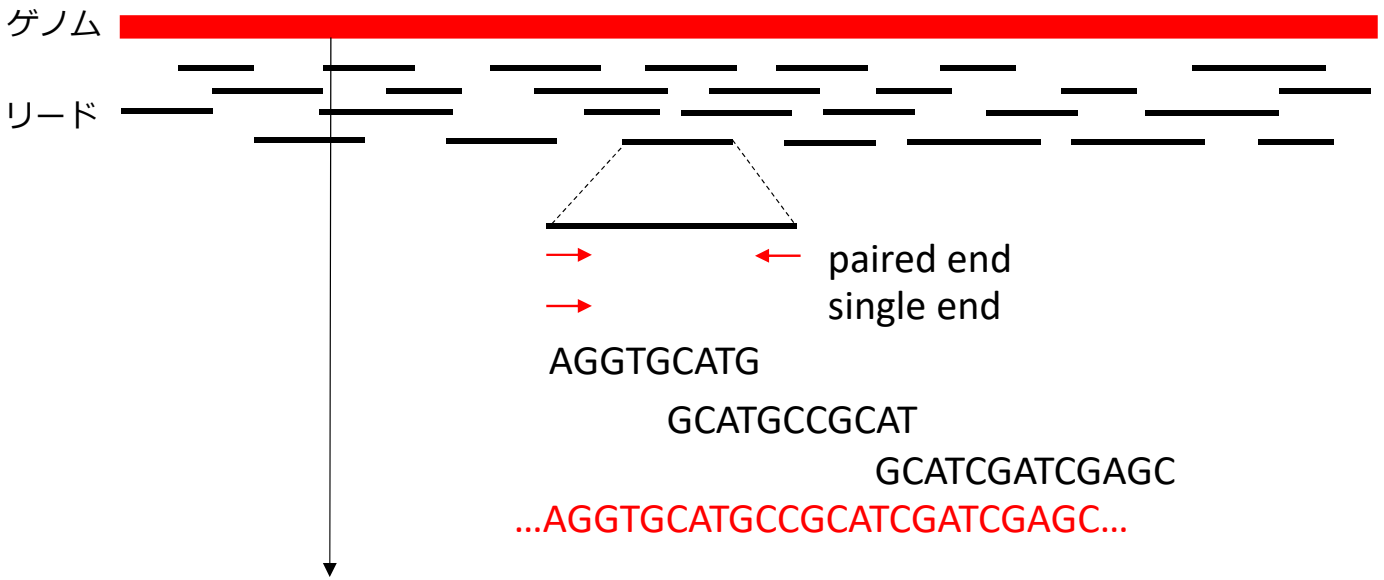
2019年度
第5回バイオインフォマティクス実習

先端医科学研究センター　バイオインフォマティクス解析室
中林潤

Next Generation Sequencer (NGS)

