20.109 MOD1 – DNA ENGINEERING
Fall 2010

Exploiting science for engineering: BRCA2 targeted therapies

Orsi Kiraly
Engelward lab

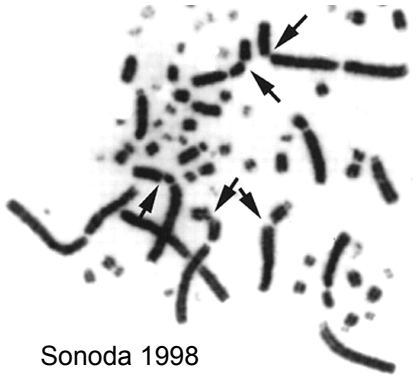
Homologous recombination is important

- **No HR**

chromosomal aberrations

cell death

normal



Sonoda 1998

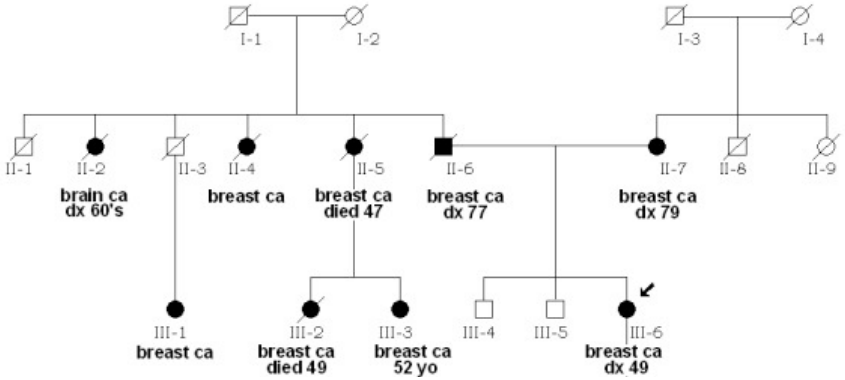
Rad51-/-
no HR

- **Faulty HR**

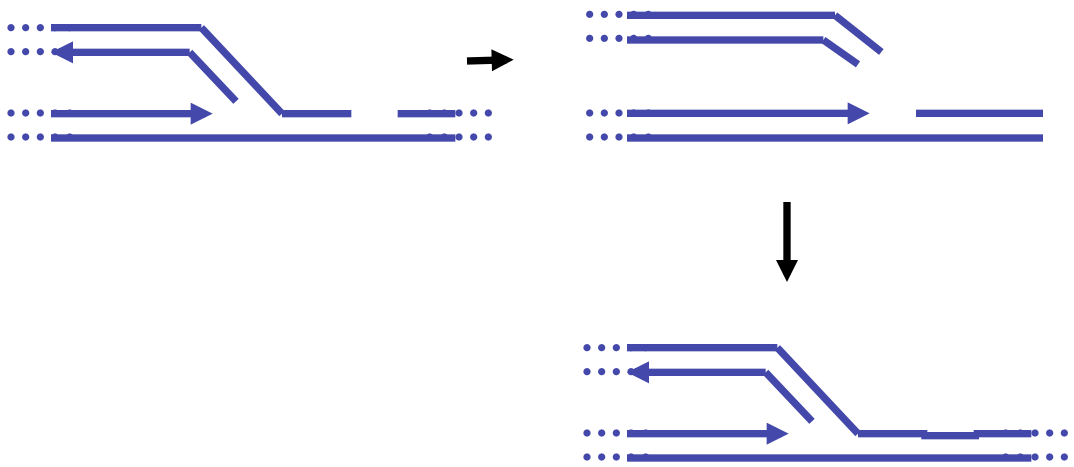
premature aging

WS patient age 15 yrs | WS patient age 48 yrs

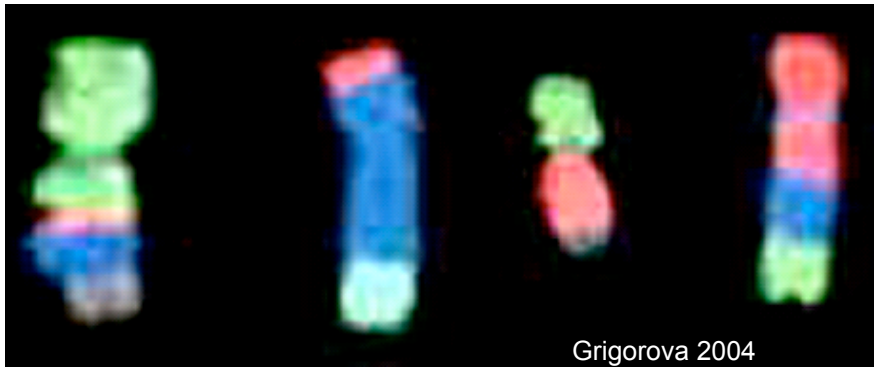
cancer



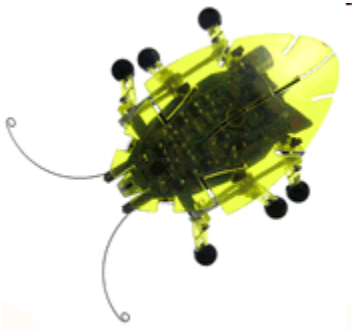
Homologous recombination is critical for repair of broken replication forks

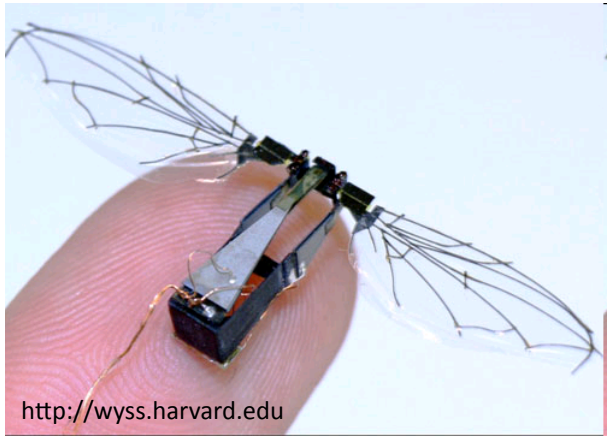


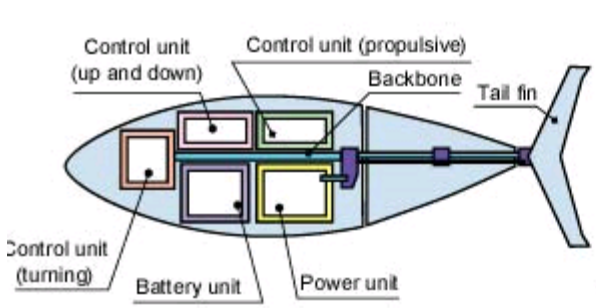
Homologous recombination is critical for repair of broken replication forks

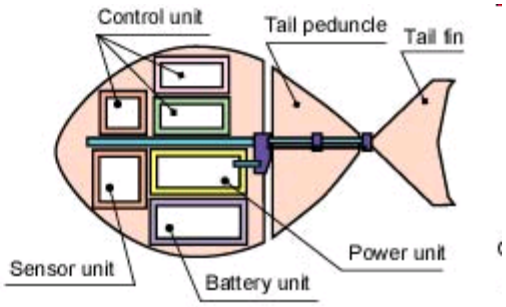


Understanding biology leads to new technologies





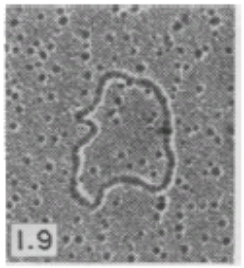




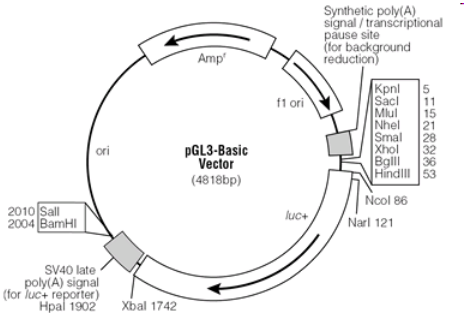
http://www.nmri.go.jp

Recombinant DNA toolbox is from nature

Plasmids



Radloff 1967 PNAS



www.promega.com

Heat stable DNA polymerases

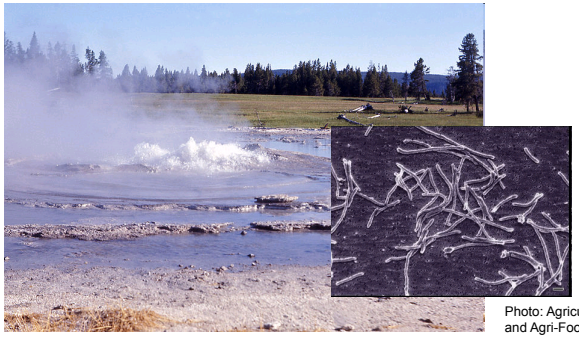
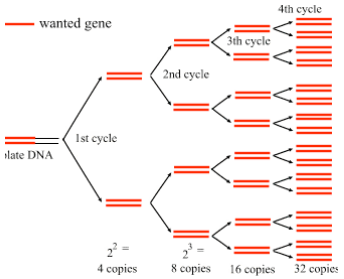


