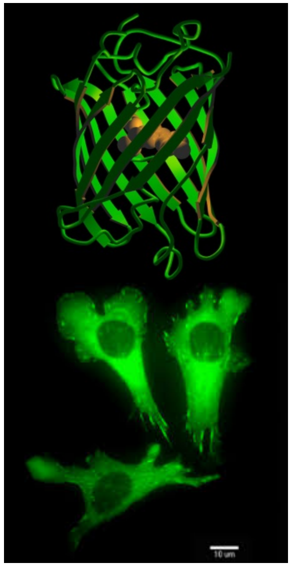
Module 1: DNA Engineering

Leona Samson – Lecture 6

Experiments and lectures based upon research in Prof. Bevin Engelward's laboratory

What experimental question will you ask in Module 1?

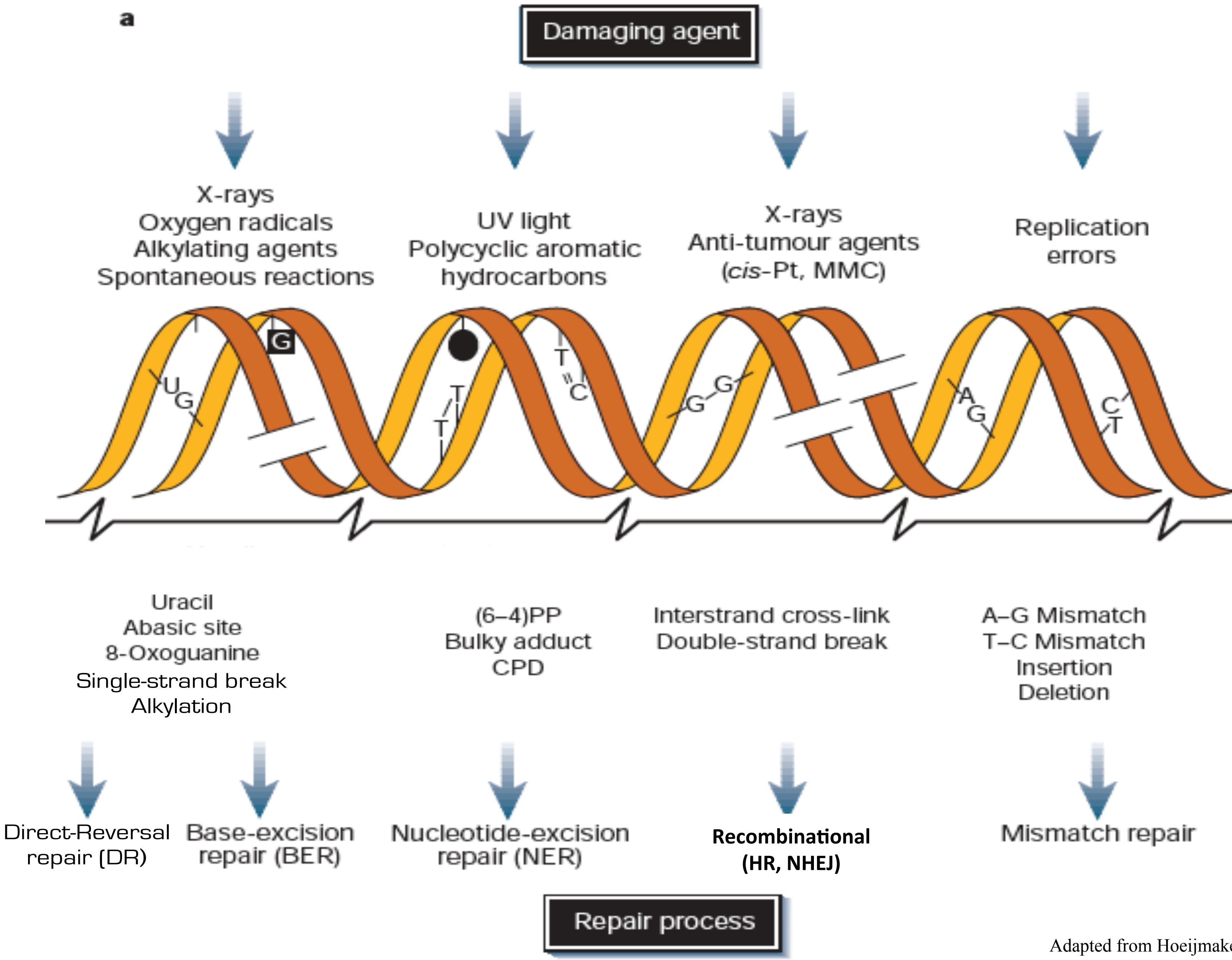


What conditions affect the frequency of DNA repair by homologous recombination in mouse embryonic stem cells?

This raises the following questions

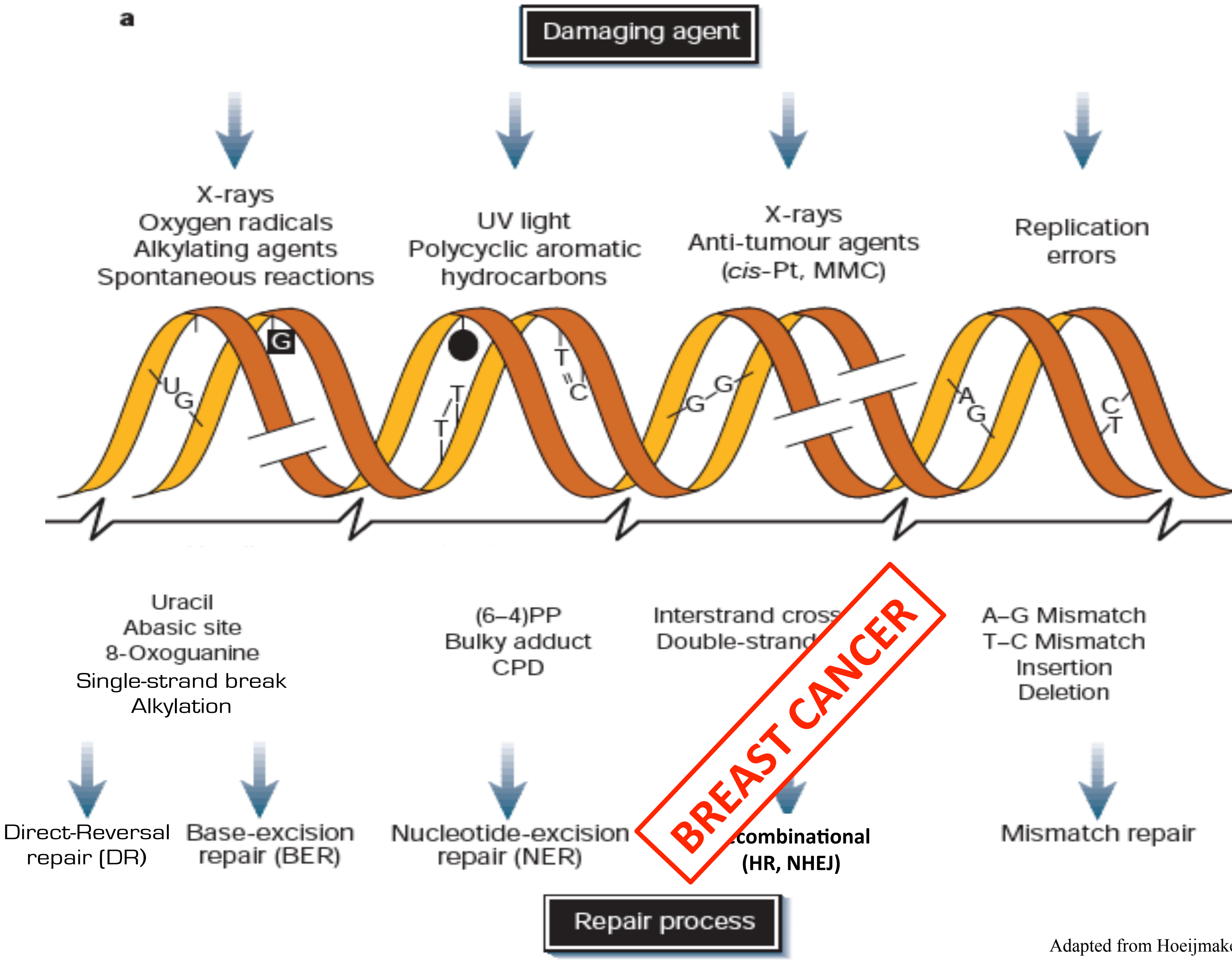
- How does DNA get damaged?
- What is DNA repair?
- Why does DNA repair exist?
- Why do we care about how efficient DNA repair is?
- How does one actually measure DNA repair?

DNA Damage and Repair



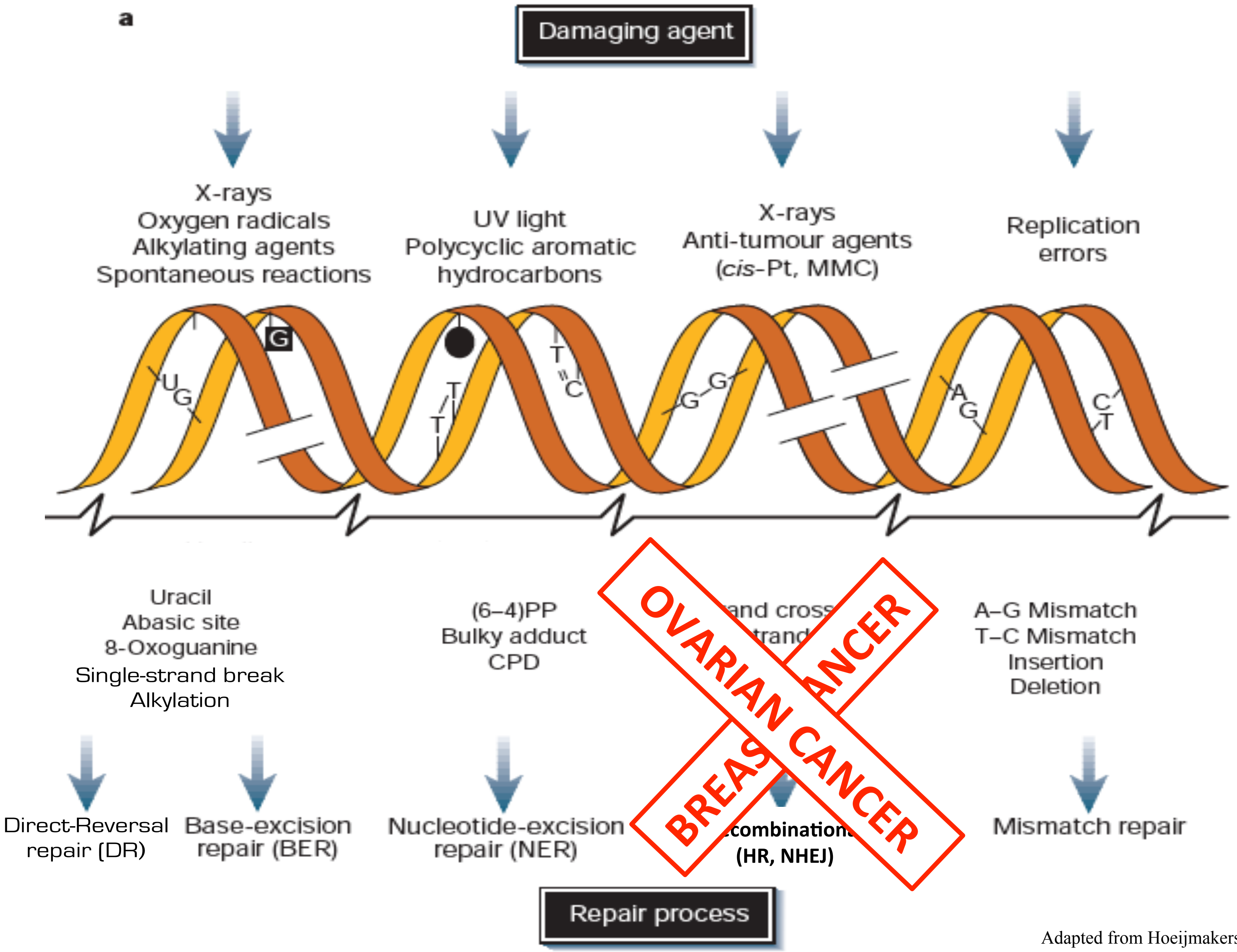
Adapted from Hoeijmakers **2001** *Nature*

DNA Damage and Repair



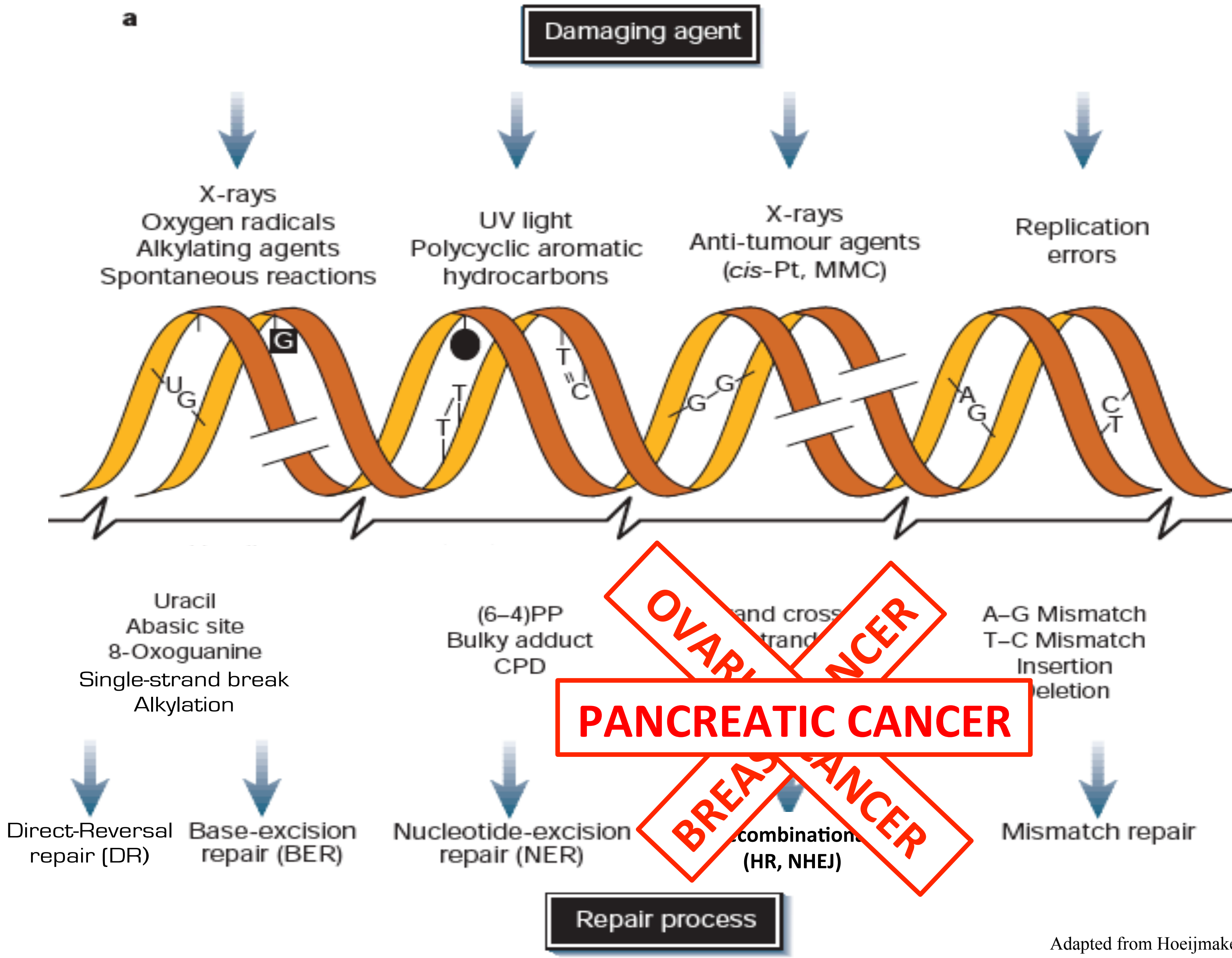
Adapted from Hoeijmakers **2001** *Nature*

DNA Damage and Repair



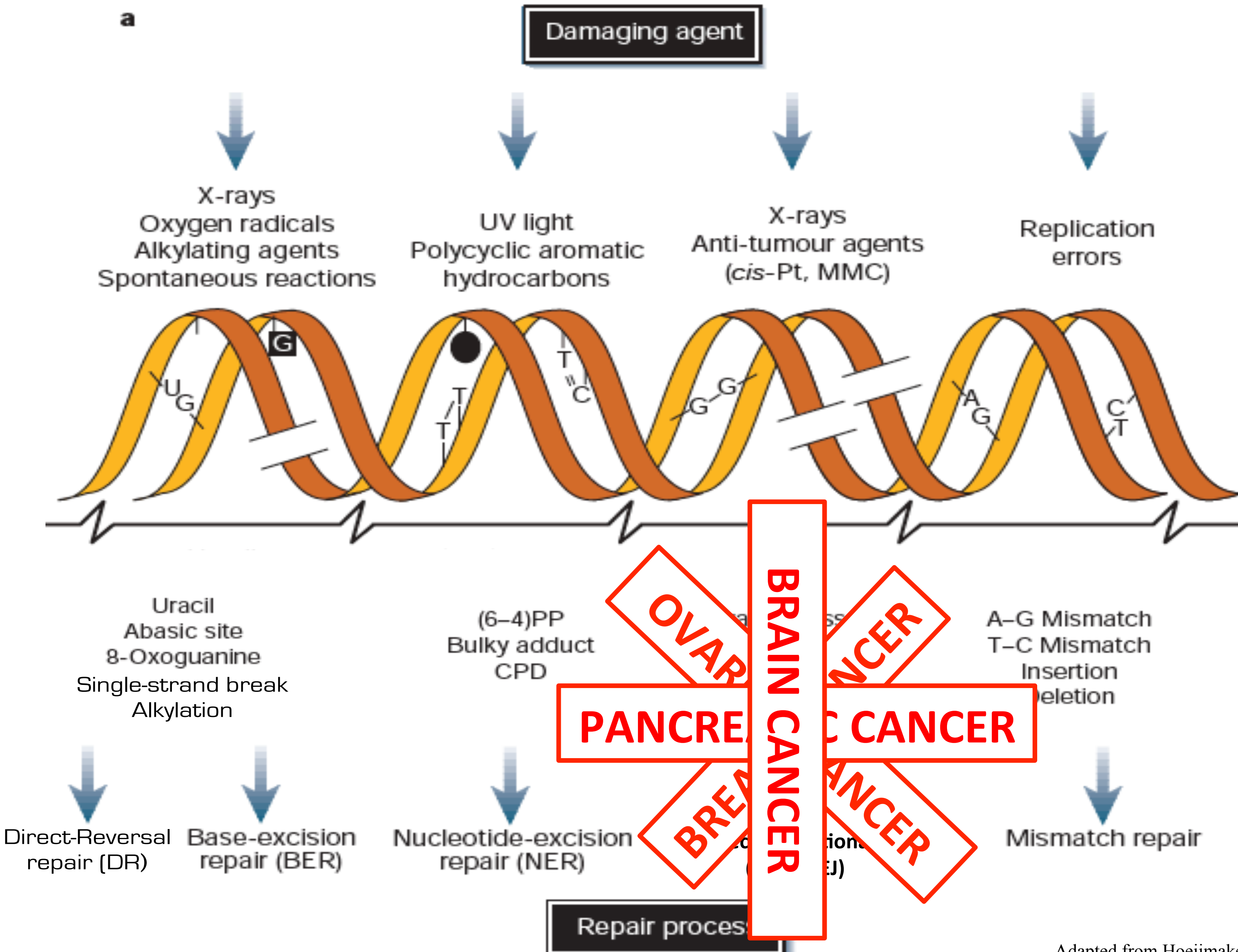
Adapted from Hoeijmakers **2001** *Nature*

DNA Damage and Repair



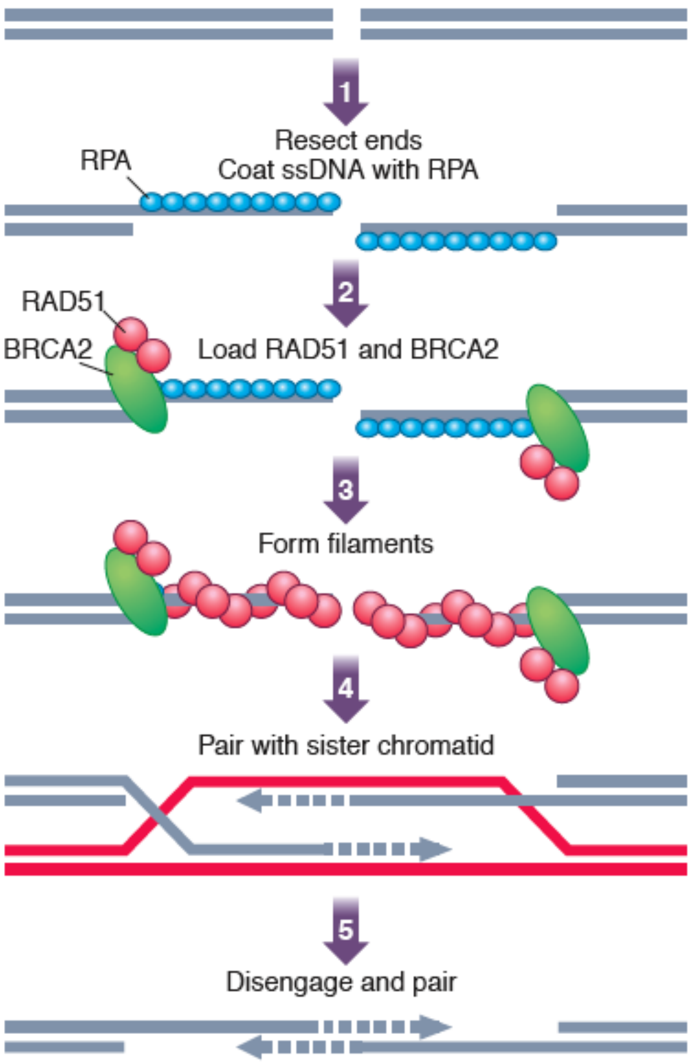
Adapted from Hoeijmakers **2001** *Nature*

DNA Damage and Repair



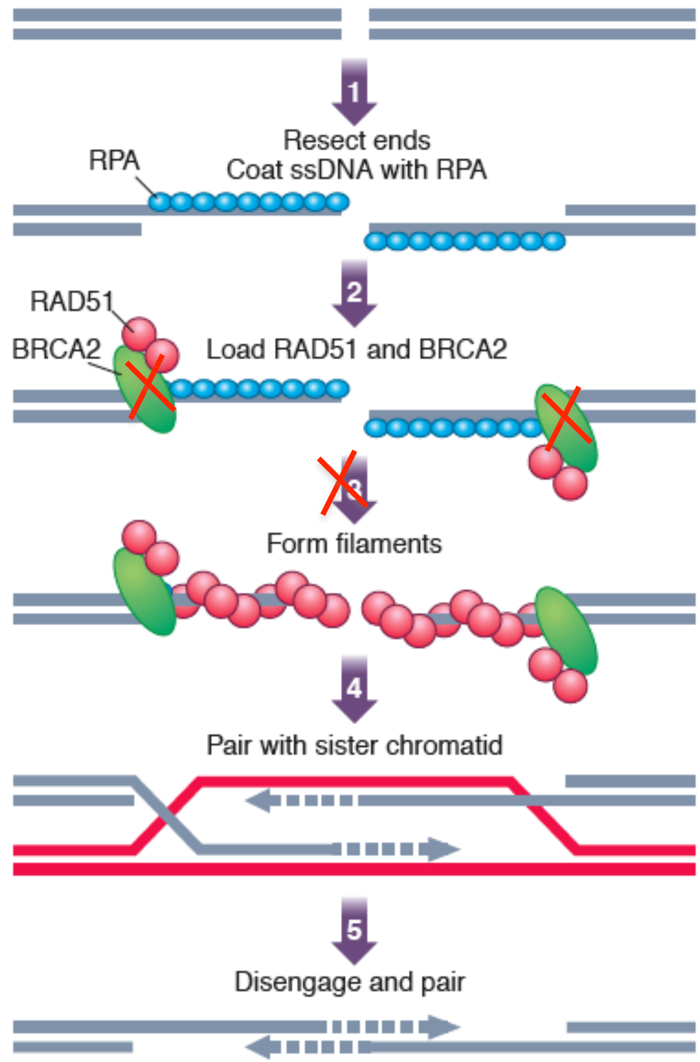
Adapted from Hoeijmakers **2001** *Nature*

