Module 1: DNA Engineering
M1D1 Lecture

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

Some lecture slides from

Prof. Engelward and Samson

- Lectures in Module 1
  - Biology and engineering of DNA damage and repair
  - Preview of experiments each day

- Today: M1D1 Lecture
  - Preview of module and underlying biology

- Today/Tomorrow: M1D1 Lab
  - PCR basics
  - Rational primer design

What experimental question will you ask in Module 1?

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

Julian Voss-Andreae
(photographer, sculptor)

This raises the following questions

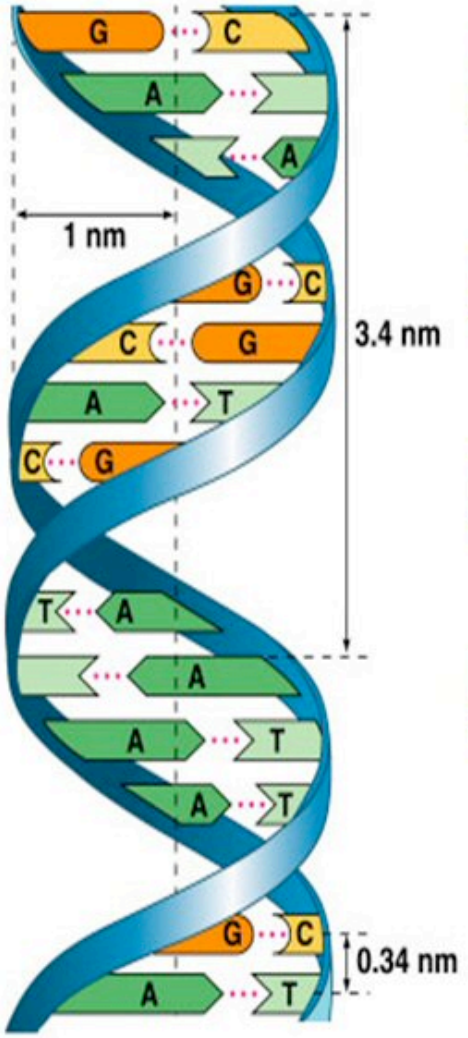
- How does DNA get damaged?
- What is DNA repair?
- Why does DNA repair exist?
- Why do we care about how efficient DNA repair is?
- How does one actually measure DNA repair?

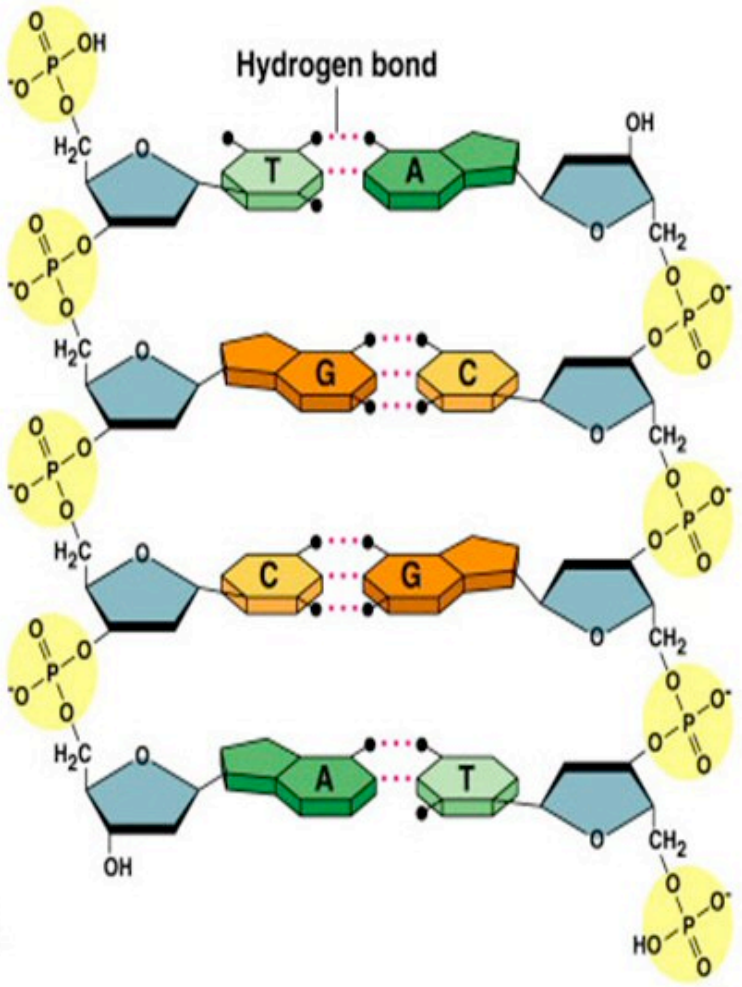
Key Experimental Methods for Module 1

- Construction of truncated eGFP gene – Cloning
  - PCR, Restriction Enzymes, Ligation/ Transformation, Bacterial culture
- Mammalian tissue cell culture
- Transfecting plasmids into mammalian cells
- Flow cytometry to measure DNA repair
- Statistical analysis of biological data

