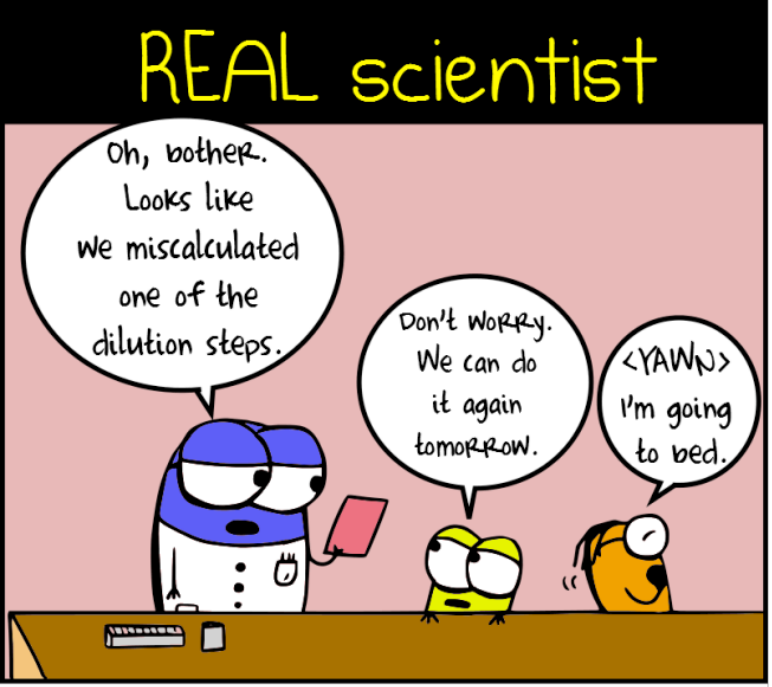
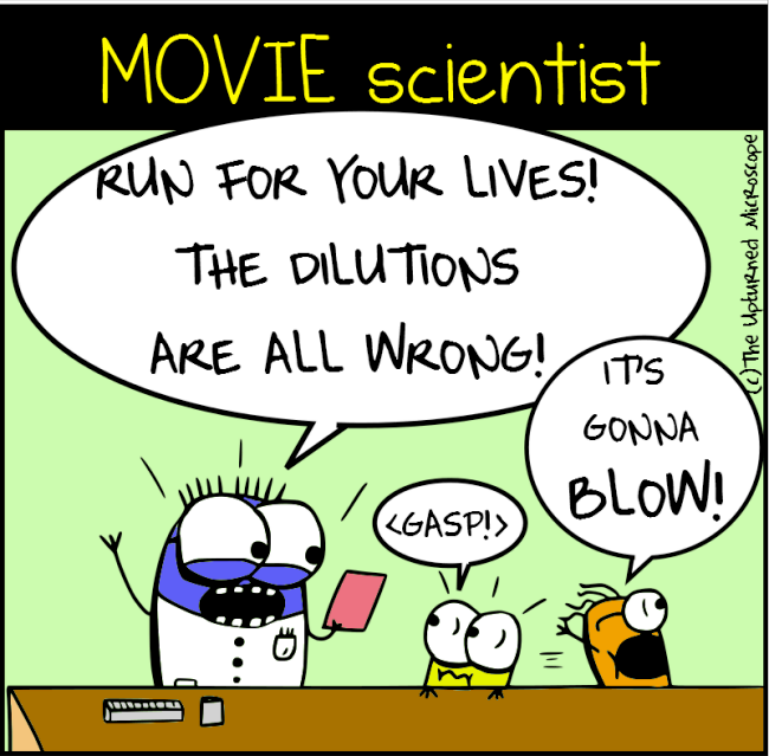




We do *real* science

- We participate in an authentic research experience
- We develop and interrogate hypotheses
- We generate and analyze novel data
- And…

…we make mistakes!

simplest view, broken DNA = no fluorescent signal, and repaired DNA = green fluorescent signal. However, it is important to consider the possibility that one cell population simply took up more plasmid DNA than another. In more technical terms, what if the *transfection efficiency* is higher for one cell type than for the other, and therefore the repair rate artificially appears higher? To control for this we co-transfected with intact pMAX_mCherry, which serves as a transfection control. Using the transfection control data, we will normalize for differences in DNA uptake. It is also important to think about the uptake of pMAX_mCherry compared to the uptake of pMAX_EGFP_MCS. What if the plasmids are taken up at different frequencies, or successfully expressed at different frequencies and/or signal intensities? Here is where the dual intact control is useful. It shows us the typical ratio of mCherry:EGFP uptake and expression, which we can use as a secondary normalization. Note that we use pMAX_EGFP as a control rather than pMAX_EGFP_MCS, because the latter will have very low expression that is not representative of the repaired construct: the nonsense insert separates the promoter and gene by too great a distance for robust expression.

…we make mistakes!

As is written on the M2D5 wiki page, you were given pMAX_EGFP_MCS for the intact control.

Why is this problematic for our experiments??

Also, experiments misbehave!

Ideally, you want to count 10,000 – 20,000 events from each sample via flow cytometry.

	H	I	
	Date Analyzed	Live Cells #Events	Li
-04	3/30/16 16:58	2701	
-51	3/30/16 16:58	2727	
49	3/30/16 16:59	879	
-b7	3/30/16 16:59	842	
-3a	3/30/16 16:59	210	
48	3/30/16 16:59	371	
-14	3/30/16 16:59	496	
-b1	3/30/16 16:59	539	

At what steps might we have lost our cells??

What are the lessons learned?

CANDIDATE INTERVIEW

June 16th 2009, 8am!

Developing Novel Methods to Measure DNA Repair Capacity in Human Populations

Leona D. Samson

MIT

Biological Engineering Department

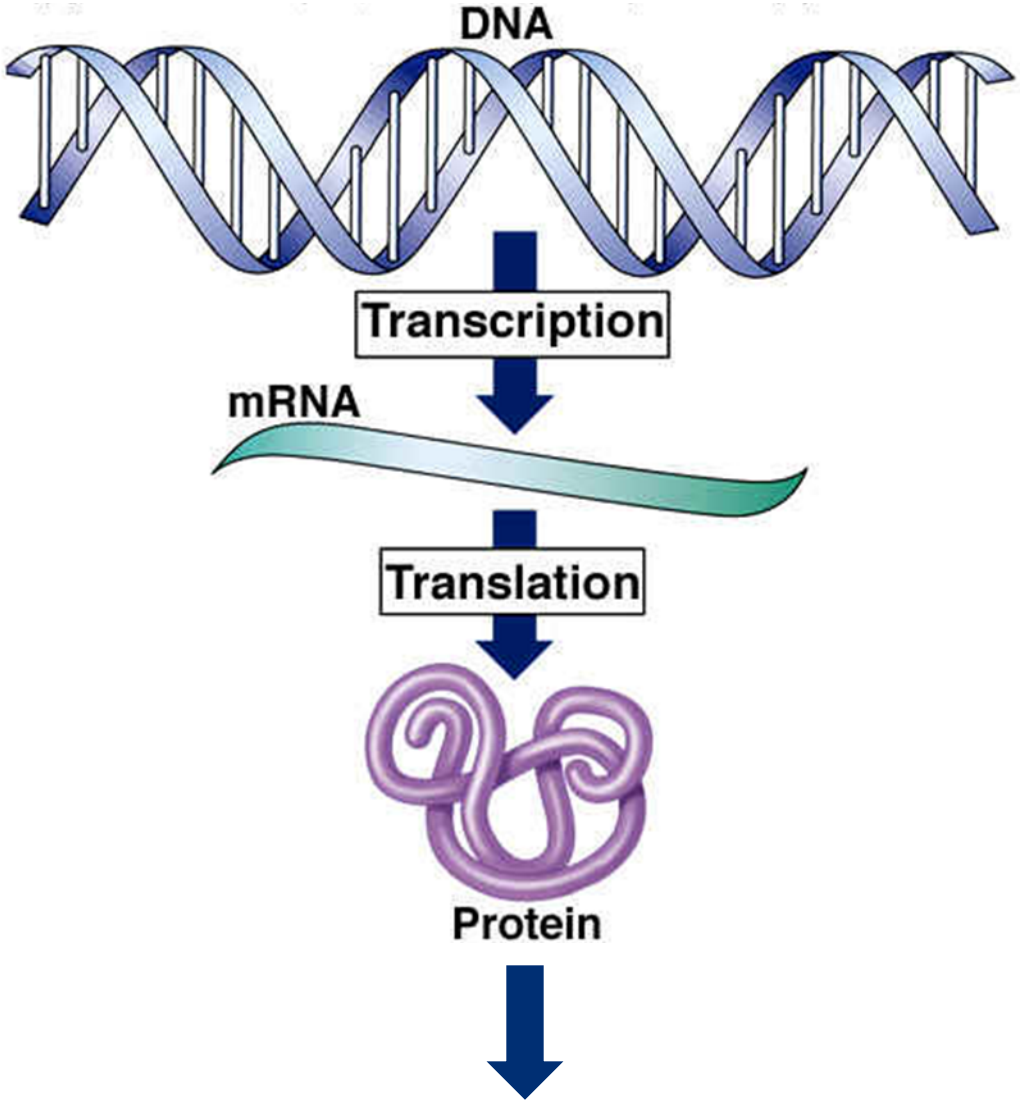
Biology Department

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

FUNCTION

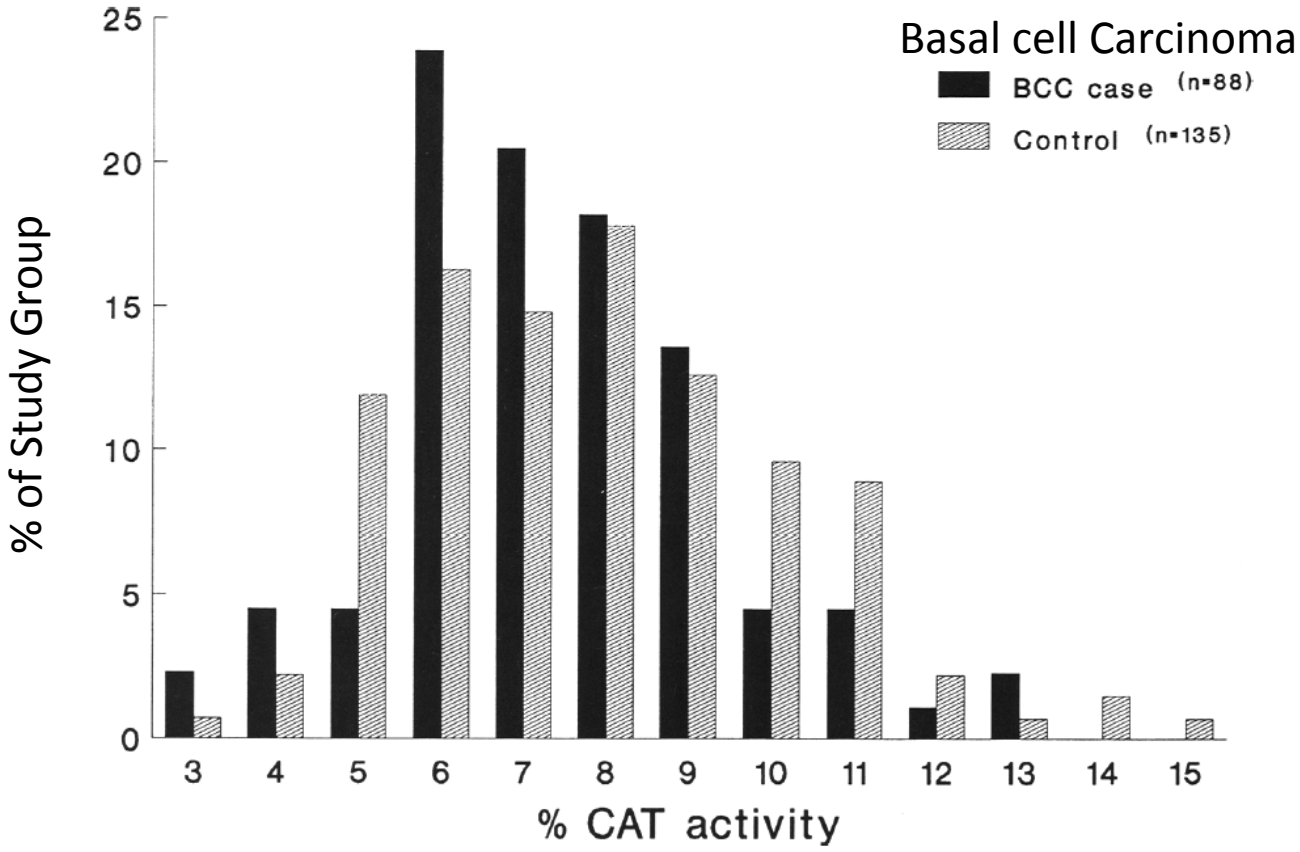
In vitro / In vivo
Functional Assays

The Proposal was based on the Pioneering work of:

Dr. Lawrence Grossman
(1924–2006)

Dr. Qingyi Wei

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

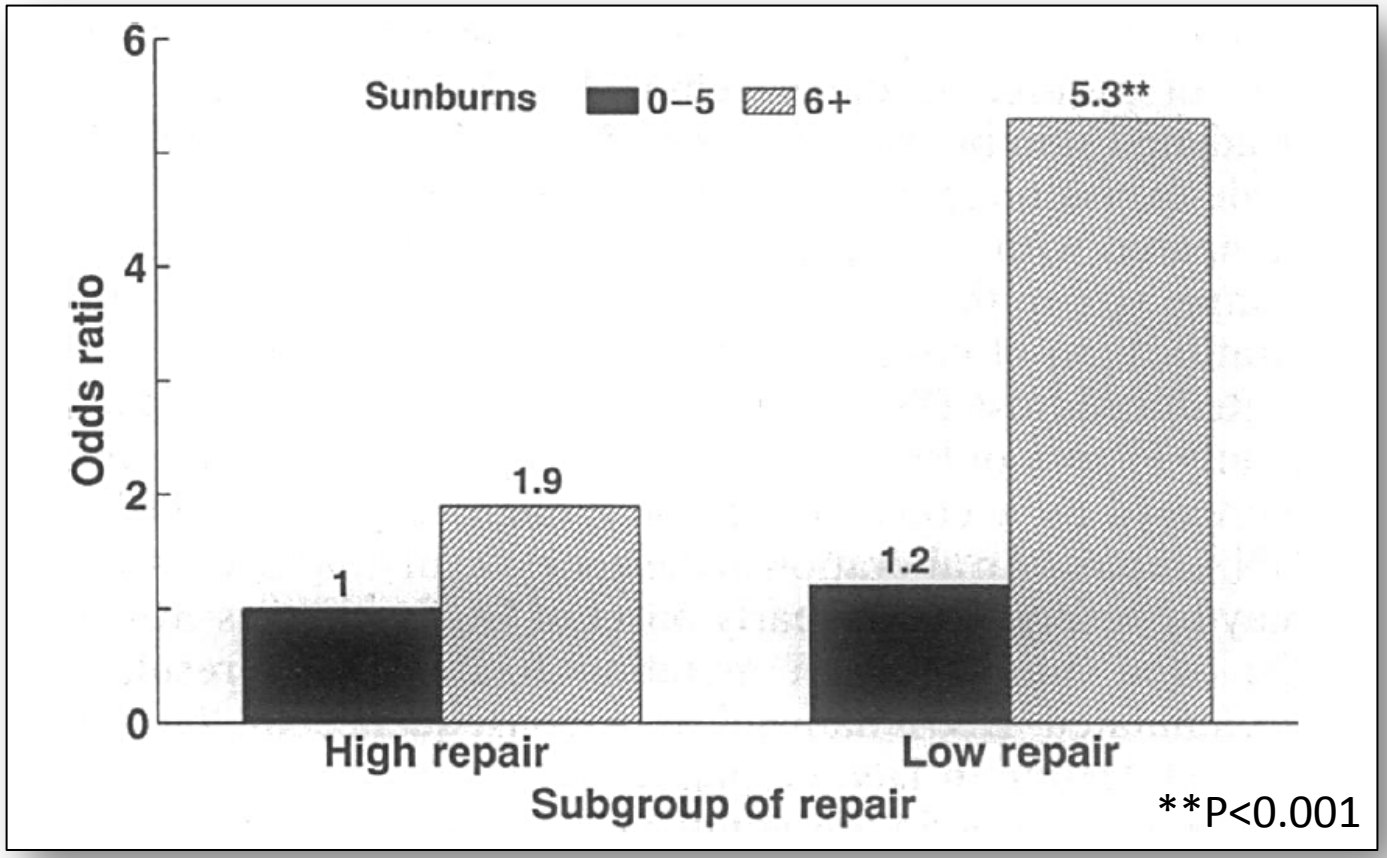


LOW Repair HIGH Repair

[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.

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

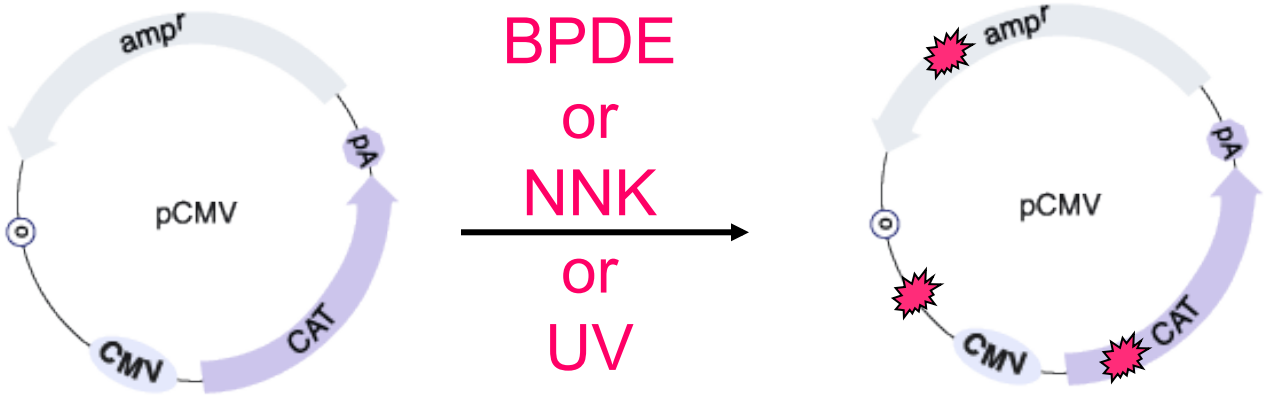


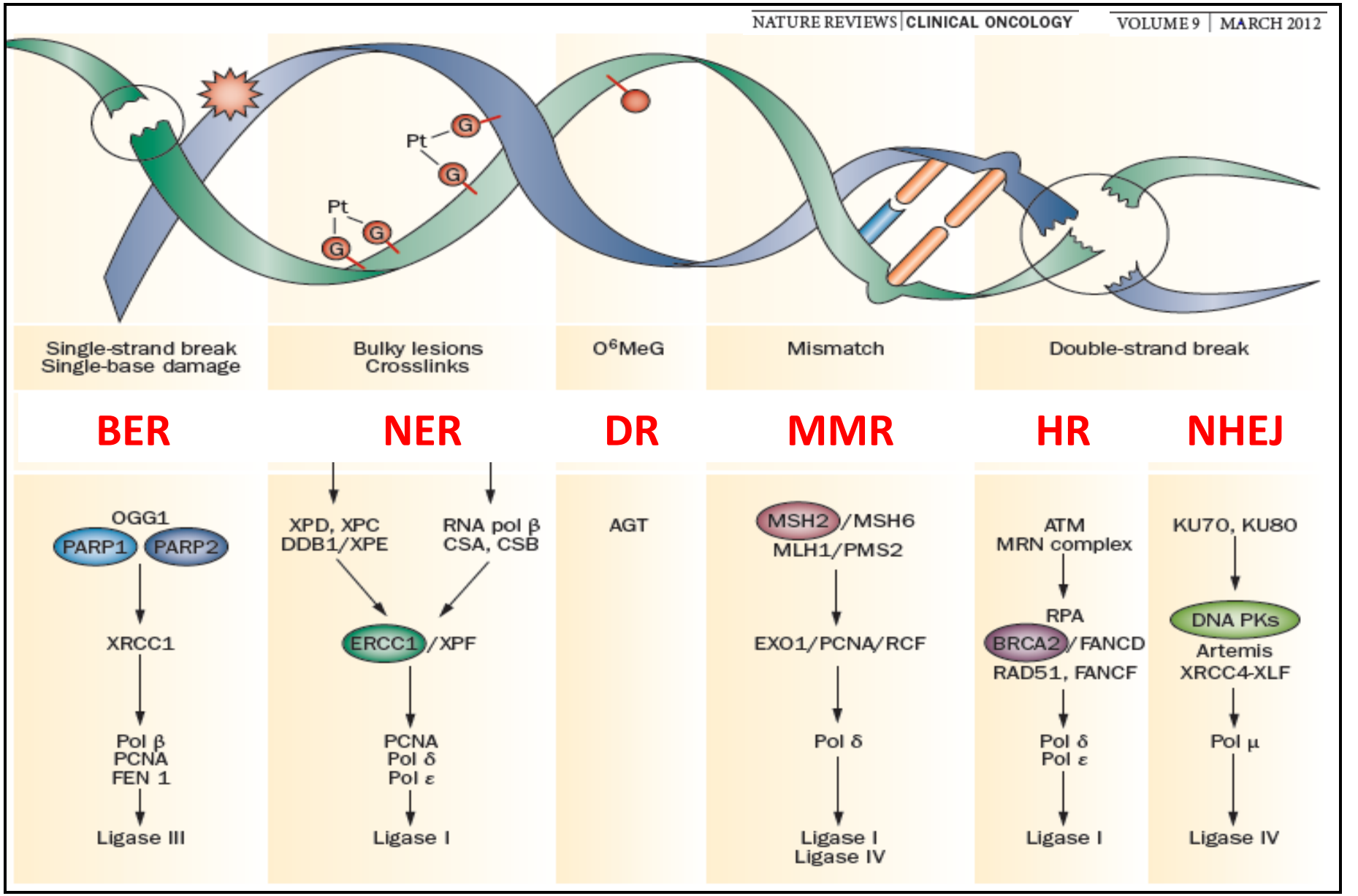
TABLE III – HCR-DRC FOR RISK OF CANCERS

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]

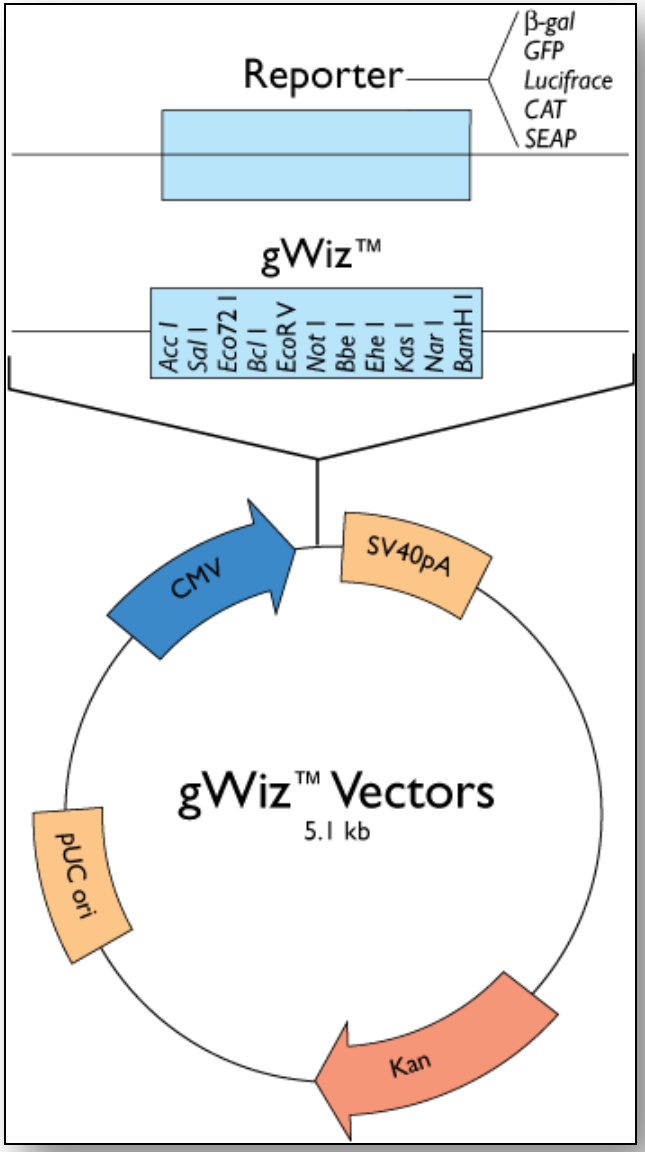
BPDE, benzo(a)pyrene diol epoxide; UV, ultraviolet; SCCHN, squamous cell carcinoma of head and neck; BCC, basal cell carcinoma; SCC, squamous cell carcinoma; CM, cutaneous melanoma.

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

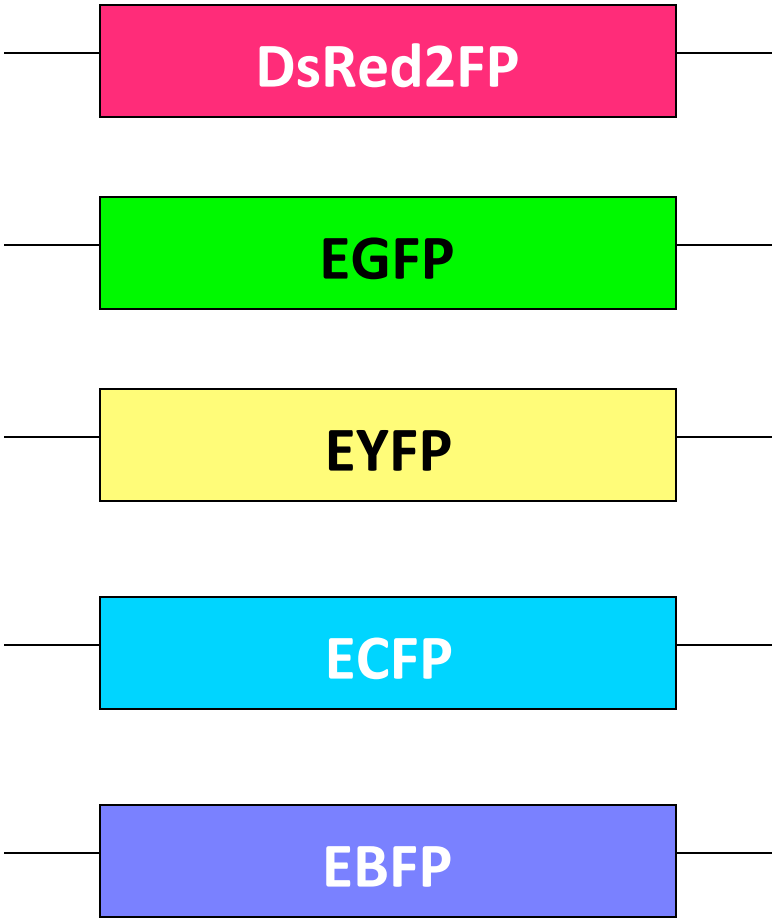
Six Major DNA Repair Pathways



Reactivation of damaged DNA – multiplexed

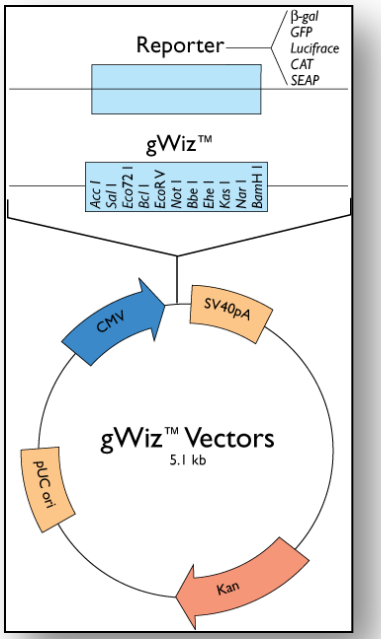


Each Fluorescent Protein gene will harbor a different type of DNA damage

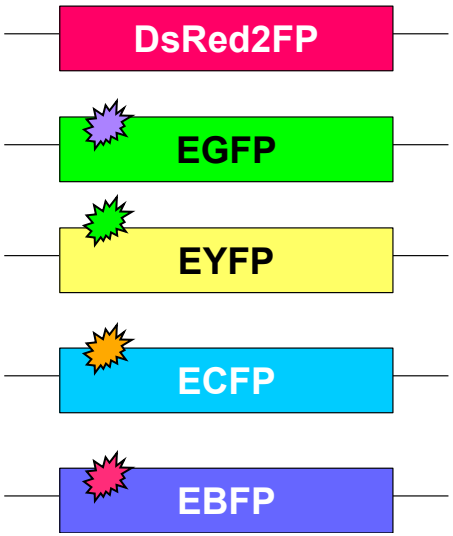


http://www.genlantis.com/corp/images/gWiz_vectmap.gif

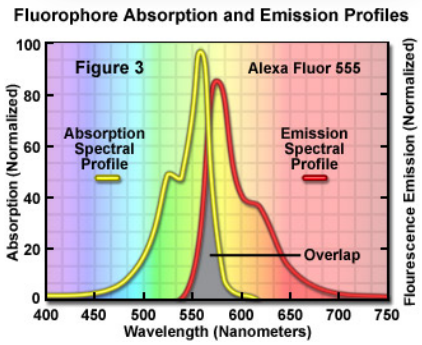
Reactivation of damaged DNA – multiplexed