DNA Double Strand Break (DSB) Repair

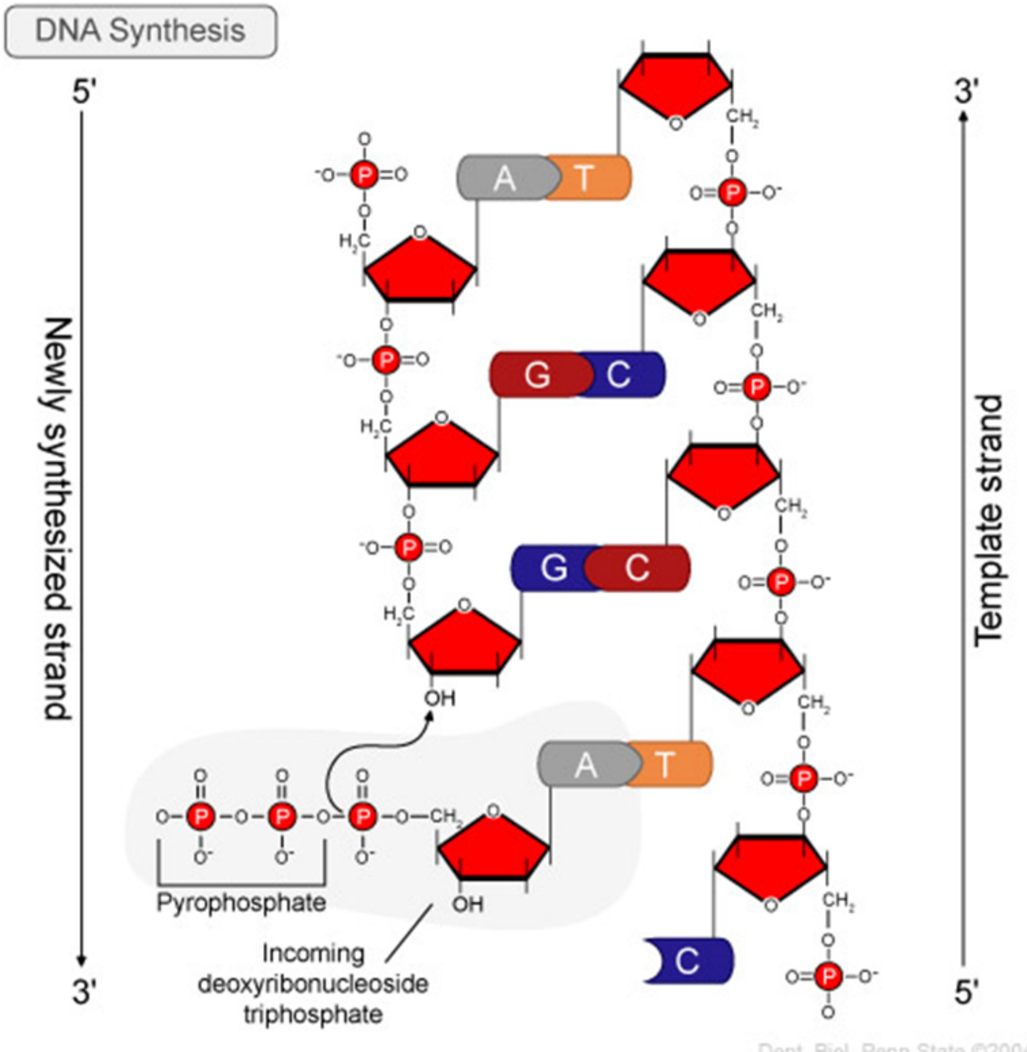


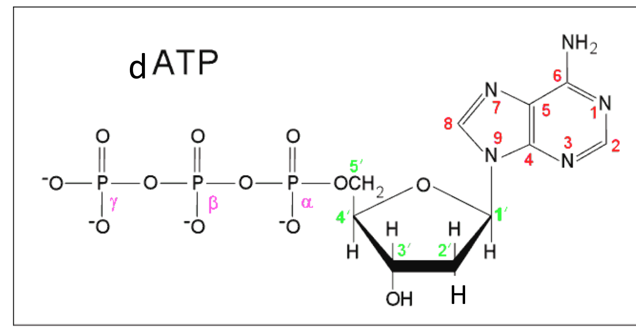
~60% Chance of Breast Cancer

BRCA2 Mutation



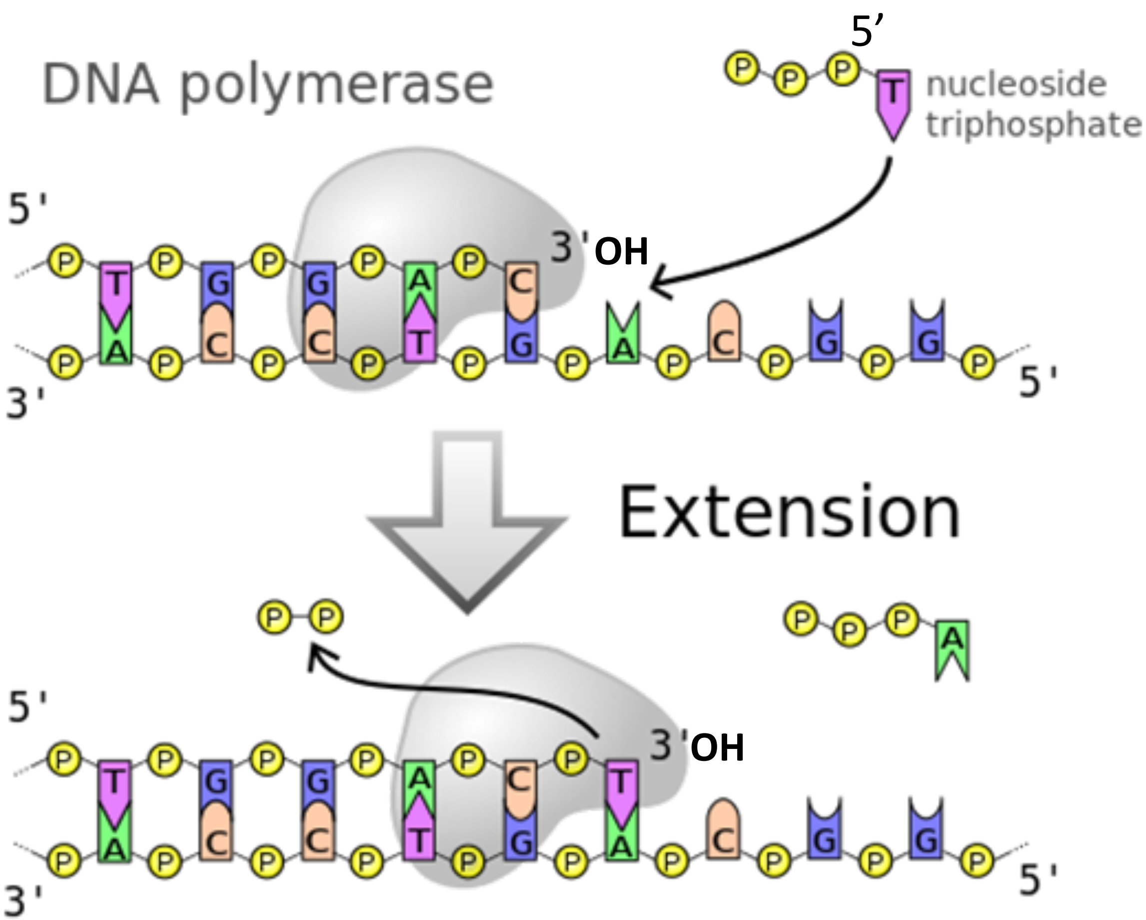
DNA Polymerase can only make new DNA in the 3' to 5' direction

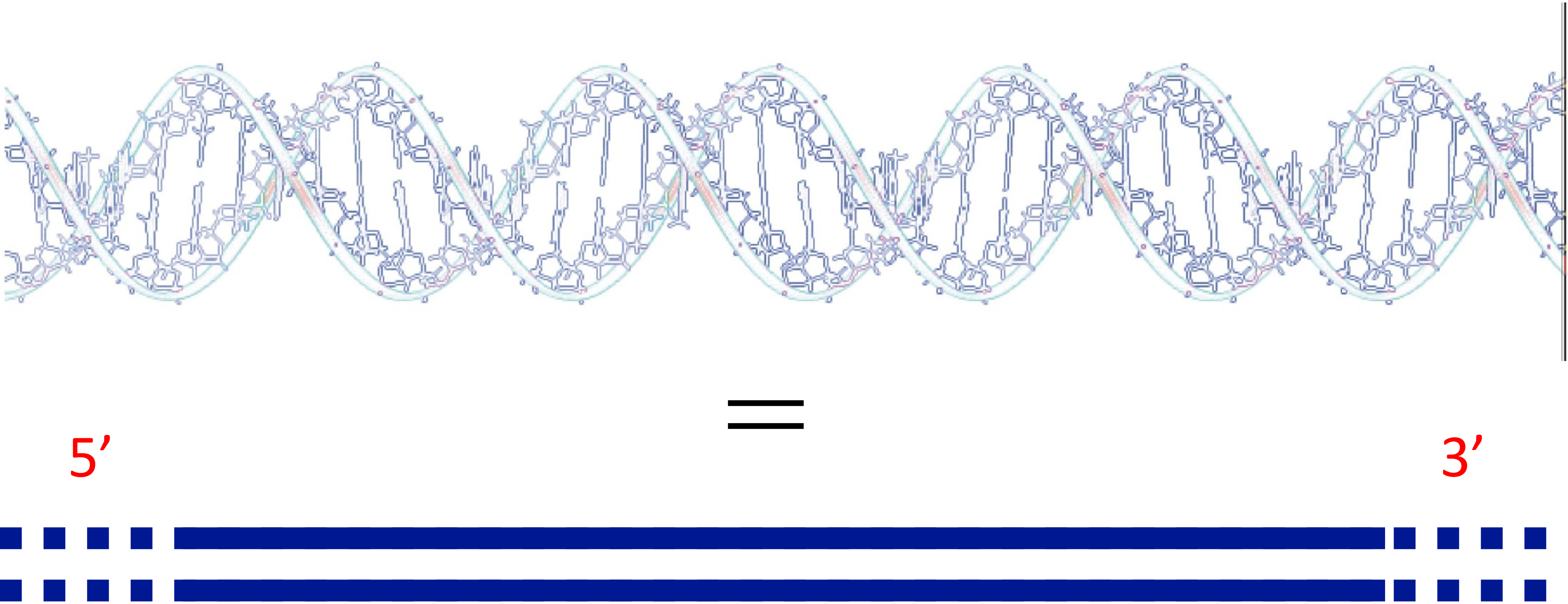


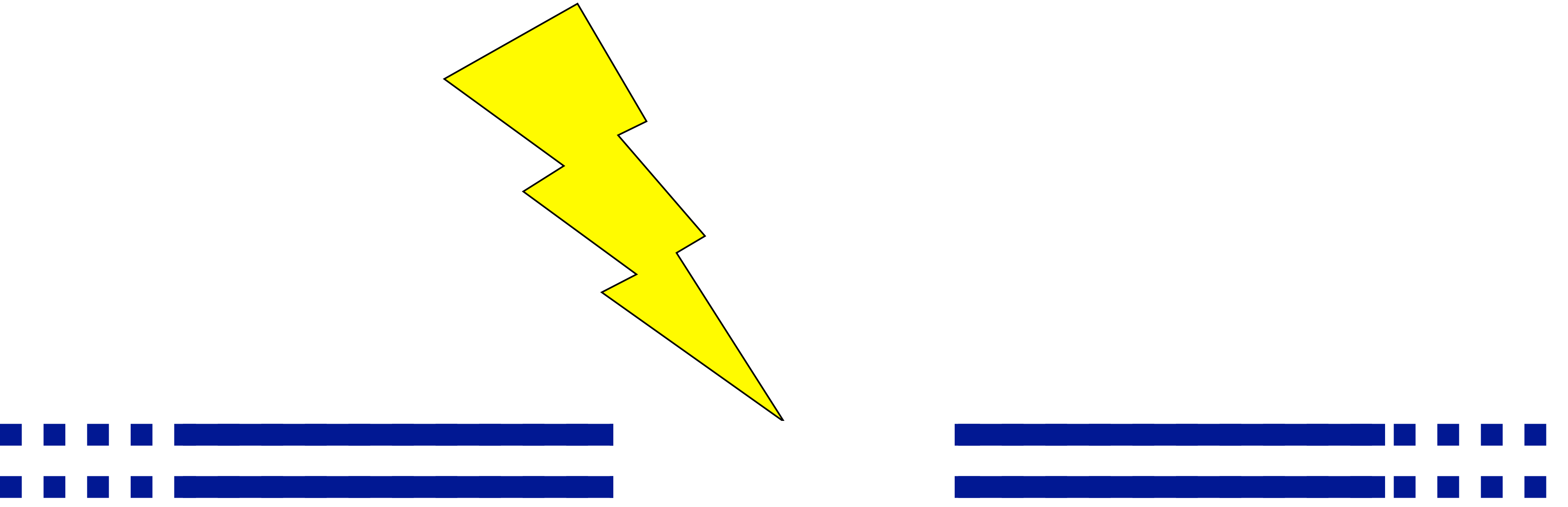


http://mbotc.tumblr.com/post/14905572540/the-nature-of-dna-polymerase

DNA Polymerase can only make new DNA in the 3' to 5' direction




5’
3’

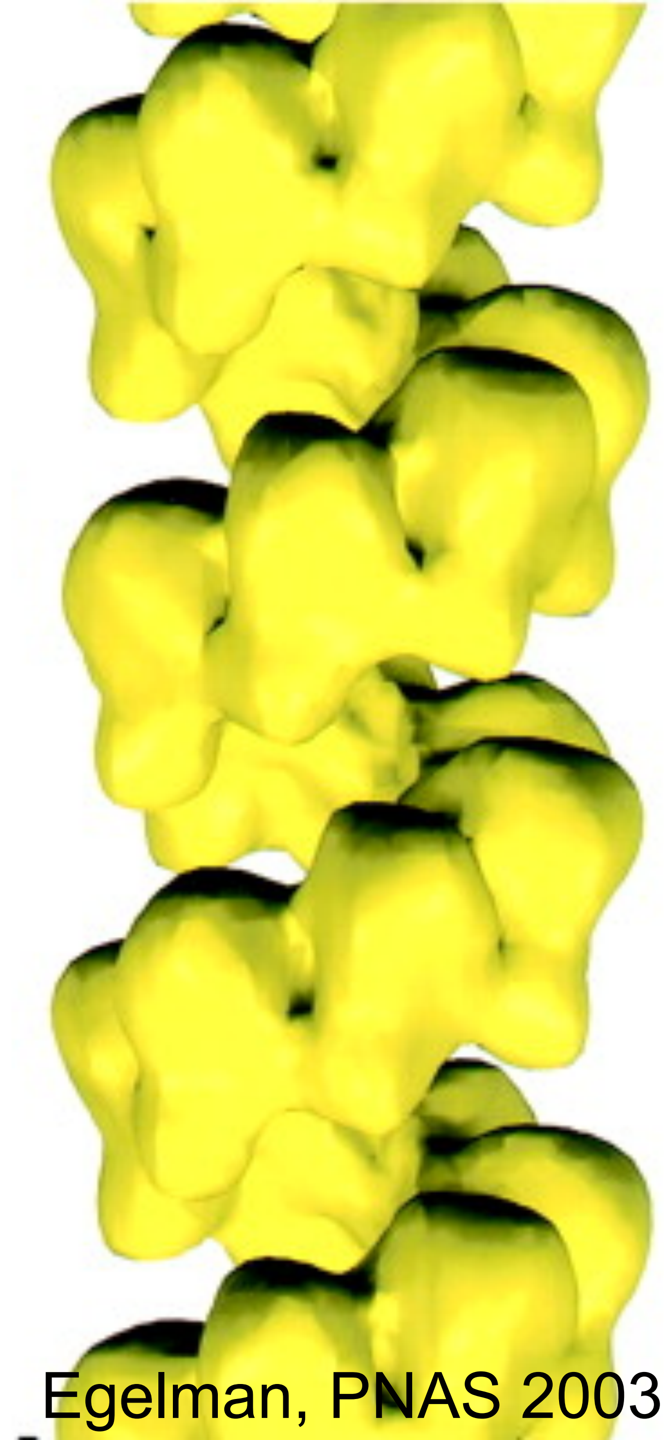


Imagine HR is initiated by the fragment on the left….

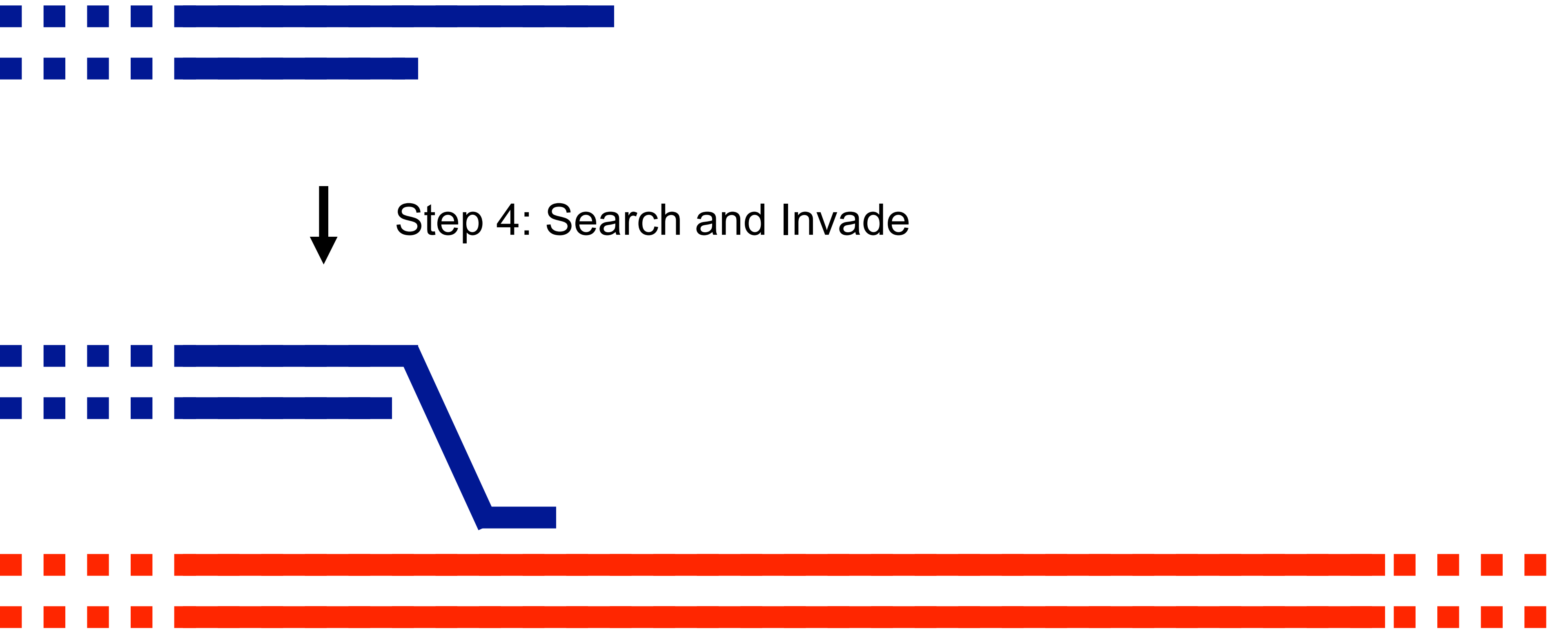
Step 1: A double stranded end has been created

Step 2: Resect the end to create a 3’ overhang

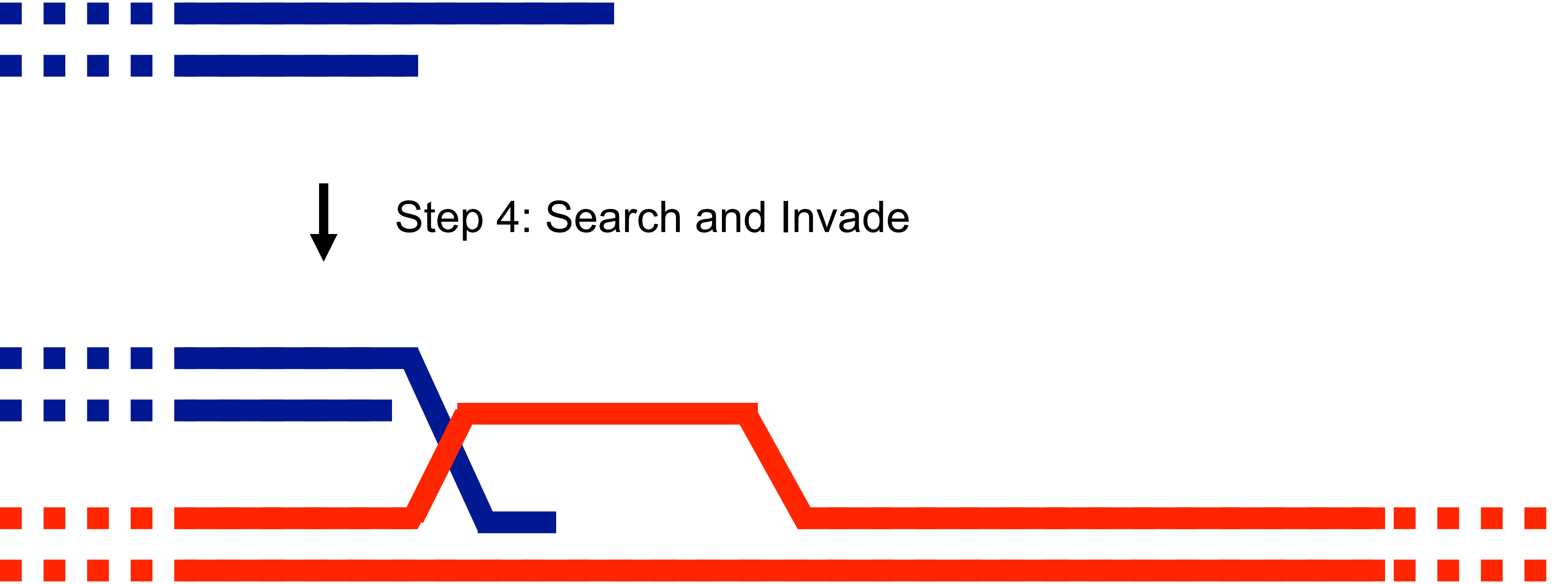
Step 3: Create a nucleoprotein filament capable of homology searching



