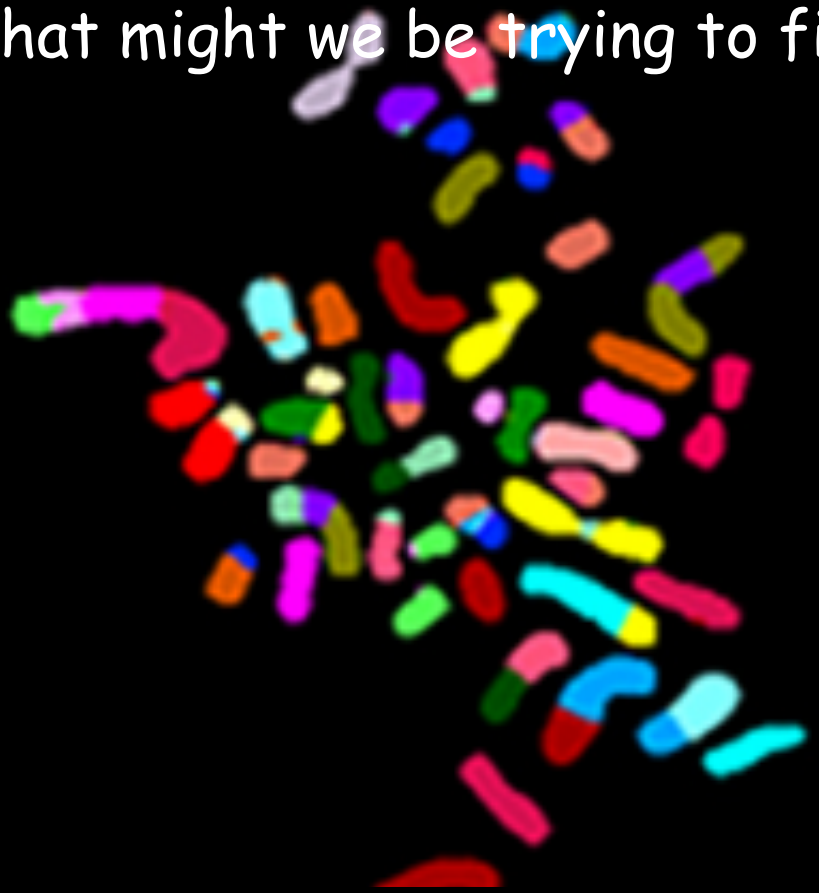
What's does the cancer genome look like and what might we be trying to find?

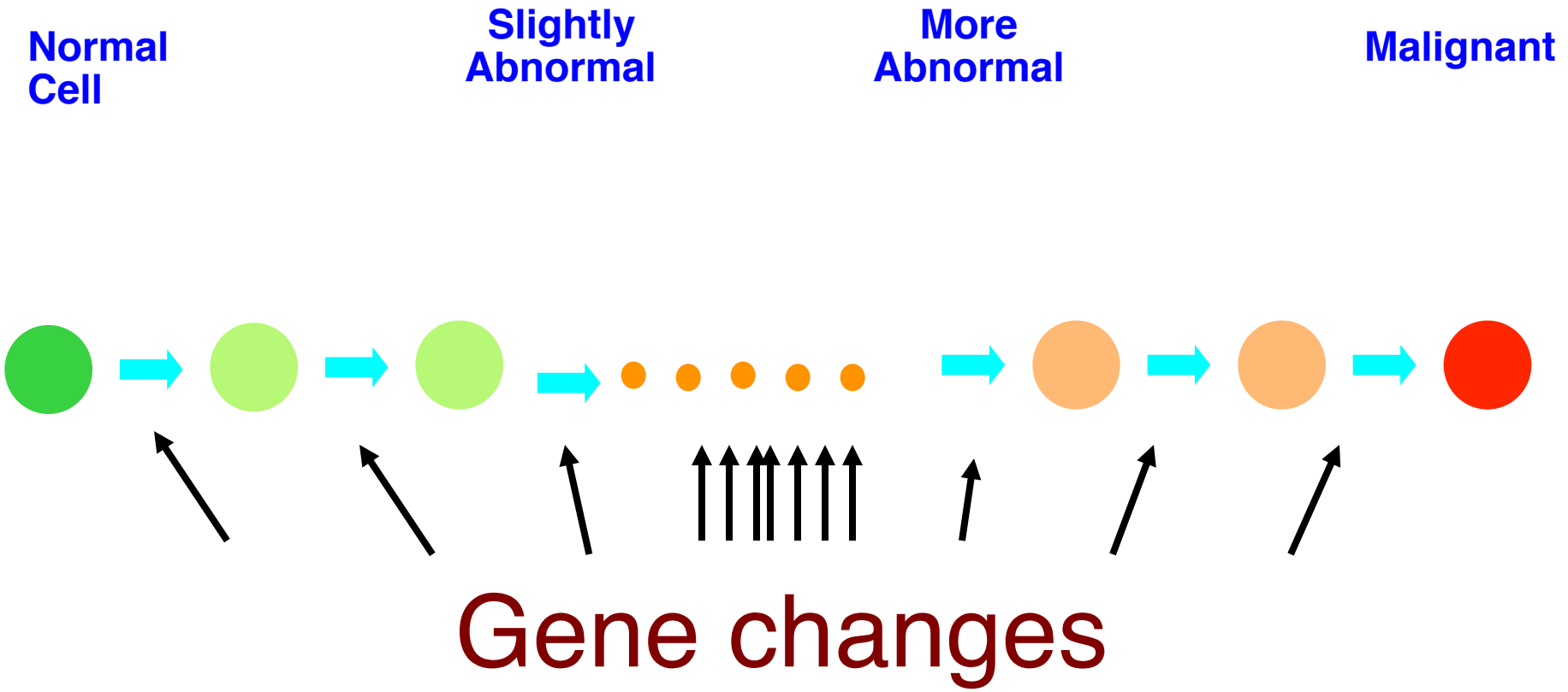


Paul Edwards

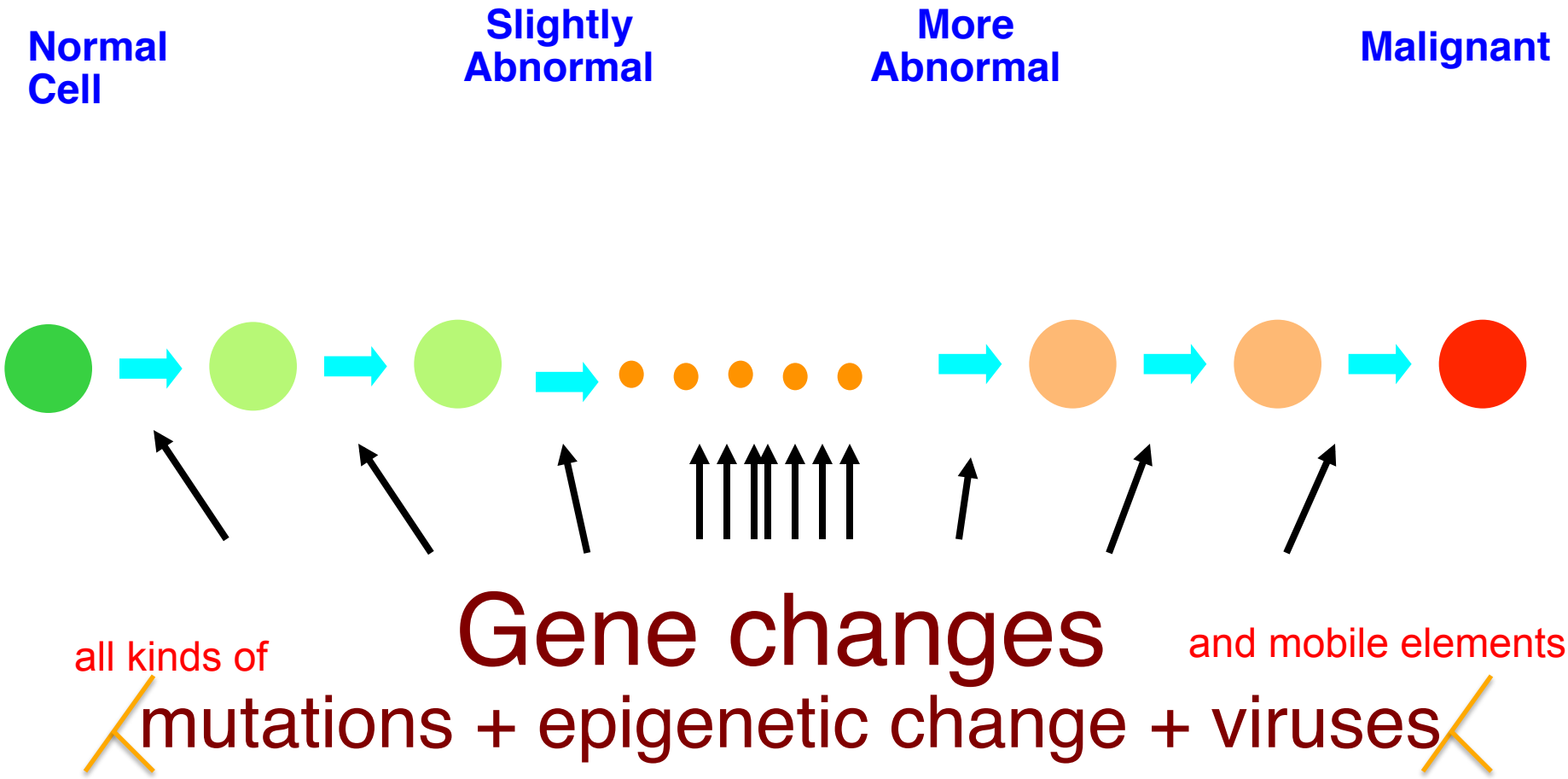
Department of Pathology and CRUK Cambridge Institute, University of Cambridge

