Module 2 – Lecture 2 & 3 Gene Expression Engineering (March 14 & 16, 2017)




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Maxine Jonas

Rob Wilson

Leona Samson (Lectures)

What experimental question will you ask in Module 2?

How does DNA repair affect the ability of cancer chemotherapy drugs to kill cancer cells?

How does cancer chemotherapy affect gene expression?

This raises the following questions

- How does DNA get damaged?
- What is DNA repair?
- Why does DNA repair exist?

Key Experimental Methods for Module 2

- Grow human cancer cells in tissue cell culture
- Monitor specific protein levels by Western blot
- Kill cancer cells with chemotherapy drugs
- Engineer the inhibition of DNA Repair pathways
- Monitor changes in a gene's expression (qPCR)
- Analyze RNAseq dataset measuring expression of ~ 20,000 genes (BIG DATA!)
- Statistical analysis of all biological data

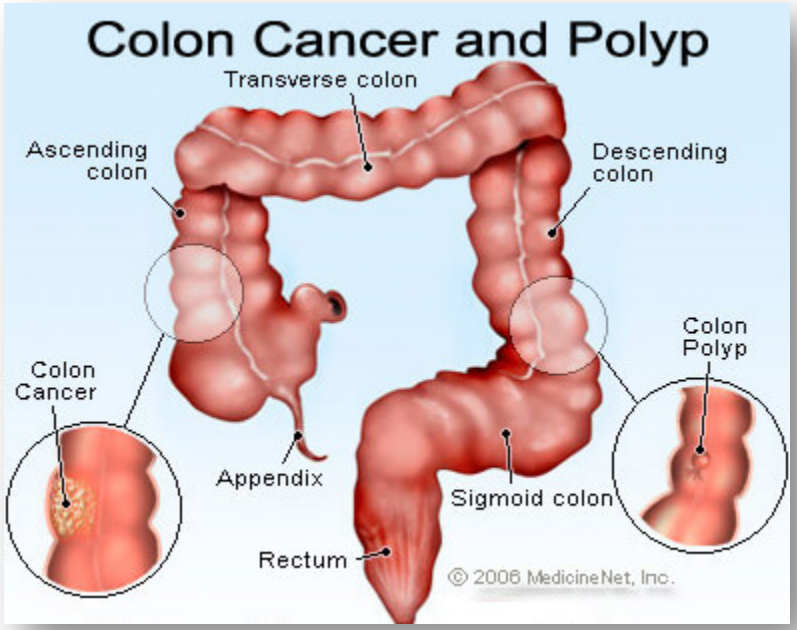
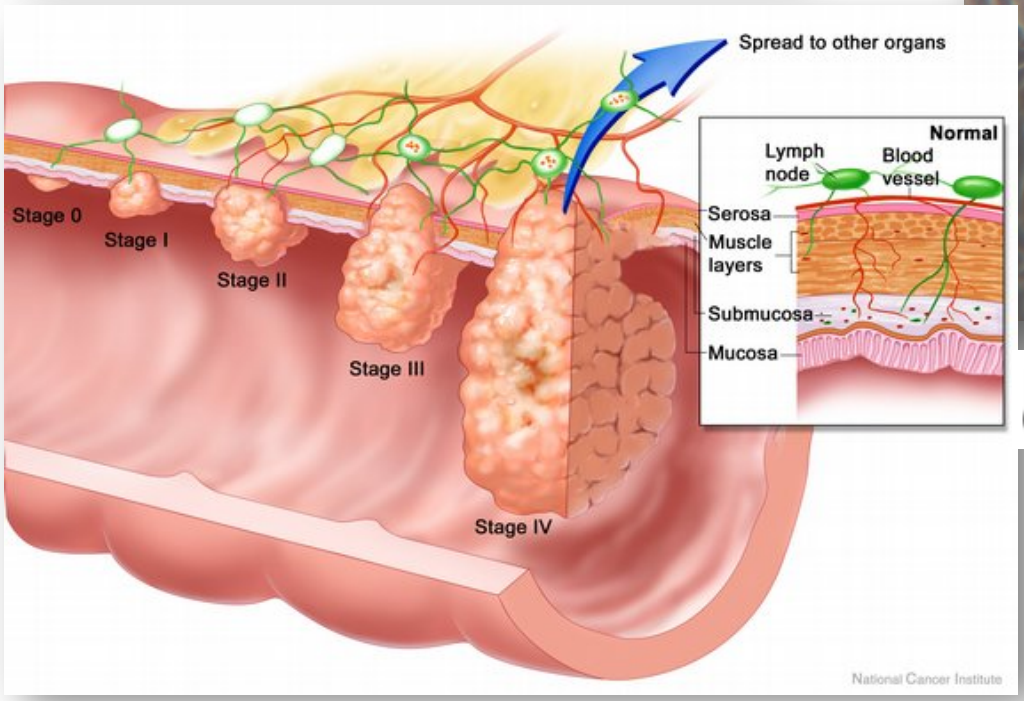


Figure from Lizzie Ngo

Graduate Student
Engelward Lab
& Samson Lab

DLD-1 cells



[CANCER RESEARCH 39, 1020–1025, March 1979]
0008-5472/79/0039-0000$02.00

https://www.google.com/search?q=colon+cancer&rlz=1T4GGHP_enUS635US636&source=lnms&tbm=isch&sa=X&ved=0ahUKEwj1hMLwrsfSAhWX0YMKHSFlB-EQ_AUICCgB&biw=1451&bih=669&dpr=1.75#imgrc=3X3SToZHyQtBfM:

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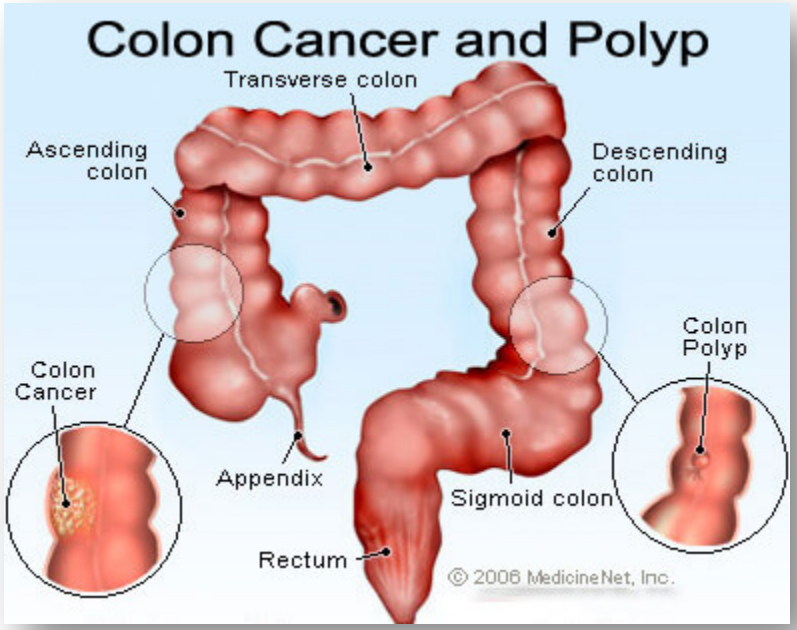

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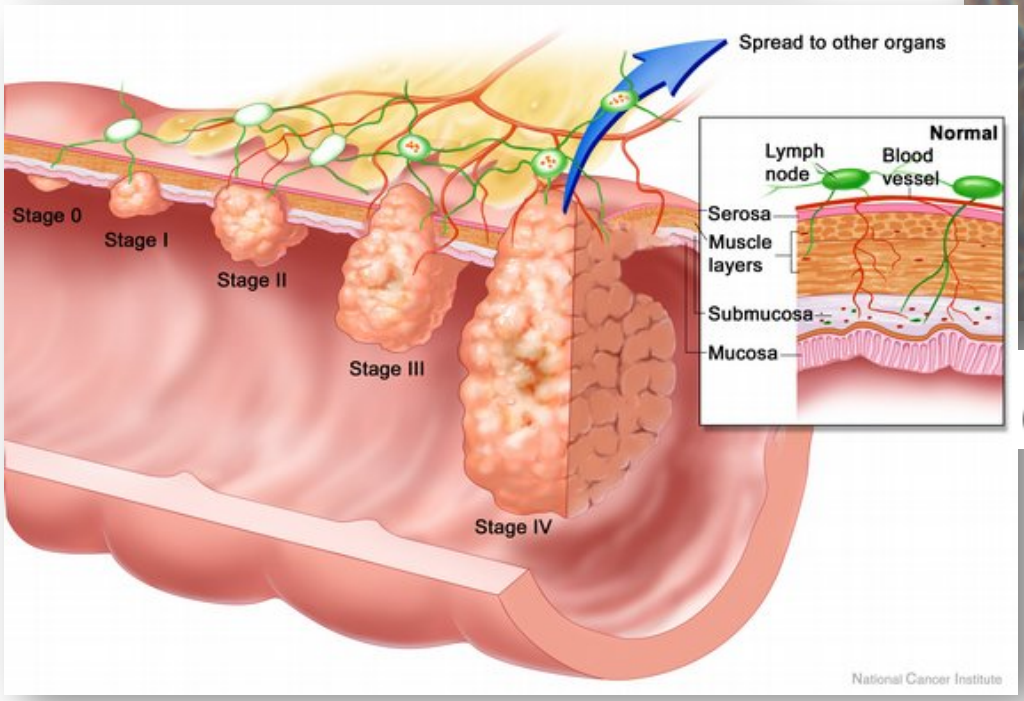
DLD-1 cells
BRCA2 -/- cells



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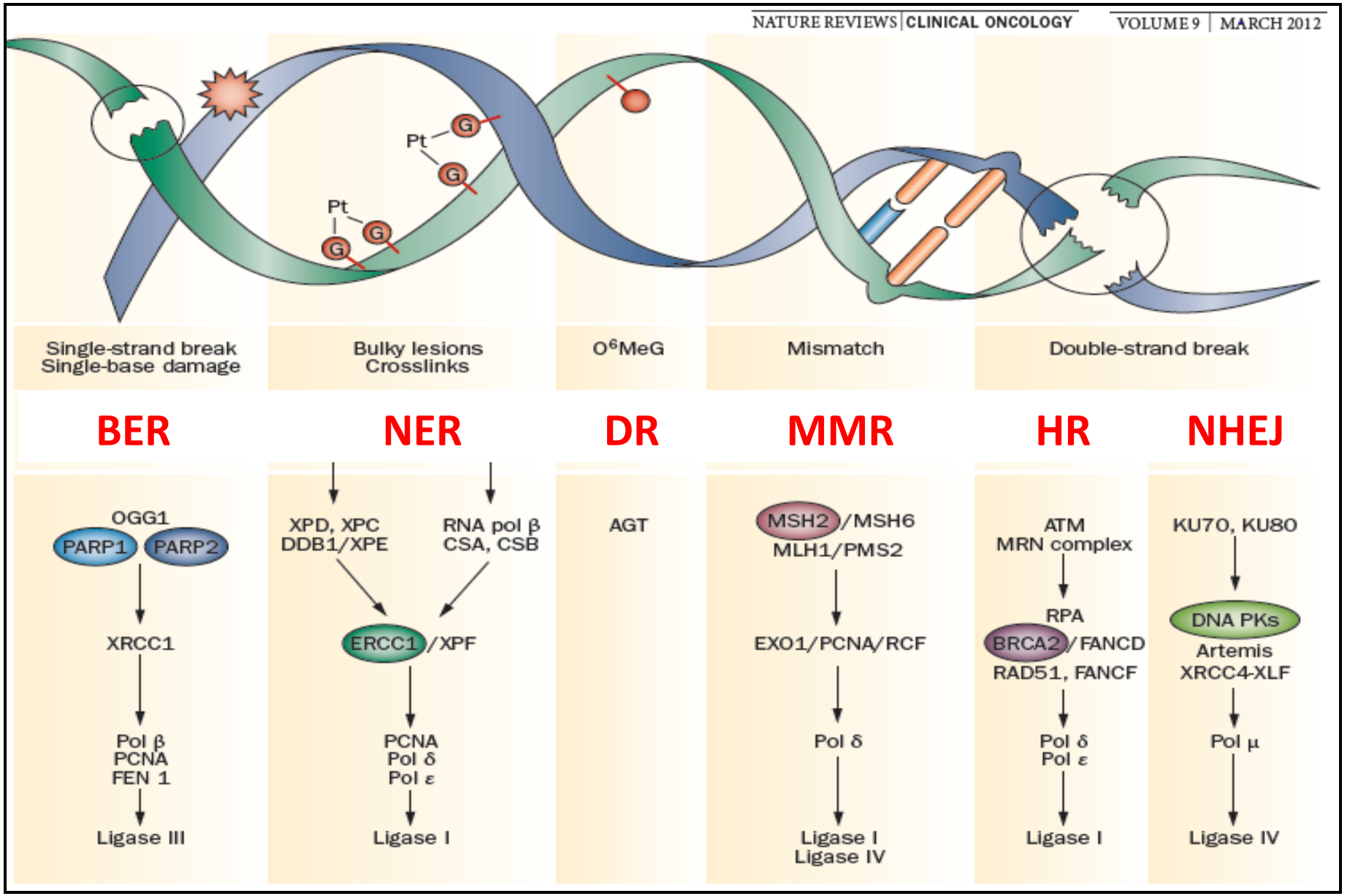
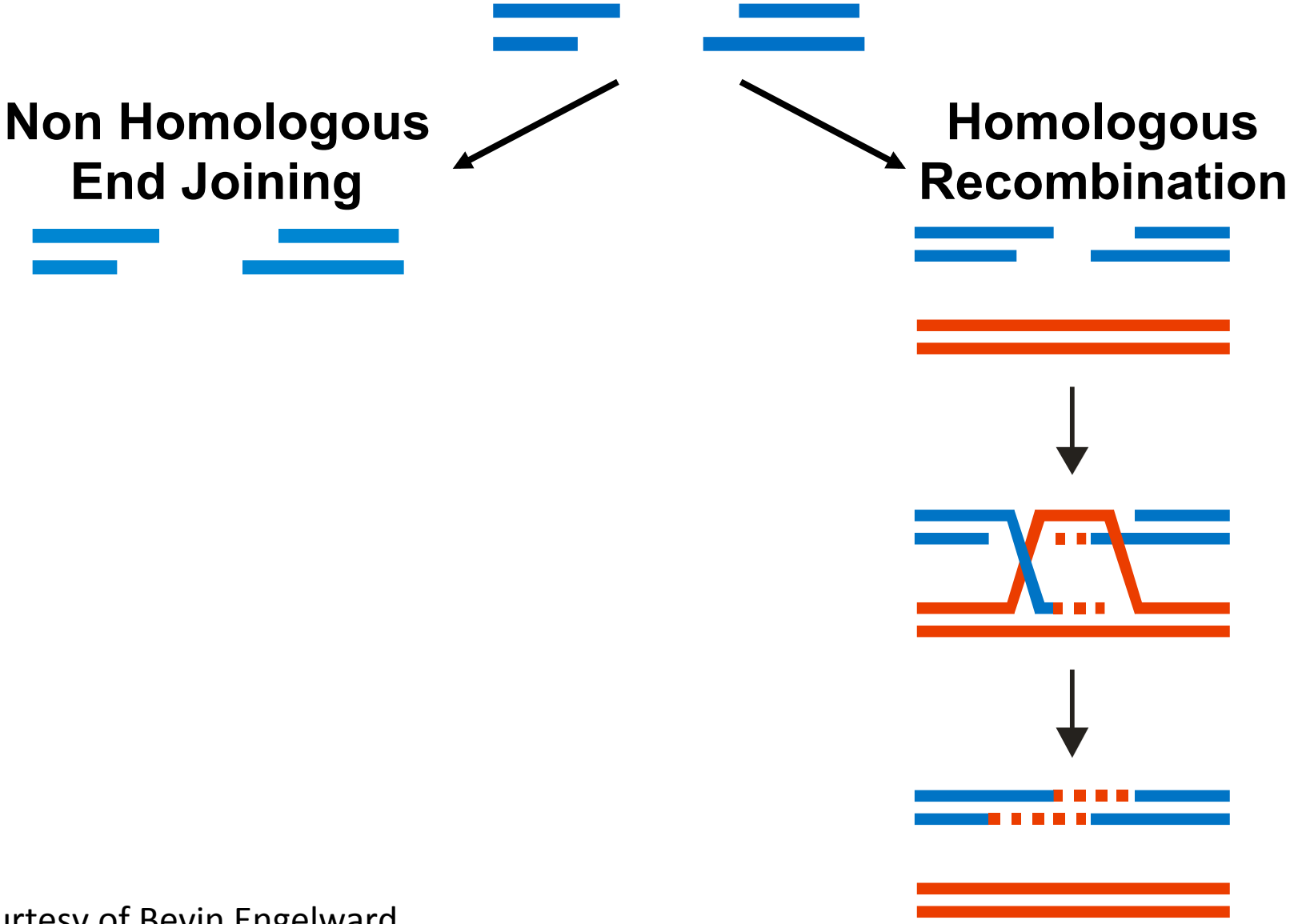
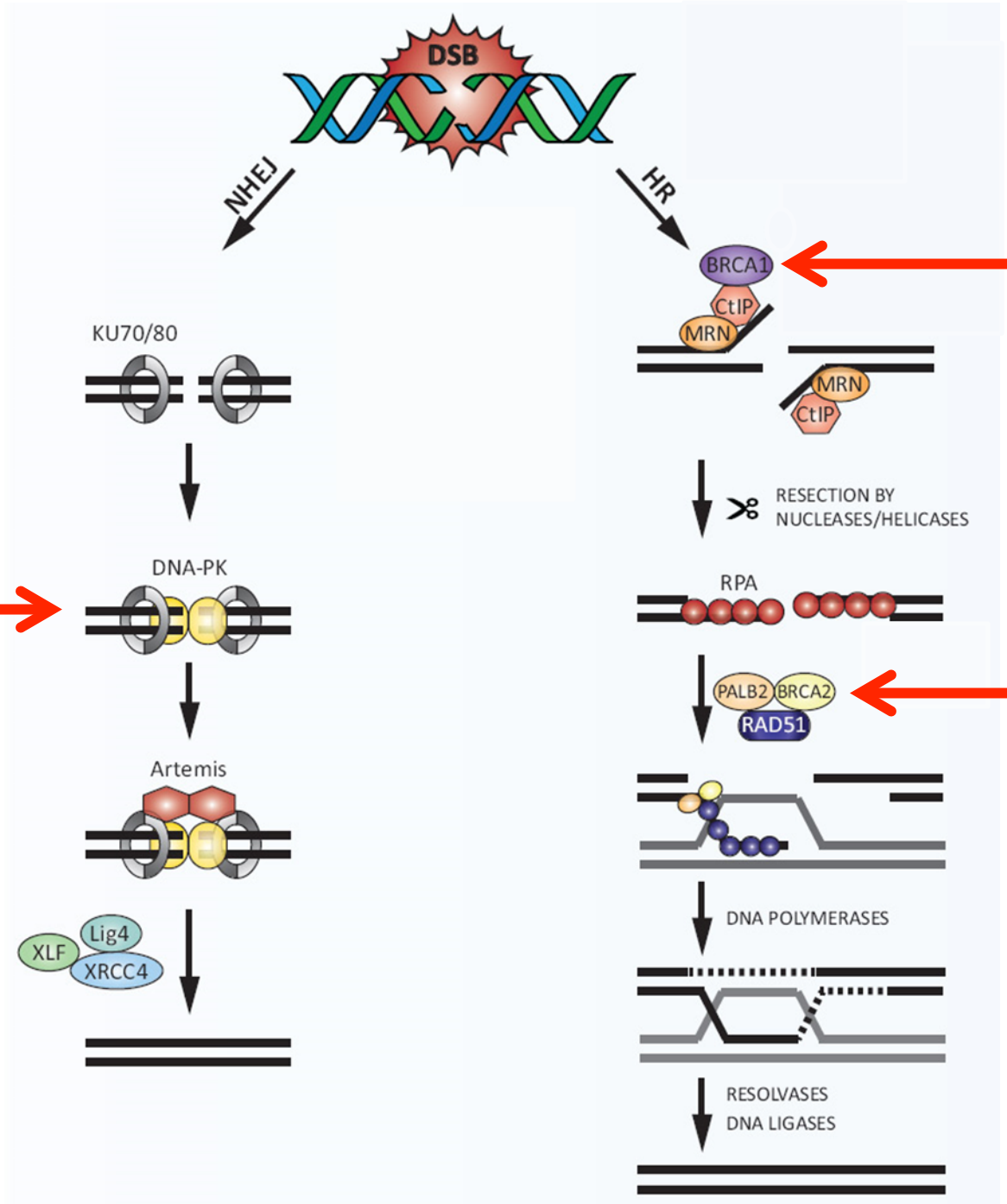
Six Major DNA Repair Pathways



DNA double-strand break repair



Courtesy of Bevin Engelward



DNA-PK

BRCA1

BRCA2

Non-Homologous End Joining

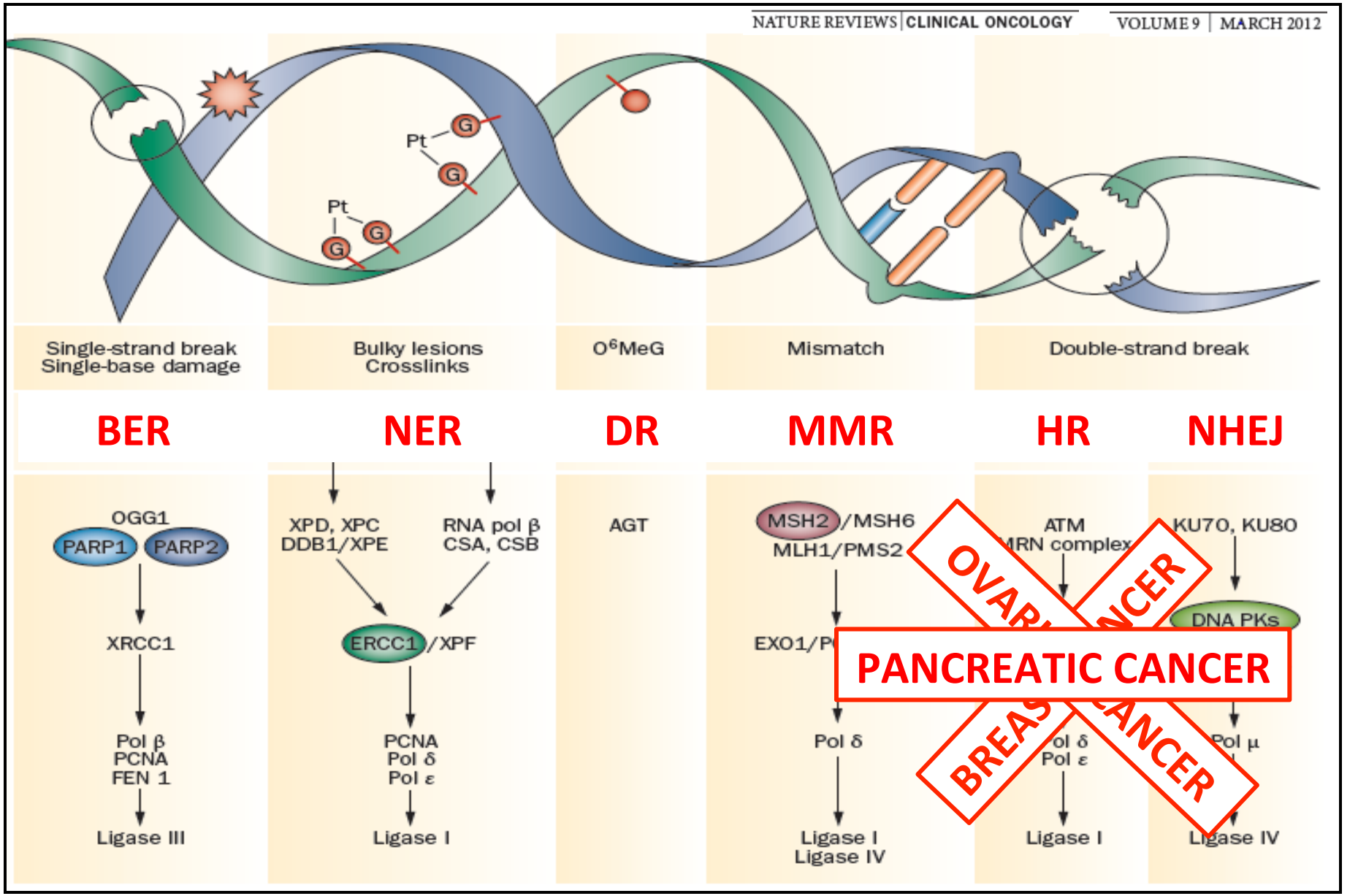
http://web.mit.edu/engelward-lab/animations/NHEJ.html

