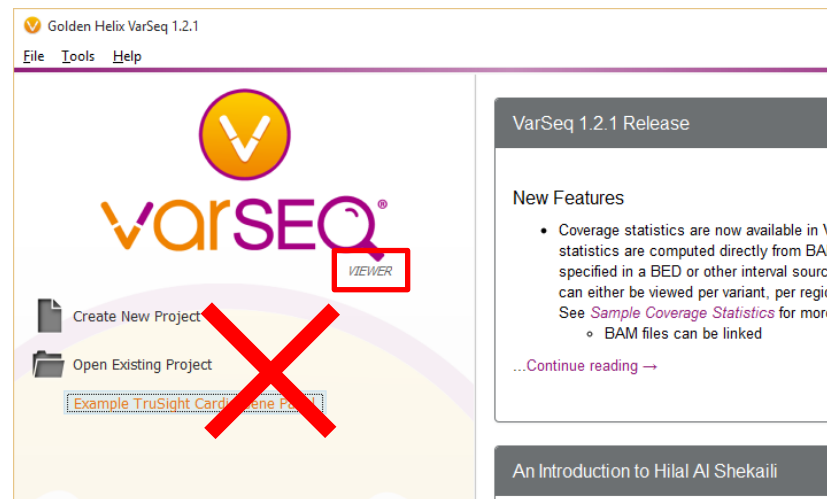
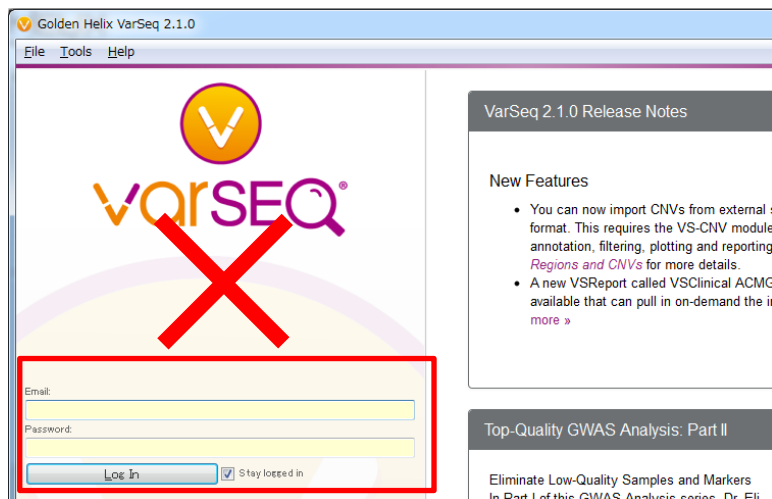
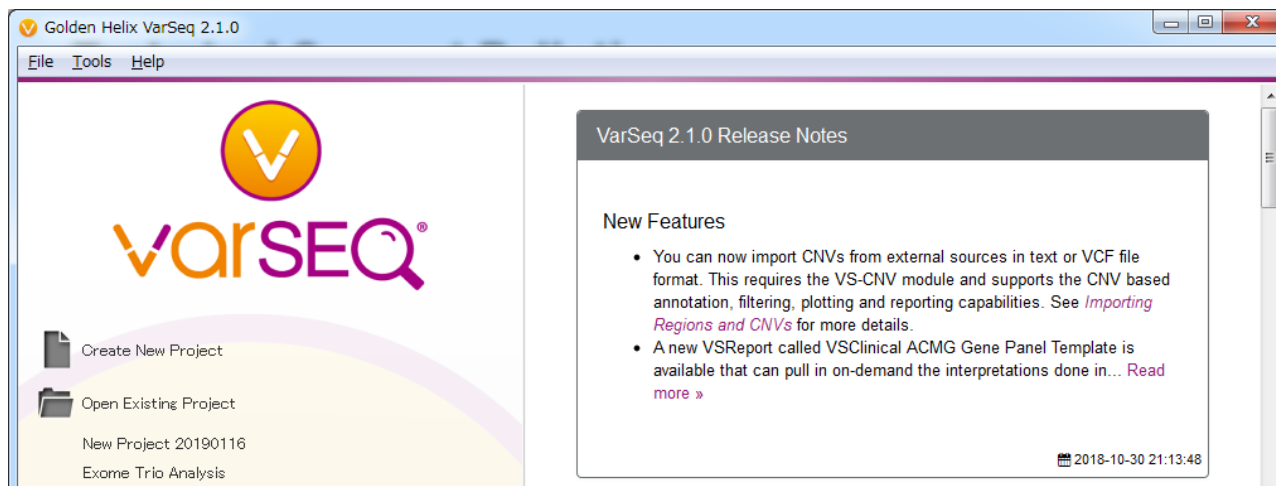


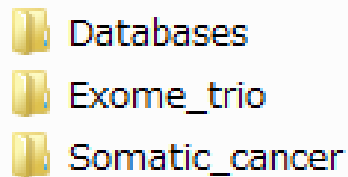
2019年2月15日 臨床ゲノム情報解析ハンズオントレーニング

 **VarSeq**
(クリニカルシーケンス編)

フィルジエン株式会社 バイオサイエンス部
(biosupport@filgen.jp)

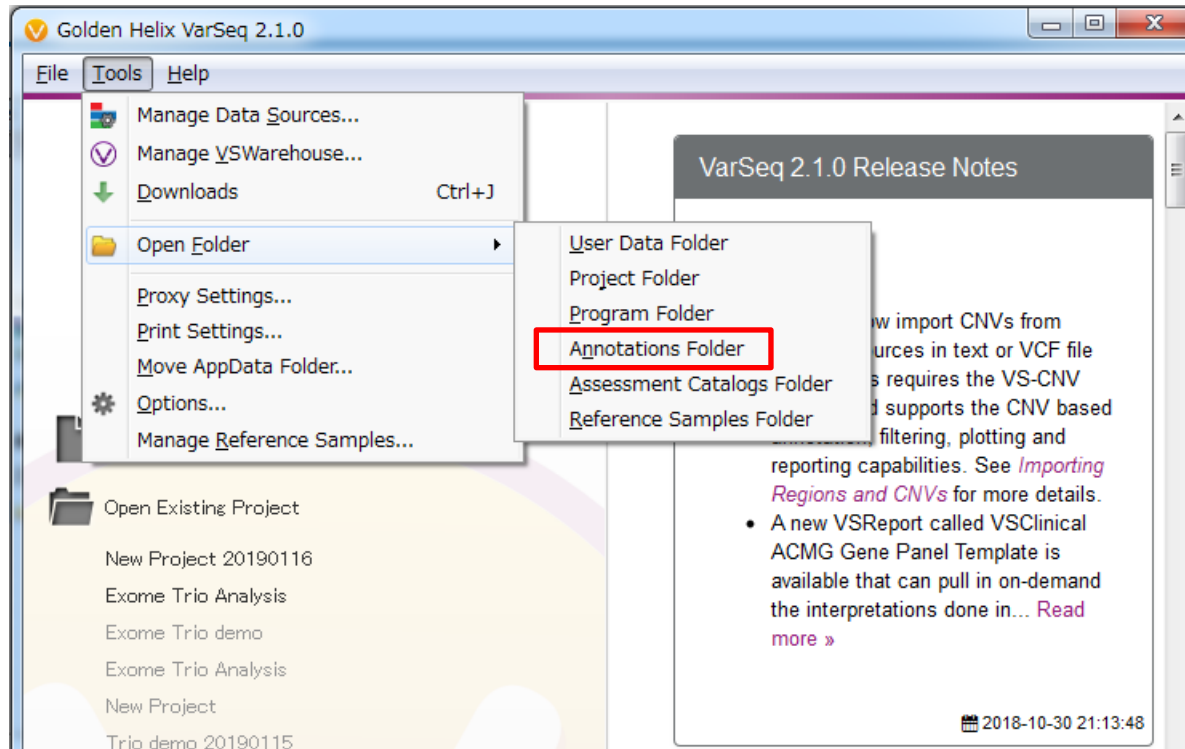


- VarSeqを起動した際に、上の図のように表示されていることをご確認ください。下の図のように、ログイン画面や、「Viewer」と表示されている状態では、ソフトウェアを使用できません。



- ✓ **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。



- 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。

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

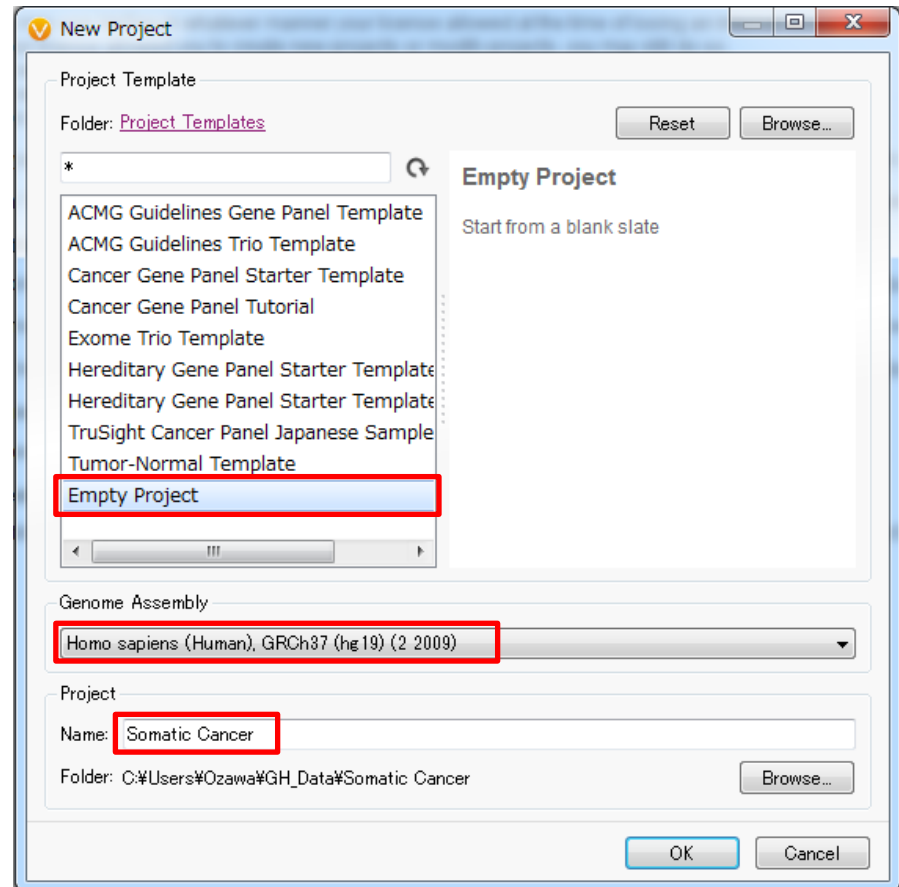
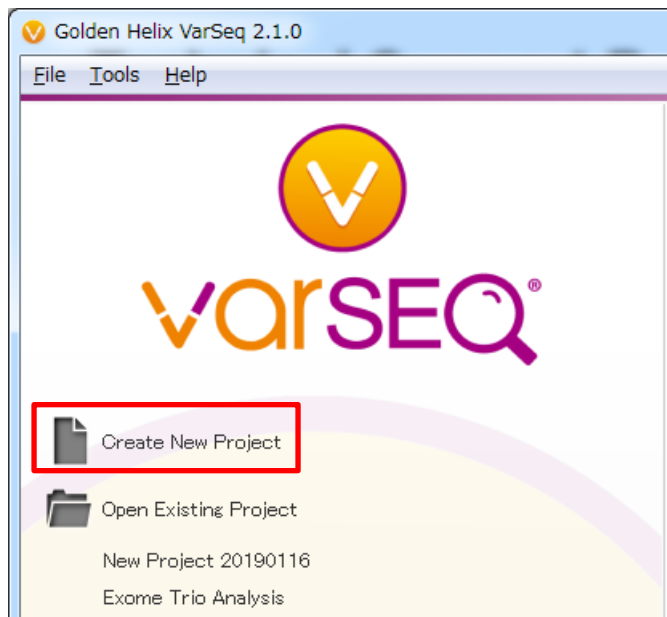
手順3： フィルタリング

- 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

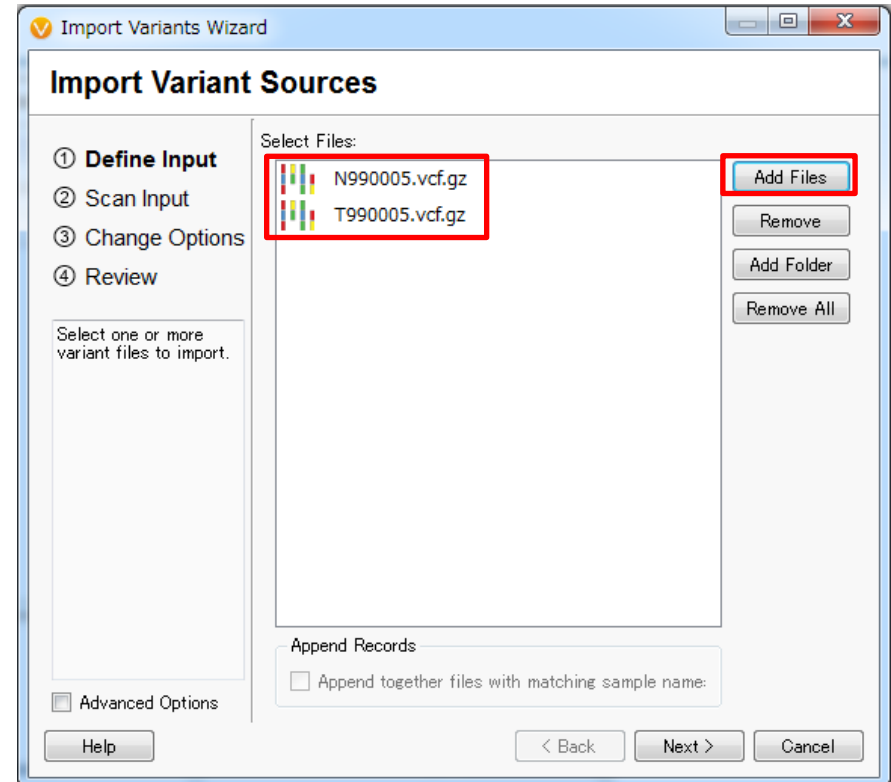
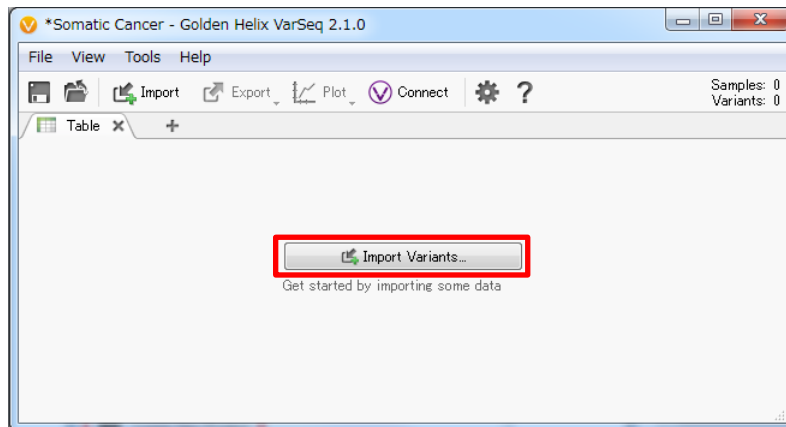
手順4： レポート作成

