ISPRO
Istituto per lo studio, la prevenzione e la rete oncologica

SST Servizio Sanitario della Toscana

mutations: from evolution to cancer

Silvo Conticello

Core Research Laboratory

“nothing in biology makes sense except in the light of evolution”

Theodosius Dobzhansky (1976)

- **1859 Charles Darwin**
  natural selection

- **1866 Gregor Mendel**
  inheritance

- **1902 Theodor Boveri**

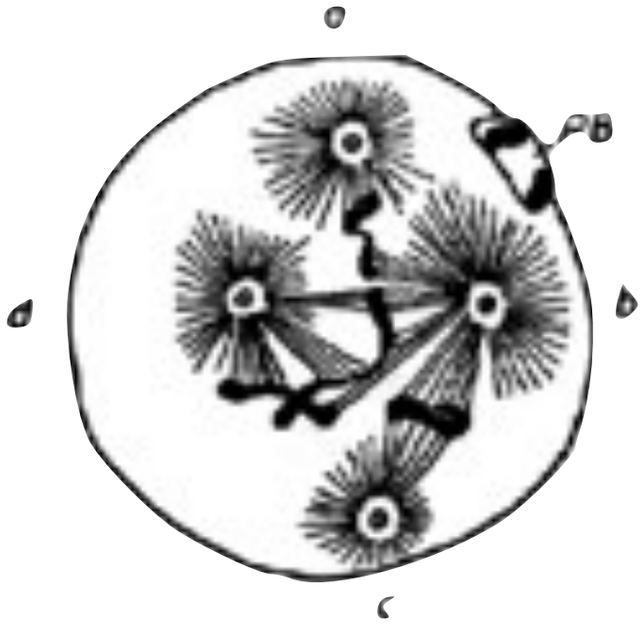
The tumour problem is a **cell problem**…

Tumours might be the consequence of a certain **abnormal chromosome** constitution…

Inhibitory mechanisms that have to be eradicated before unrestrained multiplication can take place… A tumour cell that proliferated without restraint would be generated if these `**inhibitory chromosomes**' were eliminated…

Concerning the Origin of Malignant Tumours (1914)

- 1859 **Charles Darwin**
  natural selection
- 1866 **Gregor Mendel**
  inheritance
- 1902 **Theodor Boveri**



Concerning the Origin of Malignant Tumours (1914)

- **1859 Charles Darwin**
  natural selection

- **1866 Gregor Mendel**
  laws of inheritance

- **1902 Theodor Boveri**
  the chromosomes

- **1943 Luria & Delbruck**
  is selection random?

- **1944 Oswald Avery**
  the information is in DNA

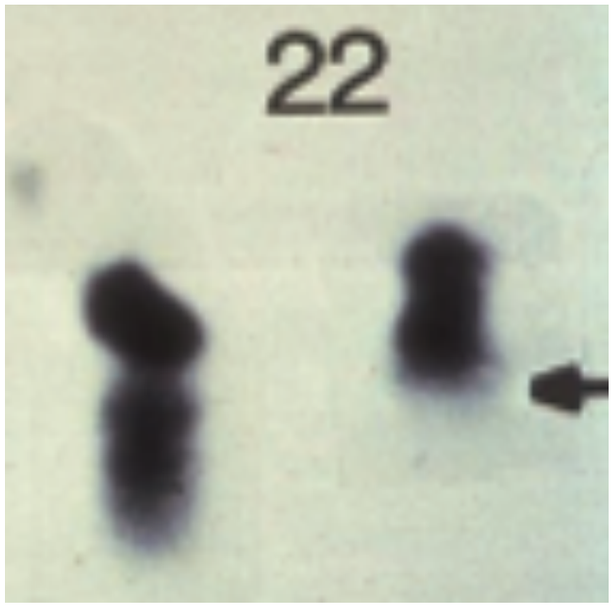
- **1953 Watson & Crick**
  the structure of DNA provides the basis for inheritance

- **1956 Hin Tjio & Levan**
  the number of human chromosomes

- **1960 Peter Nowell & David Hungerford**
  the first genetic alteration in cancer

Chronic Myeloid Leukemia is defined by genetic alterations

cancer is a clonal disease



Philadelphia Chromosome

- **1960 Peter Nowell & David Hungerford**
  the first genetic alteration in cancer

The clonal evolution of tumor cell populations

- cancer derives from a progression of mutations
- cancer evolution is a darwinian process
- “each patient's cancer may require individual specific therapy”
- “even this may be thwarted by emergence of a genetically variant subline resistant to the treatment”

– 1976 **Peter Nowell**

evolution does not have a purpose

the only evolutionary success is passing your genes

bacteria

~3.5 billion years

humans

evolution does not have a purpose

the only evolutionary success is passing your genes

bacteria → 30 min / generation

unicellular,
asexual reproduction

humans → 20 years / generation

multicellular,
highly complex,
sexual reproduction

evolution does not have a purpose

the only evolutionary success is passing your genes

cancer → hours / generation

unicellular,
asexual reproduction

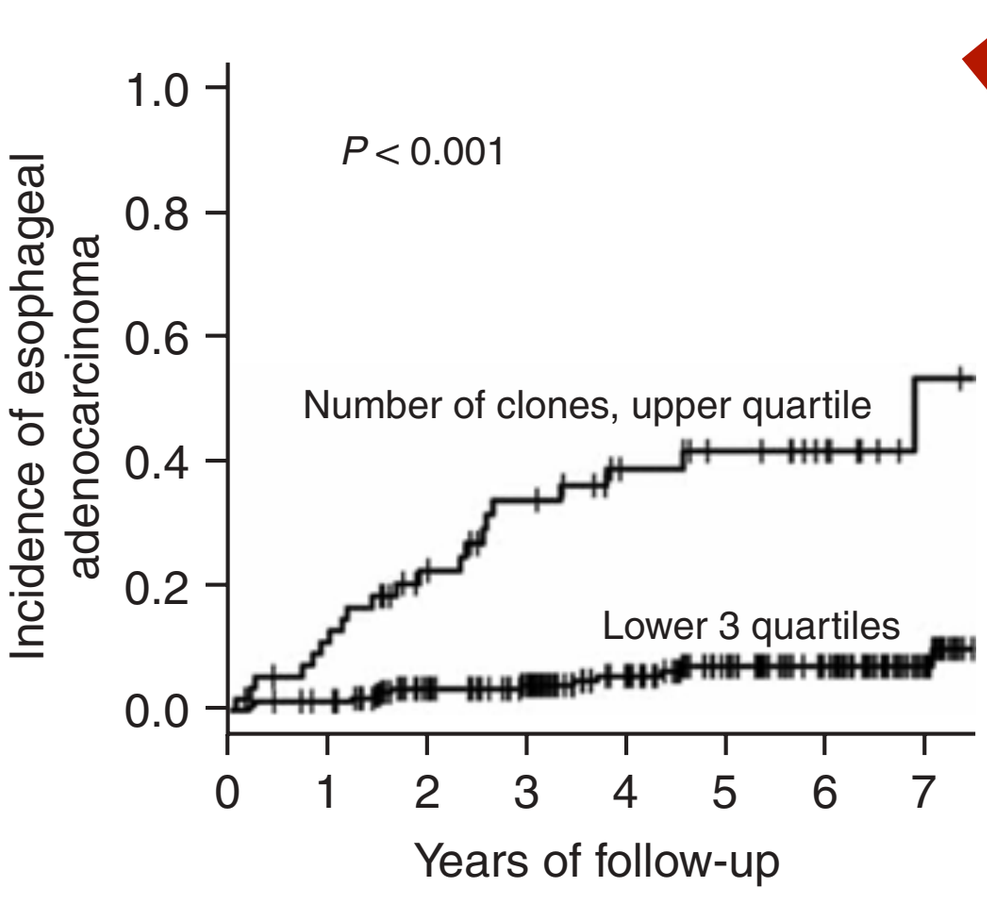
humans → 20 years / generation

multicellular,
highly complex,
sexual reproduction

evolution does not have a purpose

the only evolutionary success is passing your genes

Barrett's Oesophagus *(displasia)*



1.4-folds risk increase per every genetically different clone

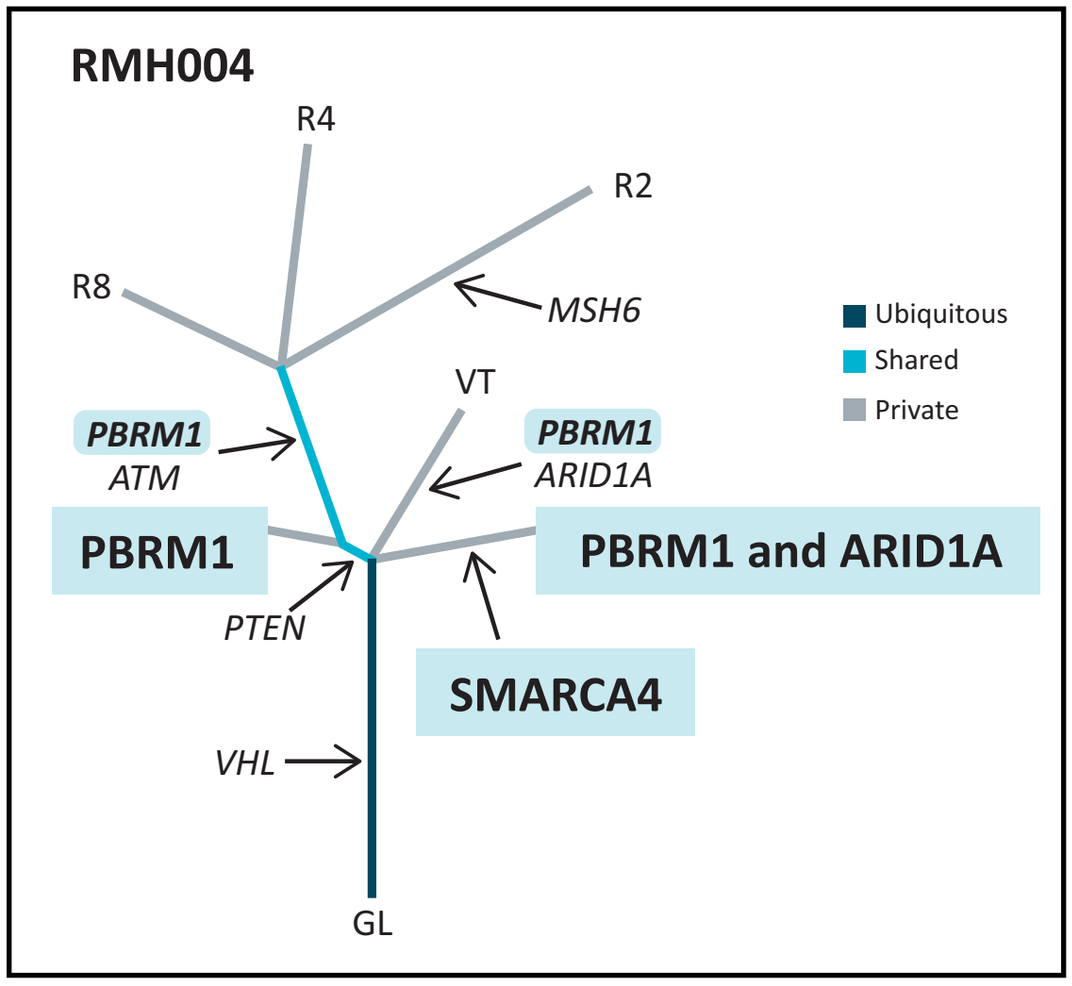
Oesophageal Adenocarcinoma

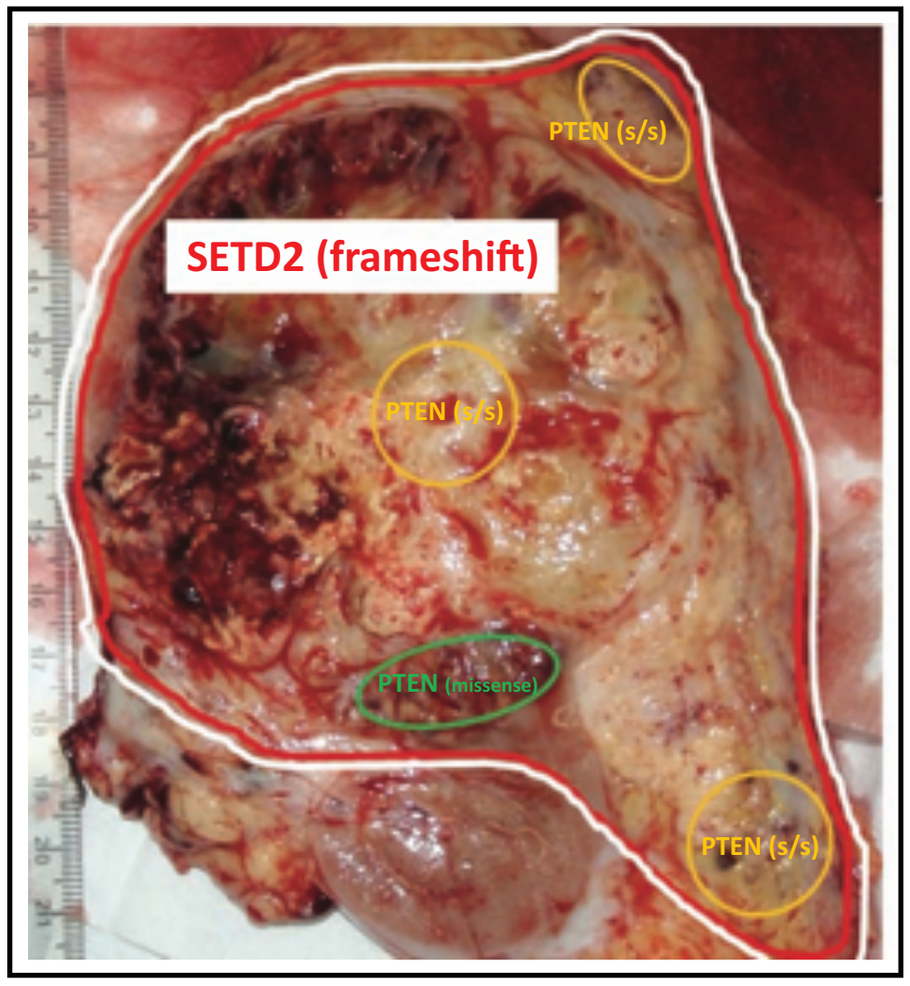
Maley et al, 2006

evolution does not have a purpose

the only evolutionary success is passing your genes

renal cell carcinoma





Hiley & Swanton, 2014

evolution has eyes only for today

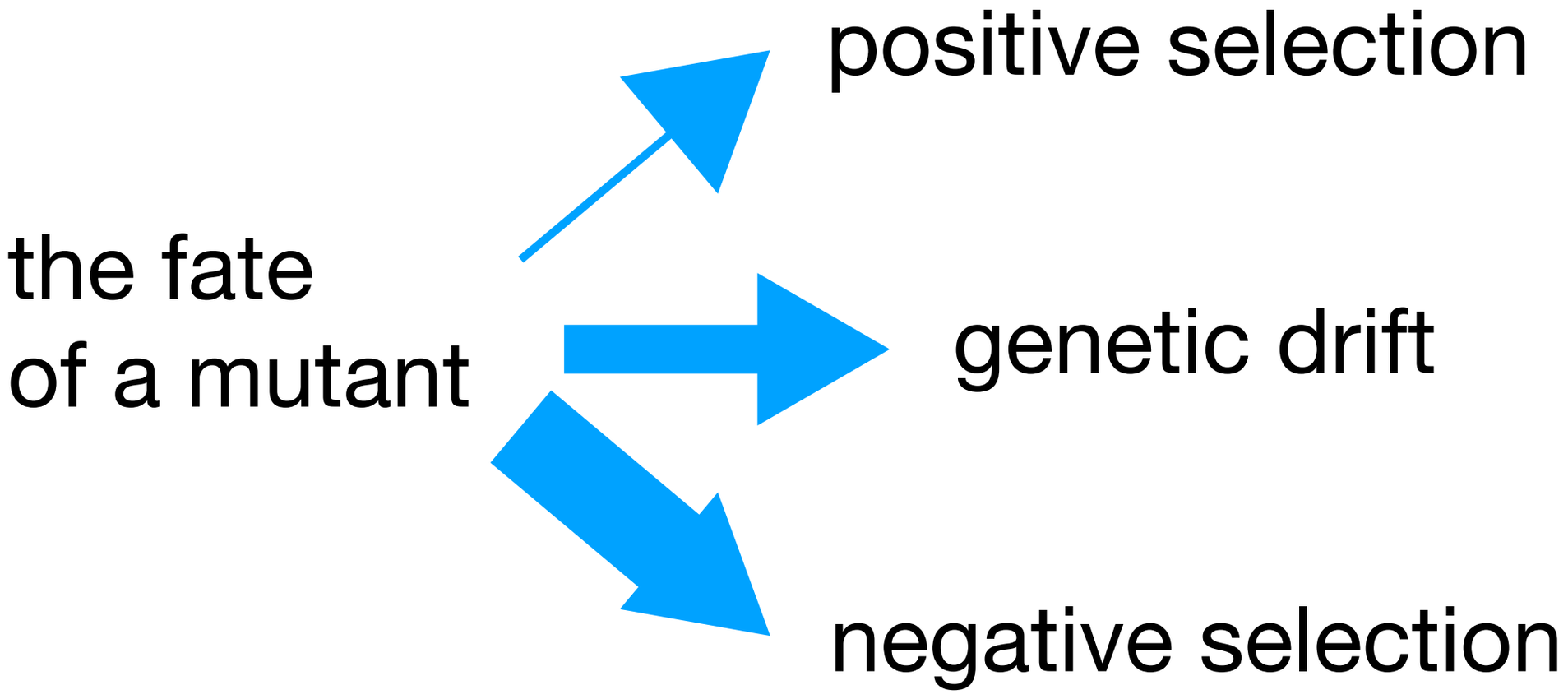
Survival of the fittest

among what is available…

- appendicitis
- menstrual cycle
- allergies
- pale skin
  > skin cancer
- hormones
  > breast cancer
  > prostate cancer
- BRCA1 in Ashkenazi Jews
  > breast cancer
- (*delayed infections in the 1st year*
  > *childhood leukemia)*

Survival of the fittest

among what is available…



selective advantage: 0.004

Bozic et al, 2010

Oncogenes	Tumor Suppressor genes
Src	*RB1*
Ras	*TP53*
Myc	*APC*
Wnt	*MLH1, MSH2, MSH6*
BCL-2	*BRCA1, BRC2*
BCR-ABL1	*WT*
	NF1, NF2

Oncogenes

DOMINANT

Src

Ras

Myc

Wnt

BCL-2

BCR-ABL1

Tumor Suppressor genes

RECESSIVE

RB1

TP53

APC

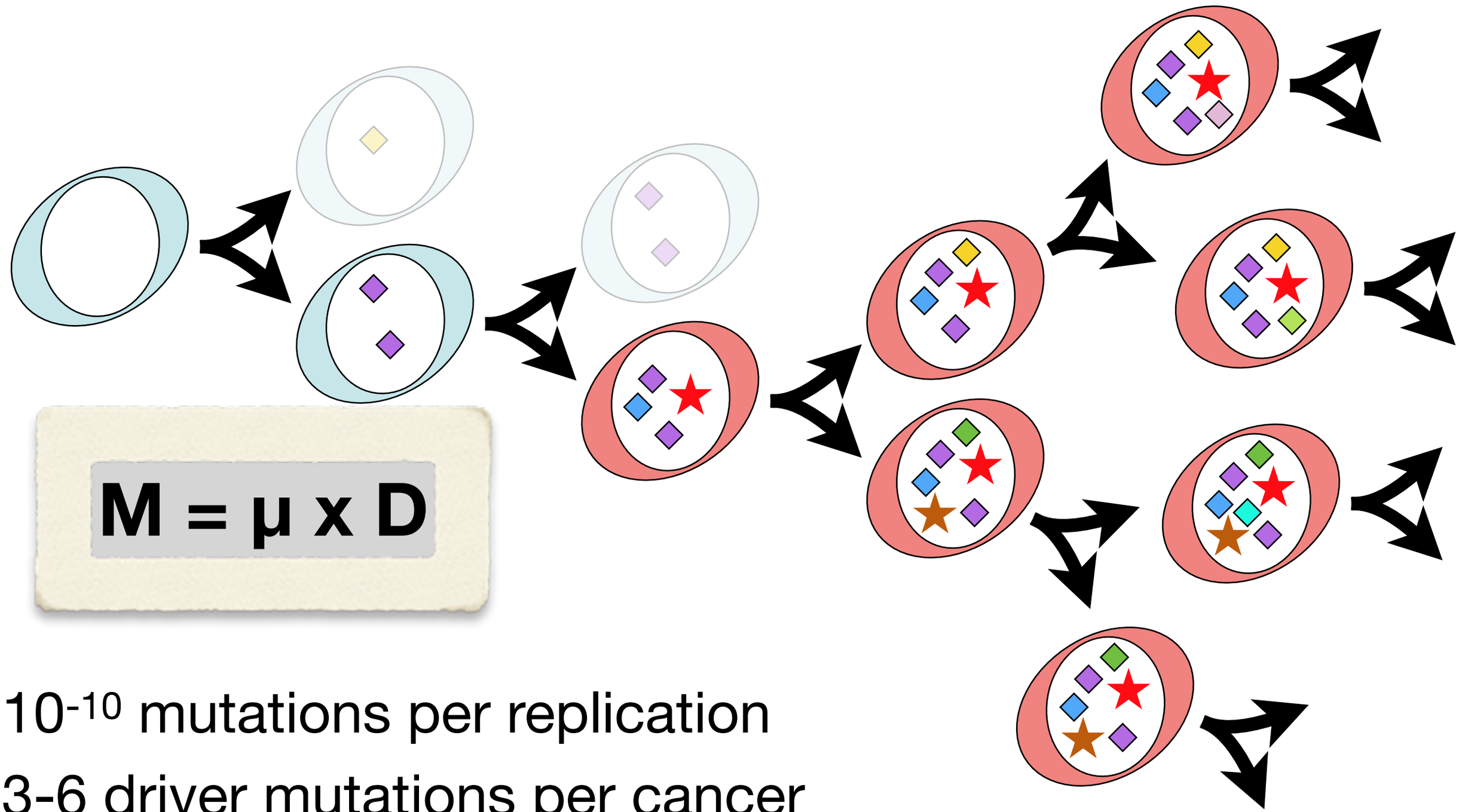
MLH1, MSH2, MSH6

BRCA1, BRC2

WT

NF1, NF2

Drivers & Passengers



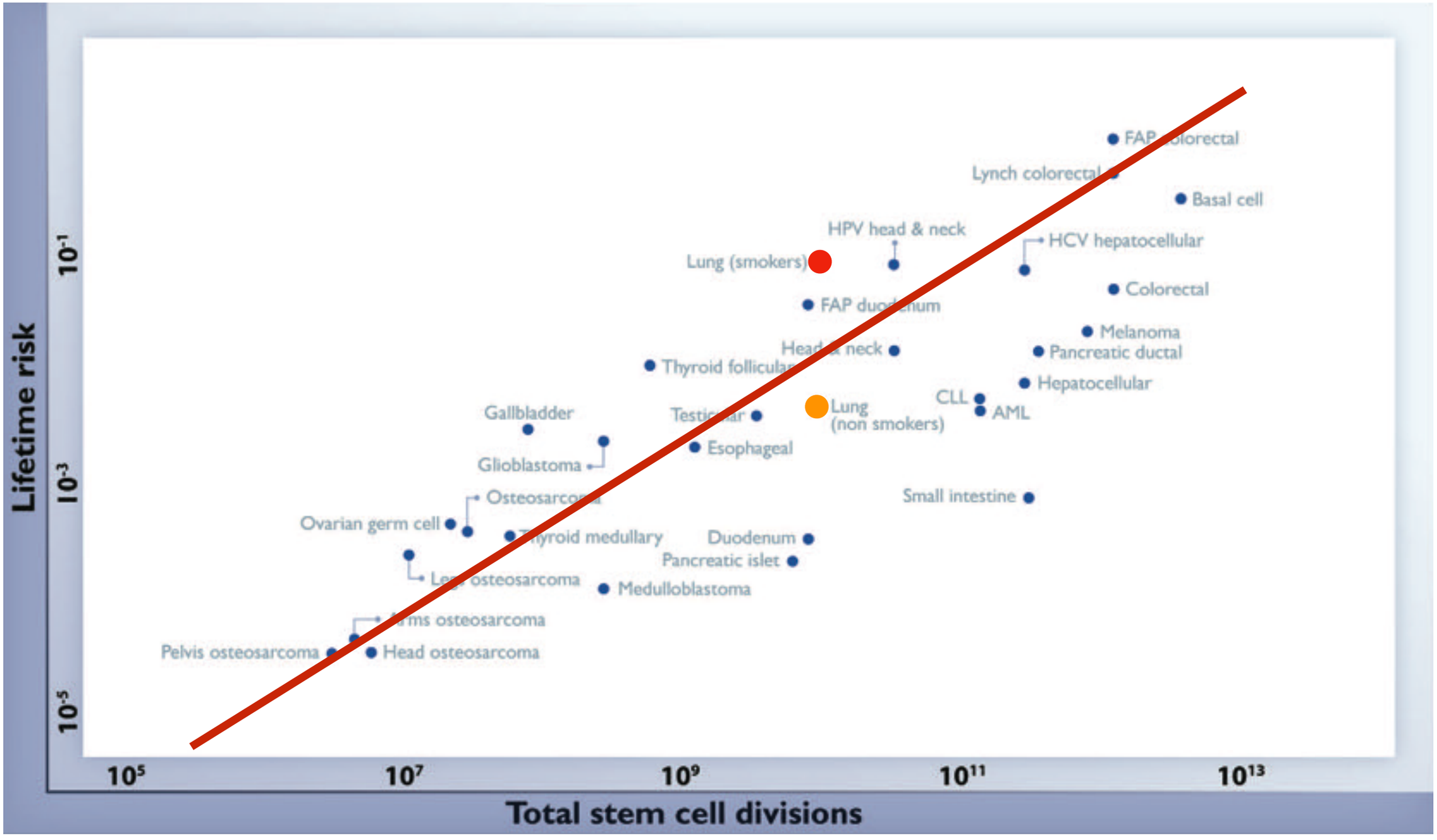
$M = \mu \times D$

10^{-10} mutations per replication

3-6 driver mutations per cancer

100-100,000 passenger mutations per cancer

Risk vs Stem Cell Divisions



Tomasetti & Vogelstein, 2015

The Consensus Coding Sequences of Human Breast and Colorectal Cancers

Tobias Sjöblom,[1*] Siân Jones,[1*] Laura D. Wood,[1*] D. Williams Parsons,[1*] Jimmy Lin,[1] Thomas D. Barber,[1†] Diana Mandelker,[1] Rebecca J. Leary,[1] Janine Ptak,[1] Natalie Silliman,[1] Steve Szabo,[1] Phillip Buckhaults,[2] Christopher Farrell,[2] Paul Meeh,[2] Sanford D. Markowitz,[3] Joseph Willis,[4] Dawn Dawson,[4] James K. V. Willson,[5] Adi F. Gazdar,[6] James Hartigan,[7] Leo Wu,[8] Changsheng Liu,[8] Giovanni Parmigiani,[9] Ben Ho Park,[10] Kurtis E. Bachman,[11] Nickolas Papadopoulos,[1] Bert Vogelstein,[1‡] Kenneth W. Kinzler,[1‡] Victor E. Velculescu[1‡]

