GENETIC STABILITY OF HUMAN PLURIPOTENT STEM CELLS

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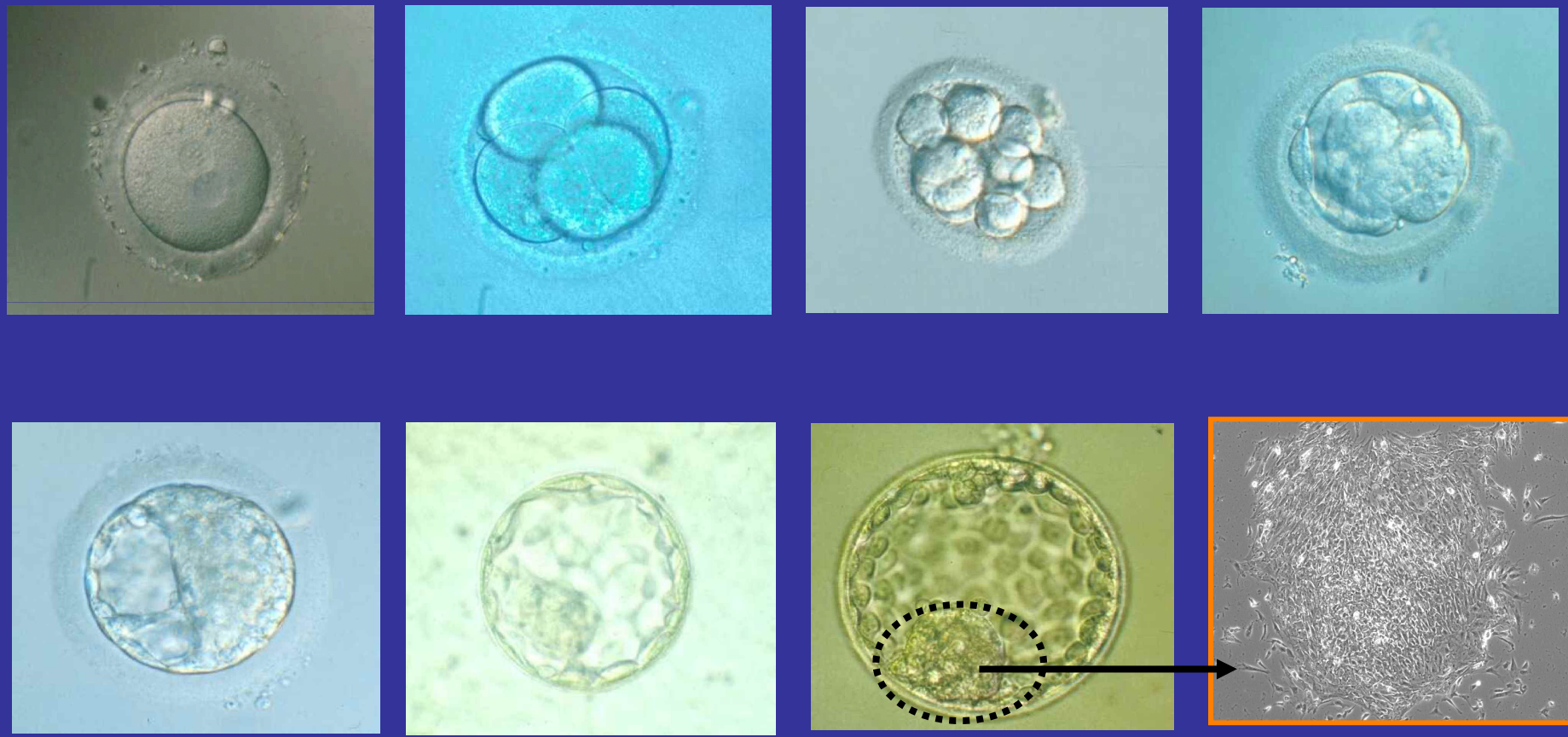
TYPES/SOURCES OF STEM CELLS & PROGENITOR CELLS

ONTOGENY: "Measured as" stage of development where they are obtained
Embryonic: derived from the inner cell mass of the blastocyst (< day 14)
Fetal: derived throughout the gestation period (beyond day 14-16)
Neonatal: derived from Umbilical Cord Blood or placenta (at birth)
Adults: derived from somatic adult tissues:

- Bone marrow
- Brain
- Skin
- Colon
- Fat
- Mammary Gland

HIERARCHY: "Measured as" pluripotency capacity
Stem Cell: capable of long-term proliferation through symmetrical divisions and multi-lineage differentiation.
Progenitor Cell: capable of long-term proliferation through symmetrical divisions but show reduced non-multi-lineage differentiation potential.
Differentiated Cell: Mature and Functional Cell unable to differentiate anymore

HUMAN EMBRYONIC STEM CELLS DERIVATION



ESCs and iPS CELLS: PROPERTIES, HOPES AND CAUTION!!

- ✓ **Pluripotent:** ability to originate any tissue representing the 3 germ layers (ecto-, meso- & endoderm).
- ✓ **High proliferative capacity:** in vitro e in vivo while maintaining the undifferentiated state.
- ✓ **Robust differentiation capacity:** in vitro and in vivo :
  - in vitro----> i.e: embryoid bodies
  - in vivo----> teratomas formation in immunedeficient mice

POTENTIAL APPLICATIONS OF hESCs & iPS cells

- ✓ Cell Therapy
- ✓ Developmental Biology
- ✓ Drug screening
- ✓ Cancer Biology

Molecular Cytogenetics

Conventional Cytogenetics

Molecular Cytogenetics

Microscopic Study aimed at analyzing the chromosomal DNA Content within the Cell

G-Banding Karyotype

Cytogenetic Techniques

CONVENCIONAL CYTOGENETICS

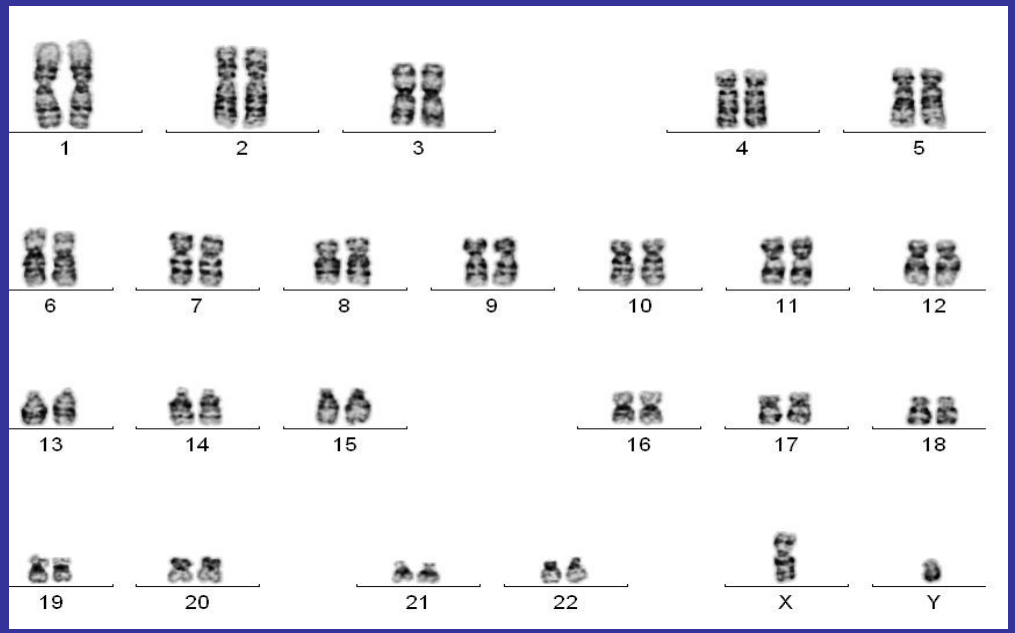
- G-Banding Karyotyping

MOLECULAR CYTOGENETICS

- FISH
- Spectral Karyotyping (SKY)
- Comparative Genomic Hibridization (CGH).
- Single Nucleotide Polymorphisms (SNPs)
- Loss of Homo/Heterozygousity
- Uniparental Disomy (LOH-UPD)

Conventional Cytogenetics

- To study chromosome alterations in metaphases. It requires cell division and fresh tissue
- Allows detection of both numeric and structural alterations throughout the genome.
- G-banding consists of a Giemsa staining after an enzymatic digestion. Dark bands represent spots rich on A-T nt while clear bands identify regions rich on G-C.

