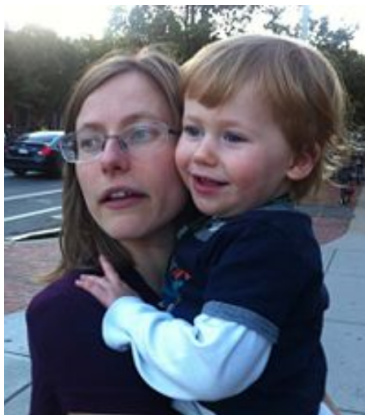
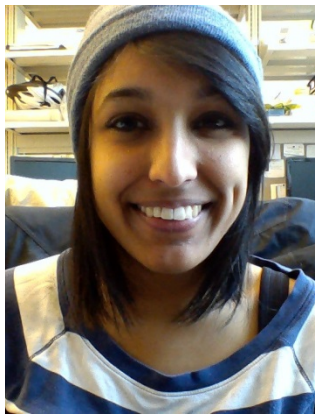
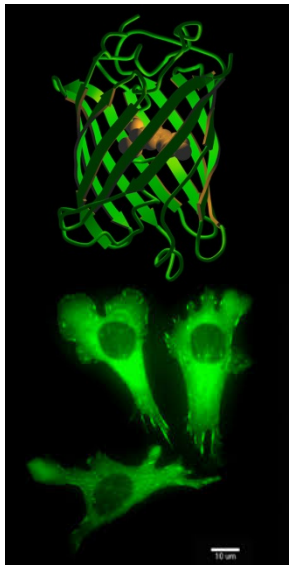
20.109 Spring 2014 Mod 2 – Lecture 6
System Engineering and Protein Foundations



Agi Stachowiak
Shannon Hughes
Aneesh Ramaswamy
Suhani Vora (TA)
Leona Samson (Lectures)
Zachary Nagel (help with development)

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

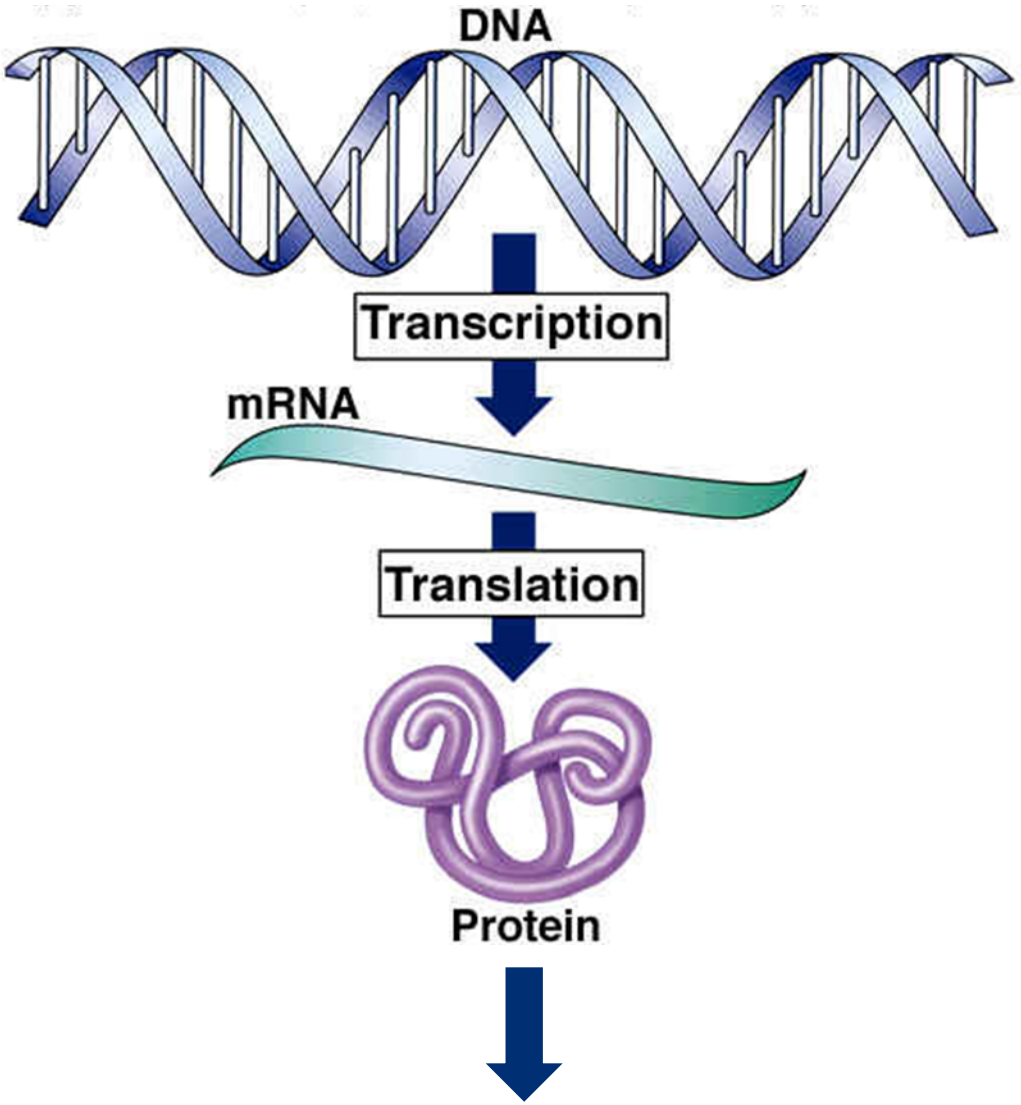
Biology Department

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

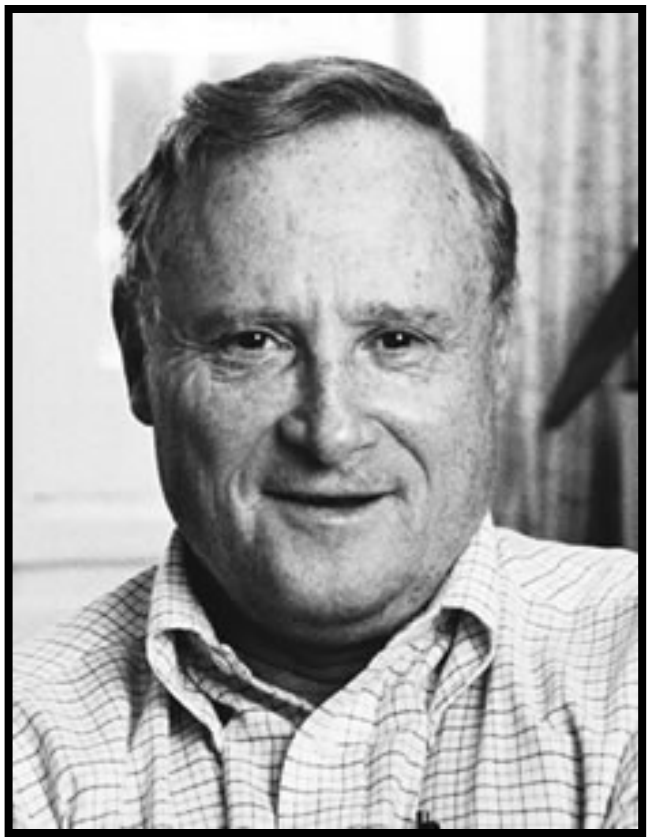
Proteomic Analyses

In vitro / In vivo

FUNCTION

Functional Assays

The Proposal was based on the Pioneering work of:

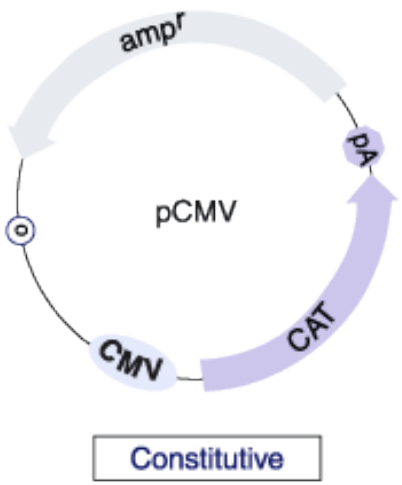


Dr. Lawrence Grossman
(1924–2006)

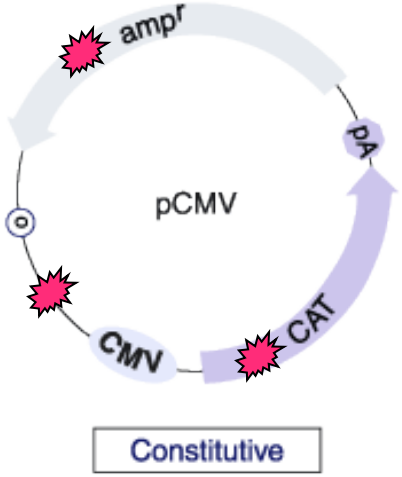
Dr. Qingyi Wei

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

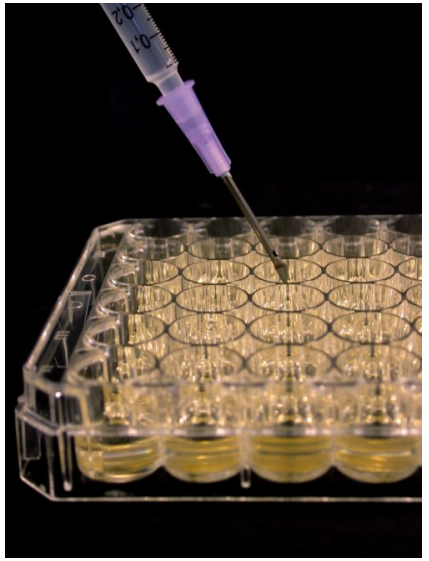
Athas & GROSSMAN
Cancer Res. 1991

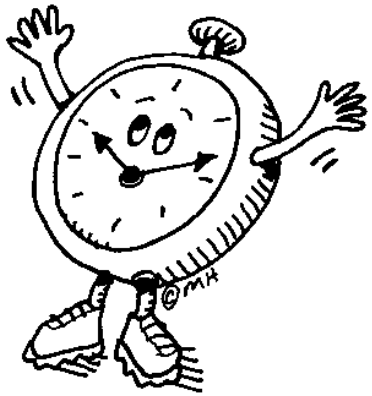


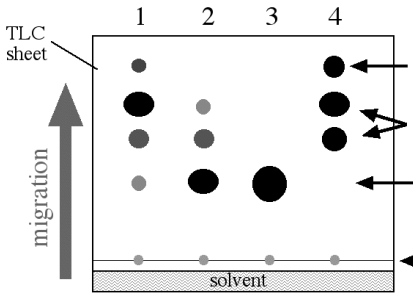
+ UV light



Transient transfection peripheral blood lymphocytes

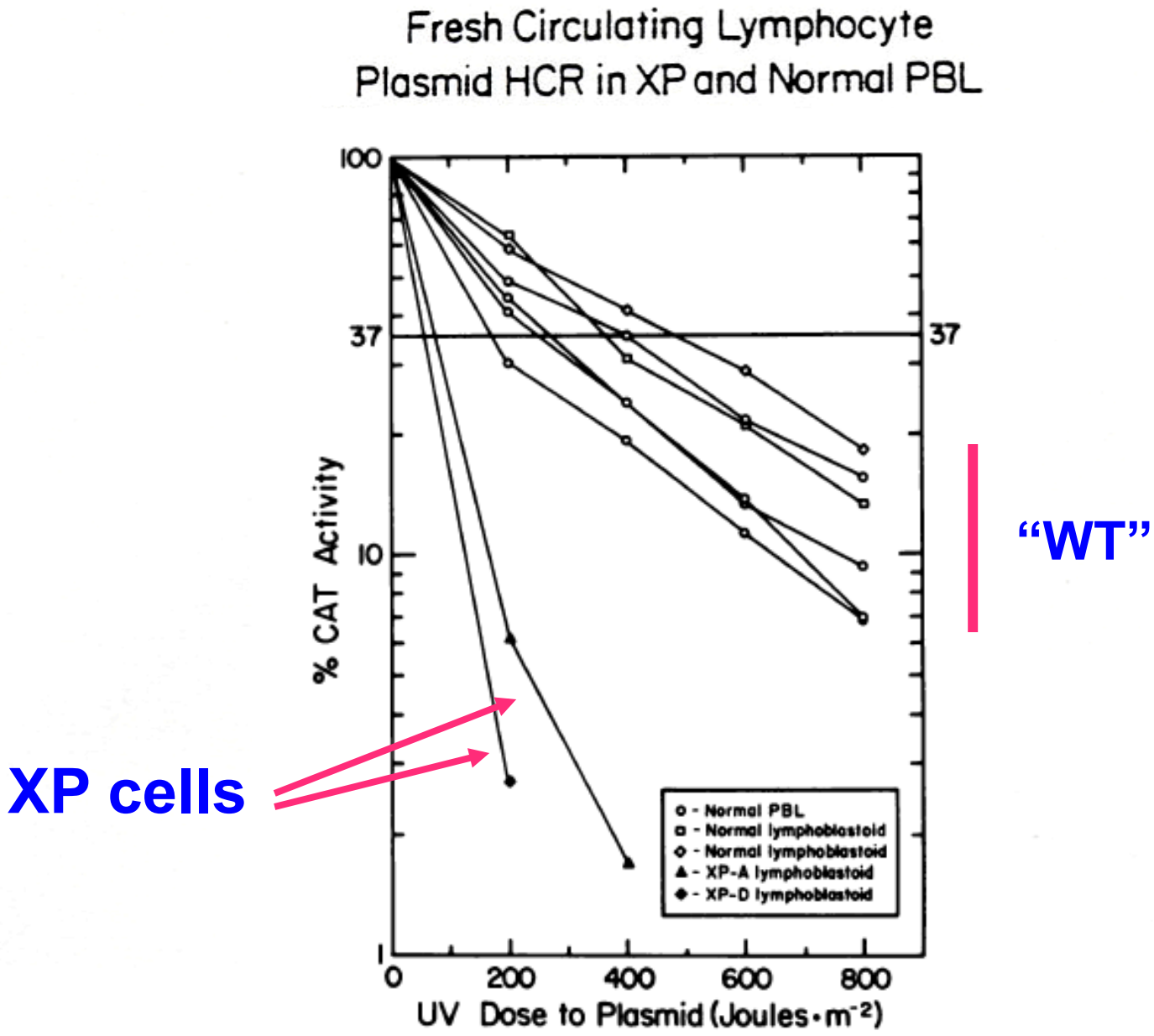






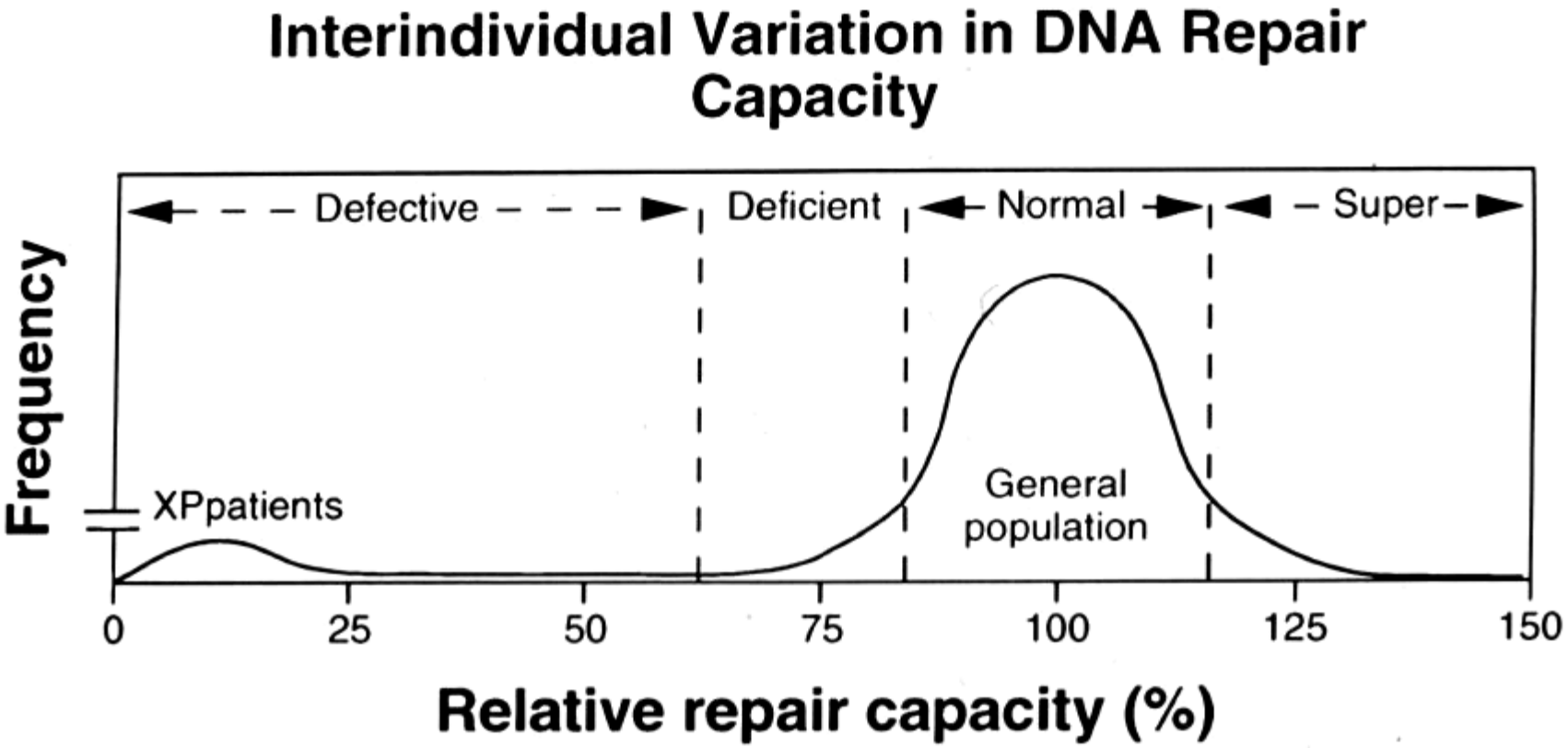
Time to repair

CAT Assay



Athas & GROSSMAN

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

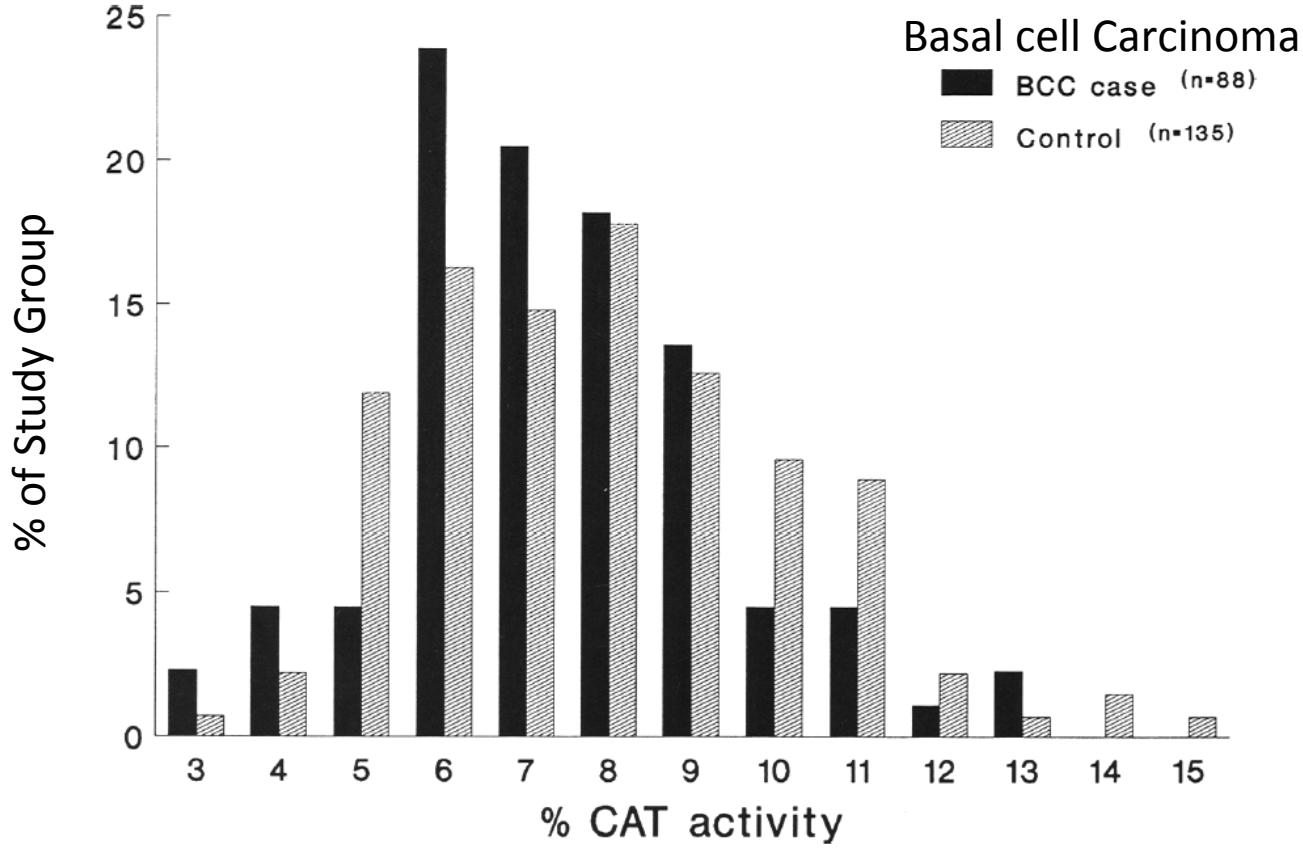


Adapted from **GROSSMAN and Wei (1995)** Clinical Chem 41: 1854-1863

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

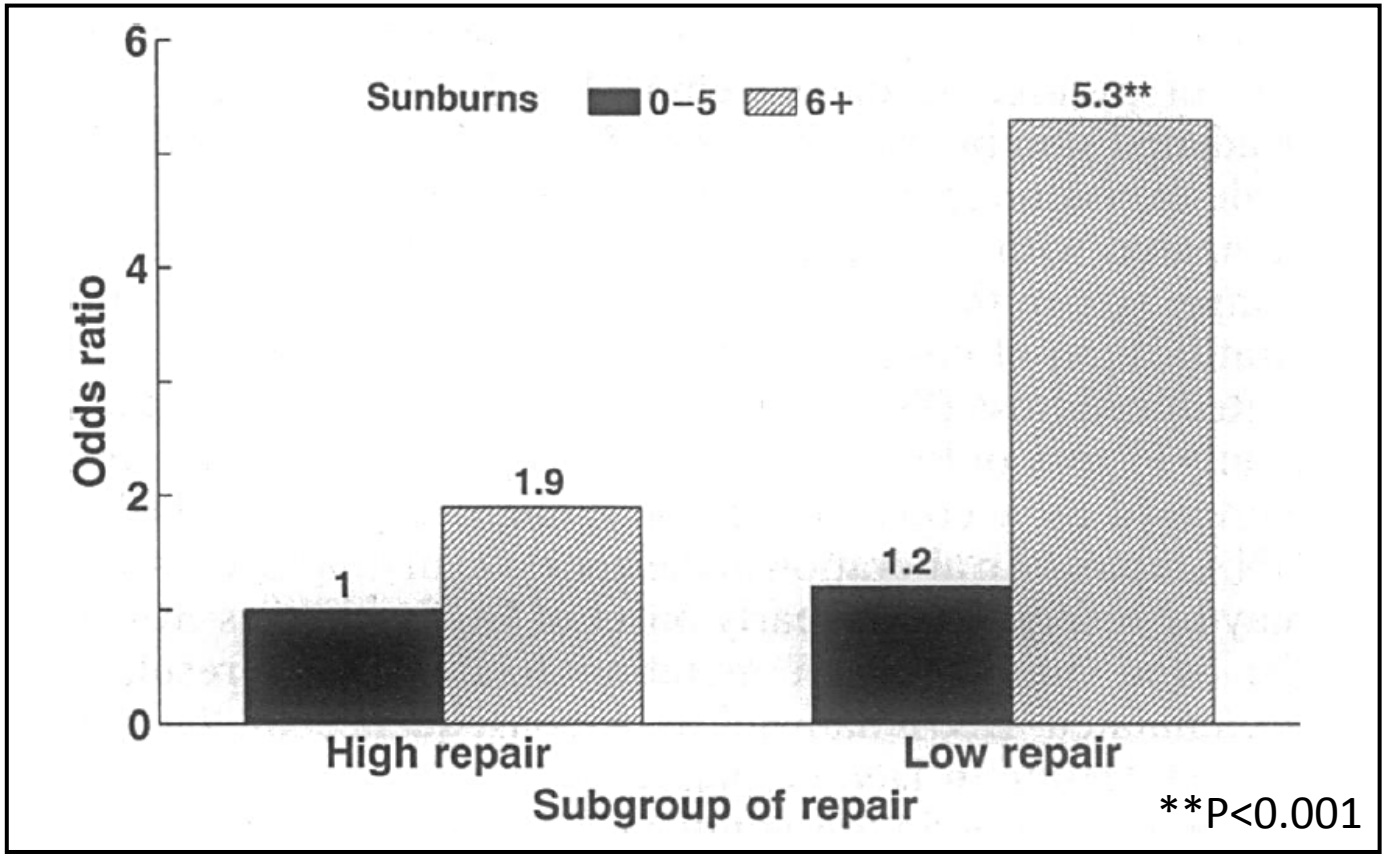


LOW Repair HIGH Repair

[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.

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

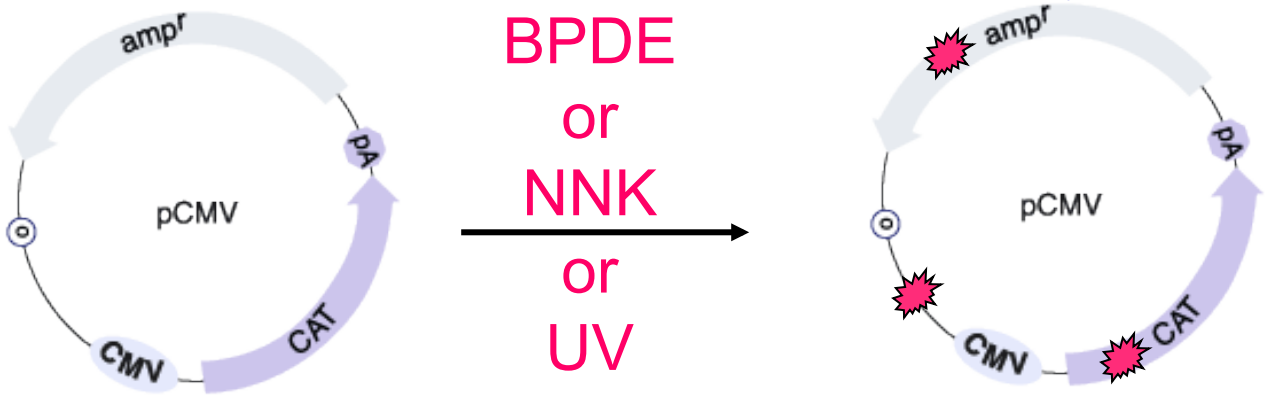


TABLE III – HCR-DRC FOR RISK OF CANCERS

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)

CANDIDATE INTERVIEW

June 16th 2009, 8am!

