

20.109 Spring 2015 Module 2 – Lecture 2

System Engineering and Protein Foundations



Shannon Hughes

Noreen Lyell

Leslie McLain

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Leona Samson (Lectures)

Zachary Nagel (help with development) Alex Chaim



What experimental question will you ask in Module 2?

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

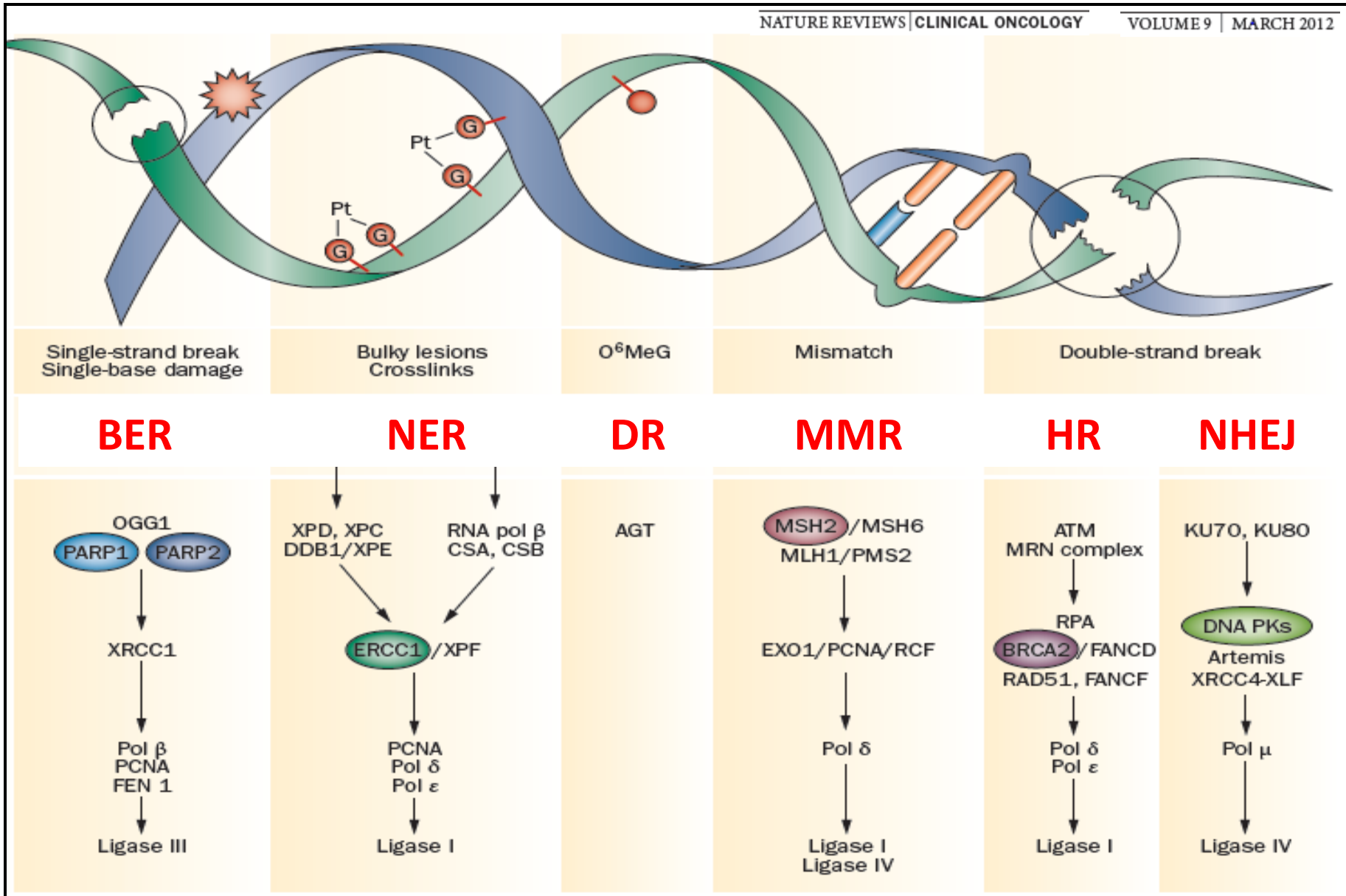


This raises the following questions

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

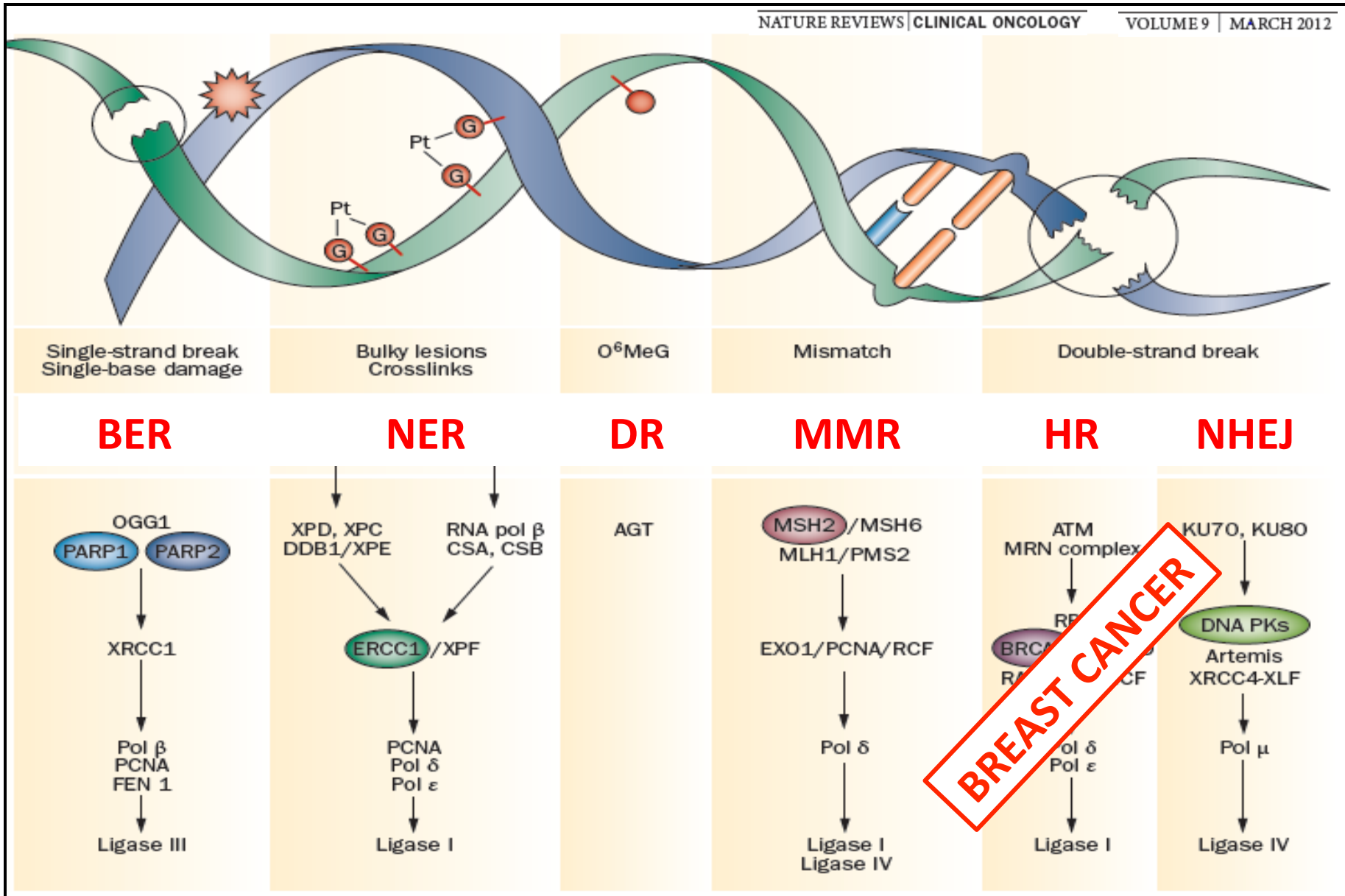
Six Major DNA Repair Pathways

NATURE REVIEWS | CLINICAL ONCOLOGY | VOLUME 9 | MARCH 2012

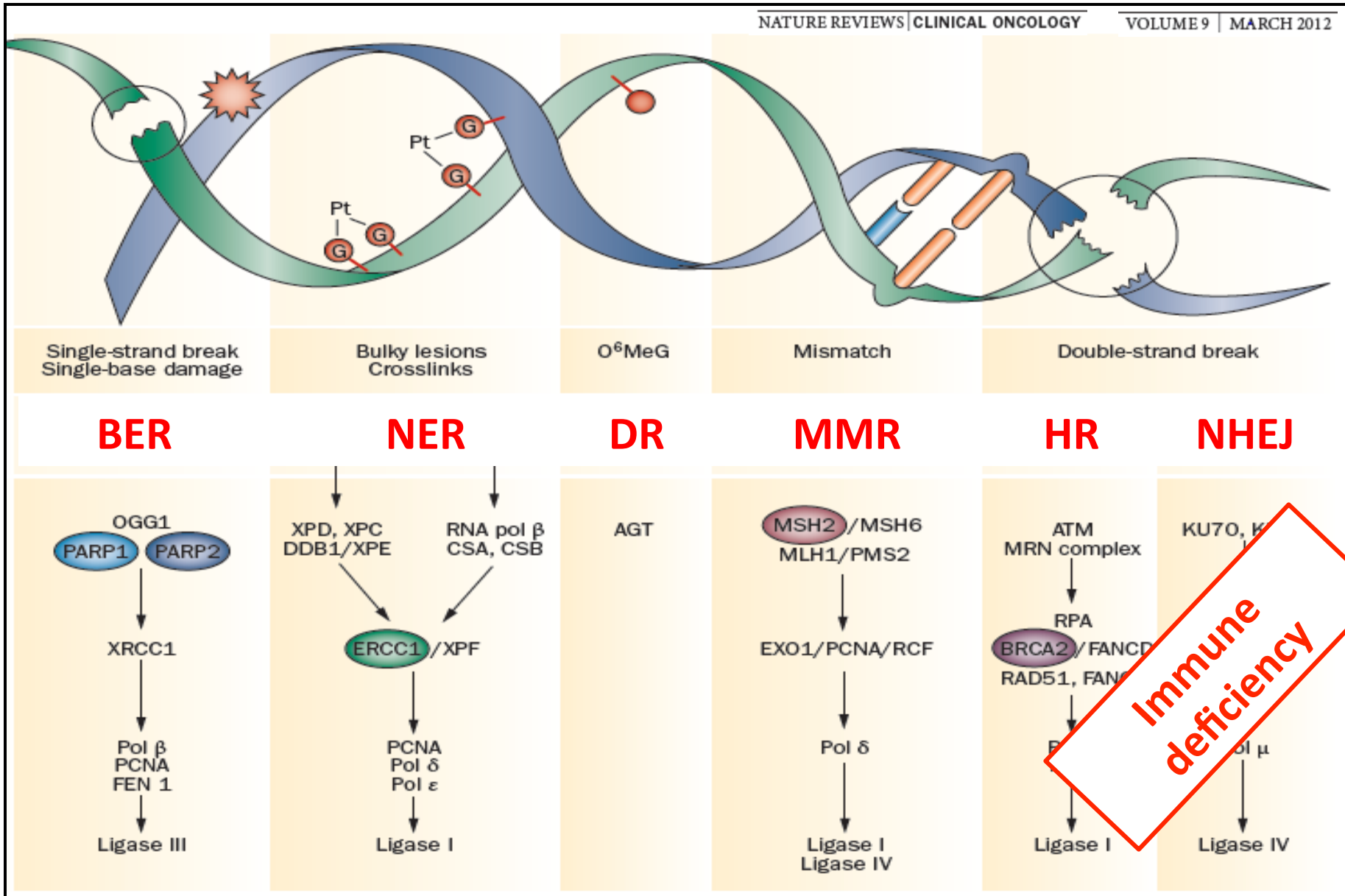


Six Major DNA Repair Pathways

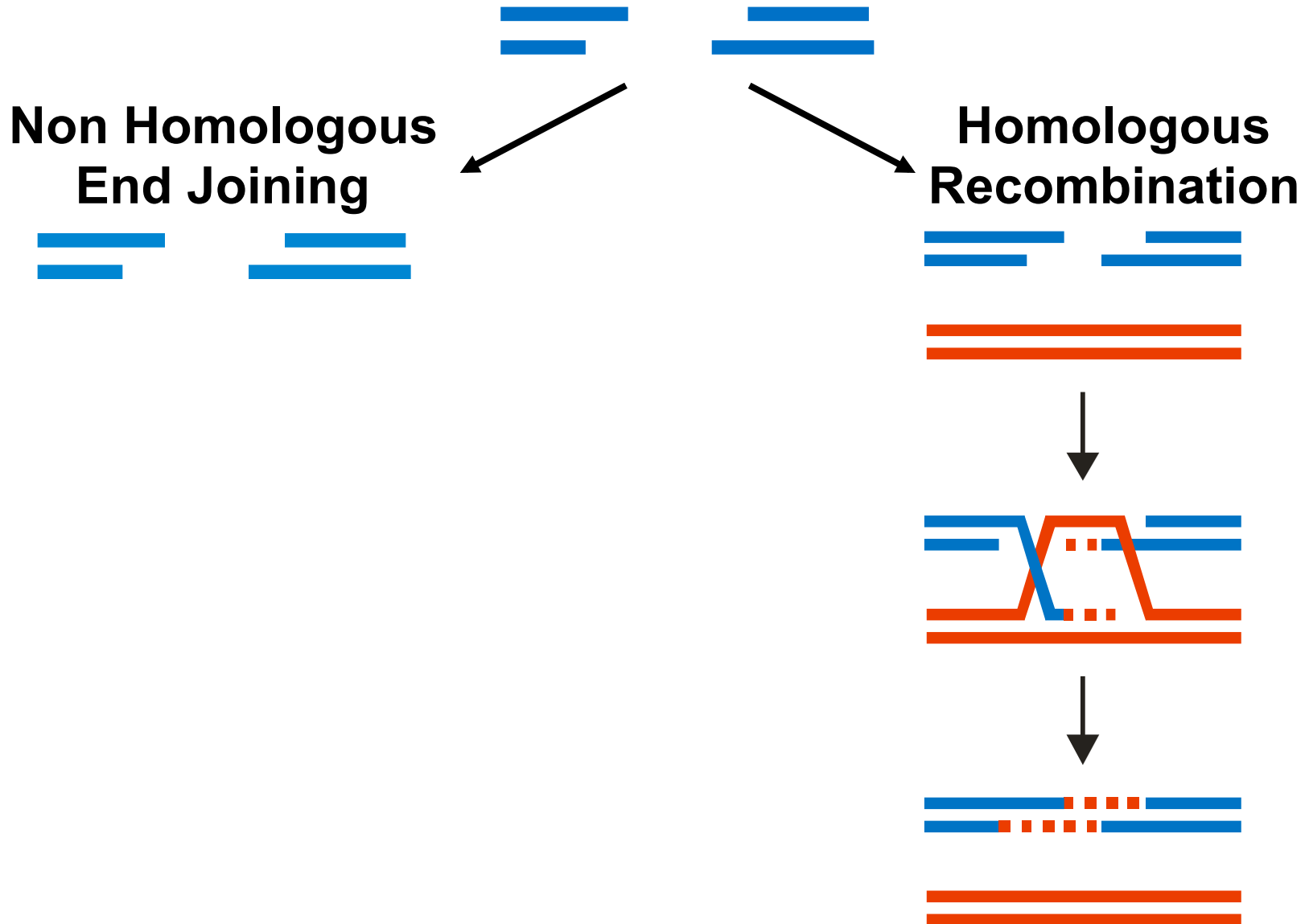
NATURE REVIEWS | CLINICAL ONCOLOGY | VOLUME 9 | MARCH 2012



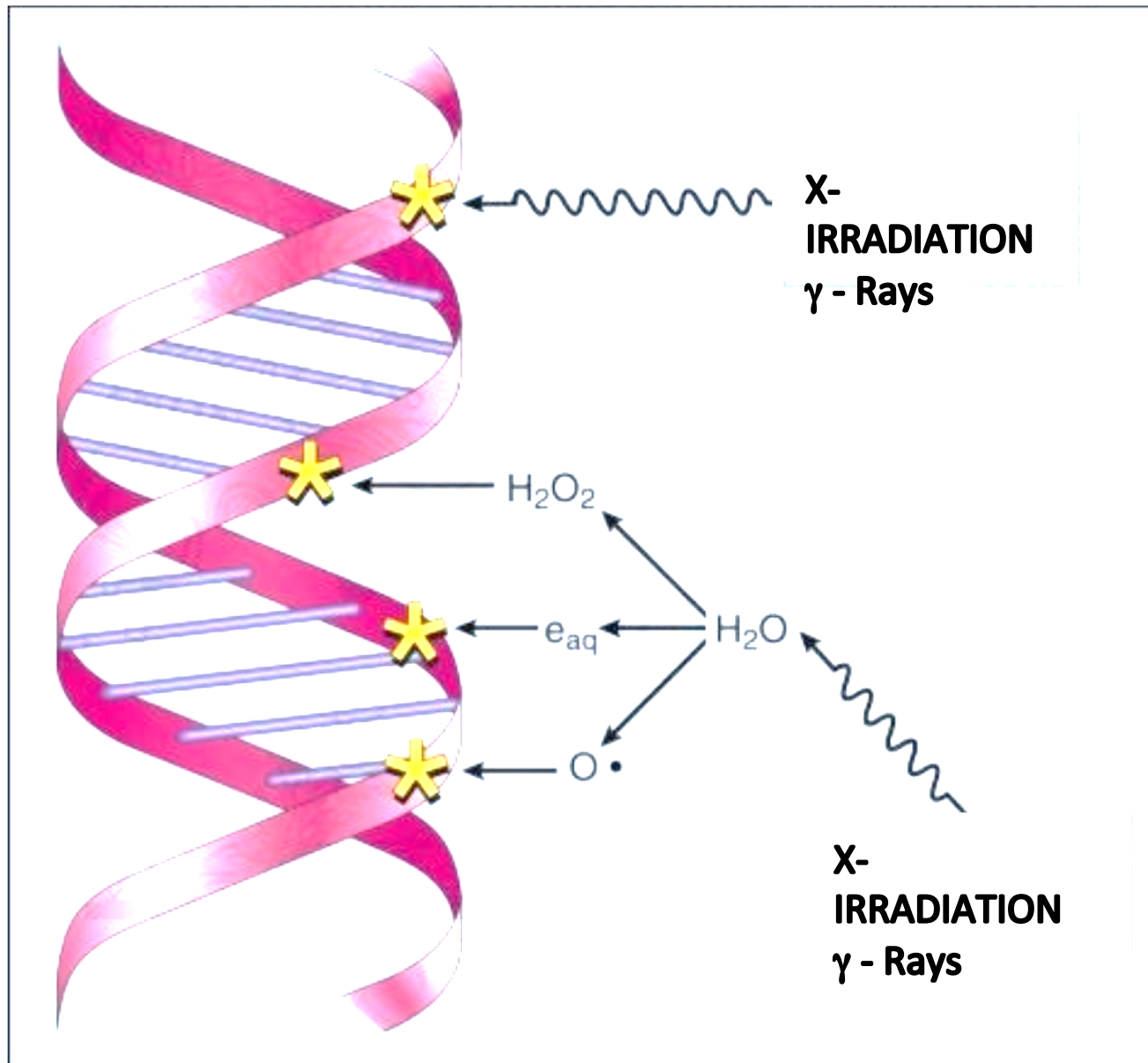
Six Major DNA Repair Pathways



