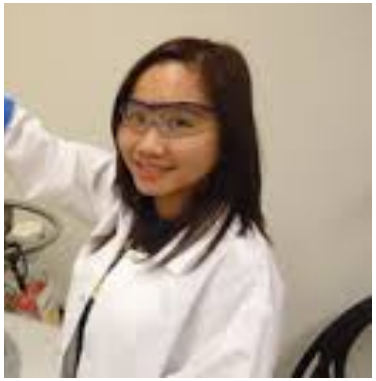
20.109 Spring 2015 Module 2 – Lecture 6
System Engineering and Protein Foundations

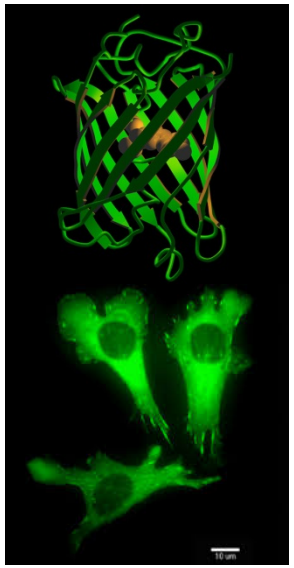


Shannon Hughes
Noreen Lyell
Leslie McLain
Nova Pishesha (TA)
Leona Samson (Lectures)

Zachary Nagel (help with development) Alex Chaim

What experimental question will you ask in Module 2?



How efficiently does DNA repair by the Non Homologous End Joining (NHEJ) pathway act on DNA damage with different topologies?

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 efficiency?

CANDIDATE INTERVIEW

June 16th 2009, 8am!

Developing Novel Methods to Measure DNA Repair Capacity in Human Populations

Leona D. Samson

MIT

Biological Engineering Department

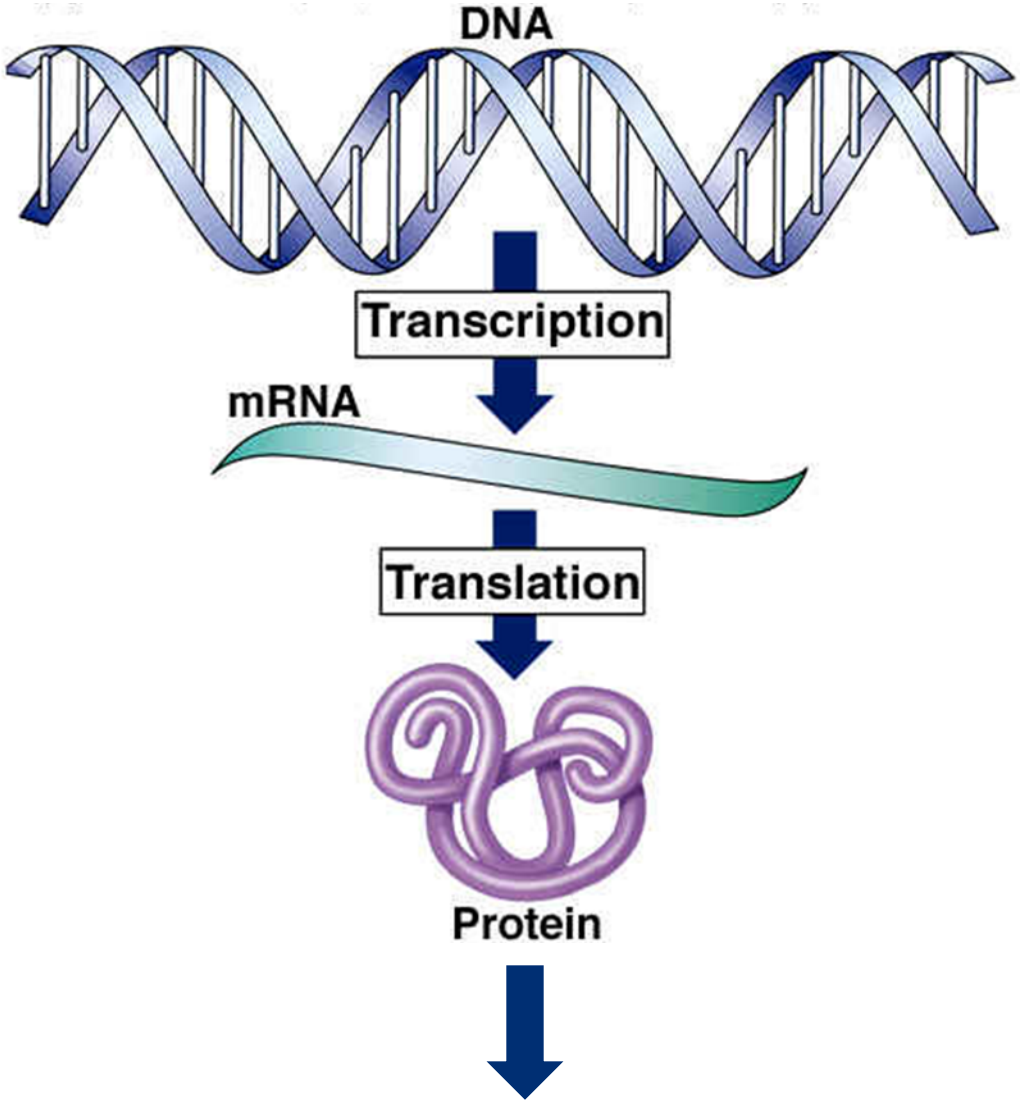
Biology Department

Center for Environmental Health Sciences

Koch Institute for Integrative Cancer Research

Computational and Systems Biology Initiative

Broad Institute (Harvard and MIT)



SNPs – GWAS
Genome sequencing

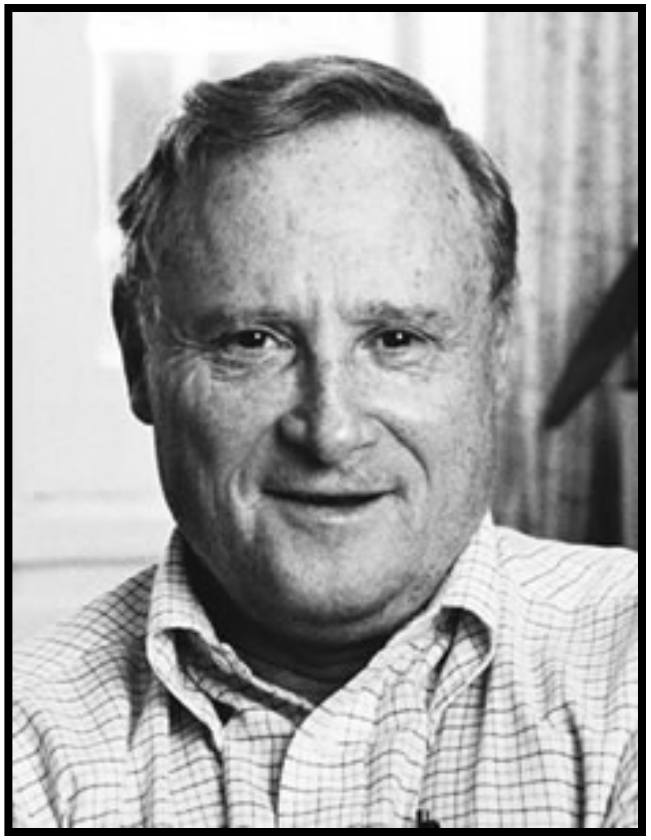
mRNA (miRNA, lncRNA)
Profiling
Exome Sequencing

Proteomic Analyses

In vitro / In vivo
Functional Assays

FUNCTION

The Proposal was based on the Pioneering work of:

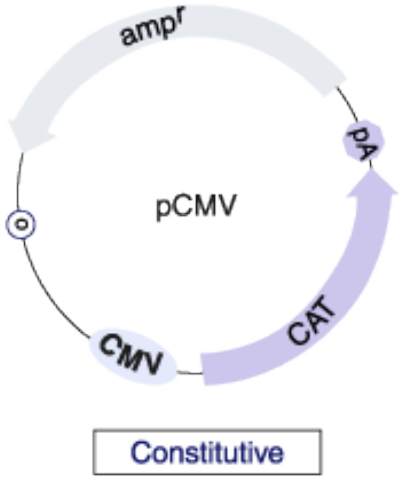


Dr. Lawrence Grossman
(1924–2006)

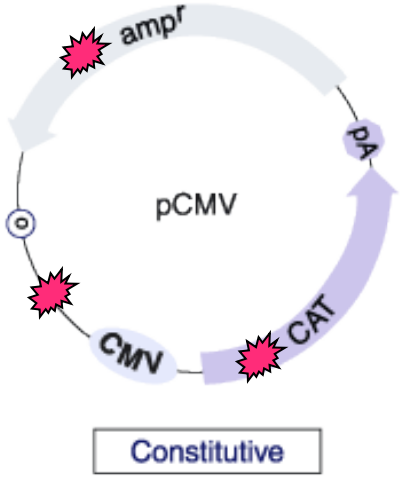
Dr. Qingyi Wei

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

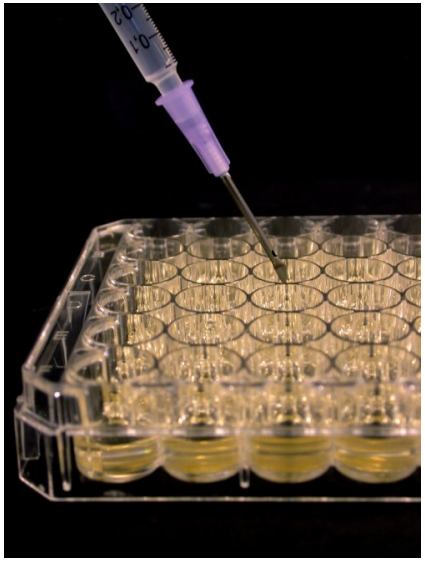
Athas & GROSSMAN
Cancer Res. 1991

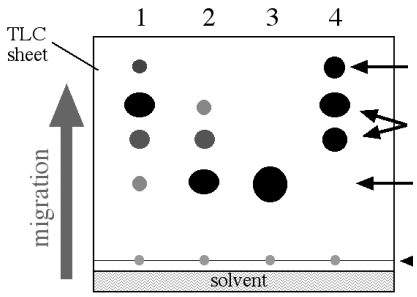


+ UV light



Transient transfection peripheral blood lymphocytes

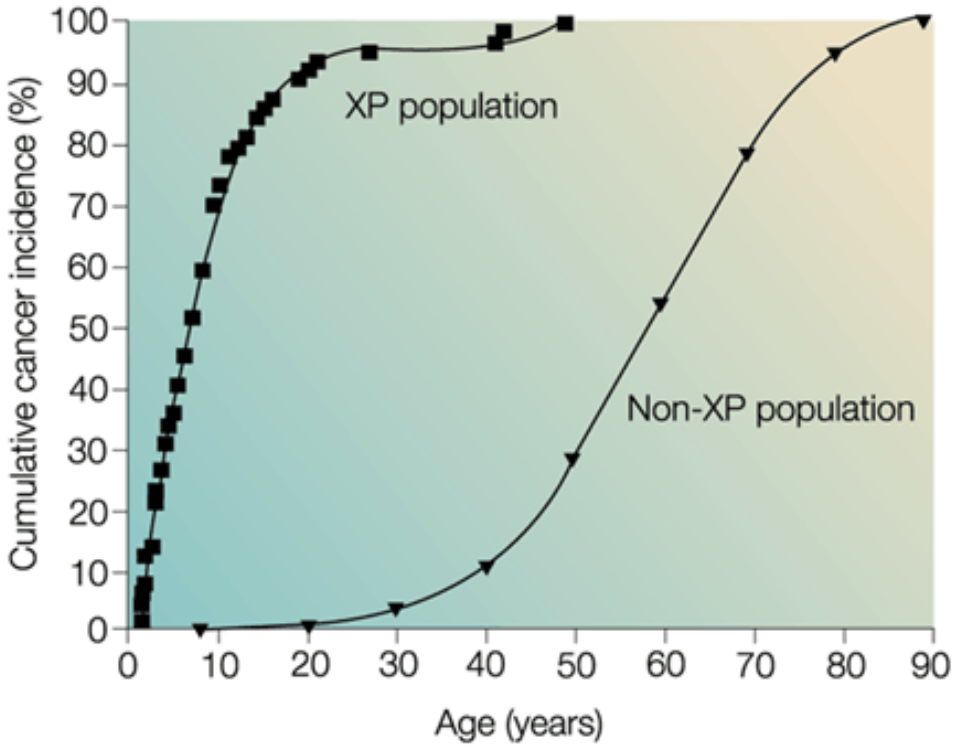




Time to repair

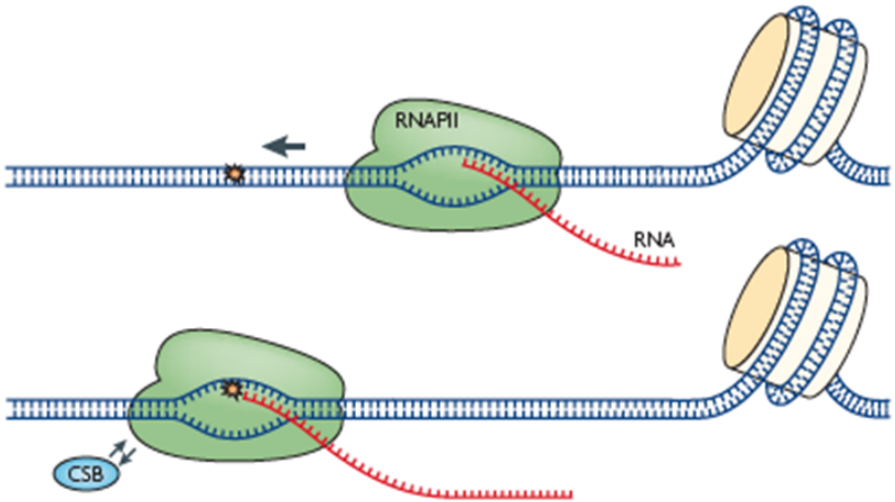
CAT Assay

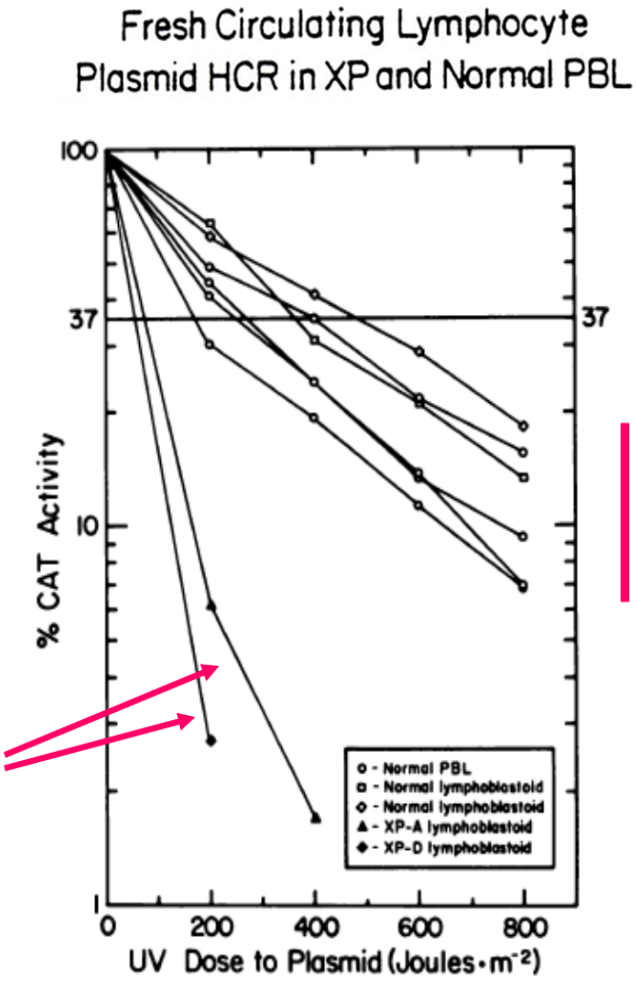
Lack of NER accelerates the onset of skin cancer in Xerderma Pigmentosum patients



1, 22-33 (October 2001)
Nature Reviews | Cancer

RNA Polymerase II is exquisitely sensitive to DNA lesions



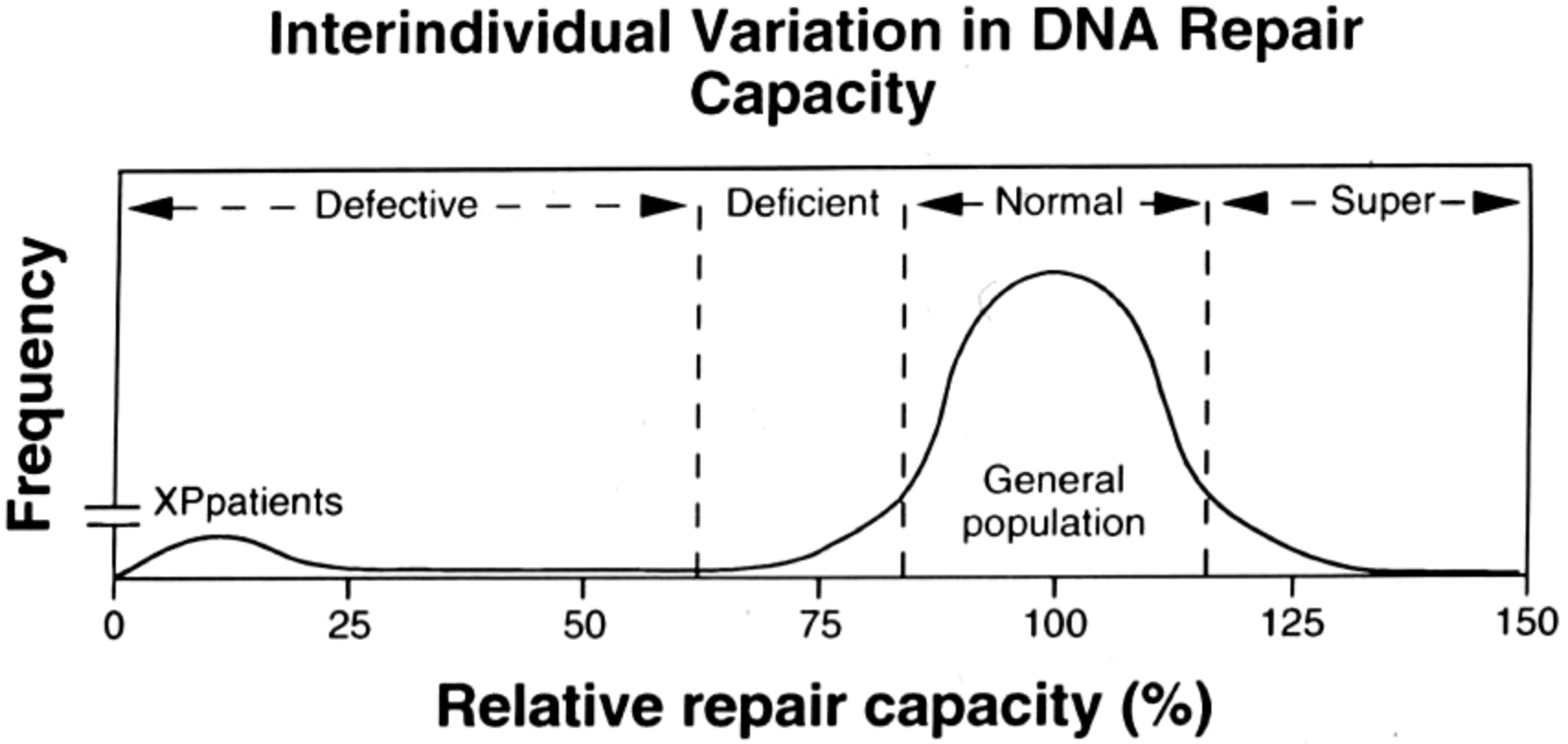


'WT" for NER

XP cells NER deficient

Nature Reviews Molecular Cell Biology 9, 958-970

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

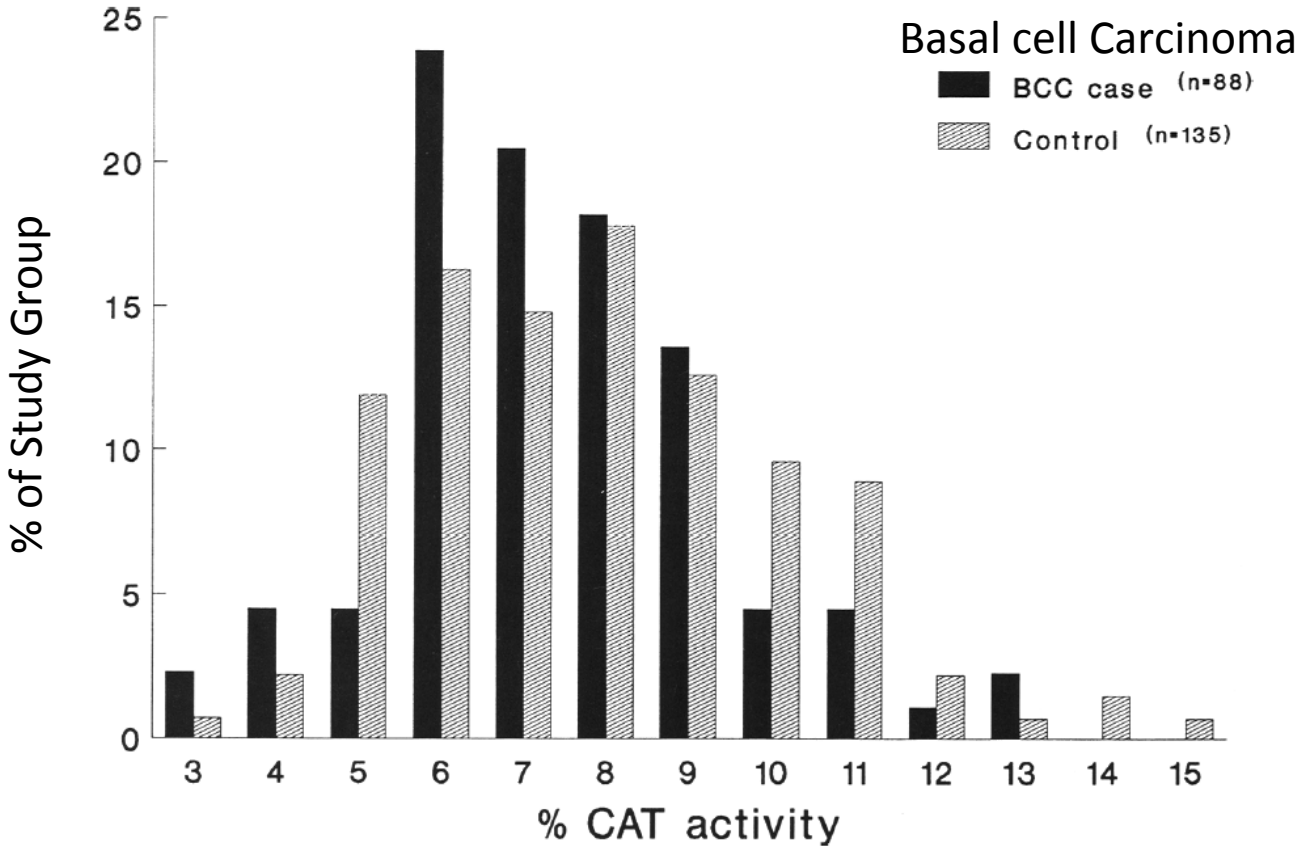


Adapted from **GROSSMAN and Wei (1995)** Clinical Chem 41: 1854-1863

XP frequency = ~1:250,000 giving theoretically ~28,000 cases worldwide with 2,000-fold increased skin cancer risk

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

Case-Control Study monitoring DNA Repair Capacity (DRC) by Host Cell Reactivation (HCR) of plasmids containing DNA damage

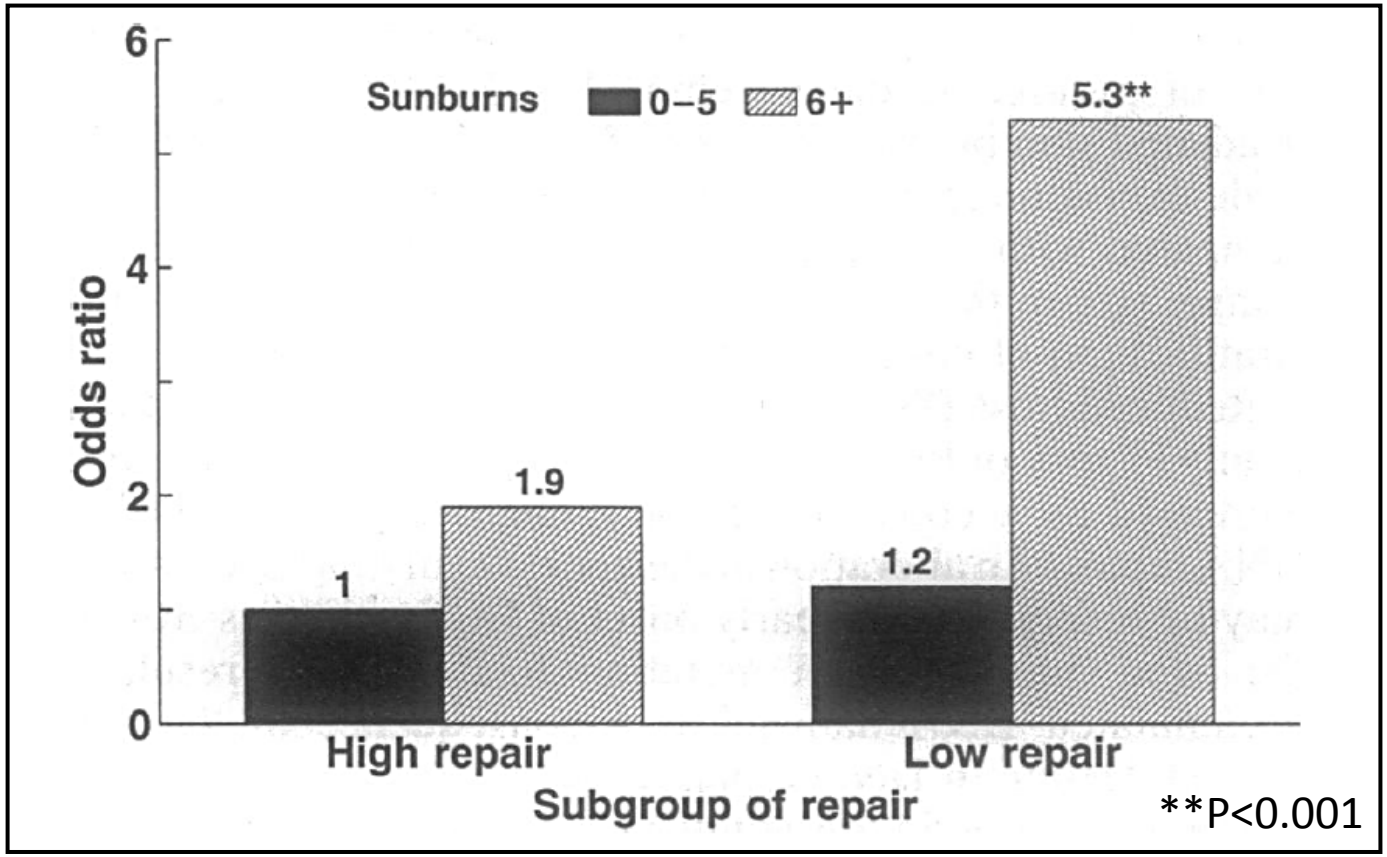


LOW Repair HIGH Repair

[CANCER RESEARCH 54, 437-44(i, January 15, 1994]

