

MOD1 – DNA ENGINEERING

Engelward, Fall 09

Day 8

Nobel Prizes

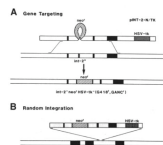
Module in Review:
Experimental Approaches &
Biological Concepts

Flow Cytometry


MARIO'S TRANSGENIC TECHNOLOGY "Knocks Out" Nobel Prize



Mario Capecchi, Nobel Prize 2007



A collage for the 2009 Nobel Prize in Physiology or Medicine. It features a gold Nobel medal, a portrait of Jack W. Szostak, and a photograph of the Greider family (Terry L. Orr-Weaver, Rodney J. Rothstein, and Franklin W. Stahl). Arrows point from the names Szostak, Blackburn, and Greider to the word 'Telomerase'. Below this is a diagram of the 'Szostak Model' for telomerase activity, showing the interaction of the RNA subunit with DNA and the protein subunit with RNA. A caption at the bottom identifies the scientists: Jack W. Szostak, Terry L. Orr-Weaver, Rodney J. Rothstein and Franklin W. Stahl. A portrait of Terry Orr Weaver (MIT!) is also included.



2009






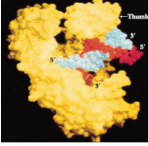


Photo: MRC Laboratory of Molecular Biology
Venkatraman Ramakrishnan



Credits: Michael Harsland/Yale University
Thomas A. Steitz



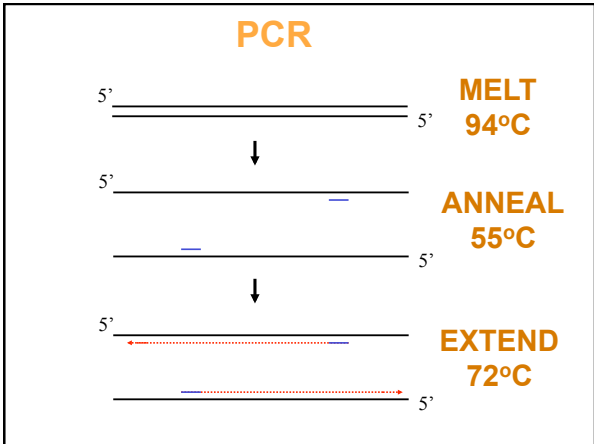
Credits: Micheline Peled/Carlsberg
Ada E. Yonath

