4D-Genomics: The genome dynamics and constraint in biology

Henry Heng

Center for Molecular Medicine and Genetic;
Department of Pathology
Wayne State University School of Medicine

Embryo Physics Course

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What is the 4-Dimensional-Genomics ?

3 D genome (gene content + genomic topology)

plus

1D time (evolutionary process)

Heng et al, 2013, Cytoget Gen Res
Horne et a, 2013, Syst Biol Reprod Med

Genome is not just a bag of genes

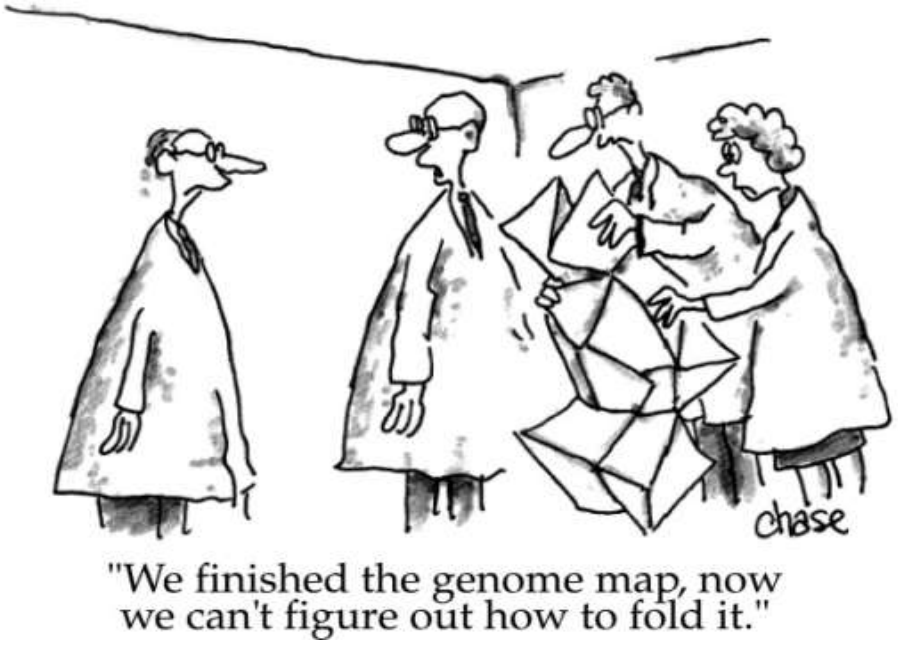
What defines the inheritance
genes vs. genome

Evolution is not just a stepwise Darwinian process

Punctuated vs. stepwise: not a
simple story of accumulating changes over time

Promise and Challenges

Advanced Technologies: sequence them all



…but the gene based new info challenges the

Gene Paradigm Itself

Where to look for molecular causes and have we missed the target?

- For most traits, the majority of the heritability remains unexplained. Missing heritability?

- Key (common driver) gene mutations cannot be found for many common/complex diseases

- Everything is involved and nothing is very important (>10,000 different genetic variants for Schizophrenia)

When identified, not very useful clinically

101 of well characterized genetic markers were found to not be useful in predicting heart disease in a clinical setting (among 19,000 women who had been monitored for 12 years), despite the fact that all these genetic variants had been statistically linked to heart disease in various genome-scanning studies.

In contrast, asking about the family history had better prediction success (JAMA)

SOS: We had major problems:

"…Bert Vogelstein has watched first-hand as complexity dashed one of the biggest hopes of the genome era: that knowing the sequence of healthy and diseased genomes would allow researchers to find the genetic glitches that cause disease, paving the way for new treatments. An individual patient's cancer has many mutations, but they differ between individuals. So the search for drug targets has shifted away from individual genes…" Nature 2010 646: 664-667

REALITY

All of those and many more are involved,
yet most really don't matter
(we all have over 300 gene mutations)

WHY?

Current concept of 1 D genetics is flawed
(Gene mediated genetic determinism and reductionism)

Heng 2014 Debating Cancer (in press)

Challenges for gene theory

- Individual gene's function is differently defined by the system/environment interaction (multiple function and moonlighting protein)
- No gene is an island
- Most of the gene mutations are low penetration
- There is no 'good' or "bad" genes for many diseases (P53 gene mutation story)
- Gaps between known function of gene mutation and clinical reality

What defines inheritance?

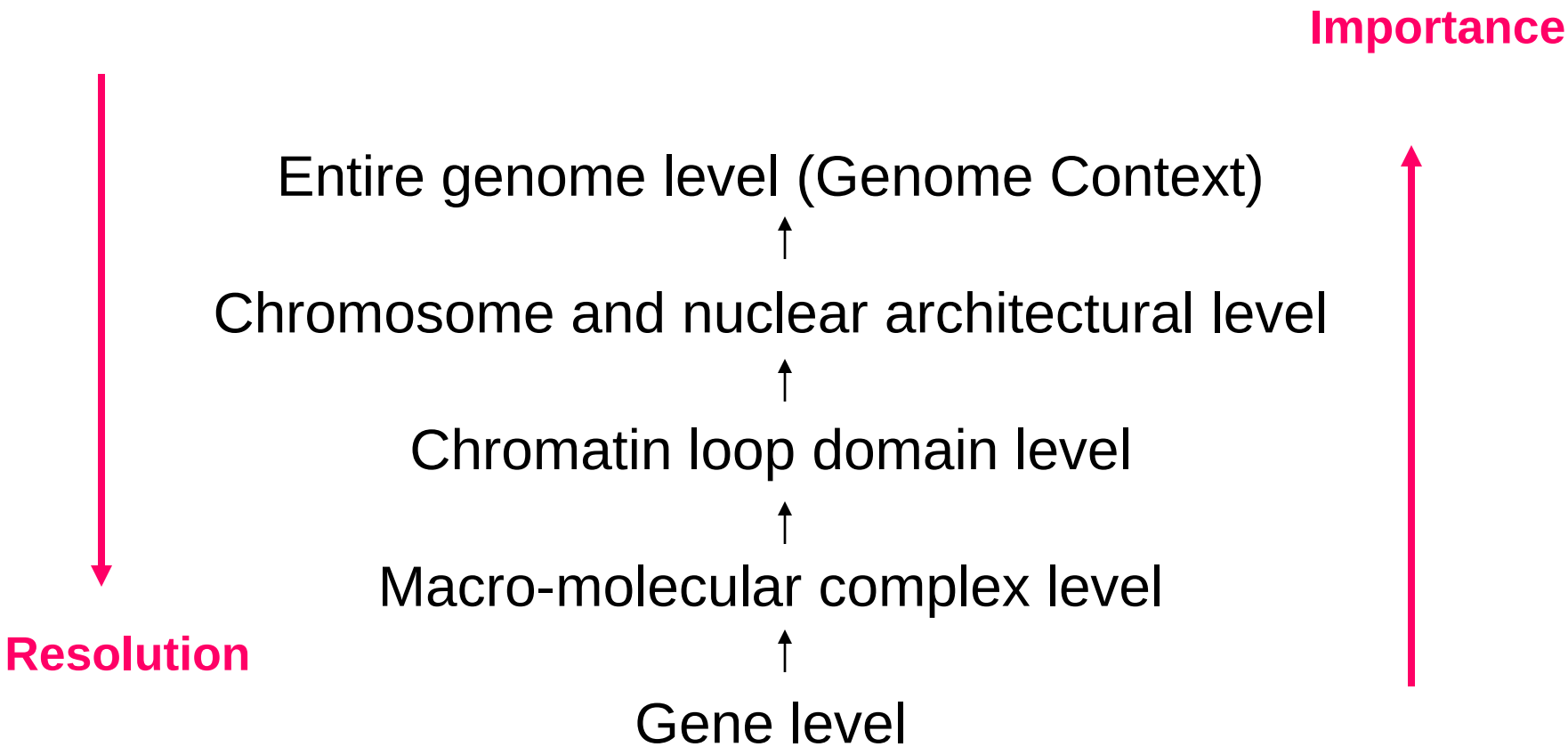
DNA dogma "Gene-Protein-Phenotype"

Collective function of multiple genes VS. "Missing heritability"
(for the majority of traits, most heritability remains unexplained)

Gene function is genome context dependent
Multiple sub systems (nuclear and mt)

Have we missed the key level of genetic organization?

Genome organization (system) is more important than genes (parts)



Heng 2008 JAMA;
Heng 2013 in: Handbooks of Systems and Complexity in Health

The main function of chromosomes

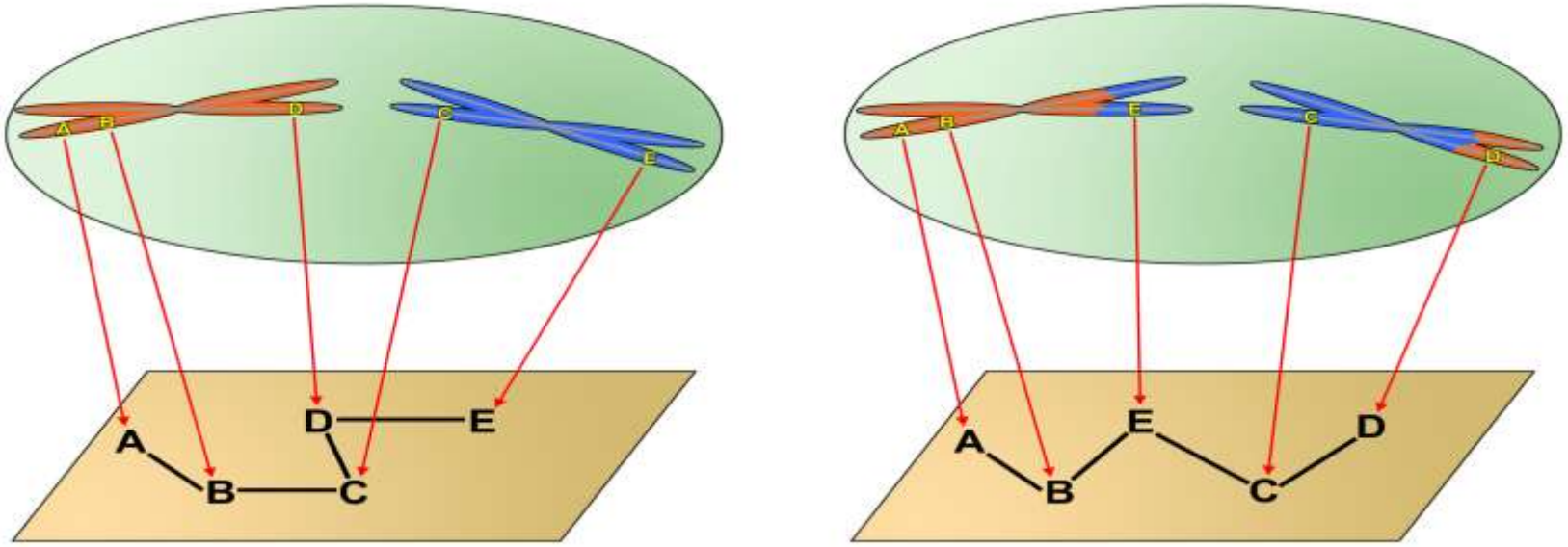
Gene-centric theory: To pass genetic material (subordinate to the gene master)

Genome theory: Defines a new type of genetic information called system inheritance

1. Defines a genetic network.
2. Ensures the maintenance of system inheritance by preserving the karyotype (genome topology)

Heng 2009 BioEssays

Genome context/genomic topology, not specific genes (when there are sufficient genes for the complexity), defines the organization of a genetic network



Chromosomes, not genes, define system inheritance

Chromosomes define the genetic interaction among genes

Heng 2009, BioEssays
Heng et al, 2011, Genomics
Heng et al, 2013, Can Metastasis Rev

Supporting Evidence:

A novel trait can evolve through genomic rearrangement and gene amplification (Blount et al, Nature, 2012)

The main function of sexual reproduction is to maintain the system inheritance by preserving karyotype rather than increasing gene level diversity (Heng, Genome, 2007; Gorelick and Heng, Evolution, 2011)

The linkage between genome alteration (nuclear and mt genomes) and diseases (as well as organismal macro-evolution) is common (Wallace, JCI, 2013; Heng et al, Cytogent Gen Res, 2013)