Photo: Agriculture and Agri-Food, Canada



http://users.ugent.be/~avierstr/principles/pcr.html

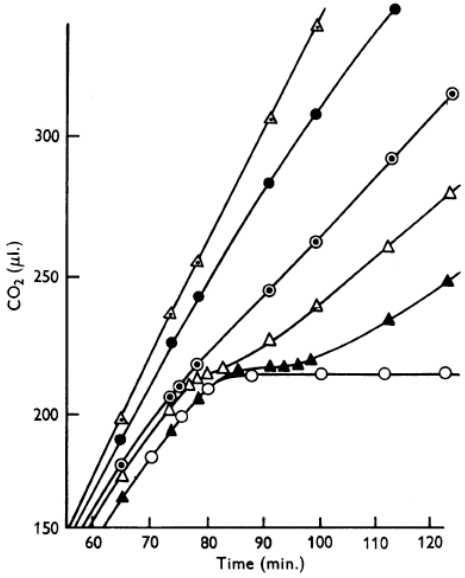
Restriction endonucleases

Lydia Dorner and Ira Schildkraut, New England Biolabs

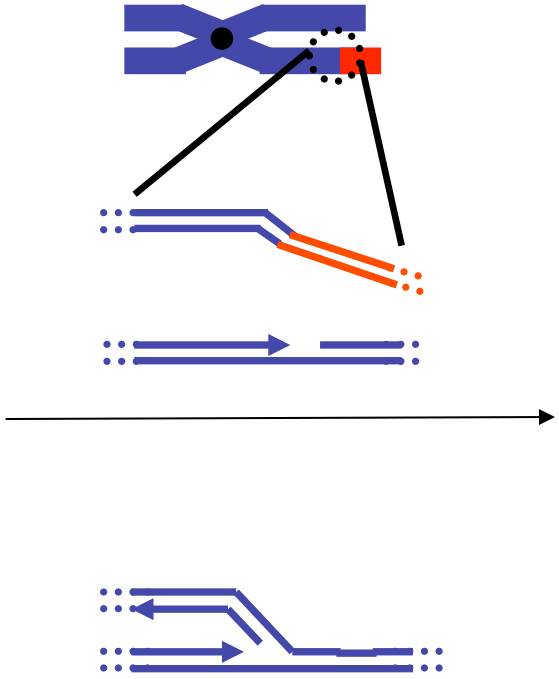
www.fermentas.com


OBSERVATION

Understanding science leads to new technologies: BRCA2 targeted therapy



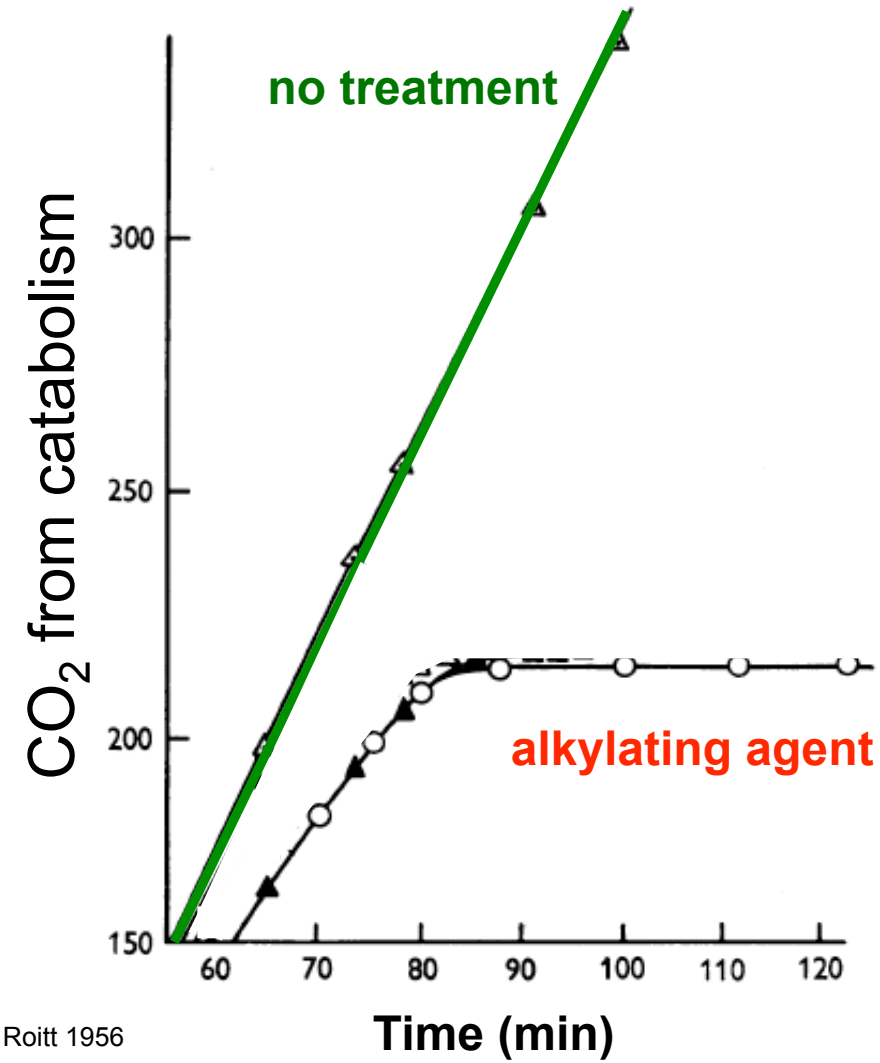
Roitt 1956



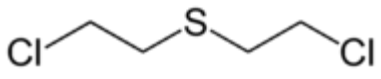
PARP Inhibitors Represent New Direction in Cancer Treatment

