CELLULES SOUCHES PLURIPOTENTES INDUITES (iPS) : APPLICATIONS AUX PATHOLOGIES GÉNÉTIQUES ET LIMITES

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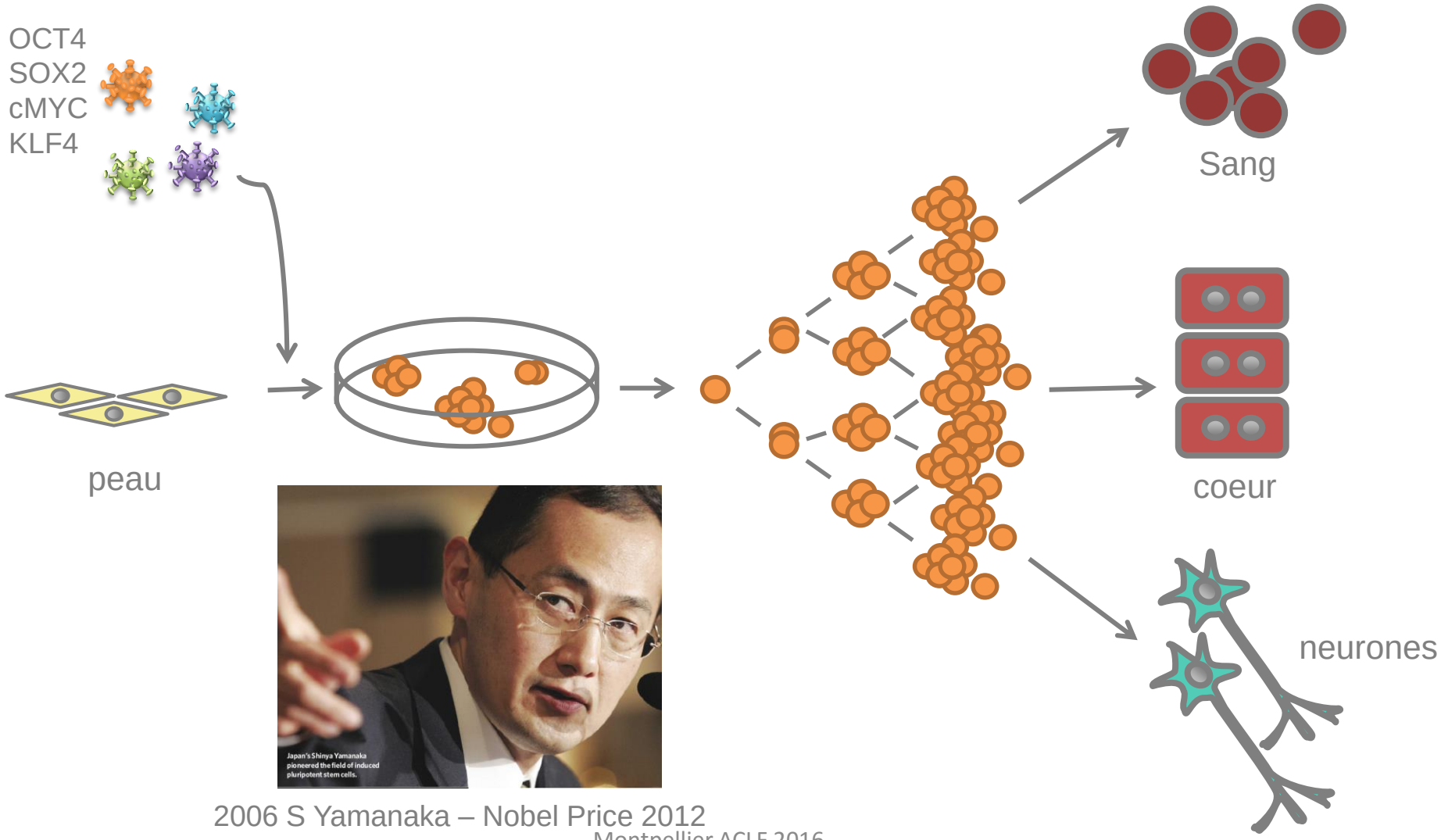
CELLULES SOUCHES PLURIPOTENTES

Induced pluripotent stem cells (iPS)



2006 S Yamanaka – Nobel Price 2012

K. Takahashi and S. Yamanaka. *Cell*, 126:663, 2006.

Applications

1. Modélisation in vitro du développement normal et des maladies génétiques
2. Une source illimité de cellules pour la thérapie cellulaire potentiellement autologues
3. Un moyen de rajeunir des cellules âgées voire sénescentes

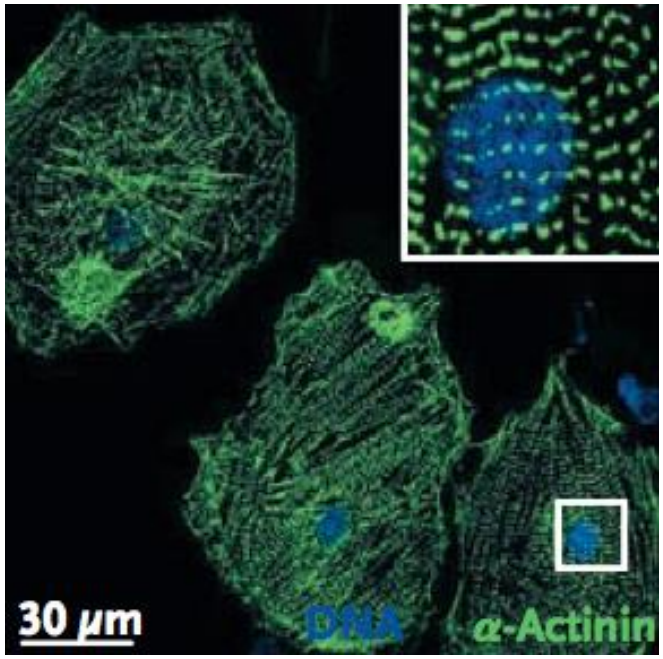
MODELLISATION DE PATHOLOGIES GENETIQUES

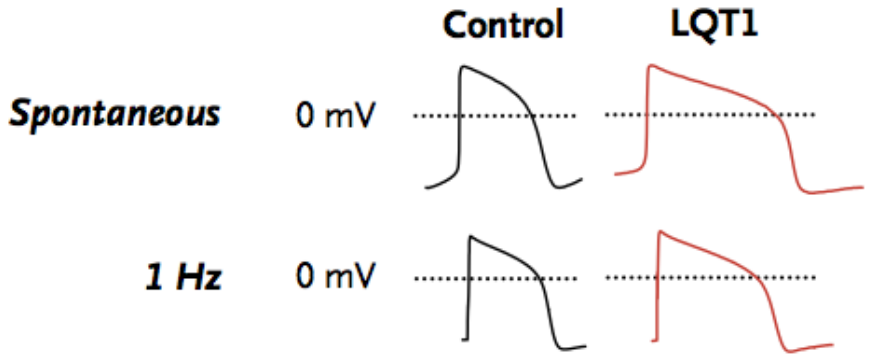
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Patient-Specific Induced Pluripotent Stem-Cell Models for Long-QT Syndrome

Alessandra Moretti, Ph.D., Milena Bellin, Ph.D., Andrea Welling, Ph.D., Christian Billy Jung, M.Sc., Jason T. Lam, Ph.D., Lorenz Bott-Flügel, M.D., Tatjana Dorn, Ph.D., Alexander Goedel, M.D., Christian Höhnke, M.D., Franz Hofmann, M.D., Melchior Seyfarth, M.D., Daniel Sinnecker, M.D., Albert Schömig, M.D., and Karl-Ludwig Laugwitz, M.D.



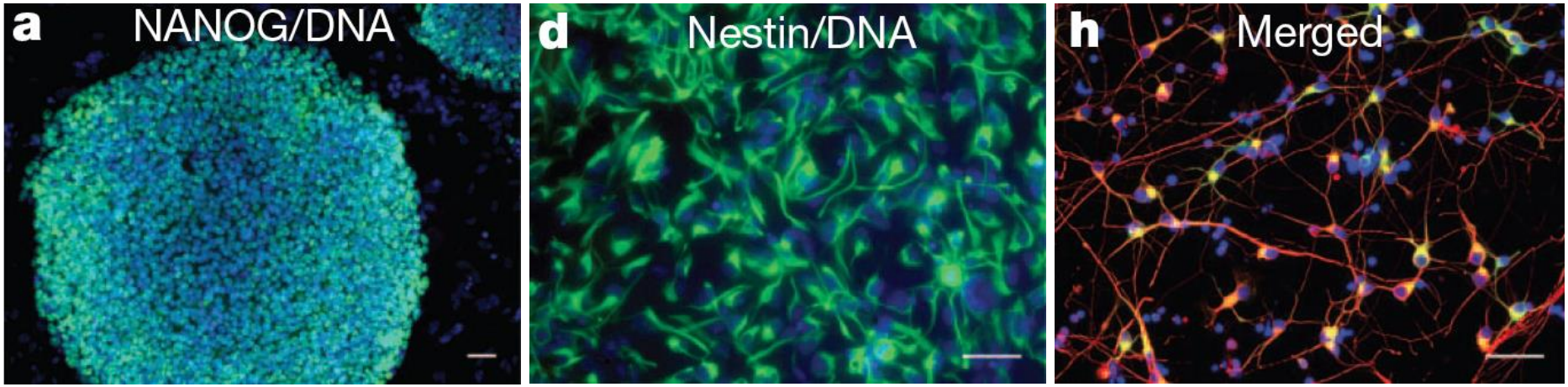


Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells

Mason A. Israel[1,2], Shauna H. Yuan[1,3], Cedric Bardy[4], Sol M. Reyna[1,2], Yangling Mu[4], Cheryl Herrera[1], Michael P. Hefferan[5], Sebastiaan Van Gorp[6], Kristopher L. Nazor[7], Francesca S. Boscolo[8], Christian T. Carson[9], Louise C. Laurent[8], Martin Marsala[5,10], Fred H. Gage[4], Anne M. Remes[11], Edward H. Koo[3] & Lawrence S. B. Goldstein[1,3]

BetaIII Tubulin

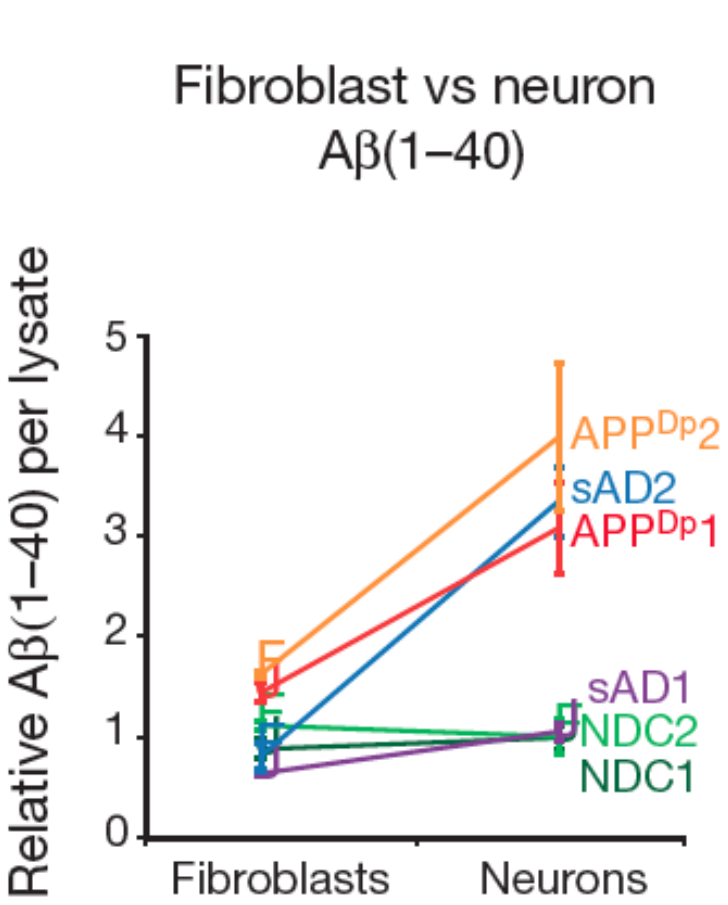
MAP2

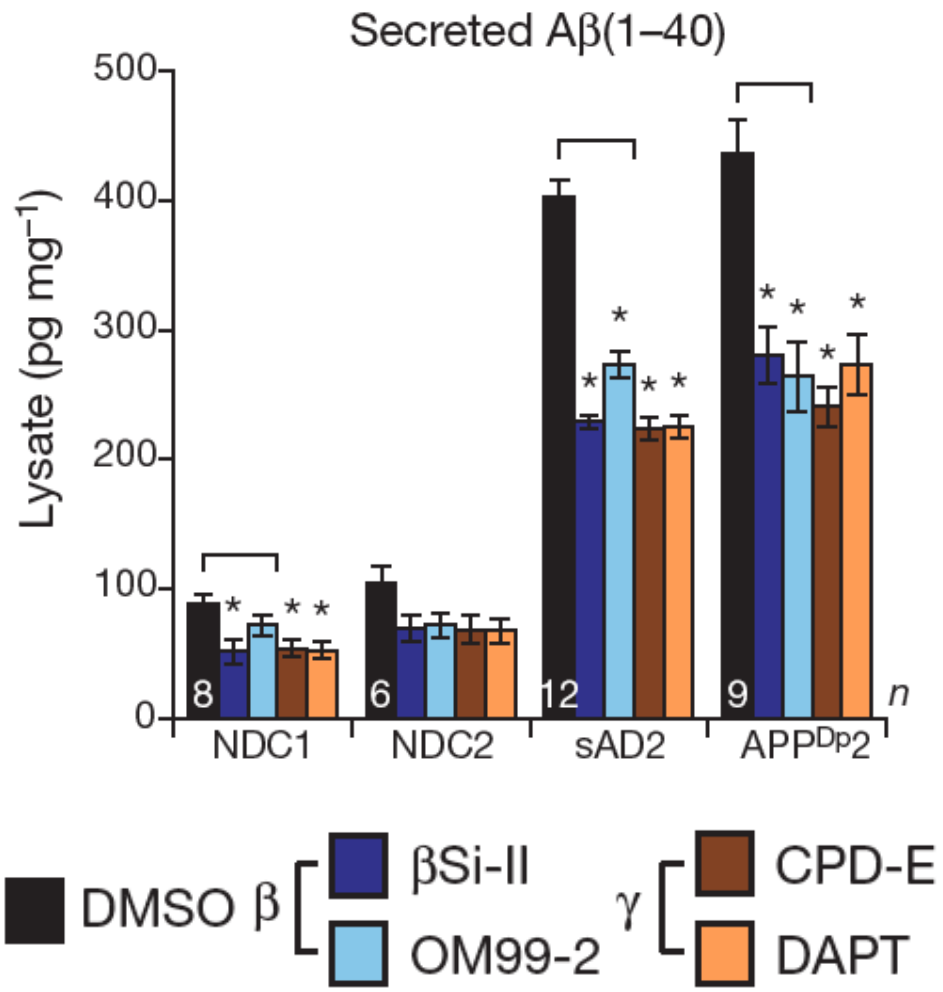


iPS — Progéniteurs neuronaux — Neurones

11j — 21j

Israel MA et al. *Nature*, 2012, *482*: 216–220





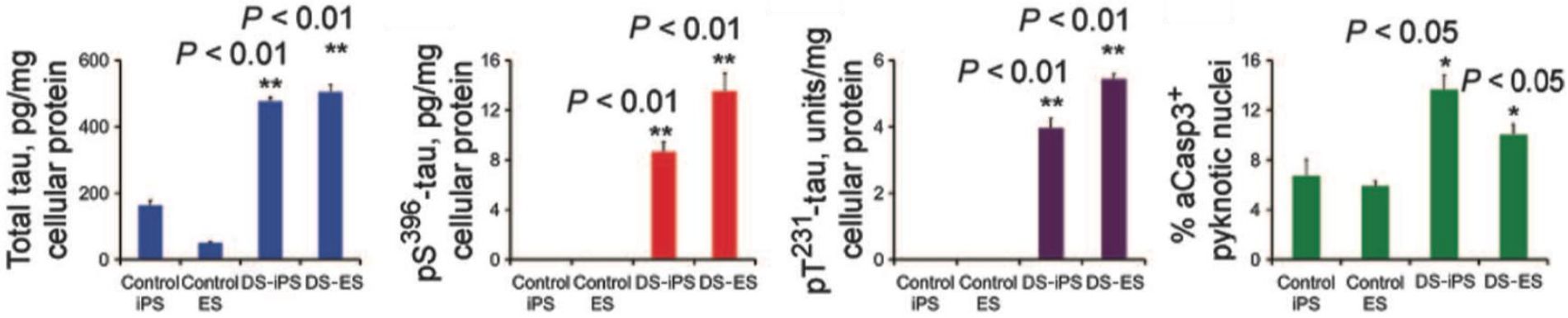
Israel MA et al. *Nature*, 2012, *482*: 216–220