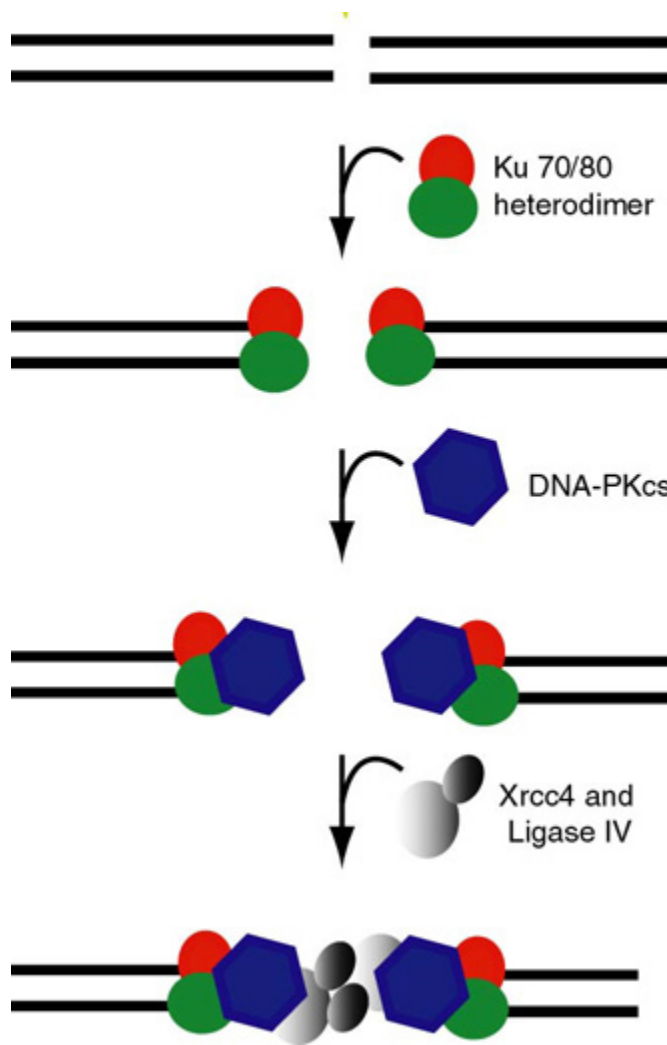
DNA double-strand break repair



X-rays are extremely good at inducing DNA Double Strand Breaks (DSBs)



Non-Homologous End Joining (NHEJ)



Ku70

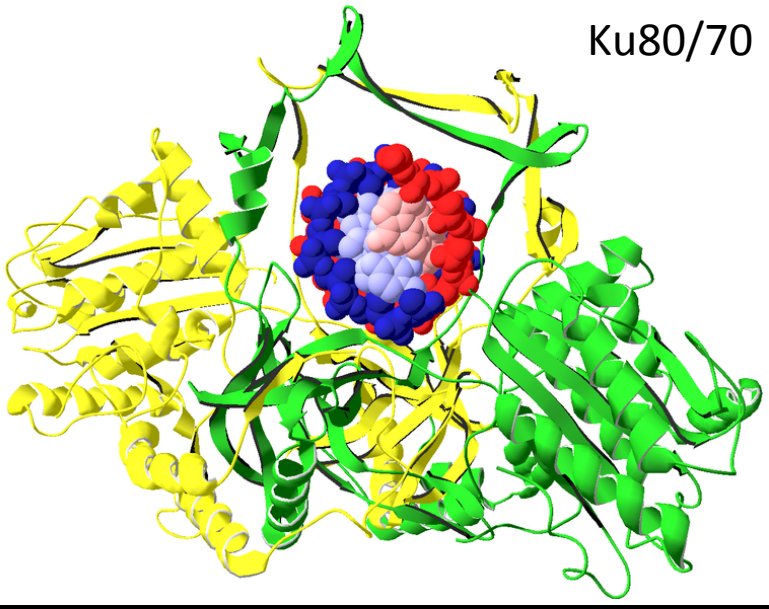
Ku80

DNA-PKcs

Xrcc4

Ligase IV

Ku80/70



double-strand break in DNA



END RECOGNITION BY
Ku HETERODIMERS



ADDITIONAL PROTEINS



PROCESSING OF DNA ENDS

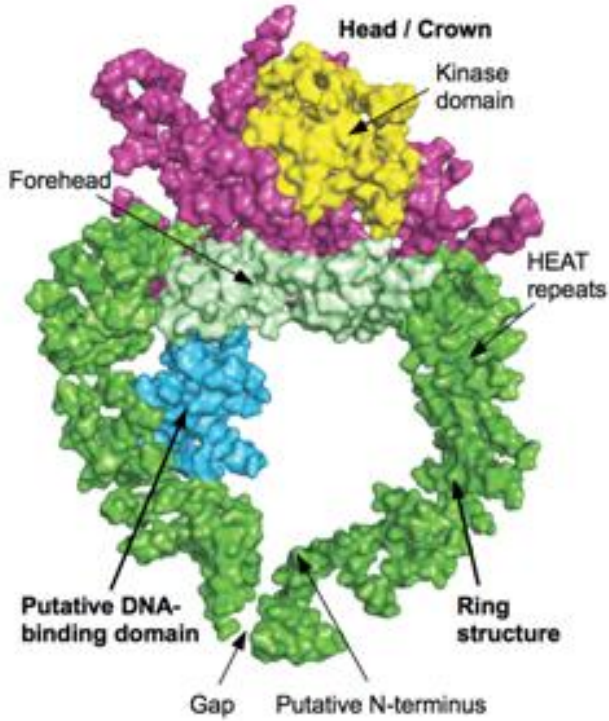
LIMITED REPAIR SYNTHESIS

LIGATION



repaired DNA has generally suffered a deletion
of nucleotides

DNA-
PKcs



Key Experimental Methods for Module 1

- Mammalian tissue cell culture
- Monitoring protein level by Western blot
- Generating plasmids with DNA damage
- Transfecting plasmids into mammalian cells
- Using fluorescent proteins as reporters of biological processes
- Flow cytometry to measure DNA repair
- Statistical analysis of biological data