Metabolism is suppressed by alkylating agent

In tumor cells



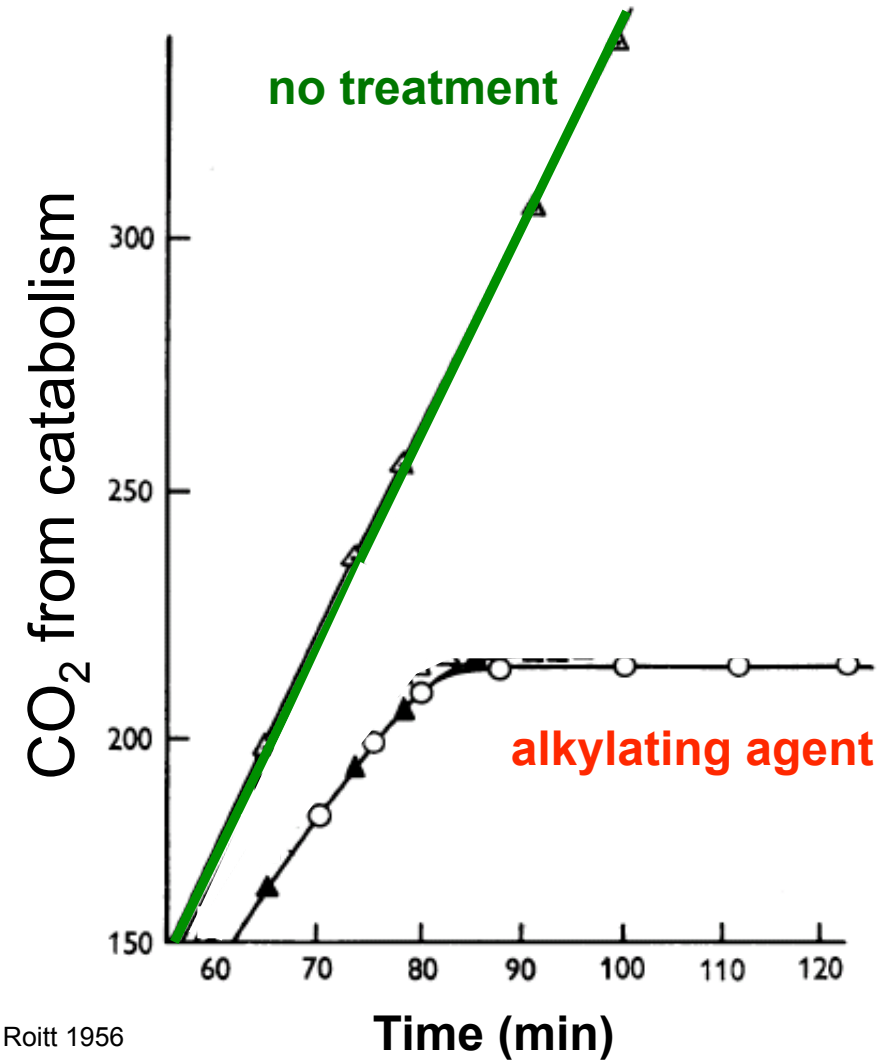
Roitt 1956



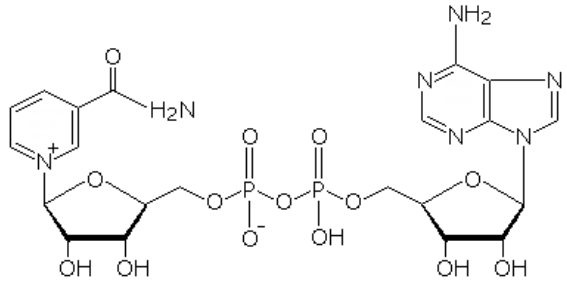
http://www.dugway.army.mil

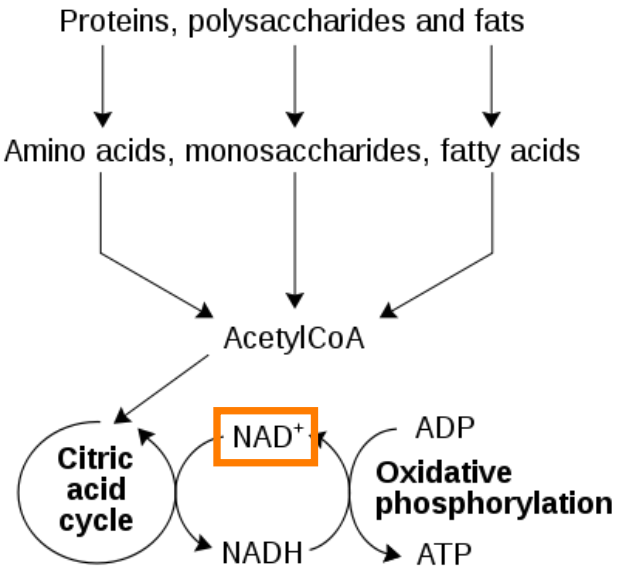
NAD levels are lowered by alkylating agent

In tumor cells



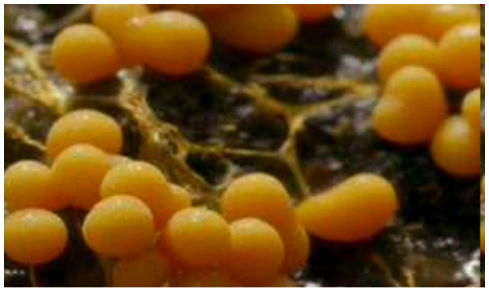
Roitt 1956



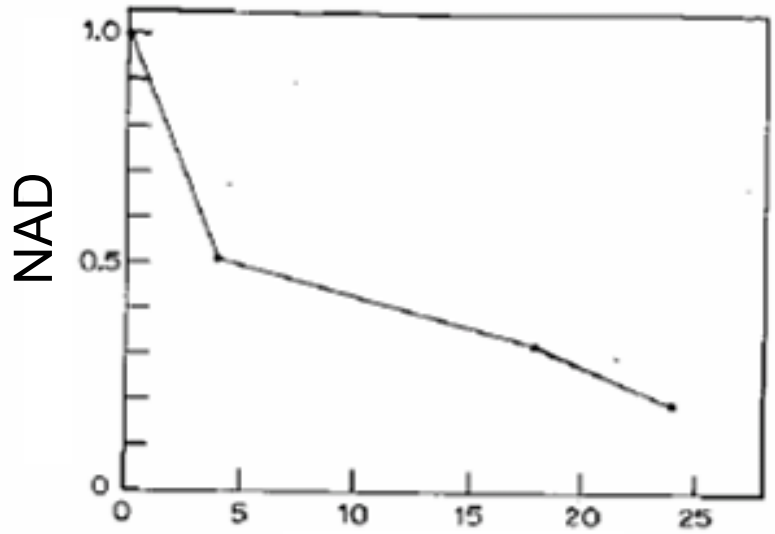


NAD levels are lowered by alkylating agent

In slime mold



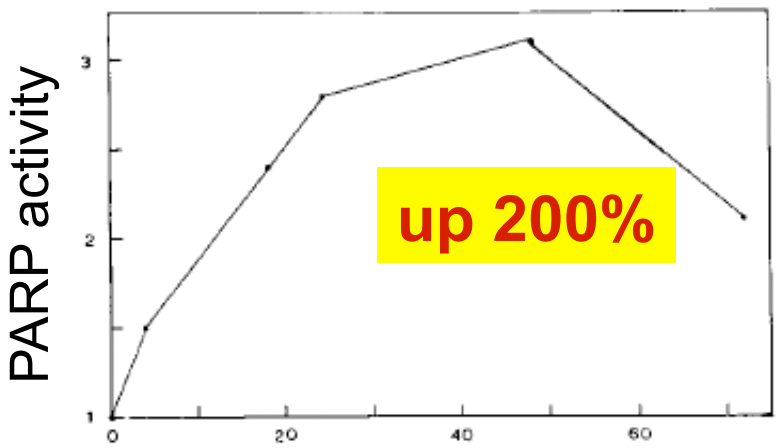
Why?