Developing Novel Methods to Measure DNA Repair Capacity in Human Populations

Leona D. Samson

MIT

Biological Engineering Department

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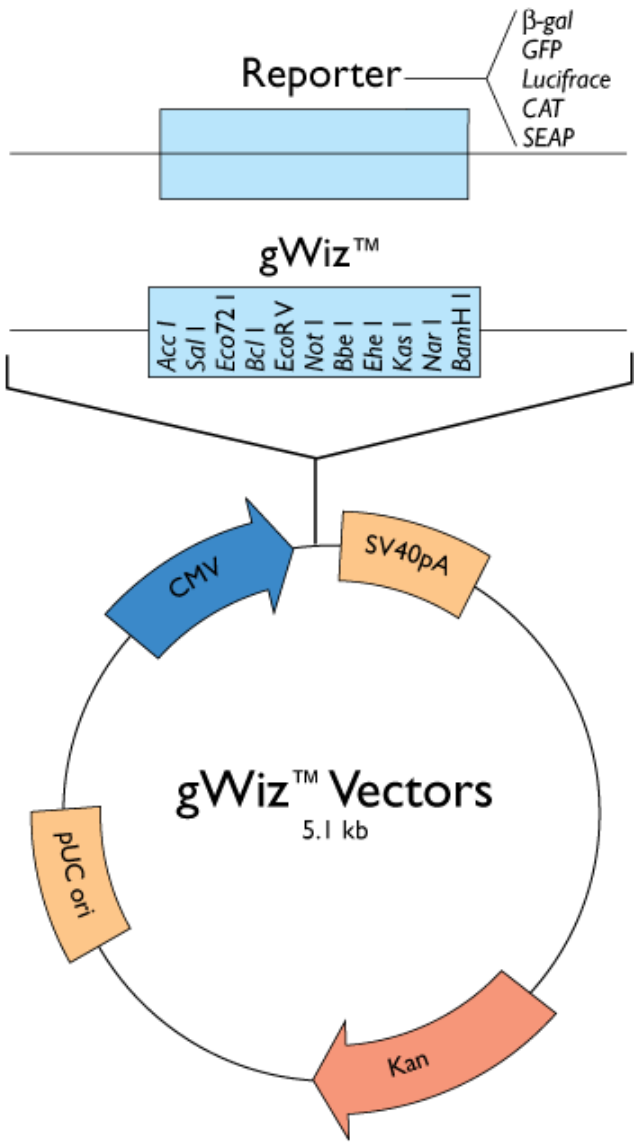
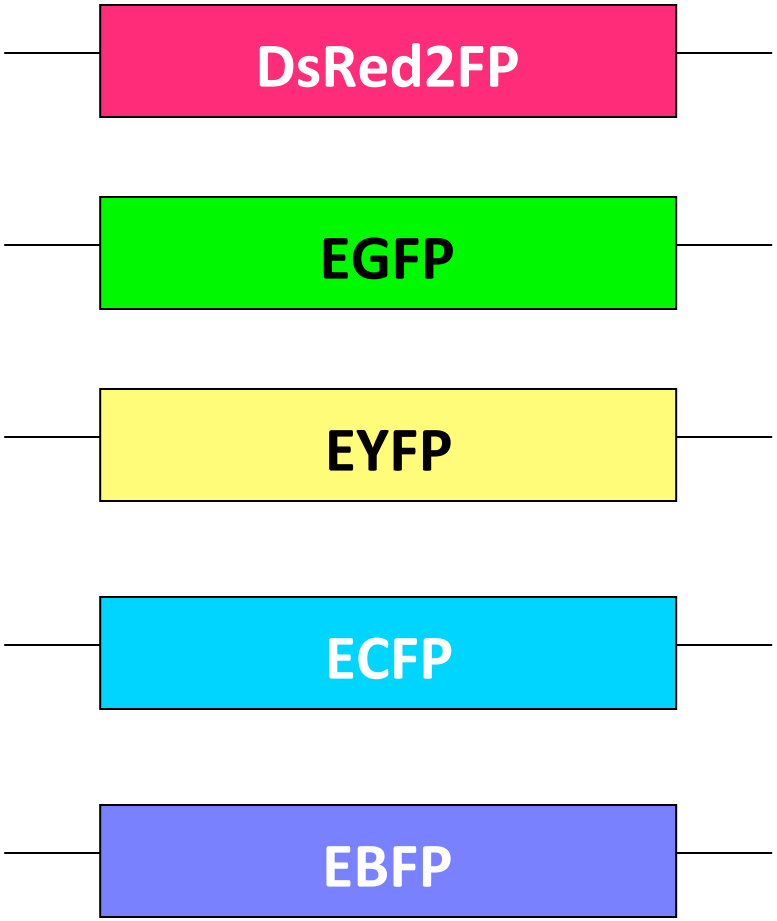
Broad Institute (Harvard and MIT)

Reactivation of damaged DNA - multiplexed

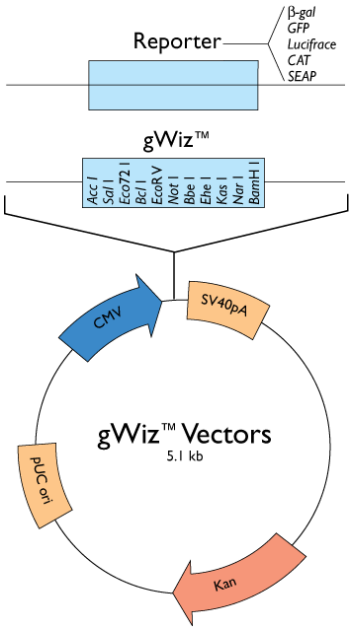


http://www.genlantis.com/corp/images/gWiz_vectmap.gif

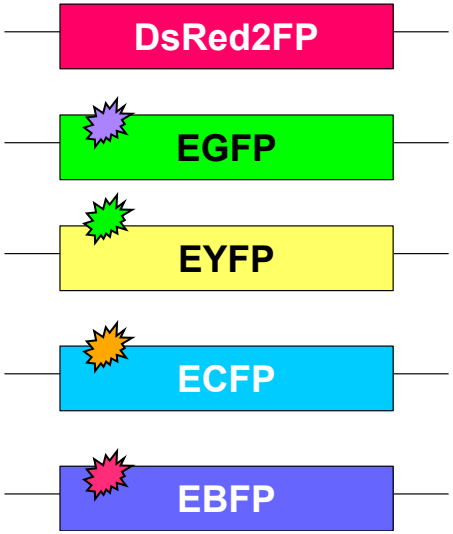
Each Fluorescent Protein gene will harbor a different type of DNA damage



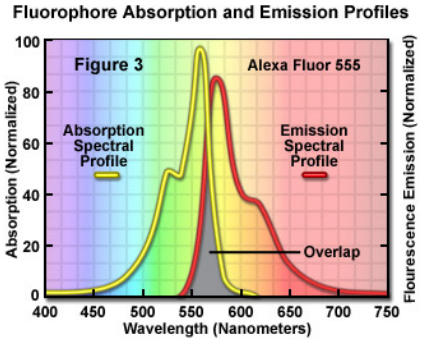
Reactivation of damaged DNA - multiplexed



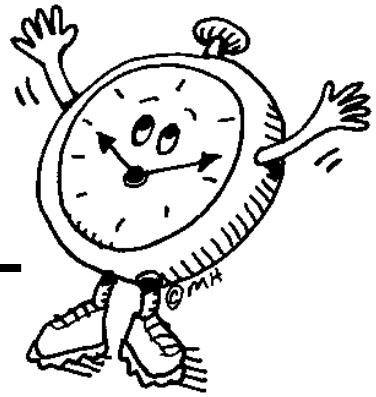
+ different DNA lesions



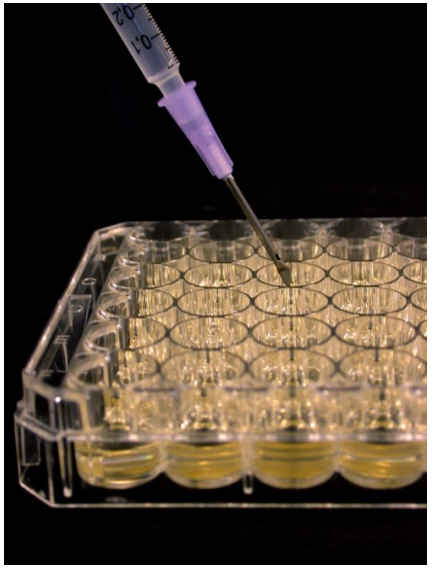
Transient transfection of mixture



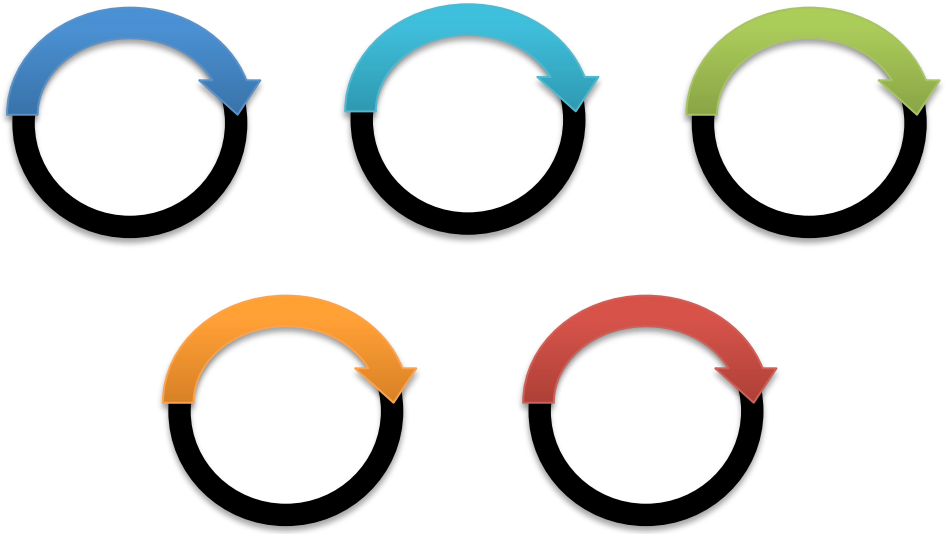
Fluorescence quantitation

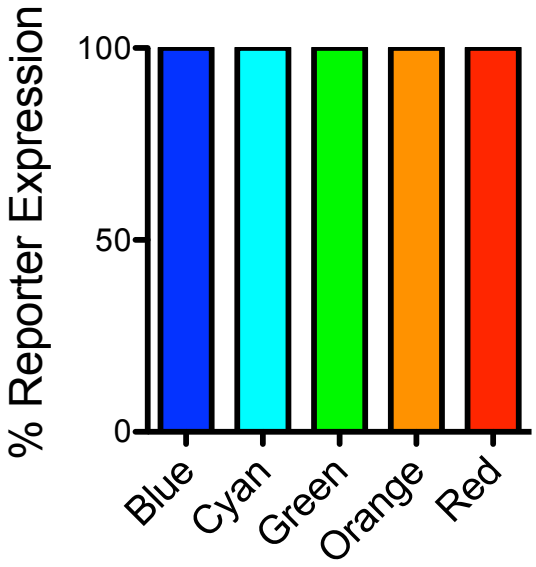


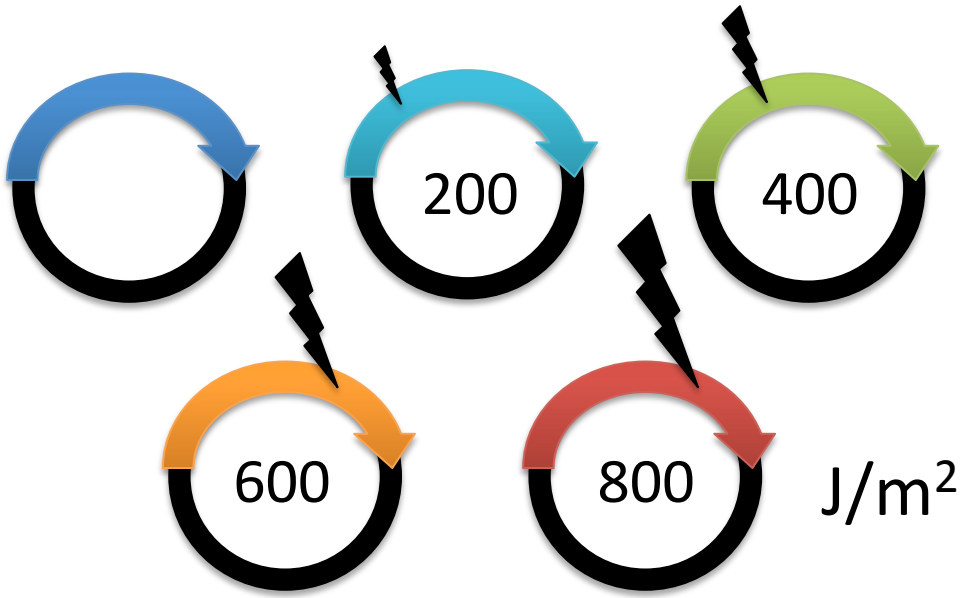
Time to repair

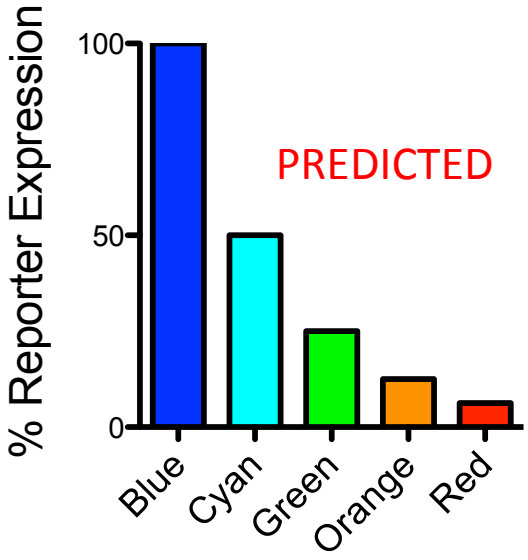


Before trying different damages - tried different doses of the same damage (UV)

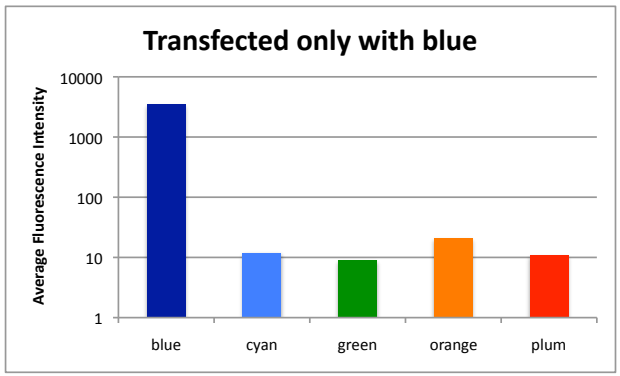


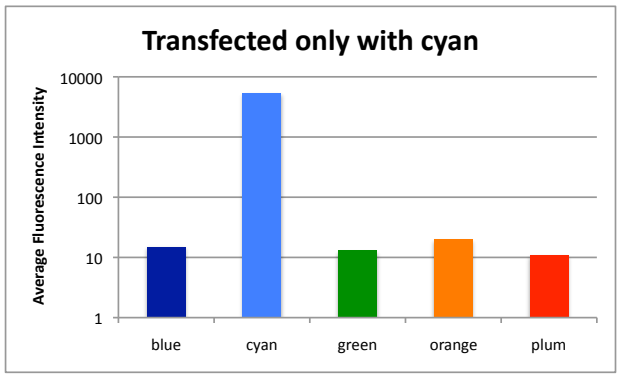


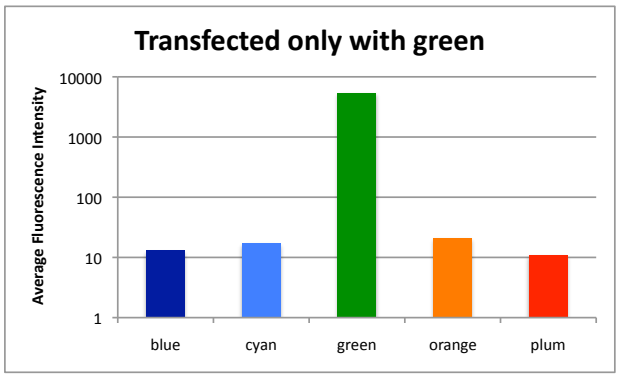


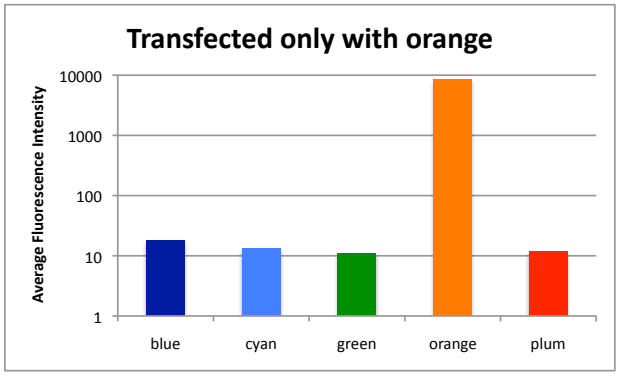


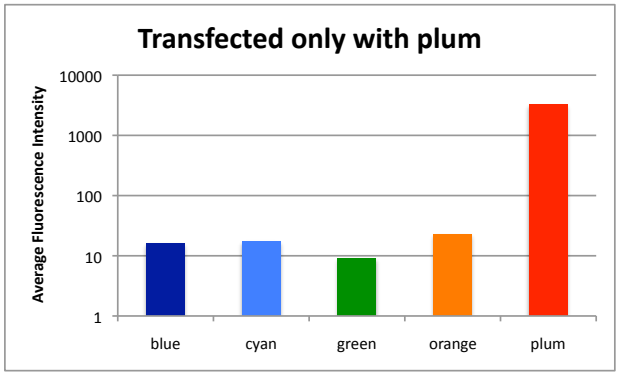
Sanity Check: Is it even feasible detect 5-colors independently?:



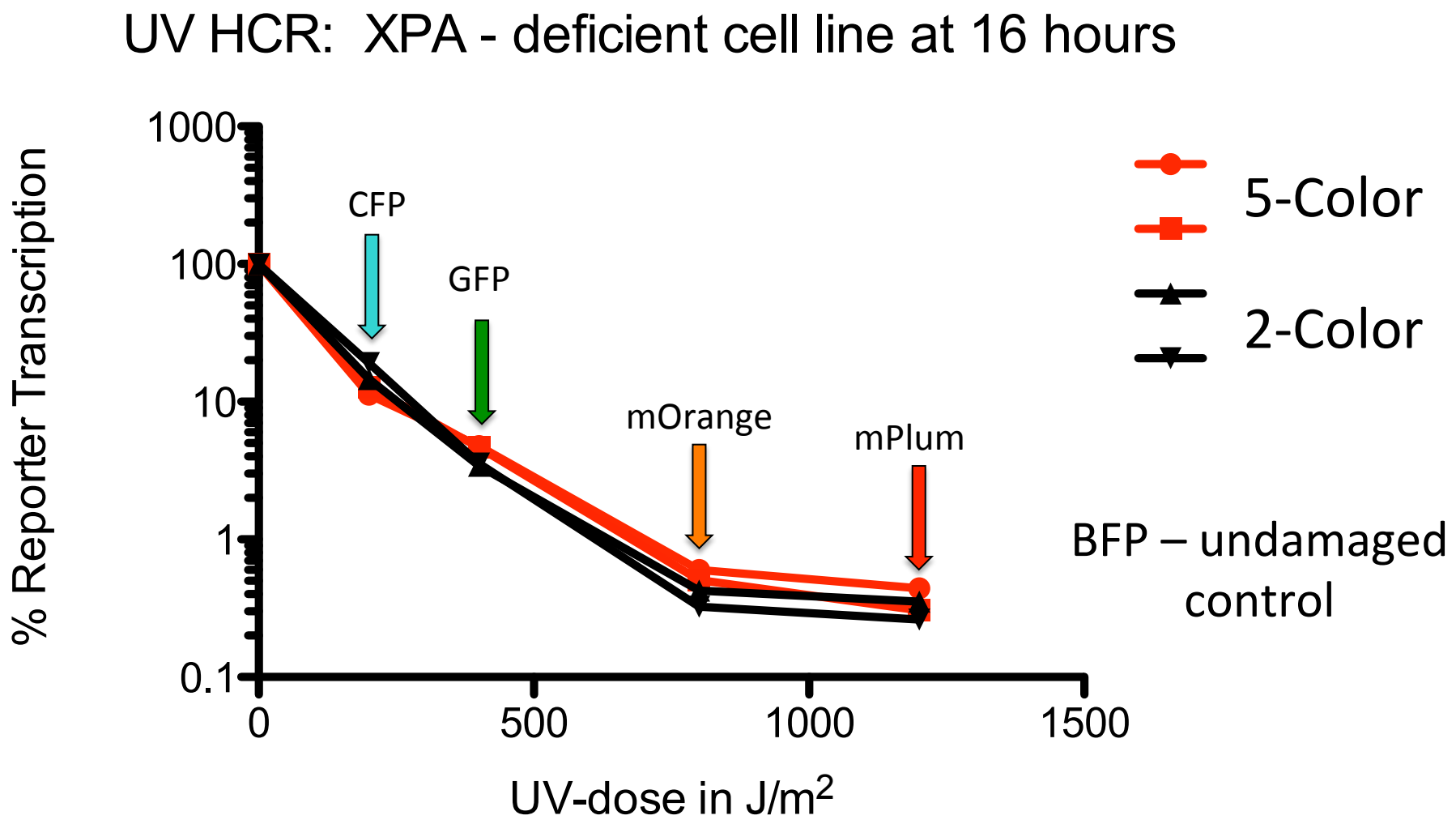






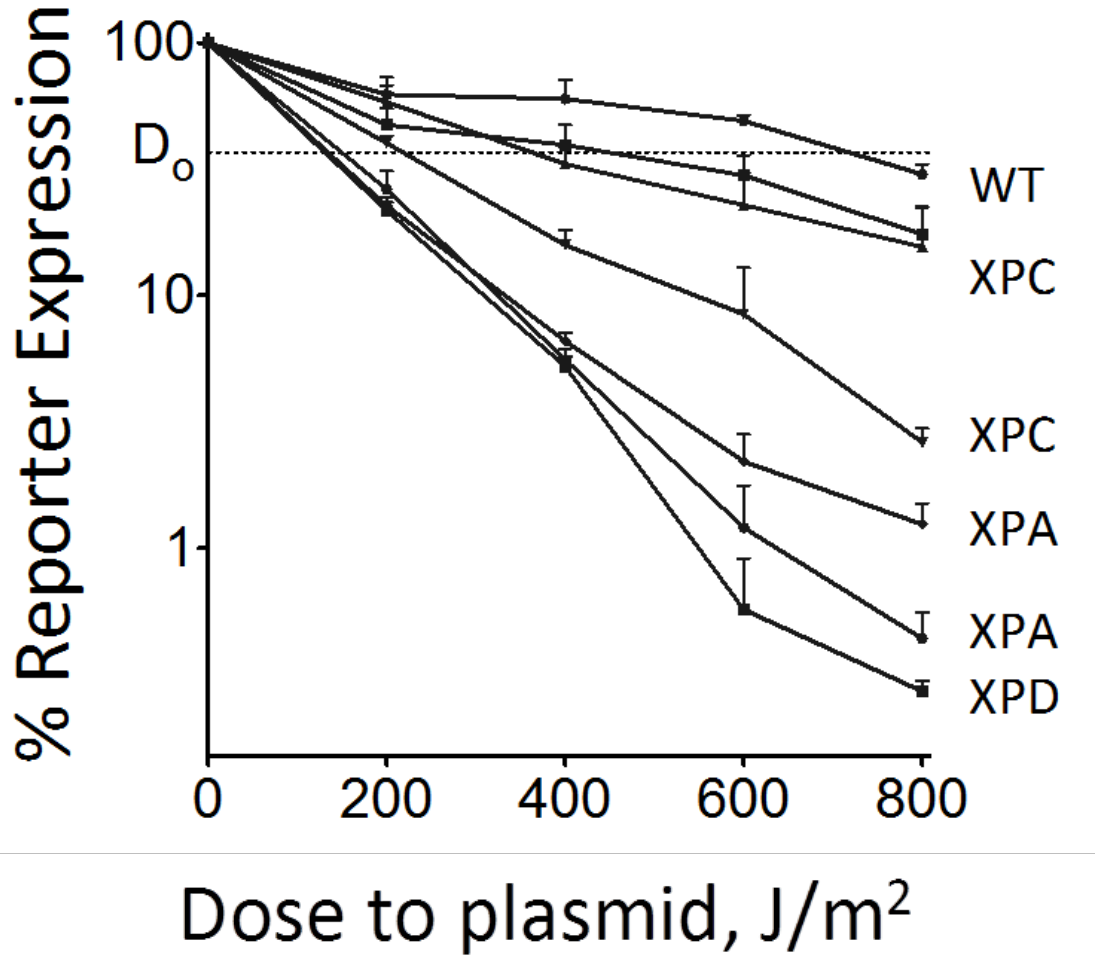


2-color versus 5-color HCR of UV-irradiated plasmids



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	

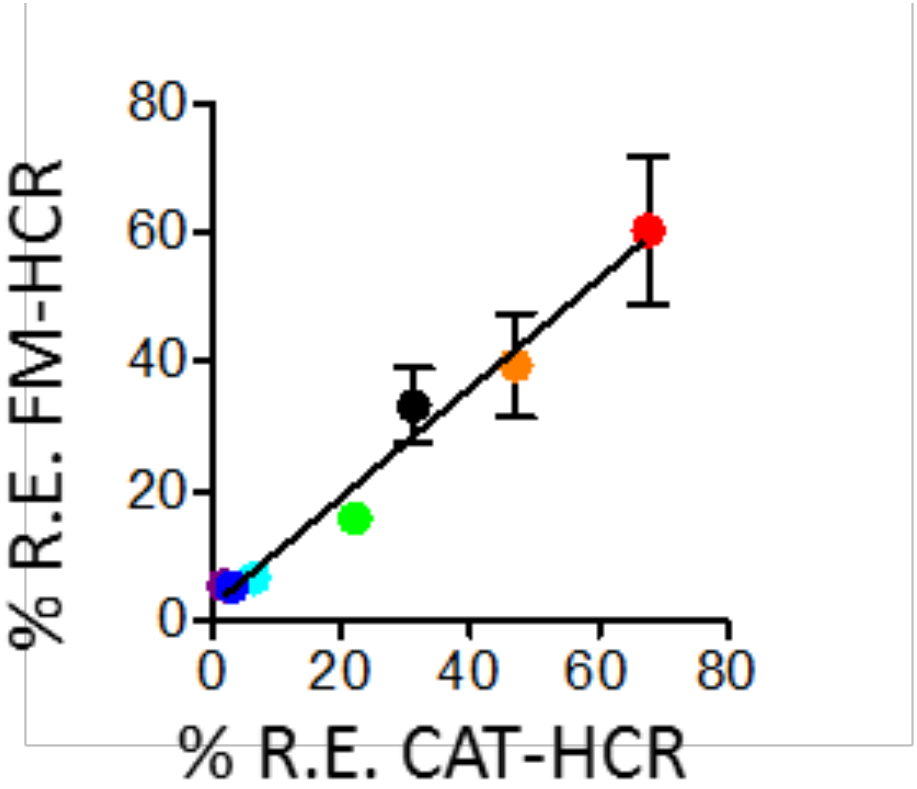
[a] CI, confidence interval.

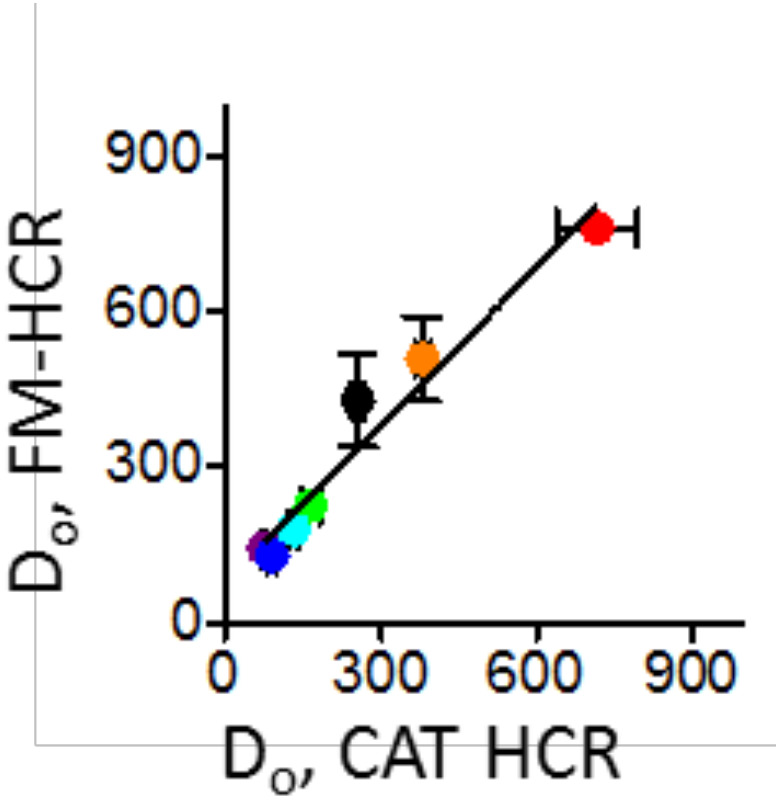
How does our FM-HCR data stack up against CAT-HCR, Grossman *et al.*, > 20 years ago?