Science Translational Medicine
AAAS

A Human Stem Cell Model of Early Alzheimer's Disease Pathology in Down Syndrome

Yichen Shi, *et al.*

Sci Transl Med **4**, 124ra29 (2012);
DOI: 10.1126/scitranslmed.3003771



ESSAIS CLINIQUES

Table 1 | **hESC and iPSC-based products in clinical trials**

Cell type (product name)	Company or group	Trial location	Disease	Stage of trial	Cell delivery	Status of trial
hESC-derived RPE (MA09-hRPE)	Ocata Therapeutics	United States	Dry AMD	Phase I/II	Cell suspension	Active, not recruiting
hESC-derived RPE (MA09-hRPE)	Ocata Therapeutics	United States	Stargardt	Phase I/II	Cell suspension	Active, not recruiting
hESC-derived RPE (MA09-hRPE)	Ocata Therapeutics	United Kingdom	Stargardt	Phase I/II	Cell suspension	Recruiting
hESC-derived RPE (MA09-hRPE)	CHABiotech (licensed from Ocata)	Korea	Dry AMD	Phase I/II	Cell suspension	Recruiting
hESC-derived RPE (MA09-hRPE)	CHABiotech (licensed from Ocata)	Korea	Stargardt	Phase I	Cell suspension	Active, not recruiting
hESC-derived RPE (MA09-hRPE)	University of California, Los Angeles (with Ocata's cells)	United States	MMD	Phase I/II	Cell suspension	Not yet recruiting
iPSC-derived RPE (autologous)	Rikagaku Kenkyūsho (RIKEN) Institute	Japan	Wet AMD	Phase I	Monolayer sheet (no membrane)	On hold
hESC-derived RPE (PF-05206388)	Pfizer	United Kingdom	Wet AMD	Phase I	Membrane-immobilized monolayer sheet	Recruiting
hESC-derived RPE (Opregen)	Cell Cure Neuroscience	Israel	Dry AMD	Phase I/II	Cell suspension	Recruiting
hESC-derived $CD15^{+}ISL\text{-}1^{+}$ cardiac progenitors	Assistance publique, Hôpitaux de Paris	France	Severe heart failure	Phase I	Cells embedded in fibrin patch	Recruiting
hESC-derived pancreatic endoderm (VC-01)	Viacyte	United States	Type I diabetes	Phase I/II	PEC-01 cells encapsulated in a medical device	Recruiting
hESC-derived oligodendrocyte progenitors (AST-OPC1)	Asterias Biotherapeutics	United States	Spinal cord injury	Phase I	Cell suspension	Completed (took over from Geron)
hESC-derived oligodendrocyte progenitors (AST-OPC1)	Asterias Biotherapeutics	United States	Spinal cord injury	Phase I/II	Cell suspension	Recruiting

AMD, age-related macular degeneration; hESC, human embryonic stem cell; iPSC, induced pluripotent stem cell; MMD, myopic macular degeneration; RPE, retinal pigment epithelium.

Kimbrel EA et al. *Nature Reviews Drug Discovery*, 2015 *14*: 681–692.

European Heart Journal Advance Access published May 19, 2015

European Heart Journal
doi:10.1093/eurheartj/ehv189

EHJ BRIEF COMMUNICATION
Heart failure/cardiomyopathy

Human embryonic stem cell-derived cardiac progenitors for severe heart failure treatment: first clinical case report

Philippe Menasché[1,2,3]*, Valérie Vanneaux[4,5], Albert Hagège[2,3,6], Alain Bel[1], Bernard Cholley[2,7], Isabelle Cacciapuoti[4,5], Alexandre Parouchev[4,5], Nadine Benhamouda[8], Gérard Tachdjian[9], Lucie Tosca[9], Jean-Hugues Trouvin[10,11], Jean-Roch Fabreguettes[12], Valérie Bellamy[3], Romain Guillemain[8], Caroline Suberbielle Boissel[13], Eric Tartour[2,3,8], Michel Desnos[2,3,6], and Jérôme Larghero[4,5,14]

Menasche P et al. *European Heart Journal*, 2015 *36*: 2011–2017

LIMITES

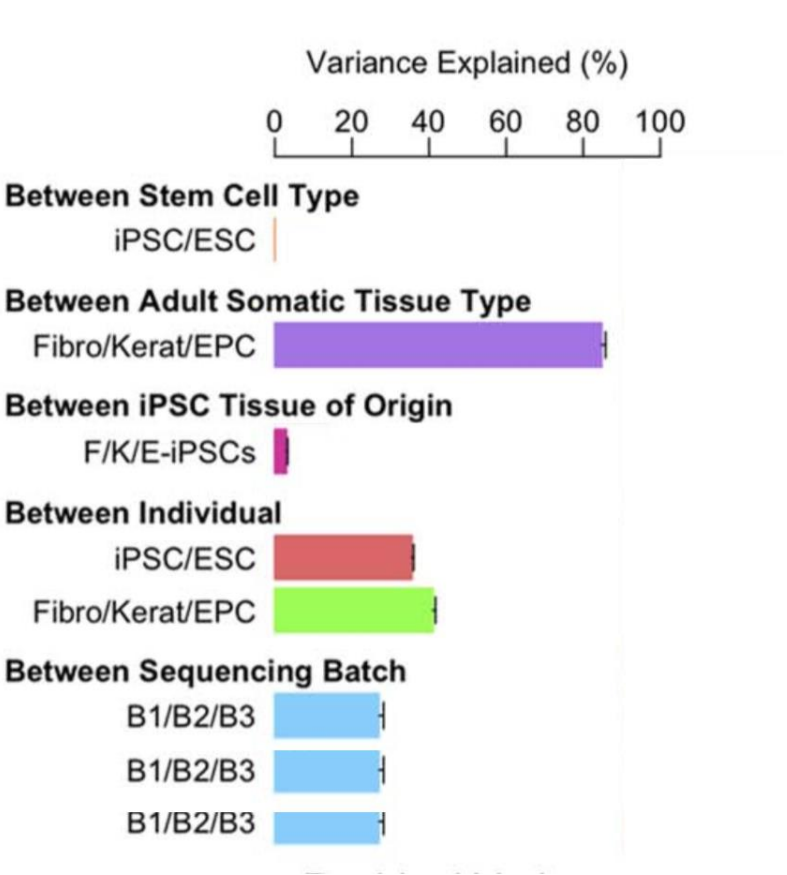
iPS = ES ?

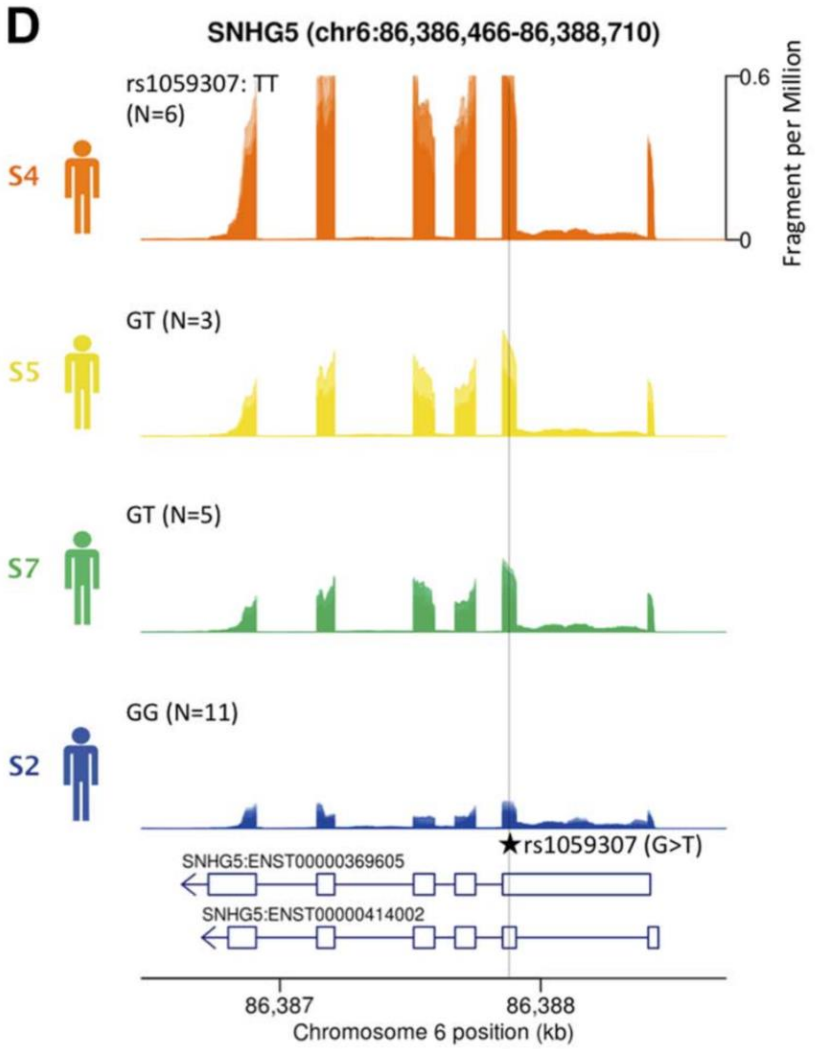
- Anomalies épigénétiques
- Anomalies génétiques secondaires à la reprogrammation

- N'est pas retrouvé par tous (sauf mutagénèse insertionnelle!): dépend du protocole de reprogrammation?

Q. Bai, R. Desprat, B. Klein, J.-M. Lemaitre, and J. De Vos, "Embryonic stem cells or induced pluripotent stem cells? A DNA integrity perspective.," Curr Gene Ther, vol. 13, pp. 93–98, Apr. 2013.

L’essentiel de la variabilité vient du fond génétique





[1] F. Rouhani,et al., “Genetic background drives transcriptional variation in human induced pluripotent stem cells.,” PLoS Genet, vol. 10, no. 6, p. e1004432, Jun. 2014.

iPS = ES !

ARTICLES

nature biotechnology

A comparison of genetically matched cell lines reveals the equivalence of human iPSCs and ESCs

Jiho Choi[1–3,10], Soohyun Lee[4,10], William Mallard[5,6], Kendell Clement[2,5,6], Guidantonio Malagoli Tagliazucchi[4,7], Hotae Lim[8], In Young Choi[8], Francesco Ferrari[4], Alexander M Tsankov[2,5,6], Ramona Pop[2,5,6], Gabsang Lee[8], John L Rinn[5,6,9], Alexander Meissner[2,5,6], Peter J Park[4] & Konrad Hochedlinger[1–3]

NATURE BIOTECHNOLOGY VOLUME 33 NUMBER 11 NOVEMBER 2015

ANOMALIES GENETIQUES INDUITES PAR LA CULTURE

Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells

Jonathan S Draper[1,6], Kath Smith[2,6], Paul Gokhale[1], Harry D Moore[3], Edna Maltby[2], Julie Johnson[4], Lorraine Meisner[4], Thomas P Zwaka[5], James A Thomson[5] & Peter W Andrews[1]

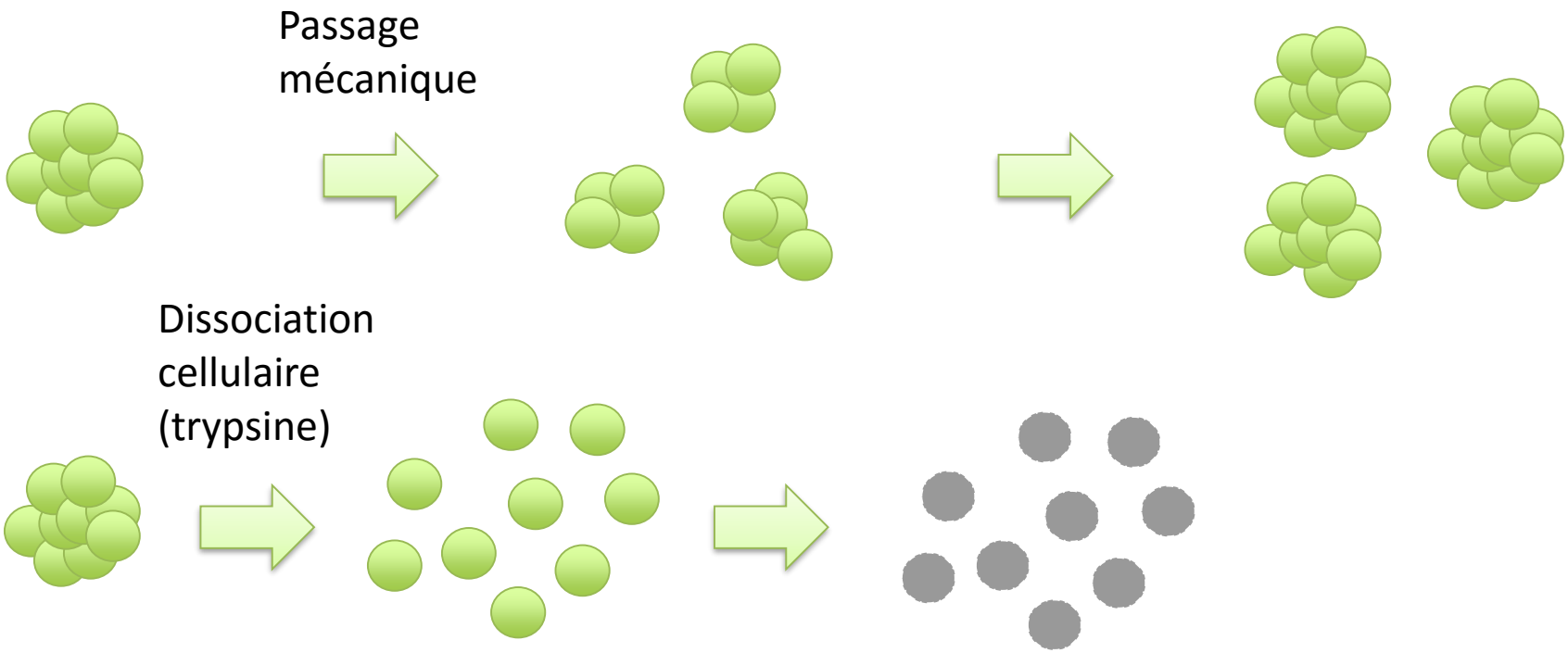
We have observed karyotypic changes involving the gain of chromosome 17q in three independent human embryonic stem (hES) cell lines on five independent occasions. A gain of chromosome 12 was seen occasionally. This implies that increased dosage of chromosome 17q and 12 gene(s) provides a selective advantage for the propagation of undifferentiated hES cells. These observations are instructive for the future application of hES cells in transplantation therapies in which the use of aneuploid cells could be detrimental.

