COMBINED MICROARRAY-BASED ANALYSES IN ACUTE MYELOID LEUKEMIA: FROM BIOLOGICAL RESEARCH TO CLINICAL APPLICATIONS

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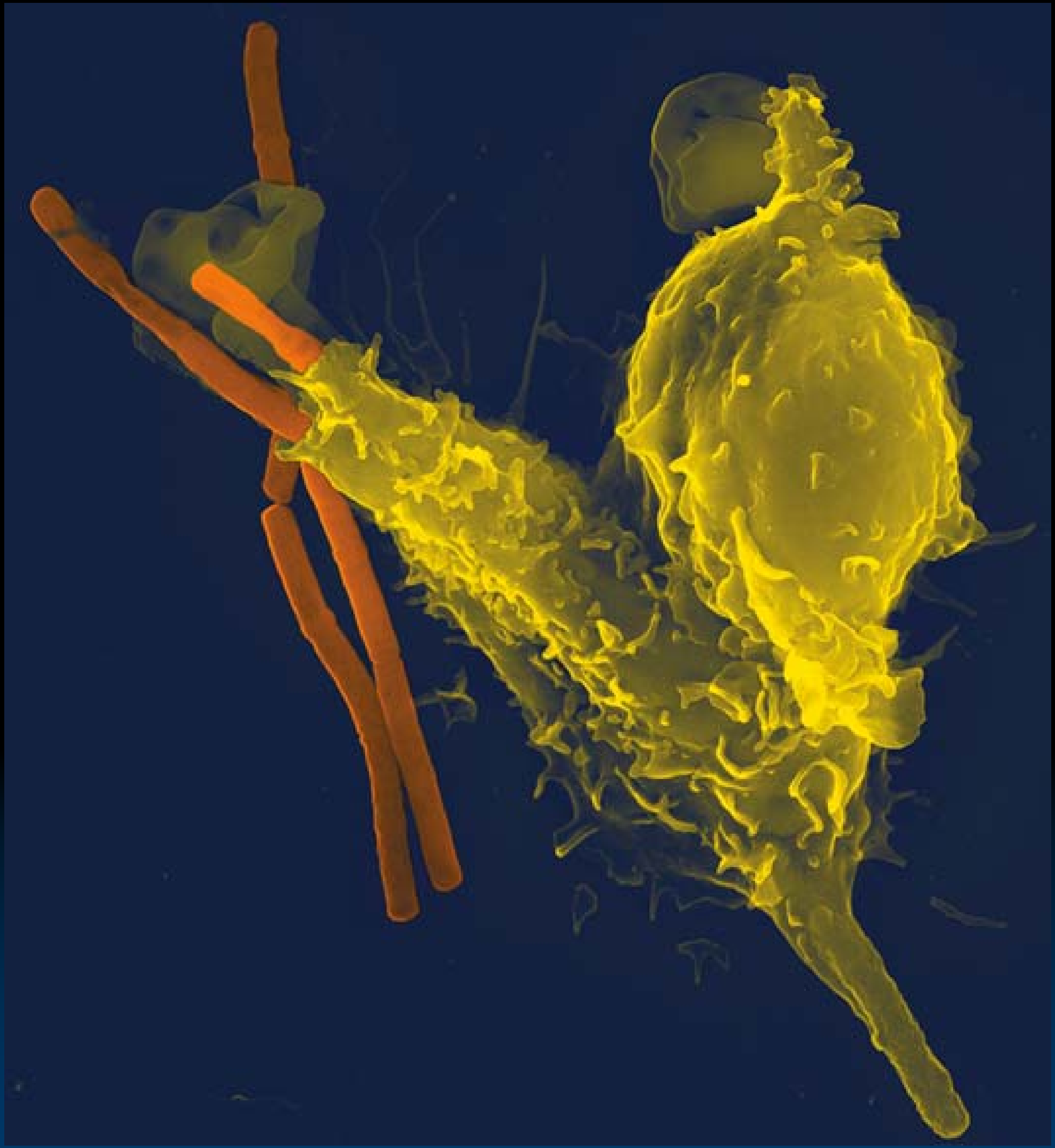
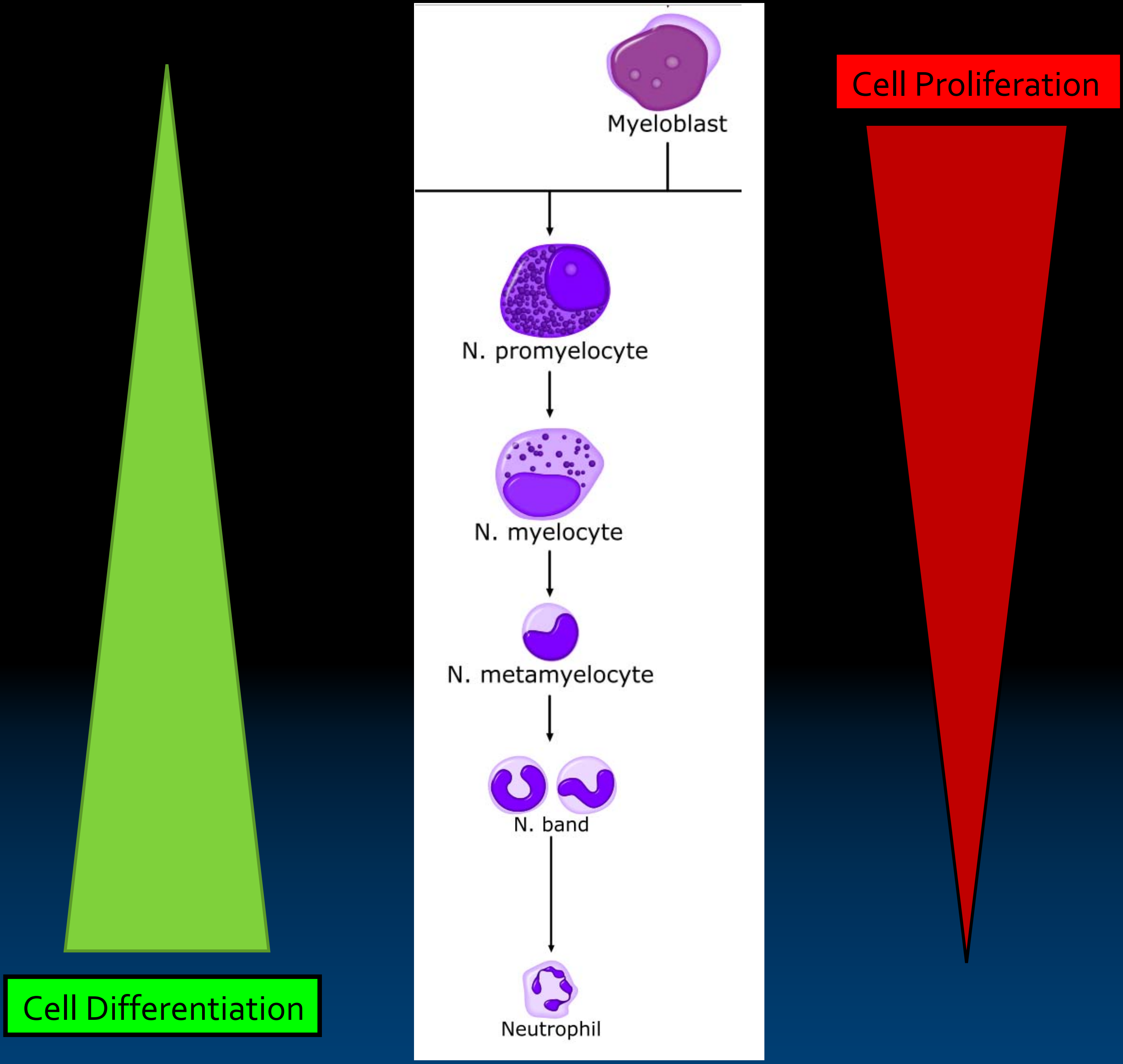
Angers - France