- 抽出変異情報を用いてレポートの作成

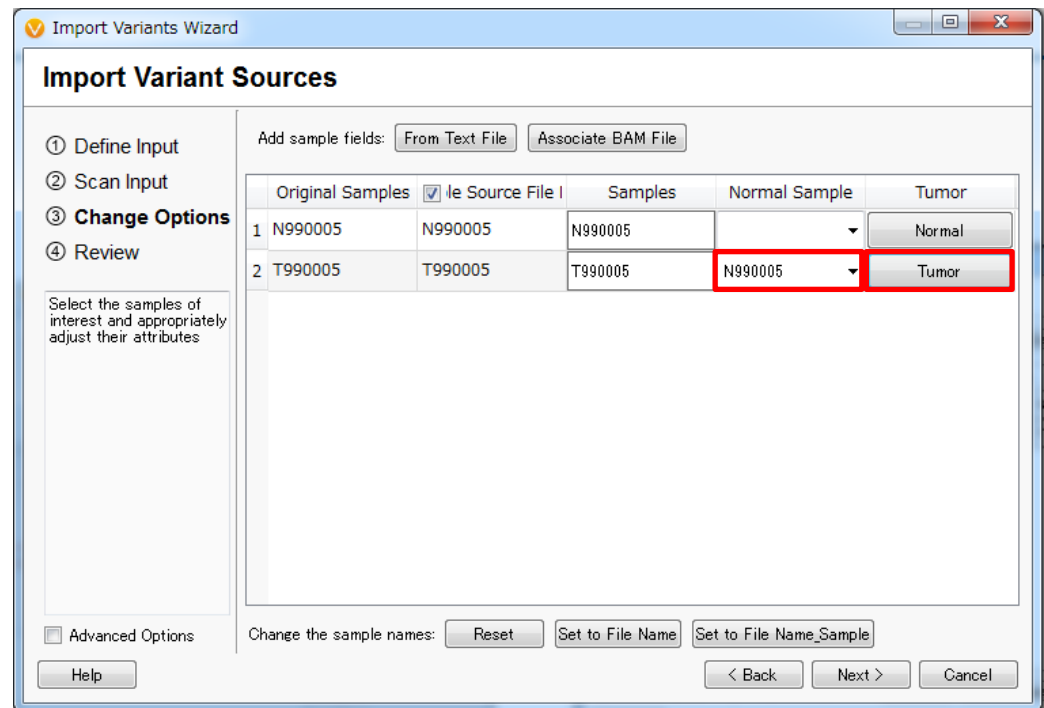
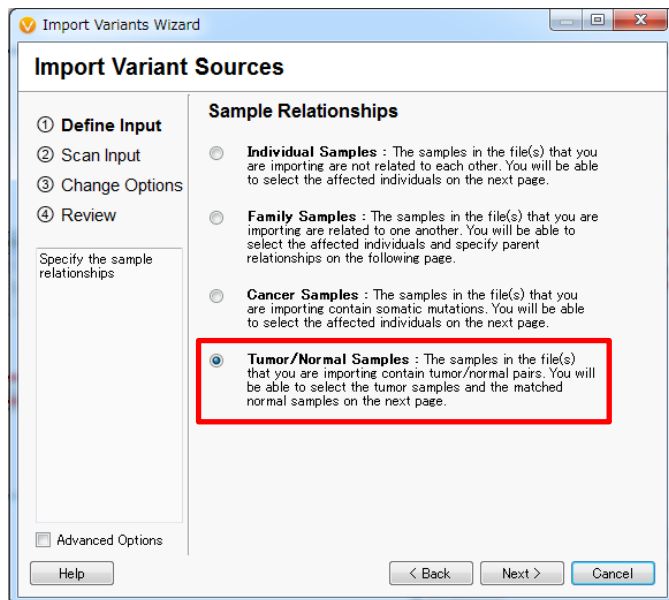
手順1. サンプルデータのインポート



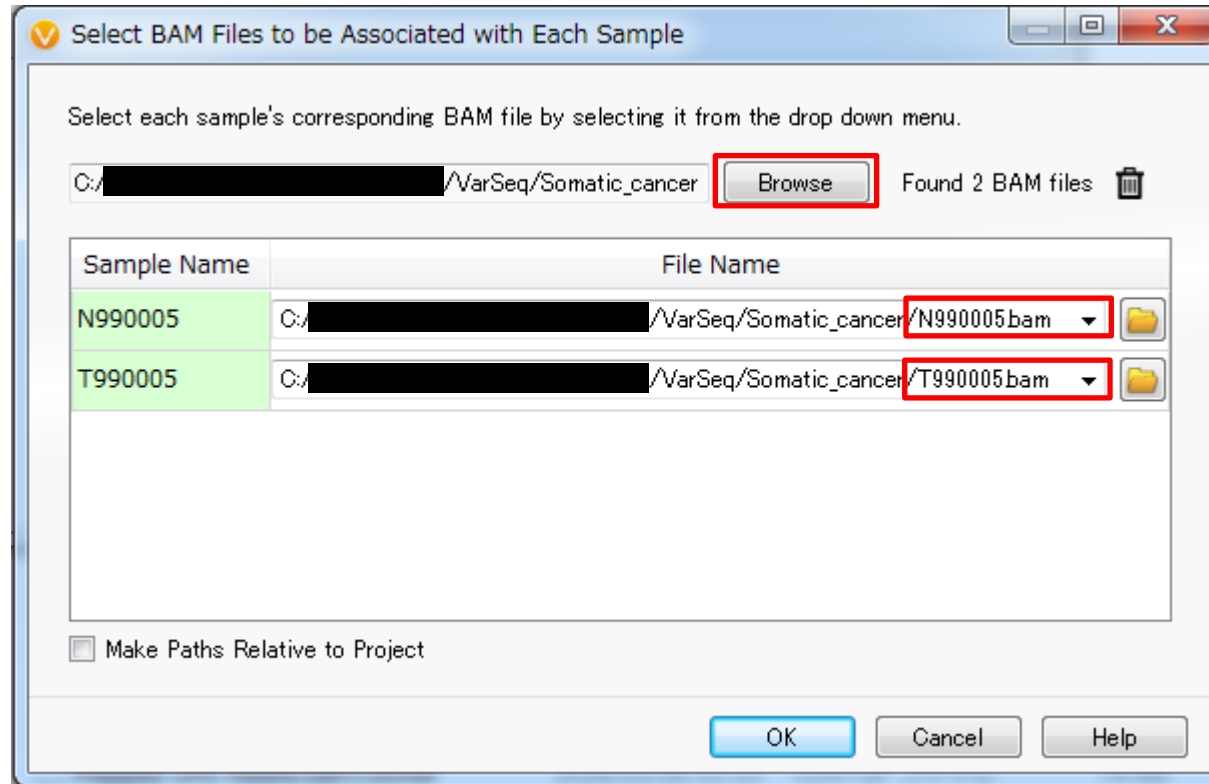
1. メイン画面の「Create New Project」をクリック
2. 任意のプロジェクト名を入力し、またProject Templateに「Empty Project」、Genome Assemblyが「Homo sapiens (Human), GRCh37 (hg19) (2 2009)」となっていることを確認したら「OK」をクリック



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、**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



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「N990005」、Tumorフィールドに「Tumor」を選択
8. Add sample fieldsの「Associate BAM File」をクリック



- 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 Sources

① Define Input
② Scan Input
③ **Change Options**
④ Review

Select the samples of interest and appropriately adjust their attributes

Advanced Options

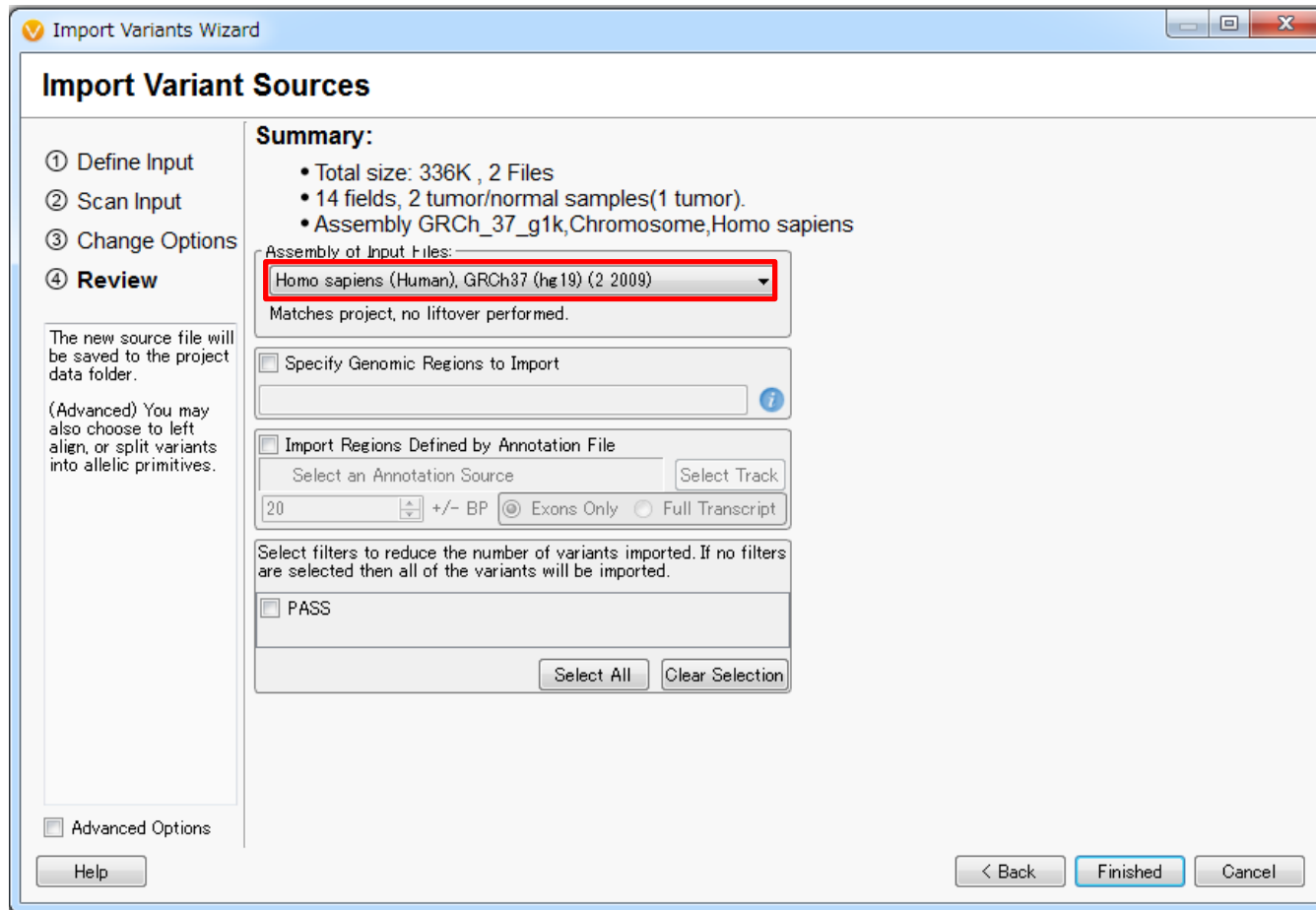
Help

Add sample fields:

	Original Samples	<input checked="" type="checkbox"/> Use Source File	Samples	Normal Sample	Tumor	<input checked="" type="checkbox"/> BAM Path
1	N990005	N990005	N990005		Normal	C:/[REDACTED]/VarSeq/Somatic_cancer/N990005.bam
2	T990005	T990005	T990005	N990005	Tumor	C:/[REDACTED]/VarSeq/Somatic_cancer/T990005.bam

Change the sample names:

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



11. Assembly of Input Files(こ、「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

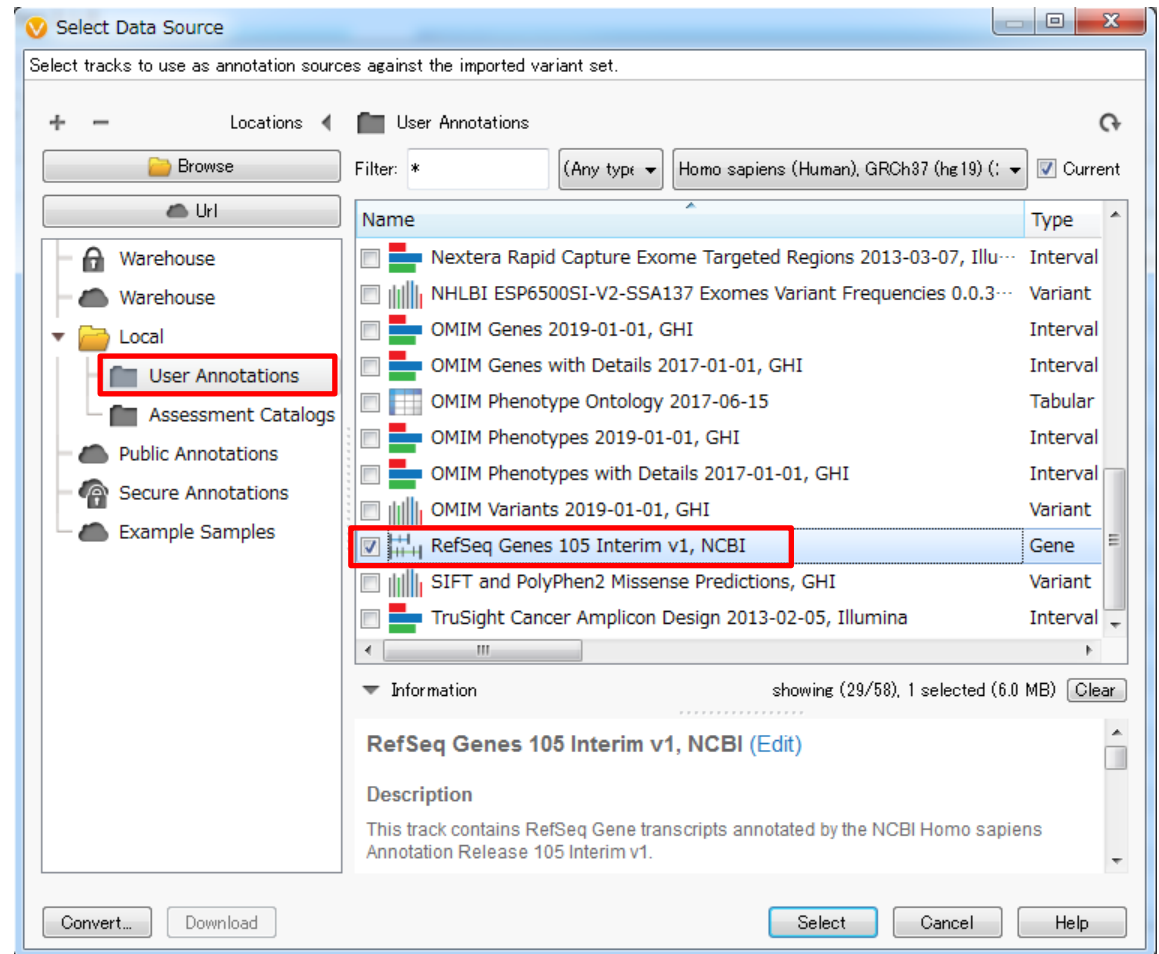
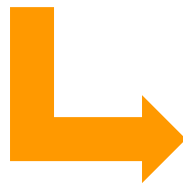
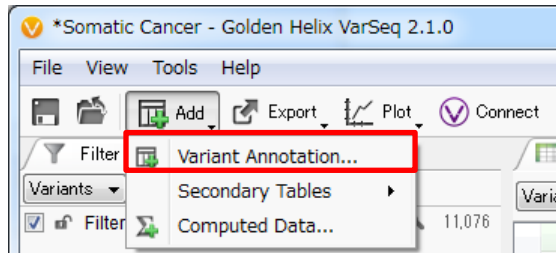
Filter Variants 11,076

Filter Variants: T990005

Variant Info			Tumor (T990005)			Normal (N990005)		
Chr:Pos	Ref/Alt	Identifier	Read Depths (DP)	0/1 Genotypes (GT)	AF	Read Depths (DP)	0/1 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
3:1424718	G/A	rs2291101	250	0/1	0.473896	213	0/1	0.43128
3:1424745	C/T	rs4684146	226	0/1	0.451327	176	0/1	0.4375
3:1424850	T/G	rs2291100	182	0/1	0.5	99	0/1	0.585859
3:1637940	G/A	rs900244	9	0/1	0.375	8	0/1	0.5
3:1771749	A/C	rs6442664	20	1/1	1	18	1/1	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



