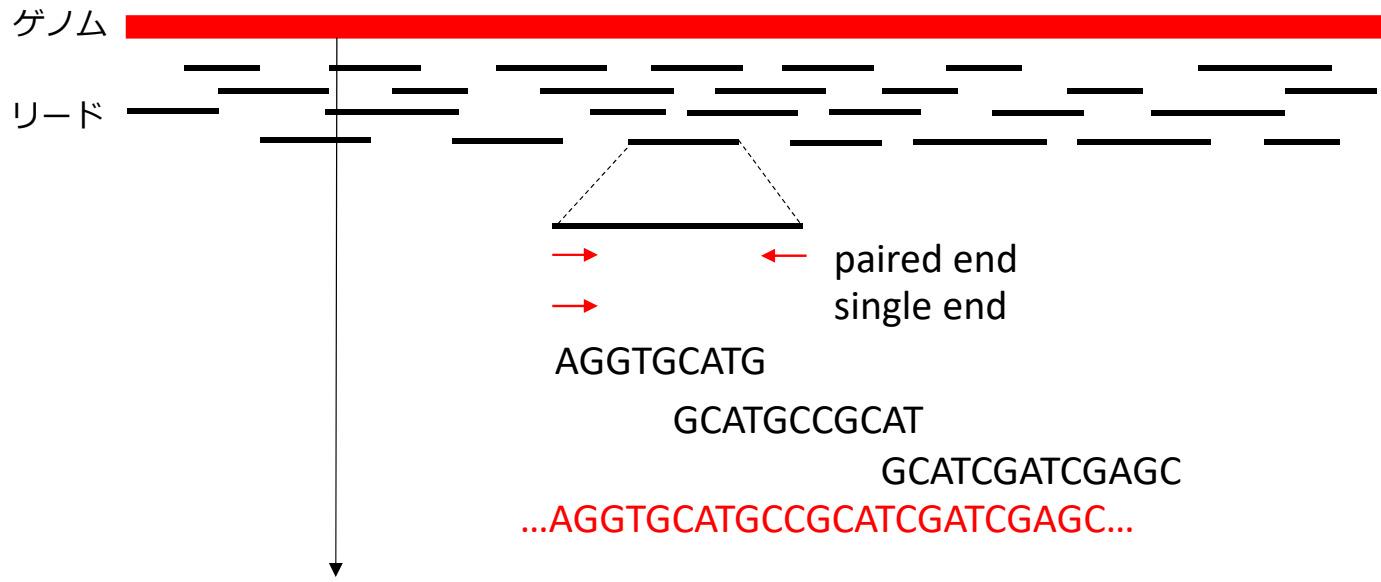


2019年度
第5回バイオインフォマティクス実習

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

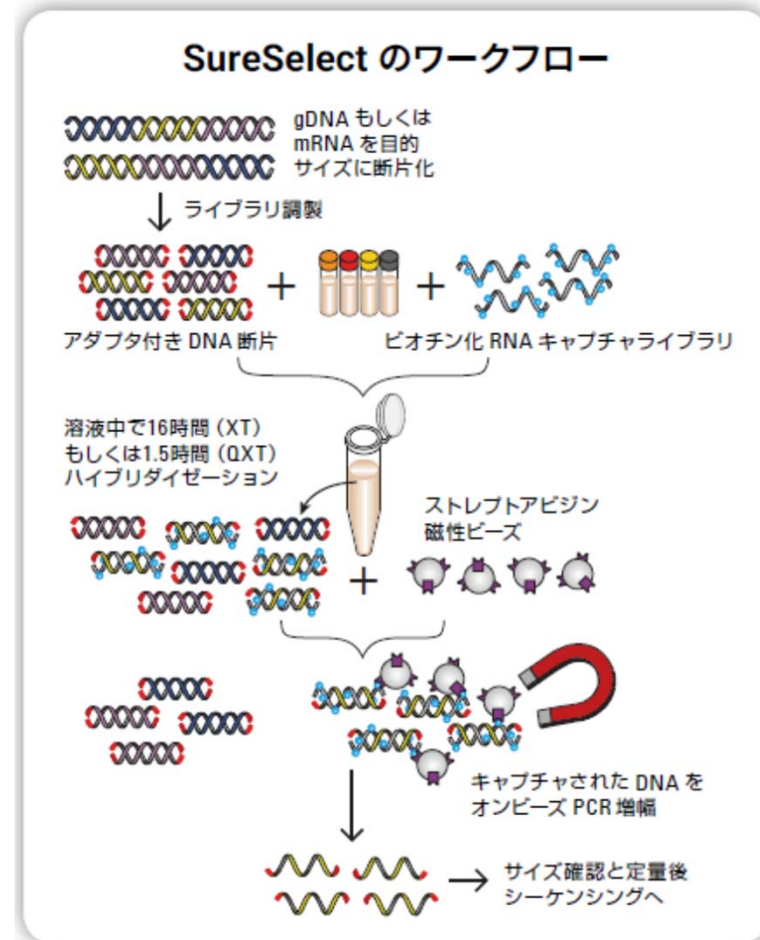


カバー率 = $(N \times L) / G$: 1塩基当りの断片数
リード数 : N
リード長 : L
