MOD1 – DNA ENGINEERING

Engelward, Fall 2009

Day 3

Why HR Matters to Normal People: BRCA2 & Cancer

- -Cloning the gene
- -Assigning function
- -Why carriers get cancer
- -Next Time: Using scientific knowledge for engineering

Agarose Gels & Ligation Preparation

- -How do we 'look' at DNA?
- -Gel purification

Mod1 Overview: Methods and Logic

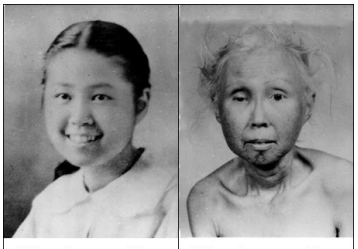
- -Getting the right molar ratio in your reaction

Background & Significance:

"Homology-Directed Repair"
for double strand breaks

Why you owe Your Youthfulness to Homologous Recombination…

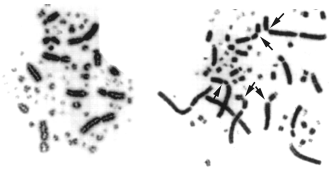
Loss of Helicase → Faulty Recomb.



WS patient age 15 yrs | WS patient age 48 yrs

Werner's Syndrome

Why you owe Your Life to Homologous Recombination…

Normal  Rad51-/-

Turn Off Homologous Recombination
→ Chromosomes Fall Apart

Sonada *et al.*, *EMBO J.* **17,** 598–608 (1998).

Why you owe Your Health to Homologous Recombination…

Cloning BRCA2

Step 1: Define the Disease

- 1/9 women get breast cancer
- 'sporadic' vs 'familial'
- Question: might there be shared genetic traits among those with *early-onset-disease*

Cloning BRCA2

Criteria for Identify Potential Carriers

Early-onset breast cancer (before menopause) in several relatives over different generations;

Relatives who have breast cancer in both breasts;

Male relatives with breast cancer.

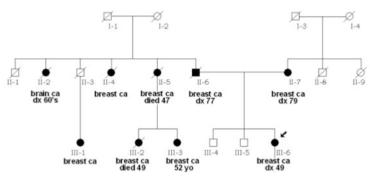
Cloning BRCA2

Step 2: Create Pedigrees

• Identify families with multiple cases of early onset breast cancer

• Create a record of the family history of disease

• Obtain DNA samples



Cloning BRCA2

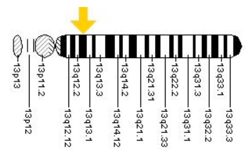
Step 3: Identify a Chromosomal Locus Associated with Disease