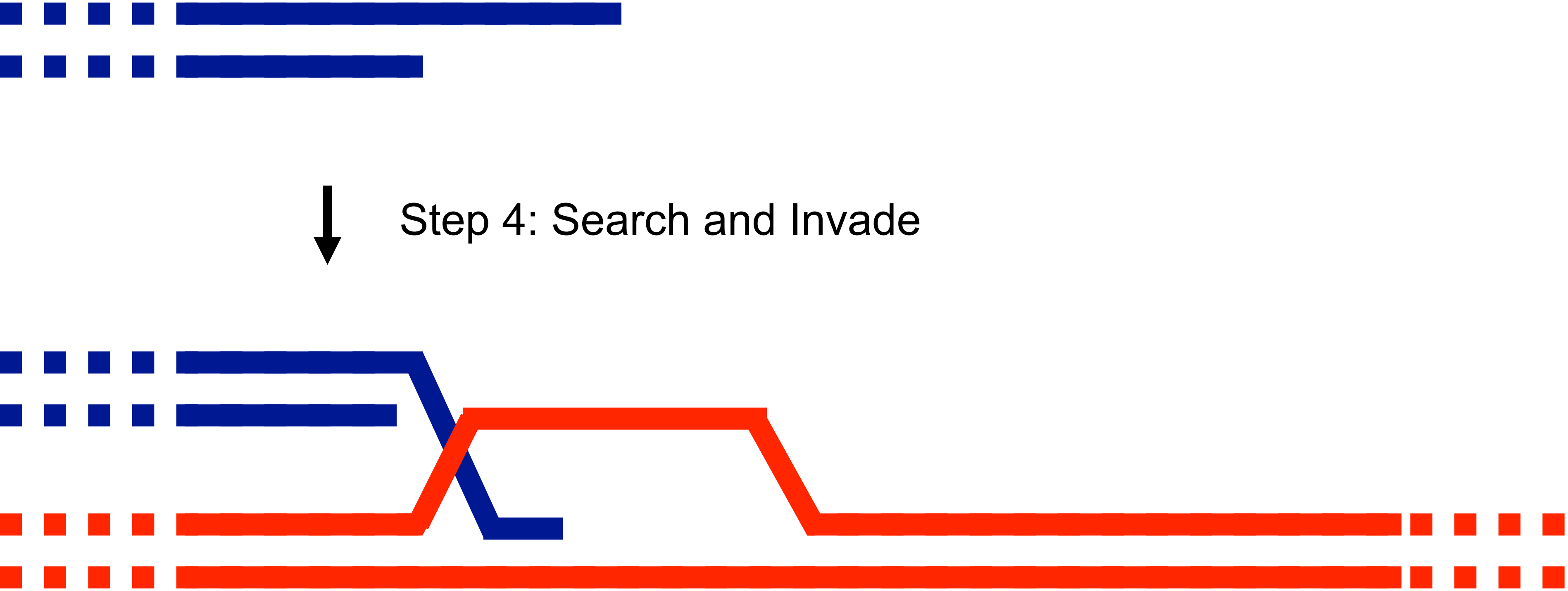
Egelman, PNAS 2003


Step 4: Search and Invade

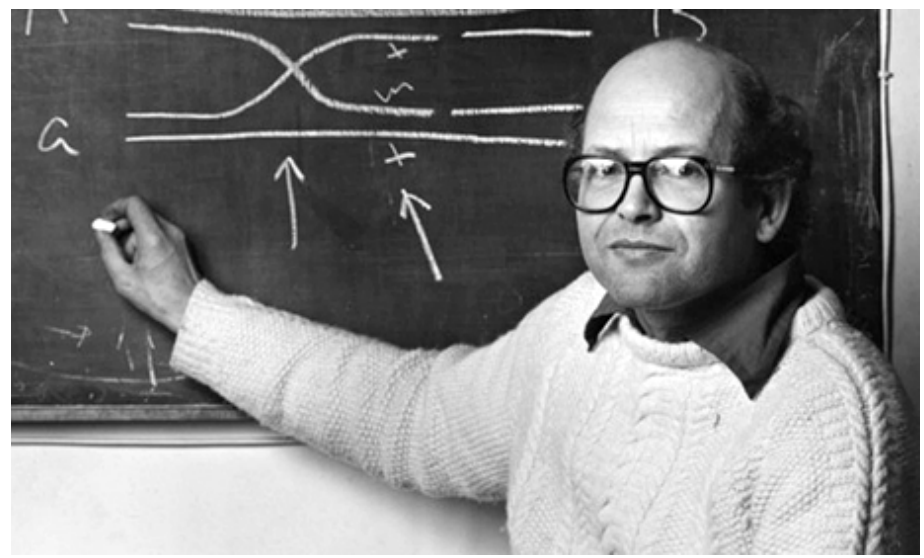

Step 4: Search and Invade



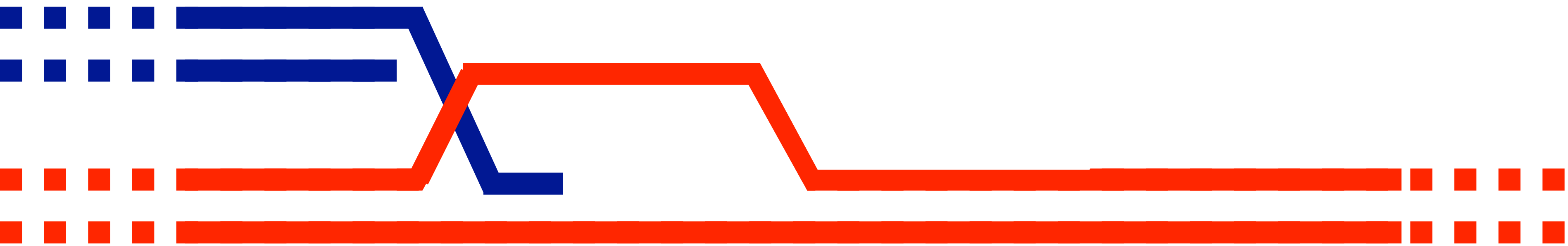
This DNA crossover structure is called a "Holliday Junction"



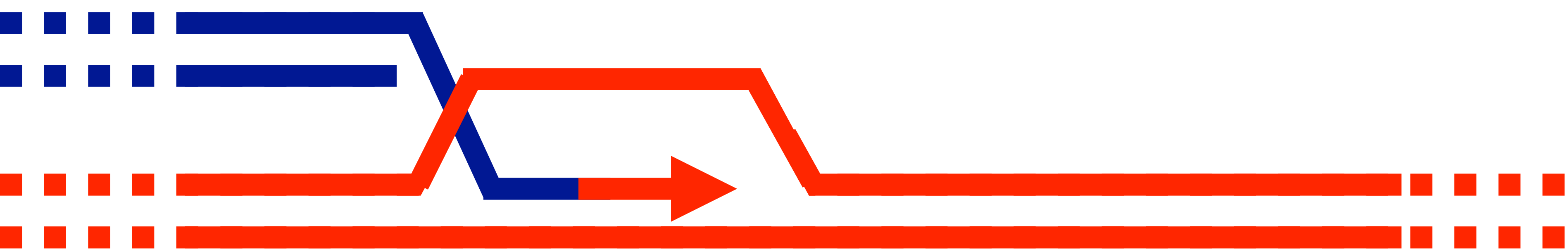
Robin Holliday
1932-2014

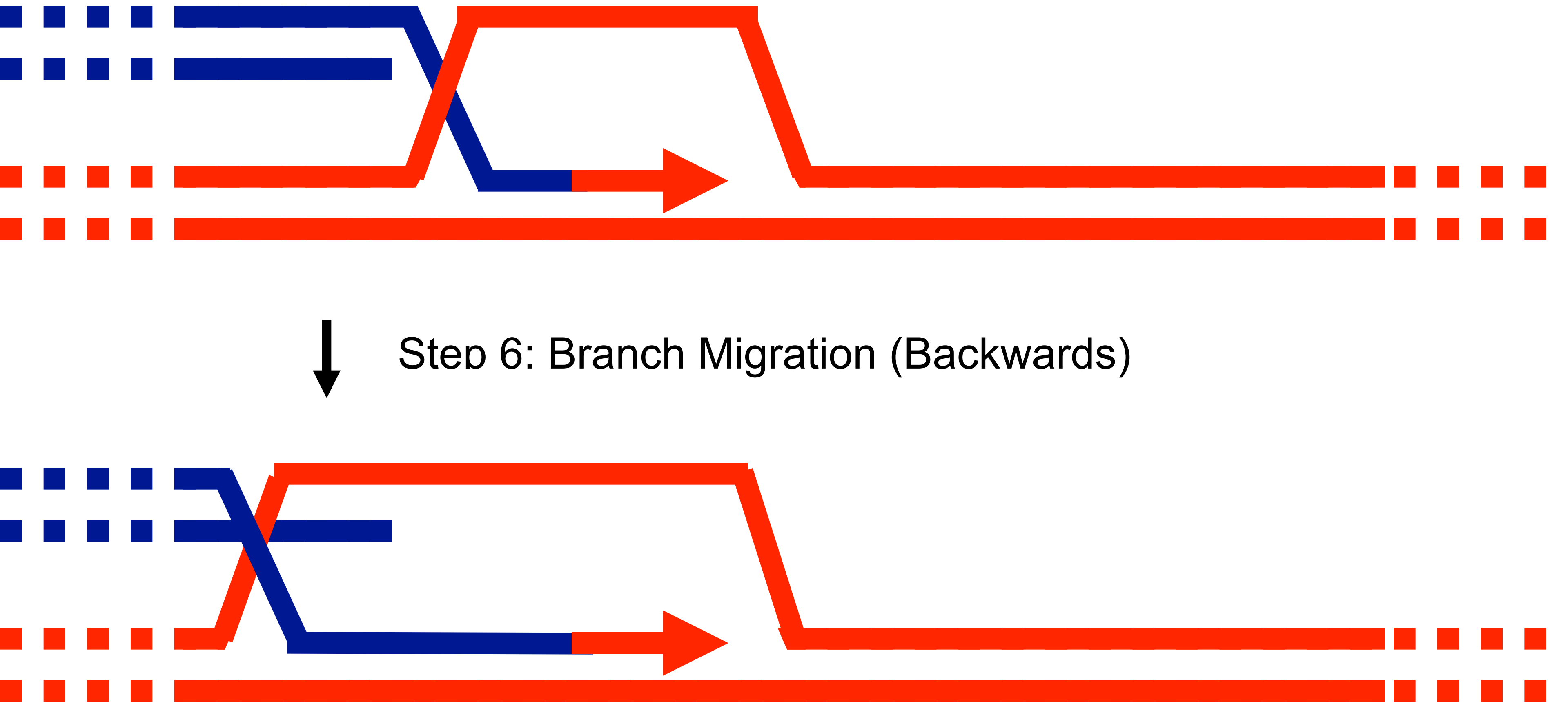


Step 4: Search and Invade

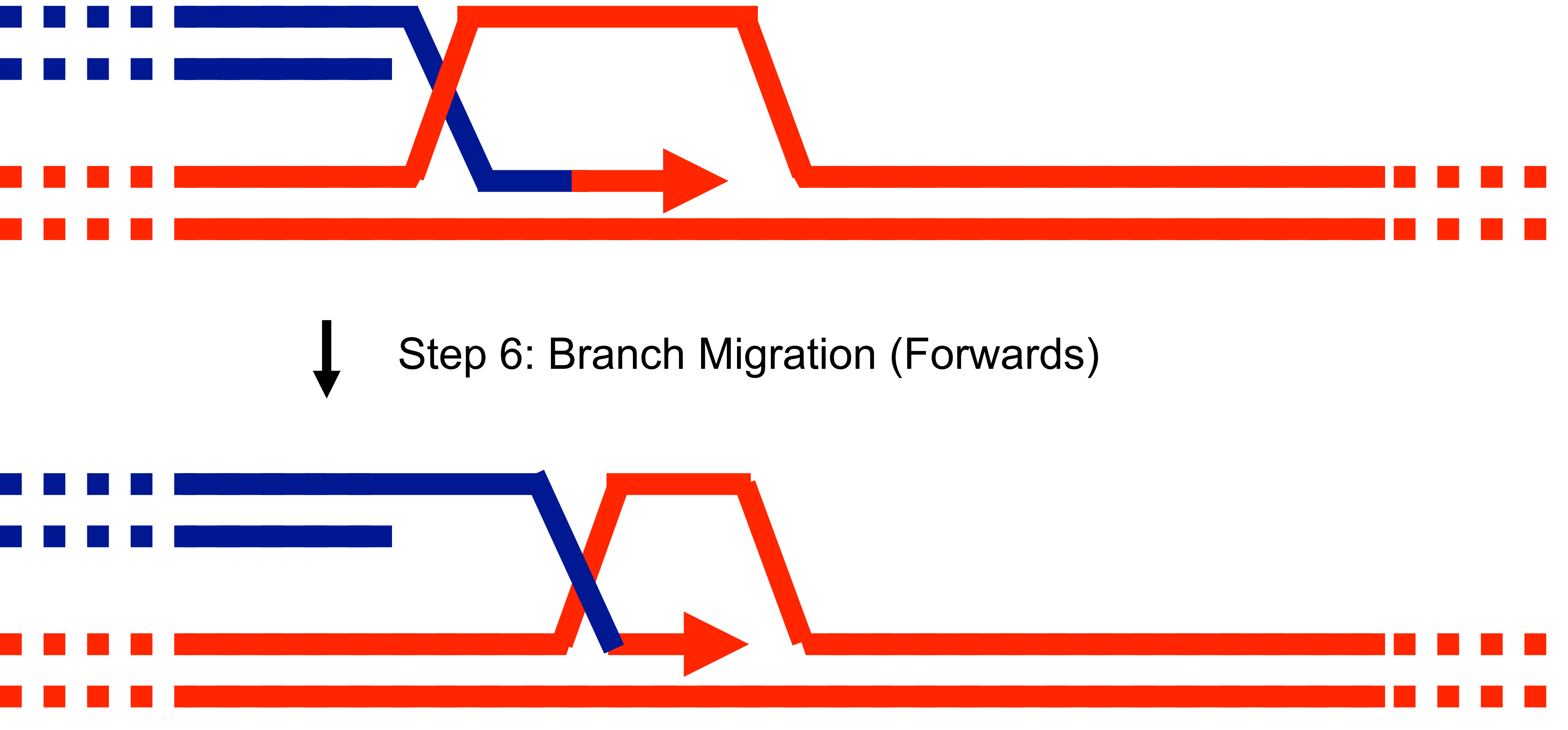


Step 5: Polymerize DNA using invading strand with 3' OH as a primer and the homologous donor DNA as a template

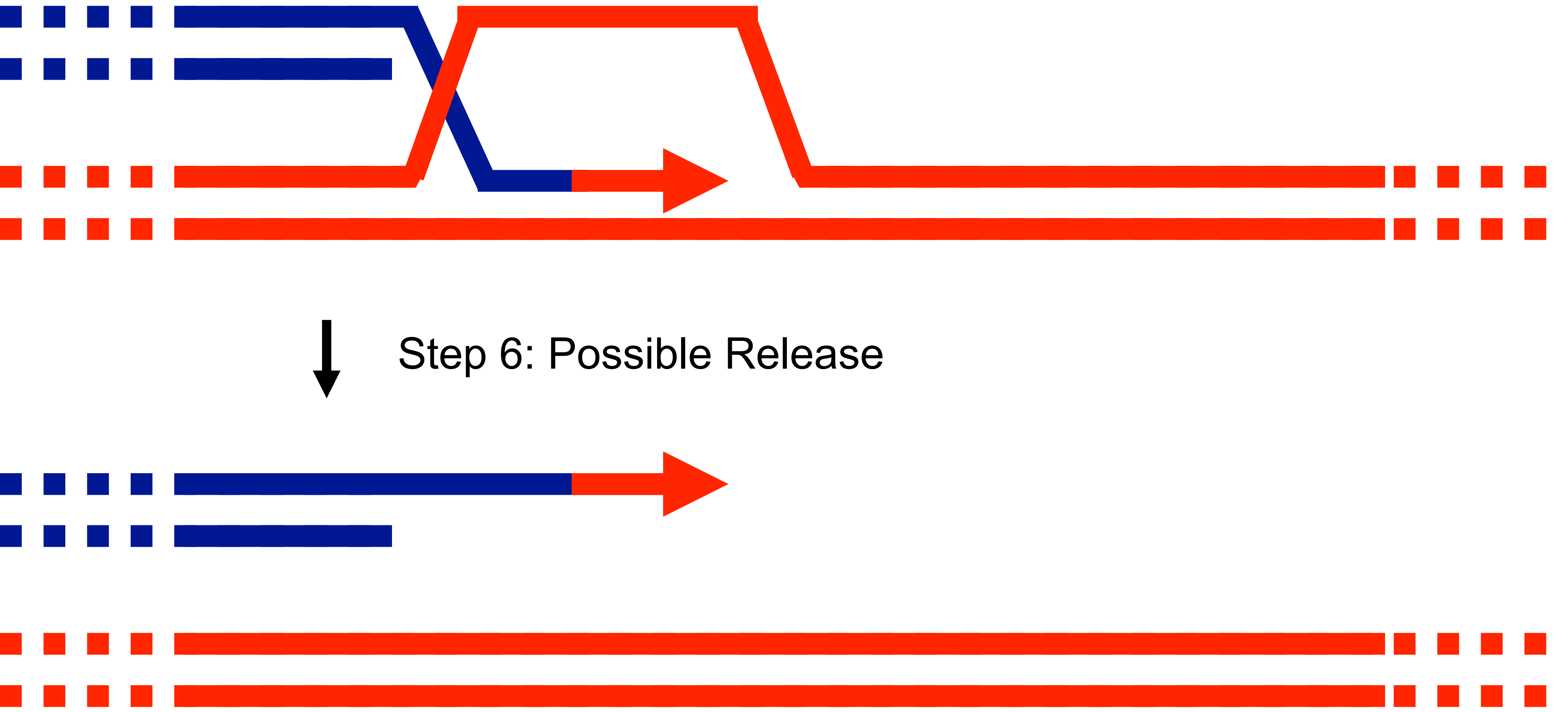


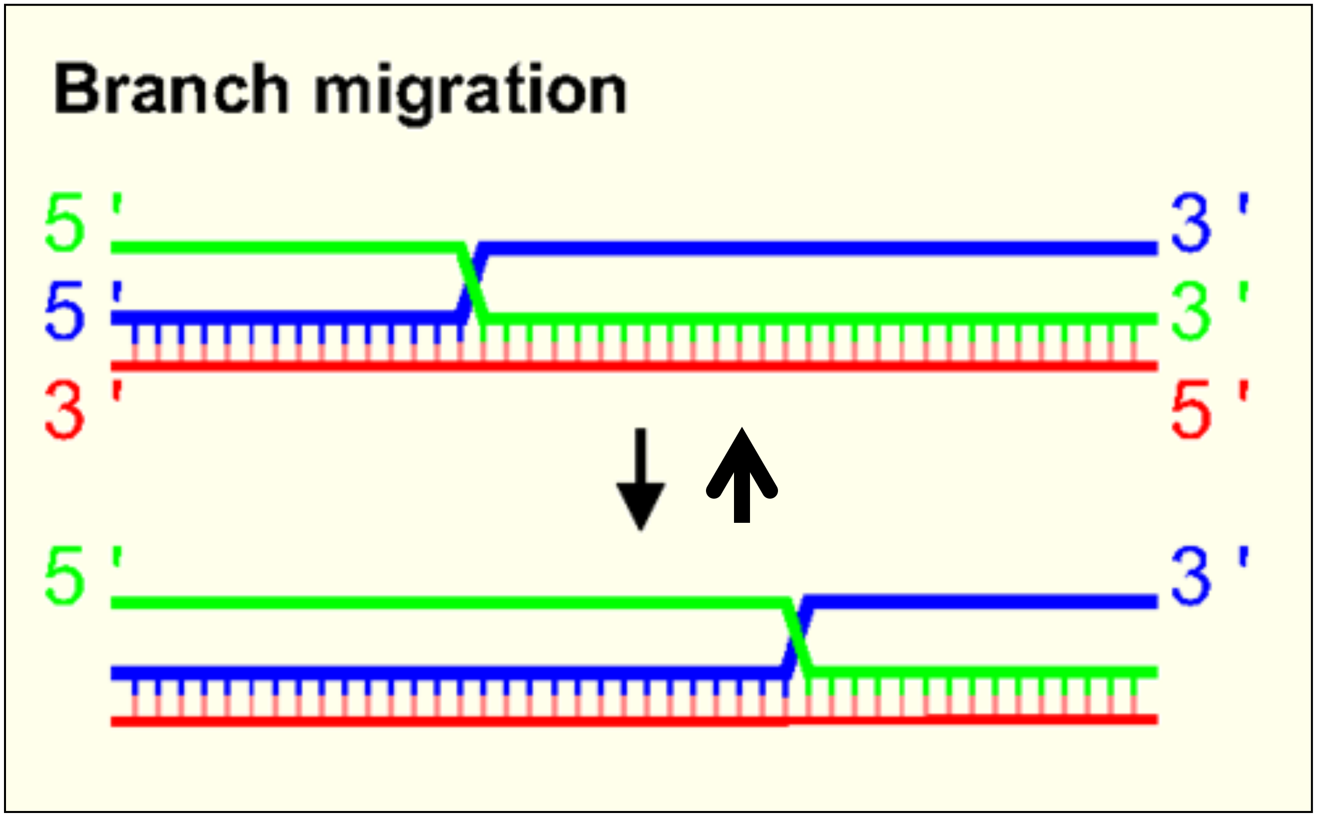


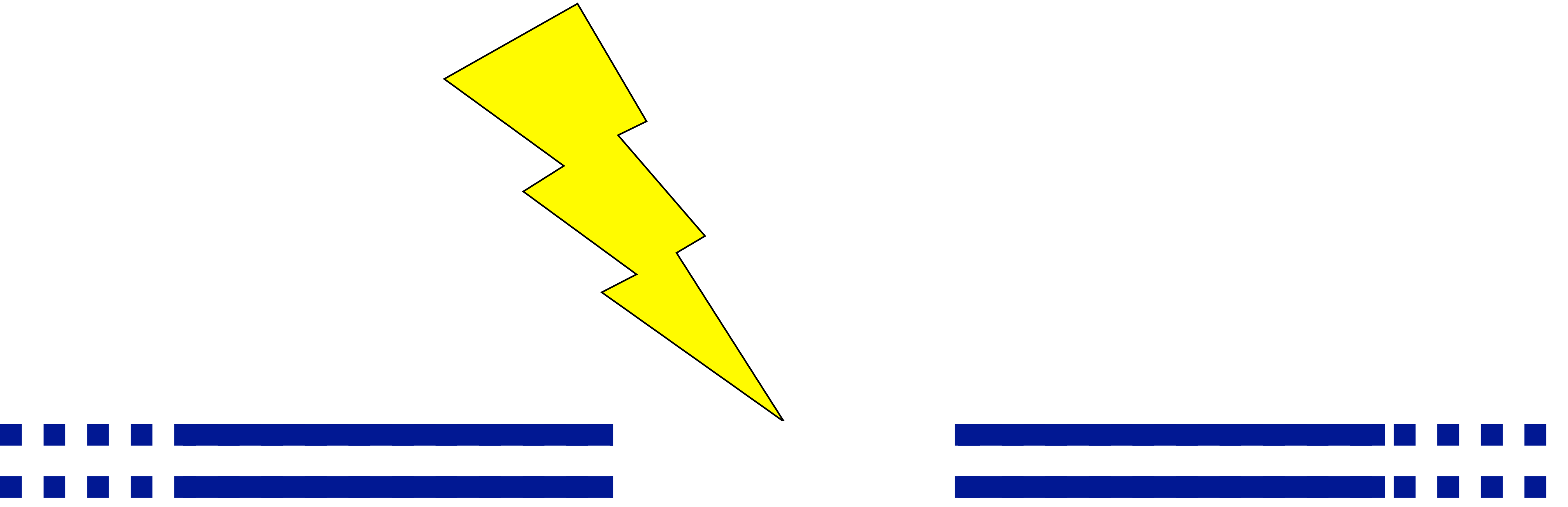
Step 6: Branch Migration (Backwards)



Step 6: Branch Migration (Forwards)

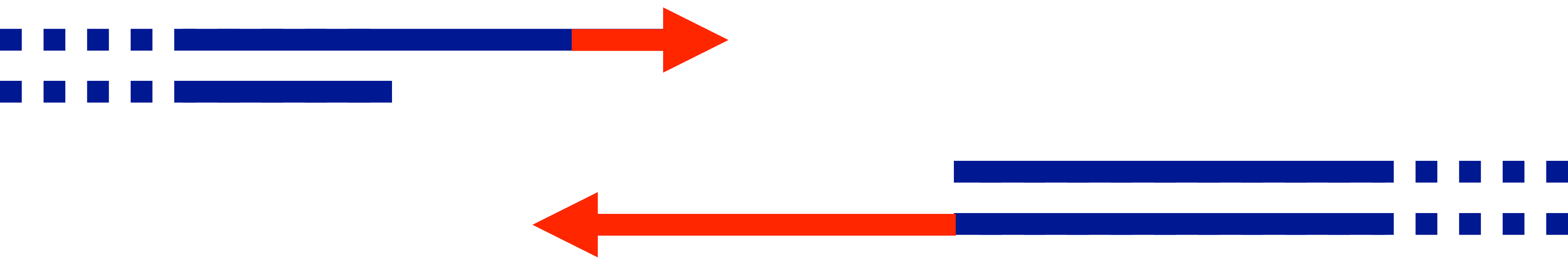

Step 6: Possible Release


Branch migration
5'
3'
5'
3'
3'
5'
5'
3'

