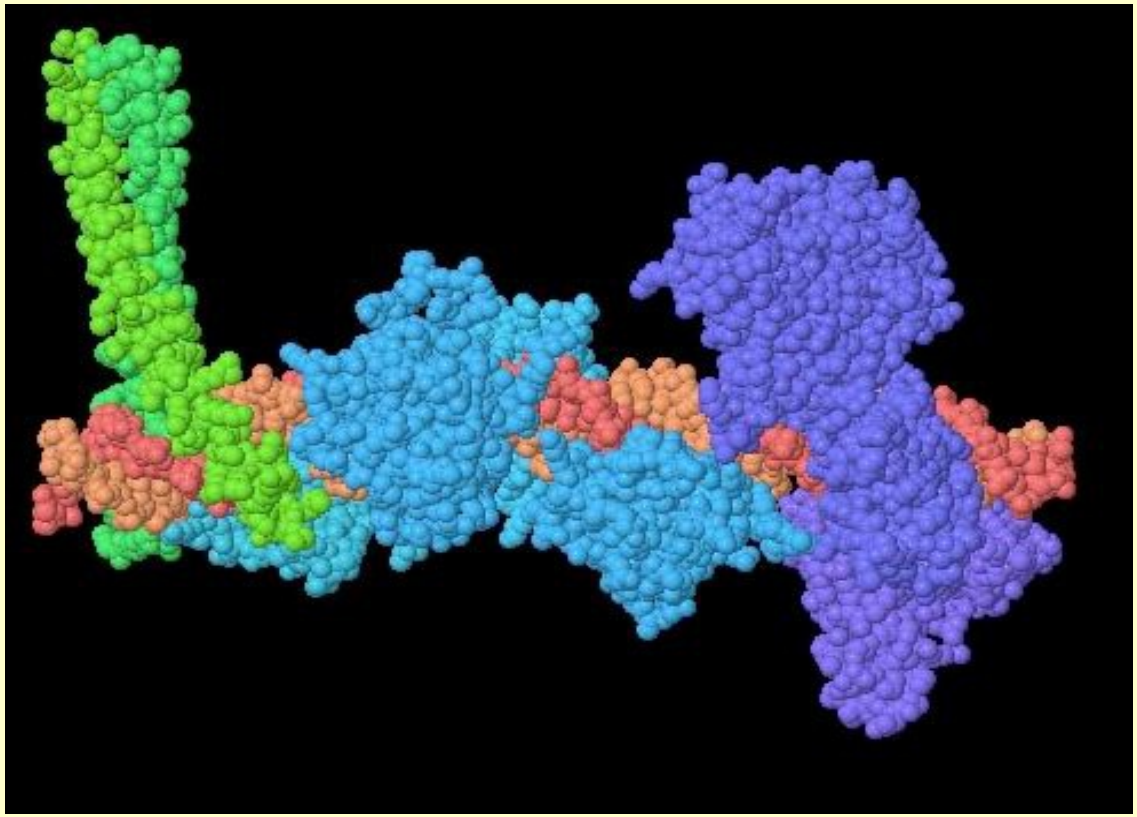
Your Genes and Your Health

http://bio84.stanford.edu/

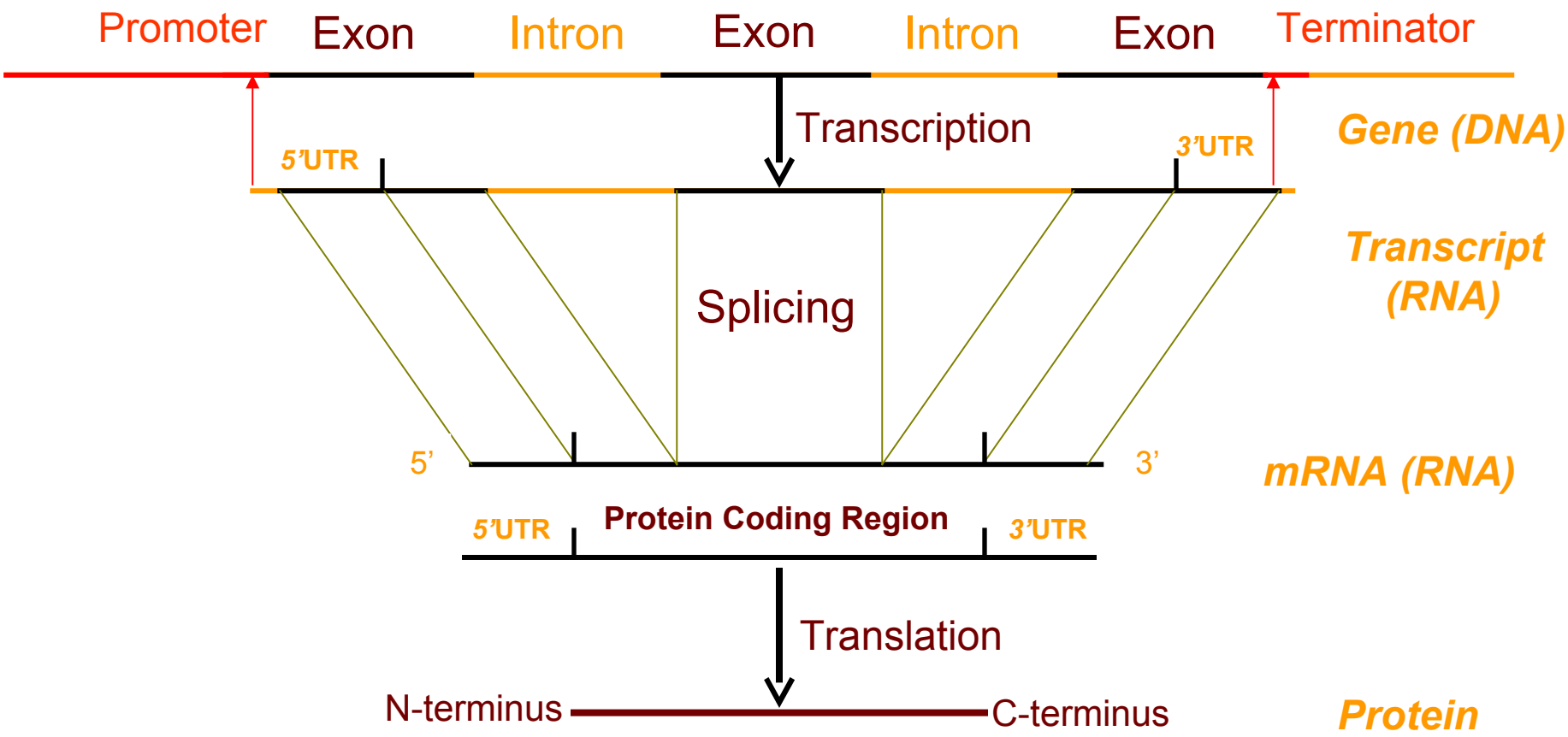
Stanford Continuing Studies

http://continuingstudies.stanford.edu/



Doug Brutlag, Professor Emeritus
Biochemistry & Medicine (by courtesy)
Stanford University School of Medicine

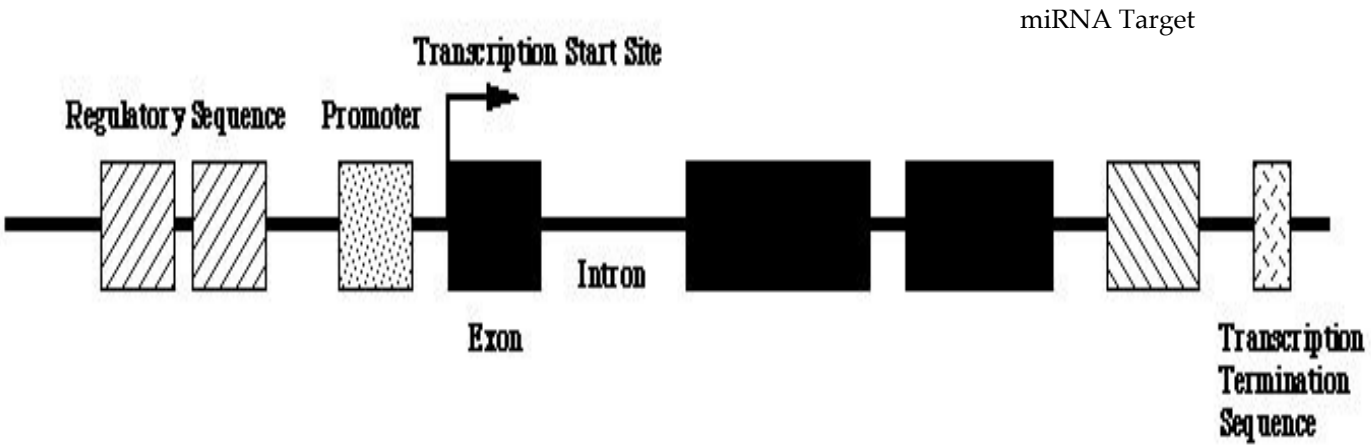
A Human Gene and Transcription to RNA and Translation to Protein



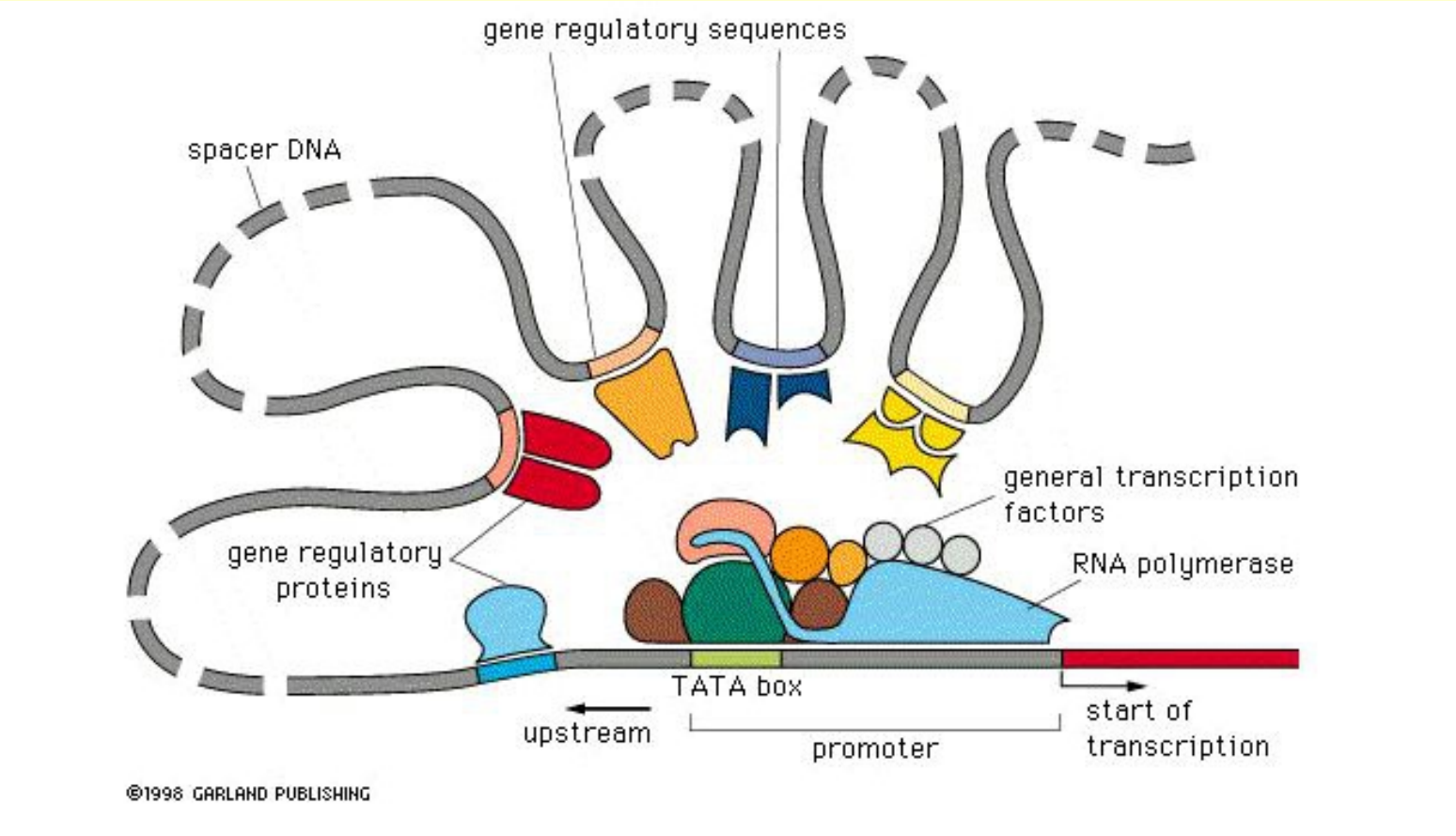
Gene Regulatory Mechanisms

- Transcriptional Mechanisms
  - Type of promoters & RNA polymerases
  - Control of transcription by transcription factors and TFBS
- RNA processing
  - 5′ capping & 3′ poly-adenylation
  - RNA degradation rates
  - Splicing and alternative splicing
- Translational Mechanisms
  - Micro RNAs (miRNAs) inhibit translation and degrade mRNA
  - Silencer RNAs (siRNAs or RNAi) degrading mRNA
- Epigenetic Mechanisms
  - DNA methylation
  - Histone modifications: acetylation, methylation, phosphorylation, etc.
  - Chromatin remodeling

