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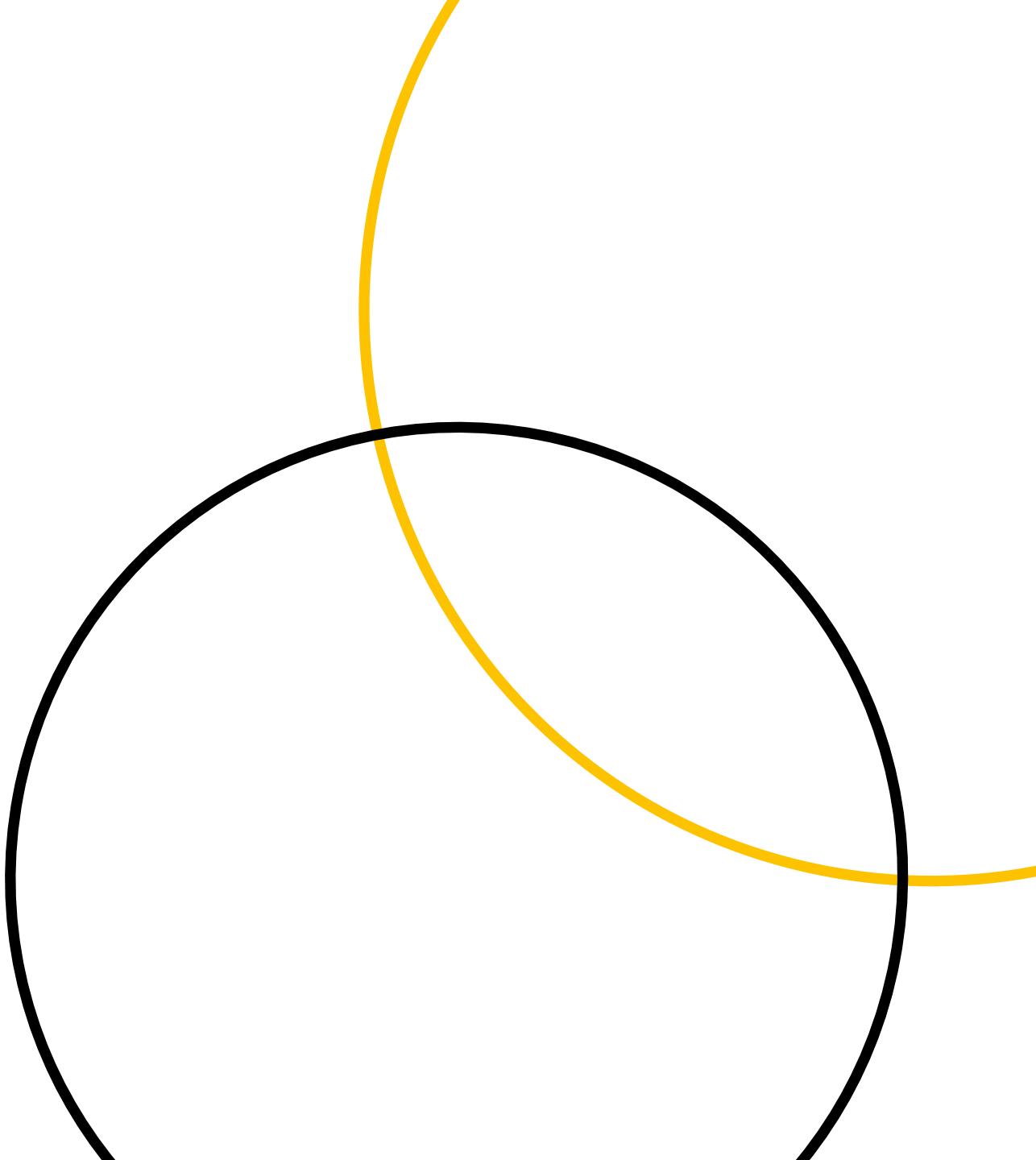
Dr Jia-Wern Pan

Cancer Research Malaysia

Introduction to mutational signatures

(adapted from Dr Marcos Díaz Gay, WCS CGA 2023)

April 2025



Recap: Variant Calling

- What does a VCF file contain?
- How does the data in a VCF file help a patient?
- How do you aggregate VCF files from multiple patients?

Why does X patient have X variants?

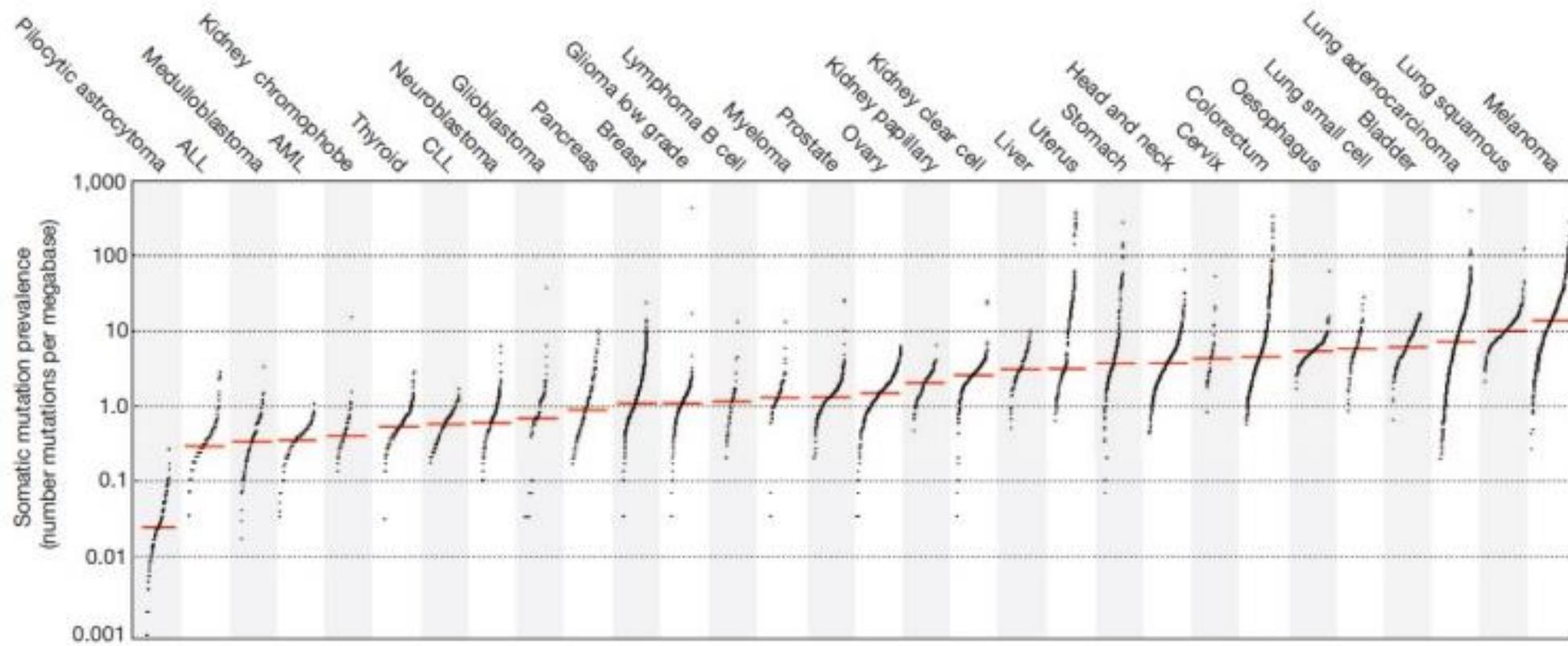
Mutational signature analysis is an attempt to determine the causal sources (aetiology) of somatic variants and mutations in an individual or set of samples

Discuss: What are common (or rare) causes of somatic mutations? How might these differ across cancer types?

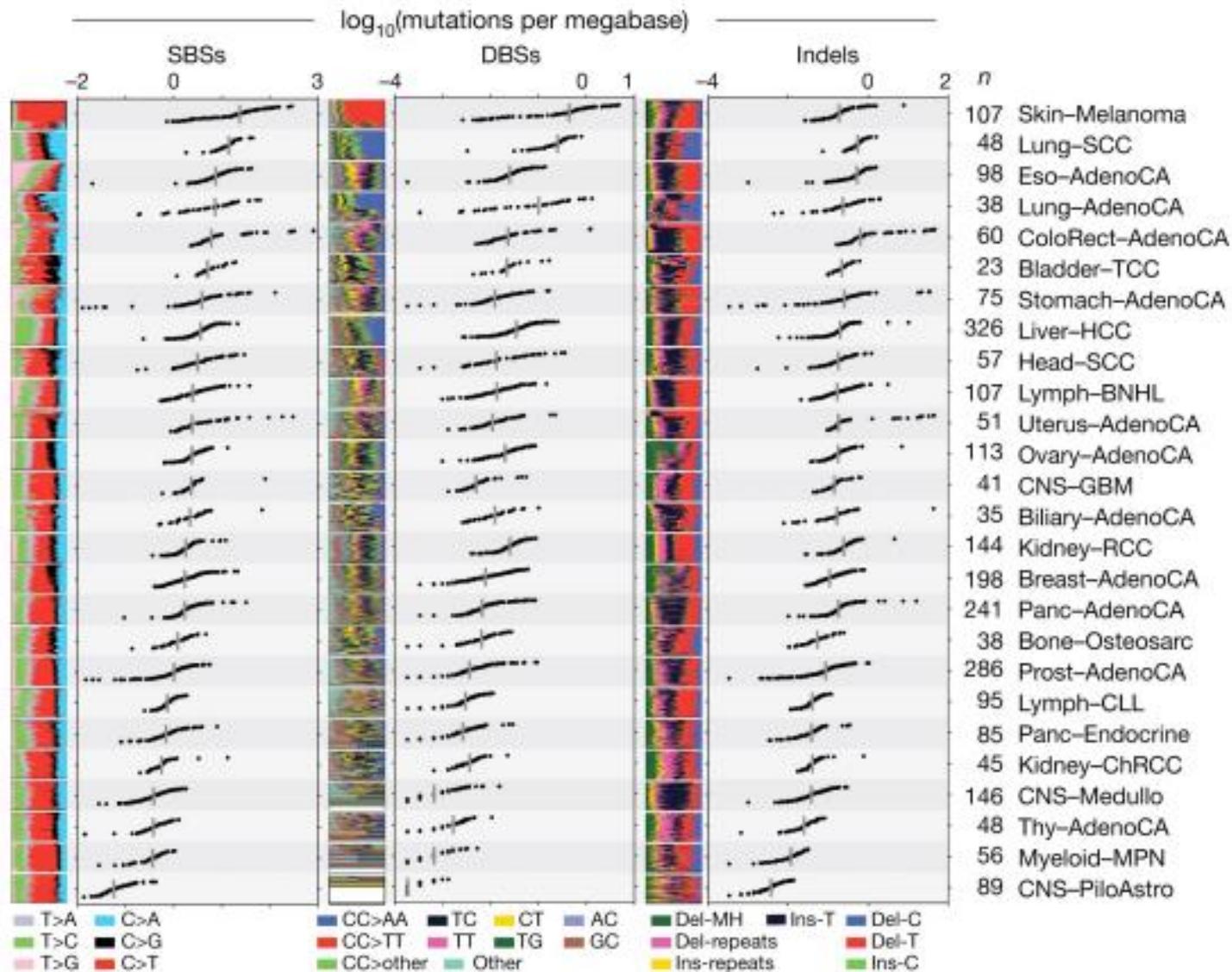
Well-known carcinogens and carcinogenic processes

- Aging
- UV light
- DNA repair deficiencies
- Tobacco smoke
- Alcohol
- Asbestos
- Bacteria i.e. *Helicobacter pylori*
- Viruses i.e. HPV, EBV

The burden of somatic mutations is highly variable across different types of tumours

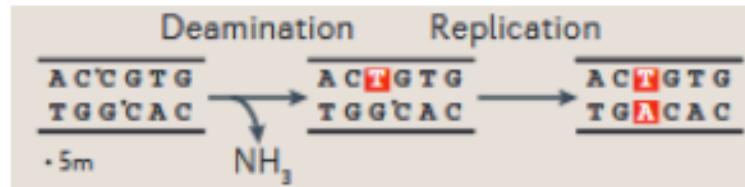
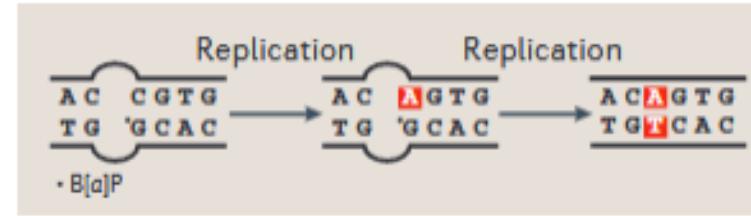


The burden of somatic mutations is highly variable across different types of tumours



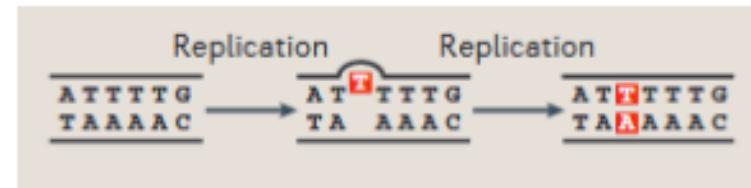
Different sources of DNA damage have different patterns of mutations