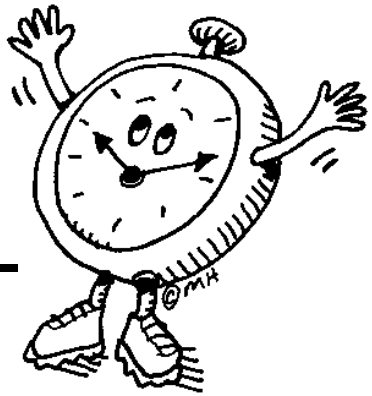


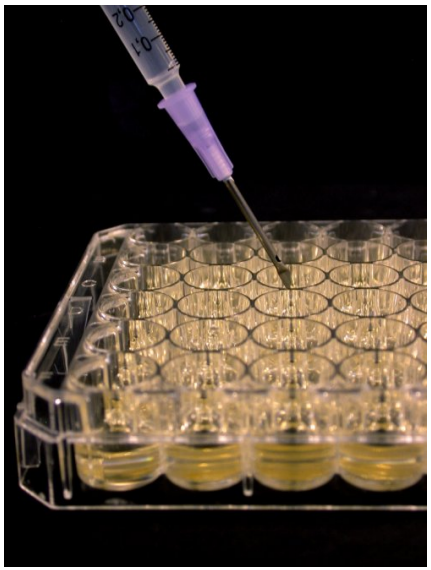
+ different DNA lesions



Transient transfection of mixture



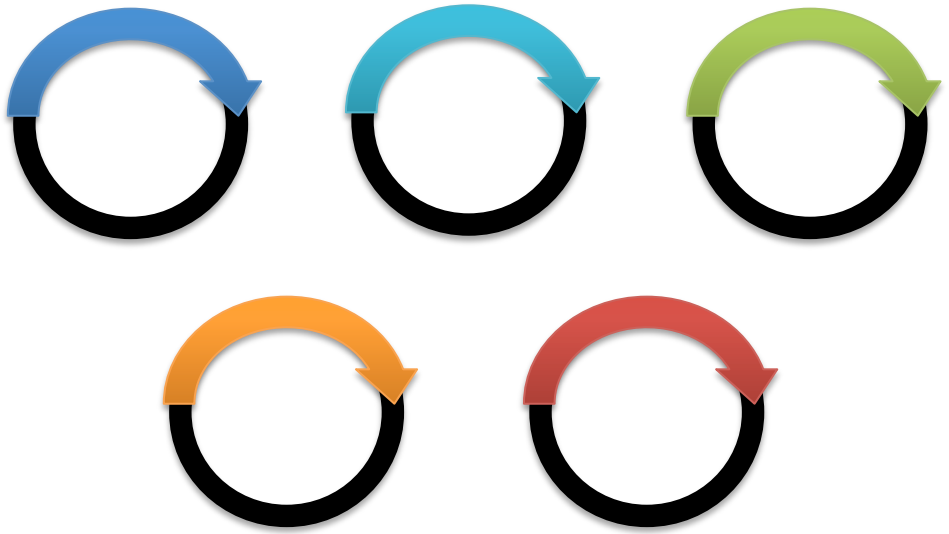


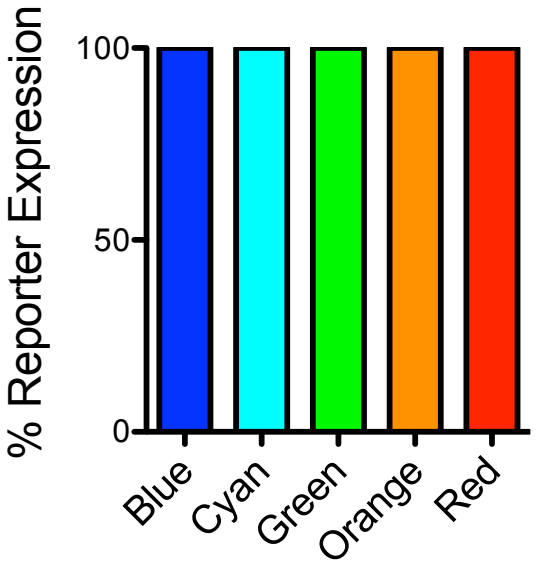


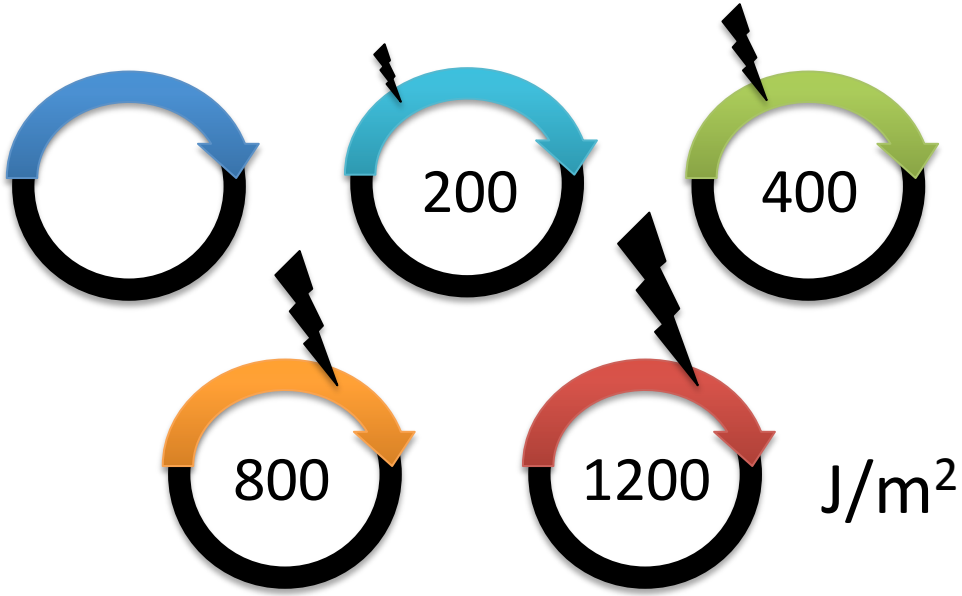
Fluorescence quantitation

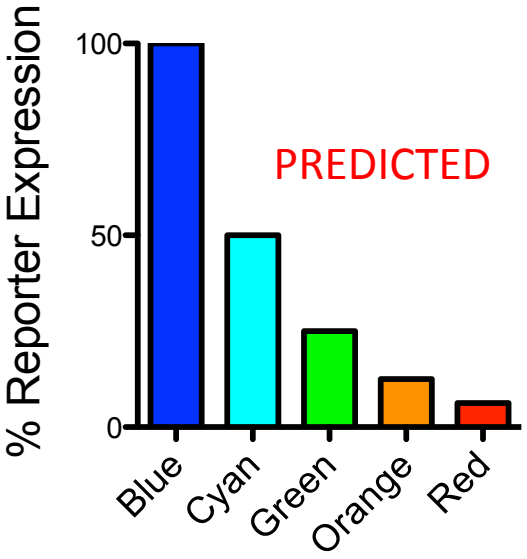
Time to repair

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

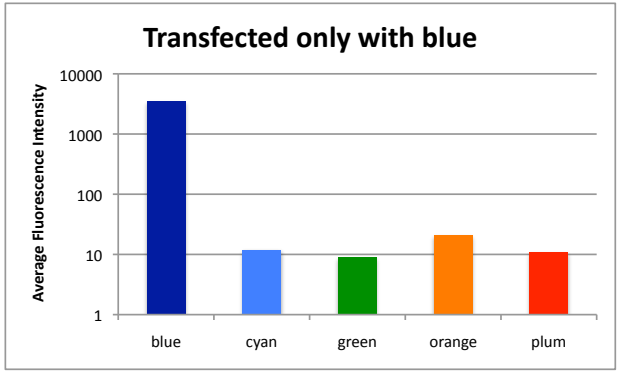


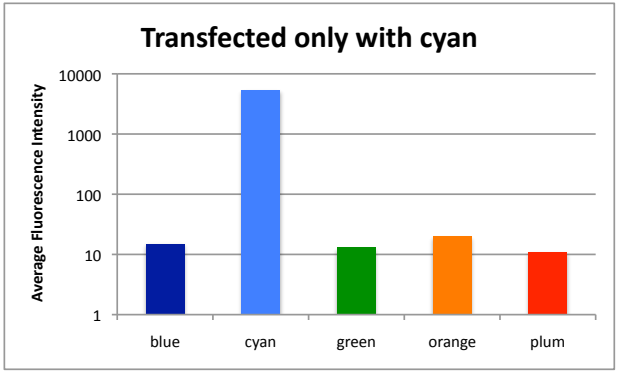


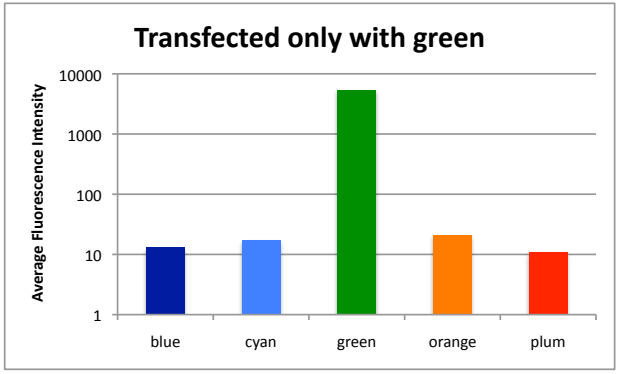


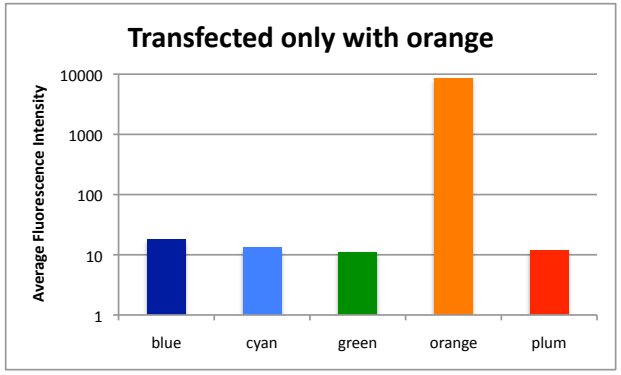


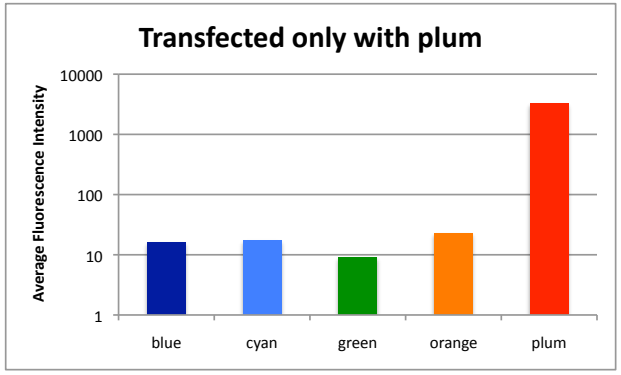
Sanity Check: Is it even feasible detect 5-colors independently?:



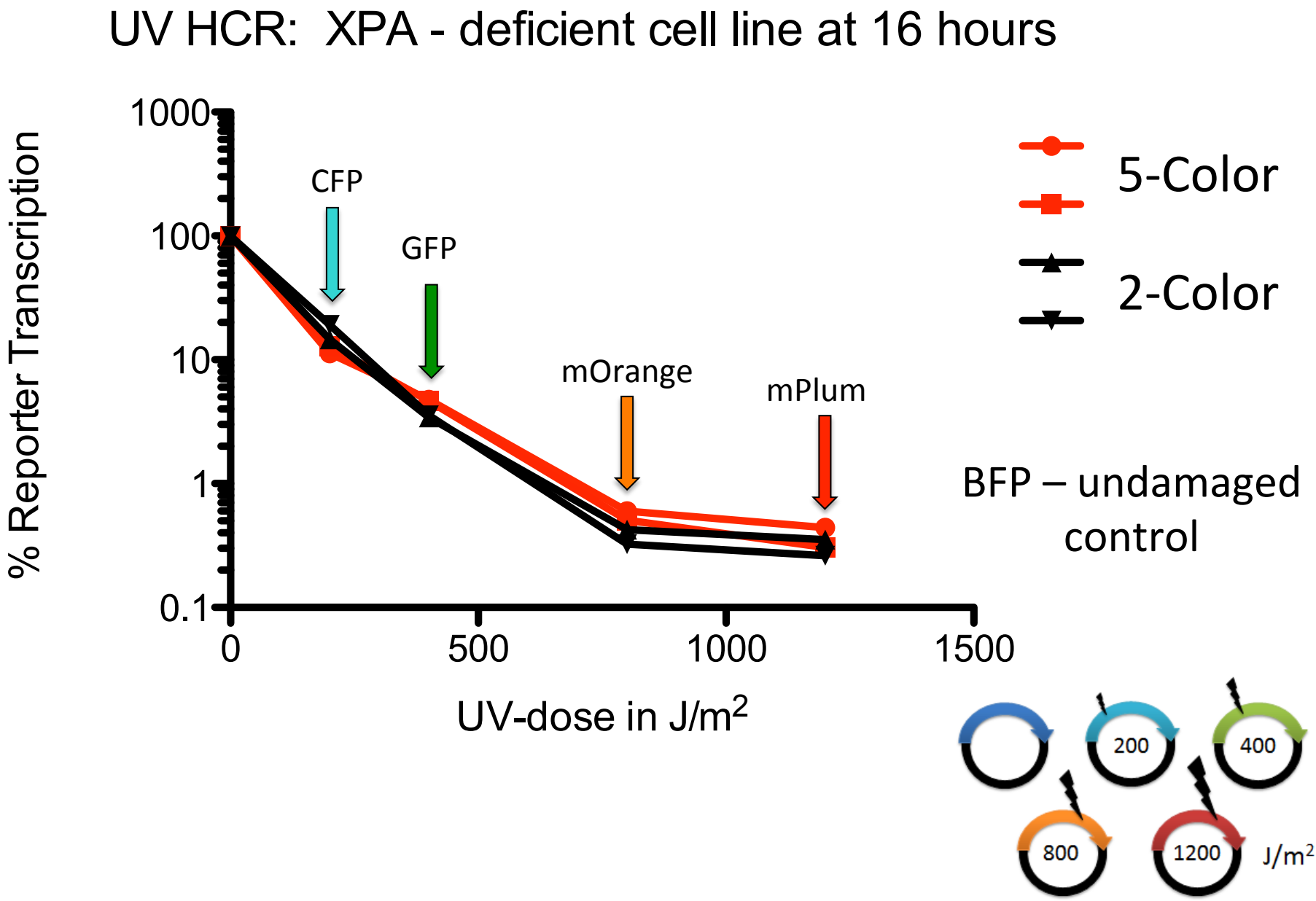






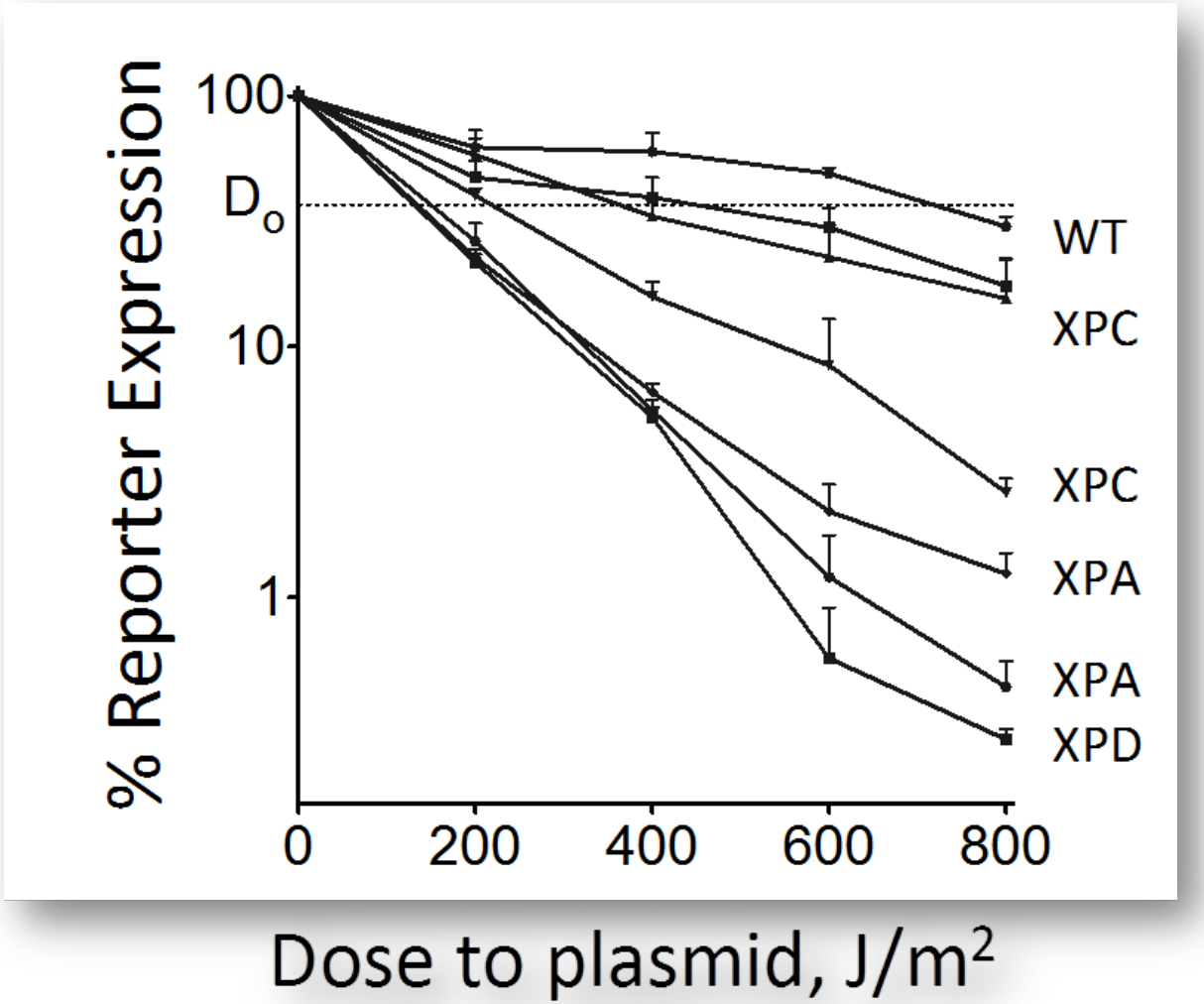


2-color versus 5-color HCR of UV-irradiated plasmids



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	

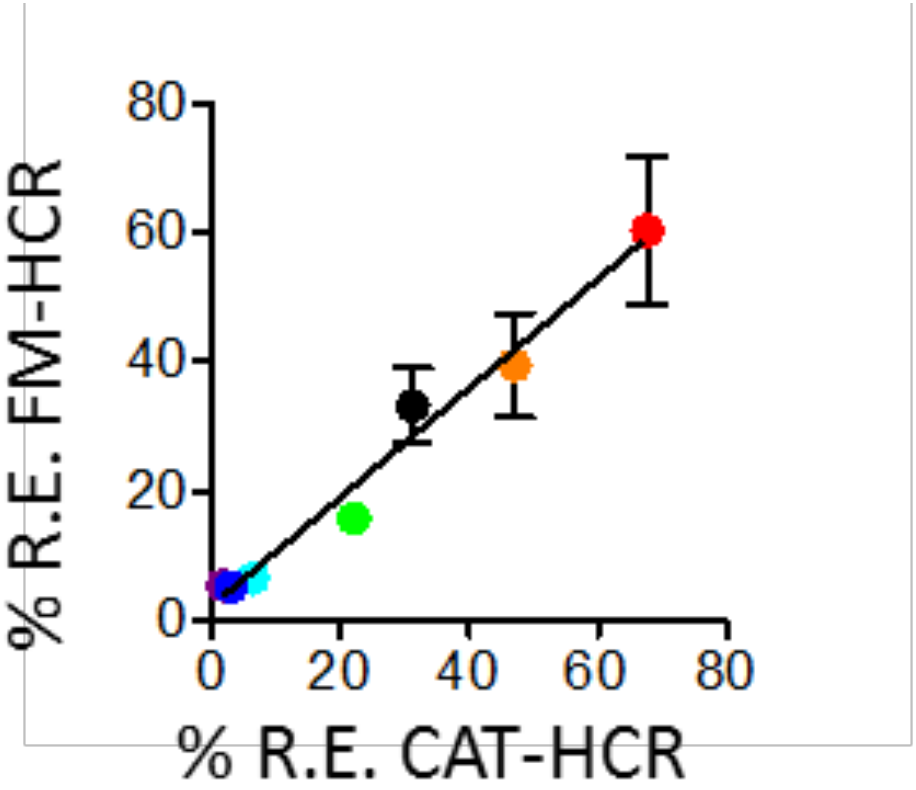
[a] CI, confidence interval.

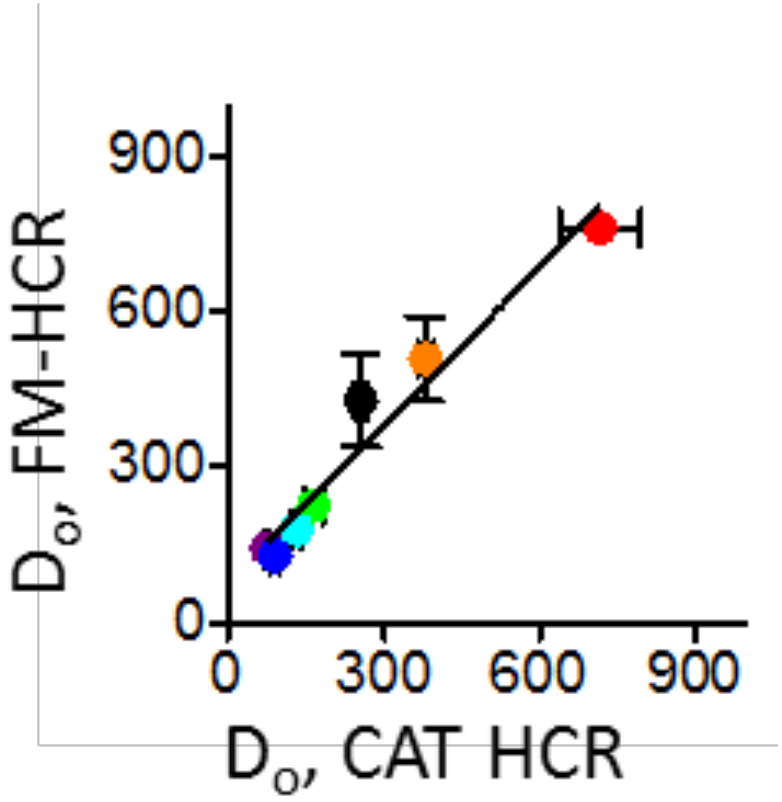
How does our FM-HCR data stack up against CAT-HCR, Grossman *et al.*, > 20 years ago?