Less NAD is made? No

More NAD is degraded? No

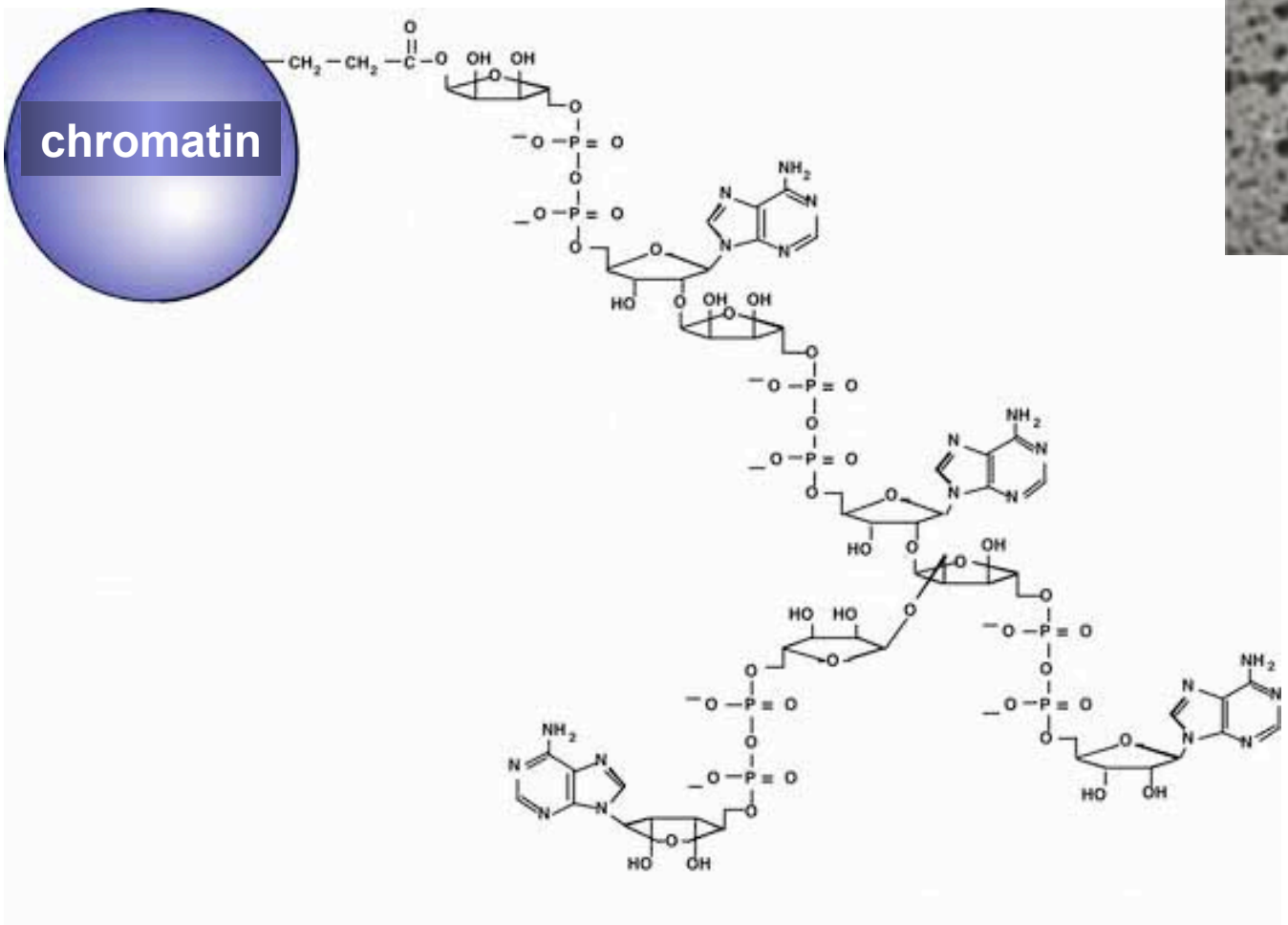
Used up to build something?? **Yes!**

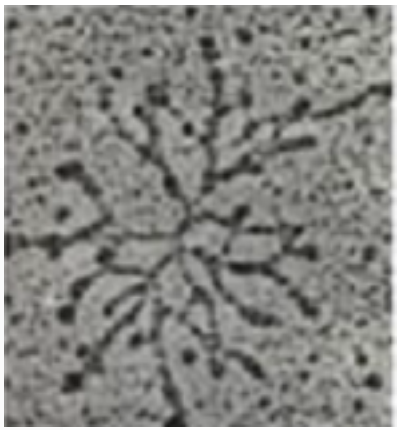


Poly(ADP-ribose) polymerase (PARP)

Whish 1975

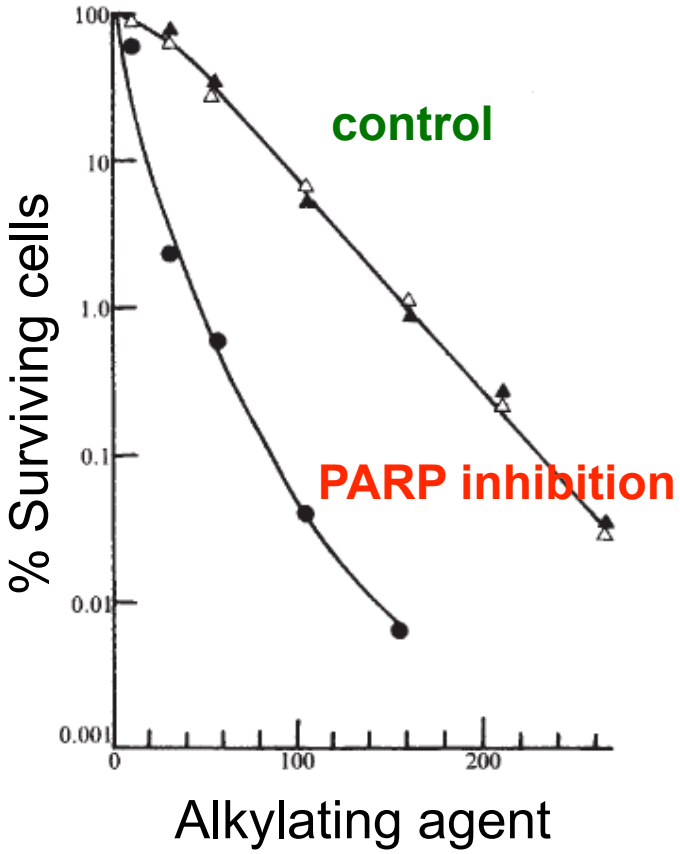
NAD is a precursor for poly(ADP-ribose)





What is the role of poly(ADP-ribosylation)?

Inhibit it, see what happens:



Durkacz 1980
Mouse leukemia cells

Inhibition of PARP affects survival after treatment with DNA damaging agent

⇩

PARP has a role in DNA repair

What is the role of poly(ADP-ribosylation)?

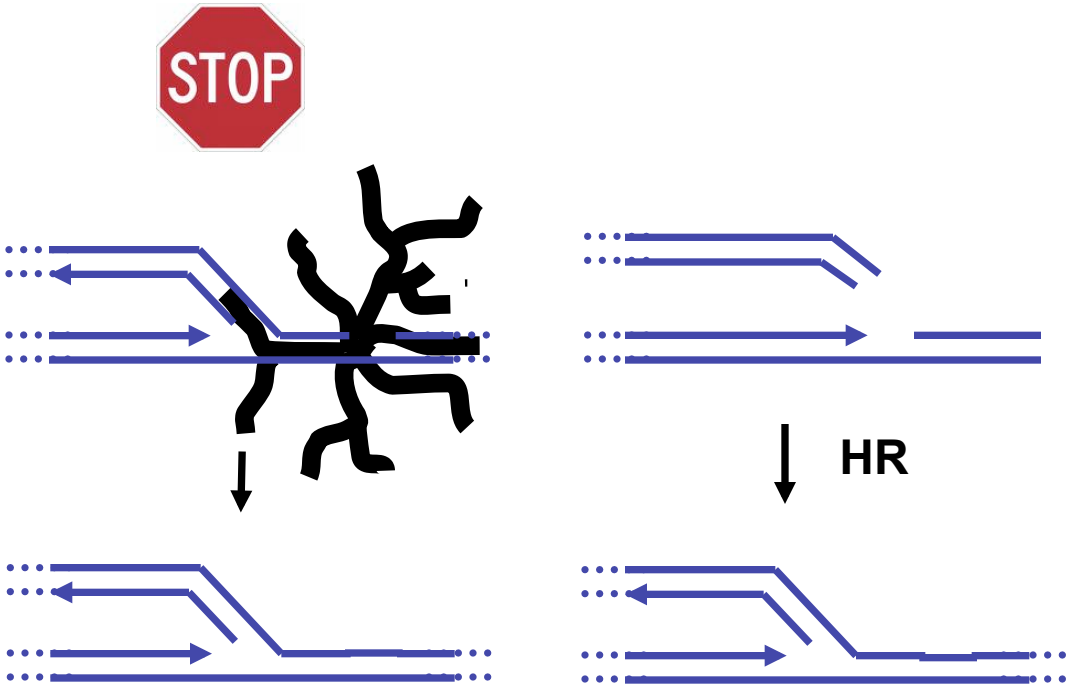
Inhibit it, see what happens:

More HR

Waldman 1991

More single strand breaks

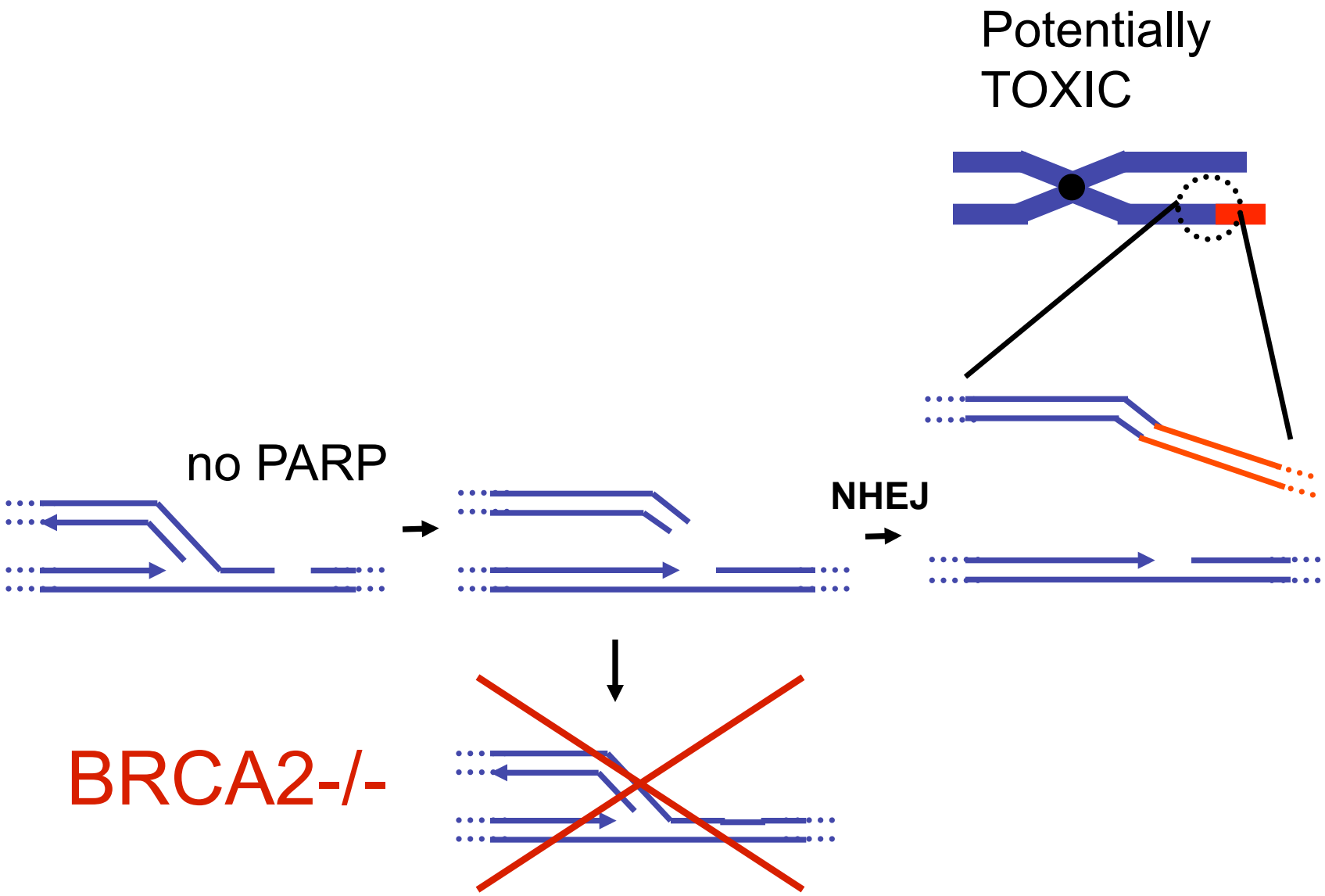
Boulton 1999



In the absence of PARP, HR is needed to fix broken fork

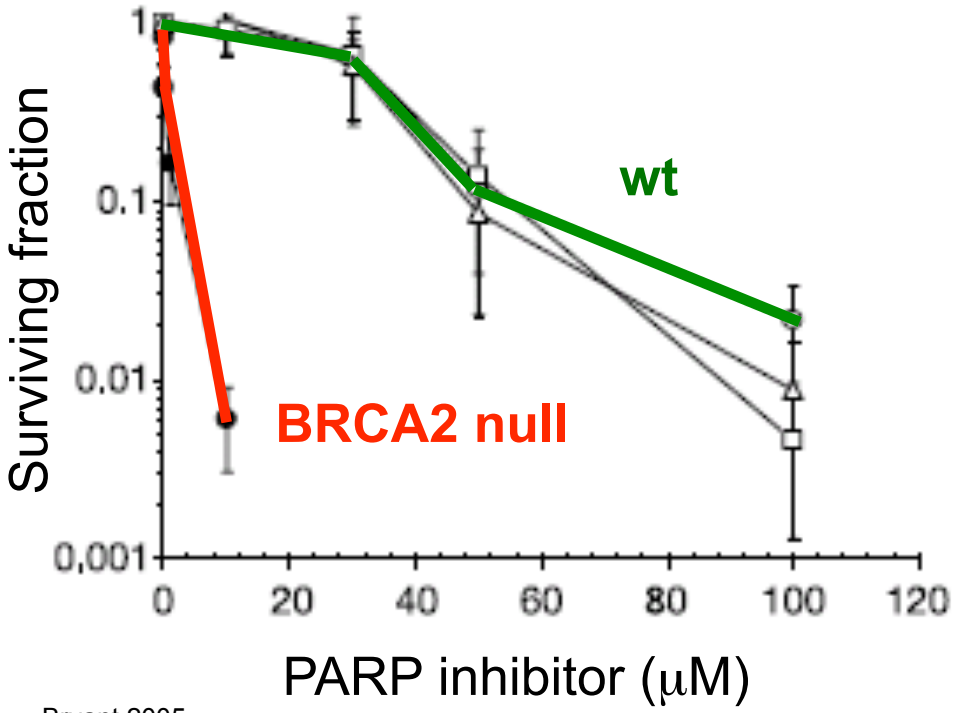
What about cancer cells that can't do HR?

What about cancer cells that can't do HR?

