MOD1 – DNA ENGINEERING

Engelward, Fall 09

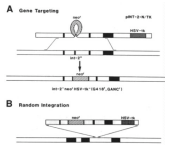
Day 8

Nobel Prizes

Module in Review: Experimental Approaches & Biological Concepts

Flow Cytometry

MARIO'S TRANSGENIC TECHNOLOGY
"Knocks Out"
Nobel Prize



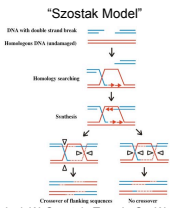
Mario Capecci, Nobel Prize 2007

2009

Szostak

Blackburn (Greider)

Telomerase



Jack W. Szostak, Terry L. Orr-Weaver,
Rodney J. Rothstein and Franklin W. Stahl

Terry Orr Weaver (MIT!)

2009

Photo: MRC Laboratory of Molecular Biology

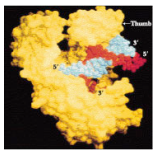
Venkatraman Ramakrishnan

Credits: Michael Marsland/Yale University

Thomas A. Steitz

Credits: Micheline Pelletier/Corbis

Ada E. Yonath



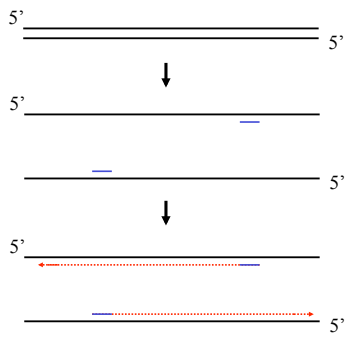
Rib-X PHARMACEUTICALS

Science ➔ Engineered Solutions

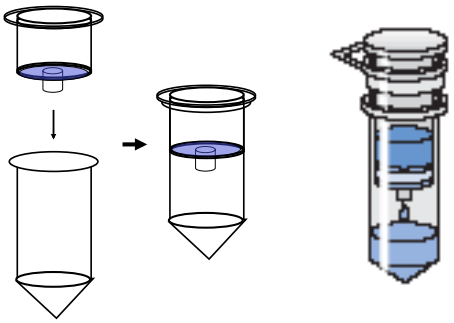
Module in Review

Experimental Approaches

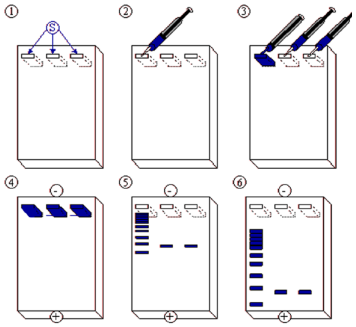
PCR



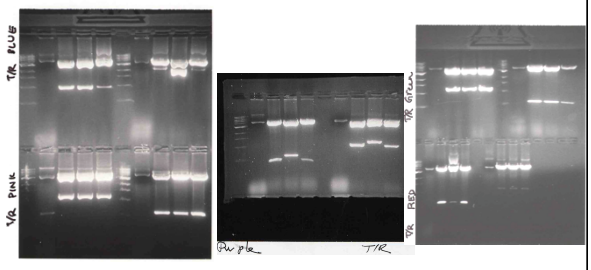
DNA Vectors & Purification



Gel Electrophoresis



Restriction Mapping & Data Analysis



DNA Engineering: Ligation

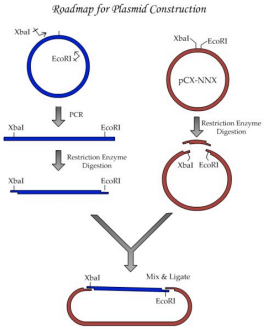
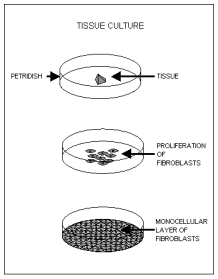
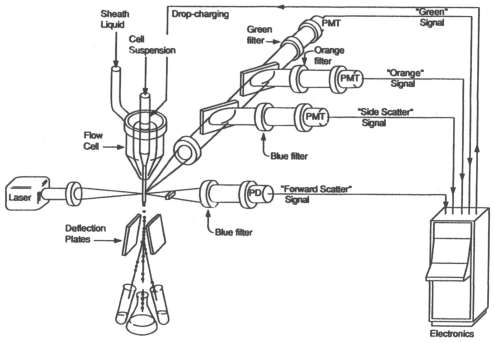