DNAを断片化して配列を読む→参照ゲノムにマッピング→配列を再構成する

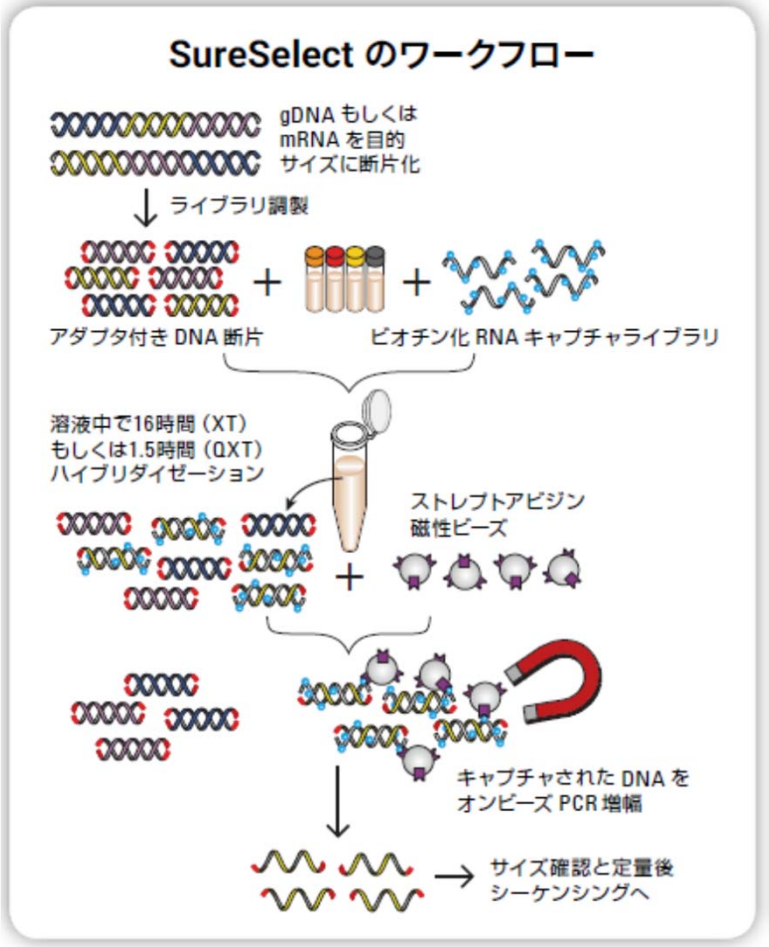


カバー率 = *(N × L) / G : 1*塩基当りの断片数
リード数 : *N*
リード数 : *L*
ゲノム長 : *G*

カバー率が十分でないと正確な配列情報が得られない。

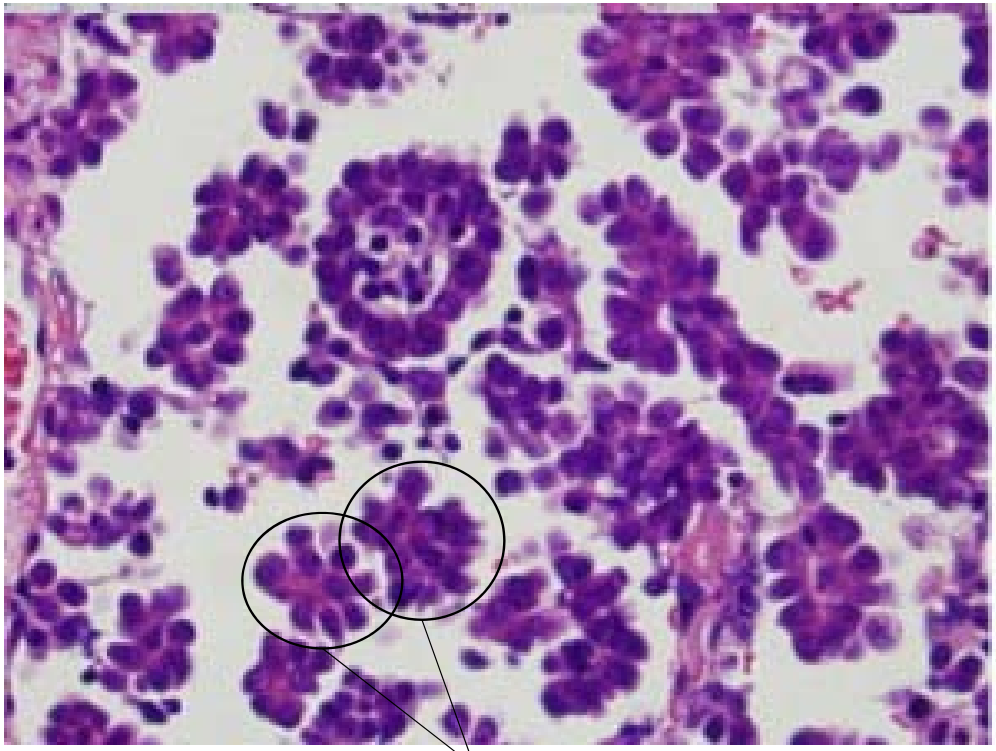
Whole-Exome Sequencing (WES) 解析

- Exon : 1~2% of the genome
- SureSelectXT Human All Exon Kit V6 (Agilent)



Agilent ®

Micropapillary Predominant Adenocarcinoma (MPA)