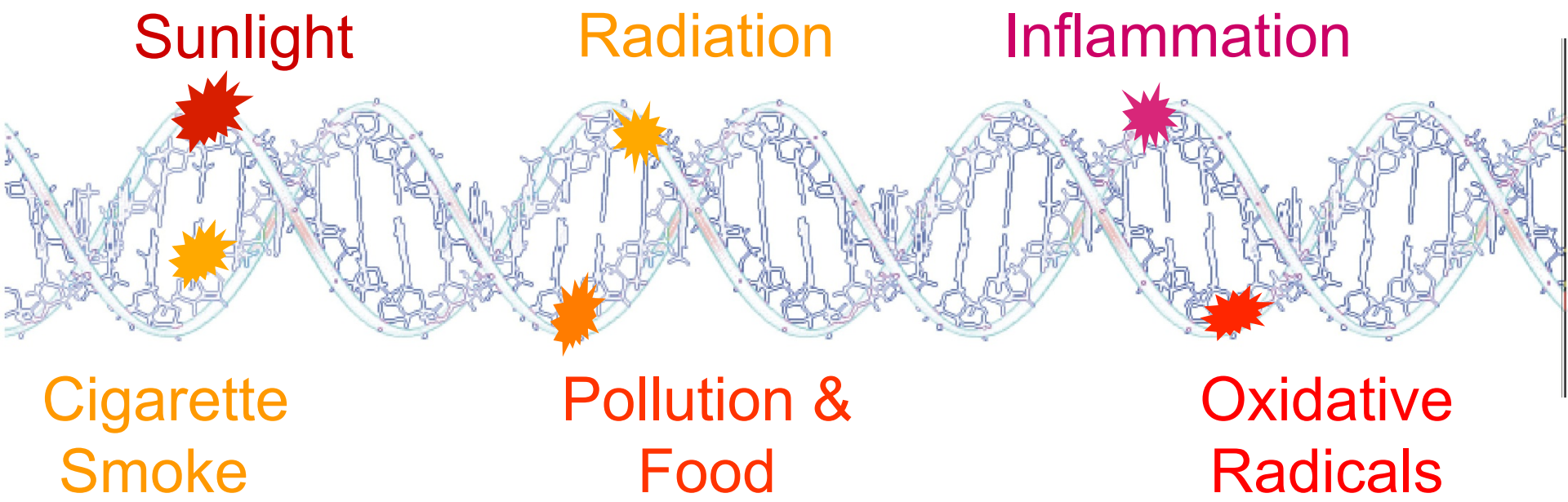
DNA structure – stable, but vulnerable

DNA structure – stable, but vulnerable





DNA is constantly being damaged by endogenous and exogenous agents

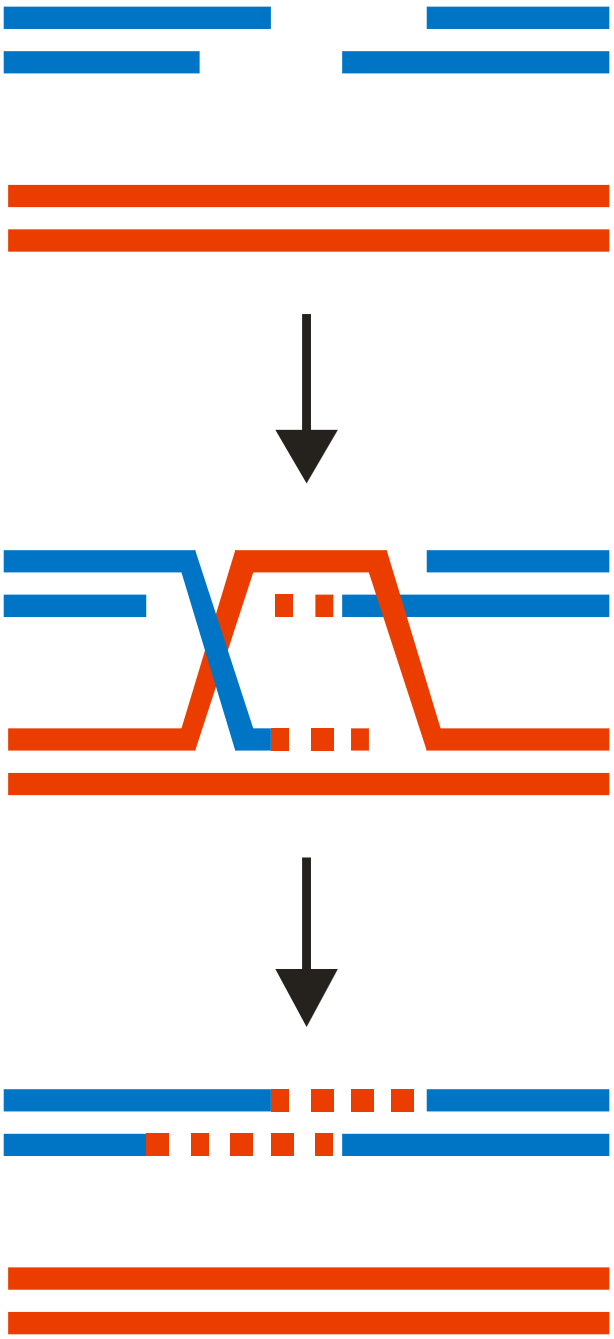


Courtesy of Bevin Engelward

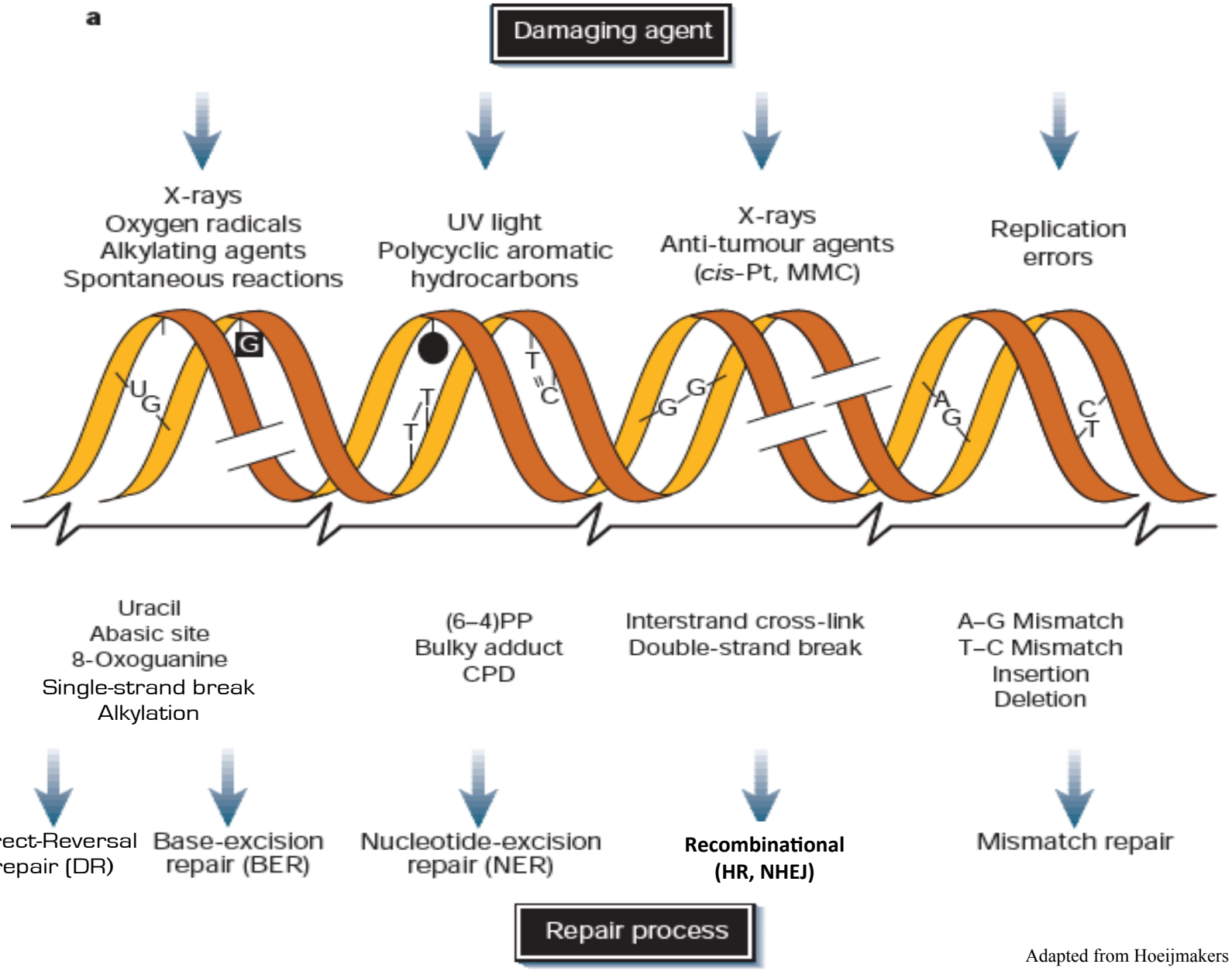
What’s the big deal?

The main DNA repair pathways:

Homologous Recombination

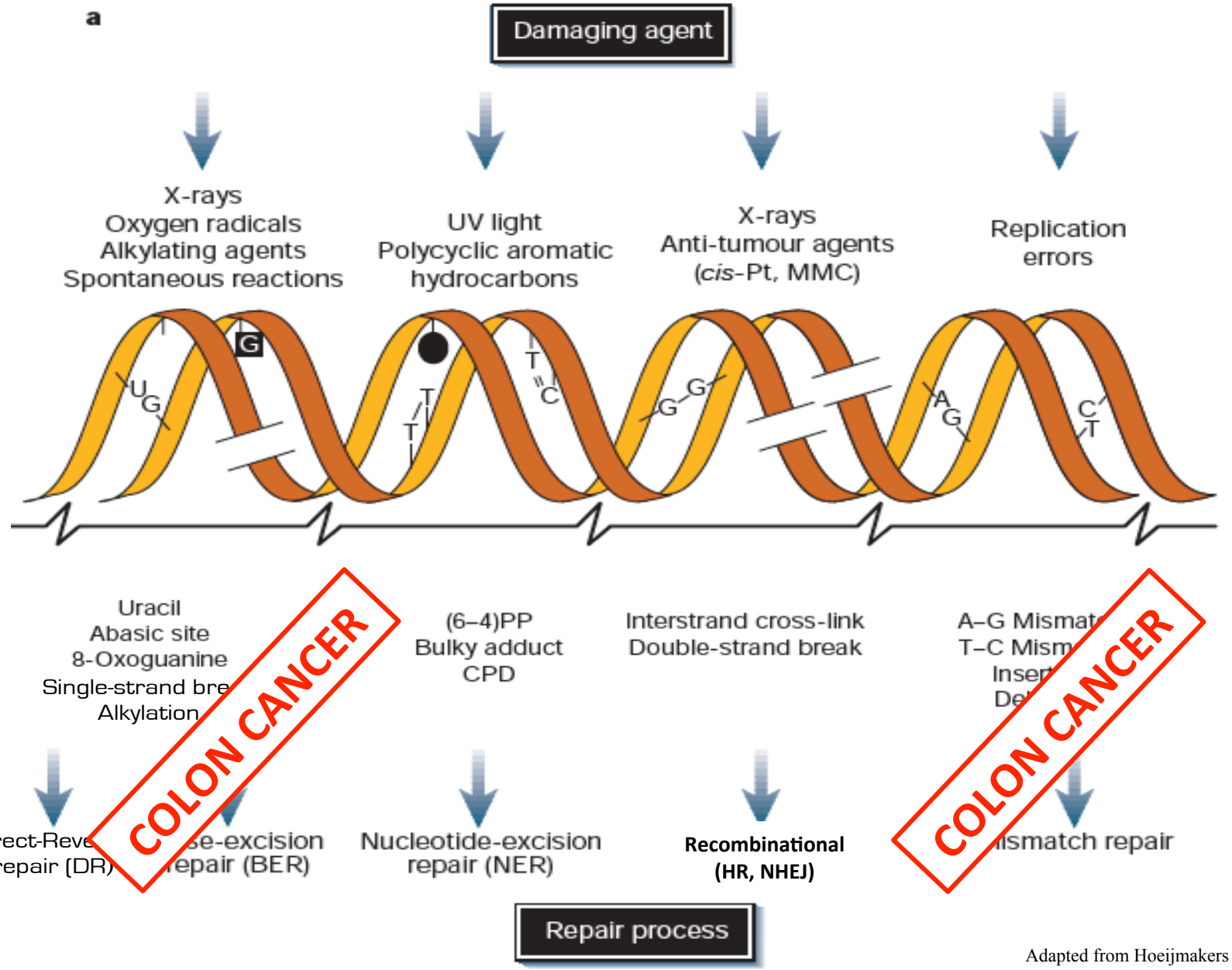


DNA Damage and Repair



Adapted from Hoeijmakers **2001** *Nature*

DNA Damage and Repair



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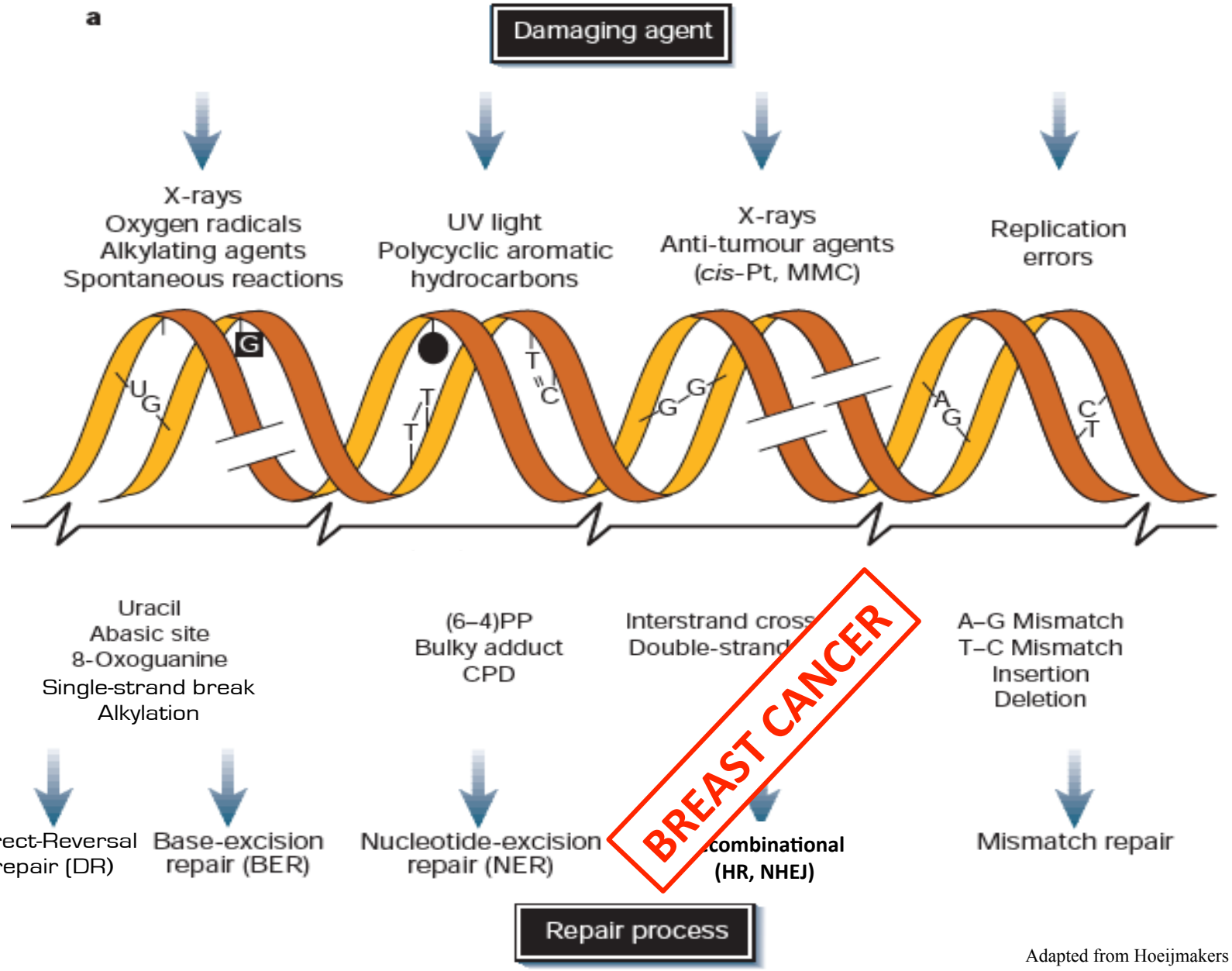
DNA Damage and Repair