1. How does cancer develop?

Development of a cancer

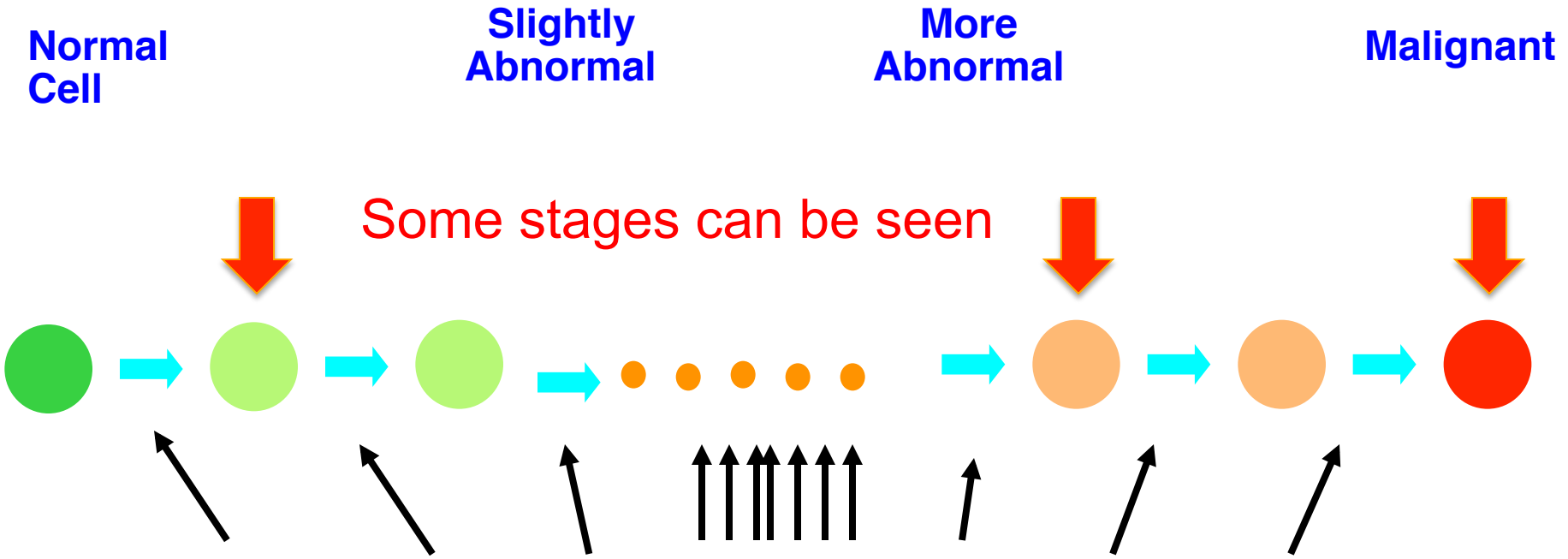


Development of a cancer



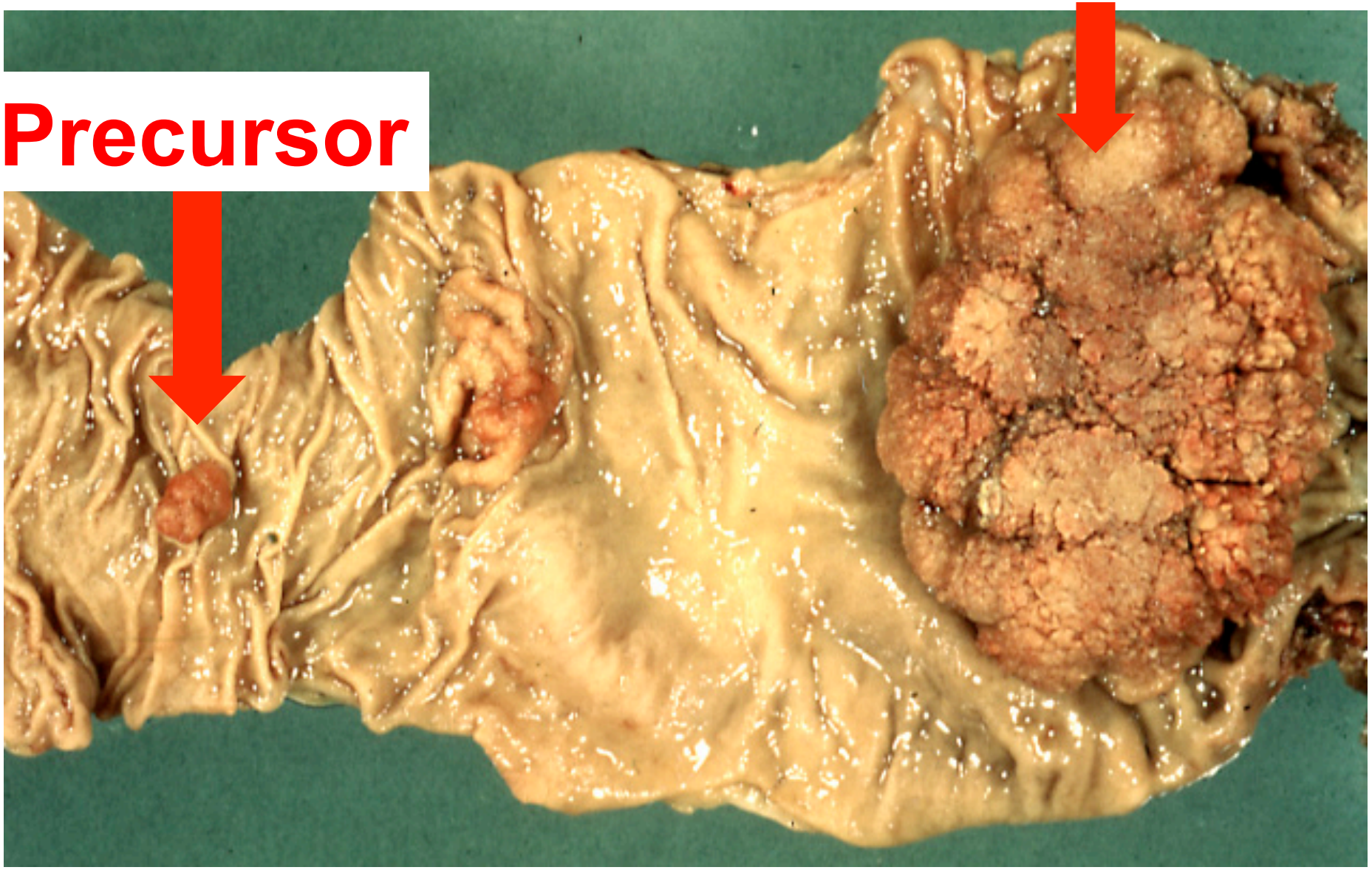
Cancer develops in multiple stages

Development of a cancer

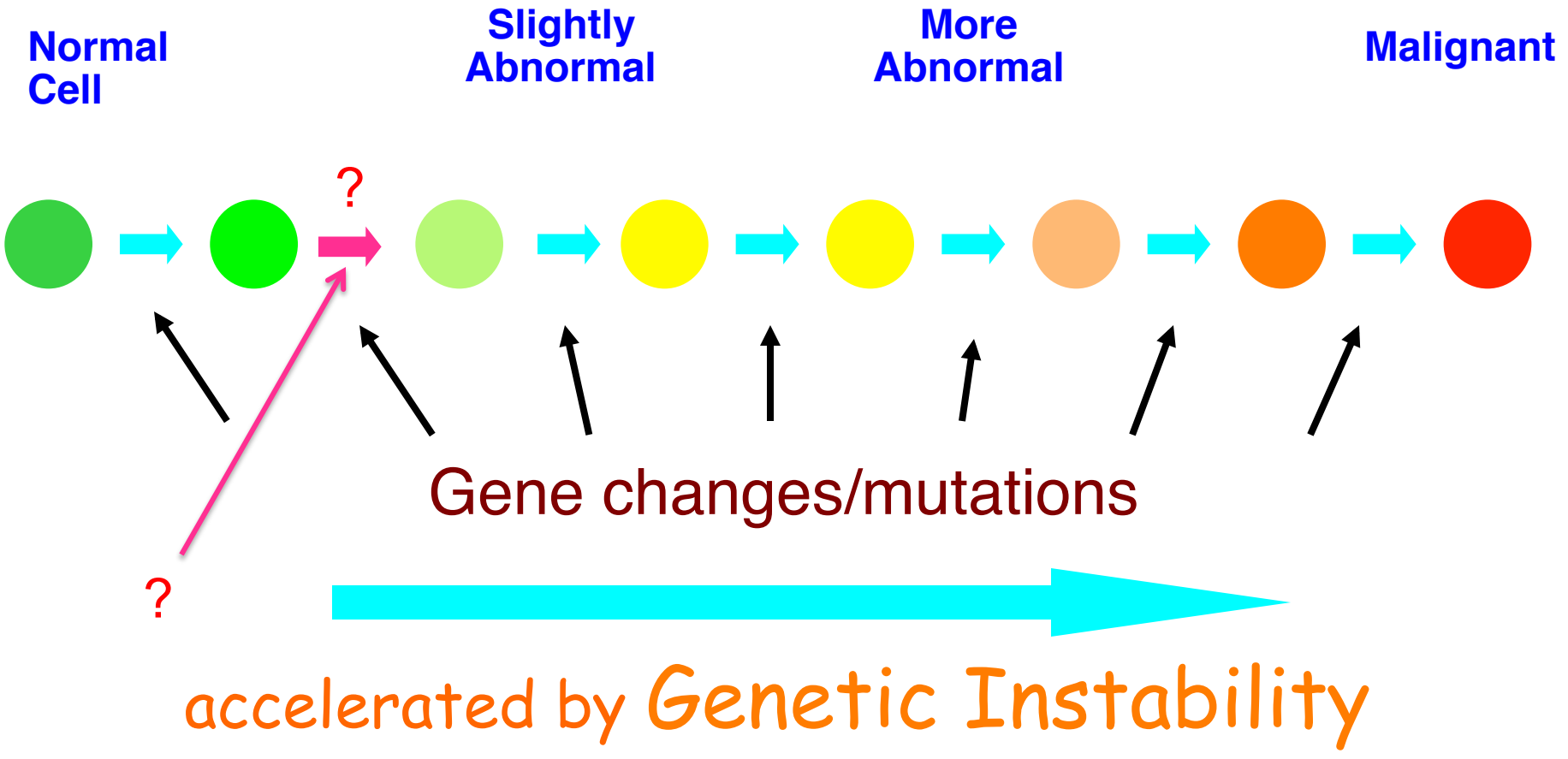


Gene changes

Colon/rectum cancer: malignant

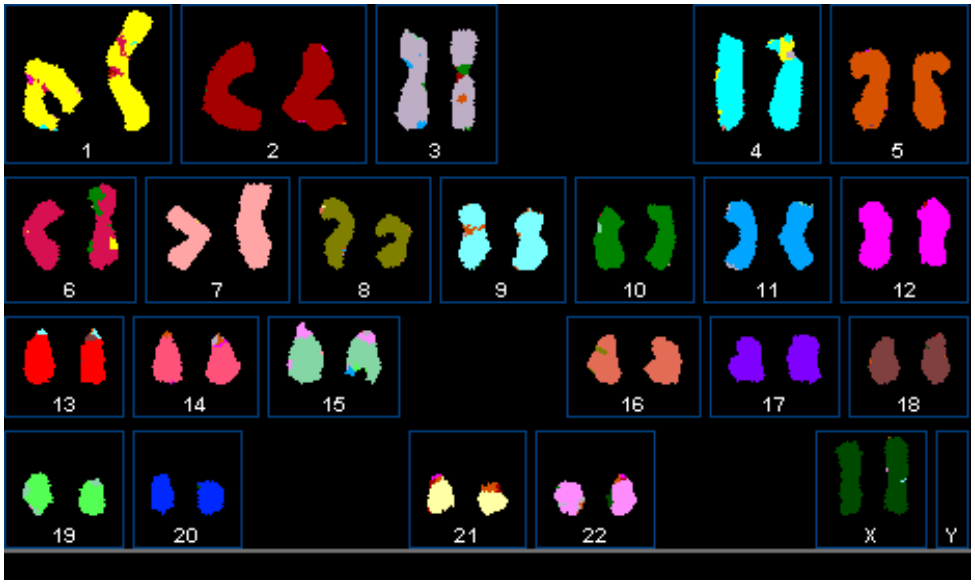


Development of a cancer



Many different types of Genetic Instability

Tumour A

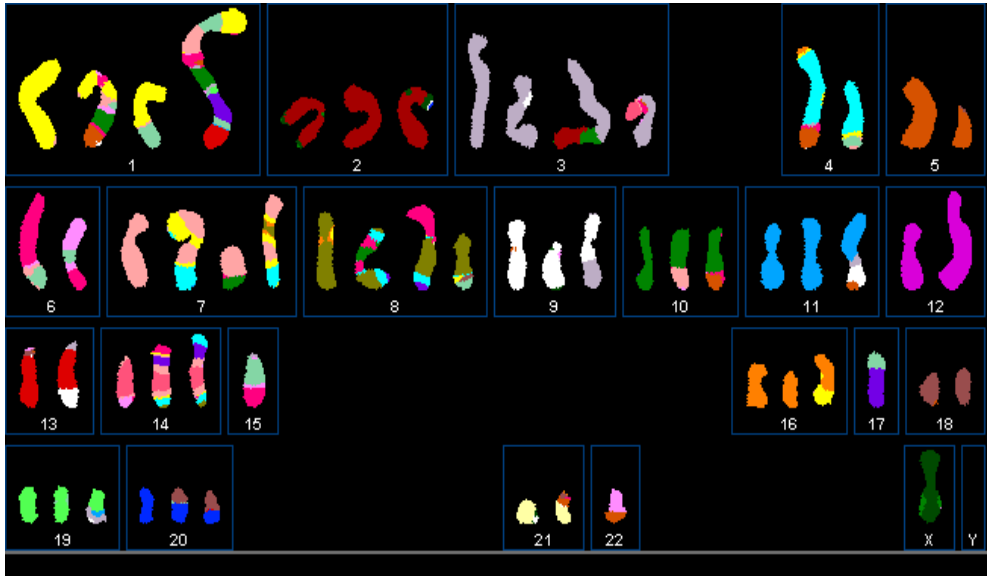


chromosomes stable

Sequence instability

100X rate sequence mutations

Tumour B



chromosome instability 'CIN'

Sequences (nearly) stable

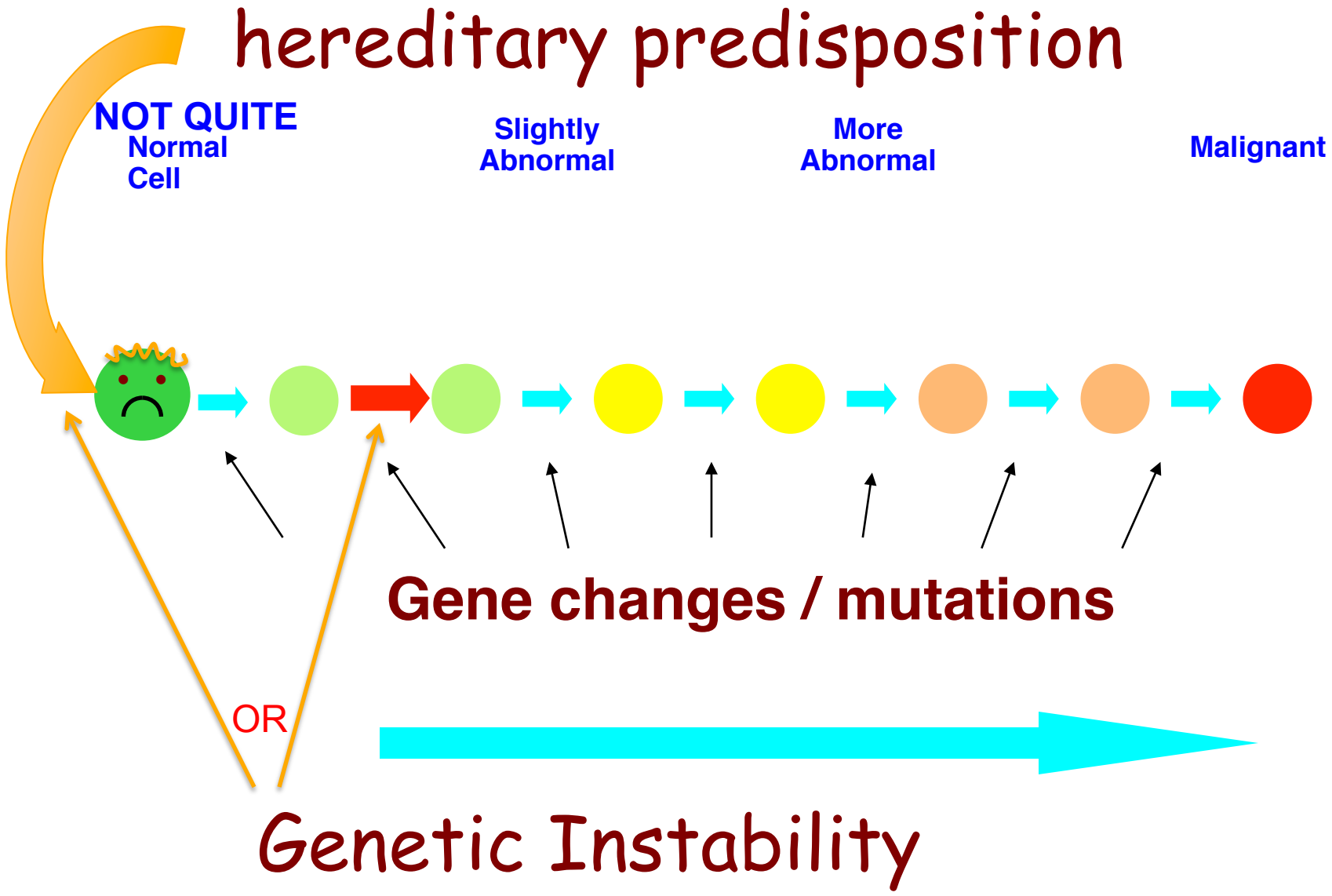
How could genetic instability come about?

	Sequence instability		chromosome instability	
Failure to repair DNA damage	√	e.g. mismatch repair	√	e.g. BRCA1, BRCA2
Errors in replication or mitosis	√	e.g. polymerase epsilon mutant	√	e.g. lagging chromosomes

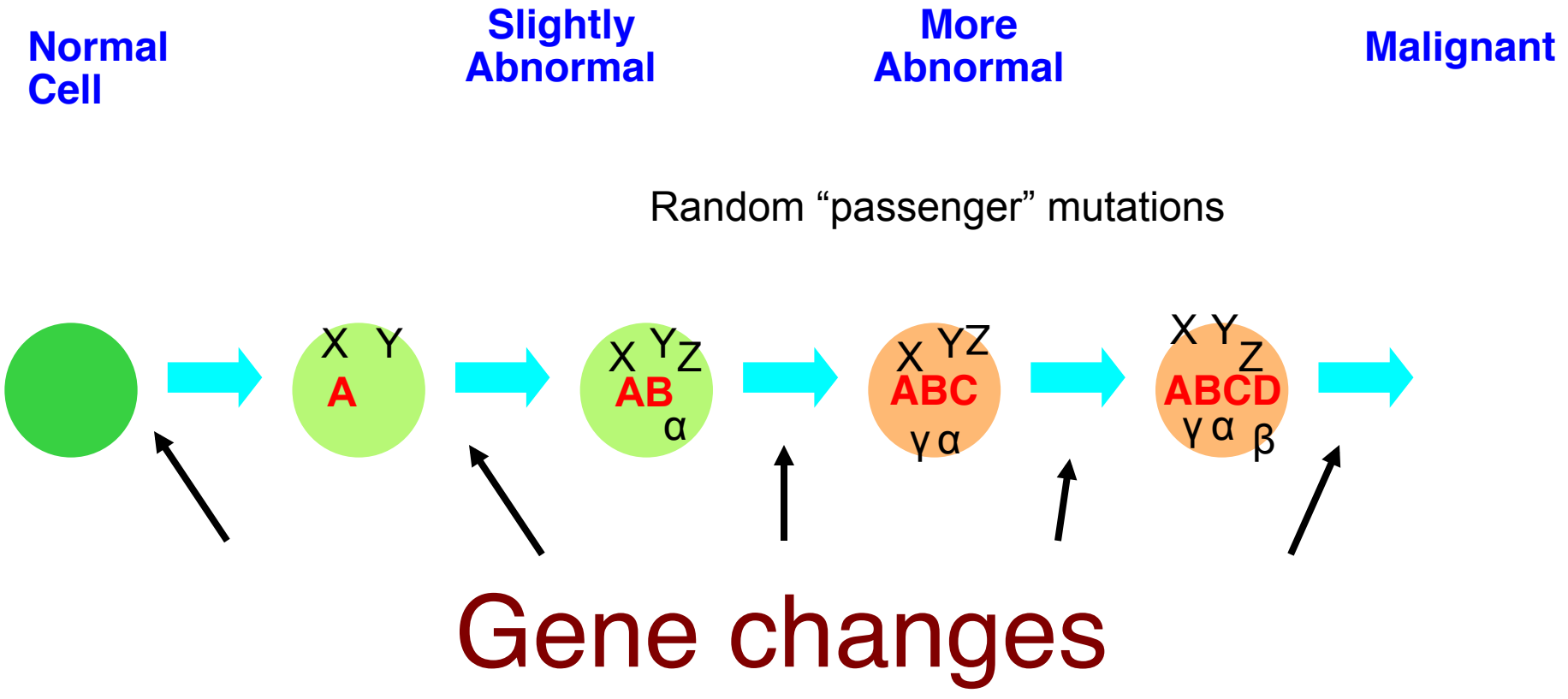
.....and there is probably ***Epi***genetic instability as well

e.g. DNMT, IDH mutations

=> Genetic instability may determine the pattern of mutation and be a target for therapy, so it's one of the things we can look for


hereditary predisposition
NOT QUITE
Normal
Cell
Slightly
Abnormal
More
Abnormal
Malignant
Gene changes / mutations
OR
Genetic Instability

Passengers versus Drivers



We usually distinguish gain of function and loss of function mutations:
Oncogenes and Tumour Suppressor genes

Definitions vary but one is:

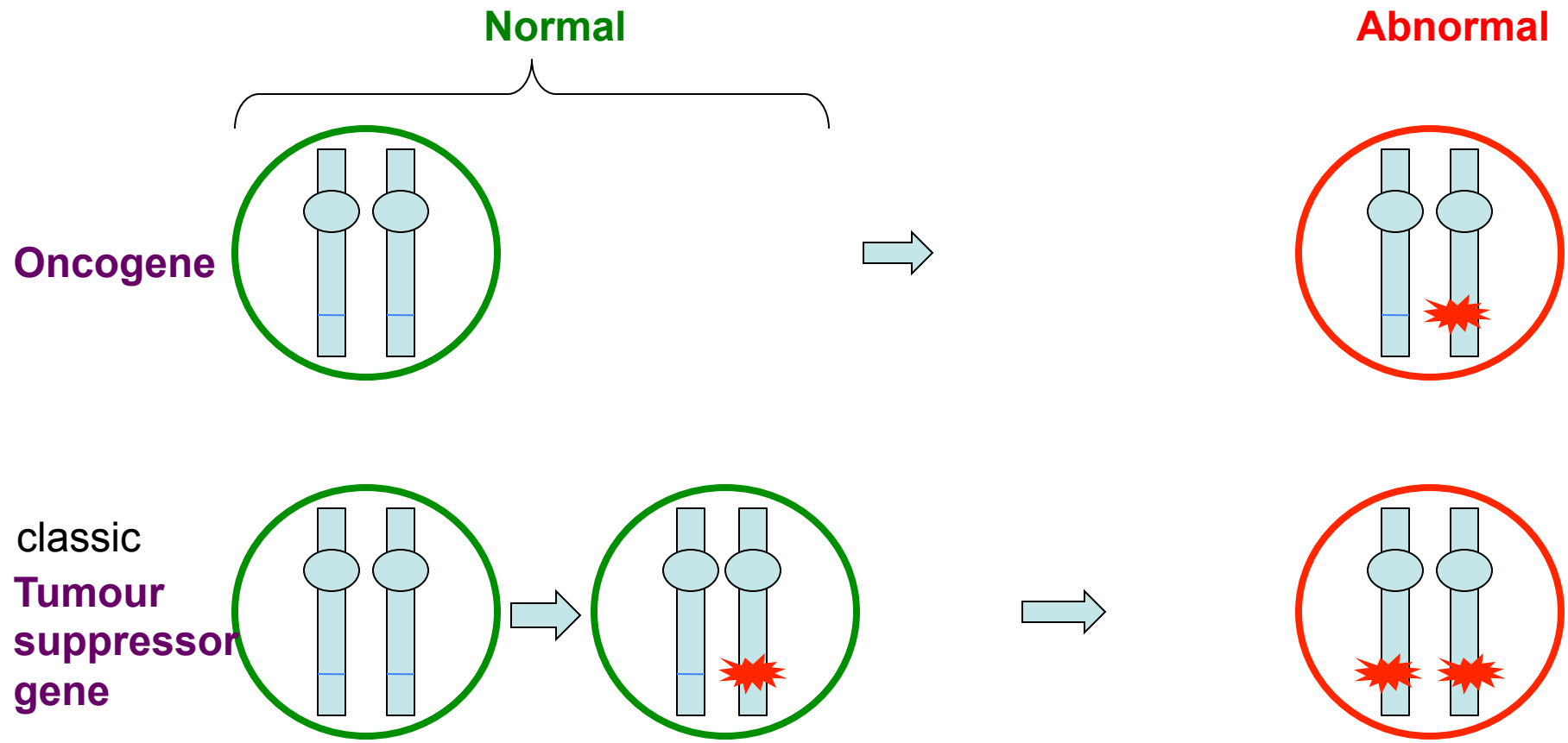
Oncogene mutations are overactivity mutations

