

MOD1 – DNA ENGINEERING

Engelward, Spring 2009

Day 4

Going from Understanding to Solutions

- Exploiting Understanding for Drug Design
- Engineered Approaches for Drug Delivery

DNA Ligase

Mod1 Overview: Methods and Logic

- Logic for steps so far
- Your data
- Looking ahead...

Background & Significance:

“Homology-Directed Repair”
for double strand breaks

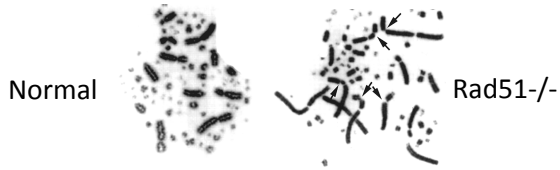
Why you owe Your Youthfulness to Homologous Recombination...

Loss of Helicase → Faulty Recomb.



Werner's
Syndrome

Why you owe Your Life to Homologous Recombination...



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

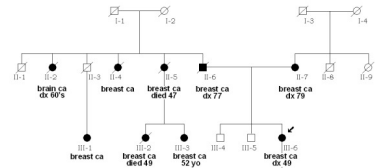
Why you owe better Cancer Treatments to HR...



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

mRNA = 11386 nts

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1 gggacgcg cttcgaac taggcggc aggcggcc gctctgcac tctctgect
61 ctctgcac tgggtgct ttggcgcg tggcgcgc cgggagag cgtgaggga
121 cagatttg accggcgg ttgtgcng cttaactcgg ccaaaaaa actgcacctc
181 tggagggac ttattacca agctttgng gaatctgta ggtaaaag cctattgat
241 ccaagagag gccacaatt ttgaattt ttaagacag ctgcacaa gcagattag
301 gaccatag tcttaattg ttgaagaac ttctcaga agctccacc tataattg
361 aacctcaga agactcga cataaaaa acattacga accaaacta ttanaact
421 cacaaggaa accacttat aatcagctg ctcaactc ataatatt aaagcaag
481 gctgactc gcccgctac caatcctg taagaatt agataatt aatfagaact
541 tagaaggaa tgttccca atgacala aaagtctg cacagtaa actaaaatg
601 atcaagcag tgatgttc tgcacact taattctg tctagtga agtctcttg
661 ttacaatg tacatgta acacacaa gagataag agtctatg ggaattgt
721 ttacacac aaatttgg aaggctctc agacacaaa acatattct 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."

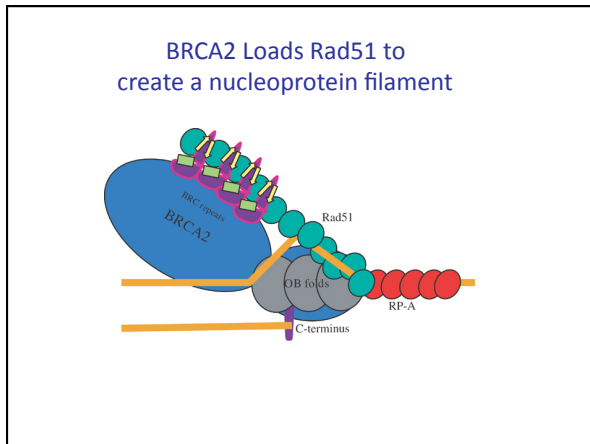
Now what??