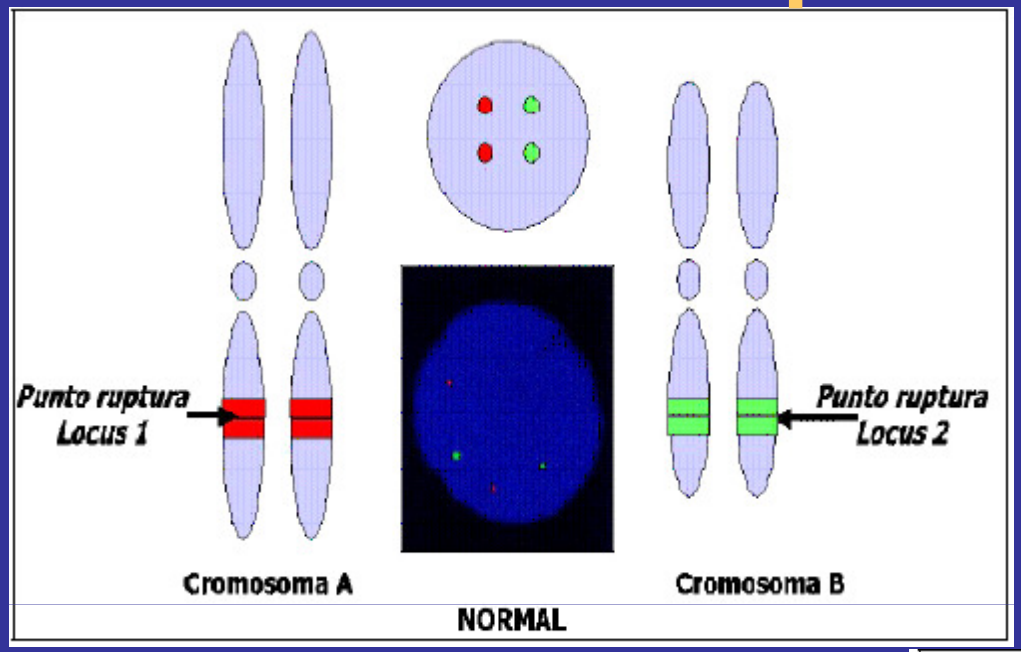


Molecular Cytogenetics
iFISH (Fluorescence In Situ Hybridization)

- Uses a DNA fluorochrome-labelled specific against a genome-specific gene/allele.
- It detects only what we are searching. It is currently used to validate the presence or absence of fusion genes or chromosomal rearrangements.
- Two main types of probes:

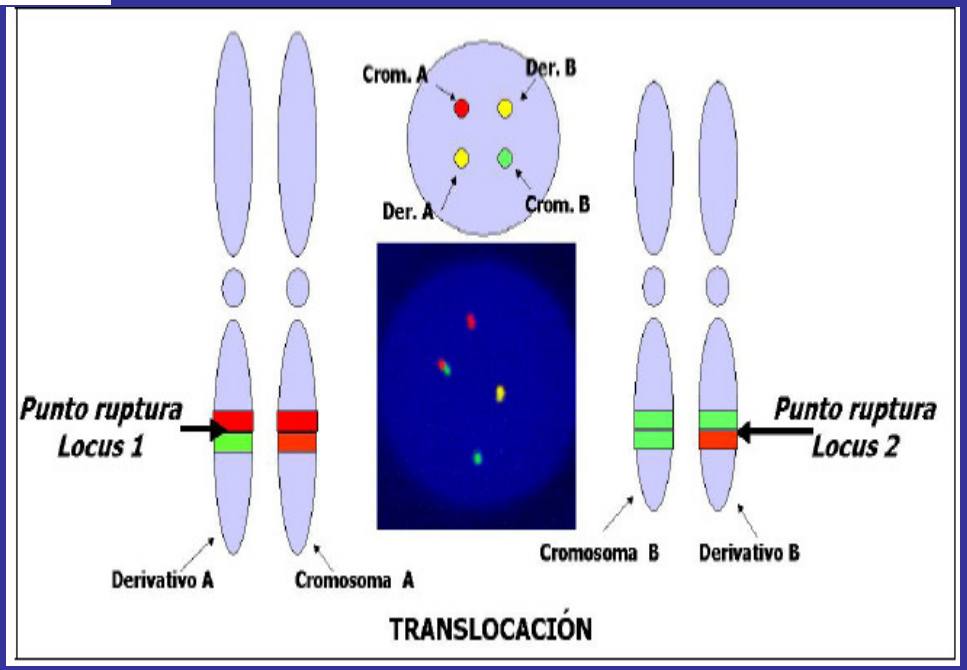
i) i) allele-specific
ii) ii) split-apart
iii) iii) centromeric
iv) iv) subtelomeric