Environmental exposures
Tobacco smoking or chewing



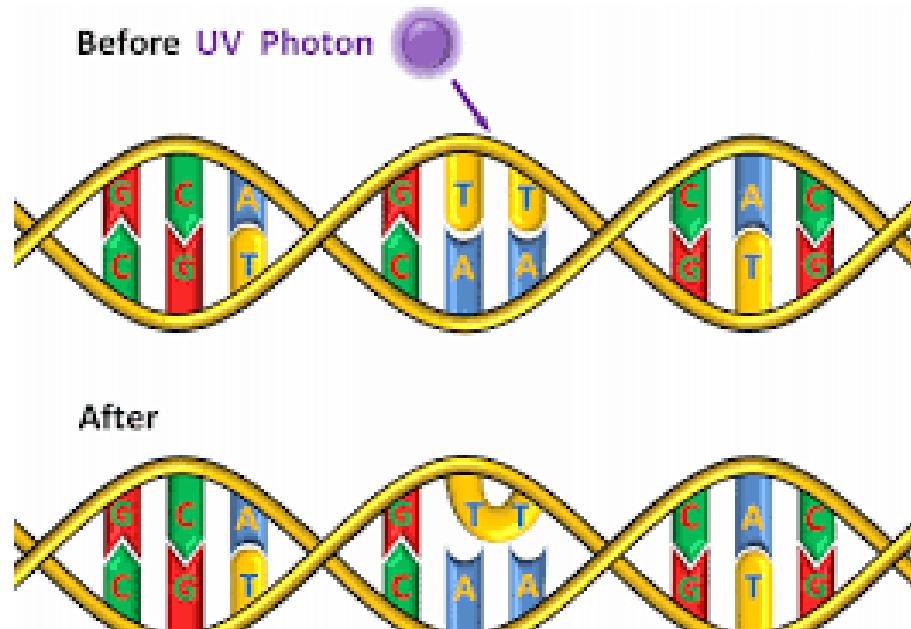
Normal cellular activities
Spontaneous deamination of methylated cytosines

Failure in DNA replication or repair
Aberrant mismatch repair pathway

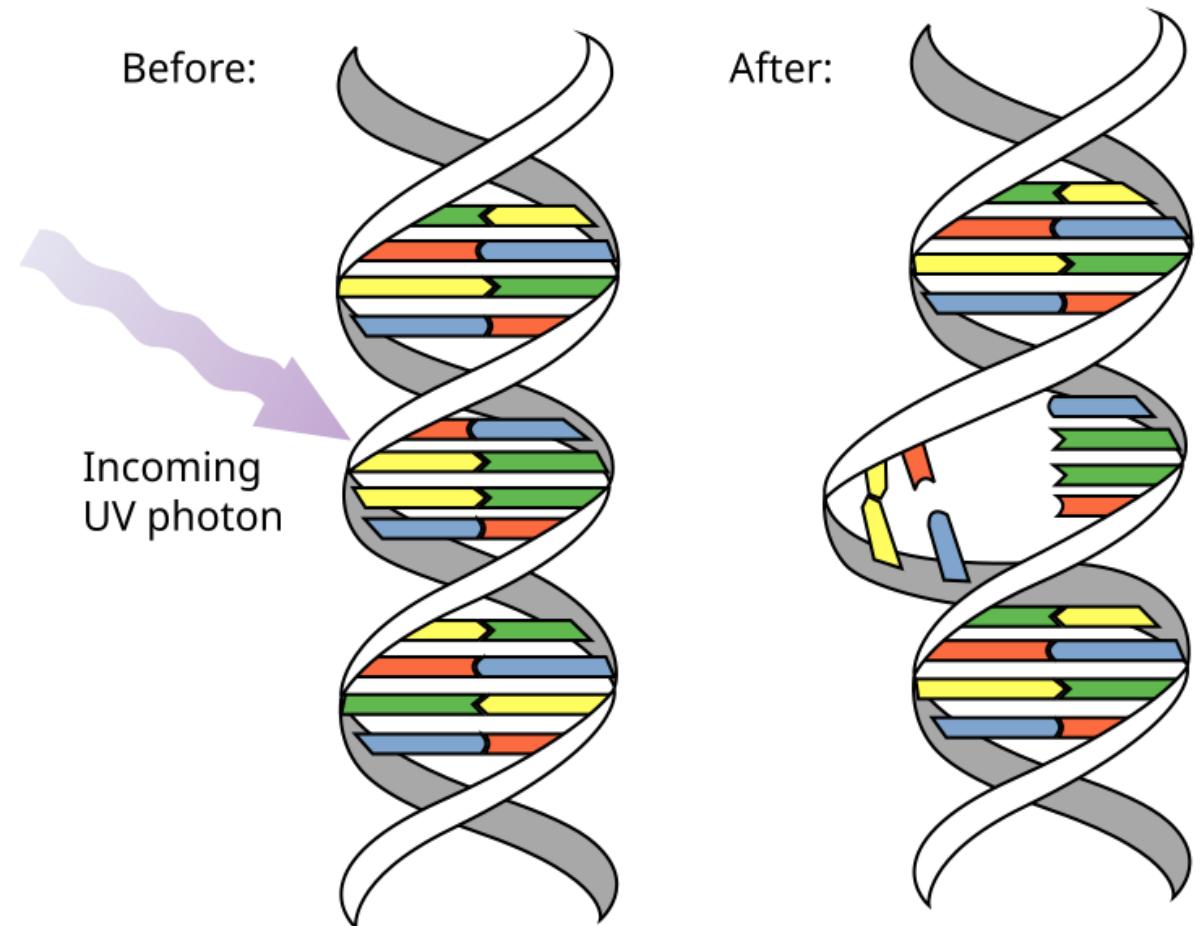


UV radiation

Thymine dimers



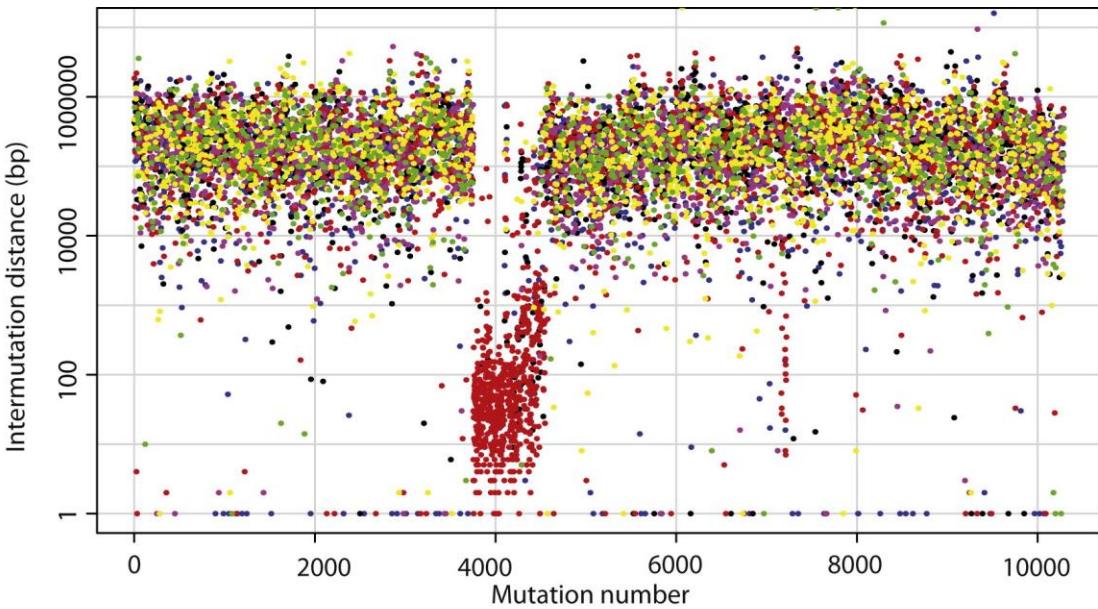
Schmid et al. 2017



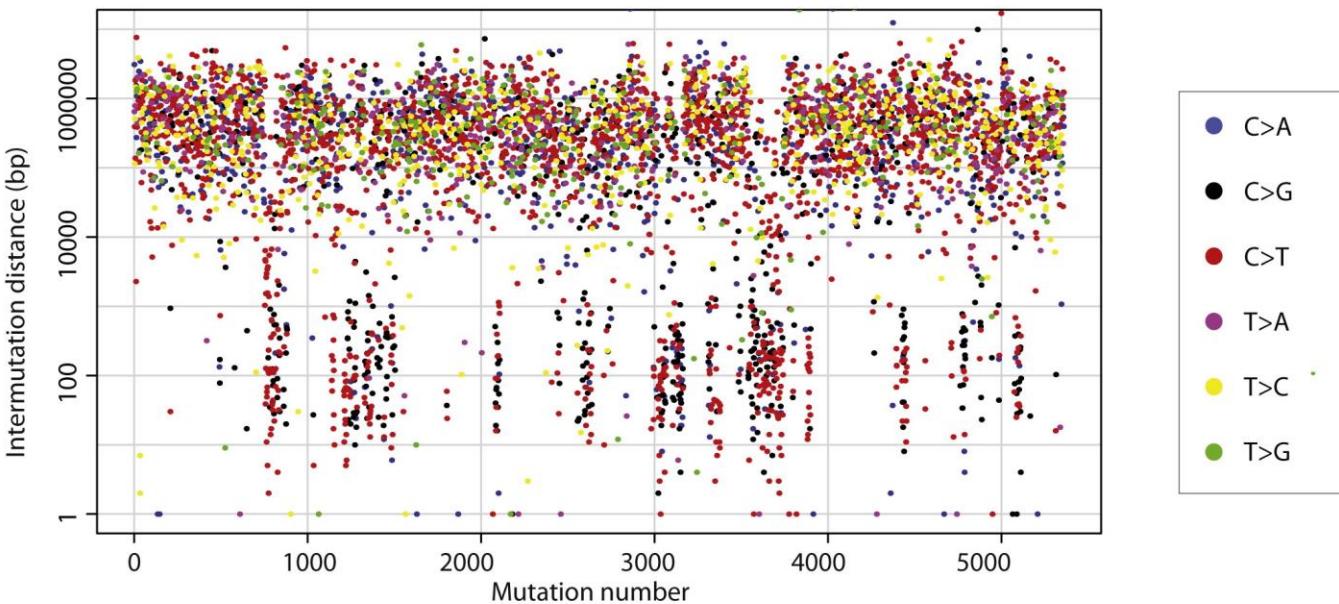
Kataegis

Localized somatic
hypermutation
associated with
APOBEC enzymes and
TLS DNA polymerases

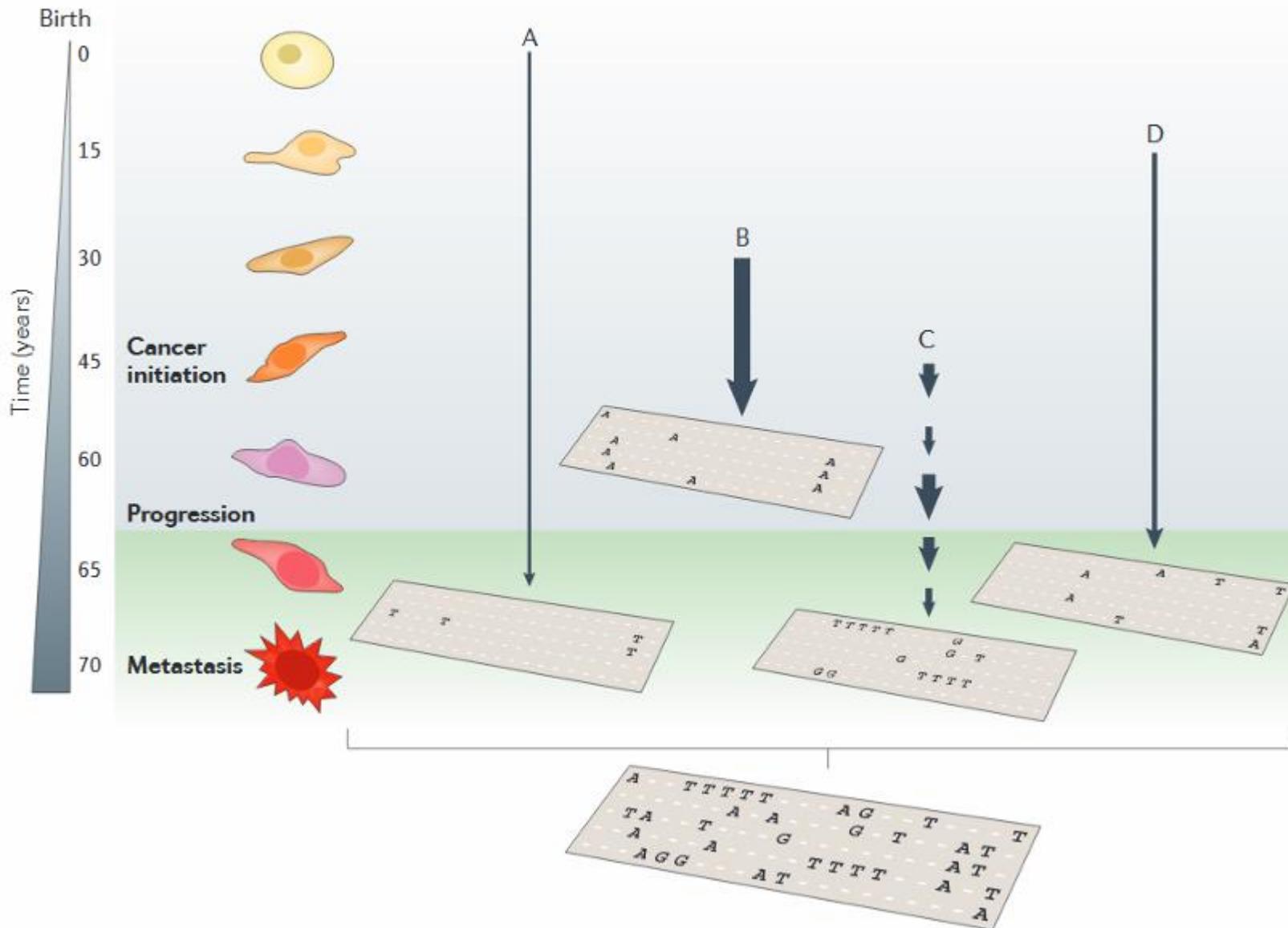
A
PD4107a
germline BRCA1



B
PD4103a
ER +ve, HER2 -ve



Multiple mutational processes can occur simultaneously

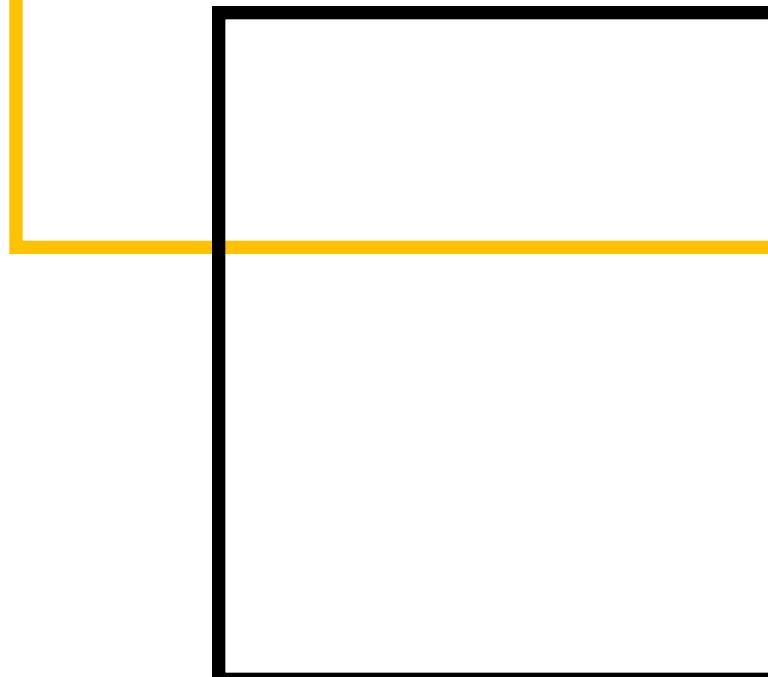


Mutational processes accumulate over the life history of each cell



Looking for patterns of somatic mutations in the cancer genome

How do we actually find and discover mutational signatures from genomic data?