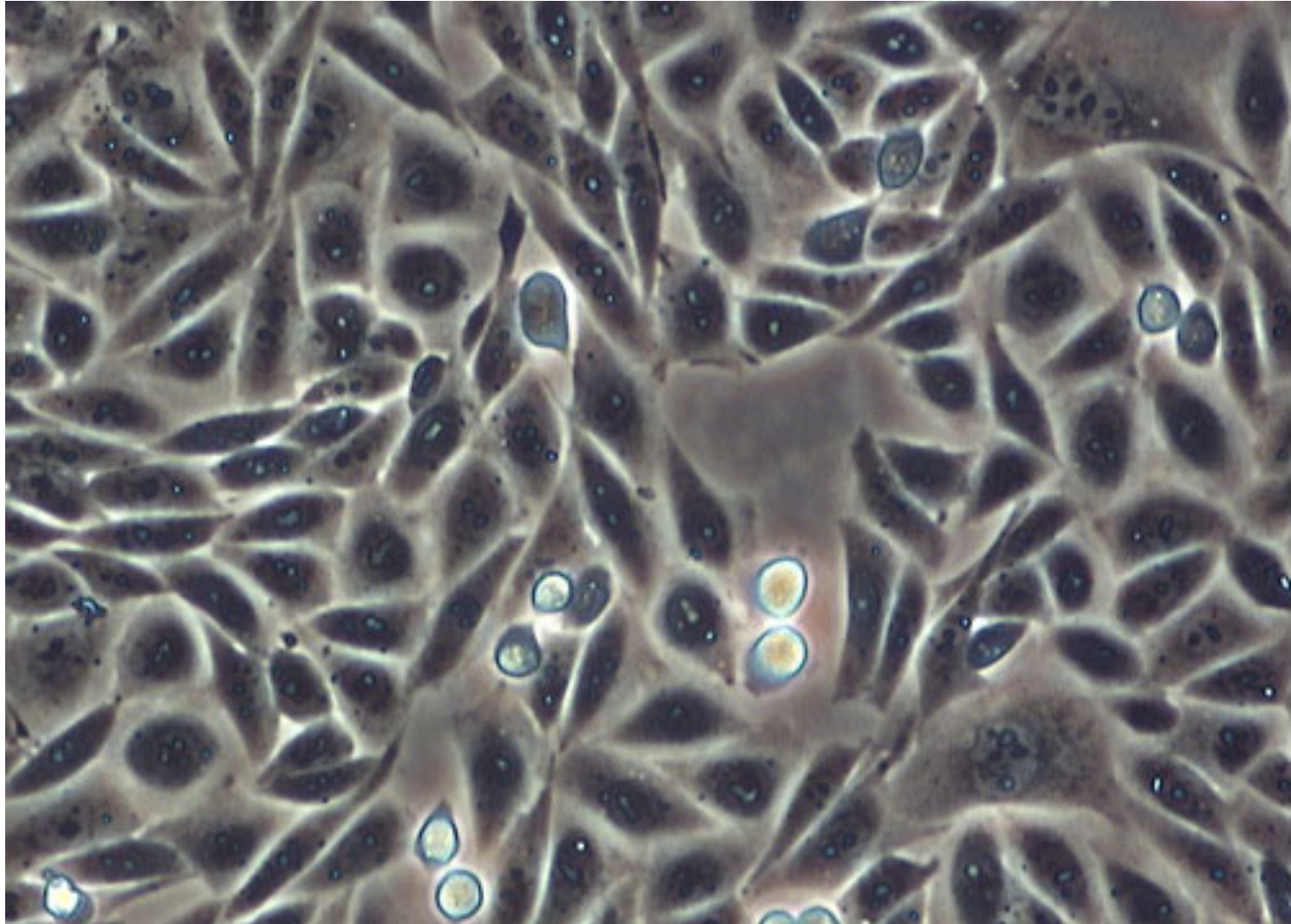


Key Experimental Methods for Module 1

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Chinese Hamster Ovary (CHO) cells are immortal
– they can grow indefinitely



Chinese Hamster Ovary (CHO) cells are immortal – they can grow indefinitely

- Isolating X-ray-sensitive (xrs) CHO cells
- Xrs cells are deficient in NHEJ
- Detecting NHEJ proteins by Western Blot

Isolating X-ray-sensitive (xrs) CHO cells

Professor Penelope Jeggo



~ Forty years ago (1975)



Age 52



Age 22



Age 25

~2.5 years ago (summer of 2012)



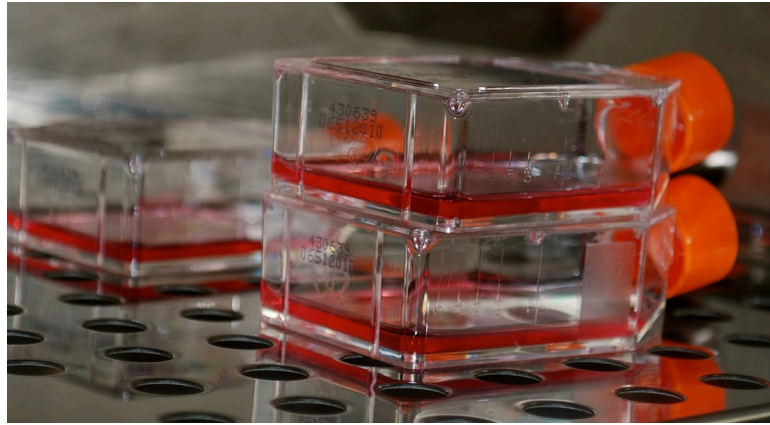
Age 90



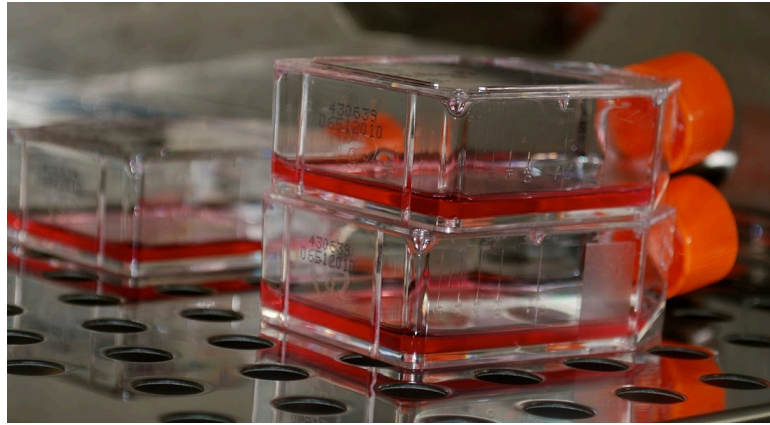
Age 60



Age 64



How would you go about
isolating X-ray **resistant**
cell lines?



How would you go about
isolating X-ray **sensitive**
cell lines?

Generate population of CHO cells with a high frequency of mutations



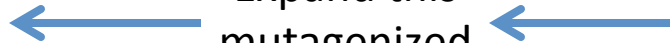
Several million
CHO-K1 cells

Add potent mutagen



70% cells die.
The 30% of cells
that live have a
high frequency of
mutants with
mutations
randomly
located across
the genome

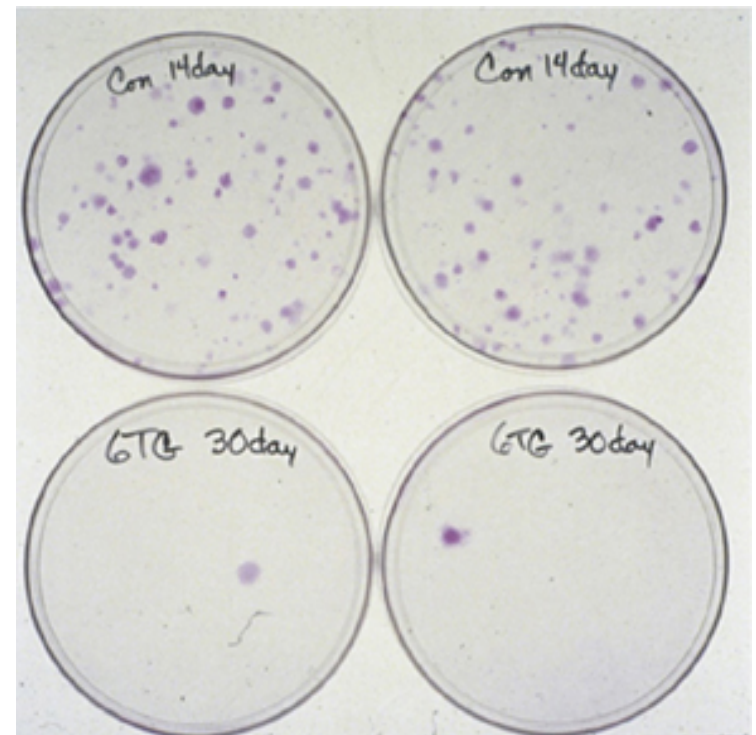
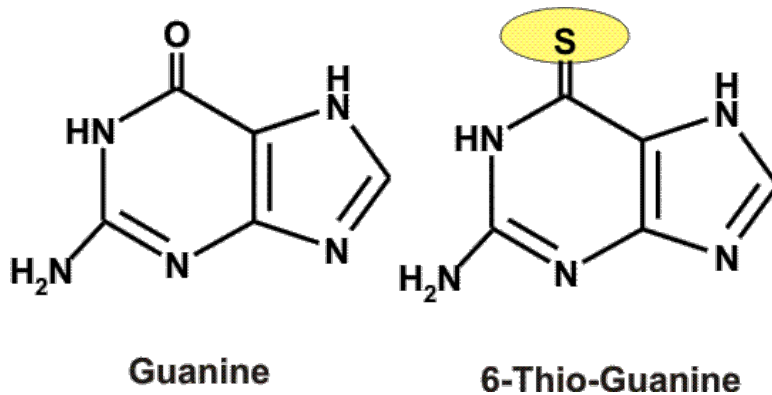
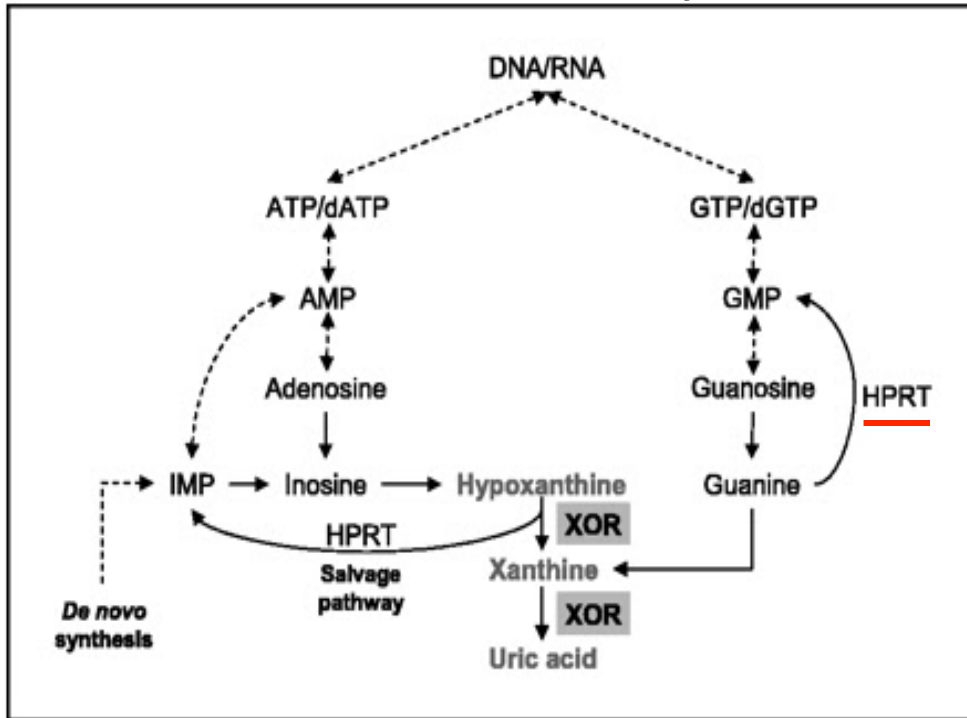
Expand this
mutagenized
population