Synthesis-Dependent Strand Annealing (Homologous Recombination)

http://web.mit.edu/engelward-lab/animations/SDSA.html

Engelward lab Animations

Six Major DNA Repair Pathways



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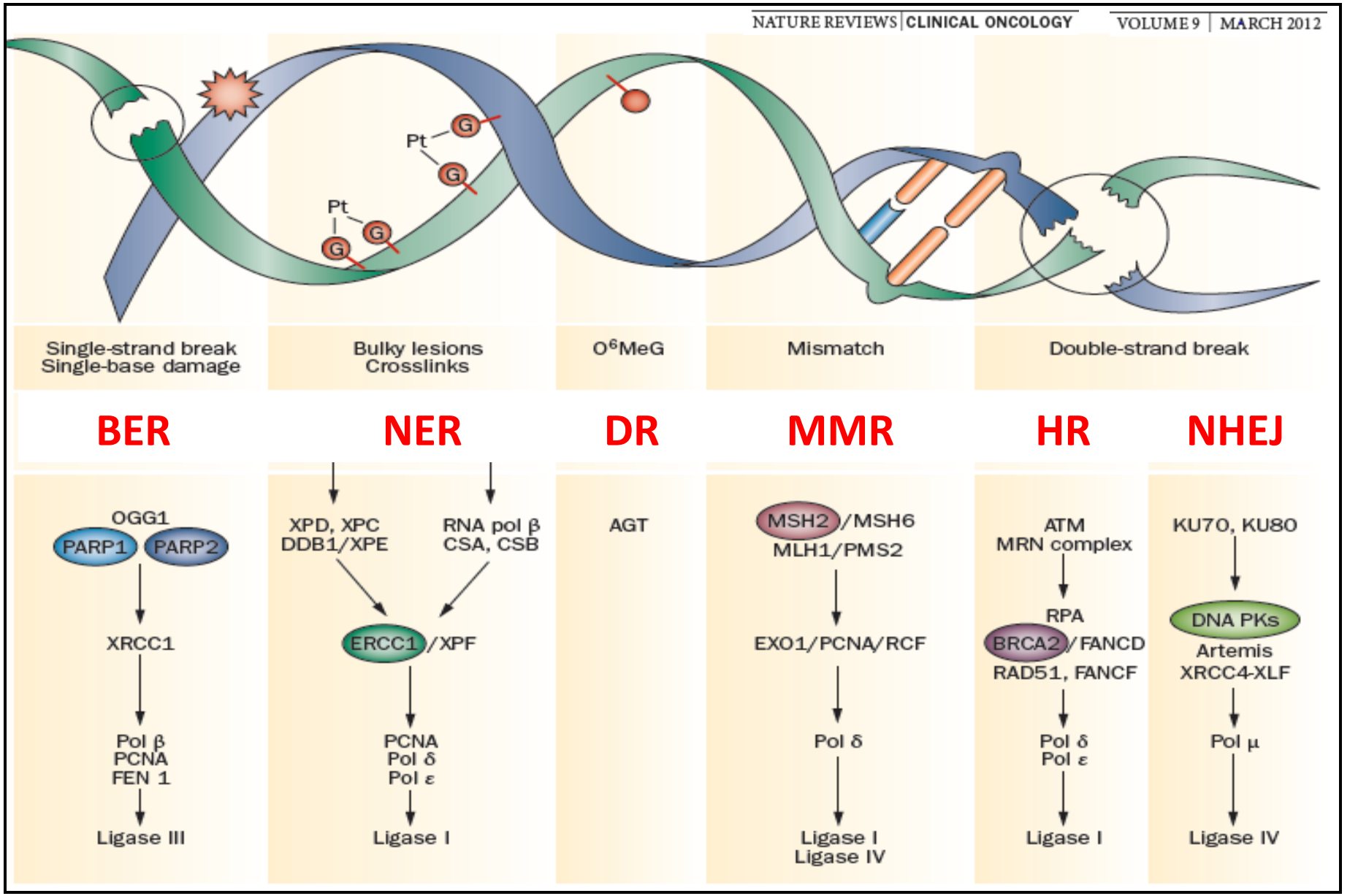
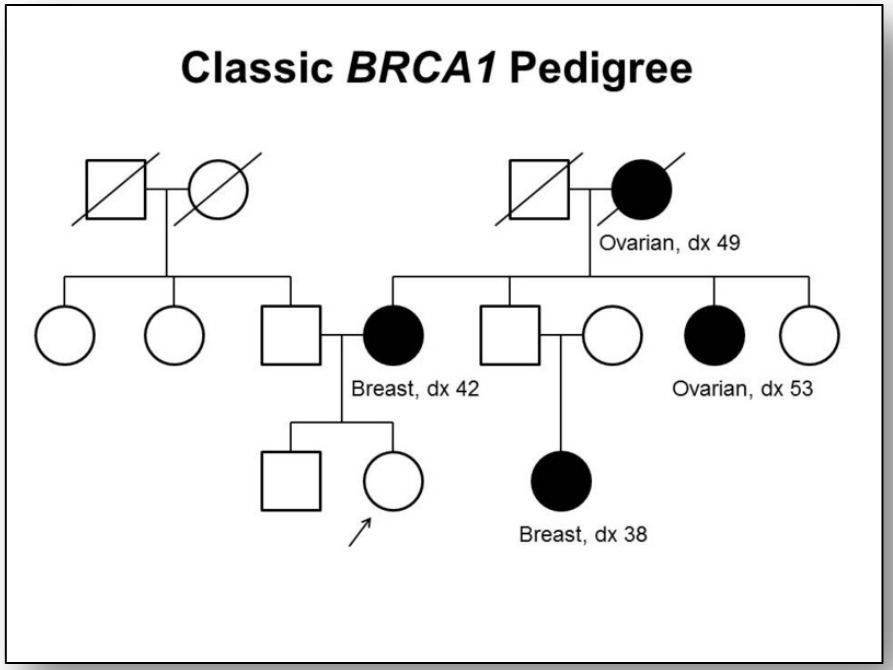
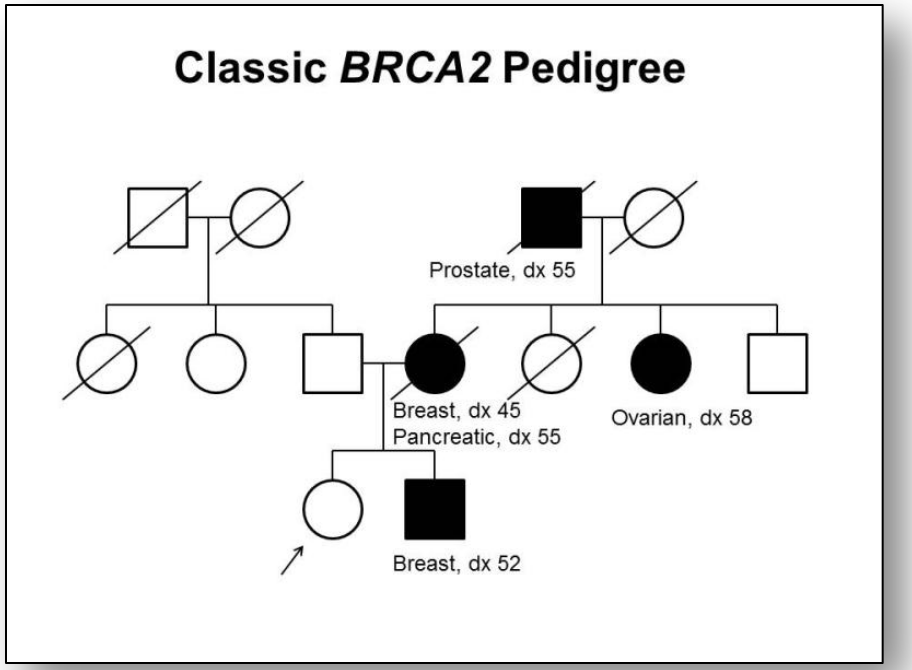


Mary-Claire King – Established genetic susceptibility to Breast Cancer

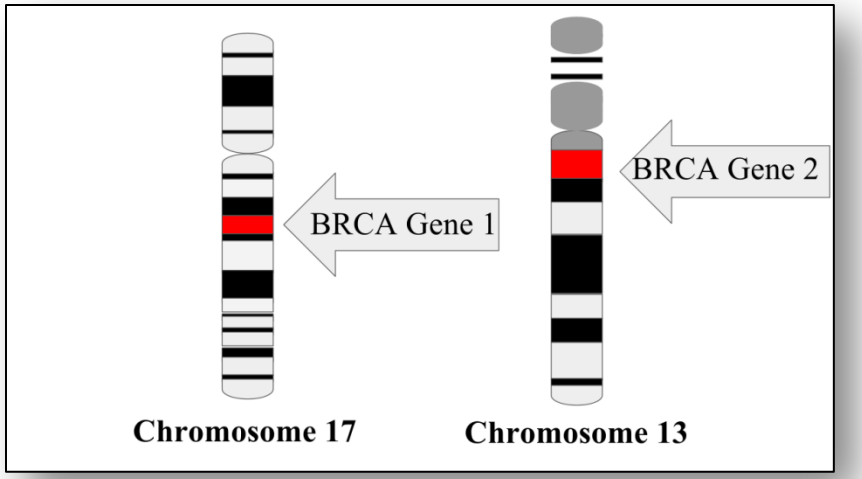
1974 – 1990 mapping gene to Chromosome 17 in Breast Cancer Families (17 years!!)
1994 BRCA1 gene was pinpointed on Chr 17
1994 BRCA2 gene was pinpointed on Chr 13

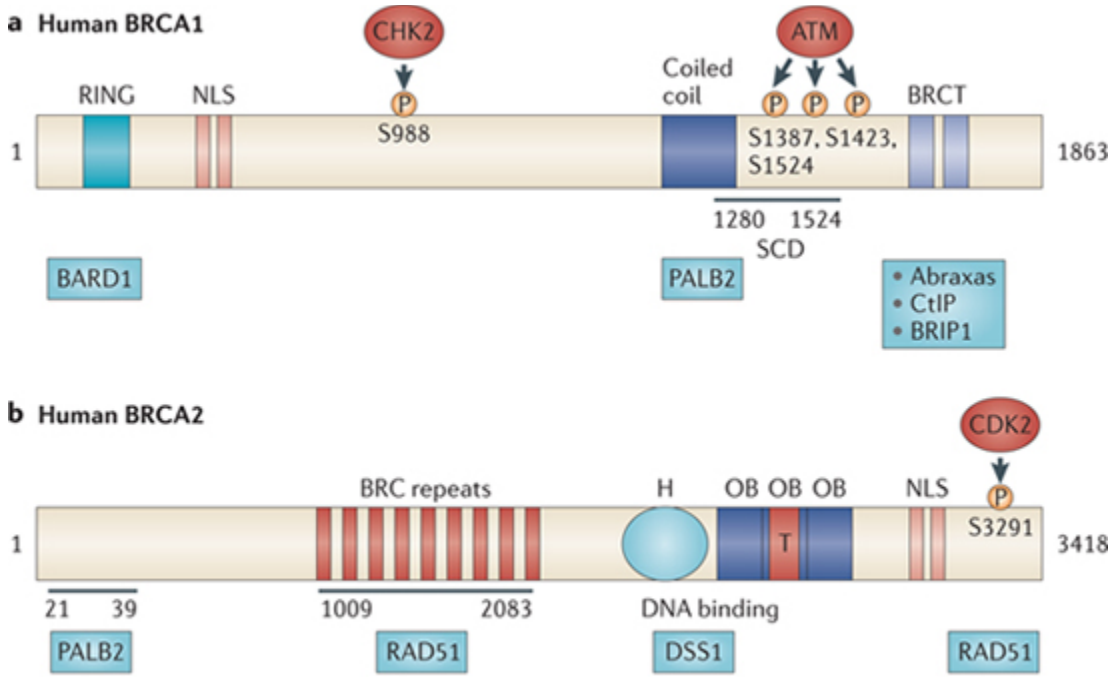
Mary-Claire King – Established genetic susceptibility to Breast Cancer



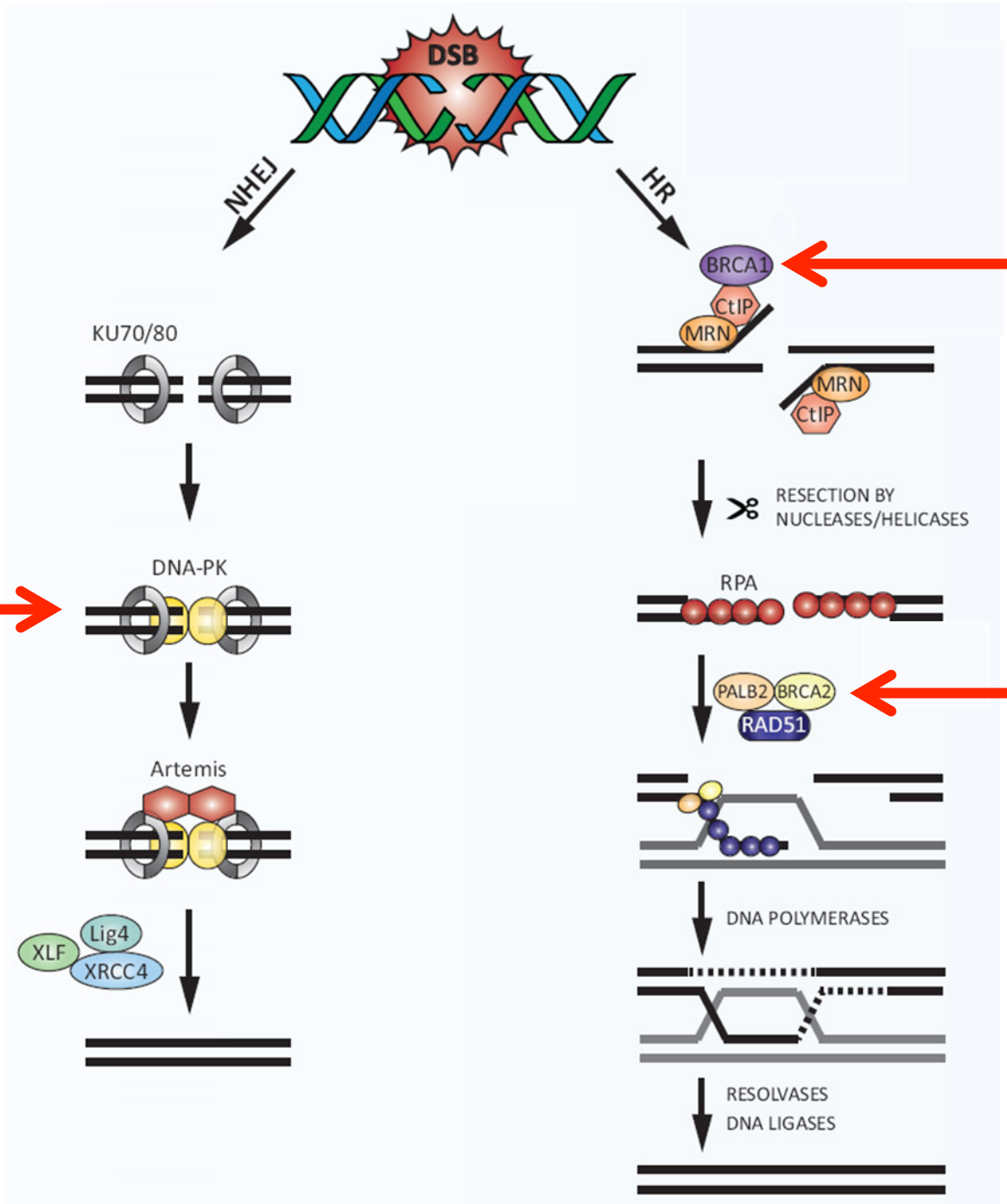


https://www.dnalc.org/view/15126-Using-pedigress-in-the-hunt-for-BRCA1-Mary-Claire-King.html





Nature Reviews | Cancer

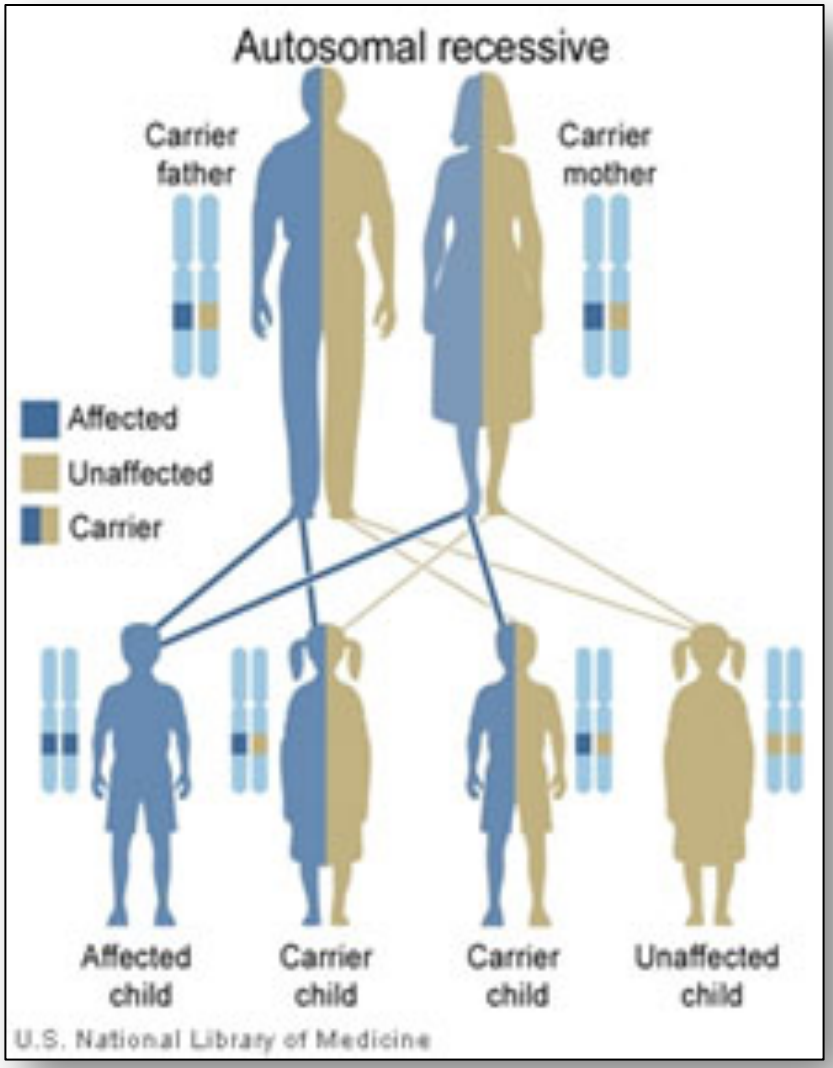


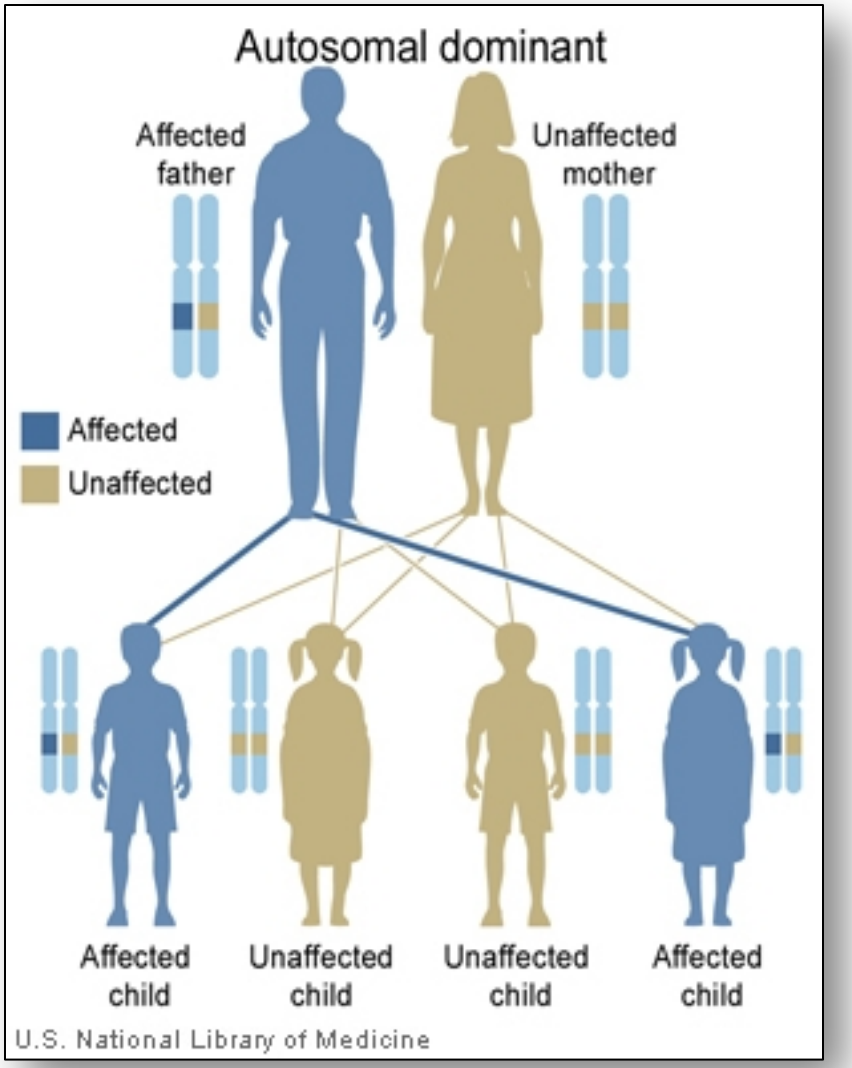
DNA-PK

BRCA1

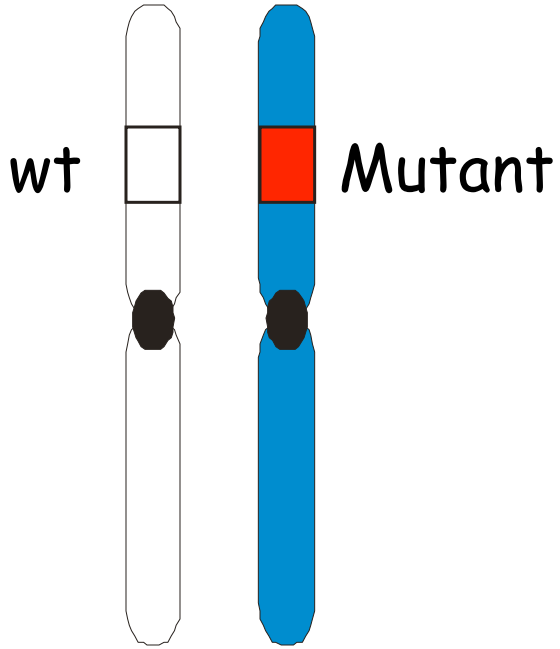
BRCA2

BRCA1 and *BRCA2* mutations behave as autosomal dominant





How is the second BRCA1/2 allele rendered non-functional?

