Genómica Funcional en Investigación Biomédica

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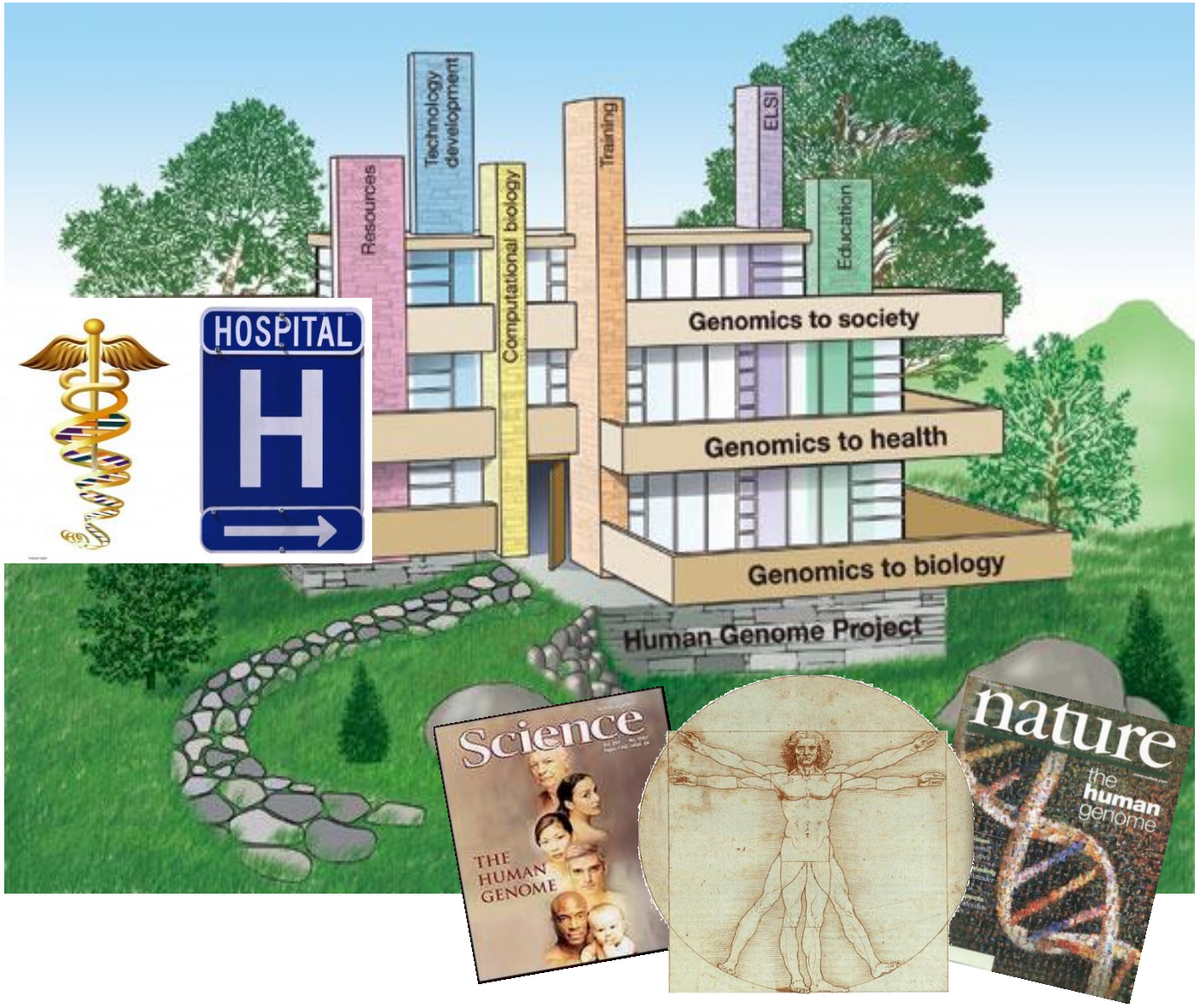
FACULTAT DE FARMÀCIA

SEMINARIS TECNOLÒGICS A LA FACULTAT DE FARMÀCIA 2012

Functional Genomics-Personalized Medicine



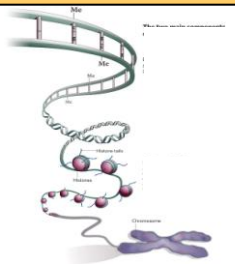
modified from Francis S. Collins, et.al.***Nature*** **422**, 835-847(24 April 2003)

FUNCTIONAL GENOMICS

GENOMICS TOOLS

Genomics

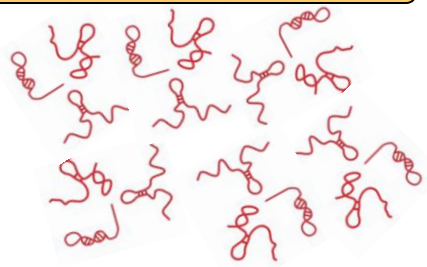
(1) Genome



DNA sequence
Structural genomics
Epigenetics

Transcriptomics

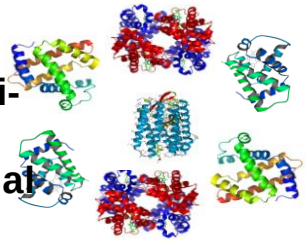
(2) Transcriptome



mRNA and
non-coding RNA

Proteomics

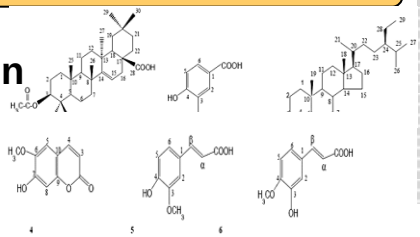
(3) Proteome



Protein expression
Post-translational modi-fications
Structural and functional analysis

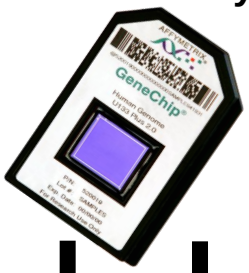
Metabolomics

(3) Metabolome



Profile and function of metabolites

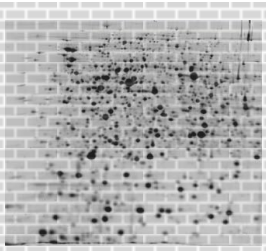
DNA microarrays

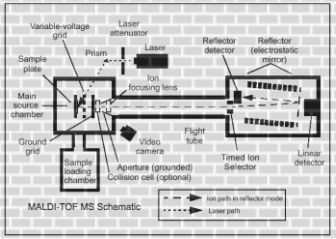


DNA sequence (mutations)
DNA variation (SNPs, CNVs)

Structural genomics
Histone modifications
DNA methylation

Gene expression
Splicing variants

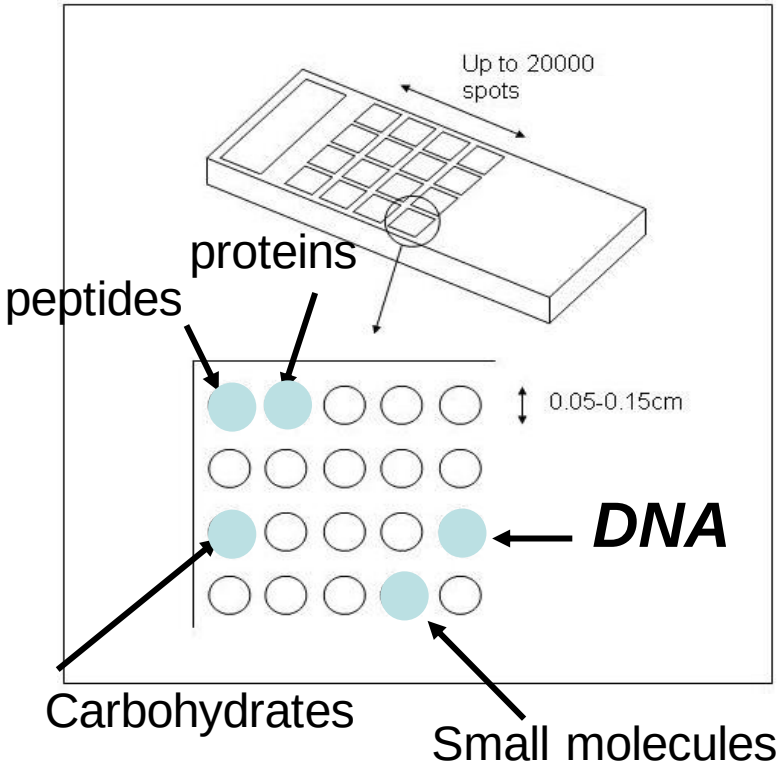




Microarray: Universal Biochemistry Platform

A microarray is a compact device that contains a large number of well-defined immobilized capture molecules assembled in an addressable format.

Synthetic oligos, PCR products, proteins, antibodies, carbohydrates etc….



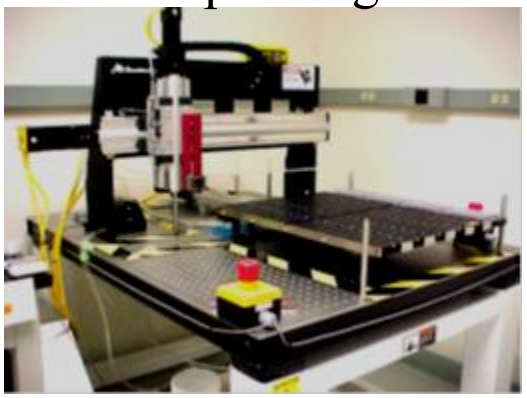
a) You can expose an unknown (test) sample on it and then examine where the molecule was captured.

b) You can then derive information on identity and amount of captured molecule.

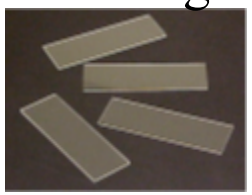
Array Life Cycle

amplifying

printing



coating

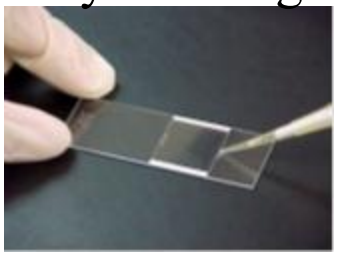


Biological Question ?

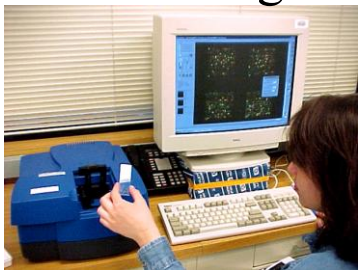
processing

labelling

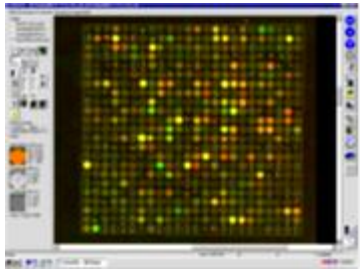
hybridizing



scanning



analyzing

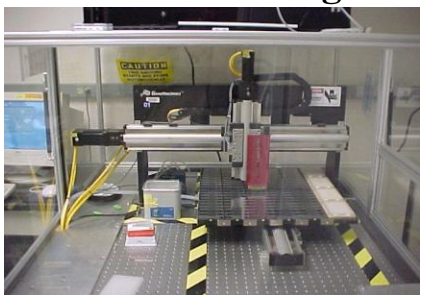


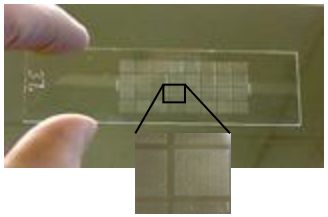
from D. Petersen ATC/NCI

Types of Array

cDNA

Robotic Printing





Oligonucleotides

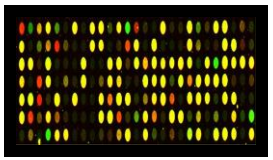
Long oligos (50-80 nt)

Short oligos (<30 nt)

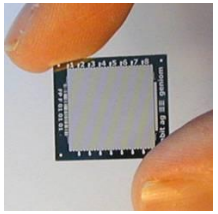
Long oligos (50nt) address seq (29 nt)

Synthesis *in situ*

Ink Jet Technology (60nt, Agilent)

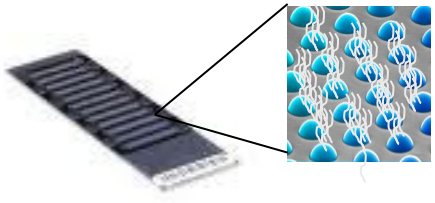


Maskless (60-70 nt Nimblegen)



Photolithography (25 nt, Affymetrix)

Bead-random Arrays (Illumina)



Cy3

Cy5

Cy3

Cy5

SAPE (Streptavidin Phycoerythrin)

Biotin

SA_Cy3 (Streptavidin Cyanine 3)

Biotin

Detection

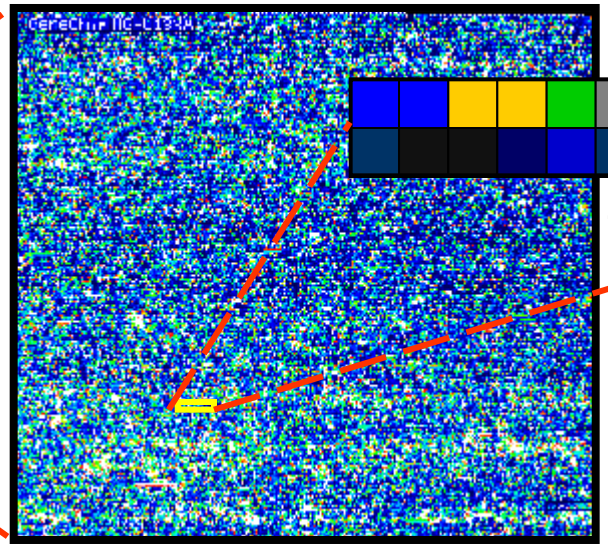
Two-color

One-color

GeneChip® and Probe selection

Up to ~1,400,000 features/chip

1) Probe Array



1.28cm

2) Probe set

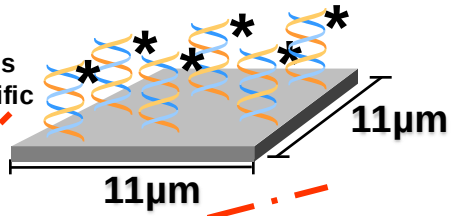
Each Probe Set contains 11 Probe Pairs (PM:MM) of different probes

3) Probe pair

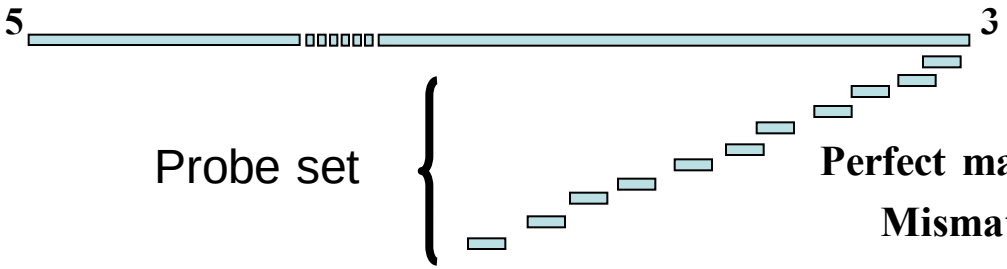
Each Perfect Match (PM) and Mismatch (MM) Probe Cells are Associated by pairs

4) Probe cell

Each Probe Cell contains $5x10^{5-6}$ copies of a specific probe

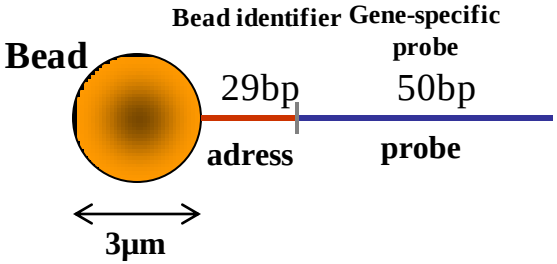


Probe selectiom

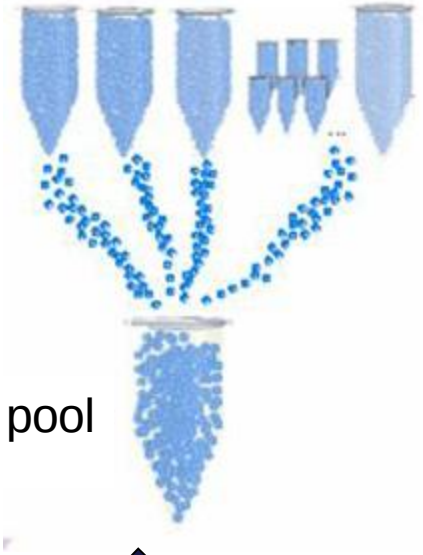


11-20 Oligonucleotides/ gene

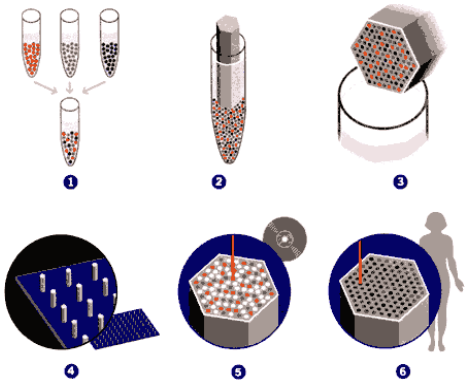
BeadArrays: assembly of a random array



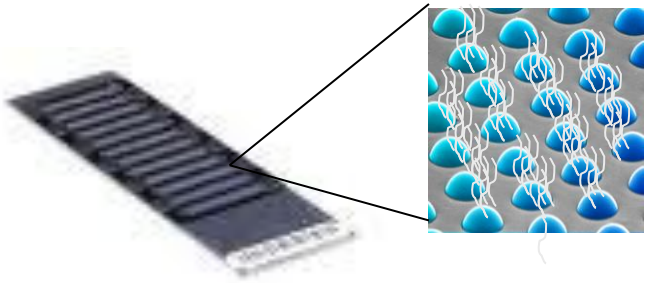
n-thousands bead types



Randomly array beads into wells



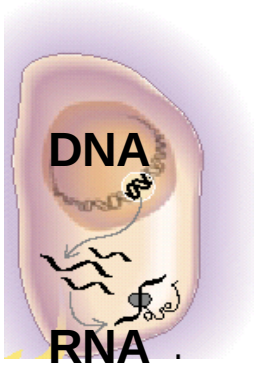
Decode each bead using hybridisation to address sequence



DMAP Files
The BeadChip decode map files (.dmap)

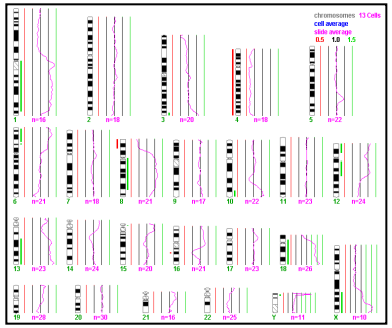
48000 probe
average 30x
99.99 % of probes are represented in each array

DNA microarrays applications



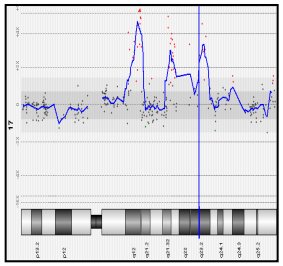
CGH arrays

Copy number analysis →

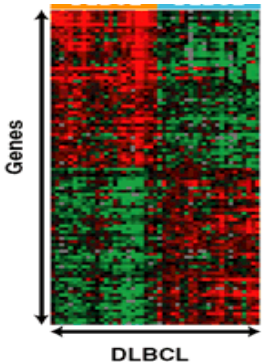


Promotor arrays

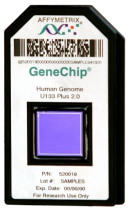
DNA regulation Epigenetics →



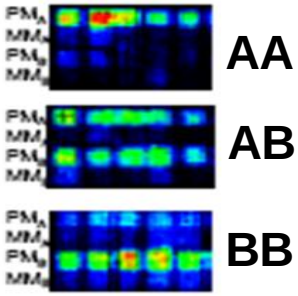
Expression arrays



SNPs arrays

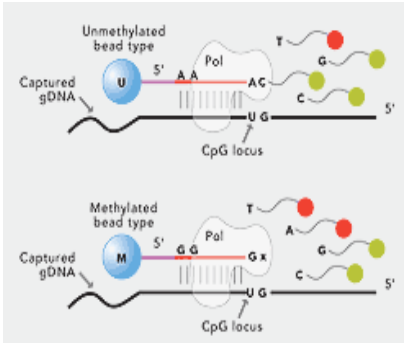


Genotyping →



CpG arrays

DNA methylation →



Gene Expression Profiling

Two color array

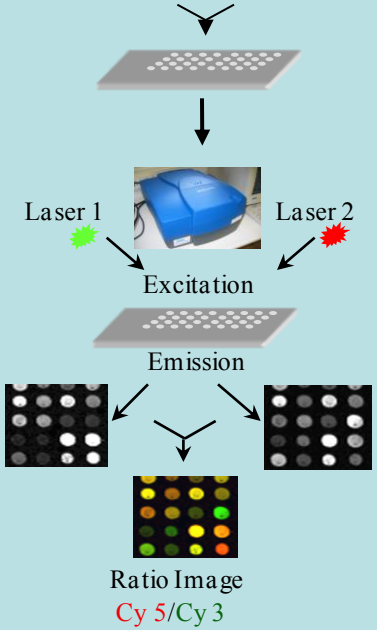
Test-RNA

Reference-RNA

Reverse Transcription
IVT
(labeling with Cy3 and Cy5)

Cy3-labeled cRNA

Cy5-labeled cRNA



RNA Isolation

Target Labeling

Hybridization
Washing
Streptavidine-PE Staining

Scanning

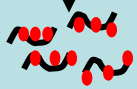
Analysis

Data Mining/ Bioinformatics

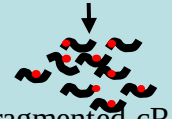
Single color array

Test-RNA

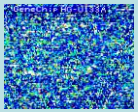
Reverse Transcription
IVT



Biotinilated cRNA



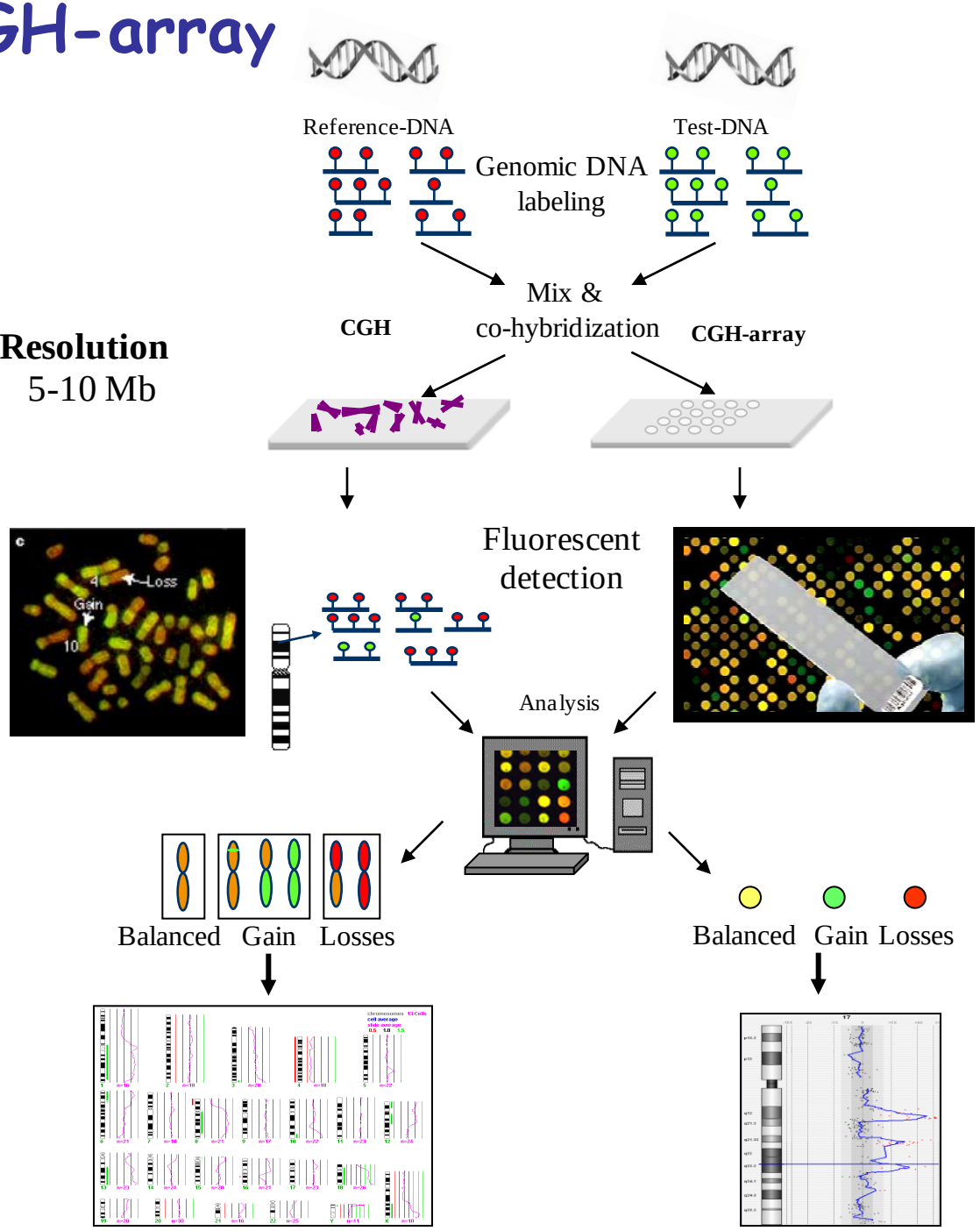
Fragmented cRNA



Signal
Present Call

PJares, Ultrastruct Pathol 2006

CGH-array



Resolution
5-10 Mb

Resolution

BAC-arrays:
Printing of 32.433 overlapping BAC clones >100Kb

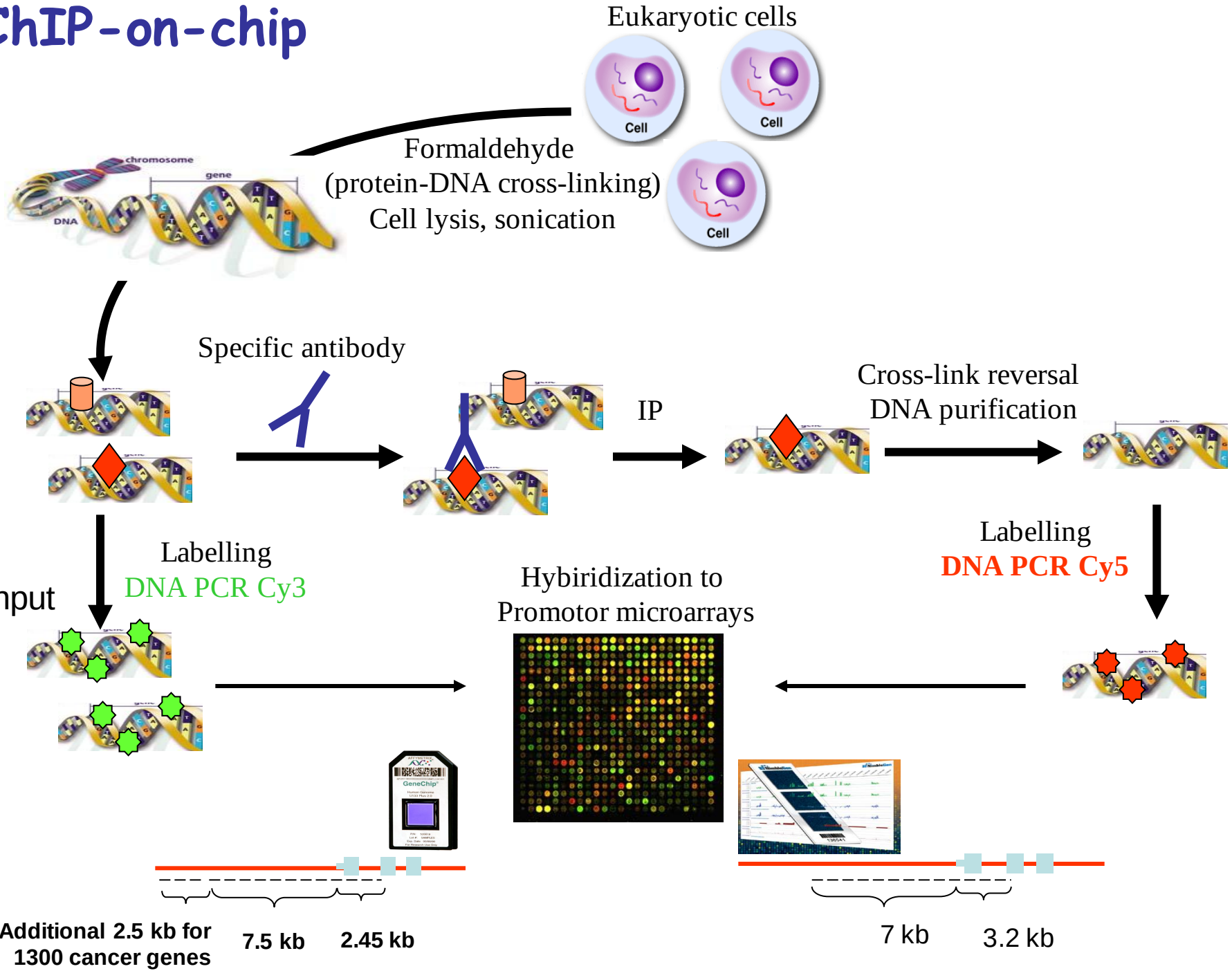
Agilent CGH array;
244k 60mer ;
8.9 kb median probe spacing