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

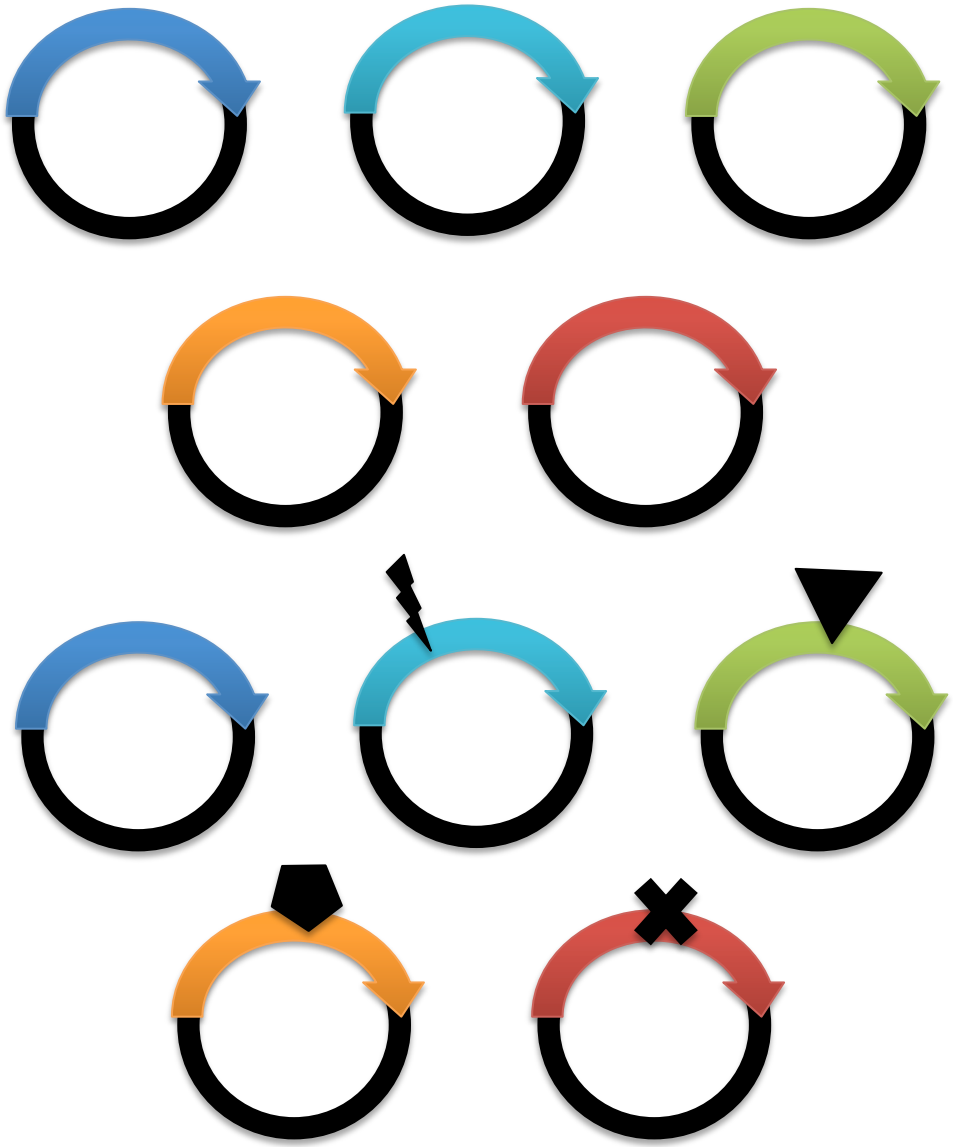


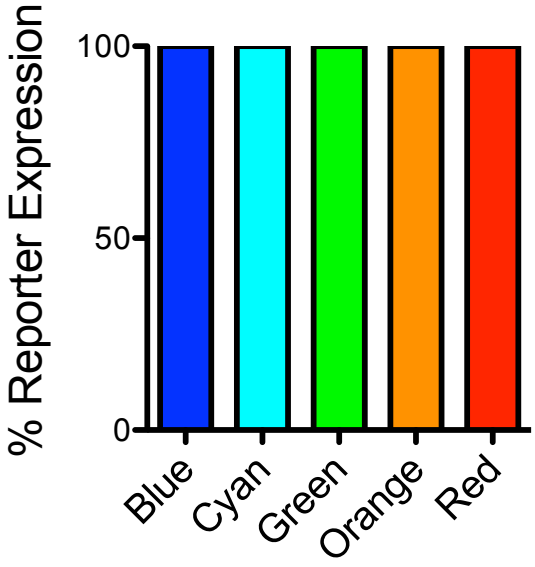


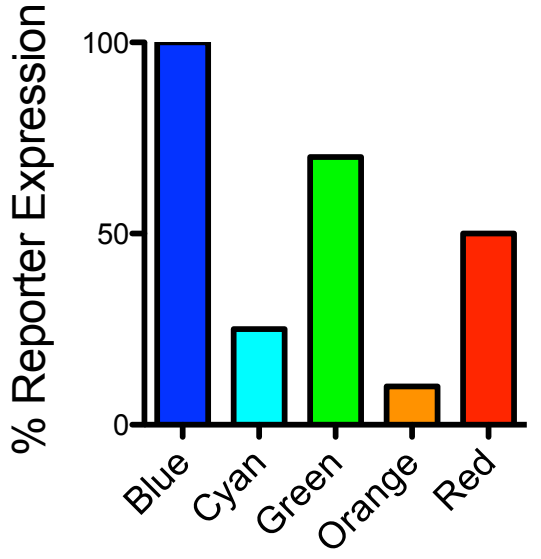
$R^2 = 0.92$, $p = 0.0006$

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

5 color HCR assay applications







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

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

DNA Repair Strategies

- Direct Reversal

  Methyltransferase, Oxidative demethylase

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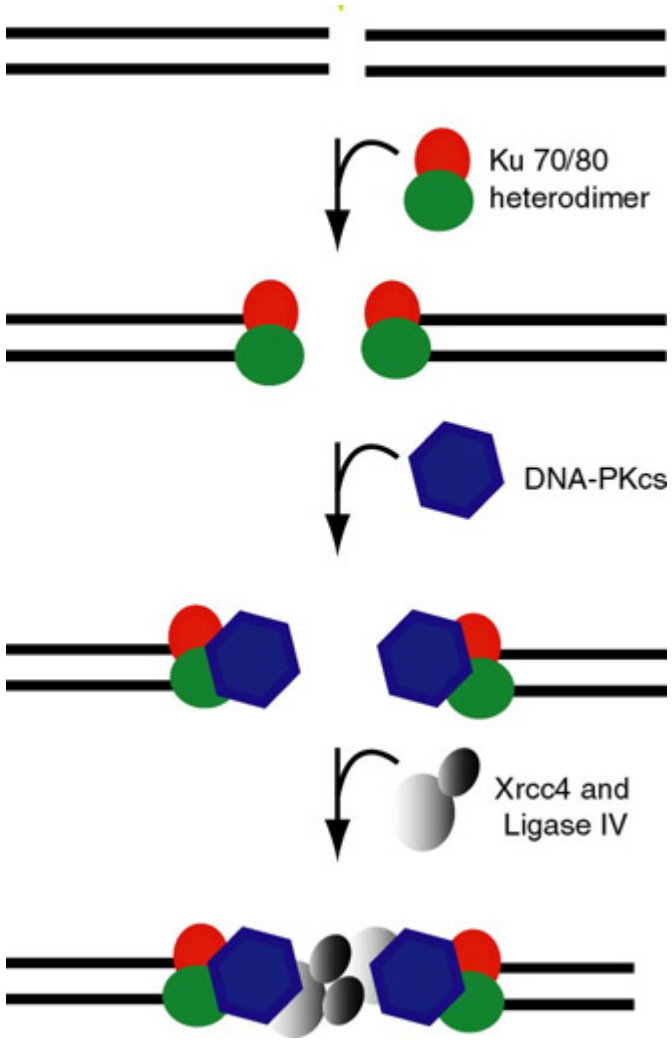
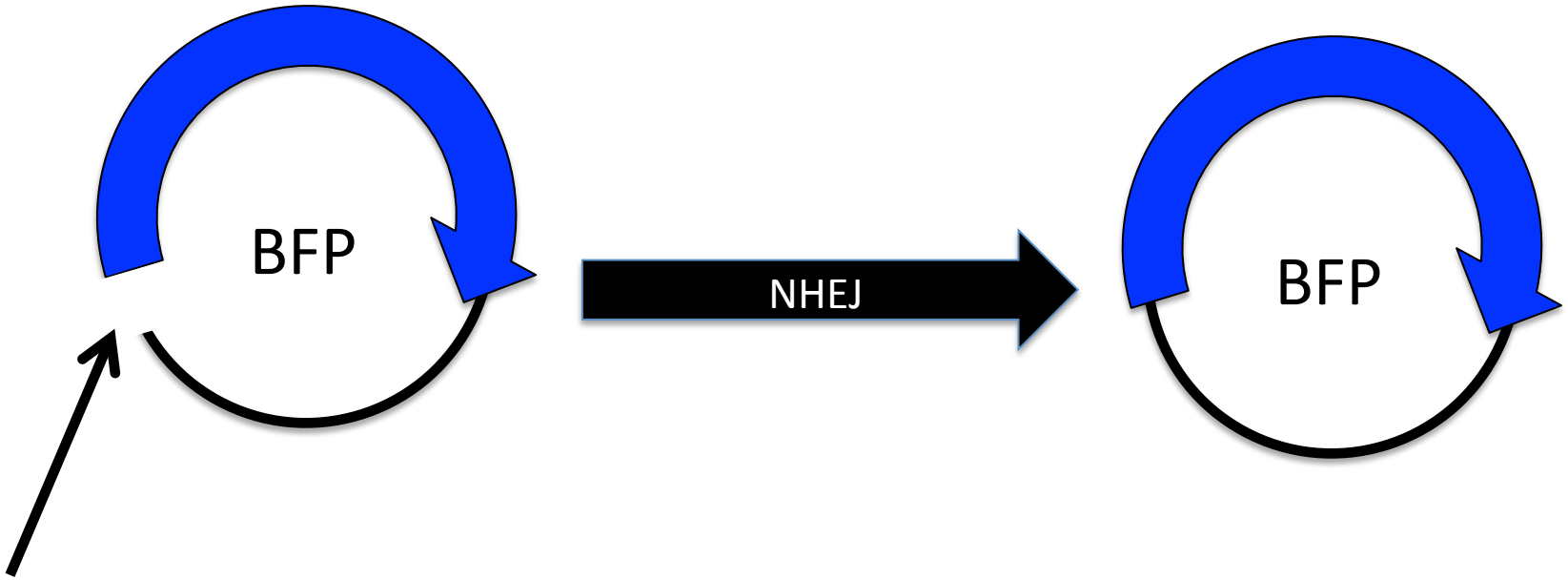
Non-Homologous End Joining (NHEJ) DNA Double Strand Break Repair



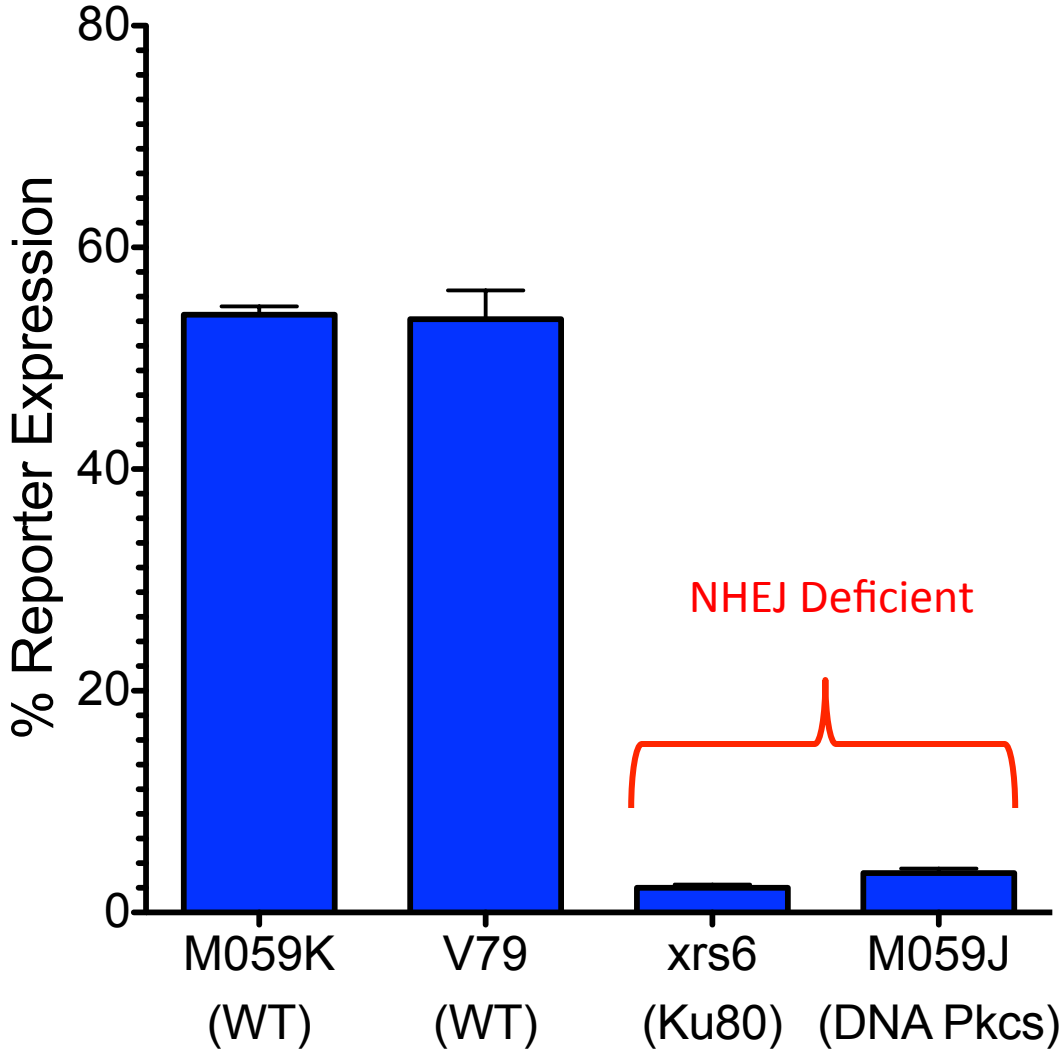
http://atlasgeneticsoncology.org/Deep/DoubleStrandBreaksID20008.html

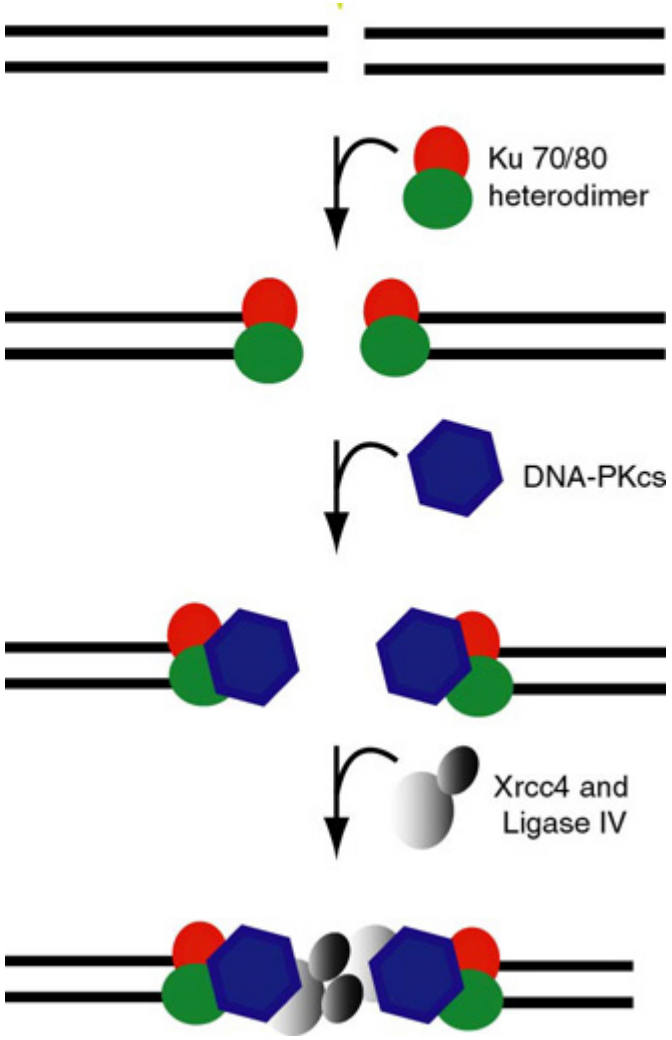
Non-Homologous End Joining (NHEJ)



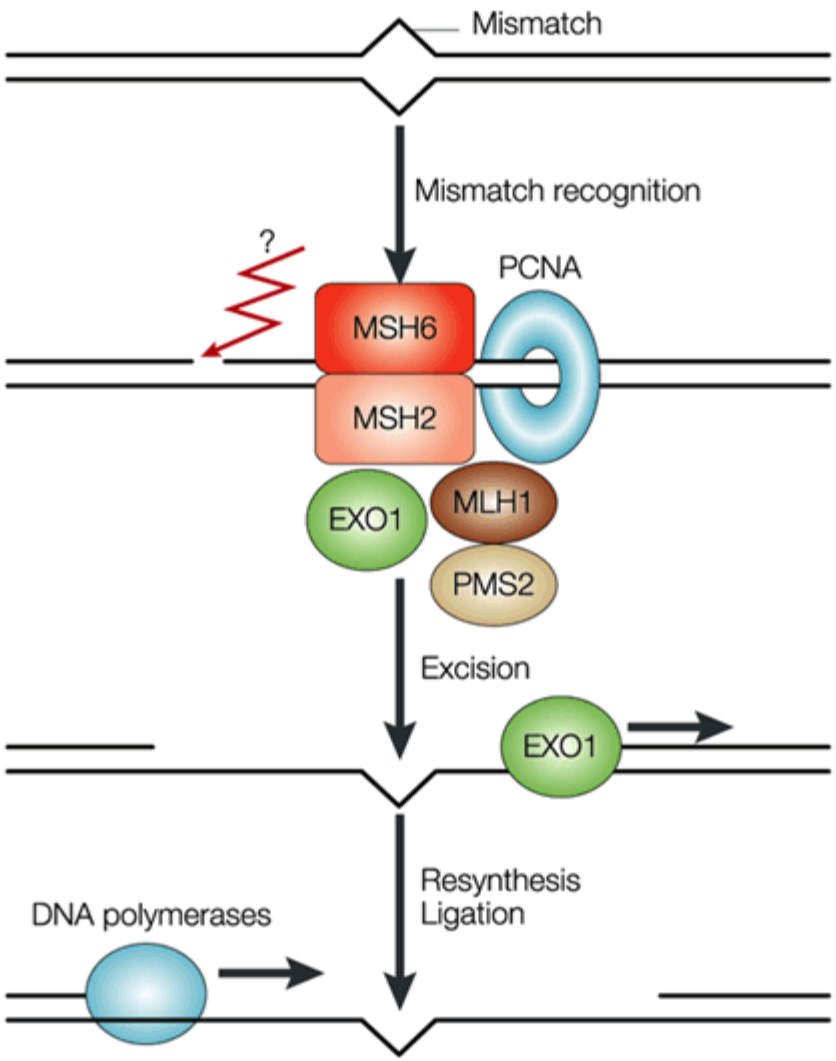
Substrate contains a blunt-end DSB in the 5’ UTR

NHEJ HCR in WT and NHEJ defective cells:





DNA Mismatch Repair



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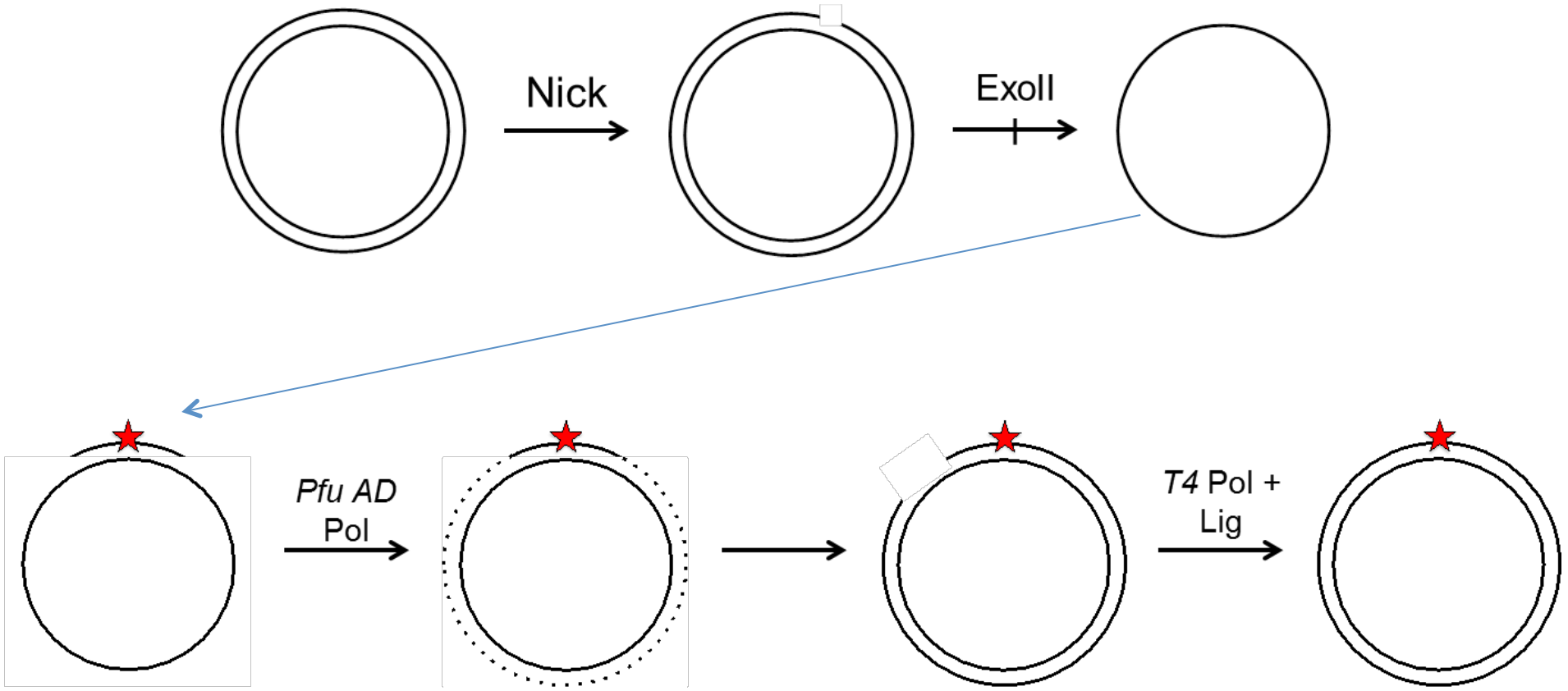
https://www.google.com/search?q=dna+mismatch+repair&tbm=isch&tbo=u&source=univ&sa=X&ei=pipkUraXMun-4AOAloGgBw&ved=0CDkQsAQ&biw=1067&bih=501&dpr=1#facrc=_&imgdii=_&imgrc=a7bj74c8D9MBtM%3A%3BYVLE34T6L3YPAM%3Bhttp%253A%252F%252Fwww.nature.com%252Fnri%252Fjournal%252Fv2%252Fn8%252Fimages%252Fnri858-i1.gif%3Bhttp%253A%252F%252Fwww.nature.com%252Fnri%252Fjournal%252Fv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

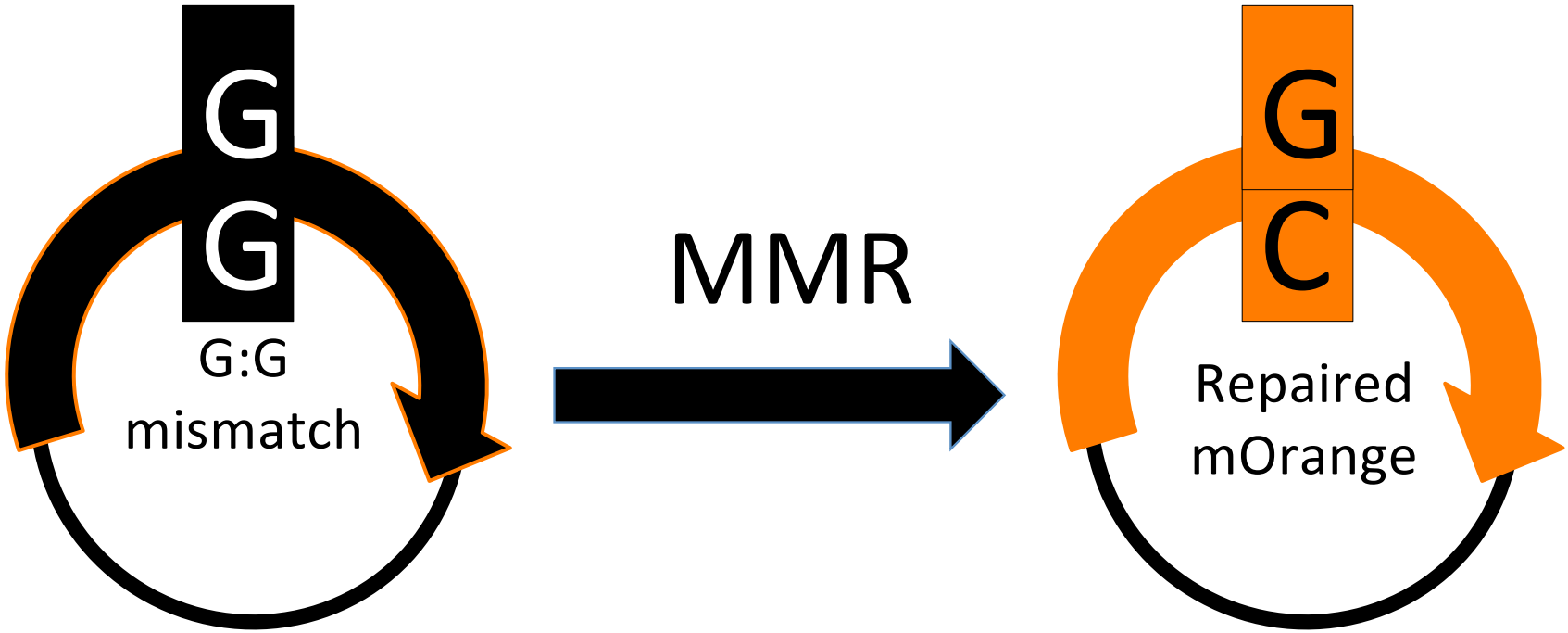
How to build a site-specific reporter?
"Primer-Extension"



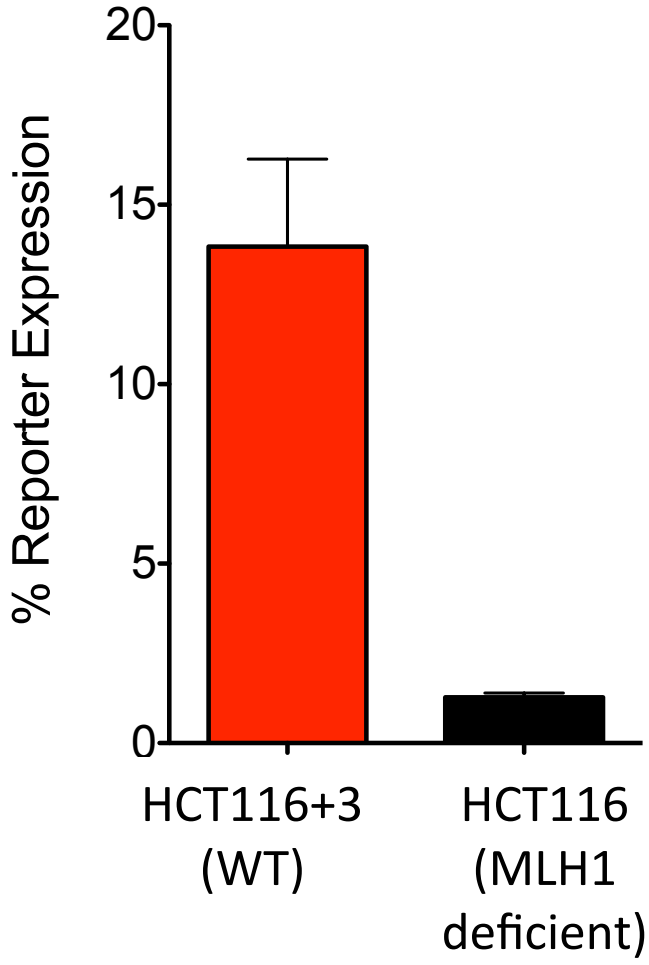
Original protocol from Baerenfaller *et al.* 2006 *Meth in enzymology*

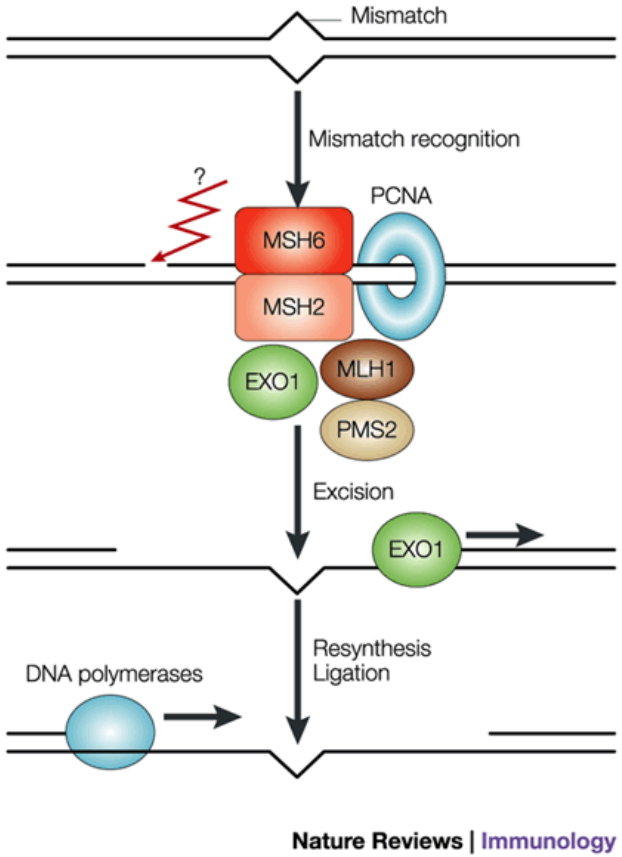
Modified and optimized by Alex Chaim, Zachary Nagel and Patrizia Mazzucato

DNA Mismatch Repair (MMR)

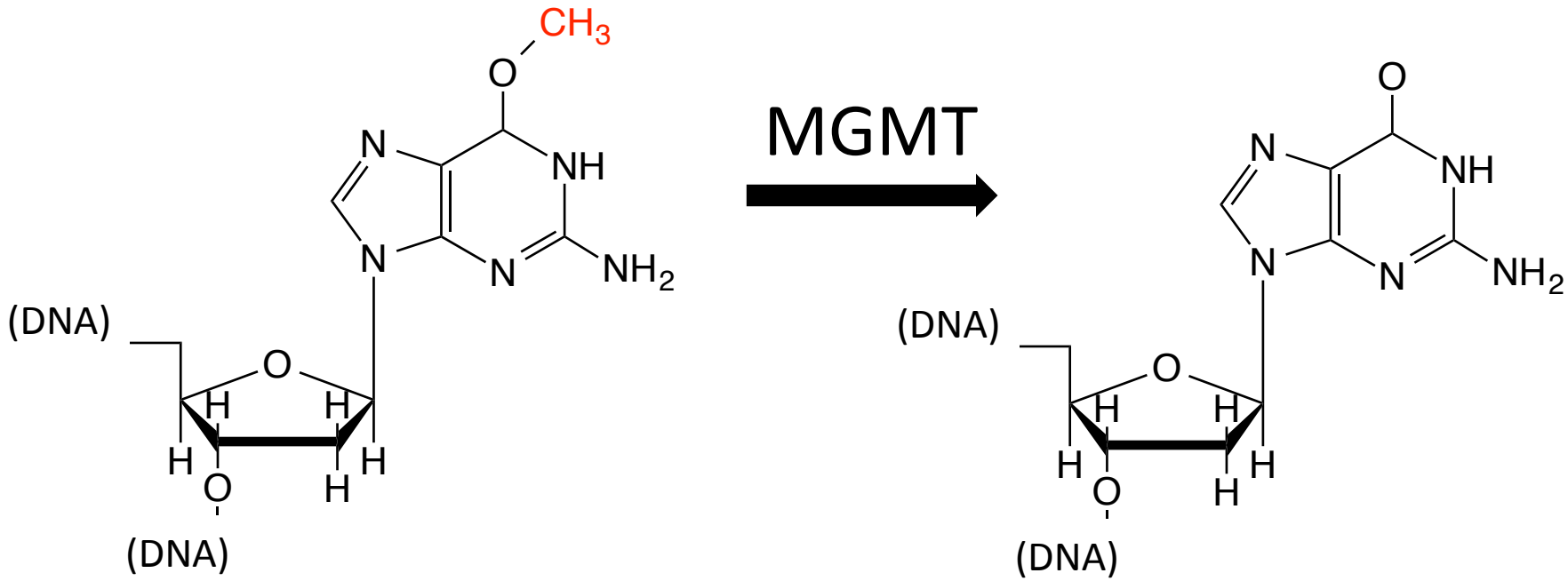


MMR assay distinguishes between proficient and deficient cell lines:



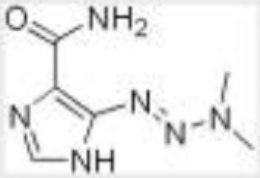
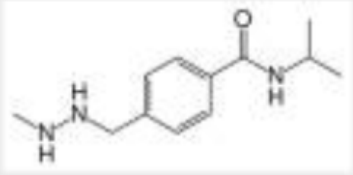
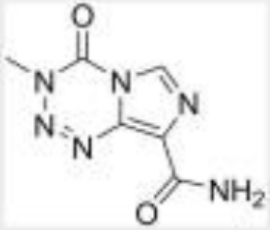
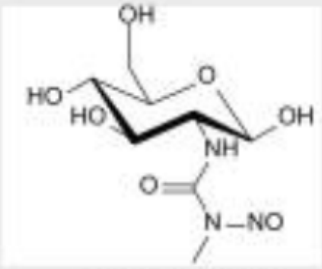
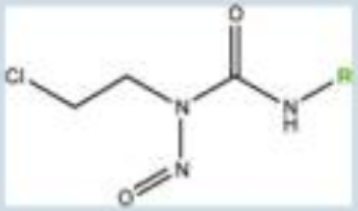
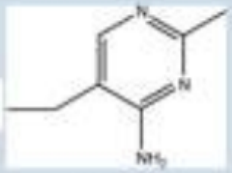
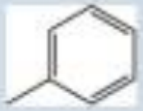
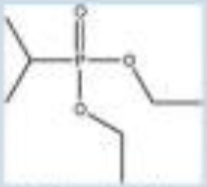


