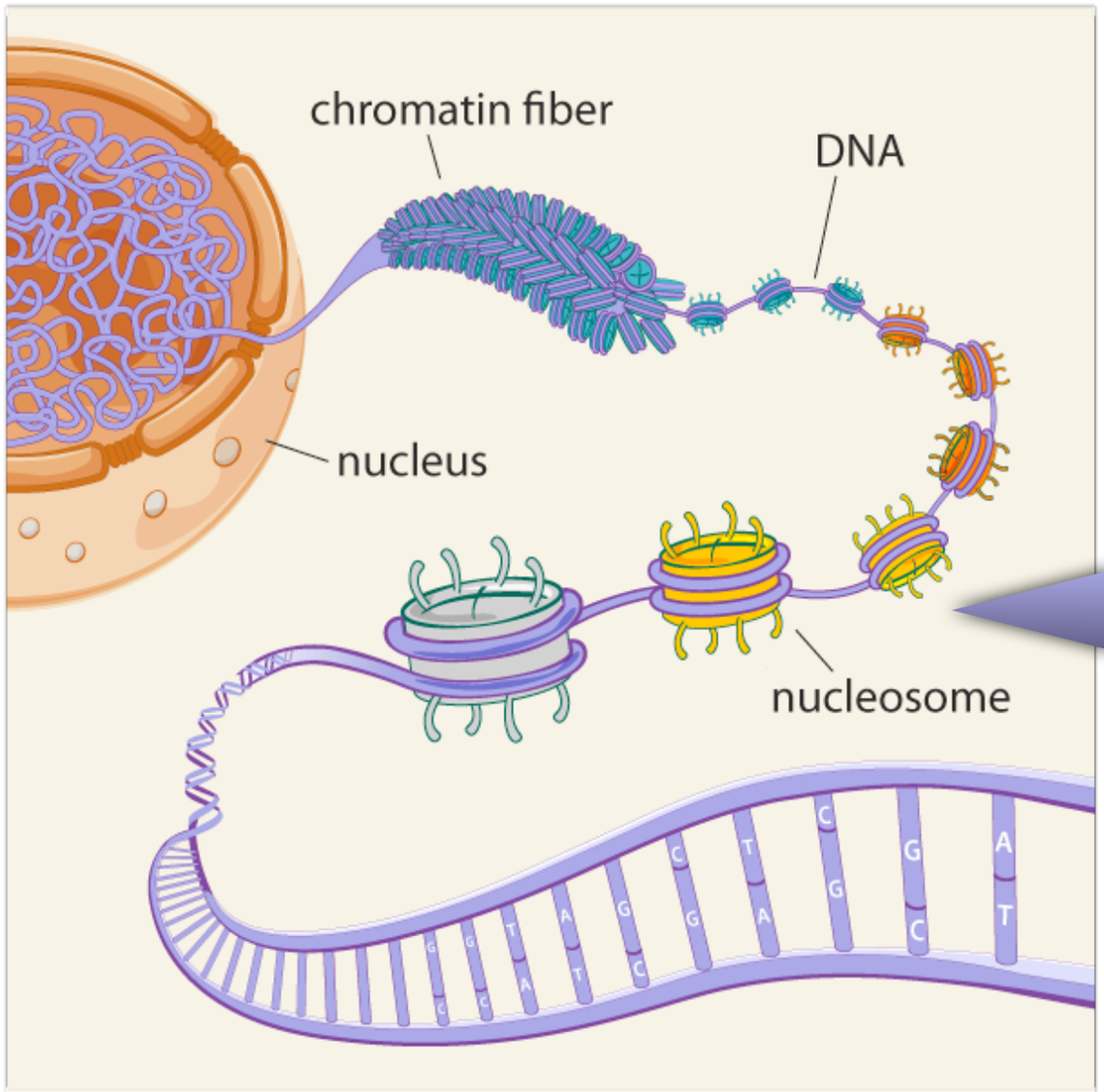
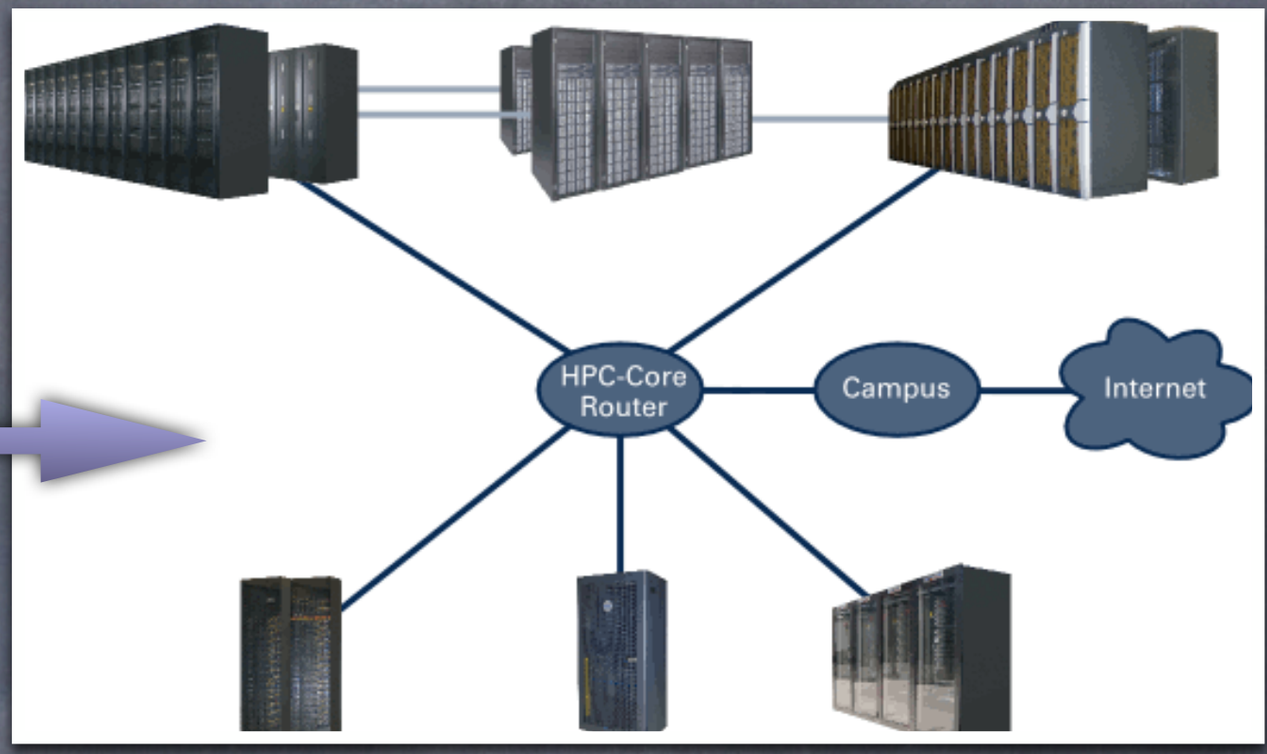


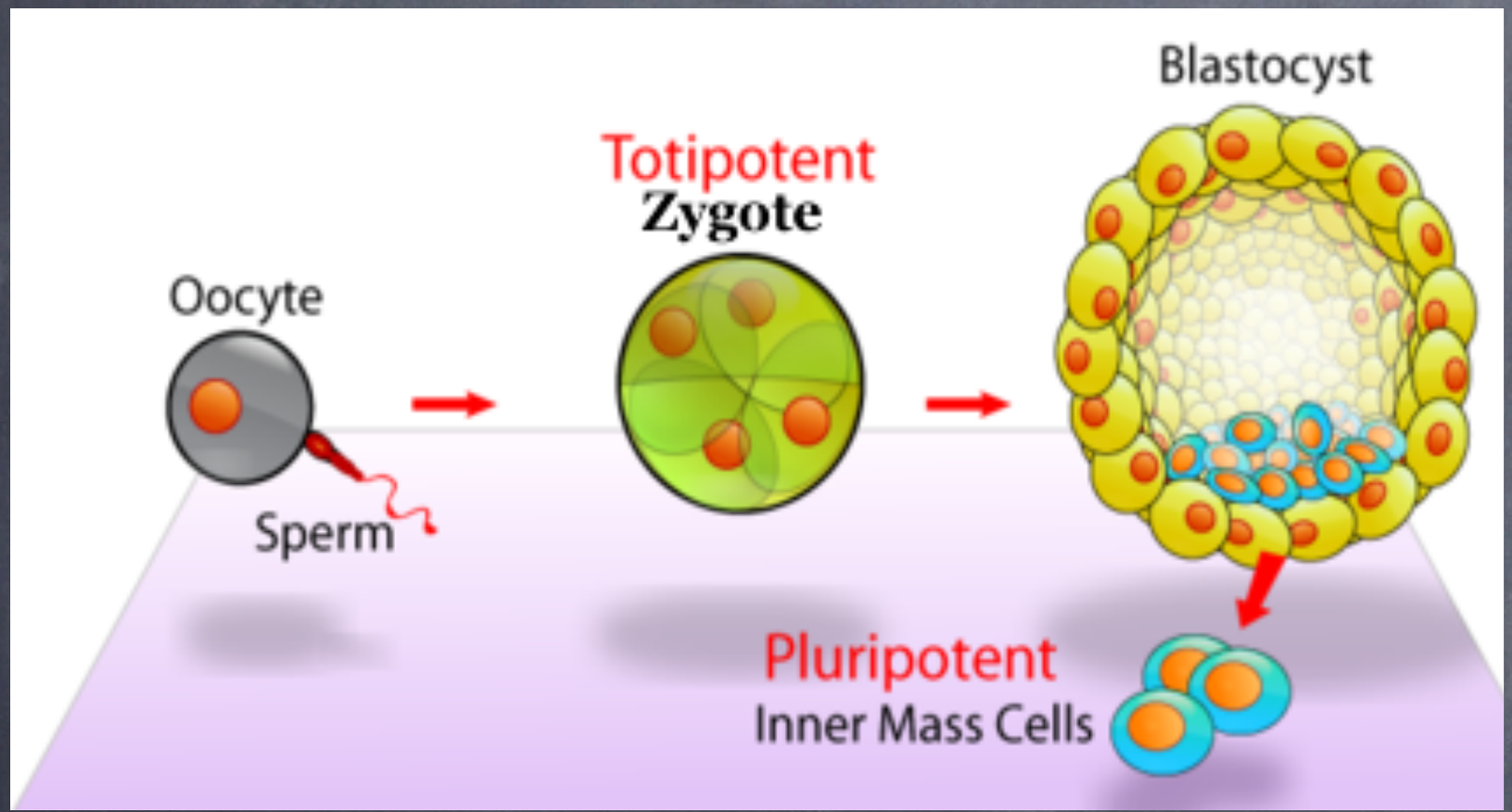


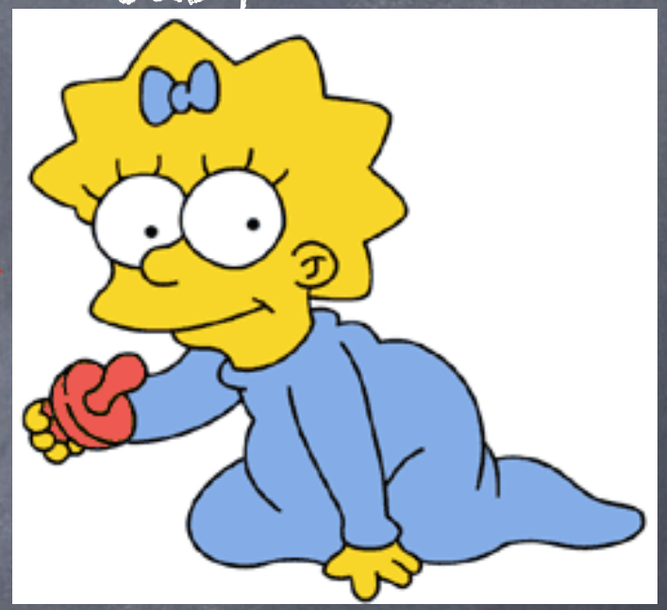
Hacking the Epigenome: Reprogramming at its best

- Chromatin, iPS cells and HPC -

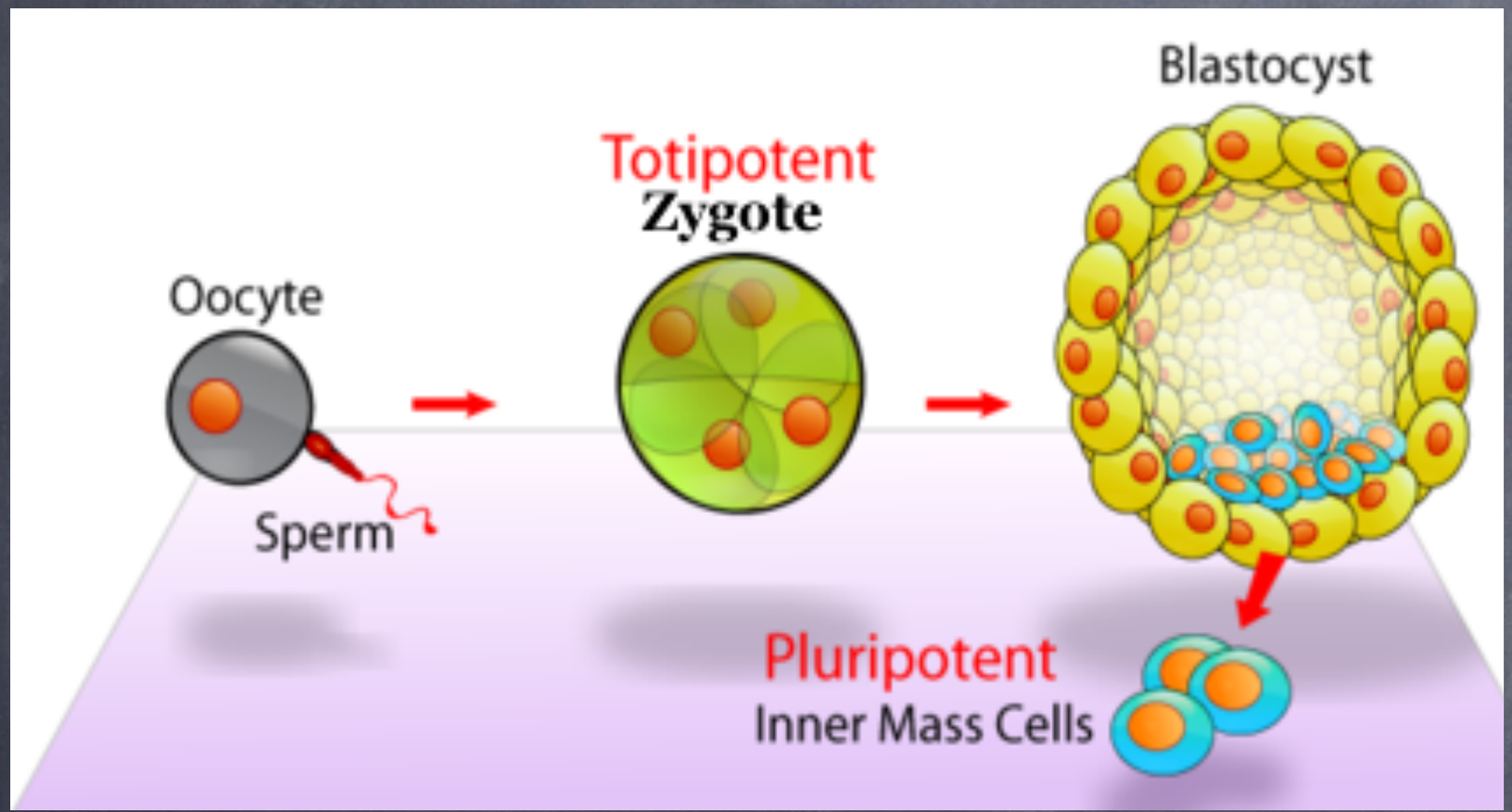
Christian Pflüger, Cairns Lab
University of Utah, Huntsman Cancer

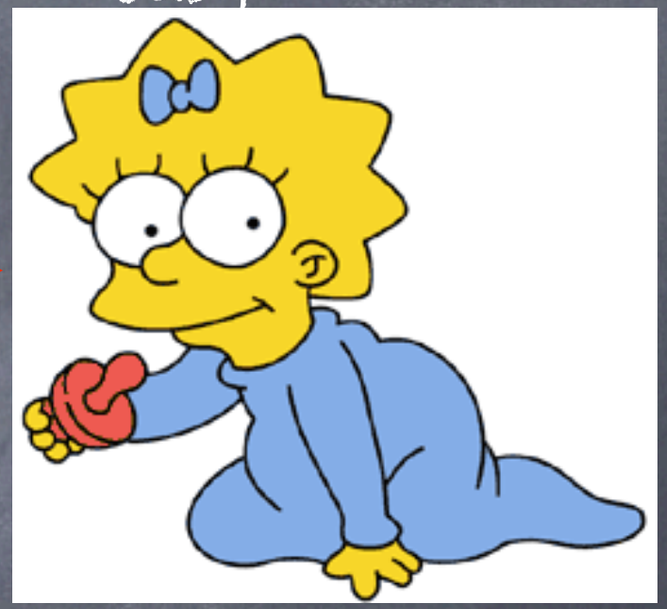
Embryonic stem cells (ES cells) and applications

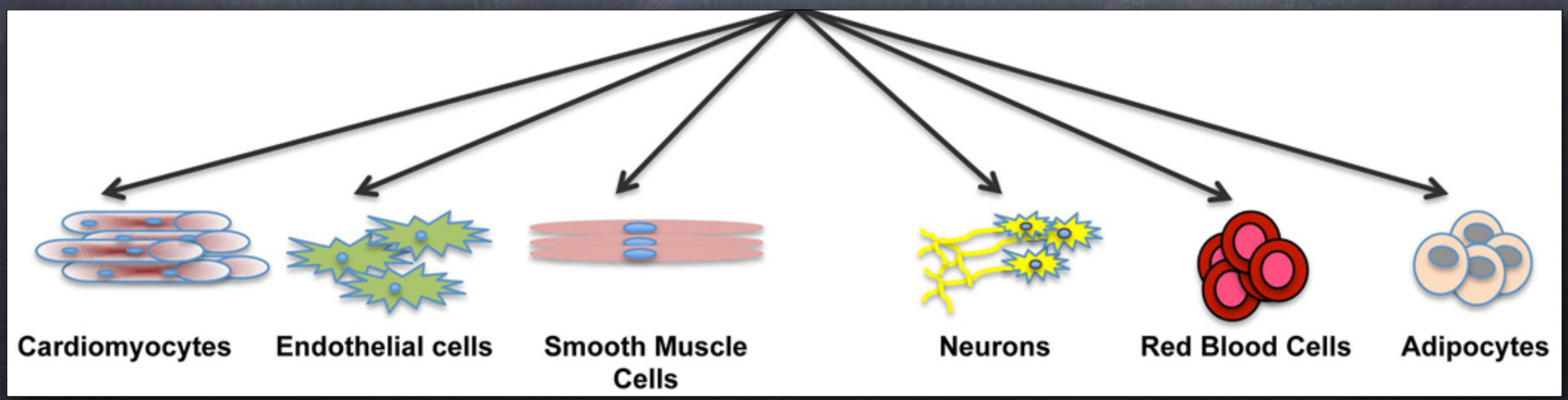




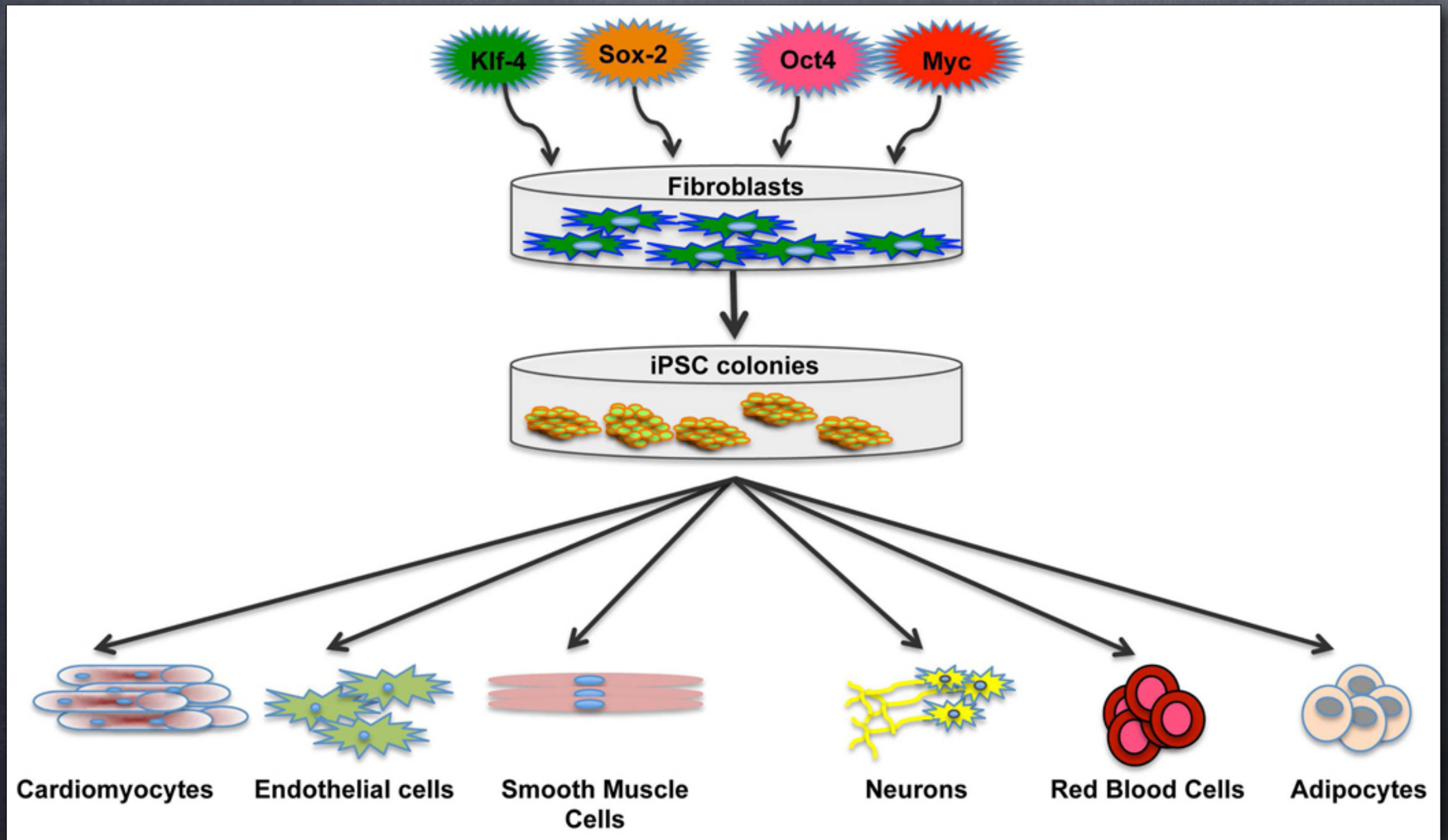
Embryonic stem cells (ES cells) and applications





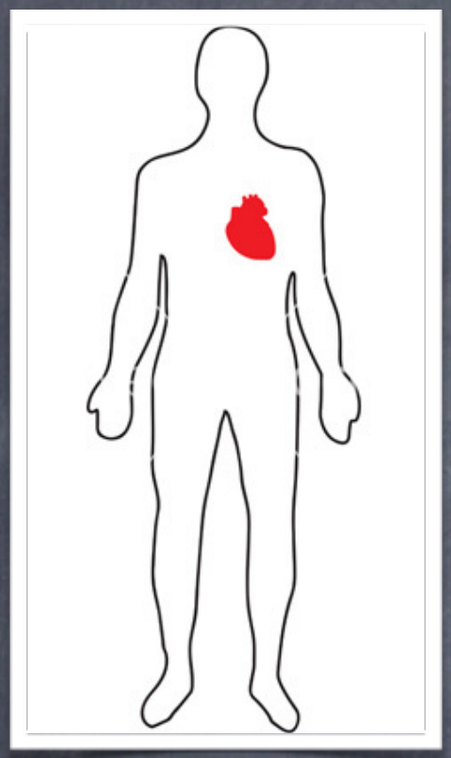


Induced pluripotent stem cells (iPS) generation and applications



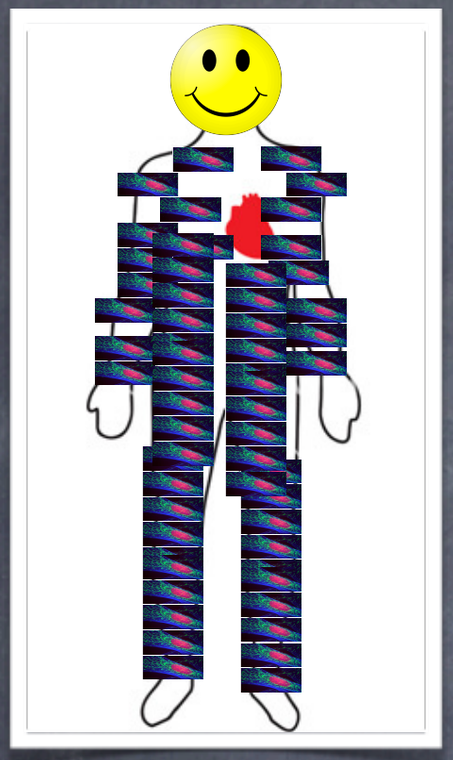
Nsair, A. & MacLellan, W. R.,Advanced Drug Delivery Reviews 63, 324-330 (2011).