NATURE BIOTECHNOLOGY VOLUME 22 NUMBER 1 JANUARY 2004

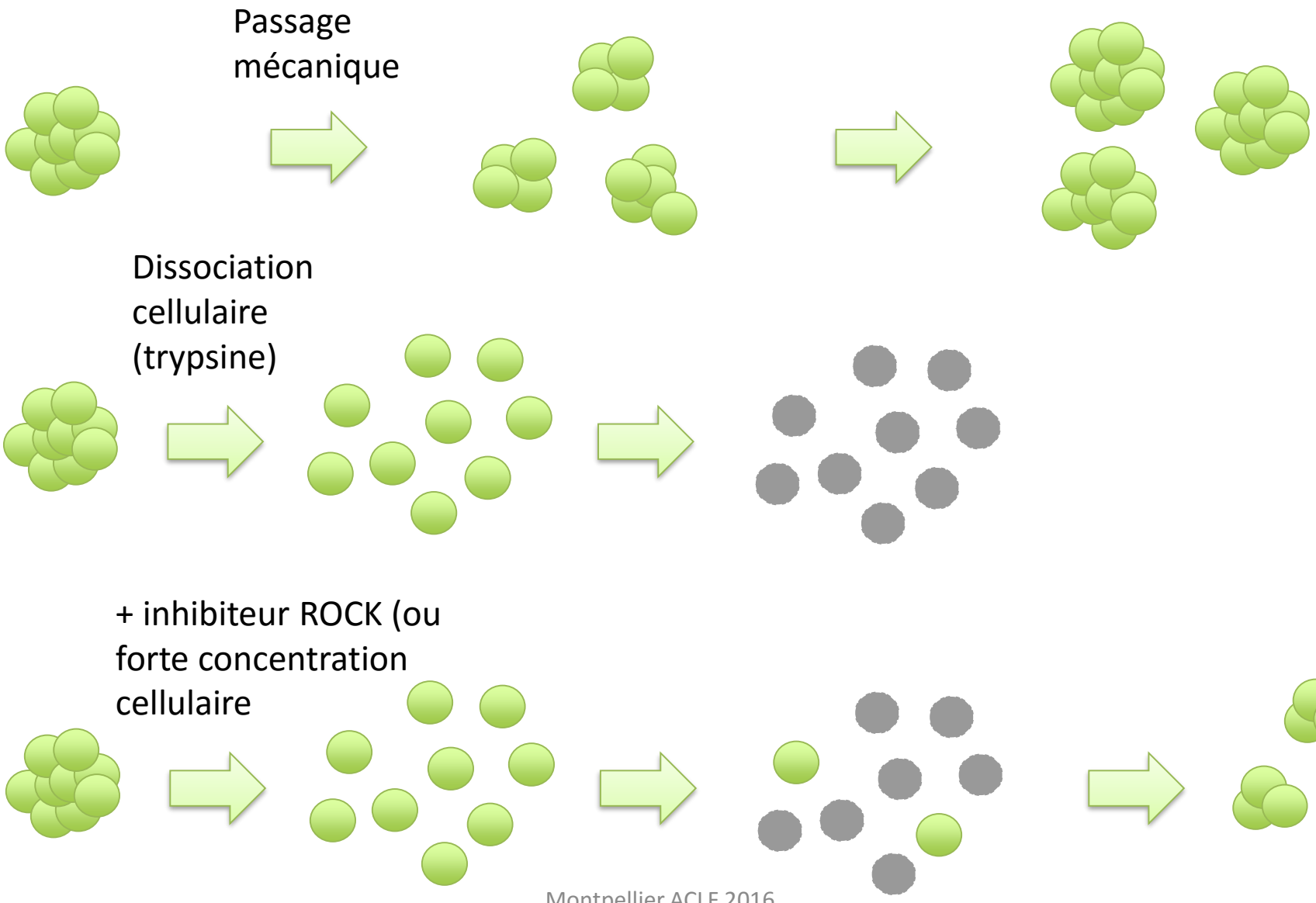
1. Chronologie et déterminants de cette instabilité
    1. A quelle vitesse les anomalies apparaissent-elles?
    2. Impact des conditions de culture (passage)
    3. Anomalies de caryotype vs infra-caryotypique?
2. Quelles anomalies génétiques?
3. Conséquences?

CHRONOLOGIE ET DETERMINANTS

Impact de la dissociation cellulaire : “adaptation”

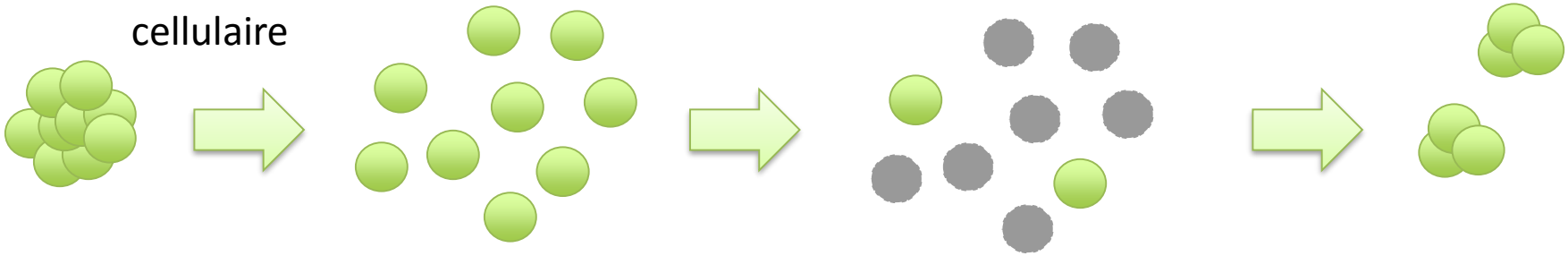


Impact de la dissociation cellulaire : “adaptation”



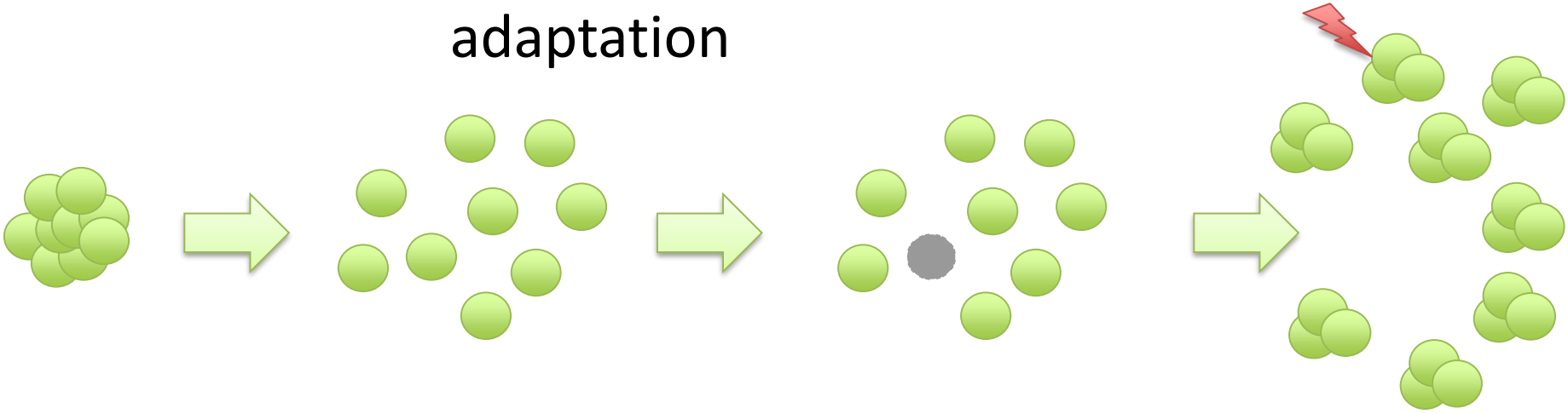
“Adaptation” à la dissociation cellulaire

+ inhibiteur ROCK (ou forte concentration cellulaire



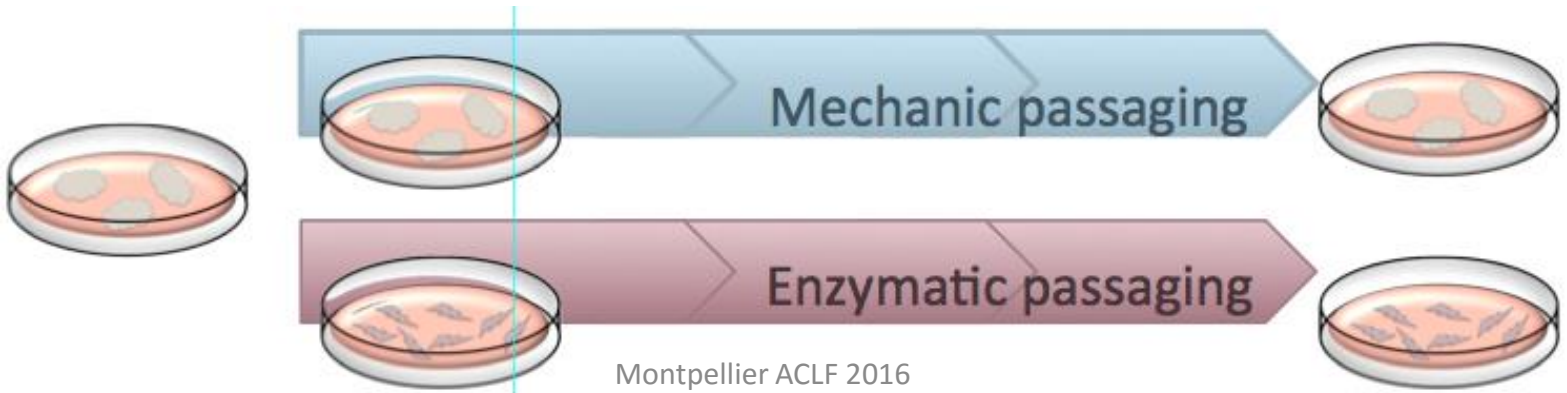
Après 5 à 10 passages : adaptation

Anomalies génétiques



ETUDE DES ANOMALIES GENETIQUES INDUITES PAR LE STRESS DE LA DISSOCIATION CELLULAIRE

- Comparaison d'un passage mécanique vs. enzymatique
- 3 lignées ES humaines à passage précoce:
  - HD129: p16 et HD291: p13; HS306: p25
- Trois techniques d'analyse génétique:
  - Caryotype
  - Puces à AND SNP 6.0 (Affymetrix)
  - NGS



Anomalies caryotypiques

Cell line	Passage	Karyotype [Number of metaphases]
HD129	p16+MP5	46, XY [13]
HD129	p16+MP9	46, XY [14]
HD291	p13+MP15	46, XY [11]
HD291	p13+MP30	46, XY [15]
HD291	p13+MP44	46, XY [20]
HS306	p25+MP10	46, XX [17]

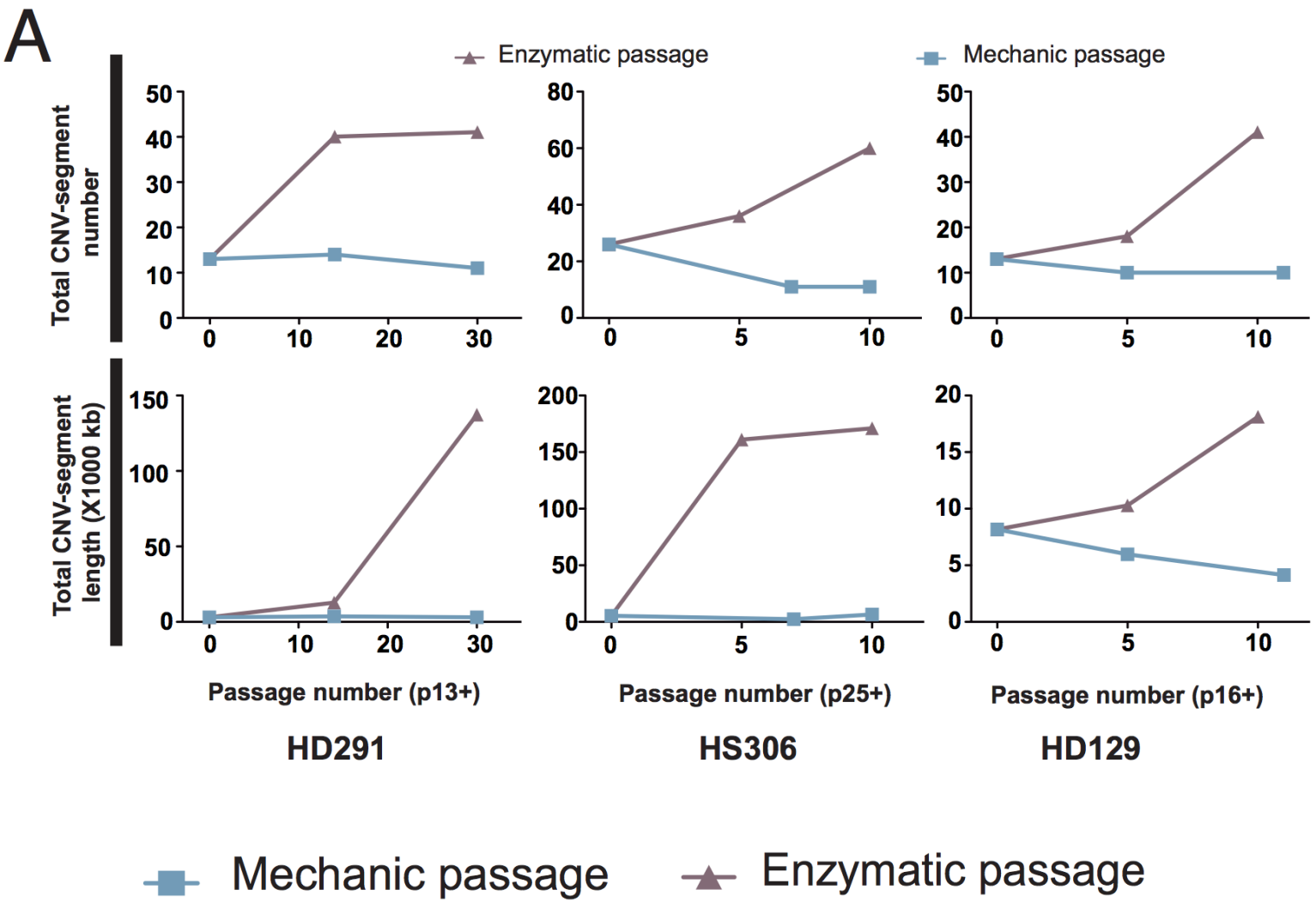
Bai et al. Stem Cell Dev, 2015

Anomalies caryotypiques

Cell line	Passage	Karyotype [Number of metaphases]
HD129	p16+MP5	46, XY [13]
HD129	p16+MP9	46, XY [14]
HD129	p16+EP9	mos 47, XY, +20 [8]/46, XY, -13, +20 [1]/ 46, XY [47]
HD291	p13+MP15	46, XY [11]
HD291	p13+MP30	46, XY [15]
HD291	p13+MP44	46, XY [20]
HD291	p13+EP34	47, XY, +12 [14]
HD291	p13+EP30MP17	47, XY, +12 [15]
HS306	p25+MP10	46, XX [17]
HS306	p25+EP5	mos 46, XX, i(7)(q10) [12]/ 47, XX, i(7)(q10), +3q, del(10q)? [1]/47, XX, dble i(7)(q10), +7 [3]/46, XX, i(7)(q10), del(9q) [2]/46, XX, i(7)(q10), del(9ter), del(10q) [2]//47, XX, i(7)(q10), +20 [1]
HS306	p25+EP11	46, XX, i(7)(q10) [17]

Bai et al. Stem Cell Dev, 2015

Copy Number Variation (CNV)



Bai et al. Stem Cell Dev, 2015

NGS

- Analyse en cours
  - Peu de mutations
  - Hasard ou déterminisme de ces mutations de novo?