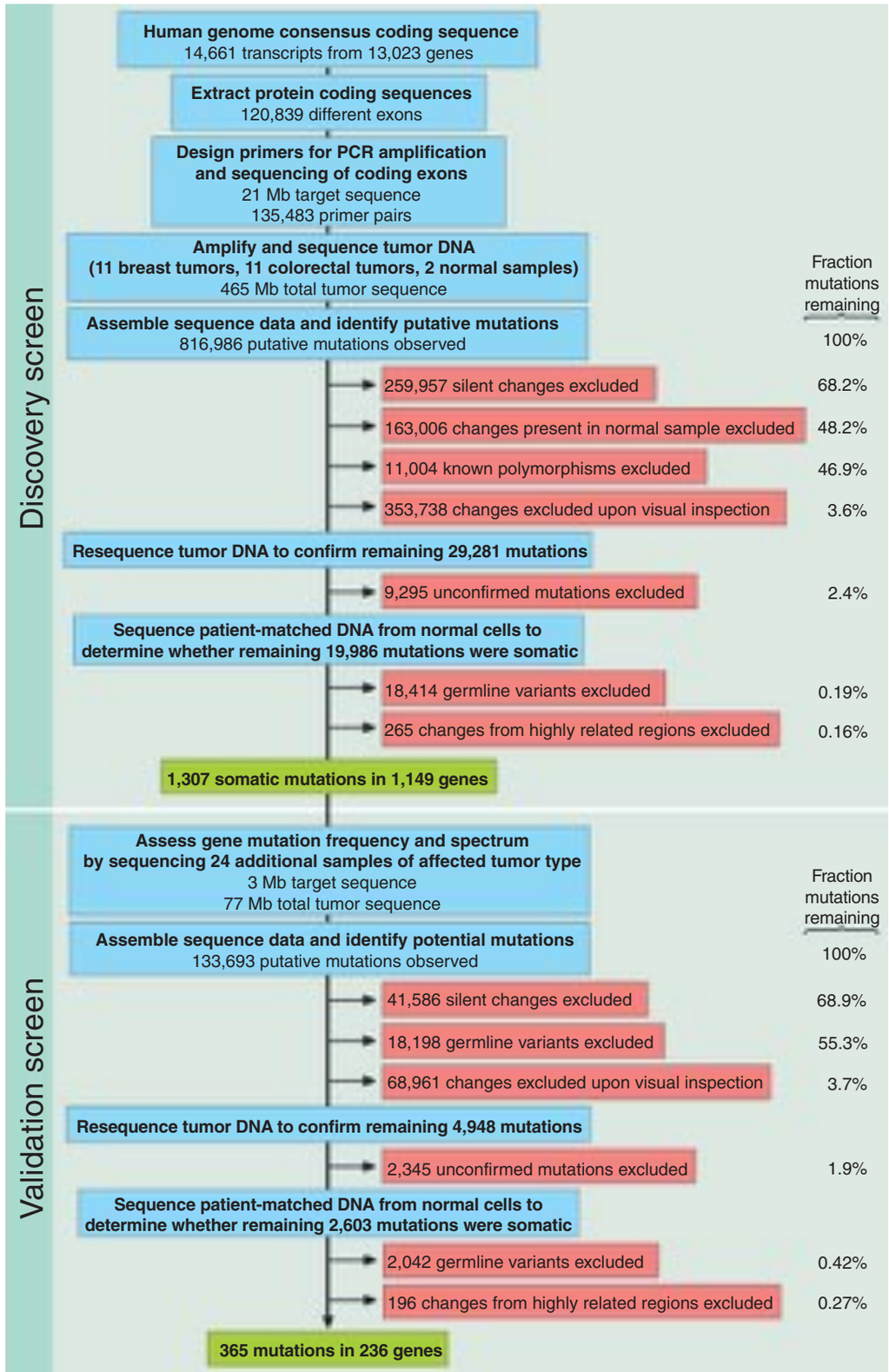
13 OCTOBER 2006 VOL 314 **SCIENCE**

11 Breast cancers

13 Colorectal Carcinomas

Targeted Sequencing

365 Mutations in 236 genes



NATURE | Vol 456 | 6 November 2008

DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome

Timothy J. Ley[1,2,3,4]*, Elaine R. Mardis[2,3]*, Li Ding[2,3], Bob Fulton[3], Michael D. McLellan[3], Ken Chen[3], David Dooling[3], Brian H. Dunford-Shore[3], Sean McGrath[3], Matthew Hickenbotham[3], Lisa Cook[3], Rachel Abbott[3], David E. Larson[3], Dan C. Koboldt[3], Craig Pohl[3], Scott Smith[3], Amy Hawkins[3], Scott Abbott[3], Devin Locke[3], LaDeana W. Hillier[3,8], Tracie Miner[3], Lucinda Fulton[3], Vincent Magrini[2,3], Todd Wylie[3], Jarret Glasscock[3], Joshua Conyers[3], Nathan Sander[3], Xiaoqi Shi[3], John R. Osborne[3], Patrick Minx[3], David Gordon[8], Asif Chinwalla[3], Yu Zhao[1], Rhonda E. Ries[1], Jacqueline E. Payton[5], Peter Westervelt[1,4], Michael H. Tomasson[1,4], Mark Watson[3,4,5], Jack Baty[6], Jennifer Ivanovich[4,7], Sharon Heath[1,4], William D. Shannon[1,4], Rakesh Nagarajan[4,5], Matthew J. Walter[1,4], Daniel C. Link[1,4], Timothy A. Graubert[1,4], John F. DiPersio[1,4] & Richard K. Wilson[2,3,4]

NATURE | Vol 461 | 8 October 2009

Mutational evolution in a lobular breast tumour profiled at single nucleotide resolution

Sohrab P. Shah[1,2]*, Ryan D. Morin[3]*, Jaswinder Khattra[1], Leah Prentice[1], Trevor Pugh[3], Angela Burleigh[1], Allen Delaney[3], Karen Gelmon[4], Ryan Guliany[1], Janine Senz[2], Christian Steidl[2,5], Robert A. Holt[3], Steven Jones[3], Mark Sun[1], Gillian Leung[1], Richard Moore[3], Tesa Severson[3], Greg A. Taylor[3], Andrew E. Teschendorff[6], Kane Tse[1], Gulisa Turashvili[1], Richard Varhol[3], René L. Warren[3], Peter Watson[7], Yongjun Zhao[3], Carlos Caldas[6], David Huntsman[2,5], Martin Hirst[3], Marco A. Marra[3] & Samuel Aparicio[1,2,5]

NATURE | Vol 463 | 14 January 2010

A small-cell lung cancer genome with complex signatures of tobacco exposure

Erin D. Pleasance[1], Philip J. Stephens[1], Sarah O'Meara[1,2], David J. McBride[1], Alison Meynert[3], David Jones[1], Meng-Lay Lin[1], David Beare[1], King Wai Lau[1], Chris Greenman[1], Ignacio Varela[1], Serena Nik-Zainal[1], Helen R. Davies[1], Gonzalo R. Ordoñez[1], Laura J. Mudie[1], Calli Latimer[1], Sarah Edkins[1], Lucy Stebbings[1], Lina Chen[1], Mingming Jia[1], Catherine Leroy[1], John Marshall[1], Andrew Menzies[1], Adam Butler[1], Jon W. Teague[1], Jonathon Mangion[2], Yongming A. Sun[4], Stephen F. McLaughlin[5], Heather E. Peckham[5], Eric F. Tsung[5], Gina L. Costa[5], Clarence C. Lee[5], John D. Minna[6], Adi Gazdar[6], Ewan Birney[3], Michael D. Rhodes[4], Kevin J. McKernan[5], Michael R. Stratton[1,7], P. Andrew Futreal[1] & Peter J. Campbell[1,8]

A comprehensive catalogue of somatic mutations from a human cancer genome

Erin D. Pleasance[1]*, R. Keira Cheetham[2]*, Philip J. Stephens[1], David J. McBride[1], Sean J. Humphray[2], Chris D. Greenman[1], Ignacio Varela[1], Meng-Lay Lin[1], Gonzalo R. Ordóñez[1], Graham R. Bignell[1], Kai Ye[3], Julie Alipaz[4], Markus J. Bauer[2], David Beare[1], Adam Butler[1], Richard J. Carter[2], Lina Chen[1], Anthony J. Cox[2], Sarah Edkins[1], Paula I. Kokko-Gonzales[2], Niall A. Gormley[2], Russell J. Grocock[2], Christian D. Haudenschild[5], Matthew M. Hims[2], Terena James[2], Mingming Jia[1], Zoya Kingsbury[2], Catherine Leroy[1], John Marshall[1], Andrew Menzies[1], Laura J. Mudie[1], Zemin Ning[1], Tom Royce[4], Ole B. Schulz-Trieglaff[2], Anastassia Spiridou[2], Lucy A. Stebbings[1], Lukasz Szajkowski[2], Jon Teague[1], David Williamson[5], Lynda Chin[6], Mark T. Ross[2], Peter J. Campbell[1], David R. Bentley[2], P. Andrew Futreal[1] & Michael R. Stratton[1,7]

10 FEBRUARY 2011 | VOL 470 | NATURE | 215

The genomic complexity of primary human prostate cancer

Michael F. Berger[1]†*, Michael S. Lawrence[1]*, Francesca Demichelis[2,3]*, Yotam Drier[4]*, Kristian Cibulskis[1], Andrey Y. Sivachenko[1], Andrea Sboner[5,6], Raquel Esgueva[2], Dorothee Pflueger[2], Carrie Sougnez[1], Robert Onofrio[1], Scott L. Carter[1], Kyung Park[2], Lukas Habegger[6], Lauren Ambrogio[1], Timothy Fennell[1], Melissa Parkin[1], Gordon Saksena[1], Douglas Voet[1], Alex H. Ramos[1,7], Trevor J. Pugh[1,7,8], Jane Wilkinson[1], Sheila Fisher[1], Wendy Winckler[1], Scott Mahan[1], Kristin Ardlie[1], Jennifer Baldwin[1], Jonathan W. Simons[9], Naoki Kitabayashi[2], Theresa Y. MacDonald[2], Philip W. Kantoff[7,8], Lynda Chin[1,7,8,10], Stacey B. Gabriel[1], Mark B. Gerstein[5,6,11], Todd R. Golub[1,12,13,14], Matthew Meyerson[1,7,8,14], Ashutosh Tewari[15], Eric S. Lander[1,7,16], Gad Getz[1], Mark A. Rubin[2] & Levi A. Garraway[1,7,8,14]

National Cancer Institute

CANCER GENOME ANATOMY PROJECT

The Cancer Genome Project

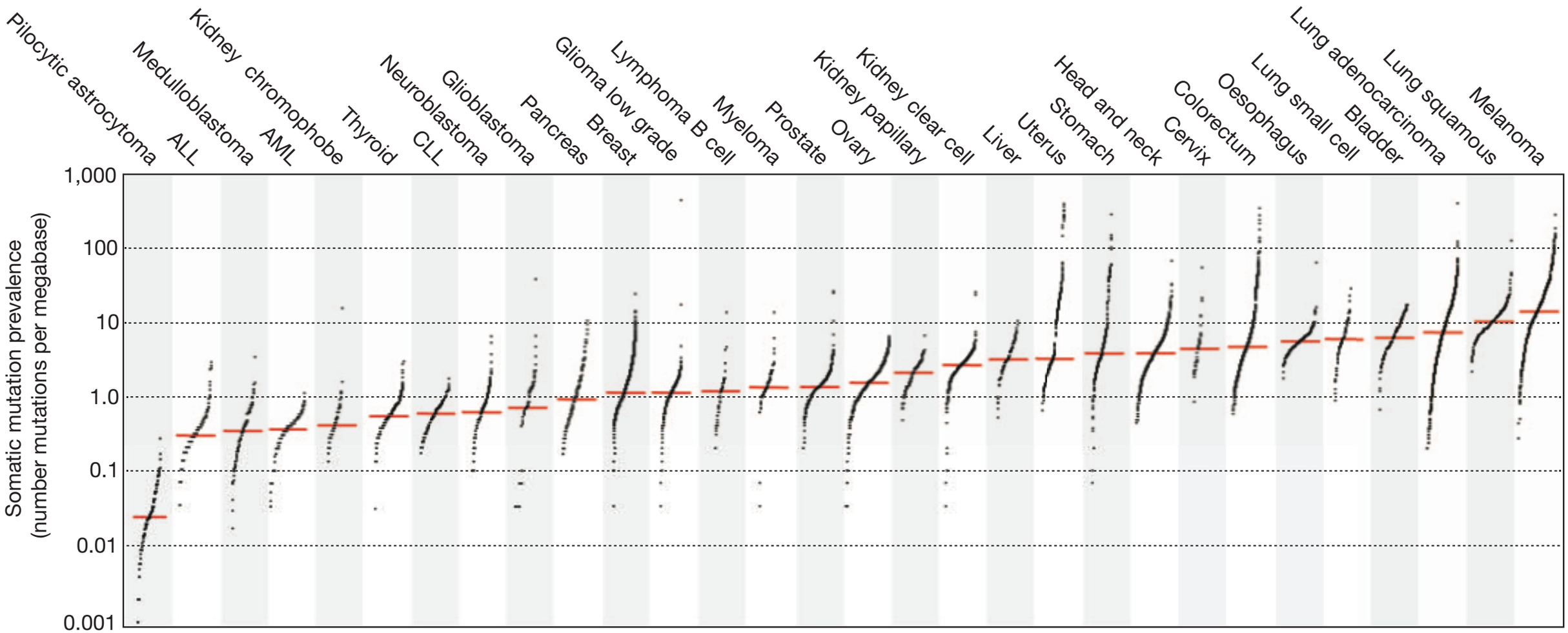
COSMIC
Catalogue of somatic mutations in cancer

The Cancer Genome Atlas

International Cancer Genome Consortium

90 projects

A pile of genetic alterations



Which genes drive the cancer?

Driver & Passenger Mutations

Which processes drive the onset of mutations?

Drivers & Passengers

Which genes drive the cancer?

- frequency

  alterations in p53: 5%-50% in most tumors

- effects of the mutation

  inactivation of Protein Kinases

- timing of the mutations

  ovarian cancer

- network analysis

  medulloblastoma classification

- improbability/conservation of the mutation

  ras-related genes

How probable are driver mutations?

SYNONYMOUS Mutations

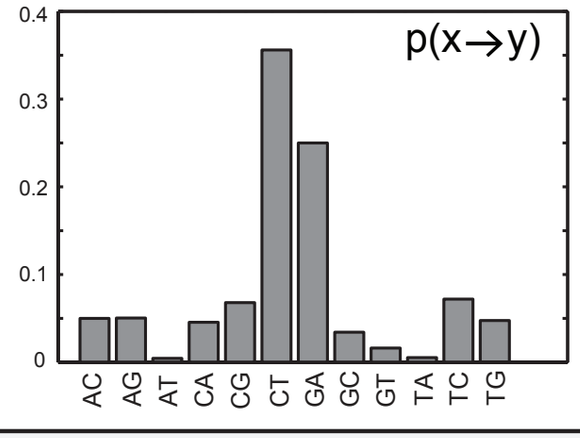
11000 pazienti

Cancer SNVs (DATASET)

2x10^6 mutazioni

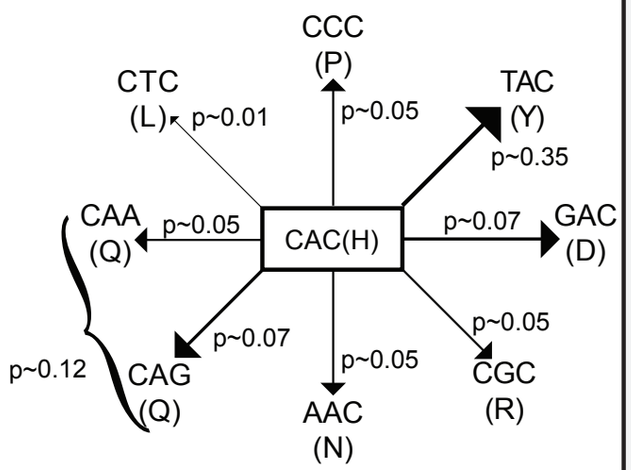
NON-SYNONYMOUS Mutations

Nucleotide to Nucleotide



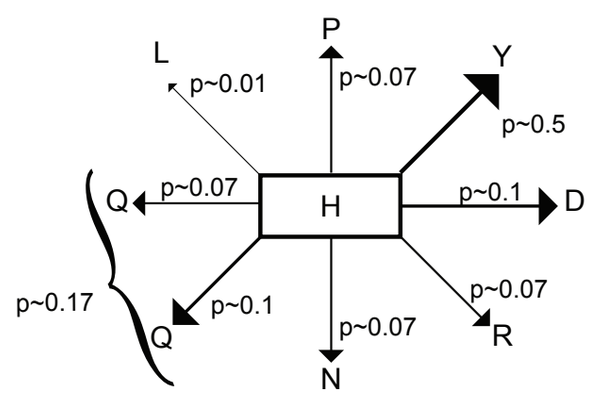
Mutational Background Model
(one for each tumor type)

Codon to Codon
(e.g. CAC codon for Histidine)



conversion

Amino Acid to Amino Acid



Mutational Divergence

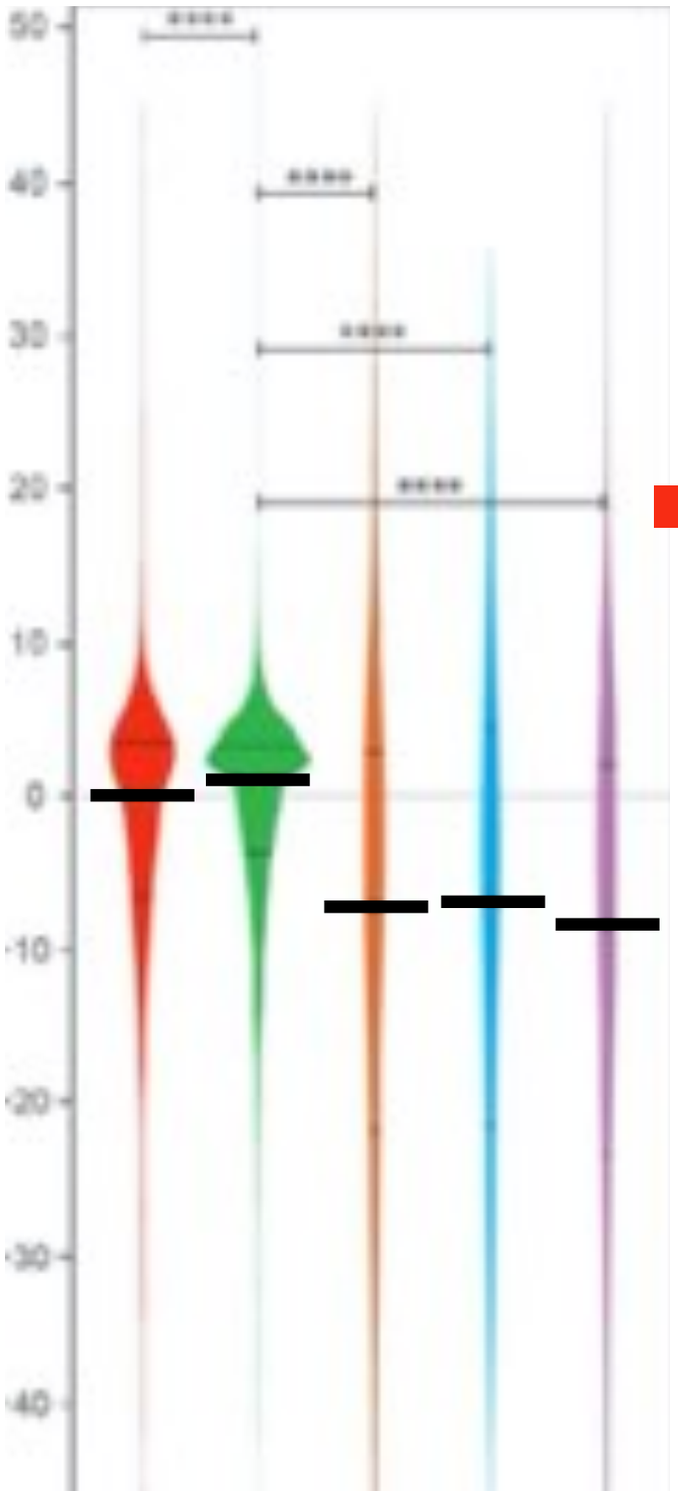
$$S_{mut} = \sum_{i=1}^{n} log_{10} p_i^{bkg}$$

Mutational Entropy

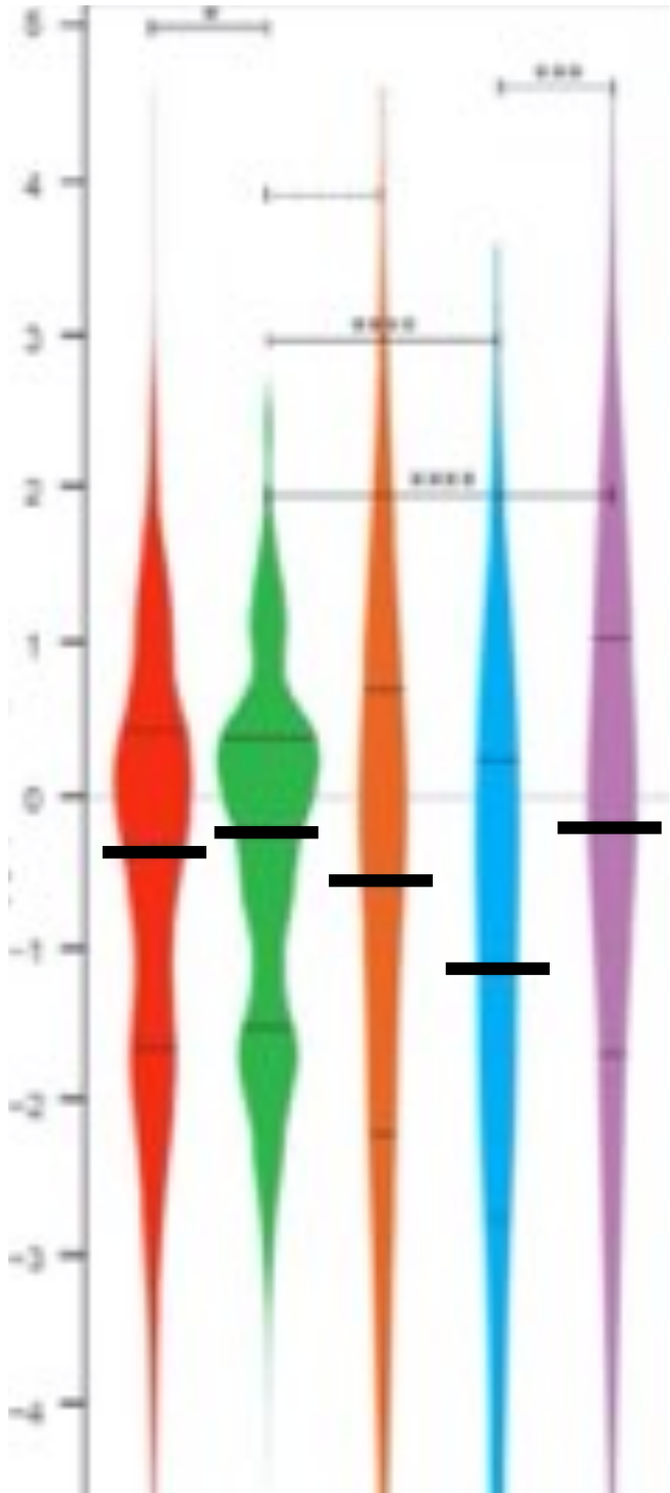
$$H = -\sum_{i=x,y,z..} f_i log_2 f_i$$

How probable are driver mutations?

