

# MOD1 – DNA ENGINEERING

Engelward, Fall 2009

Day 6

- Lecture 1: Intro to importance of HR  
Polymerases & PCR
- Lecture 2: How HR works  
Overview of experiments & discussion of controls (single digests)
- Lecture 3: Why understanding matters: HR & BRCA2  
Overview; Running an agarose gel; Purification from a gel; Discussion of controls
- Lecture 4: Exploiting Scientific Understanding for Engineering:  
Achilles heel/Parp Inhibitors & Drug delivery  
Ligase and transformation & Data discussion (purified fragments)
- Lecture 5: DNA Engineering in Mammals: Gene Targeting & Transgenics (Sonoda prep)  
Overview & Strategies for DNA analysis
- Lecture 6: DNA Engineering in Mammals: Knock Outs & Conditional Expression (Sonoda Prep)  
Mammalian Cell Culture**
- Lecture 7: DNA Engineering Reveals HR Function: Discussion of Sonoda
- Lecture 8: Flow Cytometry: How it works and how to do it

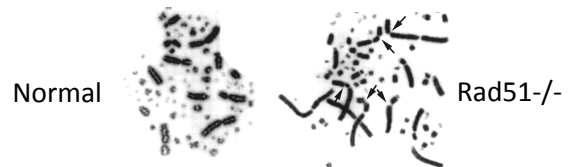
## Going from Understanding to Engineered Solutions

- Exploiting Understanding of HR  
for genetic engineering
- Conditional Expression

## Mammalian Cell Culture: Methods and Logic

- Fundamentals & How To

## Why you owe Your Life to Homologous Recombination...



*But how do we that cells cannot survive without HR?*

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

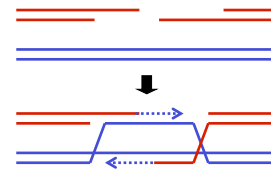
To understand how the experiments were done to show that HR is essential, you need to understand:

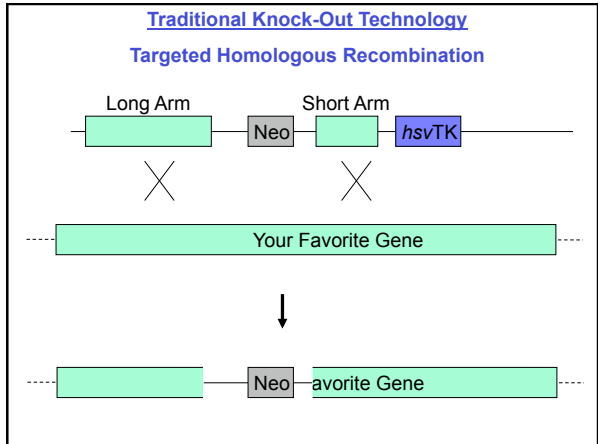
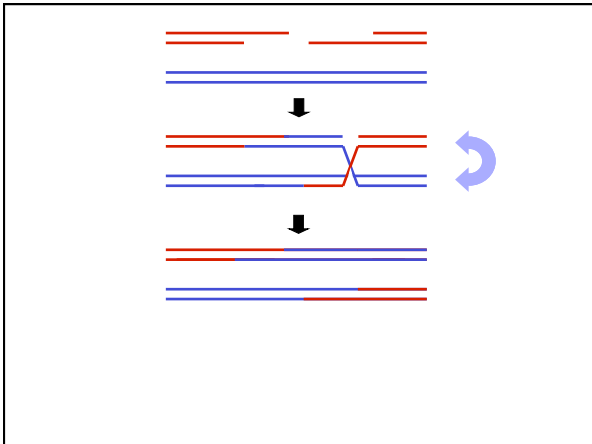
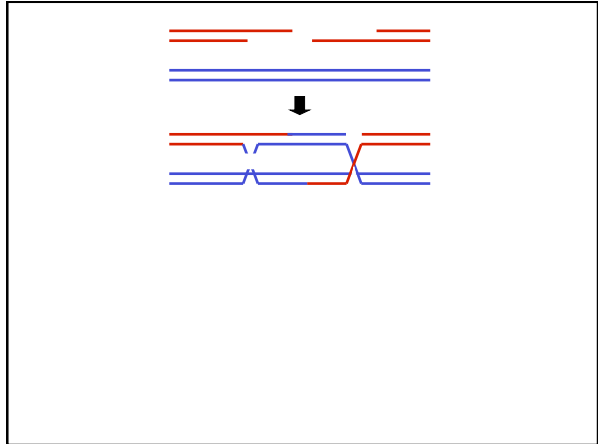
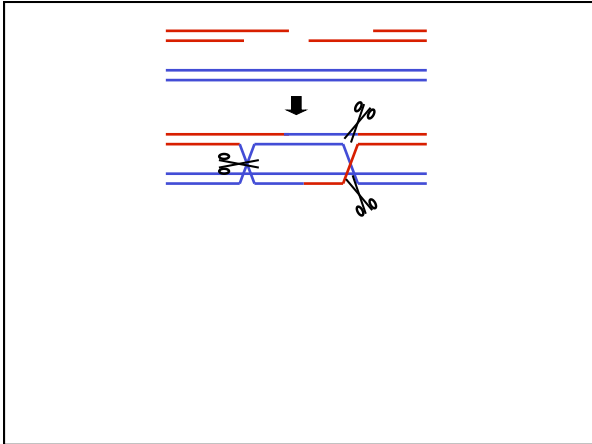
- a) Gene Targeting
- b) Conditional Expression
- c) Cell Cycle Analysis by Flow Cytometry