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

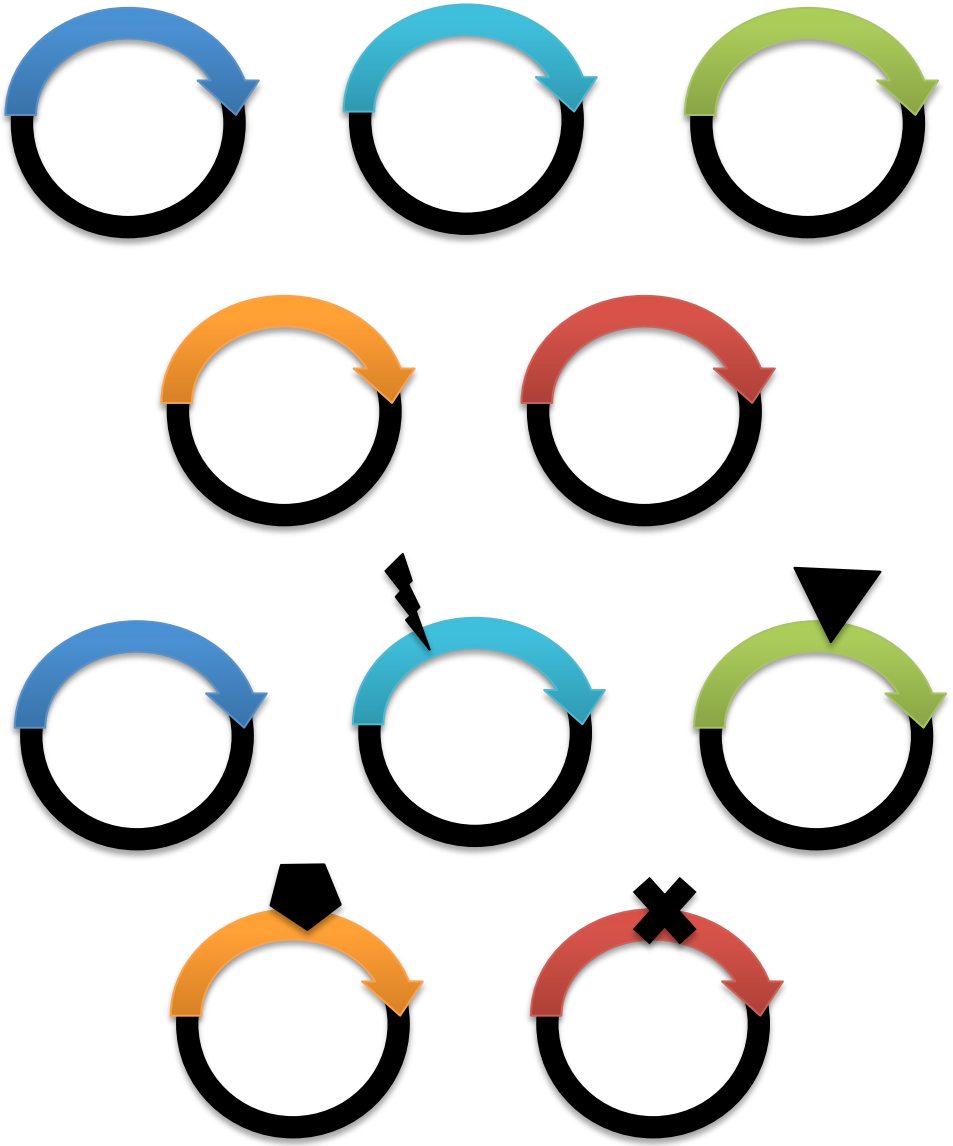


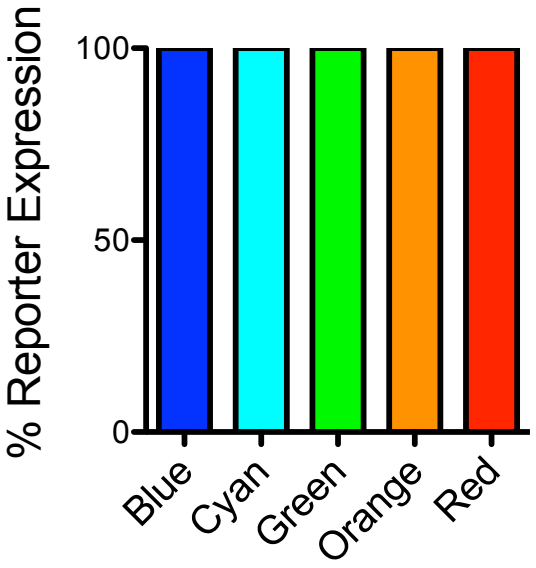


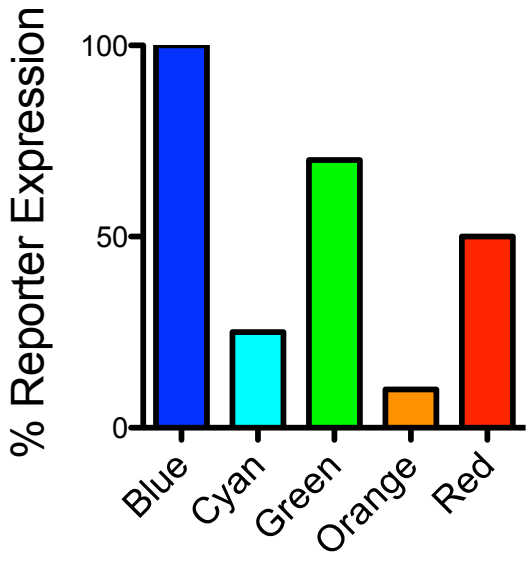
$R^2 = 0.92$, $p = 0.0006$

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

5 color HCR assay applications

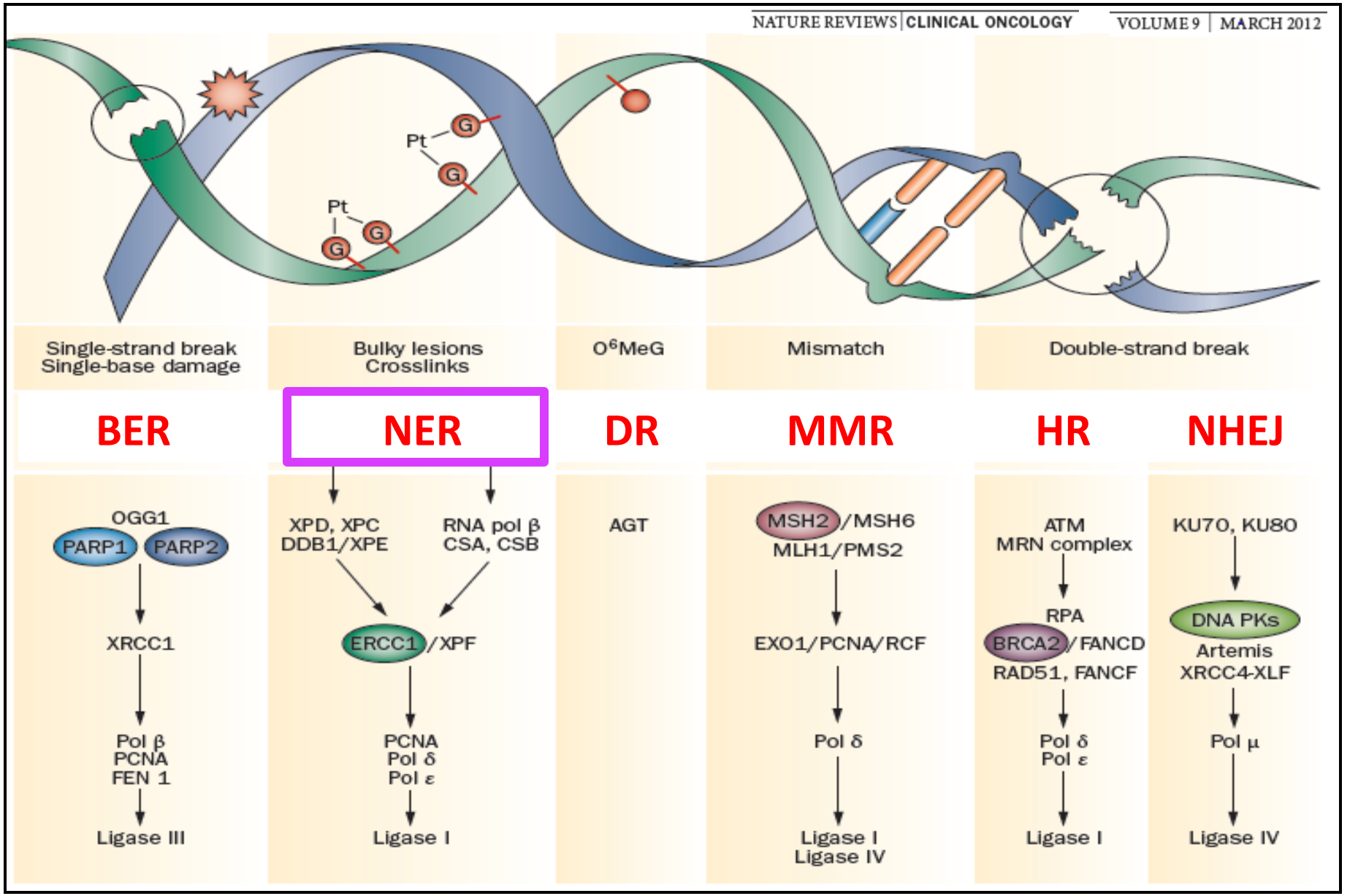




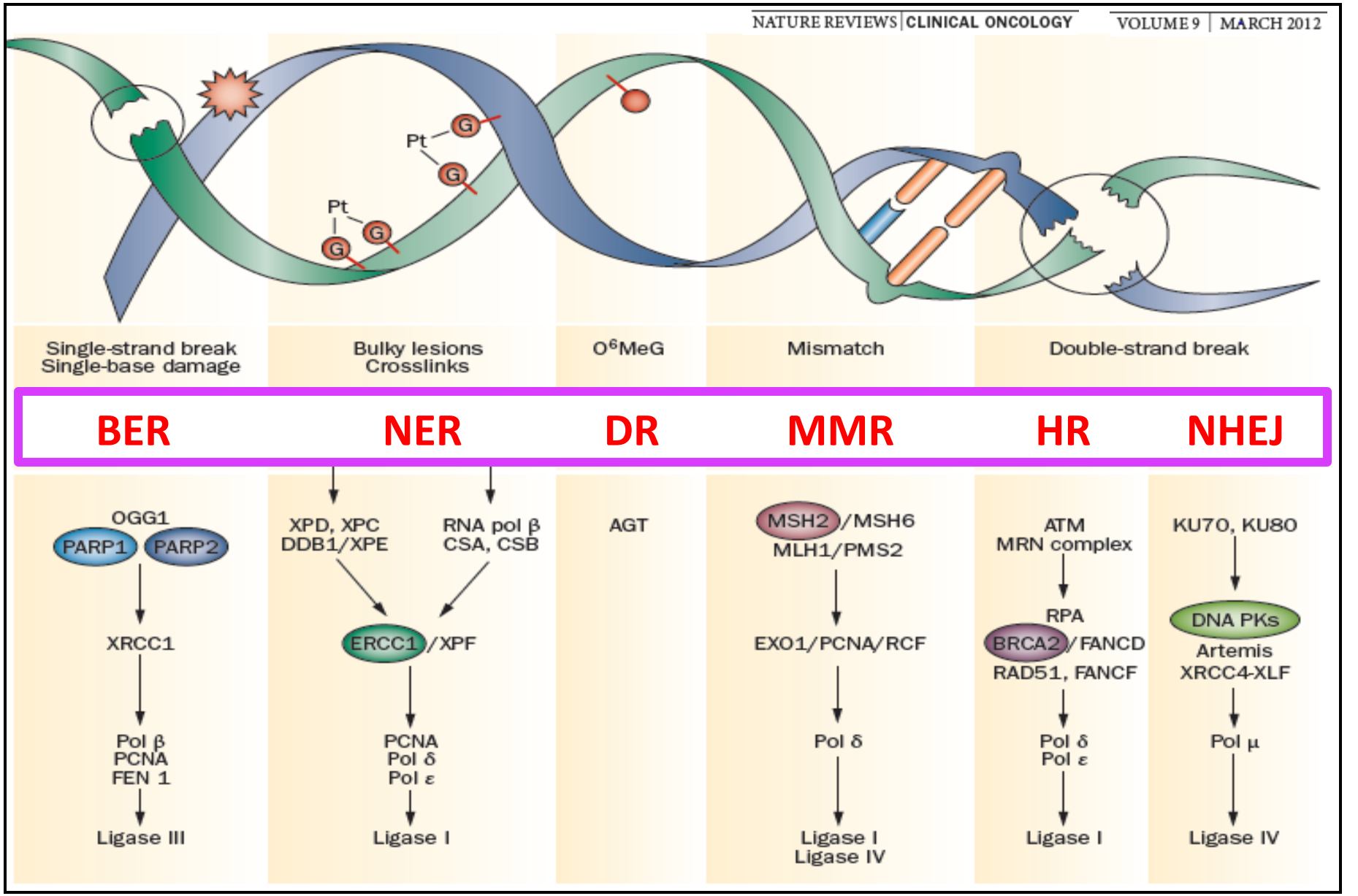


5-color HCR developed by **Dr. Zachary Nagel**

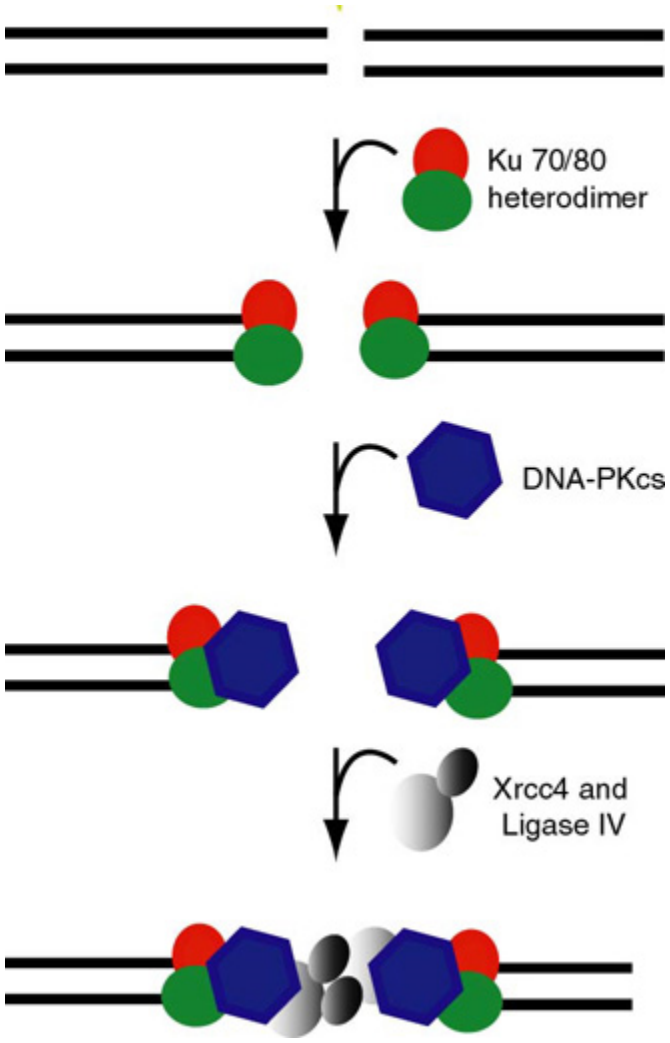
Six Major DNA Repair Pathways



Six Major DNA Repair Pathways

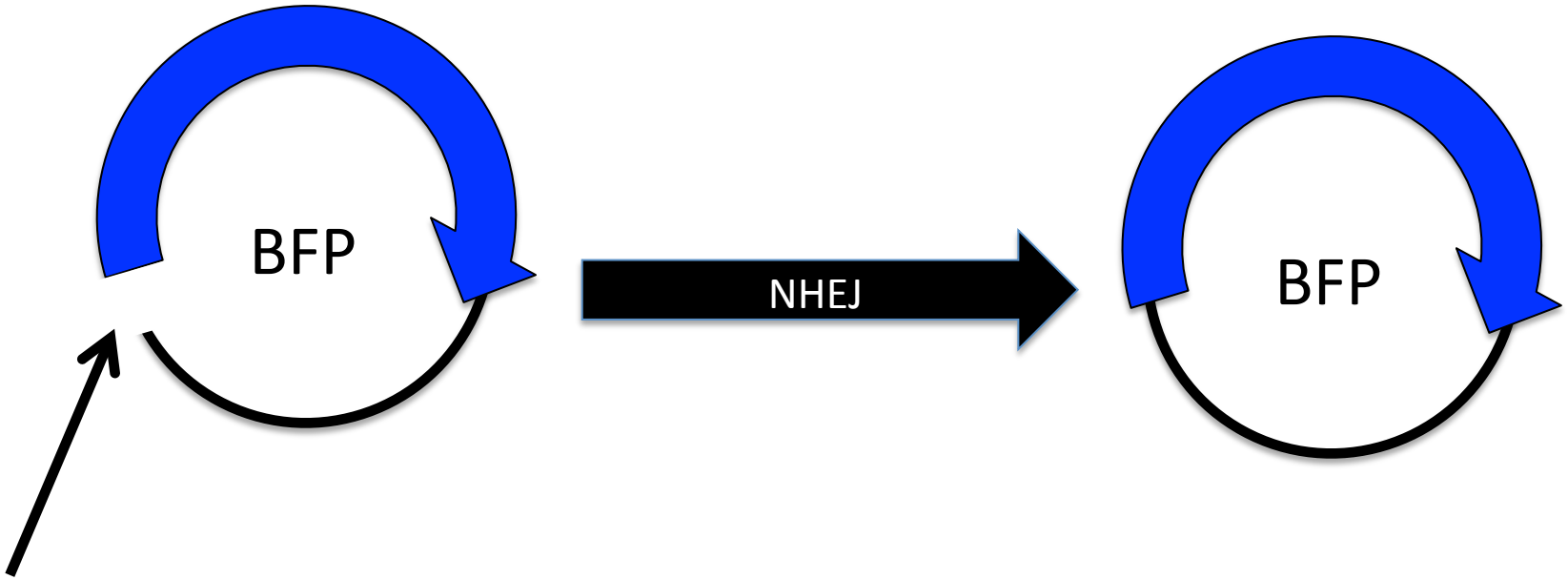


Non-Homologous End Joining (NHEJ) DNA Double Strand Break Repair



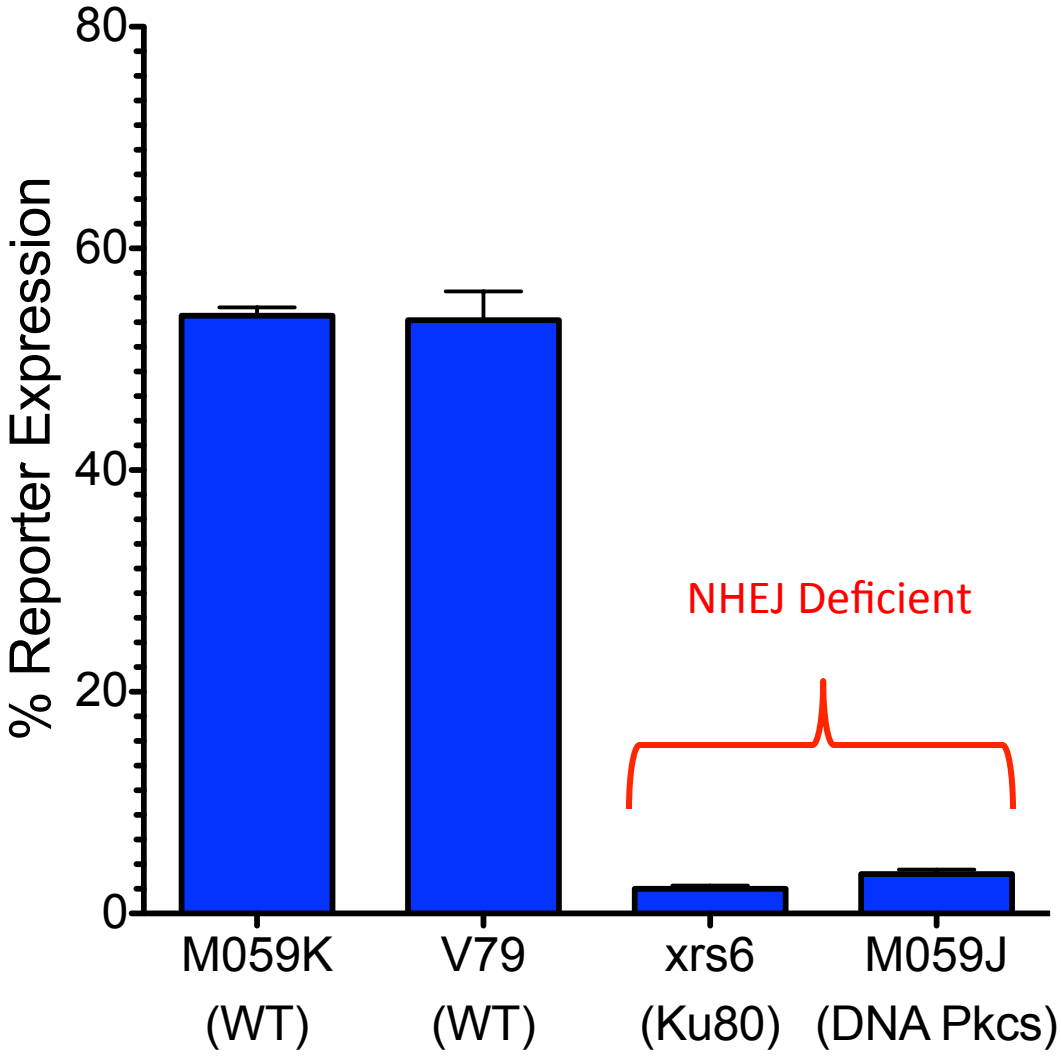
http://atlasgeneticsoncology.org/Deep/DoubleStrandBreaksID20008.html

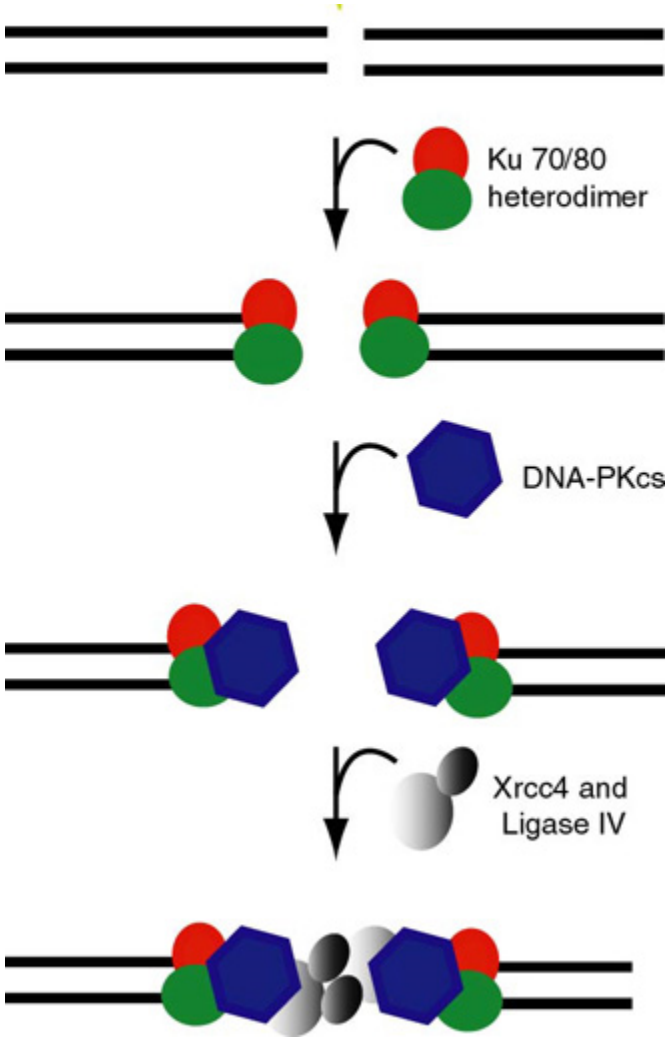
Non-Homologous End Joining (NHEJ)

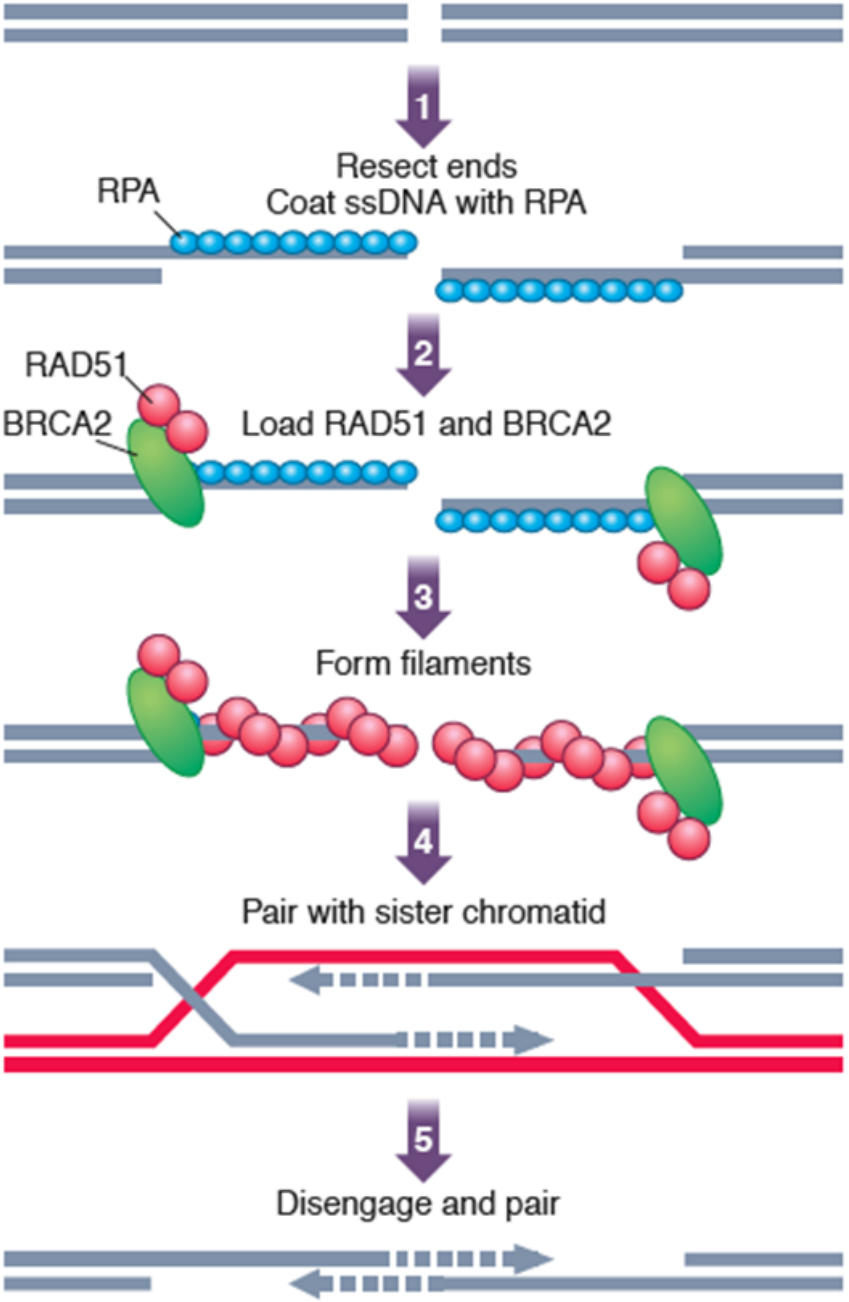


Substrate contains a blunt-end DSB in the 5’ UTR

NHEJ HCR in WT and NHEJ defective cells:

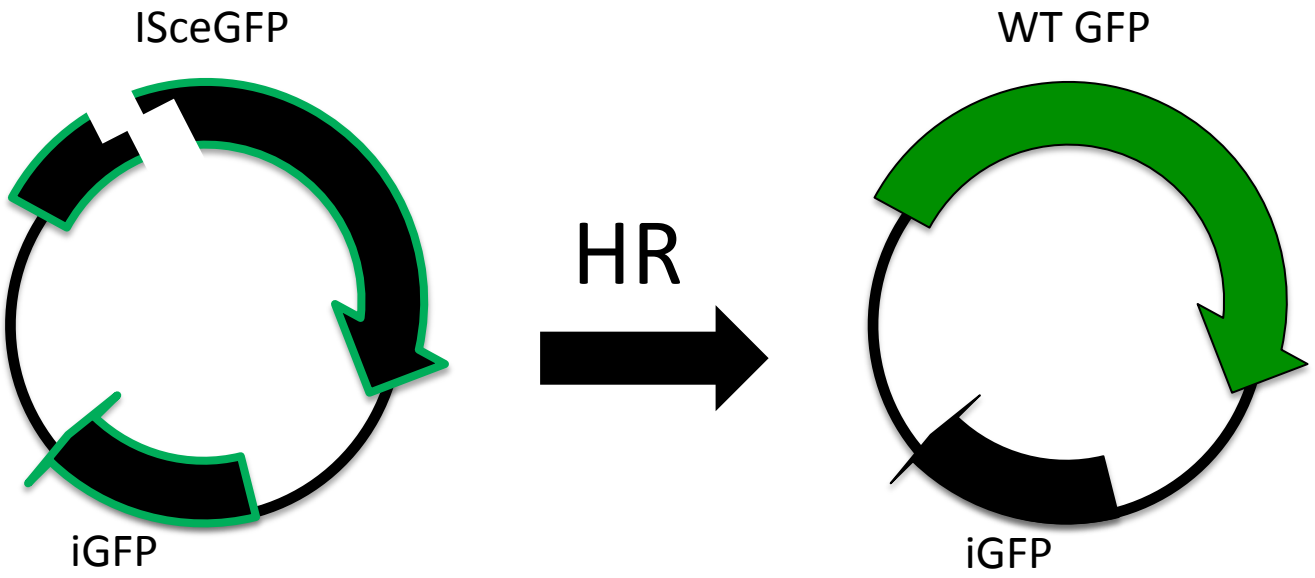






DNA DSB Repair by Homologous Recombination

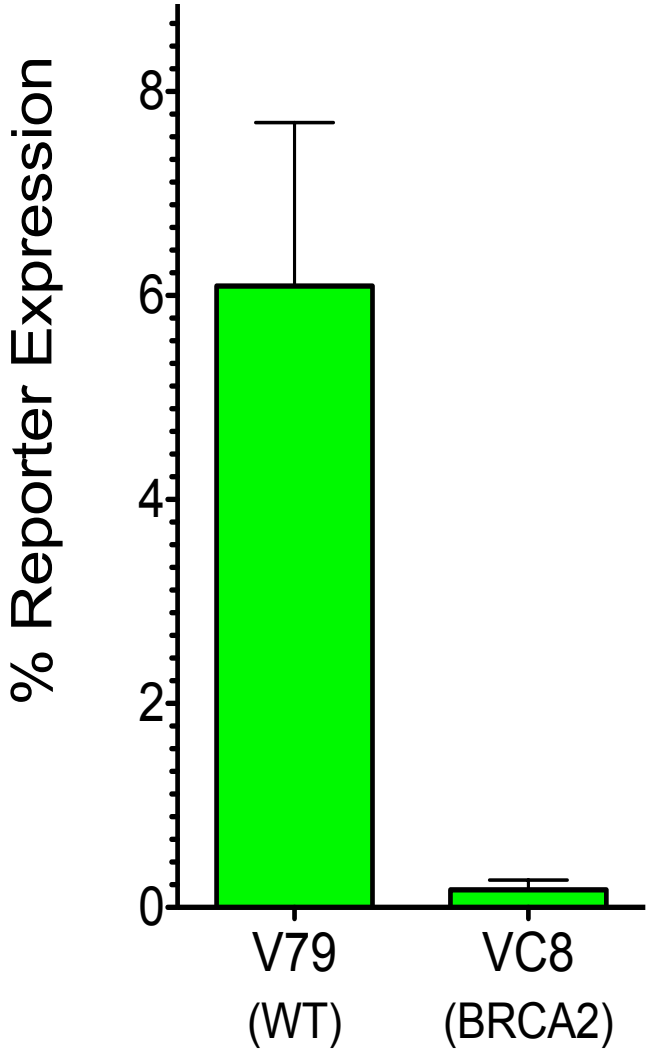
DNA Double Strand Break Repair Homologous Recombination (HR)

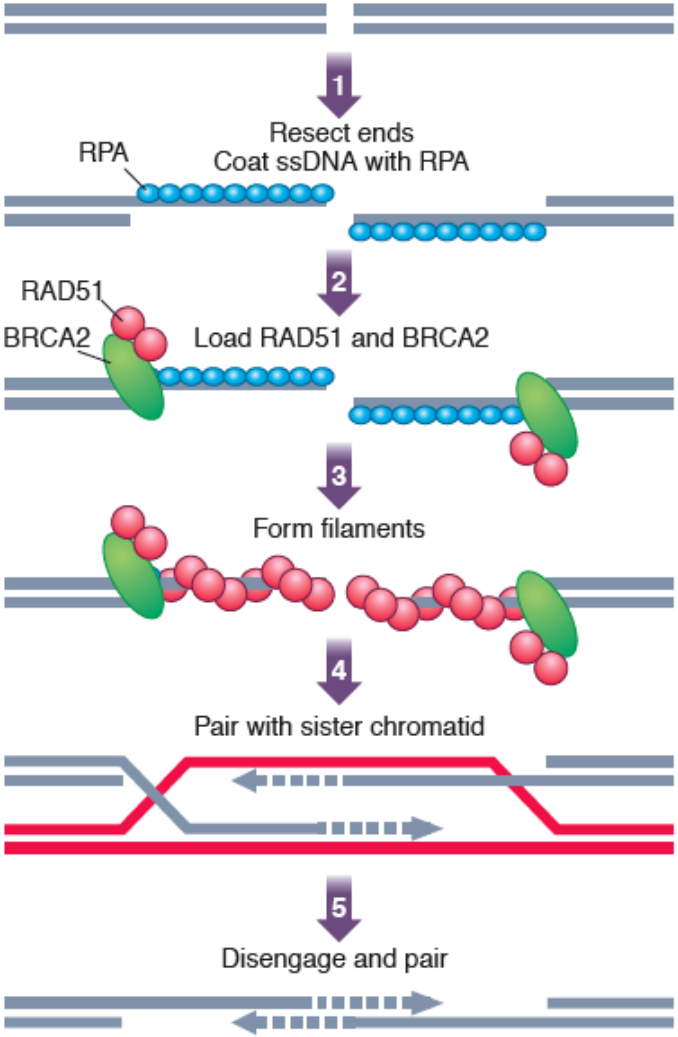


Adapted from Maria Jasin

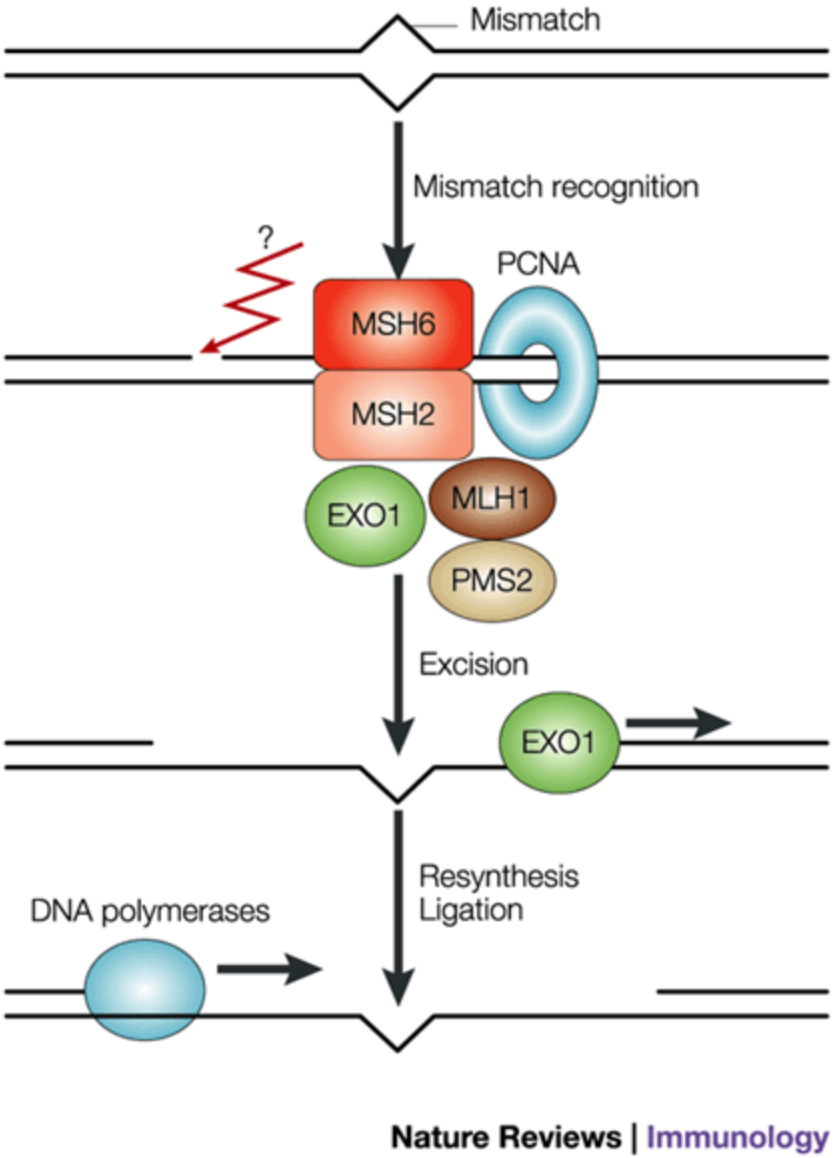
Pierce et al. (1999) Genes and Development (13) 2633-2638