Figure by Justin Lo

Mammalian Cell Culture



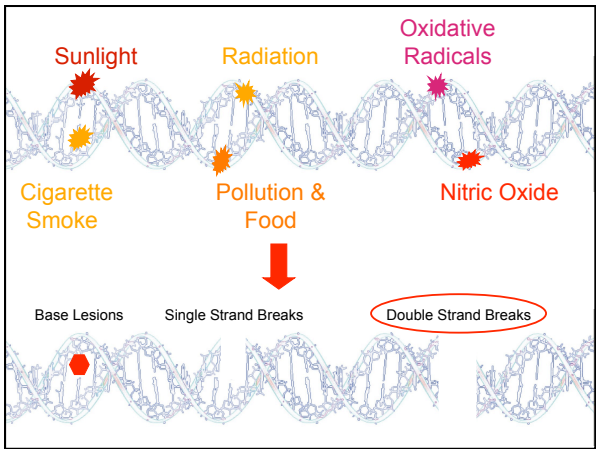
Flow Cytometry

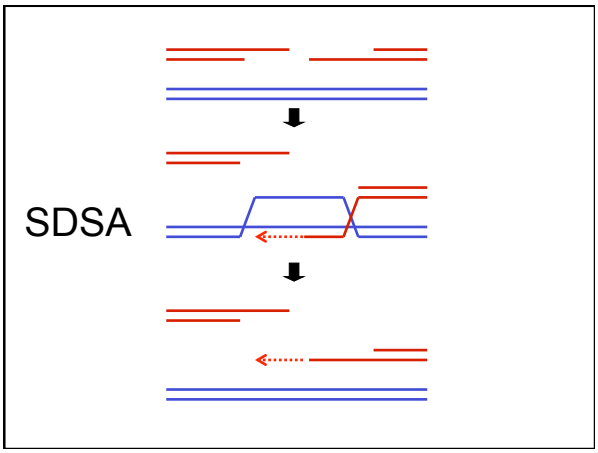


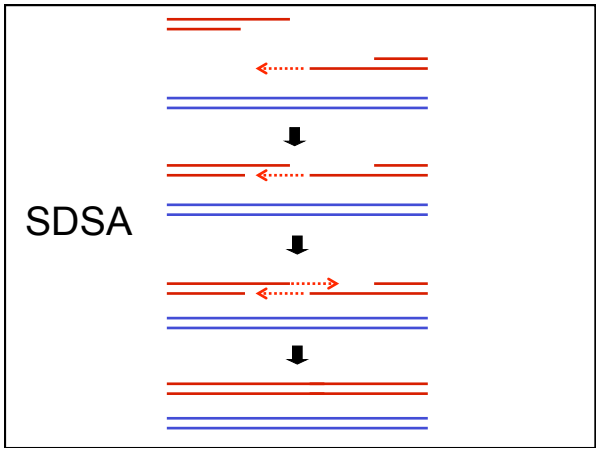
Flow Cytometry: First Principles, Alice L. Givan, 199