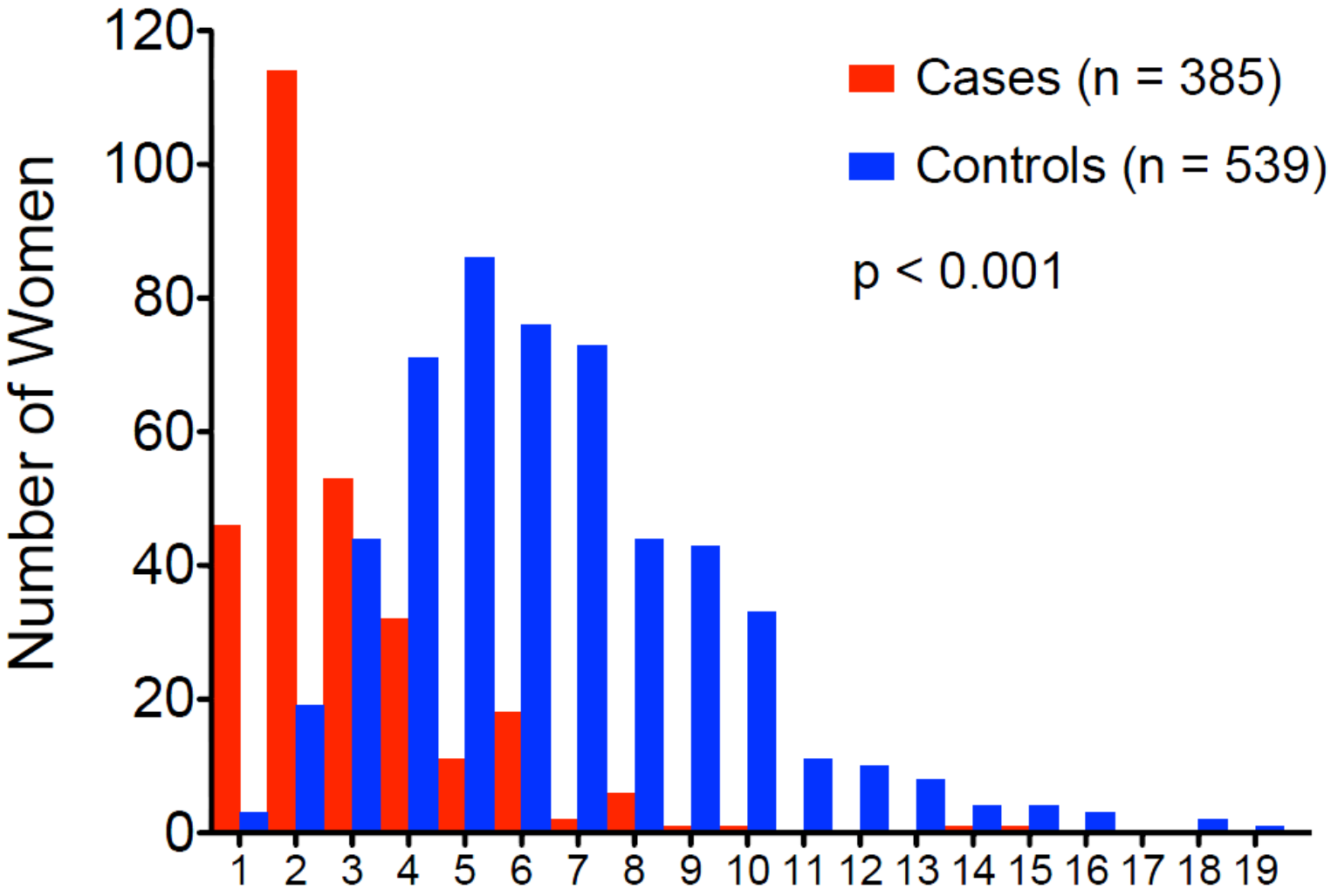
Qingyi Wei, Genevieve M. Matanoski, Evan R. Farmer, Mohammad A. Hedayati, and Lawrence GROSSMAN

Low NER Repair status combined with excessive sun exposure is very dangerous



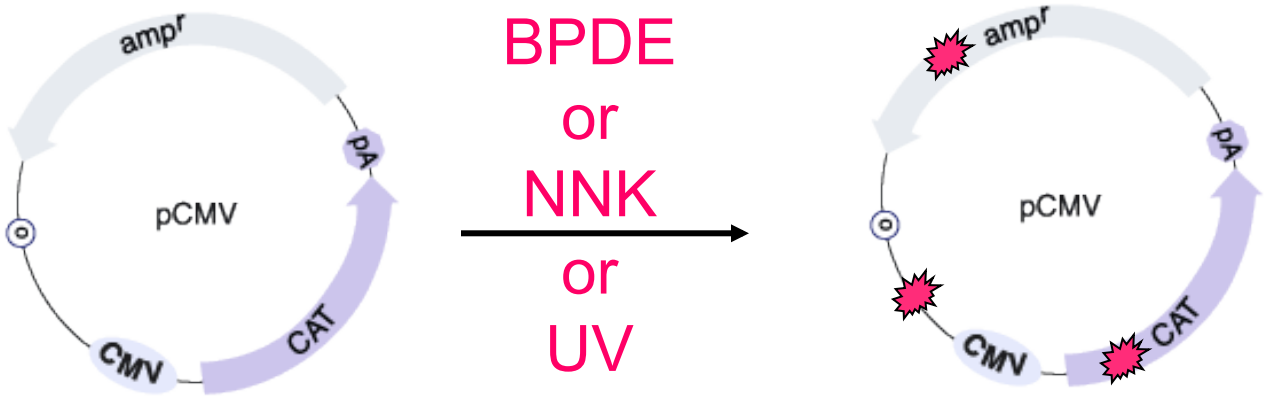
Wei Q, Matanoski GM, Farmer ER, Hedayati MA, **GROSSMAN L.** Proc Natl Acad Sci U S A. 1993 90:1614-8.

DNA Repair Capacity (DRC) in **Breast Cancer** versus **Control** Patients



Matta *et al. BMC Cancer* 2012, **12**:490
http://www.biomedcentral.com/1471-2407/12/490

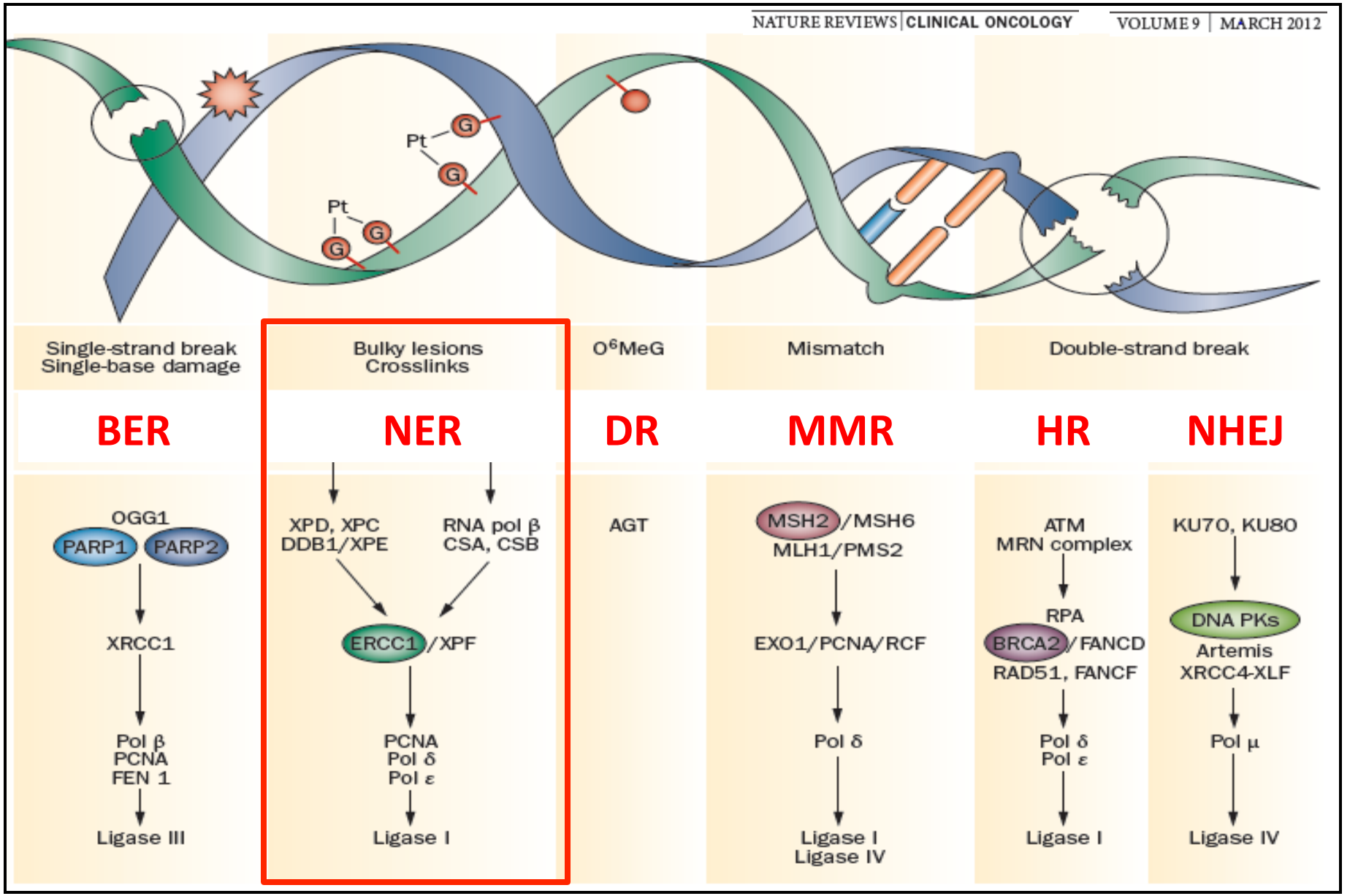
Many cancer case/control studies have monitored Nucleotide Excision Repair (NER)



Damage	Cancer	Increased Risk Low v High Repair	Source
BPDE	Lung	5.7	Wei, 1996
"	Head & Neck	2.2	Cheng, 2005
"	Breast	3.4	Shi, 2004
NNK	Lung	3.2	Wang, 2007
UV	Skin	2.0	Wei, 2002
"	Melanoma	2.0	Wei, 2002
"	Breast	>10	Matta, 2012

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

Six Major DNA Repair Pathways



CANDIDATE INTERVIEW

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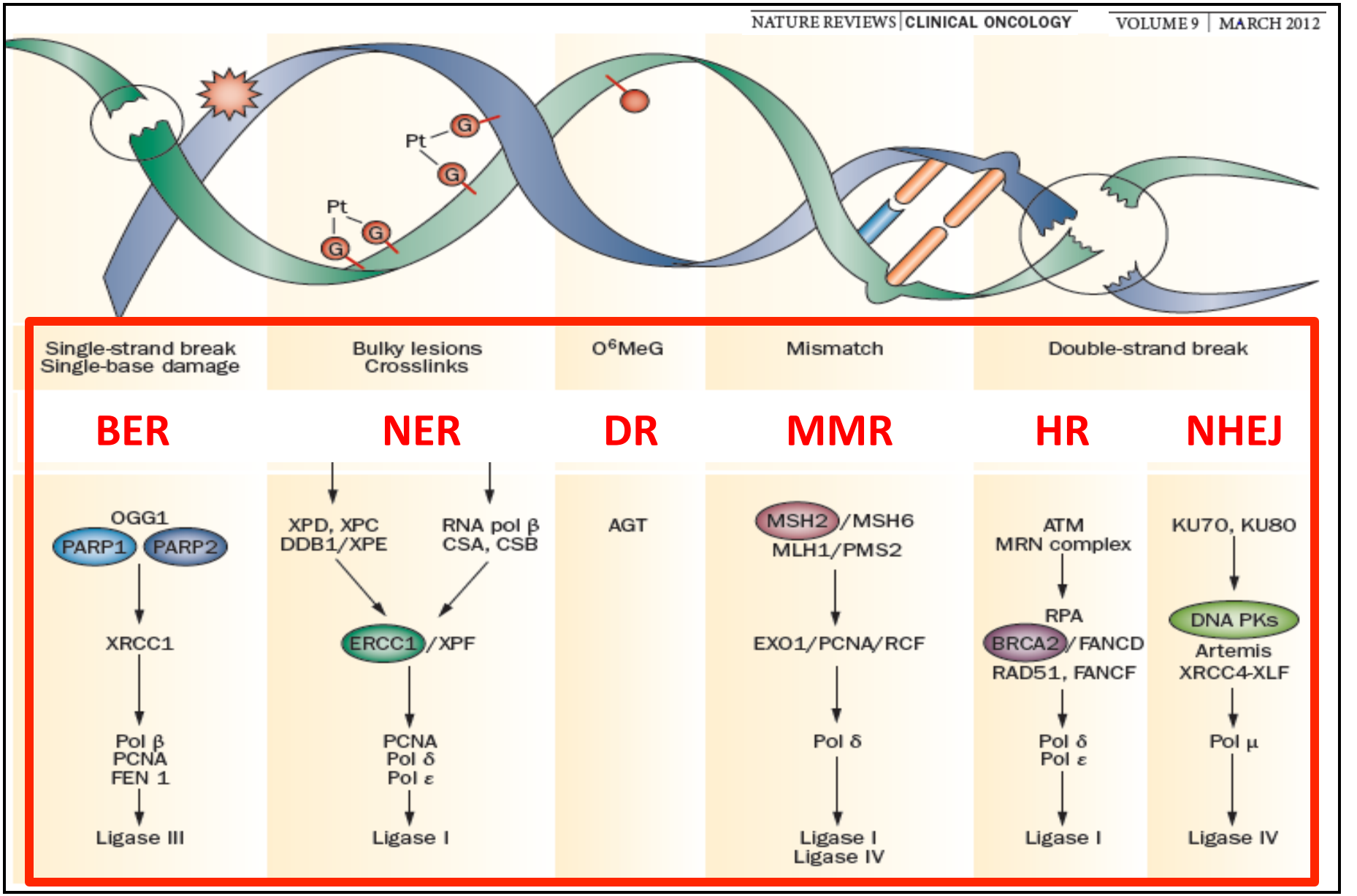
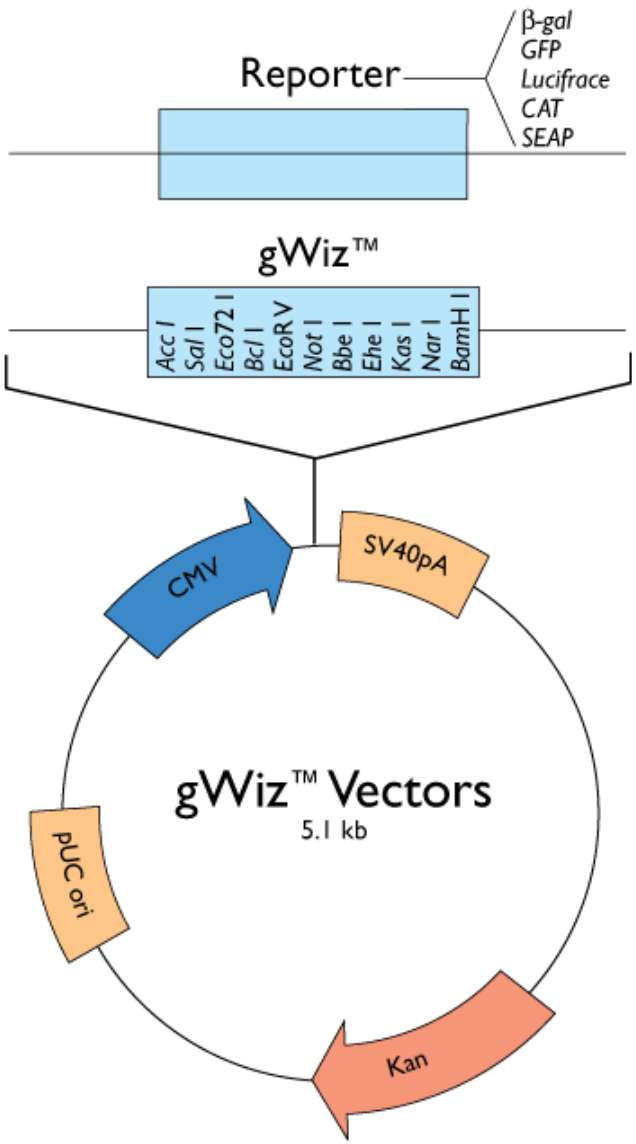
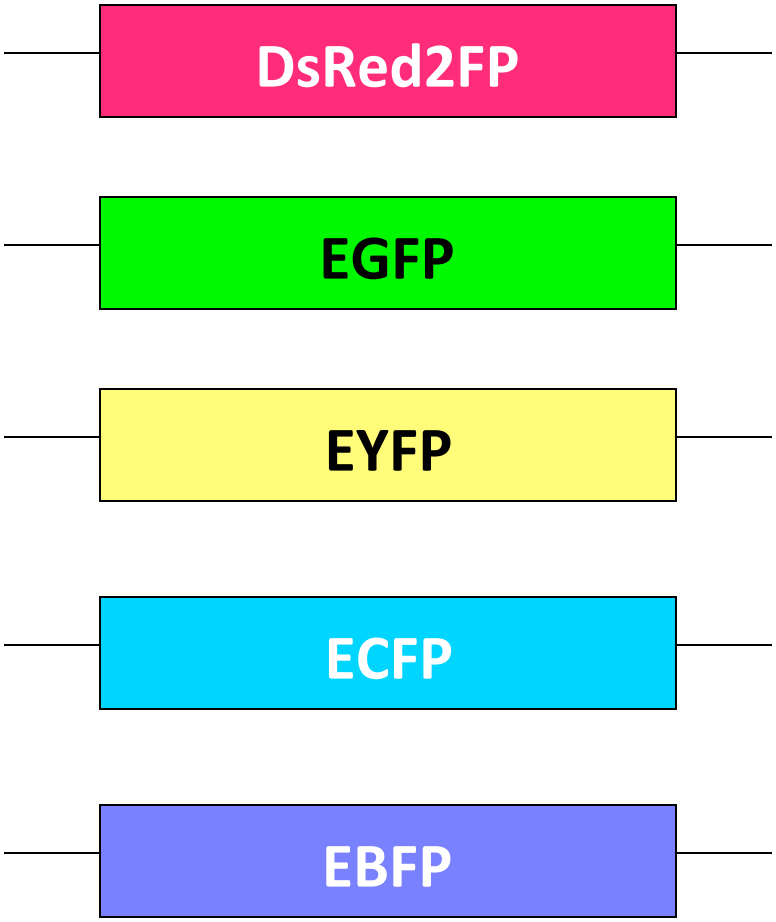
Six Major DNA Repair Pathways



Reactivation of damaged DNA – multiplexed

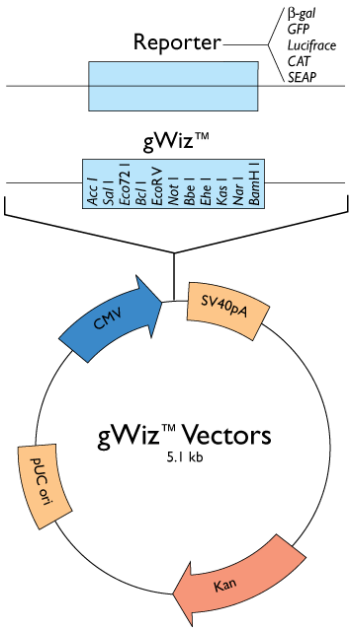


Each Fluorescent Protein gene will harbor a different type of DNA damage

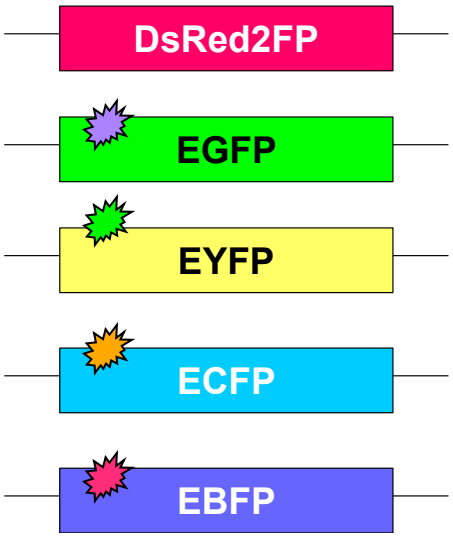


http://www.genlantis.com/corp/images/gWiz_vectmap.gif

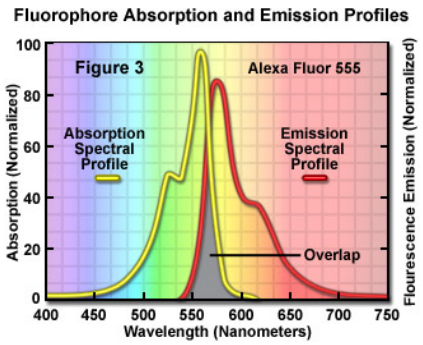
Reactivation of damaged DNA - multiplexed