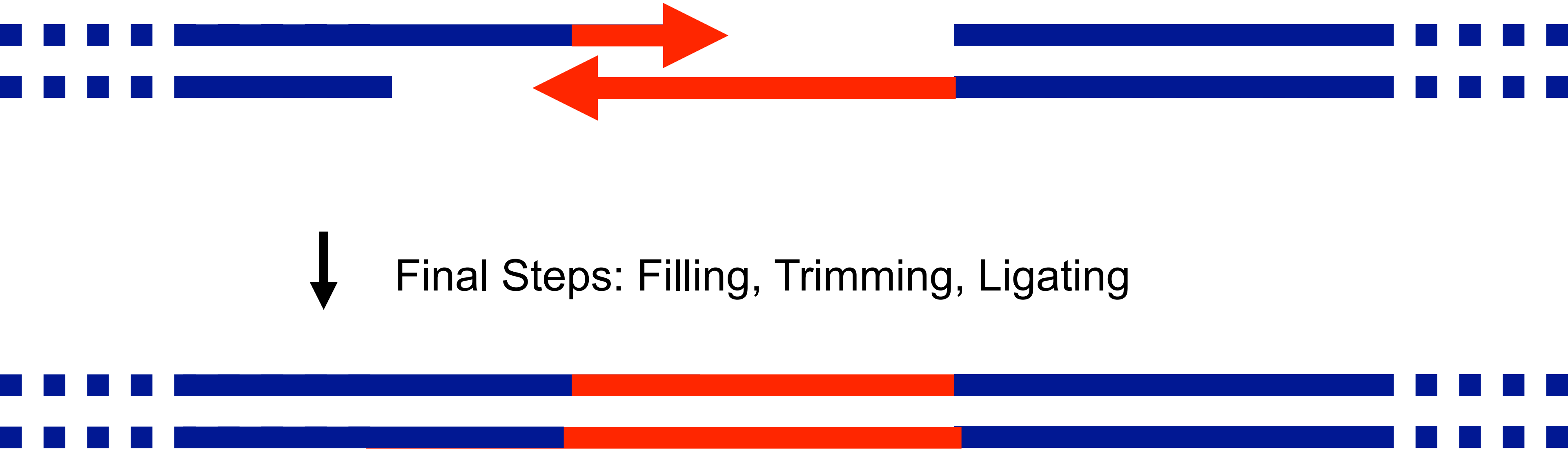


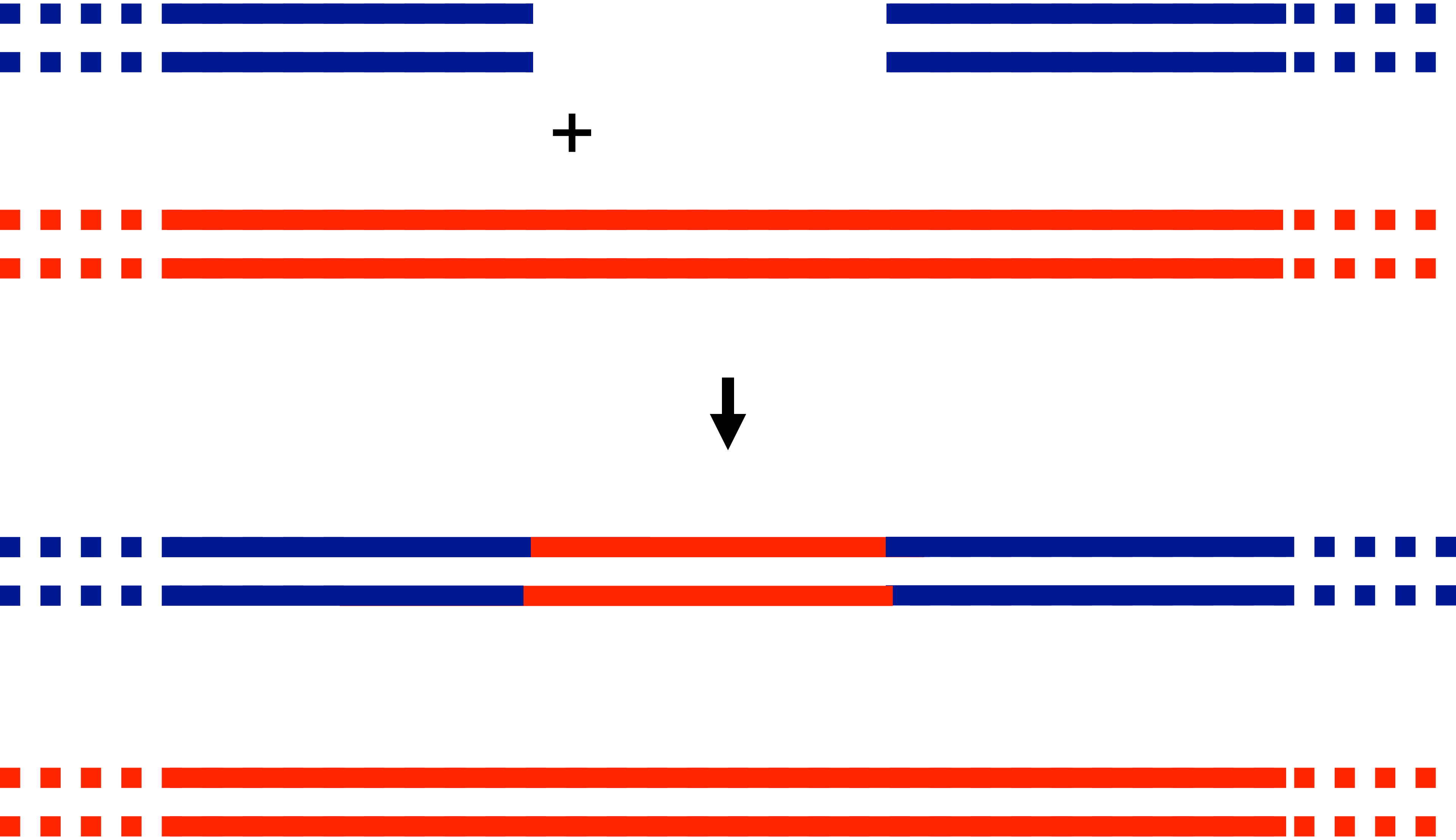
This process started with a two-ended DSB...

Now let's imagine the same thing happened at the other end…

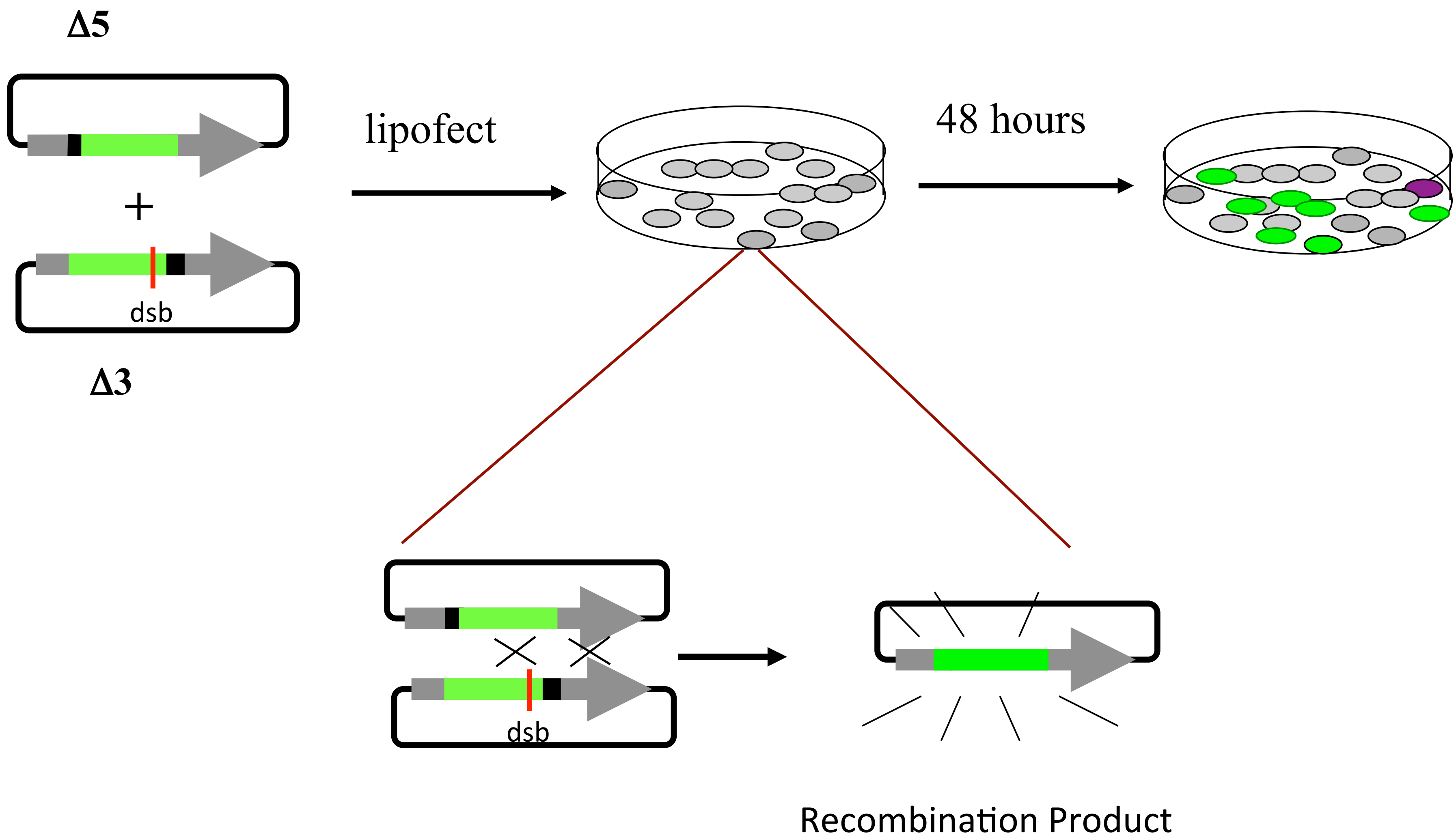


Annealing


Final Steps: Filling, Trimming, Ligating



A Plasmid-Based Assay for Homologous Recombination in Mammalian Cells

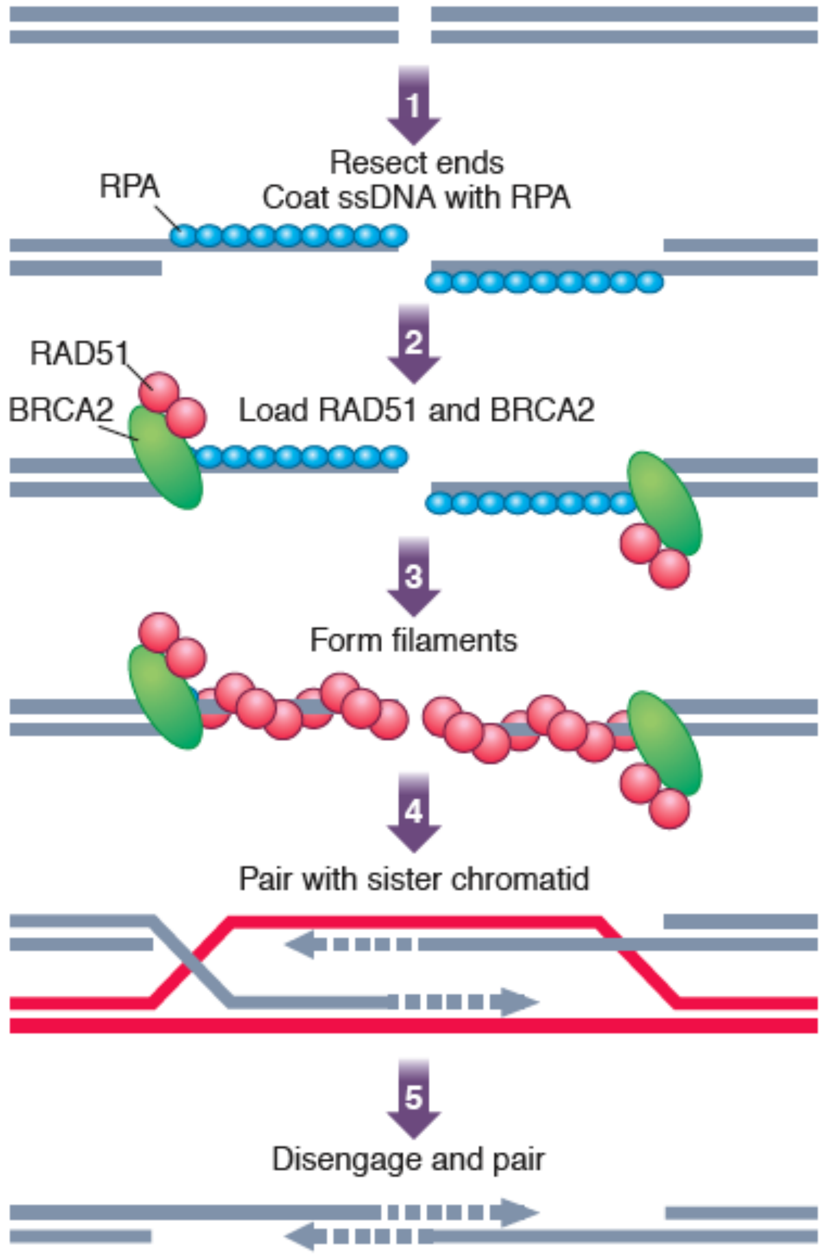


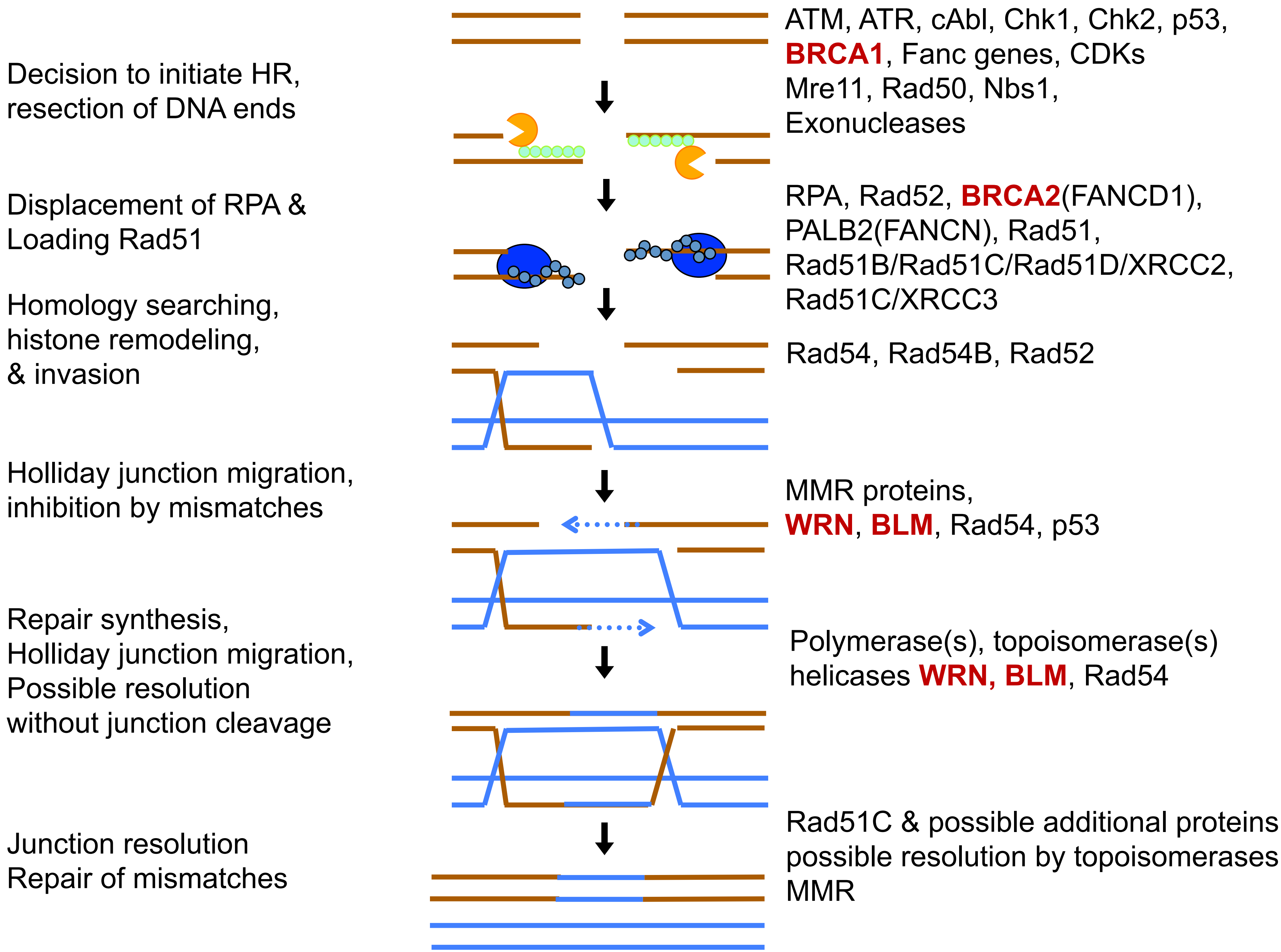
Bevin Engelward

http://web.mit.edu/engelward-lab/animations/NHEJ.html
Non Homologous End Joining

http://web.mit.edu/engelward-lab/animations/SDSA.html
DSB repair using Homologous Recombination

http://web.mit.edu/engelward-lab/animations/forkHR.html
Repair of a collapsed Replication Fork


1
Resect ends
Coat ssDNA with RPA
RPA
2
RAD51
BRCA2
Load RAD51 and BRCA2
3
Form filaments
4
Pair with sister chromatid
5
Disengage and pair


Decision to initiate HR,
resection of DNA ends
ATM, ATR, cAbl, Chk1, Chk2, p53,
BRCA1, Fanc genes, CDKs
Mre11, Rad50, Nbs1,
Exonucleases
Displacement of RPA &
Loading Rad51
RPA, Rad52, BRCA2(FANCD1),
PALB2(FANCN), Rad51,
Rad51B/Rad51C/Rad51D/XRCC2,
Rad51C/XRCC3
Homology searching,
histone remodeling,
& invasion
Rad54, Rad54B, Rad52
Holliday junction migration,
inhibition by mismatches
MMR proteins,
WRN, BLM, Rad54, p53
Repair synthesis,
Holliday junction migration,
Possible resolution
without junction cleavage
Polymerase(s), topoisomerase(s)
helicases WRN, BLM, Rad54
Junction resolution
Repair of mismatches
Rad51C & possible additional proteins;
possible resolution by topoisomerases;
MMR

Homologous Recombination Repairs DNA

Werner Syndrome (WS)

* develop normally in early age
* premature aging starting at puberty
* short stature
* leg ulceration
* soft-tissue calcification
* average life span = 47
* cancer and cardiovascular diseases are primary cause of death

Defects in HR Promote Aging, Cancer, & other Diseases

Homologous Recombination Repairs DNA

Werner Syndrome (WS)

* develop normally in early age
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Defects in HR Promote Aging, Cancer, & other Diseases

Homologous Recombination Repairs DNA

Bloom Syndrome (BS)

* sun-sensitive skin
* dwarfism
* immune deficiencies
* male infertility
* female subfertile
* cancer as primary cause of death before age of 30

Werner Syndrome (WS)

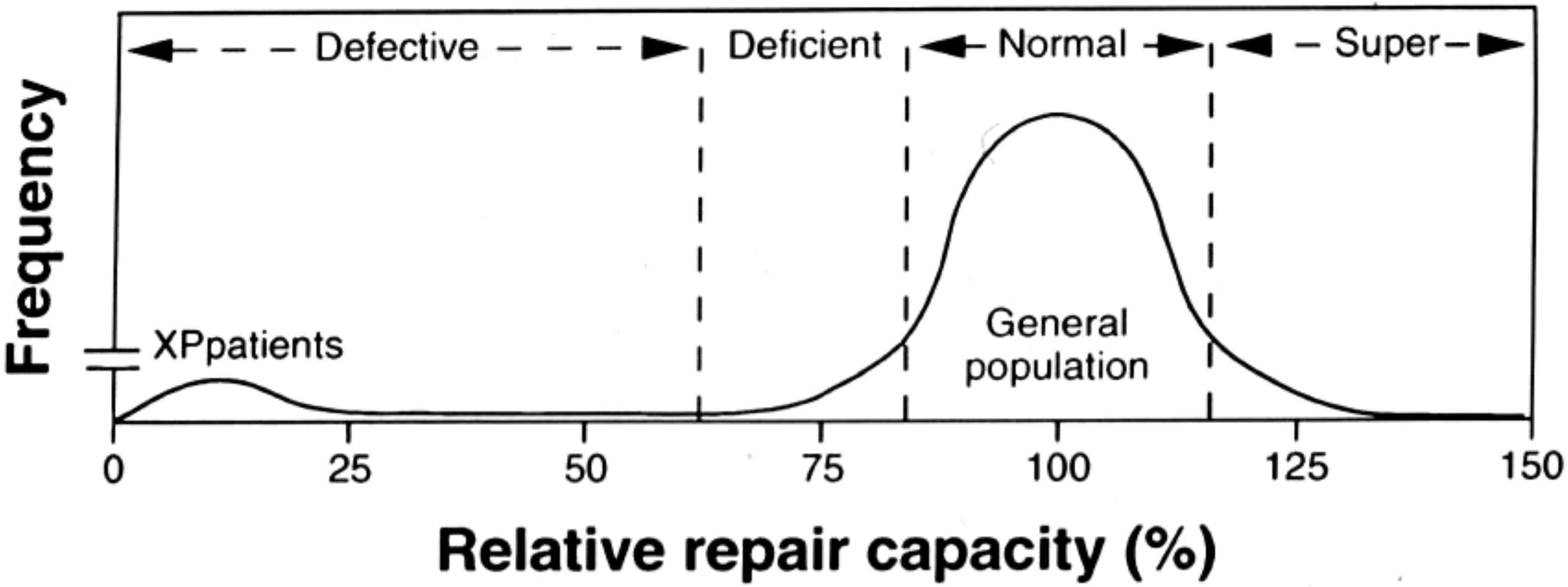
* develop normally in early age
* premature aging starting at puberty
* short stature
* leg ulceration
* soft-tissue calcification
* average life span = 47
* cancer and cardiovascular diseases are primary cause of death

Rothmund-Thomson Syndrome (RTS)

* sun-sensitive
* hyper-pigmentation of skin
* short stature
* bone abnormality
* cancer predisposition, especially osteosarcoma

Defects in HR Promote Aging, Cancer, & other Diseases

Interindividual Variation in DNA Repair Capacity



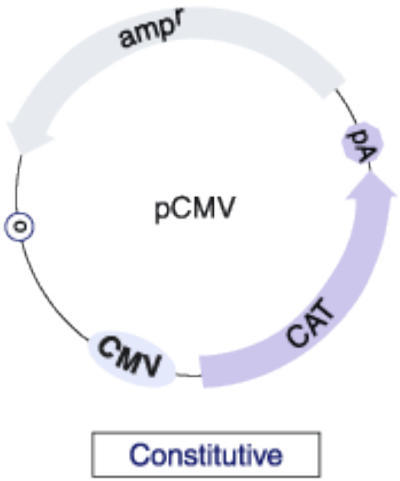
Adapted from **GROSSMAN and Wei (1995)** Clinical Chem 41: 1854-1863

XP frequency = ~1:250,000 giving a theoretical maximum of ~28,000 cases worldwide with 2,000-fold increased risk

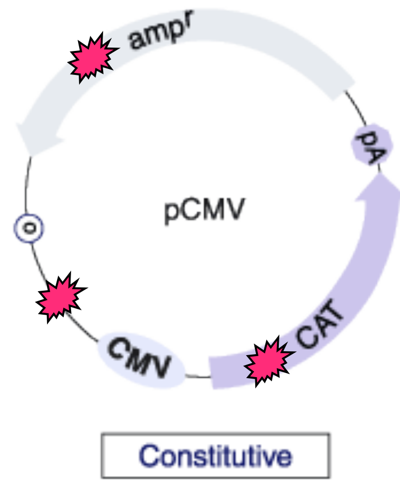
Even if just 1% of the population is relatively repair deficient, could have tens of millions with several-fold increased risk

Reactivation of UV damaged DNA by Host cell Reactivation (HCR)