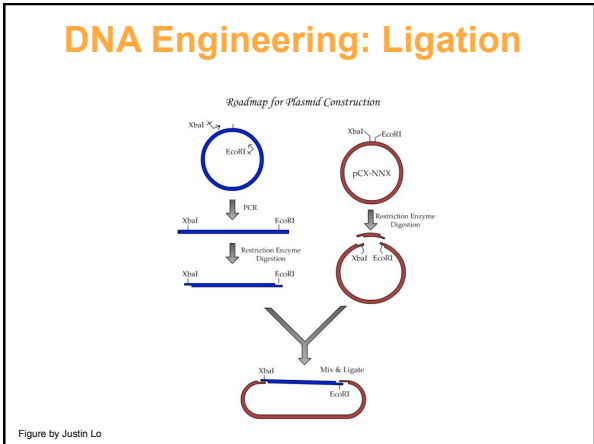
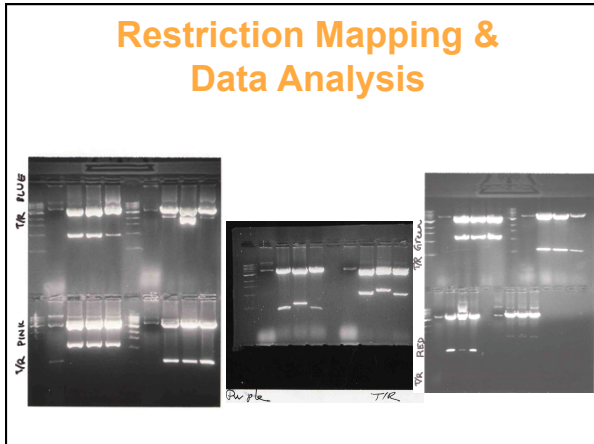
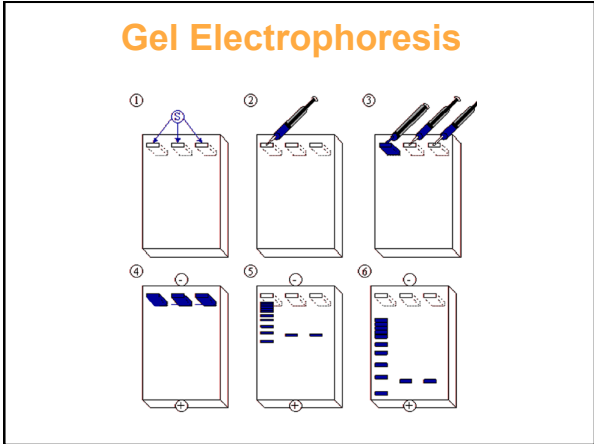
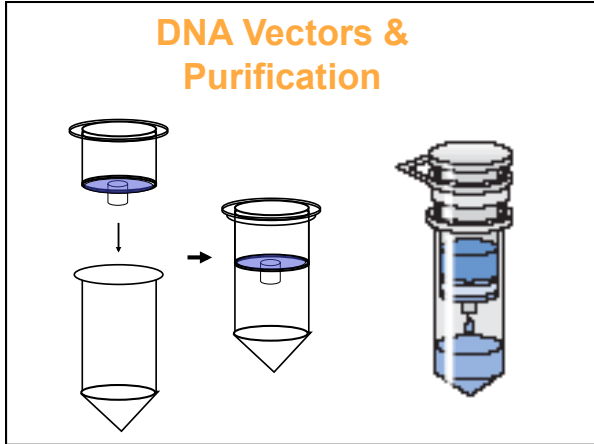




