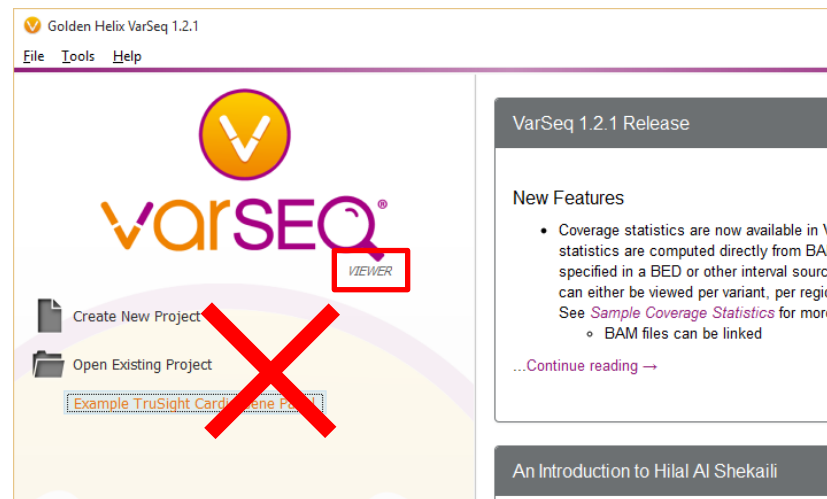
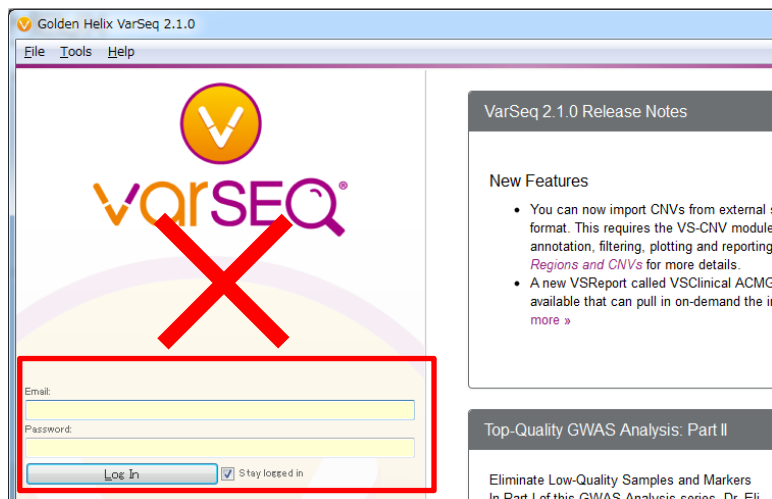
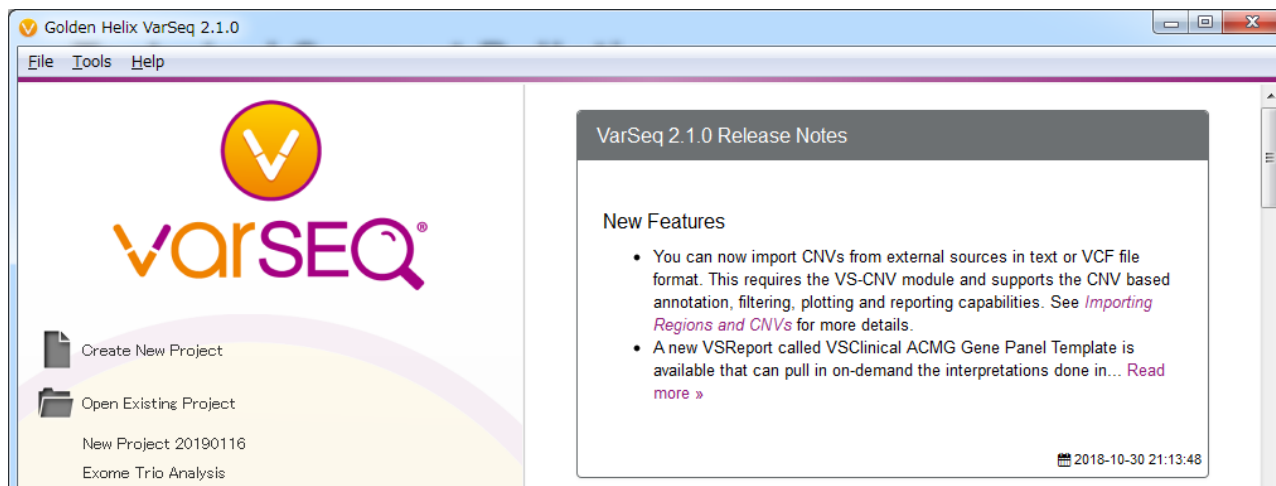
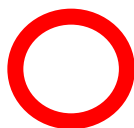


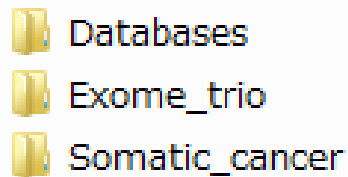
2019年11月15日 臨床ゲノム情報解析ハンズオントレーニング

 **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)

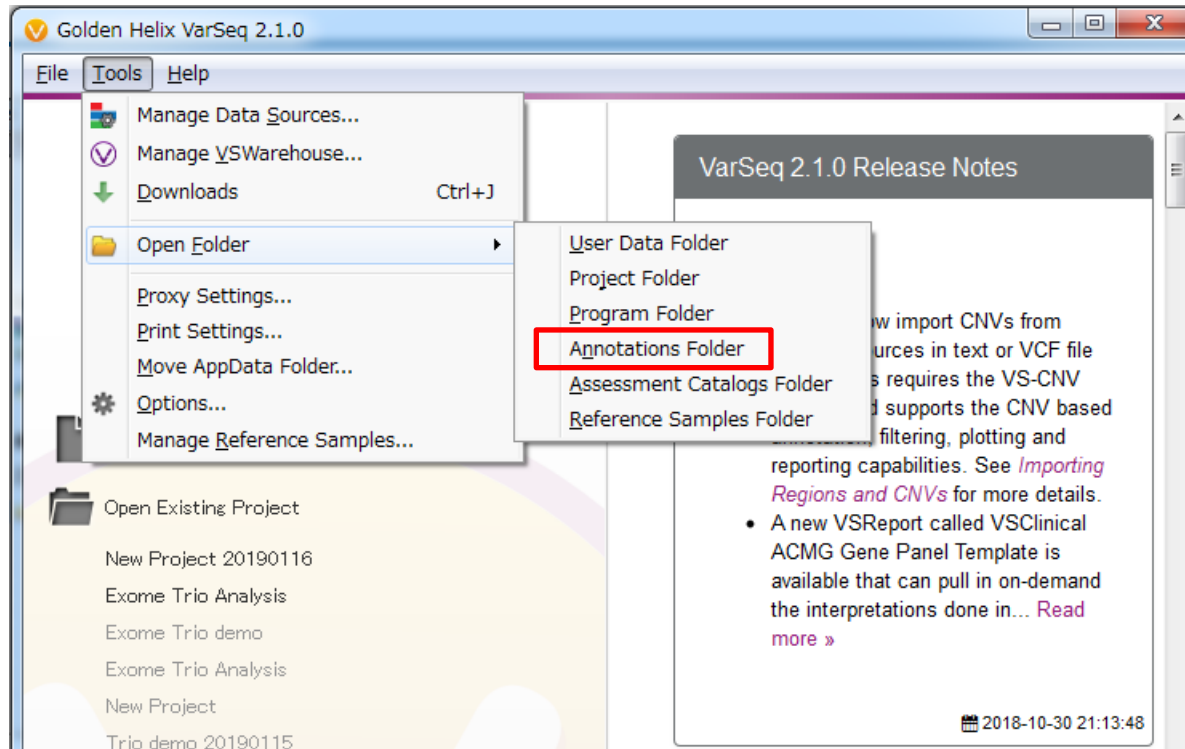


- VarSeqを起動した際に、上の図のように表示されていることをご確認ください。下の図のように、ログイン画面や、「Viewer」と表示されている状態では、ソフトウェアを使用できません。



- ✓ **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

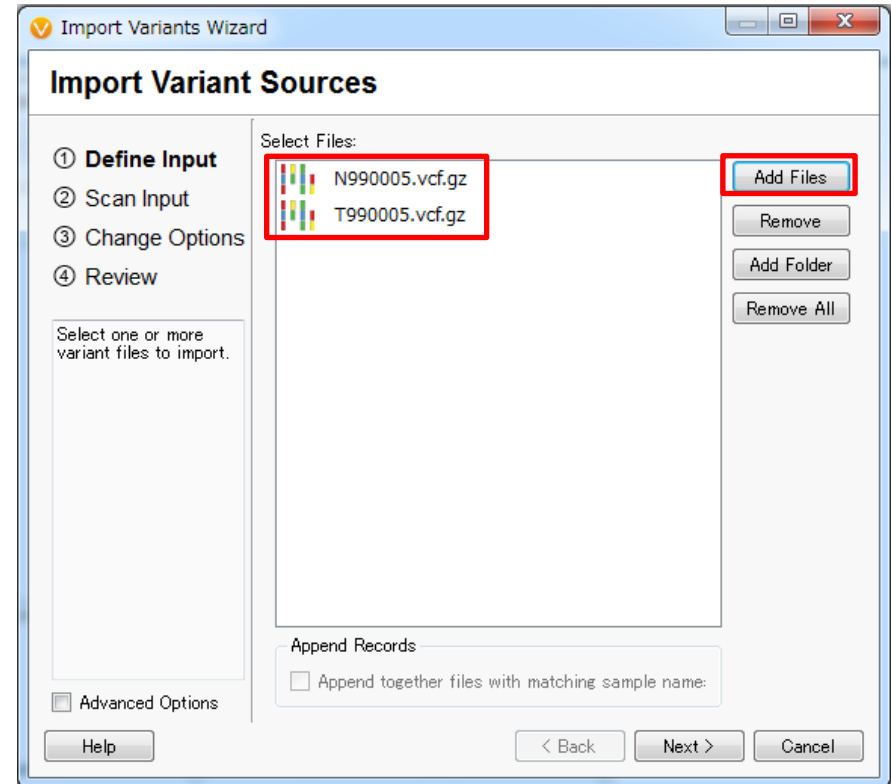
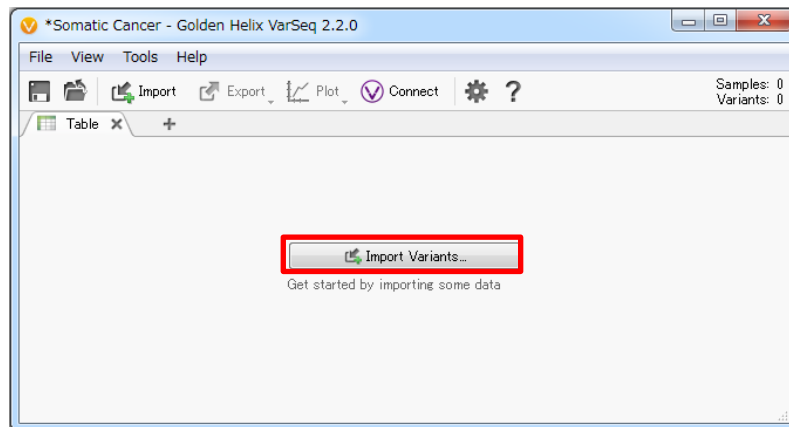
手順3 : フィルタリング

- 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

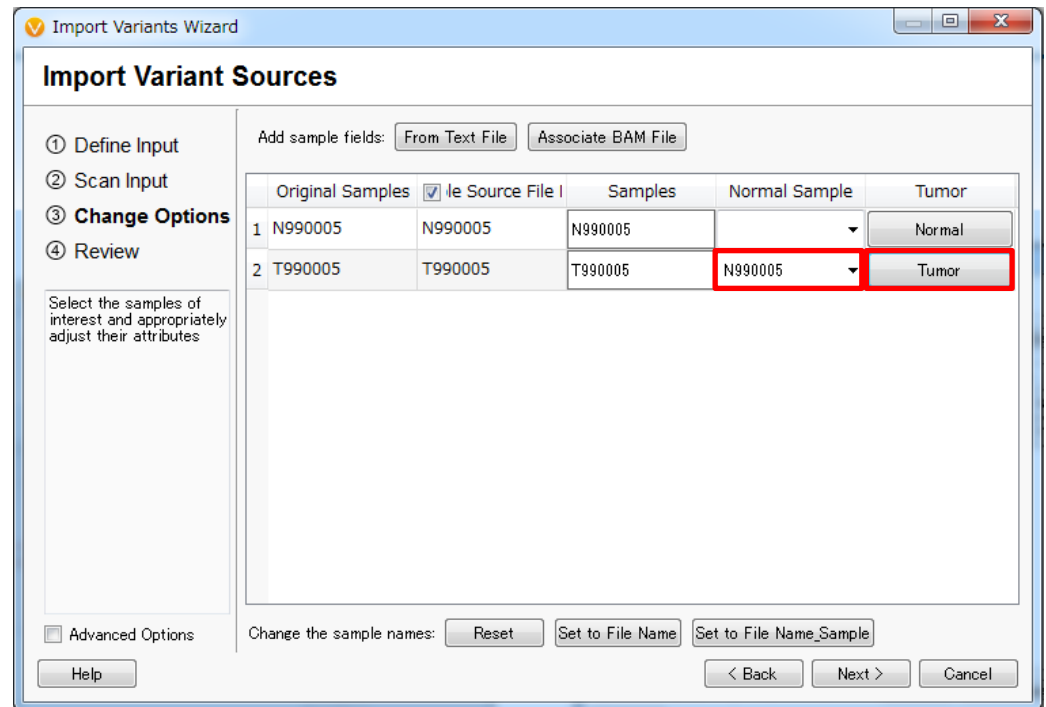
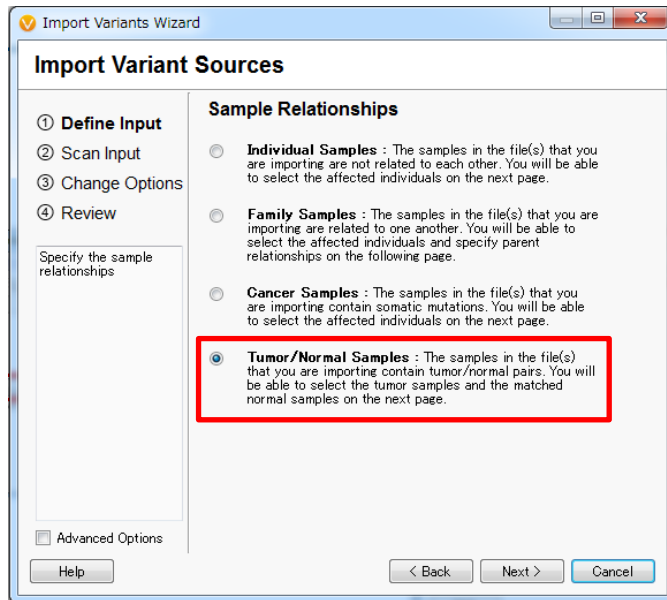
手順4 : レポート作成