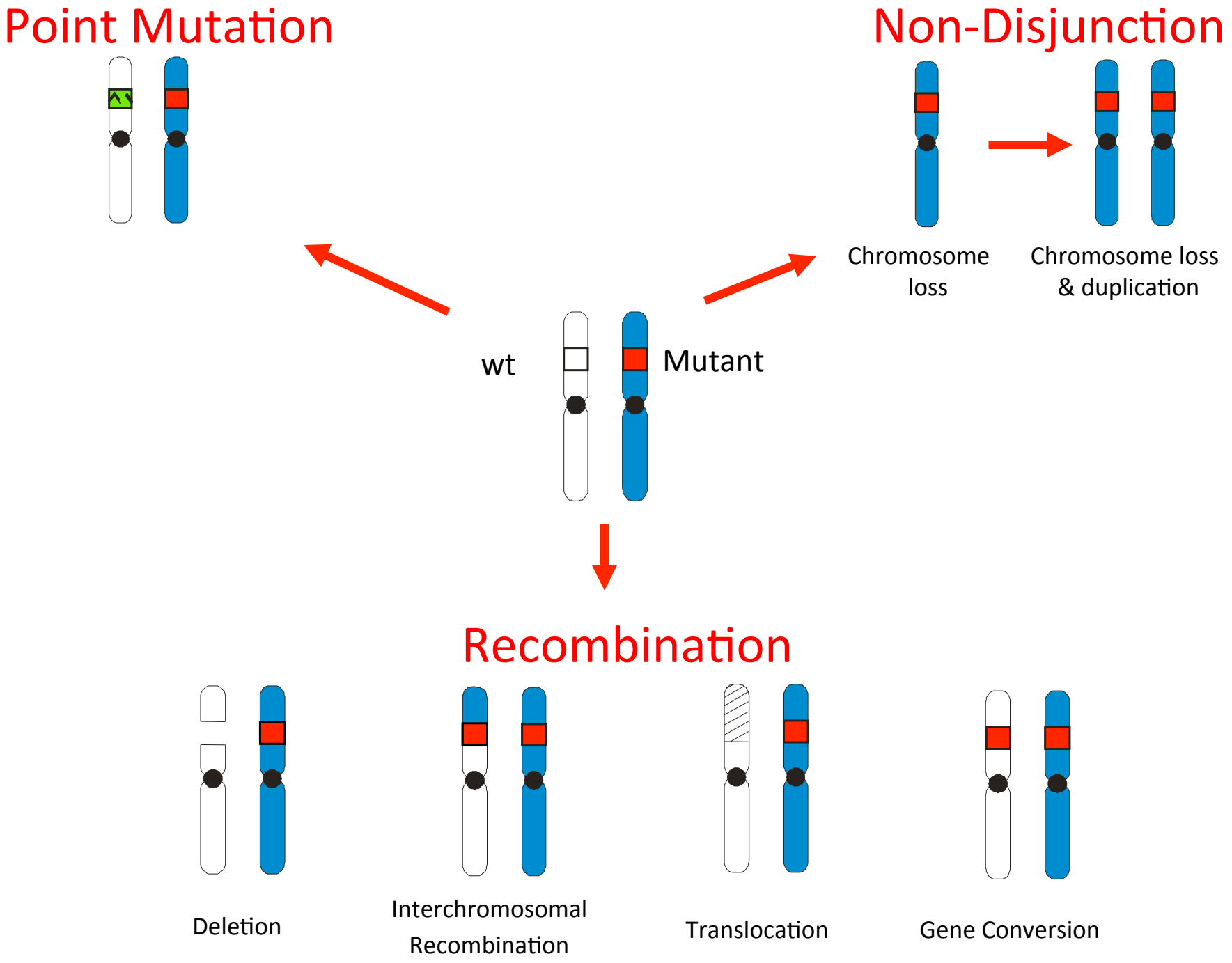


Heterozygous for BRCA mutation

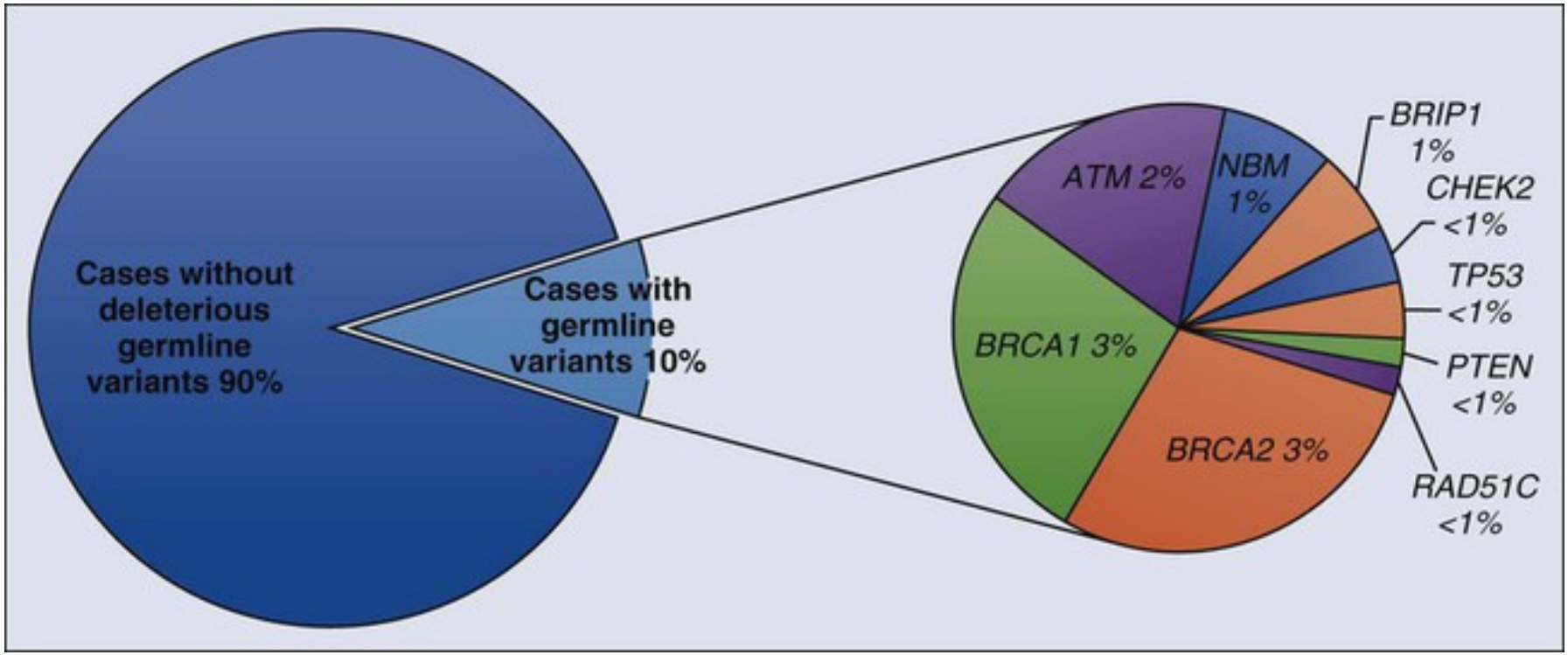
Loss of Heterozygosity

LOH

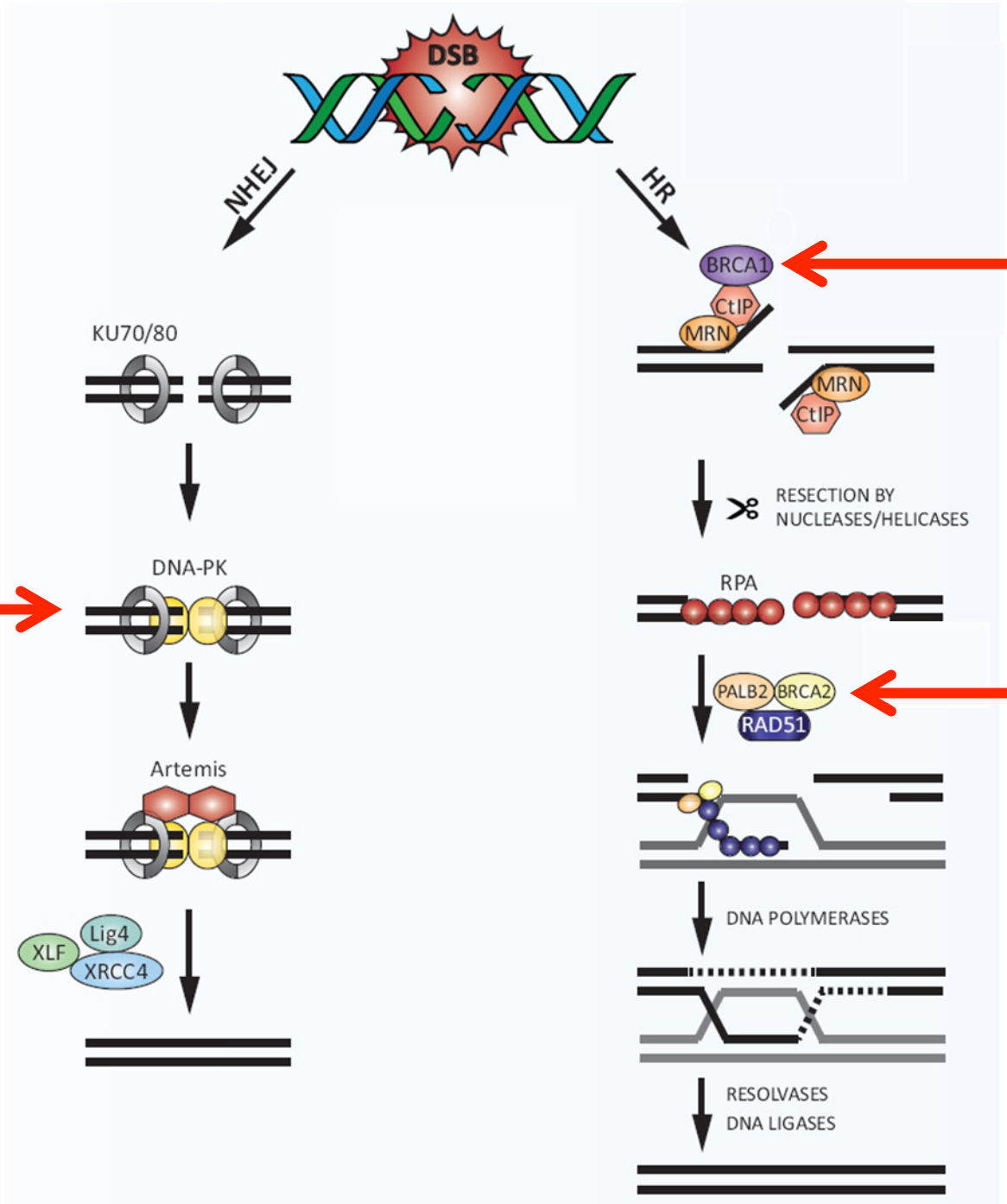
This can happen is several ways


Point Mutation
Non-Disjunction
Chromosome loss
Chromosome loss & duplication
wt
Mutant
Recombination
Deletion
Interchromosomal Recombination
Translocation
Gene Conversion

Familial Breast Cancers ~ 10%
Sporadic Breast Cancers ~ 90%



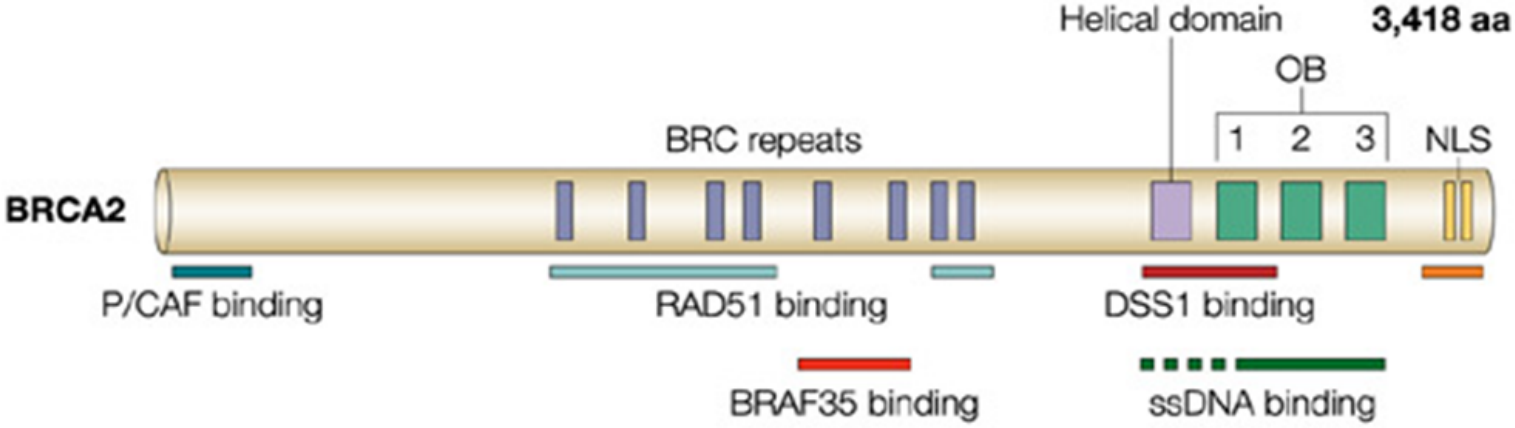
Total breast cancer cases (n = 507) analyzed in The Cancer Genome Atlas with deleterious germline variants. (Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature 2012;490:61–70.) Kasmintan A. Schrader, Ravi Sharaf, Shaheen Alanee and Kenneth Offit
http://clinicalgate.com/genetic-factors-hereditary-cancer-predisposition-syndromes-2/

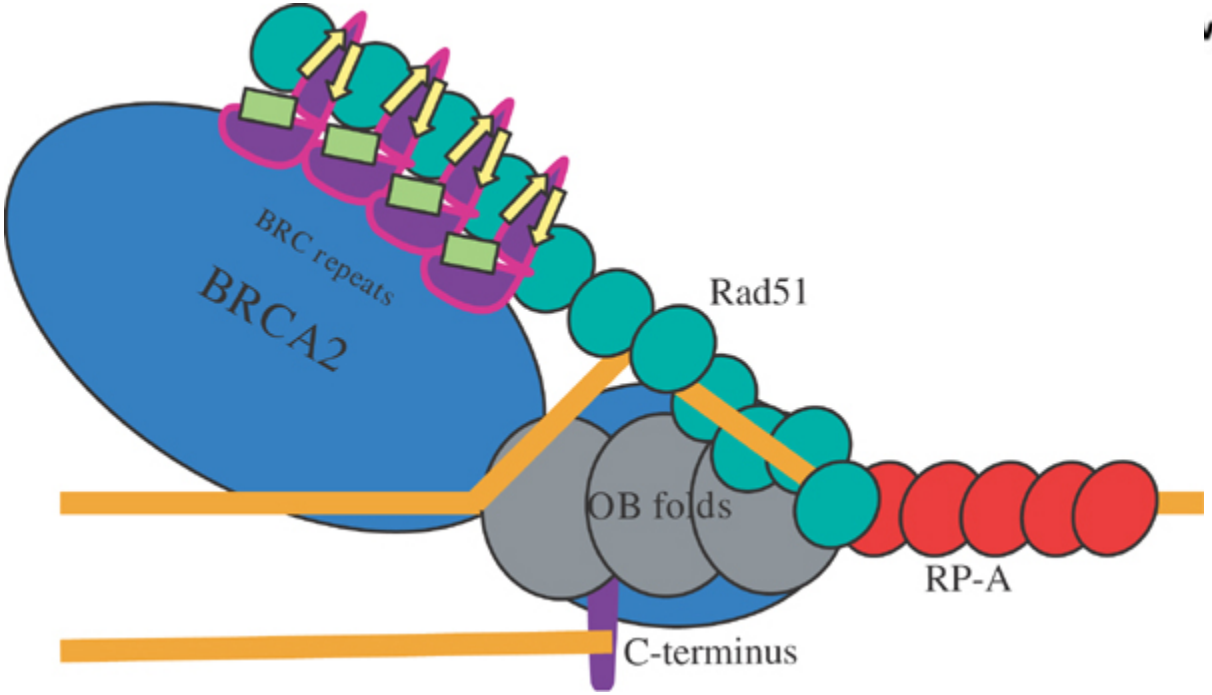


DNA-PK

BRCA1

BRCA2





http://www.nature.com/onc/journal/v22/n37/fig_tab/1206678f3.html

GeneArt™ Engineered Cell Models from Thermo Fisher Scientific

The largest collection of ready-to-go CRISPR engineered cell lines

The GeneArt Engineered Cell Models have been engineered using the latest genome editing tools including CRISPRs and rAAV editing to create both knockout and knock-in models. Each cell line ships with the unedited, isogenic parental cell lines providing the perfect control.

GeneArt Engineered Cell Models provide researchers with instant access to a collection of thousands of affordable, pre-engineered cell lines that will accelerate their research programs and eliminate the risk, cost, and time associated with developing their own cell lines or commissioning custom cell line development program.

Get Quote

Get Quote from Thermo Fisher Scientific for GeneArt™ Engineered Cell Models

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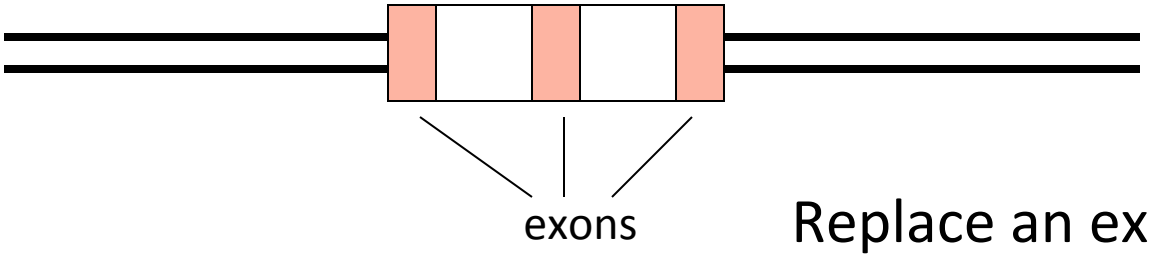
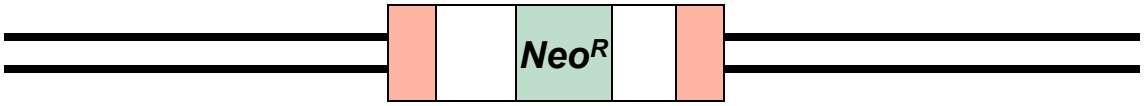
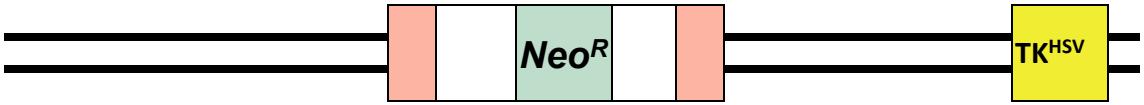
Gene X e.g., *BRCA2* gene – in the test tube



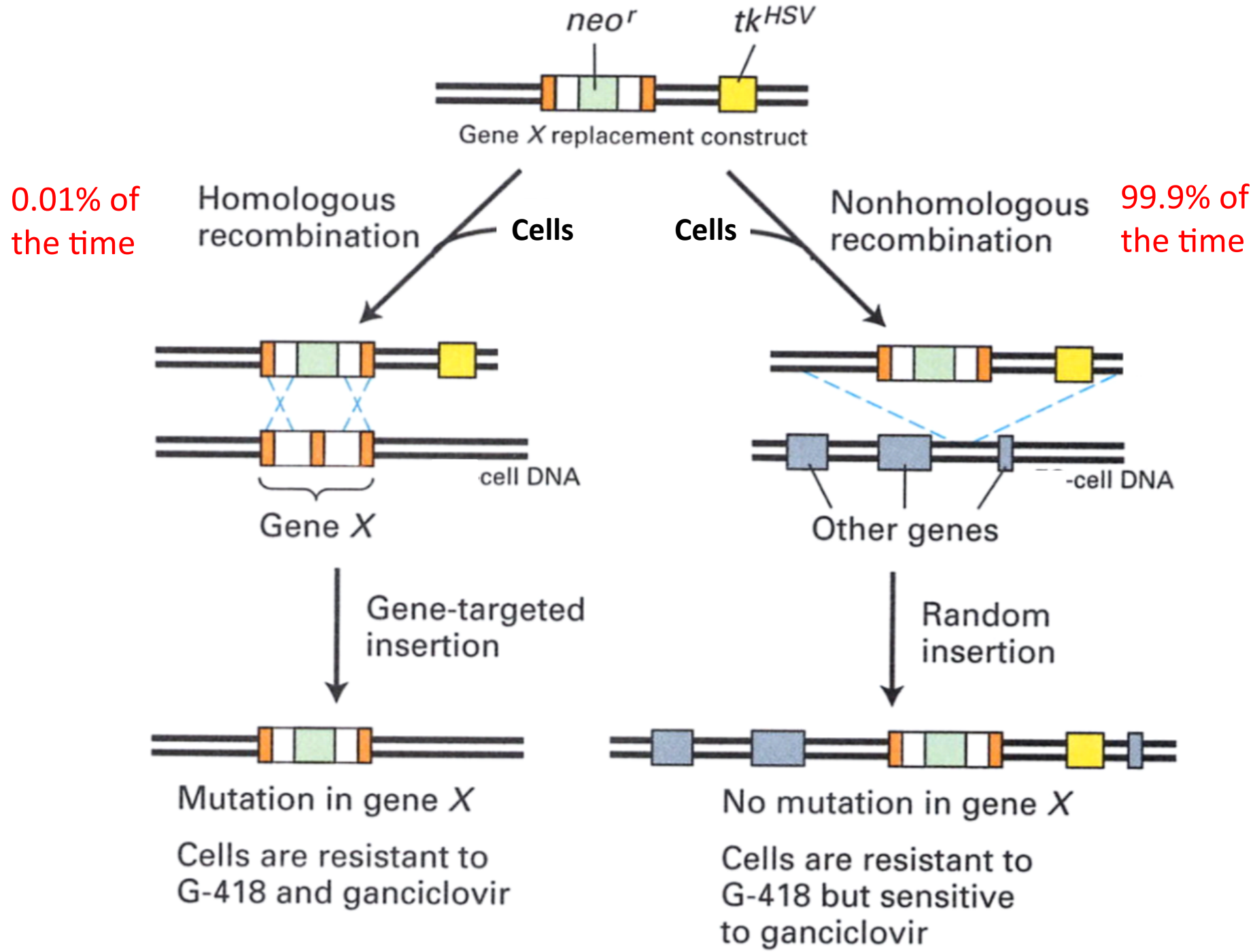
Replace an exon with a positive selectable marker, e.g., *Neo*^{R}