O^6-Methylguanine DNA Methyltransferase MGMT

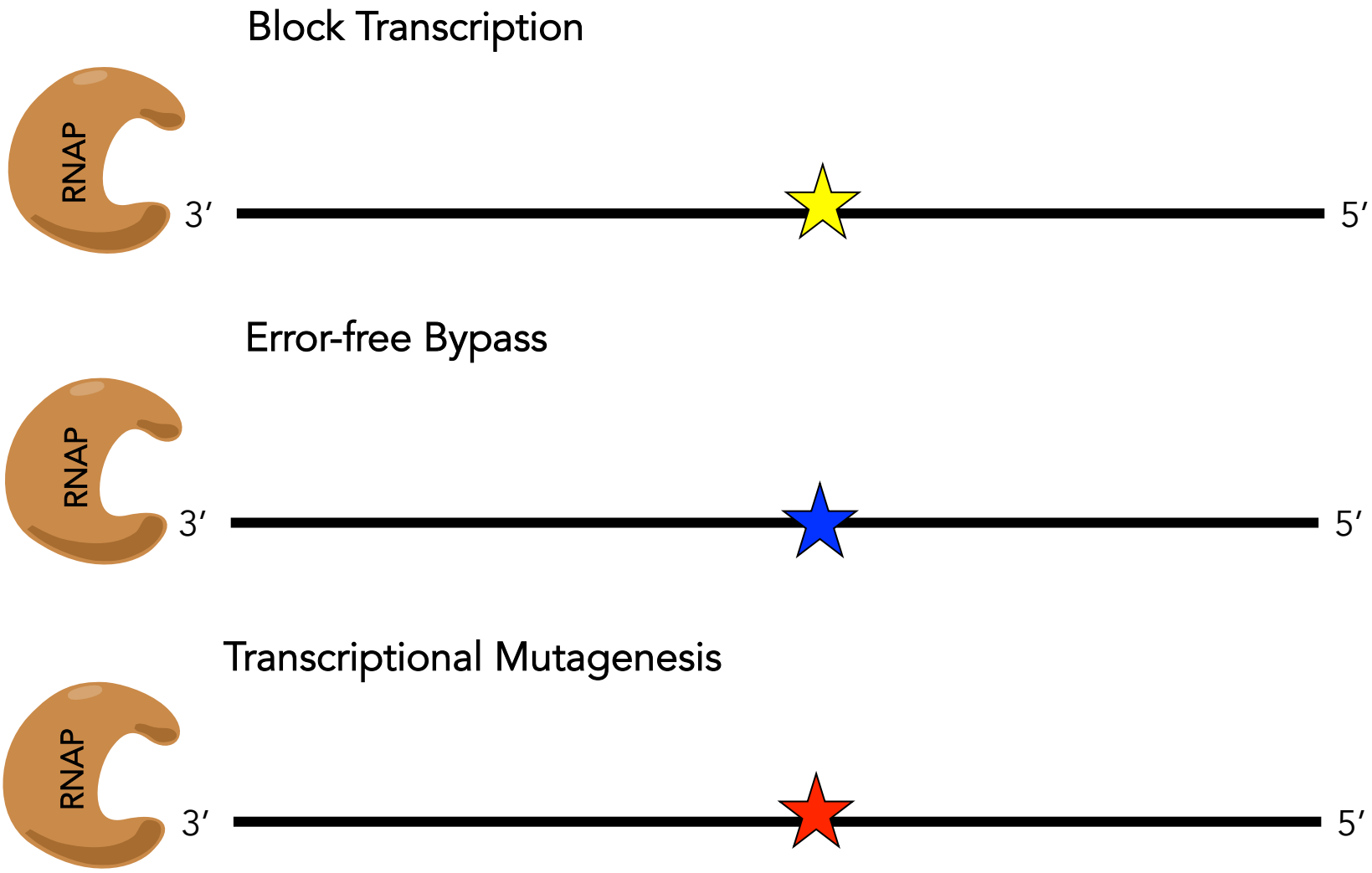


Many Cancer Chemotherapy Drugs induce this lesion in DNA

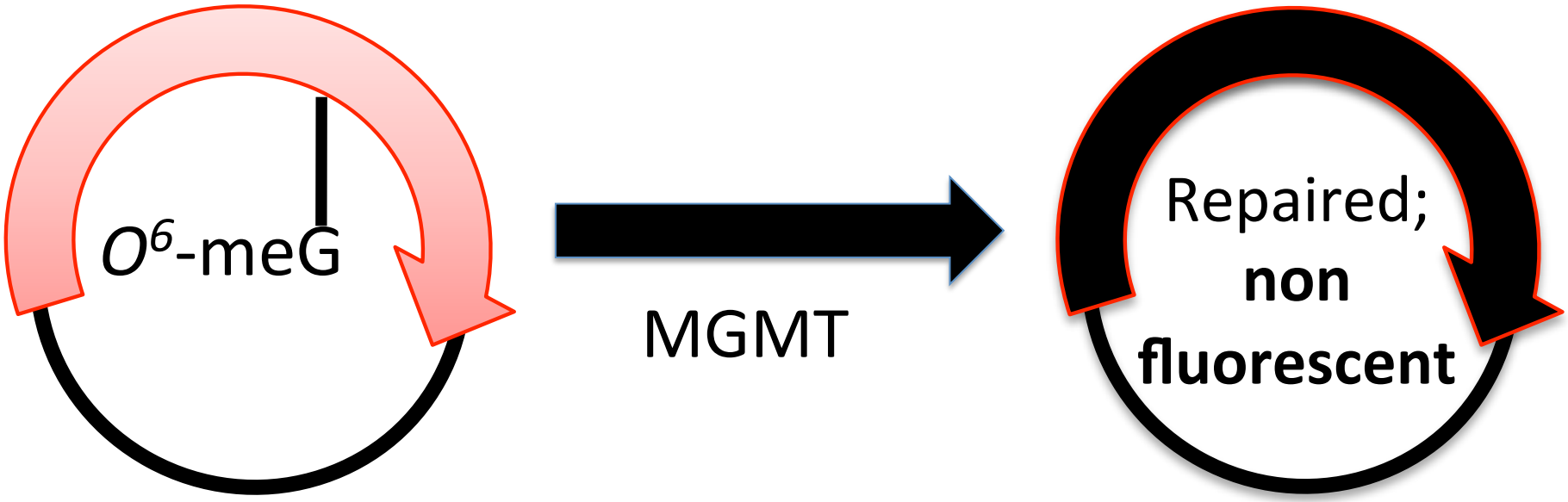
Alkylating agents used in the cancer clinic

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				
	R= 				7-alkylG O^6Cl-ethylG N1,O^6-EG G-C x-link G-G x-link
ACNU (Nimustine) Brain tumors Solid tumors	**BCNU (Carmustine)** Brain tumors Lymphomas Melanoma	**CCNU (Lomustine)** Brain tumors Lymphomas Melanoma	**Fotemustine** Metastatic melanoma	

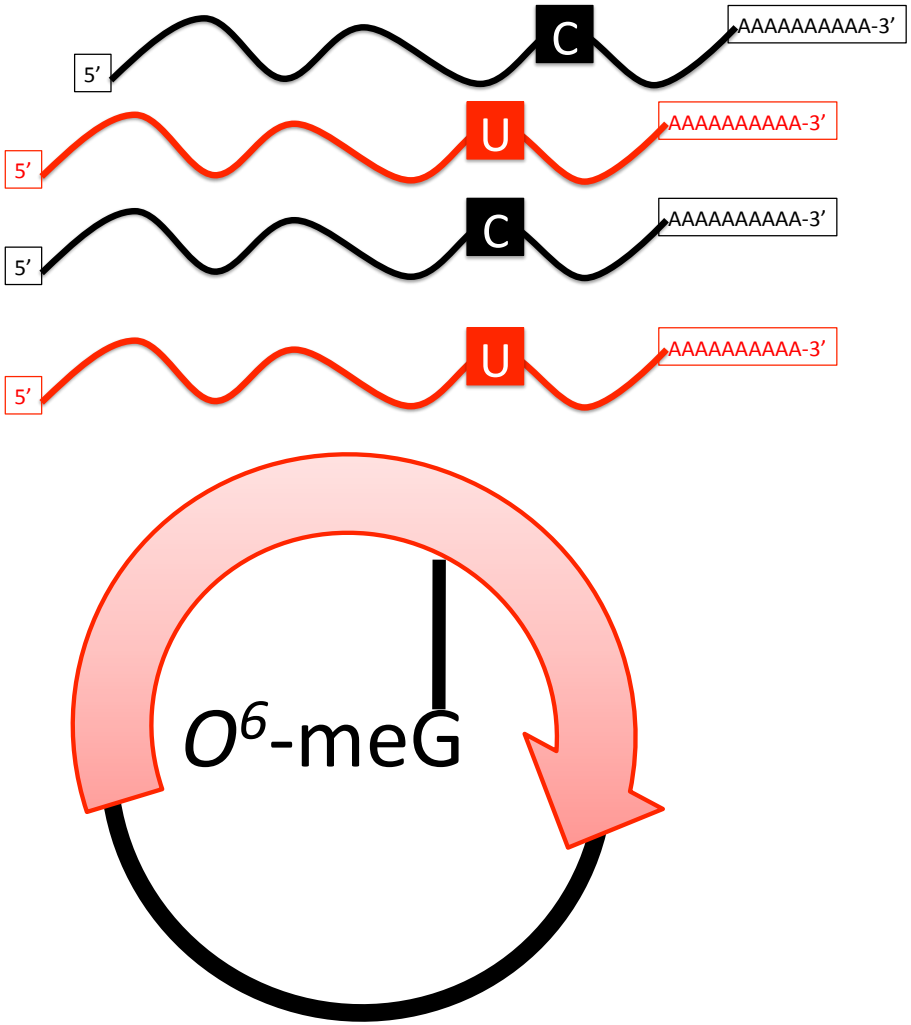
DNA lesions from an RNA polymerase perspective



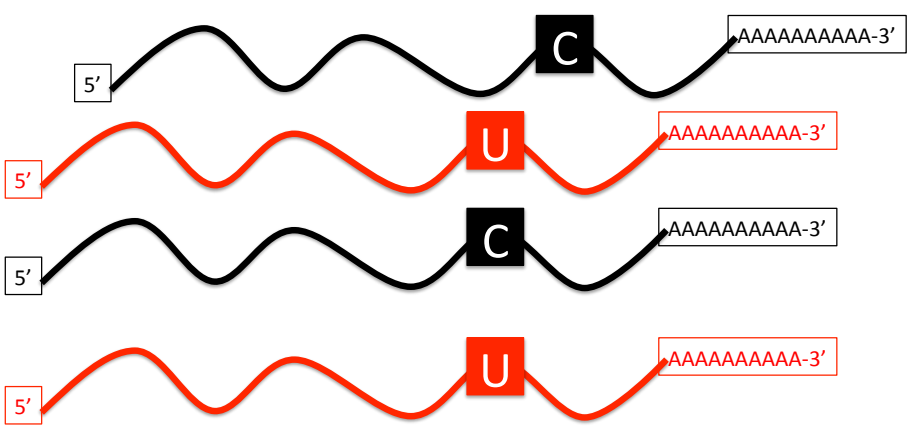
Direct Reversal of O^6-Methylguanine

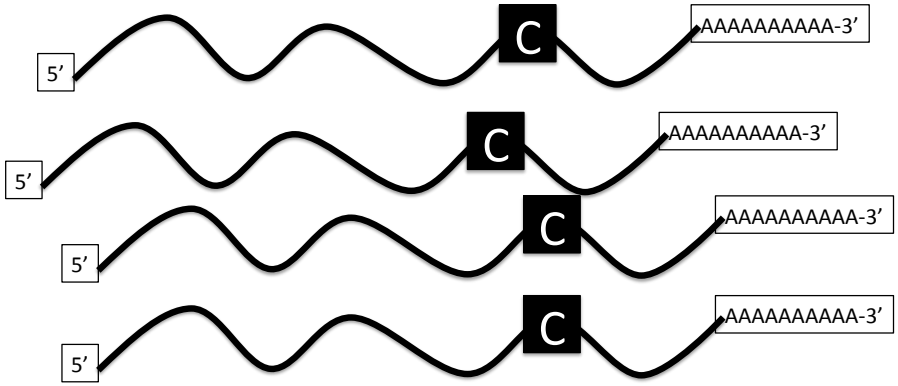


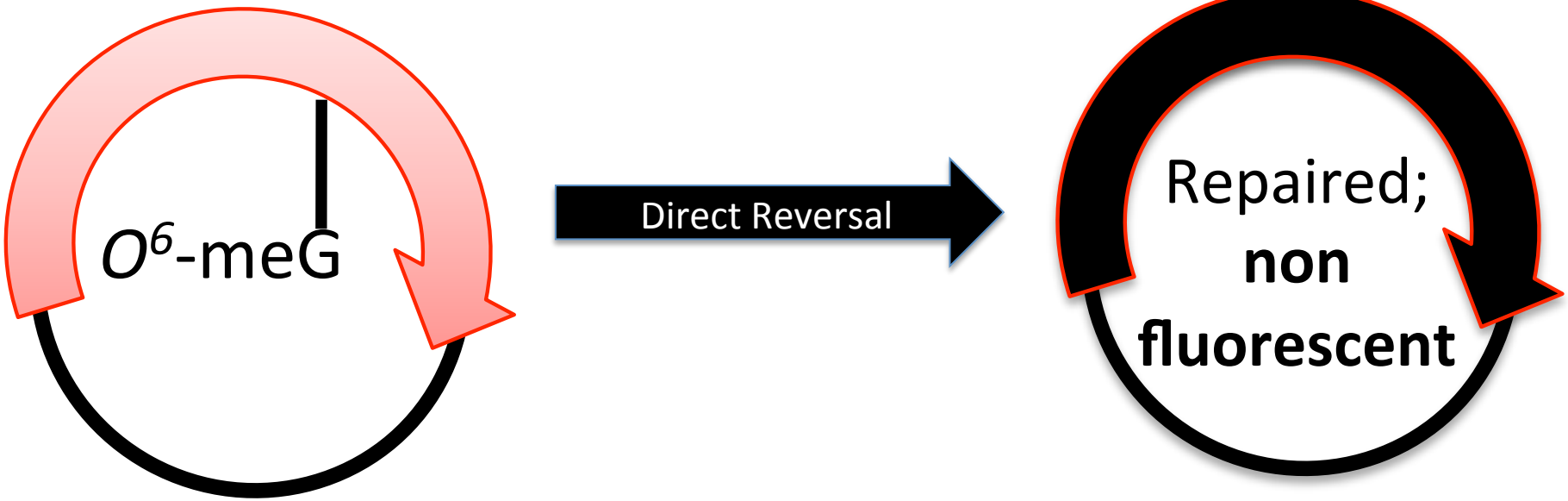
Direct Reversal of O^6-Methylguanine



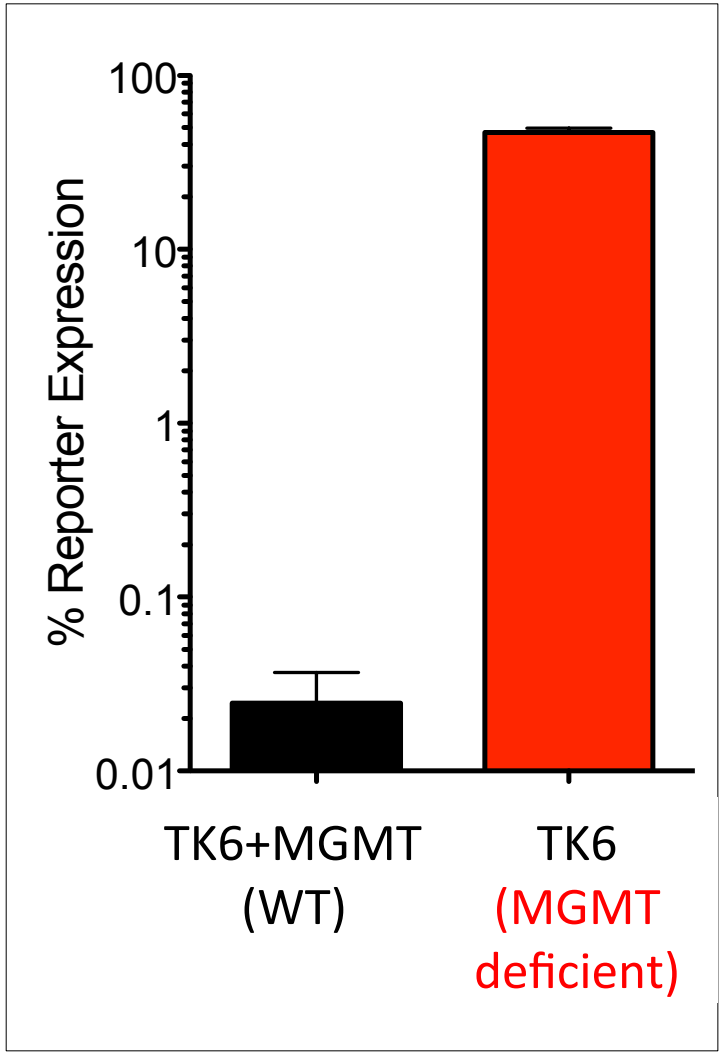
Direct Reversal of O^6-Methylguanine

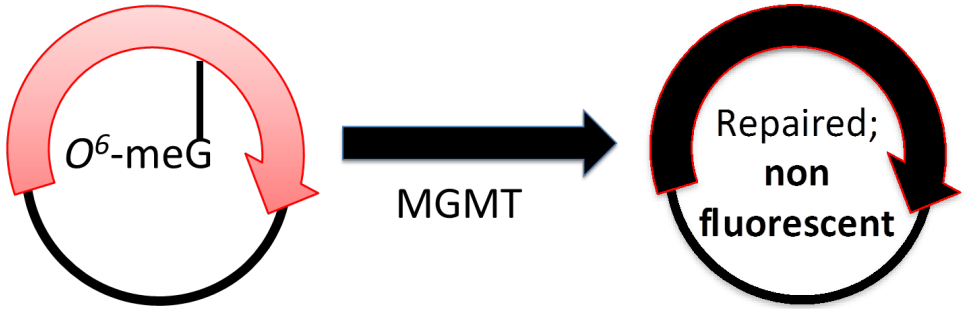






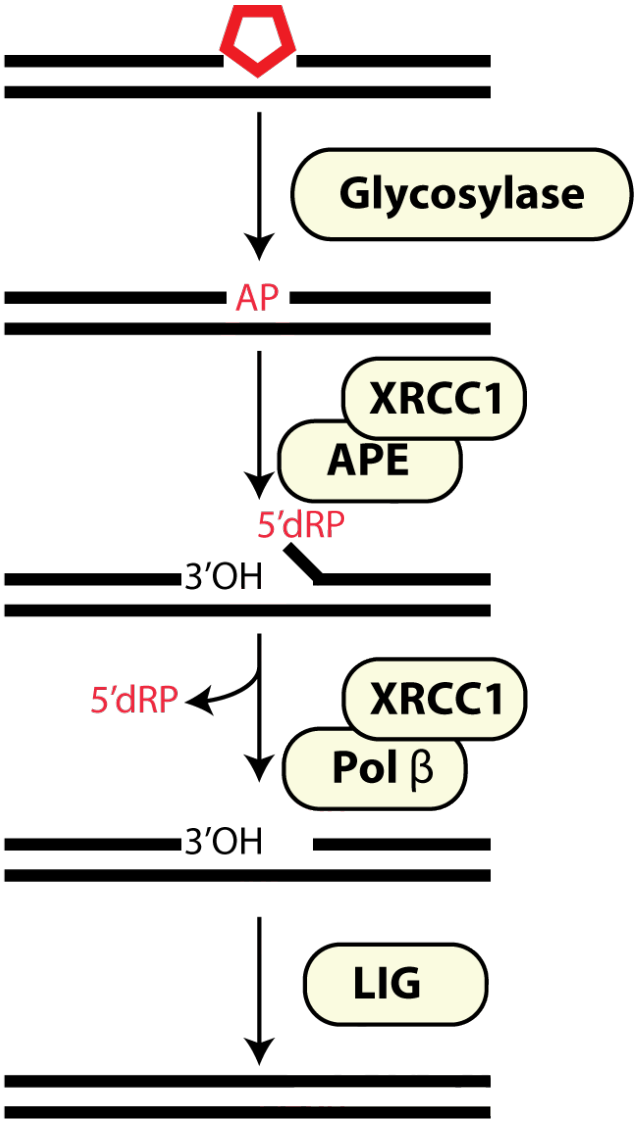
MGMT deficient cells are distinguished by a high level of reporter expression:





Base Excision Repair (BER)

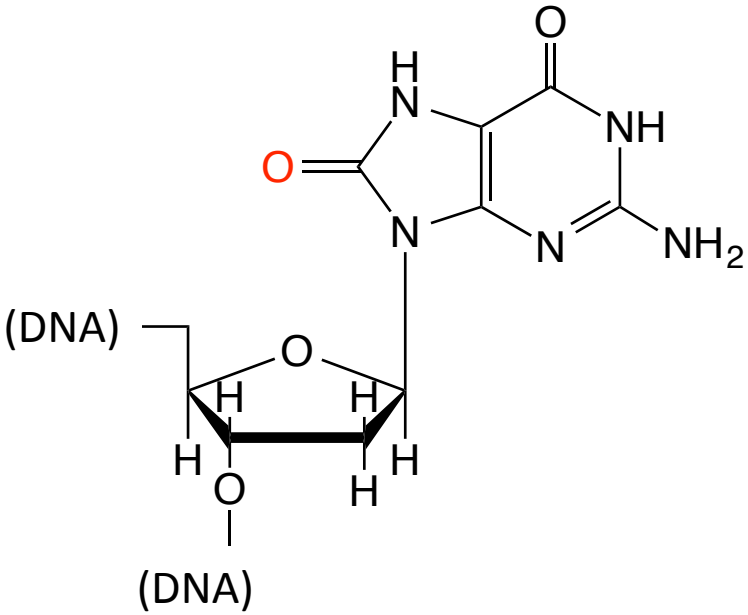
For oxidized, deaminated and alkylated base damage



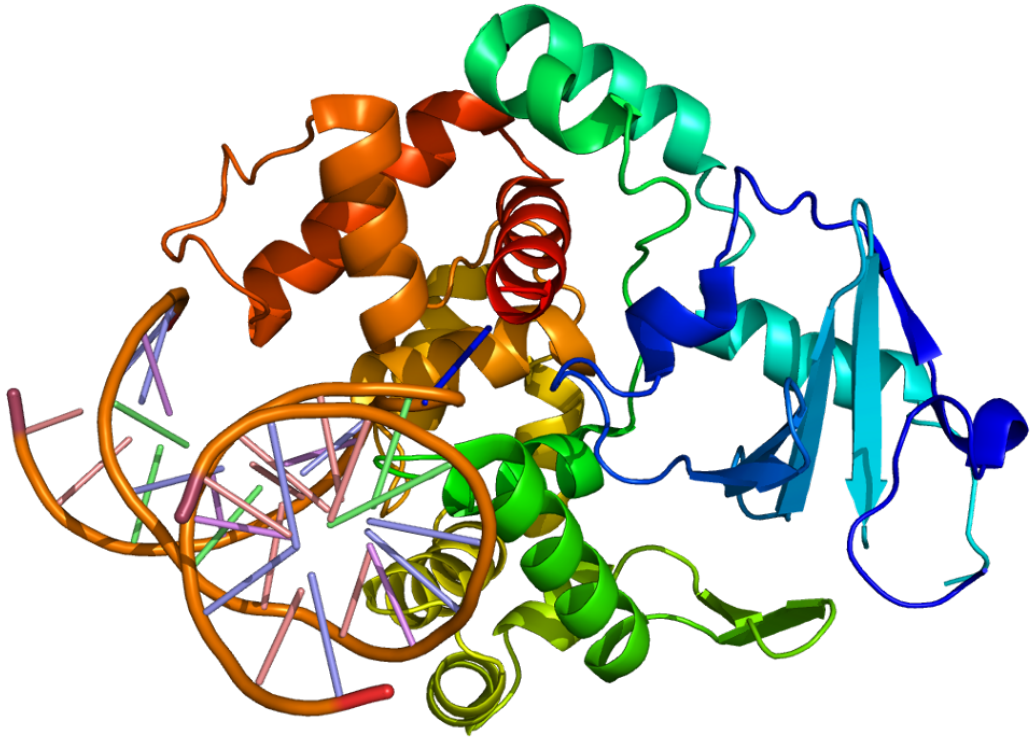
DNA Glycosylases

Bifunctional (β elimination)	OGG1	8-oxoguanine DNA glycosylase
	NTHL1	nth endonuclease III-like 1
	NEIL 3	Nei endonuclease VIII-like 3
Monofunctional	UNG	Uracil DNA glycosylase
	SMUG1	Single-strand-selective monofunctional uracil DNA glycosylase 1
	TDG	Thymine DNA glycosylase
	MBD4	Methyl-CpG binding domain protein 4
	AAG	Alkyladenine DNA glycosylase
	MUTYH	mutY homologue
Bifunctional (β,δ elimination)	NEIL 1	Nei endonuclease VIII-like 1
	NEIL 2	Nei endonuclease VIII-like 2