Allele-specific probes

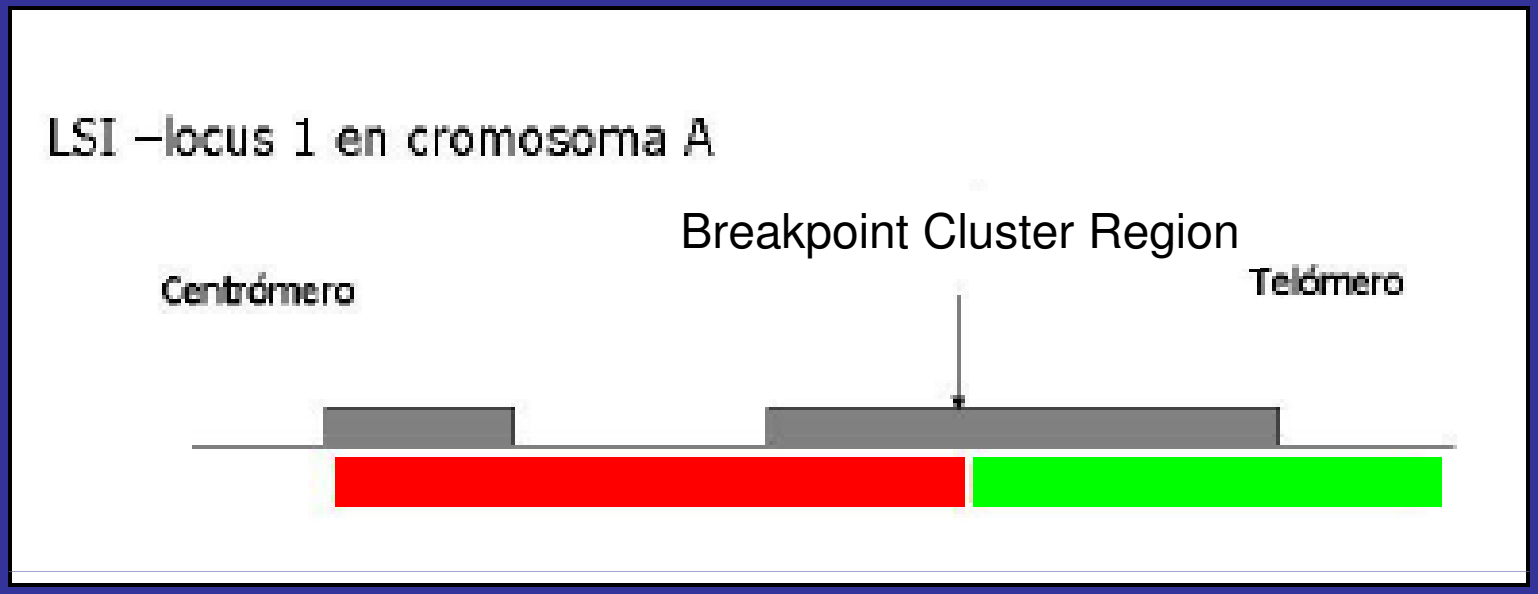


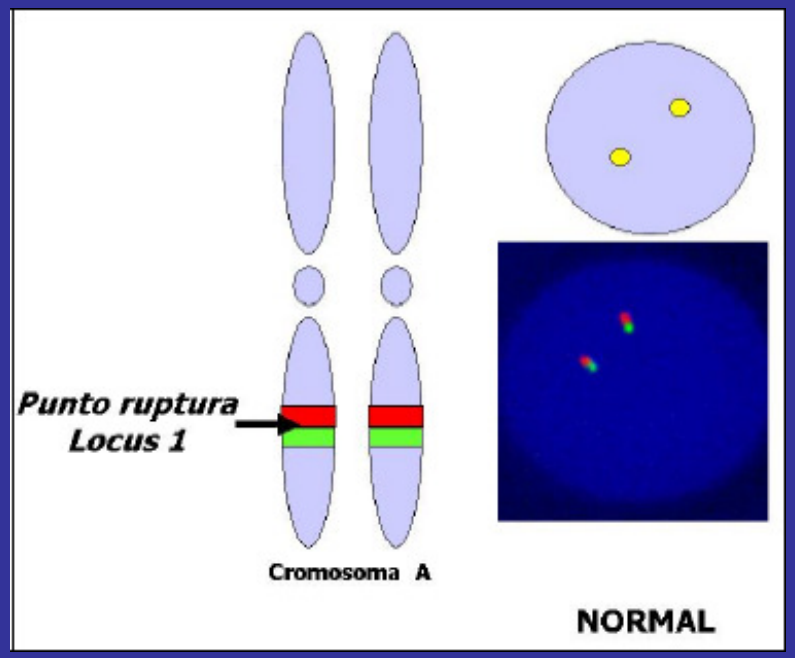
Absence of Rearrangement

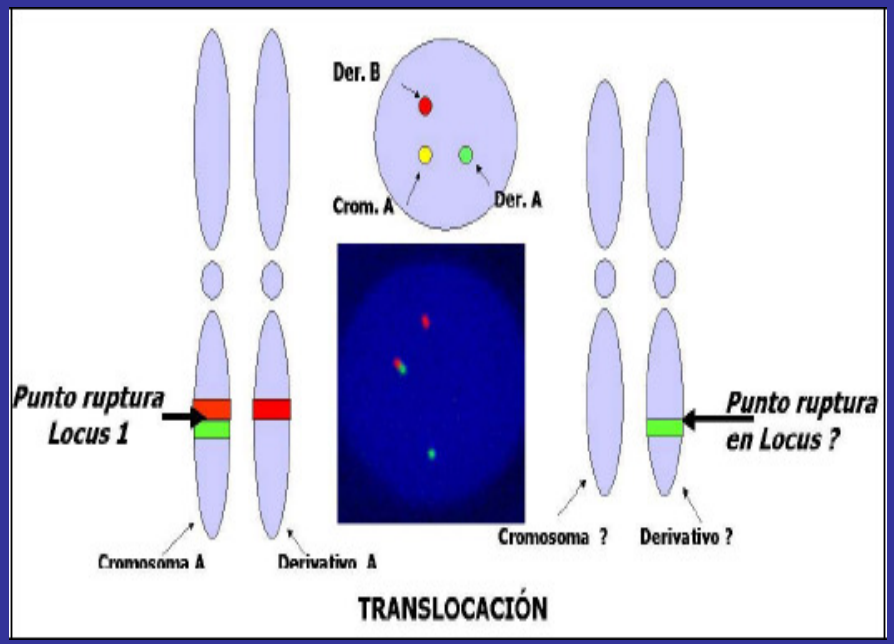
Presence of balanced Rearrangement

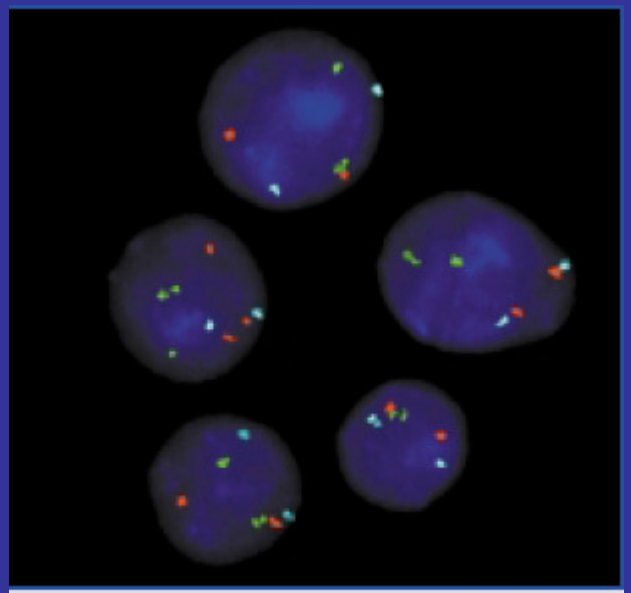


Split-apart Probes





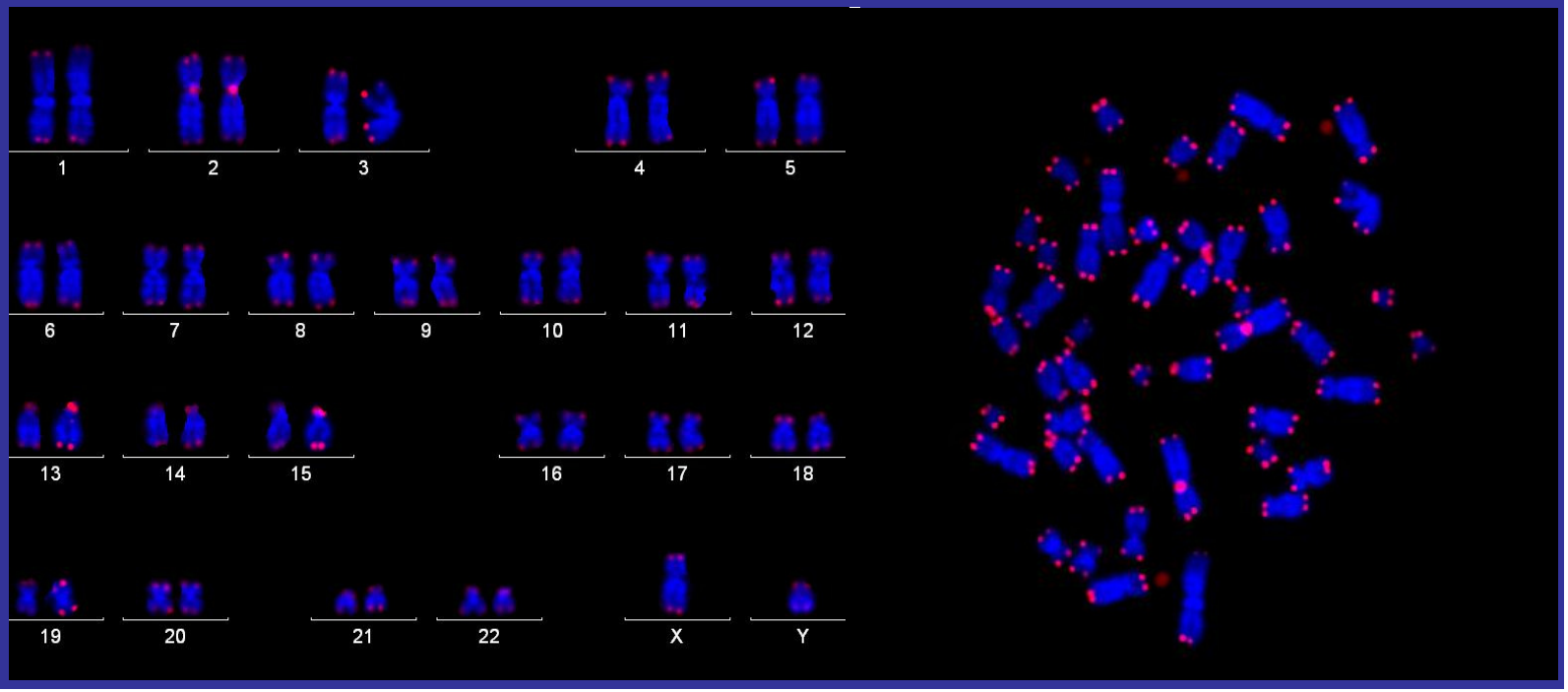




Interphase with three probes. *Red - 8q24 Cmyc. Cyan - centromere 8. Green - centromere 14.*

Centromeric Probes

Telomeric Probes



Molecular Cytogenetics
SKY (Spectral Karyotyping)

Based on chromosome painting through multiple flurochromes.

Advantages

Facilitates information about all chromosomes

Very uselful for complex karyotypes

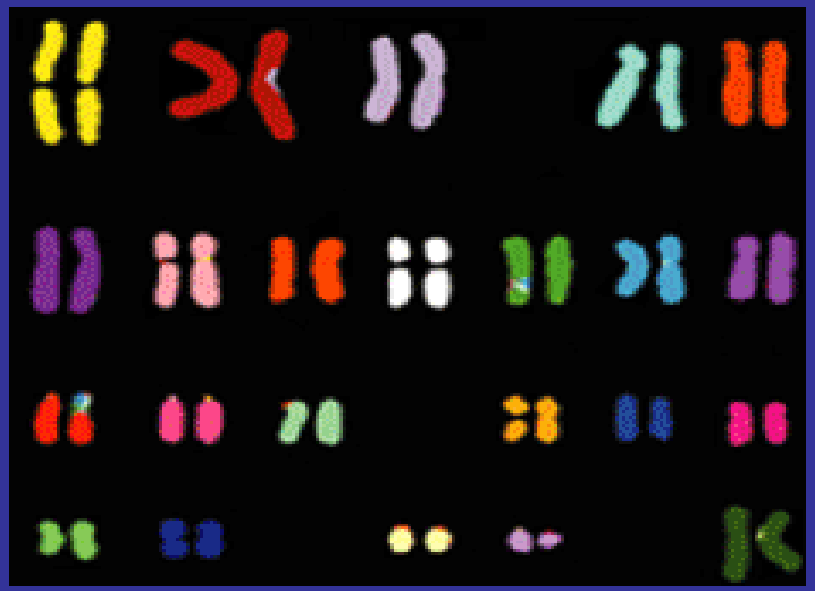
Very useful to determine the origin of the translocations tough to see by conventional G-banding