NimbleGen HD-arrays
Long oligos probes
~2,100,000 (50mer-85mer)
1.1kb median probe spacing

Illumina human 1Mduo
1,200,000 loci per bead chip
1.5 kb median marker spacing

Affymetrix SNP array 6.0
1.800,000, intermarker distance 696bp

ChIP-on-chip


Eukaryotic cells
Cell
Cell
Cell
Formaldehyde
(protein-DNA cross-linking)
Cell lysis, sonication
chromosome
gene
DNA
Specific antibody
IP
Cross-link reversal
DNA purification
Labelling
DNA PCR Cy5
Labelling
DNA PCR Cy3
input
Hybiridization to
Promotor microarrays
GeneChip
Additional 2.5 kb for
1300 cancer genes
7.5 kb
2.45 kb
7 kb
3.2 kb

SNP Arrays

SNP Allele A= **A**

SNP Allele B= **G**

SNP

….TAGCCATCGGTA**N**GTACTCAATGAT….

A

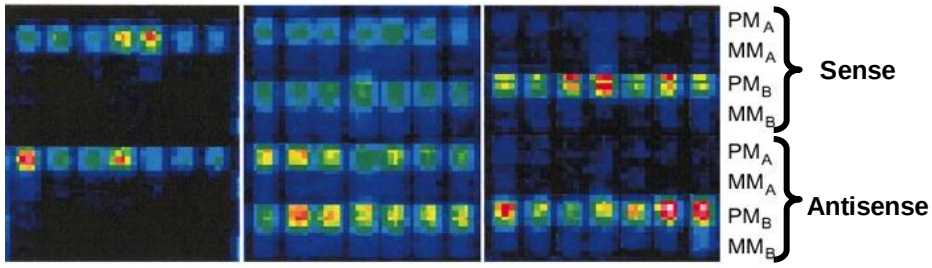
G

Affymetrix Genotyping Array Design

Sequence-Specific Oligonucleotide (SSO) Probes

ATCGGTAGCCAT**T**CATGAGTTACTA Allele A

ATCGGTAGCCAT**C**CATGAGTTACTA Allele B

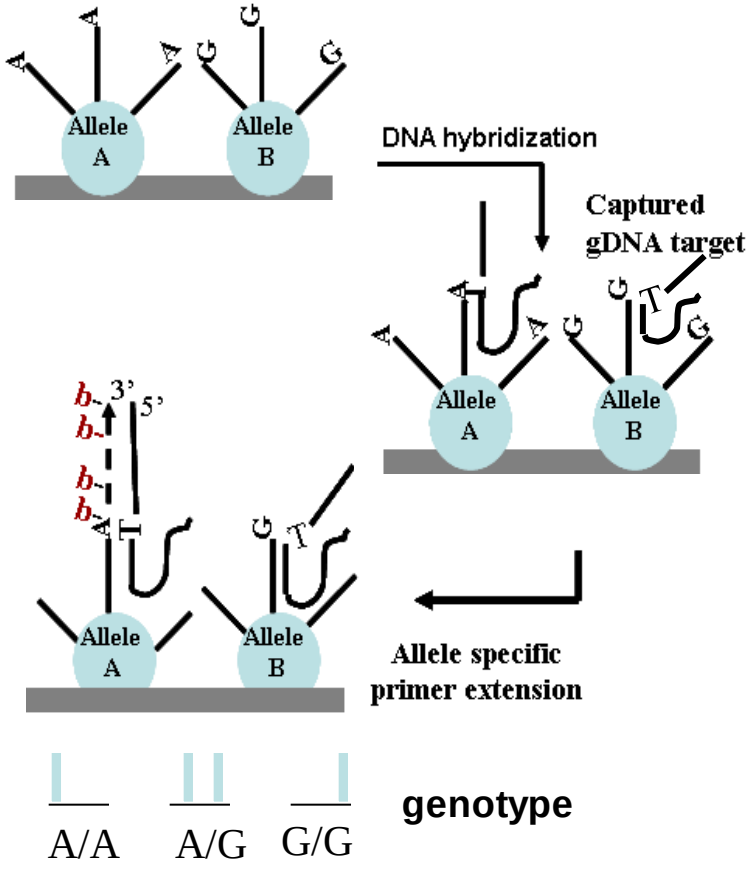


Position: -4 -2 -1 0 +1 +3 +4

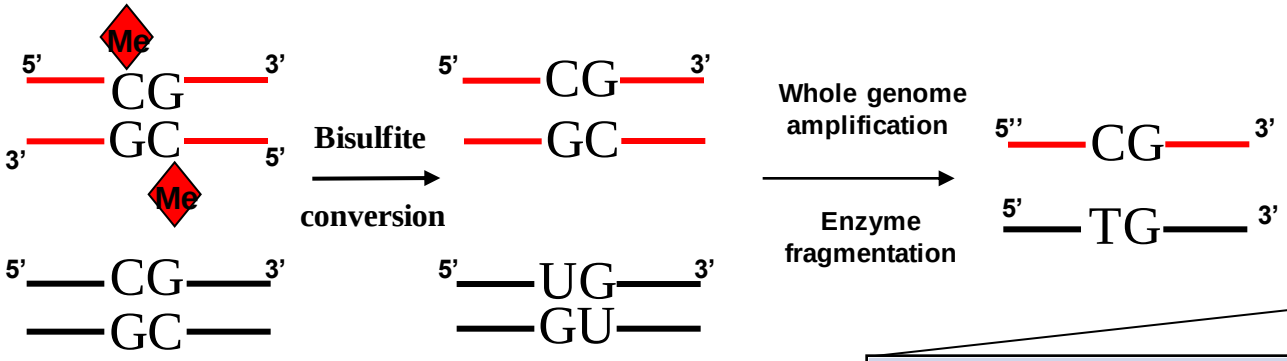
AA AB BB **genotype**

Infinium Illumina SNP

Allele-specific primer extension (ASPE)



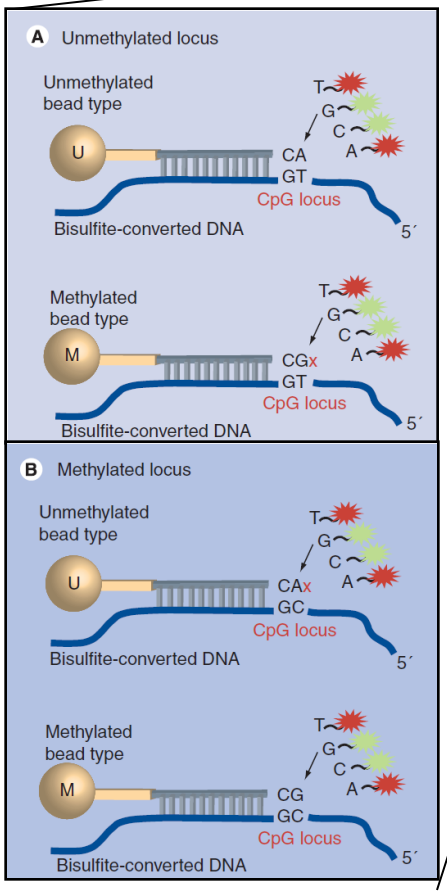
CpG Array: Infinium Methylation Assay



Hybridization

Human
Methylation 27 k

N=12 samples



Allele-specific primer
annealling.
Single base extension

Unmethylated
DNA

3' GT 5'

Methylated
DNA

3' GC 5''

Stainned and
scanned

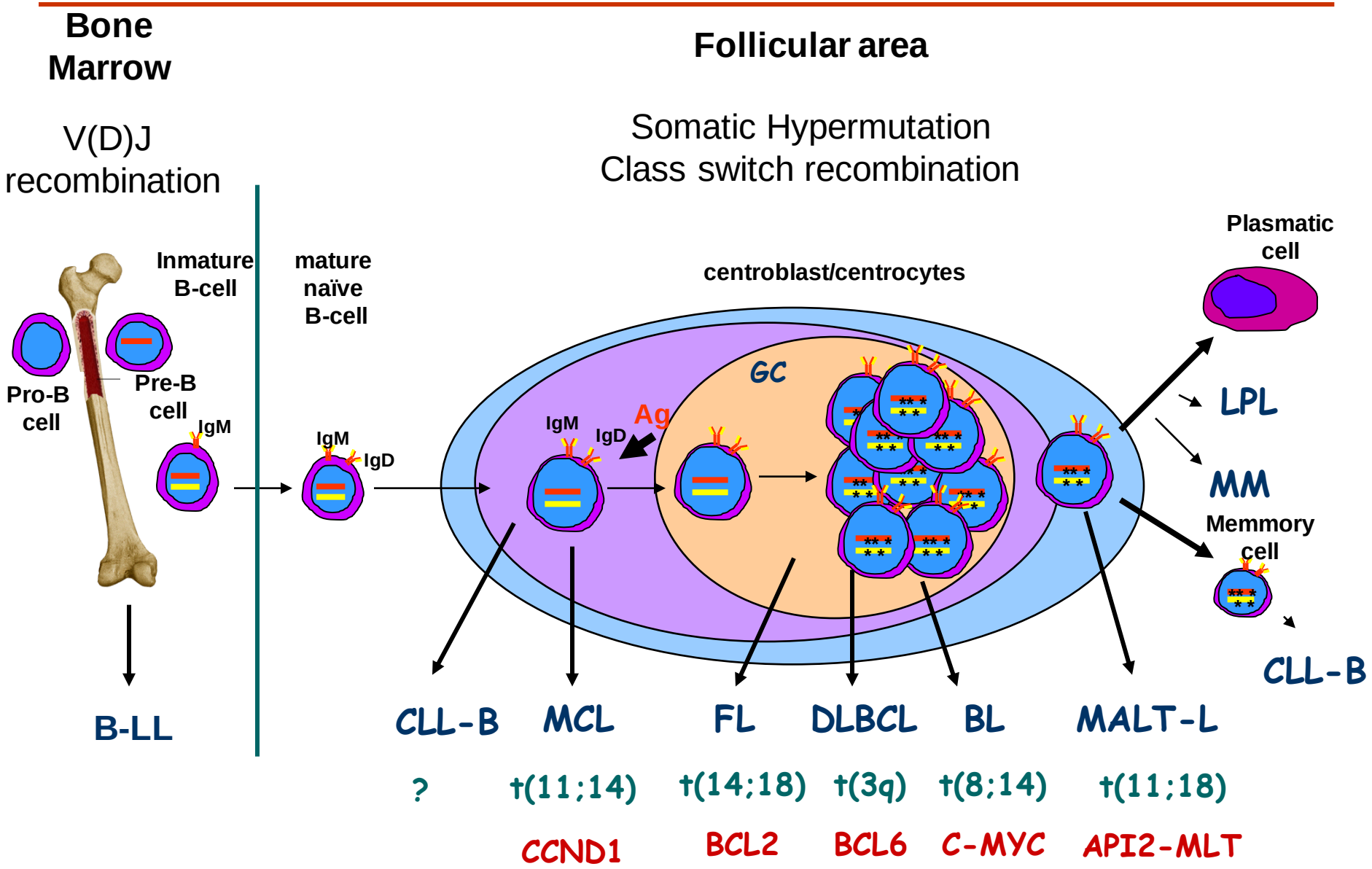
β
values

$$\frac{\text{Intensity [M]}}{\text{Intensity [M] + [U]}}$$

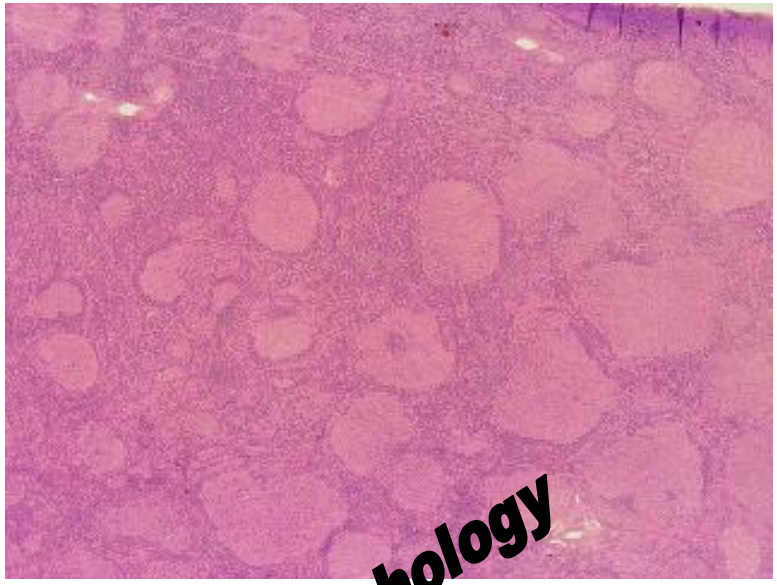
(0-1)

B-Cell NHL Pathogenesis:

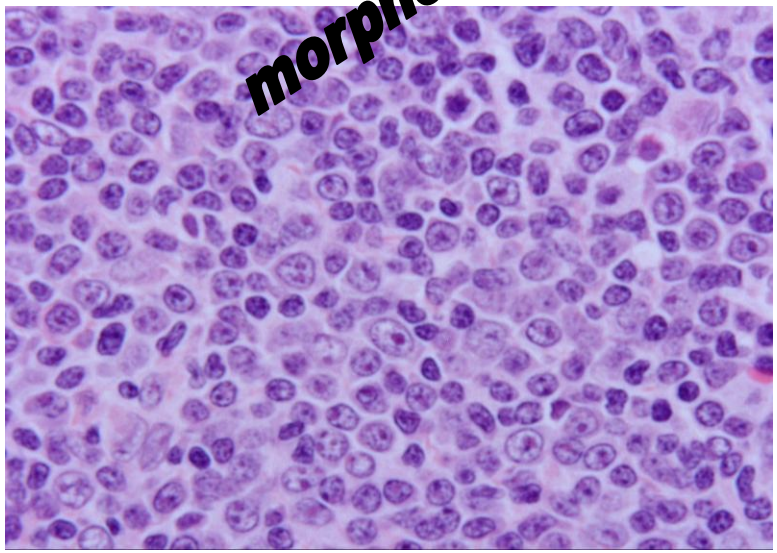
Cellular origens, primary genetic alterations and target genes

