

MOD1 – DNA ENGINEERING

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Spring 2008

Day 6

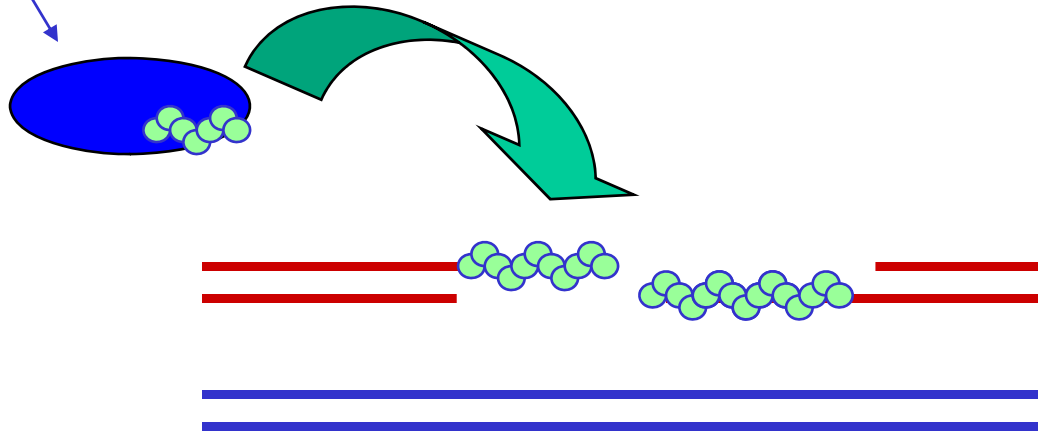
The role of homologous recombination in rescuing cells from chemotherapeutics.

Culturing Mammalian Cells

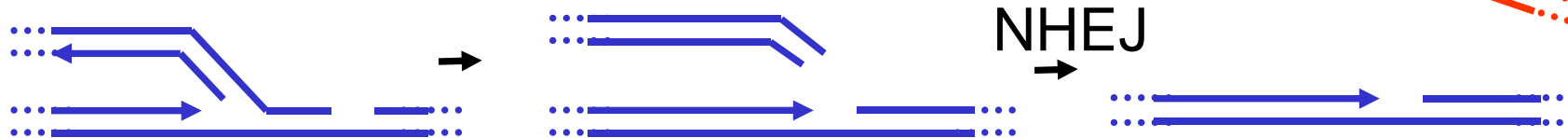
Roles of Homologous Recombination:

**Preventing Cancer
& Affecting Treatment**

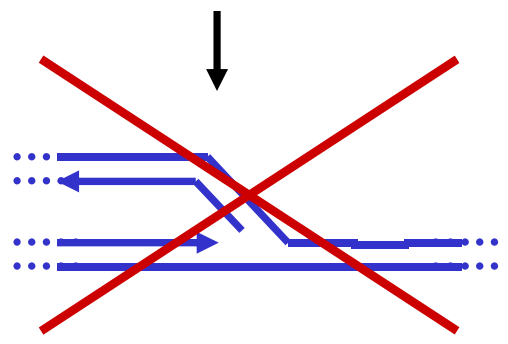
BRCA2



BRCA2 Loads Rad51



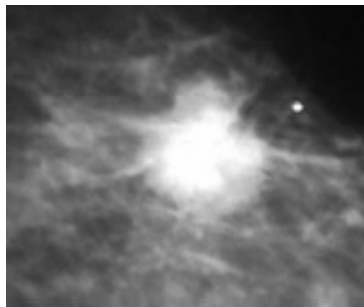
BRCA2-/-



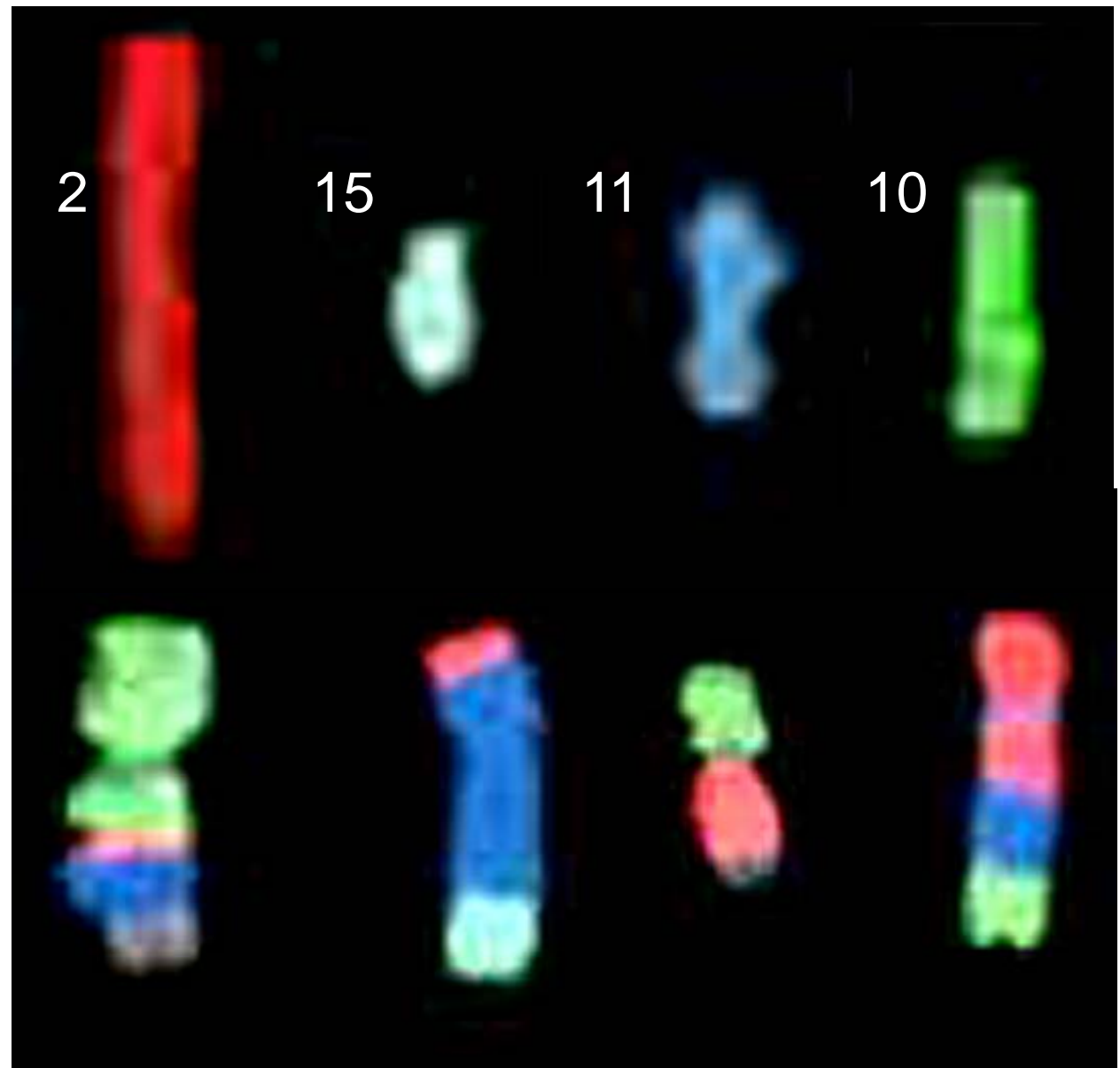
Normal Human
Chromosomes



BRCA2 -/-
Chromosomes



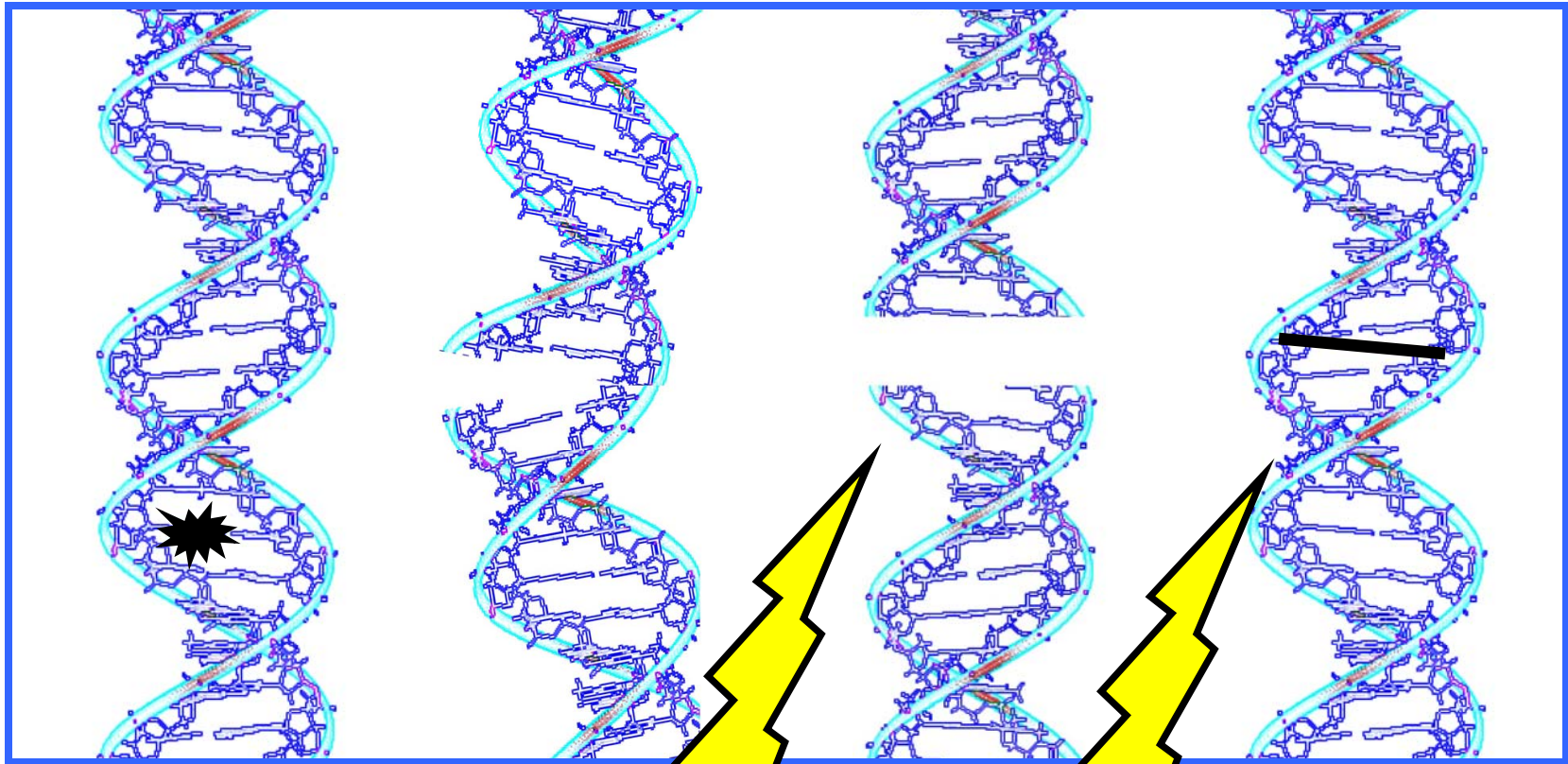