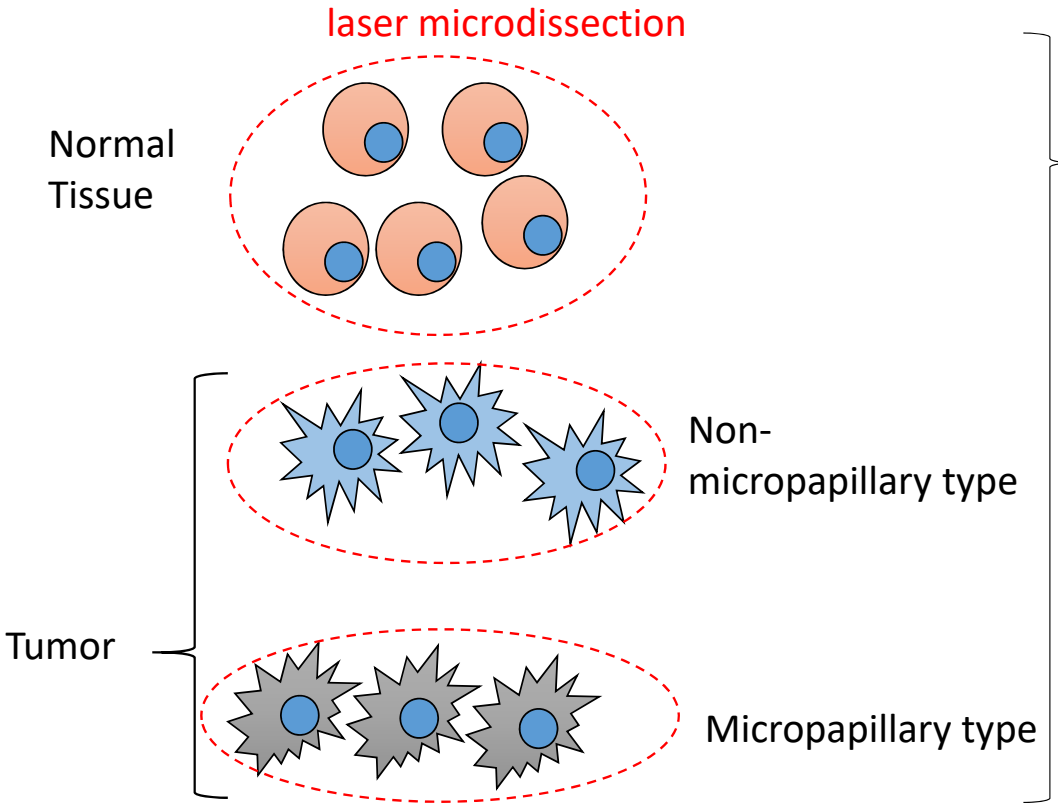


papillary tufts without fibrovascular core

https://www.pathologyoutlines.com/topic/lungtumormicropapillary.html

Sample preparation

82y female non-smoker
frozen specimen



	total DNA (ng)
normal	153.7
non-micropapillary	10.3
micropapillary	195.7

10ng

Whole Exome Sequencing

Mapping

	Total Reads (100bp paired end)	Overall Mapping Rate	Coverage
Normal	58,037,525	98.13%	193×
Non- Micropapillary	62,830,751	97.69%	207×
Micropapillary	64,318,300	97.47%	210×

Coverage = mapped read counts × 2 × 100 / (3,000,000,000 × 0.02)