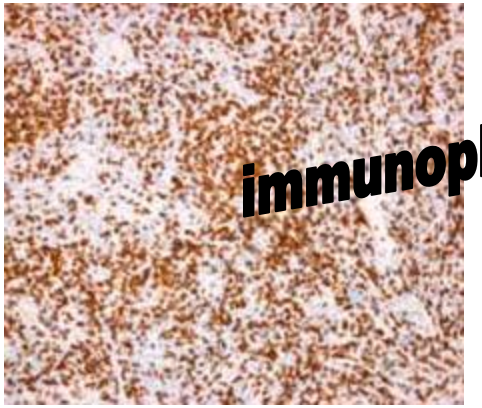


Diagnosis in Hematopathology
From Morphology to an Integrated Diagnosis

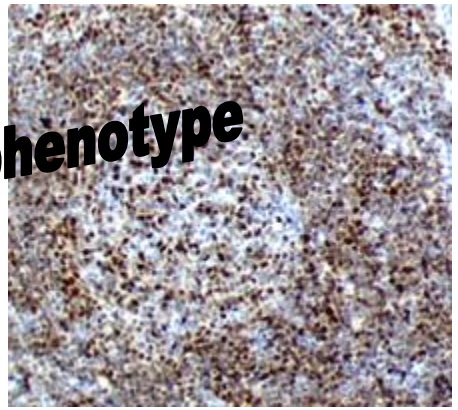


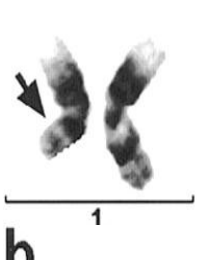
morphology



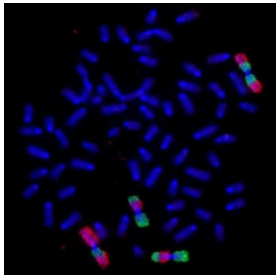


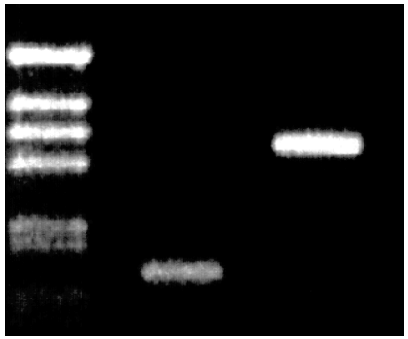
immunophenotype

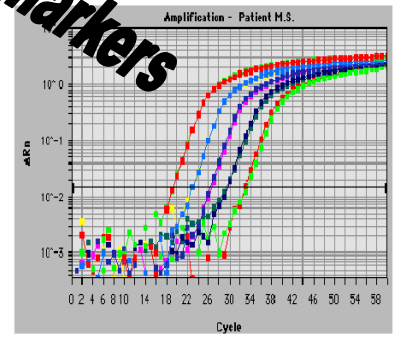




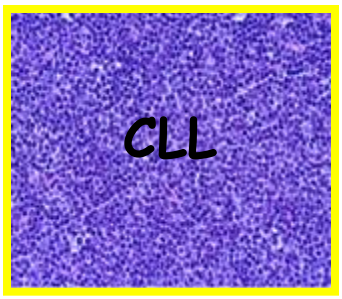
molecular markers

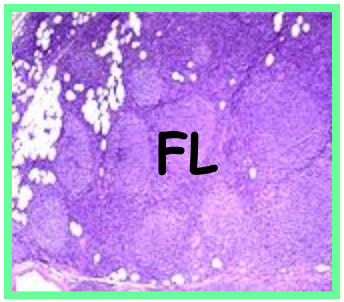


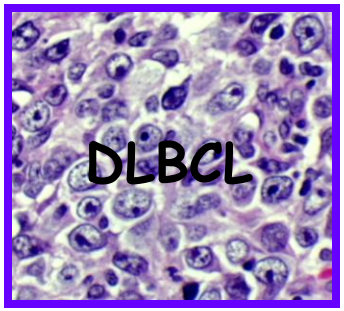


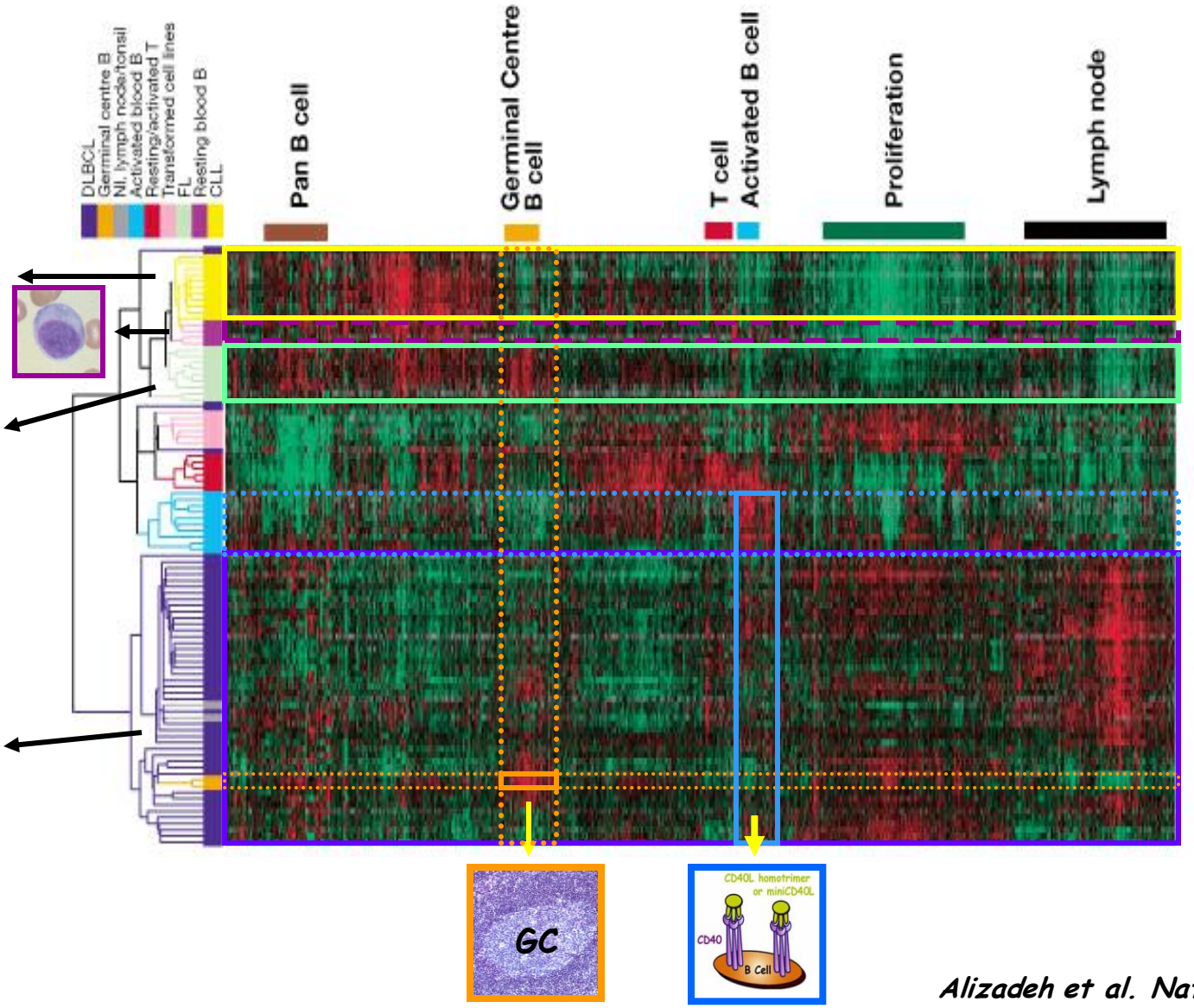


Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling



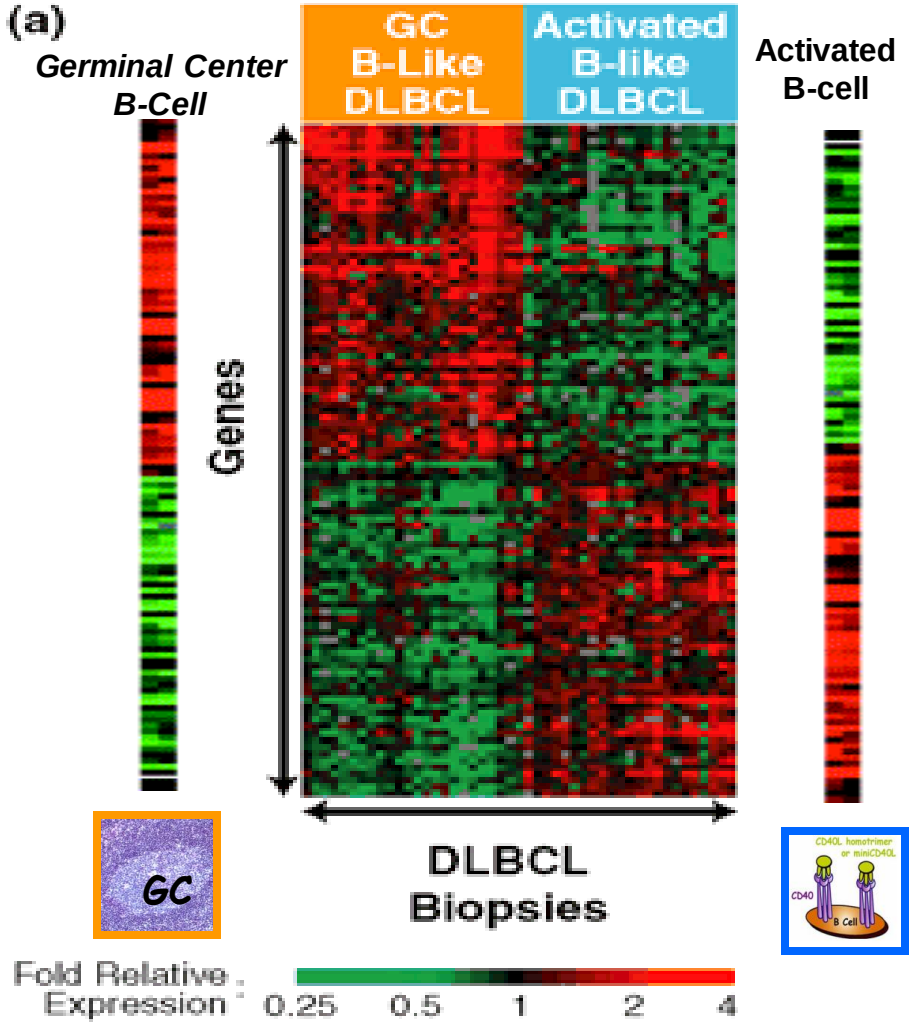


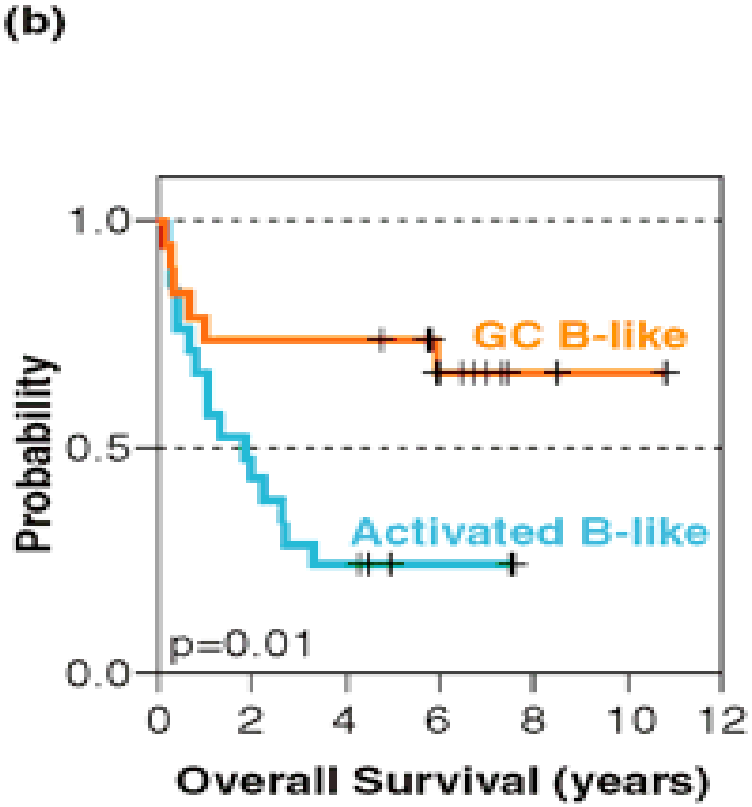




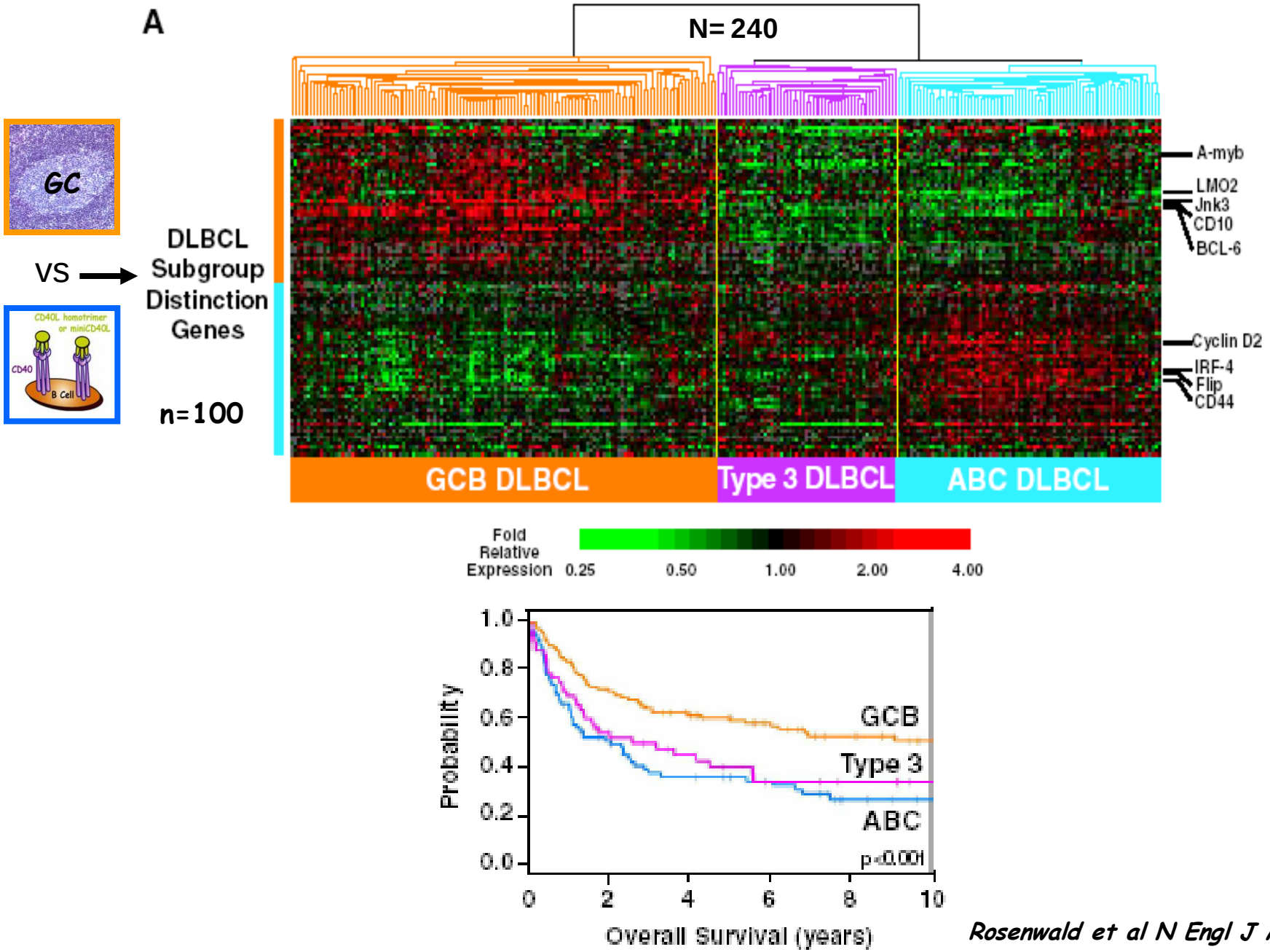
Alizadeh et al. Nature 2000

Diffuse large B cell lymphoma (DLBCL) comprises at least two distinct diseases



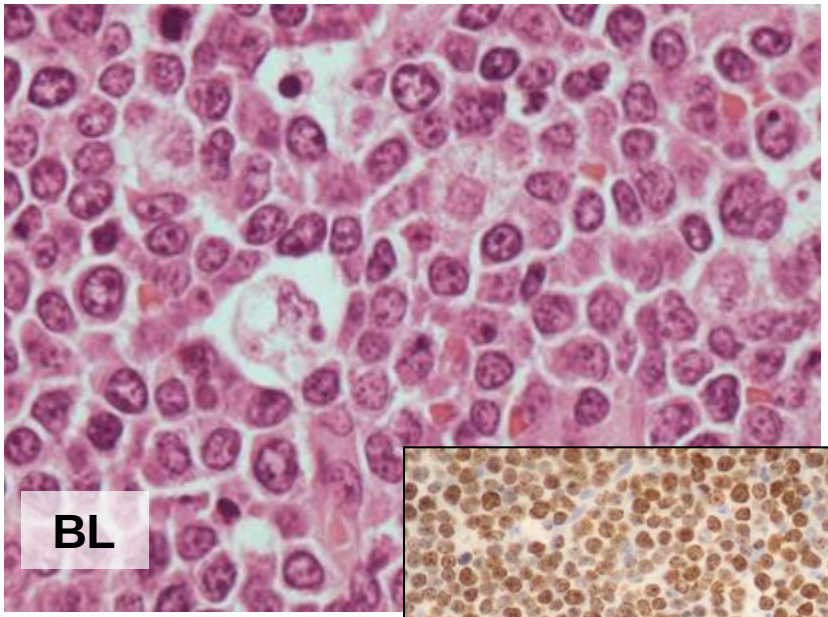


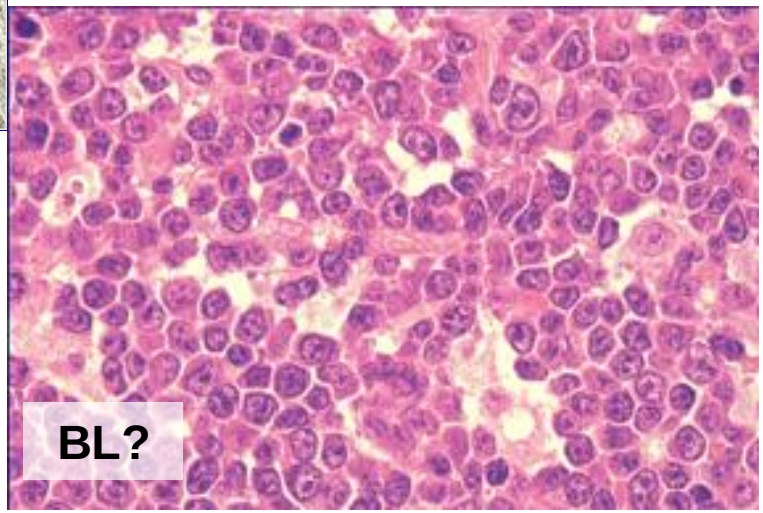
Modified from Louis M. Staudt Annu. Rev. Med. 2002

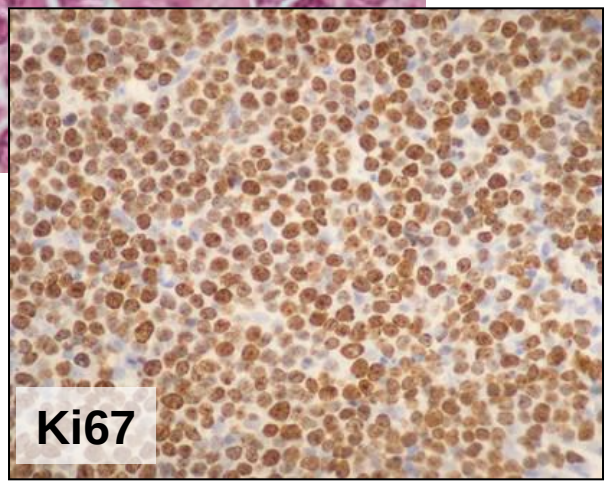


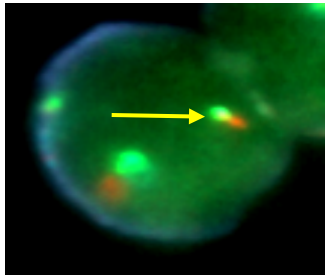
Rosenwald et al N Engl J Med 2001

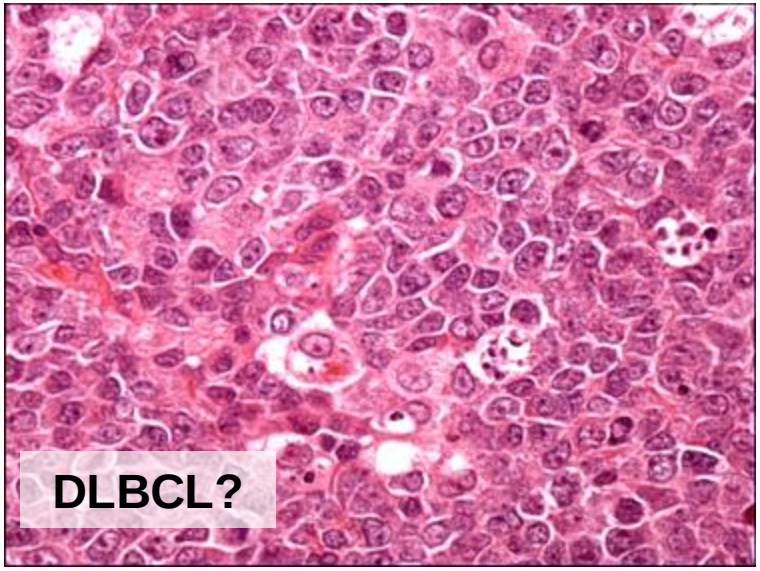
To be or not to be …



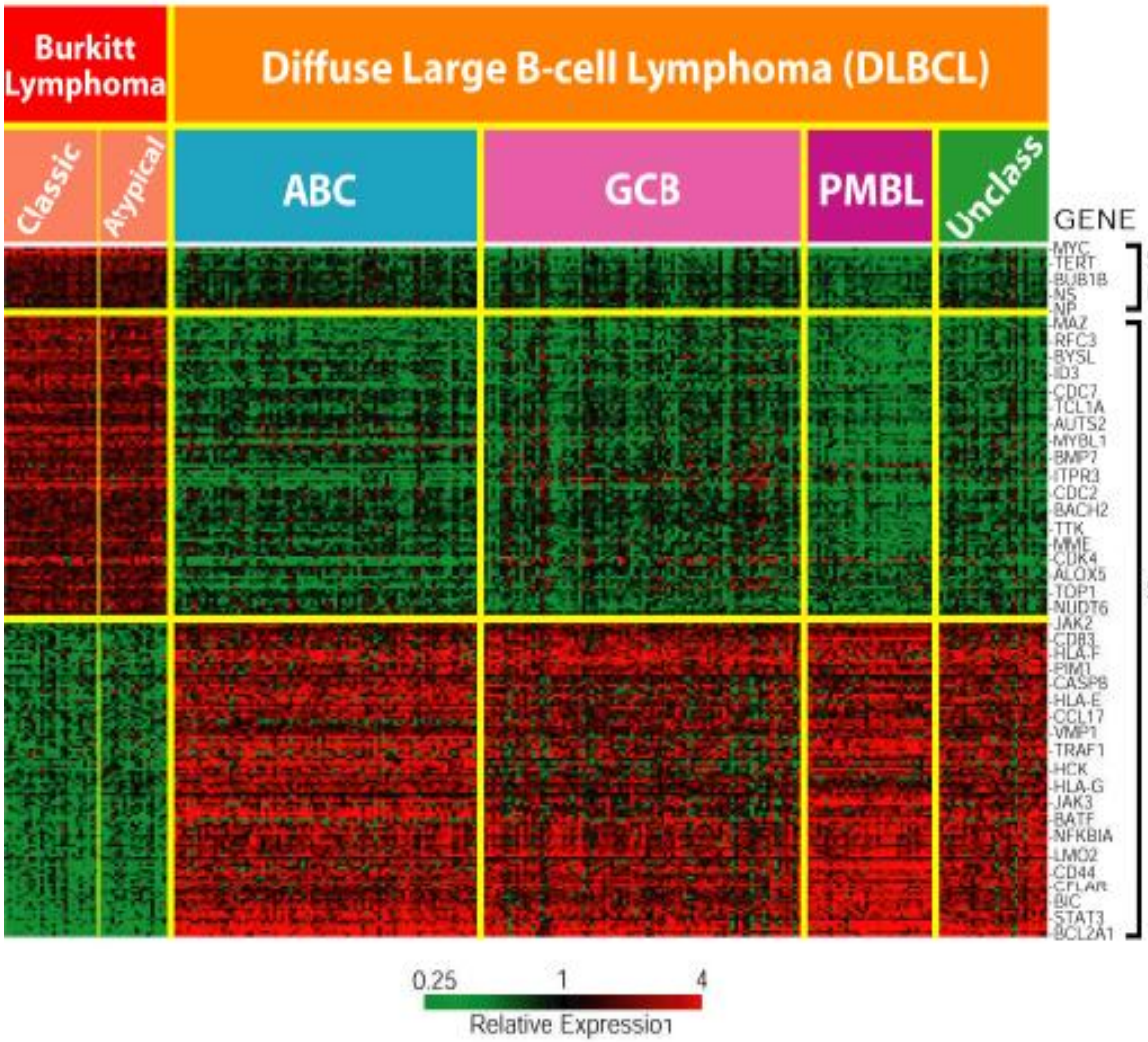








Gene Expression Patterns in Burkitt Lymphoma



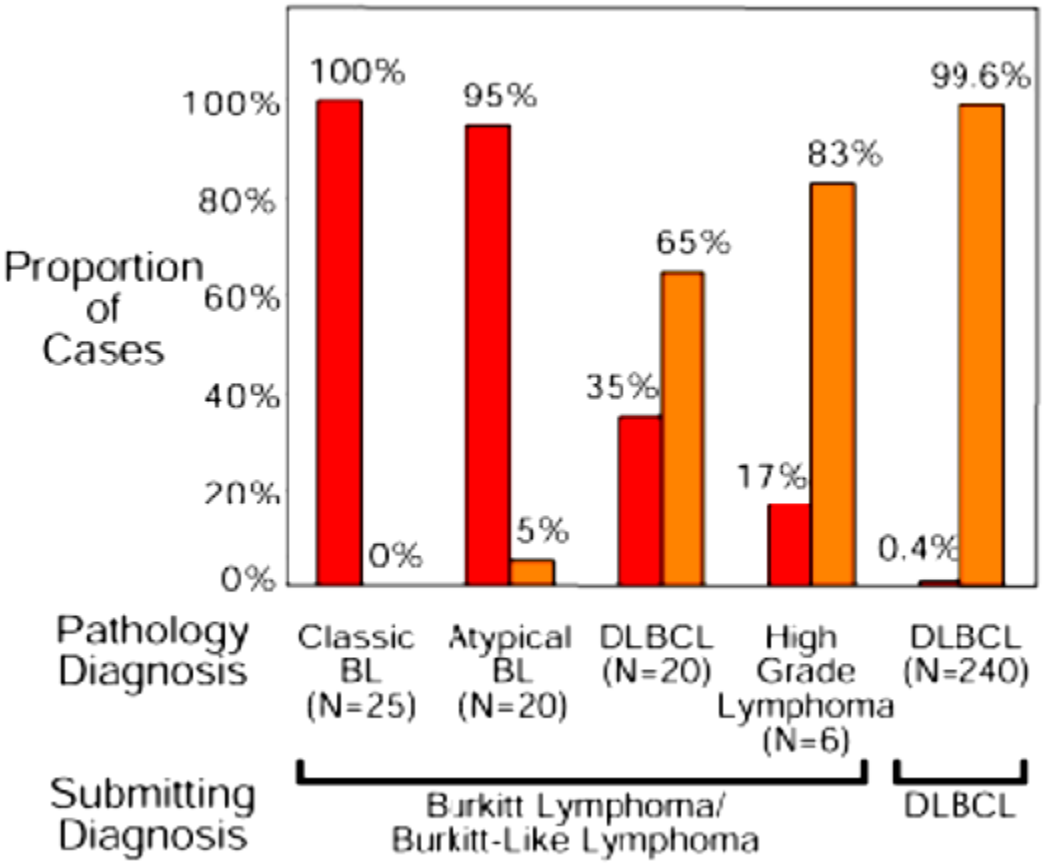
Step 1: Comparison for 21 MYC target genes as defined by MYC transfection experiment in DLBCL

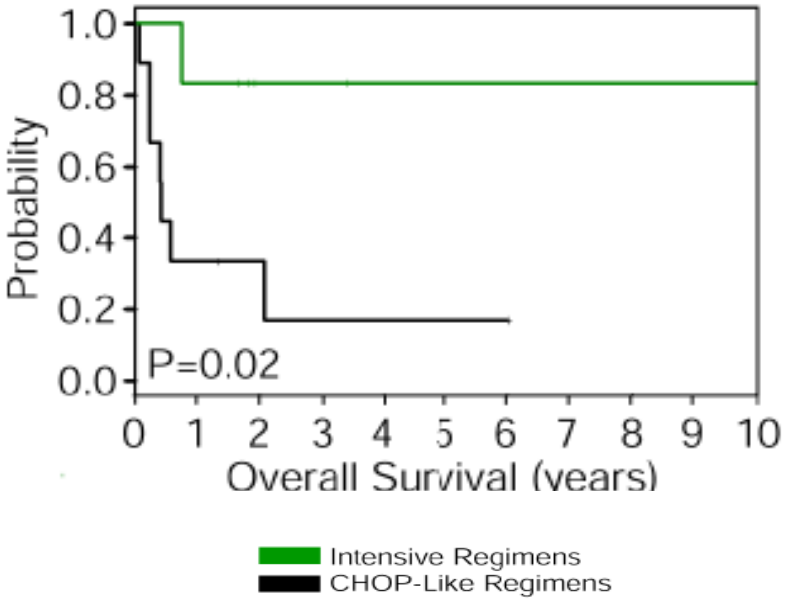
Step 2: Separate comparisons of BL with DLBCL-ABC, GCB and primary mediastinal B-cell lymphoma (PMBL)

Step 3: Construction of BL predictor; also quantitative predictor (0-100%)

Dave S et al N Engl J Med 2006

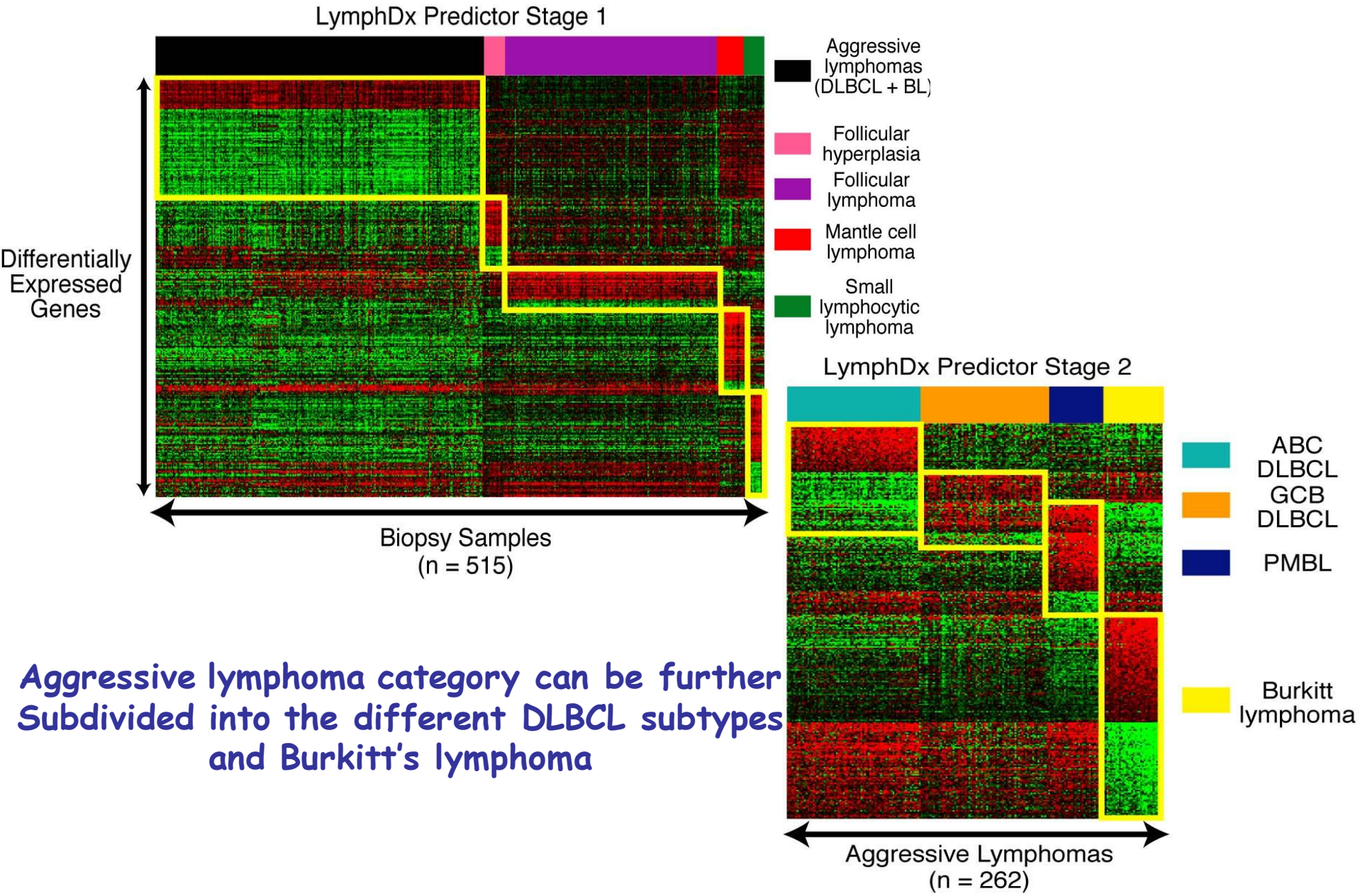
Molecular versus Pathology Diagnosis





Sandeep D et al N Engl J Med 2006

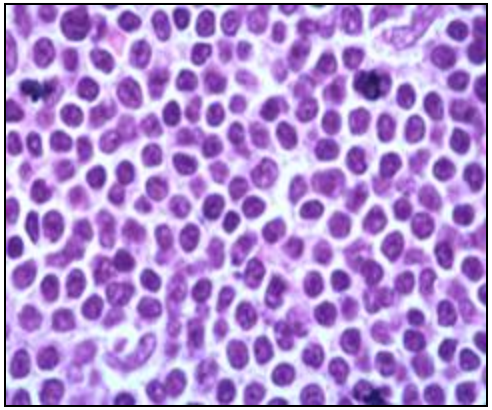
Gene expression profiles of the major classes of B-cell NHL



