The mutations that drive cancer

Paul Edwards

Department of Pathology, Hutchison-MRC centre
and Cancer Research UK Cambridge Institute, University of Cambridge

Previously on Cancer.....

- The 'Hallmarks of Cancer'

(Hanahan & Weinberg, 2000, revised 2011)

Abnormal proliferation and survival control:

- Independence of positive growth signals
- Resistance to negative signals
- Resistance to Apoptosis, cycle arrest
- Abnormal (often blocked) Differentiation
- Immortality/resistance to stress arrest

Genetic Instability

~~**Metastasis ?**~~

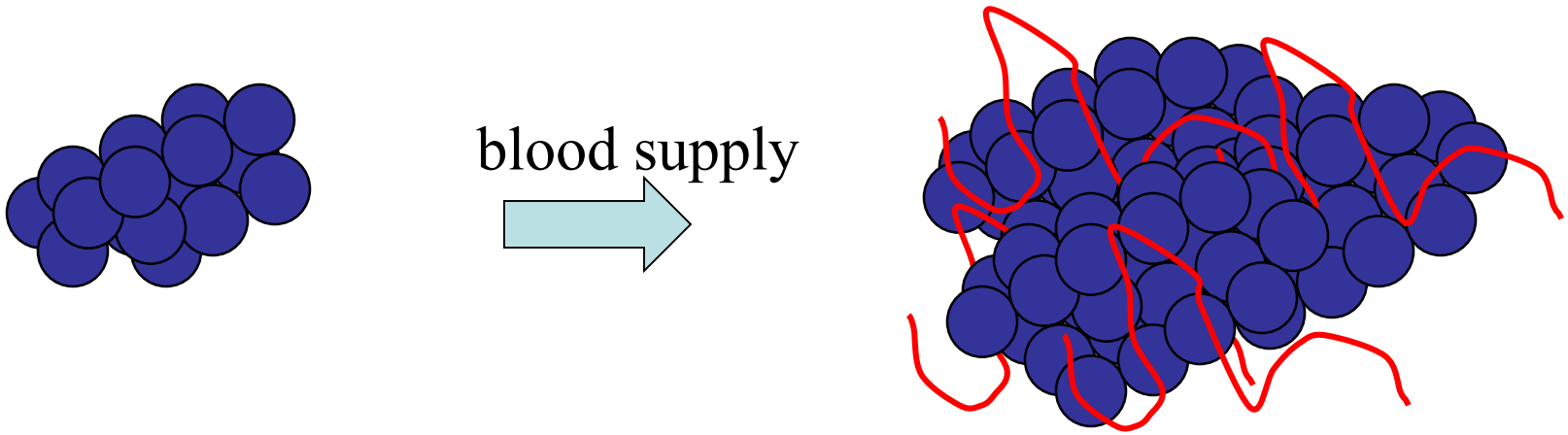
Angiogenesis ?

Metabolic changes?

Immune response?

Angiogenesis?

Tumours need new blood supply to expand.



The Angiogenesis proposition (Judah Folkman, championed by Hanahan)

Tumours need to make angiogenic factors

BUT
Normal cells make angiogenic factors when needed. Do they need anything more?
Anyway – potential therapy target

Angiogenesis

The Angiogenesis proposition: (Judah Folkman, followed up by Hanahan)

…that tumours have to elicit new blood vessel formation in order to grow and perhaps spread, and therefore they have to produce angiogenic factors

My opinion:

Important to distinguish between angiogenesis as a natural response to tumour need (which presumably occurs) and abnormal property of tumour cells (which remains to be clearly established)

In animal models increased angiogenesis can be associated with onset of malignancy, but is this a normal response by vasculature to increased tumour turnover or abnormal acquired property of tumour?

i.e. not at all understood.

Malignant cells have acquired a number of properties

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- **Genetic Instability**
- **Metastasis ?**
- ***Angiogenesis ?***
- ***Metabolic changes?***
- **Immune response?**

Is there an immune response to tumours?

Profoundly controversial

Long and dismal history of artefact and confusion

Never much evidence that the immune response restrains tumour growth

- immune suppressed humans and mice do not get much more cancer, and most of this is virus-induced*

- no evidence of selection against neoantigens**

- BUT therapy that blocks self-tolerance <u>sometimes</u> works

- produces 'autoimmune' attack on some tumours with very high mutation burden

???? Immmune system is tolerised to most tumours,

but tolerance fails for a few hypermutated tumours ???

**Engels EA et al (2011) JAMA **Martincorena et al (2017) Cell*

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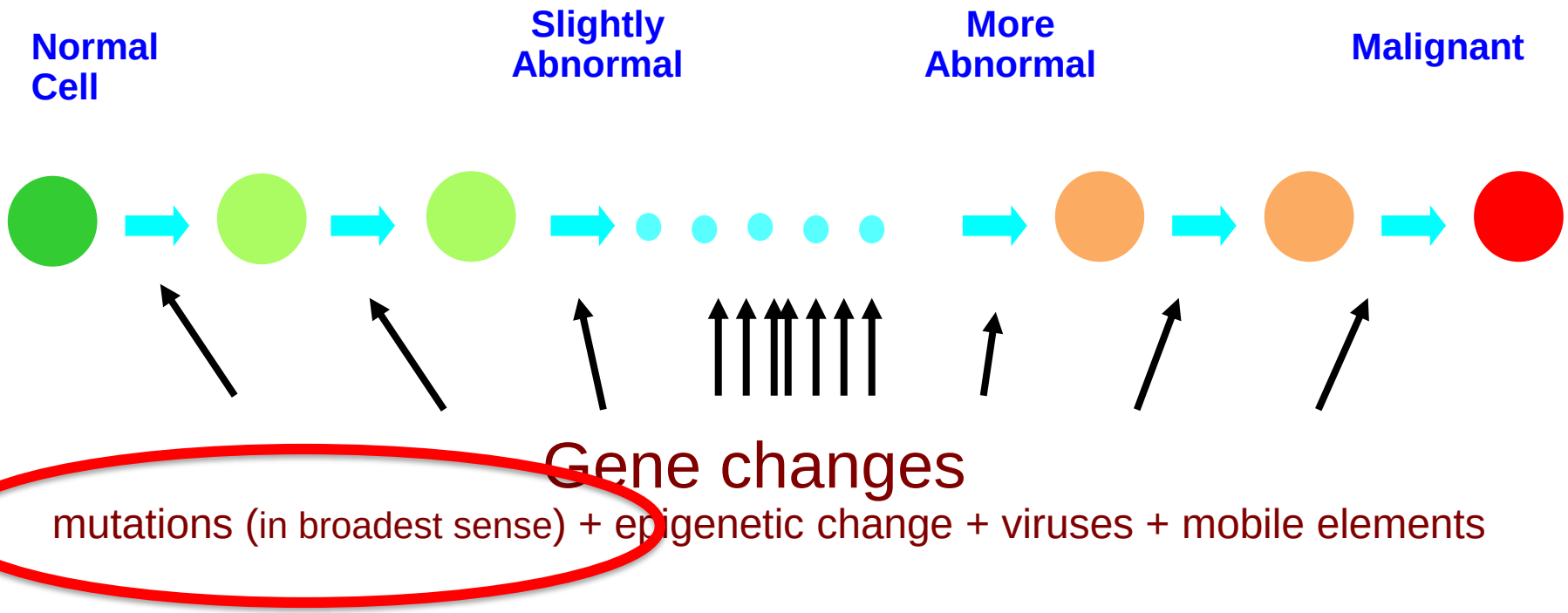
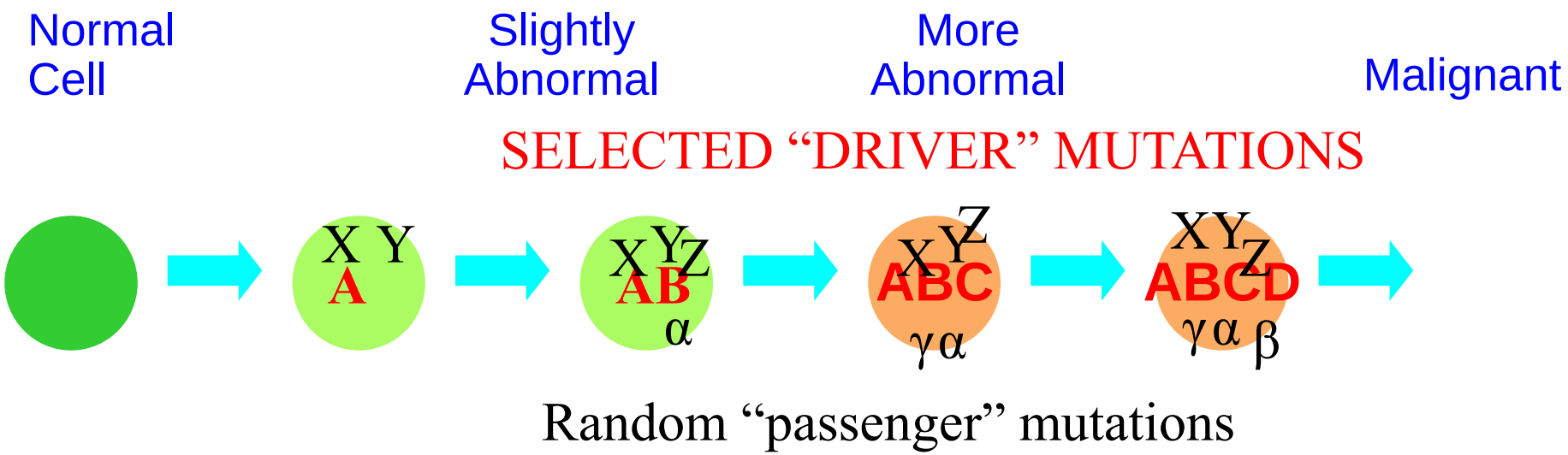
- **Metastasis ?**
- **Angiogenesis ?**
- **Metabolic changes?**
- **Immune response?**

None of these are really understood

Previously on Cancer.....



Passenger versus Driver Mutations



How many genes are altered in cancer?

**Martincorena et al (2017) Cell*

$$\text{Ratio} \quad \frac{\text{mutations that alter amino acids}}{\text{mutations that don't}} \quad \Rightarrow \quad \text{selection}$$

-> ~10 small mutations selected in colorectal cancer

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**also:

- only about half of these small mutations have been found so far
- no selection against new proteins (no selection by immune system)

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** Pancancer summary paper, in press*

+ around 3-4 structural mutations

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+ around 3-4 structural mutations

=> more than 10 mutated genes/carcinoma

* also,

colorectal	~ 3X	more **small** mutations than **structural**
breast, ovary	~ 3X	more **structural** mutations than **small**

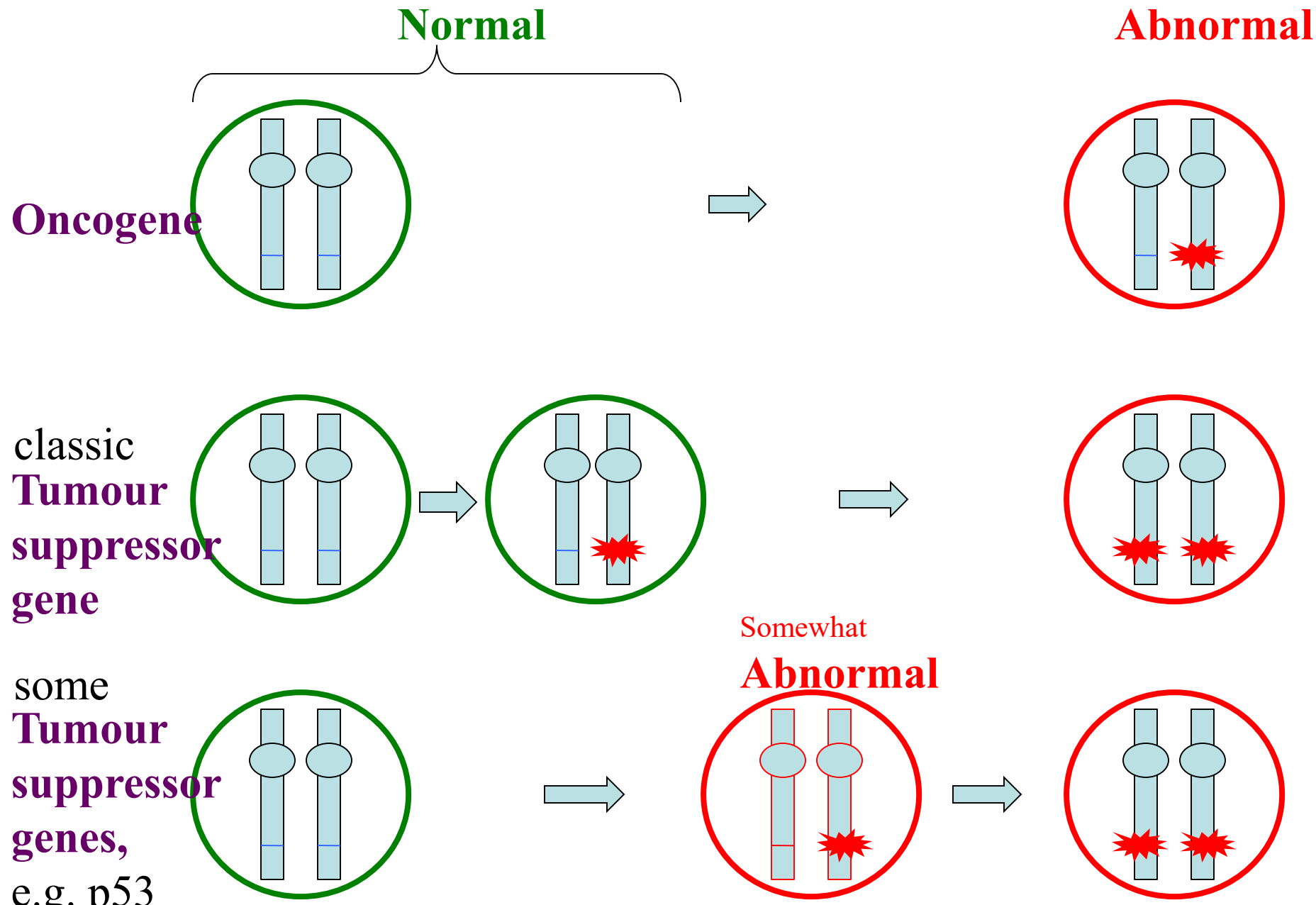
Oncogenes and Tumour Suppressor genes

Definitions vary but one is:

Oncogene mutations are overactivity mutations

Tumour Supppressor Gene mutations are loss of function mutations

Oncogenes versus Tumour Suppressor Genes



Oncogenes and Tumour Suppressor genes

Definitions vary but one is:

Oncogene mutations are overactivity mutations

- dominant in the cell, I.e. only one copy mutated

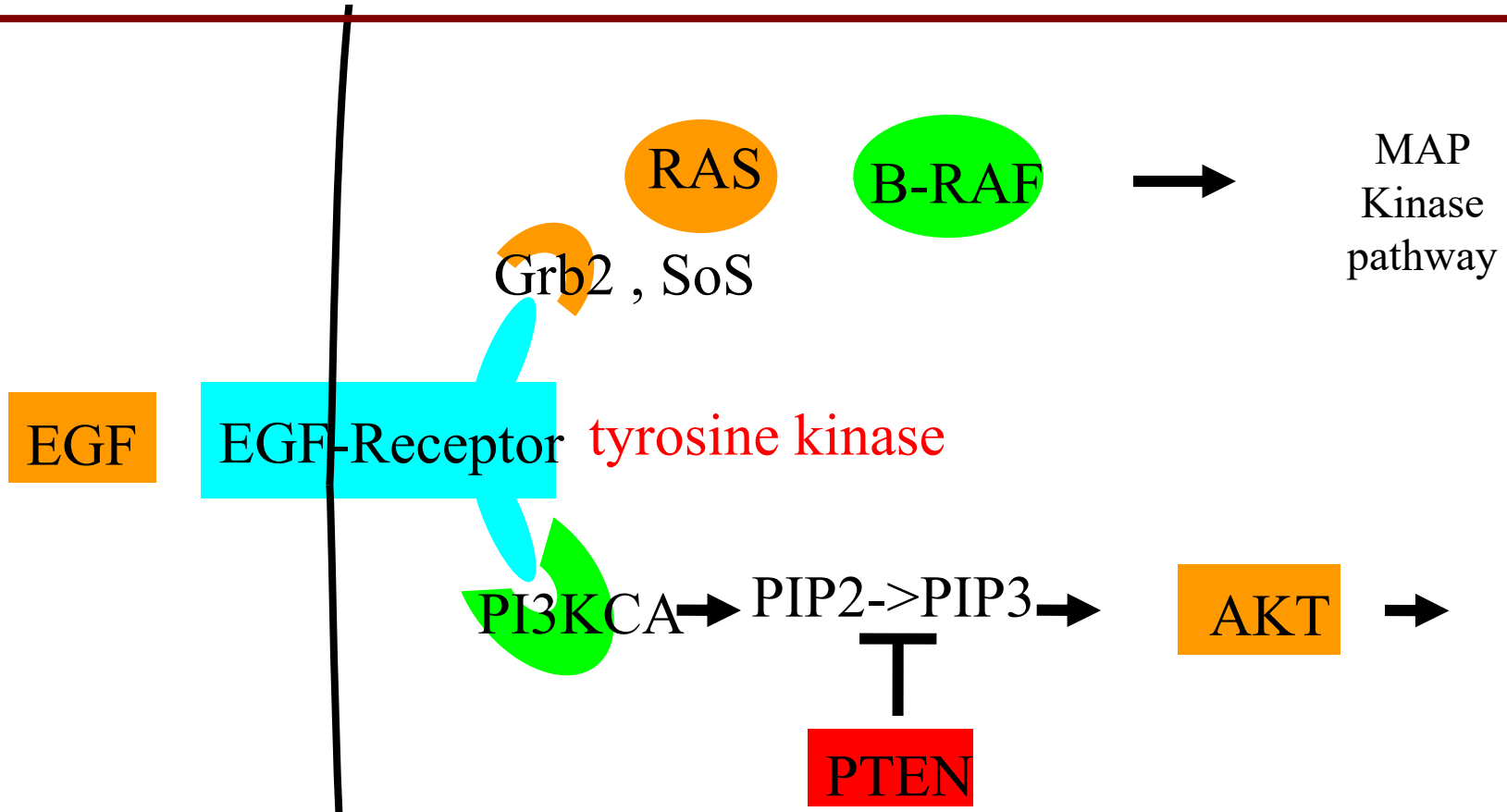
Tumour Supppressor Gene mutations are loss of function mutations