• Analyze polymorphisms among family members

• Find chromosome regions that cosegregate with disease

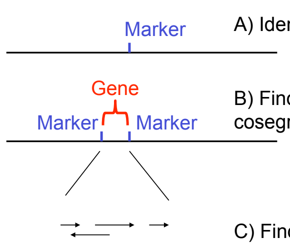
• Look for commonality among pedigrees

• Identify a shared locus that cosegregates with disease among multiple families



Cloning BRCA2

Step 4: Identify the Disease-Causing Gene



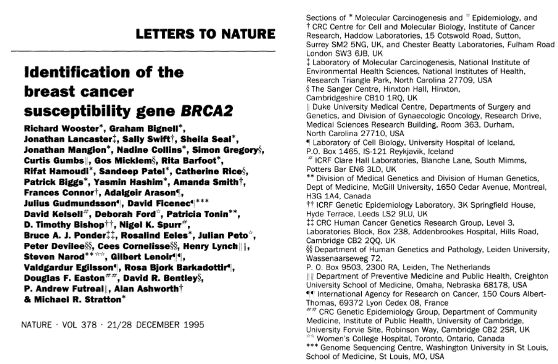
A) Identify first linkage

B) Find an additional cosegregating marker

C) Find ORFs in this region

D) Sequence ORFs of patients – look for mutations in multiple families

Cloning BRCA2



LETTERS TO NATURE

Identification of the breast cancer susceptibility gene *BRCA2*

Richard Wooster*, Graham Bignell*, Jonathan Lancaster‡, Sally Swift†, Sheila Seal*, Jonathan Mangion*, Nadine Collins*, Simon Gregory§, Curtis Gumbs‖, Gos Micklem§, Rita Barfoot*, Rifat Hamoudi*, Sandeep Patel*, Catherine Rice§, Patrick Biggs*, Yasmin Hashim*, Amanda Smith†, Frances Connor†, Adalgeir Arason¶, Julius Gudmundsson¶, David Ficenec¶* David Kelsell#, Deborah Ford☆, Patricia Tonin**, D. Timothy Bishop††, Nigel K. Spurr#, Bruce A. J. Ponder‡‡, Rosalind Eeles*, Julian Peto☆, Peter Devilee§§, Cees Cornelisse§§, Henry Lynch‖‖, Steven Narod**☆☆, Gilbert Lenoir¶¶, Valdgardur Egilsson¶, Rosa Bjork Barkadottir¶, Douglas F. Easton##, David R. Bentley§, P. Andrew Futreal‖, Alan Ashworth† & Michael R. Stratton***

NATURE · VOL 378 · 21/28 DECEMBER 1995

Sections of * Molecular Carcinogenesis and ☆ Epidemiology, and † CRC Centre for Cell and Molecular Biology, Institute of Cancer Research, Haddow Laboratories, 15 Cotswold Road, Sutton, Surrey SM2 5NG, UK, and Chester Beatty Laboratories, Fulham Road London SW3 6JB, UK
‡ Laboratory of Molecular Carcinogenesis, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina 27709, USA
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¶ Laboratory of Cell Biology, University Hospital of Iceland, P.O. Box 1465, IS-121 Reykjavik, Iceland
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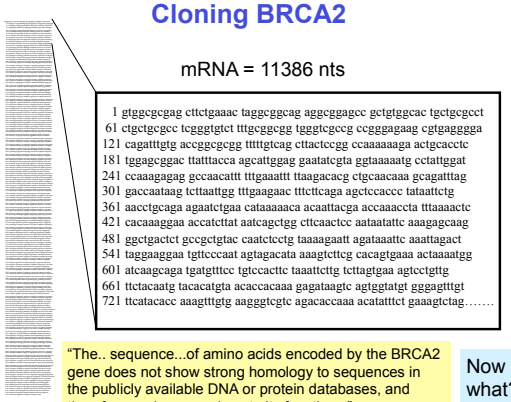
‖‖ Department of Preventive Medicine and Public Health, Creighton University School of Medicine, Omaha, Nebraska 68178, USA
¶¶ International Agency for Research on Cancer, 150 Cours Albert-Thomas, 69372 Lyon Cedex 08, France
CRC Genetic Epidemiology Group, Department of Community Medicine, Institute of Public Health, University of Cambridge, University Forvie Site, Robinson Way, Cambridge CB2 2SR, UK
☆☆ Women's College Hospital, Toronto, Ontario, Canada
*** Genome Sequencing Centre, Washington University in St Louis, School of Medicine, St Louis, MO, USA

Cloning BRCA2



mRNA = 11386 nts

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  61 ctgctgcgcc tcgggtgtct tttgcggcgg tgggtcgccg ccgggagaag cgtgagggga
 121 cagatttgtg accggcgcgg ttttgtcag cttactccgg ccaaaaaaga actgcacctc
 181 tggagcggac ttatttacca agcattggag gaatatcgta ggtaaaaatg cctattggat
 241 ccaaagagag gccaacattt tttgaaattt ttaagacacg ctgcaacaaa gcagatttag
 301 gaccaataag tcttaattgg tttgaagaac tttcttcaga agctccaccc tataattctg
 361 aacctgcaga agaatctgaa cataaaaaca acaattacga accaaaccta tttaaaactc
 421 cacaaaggaa accatcttat aatcagctgg cttcaactcc aataatattc aaagagcaag
 481 ggctgactct gccgctgtac caatctcctg taaaagaatt agataaattc aaattagact
 541 taggaaggaa tgttcccaat agtagacata aaagtcttcg cacagtgaaa actaaaatgg
 601 atcaagcaga tgatgtttcc tgtccacttc taaattcttg tcttagtgaa agtcctgttg
 661 ttctacaatg tacacatgta acaccacaaa gagataagtc agtggtatgt gggagtttgt
 721 ttcatacacc aaagtttgtg aagggtcgtc agacaccaaa acatatttct gaaagtctag.....
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"The.. sequence...of amino acids encoded by the BRCA2 gene does not show strong homology to sequences in the publicly available DNA or protein databases, and therefore we have no clues to its functions."

Now what??

Characterizing BRCA2

3418 amino acids; over 10 kb long

Now that you know the gene, how do you figure out what it is doing?

Ask the right questions…

When is it expressed in the cell cycle?