Evolution

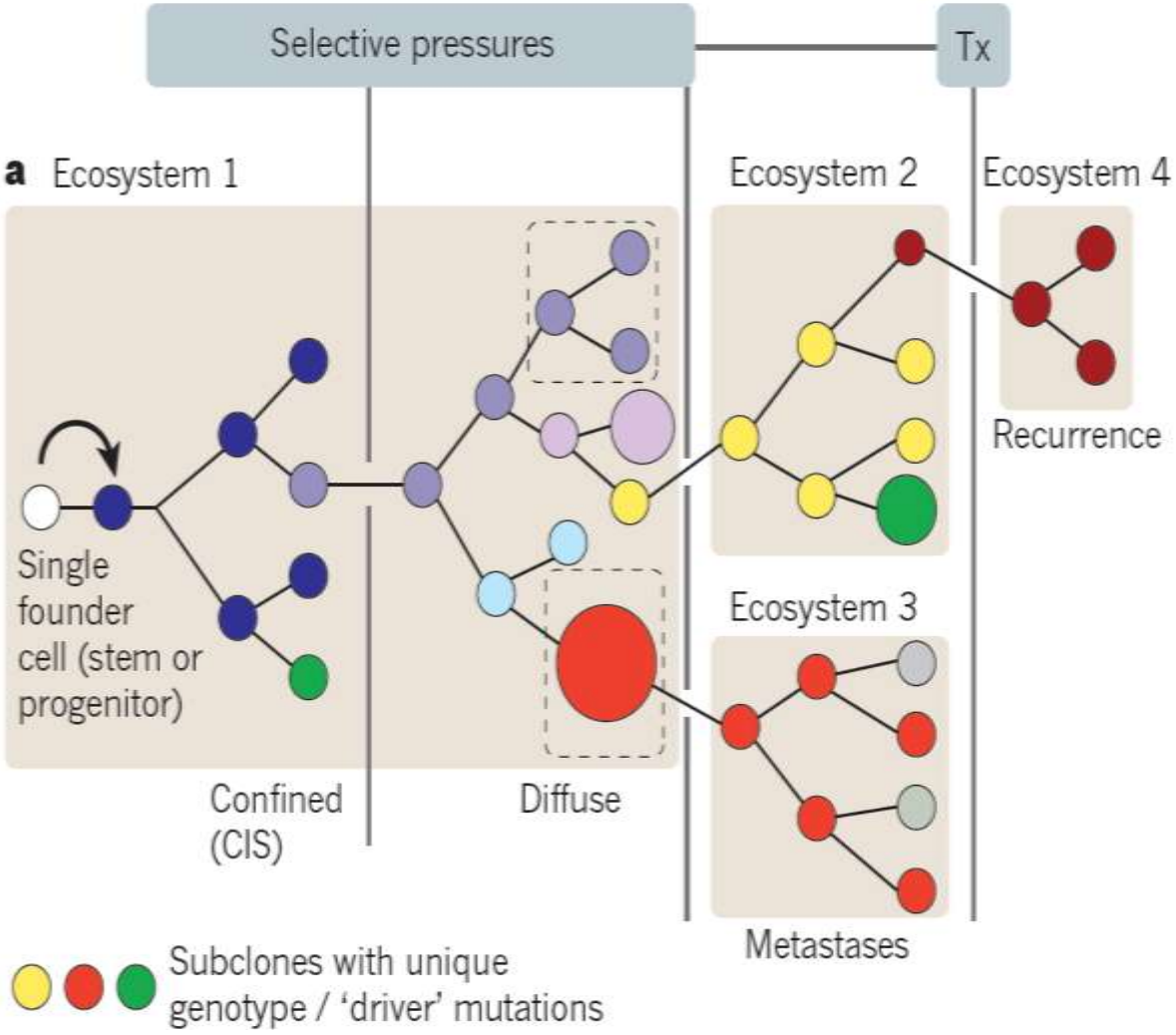
Three Key Conditions for Evolution

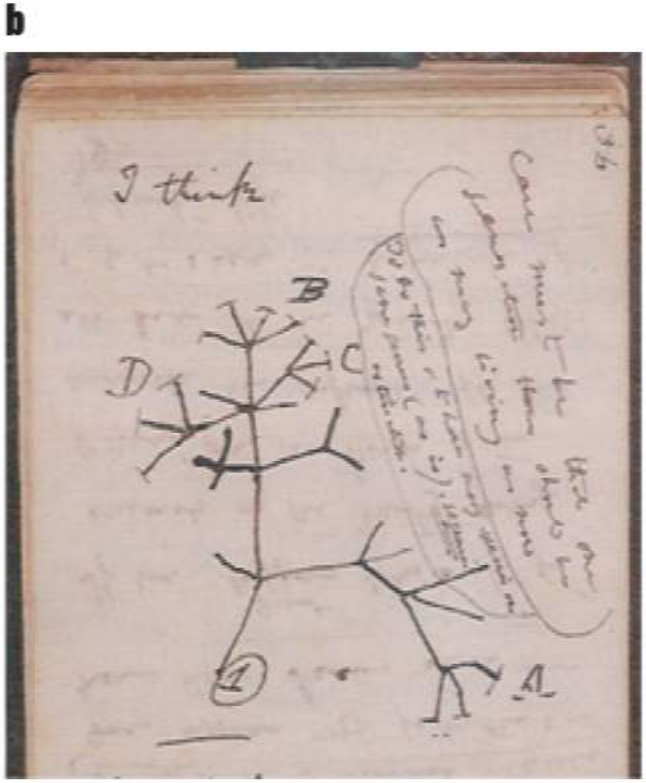
- There must be variation in the population.
- That variation must be heritable.
- That variation must affect survival or reproduction.

Heng 2007 BioEssays

Heng 2009 BioEssays

Clonal Evolution





Nature. 2012 Jan 18;481(7381):306-13

Important questions

- There must be variation in the population.

  But what types: gene mutations

  epigenes or genome variation ?

- That variation must be heritable.

  But what defines heritance: Gene or Genome ?

- That variation must affect survival or reproduction.

  But by stepwise or sudden jump?

It is the genome, stupid!

Genome alteration changes dominates

Genome defines the system inheritance

Punctuated genome change is the non-clonal, macro-evolution

Facts do not matter ?

- Most different species display different karyotypes (over 95%)
- Major evolutionary changes are detected from the genome level
- No specific genes have been identified responsible for speciation yet

- But we all believe genes are the key and chromosomal changes are incidental

System inheritance is not due to the gene, but the genome!

Human vs. Chimp

One chromosomal fusion, 5 inversions

Human vs. Mouse

250 chromosomal re-organizations

Sponges have 18,000 genes

Key: where the gene is located within the genome matters!

Most mammals have similar genes but different karyotypes

There is no fixed cancer genome

Most cancer cells are different with altered genomes, with diverse gene mutations

Yet, most species with sexual reproduction display the same genomes

What is the key difference between cancer and organismal evolution?

Watch evolution in action

Individual cell and population

Both gene and genome level

Focus on system heterogeneity rather than averaging profiles

Pattern of evolution (fast punctuated or gradual stepwise or both?)

Tracing cancer progression: stochastic evolution

Normal Cell > > > > > Cancer

Early passages > > > > Late passages

(Li-Fraumeni fibroblast model)

Dynamic genome patterns during characterized multiple stages of progression

(in vitro immortalization model: pre-immortal, crisis, post-immortal and cell lines)

Stepwise: Share common changes

Stochastic: Do not share

Spectral karyotyping: SKY

<u>Components</u>

1. CCD camera
2. Interferometer
3. SKY filter
4. Computer
5. Microscope
6. SkyPaint
7. Camera controller
8. OPD Scanner controller
9. Monitor

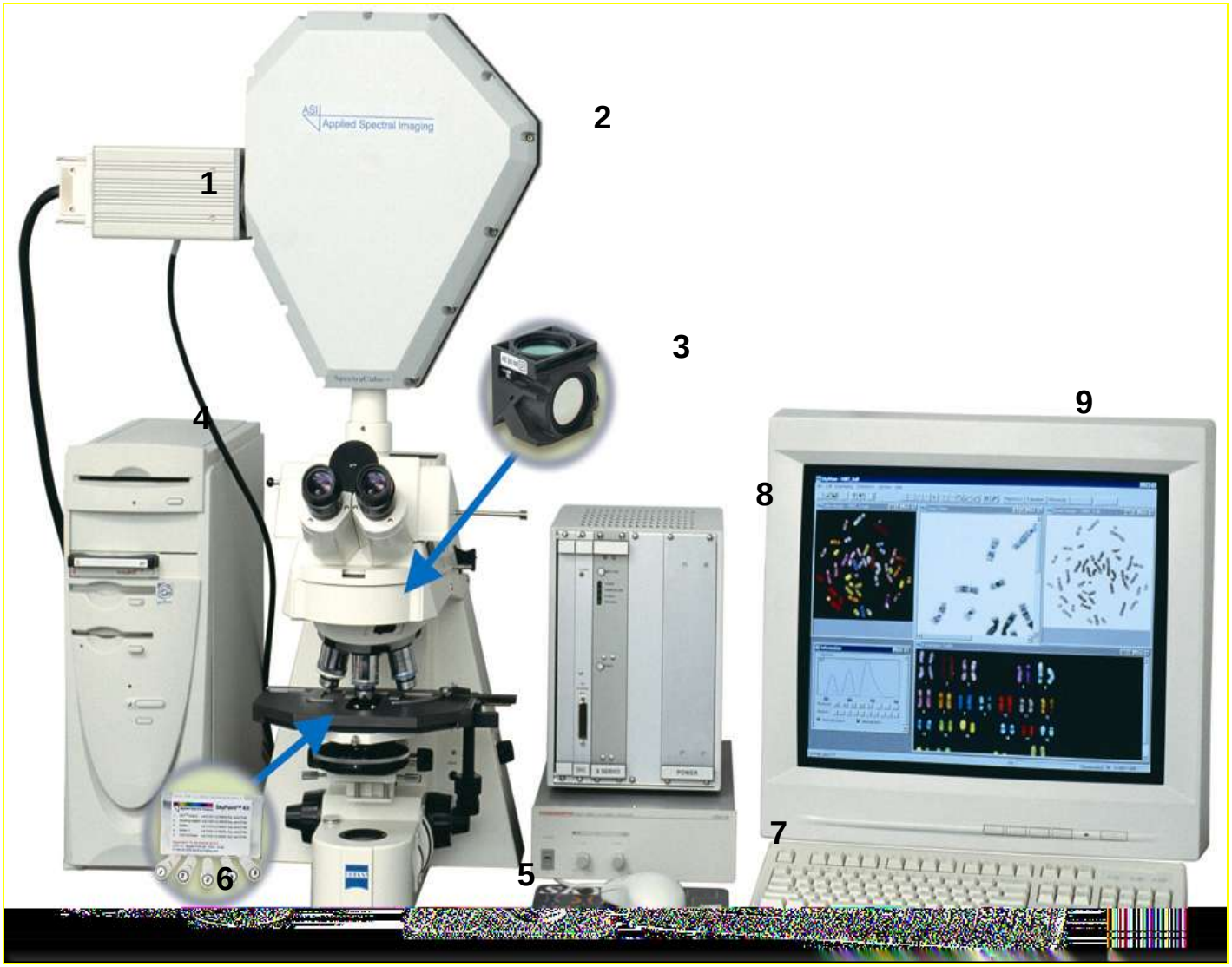


Image Analysis

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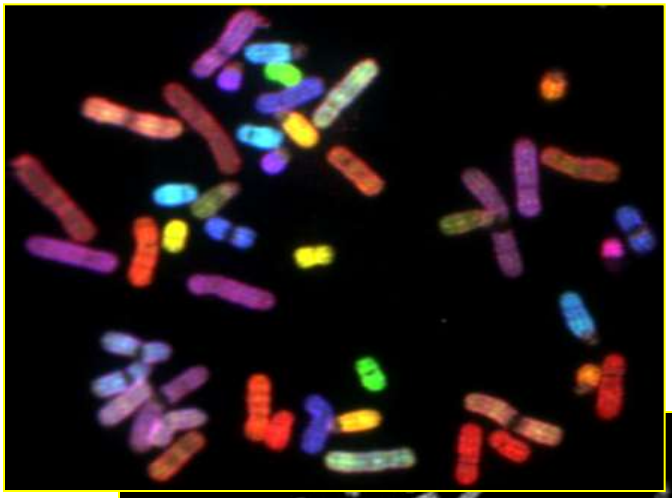

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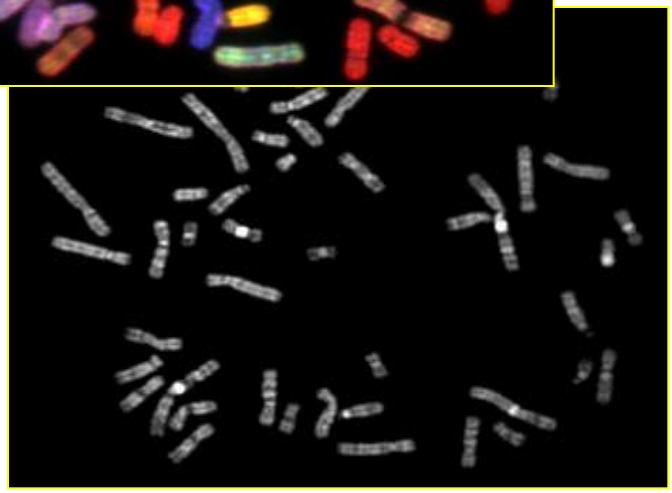
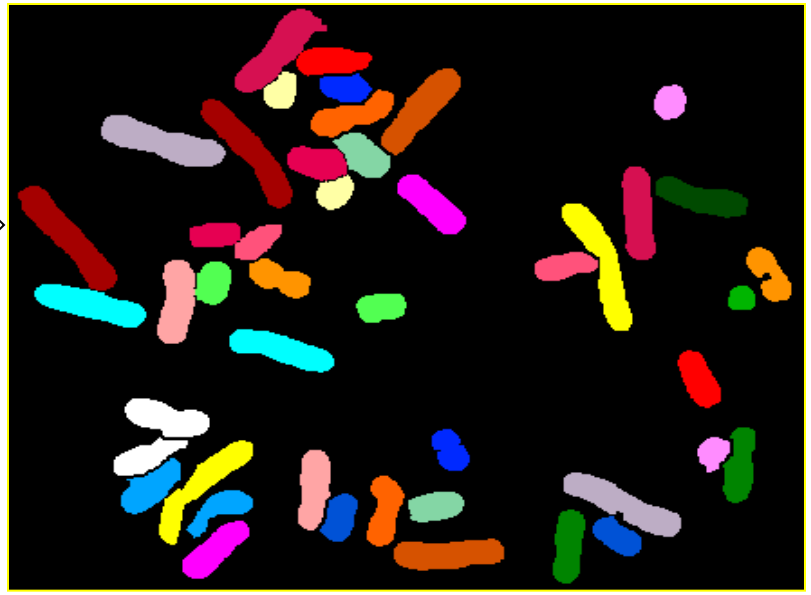
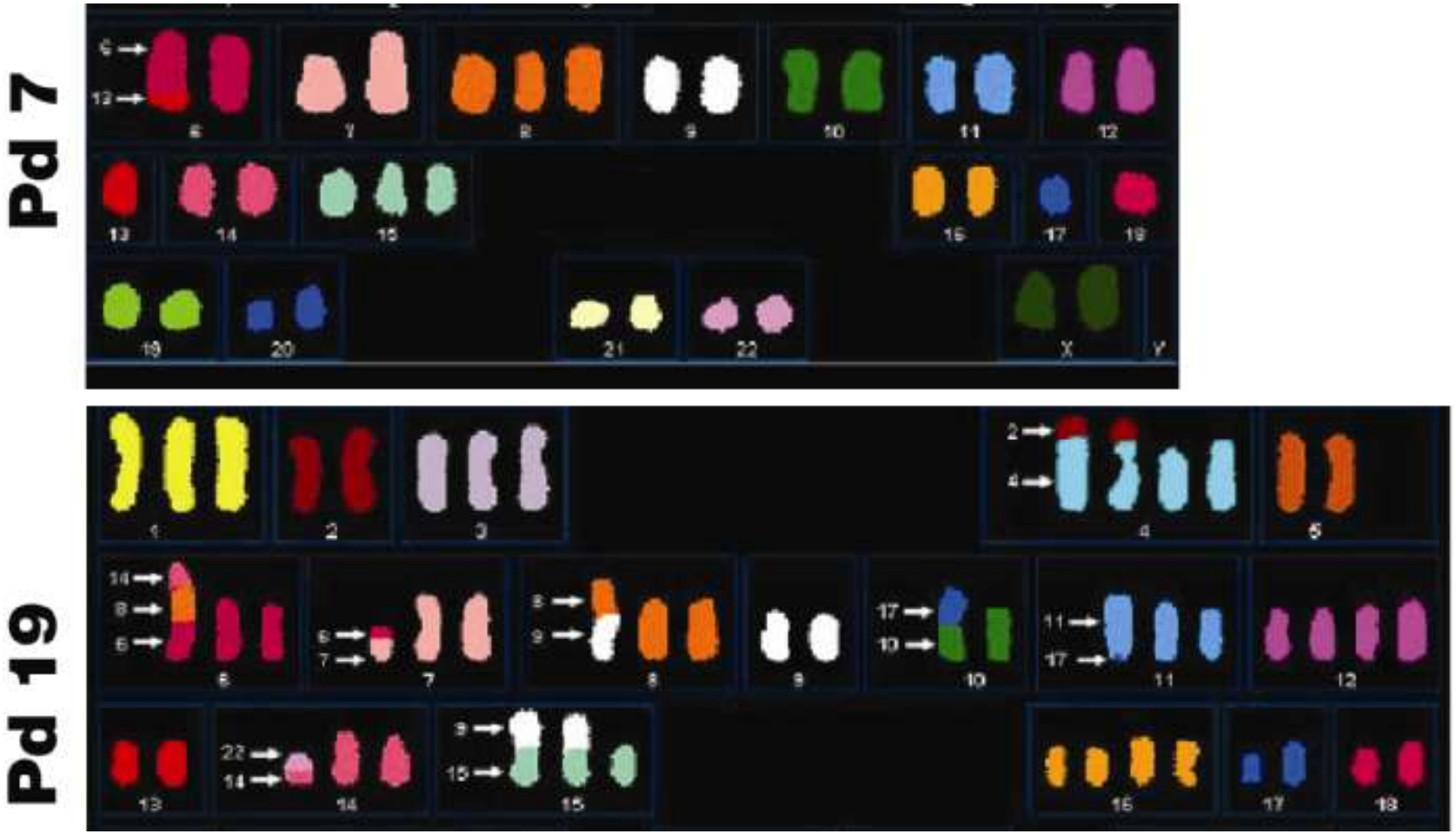