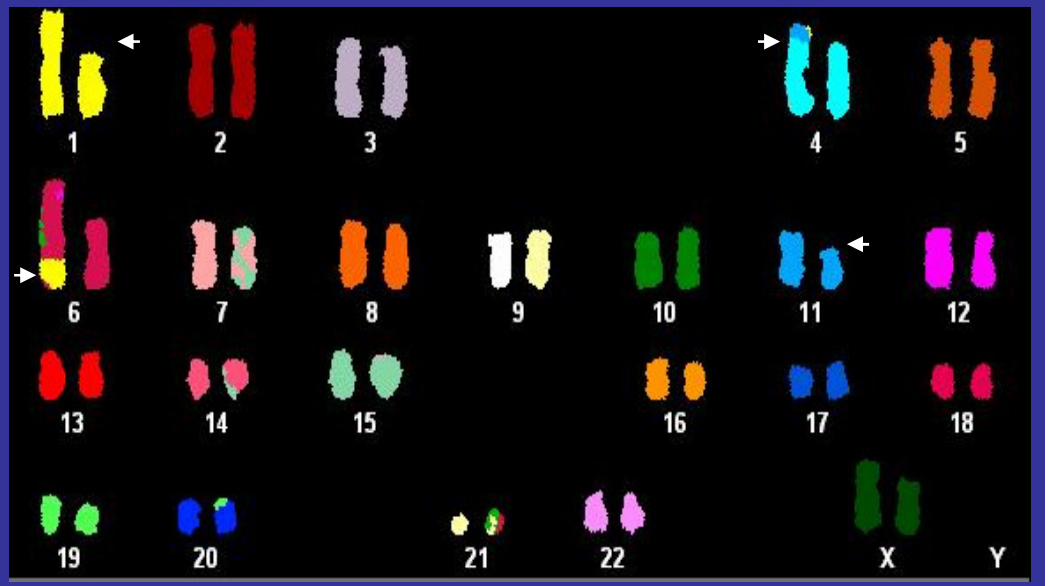
Disadvantages

Requires cell division

Unable to detect intra-chromosomic structural alterations

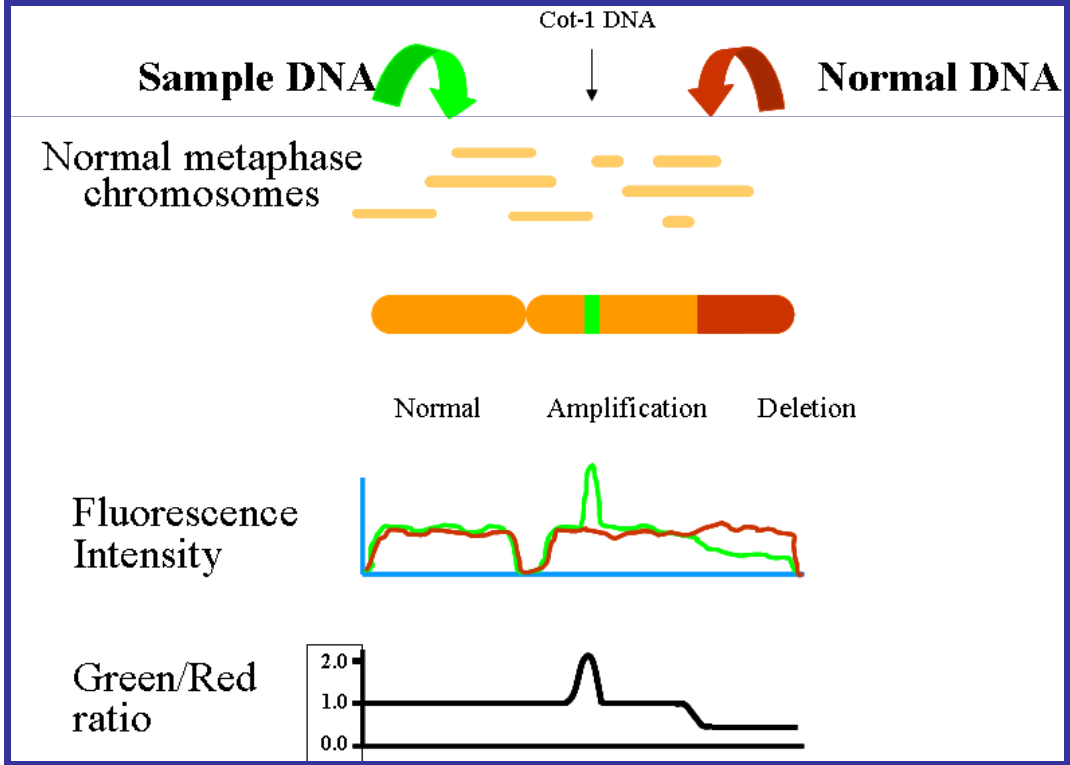
Unable to detect structural alterations < 1Mb.

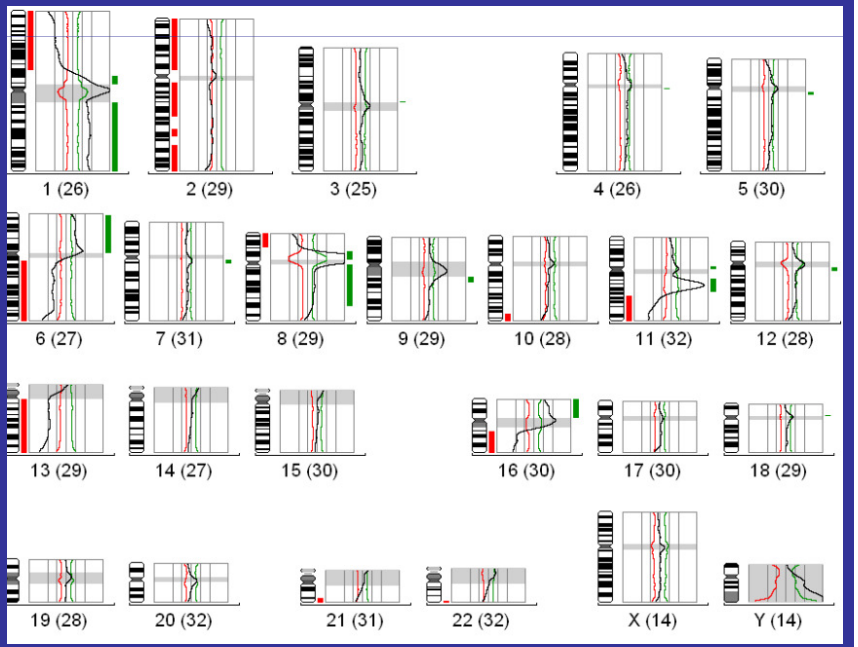


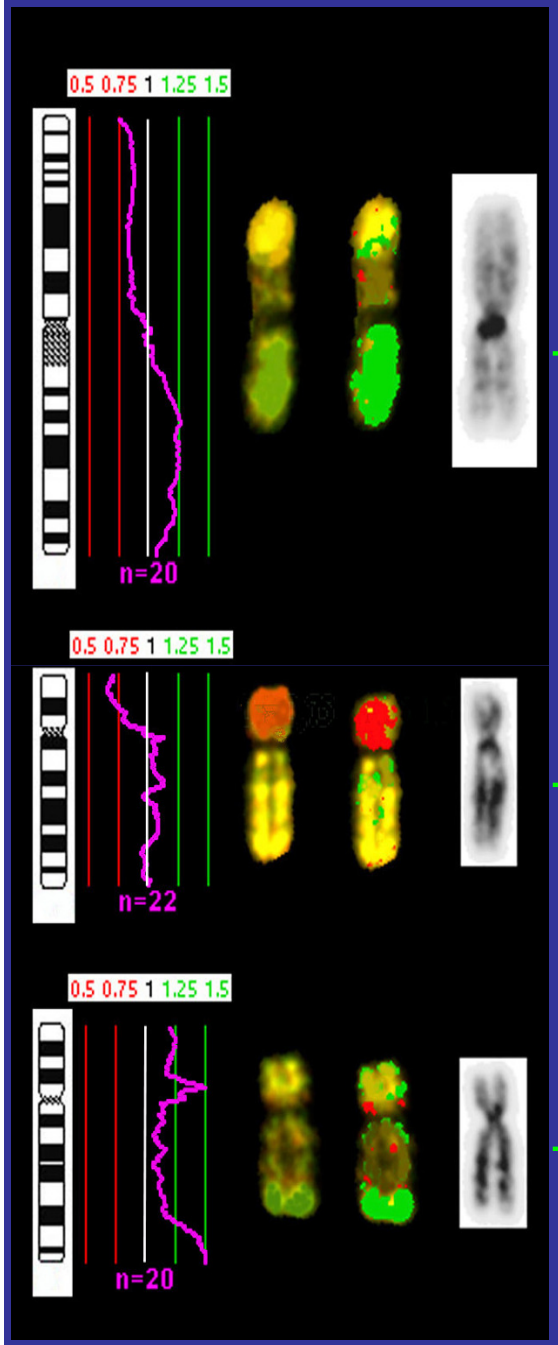


Molecular Cytogenetics
CGH (Comparative Genomic Hybridization)

- It is a technique derived from iFISH based on the competitive hybridization of two DNAs: Reference DNA and target DNA labelled with different fluorochromes.
- It allows to detect gains and losses of small pieces of DNA.
- It does not require cells in division.







DNA gains

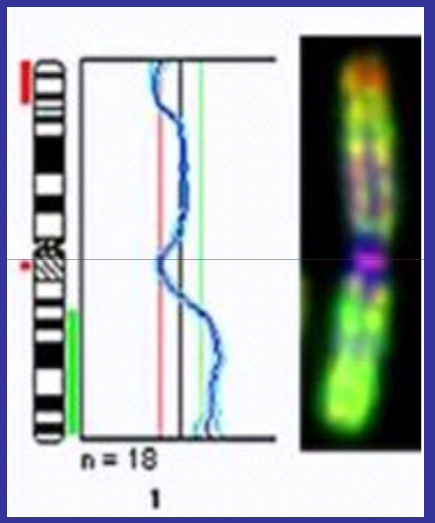
Target DNA/Reference DNA **>1.25**

DNA losses

Target DNA/Reference DNA **<0.75**

Amplifications

Target DNA/Reference DNA **>1.50**



Loss

Gain

CGH: Advantages and Disadvantages

Advantages

It does not require cells in division.

Allows to have a quick overview of the whole genome in a single experiment.

Allows studies on fresh, frozen and paraffined material.