ゲノム長 : G

カバー率が十分でないと正確な配列情報が得られない。

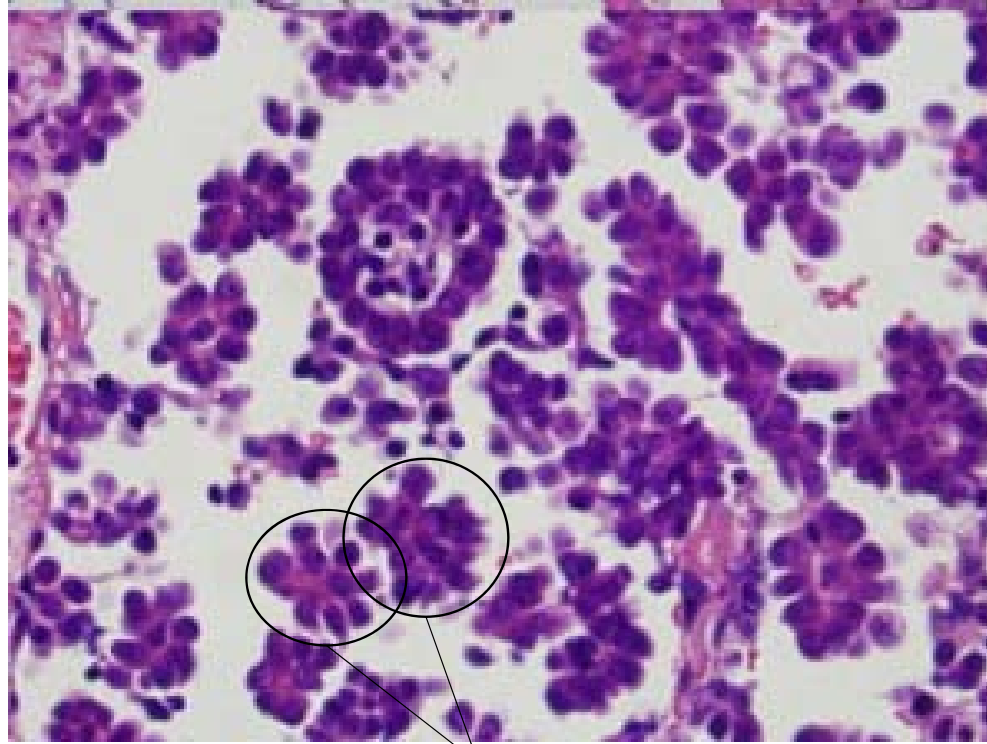
Whole-Exome Sequencing (WES) 解析

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



Agilent®

Micropapillary Predominant Adenocarcinoma (MPA)