DAPI Image



Classified Image

SKY karyotyping to trace all CCAs and NCCAs



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	3	t(1;17)	t(3;17)	t(6;13)								
	4											t(2;22) t(8;10) t(10;12) t(15;10)
	5	t(1;17)	t(3;17)	t(6;13)								
Pd 14	1		t(10;20)	t(9;22)								
	2		t(10;20)									t(2;22) t(7;8) t(10;4) t(15;4) t(16;7)
	3	t(14;22)	t(10;20)	t(9;22)								t(11;8)
	4		t(10;20)	t(9;22)								t(2;14) t(5;19) t(7;22) t(17;19)
	5	t(14;22)	t(10;20)	t(9;22)								t(14;12) t(15;5) t(19;13)
Pd 19	1	t(14;22)										t(5;9) t(7;19) t(15=15) t(15;10;15) t(15;10;15;10)
	2	t(14;22)										t(2;20) t(11;19) t(15;20;10)
	3											t(6;15) t(15;9) t(X;20) t(5;22;14) t(6=6;15) t(9;X;20) t(14;15;5) t(15;12;20) t(19;10;20) t(20;9;8) t(22;5;20) t(X;2;20) t(5;8;9;20) t(14;22;14;2) t(20;10;5;20;9;7)
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	5	t(14;22)										t(2;22) t(3;13) t(7;5) t(17;19) t(5;15;9) t(10;5;22) t(22;15;22) t(X;2;22) t(9;20;17;5)
Pd 24	1											t(2;20) t(7;13) t(8;20) t(9;22) t(14;18) t(15=15) t(19;5) t(20;7) t(20;22) t(21;10) t(18;2;5) t(20;10;14) t(20=20;22) t(15;18;10;15)
	2	t(15;14)										t(1;17) t(3;15) t(4;9) t(8;13) t(8;21) t(11;8) t(15;22) t(19;3) t(21;8) t(X;21) t(11;8;21) t(14;8;X) t(17;13;X;8;13;17)
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Pd 54	1	t(4;1)	t(15;6)	t(7;19)		t(1;5;10)	t(4;22;8;5)	t(5;15;8;5)	t(7;4;15;8;5)			t(8;4) t(11;21) t(17;9) t(18;21) t(3;18;21)
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	4	t(4;1)	t(15;6)	t(7;19)	t(9;22;8)	t(1;5;10)	t(4;22;8;5)	t(5;15;8;5)	t(7;4;15;8;5)			
	5	t(4;1)	t(15;6)	t(7;19)	t(9;22;8)	t(1;5;10)	t(4;22;8;5)	t(5;15;8;5)	t(7;4;15;8;5)			
Pd 109	1	t(4;1)		t(7;19)	t(9;22;8)	t(1;5;10)	t(4;22;8;5)	t(5;15;8;5)				
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	3	t(4;1)	t(15;6)	t(7;19)	t(9;22;8)		t(4;22;8;5)	t(5;15;8;5)	t(7;4;15;8;5)			t(10;11) t(13;X) t(15;10) t(5;8;15;8)
	4	t(4;1)	t(15;6)	t(7;19)	t(9;22;8)	t(1;5;10)	t(4;22;8;5)		t(7;4;15;8;5)			t(7;1) t(12;6) t(14;17)
	5	t(4;1)	t(15;6)	t(7;19)	t(9;22;8)	t(1;5;10)	t(4;22;8;5)	t(5;15;8;5)	t(7;4;15;8;5)			t(X;5)
Pd 236	1	t(4;1)		t(7;19)	t(9;22;8)	t(1;5;10)	t(4;22;8;5)	t(5;15;8;5)	t(7;4;15;8;5)	t(6;16)	t(6;20)	t(3;11) t(12;19) t(15;20) t(18;20) t(19;1)
	2	t(4;1)		t(7;19)	t(9;22;8)	t(1;5;10)	t(4;22;8;5)	t(5;15;8;5)	t(7;4;15;8;5)	t(6;16)	t(6;20)	t(17;11) t(15=15;6)
	3		t(15;6)	t(7;19)		t(1;5;10)	t(4;22;8;5)	t(5;15;8;5)		t(6;16)		t(1;7) t(1;17) t(8;2) t(18;20) t(7;12=12;20) t(4;5;8;22;4;1)
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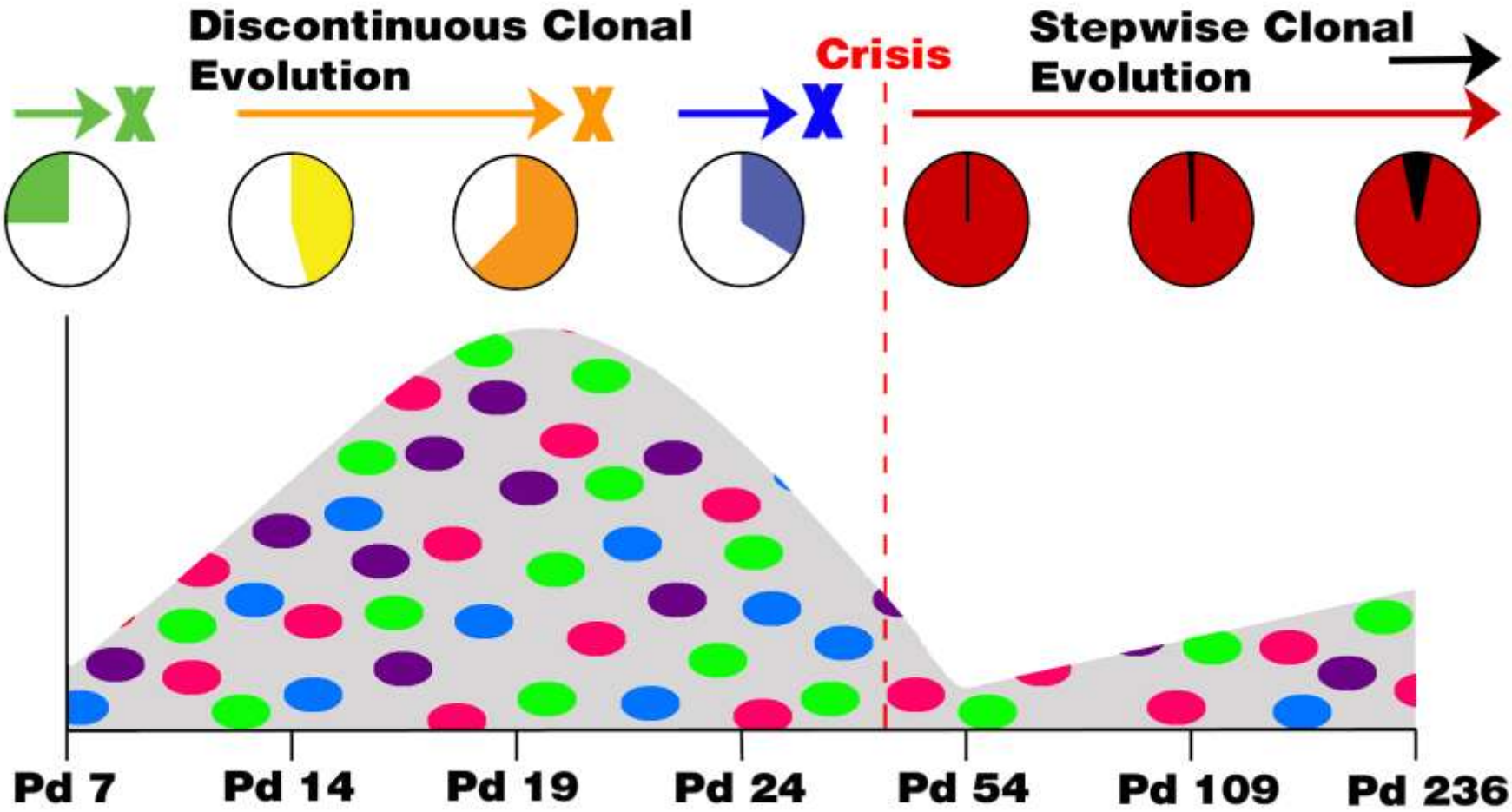
	5	t(4;1)	t(15;6)	t(7;19)	t(9;22;8)	t(1;5;10)	t(4;22;8;5)		t(7;4;15;8;5)	t(6;16)	t(6;20)	t(2;8) t(21;16) t(1;15;18)

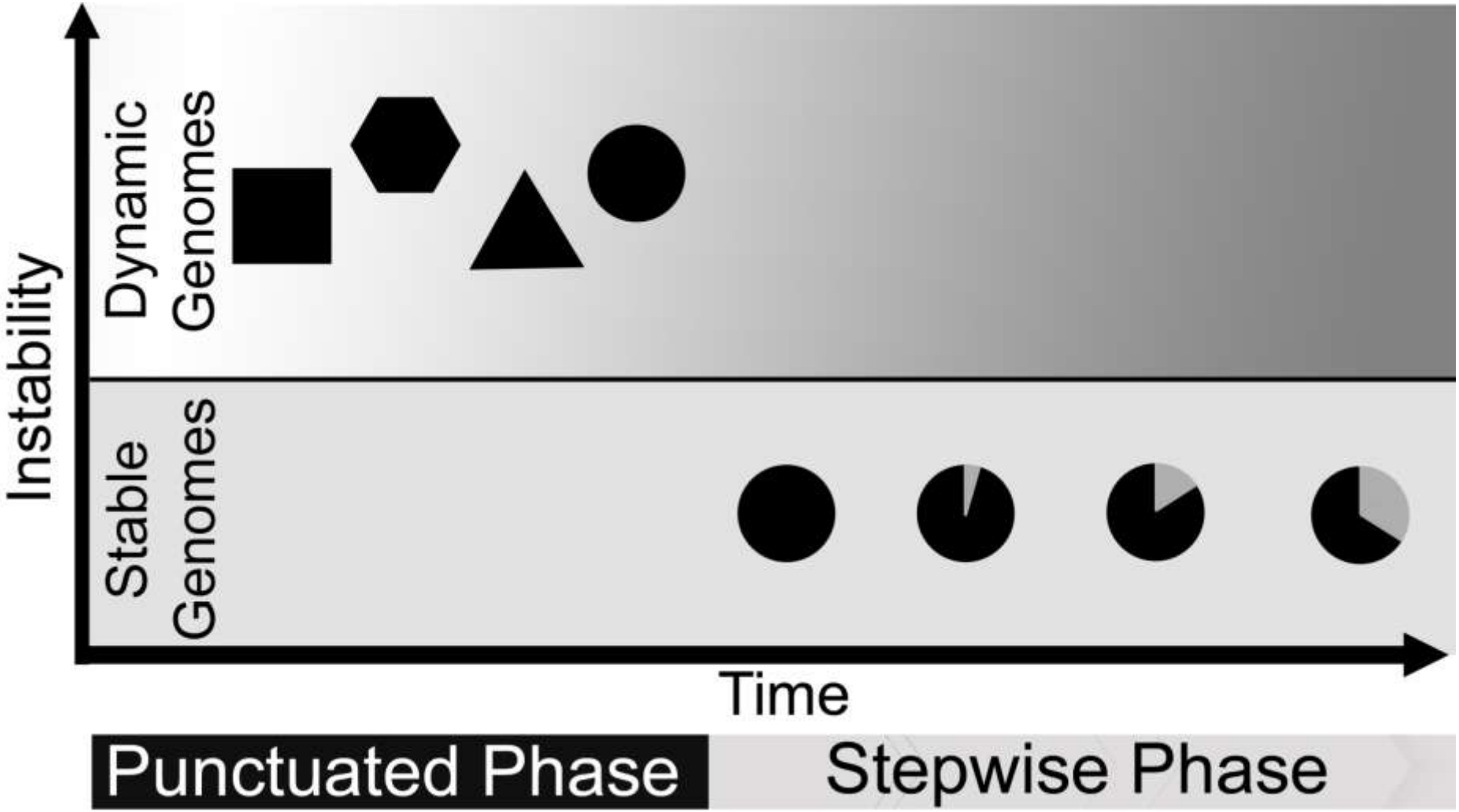
Patterns of NCCAs and CCAs during the immortalization process

Early cancer progression is not stepwise but punctuated
The pattern of evolution is determined by the system stability
Chemo-treatment switches evolutionary phases