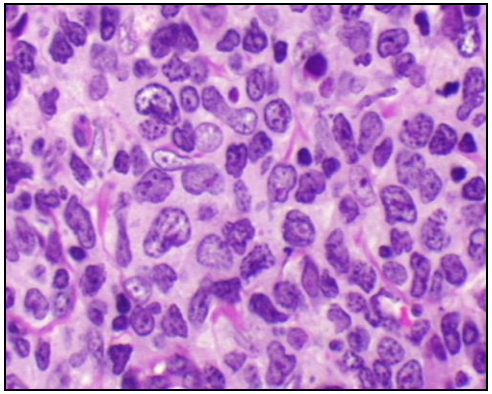
Aggressive lymphoma category can be further Subdivided into the different DLBCL subtypes and Burkitt's lymphoma

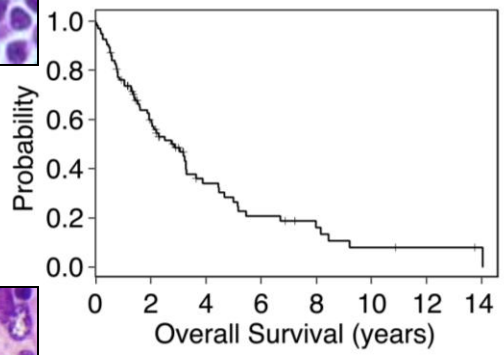
Mantle Cell Lymphoma
t(11;14) Translocation and Cyclin D1

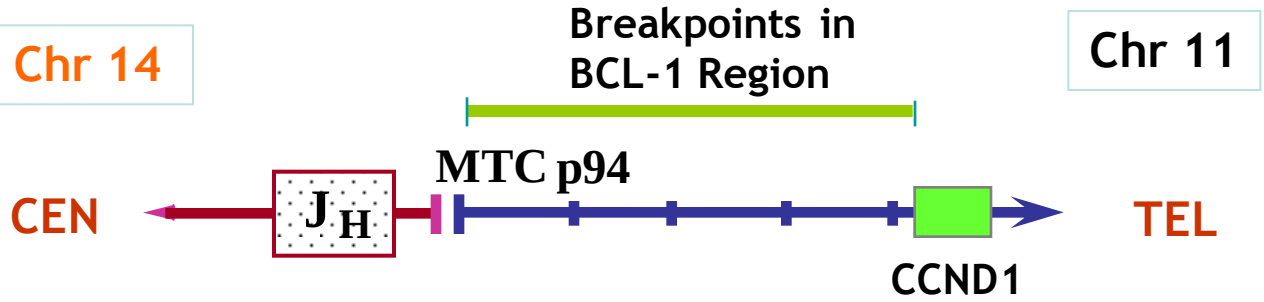
Classical

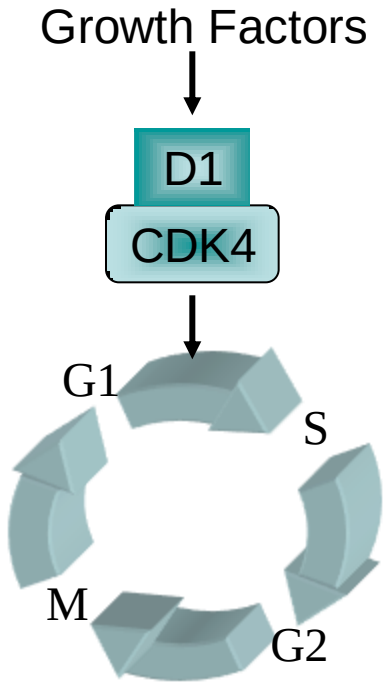


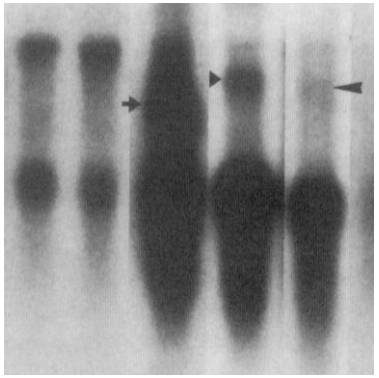
Blastic

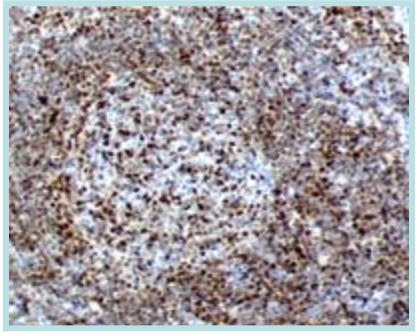




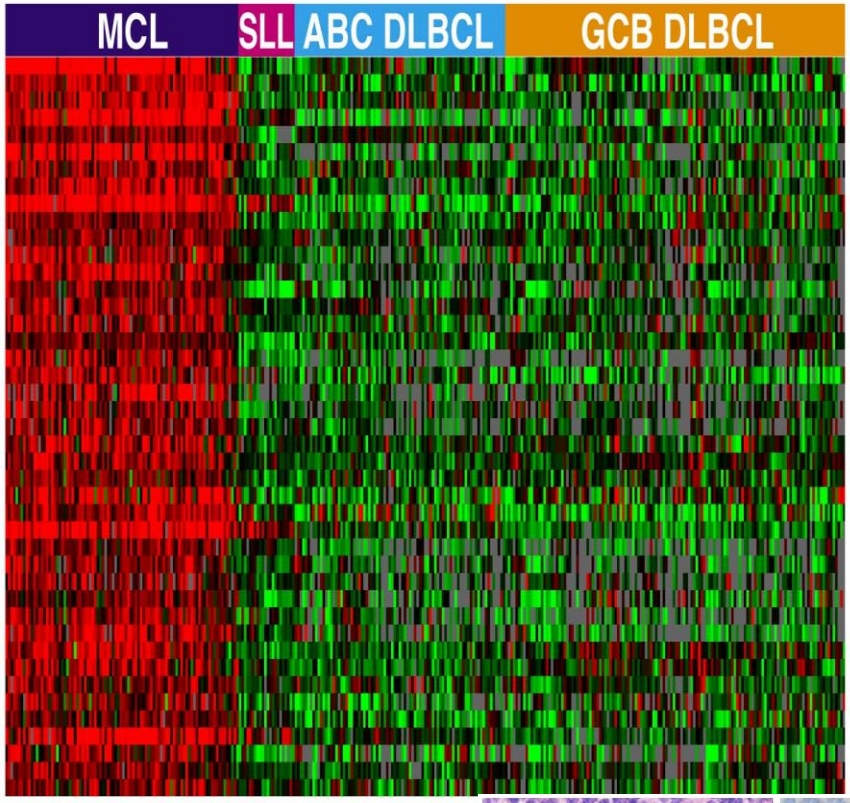


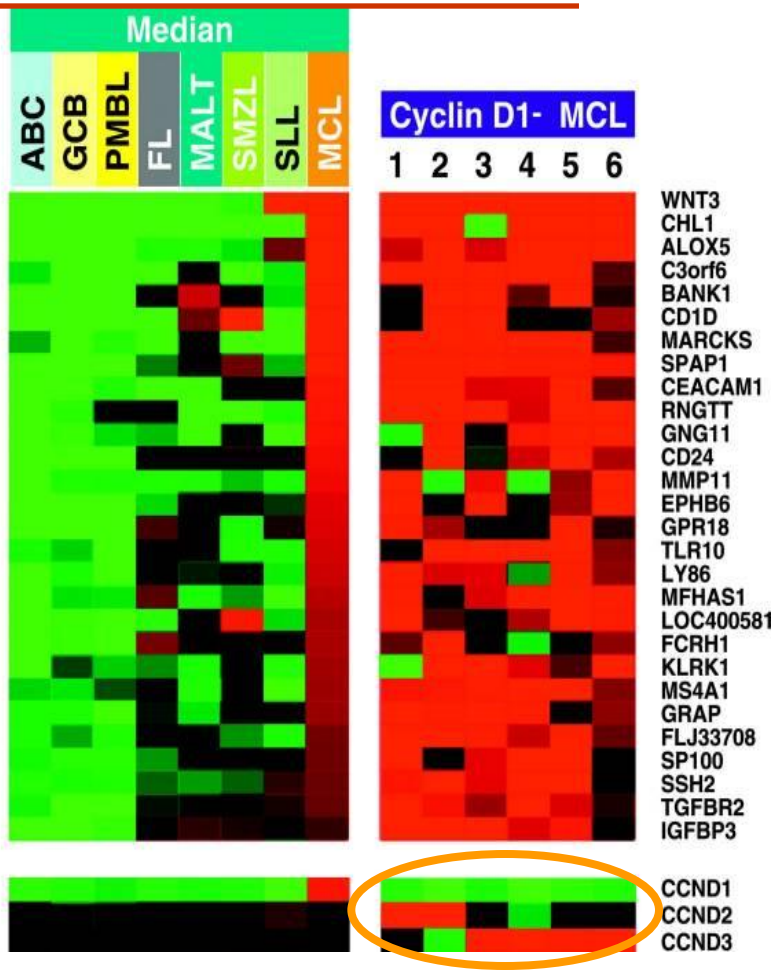


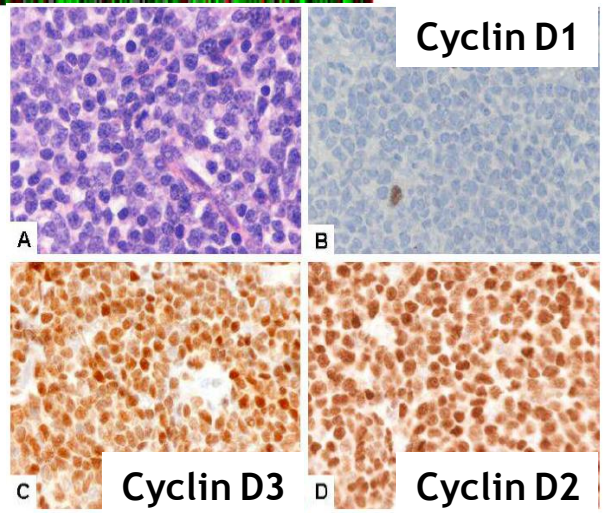




Cyclin D1 Negative MCL Variant



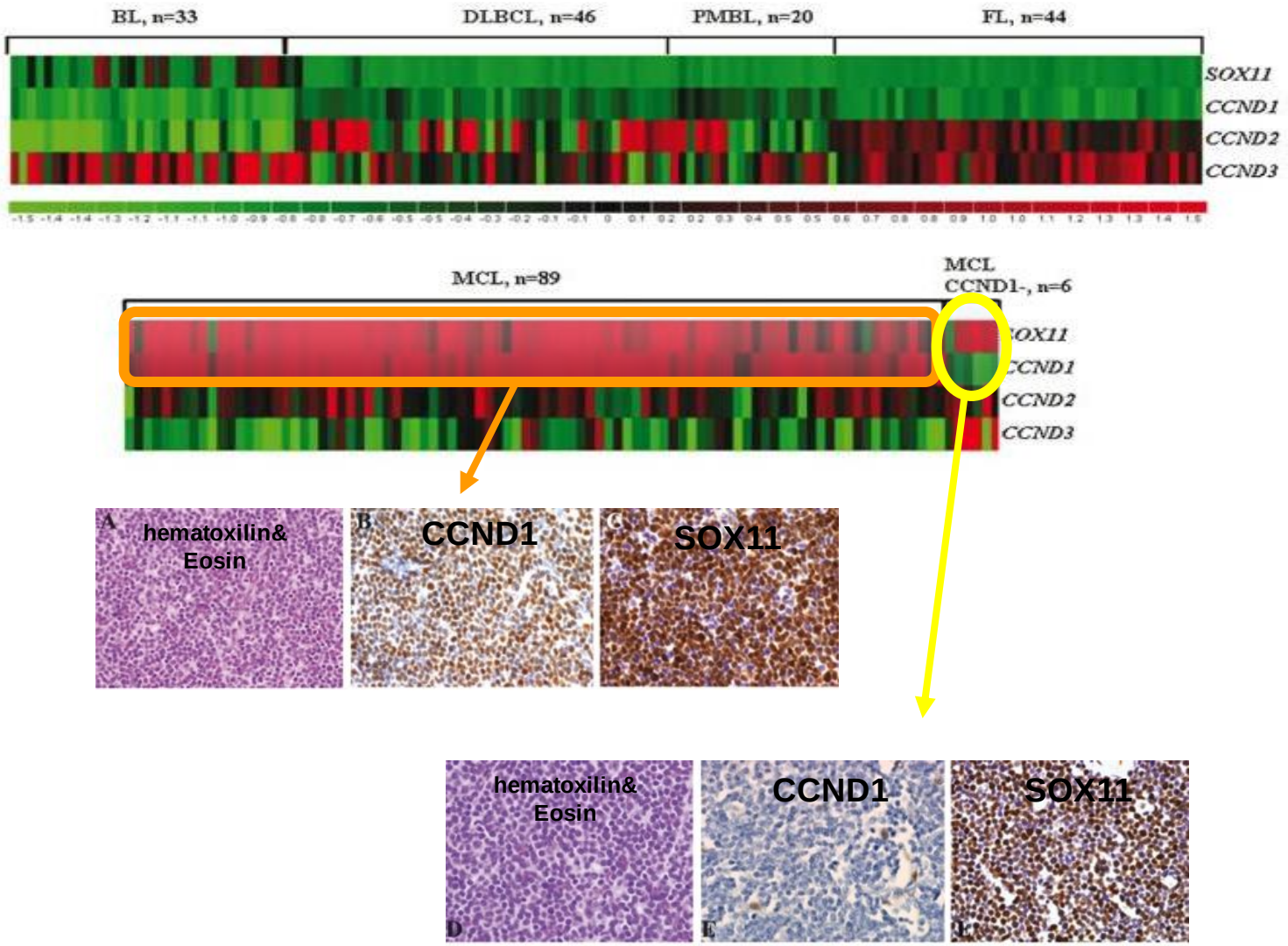




BLOOD, 15 DECEMBER 2005 • VOLUME 106, NUMBER 13

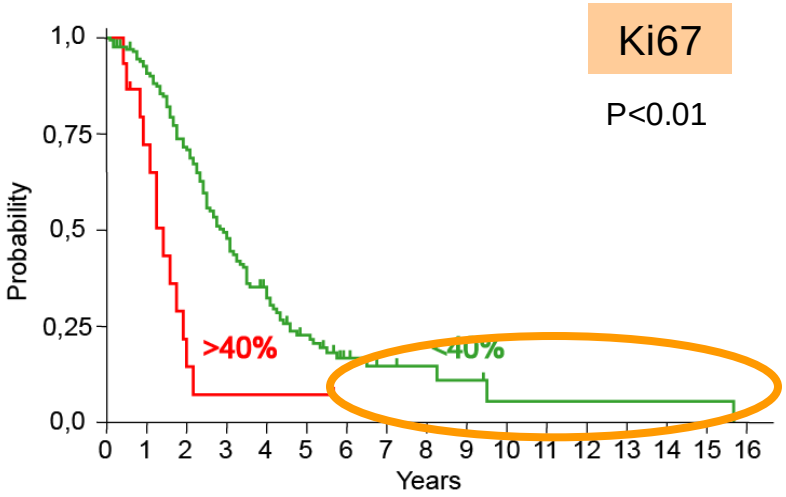
Fu K et al, Blood 2005

SOX11 expression is highly specific for MCL and identifies Cyclin D1-negative subtype



Ana Mozos et al., haematologica 2009

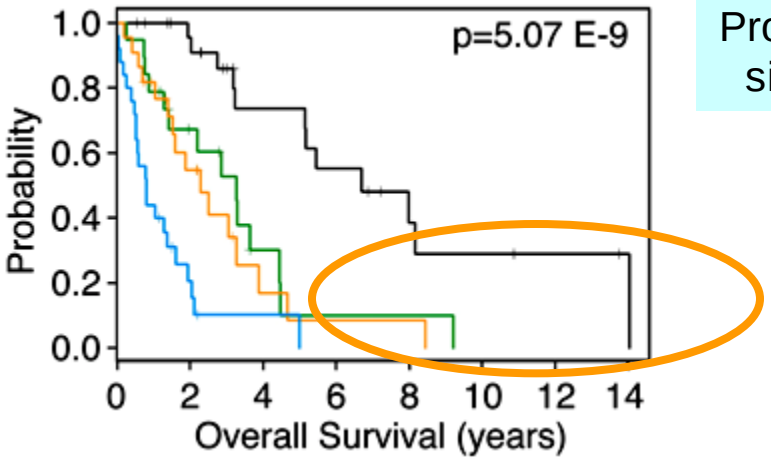
MCL prognosis



Ki67

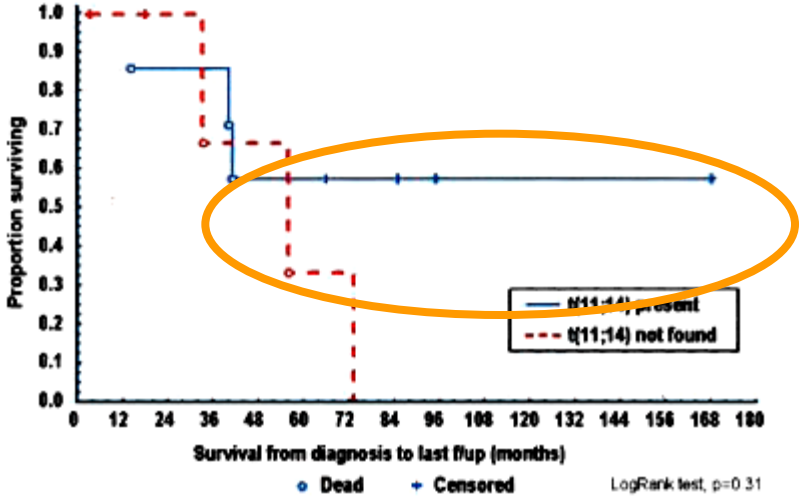
P<0.01

Tiemman et EMCL, Br J Haematol 2005; 131(1):29-38



Proliferation signature

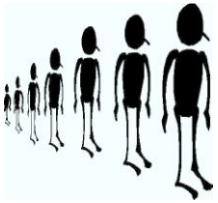
Rosenwald A et al, Cancer Cell 2003, Feb;3(2):185-97

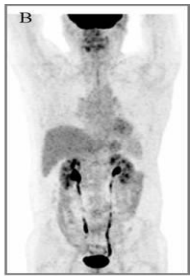


Ruchlemer R et al, Br J Haematol 2004

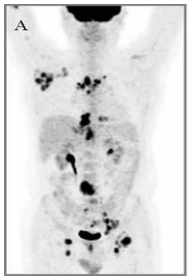
Study design

Indolent MCL



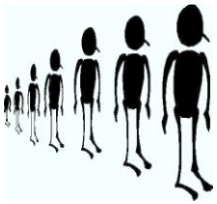


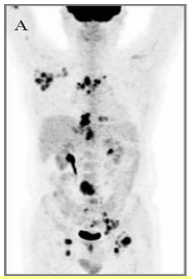
2 years



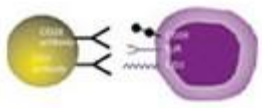
12 patients with indolent MCL (iMCL) not treated with chemotherapy and did not have evidence of clinical progression during > 2 years.
Detection t(11;14) and overexpression CCND1
Initial diagnosis: SMZL (4), CLL (2),
Leukemic Lymphoid neoplasm (NOS) (4), "in situ" MCL (2)

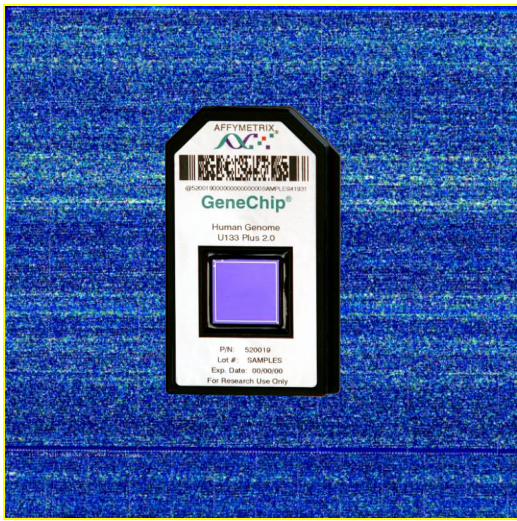
Conventional MCL





CD19

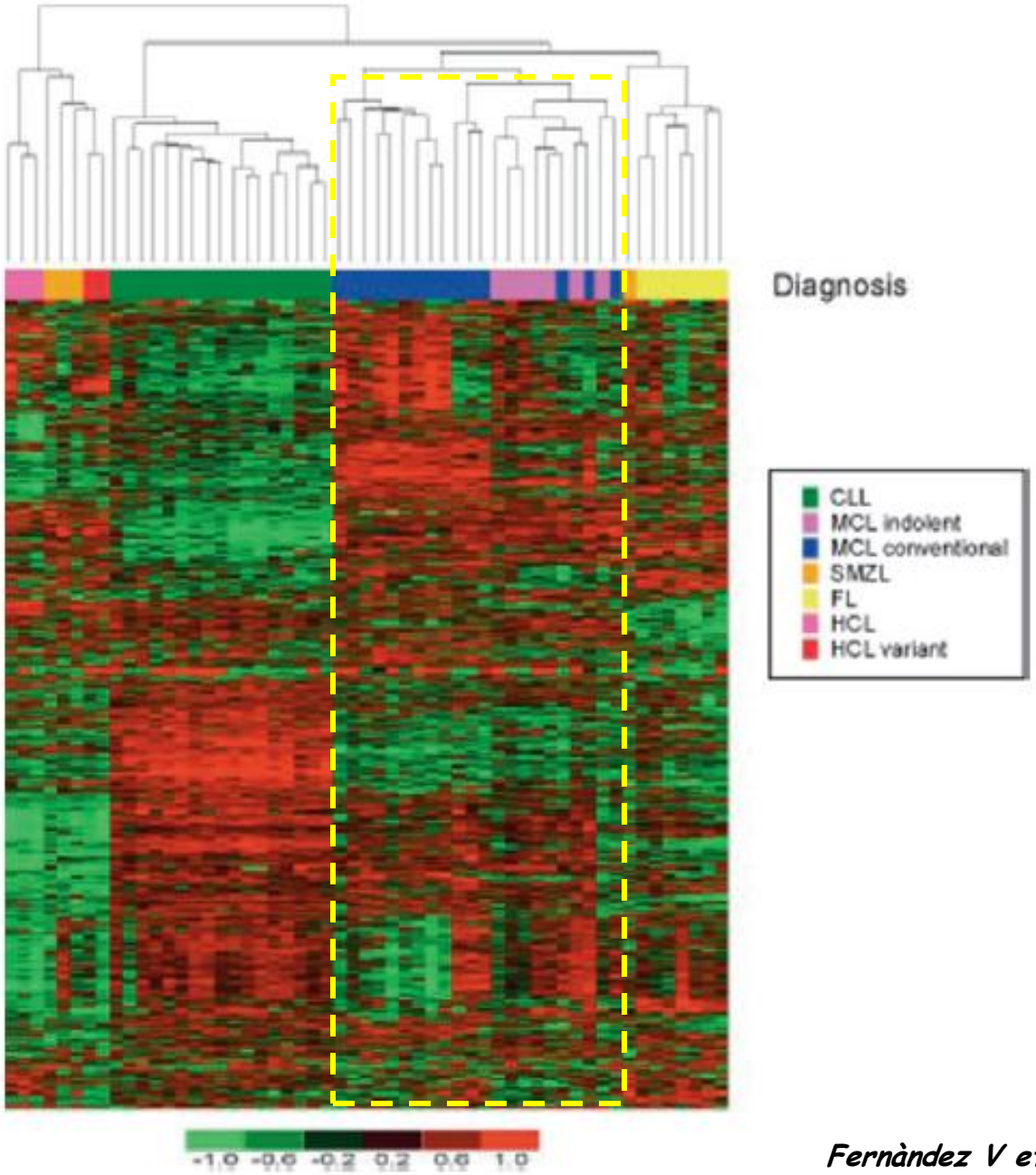




15 patients with conventional MCL (cMCL) that required chemotherapy at diagnosis

Generalized Polyadenopaty; ECOG>2
Evidence of disease progression at diagnosis
Median follow-up 15 months, range 0.3-79