www.rctradiology.com



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

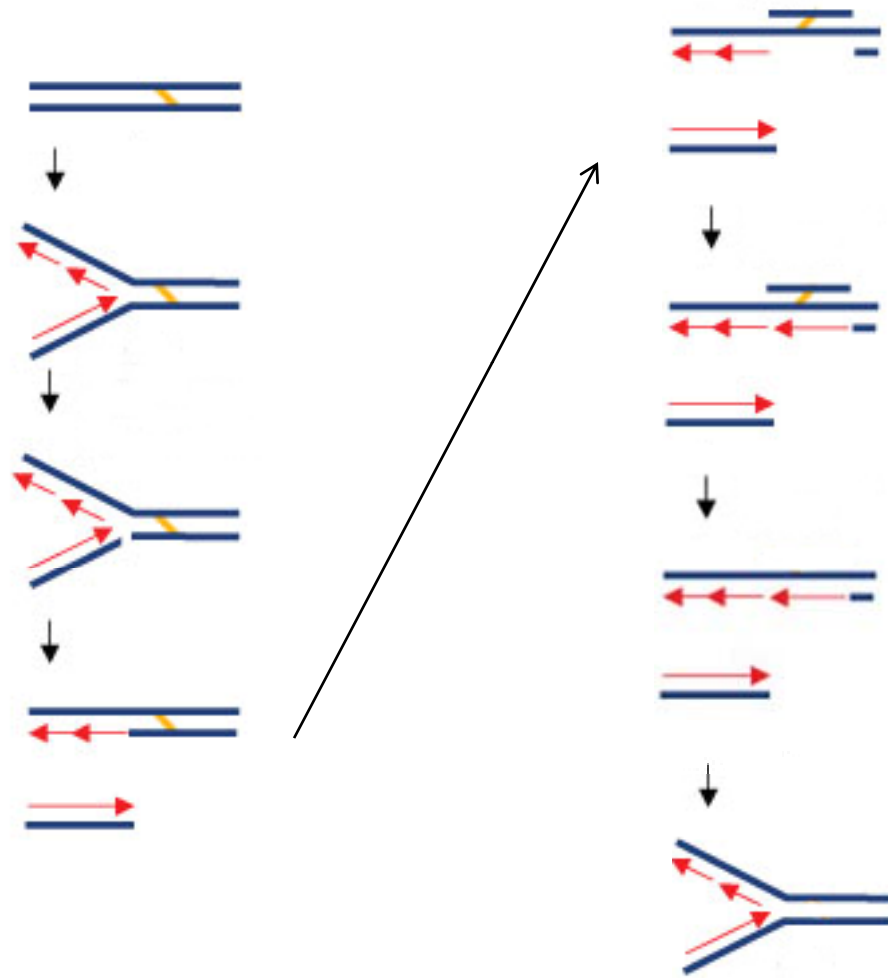
Homologous Recombination

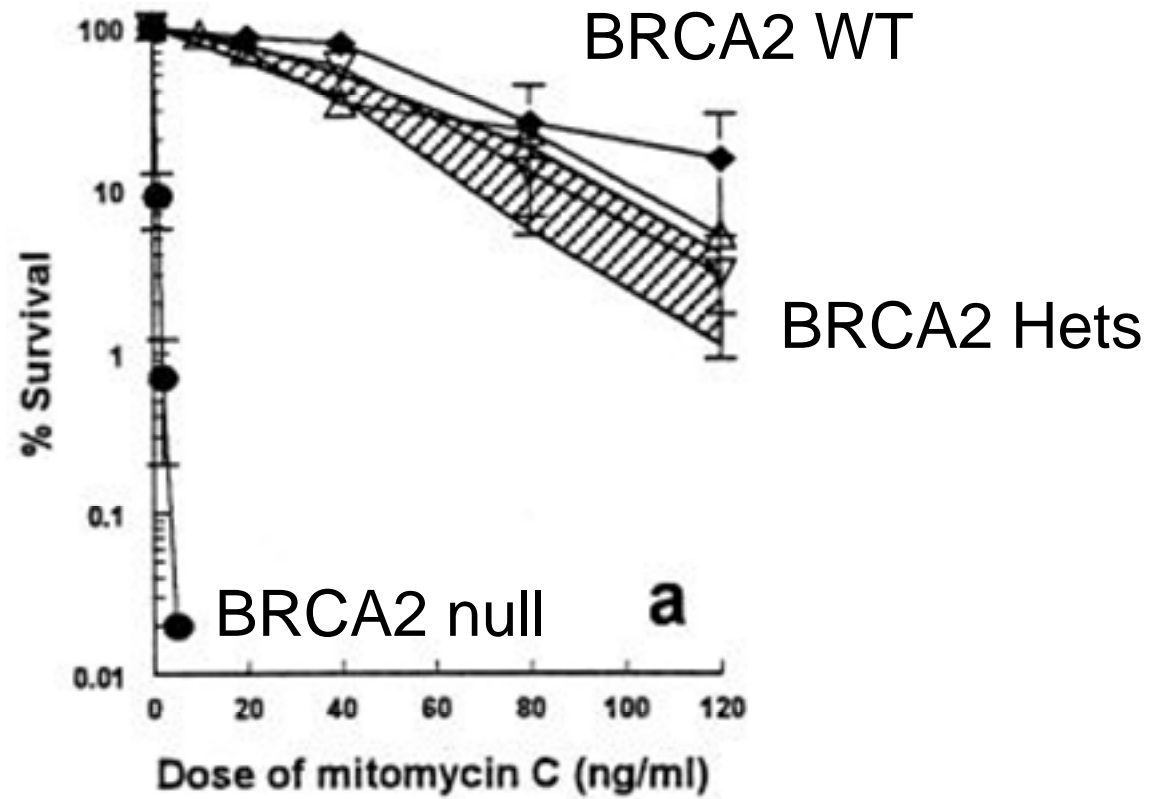
Toxicity



Radiation & Chemotherapy

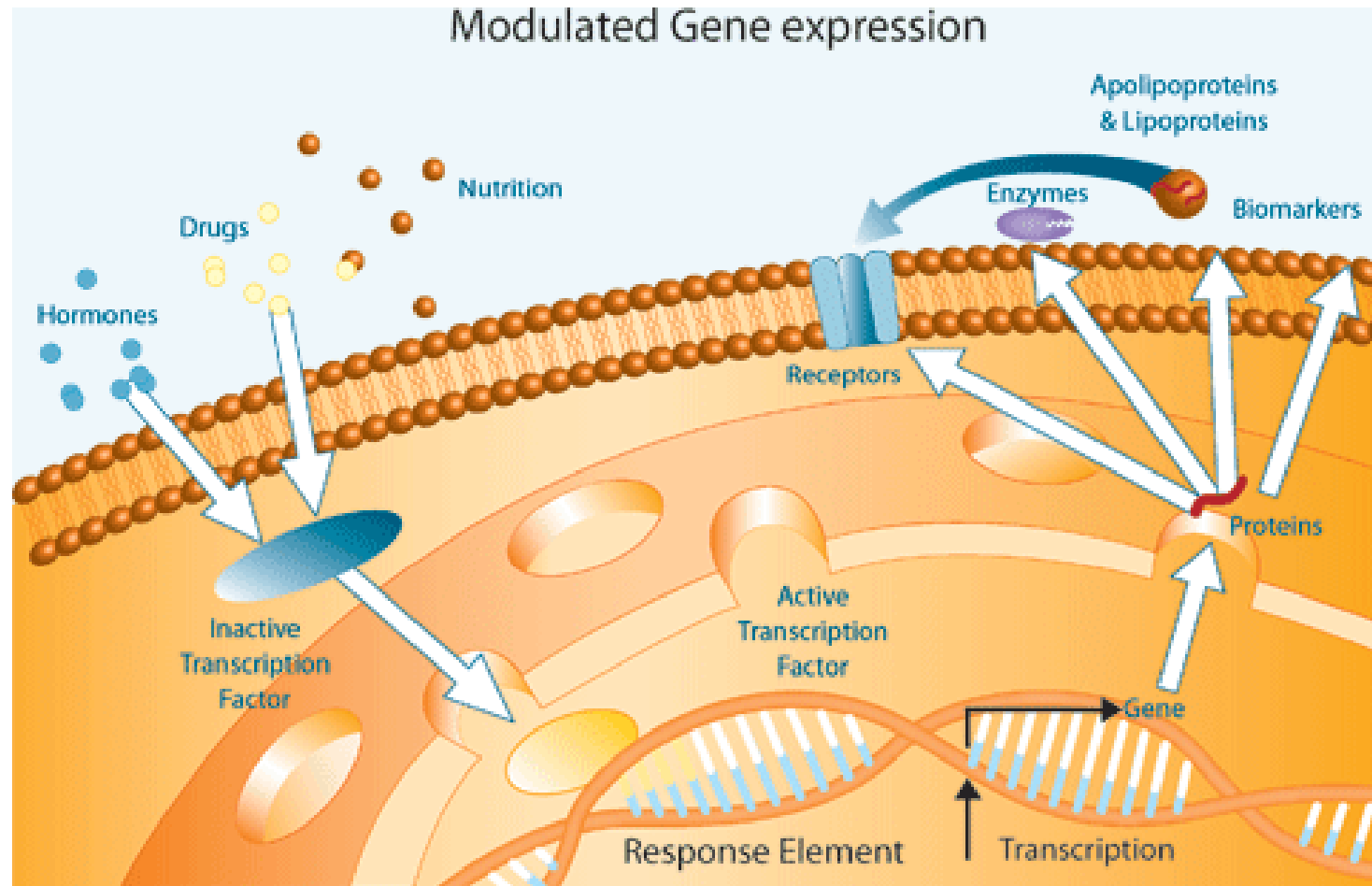
Replication Fork Animation
By Tet Matsuguchi