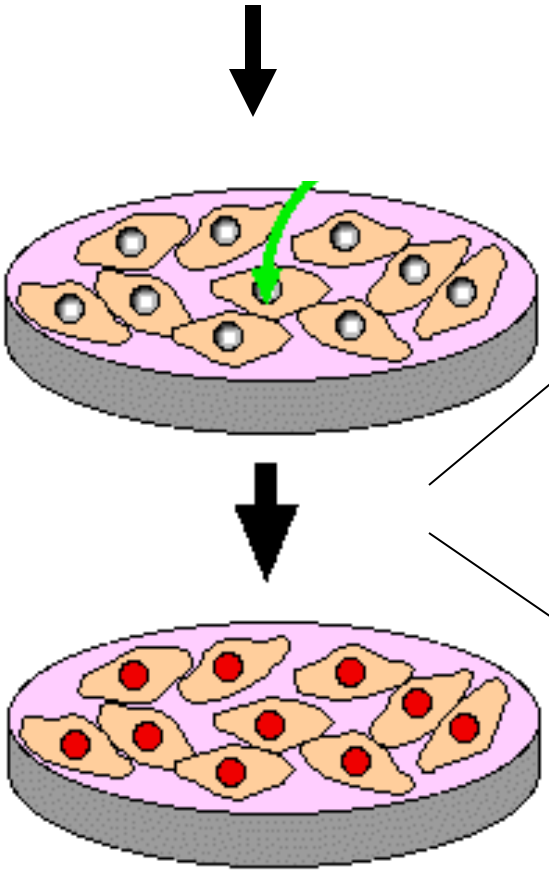
Mutated or "Knocked out" gene construct



Add some distance away, a negative selectable marker, e.g., TK^{HSV}


neo^r
tk^HSV
Gene X replacement construct
0.01% of the time
Homologous recombination
Cells
Cells
Nonhomologous recombination
99.9% of the time
cell DNA
Gene X
-cell DNA
Other genes
Gene-targeted insertion
Random insertion
Mutation in gene X
Cells are resistant to G-418 and ganciclovir
No mutation in gene X
Cells are resistant to G-418 but sensitive to ganciclovir

Targeting Construct

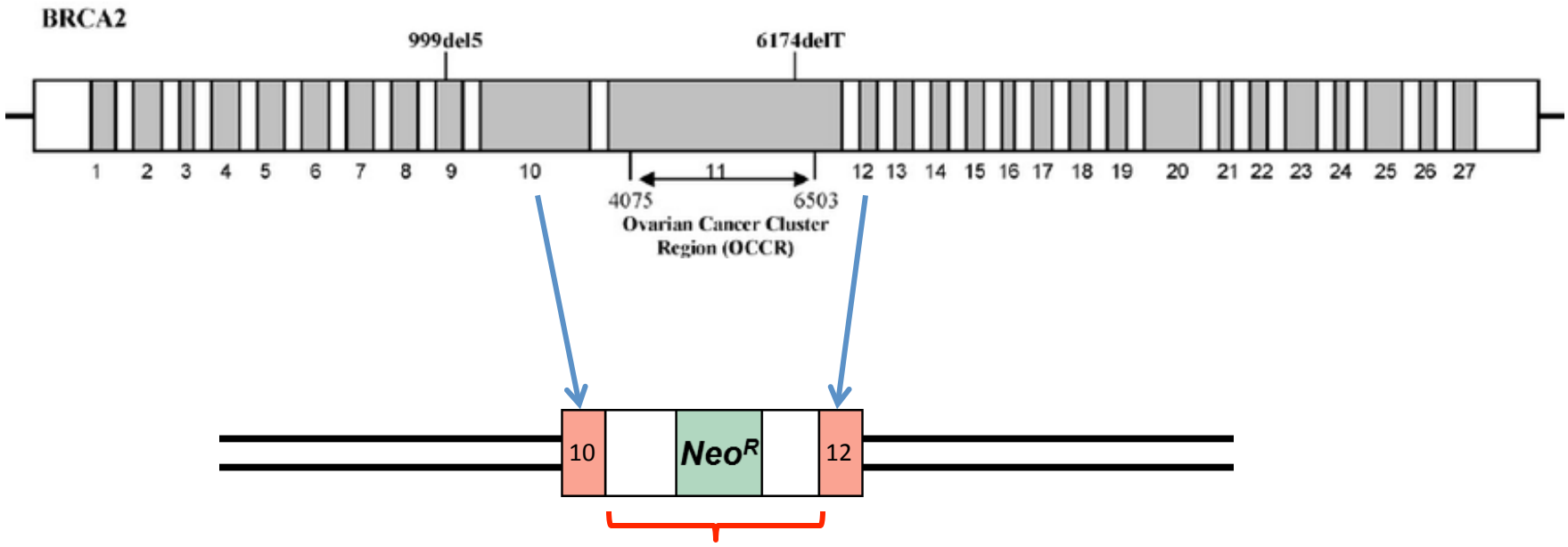


Select for the genetically altered cells you want

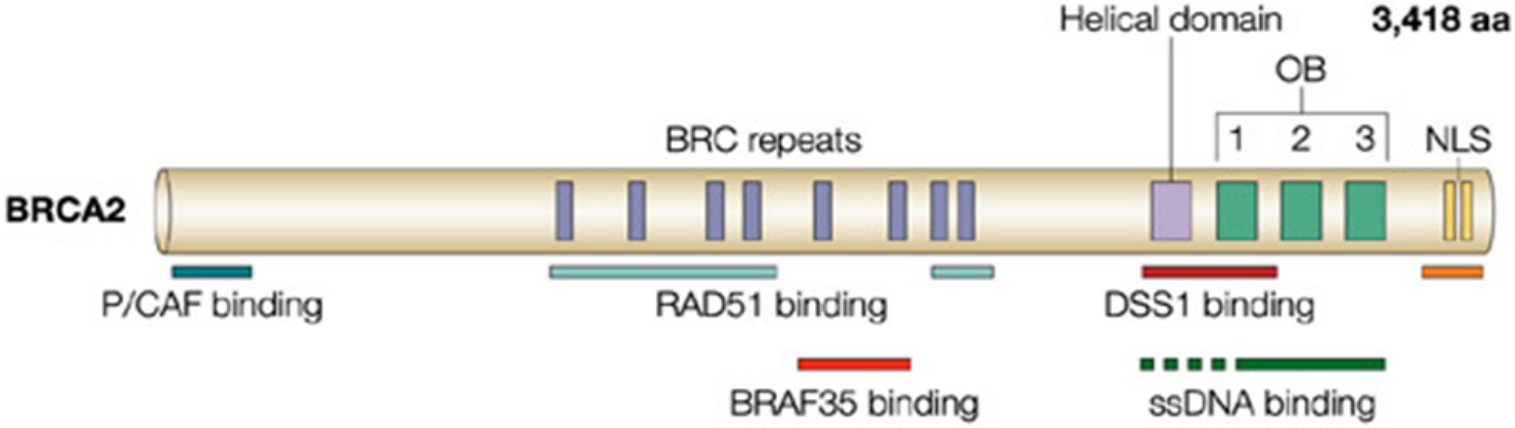
Select **for** the Neo^R gene and **against** the TK^{HSV} gene using **Neomycin** and **Gangcyclovir**

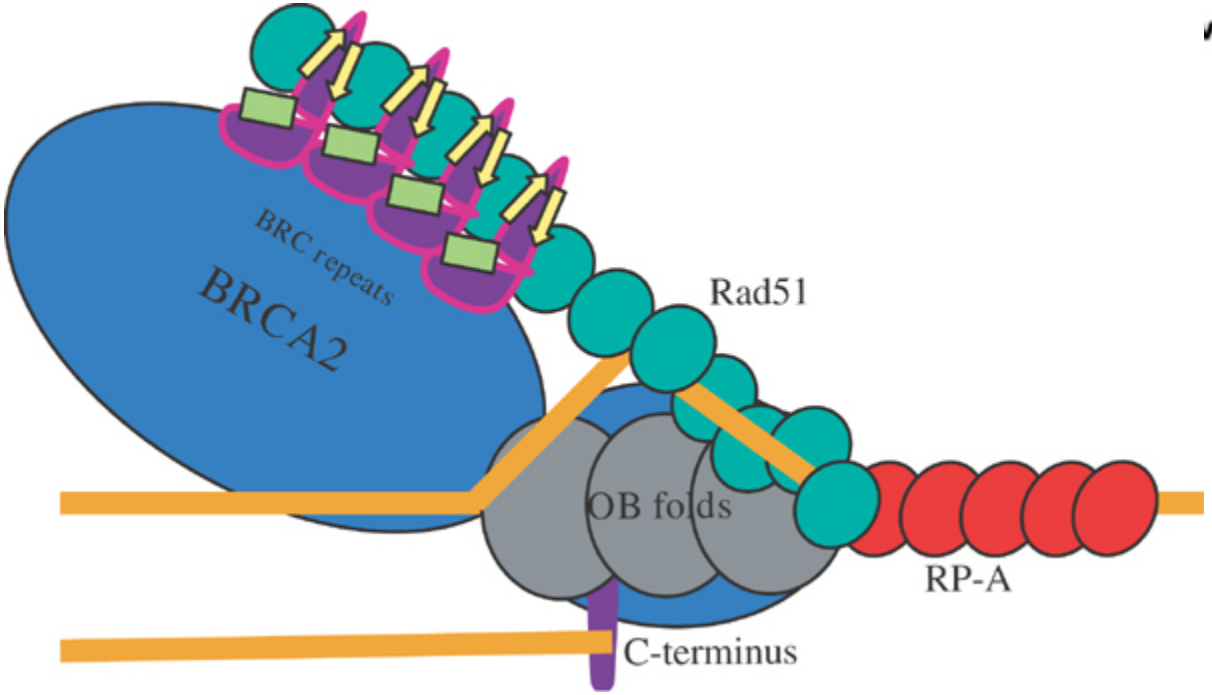
The only cells to survive have undergone a targeted homologous recombination event at the gene of interest

BOTH *BRCA2* alleles have exon 11 replaced with a selectable marker gene (for Neomycin resistance)



Exon 11 replaced with selectable marker





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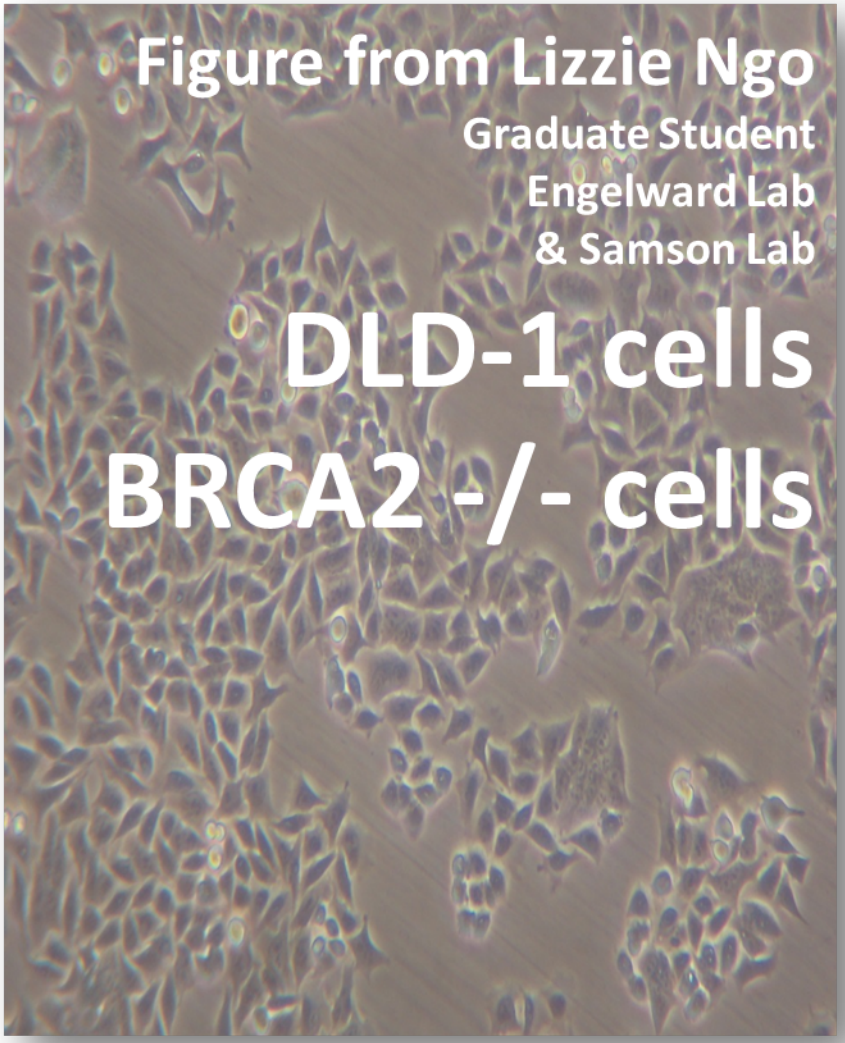

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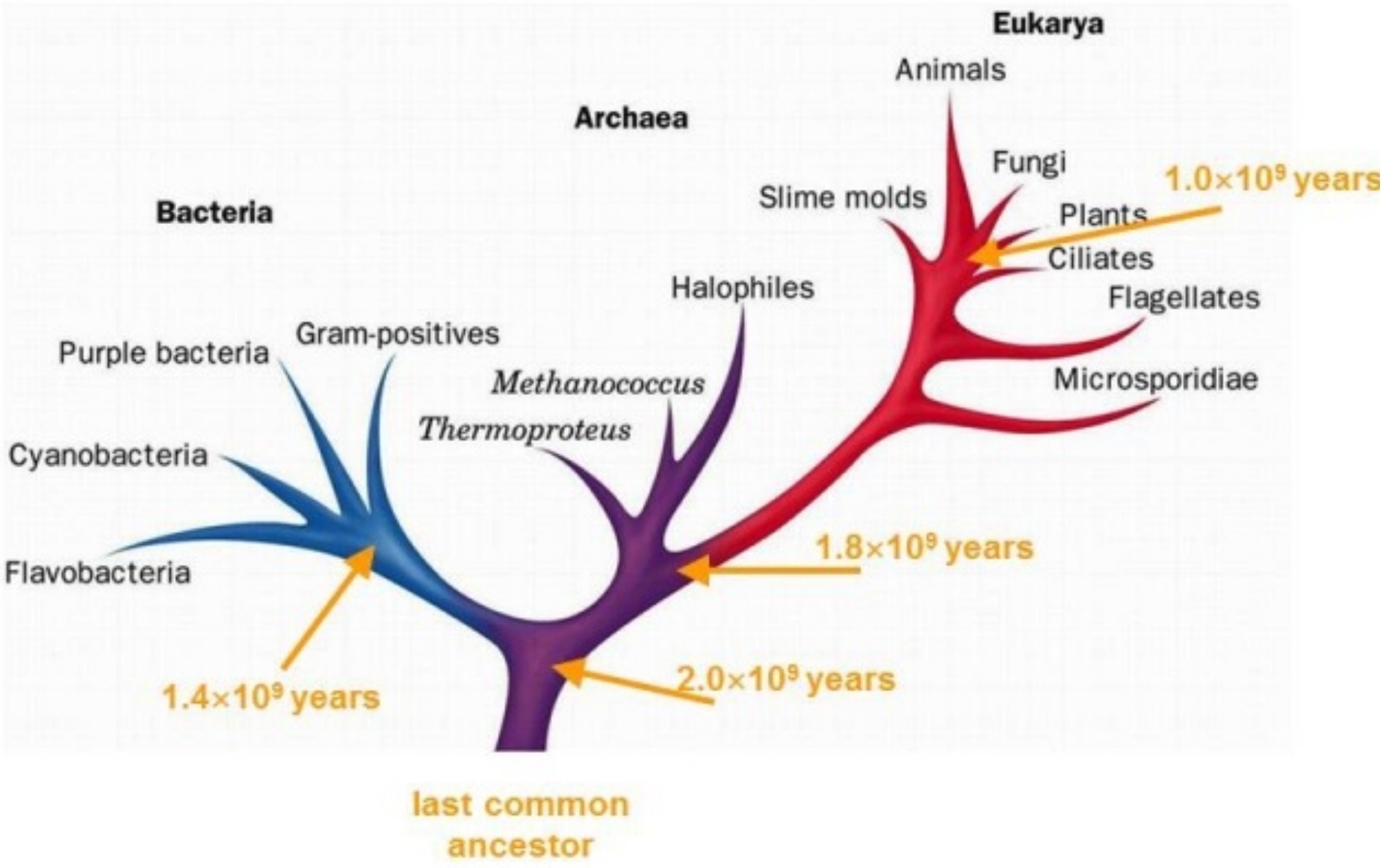
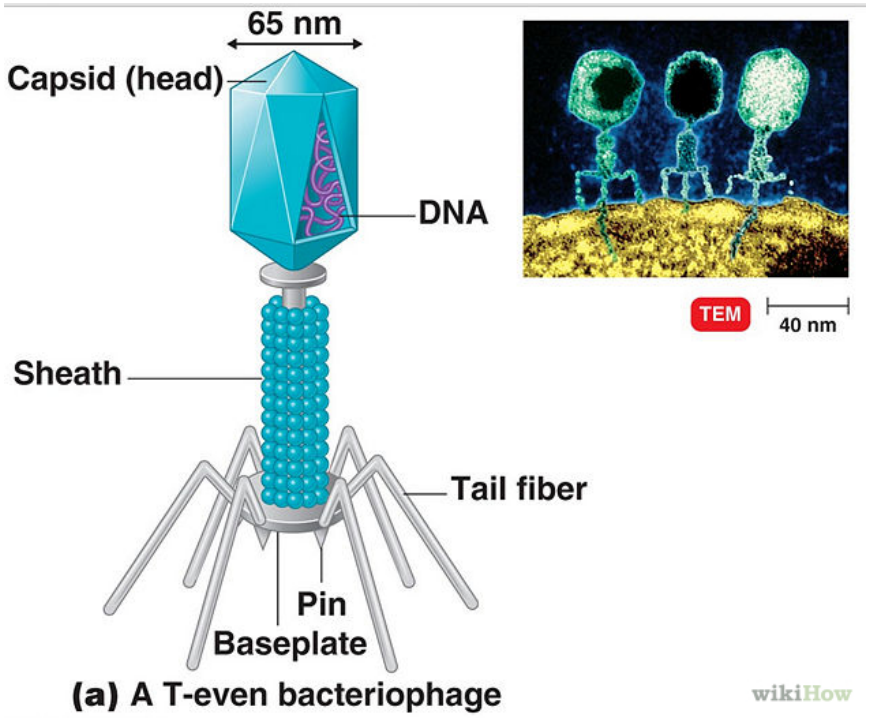
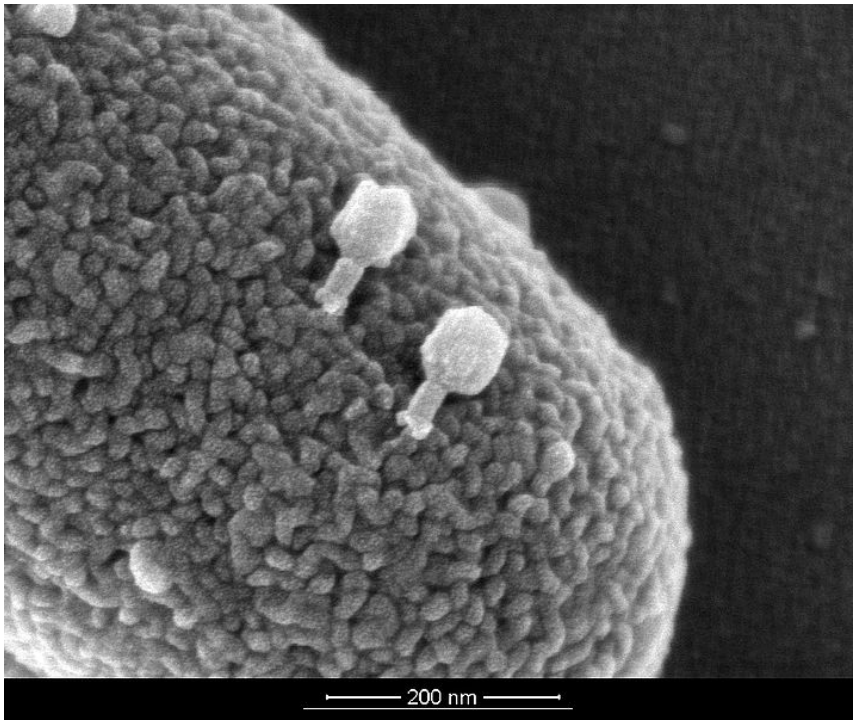
All known life forms are based on DNA