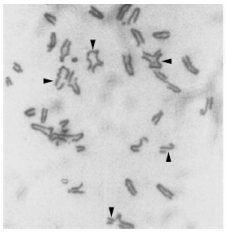
-expression is highest during early S phase

Who does it interact with?

-Rad51

What happens when you knock it out?

Characterizing BRCA2



Patel *et al.*, Mol. Cell, 1998

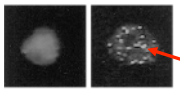
Spontaneous chromosome aberrations accumulate in BRCA2-/- cells

Characterizing BRCA2

BRCA2 mutant cells fail to form Rad51 foci in response to DNA damage

Cont. γIR

Normal BRCA2

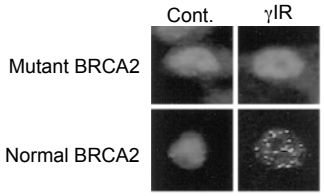


Yuan et al., Cancer Res. 1999

Rad51 Clusters form at DSBs

Characterizing BRCA2

BRCA2 mutant cells fail to form Rad51 foci in response to DNA damage

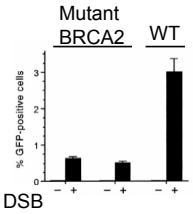


Yuan et al., Cancer Res. 1999

Characterizing BRCA2

BRCA2 mutant cells have reduced levels of damage-induced homologous recombination

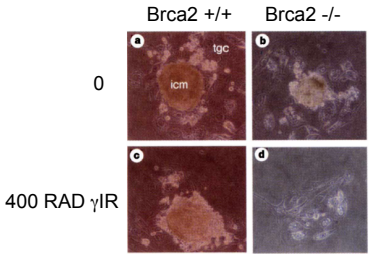
The Jasin Lab uses assays just like yours!



Moynahan et a.., Mol. Cell 2001

Characterizing BRCA2

BRCA2 mutant cells are highly sensitive to γIR

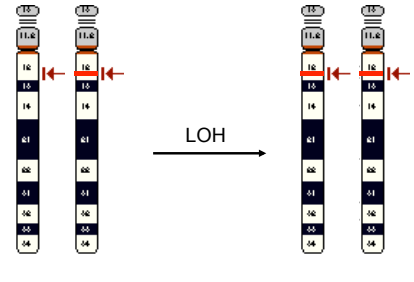


S. K. Sharan *et al.*, Nature 1997

Characterizing BRCA2

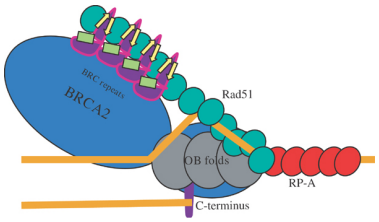
Most Cells in a BRCA2 Carrier

Breast Cancer Cells

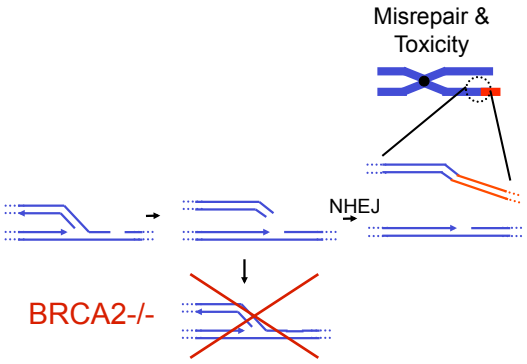


WT **MUT** **MUT** **MUT**

BRCA2 Loads Rad51 to create a nucleoprotein filament



BRCA2 is critical for repair of broken forks

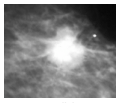


Misrepair

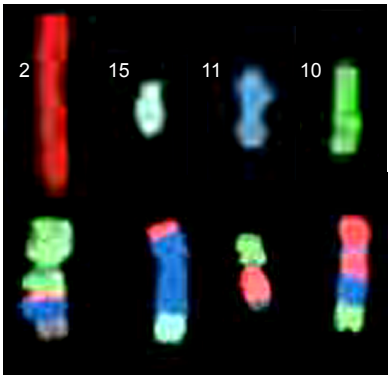
Normal Human Chromosomes

↓

BRCA2 -/- Chromosomes



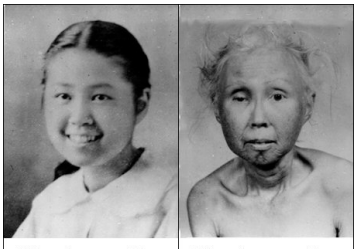
www.rctradiology.com



Raw data from Grigorova *et al.*, Cytogen. and Gen. Res. 104:333-340 (2004)

Why you owe Your Youthfulness to Homologous Recombination…

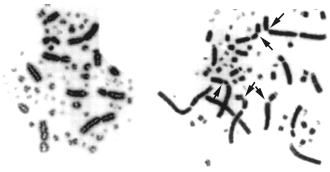
Loss of Helicase → Faulty Recomb.