Conclusion 1

- Instabilité génétique des CSP:
  - Fortement dépendant du type de passage
  - Peut-être très rapide (5 passages)
  - Anomalies caryotypiques et CNV

  - NB : anomalies de caryotype récurrent mais plutôt tardif, CNV non récurrent et précoce

QUELLES ANOMALIES GENETIQUES?

Nicolas Girault, Master 2

Said Assou, IR
Anthony Boureux, MCU
Thérèse Commes, PU
John De Vos, PUPH

Seadb.org (unpublished)

SEA database

The Stem cell gEnetic Abnormalities database

Home | Advanced search | Genome Browser | Tools | Downloads | News | Help | Maintenance actions | Contact | My account | Log out

Welcome in SEA database

Pluripotent stem cells such as Induced pluripotent stem cells (iPSCs) or embryonic stem cells (ESCs), as well as other stem cell, hold great promise for biomedical research and medical applications. However, stem cells might harbor genetic aberrations. Therefore, there is a need of guidelines to define the acceptable level of genomic integrity of stem cells for biomedical applications. SEA database is designed to store and display genetic abnormalities that have been reported in stem cells. The collection and computing of such abnormalities are a necessary step to propose guidelines for acceptable genomic integrity of stem cells.

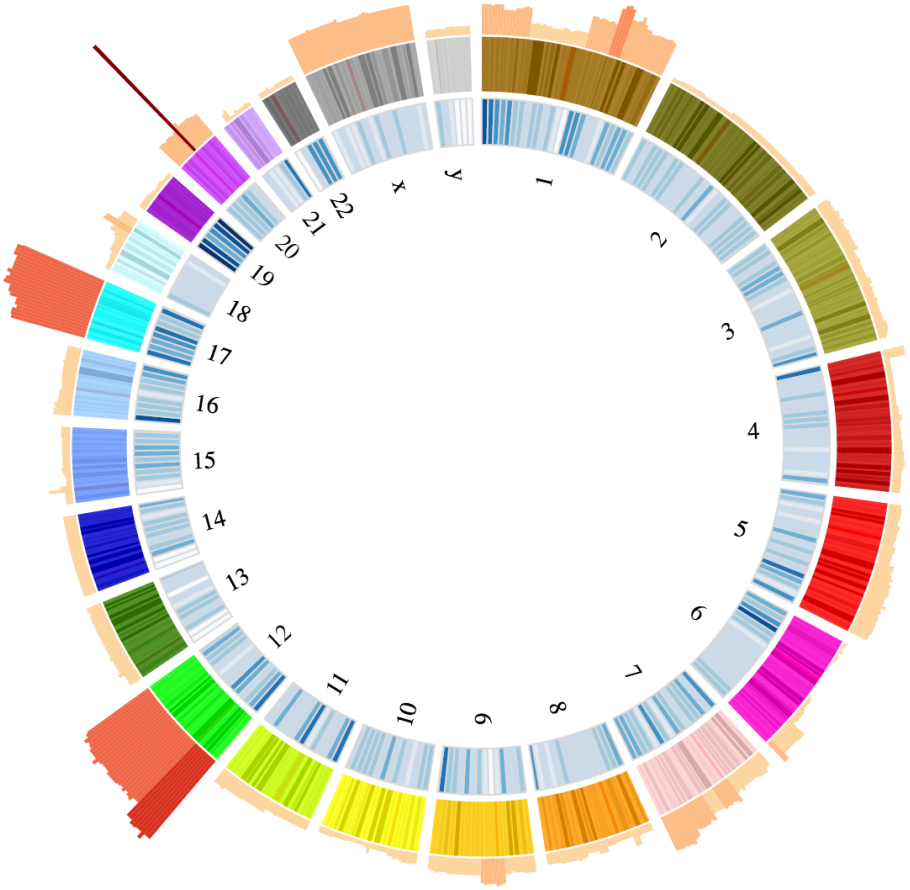
SEA database contains 101 studies, 905 samples and 405735 abnormalities and variants!

Search SEA

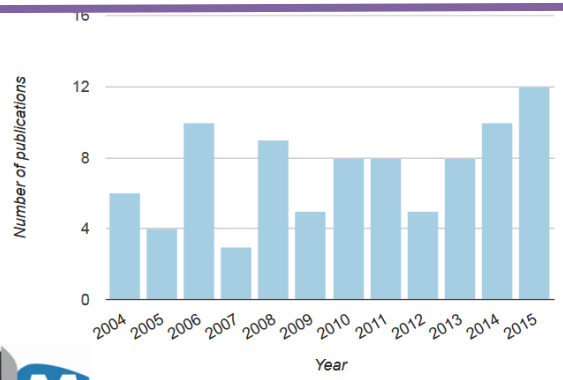
Search on genome browser. e.g. NANOG, 20:28400001..31500000

GO

Click and browse SEA abnormalities



SEAdb



Inventaire de ces anomalies dans les CSP

SEAdb

Extraction

101 Etudes

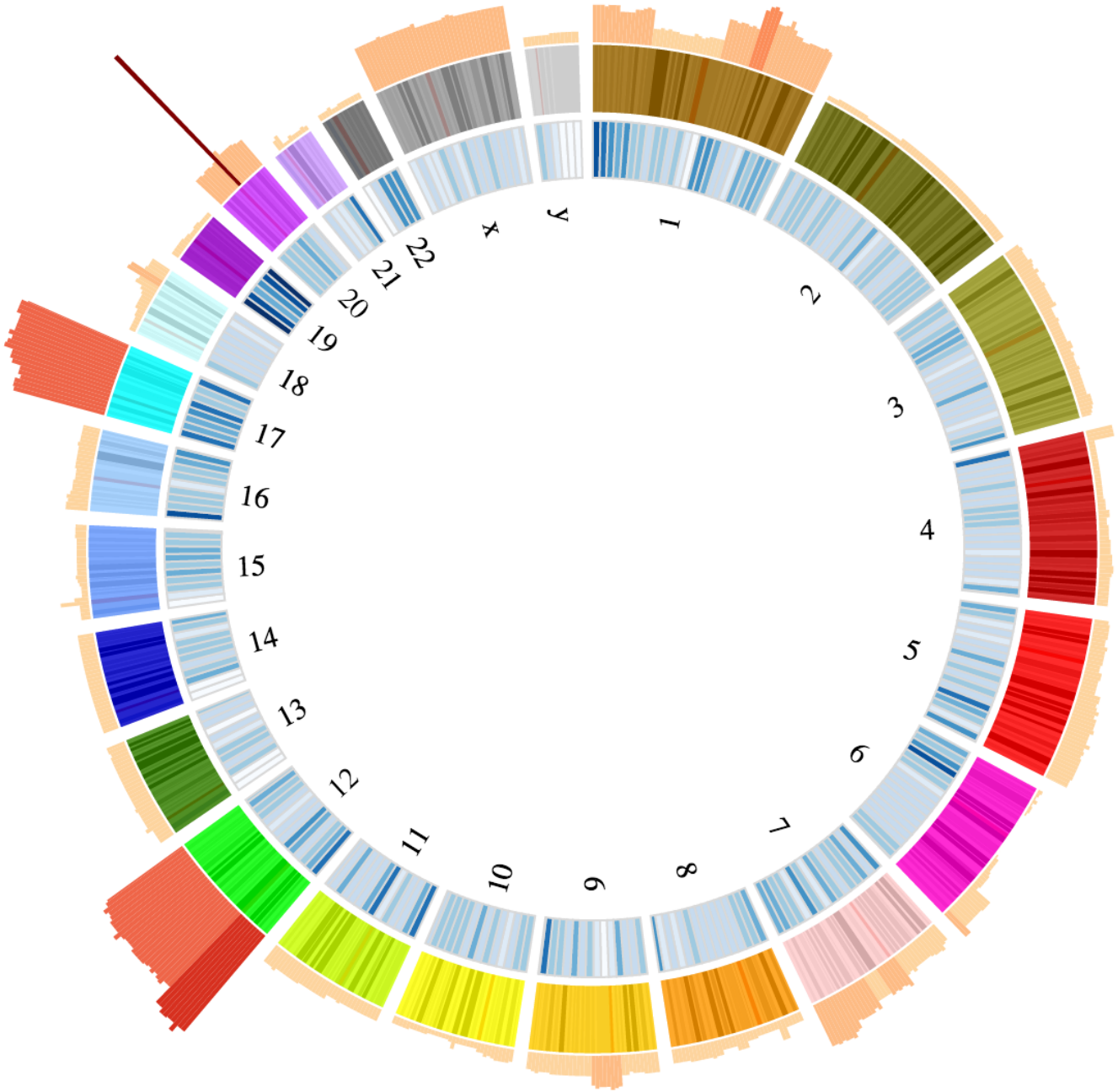
- Auteur
- PMID
- Année
- Journal
- Equipe

905 Echantillons

- Type d'échantillon
- Paramètre de culture
- Méthode de détection

405735 Variants

- Trisomy
- SNV
- CNV
- LOH
- Délétion
- Translocation
- Duplication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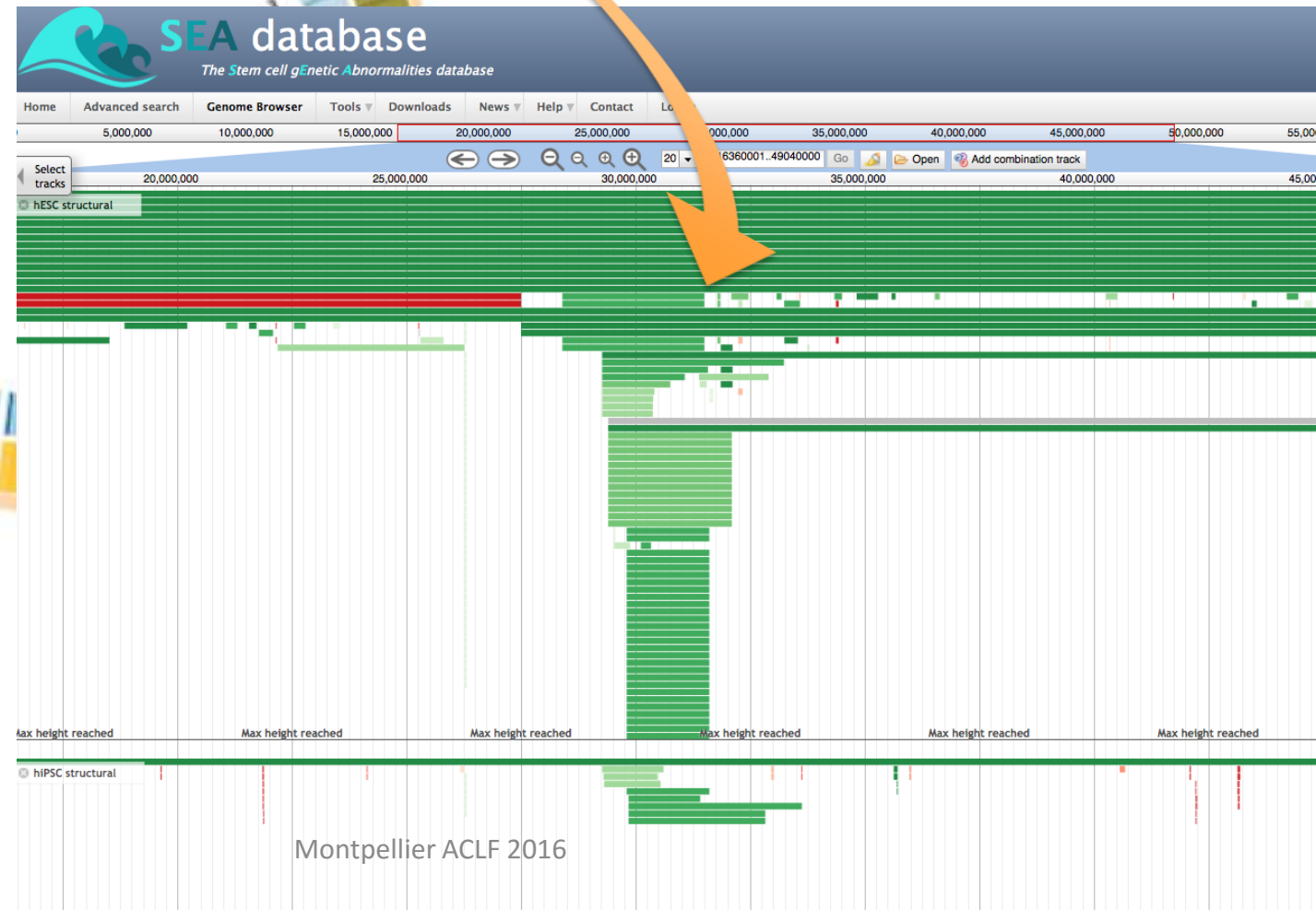
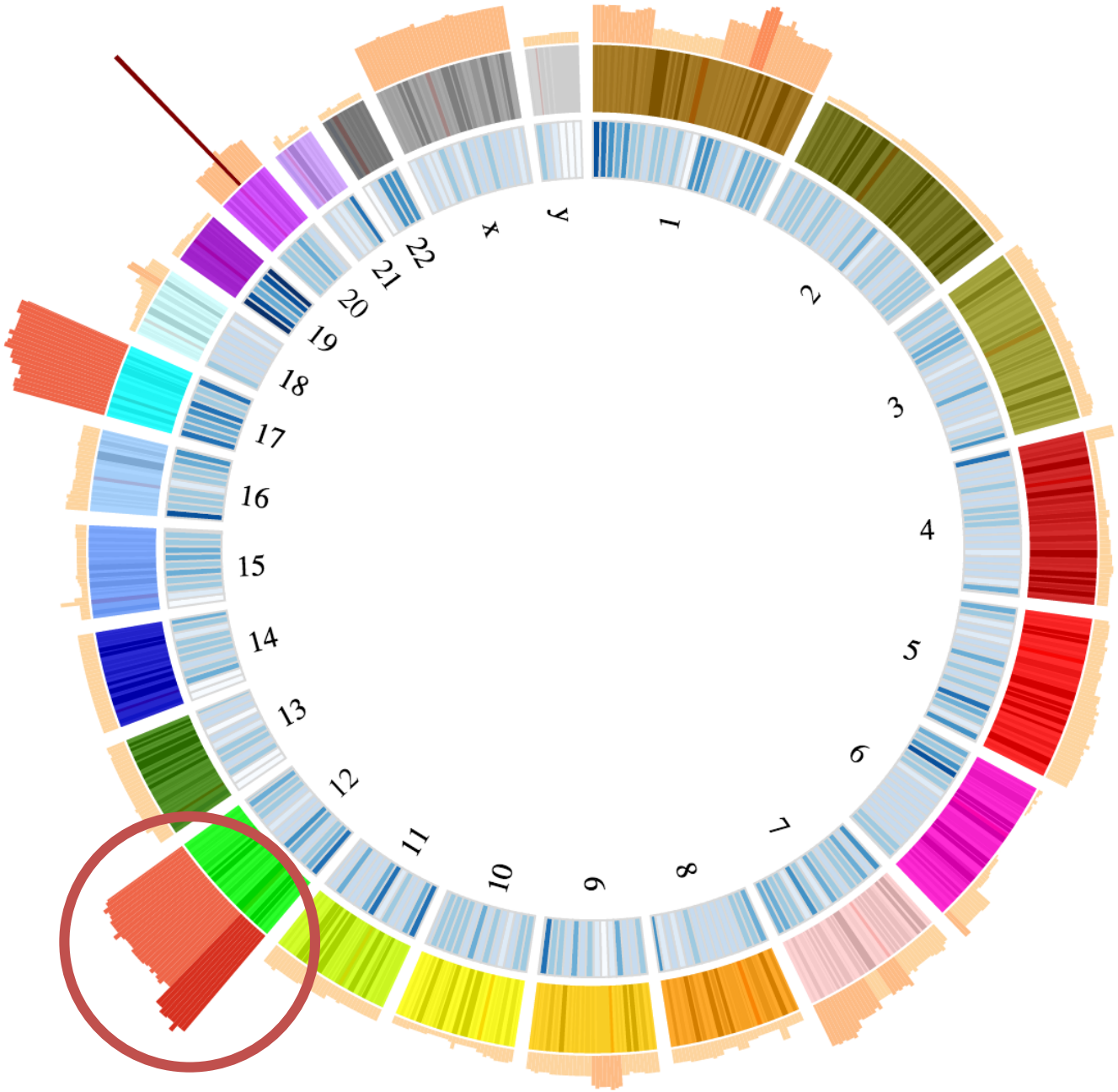
Zoom via a genome browser