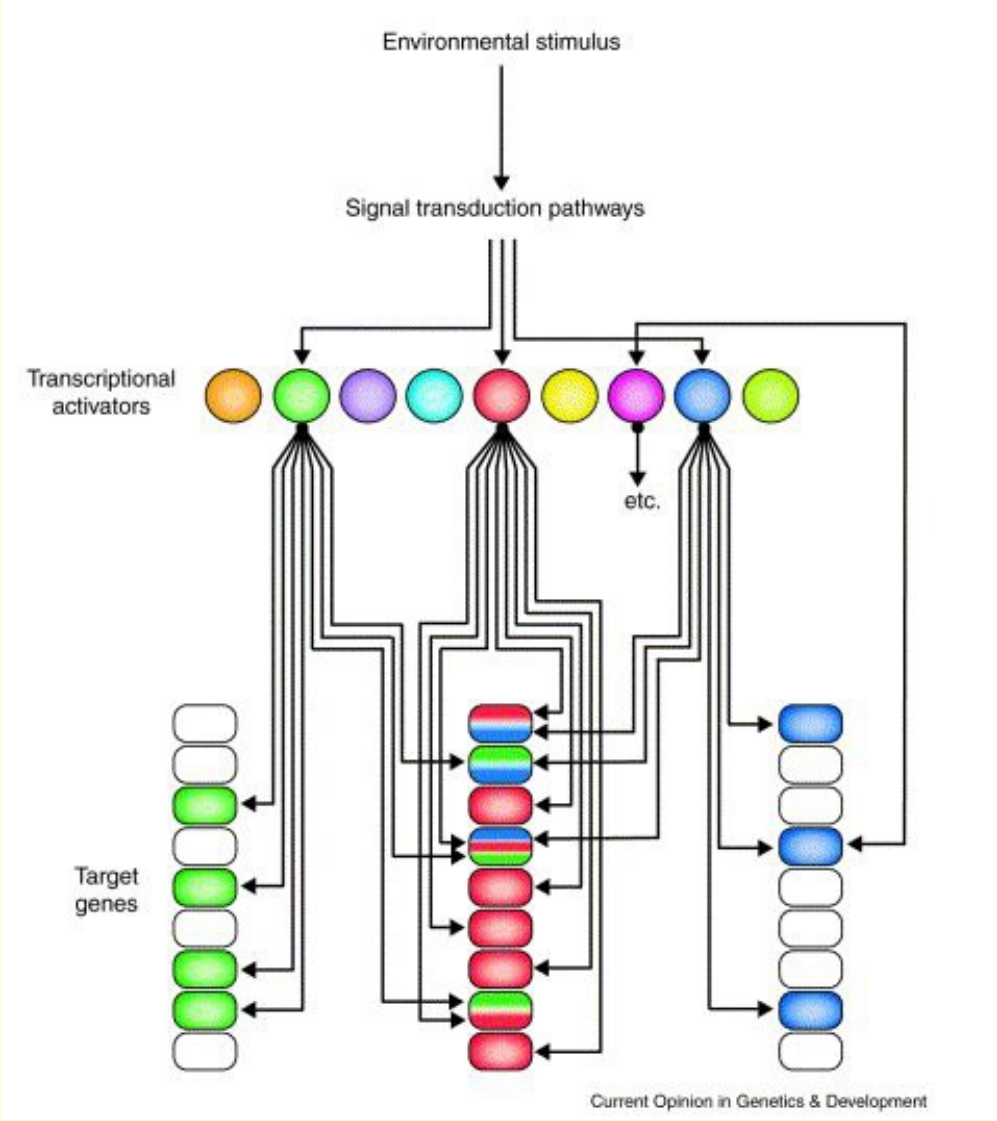
Eukaryotic Gene Structure



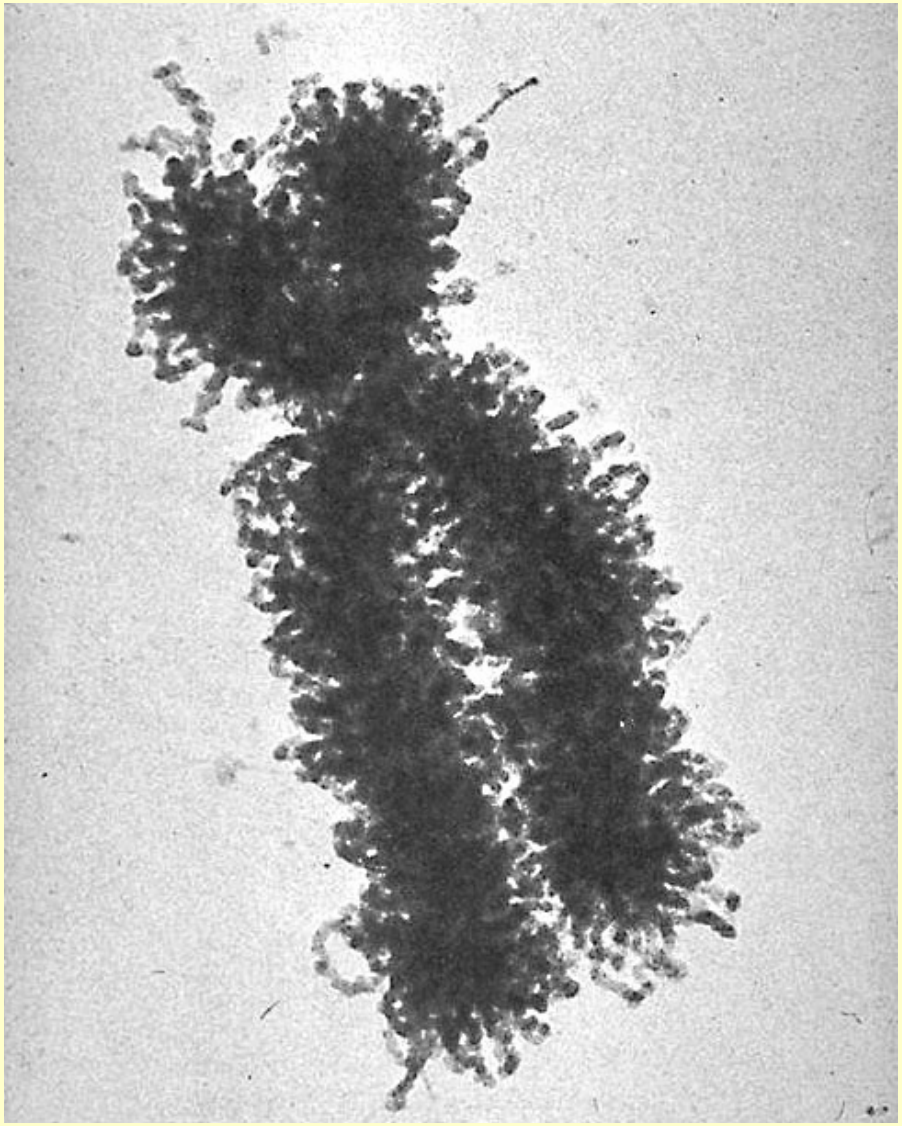
Multiple Enhancer Sequences



Gene Expression Regulatory Network



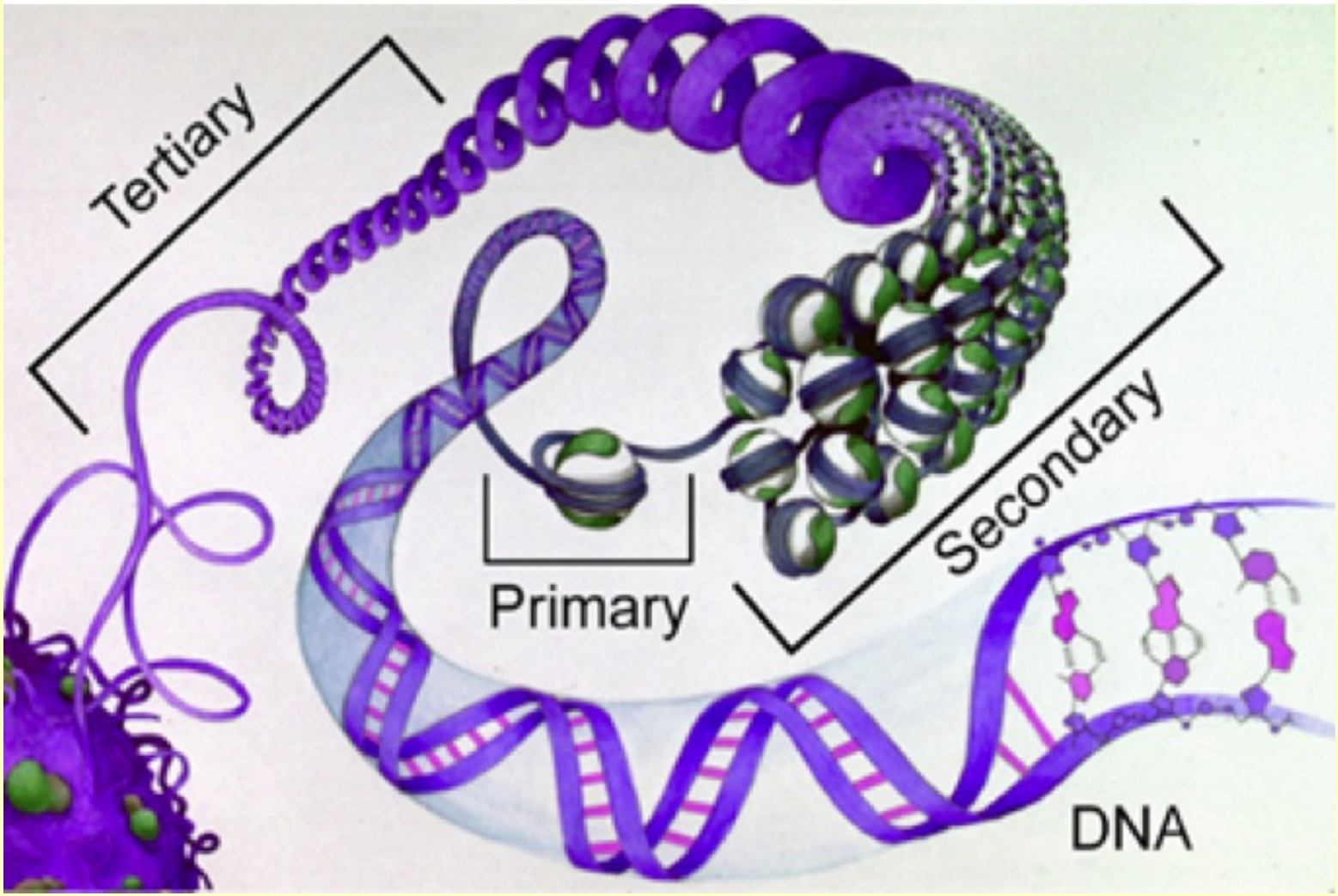
Human Mitotic Chromosome



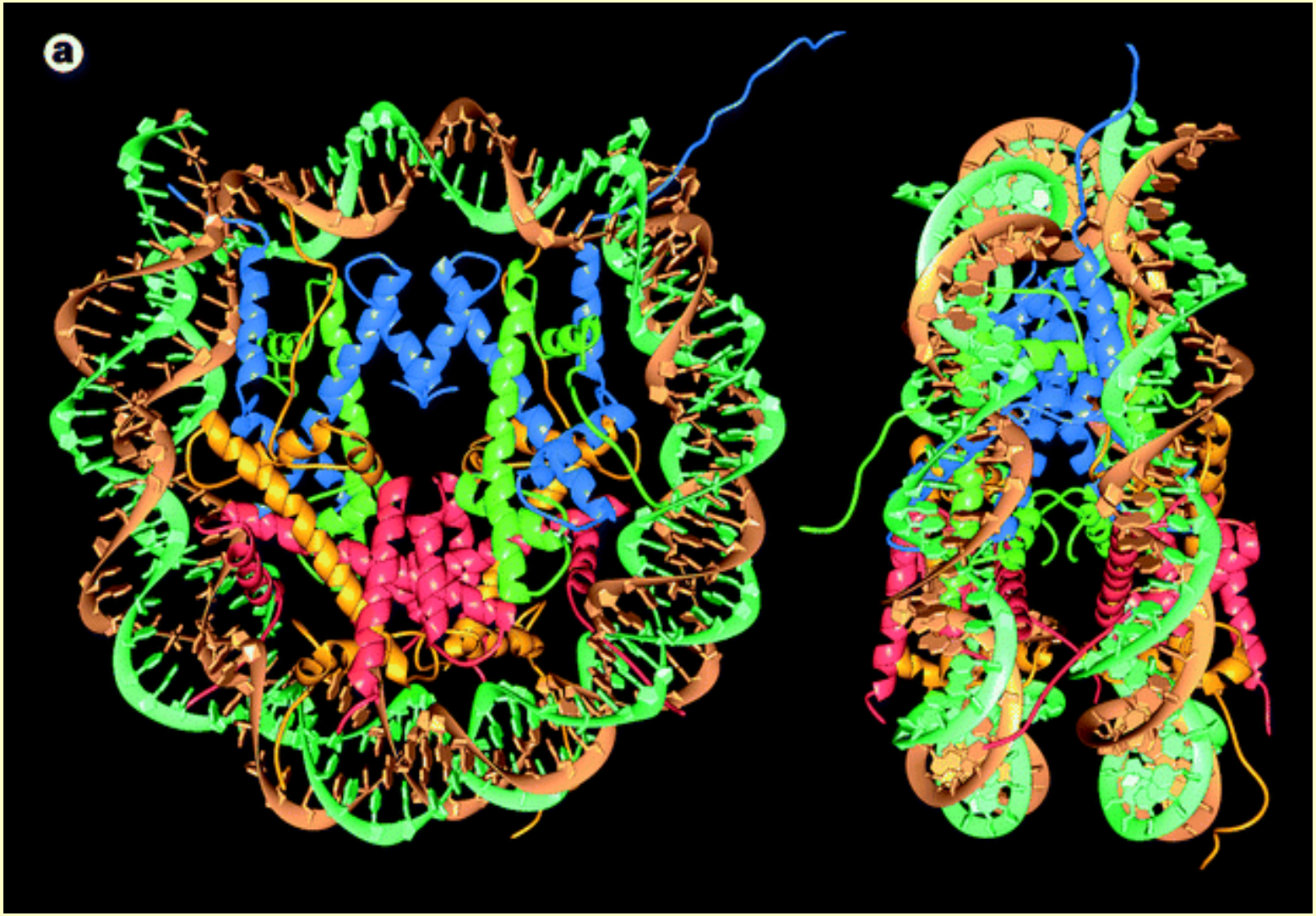
DNA in a Human Chromosome

DNA in a Human Chromosome

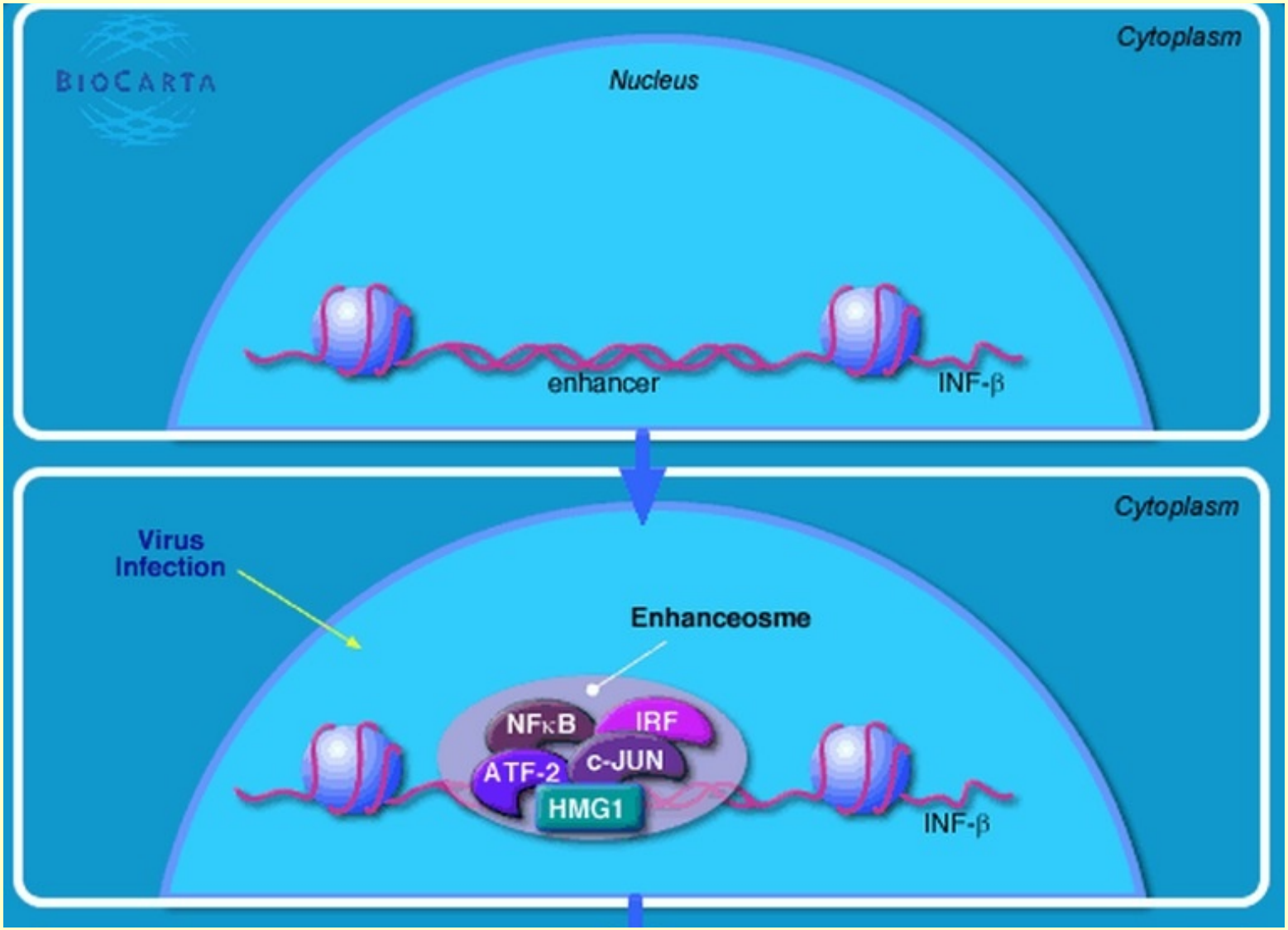
Three Levels of Folding of DNA in Chromatin



Folding of DNA in Nucleosome

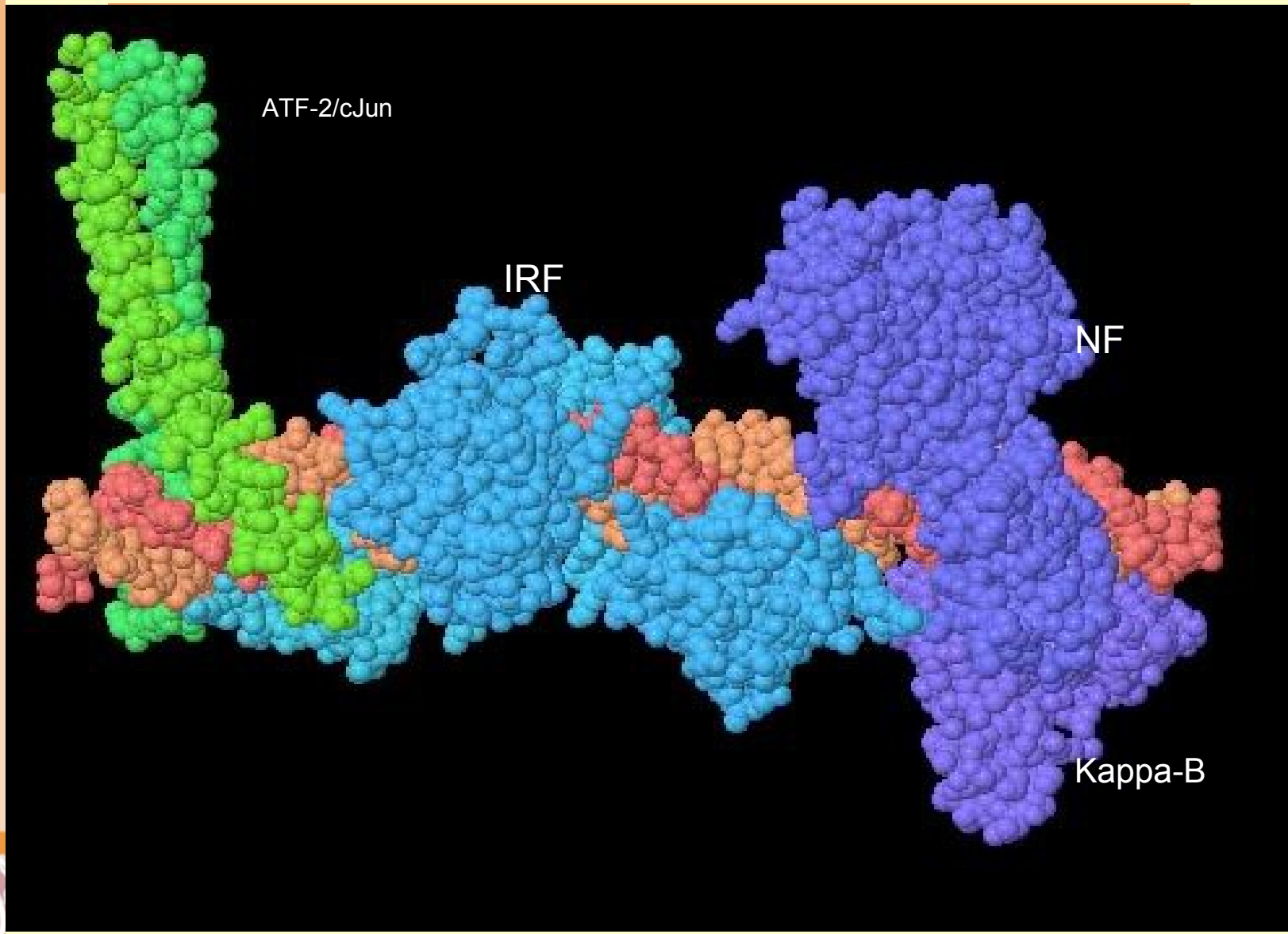


Interferon-Beta Gene Enhancer in Macrophage Cells



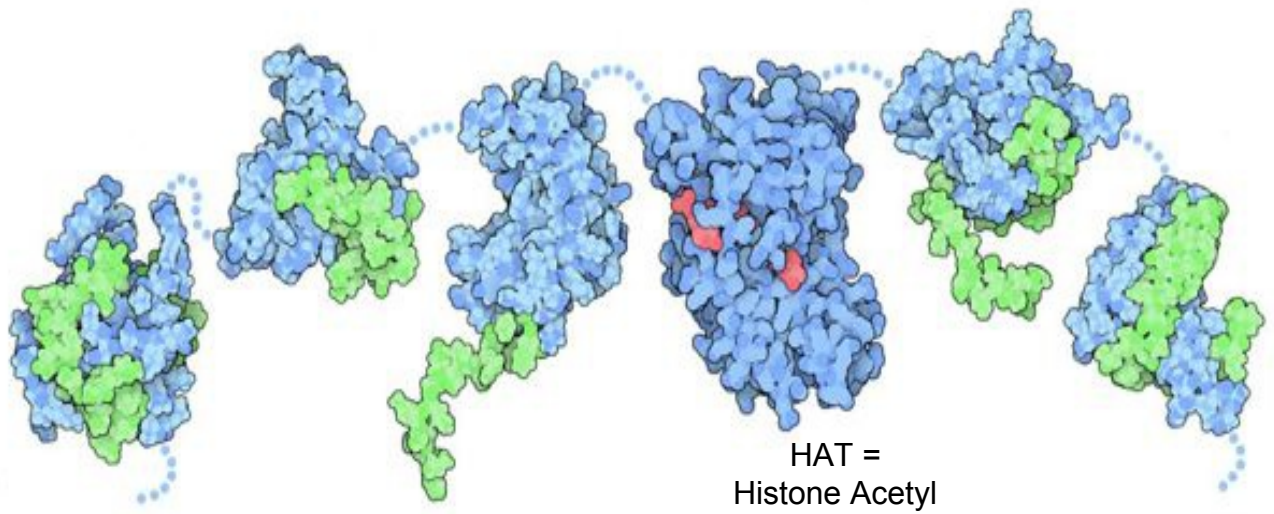
Enhanceosome: Exploring the Structure

http://www.rcsb.org/pdb/education_discussion/molecule_of_the_month/download/Enhanceosome.pdf



Enhanceosome: Integrating the Signal

http://www.rcsb.org/pdb/static.do?p=education_discussion/molecule_of_the_month/pdb122_2.html