WS patient age 15 yrs | WS patient age 48 yrs

Werner's Syndrome

Why you owe Your Life to Homologous Recombination…

Normal  Rad51-/-

Turn Off Homologous Recombination
→ Chromosomes Fall Apart

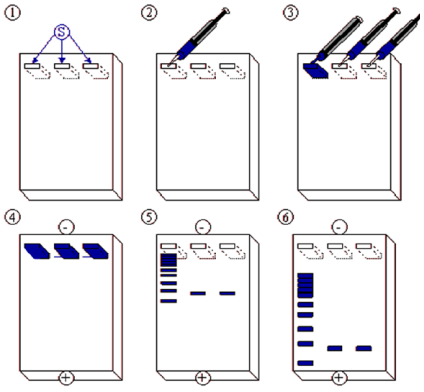
Sonada *et al.*, *EMBO J.* **17,** 598–608 (1998).

Why you owe Your Health to Homologous Recombination…

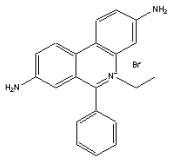
Defective Homologous Recombination
→ Cancer

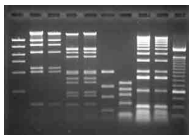
Agarose Gels & Gel Purification

- How do we 'look' at DNA?
- How do we get our DNA out of a gel?

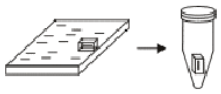


How can you see your DNA?



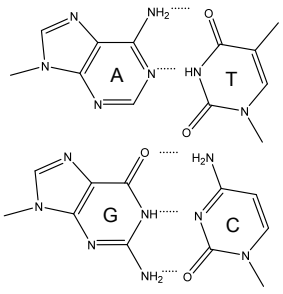


Gel Purification



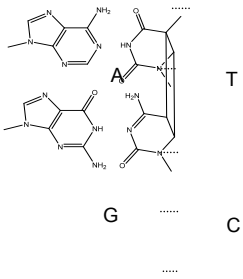
Why do you need to cut out your band fairly quickly?

Sunlight Damages DNA



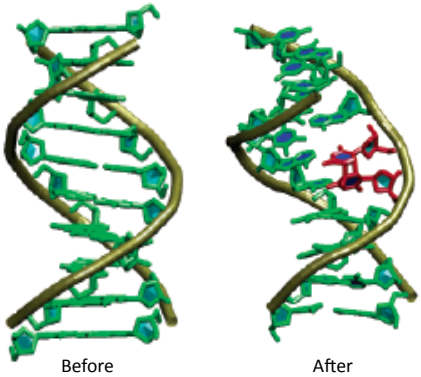
Normal Base Pairs

Sunlight Damages DNA



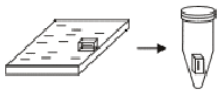
Sunlight-Induces Crosslinks Between Bases

Sunlight Damages DNA



Before After

Gel Purification

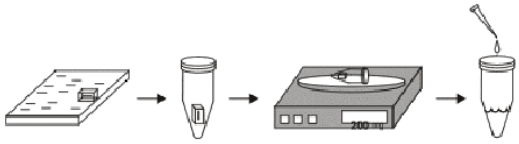


Why do you need to cut out your band fairly quickly?

You will need to dissolve the gel to get the DNA out.. You do this by adding 3 volumes of a gel-dissolving solution.

What does it mean to 'add 3 volumes'?

How can you estimate the volume of your gel slice?



Agarose Gels – How do we 'look' at DNA?