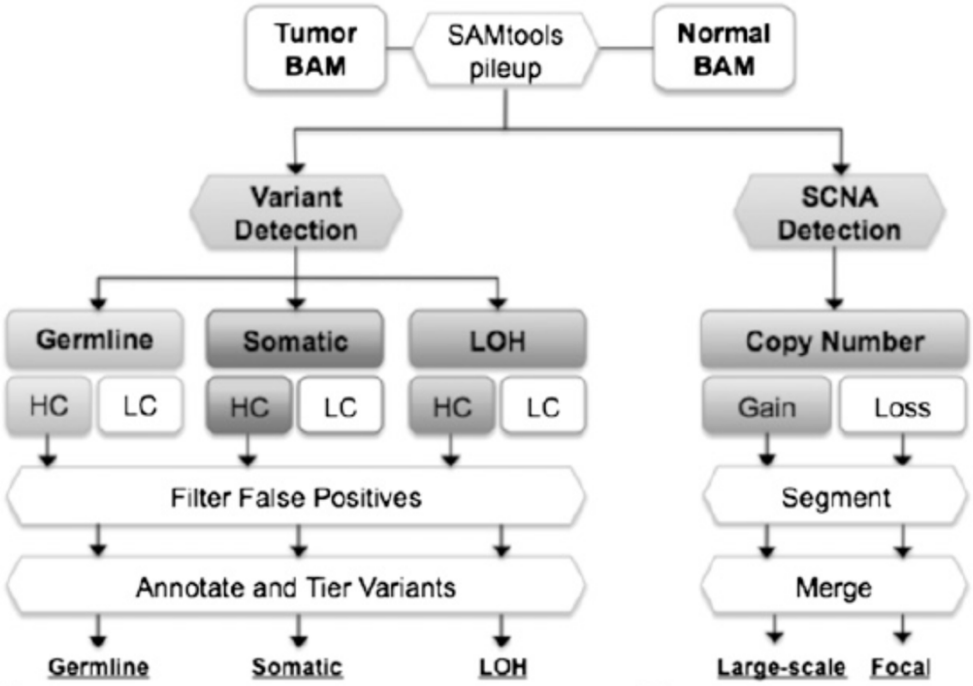
Estimation of the Somatic Mutations

- VarScan2

VarScan 2: Somatic mutation and copy number alteration discovery in cancer by exome sequencing.

Koboldt, D., et al. ***Genome Res.*** 2012 22:568-576

A strategy for estimation of the somatic mutations

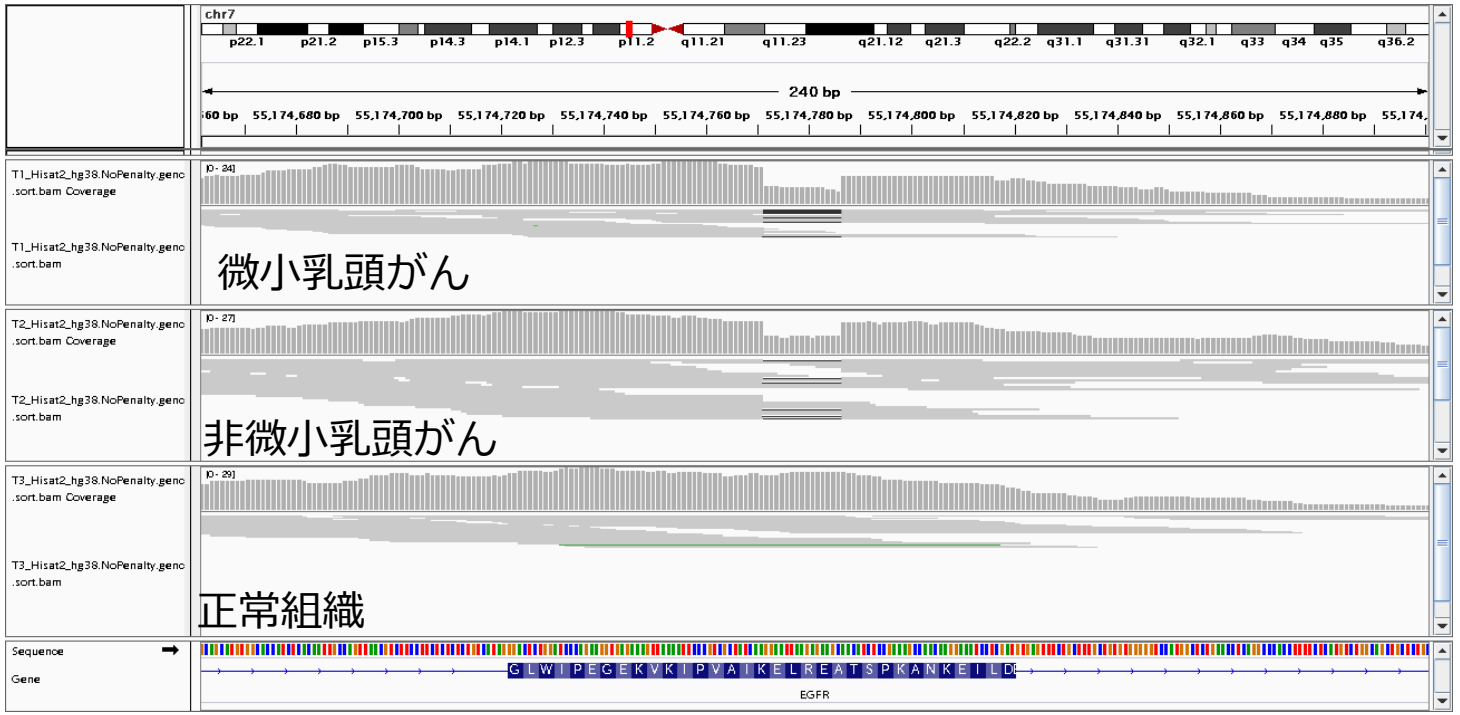


VarScan 2: Somatic mutation and copy number alteration discovery in cancer by exome sequencing.
Koboldt, D., et al.***Genome Res.*** 2012 22:568-576