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

Variants: 11,076 x +

Variants Filter Variants: T990005

Variants: 11,076

Variant Info		Tumor (T990005)		Normal (N990005)		RefSeq Genes 105 Interim v1, NCBI	
Chr:Pos	Ref/Alt	Read Depths (DP)	AF	Read Depths (DP)	AF	Gene Names	Sequence Ontology (Combined)
5:34811154	G/T	163	0.429448	107	0.542056	RAI14	missense_variant
5:34838930	-/G	?	?	5	1	?	intergenic_variant
5:34840841	A/G	6	0.5	?	?	TTC23L	missense_variant
5:34925328	T/A	21	0.428571	?	?	BRIX1	splice_region_variant
5:34925329	-/A	?	?	17	0.529412	BRIX1	splice_acceptor_variant
5:34998778	C/A	36	0.361111	32	0.46875	AGXT2	splice_donor_variant
5:35010138	A/G	120	0.991667	68	0.985294	AGXT2	synonymous_variant
5:35033605	G/A	117	1	104	0.990385	AGXT2	missense_variant
5:35037115	C/T	118	0.589744	91	0.637363	AGXT2	missense_variant
5:35039486	C/T	86	0.360465	54	0.444444	AGXT2	missense_variant
5:35641582	A/C	217	0.56682	132	0.522727	SPEF2	missense_variant
5:35644621	T/C	70	0.442857	38	0.368421	SPEF2	synonymous_variant
5:35654711	C/T	201	0.49	133	0.398496	SPEF2	synonymous_variant
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
5:35709095	C/T	132	0.992424	84	1	SPEF2	missense_variant
5:35709184	G/C	111	1	59	1	SPEF2	missense_variant
5:35709853	C/T	3	1	?	?	SPEF2	intron_variant
5:35710038	C/T	2	1	2	1	SPEF2	intron_variant
5:35710330	G/A	3	1	?	?	SPEF2	intron_variant
5:35710386	A/G	3	1	?	?	SPEF2	intron_variant
5:35710728	C/T	3	1	?	?	SPEF2	intron_variant
5:35713007	T/G	81	1	57	1	SPEF2	intron_variant
5:35753715	T/C	248	0.995968	244	1	SPEF2	intron_variant
5:35910529	C/T	173	1	122	1	CAPSL	missense_variant
5:35921069	T/C	250	1	249	1	CAPSL	intron_variant
5:35953697	A/G	2	1	3	1	UGT3A1	3_prime_UTR_variant
5:35954588	C/T	103	0.990196	65	0.984615	UGT3A1	splice_region_variant
5:35960841	T/C	5	0.6	?	?	UGT3A1	intron_variant
5:35962984	A/G	3	1	3	1	UGT3A1	missense_variant
5:36177269	C/A	130	0.457364	63	0.47619	SKP2	intron_variant

5:35033605 - G/A (1bp sub)

Sequence Ontology (Combined): missense_variant
[rs180749](#)

5:35033605 - G/A (1bp sub)

Variant Info

Chr:Pos: 5:35033605
Ref/Alt: G/A
Identifier: rs180749

Show 3 hidden fields

Sample Fields

Sample	Normal (N990005)	Tumor (T990005)
Read Depths (DP)	104	117
AF	0.990385	1

Show 2 hidden fields

RefSeq Genes 105 Interim v1, NCBI

Gene Names	AGXT2
Sequence Ontology (Combined)	missense_variant
Effect (Combined)	Missense
N of 4 Predicted Splicing Disrupted (Combined)	?
Predicted Splicing Disrupted (Combined)	?
Transcript Name (Clinically Relevant)	NM_031900.3
HGVS c. (Clinically Relevant)	NM_031900.3:c.635C>T
HGVS p. (Clinically Relevant)	NP_114106.1:p.Thr212Ile

Show 10 hidden fields

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

Variants: 11,076 x +

Filter Variants: T990005

Variant	Tumor (T990005)		Normal (N990005)		RefSeq Genes 105 Interim v1, NCBI		
Ref/Alt	Read Depths (DP)	AF	Read Depths (DP)	AF	Gene Names	Sequence Ontology (Combined)	Effect (Combined)
G/T	163	0.429448	107	0.542056	RAI14	missense_variant	Missense
-/G	?	?	5	1	?	intergenic_variant	Other
A/G	6	0.5	?	?	TTC23L	missense_variant	Missense
T/A	21	0.428571	?	?	BRIX1	splice_region_variant	Other
-/A	?	?	17	0.529412	BRIX1	splice_acceptor_variant	LoF
C/A	36	0.361111	32	0.46875	AGXT2	splice_donor_variant	LoF
A/G	120	0.991667	68	0.985294	AGXT2	synonymous_variant	Other
G/A	117	1	104	0.990385	AGXT2	missense_variant	Missense
C/T	118	0.589744	91	0.637363	AGXT2	missense_variant	Missense
C/T	86	0.360465	54	0.444444	AGXT2	missense_variant	Missense
A/C	217	0.56682	132	0.522727	SPEF2	missense_variant	Missense
T/C	70	0.442857	38	0.368421	SPEF2	synonymous_variant	Other
C/T	201	0.49	133	0.398496	SPEF2	synonymous_variant	Other
G/A	237	0.481013	167	0.463855	SPEF2	missense_variant	Missense
T/C	243	0.433884	143	0.507042	SPEF2	synonymous_variant	Other
C/T	132	0.992424	84	1	SPEF2	missense_variant	Missense
G/C	111	1	59	1	SPEF2	missense_variant	Missense
C/T	3	1	?	?	SPEF2	intron_variant	Other
C/T	2	1	2	1	SPEF2	intron_variant	Other
G/A	3	1	?	?	SPEF2	intron_variant	Other
A/G	3	1	?	?	SPEF2	intron_variant	Other
C/T	3	1	?	?	SPEF2	intron_variant	Other
T/G	81	1	57	1	SPEF2	intron_variant	Other
T/C	248	0.995968	244	1	SPEF2	intron_variant	Other
C/T	173	1	122	1	CAPSL	missense_variant	Missense
T/C	250	1	249	1	CAPSL	intron_variant	Other
A/G	2	1	3	1	UGT3A1	3_prime_UTR_variant	Other
C/T	103	0.990196	65	0.984615	UGT3A1	splice_region_variant	Other
T/C	5	0.6	?	?	UGT3A1	intron_variant	Other
A/G	3	1	3	1	UGT3A1	missense_variant	Missense
C/A	130	0.457364	63	0.47619	SKP2	intron_variant	Other
T/A	115	0.443478	79	0.481013	NADK2	intron_variant	Other
T/C	6	0.333333	12	0.583333	NADK2	intron_variant	Other
G/A	?	?	8	0.5	NADK2	intron_variant	Other
G/A	6	0.666667	?	?	NADK2	intron_variant	Other
T/C	6	0.666667	?	?	NADK2	5_prime_UTR_variant	Other
T/G	4	1	?	?	RANBP3L	missense_variant	Missense

Effect (Combined)

The highest priority of the effect annotations found among the variant transcript interactions.

Type: Categorical

Field: Effect (Combined)

Symbol: EffectCombined

Doc: The highest priority of the effect annotations found among the variant transcript interactions. The likely effect that the variant will have on the transcript's product. The ontologies that correspond to each effect category can be found at the bottom of this page in the documentation for the effect category.

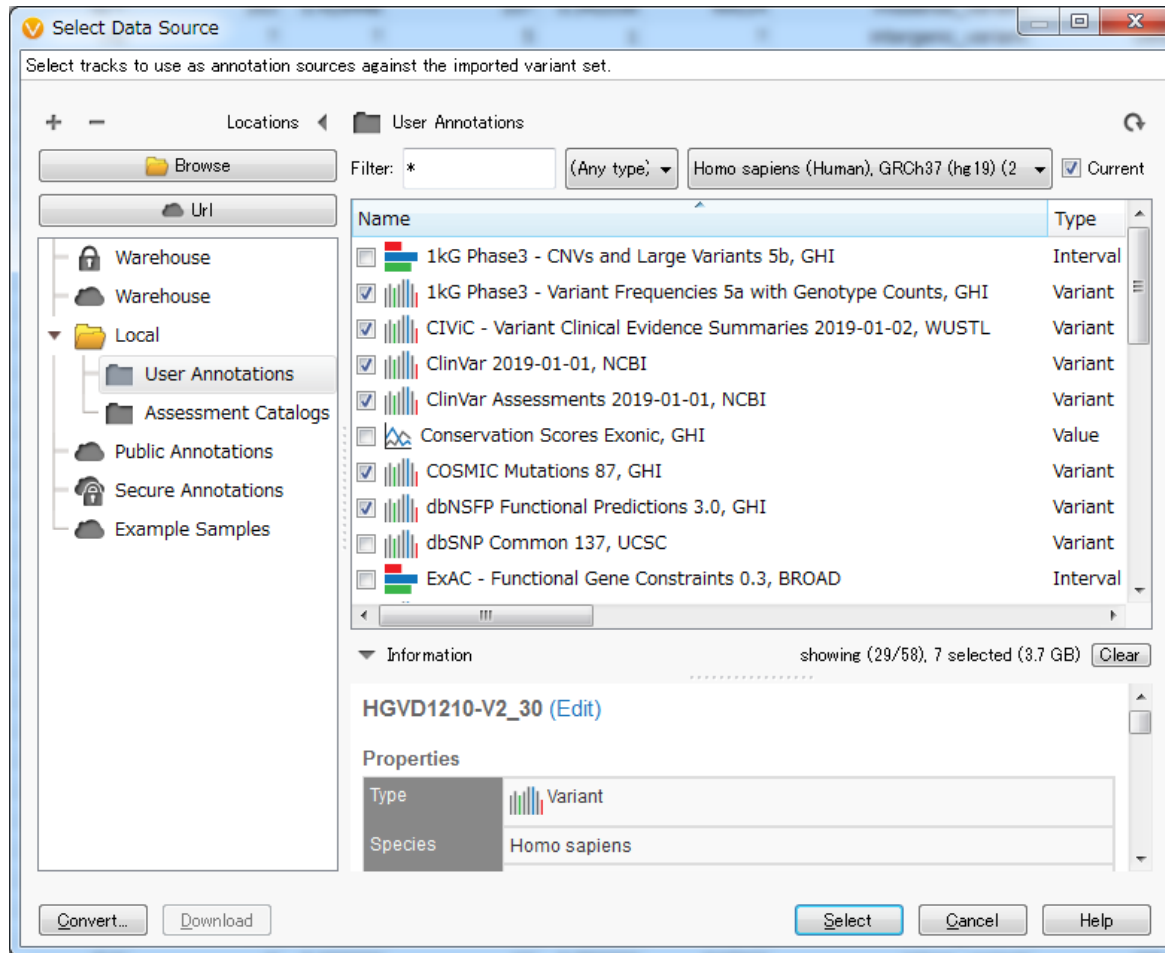
Category Counts (11,076 Records)

Category	Count	Percent
Other	9573	86.43%
Missense	1454	13.13%
LoF	49	0.44%
Total	11076	100.0%

Categories of Effect (Combined)

Other	The variant is likely to have a low or unknown effect on the transcript's functional product. These changes do not change the amino acid sequence of the protein. The ontologies included in this category are: synonymous_variant, stop_retained_variant, splice_region_variant, 3_prime_UTR_variant, 5_prime_UTR_variant, intron_variant, non_coding_exon_variant, intergenic_variant, unknown.
Missense	The variant will cause at least one amino acid to change or cause a premature start codon in the UTR5. The ontologies included in this category are: disruptive_inframe_deletion, disruptive_inframe_insertion, inframe_deletion, inframe_insertion, 5_prime_UTR_premature_start_codon_gain_variant, missense_variant.
LoF	Loss of Function. The variant is likely to cause the transcript's product to lose function. The ontologies included in this category are: transcript_ablation, exon_loss_variant, stop_lost, stop_gained, initiator_codon_variant, frameshift_variant, splice_acceptor_variant, 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



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

Variants: 11,076 x +

Filter Variants: T990005

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

Variant Info		Tumor (T990005)	Normal (N990005)
Chr:Pos	Ref/Alt	Read Depths (DP)	AF
3:238566	G/C	?	1
3:350861	A/G	5	1
3:361508	C/T	70	1481
3:391100	A/G	177	1
3:405202	A/T	19	1
3:423983	C/T	?	3846
3:439963	A/G	181	1
3:440028	T/C	230	1
3:440088	T/A	185	1
3:448063	A/G	2	?
3:449832	A/G	4	?

- Sort Ascending
- Sort Descending
- Hide
- Delete
- Plot for Current Sample
- Plot for All Samples
- Query Column Values
- Add to Filter Chain**
- Rename



Filter Variants x +

Variants ▾

Filter Variants 11,076

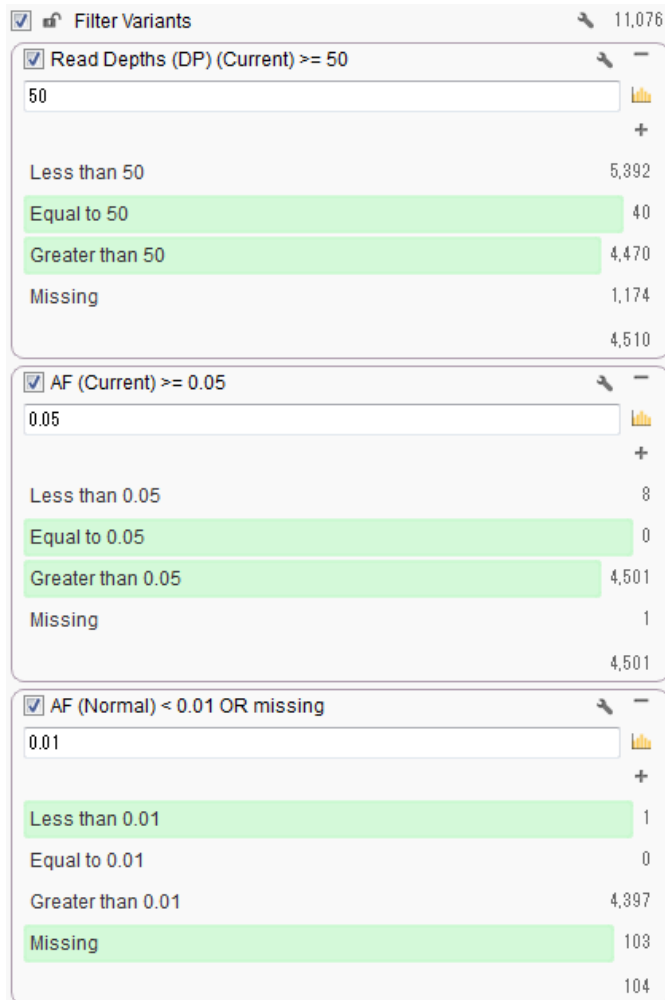
Read Depths (DP) (Current) >= 50

50

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



- Tumor (T990005)の「Read Depths (DP)」 ≥ 50
- Tumor (T990005)の「AF」 ≥ 0.05
- Normal (N990005)の「AF」 < 0.01 or Missing

1. 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 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
 - 0.01
 - Less than 0.01 3
 - Equal to 0.01 0
 - Greater than 0.01 15
 - Missing 86
- Alt_allele_freq < 0.01 OR missing 66
 - 0.01
 - Less than 0.01 3
 - Equal to 0.01 0
 - Greater than 0.01 23
 - Missing 63

- 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

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

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

3. 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

体細胞変異抽出ワークフローの作成

The image illustrates the process of creating a Somatic filter container in a software interface. It consists of three sequential screenshots connected by arrows:

- First Screenshot:** A 'Filter Variants' panel with 11,076 variants. A list of filters is shown, including '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), and 'FATHMM Prediction is PATHOGENIC' (4). A context menu is open at the bottom, with 'Add Filter Container' highlighted in a red box.
- Second Screenshot:** The same filter list is shown, but a new 'Somatic' container has been added at the bottom, highlighted with a red box.
- Third Screenshot:** The 'Somatic' container is now selected, and all the filters from the previous screenshots are nested inside it, indicating they have been moved into this container.