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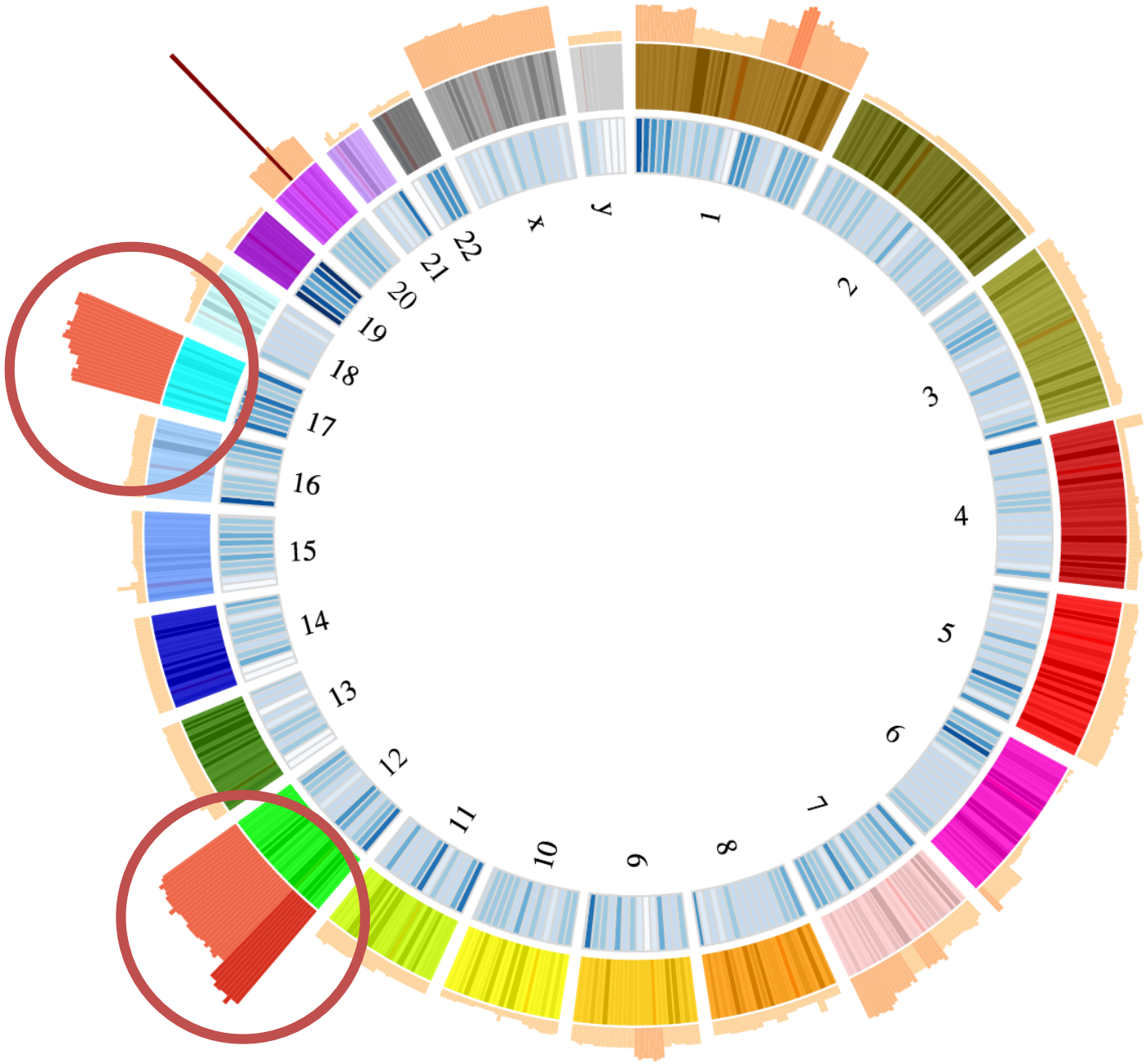
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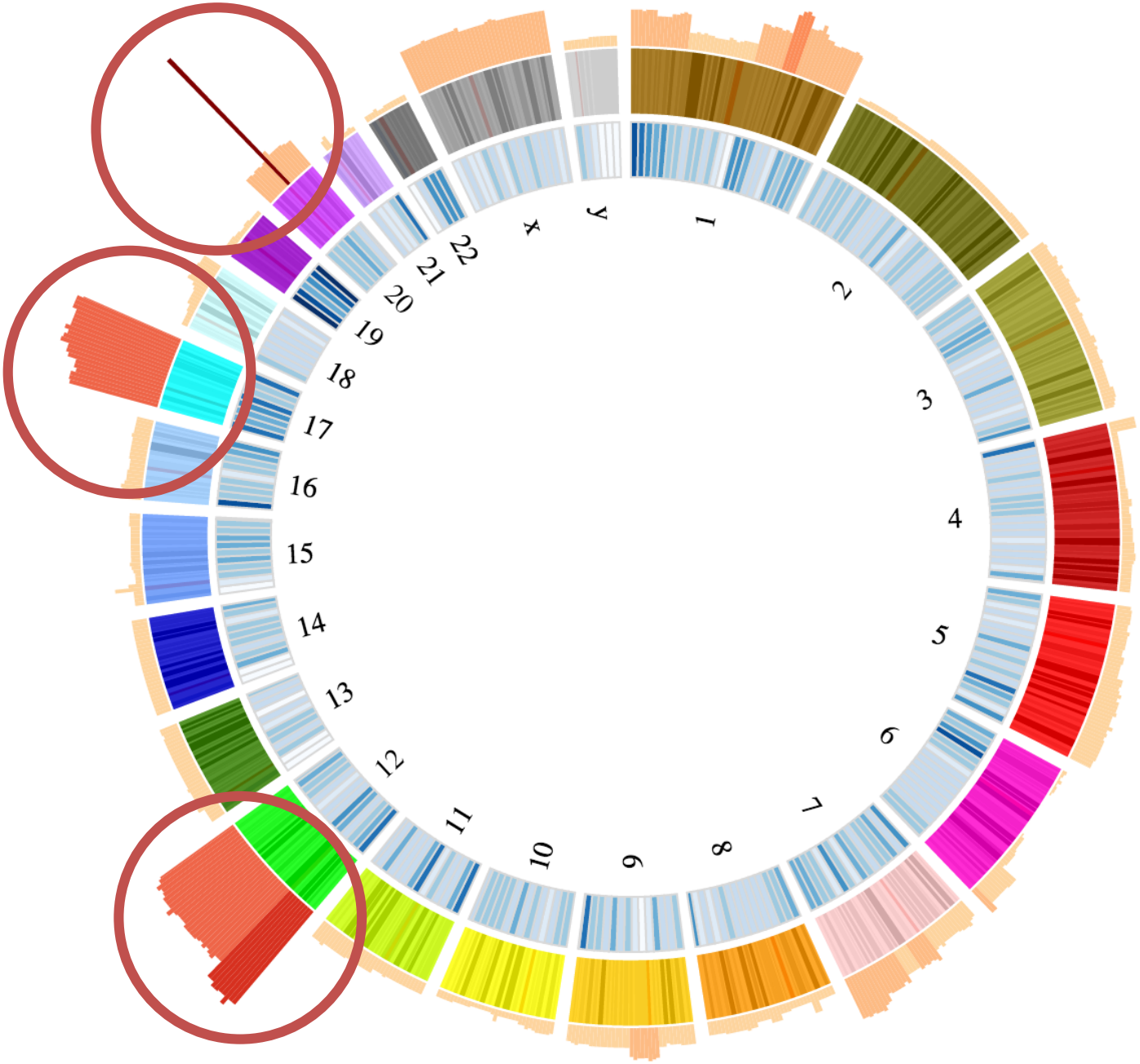
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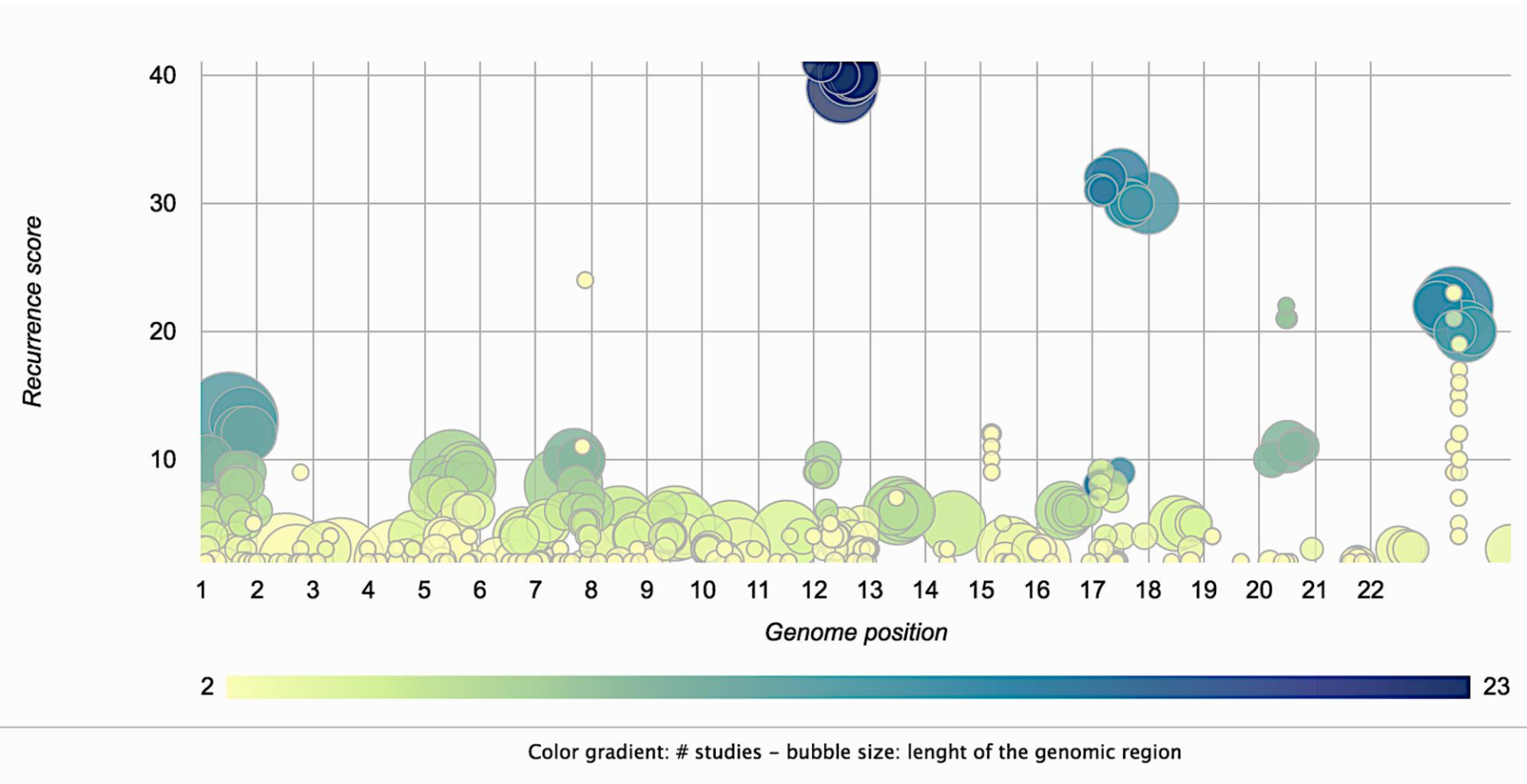
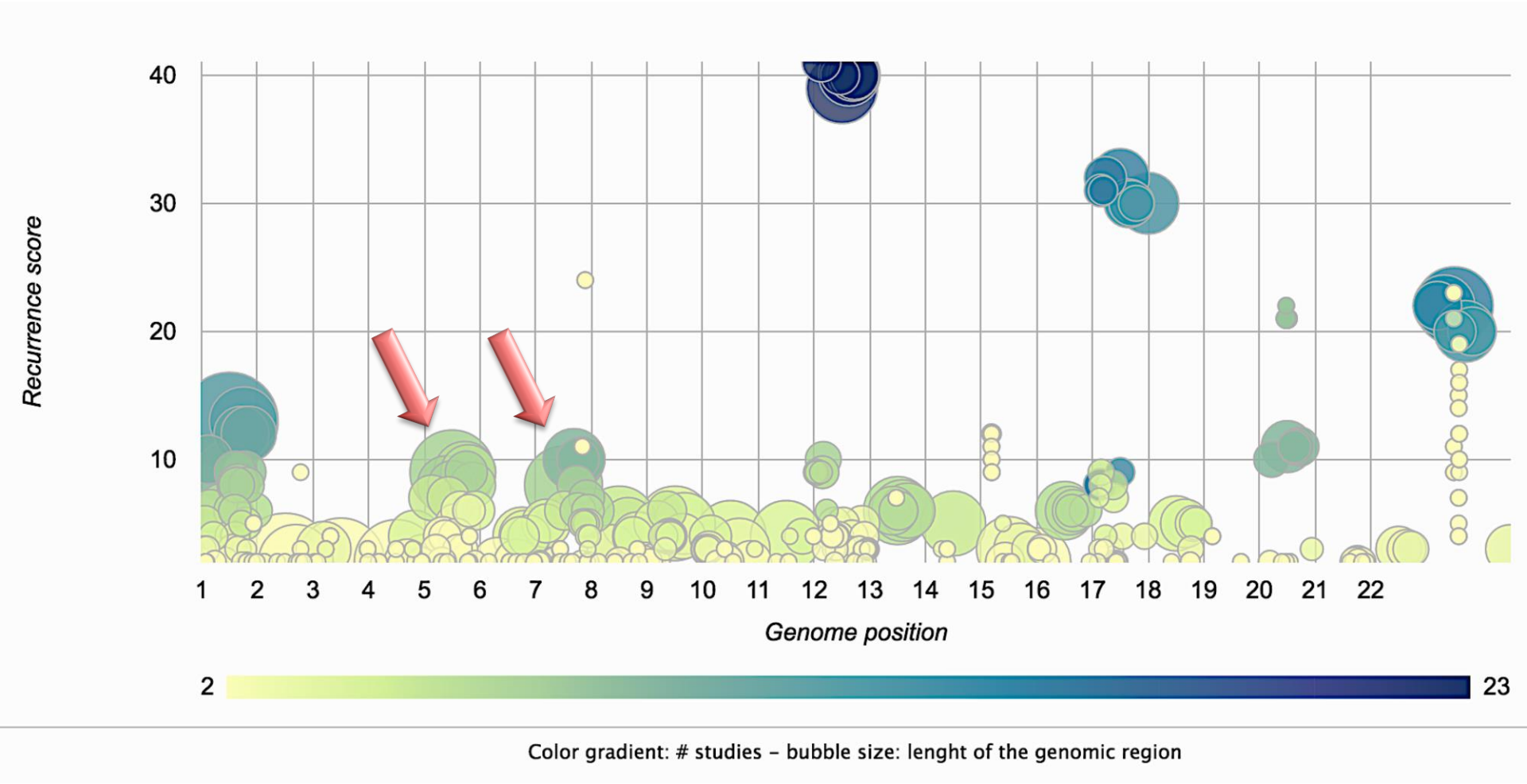
“HOTSPOT FINDER”