-Loading

-Standards

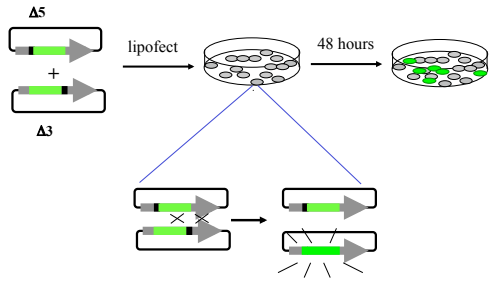
-Parameters that affect migration

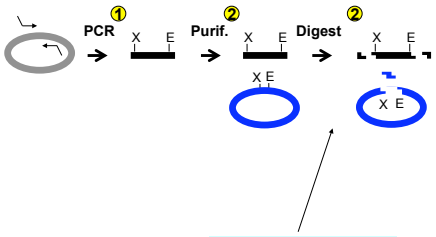
-gel concentration
-length of DNA
-tertiary structure
-effects of overloading

Overview of the Experiments in Mod1

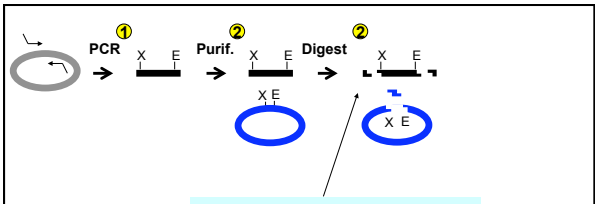
Where you are,
and where you are going

A Plasmid-Based Assay for Homologous Recombination in Mammalian Cells



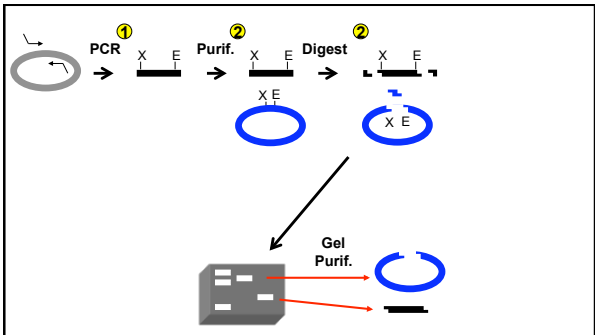


How do you know that your restriction enzymes actually cut the DNA?

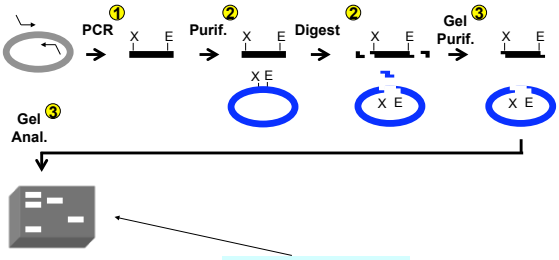


What else is in the reaction with the digested PCR product?

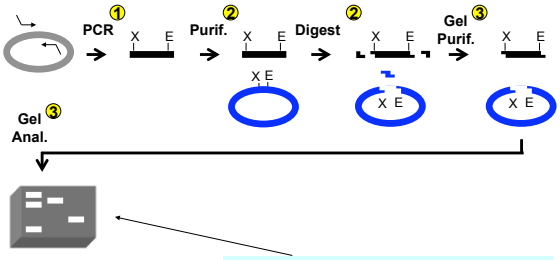
What effect could it have?



Why is it important to excise the DNA from the gel relatively quickly?

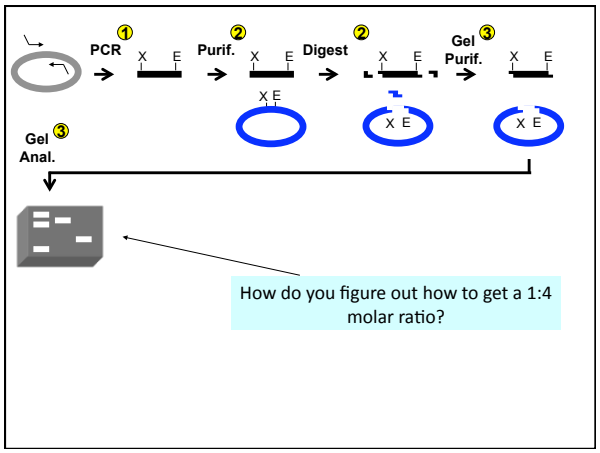


Why run this gel?



Your objective is a 1:4 vector:insert ratio – Why?

What if it was 1:100?
What if it was 100:1?



Why HR Matters to Normal People: BRCA2 & Cancer

- -Cloning the gene
- -Assigning function
- -Why carriers get cancer
- -Next Time: Using scientific knowledge for engineering

Agarose Gels & Ligation Preparation

- -How do we 'look' at DNA?
- -Gel purification

Mod1 Overview: Methods and Logic

- -Getting the right molar ratio in your reaction