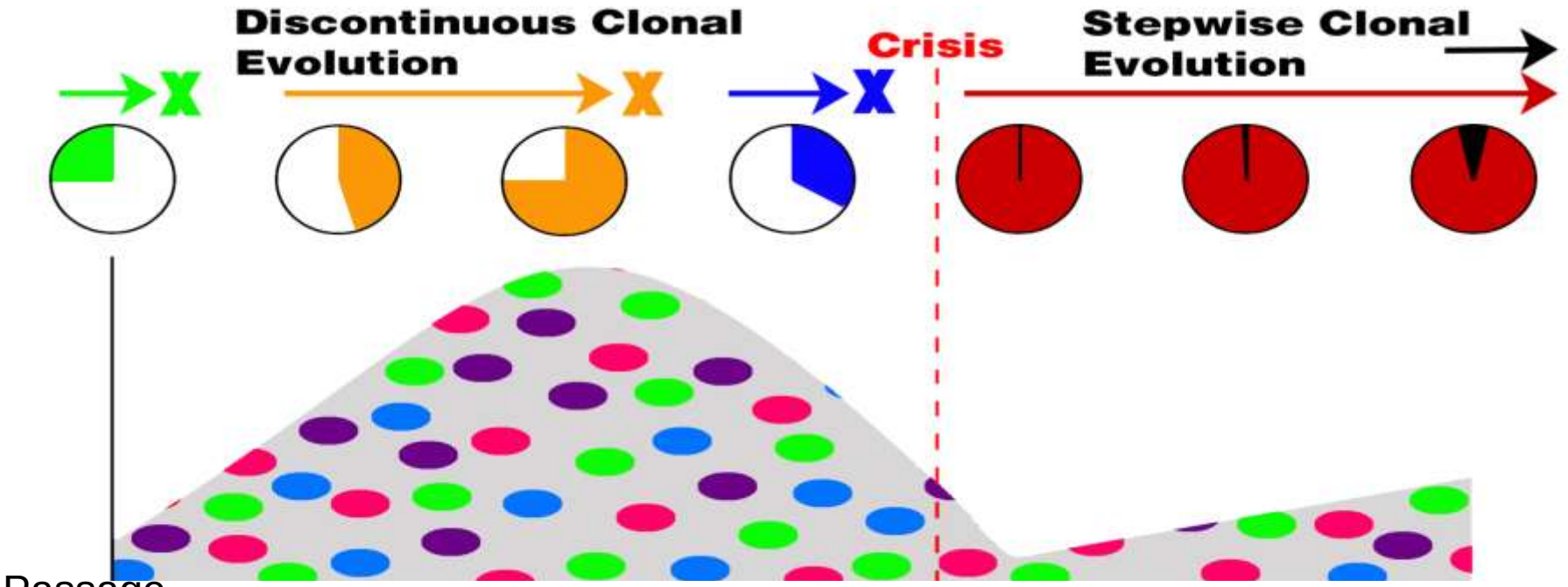
Gould's Punctuated Evolution

Darwinian evolution



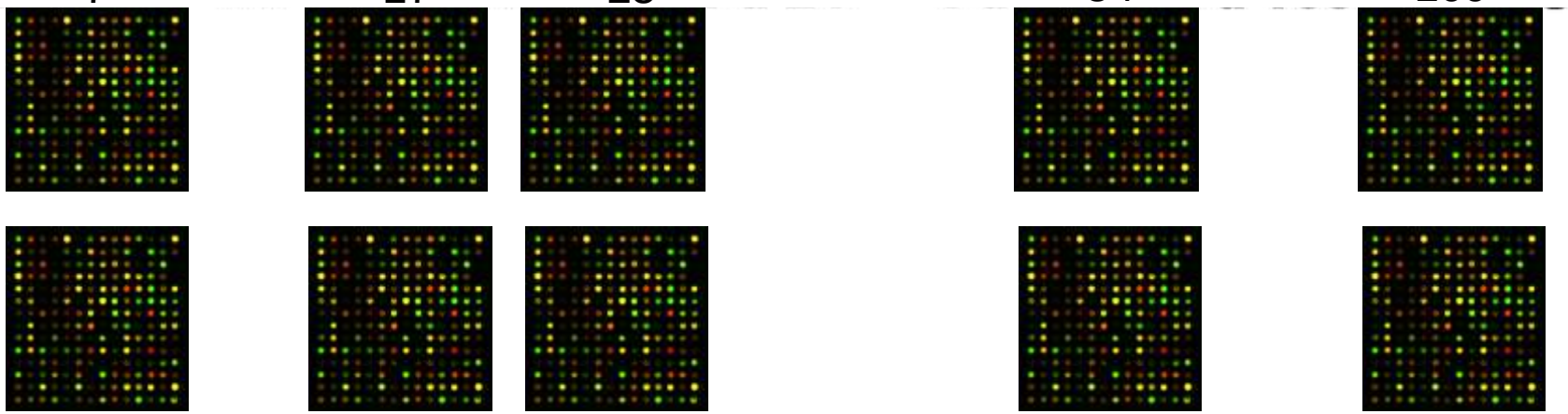

Instability
Dynamic Genomes
Stable Genomes
Time
Punctuated Phase
Stepwise Phase

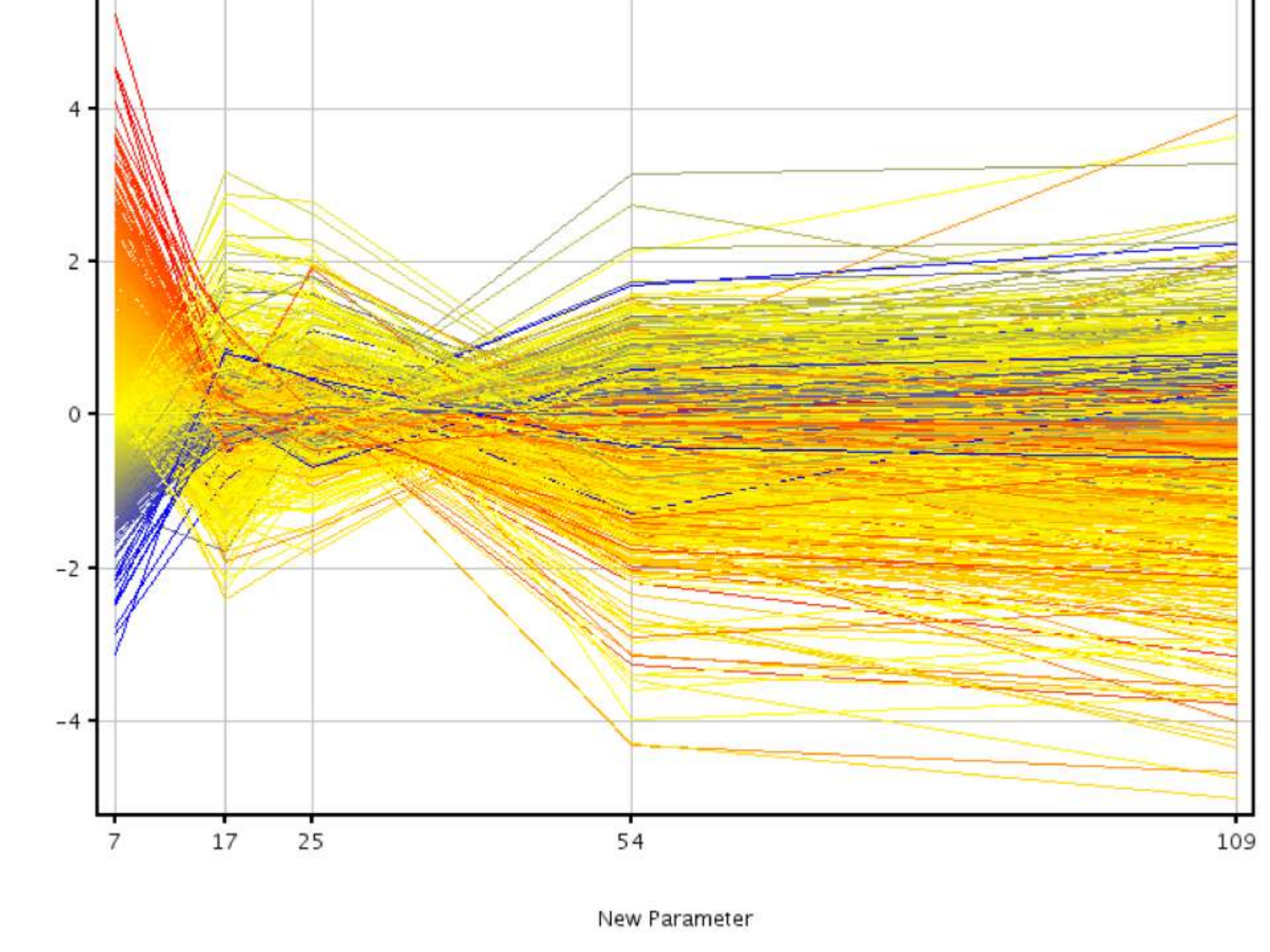
Expression study design



Passage

7 17 25 54 109

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-2
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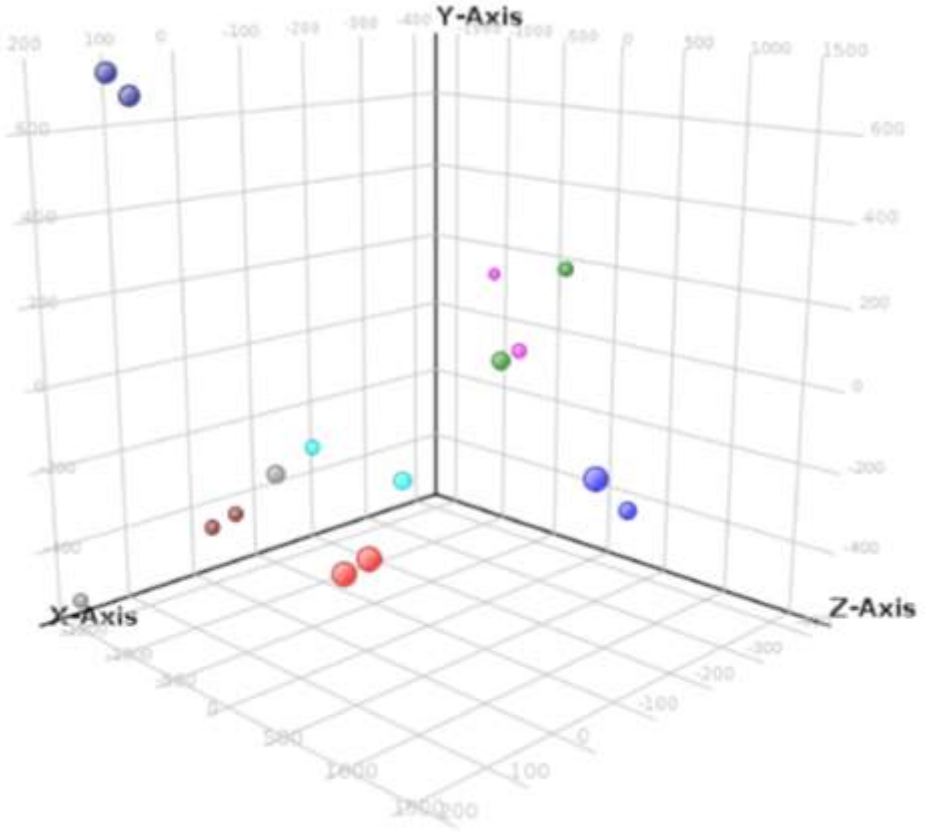
7
17
25
54
109
New Parameter

Karyotype variability impacts expression variability



Color by Passage

- ambion
- illumina
- p109
- p109 dox
- p17
- p25
- p54
- p7

Description
Algorithm: Principal Components Analysis
Parameters:
Column indices = [1-16]
Pruning option = [numPrincipalComponents, [4]]
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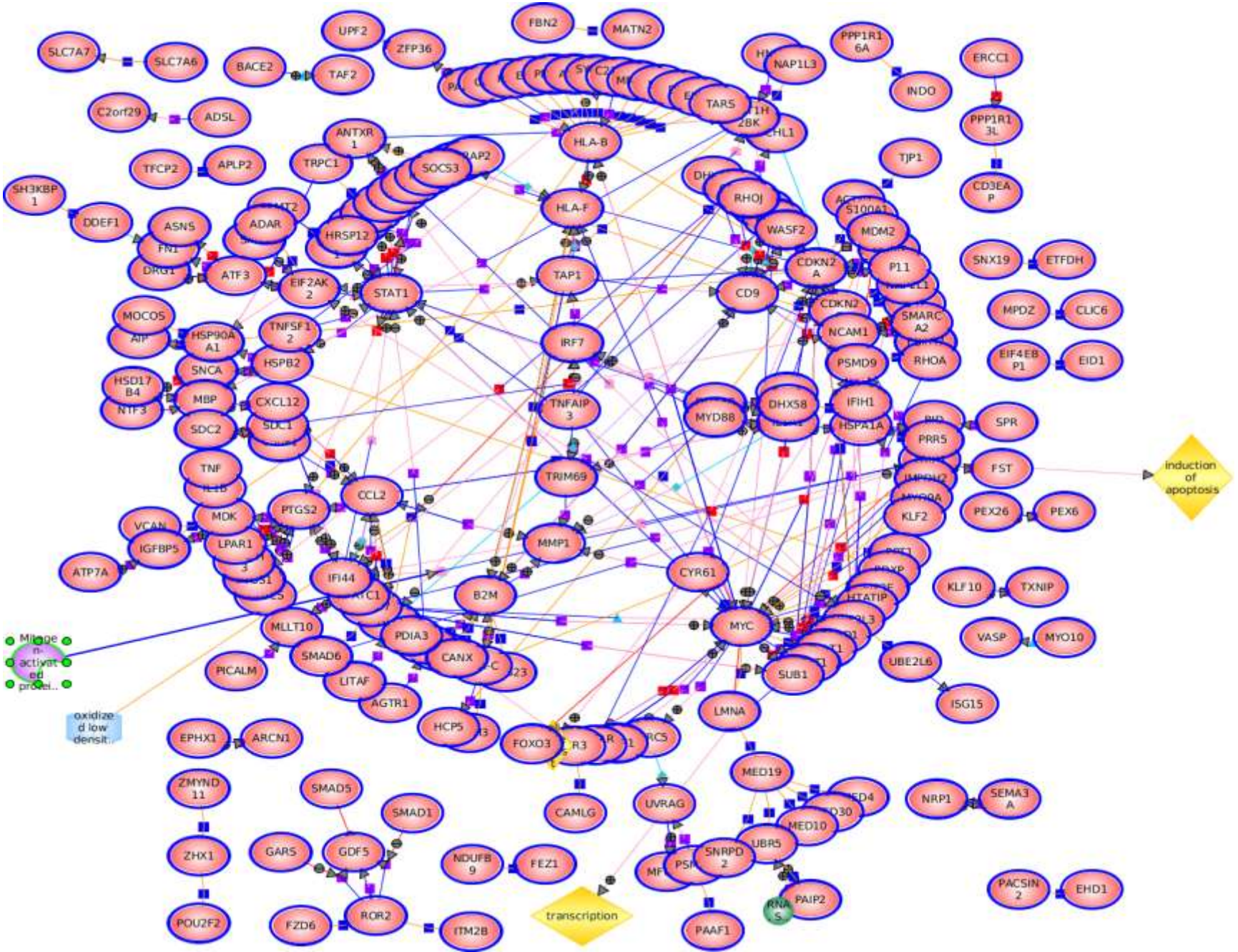
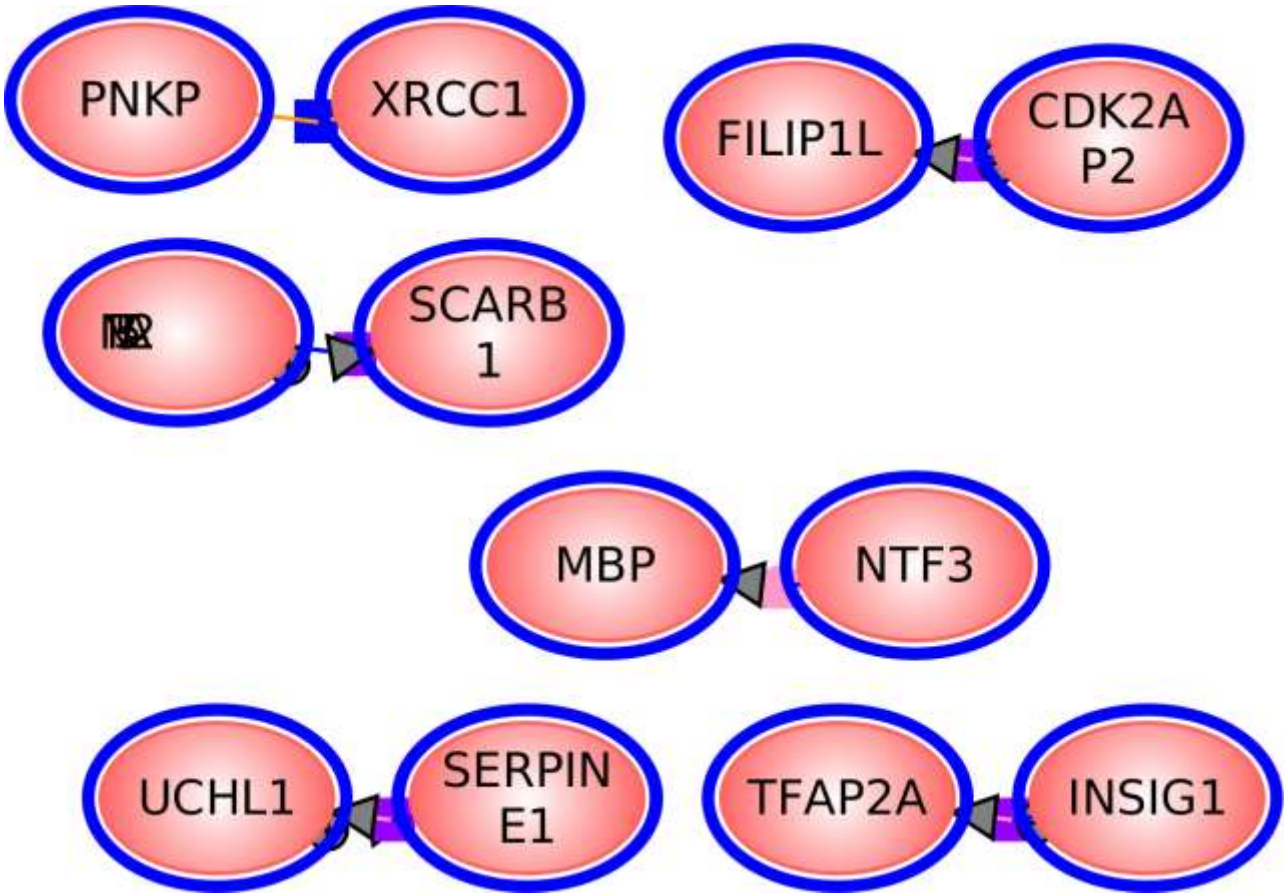
Scale = true
3-D scores = true
PCA on = Columns