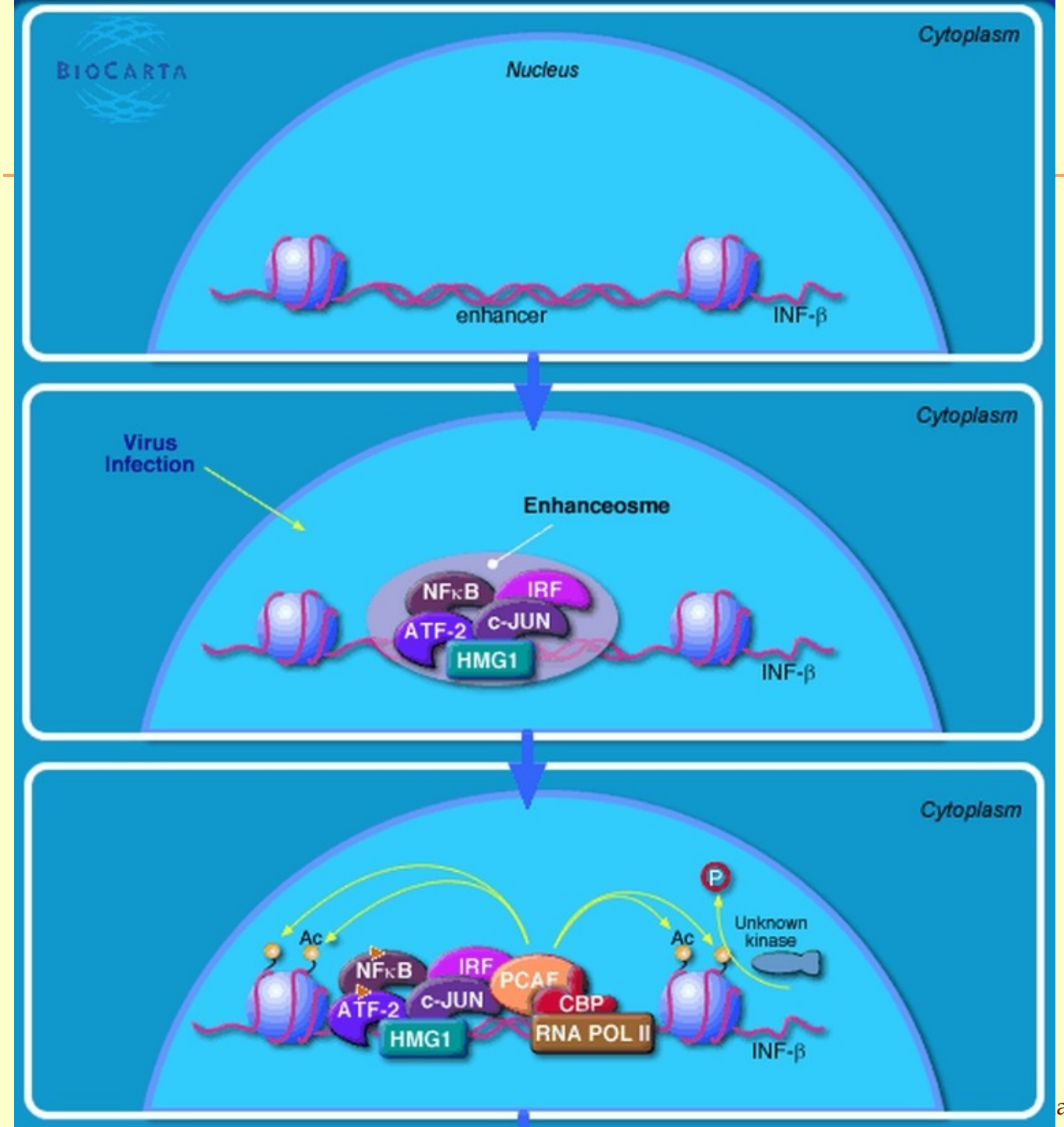


HAT =
Histone Acetyl
Transferase

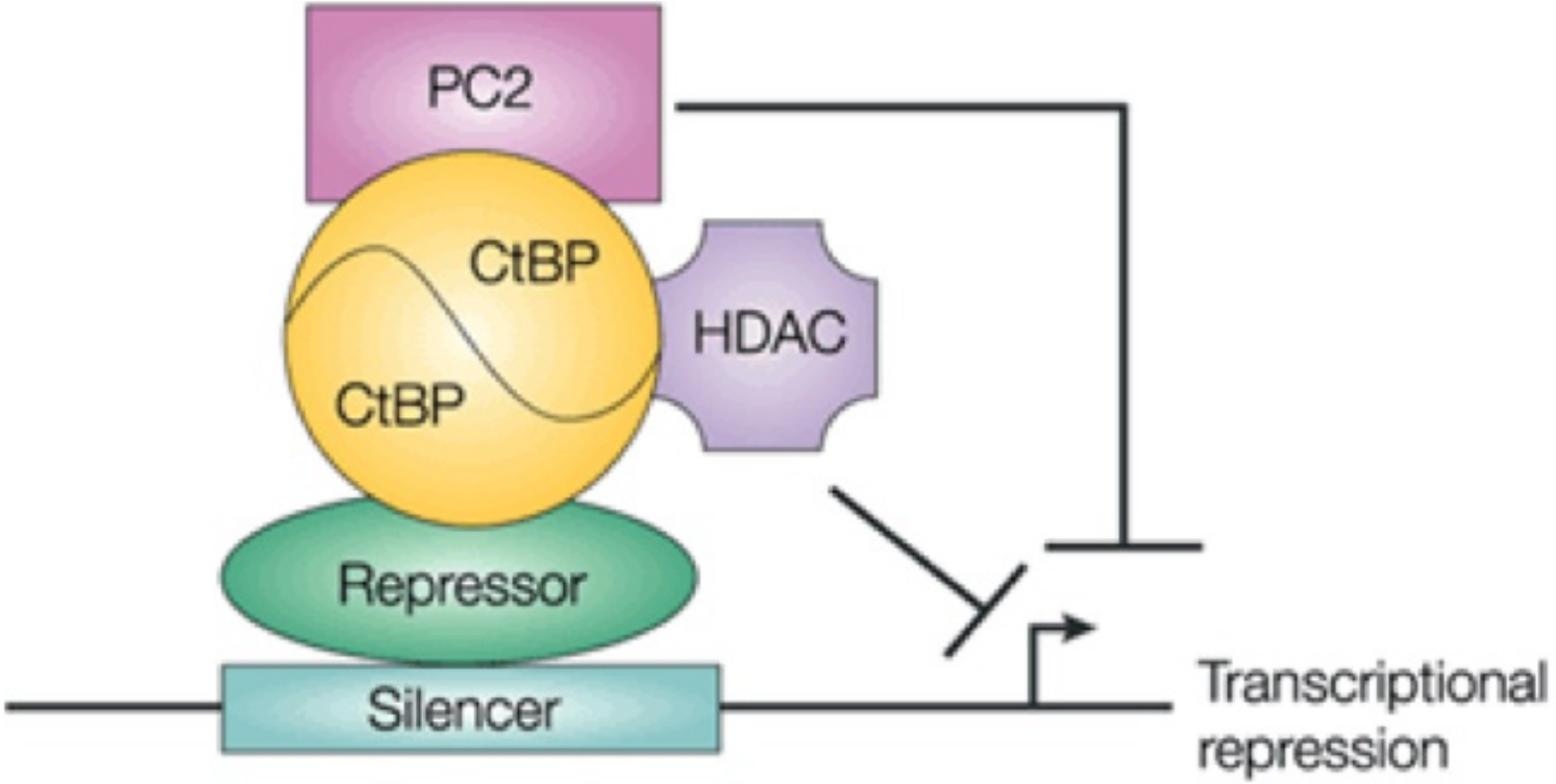
Integrating the Signal

Once the transcription factors bind to the different sites in the enhancer DNA sequence, the signal must somehow be sensed and used to activate the gene. In many cases, this is performed using CREB-binding protein or the similar protein p300. This protein is composed of many connected domains, (PDB entries **1l8c**, **1kdx**, **1jsp**, **3biy**, **2ka6** and **1kbh**), which bind to different proteins in the assembled enhanceosome. Then, a large domain in the center acts as a histone acetyltransferase, modifying histones in nucleosomes and causing them to disassemble and reveal the gene. In the intereferon-b gene, a nucleosome normally covers the start site of transcription, blocking transcription. Assembly of the enhanceosome leads to removal of this nucleosome, allowing the gene to be expressed.

Previous: **Enhanceosome** Home: **Enhanceosome** Next: **Exploring the Structure**

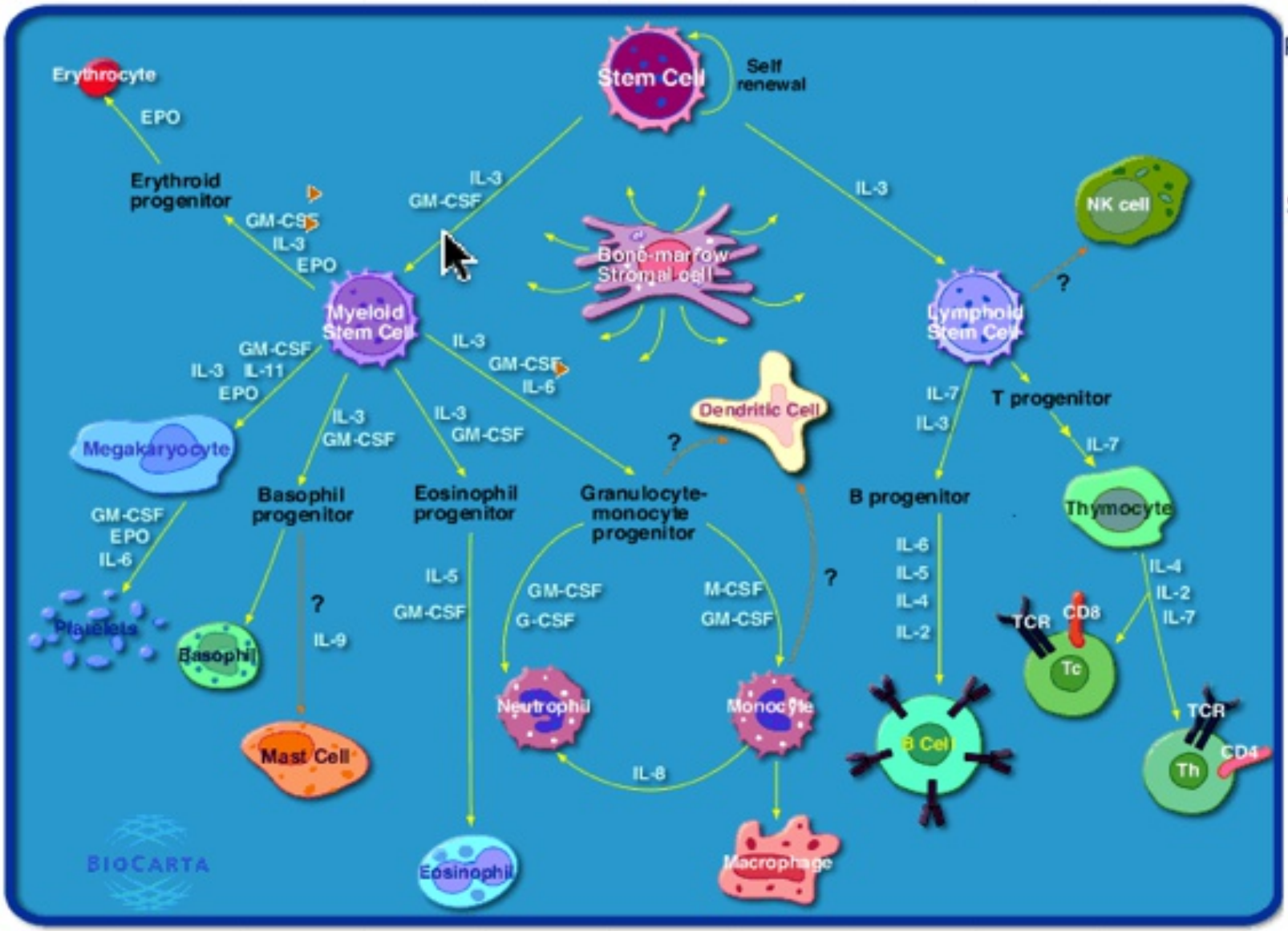

BIOCARTA
Cytoplasm
Nucleus
enhancer
INF-β
Cytoplasm
Virus
Infection
Enhanceosme
NFκB
IRF
ATF-2
c-JUN
HMG1
INF-β
Cytoplasm
Ac
P
Unknown
kinase
Ac
NFκB
IRF
PCAF
ATF-2
c-JUN
CBP
HMG1
RNA POL II
INF-β

Repressor Bound to Silencer Sites



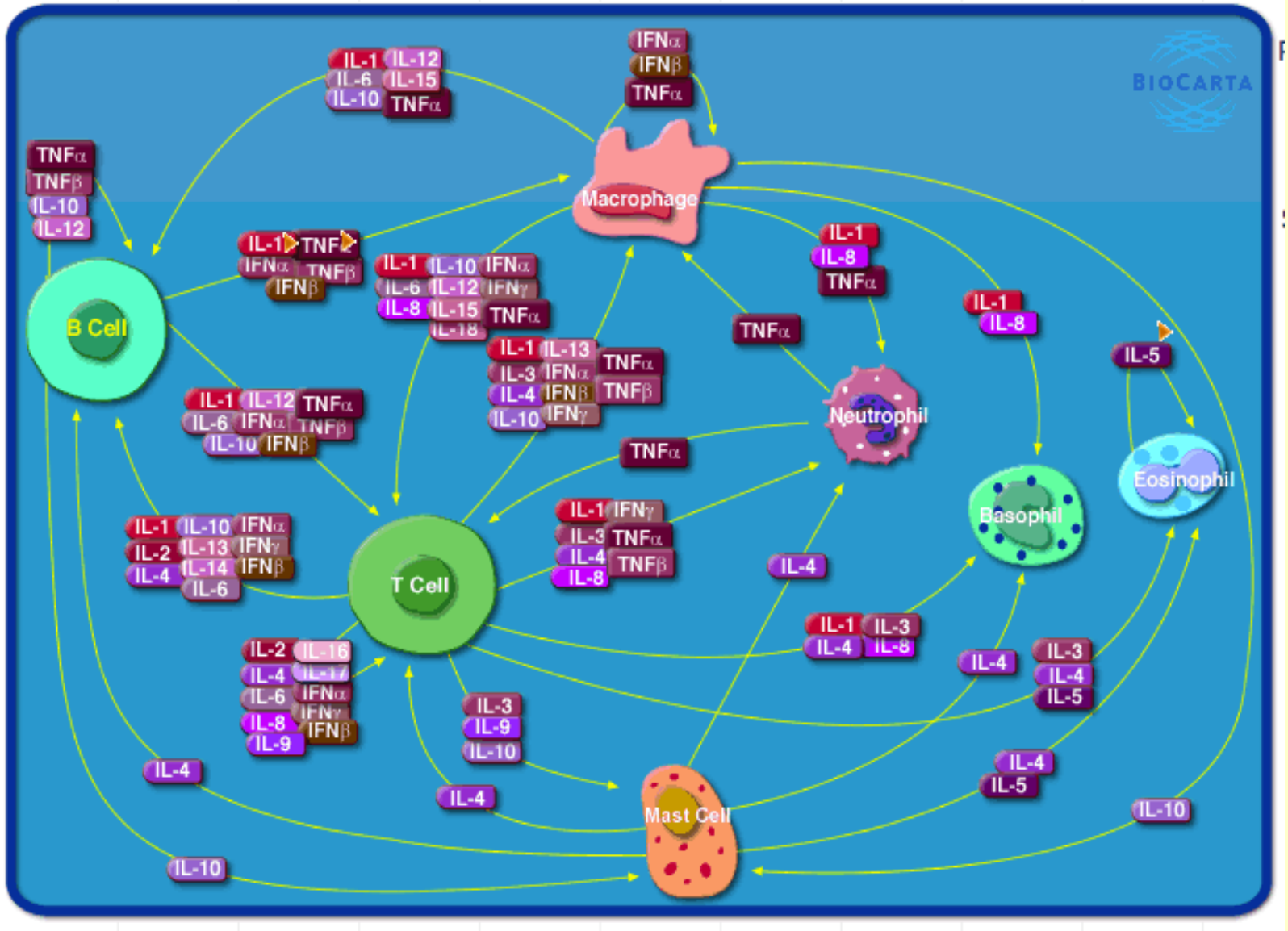
Hematopoiesis

http://www.biocarta.com/pathfiles/h_stemPathway.asp

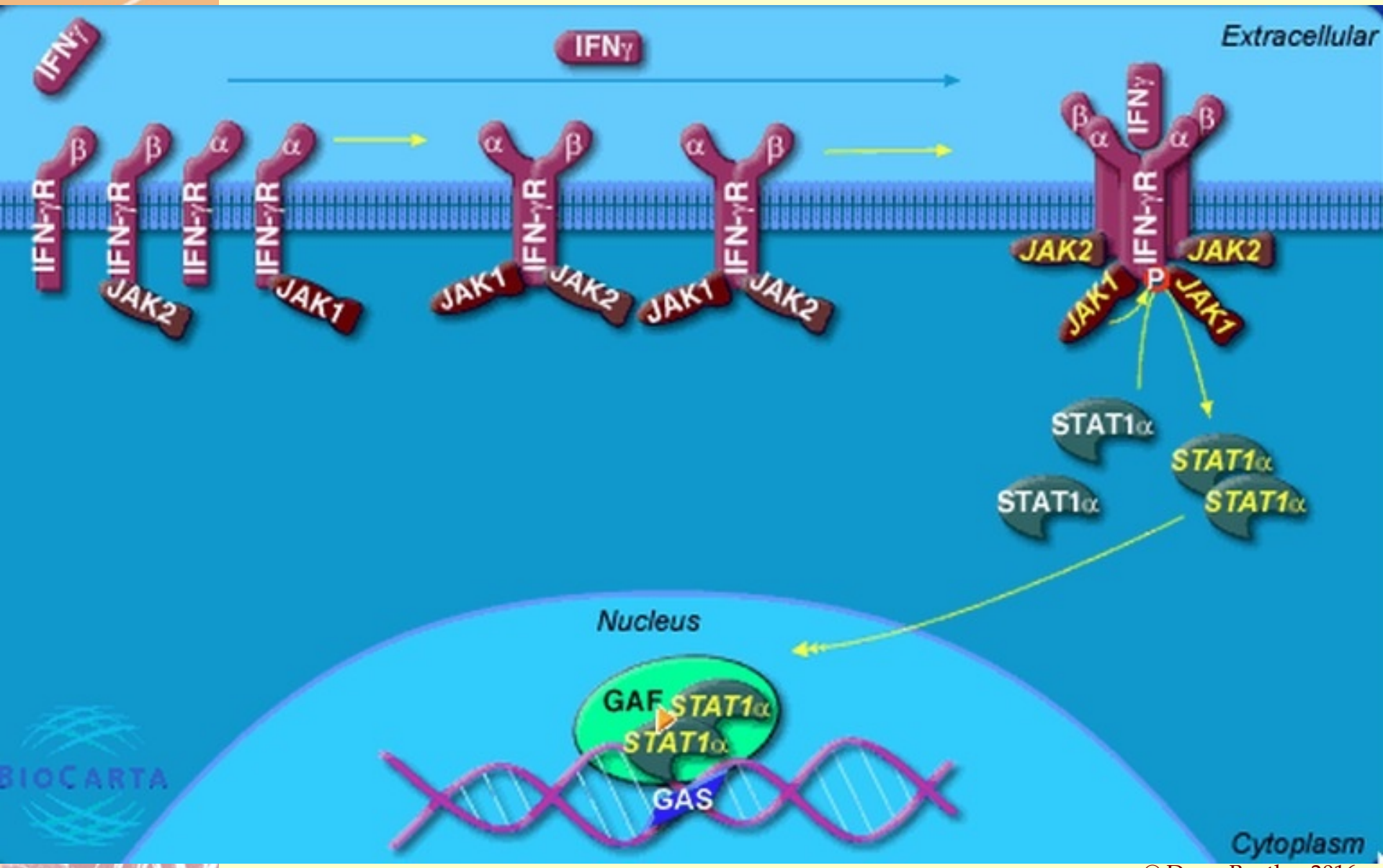


Cytokine Network

http://www.biocarta.com/pathfiles/h_cytokinePathway.asp



Interferon Signaling

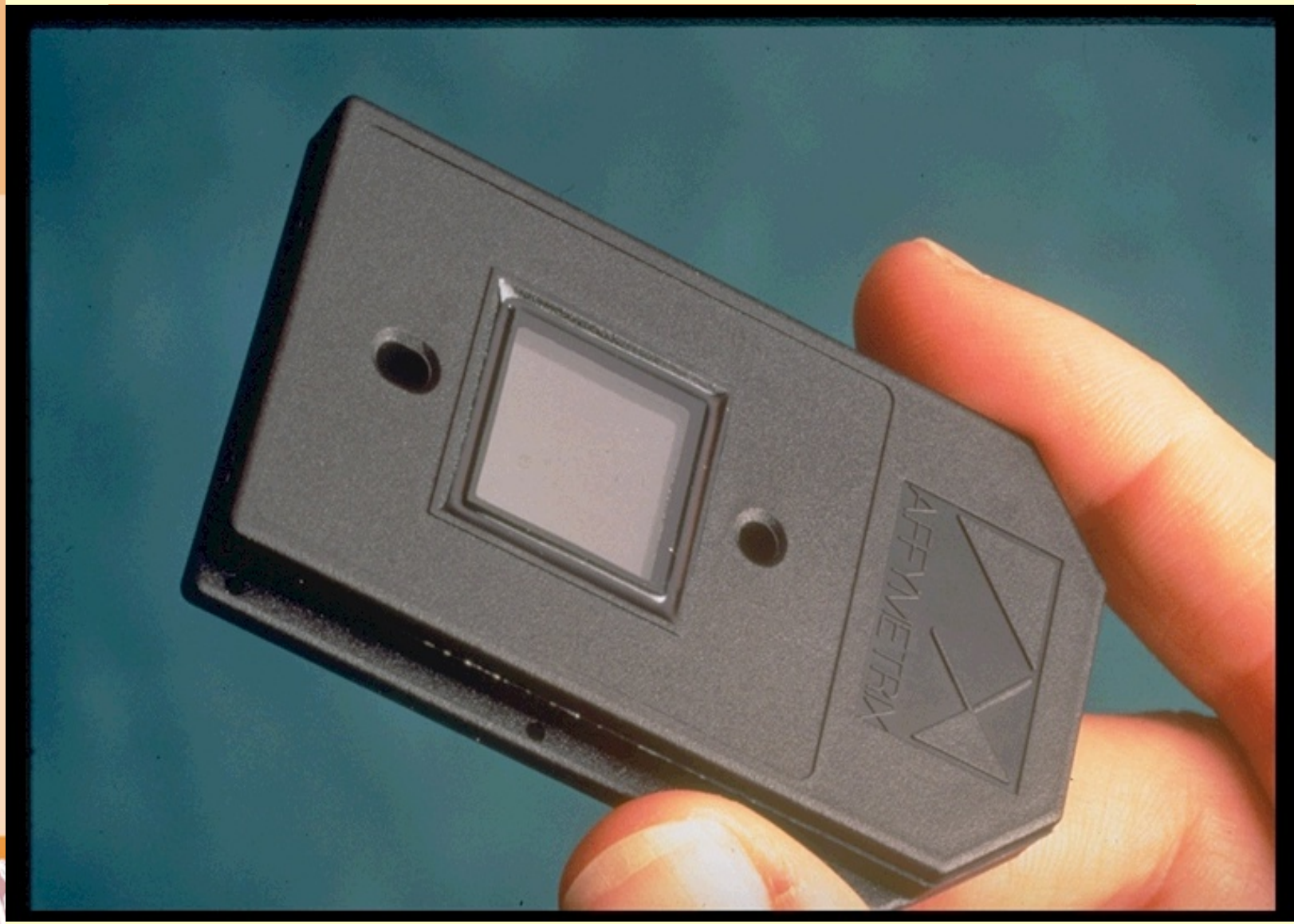


DNA Microarrays & DNA Chips Accelerate Genetic Analysis

- Parallel analyses
  - Analyze entire genomes instead of single genes
  - Analyze expression of entire genome
  - Analyze genetic polymorphisms (SNPs)
- Miniaturization
- Automation