http://biologicalphysics.iop.org/cws/article/lectures/47042

Bacteriophage (bacterial virus) infecting *E. coli*





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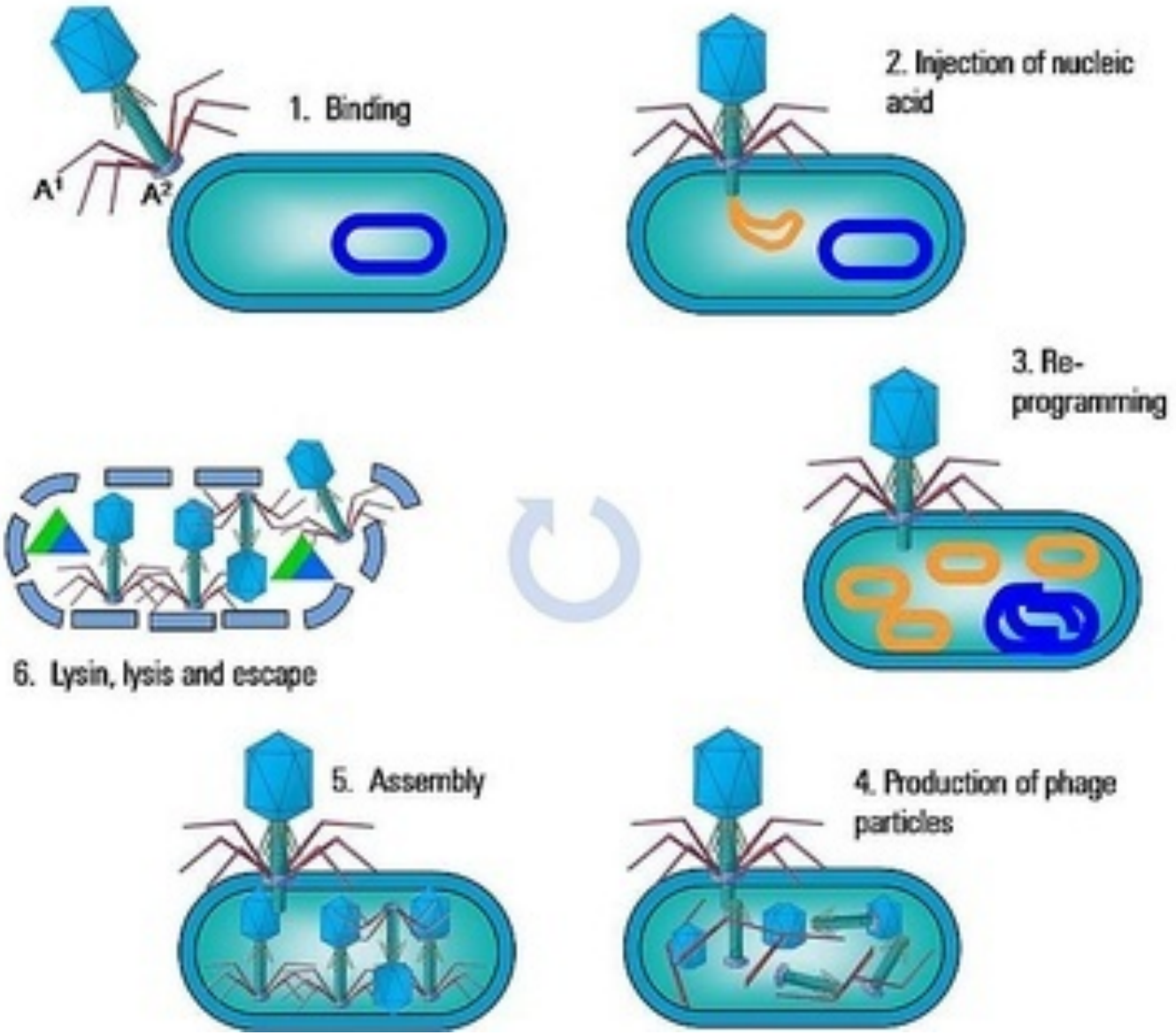
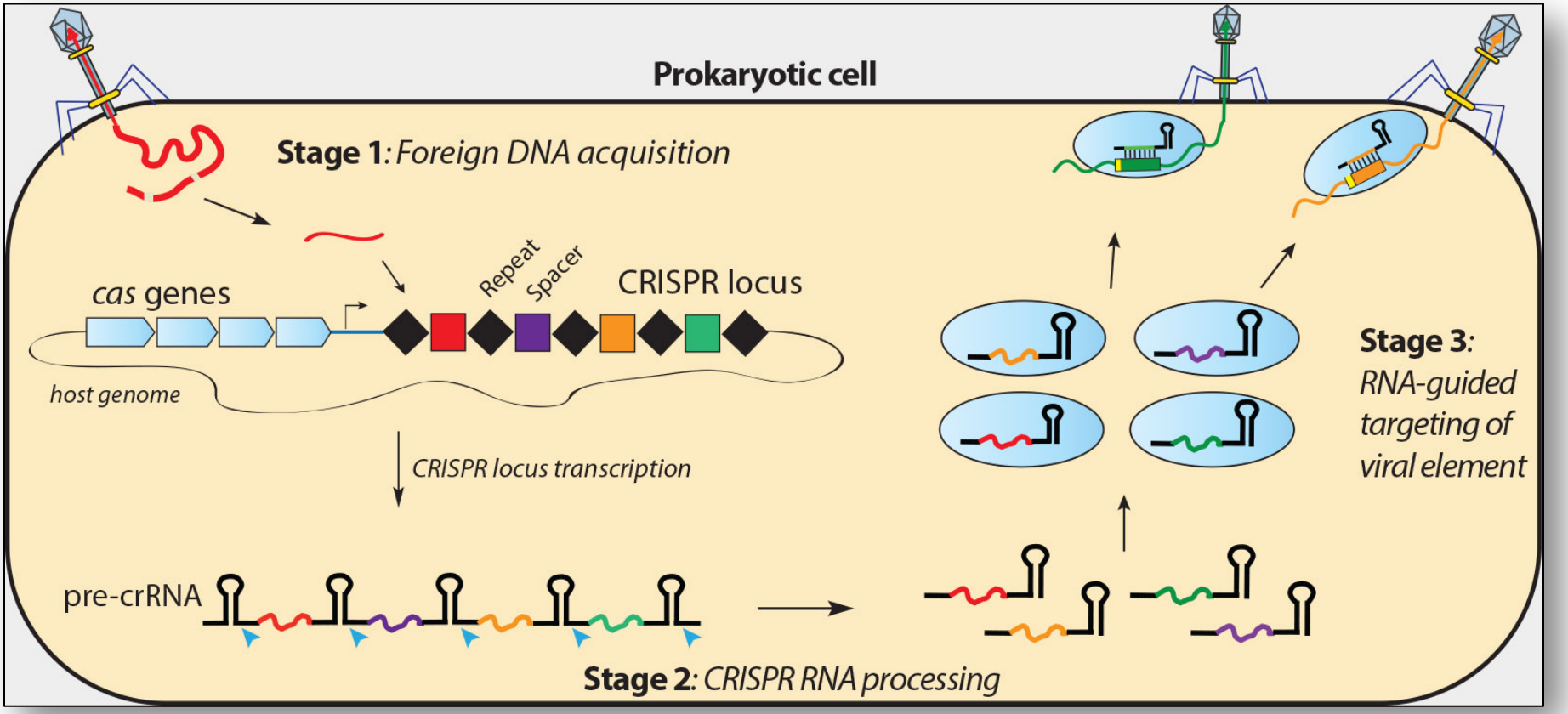
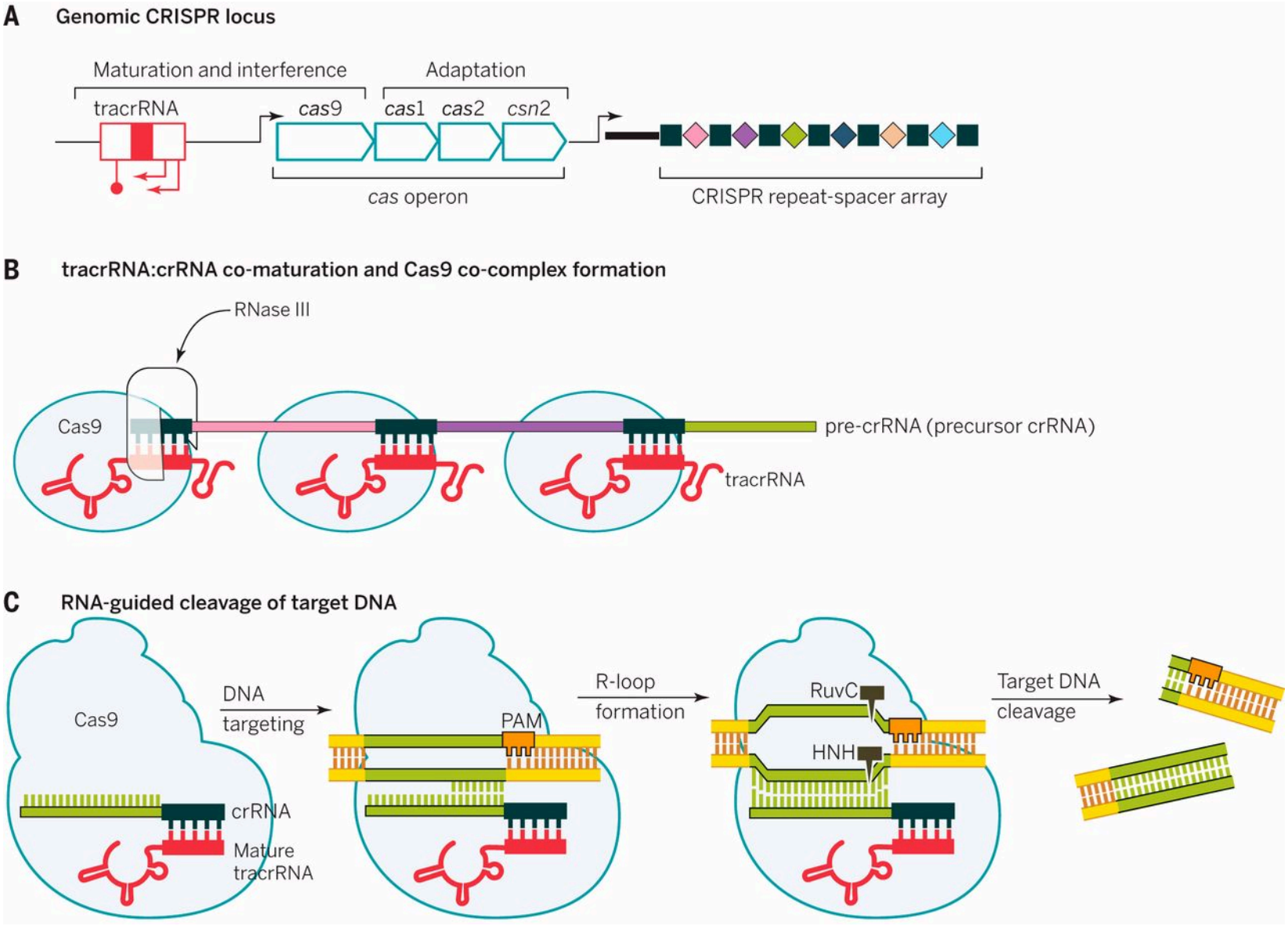


CRISPR - **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats
CAS genes – **C**RISPR **AS**sociated genes

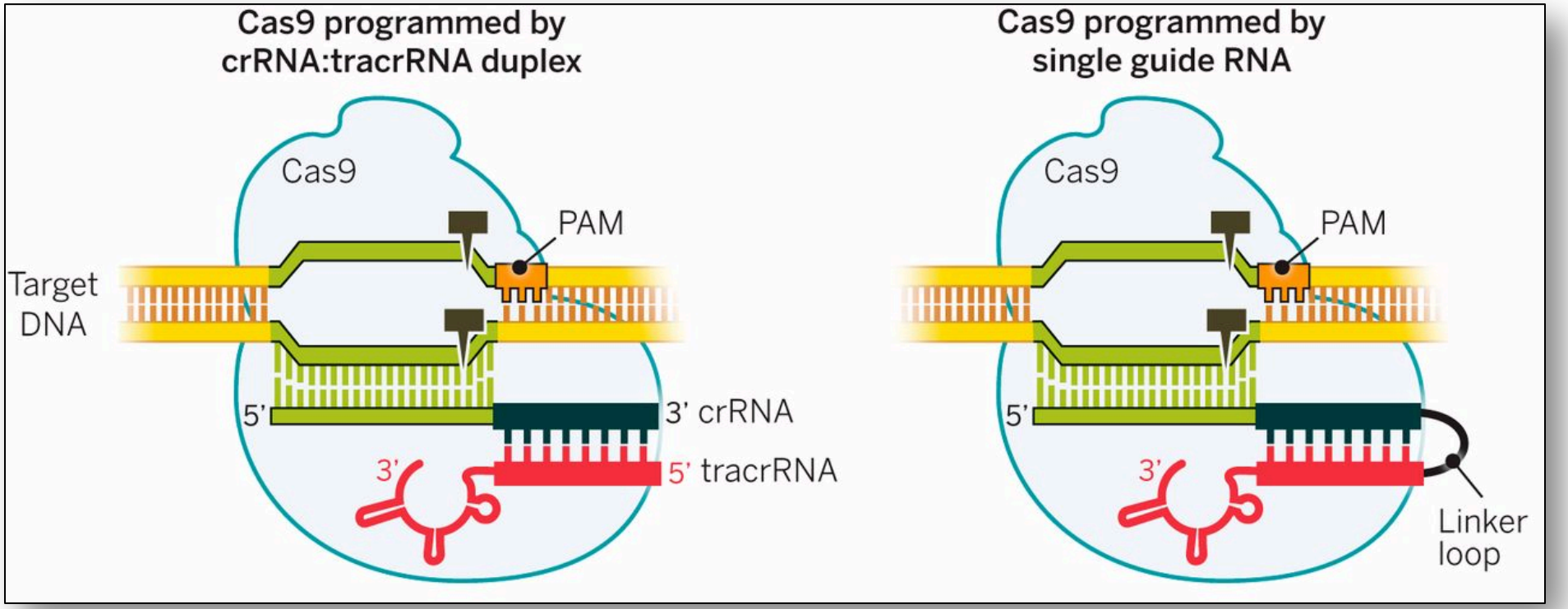


http://rna.berkeley.edu/crispr.html



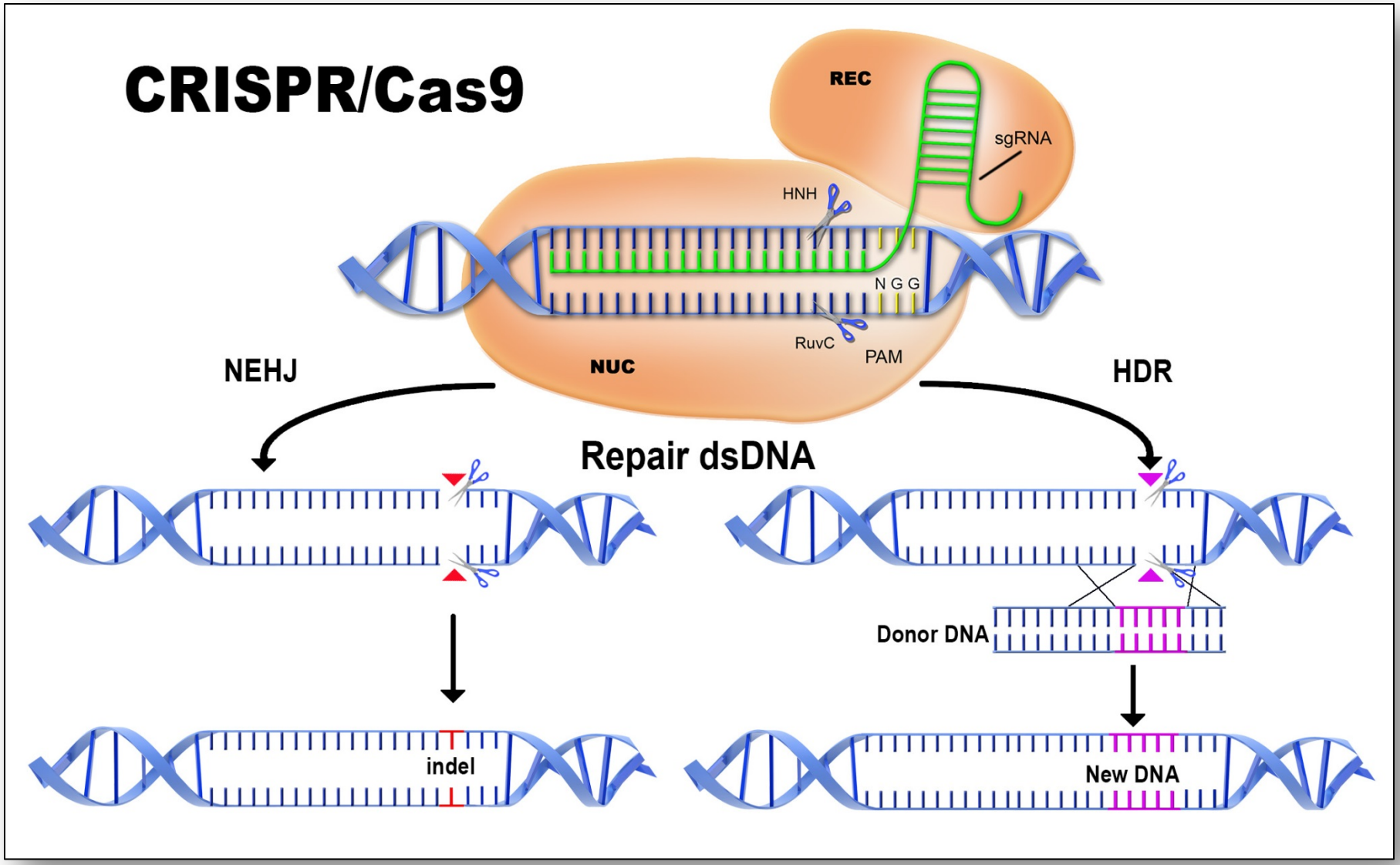
http://science.sciencemag.org/content/346/6213/1258096.figures-only

BREAKTHROUGH – fuse the crRNA and tracrRNA to make a single "Guide RNA"



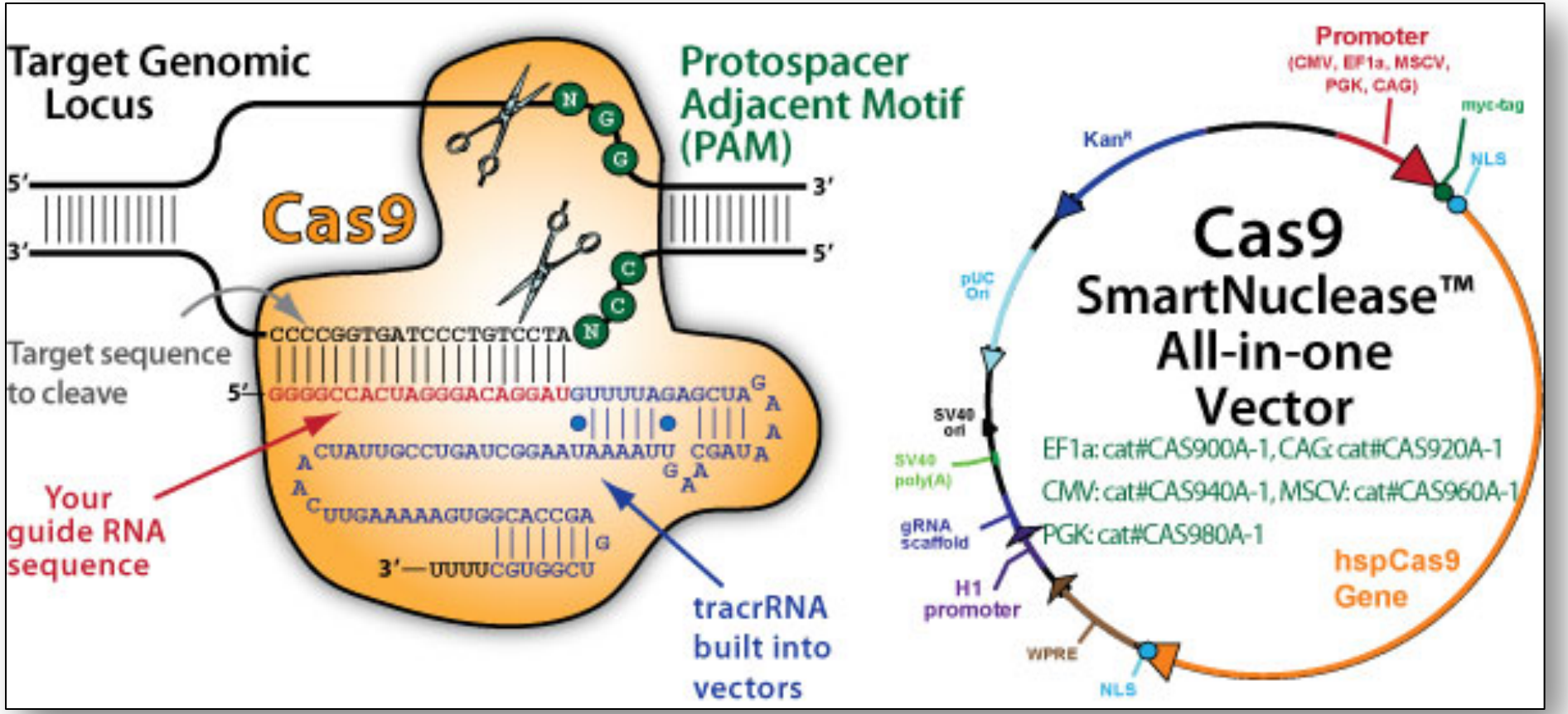
http://science.sciencemag.org/content/346/6213/1258096.figures-only

CRISPR/Cas9 can help **make** a mutation or **fix** a mutation
NHEJ vs Homologous Recombination **IN HUMAN CELLS**



http://www.timeone.ca/designer-genes-getting-crispr-and-crispr/#sthash.3wiIRuzX.dpbs

Genome Editing Kits Galore



https://www.systembio.com/crispr-cas9-plasmids

https://www.youtube.com/watch?v=2pp17E4E-O8

Awards Galore

Emmanuelle Charpentier and Jennifer Doudna

Awards Galore

Feng Zhang - MIT

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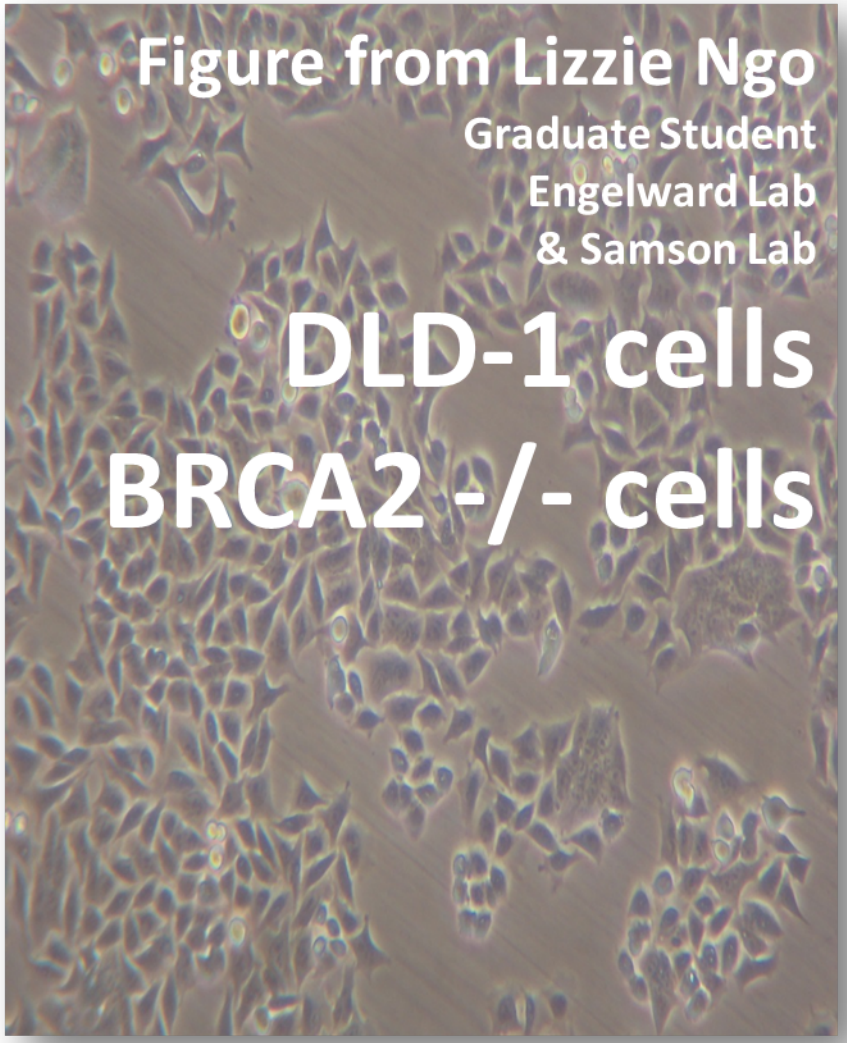
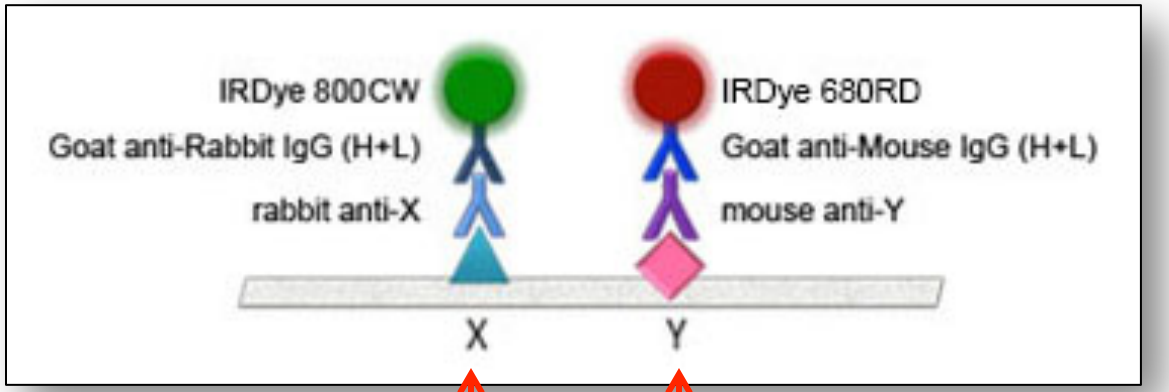