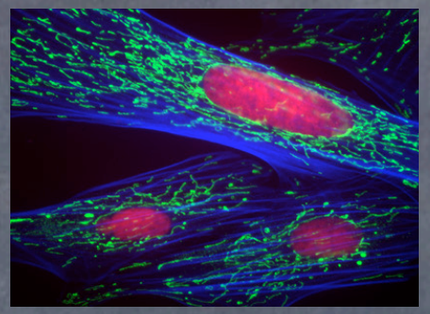
Cells and Parallel Computing



Riken Research, Invitrogen

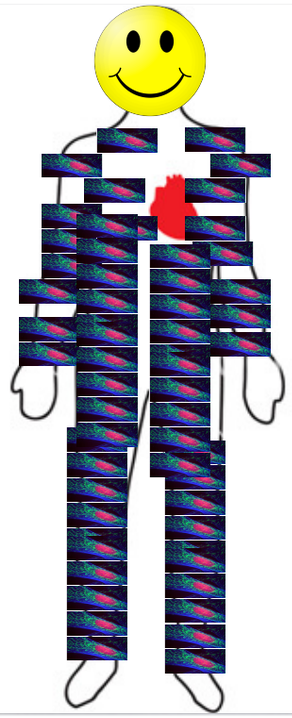
Cells and Parallel Computing

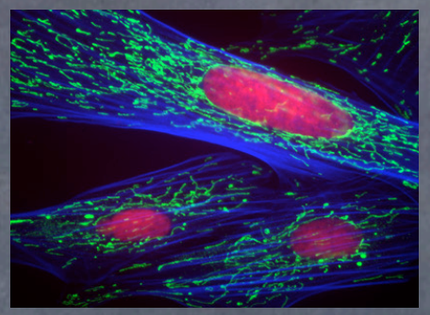


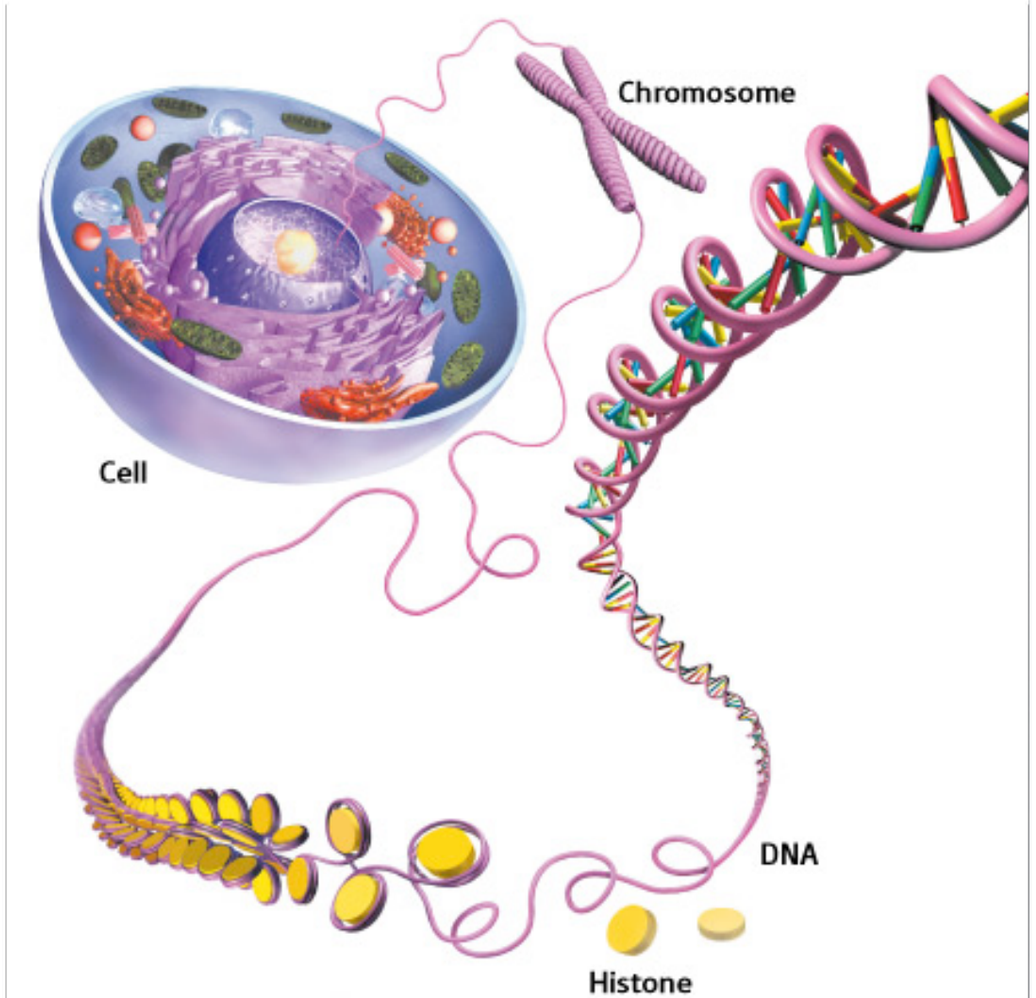


Riken Research, Invitrogen

Cells and Parallel Computing







Riken Research, Invitrogen

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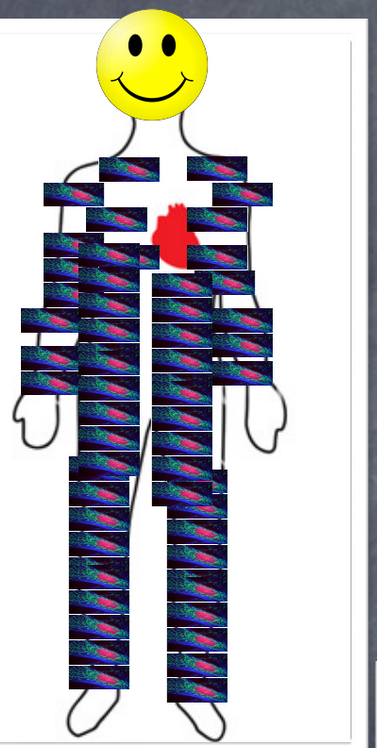
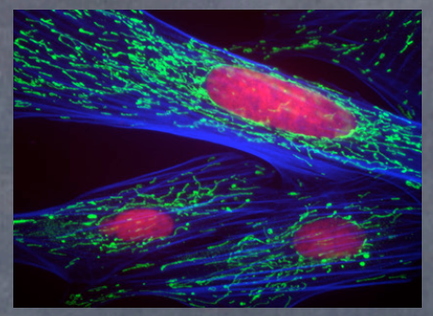
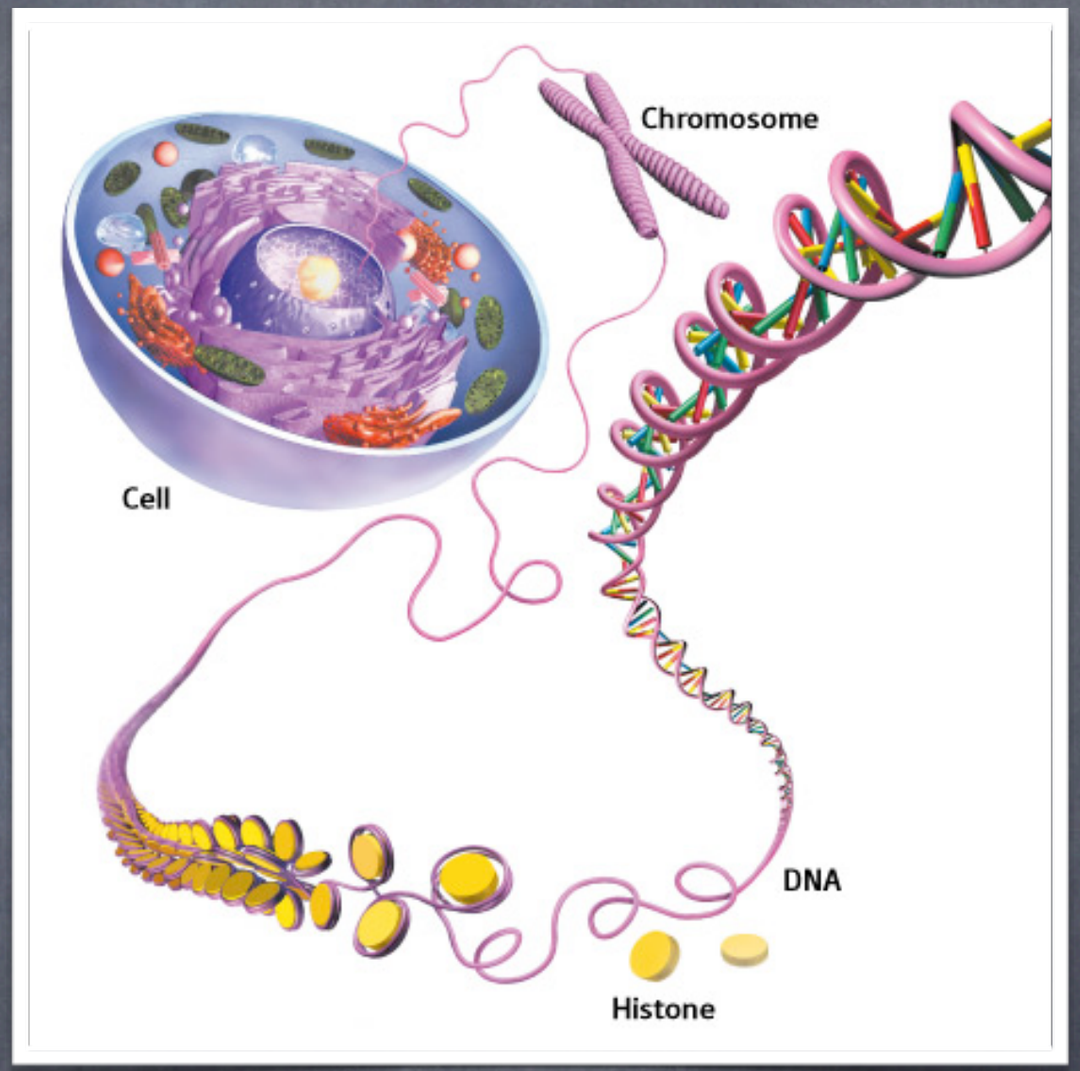
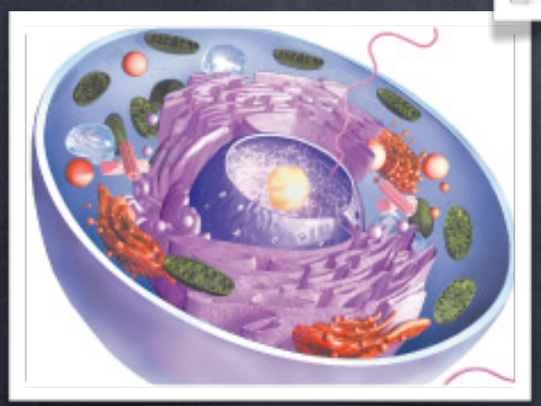


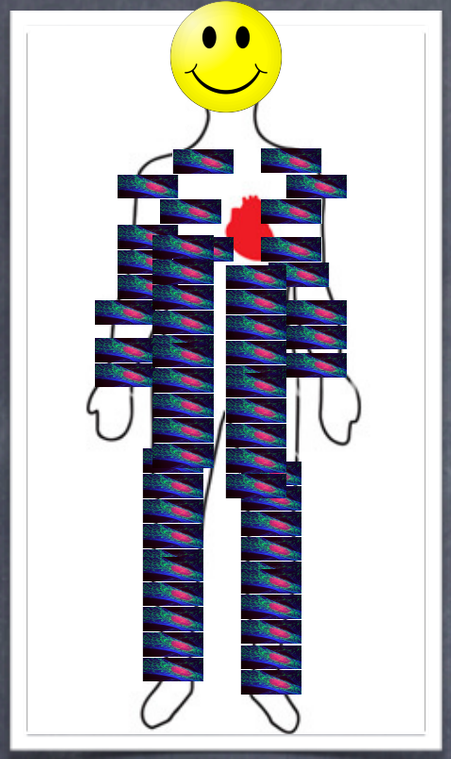


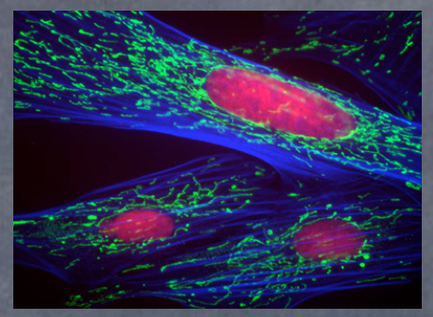


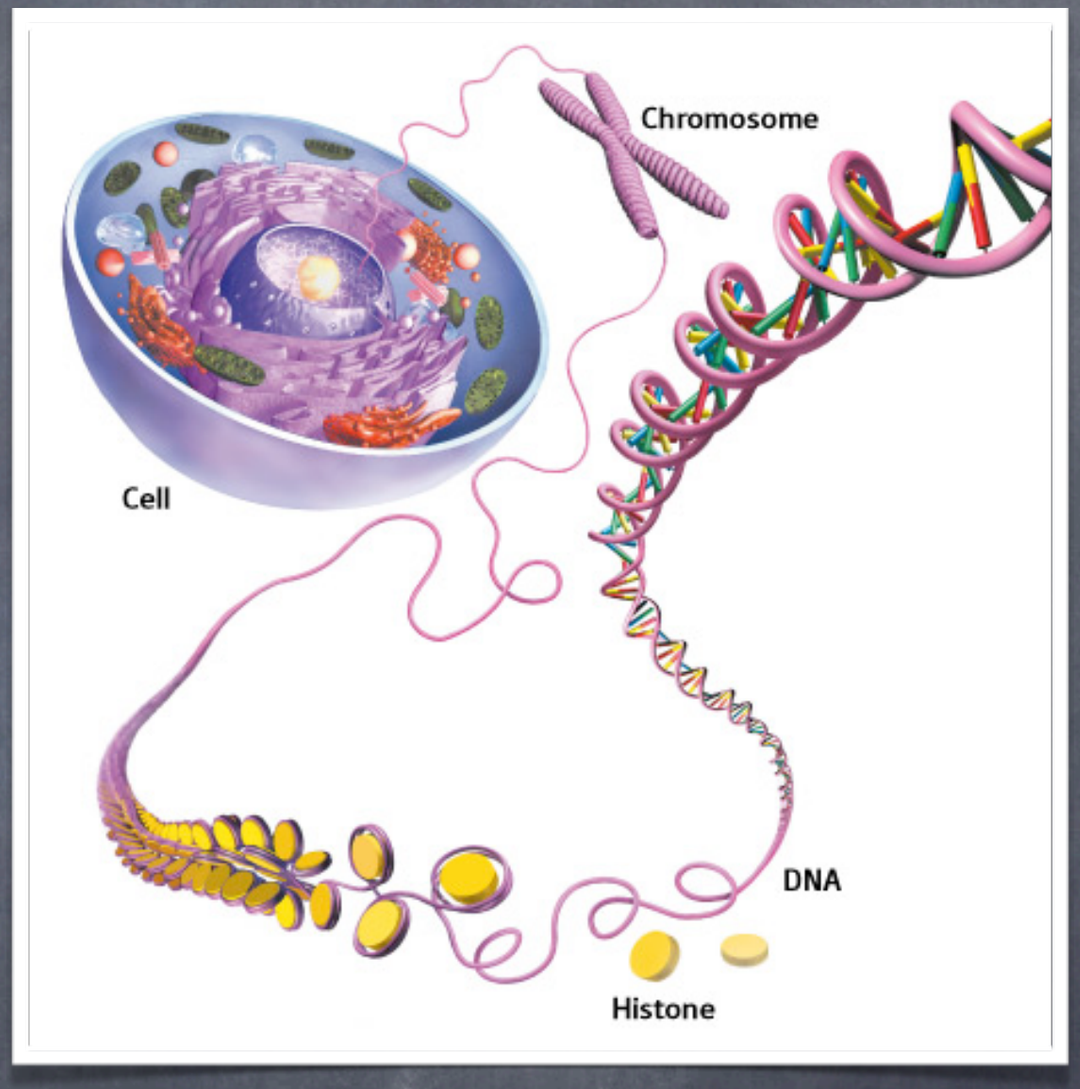


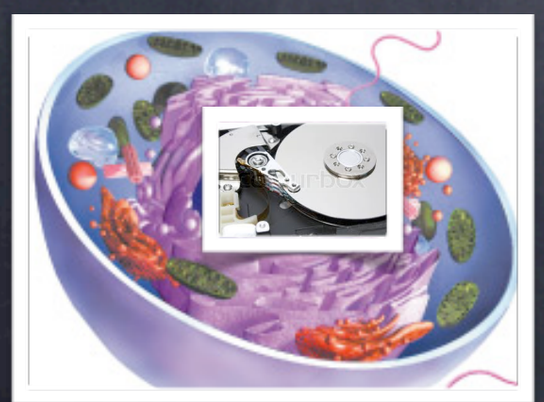
Riken Research, Invitrogen, Rodríguez-Gil, PNAS 2010

Cells and Parallel Computing



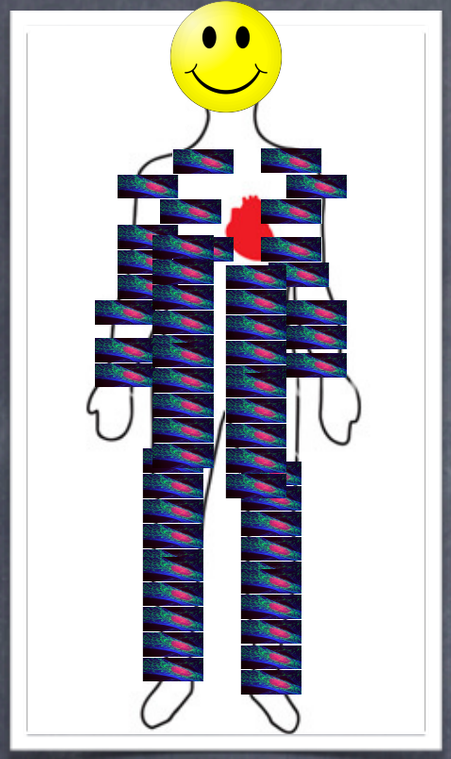


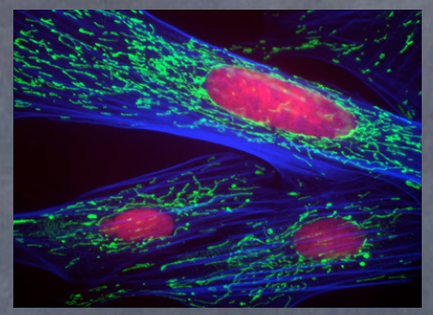


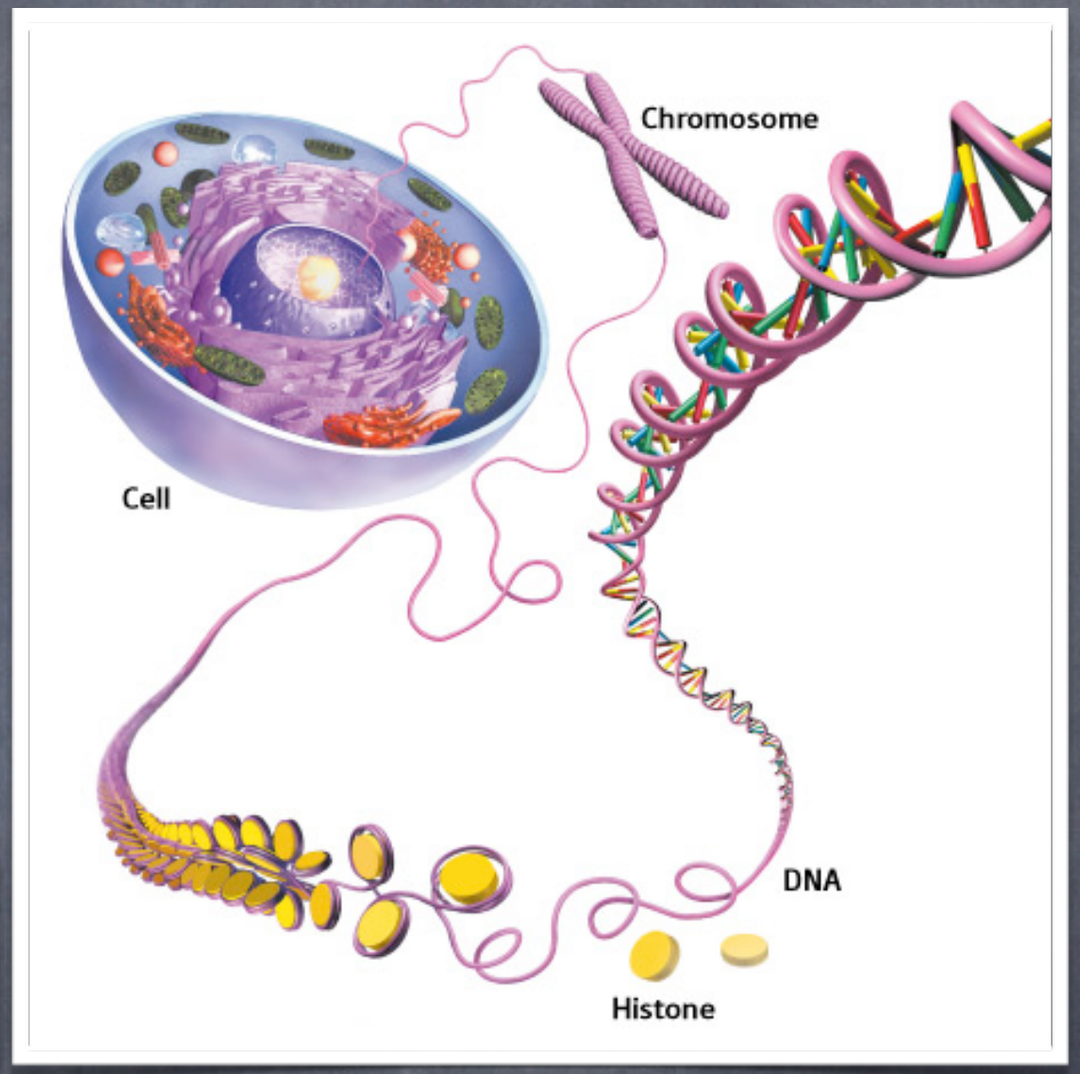


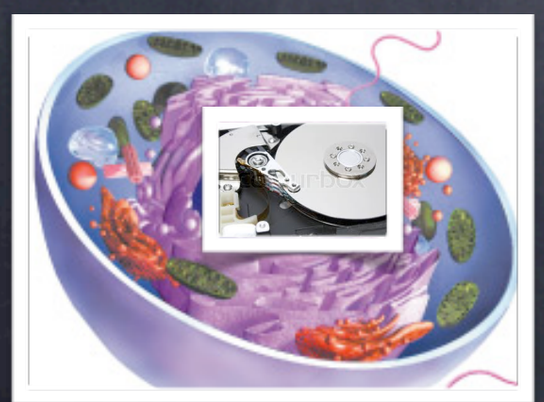
Riken Research, Invitrogen, Rodríguez-Gil, PNAS 2010

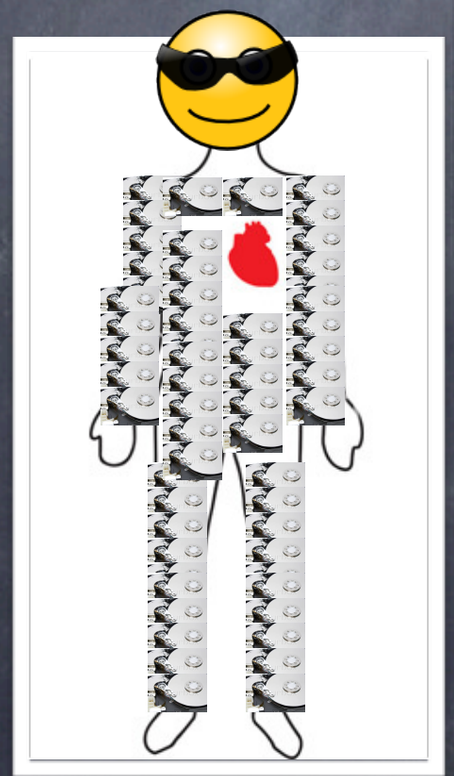
Cells and Parallel Computing











Riken Research, Invitrogen, Rodríguez-Gil, PNAS 2010

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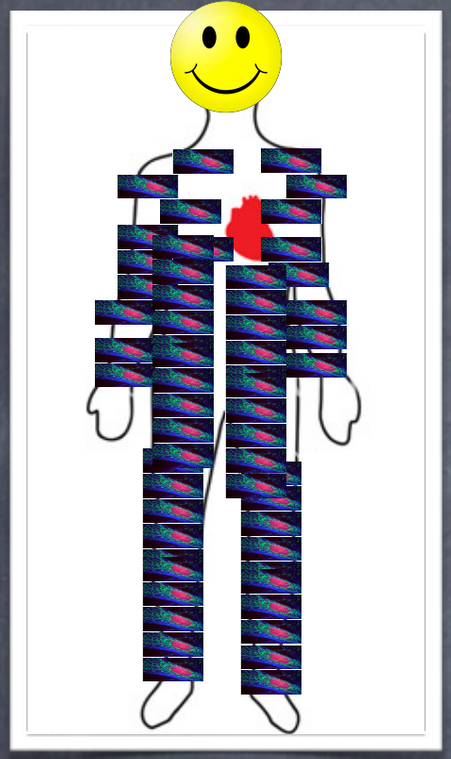