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

Filter Variants 11,076

AND Results satisfy criteria of **all** contained filters

OR Results satisfy criteria of **any** contained filter

Somatic

Read Depths (DP) (Current) > 4,510

AF (Current) >= 0.05 4,501



Filter Variants 11,076

AND Results satisfy criteria of **all** contained filters

OR Results satisfy criteria of **any** contained filter

Somatic

Read Depths (DP) (Current) > 4,510

AF (Current) >= 0.05 4,501

AF (Normal) < 0.01 OR missir 104

Allele Frequencies < 0.01 OR 89

Alt_allele_freq < 0.01 OR mis: 4

Variant Int

Chr:Pos
3:178936082
4:66280142
5:137519659

Enabled

Inverted

AND

OR

Lock

Add Filter

Add Filter Container



Filter Variants 11,076

AND Results satisfy criteria of **all** contained filters

OR Results satisfy criteria of **any** contained filter

Somatic

Germline

Read Depths (DP) (Current) > 4,510

AF (Current) >= 0.05 4,501

AF (Normal) < 0.01 OR missir 104

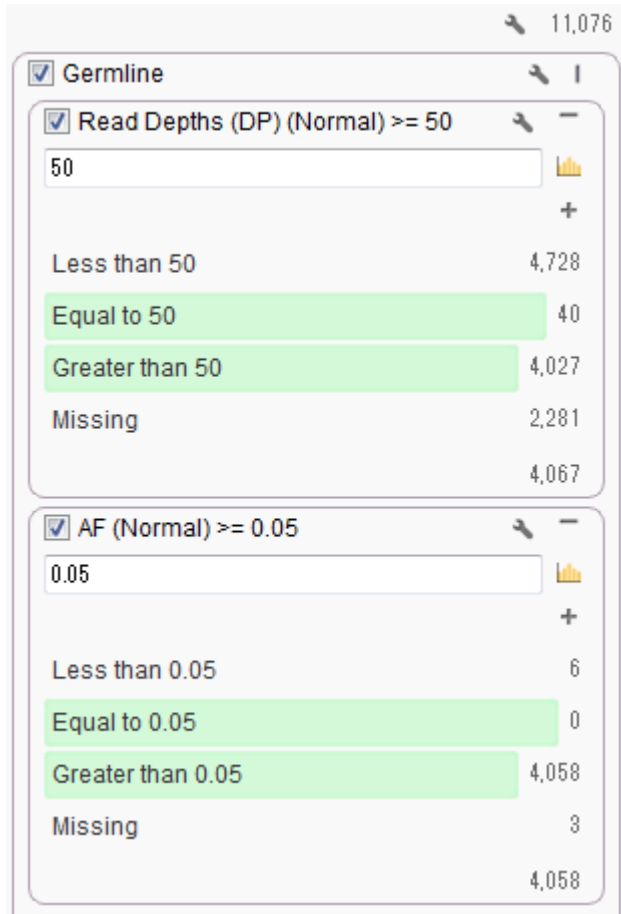
Allele Frequencies < 0.01 OR 89

Alt_allele_freq < 0.01 OR mis: 66

In COSMIC? is true 7

FATHMM Prediction is PATHOC 4

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



- Normal (N990005)の「Read Depths (DP)」 ≥ 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

The screenshot shows a variant filtering interface with the following filters and results:

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

- 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

Filter	Count
Germline	1
Read Depths (DP) (Normal) >= 50	4,067
AF (Normal) >= 0.05	4,058
Allele Frequencies < 0.01 OR missing	381
Alt_allele_freq < 0.01 OR missing	240
Effect (Combined) is (LoF, Missense)	69
LoF	8
Missense	61
Other	171
Missing	0
Classification is (Likely Pathogenic, Pathogenic)	1
Benign	0
Conflicting	2
Likely Benign	2
Likely Pathogenic	0
Other	0
Pathogenic	1
Uncertain Significance	0
Missing	64

- 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

The screenshot displays the 'Filter Variants' interface. On the left, there are two columns of filter settings:

- Somatic Filters:**
 - Read Depths (DP) (Current) ≥ 50 : 4,510
 - AF (Current) ≥ 0.05 : 4,501
 - AF (Normal) < 0.01 OR missing: 104
 - Alt_allele_freq < 0.01 OR missing: 80
 - Allele Frequencies < 0.01 OR missing: 66
 - In COSMIC? is true: 7
 - FATHMM Prediction is PATHOGENIC: 4
- Germline Filters:**
 - Read Depths (DP) (Normal) ≥ 50 : 4,067
 - AF (Normal) ≥ 0.05 : 4,058
 - Alt_allele_freq < 0.01 OR missing: 1,357
 - Allele Frequencies < 0.01 OR missing: 240
 - Effect (Combined) is (LoF, Missense): 69
 - Classification is (Likely Pathogenic, Pathogenic): 1

On the right, the 'Variants: 5' table shows the following data:

Variant Info	Tumor (T990005)			Normal (N990005)		Gene Names
	Chr:Pos	Ref/Alt	Read Depths (DP)	AF	Read Depths (DP)	
3:178936082	G/A	177	0.107345	?	?	PIK3CA
4:66280142	G/A	243	0.127572	?	?	EPHA5
4:147560457	T/C	63	0.126984	?	?	POU4F2
5:131973850	C/T	95	0.568421	81	0.592593	RAD50
5:137519659	T/C	246	0.150407	?	?	KIF20A

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

Variants: 5 x +

Filter Variants: T990005

Variant Info	Tumor (T990005)		Normal (N990005)		RefSeq Genes 105 Interim v1, NCBI				
	Chr:Pos	Ref/Alt	Read Depths (DP)	AF	Read Depths (DP)	AF	Gene Names	Sequence Ontology (Combined)	Effect (Combined)
3:178936082	G/A	177	0.107345	?	?	?	PIK3CA	missense_variant	Missense
4:66280142	G/A	243	0.127572	?	?	?	EPHA5	missense_variant	Missense
4:147560457	T/C	63	0.126984	?	?	?	POU4F2	synonymous_variant	Other
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

3:178936082 - G/A (1bp sub) x

AF: 0.107345

[rs121813273](#)

COSMIC Mutations 87, GHI

Ref/Alt	G/A
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
Gene Name	PIK3CA
HGNC ID	?
Pubmed ID	27149842, 17947469, 27135926, 22357840, 19671852, 28481359, 26184520, 27901576, 16353168, 21533174, [more]
Study ID	?
Sample Count	535
Oncotree Tissue Type	Breast (BREAST), Bowel (BOWEL), Bladder/Urinary Tract (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, 3, 3, 2, 1, 1
Primary Site	Breast, Large intestine, Urinary tract, Lung, Upper aerodigestive tract, Oesophagus, Cervix, [more]
Primary Site Counts	183, 98, 55, 35, 21, 18, 17, 17, 12, 11, 10, 9, 9, 6, 5, 5, 4, 3, 3, 2, 2, 2, 1, 1, 1
Site Subtype	Bladder, Colon, Brain, Caecum, Mouth, Anus, Head neck, Bile duct, Upper urinary tract, Pharynx, [more]
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
Primary Histology	Carcinoma, Glioma, Germ cell tumour, Malignant melanoma, Liposarcoma, Thymic carcinoma, Other, Rhabdomyosarcoma, Sarcoma, Haemangioblastoma

2. 詳細データ表示スペースより、各変異のアノテーション情報を確認する

The image shows the Golden Helix VarSeq 2.1.0 interface. On the left, the 'Plot' menu is open, with 'Plot BAM for Current Sample' and 'Plot BAM for Current Normal' highlighted in a red box. Below the menu, the genome browser view is shown for 'Homo sapiens (Human), GRCh37 (hg19) (2 2009)'. The browser displays several tracks: 'Current Normal Read Alignment' (Coverage and Pile-up), 'Current Sample Read Alignment' (Coverage and Pile-up), and 'RefSeq Genes 105 Interim v1, NCBI'. An orange arrow points from the menu to the browser view.

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
2. 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

The screenshot displays a genomic browser interface with the following components:

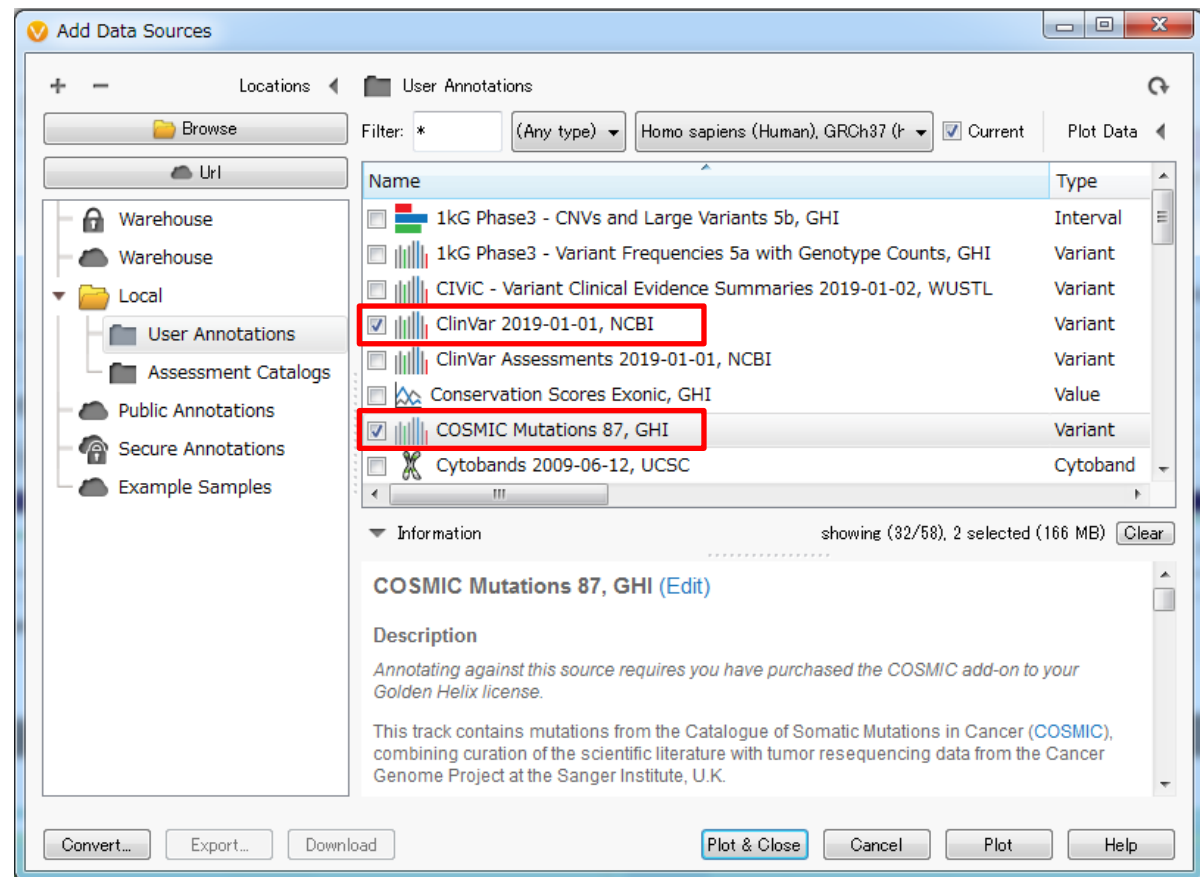
- Variants Table:** A table showing variant information for Tumor (T990005) and Normal (N990005) samples. The table includes columns for Chromosome, Position, Reference/Alternative alleles, Depth (DP), Allele Frequency (AF), Gene Name, and predicted effects.
- Genome Browser:** A central track showing the genomic region from 178,936,072 to 178,936,091 on Chromosome 3. It includes tracks for Normal and Sample Read Alignment, Coverage, and RefSeq Genes (PIK3CA, PL, S, E, I, T, E).
- Console:** A red-bordered window on the right showing detailed alignment data for Chr3: 178,936,082. It includes a table for Matches / Mismatches / Deletions and a table for Insertions.

Variant Info		Tumor (T990005)		Normal (N990005)		RefSeq Genes 105 Interim v1, NCBI				
Chr:Pos	Ref/Alt	DP	AF	DP	AF	GeneNa...	SequenceOntologyCombined	EffectCombined	Nof4PredictedSplicingDisruptedCo...	PredictedSplicingDis...
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 Predicted Splicing Disrupted	

Matches / Mismatches / Deletions				
Type	Base	Count	% of Total	Mean Quality
(match)	G	153	89.0	36.2
(mismatch)	A	19	11.0	34.6
Total		172	100	36.0

Insertions			
Base(s)	Count	% of Total	Mean Quality
Non-Insertions	177	100.0	?
Total	177	100	?

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



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

The screenshot displays the Genome Browser interface for the PIK3CA gene region on chromosome 3 (178,936,072 - 178,936,091). The tracks shown are:

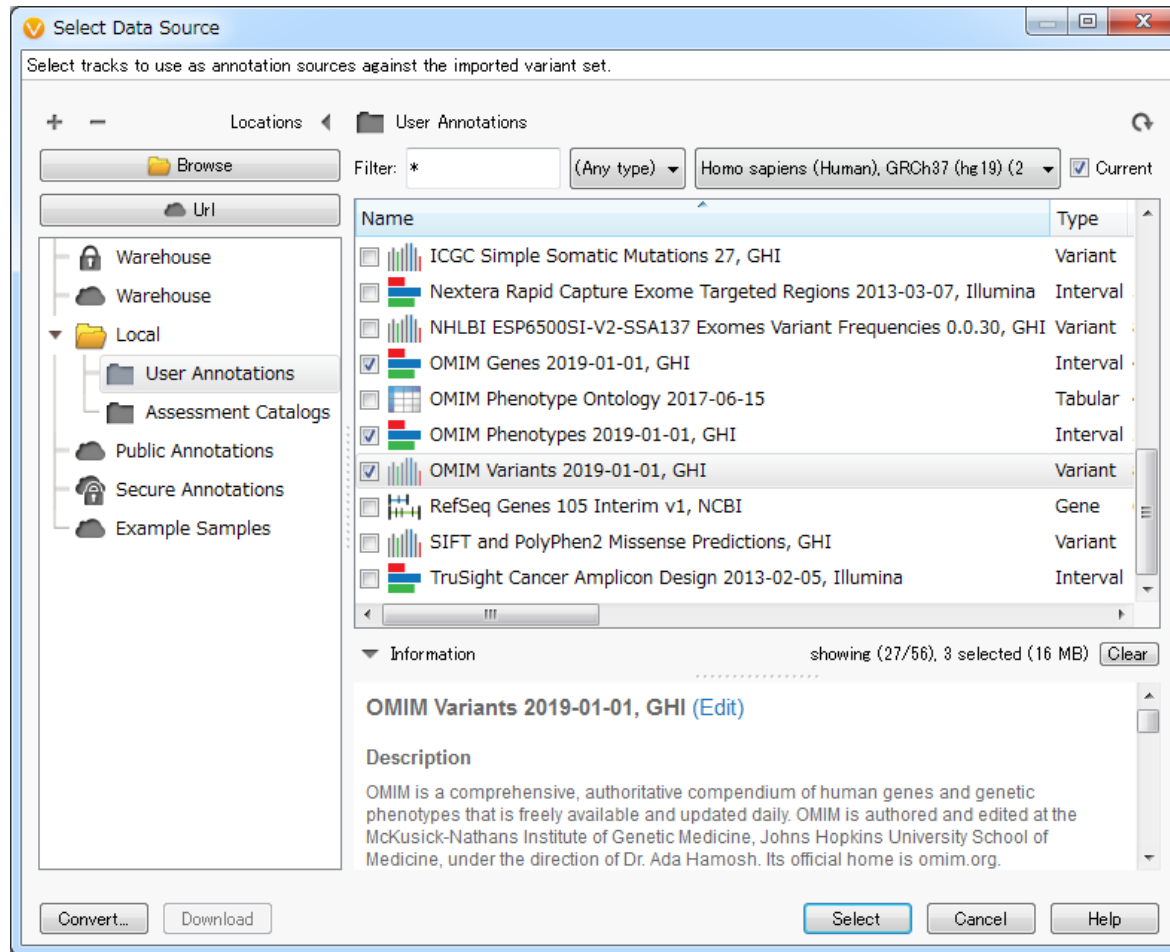
- ClinVar 2019-01-01, NCBI:** Shows a PIK3CA mutation with a coverage of 1.
- COSMIC Mutations 87, GHI:** Shows multiple PIK3CA mutations with varying coverage levels.
- Current Normal Read Alignment:** Shows a read alignment with a coverage of 250. The sequence is C C T C T C T C T G A A A T C A C T G A.
- Current Sample Read Alignment:** Shows a read alignment with a coverage of 350. The sequence is C C T C T C T C T G A A A T C A C T G A.
- RefSeq Genes 105 Interim v1, NCBI:** Shows the PIK3CA gene structure with exons labeled P, L, S, E, I, T, E.