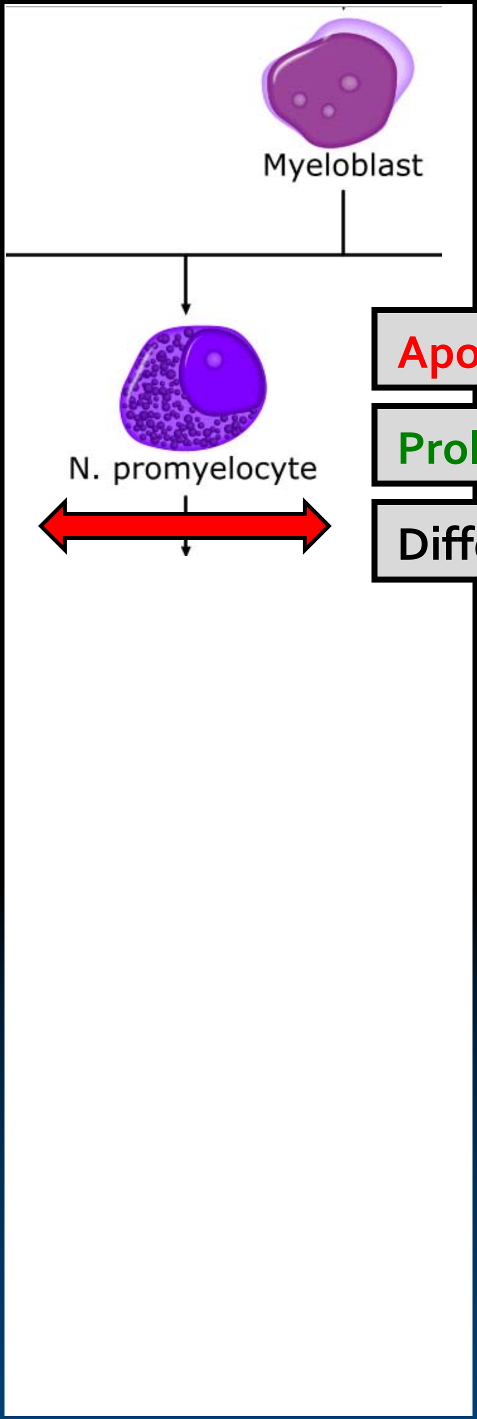
Granulocytes
Or
Neutrophils



Cell Proliferation

Cell Differentiation

In AMLs…



Apoptosis -

Proliferation +

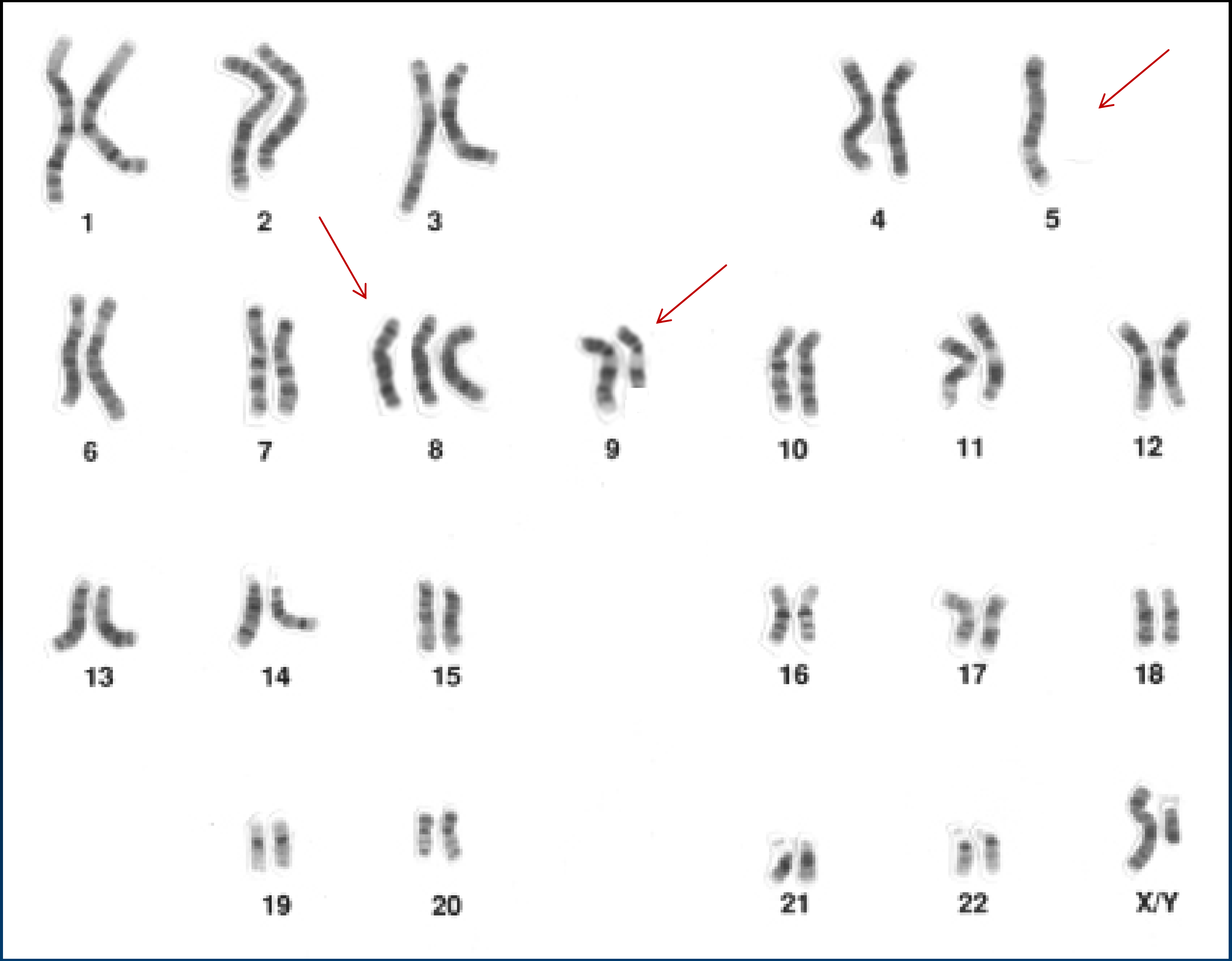
Differentiation blockade

Genome and AML

- Chromosomal abnormalities
    - Complete or partial loss of a chromosome (monosomy, deletion,…)
    - Complete or partial gain of a chromosome (trisomy, amplification,…)
    - Translocations
    - Inversions…
- Mutations in oncogenes / tumor suppressor genes