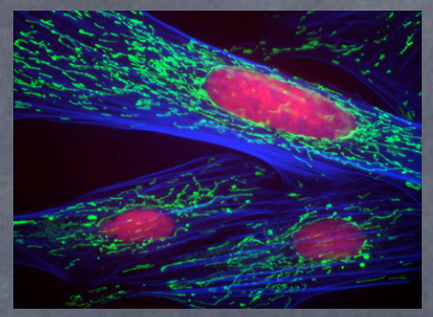

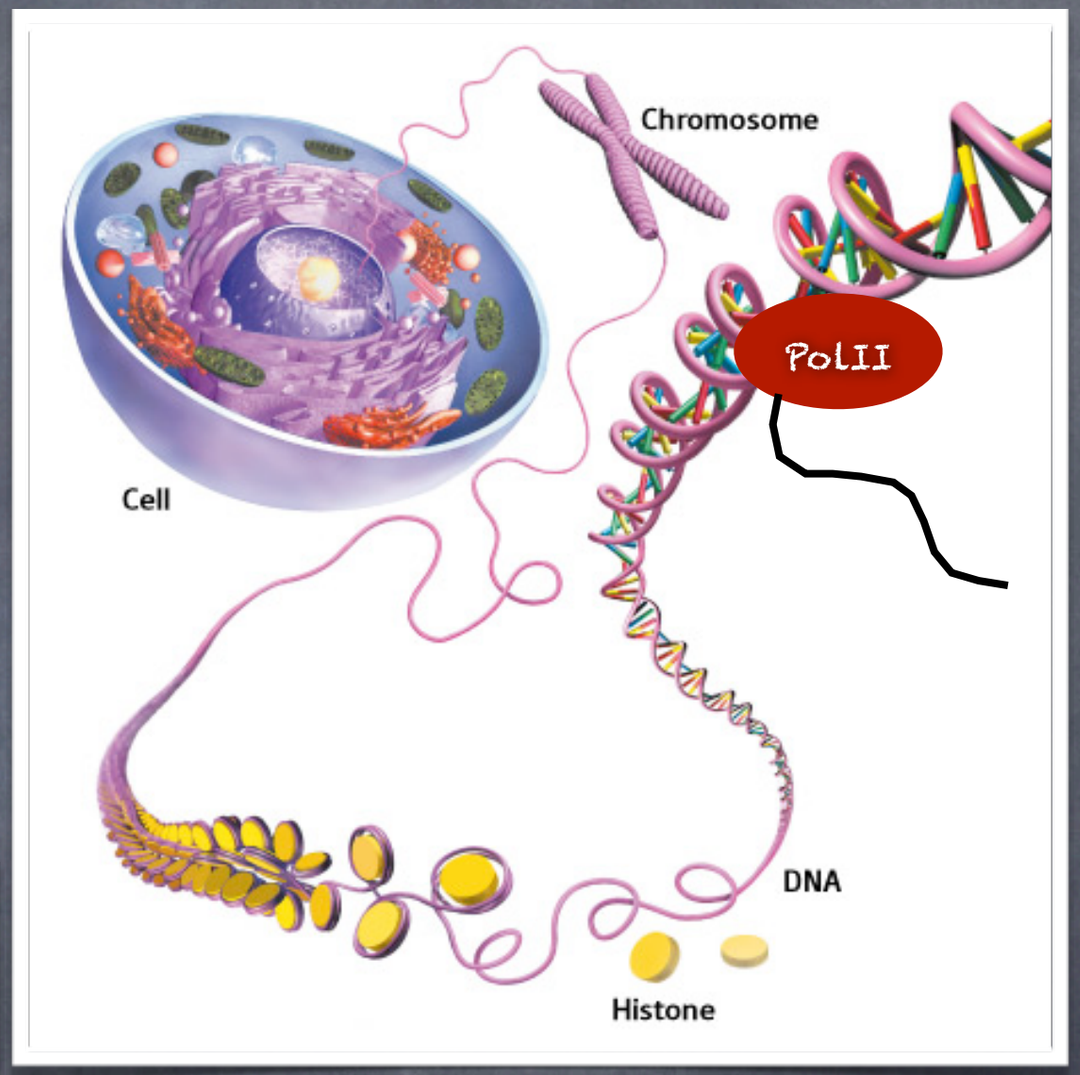

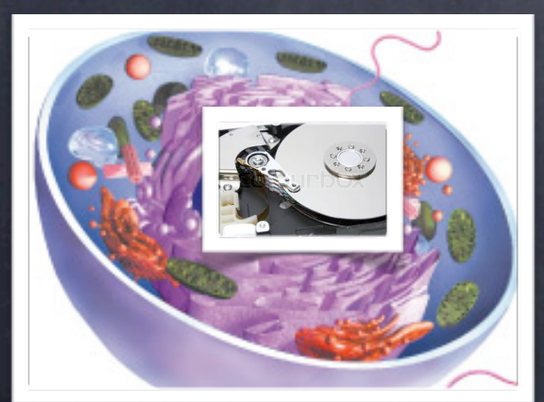

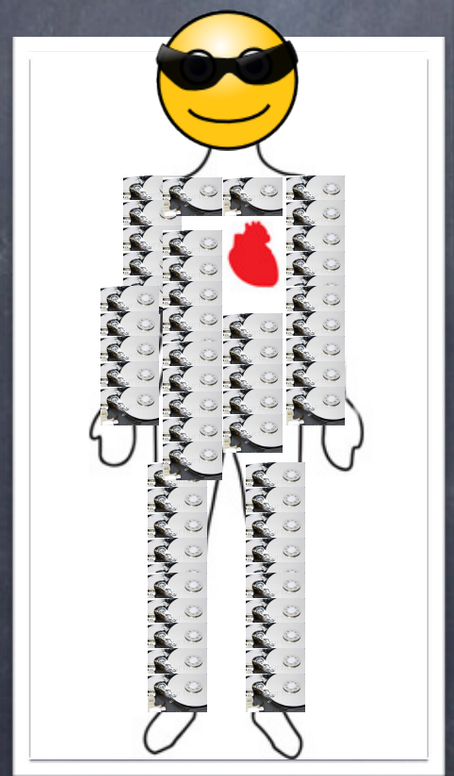

Riken Research, Invitrogen, Rodríguez-Gil, PNAS 2010

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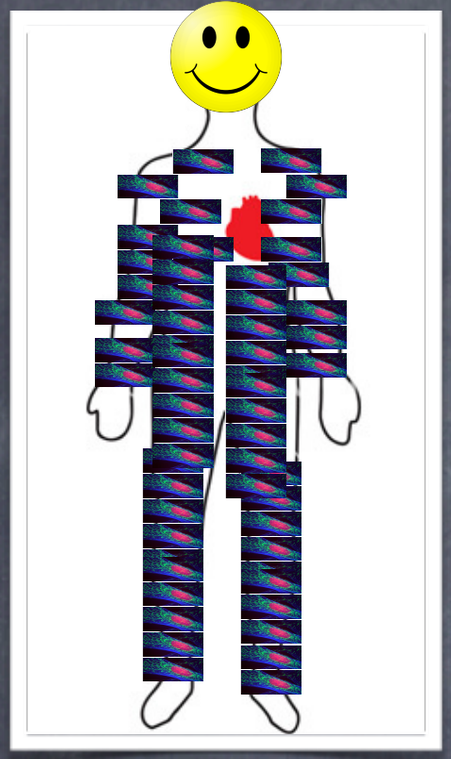
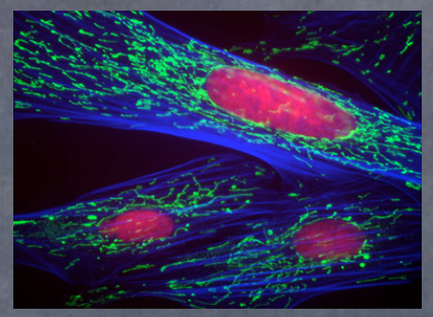
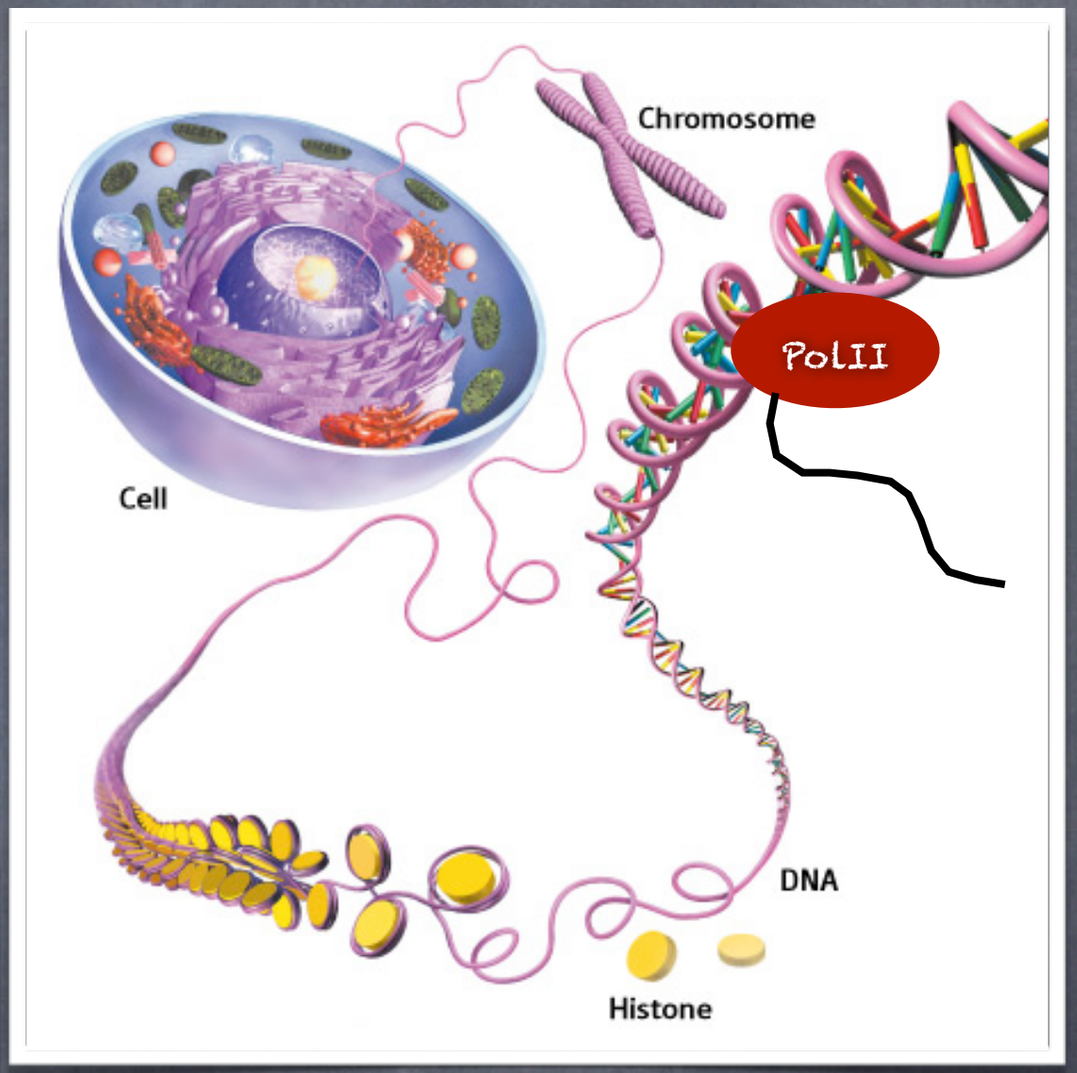
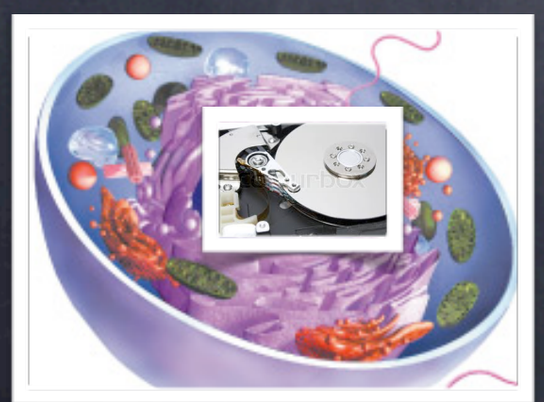
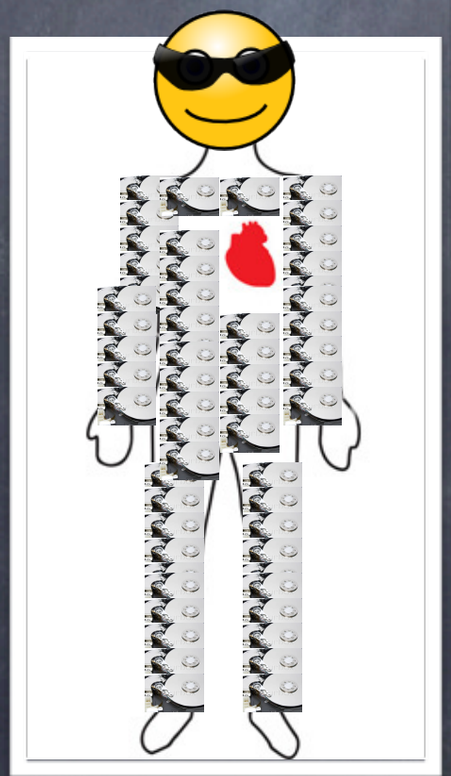








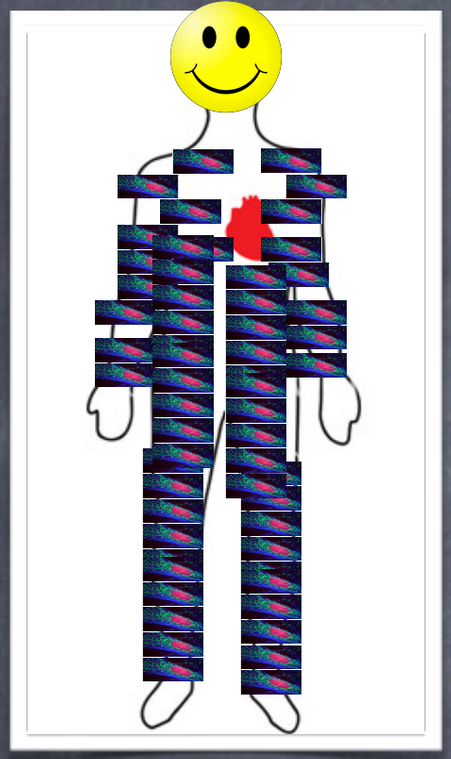


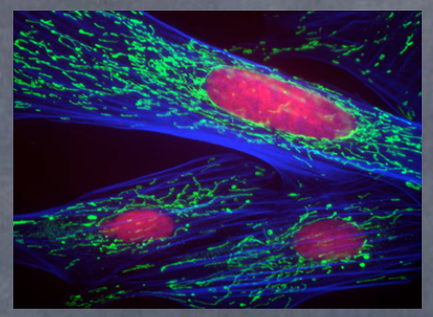
Sequoia (BlueGene/Q):

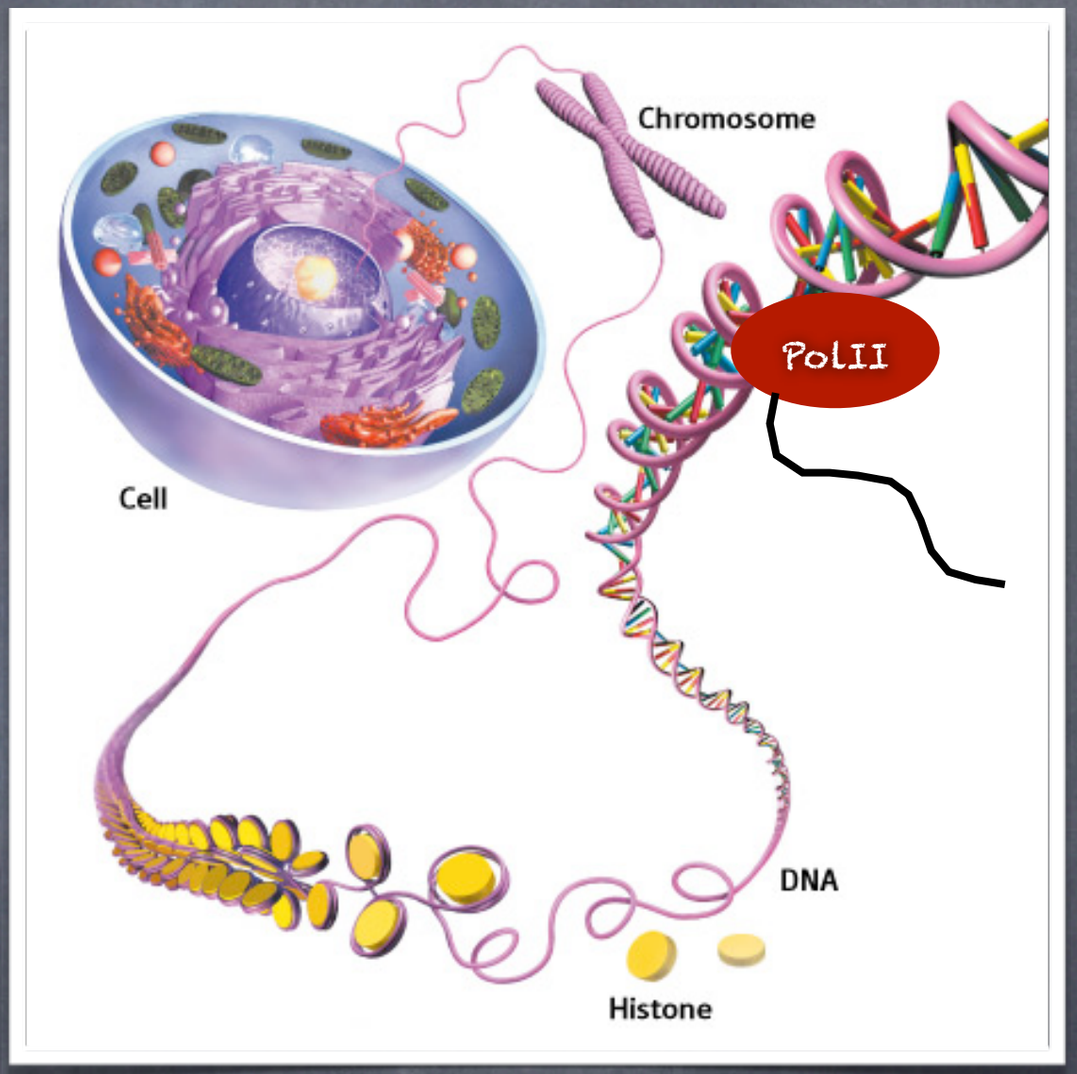
cores: 1,572,864

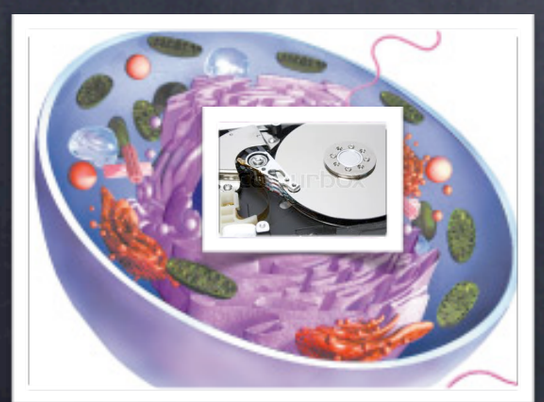
Riken Research, Invitrogen, Rodríguez-Gil, PNAS 2010

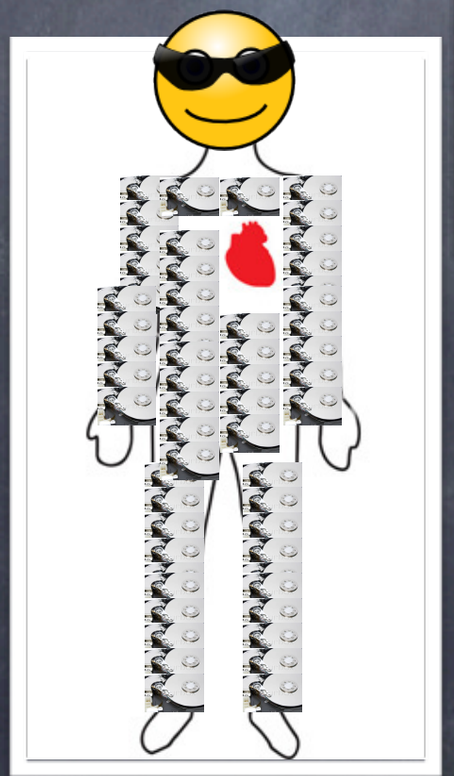
Cells and Parallel Computing











Sequoia (BlueGene/Q):

cores: 1,572,864

Human body: 10-100 trillion cells

18,000 active Polymerase II molecules / cell

Riken Research, Invitrogen, Rodríguez-Gil, PNAS 2010

Central Dogma in molecular biology:

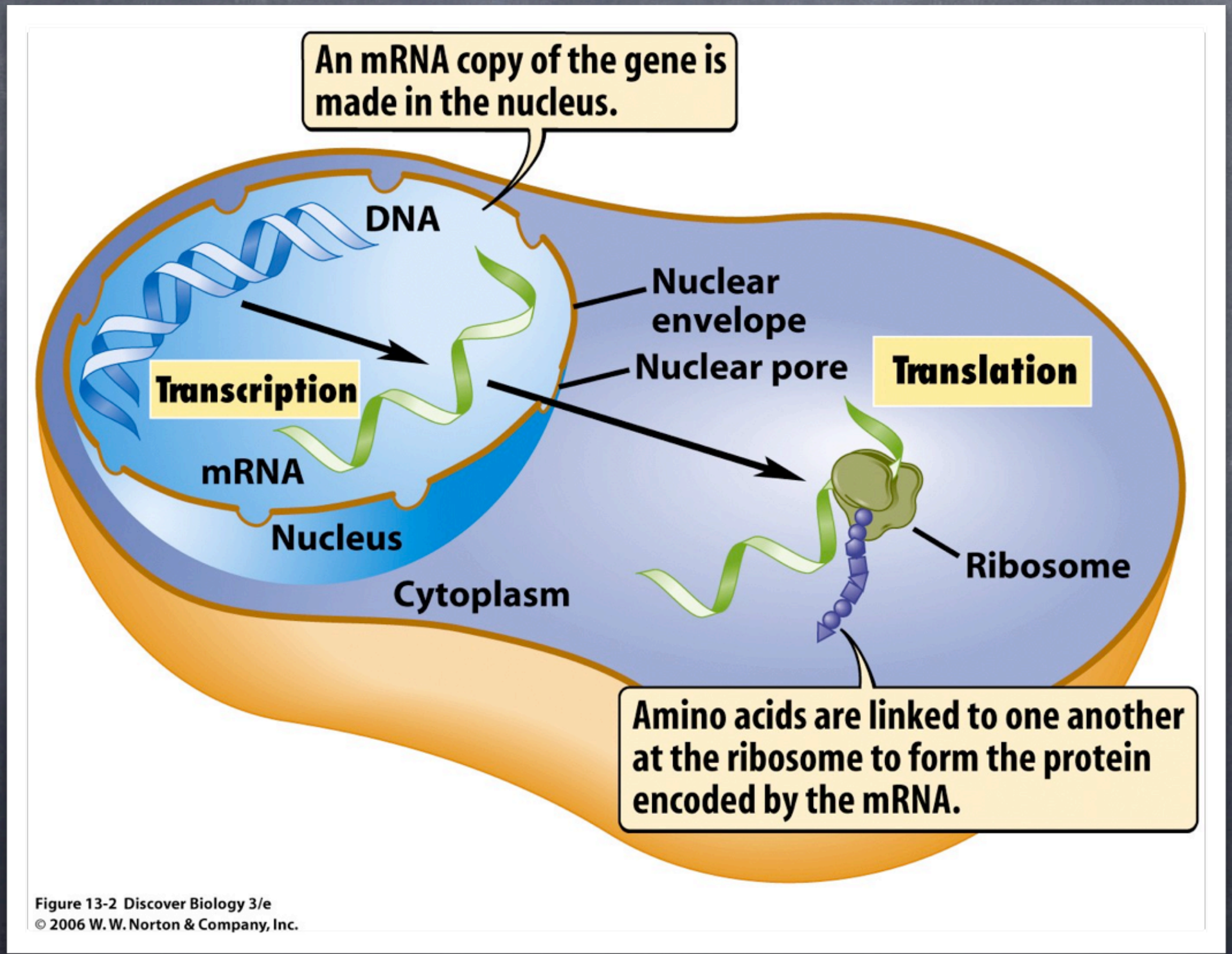
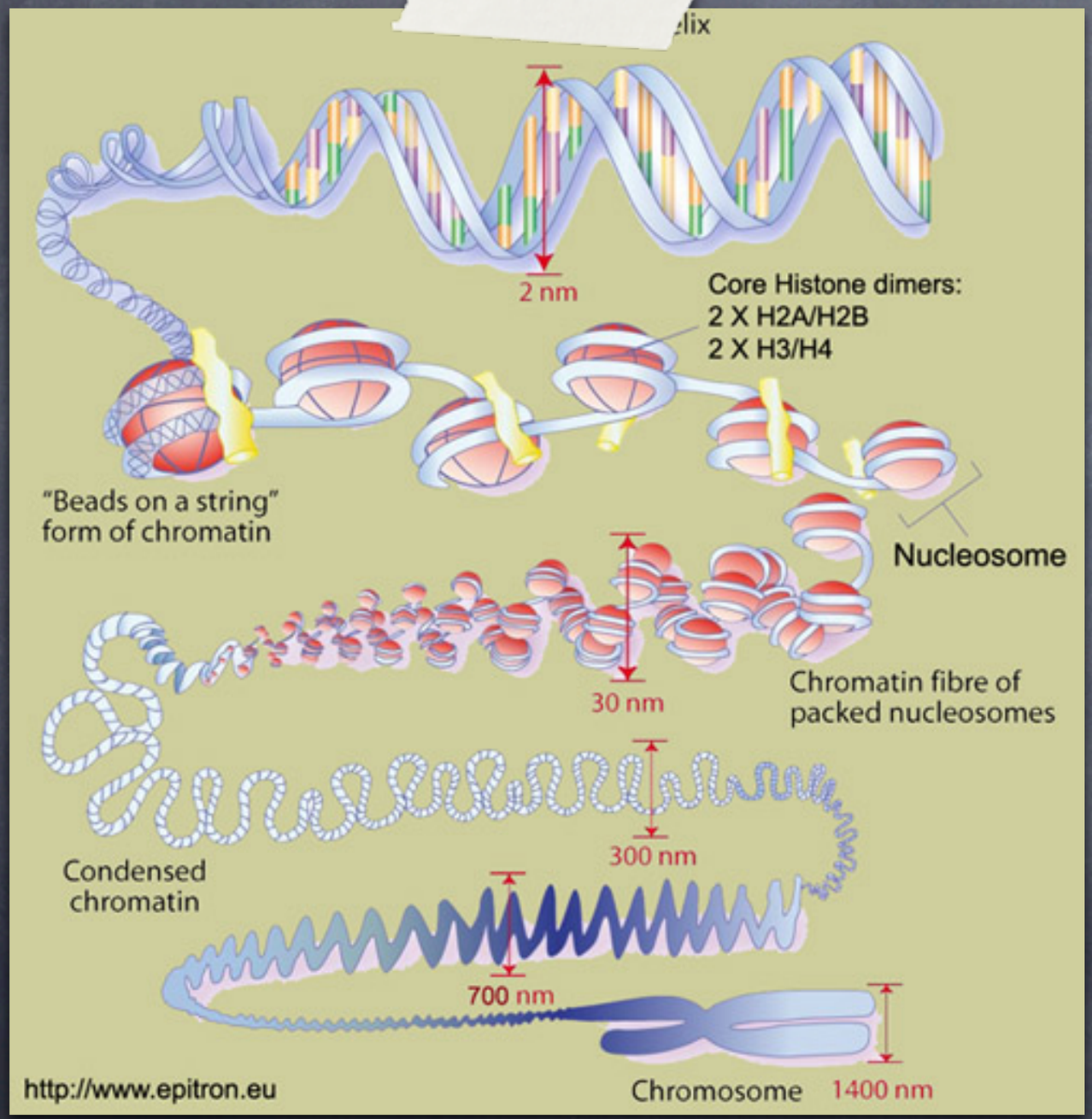
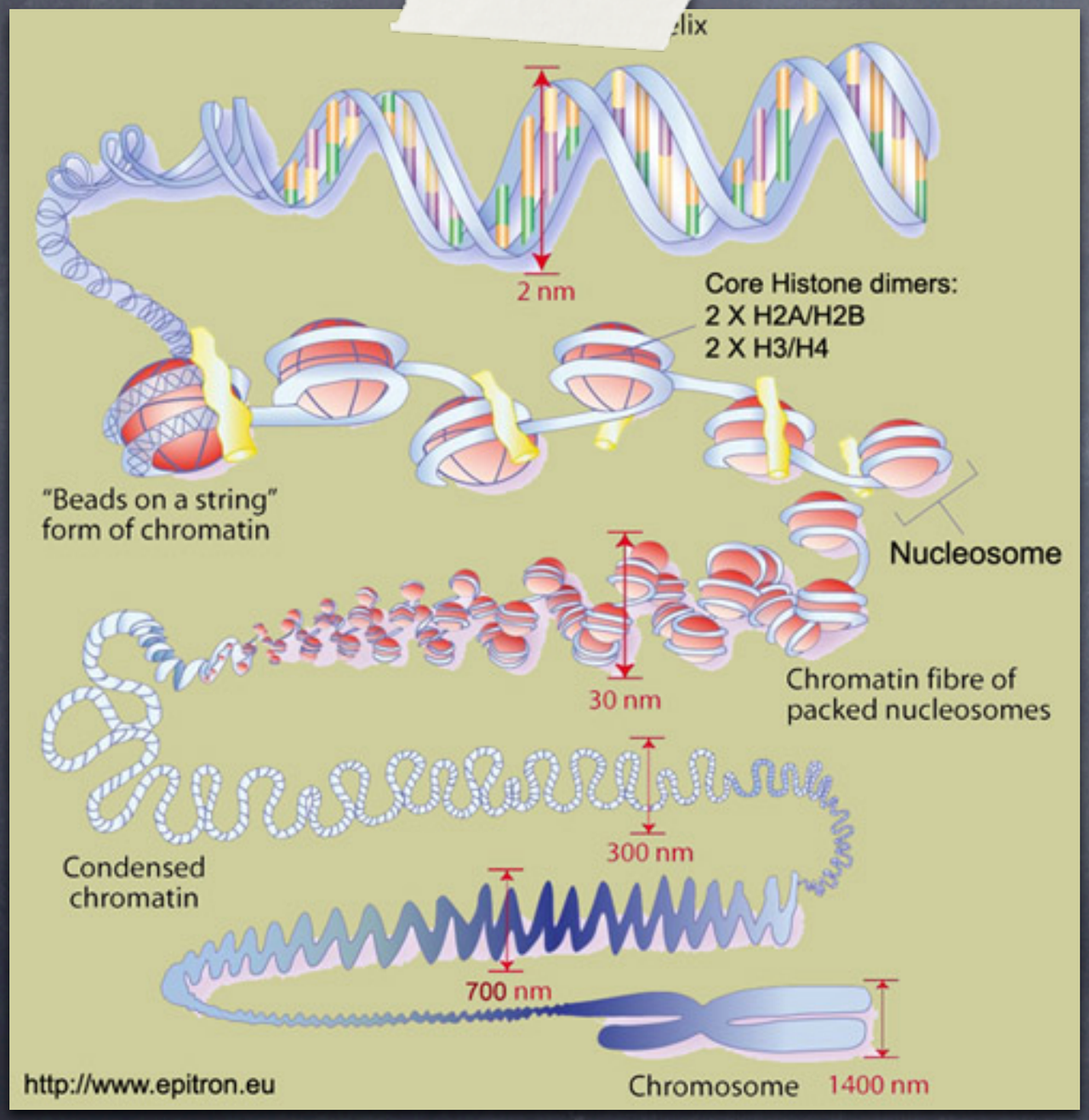