- 抽出変異情報を用いてレポートの作成

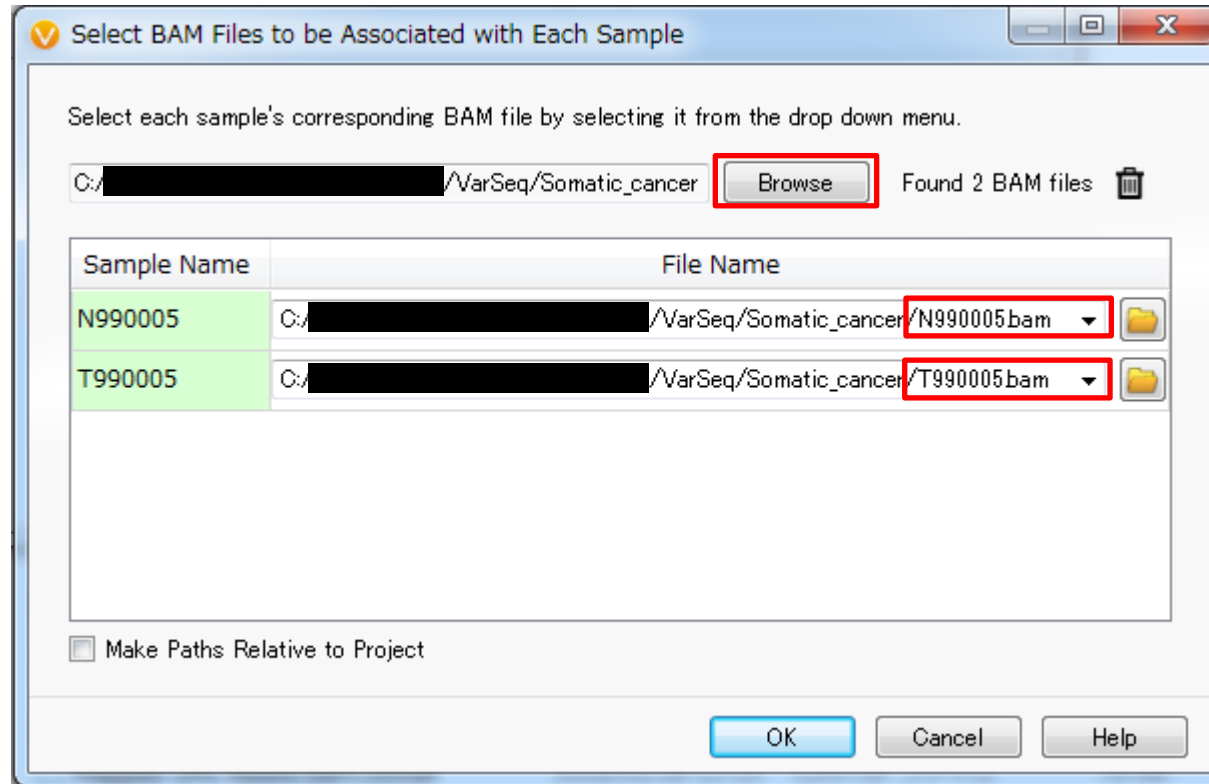
手順1. サンプルデータのインポート



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

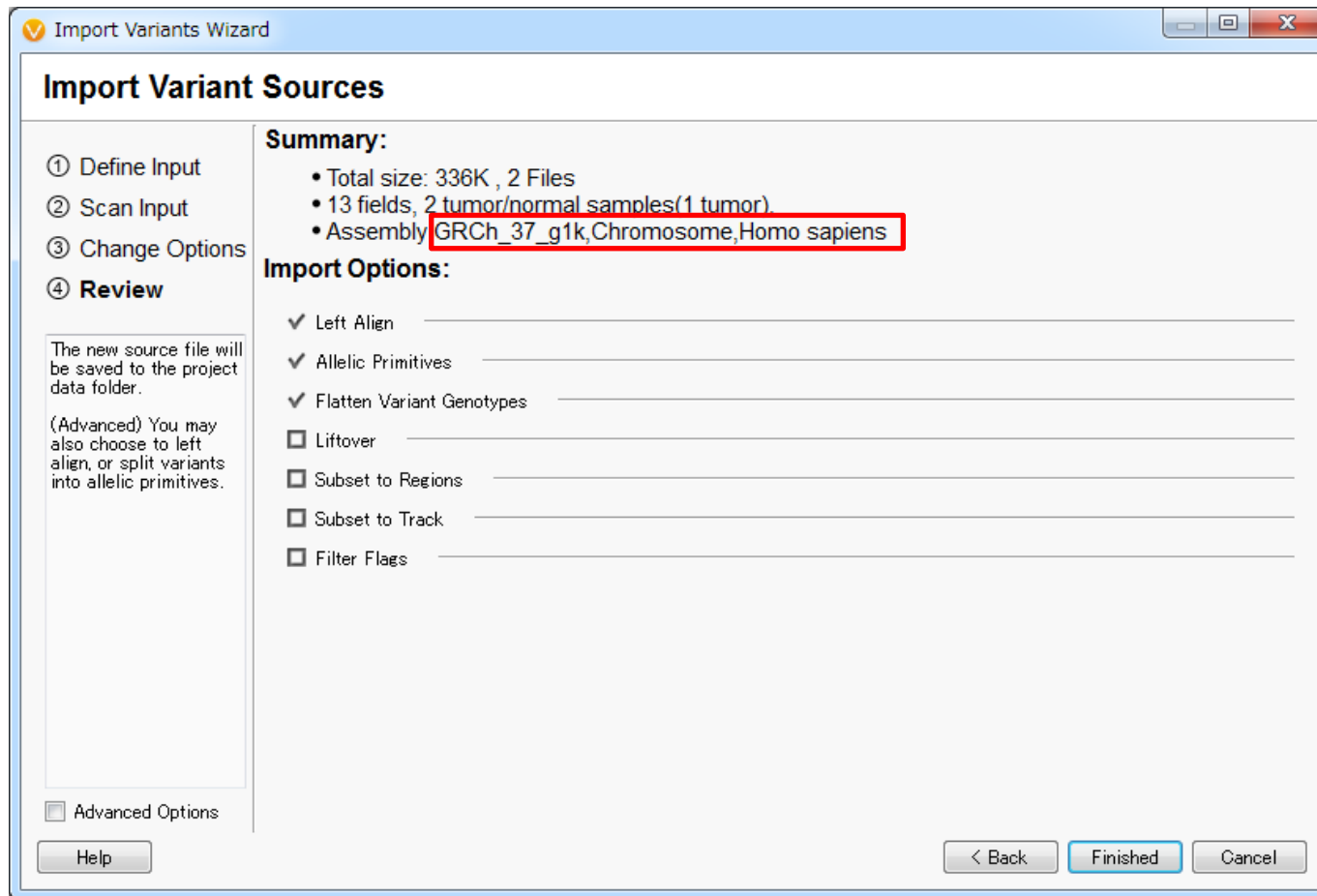
Help

Add sample fields:

	Original Samples	<input checked="" type="checkbox"/> File 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に、「GRCh_37_g1K, Chromosome, Homo sapiens」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 2.2.0

File View Tools Help

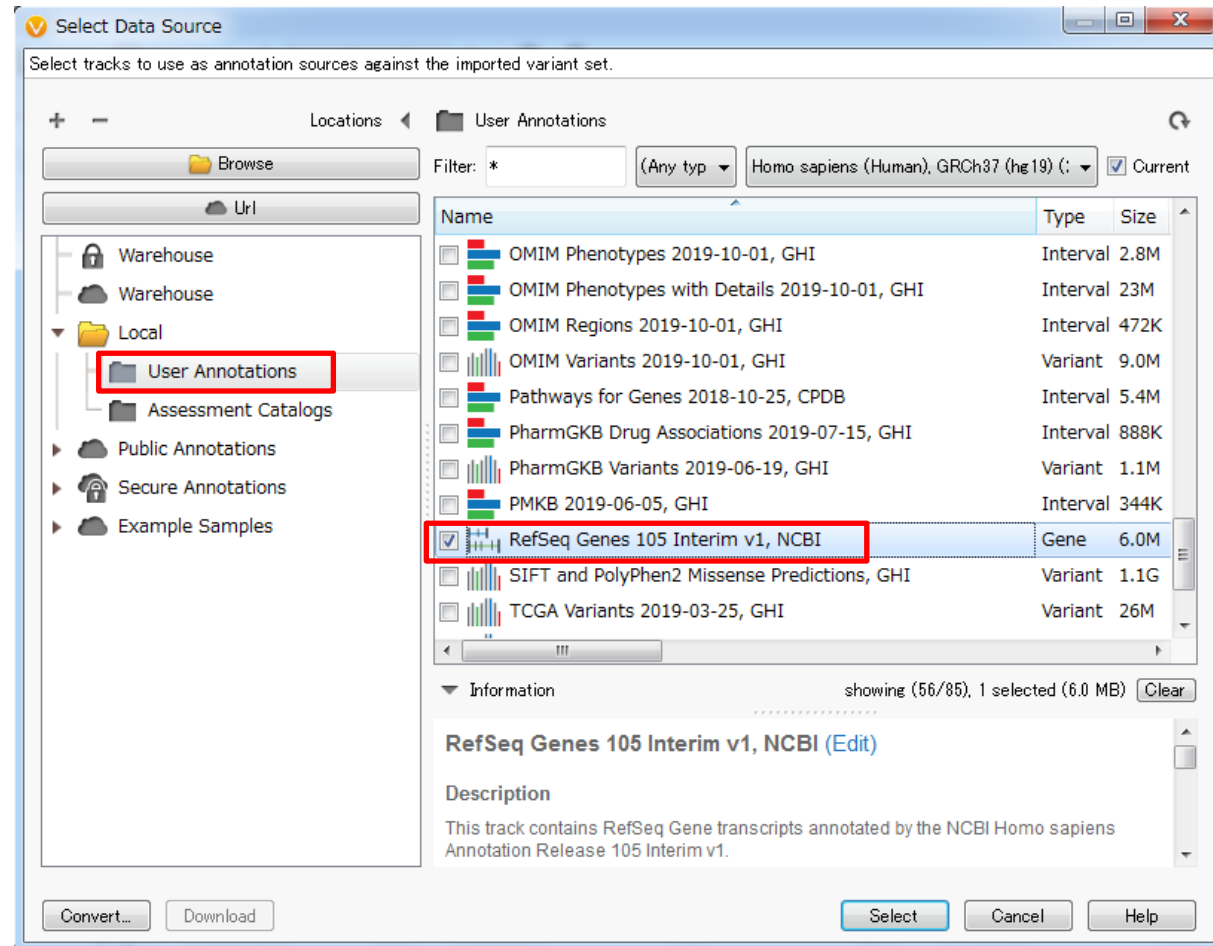
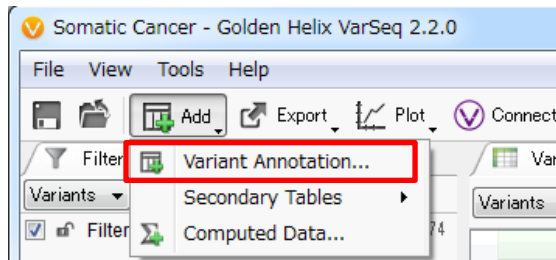
Connect Tumor (T990005) Samples: 2 Variants: 11,074

Filter Variants Variants: 11,074 Filter Variants: T990005 Variants: 11,074

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	GTTTT/-	?	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
3:2404094	C/G	rs1502572	10	1/1	1	15	1/1	1
3:2404329	C/T	rs1847169	34	1/1	1	15	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 +

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)
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.