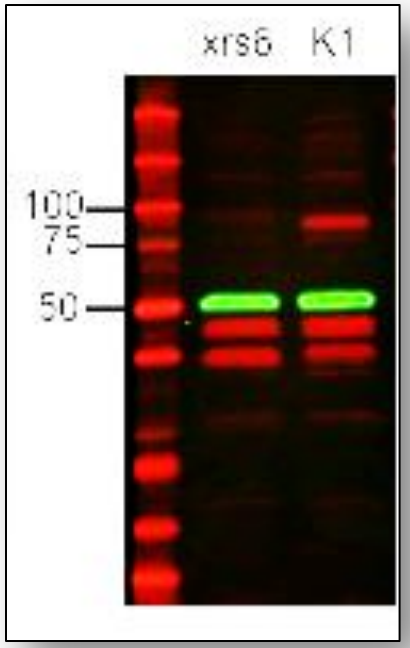
It is **essential** to confirm the identity of the cells you are working with

Western Blot Analysis



α- tubulin

Ku80
(Or in your case, BRCA2)



Ku80

α- tubulin

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- Engineer the inhibition of DNA Repair pathways
- Monitor changes in a gene's expression (qPCR)
- Analyze RNAseq dataset measuring expression of ~ 20,000 genes (BIG DATA!)
- Statistical analysis of all biological data

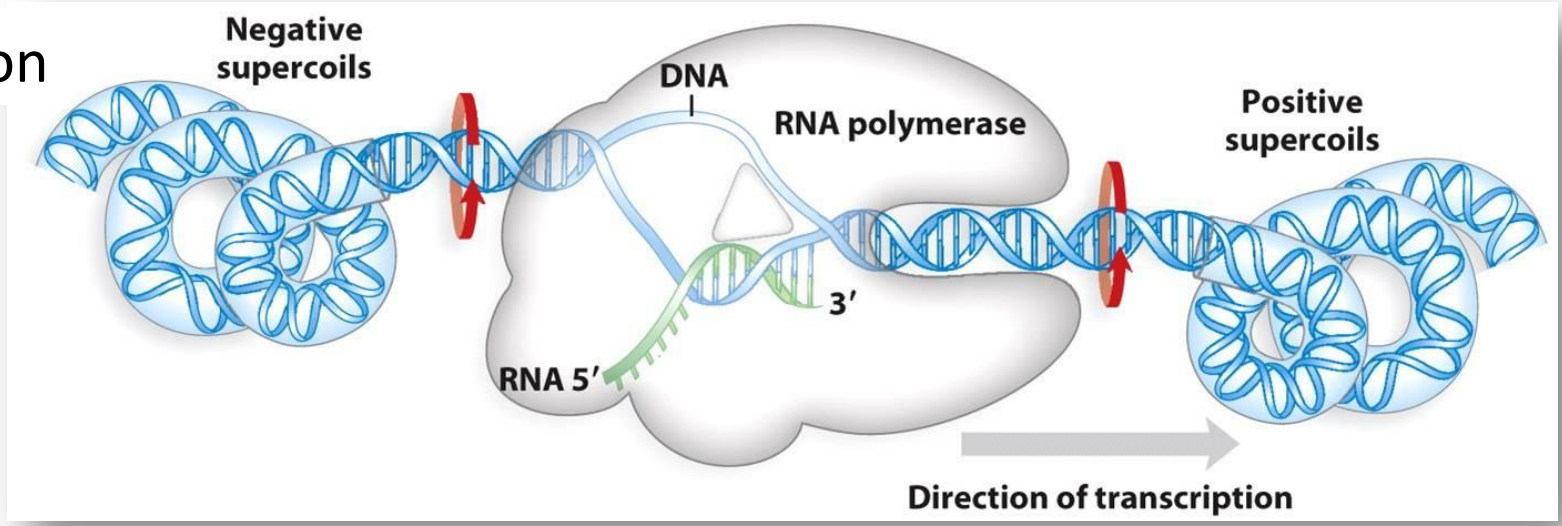
Some cancer Chemotherapy agents and all Radiotherapies

CAUSE DNA DAMAGE

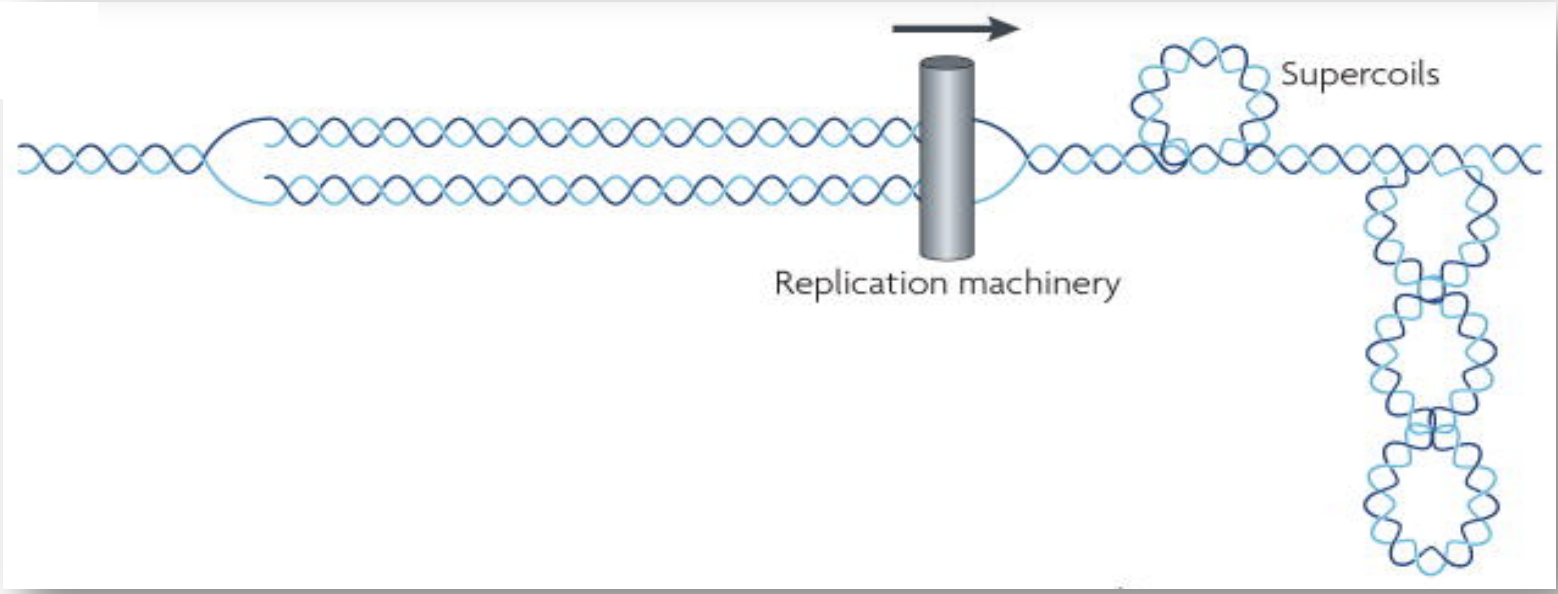
Etoposide induces DNA double strand breaks (DSBs) by inhibiting an enzyme that undoes the supercoiling of DNA

DNA Replication and RNA Transcription cause DNA SUPERCOILING

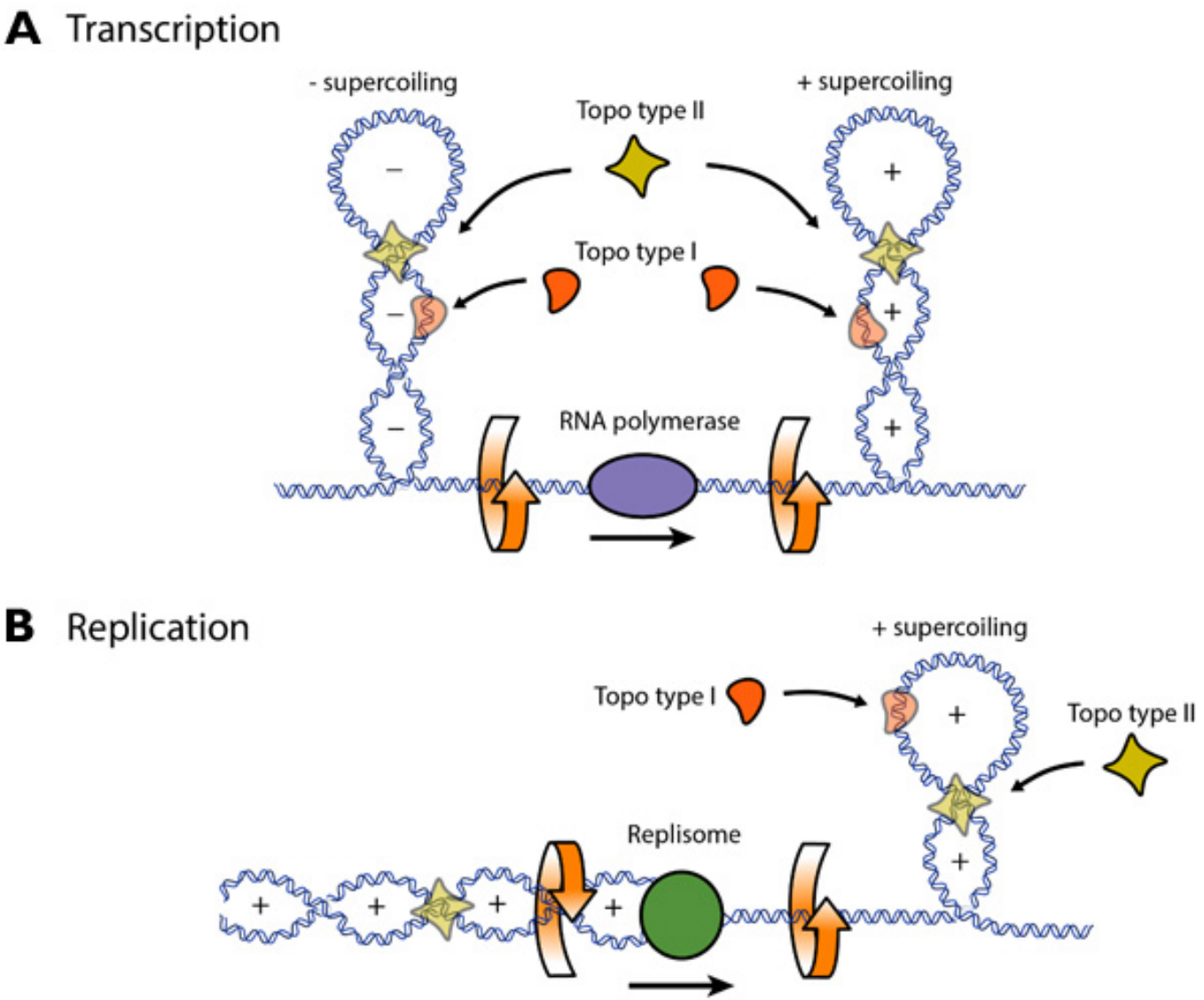
Transcription



Replication



RNA Transcription and DNA Replication cause DNA SUPERCOILING

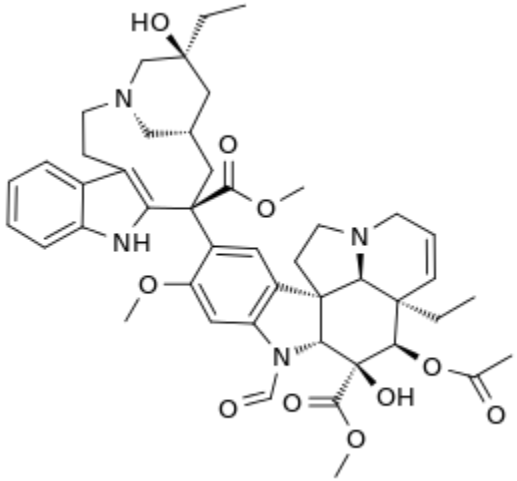


https://www.google.com/search?q=transcription+and+supercoiling&rlz=1T4GGHP_enUS635US636&source=lnms&tbm=isch&sa=X&ved=0ahUKEwiht7nTmcrSAhUC8IMKHR31CPoQ_AUICSgC&biw=1451&bih=689#imgrc=pBDGWOKz0axQVM:

Topoisomerase I and II

https://www.youtube.com/watch?v=EYGrElVyHnU

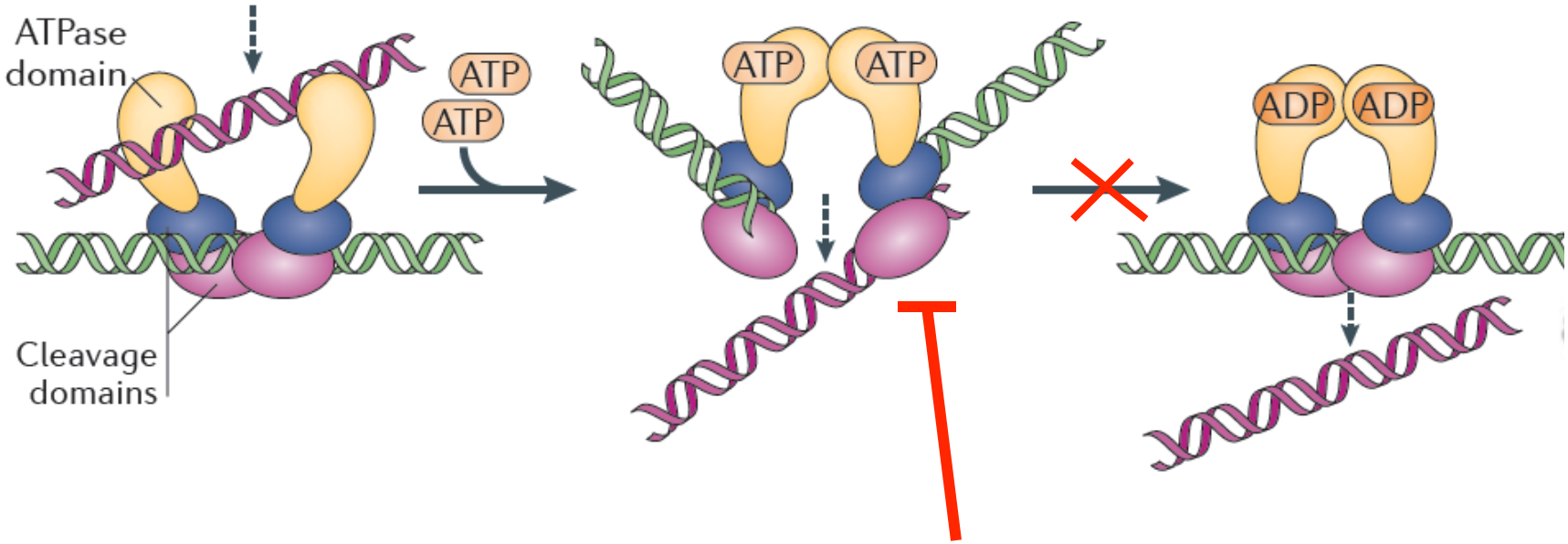
Etoposide – Inhibits Topoisomerase II

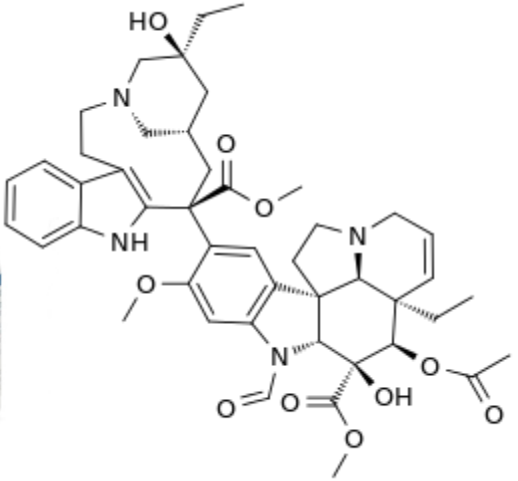


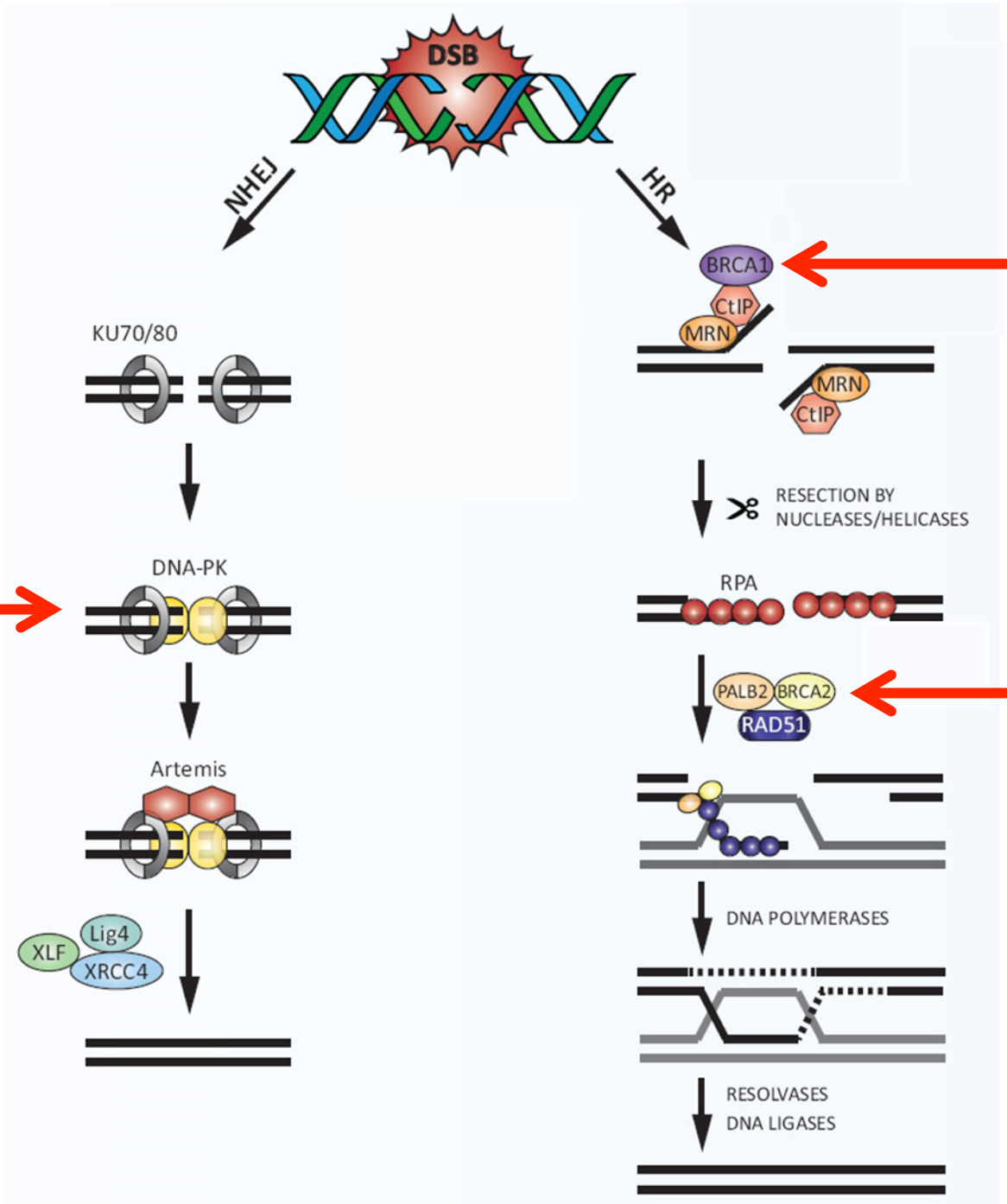
Etoposide is a semisynthetic derivative of PODOPHYLLOTOXIN from the rhizome of the wild mandrake (*Podophyllum peltatum*). Used for Cancer Chemotherapy

The wild Mandrake, with Rhizome at the bottom (at the bottom)