Figure 13-2 Discover Biology 3/e

Chromatin packaging in the nucleus



Chromatin packaging in the nucleus



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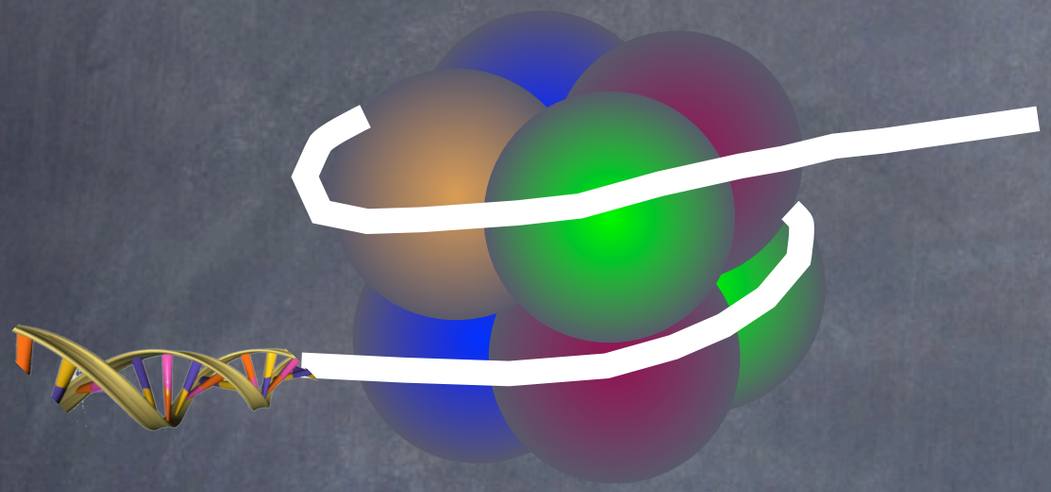
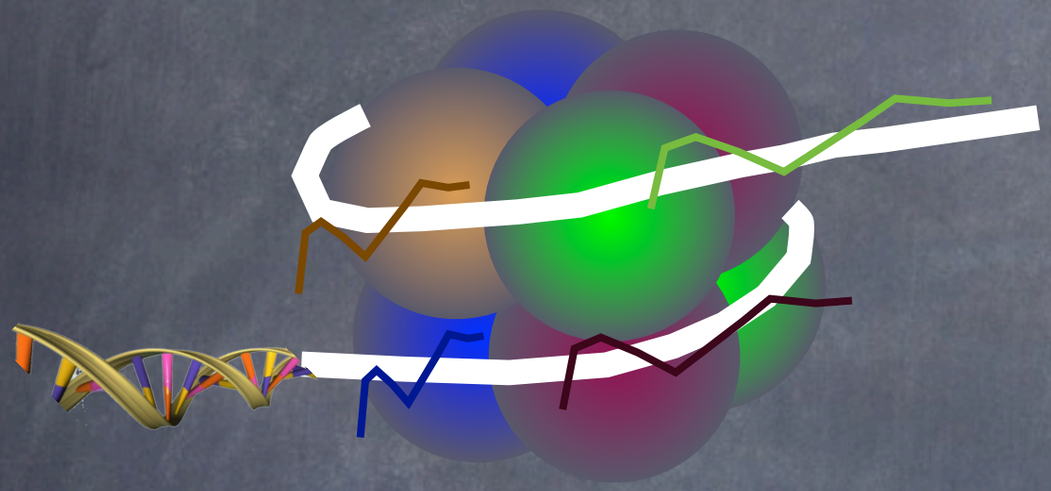
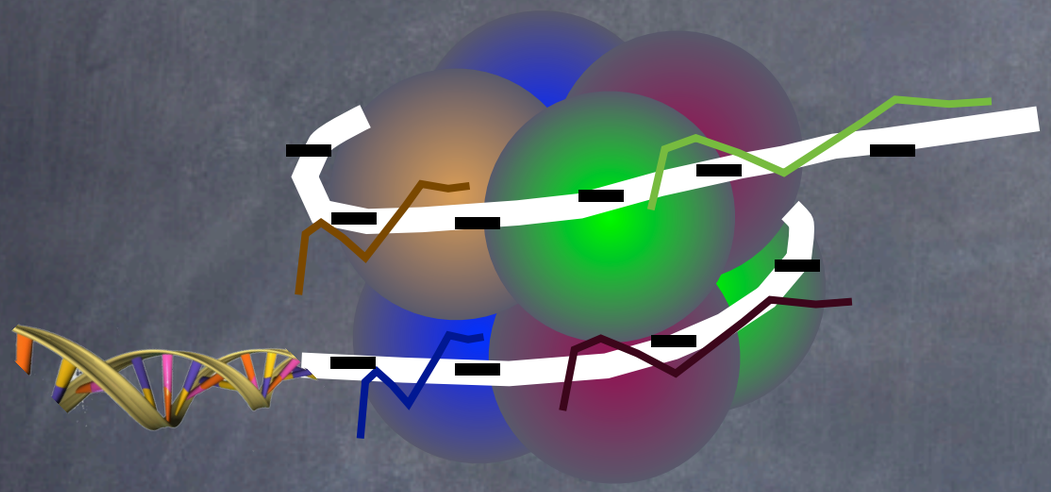
DNA is packaging, accessibility and readability



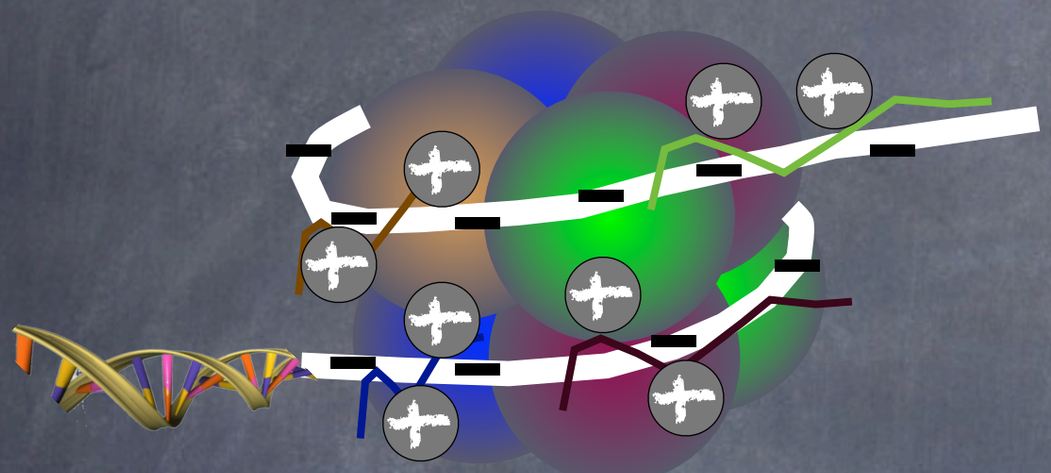
DNA is packaging, accessibility and readability



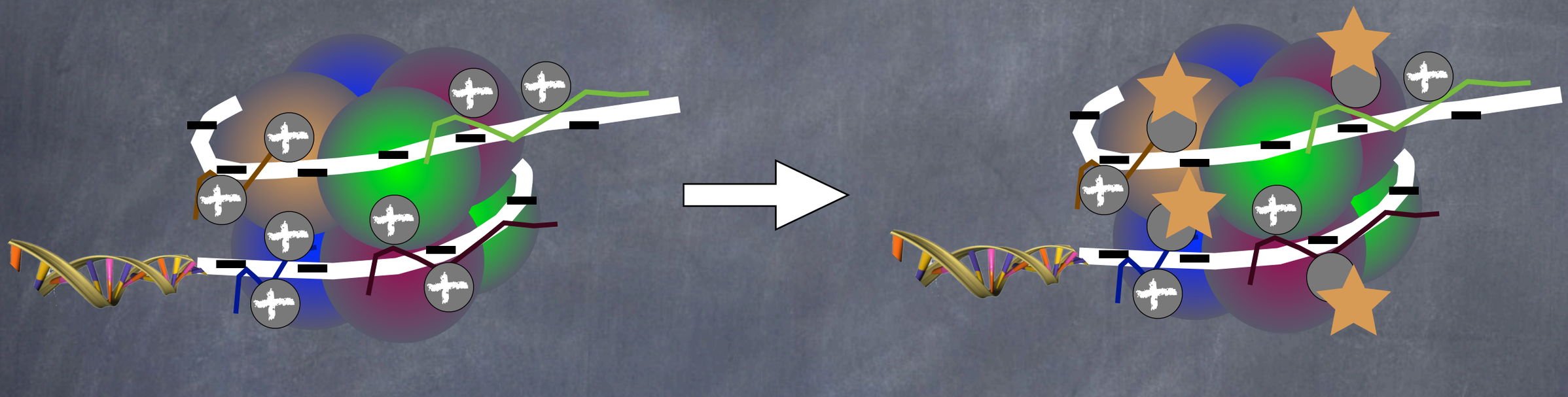
DNA is packaging, accessibility and readability



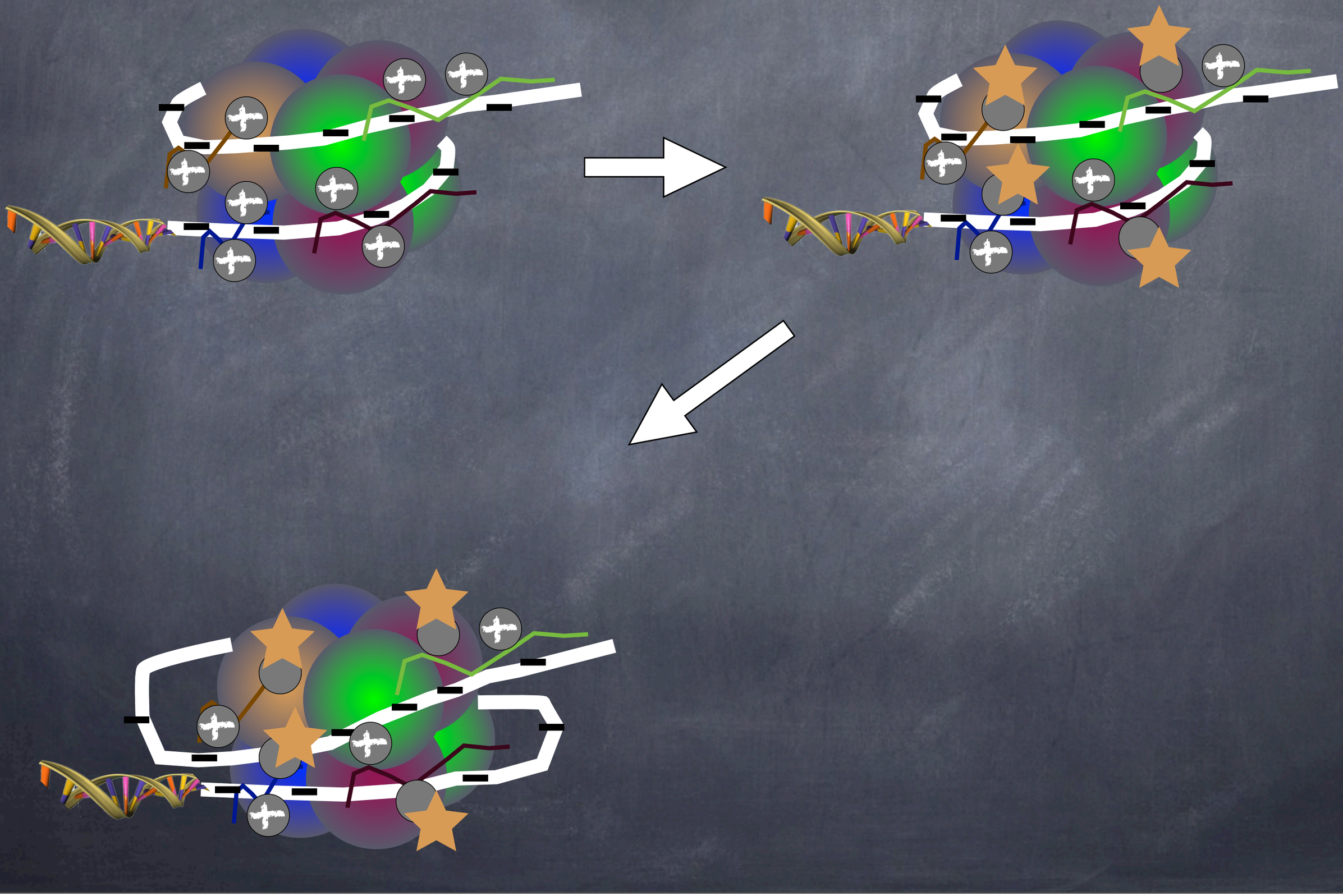
DNA is packaging, accessibility and readability



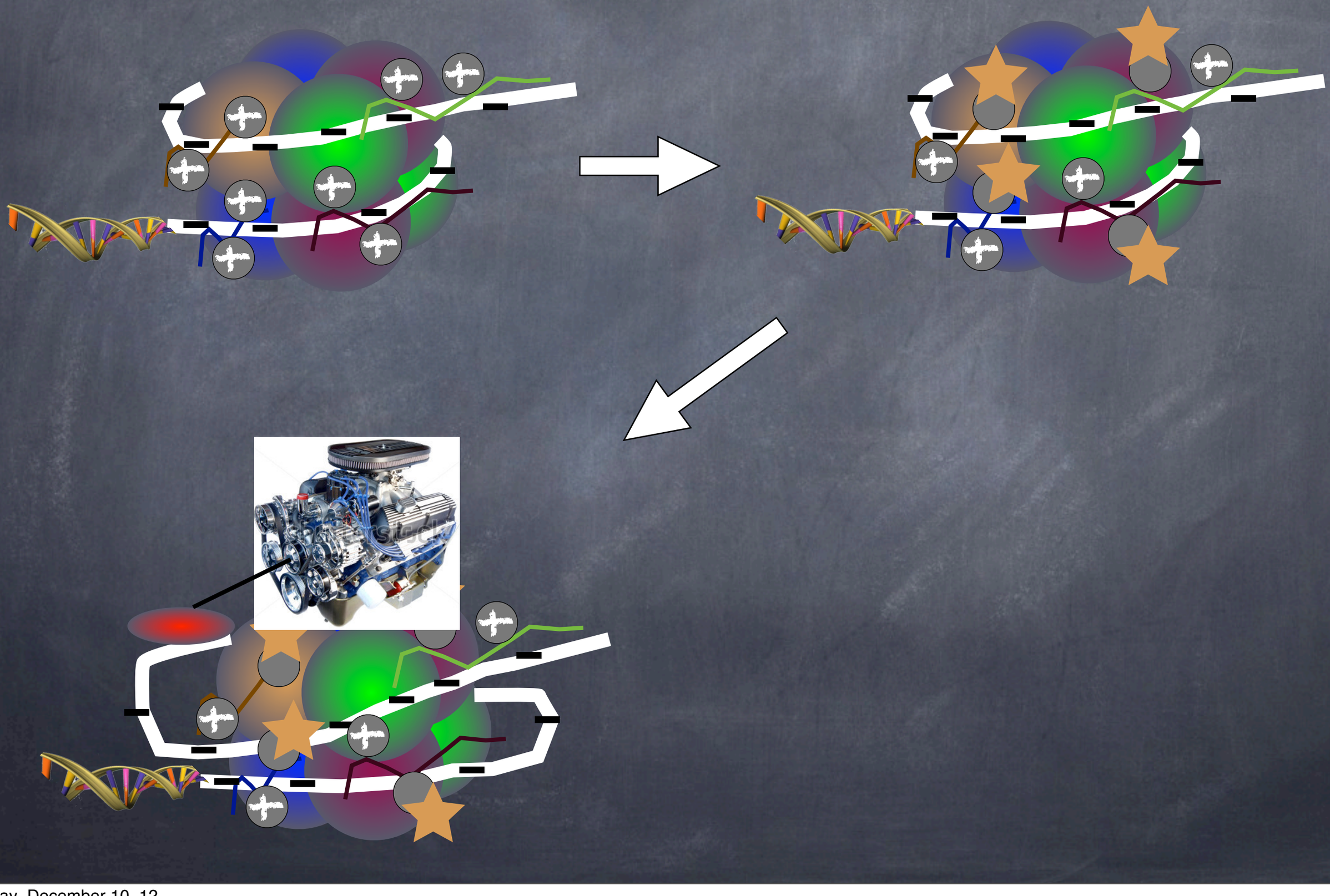
DNA is packaging, accessibility and readability



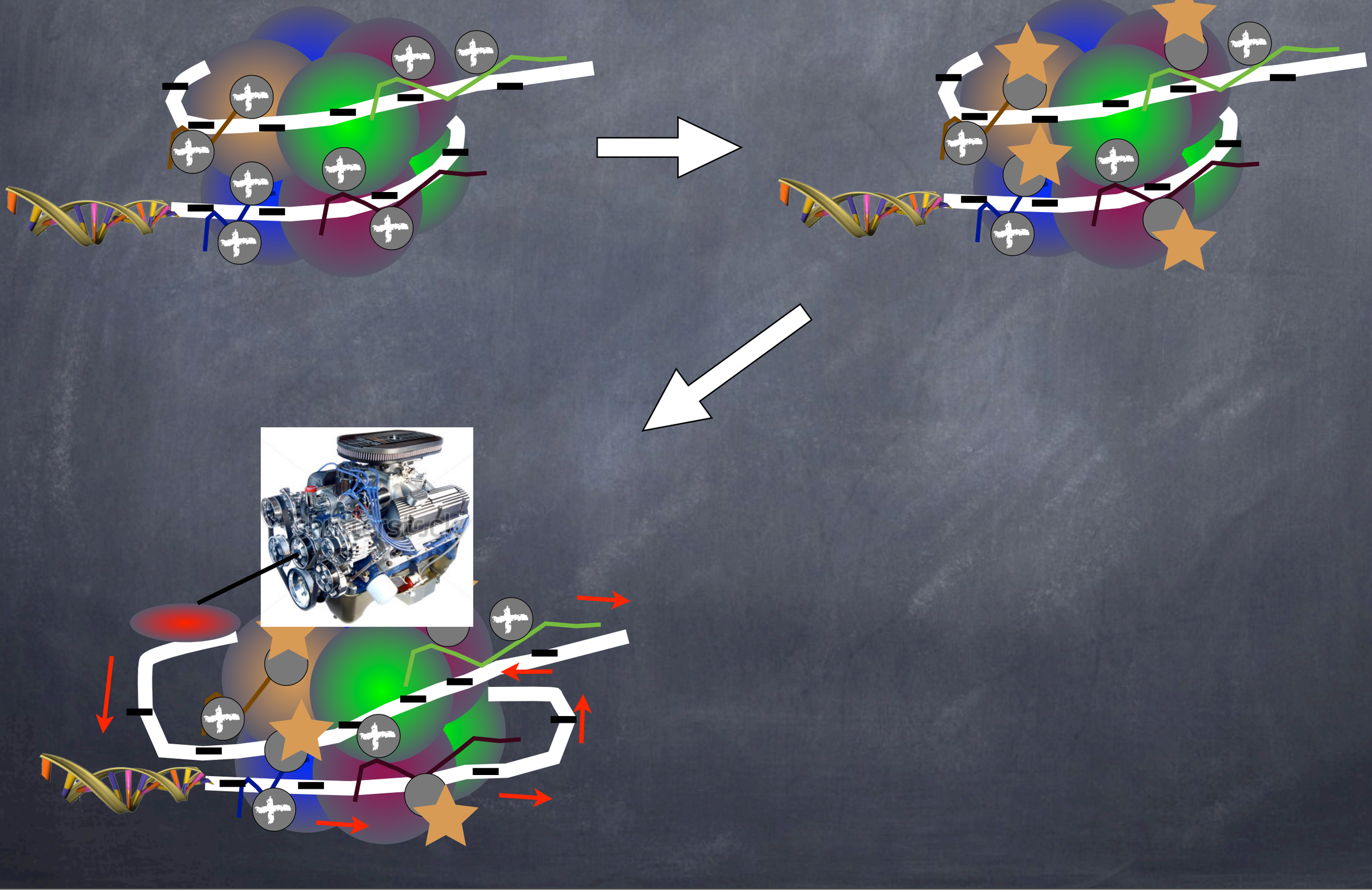
DNA is packaging, accessibility and readability



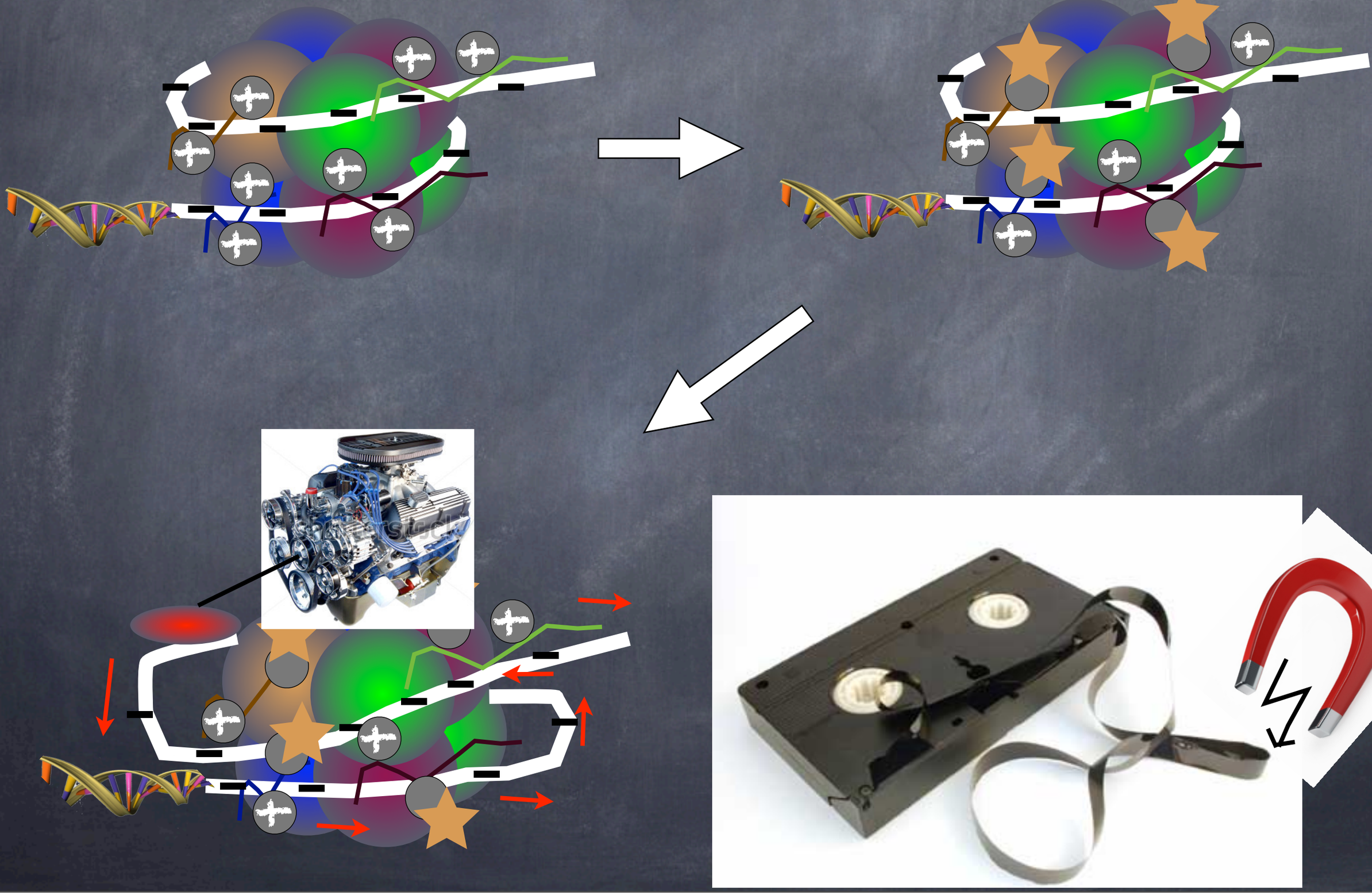
DNA is packaging, accessibility and readability



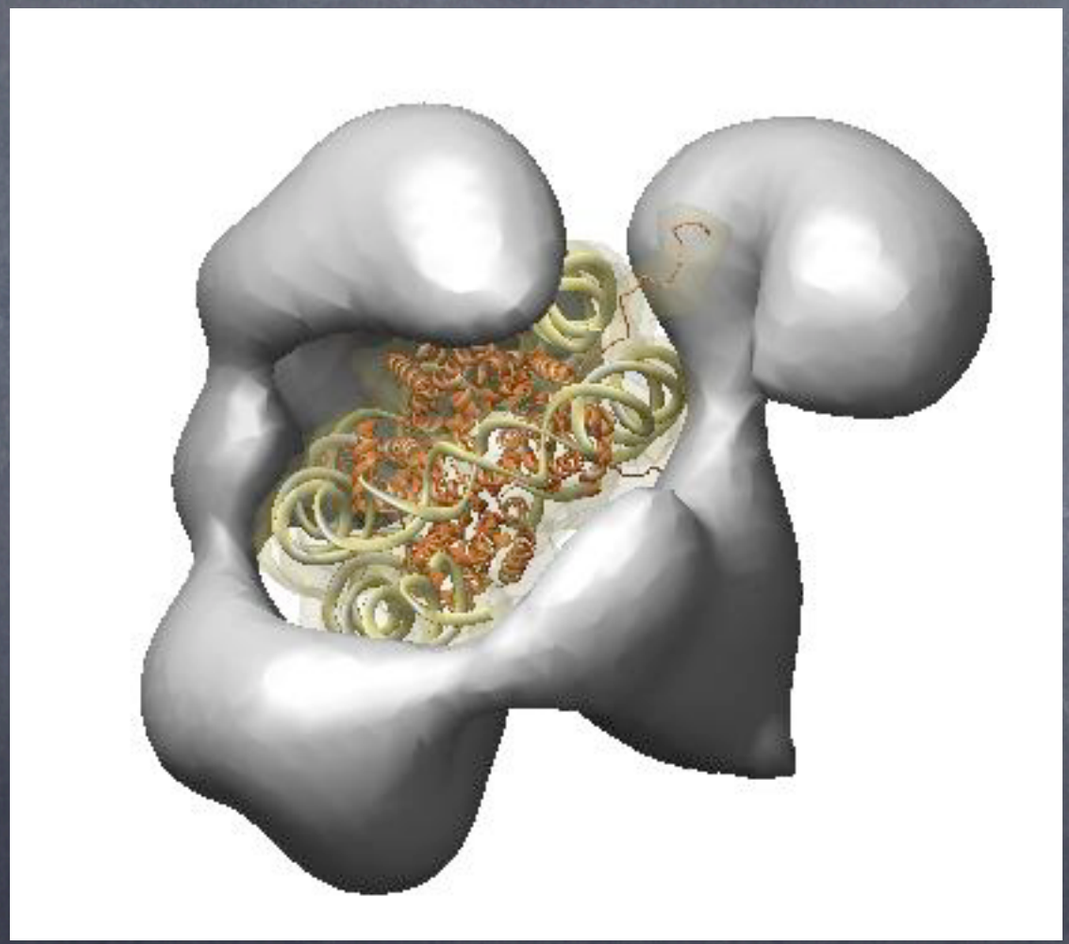
DNA is packaging, accessibility and readability