Looking for patterns of somatic mutations in the cancer genome

The first step is to classify or group different somatic mutations into categories

Discuss: What are different ways that you can group somatic mutations occurring at different positions throughout the genome?

Classification of SNVs

- Transitions versus transversions
- Synonymous versus non-synonymous
- Location/position
- Base substitutions
- **Mutational context**

Single base substitution

.....ATCGGGAAAT**C**GGACCCGATG.....
 ↓
.....ATCGGGAAAT**T**GGACCCGATG.....

Trinucleotide context of the single base substitution

.....ATCGGGAA**TCG**GACCCGATG.....
 ↓
.....ATCGGGAA**TTG**GACCCGATG.....

Trinucleotide context of the single base substitution

.....ATCGGGAA**TCG**GACCCGATG.....
 ↓
.....ATCGGGAA**TTG**GACCCGATG.....

.....ATCGGGAA**ACG**GACCCGATG.....
 ↓
.....ATCGGGAA**ATG**GACCCGATG.....

.....ATCGGGAA**ACC**GACCCGATG.....
 ↓
.....ATCGGGAA**ATC**GACCCGATG.....

C>T

C>A

C>G

T>A

T>C

T>G

6 classes of base substitutions

6 classes of base substitutions

C>T
C>A
C>G
T>A
T>C
T>G

ACA>ATA
ACC>ATC
ACG>ATG
ACT>ATT
CCA>CTA
CCC>CTC
CCG>CTG
CCT>CTT
GCA>GTA
GCC>GTC
GCG>GTG
GCT>GTT
TCA>TTA
TCC>TTC
TCG>TTG
TCT>TTT

16 different
trinucleotide
contexts per class

6 classes of base substitutions

C>T

C>A

C>G

T>A

T>C

T>G

A**CA**>ATA ATA>AAA
ACC>ATC ATC>AAC
ACG>ATG ATG>AAG
ACT>ATT ATT>AAT
CCA>CTA CTA>CAA
CCC>CTC CTC>CAC
CCG>CTG CTG>CAG
C**CT**>CTT CTT>CAT
G**CA**>GTA GTA>GAA
GCC>GTC GTC>GAC
GCG>GTG GTG>GAG
G**CT**>GTT GTT>GAT
TCA>TTA TTA>TAA
TCC>TTC TTC>TAC
TCG>TTG TTG>TAG
T**CT**>TTT TTT>TAT

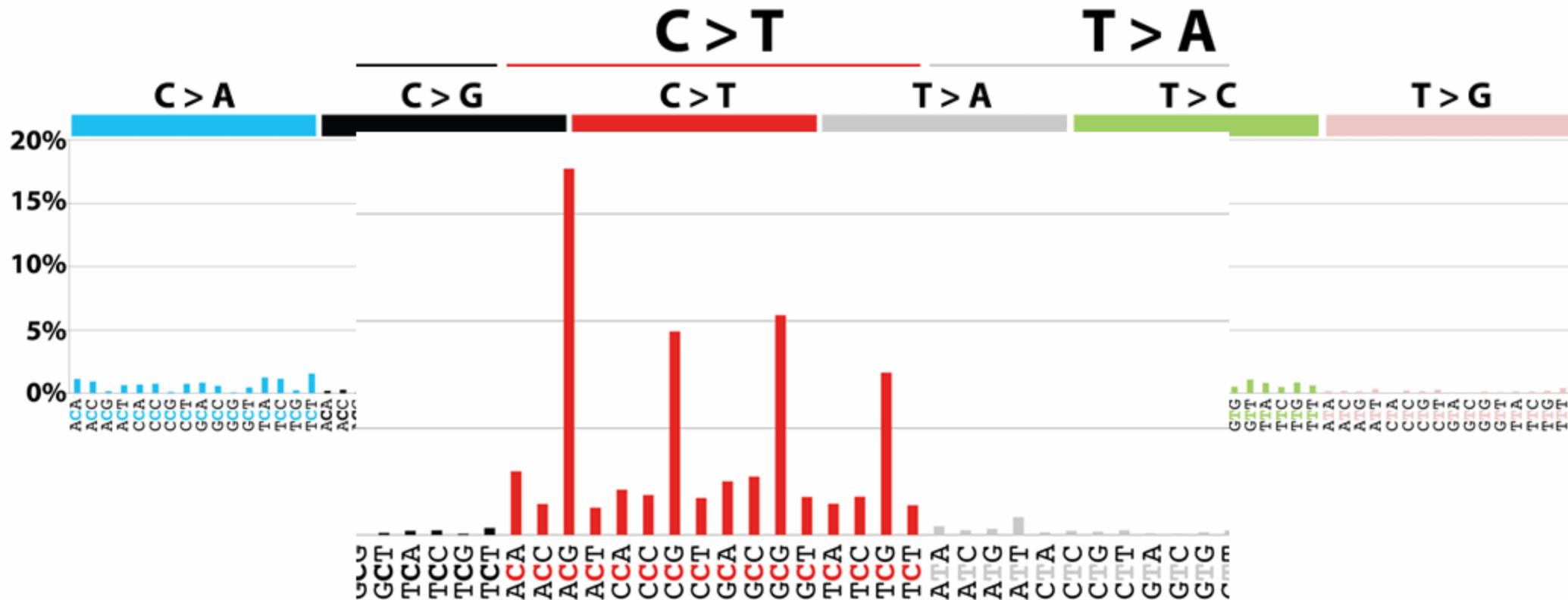
A**CA**>AAA ATA>ACA
ACC>AAC ATC>ACC
ACG>AAG ATG>ACG
ACT>AAT ATT>ACT
CCA>CAA CTA>CCA
CCC>CAC CTC>CCC
CCG>CAG CTG>CCG
C**CT**>CAT CTT>CCT
G**CA**>GAA GTA>GCA
GCC>GAC GTC>GCC
GCG>GAG GTG>GCG
G**CT**>GAT GTT>GCT
TCA>TAA TTA>TCA
TCC>TAC TTC>TCC
TCG>TAG TTG>TCG
T**CT**>TAT TTT>TCT

A**CA**>AGA ATA>AGA
ACC>AGC ATC>AGC
ACG>AGG ATG>AGG
ACT>AGT ATT>AGT
CCA>CGA CTA>CGA
CCC>CGC CTC>CGC
CCG>CGG CTG>CGG
C**CT**>CGT CTT>CGT
G**CA**>GGA GTA>GGA
GCC>GGC GTC>GGC
GCG>GGG GTG>GGG
G**CT**>GGT GTT>GGT
TCA>TGA TTA>TGA
TCC>TGC TTC>TGC
TCG>TGG TTG>TGG
T**CT**>TGT TTT>TGT