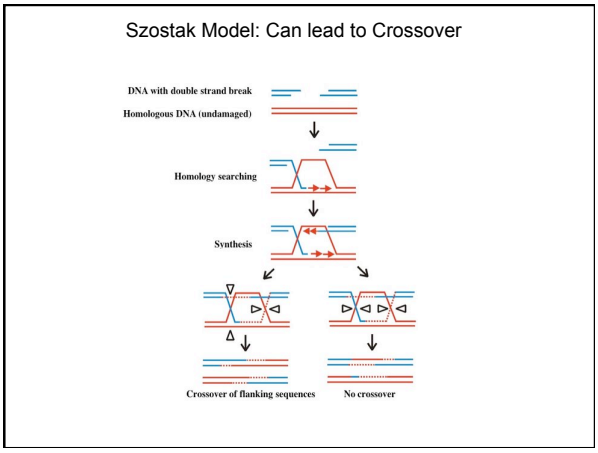
Biological Principles

DNA Damage & Repair via Homologous Recombination


Sunlight
Radiation
Oxidative Radicals
Cigarette Smoke
Pollution & Food
Nitric Oxide
Base Lesions
Single Strand Breaks
Double Strand Breaks

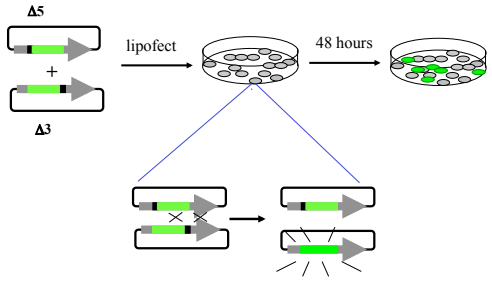

SDSA


SDSA


Szostak Model: Can lead to Crossover
DNA with double strand break
Homologous DNA (undamaged)
Homology searching
Synthesis
Crossover of flanking sequences
No crossover

Engineering an Assay for Homologous Recombination

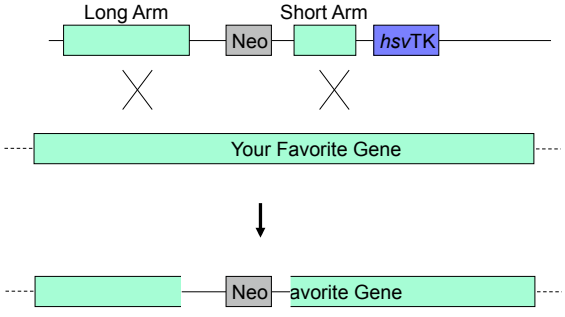
A Plasmid-Based Assay for Homologous Recombination in Mammalian Cells



Exploiting Homologous Recombination for Gene Targeting

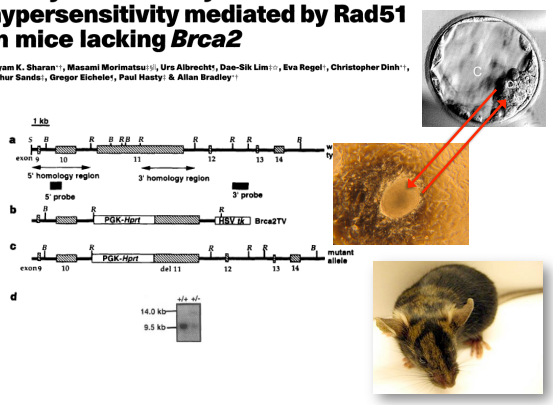
Traditional ES Knock-Out Technology

Targeted Homologous Recombination



Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking *Brca2*

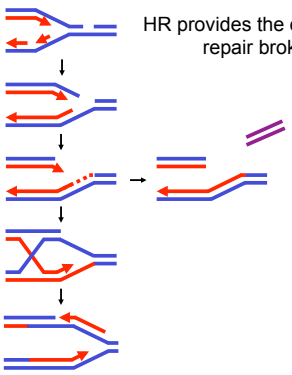
Shyam K. Sharan*†, Masami Morimatsu‡§||, Urs Albrecht¶, Dae-Sik Lim‡☆, Eva Regel†, Christopher Dinh*†, Arthur Sands‡, Gregor Eichele¶, Paul Hasty‡ & Allan Bradley*†



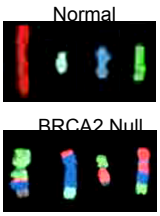
Genomic Instability:

BRCA2: Without homologous recombination, cells suffer genomic instability

Too Little HR: Mis-Repair of Broken Forks



HR provides the only pathway to accurately repair broken replication forks



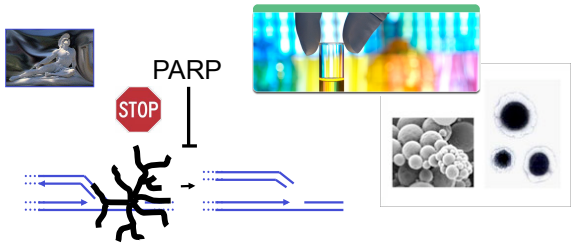
Data from Grigorova *et al.*, Cytogen. Gen. Res. 104:333 (2004)

From Science To Engineered Solutions

Two Great Bioengineering Accomplishments

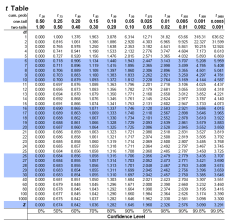
Exploiting a tumor's Achilles' Heel

Creating new drug delivery technology



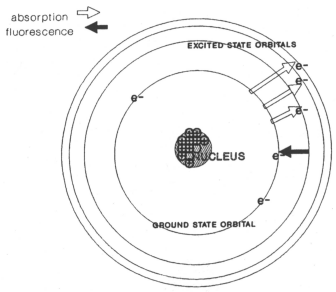
Basic Principles of Key Statistics

Normal Distribution
Standard Deviation
Confidence Interval
Student's *t*-Test



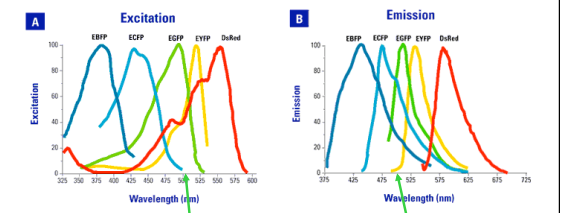
Flow Cytometry

Principle of Fluorescence Activation



Flow Cytometry: First Principles, Alice L. Givan, 19

See Specific Colors By Restricting Ex and Em:



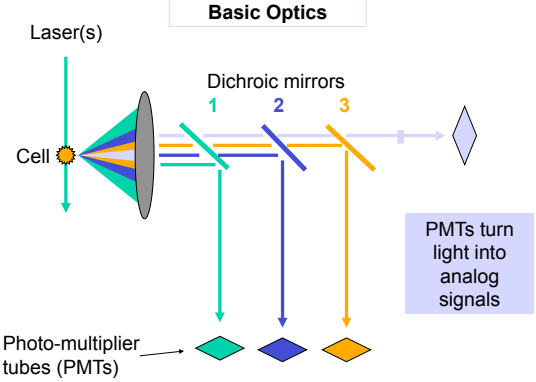
Your laser is 488 nm

For Green, Can excite using <510 nm

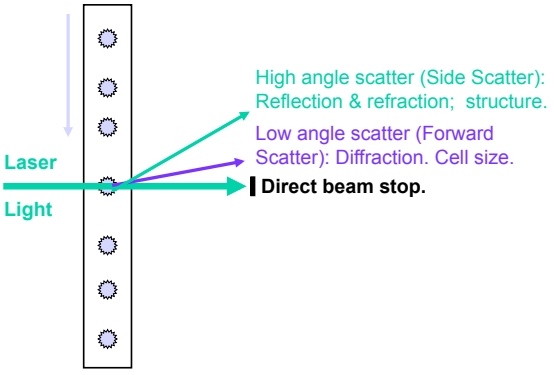
For Green, Can capture emission when >510 nm