Science → Engineered Solutions

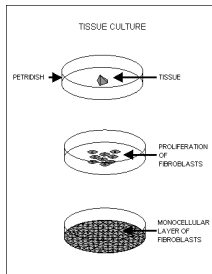
Module in Review

Experimental Approaches

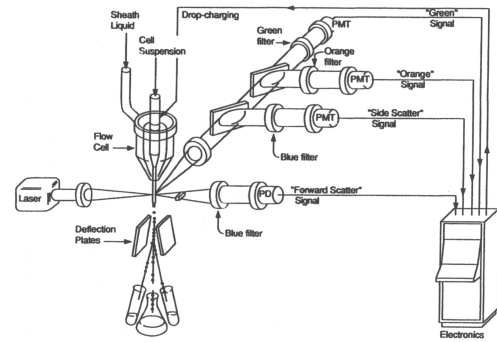




Mammalian Cell Culture



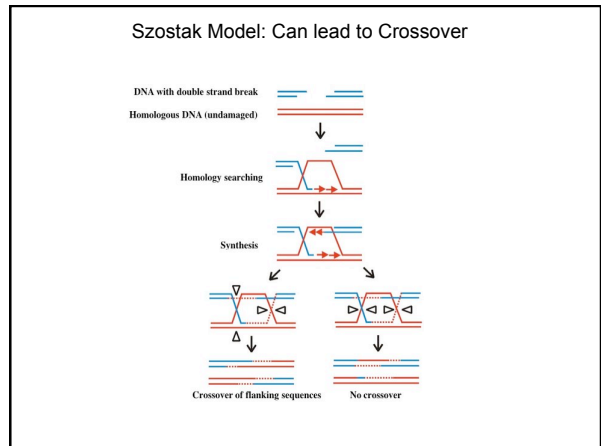
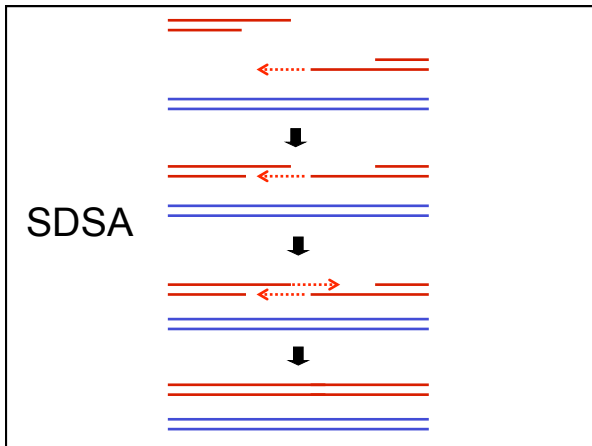
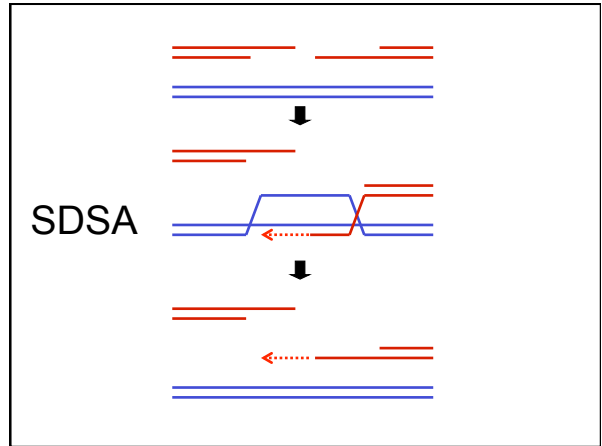
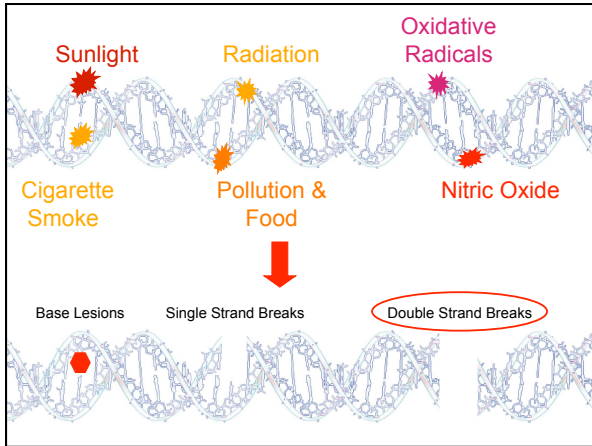
Flow Cytometry



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

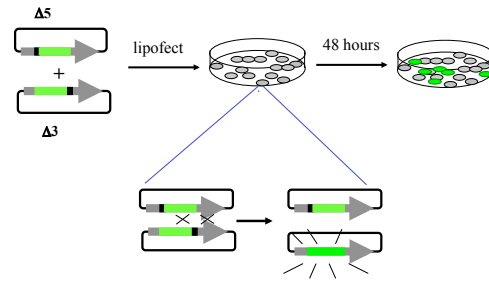
Biological Principles

DNA Damage & Repair via Homologous Recombination



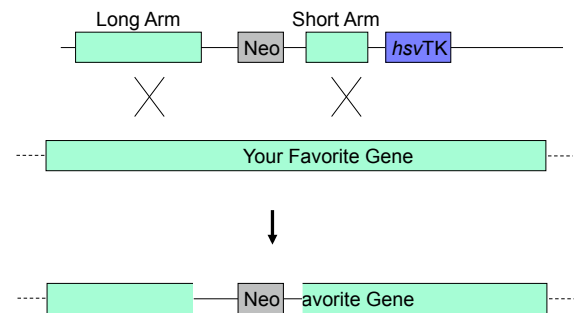
Engineering an Assay for Homologous Recombination

A Plasmid-Based Assay for Homologous Recombination in Mammalian Cells



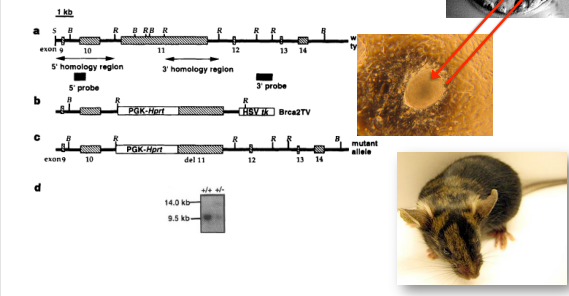
Exploiting Homologous Recombination for Gene Targeting