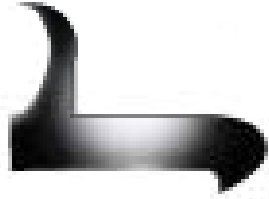


Tet-Repressible Expression

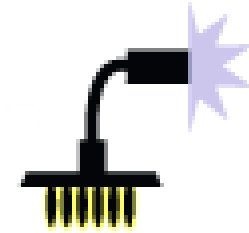
Modulated Gene expression



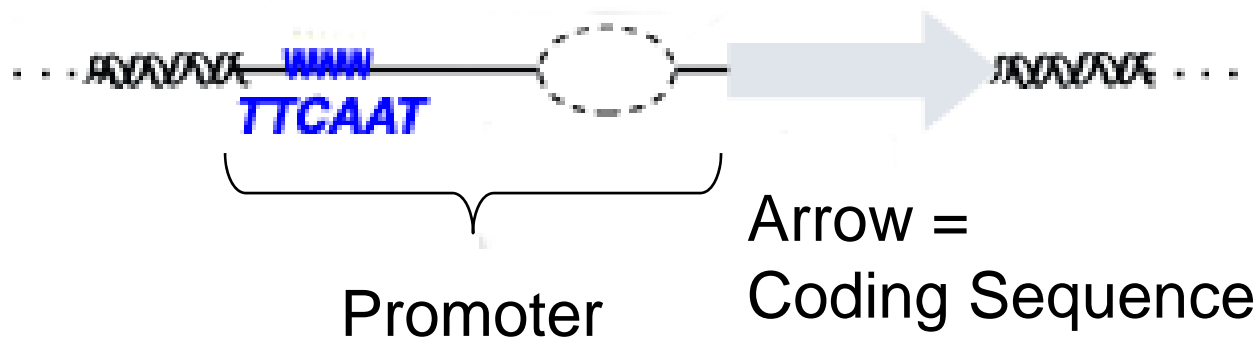
Tet-Repressible Expression



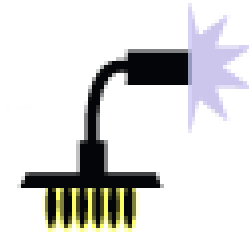
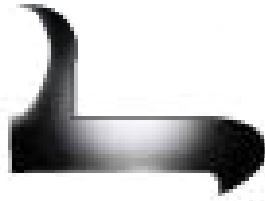
RNA Polymerase



Transcription Factor

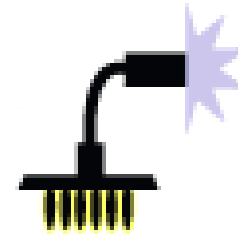
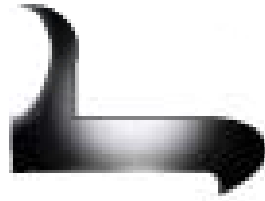


Tet-Repressible Expression



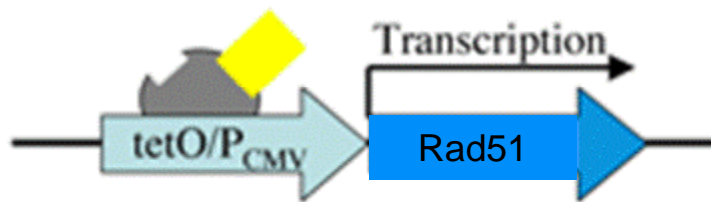
Expression is Off

Tet-Repressible Expression



Expression is On

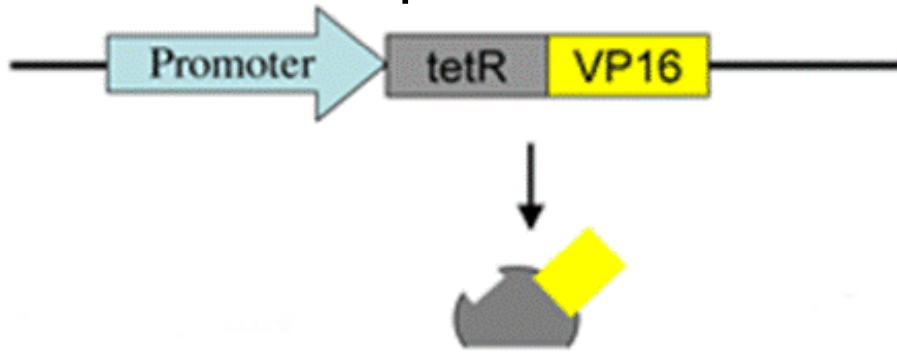
Tet-Repressible Expression



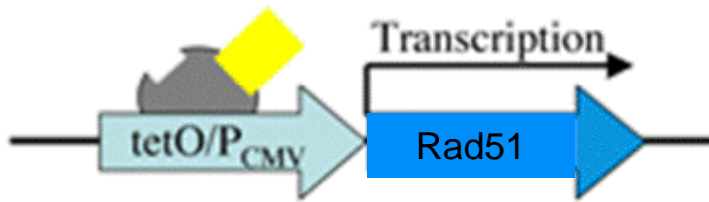
Expression On

Tet-Repressible Expression

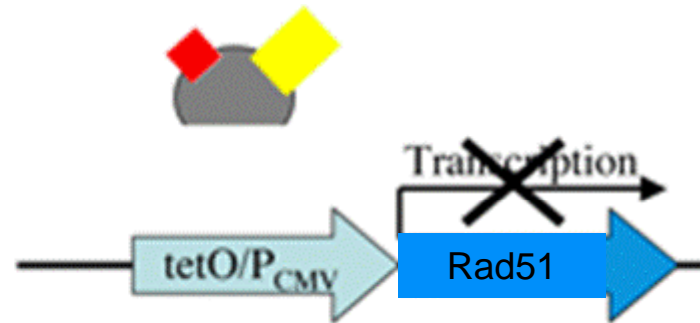
Vector for Expression of Transcription Factor



Add Doxycycline
(= a tetracycline analog)