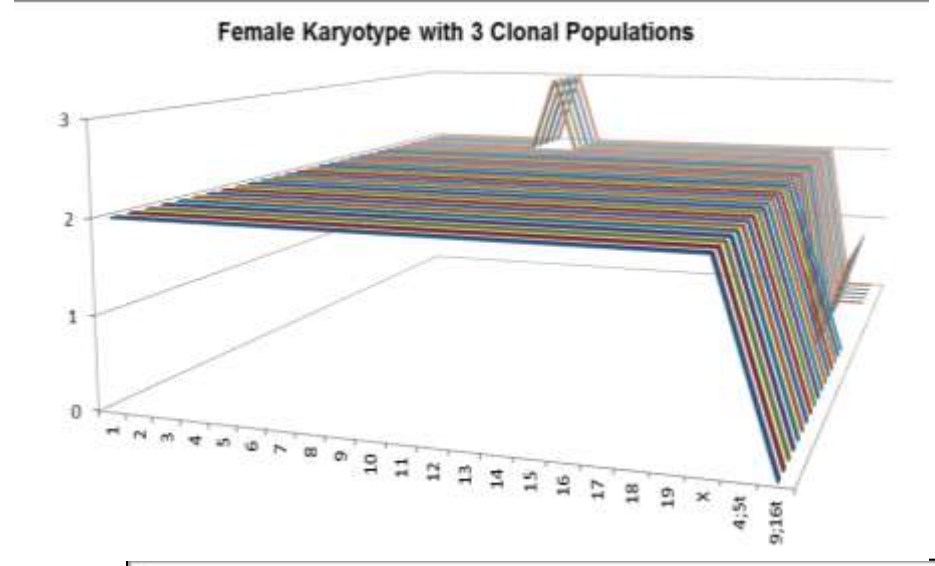
Principal component analysis demonstrates that replicates from stages with stable karyotypes have more similar expression

FUNCTIONAL NETWORKS PASSAGE 25 TO 54



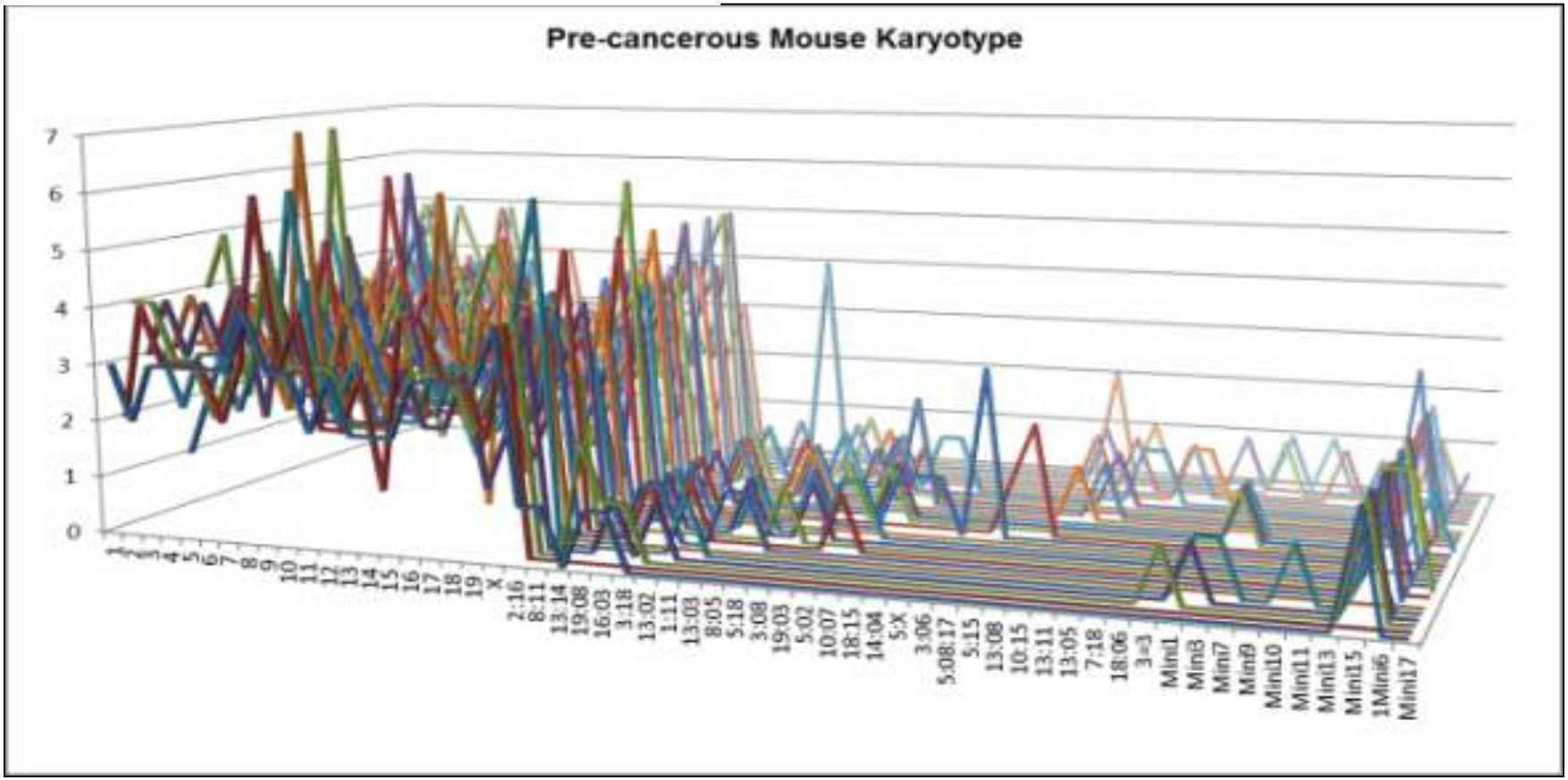
FUNCTIONAL NETWORKS PASSAGE 54 TO 109



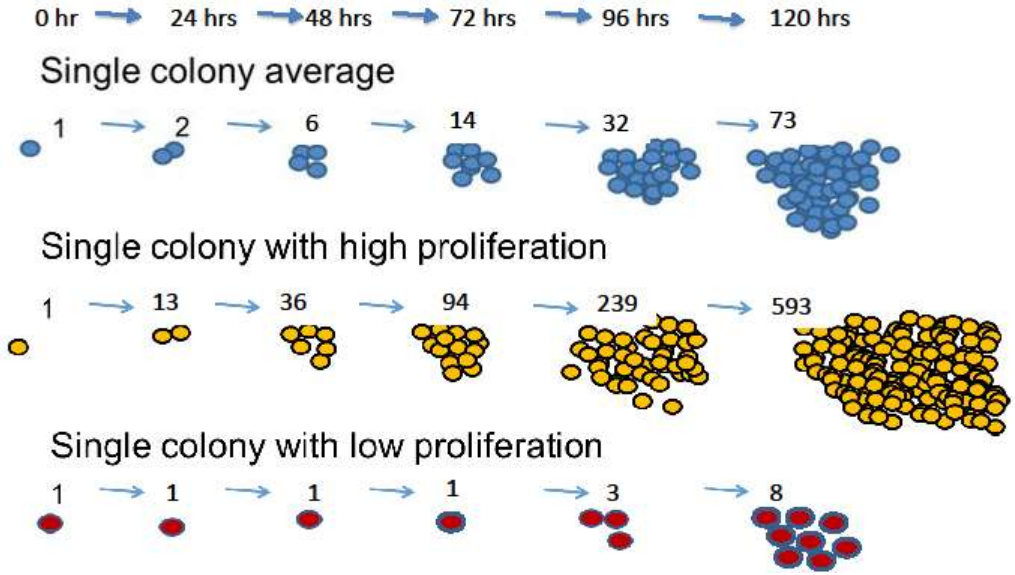


Population view

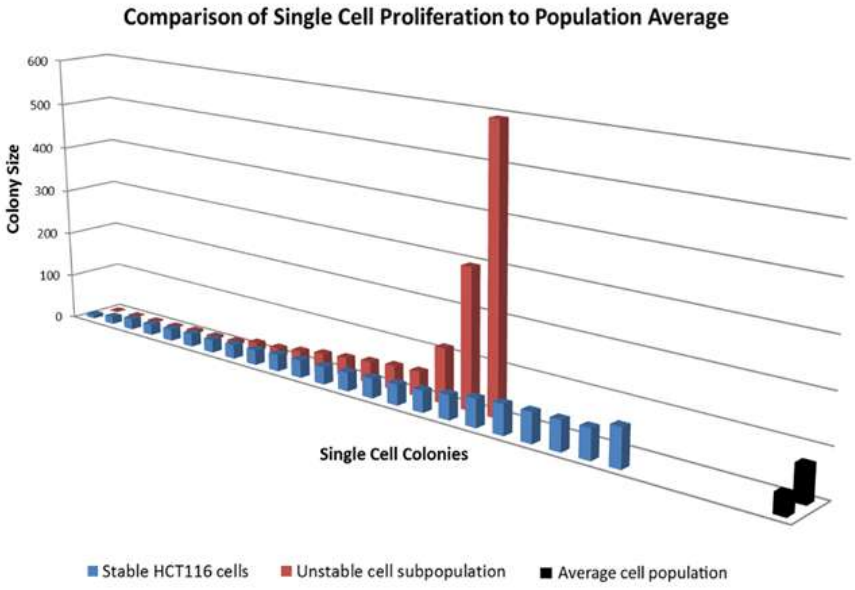
Mouse ovarian surface epithelial transformation model



Average is a poor measure for unstable cell populations



- Average is accurate for measuring clonal cell populations
- Average is a poor measure for measuring unstable cell populations



Abdallah et al.. Cell Cycle 2013

The increased acceptance of concept of macro-punctuated evolution of cancer

LETTER

doi:10.1038/nature09807

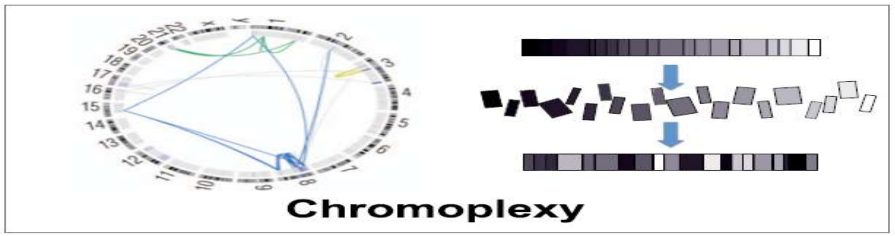
Tumour evolution inferred by single-cell sequencing

Nicholas Navin[1,2], Jude Kendall[1], Jennifer Troge[1], Peter Andrews[1], Linda Rodgers[1], Jeanne McIndoo[1], Kerry Cook[1], Asya Stepansky[1], Dan Levy[1], Diane Esposito[1], Lakshmi Muthuswamy[3], Alex Krasnitz[1], W. Richard McCombie[1], James Hicks[1] & Michael Wigler[1]

Cell

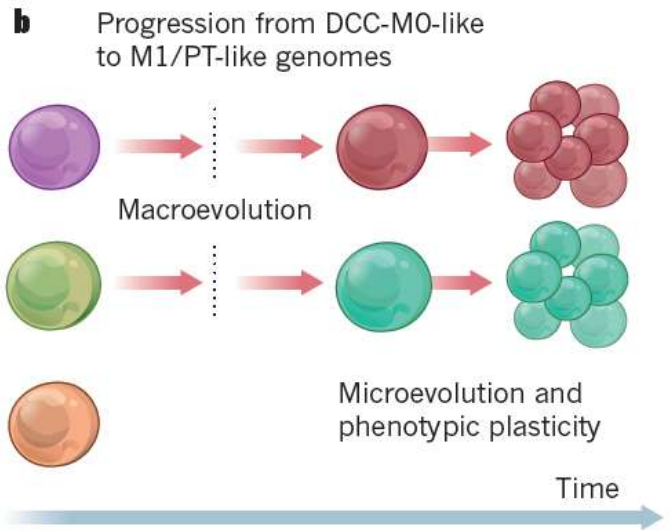
Massive Genomic Rearrangement Acquired in a Single Catastrophic Event during Cancer Development

Philip J. Stephens,[1] Chris D. Greenman,[1] Beiyuan Fu,[1] Fengtang Yang,[1] Graham R. Bignell,[1] Laura J. Mudie,[1] Erin D. Pleasance,[1] King Wai Lau,[1] David Beare,[1] Lucy A. Stebbings,[1] Stuart McLaren,[1] Meng-Lay Lin,[1] David J. McBride,[1] Ignacio Varela,[1] Serena Nik-Zainal,[1] Catherine Leroy,[1] Mingming Jia,[1] Andrew Menzies,[1] Adam P. Butler,[1] Jon W. Teague,[1] Michael A. Quail,[1] John Burton,[1] Harold Swerdlow,[1] Nigel P. Carter,[1] Laura A. Morsberger,[2] Christine Iacobuzio-Donahue,[2] George A. Follows,[3] Anthony R. Green,[3,4] Adrienne M. Flanagan,[5,6] Michael R. Stratton,[1,7] P. Andrew Futreal,[1] and Peter J. Campbell[1,3,4,*]