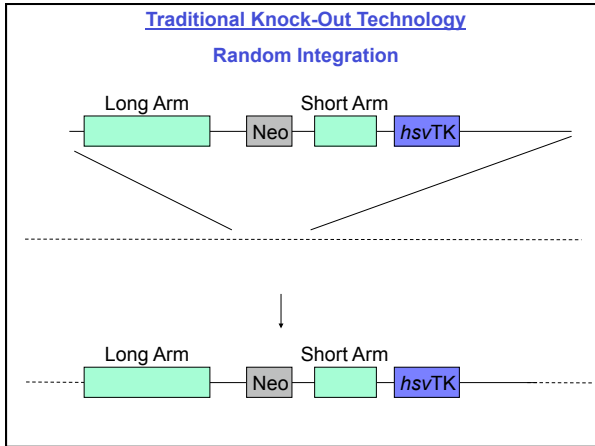
## Gene Targeting

Gene Targeting is all about exchanging DNA...

But how do we swap one piece for another?

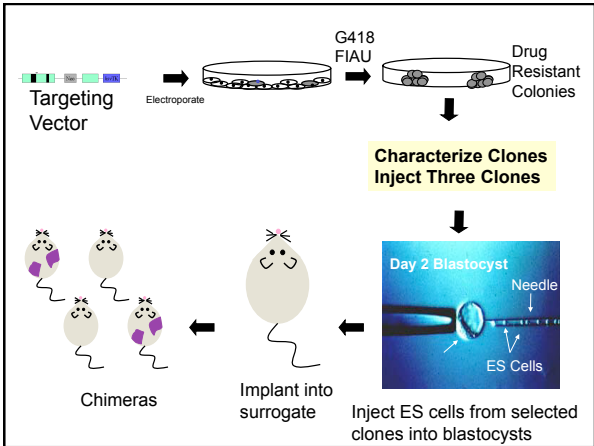
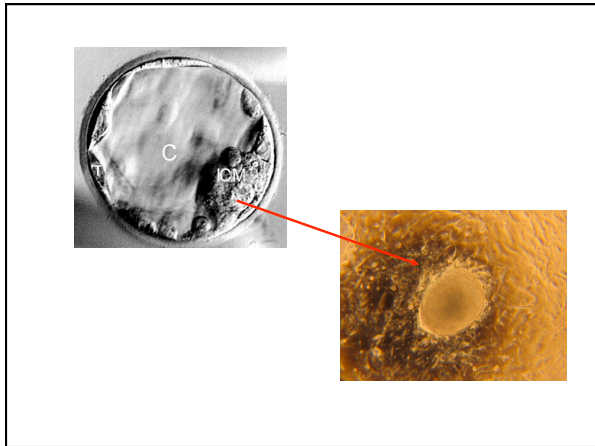