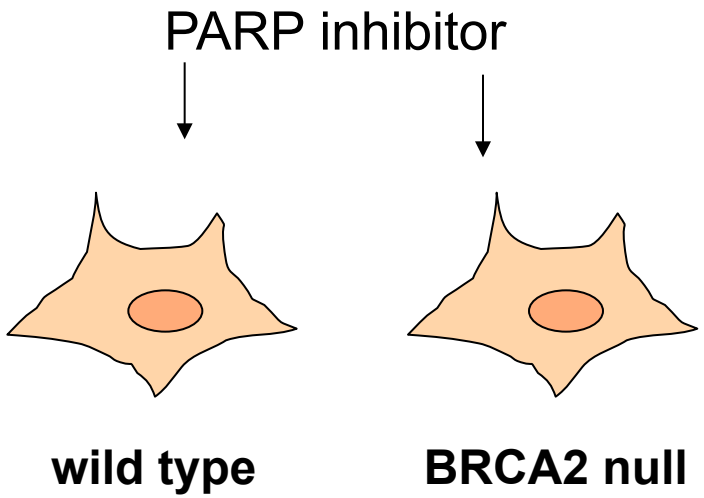


BRCA2 null cells are extremely sensitive to PARP inhibitors

In cells

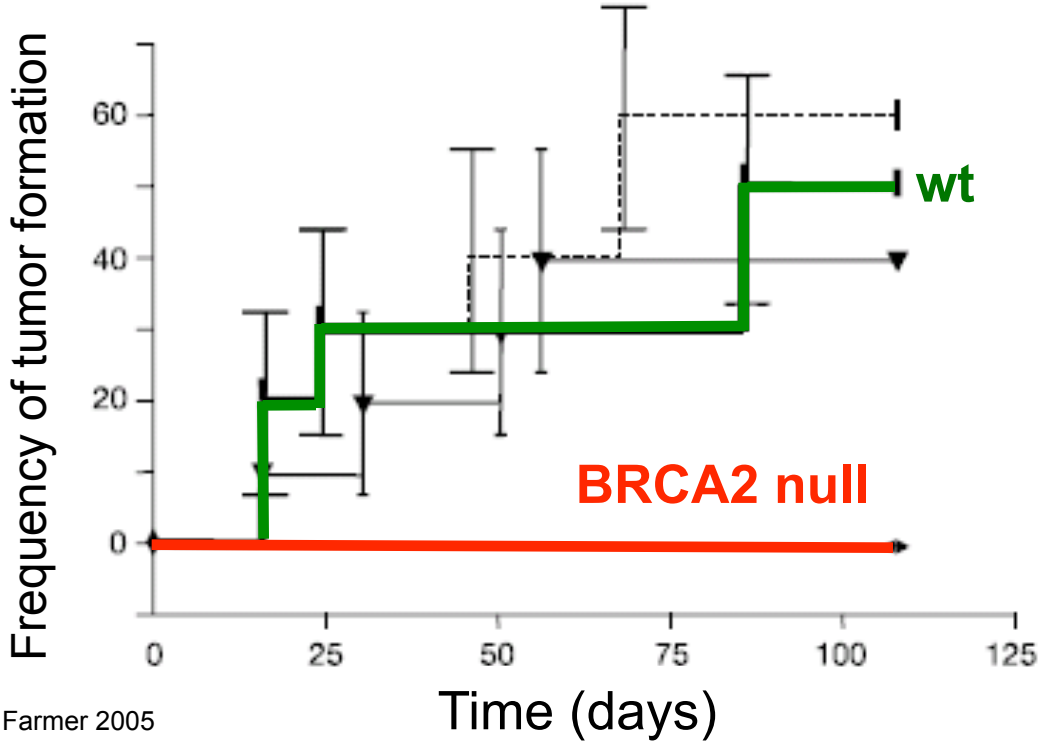


Bryant 2005

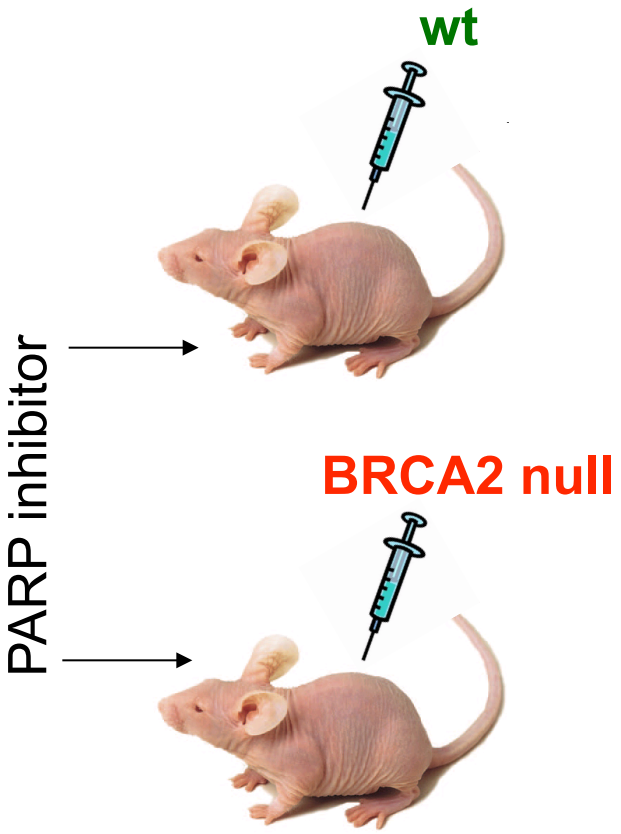


BRCA2 null cells are extremely sensitive to PARP inhibitors

In mice



Farmer 2005

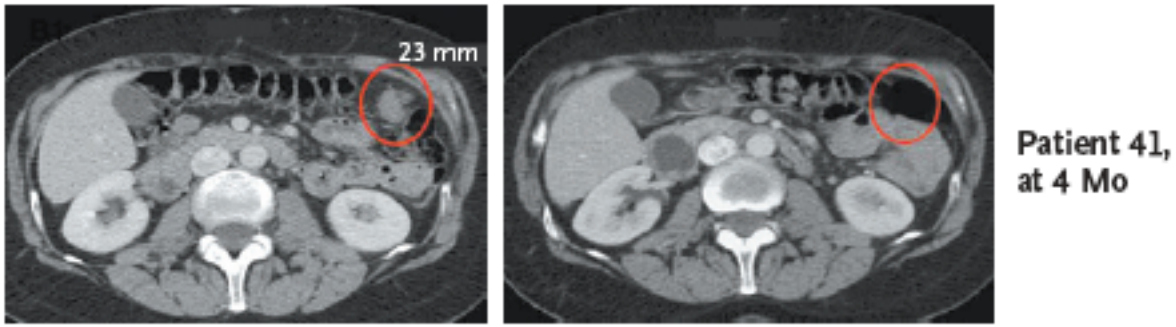


TARGETED to tumor by virtue of the tumor's own mutation!

Inhibition of Poly(ADP-Ribose) Polymerase in Tumors from *BRCA* Mutation Carriers

Peter C. Fong, M.D., David S. Boss, M.Sc., Timothy A. Yap, M.D., Andrew Tutt, M.D., Ph.D., Peijun Wu, Ph.D., Marja Mergui-Roelvink, M.D., Peter Mortimer, Ph.D., Helen Swaisland, B.Sc., Alan Lau, Ph.D., Mark J. O'Connor, Ph.D., Alan Ashworth, Ph.D., James Carmichael, M.D., Stan B. Kaye, M.D., Jan H.M. Schellens, M.D., Ph.D., and Johann S. de Bono, M.D., Ph.D.

advanced ovarian cancer

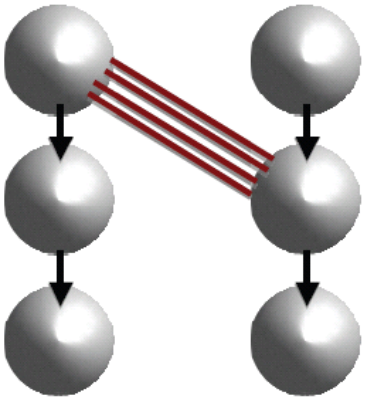


Patient 41, at Baseline

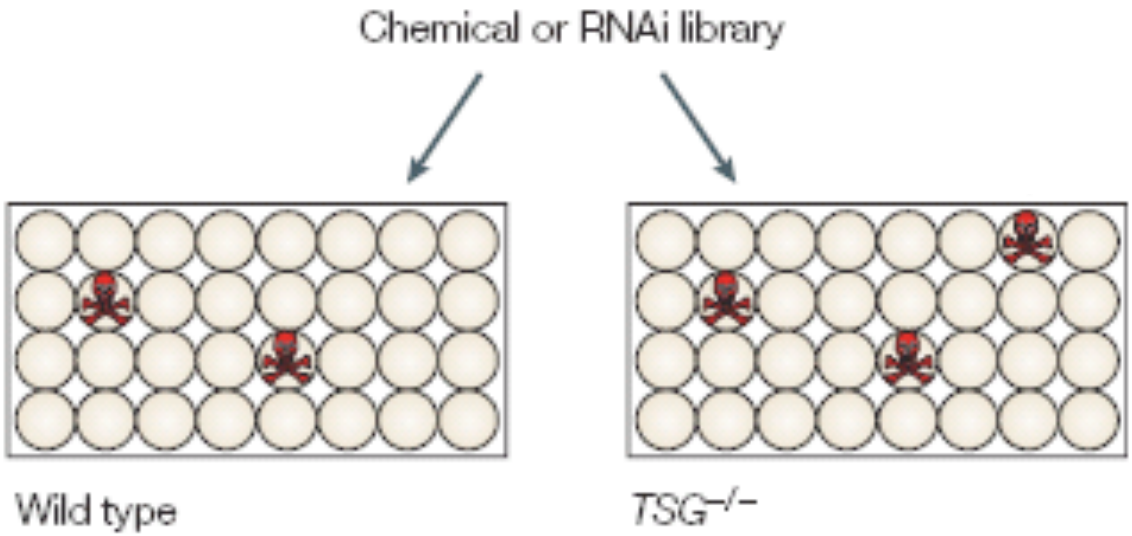
Patient 41, at 4 Mo

Subgroup and Dose	Total No. of Patients	Partial or Complete Radiologic Response
All patients	60	9
Patients with *BRCA1* or *BRCA2* ovarian, breast, or prostate cancer‡	19	9 (8 with ovarian cancer, 1 with breast cancer)