Mutational Likeliness

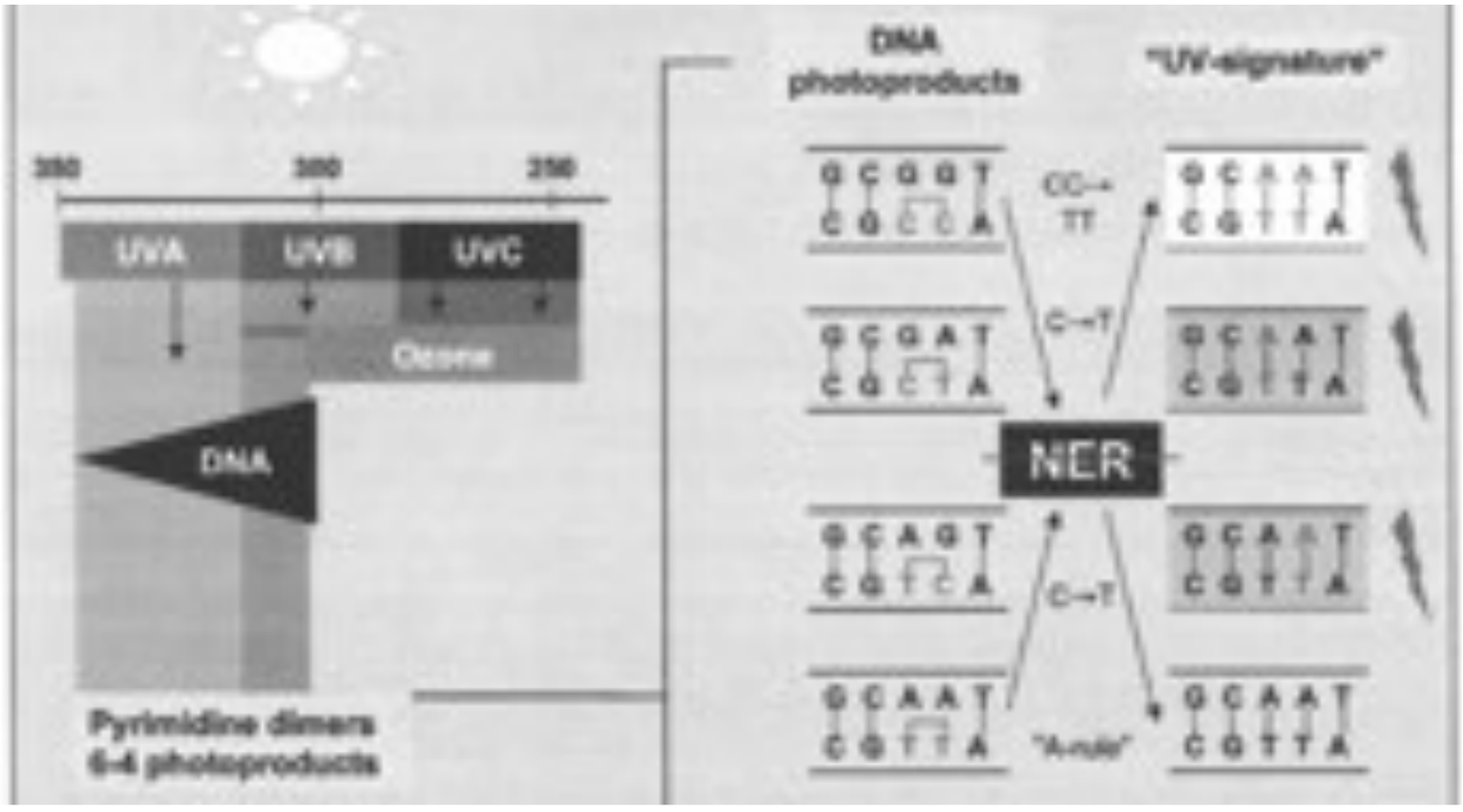


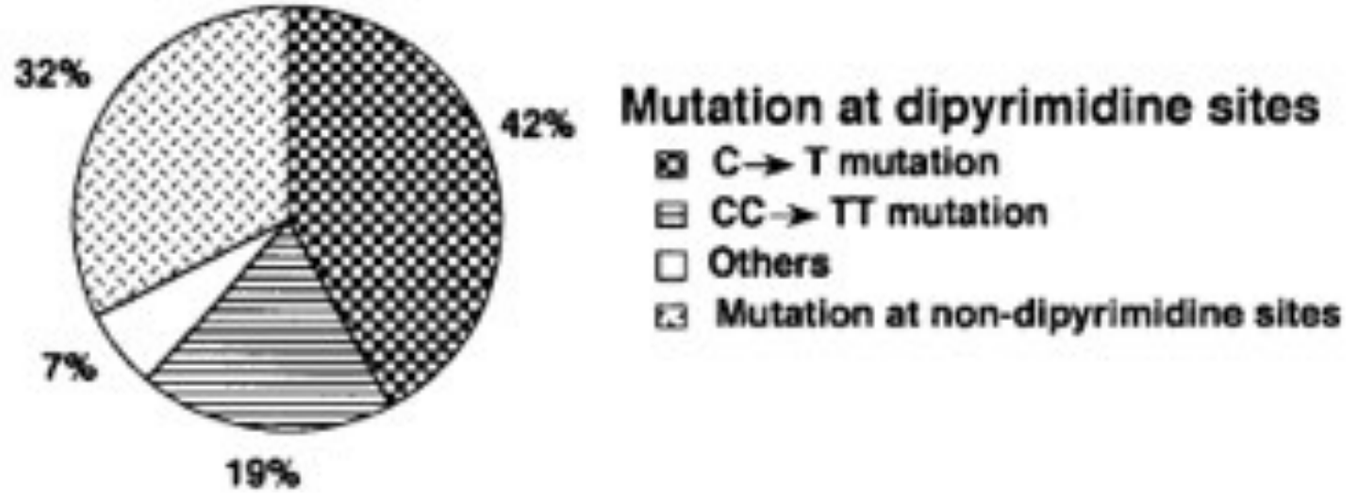
Mutational Entropy



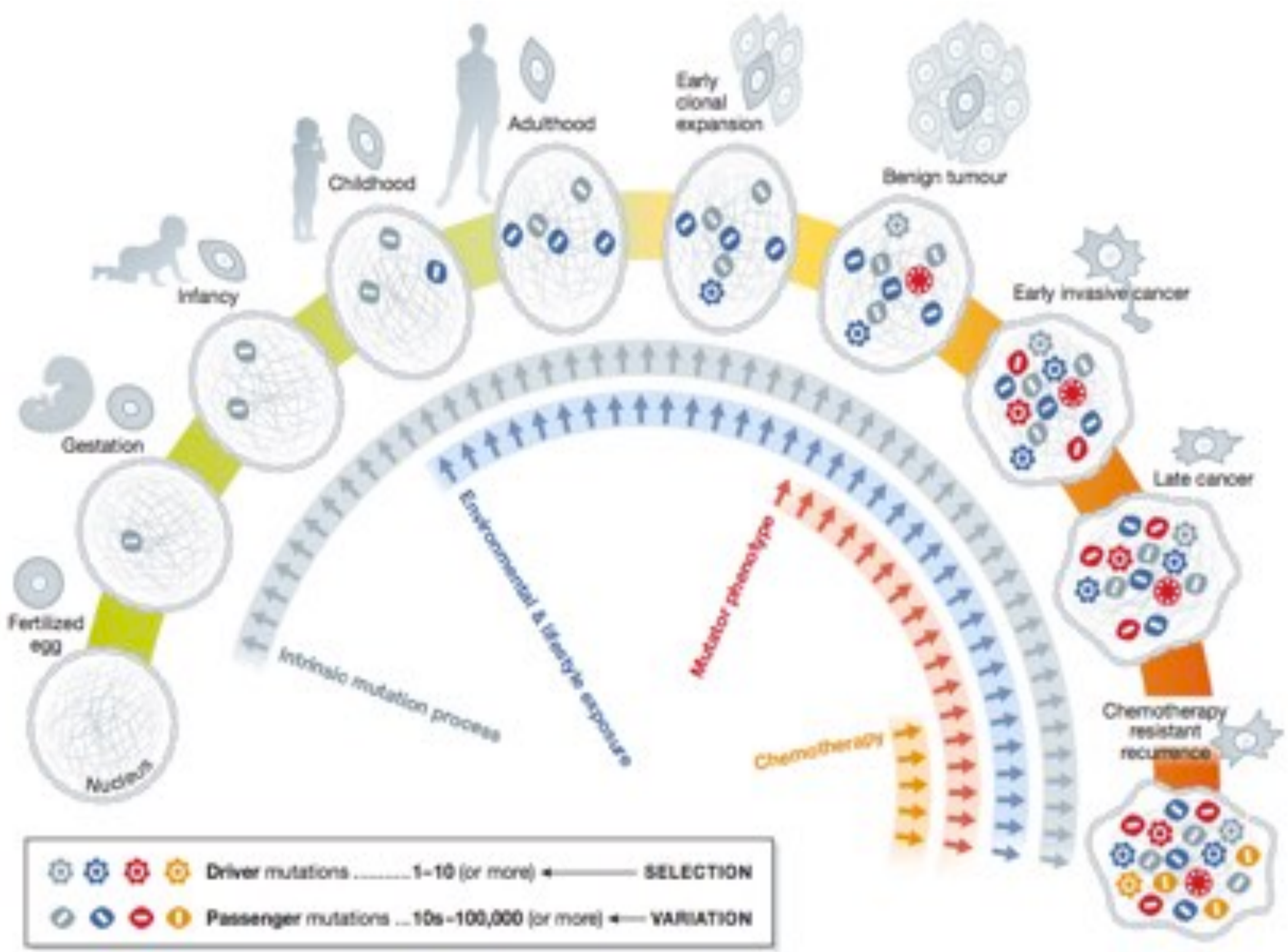
Mutational Signatures

Which processes drive the onset of mutations?



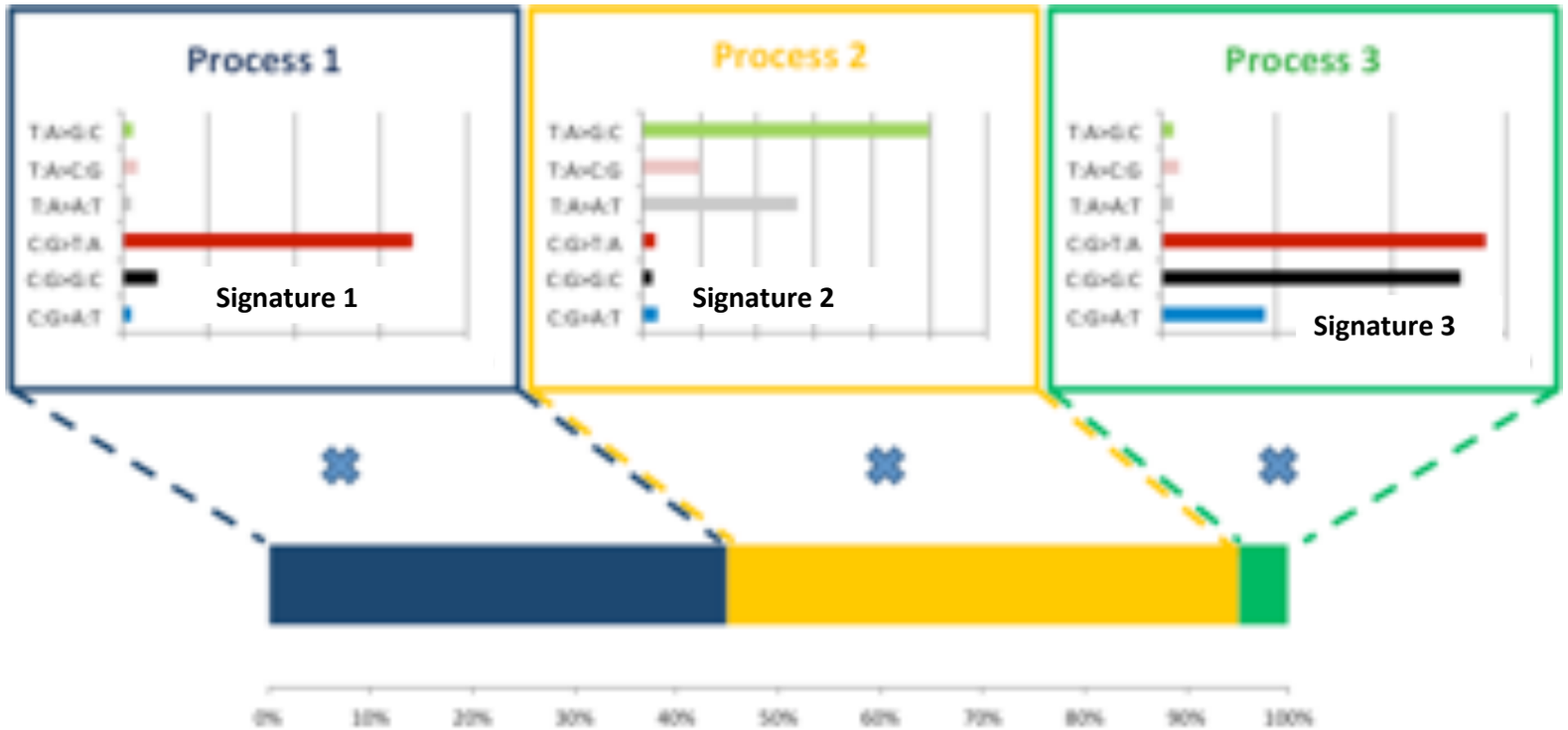


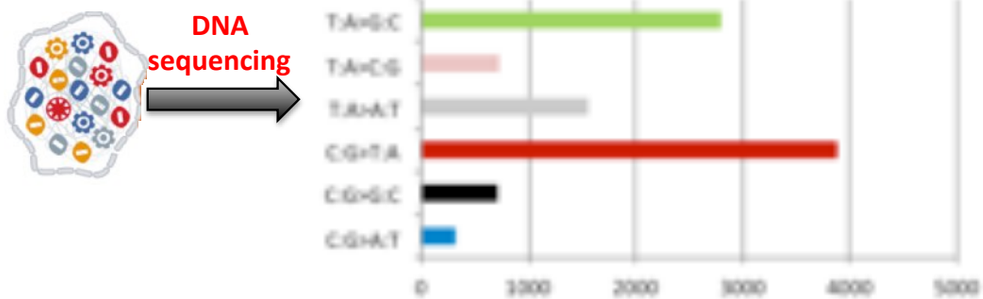
Mutational Signatures



Mutational Signatures

Non-negative Matrix Factorization





Ludmil Alexandrov

2013

Mutational Signatures

C>T

C>A

C>G

T>A

T>C

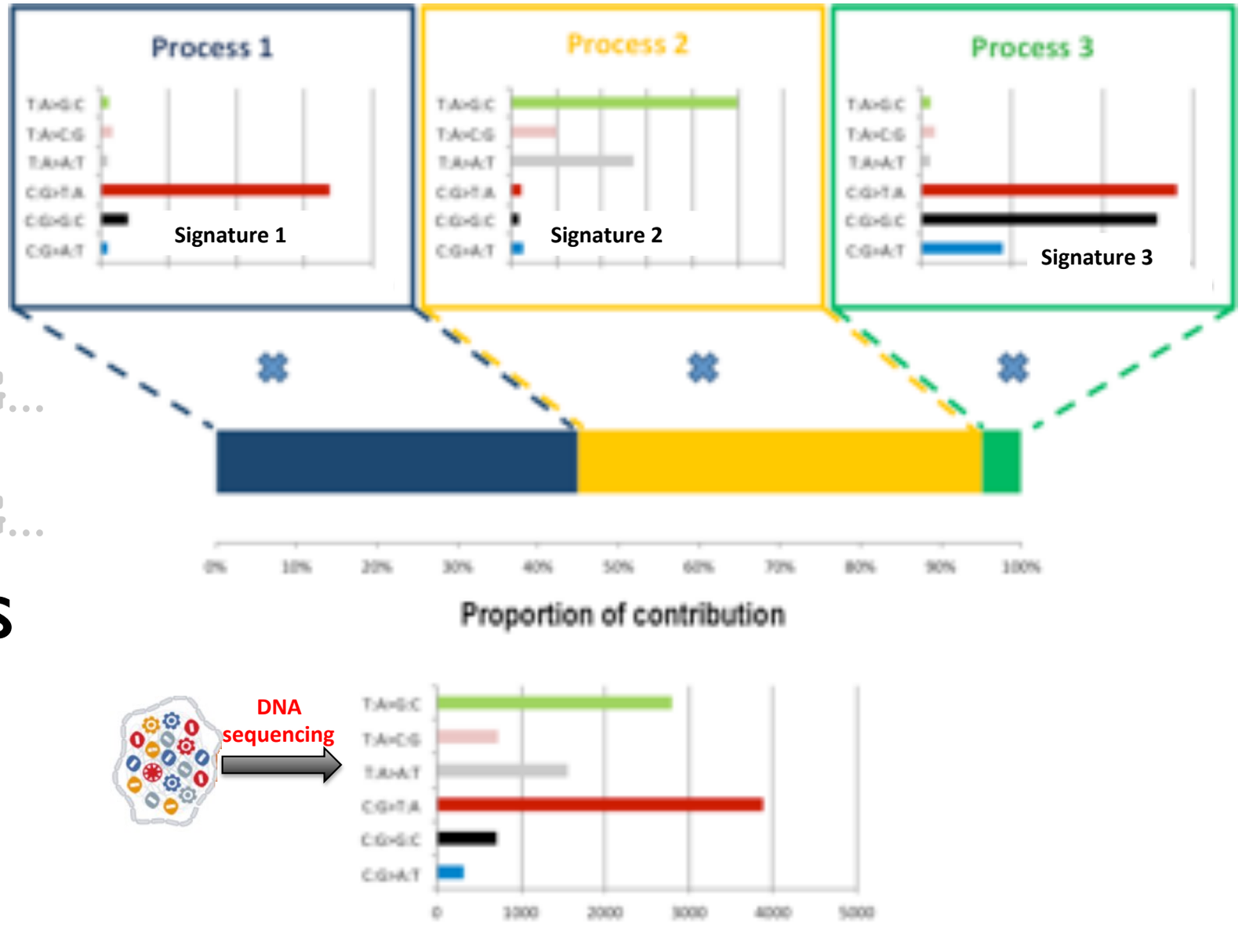
T>G

...AAA**T**C**G**CTG...

...AAA**TT**G**C**TG...

6 classes 16 contexts

96 bins



Ludmil Alexandrov

2013

Mutational Signatures

C>T

C>A

C>G

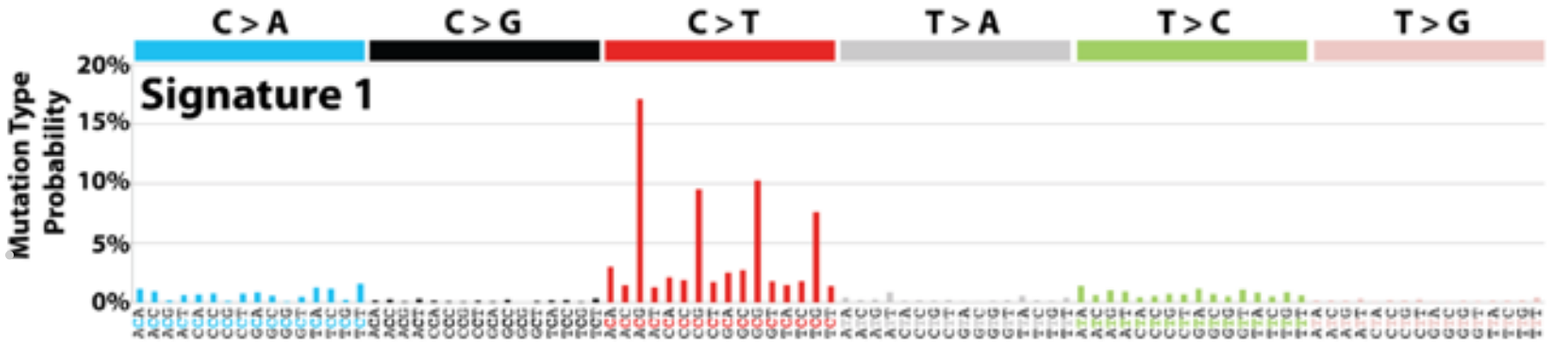
T>A

T>C

T>G

...AAATCGCTG...

...AAATTGCTG...