Etoposide – Inhibits Topoisomerase II





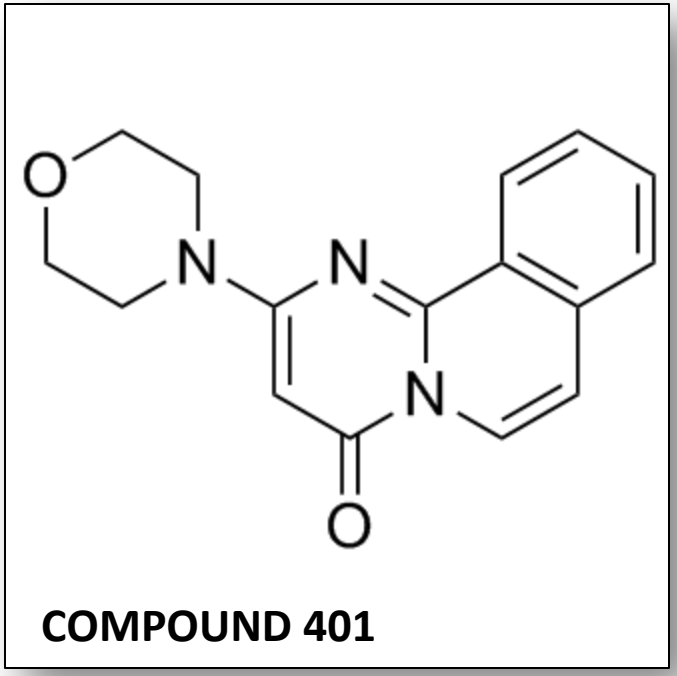


DNA-PK

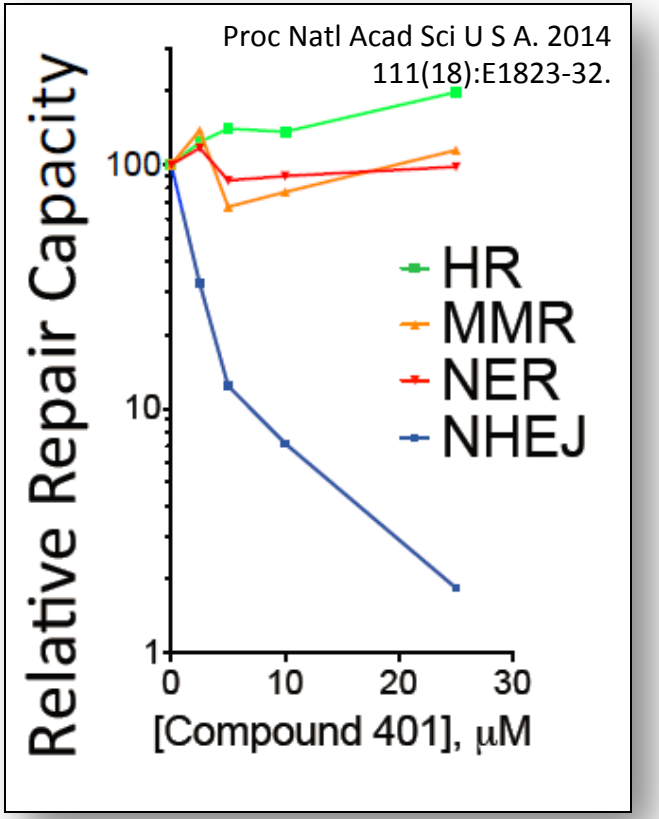
BRCA1

BRCA2

COMPOUND 401 – "Selective" Inhibitor of DNA-PK and NHEJ



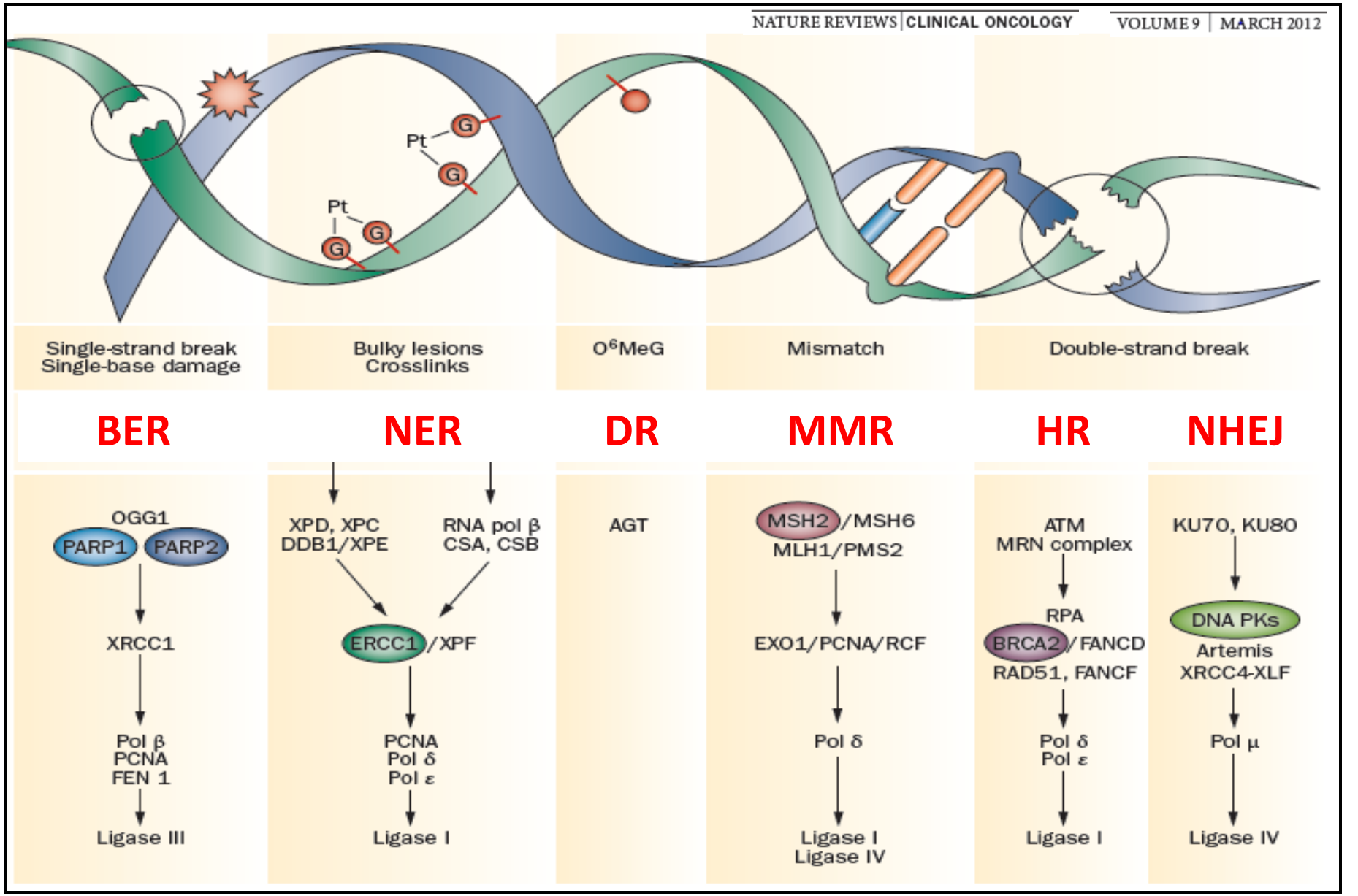
COMPOUND 401



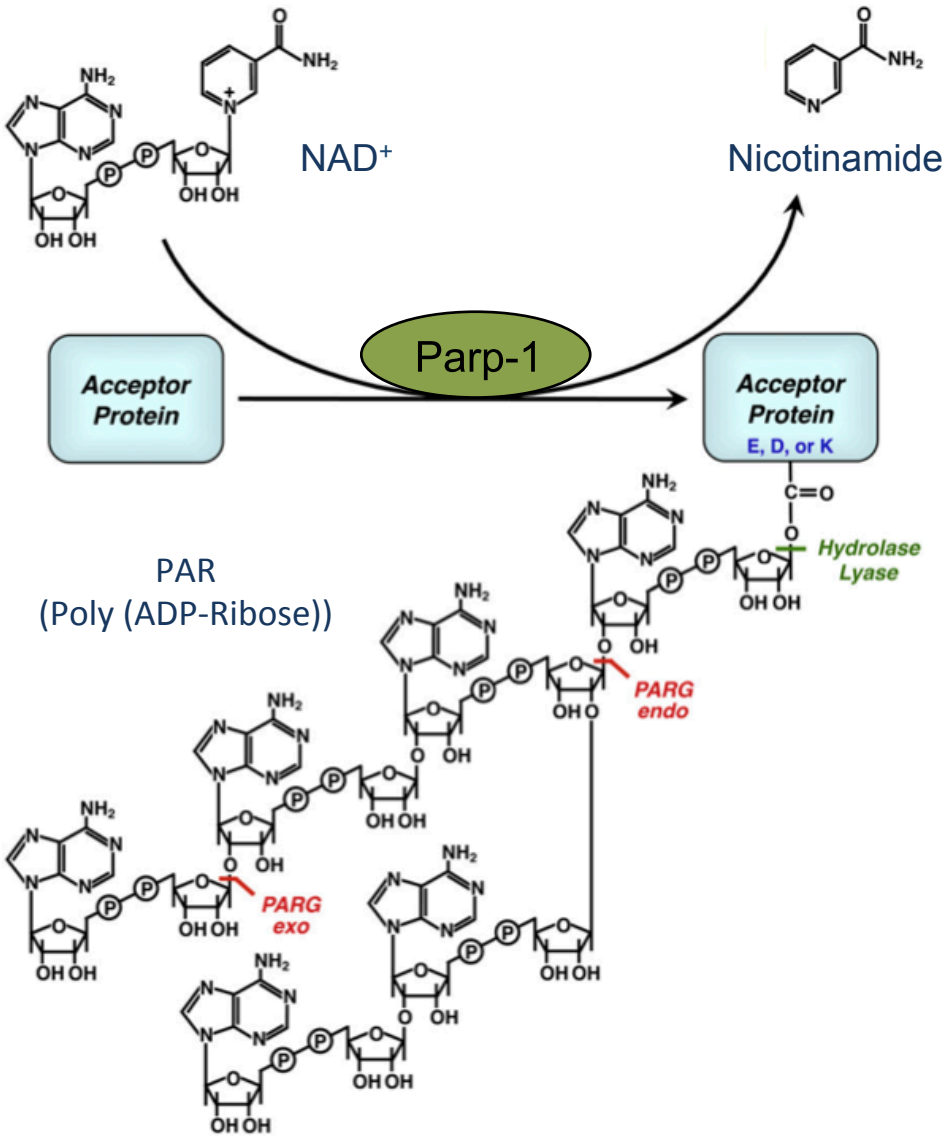
Biological Activity of COMPOUND 104

Reversible and selective inhibitor of DNA-dependent protein kinase (DNA-PK) and mammalian target of rapamycin (mTOR) (IC_{50} values are 0.28 and 5.3 μM respectively). Displays little affinity for other commonly studied kinases including PI 3-K, ATM and ATR (IC_{50} values are all > 100 μM).

Six Major DNA Repair Pathways

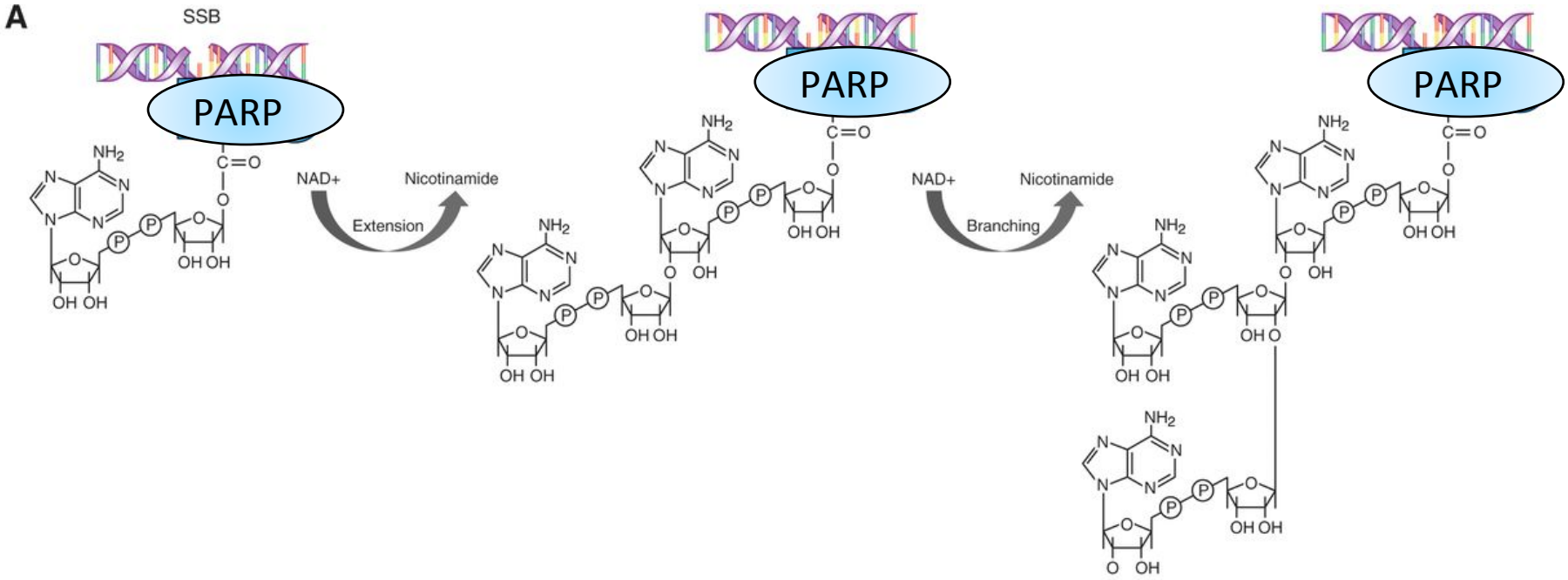


Poly (ADP-Ribose) Polymerase - PARP

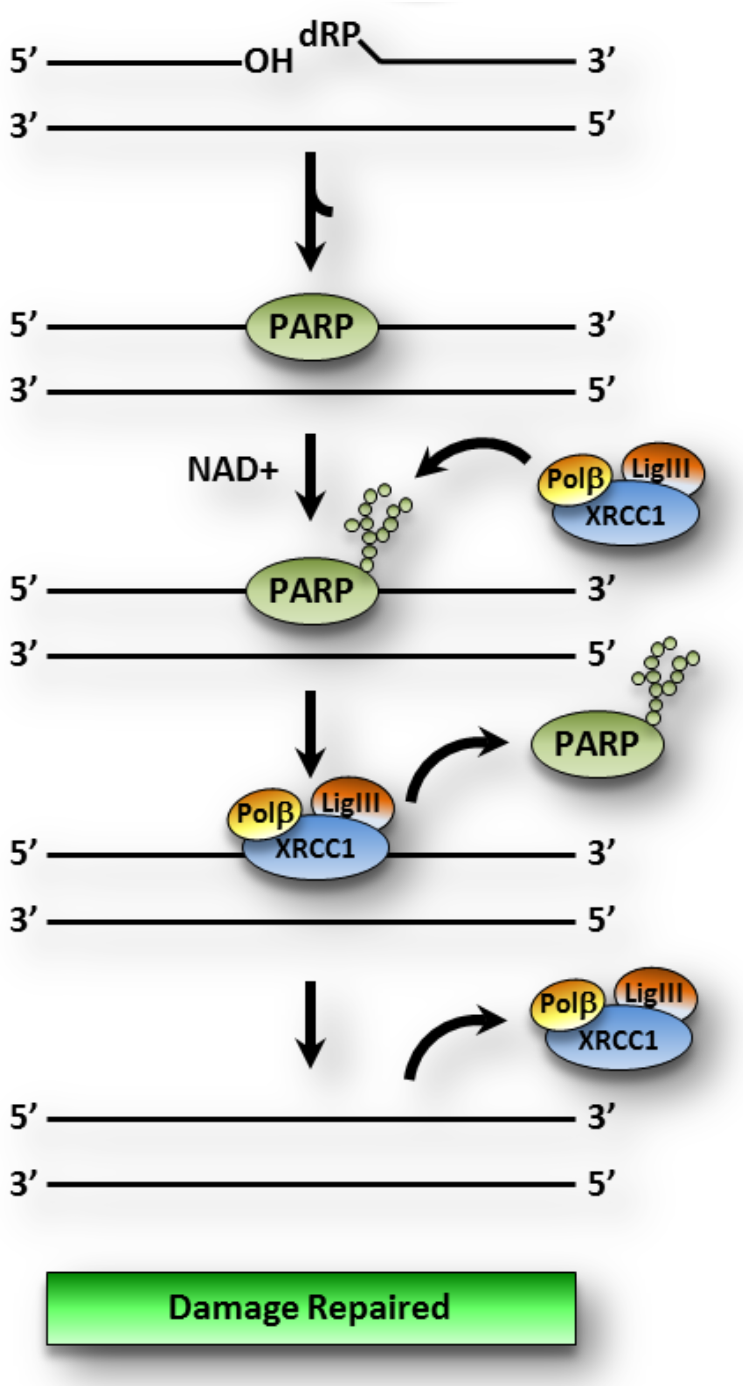


Krishnakumar, *et al.* (2010)

Poly (ADP-Ribose) Polymerase – PARP is activated at DNA Single Strand Breaks (SSBs)



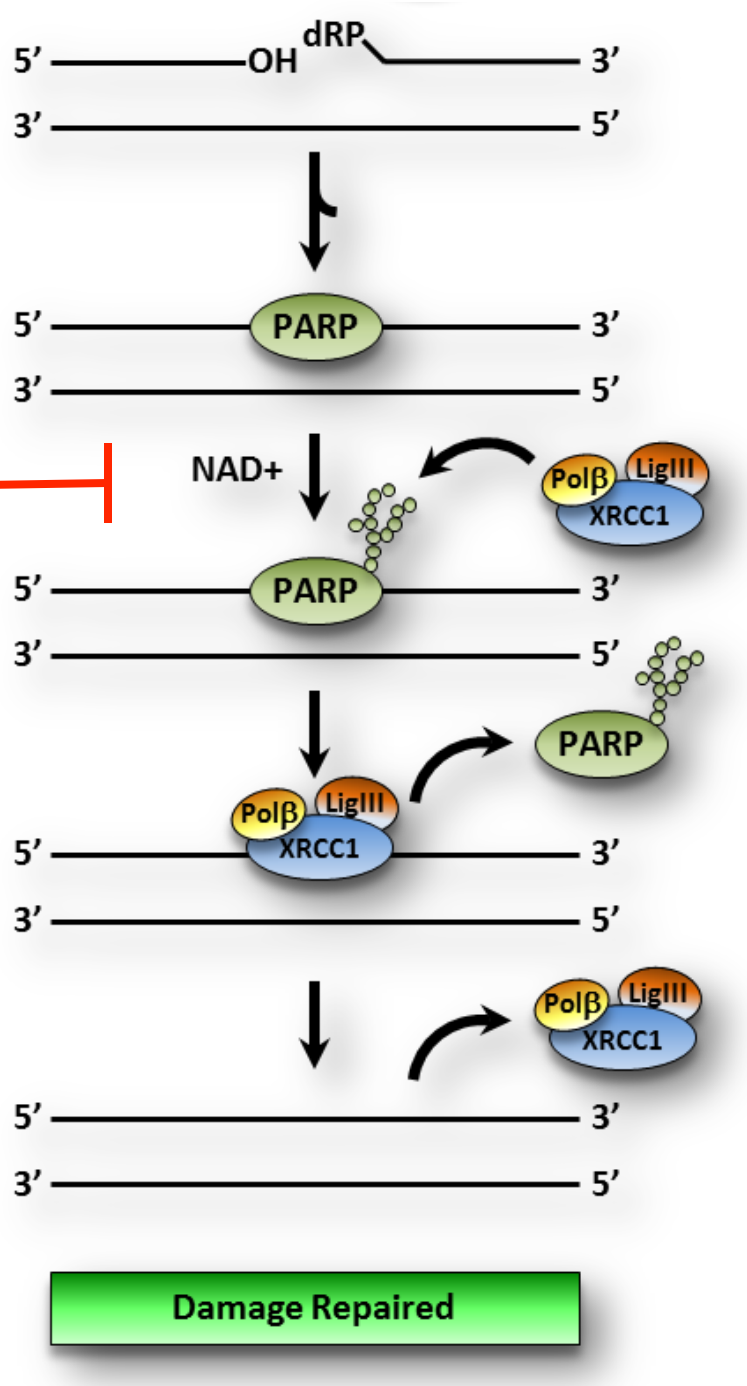
DNA Single Strand Break (SSB) Repair



Joshua J Corrigan

DNA Single Strand Break (SSB) Repair

Inhibit this step

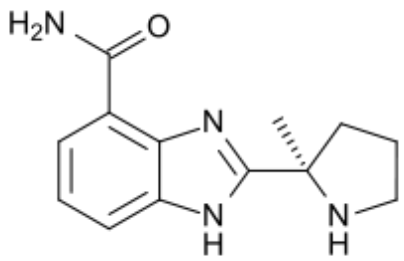


Joshua J Corrigan

Parp Inhibitors in Clinical Trials

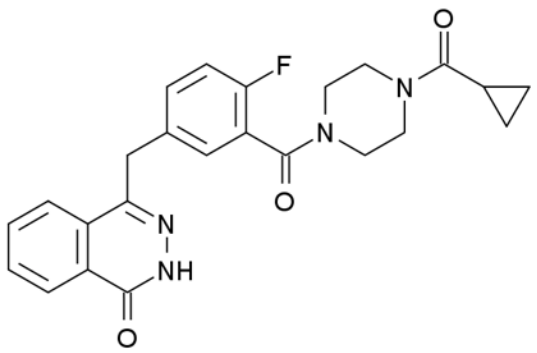
Veliparib

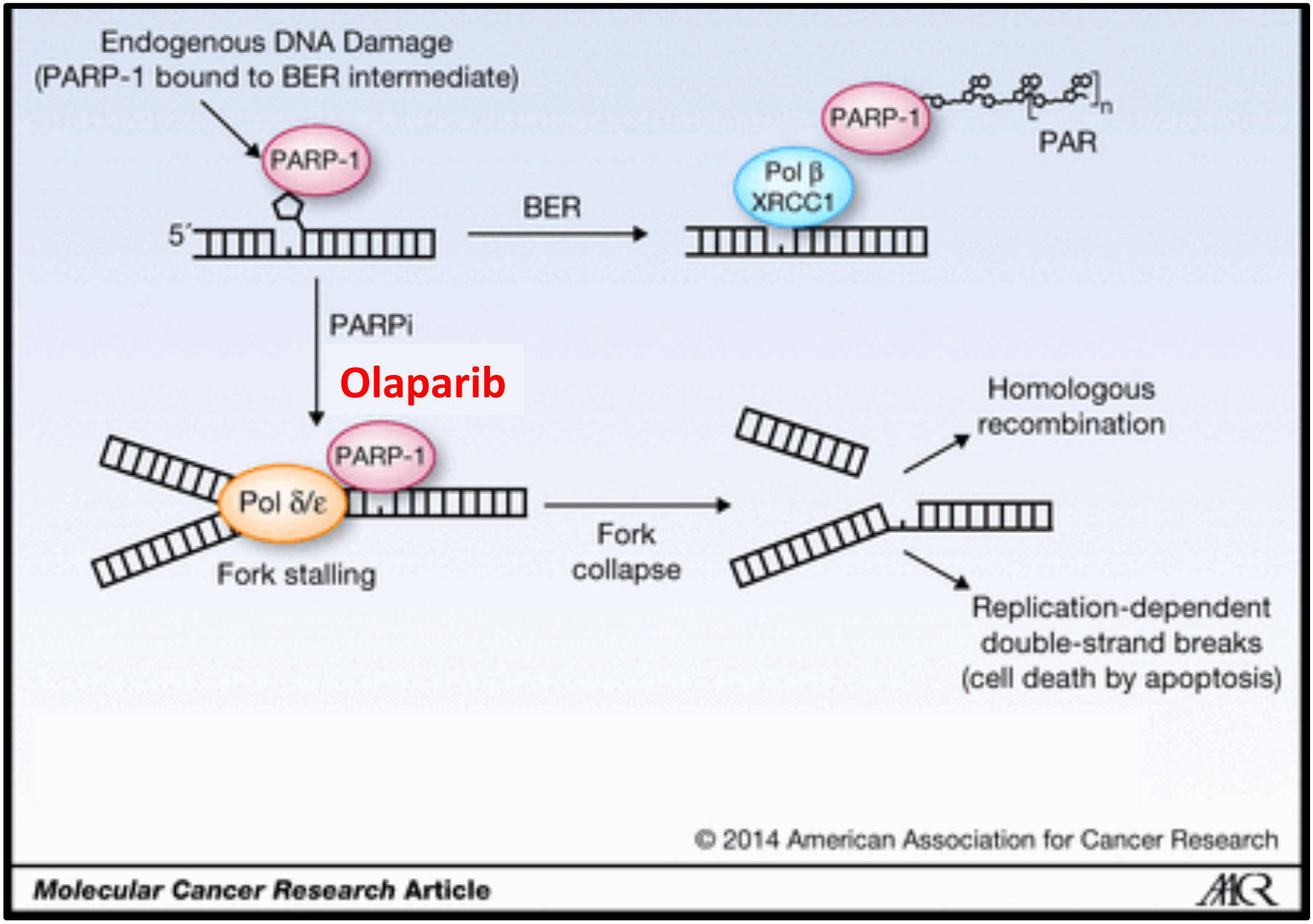
- ABT-888 (AbbVie)
- Competes for NAD+ binding site
- Less DNA trapping