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

Tumour Suppressor Gene mutations are loss of function mutations

- generally both copies are mutated, recessive in the cell

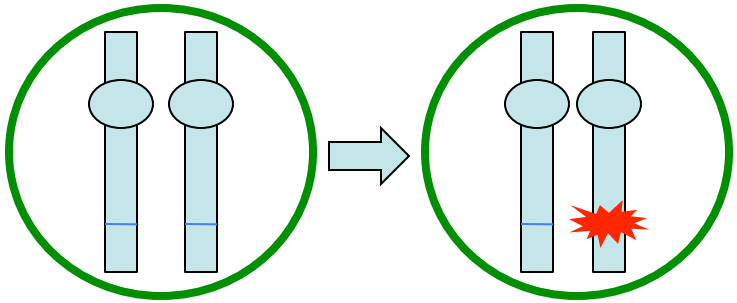
Oncogenes versus Tumour Suppressor Genes



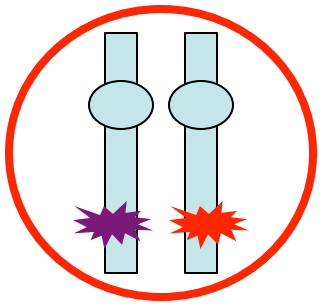
Tumour Suppressor Genes – loss of two copies often results in **loss of heterozygosity "LOH"**,

i.e. region around tumour suppressor becomes homozygous

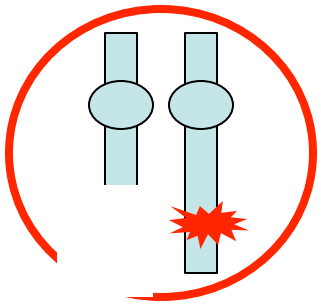
Tumour suppressor gene



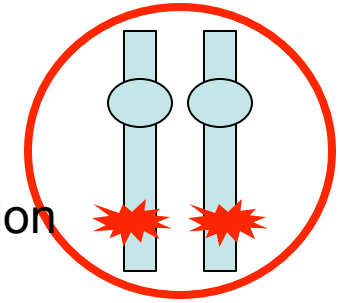
2 mutations



loss of normal



copy mutant region



loss of heterozygos "LOH"

2. What do cancer mutations look like?

Small-scale changes

STRUCTURAL changes

-Deletion

-Duplication, Tandem or Foldback

-Amplification (lot of copies of gene)

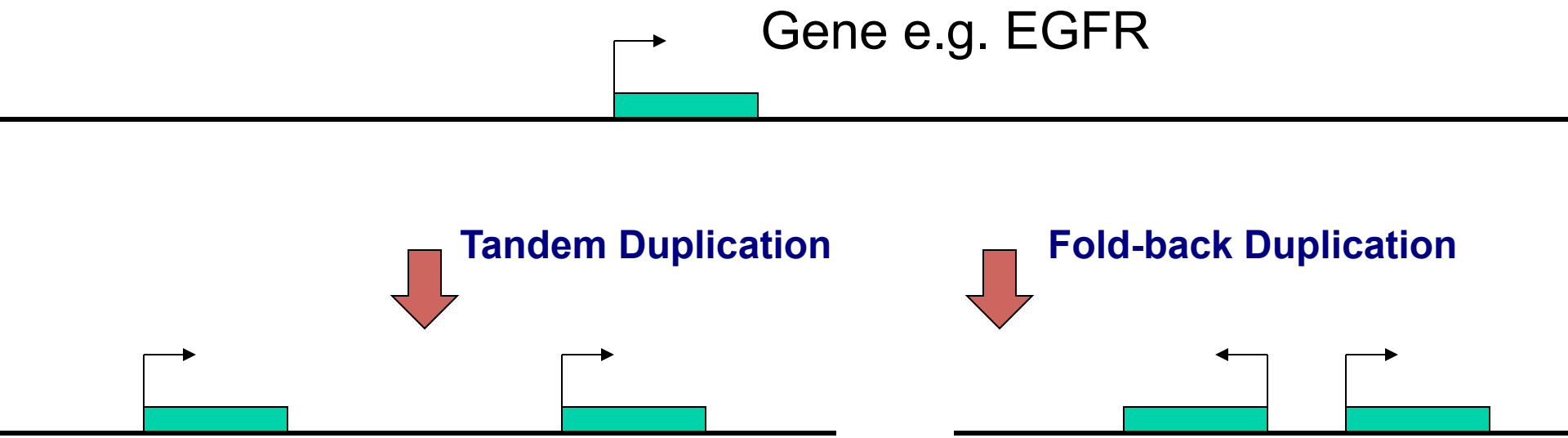
-Inversion

-Chromosome translocation

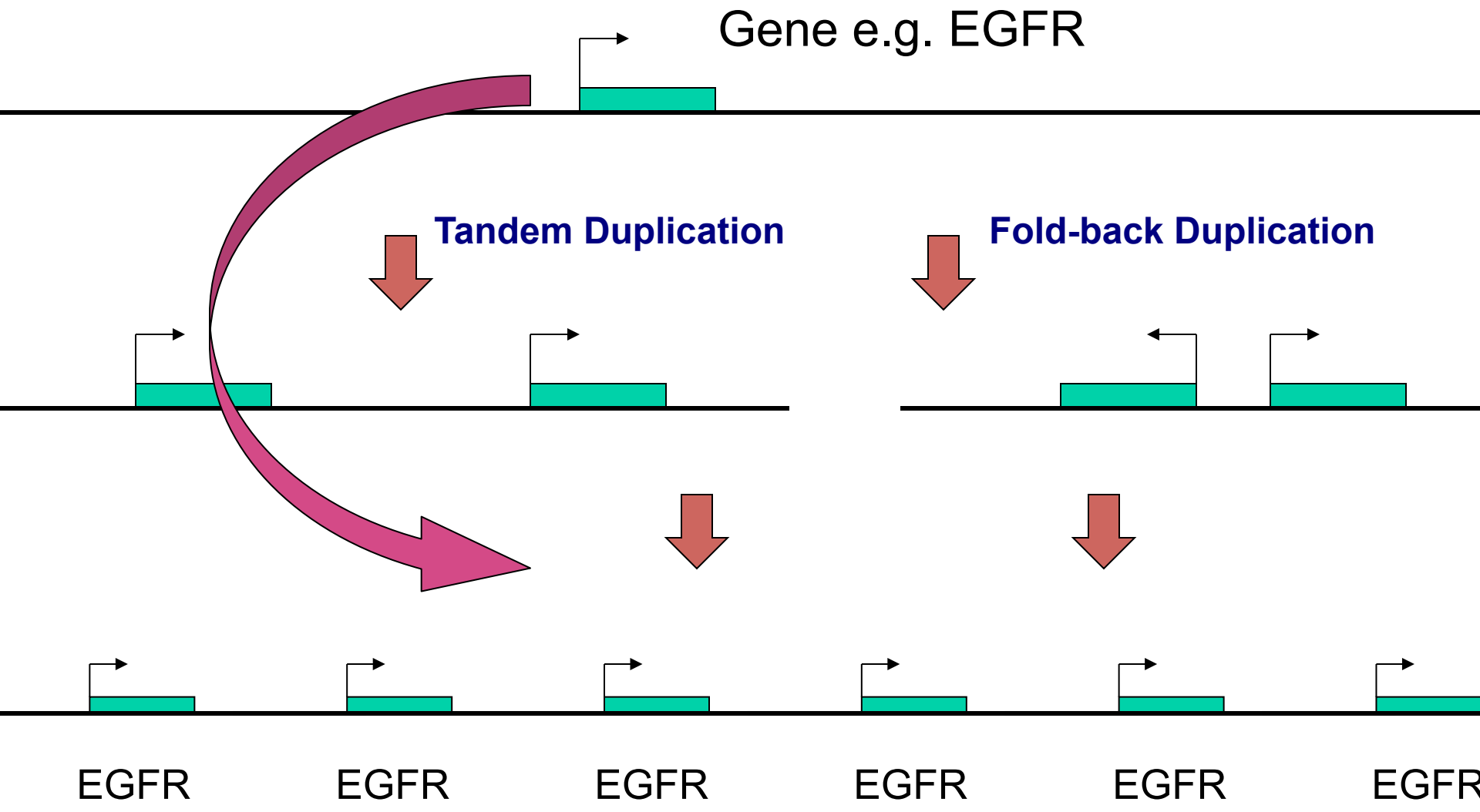
-Mobile element insertion

Large-scale changes

Duplication and amplification

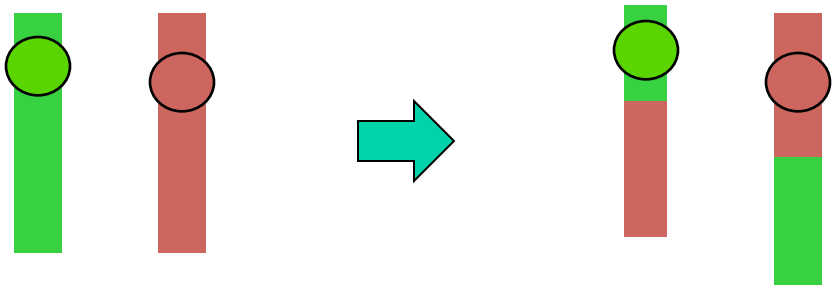


Duplication and amplification



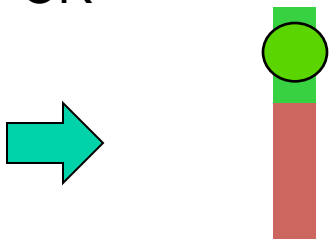
'Amplified' gene

Chromosome translocation



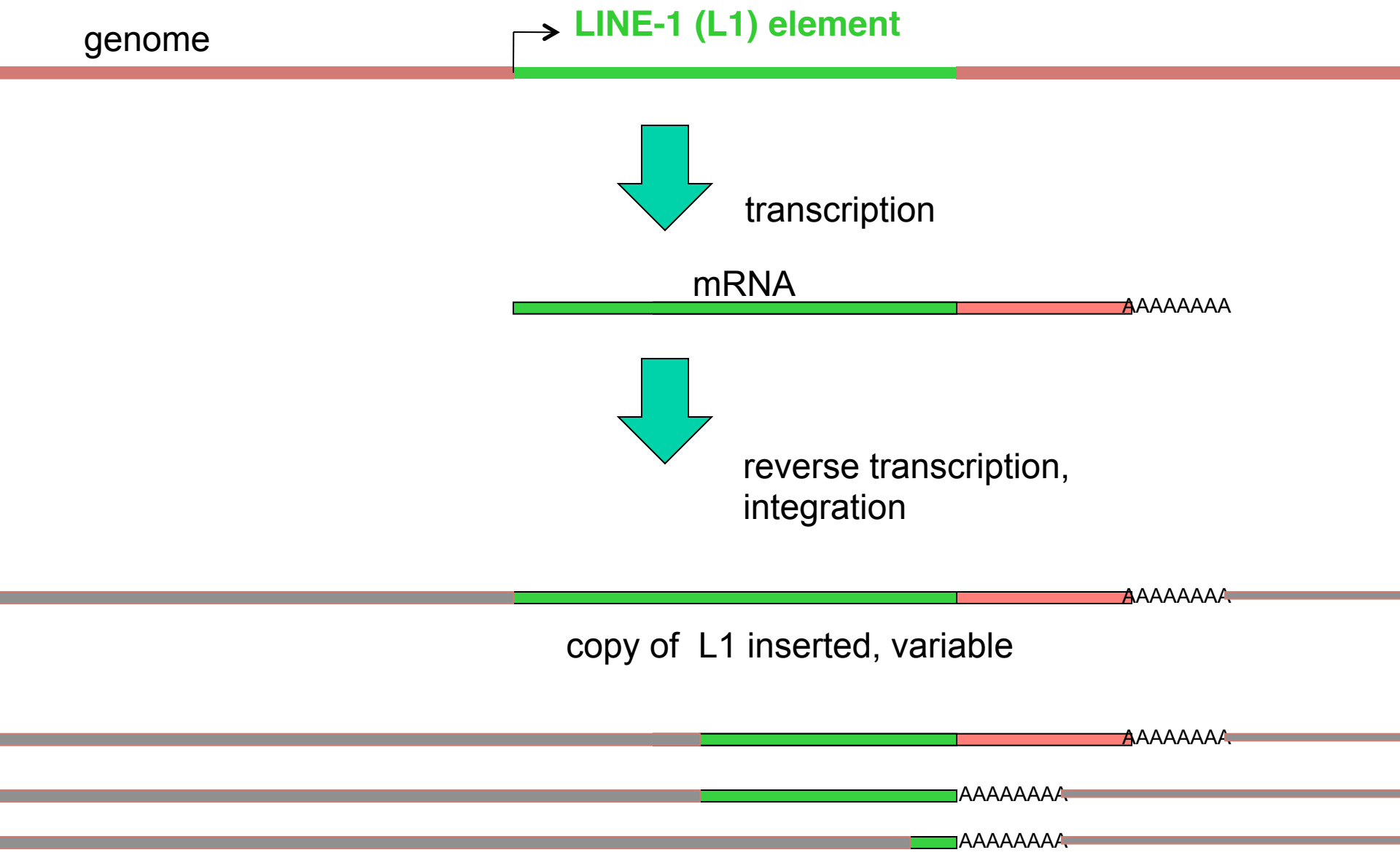
reciprocal translocation

OR



unbalanced translocation

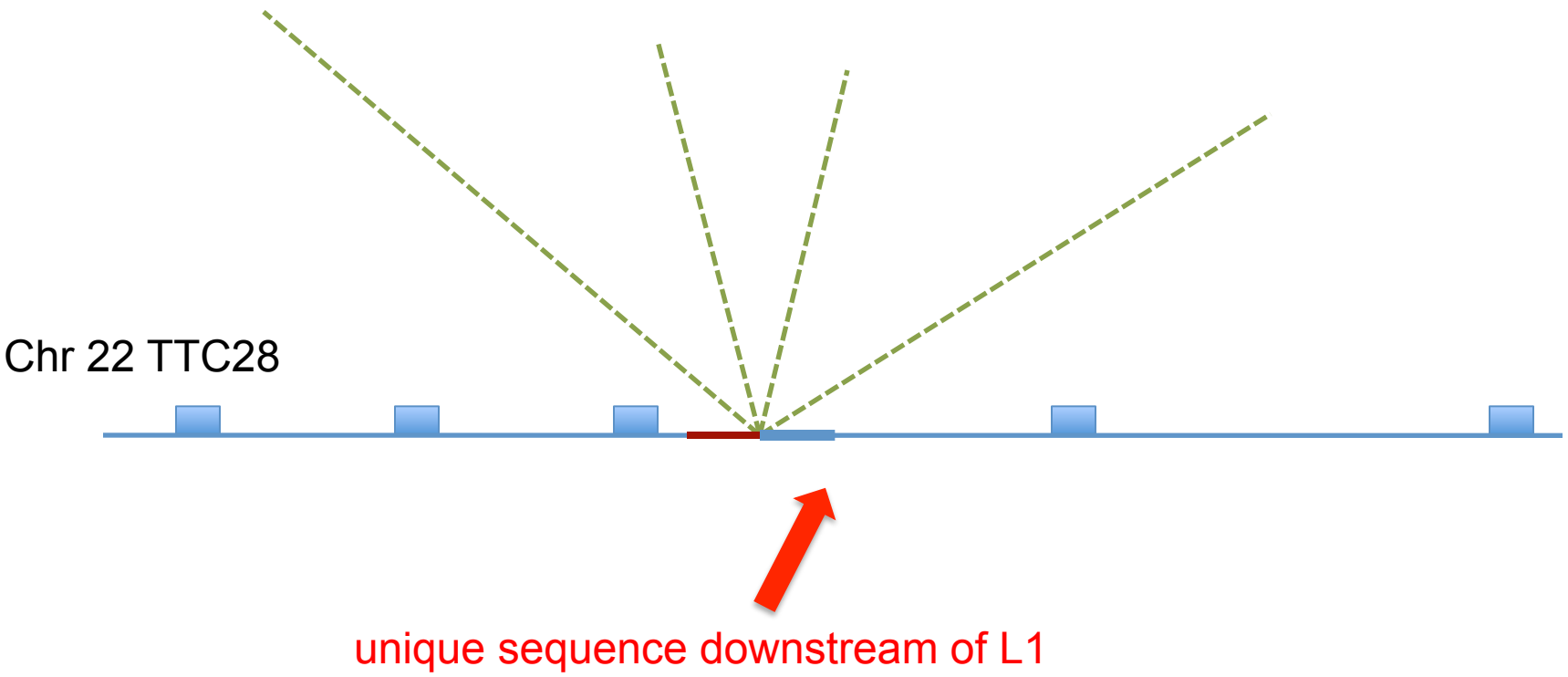
Mobile element insertion



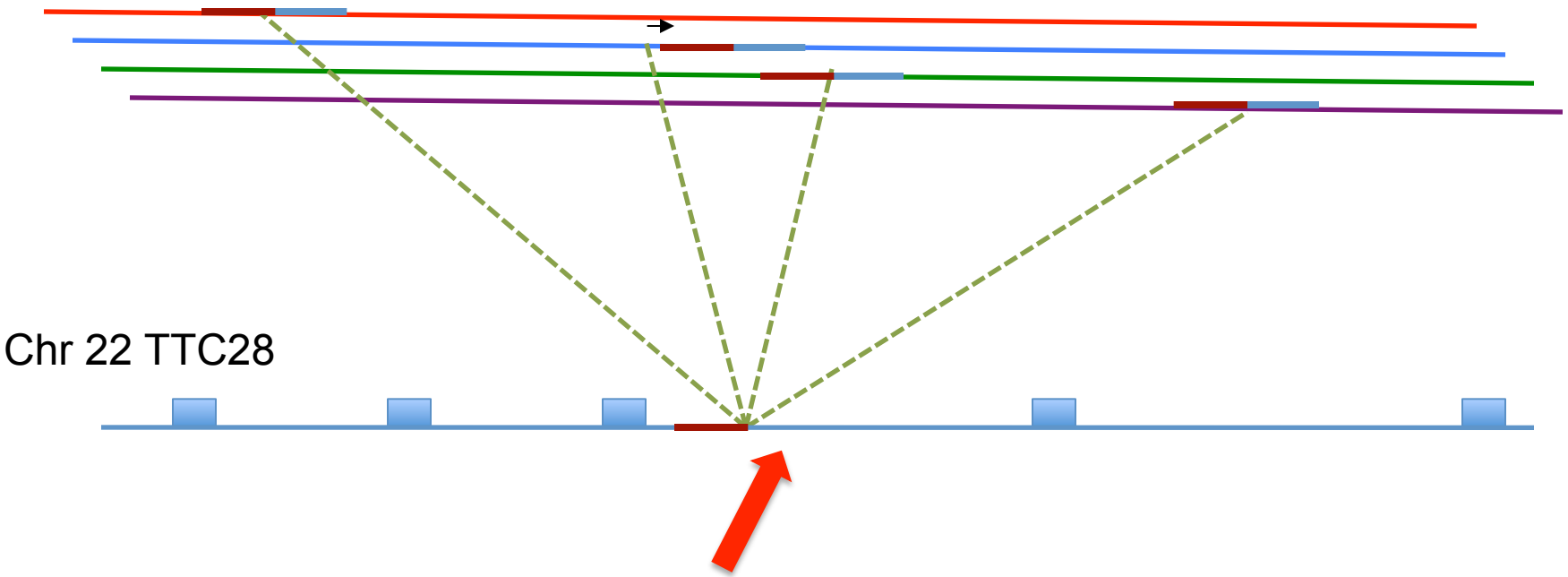
L1 transduction looks like multiple translocations