Characterizing BRCA2

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

	Cont.	γIR
Mutant BRCA2		
Normal BRCA2		

Yuan et al., Cancer Res. 1999



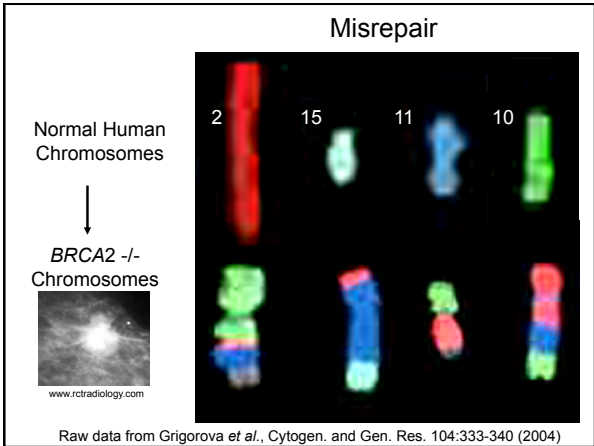
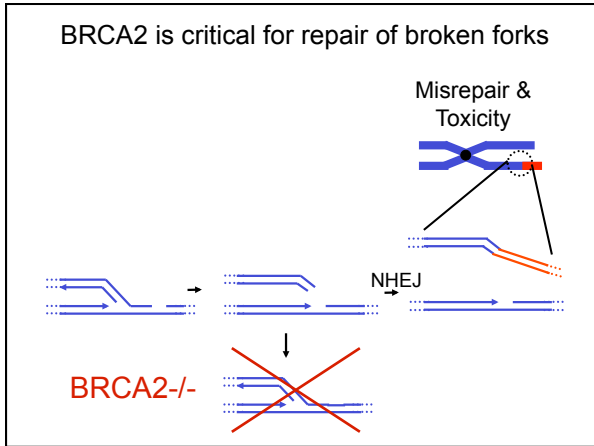
Characterizing BRCA2

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

The Jasin Lab uses assays just like yours!

DSB	Mutant BRCA2		WT	
	-	+	-	+
% GFP-positive cells	~0.8	~0.6	~0.8	~3.0

Moynahan et al., Mol. Cell 2001

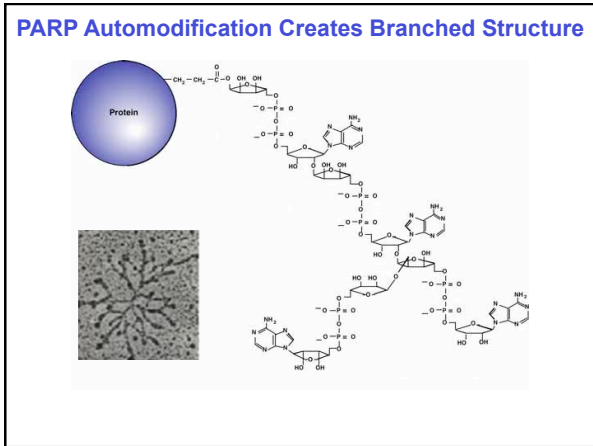
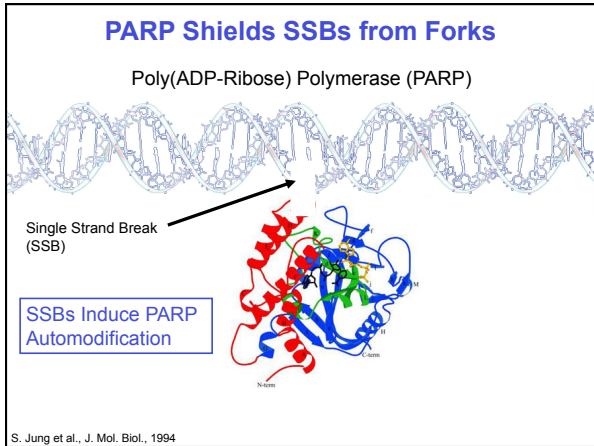
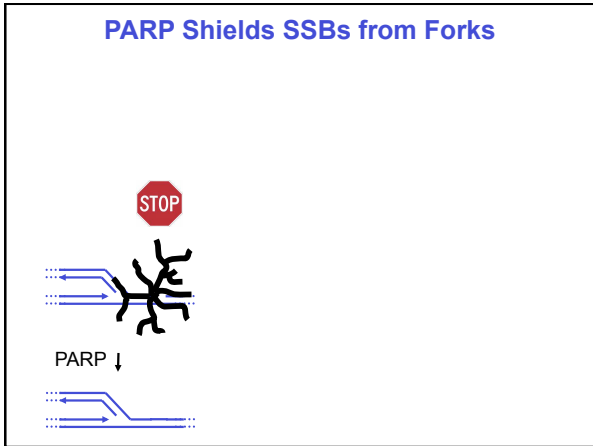
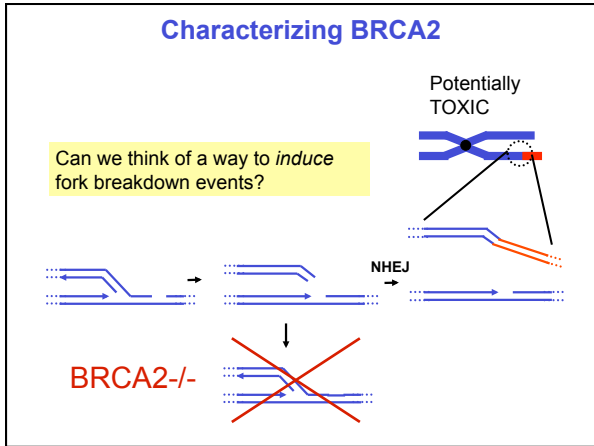