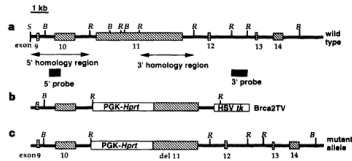
**Genetic Engineering in Mice:**

- 1) Gene Targeting (exploiting HR)
- 2) Transgenics
- 3) Modern Genetic Engineering: Conditional Expression

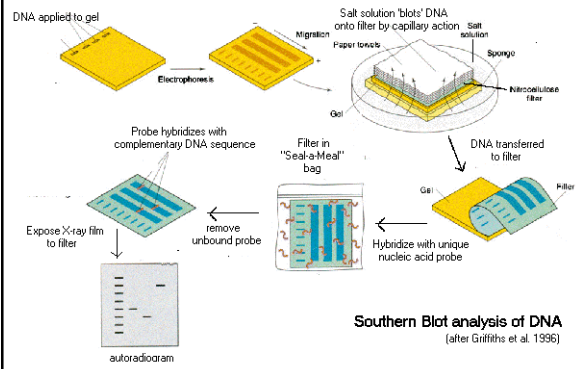


## Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking *Brca2*

Shyam K. Sharan<sup>1</sup>, Masami Morimatsu<sup>1,2</sup>, Urs Albrecht<sup>1</sup>, Dae-Sik Lim<sup>1,3</sup>, Eva Regel<sup>1</sup>, Christopher Dinh<sup>1</sup>, Arthur Sands<sup>1</sup>, Gregor Eicheler<sup>1</sup>, Paul Hasty<sup>1</sup> & Allan Bradley<sup>1</sup>