Diagnosis Using DNA Arrays

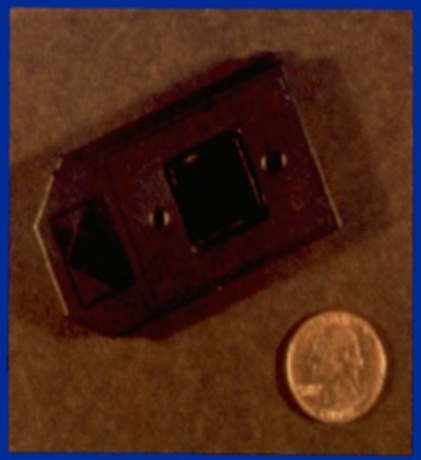
http://www.affymetrix.com/



Affymetrix Arrays

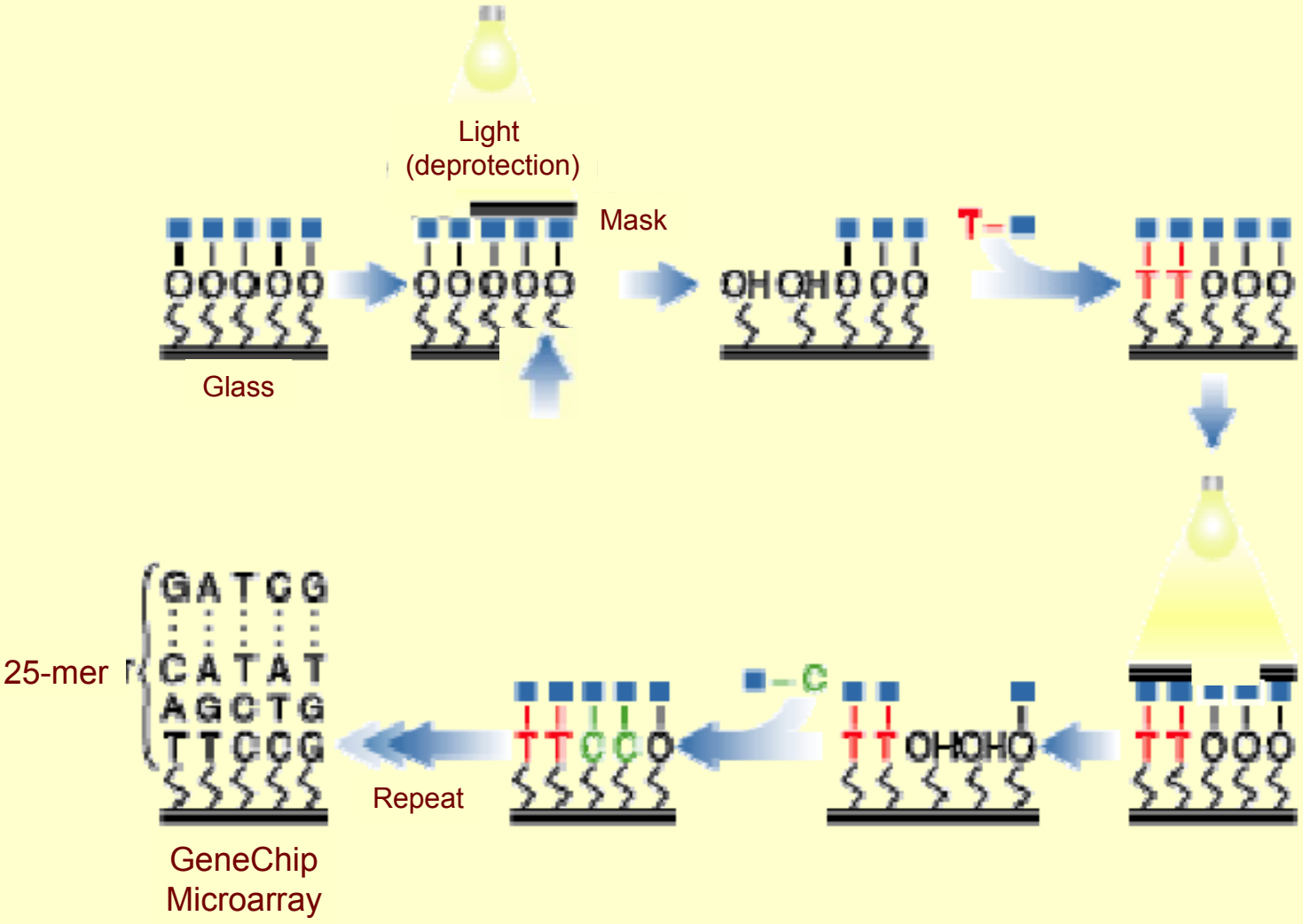
http://www.affymetrix.com/

- High-density grid of DNA sequences.
- Any collection of 25mers (1,200,000) can be synthesized in 100 steps.
- The location and identity of each sequence on the glass surface is known.



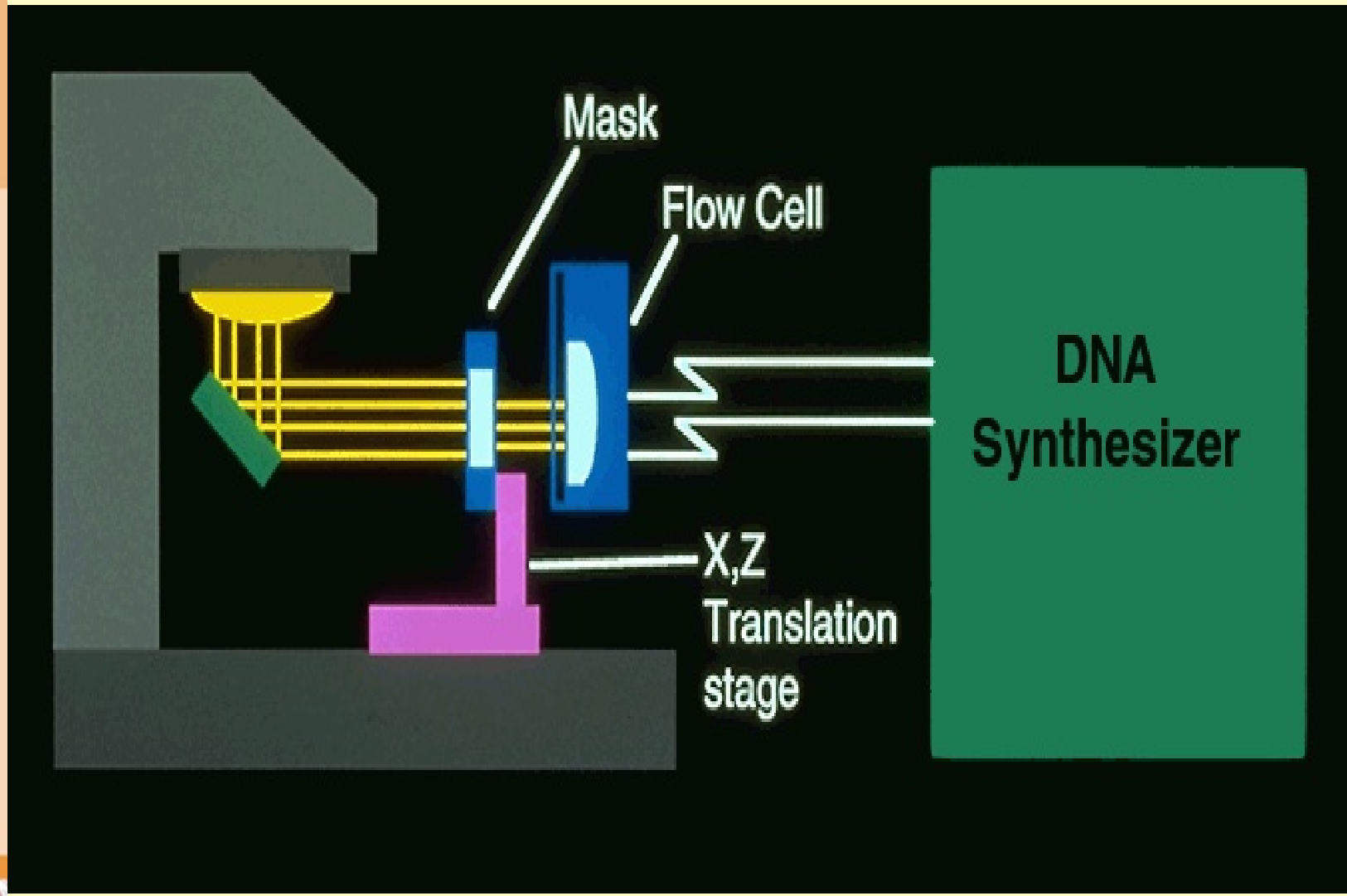
Light Directed Oligonucleotide Synthesis