The console window on the right provides detailed information for the selected mutation:

Field	Value
Ref/Alt	G/A
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
Gene Name	PIK3CA
HGNC ID	?
Pubmed ID	27149842, 17947469, 27135926, 22357840, 19671852, 28481359, 26184520, 27901576

- 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
- 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



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

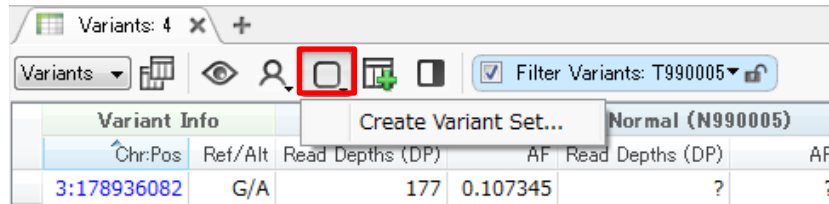
Variants: 5 x +

Variants Filter Variants: T990005

OMIM 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 ...	<p>Receptor protein tyro...	
POU4F2	4q31.2	113725	5458	8995448,1...	True	POU DOMAIN, CLASS 4, TRA...	POU-DOMAIN TRANSCRIPTION ...	<p>POU4F2 is a member ...	
RAD50	5q31	604040	10111	19487811,...	True	RAD50, S. CEREVISIAE, HOM...	?		?
KIF20A	5q31	605664	10112	10233894,...	True	KINESIN FAMILY MEMBER 20...	RAB6-INTERACTING PROTEIN ...	<p>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

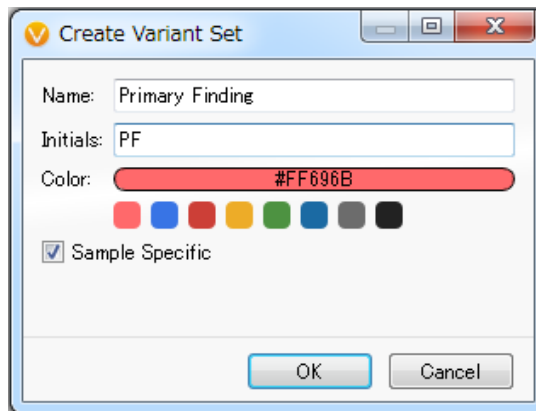
変異セットの作成



Variants: 4 x +

Filter Variants: T990005

Variant Info		Create Variant Set...		Normal (N990005)	
Chr:Pos	Ref/Alt	Read Depths (DP)	AF	Read Depths (DP)	AF
3:178936082	G/A	177	0.107345	?	?



Create Variant Set

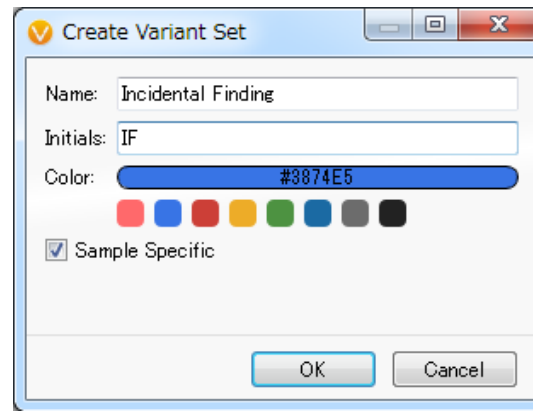
Name: Primary Finding

Initials: PF

Color: #FF696B

Sample Specific

OK Cancel



Create Variant Set

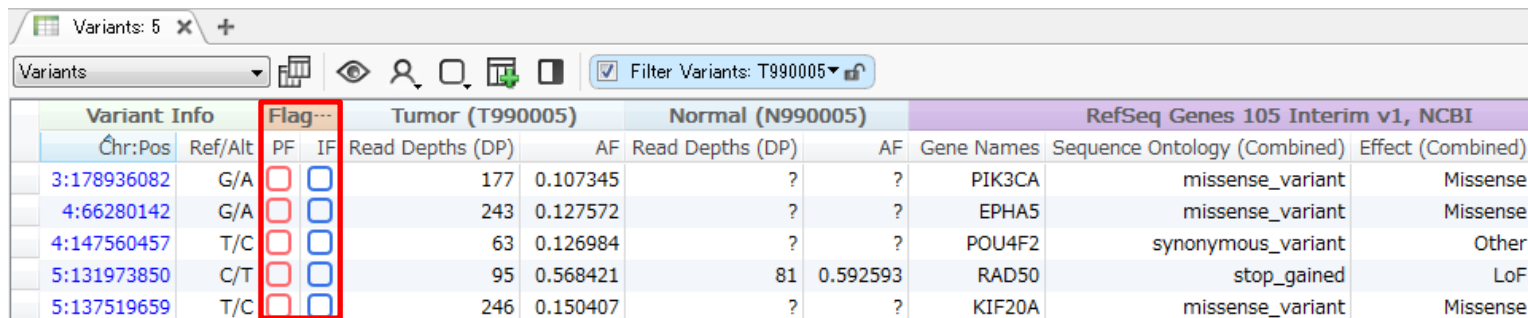
Name: Incidental Finding

Initials: IF

Color: #3874E5

Sample Specific

OK Cancel



Variants: 5 x +

Filter Variants: T990005

Variant Info		Flag...		Tumor (T990005)		Normal (N990005)		RefSeq Genes 105 Interim v1, NCBI		
Chr:Pos	Ref/Alt	PF	IF	Read Depths (DP)	AF	Read Depths (DP)	AF	Gene Names	Sequence Ontology (Combined)	Effect (Combined)
3:178936082	G/A	<input checked="" type="checkbox"/>	<input type="checkbox"/>	177	0.107345	?	?	PIK3CA	missense_variant	Missense
4:66280142	G/A	<input checked="" type="checkbox"/>	<input type="checkbox"/>	243	0.127572	?	?	EPHA5	missense_variant	Missense
4:147560457	T/C	<input checked="" type="checkbox"/>	<input type="checkbox"/>	63	0.126984	?	?	POU4F2	synonymous_variant	Other
5:131973850	C/T	<input checked="" type="checkbox"/>	<input type="checkbox"/>	95	0.568421	81	0.592593	RAD50	stop_gained	LoF
5:137519659	T/C	<input checked="" type="checkbox"/>	<input type="checkbox"/>	246	0.150407	?	?	KIF20A	missense_variant	Missense

1. 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
2. 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

Variants: 5

Filter Variants: T990005

Variant Info		Flag...		Tumor (T990005)		Normal (N990005)		Gene Names
Chr:Pos	Ref/Alt	PF	IF	Read Depths (DP)	AF	Read Depths (DP)	AF	
3:178936082	G/A	<input checked="" type="checkbox"/>	<input type="checkbox"/>	177	0.107345	?	?	PIK3CA
4:66280142	G/A	<input type="checkbox"/>	<input type="checkbox"/>	243	0.127572	?	?	EPHA5
4:147560457	T/C	<input type="checkbox"/>	<input type="checkbox"/>	63	0.126984	?	?	POU4F2
5:131973850	C/T	<input type="checkbox"/>	<input checked="" type="checkbox"/>	95	0.568421	81	0.592593	RAD50
5:137519659	T/C	<input type="checkbox"/>	<input type="checkbox"/>	246	0.150407	?	?	KIF20A



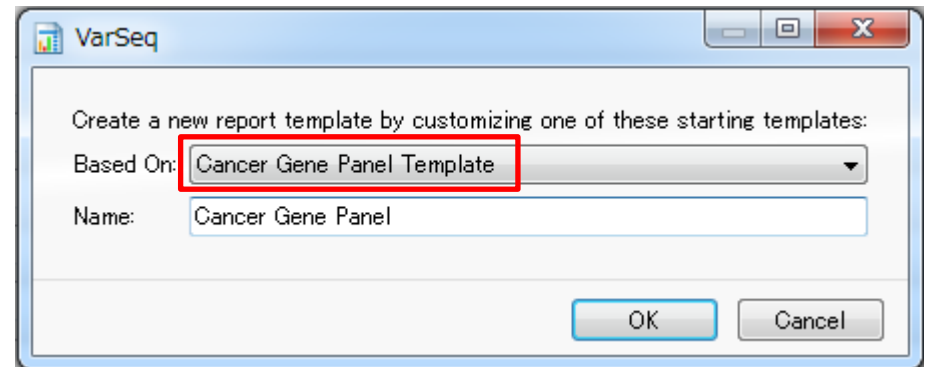
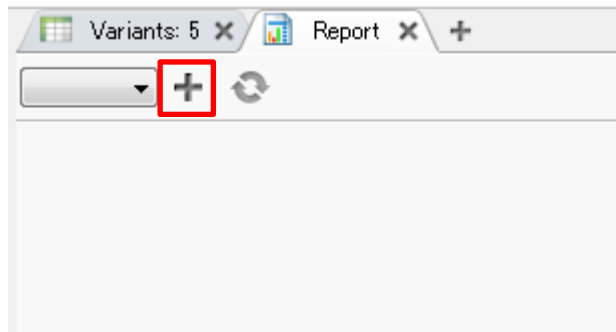
Variants: 5

Filter Variants: T990005

Variant Info		Flag...		Tumor (T990005)		Normal (N990005)		Gene Names
Chr:Pos	Ref/Alt	PF	IF	Read Depths (DP)	AF	Read Depths (DP)	AF	
3:178936082	G/A	<input checked="" type="checkbox"/>	<input type="checkbox"/>	177	0.107345	?	?	PIK3CA
4:66280142	G/A	<input type="checkbox"/>	<input type="checkbox"/>	243	0.127572	?	?	EPHA5
4:147560457	T/C	<input type="checkbox"/>	<input type="checkbox"/>	63	0.126984	?	?	POU4F2
5:131973850	C/T	<input type="checkbox"/>	<input checked="" type="checkbox"/>	95	0.568421	81	0.592593	RAD50
5:137519659	T/C	<input type="checkbox"/>	<input type="checkbox"/>	246	0.150407	?	?	KIF20A

- Assessment Catalog
- Filter
- GenomeBrowse
- Log
- Note
- Report**
- Table
- Web Browser
- VS Clinical

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

The screenshot shows a web application interface for creating a report. The browser tabs indicate 'Variants: 4' and 'Cancer Gene Panel'. The main content area is titled 'Cancer Gene Panel' and contains the following sections:

- Patient Information:** Name: T990005, Gender: Male, Date of Birth: 01/20/2019, Id: 1234.
- Reference Information:** Physician: <Insert Text>, Institution: <Insert Text>, Case Id: <Insert Text>.
- Sample Information:** Sample Site: <Insert Text>, Collection Method: <Insert Text>, Sample Type: <Insert Text>, Panel Coverage: , Avg. Read Depth: , Collection Date: 01/20/2019, Receipt Date: 01/20/2019, Report Date: 01/20/2019.
- Patient Result:** Result: Positive, Comment: Mutations with an establish somatic link detected.

A context menu is open in the top right corner, with the following options:

- Configure Report Template (highlighted with a red box)
- Reload Report Template
- Developer JavaScript Console
- Open Report Template Folder


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

The screenshot shows a 'Set Report Parameters' dialog box with two main sections: 'Lab Information' and 'Test Information'. The 'Lab Information' section includes fields for Name, Address, City, State, Zip Code, Phone Number, Fax Number, and Logo File. The 'Test Information' section includes fields for Test, Indication, Background, Method, and Limitations, each with a rich text editor toolbar (B, I, U, link). The 'Reportable Genes' field at the bottom is highlighted with a red box and contains the text: APC, CASP10, CDH1, CHEK2, ERBB2, FGFR2, IRF1, KLF6, KRAS, MSH3, MUTYH, PIK3CA. The dialog box has 'OK' and 'Cancel' buttons at the bottom right.

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

The screenshot shows the 'Cancer Gene Panel' interface with two variant entries. The first entry is for PIK3CA (3:178936082 G/A), classified as Pathogenic, with an interpretation of 'B I U' and a note about CLOVE Syndrome. The second entry is for RAD50 (5:131973850 C/T), classified as 'B I U', with a note about inheritance patterns. Red boxes highlight the dropdown menus for 'Primary Finding' and 'Incidental Finding' in both sections. A 'Report Signoff' section at the bottom has a 'Verify' checkbox that is unchecked.

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



Provider Information

Physician
Institution
Case Id

Phone:
Fax:

Patient Information

Name T990005
Gender Male
Date of Birth 1/20/2019
Id 1234

Sample Information

Sample Site
Sample Type
Collection Method
Panel Coverage

Avg. Read Depth
Collection Date 1/20/2019
Receipt Date 1/20/2019
Report Date 1/20/2019

Results

Positive: Mutations with an establish somatic link detected.

Affected Genes

APC
(0)

CASP10
(0)

CDH1
(0)

CHEK2
(0)

ERBB2
(0)

FGFR2
(0)

IRF1
(0)

KLF6
(0)

KRAS
(0)

MSH3
(0)

MUTYH
(0)

PIK3CA
(1)

Primary Findings

Gene	Zygoty	Variant	Exon	Pathogenicity
PIK3CA	?	NM_006218.3:c.1624G>A(NP_006209.2:p.Glu542Lys)	10	Pathogenic

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

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

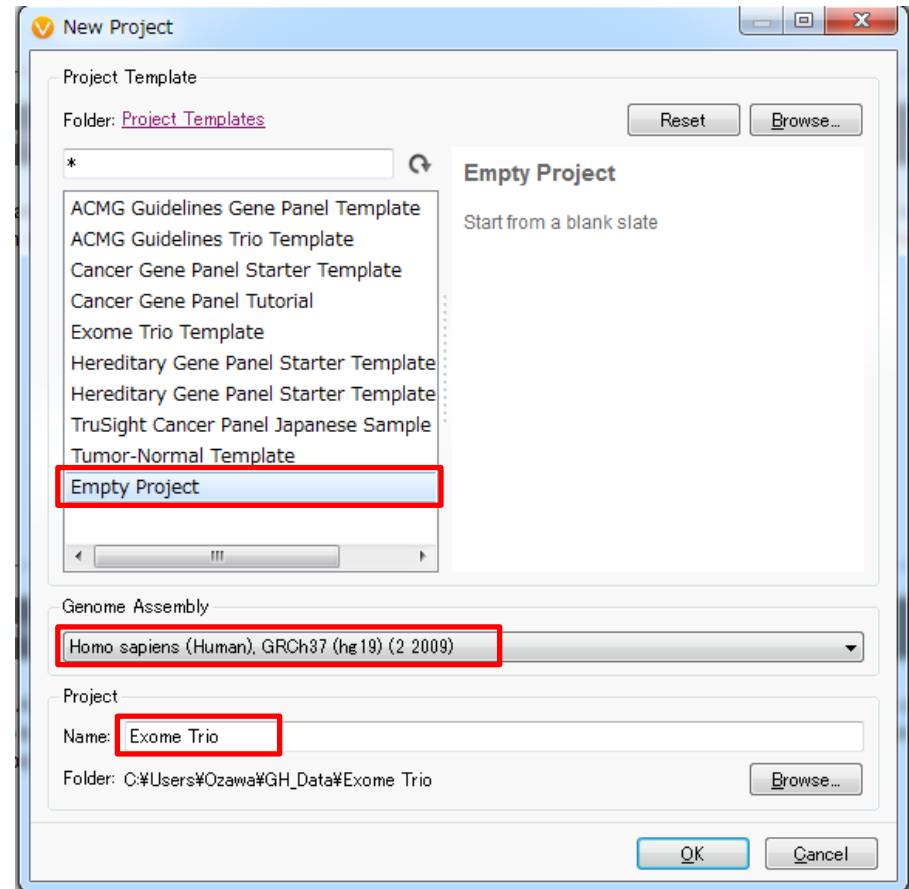
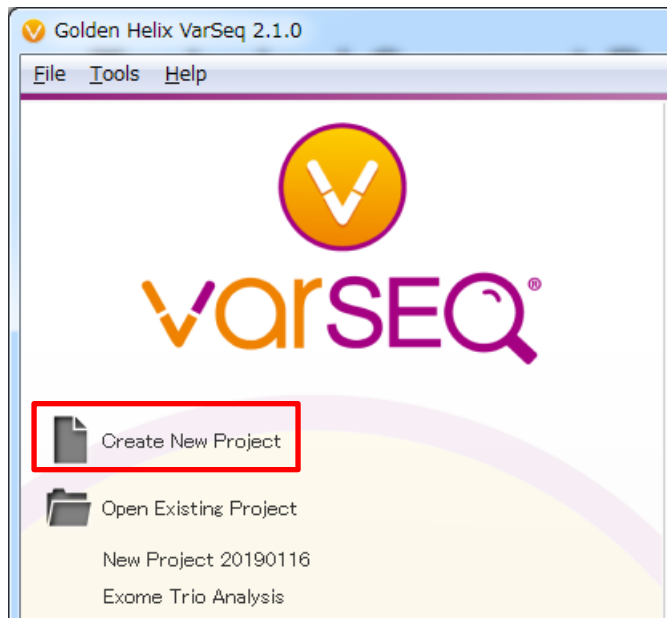
手順2 : アノテーション付加