Normal vs Non-micropapillary
Normal vs MPA

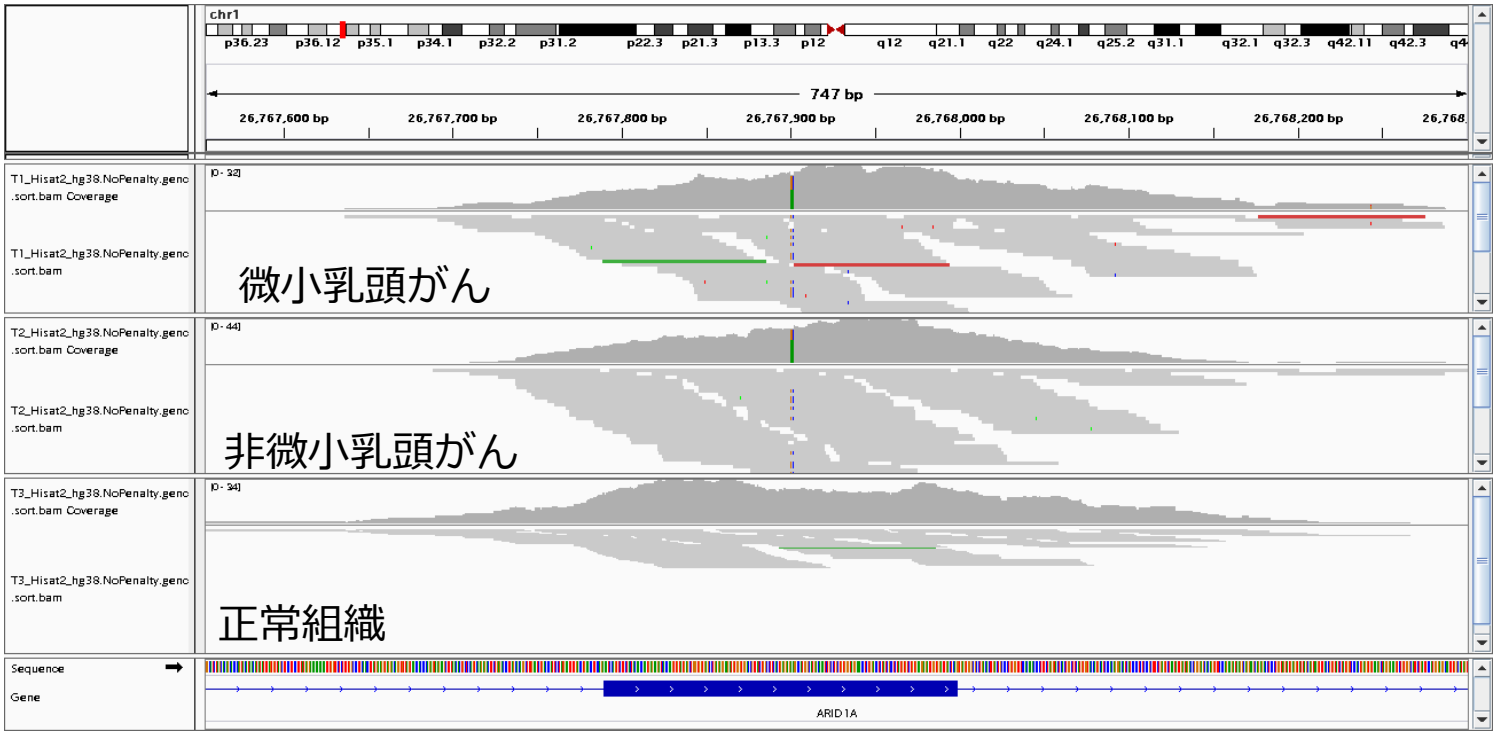
Whole-Exome Sequencing (WES) 解析

EGFR deletion



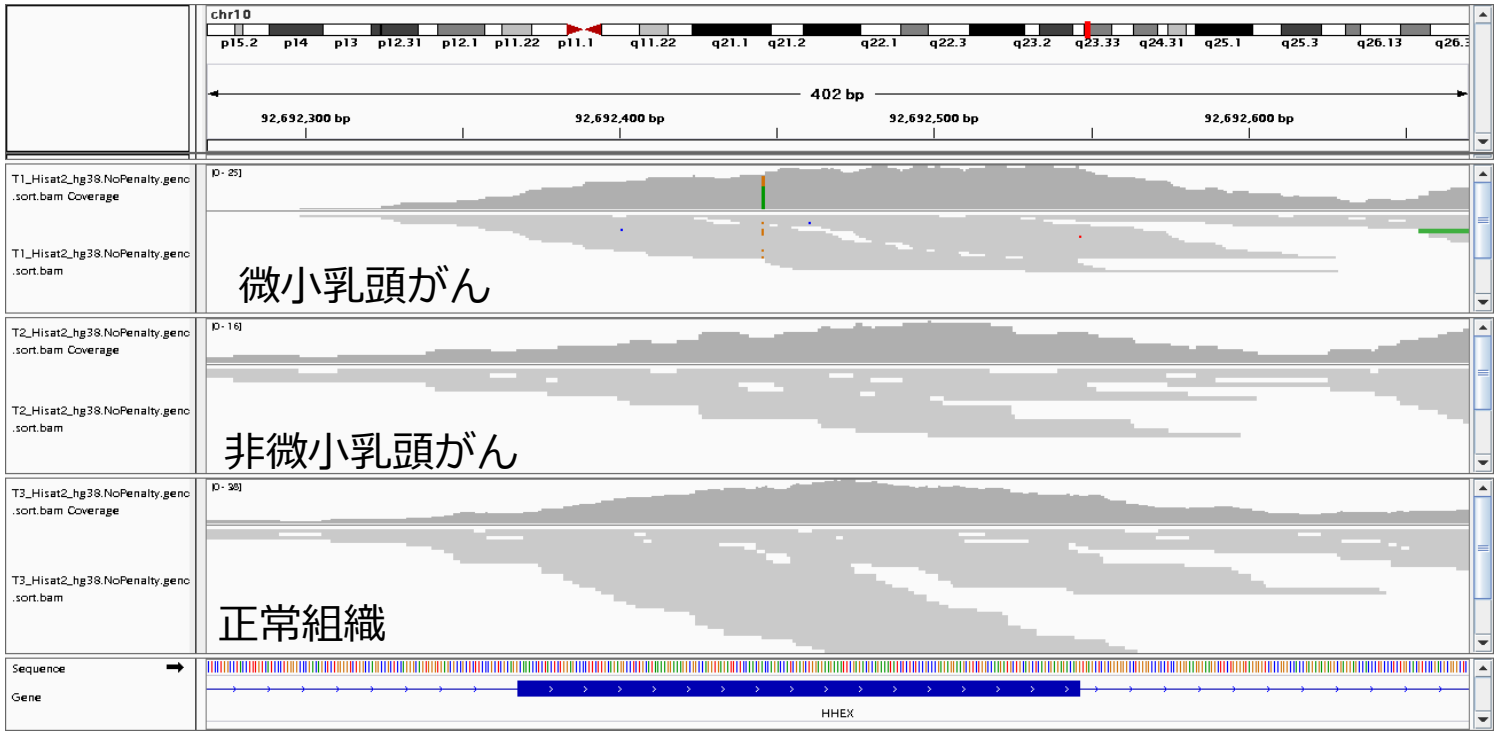
pileup format

ARID1A snp