http://www.youtube.com/watch?v=ui4BOtwJEXs&feature=related



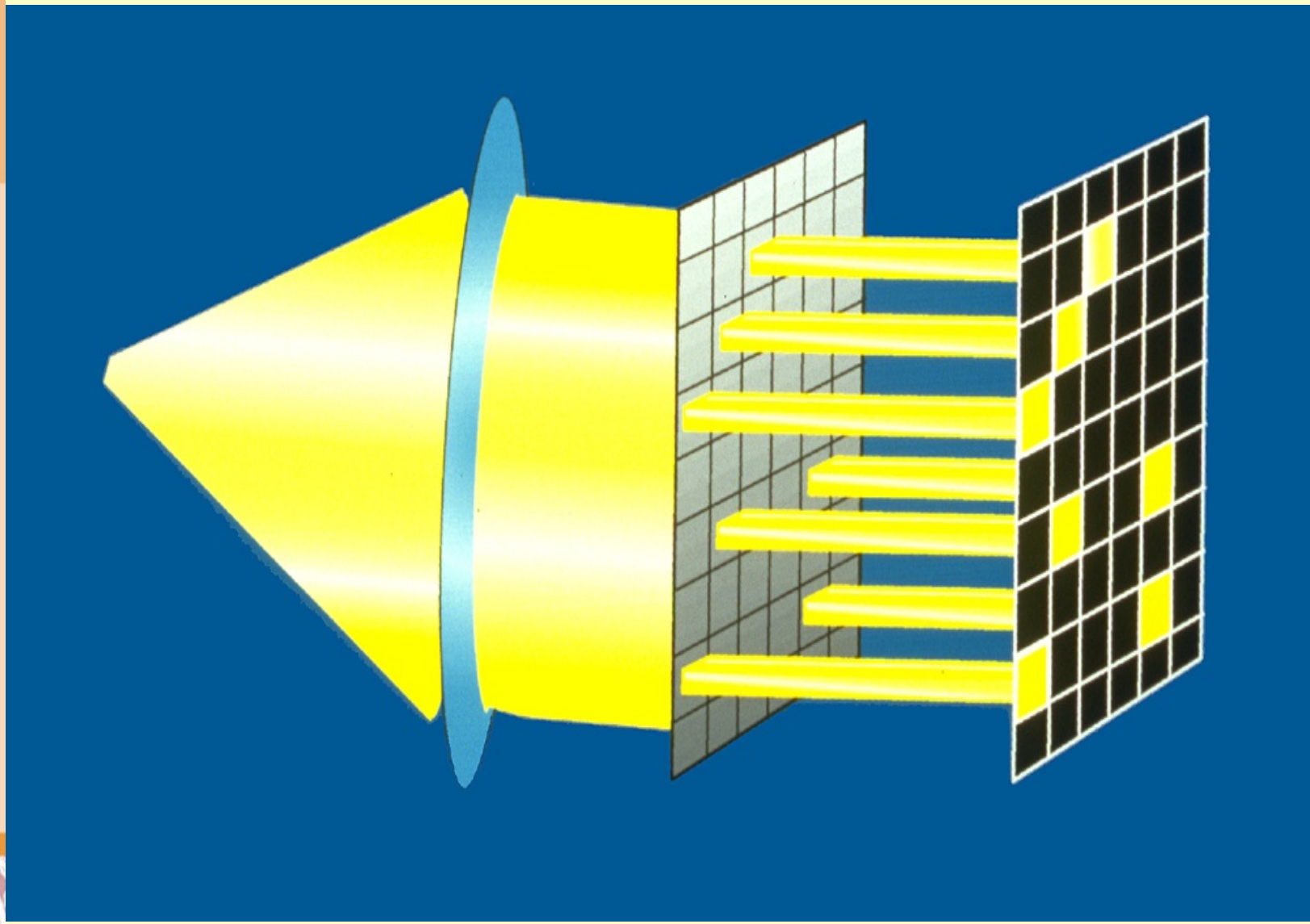
Automated DNA Chip Synthesis

http://www.affymetrix.com/

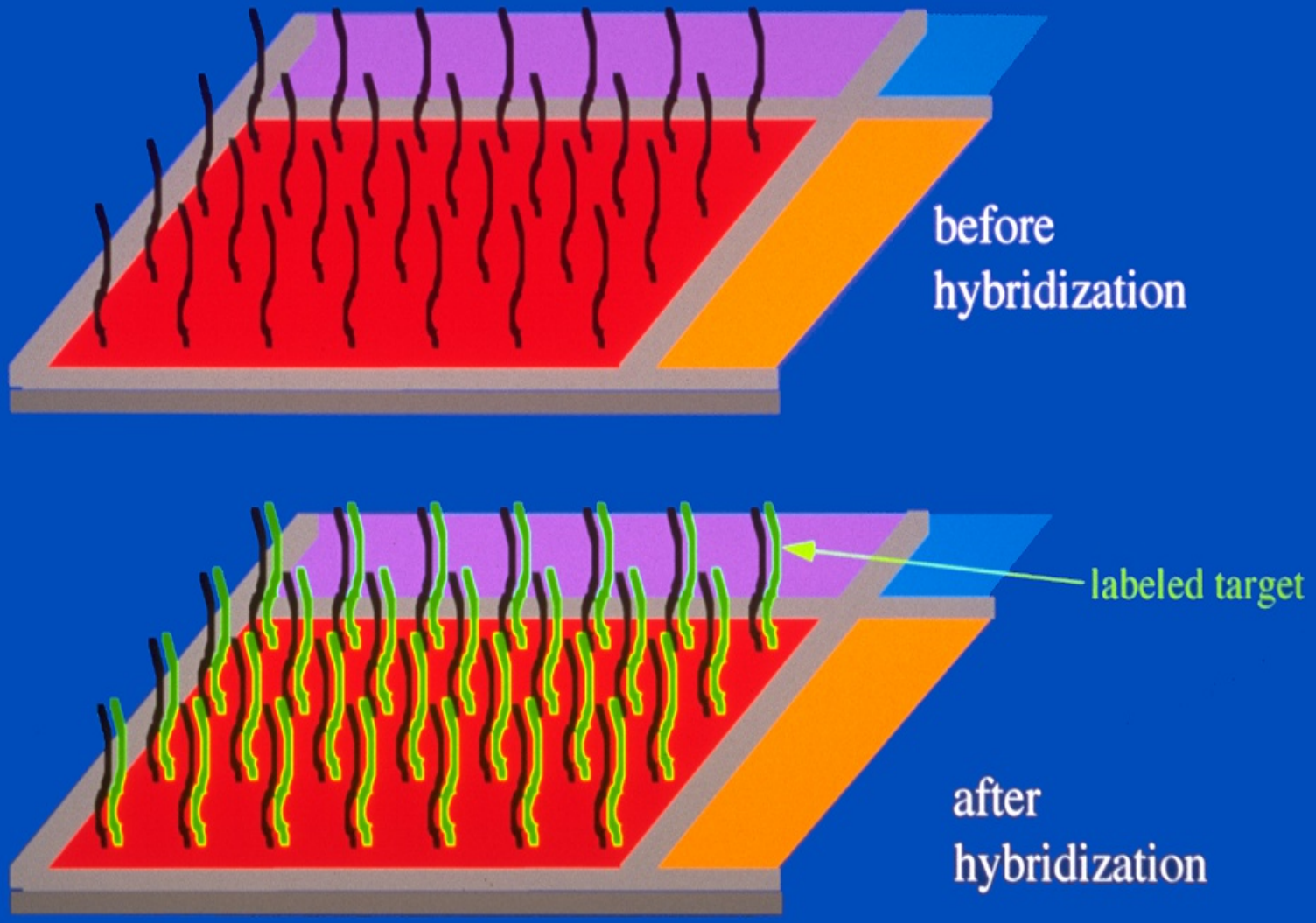


Photolithography Masks

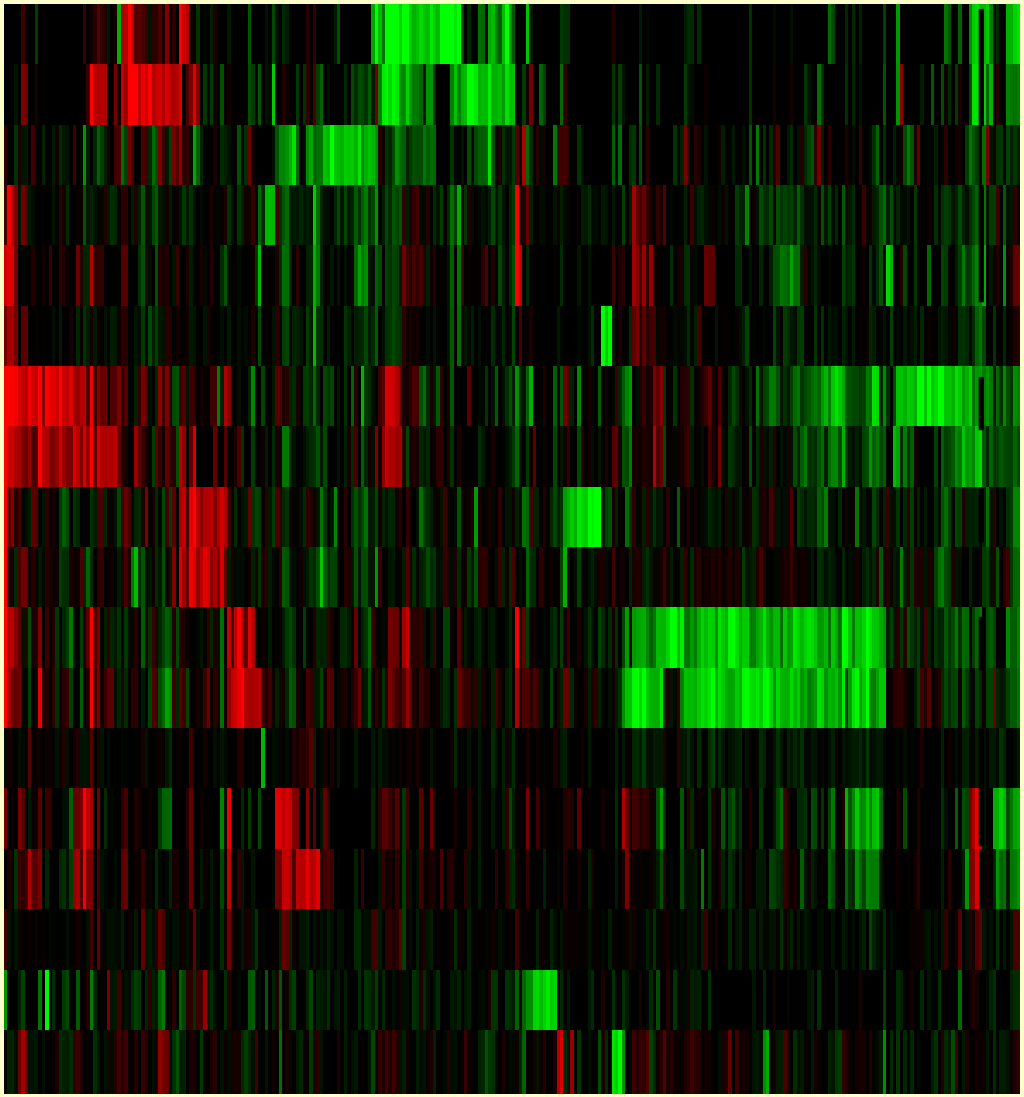
http://www.affymetrix.com/



Hybridization & Detection



Human Gene Expression Signatures



T Cells Signaling

DNA Damage

Fibroblast Stimulation

B Cells Signaling

CMV Infection

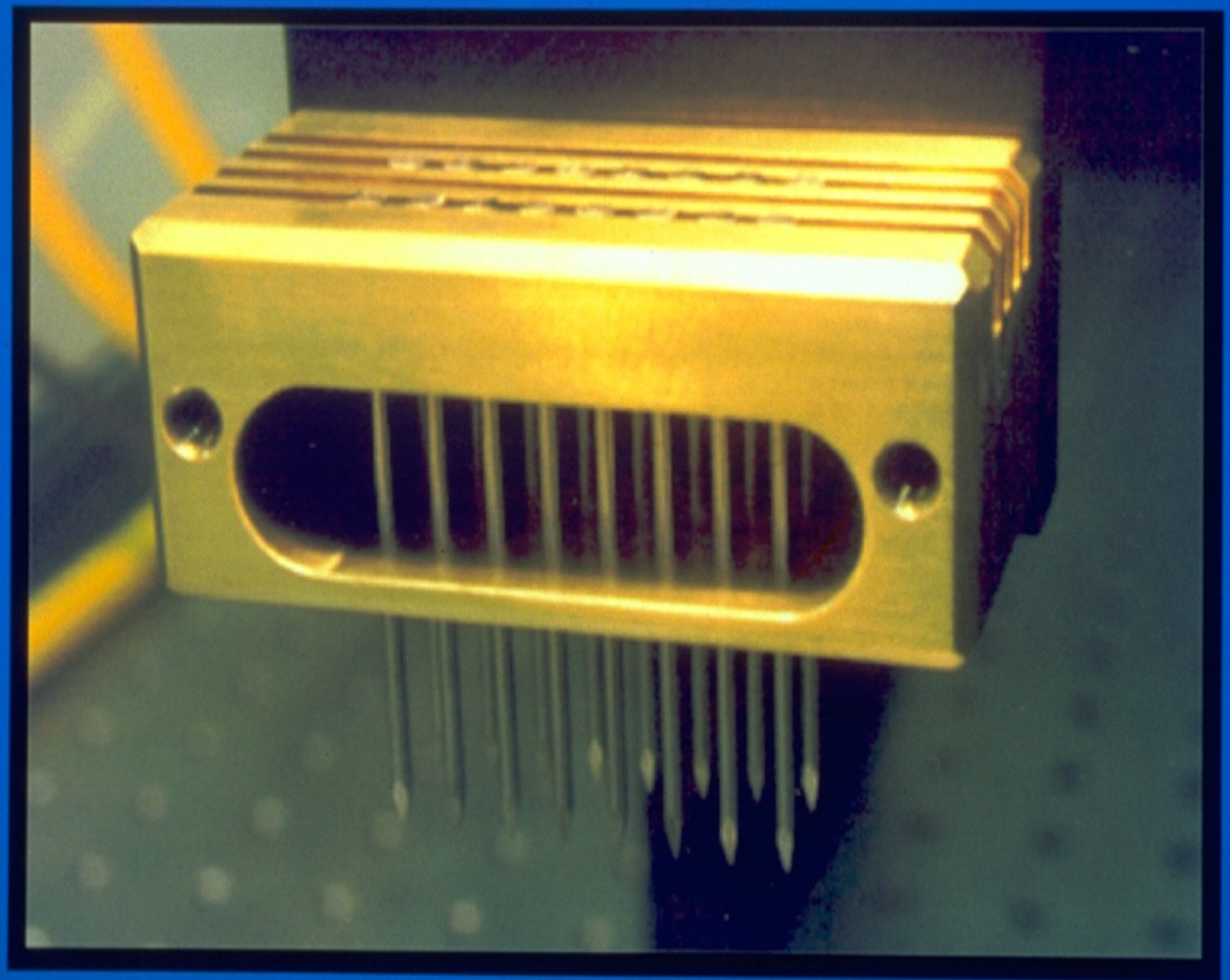
Anoxia

Polio Infection
Cell Signaling IL4
Growth Hormone

Microarrayer in Pat Brown's Lab

http://cmgm.stanford.edu/pbrown/

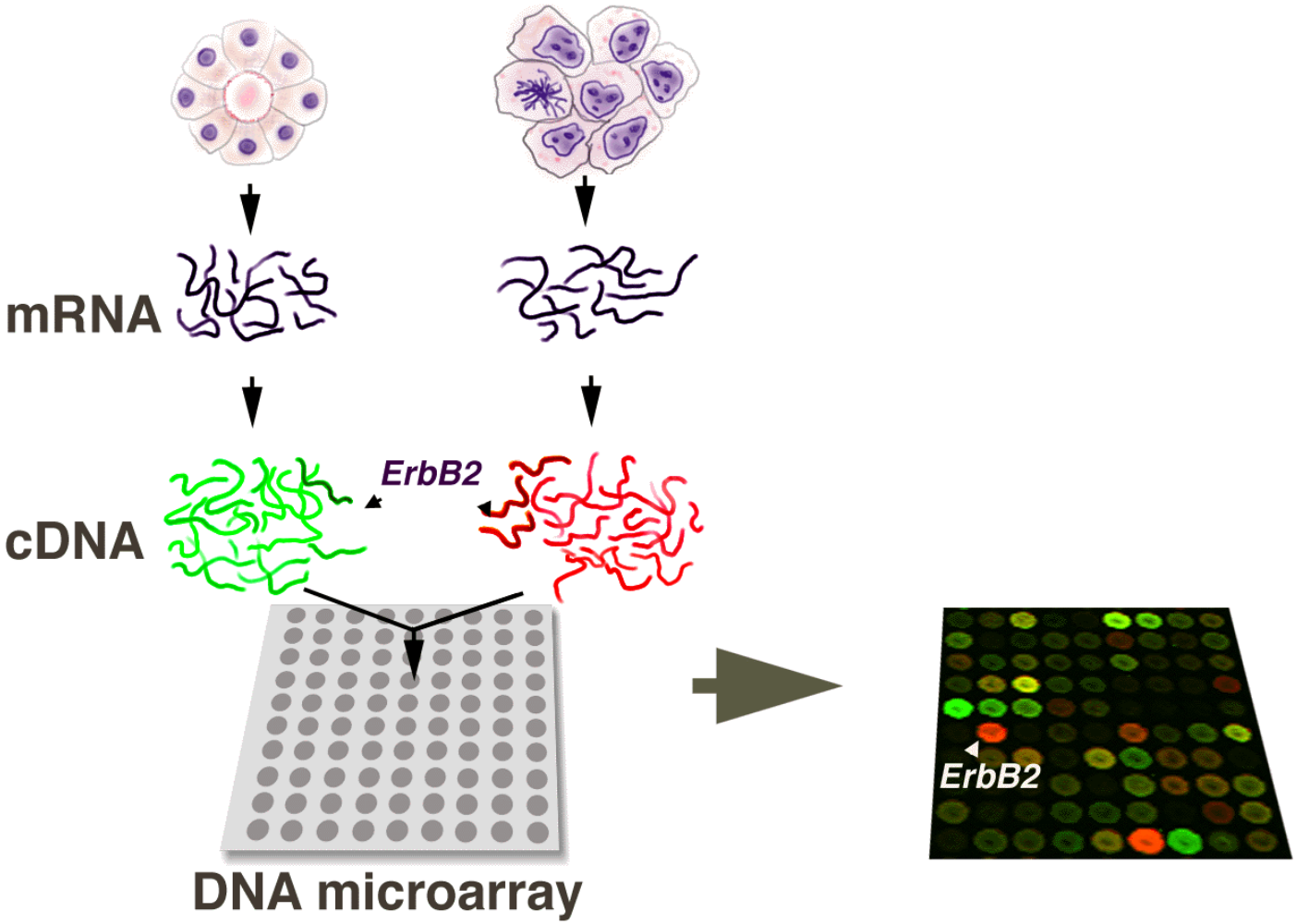
High Precision DNA Printing



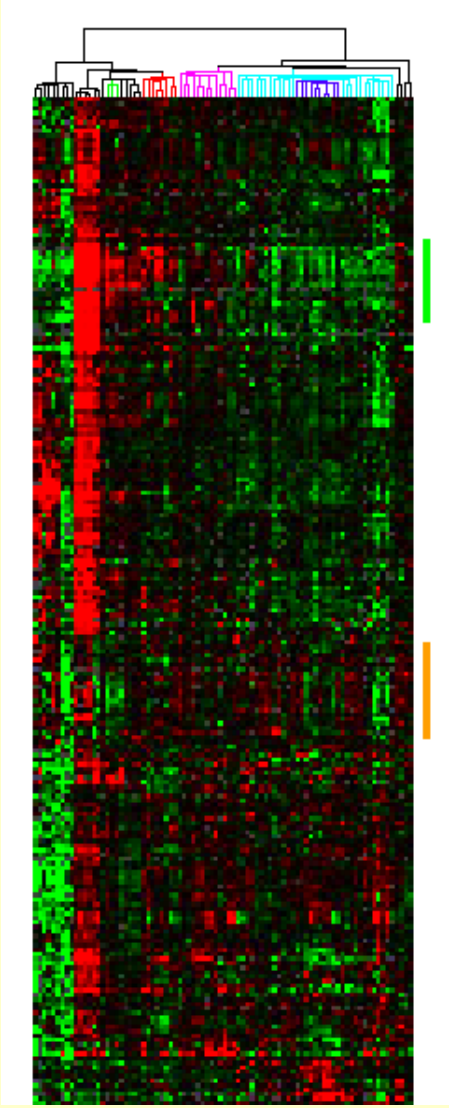
Mechanical Spotting Microarrays

http://www.arrayit.com/

DNA Microarray cDNA Labeling

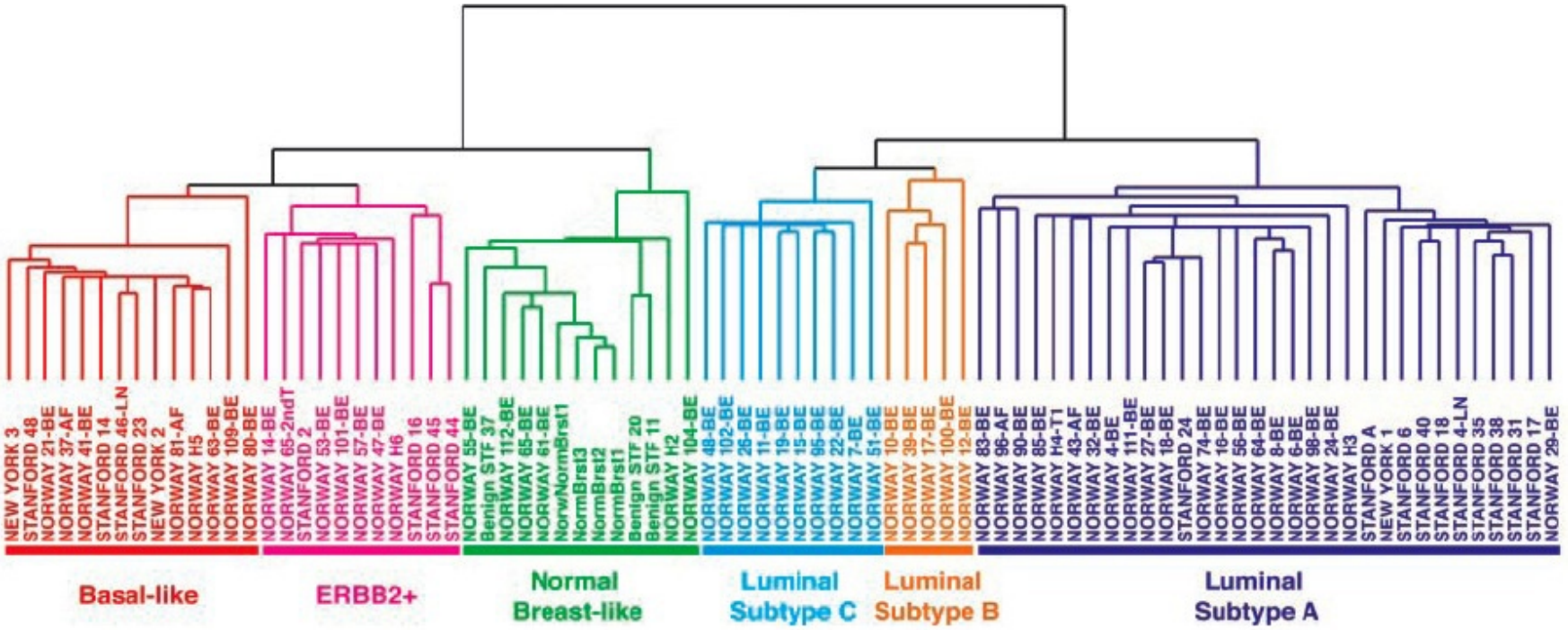


Breast Cancers Classified by 451 Gene Expression Assays



Sorlie et al., PNAS 98, 10869-10874.

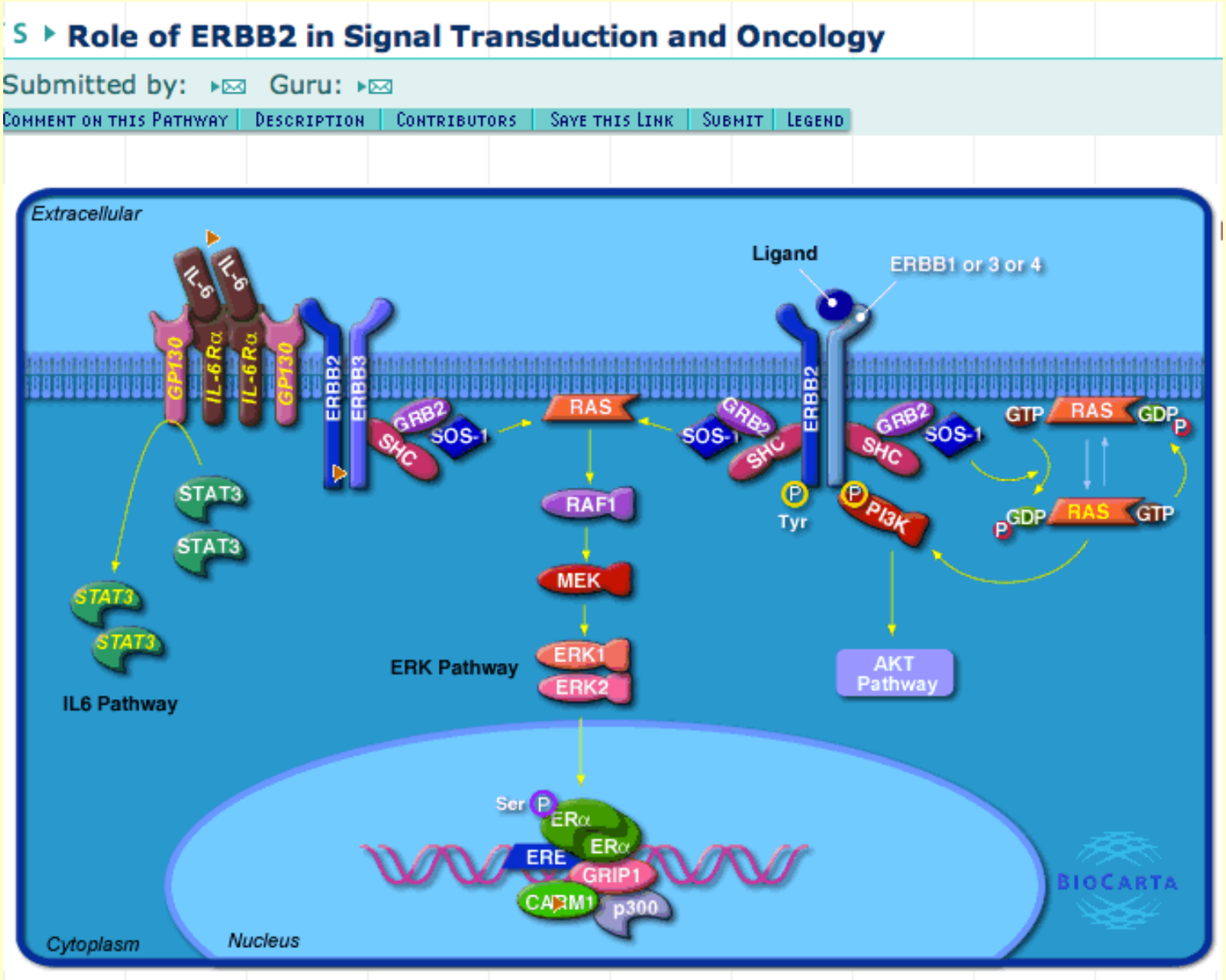
Breast Cancers Classified by 451 Gene Expression Assays



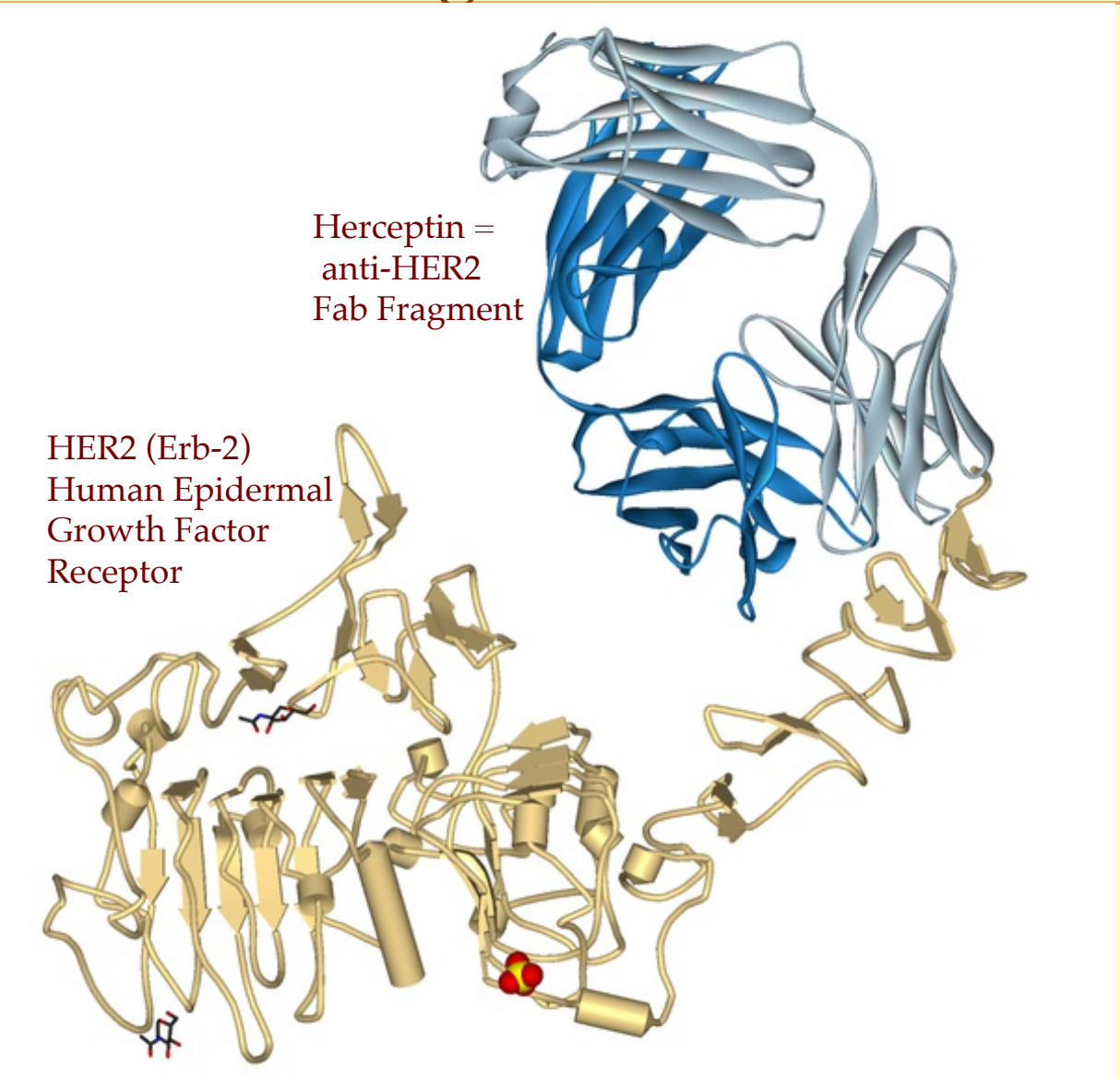
Sorlie et al., PNAS 98, 10869-10874.