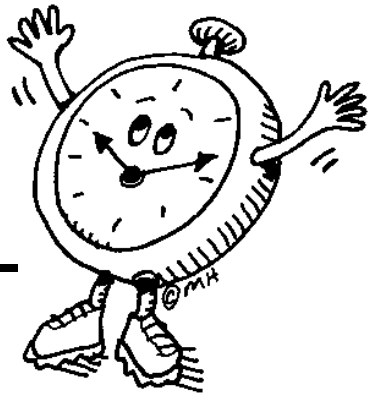


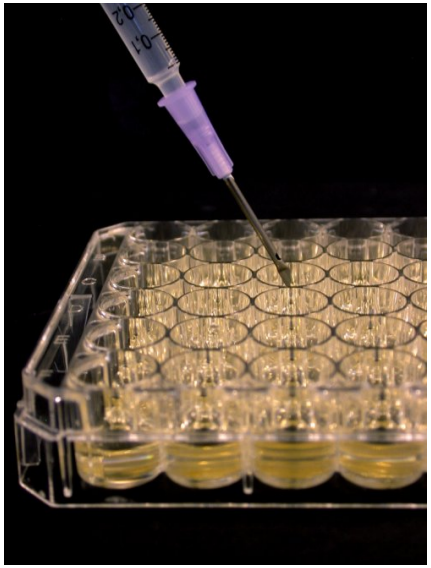
+ different DNA lesions



Transient transfection of mixture



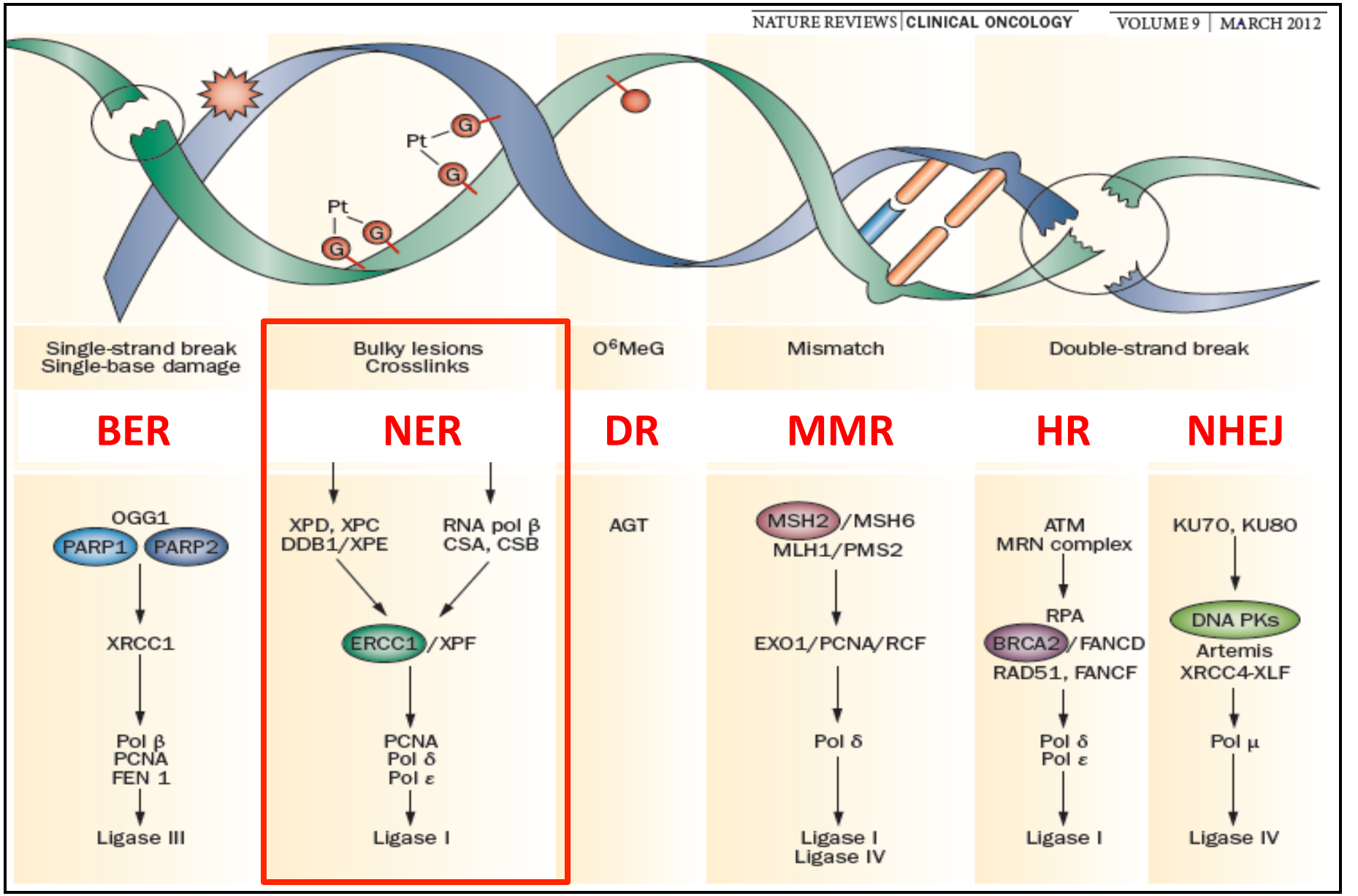




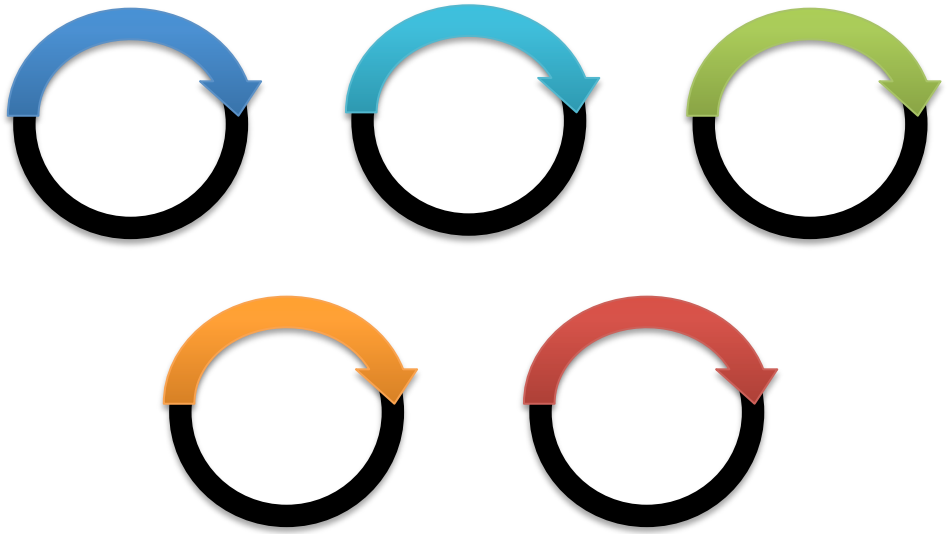
Fluorescence quantitation

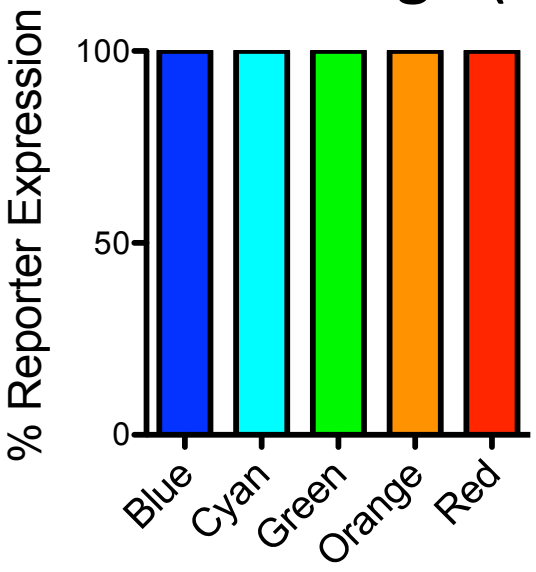
Time to repair

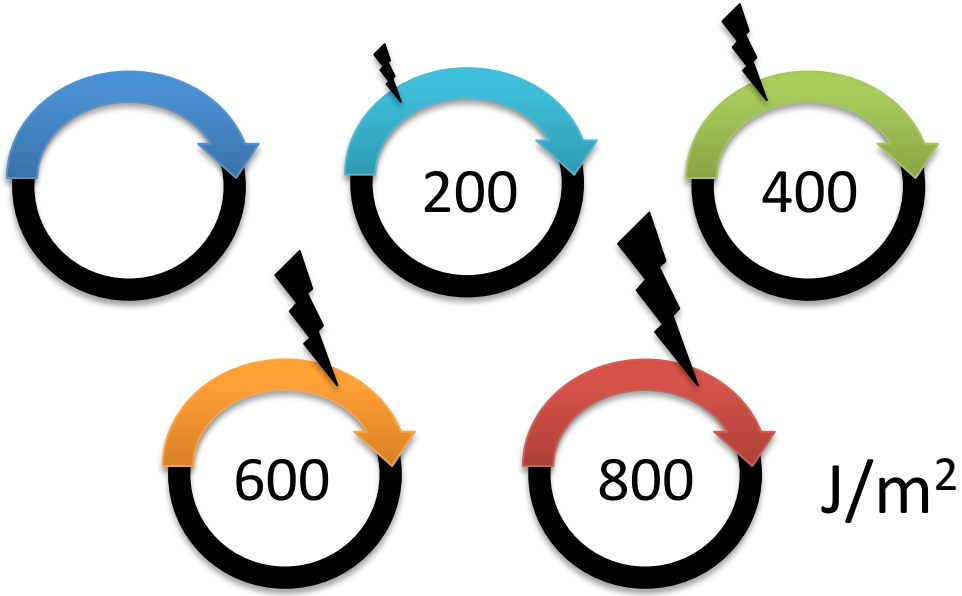
Six Major DNA Repair Pathways

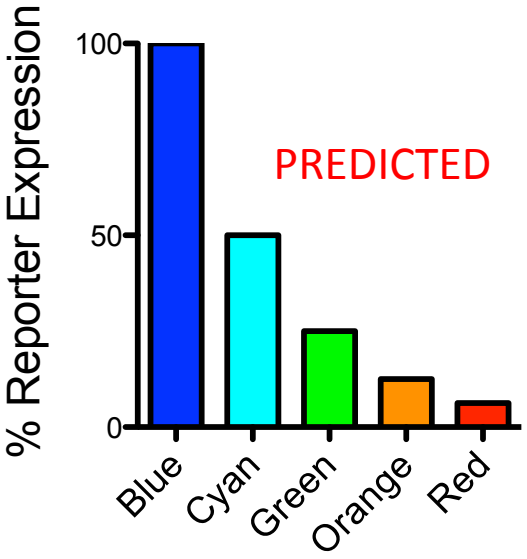


Before trying different damages - tried different doses of the same damage (UV)

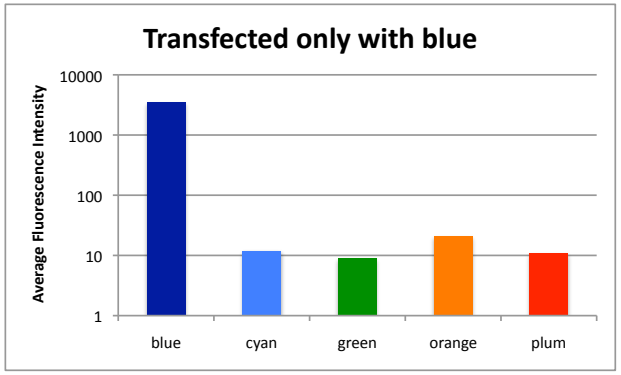


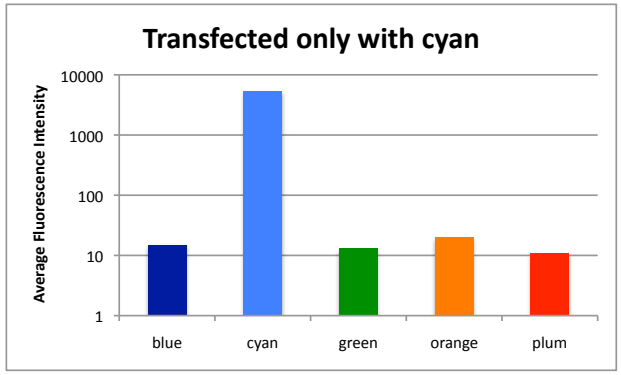


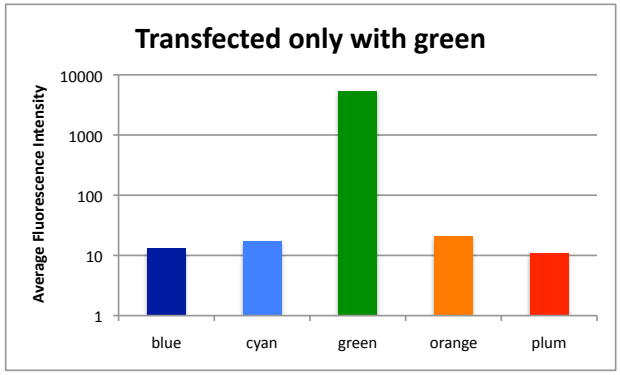


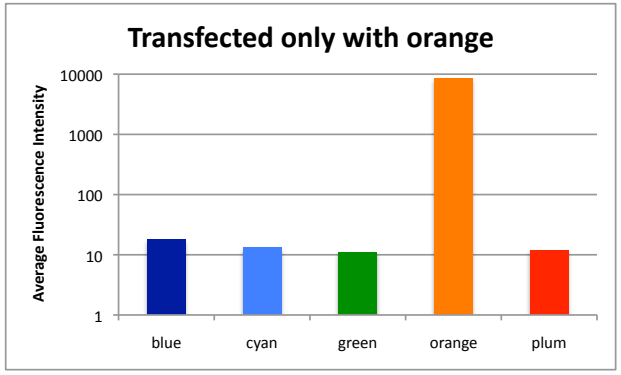


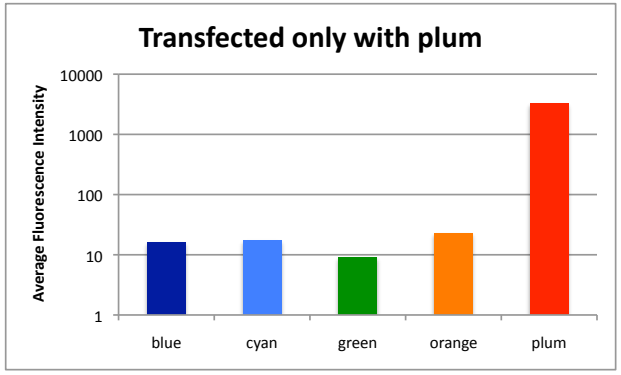
Sanity Check: Is it even feasible detect 5-colors independently?:



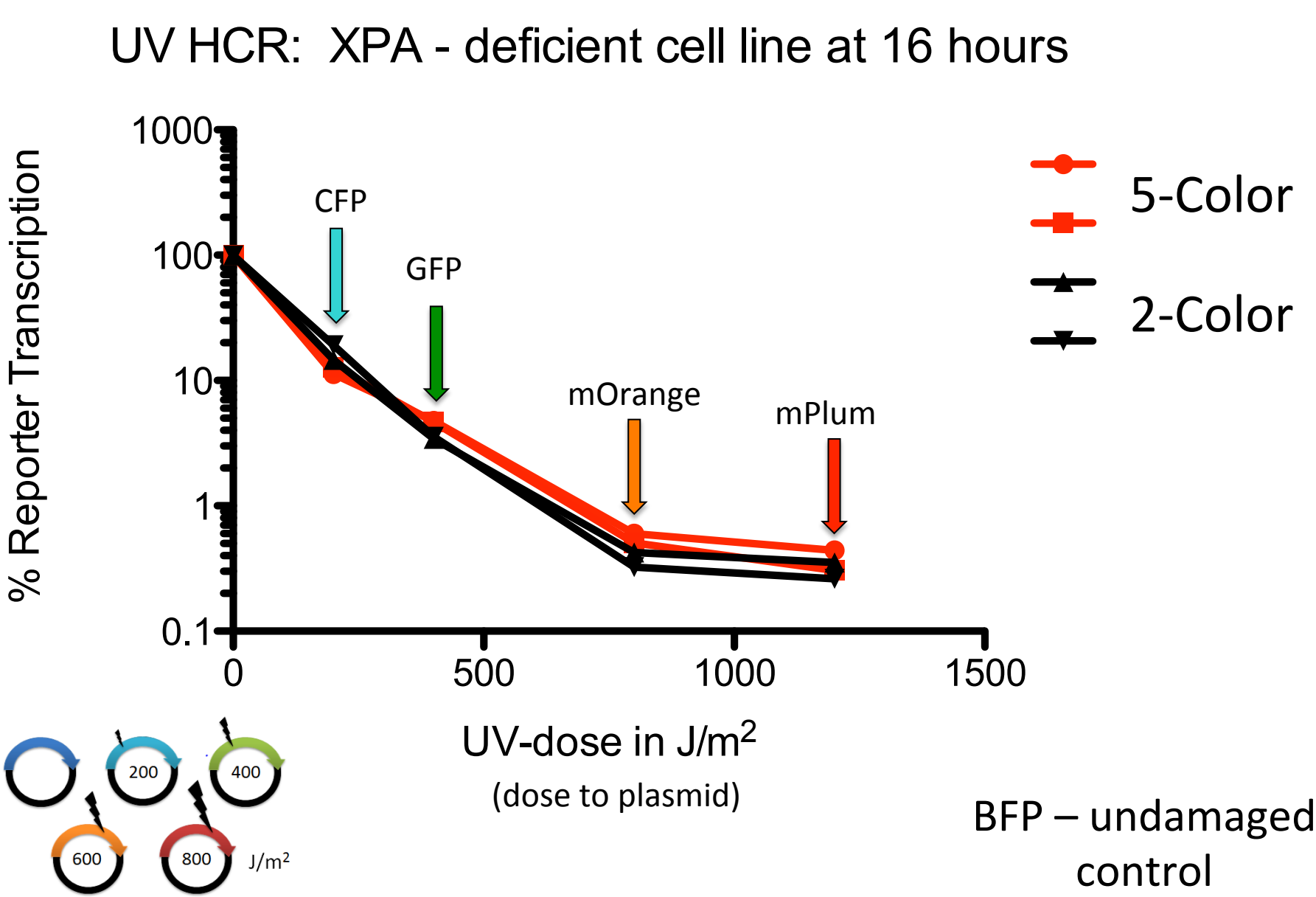






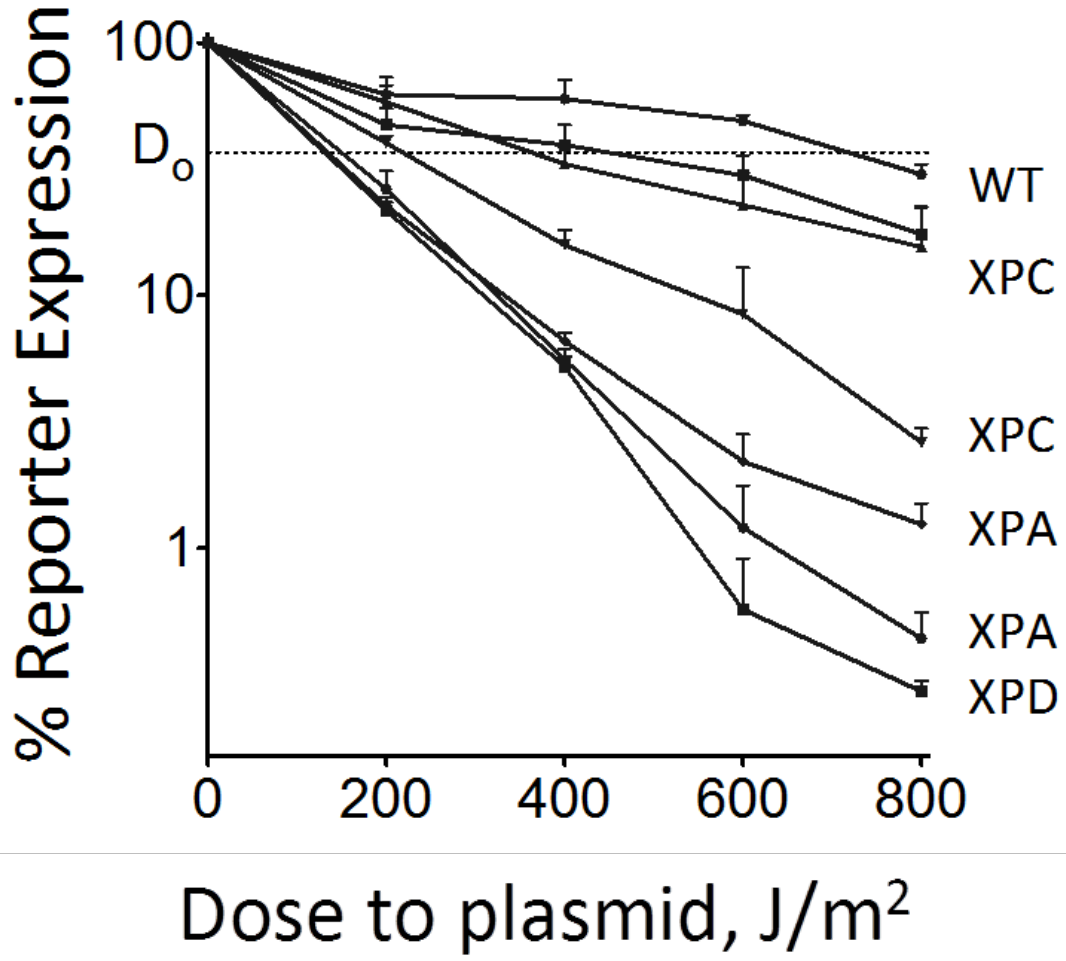


2-color versus 5-color HCR of UV-irradiated plasmids



FM-HCR for UV damaged Plasmids

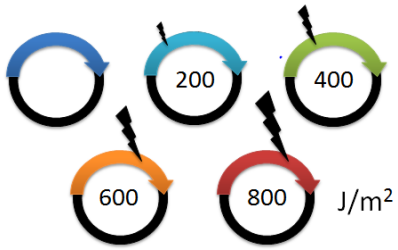
(Nucleotide Excision Repair)



FM-HCR

Fluorescence
Multiplexed

Host
Cell
Reactivation



Zachary Nagel & Siobhan McRee

Development and field-test validation of an assay for DNA repair in circulating human lymphocytes. Cancer Res.1991 51:5786-93.

Athas, Hedayati, Matanoski, Farmer & GROSSMAN

Table 2 *Phenotype and plasmid HCR response (D_0 and $\%CAT_{300}$) in XP homozygote, and apparent normal lymphoblastoid cell lines*

Cell line	Phenotype	Mean ± SD	*n*	95% CI[a]	$\%CAT_{300}$[b]
GM0536	Apparent normal	385 ± 60	3	235–534	59.4
GM0892	Apparent normal	595 ± 22	4	559–630	57.1
GM1953	Apparent normal	717 ± 78	3	523–91	67.7
GM1989	Apparent normal	594 ± 76	3	406–783	58.0
GM3657	Apparent normal	381 ± 15	4	357–405	47.0
GM2250	XP-A homozygote	90 ± 9	3	67–112	3.0
GM2344	XP-A homozygote	132 ± 9	3	110–155	6.4
GM2345	XP-A homozygote	90 ± 9	3	69–111	3.0
GM2246	XP-C homozygote	165 ± 19	4	134–195	22.1
GM2249	XP-C homozygote	256 ± 12	5	241–270	31.2
GM2253	XP-D homozygote	75 ± 5	3	62–88	1.8
GM2485	XP-D homozygote	97 ± 12	3	67–125	4.5
GM2450	XP-E homozygote	312 ± 42	5	260–364	

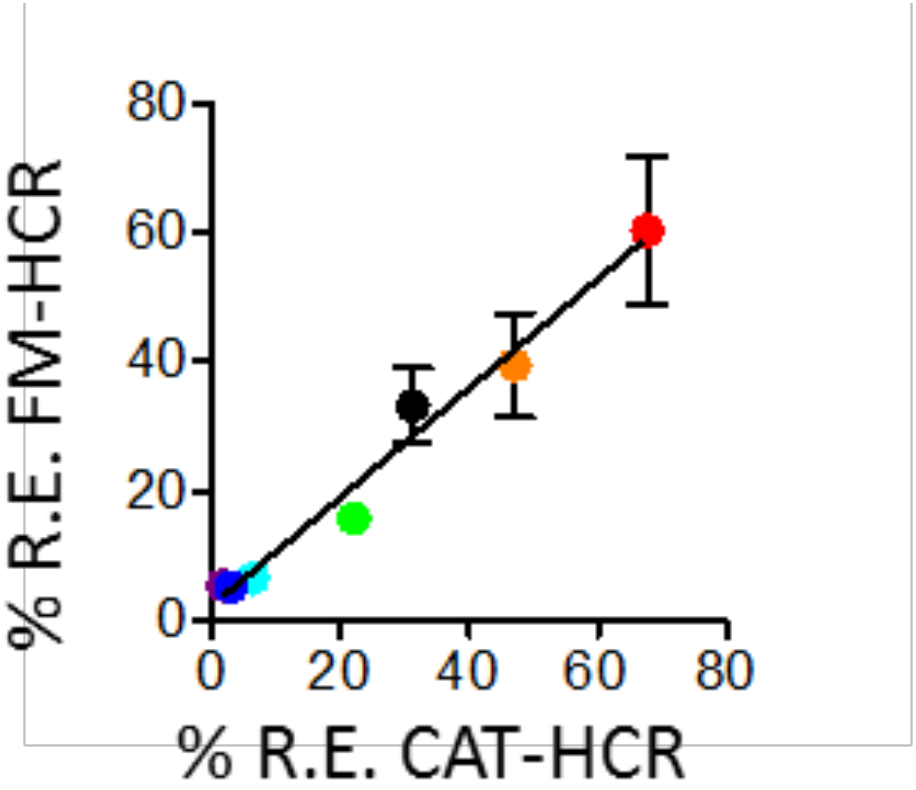
[a] CI, confidence interval.

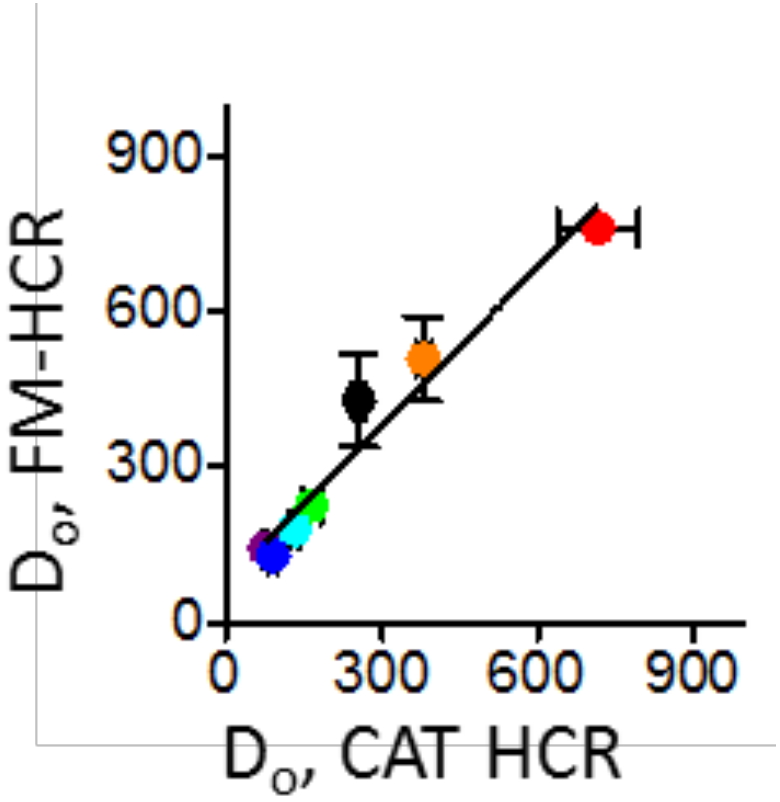
How does our FM-HCR data stack up against CAT-HCR, Grossman *et al.*, > 20 years ago?

Cancer Research. 1991; **51** (21): 5786-93

How does our FM-HCR data stack up against CAT-HCR, Grossman *et al.*, > 20 years ago?