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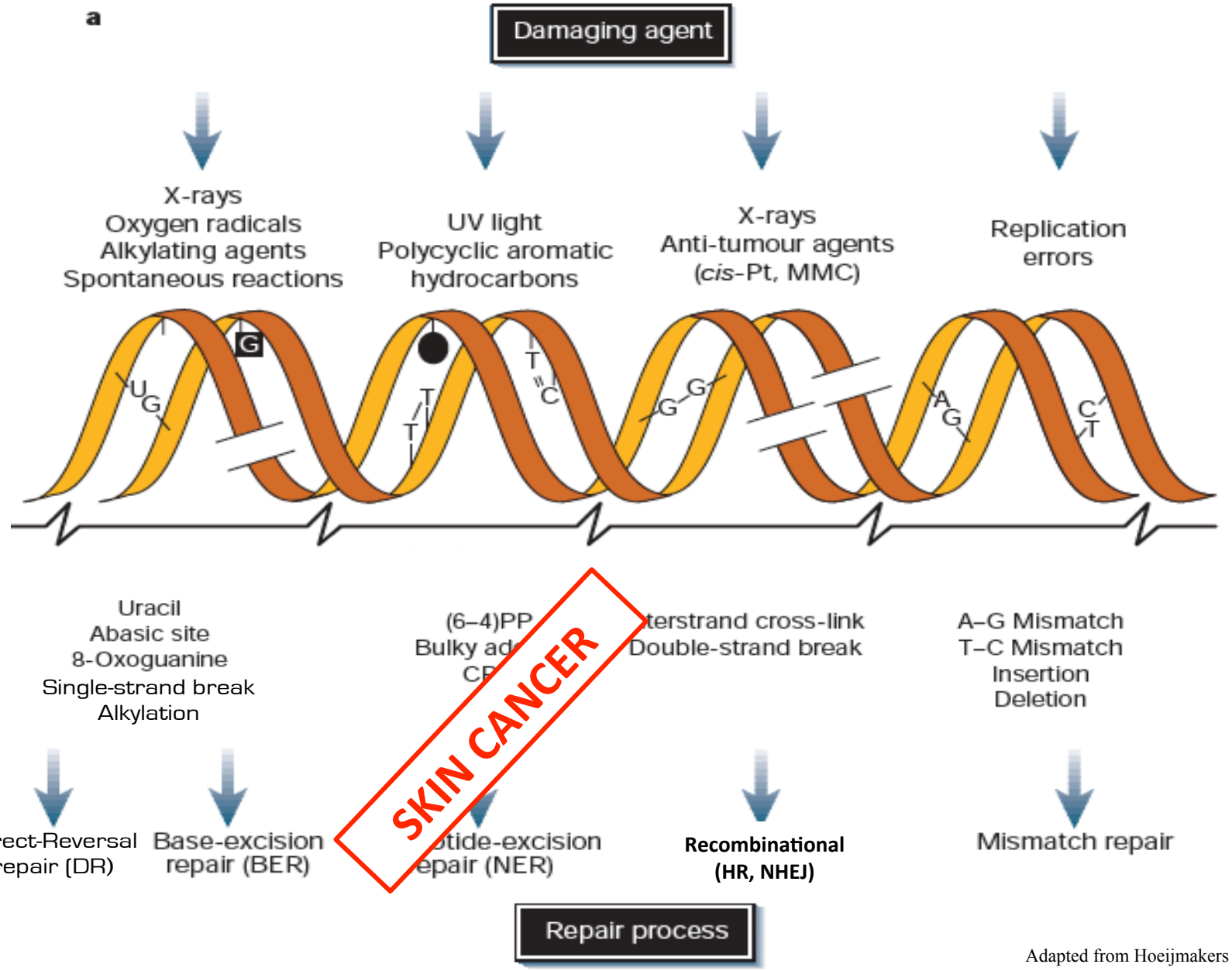
DNA Damage and Repair

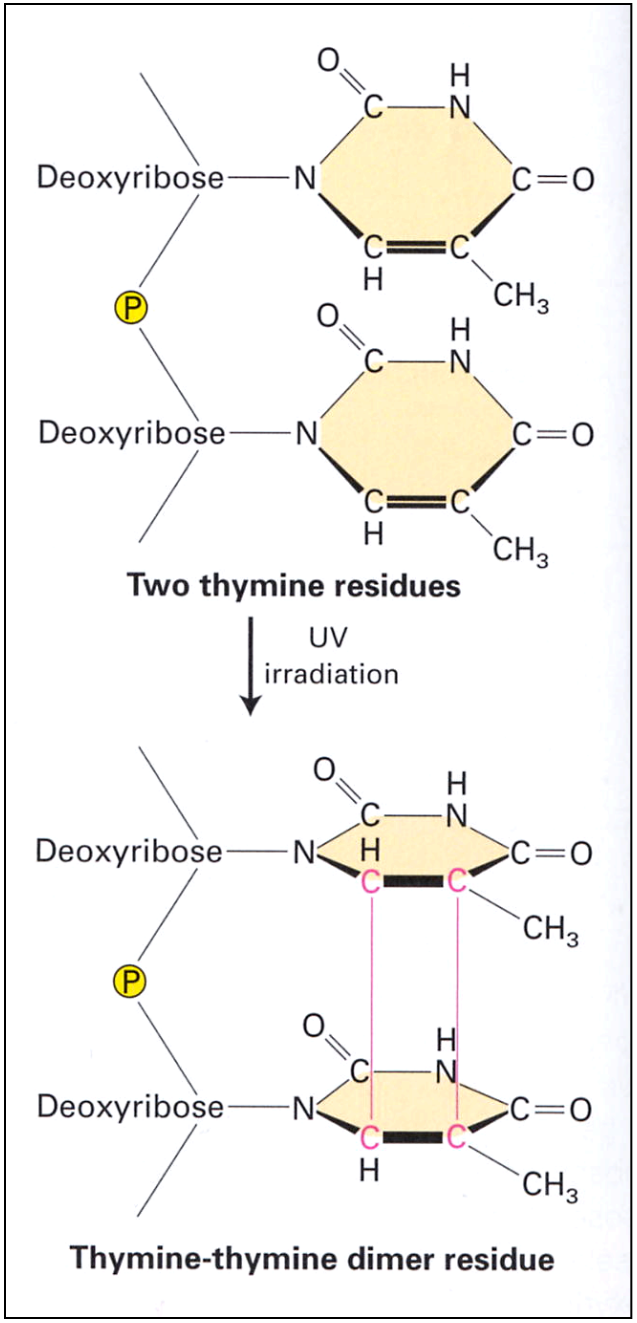


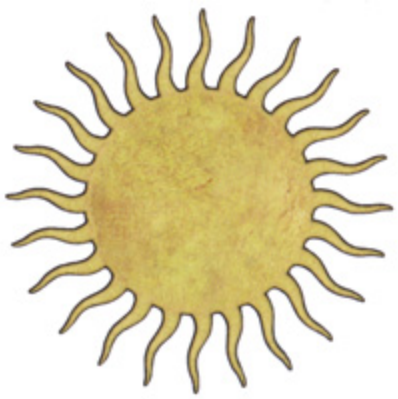
Adapted from Hoeijmakers **2001** *Nature*

What are the known risk factors for Skin Cancer?

*Joke shamelessly borrowed from Leona Samson

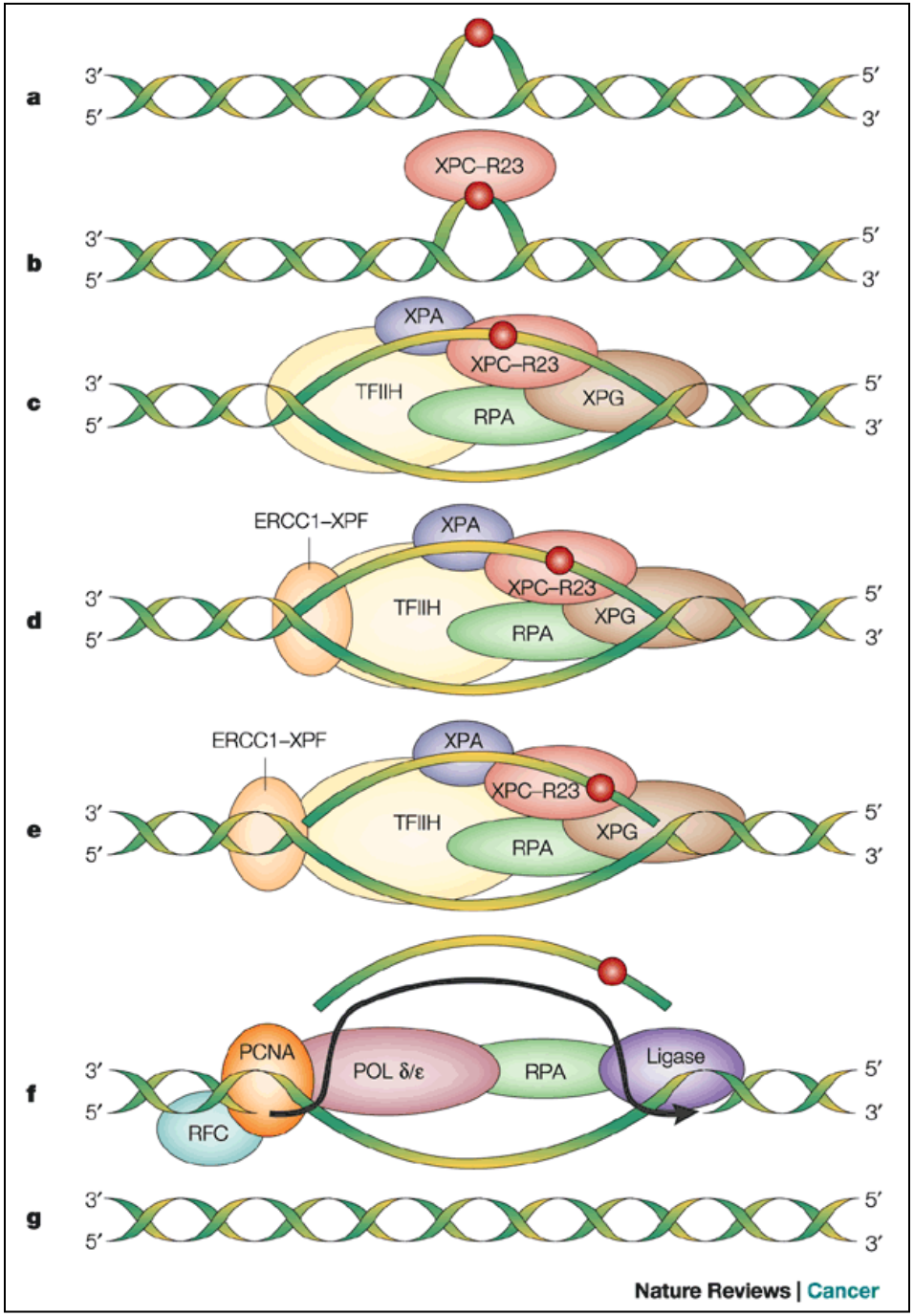
What are the known risk factors for Skin Cancer?