ERB-B2 in Signal Transduction & Oncology

http://www.biocarta.com/pathfiles/h_her2Pathway.asp

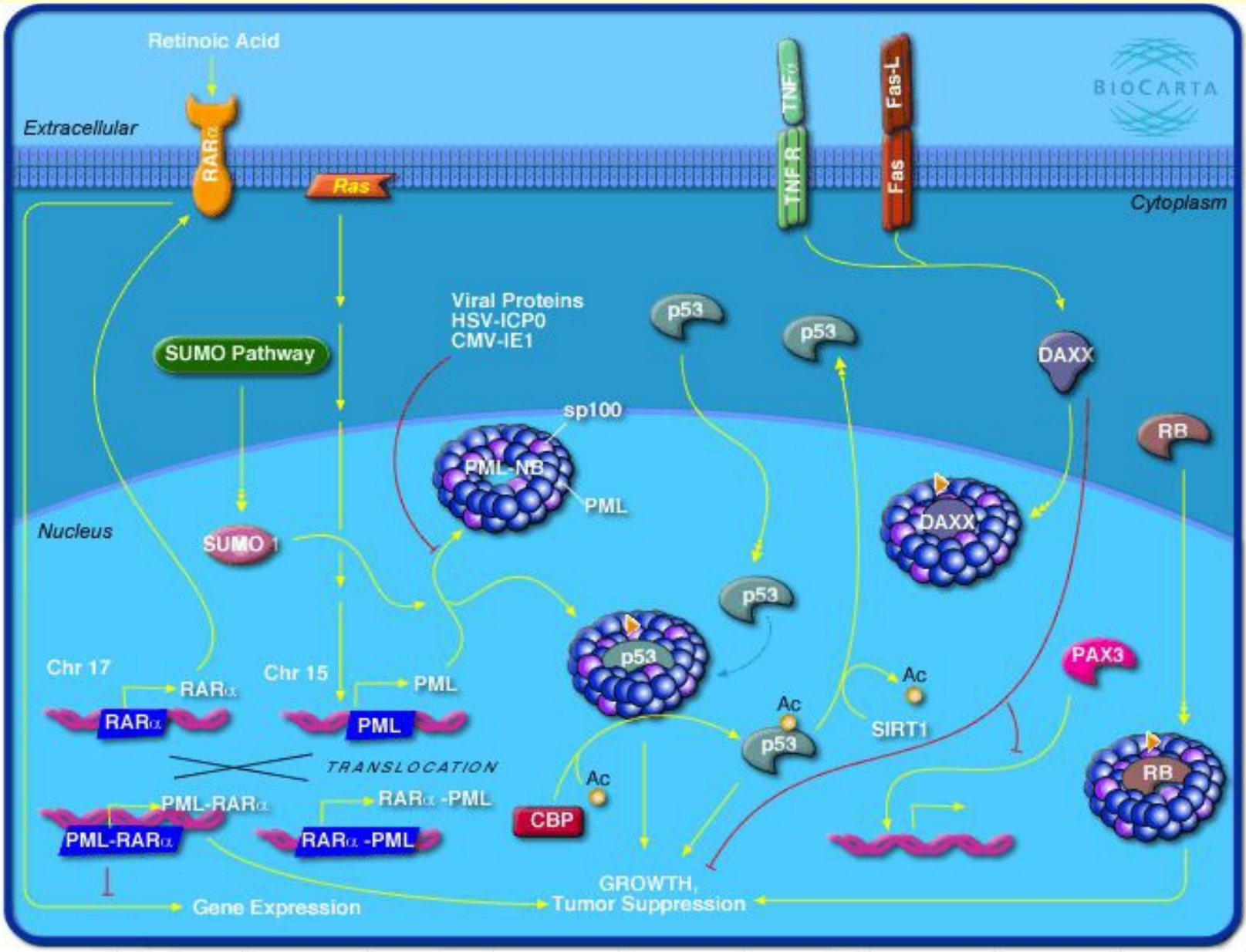


Herceptin binds to HER2 Blocking Cell Growth



Regulation of tumor supression by PML

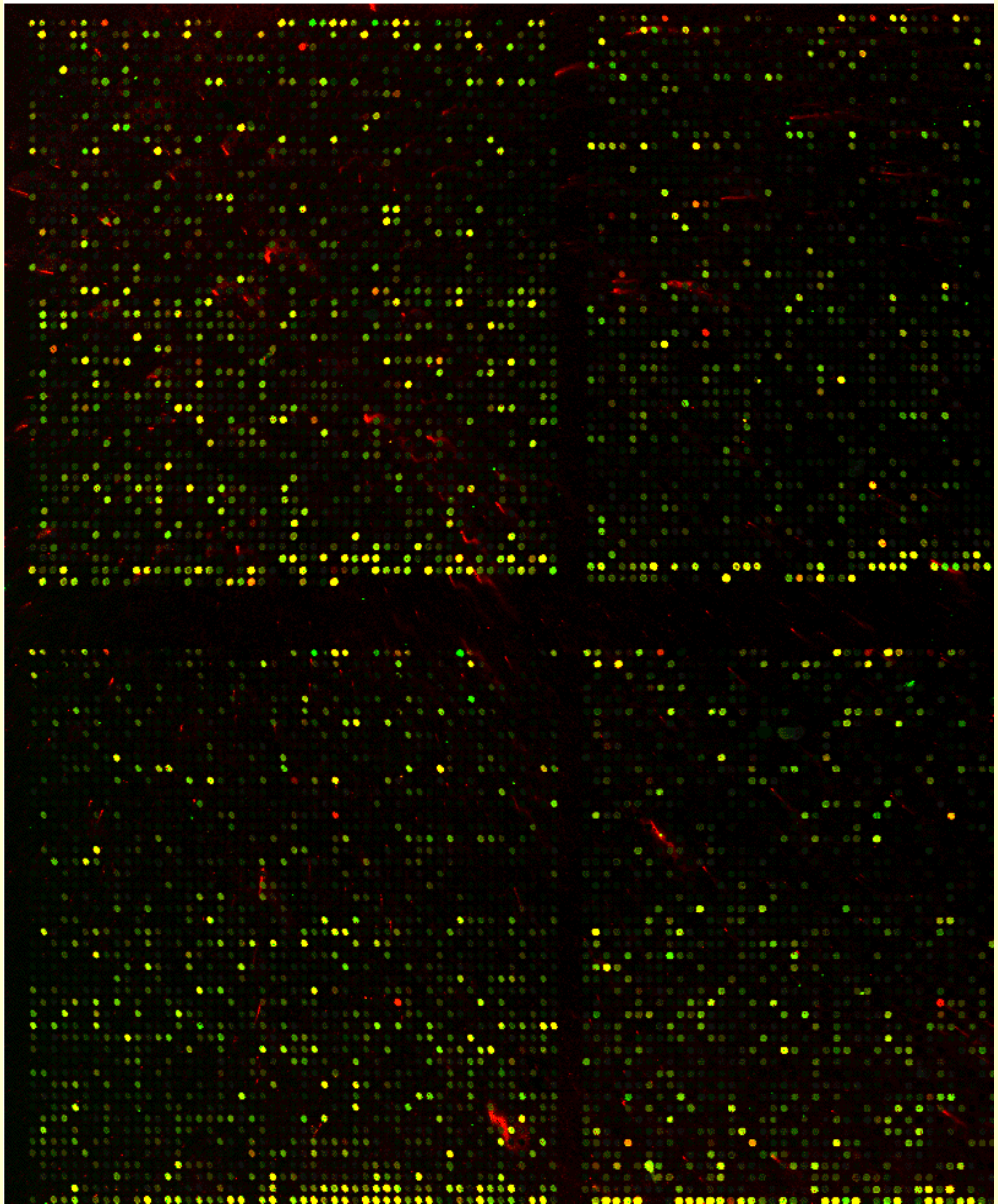
http://www.biocarta.com/pathfiles/h_pmlPathway.asp

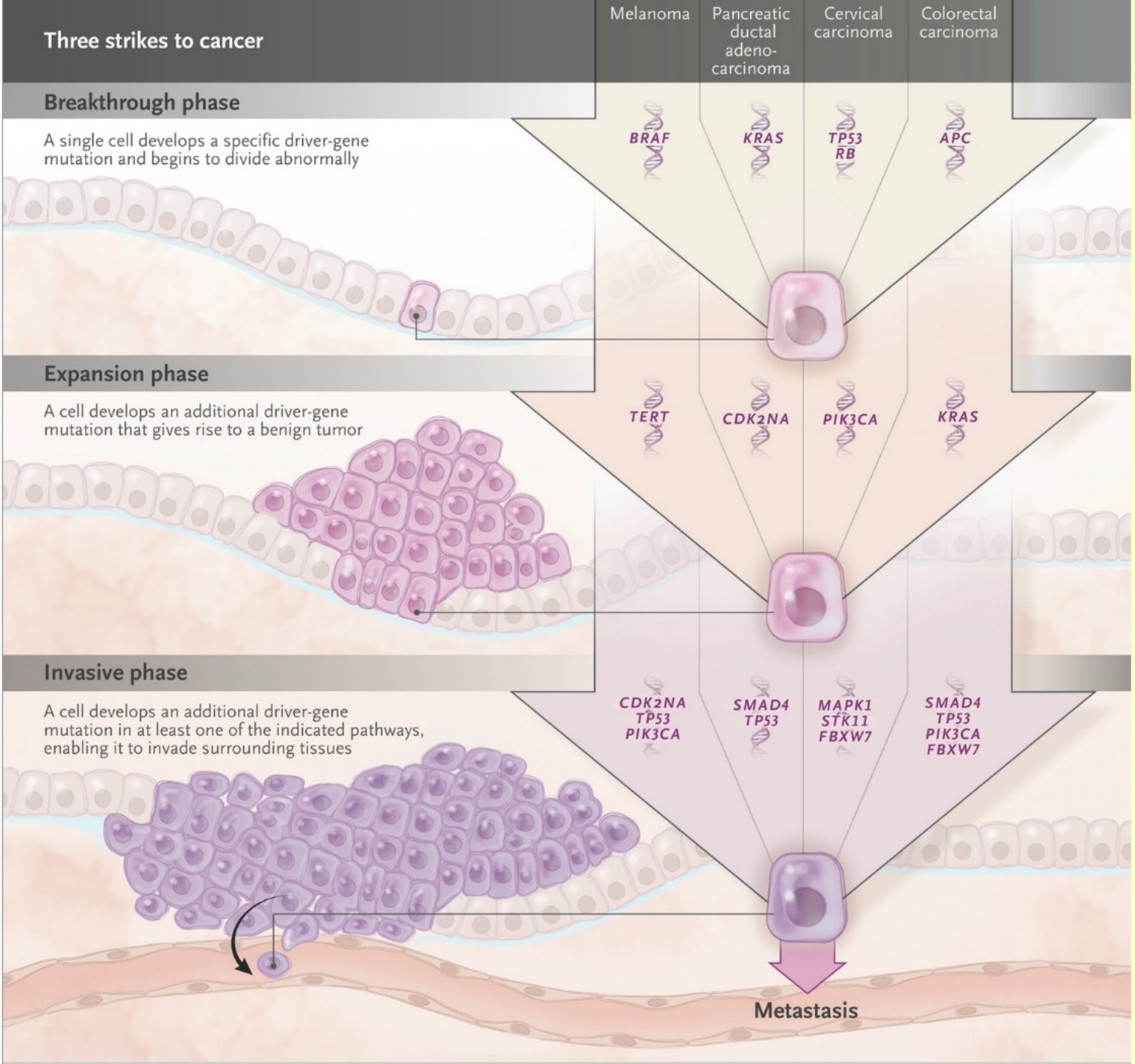


Acute Promyelocytic Leukemia

Tumor cDNA + Retinoic Acid (24 hr)

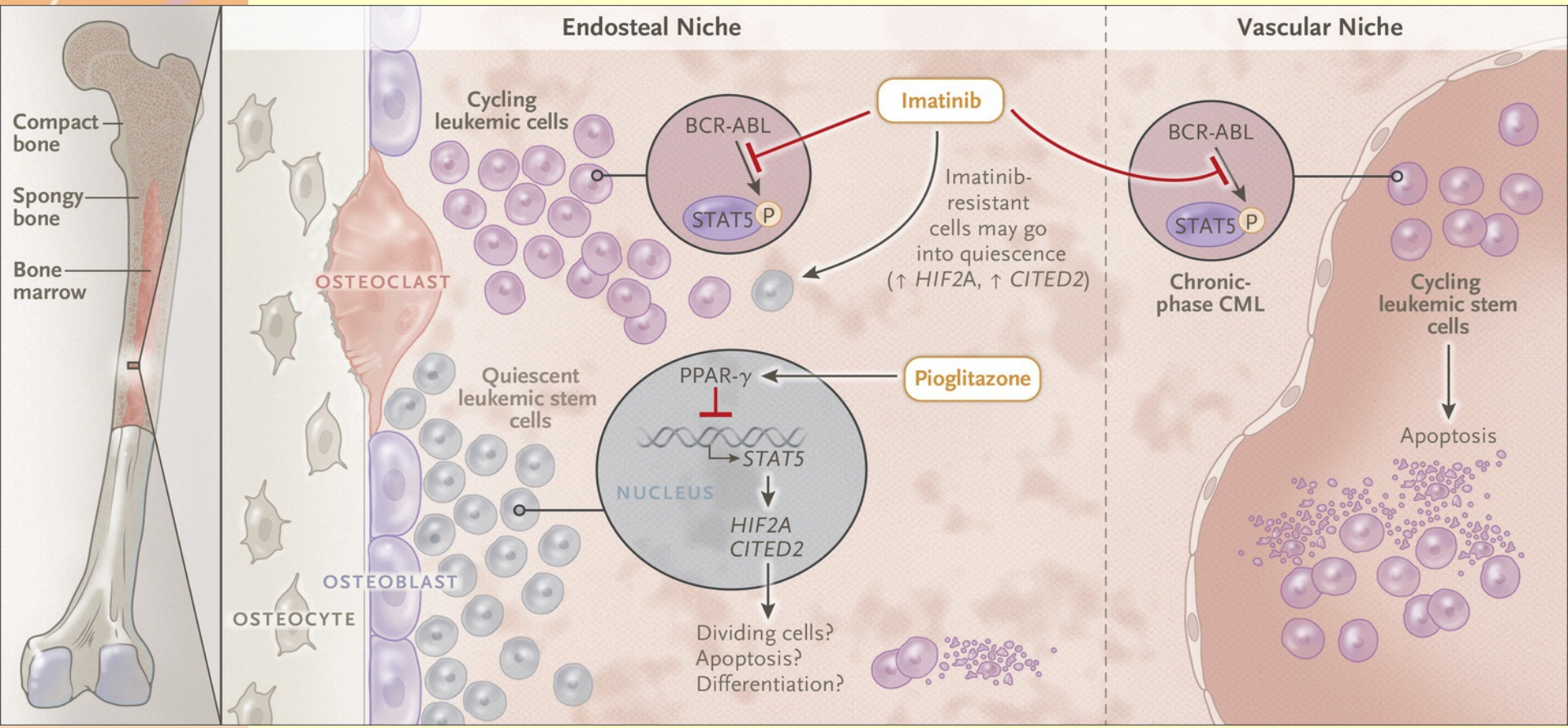
(Doug Ross & Pat Brown)





Examples of the genetic alterations leading to four representative cancer types are shown. Each gene symbol denotes a pathway. For example, APC denotes the pathway regulated by APC. A "mutation" in a pathway can be achieved by genetic or epigenetic inactivation of both alleles of a tumor-suppressor gene or by genetic activation of an oncogene in that pathway. Pathogenic strains of human papillomavirus initiate the breakthrough phase by disabling both the TP53 and RB pathways

Targeting Chronic Myeloid Leukemia (CML) Cells with a Double Whammy.



Egan JM. N Engl J Med 2015;373:1973-1975.

PathWork Diagnostics

http://www.pathworkdx.com/

Predicting Tissue of Origin for Cancers of Unknown Primary

PathWork Oncology Suite: Site of Origin for Solid Metastatic Tumors

- 1844 tumors tested one at a time versus all 18 tissues of origin
- Tests chosen based on the NCCN guidelines for treatment
- Retrospective study on well characterized patient samples
- Uses PathChip (functionally similar to Affymetrix HU-133A GeneChip)
  - 604 specimens used for training
  - 636 specimens used for test
  - 604 specimens in reserve for final validation
- Reproducibility from lab to lab
- Performance based on sensitivity (> 70%) & accuracy (> 95%)

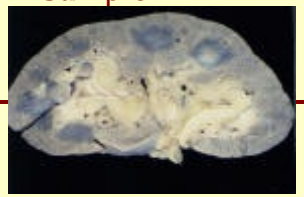
Pathwork Diagnostics

Datasets

Class	Code	Training	Test
Bladder	BLA	9	11
Breast	BRE	114	125
Central Nervous System	CNS	10	9
Colorectal	COL	67	76
Gastroesophageal	GAS	27	29
Squamous	SQU	32	22
Hepatocellular	HEP	9	12
Kidney	KID	40	42
Lymphoma (NHL)	LYM	54	59
Melanoma	MEL	13	13
Mesothelioma	MES	16	15
Non-Small Cell Lung	NSC	47	53
Ovarian	OVA	54	57
Pancreatic	PAN	22	19
Prostate	PRO	33	33
Sarcoma	SAR	31	32
Germ Line	GER	9	8
Thyroid	THY	17	21

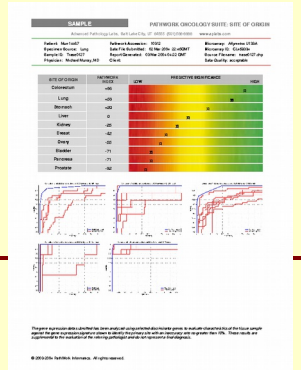
Production Data Flow

Sample



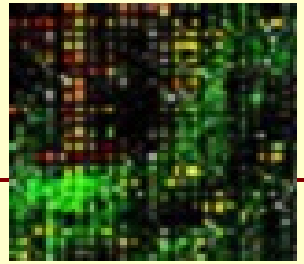
Clinical Laboratory

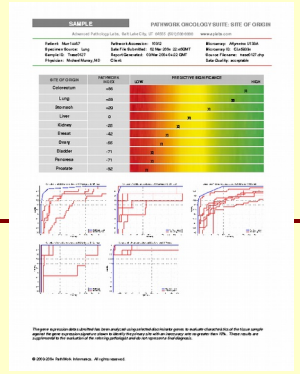
- Receive sample
- Process specimen, mRNA extraction and microarray hybridization
- Submit data file to PathWork for processing



- Deliver report to oncologist

Microarray data file





PDF Report

INTERNET

Pathwork Diagnostics

- Receive microarray data file
- Check file and data quality
- Run model against microarray data file
- Generate report file
- Deliver report file back to clinical lab

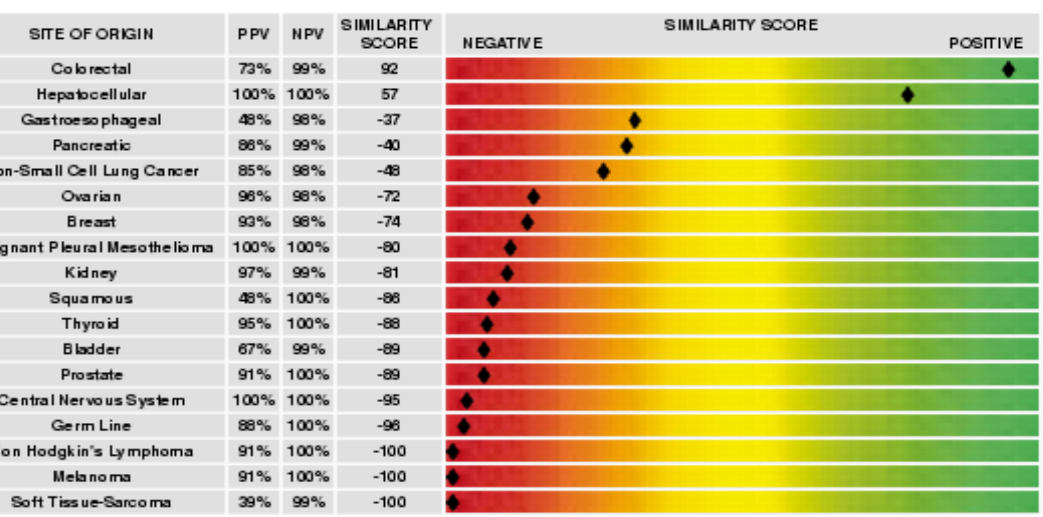
Pathwork Diagnostics

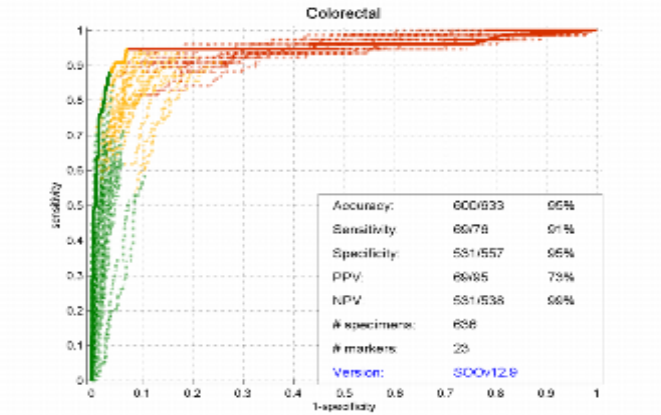
DEMONSTRATION EXAMPLE REPORT

PATHWORK ONCOLOGY SUITE: SITE OF ORIGIN
GENE EXPRESSION-BASED MOLECULAR DIAGNOSTIC

Patient ID: Liv18350	Client Name:	Data Quality: Acceptable
Specimen Source: Liver	Client ID:	Microarray: Affymetrix U133A
Medical Record:	Order Pathologist:	Microarray ID: CL18350hg133a11
Case Number:	Treating Physician:	Source Filename: 18350hg133a11.cel
PathWork Accession: SOO7982	Date/Time Processed: 29-Jul-2005 11:54-PDT	Version: SOOv12.9

SITE OF ORIGIN	PPV	NPV	SIMILARITY SCORE
Colorectal	73%	99%	92
Hepatocellular	100%	100%	57
Gastroesophageal	48%	98%	-37
Pancreatic	86%	99%	-40
Non-Small Cell Lung Cancer	85%	98%	-48
Ovarian	96%	98%	-72
Breast	93%	98%	-74
Malignant Pleural Mesothelioma	100%	100%	-80
Kidney	97%	99%	-81
Squamous	48%	100%	-86
Thyroid	95%	100%	-88
Bladder	67%	99%	-89
Prostate	91%	100%	-89
Central Nervous System	100%	100%	-95
Germ Line	88%	100%	-96
Non Hodgkin's Lymphoma	91%	100%	-100
Melanoma	91%	100%	-100
Soft Tissue-Sarcoma	39%	99%	-100





KEY
Sample Site: Liver
Primary Site: CO
Percent Tumor: 50-75
Pathology Morphology: Adenocarcinoma
Sample Description: PARTIAL HEPATECTOMY: METASTATIC ADENOCARCINOMA PRIMARY IN COLON. STATUS POST CHEMOTHERAPY.