Synthetic lethality and an assay to find it



Each decrease expression of a single gene



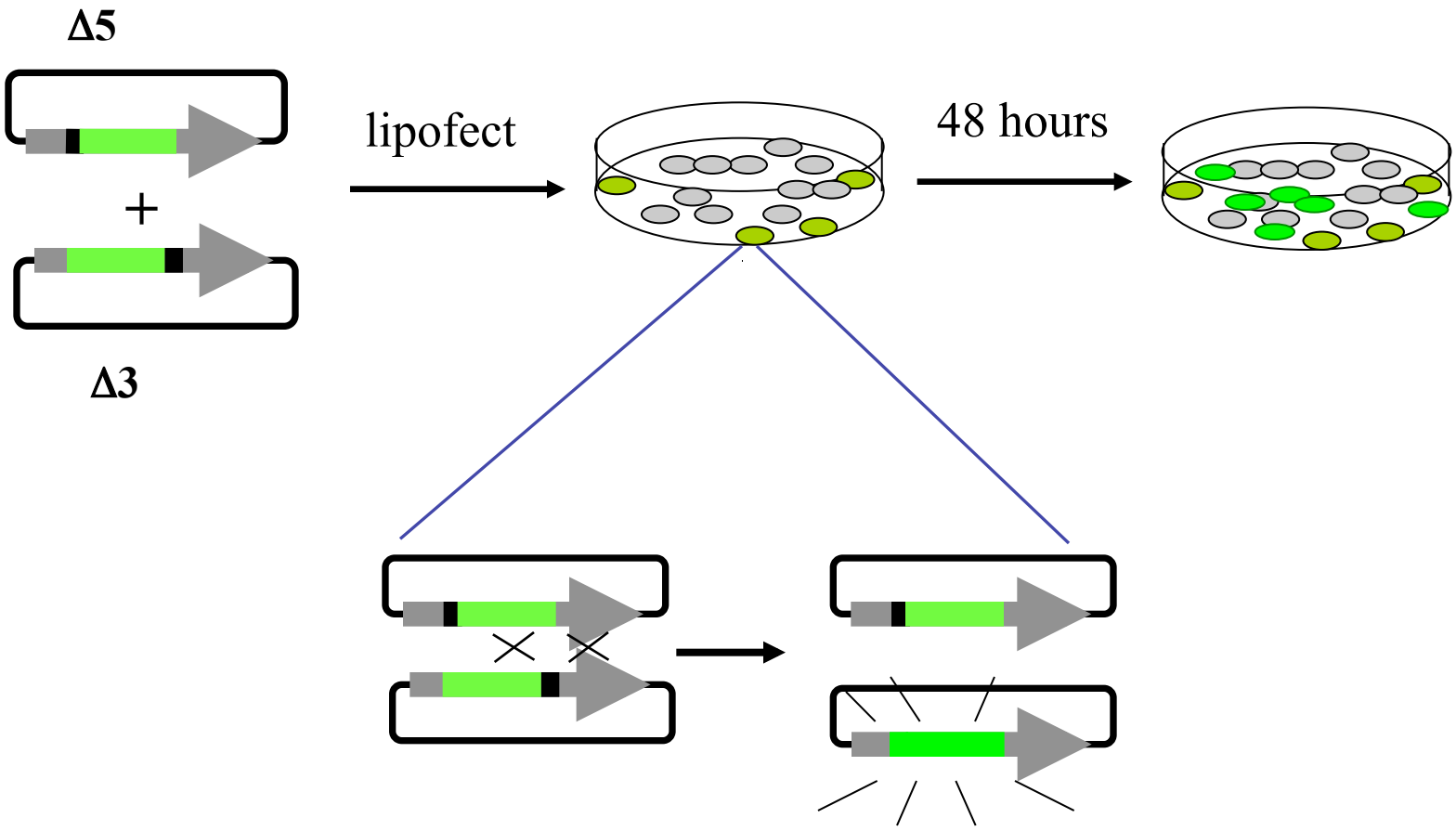
Lecture 1:	**Intro to importance of HR**
Lecture 2:	**How HR works**
Lecture 3:	**Why understanding matters: BRCA2 and HR**
Lecture 4:	**Exploiting scientific understanding for engineering: BRCA2 targeted therapies**
Lecture 5:	**Measuring HR in genotoxicity testing, using HR in genome engineering of mice**
Lecture 6:	**Journal article discussion**
Lecture 7:	**Statistics**
Lecture 8:	**Flow Cytometry: How it works and how to do it**

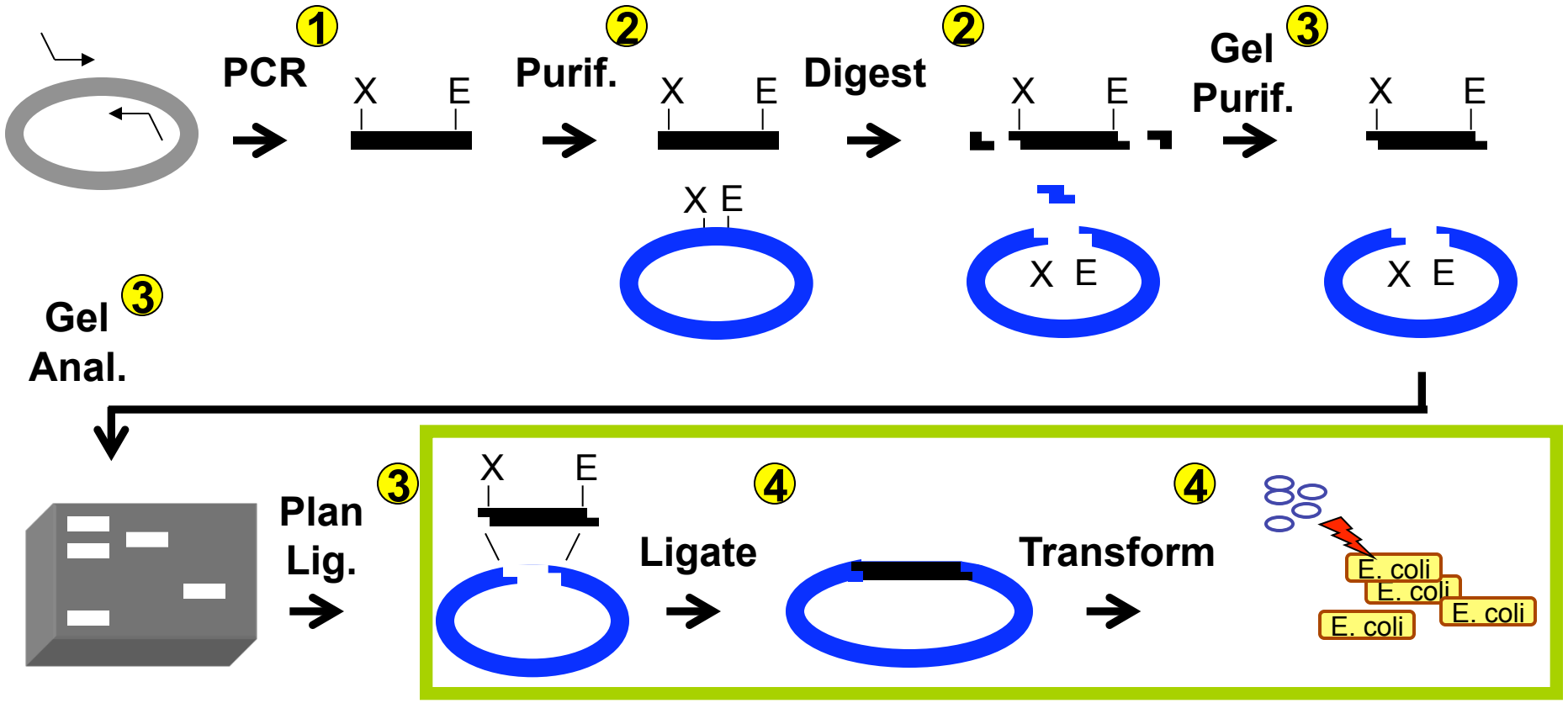
Mod 1: DNA Engineering

Engineering in vitro recombination assay

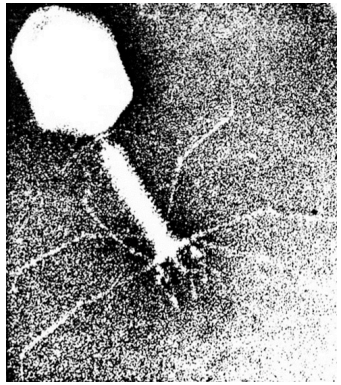
Day 4

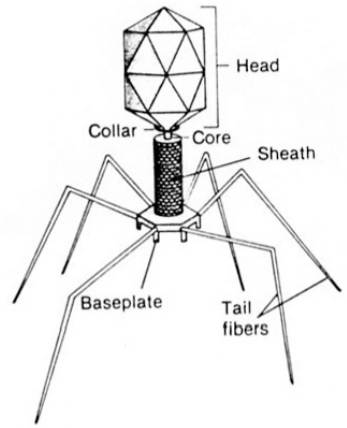
A Plasmid-Based Assay for Homologous Recombination in Mammalian Cells