Before

After

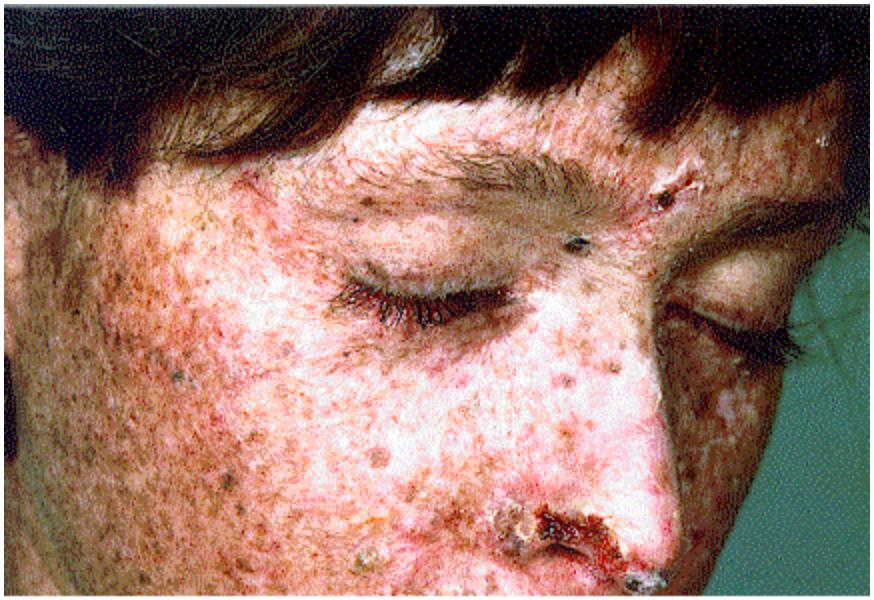


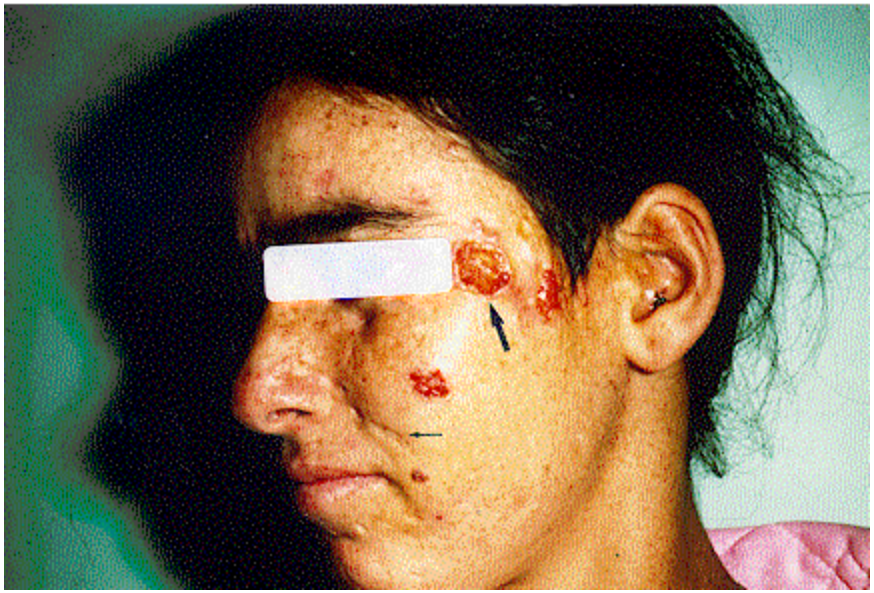
Nucleotide Excision Repair

XPA
XPB
XPC
XPD
XPE
XPF
XPG

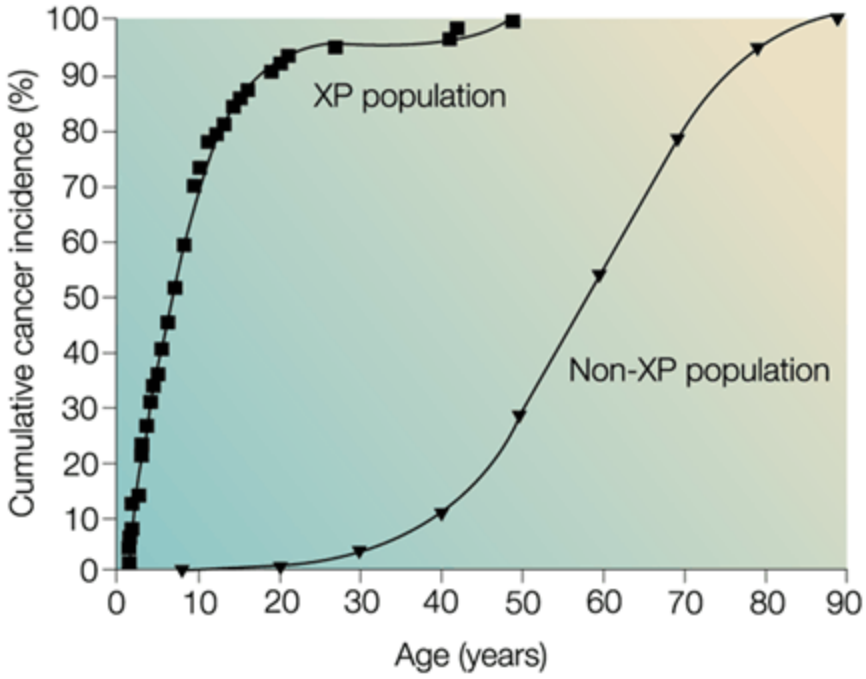
Errol C. Friedberg
Nature Reviews Cancer 1, 22-33 (2001)

Lack of DNA repair accelerates the onset of cancer





Xeroderma pigmentosum (XP)



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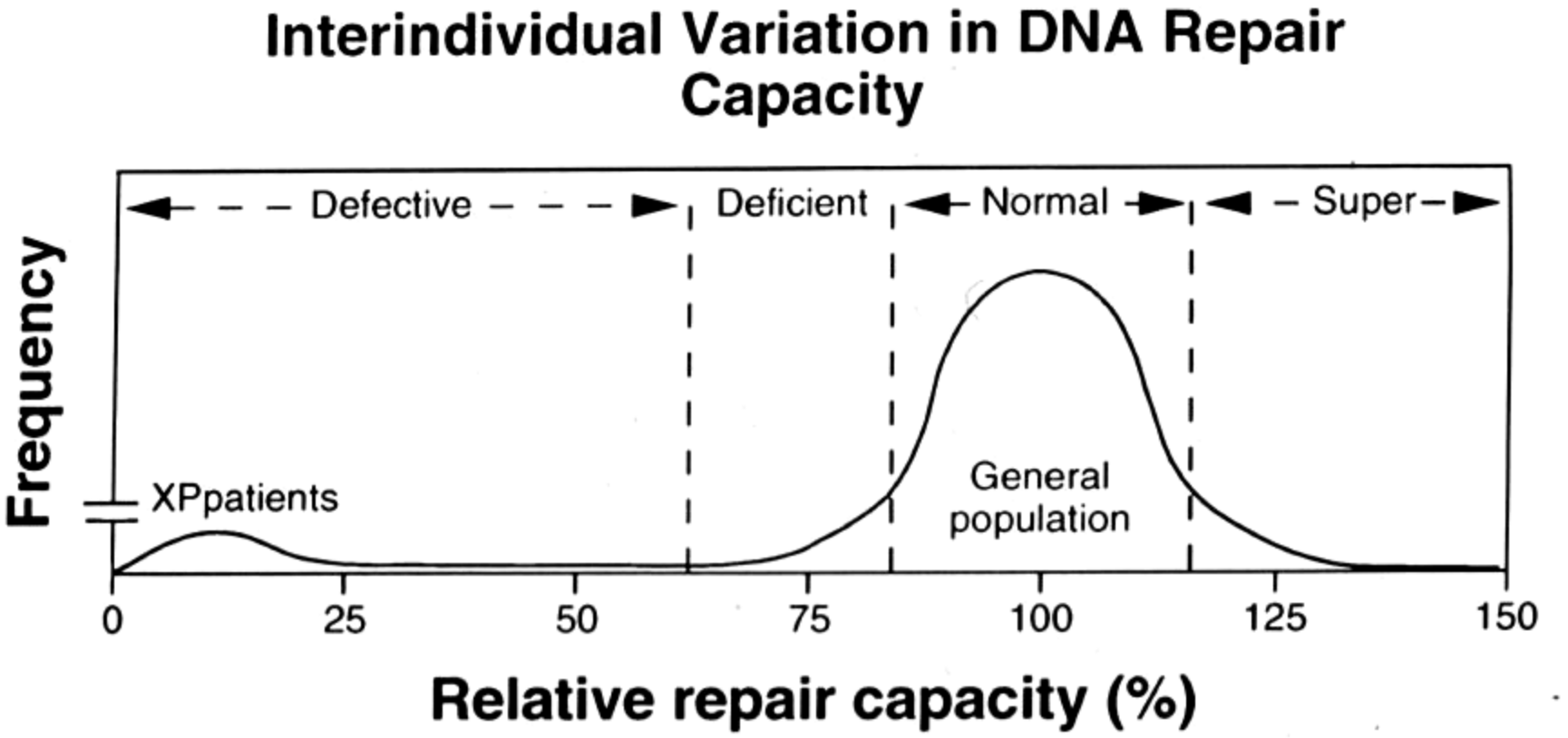
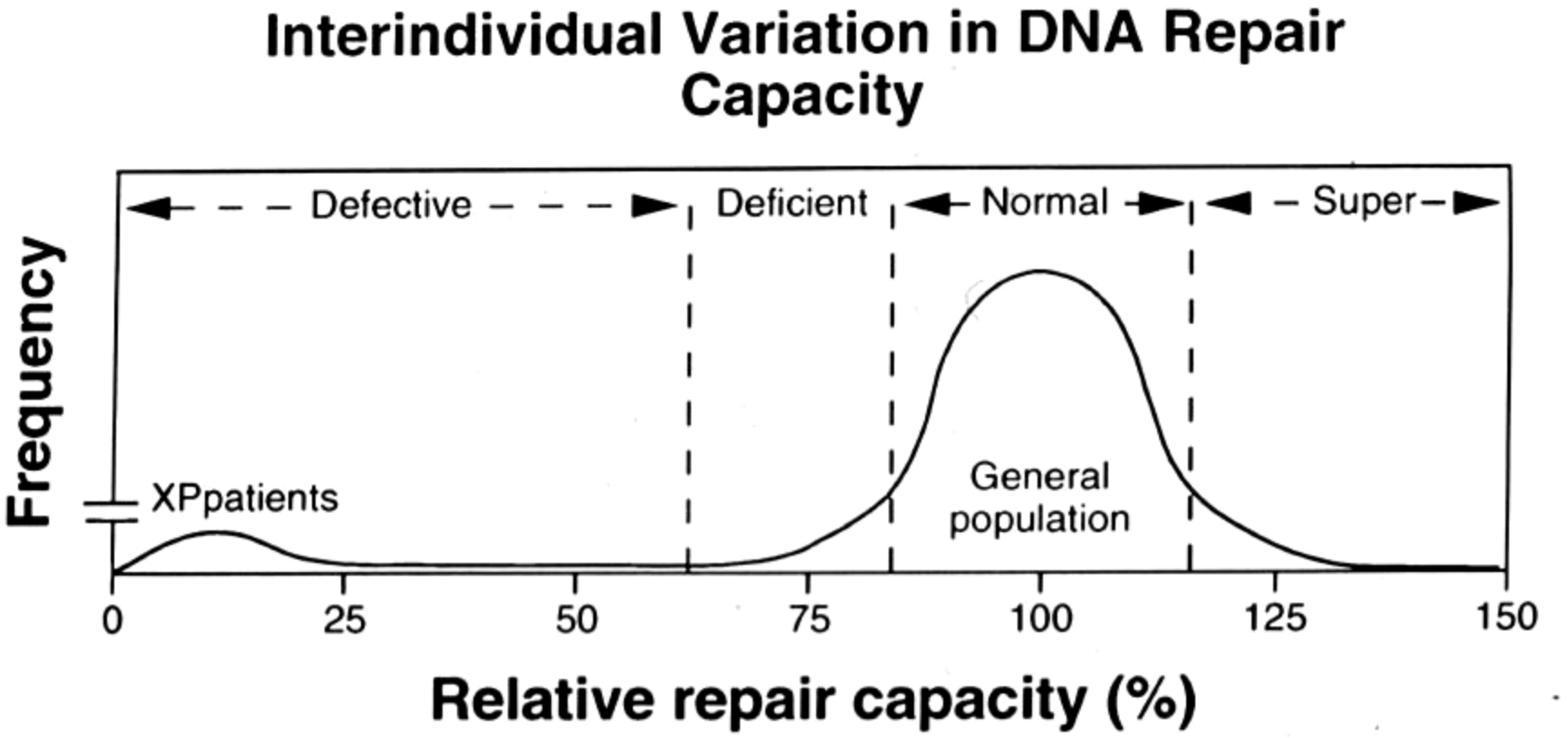