Spectra from Clontech, In

Basic Optics



Slide Adapted FromTerry Hoy, Department of Haematology, UWCM



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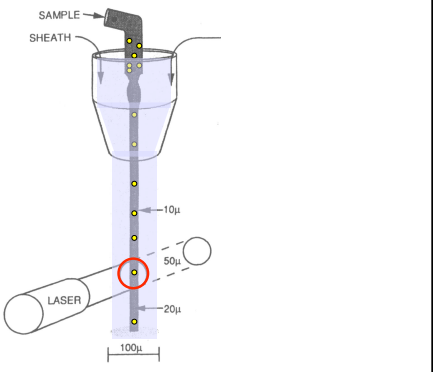
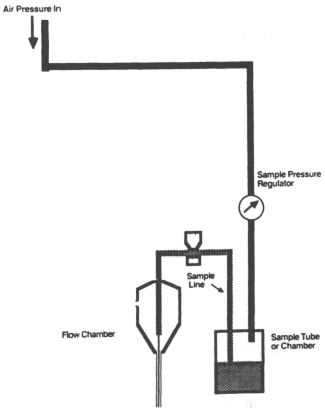


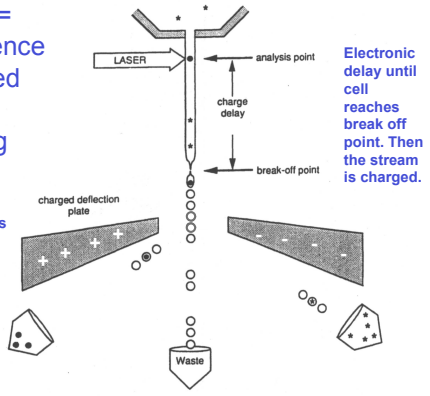
Flow Cytometry: First Principles, Alice L. Givan, 199

Sample Flow



Flow Cytometry: First Principles, Alice L. Givan, 199

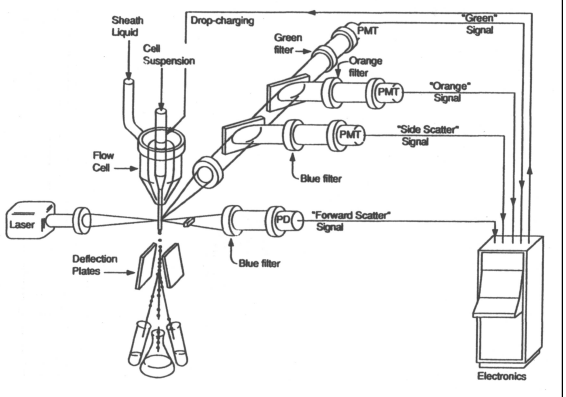
FACS = Fluorescence Activated Cell Sorting



Electronic delay until cell reaches break off point. Then the stream is charged.

Vibrating the nozzle produces a stream that breaks into droplets.

Flow Cytometry: First Principles, Alice L. Givan, 199



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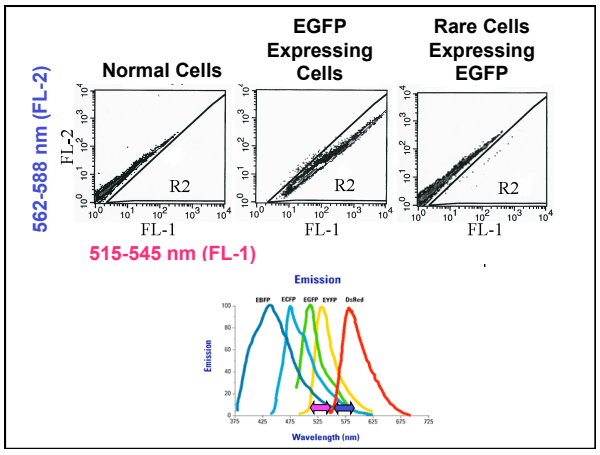
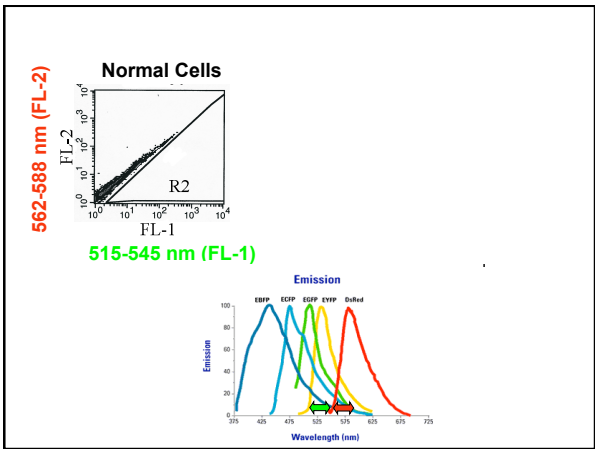