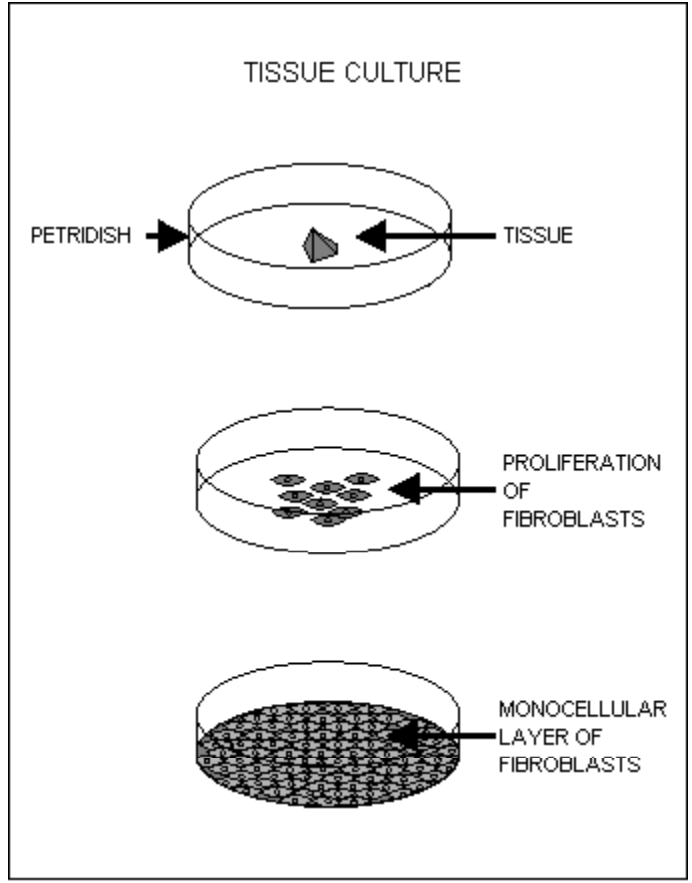


Expression On



Expression Off

Culturing Mammalian Cells



How do you grow
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 (usually in serum)

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

Salts

Amino Acids

	D 0422		
	[IX]		
COMPONENT	g/L		
INORGANIC SALTS			
CaCl ₂ ·2H ₂ O	0.265	VITAMINS	
Fe(NO ₃) ₃ ·9H ₂ O	0.0001	Choline Bitartrate	—
MgSO ₄	0.09767	Choline Chloride	0.004
KCl	0.4	Folic Acid	0.004
NaHCO ₃	3.7	myo-Inositol	0.0072
NaCl	6.4	Niacinamide	0.004
NaH ₂ PO ₄	0.109	D-Pantothenic Acid·½Ca	0.004
Succinic Acid	—	Pyridoxal·HCl	—
Sodium Succinate	—	Pyridoxine·HCl	0.004
AMINO ACIDS		Riboflavin	0.0004
L-Arginine·HCl	0.084	Thiamine·HCl	0.004
L-Cystine·2HCl	—	OTHER	
L-Glutamine	—	D-Glucose	4.3
Glycine	0.03	HEPES	—
L-Histidine·HCl·H ₂ O	0.042	Phenol Red·Na	0.0159
L-Isoleucine	0.105	Pyruvic Acid·Na	0.11
L-Leucine	0.105		
L-Lysine·HCl	0.146	ADD	
L-Methionine	—	Glucose	—
L-Phenylalanine	0.066	L-Glutamine	0.584
L-Serine	0.042	L-Cystine·2HCl	—
L-Threonine	0.095	L-Leucine	—
L-Tryptophan	0.016	L-Lysine·HCl	—
L-Tyrosine (free base)	—	L-Methionine	—
L-Tyrosine·2Na·2H ₂ O	0.10379	NaHCO ₃	—
L-Valine	0.094	NaH ₂ PO ₄	—
		Phenol Red·Na	—
		Pyruvic Acid·Na	—
		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 (CO₂) and bicarbonate anion (HCO₃⁻).

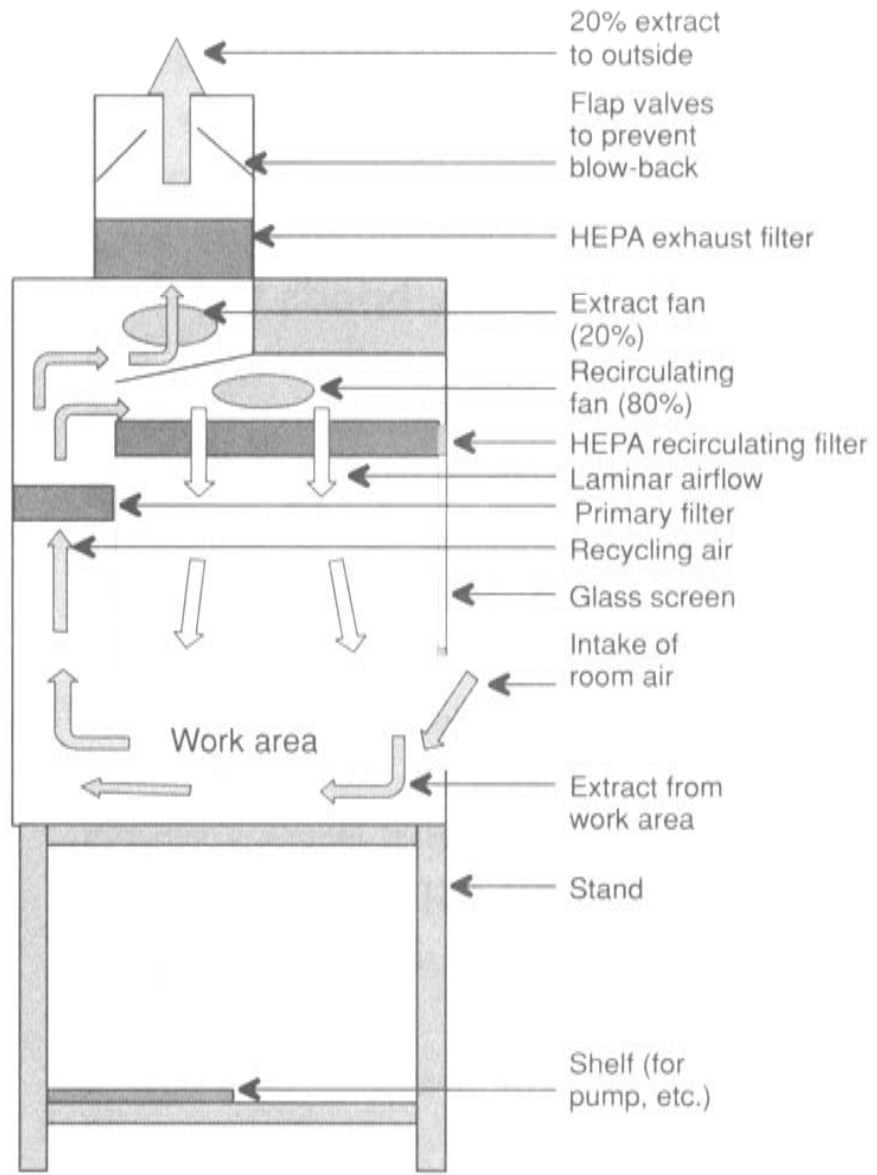


The pH is determined by the concentration of CO₂ and bicarbonate.