Base excision repair of 8-oxo-G



8-oxo-guanine



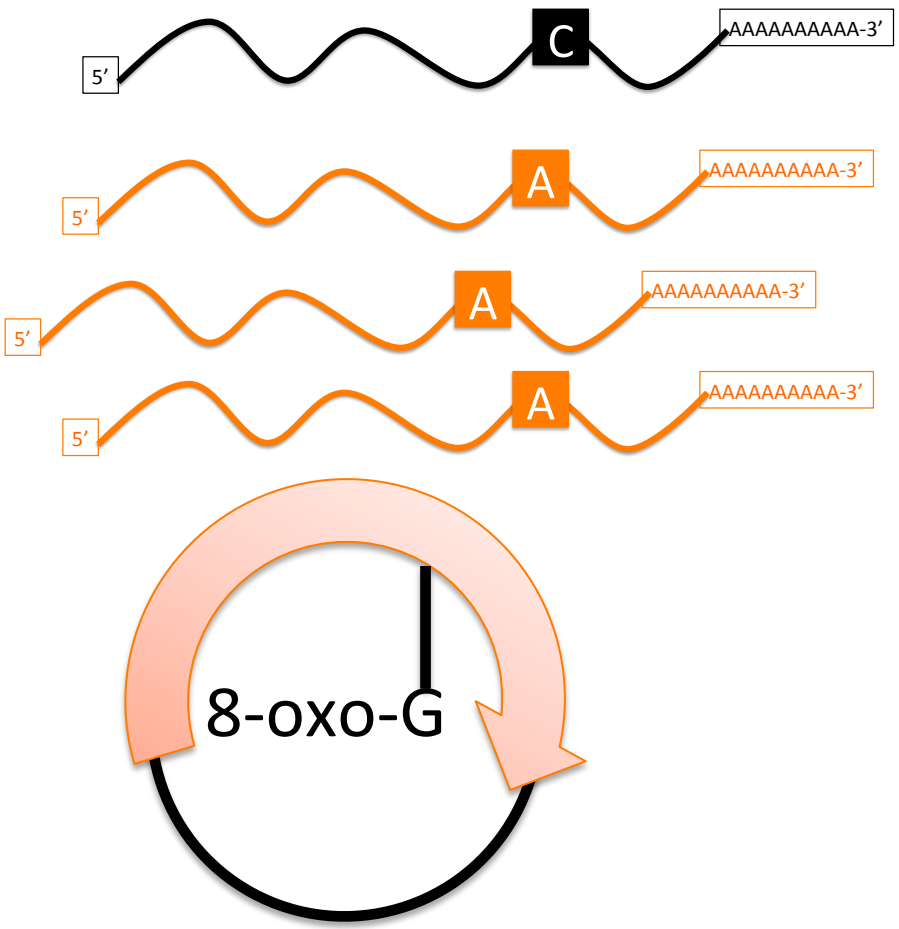
OGG1 DNA Glycosylase

Alex Chaim

Base excision repair of 8-oxo-G

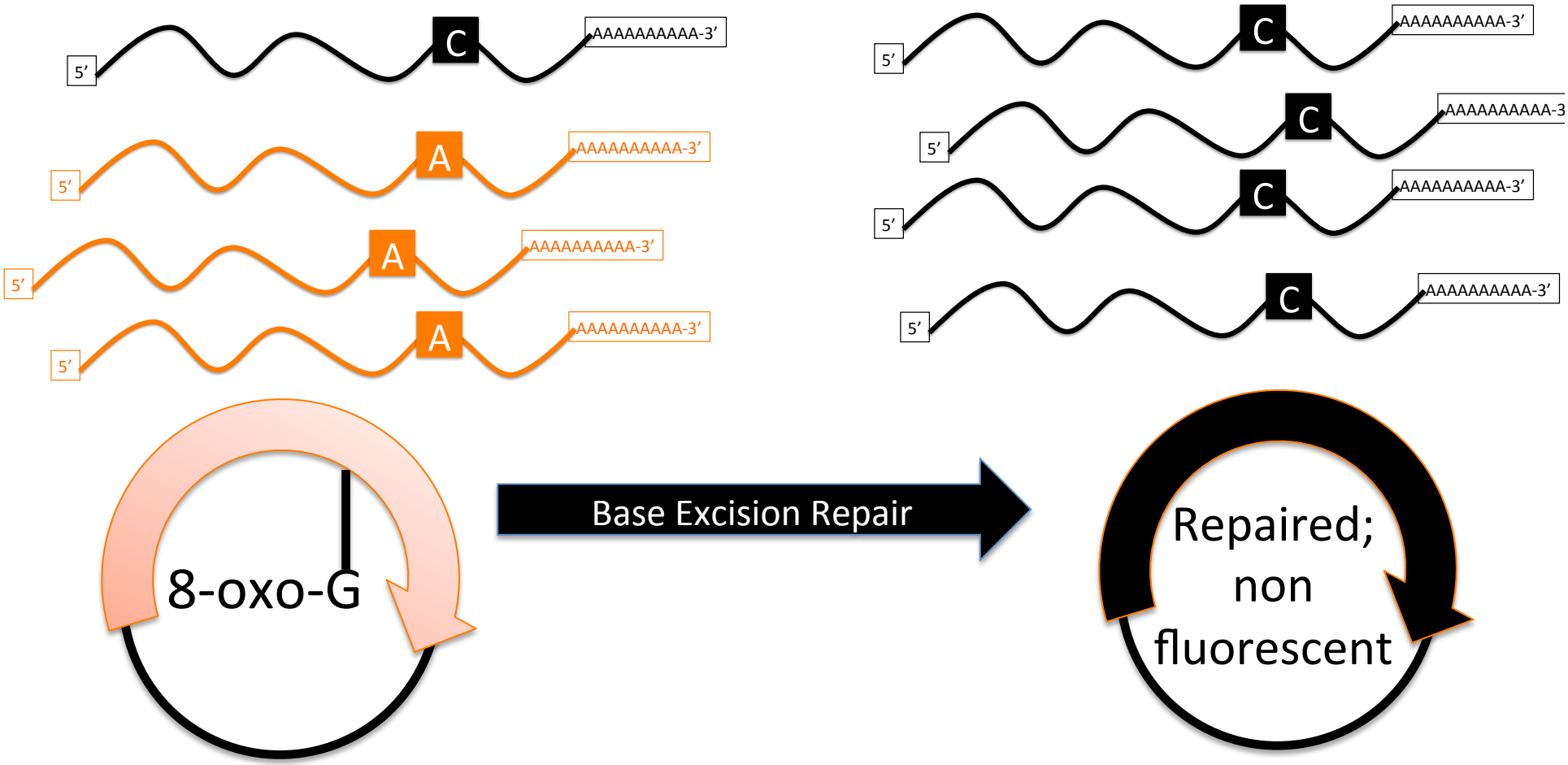
Alex Chaim

Base excision repair of 8-oxo-G



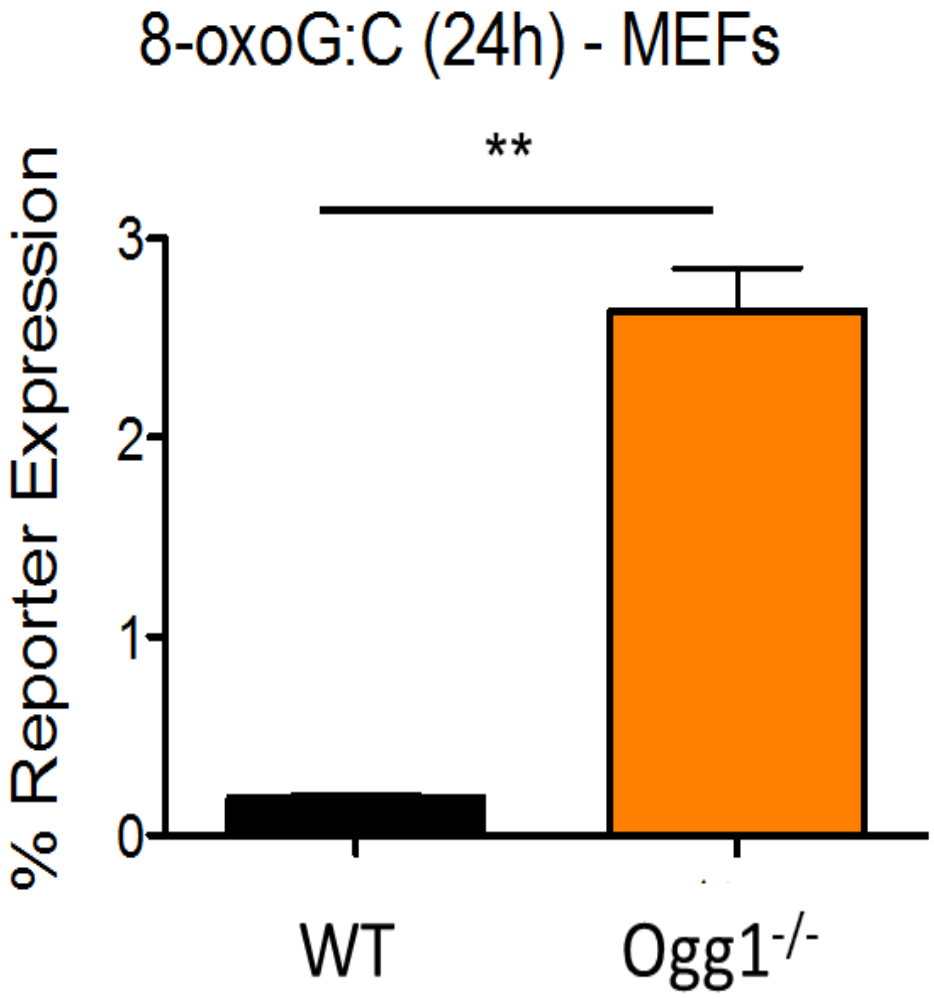
Alex Chaim

Base excision repair of 8-oxo-guanine



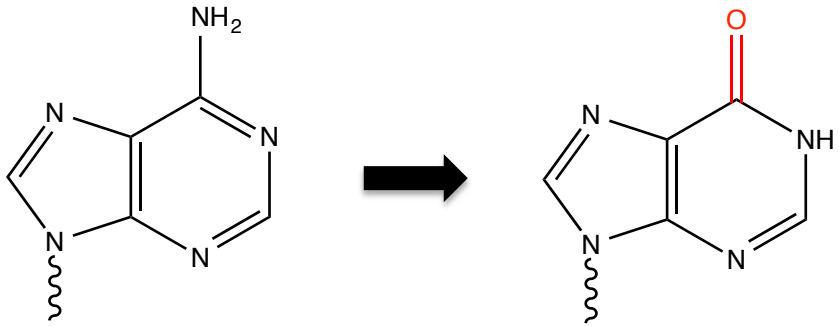
Alex Chaim

Measuring BER of 8-oxo-guanine



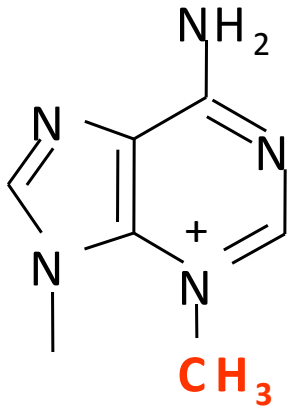
Alex Chaim

Base excision repair of Hypoxanthine (Hx)

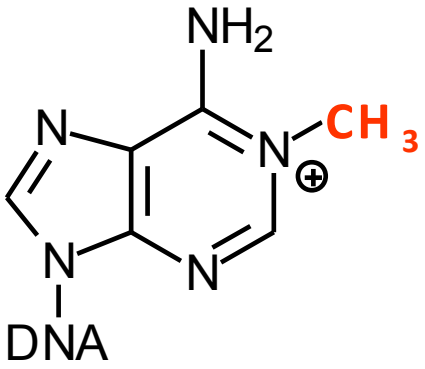


- Found in DNA
  - Product of A deamination
  - Incorporated from the nucleotide pool across C
- Highly mutagenic
  - A➔G transitions

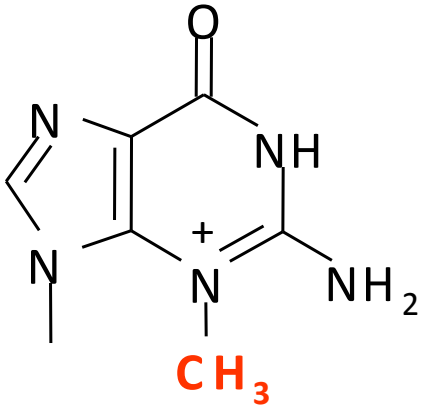
Human (AAG), and Mouse (Aag) 3MeA DNA Glycosylases Act on Many Lesions



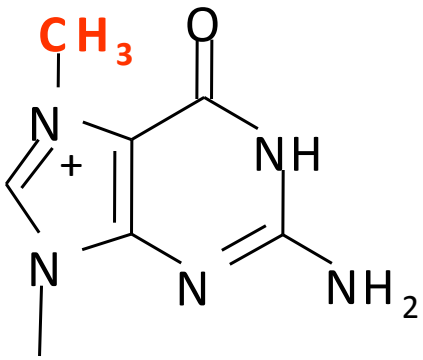
3meA



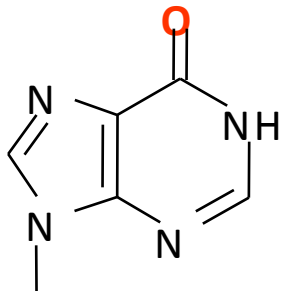
1meA



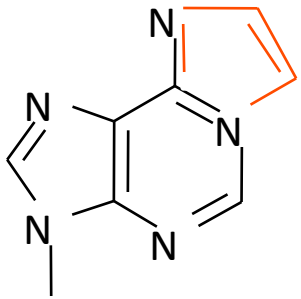
3meG



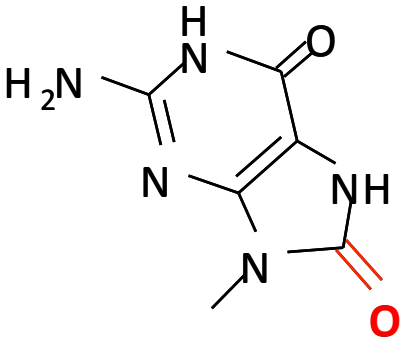
7meG



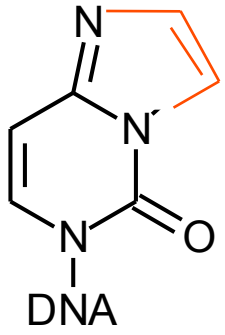
Hx



εA

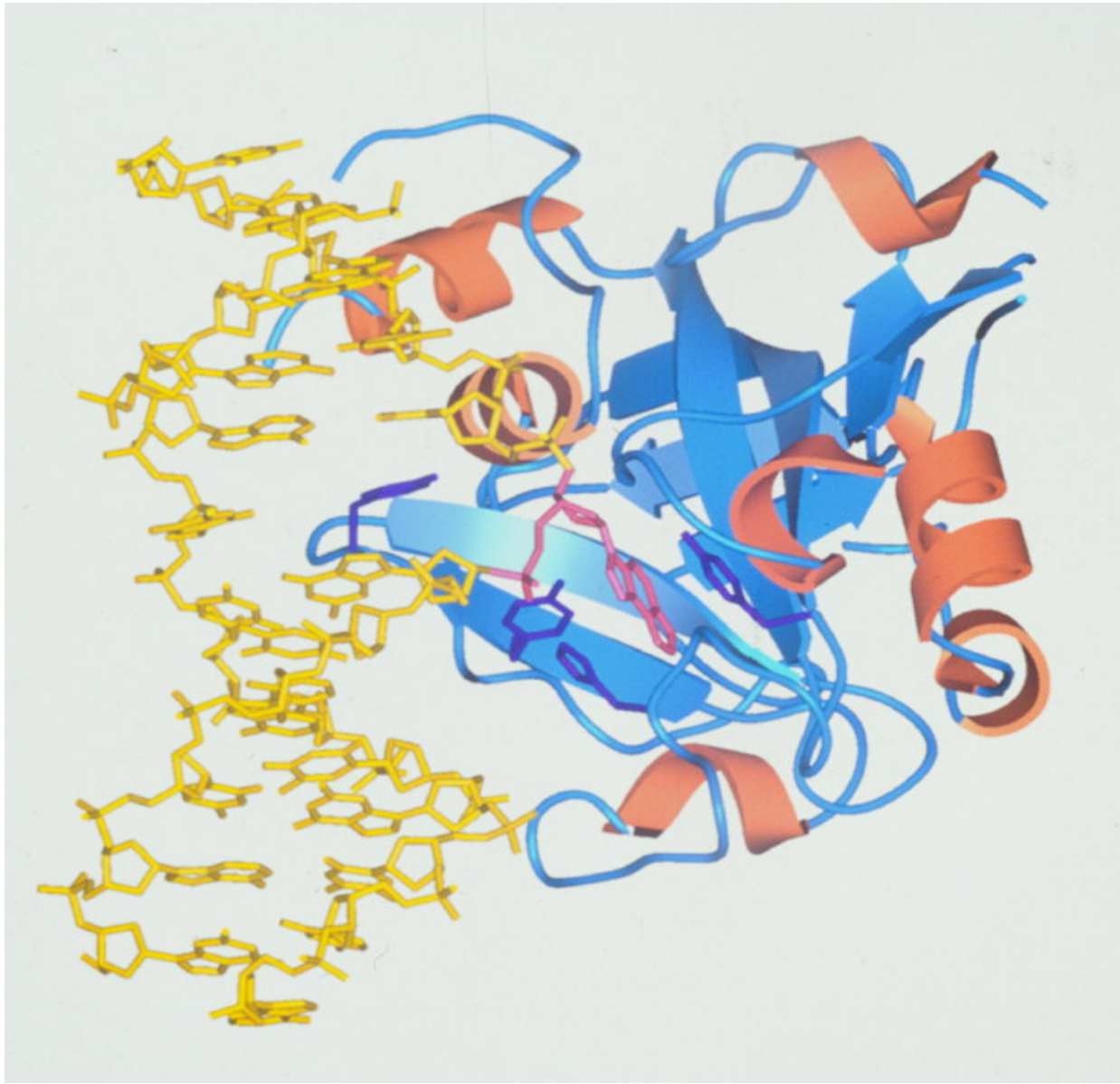


8-oxoG



εC

AAG: Alkyladenine DNA Glycosylase

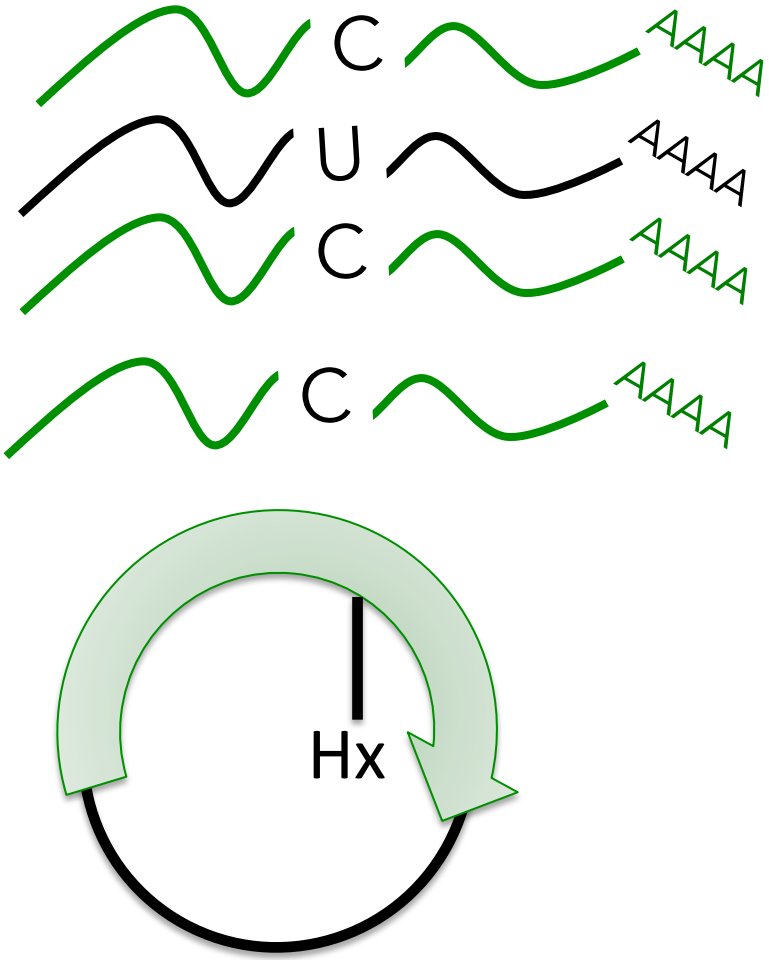


Lau et al. *PNAS*. 2000. **97**:13573-78.