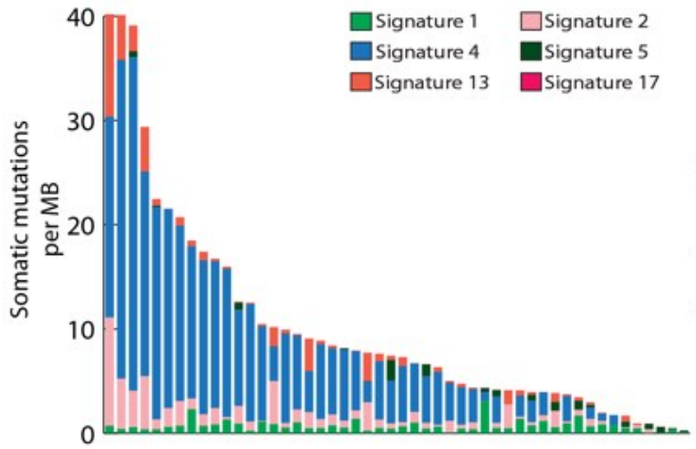
6 classes 16 contexts

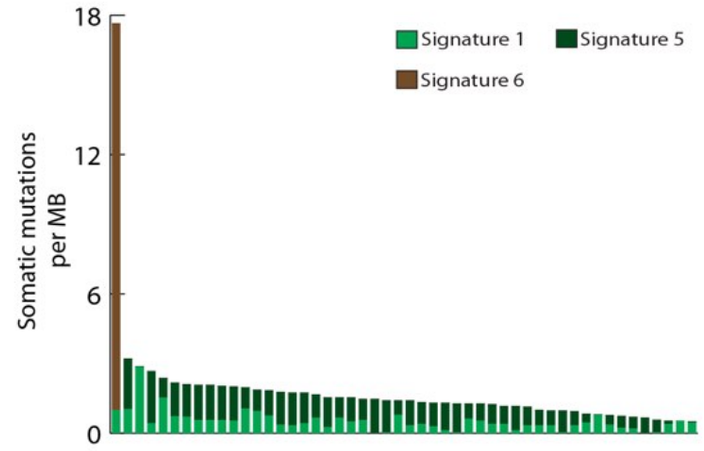
96 bins

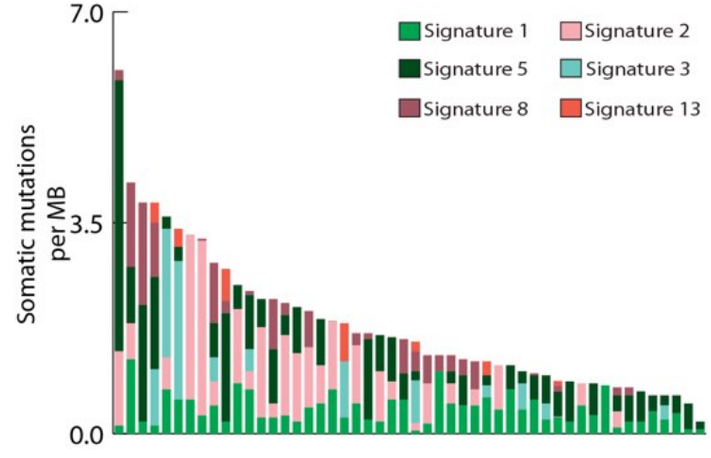
Ludmil Alexandrov

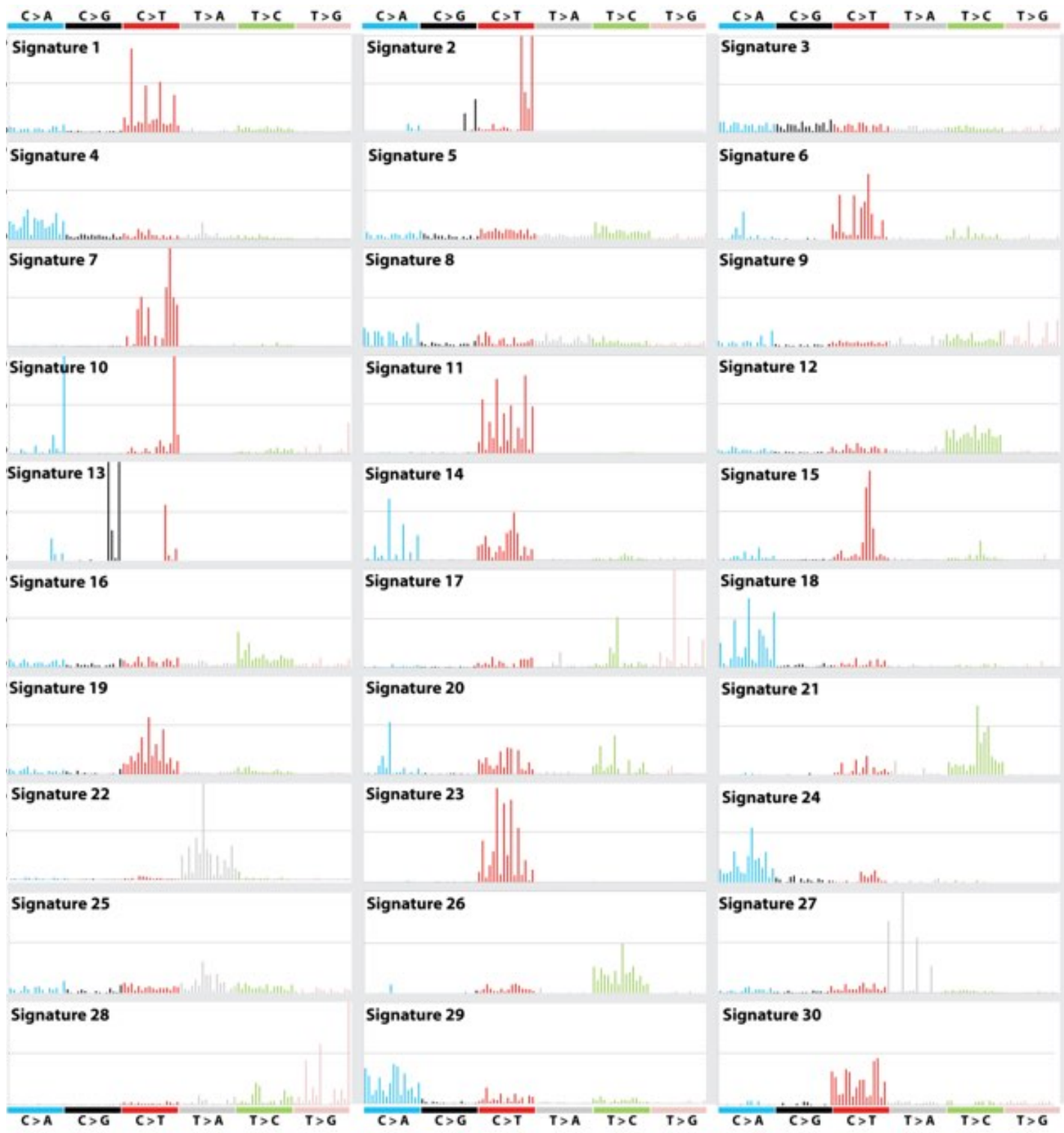
2013

Mutational Signatures









Mutational Signatures

Deamination
of 5-Methyl Cytosine

BRCA

DNA repair

Mutational Signatures

Mutational Signatures

Temozolomide

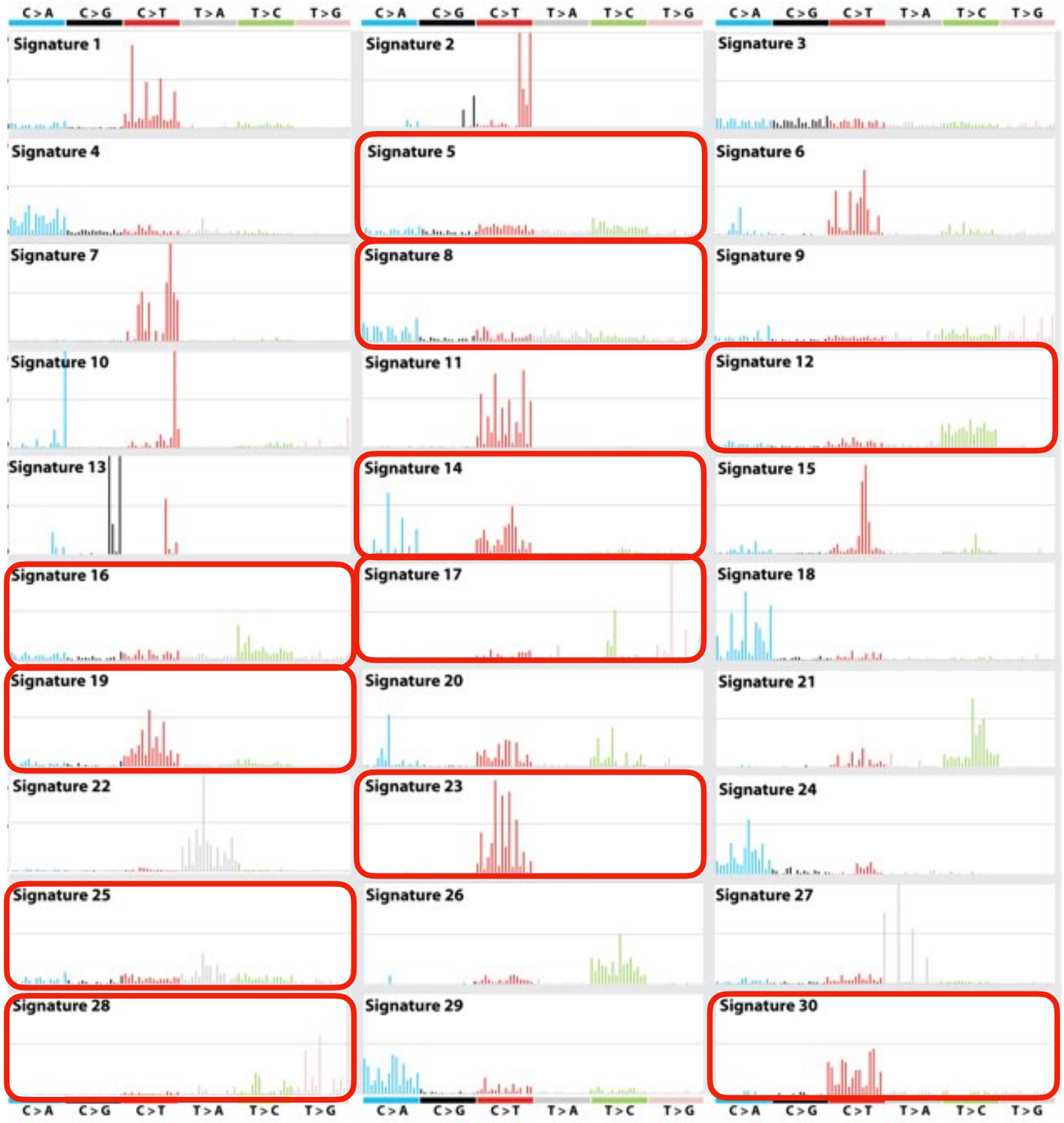
Aflatoxin

Aristolochic Acid

Psoralen

Mutational Signatures

Unknown



Mutational Signatures

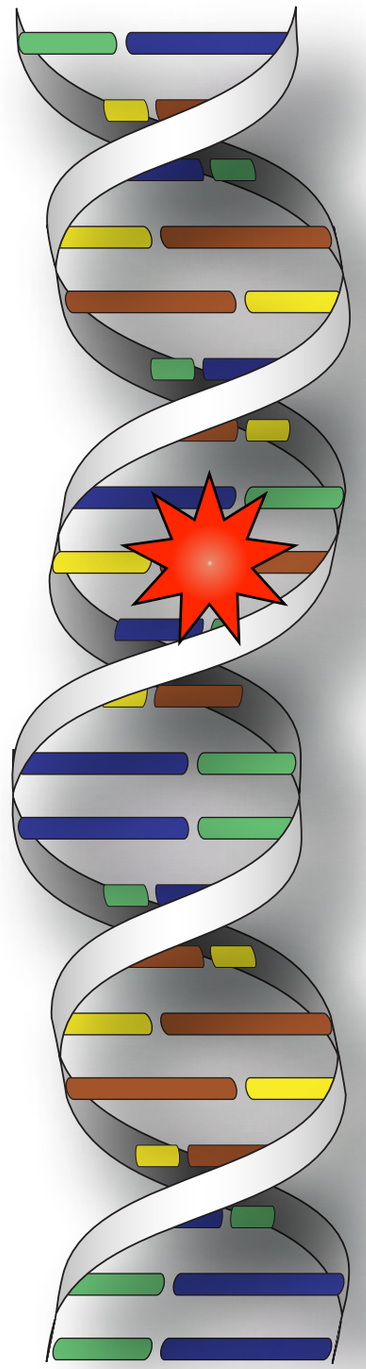
APOBEC
Mutational Signature

the (cancer) evolutionary process

- during replication
- environmental damage
  - Radiations
  - Chemicals
  - Spontaneous Breakdown

- DNA repair
  - Mismatch Repair
  - Excision Repair
  - End-Joining
  - Recombinational Repair

- AID/APOBECs



Stability

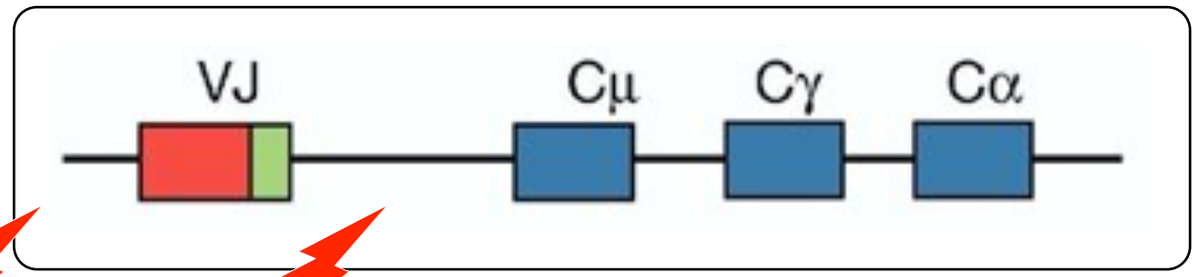
Evolution

More Evolution

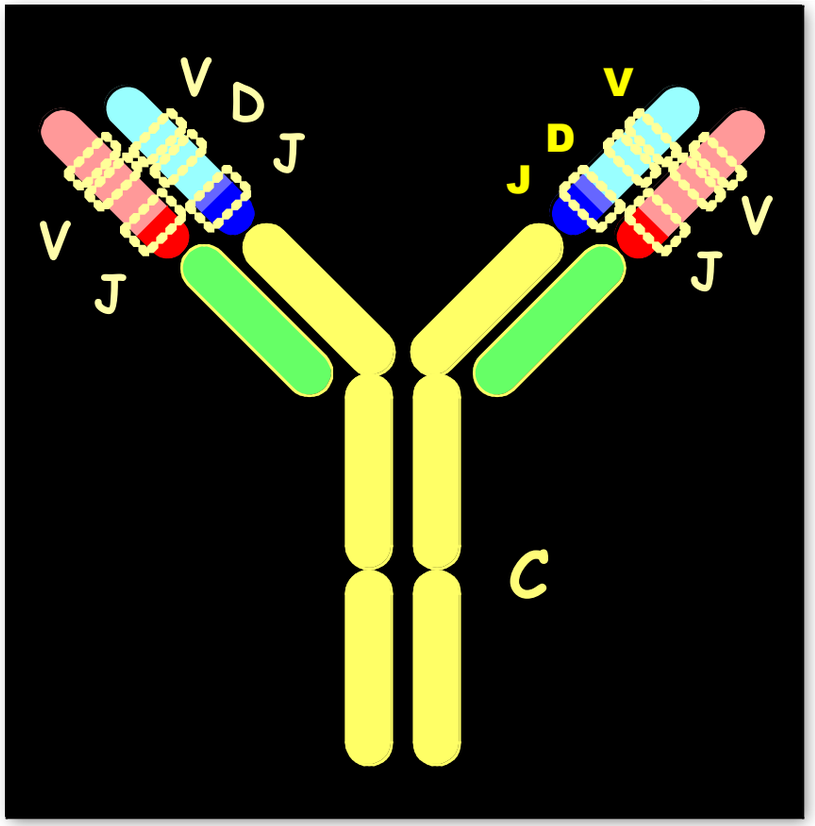
Evolution - Live!

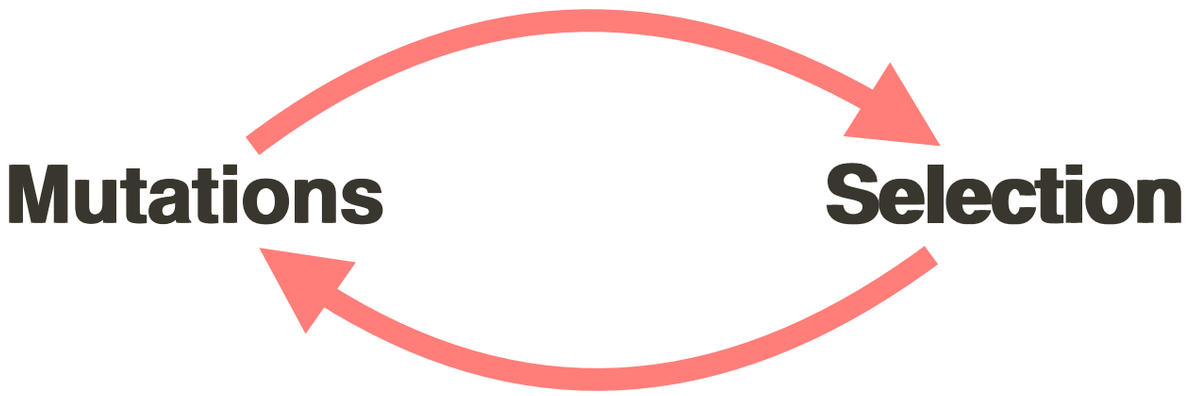
Antibody Diversification

Immunoglobulin gene

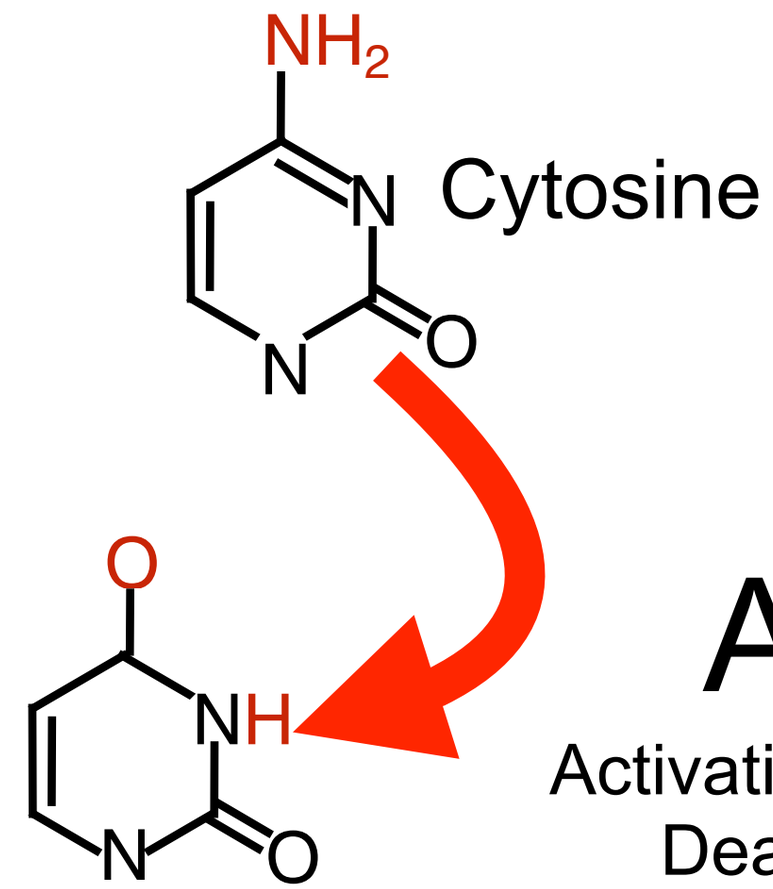


proteins



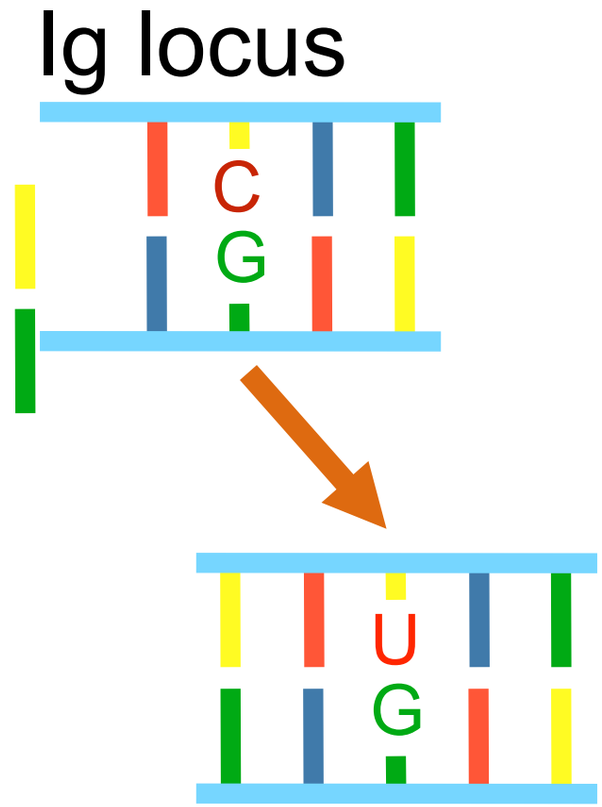


Diversification by Deamination



AID

Activation Induced Deaminase

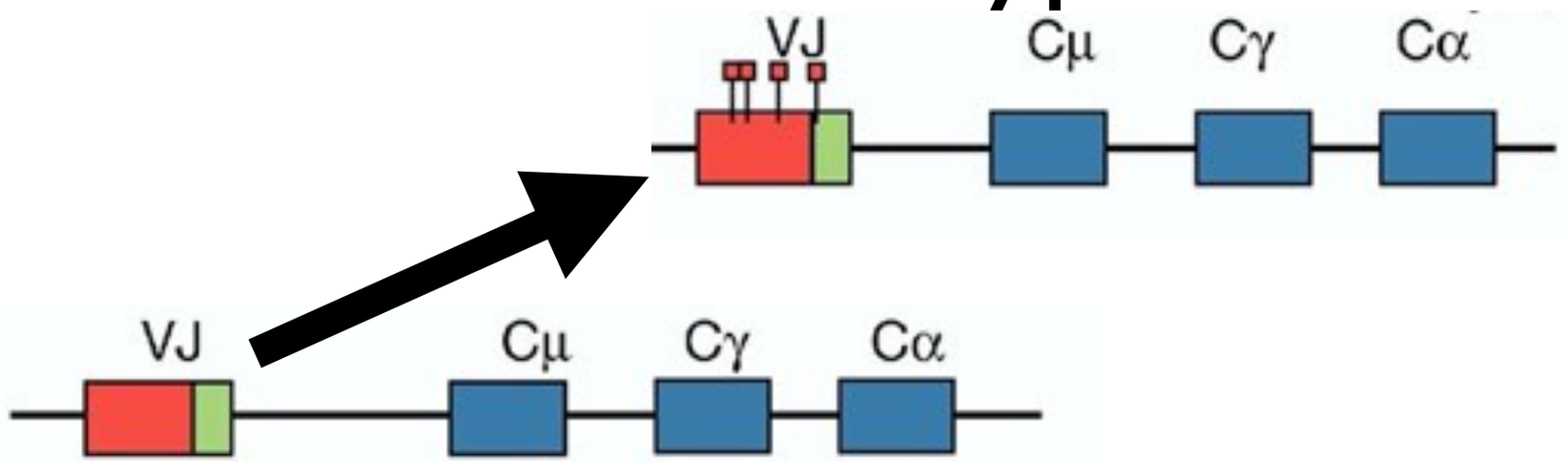


Error-Prone DNA Repair

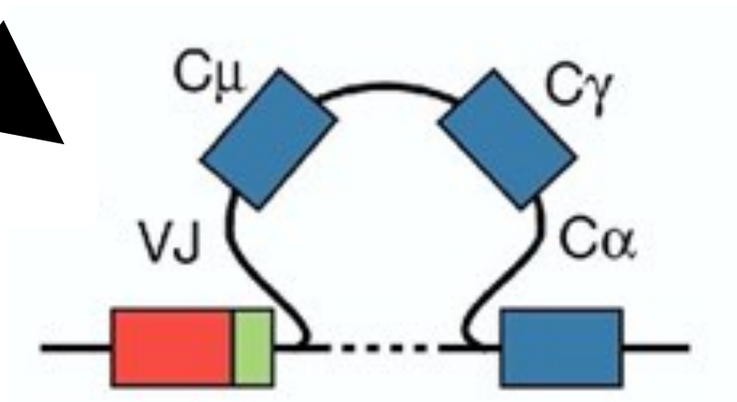
Antigen-dependent Antibody Diversification