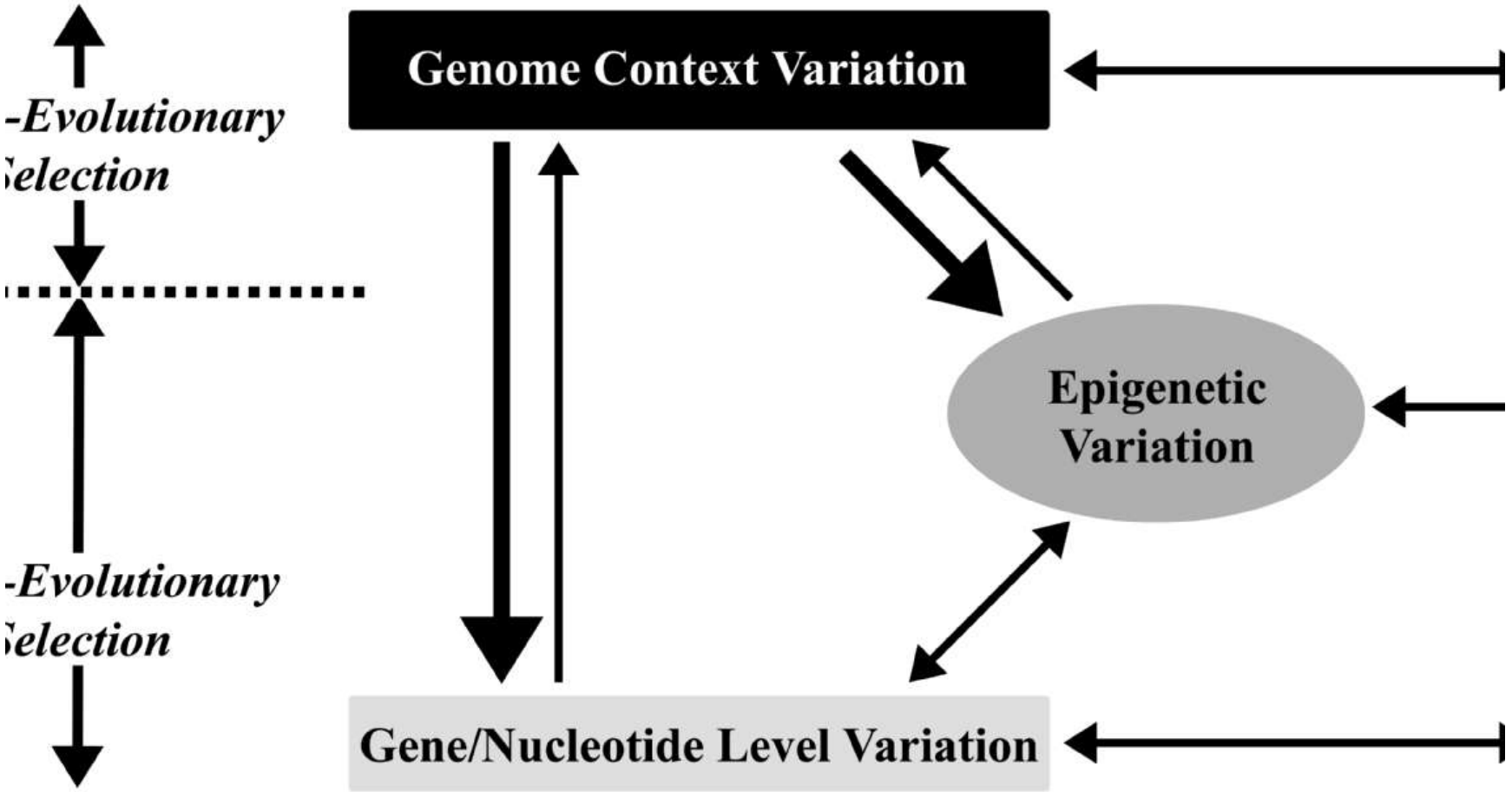
Baca et al, 2013 Cell

At DNA sequence level tumours grow by punctuated clonal expansions with few persistent intermediates



Klein CA 2013 Nature

Why focus on the measurement at the genome level?



Heng et al, 2009 JCP

Micro- and macro- evolution

- Micro-evolution: gene mutation, epigenetic alterations
- Macro-evolution: genome level alterations

Genome theory:

Macro-evolution creates system (species)

Micro-evolution modifies system (species)

(Heng 2009 BioEssays; Heng et al, 2010 J Cell Biochemistry)

Mechanism of Cancer

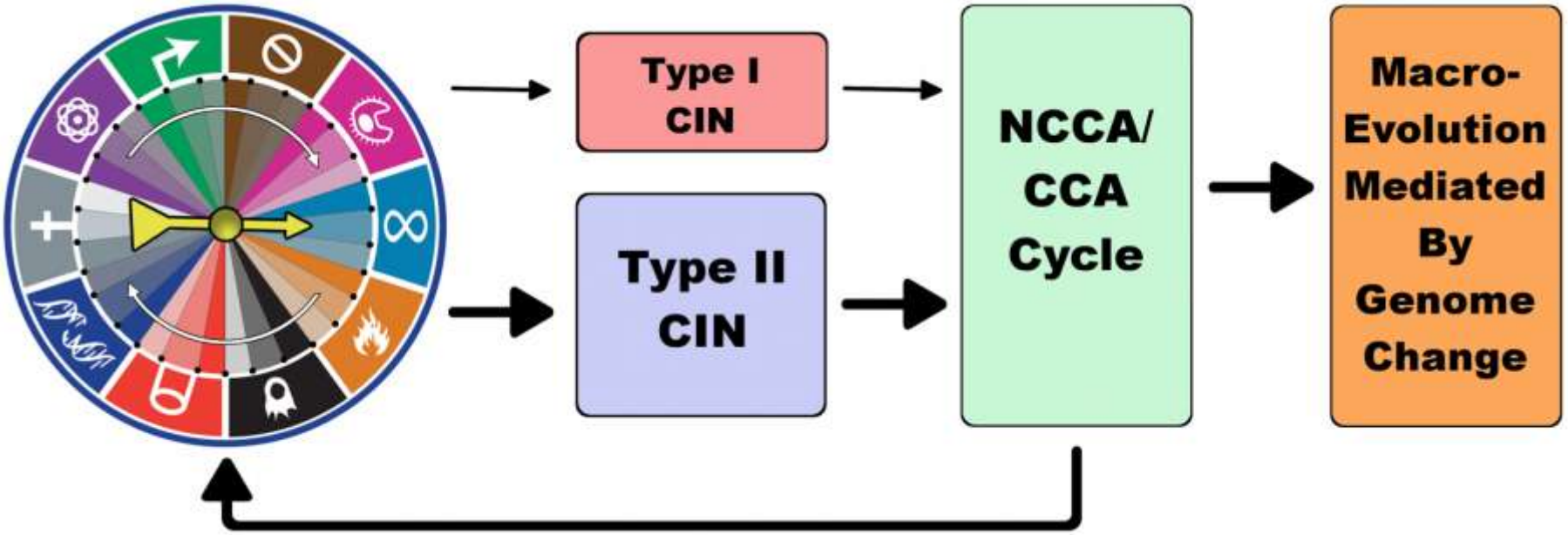
Evolutionary mechanism:

1. Stress induced system dynamics – increased stochastic changes
2. Population diversity (genome heterogeneity)
3. Natural selection based on genome package

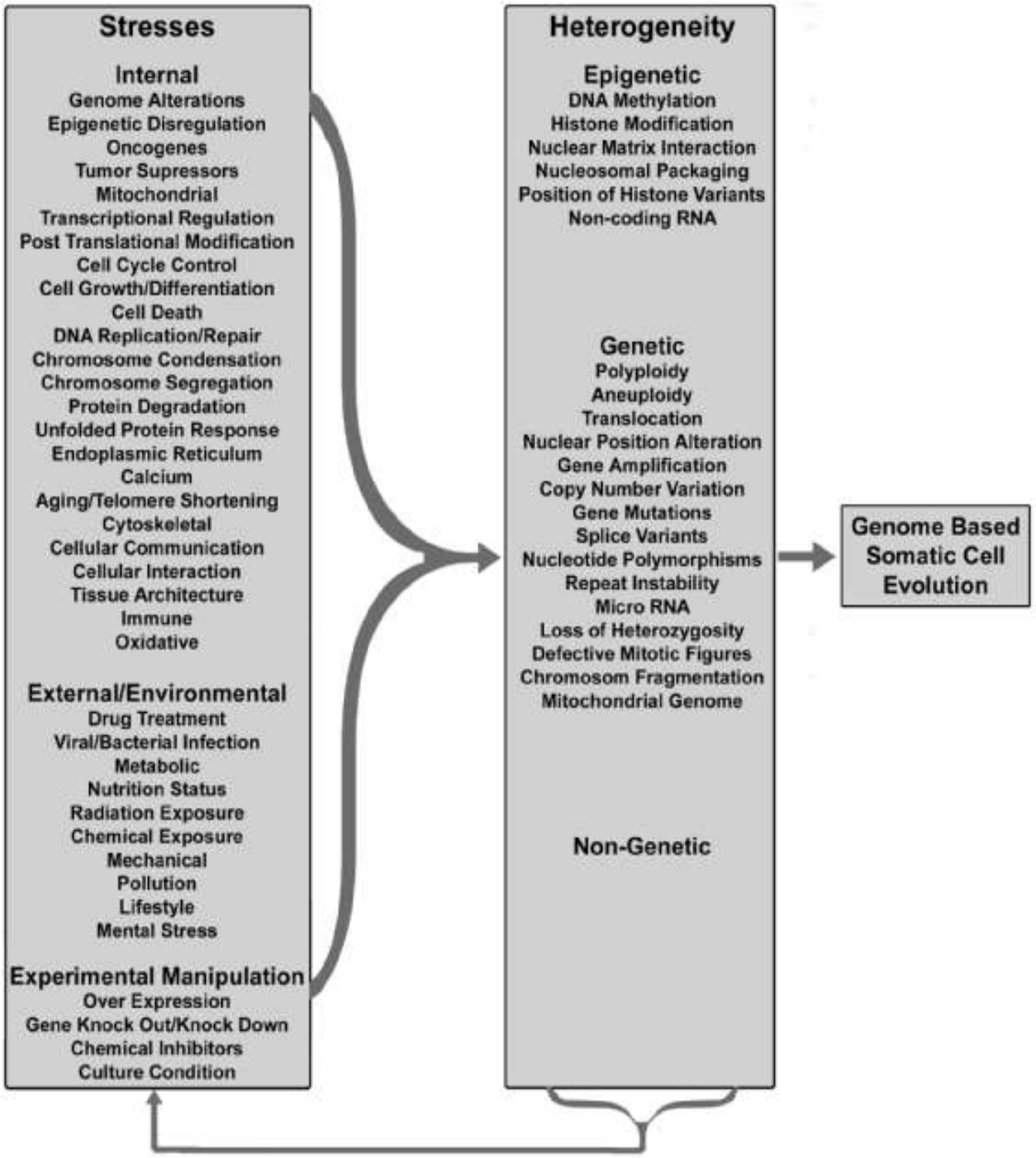
Evolutionary Mechanism $(1 \rightarrow 2 \rightarrow 3)$ =

$\sum$ Individual Molecular Mechanisms

Ye et al, 2009 JCP
Heng et al, 2010 JCB
Heng et al, 2011, Adv Can Res



Heng et al, Cancer Metastasis and review 2013



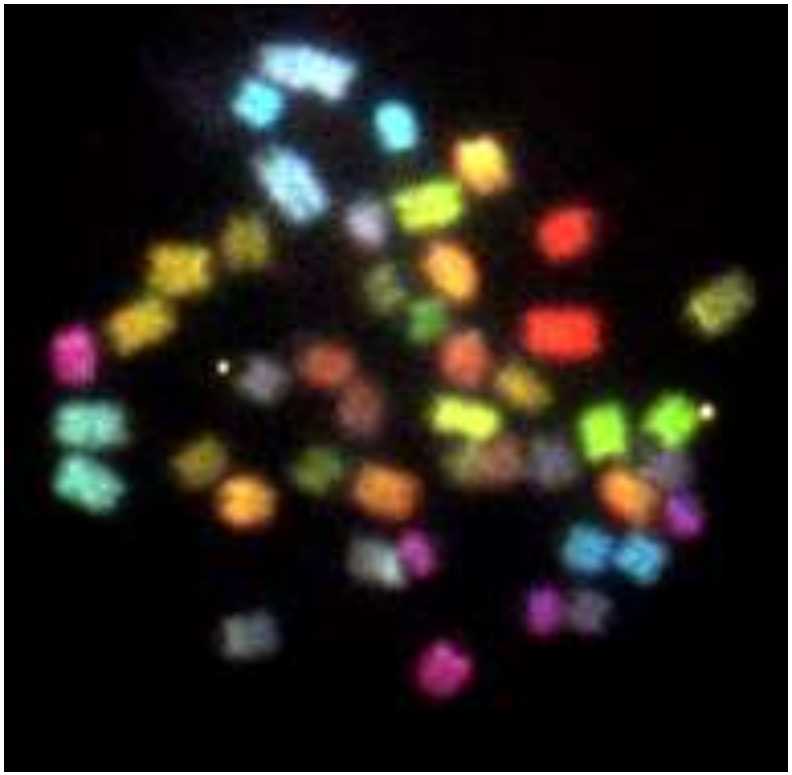
Paradox

Insignificance
of
the significant

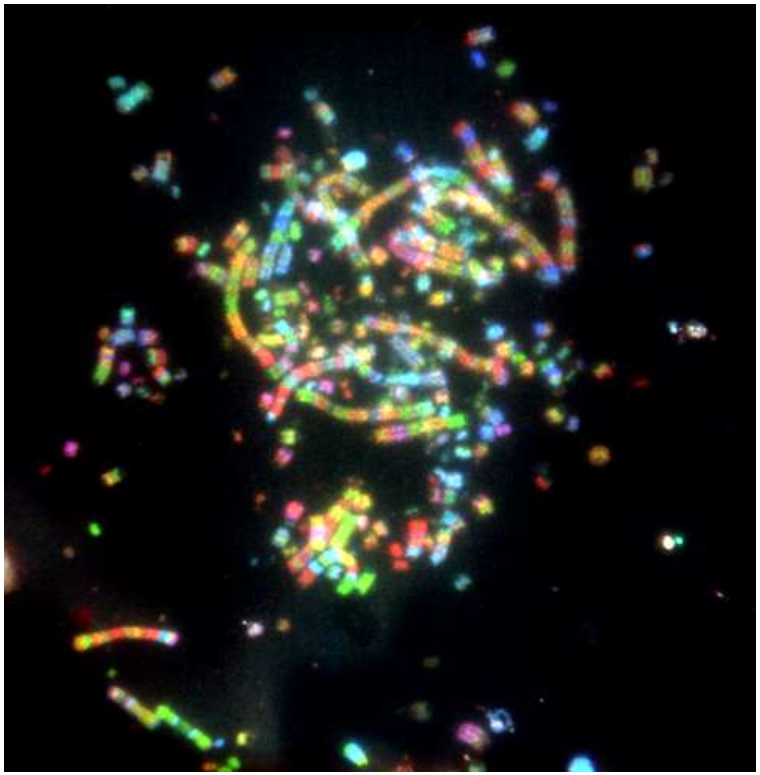
Heng et al, 2009 JCP

Chromosome defined system is the key to cancer formation and drug resistance

The pattern of dynamics can be traced!
Key : score high levels of heterogeneity (Genome chaos)