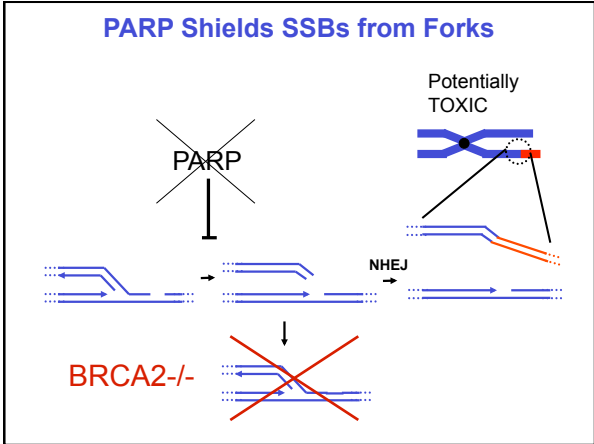
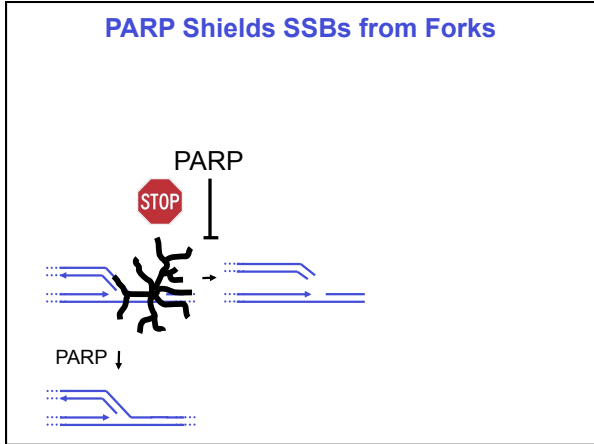
**Rational
Cancer
Chemotherapy**

How can we use our understanding to come up with better cancer treatments?

Science → Engineering Opportunities

What is the Achilles heel of a BRCA2 null tumor cell???





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-Roekink, 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.

Patient 41, at Baseline

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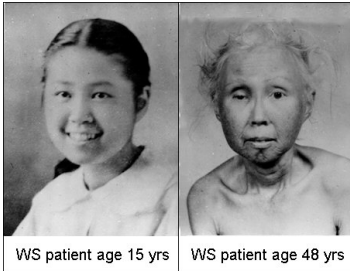
Patient 41, at Baseline

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	19	9 (8 with ovarian cancer, 1 with breast cancer)

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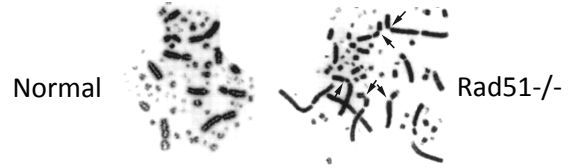


Werner's Syndrome

WS patient age 15 yrs

WS patient age 48 yrs

Why you owe Your Life to Homologous Recombination...



Turn Off Homologous Recombination
→ Chromosomes Fall Apart

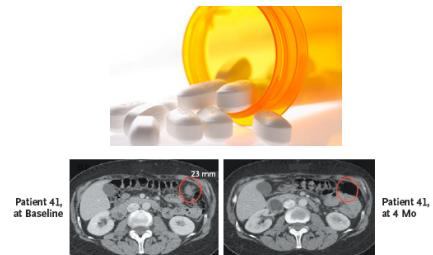
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Why you owe Your Health to Homologous Recombination...



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→ Cancer

Why you owe better Cancer Treatments to HR...