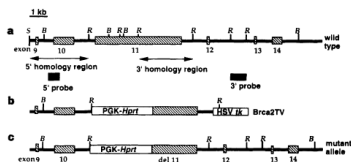


## Southern Blot Analysis

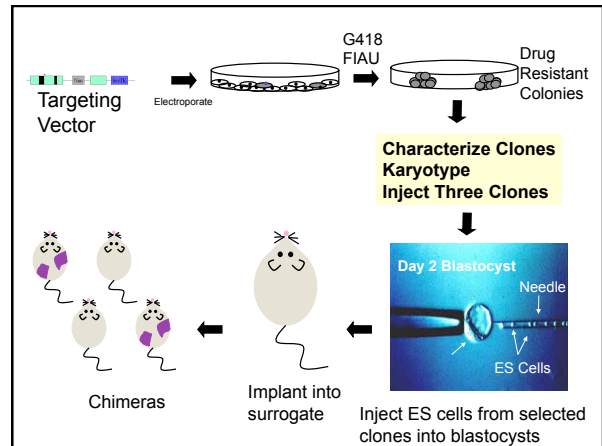


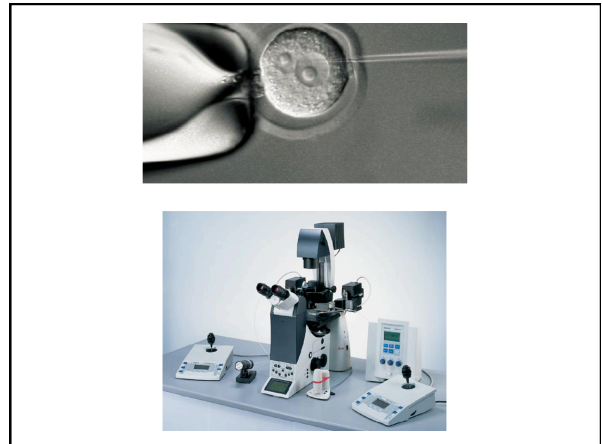
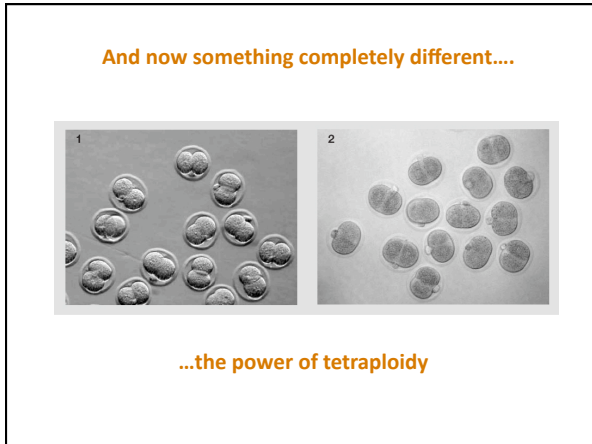
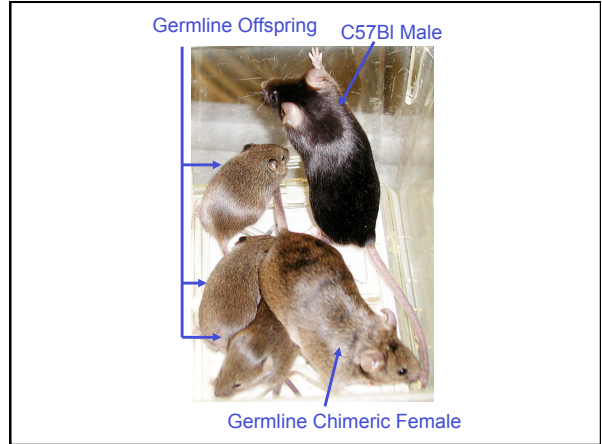
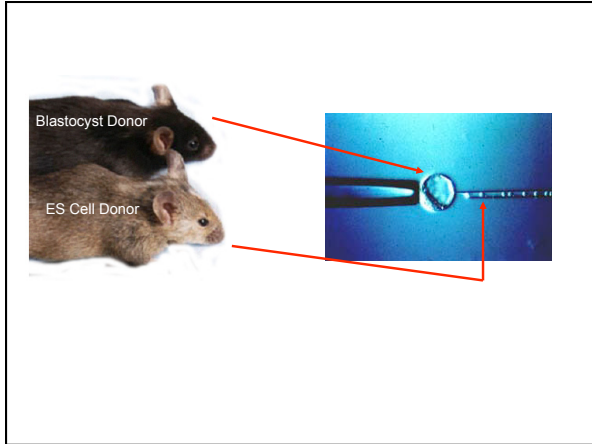
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**Figure 1** Disruption of *Brca2* gene in ES cells. **a.** Restriction map of the *Brca2* genomic fragment containing exon 9-15 is shown. Restriction sites shown here are *EcoRI* ( $\Psi$ ) and *BamHI* ( $\beta$ ). *SalI* ( $S$ ) is from the cloning vector. Double-headed arrows correspond to the 5' and 3' homology regions and the dark shaded boxes show the probes used. **b.** Restriction map of the targeting vector pBrca2TV. **c.** Expected restriction map of the mutated *Brca2* locus. A 2.8-kb genomic region is deleted and replaced by the 3.6-kb *Hprt* gene. **d.** Southern analysis to identify heterozygous ES cells by digesting genomic DNA with *BamHI*. The 3' probe detects a 9.5-kb wild-type band and a 14.0-kb mutant band. **e.** Restriction map of

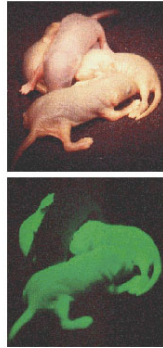




DNA integrates into the genome of the mouse at a random location.

Usually multiple copies of the transgene are integrated.

Expression patterns depend on the locus of integration.