Chr 22 TTC28

L1 transduction looks like multiple translocations



L1 transduction looks like multiple translocations



unique sequence downstream of L1

2. What do cancer mutations look like?

Small-scale changes

STRUCTURAL changes

-Deletion

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-Amplification (lot of copies of gene)

-Inversion

-Chromosome translocation

-Mobile element insertion

Large-scale changes

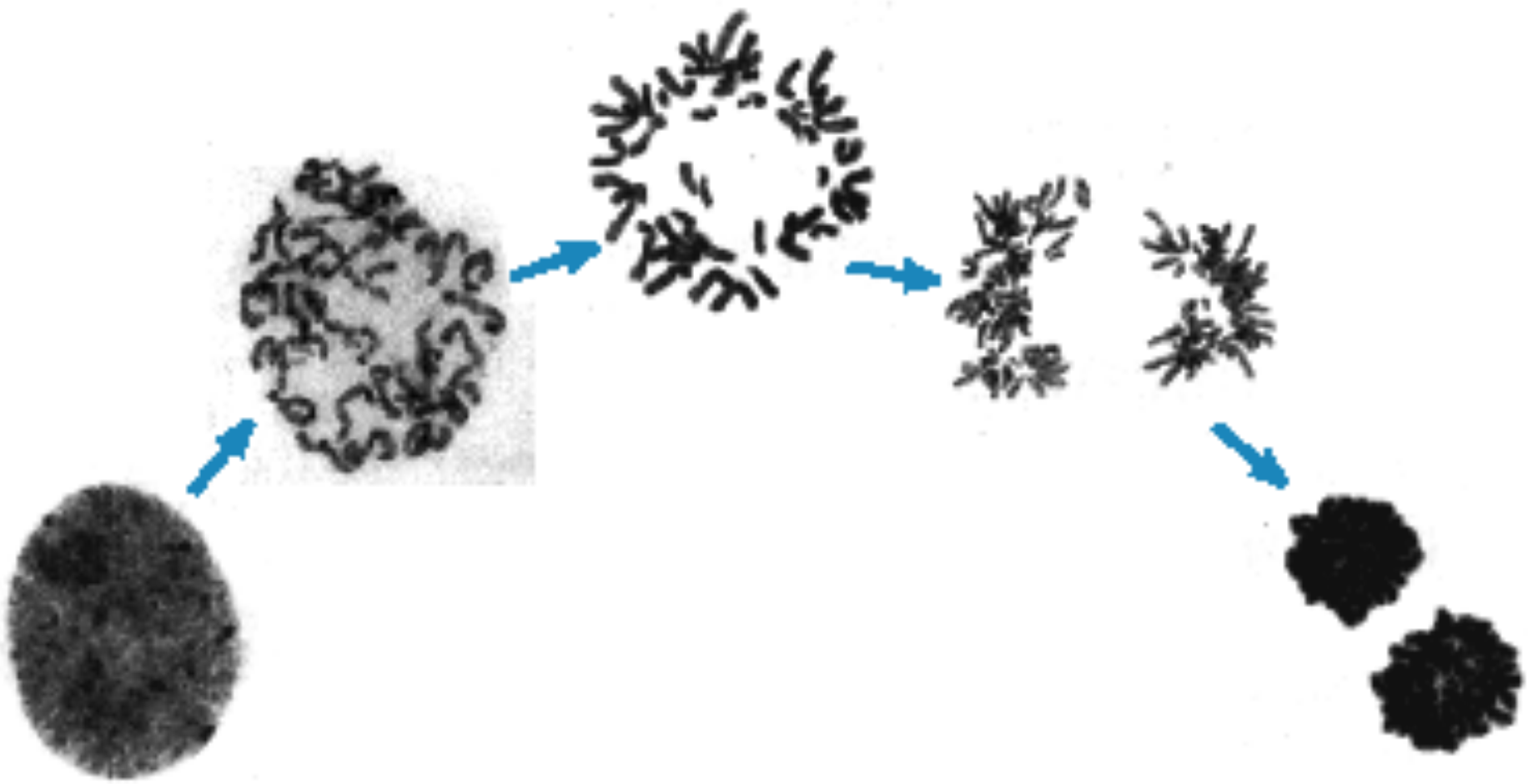
3.Methods available

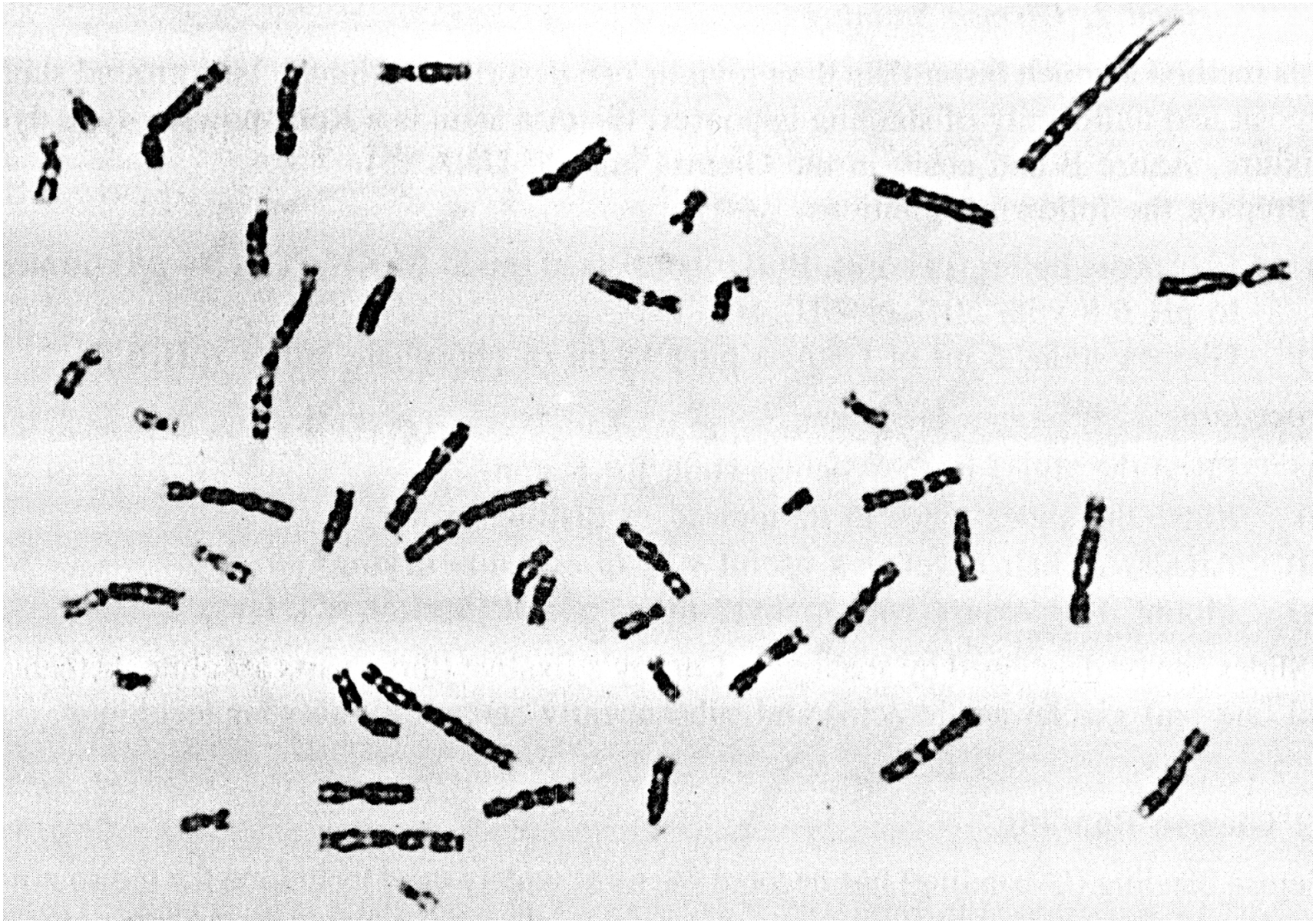
3.Methods available

Sequencing: PCR+Sanger, Illumina, long-read methods
Cytogenetics
FISH
Chromsosome sorting
Arrays: CGH, SNP arrays (mainly copy number counting)
Mapping, HAPPY mapping and 10X