Understanding → Engineered Solutions

Nanocell for Drug Delivery



LETTERS

Temporal targeting of tumour cells and neovasculature with a nanoscale delivery system

Shih-Hya Sengupta¹*, David Evanson²*, Ishan Gupta³, Gemin Zhou¹, Nicki Watson², Tanyel Kollipati² & Ram Sureshkumar¹

CERULEAN
Pharma Inc.
Pioneering the Field of Nanobiology

Back to 109....

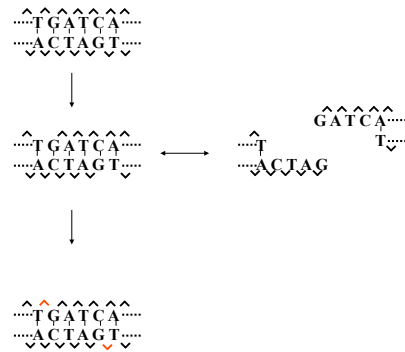
Our major goals are:

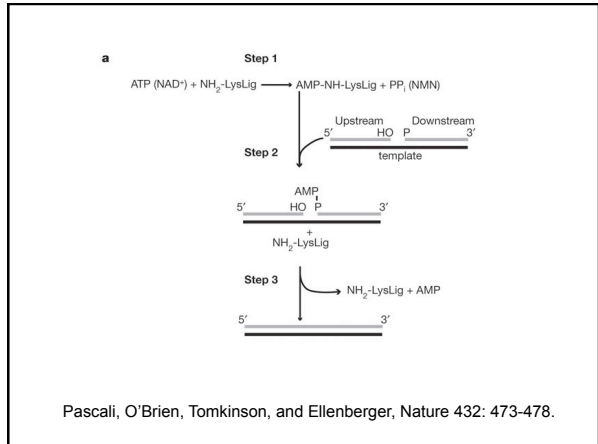
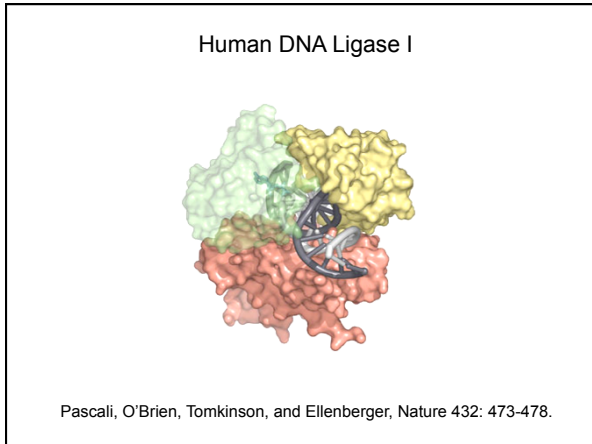
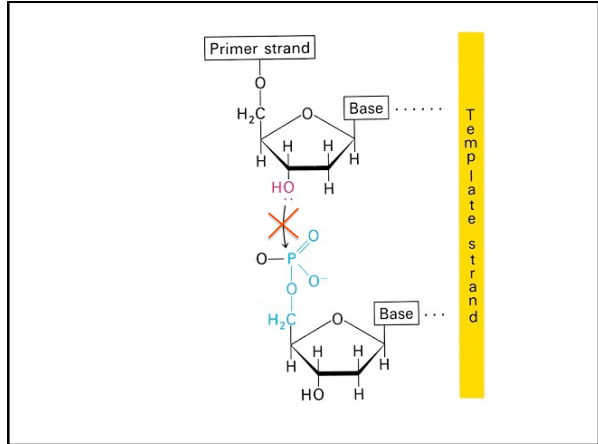
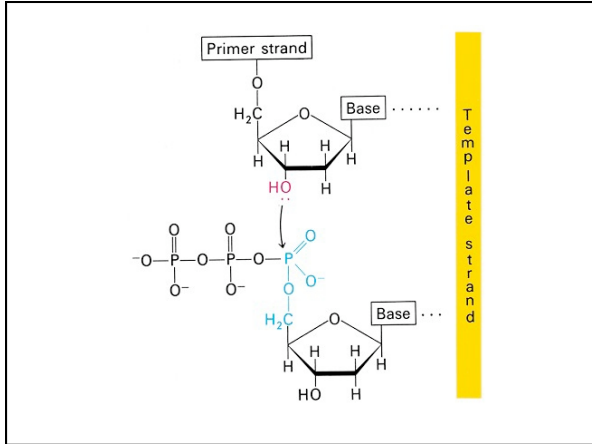
To teach strong fundamentals
in laboratory science