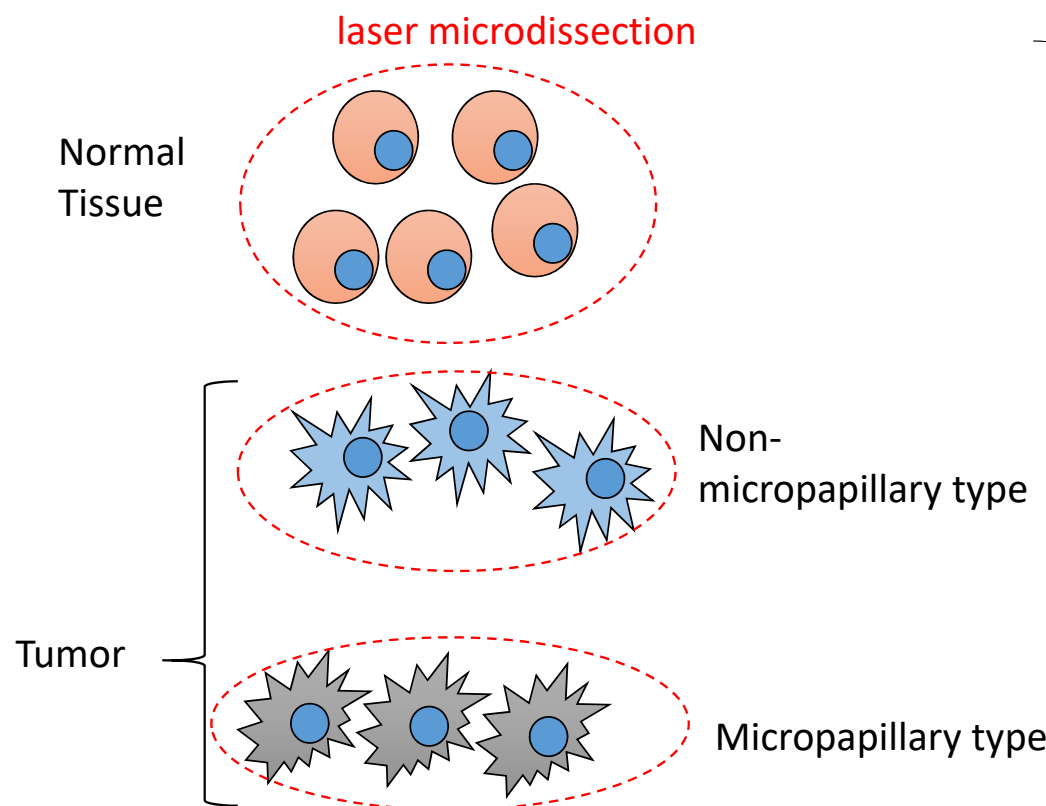


papillary tufts without fibrovascular core

<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×

$$\text{Coverage} = \text{mapped read counts} \times 2 \times 100 / (3,000,000,000 \times 0.02)$$