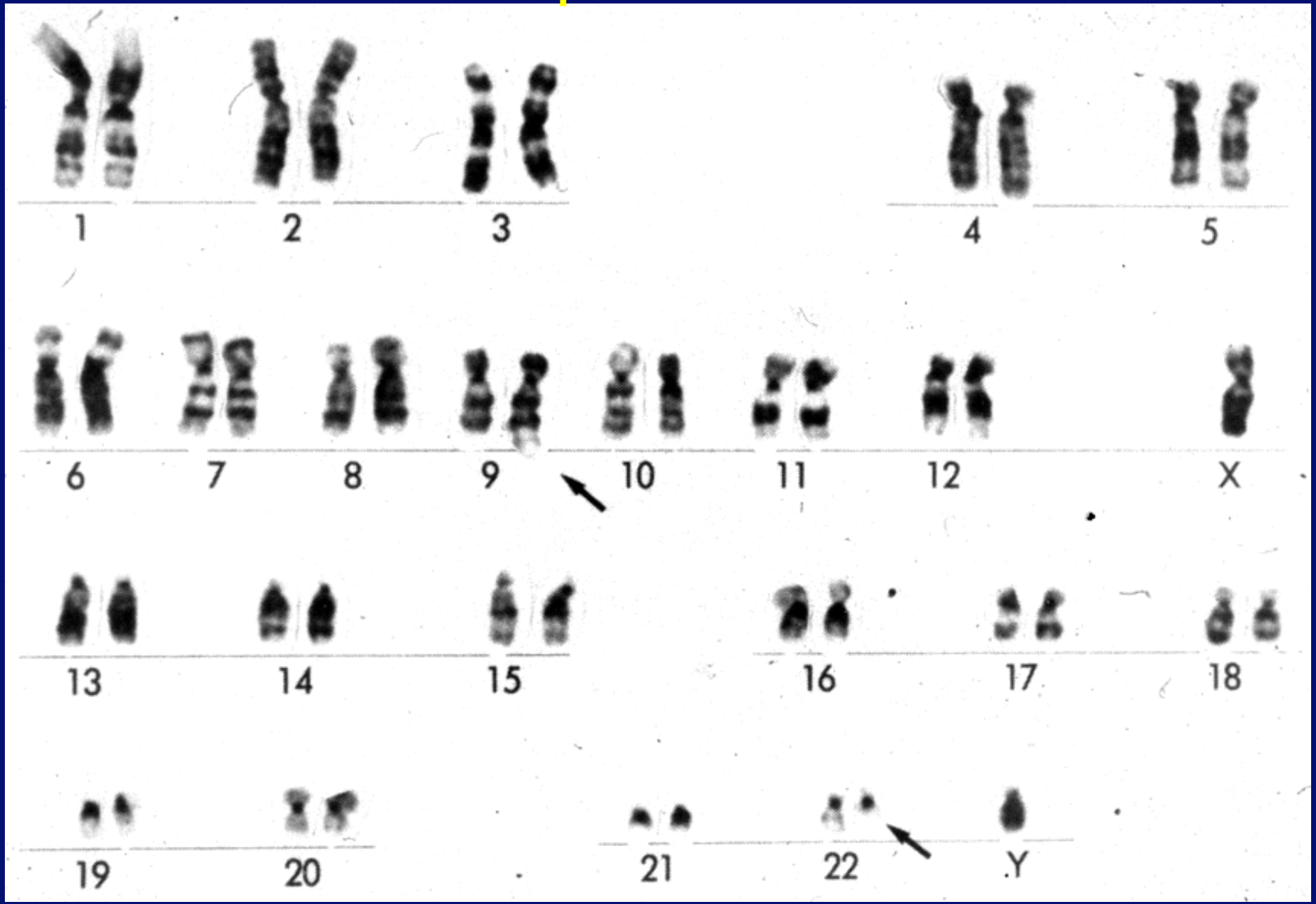
Epigenetics: bisulphite sequencing

Metaphase chromosomes

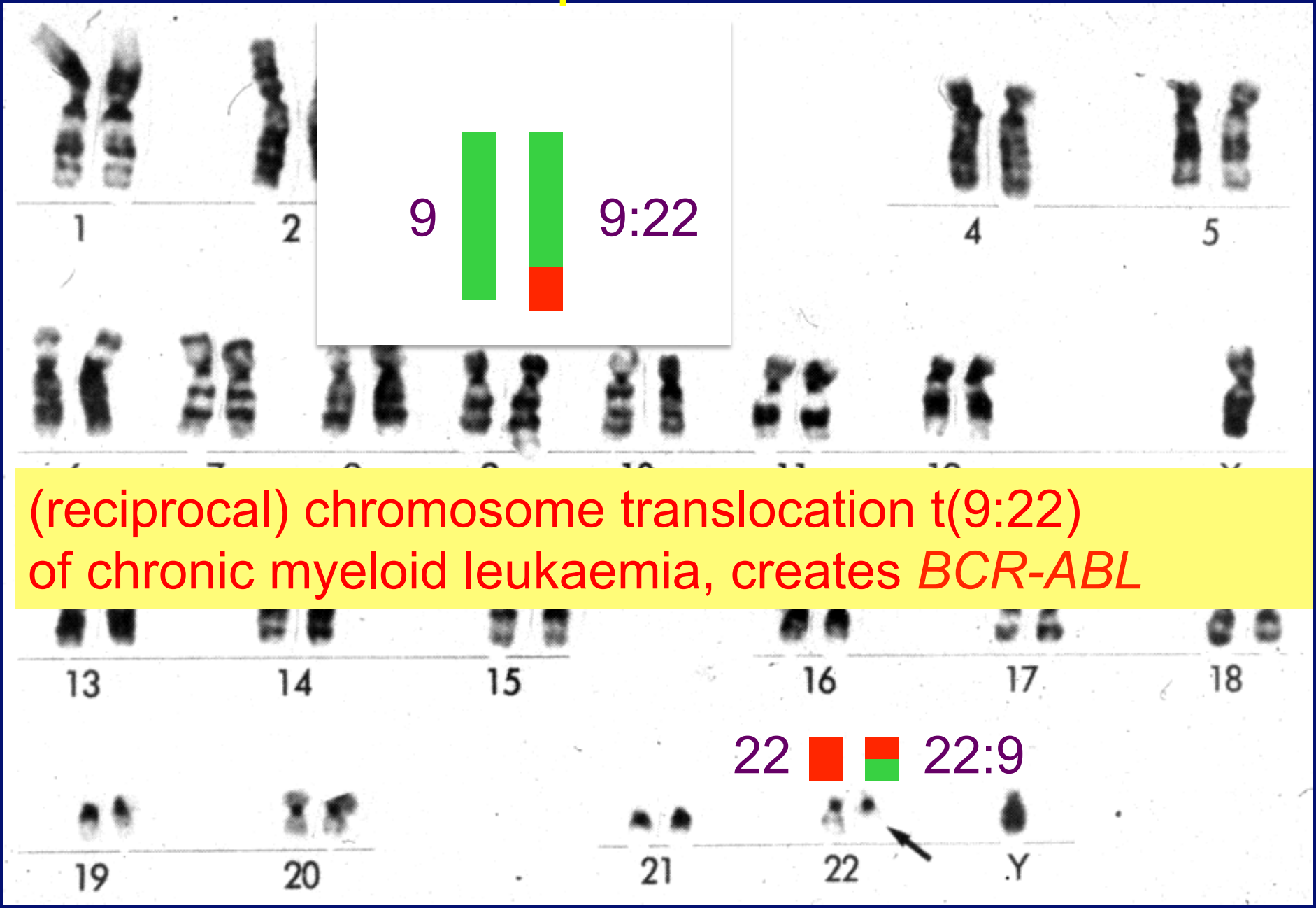




Philadelphia chromosome

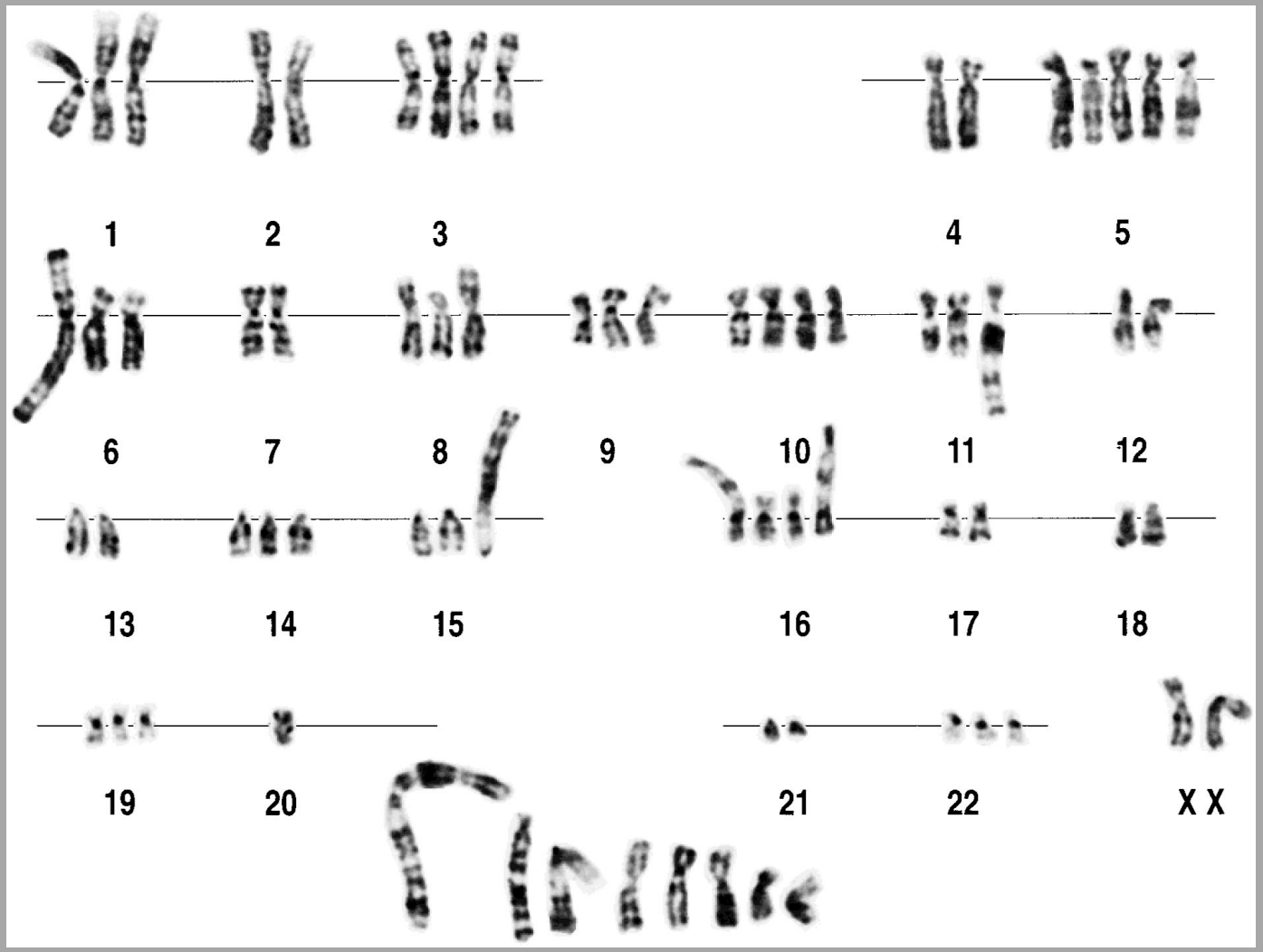


Philadelphia chromosome



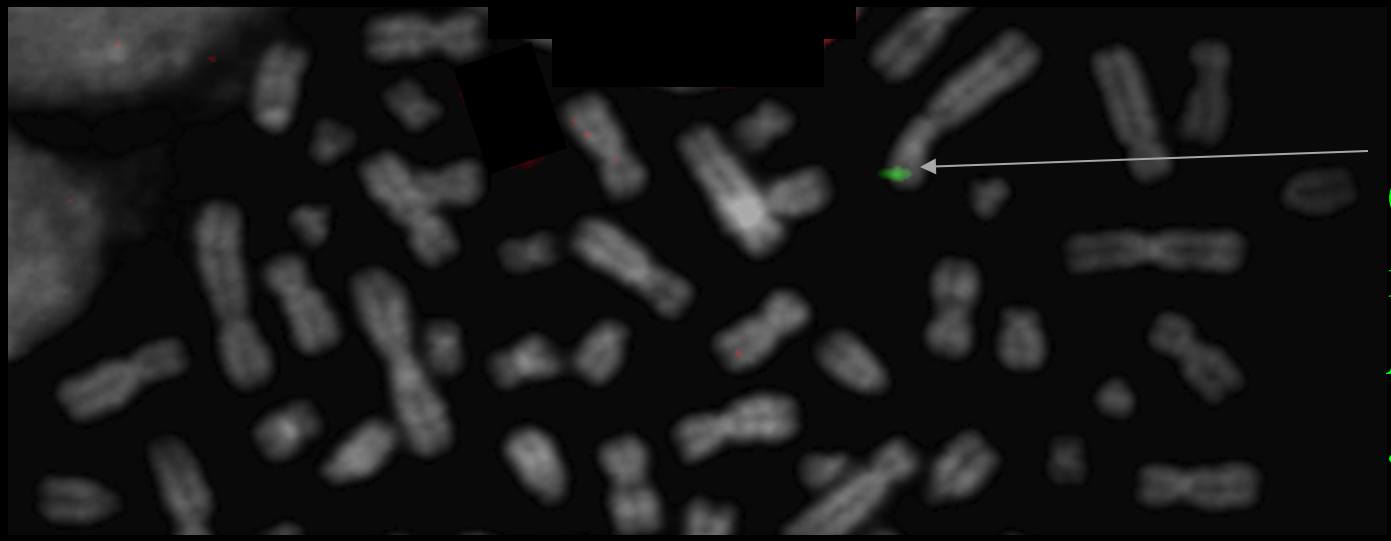
(reciprocal) chromosome translocation t(9:22)
of chronic myeloid leukaemia, creates *BCR-ABL*

Breast Cancer Karyotype, from primary culture



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

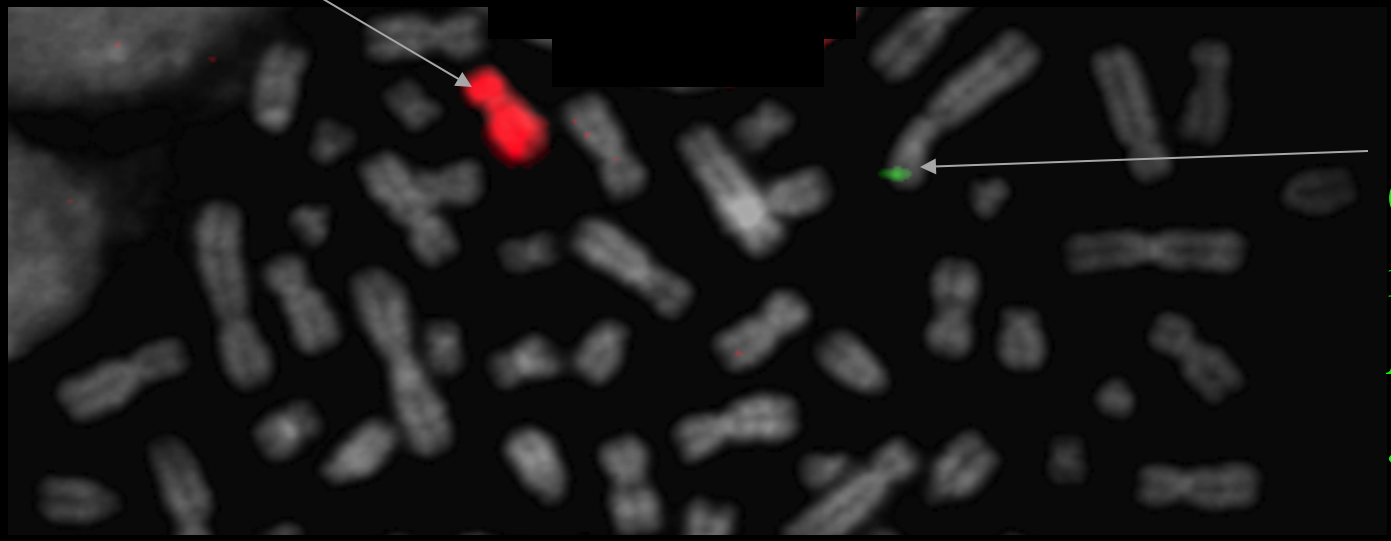
‘ FISH’ fluorescence-in situ hybridisation



100kb bit
Of Chr 2
including
N-MYC
gene

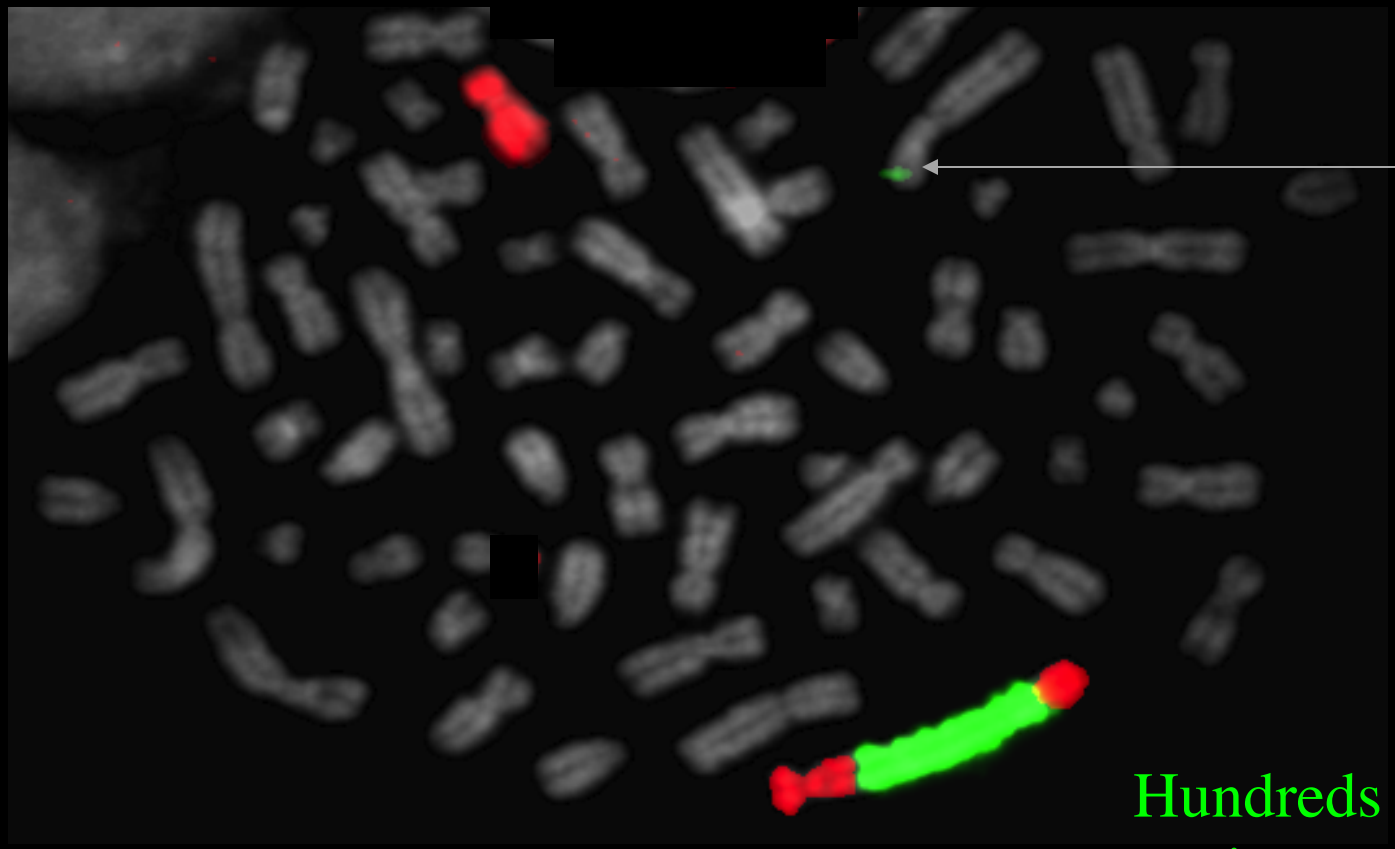
‘ FISH’ fluorescence-in situ hybridisation