- 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

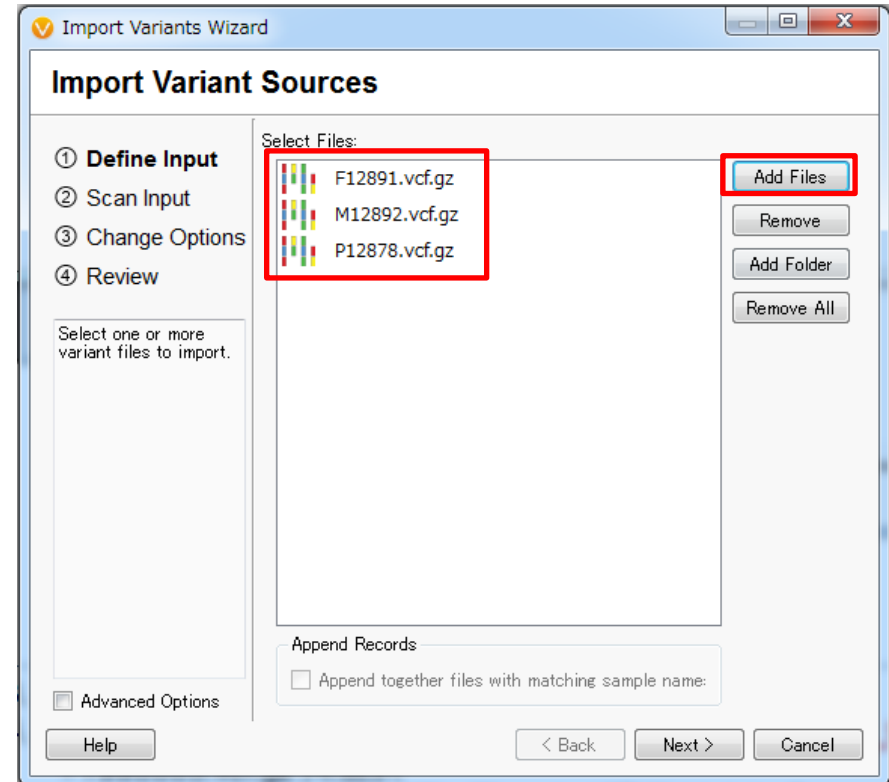
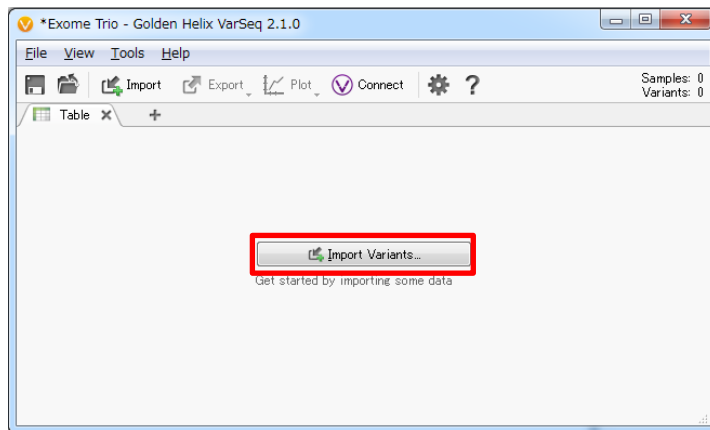
手順3 : フィルタリング

- 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

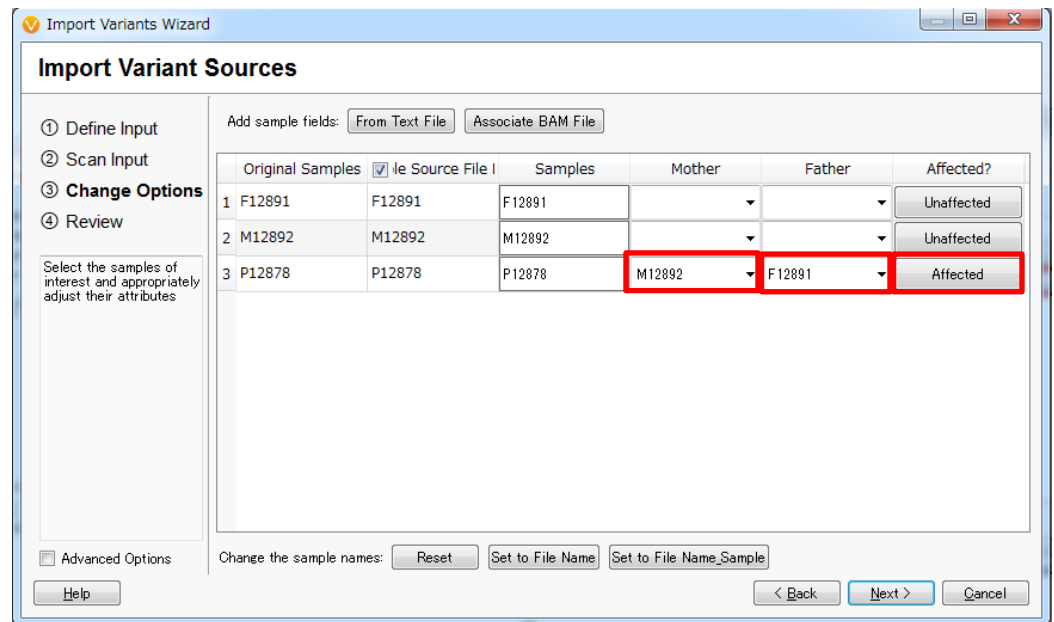
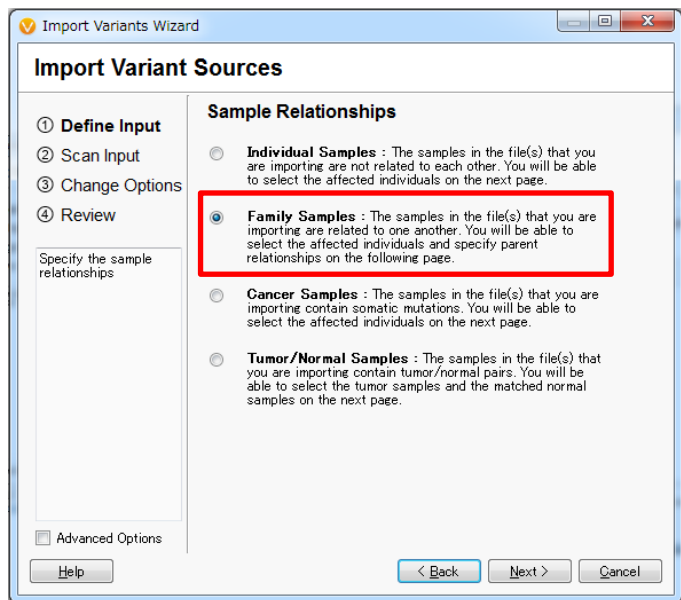
手順1. サンプルデータのインポート



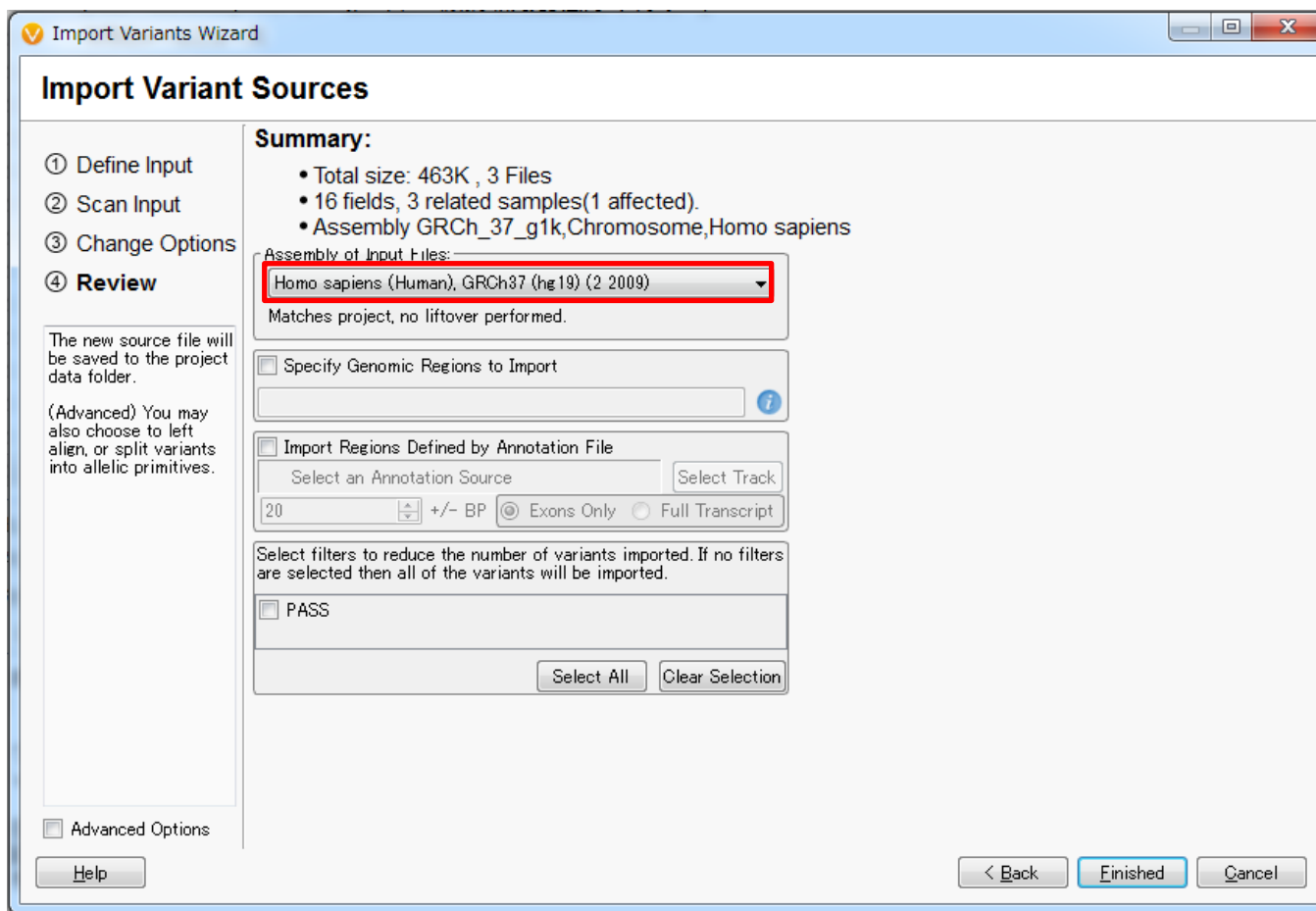
1. メイン画面の「Create New Project」をクリック
2. 任意のプロジェクト名を入力し、またProject Templateに「Empty Project」、Genome Assemblyが「Homo sapiens (Human), GRCh37 (hg19) (2 2009)」となっていることを確認したら「OK」をクリック



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



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



8. Assembly of Input Files(こ、「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 VarSeq 2.1.0

File View Tools Help

Connect Proband (P12878) ?

Samples: 3
Variants: 13,086

Filter Variants x + Variants: 13,086 x +

Filter Variants 13,086

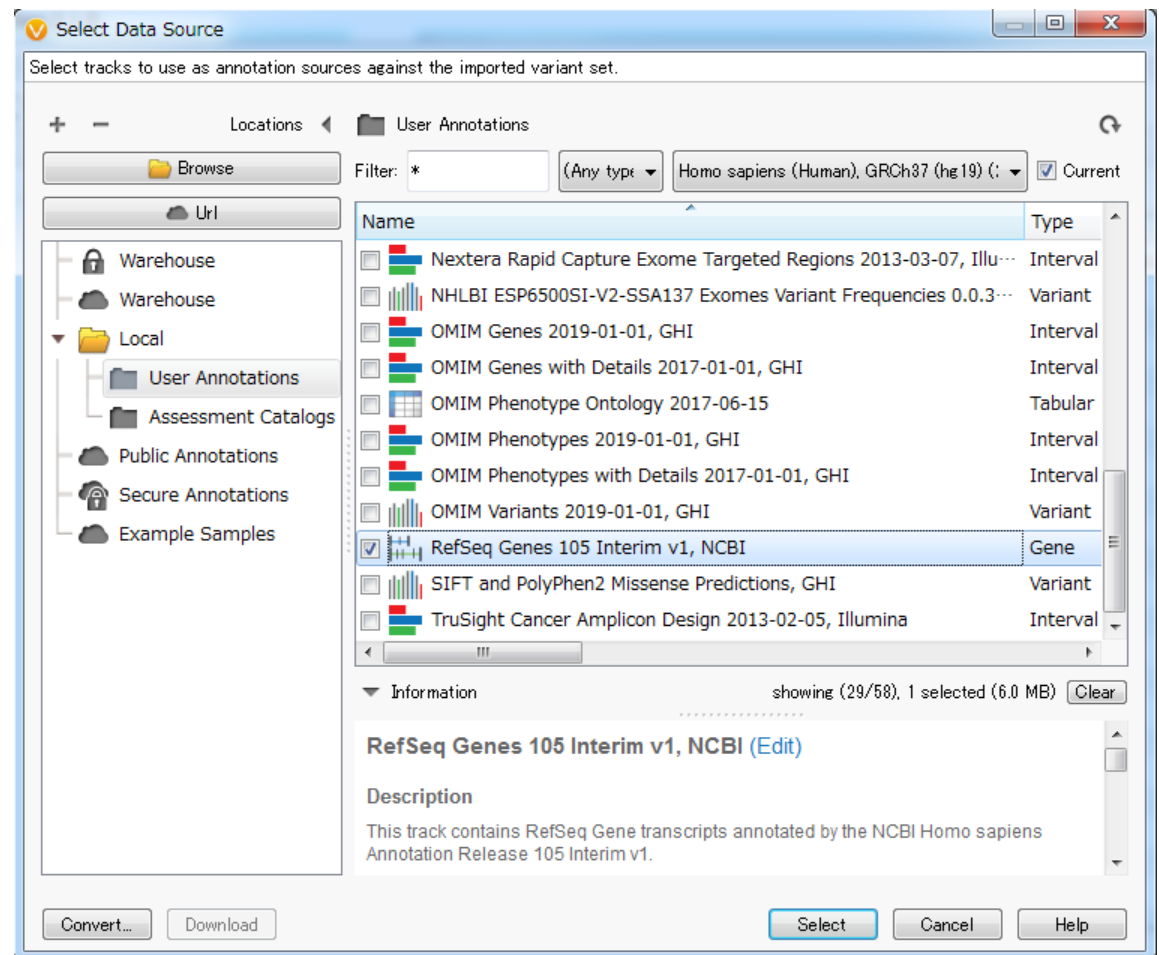
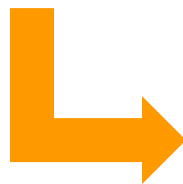
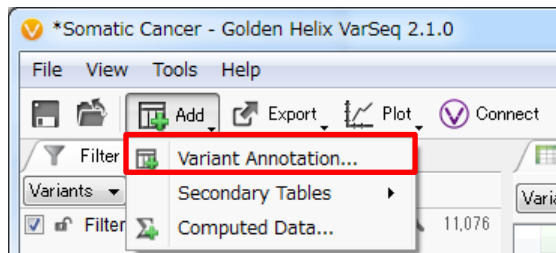
Filter Variants: P12878

Variants: 13,086

Variant Info		Proband (P12878)		Mother (M12892)		Father (F12891)	
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	T/A	0.411765	17	0.545455	55	?	?
6:304900	TA/-	1	14	0.948276	58	?	?
6:305095	C/T	?	?	0.357143	14	0.6	10
6:308331	G/A	0.333333	12	?	?	?	?
6:309718	AT/-	0.916667	12	?	?	?	?
6:311548	C/T	?	?	0.272727	11	?	?
6:311680	-/A	?	?	0.886792	58	?	?