Diversification by Deamination

Somatic Hypermutation



Class Switch Recombination



Diversification by Deamination

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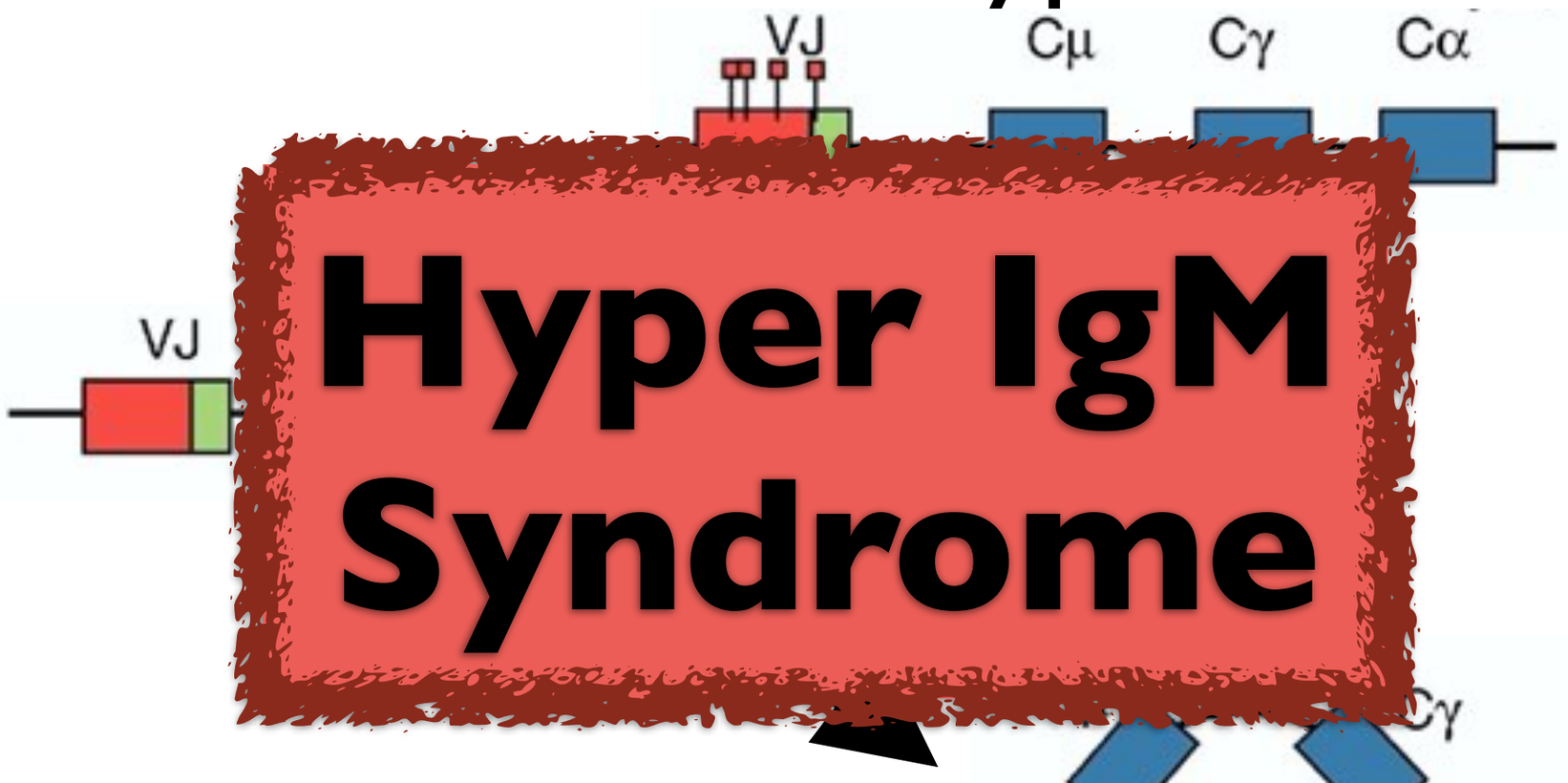
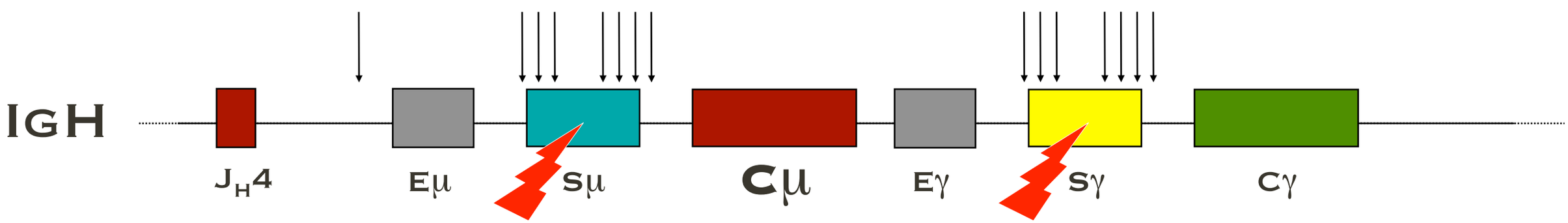


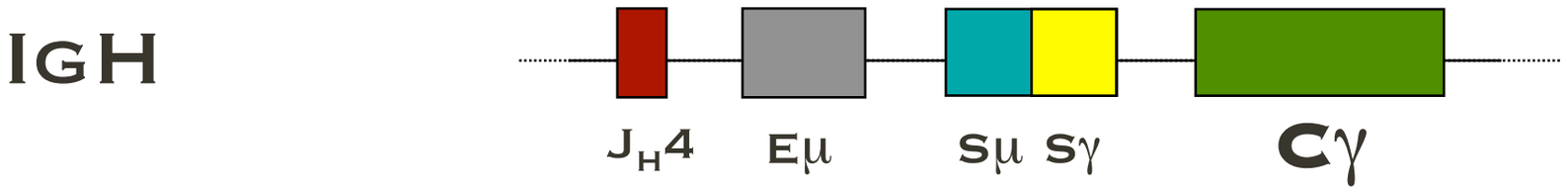
Class Switch Recombination

Physiology:

Class Switch Recombination

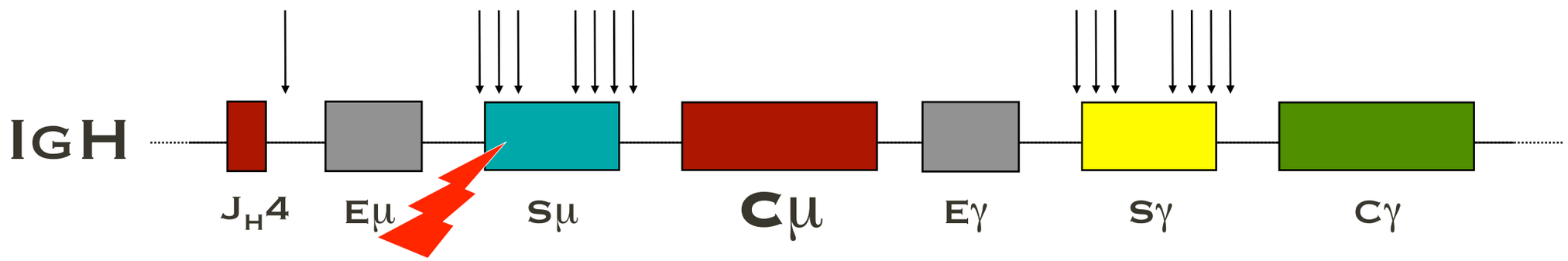


AID

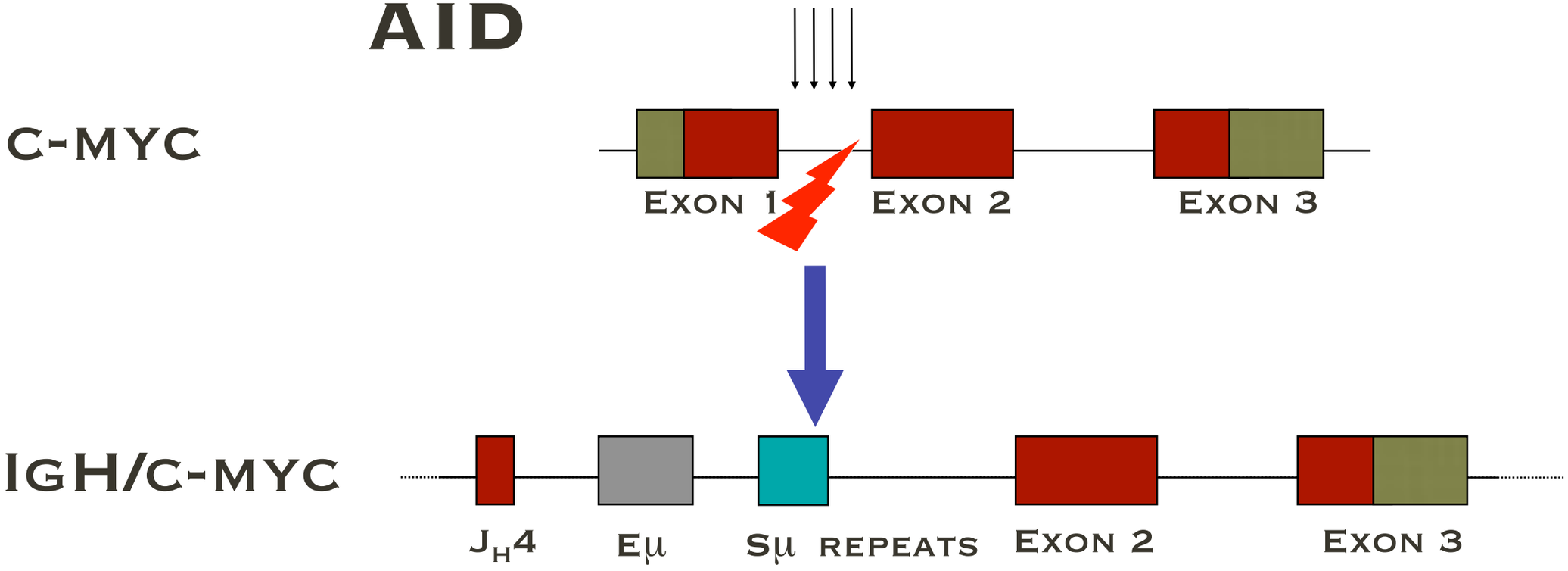


Pathology:

Chromosomal Translocations



AID



Ramiro et al. 2004; 2006

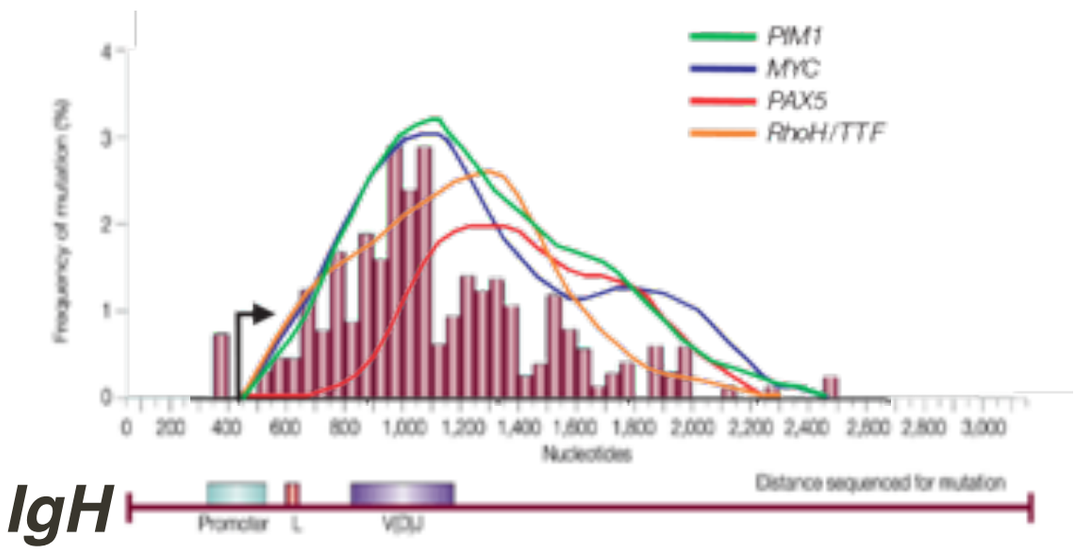
Robbiani et al. 2008; 2009

AID & Lymphomas: Chromosomal Translocations

sporadic Burkitt's Lymphoma	***c-myc,* t(8;14)**
Diffuse Large B Cell Lymphoma	***bcl-6,* t(3;14)**
B-Chronic Lymphocytic Leukemia	***bcl-3,* t(14;19)**
Lymphoplasmacytoid Lymphoma	***Pax-5,* t(9;14)**
Diffuse Large Cell Lymphoma	***lyt-10,* t(10;14)**
Extranodal Lymphoma	***MUC-1,* t(1;14)**

Chromosomal breakpoints at the Switch Regions

MUTATIONS



Pim1

	G	A	T	C
G				
A				
T	1	1		
C	4	4	45	

Pax5

	G	A	T	C
G				
A	2			
T	1	2		
C	12	5	26	

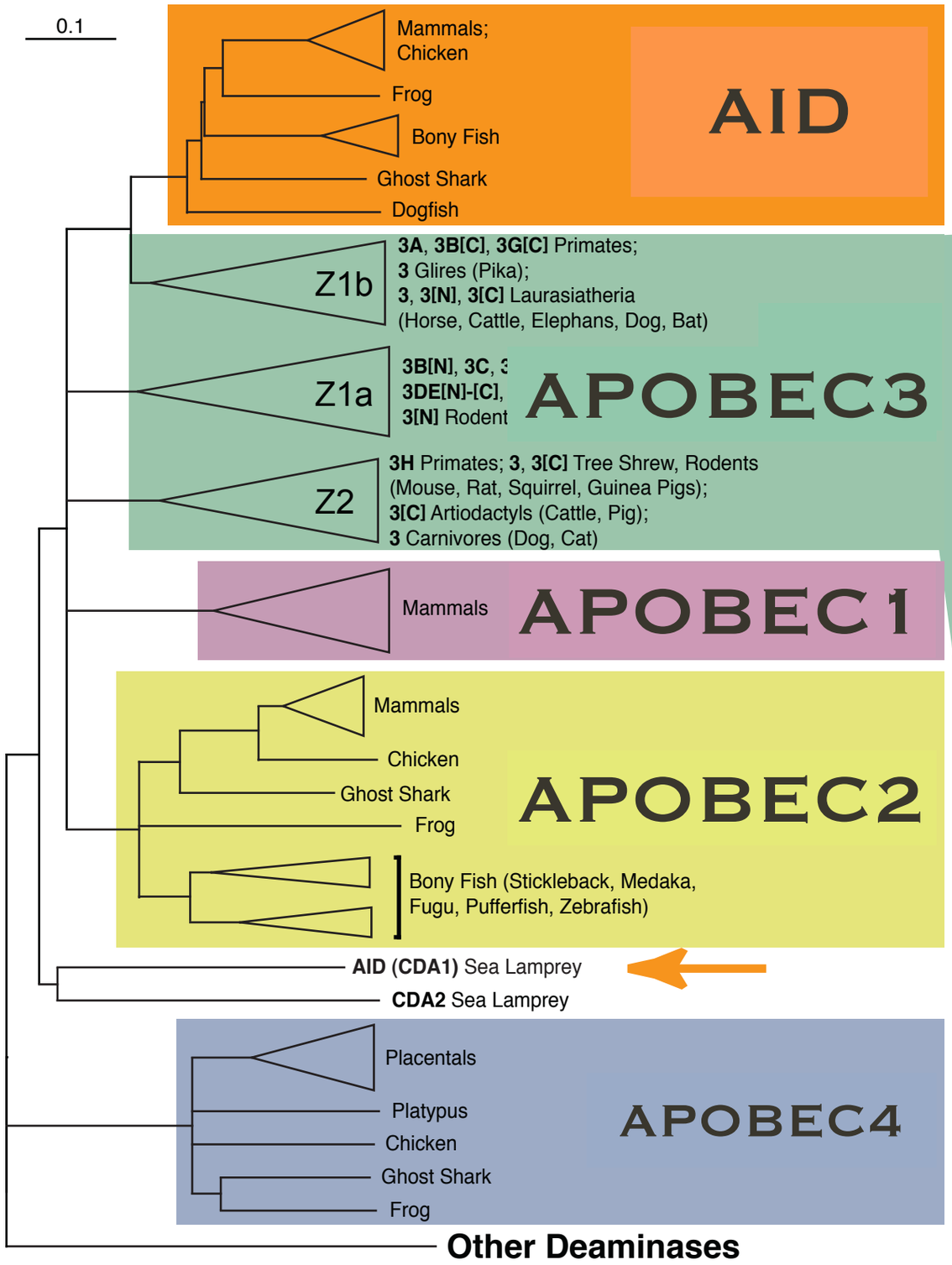
c-Myc

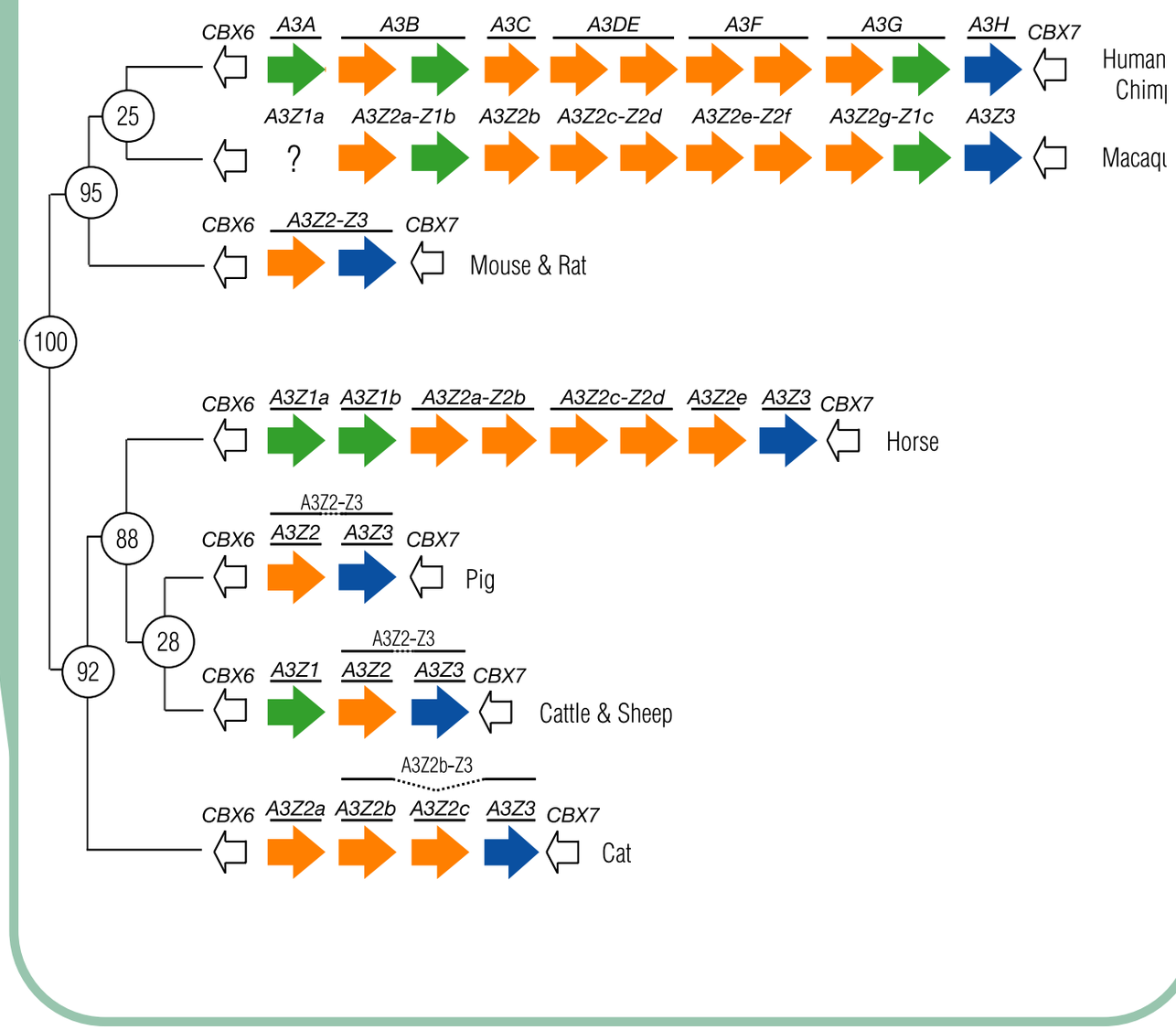
	G	A	T	C
G				
A	4			
T	5	3		
C	16	3	22	

Bcl-6

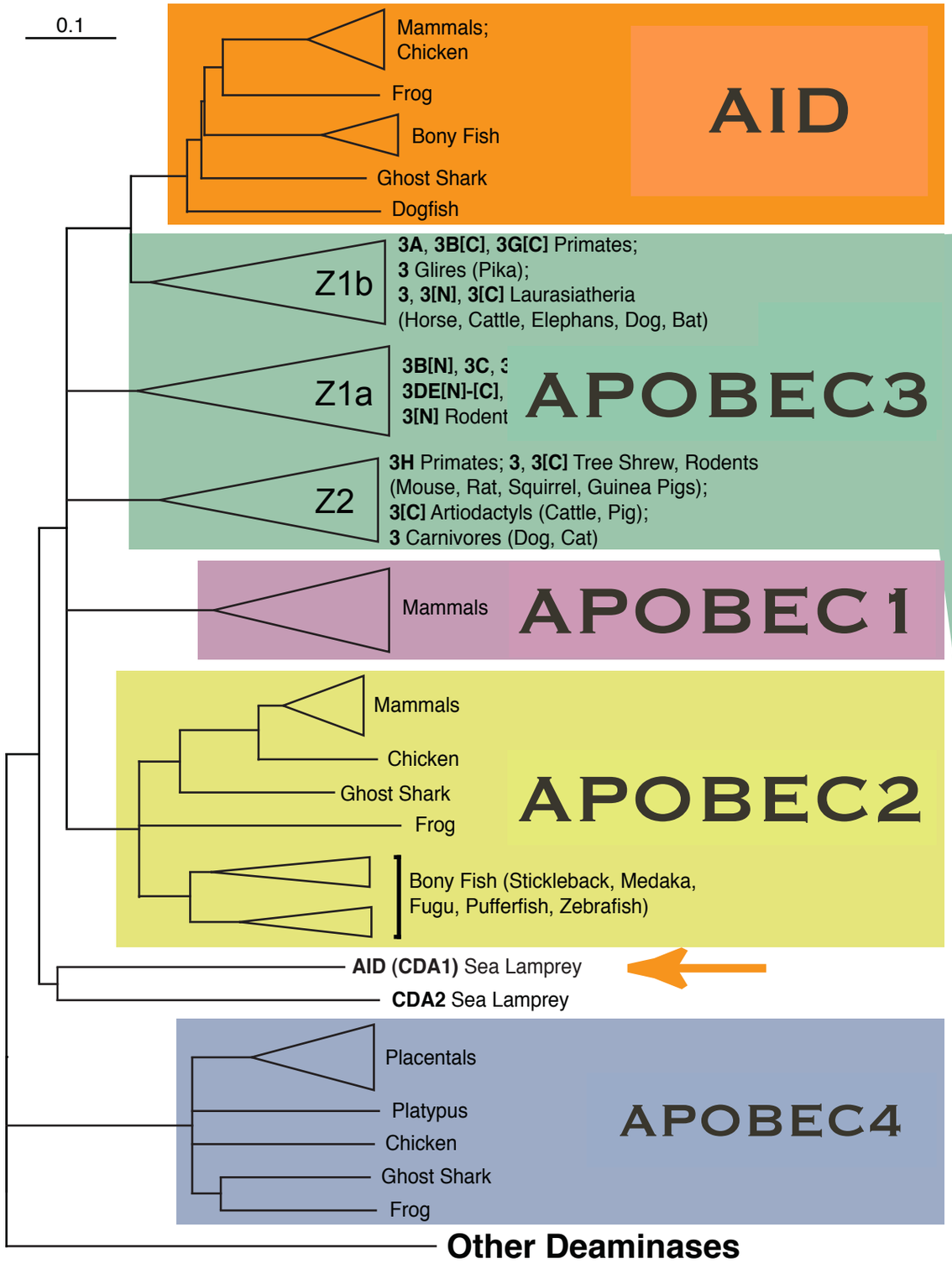
	G	A	T	C
G				
A	14			
T	8	4		
C	12	3	19	