96 classes of SNV mutations

Six classes of single-base mutations
Reported by pyrimidine

Adding 5' and 3' adjacent bases
96 possibilities considering context

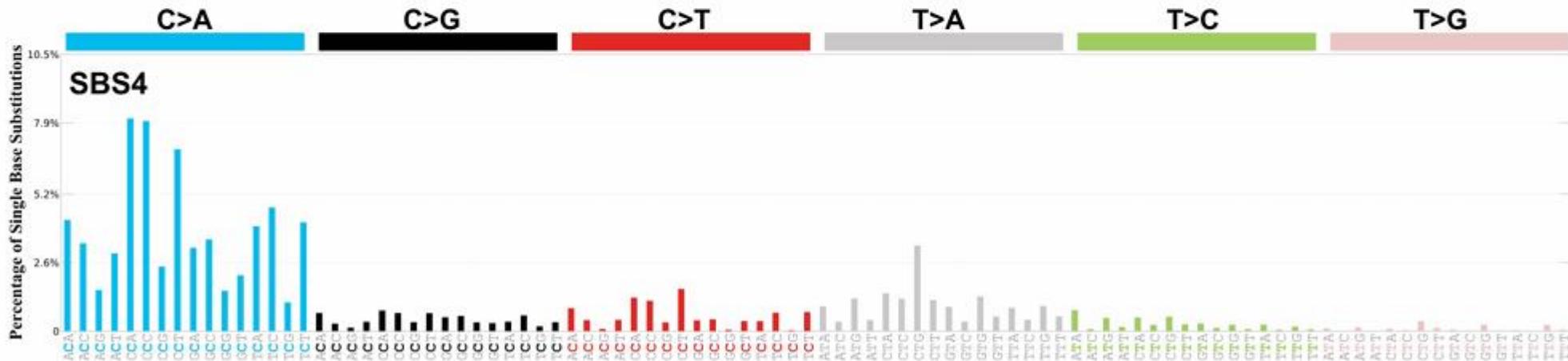




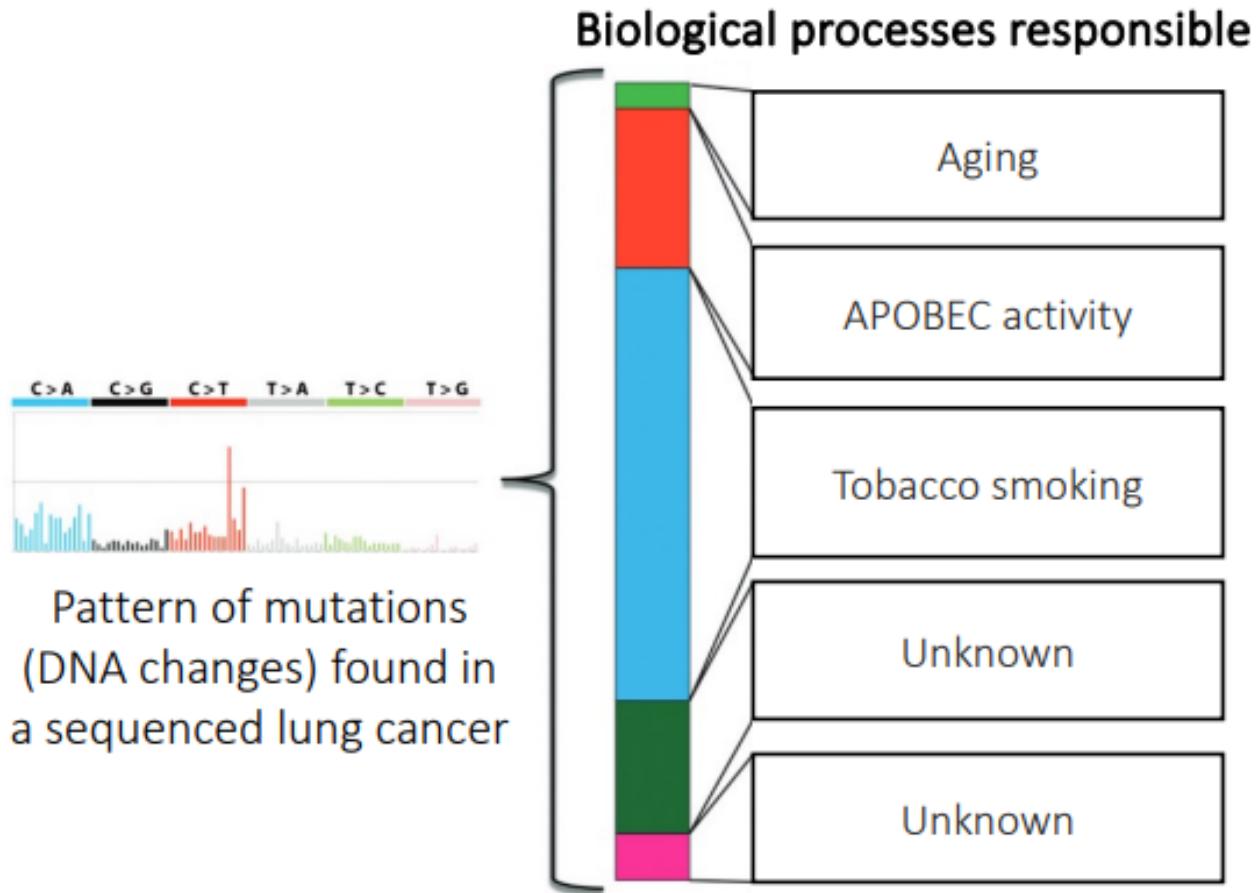
Tobacco smoking



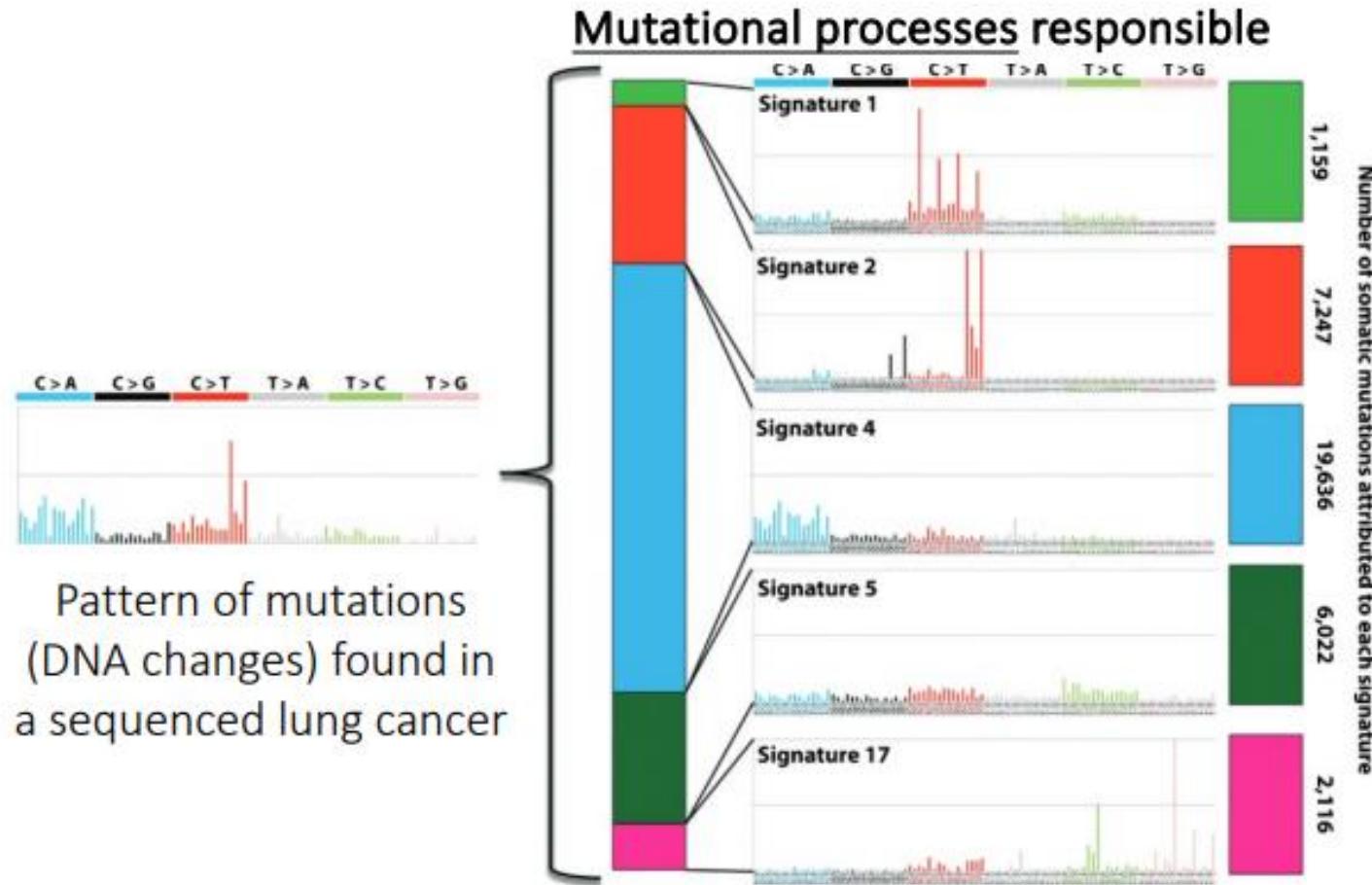
Cause of ~80% of lung cancers



The mutational profile of a tumour is the sum of all the biological processes acting on the cells in the tumour



The mutational profile of a tumour is the sum of all the mutational processes acting on the cells in the tumour



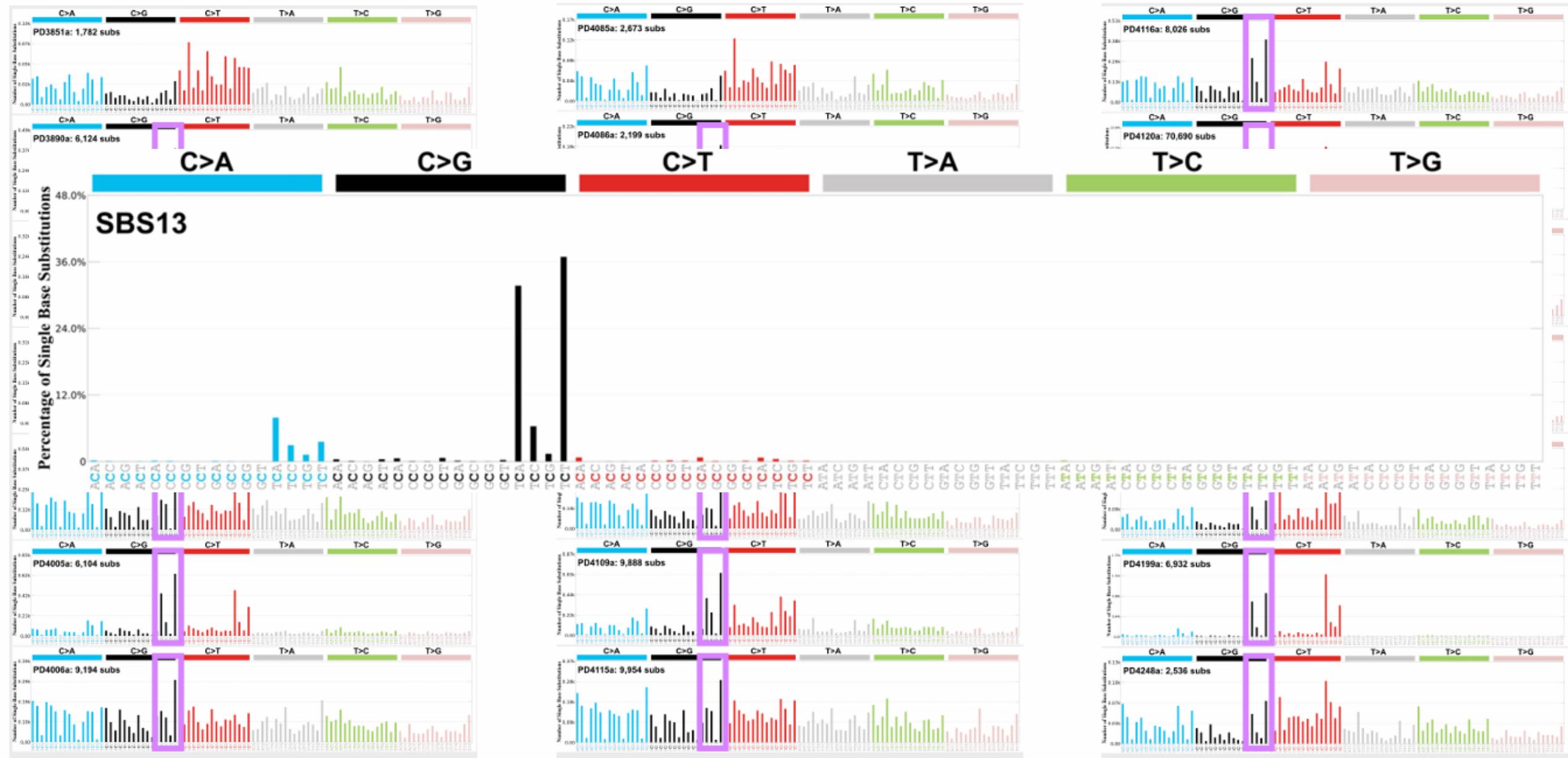
Patterns of the 96 mutation classes across individual breast tumours



Patterns of the 96 mutation classes across individual breast tumours

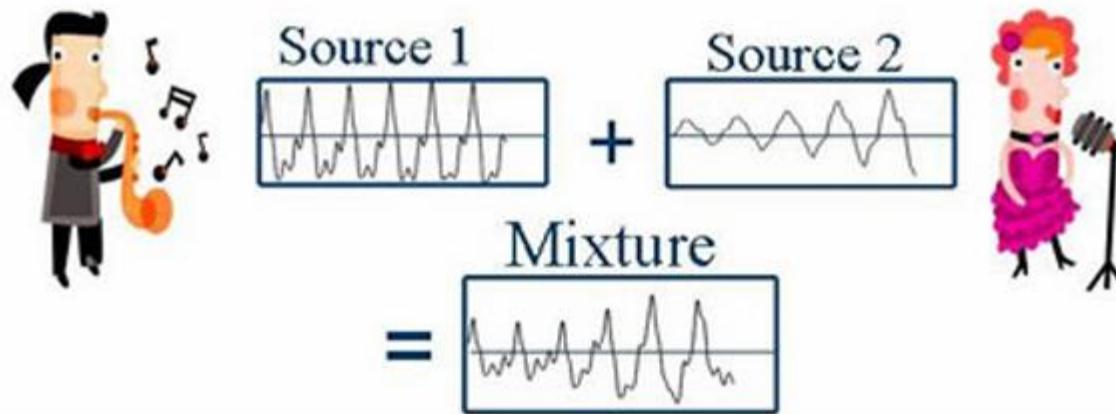


Patterns of the 96 mutation classes across individual breast tumours

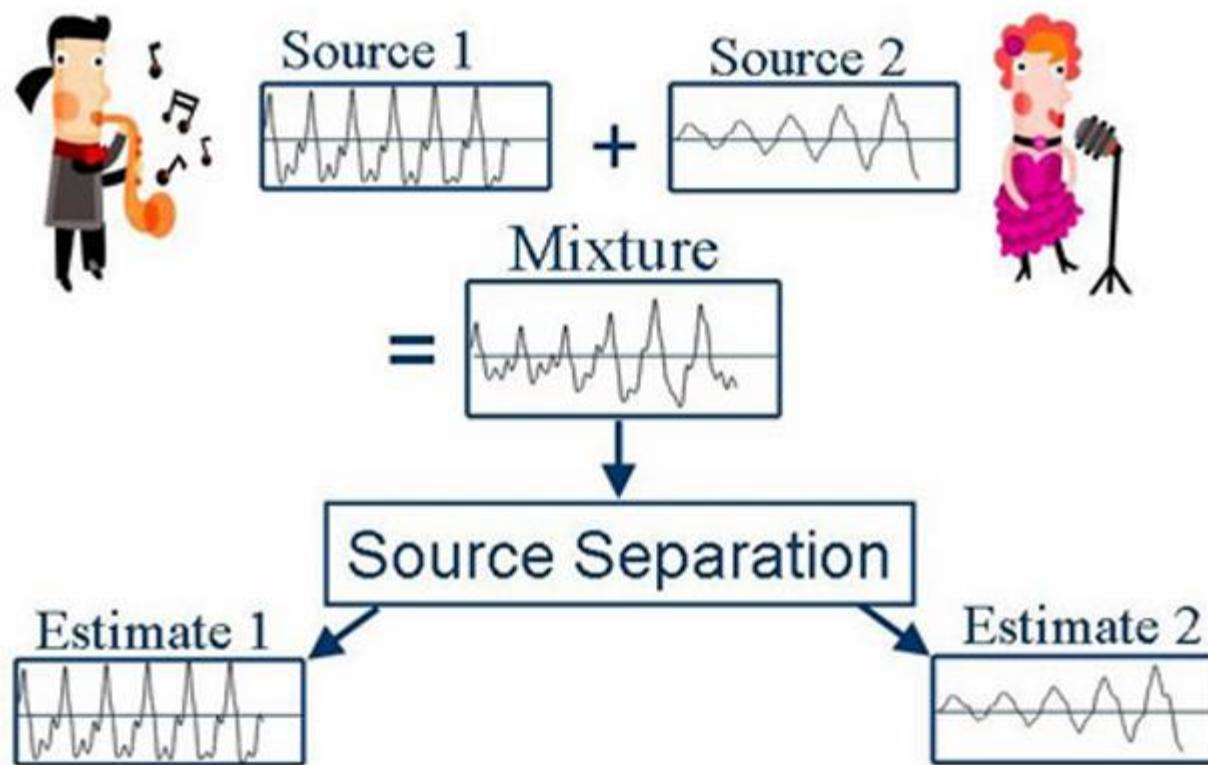


Isolating the contribution of individual signatures to an overall pattern = blind signal separation problem