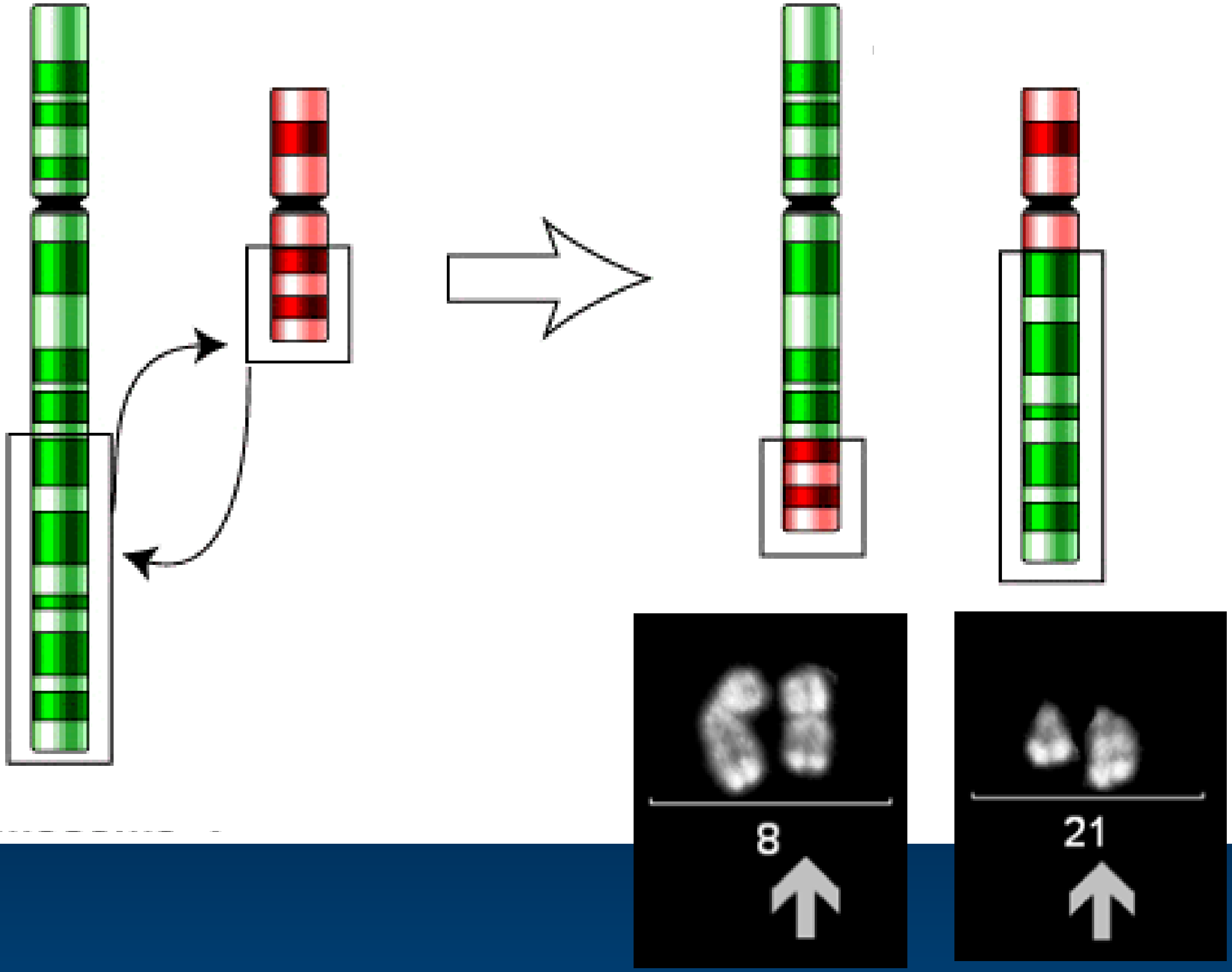
➔**Prognostic value**

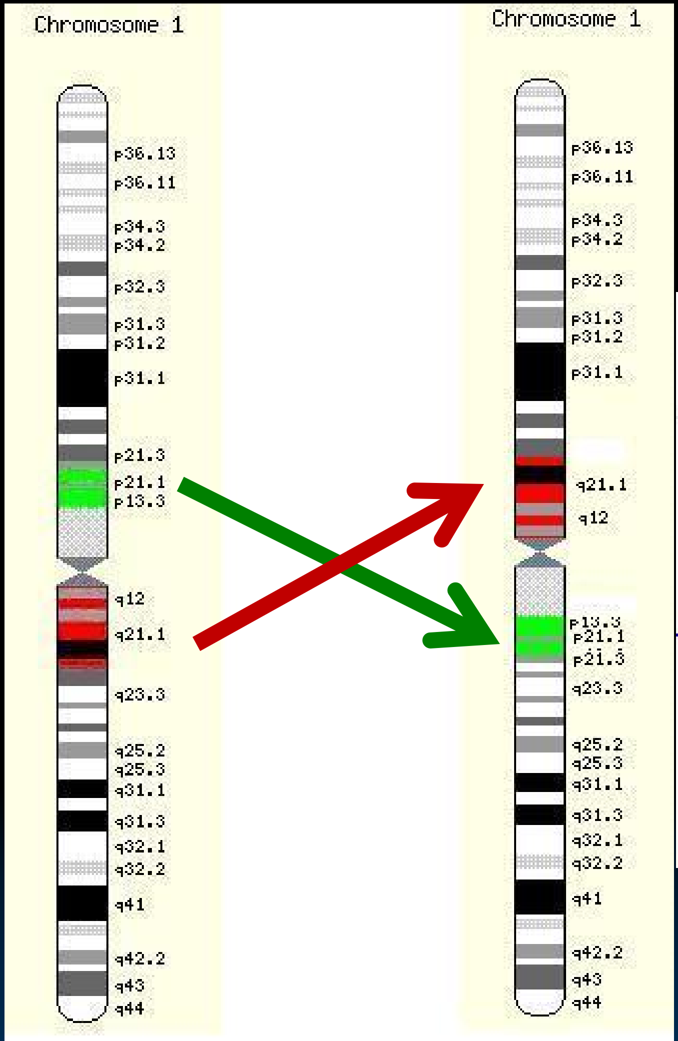
➔**Guide therapeutic strategies**


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X/Y

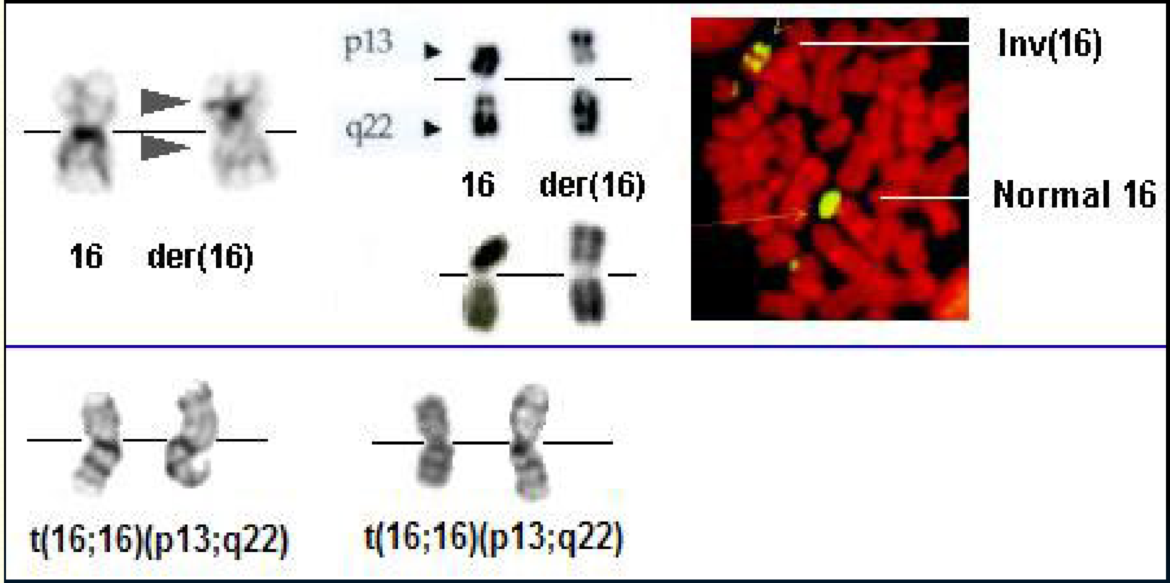
Before translocation

After translocation

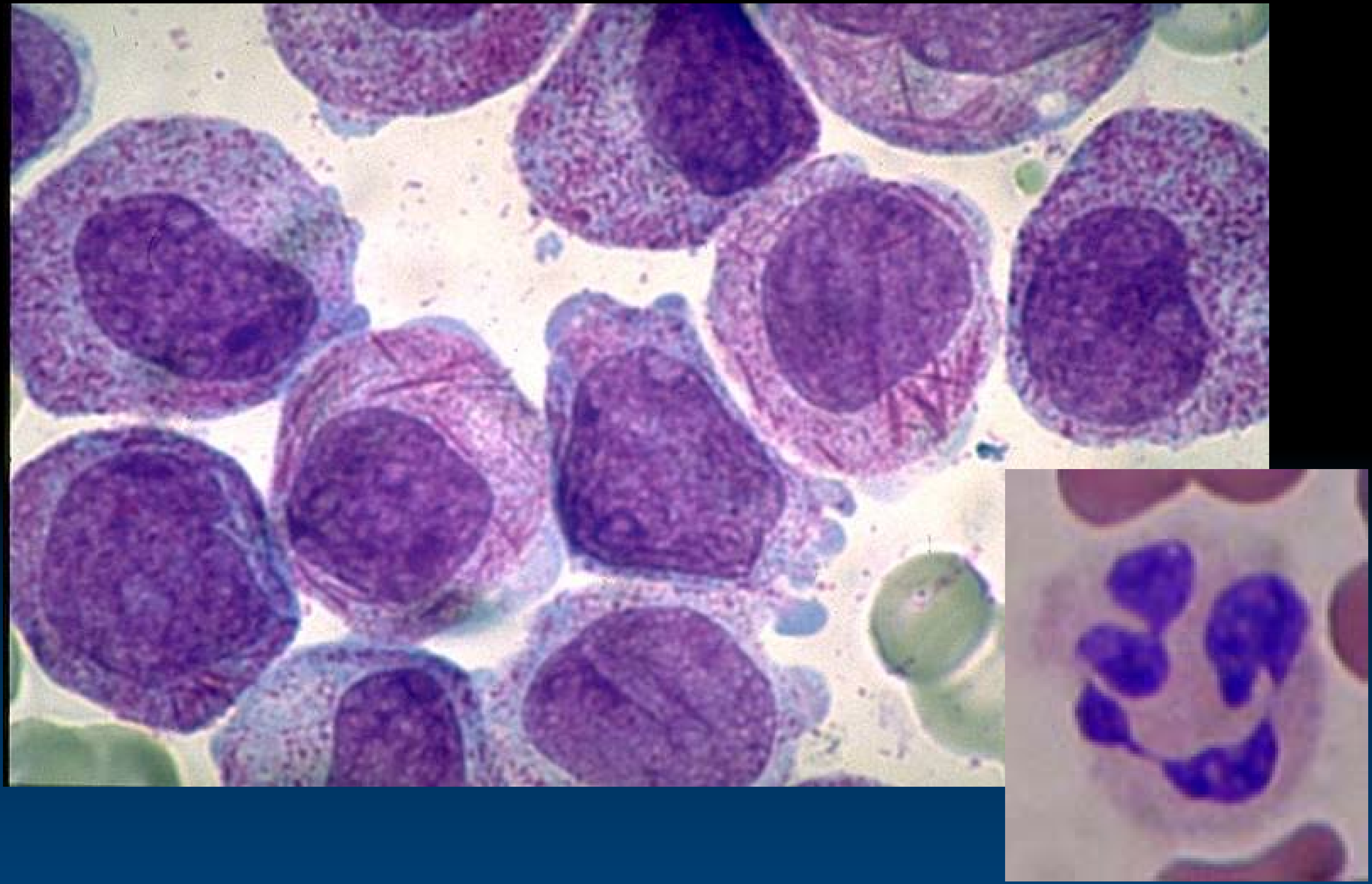




Pericentric inversion

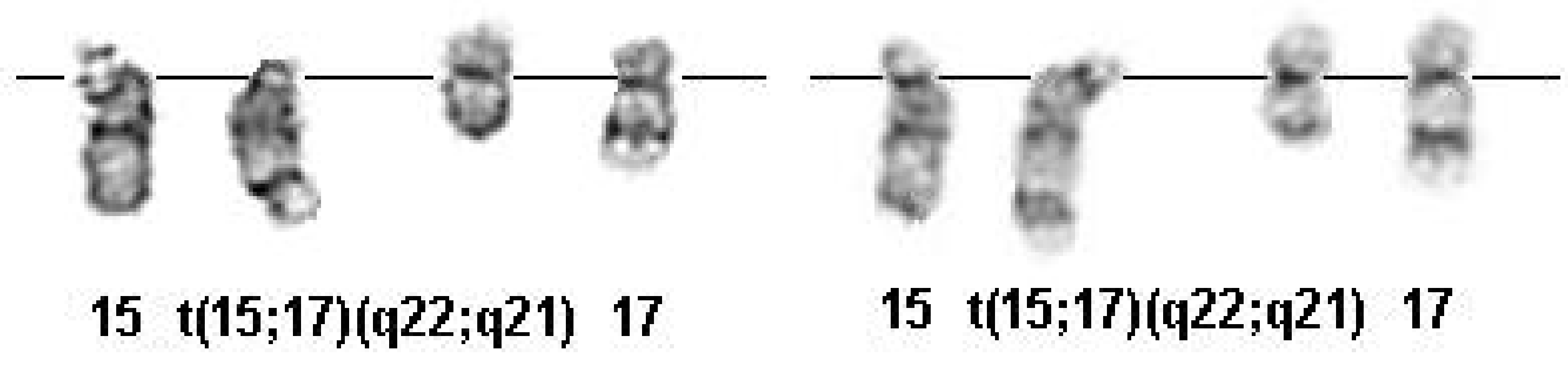


Acute Promyelocytic Leukemia, M3-AML



Acute Promyelocytic Leukemia, M3-AML

Translocation t(15;17)(q22;q21)



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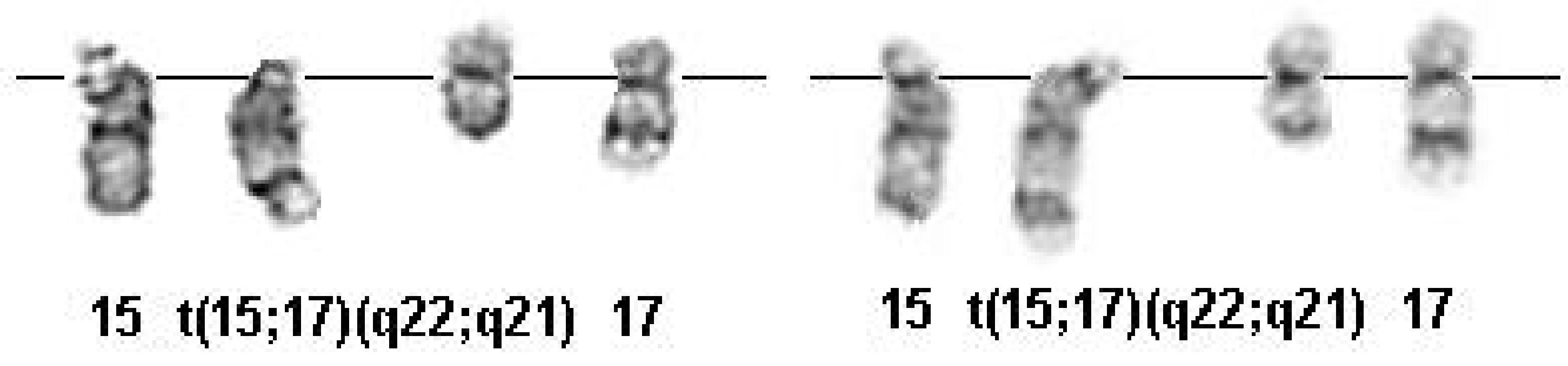
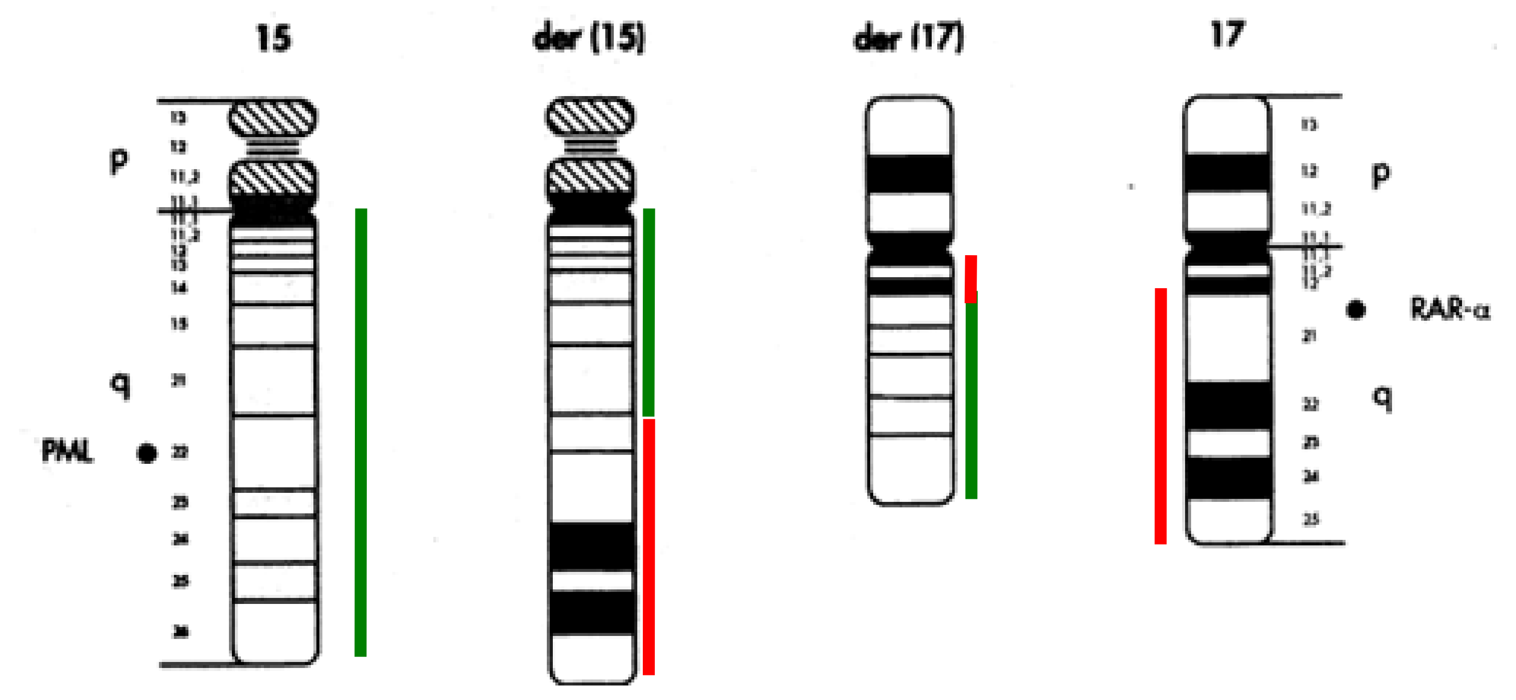
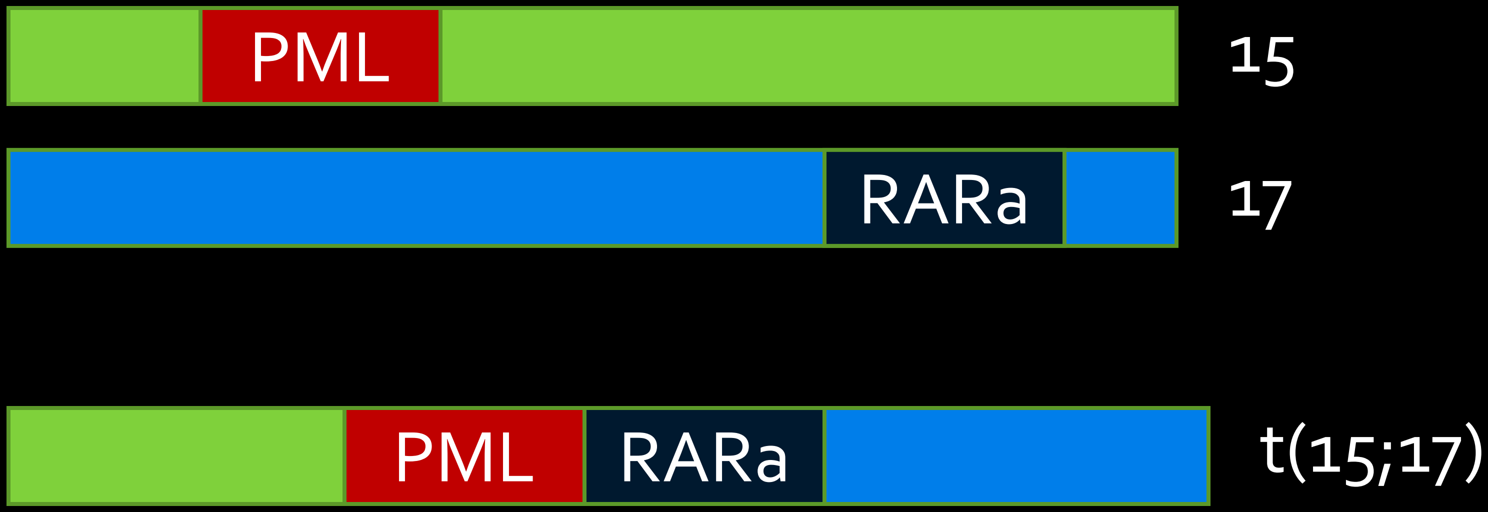