Base excision repair of Hx

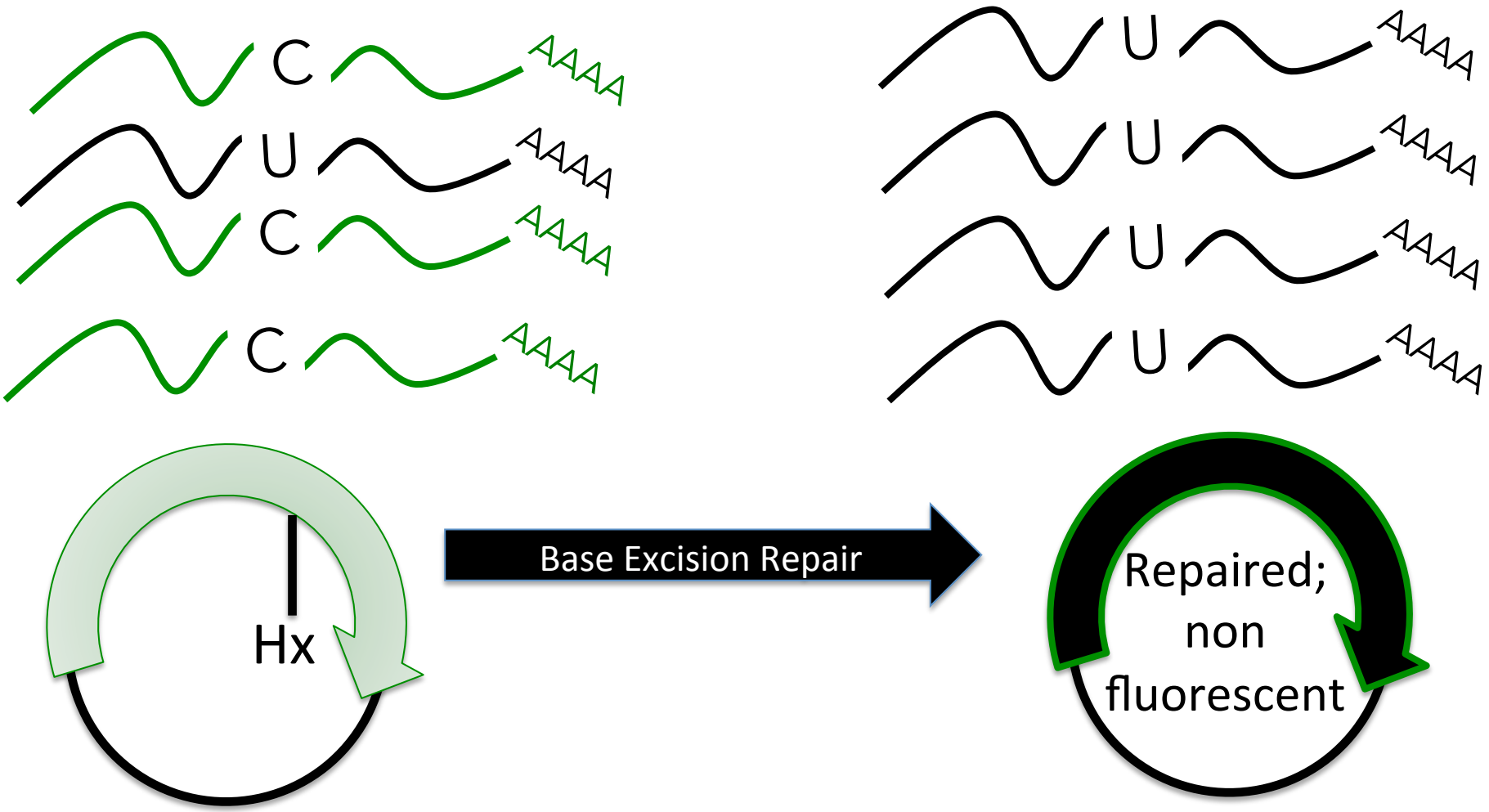
Alex Chaim

Base excision repair of Hx



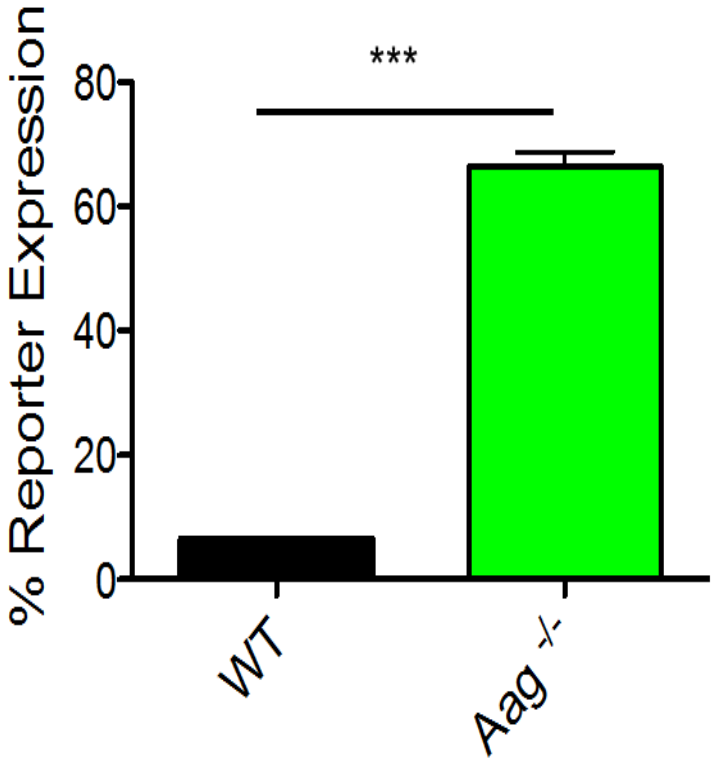
Alex Chaim

Base excision repair of Hx



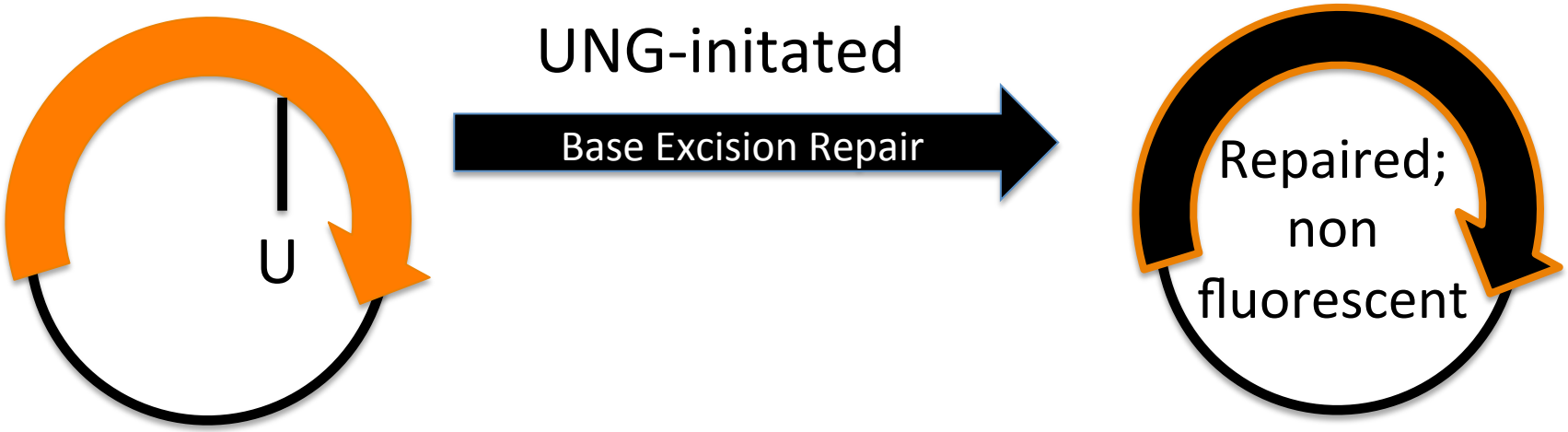
Alex Chaim

Measuring BER of Hx



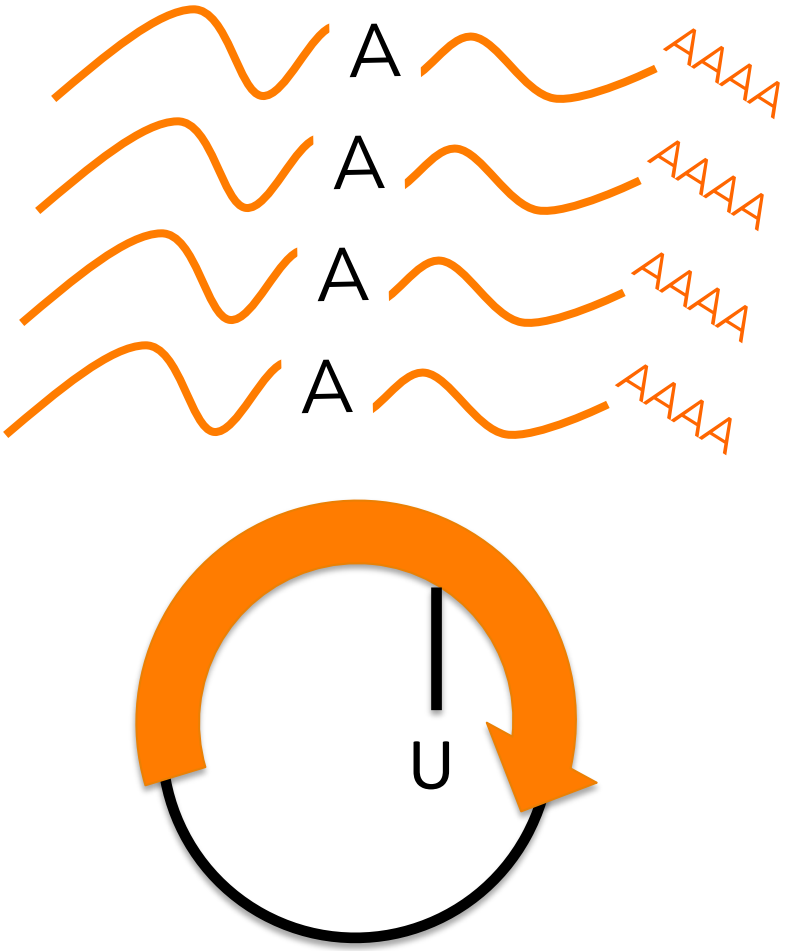
Alex Chaim

Base excision repair of Uracil



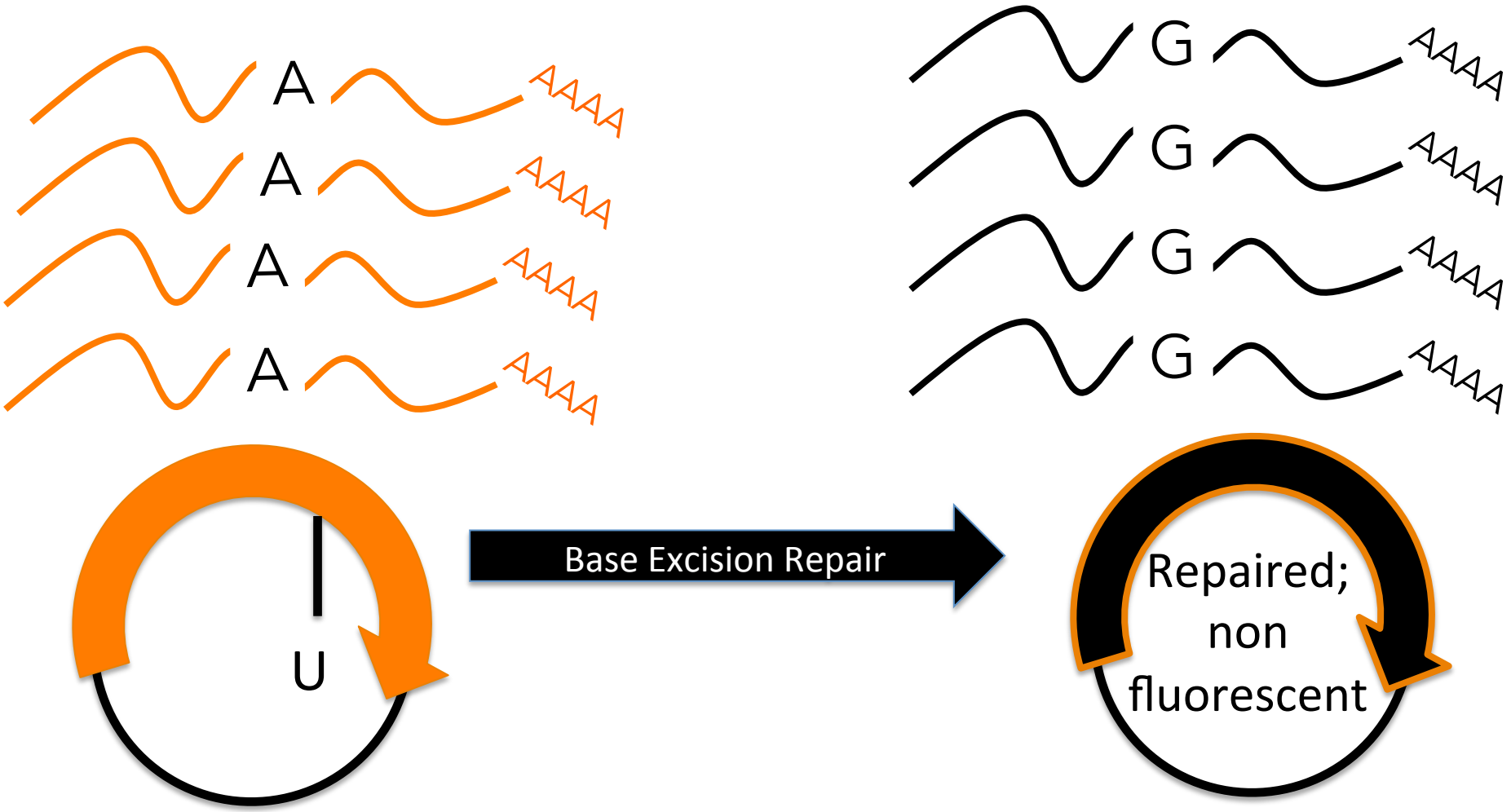
Alex Chaim

Base excision repair of Uracil



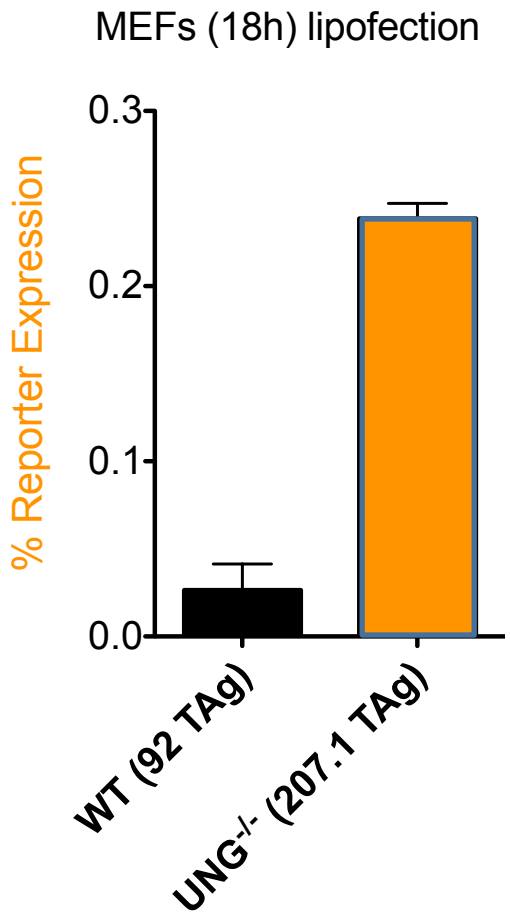
Alex Chaim

Base excision repair of Uracil



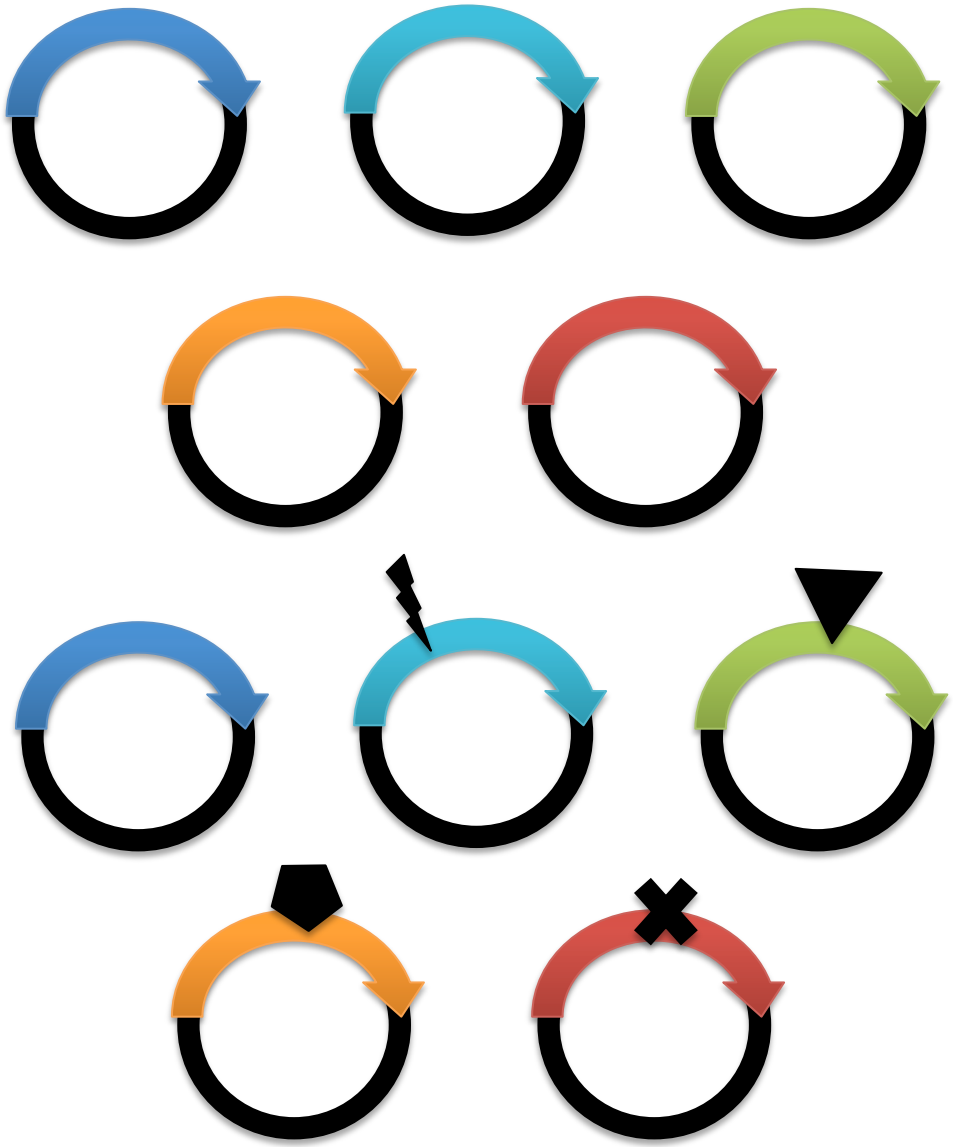
Alex Chaim

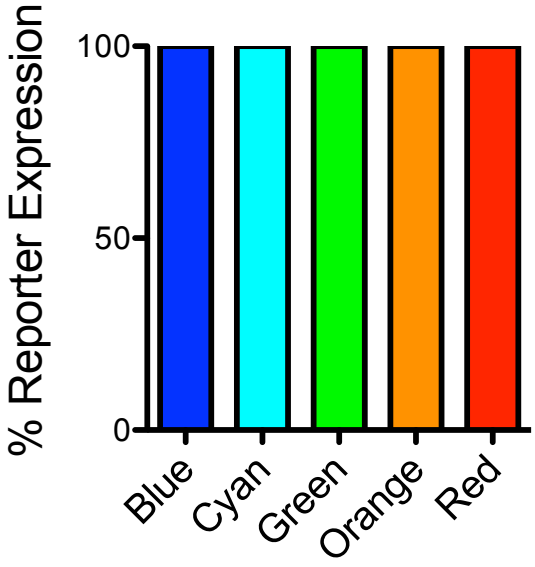
Measuring BER of Uracil

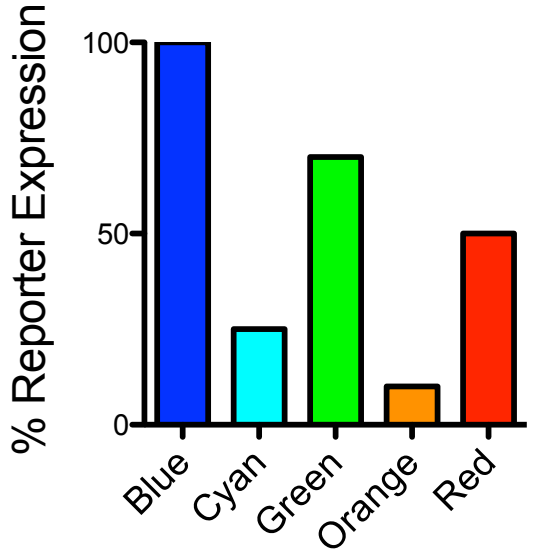


Alex Chaim

5 color HCR assay applications

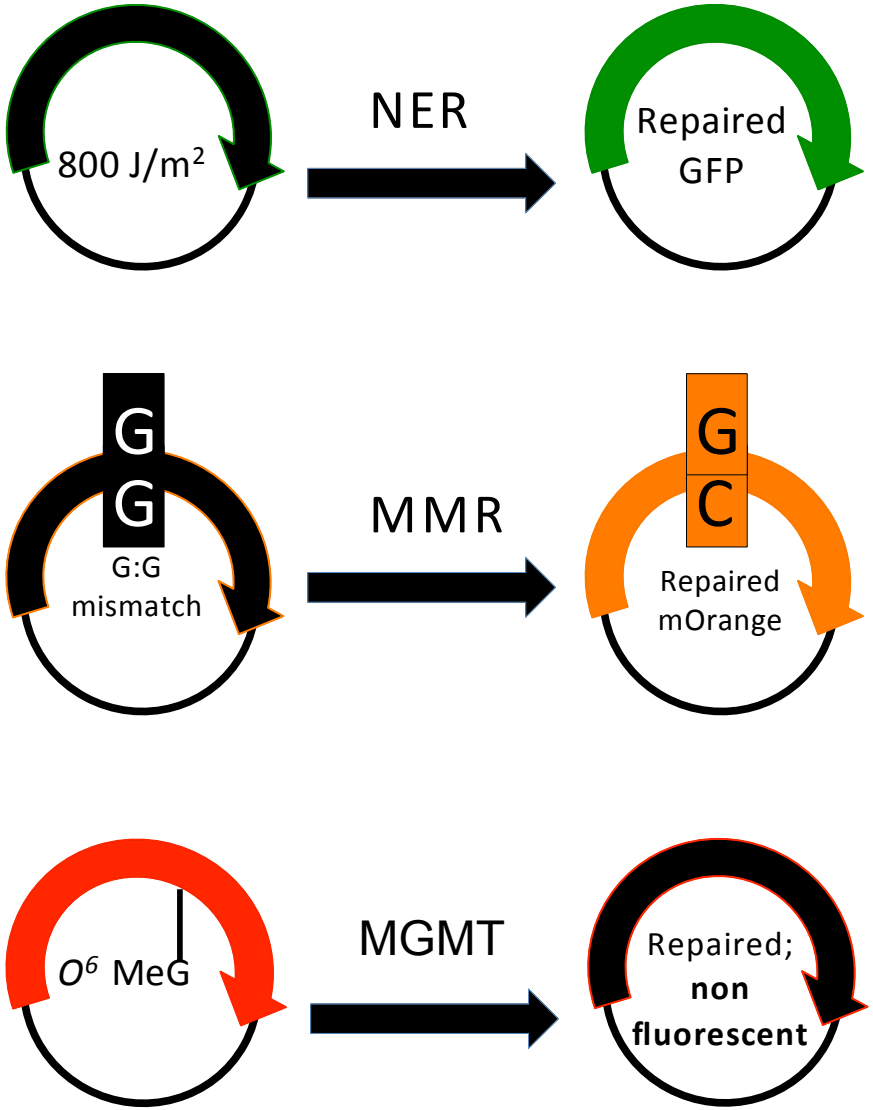




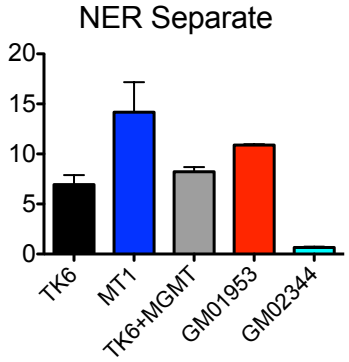


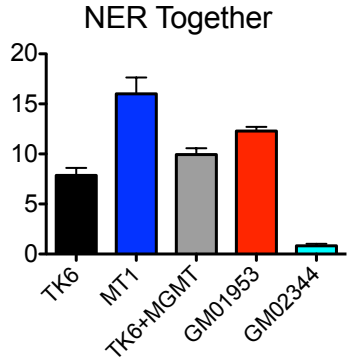
5-color HCR developed by **Dr. Zachary Nagel**

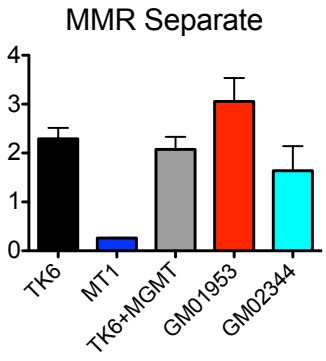
Started by Measuring DRC in 3 pathways

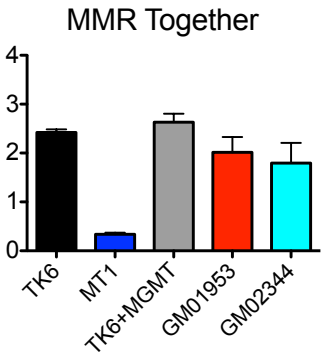


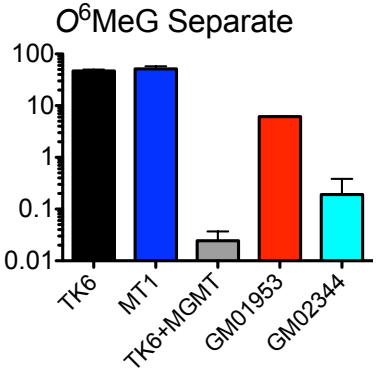
Started by Measuring DRC in 3 pathways in 5 different cell lines

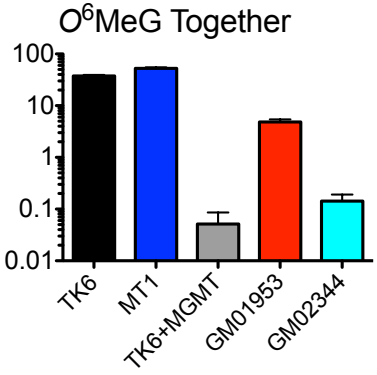












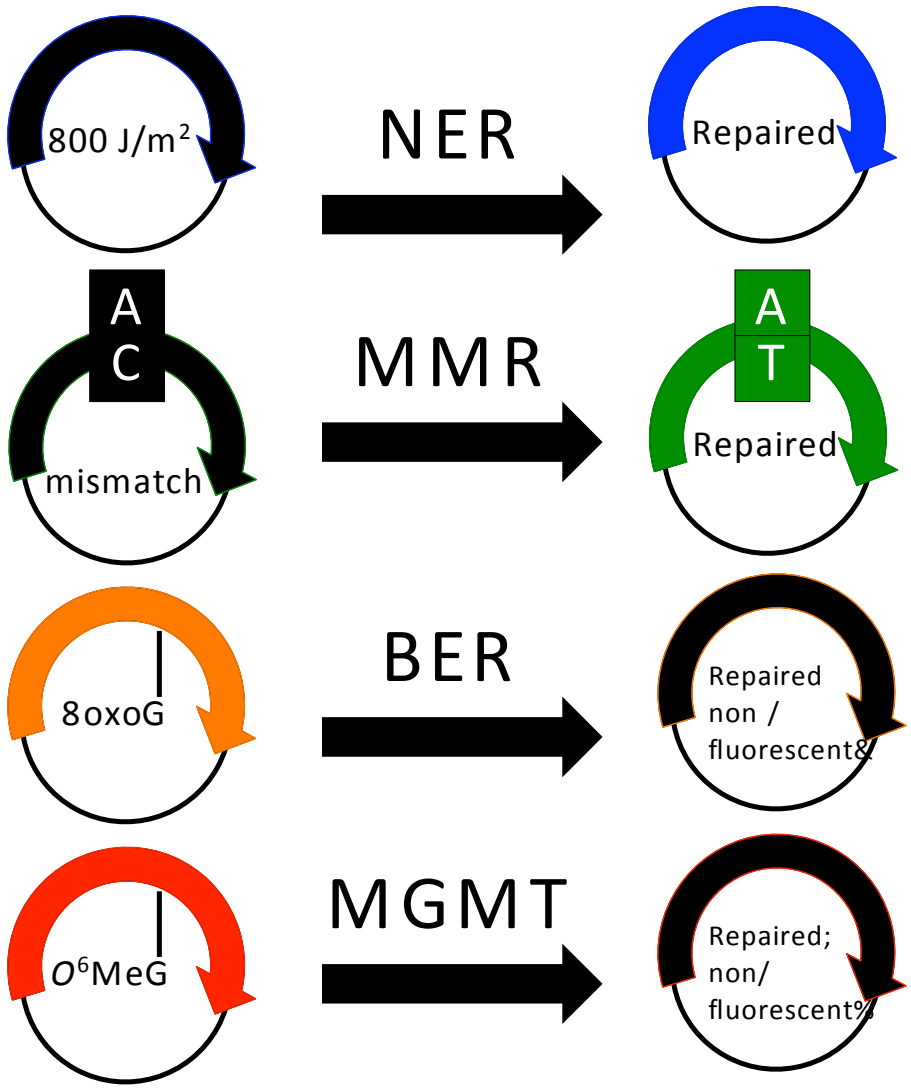
GM02344
NER-deficient (XPA)

MT1
MMR-deficient (MSH6 null)

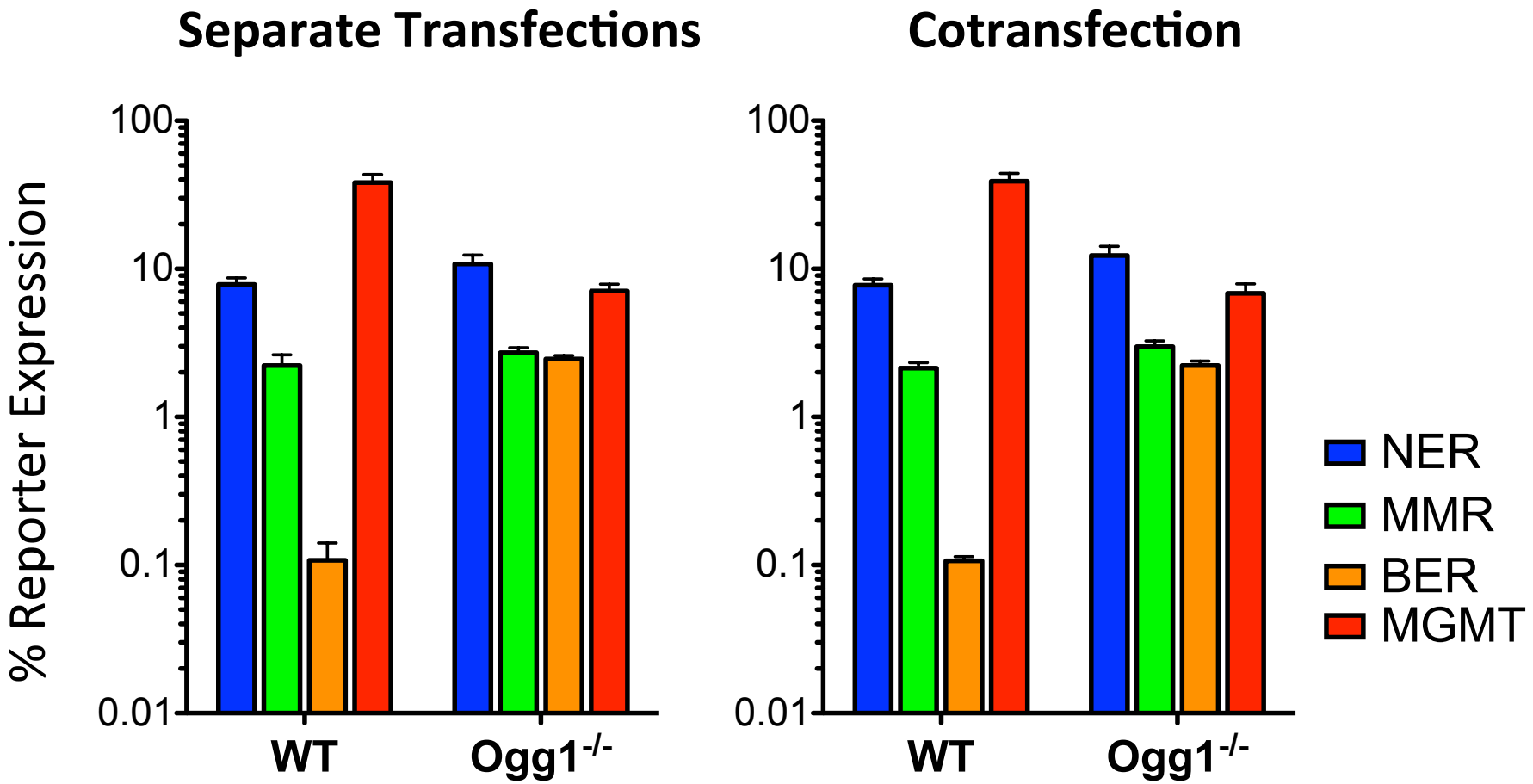
TK6+MGMT
Overexpressing MGMT

(TK6 and GM1953 “WT”)

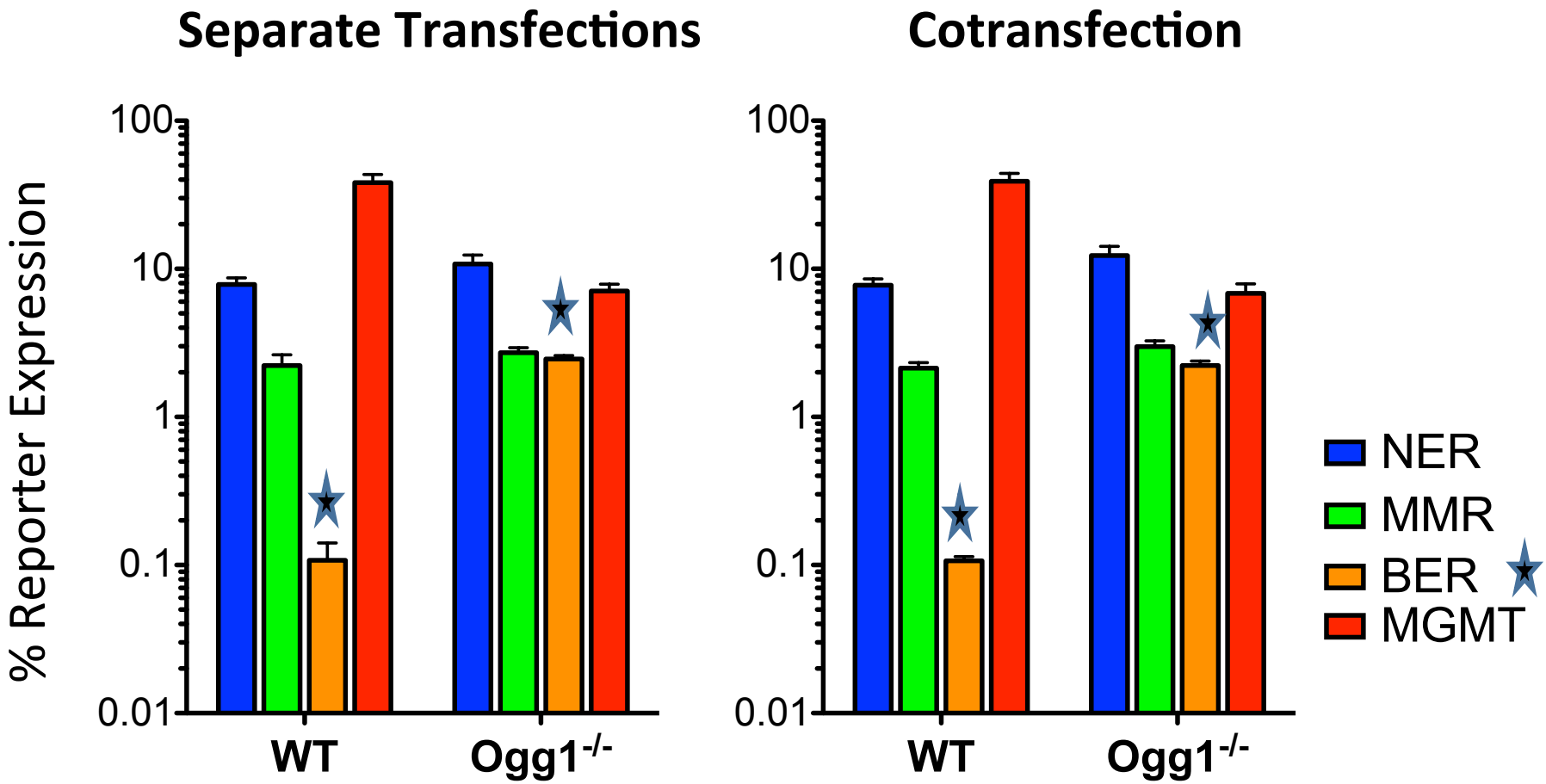
Then Measured DRC in 4 pathways in a single assay:



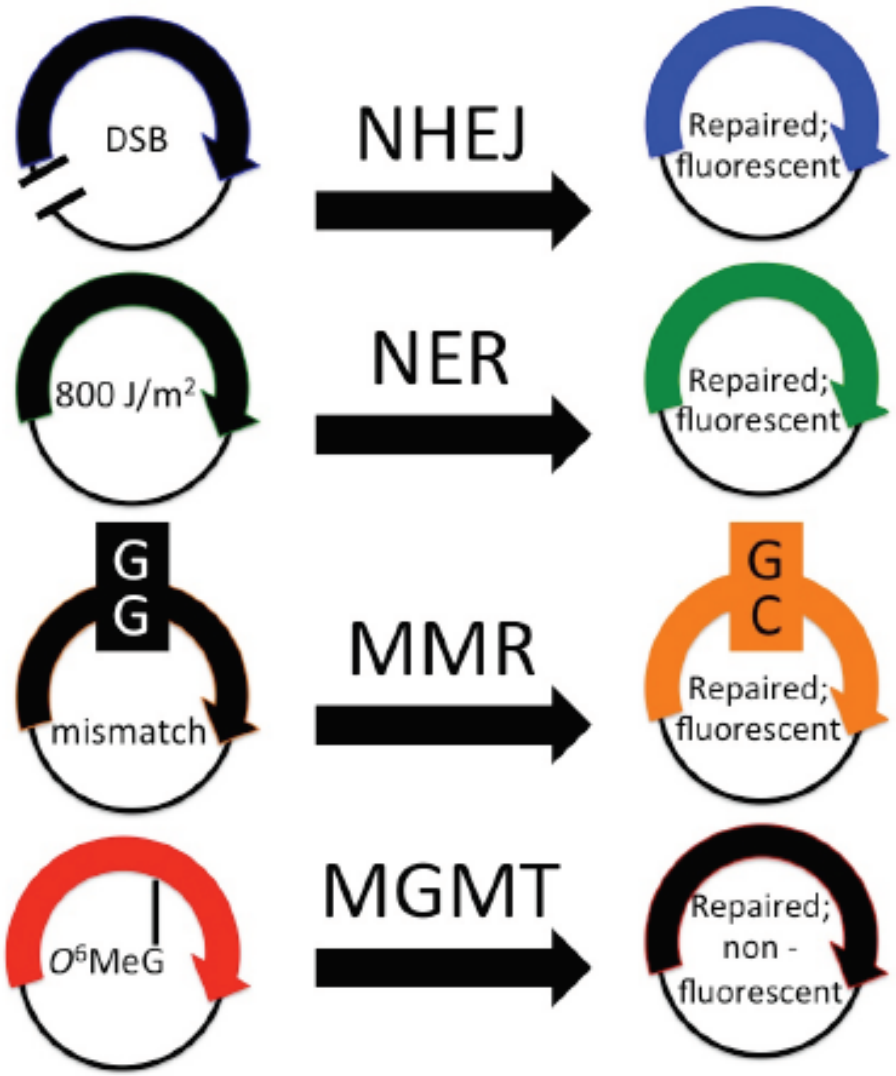
Then Measured DRC in 4 pathways in a single assay (1 of 2):



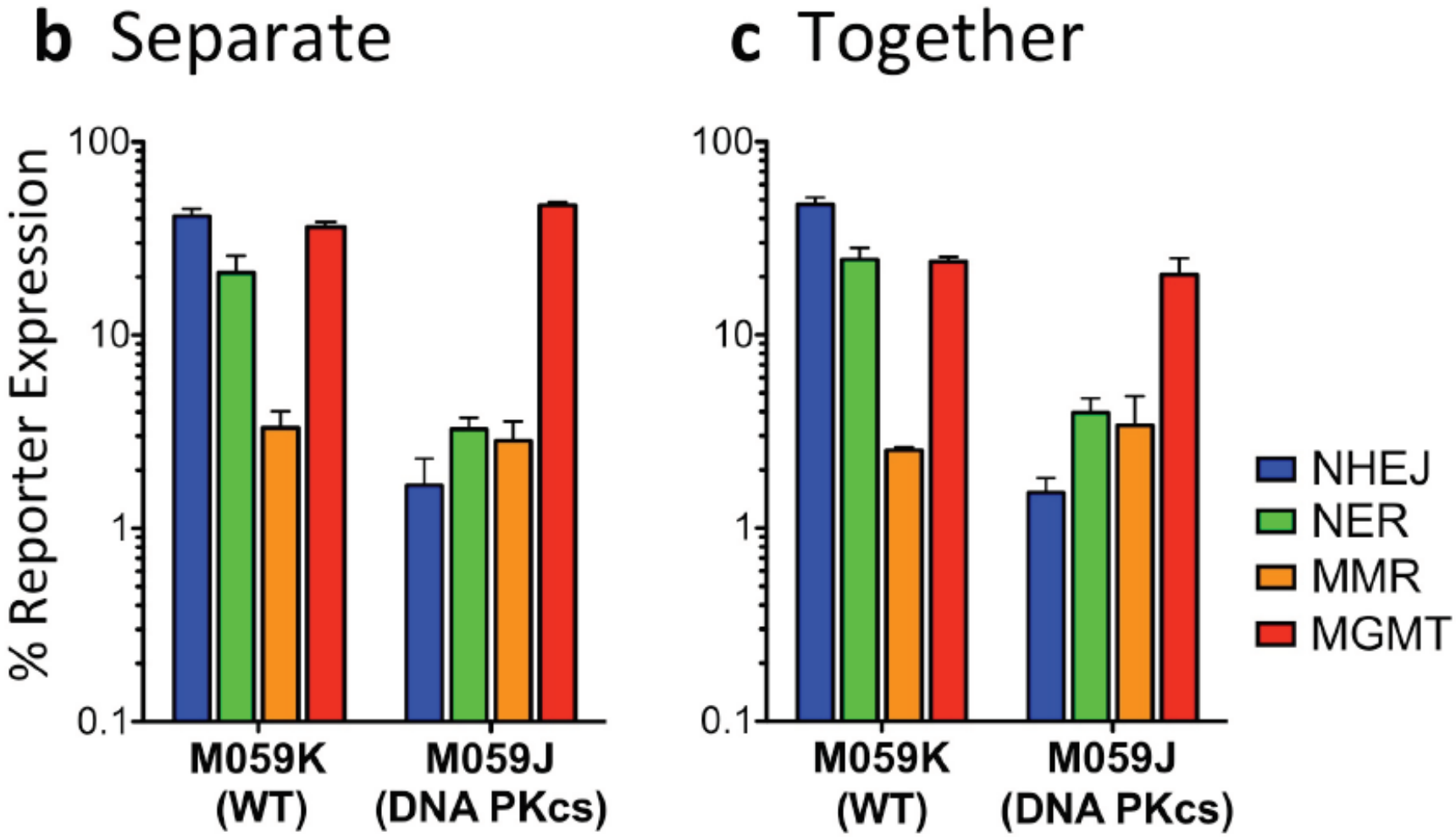
Then Measured DRC in 4 pathways in a single assay (1 of 2):