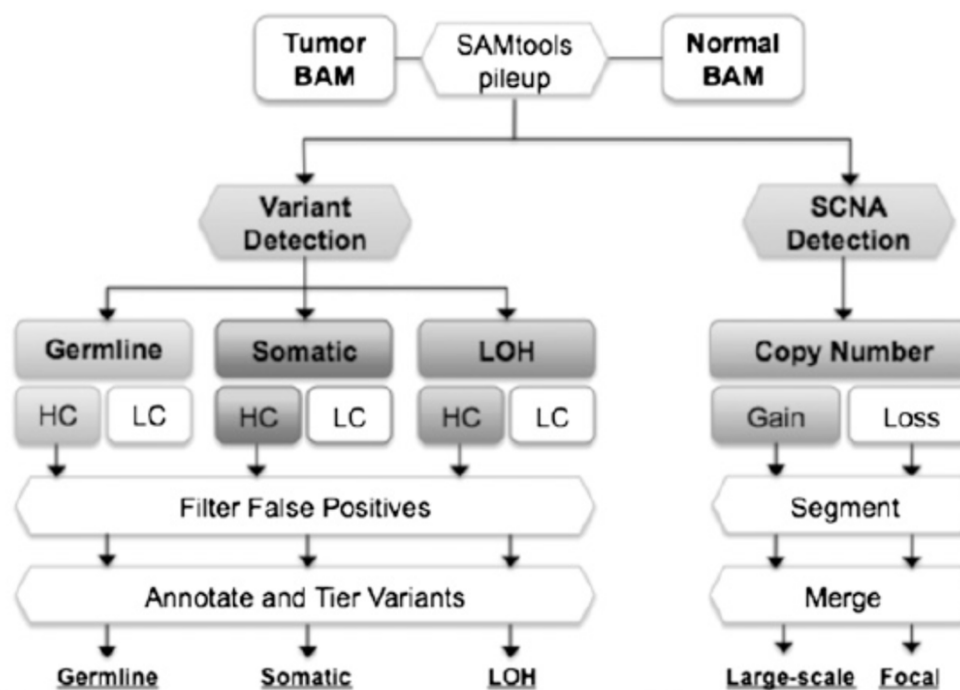
Estimation of the Somatic Mutations

- 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



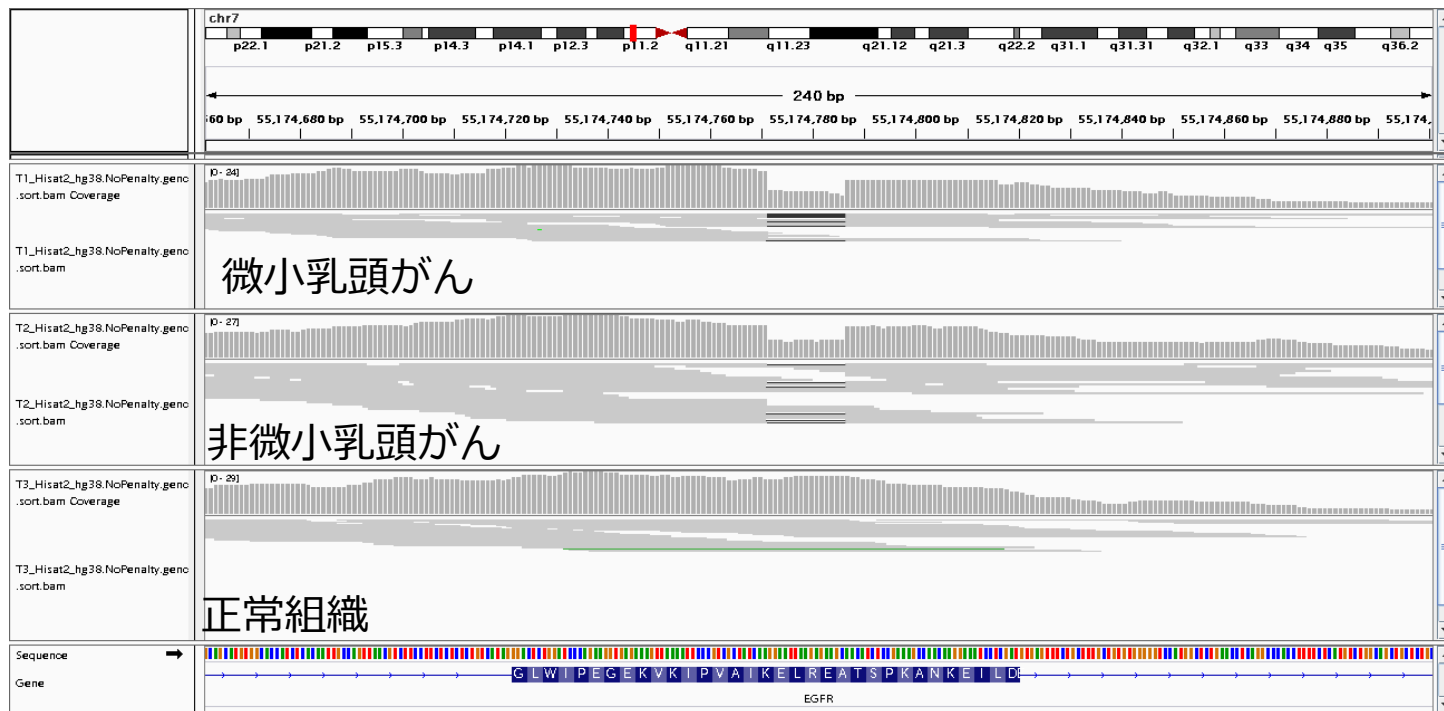
VarScan 2: Somatic mutation and copy number alteration discovery in cancer by exome sequencing.