- generally both copies are mutated, recessive in the cell

Examples of mutations

Tyrosine kinases and signalling downstream from them

Receptor Tyrosine Kinase (RTK) signalling pathways



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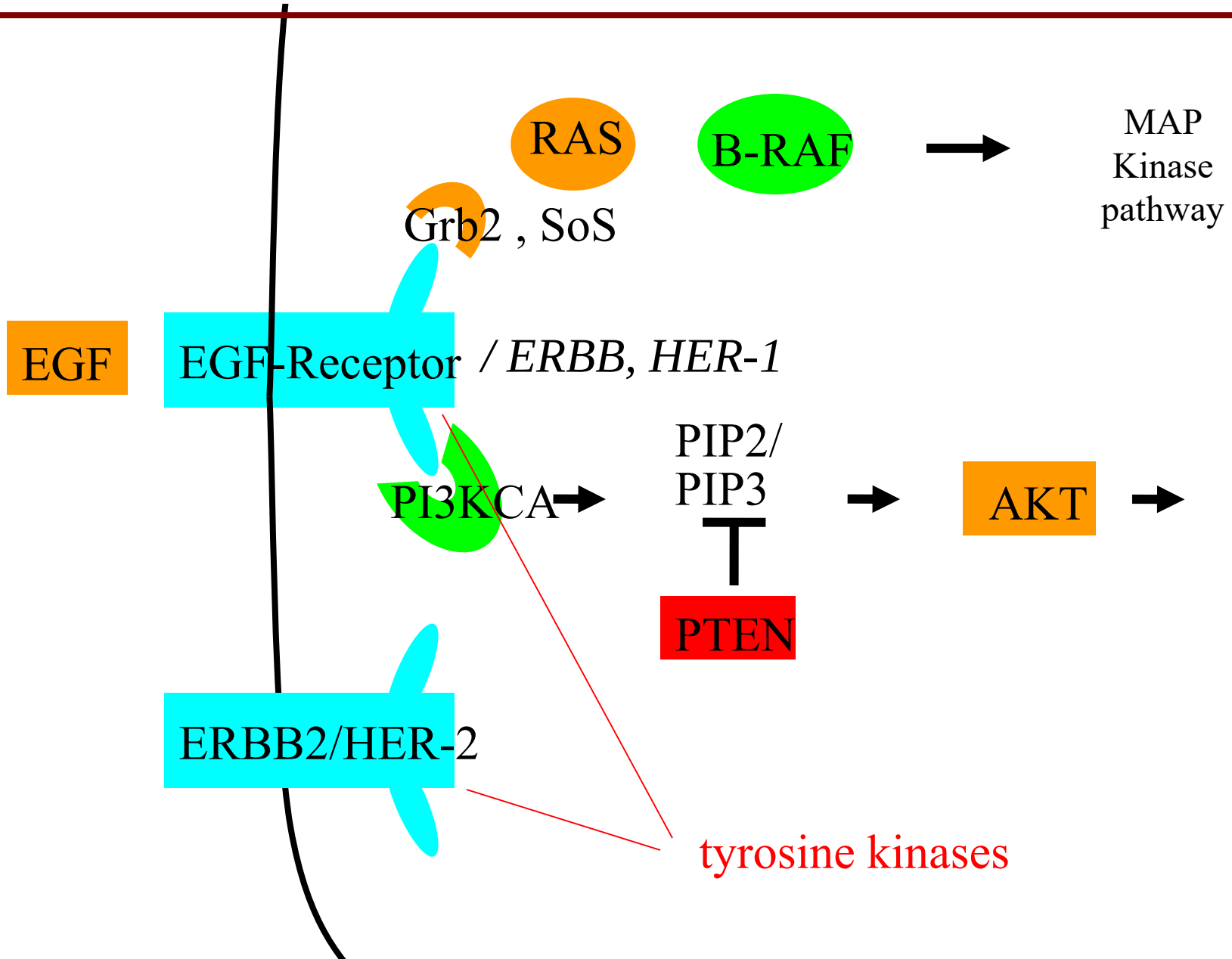
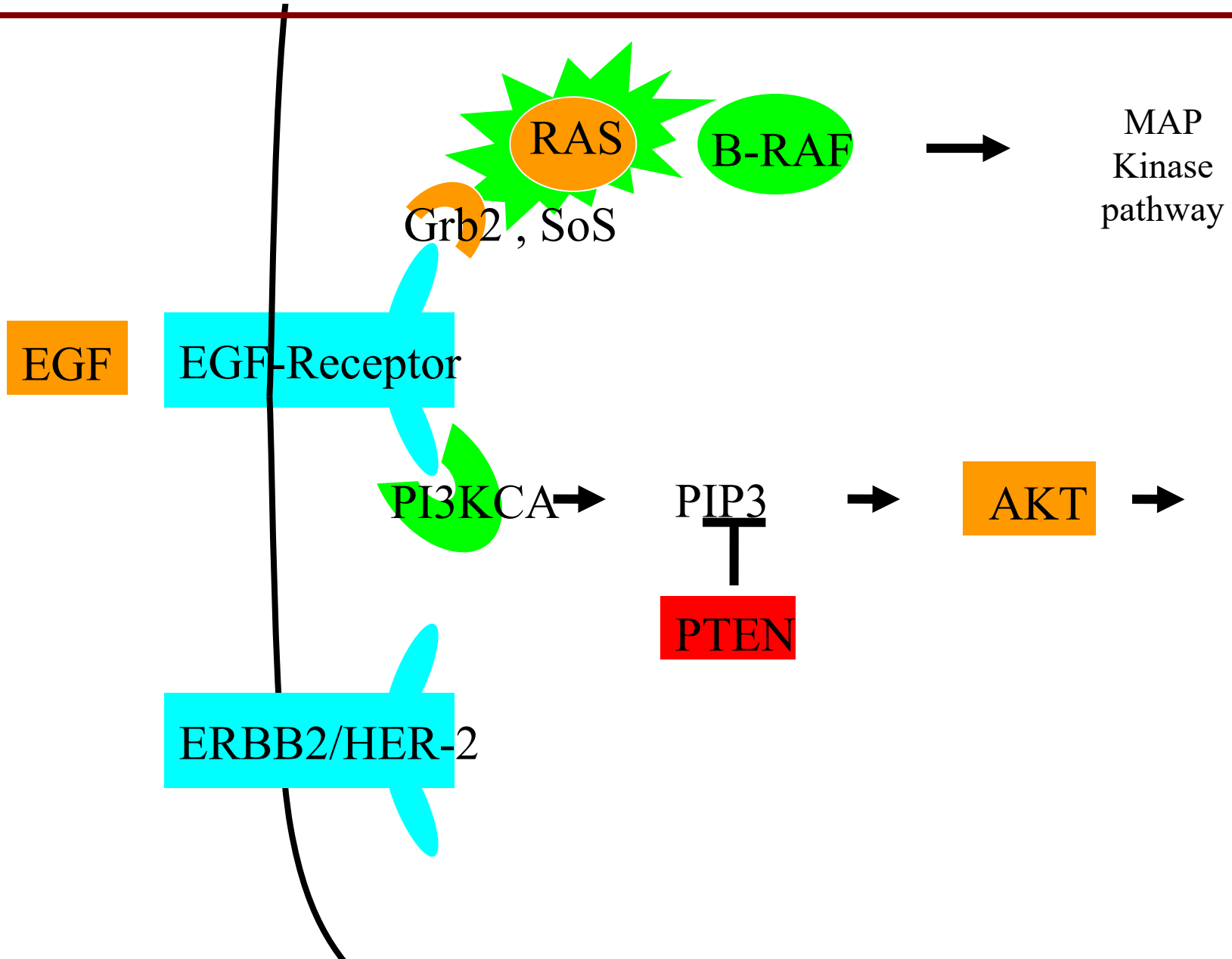


POINT Mutations in RTK signalling pathways



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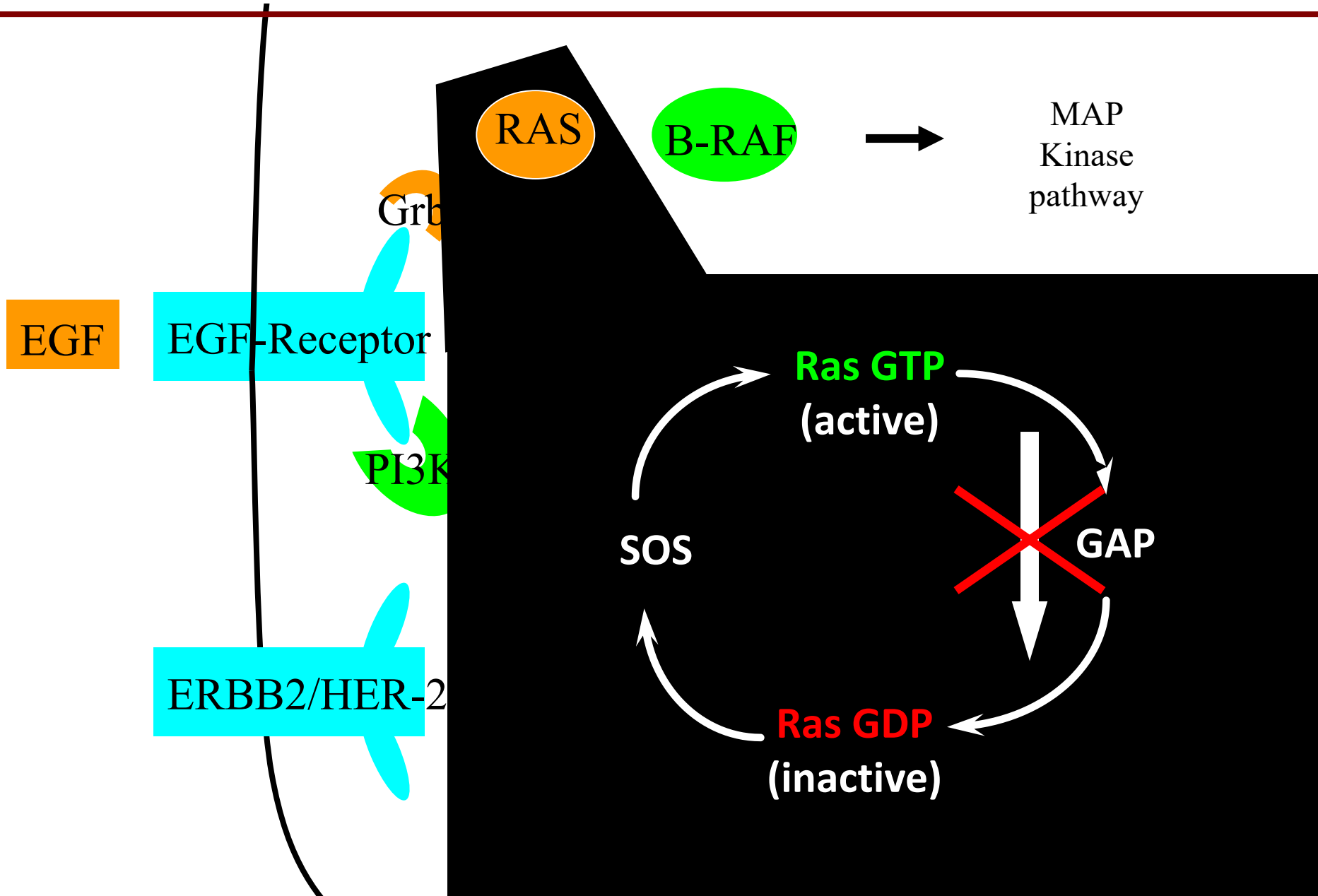

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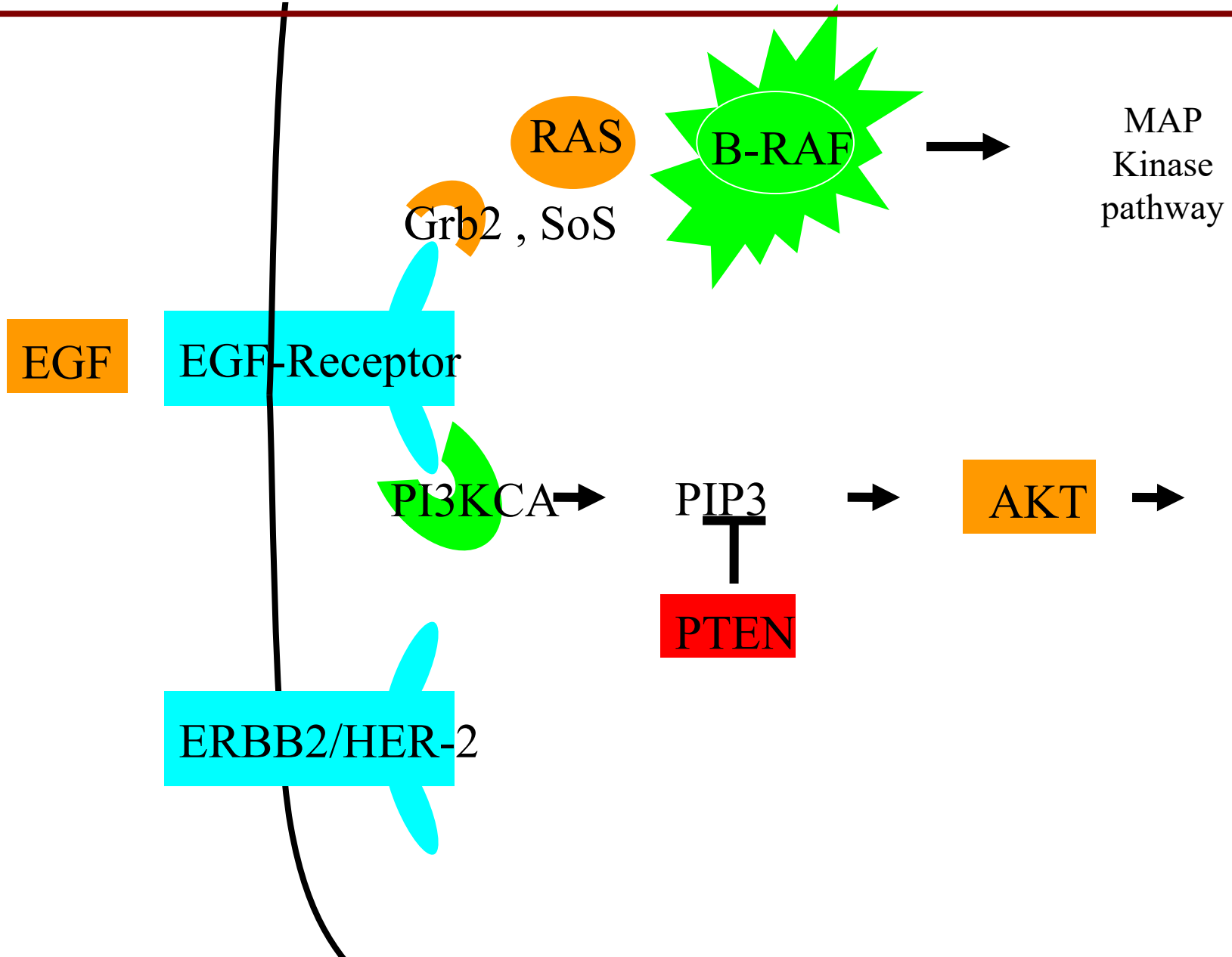
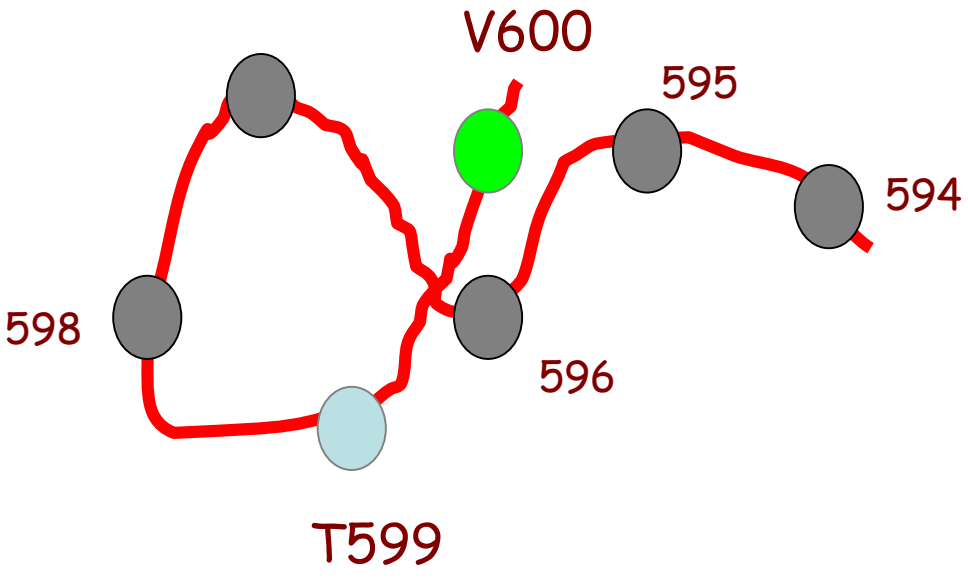


BRAF V600E activation

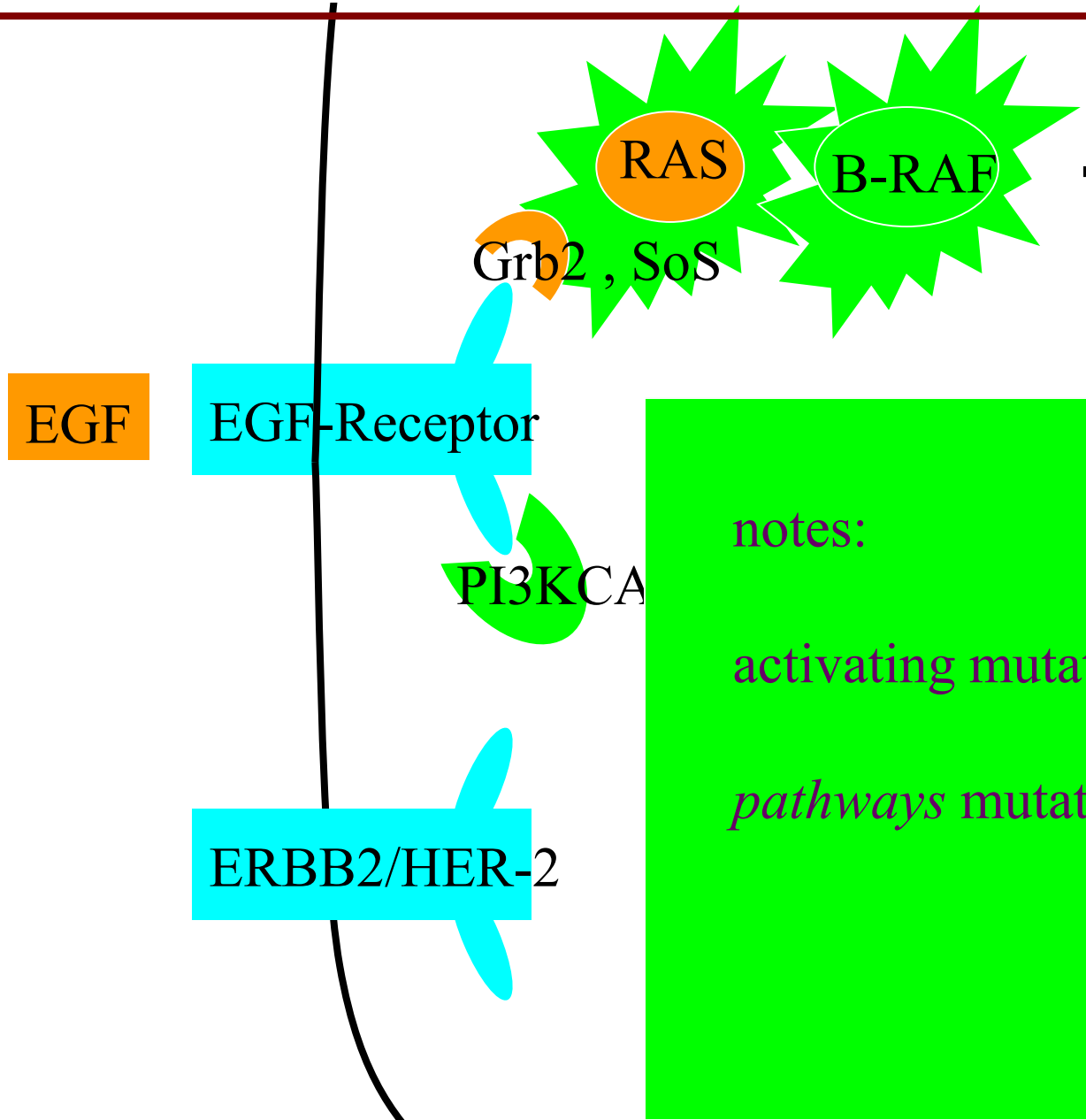
valine ⇨ glutamic acid



Activation segment is thought to move when Thr599 is phosphorylated, activating kinase. V600E where valine replaced by Gutamic acid doesn't need phosphorylation.