Traditional ES Knock-Out Technology Targeted Homologous Recombination



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

Shyam K. Sharan¹, Masami Morimatsu^{1,2}, Ura Albrecht¹, Dae-Sik Lim^{1,2}, Eva Regel¹, Christopher Dinh¹, Arthur Sands¹, Gregor Eicheler¹, Paul Hasty¹ & Allan Bradley¹

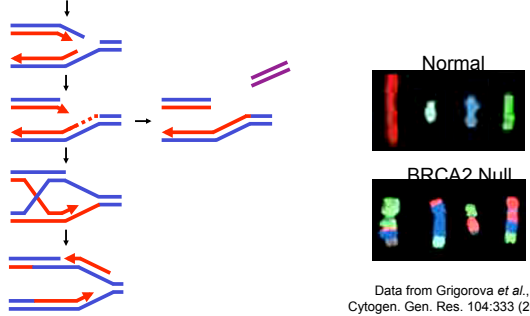


Genomic Instability:

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



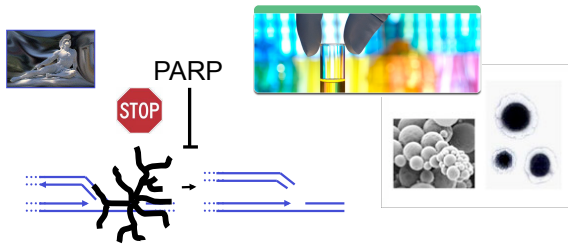
Data from Grigorova *et al.*, Cytogen. Gen. Res. 104:333 (2004)