Olaparib

- AZD-2281 (Astra Zeneca)
- Competes for NAD+ binding site
- More DNA trapping

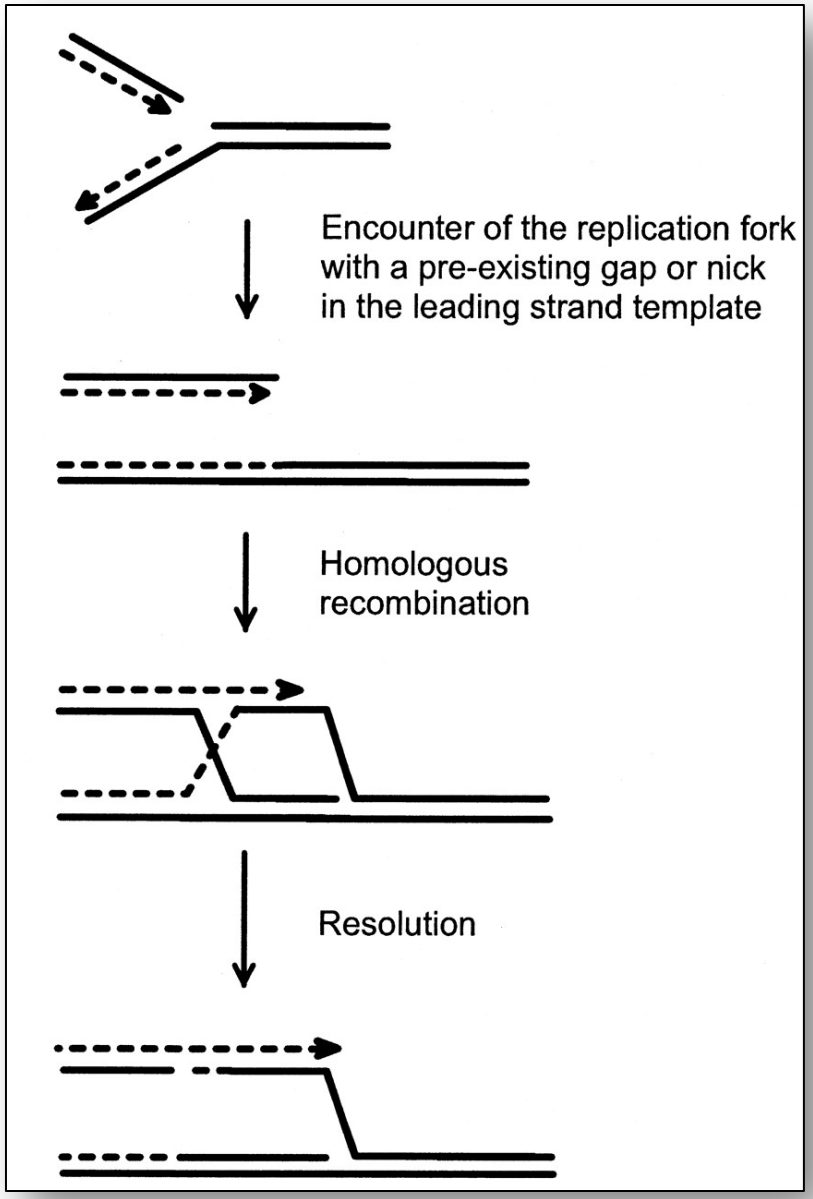




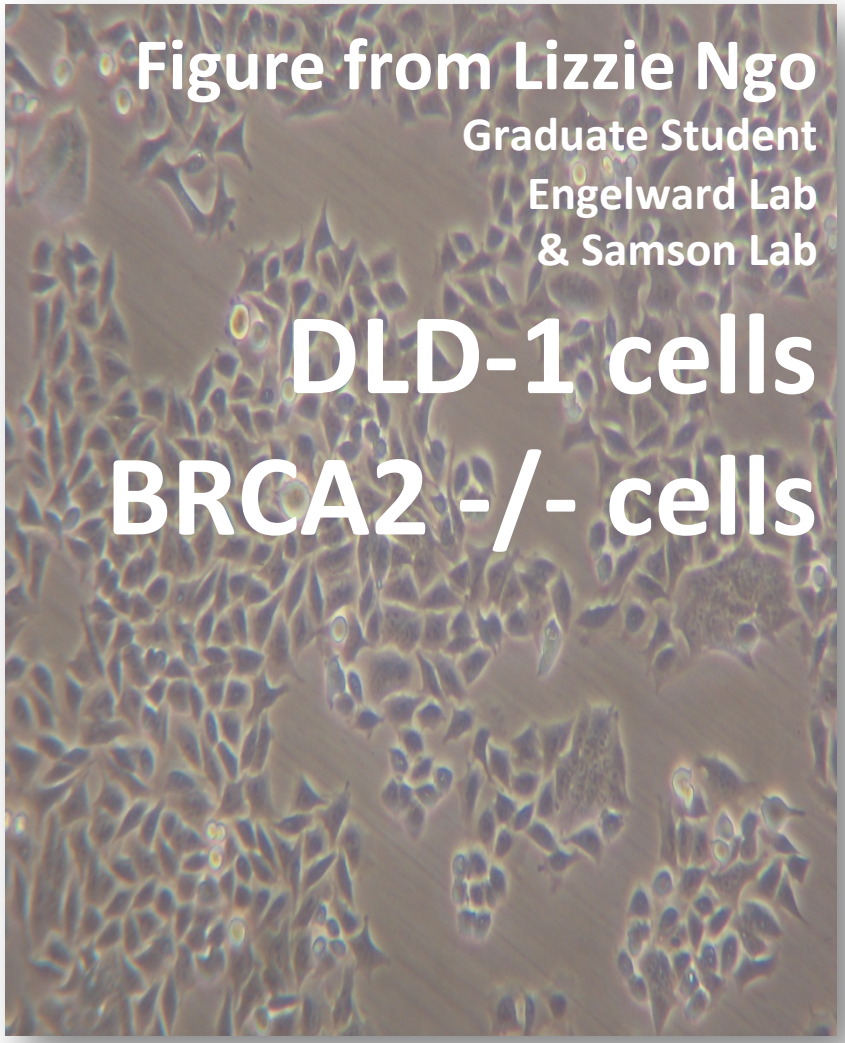
http://mcr.aacrjournals.org/content/12/8/1128

Homologous Recombination can Rescue collapsed Replication Forks – what happen in BRCA2-/- cells?

Collapsed replication fork



Here's how you will treat your cells today



[CANCER RESEARCH 39, 1020–1025, March 1979]
0008-5472/79/0039-0000$02.00

- Etoposide to inhibit TopoII leading to DNA DSBs
- Compound 401 to inhibit Non Homologous End Joining (NHEJ)
- Etoposide + Compound 401
- Olaparib to inhibit PARP1 to stabilize DNA SSBs leading to DNA DSBs at collapsed replication forks

20.109 Spring 2017 Module 2 – Lecture 2

Gene Expression Engineering (March 14th 2017)




Noreen Lyell

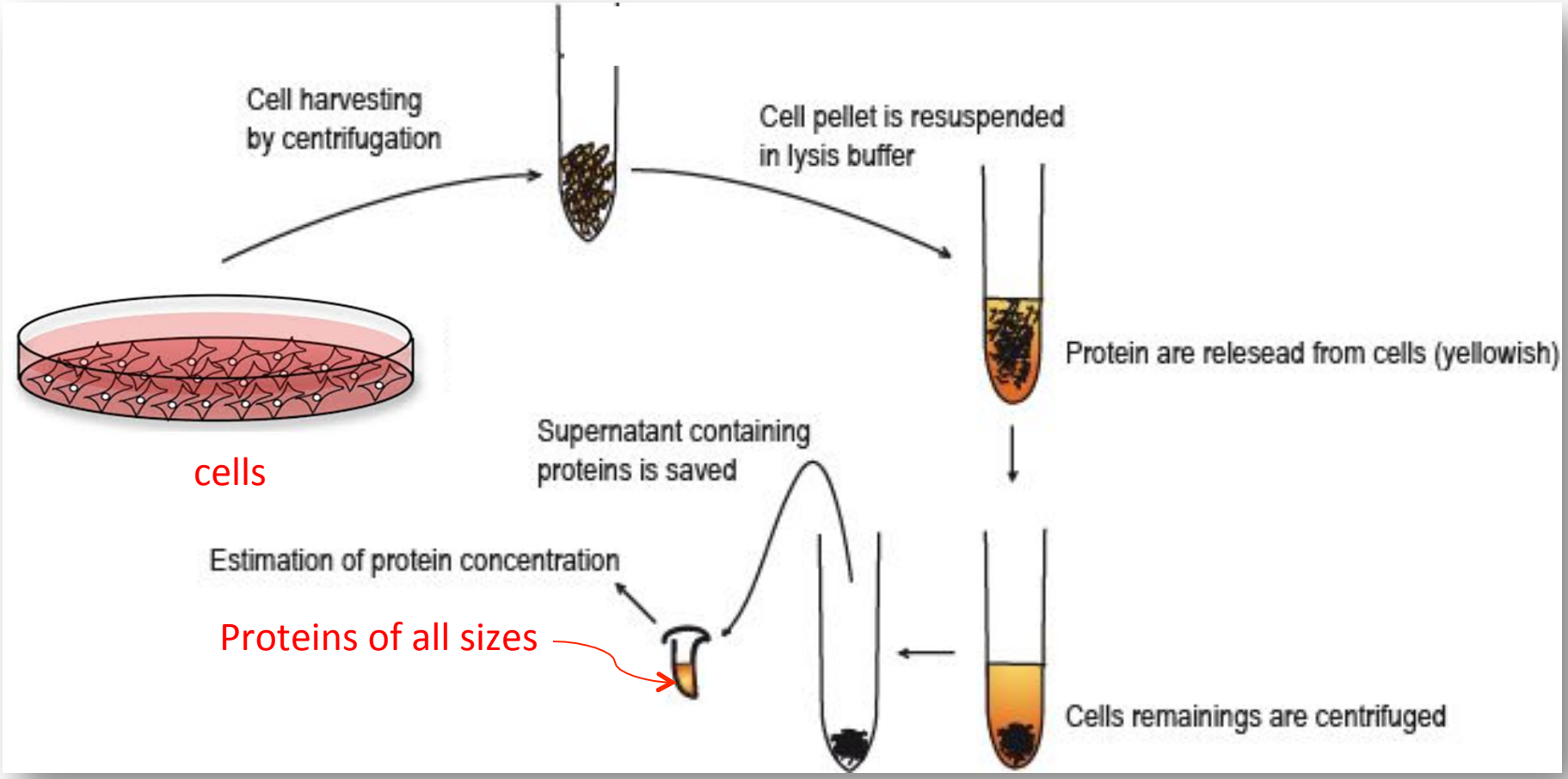
Leslie McLain

Maxine Jonas

Rob Wilson

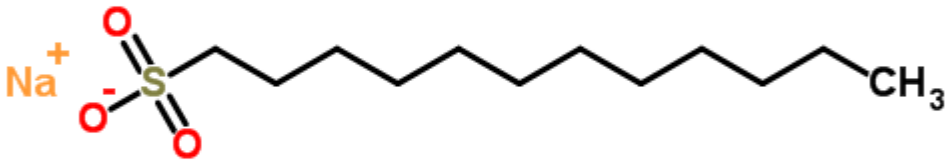
Leona Samson (Lectures)

Prepare Cell Extract for Western Blot (Immunoblot) Analysis



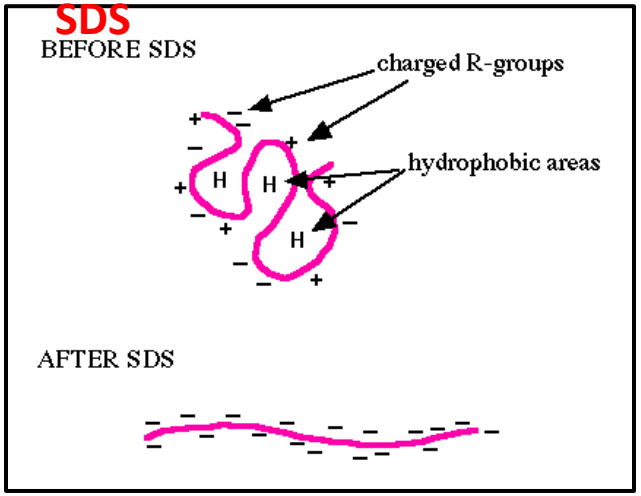
http://bio-ggs.blogspot.com/2009/11/ggs-live-western-blotting.html

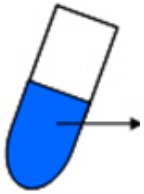
Western Blot or Immunoblot Analysis



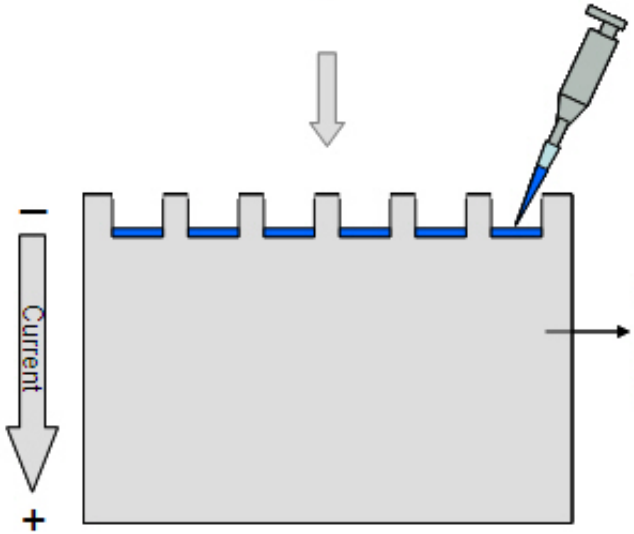
SDS – sodium dodecyl sulfate

Proteins denatured by SDS



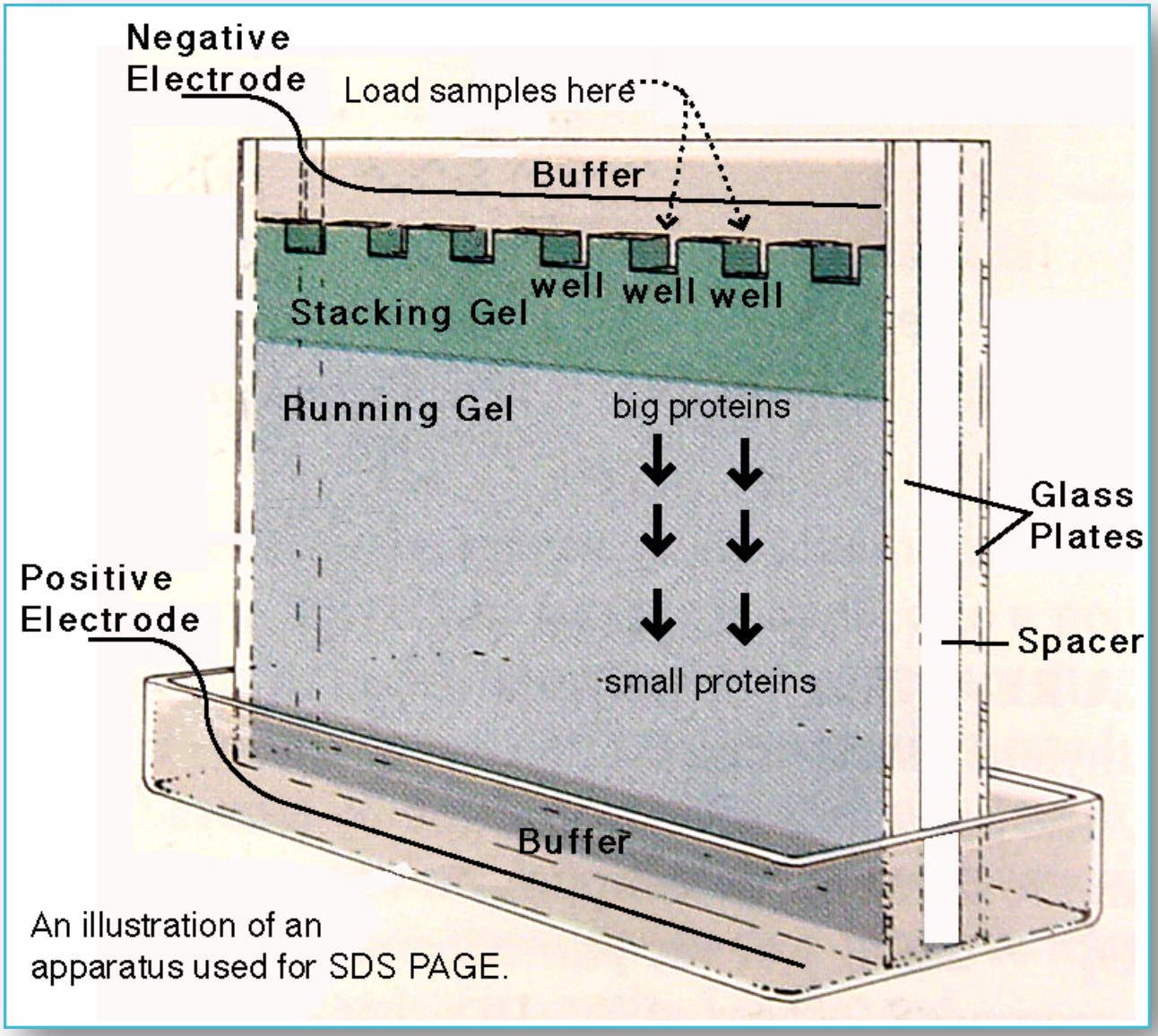


SDS to denature proteins plus blue dye – loading buffer

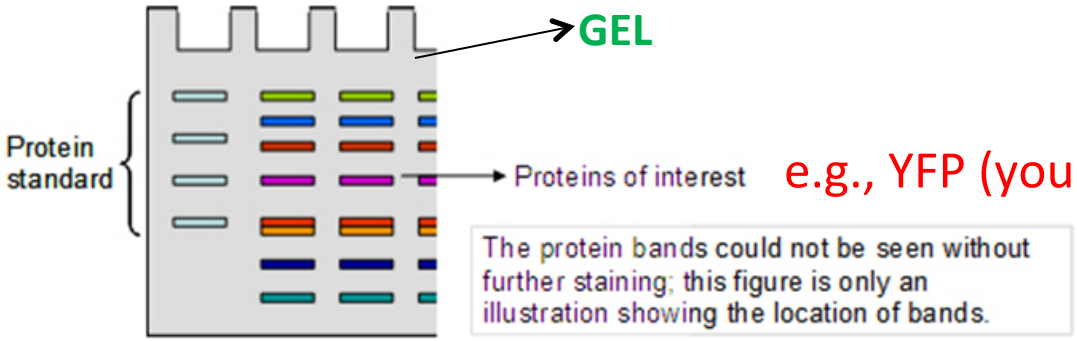


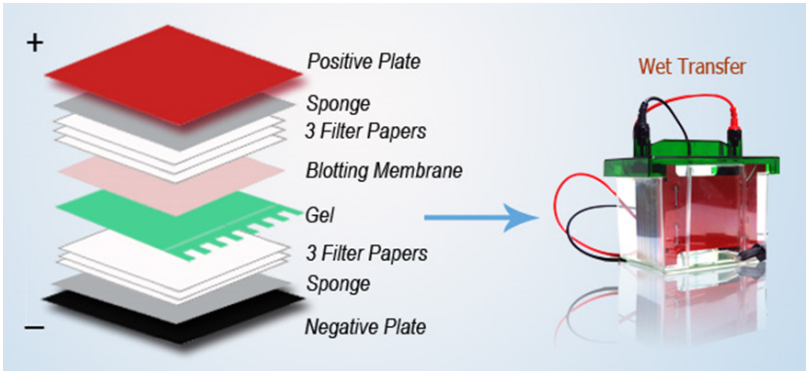
Protein samples along with a protein standard are loaded into wells; gel eletrophoresis is run by electric current.

Separating proteins on an SDS polyacrylamide gel

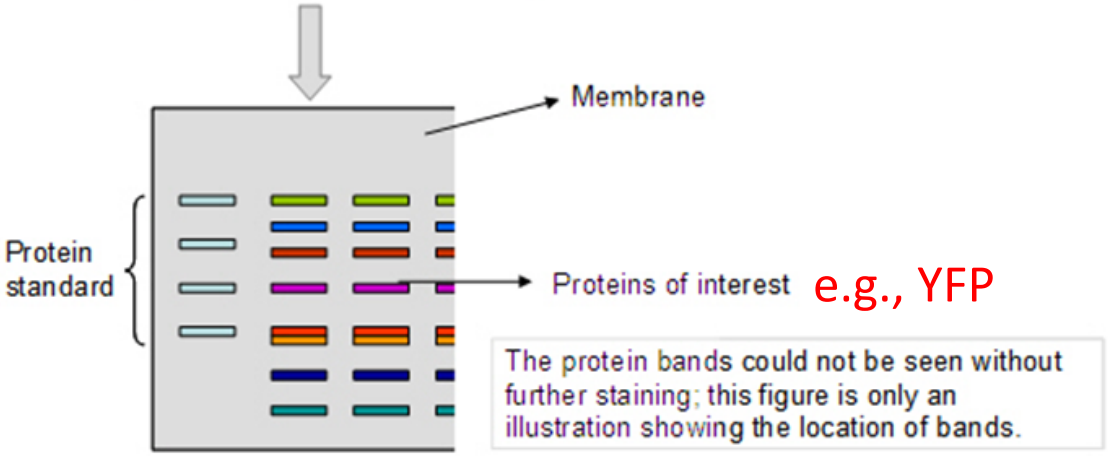


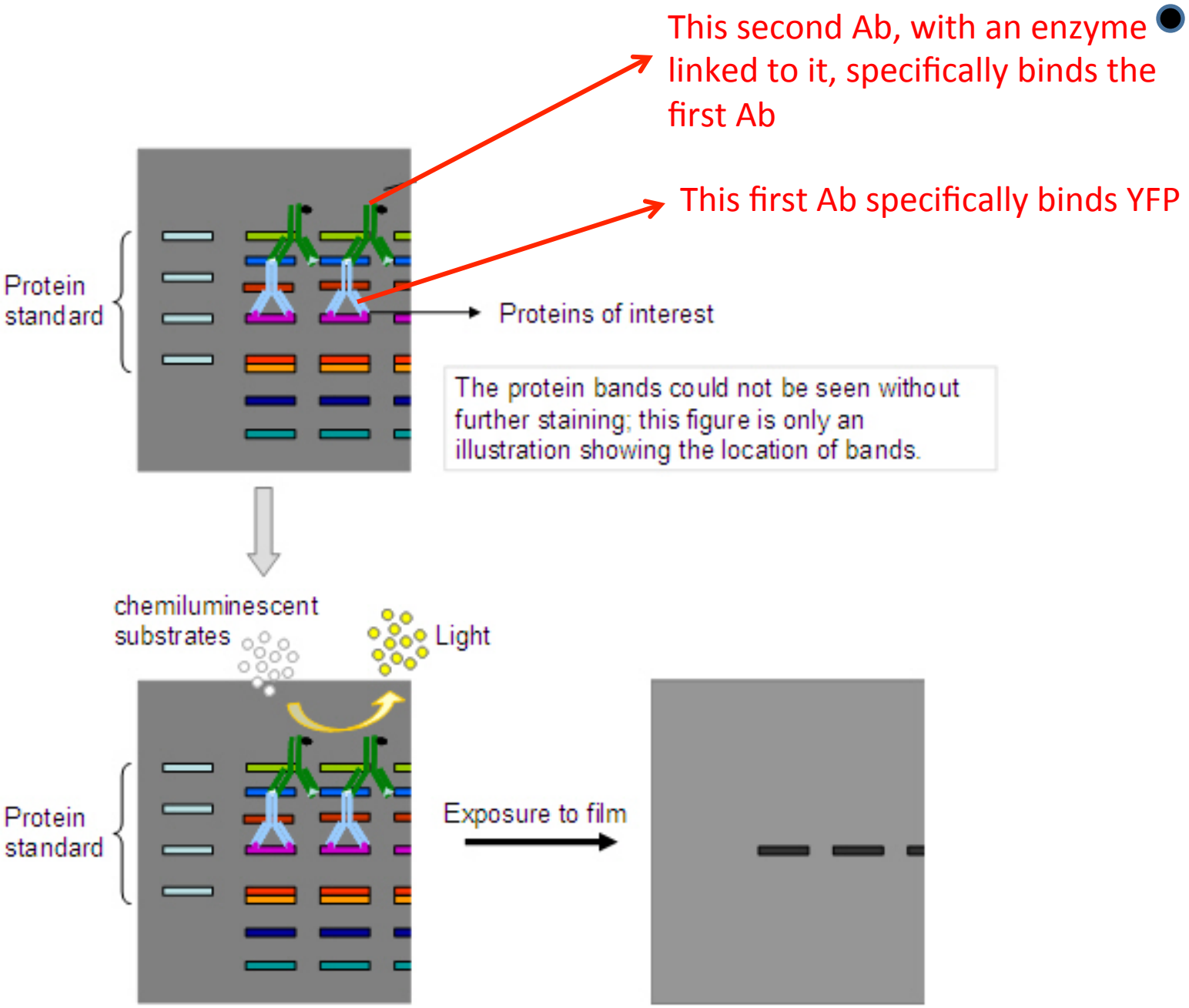
An illustration of an apparatus used for SDS PAGE.





The Separated proteins are transferred out of the gel and BLOTTED onto a nitrocellulose or other membrane




This second Ab, with an enzyme linked to it, specifically binds the first Ab
This first Ab specifically binds YFP
Protein standard
Proteins of interest
The protein bands could not be seen without further staining; this figure is only an illustration showing the location of bands.
chemiluminescent substrates
Light
Protein standard
Exposure to film

Western Blot Analysis