Wan et al, 2004 PMID 15035987

POINT Mutations in RTK signalling pathways

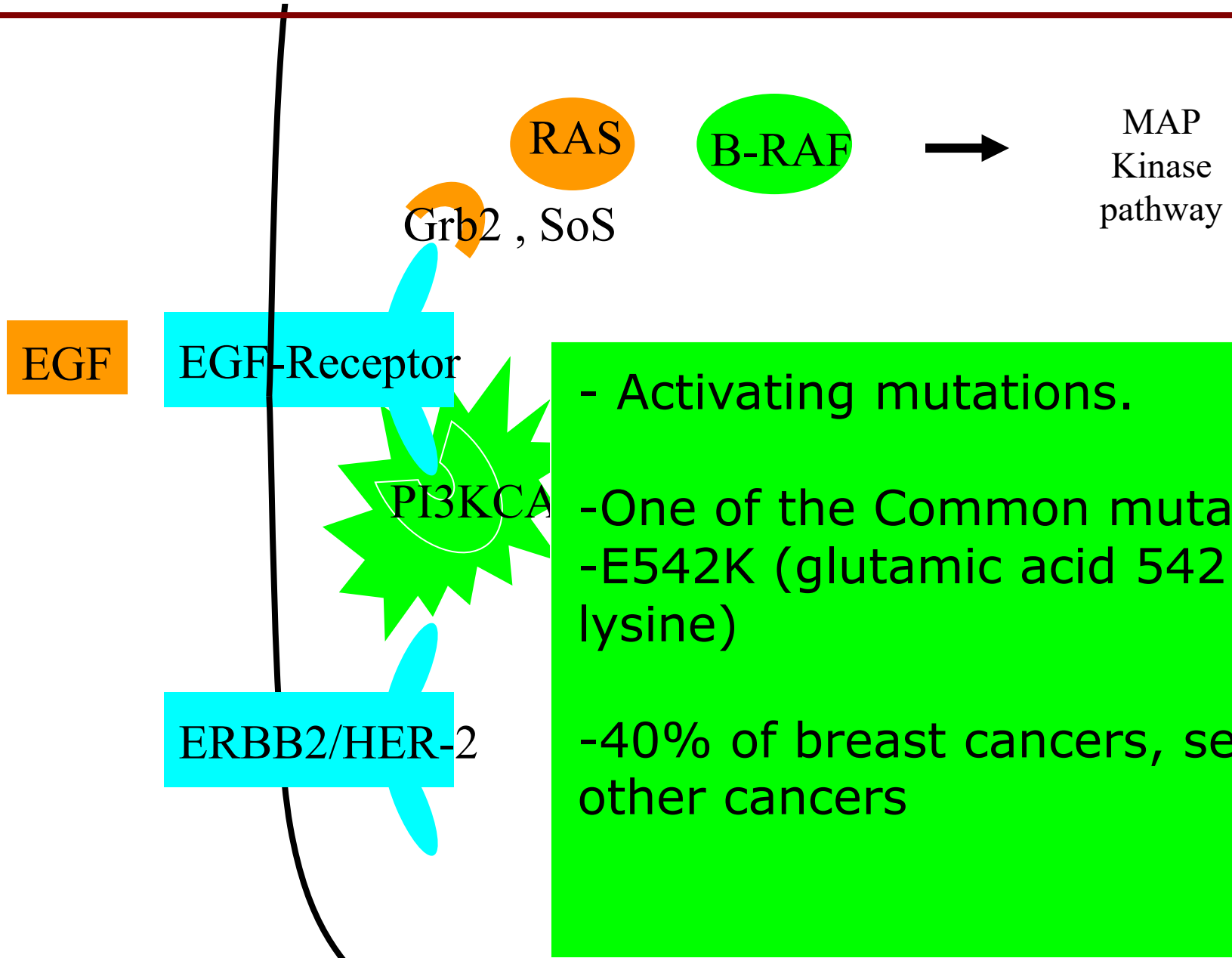


notes:

activating mutations are highly specific

pathways mutated rather than *genes*

POINT Mutations in RTK signalling pathways

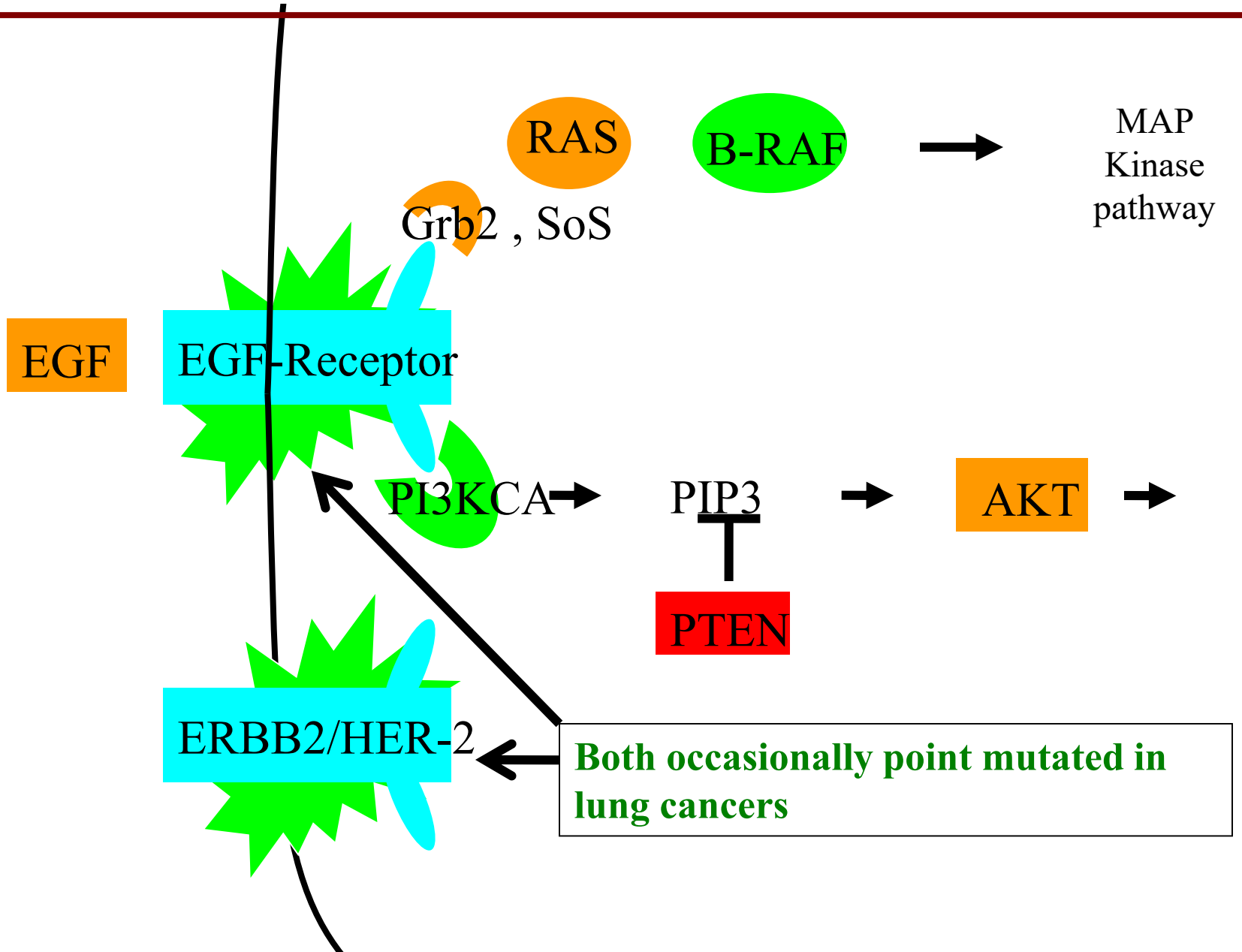


- Activating mutations.

-One of the Common mutations -E542K (glutamic acid 542 -> lysine)

-40% of breast cancers, several other cancers

POINT Mutations in RTK signalling pathways



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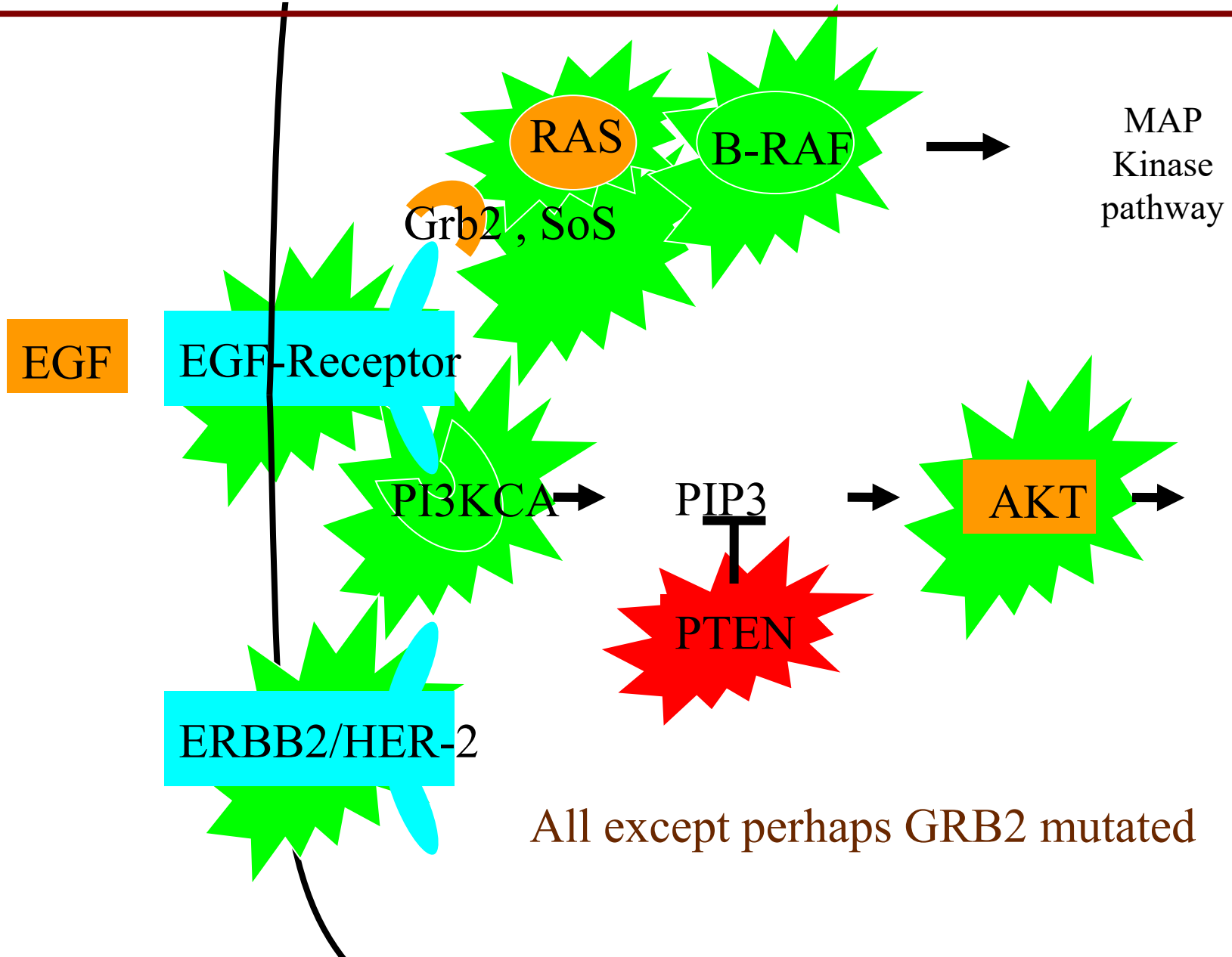
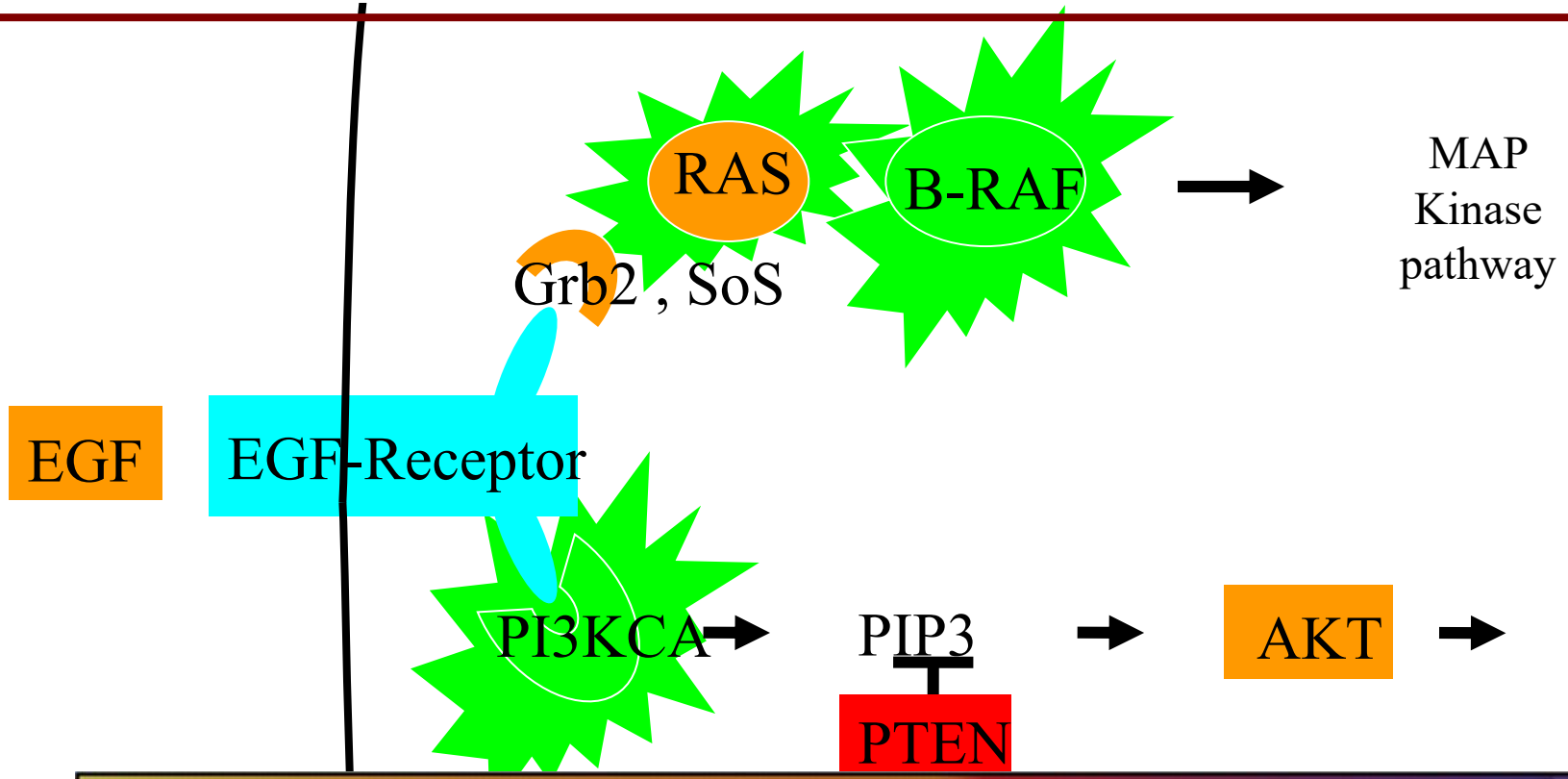
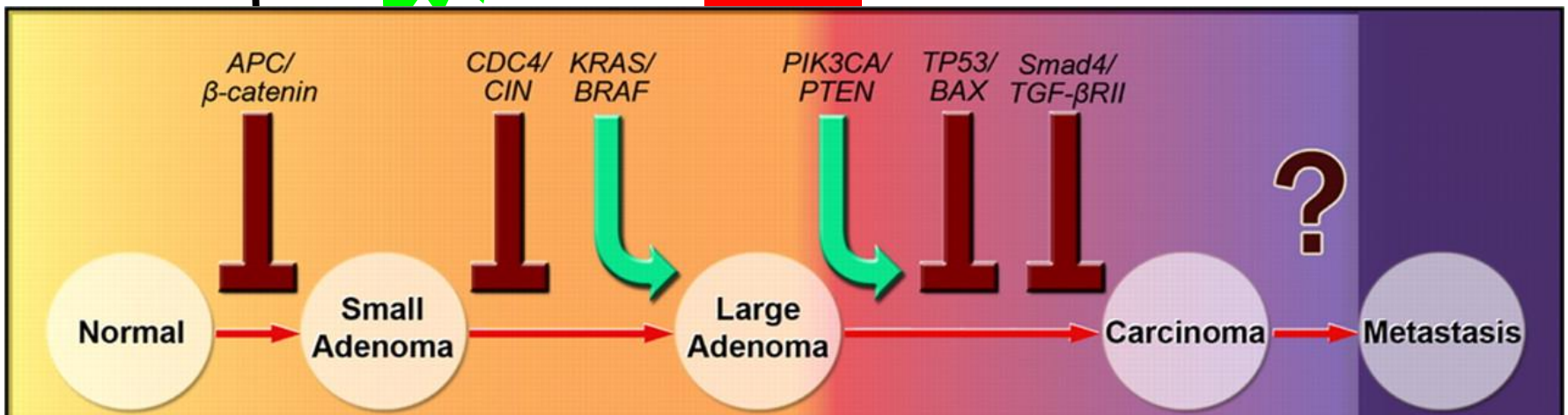


All except perhaps GRB2 mutated

POINT Mutations in RTK signalling pathways





Which genes are altered in cancer?

-all sorts of different pathways and systems

-Mitogenic signalling pathways e.g.
Wnt signalling pathway*,
Receptor tyrosine kinase pathways**
Rb control of cell cycle
Hedgehog pathway, Notch pathway, Hippo pathway...

- Inhibitory signalling, e.g.
TGF-β pathway*

-Transcription control systems
- transcription factors
- p53
- beta-catenin, MYC and ETS families
- hormone receptors ER
- chromatin / histone modifiers

-Cell adhesion, e.g. cadherin E in breast

-telomerase

-DNA repair and mitotic processes

- Carbohydrate metabolism (IDH1 in gliomas and AML)

- etc., etc.!

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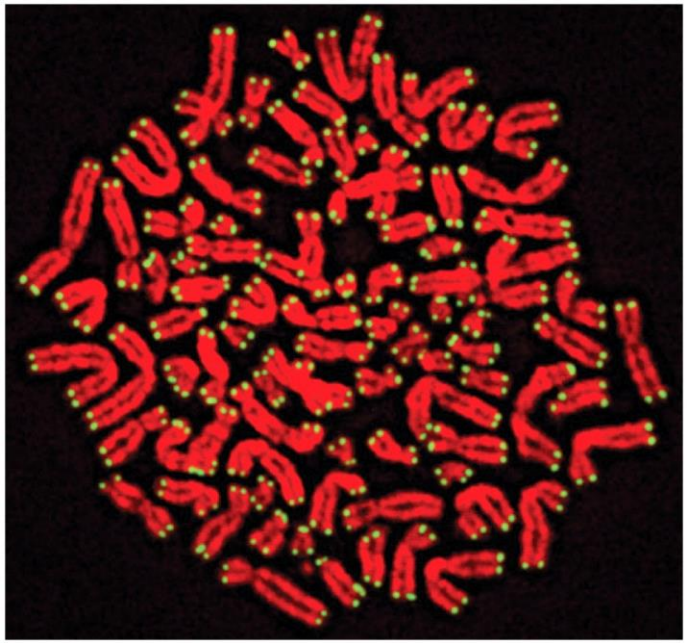

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A weird example

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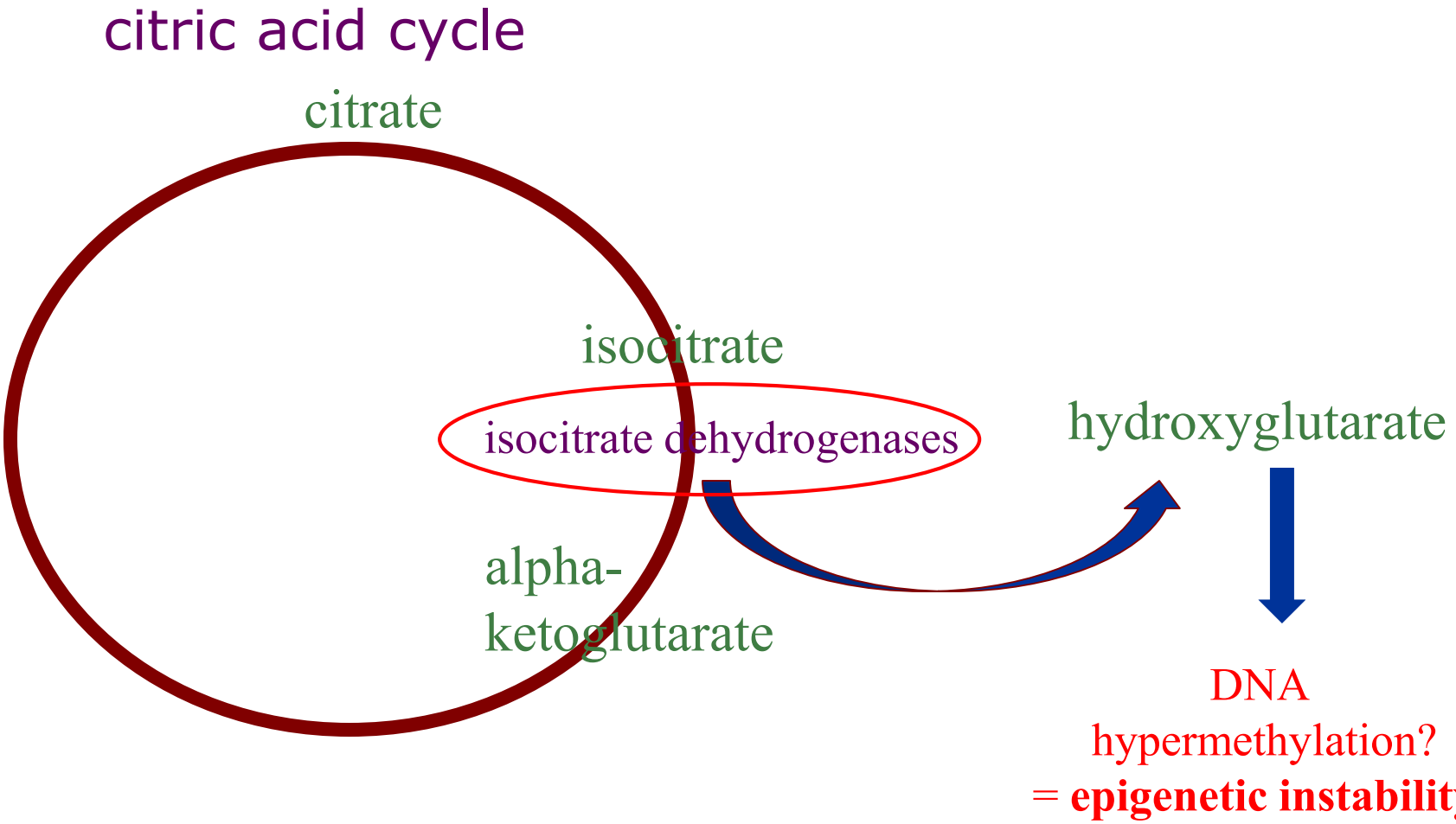


isocitrate dehydrogenase IDH1

mutated in majority glioblastomas, occasional leukaemias,

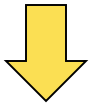
What sorts of mutations alter the genes?