Cancer Research. 1991; **51** (21): 5786-93

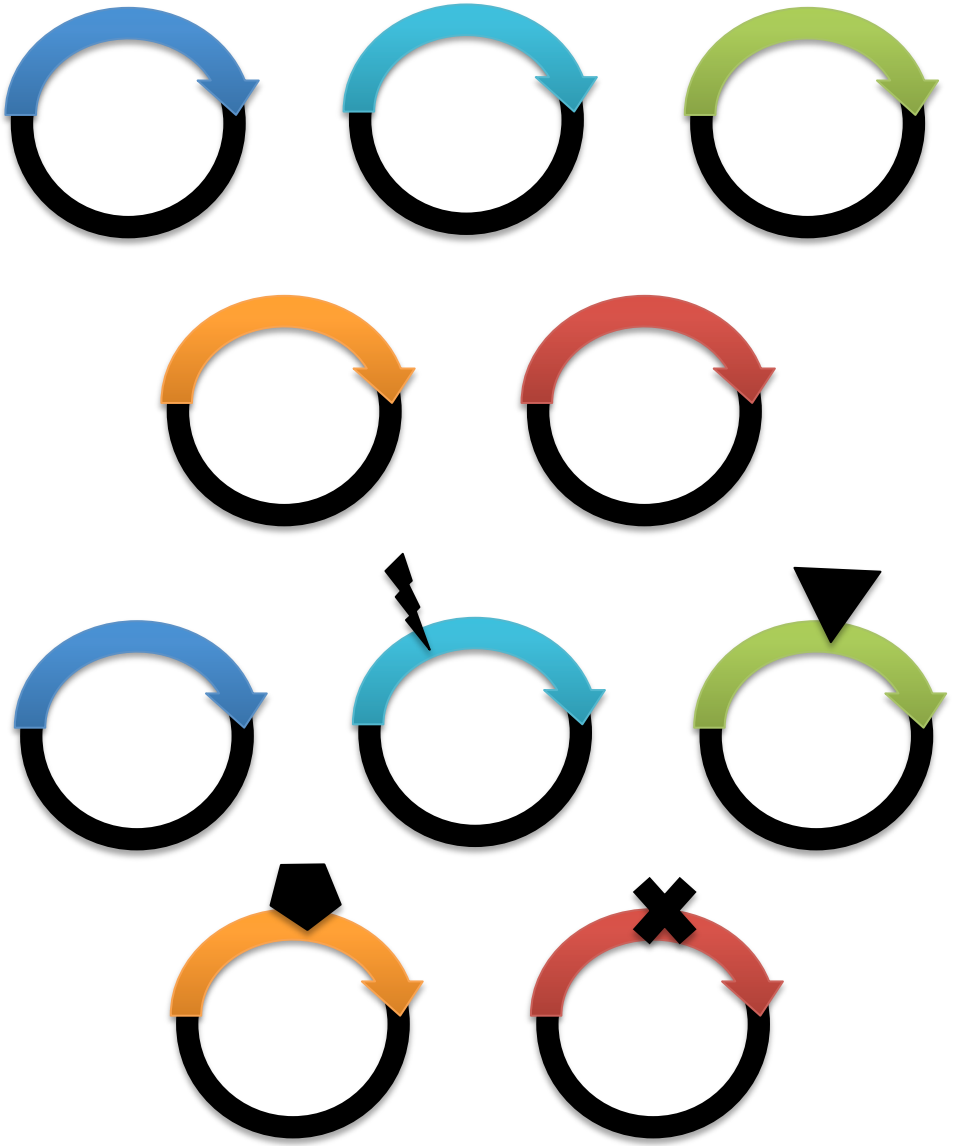


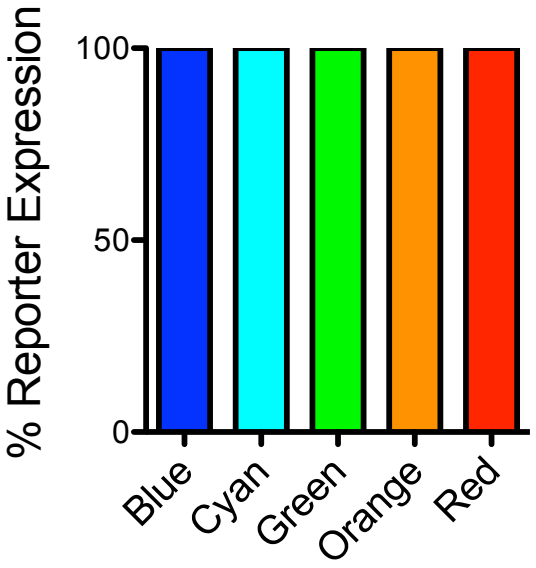


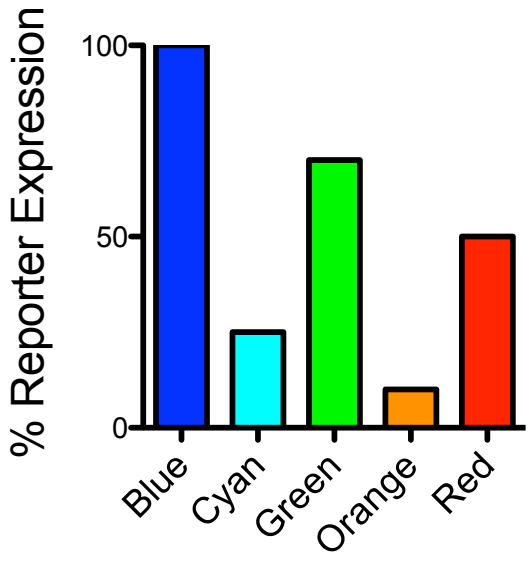
$R^2 = 0.92$, $p = 0.0006$

$R^2 = 0.92$, $p = 0.0001$

5 color HCR assay applications

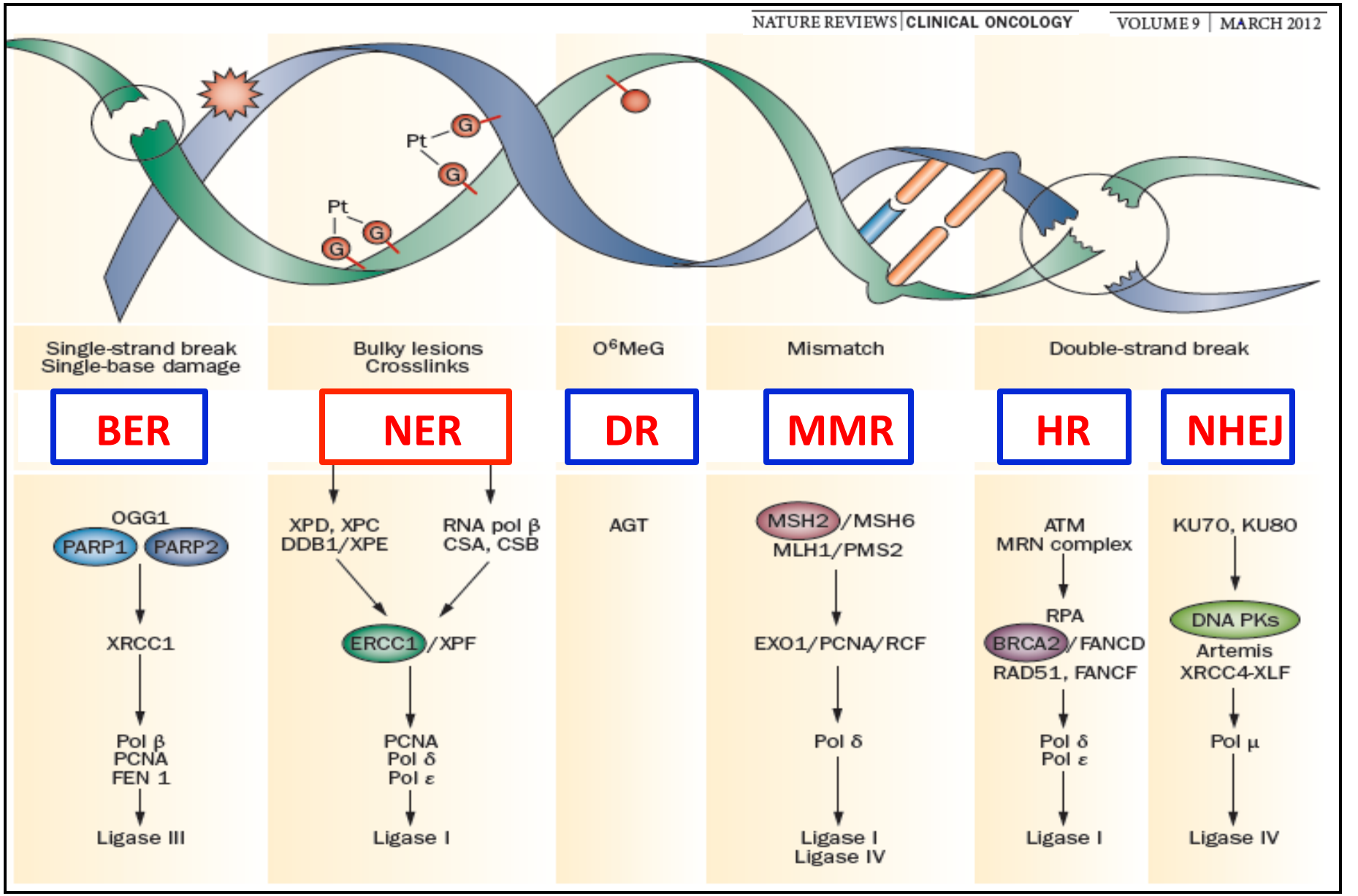




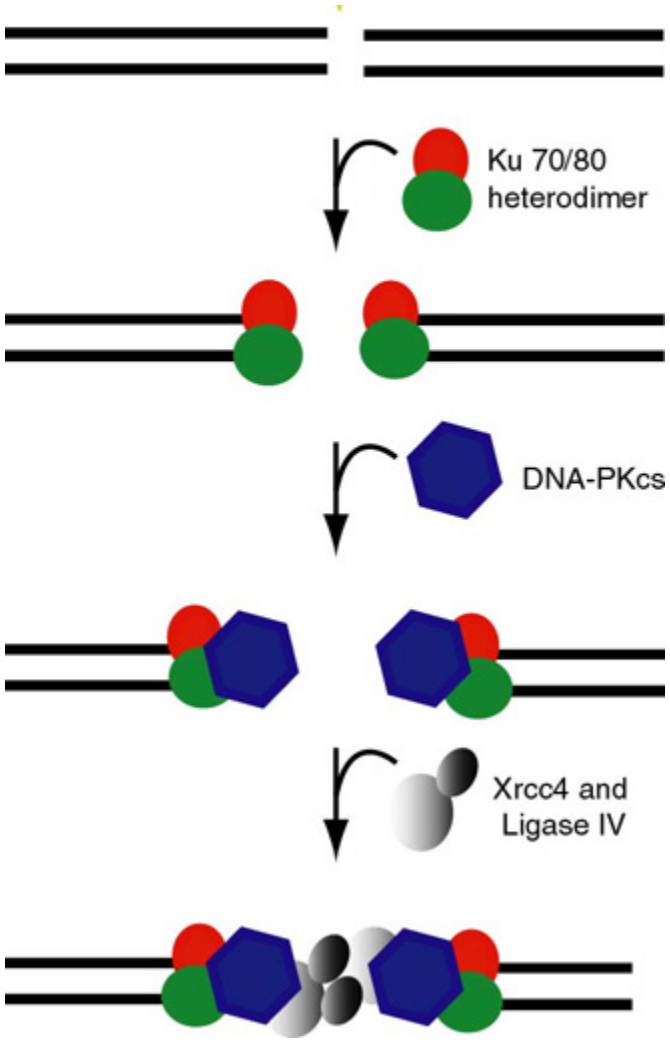


5-color HCR developed by **Dr. Zachary Nagel**

Six Major DNA Repair Pathways

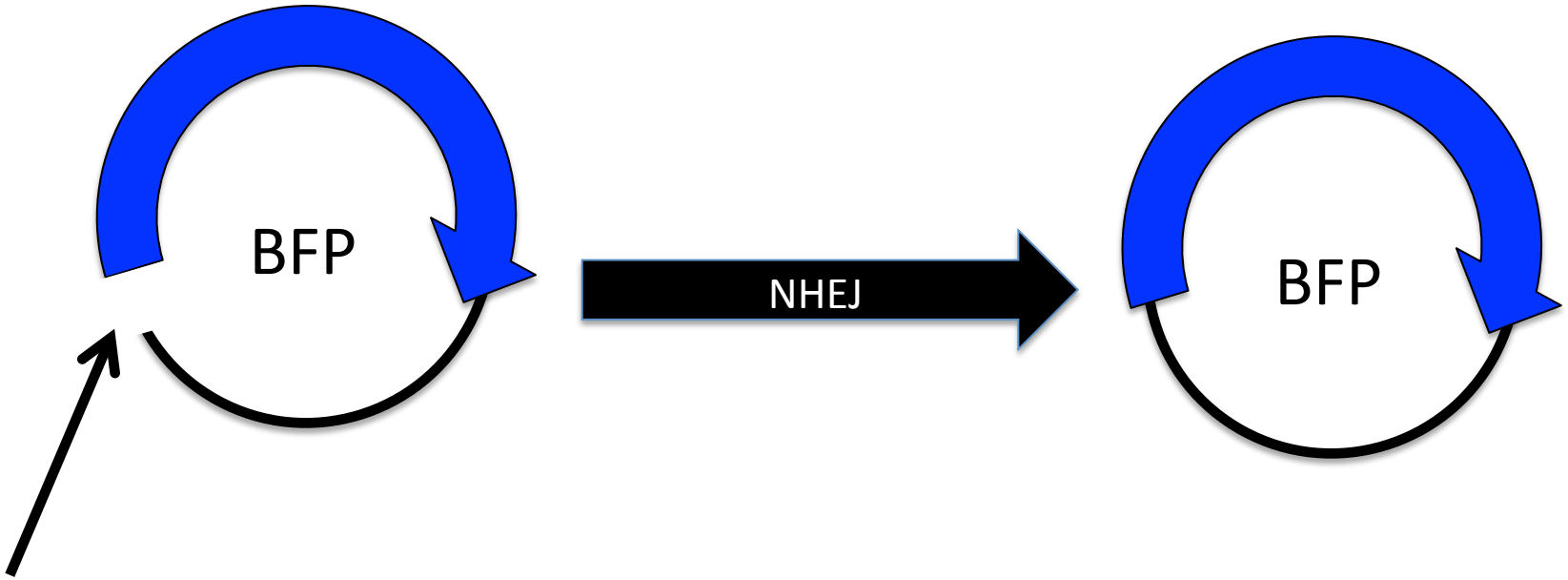


Non-Homologous End Joining (NHEJ) DNA Double Strand Break Repair



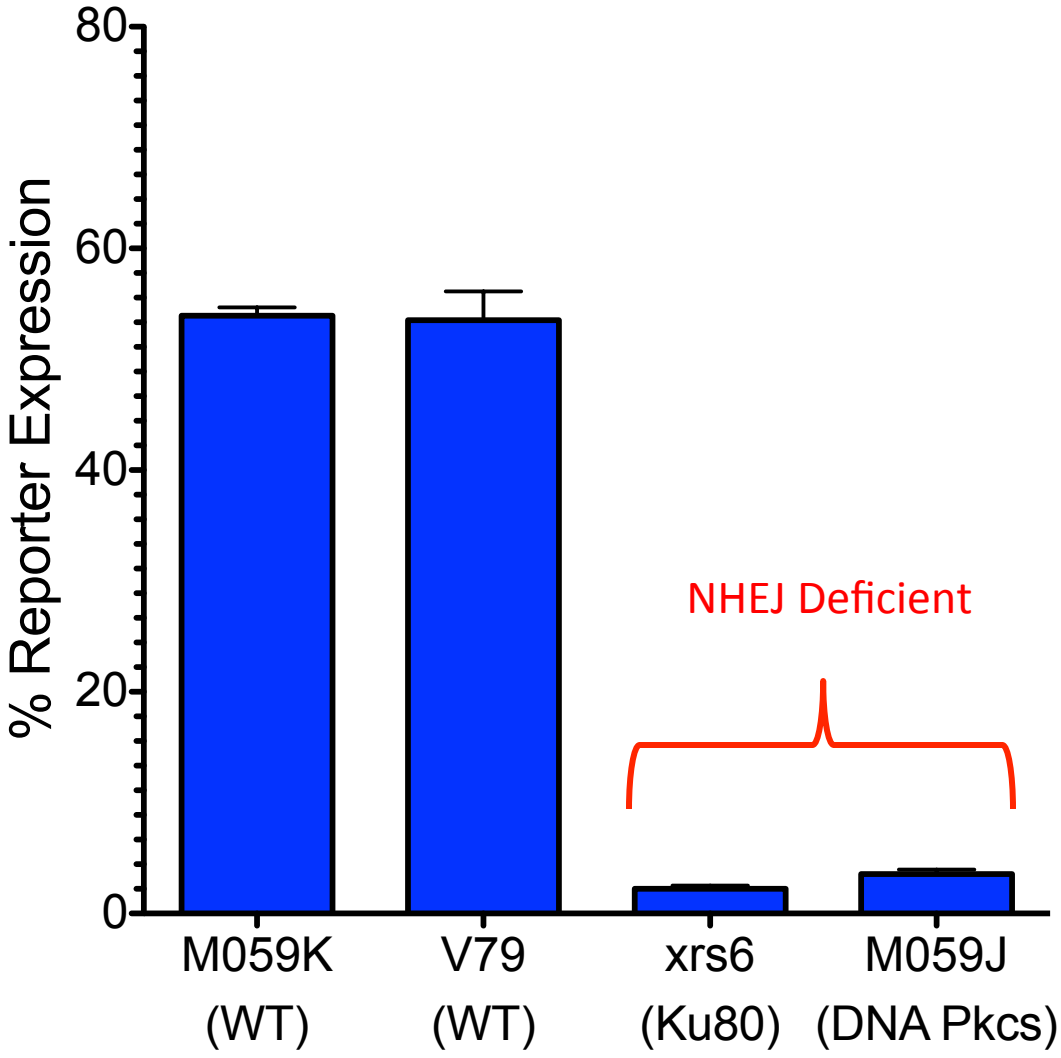
http://atlasgeneticsoncology.org/Deep/DoubleStrandBreaksID20008.html

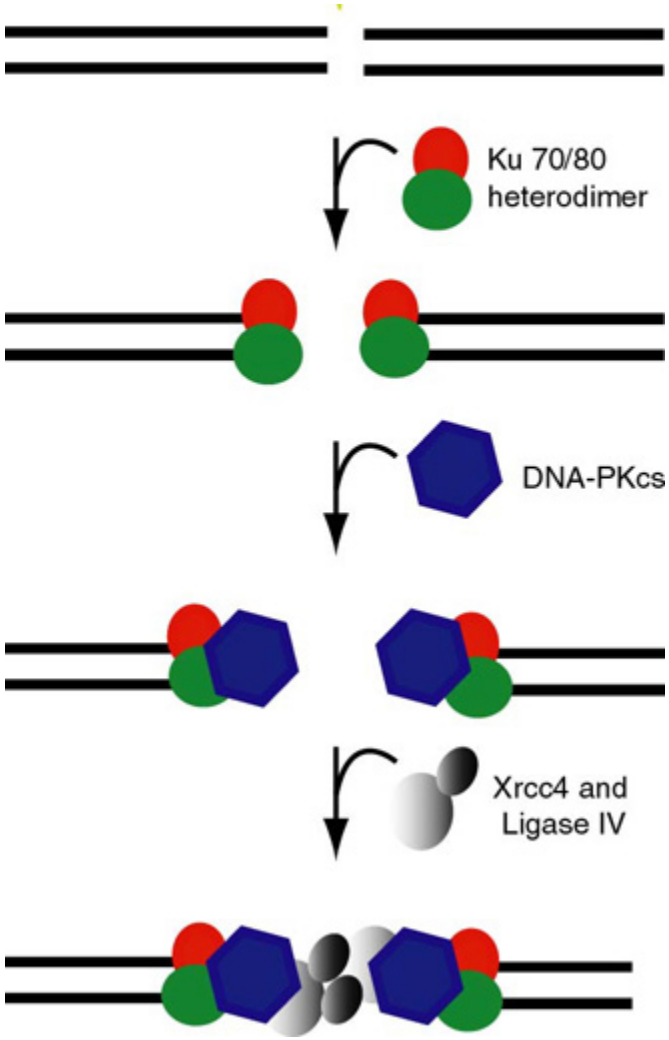
Non-Homologous End Joining (NHEJ)



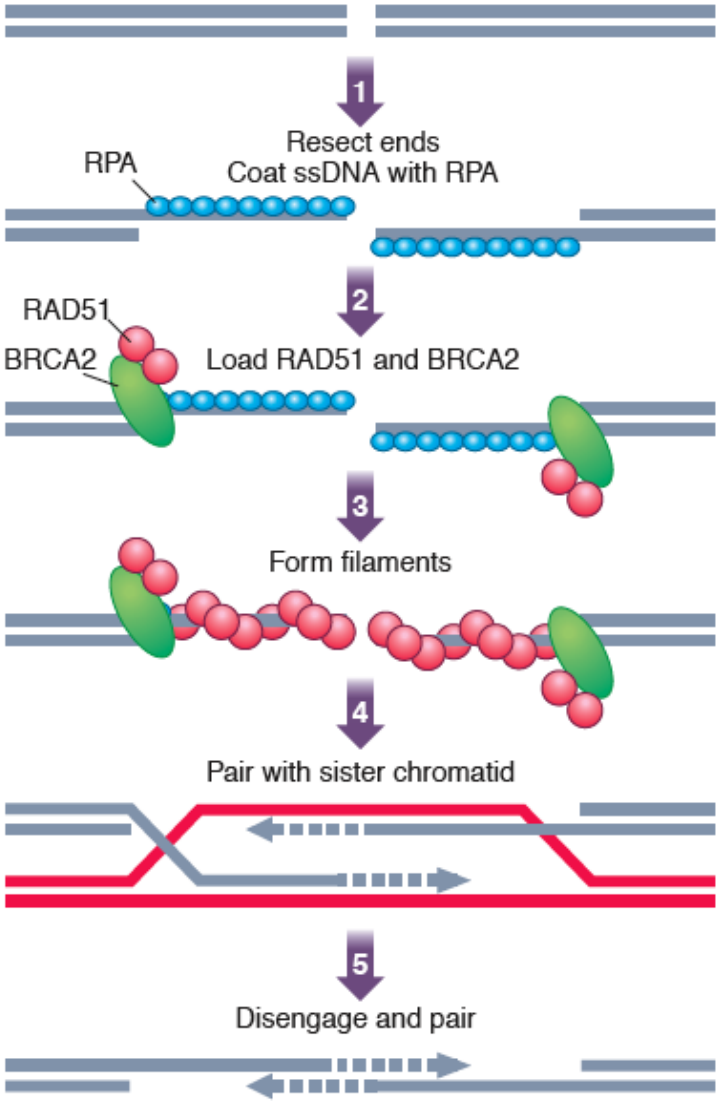
Substrate contains a blunt-end DSB in the 5’ UTR

NHEJ HCR in WT and NHEJ defective cells:

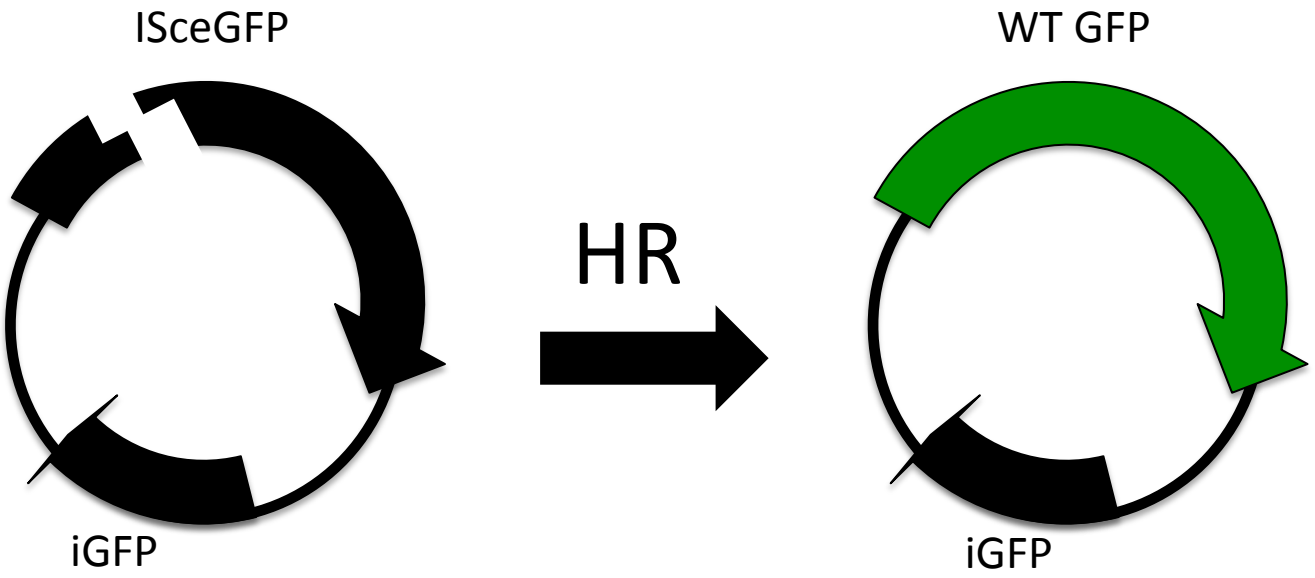




DNA Double Strand Break Repair Homologous Recombination (HR)



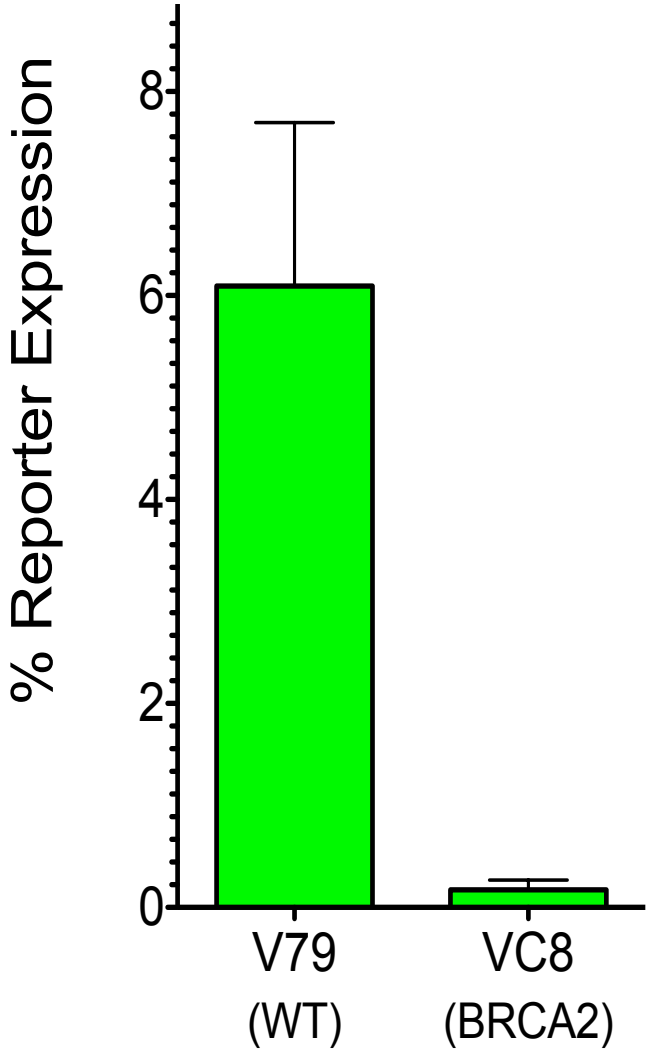
DNA Double Strand Break Repair Homologous Recombination (HR)