iMCL & cMCL clusterize together

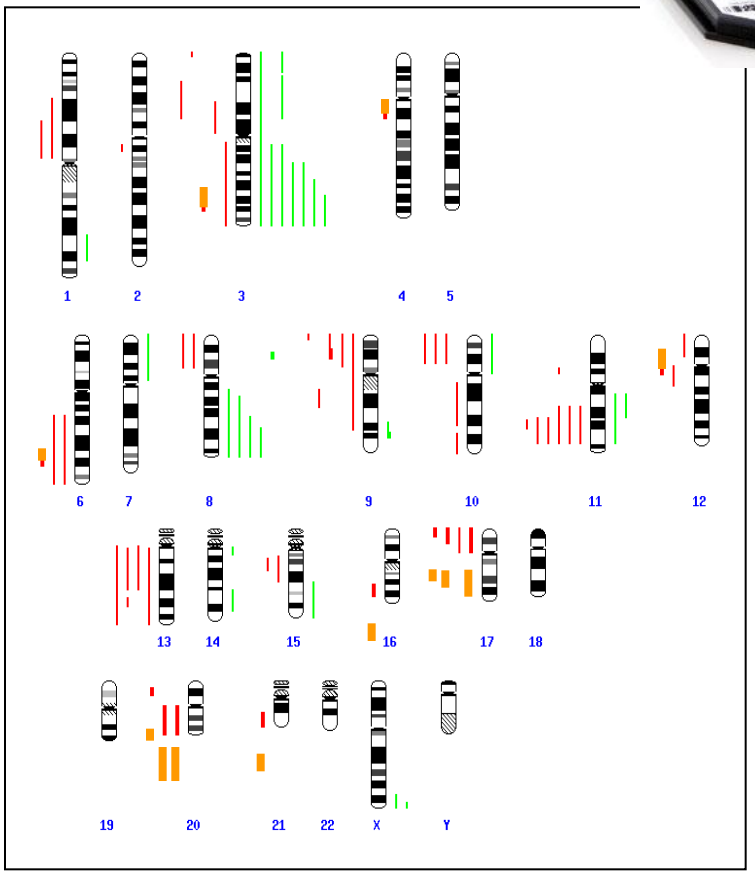


Fernàndez V et al. Cancer Res 2010

cMCL & iMCL Chromosomal Alterations by SNP Arrays

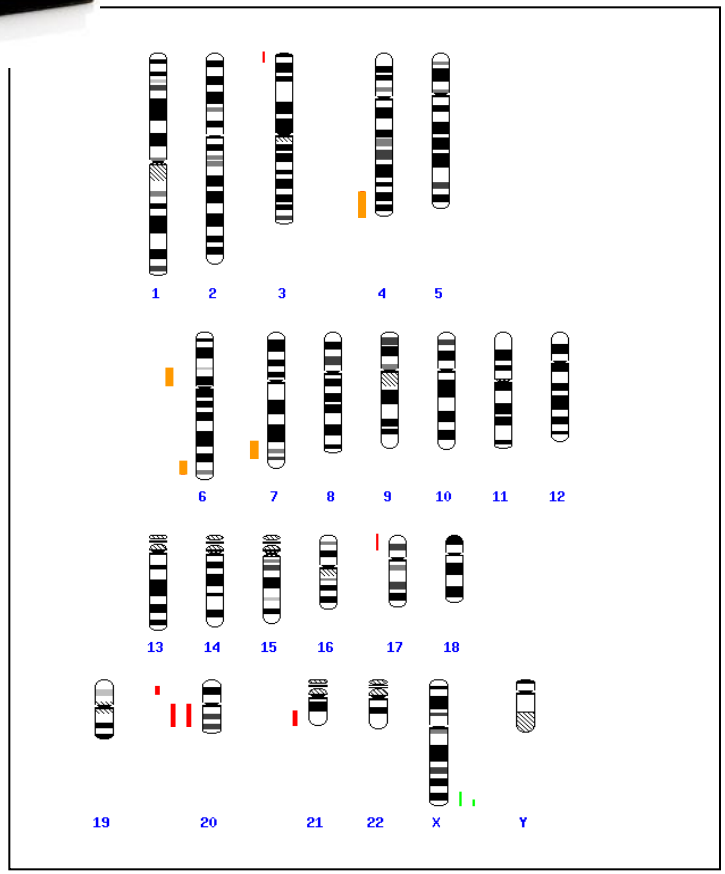
SNP 6.0

cMCL

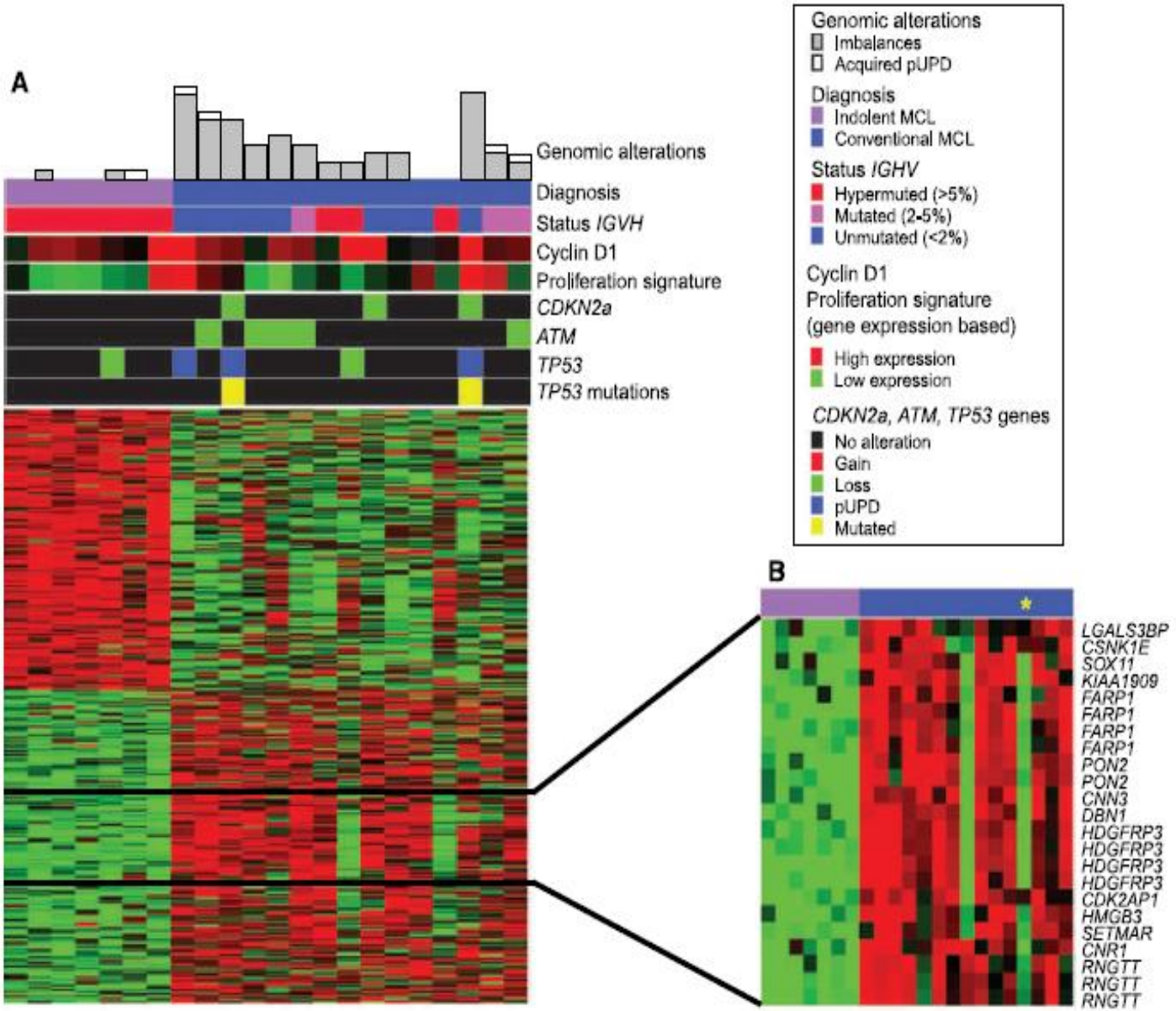


Gain
Loss
pUPD

iMCL

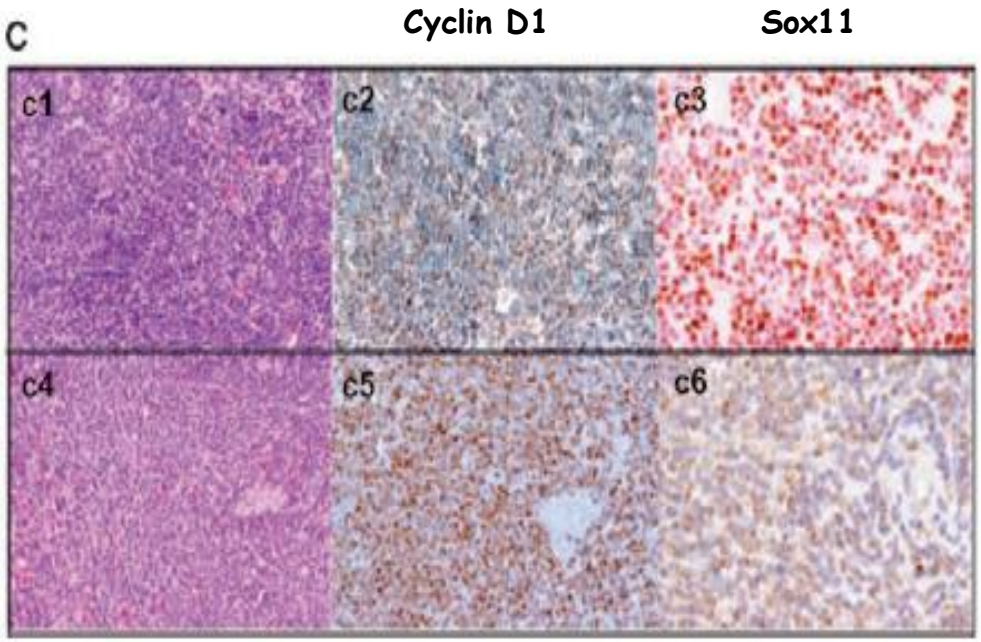


Gene Expression Profiling: conventional vs indolent MCL



Fernàndez V et al. Cancer Res 2010

SOX11 expression in a independent validation series



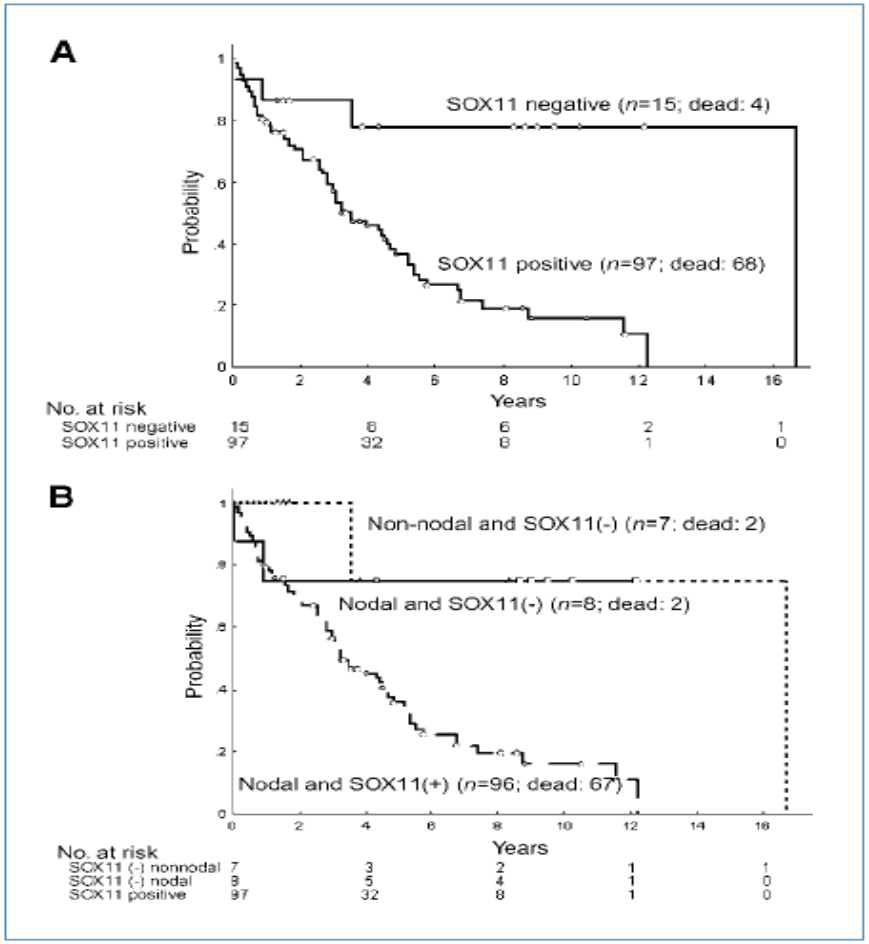
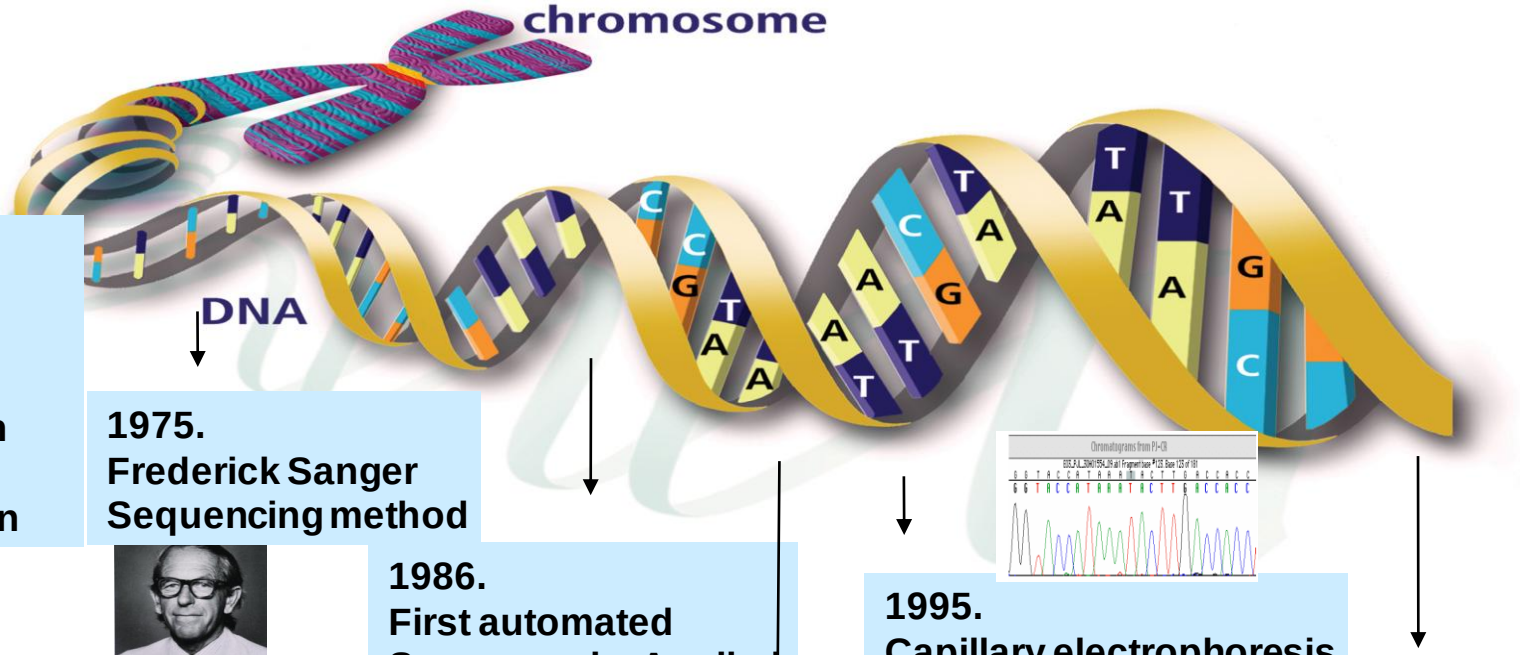


Figure 4. A, Kaplan-Meier estimates of OS of the 112 MCLs in the validation series according to SOX11 expression ($P < 0.001$). B, OS of 112 patients with MCL according to the nodal/nonnodal presentation and SOX11 expression ($P = 0.05$). The only patient with nonnodal presentation and SOX11-positive was not included.

Fernàndez V et al. Cancer Res 2010

Next Generation Sequencing



1953
DNA
double
helix
Watson
Crick
Franklin

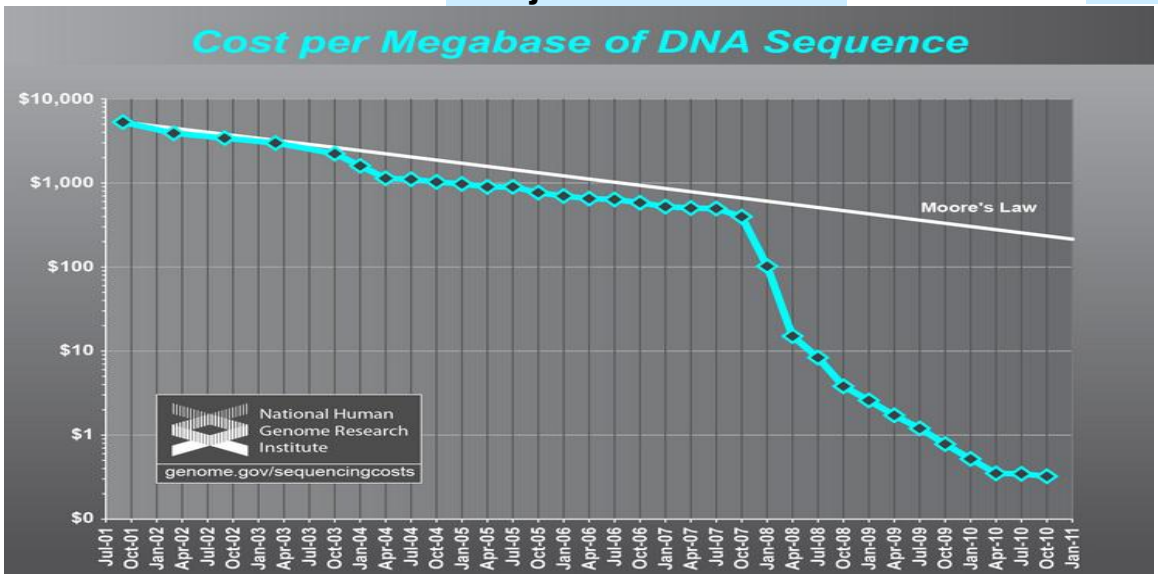
1975.
Frederick Sanger
Sequencing method

1986.
First automated
Sequencer by Applied

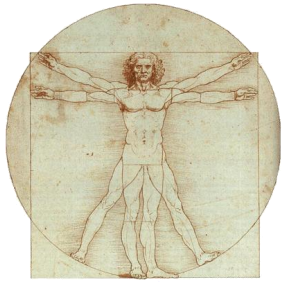
1995.
Capillary electrophoresis
Sequencer by Applied

1990.
The Human Genome
Project start

2003.
The Human Genome
Project finish

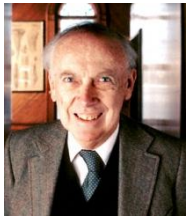


Sequencing Tecnologies Evolution

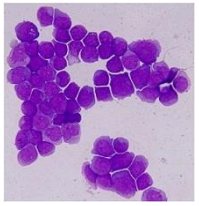


2004 Secuenciación Sanger 300 x10^6 $ > 10 años

2007 Secuenciación Sanger 100 x10^6 $ 2-4 años



2008 Next-Generation 454 1 x10^6 2 meses



2008 Next-Gen Solexa 0.25 x10^6 2 meses

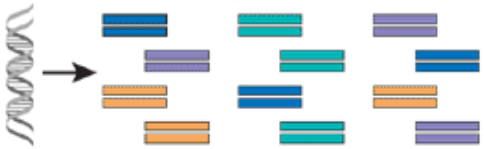
Sanger Sequencing

About 2-3 weeks

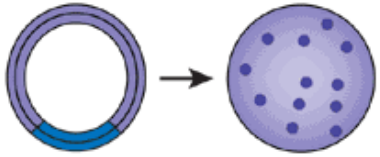
2 days

a

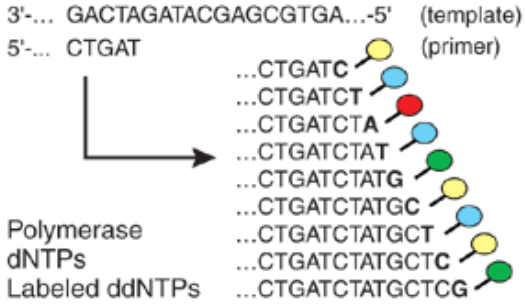
DNA fragmentation



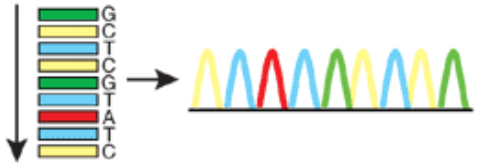
In vivo cloning and amplification



Cycle sequencing



Electrophorsesis
(1 read/capillary)



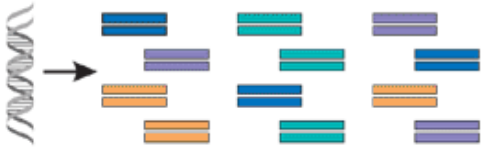
NGS

1 day

5-7 days

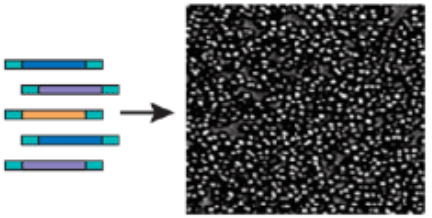
b

DNA fragmentation

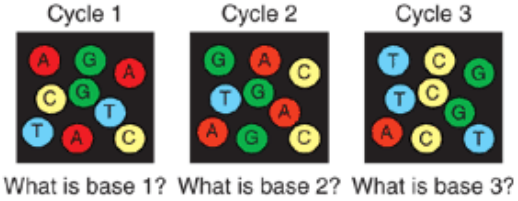


In vitro adaptor ligation

Generation of polony array



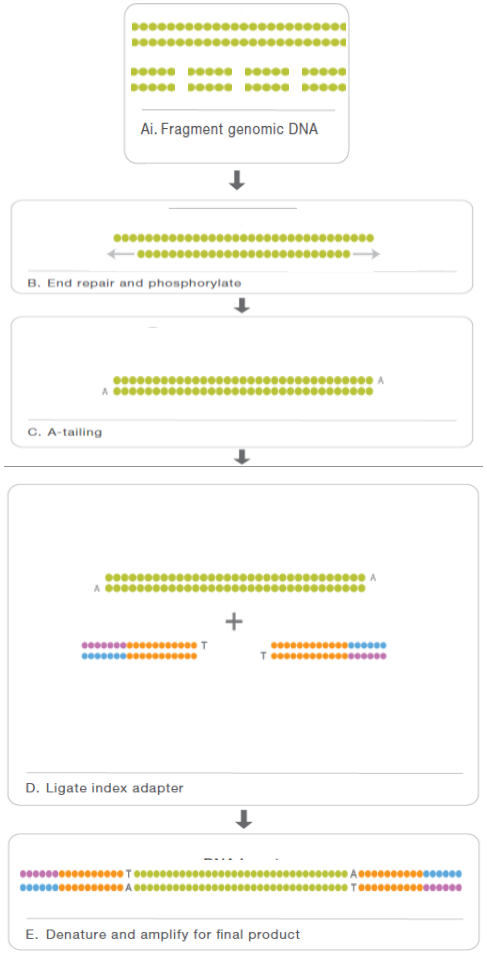
Cyclic array sequencing
(>10^6 reads/array)



Jay Shendure and Hanlee Ji, Nature 2008

Next-Generation Sequencing Technology

Sample Preparation Library Generation → **Target enrichment*** → **Cluster generation** → **Sequencing**



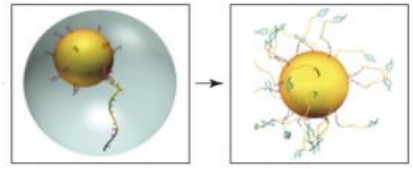
1. DNA Inmobilization

Beads

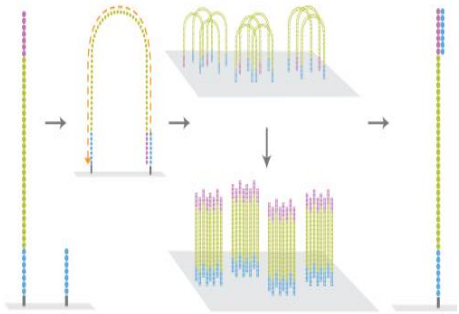
Surface

2. Amplification

- Emulsion PCR



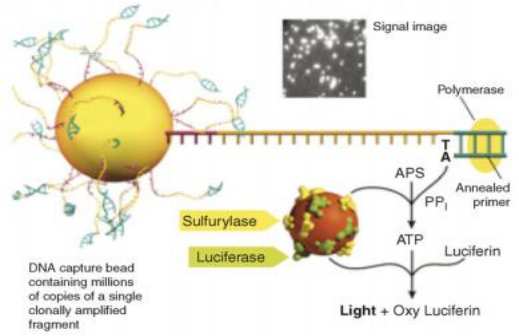
- Bridge Amplification



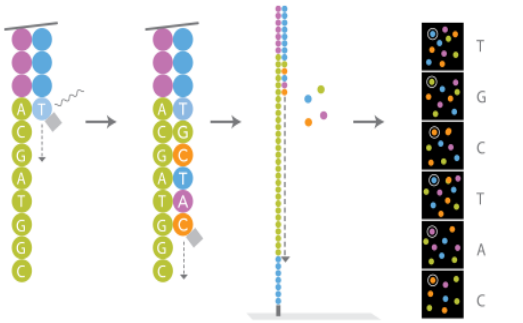
Sequencing Chemistry (SBS)