For use by CLIA-certified clinical laboratories only. The clinical interpretation of the results should be made in context of the patient's clinical history and other diagnostic tests performed by a qualified individual.

Advanced Pathology Labs
123 Diagnostic Drive, Multiplex, CA 99212 (800) 555-1212

Pathwork Diagnostics

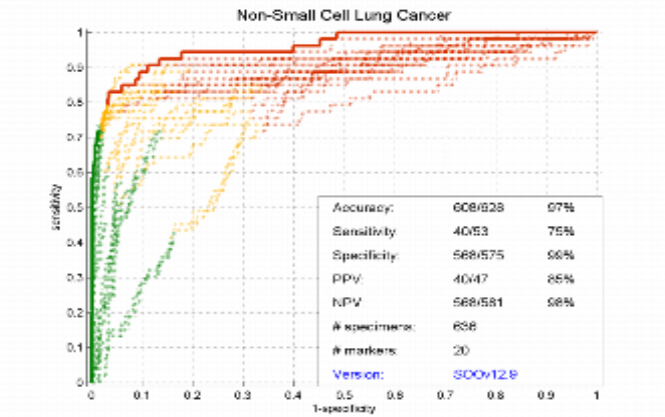
DEMONSTRATION EXAMPLE REPORT

PATHWORK ONCOLOGY SUITE: SITE OF ORIGIN
GENE EXPRESSION-BASED MOLECULAR DIAGNOSTIC

Patient ID: Lun31878	Client Name:	Data Quality: Acceptable
Specimen Source: Lung	Client ID:	Microarray: Affymetrix U133A
Medical Record:	Order Pathologist:	Microarray ID: CL31878hg133a11
Case Number:	Treating Physician:	Source Filename: 31878hg133a11.cel
PathWork Accession: SOO8030	Date/Time Processed: 29Jul2005 11:54PDT	Version: SOOv12.9

SITE OF ORIGIN	PPV	NPV	SIMILARITY SCORE
Non-Small Cell Lung Cancer	85%	98%	98
Colorectal	73%	99%	2
Gastroesophageal	48%	98%	-43
Non Hodgkin's Lymphoma	91%	100%	-56
Pancreatic	88%	99%	-57
Kidney	97%	99%	-65
Squamous	48%	100%	-68
Melanoma	91%	100%	-70
Bladder	67%	99%	-71
Ovarian	98%	98%	-71
Malignant Pleural Mesothelioma	100%	100%	-73
Thyroid	95%	100%	-74
Breast	93%	98%	-75
Central Nervous System	100%	100%	-79
Prostate	91%	100%	-89
Hepatocellular	100%	100%	-90
Germ Line	88%	100%	-91
Soft Tissue-Sarcoma	39%	99%	-93

SIMILARITY SCORE
NEGATIVE — POSITIVE



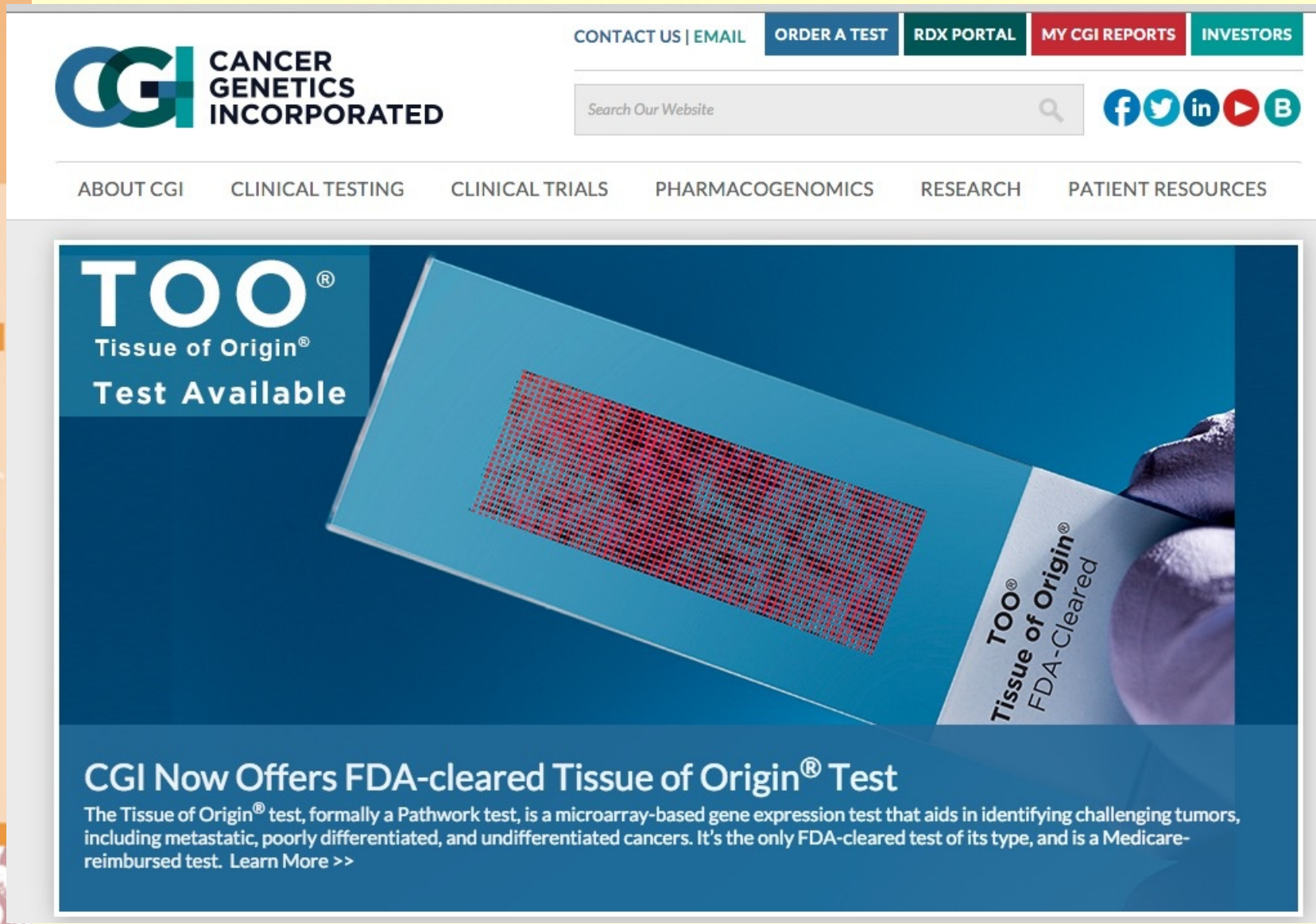
KEY
Sample Site: Lung
Primary Site: CO
Percent Tumor: 25-50
Pathology Morphology: Adenocarcinoma
Sample Description: LUL LUNG WEDGE BIOPSY: METASTATIC ADENOCARCINOMA C/W COLONIC PRIMARY DIAGNOSED 7 YEARS PRIOR; STAGING AT TIME OF ORIGINAL DIAGNOSIS UNKNOWN.

For use by CLIA-certified clinical laboratories only. The clinical interpretation of the results should be made in context of the patient's clinical history and other diagnostic tests performed by a qualified individual.

Advanced Pathology Labs
123 Diagnostic Drive, Multiplex, CA 99212 (800) 555-1212

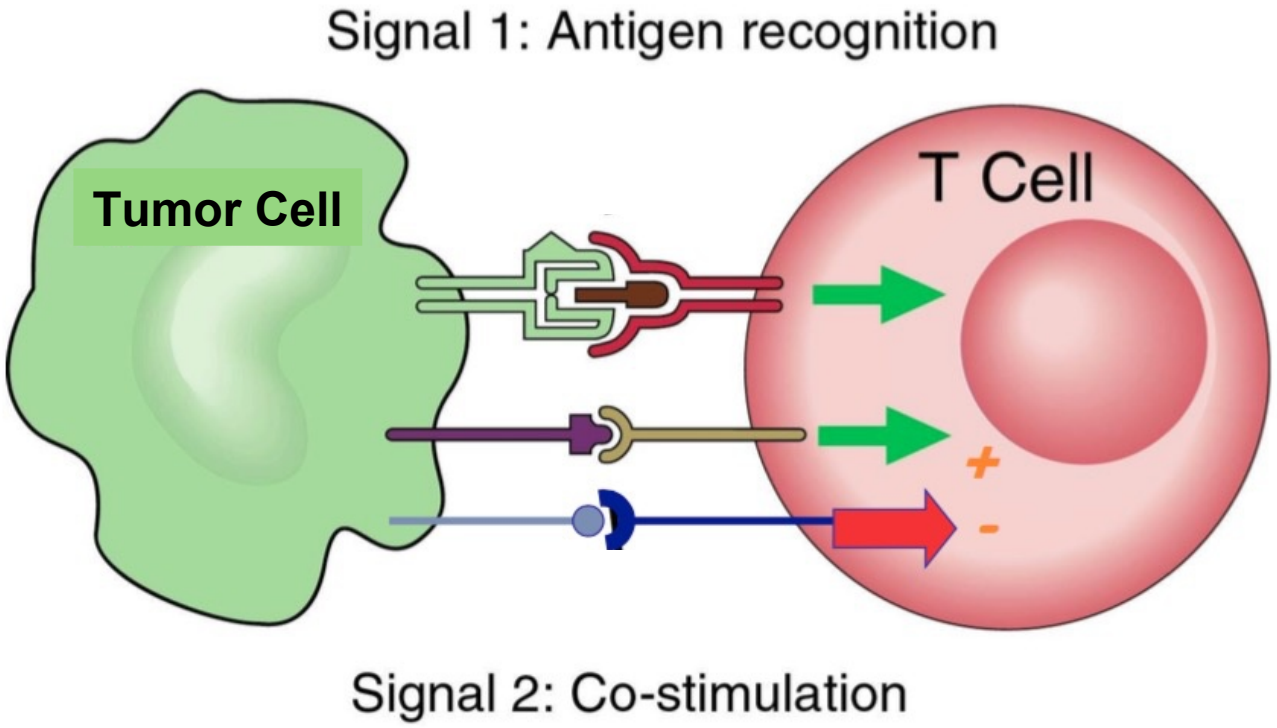
Cancer Genetics Inc.

http://www.cancergenetics.com/



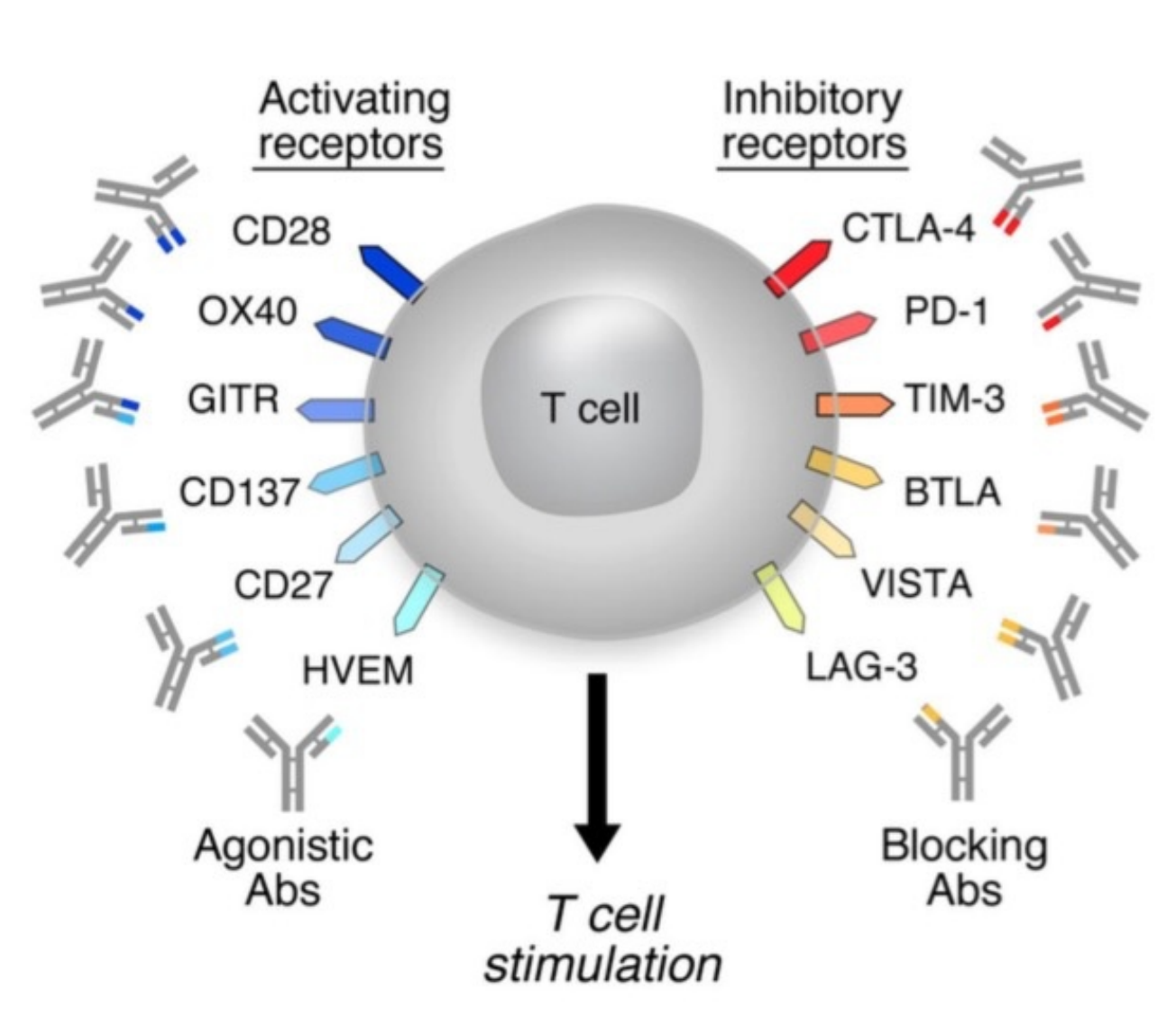
T Cell Activation Signals

T cells need 2 signals for activation



- **There are positive and negative second signals**

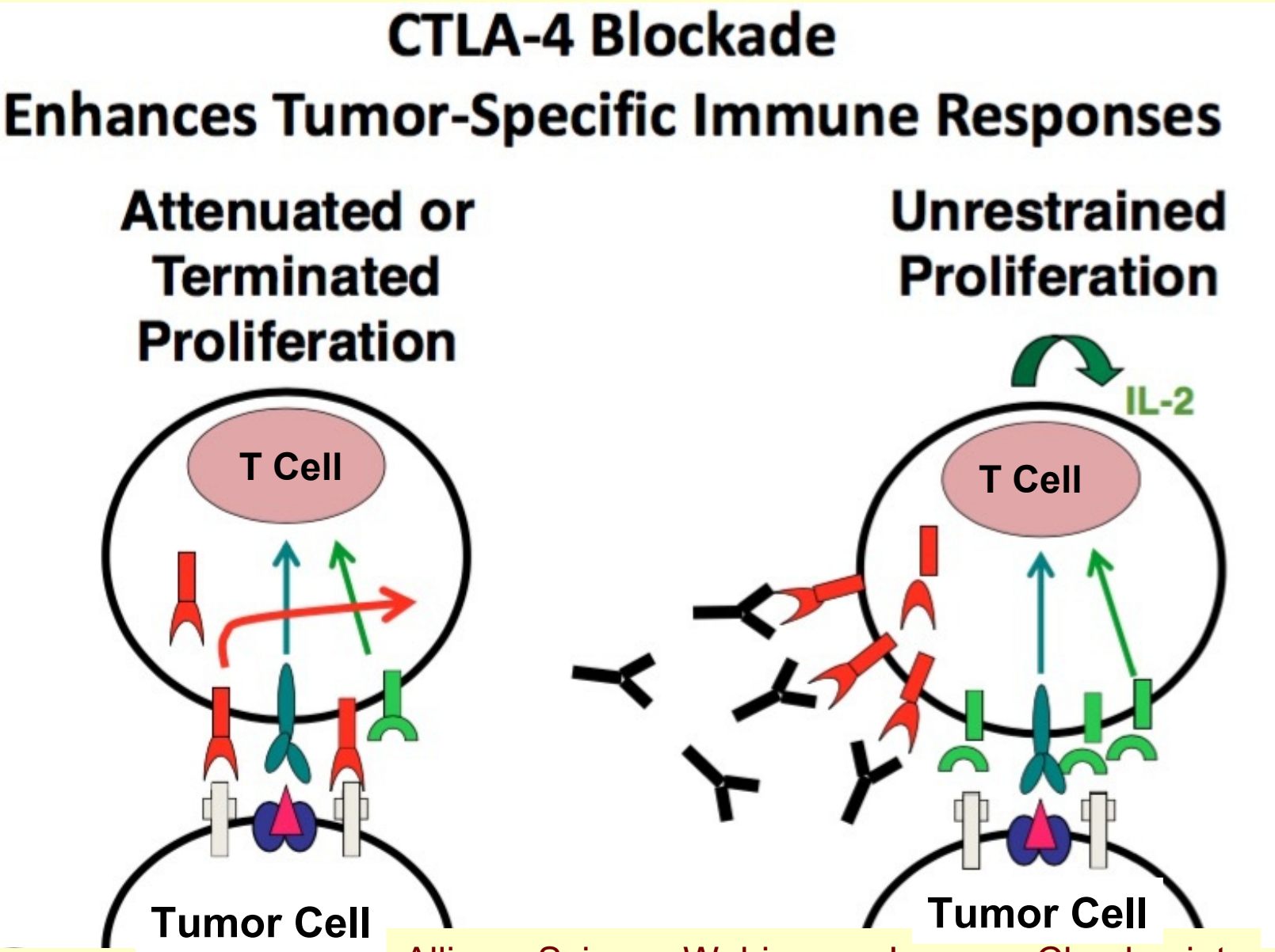
Activating and Inhibitory T Cell Signals