Adapted from **GROSSMAN and Wei (1995)** Clinical Chem 41: 1854-1863

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

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



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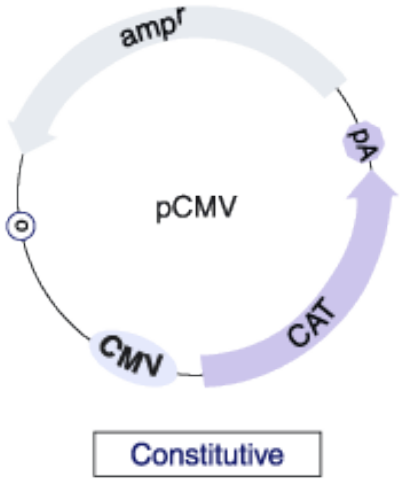
XP frequency = ~1:250,000 giving a theoretical maximum of ~28,000 cases worldwide with 2,000-fold increased risk

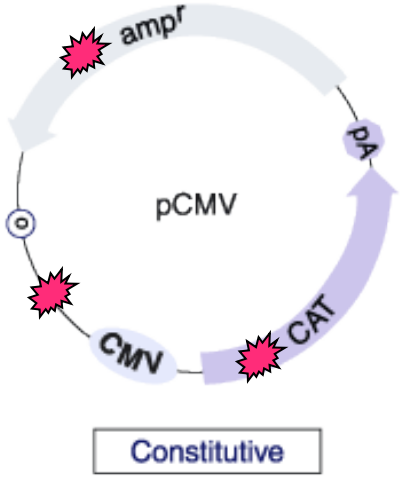
Even if just 1% of the population is relatively repair deficient, could have tens of millions with several-fold increased risk