DNA is packaging, accessibility and readability

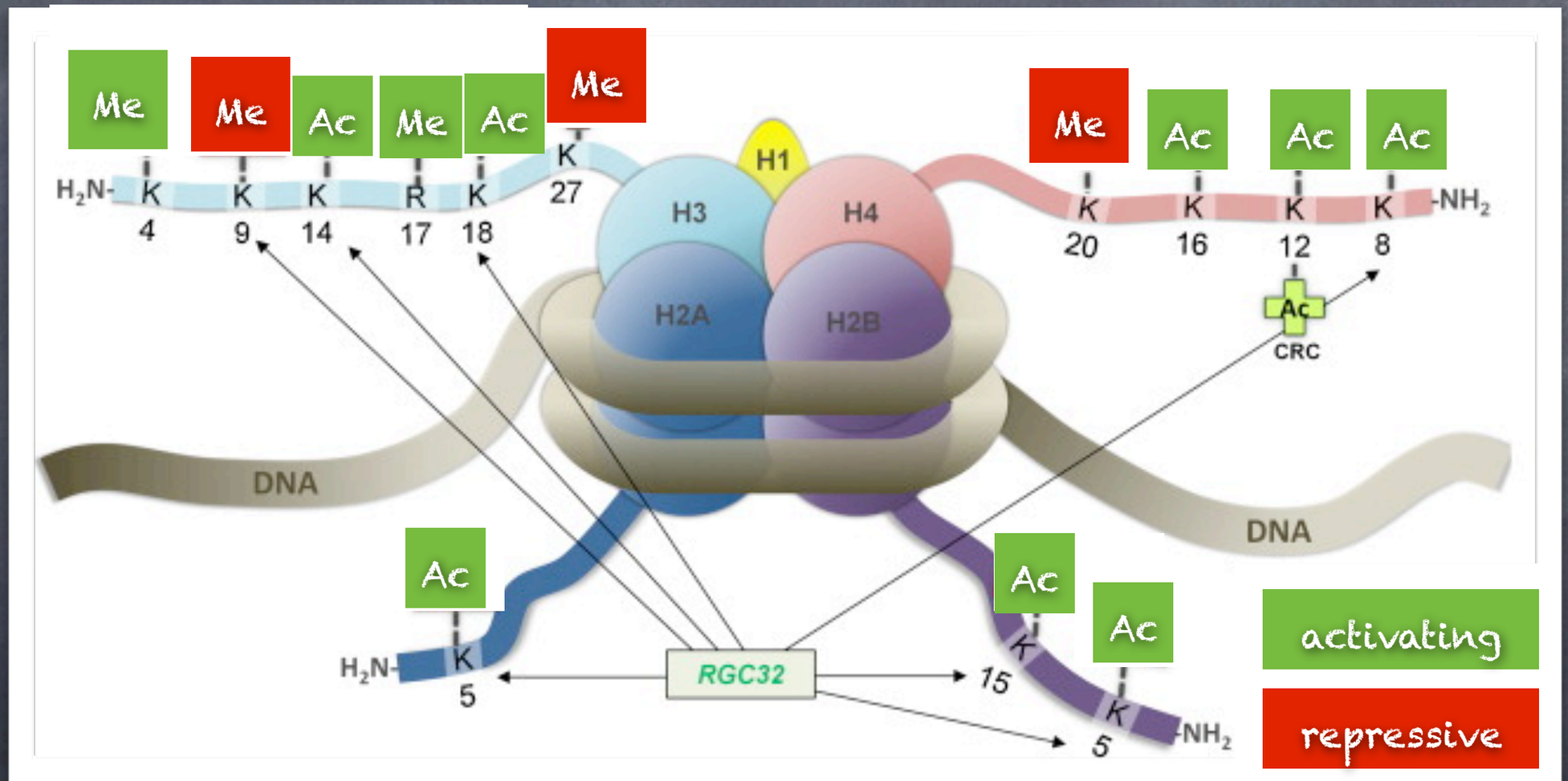


DNA remodeler RSC binding to nucleosome - modeled



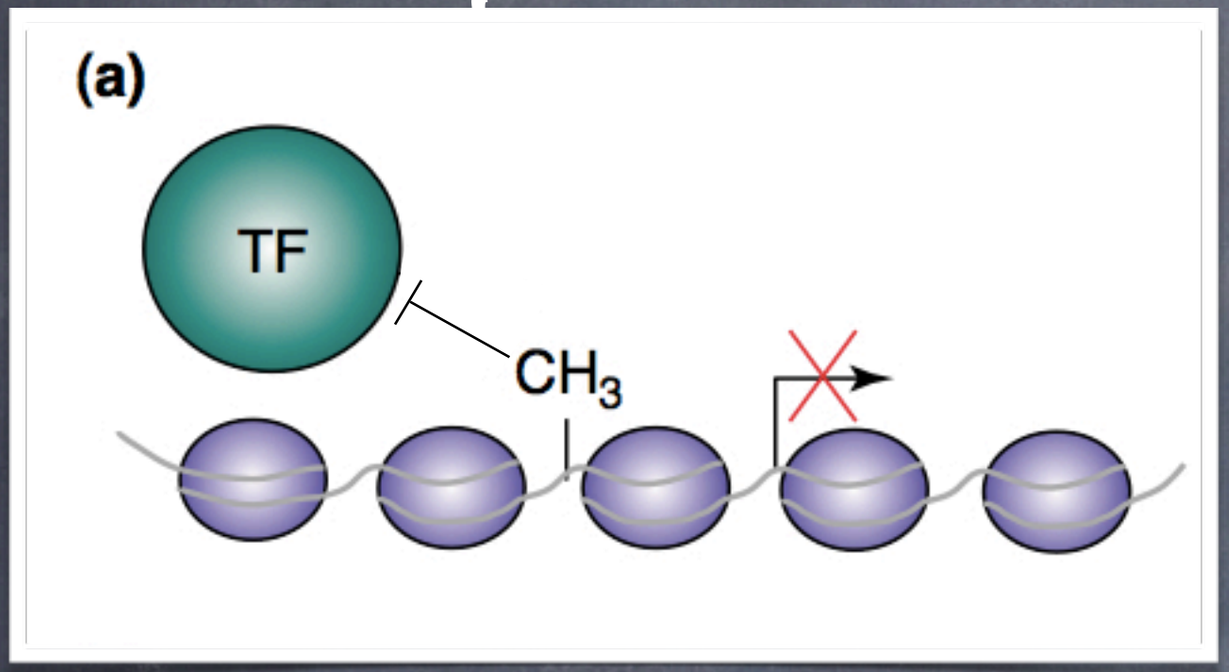
CairnsLab

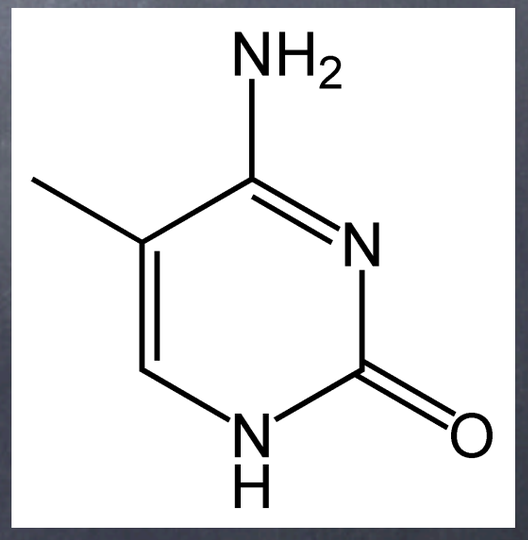
Active and repressive epigenetic marks at the nucleosome mediate transcriptional control



Gargalonis, INCBC 2012

5-methyl-cytosine (5mC) acts as a repressive chromatin mark





5-methylcytosine

Klose and Bird,Trends Biochem Sci (2006)

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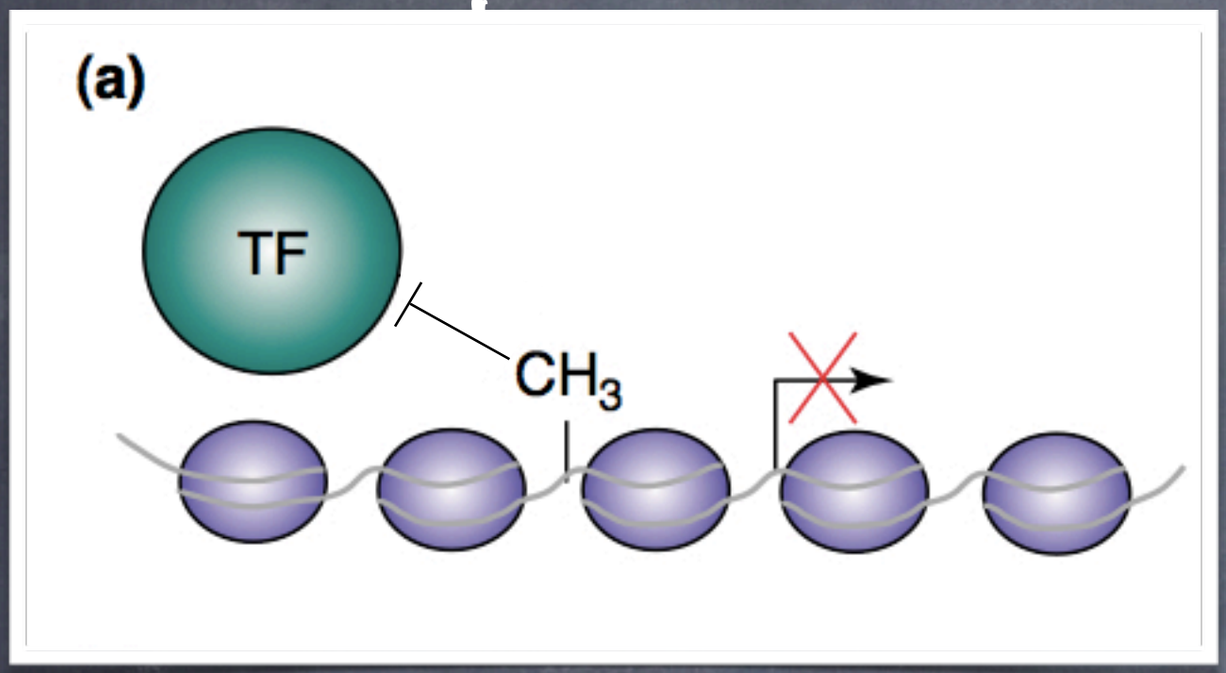
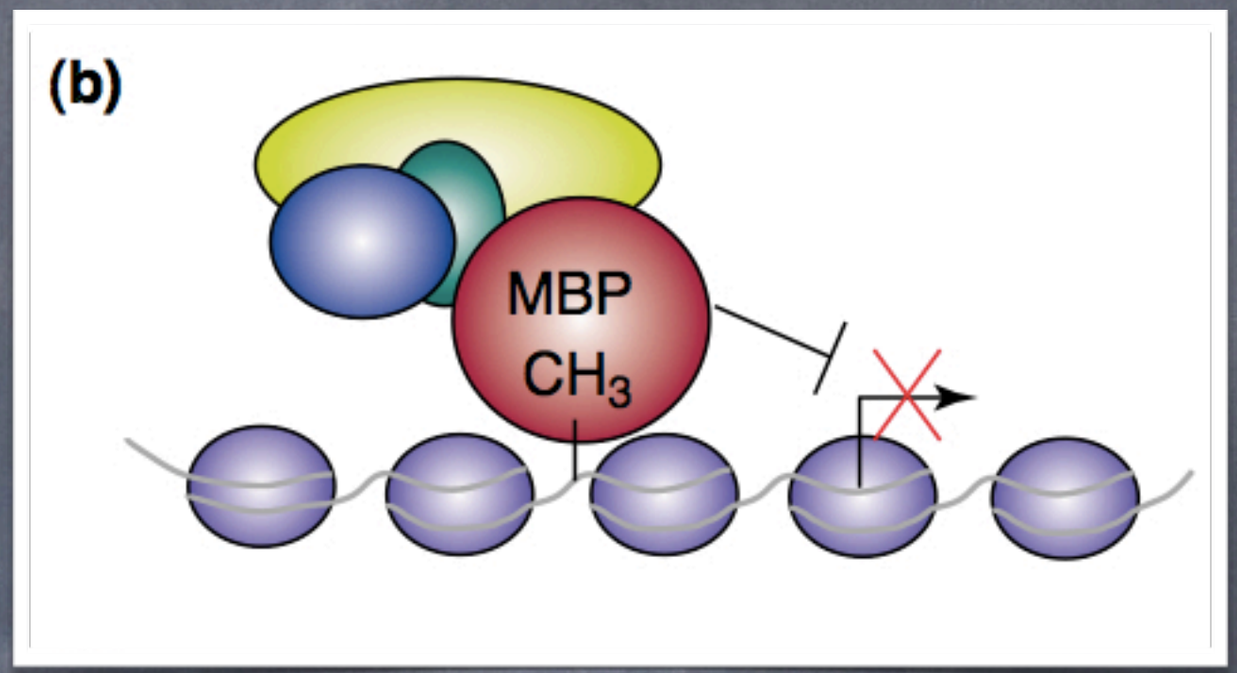
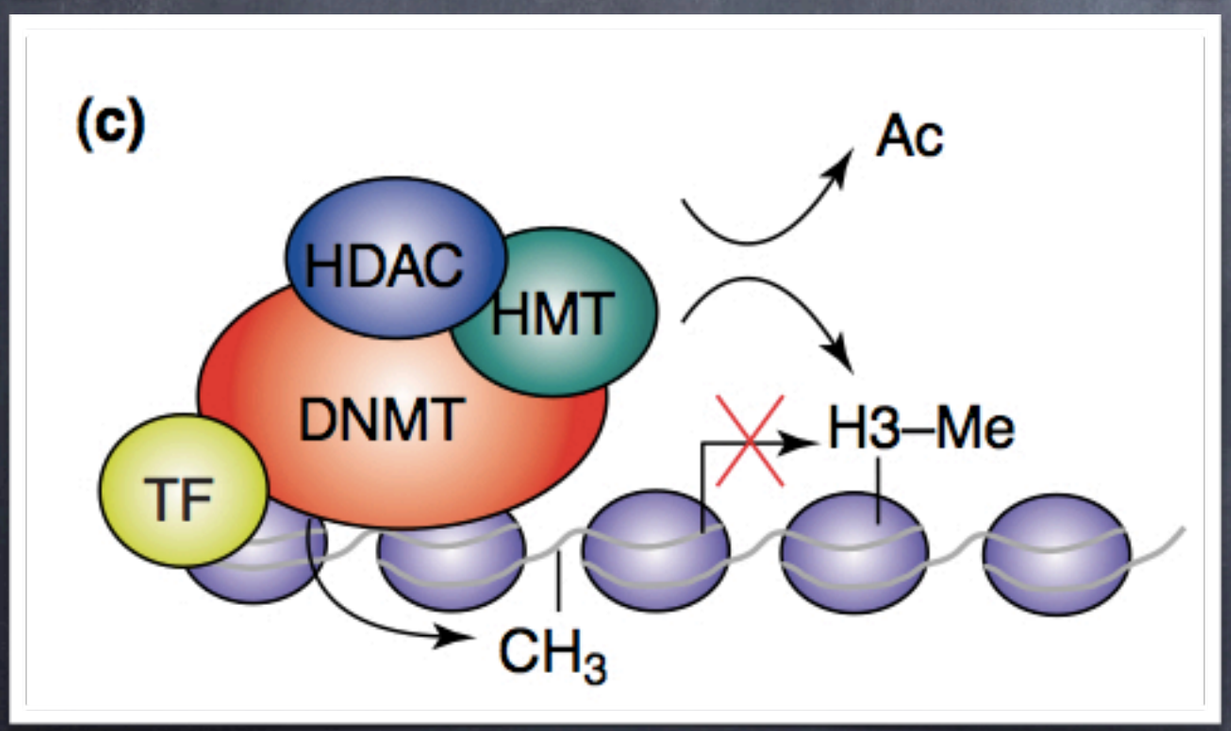
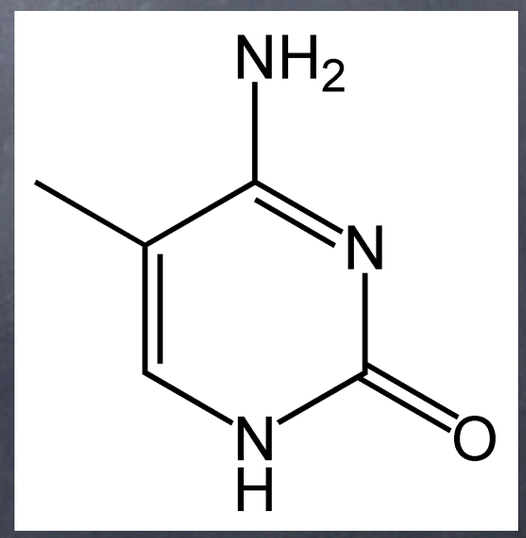




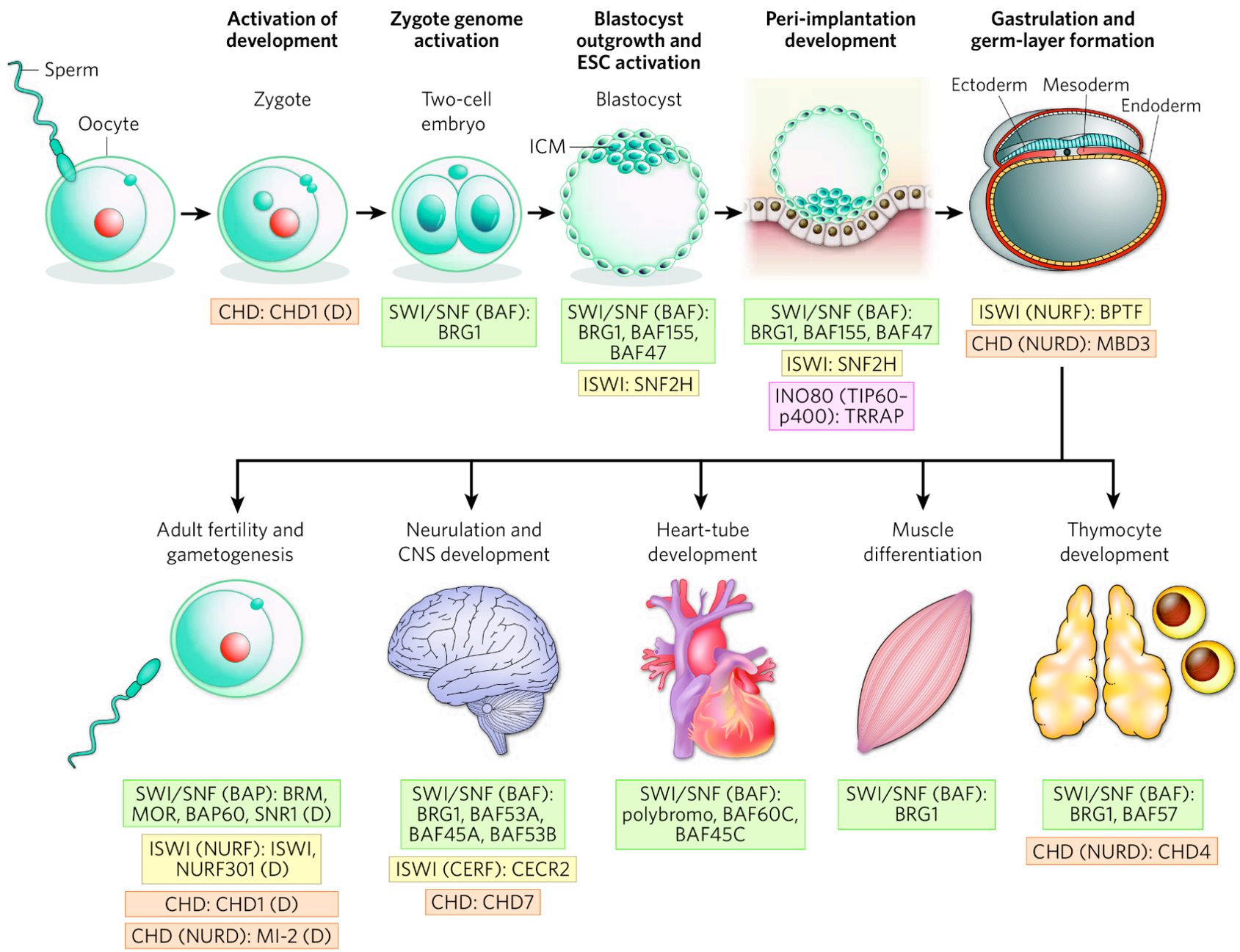




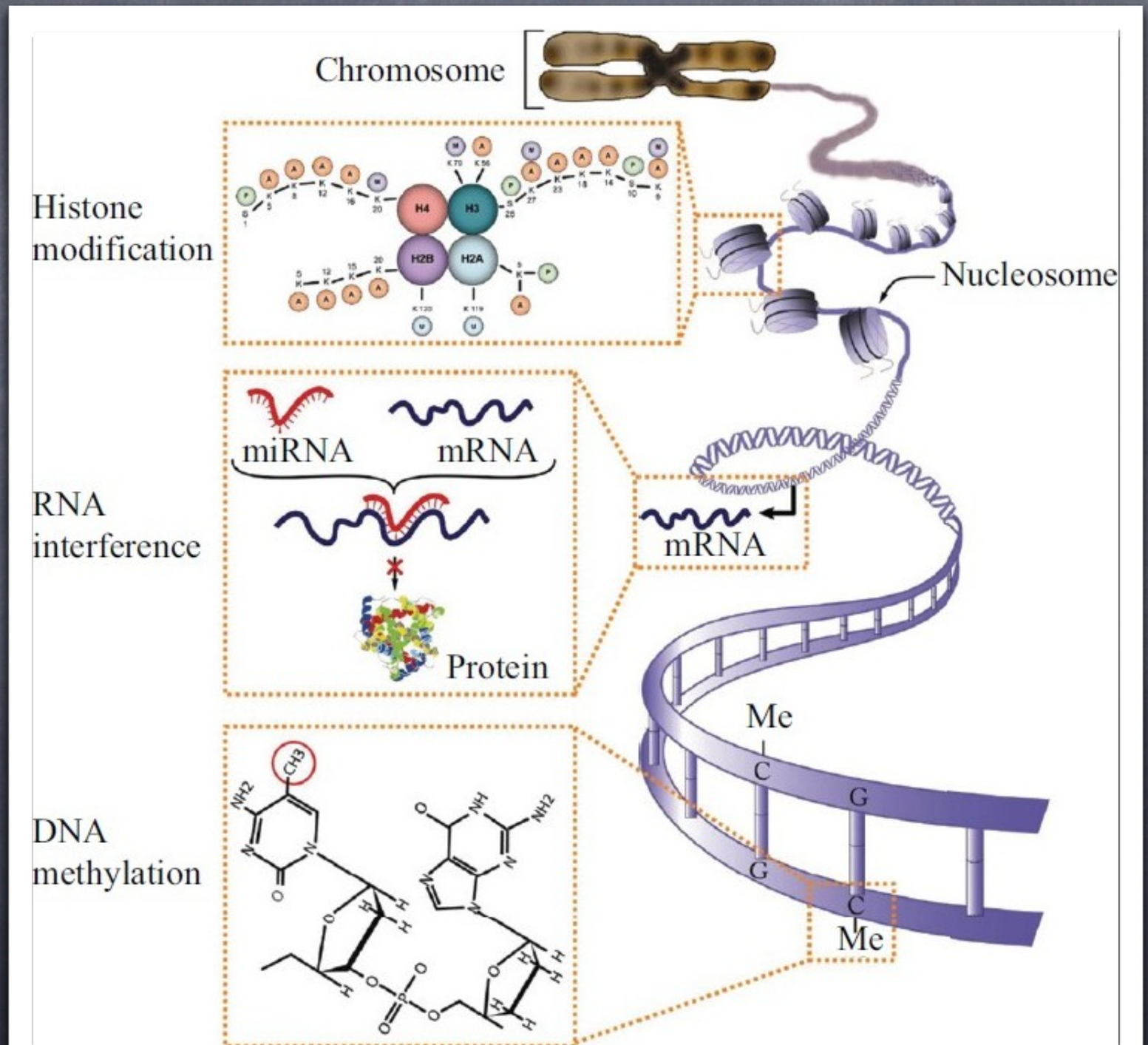
5-methylcytosine

Klose and Bird,Trends Biochem Sci (2006)

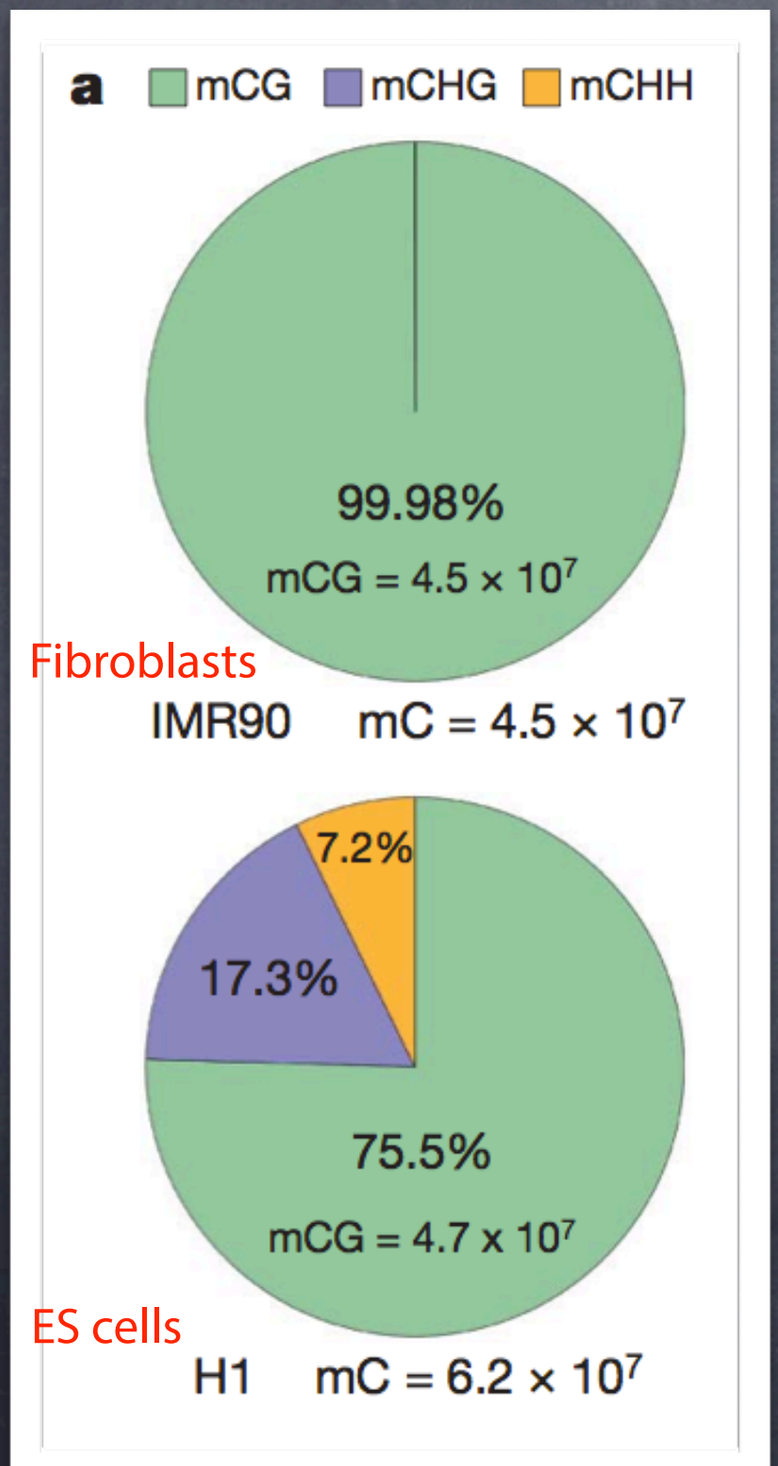
Embryo development and DNA remodeler involvement