All sorts of mutation

What sorts of mutations alter the genes?

Sequence changes, e.g.

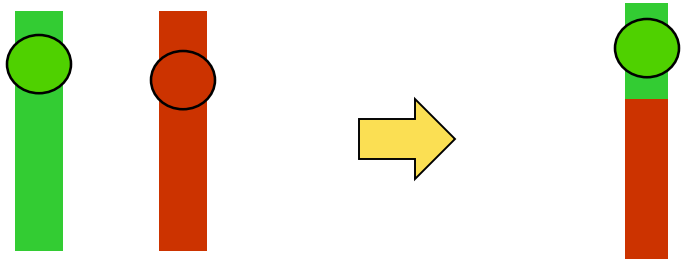
TCGAGCTATGTGTCTCTAGGTCGGT



TCGAGCTATGAGTCTCTAGGTCGGT

Small-scale changes

STRUCTURAL changes, e.g.



Large-scale changes

What sorts of mutations alter the genes?

-single base pair change

-Indel = Small insert or deletion ->frameshift

Small-scale changes

APC classic example, truncated in colon

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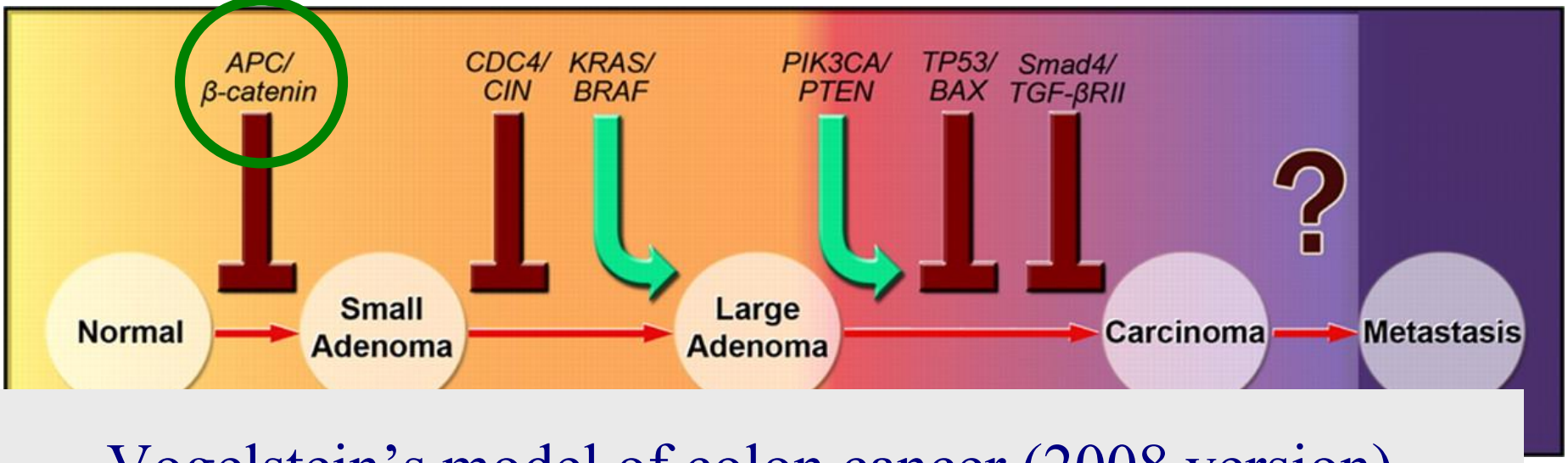

Vogelstein's model of colon cancer (2008 version)

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Small-scale changes

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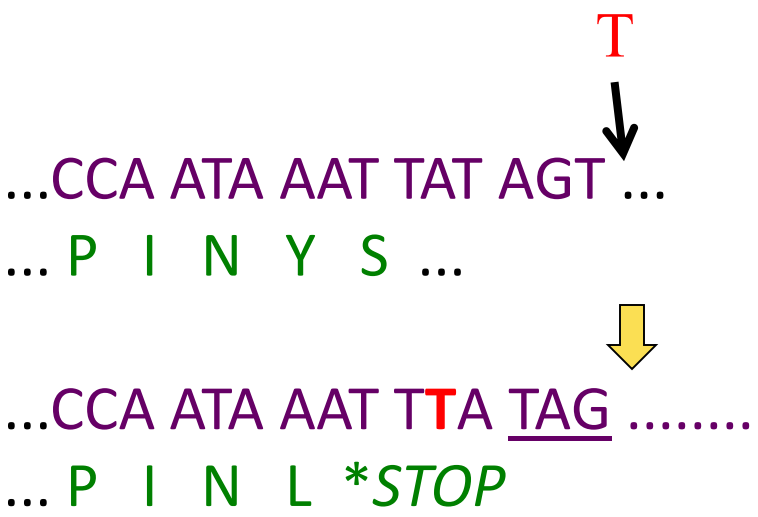


truncated APC protein

What sorts of mutations alter the genes?

-single base pair change

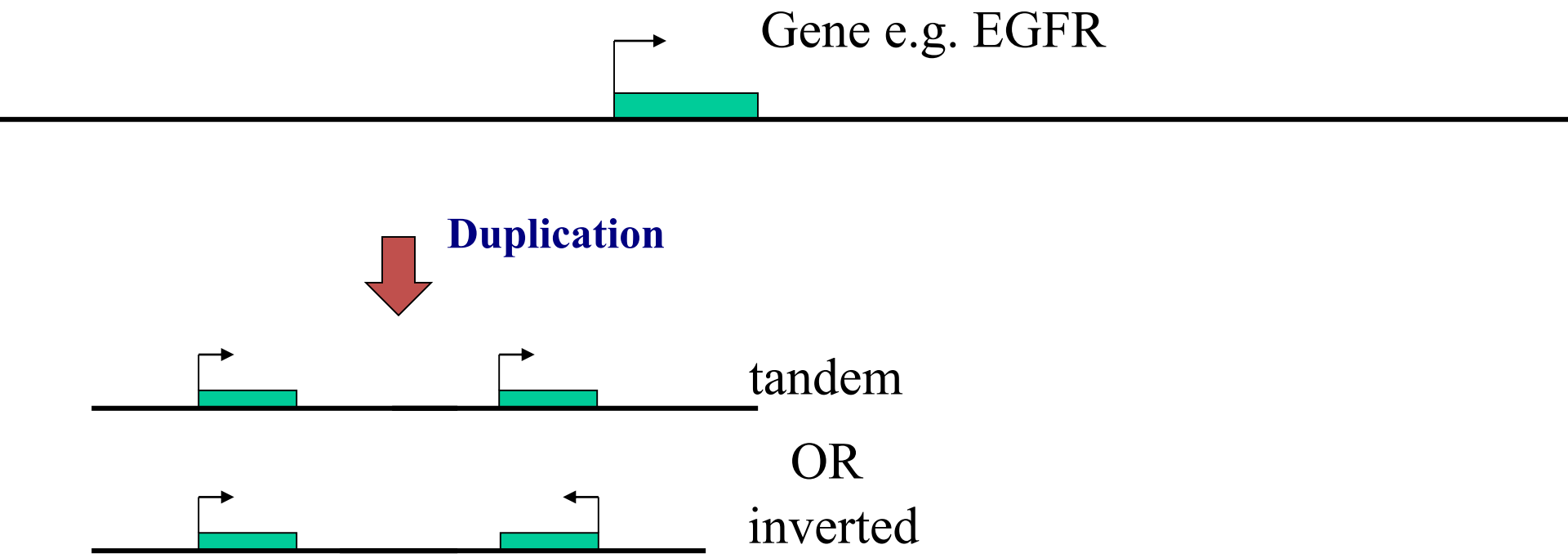
-Indel = Small insert or deletion ->frameshift
- (e.g. APC in practical)

Small-scale changes

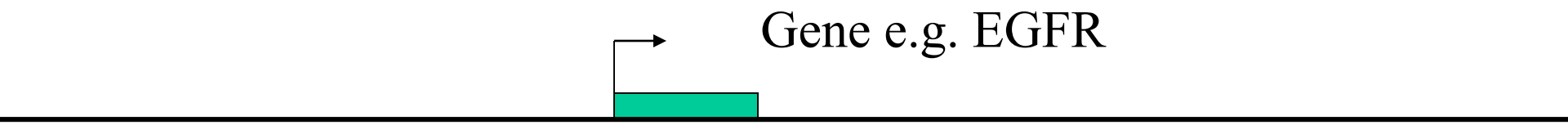
-Deletion
-Inversion
-Duplication
-Amplification
-Chromosome translocation

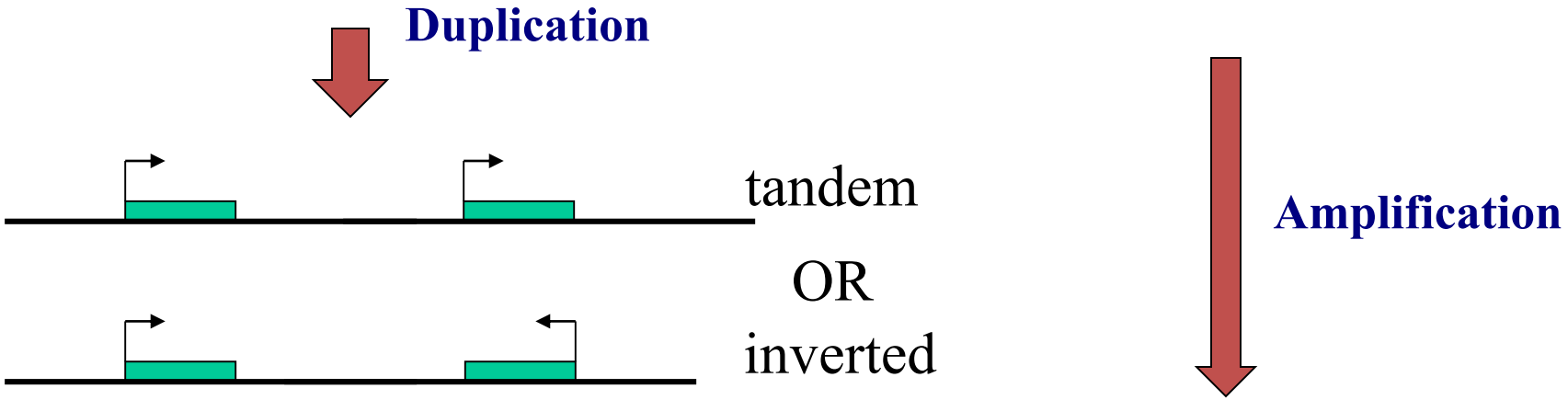
Large-scale changes

Duplication and amplification

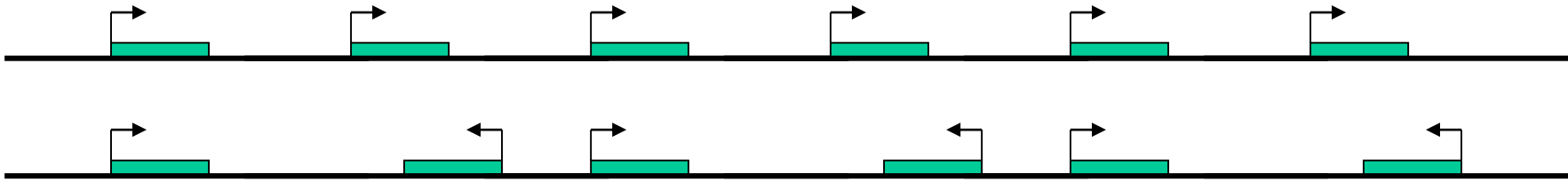


Duplication and amplification

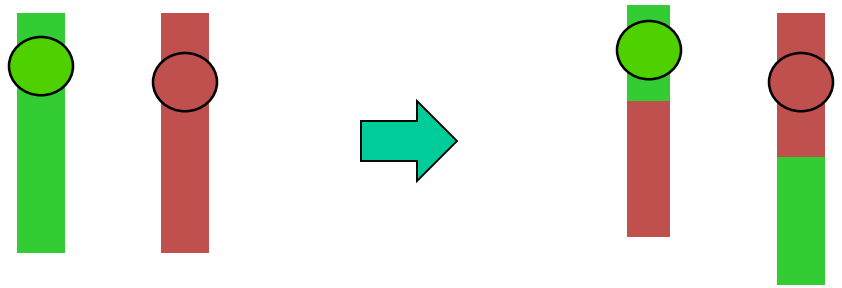




‘Amplified’ gene

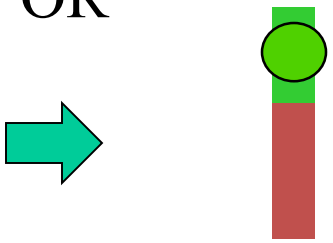


Chromosome translocation



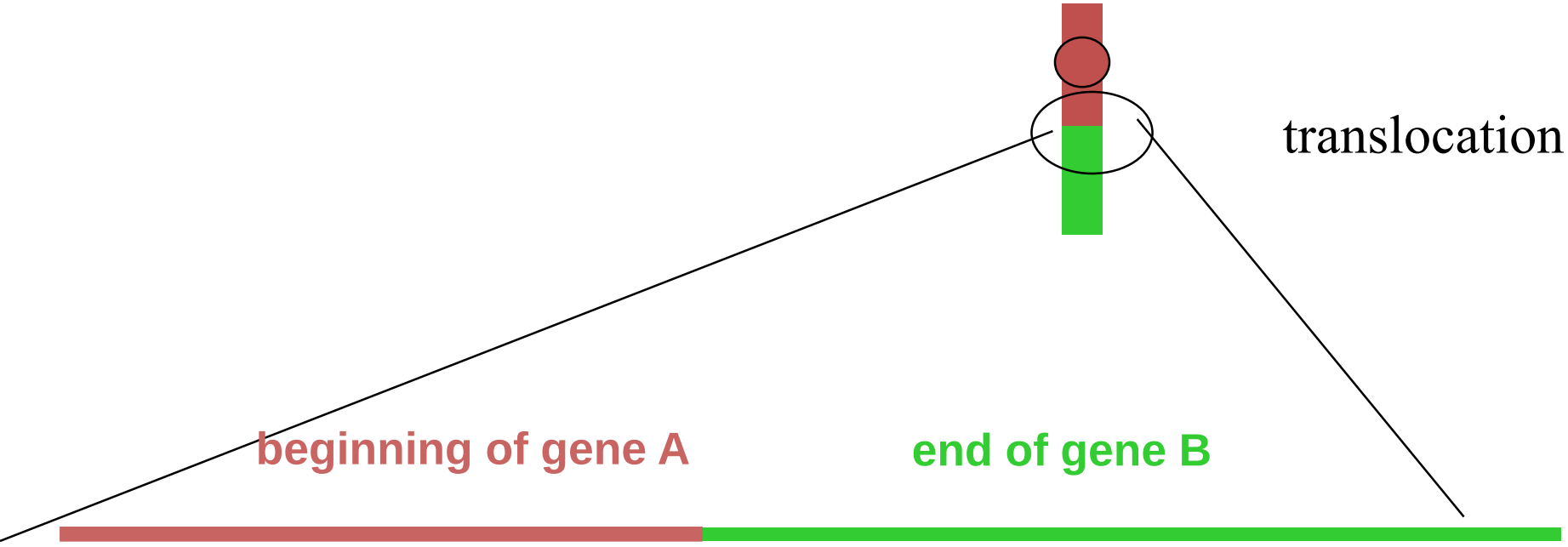
reciprocal translocation

OR



unbalanced translocation

Chromosome translocation



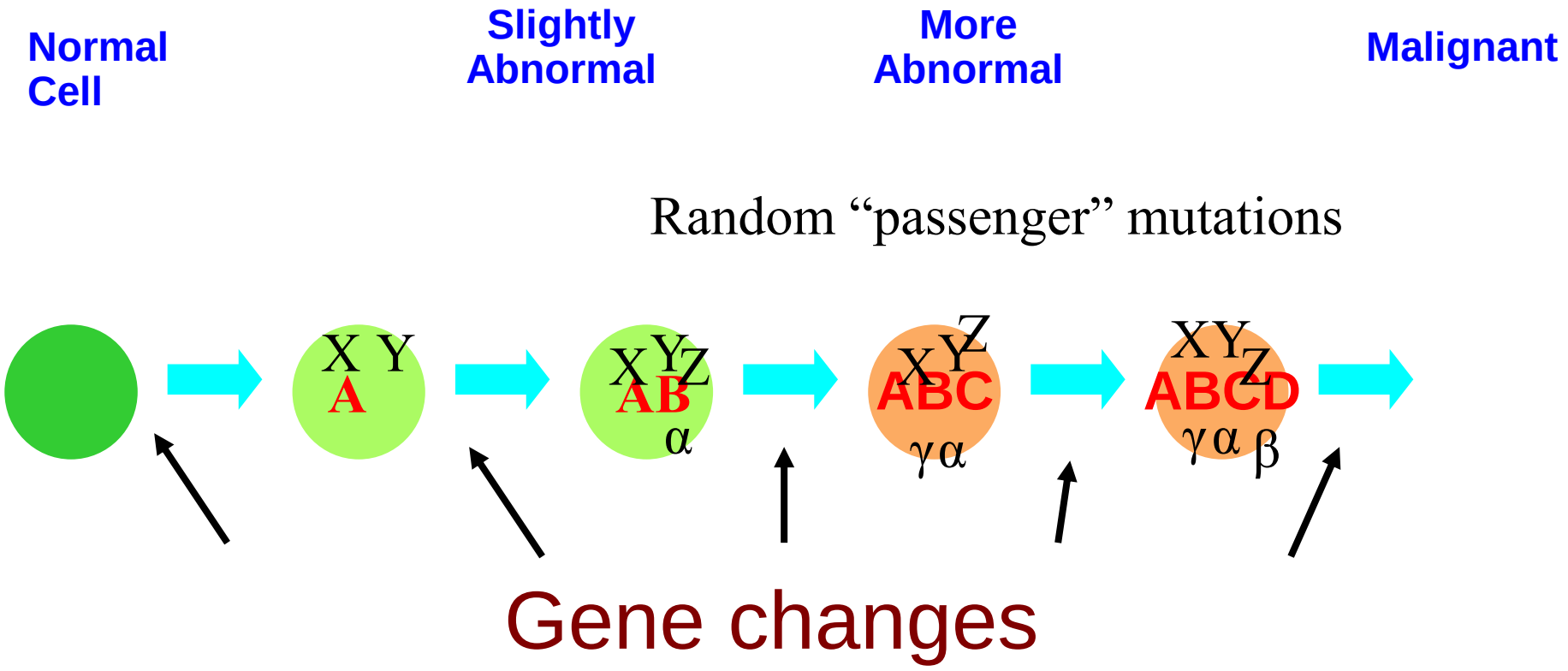
How do we study these mutations?