Mammalian Cell Incubators



Why is sterility
important?



(b) VERTICAL LAMINAR FLOW

Mammalian Cell Culture Hood

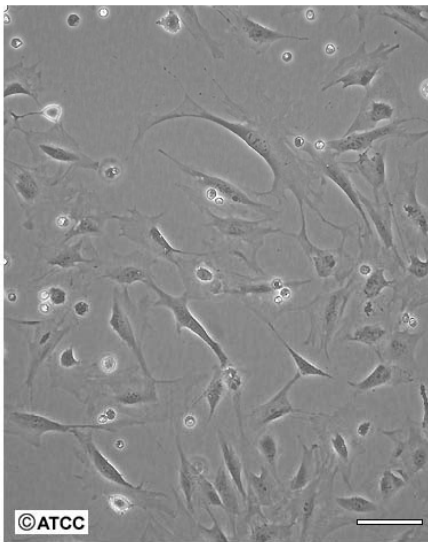


Hands-On..

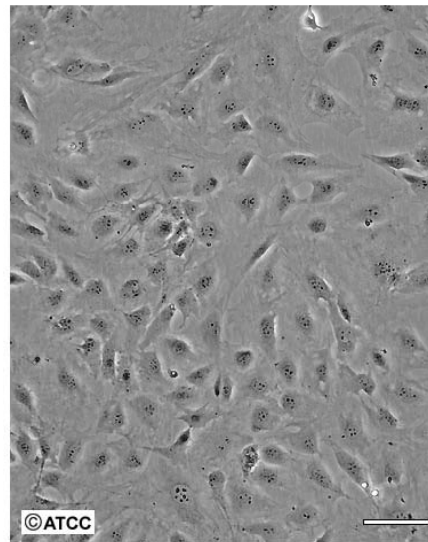
What does it mean to
“pass” your cultures?

Cell Density (cells per cm²)

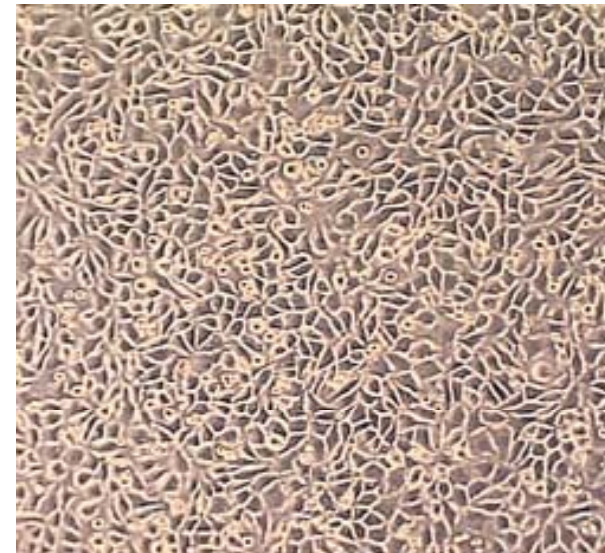
ATCC Number: **CCL-92**
Designation: **3T3 Swiss Albino**