**From Science
To Engineered Solutions**

Two Great Bioengineering Accomplishments

Exploiting a tumor's Achilles' Heel

Creating new drug delivery technology



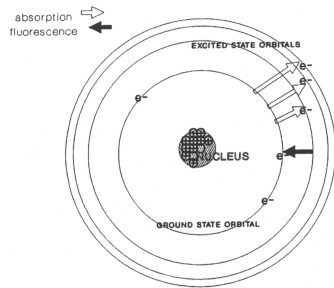
Basic Principles of Key Statistics

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

t	0.90	0.95	0.98	0.99	0.995	0.9975	0.999	0.9995	0.99975	0.9999	0.99995
0	1.28155	1.64485	2.05375	2.32635	2.57583	2.80703	3.09023	3.29053	3.43813	3.59648	3.71902
1	1.28583	1.64949	2.05818	2.33094	2.58089	2.81210	3.10383	3.30433	3.45193	3.61028	3.73282
2	1.29012	1.65413	2.06261	2.33550	2.58594	2.81731	3.11656	3.31706	3.46466	3.62301	3.74555
3	1.29441	1.65877	2.06704	2.34006	2.59099	2.82252	3.12929	3.32979	3.47736	3.63566	3.75809
4	1.29870	1.66341	2.07147	2.34462	2.59604	2.82773	3.14202	3.34252	3.49003	3.64796	3.77062
5	1.30299	1.66805	2.07590	2.34918	2.60109	2.83294	3.15475	3.35525	3.50274	3.66029	3.78318
6	1.30728	1.67269	2.08033	2.35374	2.60614	2.83815	3.16748	3.36798	3.51547	3.67262	3.79572
7	1.31157	1.67733	2.08476	2.35830	2.61119	2.84336	3.18021	3.38071	3.52816	3.68495	3.80826
8	1.31586	1.68197	2.08919	2.36286	2.61624	2.84857	3.19294	3.39344	3.54089	3.69728	3.82080
9	1.32015	1.68661	2.09362	2.36742	2.62129	2.85378	3.20567	3.40617	3.55358	3.70961	3.83334
10	1.32444	1.69125	2.09805	2.37198	2.62634	2.85899	3.21840	3.41890	3.56631	3.72194	3.84588
11	1.32873	1.69589	2.10248	2.37654	2.63139	2.86420	3.23113	3.43163	3.57904	3.73427	3.85842
12	1.33302	1.70053	2.10691	2.38110	2.63644	2.86941	3.24386	3.44436	3.59177	3.74660	3.87096
13	1.33731	1.70517	2.11134	2.38566	2.64149	2.87462	3.25659	3.45709	3.60450	3.75893	3.88350
14	1.34160	1.70981	2.11577	2.39022	2.64654	2.87983	3.26932	3.46982	3.61723	3.77126	3.89604
15	1.34589	1.71445	2.12020	2.39478	2.65159	2.88504	3.28205	3.48255	3.62996	3.78359	3.90858
16	1.35018	1.71909	2.12463	2.39934	2.65664	2.89025	3.29478	3.49528	3.64269	3.79592	3.92112
17	1.35447	1.72373	2.12906	2.40390	2.66169	2.89546	3.30751	3.50801	3.65542	3.80825	3.93366
18	1.35876	1.72837	2.13349	2.40846	2.66674	2.90067	3.32024	3.52074	3.66815	3.82058	3.94620
19	1.36305	1.73301	2.13792	2.41302	2.67179	2.90588	3.33297	3.53347	3.68088	3.83291	3.95874
20	1.36734	1.73765	2.14235	2.41758	2.67684	2.91109	3.34570	3.54620	3.69361	3.84524	3.97128
25	1.37449	1.74589	2.15119	2.42582	2.68469	2.91894	3.36023	3.56193	3.70934	3.86097	3.98691
30	1.38043	1.75253	2.15753	2.43116	2.68914	2.92239	3.36756	3.56926	3.71667	3.86830	3.99445
40	1.38882	1.76077	2.16487	2.43650	2.69359	2.92584	3.37489	3.57659	3.72400	3.87563	4.00199
50	1.39443	1.76641	2.16930	2.44094	2.69604	2.92729	3.37932	3.58102	3.72843	3.87906	4.00642
60	1.39894	1.77005	2.17273	2.44438	2.69749	2.92874	3.38275	3.58545	3.73286	3.88249	4.01085
70	1.40273	1.77269	2.17516	2.44682	2.69894	2.92919	3.38518	3.58988	3.73729	3.88592	4.01528
80	1.40602	1.77483	2.17659	2.44826	2.69939	2.92964	3.38761	3.59431	3.74172	3.88935	4.01971
90	1.40891	1.77647	2.17743	2.44910	2.69984	2.92989	3.38904	3.59674	3.74415	3.89178	4.02214
∞	1.41254	1.77835	2.17827	2.45000	2.70000	2.93000	3.39000	3.59800	3.74600	3.89300	4.02300

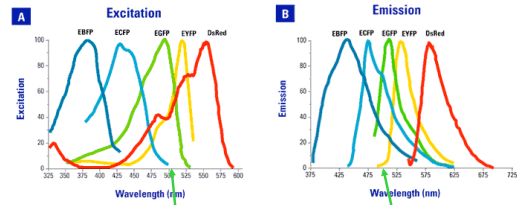
Flow Cytometry

Principle of Fluorescence Activation



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

See Specific Colors By Restricting Ex and Em:

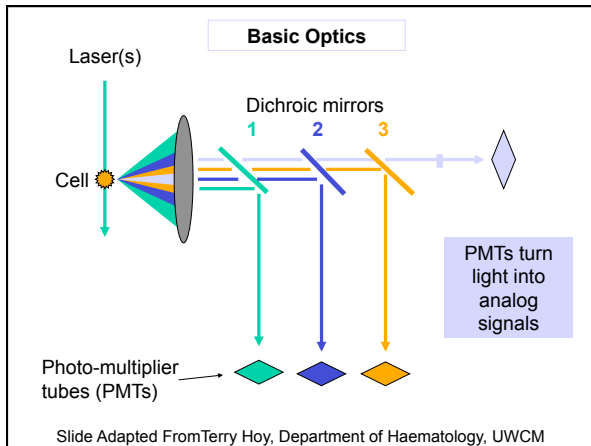


Your laser is 488 nm

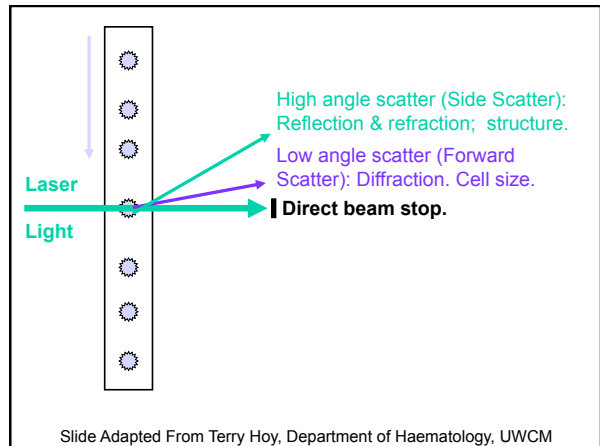
For Green, Can excite using <510 nm

For Green, Can capture emission when >510 nm

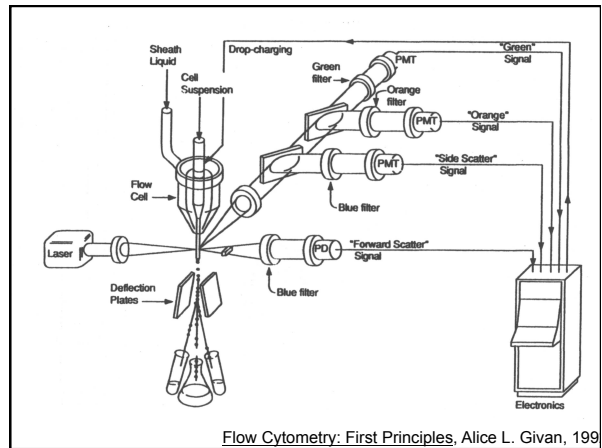
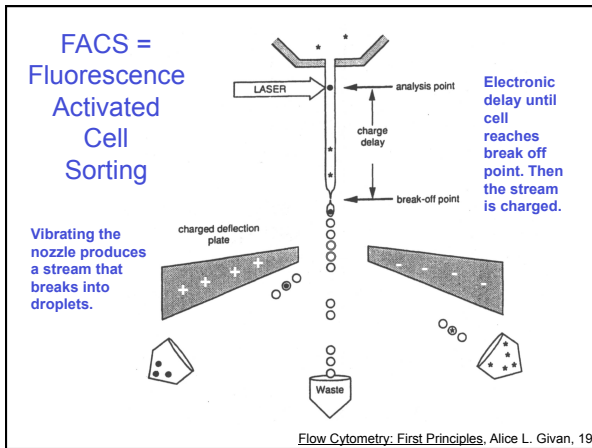
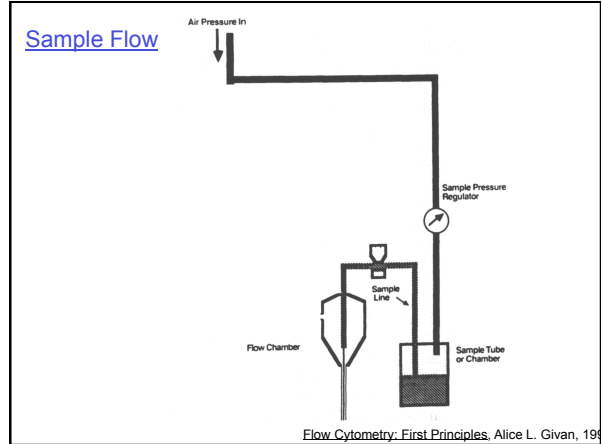
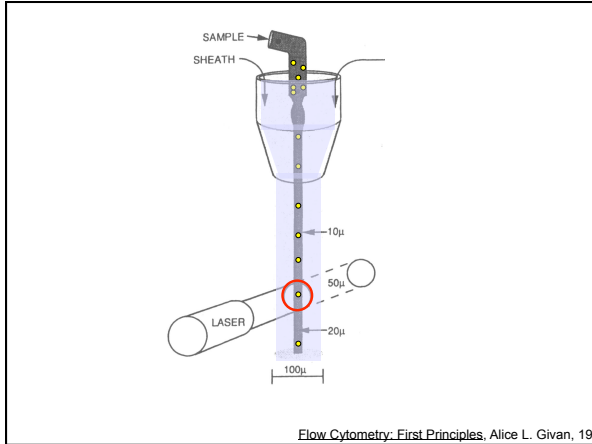
Spectra from Clontech, Inc