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

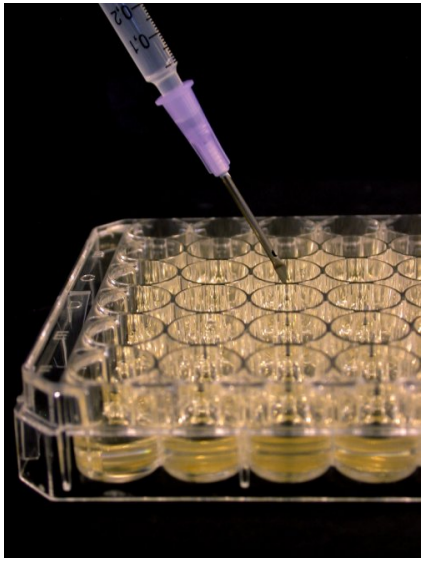
Athas & GROSSMAN
Cancer Res. 1991

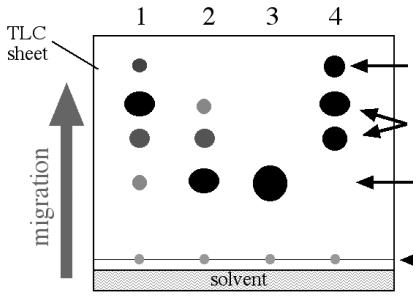


+ UV light



Transient transfection peripheral blood lymphocytes

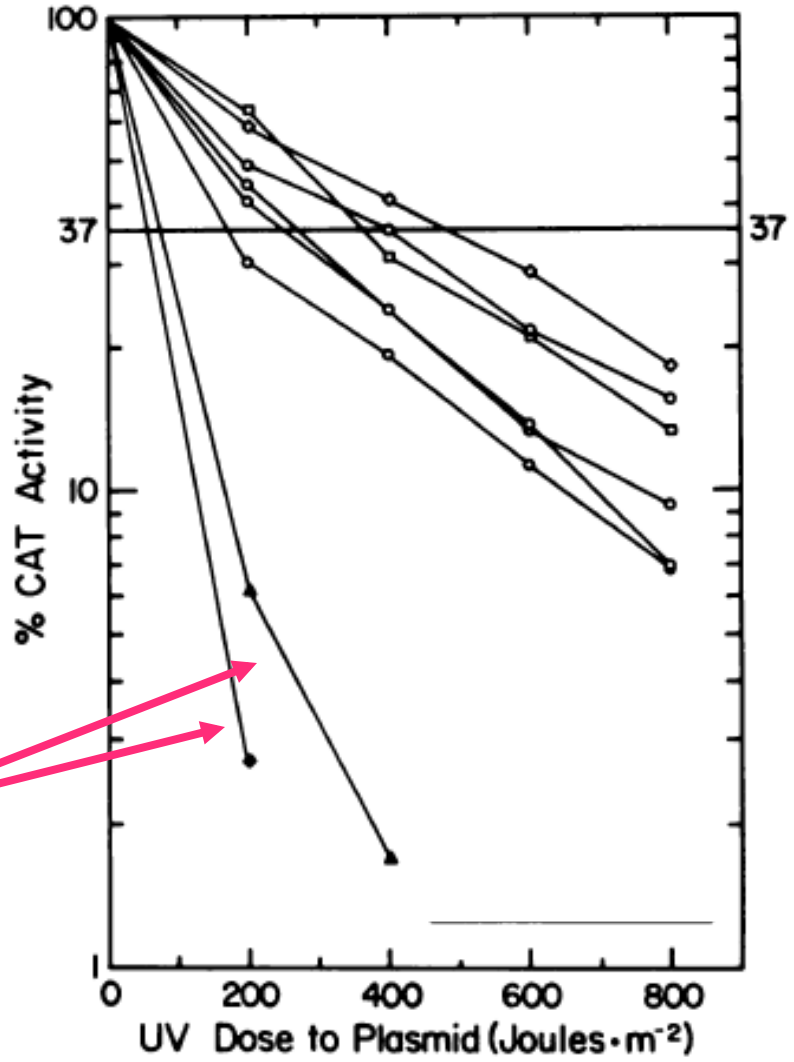




CAT Assay

Time to repair

Fresh Circulating Lymphocyte
Plasmid HCR in XP and Normal PBL

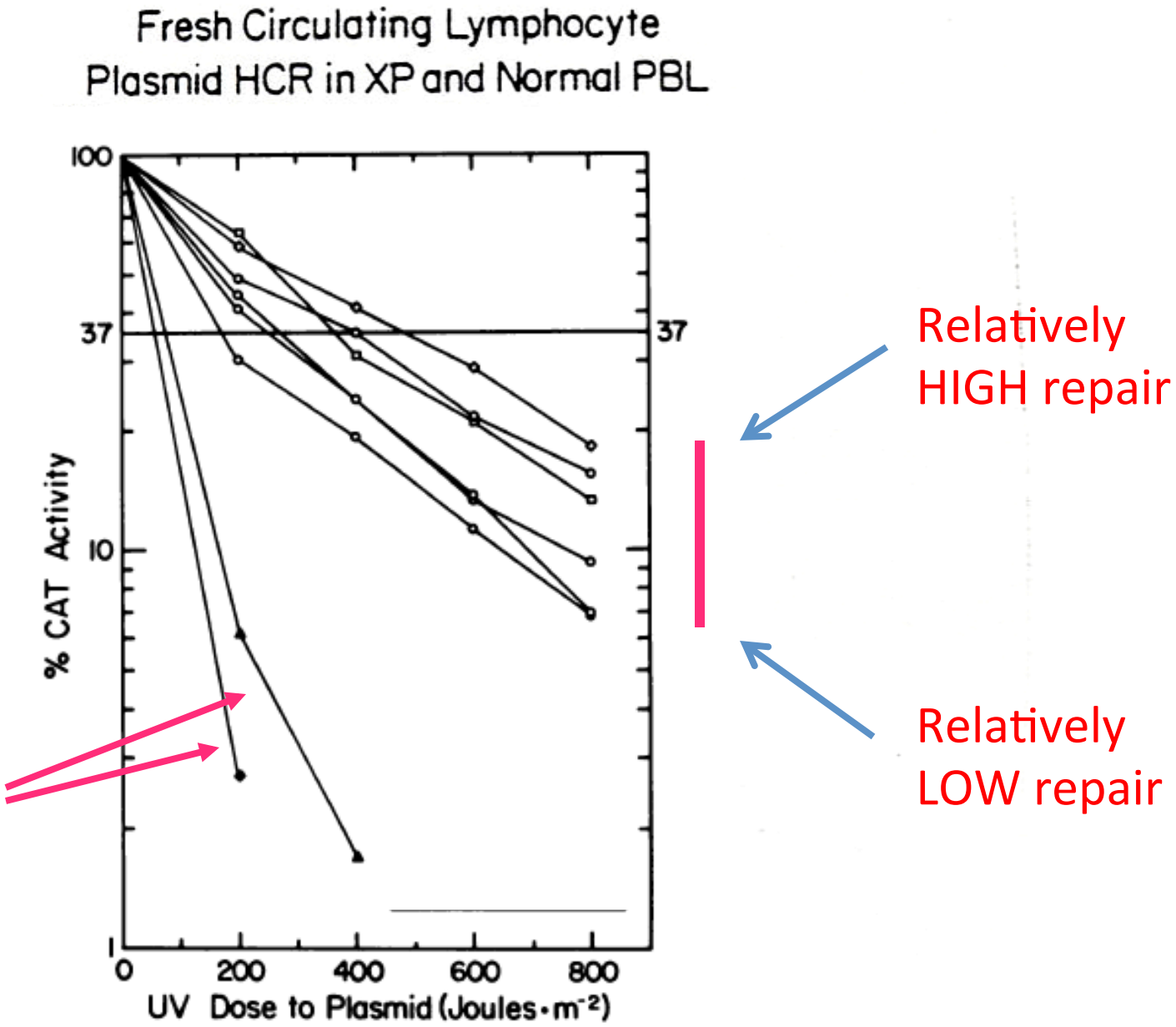


Cells from 'healthy" people

Cells from XP patients

Athas & GROSSMAN

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



Athas & GROSSMAN

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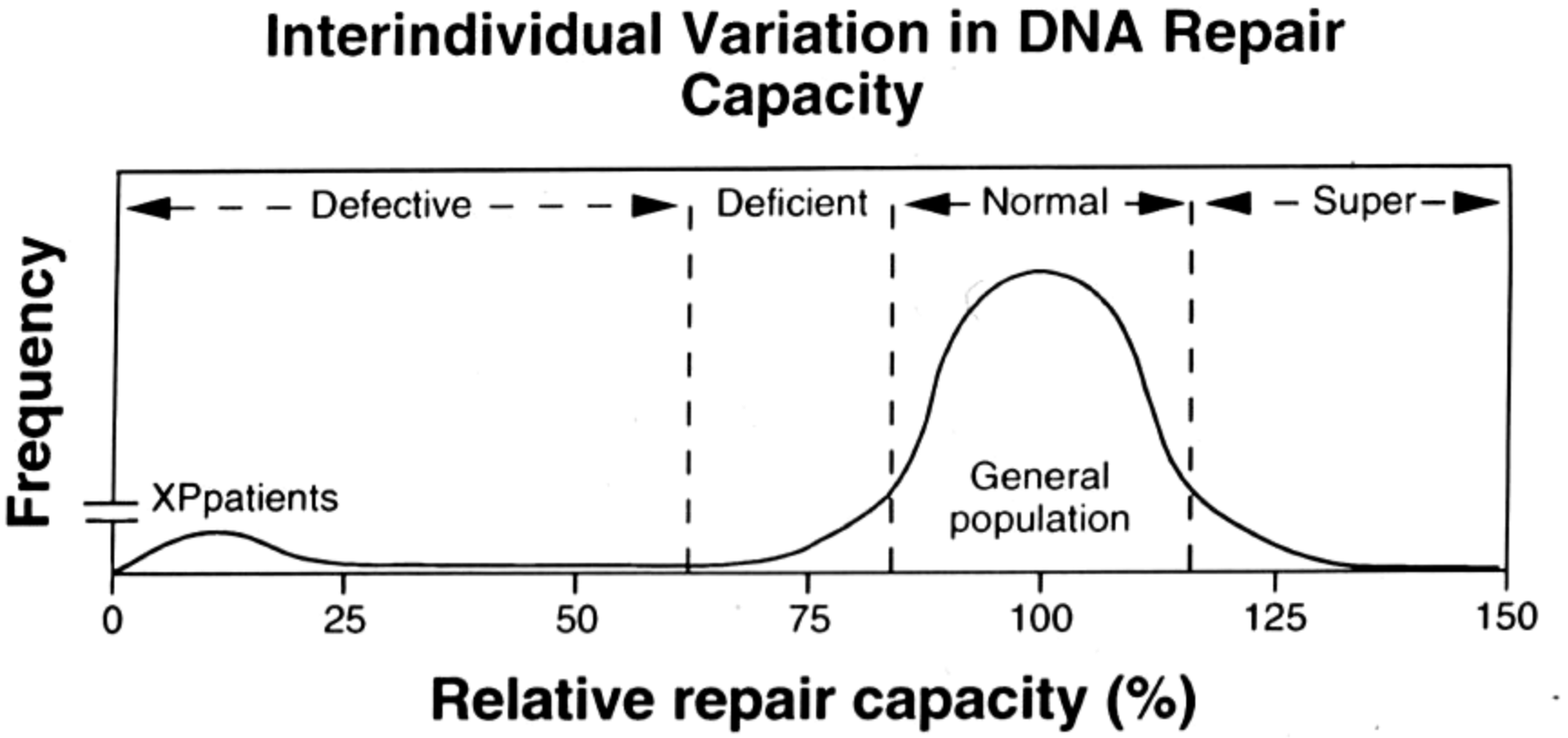

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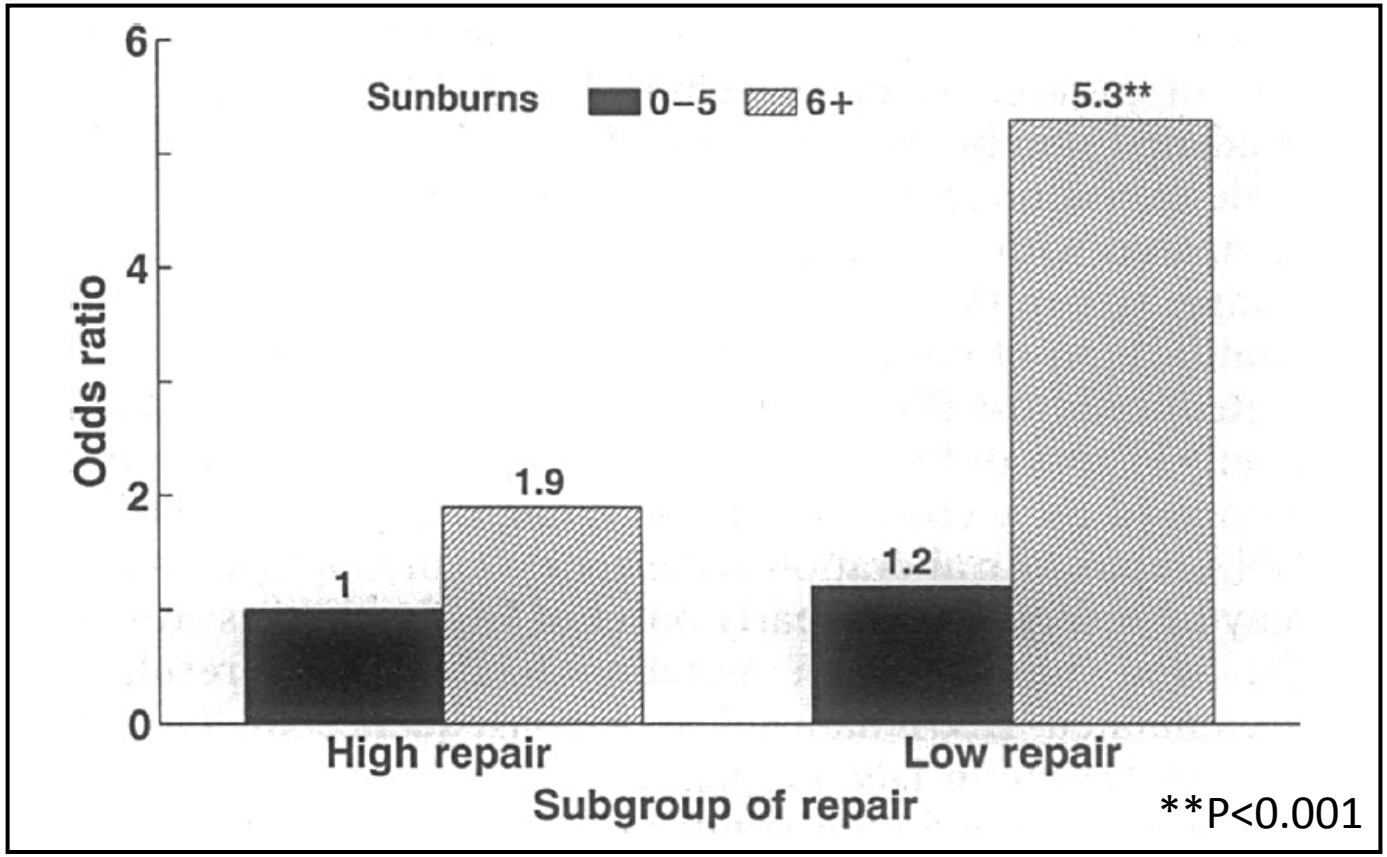
Even if just 1% of the population is relatively repair deficient, could have tens of millions with several-fold increased risk

Low NER 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.

…and the effect is gender specific.

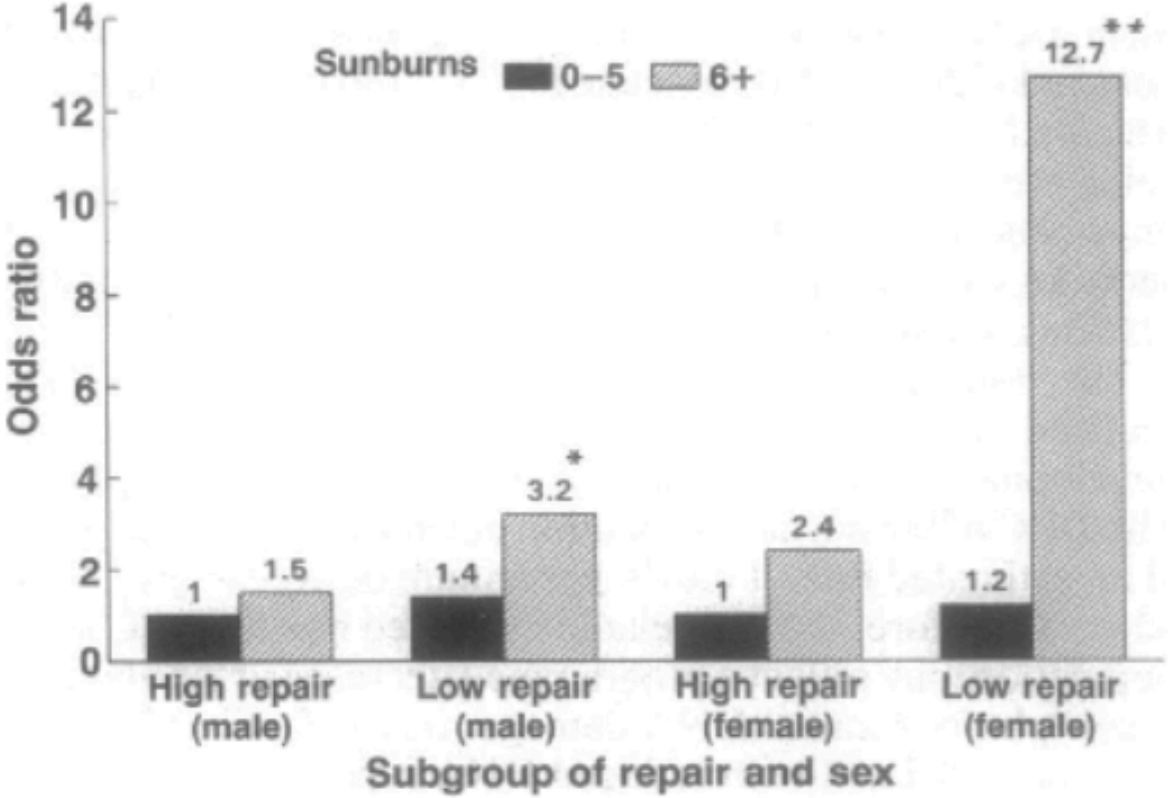


FIG. 3. Effect of DCC on risk of BCC by gender: Relationship to number of severe sunburns in a lifetime. The data in this graph are

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

Very current motivation for Mod1:

NYT – Monday, Sept 8 2014 – Winners of the Lasker Prize ("American Nobel") announced

HEALTH | Lasker Winner Calls for More Genetic Testing for Cancer

Dr. Mary-Claire King, a noted breast cancer geneticist, is one of five scientists to win the award. University of Washington

"We recommend that every woman in America of any race or ancestry be offered this opportunity when she's in the midst of childbearing or beginning childbearing," Dr. King said in an interview. "You only need to be tested once, and the vast majority of women will not have a mutation and can go about their life. The actual cost is minimal.

"But women who do learn they have a mutation that's comparable to Angelina Jolie's and confers very high risk can begin to think about what that means and be referred to a high-risk clinic" to develop a prevention plan, she said.

A common recommendation is to have the ovaries removed by age 40, because ovarian cancer is difficult to diagnose early and, also, removing the ovaries may reduce the risk of breast cancer.

Several experts in the field called the proposal a "provocative," and some said they wanted to see more evidence that a healthy woman with a genetic mutation but no breast or ovarian cancer in her family is at high risk for the diseases.

But Michael Watson, the executive director of the American College of Medical Genetics, said medicine was already "rapidly moving" toward genetic screening in healthy patients, though there are "still gaps in our

Dr. King discovered link between BRCA1 and breast cancer.

Mutations in BRCA1 & BRCA2 increase risk of breast cancer – but mutation does not guarantee cancer.

Is there a better way to decide on preemptive action other than just genetic screening?