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

Normal vs Non-micropapillary
Normal vs MPA

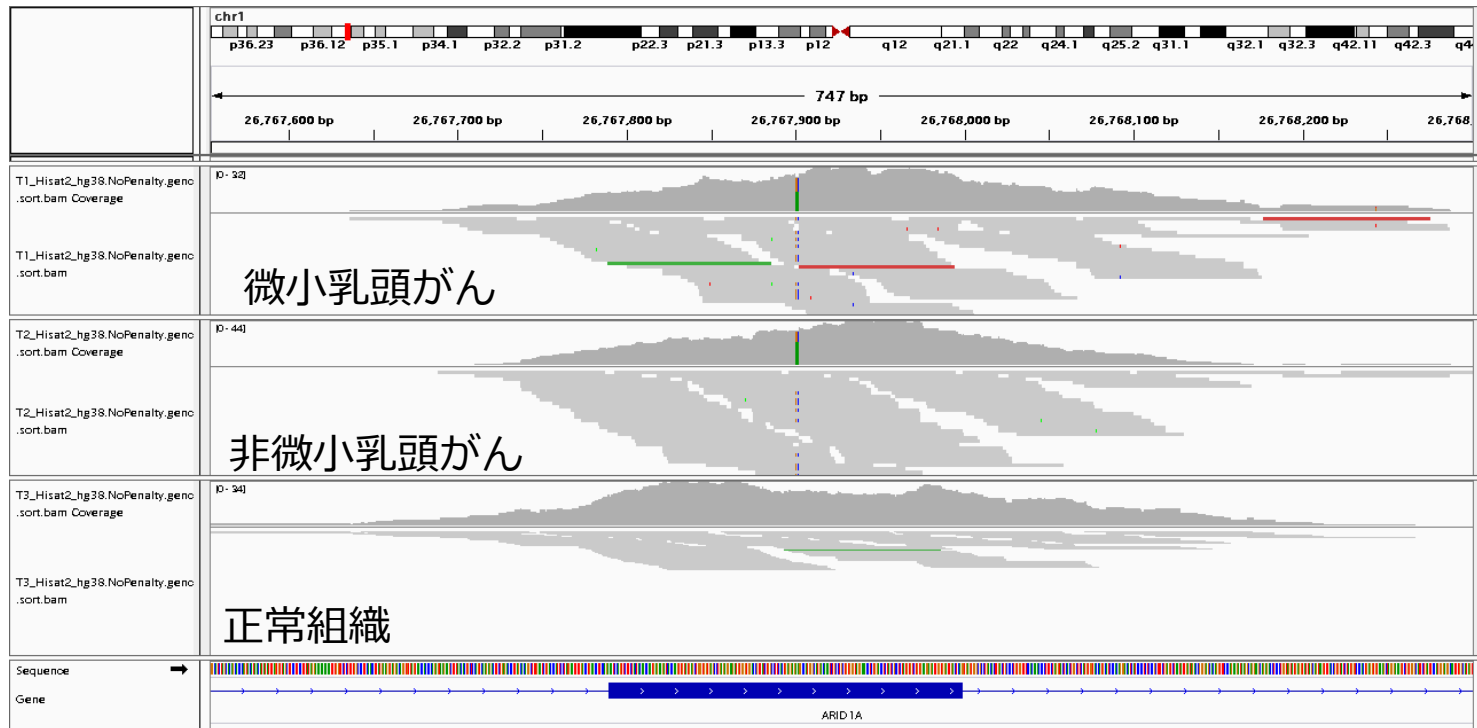
Whole-Exome Sequencing (WES) 解析

EGFR deletion



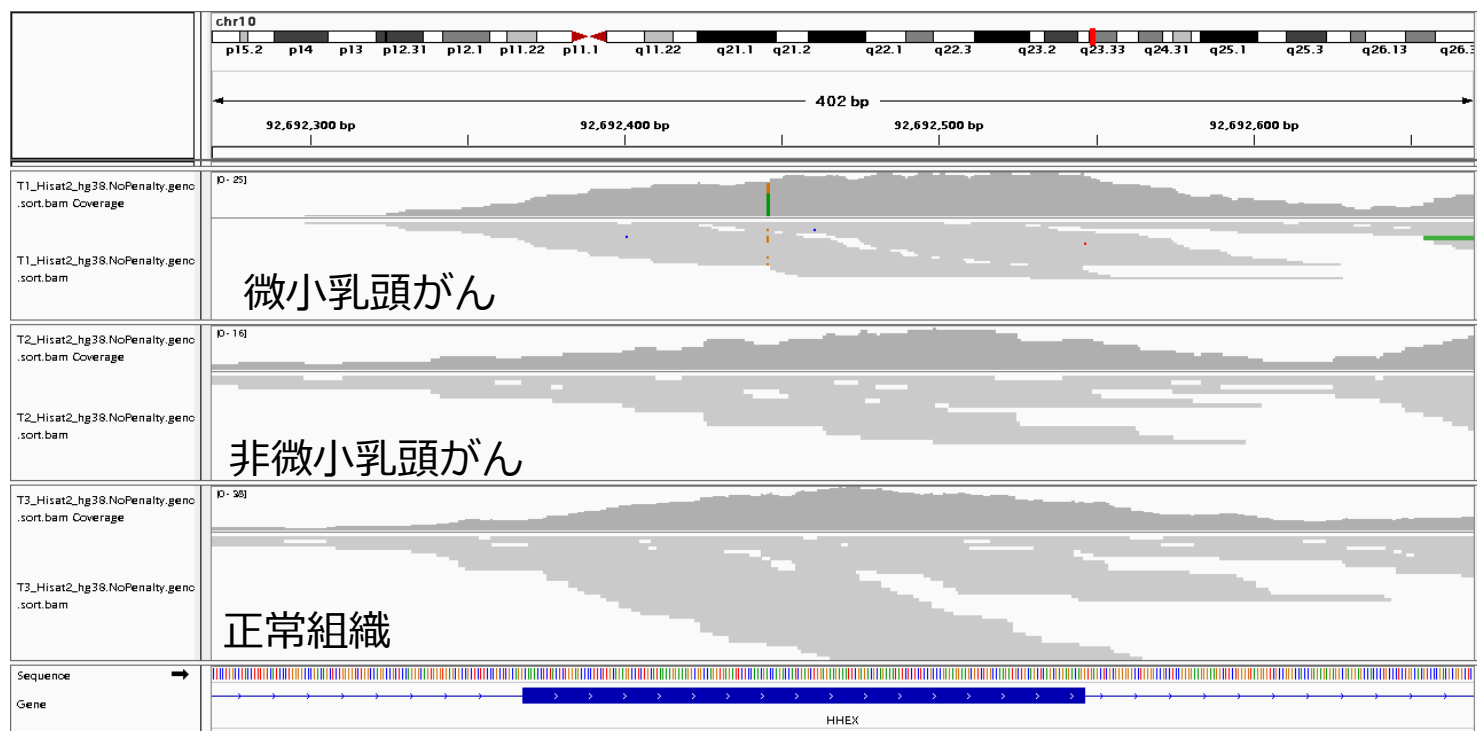
pileup format

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

The screenshot shows the Ensembl Variant Effect Predictor (VEP) web interface in a browser window. The browser's address bar displays the URL <https://asia.ensembl.org/Tools/VEP>. The page header includes the Ensembl logo and navigation links for BLAST/BLAT, VEP, Tools, BioMart, Downloads, Help & Docs, and Blog. A search bar for species is also present.

The main content area is titled "Variant Effect Predictor" and features a "New job" button and a "Clear form" button. The "Species" dropdown menu is set to "Human (Homo sapiens)". Below this, the assembly is specified as "GRCh38.p12 (If you are looking for VEP for Human GRCh37, please go to [GRCh37 website](#))".

The "Name for this job (optional):" field is empty. The "Input data:" section offers two options: "Either paste data:" with a large text area, and "Or upload file:" with a file selection button labeled "参照...". Below this, there is a field for "Or provide file URL:". Examples of input formats are listed: Ensembl default, VCF, Variant identifiers, HGVS notations, and SPDI.

At the bottom, the "Transcript database to use:" section has a radio button selected for "Ensembl/GENCODE transcripts".

The browser's taskbar at the bottom shows the Windows logo, a search bar with the text "ここに入力して検索", and several application icons. The system tray on the right indicates the time as 15:30 on 2019/05/22.