- Ligation-Based sequencing

- Polymerase-Pyrosequencing

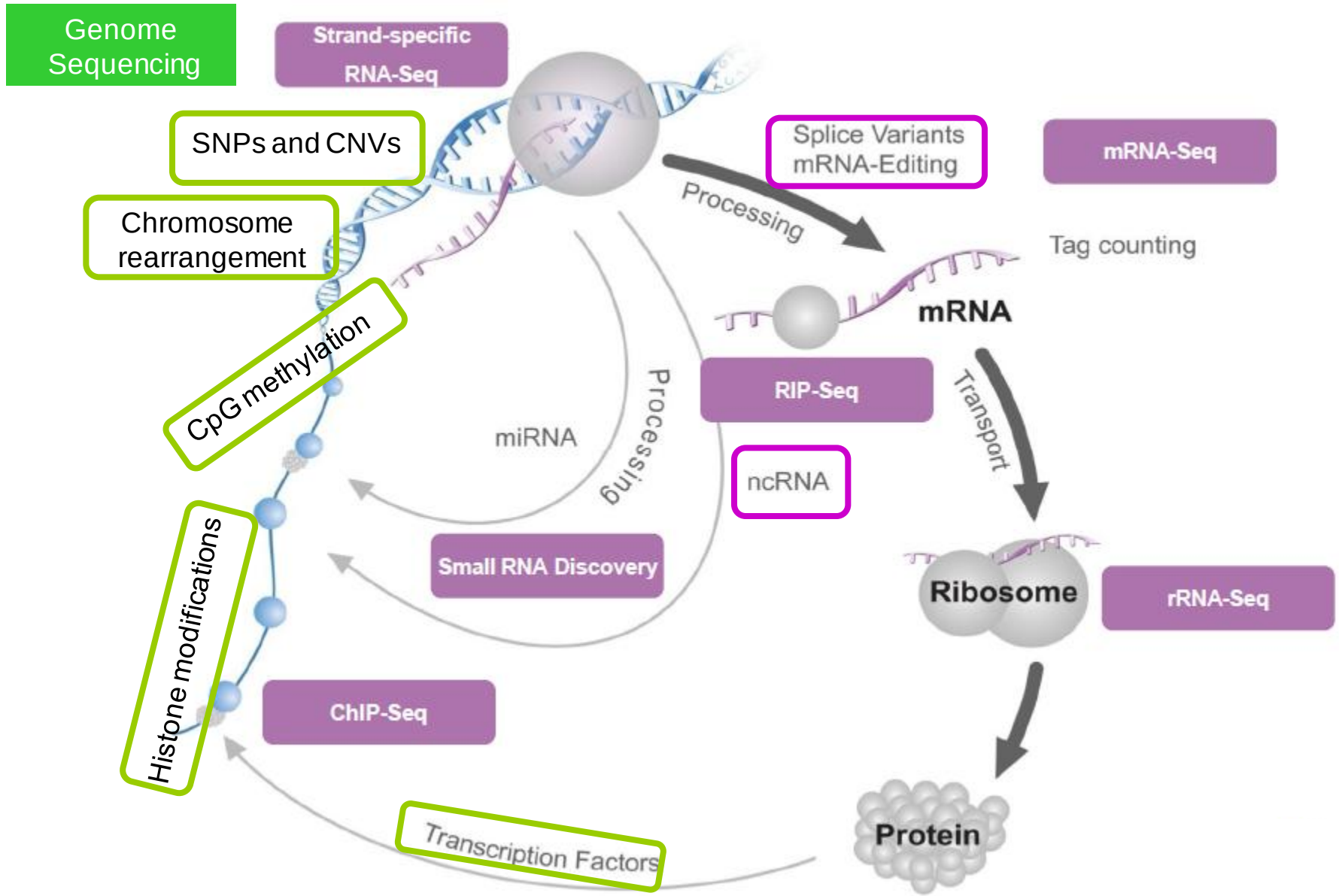


- Polymerase-Reversible terminators



Single-end / Paired-end

NGS Applications: Genomics & Transcriptomics



modified illumina

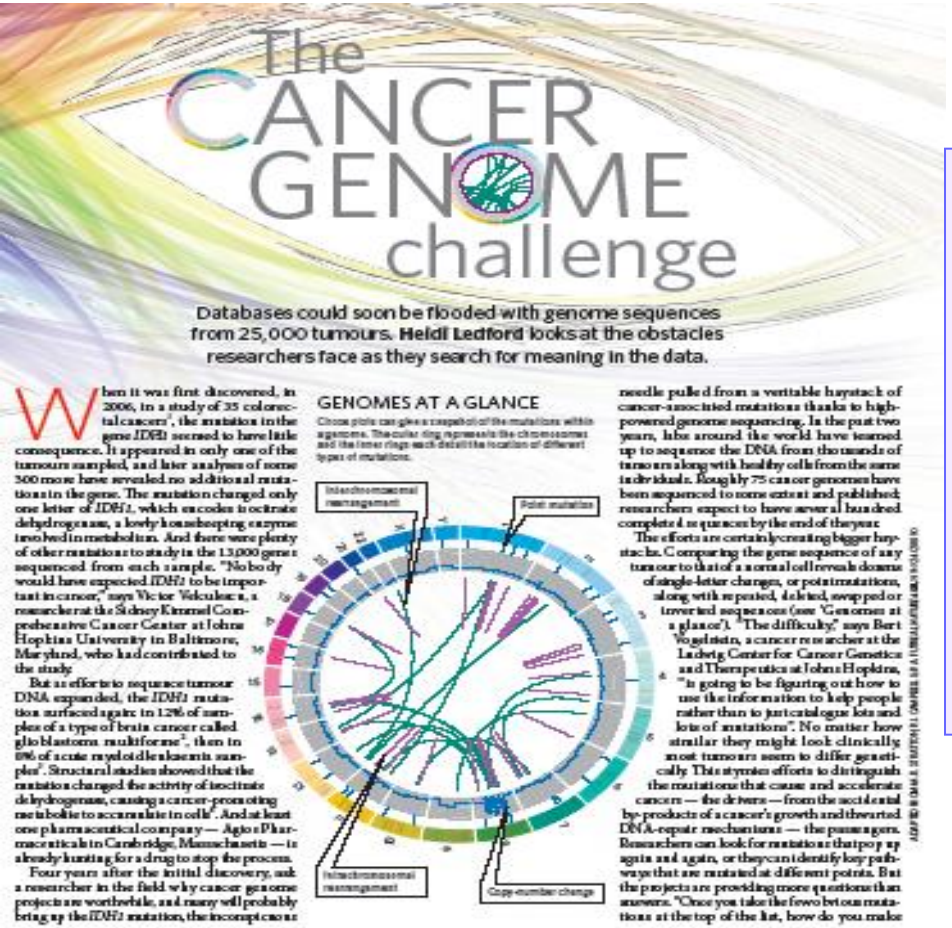
Vol 464|15 April 2010|doi:10.1038/nature08987 nature

PERSPECTIVES

International network of cancer genome projects

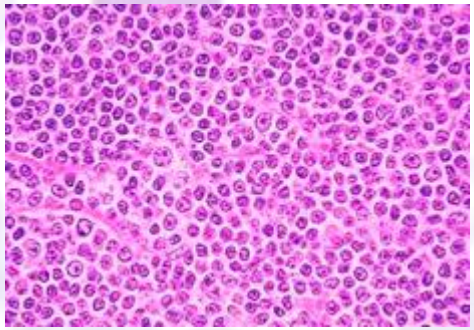
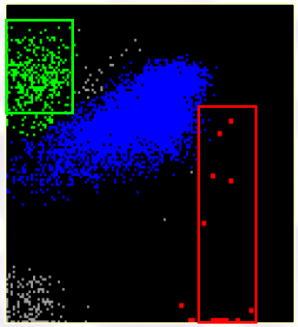
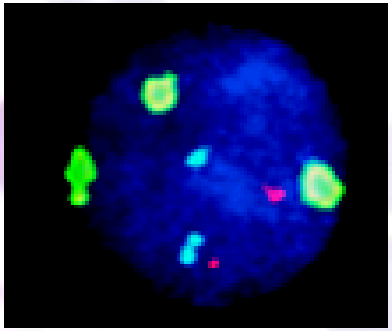
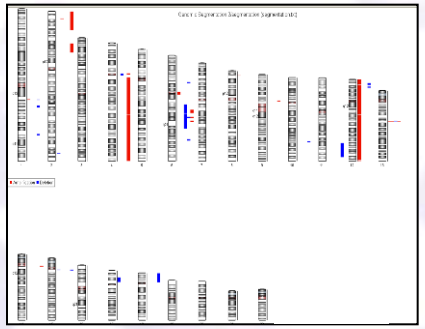
The International Cancer Genome Consortium*

The International Cancer Genome Consortium (ICGC) was launched to coordinate large-scale cancer genome studies in tumours from 50 different cancer types and/or subtypes that are of clinical and societal importance across the globe. Systematic studies of more than 25,000 cancer genomes at the genomic, epigenomic and transcriptomic levels will reveal the repertoire of oncogenic mutations, uncover traces of the mutagenic influences, define clinically relevant subtypes for prognosis and therapeutic management, and enable the development of new cancer therapies.



- **Identification of the genetic alterations present in 50 different cancer types**
- **At least 500 patients for each tumor**
- **Uncover traces of mutagenic influences**
- **New targets for diagnosis and treatment**
- **New therapies based on genomic alterations identified in tumors**

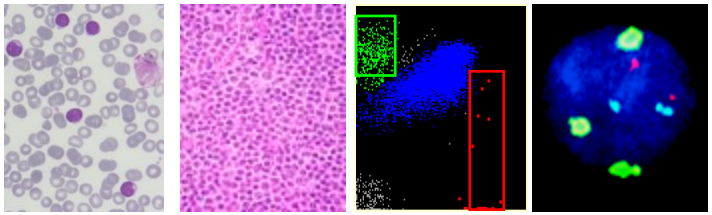
Proyecto Genoma
Leucemia Linfática Crónica

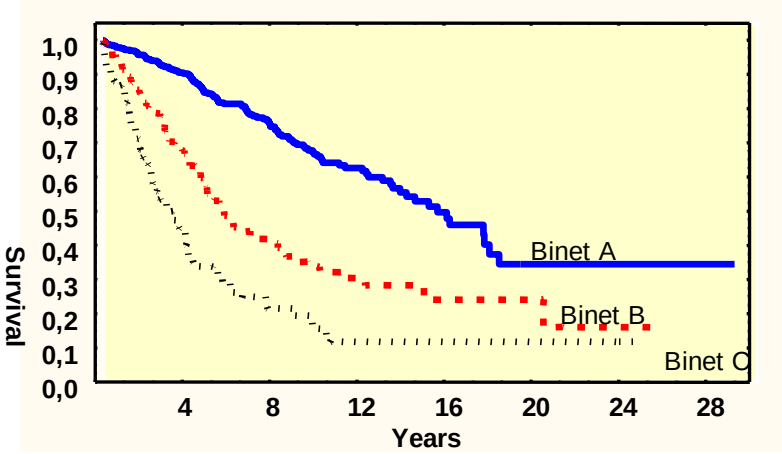





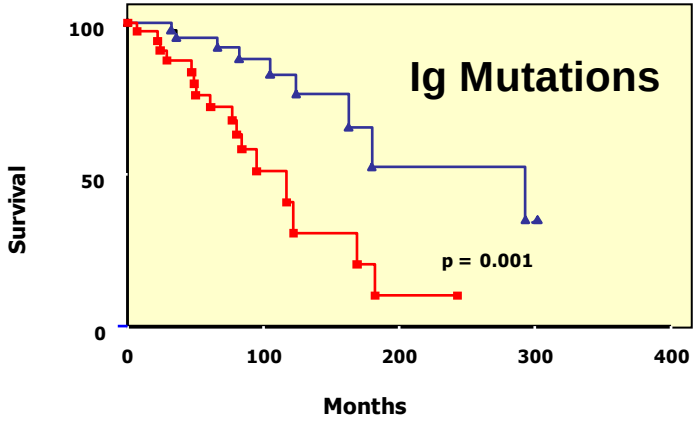
GOBIERNO DE ESPAÑA
MINISTERIO DE CIENCIA E INNOVACIÓN
Instituto de Salud Carlos III

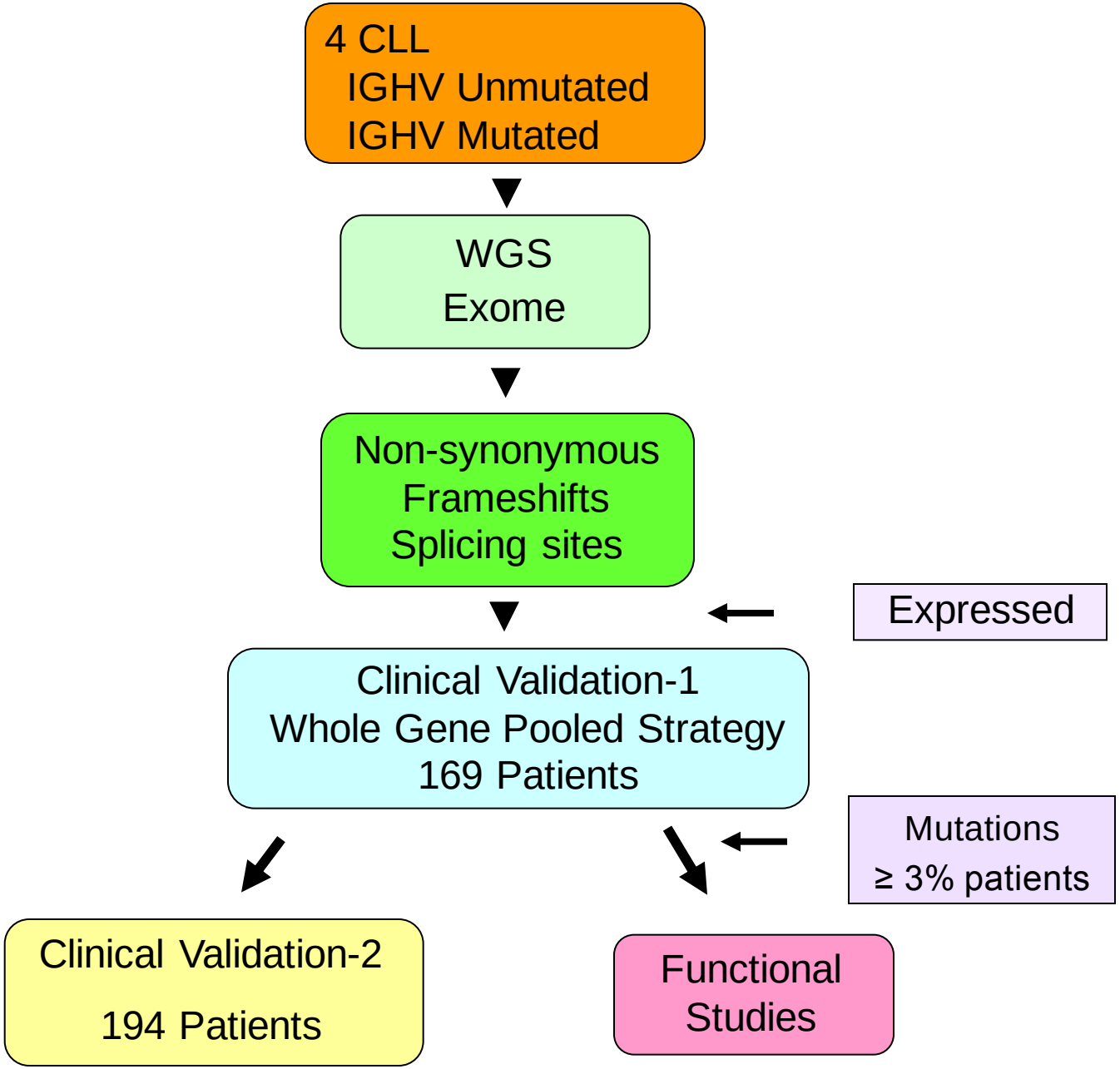
Chronic Lymphocytic Leukemia

- **Most frequent leukemia in Western countries (5-7 cases /100,000 /year)**
- **Heterogeneous disease with different stages of progression and molecular subtypes**
- **No effective therapy**
- **Pathogenesis**
  - **Unknown initiating genetic alterations**
  - **Microenvironment**
  - **Evidence of genetic predisposition**
    - **Geographic distribution**
    - **Familiar clustering**
    - **Susceptibility loci**
  - **Environmental influences?**

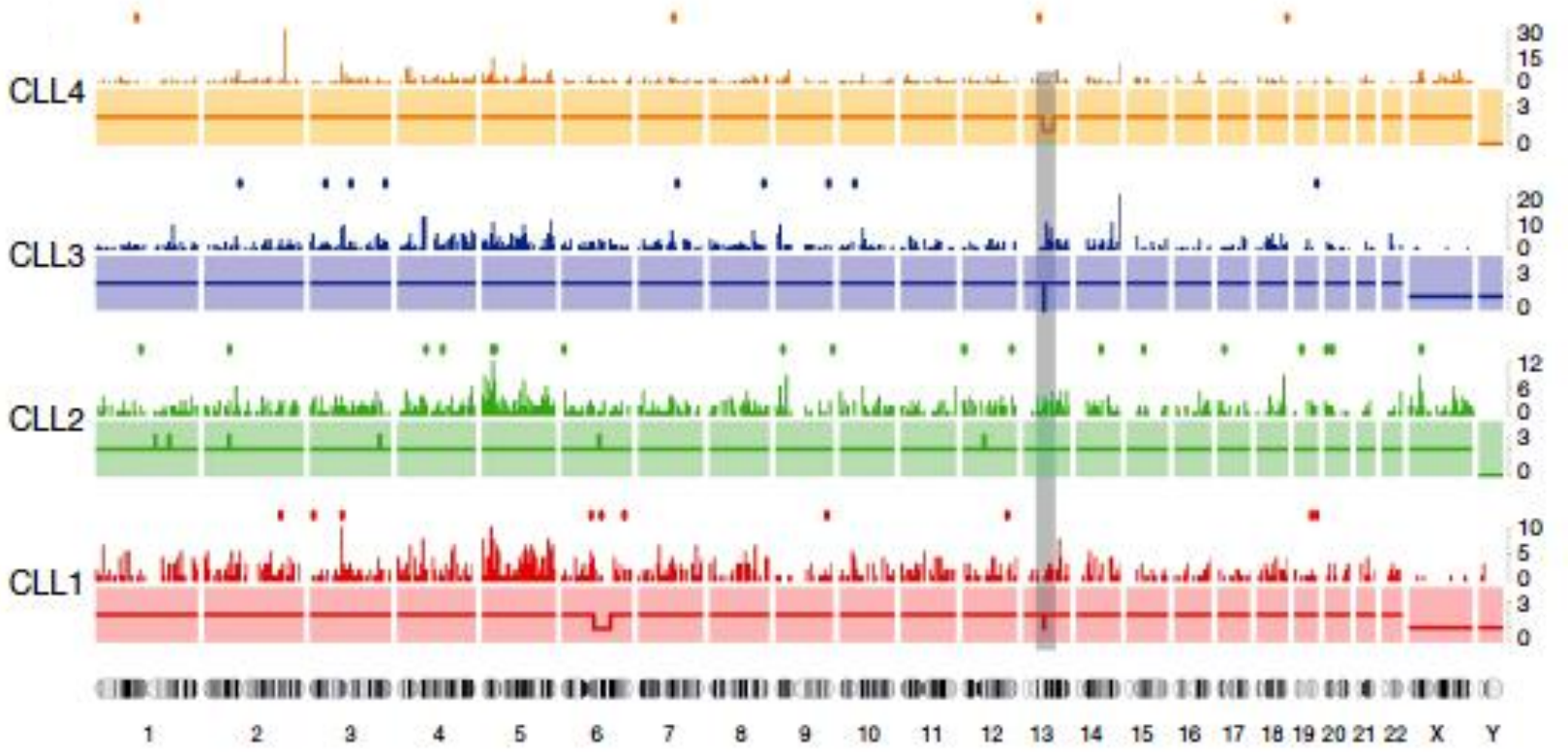







4 CLL
IGHV Unmutated
IGHV Mutated
WGS
Exome
Non-synonymous
Frameshifts
Splicing sites
Expressed
Clinical Validation-1
Whole Gene Pooled Strategy
169 Patients
Mutations
≥ 3% patients
Clinical Validation-2
194 Patients
Functional
Studies

Profile of Somatic Mutations in Four CLL Genomes



Solid lines: copy number; **Bars:** mutation density per 5 Mb
Dots: class 1 mutations (non-syn., frameshifts, splicing sites)

Puente et al, Nature 2011

Genes recurrently mutated in CLL

Gene	Mutation	Mutated cases / total	Overall frequency (%)	Frequency in *IGHV*-unmutated (%)	Frequency in *IGHV*- mutated (%)
NOTCH1	P2515Rfs*4 Q2503* F2482Ffs*2	29/255 1/255 1/255	12.2	20.4	7
MYD88	L265P	9/310	2.9	0.8	5.6
XPO1	E571K E571G	3/165 1/165	2.4	4.6	0
KLHL6	F49L/L65P L90F L58P/T64A/Q81P	3/160	1.8	0	4.5

Puente et al, Nature 2011

WGS in CLL: Summary

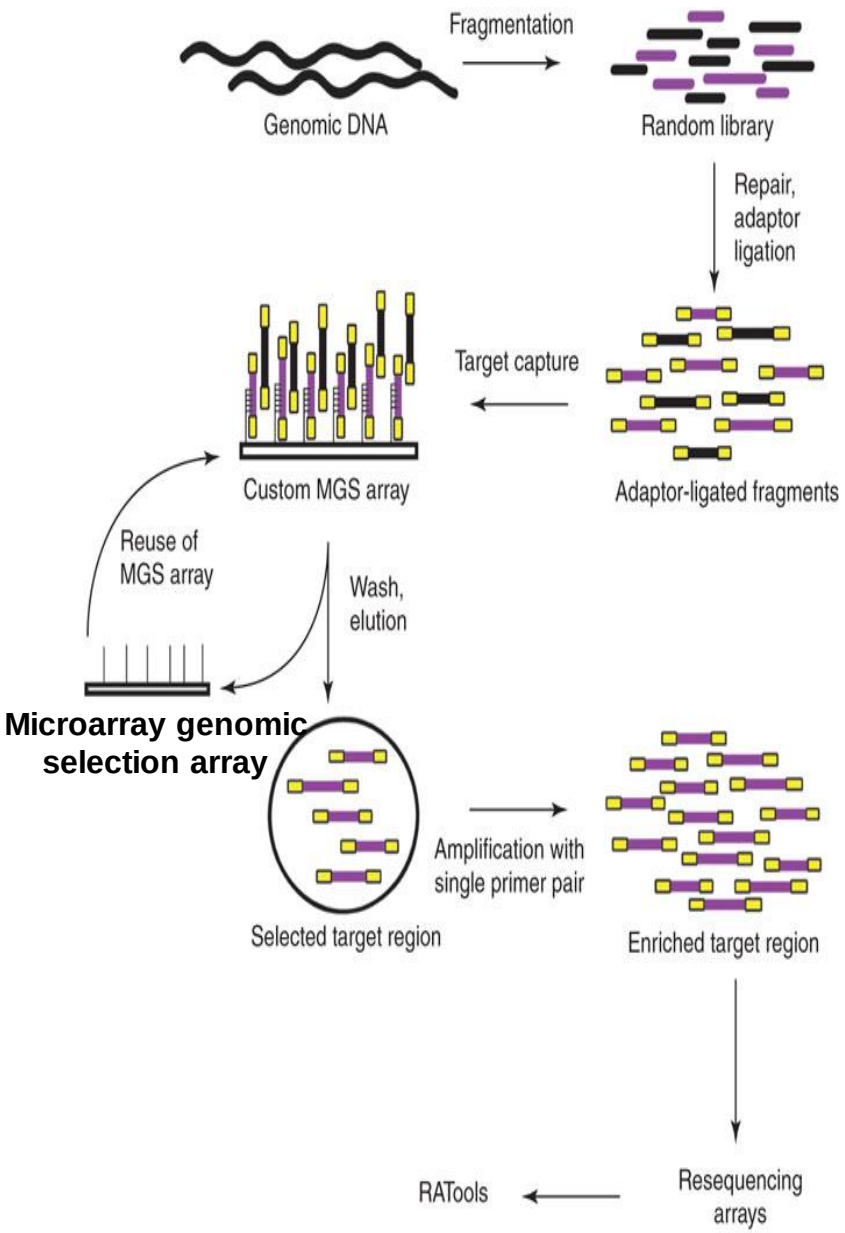
- CLL carries approximately 1 somatic mutations /MB (1000 per case)
- Identification of a potential mutational mechanism in IGHV hypermutated CLL related to the activity of DNA polymerase eta
- Identification of 46 somatic mutations in coding regions with potential functional effect. 5-20 per case
- Identification of 4 recurrent mutations with clinical implication
- NOTCH1 mutations are a frequent event in CLL
  - NOTCH1 mutations truncate and stabilize the protein and activate NOTCH1 pathway in CLL
  - Predominant in IGHV unmutated CLL and associated with high risk factors (ZAP70/CD38)
- MYD88 mutations
  - Same mutation found in ABC DLBCL
  - Activates NFkB pathway (IRAK1, STAT3)
  - Promotes a high production of several chemokines (CCL2, CCL3, CCl4, IL6, ILR1A) in response to TLR stimulation

Exome sequencing identifies recurrent mutations of the splicing factor *SF3B1* gene in chronic lymphocytic leukemia

Víctor Quesada[1], Laura Conde[2], Neus Villamor[2], Gonzalo R Ordóñez[1], Pedro Jares[2], Laia Bassaganyas[3], Andrew J Ramsay[1], Sílvia Beà[2], Magda Pinyol[4], Alejandra Martínez-Trillos[5], Mónica López-Guerra[2], Dolors Colomer[2], Alba Navarro[2], Tycho Baumann[5], Marta Aymerich[2], María Rozman[2], Julio Delgado[5], Eva Giné[5], Jesús M Hernández[6], Marcos González-Díaz[6], Diana A Puente[1], Gloria Velasco[1], José M P Freije[1], José M C Tubío[3], Romina Royo[7], Josep L Gelpí[7], Modesto Orozco[7], David G Pisano[8], Jorge Zamora[8], Miguel Vázquez[8], Alfonso Valencia[8], Heinz Himmelbauer[9], Mónica Bayés[10], Simon Heath[10], Marta Gut[10], Ivo Gut[10], Xavier Estivill[3], Armando López-Guillermo[5], Xose S Puente[1], Elías Campo[2,11] & Carlos López-Otín[1,11]