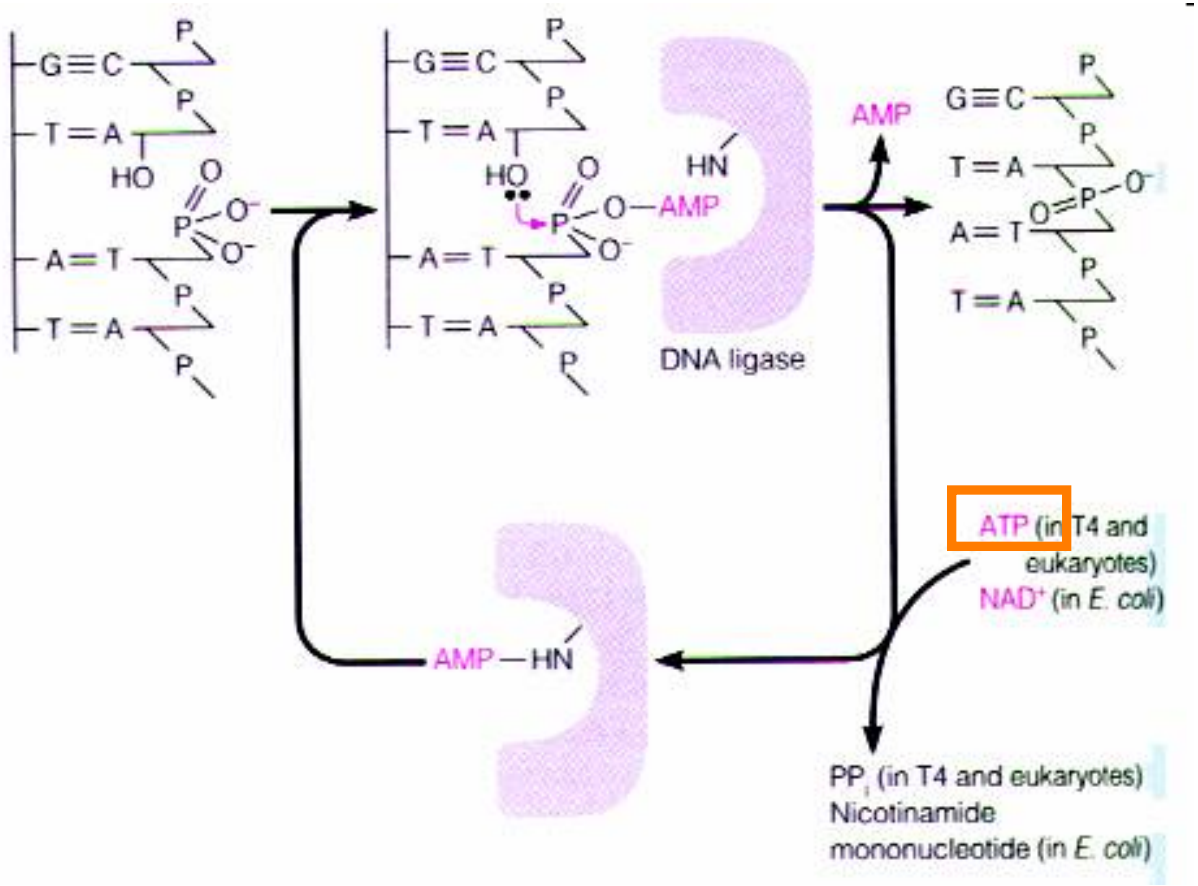

1
PCR
X
E
2
Purif.
X
E
2
Digest
X
E
3
Gel
Purif.
X
E
X E
X E
X E
3
Gel
Anal.
Plan
Lig.
3
X
E
4
Ligate
4
Transform
E. coli
E. coli
E. coli
E. coli

Ligase from T4 bacteriophage is used in recombinant DNA technology





textbookofbacteriology.net



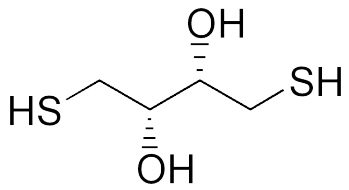
http://www.biochem.umd.edu

Your ligation reaction

- **Ligase**
- **ATP**
- **Buffer**

Water, salts, buffer system

DTT



Ligase has NO activity w/o DTT
DTT is unstable
Make single use aliquots
Vortex to suspend
SMELL

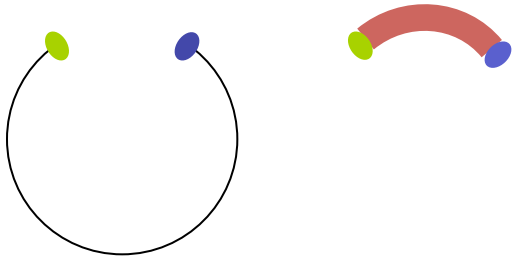
- **Vector**
- **Insert**

How much?
In what ratio?

Your ligation reaction

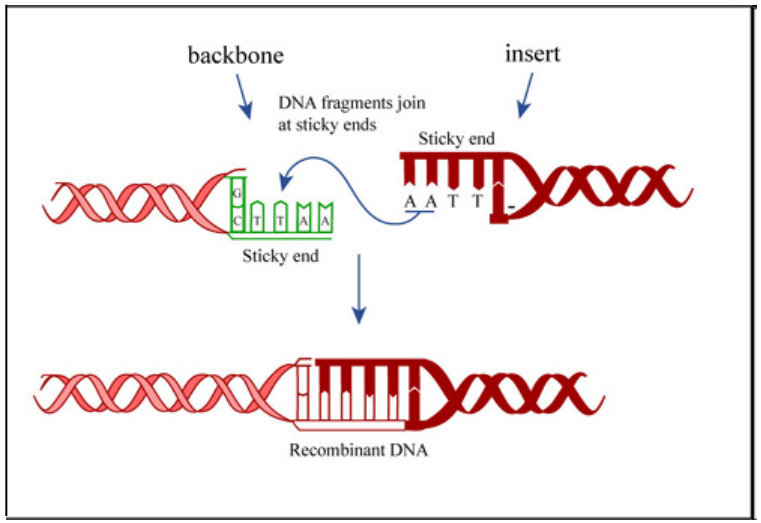
- **Vector**
- **Insert**

How much?
In what ratio?

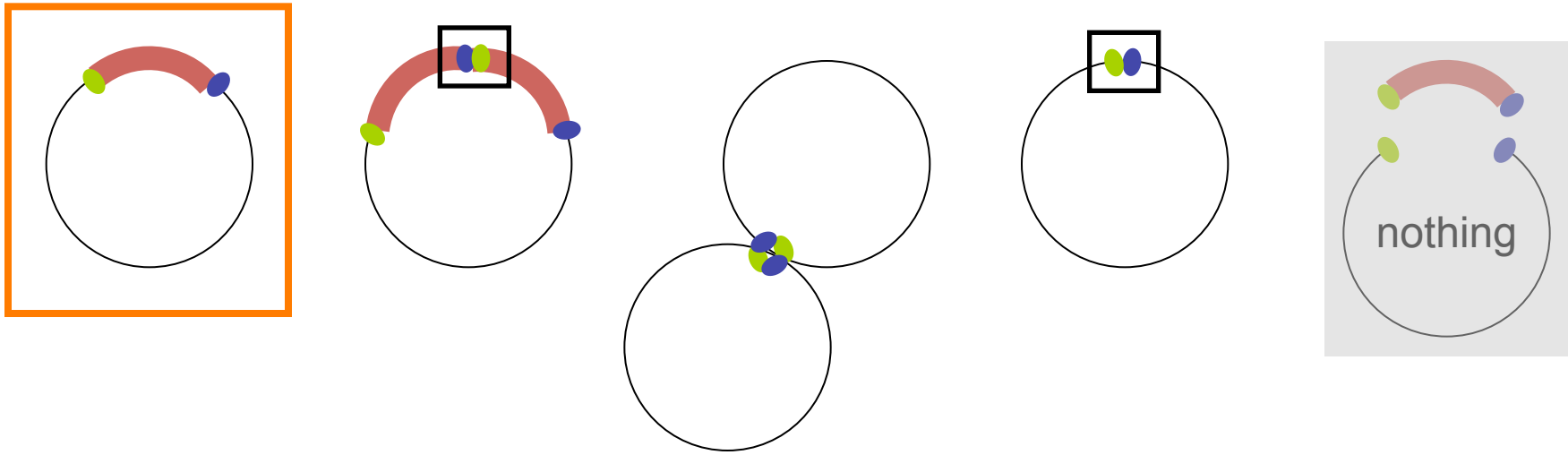


Reaction conditions

Optimal temperature
for T4 ligase is 25°C



Your ligation reaction: possible outcomes



Population of different products

Need to separate and amplify individual products to analyze and select correct one

Transforming bacteria with ligation reaction

MIX

SHOCK

PLASMID TAKEN UP

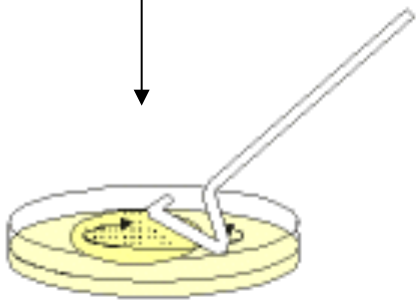
electric (10-20 kV/cm)

temperature (42°C → ice)

RECOVER

grow in rich media

SEPARATE
SELECT
AMPLIFY



separate by spreading

Proceed to grow up individual colonies and analyze ligation products

SEPARATE
SELECT
AMPLIFY

GROW UP
INDIVIDUAL
COLONIES

ISOLATE
PLASMID DNA

ANALYZE