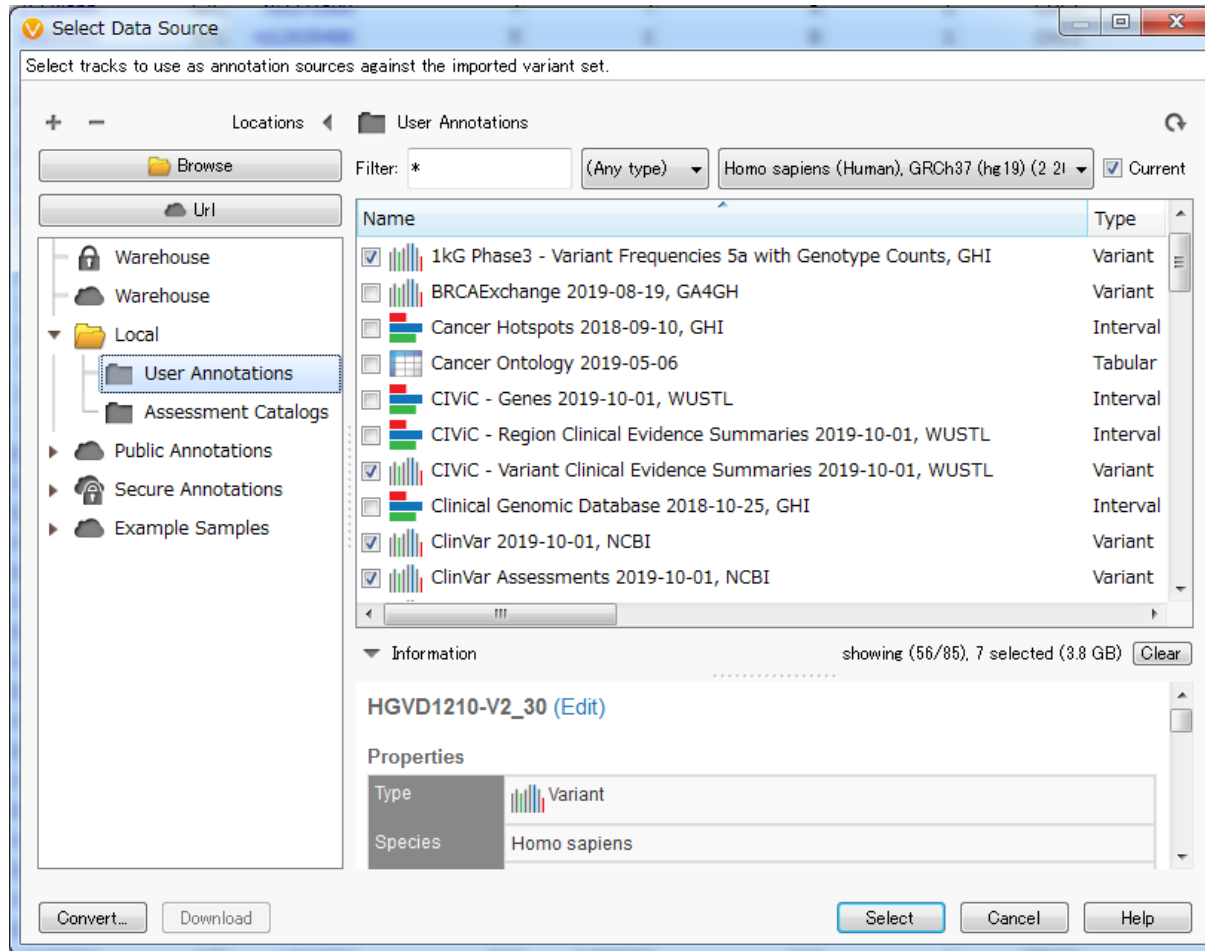
Category Counts (11,076 Records)

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
- CIViC - Variant
- ClinVar
- ClinVar Assessments
- COSMIC Mutations 89
- 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: 11,074 x +

Filter Variants Input: 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 Sur...	
A/G	25382	Benign	Benign	Benign (7) (2 Stars) Criteria Provided, Multiple Sur...	
A/G	94101	Benign	Benign	Benign (5) (2 Stars) Criteria Provided, Multiple Sur...	
T/C	94102	Benign	Benign	Benign (4) (2 Stars) Criteria Provided, Multiple Sur...	
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 Sur...	
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 ↻ 🔍 11,074

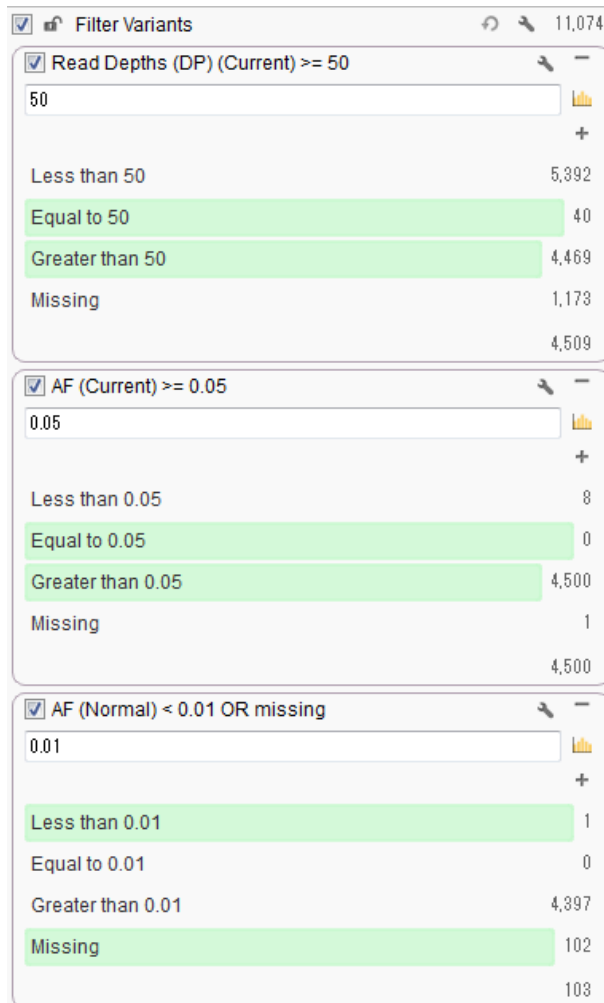
Read Depths (DP) (Current) >= 50

50 📊 +

Less than 50	5,392
Equal to 50	40
Greater than 50	4,469
Missing	1,173

4,509

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 11,074

- Read Depths (DP) (Current) >= 50 4,509
- AF (Current) >= 0.05 4,500
- AF (Normal) < 0.01 OR missing 103
- In COSMIC? is true 29
 - True 29
 - False 74
 - Missing 0
- Sample Count >= 100 1
 - 100 1
 - Less than 100 28
 - Equal to 100 0
 - Greater than 100 1
 - Missing 0

- COSMIC Mutationsの「In COSMIC?」 is TRUE
- COSMIC Mutationsの「Sample Count」 \geq 100

2. データベースに高頻度に登録されている生体に有害な変異の抽出のため、COSMIC Mutationsの「In COSMIC?」、「Sample Count」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. It consists of three sequential screenshots of a software interface. In the first screenshot, a list of filters is shown with counts: Read Depths (DP) (Current) >= 50 (4,509), AF (Current) >= 0.05 (4,500), AF (Normal) < 0.01 OR missing (103), In COSMIC? is true (29), and Sample Count >= 100 (1). A context menu is open, and 'Add Filter Container' is selected. The second screenshot shows the 'Somatic' container added to the list, highlighted with a red box. The third screenshot shows all the original filters moved into the 'Somatic' container.

3. 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
4. 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
5. 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

生殖細胞変異抽出ワークフローの作成

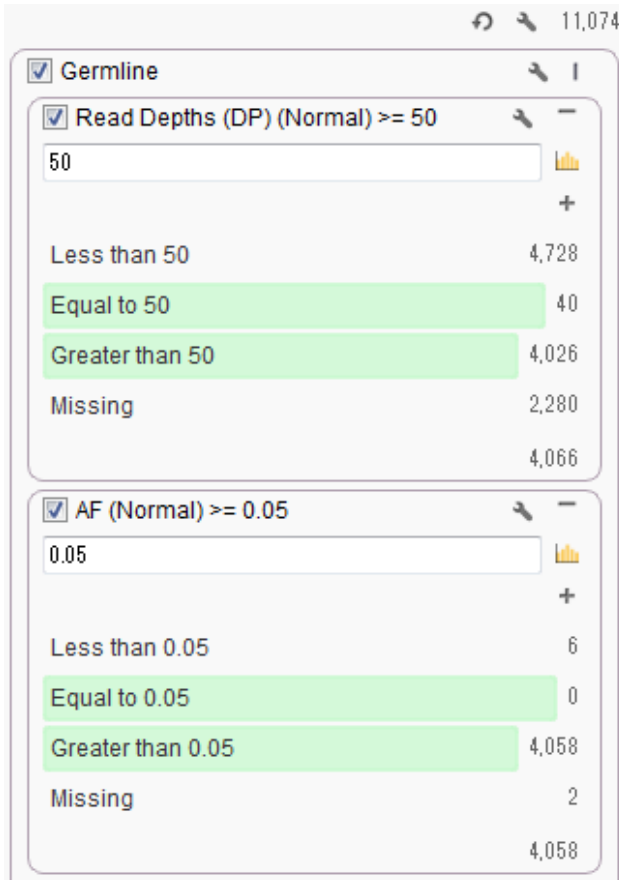
The image illustrates the steps to create a germline filter container in the Filgen software. It shows three sequential screenshots of the 'Filter Variants' configuration window.

Initial Configuration: The 'Filter Variants' window shows the 'OR' radio button selected under the logic options. The 'Somatic' container is active and contains two filters: 'Read Depths (DP) (Current) >' (4,509) and 'AF (Current) >= 0.05' (4,500).

Adding a Container: A right-click context menu is shown over the 'AF (Current) >= 0.05' filter. The 'Add Filter Container' option is highlighted with a red box.

Final Configuration: The 'Filter Variants' window now shows a new 'Germline' container added to the right of the 'Somatic' container. The 'Germline' container is highlighted with a red box. The 'Somatic' container now includes three filters: 'Read Depths (DP) (Current) >' (4,509), 'AF (Current) >= 0.05' (4,500), and 'AF (Normal) < 0.01 OR missir' (103). The 'Germline' container contains one filter: 'In COSMIC? is true' (29). The 'Sample Count' for the entire configuration is 1.

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」を選択してクリック
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

11,074

- Germline
 - Read Depths (DP) (Normal) ≥ 50 4,066
 - AF (Normal) ≥ 0.05 4,058
 - Allele Frequencies < 0.01 OR missing
 - 0.01
 - Less than 0.01 116
 - Equal to 0.01 0
 - Greater than 0.01 3,677
 - Missing 265381
 - Allele Frequencies < 0.01 OR missing
 - 0.01
 - Less than 0.01 45
 - Equal to 0.01 0
 - Greater than 0.01 141
 - Missing 195240

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

5. 人種特異的なSNPの除去のため、1kG Phase3 - Variant Frequencies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

11,074

<input checked="" type="checkbox"/> Germline	1
<input checked="" type="checkbox"/> Read Depths (DP) (Normal) >= 50	4,066
<input checked="" type="checkbox"/> AF (Normal) >= 0.05	4,058
<input checked="" type="checkbox"/> Allele Frequencies < 0.01 OR missing	381
<input checked="" type="checkbox"/> Allele Frequencies < 0.01 OR missing	240
<input checked="" type="checkbox"/> Effect (Combined) is (LoF, Missense)	-
Invalid	0
LoF	8
Missense	61
Other	171
Unknown	0
Missing	0
	69
<input checked="" type="checkbox"/> Classification is (Likely Pathogenic, Pat)	-
Association Not Found	0
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

Filter Variants 11,074

Filter Variants

Somatic 4,509

- Read Depths (DP) (Current) ≥ 50 4,509
- AF (Current) ≥ 0.05 4,500
- AF (Normal) < 0.01 OR missing 103
- In COSMIC? is true 29
- Sample Count ≥ 100 1

Germline 4,066

- Read Depths (DP) (Normal) ≥ 50 4,066
- AF (Normal) ≥ 0.05 4,058
- Allele Frequencies < 0.01 OR missing 381
- Allele Frequencies < 0.01 OR missing 240
- Effect (Combined) is (LoF, Missense) 69
- Classification is (Likely Pathogenic, Pathogenic) 1

1

1

2

Variants: 2

Filter Variants: T990005

Variant Info	Tumor (T990005)		Normal (N990005)		RefSeq Gene Names	
	Chr:Pos	Ref/Alt	Read Depths (DP)	AF		Read Depths (DP)
3:178936082	G/A	177	0.107345	?	?	PIK3CA
5:131973850	C/T	95	0.568421	81	0.592593	RAD50

1. Somaticワークフロー、Germlineワークフローでそれぞれ1つずつ変異が検出され、合計2種類の変異がテーブルに表示される

Variants: 2 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)
3:178936082	G/A	177	0.107345	?	?	PIK3CA	missense_variant	Missense
5:131973850	C/T	95	0.568421	81	0.592593	RAD50	stop_gained	LoF

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

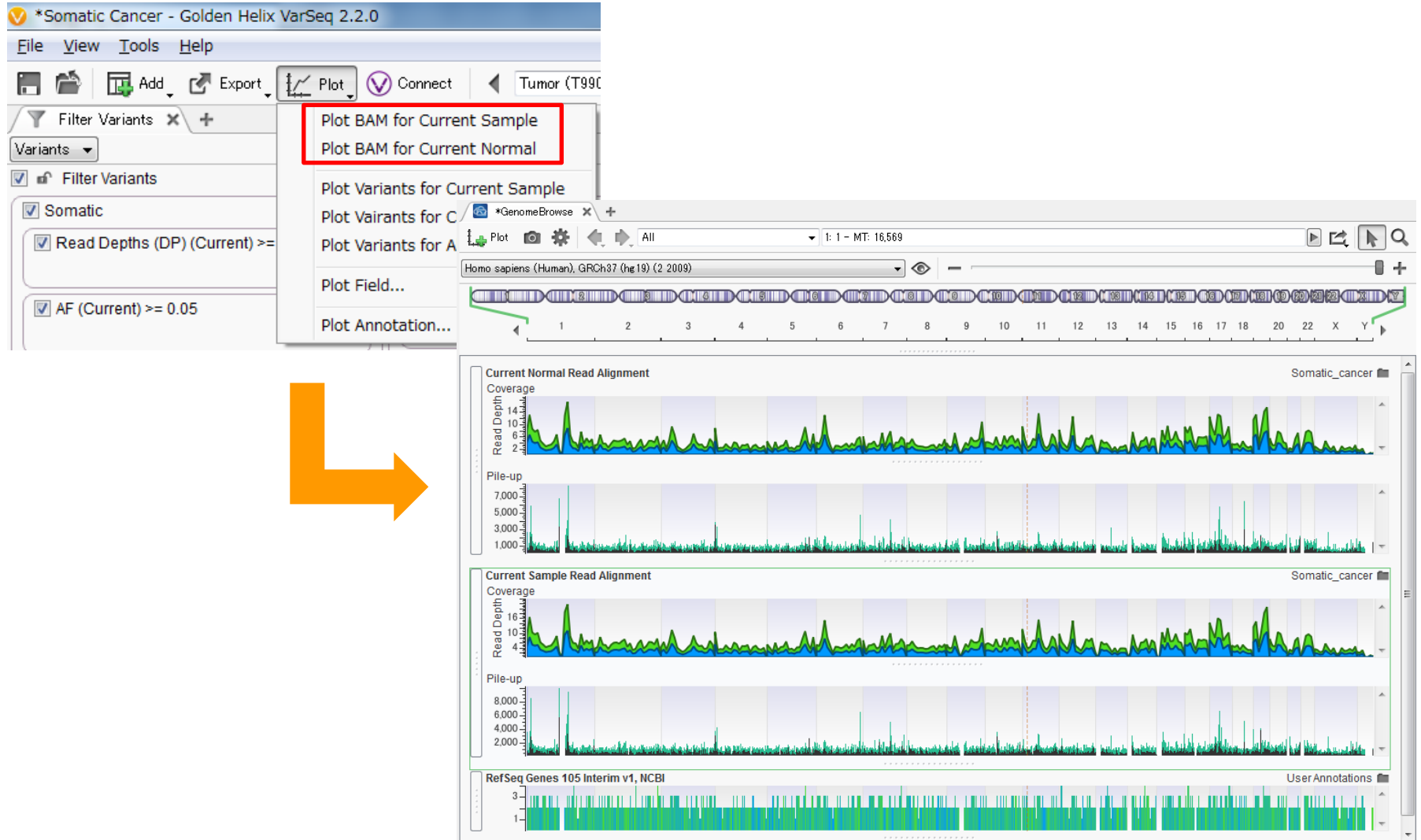
Read Depths (DP): 177

[rs121913273](#)