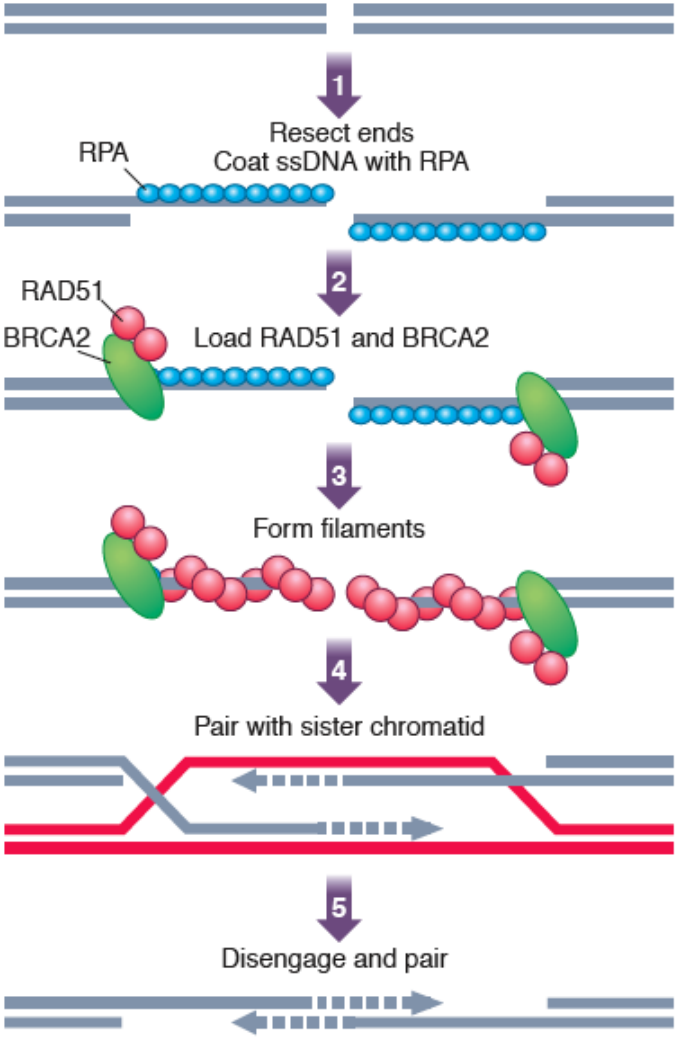


Adapted from Maria Jasin

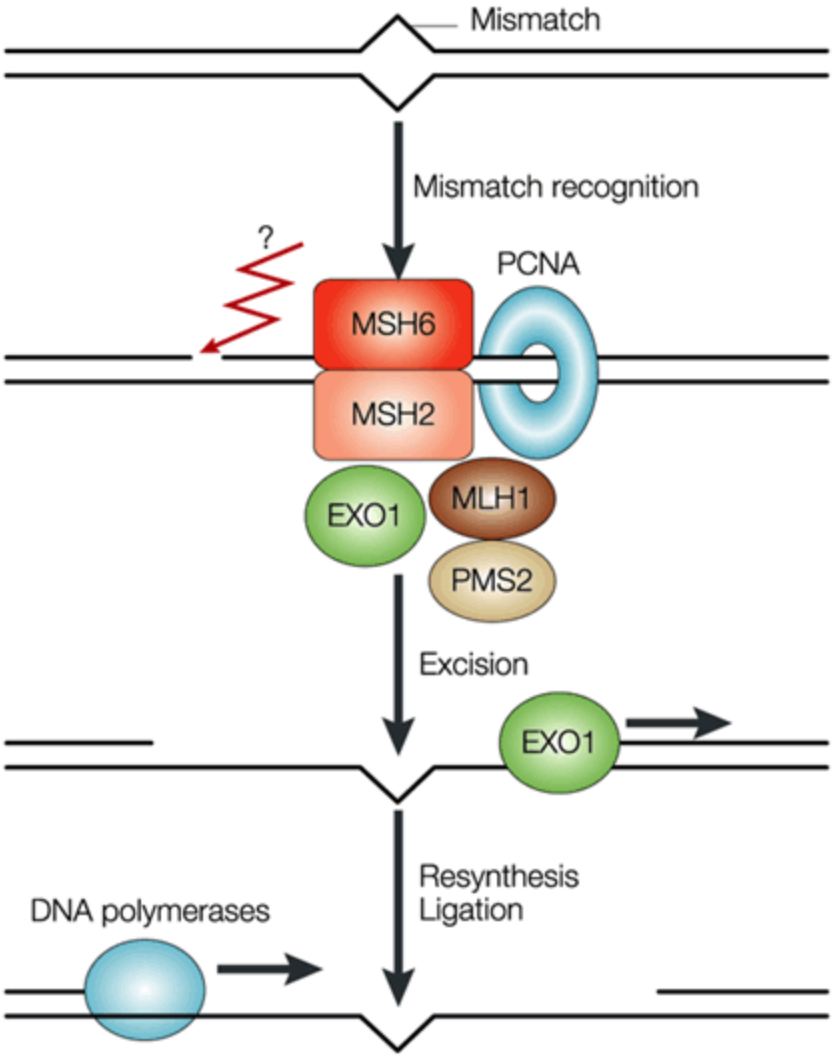
Pierce et al. (1999) Genes and Development (13) 2633-2638

FM-HCR for DSB repair by Homologous Recombination (HR)



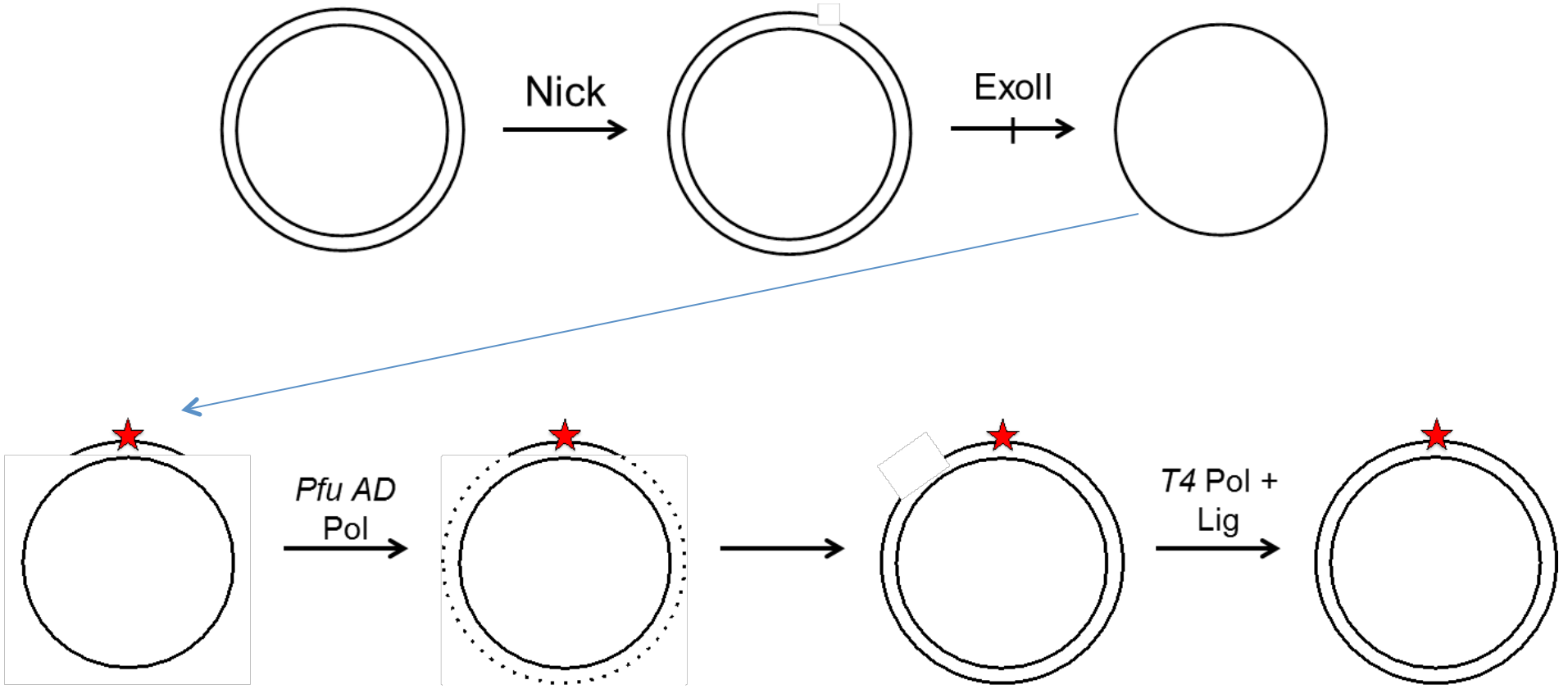


DNA Mismatch Repair (MMR)



https://www.google.com/search?q=dna+mismatch+repair&tbm=isch&tbo=u&source=univ&sa=X&ei=pipkUraXMun-4AOAloGgBw&ved=0CDkQsAQ&biw=1067&bih=501&dpr=1#facrc=_&imgdii=_&imgrc=a7bj74c8D9MBtM%3A%3BYVLE34T6L3YPAM%3Bhttp%253A%252F%252Fwww.nature.com%252Fnri%252Fjournal%252Fv2%252Fn8%252Fimages%252Fnri858-i1.gif%3Bhttp%253A%252F%252Fwww.nature.com%252Fnri%252Fjournal%252Fv2%252Fn8%252Fbox%252Fnri858_BX1.html%3B600%3B639

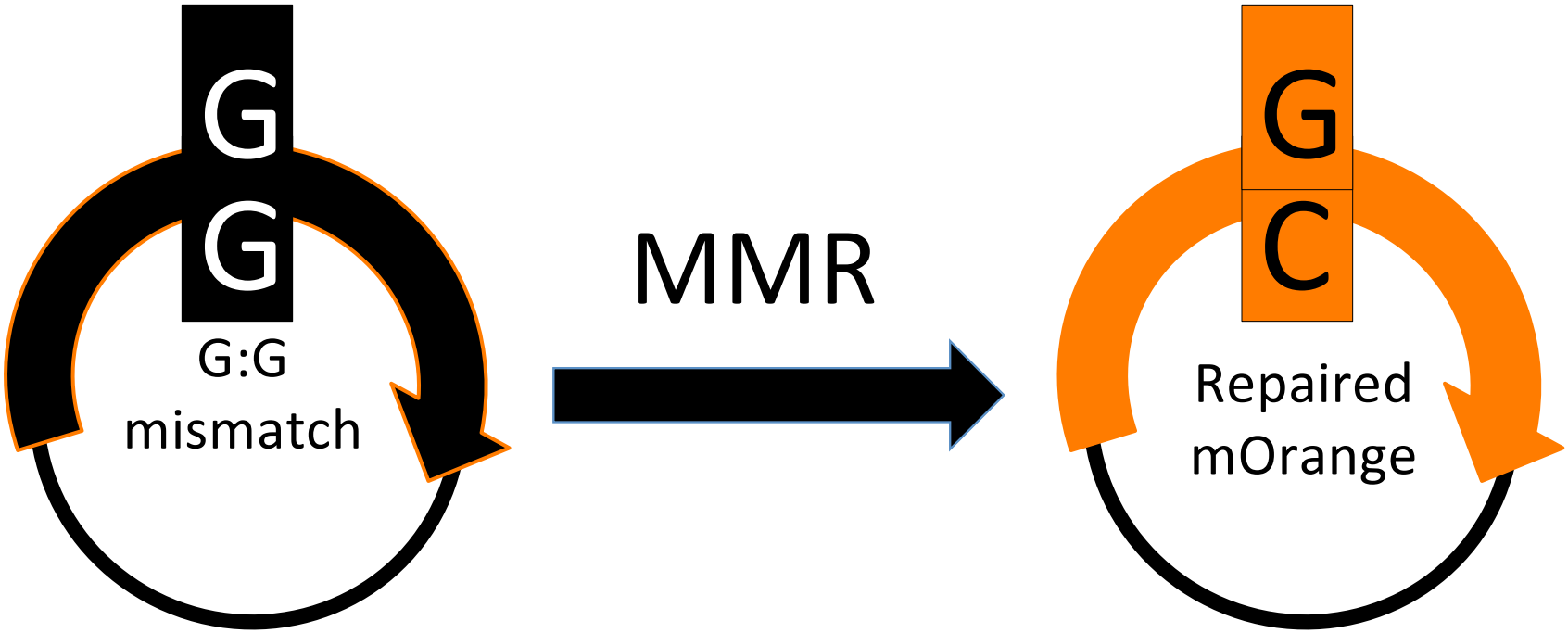
How to build a site-specific reporter? "Primer-Extension"



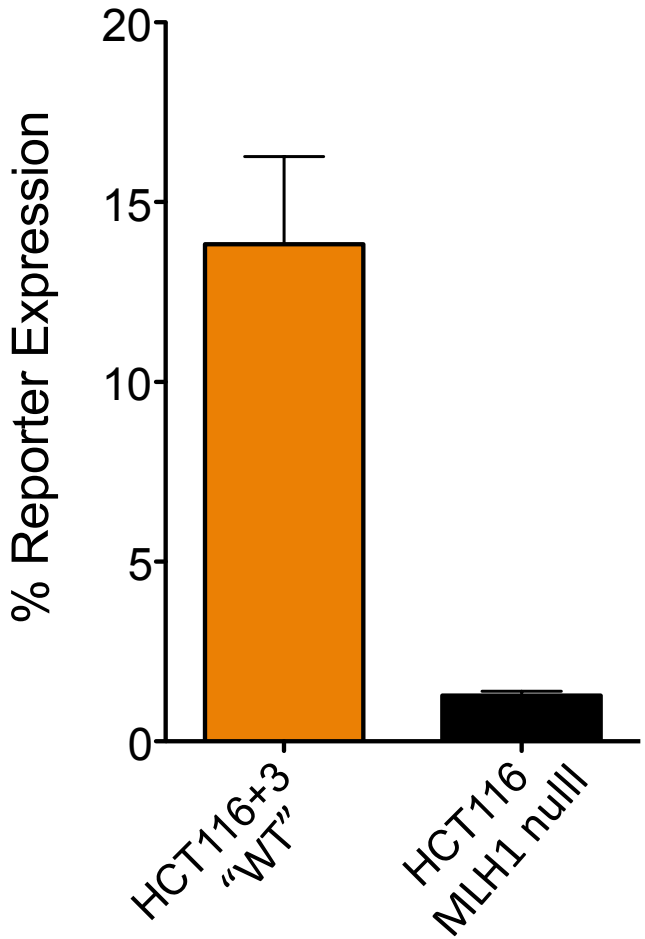
Original protocol from Baerenfaller *et al.* 2006 *Meth in enzymology*

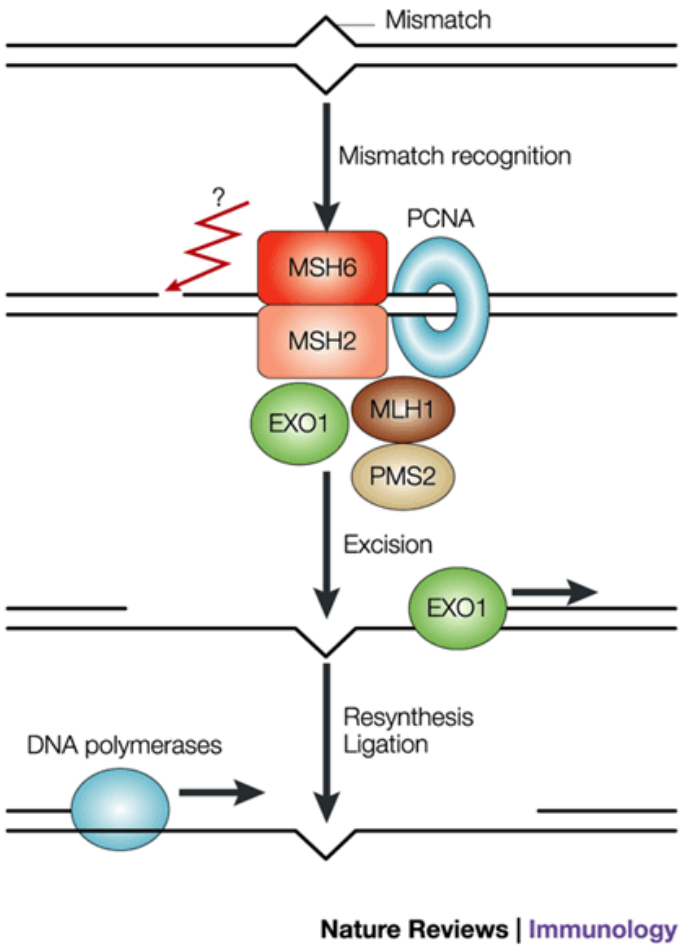
Modified and optimized by Alex Chaim, Zachary Nagel and Patrizia Mazzucato

DNA Mismatch Repair (MMR)

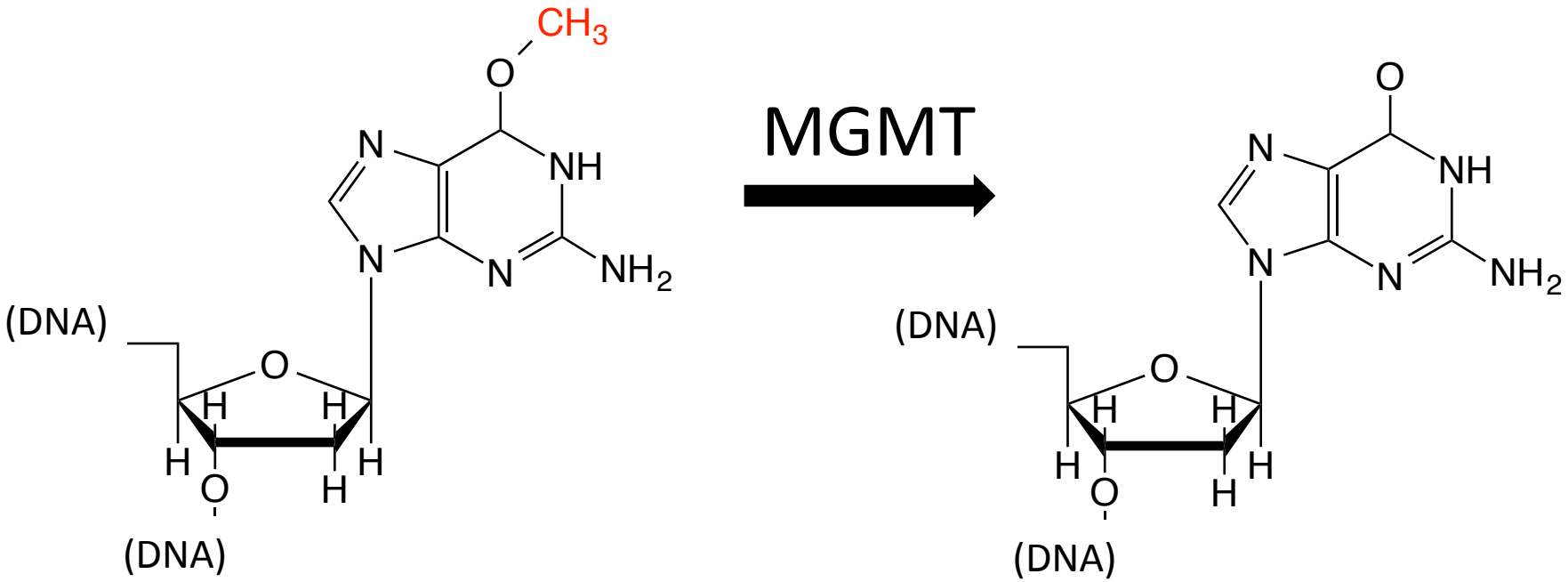


FM-HCR for DNA Mismatch Repair

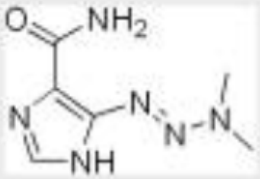
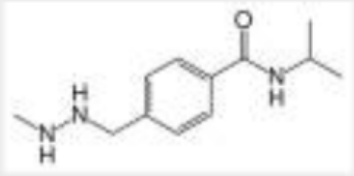
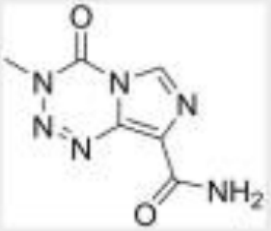
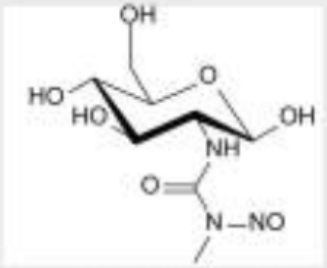
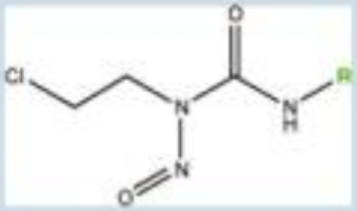
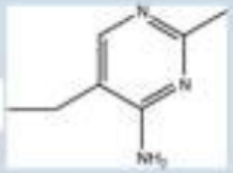
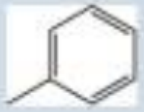
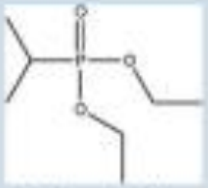




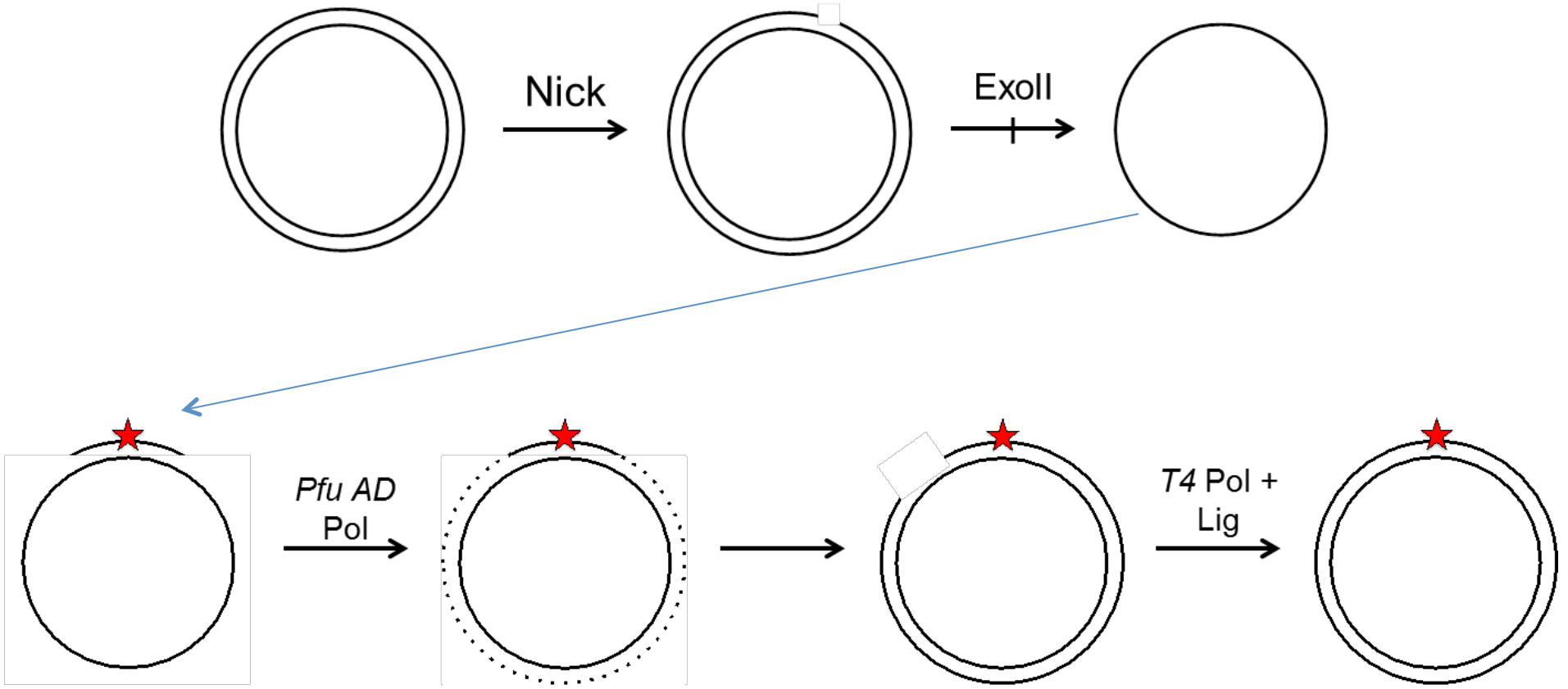
O^6-Methylguanine DNA Methyltransferase MGMT – Repairs by Direct Reversal (DR)



Alkylating agents used in the cancer clinic

a Monofunctional				Lesions[1]
Triazene			*Nitrosourea*	
				7meG 3meA O^6meG
Dacarbazine Metastatic melanoma Hodgkin's lymphoma Sarcoma	**Procarbazine** Malignant gliomas Hodgkin's lymphoma	**Temozolomide** Malignant gliomas	**Streptozotocin** Pancreatic islet cell cancer	
Chloroethylating Nitrosoureas				7-alkylG O^6Cl-ethylG N1,O^6-EG G-C x-link G-G x-link
	R= 			
	ACNU (Nimustine) Brain tumors Solid tumors	**BCNU (Carmustine)** Brain tumors Lymphomas Melanoma	**CCNU (Lomustine)** Brain tumors Lymphomas Melanoma	**Fotemustine** Metastatic melanoma