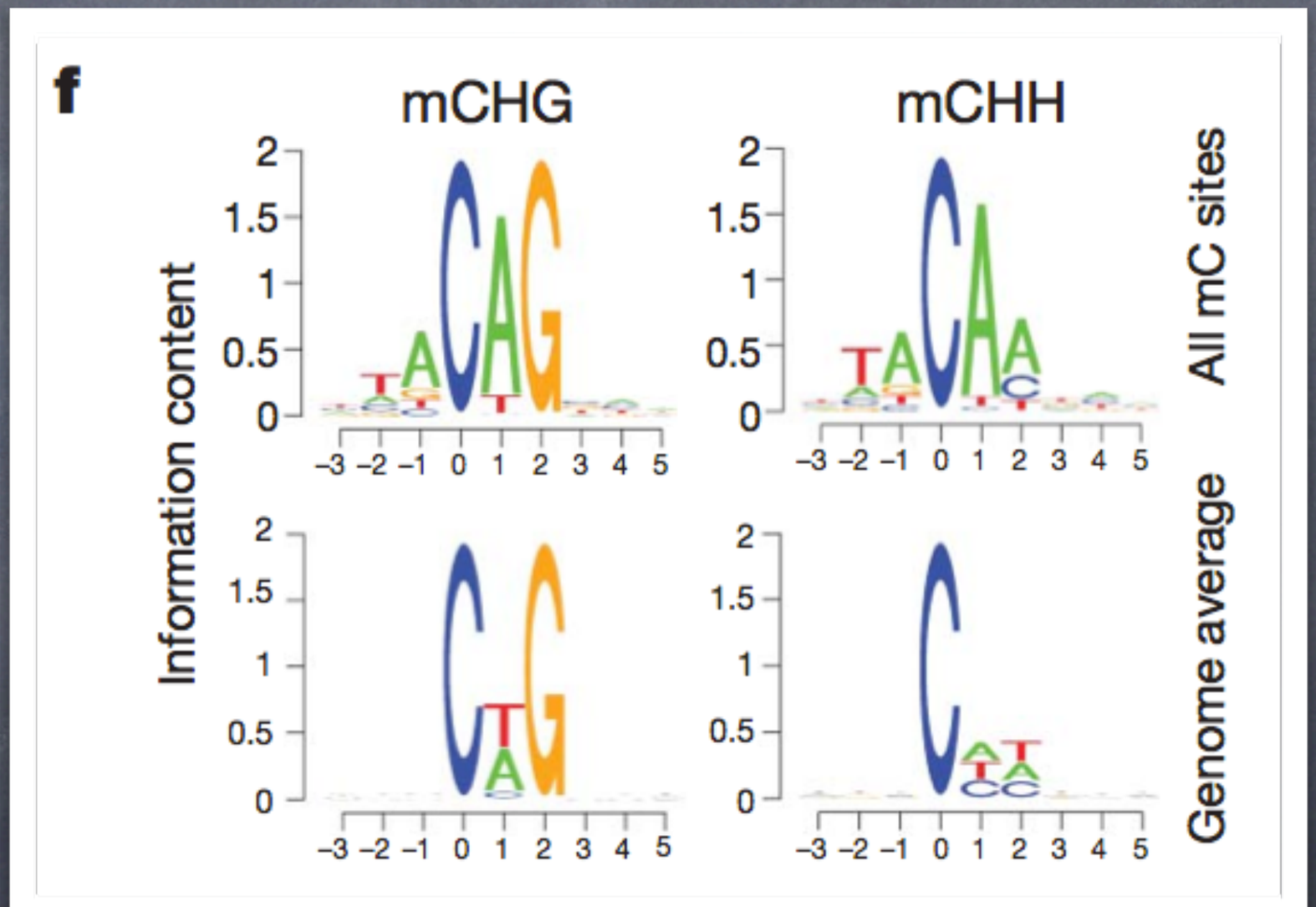


Summary: Modes of transcriptional and translational regulation



distribution of CG, CHG, and CHH cytosine methylation in human cells





Lister, R. et al., Nature (2009)

Bisulfite Sequencing

me me

5′ ATCCCTACGCGTGCCTCGA 3′

Bisulfite treat DNA

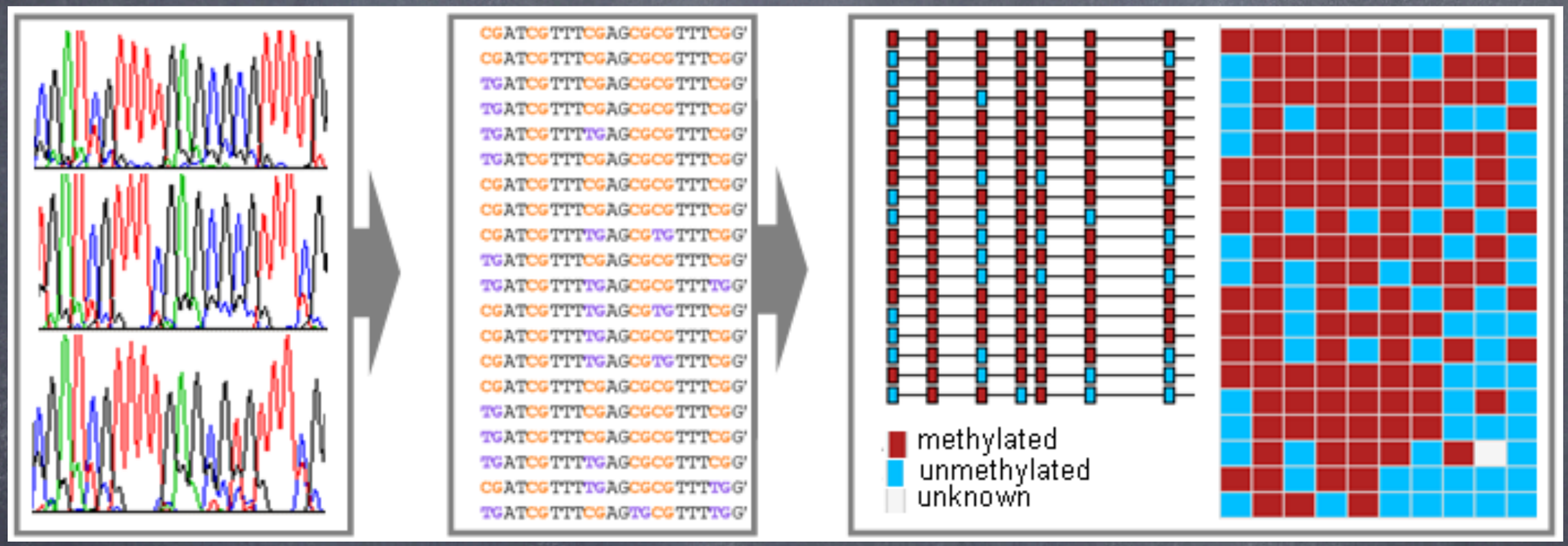
5′ ATTTTTACGCGTGTTTTGA 3′

Design bisulfite primers

Amplify region of interest by PCR

Sequencing

Bisulfite Sequencing Alignment



Bisulfite Sequencing Alignment

Multiple Sequence Alignment of the sequences included:

Include	Number	Sequence_id	Sequence
		Reference	CGATCGTTTCGAGCGCGTTTCGGTTTAATGTGAA
☑	1	P04_37_051_new5_HEK293_M13	TGATCGTTTTGAGCGCGTTTCGGTTTAATGTGAA
☑	2	P04_39_051_new5_HEK293_M13	CGATCGTTTTGAGCGTGTTTCGGTTTAATGTGAA
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☑	4	P04_41_051_new5_HEK293_M13	TGATCGTTTCGAGCGCGTTTCGGTTTAACGTGAA
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17/17 cytosines are methylated

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17/17 cytosines are methylated

3/17 cytosines are methylated

CHPC, University of Utah

- Ember Cluster Hardware Overview
- 262 Dual Socket-Six Core Nodes (3144 total cores), 14 Nodes dedicated for Bioinformatics Core
- 2.8 GHz Intel Xeon (Westmere X5660) processors
- 24 Gbytes memory per node (2 Gbytes per processor core)
- Mellanox QDR Infiniband interconnect
- Gigabit Ethernet interconnect for management

Stats on bioinformatics

- Bisulfite Sequencing alignment: 4-5 days straight computation on 14 nodes, @ 2.8Ghz 12 core Westmere for each experiment
- 101bp paired end read per lane ~80GB of data
- output: 160-180 million reads, 5x human genome coverage
- about 20x-30x coverage is needed to get enough statistical power
- reads are independent from each other and can be processed in parallel
- split into ~40 files, 2-3h computing time for each file 10-20x coverage needed
- Amazon Cloud computing: ~$600-$1000 per bisulfite run

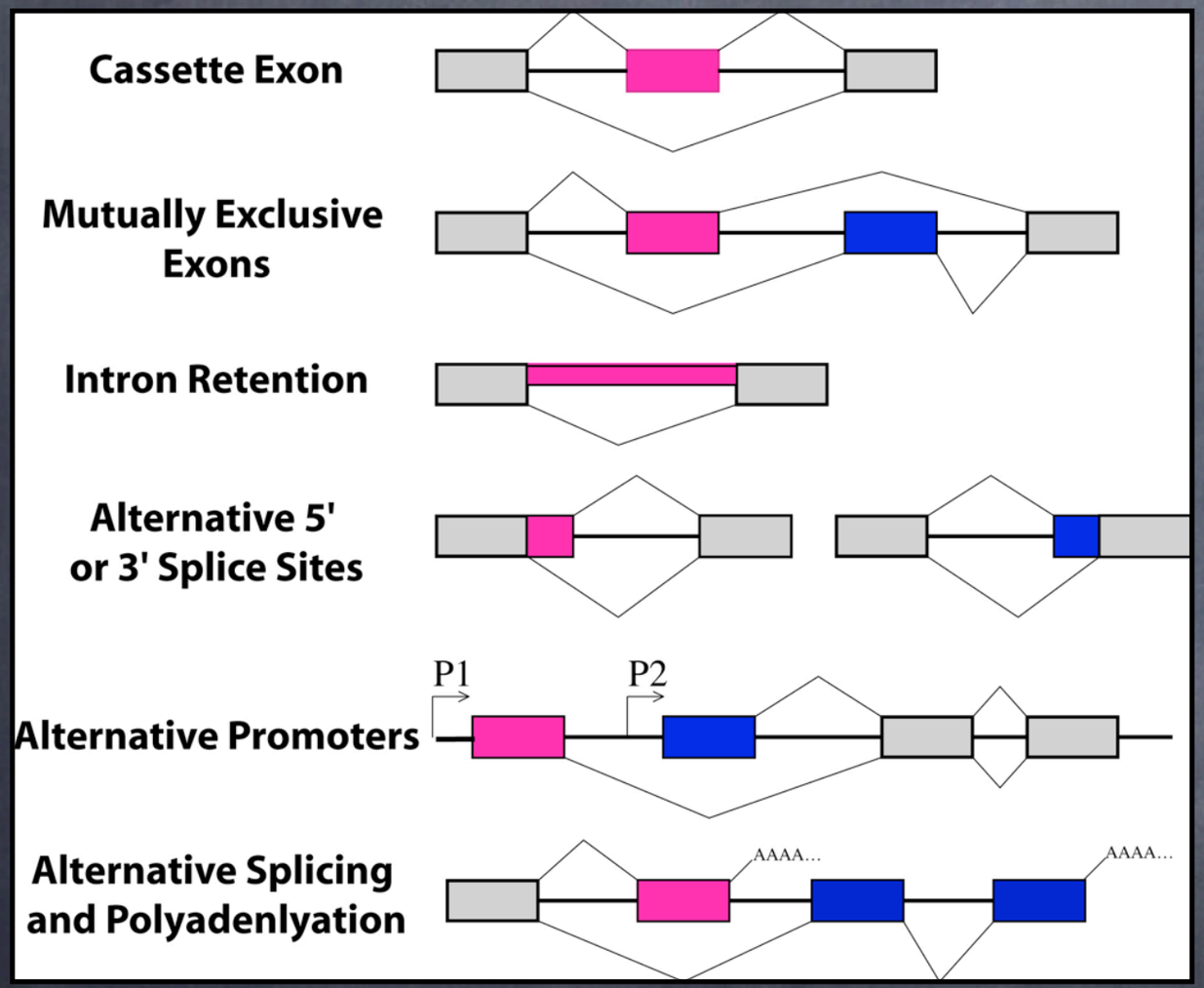
Stats on bioinformatics

- ChIP seq or RNA seq need only few hours to align to back to the reference genome, provided the splice junction file is ready
- Sequencing cost: $2000 per lane + Library preparation $200
- 2-3 jobs for alignment per week just from one person (Sue Hammoud)

Splice Junction File Generation

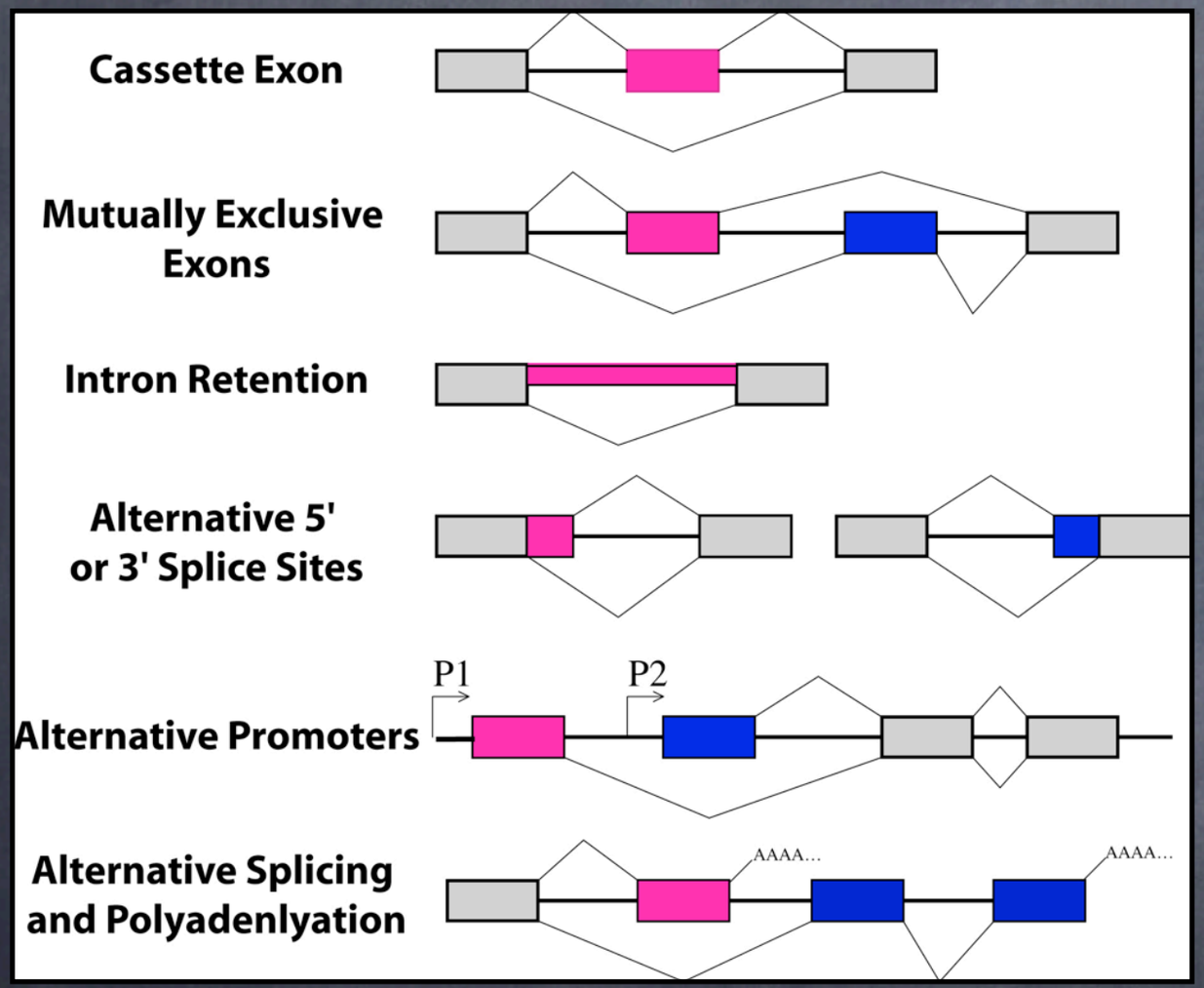
Reference Genome

ATGCTCGACGGCGTACG



wormbook.org

Splice Junction File Generation



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mRNA spliced results

wormbook.org

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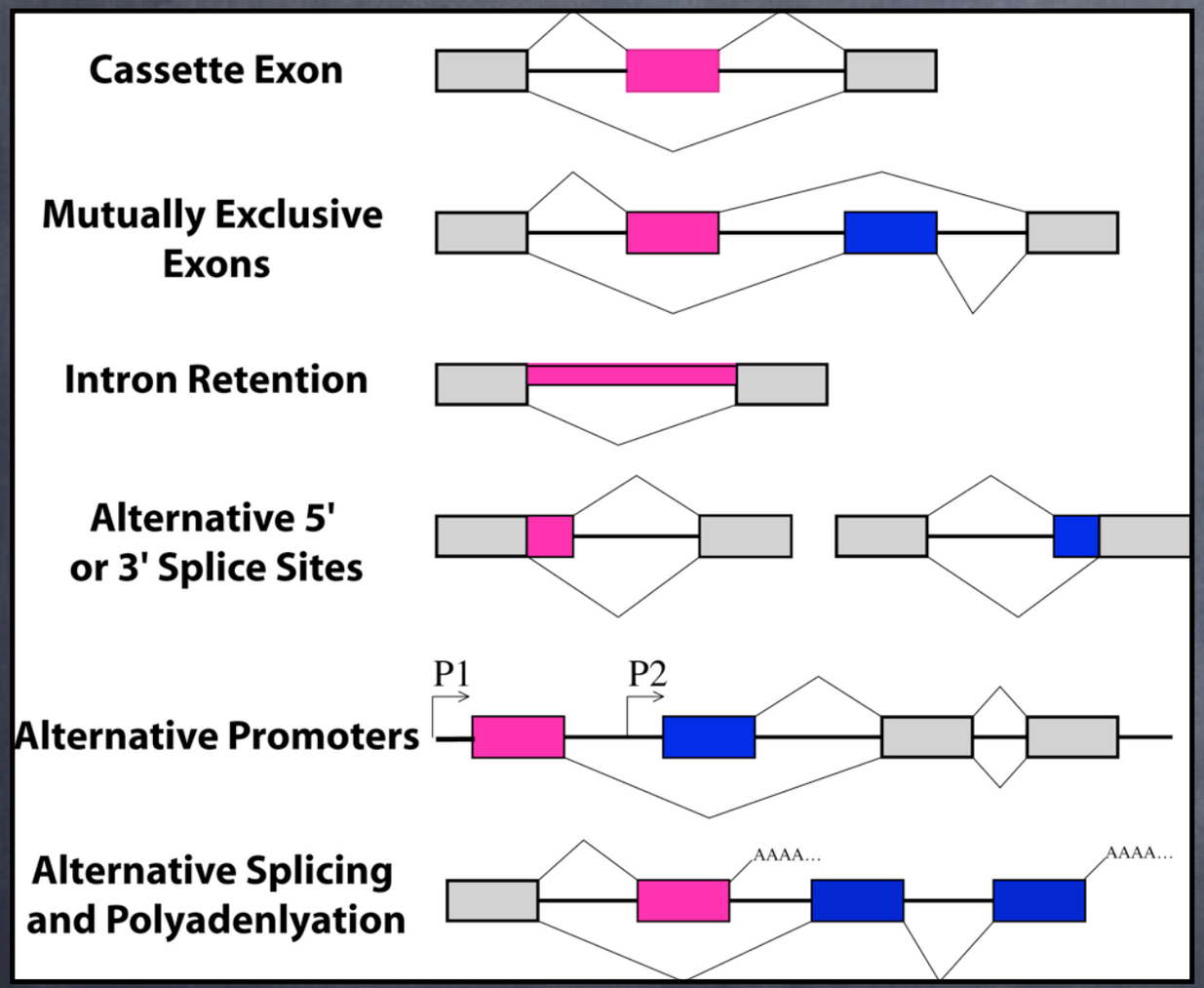

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wormbook.org

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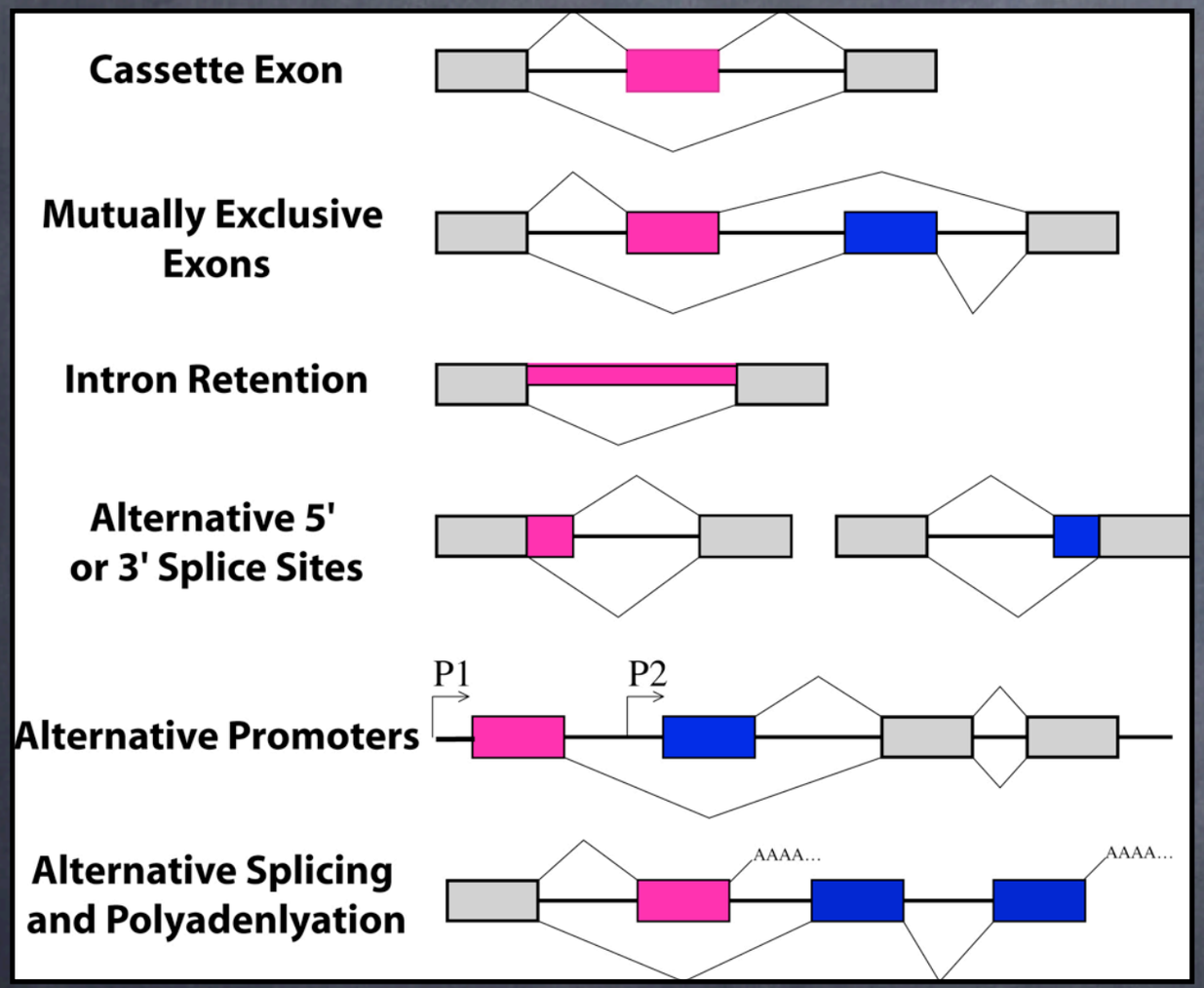


Reference Genome

ATGCTCGACGGCGTACG

mRNA spliced results

ATGCCGGCGTACG

ATGCTCGATACG

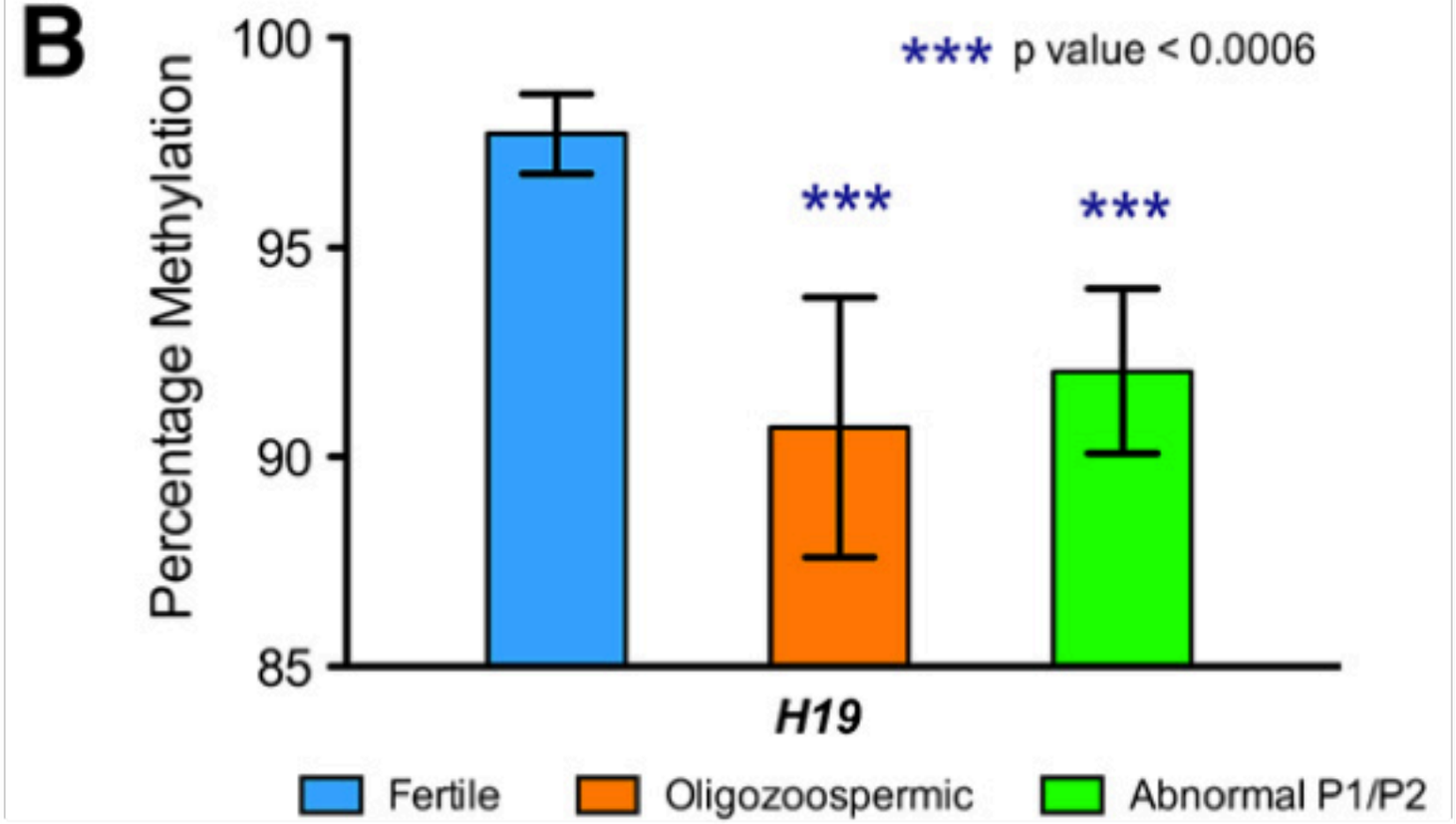
TCGATACG

ATGCTACG

wormbook.org

Using the power of bisulfite sequencing and analysis to investigate problems of infertility

DNA methylation is patterns are abnormal in infertile patients



Hammoud, S. S., Purwar, J., Pflueger, C., Cairns, B. R. & Carrell, D. T., Fertil. Steril.(2010)