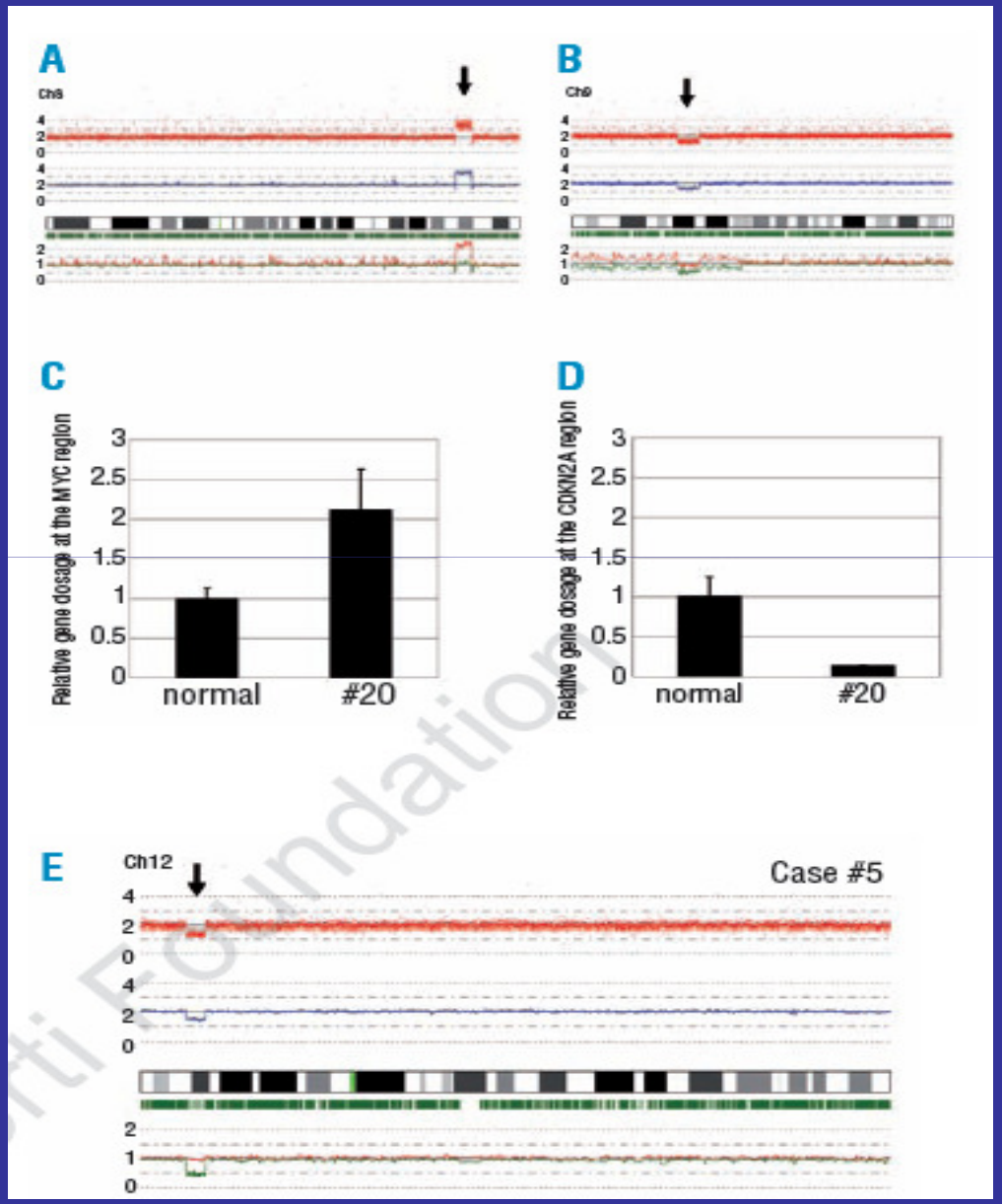
Disadvantages

The sensibility of this technique depends on the % of abnormal cells (should be > 10%).

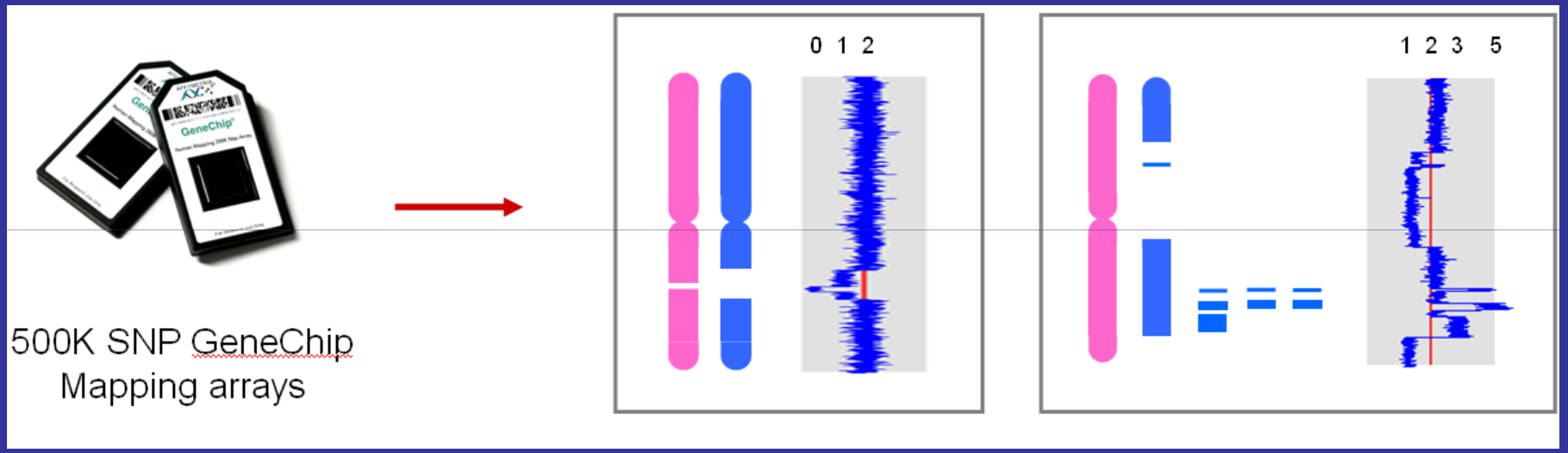
Fail to detect balanced cytogenetic alterations. It only detects numeric alterations.

Time-consuming technique

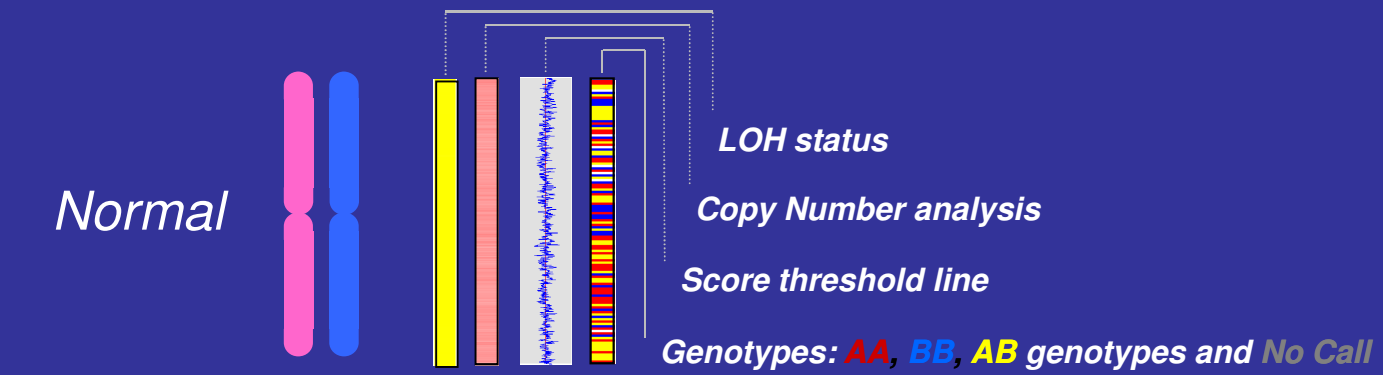
CGH Arrays

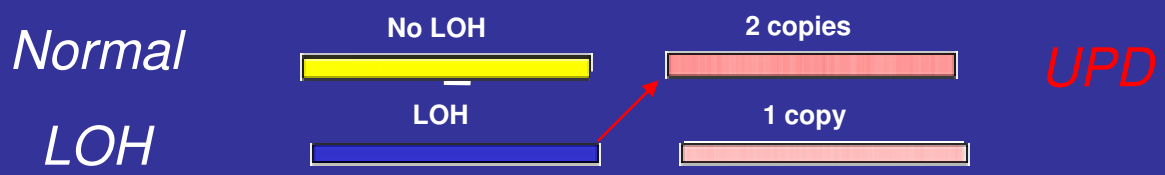

A
Ch8
B
Ch9
C
Relative gene dosage at the MYC region
3
2.5
2
1.5
1
0.5
0
normal
#20
D
Relative gene dosage at the CDKN2A region
3
2.5
2
1.5
1
0.5
0
normal
#20
E
Ch12
Case #5