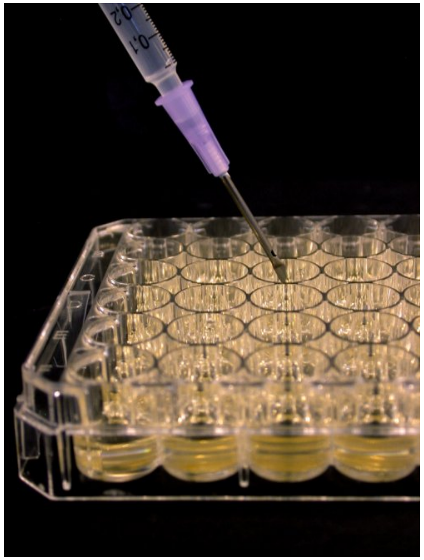
Athas & GROSSMAN
Cancer Res. 1991



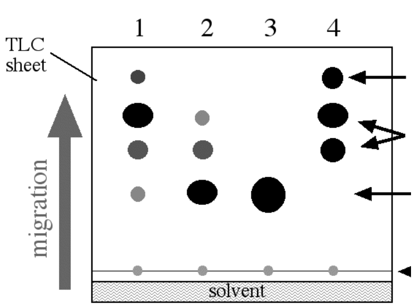
+ UV light



Transient transfection peripheral blood lymphocytes

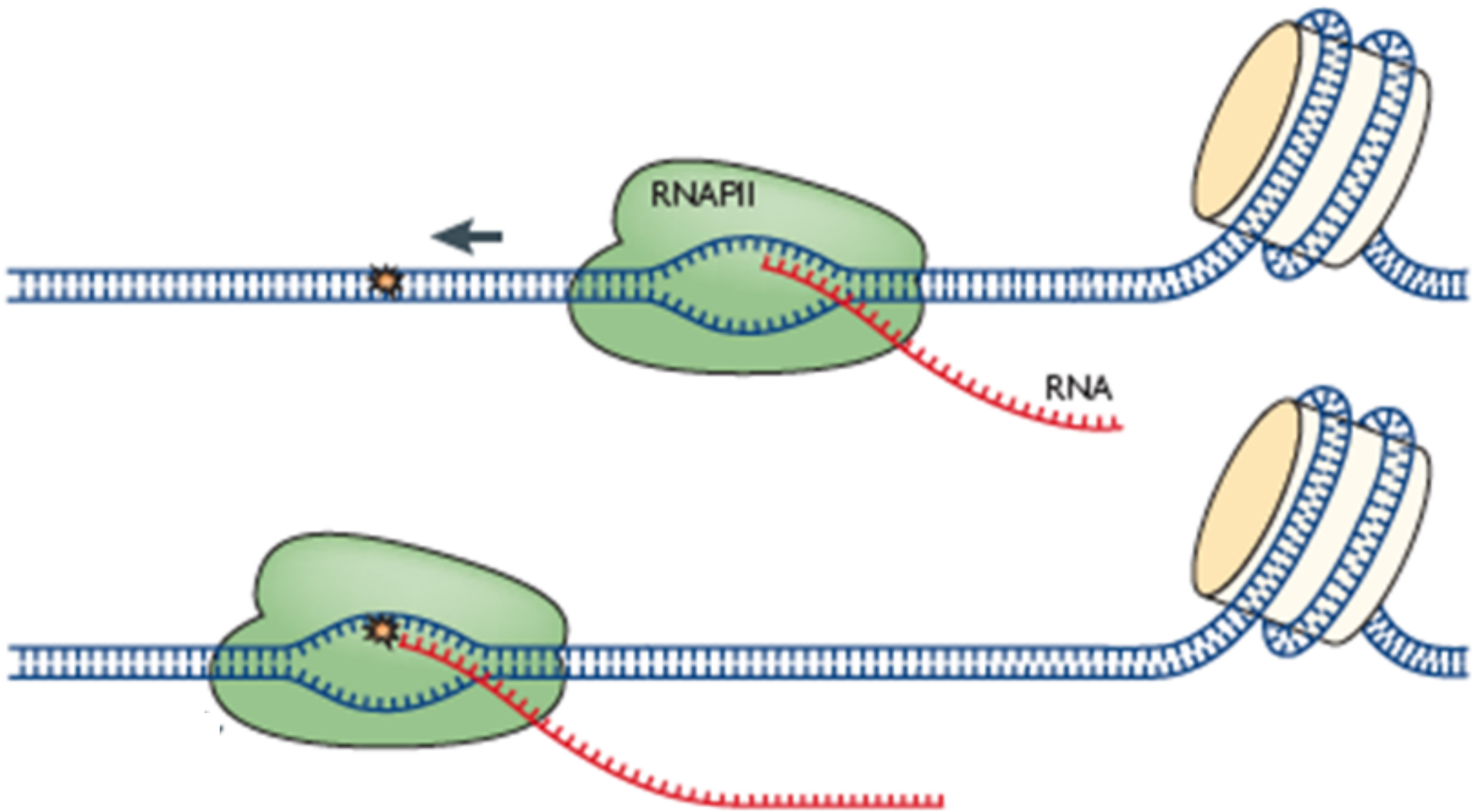


Time to repair



CAT Assay

RNA Polymerase II is exquisitely sensitive to DNA lesions



Nature Reviews Molecular Cell Biology 9, 958-970

Reactivation of UV damaged DNA by Host cell Reactivation (HCR)

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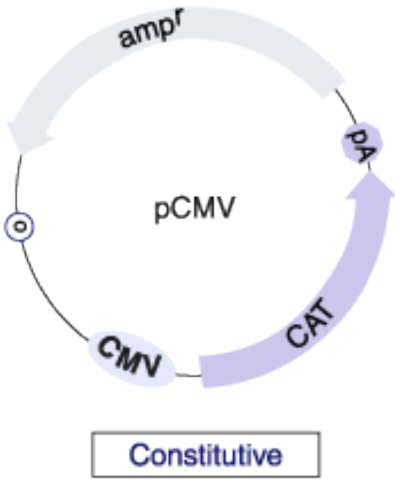
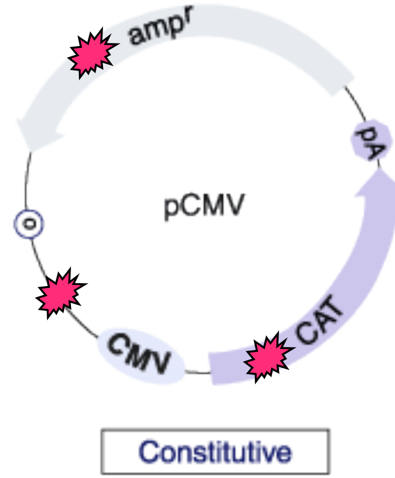


+ UV light



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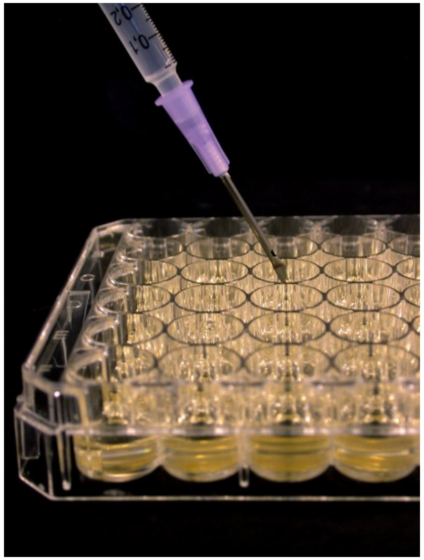
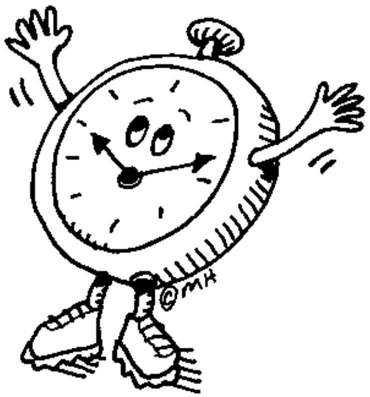
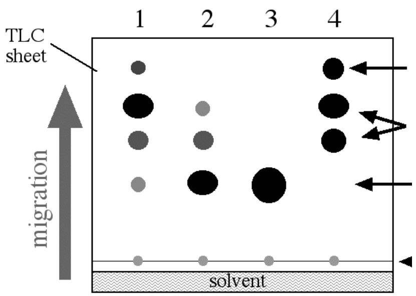
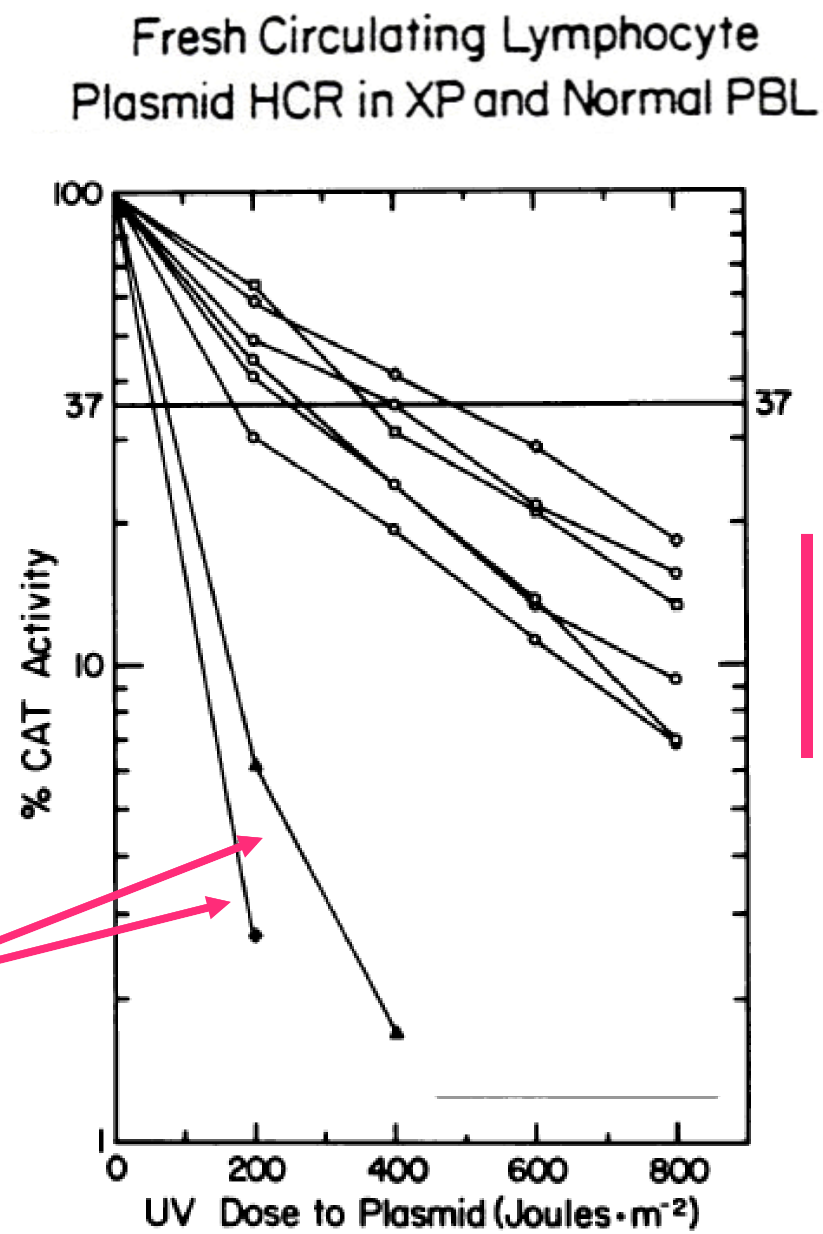




Time to repair



CAT Assay

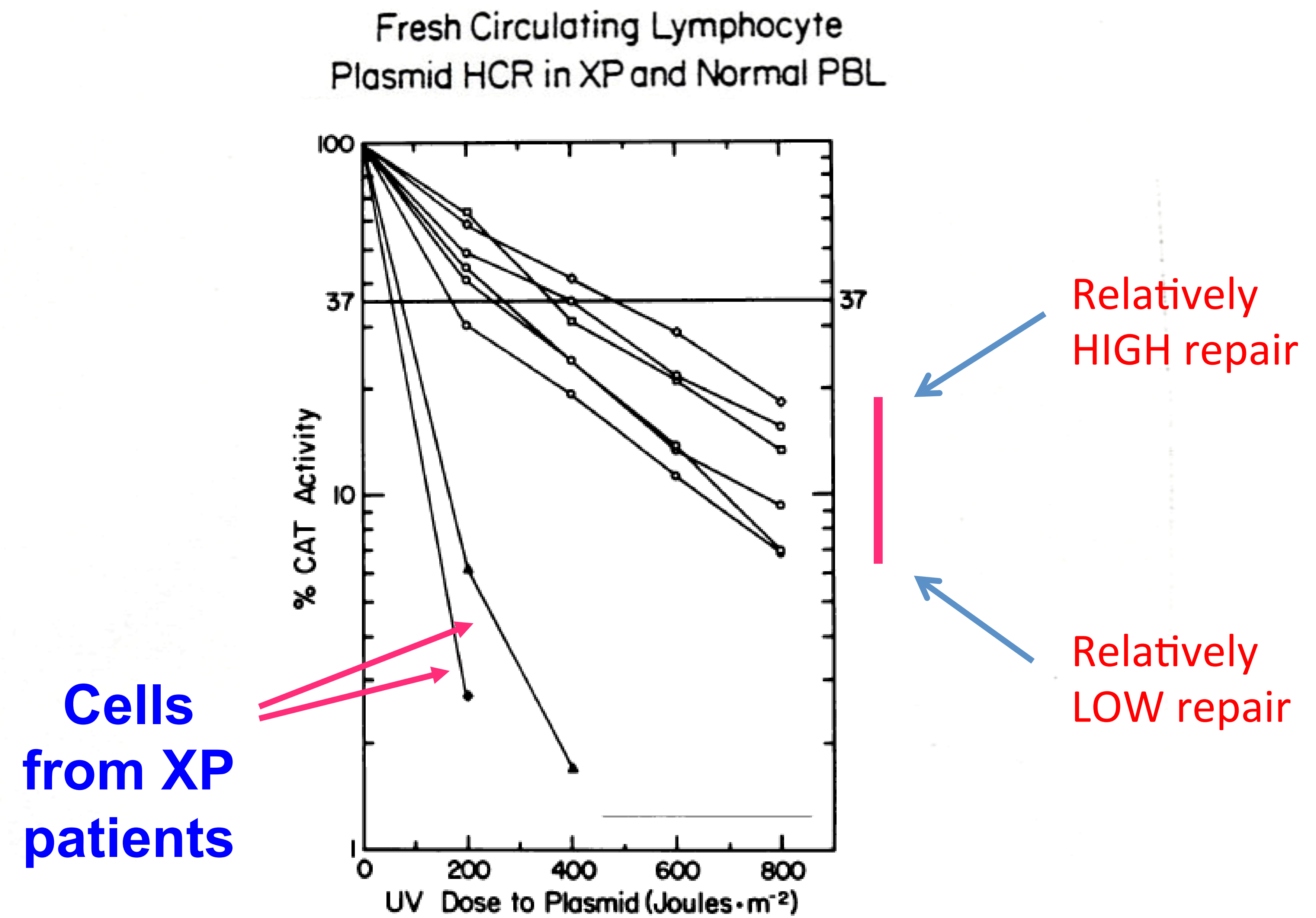


Cells from 'healthy" people

Cells from XP patients

Athas & GROSSMAN

[CANCER RESEARCH 51, 5786–5793, November 1, 1991]



Athas & GROSSMAN

[CANCER RESEARCH 51, 5786–5793, November 1, 1991]

Virtually all case/control HCR studies have monitored Nucleotide Excision Repair (NER)

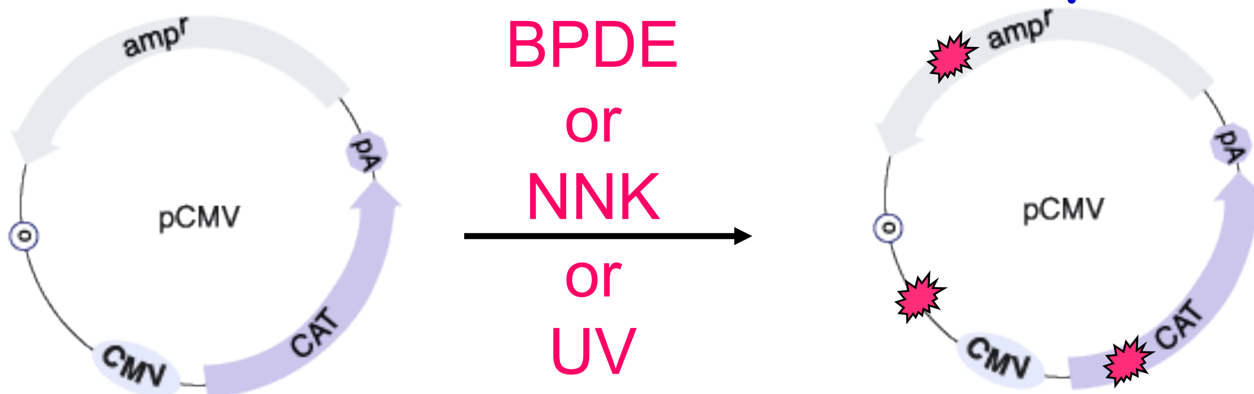


TABLE III – HCR-DRC FOR RISK OF CANCERS

Mutagen	Cancer type	Number Case/control	Risk estimate	Reference
BPDE	Lung	51/56	5.70 (2.10–15.7)	Wei *et al*. 1996[25]
	Lung, nonsmall cell	467/488	1.85 (1.42–2.42)	Shen *et al*. 2003 [58]
	Lung	764/677	1.50 (1.10–3.10)	Spitz *et al*. 2003[37]
	SCCHN	55/61	2.20 (1.02–4.77)	Cheng *et al*. 1998[61]
	Breast	69/79	3.36 (1.15–9.80)	Shi *et al*. 2004[64]
NNK	Lung, adenocarcinoma	48/45	3.21 (1.25–8.21)	Wang *et al*. 2007[59]
UV	BCC	146/333	1.62 (1.07–2.45)	Wang *et al*. 2007[63]
	SCC	109/333	1.63 (0.95–2.79)	
	CM	312/324	2.02 (1.45–2.82)	Wei *et al*. 2002[62]

BPDE, benzo(a)pyrene diol epoxide; UV, ultraviolet; SCCHN, squamous cell carcinoma of head and neck; BCC, basal cell carcinoma; SCC, squamous cell carcinoma; CM, cutaneous melanoma.

Chunying Li, Li-E. Wang and Qingyi Wei* *Int. J. Cancer:* **124**, 999–1007 (2009)

DNA Repair Strategies

- Direct Reversal

  Methyltransferase, Oxidative demethylase

- Excision Repair

  Base excision, nucleotide excision, mismatch repair

- Double strand break repair

  Homologous recombination, Non-homologous end joining

NIH DIRECTOR'S

PIONEER

A·W·A·R·D

CANDIDATE INTERVIEW

June 16th 2009, 8am!

Developing Novel Methods to Measure DNA Repair Capacity in Human Populations

Leona D. Samson

MIT

Biological Engineering Department

Biology Department

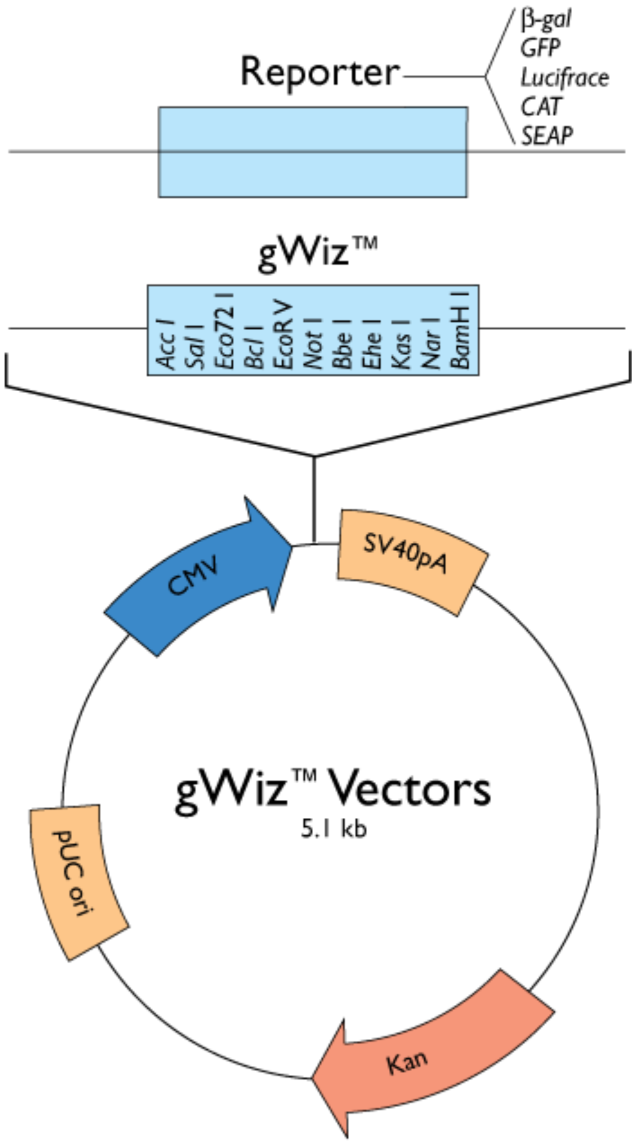
Center for Environmental Health Sciences

Koch Institute for Integrative Cancer Research

Computational and Systems Biology Initiative

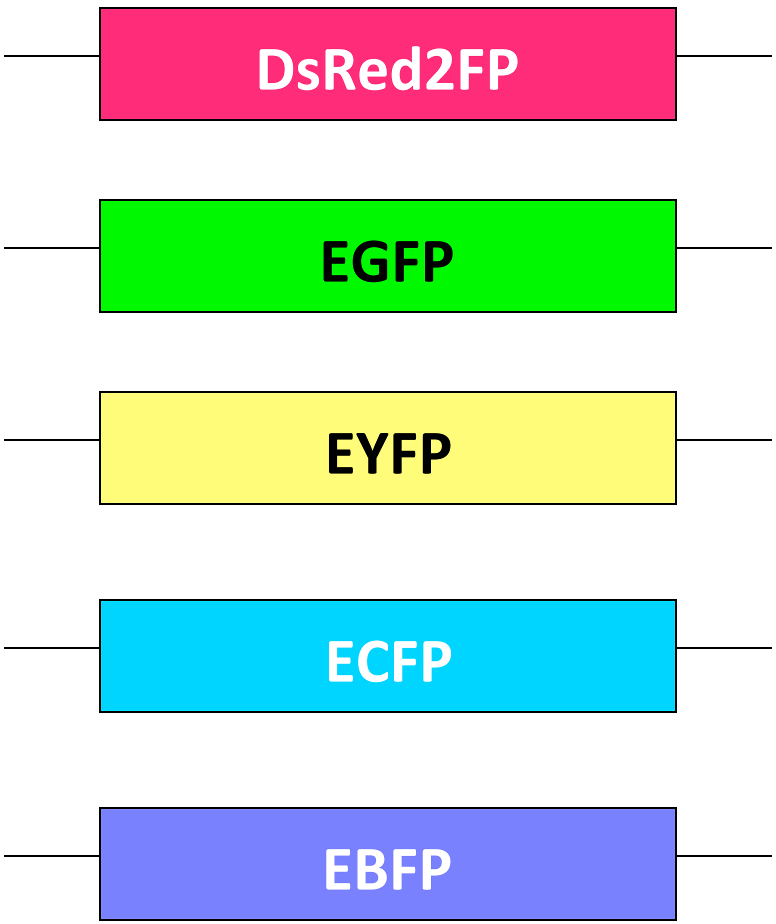
Broad Institute (Harvard and MIT)