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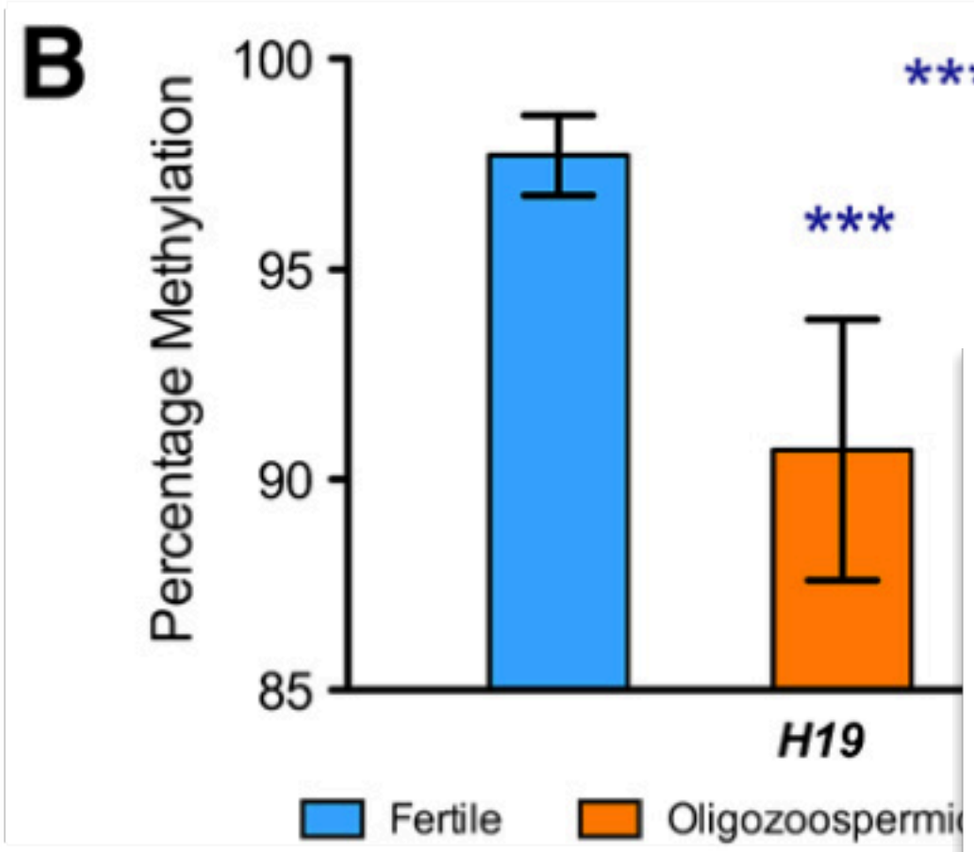
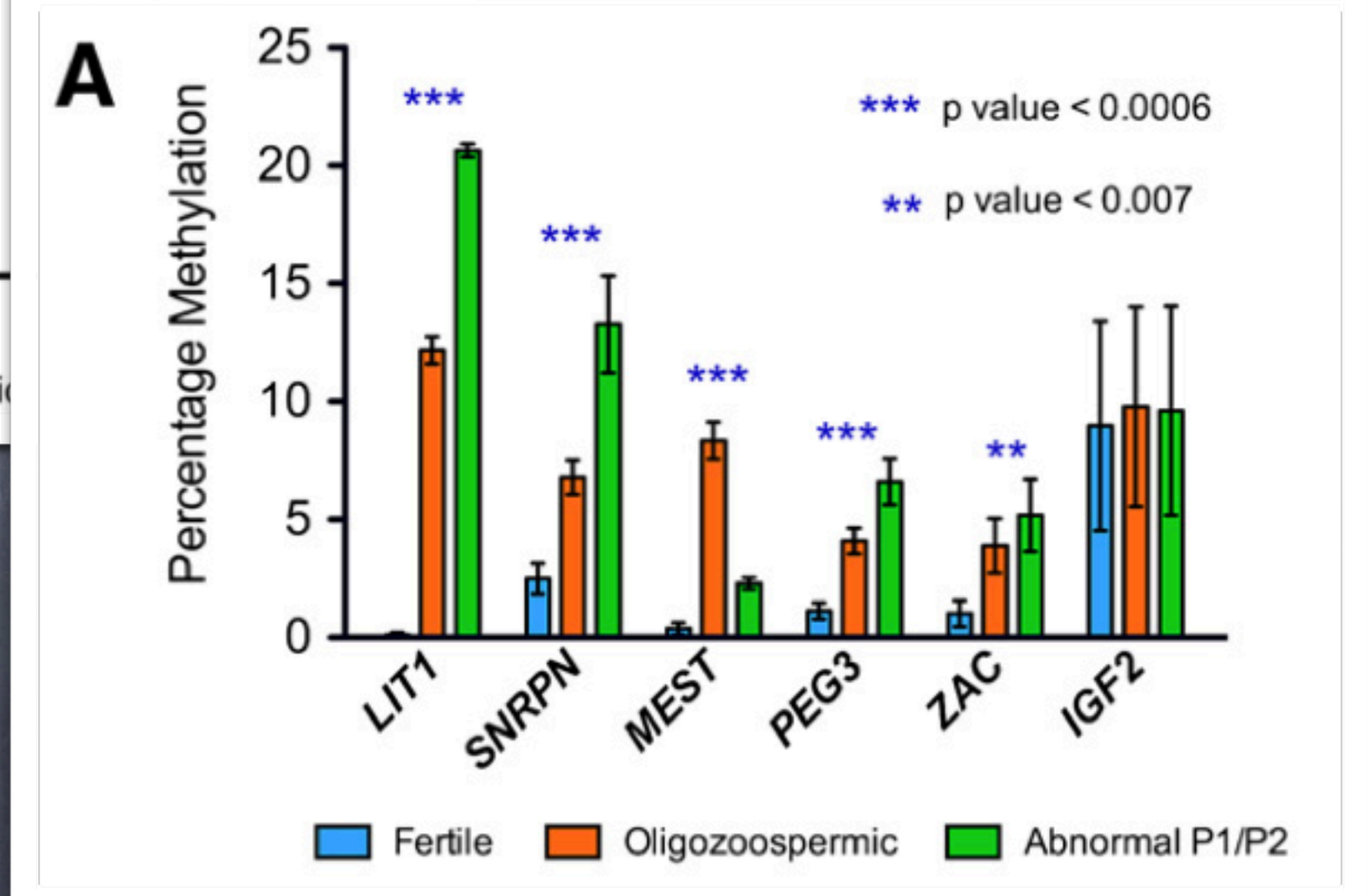
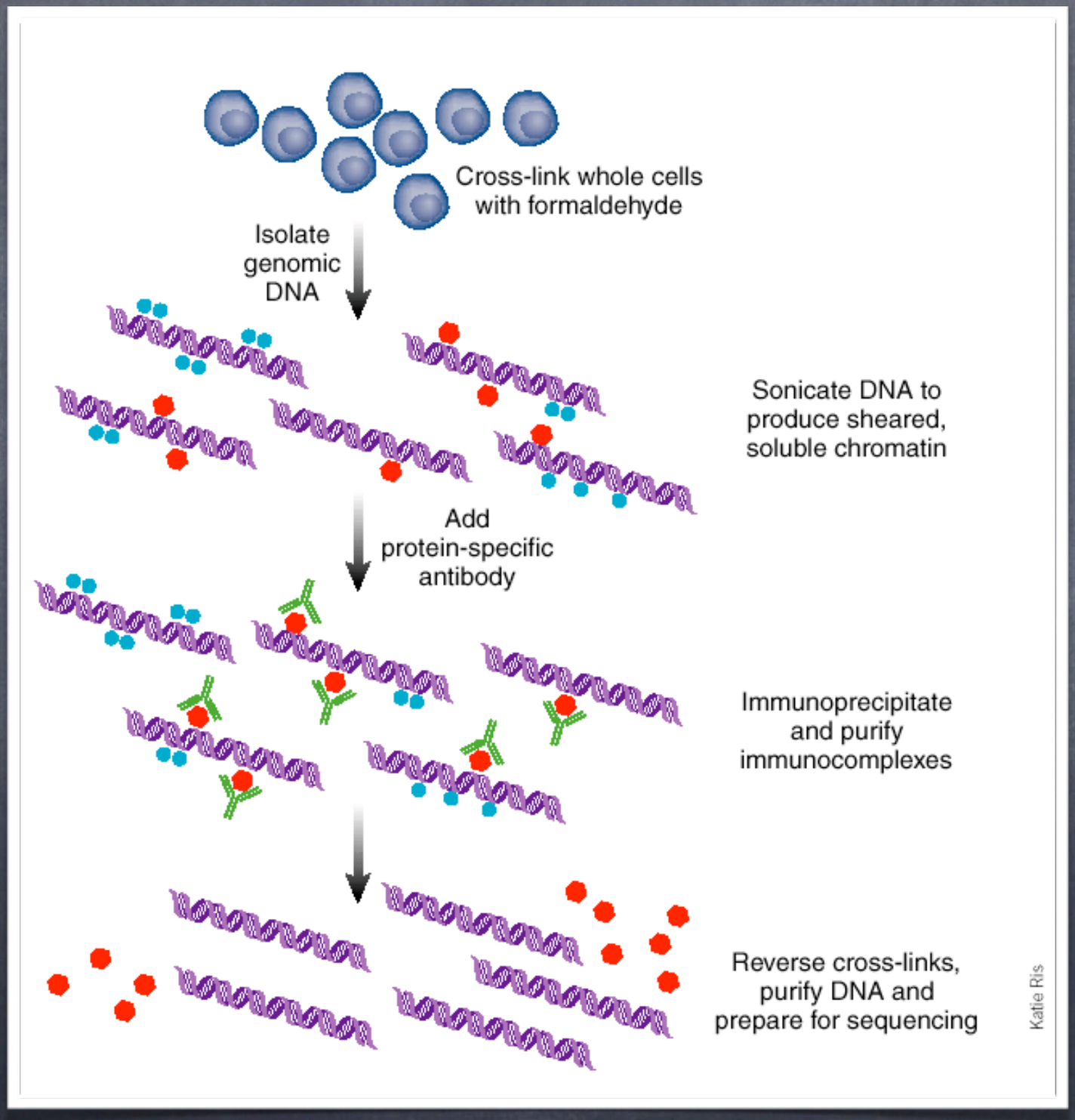




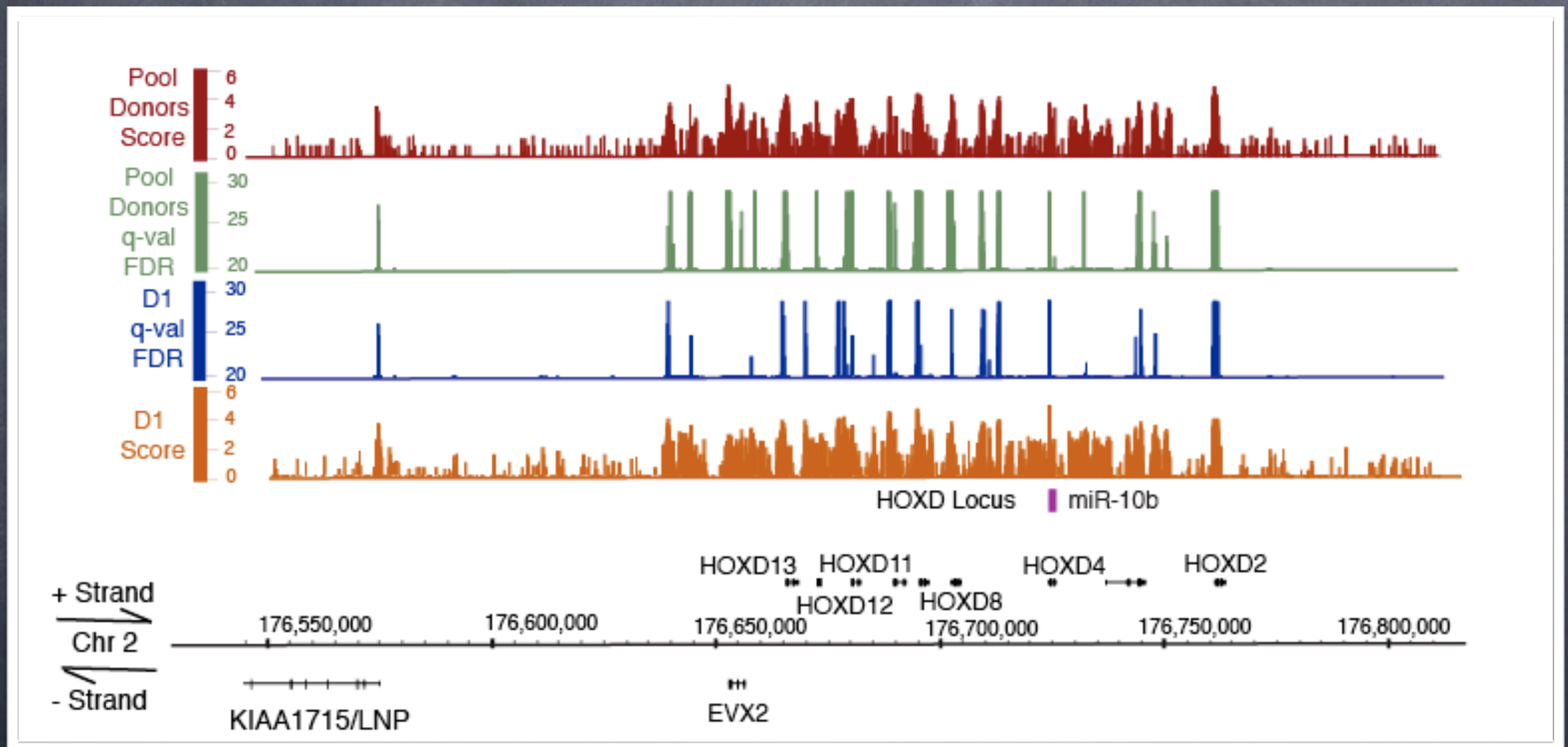
Hammoud, S. S., Purwar, J., Pflueger, C., Cairns, B. R. & Carrell, D. T., Fertil. Steril.(2010)

Method: ChiP Seq



Elaine R Mardis et al., Nature methods 2007

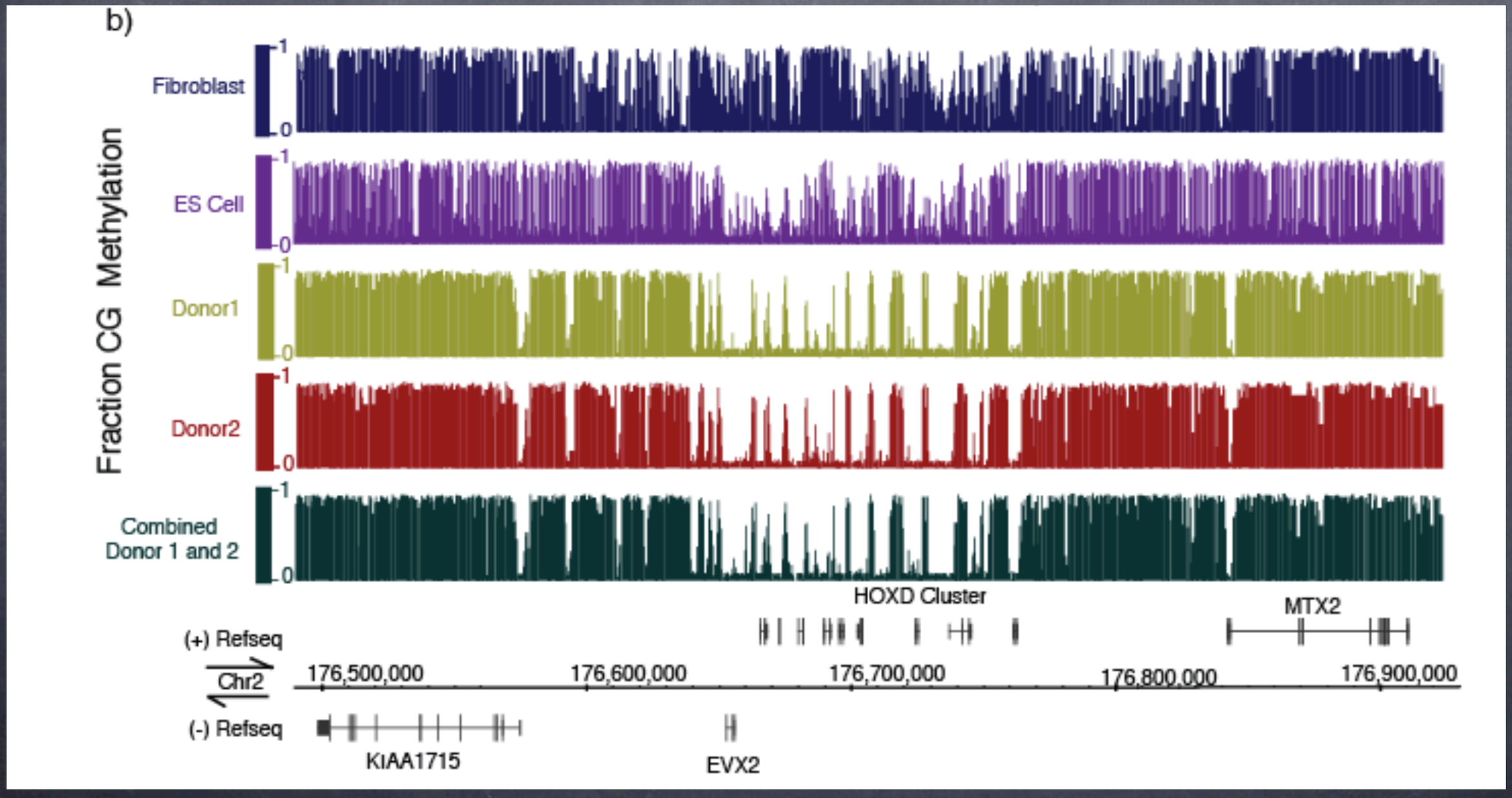
Fertile males retain nucleosomes at developmental loci in sperm



FDR 20 = 0.01, 25 = 0.003,

Hammoud et al. Nature 2009

Regions of severe hypomethylation punctuate the sperm genome



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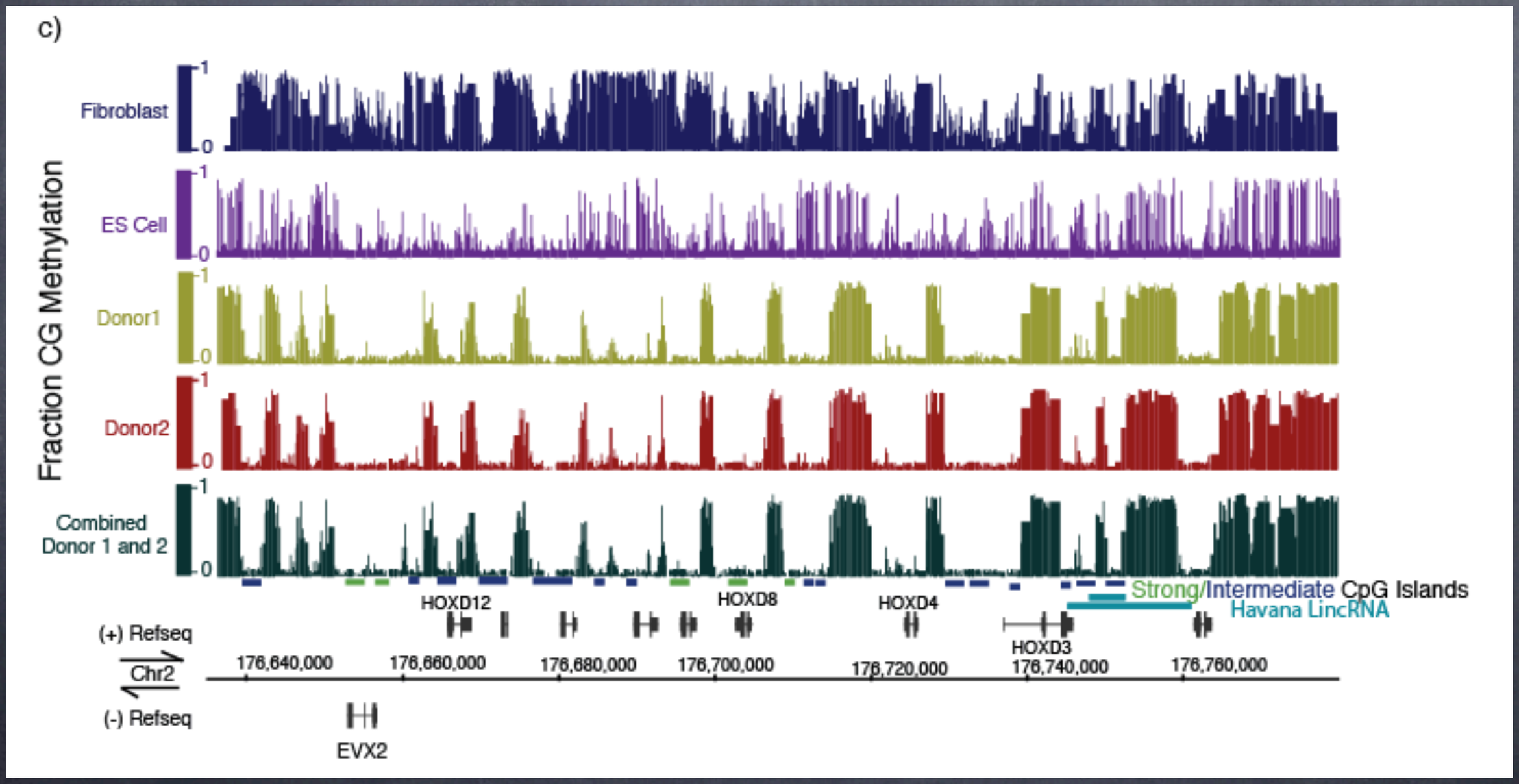
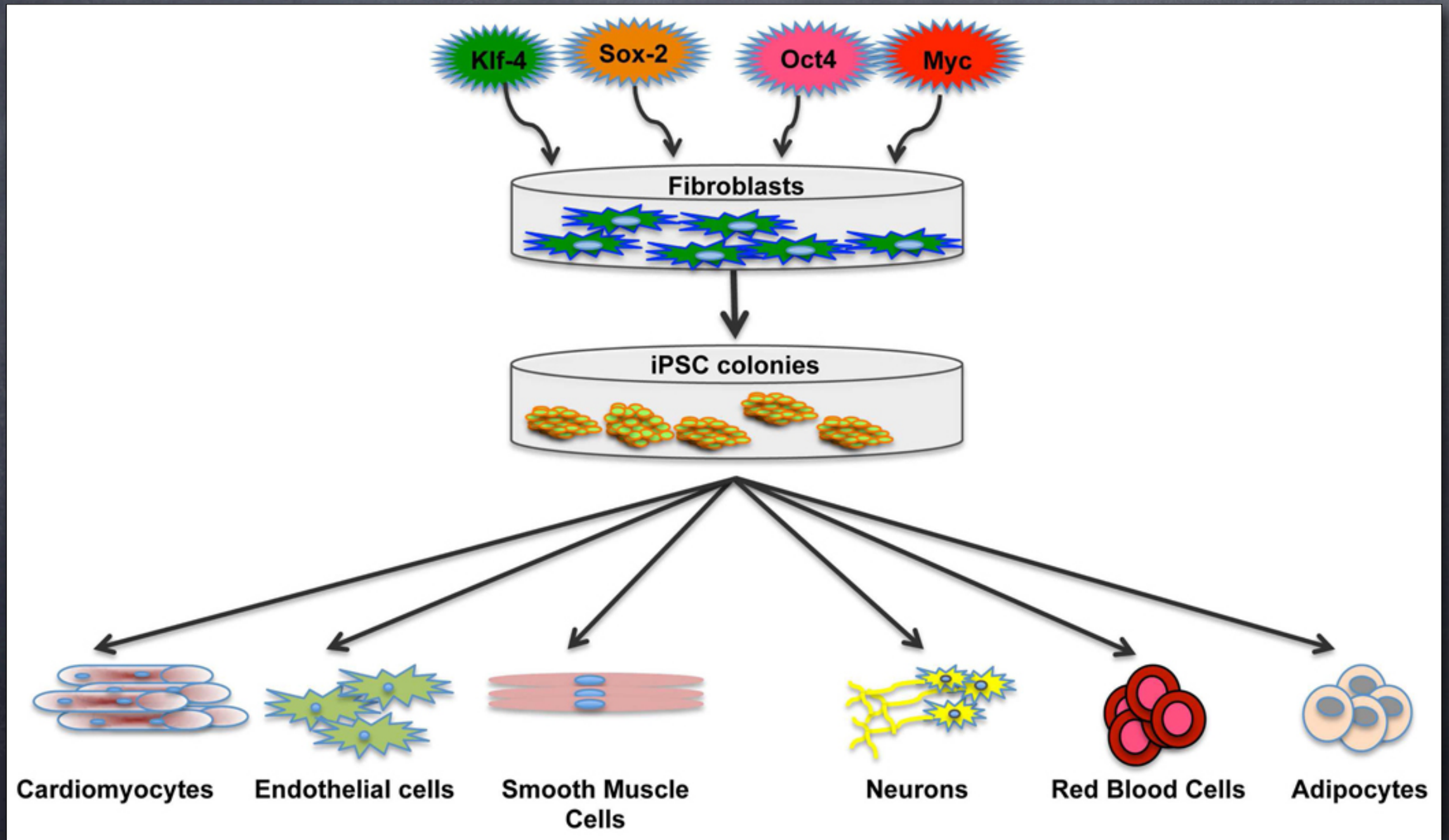


Induced pluripotent stem cells (iPS) generation and applications



Nsair, A. & MacLellan, W. R.,Advanced Drug Delivery Reviews 63, 324-330 (2011).