COSMIC Mutations 89, GHI

In COSMIC?	True
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	23548132, 26563128, 15016963, 25233892, 23317280, 27901576, 28027327, 28481359, 24062397, 21423156, [more]
Study ID	582
Sample Count	543
Samples	2719015 , 2724696 , 2716622 , 2724758 , 1651050 , 2584473 , 2719549 , 2146286 , 2234144 , 2187800 , 2719782 , [more]
Sample Primary Sites	Oesophagus, Breast, Breast, Breast, Oesophagus, Cervix, Breast, NS, Urinary tract, Genital tract, [more]
Sample Tissue Types	Esophagus/Stomach, Breast, Breast, Breast, Esophagus/Stomach, Cervix, Breast, CNS/Brain, Bladder/ [more]
Oncotree Tissue Type	Breast, Bowel, Bladder/Urinary Tract, Lung, Esophagus/Stomach, Head and Neck, Cervix, Uterus, CNS/ [more]
Oncotree Tissue Type Counts	183, 99, 65, 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 aerodigestive tract, Oesophagus, Cervix, [more]

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



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:**

Variant Info	Tumor (T990005)	Normal (N990005)	RefSeq Genes 105 Interim v1, NCBI							
Chr:Pos	Ref/Alt	DP	AF	DP	AF	Gene Names	SequenceOntologyCombined	Effect (Combined)	Nof4PredictedSplicingDisruptedCombined	Predicted Splicing Disrupted (Combin...
3:178936082	G/A	177	0.107345	?	?	PIK3CA	missense_variant	Missense	?	
5:131973850	C/T	95	0.568421	81	0.592593	RAD50	stop_gained	LoF	?	
- GenomeBrowse:** Shows a genomic track for Chromosome 3, with a zoomed-in view of the region 3:178,936,072 - 178,936,091.
- Read Alignments:** Displays 'Current Normal Read Alignment' and 'Current Sample Read Alignment' with coverage graphs and pile-up views. The sample alignment shows a G>A mismatch at position 178,936,082.
- Console:** A red-bordered window showing analysis results for Chr3:178,936,082.

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

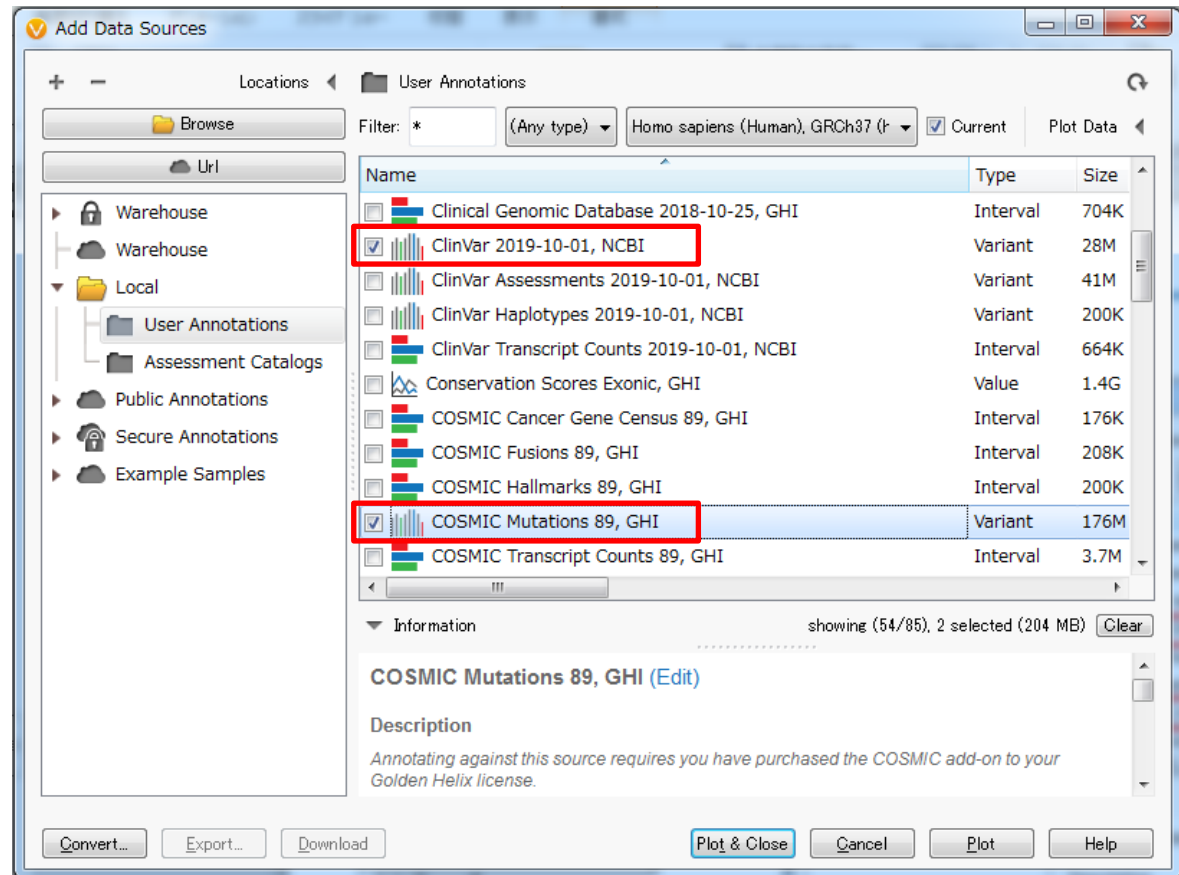
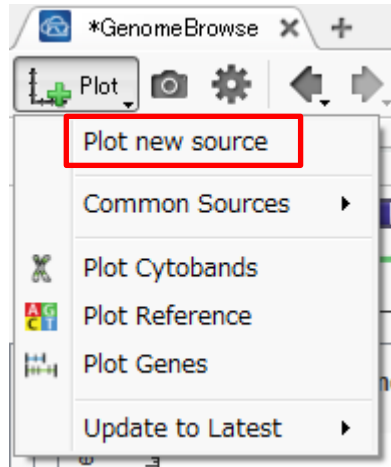
5 alignments filtered out by quality settings.

Chr3 between 178,936,081 and 178,936,082

Insertions			
Base(s)	Count	% of Total	Mean Quality
Non-Insertions	173	100.0	?
Total	173	100	?

Note: Any alignment spanning or adjacent to the insertion junction that does not have an insertion at the junction is counted as a non-insertion.
- RefSeq Genes:** Shows the PIK3CA gene structure with exons labeled P, L, S, E, I, T, E.

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

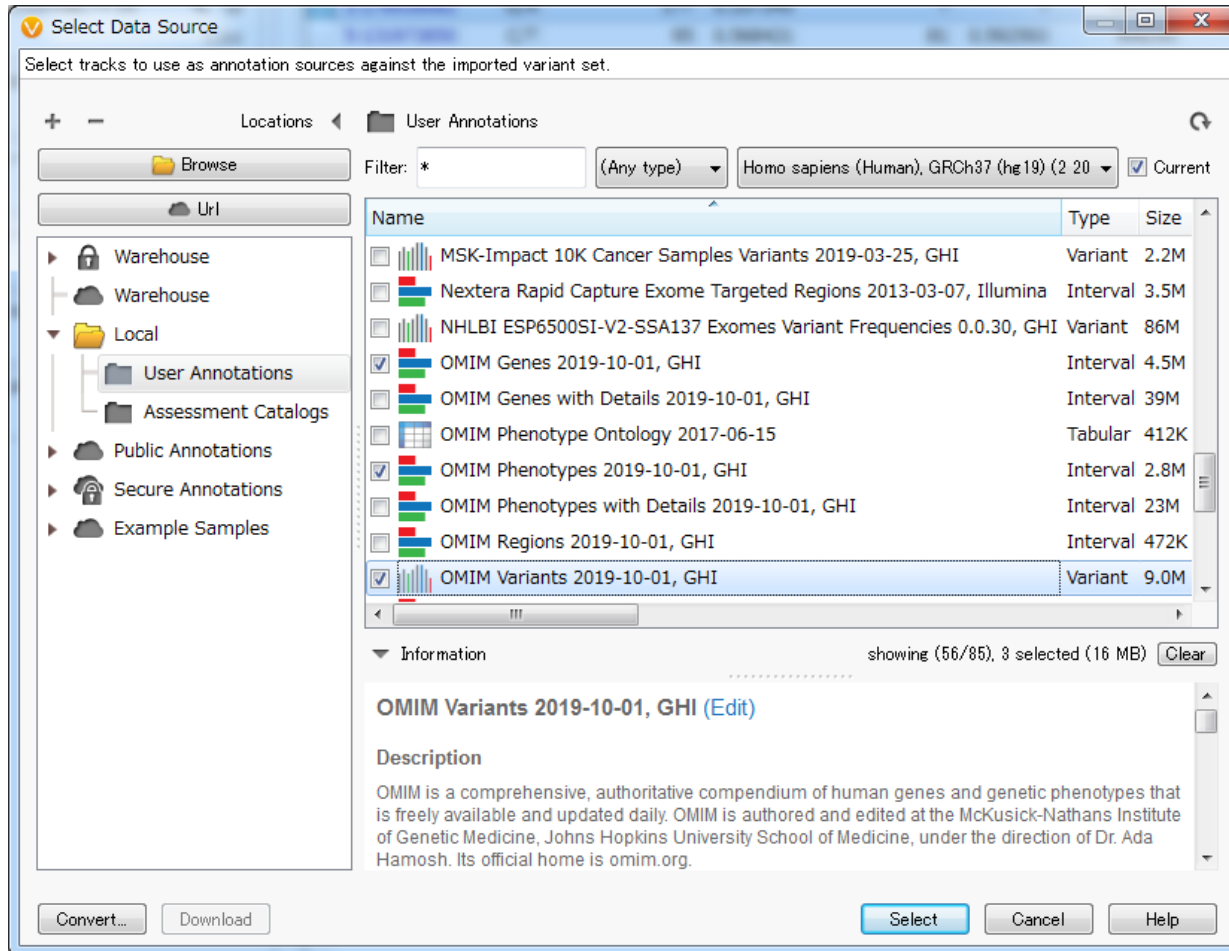


5. ゲノムブラウザーの「Plot」をクリックし、メニューより「Plot new source」を選択してクリック
6. Select Data Source画面において、データベースリストより「ClinVar」と「COSMIC Mutation」にチェックを入れ、「Plot & Close」をクリック



- 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

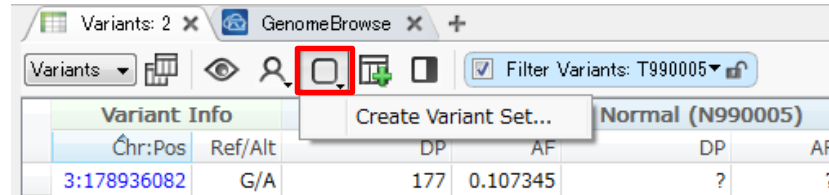
1. 再びプロジェクト画面の「Add」をクリックし、メニューより「Variant Annotation」を選択してクリック
2. Select Data Source画面において、上記3つのOMIMデータベース名にすべてチェックを入れ、「Select」をクリック

GenomeBrowse interface showing a table of OMIM Genes. The table is titled "OMIM Genes 2019-10-01, GHI". The table has 8 columns: Gene Name, Cytogenetic Location, OMIM ID, Entrez Gene ID, PubMed ID, HasPubMedID, Title, and Alternative Title(s). Two genes are listed: PIK3CA and RAD50.