Low Density



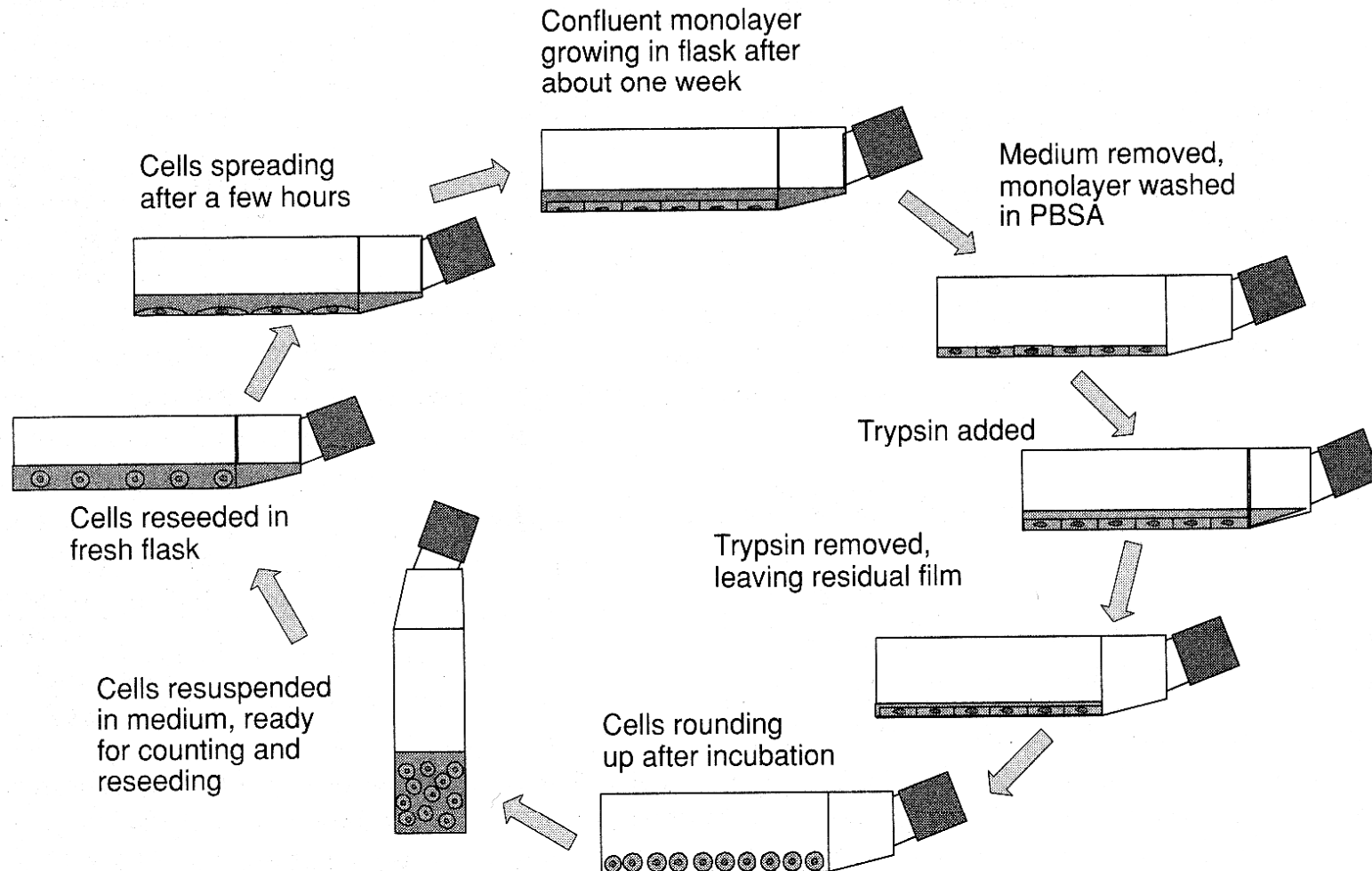
High Density



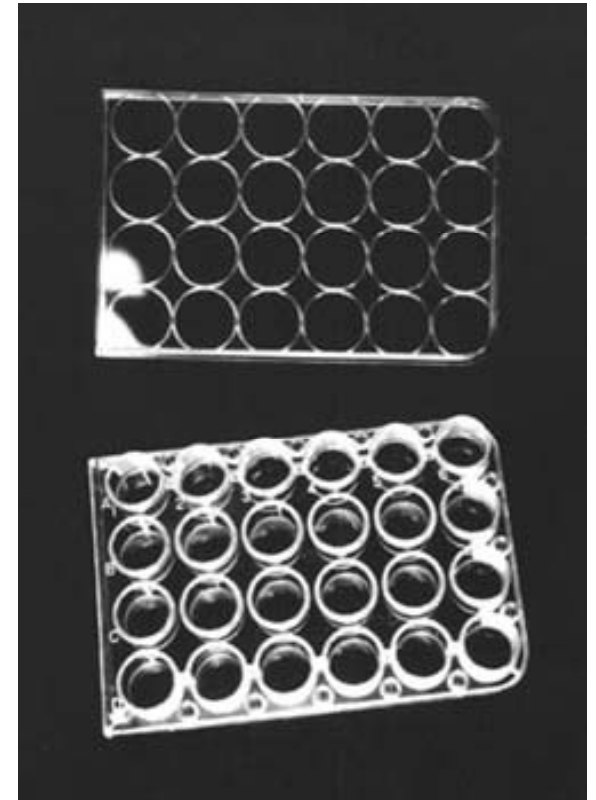
Confluent

Confluent cells are often contact inhibited

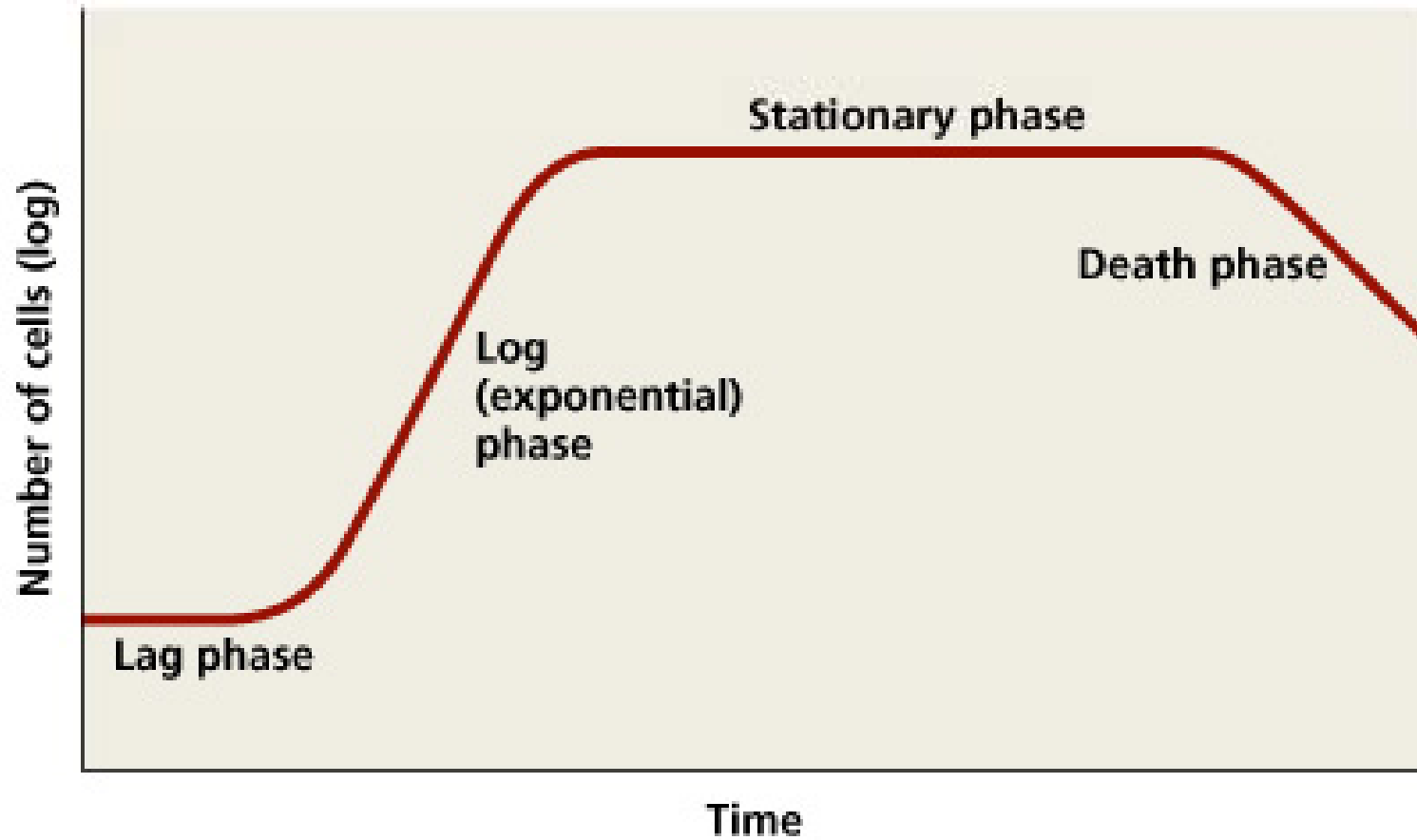
Cell Density can be kept low through “Passaging”



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



Cell Culture Growth Phases



Things you need to know about your cells...