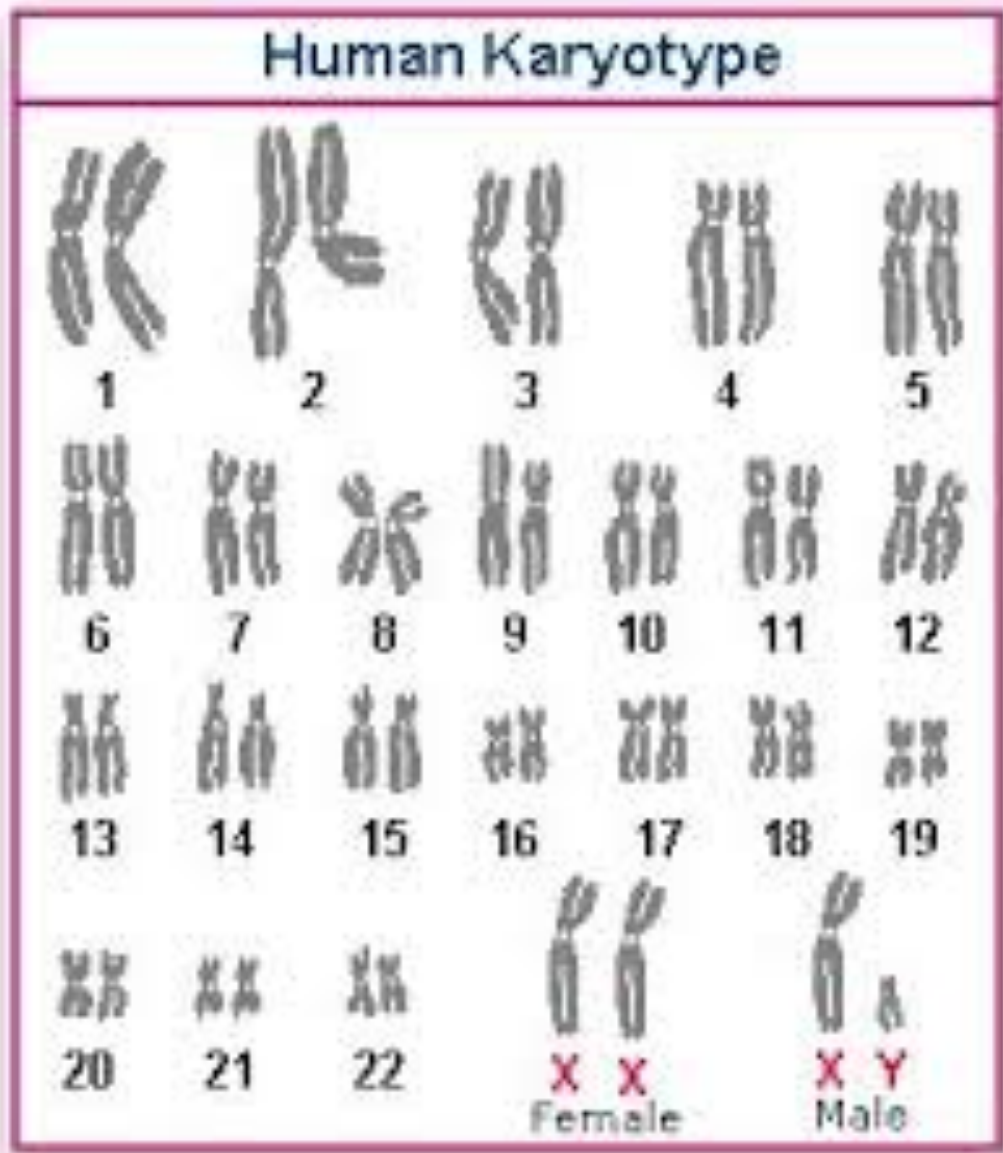


Freeze down aliquots
of mutagenized cells

Hypoxanthine phosphoribosyl transferase enzyme

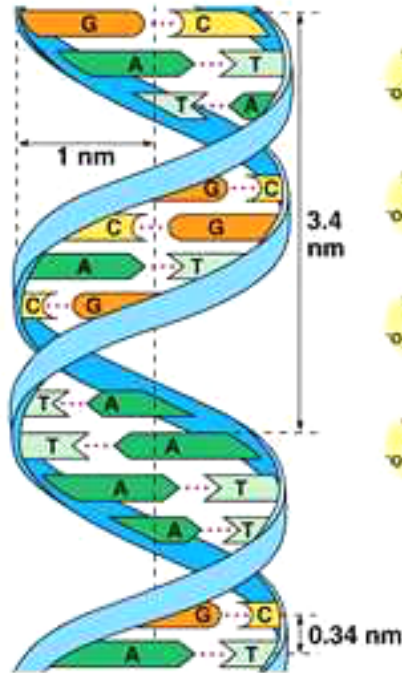
Measure the frequency of mutations at a specific gene locus – the *HPRT* gene: 1 in 10^3 or 10^{-3}





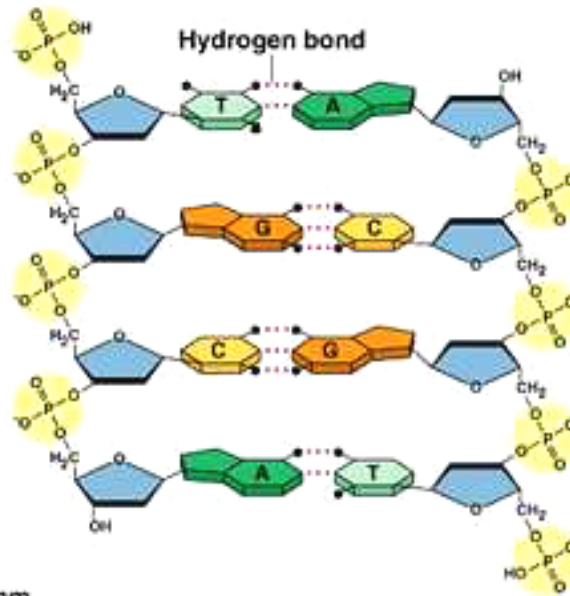
HPRT gene is on the X-chromosome

The Structure of DNA



(a)

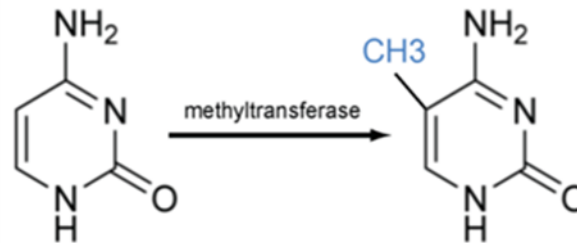
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(b)

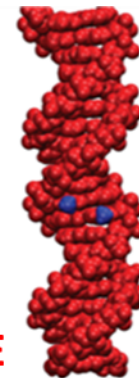


(c)

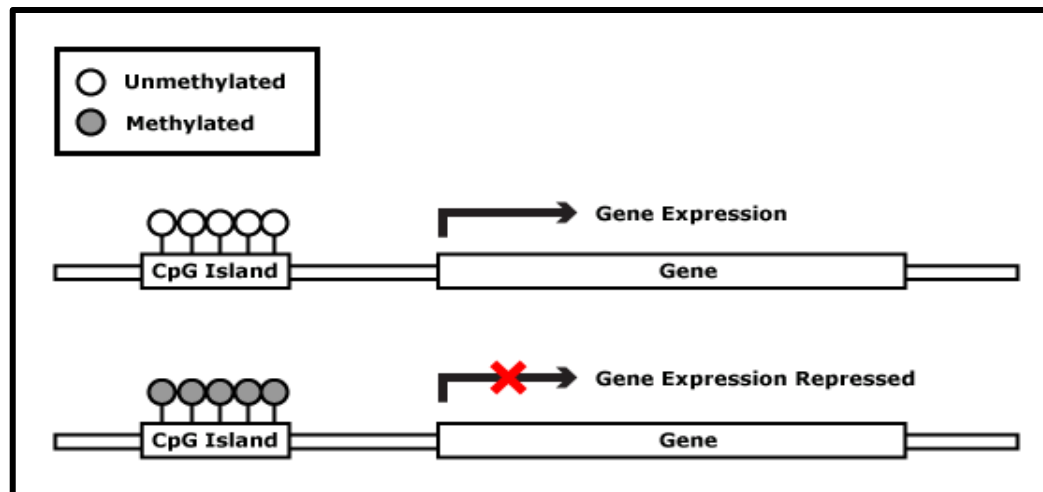
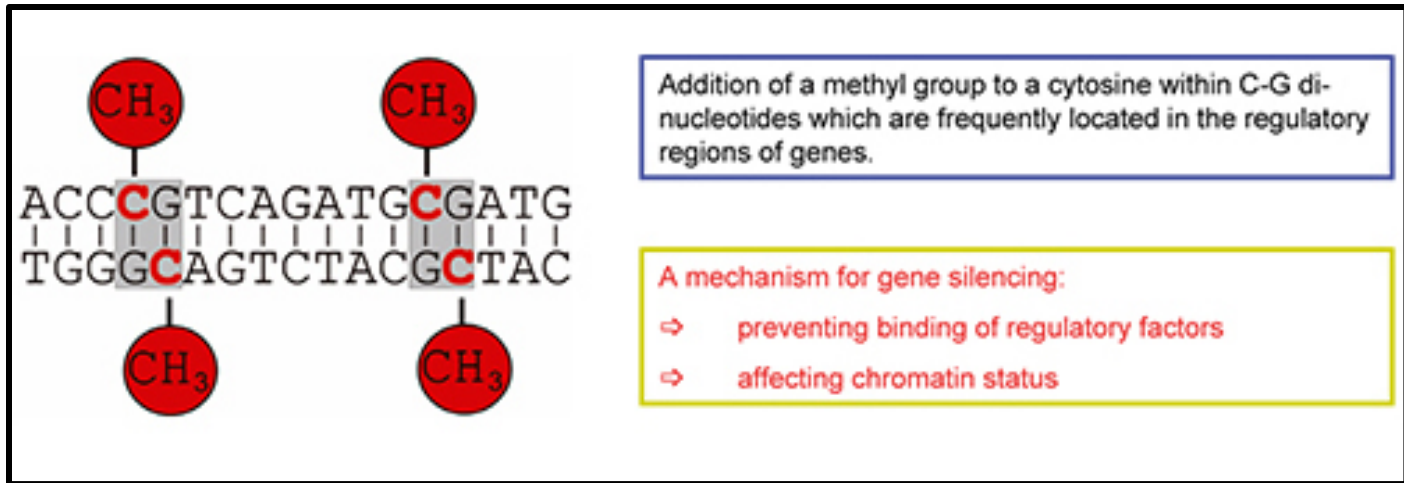


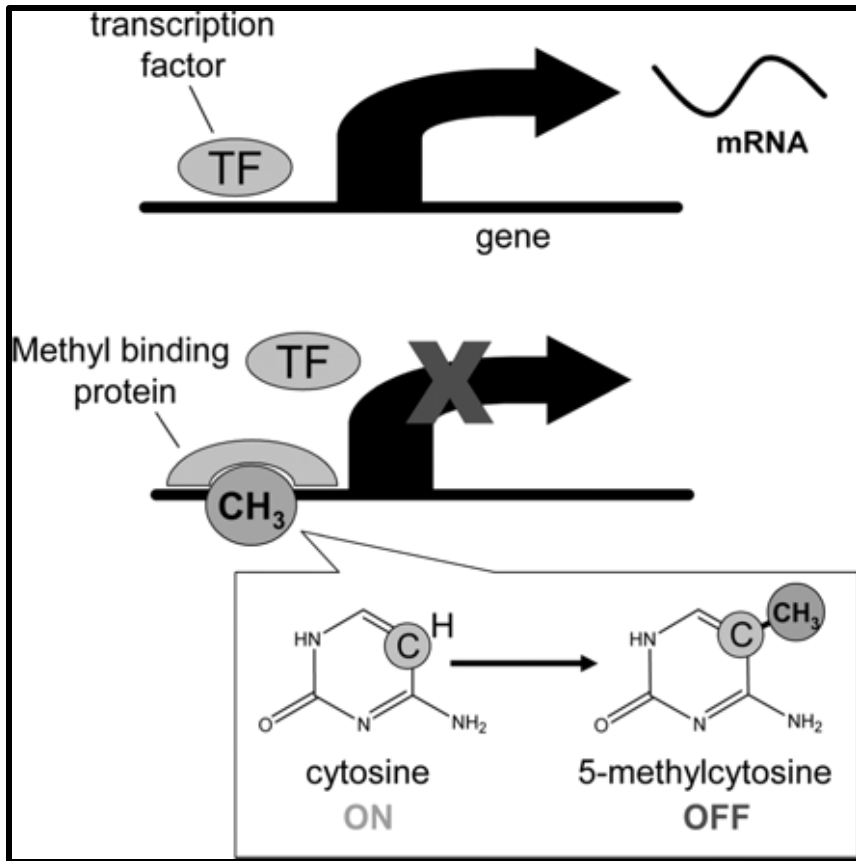
CYTOSINE

5Me-CYTOSINE



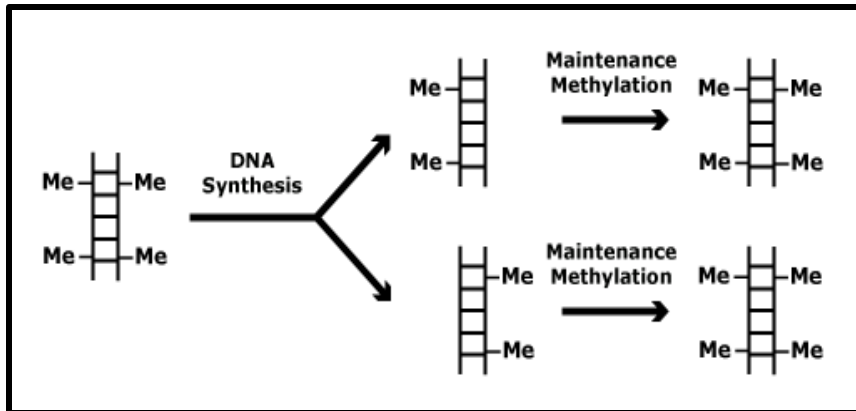
Promoter methylation at CpG silences gene expression