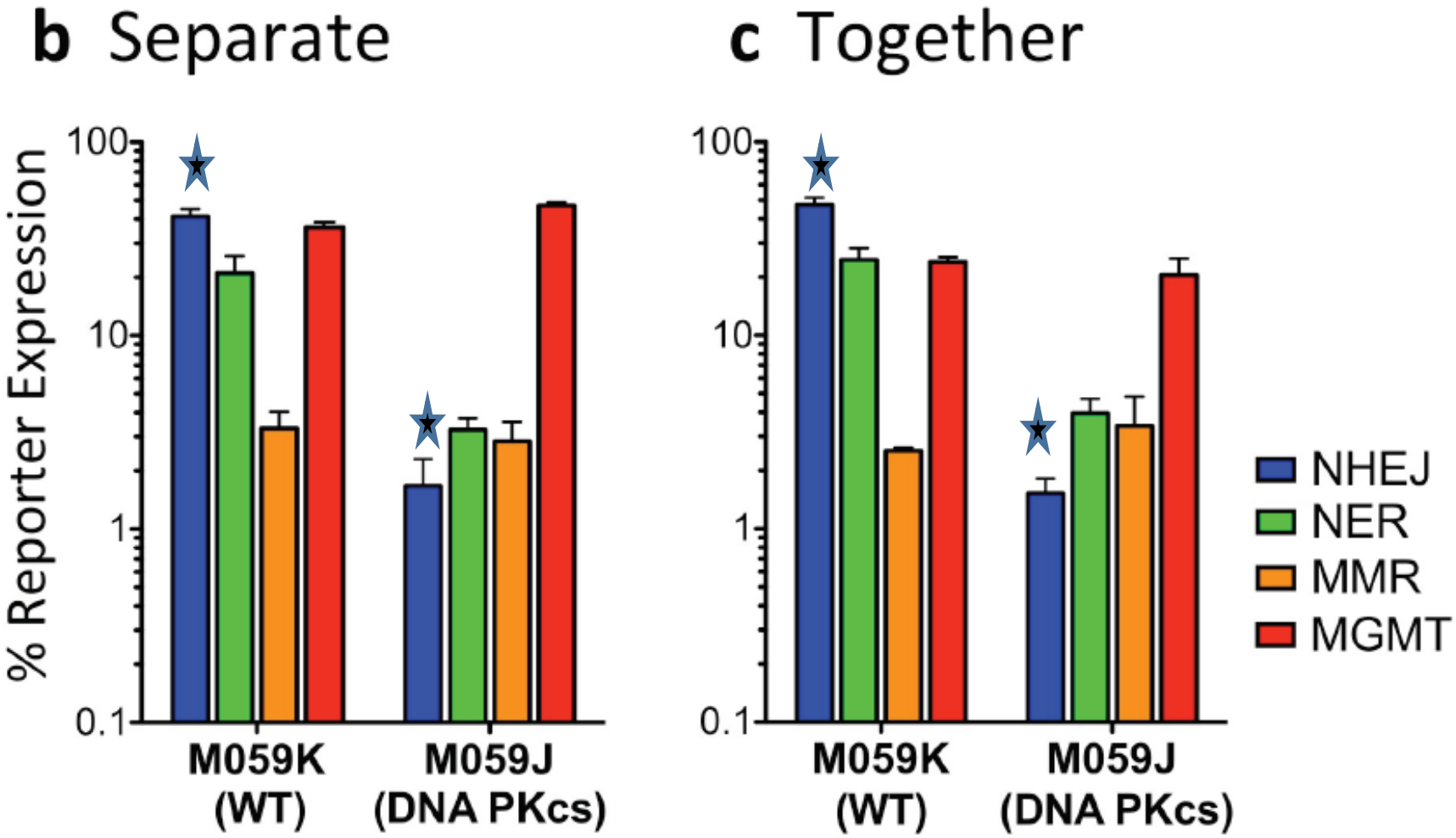
Then Measured DRC in 4 pathways in a single assay (2 of 2):



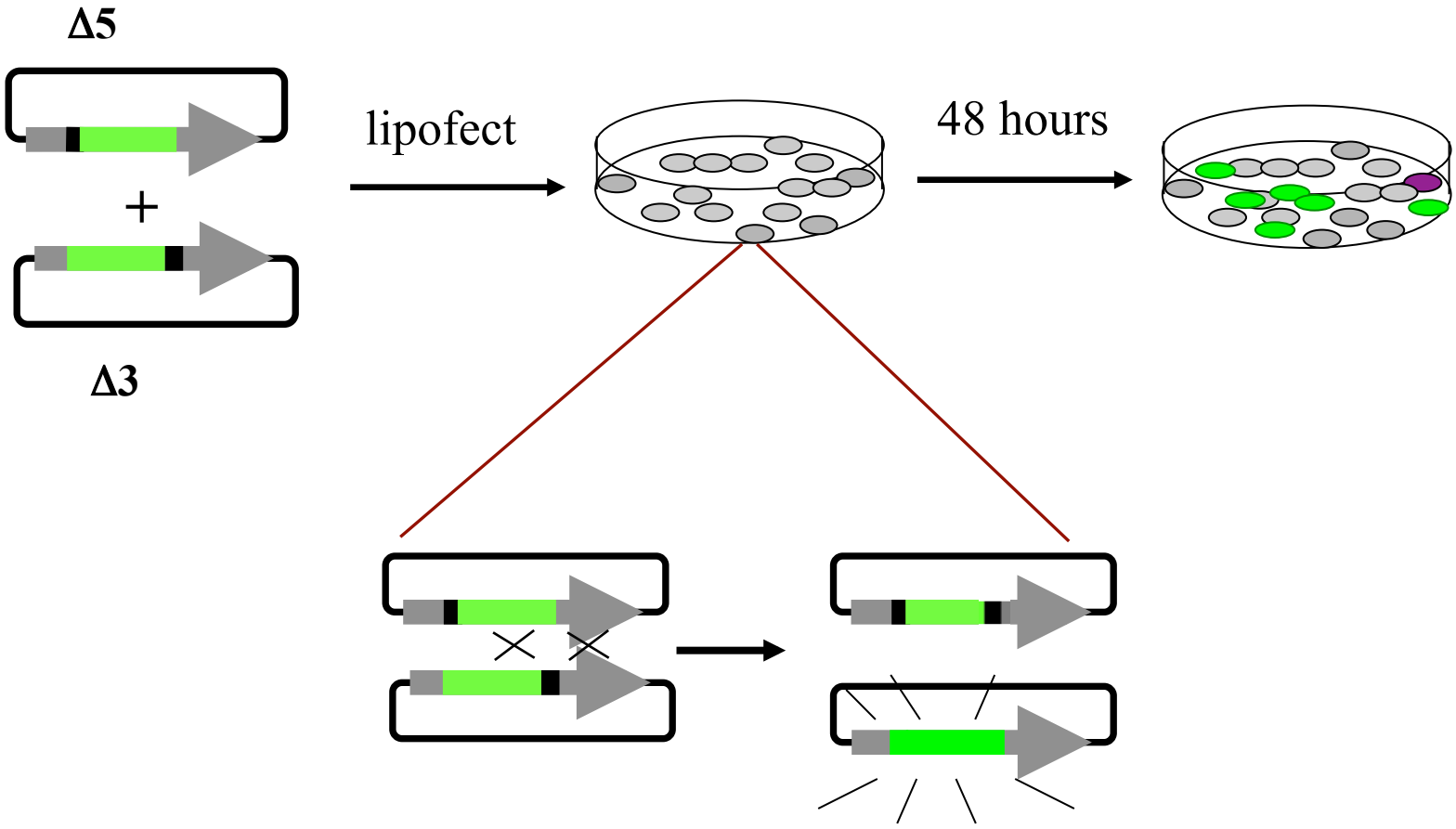
Then Measured DRC in 4 pathways in a single assay (2 of 2):



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

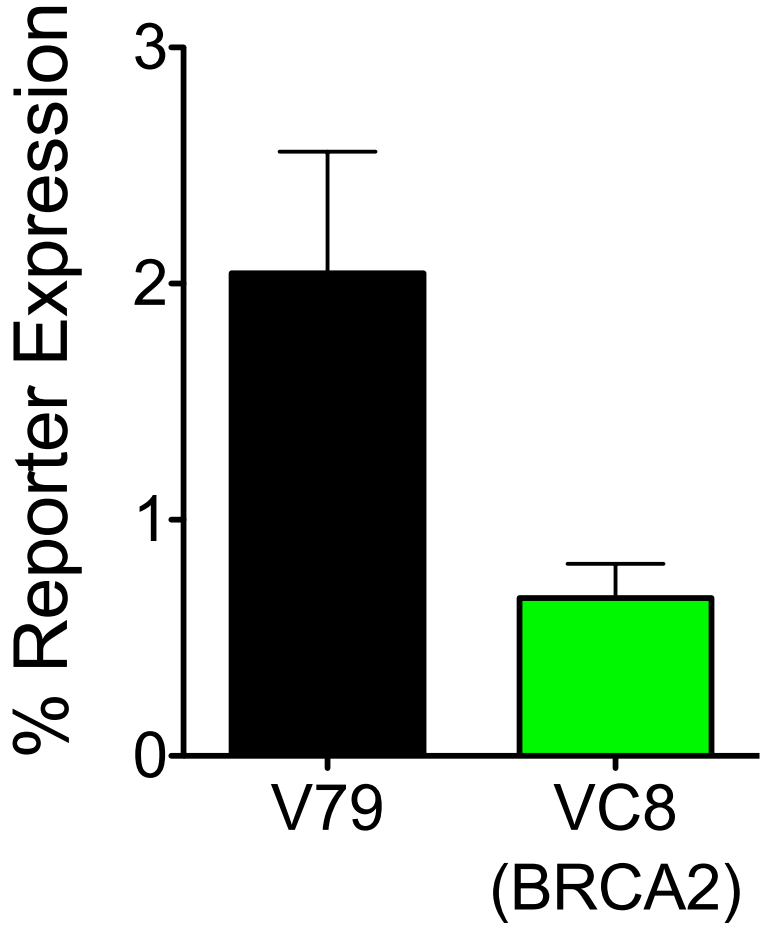


A Plasmid-Based Assay for Homologous Recombination in Mammalian Cells



Bevin Engelward

A Plasmid-Based Assay for Homologous Recombination in Mammalian Cells



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

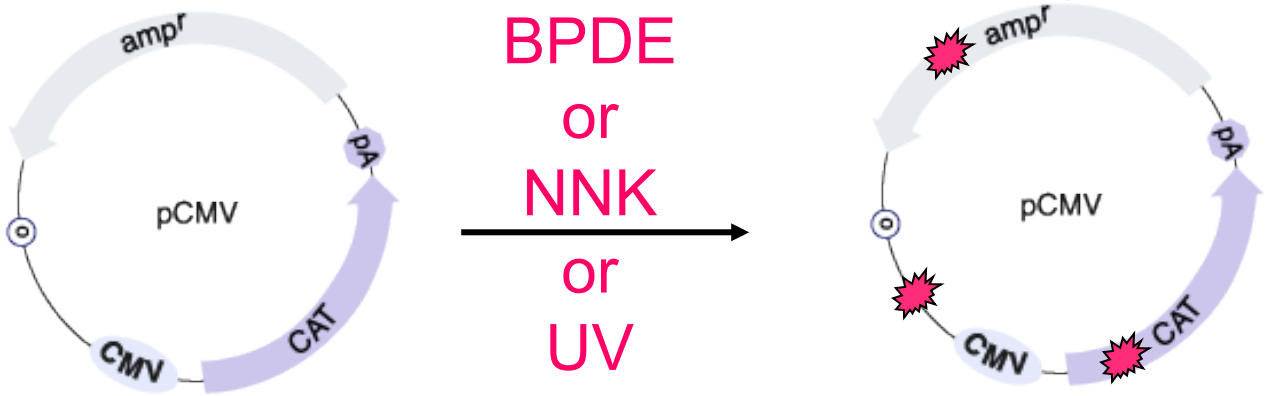


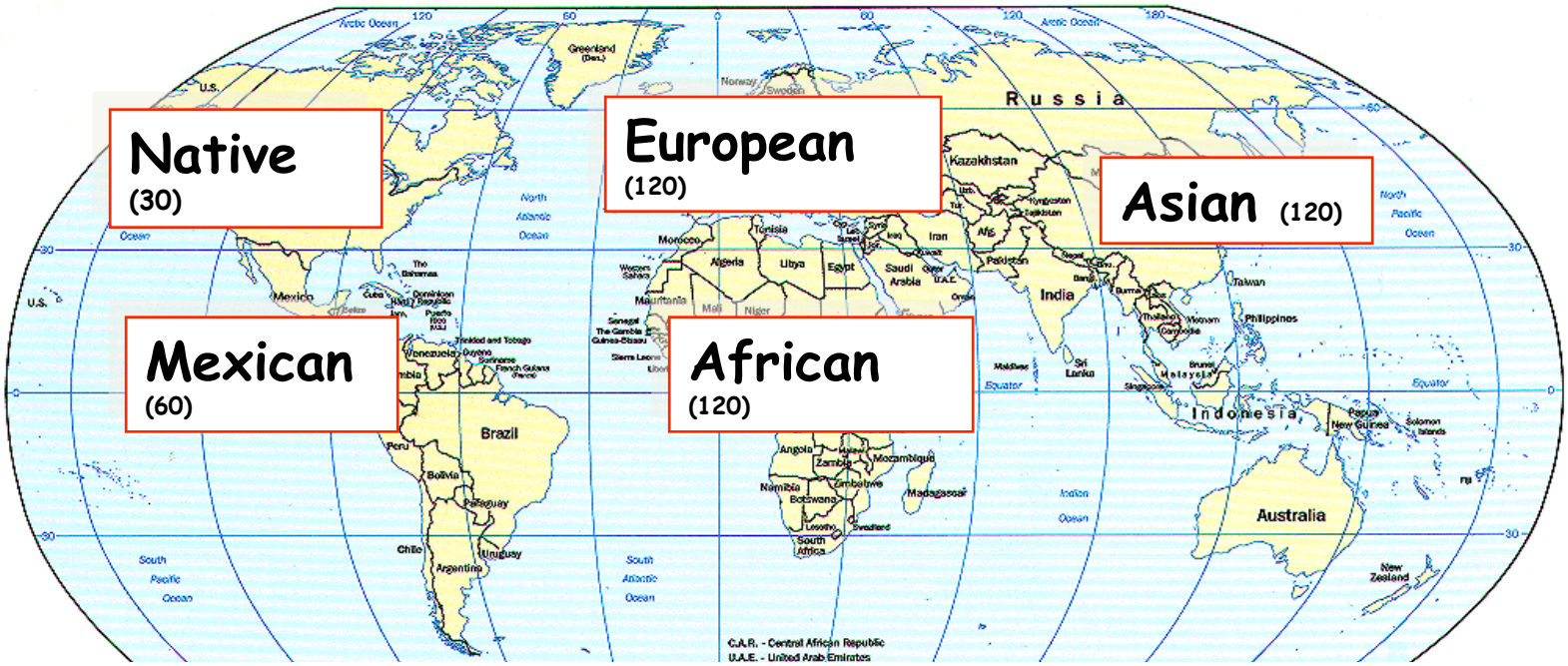
TABLE III – HCR-DRC FOR RISK OF CANCERS

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)

Coriell Lymphoblastoid Cell line collection derived from ethnically diverse HEALTHY humans

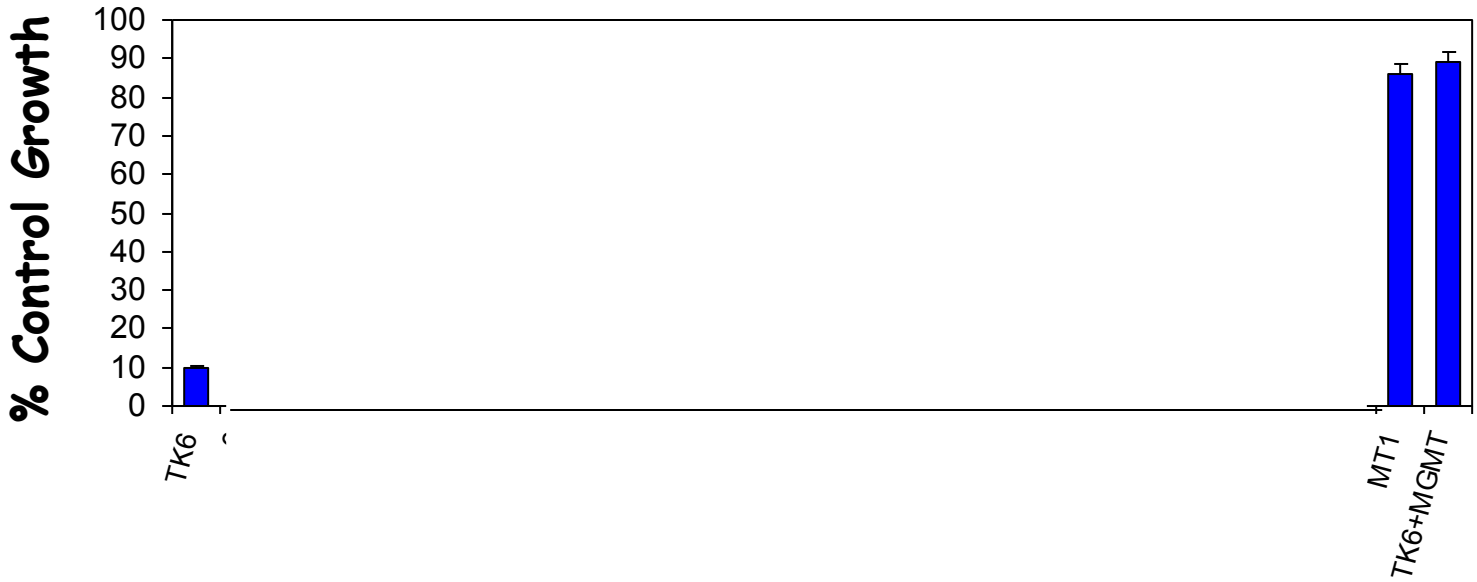


450 healthy unrelated US residents with ancestry from around the globe

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



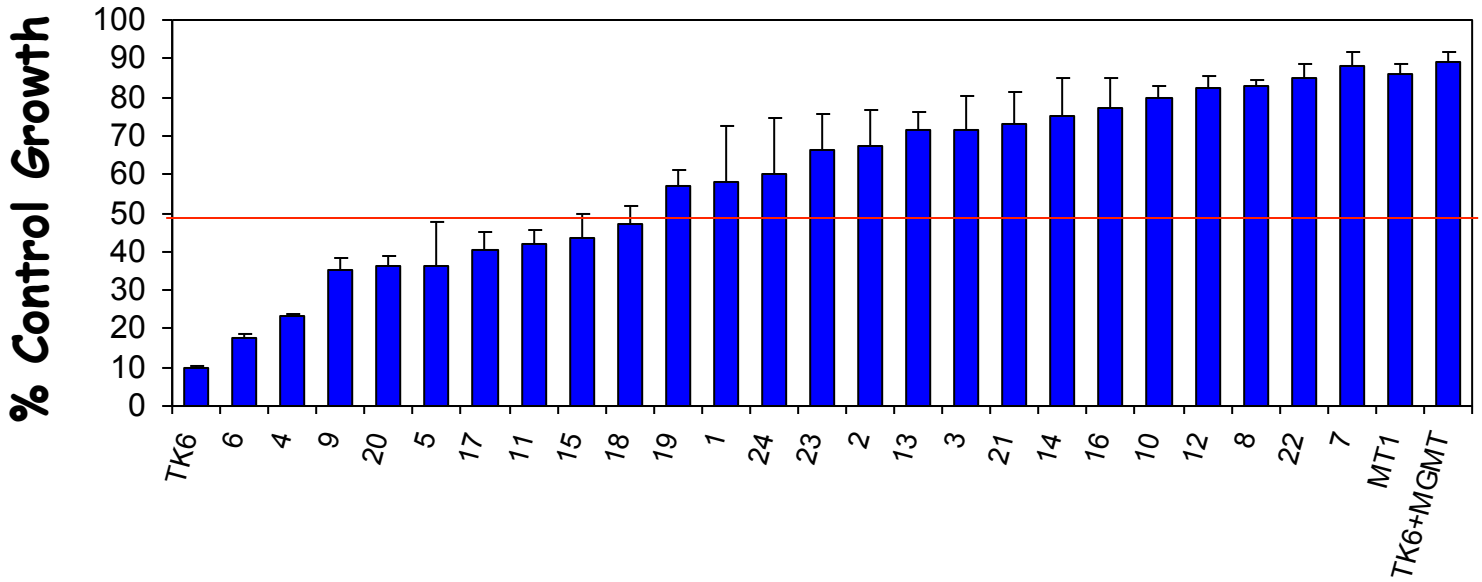
Sensitive

To MNNG

Resistant

To MNNG

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



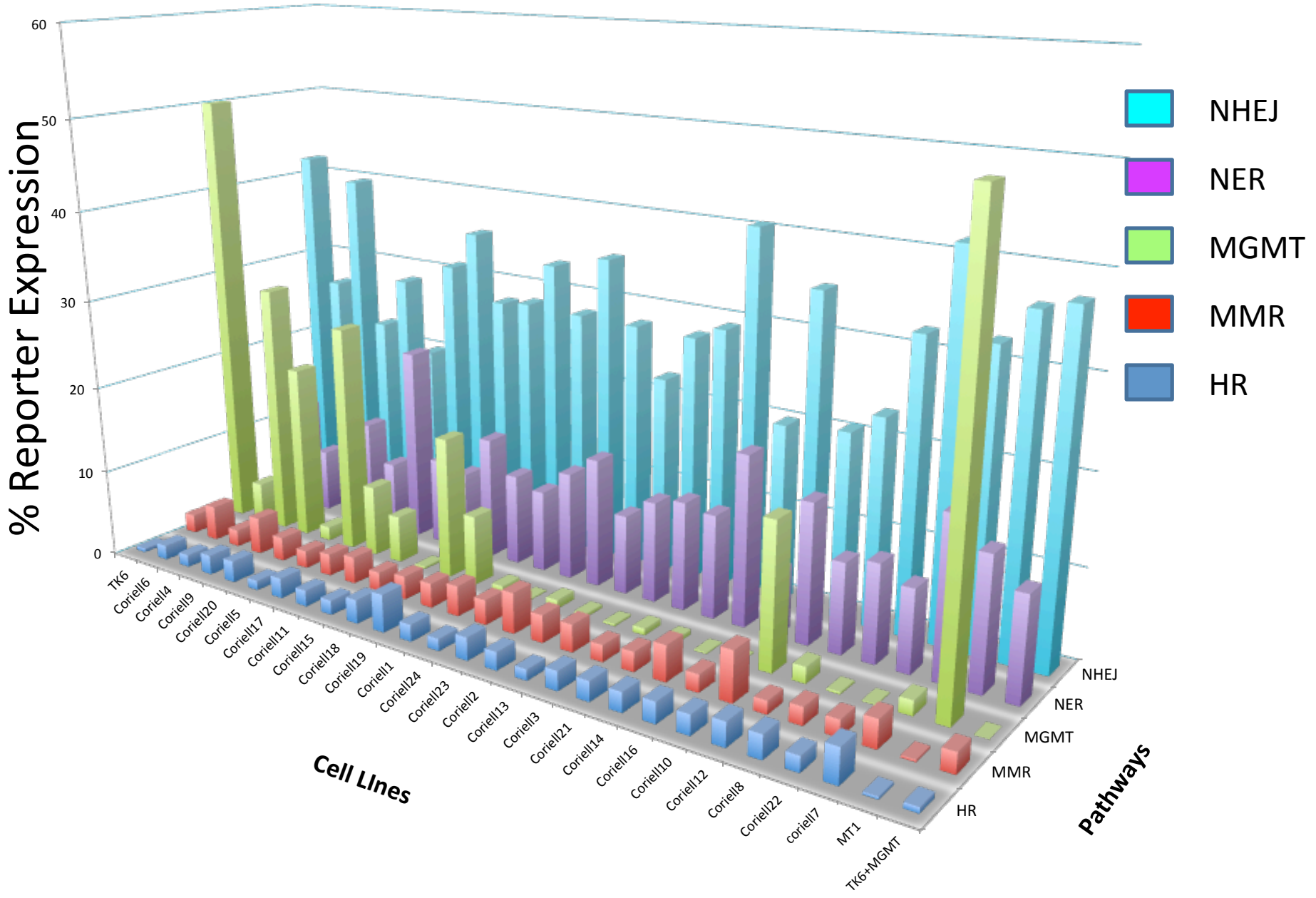
Sensitive

To MNNG

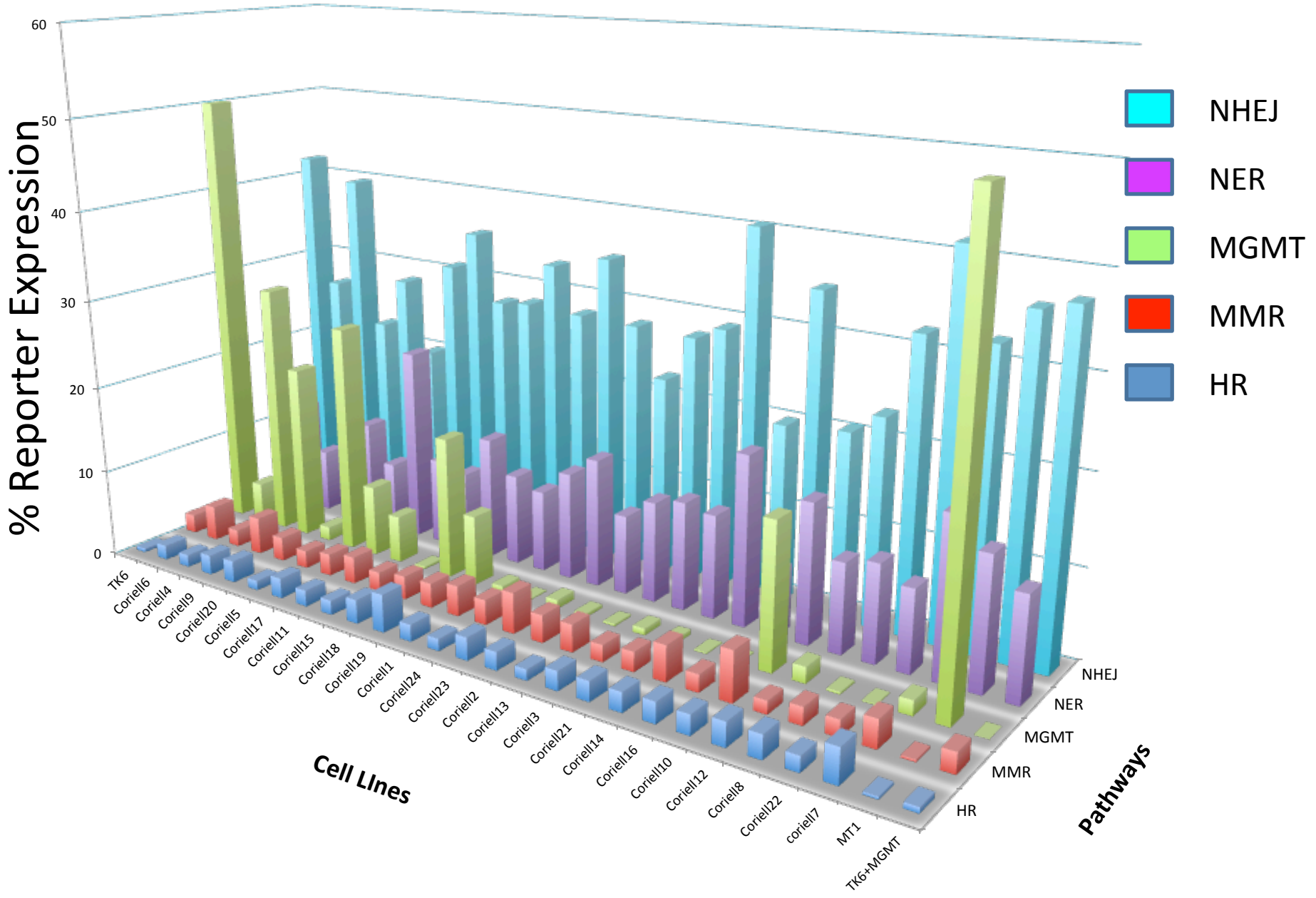
Resistant

To MNNG

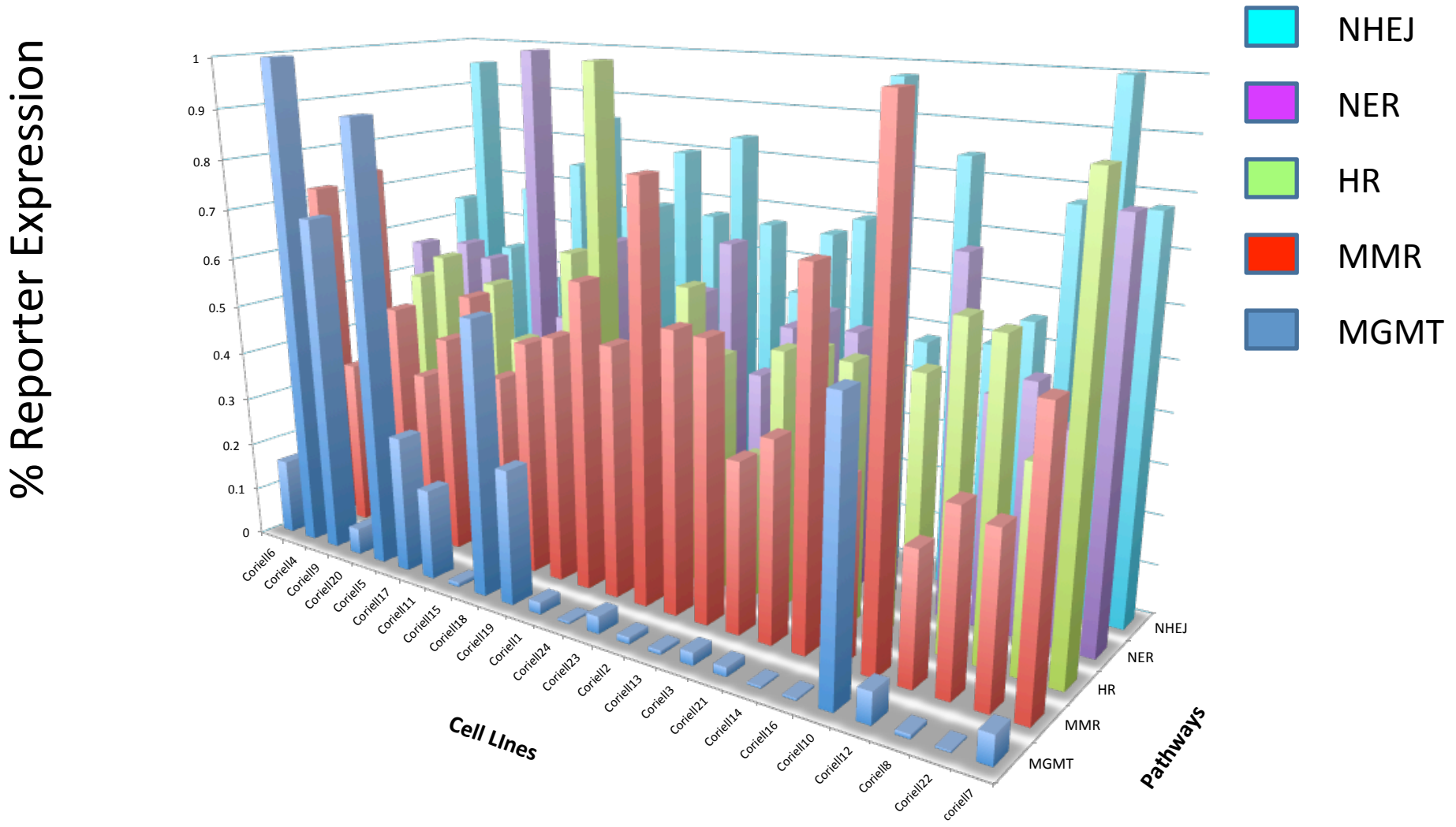
DRC vs. MNNG sensitivity



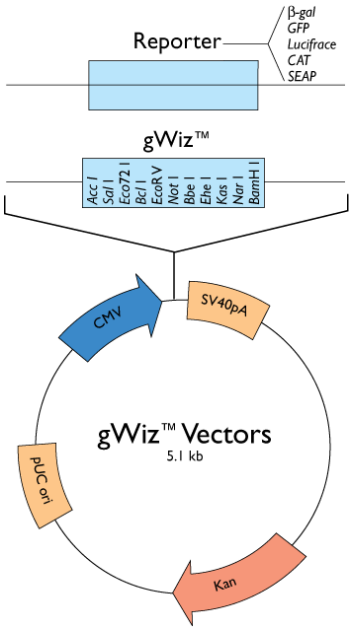
DRC vs. MNNG sensitivity



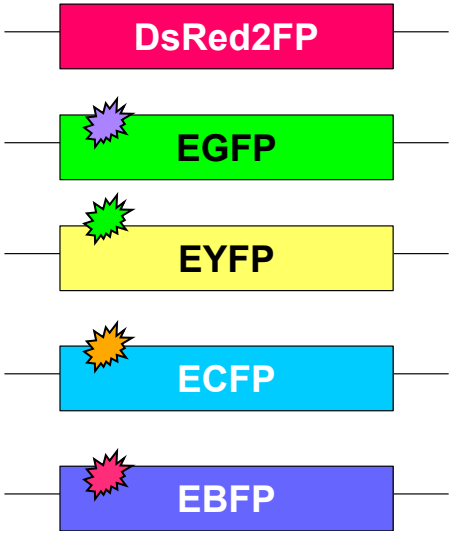
DRC vs. MNNG sensitivity



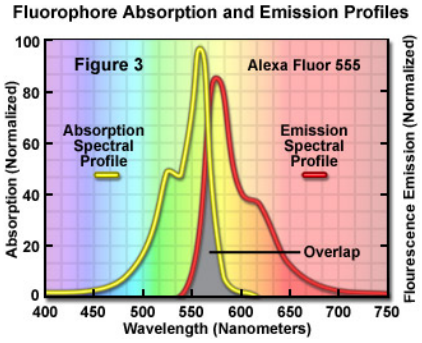
Reactivation of damaged DNA - multiplexed