How do we study the alterations/mutations?

sequencing

Small-scale changes

Passengers versus Drivers



How do we study the alterations/mutations?

sequencing

Small-scale changes

-Deletion

started with cytogenetics.....

-Inversion

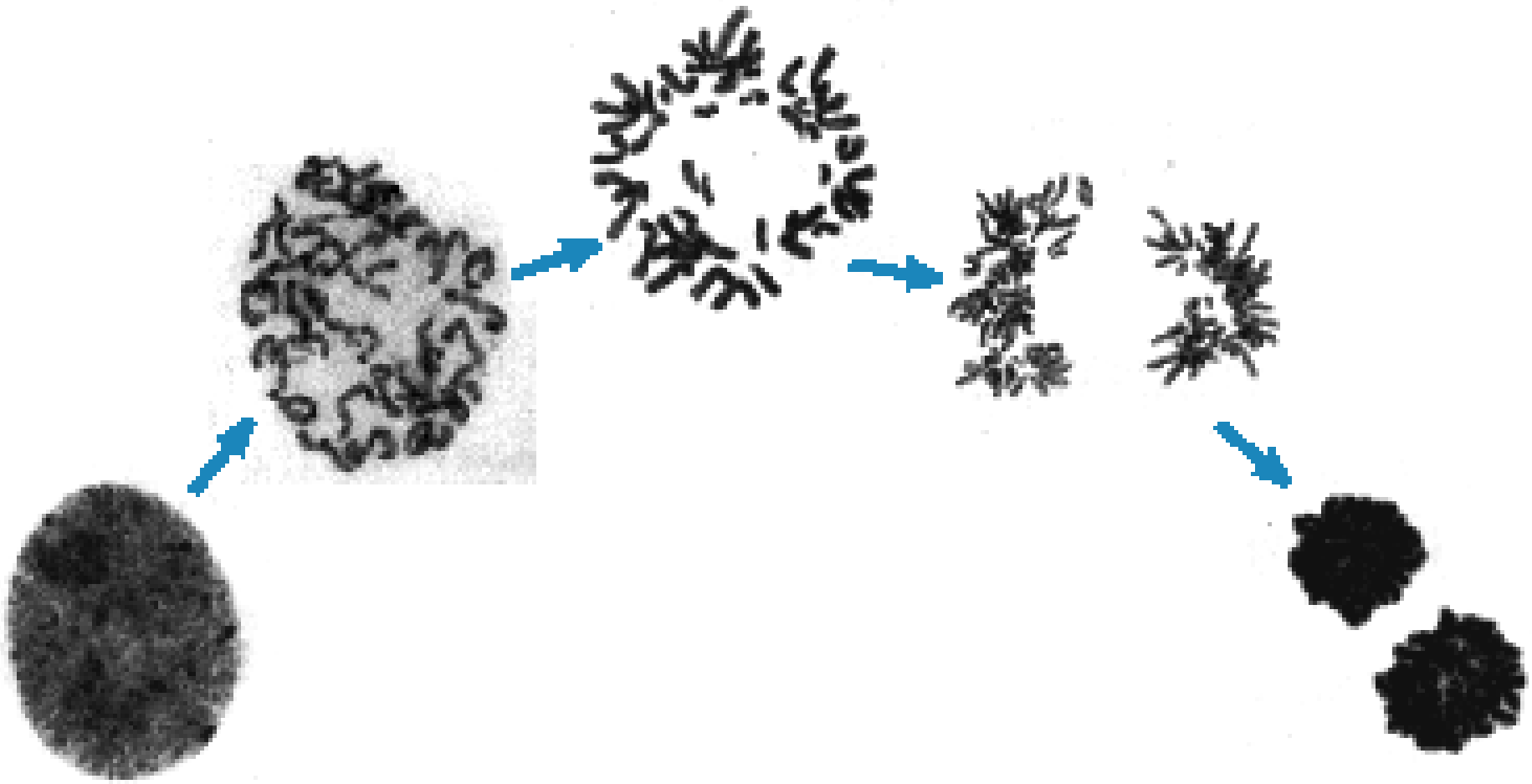
-Duplication

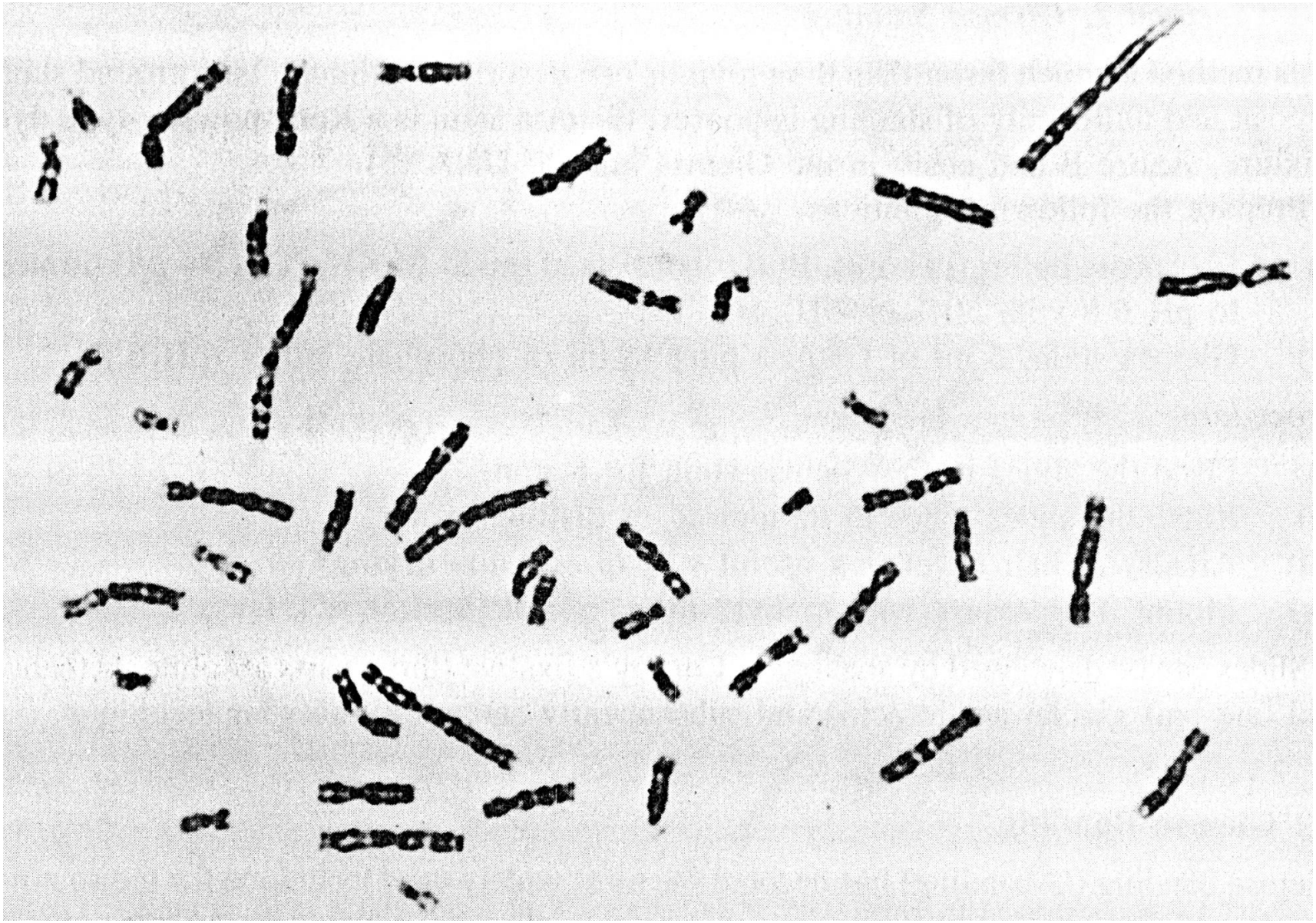
-Amplification

-Chromosome translocation

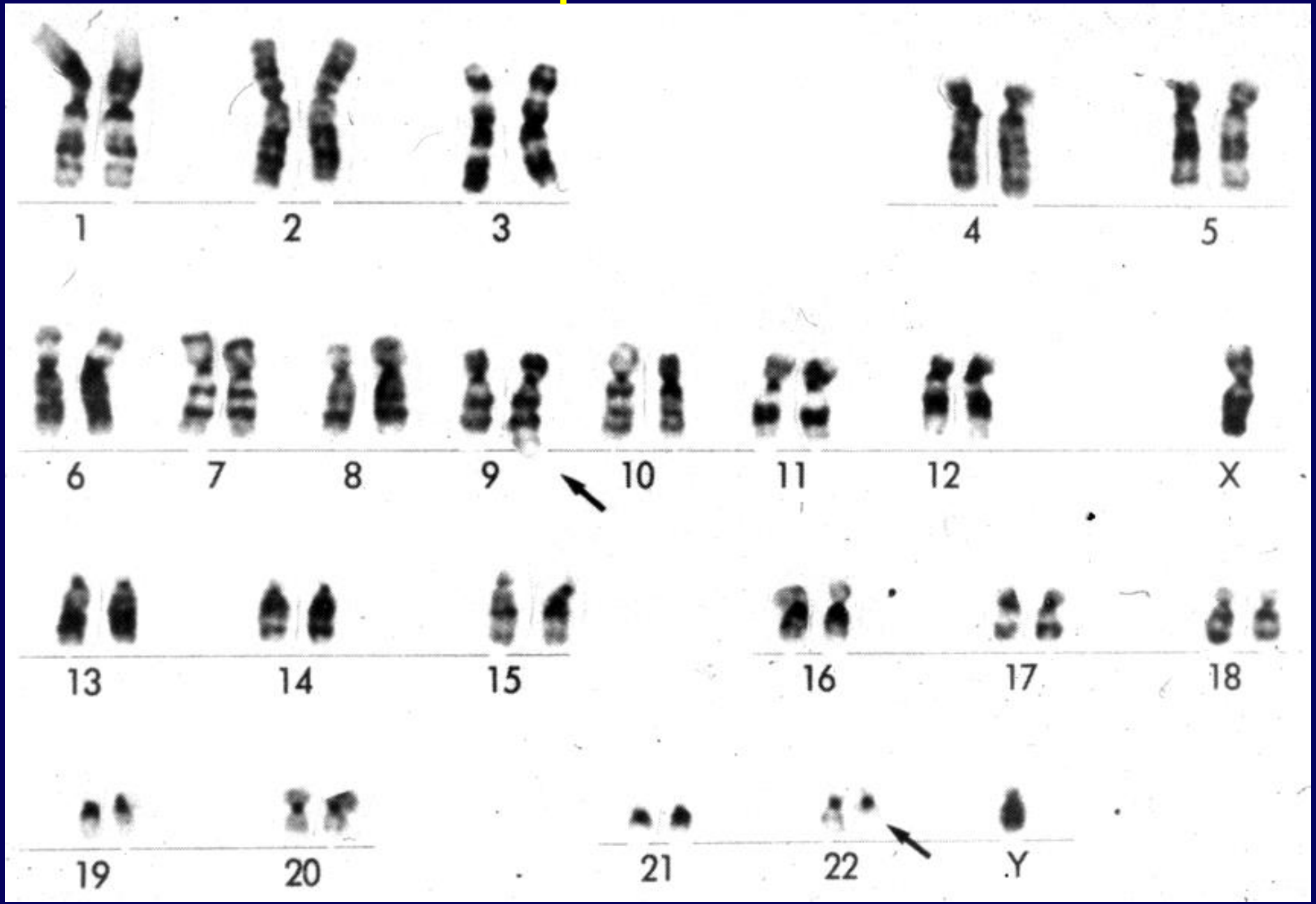
Large-scale changes

Metaphase chromosomes





Philadelphia chromosome



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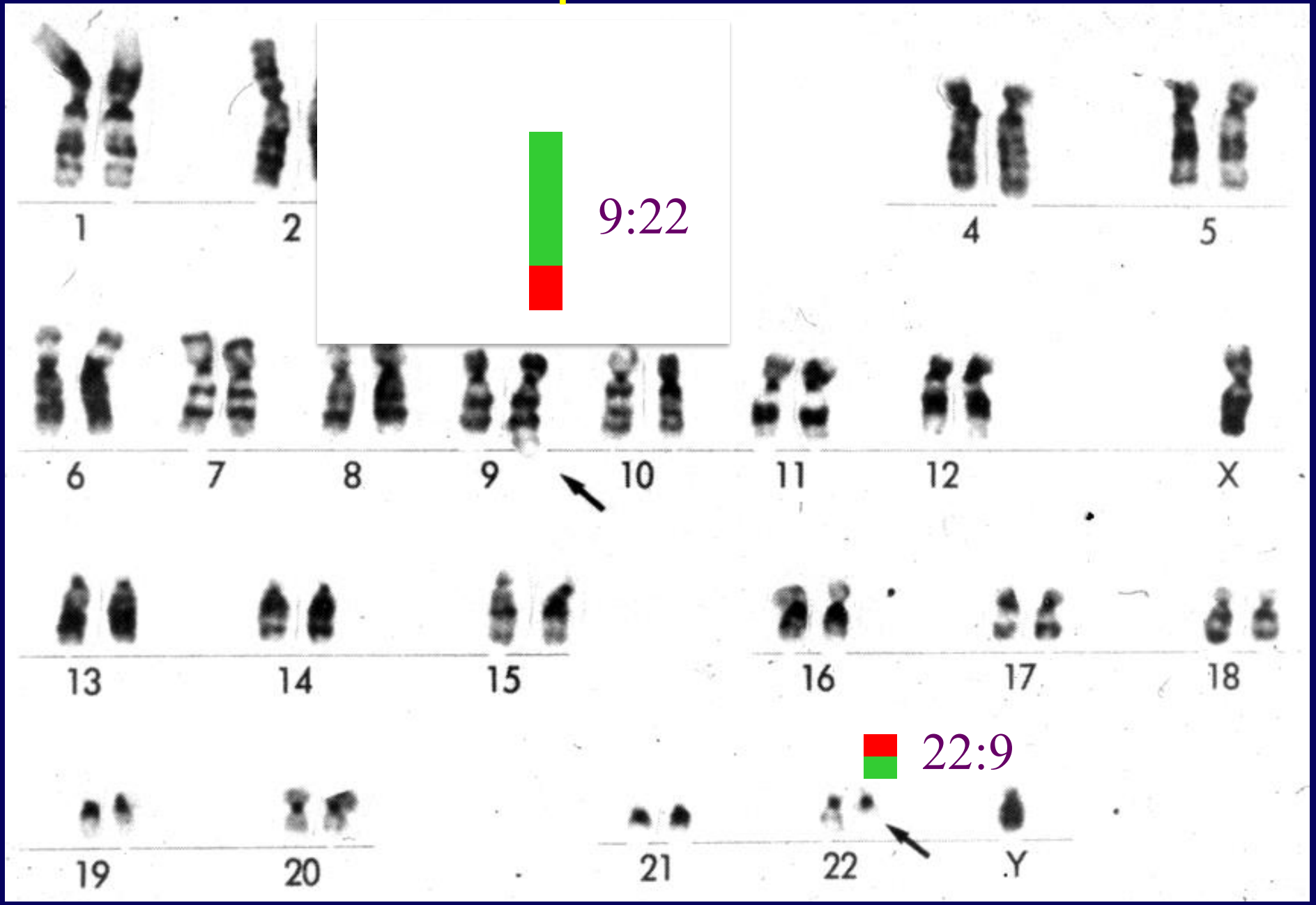
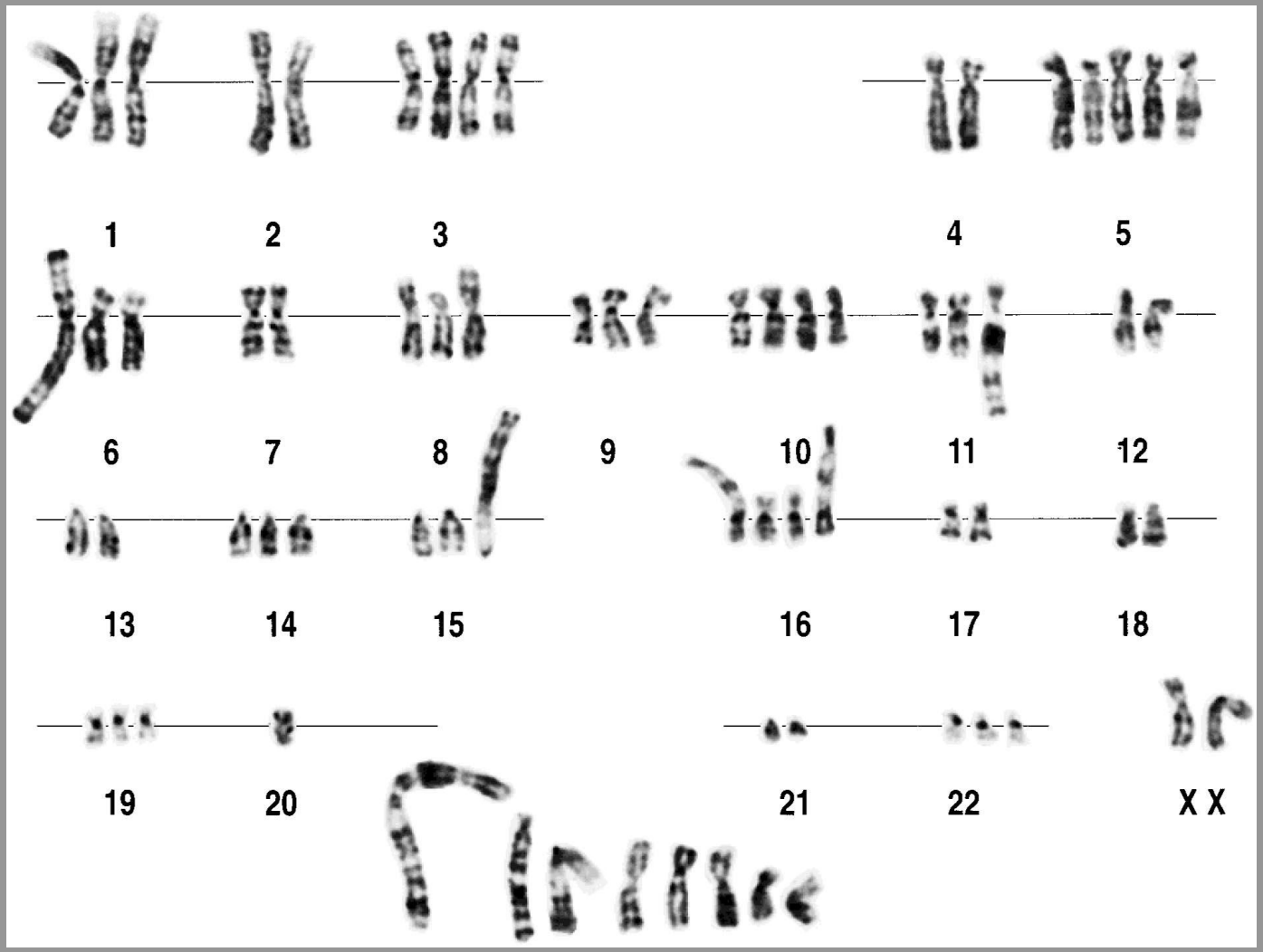
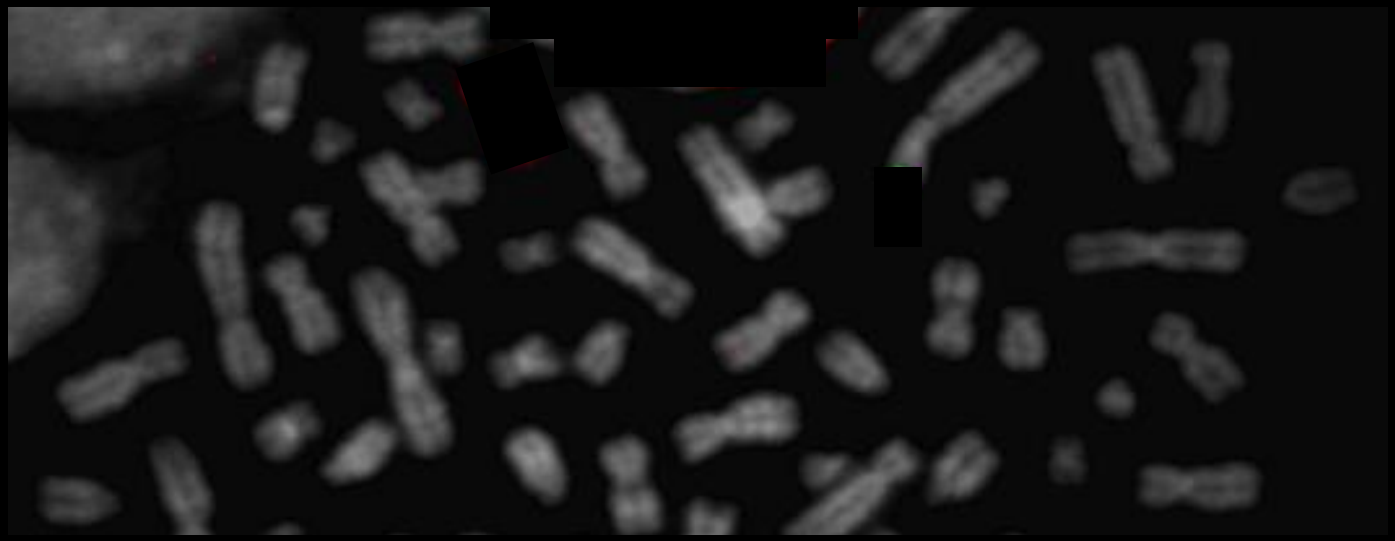