Gene Name	Cytogenetic Location	OMIM ID	Entrez Gene ID	PubMed ID	HasPubMedID	Title	Alternative Title(s)
PIK3CA	3q26.3	171834	5290	16432179,...	True	PHOSPHATIDYLINOSITOL 3-KINASE, ...	PHOSPHATIDYLINOSITOL 3-KINA...
RAD50	5q31	604040	10111	19487811,...	True	RAD50 DOUBLE-STRAND BREAK REPA...	RAD50 S. CEREVISIAE HOMOLOG O

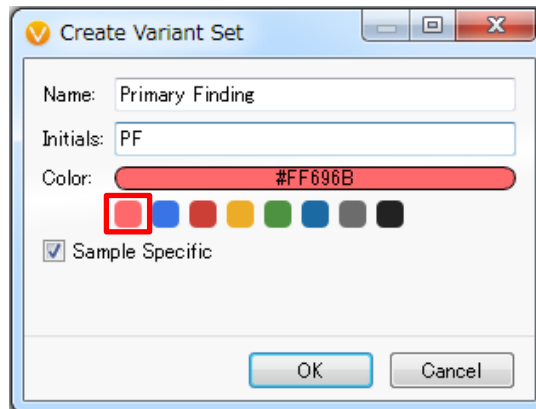
- 変異データテーブルに、選択した3種類のOMIMデータベースのアノテーション列が追加される

変異セットの作成



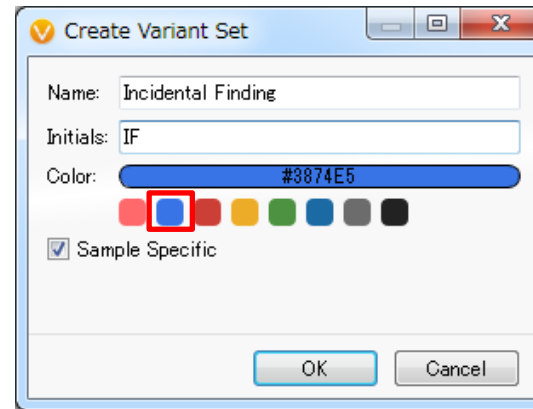
GenomeBrowse interface showing a variant table. The table has columns for Variant Info, Tumor (T990005), and Normal (N990005). The variant at 3:178936082 (G/A) is highlighted. A menu option 'Create Variant Set...' is visible above the table, with a red box around the icon.

Variant Info	Tumor (T990005)	Normal (N990005)			
Chr:Pos	Ref/Alt	DP	AF	DP	AF
3:178936082	G/A	177	0.107345	?	?



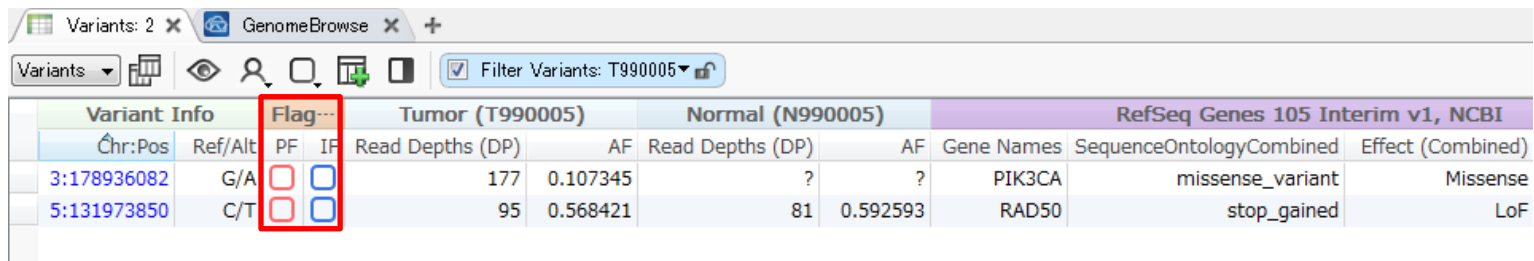
Dialog box for creating a variant set named 'Primary Finding'. The 'Color' field is set to #FF696B. A red box highlights the red color selection button.

Name: Primary Finding
Initials: PF
Color: #FF696B
 Sample Specific



Dialog box for creating a variant set named 'Incidental Finding'. The 'Color' field is set to #3874E5. A red box highlights the blue color selection button.

Name: Incidental Finding
Initials: IF
Color: #3874E5
 Sample Specific



GenomeBrowse interface showing the variant table with 'Flag' columns for PF and IF. A red box highlights the 'Flag' column headers and the corresponding checkboxes for the two variants.

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	SequenceOntologyCombined	Effect (Combined)
3:178936082	G/A	<input type="checkbox"/>	<input type="checkbox"/>	177	0.107345	?	?	PIK3CA	missense_variant	Missense
5:131973850	C/T	<input type="checkbox"/>	<input type="checkbox"/>	95	0.568421	81	0.592593	RAD50	stop_gained	LoF

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、任意のColorで作成すると、変異テーブルにフラグが表示される

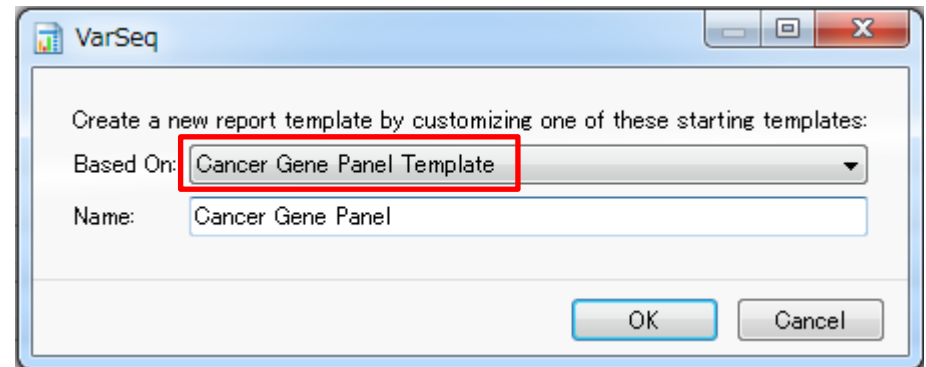
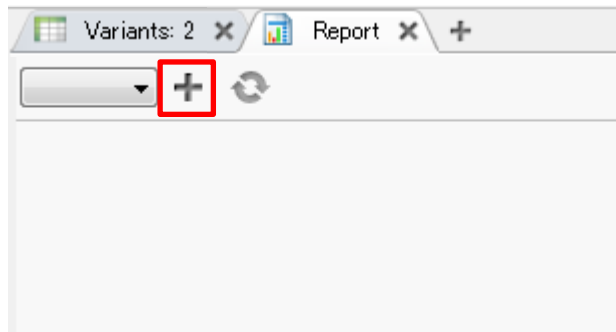
Variant Info		Flag...		Tumor (T990005)		Normal (N990005)		
Chr:Pos	Ref/Alt	PF	IF	Read Depths (DP)	AF	Read Depths (DP)	AF	Gene Names
3:178936082	G/A	<input checked="" type="checkbox"/>	<input type="checkbox"/>	177	0.107345	?	?	PIK3CA
5:131973850	C/T	<input type="checkbox"/>	<input checked="" type="checkbox"/>	95	0.568421	81	0.592593	RAD50



Variant Info		Flag...		Tu
Chr:Pos	Ref/Alt	PF	IF	Read D
3:178936082	G/A	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
5:131973850	C/T	<input type="checkbox"/>	<input checked="" type="checkbox"/>	

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

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

Variants: 2 x *GenomeBrowse x Cancer Gene Panel x +

Cancer Gene Panel + -

Configure Report Template
Reload Report Template
Developer JavaScript Console
Open Report Template Folder

Patient Information

Name: T990005
Gender: Male
Date of Birth: 11/10/2019
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: 11/10/2019
Receipt Date: 11/10/2019
Report Date: 11/10/2019


Patient Result

Result: Positive
Comment: Mutations with an establish somatic link detected.
Interpretation Summary:
Recommendations:




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 formatting options (B, I, U) and a link icon. The 'Reportable Genes' field 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


Variants: 2 x Cancer Gene Panel x +
 Cancer Gene Panel + 

Primary Findings
 Primary Finding


Variant: 3:178936082 G/A (*PIK3CA*) 
 Classification: Pathogenic 
 Interpretation: **B I U** 
 This is a Missense Variant located in the PIK3CA gene.
CLOVE Syndrome
 In a 14-year-old girl and an unrelated 1-year-old boy with CLOVE syndrome (619019, Kuroki et al. (2019)) identified co-stic...

Incidental Findings
 Incidental Finding

Variant: 5:131973850 C/T (*RAD50*) 
 Interpretation: **B I U** 
 This is a Stop Gained located in the RAD50 gene.
 This gene has been observed to exhibit ? inheritance pattern.
 It has been associated with Niimogen breakage syndrome-like disorder...

Report Signoff
 Verify: Report 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



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

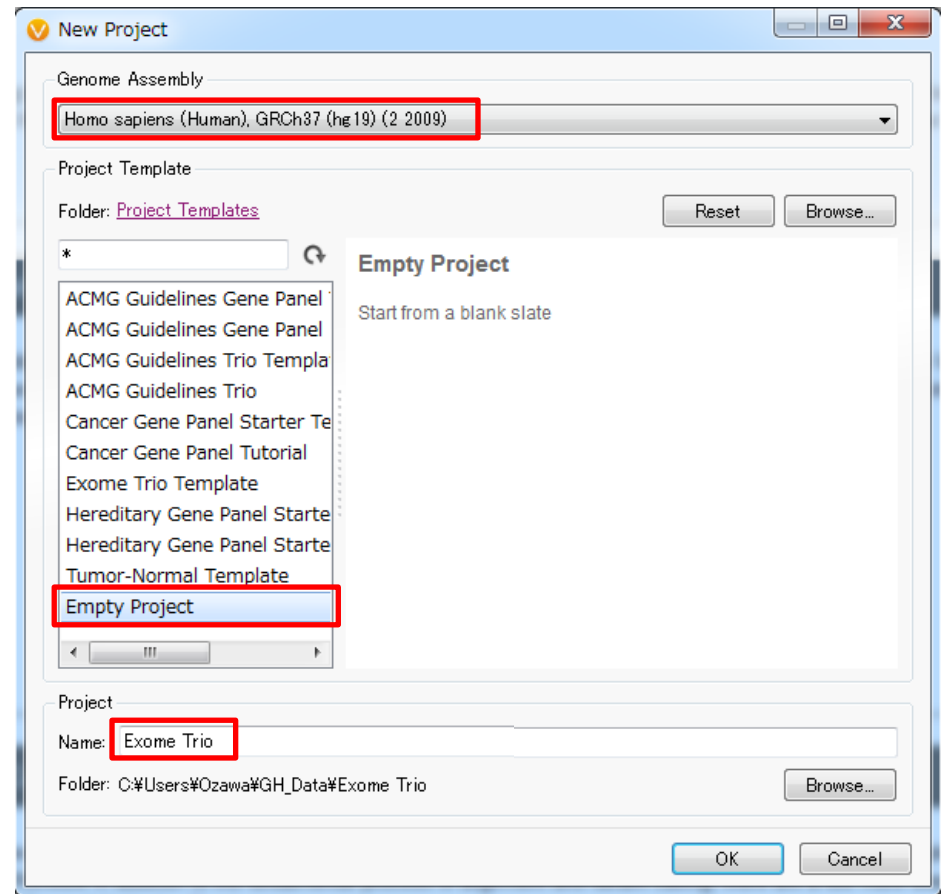
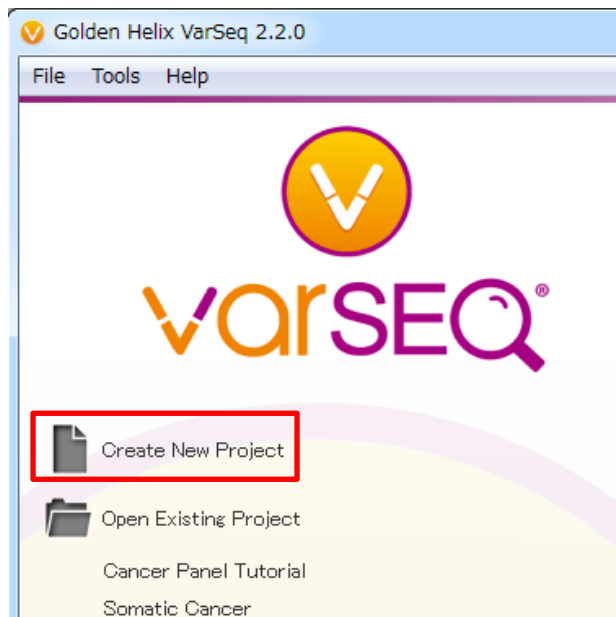
手順2 : アノテーション付加

- 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

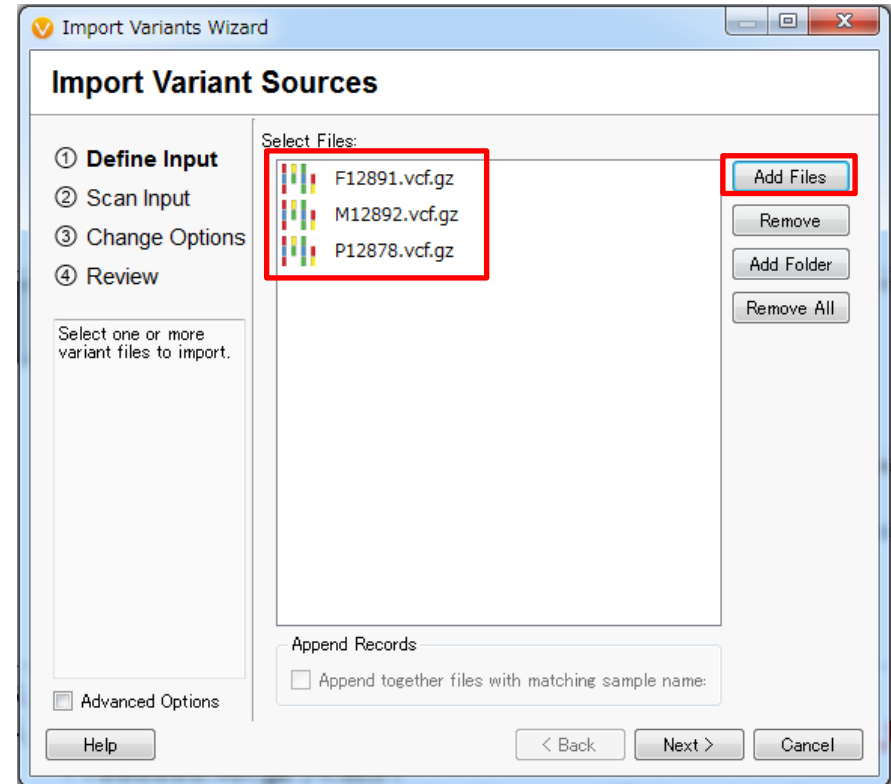
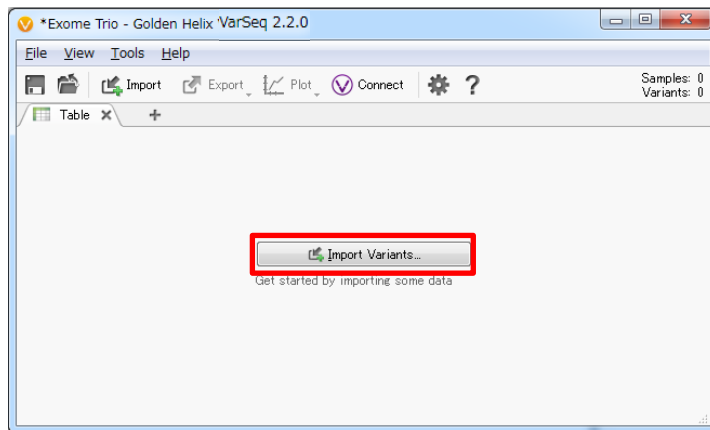
手順3 : フィルタリング