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



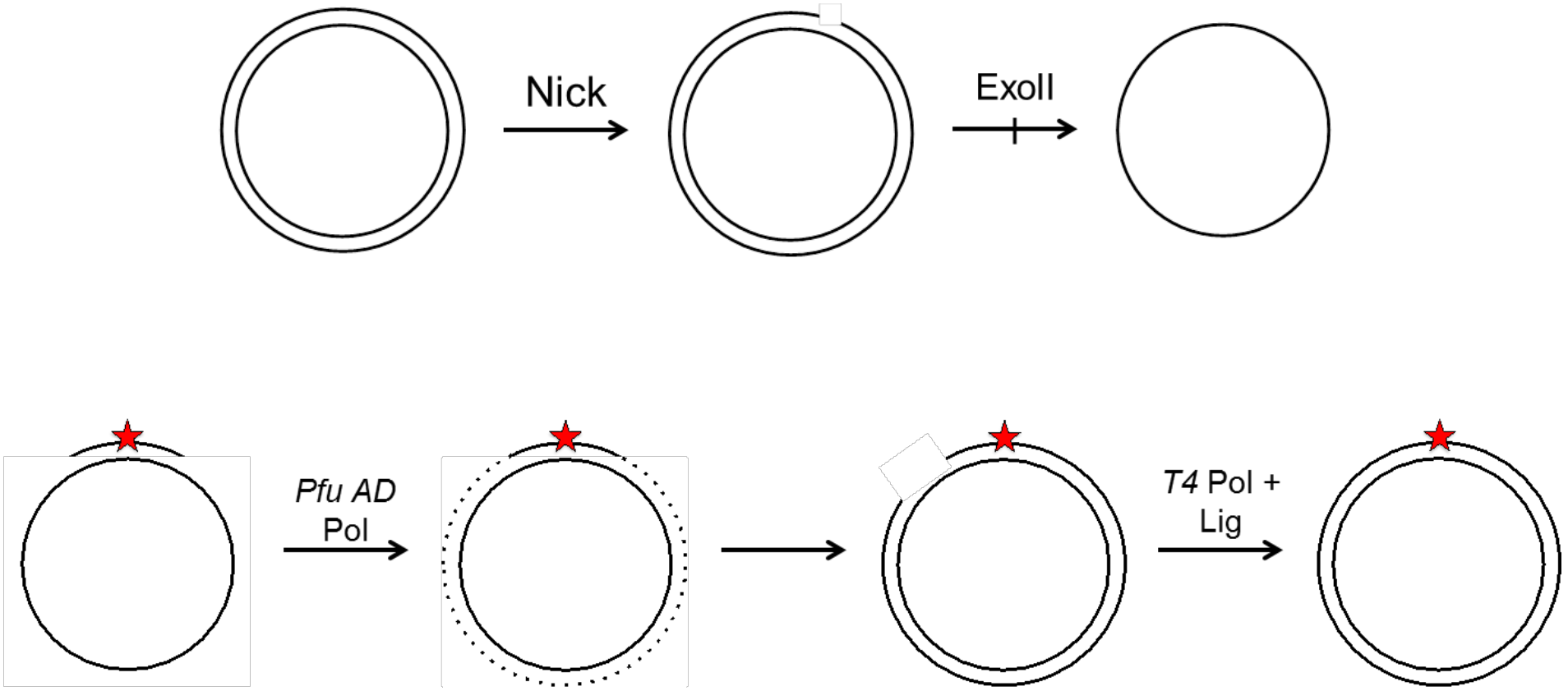


DNA Mismatch Repair



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

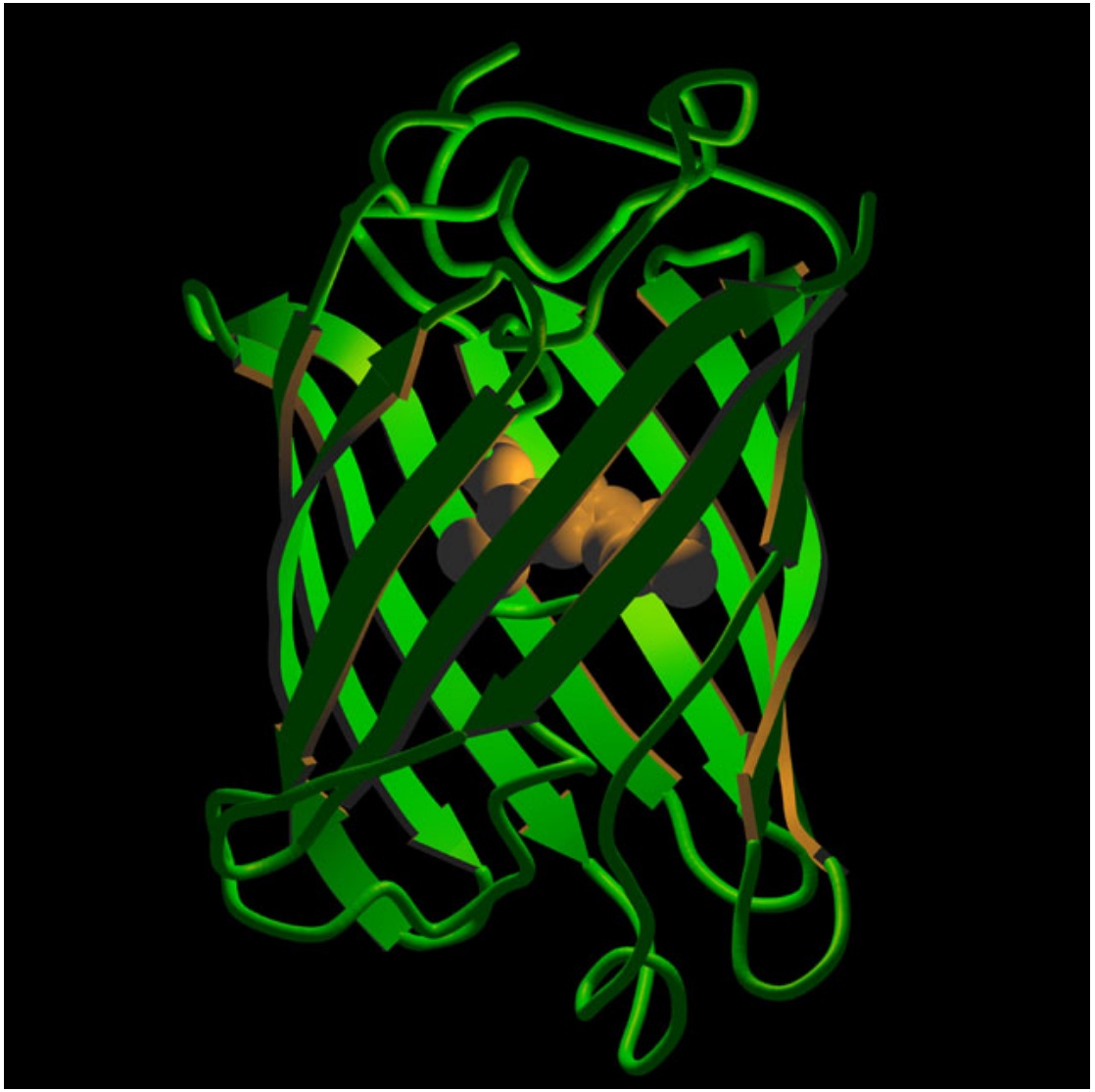
How to build a site-specific reporter?
"Primer-Extension"



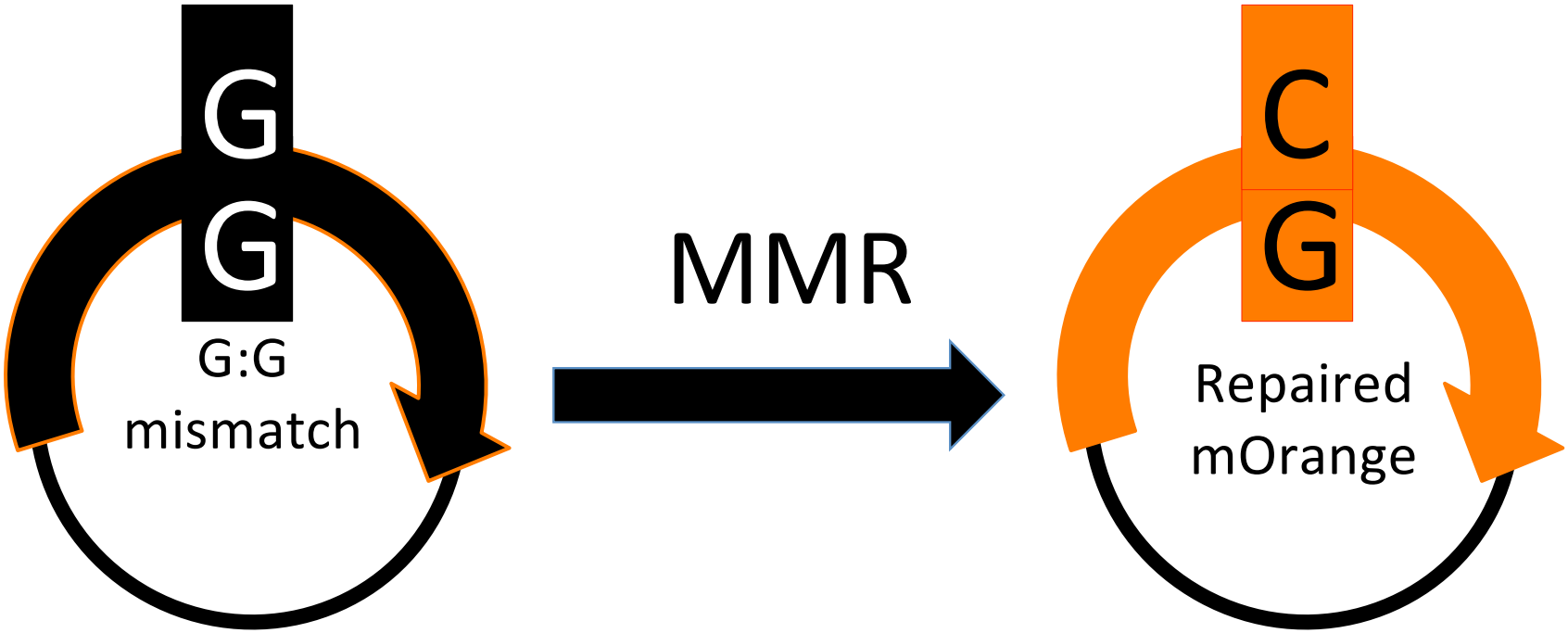
Original protocol from Baerenfaller *et al.* 2006 *Meth in enzymology*

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

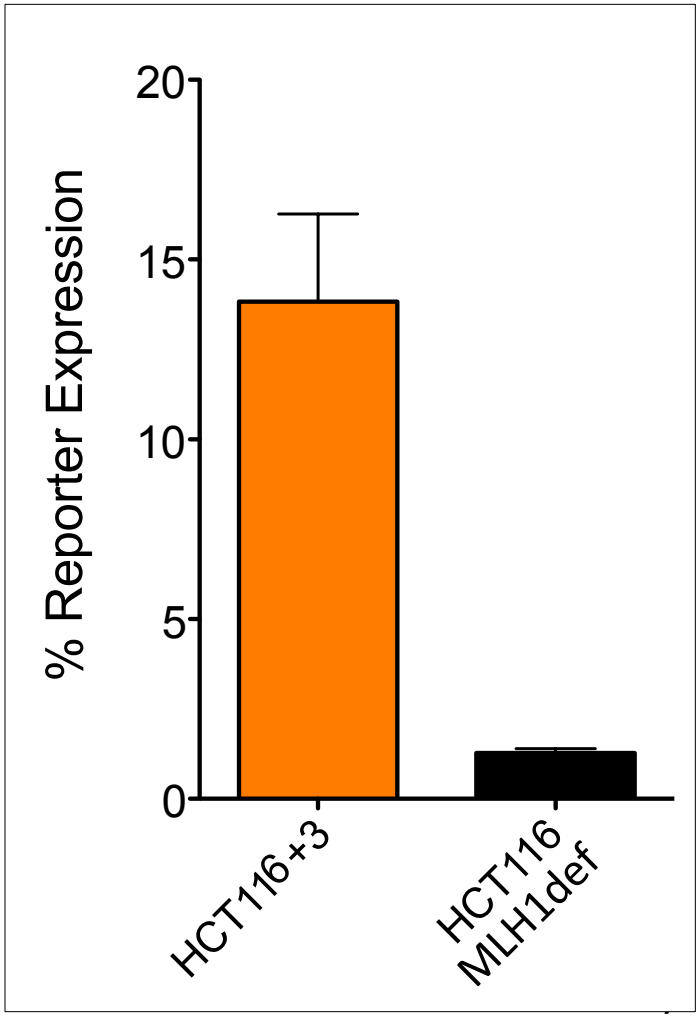
Green Fluorescent Protein (GFP) first isolated from crystal jellyfish *(Aequorea victoria).*

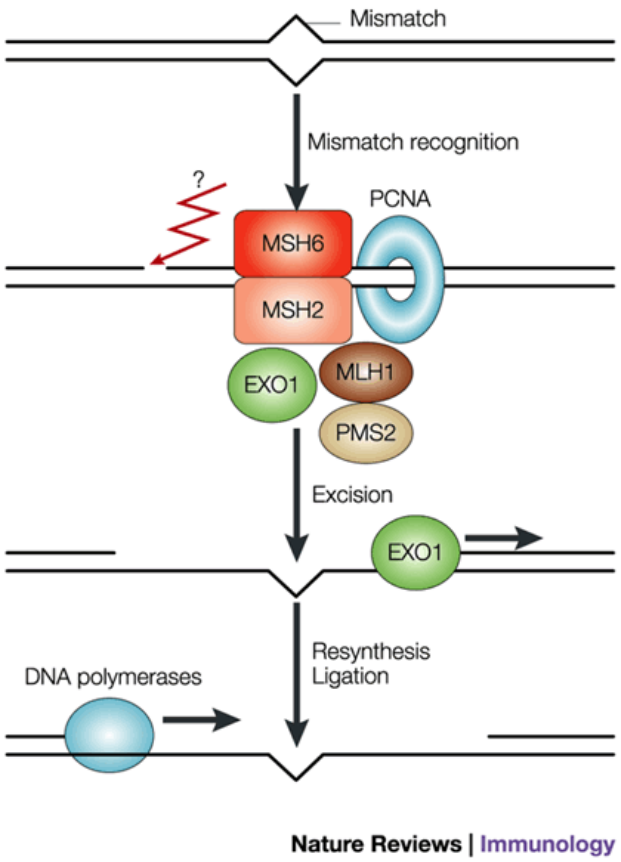


DNA Mismatch Repair (MMR)

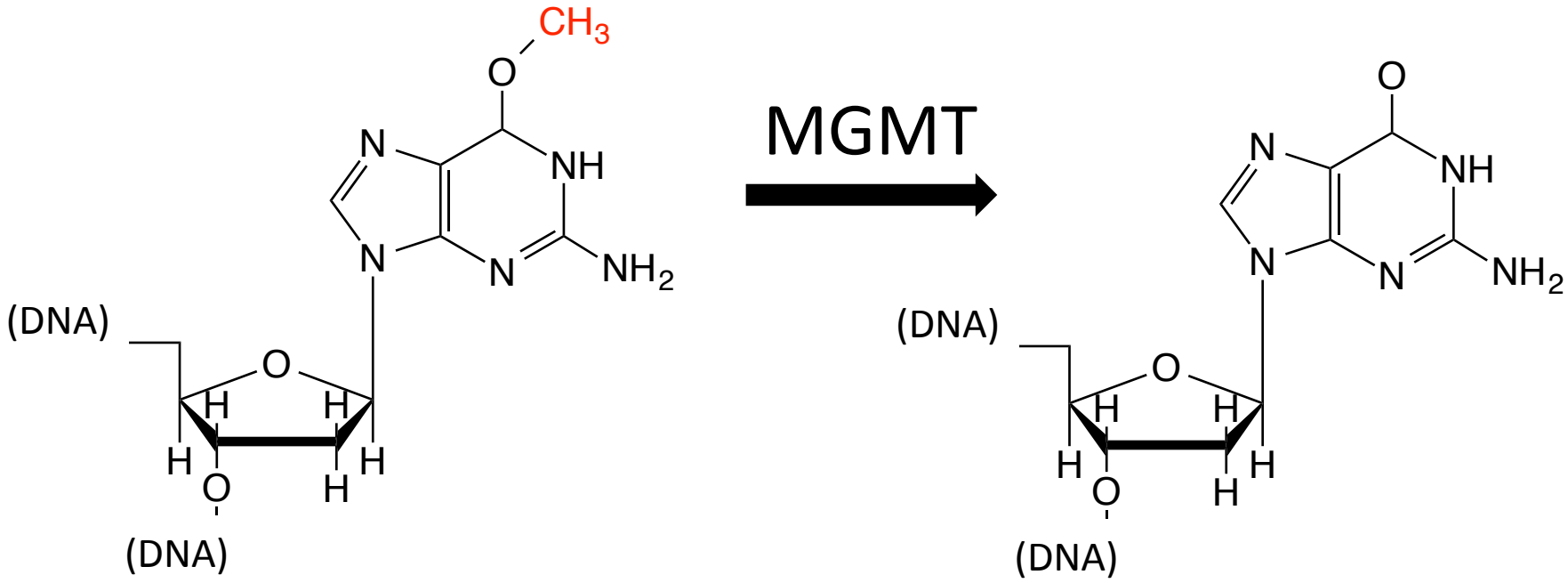


MMR assay distinguishes between proficient and deficient cell lines:



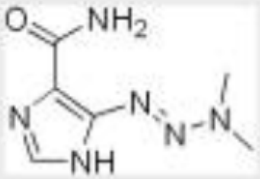
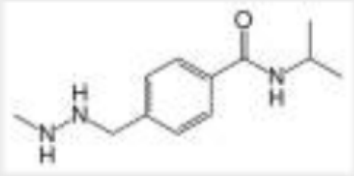
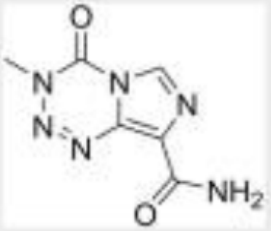
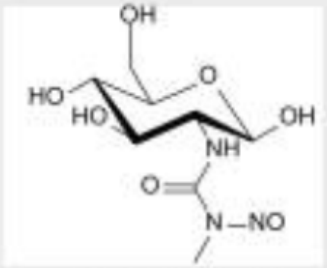
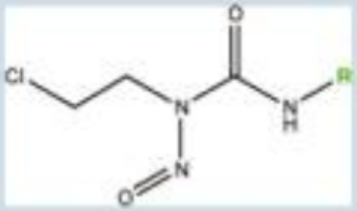
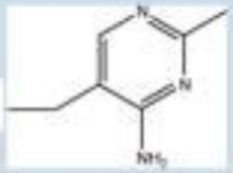
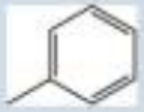
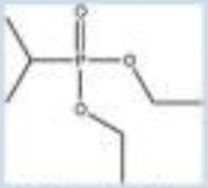


O^6-Methylguanine DNA Methyltransferase MGMT

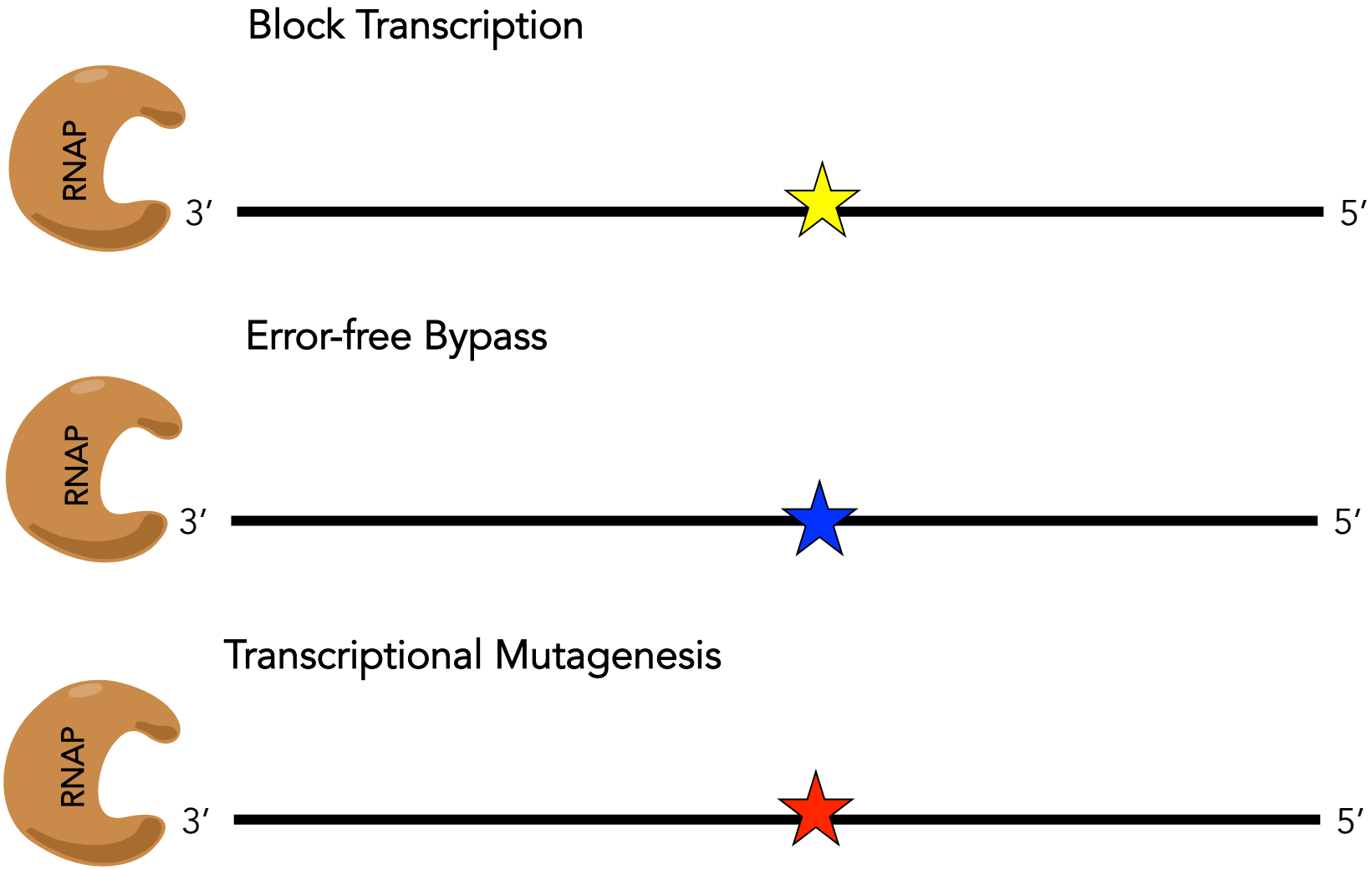


Many Cancer Chemotherapy Drugs induce this DNA lesion

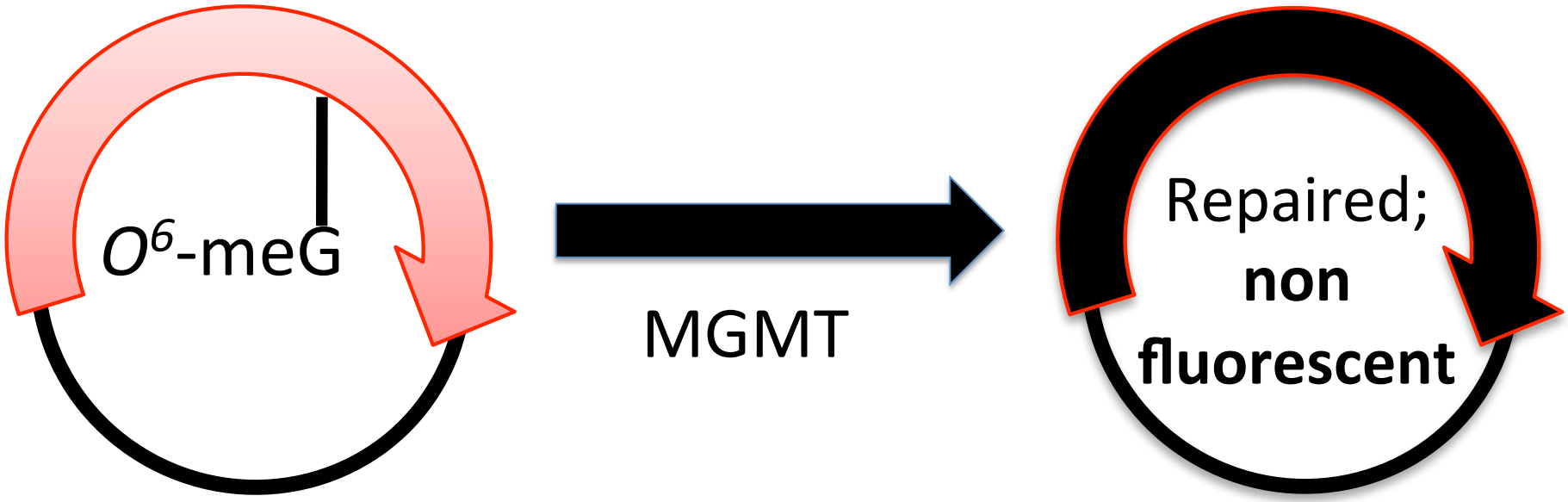
Alkylating agents used in the cancer clinic

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

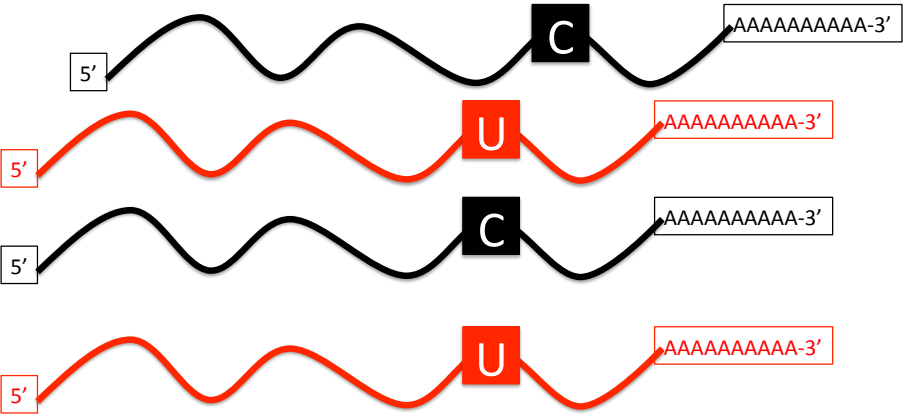
DNA lesions from an RNA polymerase perspective