Chr 12

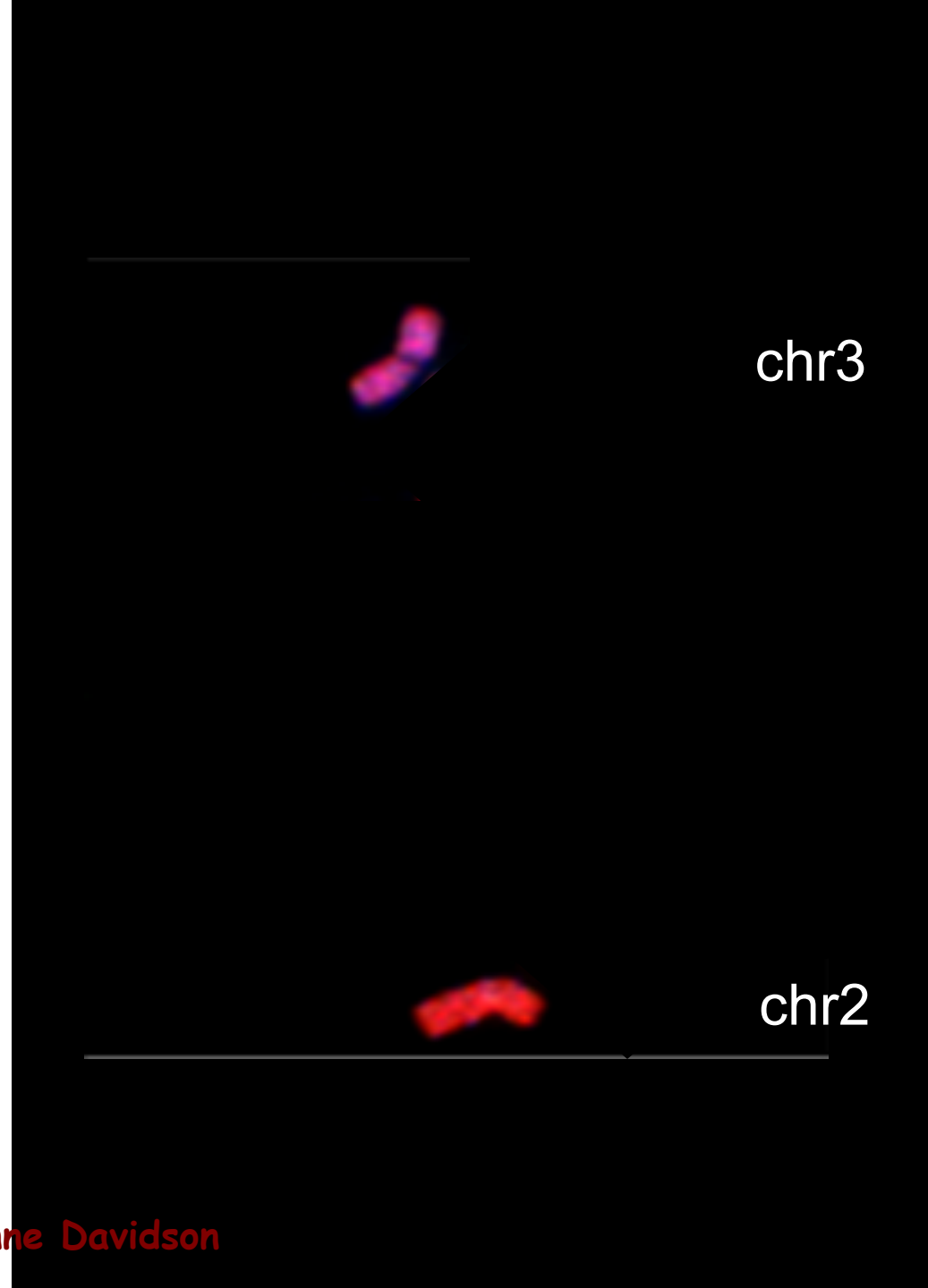


100kb bit
Of Chr 2
including
N-MYC
gene

‘Amplification’ of N-MYC

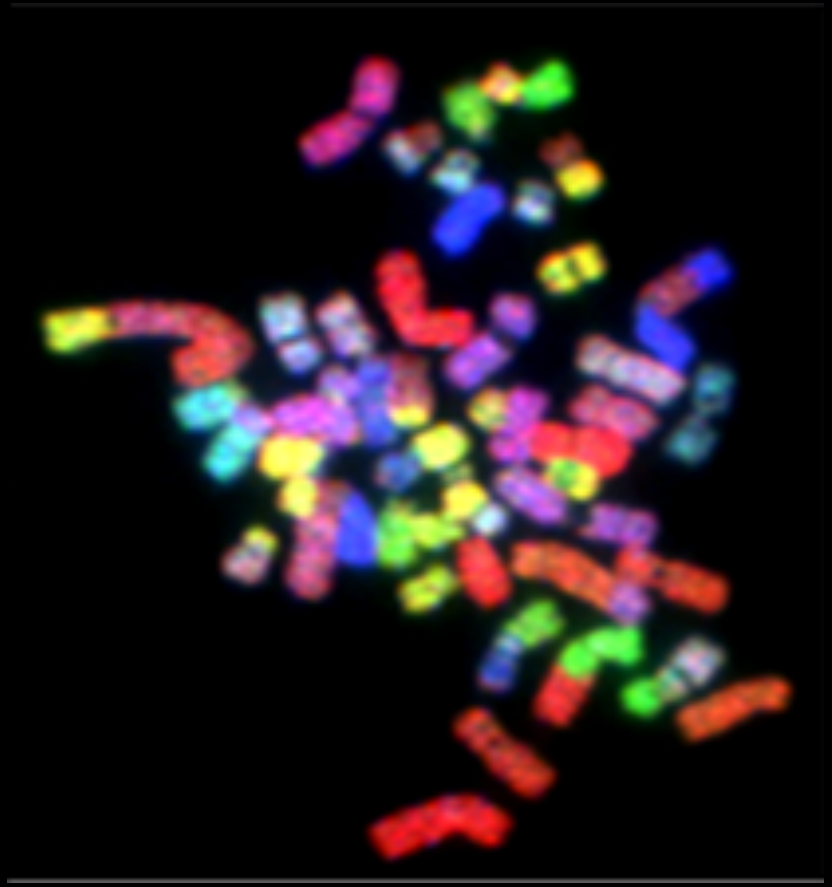


Mira Grigorova



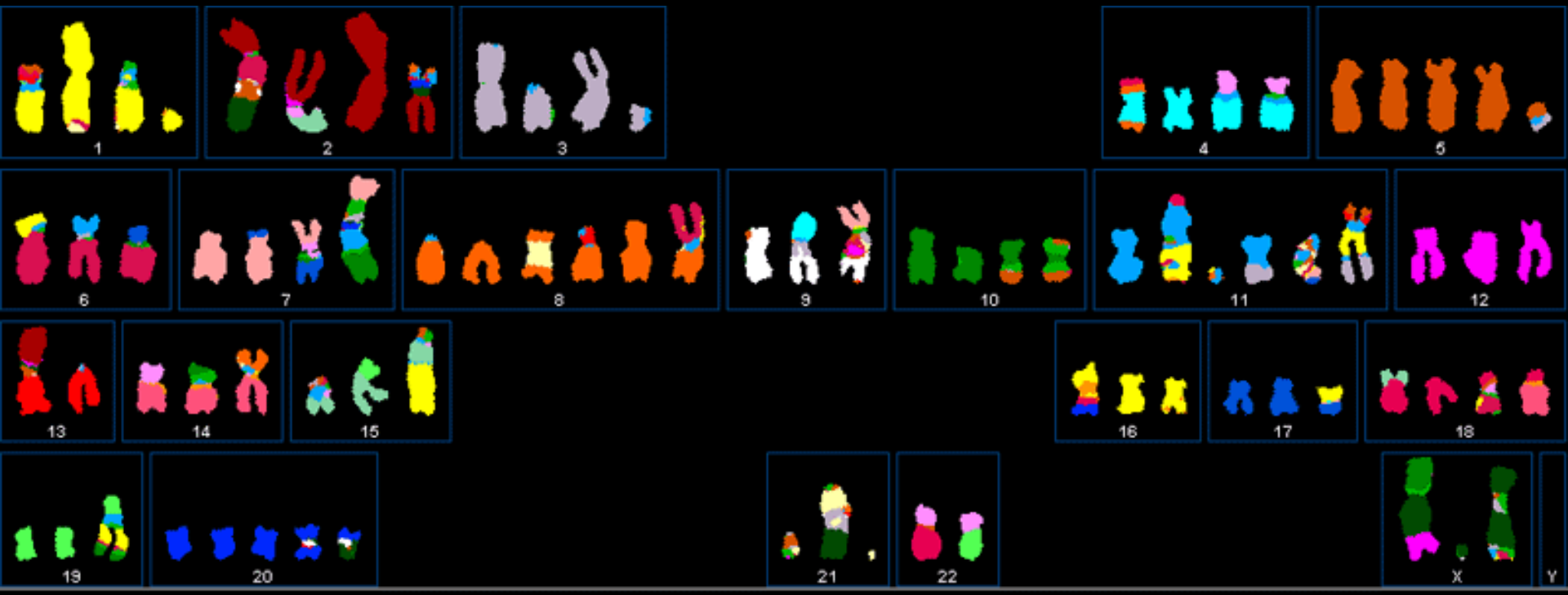
Joanne Davidson

‘SKY” or ‘M-FISH’



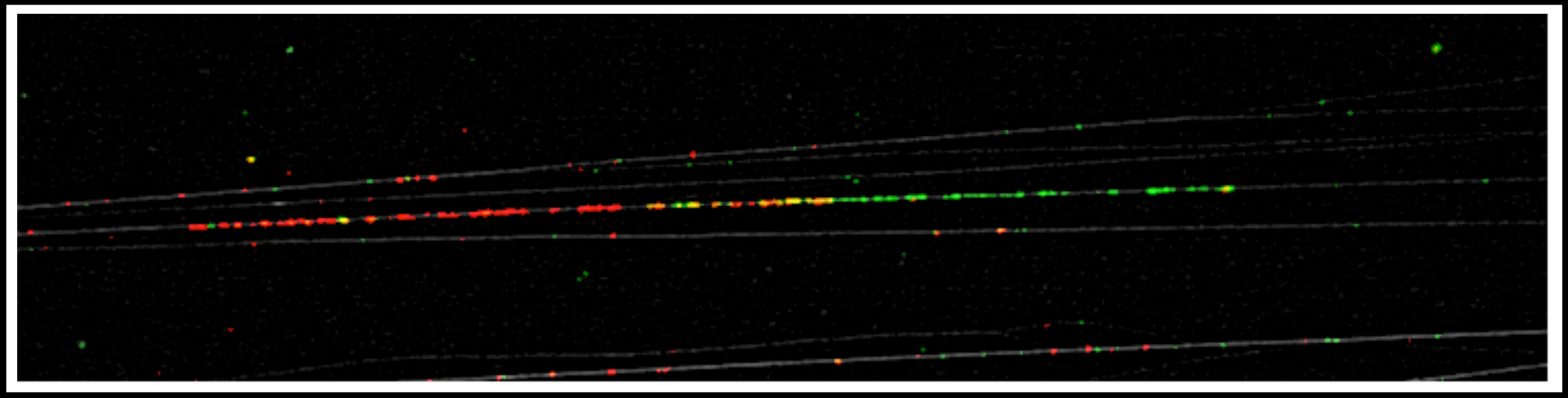
Joanne Davidson

Breast Cancer Cell Line HCC1143



Mira Grigorova

Fibre-FISH

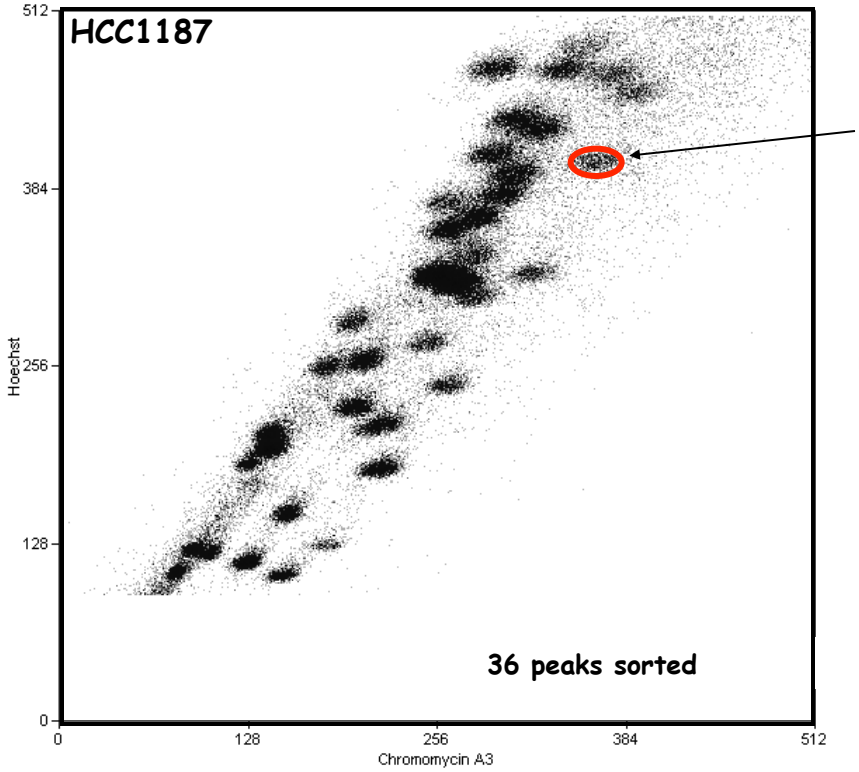


BAC A

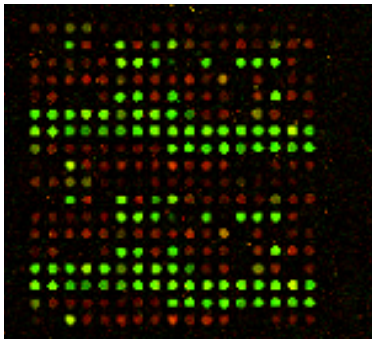
BAC B

Genome sequen

Chromosome sorting and Array Painting



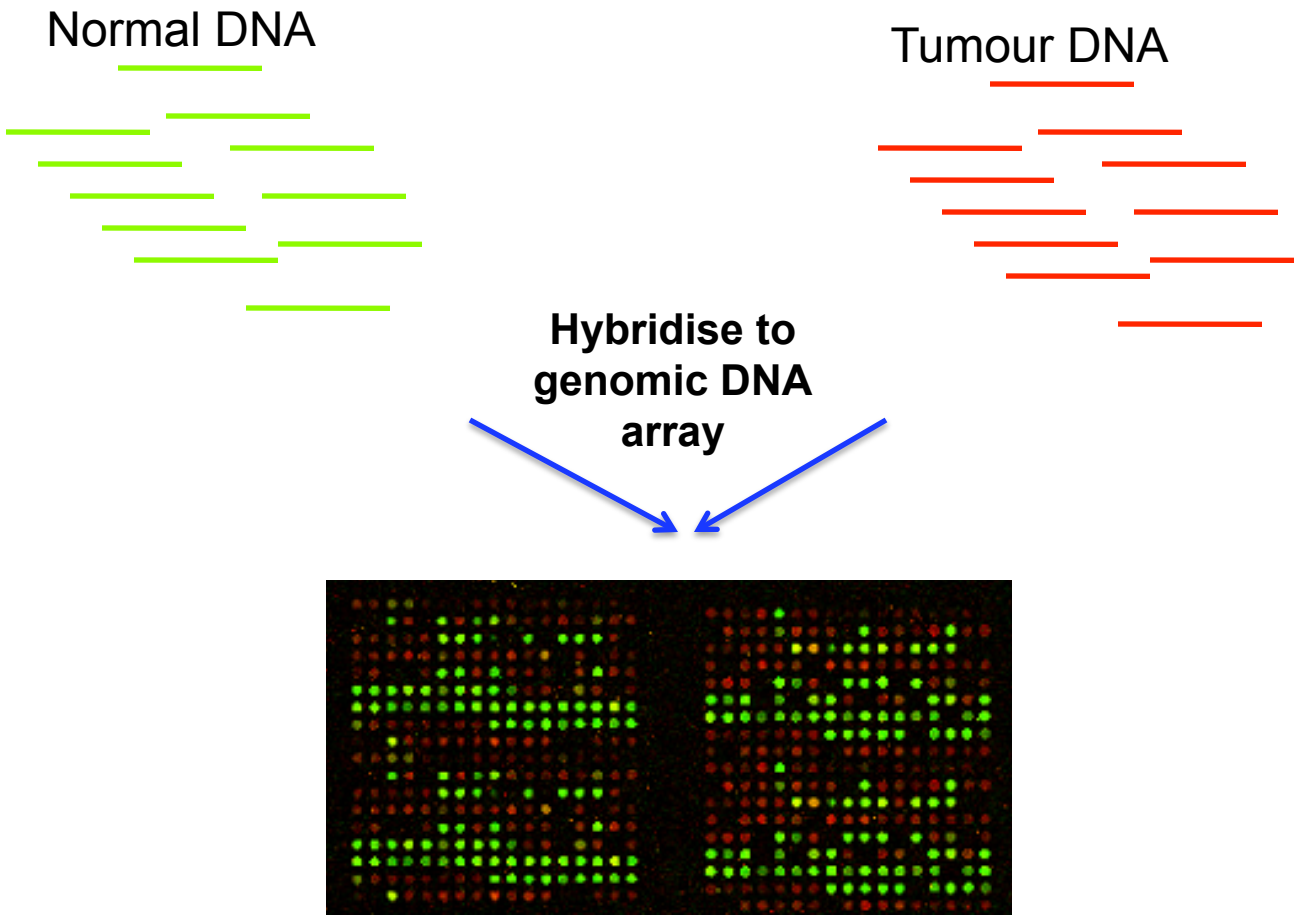
Amplification



Hybridise to genomic DNA array

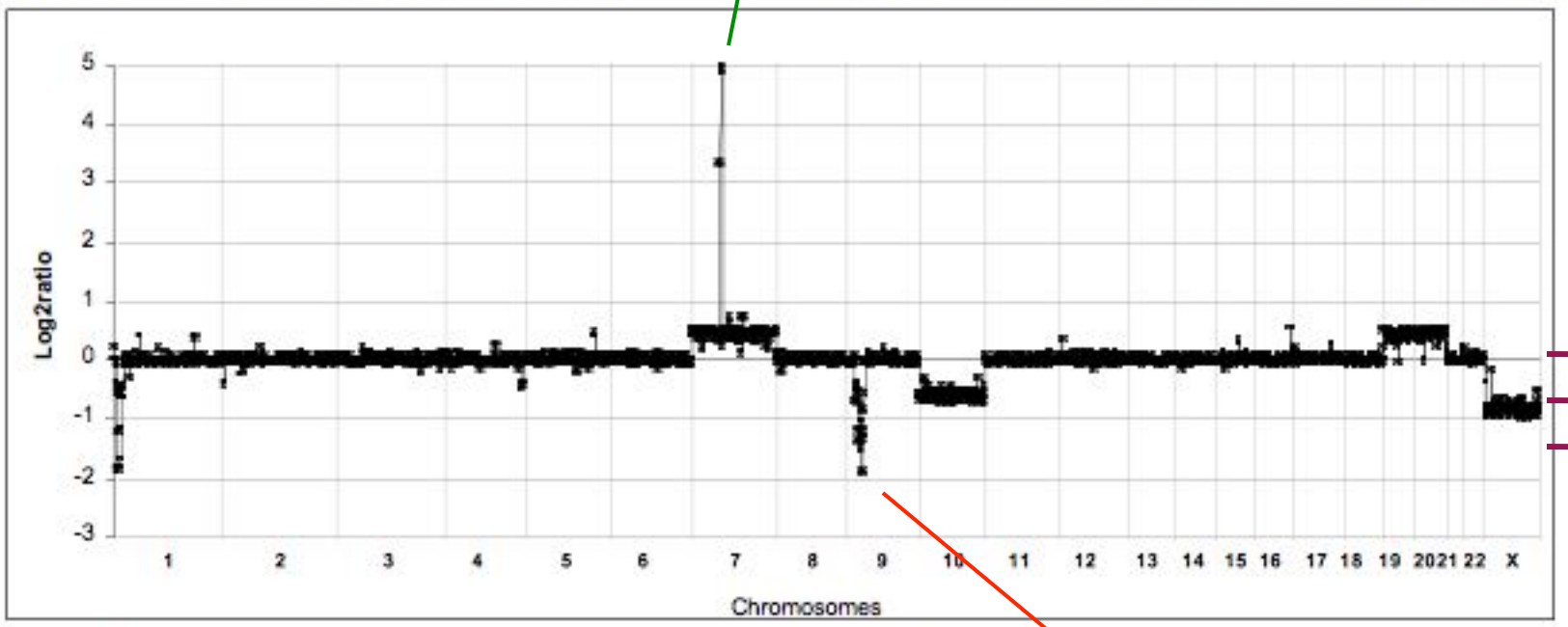
Karen Howarth

Copy number by hybridization: CGH



Search for deletions and amplifications: measure copy number

Amplification EGFreceptor (ERBB/HER-1)