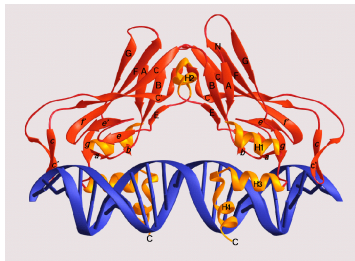


Okabe *et al.*

## Conditional Expression:

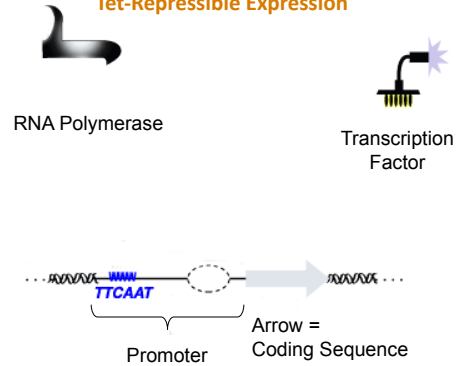
## Tet-Repressible Expression

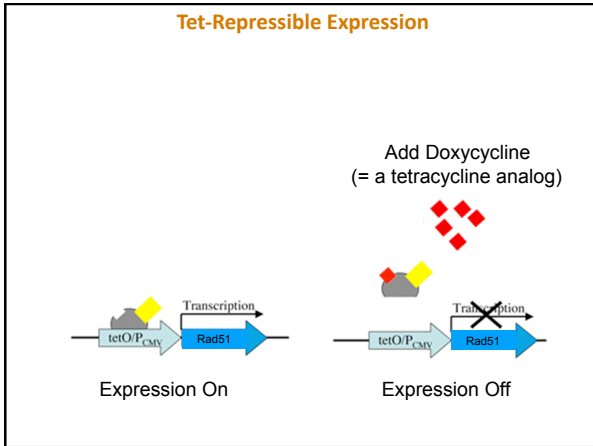
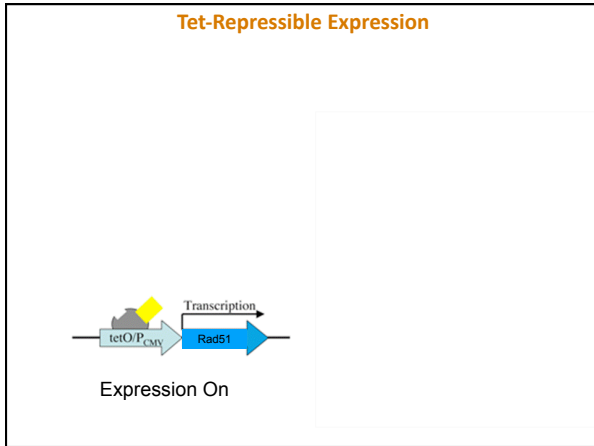
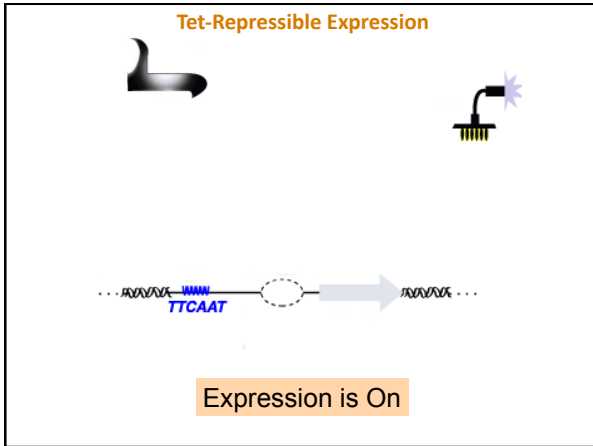
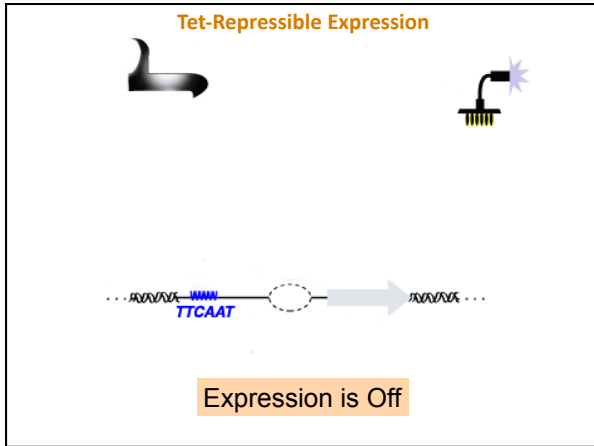
Transcription Factor