Philip Gotwals, Science Webinar on Immune Checkpoints

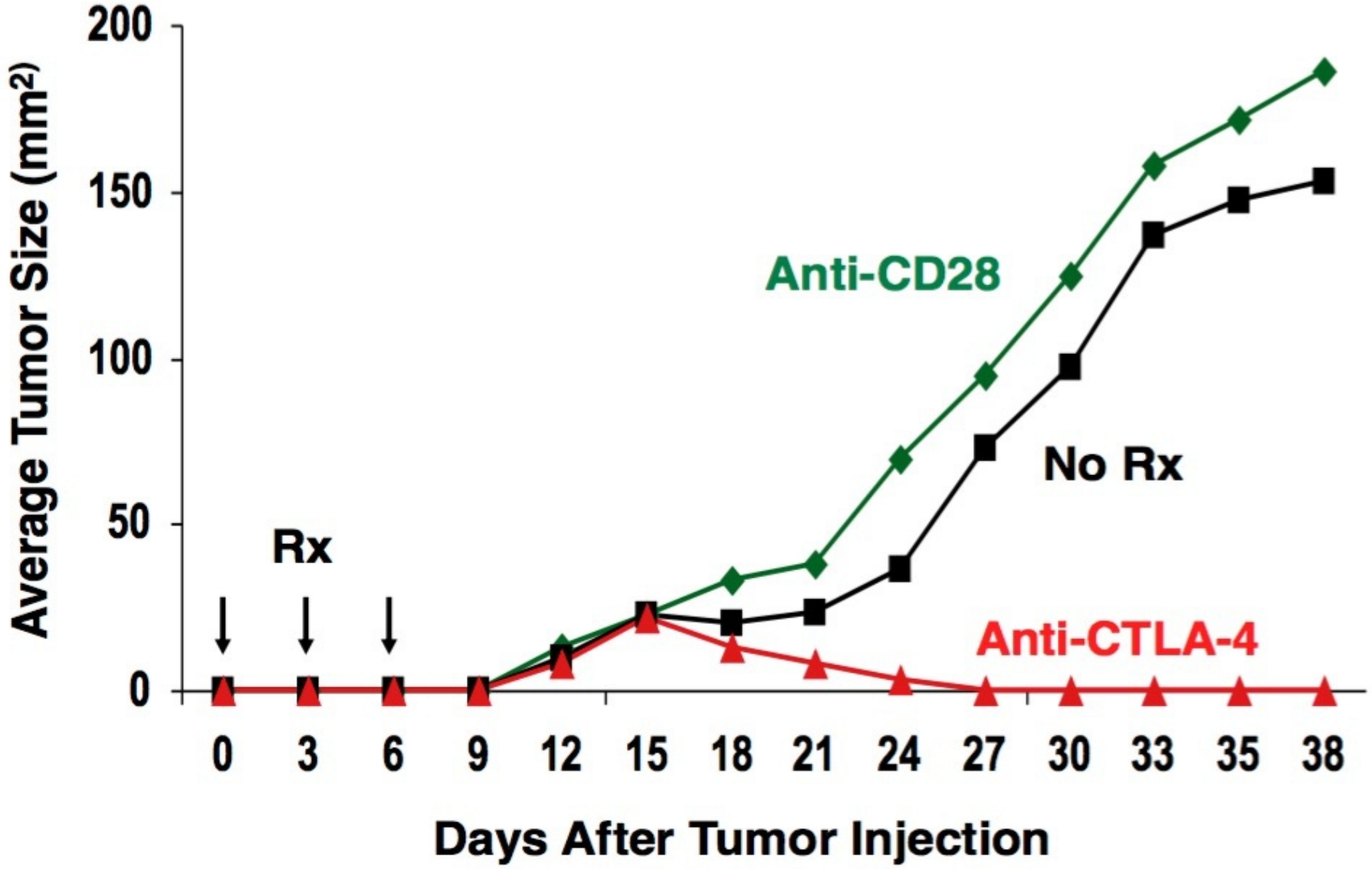
THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Anti CTLA-4 Antibody Stimulates Immune Response



THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Anti CTLA-4 Induces Colon Cancer Tumor Regression



Ipilimumab
(Medarex, Bristol-Myers Squibb)

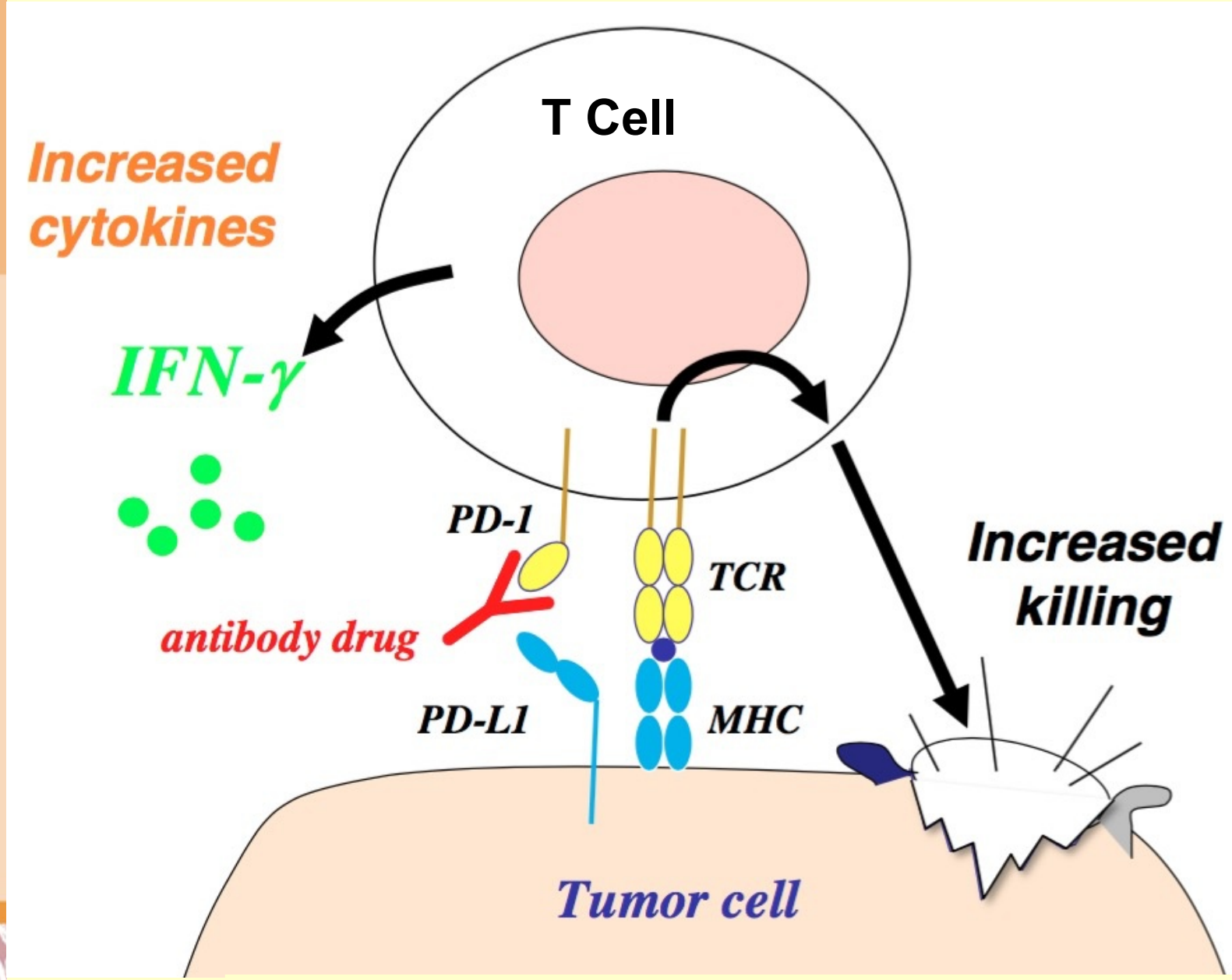
Fully human antibody to CTLA-4

>50,000 patients treated to date:

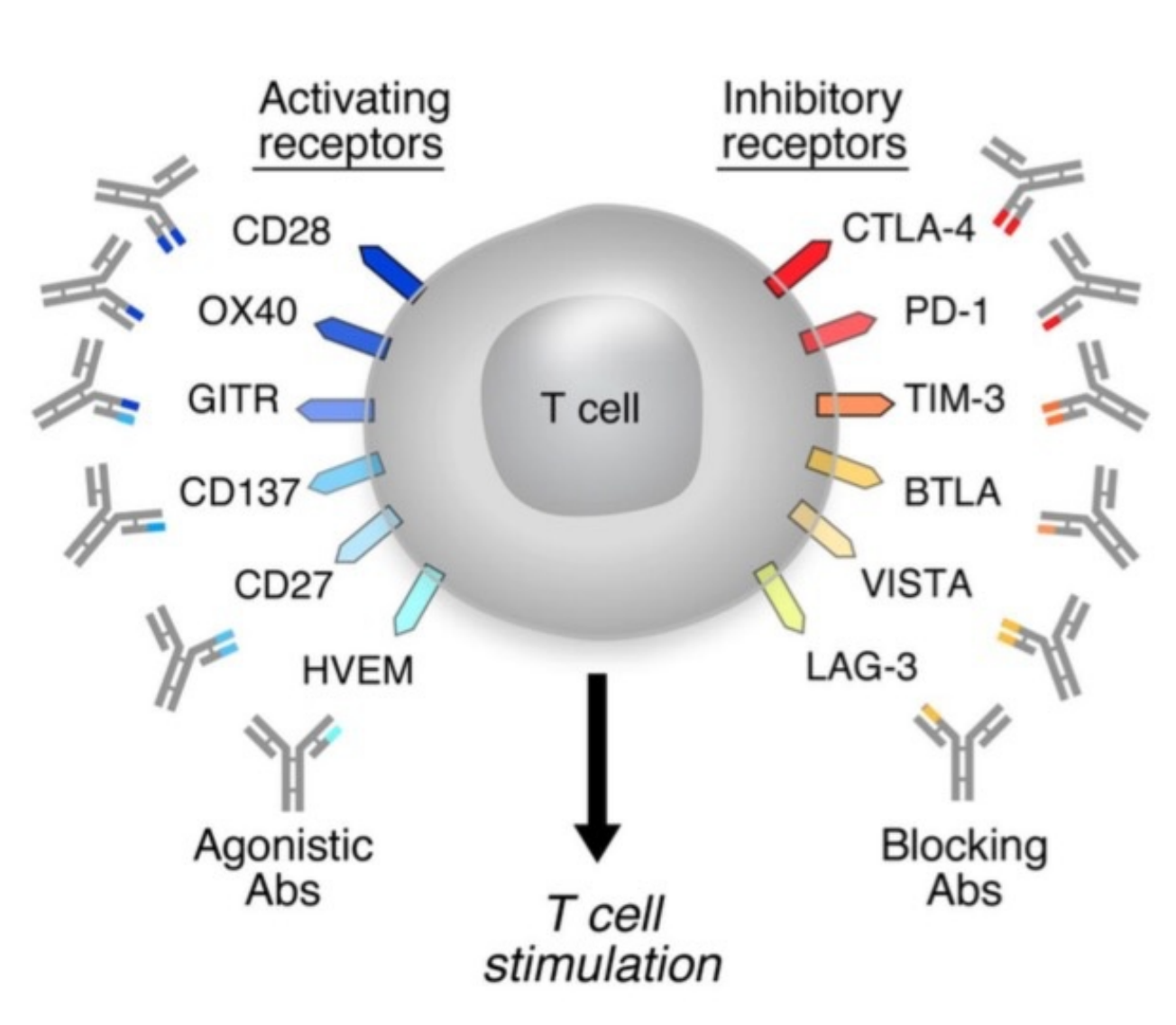
Objective responses in many tumor types, including melanoma, prostate, kidney, bladder, ovarian & lung cancer, etc.

Adverse events (colitis, hepatitis, hypophysitis, etc.) serious but generally manageable

Anti PD-1 Also Reactivates T Cells



Activating and Inhibitory T Cell Signals



Philip Gotwals, Science Webinar on Immune Checkpoints

FDA Approval of Immune Checkpoint Antibodies

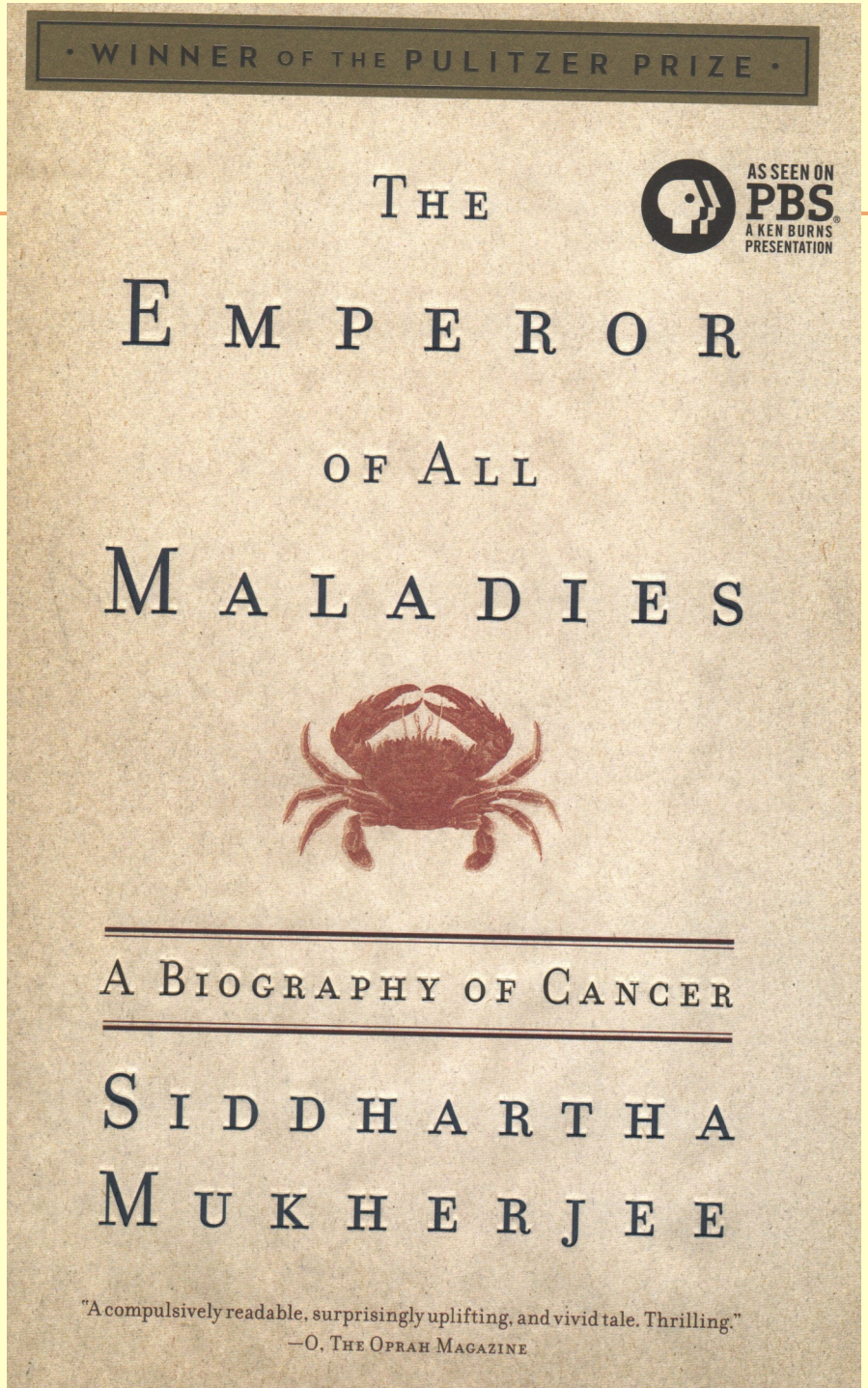
- **2011 Ipilimumab (BMS) - Melanoma**
- **2014 Pembrolizimab (Merck) – Melanoma**
- **2014 Nivolumab (BMS) – Melanoma**
- **2015 Nivolumab (BMS) – Lung**
- **2015 Ipilimumab + Nivolumab (BMS) – Melanoma**
- **2015 Pembrolizumab (Merck) – Lung**
- **2015 Ipilimumab (BMS) – Adjuvant melanoma**
- **2015 Nivolumab (BMS) – Renal cell carcinoma**

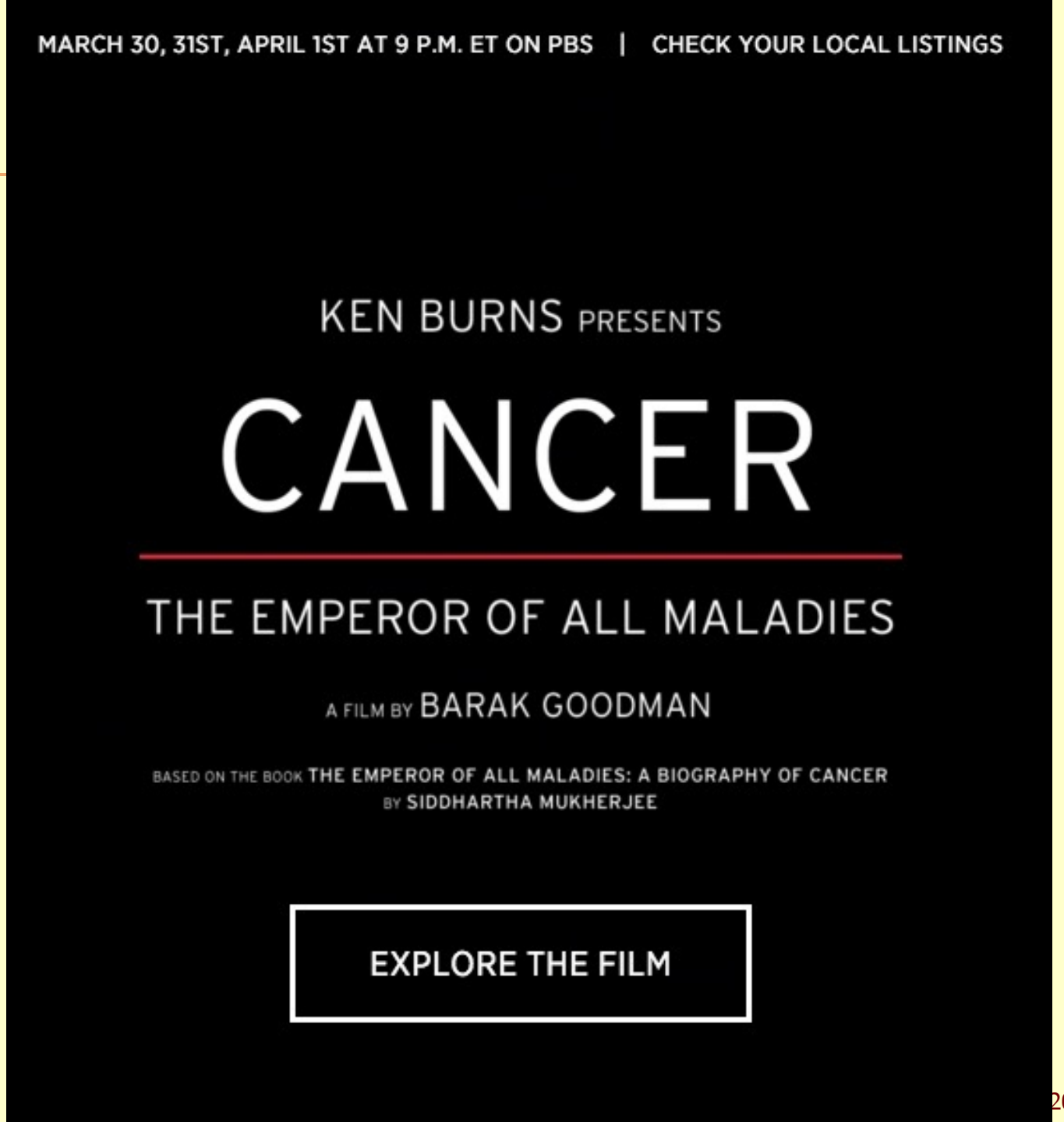
On Cancer

Understanding Jimmy Carter's Surprise Cancer Turnaround: A Conversation with Jedd Wolchok

By **Matthew Tontonoz** on **Wednesday, December 9, 2015**

Former President Jimmy Carter announced this week that he is "cancer free" after receiving treatment for advanced melanoma. Photo Credit: The Carter Center.




MARCH 30, 31ST, APRIL 1ST AT 9 P.M. ET ON PBS | CHECK YOUR LOCAL LISTINGS
KEN BURNS PRESENTS
CANCER
THE EMPEROR OF ALL MALADIES
A FILM BY BARAK GOODMAN
BASED ON THE BOOK THE EMPEROR OF ALL MALADIES: A BIOGRAPHY OF CANCER
BY SIDDHARTHA MUKHERJEE
EXPLORE THE FILM