Pileup format

HHEX snp

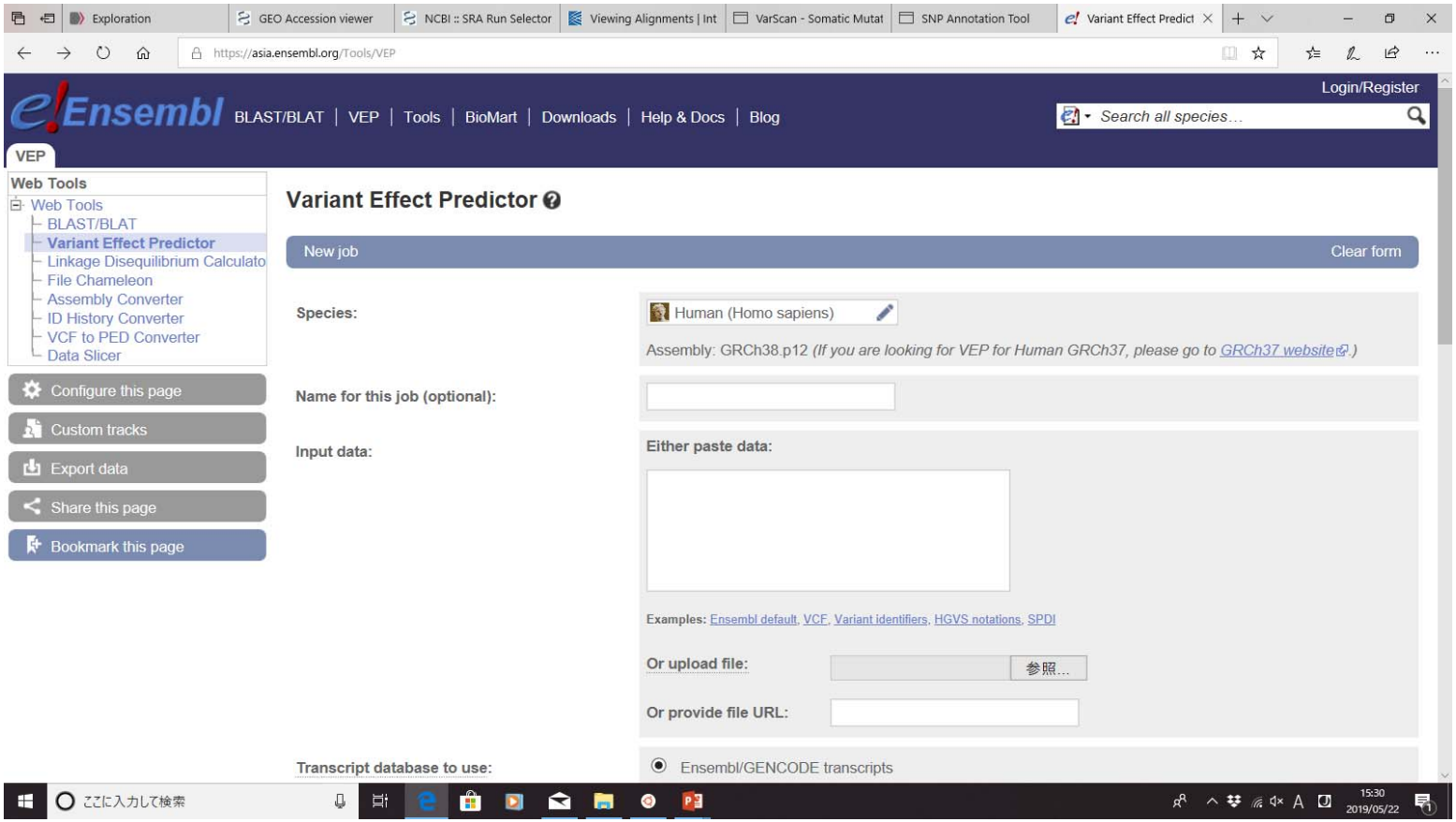


微小乳頭がん特異的体細胞変異

Variant Call Format (VCF)

#CHROM	POS	ID	REF	ALT	QUAL	FILTER
chr1	14930	2	A	G	0.042602	PASS
chr1	1455924	3	T	C	0.067266	PASS
chr1	2059684	4	G	A	0.05858	PASS
chr1	6554475	5	G	A	0.035576	PASS
chr1	10413139	6	C	T	0.055485	PASS
chr1	11668837	7	G	A	0.024802	PASS
chr1	16058680	8	G	A	0.067123	PASS
chr1	16563946	9	C	T	0.04199	PASS