Normal Cells
EGFP Expressing Cells
Rare Cells Expressing EGFP
562-588 nm (FL-2)
FL-2
FL-1
R2
515-545 nm (FL-1)
Emission
EBFP ECFP EGFP EYFP DsRed
Emission
Wavelength (nm)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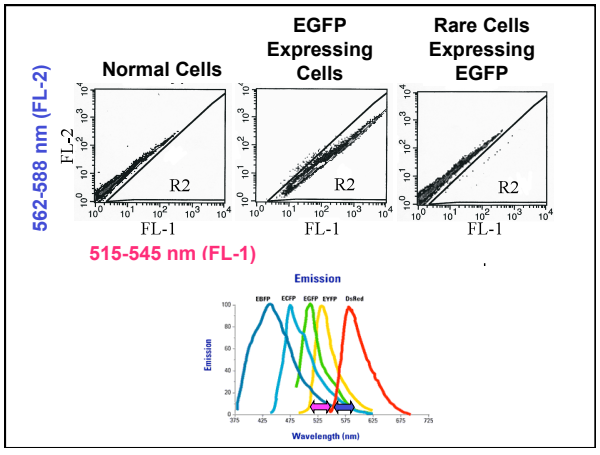
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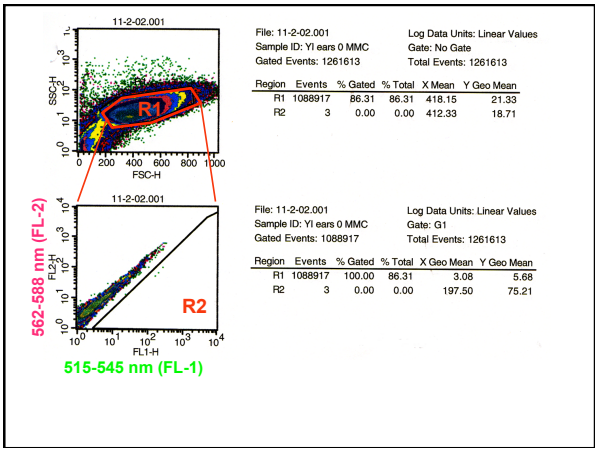
11-2-02.001
SSC-H
FSC-H
R1
File: 11-2-02.001
Sample ID: Yl ears 0 MMC
Gated Events: 1261613
Log Data Units: Linear Values
Gate: No Gate
Total Events: 1261613
Region Events % Gated % Total X Mean Y Geo Mean
R1 1088917 86.31 86.31 418.15 21.33
R2 3 0.00 0.00 412.33 18.71
11-2-02.001
562-588 nm (FL-2)
FL2-H
FL1-H
R2
515-545 nm (FL-1)
File: 11-2-02.001
Sample ID: Yl ears 0 MMC
Gated Events: 1088917
Log Data Units: Linear Values
Gate: G1
Total Events: 1261613
Region Events % Gated % Total X Geo Mean Y Geo Mean
R1 1088917 100.00 86.31 3.08 5.68
R2 3 0.00 0.00 197.50 75.21

Flow Cytometry

Flow cytometry analyzes cells one by one

Fluorescence, diffracted, and reflected light can be measured for each cell

Multiple lasers and multiple colors can be analyzed at millions of cells per minute

Resulting plots show the relative level of fluorescence of each cell for specific wave lengths (a dot is a single cell)

Flow cytometry is an analysis method, where as FACS actually sorts cells

Key Experimental Concepts for Mod1:

Nothing is 100%
Ask 'What else might be happening'?
Avoid Assumptions (Controls!)
Double Check at Every Opportunity

20.109….

Our major goals are:

To teach strong fundamentals in laboratory science

&

To inspire