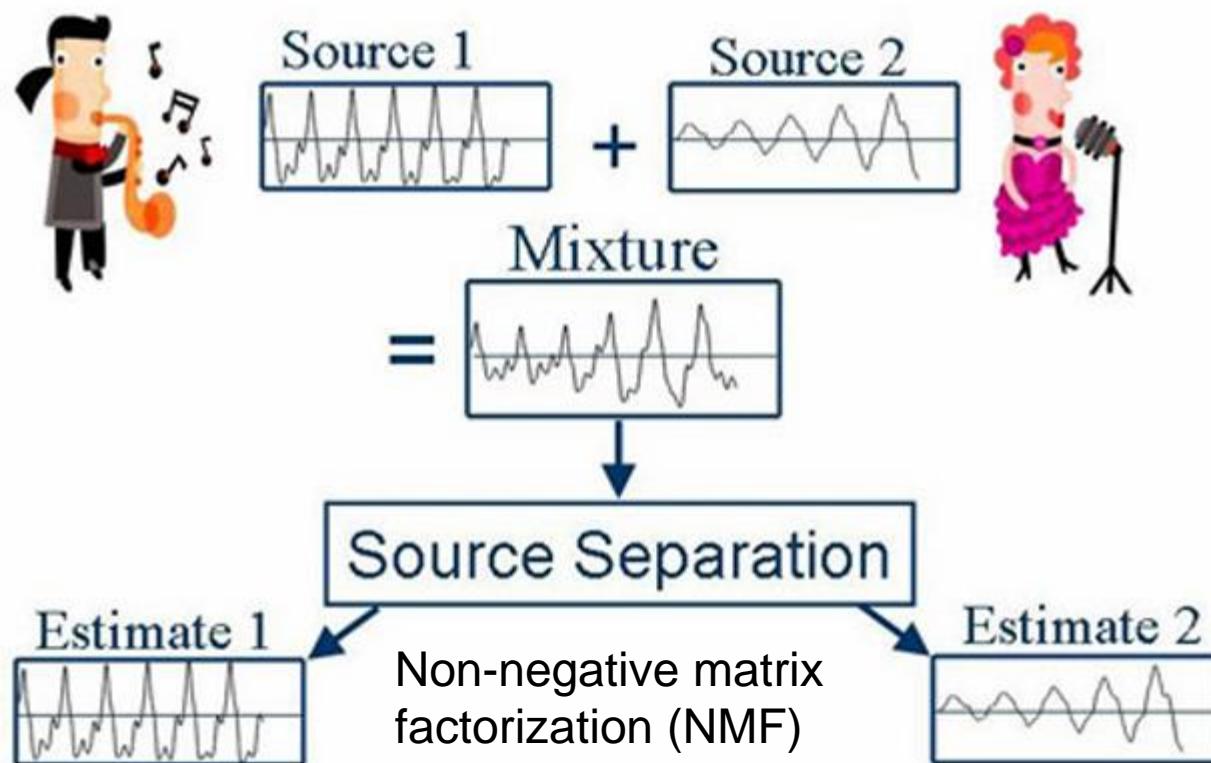
Blind signal separation problem



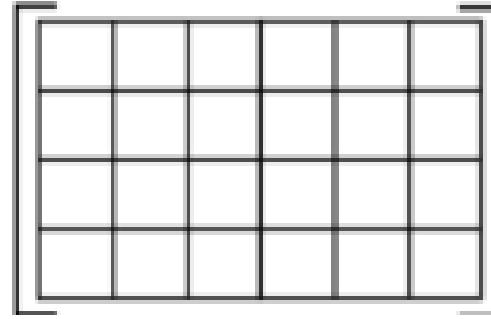
Blind signal separation problem



Blind signal separation problem

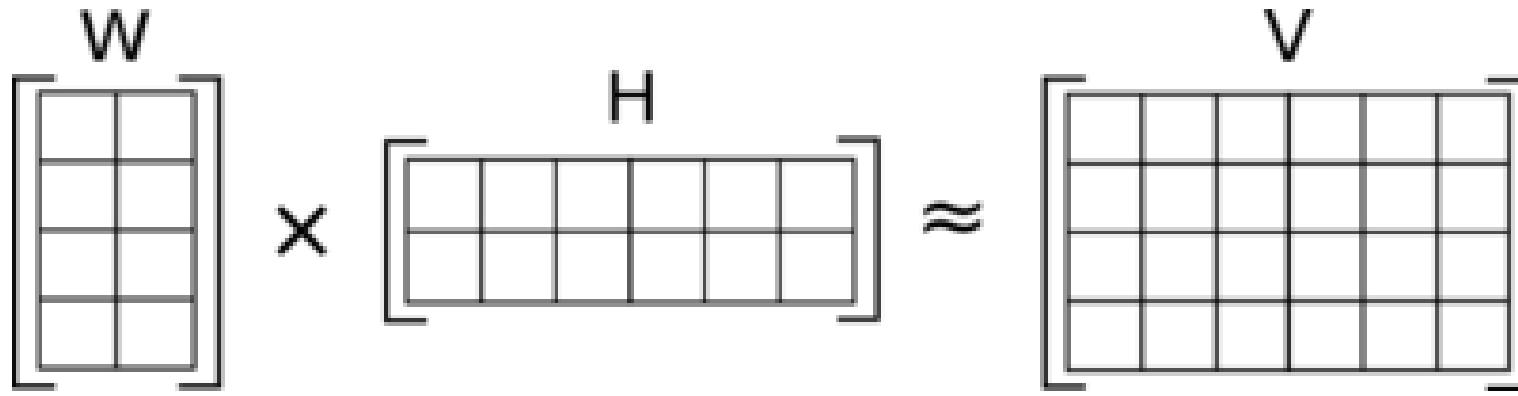


Non-negative matrix factorization (NMF)

$$V$$


A 5x5 matrix V is shown as a grid of 25 empty squares. The grid has 5 rows and 5 columns, enclosed in a double-lined rectangular frame. Above the grid, the letter V is centered.

Non-negative matrix factorization (NMF)



Constraints: W and H must contain positive values

Non-negative matrix factorization (NMF)

NMF is often implemented as a multi-step optimization/selection process:

1. Find an initial random solution for W and H
2. Compare the solution to the actual data to quantify error
3. Iterate on the solution to minimize the error value

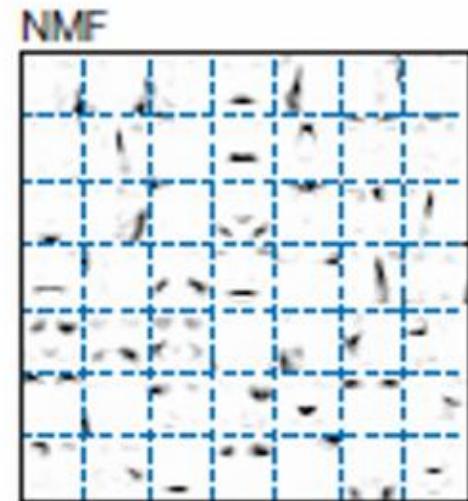
NMF to learn the components of human faces (facial recognition)

Learning the parts of objects by non-negative matrix factorization

Daniel D. Lee* & H. Sebastian Seung*†

* Bell Laboratories, Lucent Technologies, Murray Hill, New Jersey 07974, USA

† Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

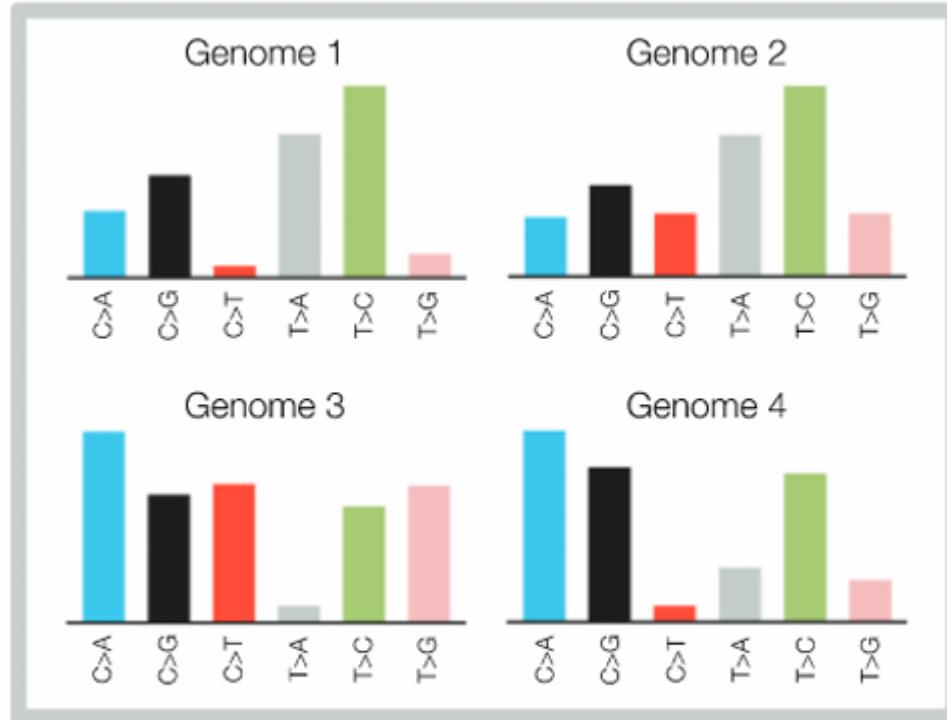


NMF to isolate different instruments from an audio file

Audio examples:

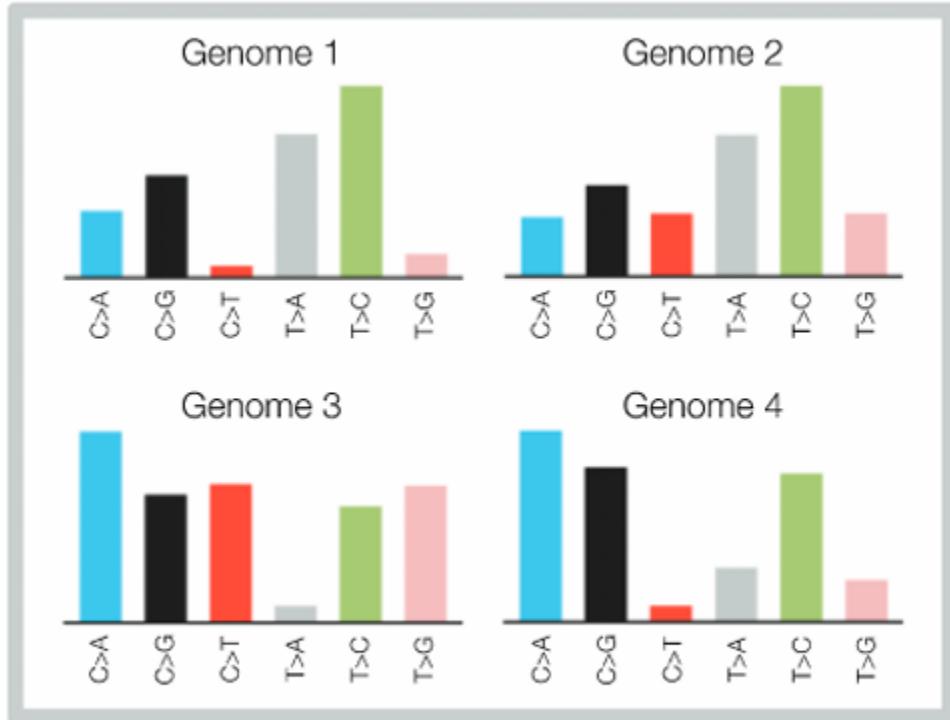
1. d-kitamura.net/demo-defNMF_en.html [[Daichi Kitamura](#)]
2. [Audio Source Separation. Unleashing the Power of Non-Negative Matrix Factorization: A Python Implementation.](#)

NMF to isolate SNV mutational signatures from a collection of genomes

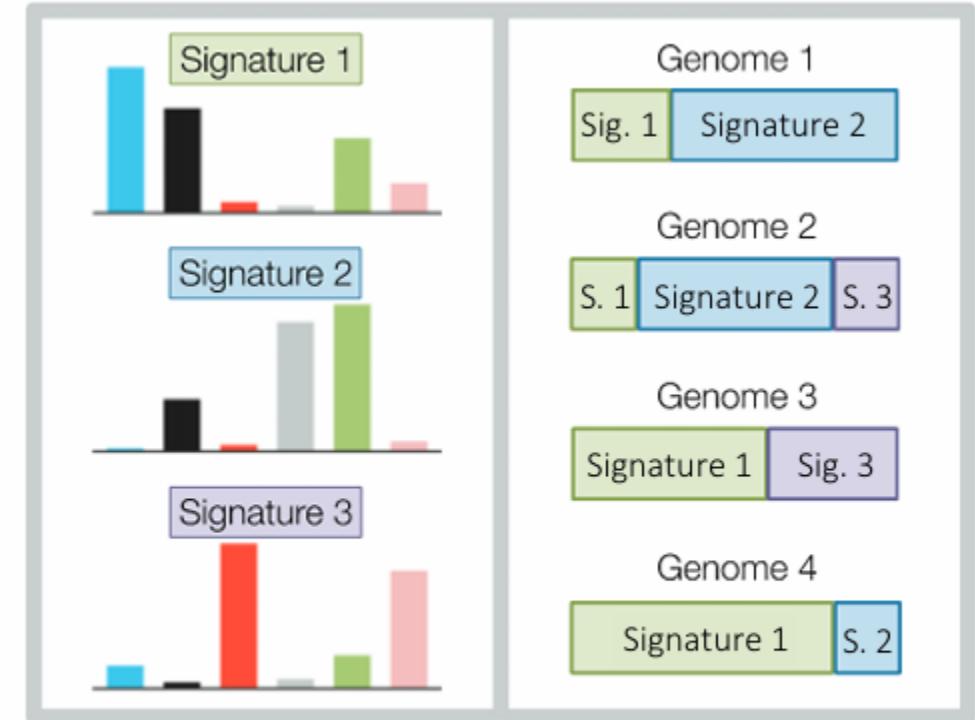


Large number of samples

NMF to isolate SNV mutational signatures from a collection of genomes



Large number of samples



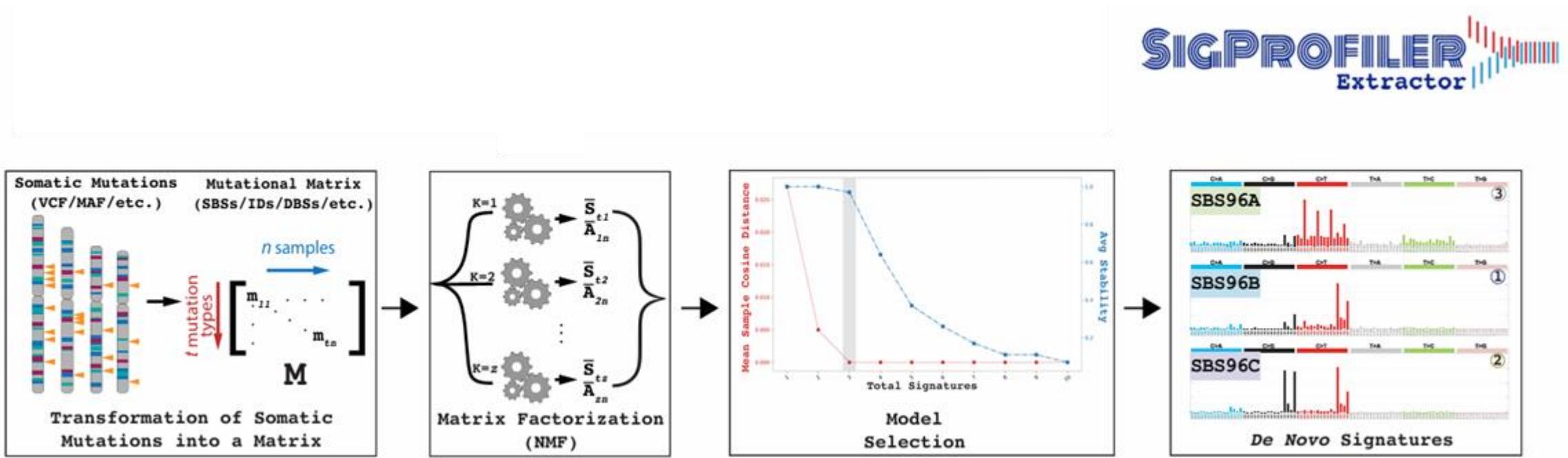
Set of consensus signatures

Considerations for mutational signature experiments

Discuss:

- What kind of sequencing strategy will give you the best data for mutational signatures, and why?
- What other things should you consider before doing a de novo mutational signature analysis? [Sample/cohort size, sequencing depth, cancer type?]