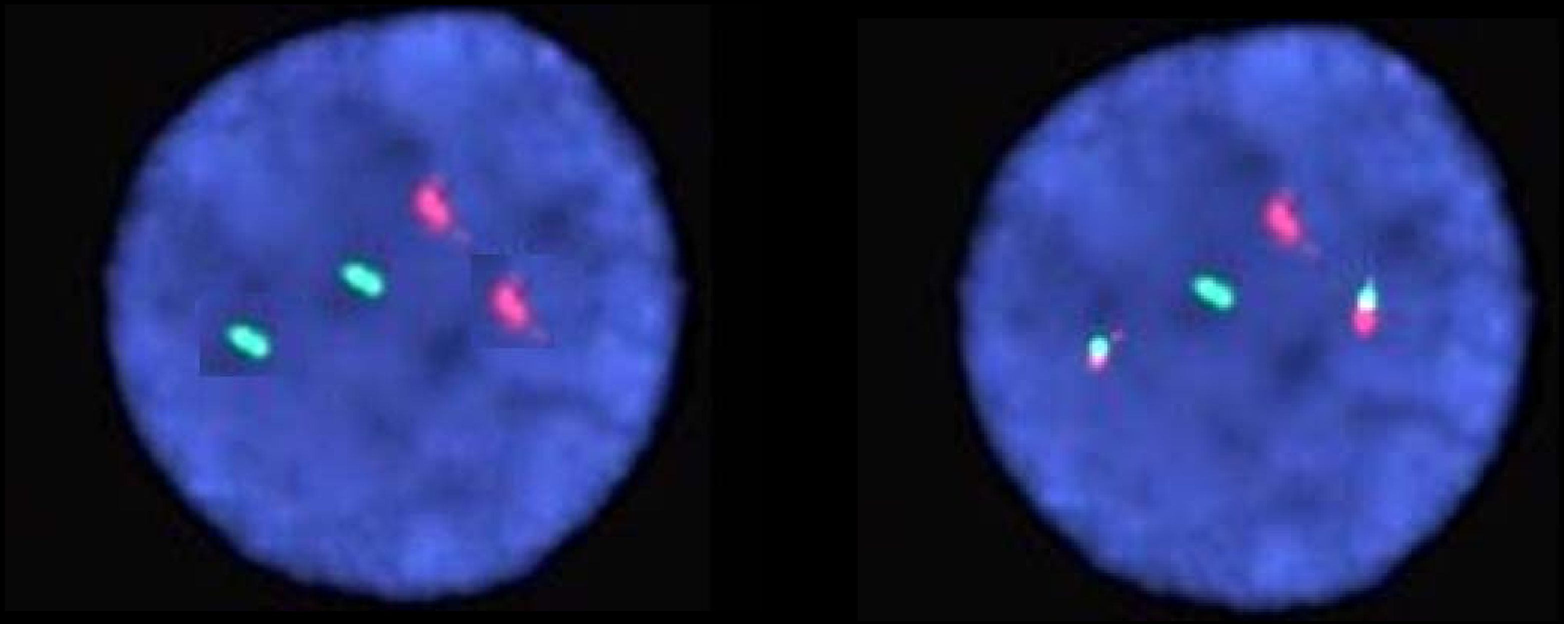




PML 15

RARa 17

PML RARa t(15;17)



Cytological Abnormalities

FAB Classification: M3 promyelocytes + Auer rods

Cytogenetic Abnormalities

Quantitatives: tri 8, del5q, del7q, mono 7, complex…

Qualitatives: t(15;17), inv(16), t(8;21), t(11q23), Ph1

Molecular Abnormalities

PML-RARA CBFB-MYH11 AML1-ETO
BCR-ABL MLL-PTD

FLT3-ITD FLT3-TKD NPM1
CEBPA KIT RAS
JAK2 WT1 RUNX1

EVI-1 ERG FLT3
BAALC MN1 …

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KIT
WT1
NPM1
RAS
RUNX1

EVI-1
BAALC
ERG
MN1
FLT3
…

Cytological Abnormalities

FAB Classification: M1

Cytogenetic Abnormalities normal karyotype

Quantitatives: none

Qualitatives: none

Molecular Abnormalities

PML-RARA
BCR-ABL
CBFB-MYH11
MLL-PTD
AML1-ETO

FLT3-ITD
CEBPA
JAK2
FLT3-TKD
KIT
WT1
NPM1
RAS
RUNX1

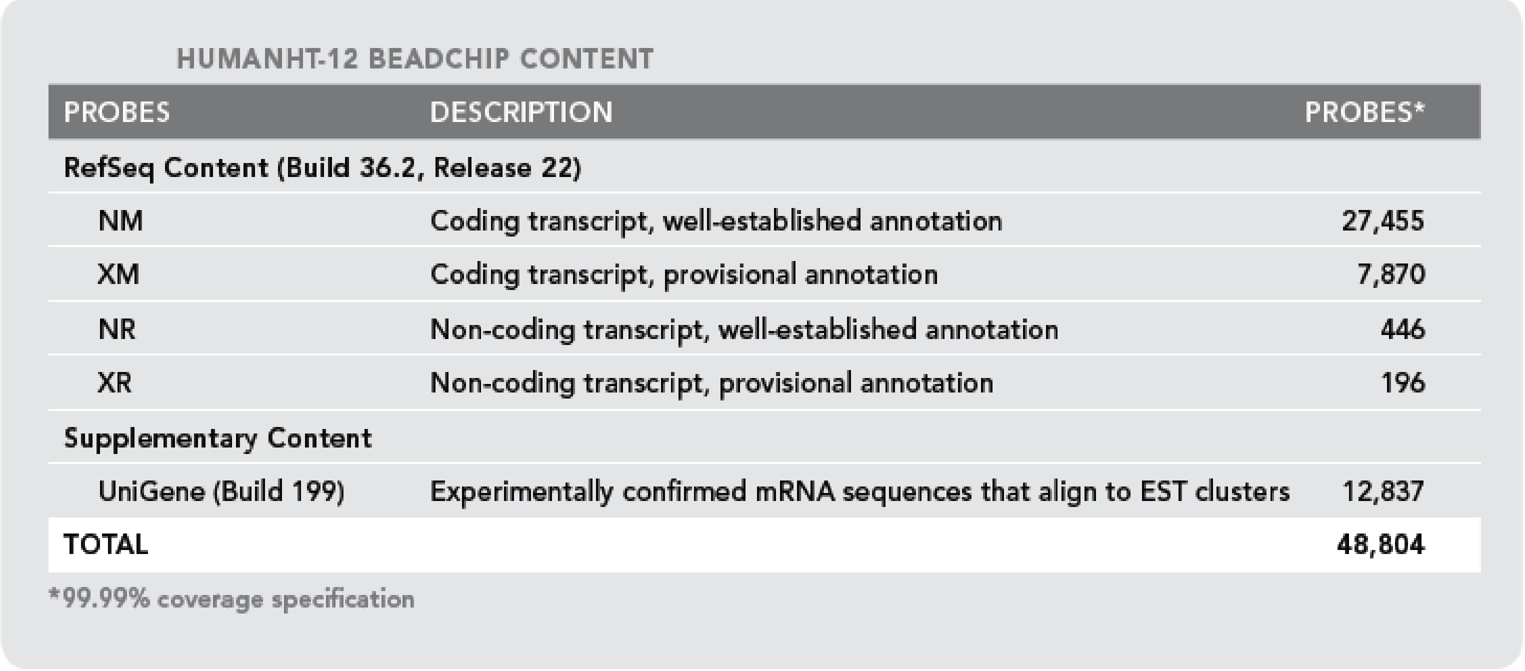
EVI-1
BAALC
ERG
MN1
FLT3
…

Combined Analysis - 1

TOWARD A CLINICAL APPLICATION OF GENE EXPRESSION PROFILING

Gene Expression: Human HT-12 v3

- Targets > 27K annotated genes, with > 48K probes.
- 50-base probe to-target complementarity and average 15-fold feature redundancy
- Starting material: 25 - **200 ng** total RNA



HUMANHT-12 BEADCHIP CONTENT

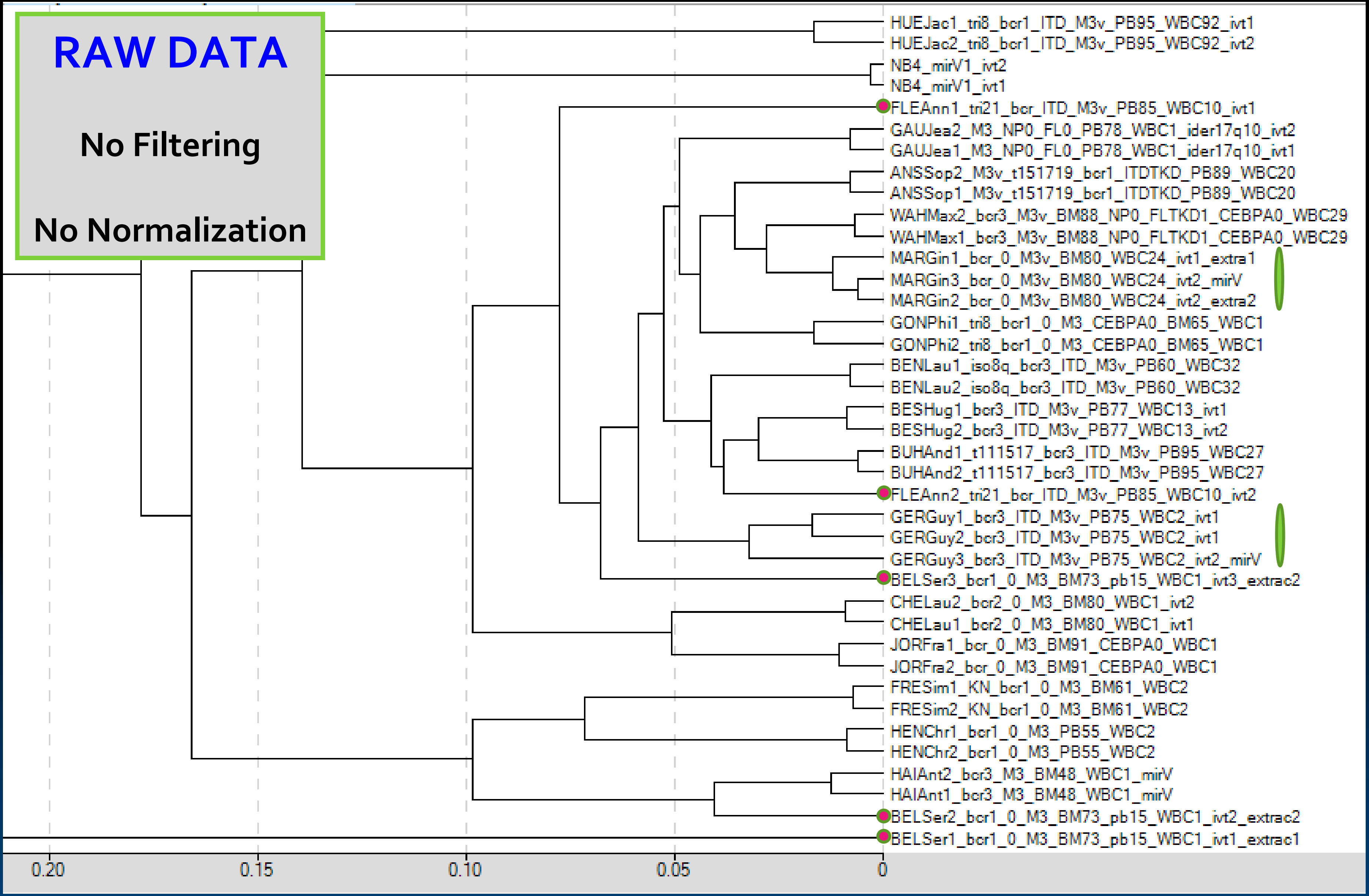
PROBES	DESCRIPTION	PROBES*
RefSeq Content (Build 36.2, Release 22)		
NM	Coding transcript, well-established annotation	27,455
XM	Coding transcript, provisional annotation	7,870
NR	Non-coding transcript, well-established annotation	446
XR	Non-coding transcript, provisional annotation	196
Supplementary Content		
UniGene (Build 199)	Experimentally confirmed mRNA sequences that align to EST clusters	12,837
TOTAL		48,804

*99.99% coverage specification

Validation – Gene Expression

- **Reproductibility**
  - Replicates (n=39 stripes)
    - **With different total RNA extraction kits**
      - **Duplicates RNEasy Mini kits QIAGEN (n=15 samples)**
      - **Triplicates QIAGEN + Mirvana kits AMBION (n=3 samples)**
    - **Duplicates on different HT-12 BeadChips**
    - **With different RNA extractions**
    - **With different batches of Illumina Total Prep Kits**
    - **At different time points**
    - **Performed by two different technicians**
- Acute Promyelocytic Leukemias – CHU Angers
  - APLs or M3-variants AMLs, n = 17 patients ➔ 37 stripes
  - NB4 cell line (n=2)

APLs - Sample Clustering



RAW DATA

No Filtering

No Normalization

HOW EFFICIENT ARE WE TO DIAGNOSE AML SUBTYPES USING GEX PROFILING ?