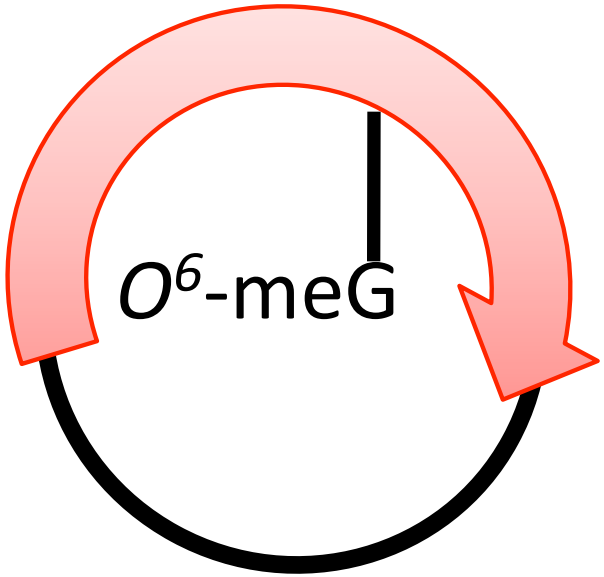


Direct Reversal of O^6-Methylguanine

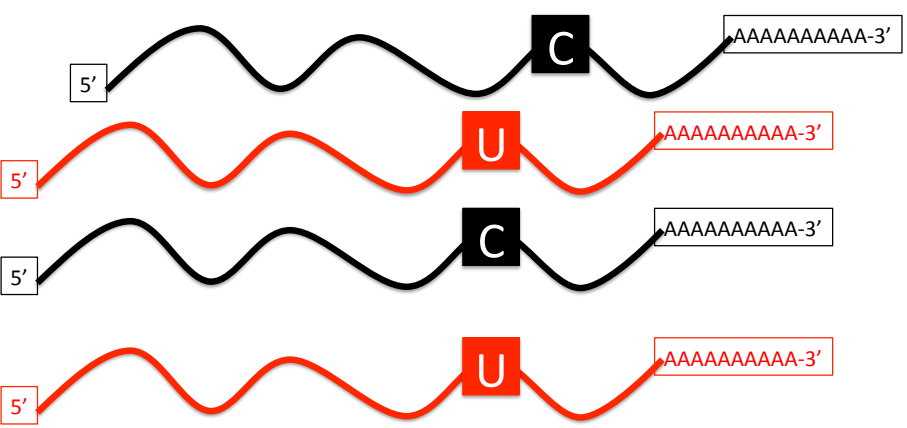


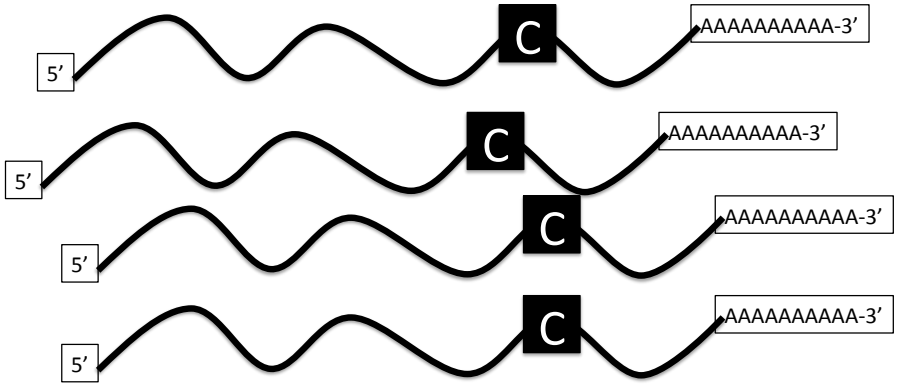
Direct Reversal of O^6-Methylguanine

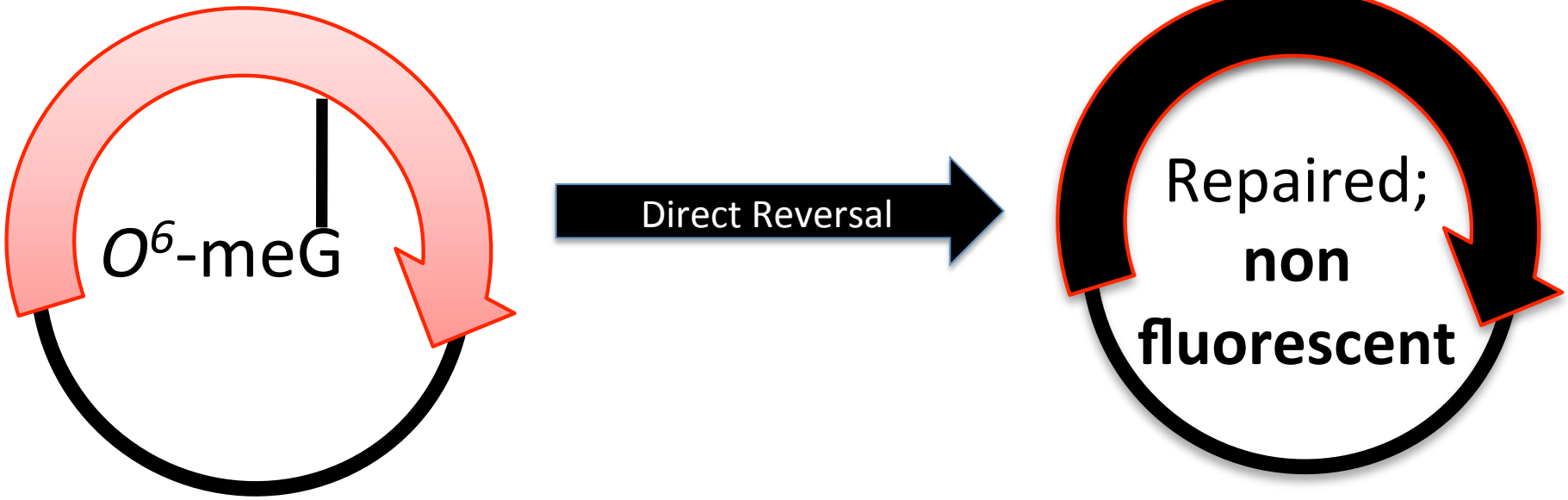




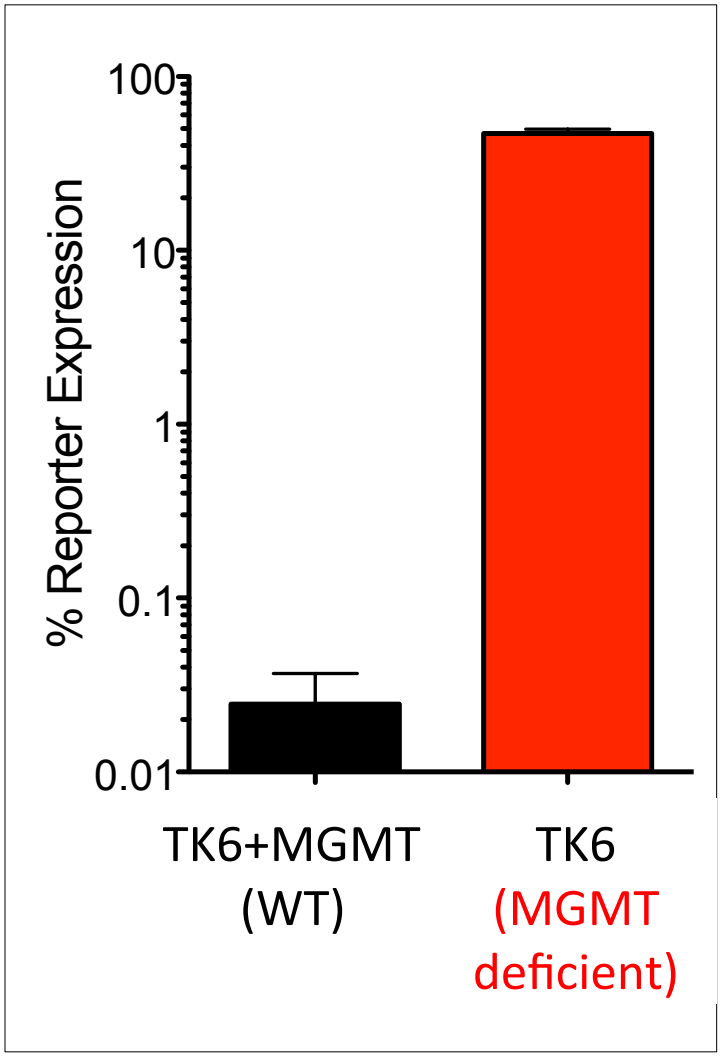
Direct Reversal of O^6-Methylguanine

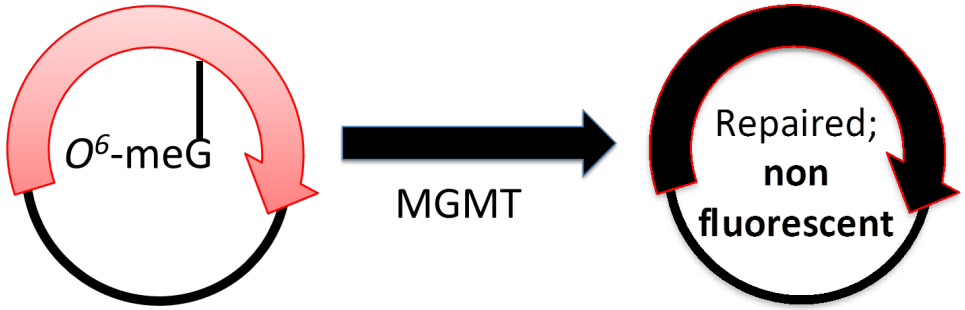




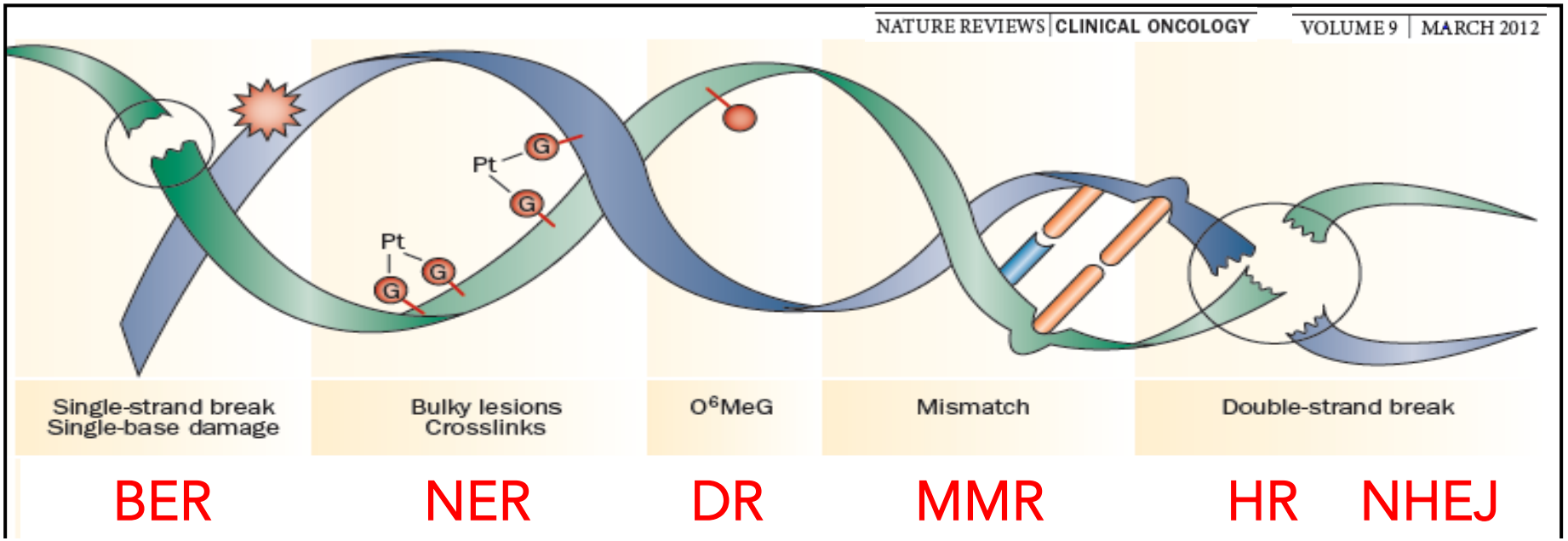


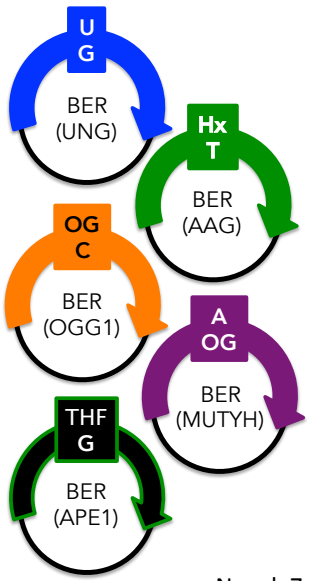
MGMT deficient cells are distinguished by a high level of reporter expression:

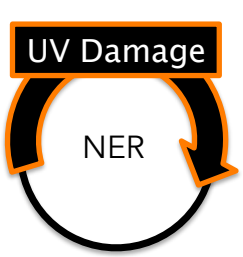


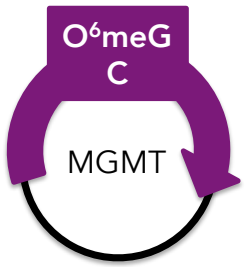


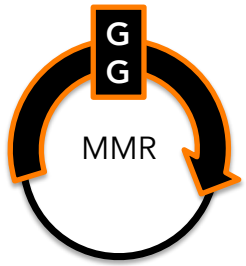
Six Major DNA Repair Pathways

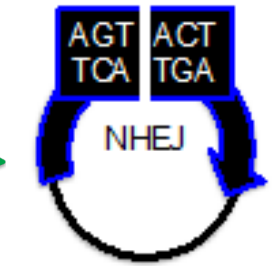












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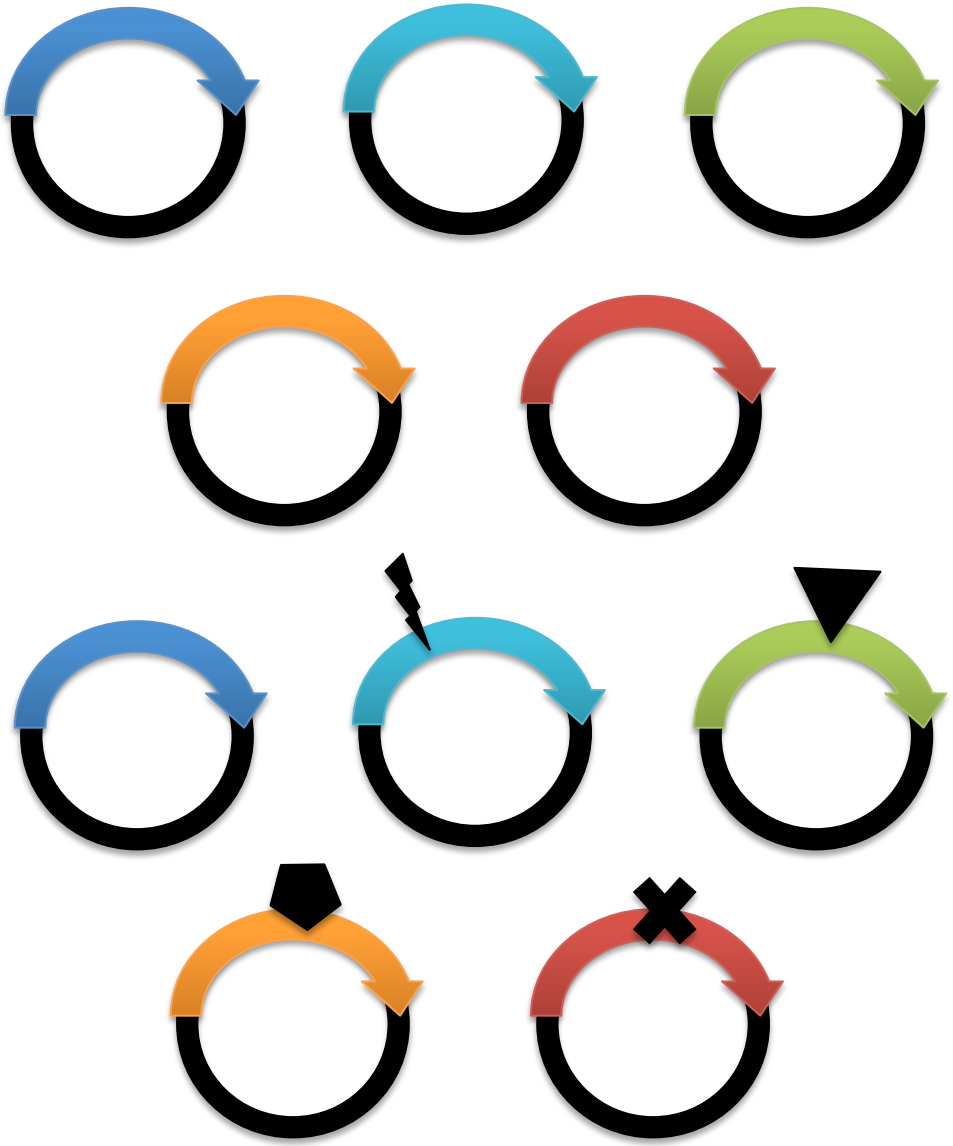
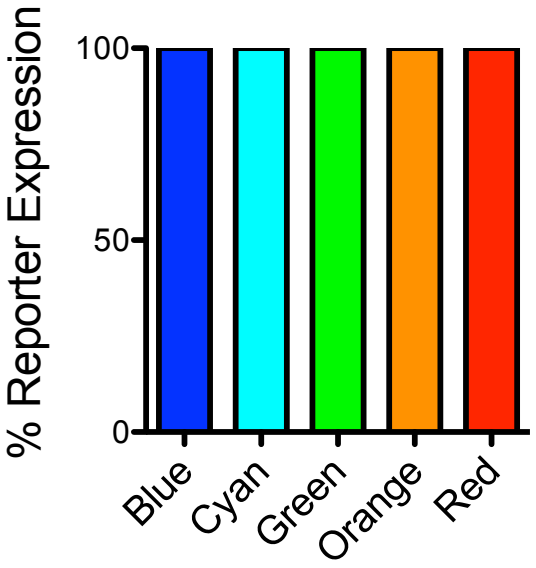
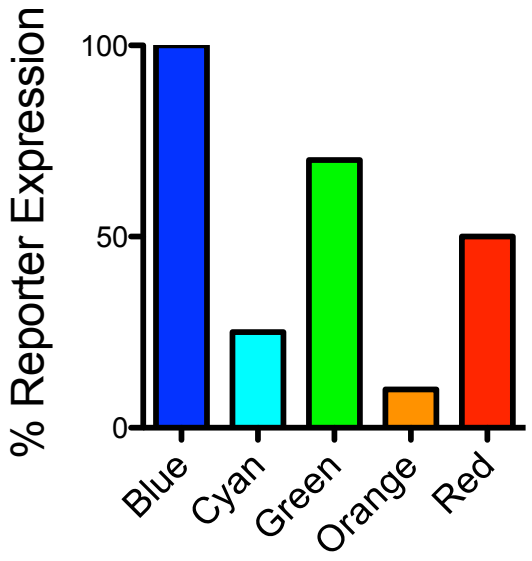
5 color HCR assay applications

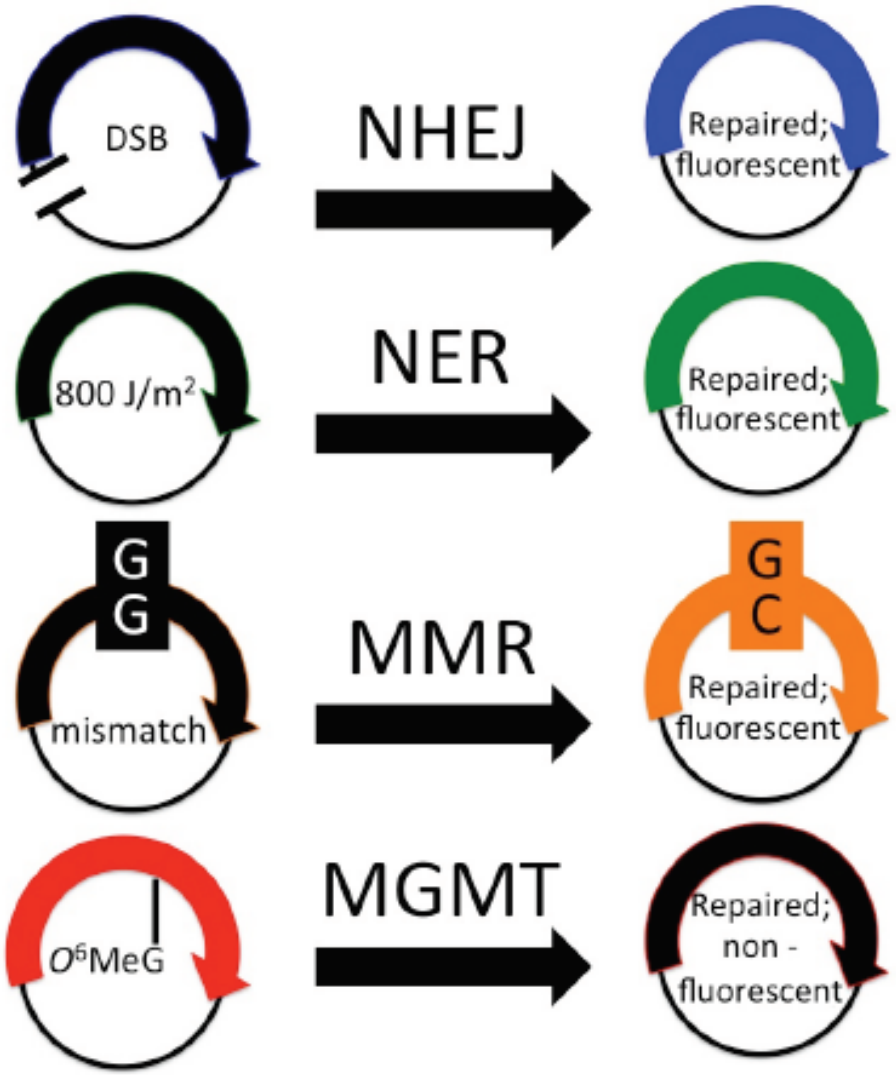




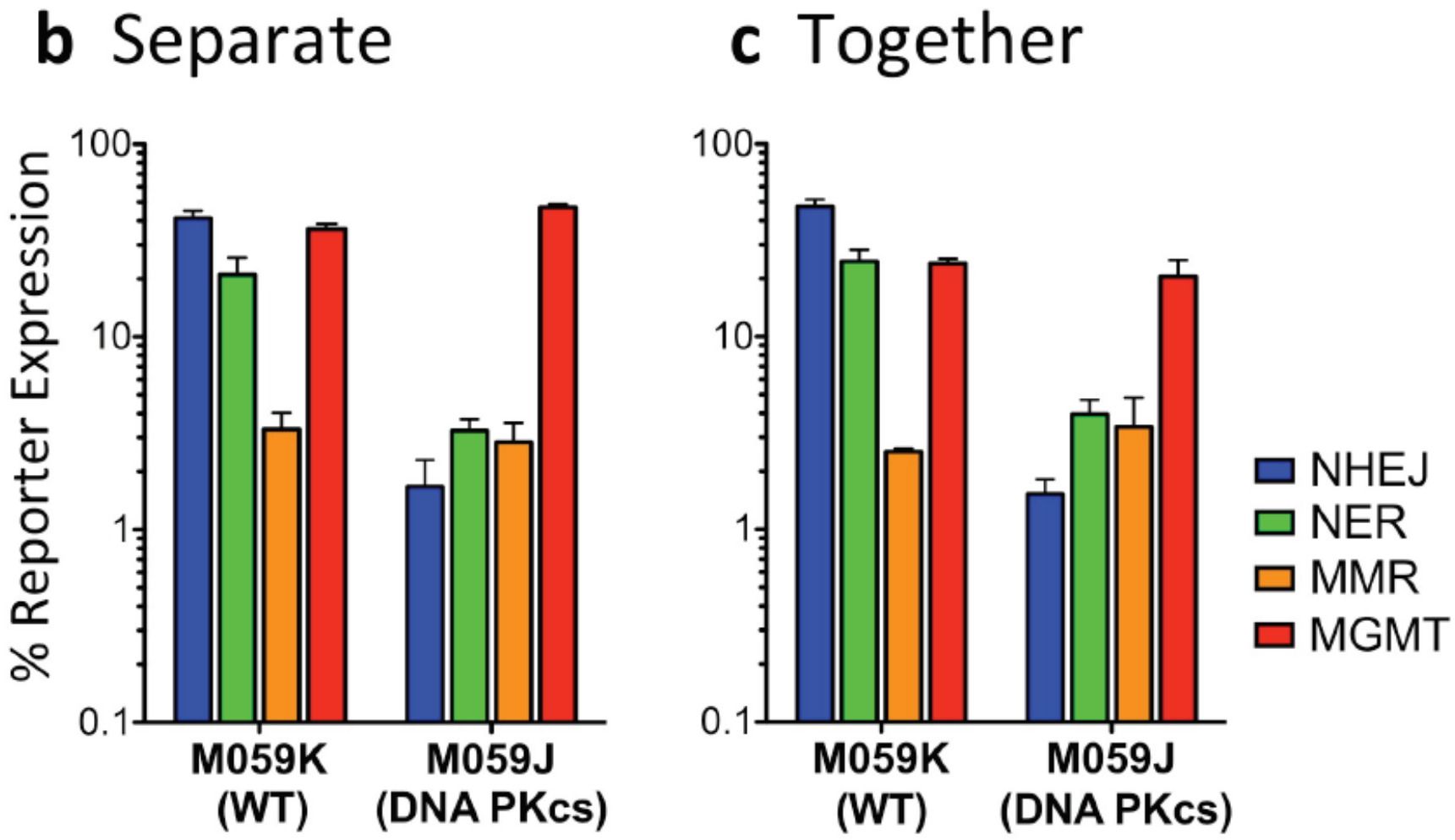


5-color HCR developed by **Dr. Zachary Nagel**

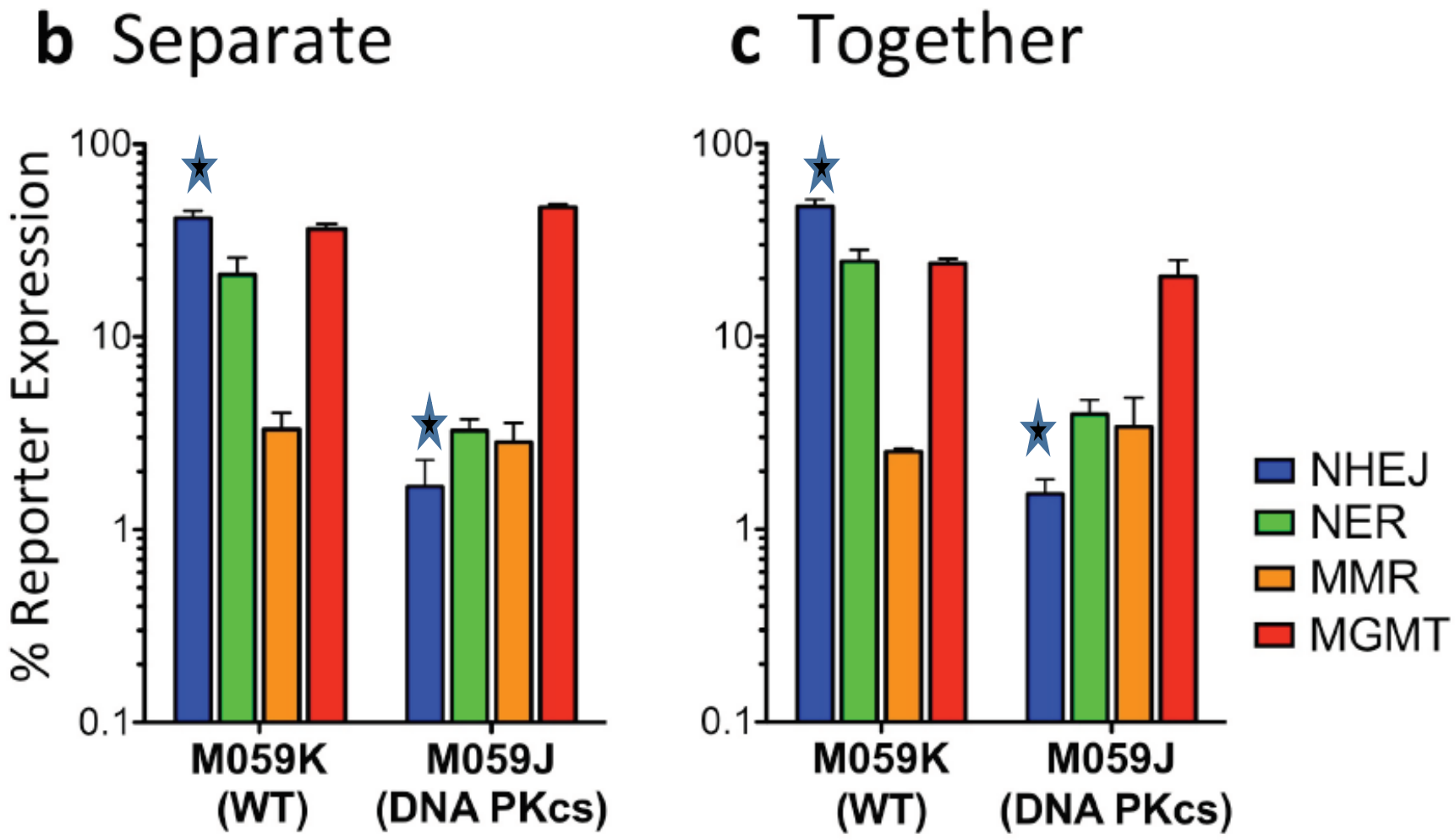
Measured DRC in 4 pathways in a single assay:



Then Measured DRC in 4 pathways in a single assay:



Then Measured DRC in 4 pathways in a single assay (2 of 2):



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

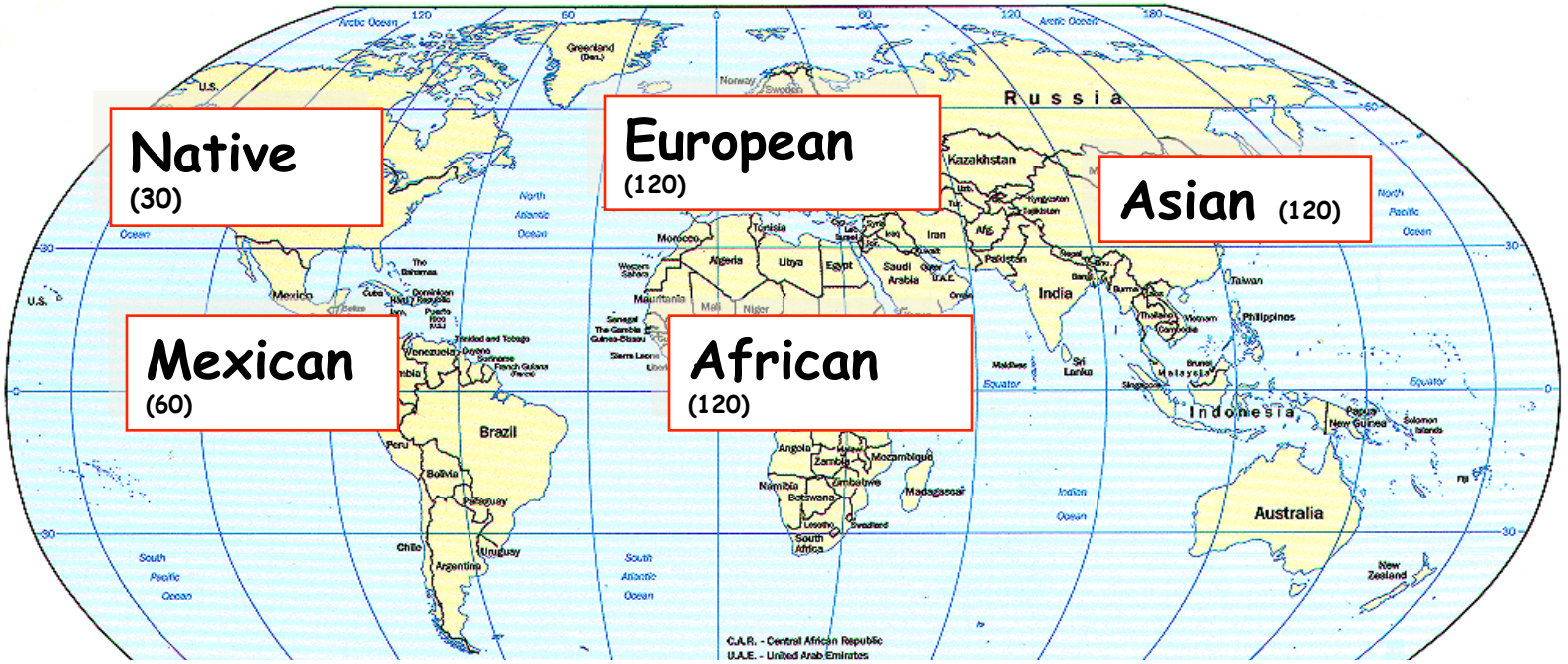
Center for Environmental Health Sciences