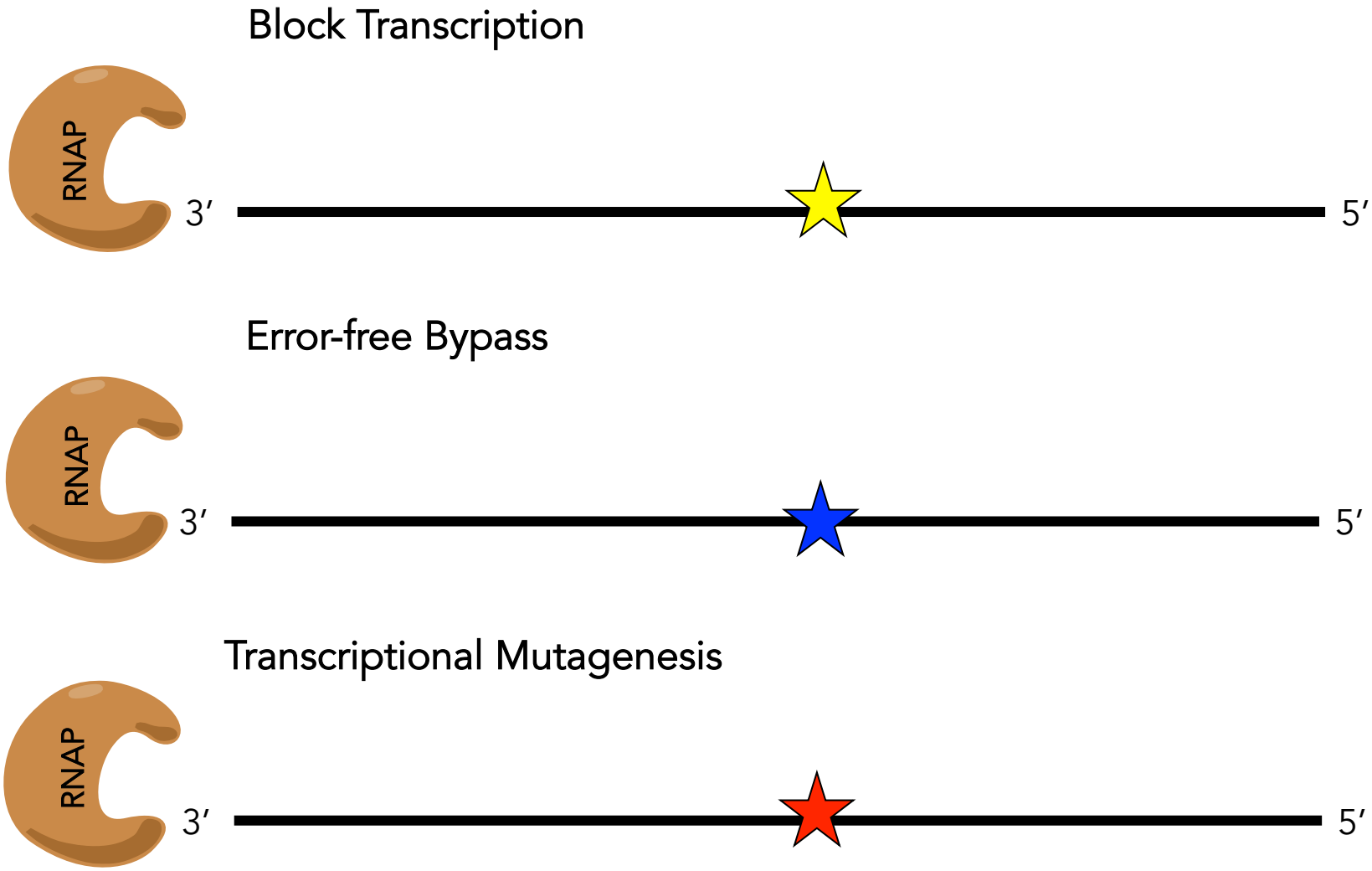
How to build a site-specific reporter? "Primer-Extension"



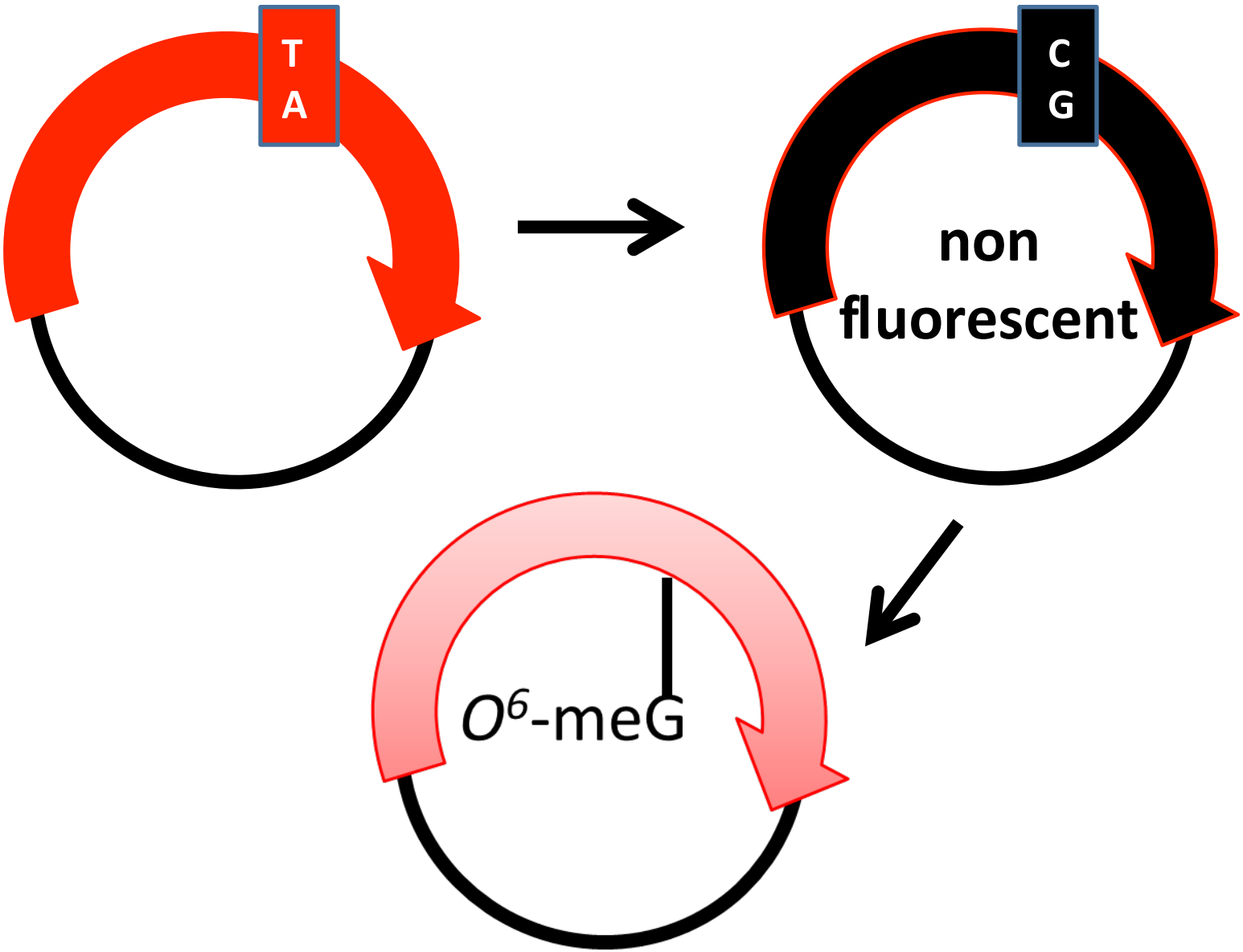
Original protocol from Baerenfaller *et al.* 2006 *Meth in enzymology*

Modified and optimized by Alex Chaim, Zachary Nagel and Patrizia Mazzucato

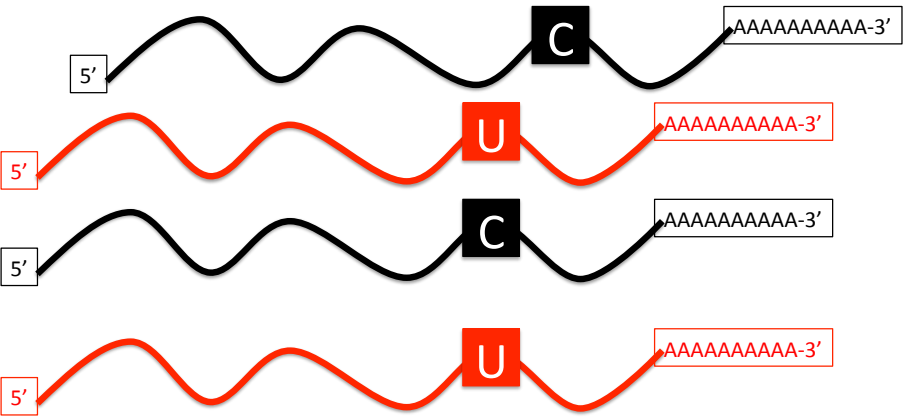
DNA lesions from an RNA polymerase perspective

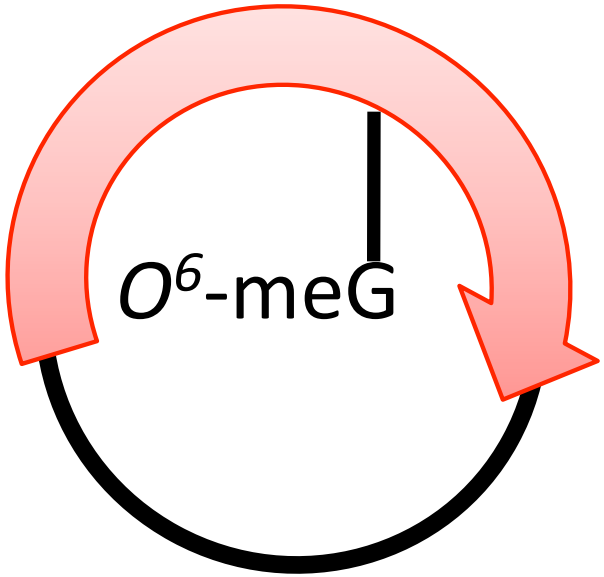


Manipulation of the RFP Reporter Gene

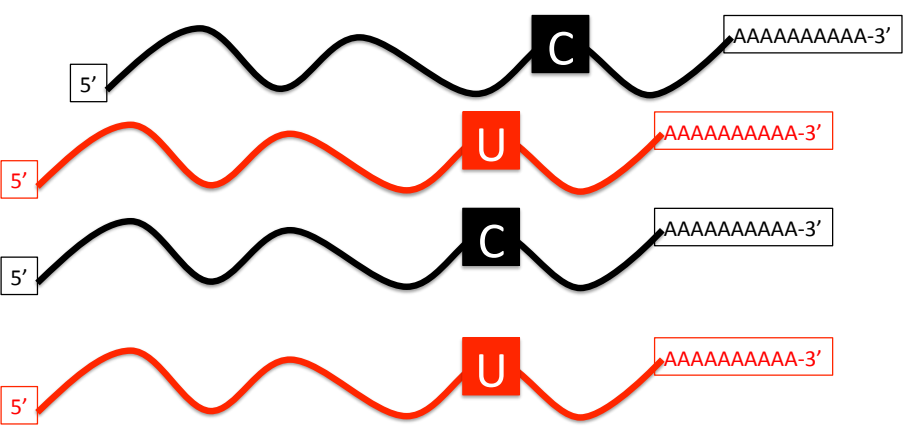


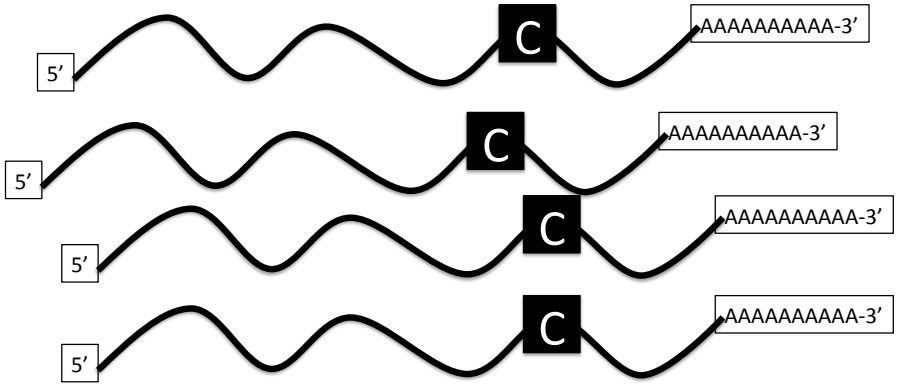
RNA Pol II occasionally inserts U at O^6-meG
Transcriptional Mutagenesis

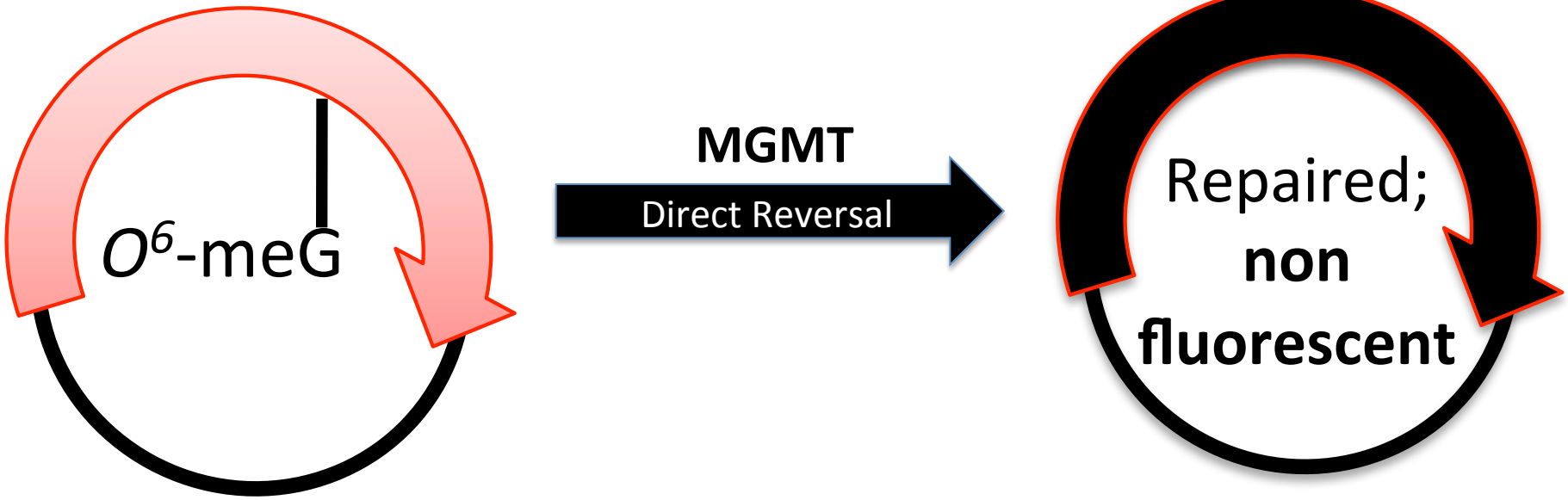




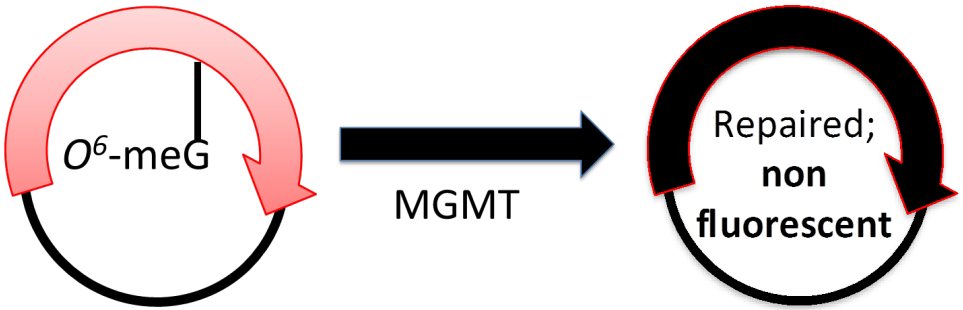
RNA Pol II occasionally inserts U at O^6-meG
Transcriptional Mutagenesis

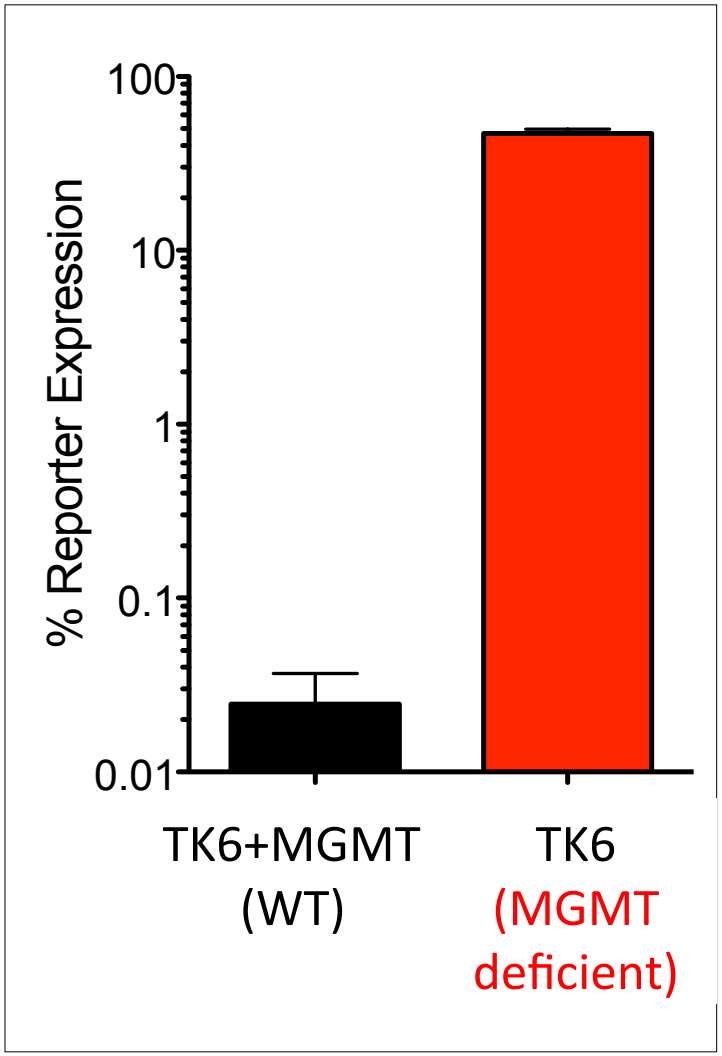






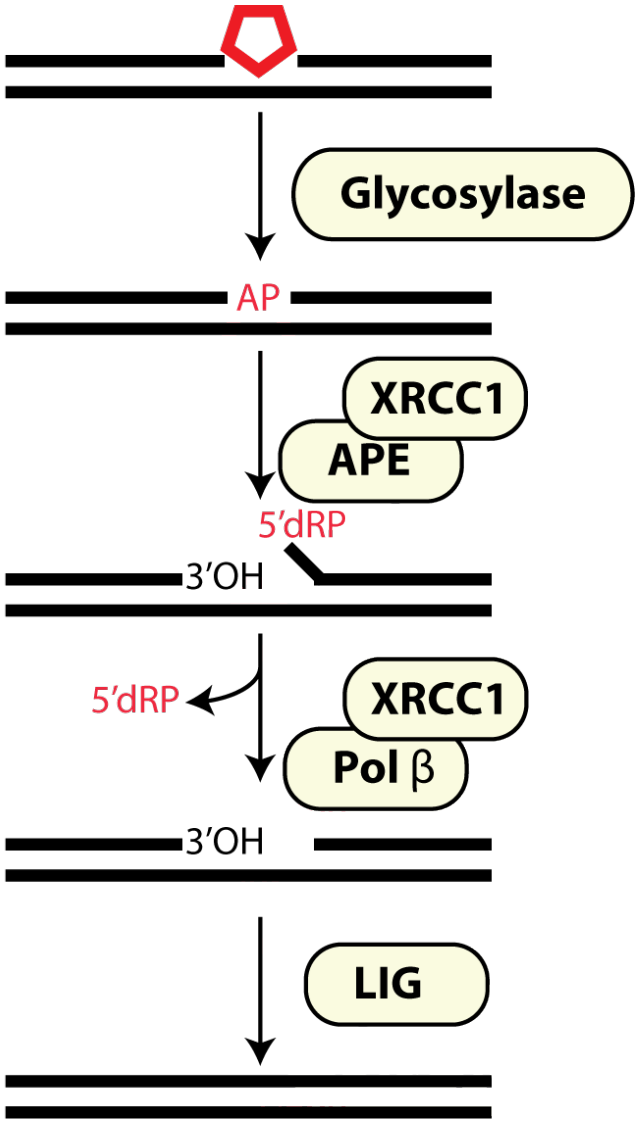
FM-HCR for O^6-MeG repair via Direct Reversal (DR) by MGMT



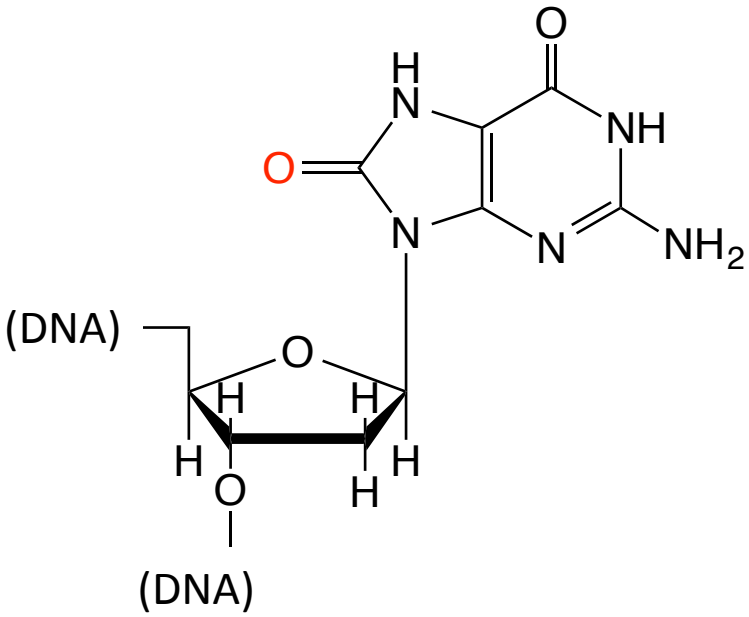


Base Excision Repair (BER)

For oxidized, deaminated and alkylated base damage



Base excision repair of 8-oxoG

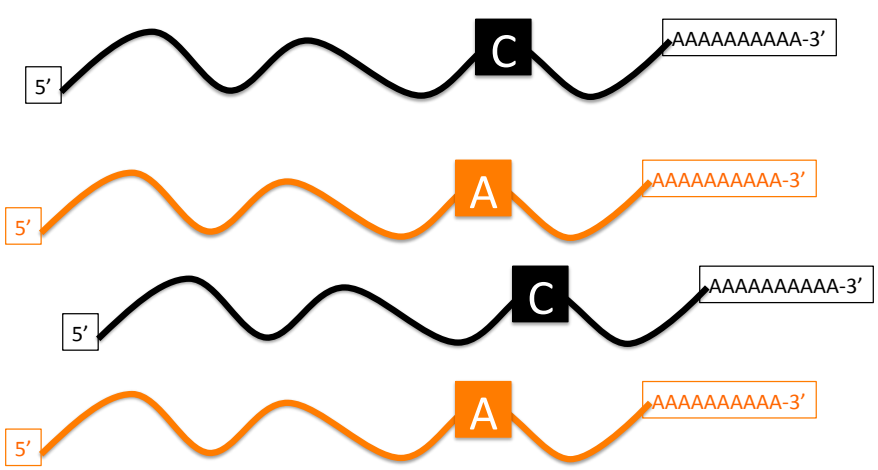


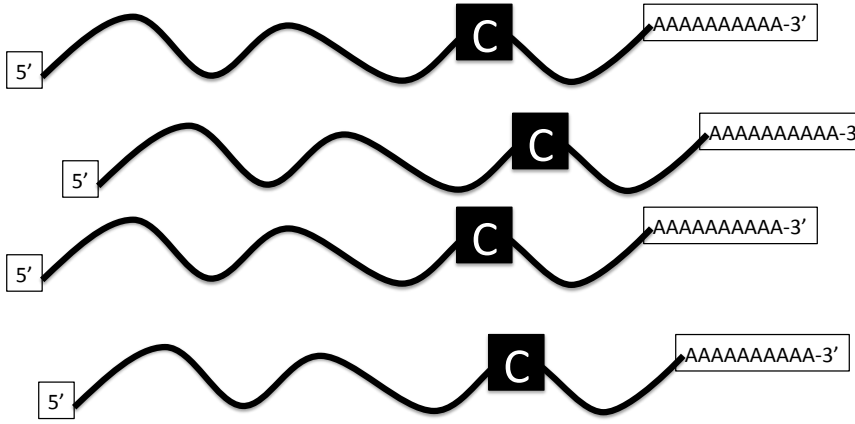
8-oxo-guanine

OGG1 DNA Glycosylase

Alex Chaim

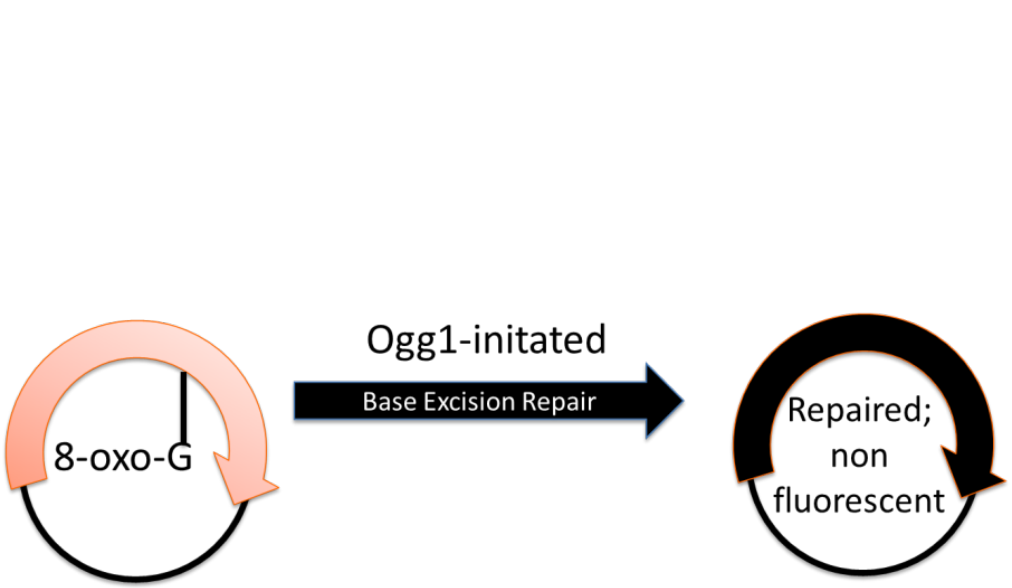
RNA Pol II occasionally inserts A at 8-oxoG
Transcriptional Mutagenesis