The issue…

- Most reported studies used AML samples containing at least 50% of leukemic blasts for class prediction analyses… usually > 80% blasts
- Definition of acute leukemia: Blasts $\geq$ 20%
- How do classifiers behave with « real life » samples containing 20% to 60% of blasts ?

…can we use microarrays in the clinics for real patients not only for publications !?

Class Prediction Analysis

- APLs vs. CBF-AMLs vs NPM1+ NK-AMLs CHU Angers + CHU Reims
  - APLs or M3-variant AMLs with t(15;17)
  - CBF-apha AMLs with t(8;21)
  - CBF-beta AMLs with inv(16) or t(16;16)
  - NPM1 mutated NK-AMLs

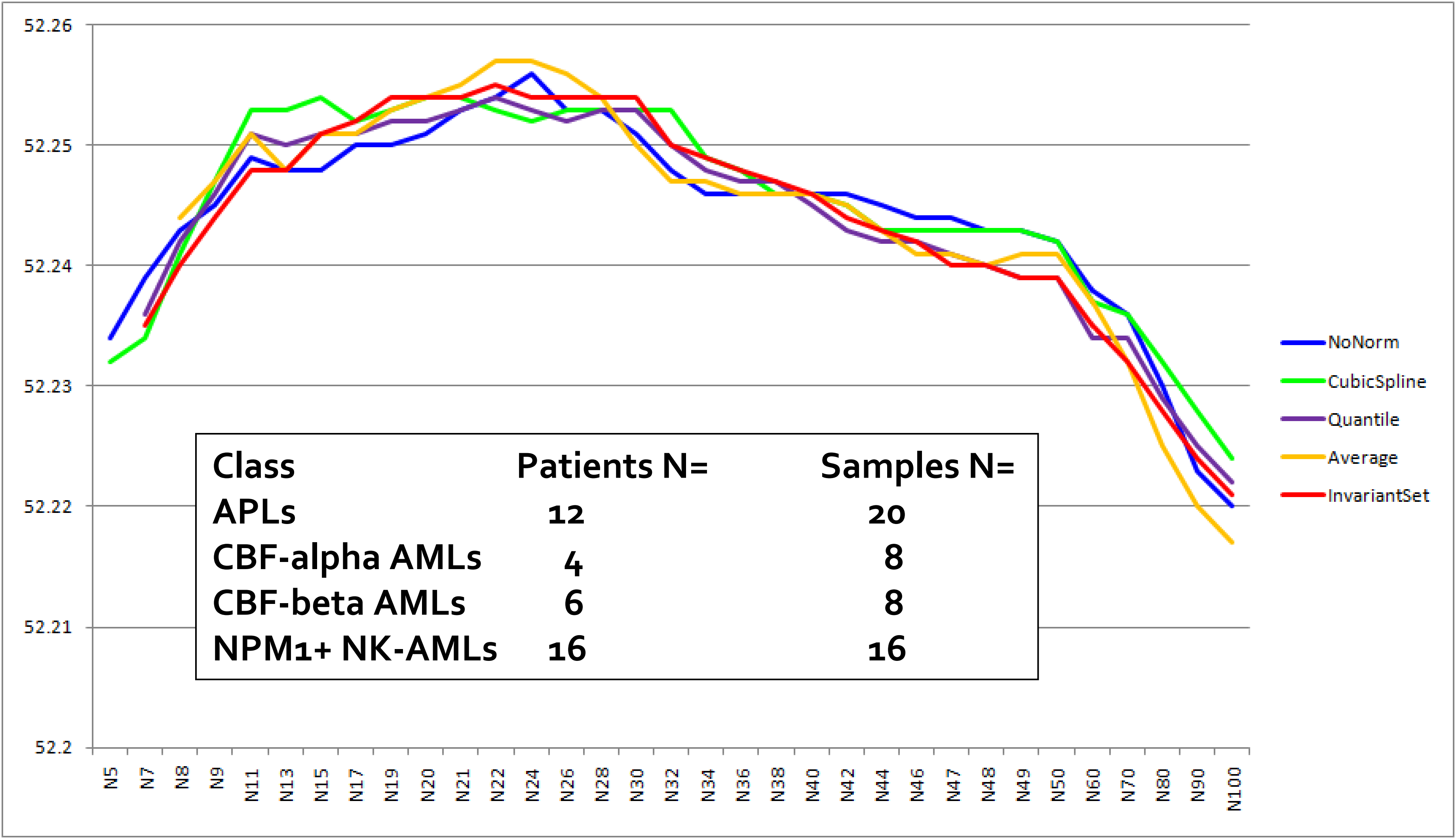
- Data from the literature or from GEO (Affymetrix)
  - Verhaak et al - Haematologica 2009
  - Ichikawa et al - British Journal of Haematology 2006
  - Gutierrez et al - Leukemia 2005
  - Vaalk et al - New England Journal of Medicine 2004
  - Kohlmann et al - Gene Chromosome & Cancer 2003
  - Debernardi et al - Gene Chromosome & Cancer 2003
  - Schoch et al - Proceedings of the NAS 2002

Method

- **Genome Studio 2009.1**
  - Processing of the signal
  - Quantile normalization
- **ArrayMiner 5.3.3. *ClassMarker Module***
  - Class prediction analysis based on Genetic Algorithms
  - Log-transformed data
  - Filter of the data
    - Threshold maximum: 50,000
    - Fold change > 1.50
    - Absolute change > 150
  - Signal-to-noise ratio > 1.50 for all markers / class
- **Qlucore Omics Explorer 2.0**
  - 3-D dynamic PCA

BUILDING OF THE CLASSIFIERS TRAINING SET (BLASTS ≥ 50%)

Fitness, normalization methods and number of features per class



Class	Patients N=	Samples N=
APLs	12	20
CBF-alpha AMLs	4	8
CBF-beta AMLs	6	8
NPM1+ NK-AMLs	16	16

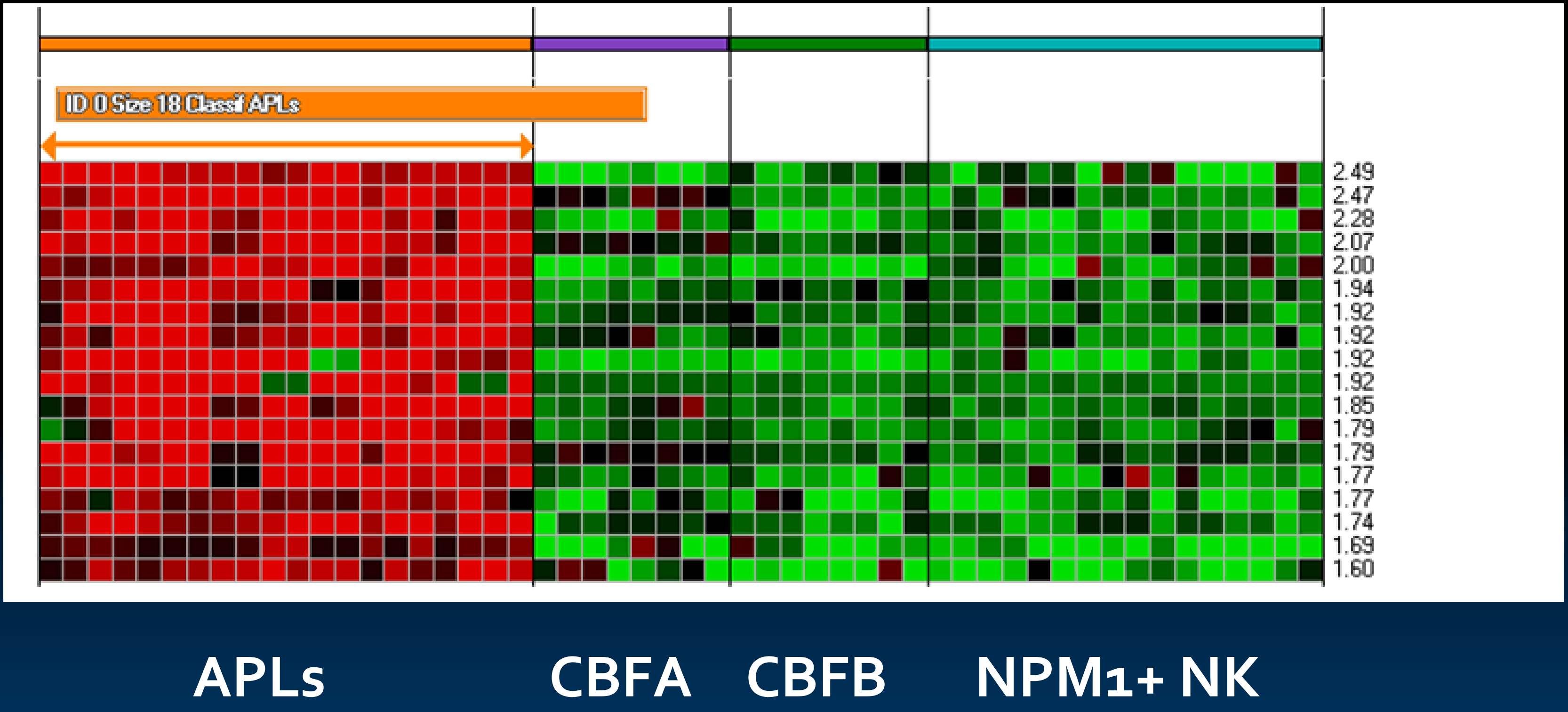
APLs

t(15;17)