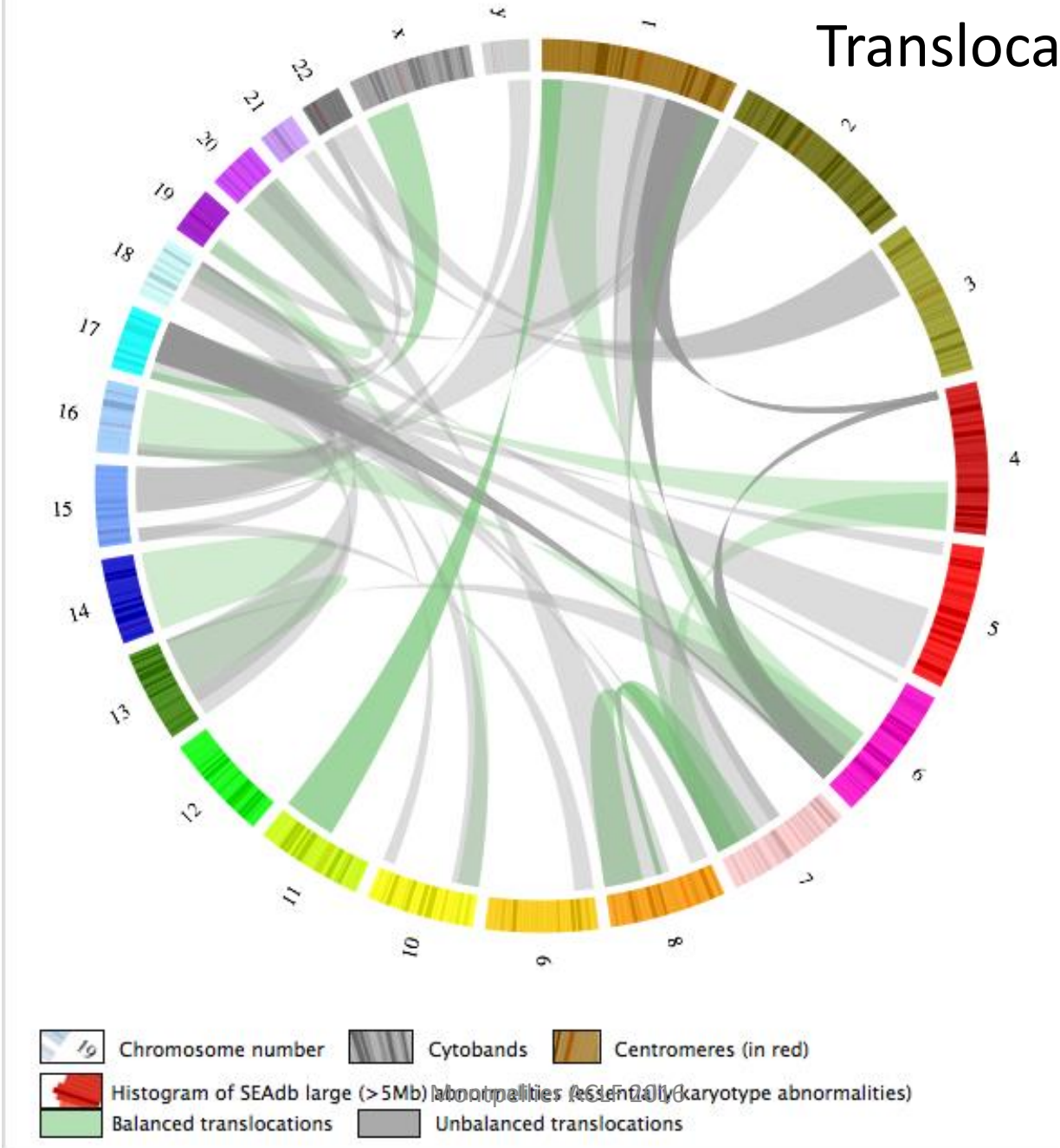
“HOTSPOT FINDER”



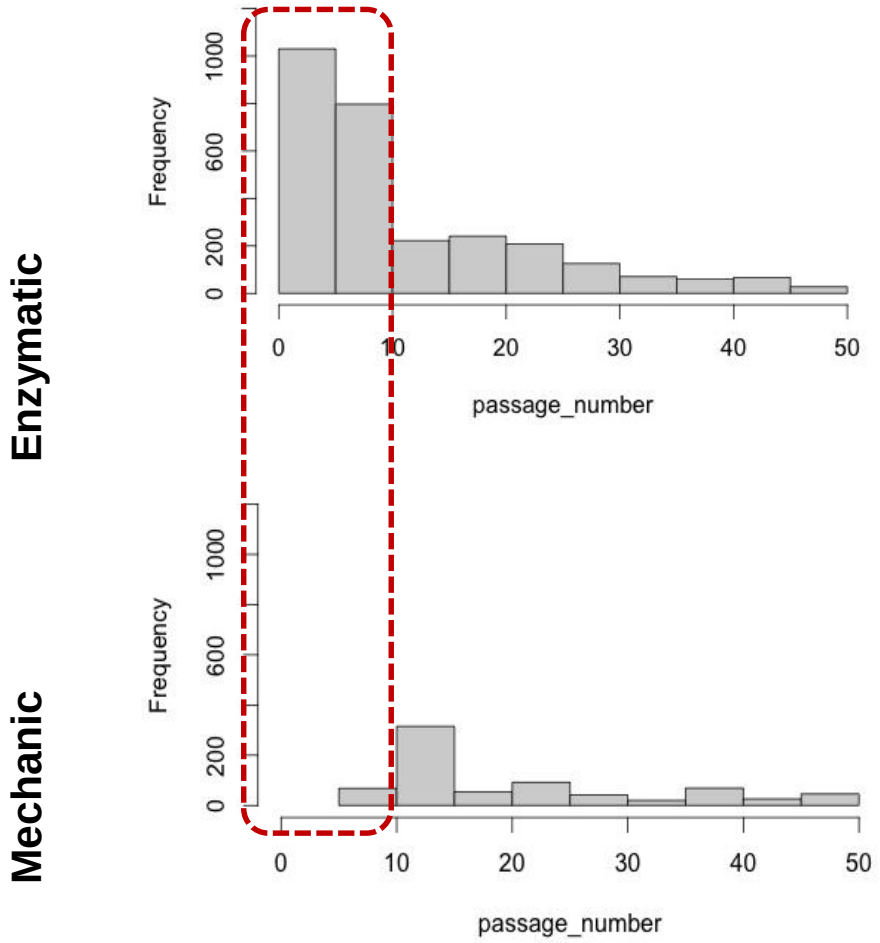
“HOTSPOT FINDER”



Translocations



Impact de la méthode de passage sur la fréquence des variants

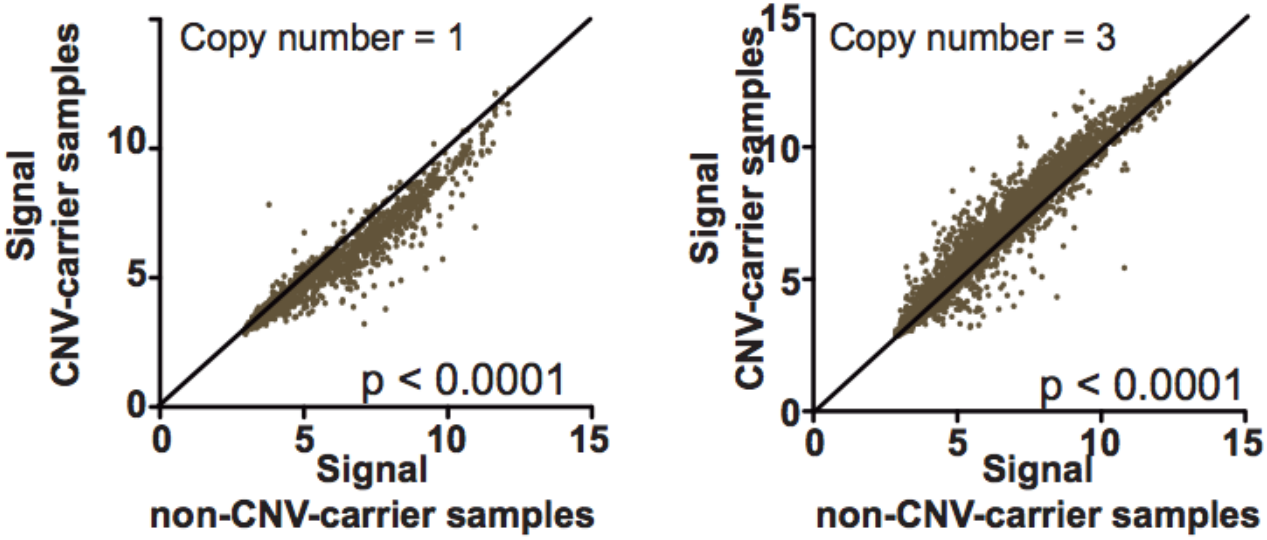


Conclusion 2

- Les principales anomalies génétiques récurrentes:
  - Aneuploïdies
  - Et des gains > 1Mb
  - Ce sont des anomalies « hyperrécurrentes »