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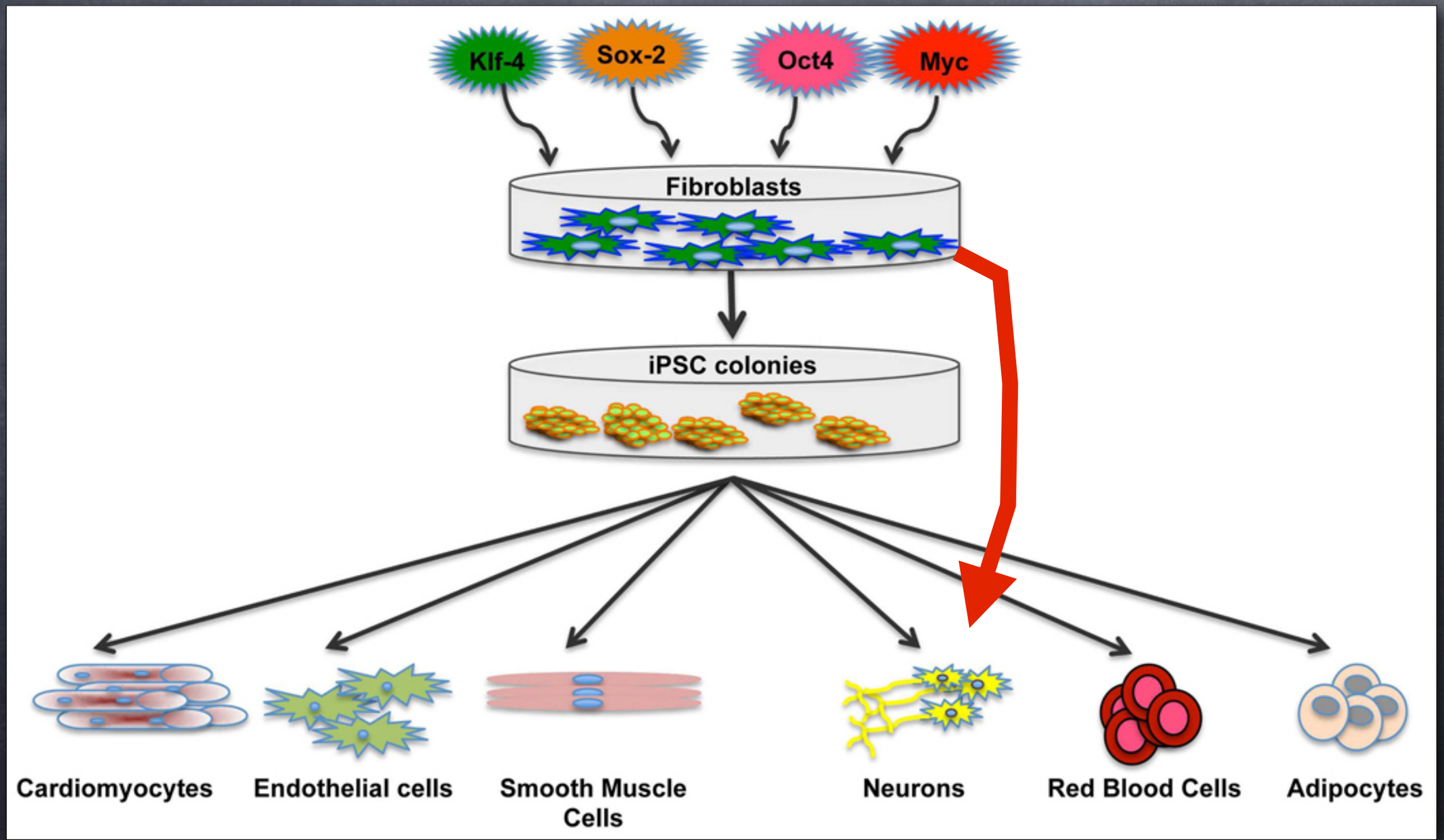
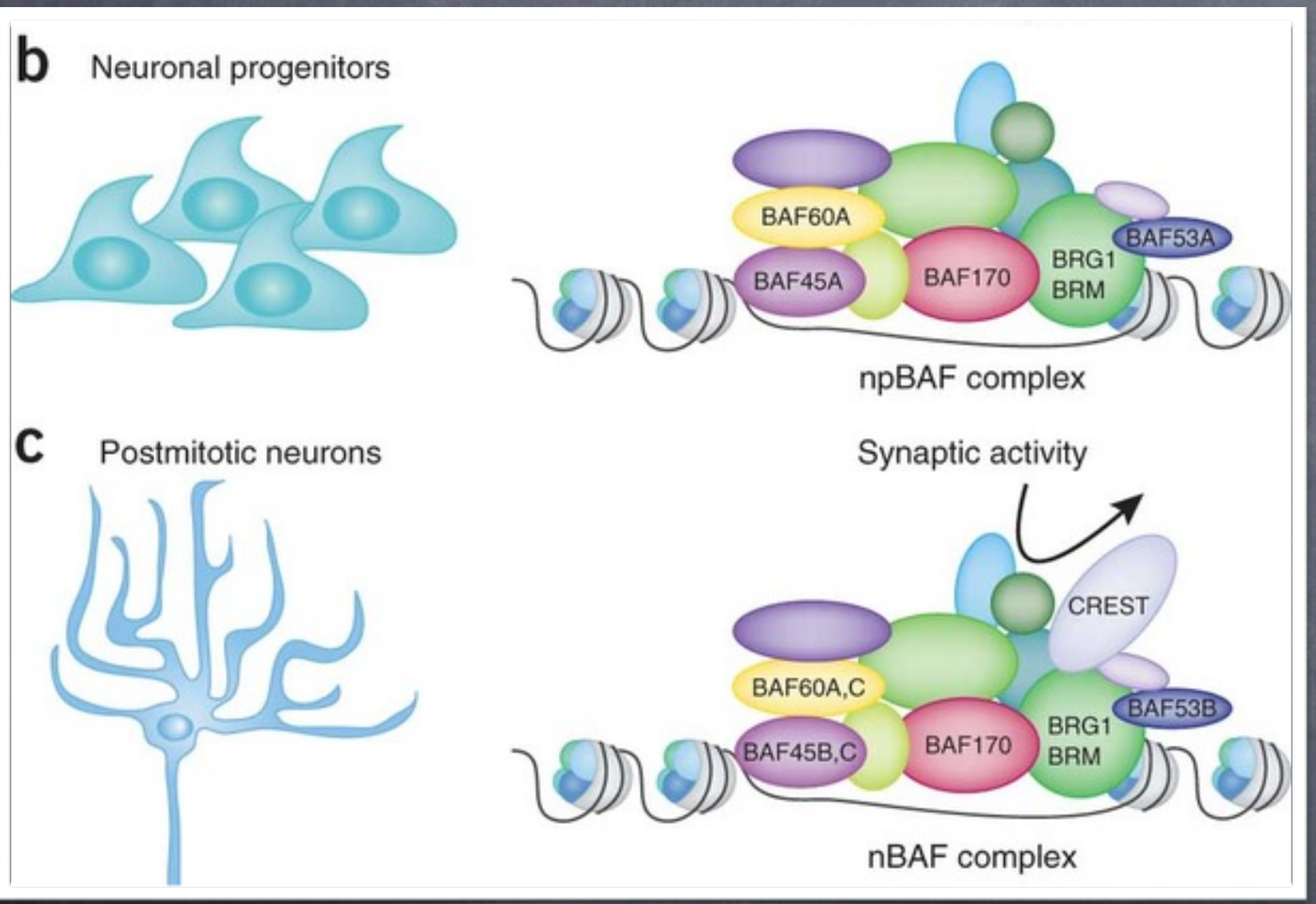


Nsair, A. & MacLellan, W. R.,Advanced Drug Delivery Reviews 63, 324-330 (2011).

Introduction - induced neurons require nBAF complex



Yoo, A. S. et al., Nature 476 (2011).

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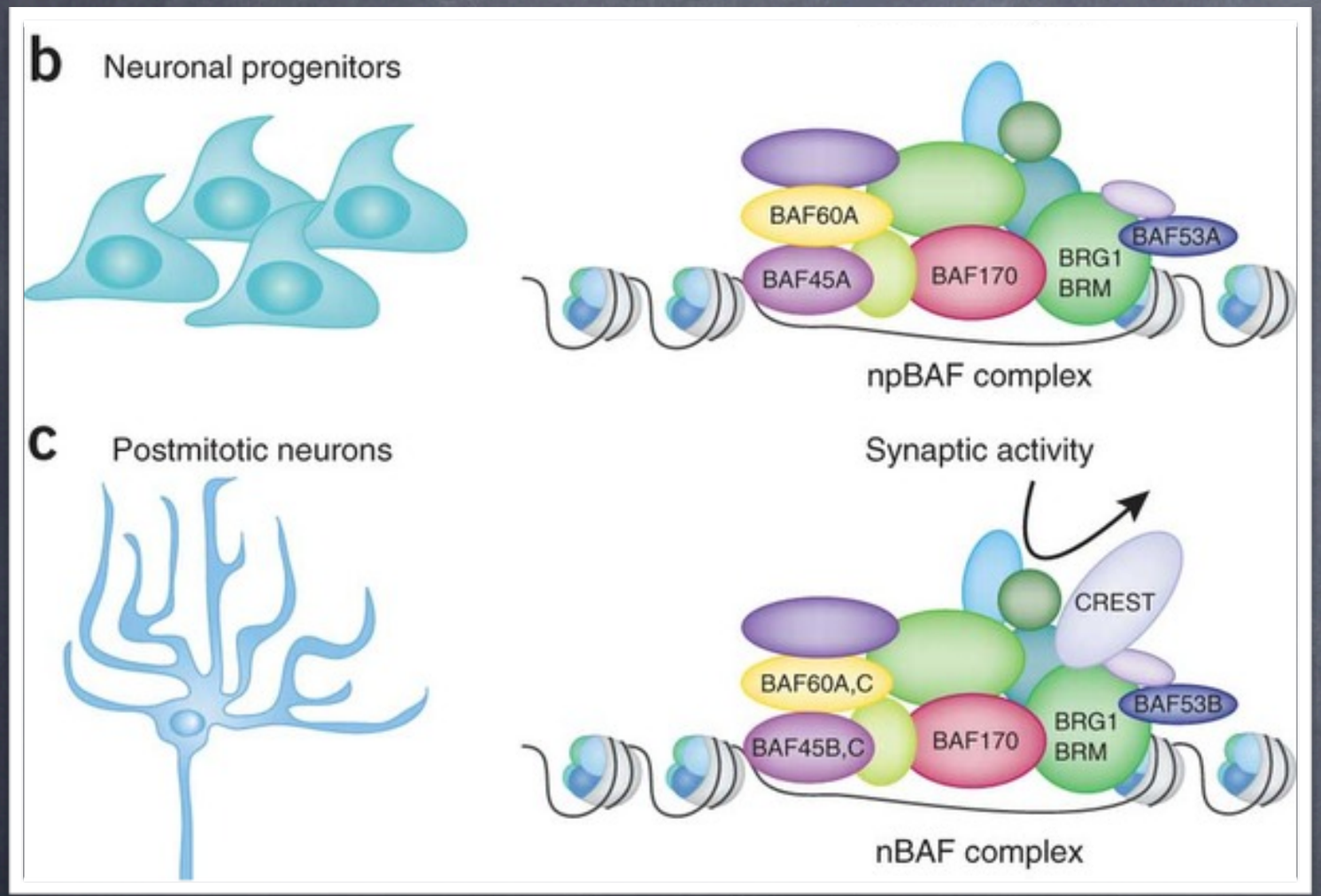
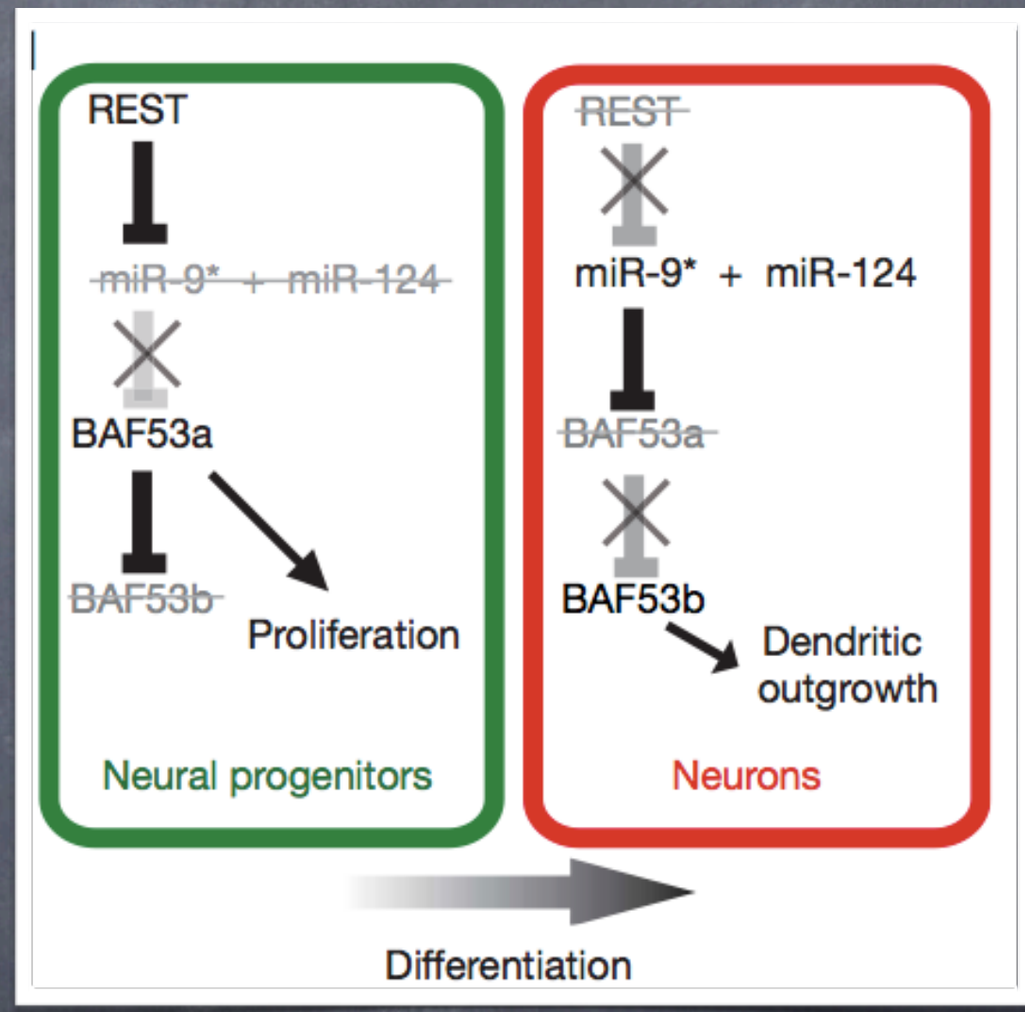
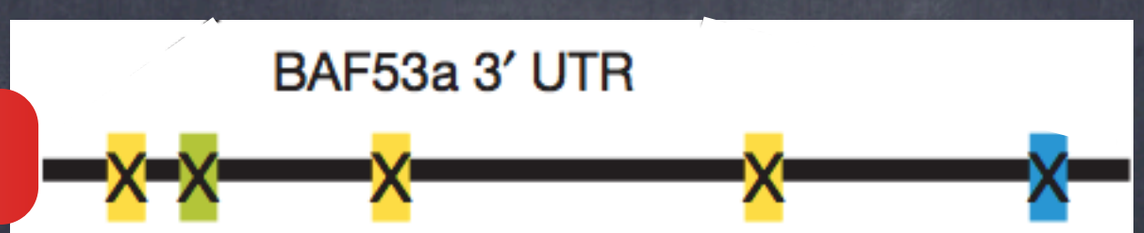


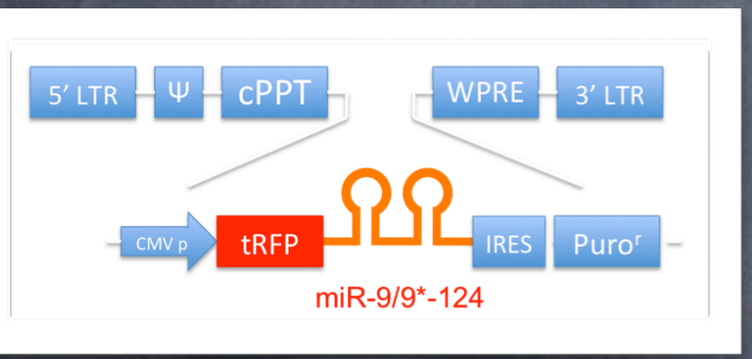


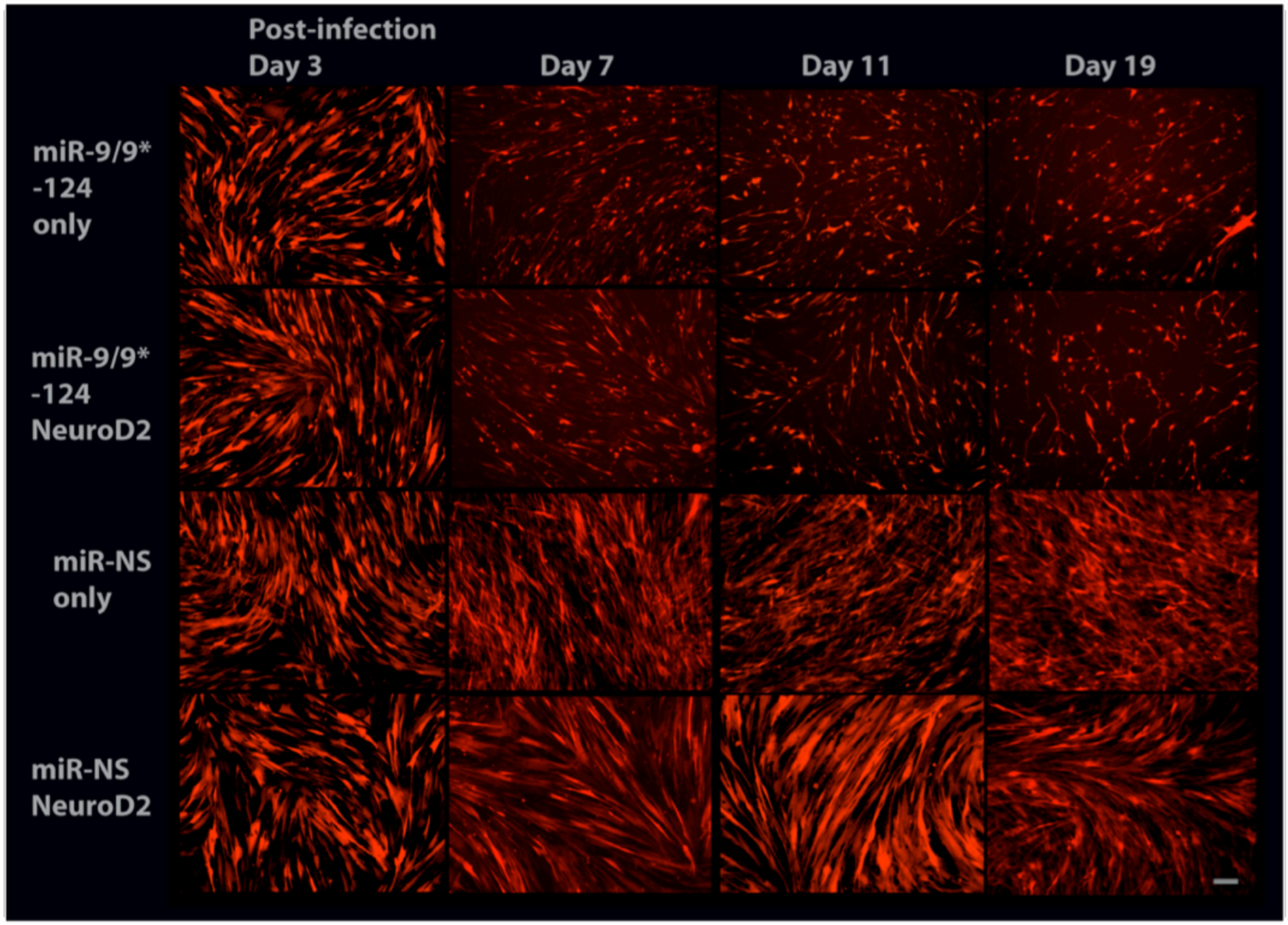


mRNA

Yoo, A. S. et al., Nature 476 (2011).

Induced Neurons can be derived from fibroblasts





Yoo, A. S. et al., Nature 476 (2011).

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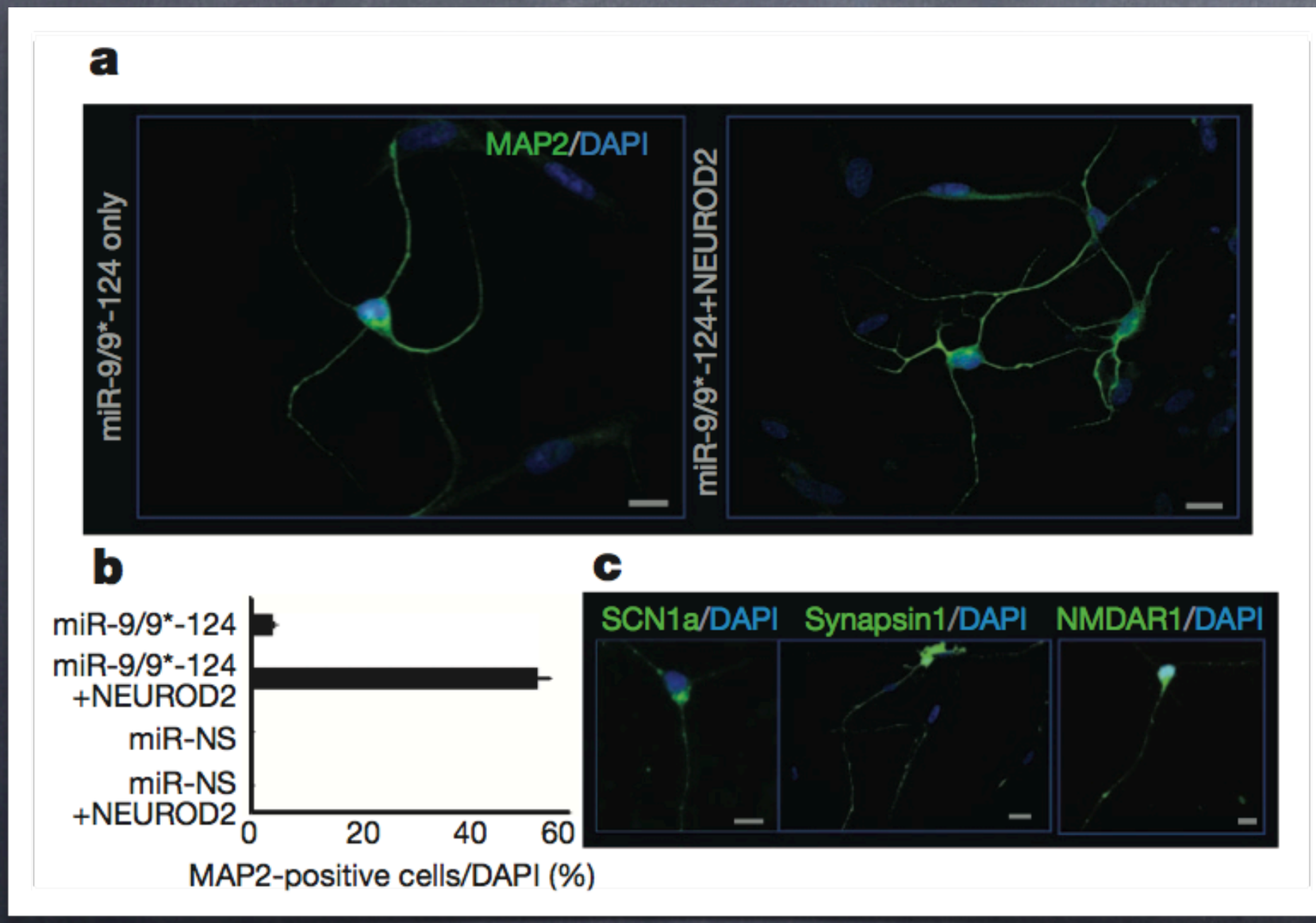

after 30 days

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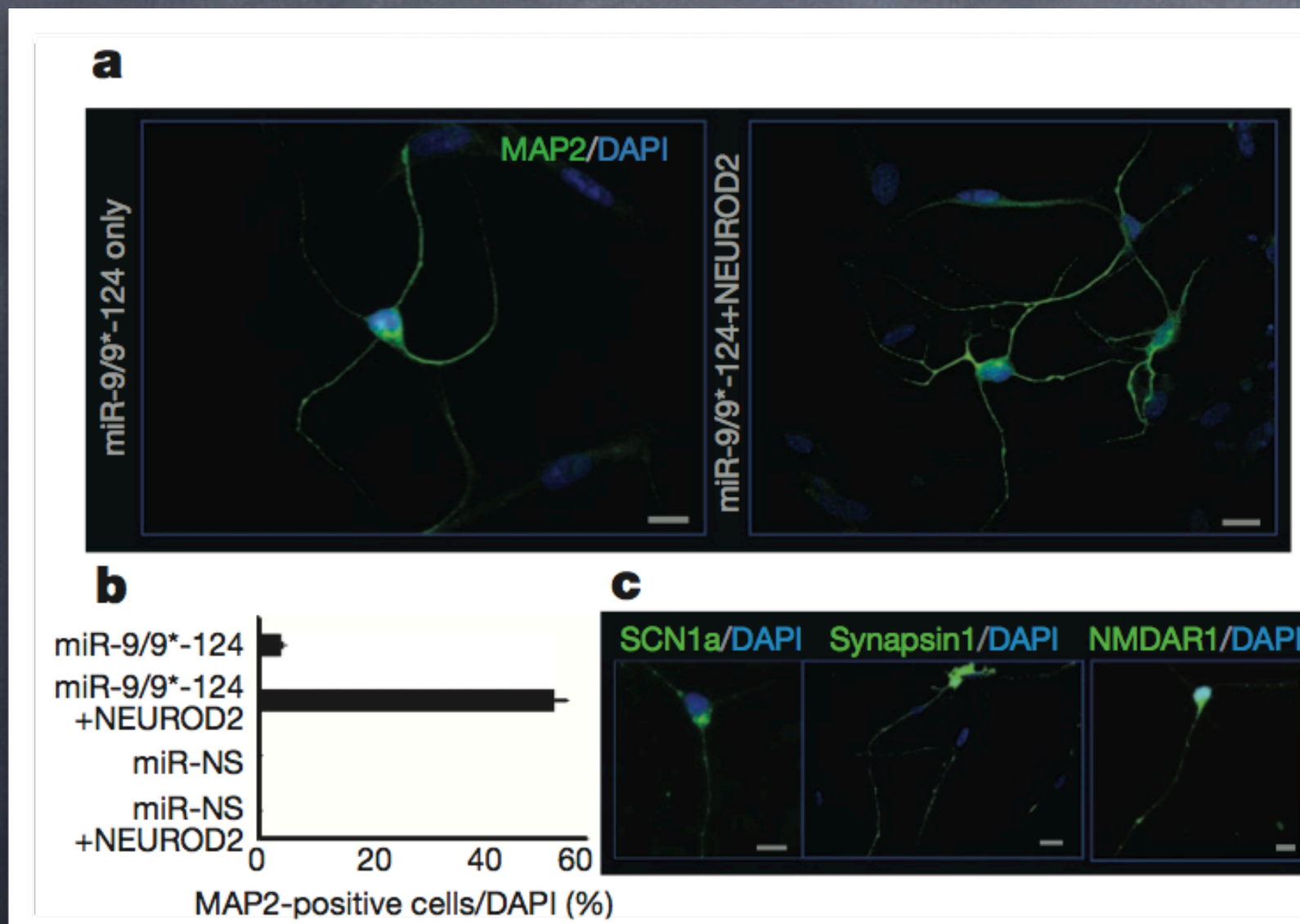

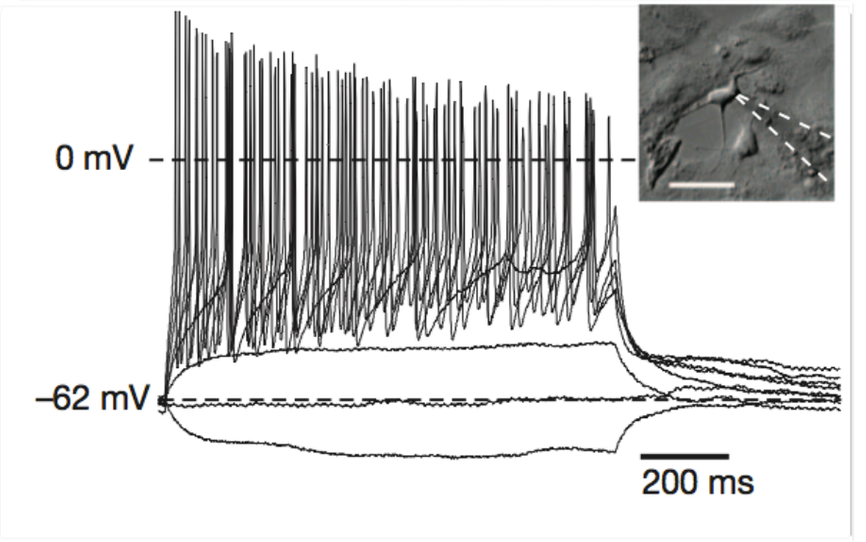

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INTERDEPARTMENTAL PROGRAMS IN

BIOLOGICAL CHEMISTRY AND MOLECULAR BIOLOGY

Timothy Parnell, Cairns' lab

Acknowledgments

- Brad Cairns
- Sue Hammoud
- Jahnvi Purwar
- All Cairns' Lab Members

Prof. Nagel and the ZIH

<u>Thesis committee</u>

Brad Cairns

Dave Jones

Chris Hill

Nick Trede

Cynthia Burrows