Blast ≥ 50%

Top-18
Markers

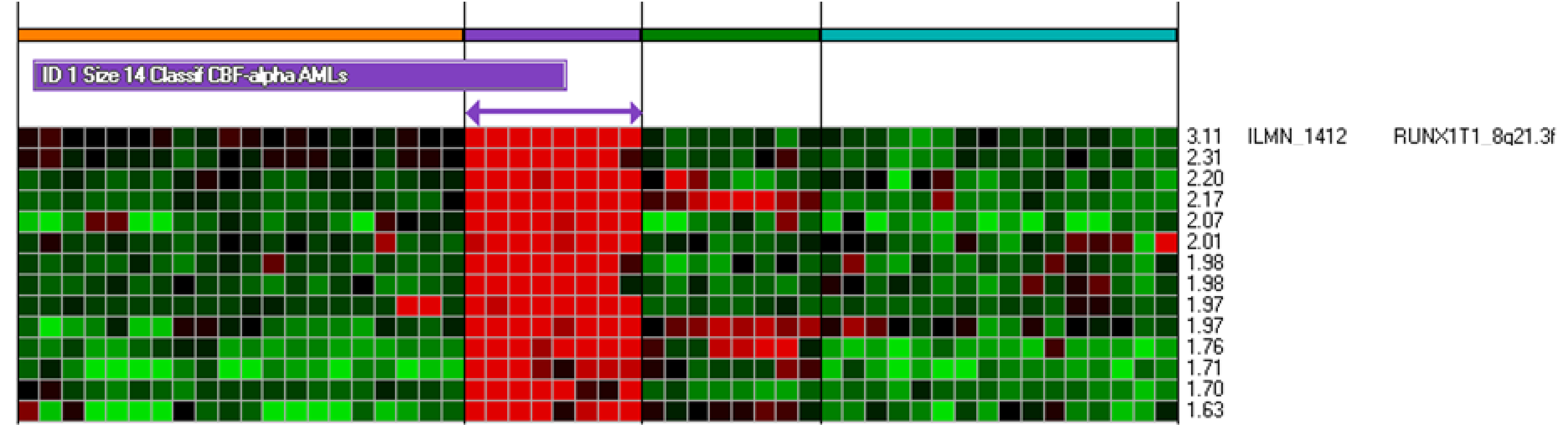
S/N ratio ≥ 1.50



CBF-alpha AMLs
t(8;21)
Blast ≥ 50%

Top-14 Markers

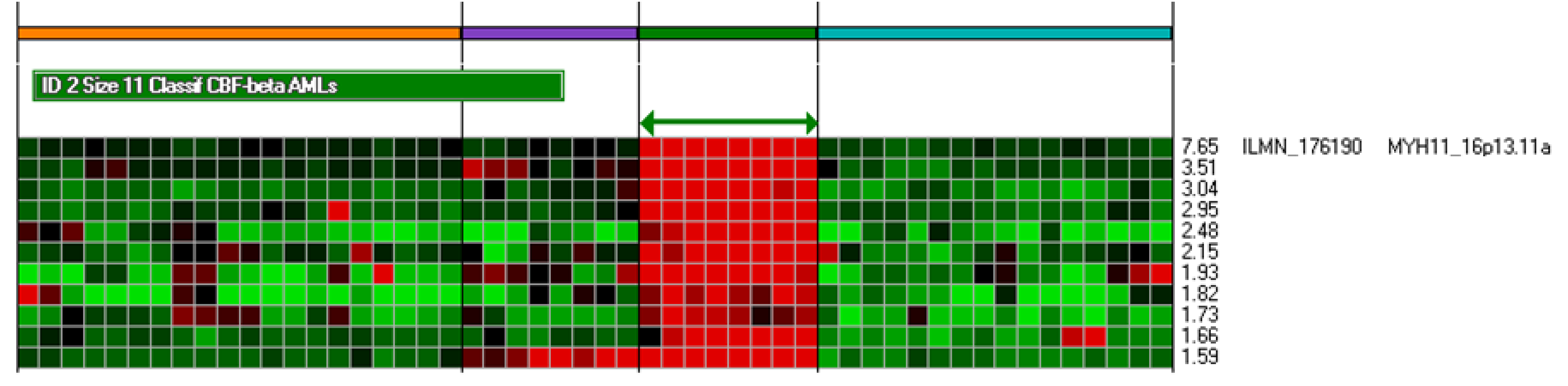
S/N Ratio ≥ 1.50



APLs CBFA CBFB NPM1+ NK

# CBF-beta AMLs
Inv(16) / t(16;16)
Blast ≥ 50%

Top-11
Markers
S/N Ratio ≥ 1.50

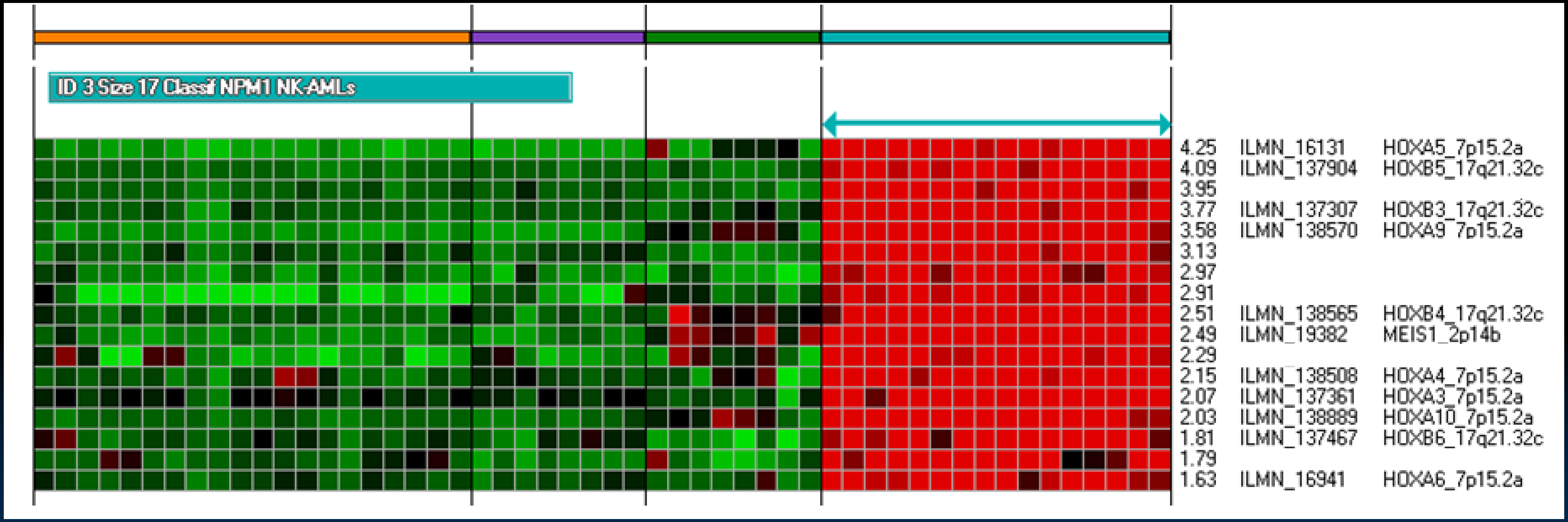


APLs CBFA CBFB NPM1+ NK

NPM1+ NK-AMLs
Blast ≥ 50%

Top-17 Markers

S/N Ratio ≥ 1.50



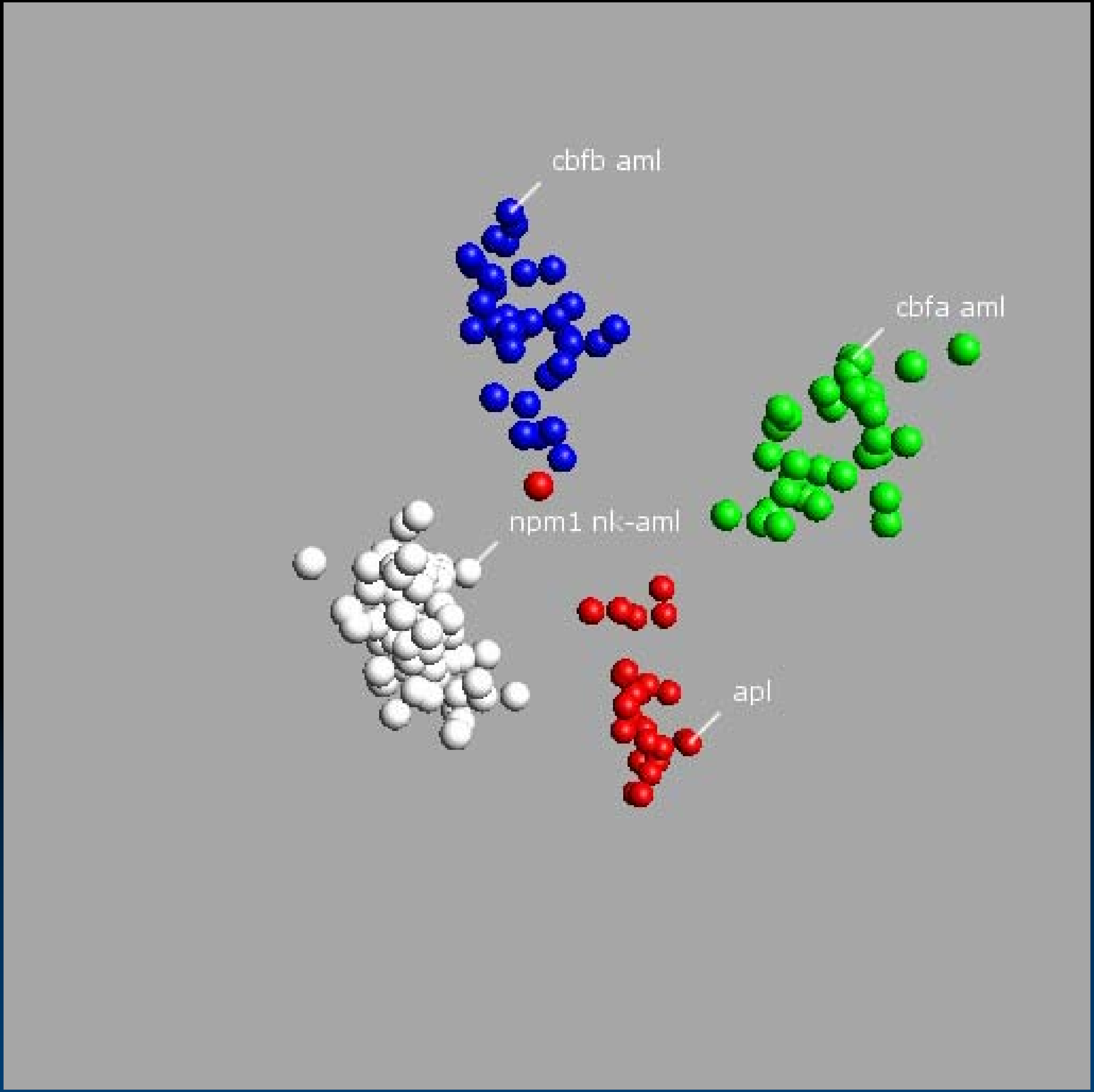
4.25	ILMN_16131	HOXA5_7p15.2a
4.09	ILMN_137904	HOXB5_17q21.32c
3.95		
3.77	ILMN_137307	HOXB3_17q21.32c
3.58	ILMN_138570	HOXA9_7p15.2a
3.13		
2.97		
2.91		
2.51	ILMN_138565	HOXB4_17q21.32c
2.49	ILMN_19382	MEIS1_2p14b
2.29		
2.15	ILMN_138508	HOXA4_7p15.2a
2.07	ILMN_137361	HOXA3_7p15.2a
2.03	ILMN_138889	HOXA10_7p15.2a
1.81	ILMN_137467	HOXB6_17q21.32c
1.79		
1.63	ILMN_16941	HOXA6_7p15.2a

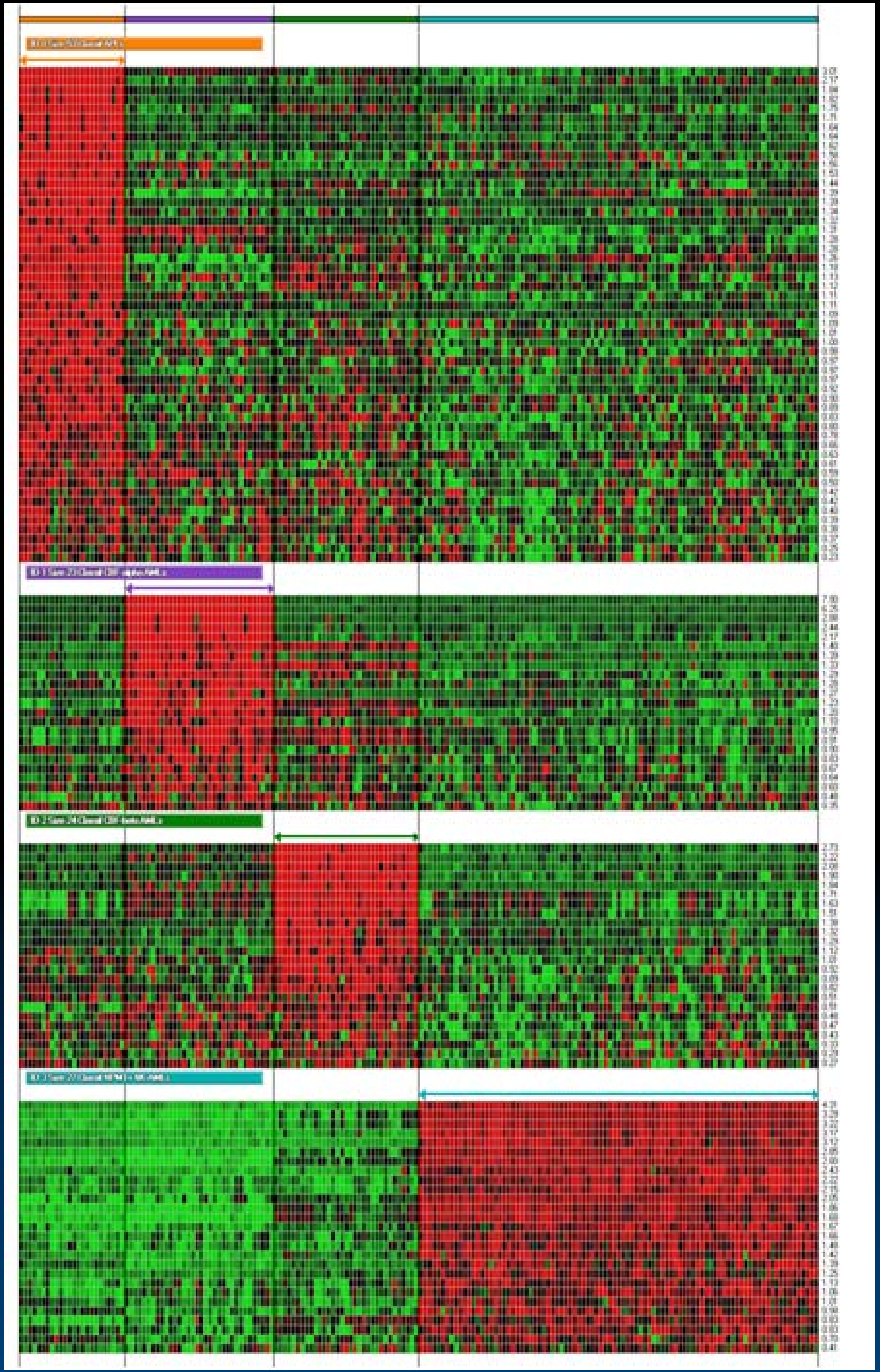
APLs CBFA CBFB NPM1+ NK

Affymetrix
Human Genome
U133 Plus 2.0

Verhaak et al, 2009
Haematologica
GEO # GSE6891

Assessment of
our 60 markers
Classifiers





APLs

t(15;17)