ALTERATION DU TRANSCRIPTOME

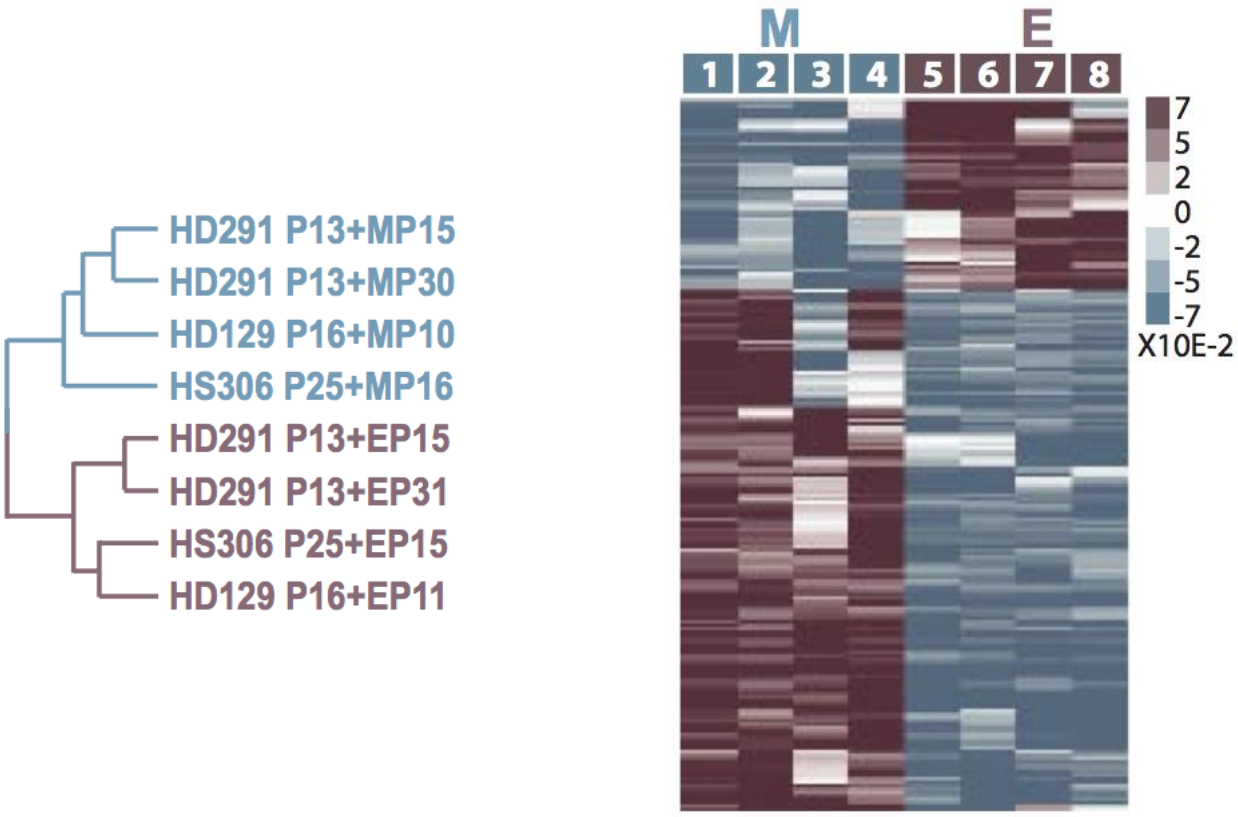
COPY NUMBER EFFECT



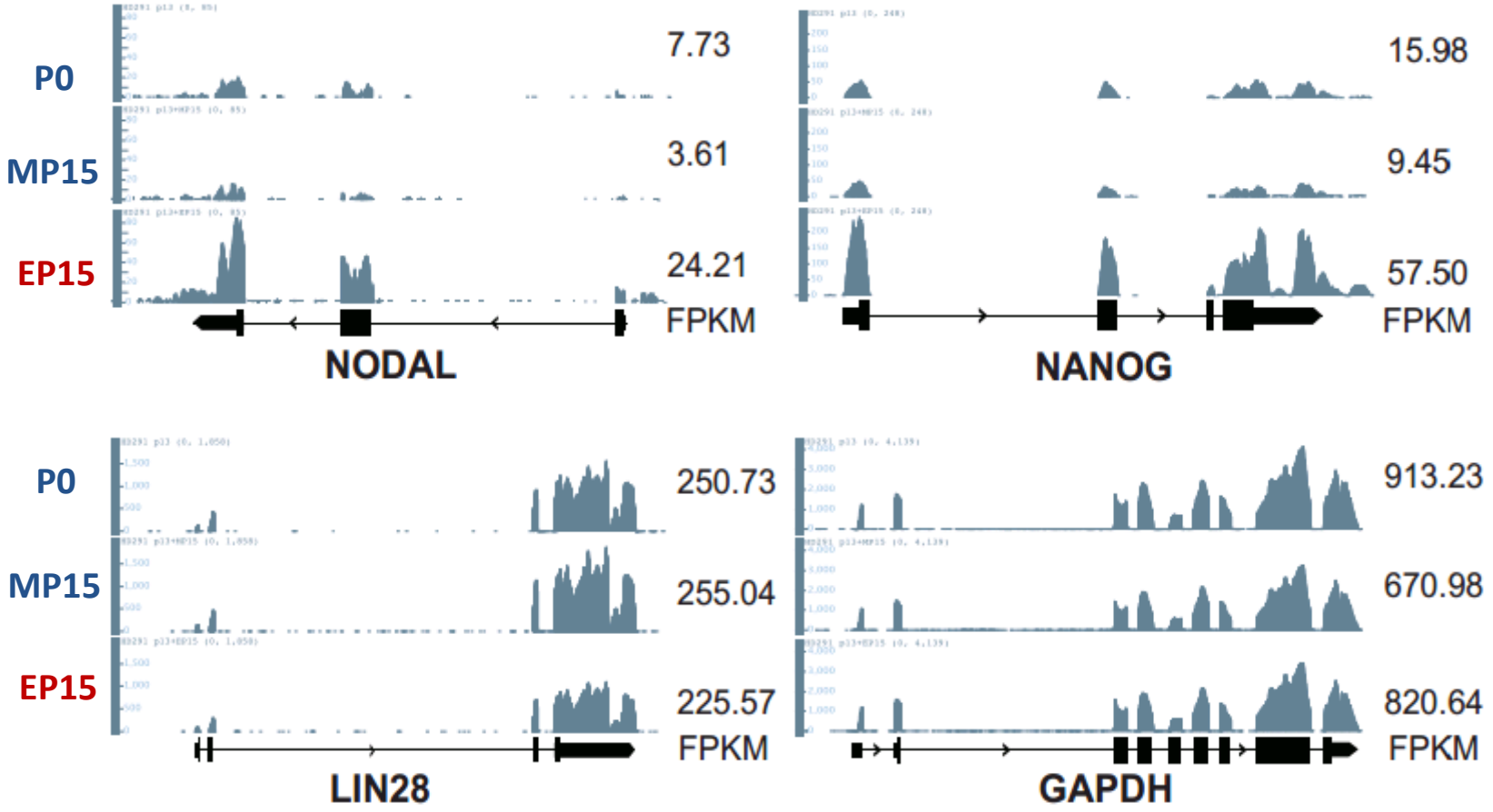
COPY NUMBER EFFECT?

- 320 probesets avec au moins un FC > 4 dans une lignée (max : 119,04)
- 22 probesets localisées sur un CNV (max : 7,8)

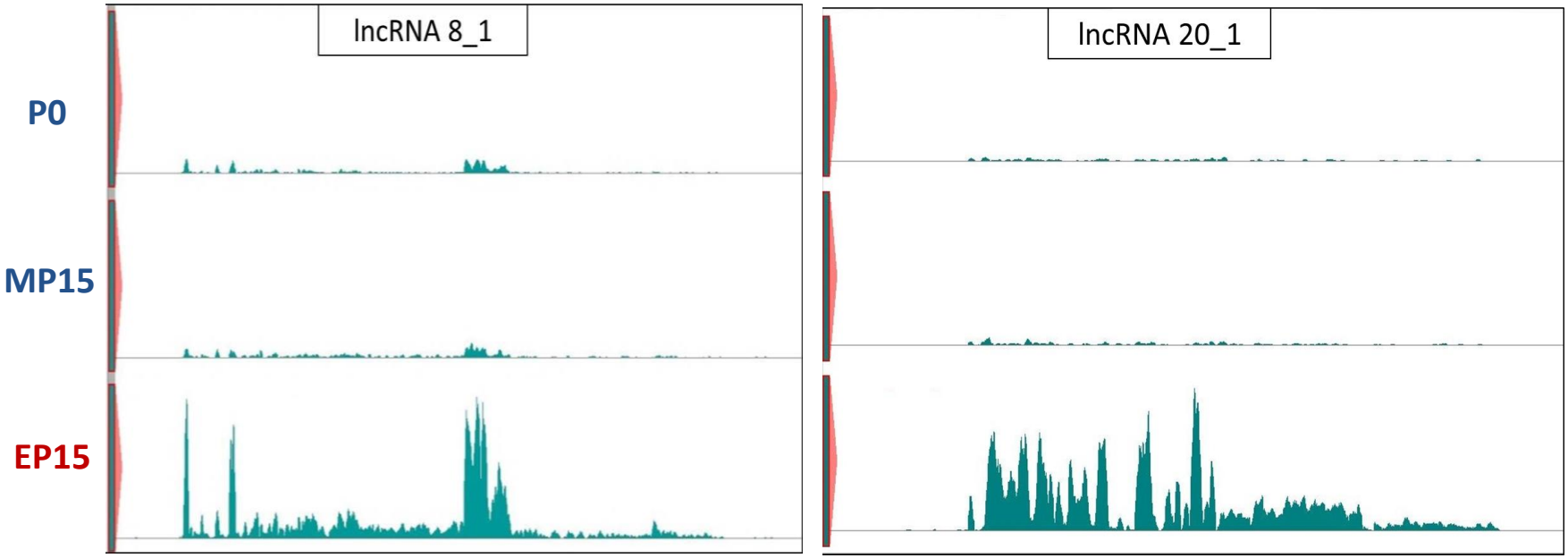
Signature transcriptionnelle caractéristique



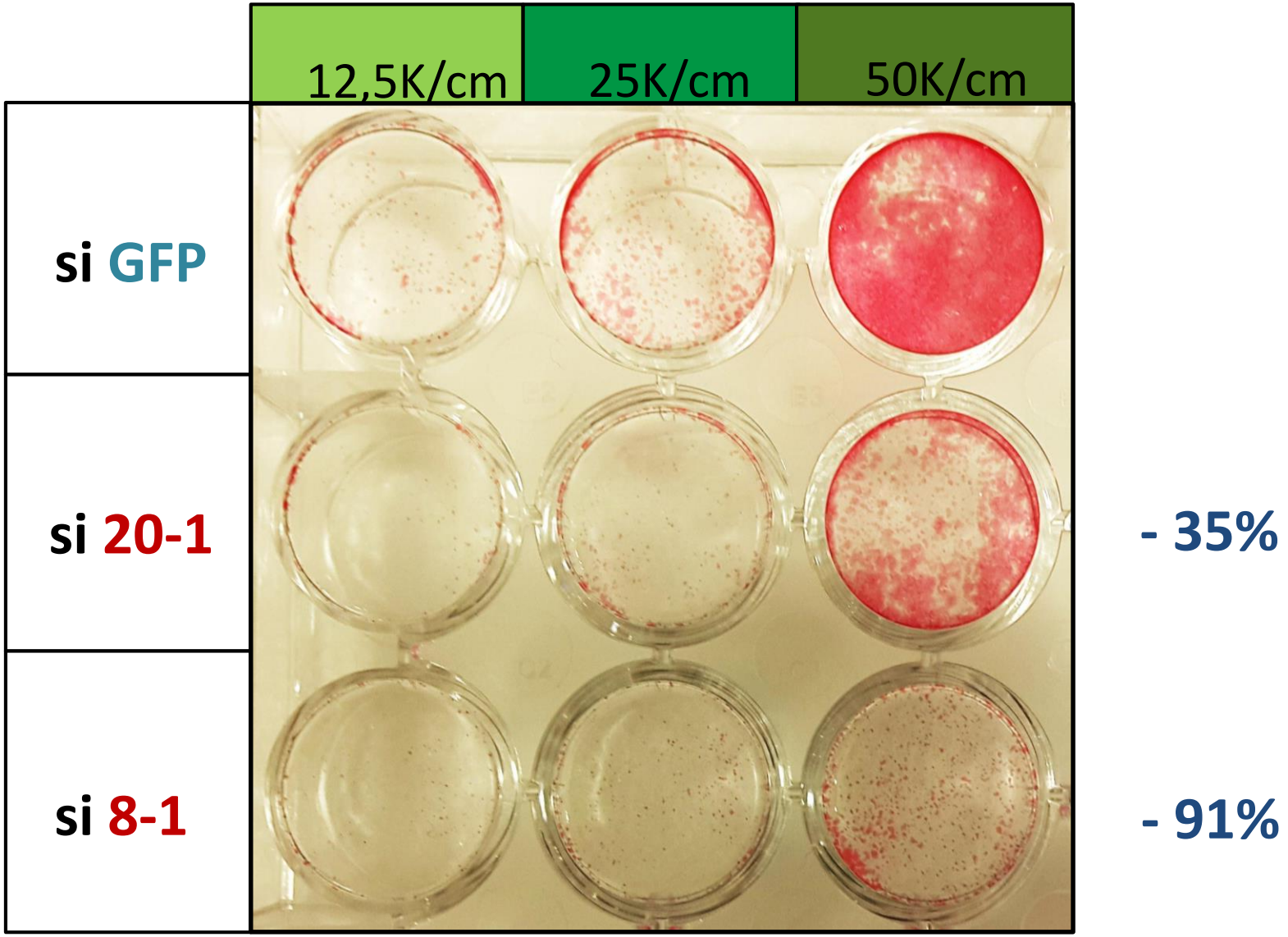
mRNAseq


P0
MP15
EP15
7.73
3.61
24.21
FPKM
NODAL
15.98
9.45
57.50
FPKM
NANOG
250.73
255.04
225.57
FPKM
LIN28
913.23
670.98
820.64
FPKM
GAPDH

Long non-coding RNA



- Certains lncRNA induits massivement (> x50)



- Knock-down par siRNA sur cellules adaptées

Conclusion 3

- Modifications transcriptome plus importantes que ne le voudraient les anomalies génétiques
- Modifications du phénotype précèdent le changement génétique?

Conclusion générale

- iPS : modèle prometteur d'étude des maladies génétiques
- Attention aux aneuploïdes induites par la culture
  - surveillance régulière de la culture (caryotype, FISH?, puces? séquençage?)
  - iPS « naïves »?

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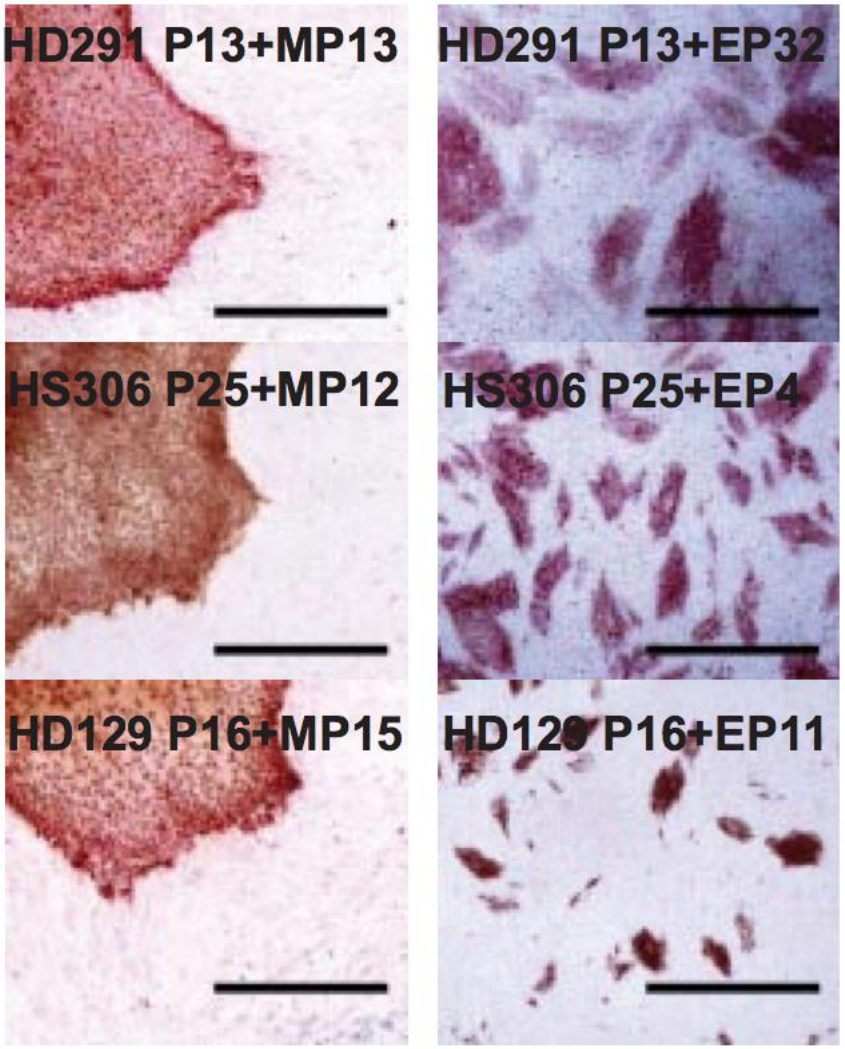
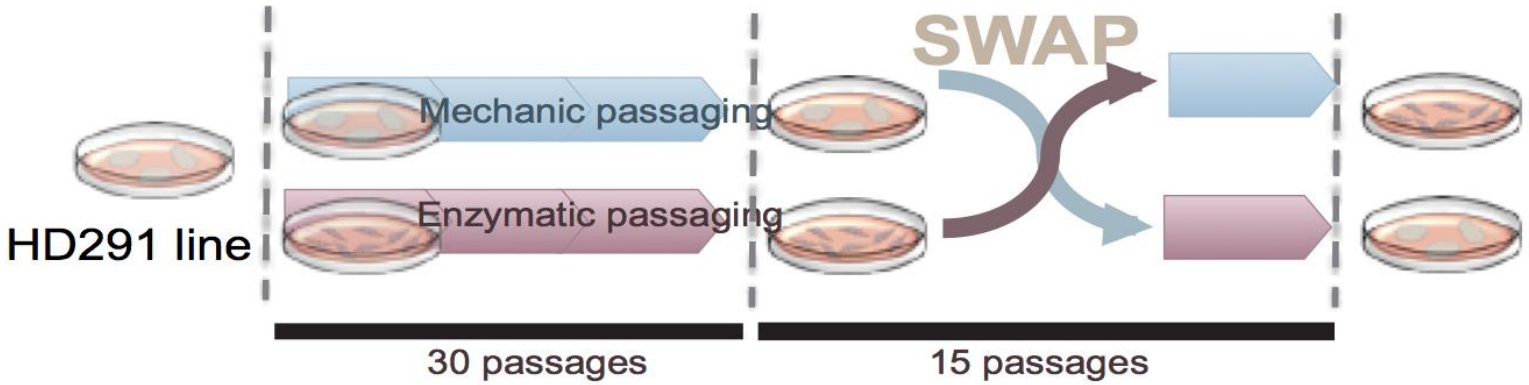