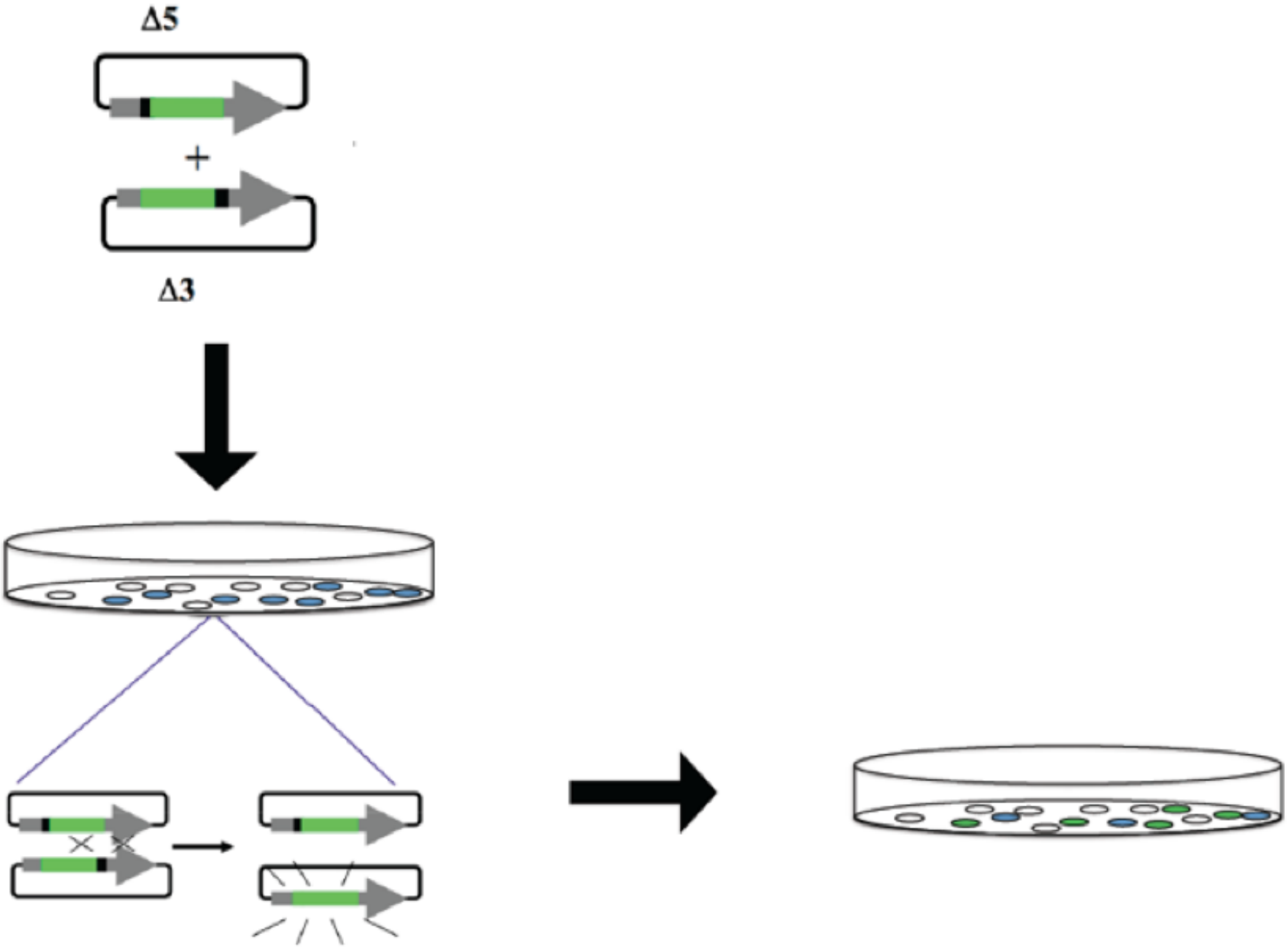
Your Experiment:

Create a plasmid that will be part of a homologous recombination sensor.

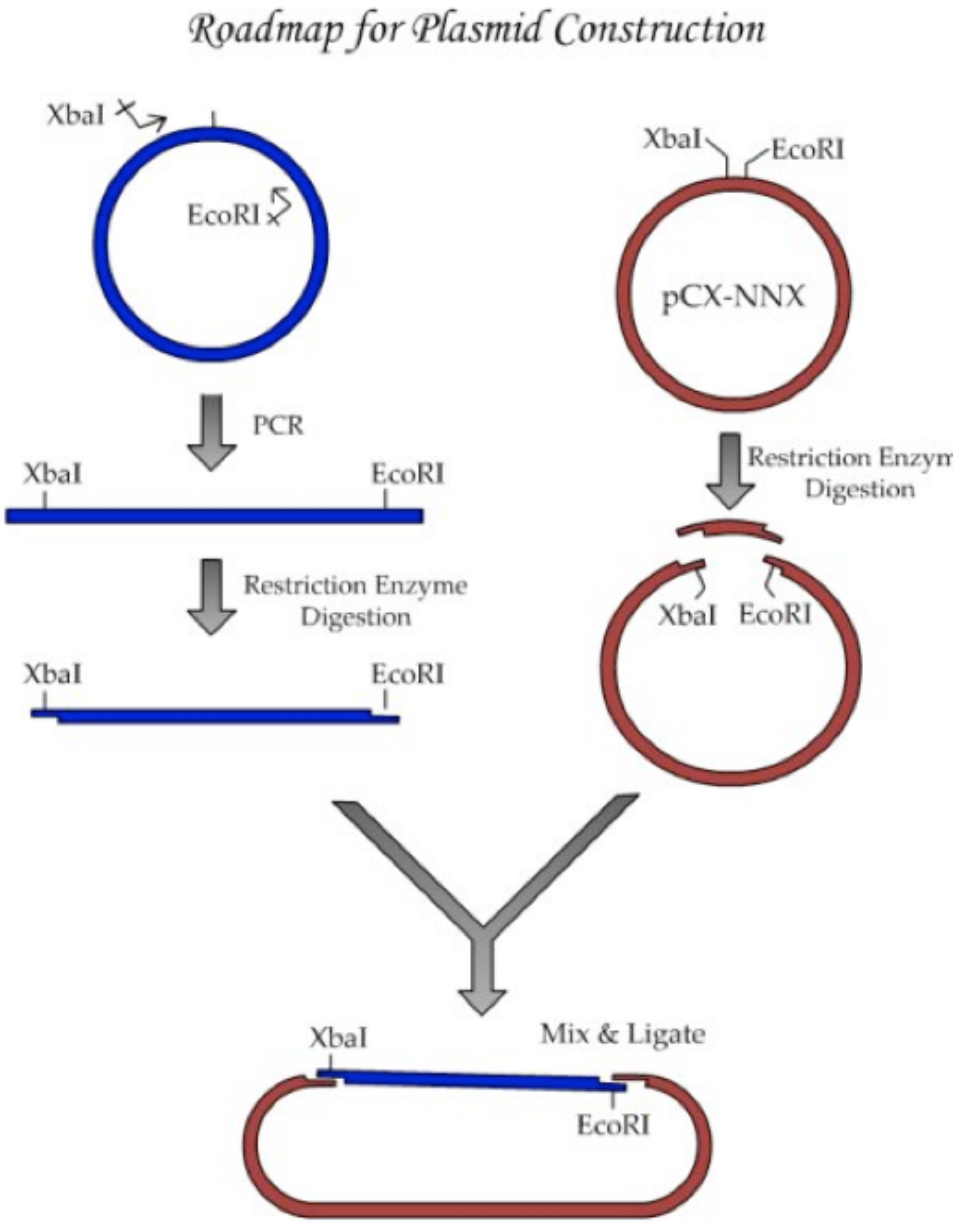
Measure the frequency of cells in which HR between two plasmids gives rise to a fluorescent cell.

Test conditions that might affect the frequency of green cells!

Your Experiment:

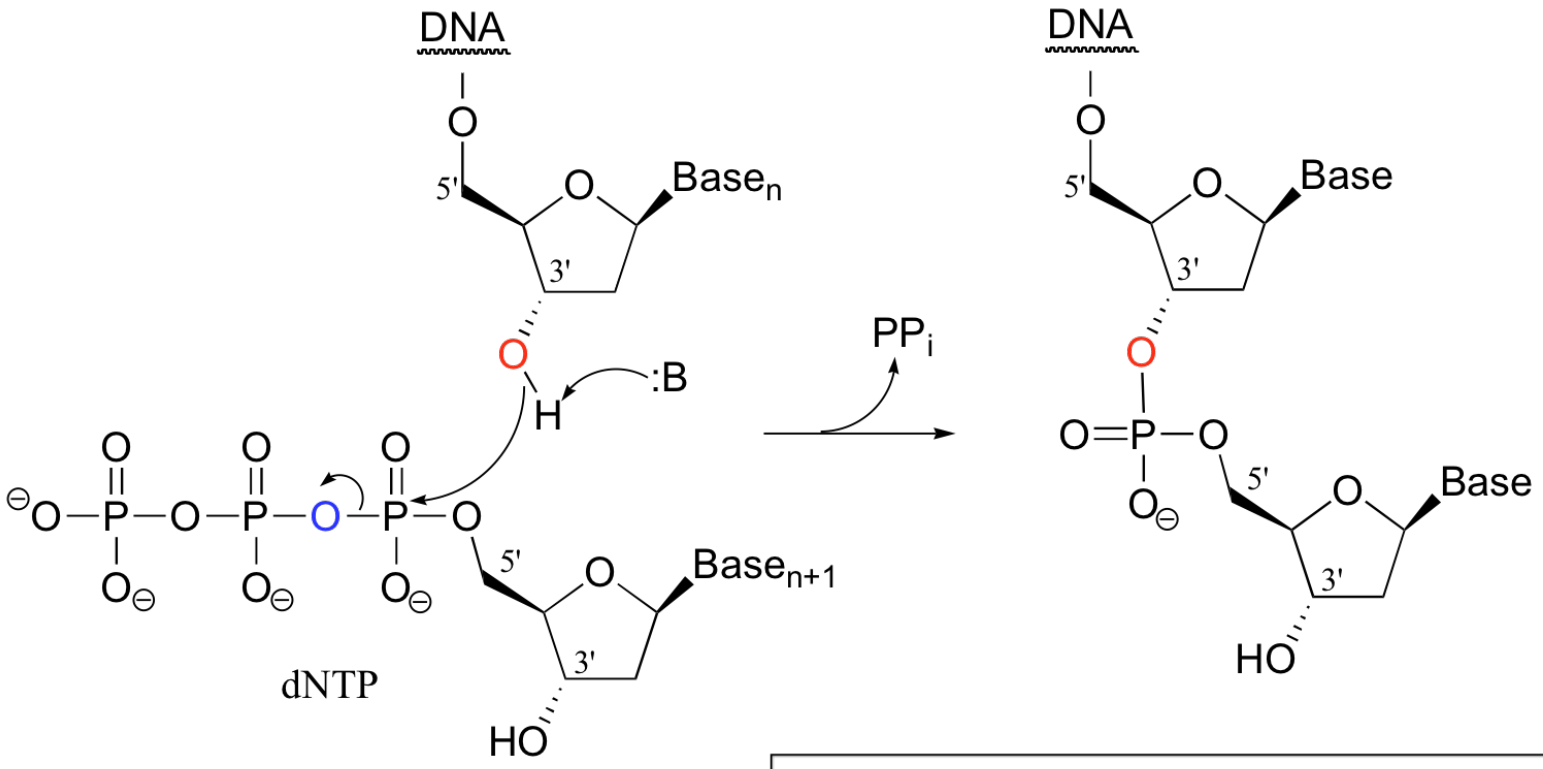


Today you will start building your system.