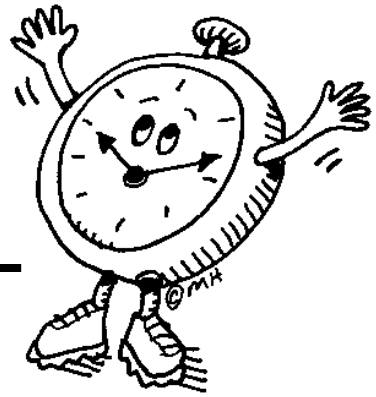
+ different DNA lesions



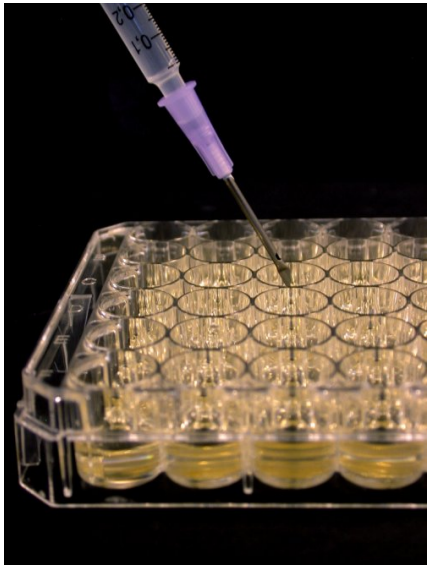
Transient transfection of mixture



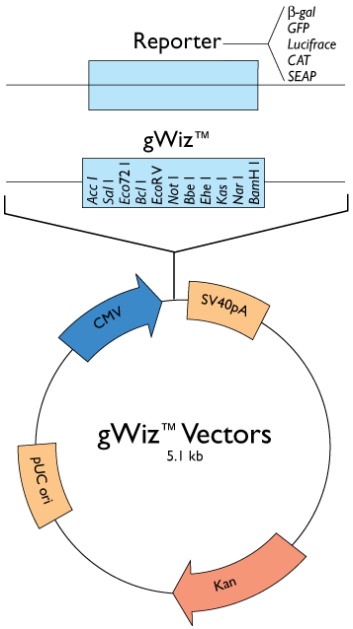
Fluorescence quantitation



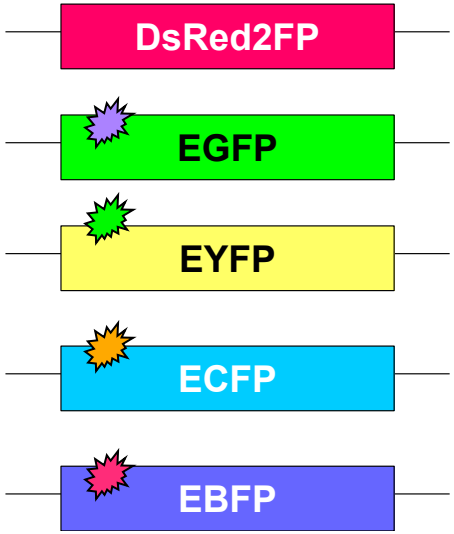
Time to repair



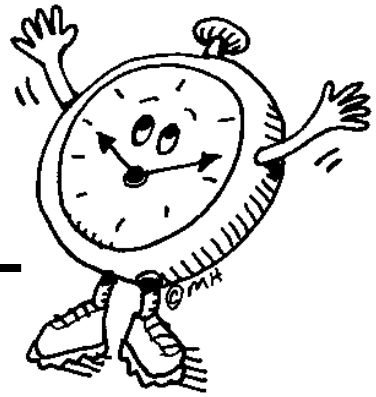
Reactivation of damaged DNA - multiplexed



+ different DNA lesions



Transient transfection of mixture



Time to repair

Next Gen Sequencing

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

The Pioneer Team

Dr. Zachary Nagel

Carrie Thompson

Dr. Anwaar Ahmad

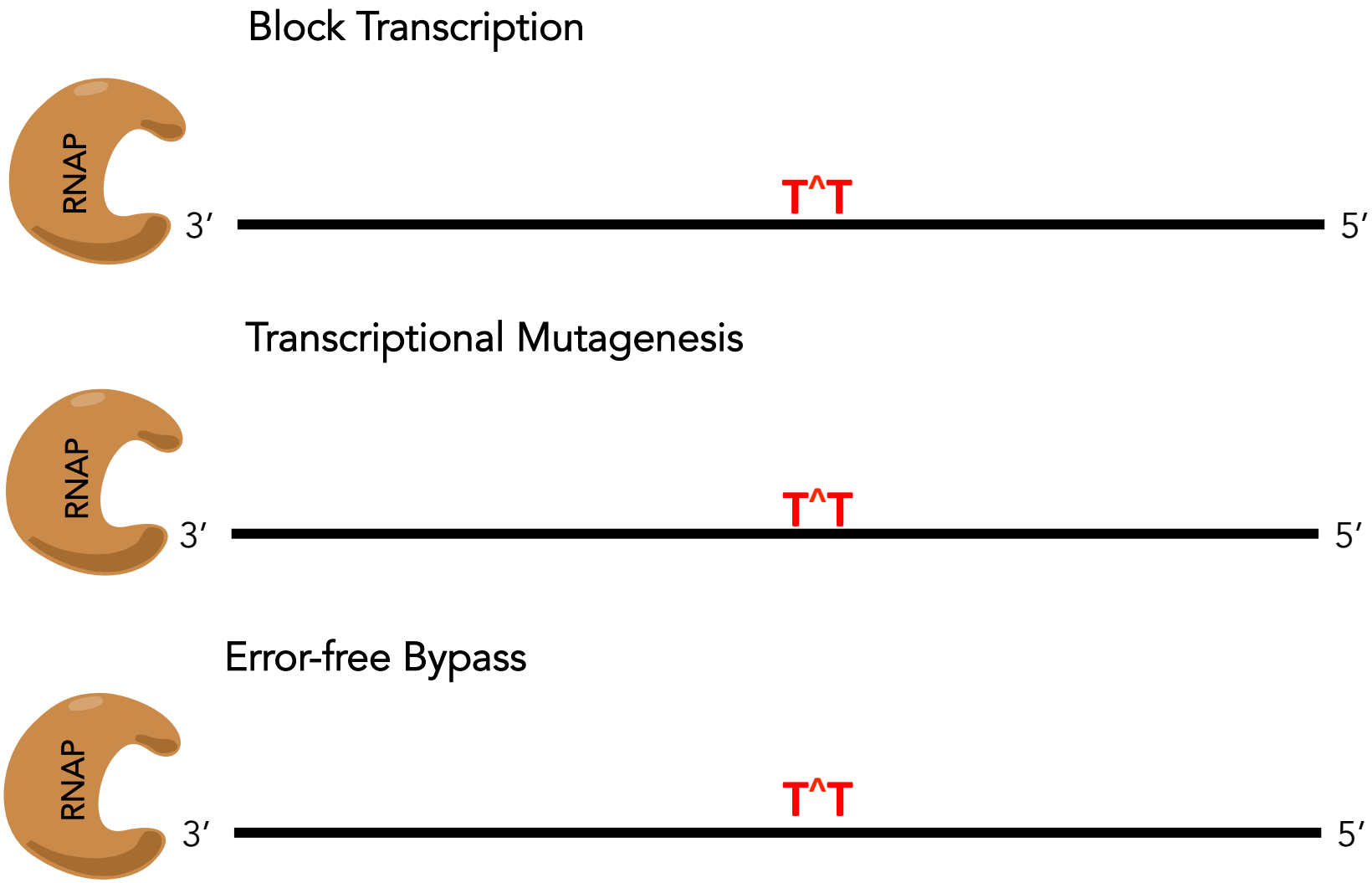
Isaac (Alex) Chaim

Patrizia Mazzucato

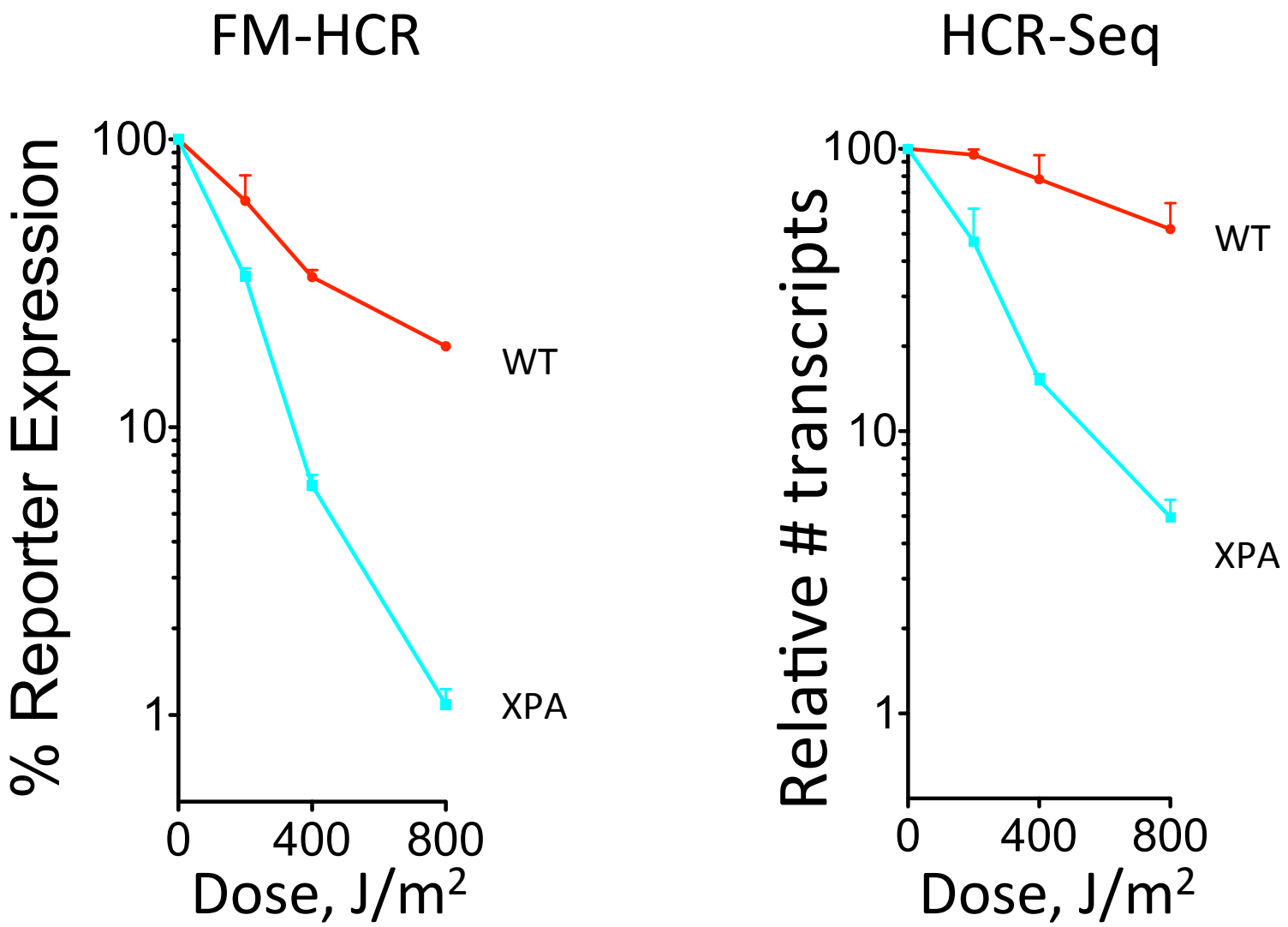
Siobhan McRee

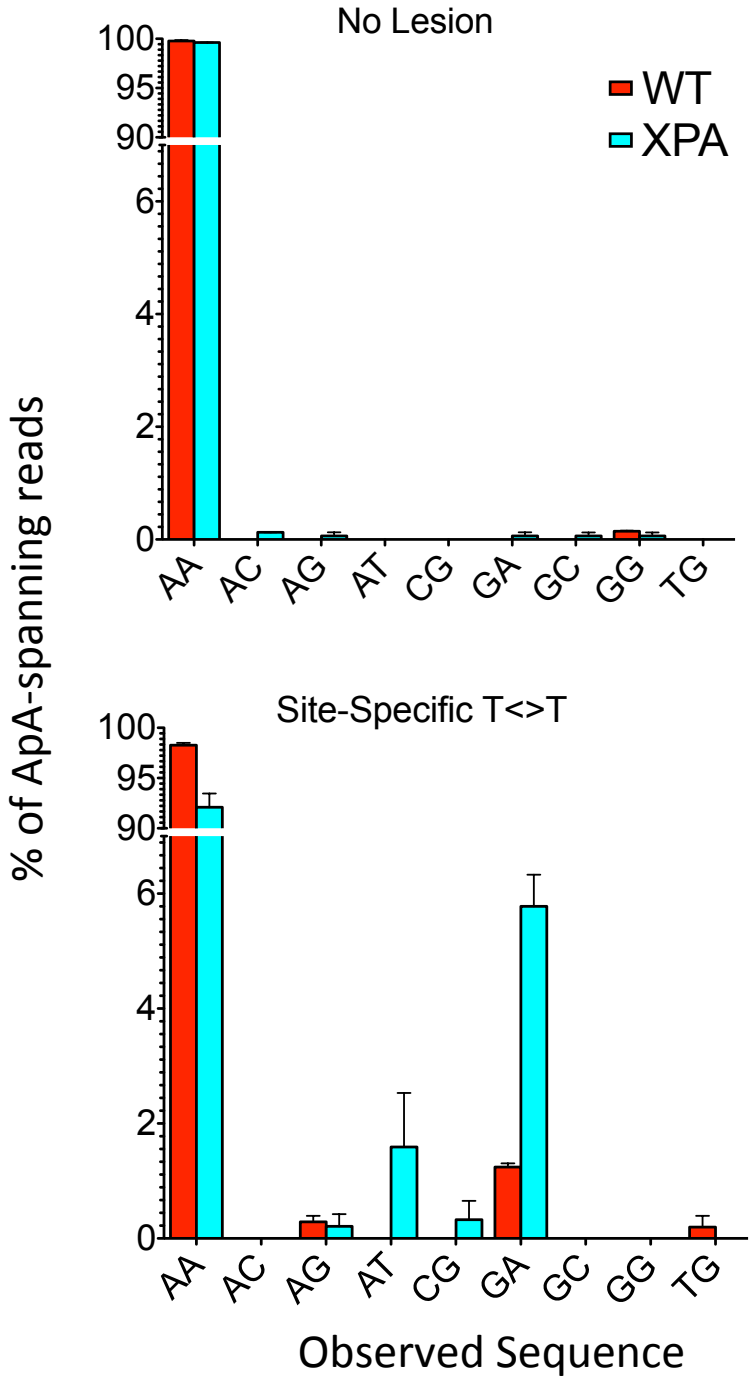
Thanks to the NIH Director's Pioneer Award & the NIEHS!!!

DNA lesions from an RNA polymerase perspective

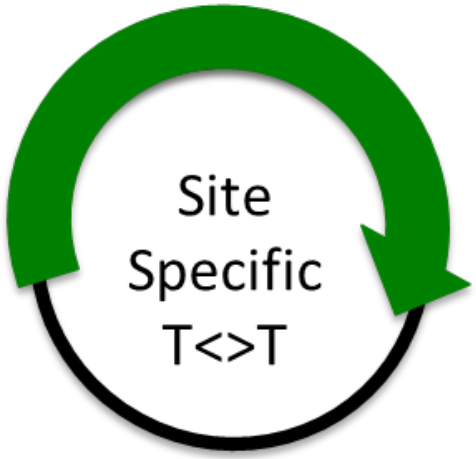


Dose-dependent reporter protein and reporter transcript expression from irradiated plasmids:





Transcriptional errors are induced by a site-specific thymine dimer



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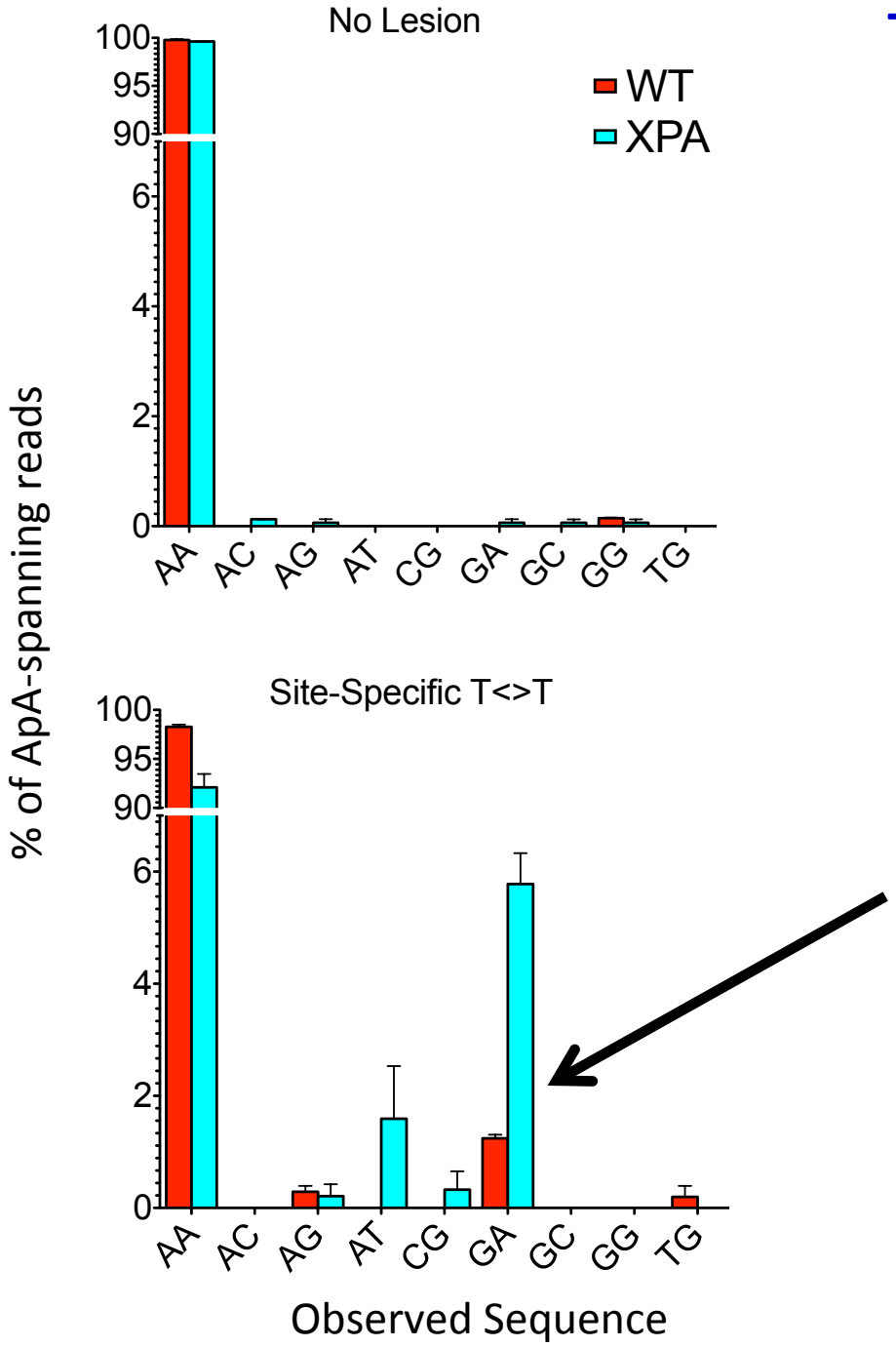
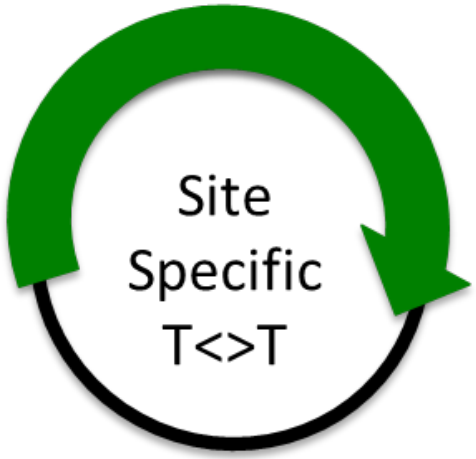
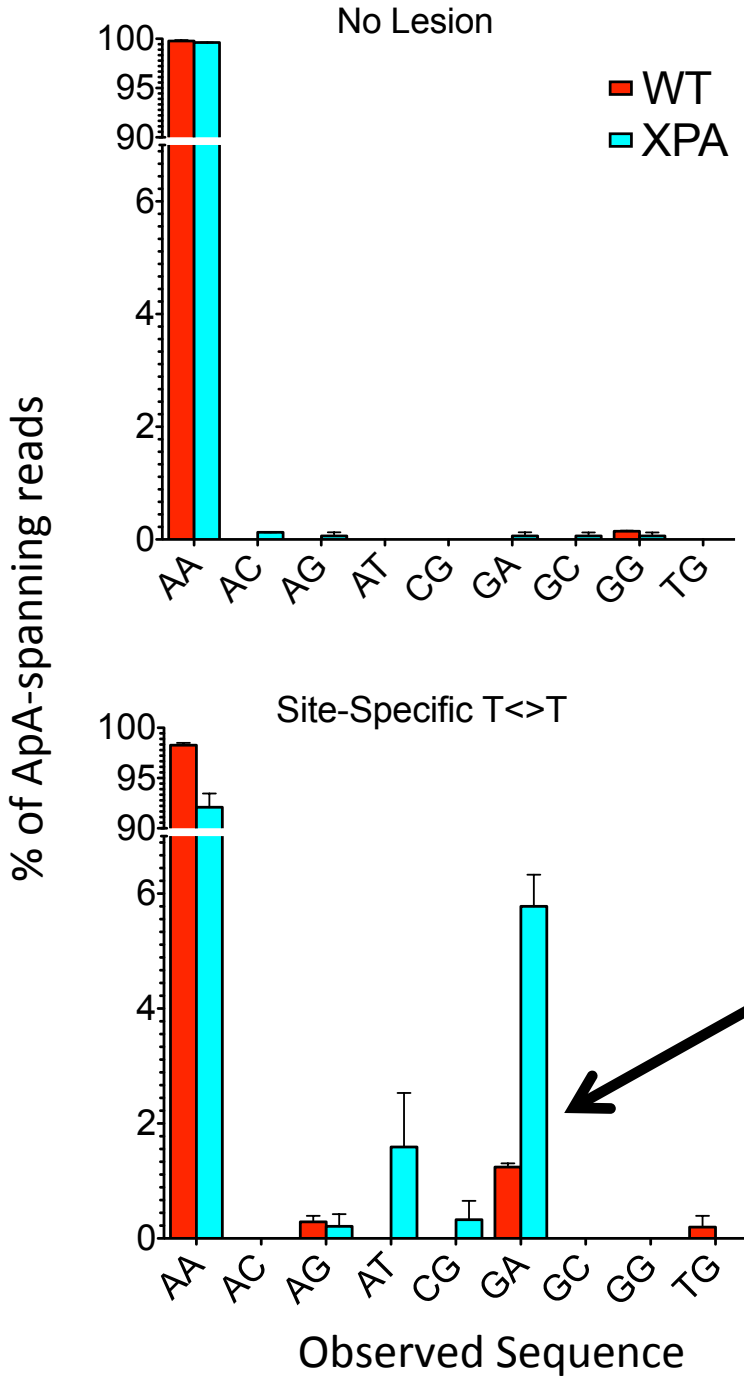
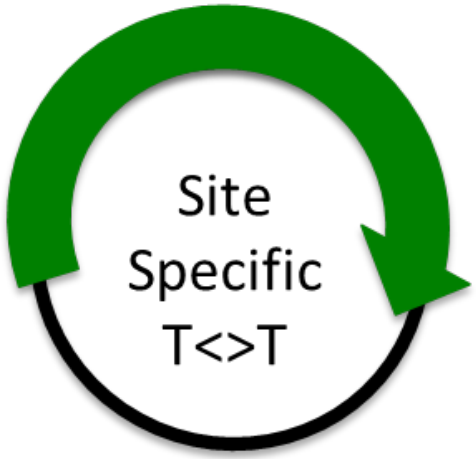




AA→GA was the most frequently observed sequence change

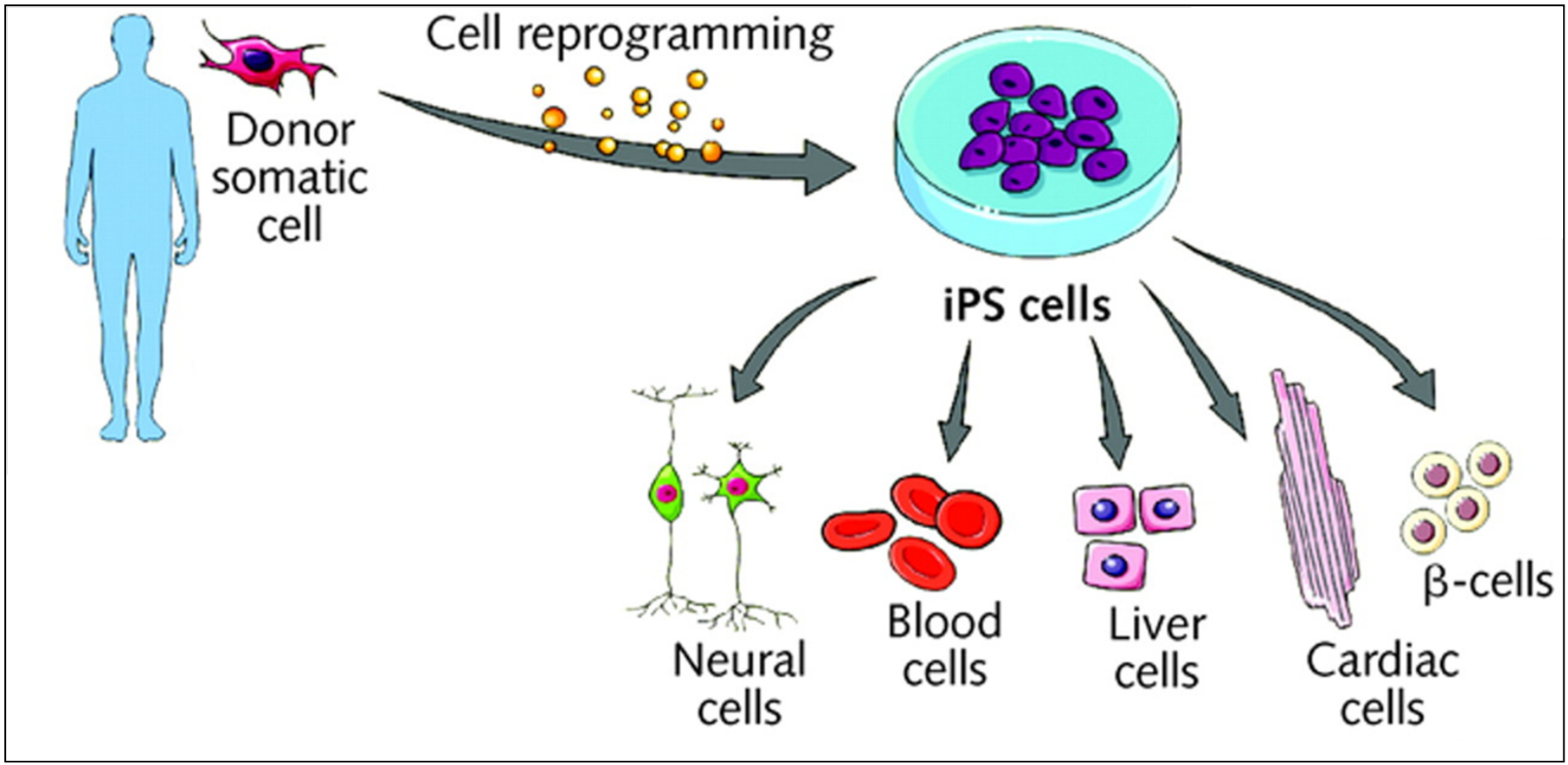
Transcriptional errors are induced by a site-specific thymine dimer





Transcriptional errors are more frequent in the NER-deficient XPA cells

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