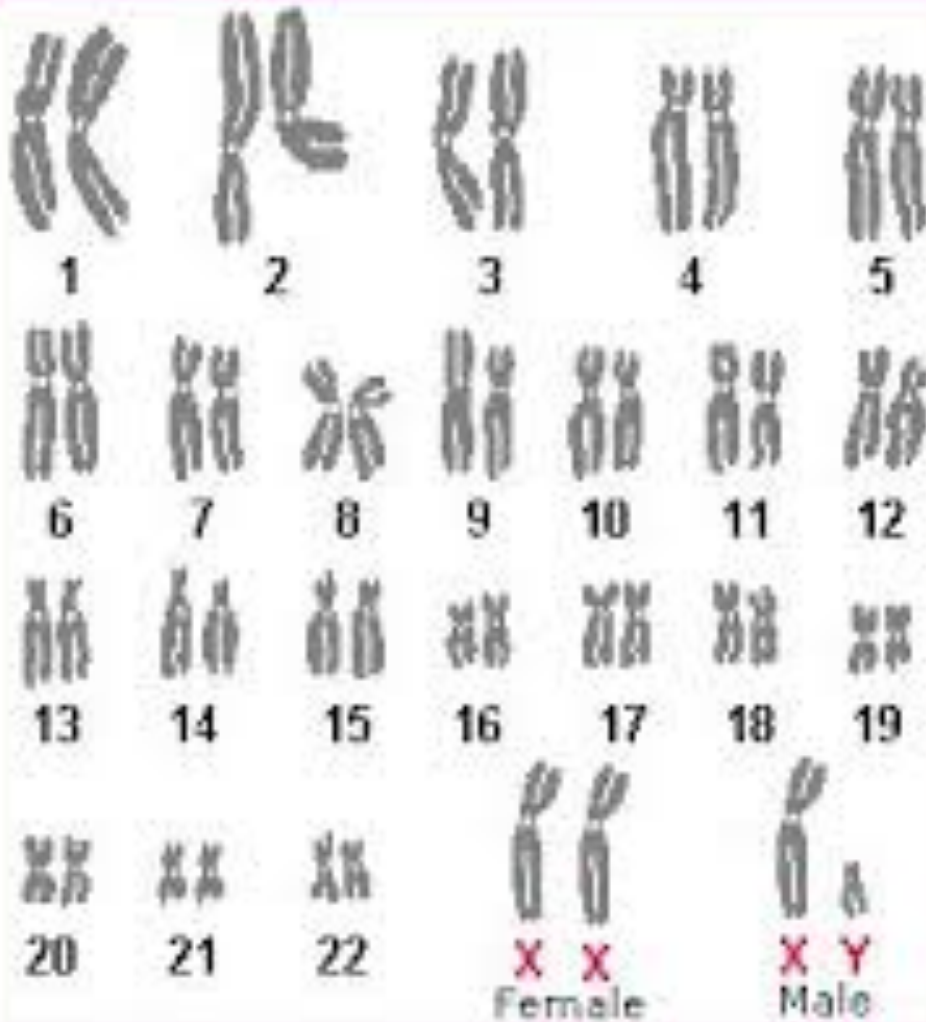
CHO cells happen to have more than the usual amount of CpG methylation

This results in extensive



“FUNCTIONAL HEMIZYGOSITY”

Human Karyotype



HPRT gene is on the X-chromosome – functional hemizyosity in female cells, actual hemizyosity in male cells

Hope that the genes for repairing X-ray induced damage are also in functionally hemizygous state

Generate population of CHO cells with a high frequency of mutations



Several million
CHO-K1 cells

Add potent mutagen



70% cells die.
The 30% of cells
that live have a
high frequency of
mutants with
mutations
randomly
located across
the genome

Expand this
mutagenized
population



Freeze down aliquots
of mutagenized cells

X-ray-sensitive mutants of Chinese hamster ovary cell line

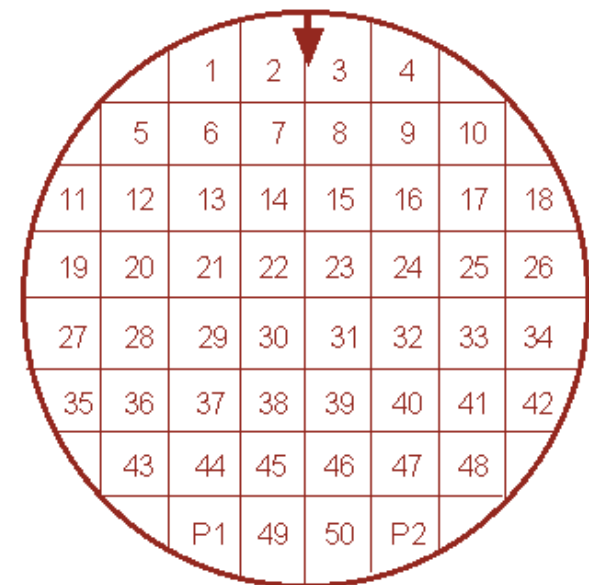
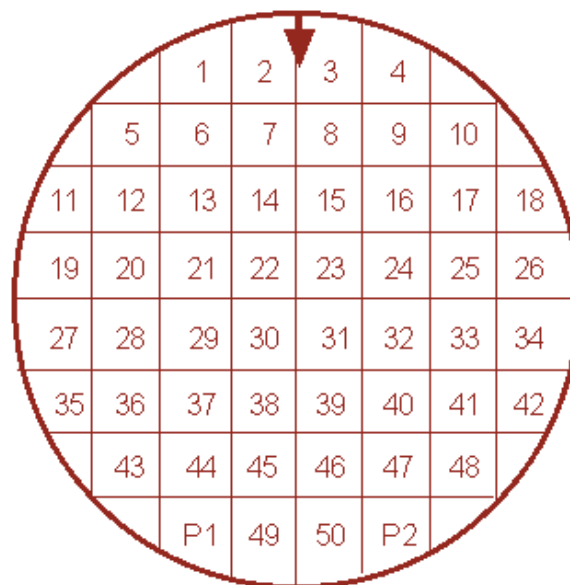
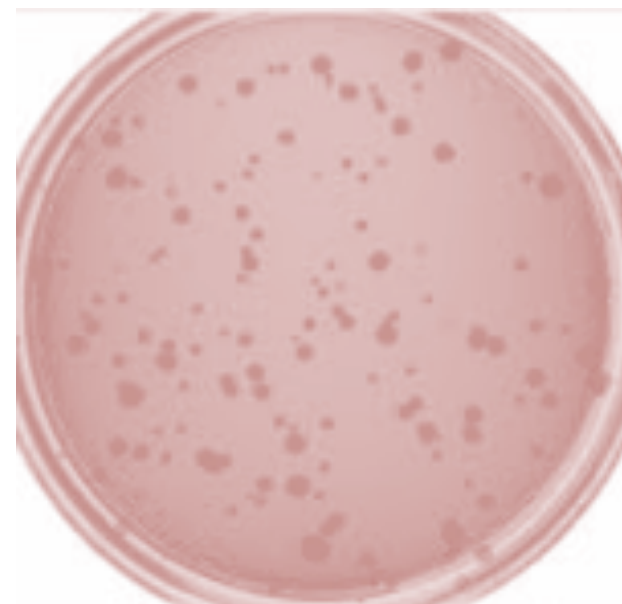
Isolation and cross-sensitivity to other DNA-damaging agents

P.A.Jeggo * and L.M. Kemp

*Genetics Division, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA
(Great Britain)*

(Received 12 April 1983)

(Accepted 15 August 1983)



X-ray-sensitive mutants of Chinese hamster ovary cell line

and cross-sensitivity to other DNA-damaging agents

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19	20	21	22	23	24	25	26
27	28	29	30	31	32	33	34
35	36	37	38	39	40	41	42
	43	44	45	46	47	48	
	P1	49	50	P2			

	1	2	3	4			
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X-ray-sensitive mutants of Chinese hamster ovary cell line

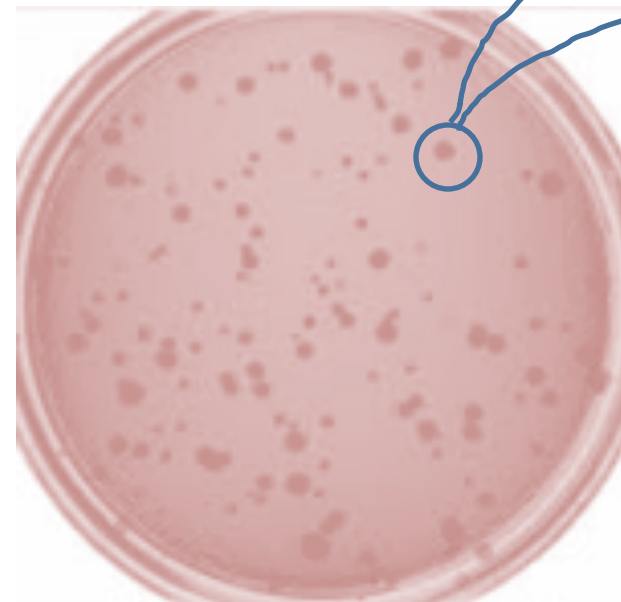
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X-RAYS

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	P1	49	50	P2			

No X-RAYS

X-ray-sensitive mutants of Chinese hamster ovary cell line

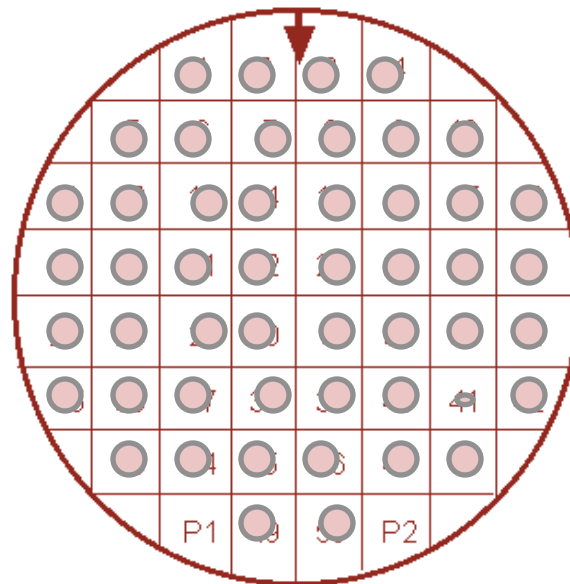
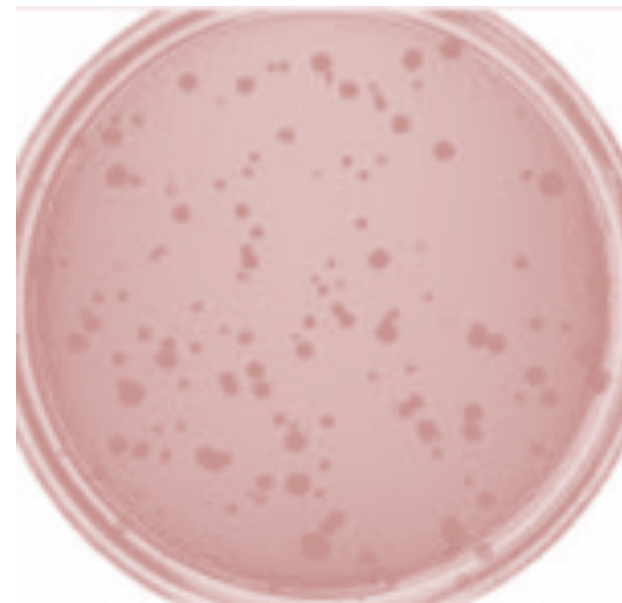
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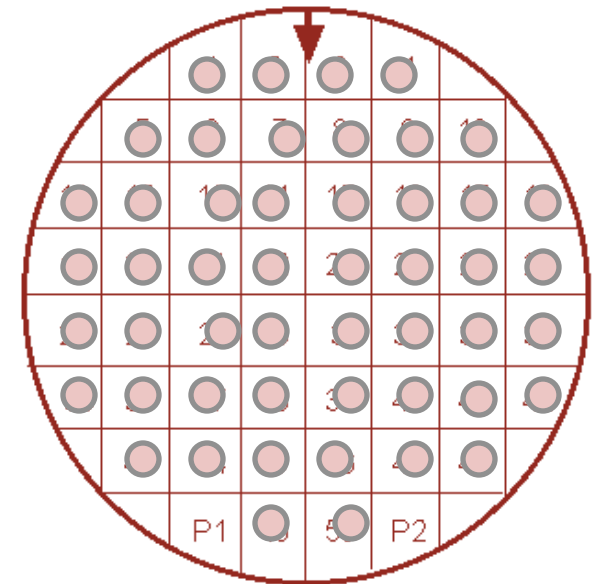
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6 days after X-RAYS



6 days after **No** X-RAYS

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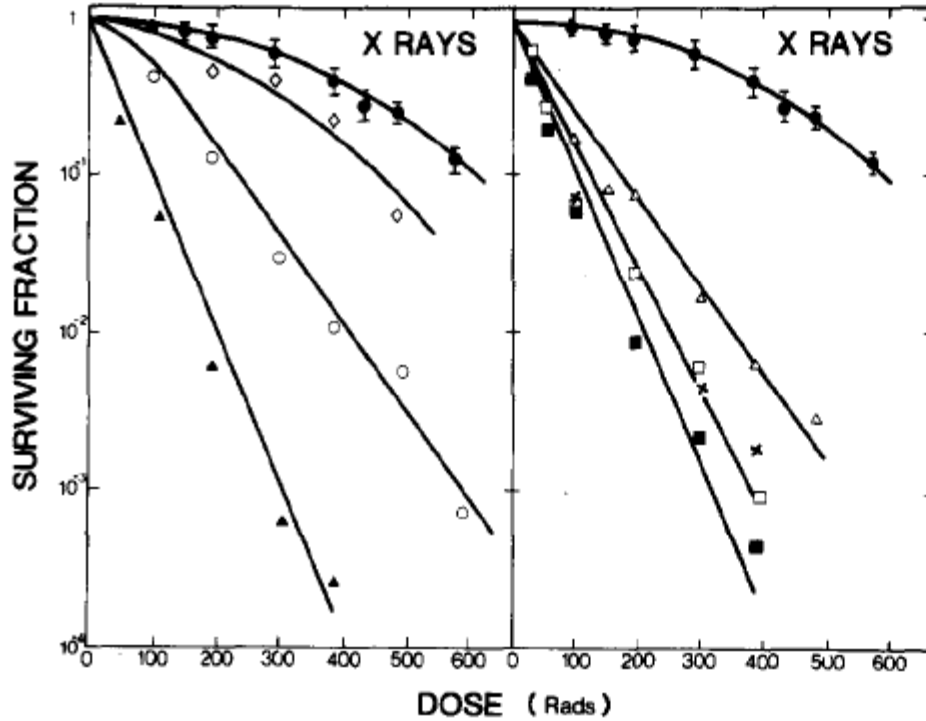


Fig. 1. X-ray survival of CHO-K1 and mutant strains: ●, CHO-K1; □, *xrs-1*; ○, *xrs-2*; ◇, *xrs-3*; △, *xrs-4*; ▲, *xrs-5*; ■, *xrs-6*; ×, *xrs-7*. The error bars when shown represent one standard deviation. Each survival curve represents the mean of at least three experiments.