SNP GeneChip Mapping Arrays



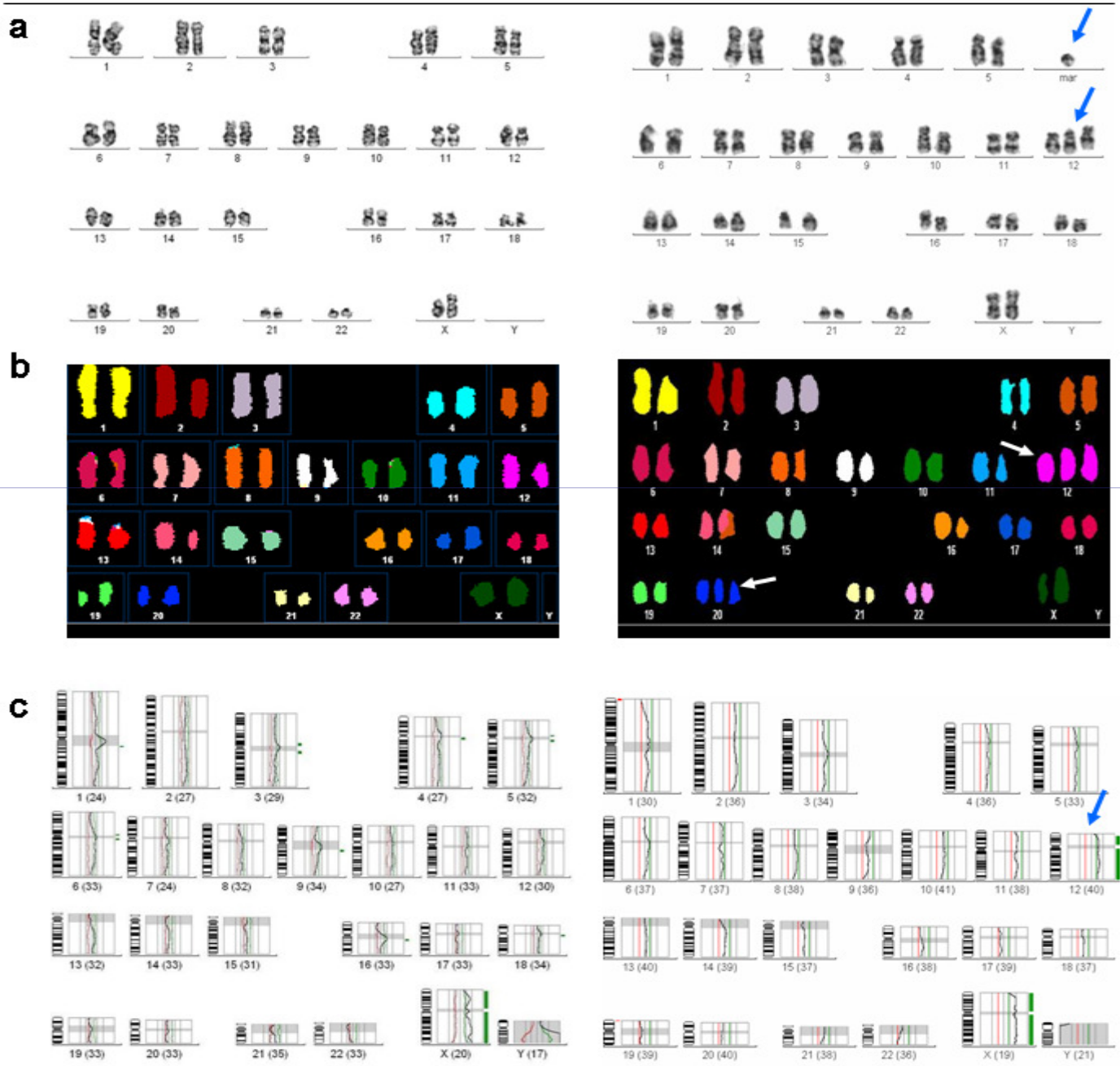
Identification by Mapping Arrays of LOH and UPD traits



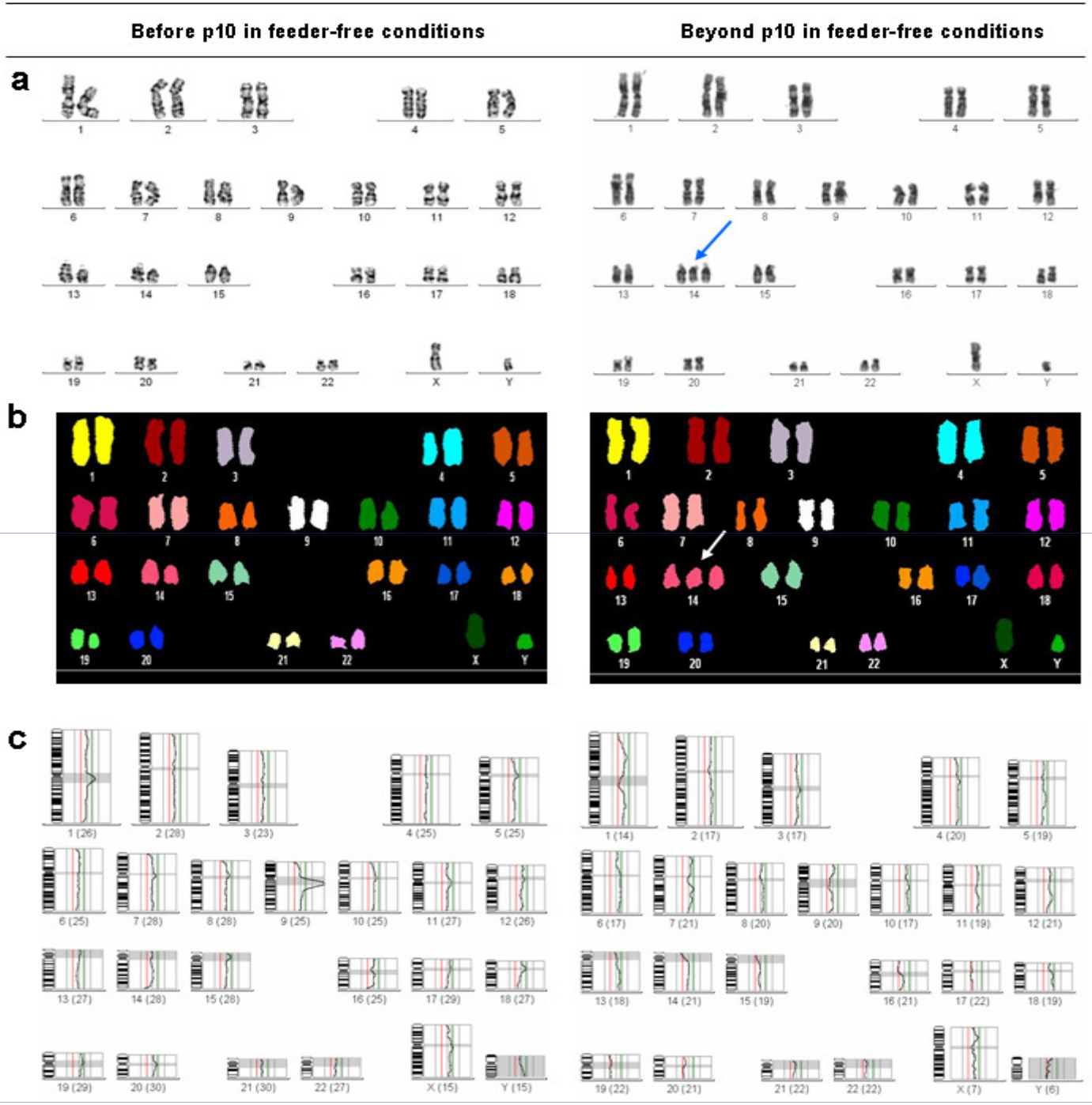


Human ESCs/iPS cell predisposition to karyotypic instability: Is a matter of culture adaptation or differential vulnerability among hESC/iPS lines due to inherent properties?