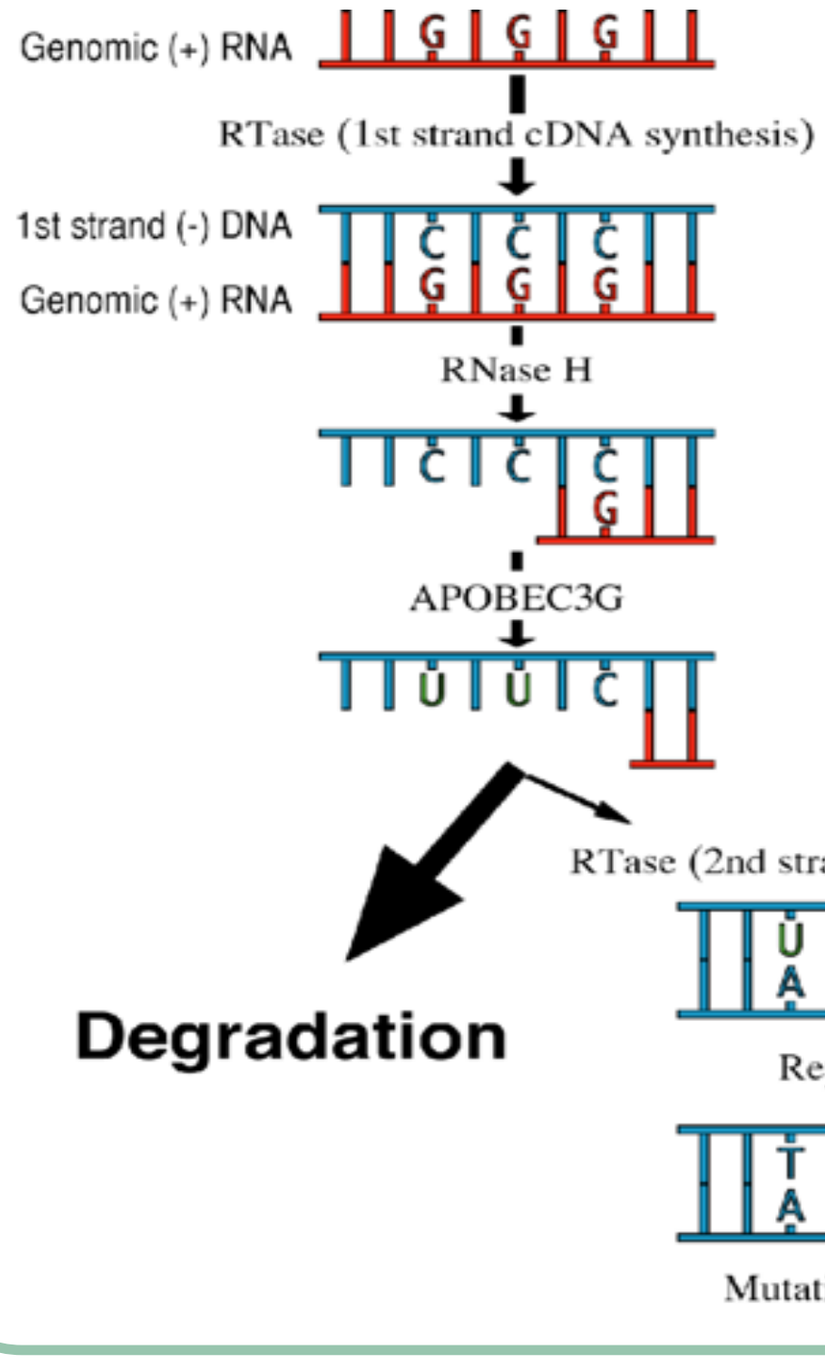
THE AID/APOBECs



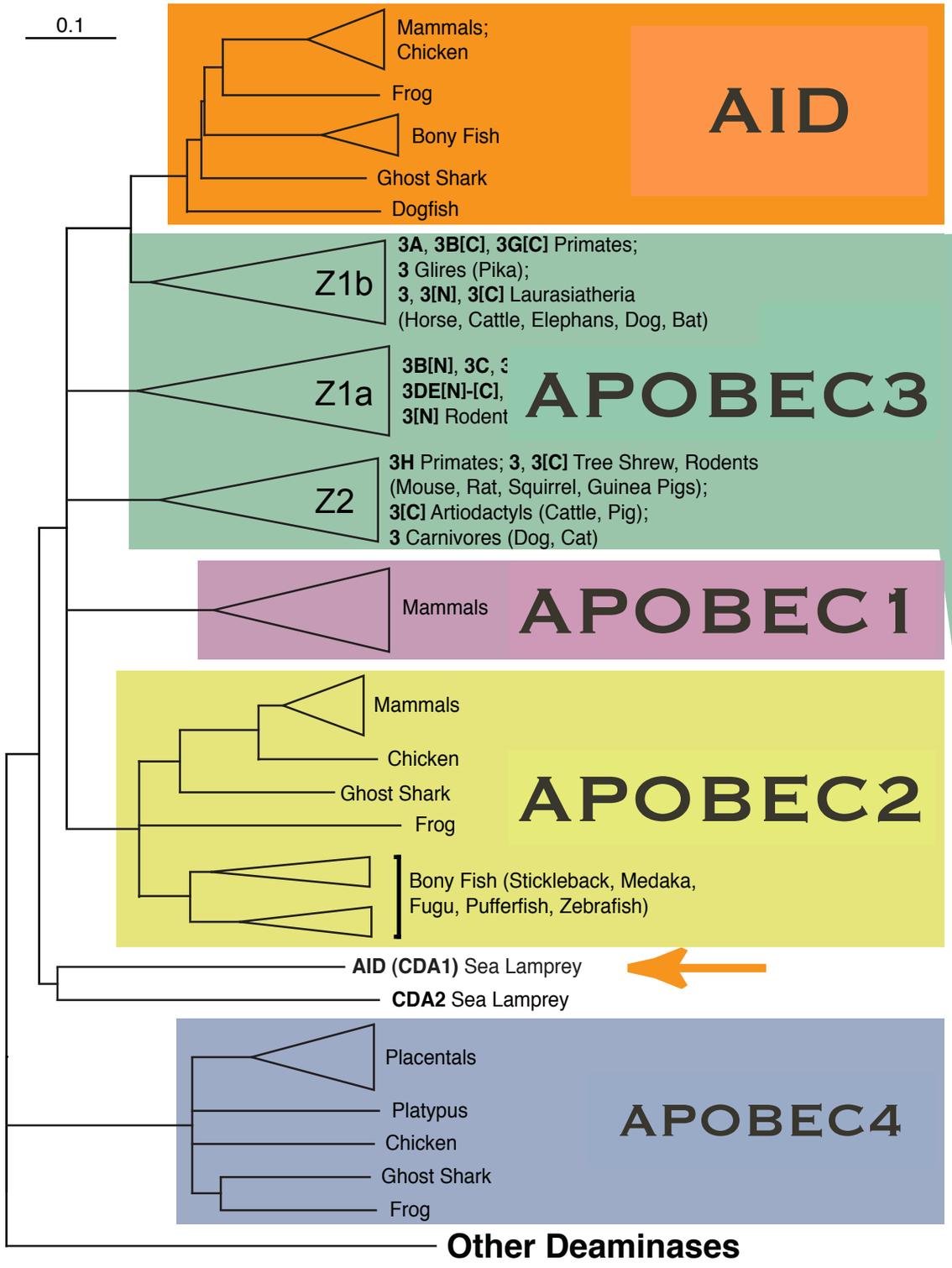


THE AID/APOBECs

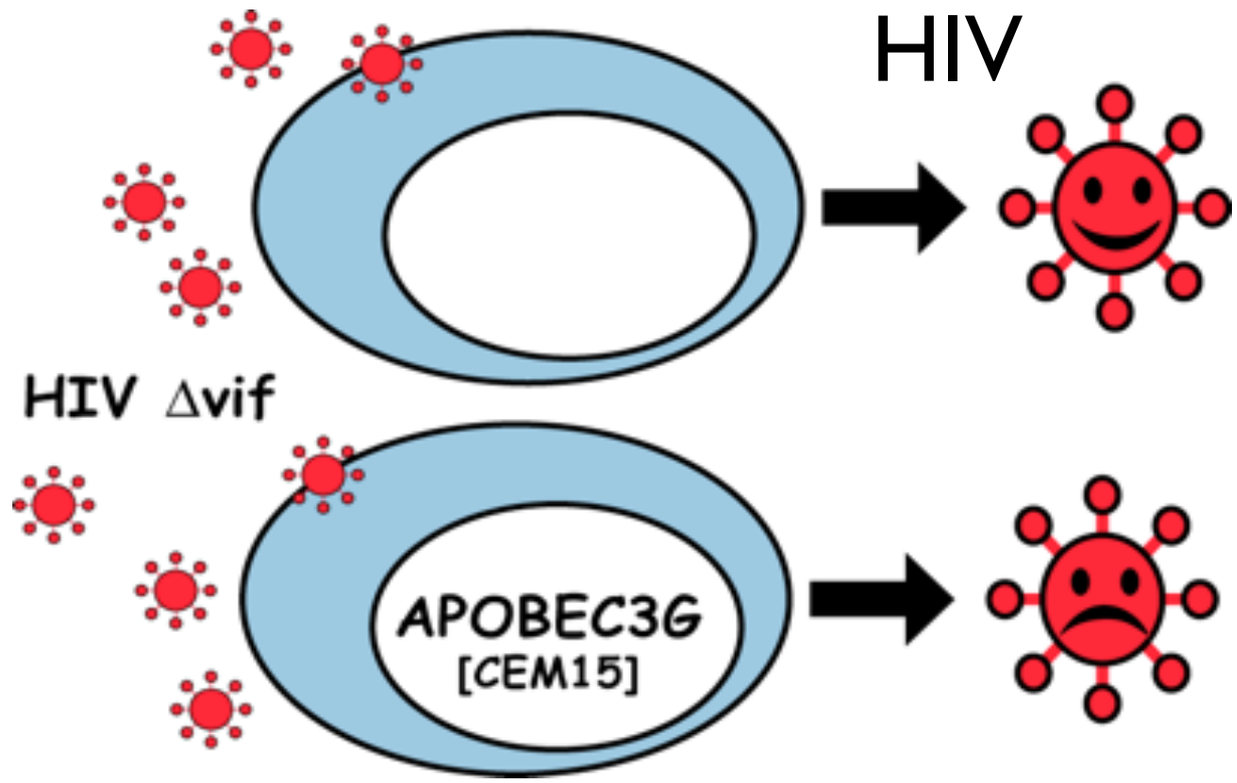




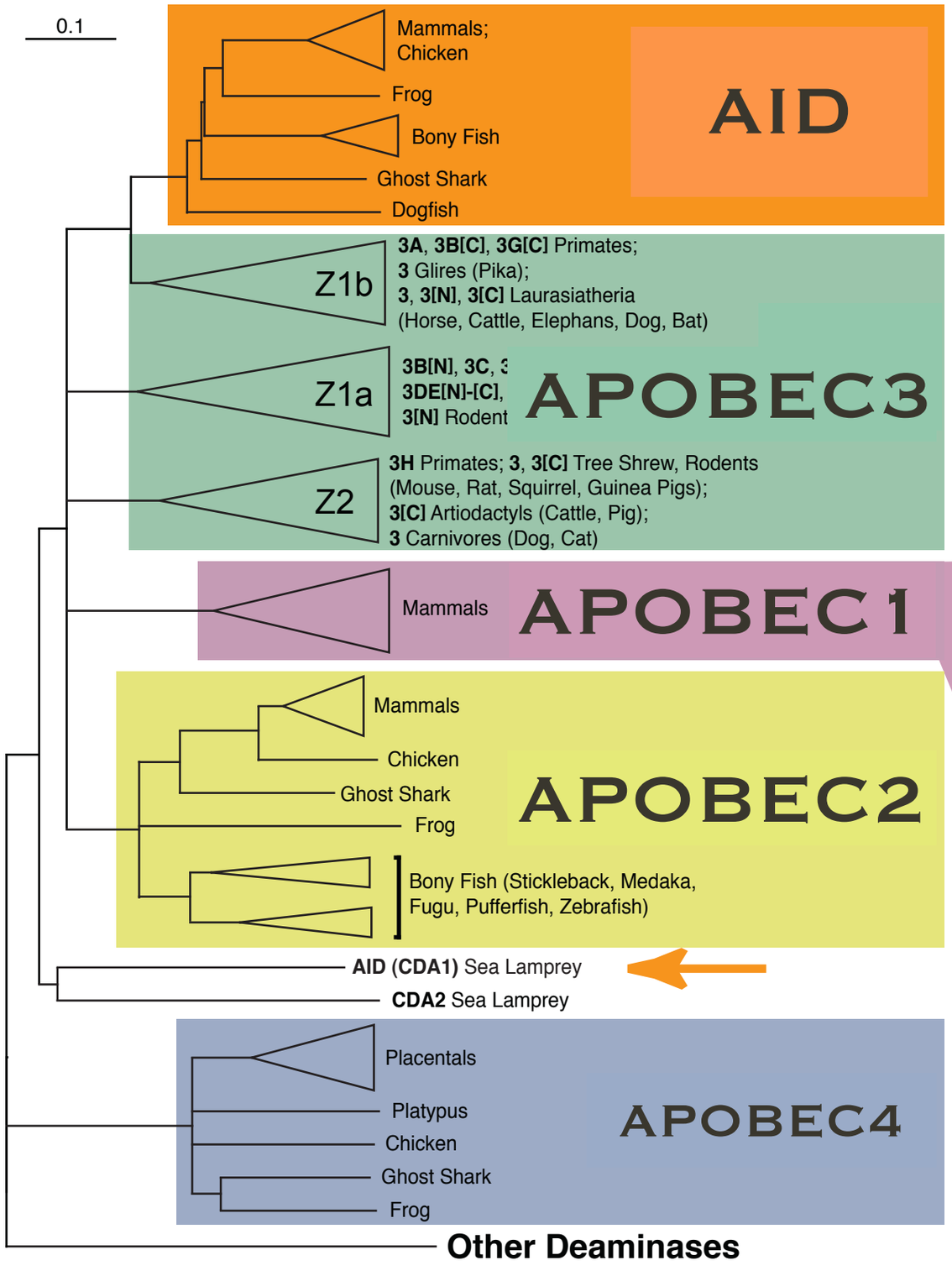
THE AID/APOBECs



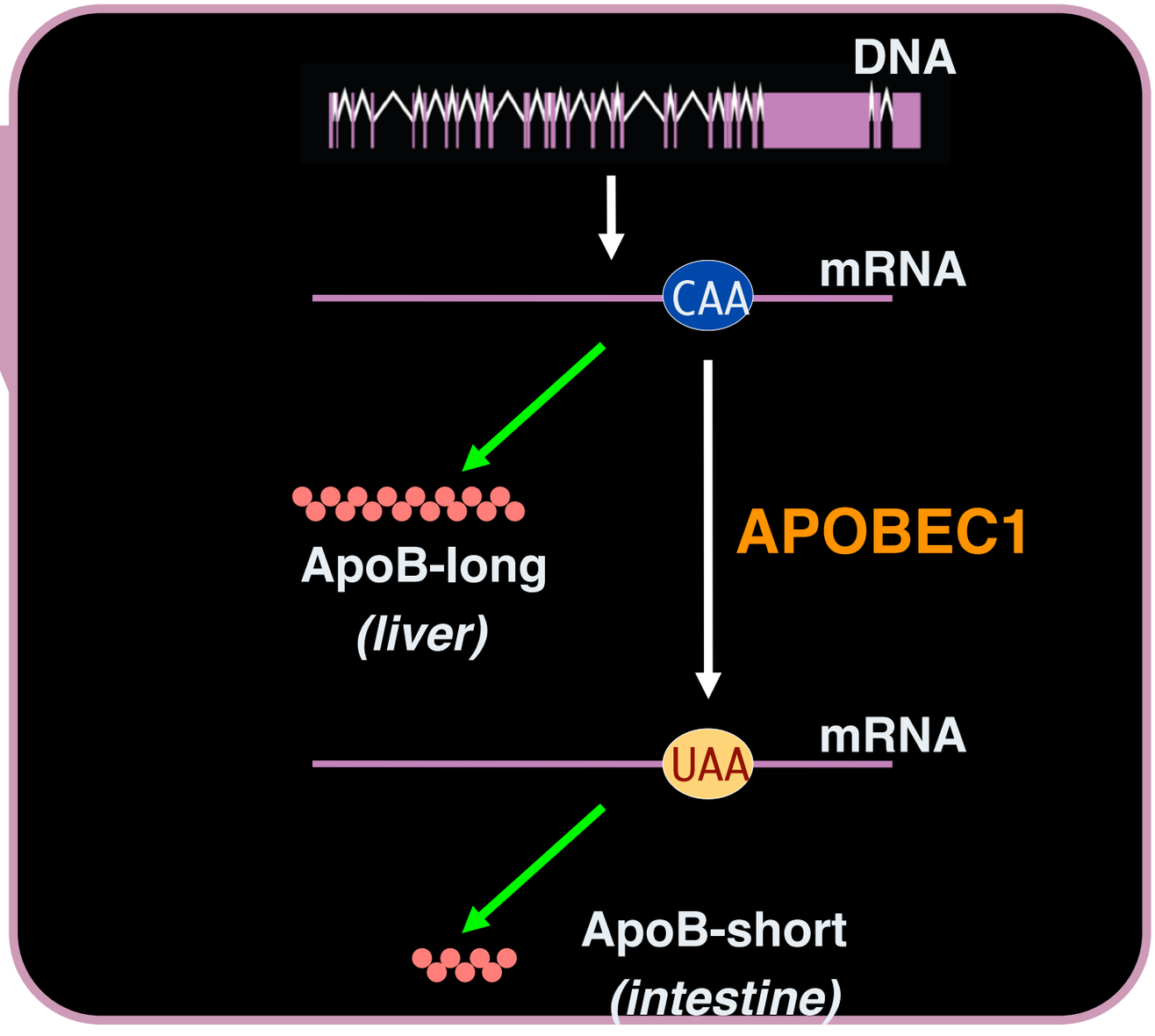
Innate Viral/Mobile Elements Restriction Pathway



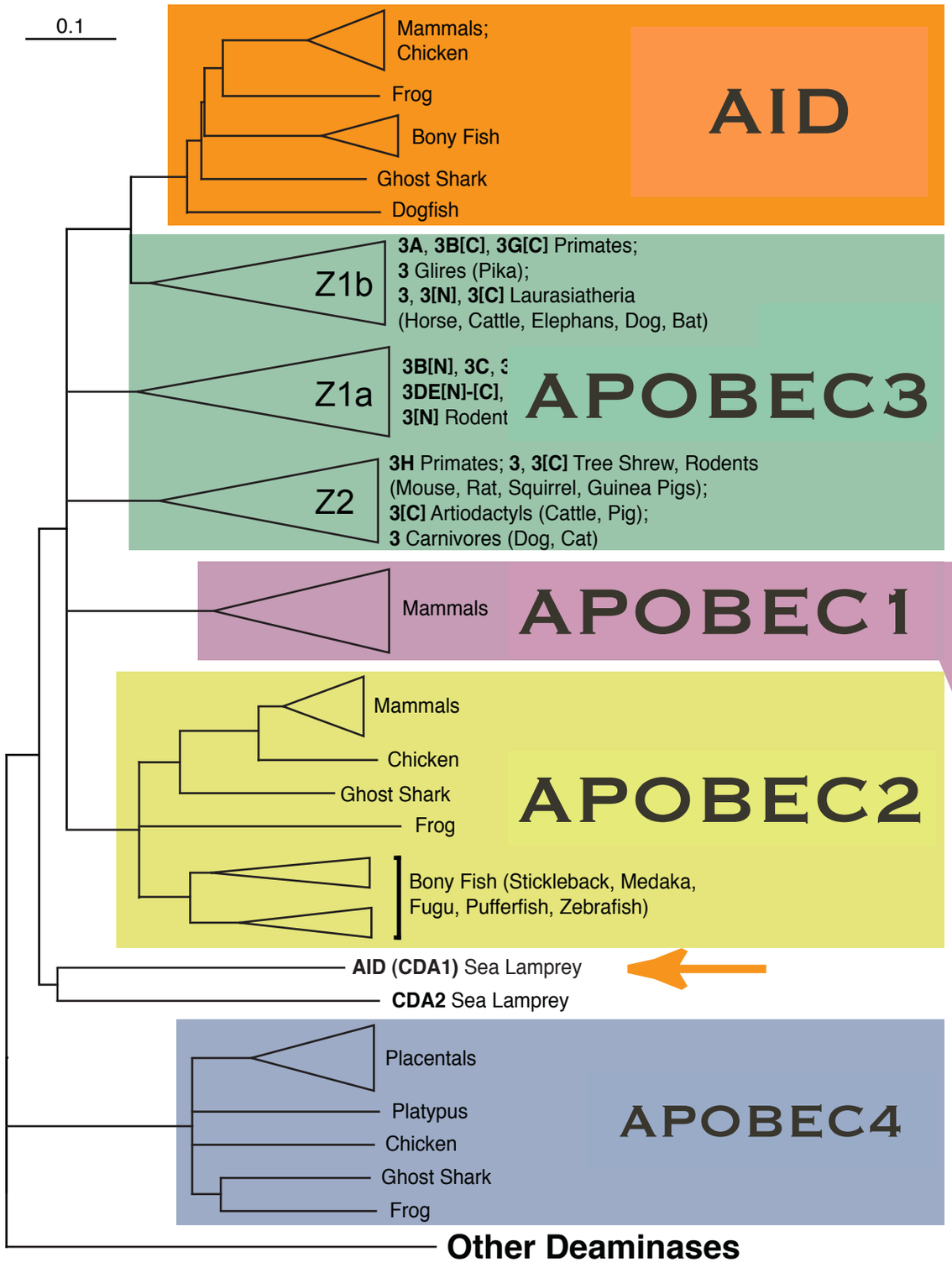
THE AID/APOBECs

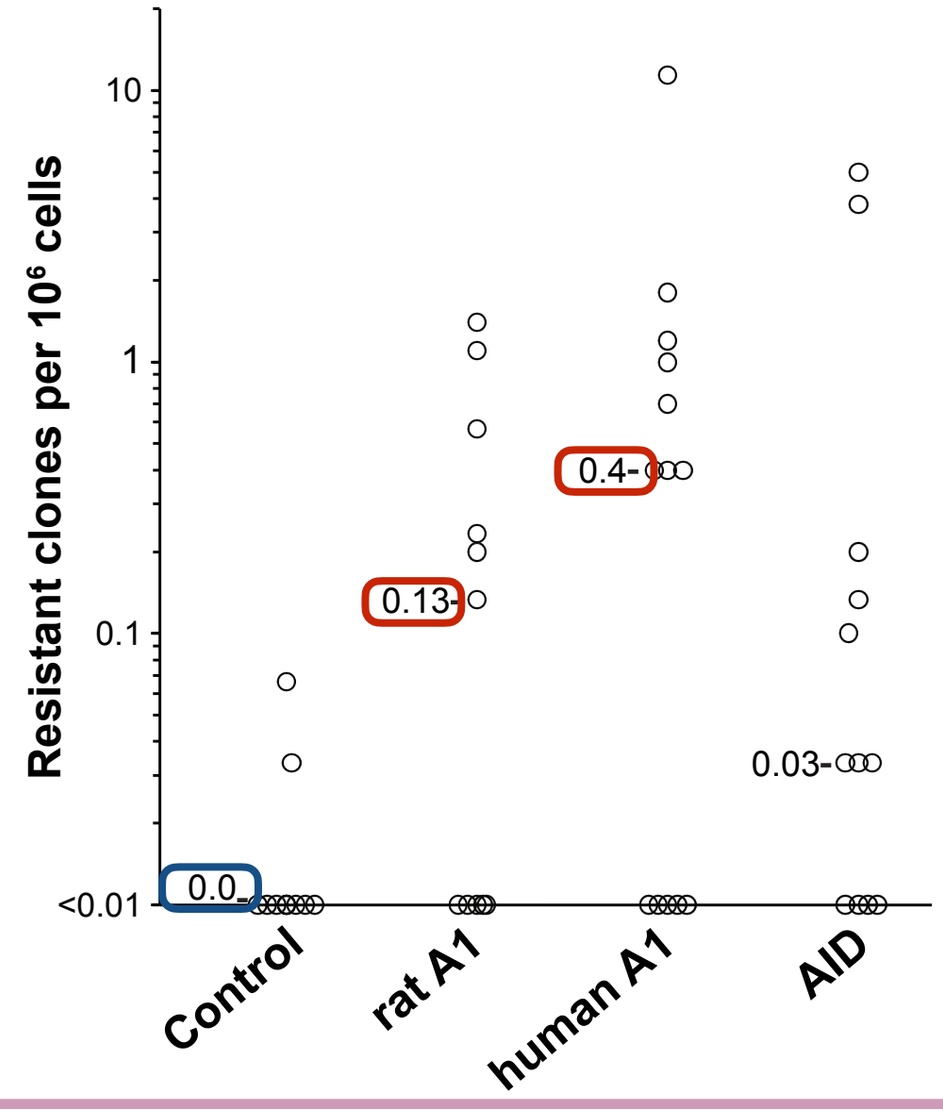


THE ODD ONE...



