	112	Heterozygous	de Novo Allele	
wissing 0	Untransmitted					6:43581563	A/C	0.588235	102	Heterozygous	de Novo Allele	
	de Novo Allele	37		<u> </u>	-	6:44108008	C/G	0.456522	46	Heterozygous	de Novo Allele	
	Missing	0	Zygosity (Father) is Heterozygous	< □		6:47649853	G/C	0.380282	71	Heterozygous	de Novo Allele	
				0		6:69685178	A/C	0.507692	65	Heterozygous	de Novo Allele	
		0.7				6:75893766	G/T	0.269565	115	Heterozygous	de Novo Allele	
0		57		─── └── ┛		6:83877723	C/A	0.34375	97	Heterozygous	de Novo Allele	
				40		6:88387622	A/C	0.407407	135	Heterozygous	de Novo Allele	
						6:110064911	C/G	0.487179	78	Heterozygous	de Novo Allele	
						6:121563477	C/A	0.471429	71	Heterozygous	de Novo Allele	

1. 複合ヘテロ接合体では3つ、de Novoアレルでは37個、劣性ホモ接合体では0個の変異が検出され、合計40個の変異がテーブルに表示される
ワークフロー解析結果の確認



Variants: 40 🗙 🔲 Variant Genes: 37 🗙 +													
Variants by Variant Genes 🚽 🖽 💿 🔍 🗔 🔯 Trio Analysis: P12878 🖬 💟 🤾 GeneRank 🗙													
	Variant Gene	Aariant Gene Compound Het Genes for Proband (P12878)			Muscular Dystrophy PhoRank			Variant Info		Proband (P12878)			
	Gene Names	Has Compound Het?	Inherited from Father	Inherited from Mother	Gene Rank	Gene Score		Chr:Pos	Ref/Alt	Variant Allele Freq	Read Depths (DP)	Zygosity	Mendel Error
	COL12A1	False	0	0	0.990279	0.736401		6:152470619	C/G	0.538462	119	Heterozygous	Transmitted
	SYNE1	True	2	1	0.986634	0.735329		6:152542036	C/G	0.352941	153	Heterozygous	de Novo Allele
	FIG4	False	0	0	0.855407	0.0306254		6:152555877	C/T	0.47191	90	Heterozygous	Transmitted
	PGM3	False	0	0	0.749696	0.0131189		6:152784621	T/C	0.482759	58	Heterozygous	Transmitted
	SYNGAP1	False	0	0	0.550425	0.00761623		6:152786454	C/T	0.421053	152	Heterozygous	de Novo Allele
	FGFR10P	False	0	0	0.470231	0.00190696							
	SLC35B3	False	0	0	0.425273	0.00136835							
	CLDN20	False	0	0	0.399757	0.00108969							
	JARID2	False	0	0	0.379101	0.000974205							
	DHX16	False	1	0	0.377886	0.000968939							
	HLA-DQB2	False	0	0	0.36695	0.000910578							
	ADGRB3	False	1	0	0.35723	0.000870164							
	SF3B5	False	0	0	0.343864	0.00080745							

- 2. Variants by Variant Genesテーブルでは、フィルタリングの結果抽出された変異に対して、画面左側に 遺伝子名、右側に該当する変異をテーブル表示
- 3. 必要に応じて、複合ヘテロ接合体のHas Compound Het?フィールドや遺伝子ランキングのGene Rank フィールドで表示を並び替え、複合ヘテロ接合体の構成変異や、表現型との関連が高い遺伝子の変異 を確認する



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