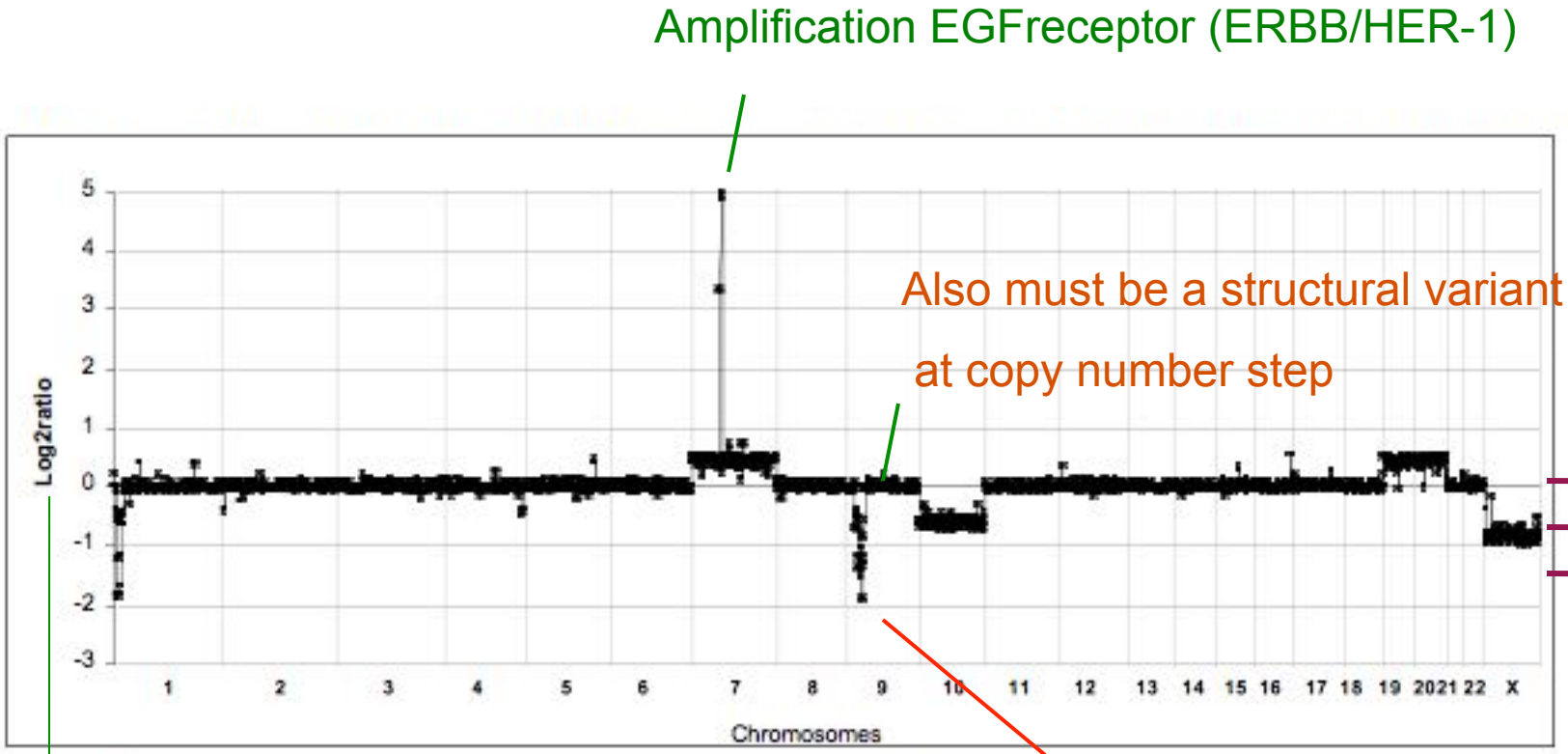


Deletion
P16/CDKN2A/INK4A

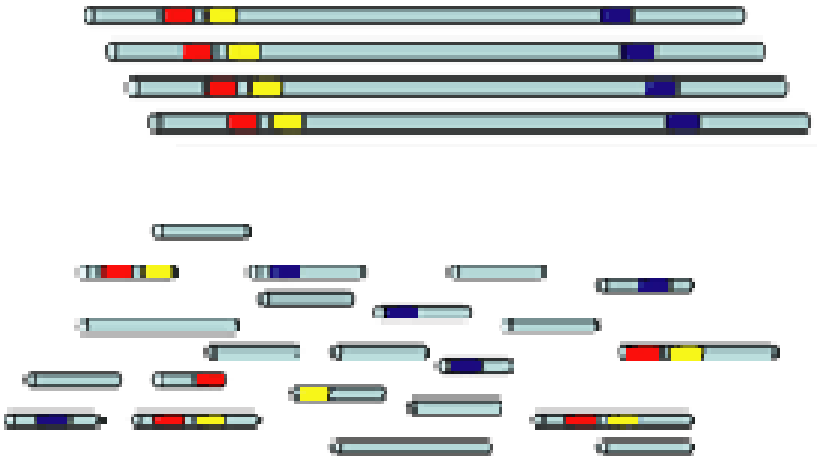
Genome position

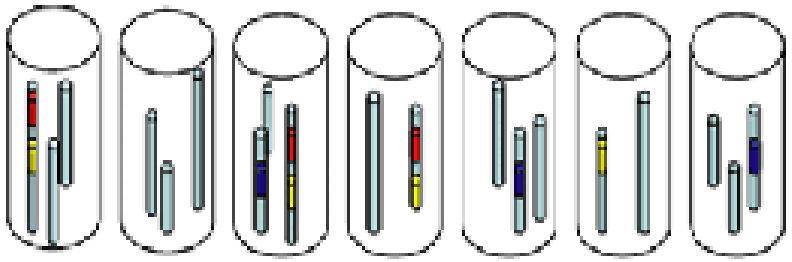
glioblastoma

Search for deletions and amplifications: measure copy number

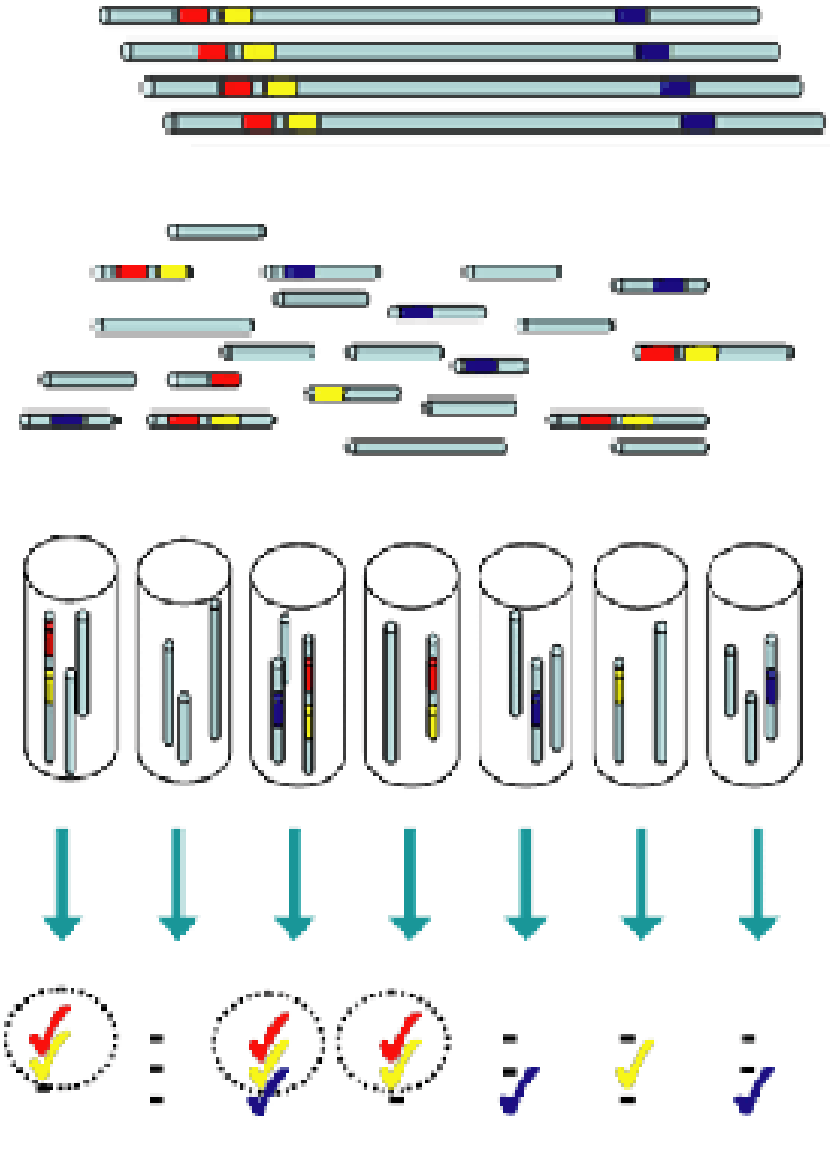


Mapping approaches





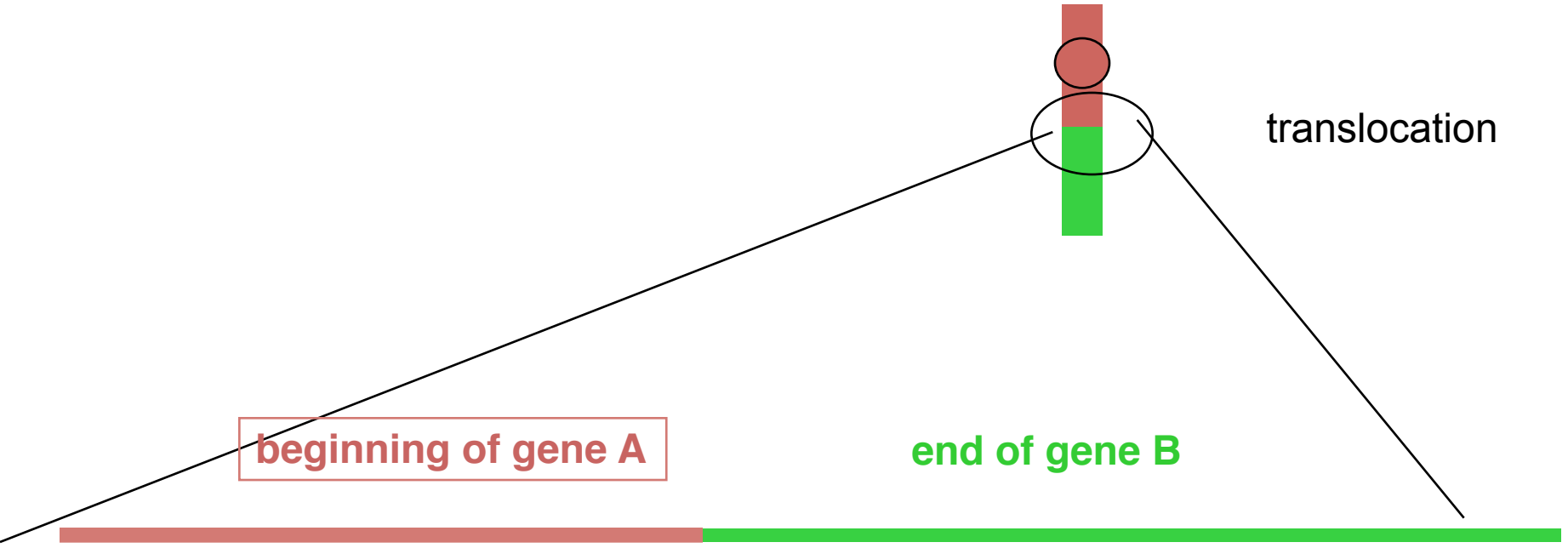
HAPPY mapping, also 10X, GAM



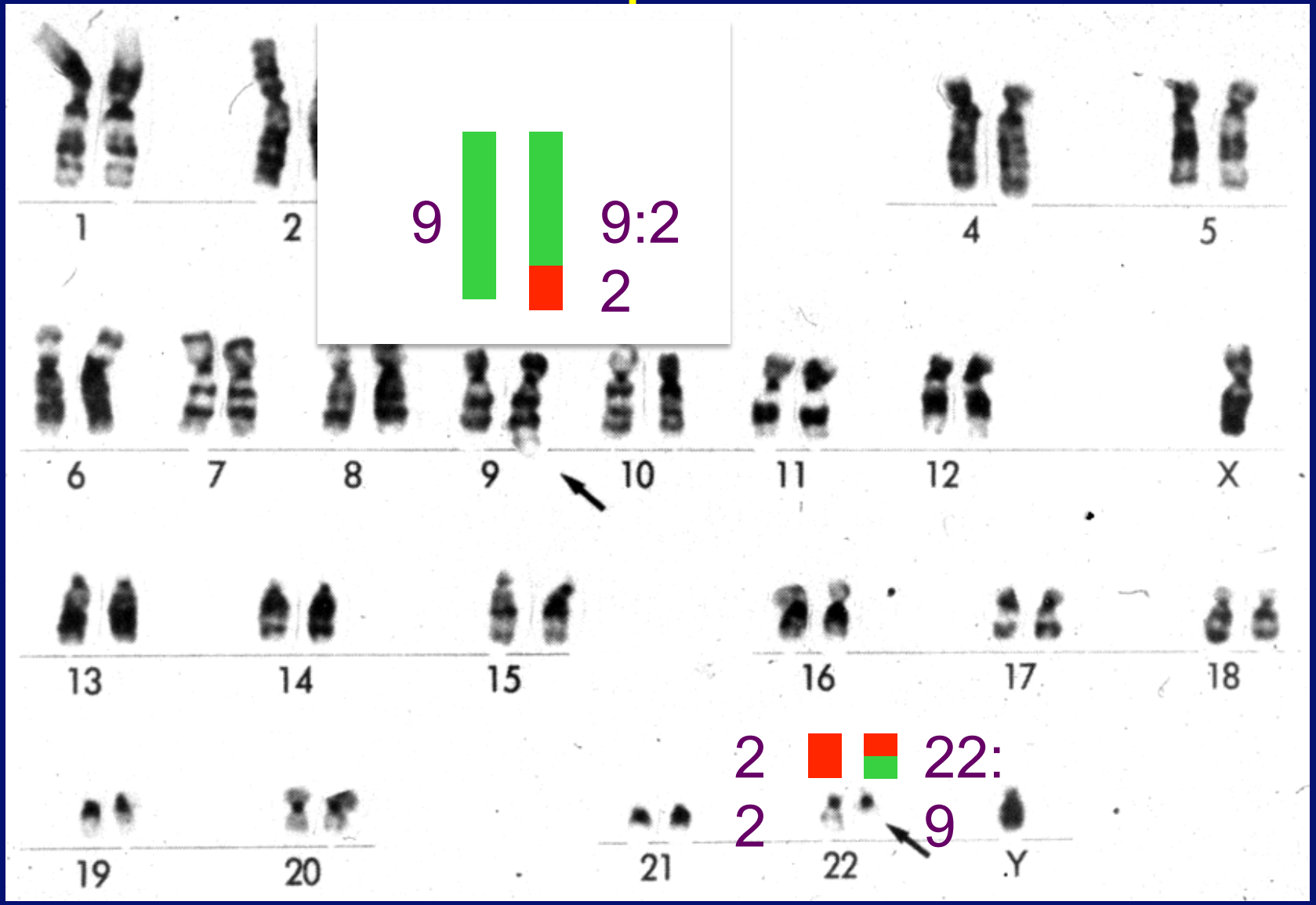
4. More on rearrangements:

Fusion genes

Chromosome translocation: classic source of fusion genes



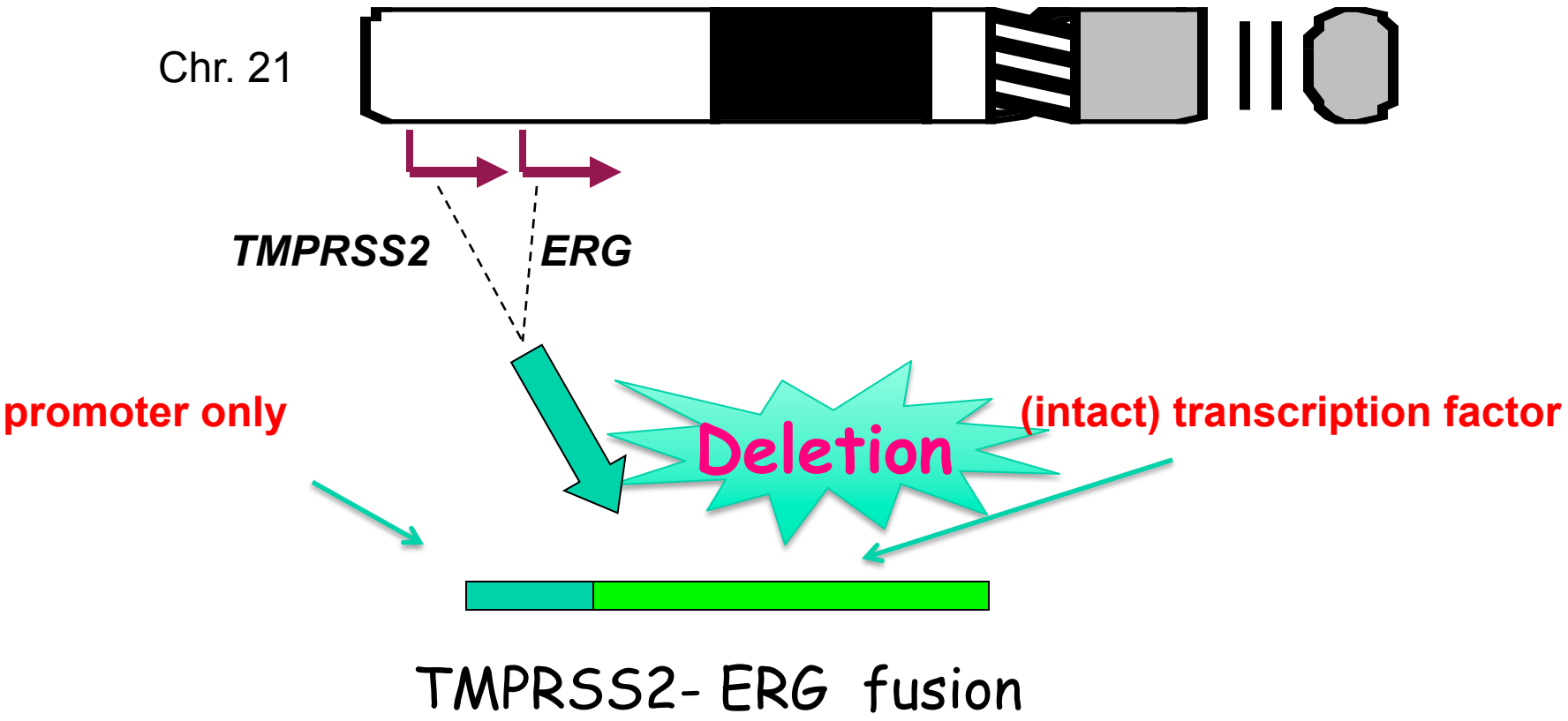
Philadelphia chromosome



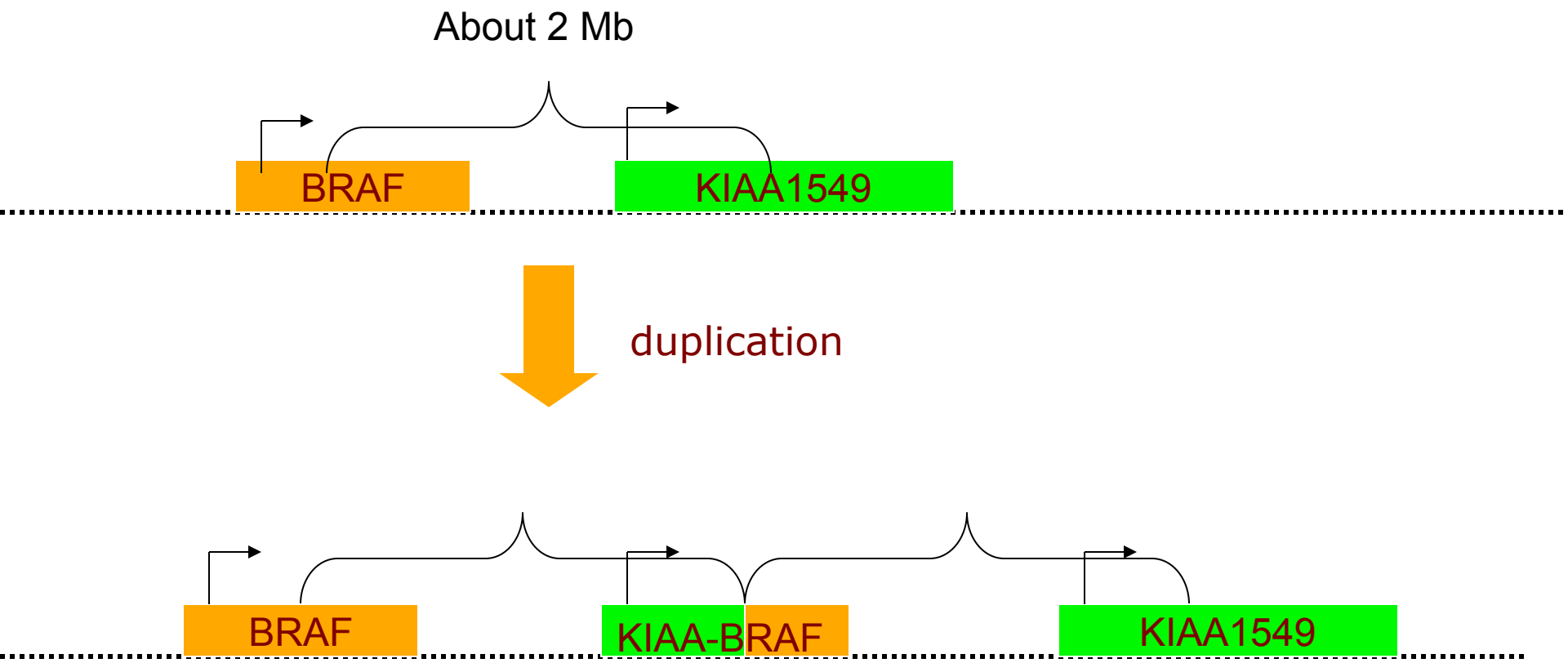
Creates BCR-ABL fusion

There are fusion genes in common epithelial cancers (not just leukaemias) and not just translocations

TMPRSS2-ERG ~50% prostate cancers



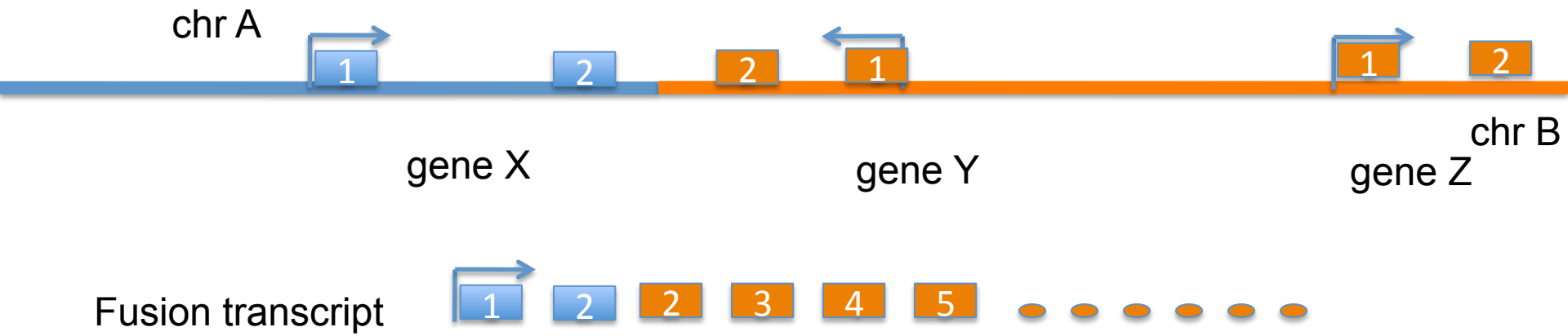
Tandem duplications causing gene fusion of BRAF



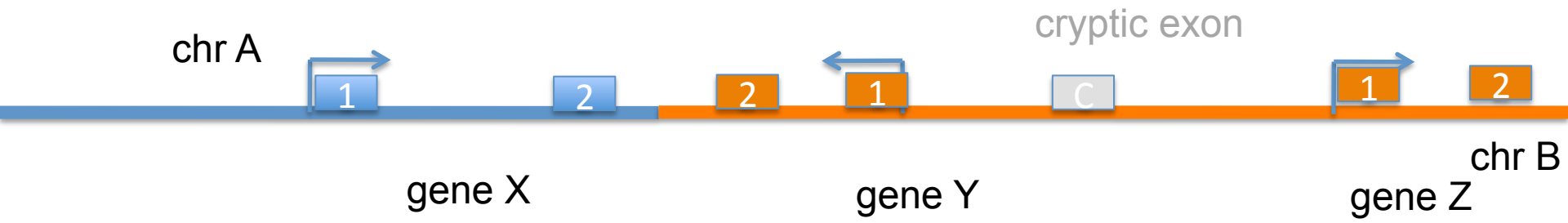
Fusions aren’t the only consequence of interest!

Most often, genes are simply disabled

Fusions may not be immediately obvious, e.g. ‘Run-through’ Fusions*



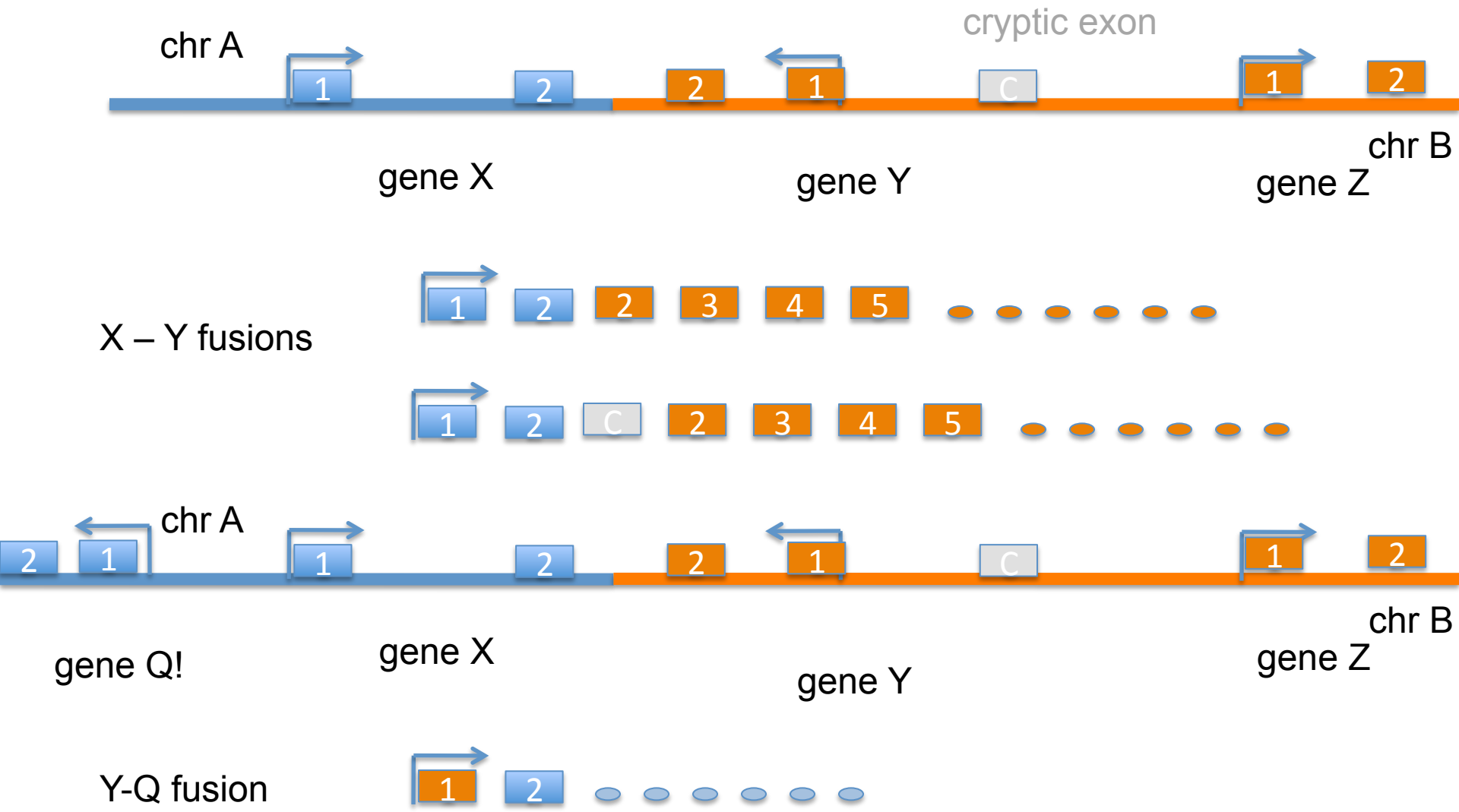
Fusions may not be immediately obvious, e.g. ‘Run-through’ Fusions*



Fusion transcript

Fusion transcript

Fusions may not be immediately obvious, e.g. ‘Run-through’ Fusions*



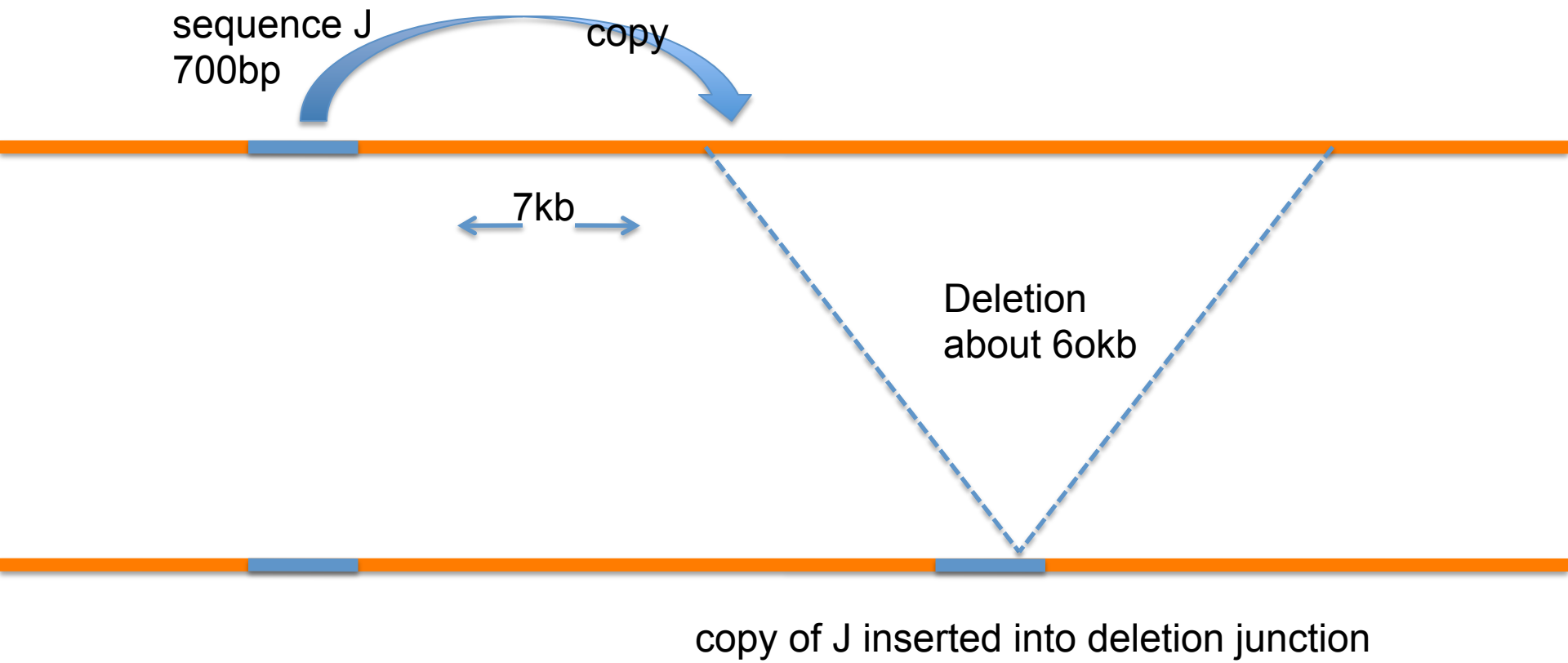
4. More on rearrangements:

Rearrangements are often complex

- Shards
- Fragile sites
- L1 insertions in rearrangement junctions
- Breakage-fusion-bridge cycles
- Chromothripsis
- Kataegis

Shards

Rearrangements often have small fragments inserted into the junctions, from somehwere else, e.g.



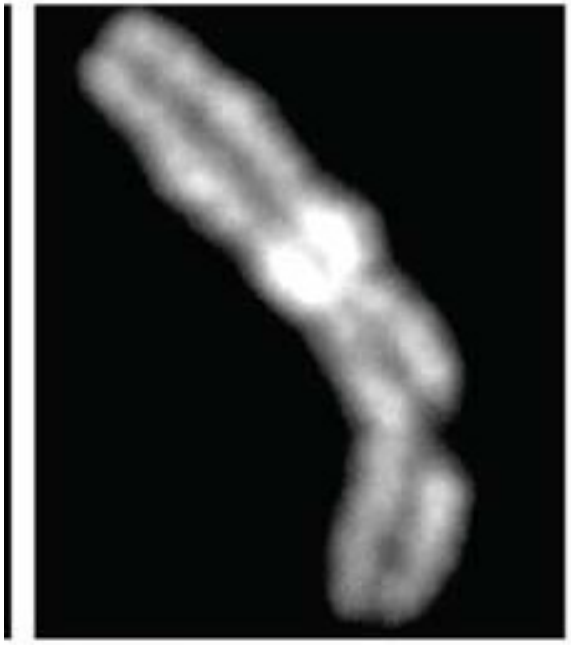
Alsop et al, Genes Chromosomes Cancer, 2008

Common Fragile Sites

Sites in the genome which are prone to breakage in cells under ‘replication stress’

Debatisse et al: regions with few replication origins

High density of rearrangements in the region, not clear whether passengers or not



Breakage-fusion-bridge cycles (BFB)

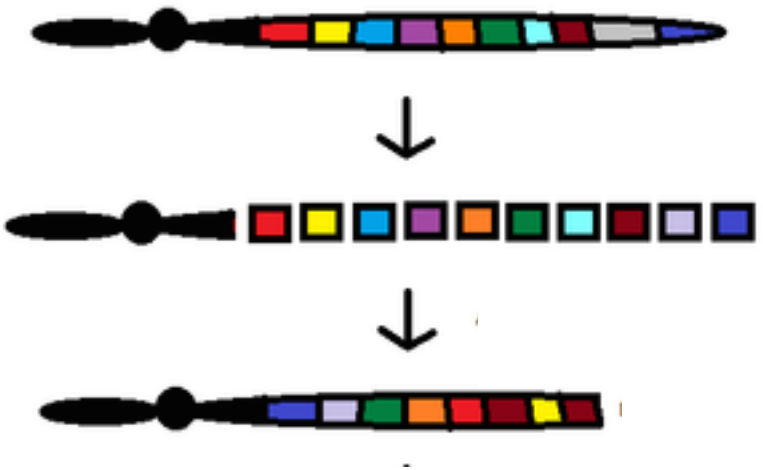
- *one* of the known mechanisms of amplification

chromosome breakage -> joining of chromatids -> dicentric chr. -> breaks again

-> repeated fold-back duplications,
amplification of region C

Chromothripsis

- Shattering and repair of a chromosome
  - or regions of (a) chromosome(s)



shattering

repair of some bits
in random order

Wikipedia

Kataegis

- Cluster of SNVs, sometimes close to a rearrangement

Kataegis rainfall plot *from Nik-Zainal et al Cell 2012*