Exome Sequencing: target enrichment

Microarray-based genomic selection



Target enrichment in solution

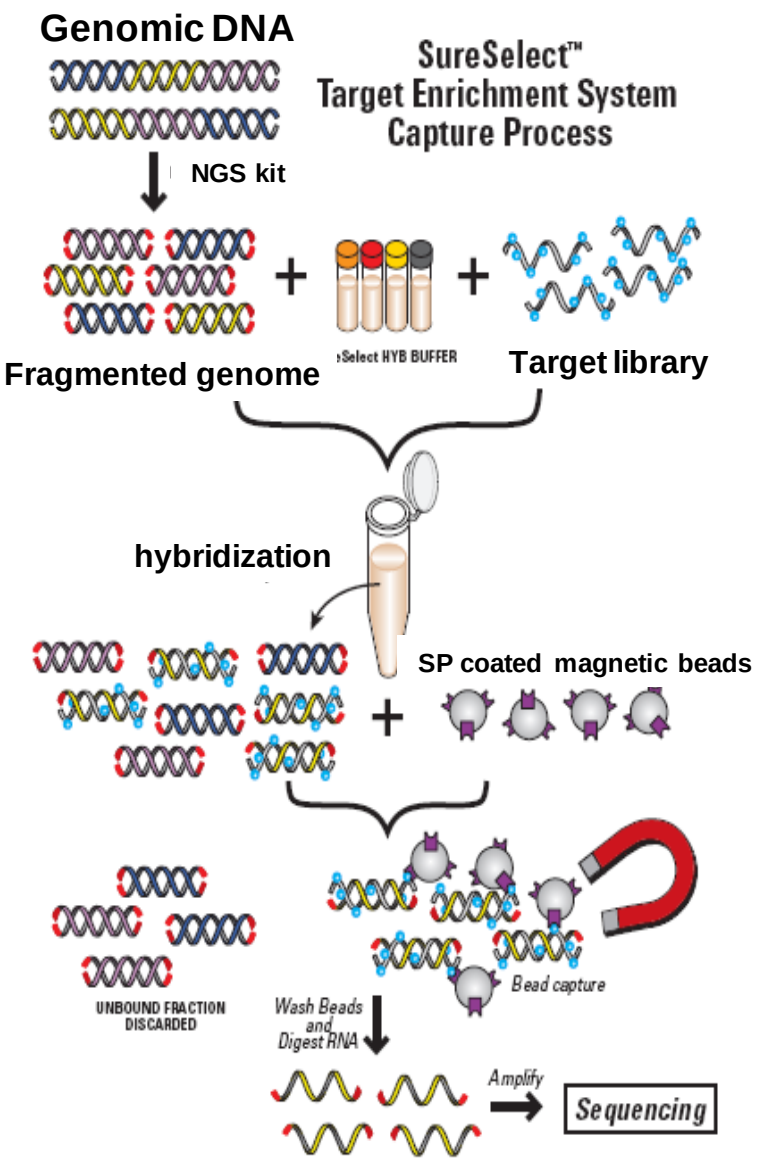
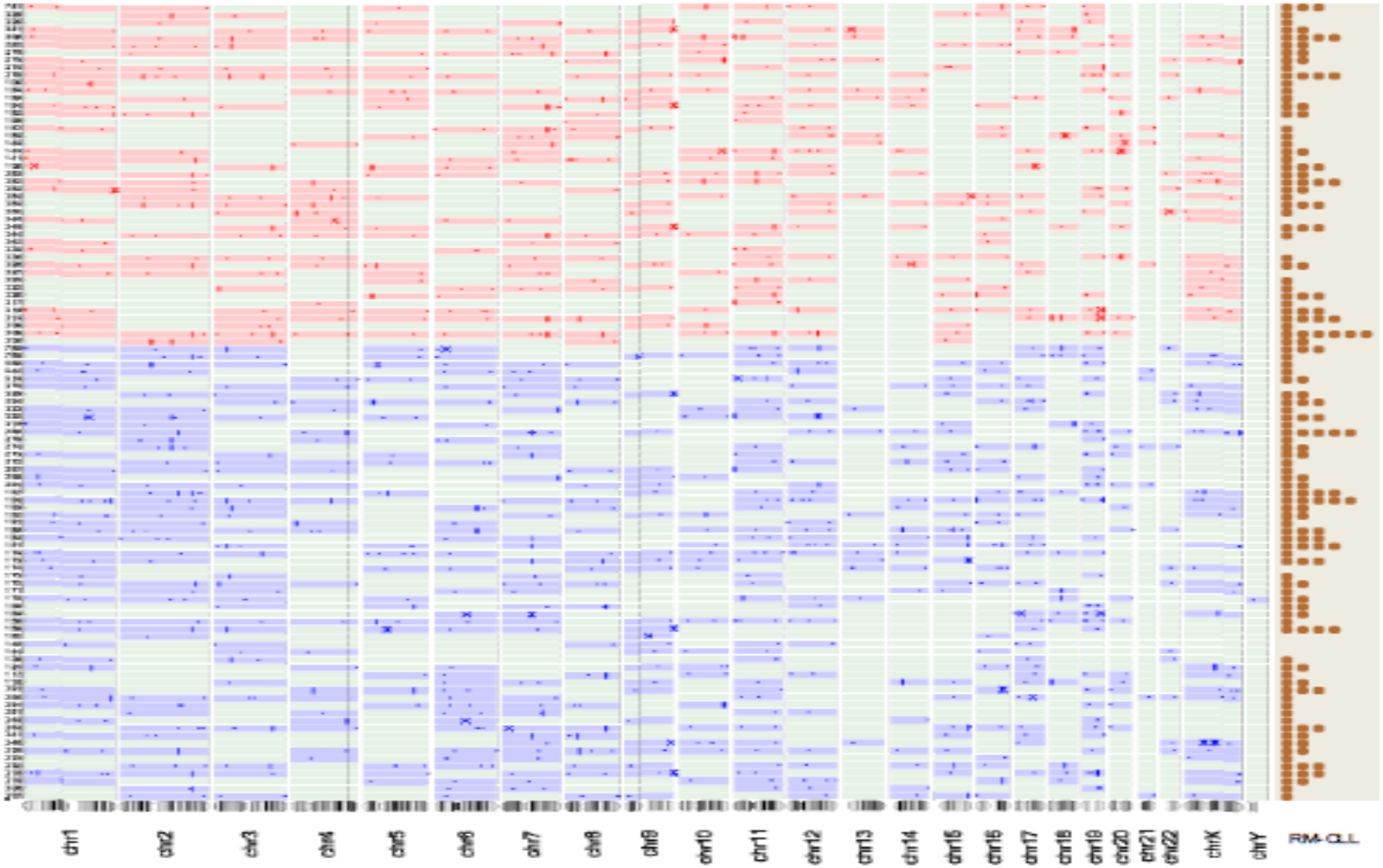


Figure 4 SureSelect Target Enrichment System Capture Process

Profile of Somatic Mutations in 105 CLL Exomes



Quesada et al, Nature Genet 2012

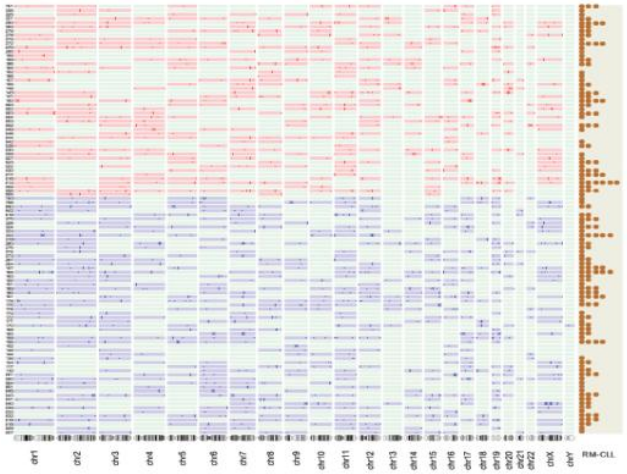
Recurrent Mutated Genes in CLL

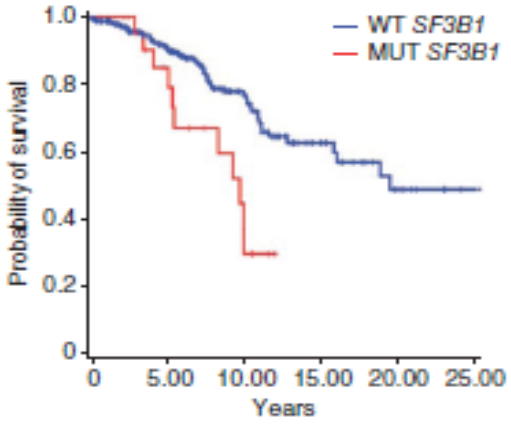
Gene	Mutated cases / total	Overall frequency (%)	Frequency in *IGHV*-unmutated (%)	Frequency in *IGHV*- mutated (%)
NOTCH1	31/255	12.2	10.1	2.8
SF3B1	27/279	9.7	20.5	7.9
POT1	5/105	4.8	11.1	0
CHD2	5/105	4.8	0	8.3
LPP1D	5/105	4.8	5.0	5.0

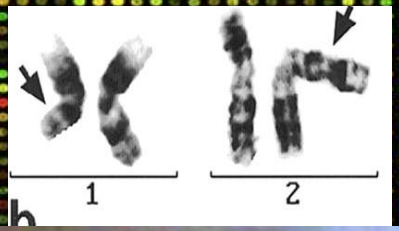
Quesada et al, Nature Genet 2012

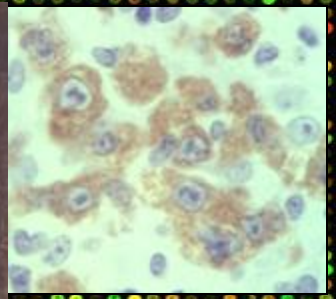
WGS in CLL: Summary

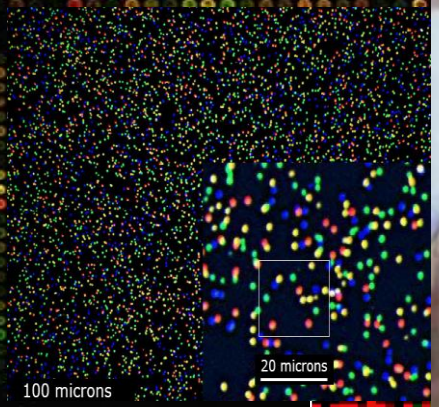
- Exome sequencing of 105 CLL patients
- Identification of 1246 somatic mutations afectting about 1100 genes.
- Mutations in 78 genes are found in more than one patient.
- The initial functional analysis showed that the genes found mutated in CLL significantly clustered in specific gene pathways including Toll-like receptor pathway, RNA splicing and processing, among others.
- The different mutations are associated to different clinical groups.
- SF3B1 mutations are identified in patients with worst prognosis.

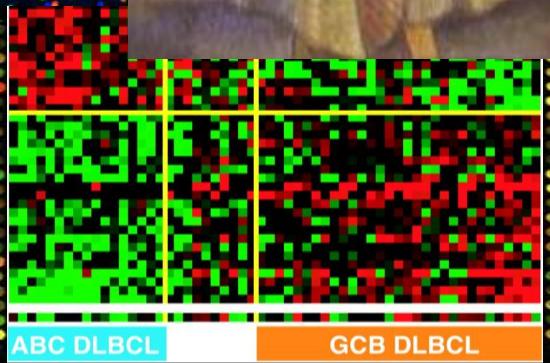


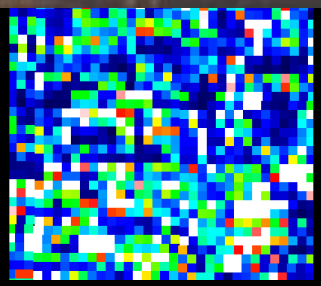


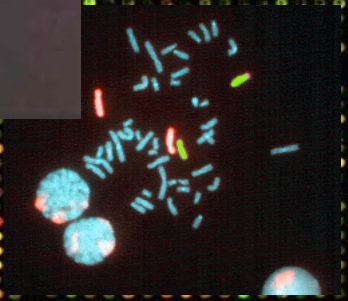

1
2




20 microns
100 microns

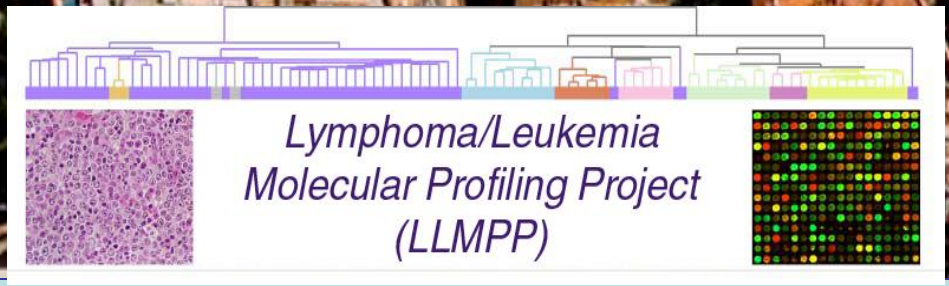

ABC DLBCL
GCB DLBCL





Hematopathology Section
Pathology Department
Hospital Clinic,
University of Barcelona

E Campo
S Beà
M Pinyol
L Colomo
A Martinez
L Hernandez
V Amador
C Arroyo
A Navarro
P Jares



Lymphoma/Leukemia Molecular Profiling Project

University of Nebraska, Omaha
National Cancer Institute, Bethesda, MD
South-West Oncology Group
British Columbia Cancer Agency, Vancouver
University of Wurzburg, Wurzburg
Radium Hospital, Oslo
St Bartolomew Hospital, London
Hospital Clinic, University of Barcelona

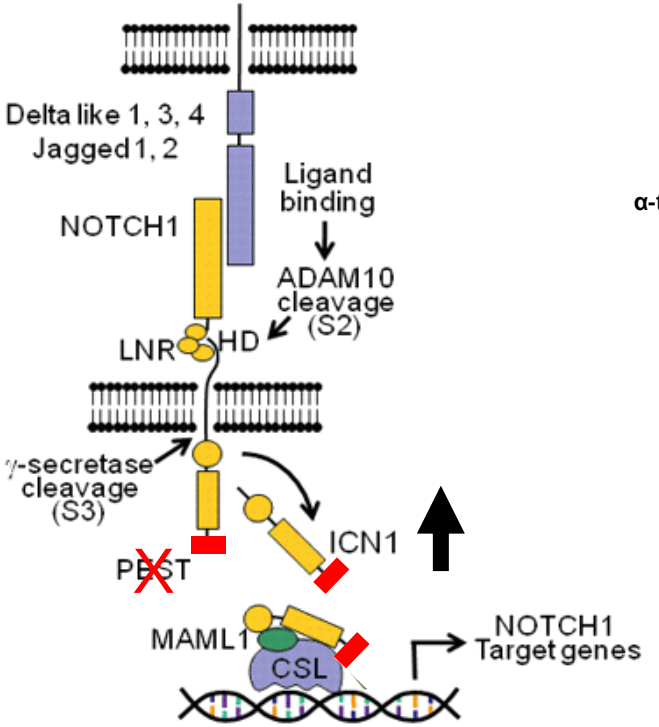
Consorcio ICGC - CLL

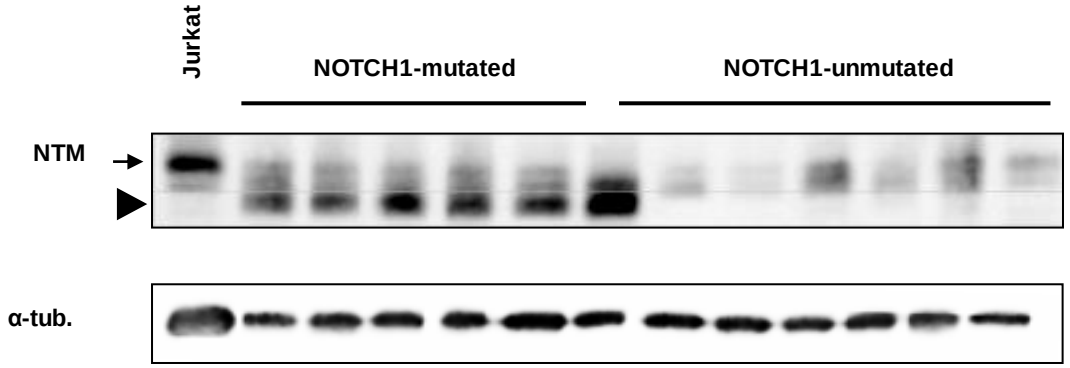
Hospital Clínico, Universidad de Barcelona
Universidad de Oviedo, IUOPA
Instituto de Investigaciones Biomédicas August Pi I Sunyer
Centro de Regulación Genómica
Instituto Catalán de Oncología
Centro Investigación Cancer, Hospital Universitario (Salamanca)
Centro Nacional Investigaciones Oncológicas
Barcelona Supercomputer Center
Universidad de Santiago de Compostela
Universidad de Deusto
Universidad Pompeu Fabra
Hospital Clinico de Valencia
Hospital General de Asturias
Hospital Marques de Valde cilla

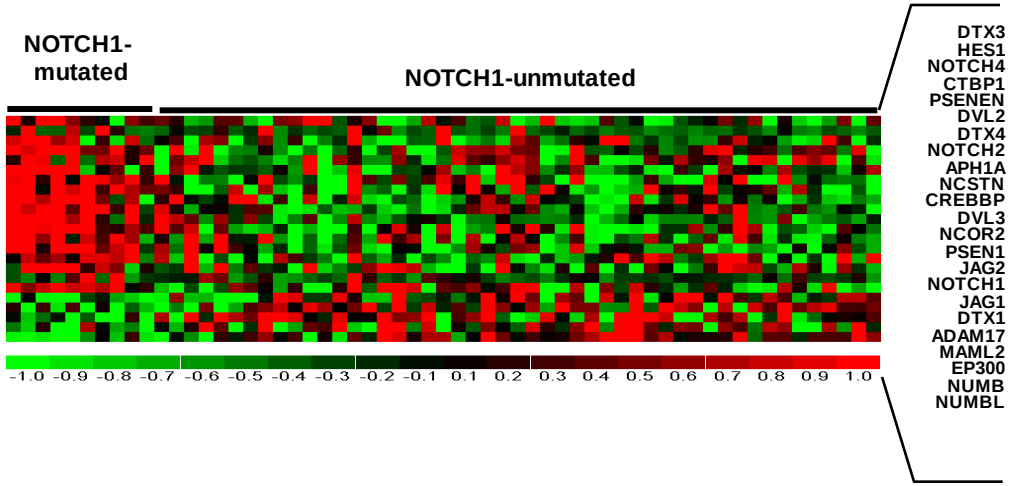
Red de Investigación Cooperativa del Cáncer (RTICC)
Redes Nacionales del Banco de DNA y de Tumores
Instituto Nacional de Bioinformática (INB)
Centro Nacional de Analisis Genómico

Ministerio de Ciencia e Innovación
Instituto de Salud Carlos III

NOTCH1 oncogenic mutations in CLL

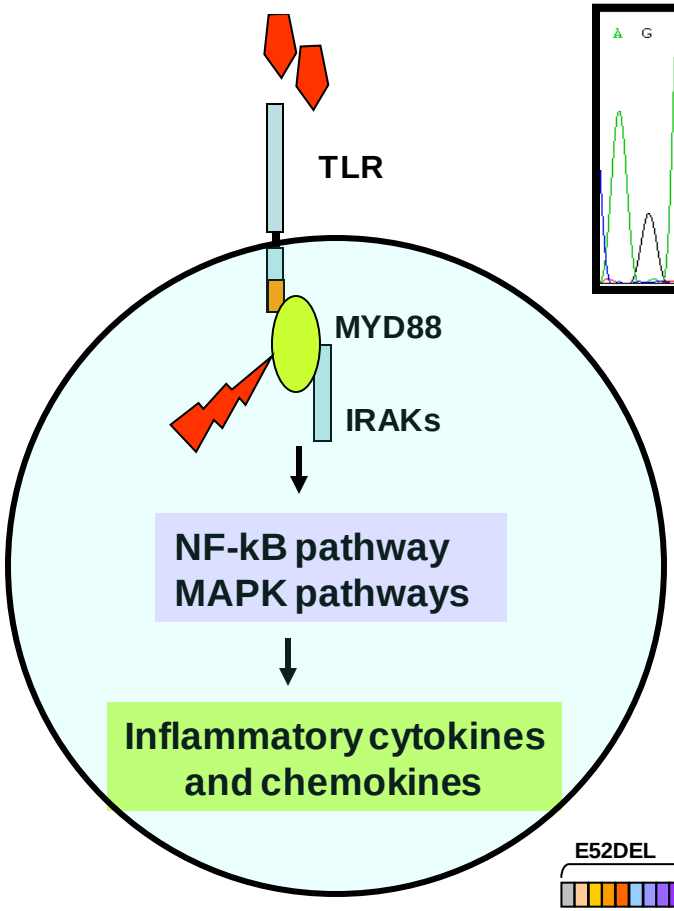


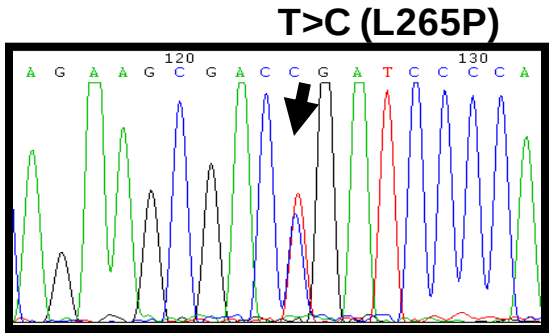


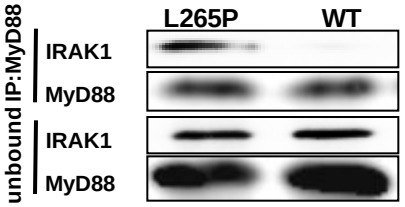


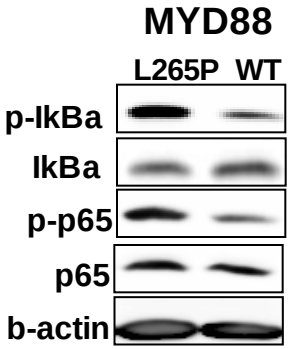
Puente et al, Nature 2011

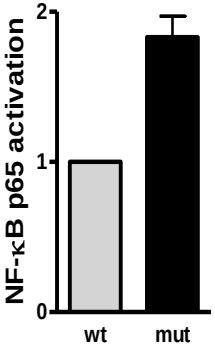
MYD88 oncogenic activation in CLL

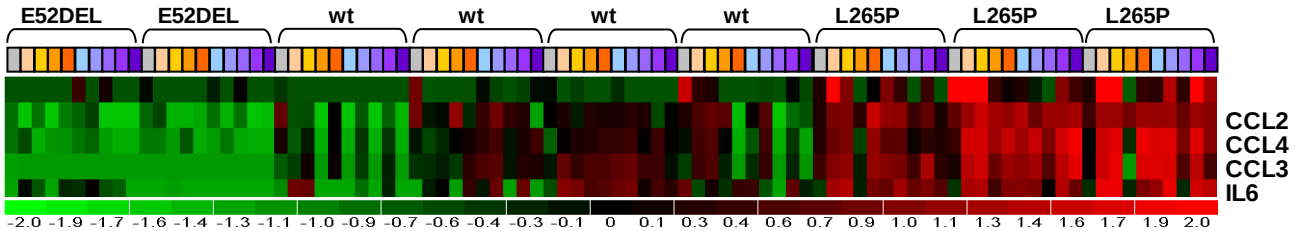




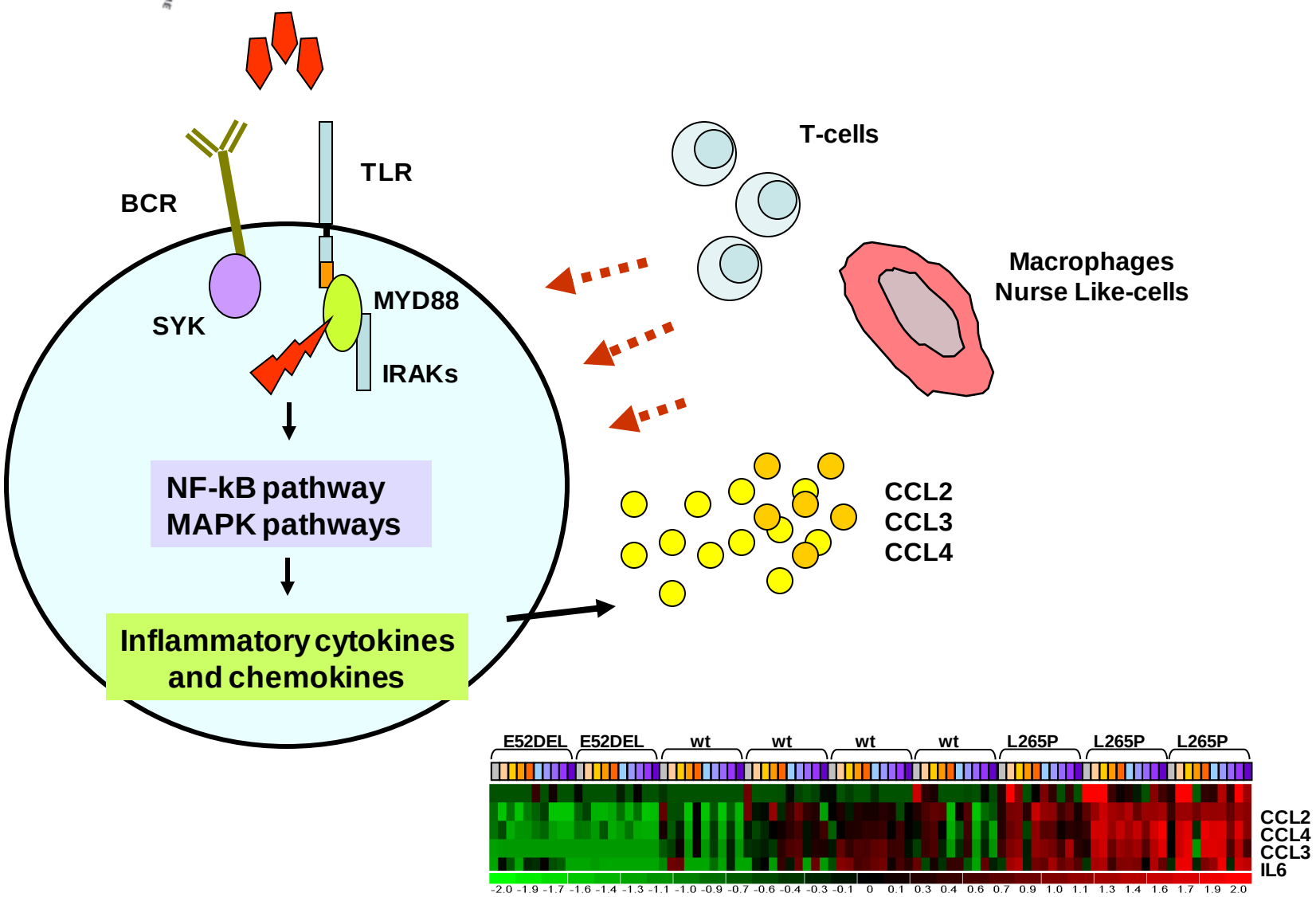








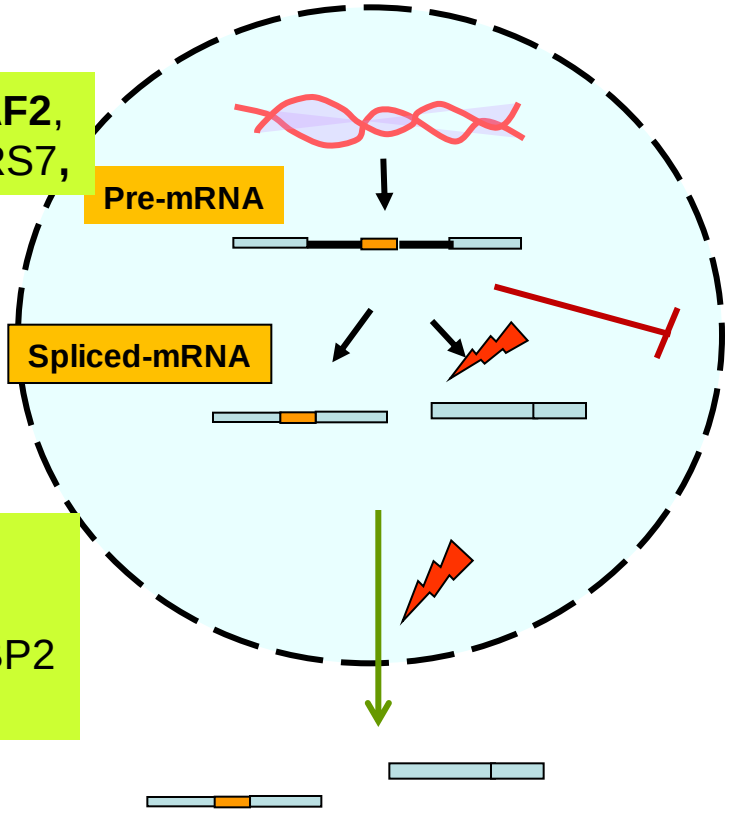
MYD88 oncogenic activation in CLL may promote a favorable microenvironment