Variant Effect Predictor

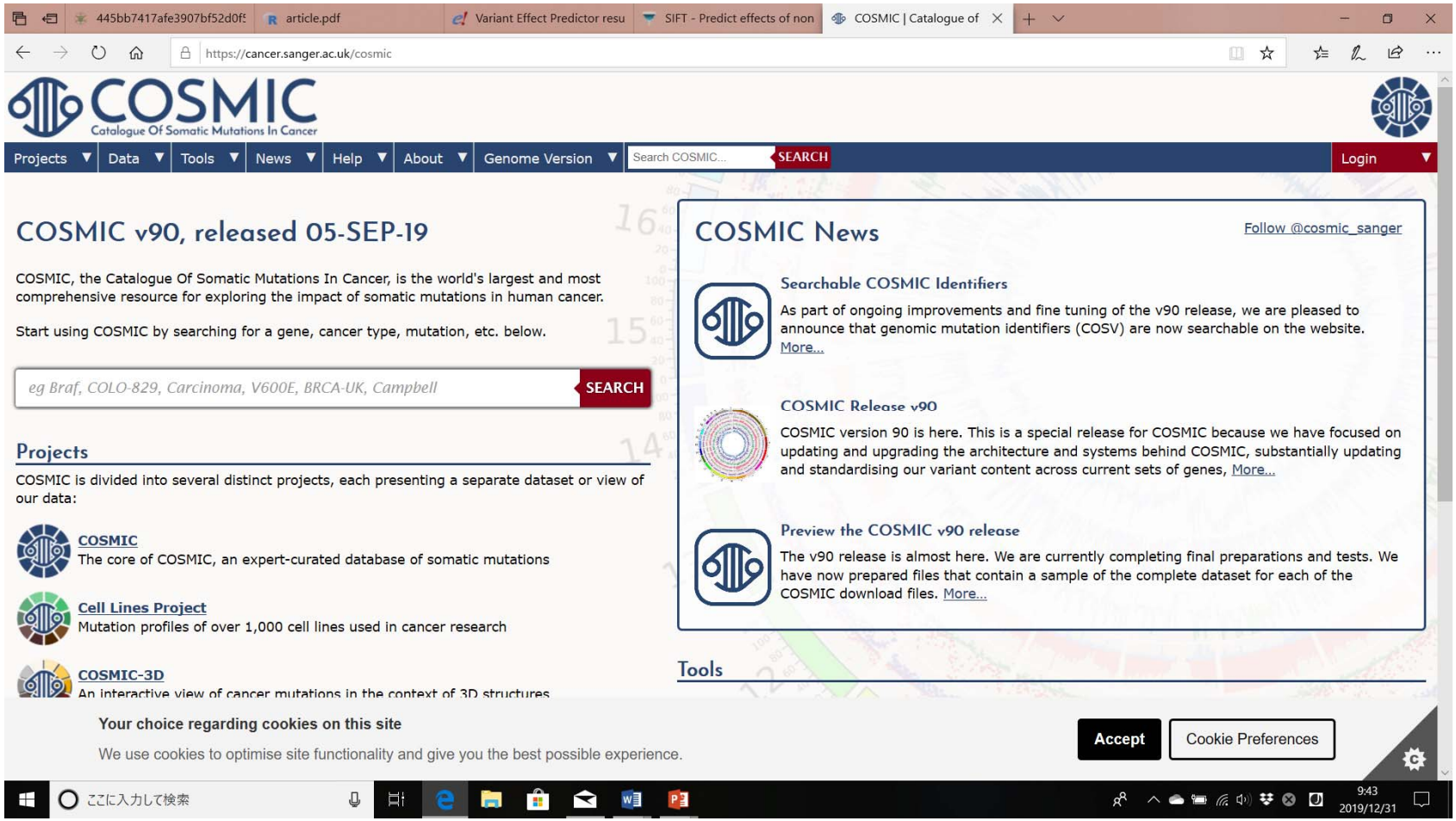


https://asia.ensemble.org/Tools/VEP

Variant Effect Predictor

- TEST.vcfファイルをデスクトップにドラッグアンドドロップしてコピー
- Variant Effect Predictorのファイルを選択ボタンでTEST.vcfを選択し，VEPにアップロード
- Runボタンをクリック

COSMIC



https://cancer.sanger.ac.uk/cosmic

アンケートにご協力ください

バイオインフォマティクス解析室HP
https://www.yokohamacu.ac.jp/amedrc/section/support/bioinfomatics2.html

第５回分アンケートのQRコード

最終アンケートのQRコード