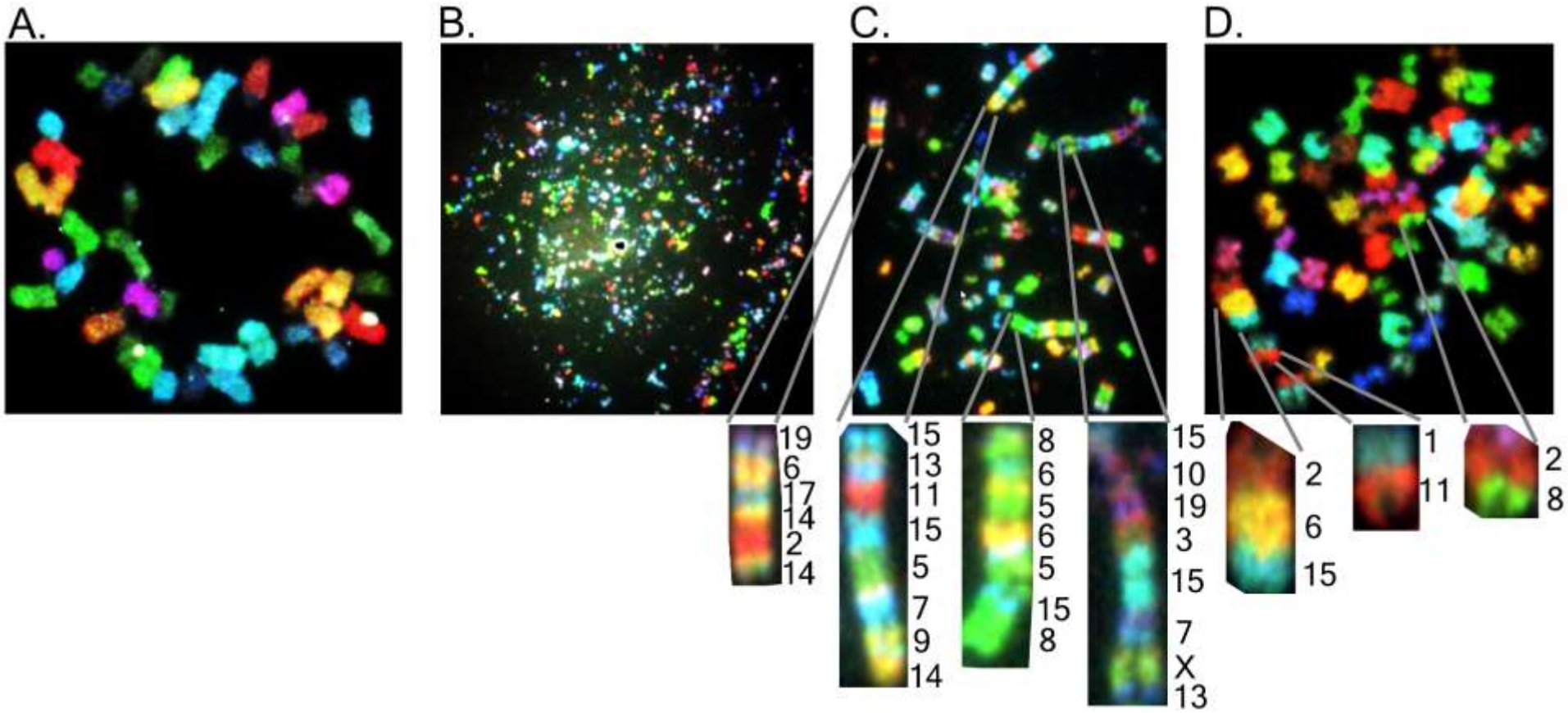


1-2
generations



Example of karyotypic chaos achieved by drug treatment

Mechanism: following the entire process of genome chaos

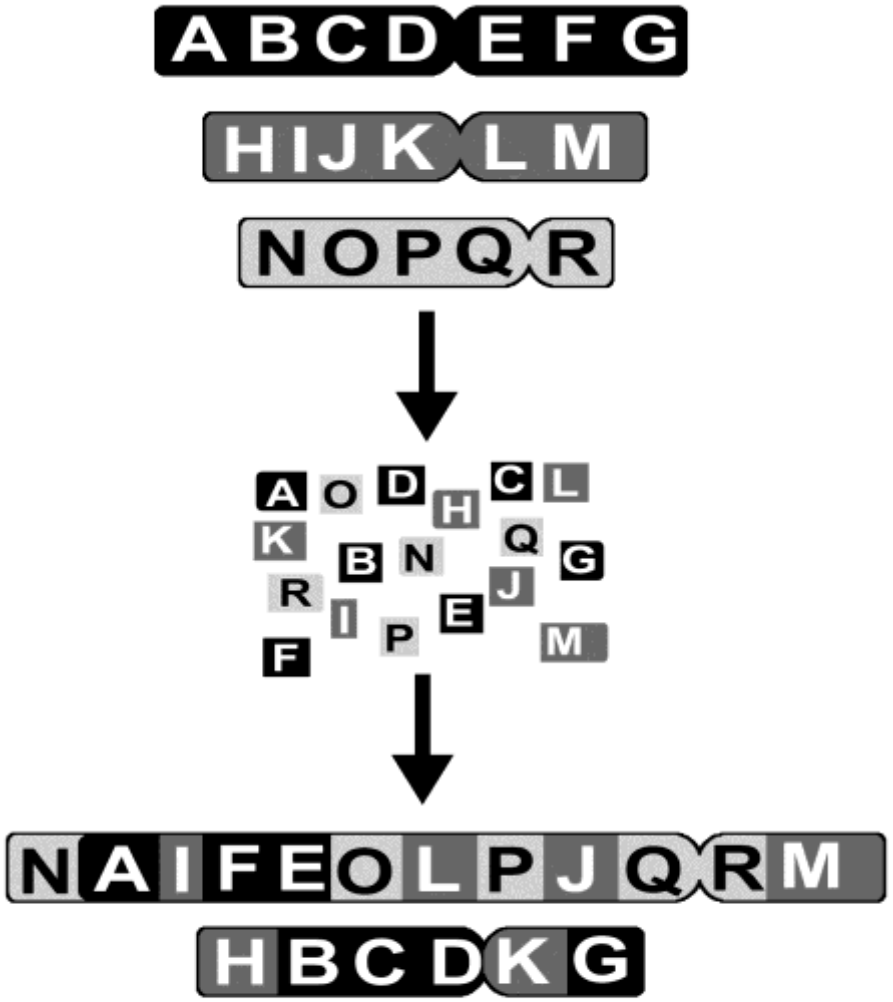


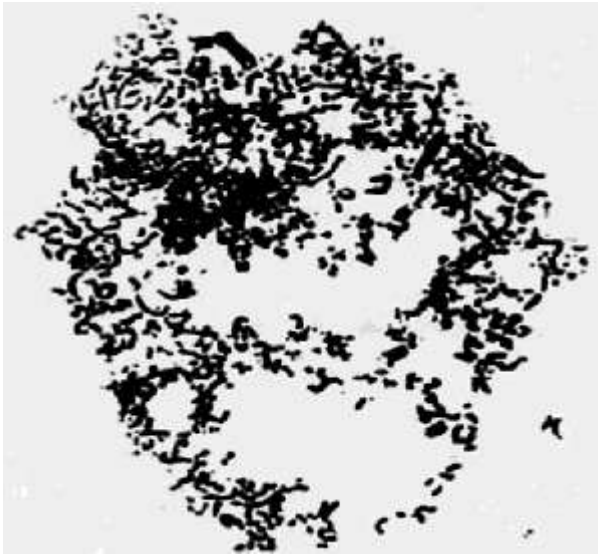
Compare multiple runs of evolution: all survivors are different!

It is not a one time event; occurs multiple times over a few week period

Mechanism of chromosome chaos:

Stress, Chromosome fragmentations, newly formed chromosomes





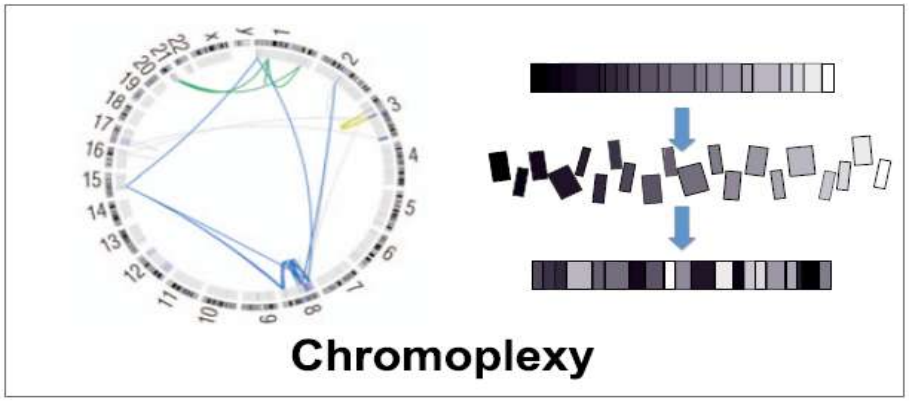
Stevens et al, 2007 Can Res;
2011 Cell Death &Diseases
Heng et al, 2011 Adv Can Res

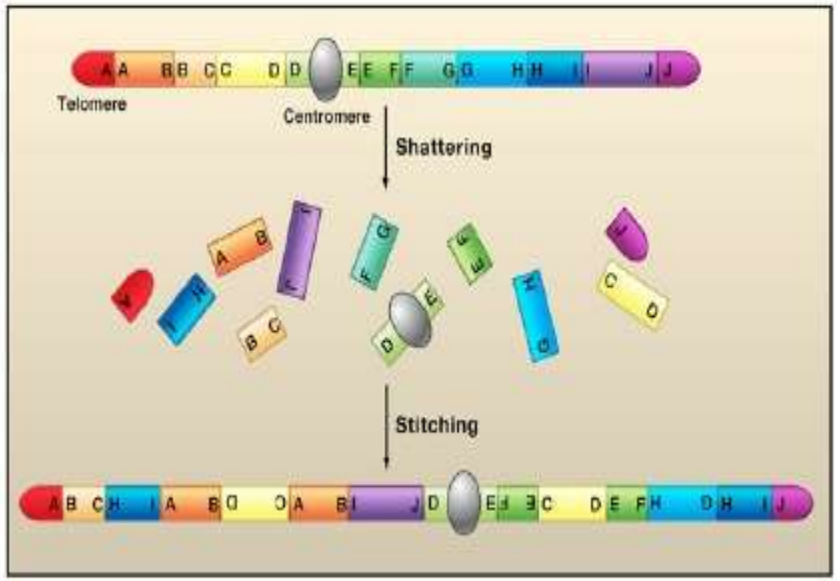
However, some consider genome chaos as artifacts of cell culture system, as it was hard to image that these cells can survive
until…molecular confirmation

Cell

Massive Genomic Rearrangement Acquired in a Single Catastrophic Event during Cancer Development

Philip J. Stephens,[1] Chris D. Greenman,[1] Beiyuan Fu,[1] Fengtang Yang,[1] Graham R. Bignell,[1] Laura J. Mudie,[1] Erin D. Pleasance,[1] King Wai Lau,[1] David Beare,[1] Lucy A. Stebbings,[1] Stuart McLaren,[1] Meng-Lay Lin,[1] David J. McBride,[1] Ignacio Varela,[1] Serena Nik-Zainal,[1] Catherine Leroy,[1] Mingming Jia,[1] Andrew Menzies,[1] Adam P. Butler,[1] Jon W. Teague,[1] Michael A. Quail,[1] John Burton,[1] Harold Swerdlow,[1] Nigel P. Carter,[1] Laura A. Morsberger,[2] Christine Iacobuzio-Donahue,[2] George A. Follows,[3] Anthony R. Green,[3,4] Adrienne M. Flanagan,[5,6] Michael R. Stratton,[1,7] P. Andrew Futreal,[1] and Peter J. Campbell[1,3,4,*]





Genome Chaos, Heng et al, 2006
Chromosome Chaos, Duesberg, 2007
Chromothripsis, Stevens et al, 2011
Chromoplexy, Baca et al, 2013

What is the difference between cancer and organismal evolution?

- Cancer is a “disease” of somatic cell evolution within the body, but they both are bio-systems

- The key difference between cancer and organismal evolution is the system dynamics (sexual reproduction ensures the genome identity). Somatic cells (without sexual filter) are more sensitive to stresses leading to genome alterations mediated cancer

- The pattern of evolutionary dynamics of cancer can offer important information on organismal evolution

Why sex?

For nearly a century sex has been biology's biggest mystery

Example of using genome theory to address key biological questions

- The paradox of sex (the persistence of sexual reproduction despite its overwhelming "cost") has been a key question in biology for 150 years

- Concept: the evolutionary benefits of genetic recombination is diversity, however, this does not make sense.

"Fact":

- Asexual = Identical genome
- Sexual = Diverse genome

Unsolved questions:

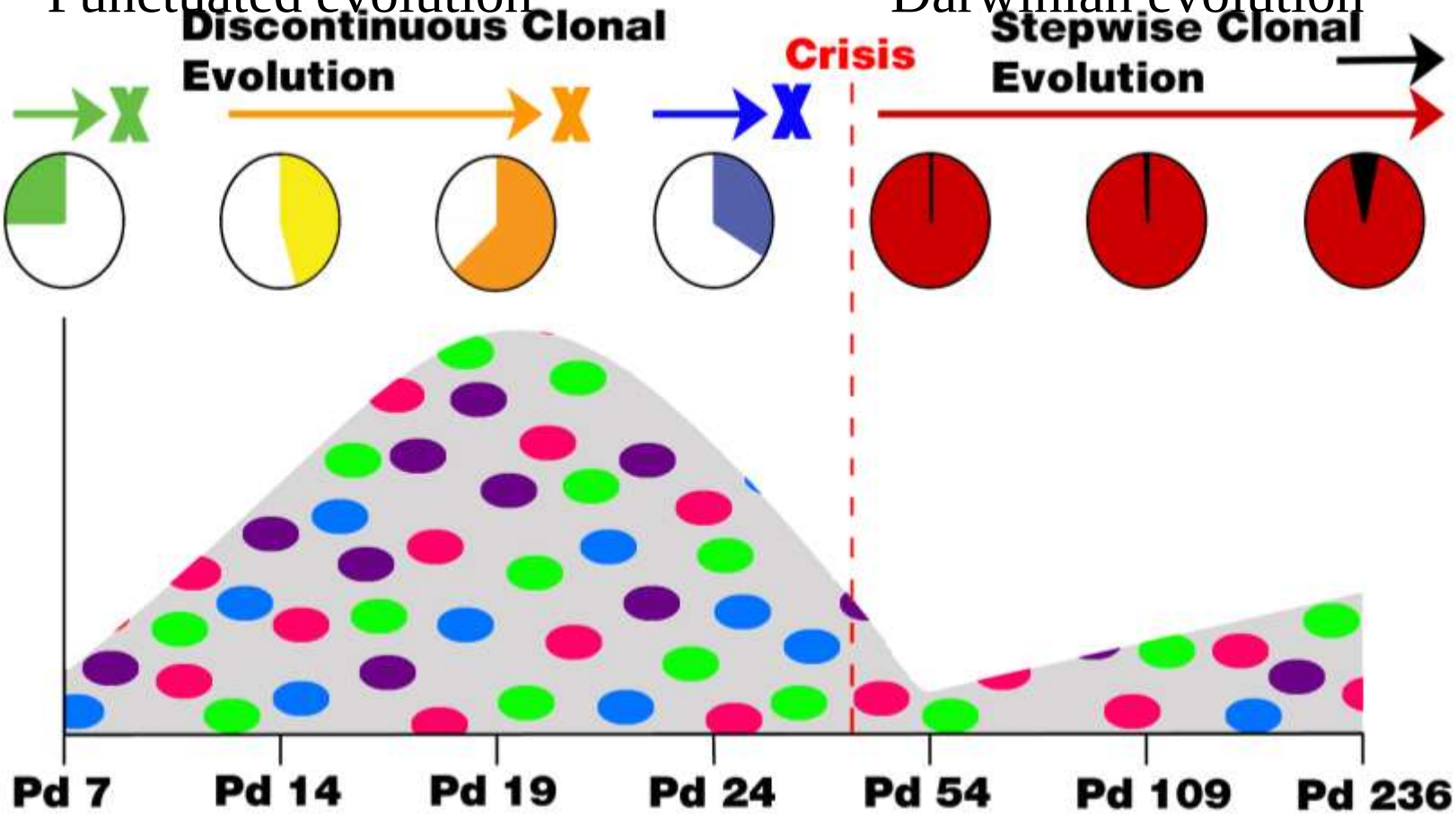
Why is there prevalence of asexual reproduction in harsh, unstable environments