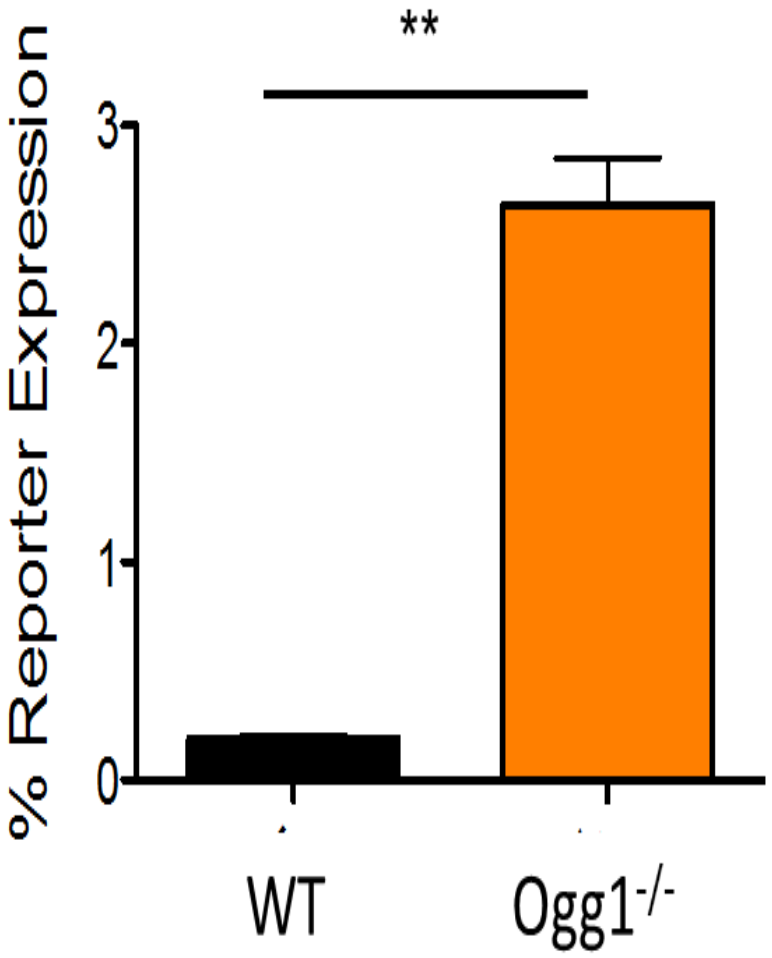




Alex Chaim

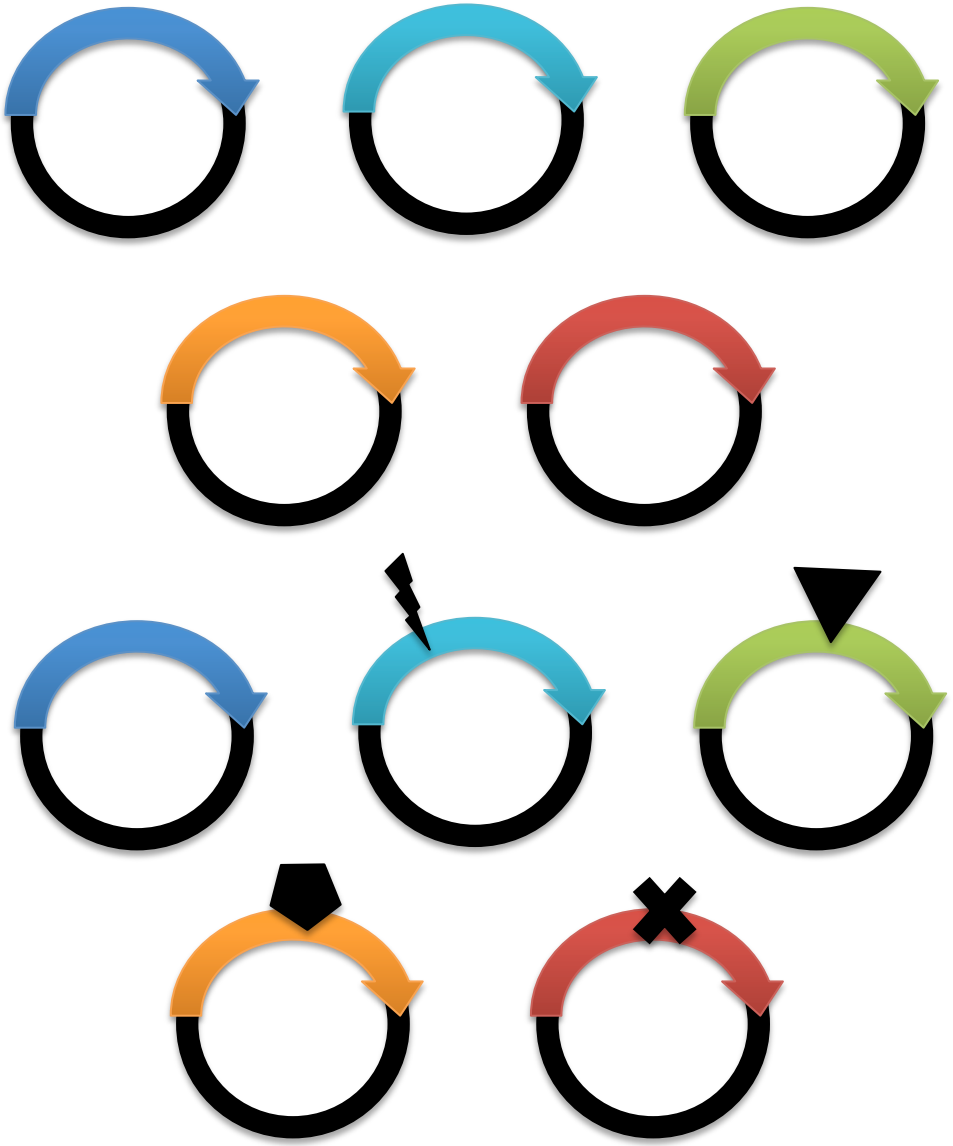
FM-HCR for Ogg1-initiated BER

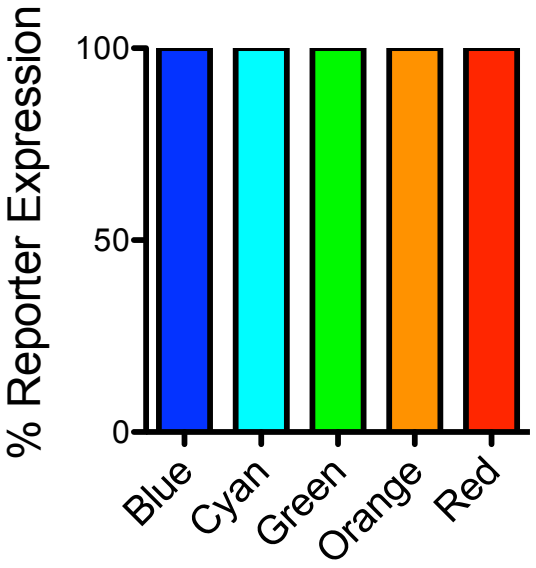


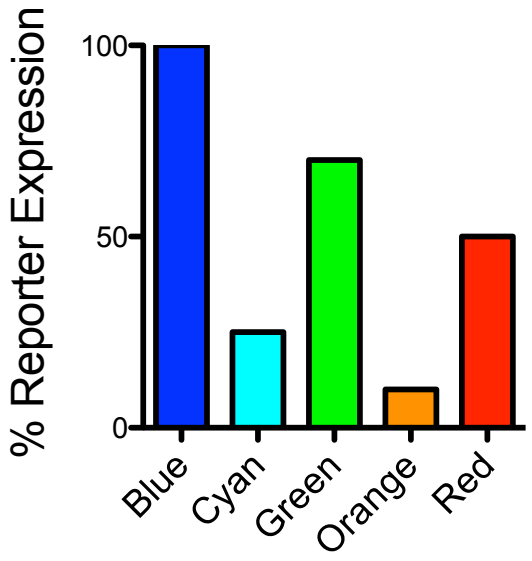


Alex Chaim

5 color HCR assay applications

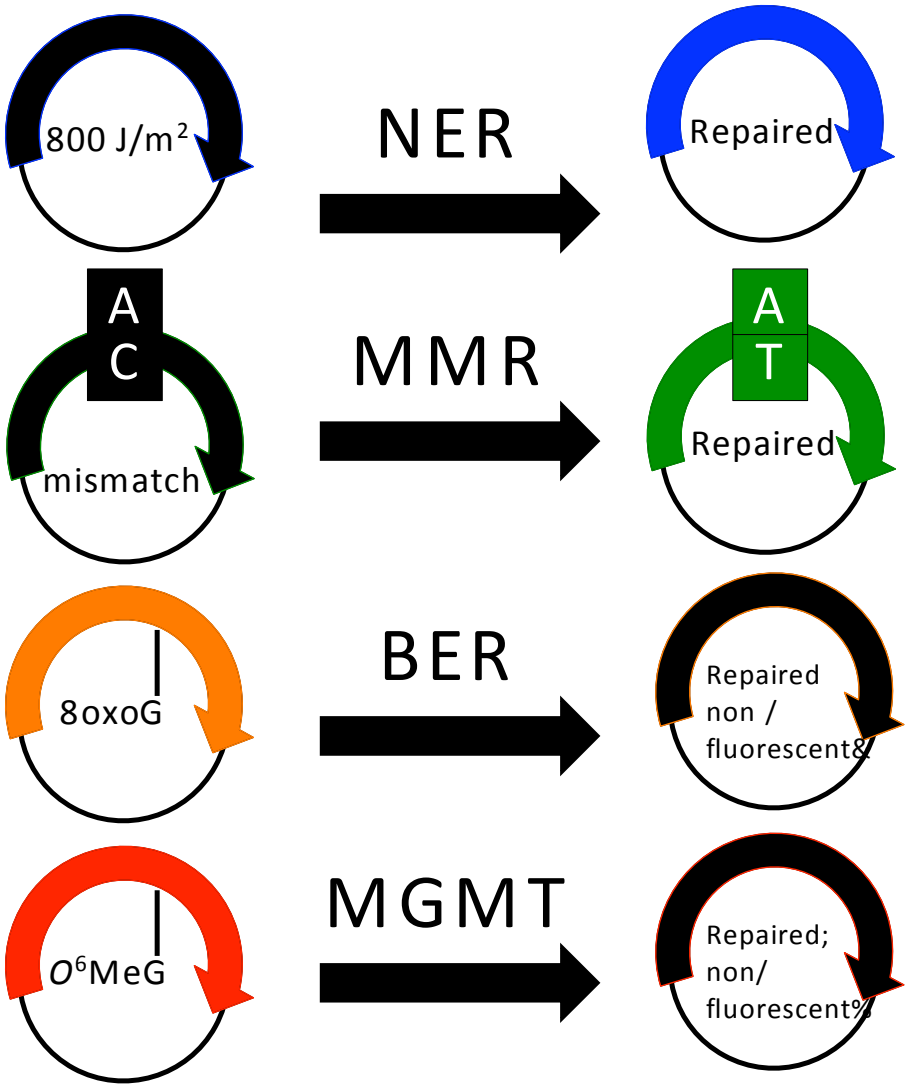




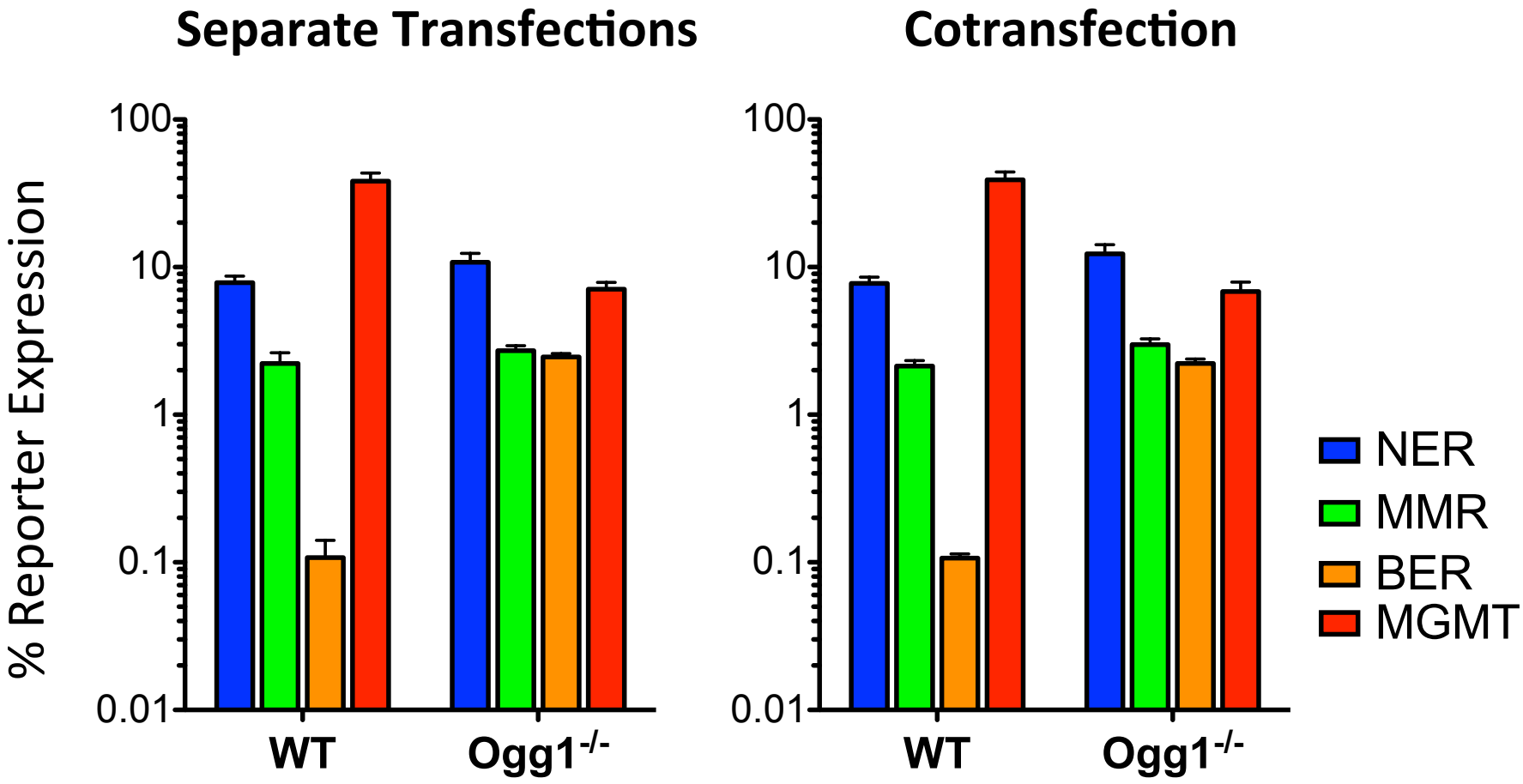


5-color HCR developed by **Dr. Zachary Nagel**

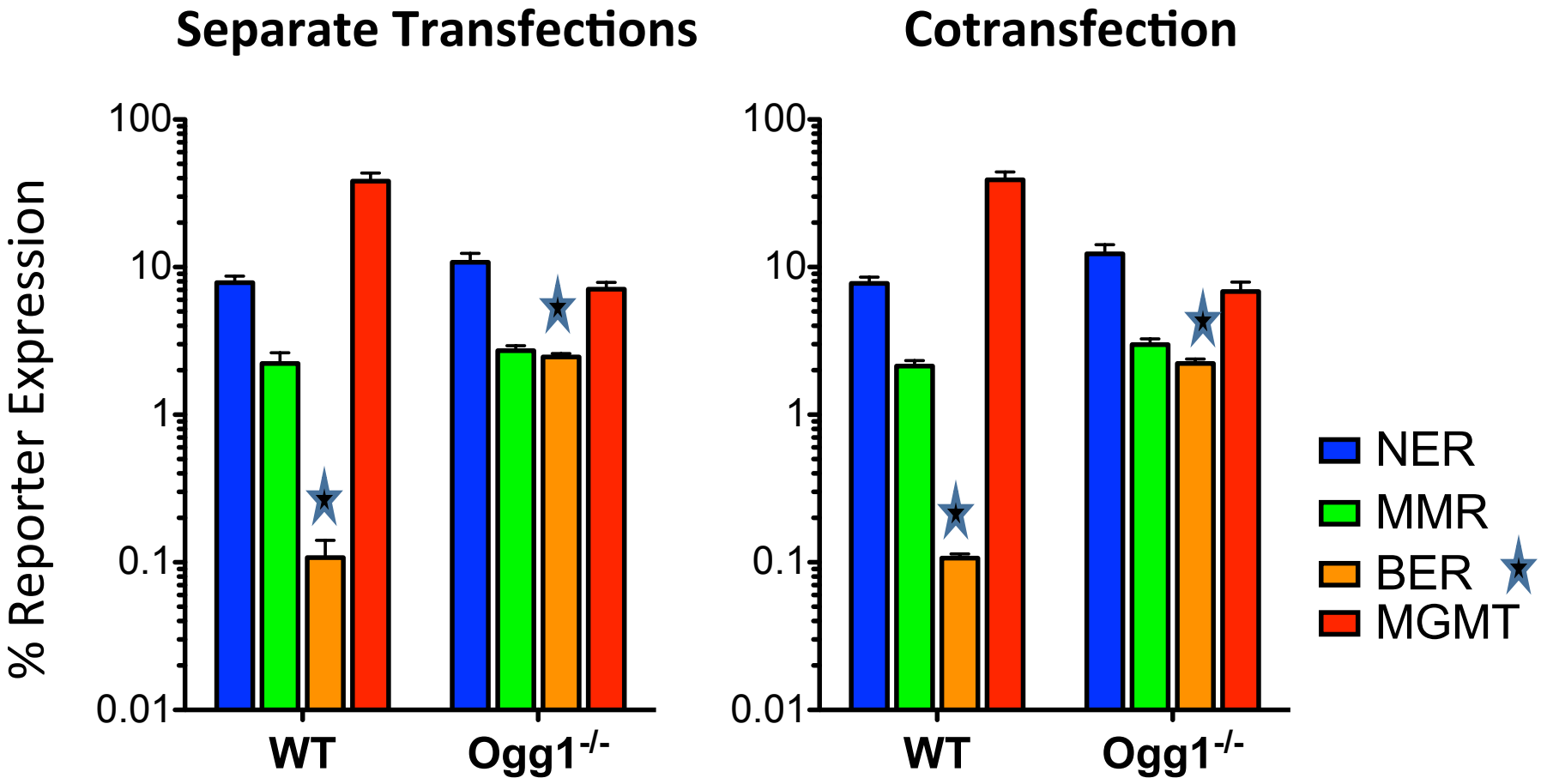
Can We Measure DRC in 4 pathways in a single assay: Example 1



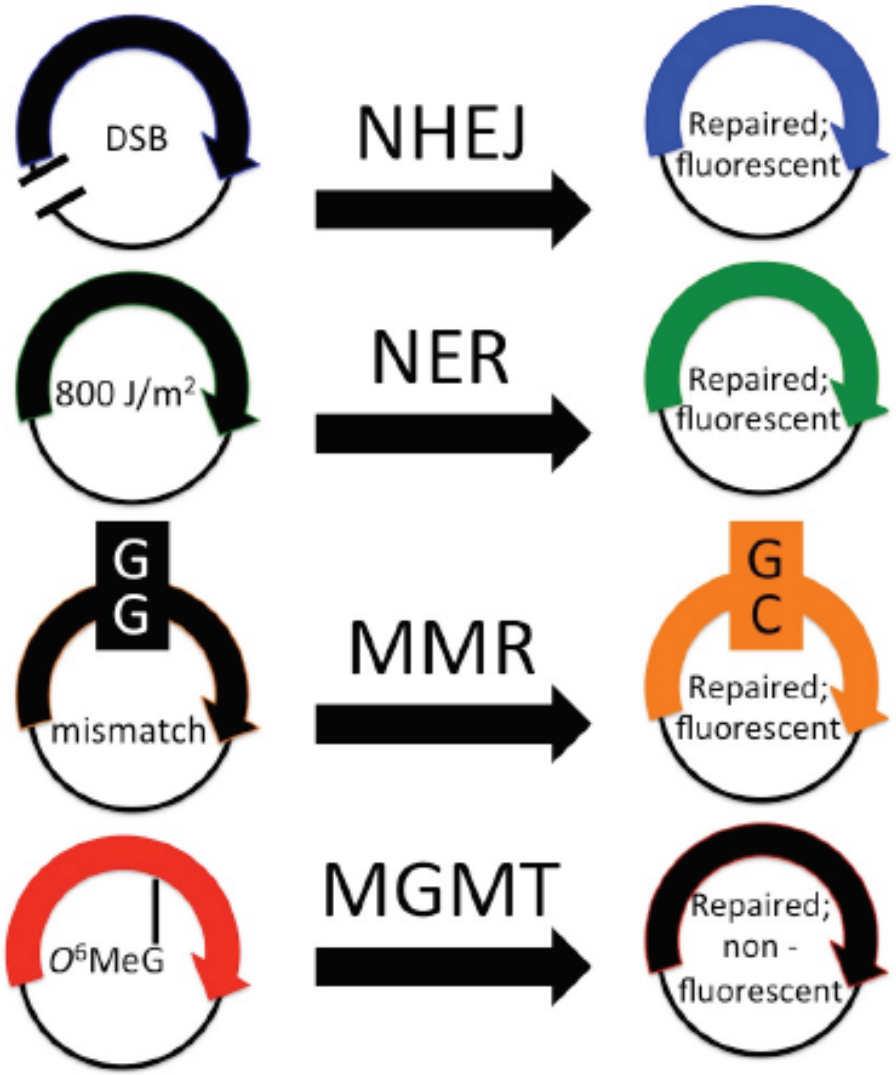
Can We Measure DRC in 4 pathways in a single assay: Example 1



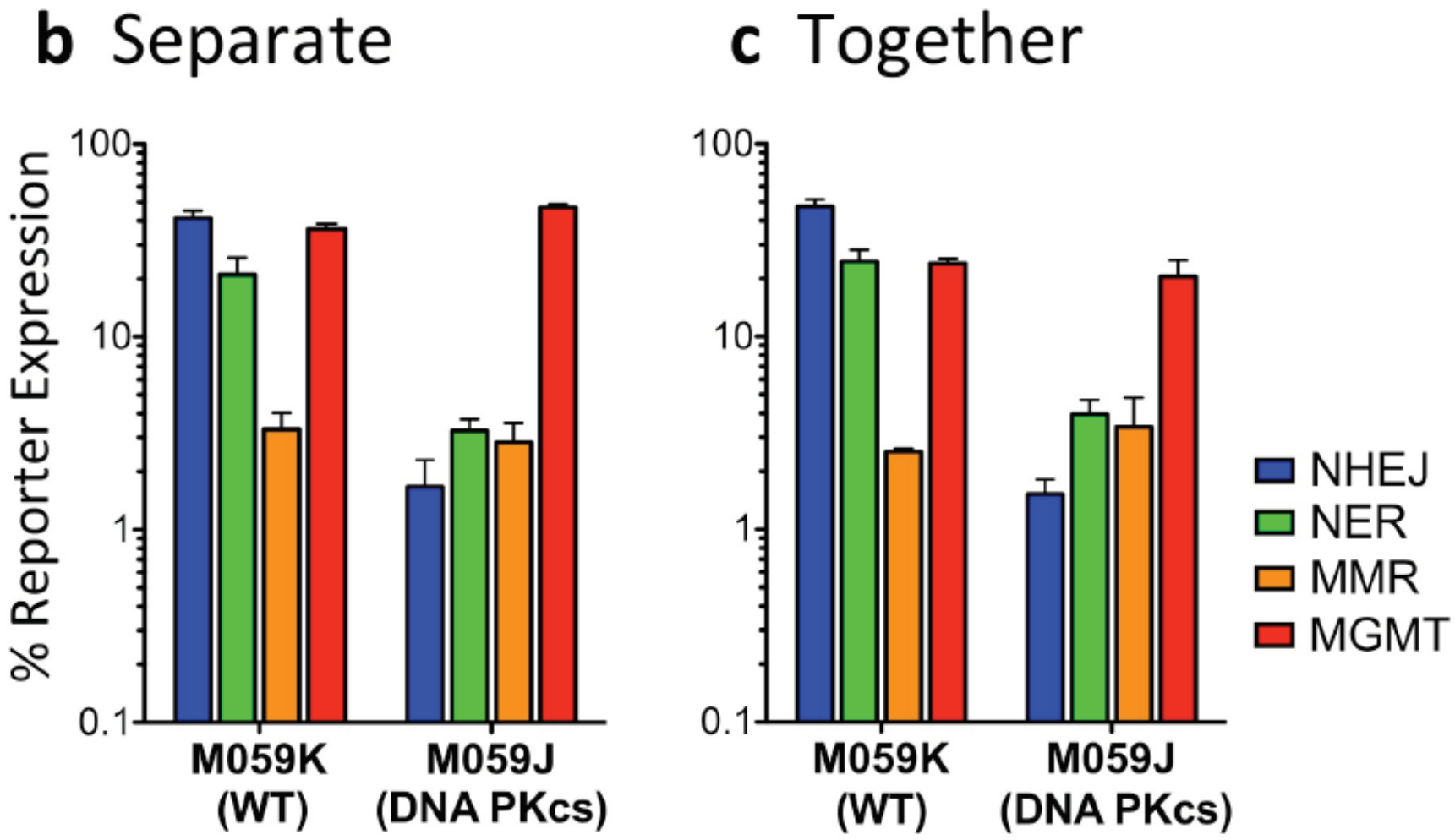
Can We Measure DRC in 4 pathways in a single assay: Example 1