- 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

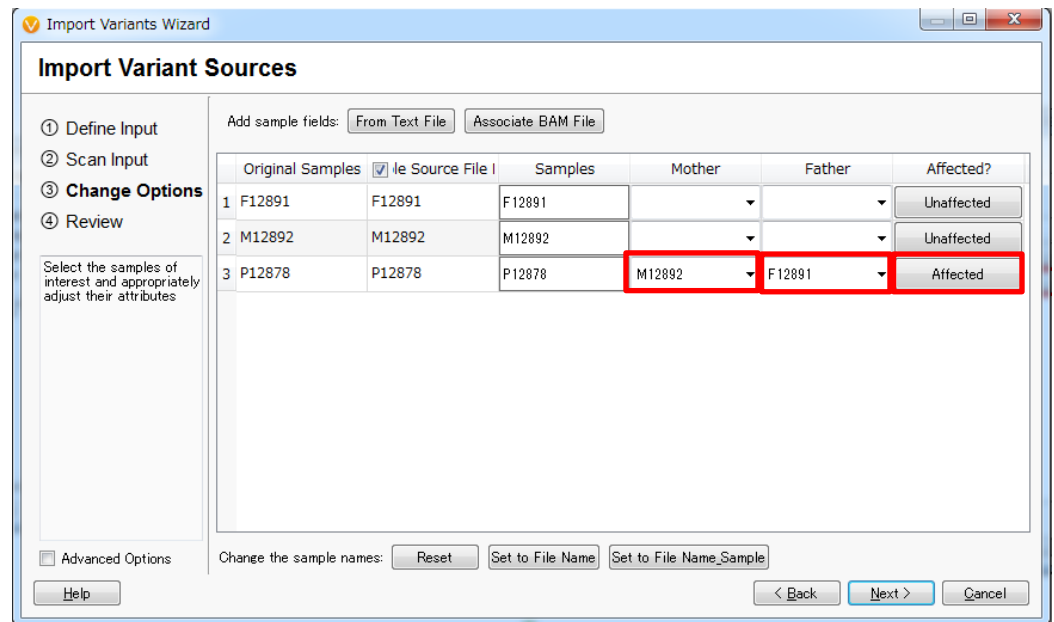
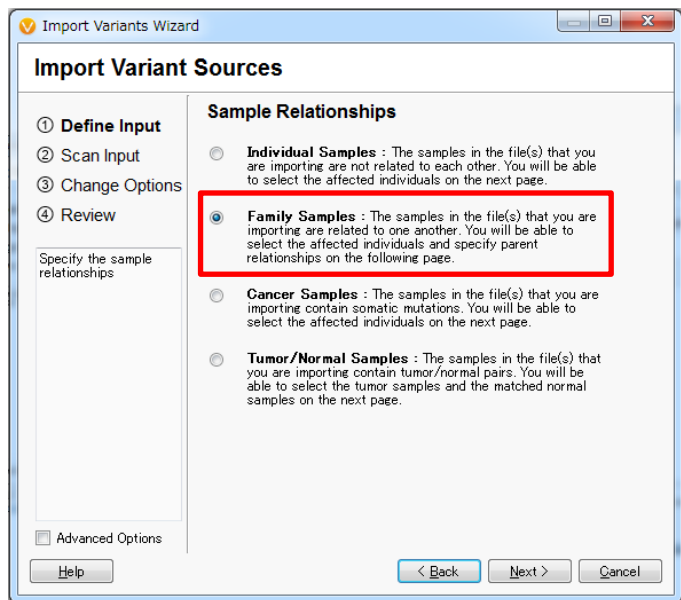
手順1. サンプルデータのインポート



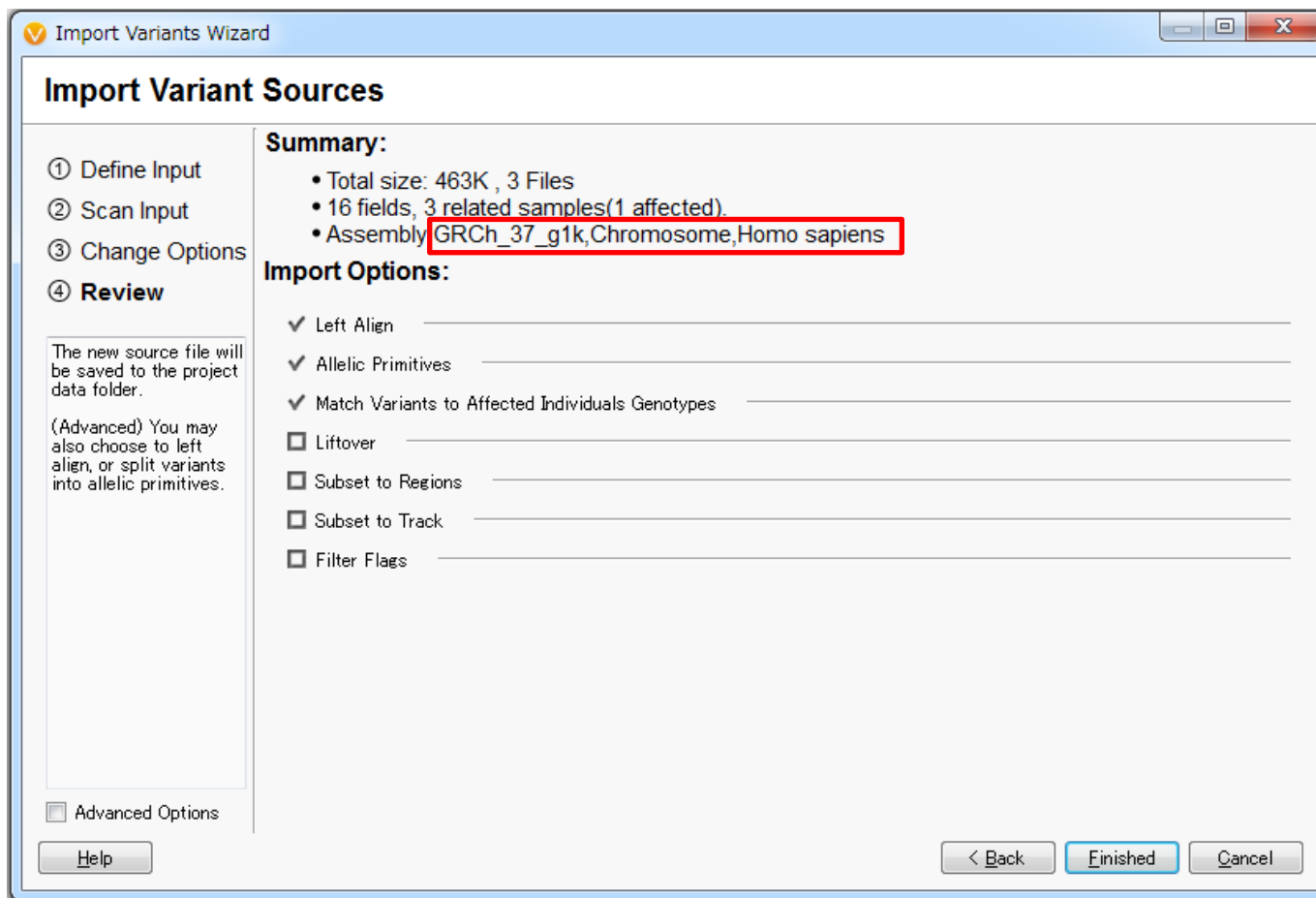
1. メイン画面の「Create New Project」をクリック
2. 任意のプロジェクト名を入力し、またProject Templateに「Empty Project」、Genome Assemblyが「Homo sapiens (Human), GRCh37 (hg19) (2 2009)」となっていることを確認したら「OK」をクリック



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に、「GRCh_37_g1K, Chromosome, Homo sapiens」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 2.2.0

File View Tools Help

Save Add Export Plot Connect Proband (P12878) ?

Filter Variants x + Variants: 13,091 x +

Filter Variants 13,091

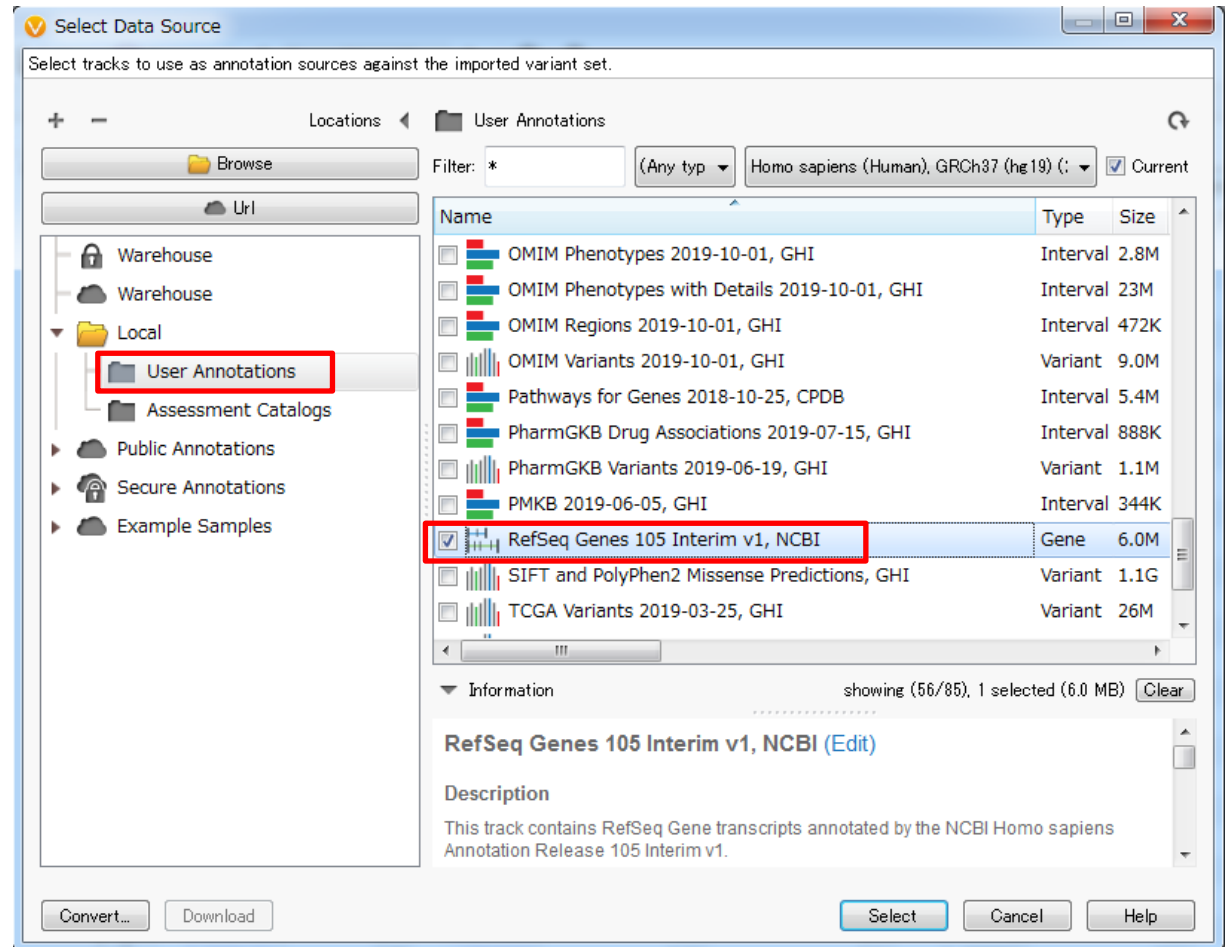
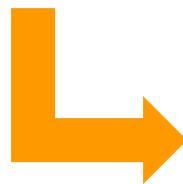
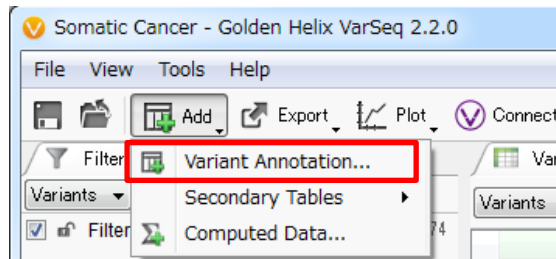
Filter Variants: P12878

Variants: 13,091

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	?	?
6:311811	C/T	?	?	0.175	240	?	?