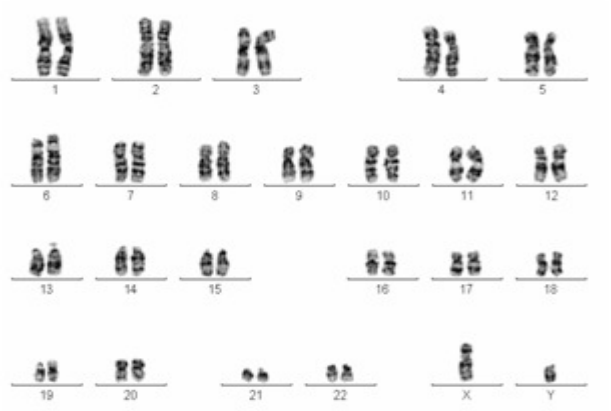
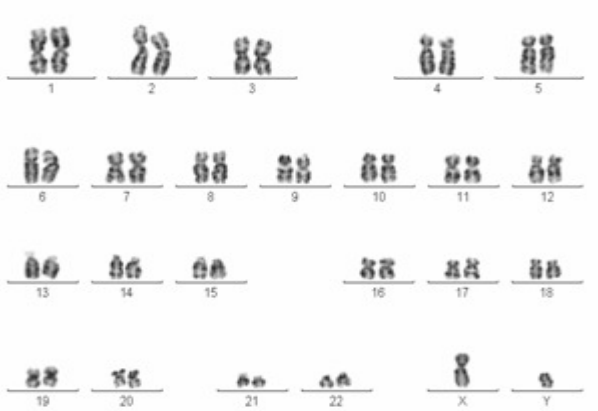
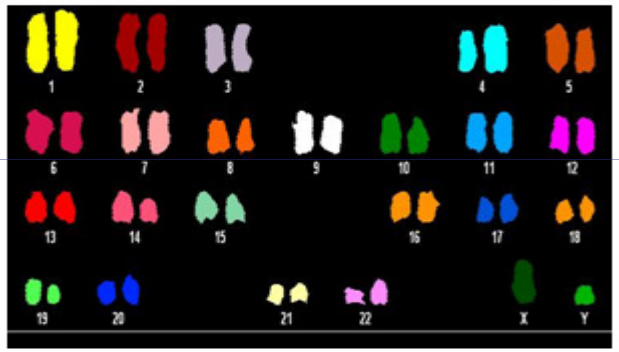
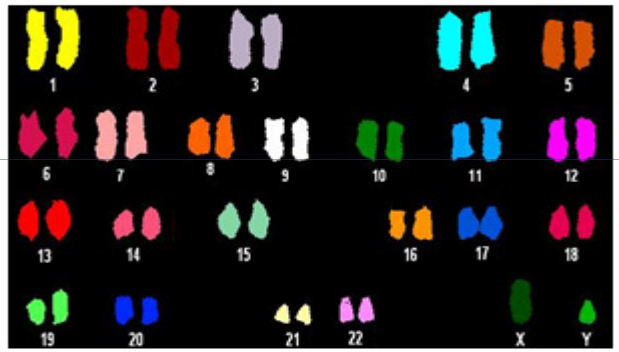
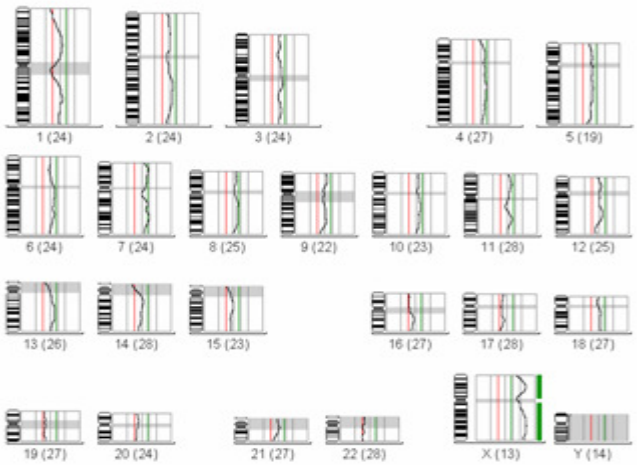
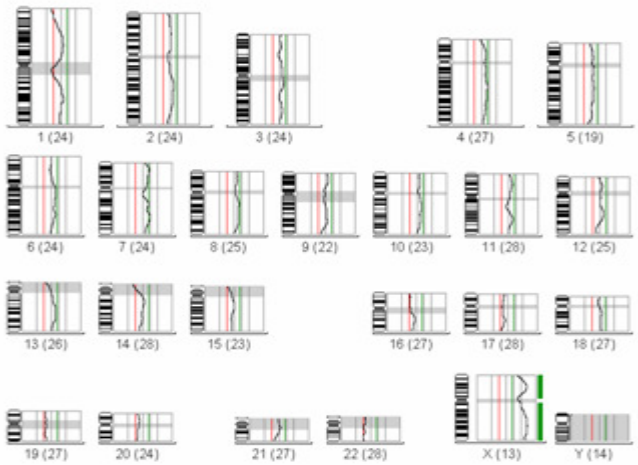
Human ESC Line HS181



Human ESC Line SHEF3



Human ESC Line SHEF1

	In Feeders (after 180 passages)	Feeder-free culture (after 215 passages)
a		
b		
c		

Summary of the karyotypic changes observed overtime in the HS181, SHEF-1 and SHEF-3 hESC lines and its potential relation to the culture conditions.

Human ESC Line	Passages under **feeders** conditions	Passages under **feeder-free** conditions	Karyotype (G-Banding)	% Mosaicism	**SKY / CGH**
HS181	71*	0	46,XX	n.a	normal/normal
	71*	10	46,XX	n.a	normal/normal
	71*	17	47,XX, +12	25 %	n.d
	71*	21	48,XX, +12, +mar	31%	48,XX, +12, +mar
	71*	30	47,XX, +12	89 %	47,XX, +12
SHEF-3	29** + 22*	0	46,XY	n.a	Normal/normal
	29** + 22*	10	47,XY+14	36%	47,XY+14
	29** + 22*	17	47,XY+14	24%	n.d
	29** + 22*	21	47,XY+14	23%	n.d
	29** + 22*	26	47,XY+14	13%	47,XY+14
SHEF-1	130** + 55*	0	46,XY	n.a	Normal/normal
	130** + 55*	10	46,XY	n.a	Normal/normal
	130** + 55*	17	46,XY	n.a	n.d
	130** + 55*	21	46,XY	n.a	n.d
	130** + 55*	30	46,XY	n.a	normal/normal

*Feeders were Human Embryonic Fibroblast (HFFs). **Feeders were Mouse Embryonic Fibroblasts (MEFs).
Abbreviations: n.a: not applicable; n.d: not done; SKY: Spectral Karyotyping; CGH: Comparative Genomic Hybridization

CONCLUSION

Significant differences among different hESC lines in regard to their chromosomal integrity.

In feeders, the 3 hESC lines were chromosomally stable up to 185 passages using either mechanical or enzymatic dissection methods.

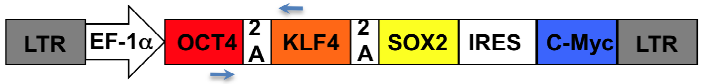
Despite the 3 hESC lines were maintained under identical conditions, each hESC line behaved differently upon being transferred to a feeder-free culture system: the 2 younger hESC lines, became chromosomally unstable shortly after being cultured in feeder-free conditions. Importantly, the mosaicism for trisomy 12 gradually increased up to 89% by p30, suggesting that this karyotypic abnormality provides a selective advantage.

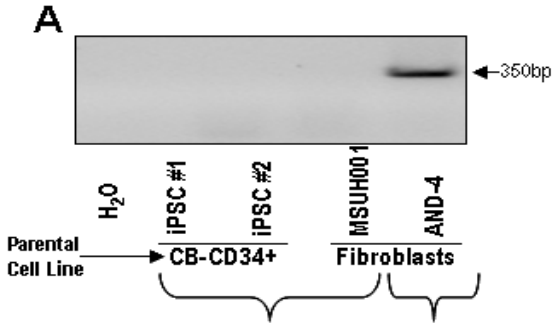
Similarly, other line also acquired a trisomy of chromosome 14 as early as p10 but this karyotypic aberration did not confer selective advantage to the genetically abnormal cells within the bulk culture and the level of mosaicism for the trisomy 14 remained/decreased overtime.

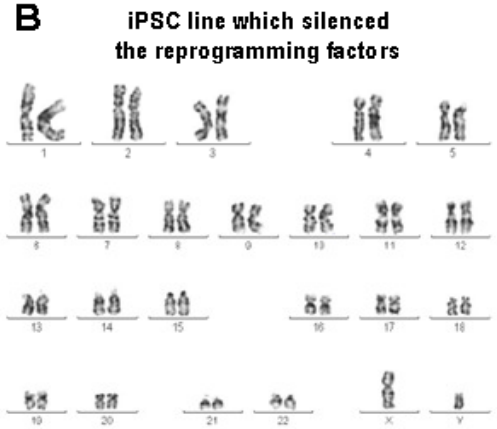
However, a much older hESC line, which was maintained for 185 passages in feeders did not undergo any numerical or structural chromosomal change after 30p in feeder-free culture and over 215 passages in total.

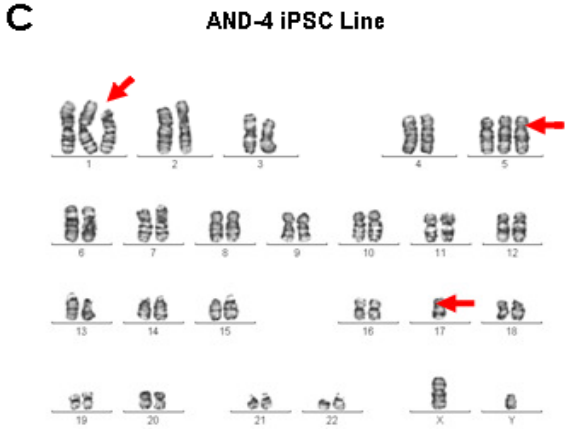
These results support the concept that feeder-free conditions may partially contribute to hESC chromosomal changes but also confirm the hypothesis that regardless of the culture conditions, culture duration or splitting methods, **some hESC lines are inherently more prone than others to karyotypic instability.**

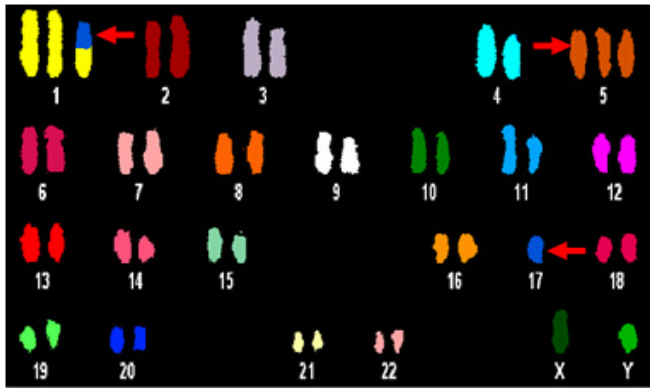
A potential link between lack of ectopic reprogramming factors silencing and propensity to genomic instability?