Koch Institute for Integrative Cancer Research

Computational and Systems Biology Initiative

Broad Institute (Harvard and MIT)

Coriell Lymphoblastoid Cell line collection derived from ethnically diverse HEALTHY humans

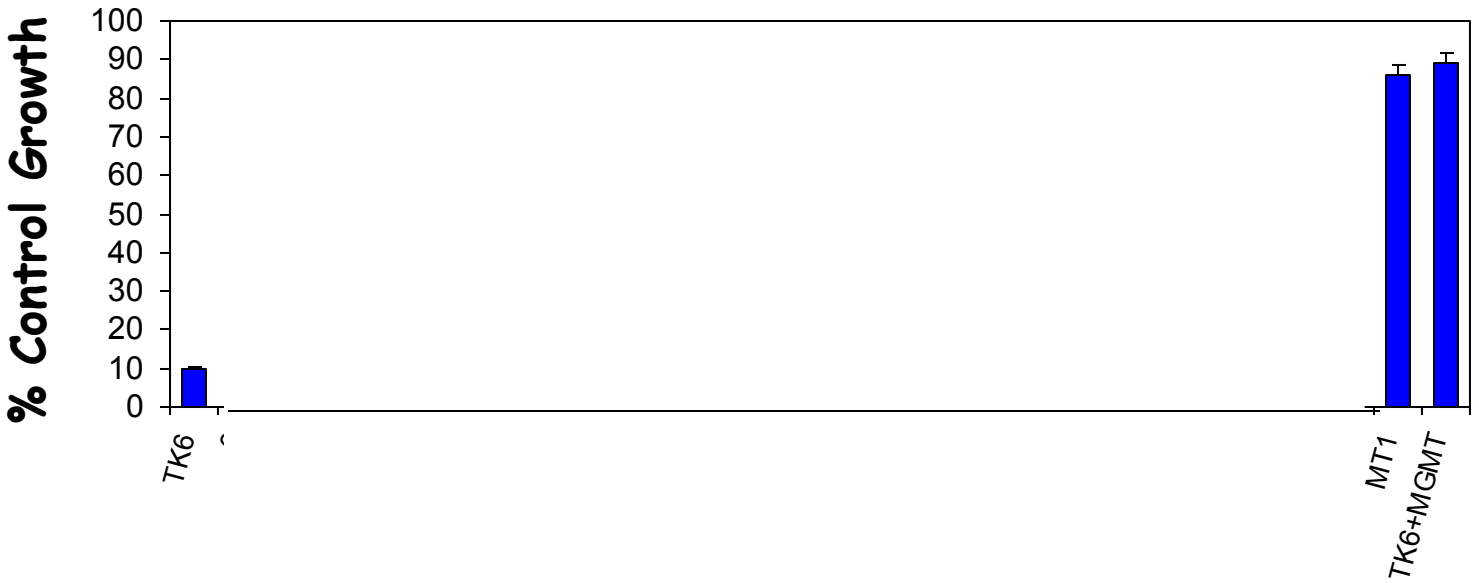


450 healthy unrelated US residents with ancestry from around the globe

Nested subsets: 90, 44, 24, 8

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

Extensive Range of Sensitivity in Cells Exposed to Alkylation Damage – Control Cell Lines



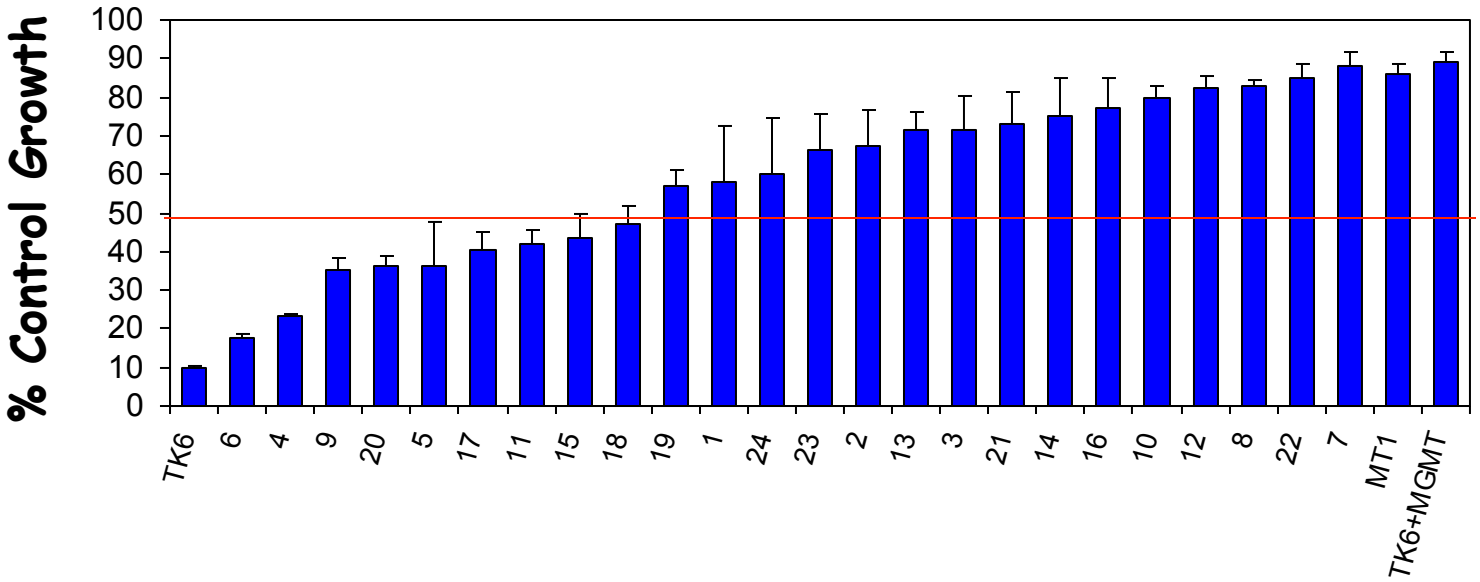
Sensitive

To MNNG

Resistant

To MNNG

Extensive Range of Sensitivity in Cells Exposed to Alkylation Damage – Corriel Cell Lines



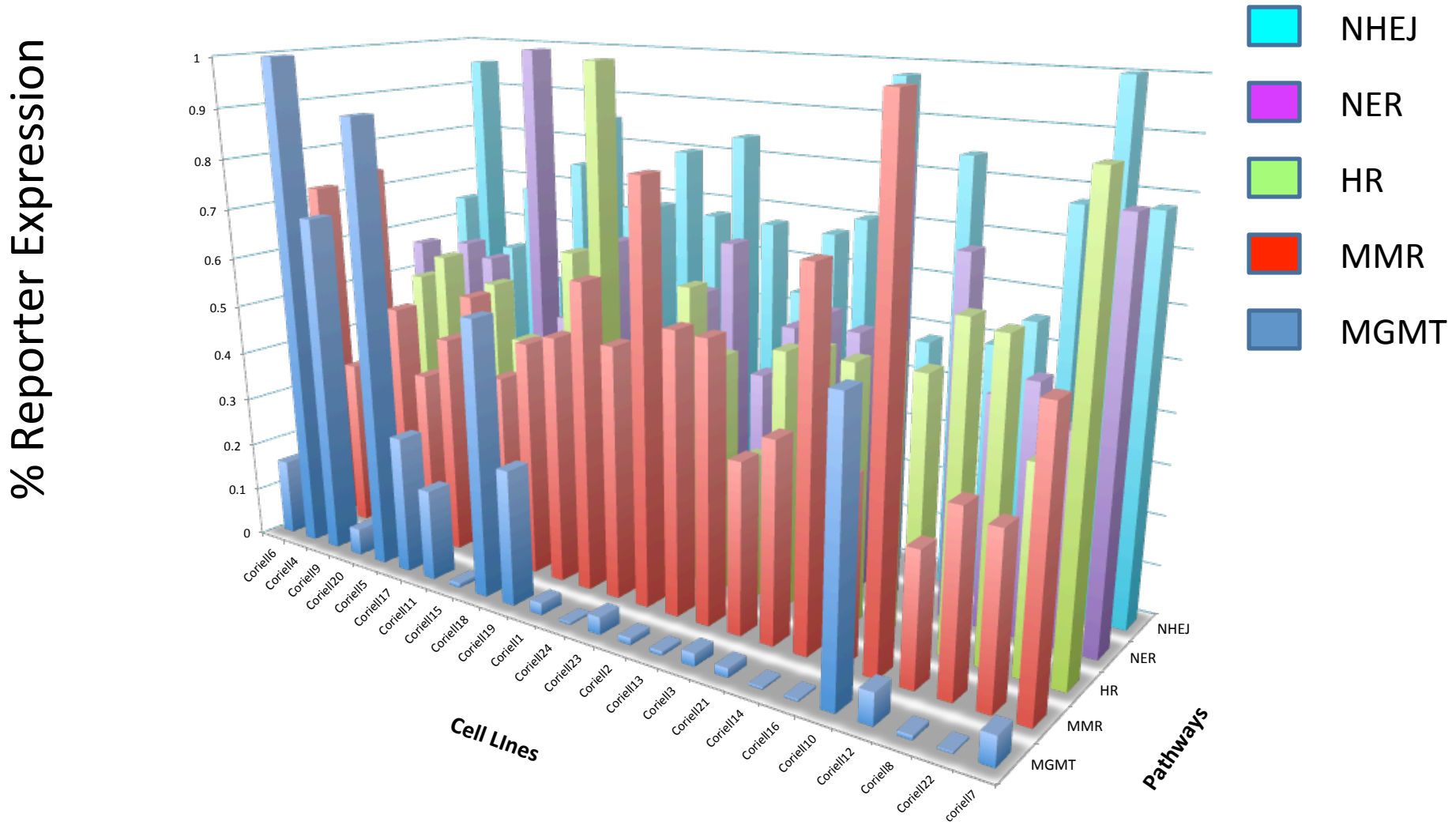
Sensitive

To MNNG

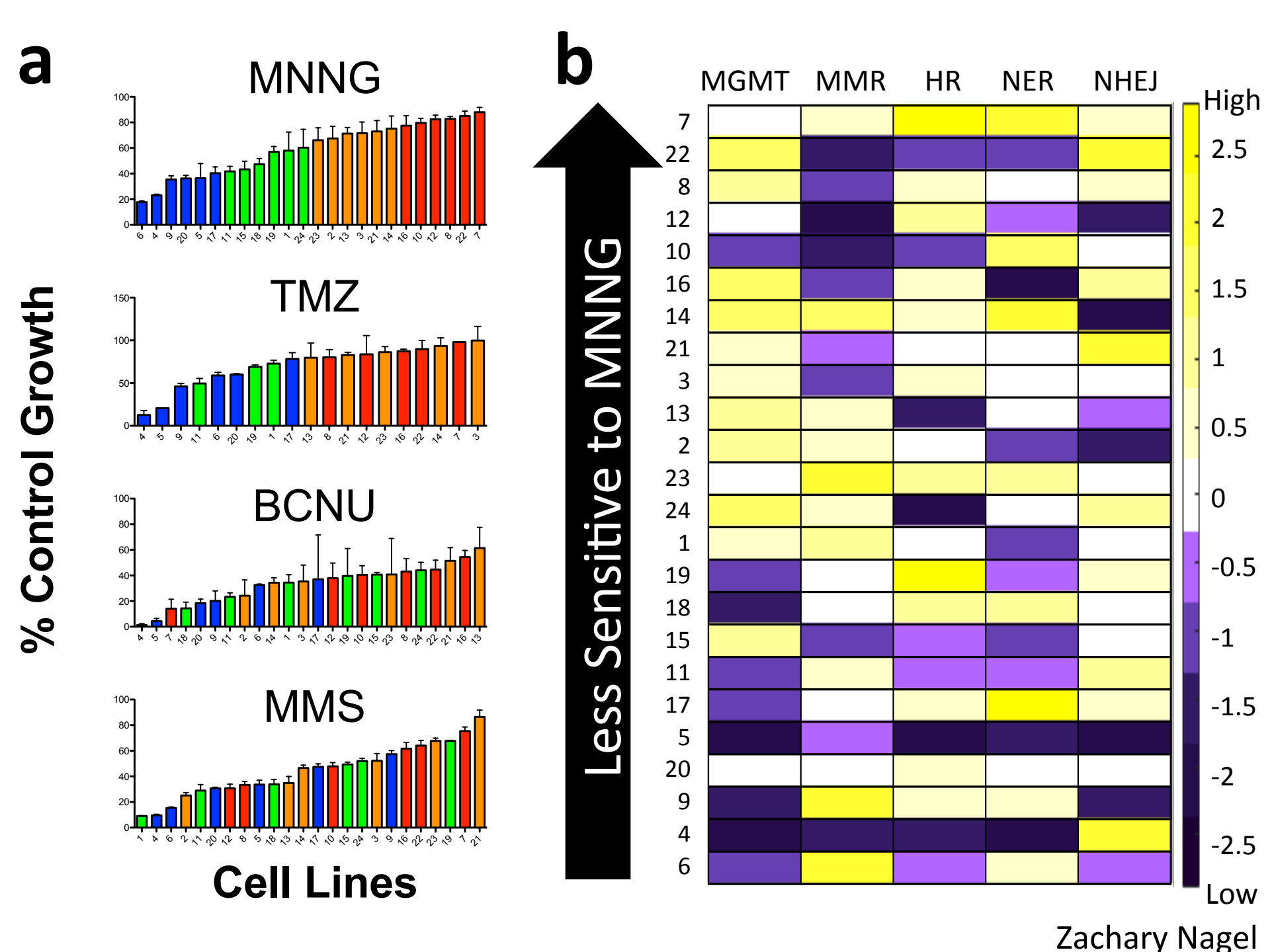
Resistant

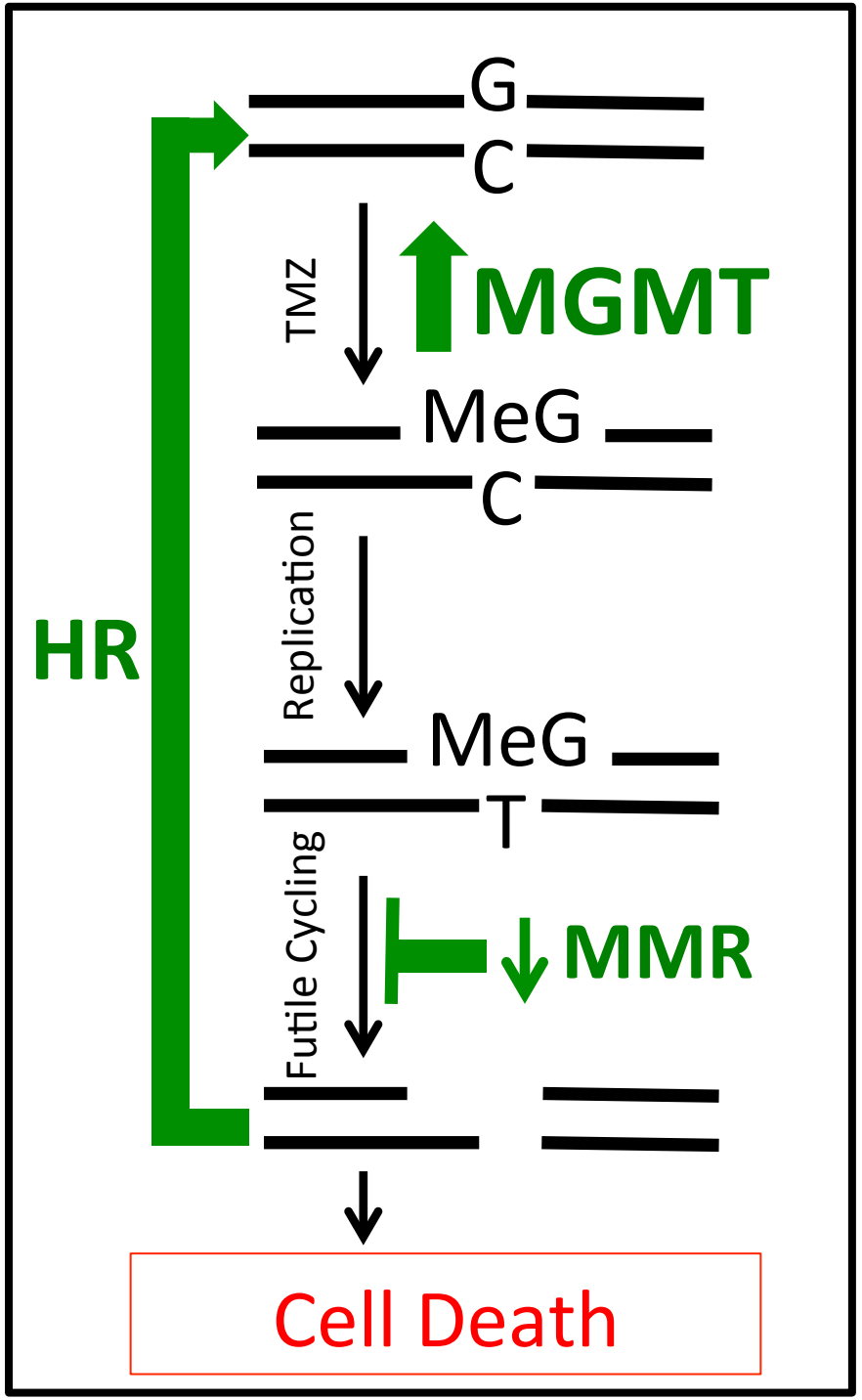
To MNNG

DRC vs. MNNG sensitivity



Zachary Nagel


a
MNNG
TMZ
BCNU
MMS
% Control Growth
Cell Lines
b
MGMT
MMR
HR
NER
NHEJ
Less Sensitive to MNNG
7
22
8
12
10
16
14
21
3
13
2
23
24
1
19
18
15
11
17
5
20
9
4
6
High
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
Low
Zachary Nagel



MATHEMATICAL MODELING using MULTIPLE LINEAR REGRESSION

For MNNG

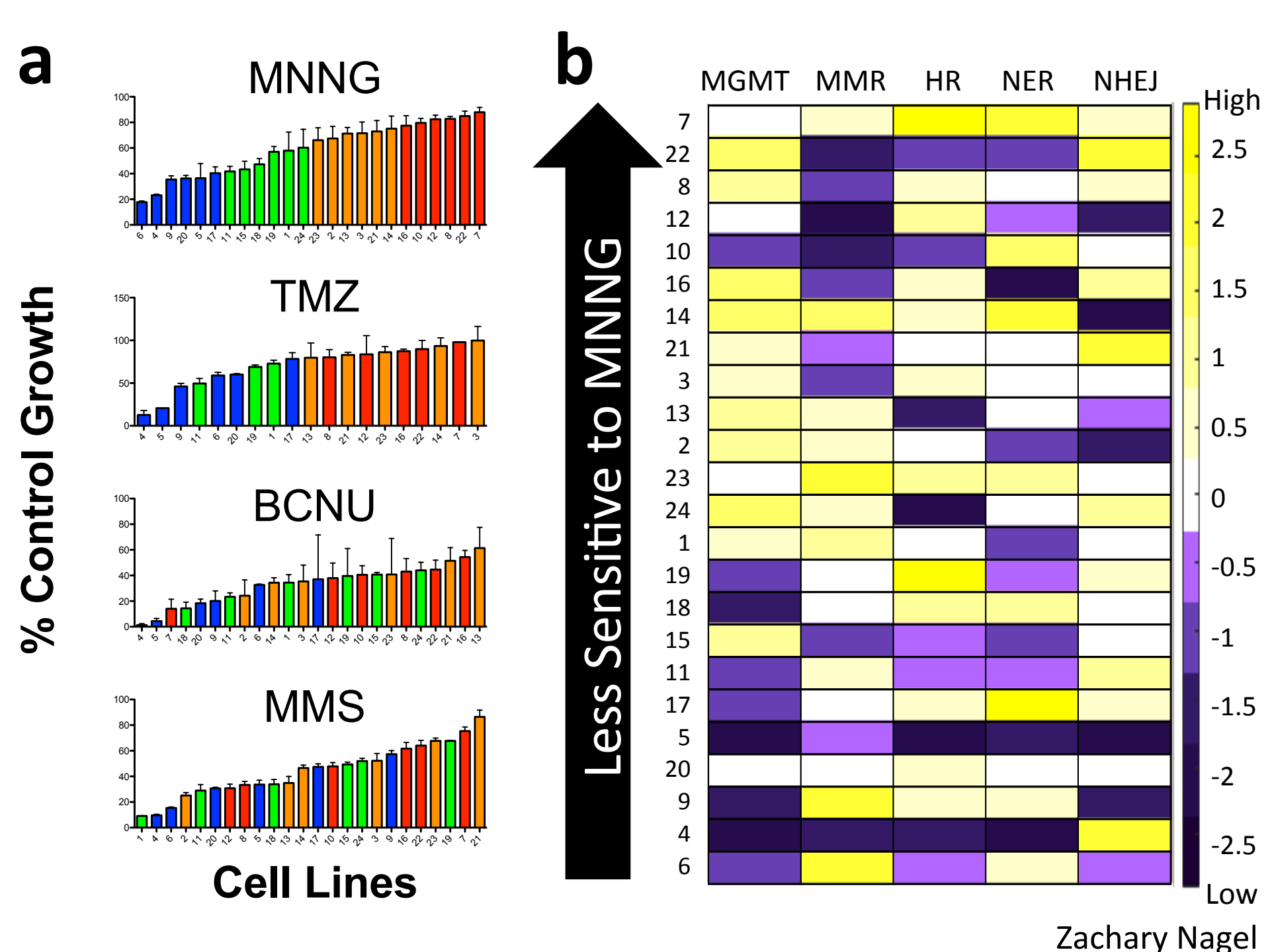
$$+\mathbf{13.3}*MGMT$$

$$+\mathbf{8.1}*HR$$

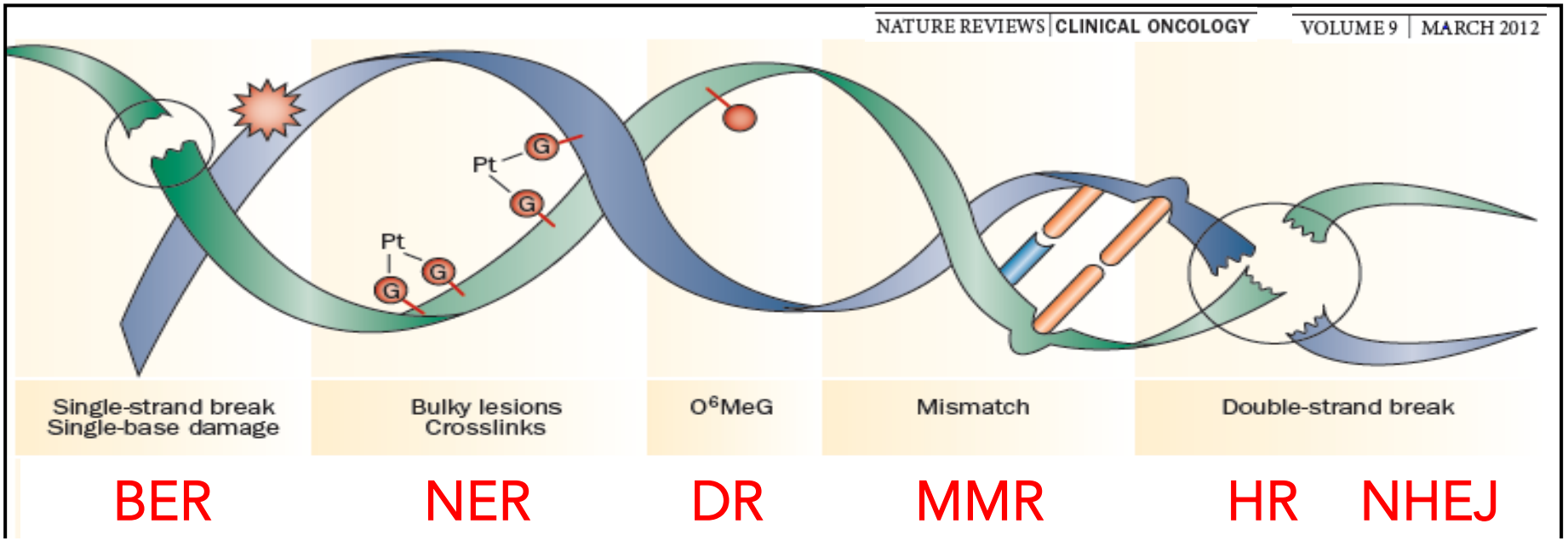
$$-\mathbf{7.0}*MMR$$

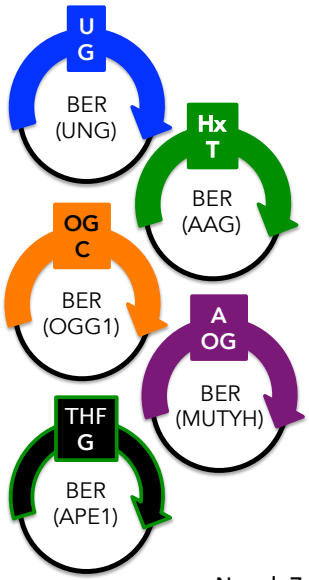
Collaboration with Lauffenburger Lab

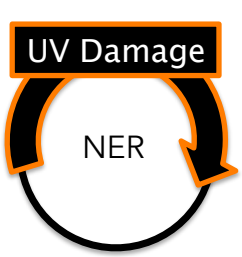
Zachary Nagel

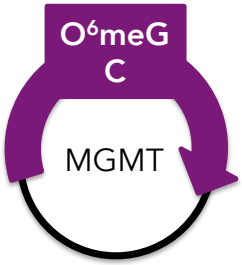

a
MNNG
TMZ
BCNU
MMS
% Control Growth
Cell Lines
b
MGMT
MMR
HR
NER
NHEJ
Less Sensitive to MNNG
7
22
8
12
10
16
14
21
3
13
2
23
24
1
19
18
15
11
17
5
20
9
4
6
High
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
Low
Zachary Nagel

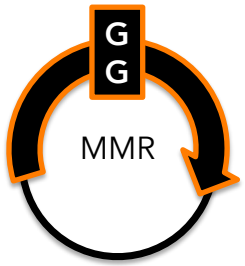
Six Major DNA Repair Pathways

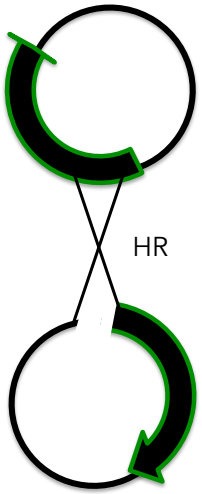


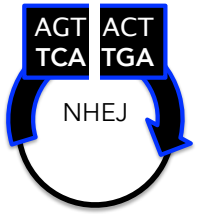












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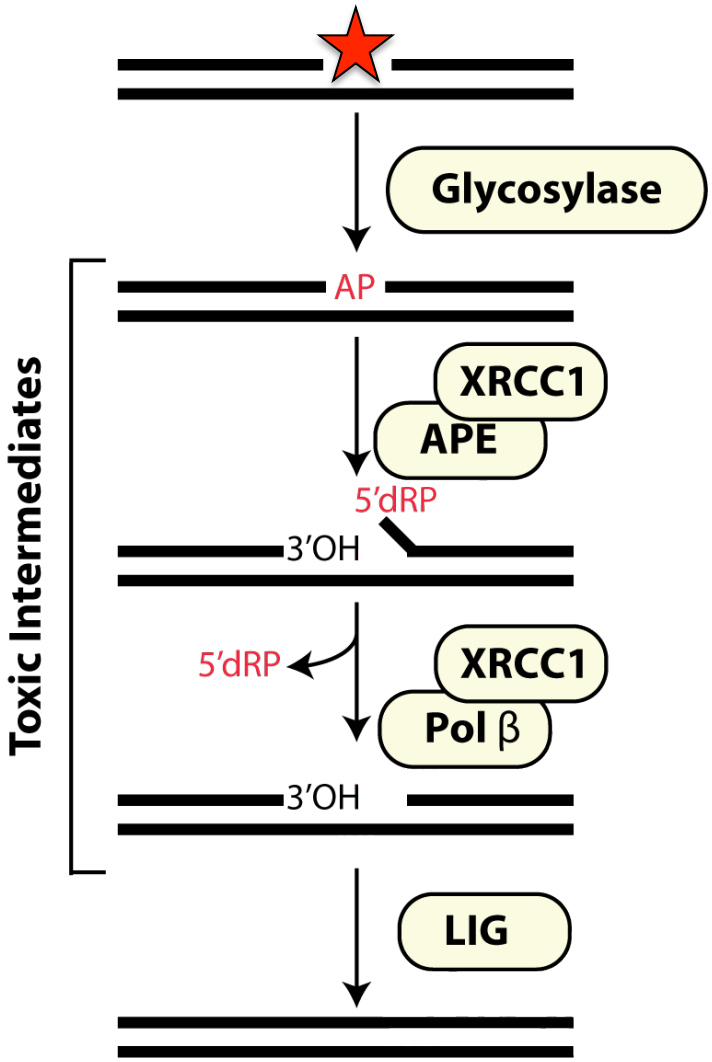
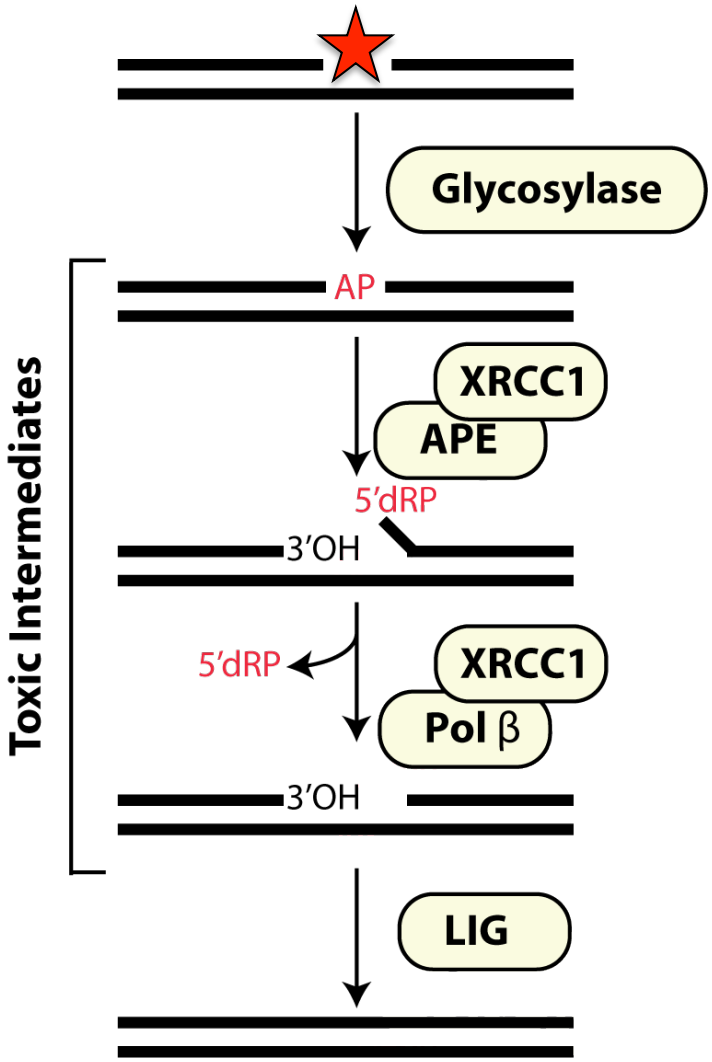
Base Excision Repair (**BER**)