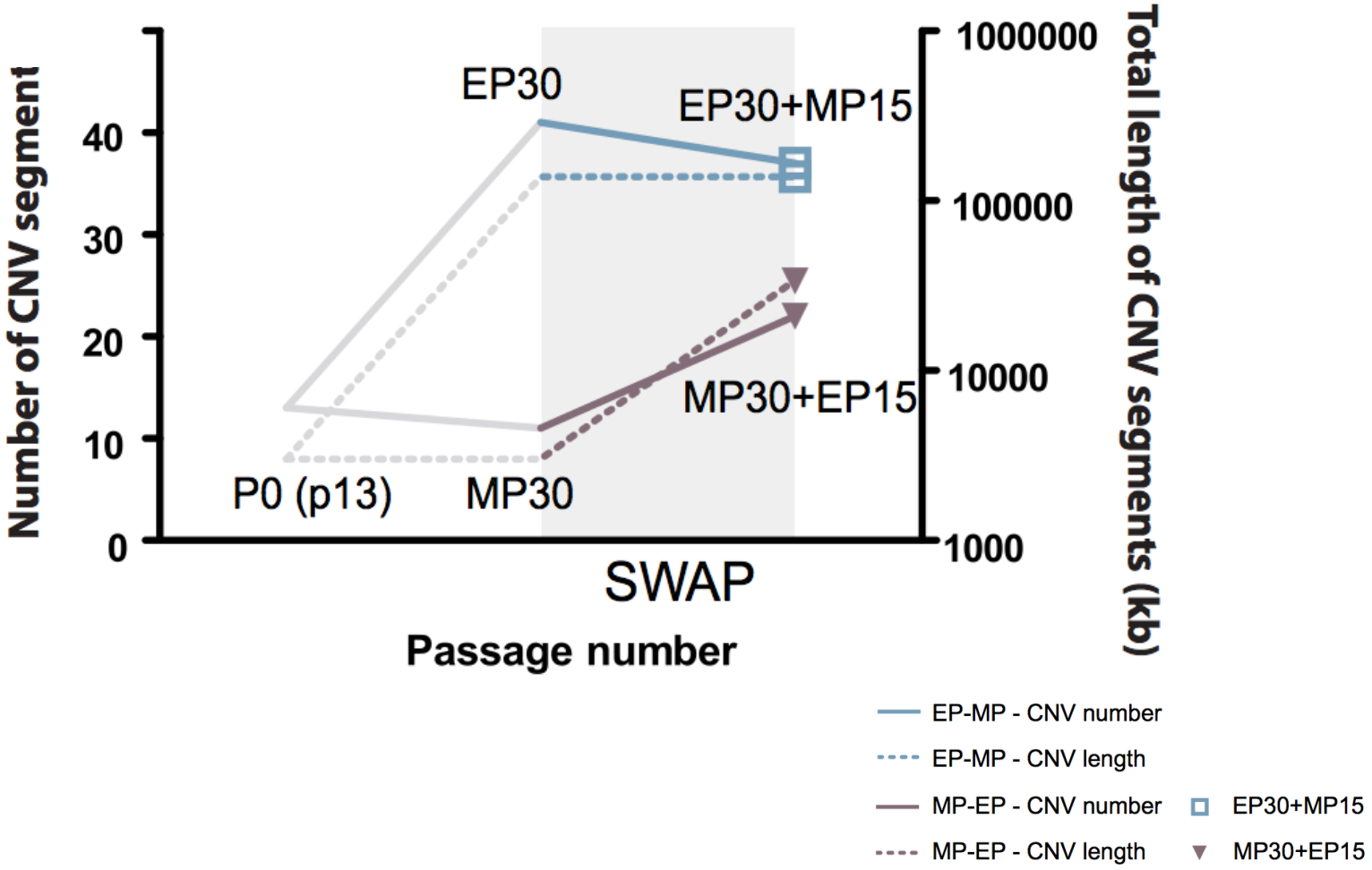
Bai et al. Stem Cell Dev, 2015

SWAP



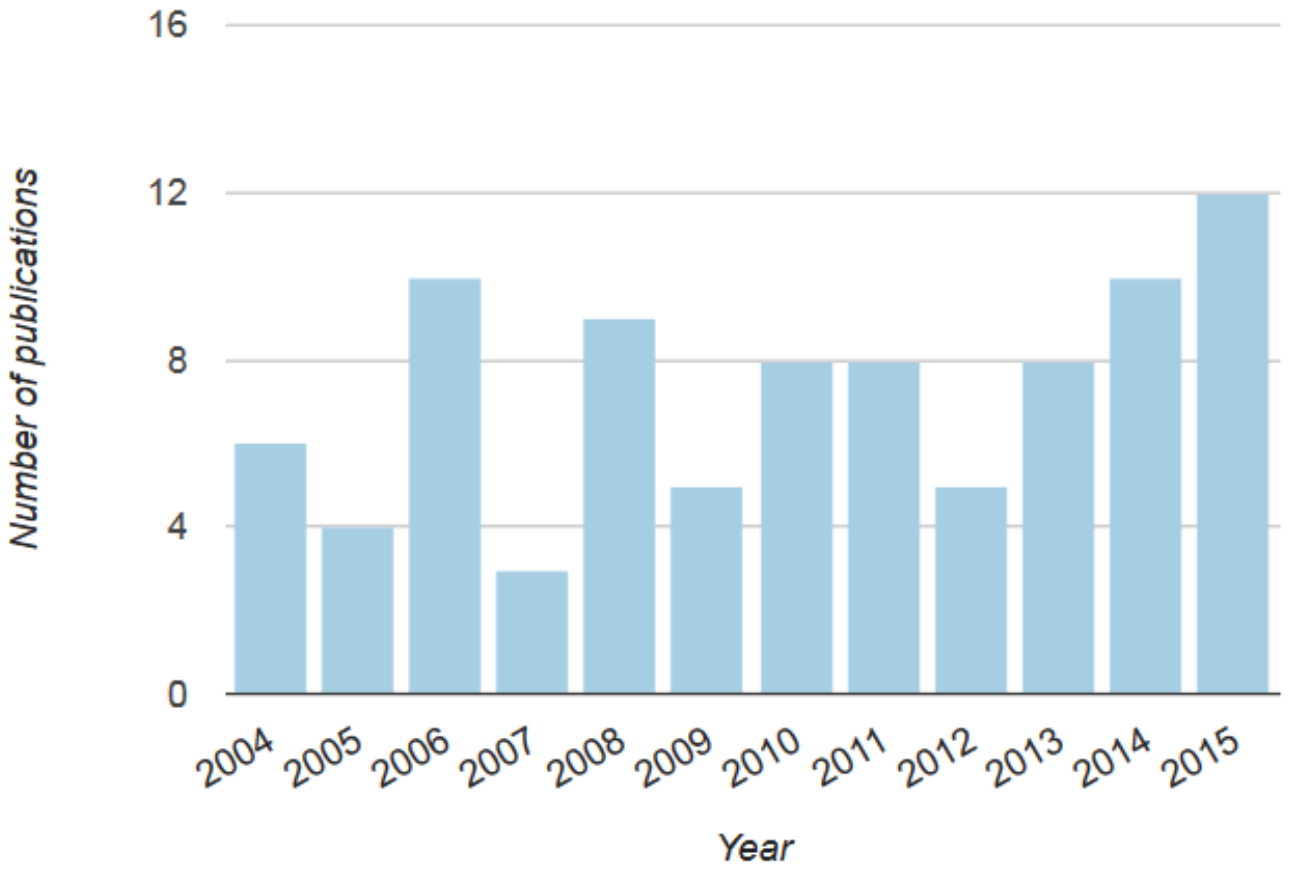
Bai et al. Stem Cell Dev, 2015

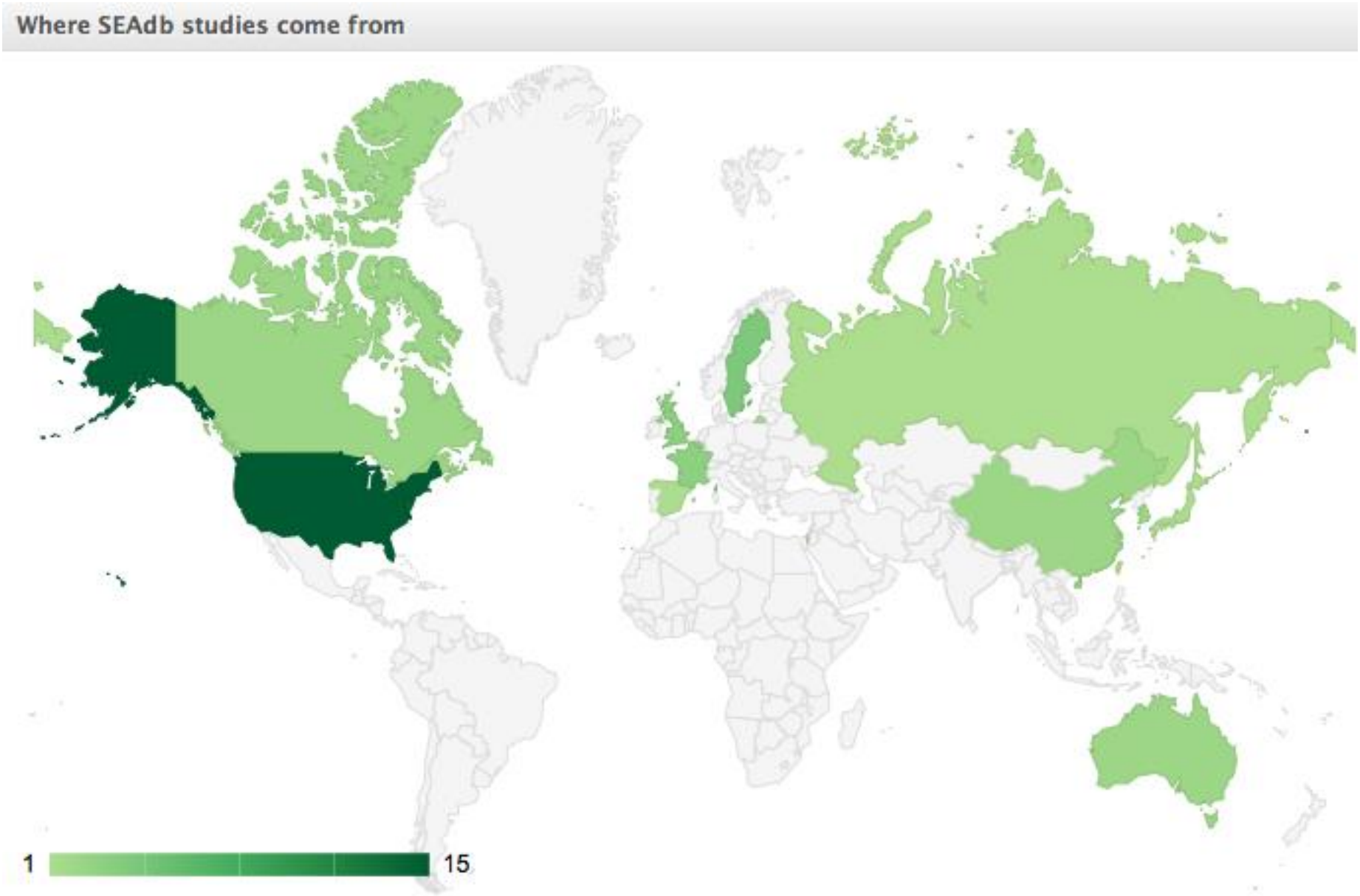
SWAP



Bai et al. Stem Cell Dev, 2015

Nombre de publications par année




Where SEAdb studies come from
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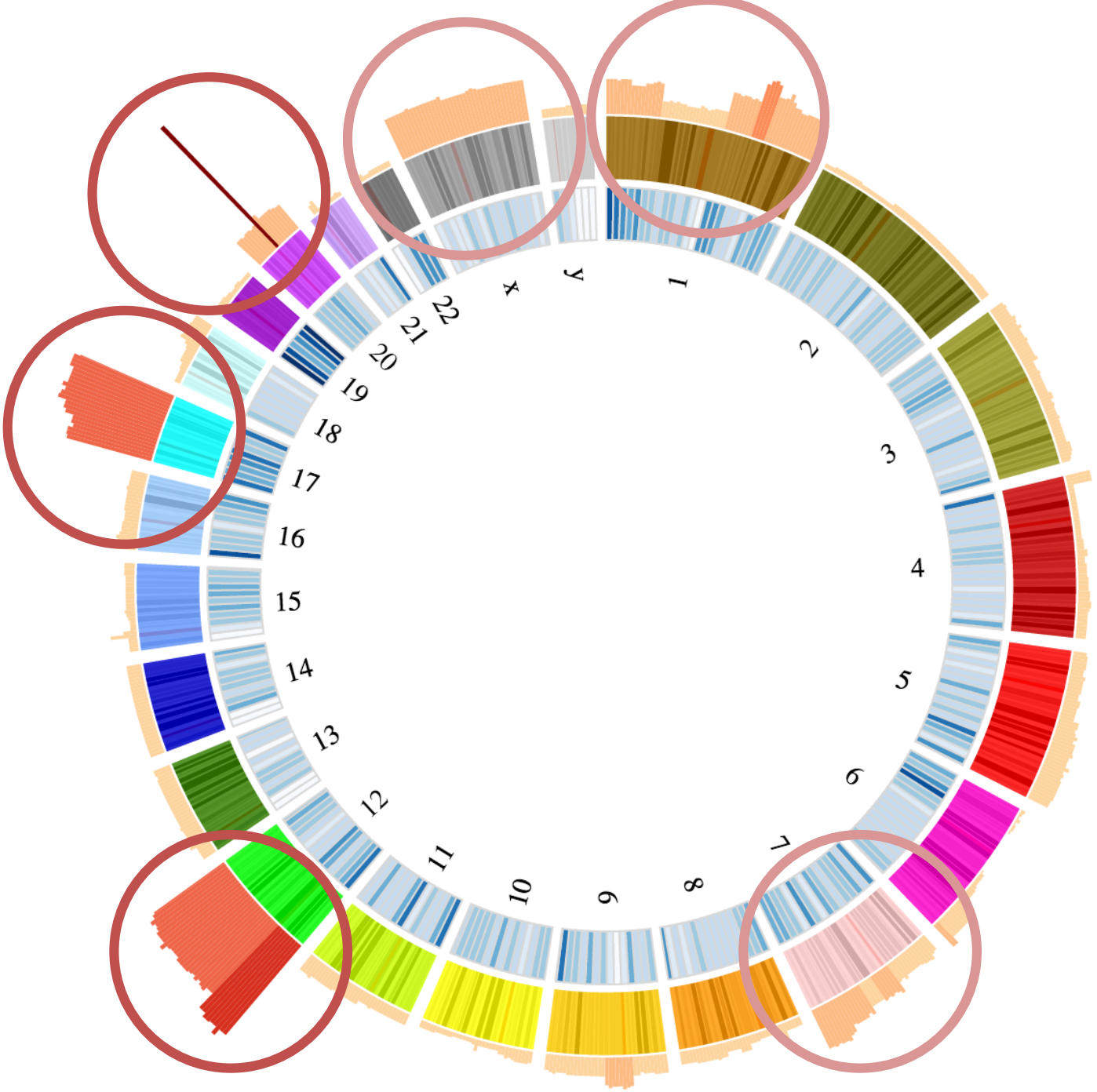
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