Lag phase

Log phase

Doubling time

Optimal Maximum Density

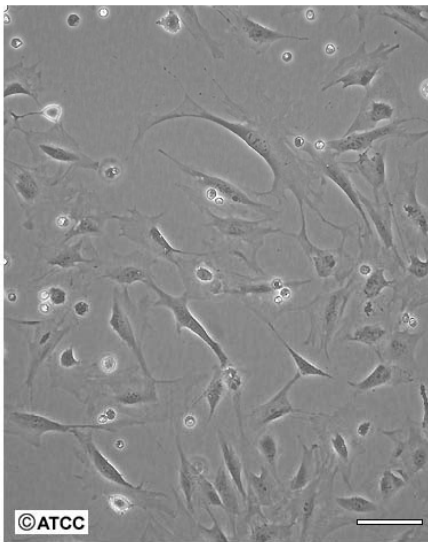
Optimal Minimum Density

Growth inhibition – arrest

Plating Efficiency

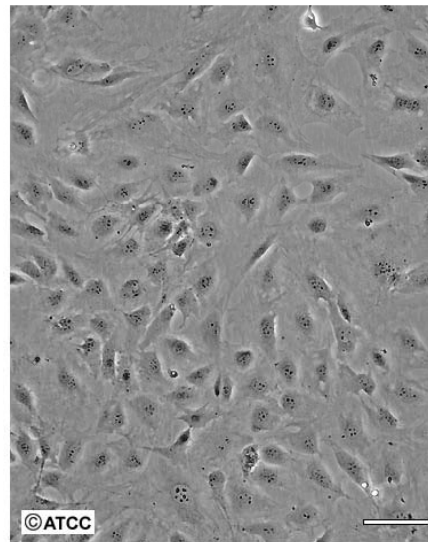
Contact Inhibition

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



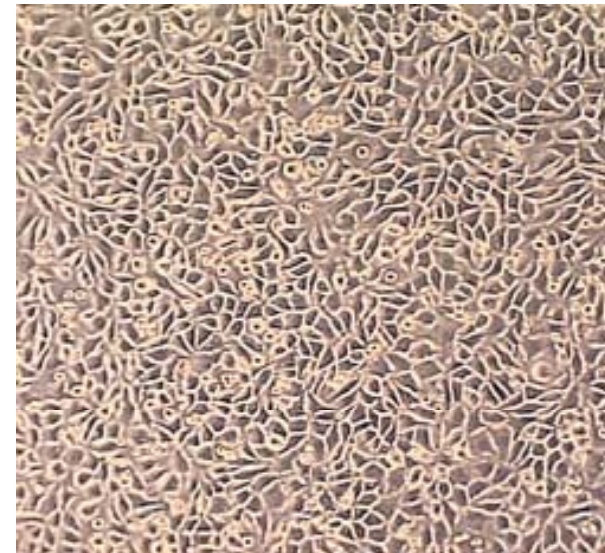
© ATCC
Low Density

Scale Bar = 100µm



© ATCC
High Density

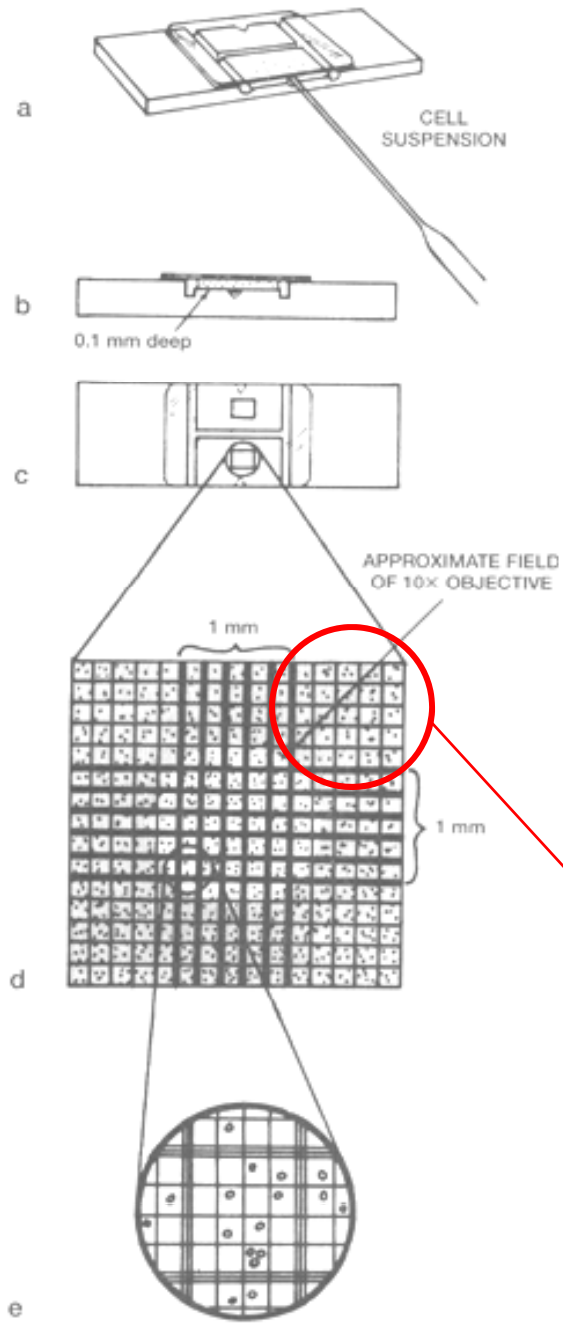
Scale Bar = 100µm



Low Density

High Density

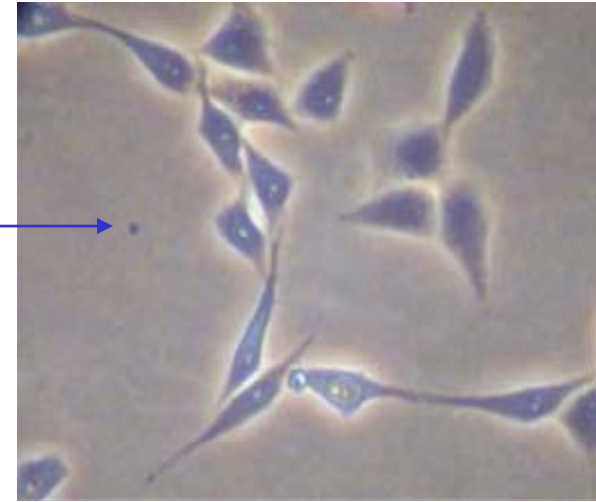
Confluent



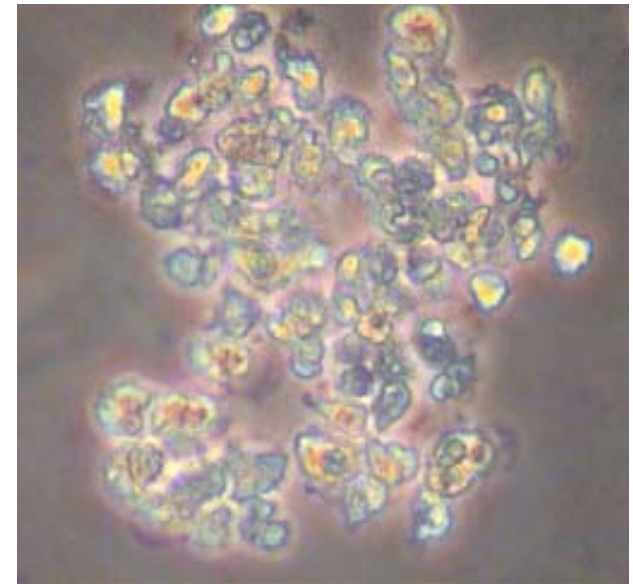
Figuring out how many cells you have in your dish...

You need to know what your cells should look like!

Healthy
Hela
Cells



Sick Cells:



The role of homologous recombination in rescuing cells from chemotherapeutics.

Culturing Mammalian Cells