AAG	Alkyladenine DNA glycosylase
MBD4	CpG binding domain protein 4
MUTYH	mutY homologue
OGG1	8-oxoguanine DNA glycosylase
NEIL 1	Nei endonuclease VIII-like 1
NEIL 2	Nei endonuclease VIII-like 2
NEIL 3	Nei endonuclease VIII-like 3
NTHL1	nth endonuclease III-like 1
SMUG1	SS uracil DNA glycosylase 1
TDG	Thymine DNA glycosylase
UNG	Uracil DNA glycosylase

I. Alex Chaim

Base Excision Repair (**BER**)



Colon Cancer

Human Molecular Genetics, 2002, Vol. 11, No. 23 ***2961–2967***

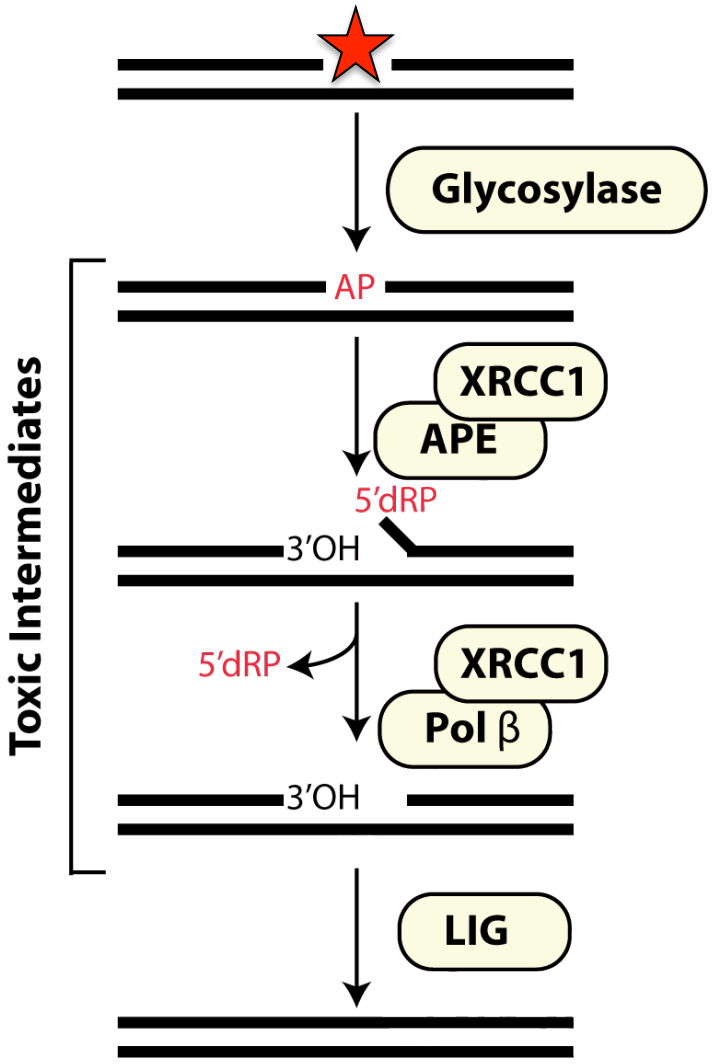
Neurodegeneration

Brain Research 855 (2000) 116–123

Immunodeficiency

NATURE IMMUNOLOGY VOLUME 4 NUMBER 10 OCTOBER 2003

Base Excision Repair (**BER**)



Colon Cancer

Human Molecular Genetics, 2002, Vol. 11, No. 23 ***2961–2967***

Neurodegeneration

Brain Research 855 (2000) 116–123

Immunodeficiency

NATURE IMMUNOLOGY VOLUME 4 NUMBER 10 OCTOBER 2003

Lung Cancer Risk

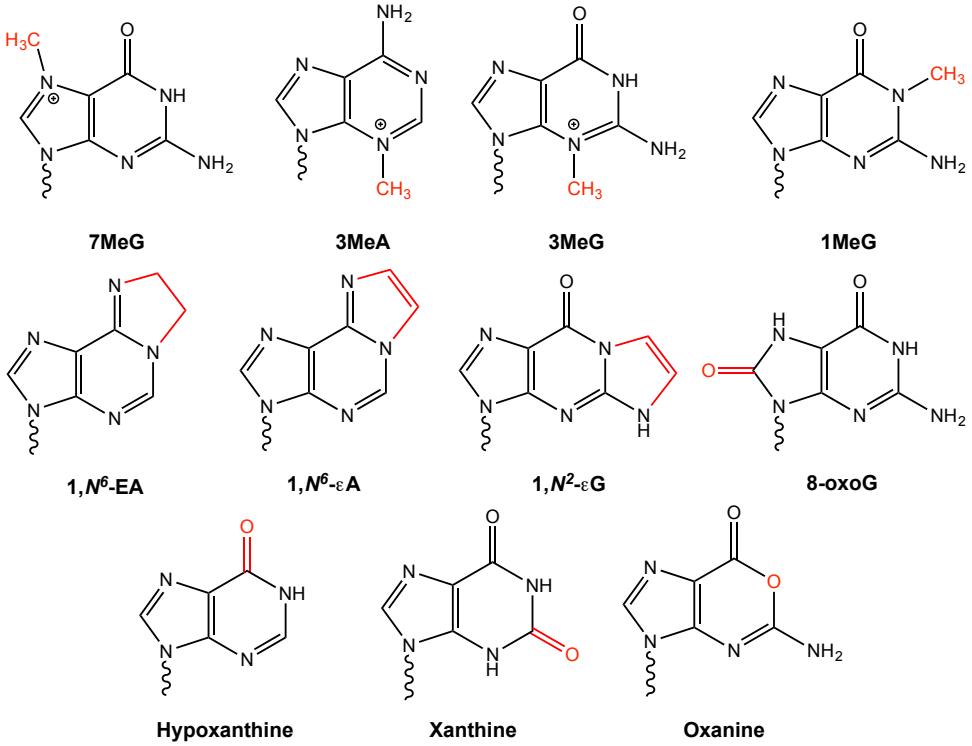
Cancer Prev Res; 7(4) April 2014

Head and Neck Cancer Risk

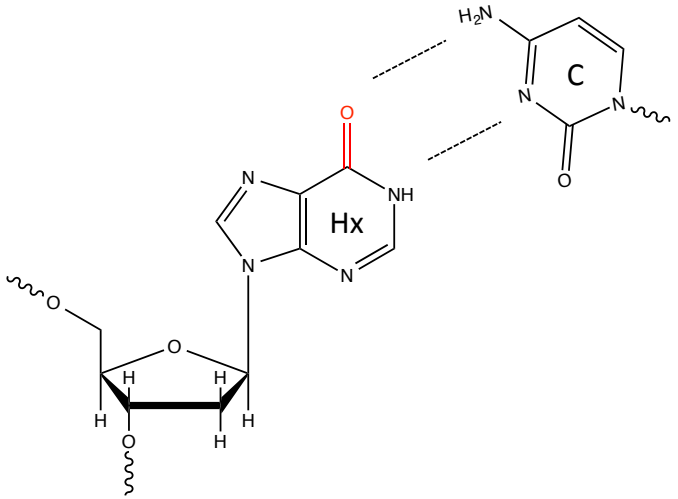
Cancer Res 2006; 66: (24). December 15, 2006

Measuring Alkyladenine DNA Glycosylase (**AAG**/MPG) activity through transcriptional mutagenesis of **Hx**

Broad substrate range

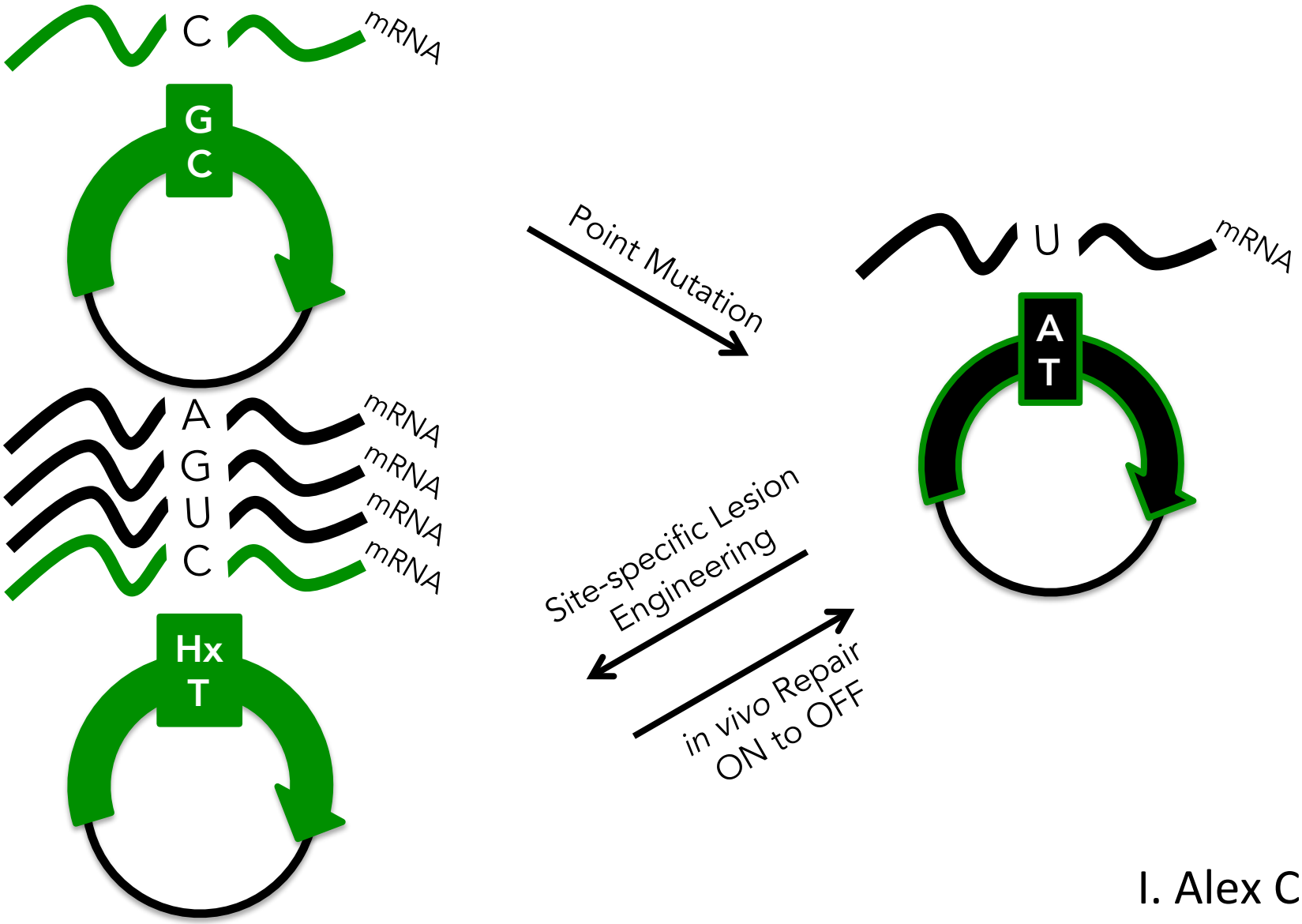


Transcriptional Mutagenesis



I. Alex Chaim

Measuring Alkyladenine DNA Glycosylase (**AAG**/MPG) activity through transcriptional mutagenesis of **Hx**



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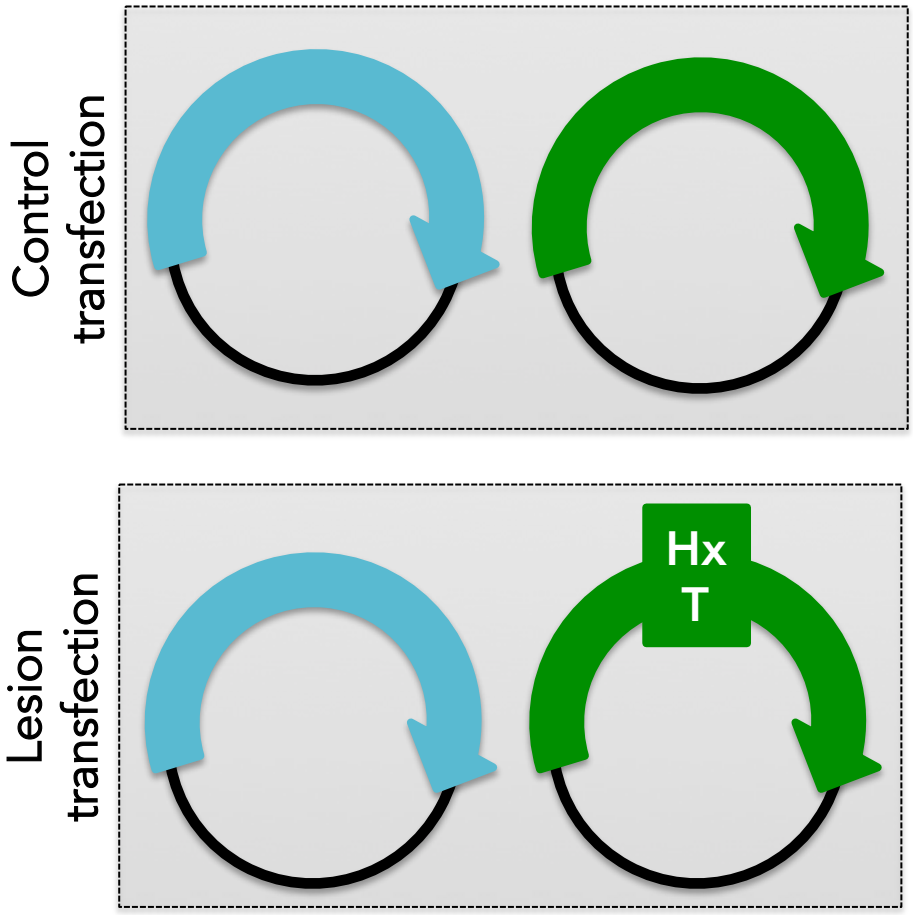
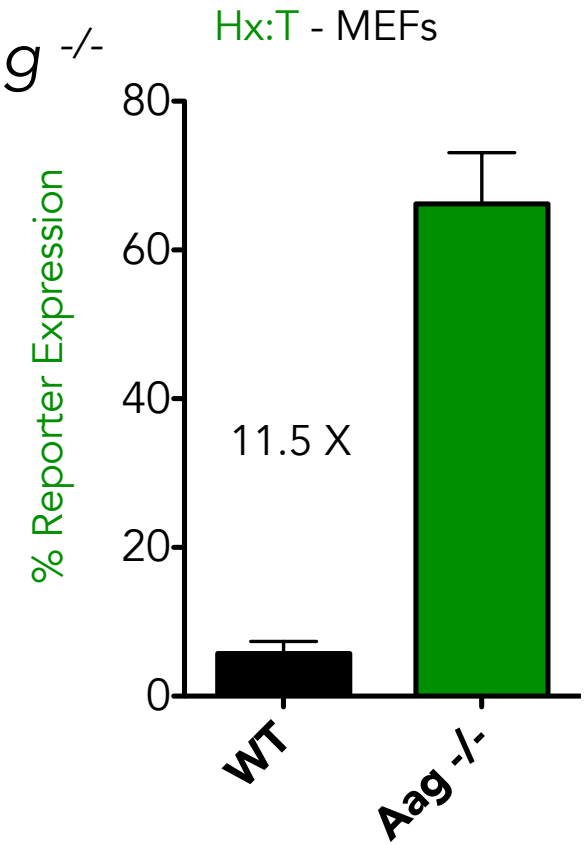
AAG's *in vivo* activity measured through transcriptional mutagenesis of **Hx**

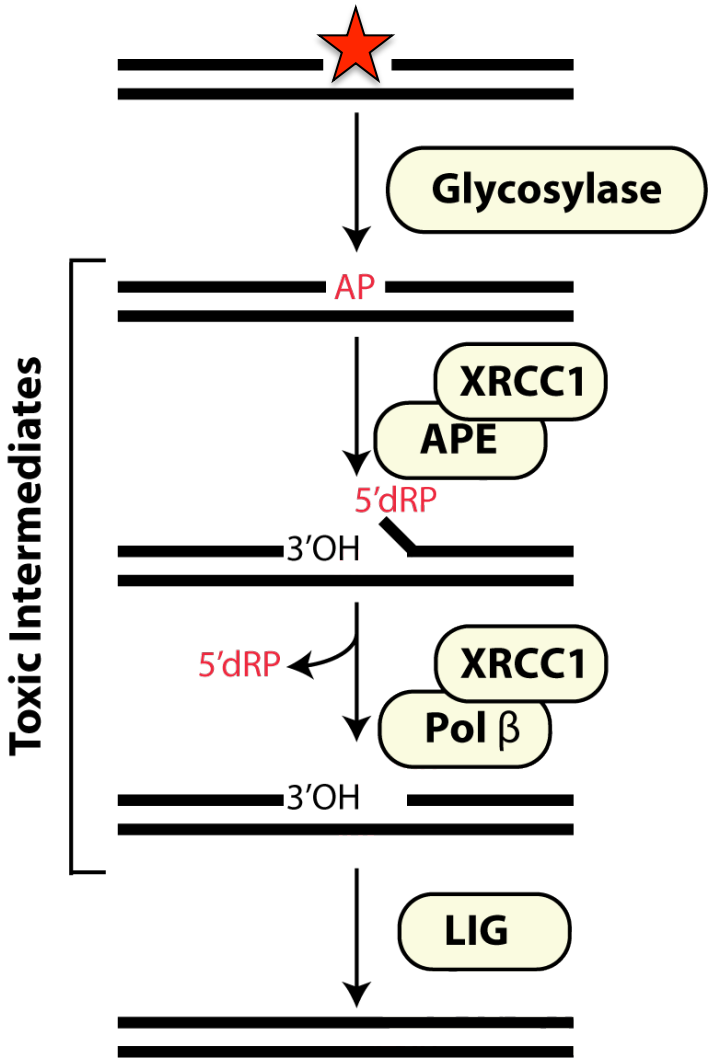


- MEFs
  - WT
  - *Aag* $^{-/-}$



MEFs = Mouse Embryonic Fibroblasts

Base Excision Repair (**BER**)



AAG	Alkyladenine DNA glycosylase
MBD4	CpG binding domain protein 4
MUTYH	mutY homologue
OGG1	8-oxoguanine DNA glycosylase
NEIL 1	Nei endonuclease VIII-like 1
NEIL 2	Nei endonuclease VIII-like 2
NEIL 3	Nei endonuclease VIII-like 3
NTHL1	nth endonuclease III-like 1
SMUG1	SS uracil DNA glycosylase 1
TDG	Thymine DNA glycosylase
UNG	Alkyladenine DNA glycosylase

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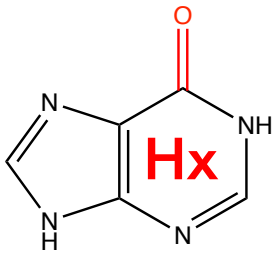
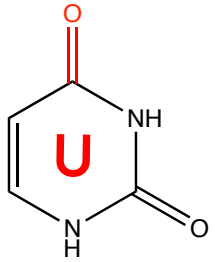
BER Substrates

AAG	MBD4	MUTYH	OGG1	SMUG1	UNG
NEIL 1	NEIL 2	NEIL 3	NTHL1	TDG	

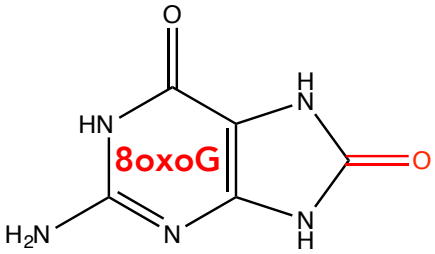
Hypoxanthine



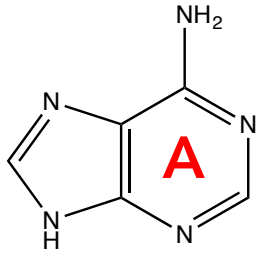
Uracil



8oxoG



A:8oxoG



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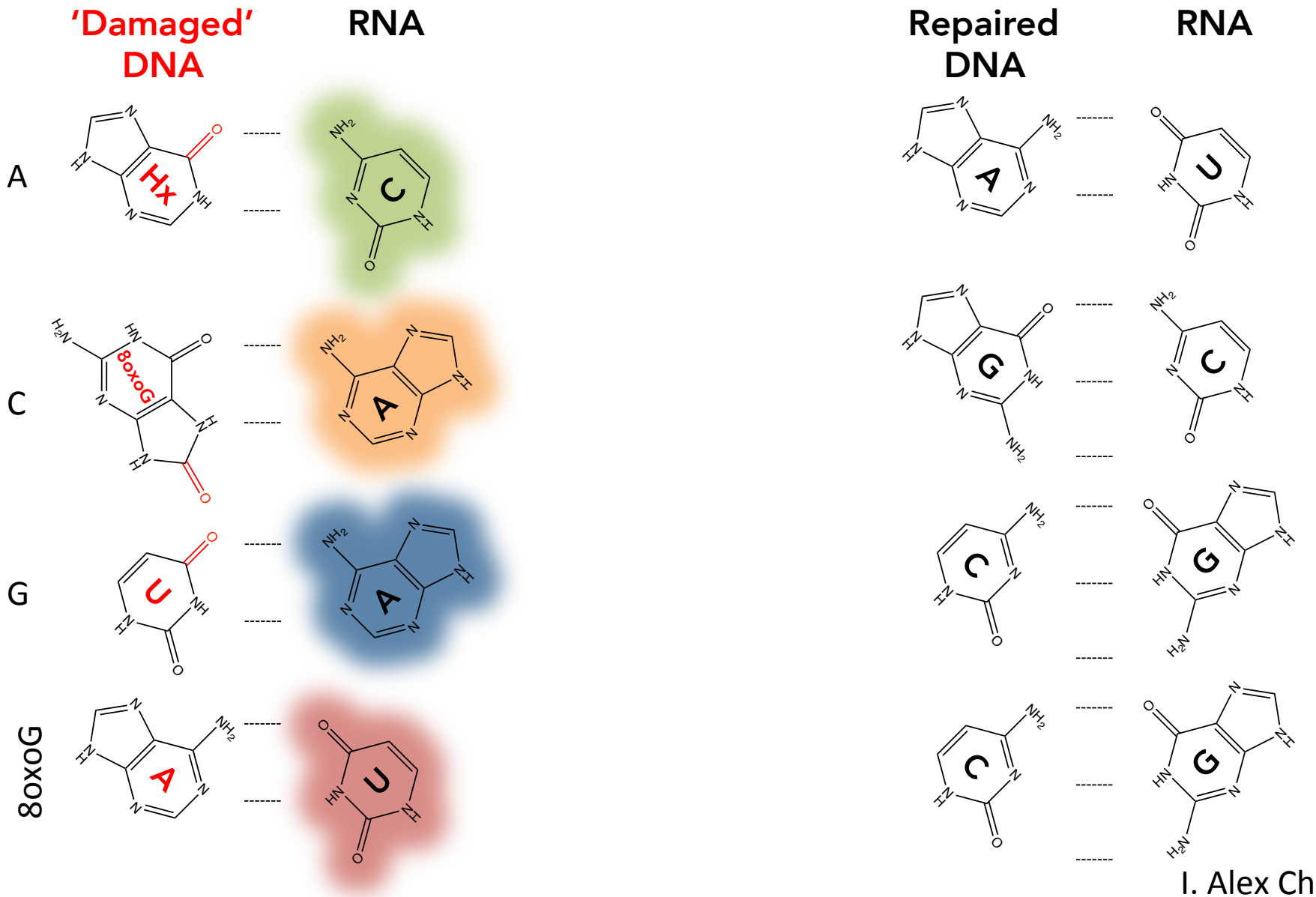
BER Substrate Reporters



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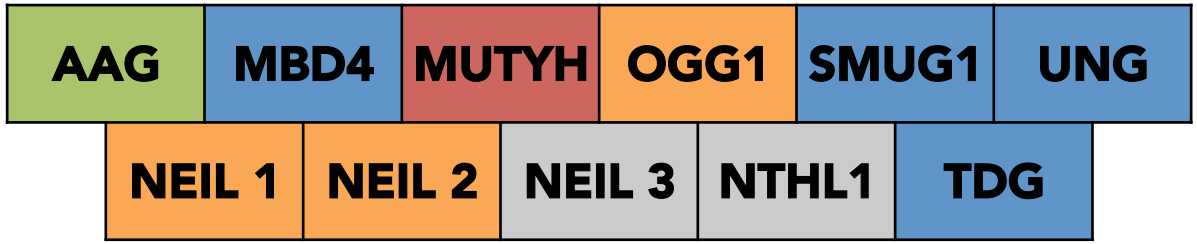
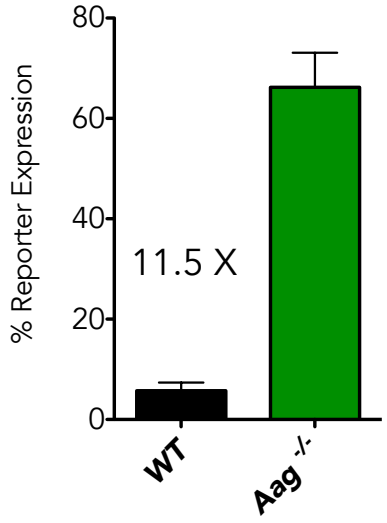
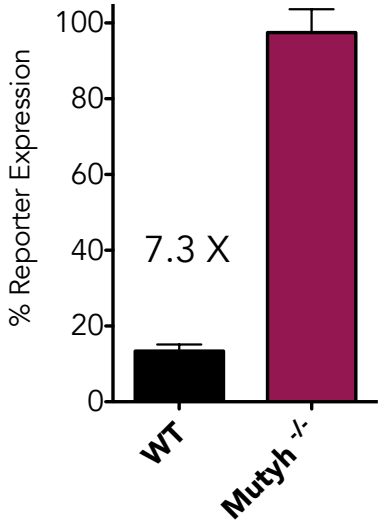
BER Substrates Reporters