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



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

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

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



- Proband (P12878)の「Read Depths (DP)」 ≥ 30
- Proband (P12878)の「Variant Allele Freq」 ≥ 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

The screenshot shows a 'Filter Variants' panel with 13,086 total variants. It contains four filter criteria, each with a sub-panel showing the number of variants that pass the filter:

- Read Depths (DP) (Current) ≥ 30** : 3,960 variants
- Variant Allele Freq (Current) ≥ 0.2** : 3,814 variants
- Allele Frequencies < 0.01 OR missing**: 566 variants total, with a breakdown:
 - Less than 0.01: 76
 - Equal to 0.01: 0
 - Greater than 0.01: 3,249
 - Missing: 490
- Alt_allele_freq < 0.01 OR missing**: 469 variants total, with a breakdown:
 - Less than 0.01: 24
 - Equal to 0.01: 0
 - Greater than 0.01: 97
 - Missing: 445

- 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

The screenshot shows a 'Filter Variants' panel with a total of 13,086 variants. The following filters are applied:

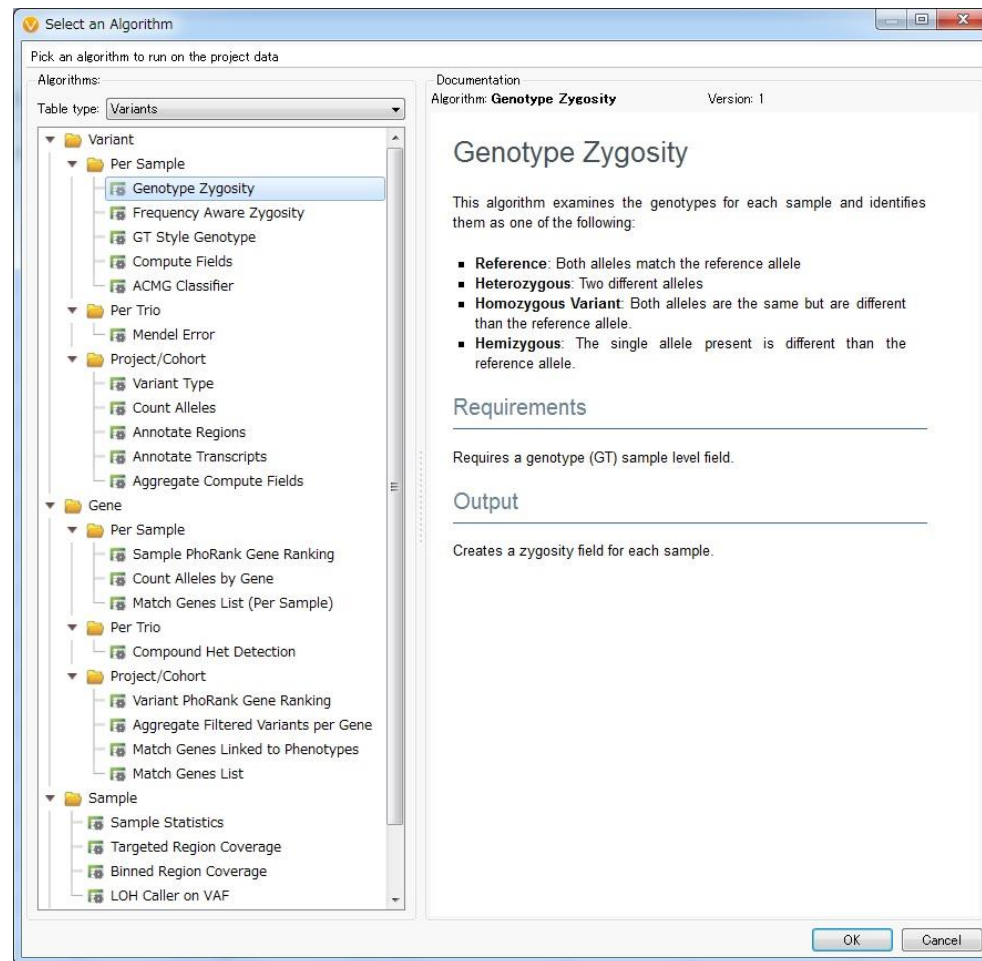
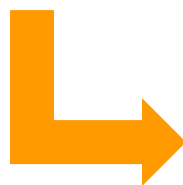
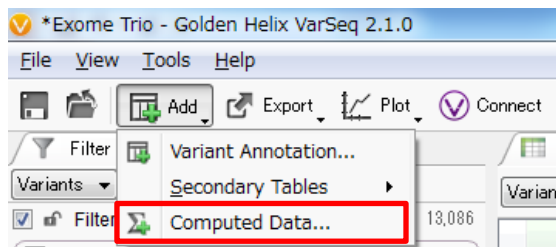
- Read Depths (DP) (Current) ≥ 30 : 3,960 variants
- Variant Allele Freq (Current) ≥ 0.2 : 3,814 variants
- Allele Frequencies < 0.01 OR missing: 566 variants
- Alt_allele_freq < 0.01 OR missing: 469 variants
- Effect (Combined) is (LoF, Missense): 134 variants

The 'Effect (Combined)' filter is expanded to show the following breakdown:

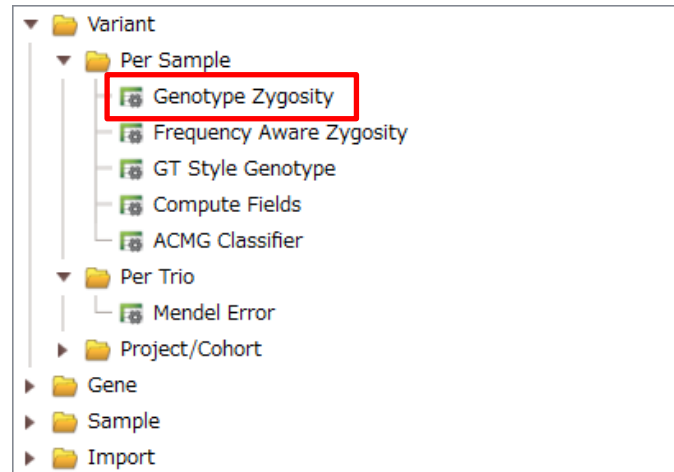
Effect	Count
LoF	8
Missense	126
Other	335
Missing	0
Total	134

- 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

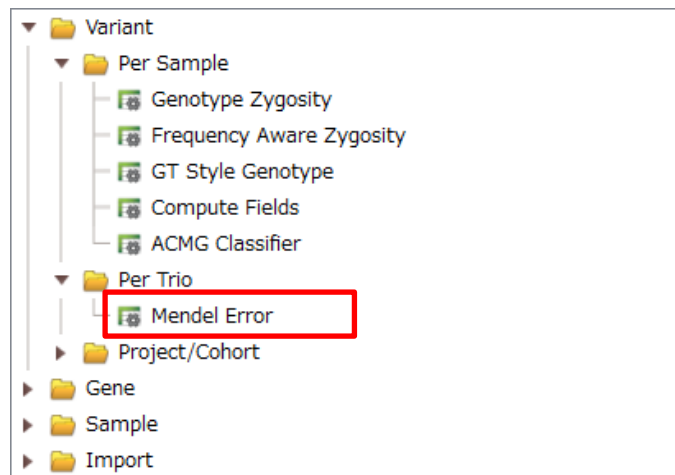


1. プロジェクト画面の「Add」をクリックし、メニューより「Computed Data」を選択してクリック
2. Select an Algorithm画面において、任意の解析アルゴリズムを選択して、「OK」をクリック
3. 解析アルゴリズムの種類によっては、計算時のパラメータなどを指定する
4. 多くの解析アルゴリズムでは、計算が終了すると、変異テーブルに計算結果のアノテーションが付加され、フィルタリングに使用できるようになる



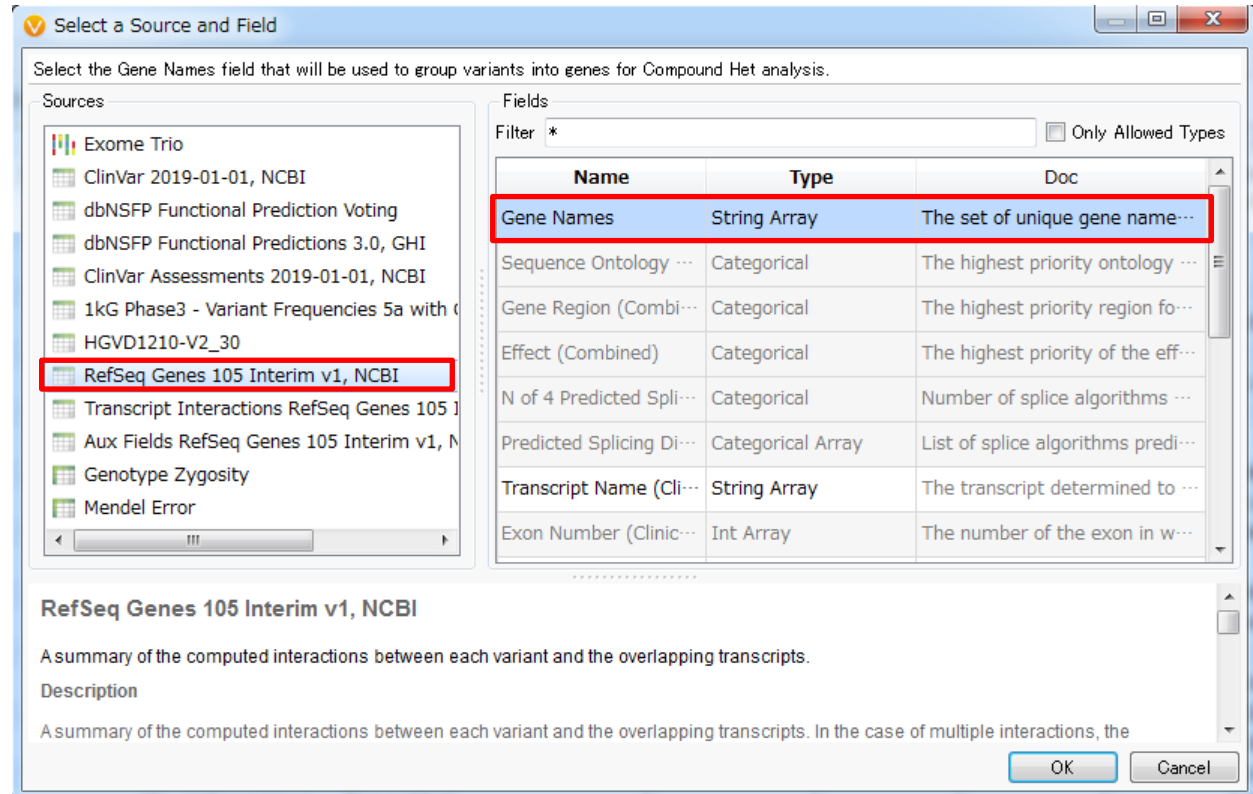
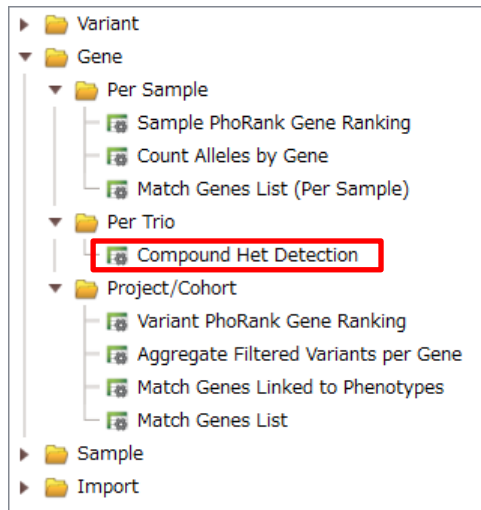
Variant Info		Proband (P12878)			Mother (M12892)			Father (F12891)		
Chr:Pos	Ref/Alt	Variant Allele Freq	Read Depths (DP)	Zygosity	Variant Allele Freq	Read Depths (DP)	Zygosity	Variant Allele Freq	Read Depths (DP)	Zygosity
6:564567	C/G	0.393443	122	Heterozygous	?	?	?	0.496552	145	Heterozygous
6:7405508	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

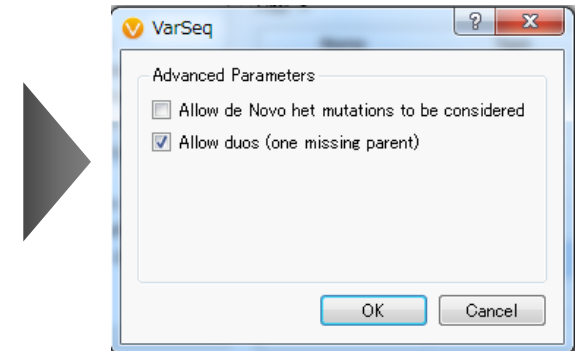
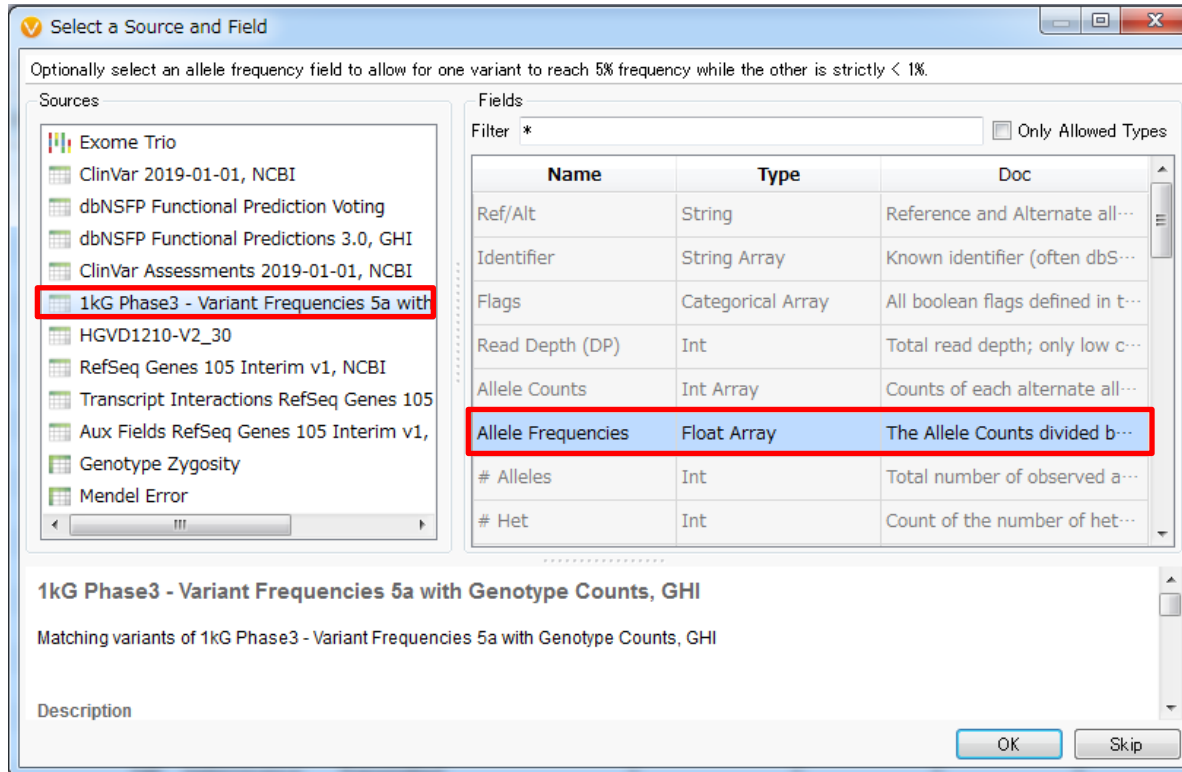