Tooth-picking and screening
9,000 colonies!!!
Isolated seven (maybe only 6?)
X-ray sensitive clones!!

Penelope Jeggo



Penelope Jeggo

Penelope Jeggo was born in Cambridge, England. She obtained a BSc Honours degree in Microbiology at Queen Elizabeth College, University of London in 1970. She then did a PhD in the Genetics Division at the National Institute for Medical Research (NIMR), London, in Robin Holliday's laboratory. Her first postdoctoral position was with John Cairns at the ICRF Mill Hill Laboratory. She then obtained a postdoctoral fellowship with Miroslav Radman at the Université Libre de Bruxelles, Belgium. From there, she returned to the Genetics Division at NIMR as a scientific research officer. In 1989, she moved to the Medical Research Council's Cell Mutation Unit (CMU) at the University of Sussex. In 2001, following closure of the CMU on the retirement of the director, Penny became a founding member of the Genome Damage and Stability Centre (GDSC), a new collaborative research centre established by the University of Sussex and the Medical Research Council.

Penny's research has focused on DNA damage responses and particularly on the repair of DNA double strand breaks (DSBs). She applied the techniques learnt during her early years working with lower organisms to isolate mammalian cell lines sensitive to ionising radiation. Using these cell lines, she characterised the major DSB repair pathway in higher organisms and, in collaborative work, showed that the DSB repair pathway also functions during V(D)J recombination, a critical process during immune development. The cell lines were also pivotal in allowing her to identify the first mammalian genes that significantly contribute to the response to radiation exposure and to V(D)J recombination. The GDSC houses the UK's largest collection of cell lines from patients with damage response disorders. Penny has exploited and extended this resource to identify patients deficient in DSB repair, as well as additional damage response genes. Such studies have provided insights into the role of the damage response pathways in human development and cancer avoidance.

She has recently discovered an important connection between the signalling response to DNA damage and the DNA repair machinery, which makes a significant contribution to the response of human cells to ionising radiation. She continues to focus on understanding the basis of human radiosensitivity.

In the interview below, Fiona Watt, Editor-in-Chief of JCS, asks Penny about her experiences as a woman in science.

FMW: How has your research career impacted on your personal life and vice versa?

PJ: I consider myself just plain lucky to have chosen a career that continues to excite and motivate me. It is this aspect of a career in science that has impacted upon my personal life in a positive way and provided the *raison d'être* to endure the hard work and the more difficult challenges. I remember the thrill of doing an undergraduate laboratory project; although my results contributed only the tiniest smidgeon to scientific knowledge, I gained immense satisfaction from it. I was lucky in having two wonderful mentors in my early days of research: Robin Holliday, in whose laboratory I studied for my PhD; and John Cairns, with whom I undertook my first postdoctoral position. In addition to being excellent scientists, they enhanced my ability to enjoy and be excited by science. I believe this is a defining criterion of a great career—if the highs are high enough, the lows can be endured.

As a young postdoc, I was lucky in having a partner who understood the joy I gained from laboratory life. Though not a research scientist, and certainly not motivated in the same way as me, he supported my needs and achievements, and encouraged my independence. Finally, he found his own job satisfaction, which resulted in us commuting between Germany and England for a couple of years.

Enjoying science as I did, and taking the opportunity to do a postdoctoral fellowship outside the UK with Miroslav Radman, I didn't worry too much about starting a family. But as my thirties progressed, the motherhood desire set in and new excitement entered my life. Tragically, my partner, who had endured



Penny Jeggo with her son, Matthew, taken in the Grange Gardens at Lewes, East Sussex around 1990.

sympathy pains during the course of my pregnancy, was diagnosed with colon cancer not long after our baby was born and died within a year. I felt let down by cancer research as a career and I might well have quit, had it not been for the support of wonderful colleagues and friends. But I recovered the fire, and before long the thrill of a good result was sustaining me through the difficult times of being a single parent in a demanding career.

A wonderful aspect of science is the ability to form friendships around the world. A few years after the death of my husband, I had the opportunity to re-establish myself and make new friends by undertaking a sabbatical in the USA. This was of huge personal benefit. Although it becomes increasingly tiring, I still love to travel and meet with my international friends and colleagues.

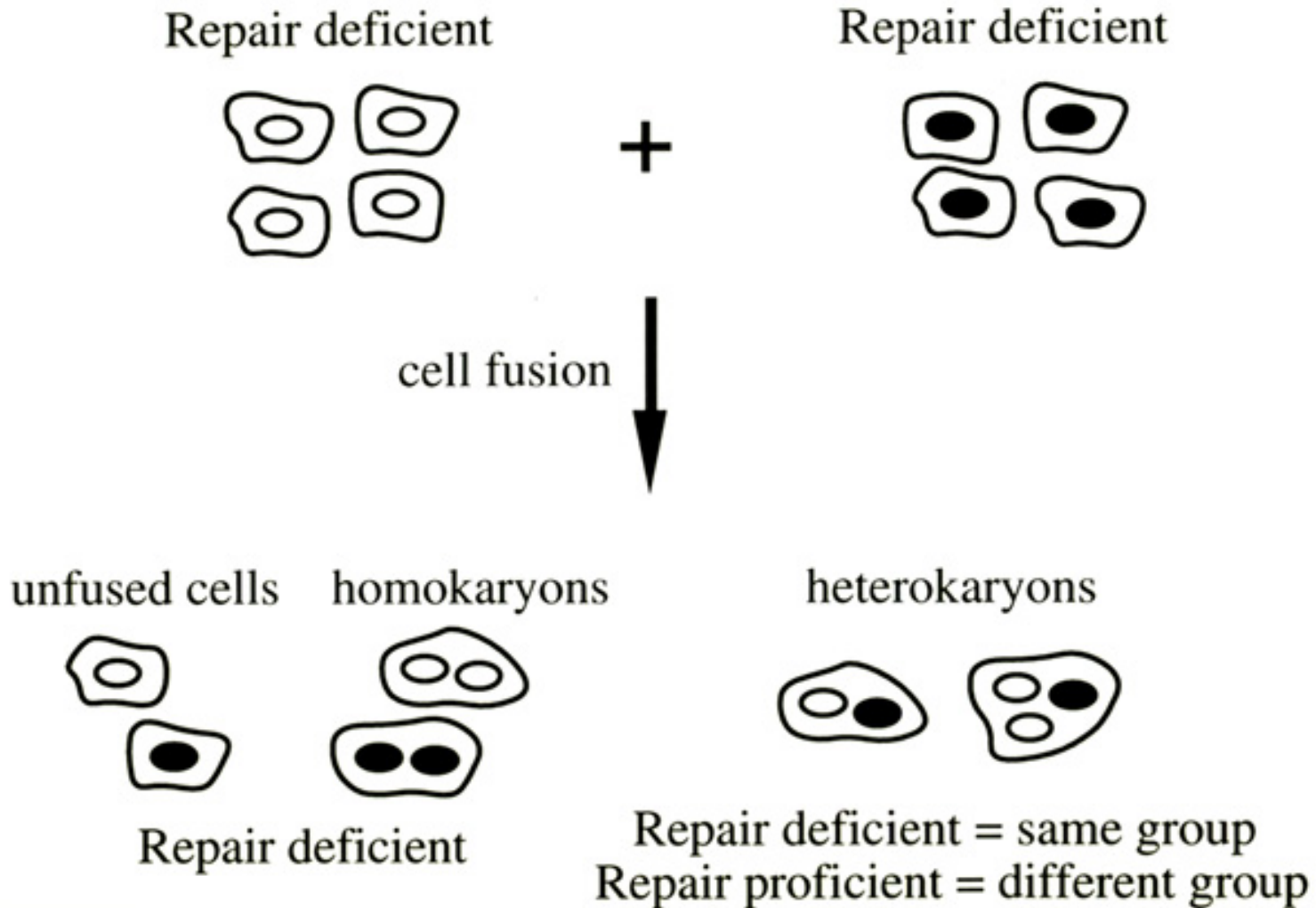
Since the start of my PhD, I have been fascinated by DNA recombination and repair. At that time, the importance of genomic stability was not well appreciated, and at ICRF (now Cancer Research UK) viruses and viral oncogenes were considered to be all important. The work in Cairns's laboratory on DNA repair was only tolerated as a concession that some basic research had to be undertaken. Those of us in the field were convinced, however, that the maintenance of genomic stability was central to cancer avoidance. I still feel thrilled by the recent conversion of many scientists to appreciate the importance of my field!

I rarely felt guilty about the limitations that a research career places on

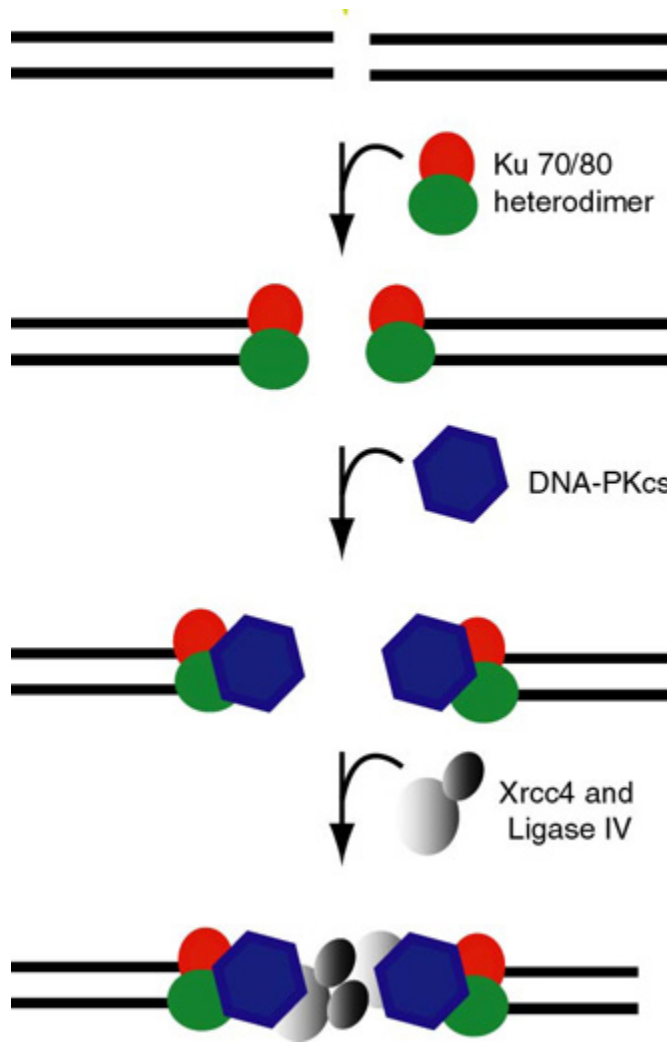
- You now have 6 or 7 mutant cell lines with similar phenotype
- How do you figure out if the cell lines have mutations in the same genes, or in different genes?

Functional Complementation

Complementation groups



Non-Homologous End Joining (NHEJ)



Ku70

Ku80

DNA-PKcs

Xrcc4

Ligase IV

What is the experiment?

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

Check list of critical materials to be used:

1. Wild type CHO-K1 cells
2. CHO xrs6 cells, deficient in Ku80 protein
3. Inhibitor of DNA-PKcs
4. Plasmids containing DNA double strand breaks of different topologies.