The Odd One...

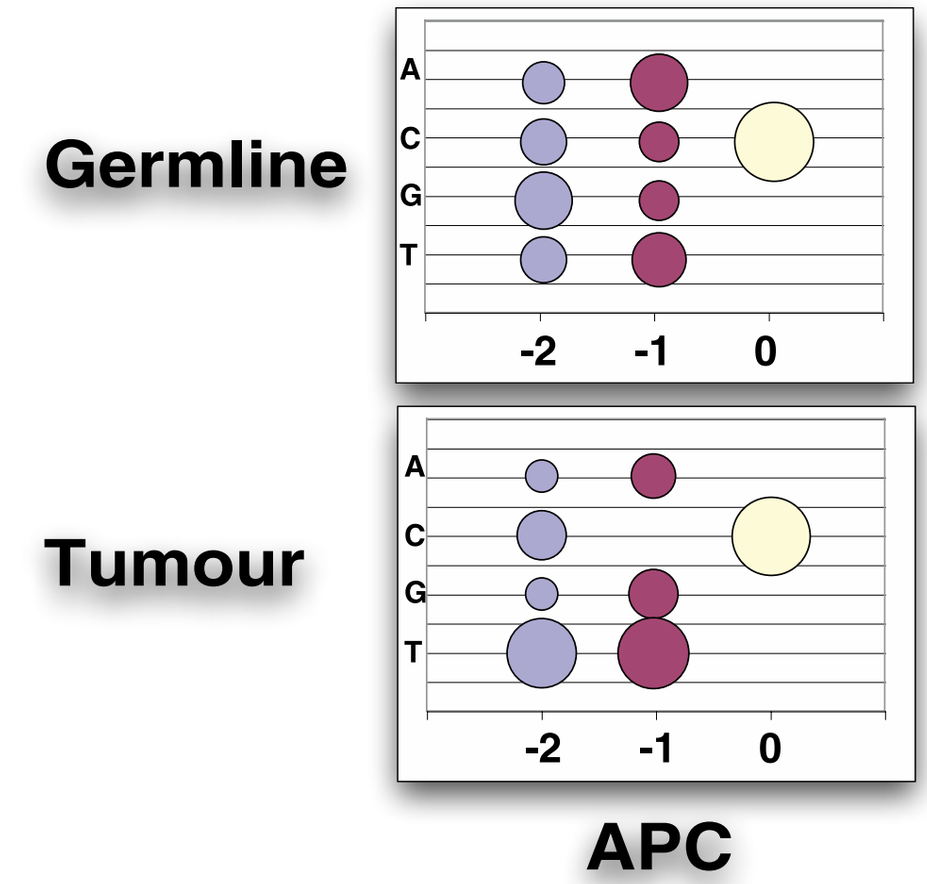

0.1
Mammals;
Chicken
Frog
Bony Fish
Ghost Shark
Dogfish
AID
Z1b
3A, 3B[C], 3G[C] Primates;
3 Glires (Pika);
3, 3[N], 3[C] Laurasiatheria
(Horse, Cattle, Elephans, Dog, Bat)
Z1a
3B[N], 3C, 3
3DE[N]-[C],
3[N] Rodent
APOBEC3
Z2
3H Primates; 3, 3[C] Tree Shrew, Rodents
(Mouse, Rat, Squirrel, Guinea Pigs);
3[C] Artiodactyls (Cattle, Pig);
3 Carnivores (Dog, Cat)
Mammals
APOBEC1
Mammals
Chicken
Ghost Shark
Frog
APOBEC2
Bony Fish (Stickleback, Medaka,
Fugu, Pufferfish, Zebrafish)
AID (CDA1) Sea Lamprey
CDA2 Sea Lamprey
Placentals
Platypus
Chicken
Ghost Shark
Frog
APOBEC4
Other Deaminases


Resistant clones per 10⁶ cells
10
1
0.1
<0.01
0.0
0.13
0.4
0.03
Control
rat A1
human A1
AID

APOBECs & Cancer

- **Mutational Signature in mutated p53 and APC genes**

Beale, 2004



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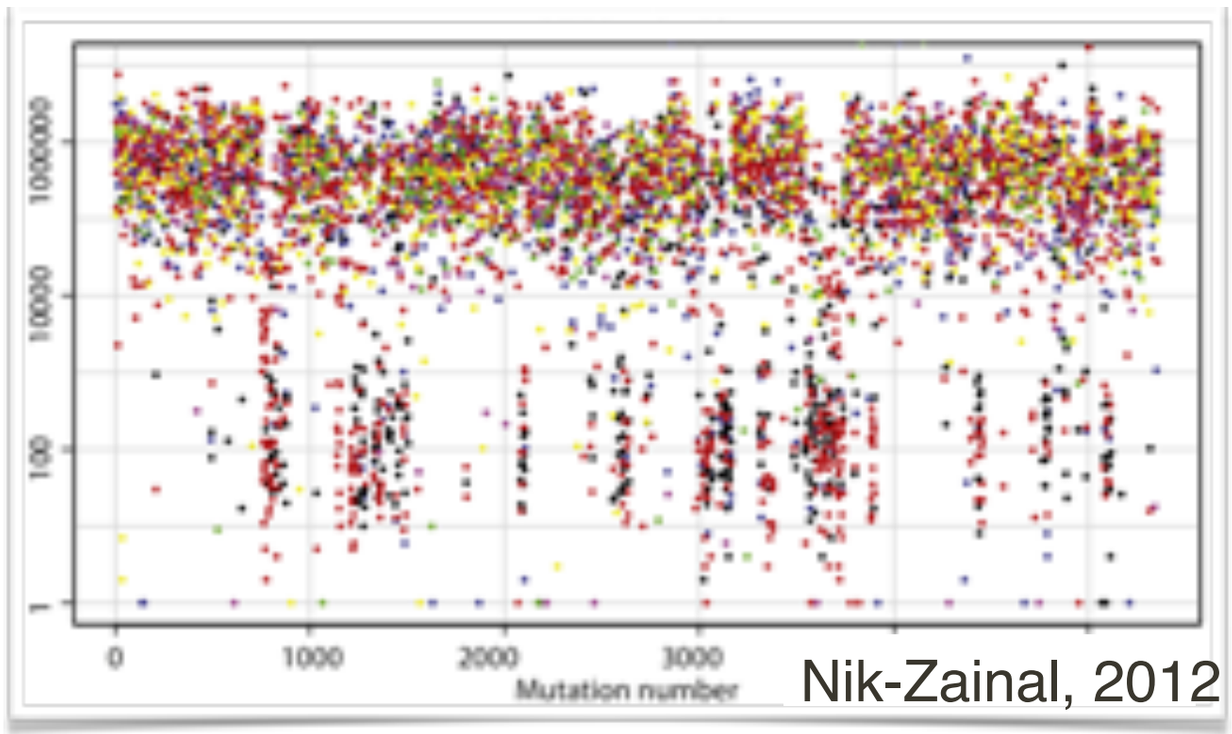
  Nik-Zainal, 2012; Roberts 2012; Taylor, 2013; ...



Nik-Zainal, 2012

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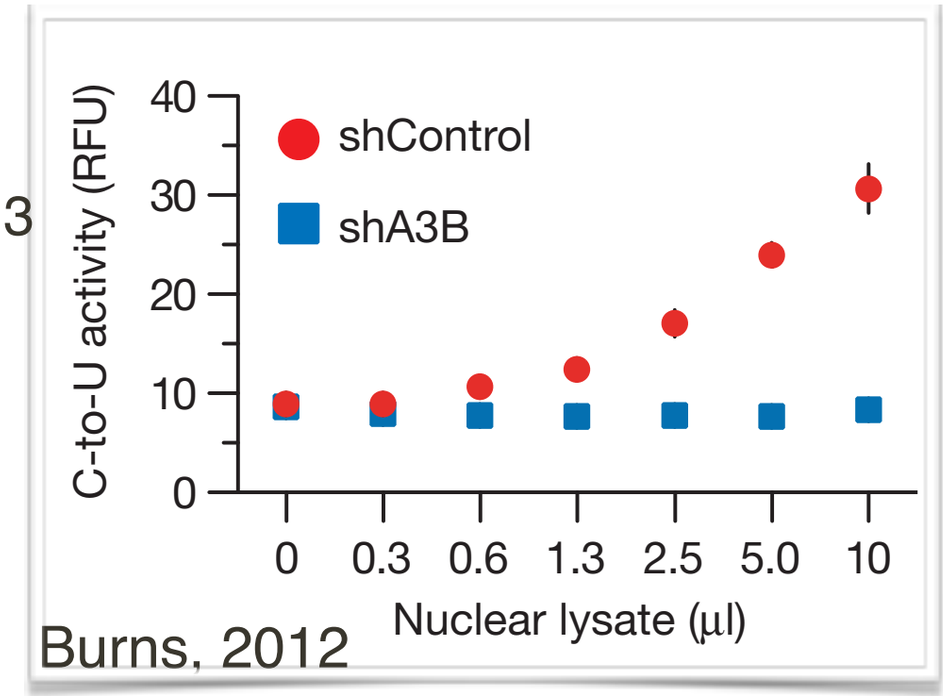

Burns, 2012

APOBECs & Cancer

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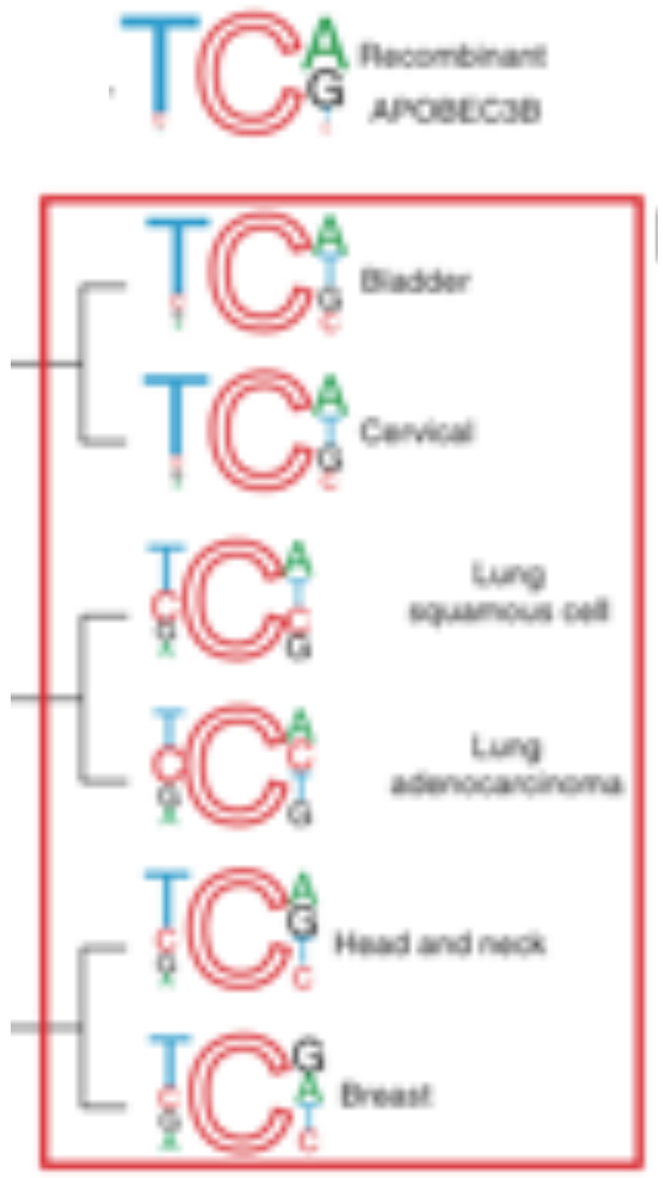
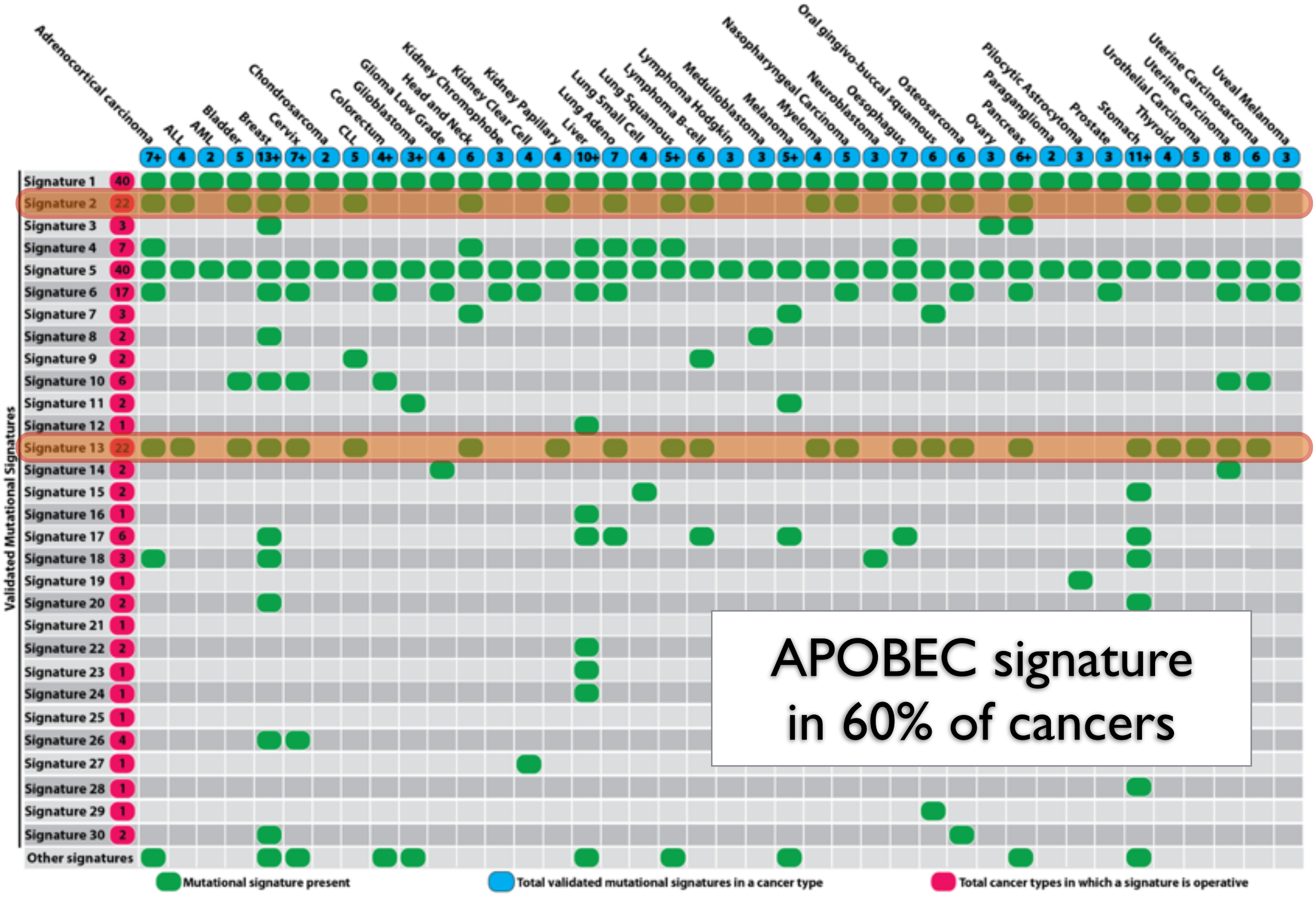
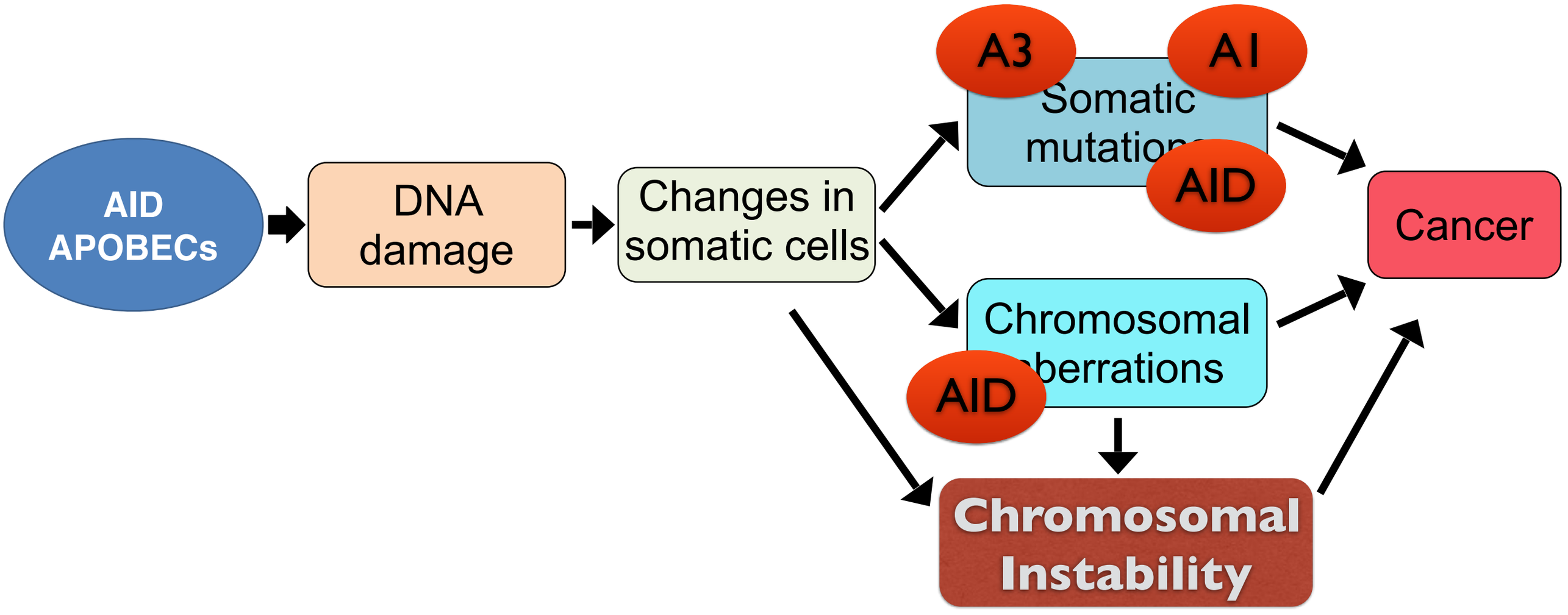
  Burns, 2013; Roberts 2013; Alexandrov, 2013; Saraconi 2014...