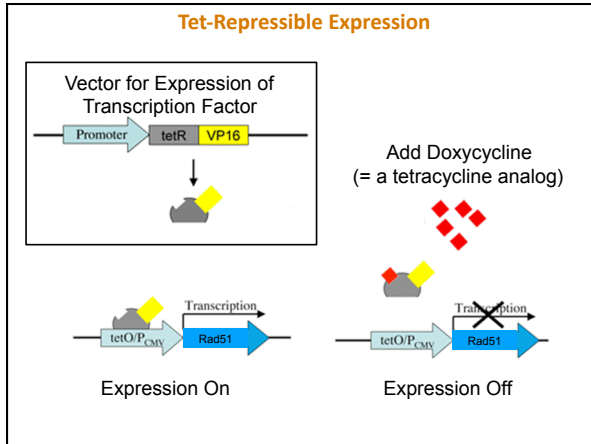
T-box transcription factor

### Tet-Repressible Expression



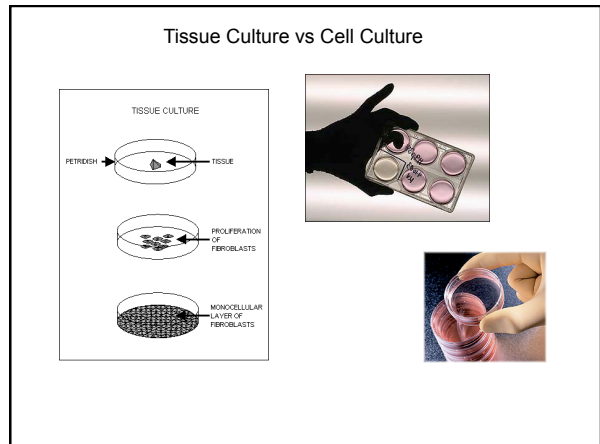






- Genetic Engineering in Mice:**
- 1) Gene Targeting (exploiting HR)
  - 2) Transgenics
  - 3) Modern Genetic Engineering: Conditional Expression

**Culturing Mammalian Cells**



Where do you  
get mammalian cells?

What do cells need to grow in culture?

- Correct Temperature
- Correct pH
- Correct Osmolality
- Amino Acids
- Glutamine (used for energy)
- Vitamins
- Glucose
- Salts
- Growth Factors (Antibiotics)
- Lipids (usually in serum)
- Minerals

Serum: Calf, Fetal, Horse, Bovine...

Typical media...

- 82 ml DMEM
- 15 ml Calf Serum
- 1.5 ml Glutamine
- 1.5 ml Penn/Strep

What is in DMEM?

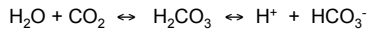
Vitamins

	D 0412	VITAMINS	
COMPONENT	10% FCS		
		Calcium Bicarbonate	—
		Calcium Chloride	0.004
		Folic Acid	0.004
		Inye-Inositol	0.0075
		Niacinamide	0.004
		D-Pantoic Acid*1/2Ca	0.004
		Pridoxal*HCl	—
		Pyridoxine*HCl	0.004
		Riboflavin	0.0004
		Thiamine*HCl	0.004
		OTHER	
		D-Glucose	4.7
		HEPES	—
		Phenol Red*Na	0.0159
		Pyruvic Acid*Na	0.11
		ADD	
		Glucose	—
		L-Glutamine	0.384
		L-Histidine	—
		L-Isoleucine	—
		L-Leucine	—
		L-Lysine*HCl	—
		L-Methionine	—
		L-Methionine*HCl	—
		L-Phenylalanine	—
		L-Serine	—
		L-Threonine	—
		L-Tryptophan	—
		L-Tyrosine (See base)	—
		Pyruvic Acid*Na	—
		L-Tyrosine*2Na*2H <sub>2</sub> O	—
		L-Valine	—
		Grams of powder required to prepare 1 L	N/A

### How do you maintain a neutral pH?

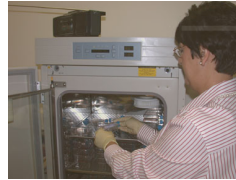
Blood pH is 7.4

The most important buffer in extracellular fluids is a mixture of carbon dioxide (CO<sub>2</sub>) and bicarbonate anion (HCO<sub>3</sub><sup>-</sup>).