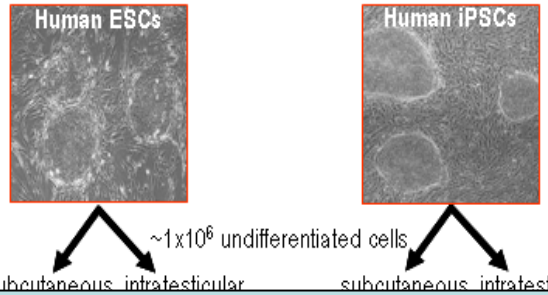






Ramos-Mejía V et al. 2010 Cell Res

Human iPSC develop teratoma more efficiently and faster than ESCs regardless the site of injection

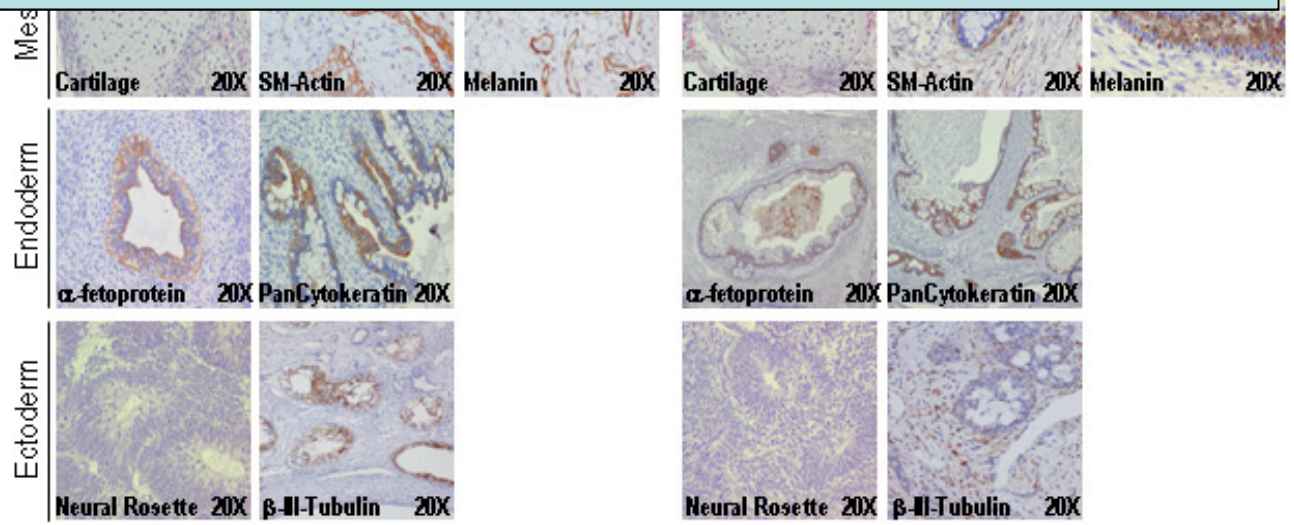


Type of pluripotent Cell Line	Inoculation Site	Teratoma Efficiency	Teratoma Latency (days)	Histological Analysis (3 germ layers staining)
Human ESCs	subcutaneous	13/16 (81%)	59±36	YES (meso, ecto & endoderm)
	intratesticular	13/14 (94%)	66±30	YES (meso, ecto & endoderm)

Are ectopic reprogramming factors with oncogenic potential (i.e: c-myc, Klf4) being re-activated during teratoma in vivo differentiation therefore promoting tumor formation?

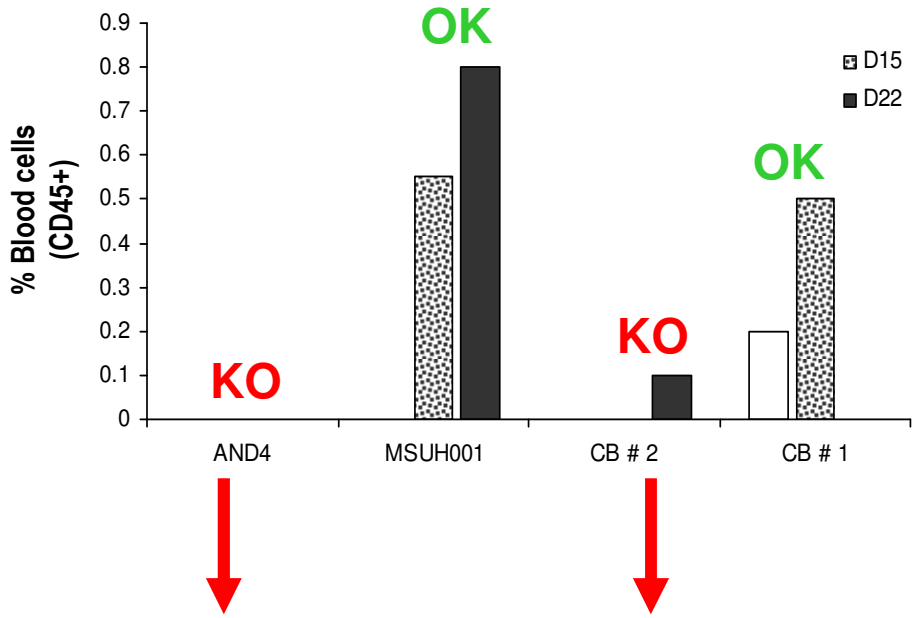
↓

CRE-mediated removal of the provirus and analyse:
-Teratoma efficiency and latency
-Karyotypic stability



Gutierréz-Aranda et al.
Stem Cells 2010

DETRIMENTAL EFFECT OF GENETIC INSTABILITY AND ECTOPIC REPROGRAMING FACTOR REACTIVATION ON iPSC DIFFERENTIATION



Genomic Instability linked to a lack of ectopic reprogramming factors

Reactivation of ectopic reprogramming factors during differentiation

Does re-activation of the ectopic reprogramming factors during differentaition (or genomic instability) impair directed differentiation?

↓

CRE-mediated removal of the provirus and analyse directed differentiation

Ramos-Mejía et al. In preparation

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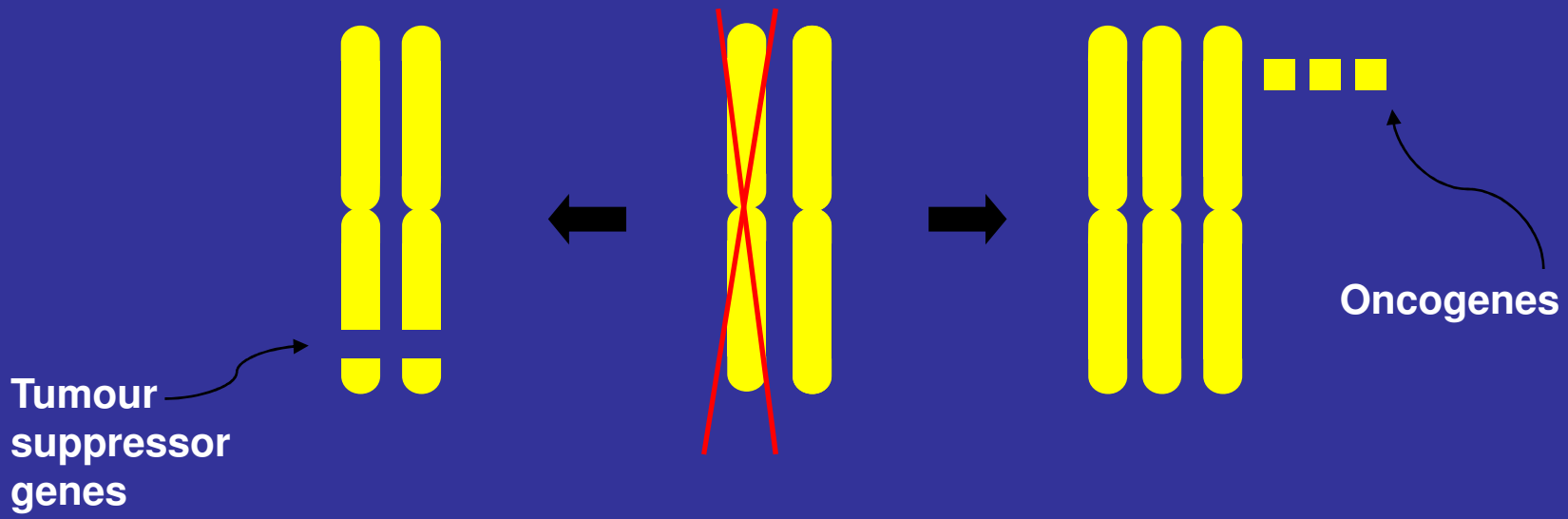
PARA LA LUCHA CONTRA LA LEUCEMIA

Copy Number Variations



Loss of Heterozygosity (LOH) and Uniparental Disomy (UPD)