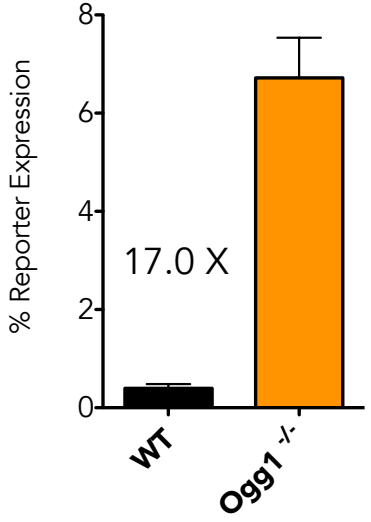
Hx:T - MEFs



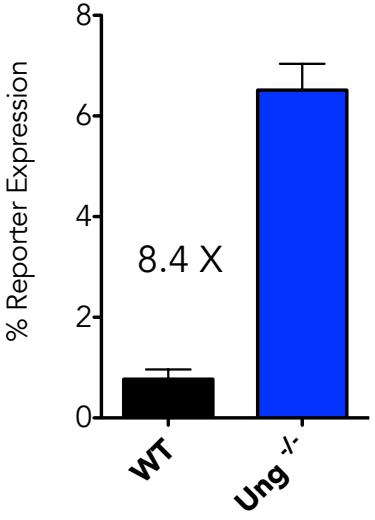
A:8oxoG - MEFs



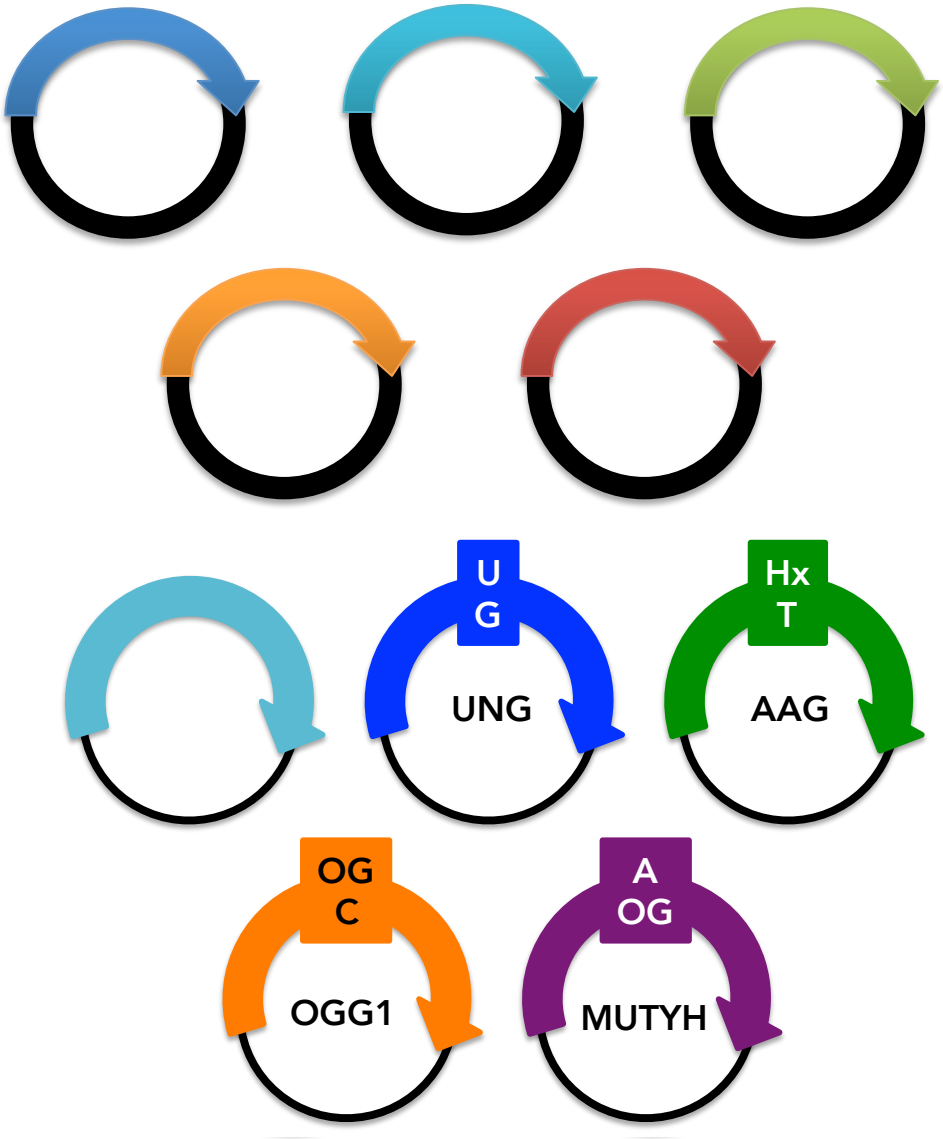
8oxoG:C - MEFs

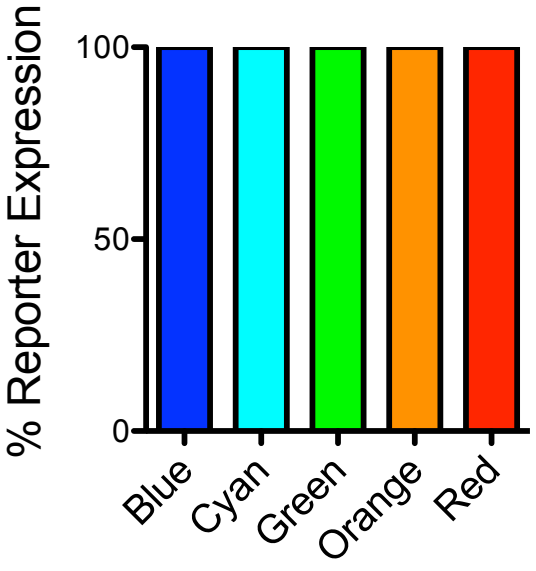


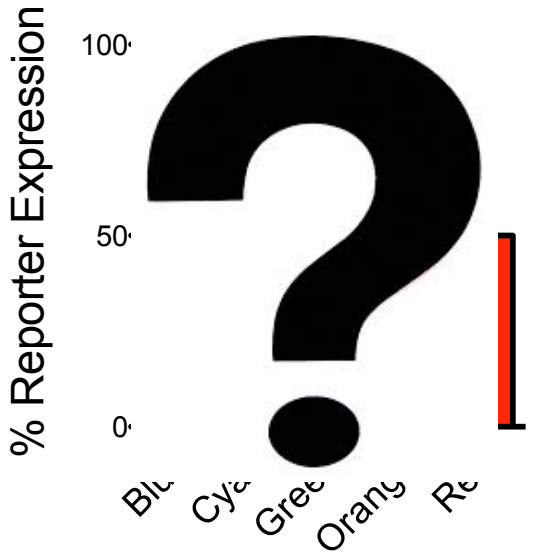
U:G - MEFs



5-color Host Cell Reactivation Assay to report on Glycosylase Activity

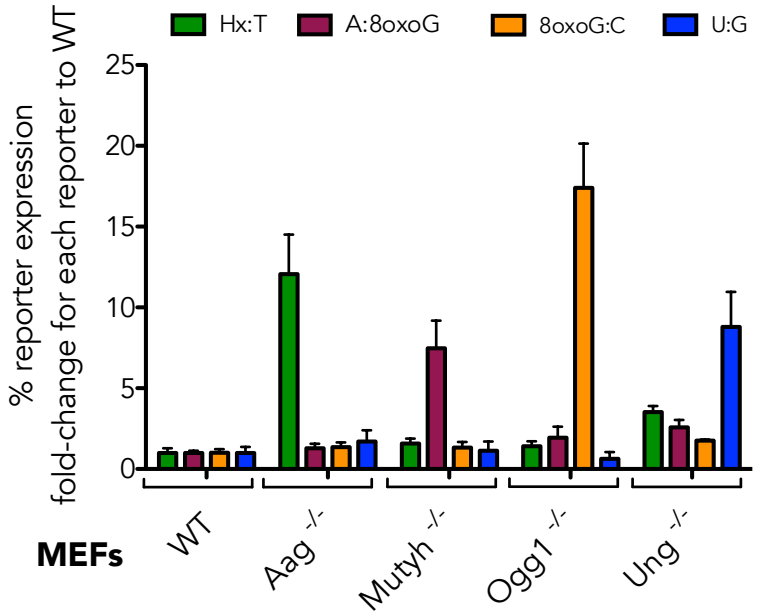


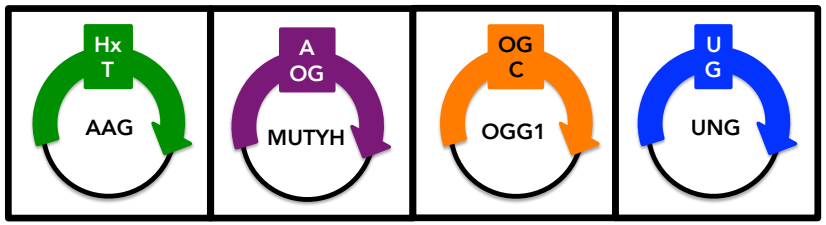




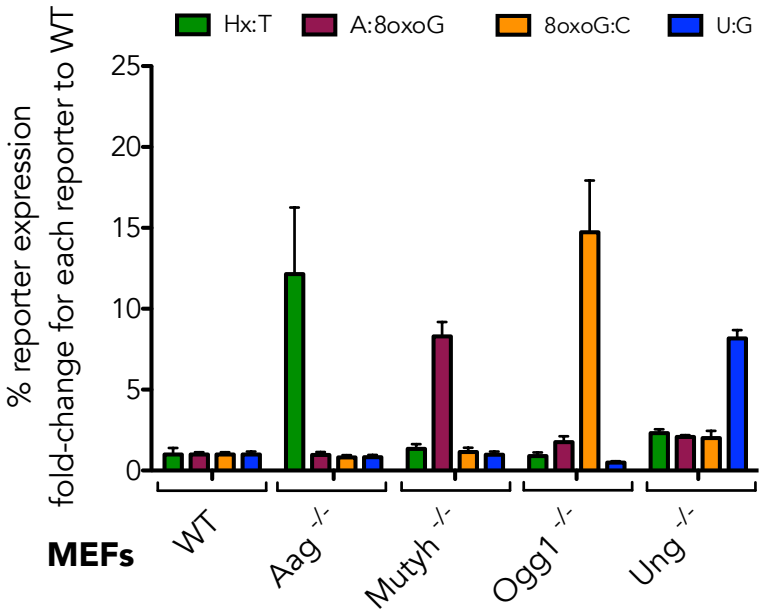
Single *versus* **Multiplexed** Assay

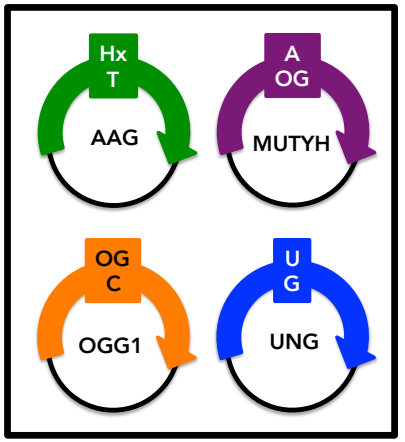
Plasmids - *Separate*



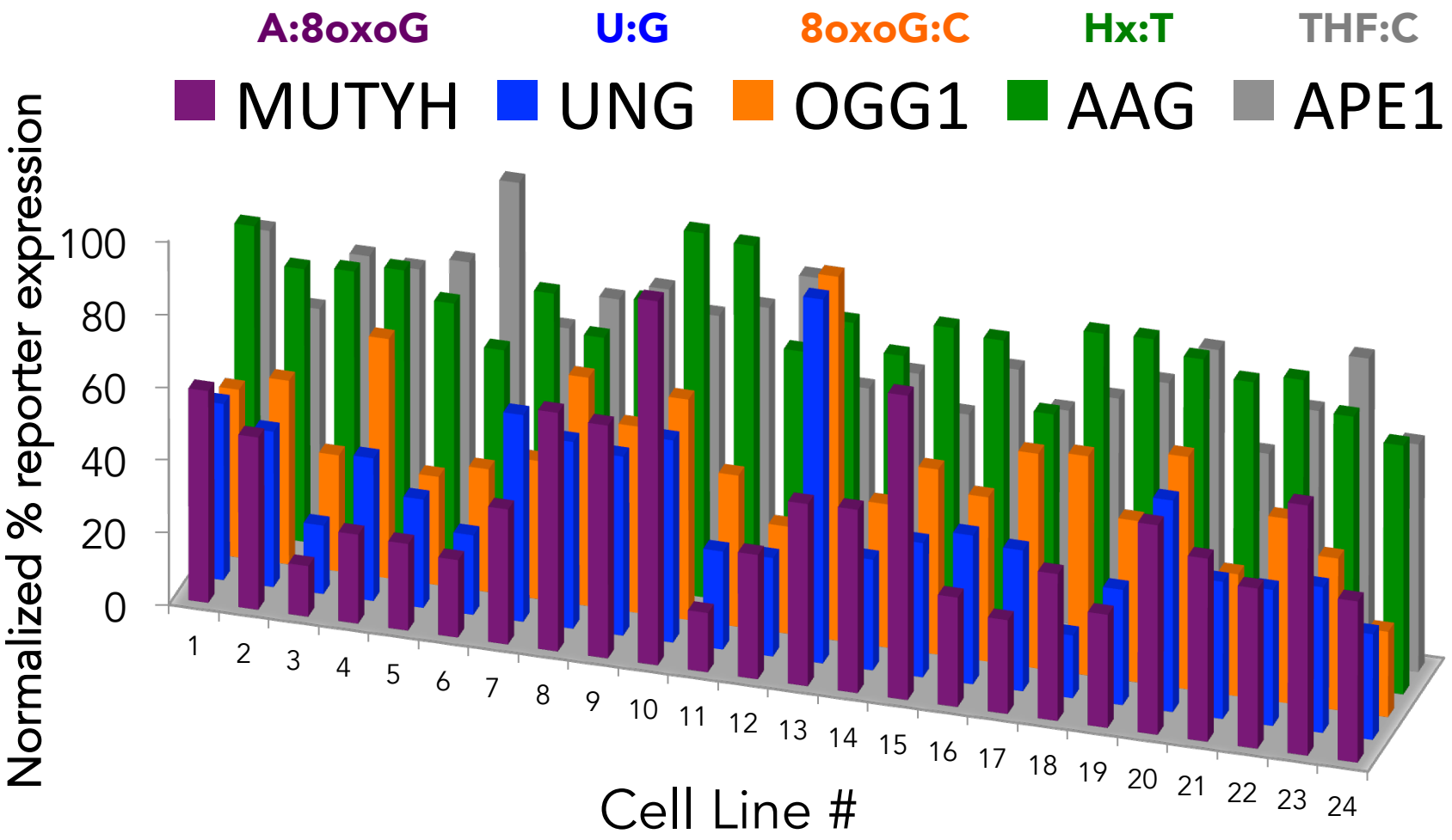


Plasmids - *Together*





BER capacity of a panel of lymphoblastoid cell lines derived from healthy individuals



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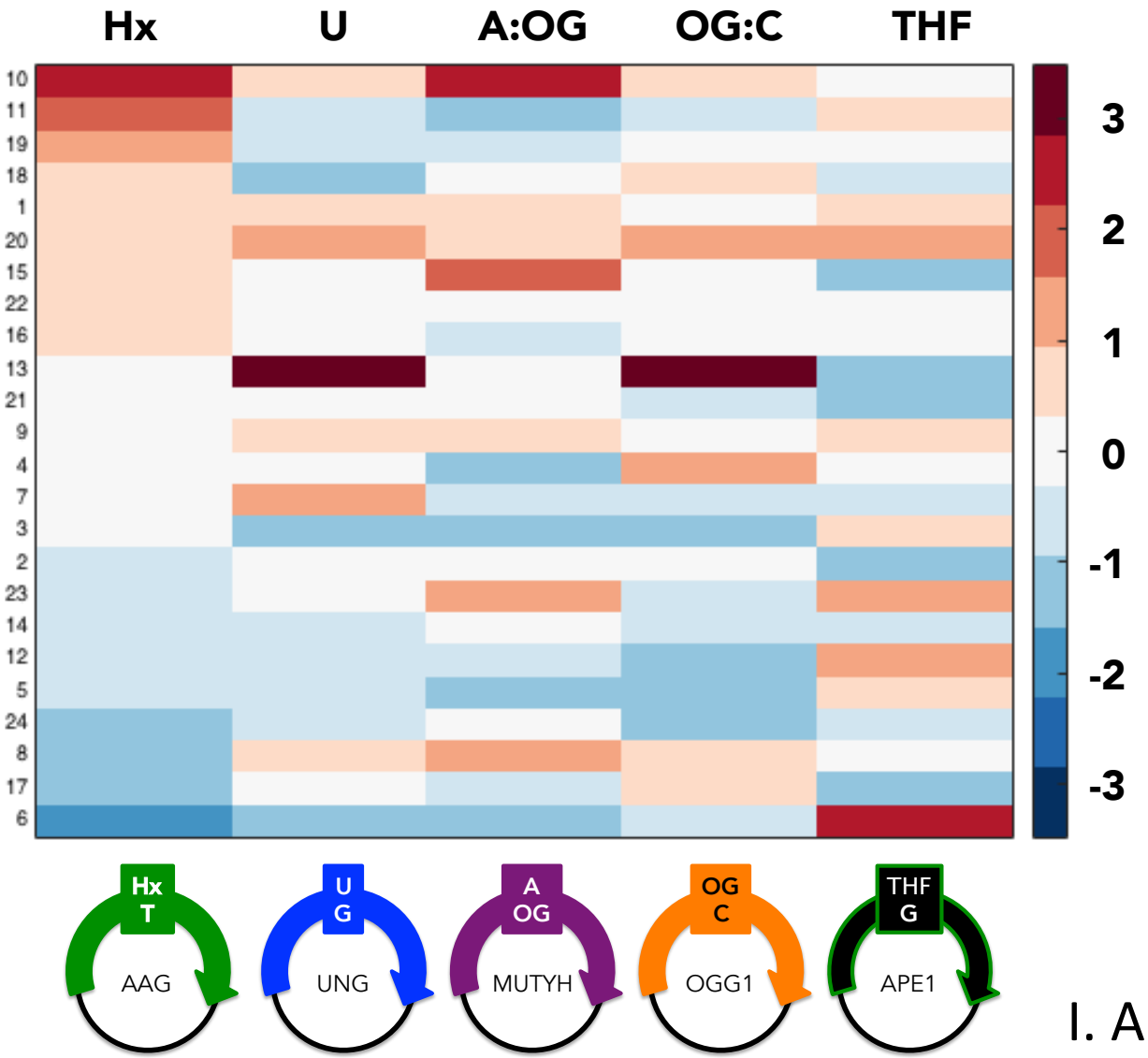
BER capacity of a panel of lymphoblastoids derived from healthy individuals



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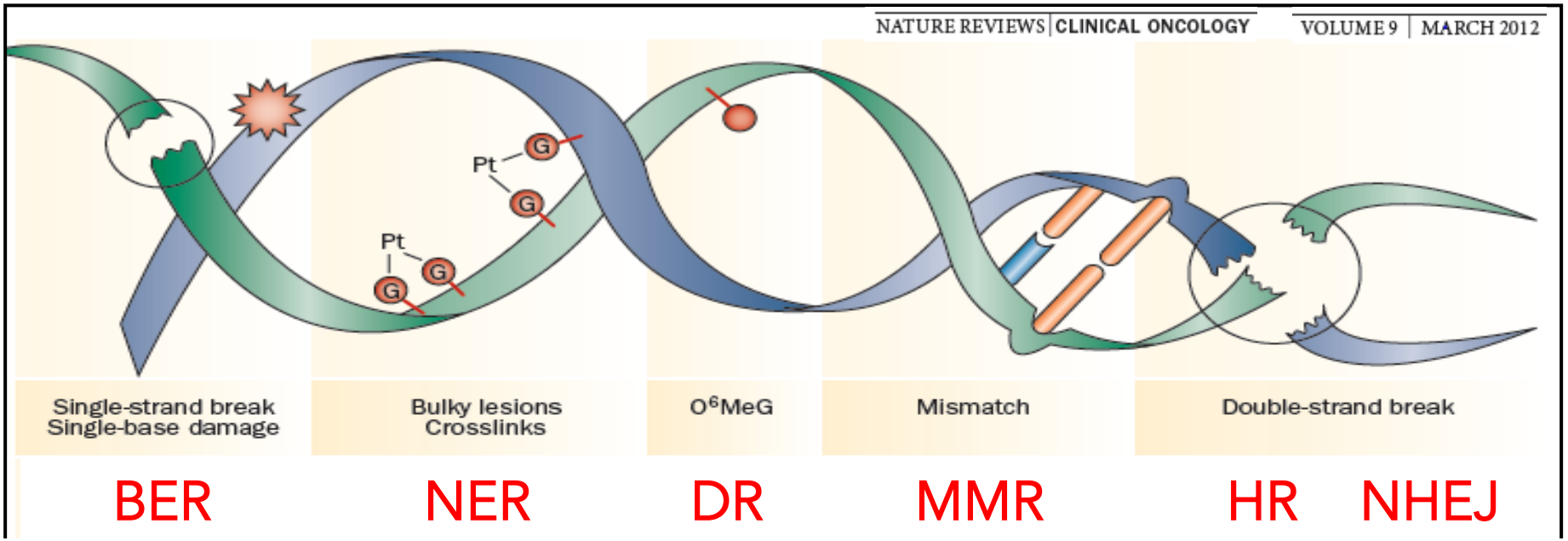
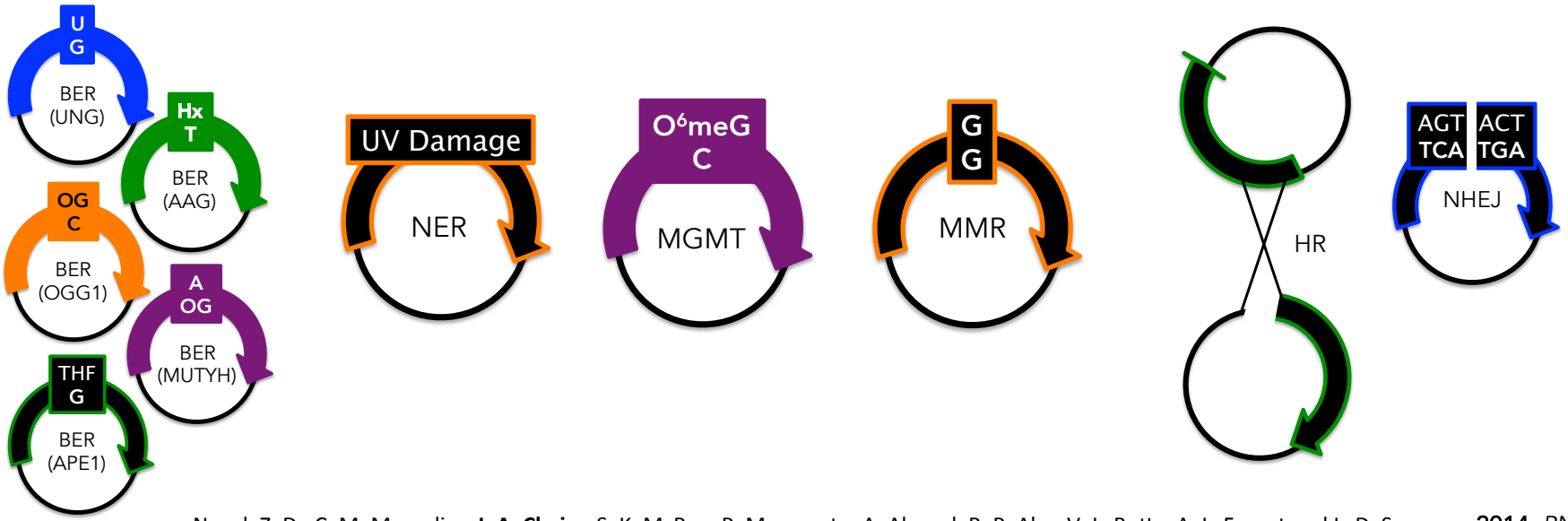
Six Major DNA Repair Pathways

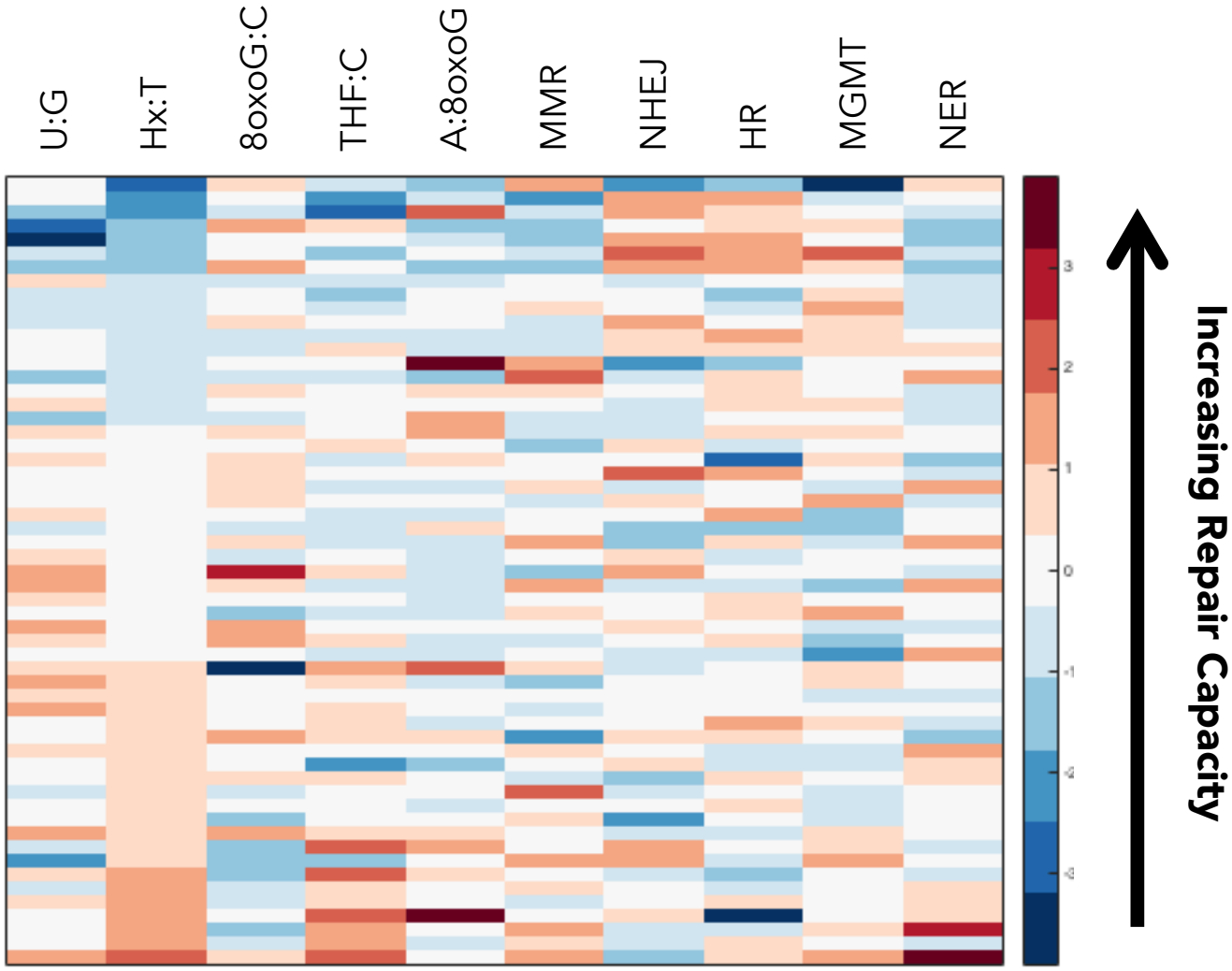


BER NER DR MMR HR NHEJ



Nagel, Z. D., C. M. Margulies, **I. A. Chaim**, S. K. McRee, P. Mazzucato, A. Ahmad, R. P. Abo, V. L. Butty, A. L. Forget and L. D. Samson ; 2014; *PNAS*

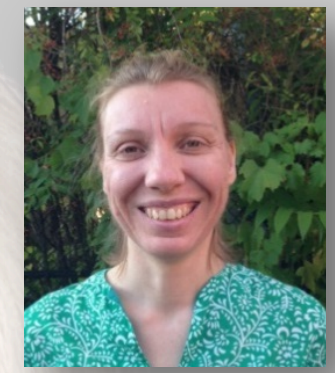
Inter-individual variability in DNA Repair Capacity



The Pioneer Team

Isaac (Alex) Chaim

Dr. Zachary Nagel



Patrizia Mazzucato

Carrie Margulies

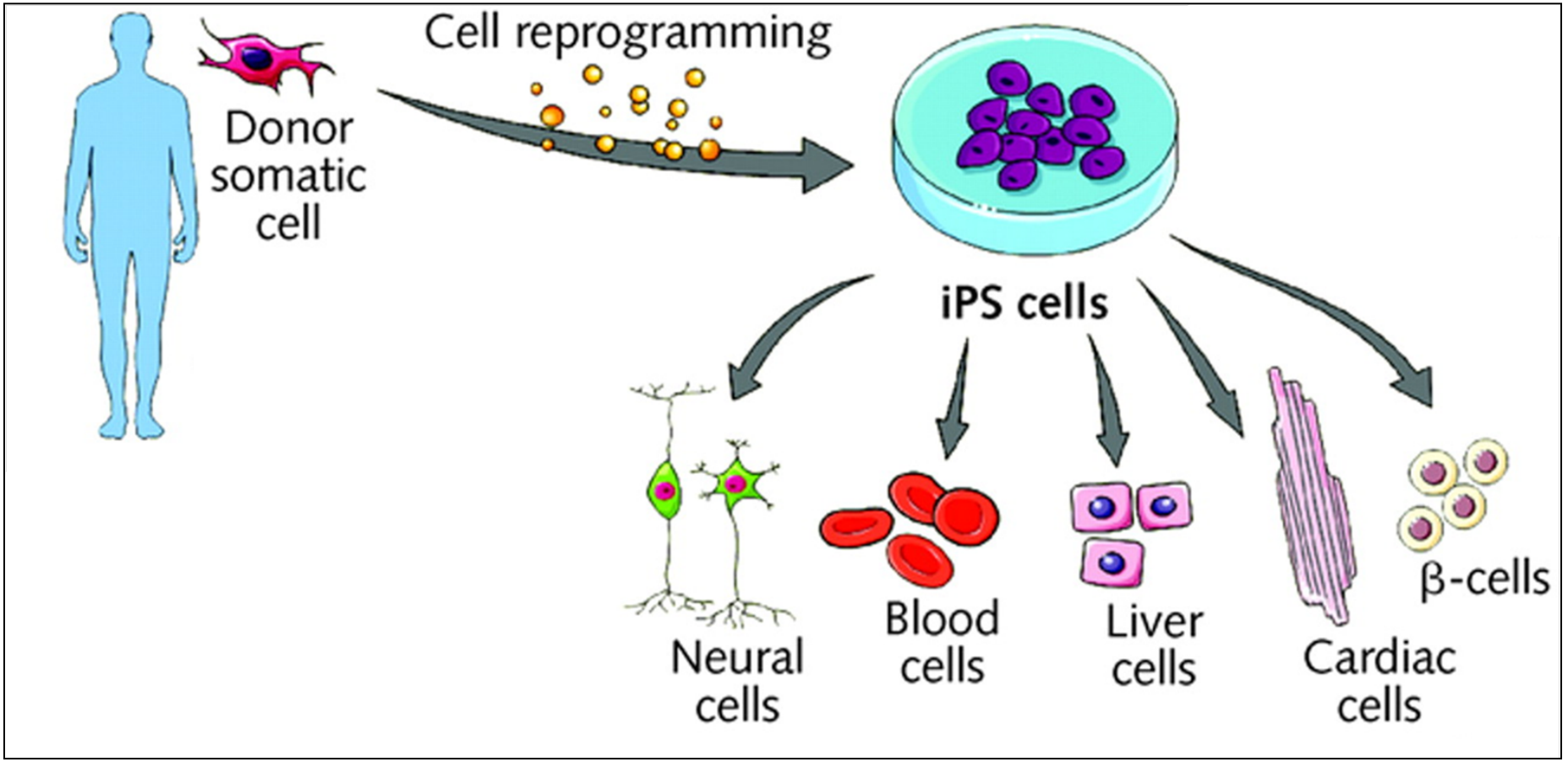
Dr. Anwaar Ahmad

Siobhan McRee

Dr. Anthony Forget

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

Ultimately measure DRC in many different cell types – ideally derived from each individual



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