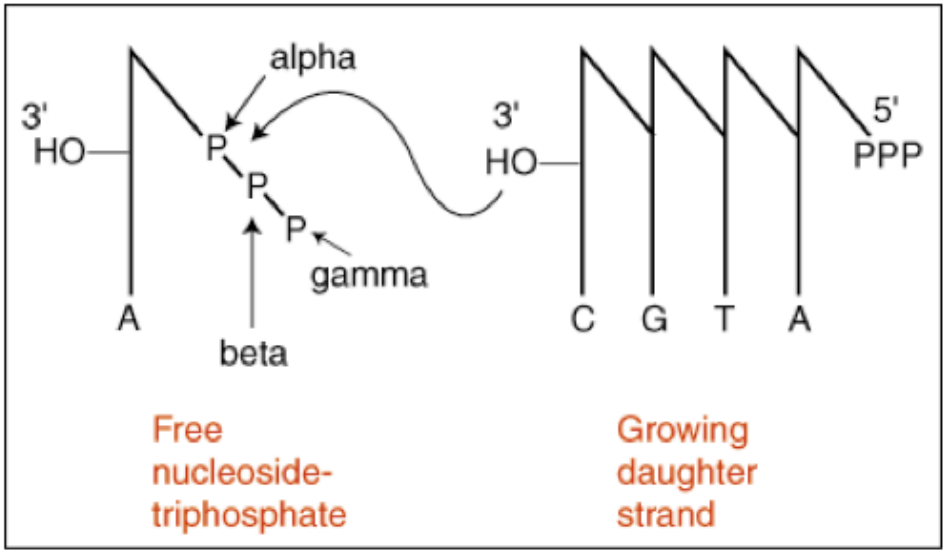


M1D1: PCR

DNA Learning Center -- Cold Spring Harbor



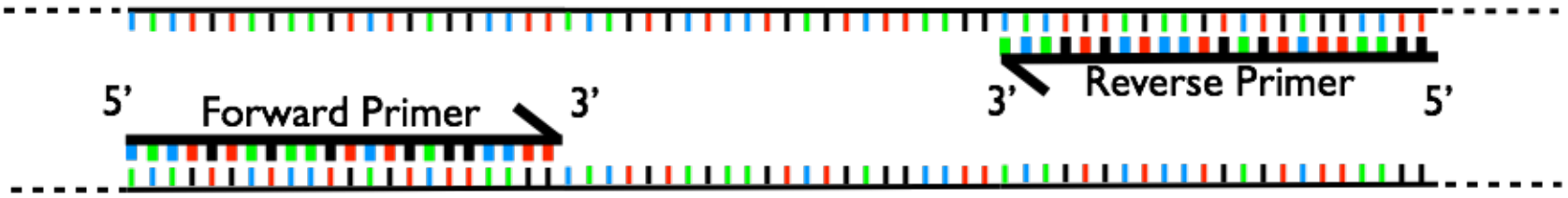
DNA Replication



http://www.sparknotes.com/biology/molecular/dnareplicationandrepair/section2.rhtml

M1D1: Primer Design

M1D1: Primer Design



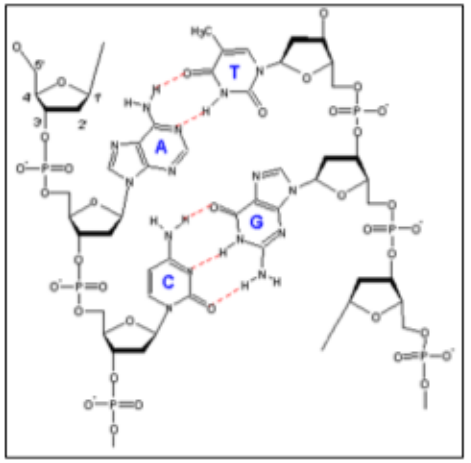
1. The <u>forward primer binds to the α-sense strand</u> (or the 'Template') and 'reads' in an intuitive direction from 5' to 3'. Look at the reverse primer and consider it's orientation.

2. Primer <u>length is important</u> to decrease the chances of off-target binding:
   - Consider that the human genome is ~ 3x10^9 bp. If we designed primers that were only 10 bp long, we might expect to find that 10bp sequence once in every 4^10 ≈ 10^6 bp -- a very risky gamble for off target binding.
   - The optimal primer length is > 16 bp for specificity. Think about why.

3. Primer <u>melting temperature should optimal</u>ly be kept between 55-60 C.
   - Tm,p is the temp ~50% of the primer is double vs. single stranded.
   - The melting temperature will be higher with increased G/C content.

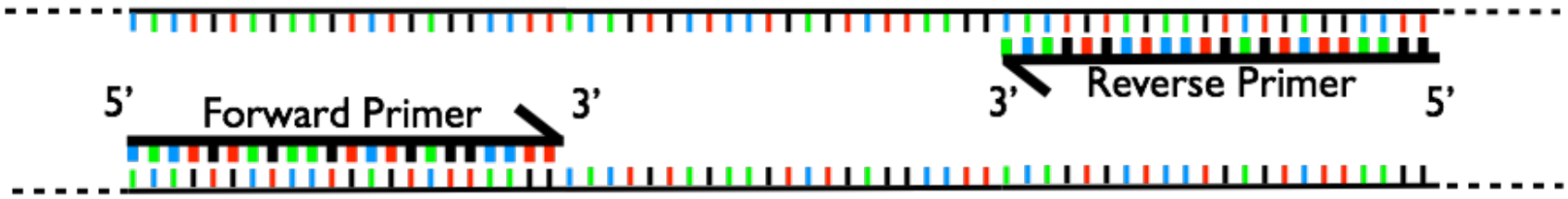
   Why? Look at the diagram of bp hydrogen bonding to the right -- which pair requires more energy to denature? *Also explains why optimal primer design calls for only 40-50% of the bp to be G/C.*

   The Tm,p is kept between 55-60C so that the annealing (hybridization) step is optimally efficient.



Thermodynamics of DNA Duplex, New Mexico State University

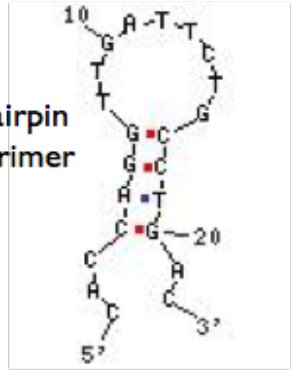
M1D1: Primer Design



4. Avoid long repeats of one type of bp (ex. ATATATA) or one bp individually -- especially TTTT -- remember the polyA tail on pre-mRNA? This can lead to non-specific priming.

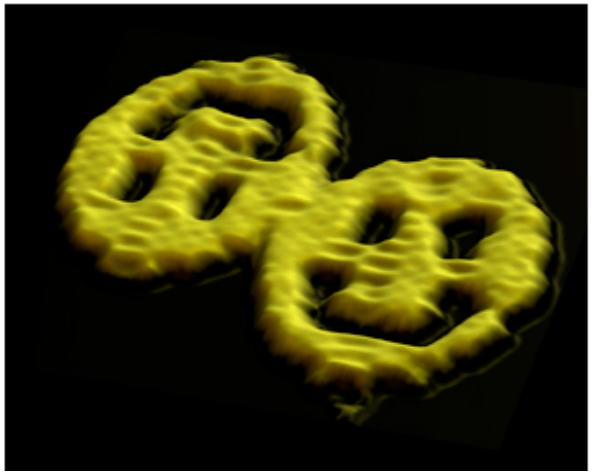
5. Consider secondary structure of your primer.
   - Does the primer have an internal sequence that can bind itself?

   If so, you can end up with a hairpin structure that will prefer (energetically speaking) to bind to itself and not your target sequence.



Making a hairpin with your primer is bad.

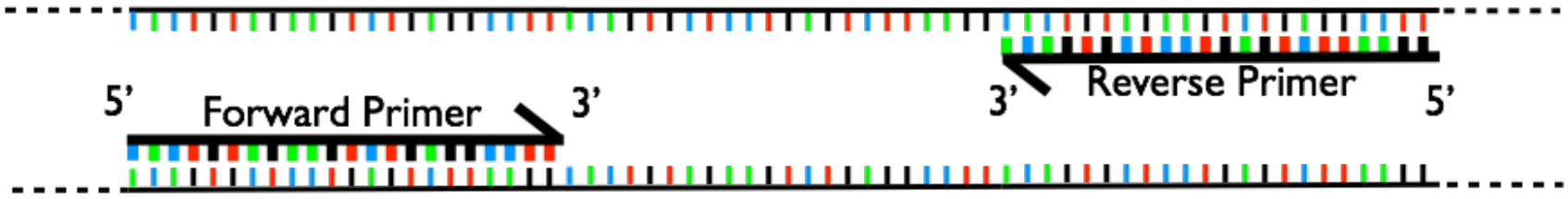
In fact, this behavior of DNA has been harvested to make higher order structures: DNA Origami is an active area of research.



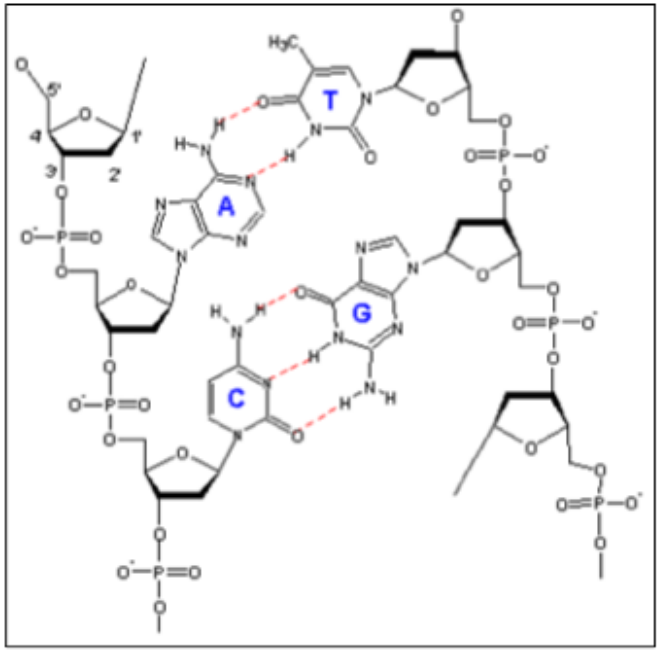
DNA Origami is cool.

Image from: http://www.dna.caltech.edu/~pwkr/

M1D1: Primer Design



6. Tip the deck in your favor: Add a GC clamp to the 3' end if possible.
   - Consider again the image below. G/C binding is more stable and can help to increase efficiency of polymerase binding at the 3' end to promote extension.
   - But don't go overboard! > 5 G/C pairs won't help you.



Thermodynamics of DNA Duplex, New Mexico State University

Image credits:

Slide 2: "Steel Jellyfish", 2006. Stainless steel, height: 4'7" (1.40 m). Location: Friday Harbor Labs, San Juan Island, WA
http://commons.wikimedia.org/wiki/File:Steel_Jellyfish_%28GFP%29.jpg

Slide 32: http://chemwiki.ucdavis.edu/Organic_Chemistry/Organic_Chemistry_With_a_Biological_Emphasis/Chapter_10%3A_Phosphoryl_transfer_reactions/Section_10.4%3A_Phosphate_diesters