CBF-alpha AMLs

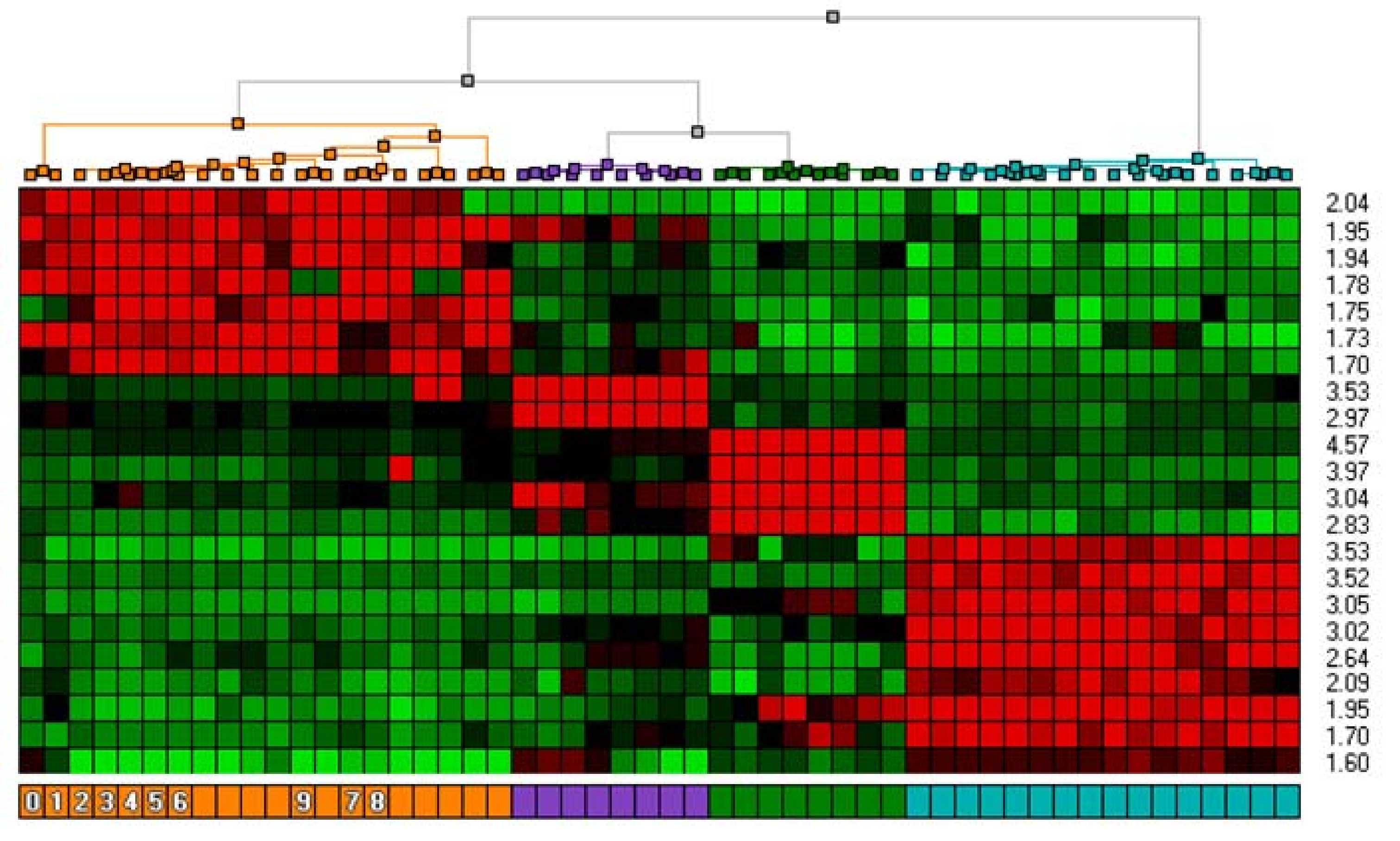
t(8;21)

CBF-beta AMLs

Inv(16) / t(16;16)

NPM1+ NK-AMLs

Illumina & Affymetrix combined
22 marker signature



APLs 7

CBFA 2

CBFB 4

NPM1+ 9

APLs **CBFA** **CBFB** **NPM1+ NK**

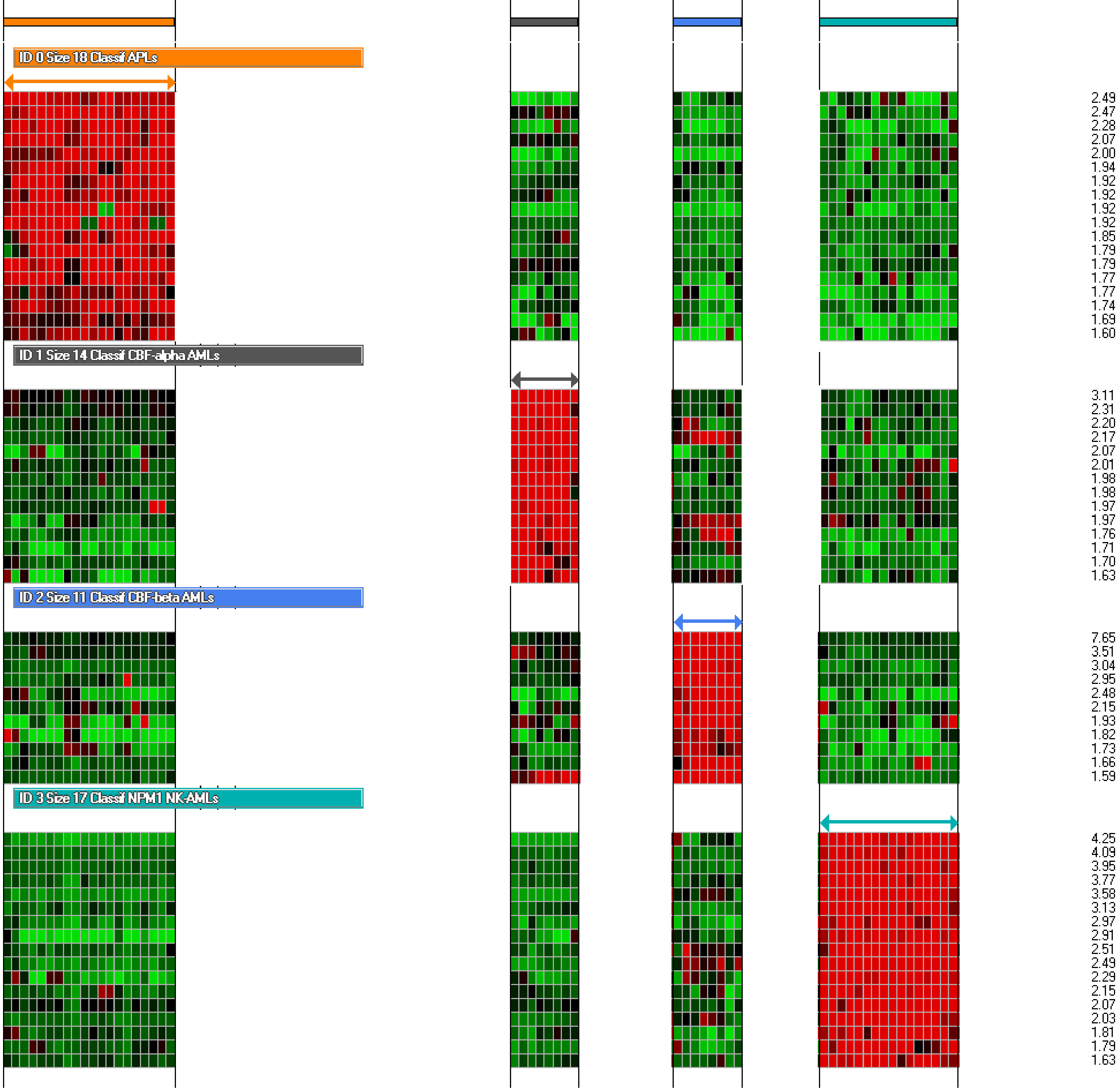
LOW LEUKEMIC BLAST LOAD SAMPLES AND AML-VARIANTS TEST SET

Two options for the test set

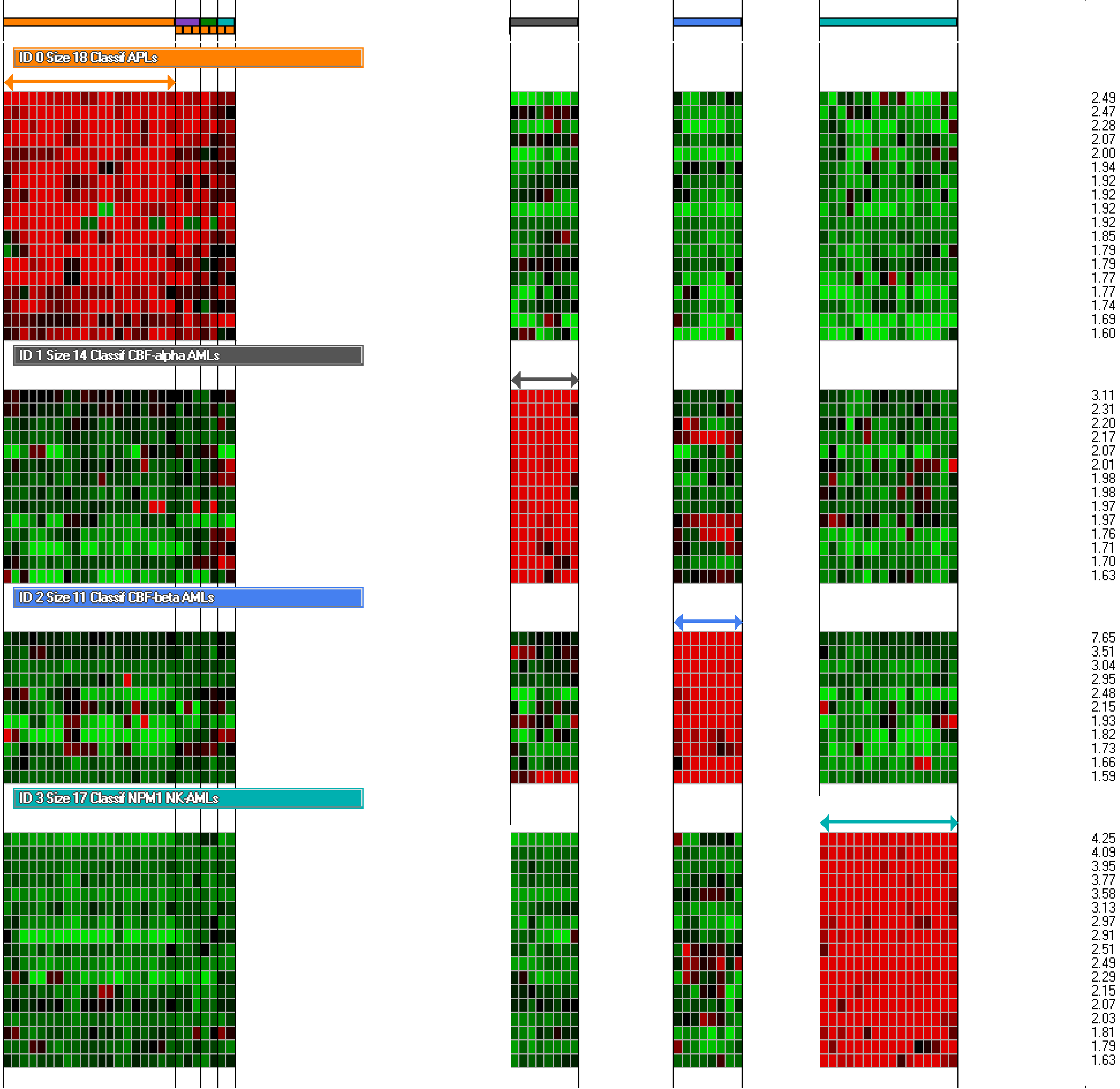
- Use samples with low blast load
  - At diagnosis
  - At relapse