Reactivation of damaged DNA - multiplexed

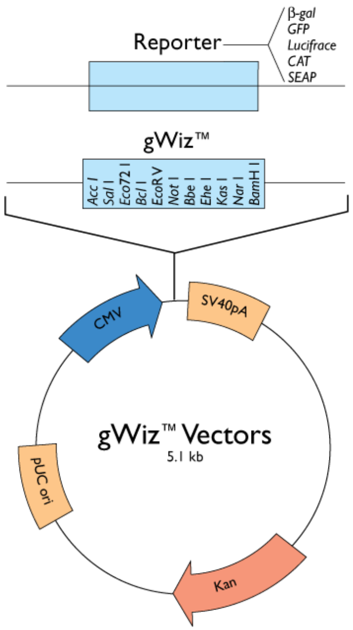


http://www.genlantis.com/corp/images/gWiz_vectmap.gif

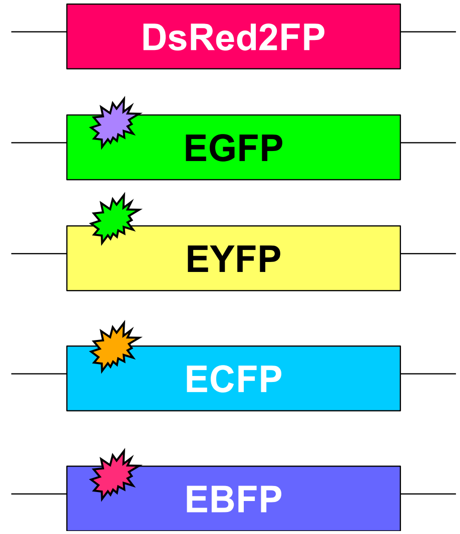
Each Fluorescent Protein gene will harbor a different type of DNA damage



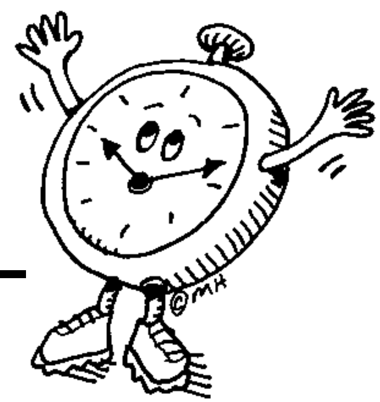
Reactivation of damaged DNA - multiplexed



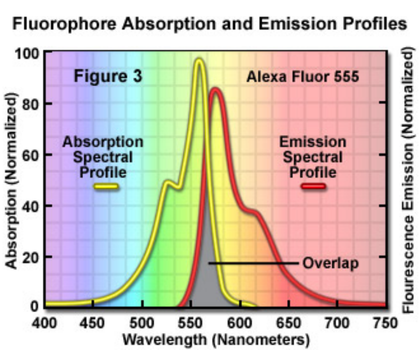
+ different DNA lesions



Transient transfection of mixture



Time to repair



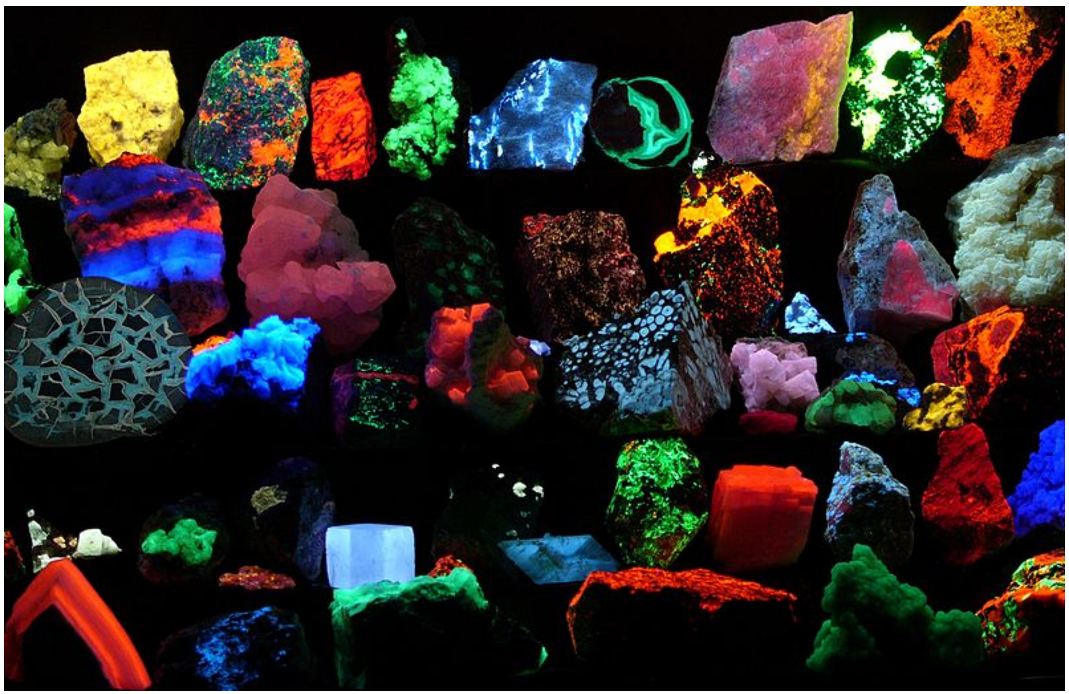
Fluorescence quantitation

fluo·res·cence

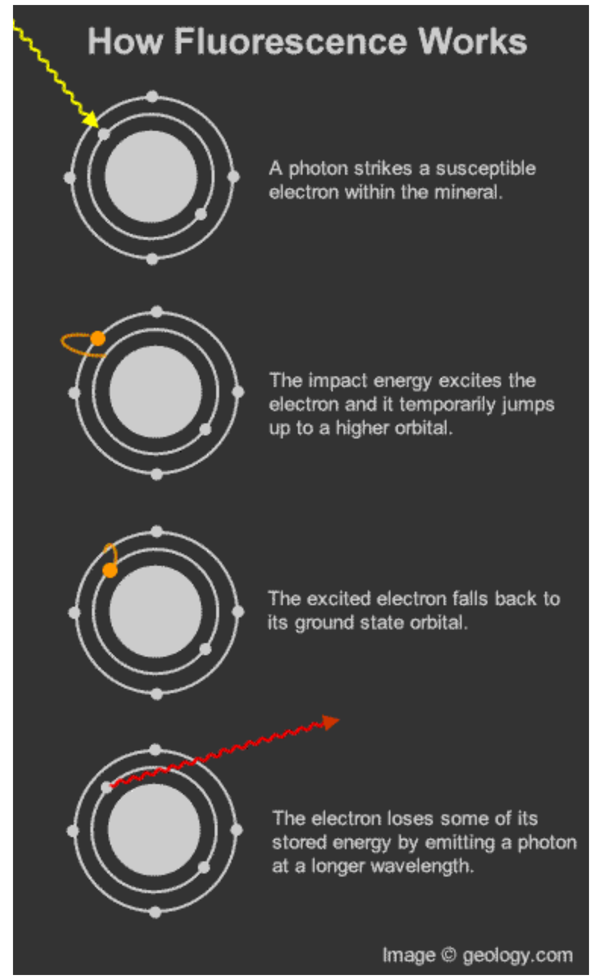
/flo͝o(ə)ˈresəns,flôrˈesəns/

noun

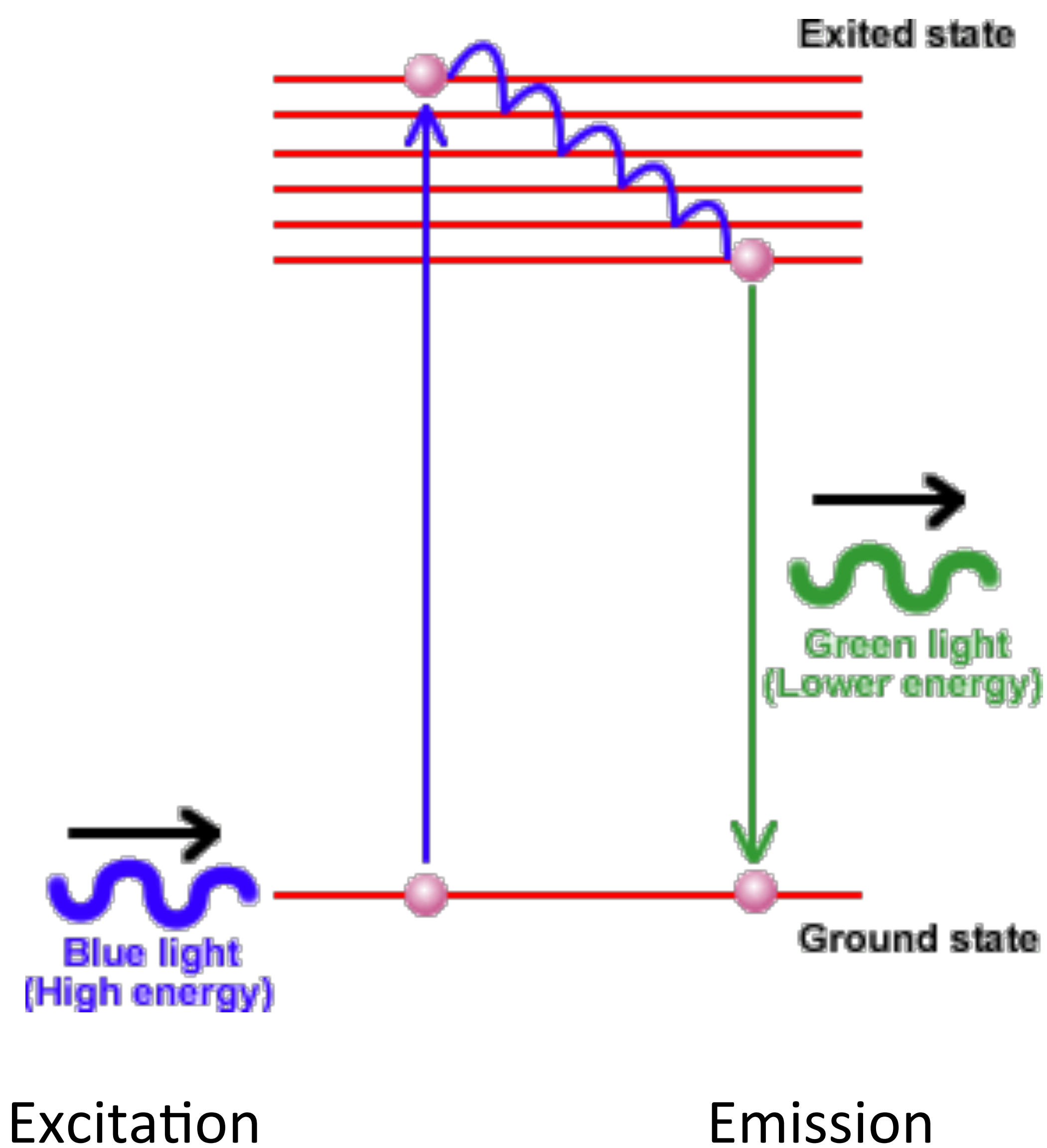
1. the visible or invisible radiation emitted by certain substances as a result of incident radiation of a shorter wavelength such as X-rays or ultraviolet light.



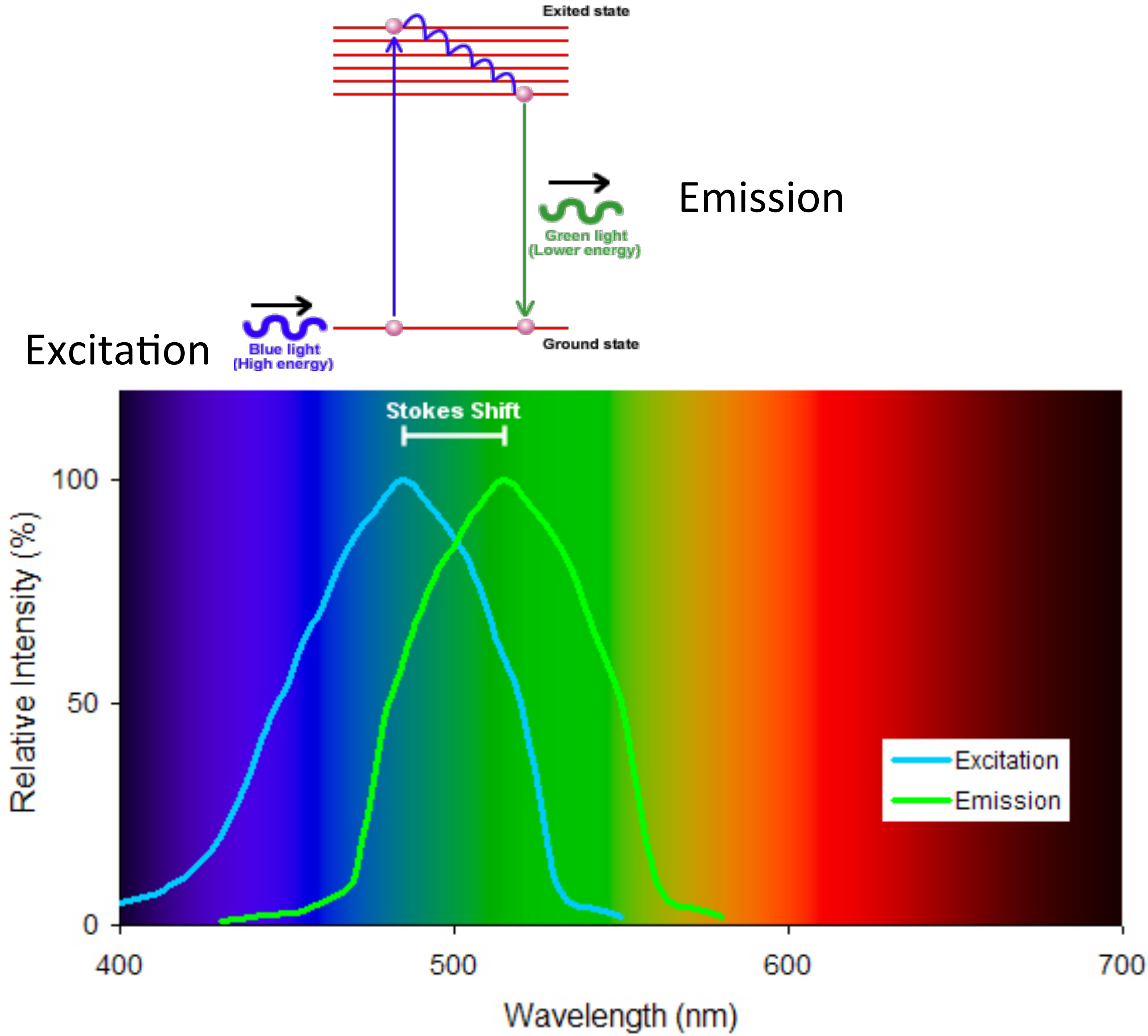
Minerals fluorescing under UV-light



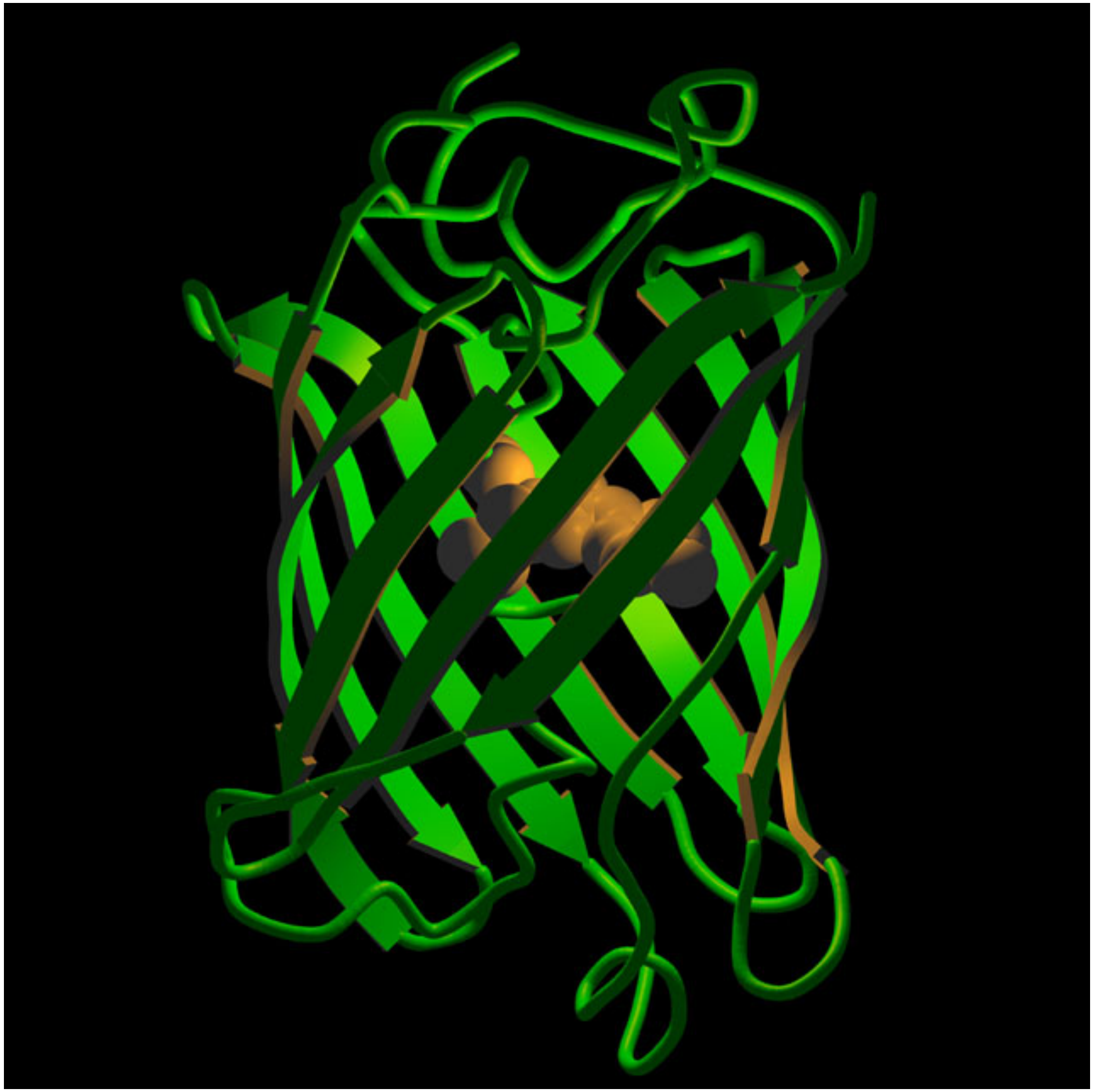
Theory of Fluorescence



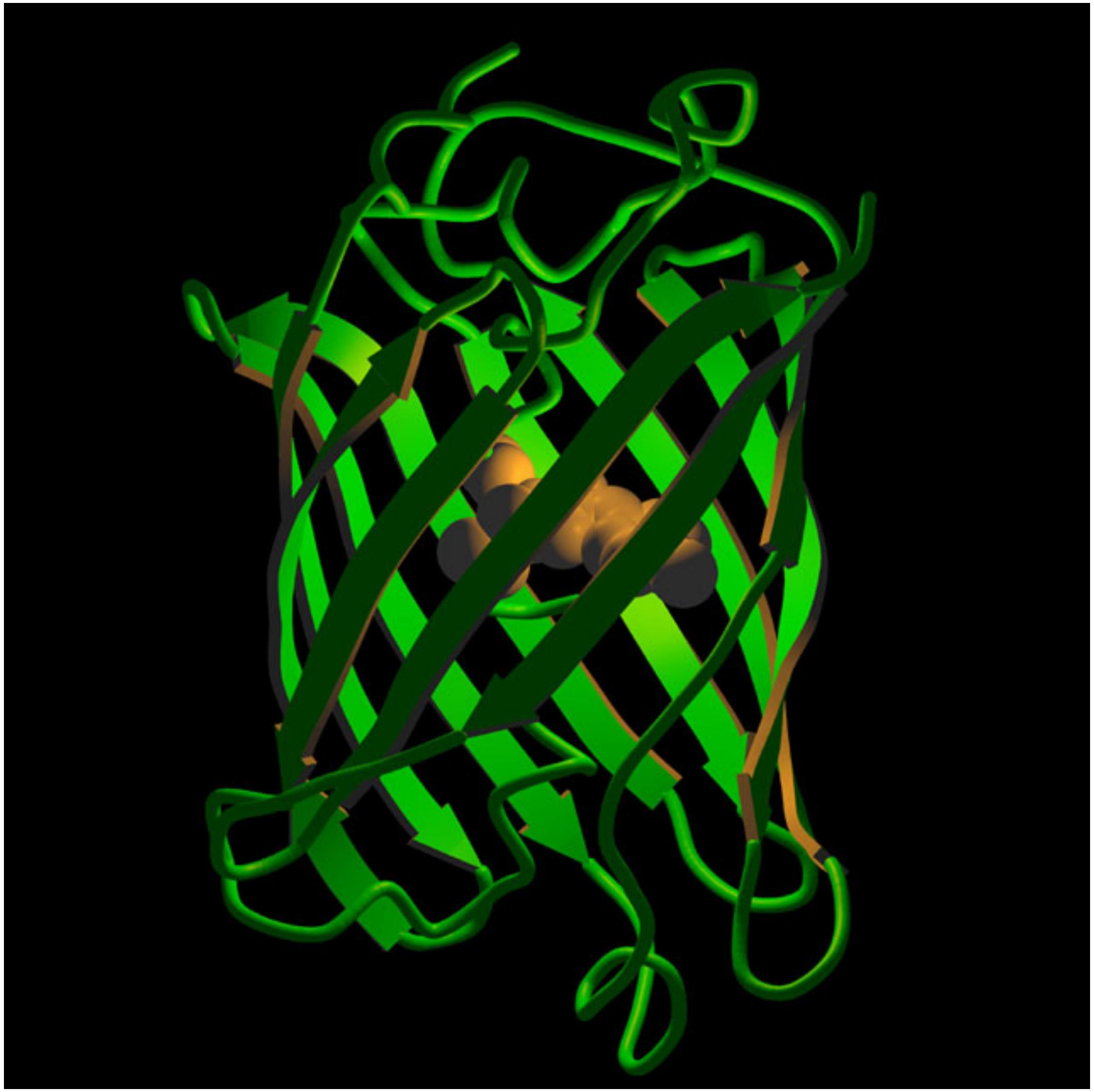
1) Electrons excited by light source
2) Electrons reach a high energy state
3) Energy loss occurs within a few nano seconds
4) Energy loss observed as fluorescent light of a longer wavelength

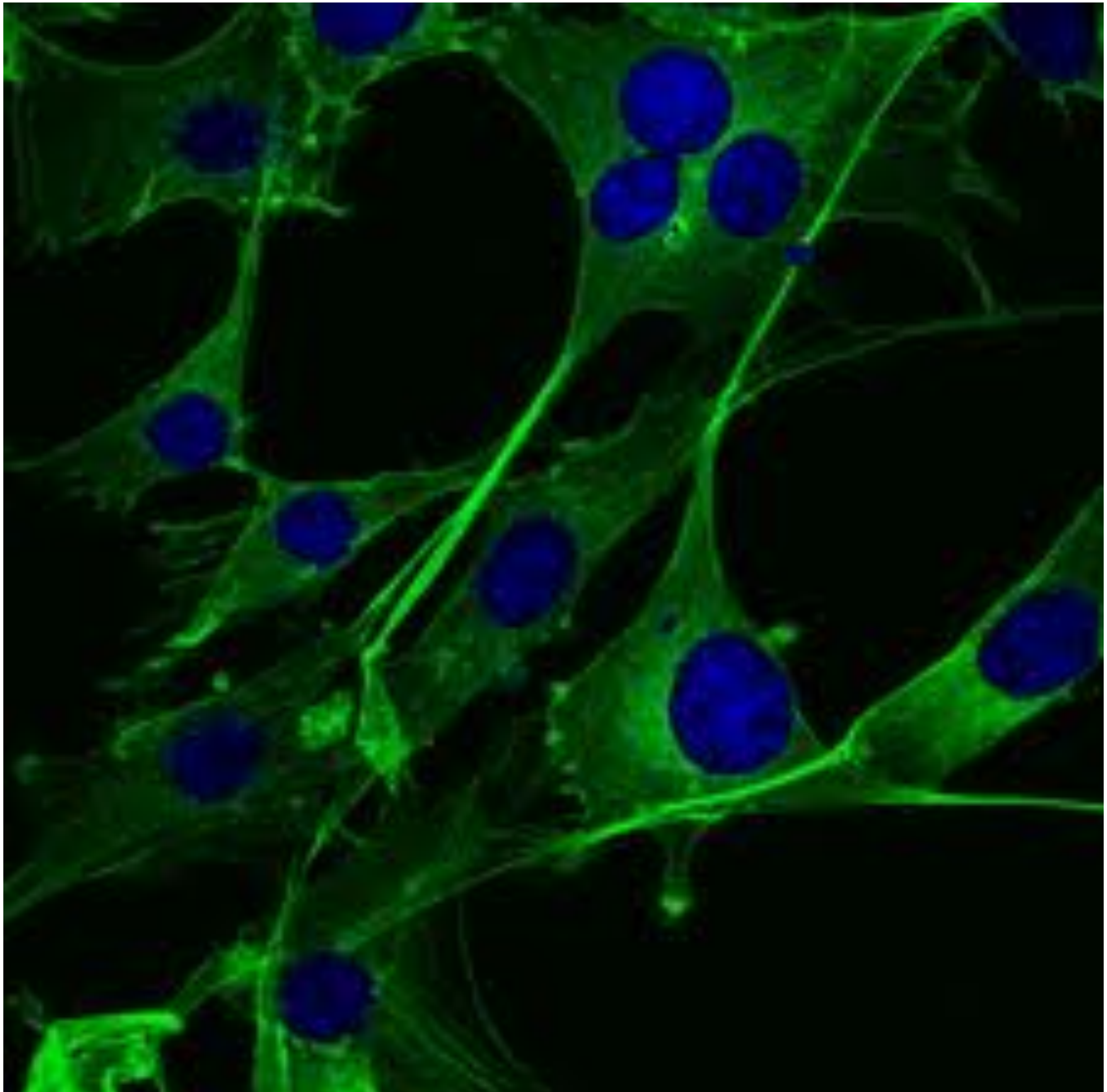

Exited state
Emission
Green light
(Lower energy)
Excitation
Blue light
(High energy)
Ground state
Stokes Shift
100
50
0
Relative Intensity (%)
400
500
600
700
Wavelength (nm)
Excitation
Emission