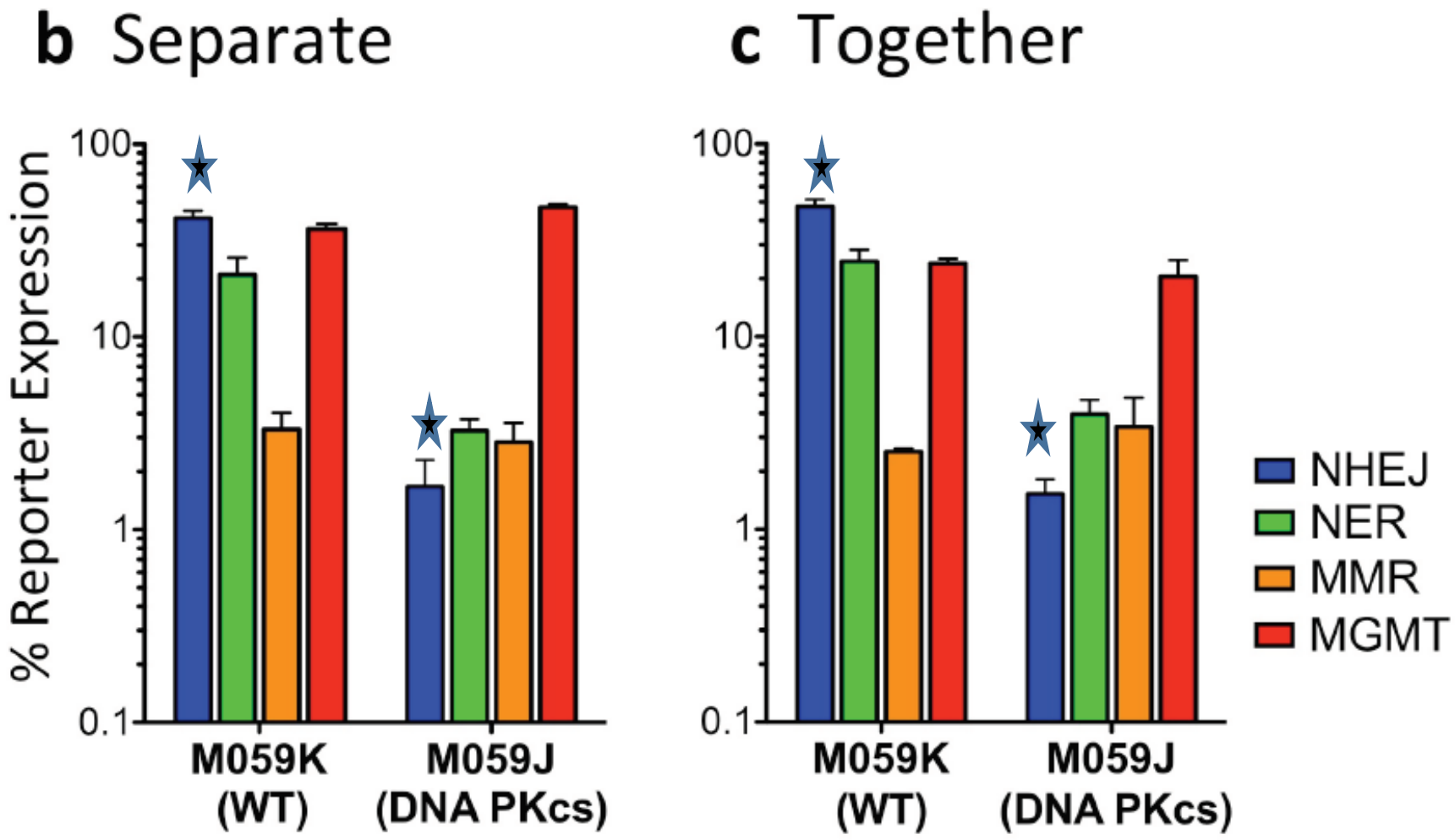
Can We Measure DRC in 4 pathways in a single assay: Example 2



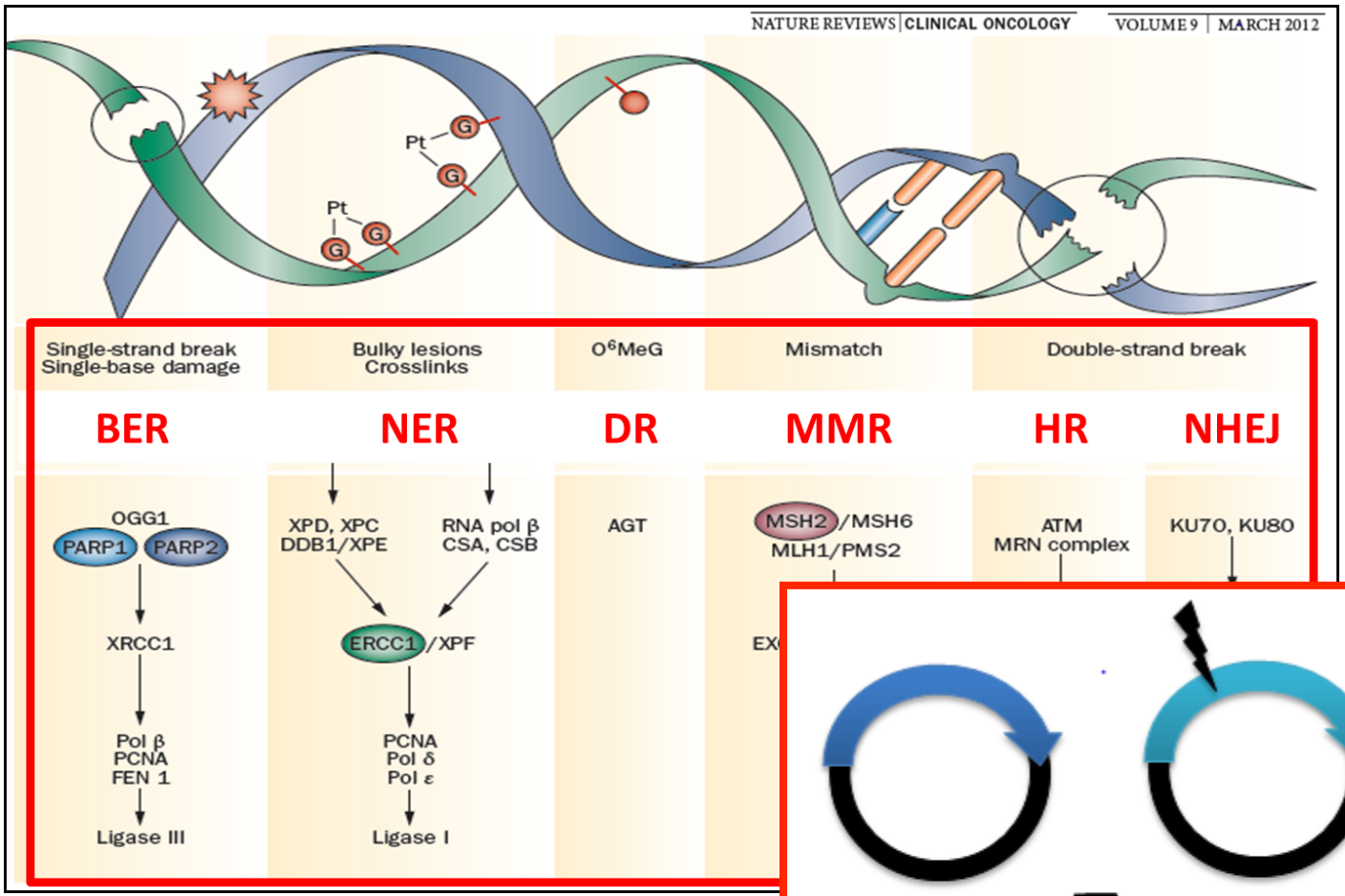
Can We Measure DRC in 4 pathways in a single assay: Example 2

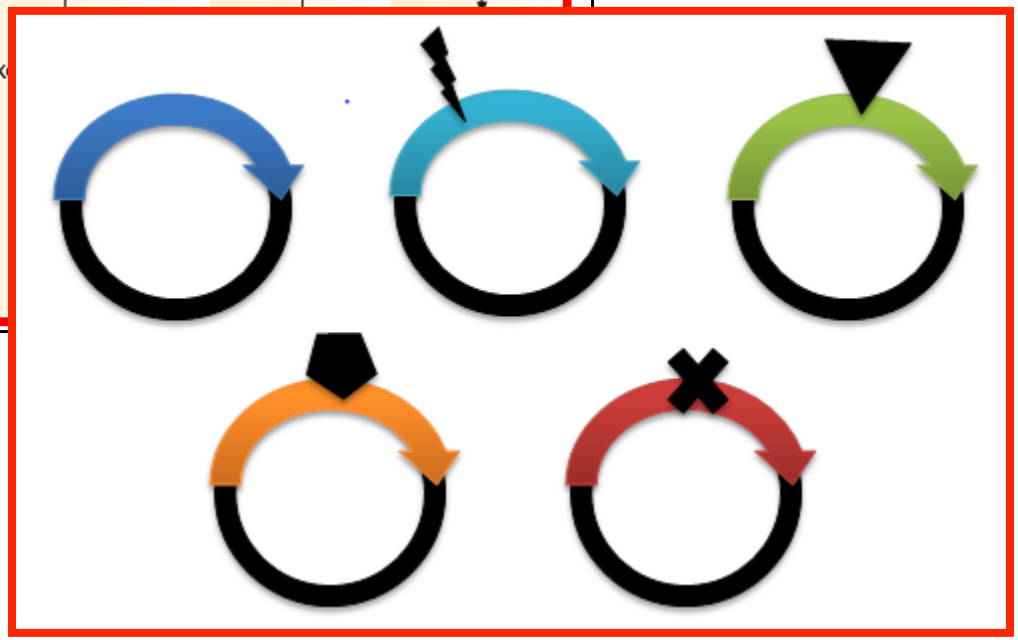


Can We Measure DRC in 4 pathways in a single assay: Example 2

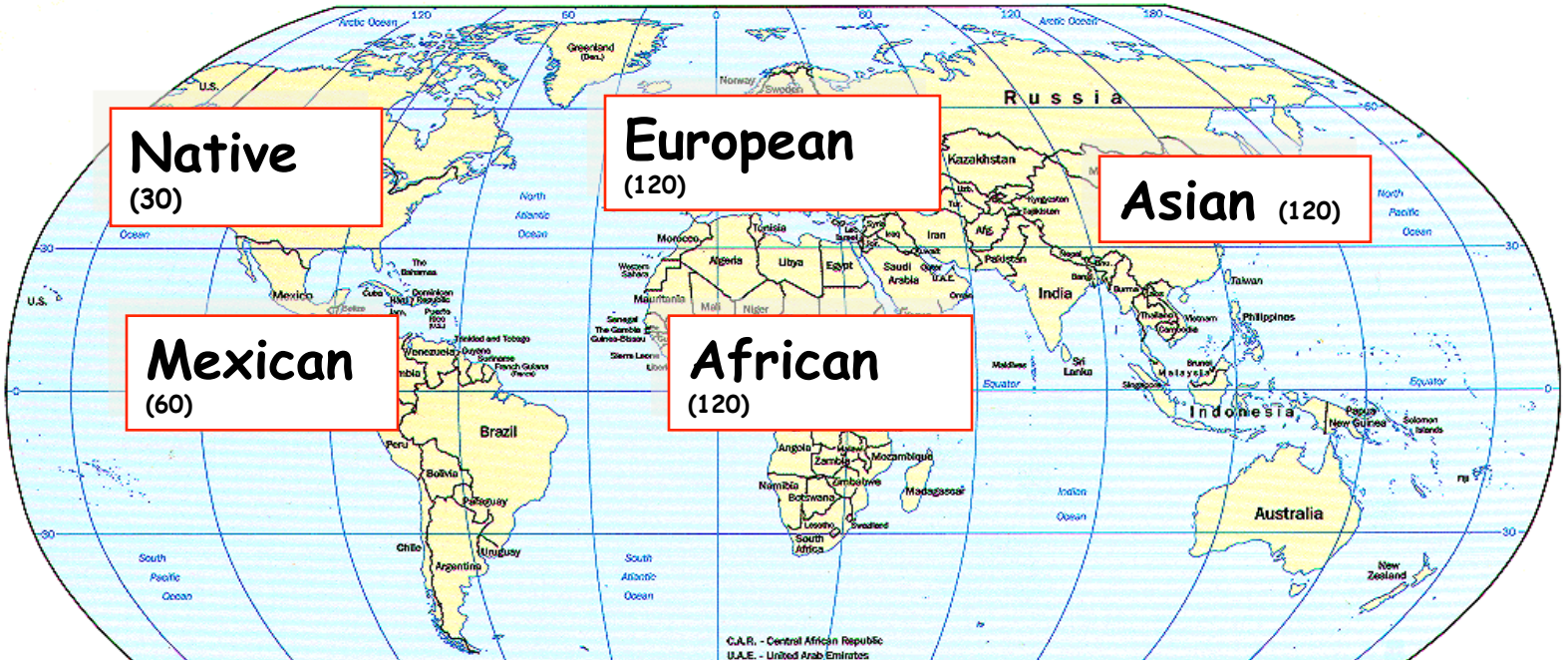


DNA Repair Capacity - Multiplexed





Coriell Lymphoblastoid Cell line collection derived from ethnically diverse HEALTHY humans

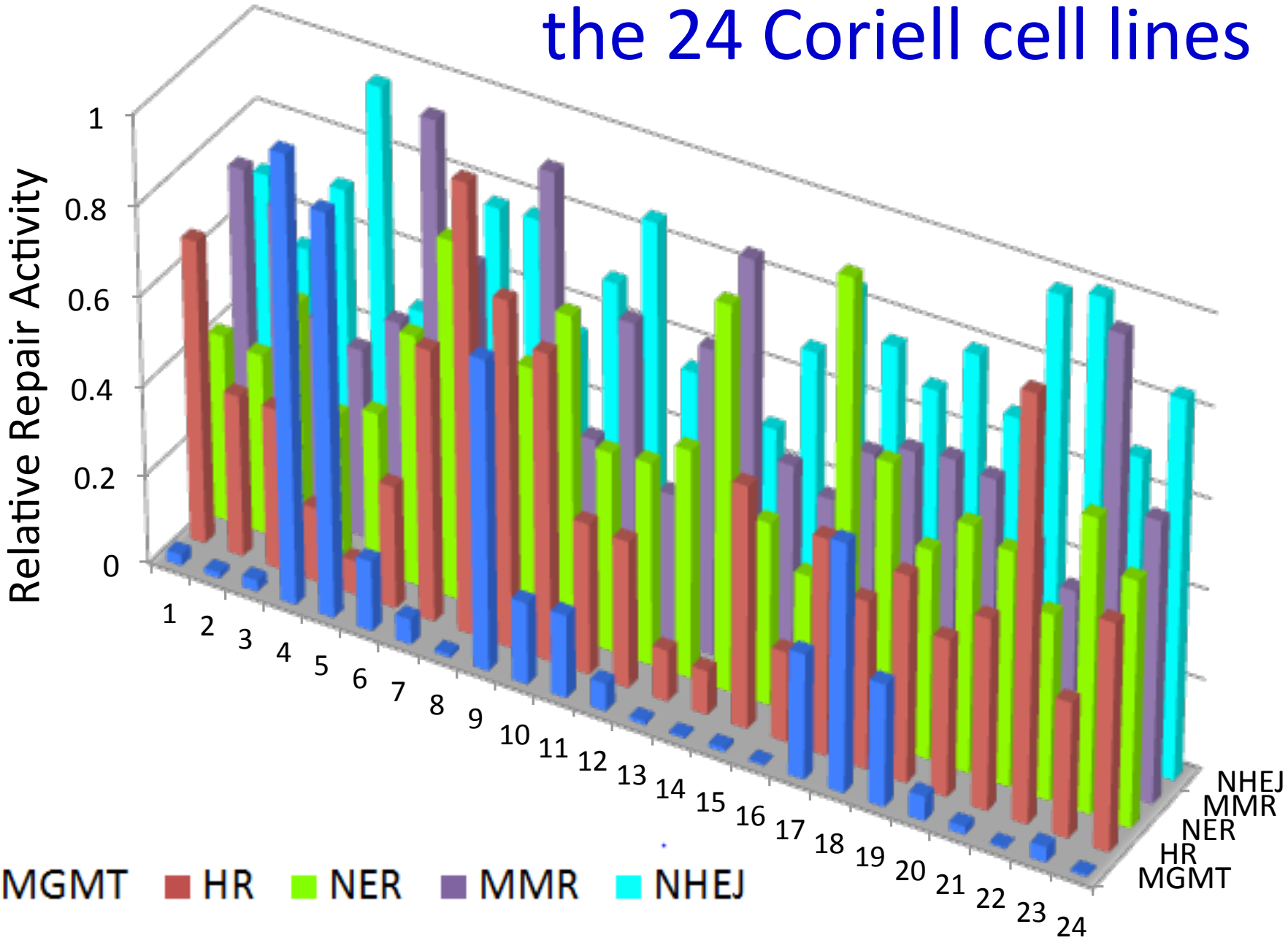


450 healthy unrelated US residents with ancestry from around the globe

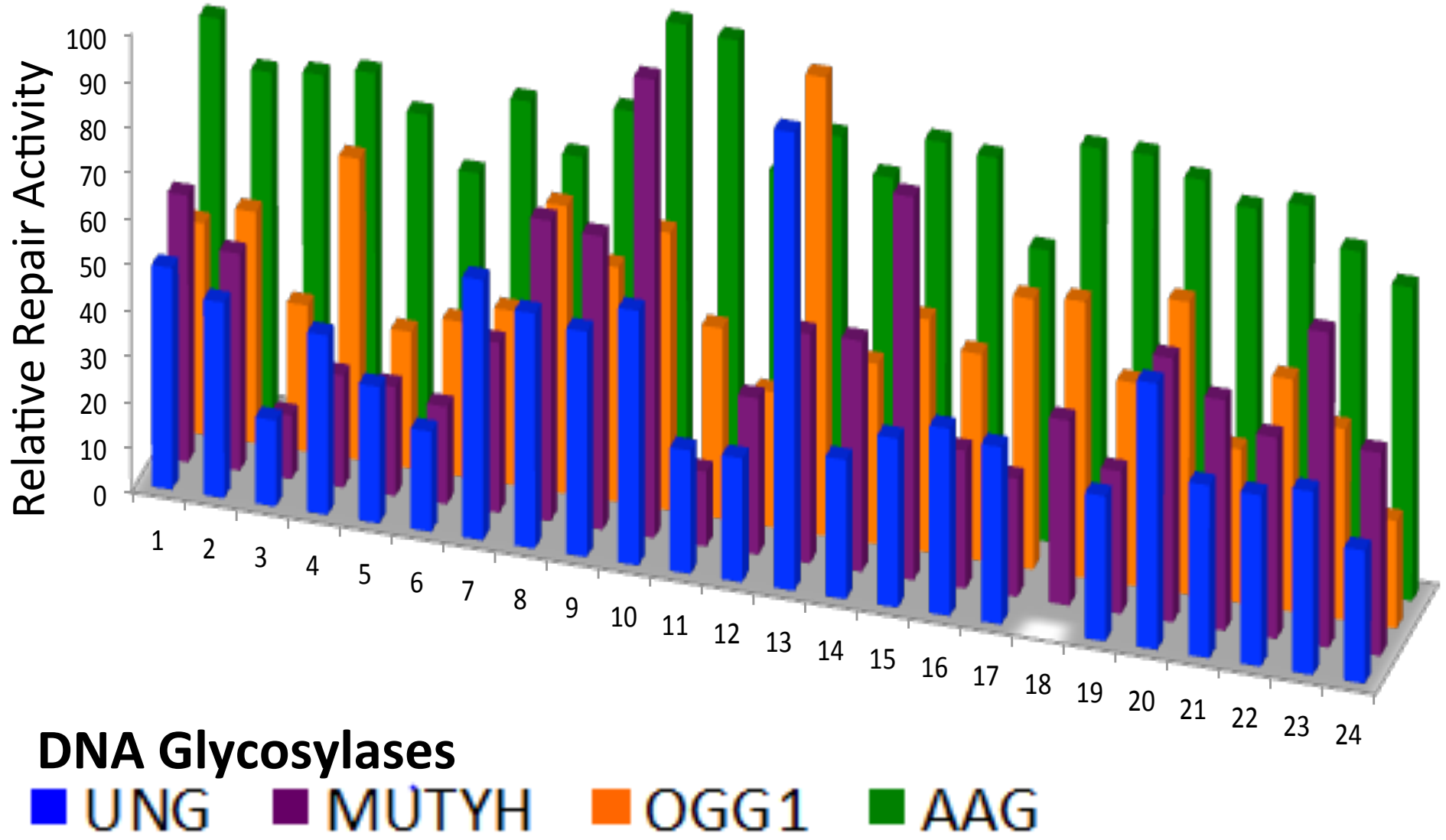
Nested subsets: 90, 44, 24, 8

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

DNA Repair Capacity in 5 pathways for the 24 Coriell cell lines



DNA Repair Capacity for BER initiated by 4 different DNA Glycosylases in the 24 Coriell cell lines

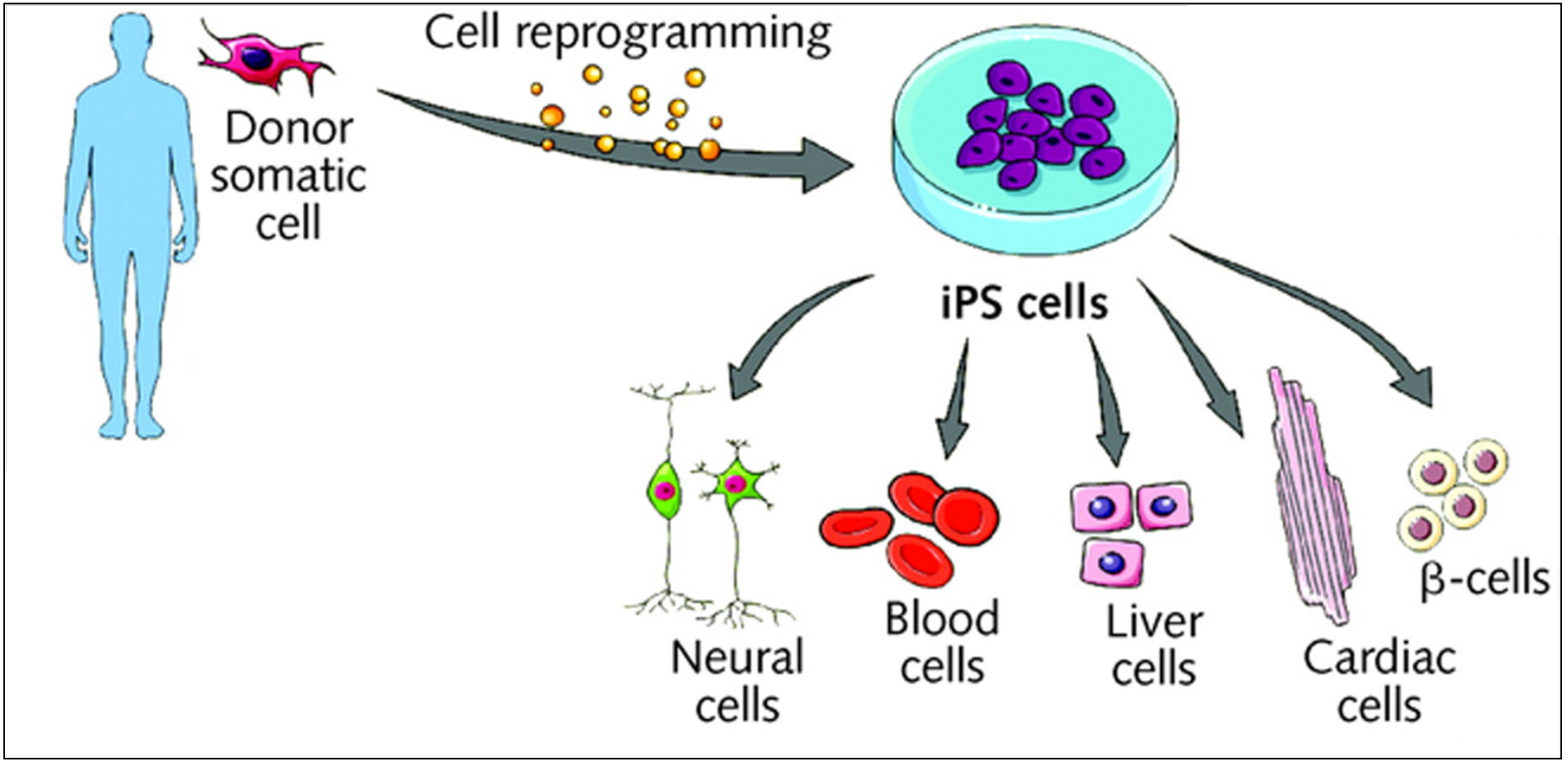


Ongoing and Future plans post-Pioneer

- Mathematical Modeling testing predictive value of FM-HCR data.
- Exploring inter-individual variations DRC in human lymphocytes.
- DRC in glioblastoma tumor cells predicting chemo/radiotherapy outcome (with Jann Sarkaria, Mayo Clinic).
- RNAseq to overcome limitations using only 5 fluorescent reporters.

- Clinical, Basic & Population Research grants under review.
- Determine whether global (DRC) correlates to:
  - (i) disease susceptibility
  - (ii) efficacy of chemo/radiotherapy
  - (iii) chemo/radiotherapy side effects
  - (iv) degenerative diseases.

Ultimately want to measure DRC for every major DNA repair activity in many different cell types – ideally derived from each individual



Modified from Power C , Rasko J E Ann Intern Med 2011;155:114-121

The Pioneer Team

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Patrizia Mazzucato

Carrie Margulies

Dr. Anwaar Ahmad

Siobhan McRee

Anthony Forget

NIEHS
National Institute of Environmental Health Sciences

NIH DIRECTOR'S PIONEER A·W·A·R·D