Breast Cancer Karyotype, from primary culture



Pandis et al (1998) Genes Chromosomes Cancer 22, 122

‘ FISH’ fluorescence-in situ hybridisation



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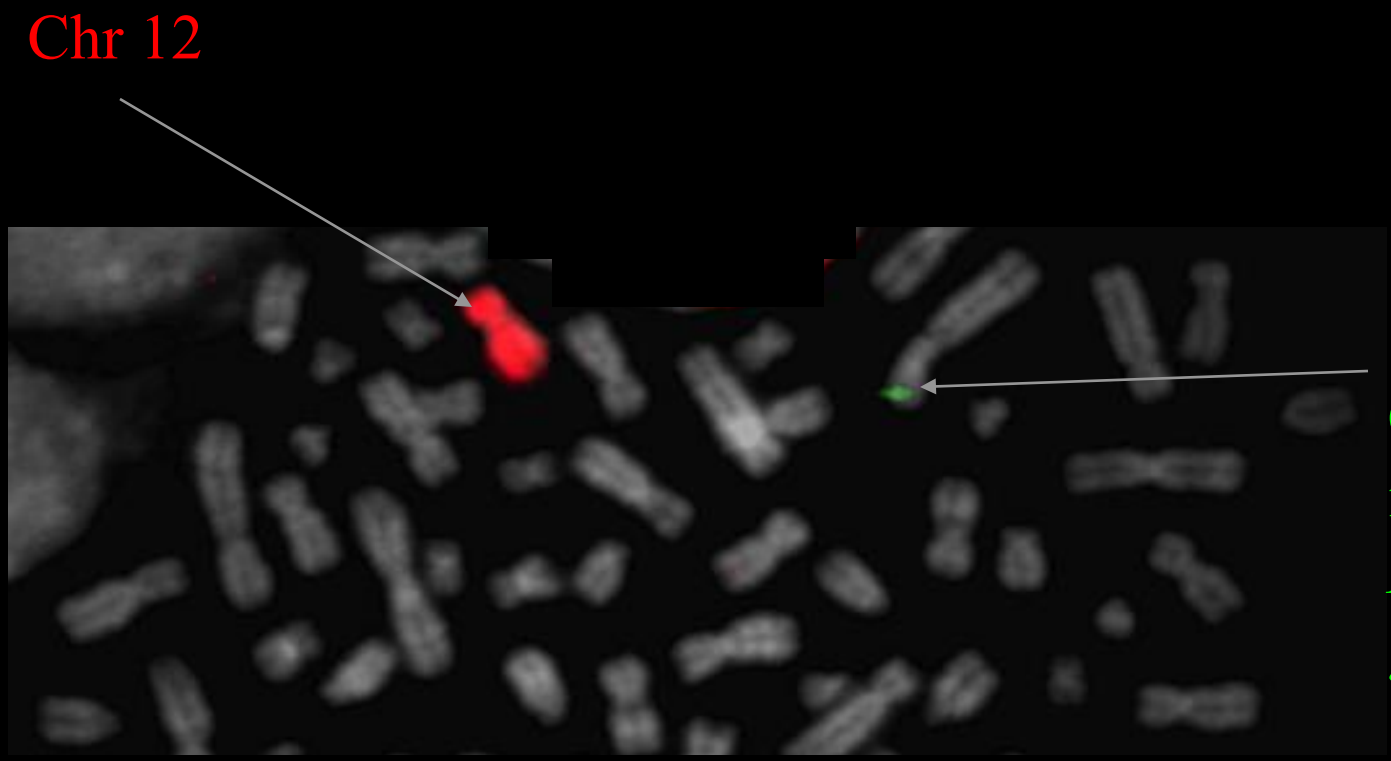
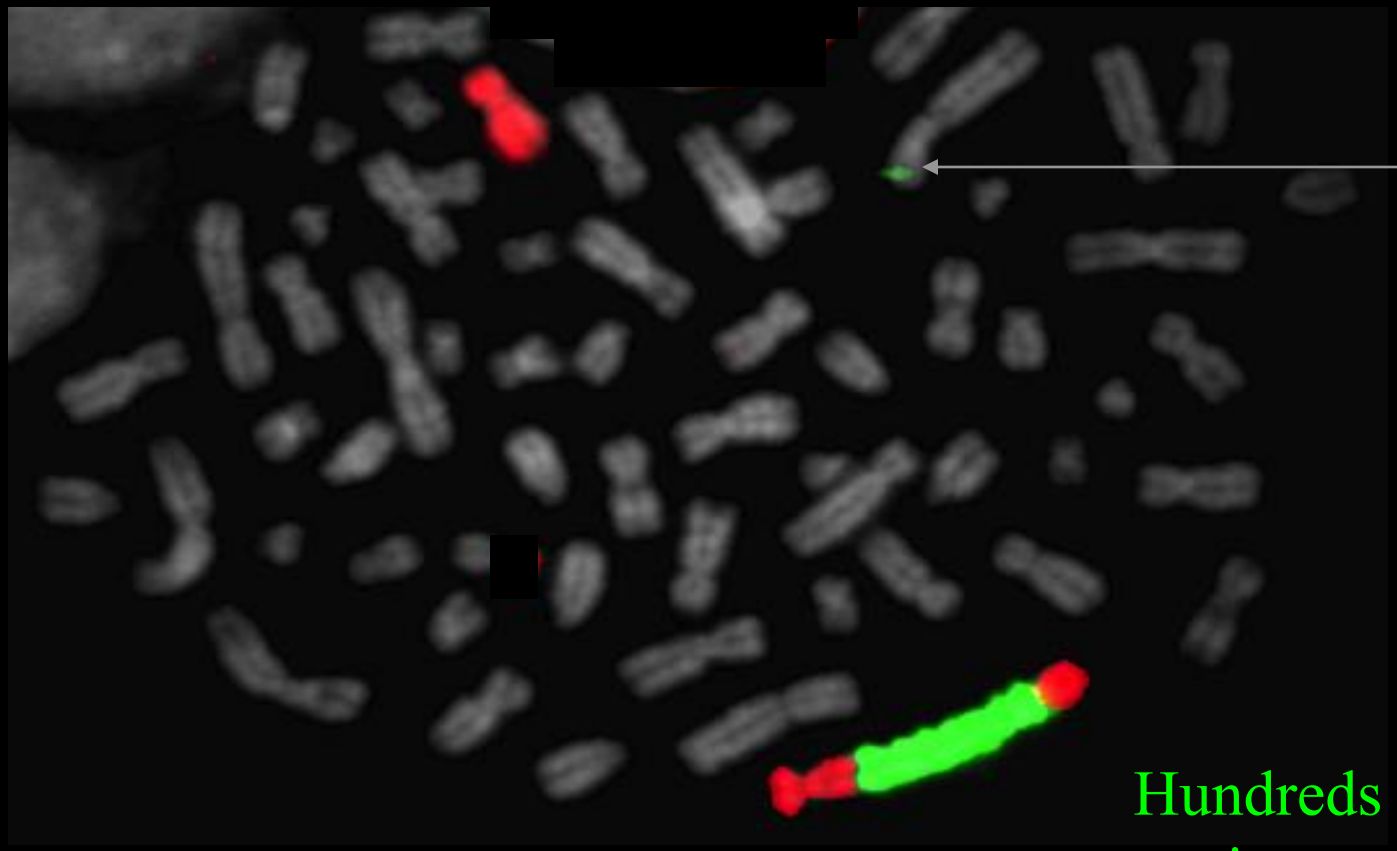


‘Amplification’ of N-MYC

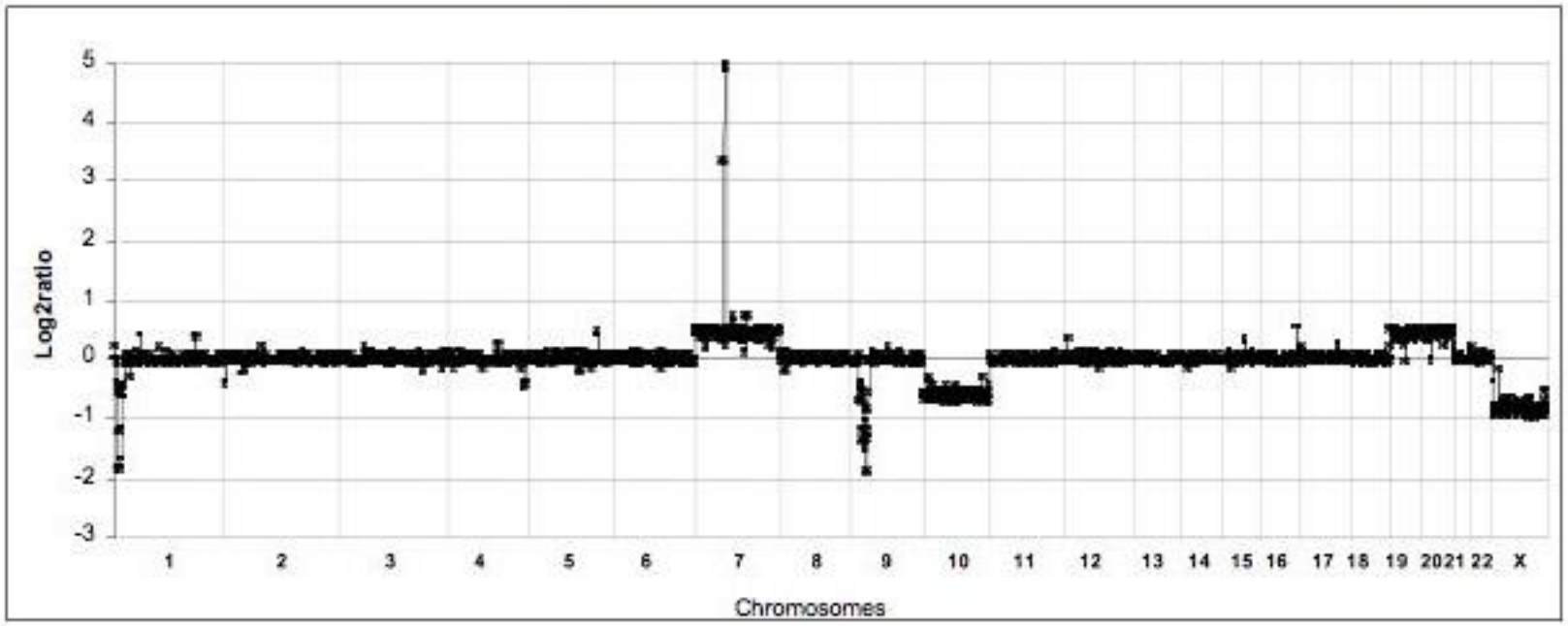


Breast Cancer Cell Line
MDA-MB-361

Joanne Davidson

Search for deletions and amplifications: measure copy number

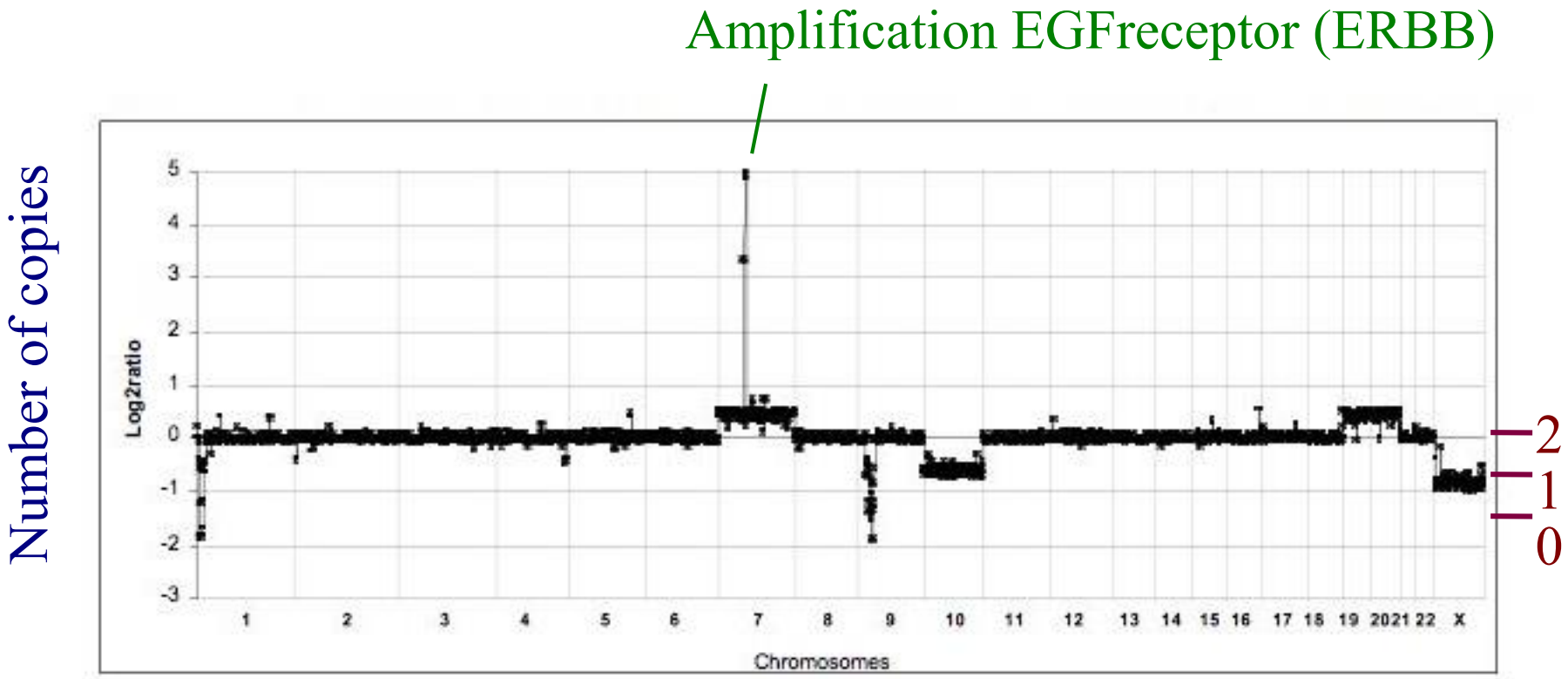
No of copies



Genome position

glioblastoma

Search for deletions and amplifications: measure copy number

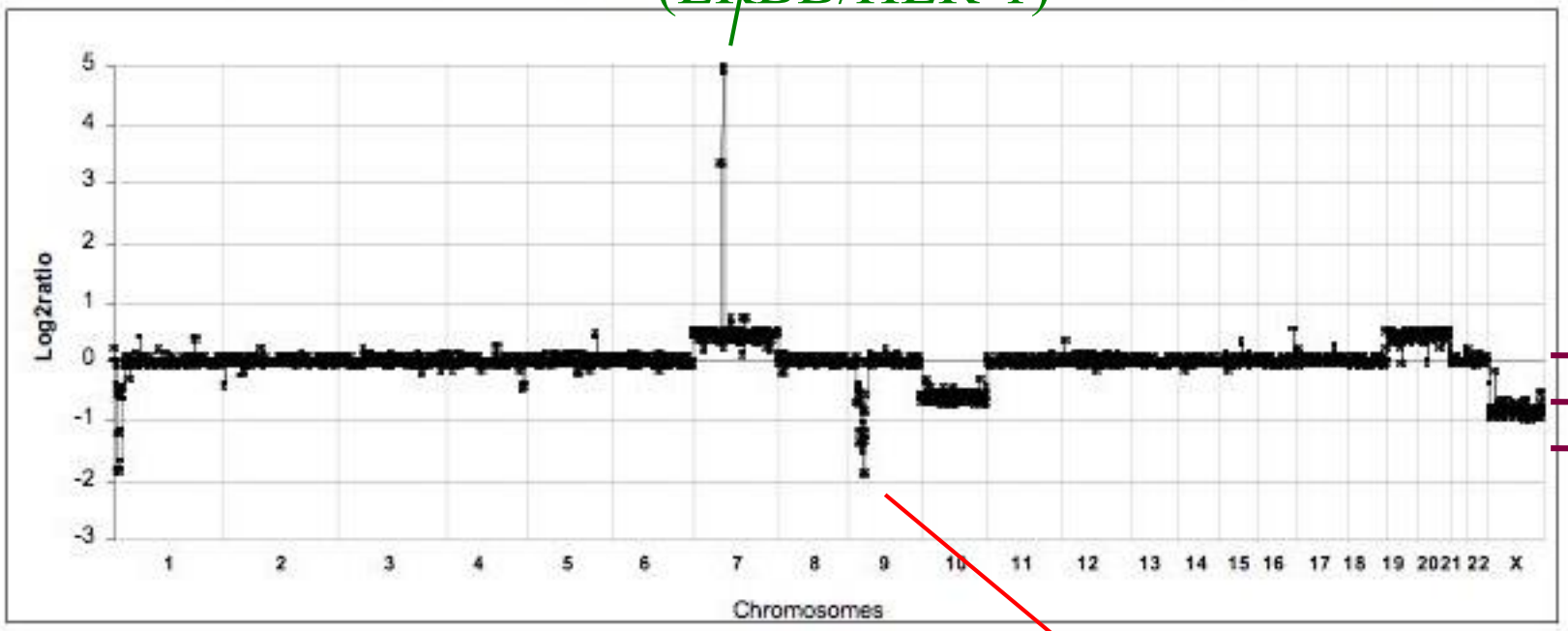


Genome position

glioblastoma

CGH Hybridization: Search for deletions and amplifications

Amplification EGFreceptor (ERBB/HER-1)



Number of copies

2
1
0

Deletion P16/CDKN2A/INK4A

implicated in senescence

Genome position

glioblastoma

Examples of alterations/mutations?