<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

The screenshot shows the COSMIC website homepage. At the top, there is a navigation bar with dropdown menus for 'Projects', 'Data', 'Tools', 'News', 'Help', 'About', and 'Genome Version'. A search bar is located to the right of these menus, and a 'Login' button is on the far right. The main content area is divided into several sections:

- COSMIC v90, released 05-SEP-19**: A section announcing the release of COSMIC v90, describing it as the world's largest and most comprehensive resource for somatic mutations. It includes a search bar with the text 'eg Braf, COLO-829, Carcinoma, V600E, BRCA-UK, Campbell' and a 'SEARCH' button.
- Projects**: A section listing three distinct projects: 'COSMIC' (the core database), 'Cell Lines Project' (mutation profiles of 1,000 cell lines), and 'COSMIC-3D' (an interactive 3D view of mutations).
- COSMIC News**: A section with three news items: 'Searchable COSMIC Identifiers', 'COSMIC Release v90', and 'Preview the COSMIC v90 release'. Each item includes a small icon and a 'More...' link.
- Tools**: A section header at the bottom of the main content area.

At the bottom of the page, there is a cookie consent banner with 'Accept' and 'Cookie Preferences' buttons. The Windows taskbar is visible at the very bottom, showing the time as 9:43 on 2019/12/31.

<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/bioinformatics2.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