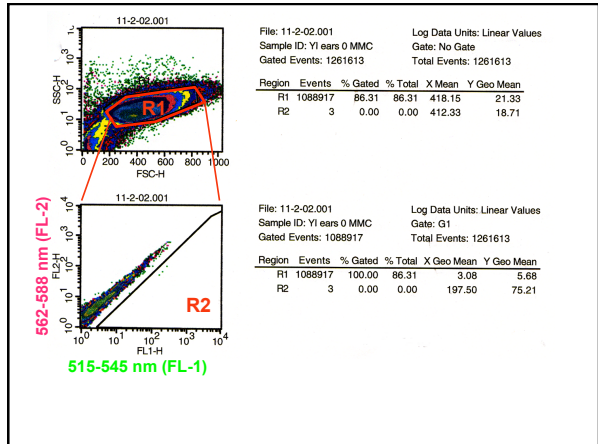
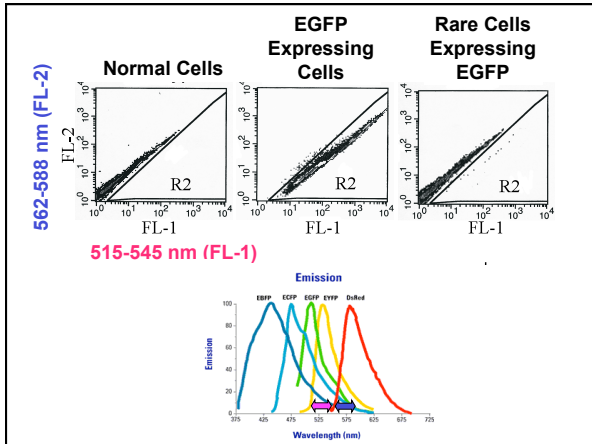
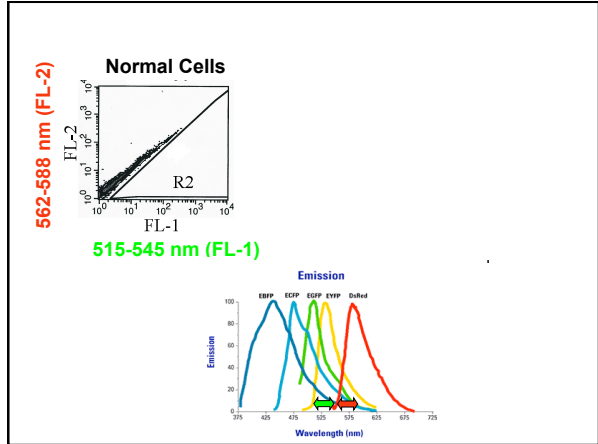
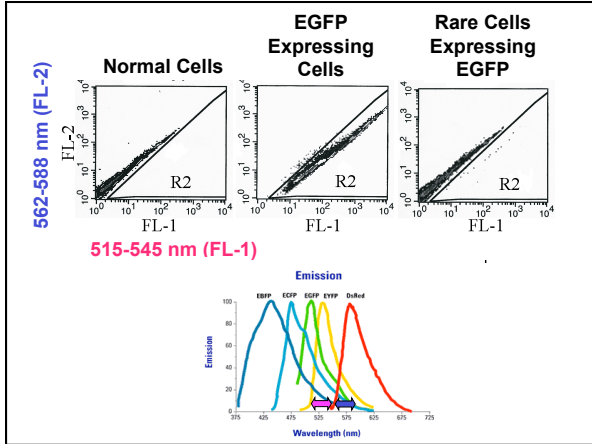


Slide Adapted From Terry Hoy, Department of Haematology, UWCM



Slide Adapted From Terry Hoy, Department of Haematology, UWCM





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