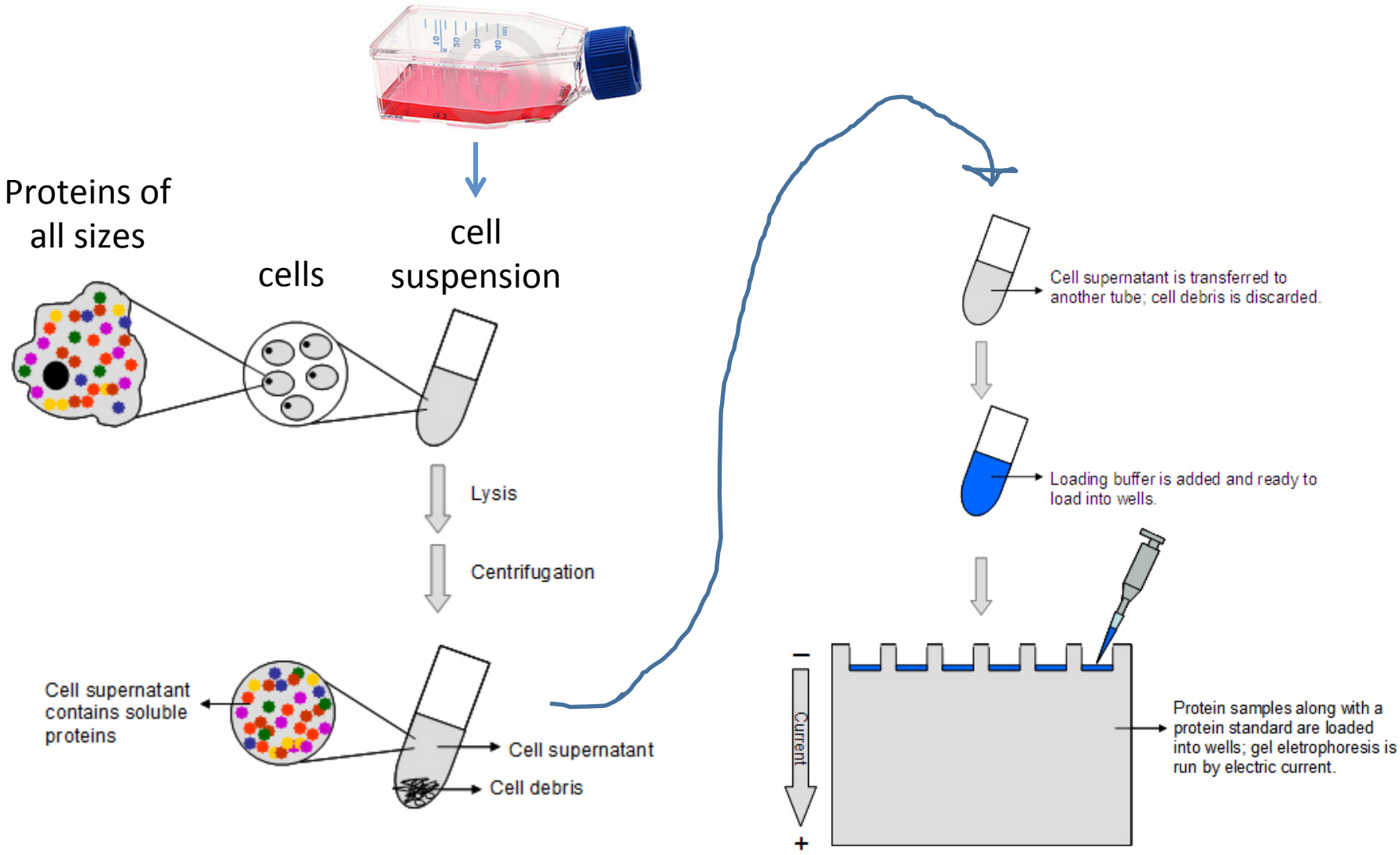
What is the experiment?

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

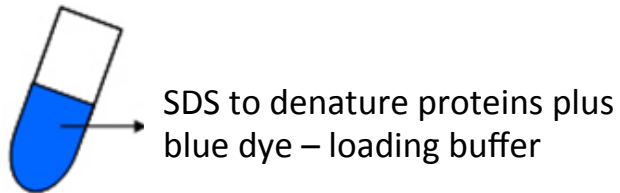
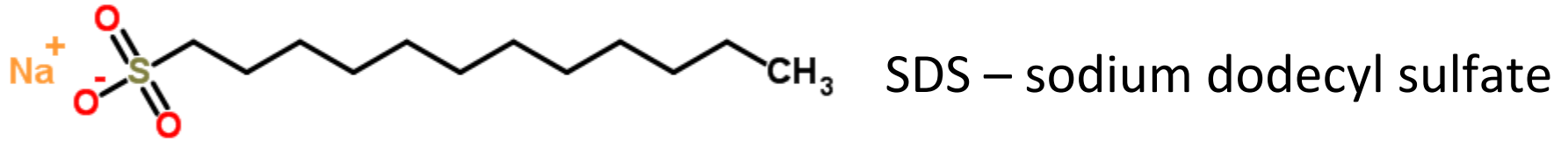
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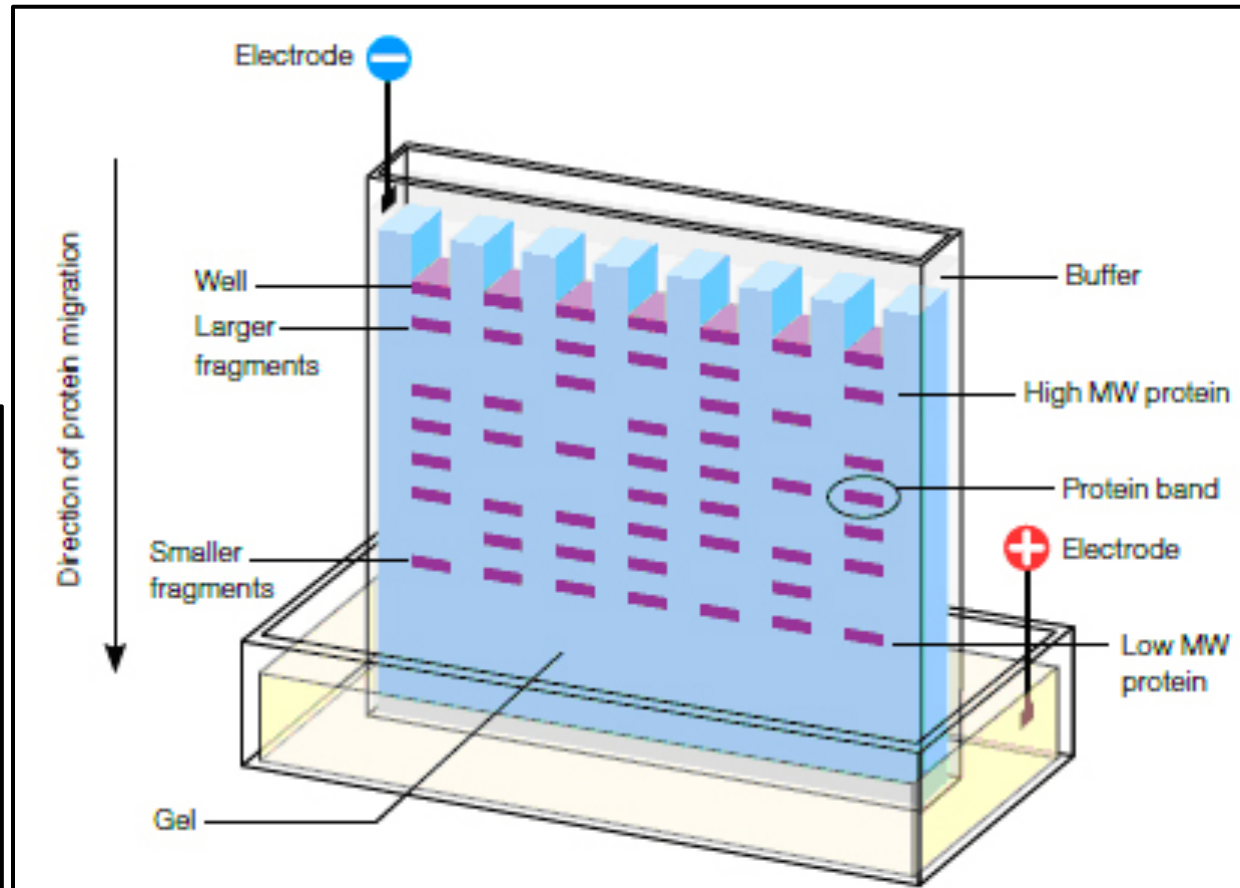
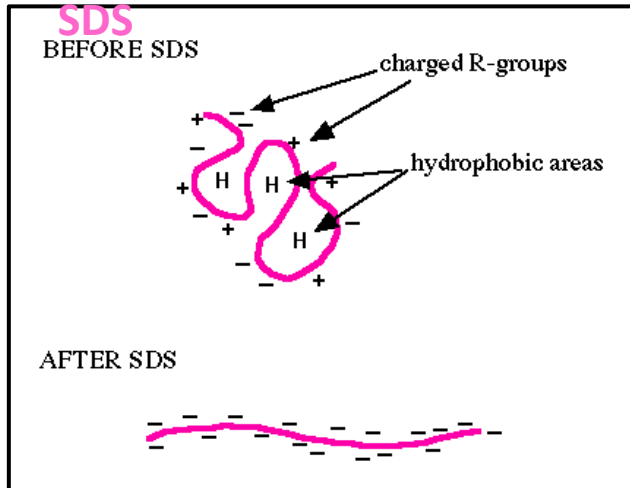
Western Blot or Immunoblot Analysis

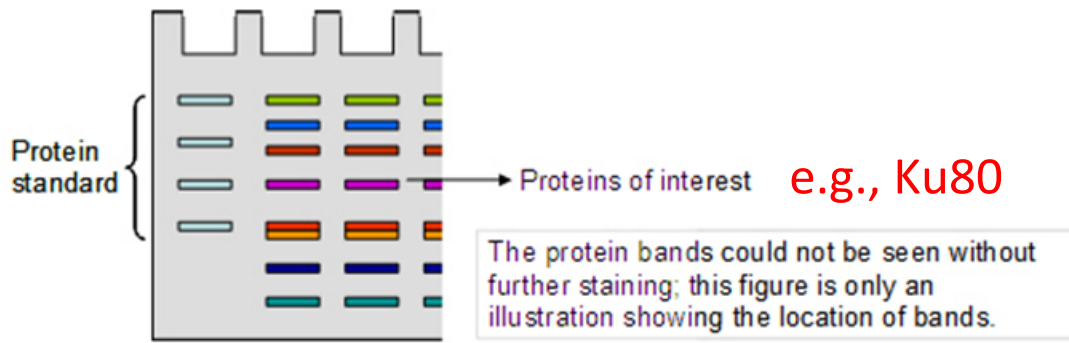


Separating proteins on an SDS polyacrylamide gel

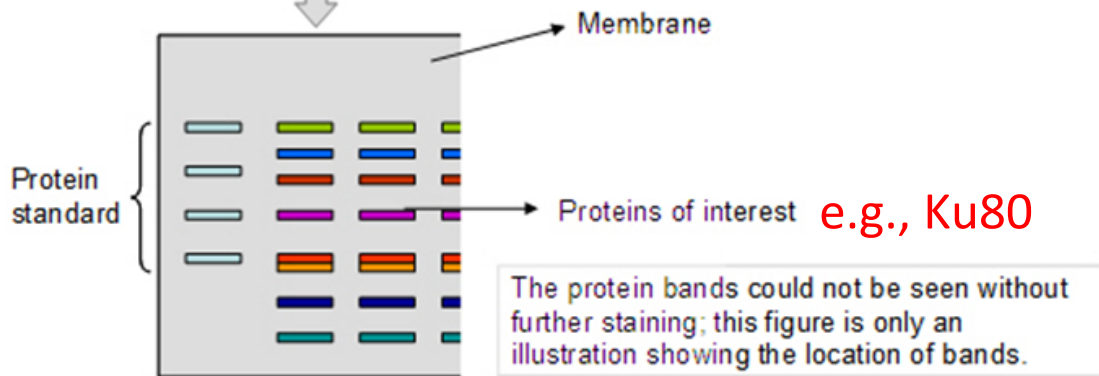
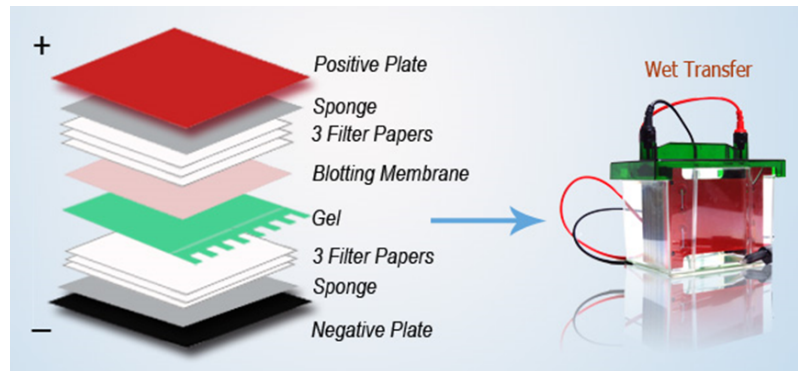