SigProfilerExtractor is a commonly used implementation of NMF to extract mutational signatures



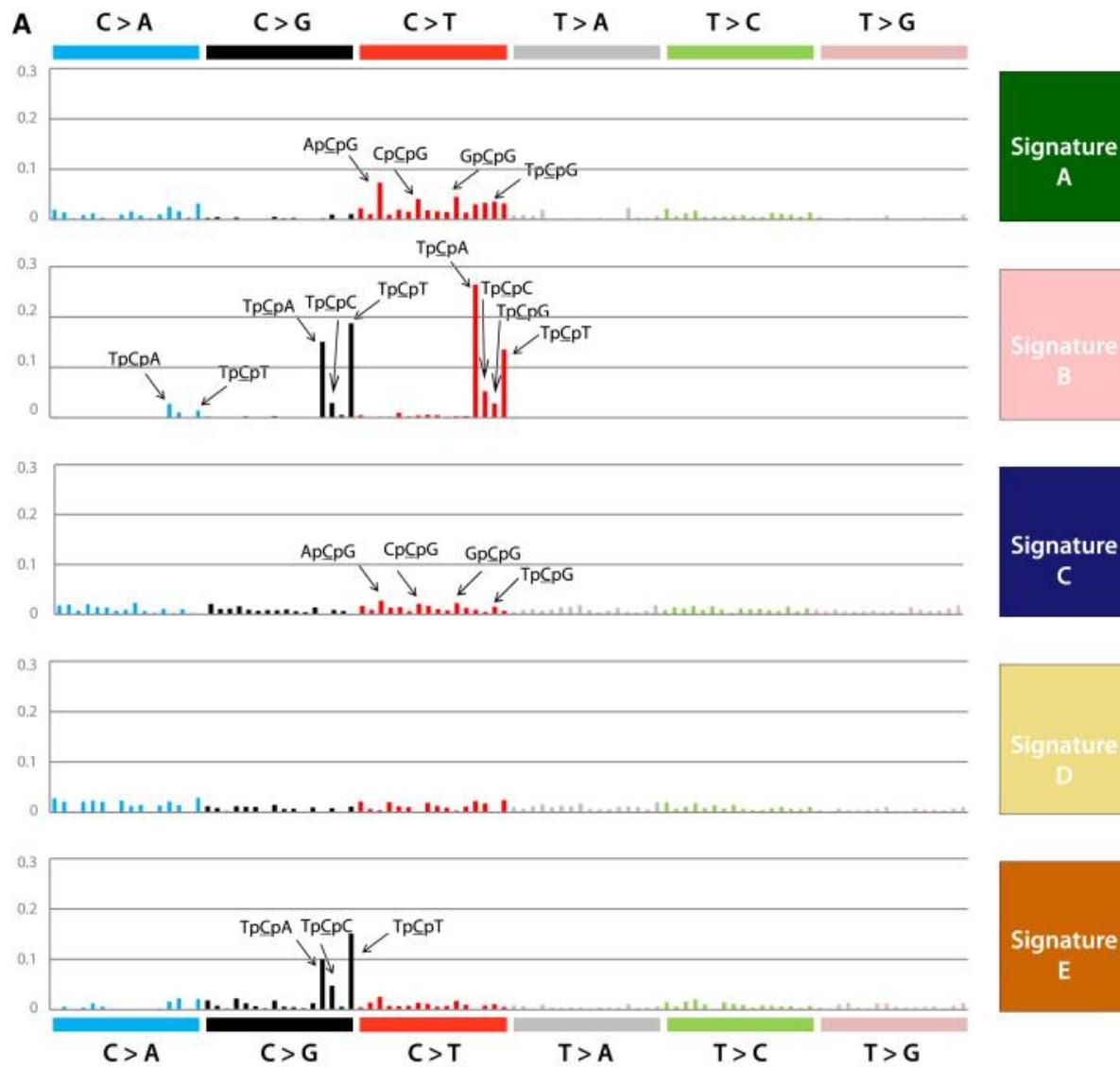
Many other tools and algorithms exist to extract mutational signatures

Tool	Platform	Factorization Approach		Selection Approach		Reference
		Method	Computational Engine	Type	Algorithm	
EMu	C++	EM	Original implementation	M/A	BIC	Fischer <i>et al.</i> 2013
Maftools	R-Bioconductor	NMF	NMF R package	M	-	Mayakonda <i>et al.</i> 2018
MutationalPatterns	R-Bioconductor	NMF	NMF R package	M	-	Blokzijl <i>et al.</i> 2018
MutSignatures	R	NMF	Brunet <i>et al.</i> 2004	-	-	Fantini <i>et al.</i> 2020
MutSpec	R/Galaxy	NMF	NMF R package	M	-	Ardin <i>et al.</i> 2016
SigFit	R	Bayesian inference	Stan R package	M/A	Elbow method	Gori <i>et al.</i> 2020
SigMiner	R	NMF/Bay. NMF	NMF R package/SA	M/A	ARD	Wang <i>et al.</i> 2021
SignatureAnalyzer	R/Python	Bayesian NMF	Original implementation	A	ARD	Kasar <i>et al.</i> 2015
SignatureToolsLib	R	NMF	NMF R package	M	-	Degasperis <i>et al.</i> 2020
SigneR	C++/R-Bioconductor	Bayesian NMF	Original implementation	M/A	BIC	Rosales <i>et al.</i> 2017
SigProfilerExtractor	Python/R	NMF	Original implementation	M/A	NMFk	Islam <i>et al.</i> 2021
SigProfiler_PCAWG	Python/MATLAB	NMF	Brunet <i>et al.</i> 2004	M	-	Alexandrov <i>et al.</i> 2013
SomaticSignatures	R-Bioconductor	NMF	NMF R package	M	-	Gehring <i>et al.</i> 2015
TensorSignatures	Python	NTF	TensorFlow	M/A	BIC	Vöhringer <i>et al.</i> 2021

Early work on mutational signatures: Breast Cancer (Nik-Zainal 2012)

21 breast cancer whole genomes

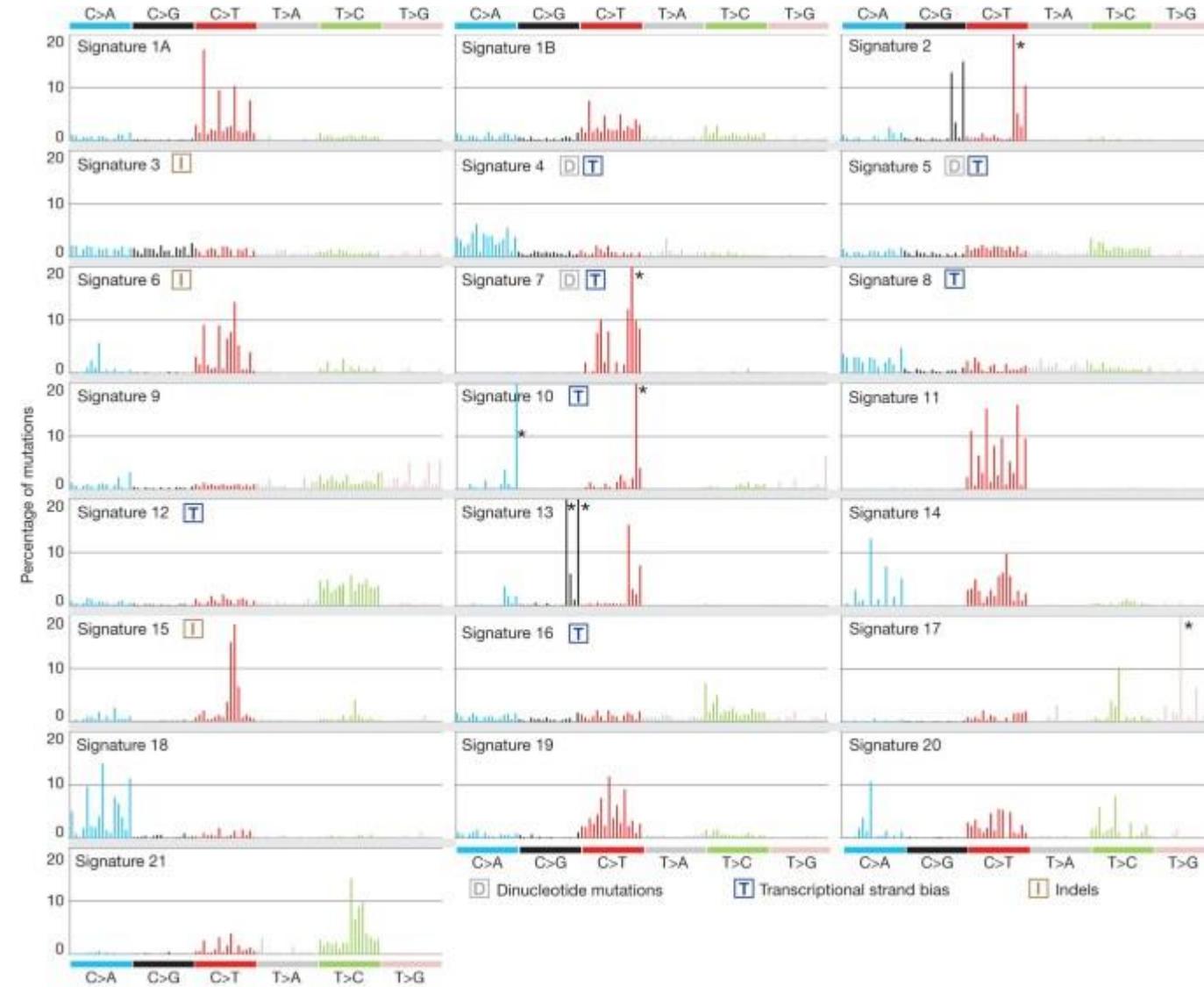
5 mutational signatures, A-E



Early work on mutational signatures: Pan-Cancer (Alexandrov 2013)

7,042 cancers (exomes + genomes from TCGA and ICGC)

21 single base substitution (SBS) mutational signatures



COSMIC Mutation Signature Database



7,042 cancers (exomes + genomes)

21 single base substitution (SBS) mutational signatures

COSMIC v1 (August 2013)

COSMIC Mutation Signature Database



COSMIC v2 (March 2015)

- 30 SBS signatures

COSMIC Mutation Signature Database



v3 (May 2019)

- 67 SBS signatures
- 11 DBS signatures
- 17 ID signatures

COSMIC Mutational Signatures Database

COSMIC
Catalogue Of Somatic Mutations In Cancer

Projects ▾ Data ▾ Tools ▾ News ▾ Help ▾ About ▾ Search COSMIC... SEARCH

Mutational Signatures (v3.3 - June 2022)

Introduction

Somatic mutations are present in all cells of the human body and occur throughout life. They are the consequence of multiple mutational processes, including the intrinsic slight infidelity of the DNA replication machinery, exogenous or endogenous mutagen exposures, enzymatic modification of DNA and defective DNA repair. Different mutational processes generate unique combinations of mutation types, termed "Mutational Signatures".

In the past few years, large-scale analyses have revealed many mutational signatures across the spectrum of human cancer types, including the latest effort by the ICGC/TCGA Pan-Cancer Analysis of Whole Genomes (PCAWG) Network (Alexandrov, L.B. et al., 2020) using data from more than 23,000 cancer patients.

About

COSMIC Mutational Signatures is a resource curated in partnership with COSMIC and Cancer Grand Challenges, and in close association with our collaborators at Wellcome Sanger Institute, the Pillay lab at University College London and the Alexandrov lab at University of California.

Partners: welcome sanger institute, CANCER GRAND CHALLENGES, COSMIC Catalogue Of Somatic Mutations In Cancer

Signature-based websites

At COSMIC Signatures we identify signatures from analysis of the PCAWG dataset and through curation of specific papers. Papers are looked at particularly (but not exclusively) when there is a specific exposure which captures signatures not present in the PCAWG dataset. Please note that this catalogue of signatures is not exhaustive or a final set, but a reference set of high confidence signatures that have been curated by experts in the field. We aim to update as comprehensively as possible as new data become available and improvements are made to extraction methodologies.

This summary includes the mutational profile, proposed aetiology and tissue distribution of each signature, as well as potential associations with other mutational signatures and how the signature has changed during iterations of analysis. Currently, four different variant classes are considered, resulting in the following sets of mutational signatures.

[SBS Signatures](#) [DBS Signatures](#) [ID Signatures](#) [CN Signatures](#)

Data downloads

Download current COSMIC Mutational Signatures version 3.3 and previous releases here.