-single base pair change

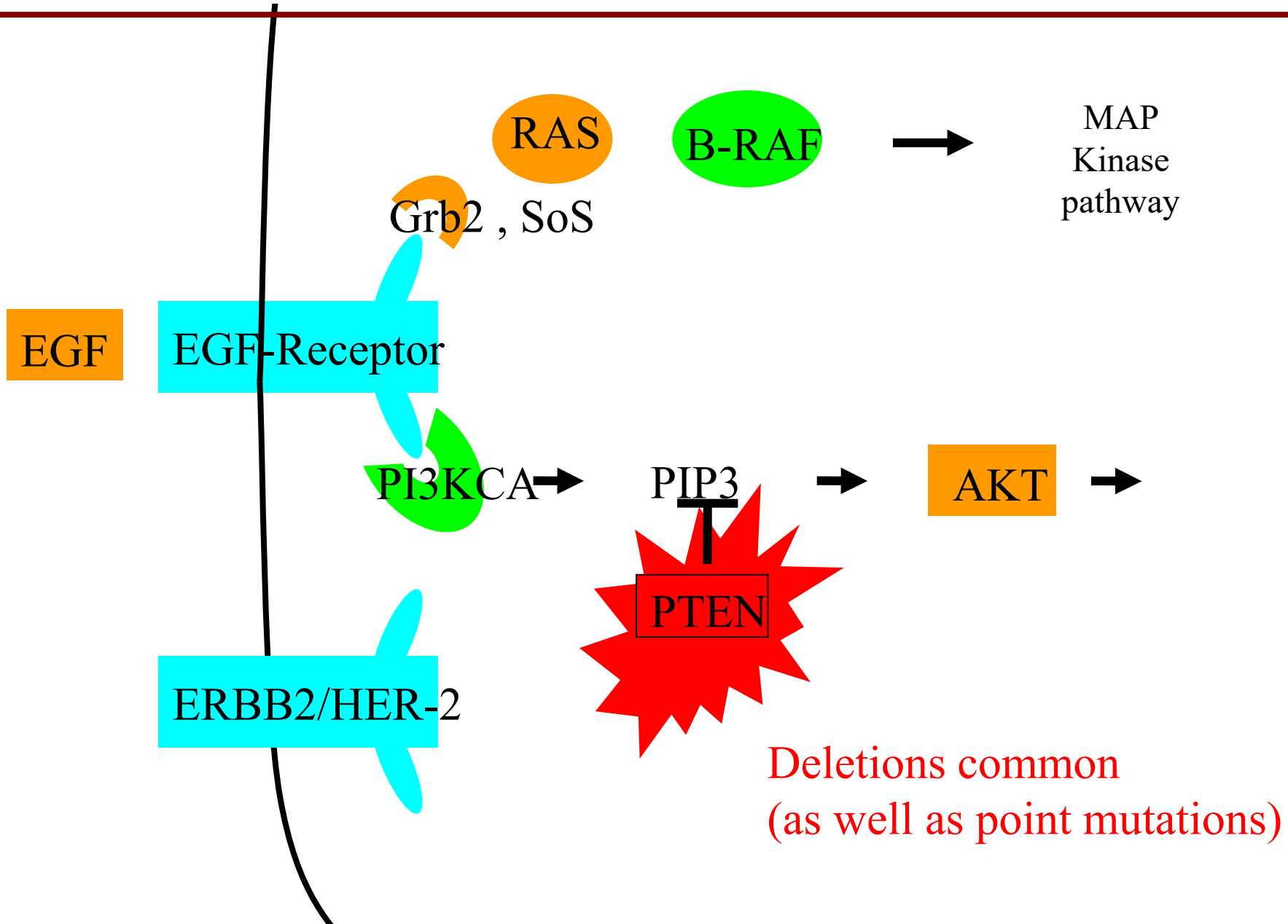
-Indel = Small insert or deletion

Small-scale changes

-Deletion
-Inversion
-Duplication
-Amplification
-Chromosome translocation

Large-scale changes

Mutations in RTK signalling pathways



What sorts of mutations alter the genes?

-single base pair change

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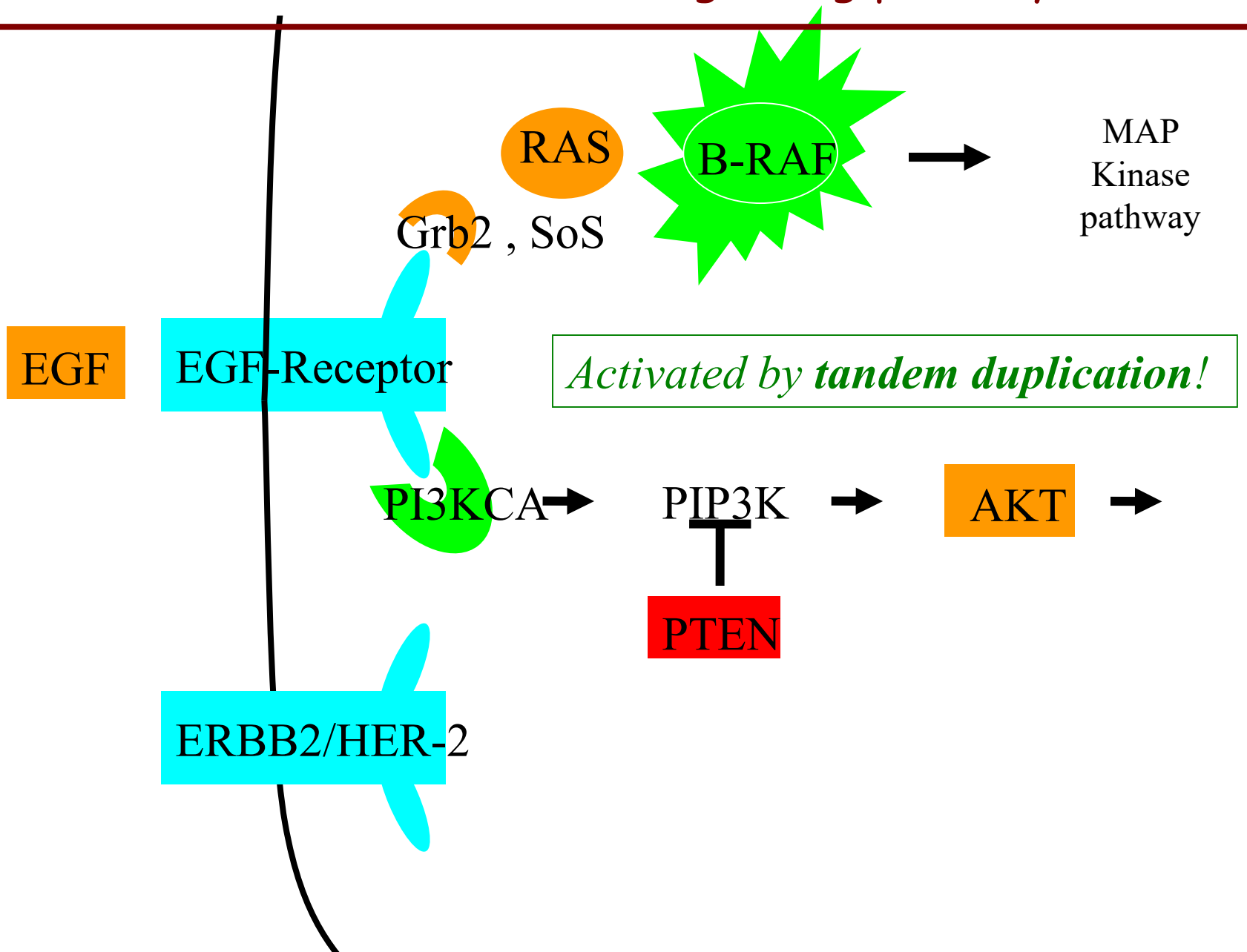
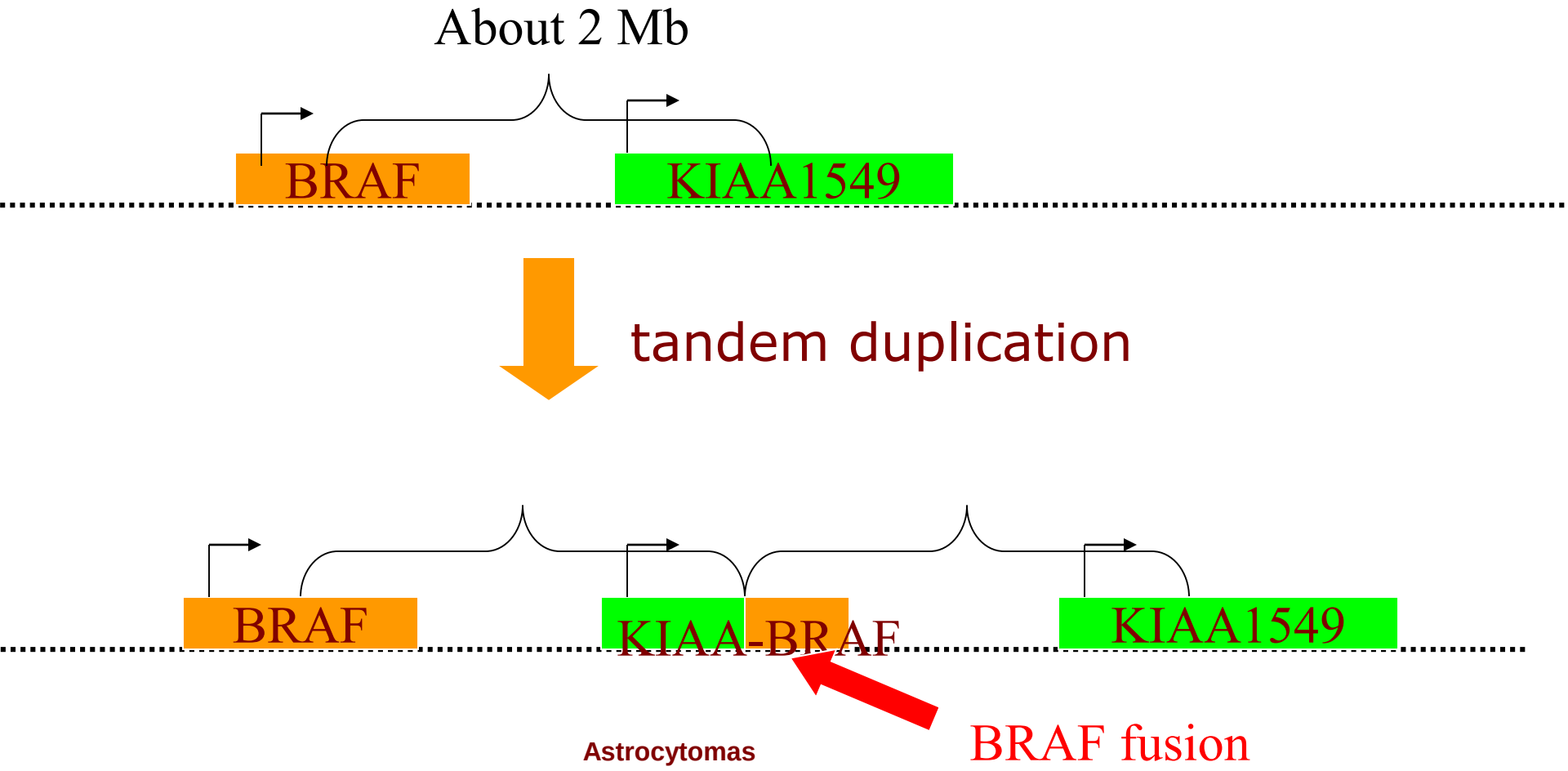
Large-scale changes

Mutations in RTK signalling pathways

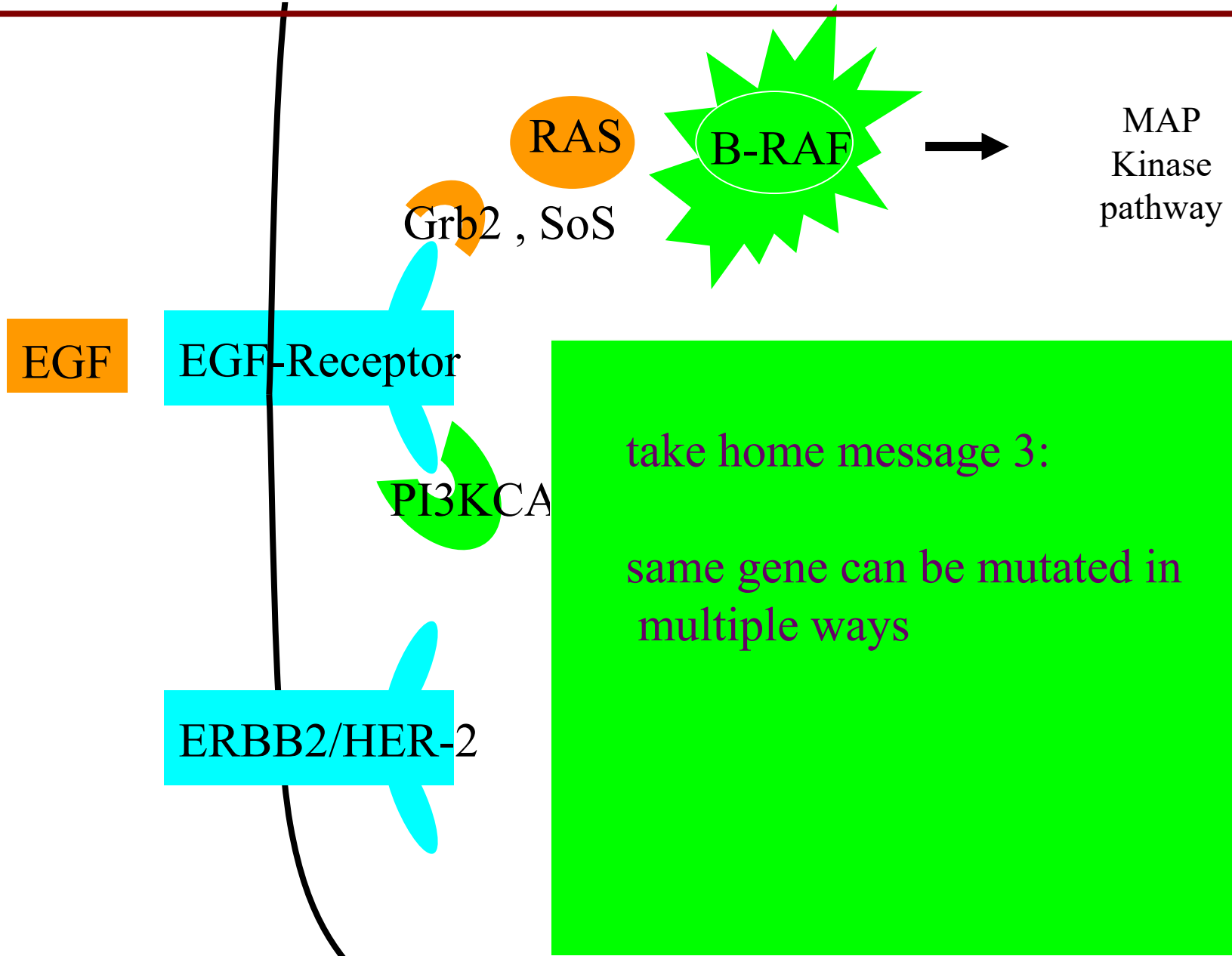


Tandem duplications causing gene fusion of BRAF



Astrocytomas

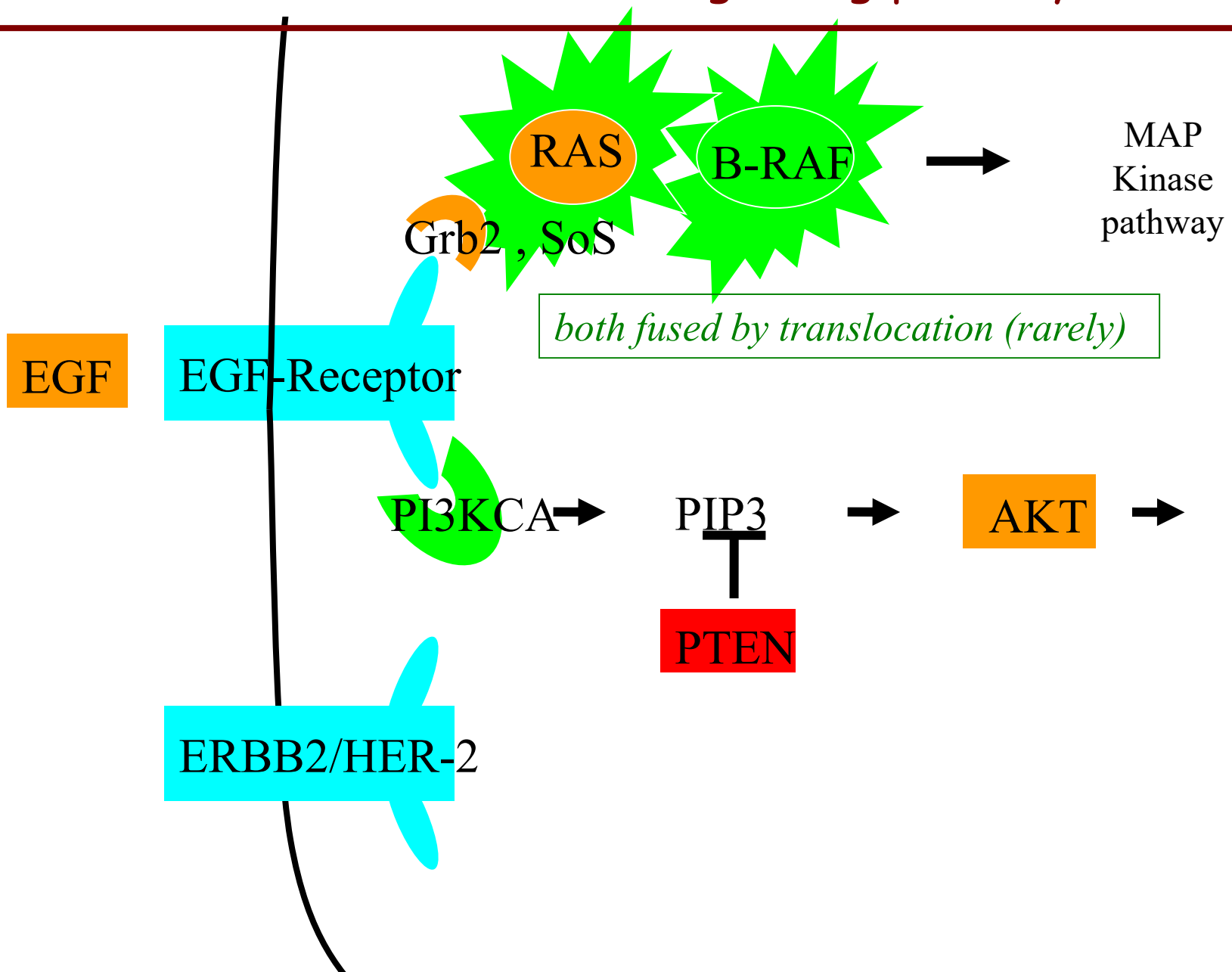
Mutations in RTK signalling pathways



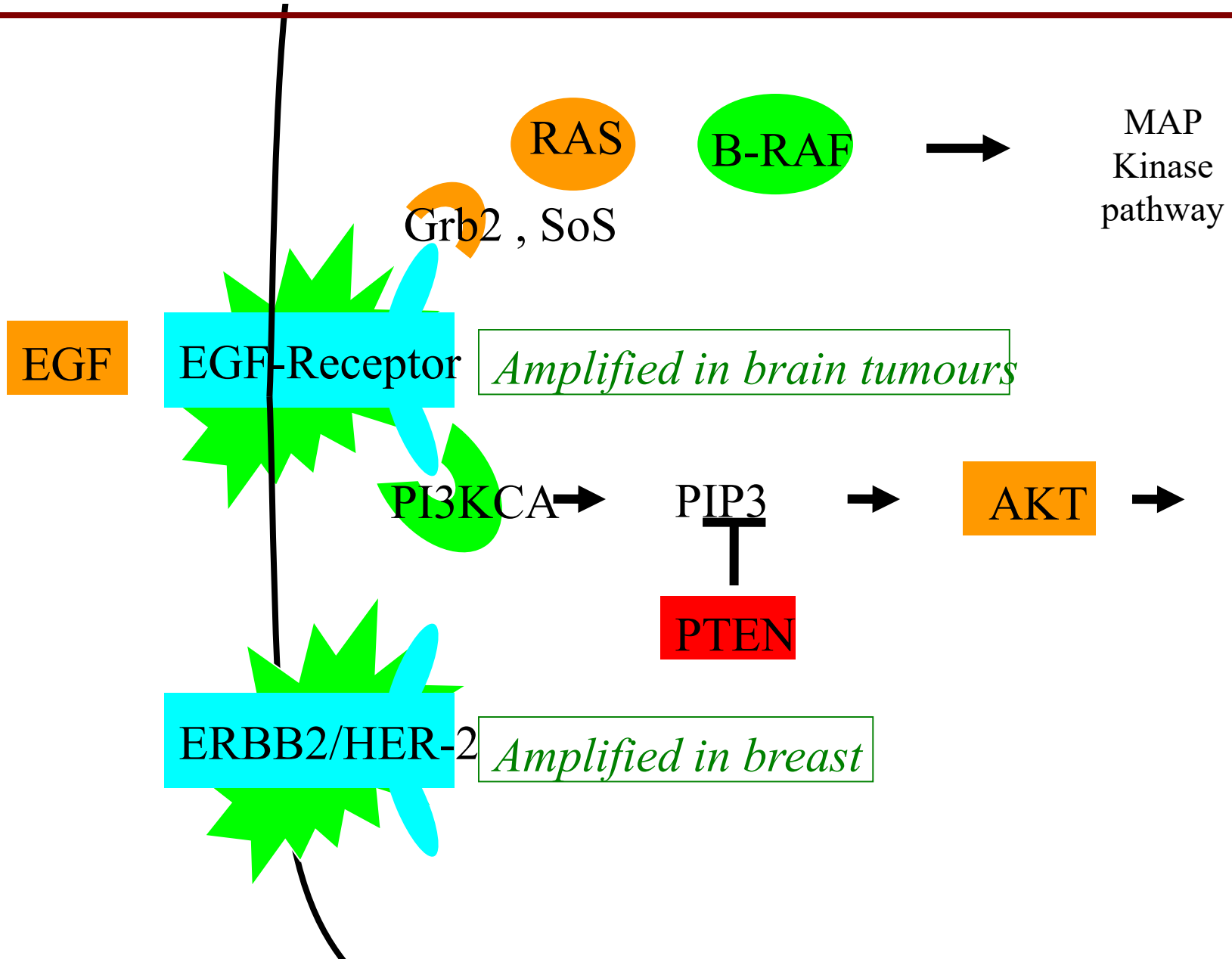
take home message 3:

same gene can be mutated in multiple ways

Mutations in RTK signalling pathways



Mutations in RTK signalling pathways



Mutations in RTK signalling pathways

The same gene can be altered by widely different mechanisms:

EGFR and ERBB2 point mutated or amplified

RAF: point mutated,
fused by tandem duplication,
fused by chromosome translocation

even RAS has been found fused by translocation (rarely)

What sorts of mutations alter the genes?