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
- 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' window with 13,091 variants. Two filters are applied: 'Read Depths (DP) (Current) >= 30' (3,963 variants) and 'Variant Allele Freq (Current) >= 0.2' (3,814 variants). Below these are two identical filter panels for 'Allele Frequencies < 0.01 OR missing'. Each panel has a search bar with '0.01' and a histogram icon. The first panel shows counts for 'Less than 0.01' (76), 'Equal to 0.01' (0), 'Greater than 0.01' (3,249), and 'Missing' (490), with a total of 566. The second panel shows counts for 'Less than 0.01' (24), 'Equal to 0.01' (0), 'Greater than 0.01' (97), and 'Missing' (445), with a total of 469.

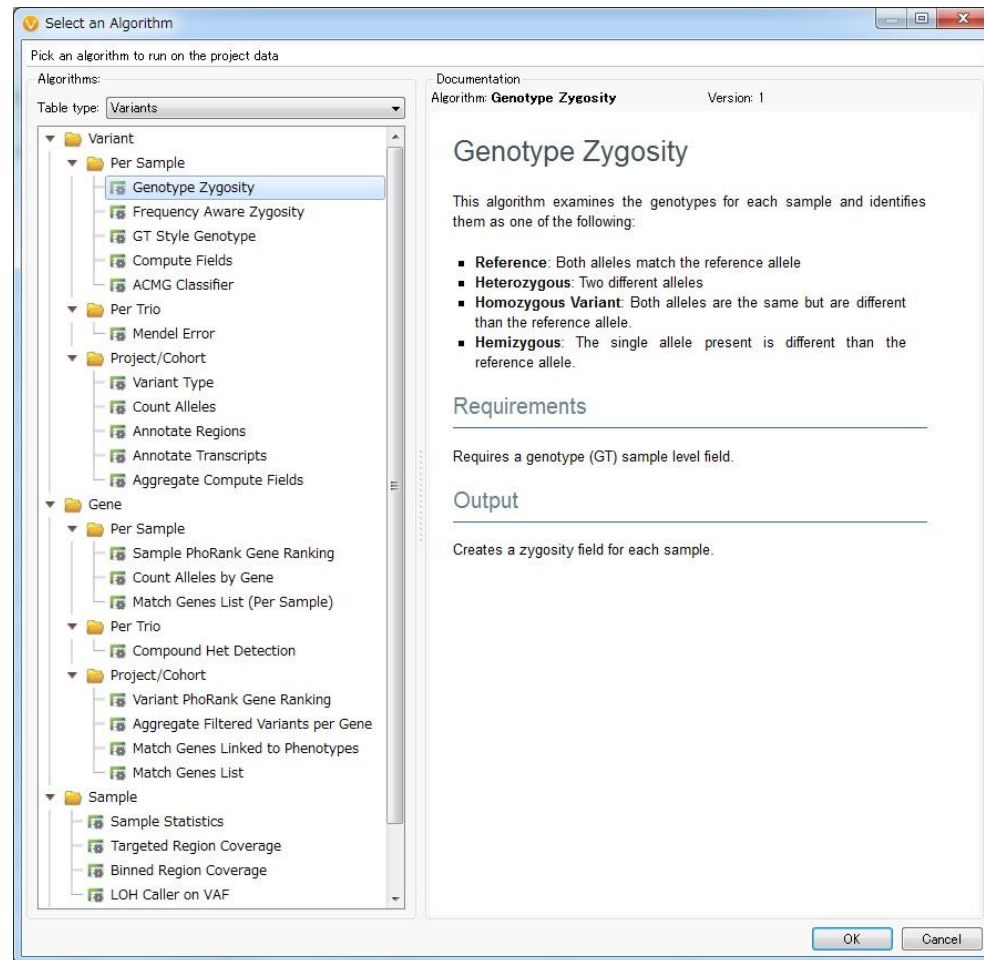
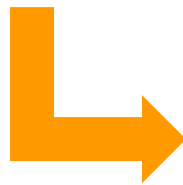
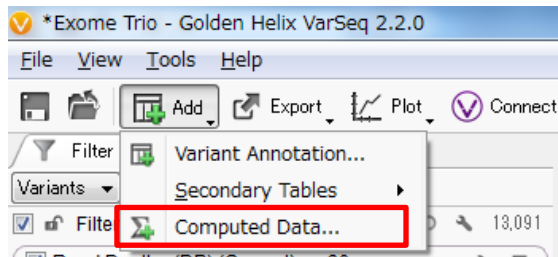
- 1kG Phase3 - Variant Frequenciesの「Allele Frequencies」 < 0.01 or Missing
- HGVD1210-V2_30の「Allele Frequencies」 < 0.01 or Missing

2. 人種特異的なSNPの除去のため、1kG Phase3 - Variant Frequenciesの「Allele Frequencies」、さらにHGVD1210-V2_30の「Allele Frequencies」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

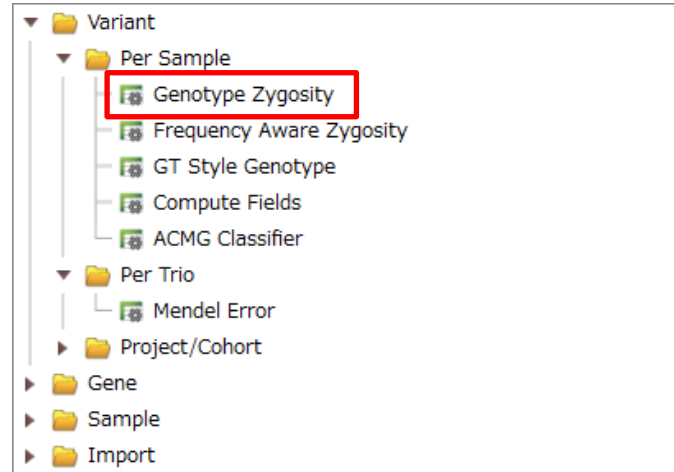
Filter Variants	Count
<input checked="" type="checkbox"/> Read Depths (DP) (Current) >= 30	3,963
<input checked="" type="checkbox"/> Variant Allele Freq (Current) >= 0.2	3,814
<input checked="" type="checkbox"/> Allele Frequencies < 0.01 OR missing	566
<input checked="" type="checkbox"/> Allele Frequencies < 0.01 OR missing	469
<input checked="" type="checkbox"/> Effect (Combined) is (LoF, Missense)	-
Invalid	0
LoF	8
Missense	126
Other	335
Unknown	0
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

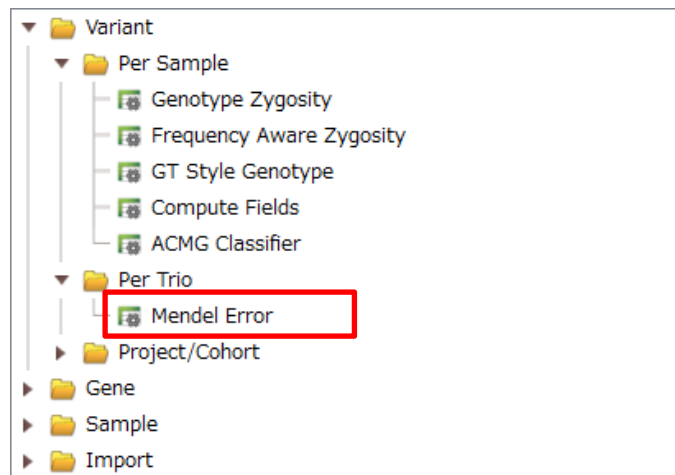


1. プロジェクト画面の「Add」をクリックし、メニューより「Computed Data」を選択してクリック
2. Select an Algorithm画面において、任意の解析アルゴリズムを選択して、「OK」をクリック
3. 解析アルゴリズムの種類によっては、計算時のパラメータなどを指定する
4. 多くの解析アルゴリズムでは、計算が終了すると、変異テーブルに計算結果のアノテーションが付加され、フィルタリングに使用できるようになる



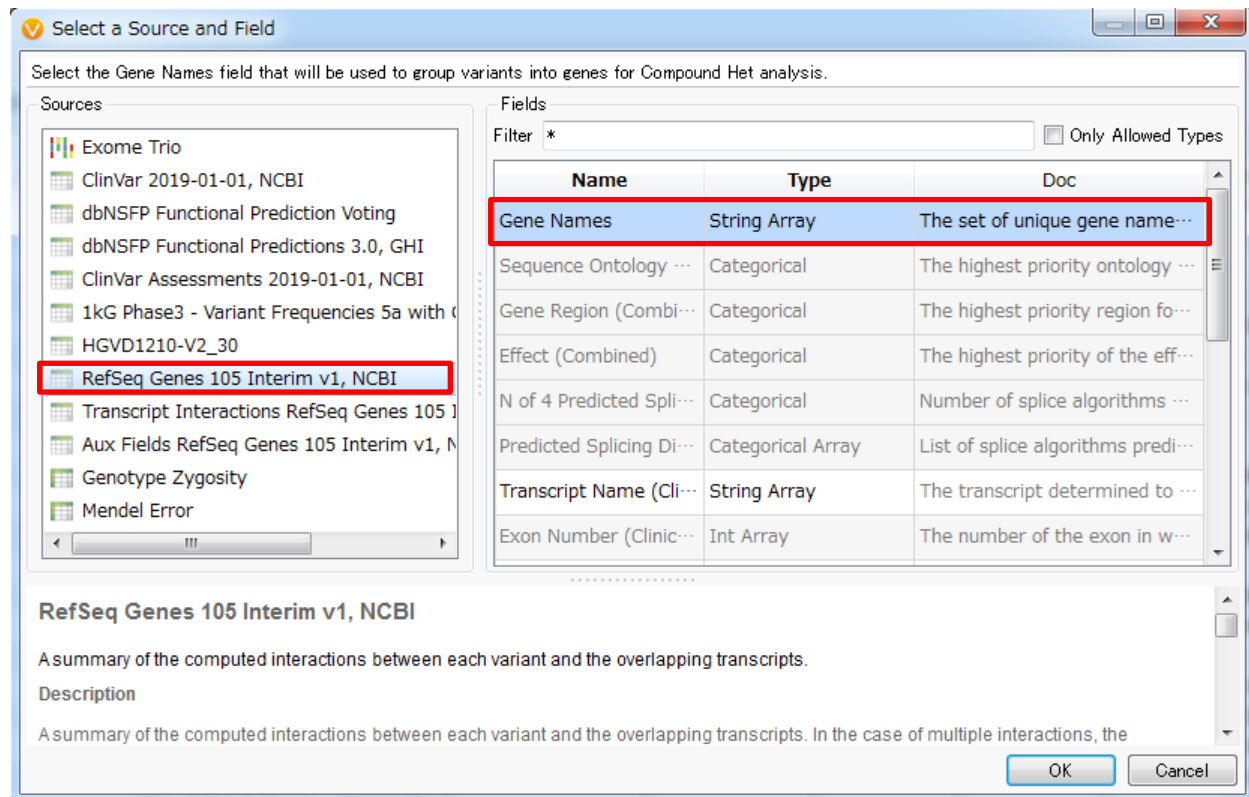
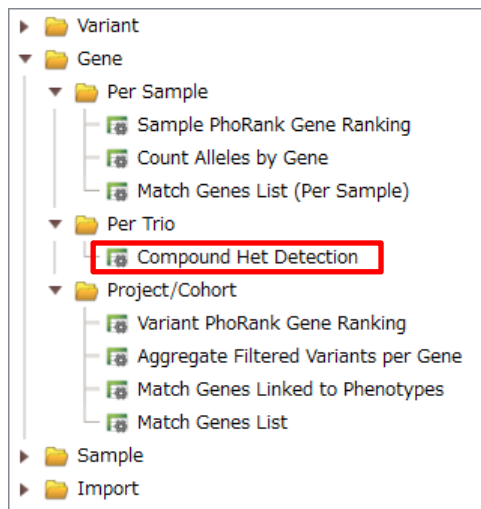
Variant Info		Proband (P12878)			Mother (M12892)			Father (F12891)		
Chr:Pos	Ref/Alt	Variant Allele Freq	Read Depths (DP)	Zygosity	Variant Allele Freq	Read Depths (DP)	Zygosity	Variant Allele Freq	Read Depths (DP)	Zygosity
6:564567	C/G	0.393443	122	Heterozygous	?	?	?	0.496552	145	Heterozygous
6:7405508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

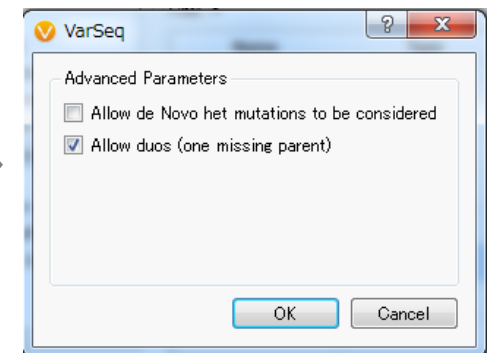
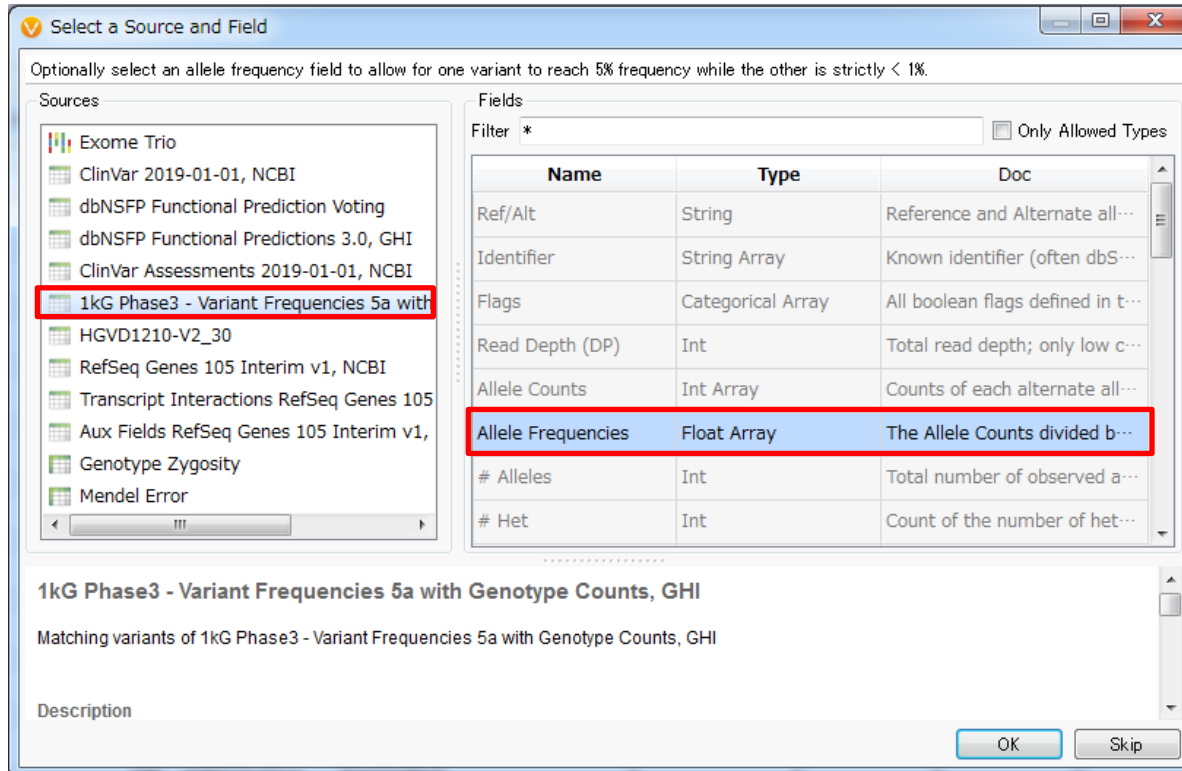