[Downloads](#)

Versions

COSMIC Mutational Signatures version 3.3 is the latest release.

Version 3 was released as part of COSMIC release v89 (May 2019), updated to version 3.1 in COSMIC release v91 (June 2020), to version 3.2 in COSMIC release v93 (March 2021) and most recently version 3.3 in COSMIC v95 (May 2022).

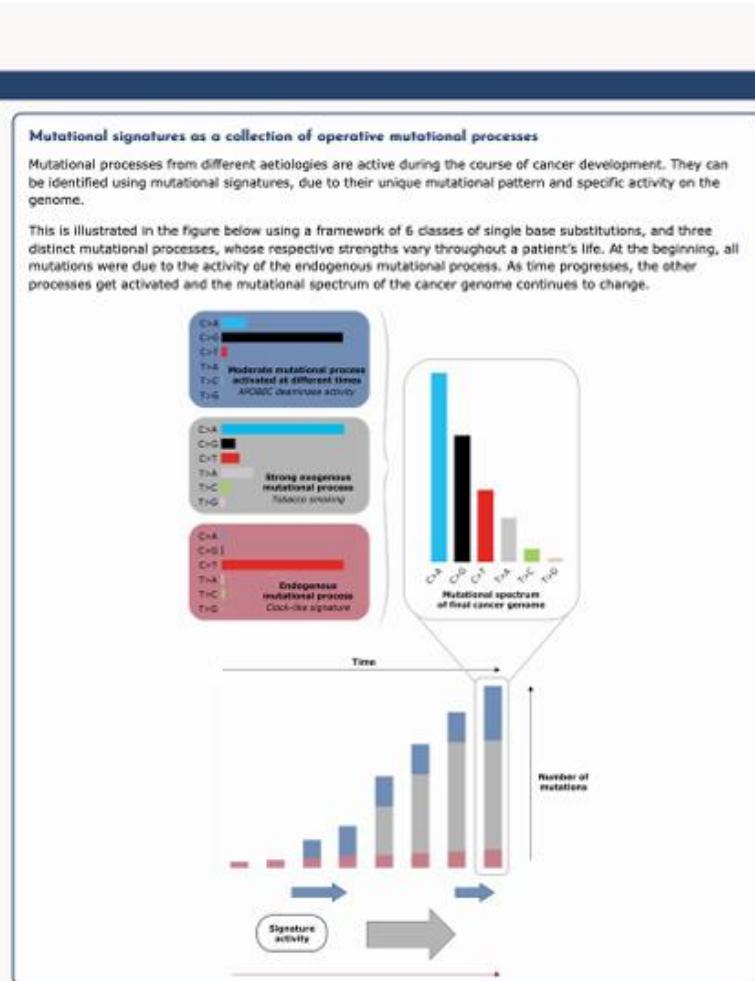
Version 2 signatures (March 2015) were part of earlier COSMIC releases can still be consulted:

[Version 2](#)

SigProfiler tools

The current set of mutational signatures has been extracted using SigProfiler, a compilation of publicly available bioinformatic tools addressing all the steps needed for signature identification. SigProfiler functionalities include mutation matrix generation from raw data and signature extraction, among others.

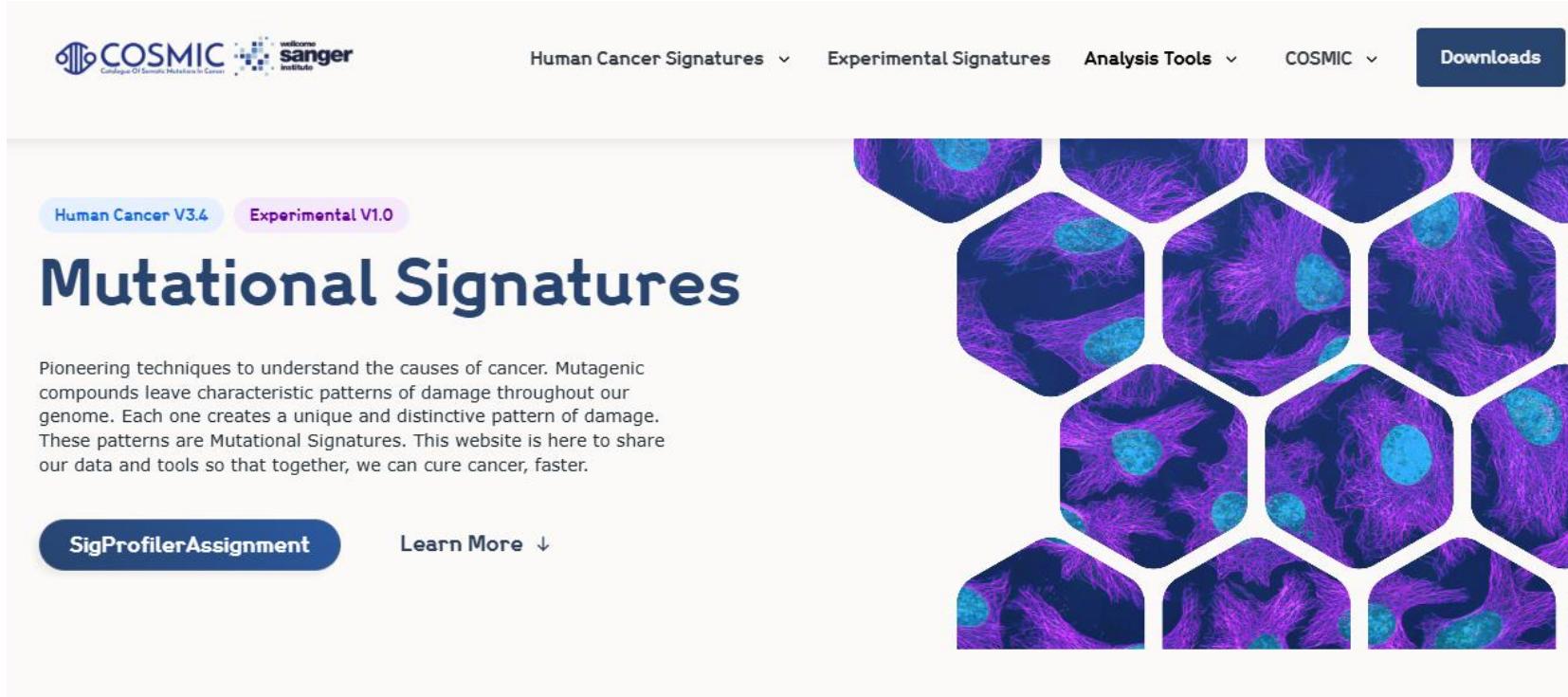
[SigProfiler Tools](#)



COSMIC v3.3 (June 2022)

- 79 SBS signatures
- 11 DBS signatures
- 18 ID signatures
- 21 CN signatures

COSMIC Mutational Signatures Database



Human Cancer Signatures ▾ Experimental Signatures Analysis Tools ▾ COSMIC ▾ Downloads

Human Cancer V3.4 Experimental V1.0

Mutational Signatures

Pioneering techniques to understand the causes of cancer. Mutagenic compounds leave characteristic patterns of damage throughout our genome. Each one creates a unique and distinctive pattern of damage. These patterns are Mutational Signatures. This website is here to share our data and tools so that together, we can cure cancer, faster.

SigProfilerAssignment Learn More ↓

October v3.4 (June 2023)

Genomics

- 99 SBS signatures
- 20 DBS signatures
- 23 ID signatures
- 25 CN signatures
- 10 SV signatures
- 5 RNA-SBS signatures

Experimental

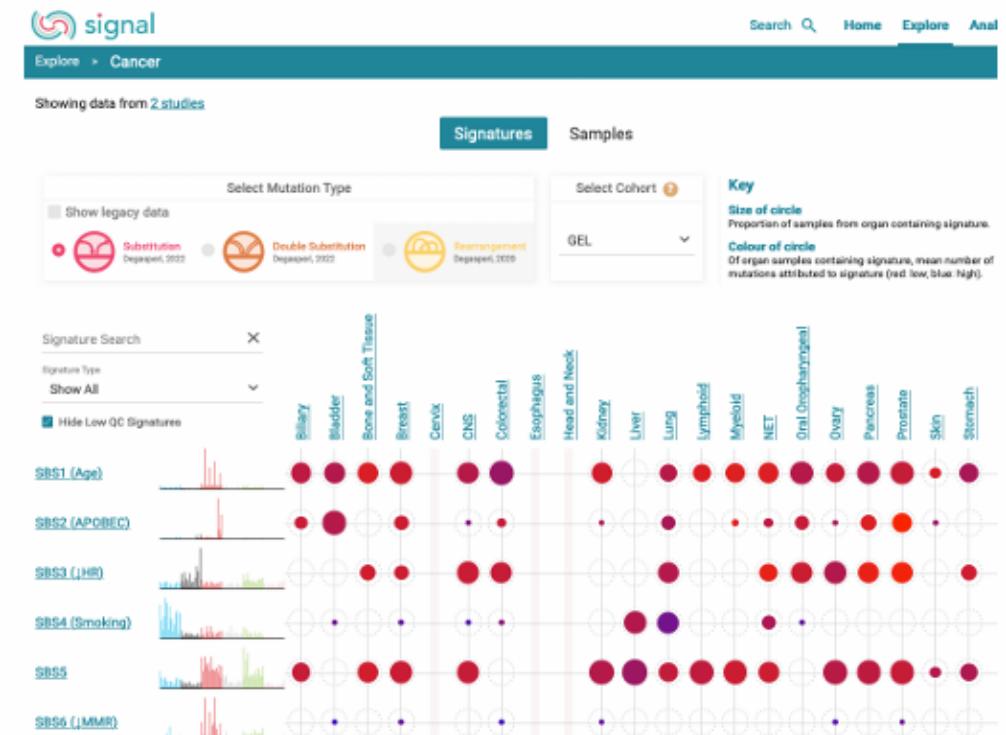
- 140 signatures

Other Mutational Signatures Databases

MUTAGENE



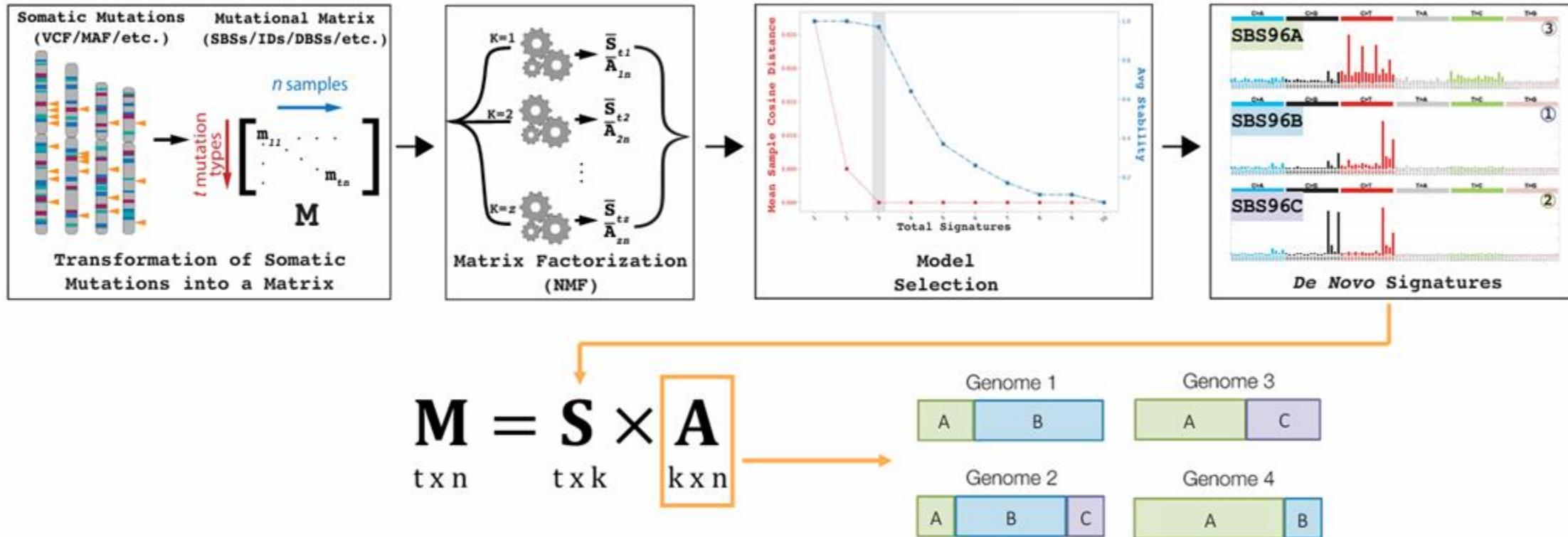
<https://www.ncbi.nlm.nih.gov/research/mutagene/>