-single base pair change

-Indel = Small insert or deletion ->

Small-scale changes

-Deletion

-Inversion

-Duplication

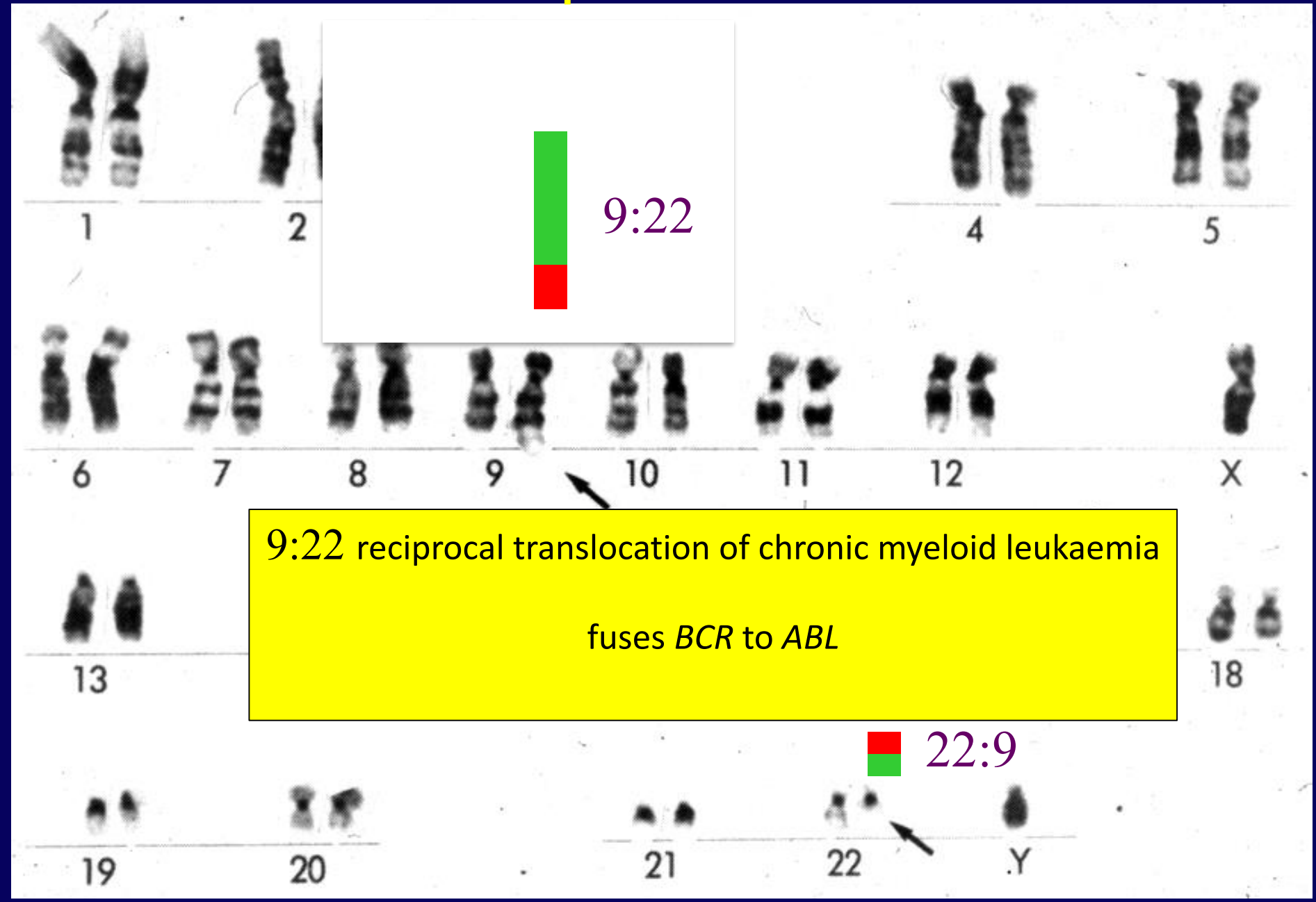
-Amplification

-Chromosome translocation

Large-scale changes

The first human cancer mutation: The Philadelphia chromosome translocation

Philadelphia chromosome



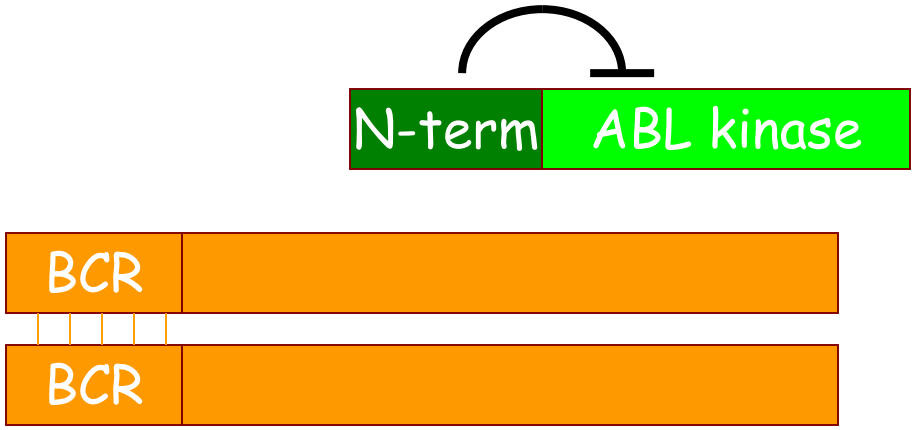
9:22 reciprocal translocation of chronic myeloid leukaemia

fuses *BCR* to *ABL*

22:9

BCR-ABL fusion protein

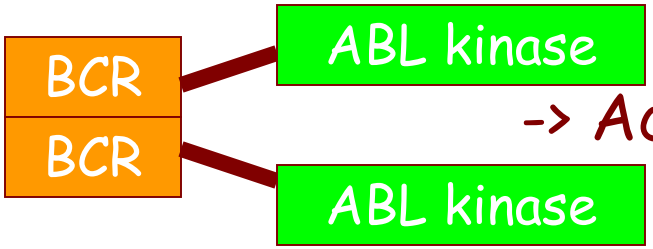
Abl is a tyrosine kinase, controlled by N-terminal domain



Normal Abl

Normal Bcr
likes to form dime

Tyrosine kinases are activated by dimerisation

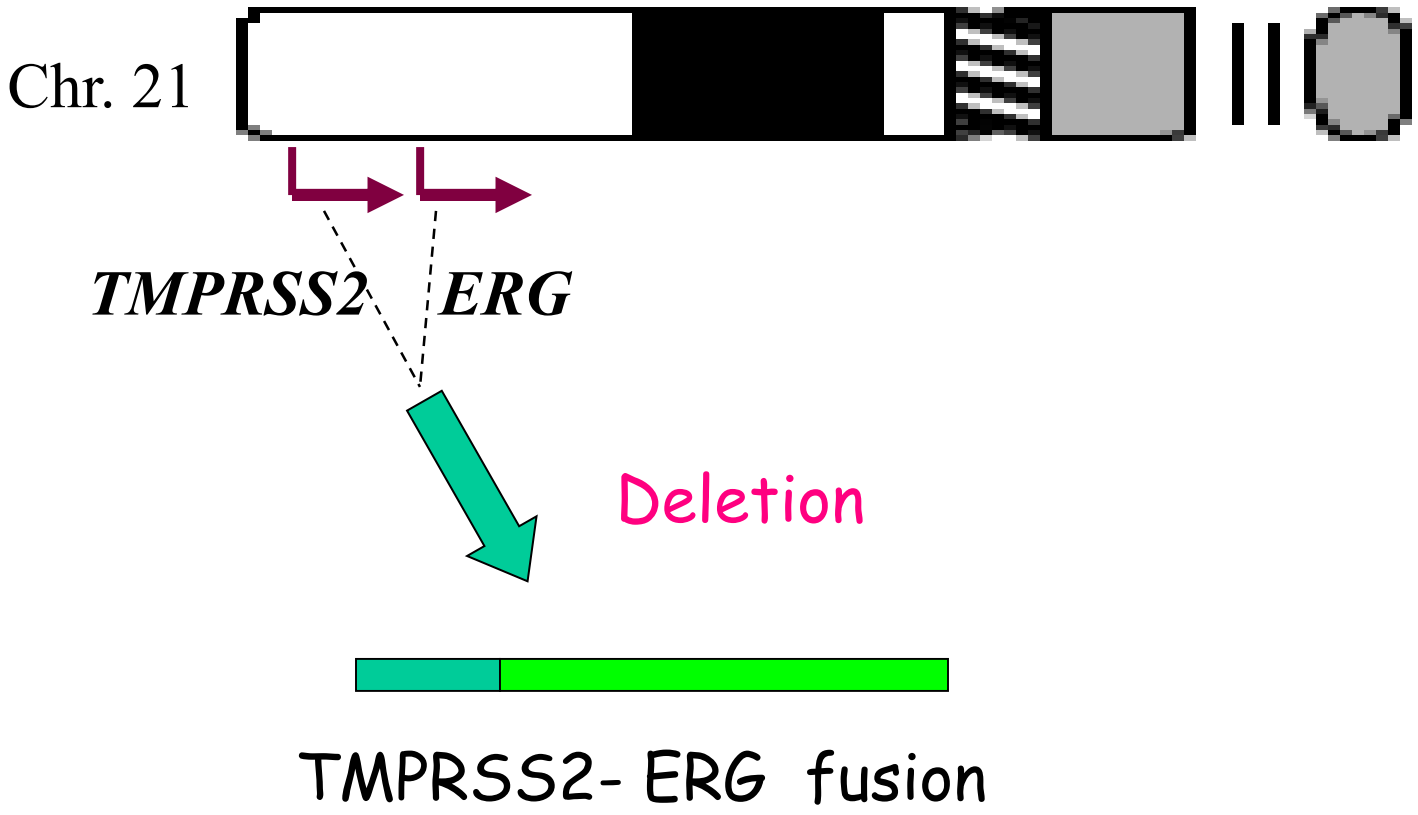


-> Activated tyrosine kinase

Iconic translocation; diagnostic of CML; target for drug Glivec/Gleevec

There are fusion genes in common epithelial cancers

TMPRSS2-ERG ~50% prostate cancers



There are fusion genes in common epithelial cancers

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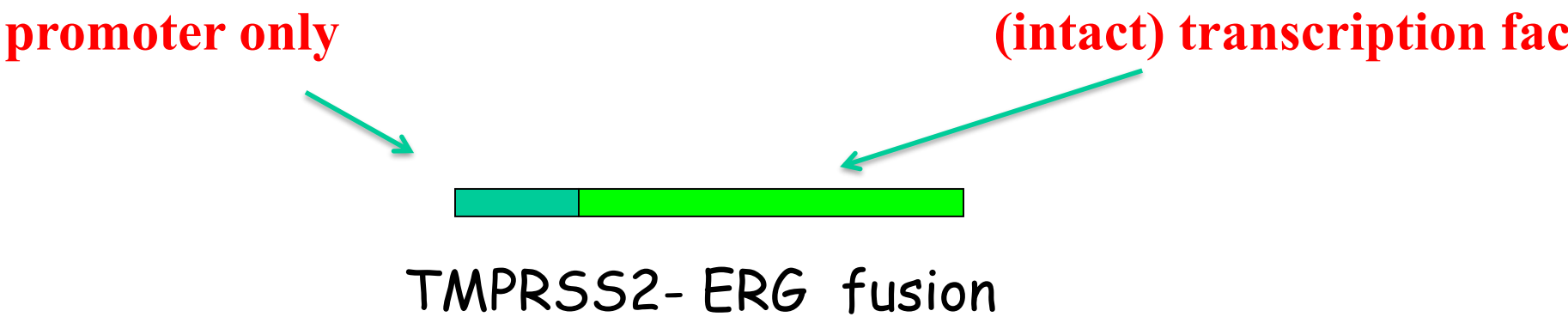


Note: Any rearrangement can form a fusion gene

-Deletion e.g. TMPRSS2-ERG

-Duplication (e.g. B-RAF)

-Inversion

-Chromosome translocation

...but also any rearrangement can inactivate a gene,
or may simply cause gene loss

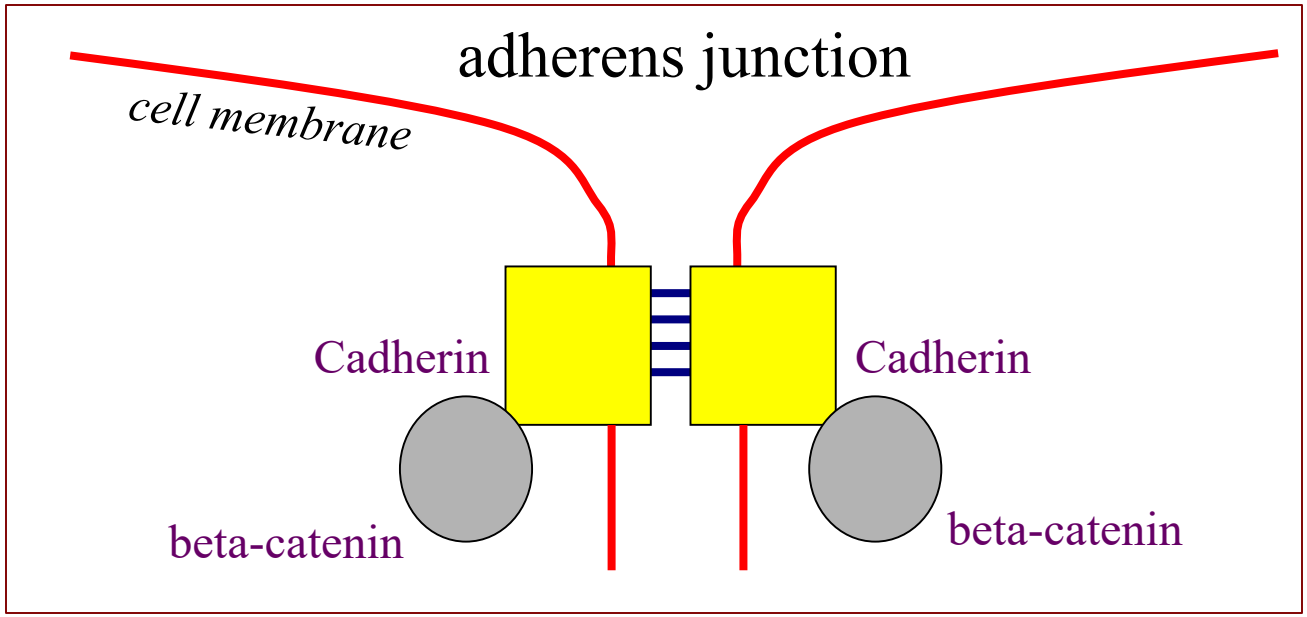
Rearrangement more often *IN*activates a gene

A rearrangement can inactivate a gene,
- by breaking it or
- by gene loss

Don’t forget **epigenetics !**

Examples of genes silenced by DNA methylation:

Gene	Function	Cancer
MLH1	mismatch DNA repair	colorectal
Cadherin E	cell-cell adhesion	lobular breast



Viruses

e.g.
several viruses encode proteins that block p53 and Rb:

Human papillomavirus(es) (HPVs) proteins E6, E7

Adenovirus E1a, E1b

and hot topic **LINE-1 retrotransposons?**

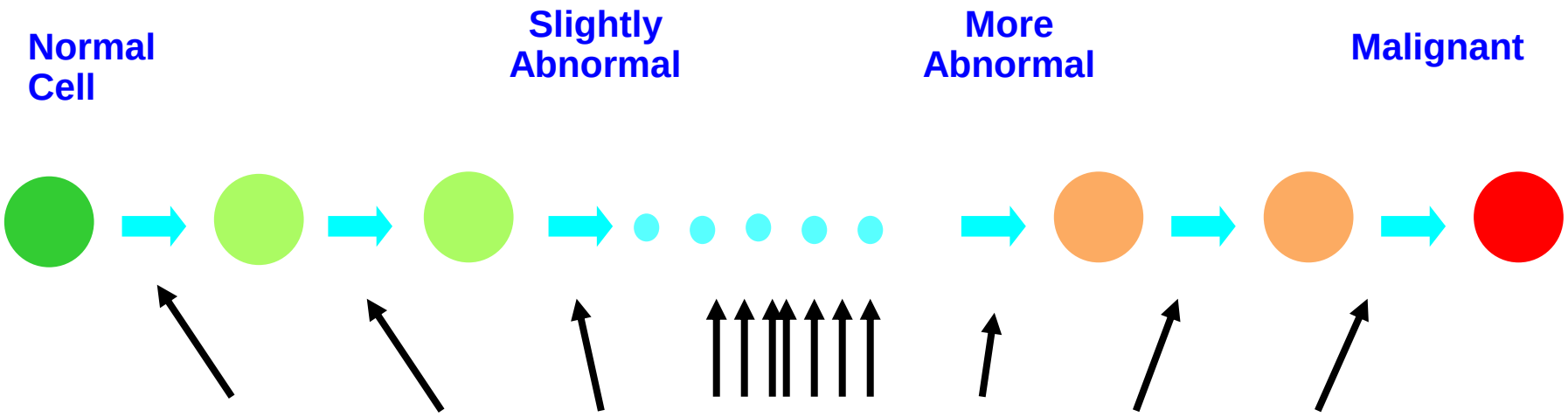
We know even less, but

LINE1 retrotransposons form 10 - 1000 new insertions in some tumours

can interrupt genes and turn genes on

watch this space!

Conclusion



> 10 Gene changes

small mutations + large mutations + epigenetic change + viruses + mobile elements

in all kinds of gene