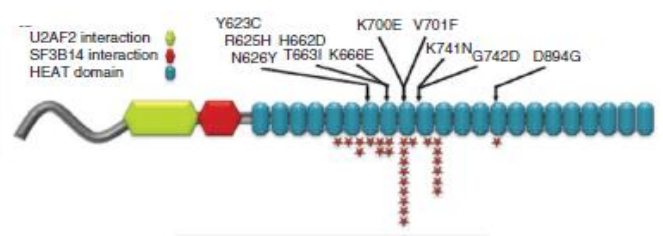
Mutacions Somàtiques en la maquinària molecular del processament del RNA en LLC

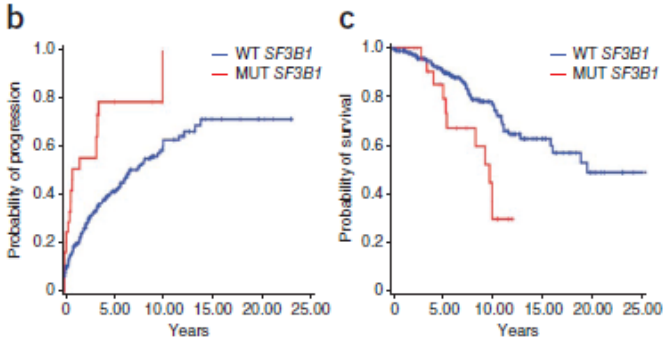
SF3B1, **U2AF2**, **SFRS**1, SFRS7**,**

XPO1, NXF1
EIF4A3,
MAGOH, NCBP2
RBMX,



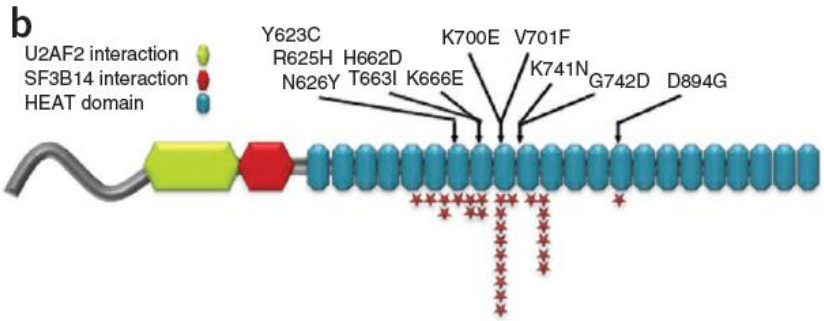
SF3B1



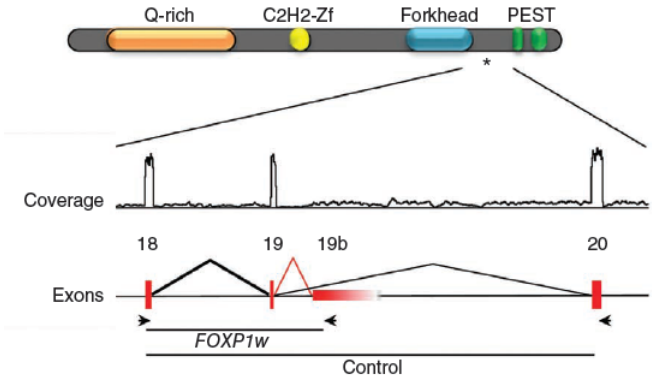


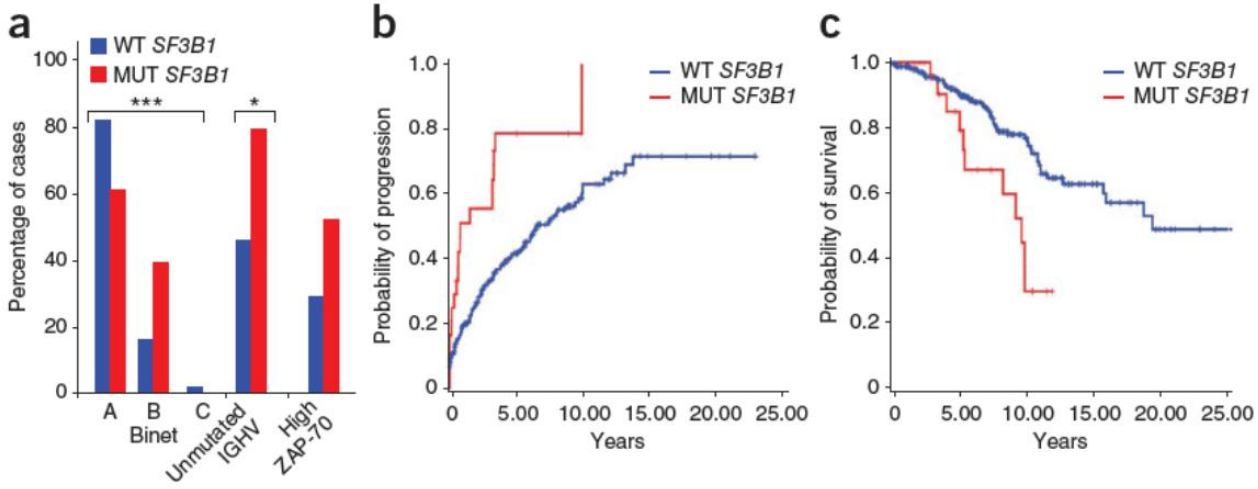
SF3B1 Mutations in CLL

SF3B1



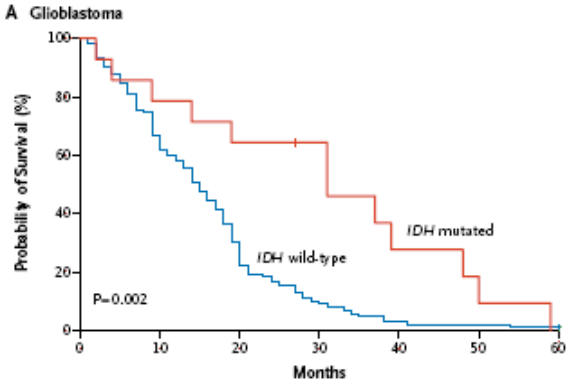
FOXP1

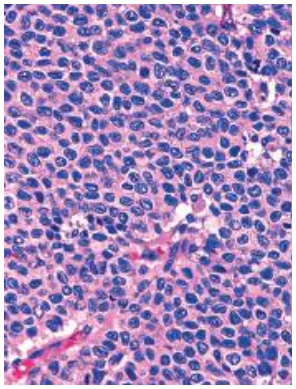


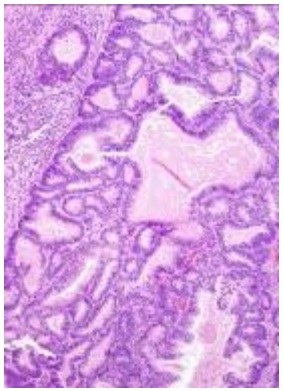


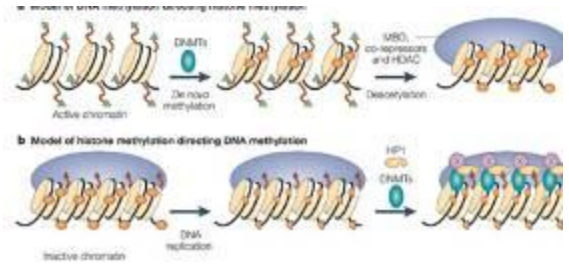
Somatic Mutations in Exome/Transcriptome

Tumor	Mutated Genes	Frequency
Low grade gliomas	IDH1/IDH2	70%
Glioblastoma		12%
Ovary		
• Granulosa Cell tumor	FOXL2	99%
• Clear cell Ca	ARID1A	46%
• Endometrioid Ca		30%
Clear Cell Renal Cancer	SETD2	
	JAR	15%
	UTX	
	PBRM1	44%







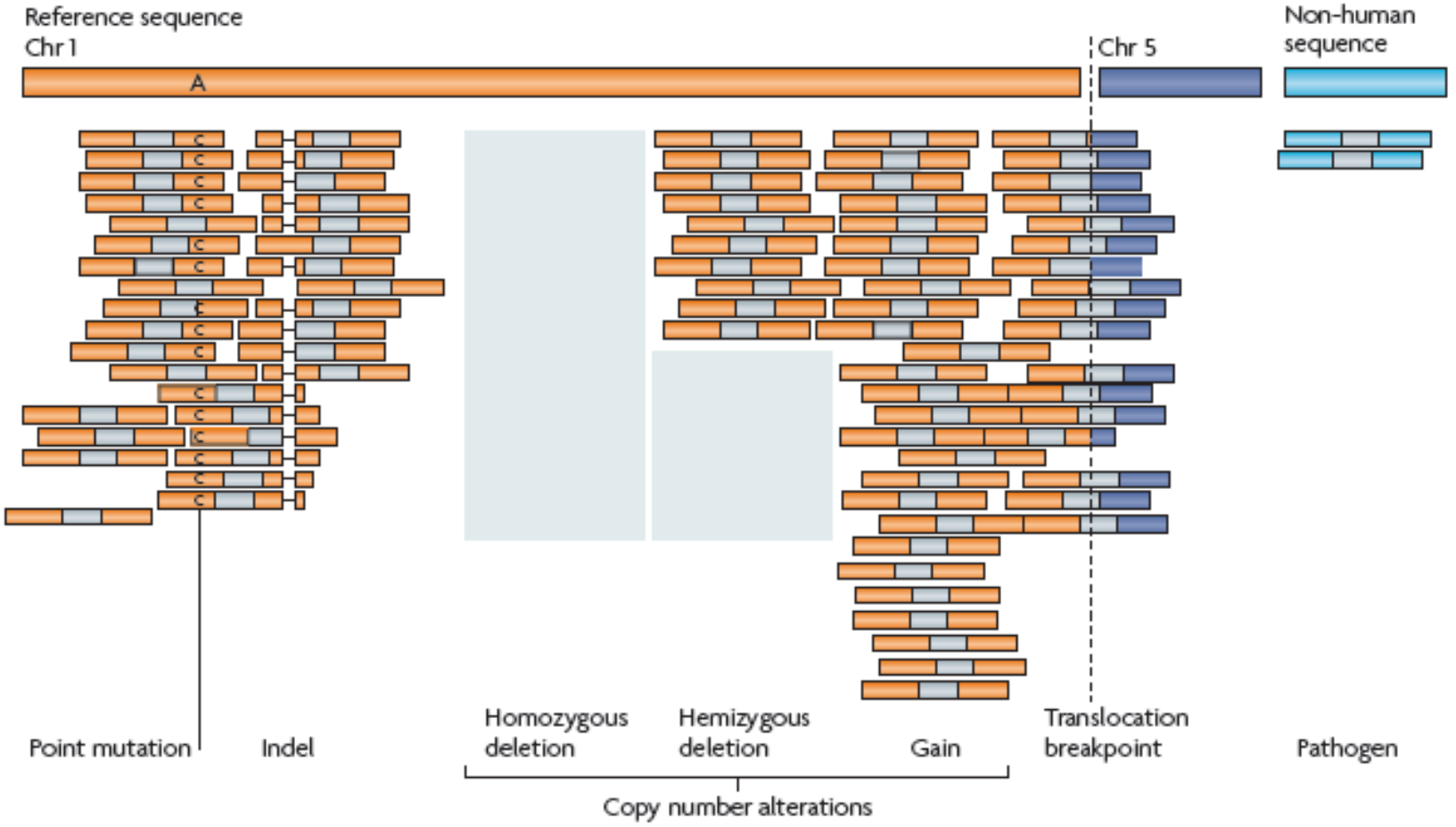


Somatic Mutations in WGS Studies

Tumor (yr)	Somatic Mutations	Non Synonymous	Clinical Validation
AML (2008,2009,2010)	**750**	**10-12**	***DNMT3A*** **(22%)** ***IDH1*** **(16%)**
Breast			***ERBB2*** **(1.5%)**
Lobular Met (2009)	**-**	**32**	***HAUS*** **(1%)**
Basal-like (2009)	**-**	**50**	
Lung			**Not-performed**
Small-cell (cell line) (2009)	**23,000**	**100**	
Non-small cell lung (2009)	**-**	**378**	
Melanoma (cell line) (2009)	**33,000**	**187**	**Not-performed**
Hepatocellular Ca HVC (2011)	**11,000**	**70**	**Not-performed**
Multiple Myeloma (2011) 20 cases	**7,500**	**35**	**10** **Different pathways**

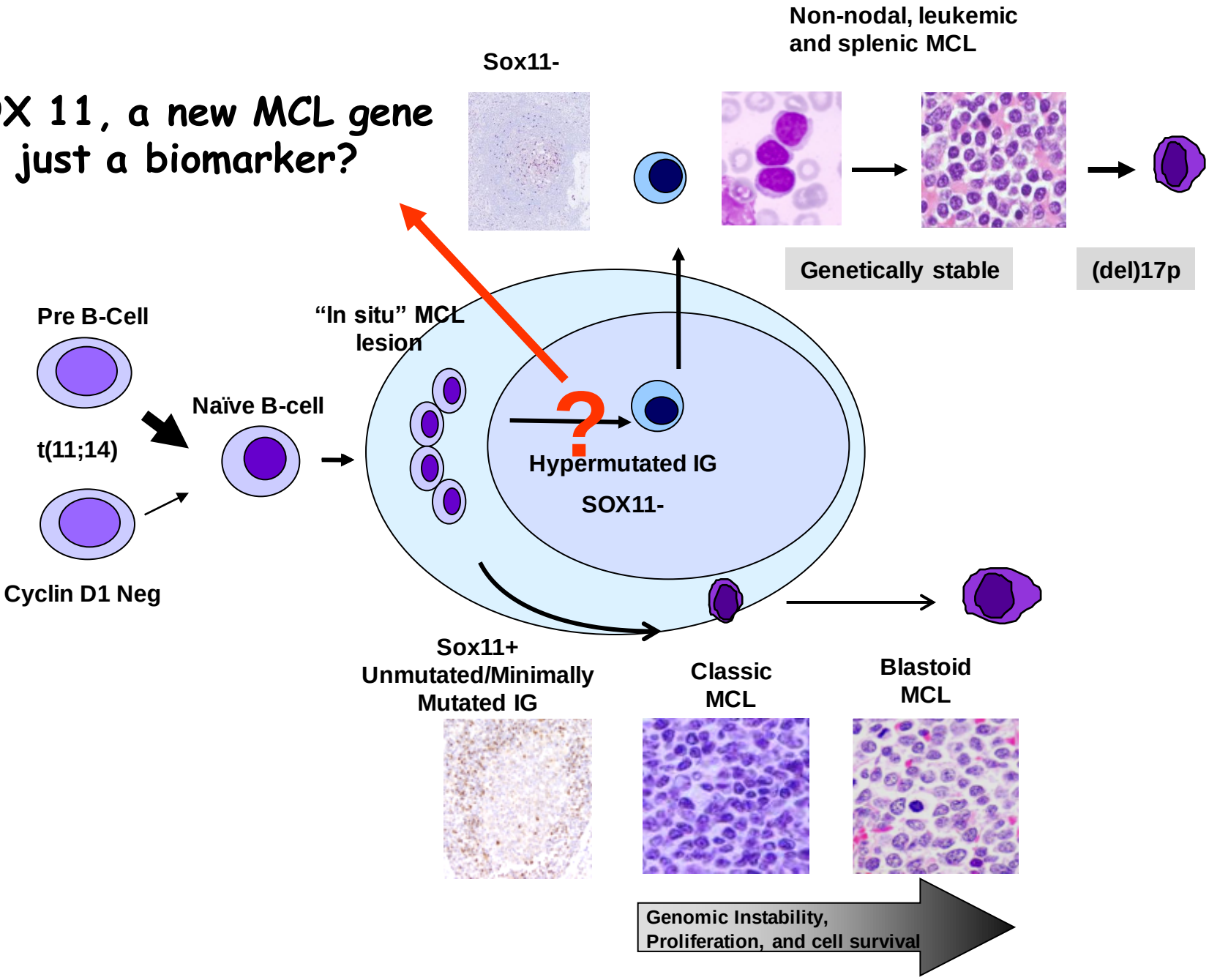
NGS Applications: Genomics

Genome alterations than can be detected by NGS



Meyerson M, et al. Nature Reviews 2010

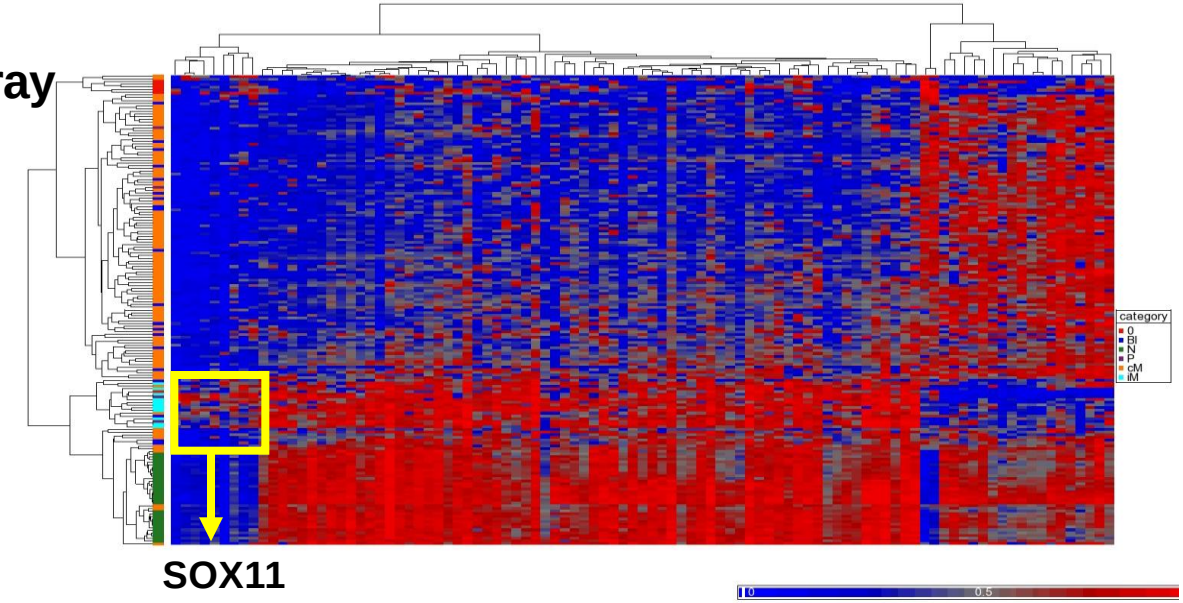
SOX 11, a new MCL gene or just a biomarker?



SOX 11, a new MCL gene or just a biomarker?

CpG methylation array

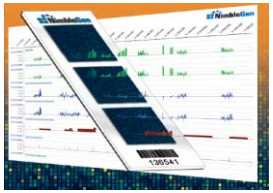
N=132 samples



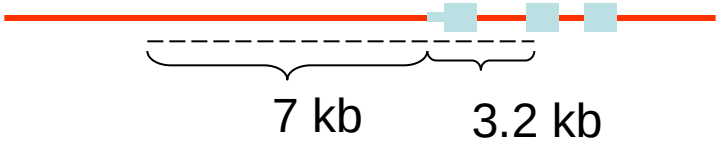
SOX11

SOX11 ChIP-on-chip

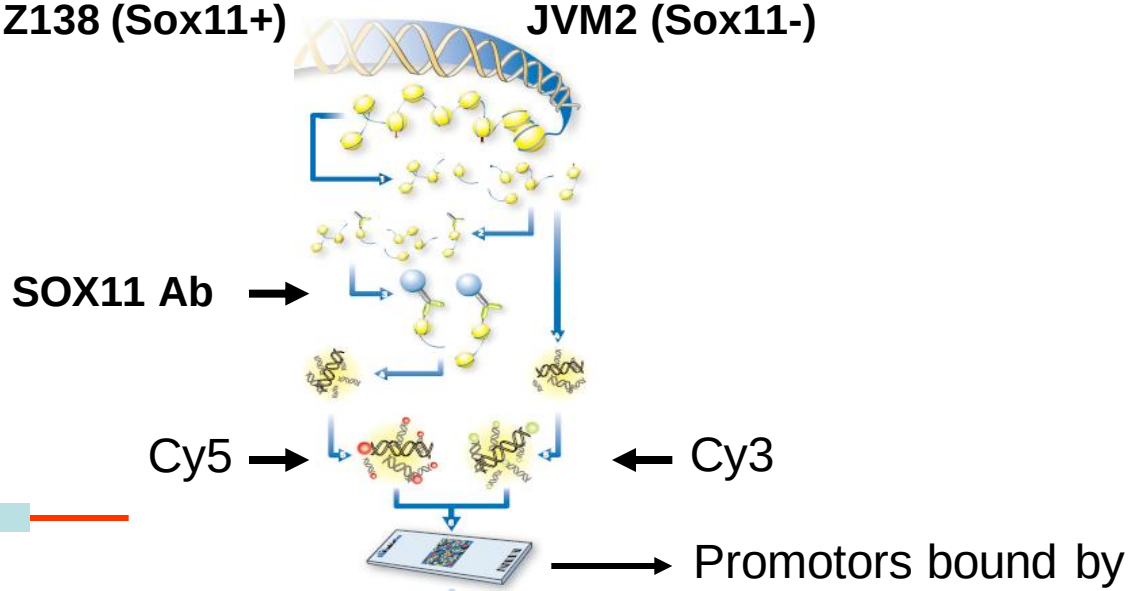
2.1 M Promoter array



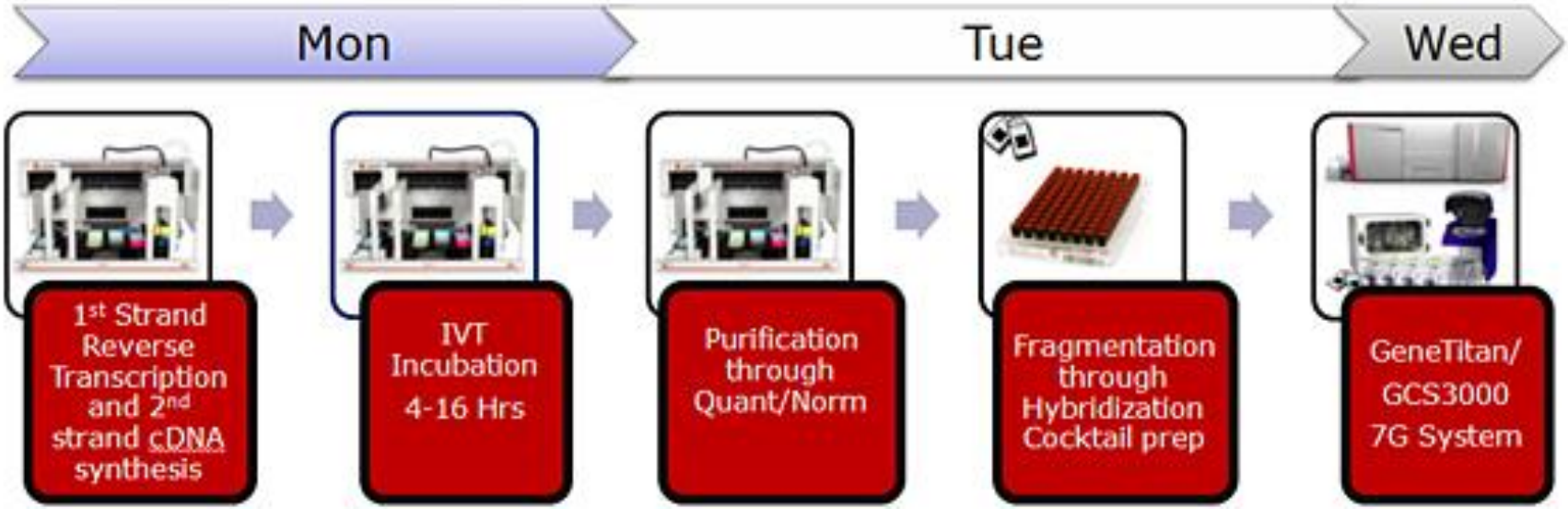
NimbleGen HD2



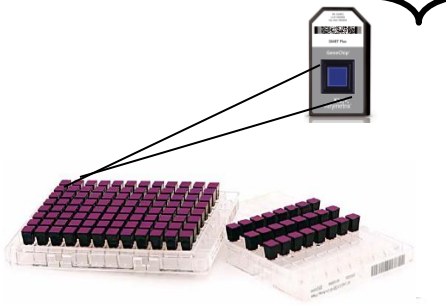
Z138 (Sox11+) JVM2 (Sox11-)



GENE TITAN MULTI-CHANNEL INSTRUMENT



Beckman Coulter Biomek FX^P Target Prep Express System



PEG arrays
16, 24, and 96

GeneTitan™ Multi-Channel Instrument

1. Presència d'unes 1000 mutacions somàtiques en el genoma de cada LLC

2. Identificació de 46 mutacions en les regions codificants del genoma, 5-20 per cas

3.Identificació de 4 mutacions recurrents amb implicacions clíniques

4.Desenvolupament d'un mètode bioinformàtic altament eficaç per detectar mutacions somàtiques