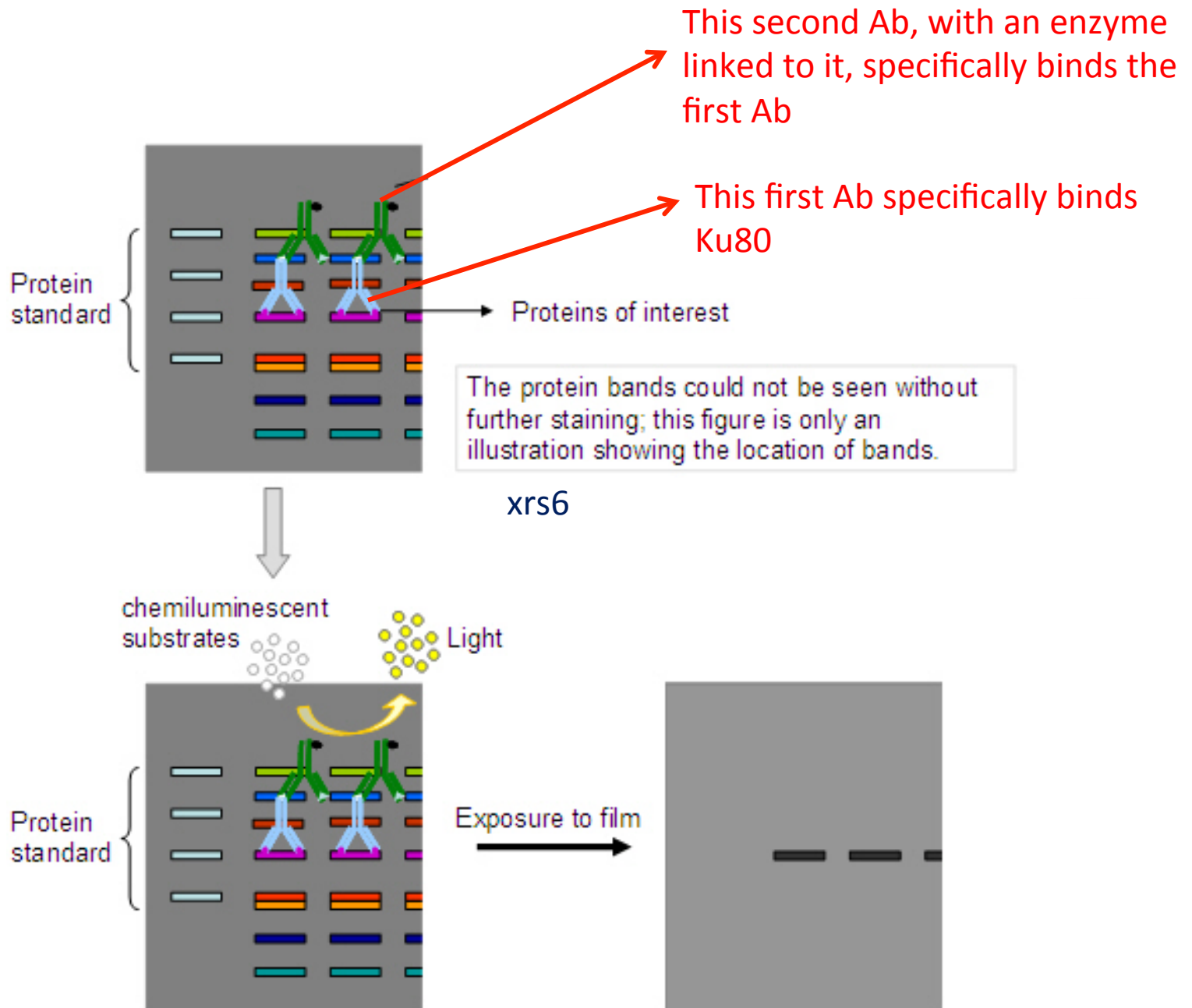
Proteins denatured by SDS



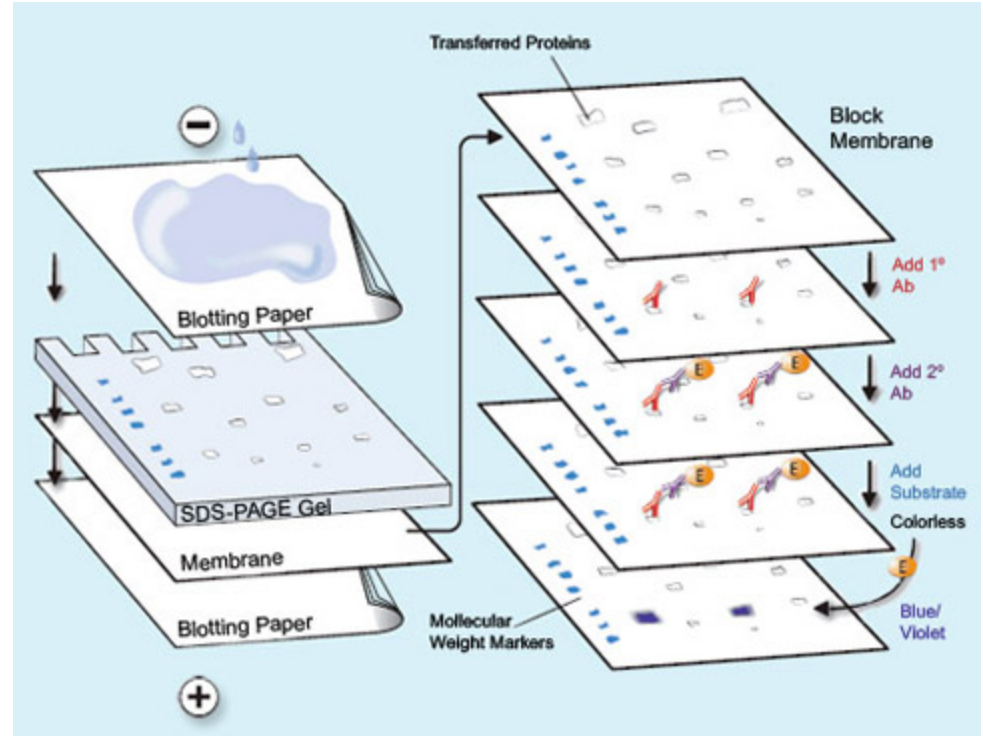
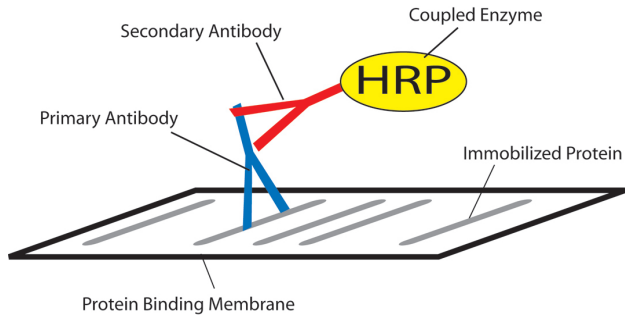


Transfer (Semi-dry transfer as an example)





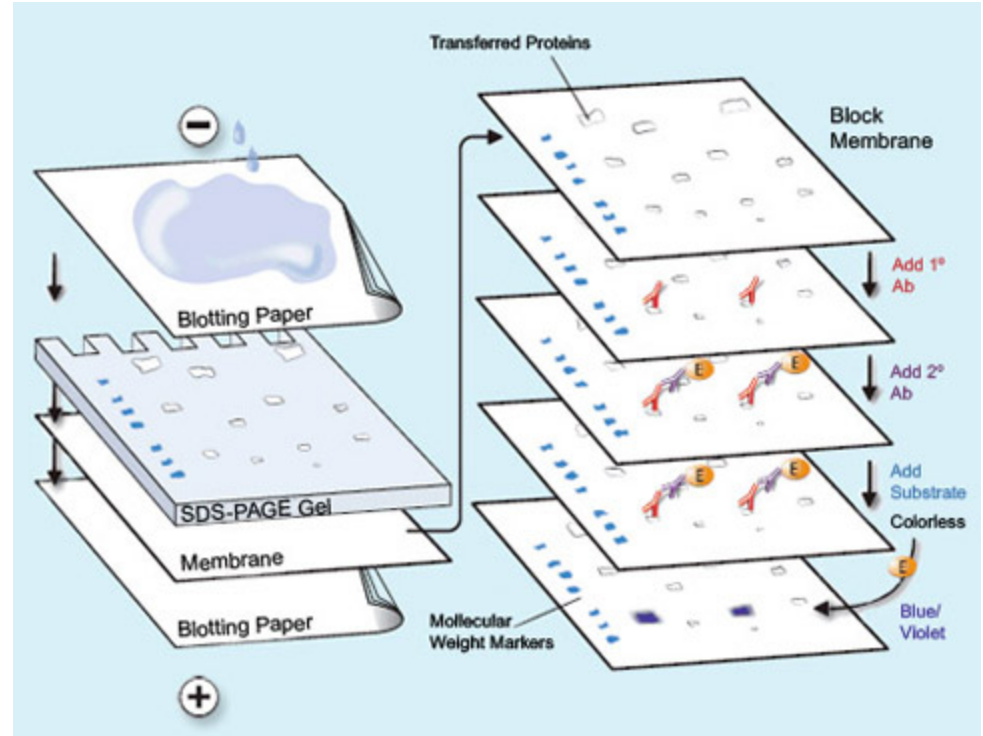
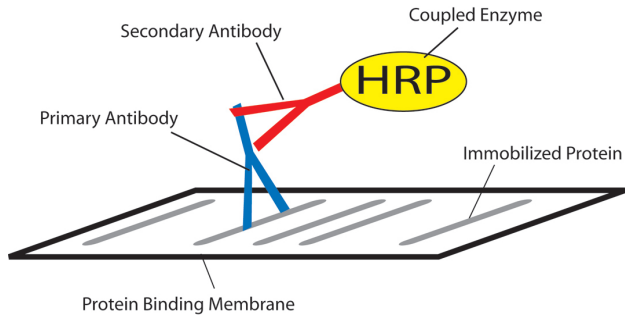
Horseradish Peroxidase



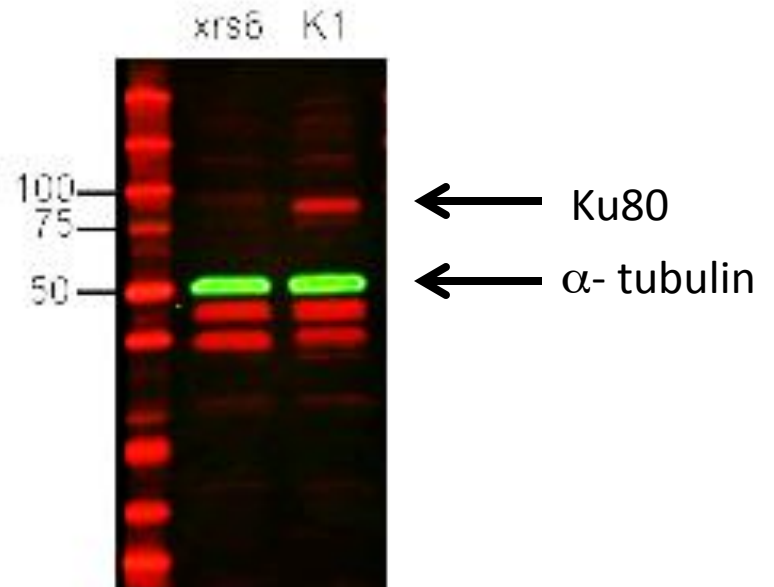
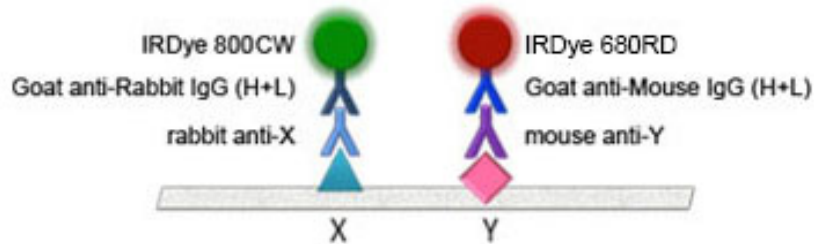
Western Blot Analysis



Horseradish Peroxidase



Western Blot Analysis

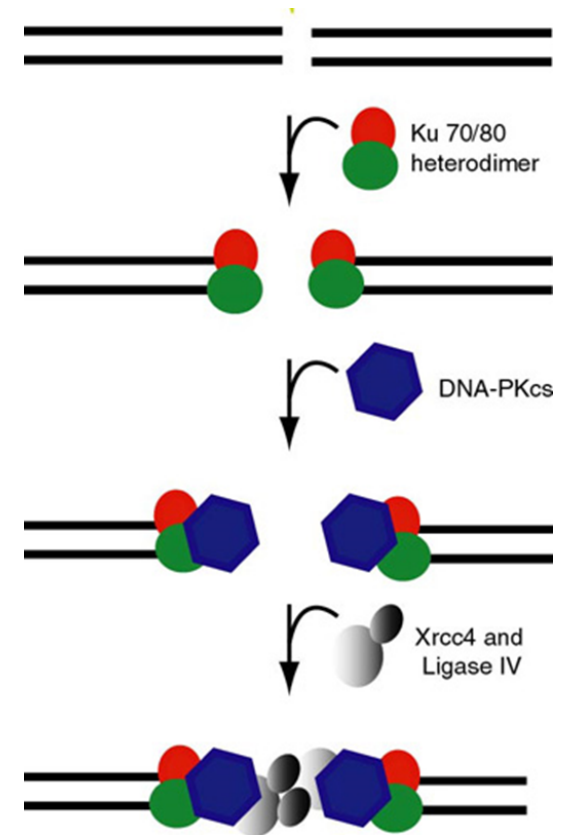


What is the experiment?

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

Check list of critical materials to be used:

1. Wild type CHO-K1 cells
2. CHO xrs6 cells, deficient in Ku80 protein
3. Inhibitor of DNA-PKcs
4. Plasmids containing DNA double strand breaks of different topologies.



Chinese Hamster Ovary (CHO) cells are immortal
– they can grow indefinitely

NEXT LECTURE

- Isolating X-ray-sensitive (xrs) CHO cells
- Xrs cells are deficient in NHEJ
- Detecting NHEJ proteins by Western
- Measuring NHEJ activity
- Using fluorescent proteins to measure biological processes....



20.109 Spring 2015 Module 2

System Engineering and Protein Foundations



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