Green Fluorescent Protein (GFP) first isolated from crystal jellyfish *(Aequorea victoria).*



Green Fluorescent Protein (GFP) first isolated from crystal jellyfish *(Aequorea victoria).*





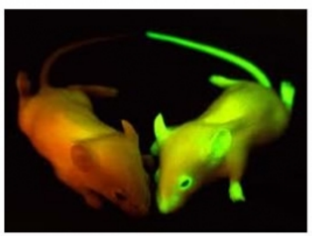
DNA – Blue

GFP - Green

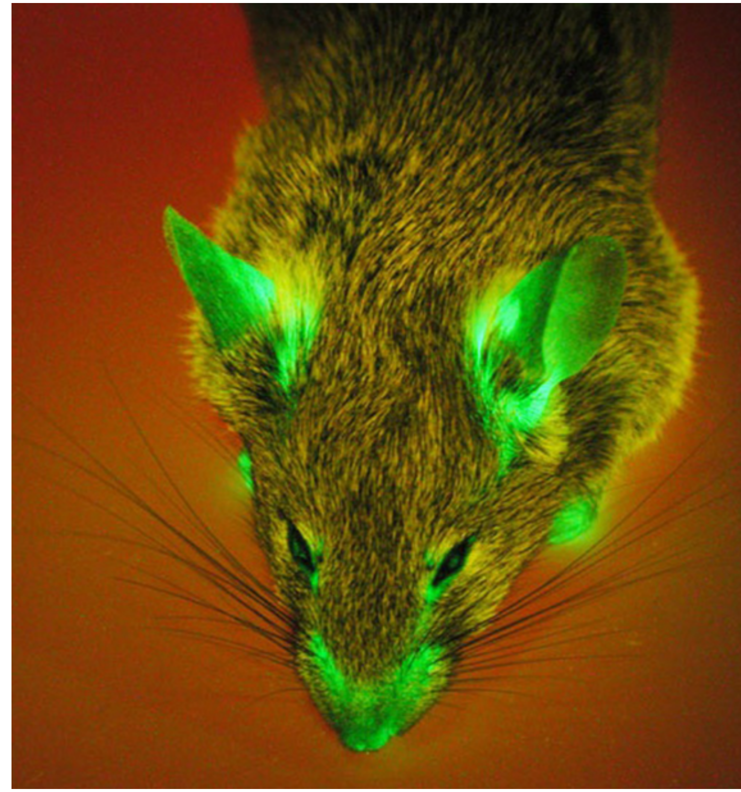


SPL

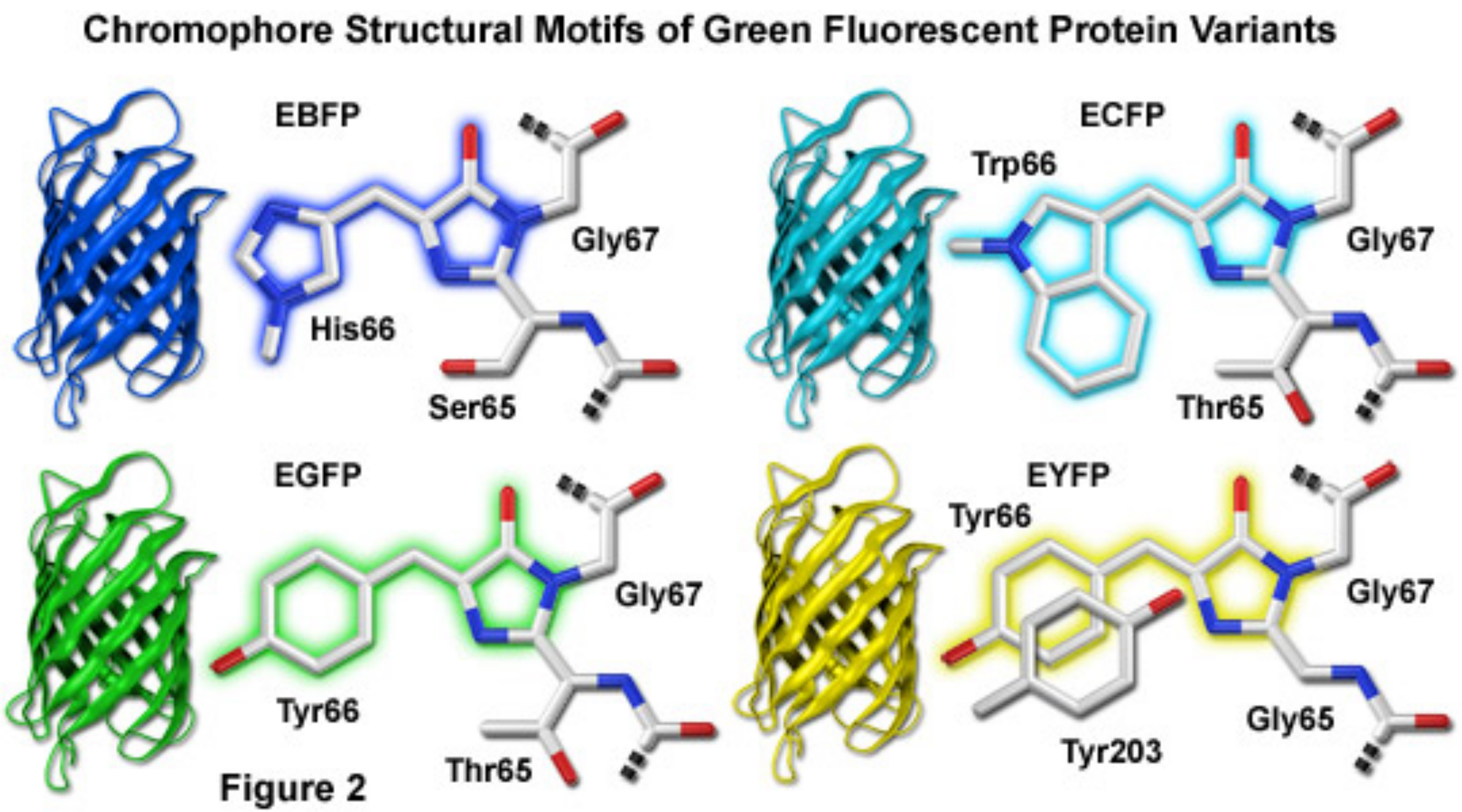






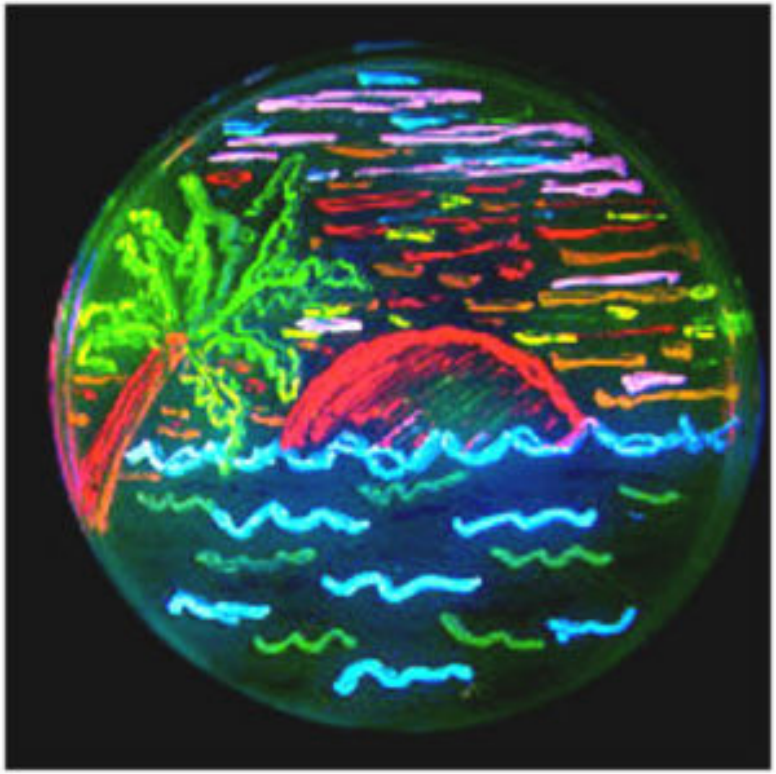



GFP modified to Enhanced GFP (EGFP) and EGFP modified to fluoresce at different wavelengths



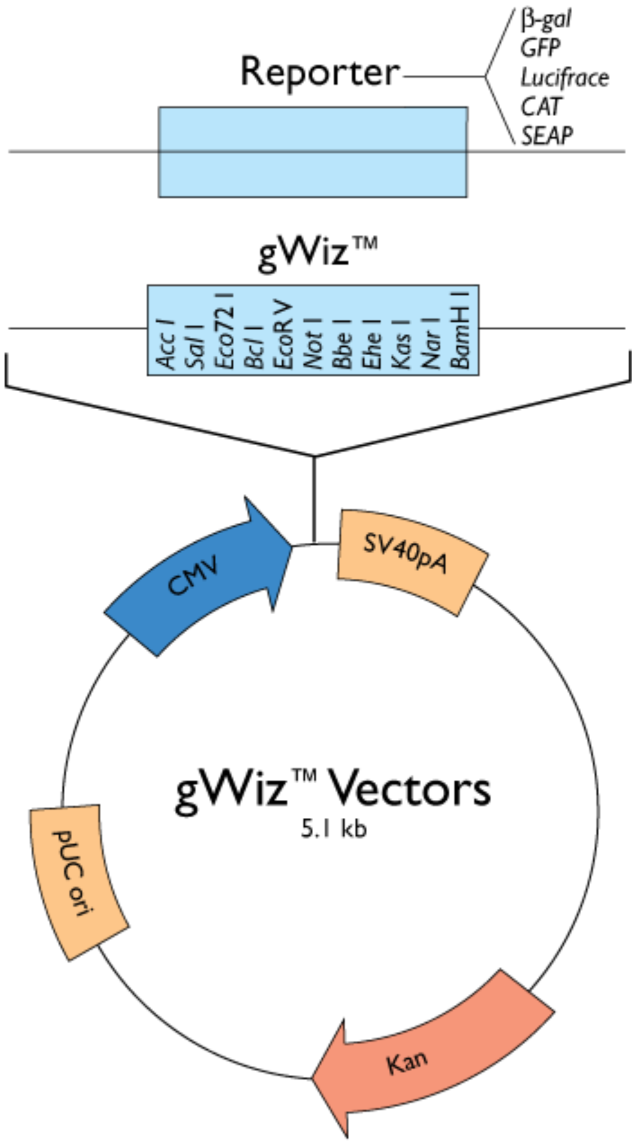
Mushroom Coral

Fluorescent Bulb Anemone (Entacmaea quadricolor)

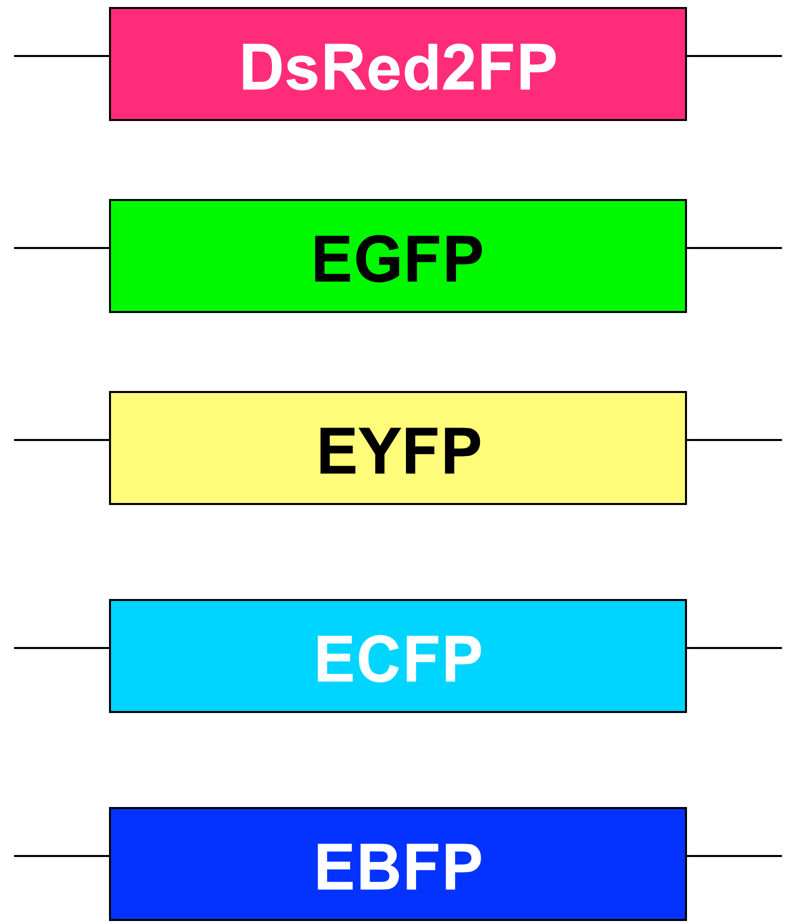


The diversity of fluorescent proteins and genetic mutations is illustrated by this San Diego beach scene drawn with living bacteria expressing 8 different colors of fluorescent proteins.

Reactivation of damaged DNA - multiplexed

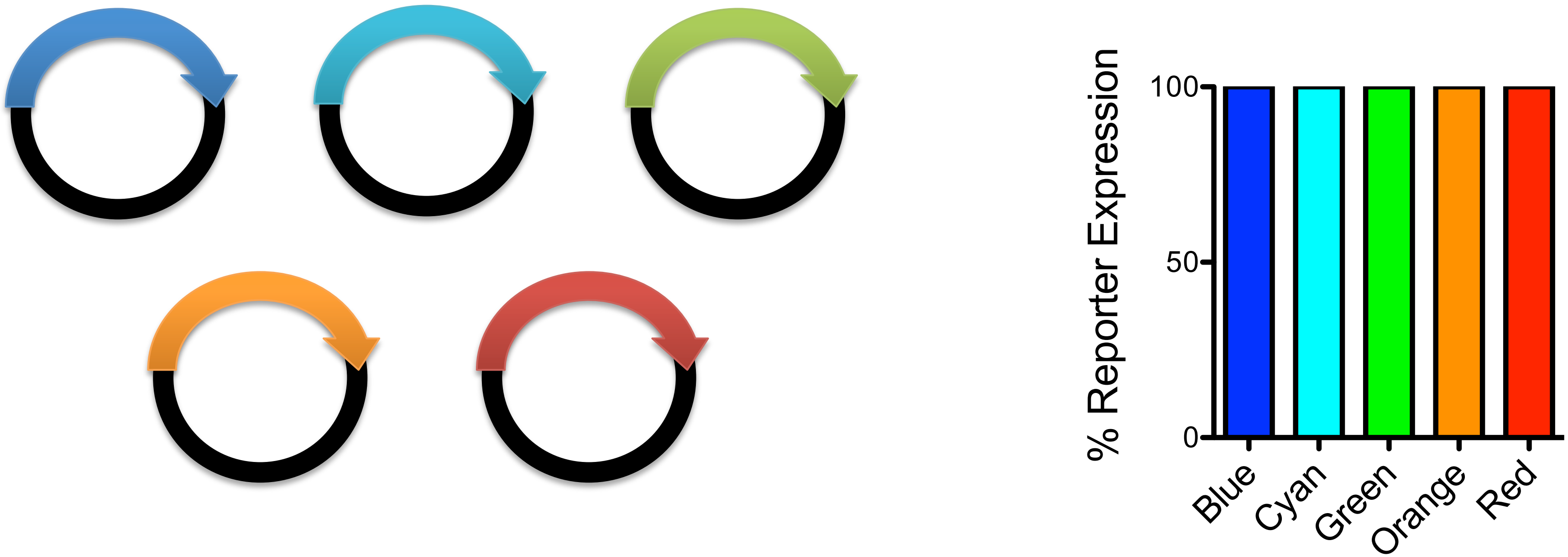


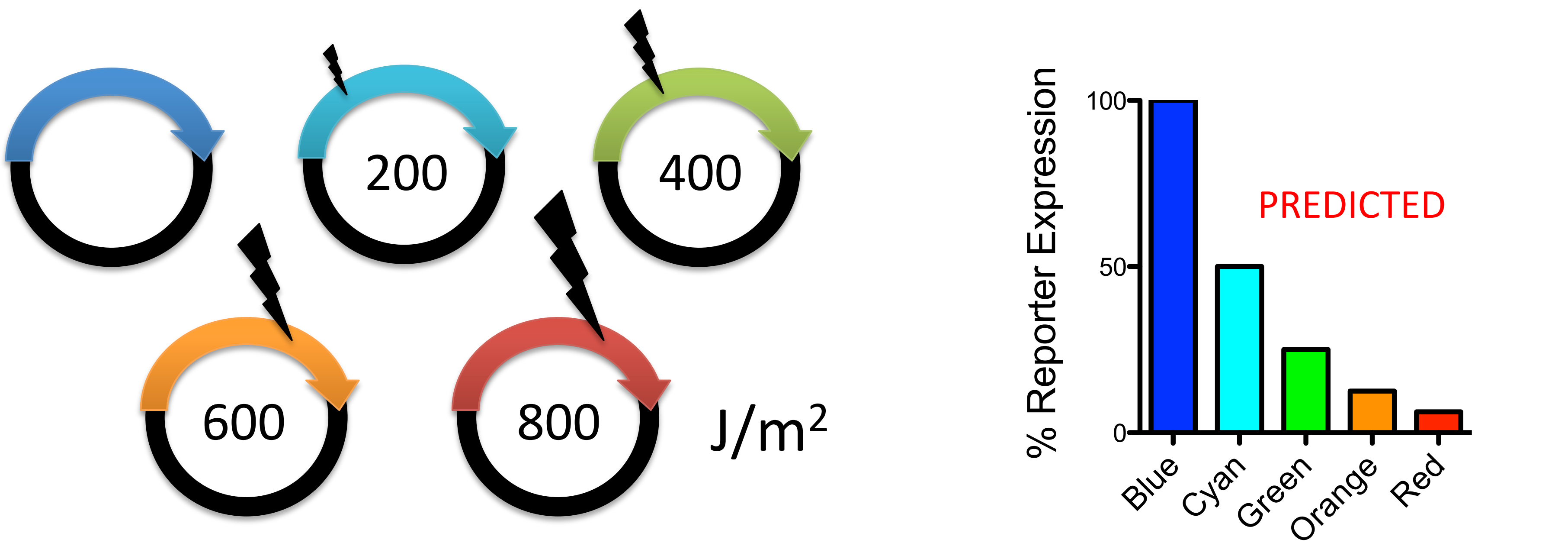
Each Fluorescent Protein gene will harbor a different type of DNA damage



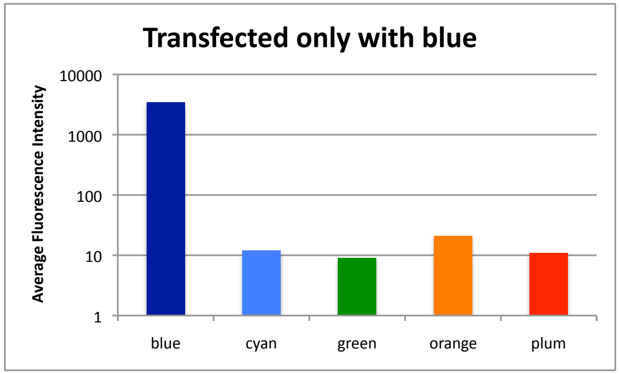
http://www.genlantis.com/corp/images/gWiz_vectmap.gif

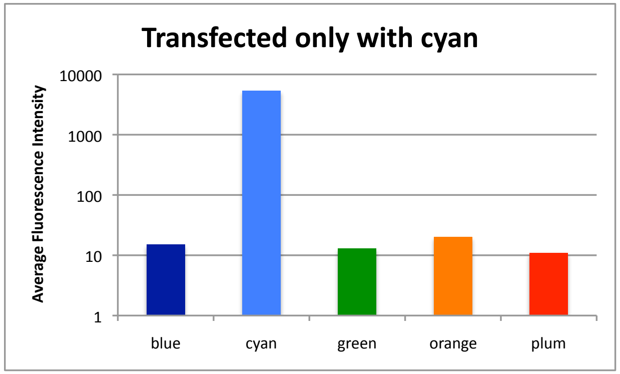
Before trying different damages - tried different doses of the same damage (UV)

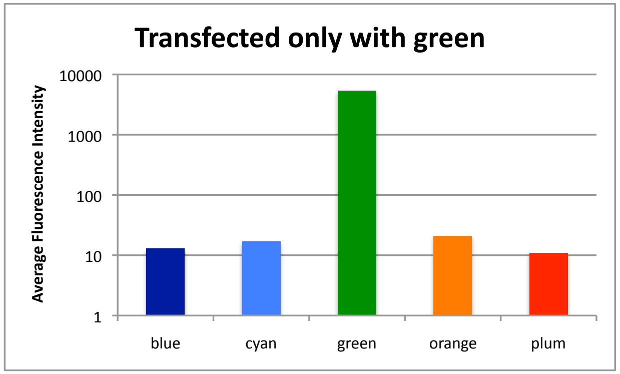


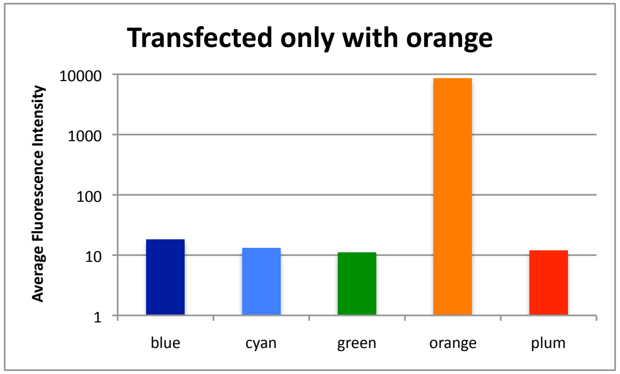


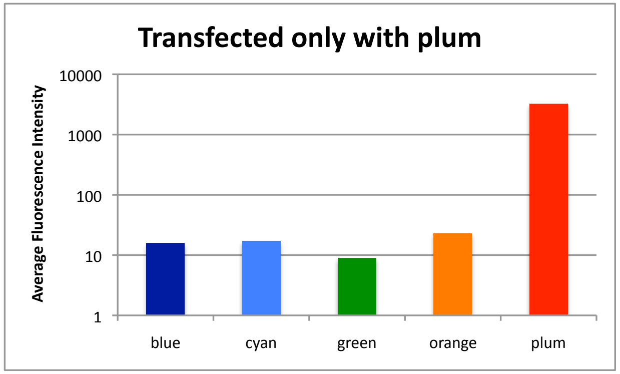
Sanity Check: Is it even feasible detect 5-colors independently?:



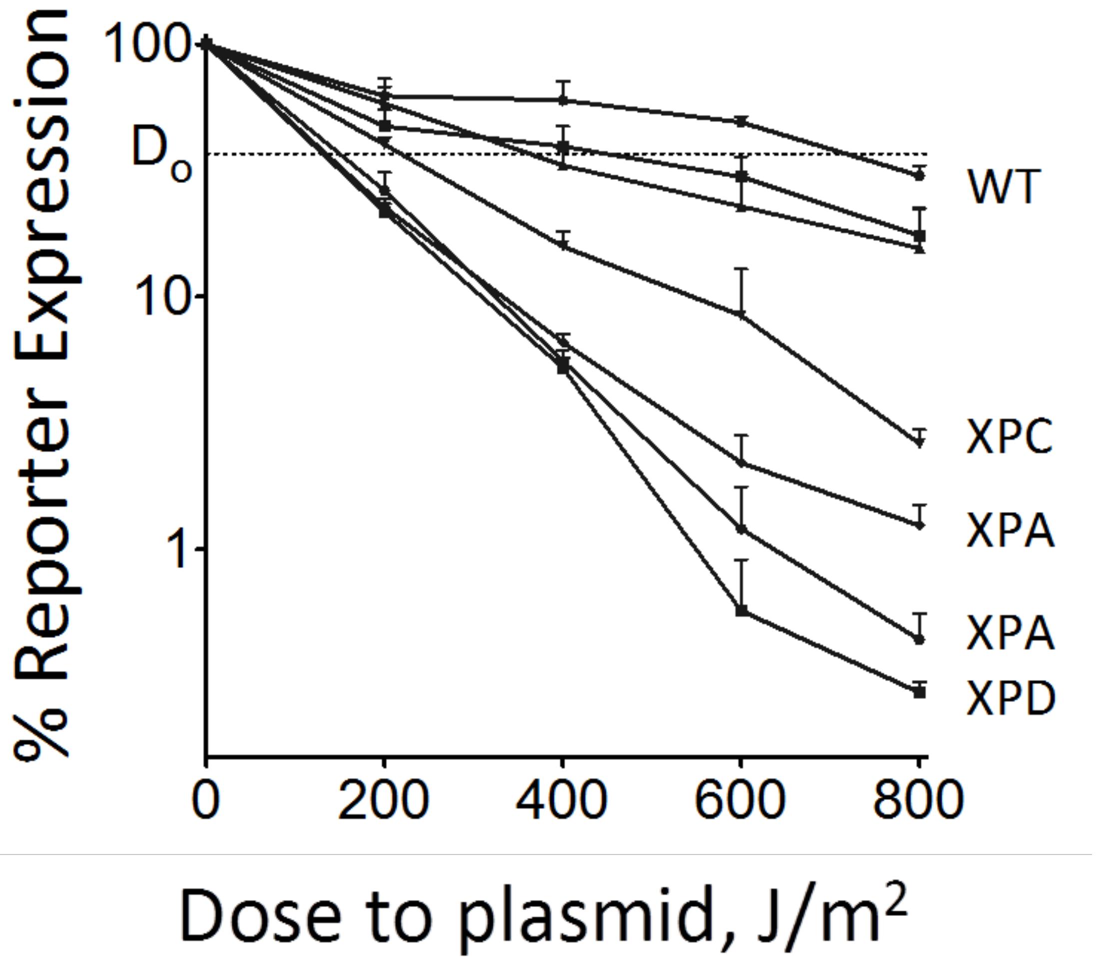








FM-HCR for UV damaged Plasmids
(Nucleotide Excision Repair)



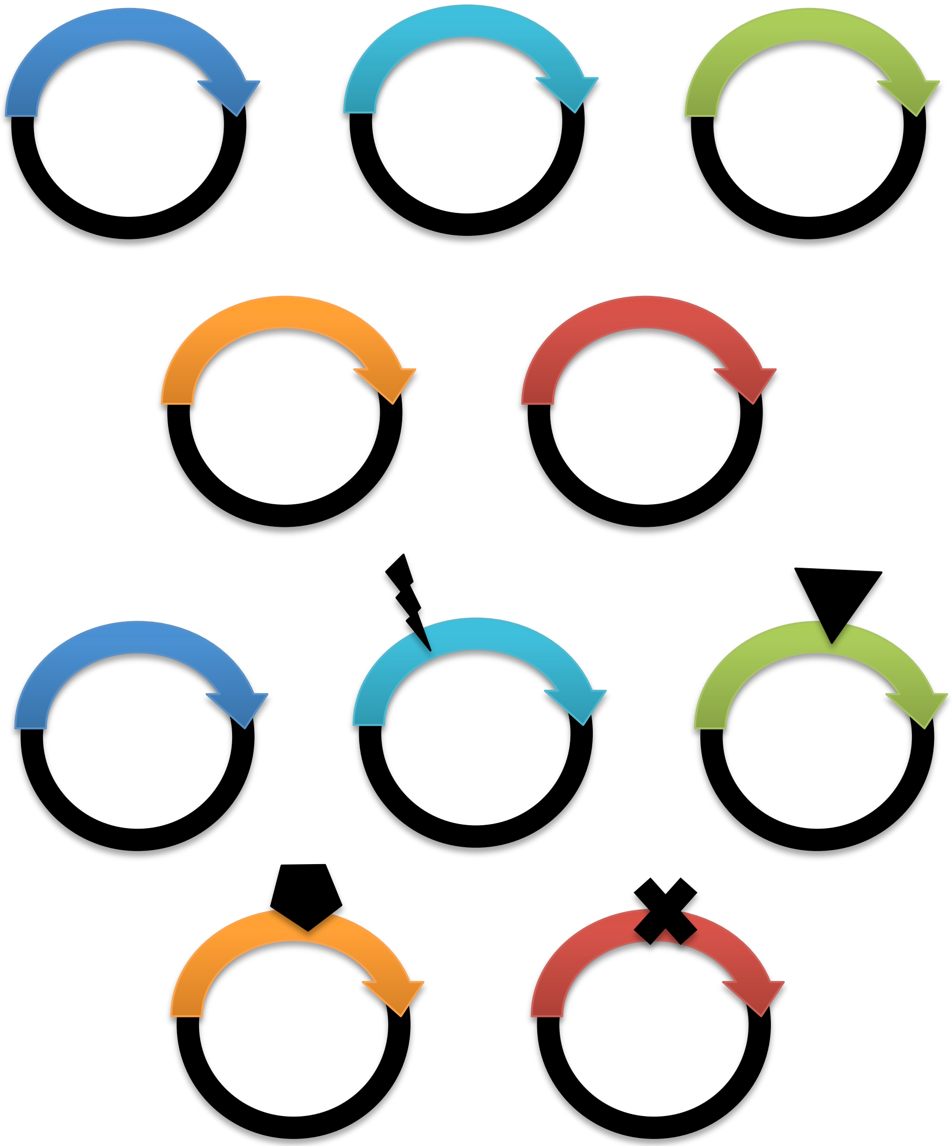
FM-HCR

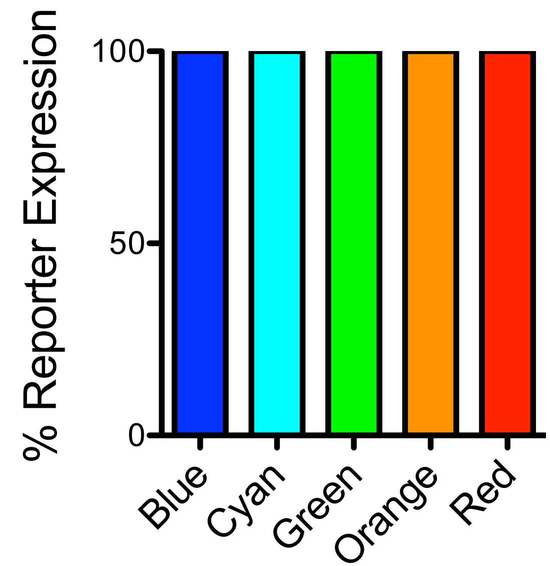
Fluorescence
Multiplexed

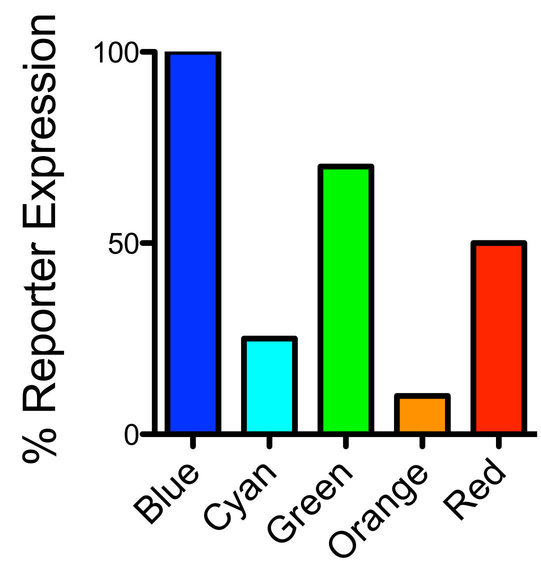
Host
Cell
Reactivation

Zachary Nagel & Siobhan McRee

5 color HCR assay applications







5-color HCR developed by **Dr. Zachary Nagel**

DNA Repair Strategies

- Direct Reversal

  Methyltransferase, Oxidative demethylase

- Excision Repair

  Base excision, nucleotide excision, mismatch repair

- Double strand break repair

  Homologous recombination, Non-homologous end joining

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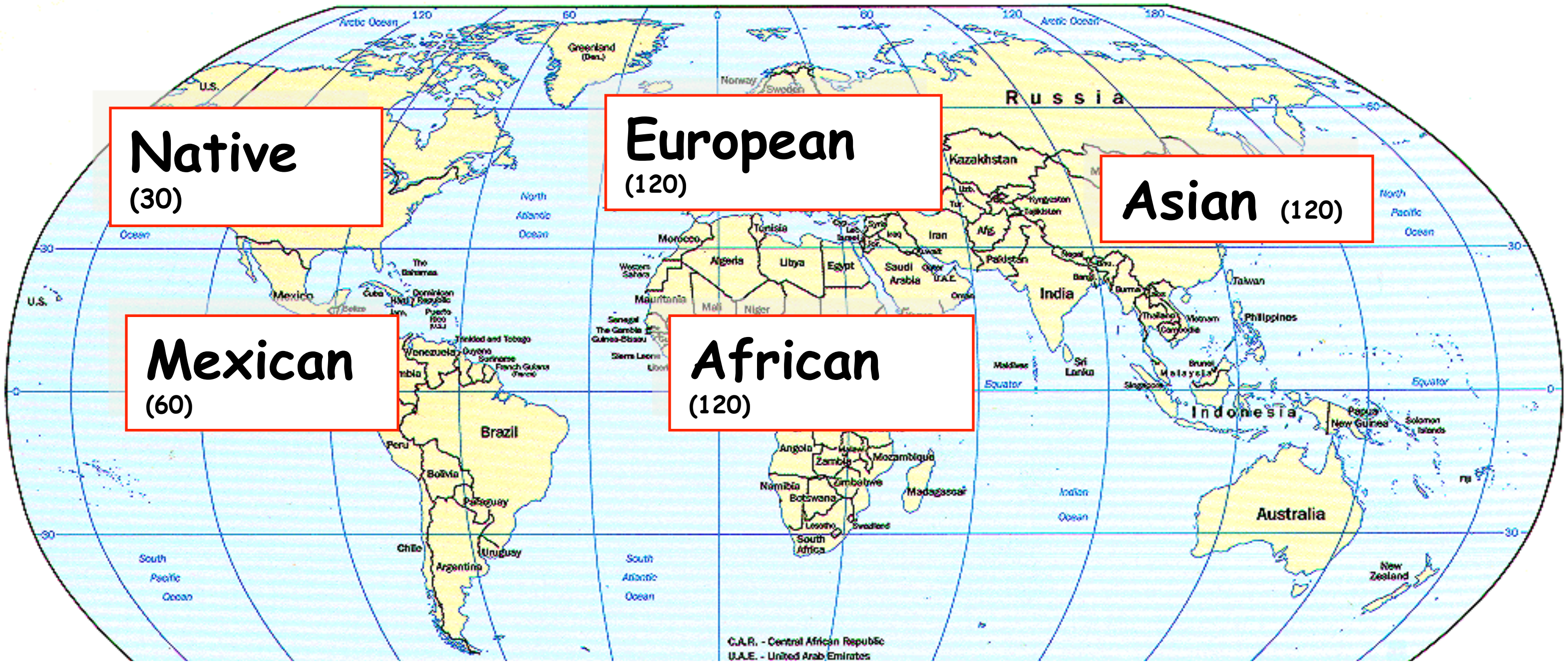
Coriell Lymphoblastoid Cell line collection derived from ethnically diverse HEALTHY humans

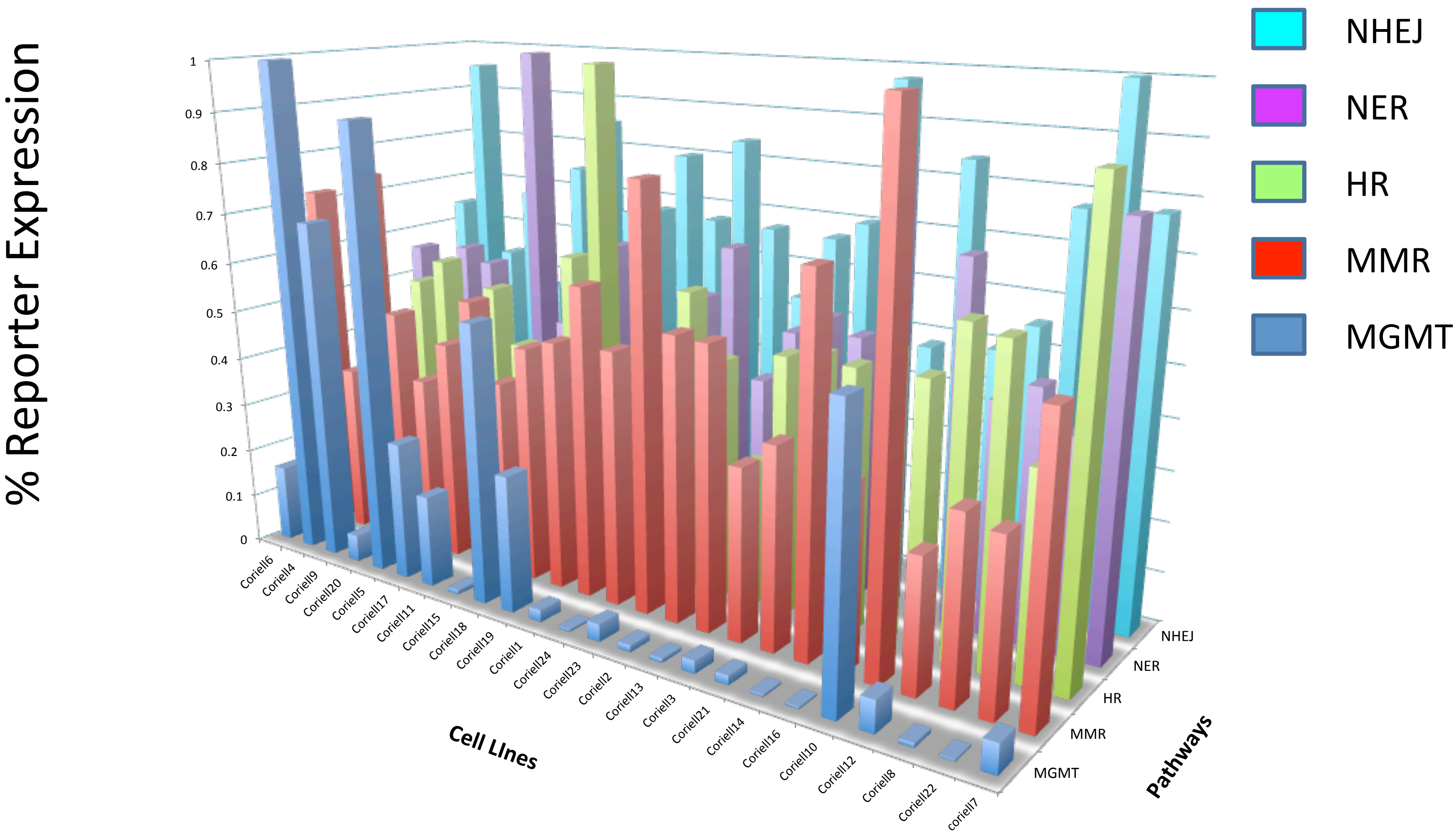


450 healthy unrelated US residents with ancestry from around the globe

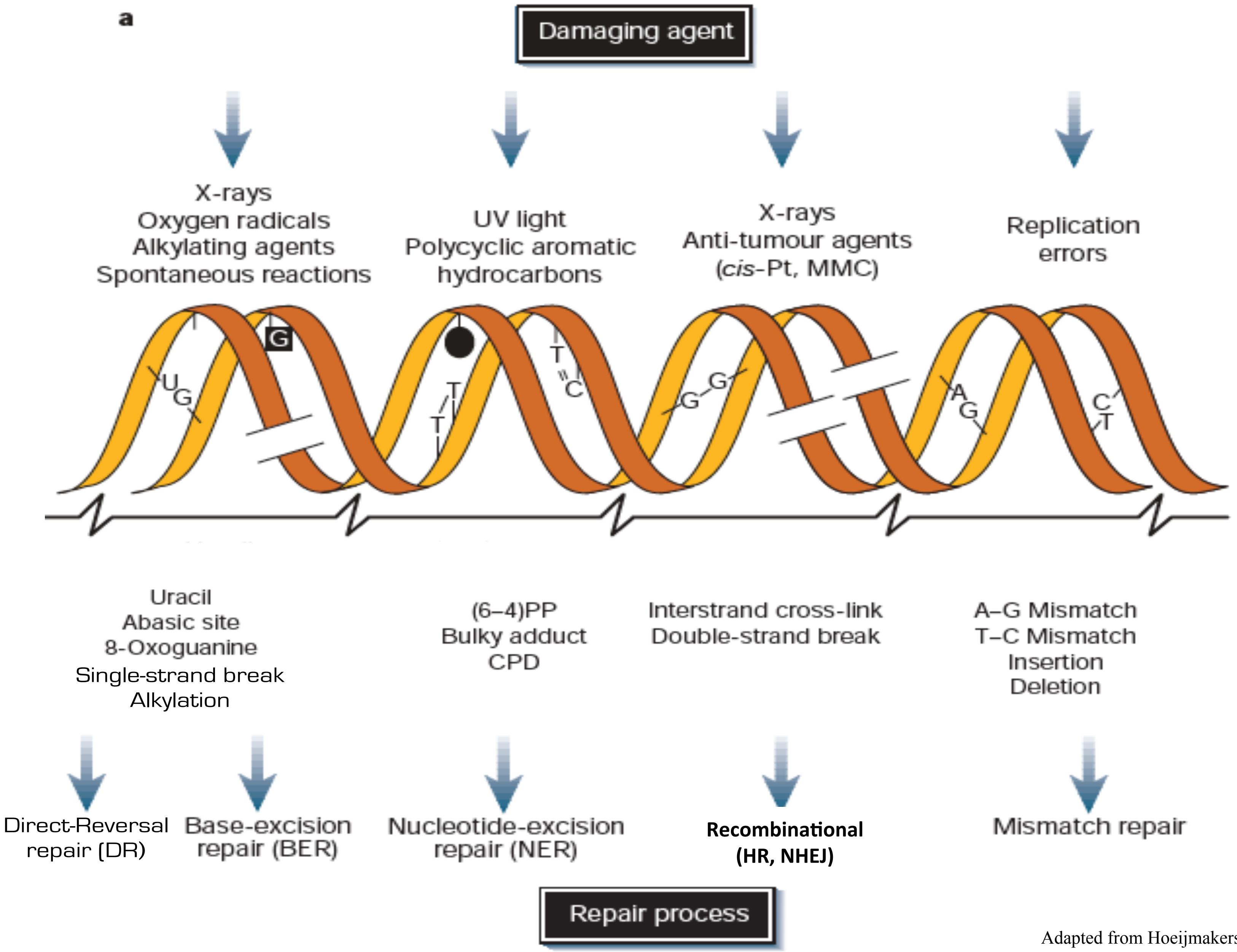
Nested subsets: 90, 44, 24, 8

Ethical reasons: no medical, phenotypic, or ethnic information is provided

DNA Repair Capacity in cells from genetically diverse healthy people

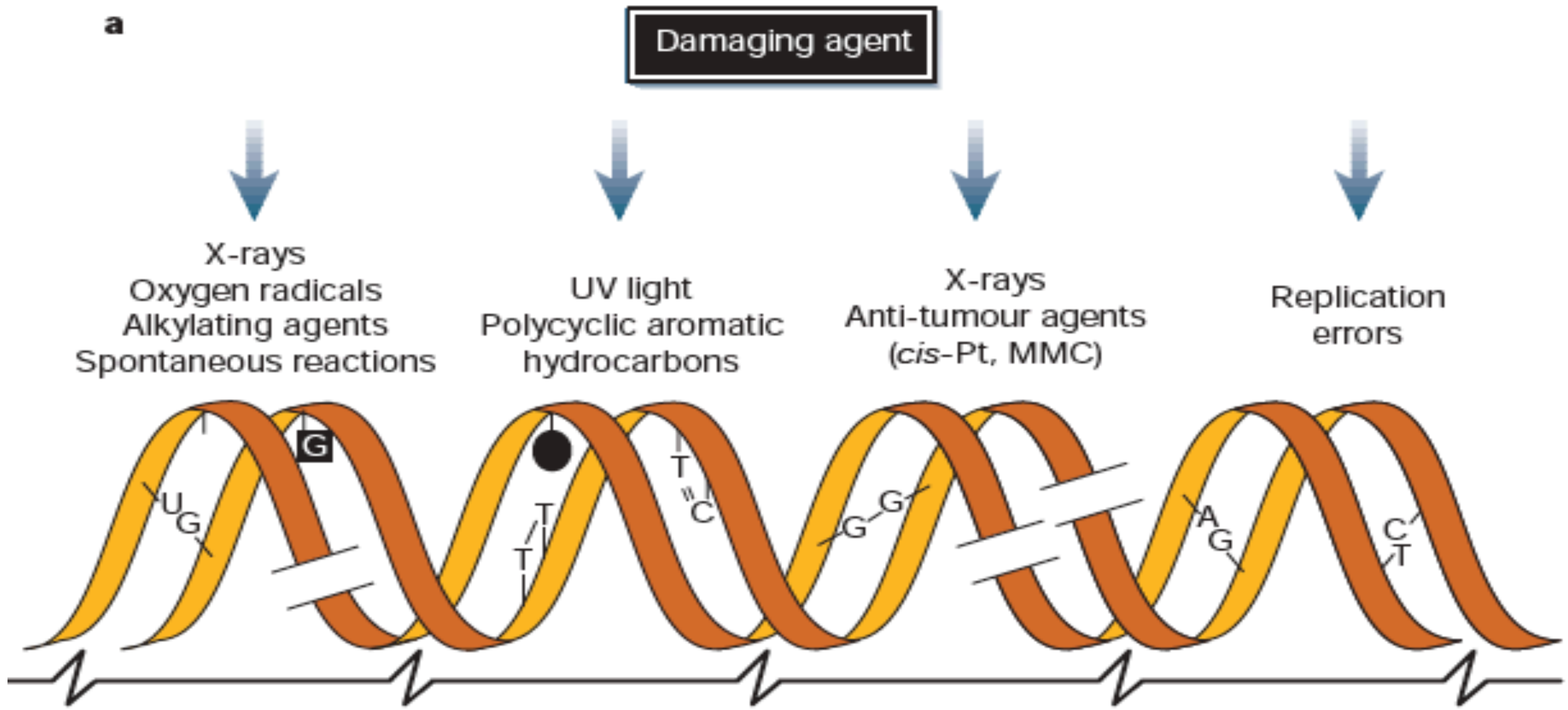


DNA Damage and Repair



Adapted from Hoeijmakers **2001** *Nature*

DNA Damage and Repair



RESPONSES of TUMOR and NON-TUMOR CELLS to CANCER RADIOTHERAPY and CHEMOTHERAPY

Adapted from Hoeijmakers **2001** *Nature*

The Pioneer Team

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Dr. Anwaar Ahmad

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Patrizia Mazzucato

Siobhan McRee

Thanks to the NIH Director’s Pioneer Award & the NIEHS!!!