Variant Info		Proband (P12878)				Mother (M12892)				Father (F12891)			
Chr:Pos	Ref/Alt	Variant Allele Freq	Read Depths (DP)	Zygosity	Mendel Error	Variant Allele Freq	Read Depths (DP)	Zygosity	Mendel Error	Variant Allele Freq	Read Depths (DP)	Zygosity	Mendel Error
6:564567	C/G	0.393443	122	Heterozygous	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



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



- 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
- 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

ワークフロー

<input checked="" type="checkbox"/> Filter Variants	13,086
<input checked="" type="checkbox"/> Read Depths (DP) (Current) >= 30	3,960
<input checked="" type="checkbox"/> Variant Allele Freq (Current) >= 0.2	3,814
<input checked="" type="checkbox"/> Allele Frequencies < 0.01 OR missing	566
<input checked="" type="checkbox"/> Alt_allele_freq < 0.01 OR missing	469
<input checked="" type="checkbox"/> Effect (Combined) is (LoF, Missense)	134
<input checked="" type="checkbox"/> Compound Het? (Current)	-
True	3
False	131
Missing	0
	134

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

遺伝子テーブル

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

- 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
- 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

The image illustrates the process of creating a Trio Analysis workflow in three stages:

- Initial State:** A 'Filter Variants' panel with 13,086 variants. Filters include: Read Depths (DP) (Current) >= 30 (3,960), Variant Allele Freq (Current) >= 0.2 (3,814), Allele Frequencies < 0.01 OR missing (566), Alt_allele_freq < 0.01 OR missing (469), Effect (Combined) is (LoF, Missense) (134), and Compound Het? (Current) is true (3 True, 131 False, 0 Missing). A context menu is open, highlighting 'Add Filter Container'.
- Trio Analysis Added:** A new 'Trio Analysis' filter is added to the list. The logic is set to 'OR' (Results satisfy criteria of any contained filter). The 'Compound Het?' filter is also visible within this container.
- Final Configuration:** The 'Compound Het?' filter is now nested inside the 'Trio Analysis' container. The 'Trio Analysis' filter remains selected with 'OR' logic.

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

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 566
- Alt_allele_freq < 0.01 OR missing 469
- Effect (Combined) is (LoF, Missense) 134

Trio Analysis

Compound Het?	Count
True	3
False	131
Missing	0
Total	3

Mendel Error (Current)	Count
MIE	1
Transmitted	96
Untransmitted	0
de Novo Allele	37
Missing	0
Total	37

40

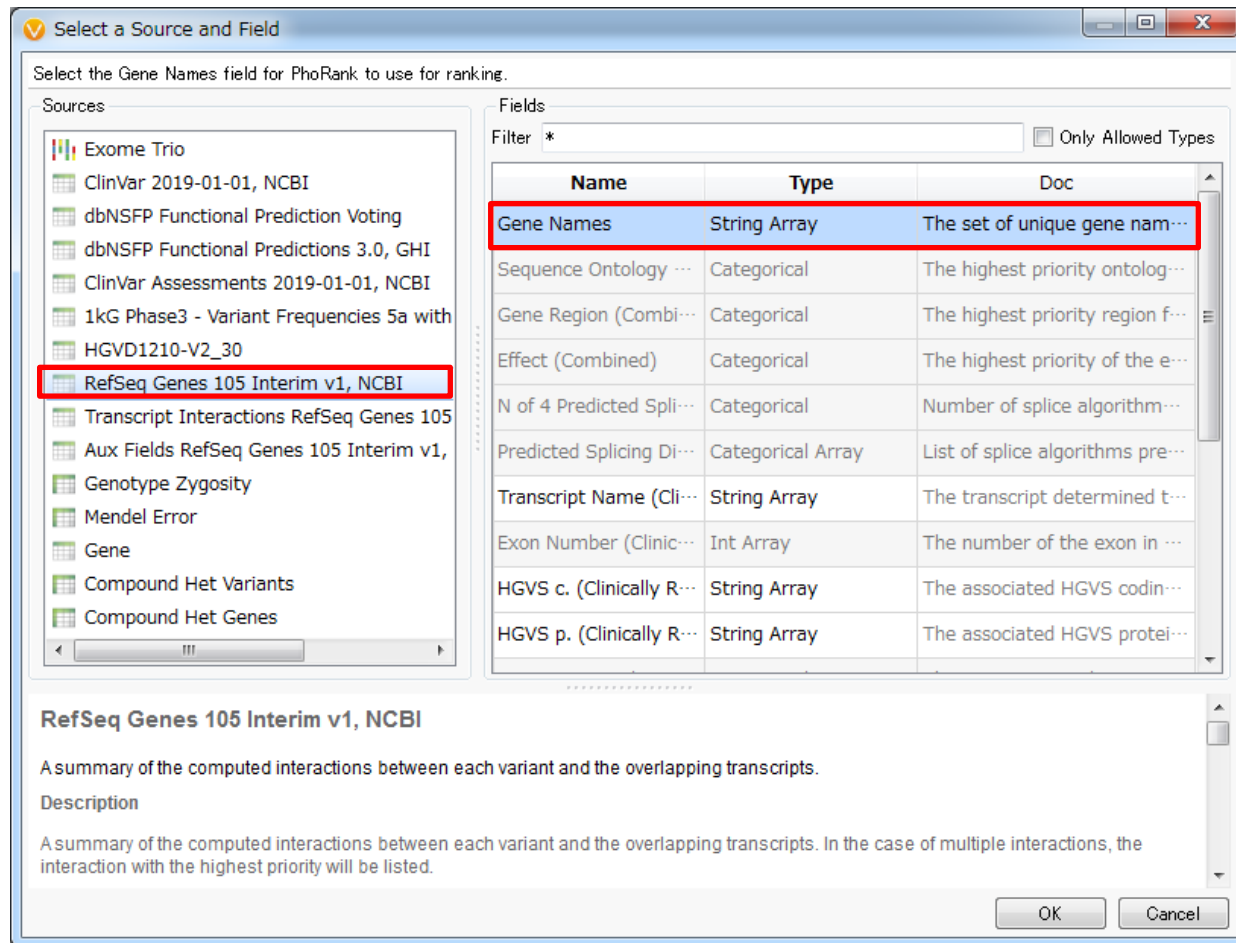
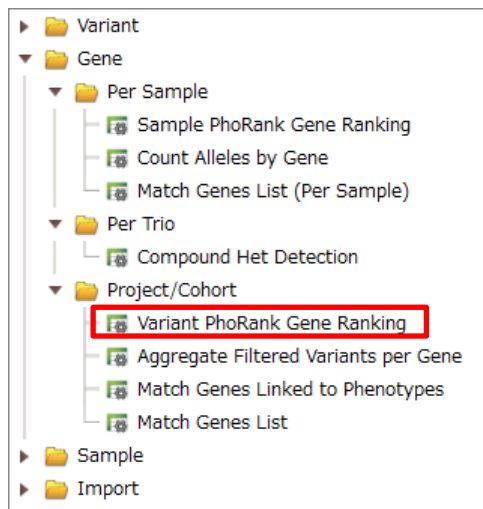
- Proband (P12878)の「Mendel Error」is de Novo Allele

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

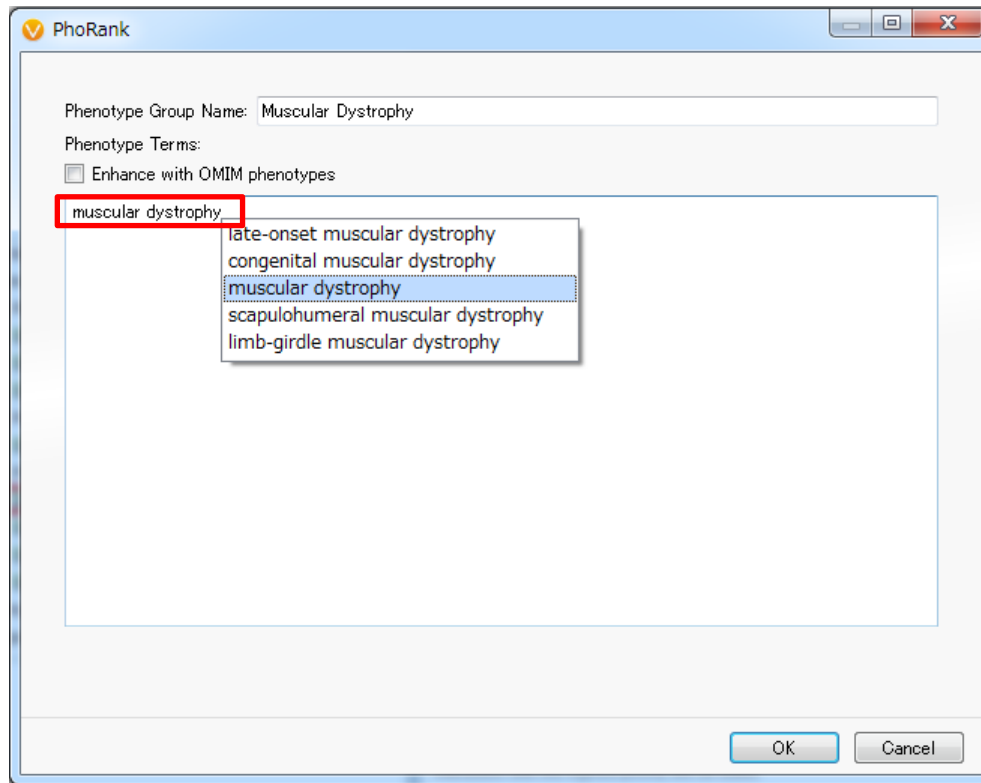
The screenshot shows the 'Trio Analysis' window with three filter containers. The 'Compound Het?' container has 3 items (True, False, Missing). The 'Mendel Error (Current)' container has 37 items (MIE, Transmitted, Untransmitted, de Novo Allele, Missing). The 'Recessive Homozygous' container is expanded to show three sub-filters: 'Zygosity (Current) is Homozygous Vari' with 133 items (Heterozygous, Homozygous Variant, Reference, Missing); 'Zygosity (Mother) is Heterozygous' with 0 items (Heterozygous, Homozygous Variant, Reference, Missing); and 'Zygosity (Father) is Heterozygous' with 0 items (Heterozygous, Homozygous Variant, Reference, Missing).

- 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



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



Muscular Dystrophy PhoRank		
Gene Rank	Gene Score	Path
0.425273	0.00136835	SLC35B3 / G...
0.221142	0.000465594	HIVEP1 / GO...
0.379101	0.000974205	JARID2 / GO...
0.281896	0.000590947	KIF13A / GO...
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 / ...

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

Filter Variants x +

Variants

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 566

Alt_allele_freq < 0.01 OR missing 469

Effect (Combined) is (LoF, Missense) 134

Trio Analysis

Compound Het? | 3

True 3

False 131

Missing 0

3

Mendel Error (Current) | 1

MIE 1

Transmitted 96

Untransmitted 0

de Novo Allele 37

Missing 0

37

Recessive Homozygous | 1

Zygosity (Current) is Homozygous Vari: 1

Zygosity (Mother) is Heterozygous 0

Zygosity (Father) is Heterozygous 0

0

40

Variants: 40 x Variant Genes: 902 x +

Variants

Trio Analysis: P12878

Variant Info			Proband (P12878)			
Chr:Pos	Ref/Alt	Variant Allele Freq	Read Depths (DP)	Zygosity	Mendel Error	
6:8428249	A/C	0.505155	194	Heterozygous	de Novo Allele	
6:12124988	T/C	0.433862	189	Heterozygous	de Novo Allele	
6:15501276	C/G	0.417722	79	Heterozygous	de Novo Allele	
6:17794528	A/C	0.381818	112	Heterozygous	de Novo Allele	
6:30622603	G/T	0.449275	69	Heterozygous	de Novo Allele	
6:31626986	C/G	0.244444	91	Heterozygous	de Novo Allele	
6:31675842	A/G	0.39375	160	Heterozygous	de Novo Allele	
6:31938841	T/C	0.4375	112	Heterozygous	de Novo Allele	
6:32135202	A/G	0.528302	54	Heterozygous	de Novo Allele	
6:32155057	T/G	0.438596	57	Heterozygous	de Novo Allele	
6:32156189	G/C	0.470588	34	Heterozygous	de Novo Allele	
6:32725625	T/C	0.321429	57	Heterozygous	de Novo Allele	
6:33410691	T/C	0.343137	102	Heterozygous	de Novo Allele	
6:34985432	G/C	0.210526	38	Heterozygous	de Novo Allele	
6:35782521	A/G	0.523077	65	Heterozygous	de Novo Allele	
6:41533574	C/A	0.6	146	Heterozygous	de Novo Allele	
6:43267651	A/G	0.5	112	Heterozygous	de Novo Allele	
6:43581563	A/C	0.588235	102	Heterozygous	de Novo Allele	
6:44108008	C/G	0.456522	46	Heterozygous	de Novo Allele	
6:47649853	G/C	0.380282	71	Heterozygous	de Novo Allele	
6:69685178	A/C	0.507692	65	Heterozygous	de Novo Allele	
6:75893766	G/T	0.269565	115	Heterozygous	de Novo Allele	
6:83877723	C/A	0.34375	97	Heterozygous	de Novo Allele	
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

Variants: 40 x Variant Genes: 37 x +

Variants by Variant Genes Trio Analysis: P12878 GeneRank

Variant Gene	Compound Het Genes for Proband (P12878)				Muscular Dystrophy PhoRank		Variant Info					
	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	0.990279	0.736401	6:152470619	C/G	0.538462	119	Heterozygous	Transmitted
SYNE1	True	2	1	1	0.986634	0.735329	6:152542036	C/G	0.352941	153	Heterozygous	de Novo Allele
FIG4	False	0	0	0	0.855407	0.0306254	6:152555877	C/T	0.47191	90	Heterozygous	Transmitted
PGM3	False	0	0	0	0.749696	0.0131189	6:152784621	T/C	0.482759	58	Heterozygous	Transmitted
SYNGAP1	False	0	0	0	0.550425	0.00761623	6:152786454	C/T	0.421053	152	Heterozygous	de Novo Allele
FGFR10P	False	0	0	0	0.470231	0.00190696						
SLC35B3	False	0	0	0	0.425273	0.00136835						
CLDN20	False	0	0	0	0.399757	0.00108969						
JARID2	False	0	0	0	0.379101	0.000974205						
DHX16	False	1	0	0	0.377886	0.000968939						
HLA-DQB2	False	0	0	0	0.36695	0.000910578						
ADGRB3	False	1	0	0	0.35723	0.000870164						
SF3B5	False	0	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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