<https://signal.mutationalsignatures.com/>

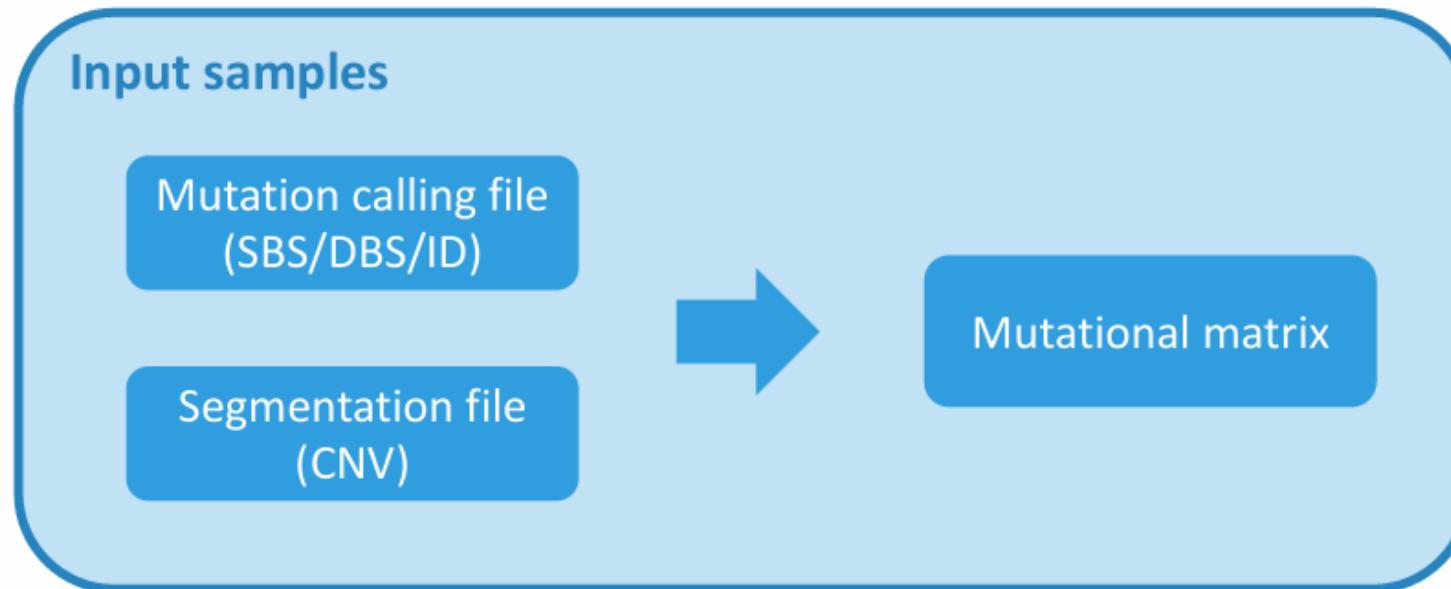
SigProfiler is an open-source toolkit for mutational signatures analyses

SIGPROFILER
Extractor



SIGPROFILER
Assignment

SigProfilerAssignment assigns mutational signature weights to individual samples



$$M = S \times A$$

$t \times n$ $t \times k$ $k \times n$

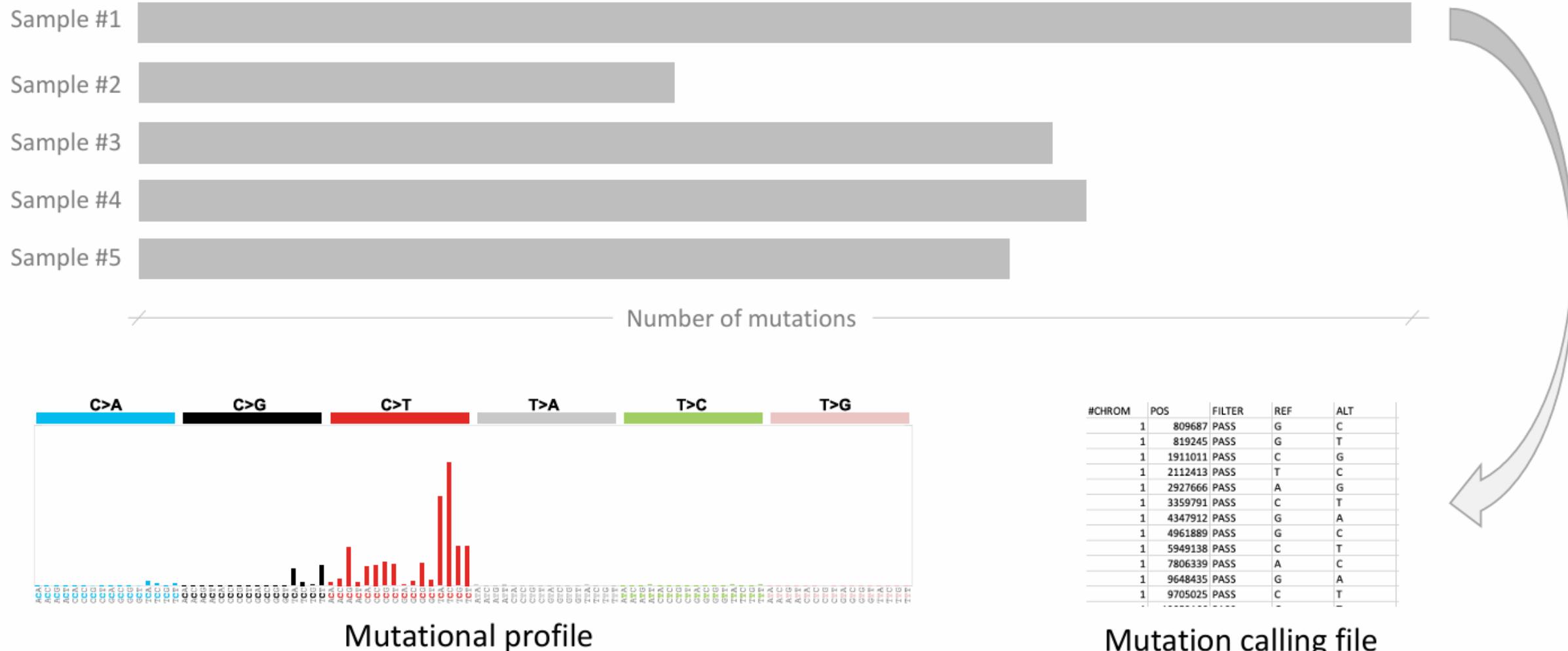
The equation $M = S \times A$ is shown in blue and red text. To the left of the equals sign, M is in blue and $t \times n$ is in blue below it. To the right of the equals sign, S is in red and $t \times k$ is in red below it. To the right of the times sign, A is in black and $k \times n$ is in black below it. The matrix A is enclosed in a black rectangular border.



t mutational contexts
n samples
k signatures

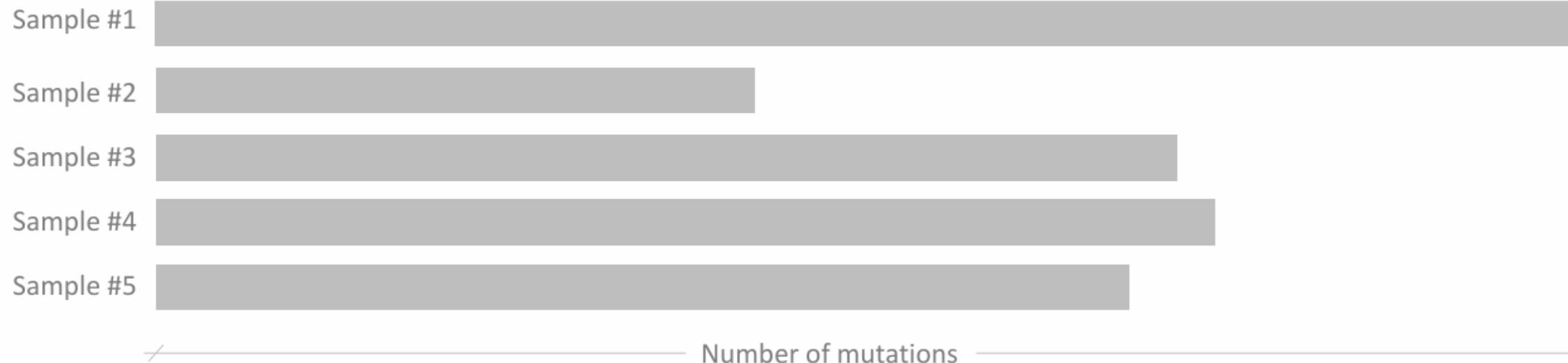
Assigning reference signatures to individual samples

M



Assigning reference signatures to individual samples

M

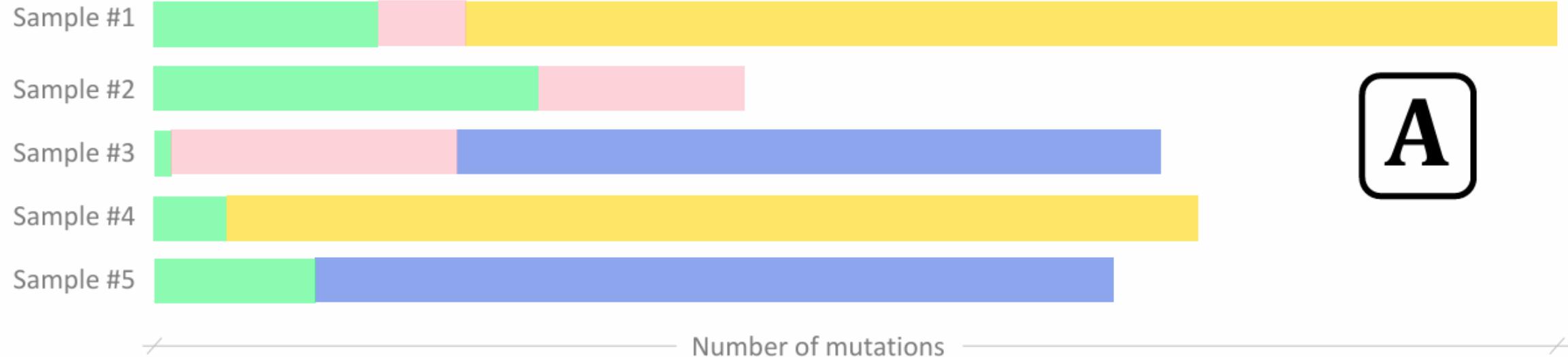


S

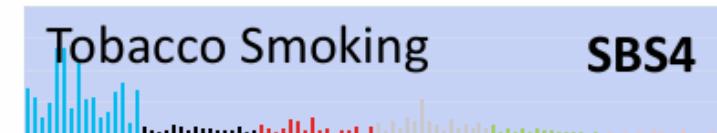
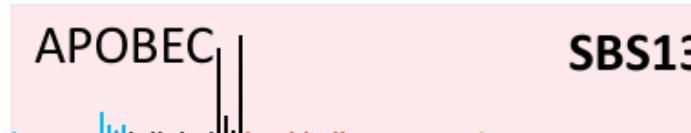


Assigning reference signatures to individual samples

M



S



Assigning reference signatures to individual mutations

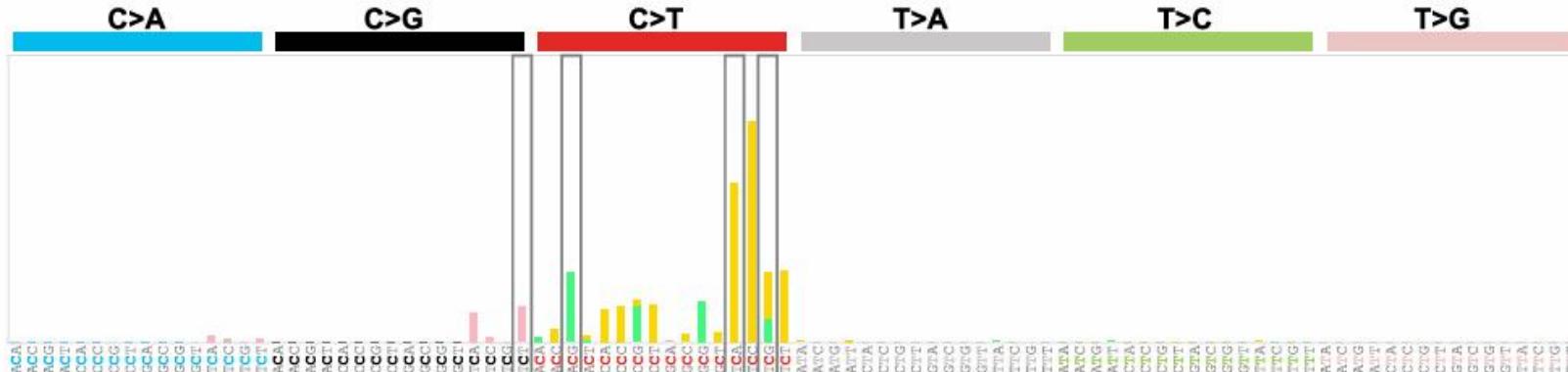


Assigning reference signatures to individual mutations



$$P(\text{Signature} \mid \text{Mut. context}) = \frac{P(\text{Mut. context} \mid \text{Signature}) \times P(\text{Signature})}{P(\text{Mut. context})}$$

Assigning reference signatures to individual mutations



CHROM	POS	REF	ALT	Most Prob. Signature	Prob.
1	221436661	G	A	SBS1	99.9%
3	178936091	G	A	SBS7a	99.5%
4	119785075	C	G	SBS13	98.6%
6	162294115	C	T	SBS7a	70.1%
...

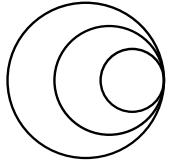
Many other tools and algorithms exist to assign mutational signatures and their weights to individual samples

Tool	Platform	Refitting Approach		Reference
		Method	Computational Engine	
deconstructSigs	R	Non-negative linear regression	Original implementation	Rosenthal <i>et al.</i> 2016 Genome Biology
MSA	Python / Nextflow	NNLS	Original implementation / Scipy python package	Senkin 2021 BMC Bioinformatics
MutationalPatterns (standard)	R	NNLS	Pracma R package	Blokzijl <i>et al.</i> 2018 Genome Medicine
MutationalPatterns (strict)	R	NNLS	Original implementation / Pracma R package	Manders <i>et al.</i> 2022 BMC Genomics
sigLASSO	R	Lasso regression	Original implementation / glmnet R package	Li <i>et al.</i> 2020 Nature Communications
SignatureToolsLib	R / Web app	Non-negative linear regression	NNLM R package	Degasperi <i>et al.</i> 2022 Science
SigProfilerAssignment	Python / R / Web app	NNLS	Original implementation / Scipy python package	Díaz-Gay <i>et al.</i> 2023 bioRxiv

NNLS: non-negative least squares

Summary and takeaways

- Mutational signatures are patterns of mutations in the genome associated with a distinct biological source or process
- SBS mutational signatures were discovered by applying NMF to 96-class mutation profiles of large genomics cohorts
- The SigProfiler toolkit can conduct both de novo extraction of mutational signatures as well as assignment of known signatures to individual samples



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