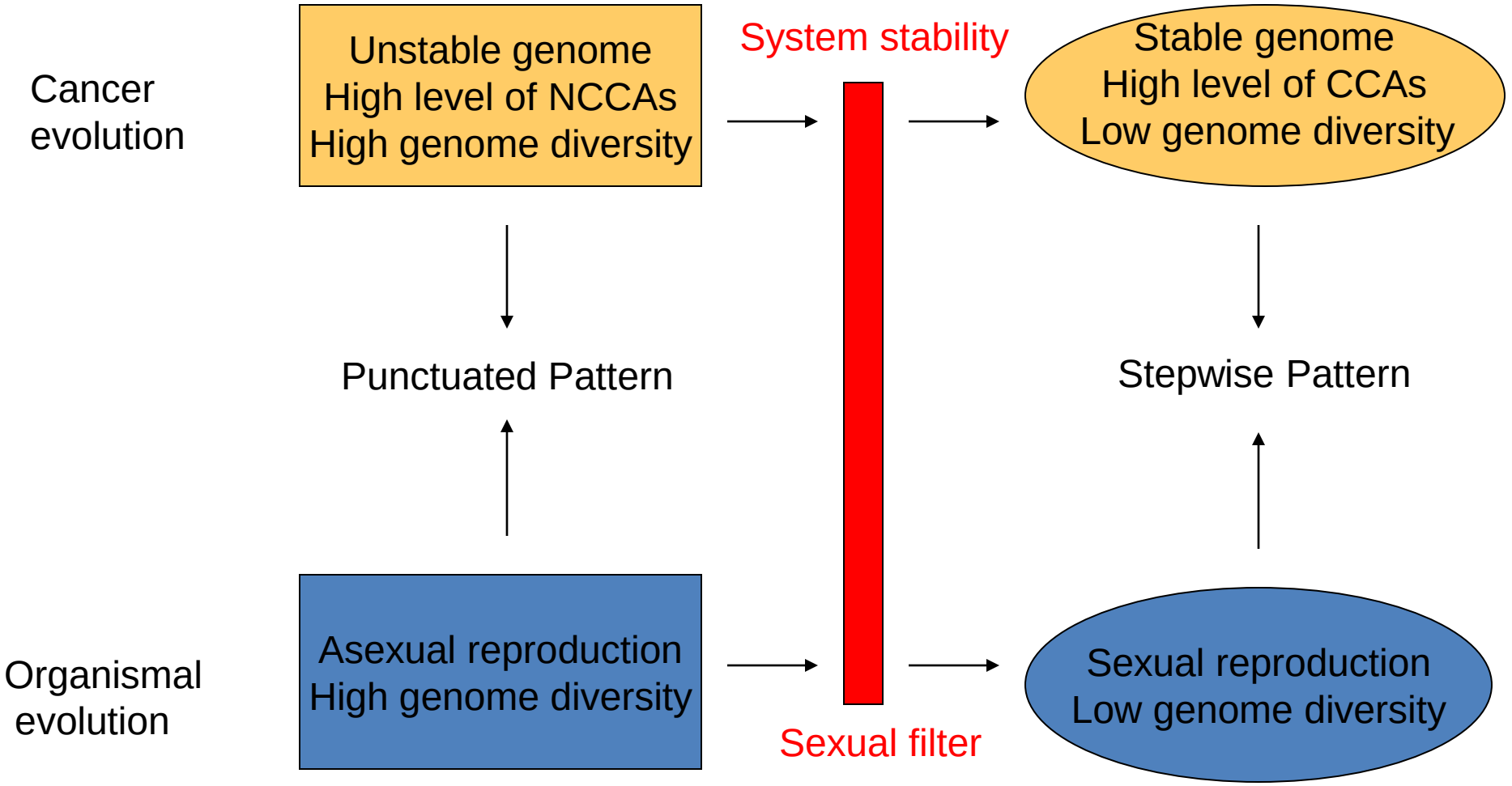
Giving existence diversity, why sexual population display slow evolution

What is the purpose of sex without genetic mixing
(for species with self sex)

Asexual reproduction
Punctuated evolution

Sexual reproduction
Darwinian evolution





Asexual = Identical genome?

Sexual = Diverse genome ?

Let's switch!

In fact, asexual reproduction displays high levels of genome diversity

Genome diversity:

9 strains of E.coli 40%–55% of genes

Human 0.1%

Rotifer (evolutionary scandal):

Bdelloidea 36-73% (asexual)

Monogonnta 0-2.4% (sexual)

Yeast: Asexual phase with high level of aneuploidy

The function of sexual reproduction = “Filter” to keep the genome pure at following stages:

Meiosis-Fertilization-Early development-Infant mortality-Infertility

Each step filters out the genome alterations (the majority of spontaneously aborted early human embryos display chromosomal abnormalities)

Genes and chromosomes display drastically different functions

Genome level, reduces change, gene level increase change

The genome defines the species, the gene modifies a species

Heng HH, Genome 517-524, 2007

The evolution of meiosis from mitosis.

Wilkins AS, Holliday R.

Genetics. 2009 Jan;181(1):3-12.

"The conclusion is surprising: the initial function of chromosome pairing was to *limit*, not enhance, recombination".

"A similar general conclusion, from a consideration of cancer cells, has been proposed by HENG (2007)."

SEX REDUCES GENETIC VARIATION: A MULTIDISCIPLINARY REVIEW

Gorelick and Heng, Evolution, 2011 65:1088-98

- Sex reduces genetic variation particularly at the genome level
- The genome is responsible for evolutionary constraint
- Small accumulations at the gene level will not lead to genome alteration (man is man)

What is new?

Non-Clonal Evolution

Two phases of cancer evolution defined by instability

Genome re-organization through Genome Chaos

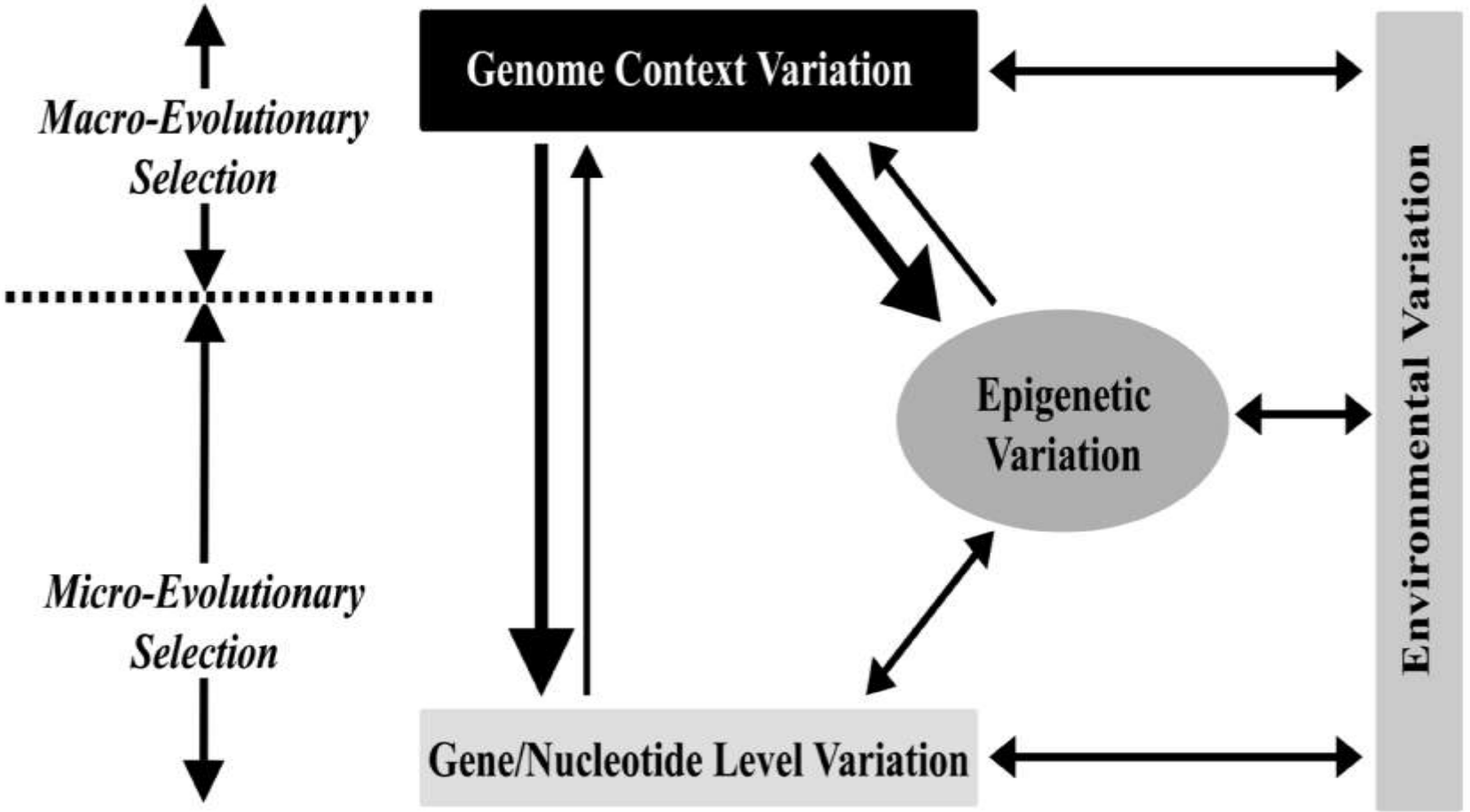
Measure instability by random genome changes (noise)

Importance of gene mutation vs. chromosome aberration

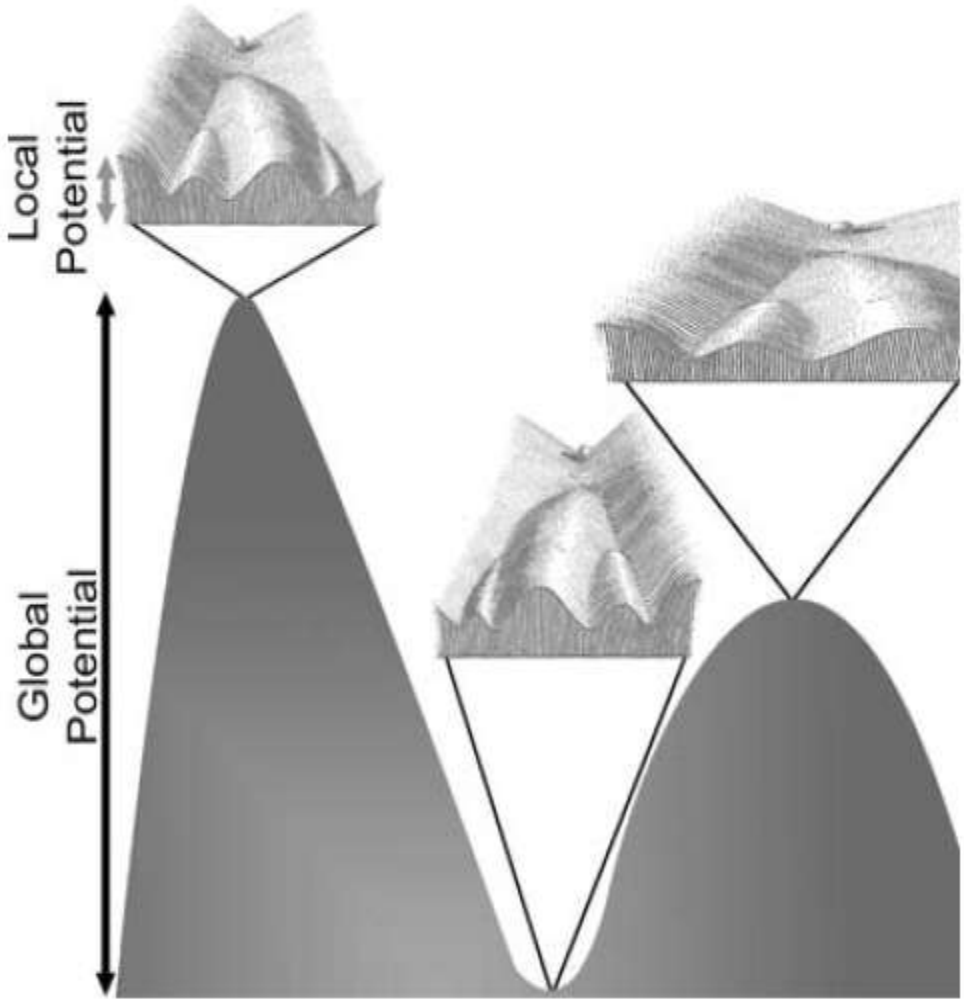
Referred as Heng-Duesberg causality

Advances in Cancer Res 112: 281-348

How about epigenetic variation



Multiple level of genetic/nongenetic landscape model



- The genetic landscape can be broken down to two levels of evolutionary potential.

Local potential refers to adaptation potential provided primarily by gene-level or nongenetic changes. While important for many biological processes such as development, local adaptive landscapes do not typically drive the evolutionary process of cancer.

The global potential of the evolutionary landscape (speciation or cancer) is derived primarily by genome level change that drives macroevolution.

Now we understand that, the key is to separate genes/epigenes and genomes when studying evolution dynamics and constraint

At the species level, sex eliminates most of the big changes, bringing the genome system to the same genome context, so that the same species does not gradually evolve into another type
(by genome chaos)

This balance of dynamic genes and constraint of genome are the main players of evolution, which solves a key paradox of evolution: short term adaptation (by gene mutations/epigenetic regulation) and long term stasis (by preserving the genome)

The mechanism of separating germ line and somatic cell ensure such balance.

Steve Krawetz
Alan Wang
Prem Reddy
Markku Kurkinen
Root Gorelick
G-S Wu
Y-M Xie
Wei Zen Wei
Lydia Choi
Faz Sarkar
Christine Ye
Joshua Liao
J Wang
Hao Ying
Ed Golenberg
Markus Friedrich
William Moore
Derek Wildman
M-Z Yang
Gary Gibson
KZ-Zhang
S Savansan

Gloria Heppner
Barbara Spyropoulos
Mina Bissell
Lap-Chee Tsui

Harry Rubin
Uta Franke
O.J. Miller
Peter Moens
Jim Davis
Donald Coffey
Gary Stein

Heng's group

Guo Liu
Steve Bremer
Joshua Stevens
Steven Horne
Lesley Lawrenson
Batoul Abdallah

SeeDNA Inc.

Karen Ye
Susan Zheng

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