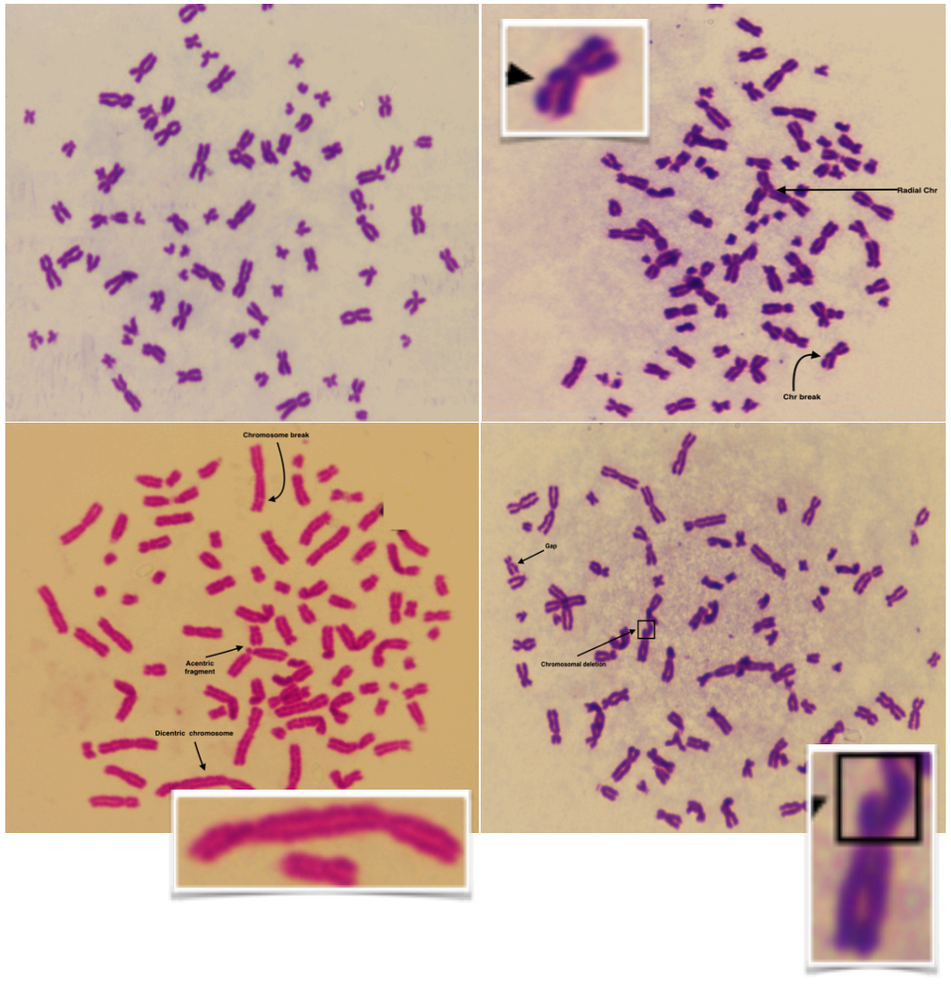


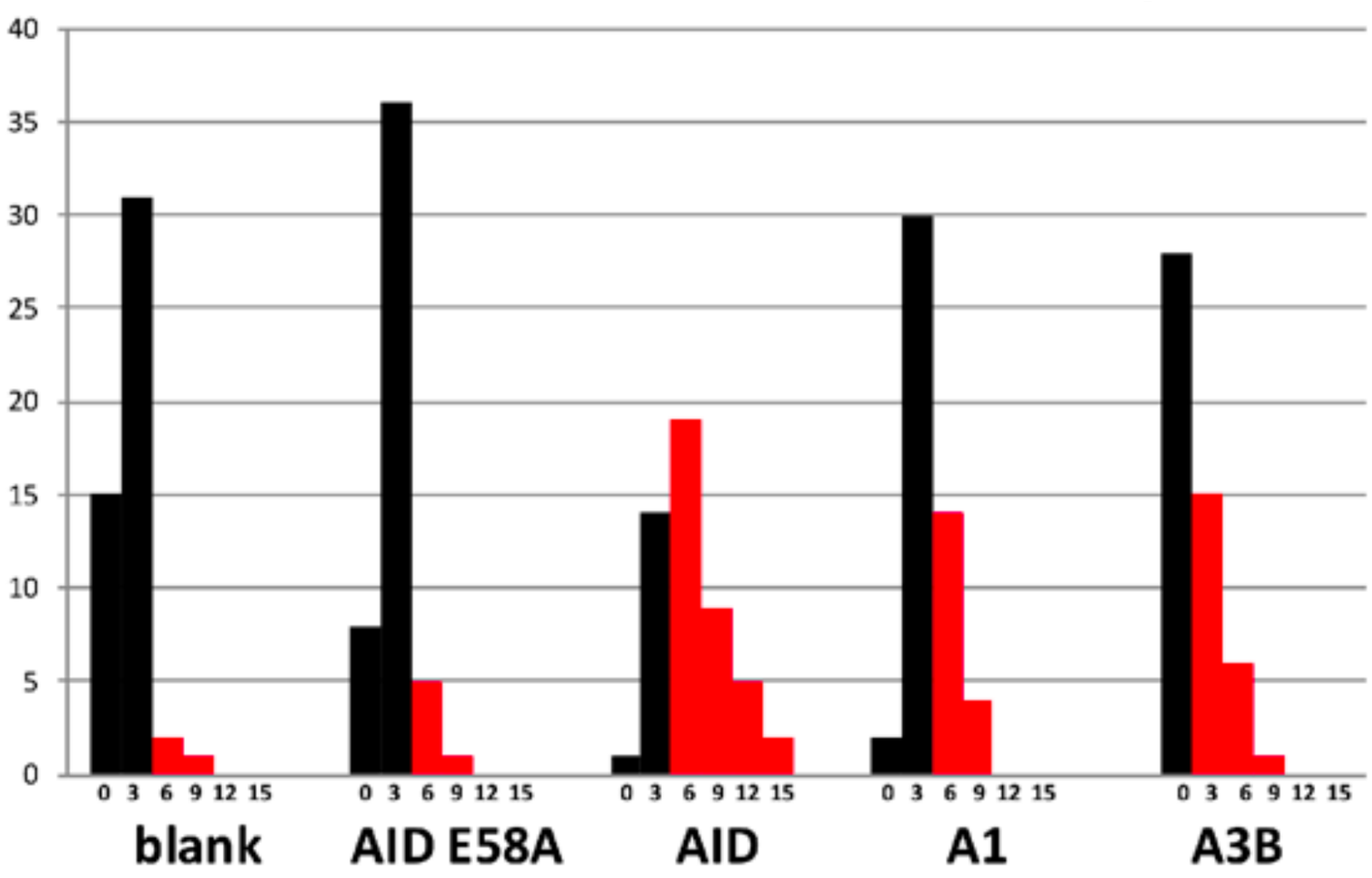
Burns, 2013


Adrenocortical carcinoma
ALL
AML
Bladder
Breast
Cervix
Chondrosarcoma
CLL
Colorectum
Glioblastoma
Glioma Low Grade
Head and Neck
Kidney Chromophobe
Kidney Clear Cell
Kidney Papillary
Liver
Lung Adeno
Lung Small Cell
Lung Squamous
Lymphoma B-cell
Lymphoma Hodgkin
Medulloblastoma
Melanoma
Nasopharyngeal Carcinoma
Myeloma
Neuroblastoma
Oesophagus
Oral gingivo-buccal squamous
Osteosarcoma
Ovary
Pancreas
Paraganglioma
Pilocytic Astrocytoma
Prostate
Stomach
Urothelial Carcinoma
Thyroid
Uterine Carcinoma
Uterine Carcinosarcoma
Uveal Melanoma
7+ 4 2 5 13+ 7+ 2 5 4+ 3+ 4 6 3 4 4 10+ 7 4 5+ 6 3 3 5+ 4 5 3 7 6 6 3 6+ 2 3 3 11+ 4 5 8 6 3
Signature 1 40
Signature 2 22
Signature 3 3
Signature 4 7
Signature 5 40
Signature 6 17
Signature 7 3
Signature 8 2
Signature 9 2
Signature 10 6
Signature 11 2
Signature 12 1
Signature 13 22
Signature 14 2
Signature 15 2
Signature 16 1
Signature 17 6
Signature 18 3
Signature 19 1
Signature 20 2
Signature 21 1
Signature 22 2
Signature 23 1
Signature 24 1
Signature 25 1
Signature 26 4
Signature 27 1
Signature 28 1
Signature 29 1
Signature 30 2
Other signatures
Validated Mutational Signatures
APOBEC signature
in 60% of cancers
Mutational signature present
Total validated mutational signatures in a cancer type
Total cancer types in which a signature is operative

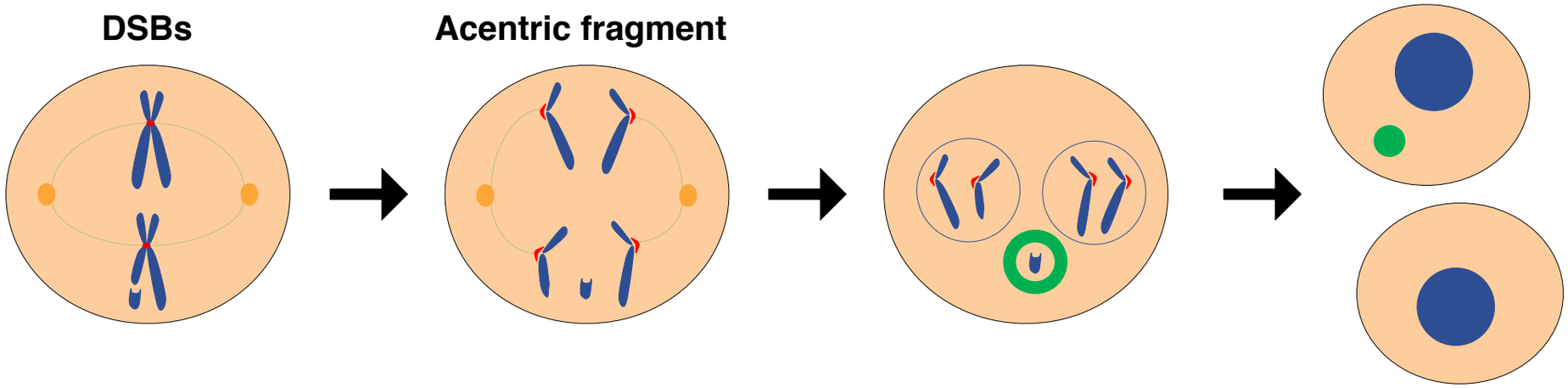

AID
APOBECs
DNA
damage
Changes in
somatic cells
A3
AI
Somatic
mutations
AID
Cancer
Chromosomal
aberrations
AID
Chromosomal
Instability

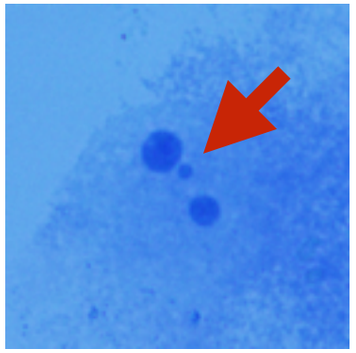
Chromosomal Aberrations

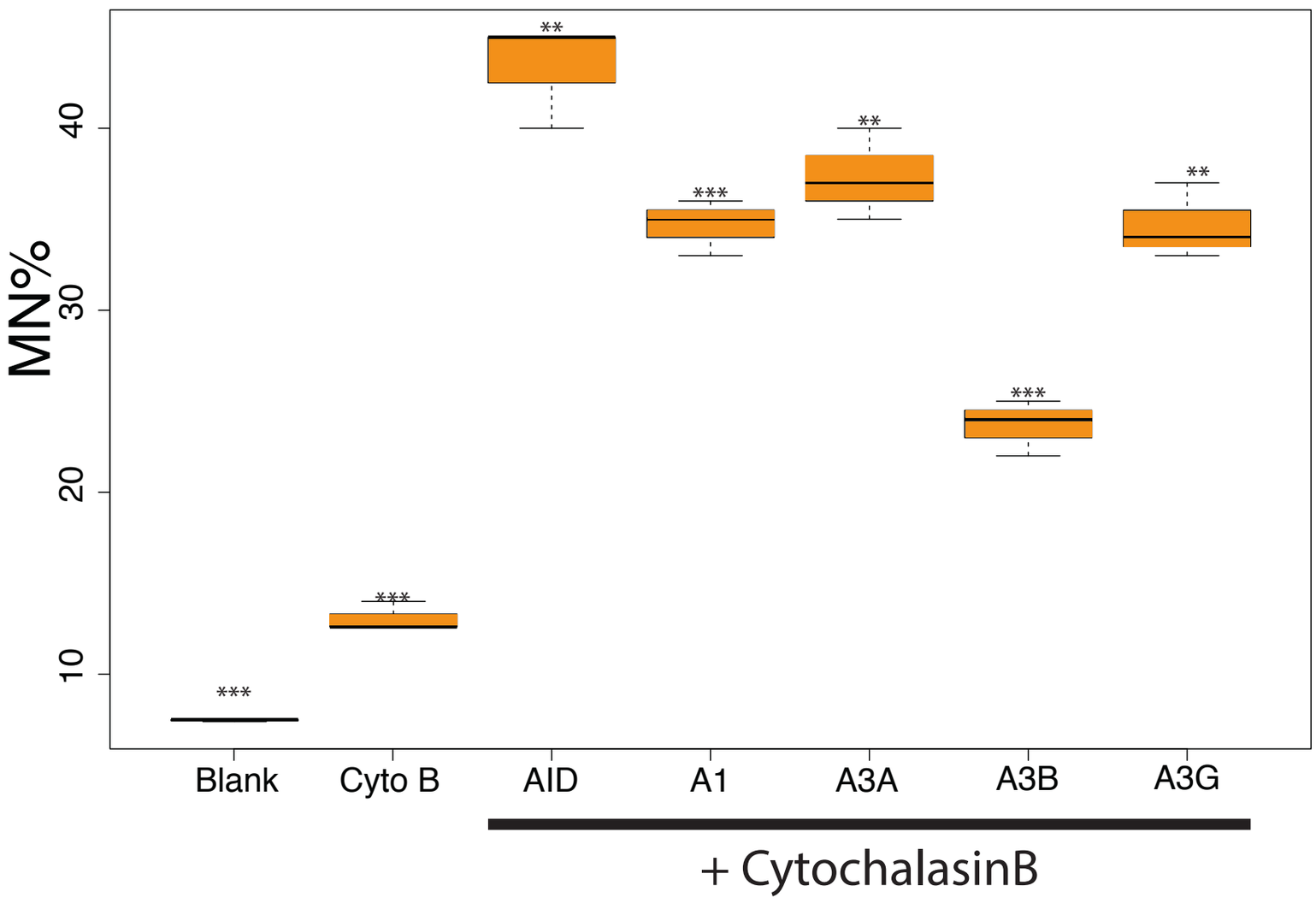




Micronuclei Formation







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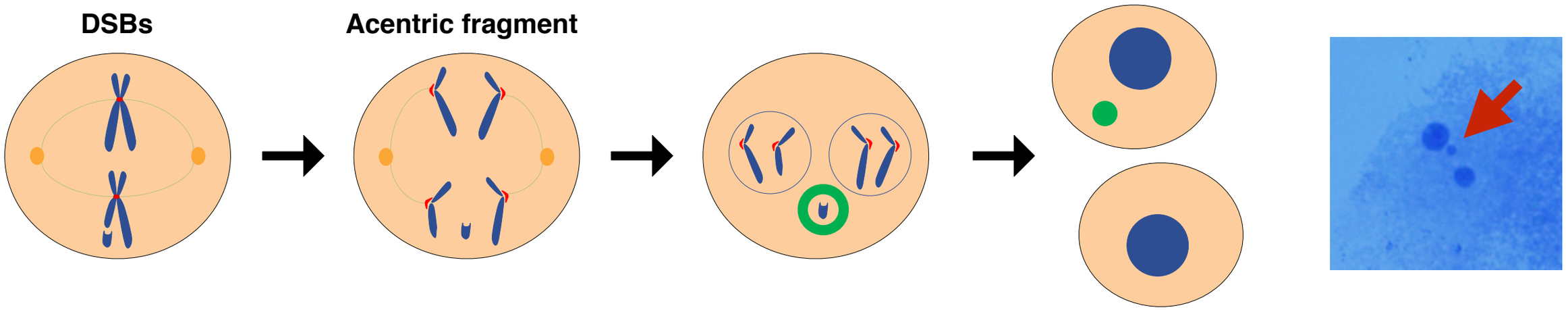
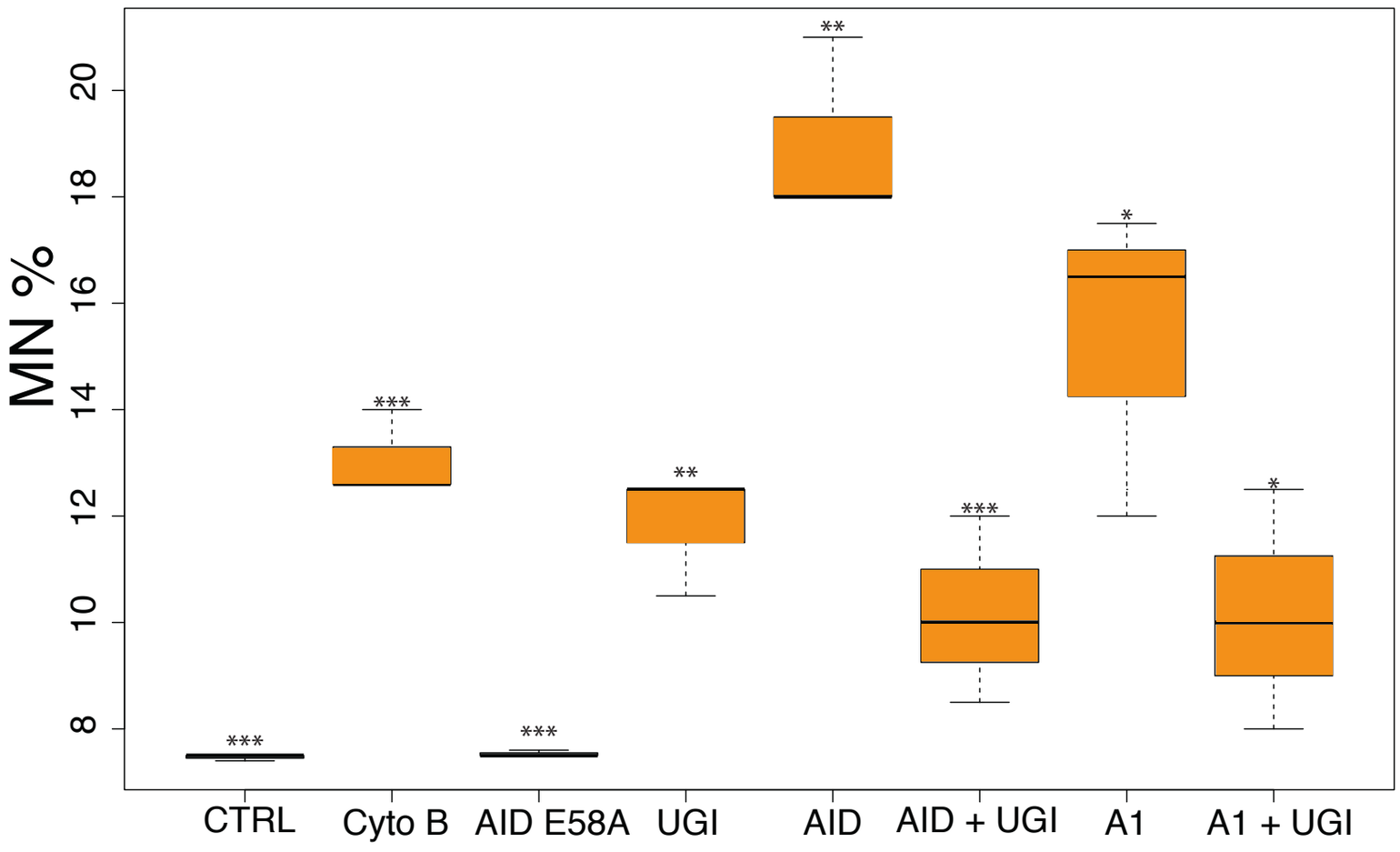
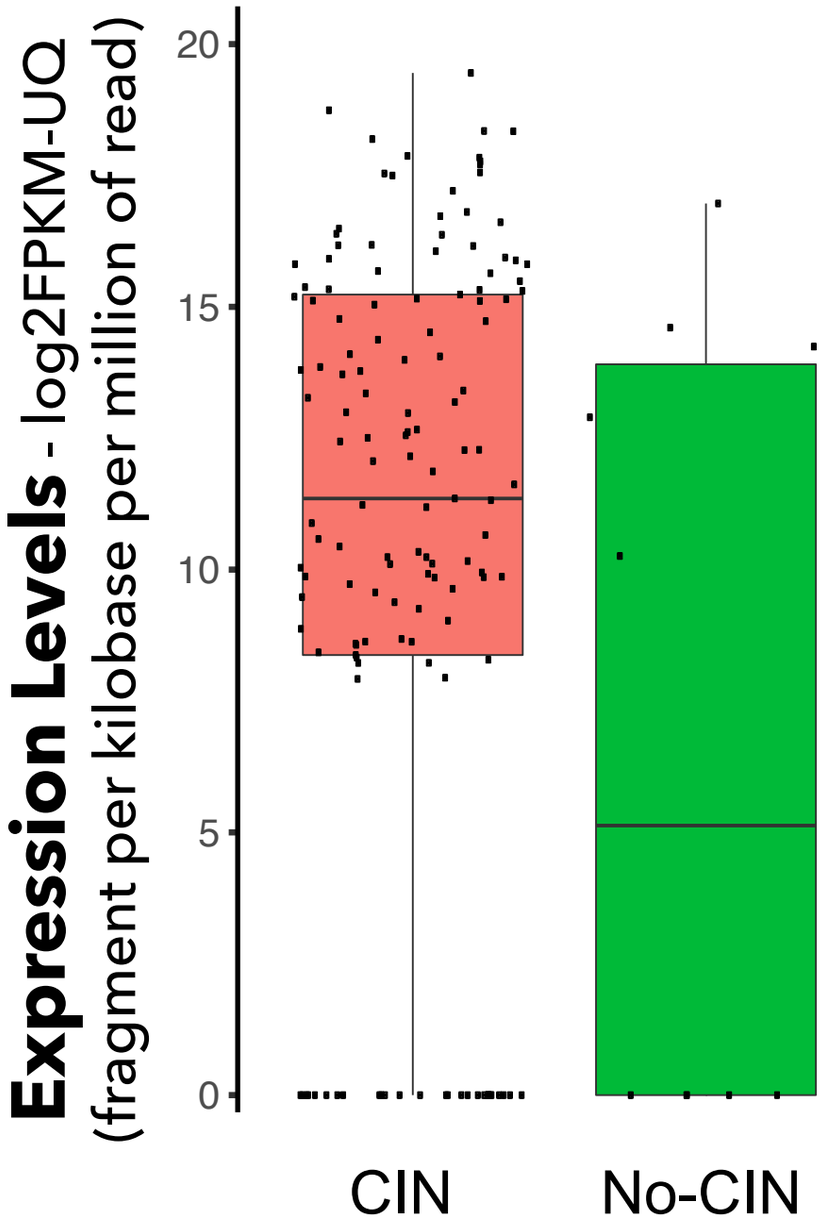


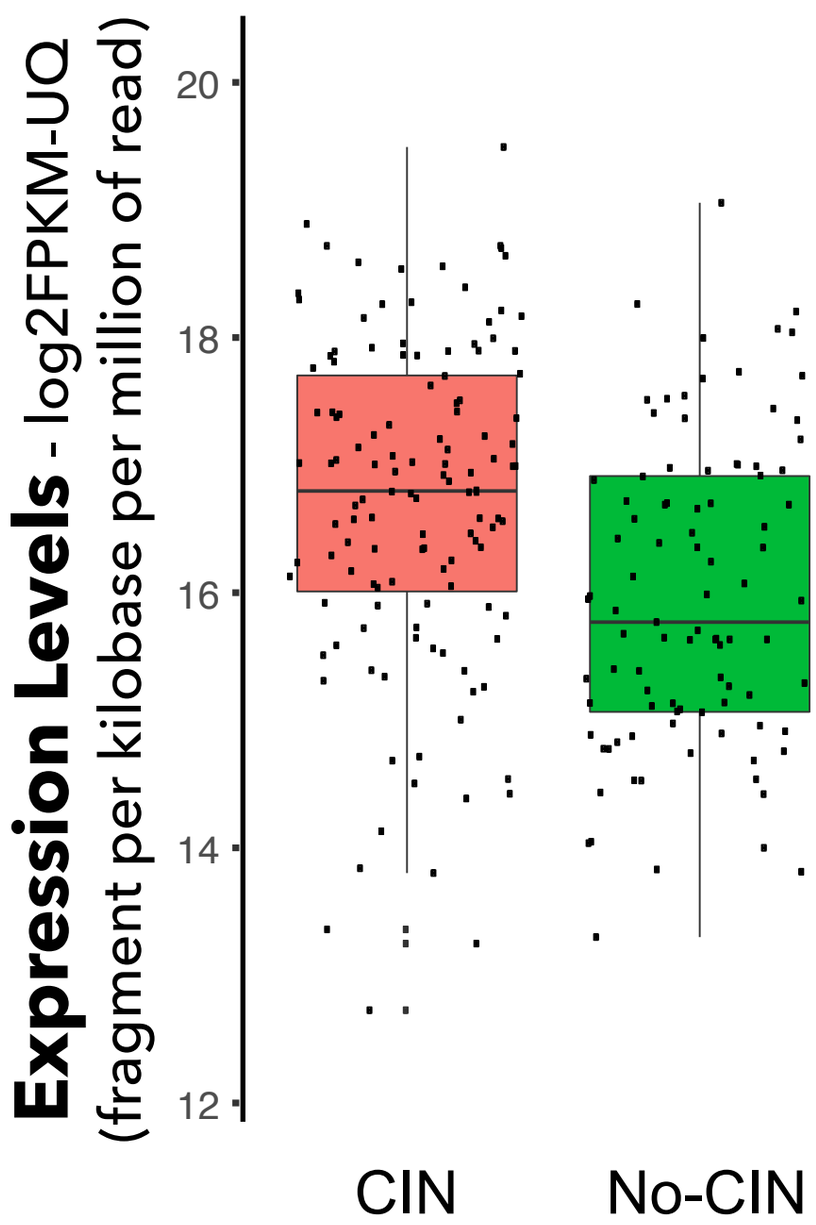


Correlation between CIN and APOBEC expression

Esophageal Adenocarcinoma



Stomach Cancer



$$M = \mu \times D$$

inheritance

chance

environment

genetic drift

negative

darwinian

selection

drive to growth

therapy

cancer

“nothing in ~~biology~~ makes sense except in the light of evolution”