&

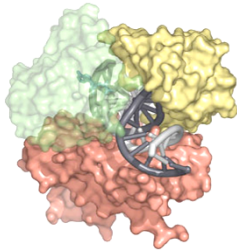
To inspire

DNA Ligase





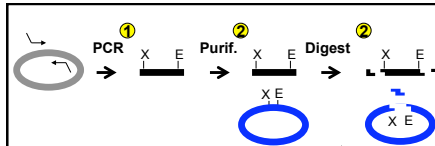
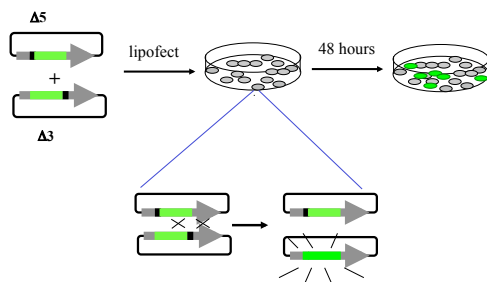
Human DNA Ligase I



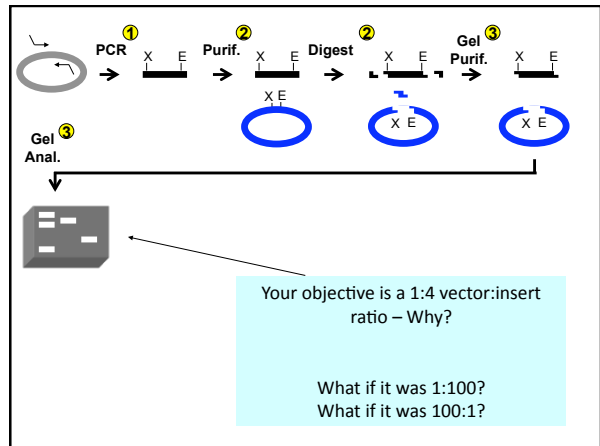
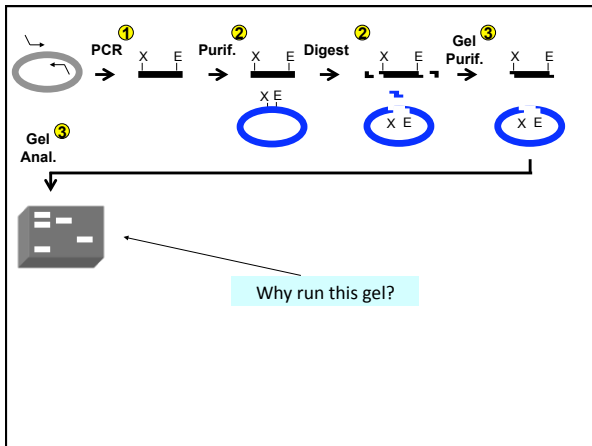
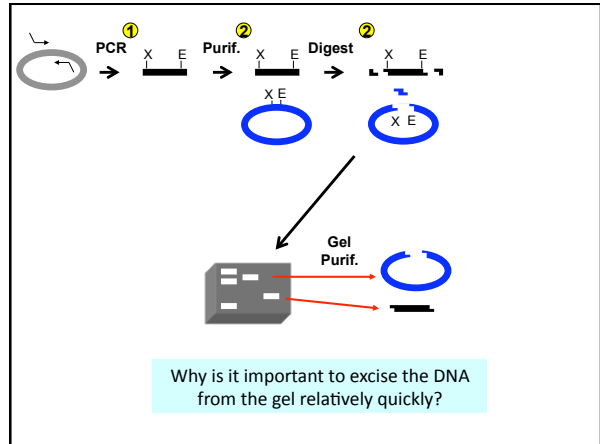
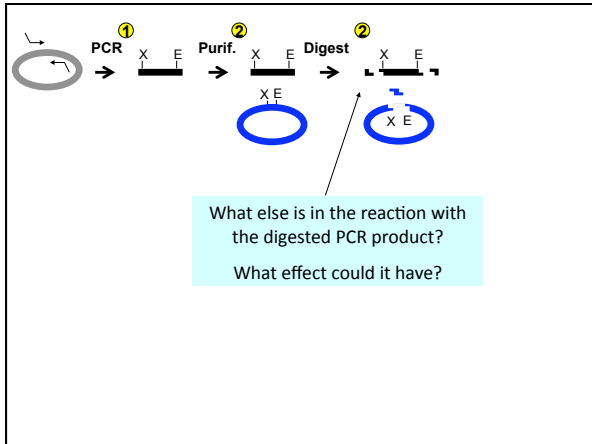
Pascali, O'Brien, Tomkinson, and Ellenberger, Nature 432: 473-478.