Variant Info		Proband (P12878)				Mother (M12892)				Father (F12891)			
Chr:Pos	Ref/Alt	Variant Allele Freq	Read Depths (DP)	Zygosity	Mendel Error	Variant Allele Freq	Read Depths (DP)	Zygosity	Mendel Error	Variant Allele Freq	Read Depths (DP)	Zygosity	Mendel Error
6:564567	C/G	0.393443	122	Heterozygous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

Filter Variants 13,091

- Read Depths (DP) (Current) >= 30 3,963
- Variant Allele Freq (Current) >= 0.2 3,814
- Allele Frequencies < 0.01 OR missing 566
- Allele Frequencies < 0.01 OR missing 469
- Effect (Combined) is (LoF, Missense) 134
- Compound Het? (Current)**
 - True 3
 - False 131
 - Missing 0

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

Trio解析ワークフローの作成

The image shows three sequential screenshots of a software interface for creating a Trio Analysis filter container. The interface displays a list of filters under the heading 'Filter Variants' with a total count of 13,091. The filters are: Read Depths (DP) (Current) >= 30 (3,963), Variant Allele Freq (Current) >= 0.2 (3,814), Allele Frequencies < 0.01 OR missing (566), Allele Frequencies < 0.01 OR missing (469), Effect (Combined) is (LoF, Missense) (134), and Compound Het? (Current) is true (3). In the first screenshot, a context menu is open over the 'Compound Het?' filter, with 'Add Filter Container' highlighted. In the second screenshot, a new 'Trio Analysis' filter container has been added, and the 'OR' radio button is selected. In the third screenshot, the 'Compound Het?' filter has been dragged into the 'Trio Analysis' container.

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

The screenshot shows a 'Filter Variants' panel with the following filters and counts:

- Read Depths (DP) (Current) >= 30: 3,963
- Variant Allele Freq (Current) >= 0.2: 3,814
- Allele Frequencies < 0.01 OR missing: 566
- Allele Frequencies < 0.01 OR missing: 469
- Effect (Combined) is (LoF, Missense): 134

The 'Trio Analysis' section is expanded to show two sub-sections:

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

Filter	Count
Mendel Error (Current)	
MIE	1
Transmitted	95
Untransmitted	0
de Novo Allele	38
Missing	0
Total	38

Total count for the selected filters: 41

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

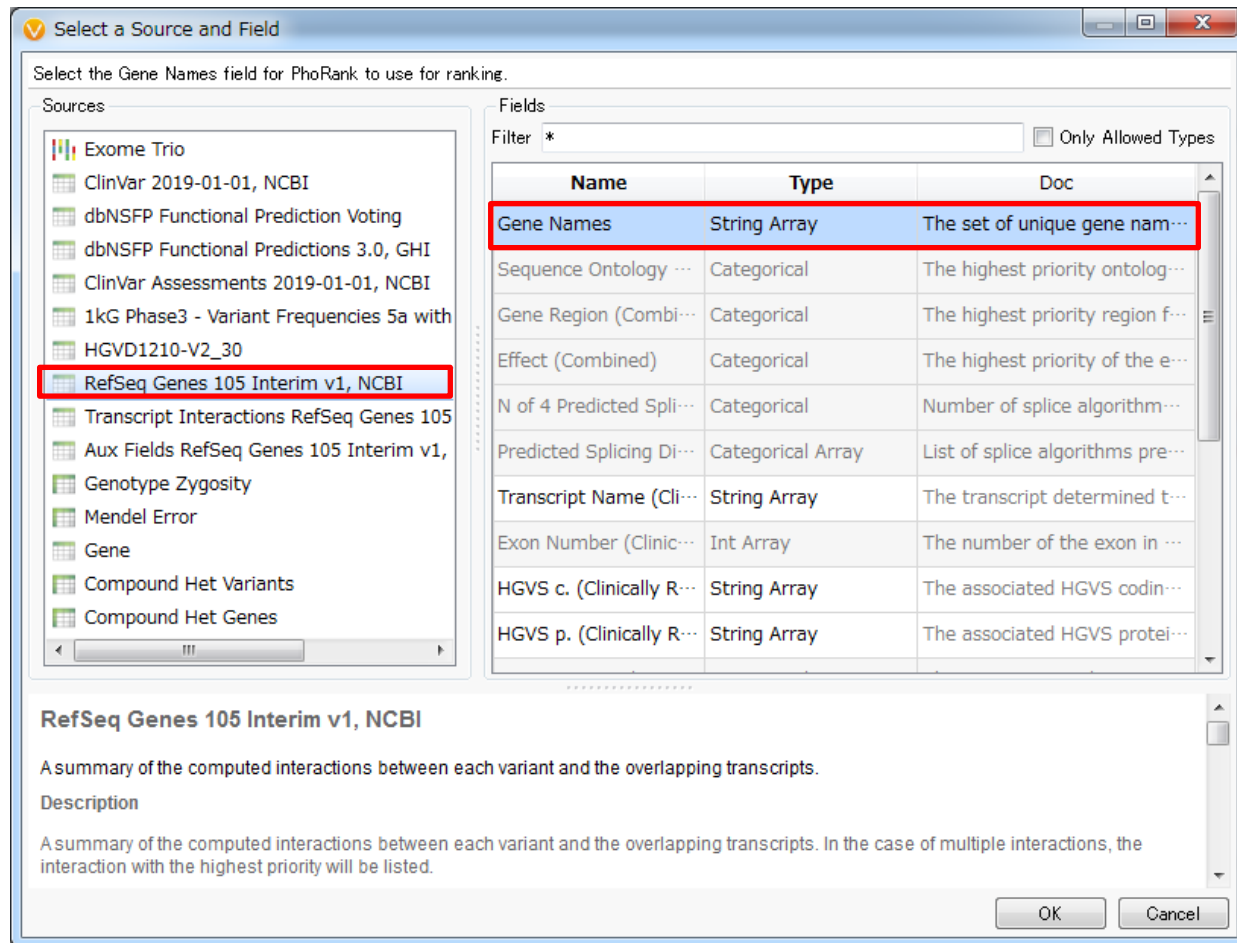
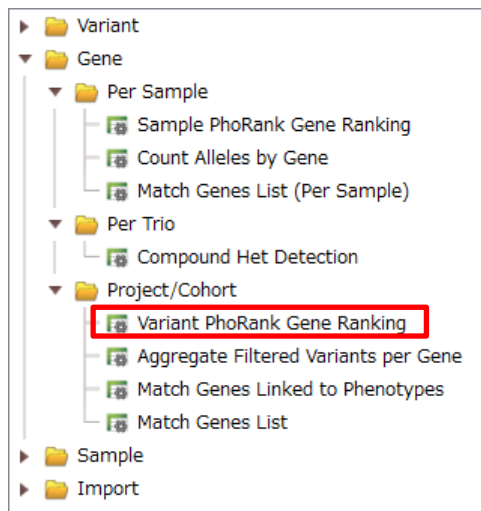
4. Proband (P12878サンプル) の「Mendel Error」のフィールドのコンテナをつくり、上記のとおり検索条件を指定する

The screenshot shows the 'Trio Analysis' window with three filter containers. The first container, 'Compound Het?', has 'True' selected with a count of 3. The second container, 'Mendel Error (Current)', has 'de Novo Allele' selected with a count of 38. The third container, 'Recessive Homozygous', has three sub-filters: 'Zygoty (Current) is Homozygous Vari...', 'Zygoty (Mother) is Heterozygous', and 'Zygoty (Father) is Heterozygous'. In the first sub-filter, 'Homozygous Variant' is selected with a count of 1. In the second sub-filter, 'Heterozygous' is selected with a count of 0. In the third sub-filter, 'Heterozygous' is selected with a count of 0. The total count for the Recessive Homozygous container is 41.

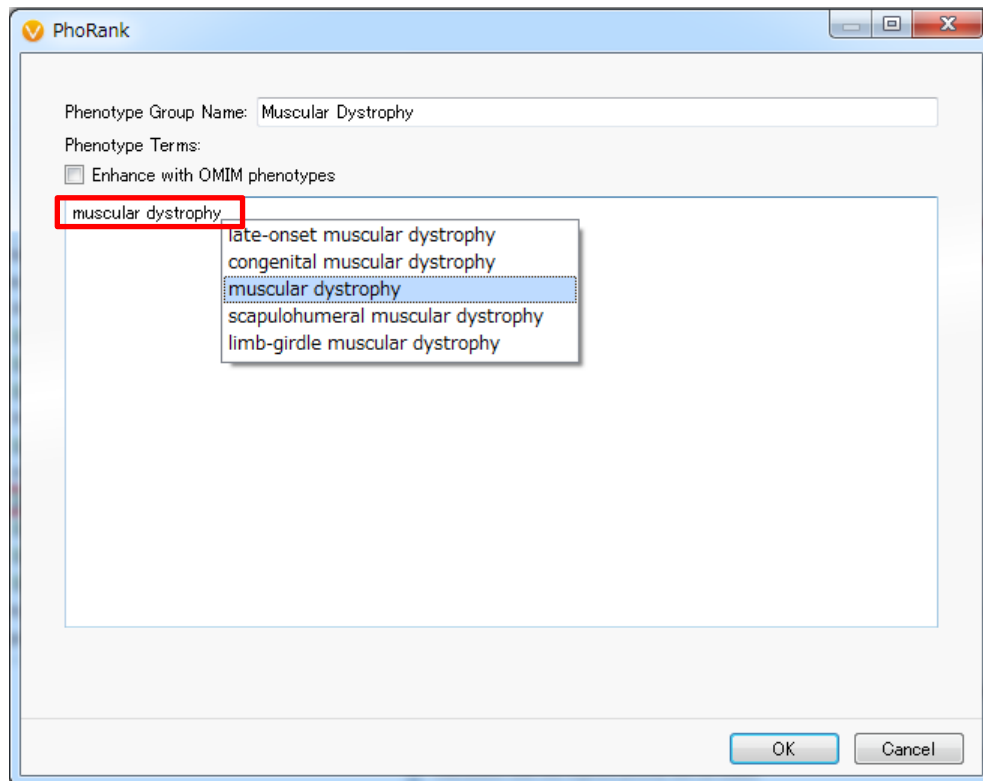
Filter	Sub-filter	Count
Compound Het?	True	3
	False	131
	Missing	0
Mendel Error (Current)	MIE	1
	Transmitted	95
	Untransmitted	0
	de Novo Allele	38
Missing	0	
Recessive Homozygous	Zygoty (Current) is Homozygous Vari...	
	Hemizygous	0
	Heterozygous	133
	Homozygous Variant	1
	Reference	0
	Missing	0
	Zygoty (Mother) is Heterozygous	
	Hemizygous	0
	Heterozygous	0
	Homozygous Variant	0
	Reference	0
	Missing	1
Zygoty (Father) is Heterozygous		
Hemizygous	0	
Heterozygous	0	
Homozygous Variant	0	
Reference	0	
Missing	0	

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

- 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
- 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









The screenshot displays a variant analysis interface. On the left, a 'Filter Variants' panel shows several filters applied, including 'Read Depths (DP) (Current) >= 30' (3,963 variants), 'Variant Allele Freq (Current) >= 0.2' (3,814 variants), and 'Allele Frequencies < 0.01 OR missing' (566 variants). The 'Trio Analysis' section is expanded, showing counts for 'Compound Het?' (True: 3, False: 131, Missing: 0), 'Mendel Error (Current)' (MIE: 1, Transmitted: 95, Untransmitted: 0, de Novo Allele: 38, Missing: 0), and 'Recessive Homozygous' (Zygosity (Current) is Homozygous Vari: 1, Zygosity (Mother) is Heterozygous: 0, Zygosity (Father) is Heterozygous: 0). Red boxes highlight the counts 3, 38, 0, and 41 in this section.

On the right, a table titled 'Variants: 41' and 'Variant Genes: 902' shows a list of variants for 'Proband (P12878)'. The table has columns for 'Chr.Pos', 'Ref/Alt', 'Variant Allele Freq', 'Read Depths (DP)', 'Zygosity', and 'Mendel Error'. The variants listed are all 'de Novo Allele' and 'Heterozygous'.

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
6:26108282	C/A	0.245902	62	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38個、劣性ホモ接合体では0個の変異が検出され、合計41個の変異がテーブルに表示される

Variants: 41 x Variant Genes: 38 x +

Variants by Variant Genes       Trio Analysis: P12878  GeneRank 

Variant 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	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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