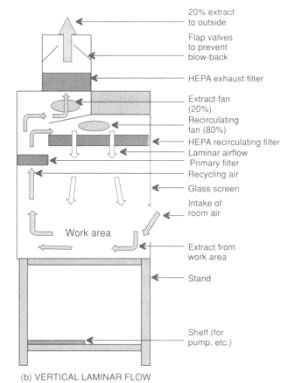
The pH is determined by the concentration of CO<sub>2</sub> and bicarbonate.

### Where do you grow your cells?



### Why is sterility important?

### How do you maintain sterility?



(b) VERTICAL LAMINAR FLOW

From Freshney's "Culture of Mammalian Cells"

## Mammalian Cell Culture Hood

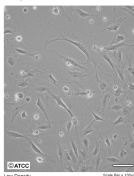


## Hands-On..

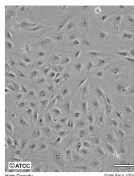
What does it mean to  
“pass” your cultures?

## Cell Density (cells per cm<sup>2</sup>)

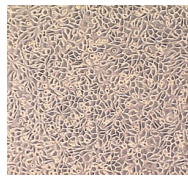
ATCC Number: CCL-92  
Designation: 3T3 Swiss Albino



Low Density

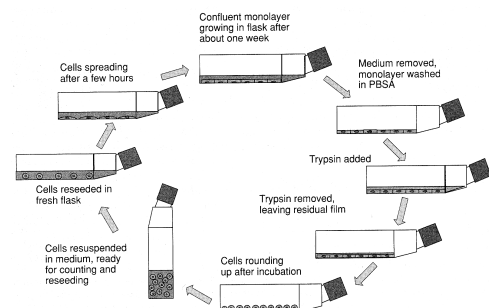


High Density

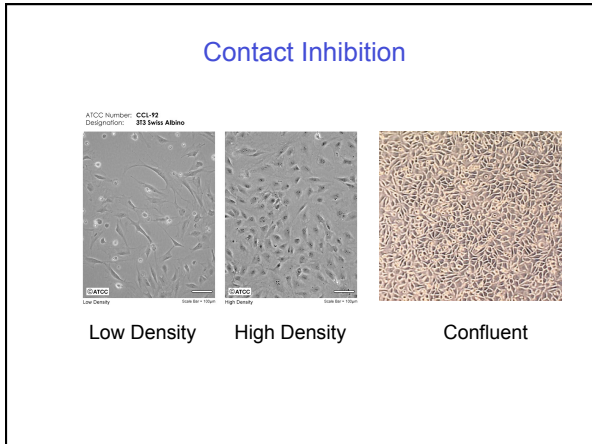
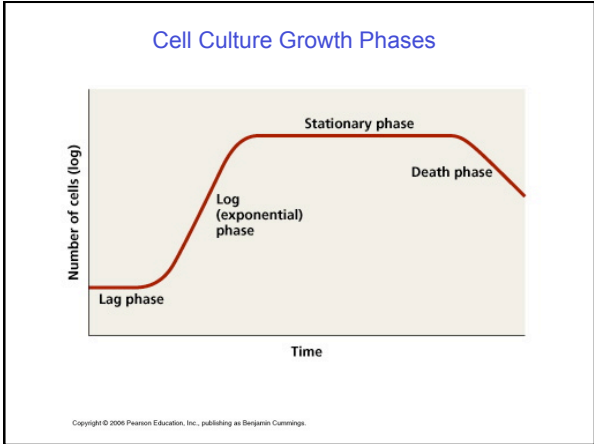


Confluent

## Cell Density can be kept low through “Passaging”



Trypsin is used to release cells from the surface of the dish



**Going from Understanding to Engineered Solutions**

- Exploiting Understanding of HR for genetic engineering
- Conditional Expression

**Mammalian Cell Culture: Methods and Logic**

- Fundamentals & How To