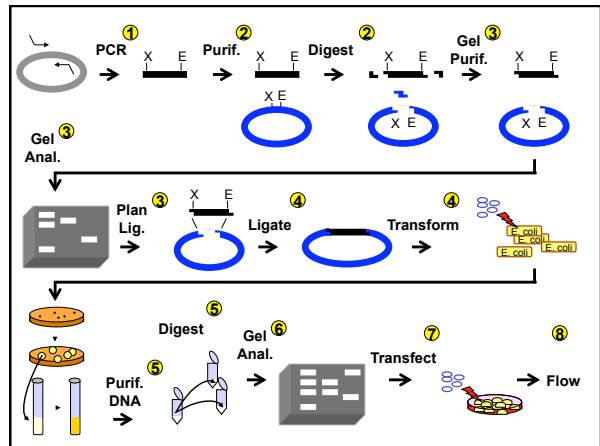
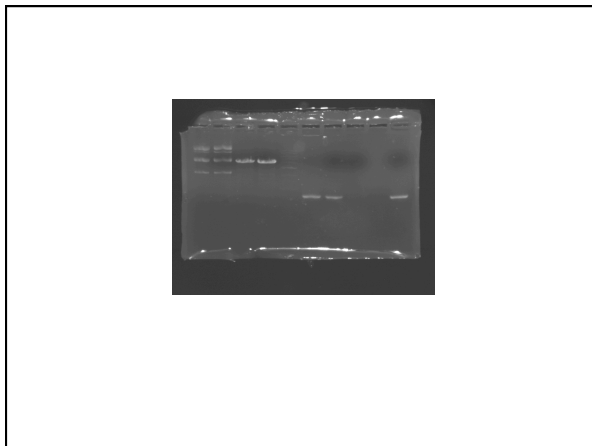
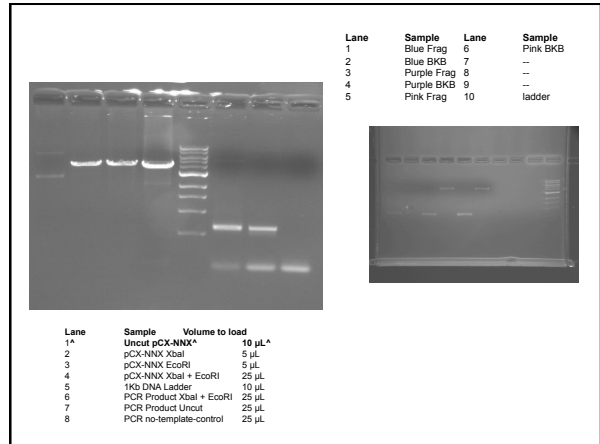
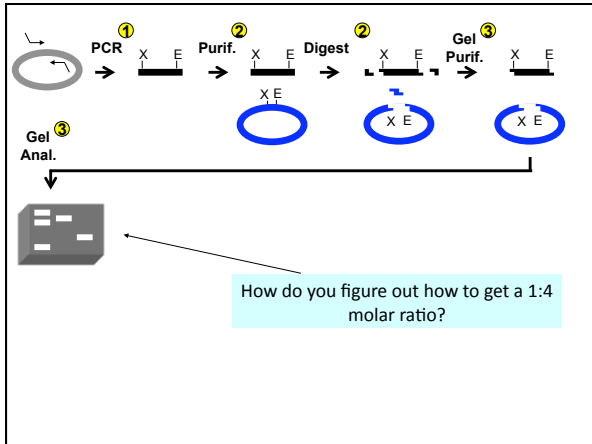
Methods & Logic For Mod1

A Plasmid-Based Assay for Homologous Recombination in Mammalian Cells



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





Going from Understanding to Solutions

- Exploiting Understanding for Drug Design
- Engineered Approaches for Drug Delivery

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