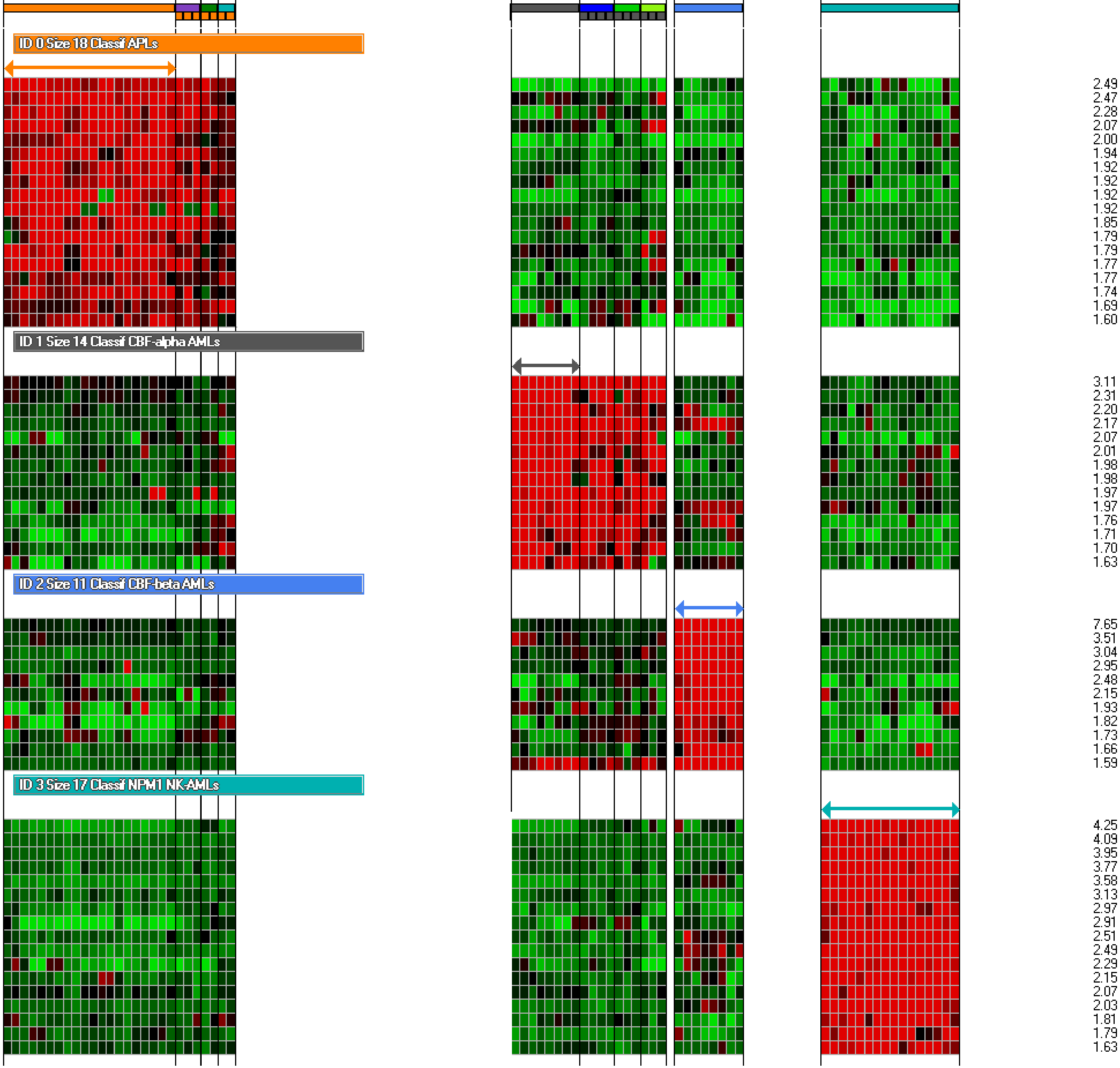
- Use samples with high blast load but diluted in a pool of normal bone marrow samples
  - Dilution at 50% and at 75% of the AML samples

Start with 80-100% blasts ➔ 20-25% leukemic blasts


ID 0 Size 18 Classif APLs
2.49
2.47
2.28
2.07
2.00
1.94
1.92
1.92
1.92
1.92
1.85
1.79
1.79
1.77
1.77
1.74
1.69
1.60
ID 1 Size 14 Classif CBF-alpha AMLs
3.11
2.31
2.20
2.17
2.07
2.01
1.98
1.98
1.97
1.97
1.76
1.71
1.70
1.63
ID 2 Size 11 Classif CBF-beta AMLs
7.65
3.51
3.04
2.95
2.48
2.15
1.93
1.82
1.73
1.66
1.59
ID 3 Size 17 Classif NPM1 NK-AMLs
4.25
4.09
3.95
3.77
3.58
3.13
2.97
2.91
2.51
2.49
2.29
2.15
2.07
2.03
1.81
1.79
1.63

Fine Down to 11% blasts For APLs





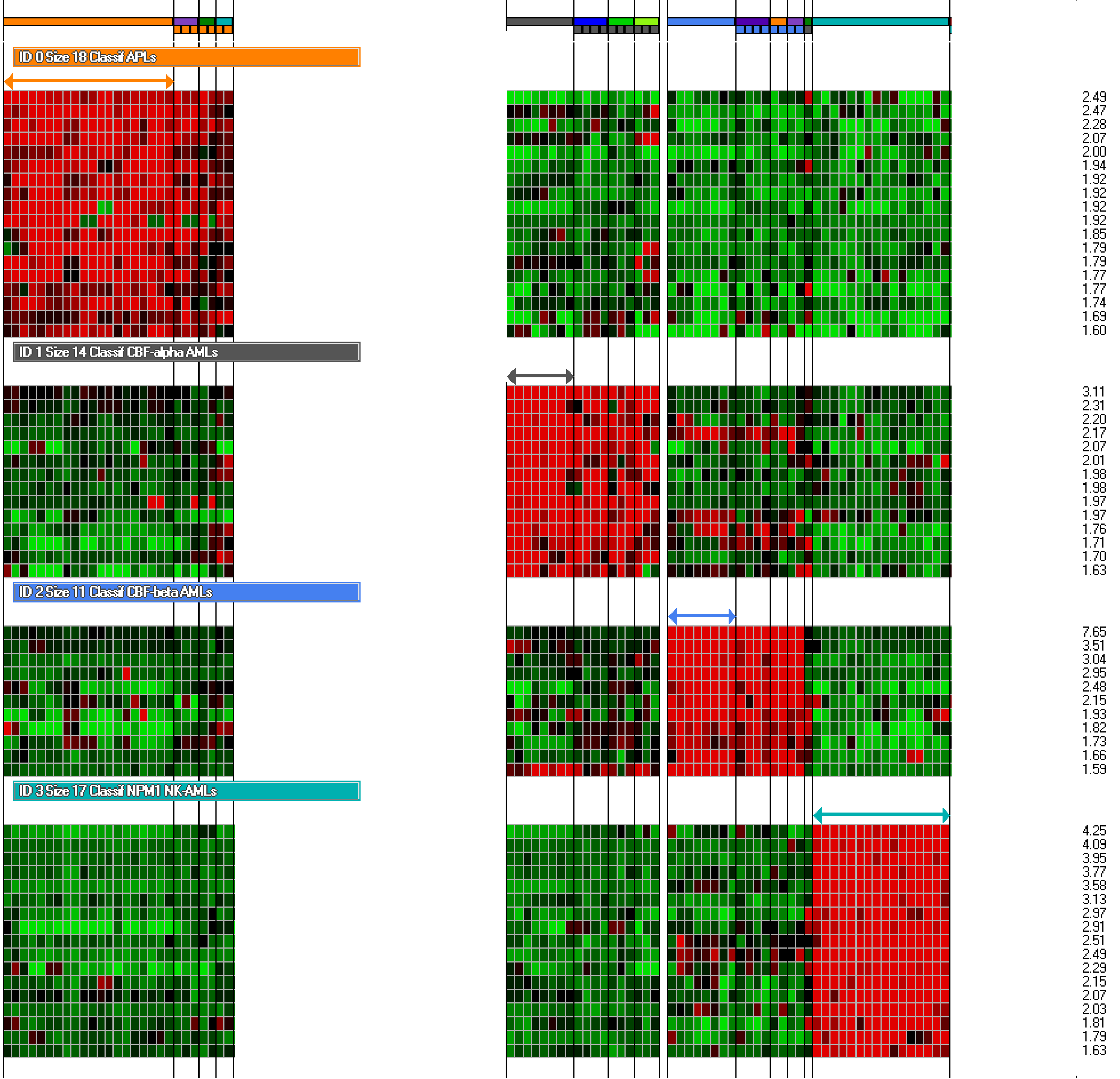
Fine

Down to

16% blasts

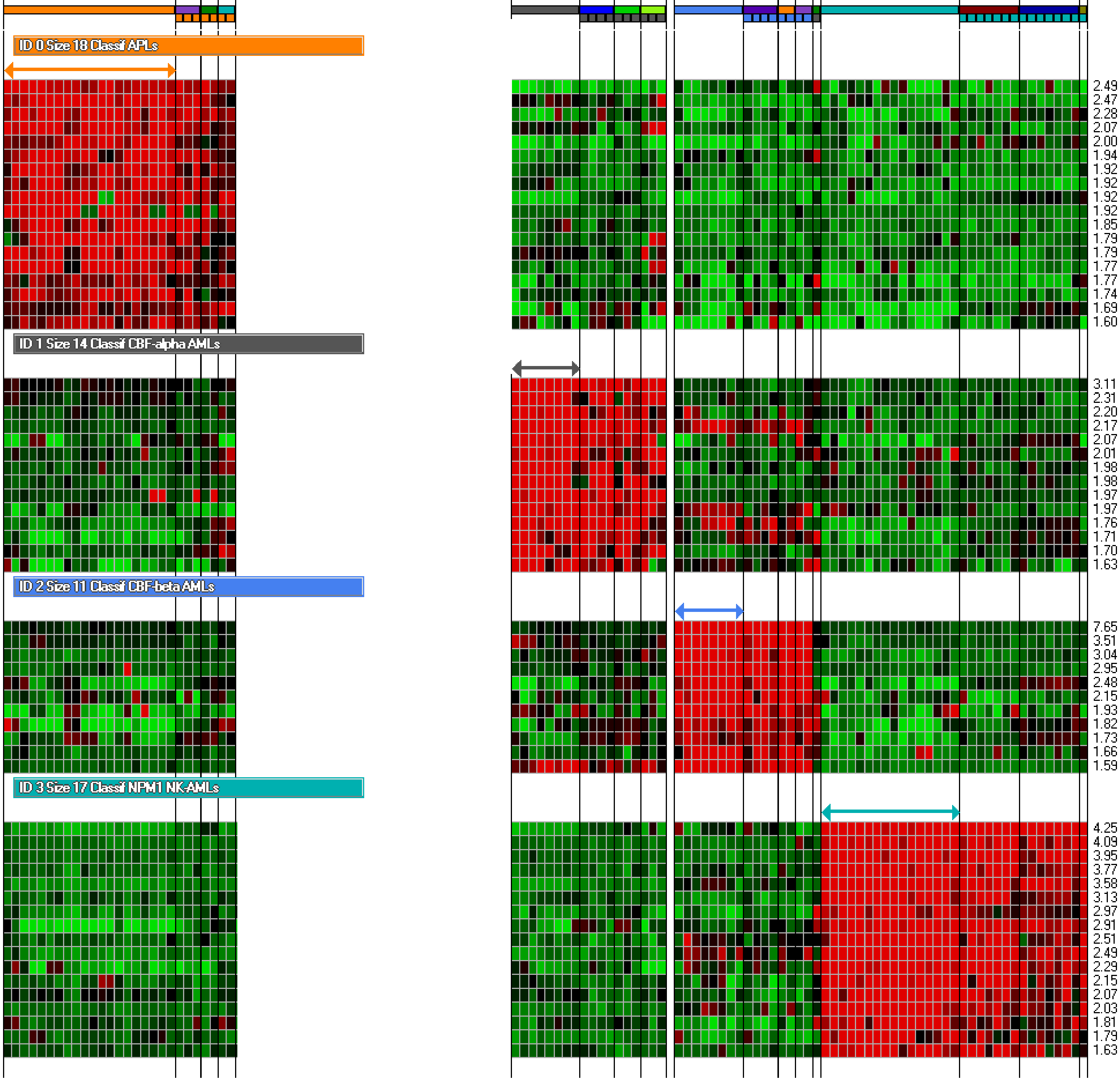
For

CBFA AMLs

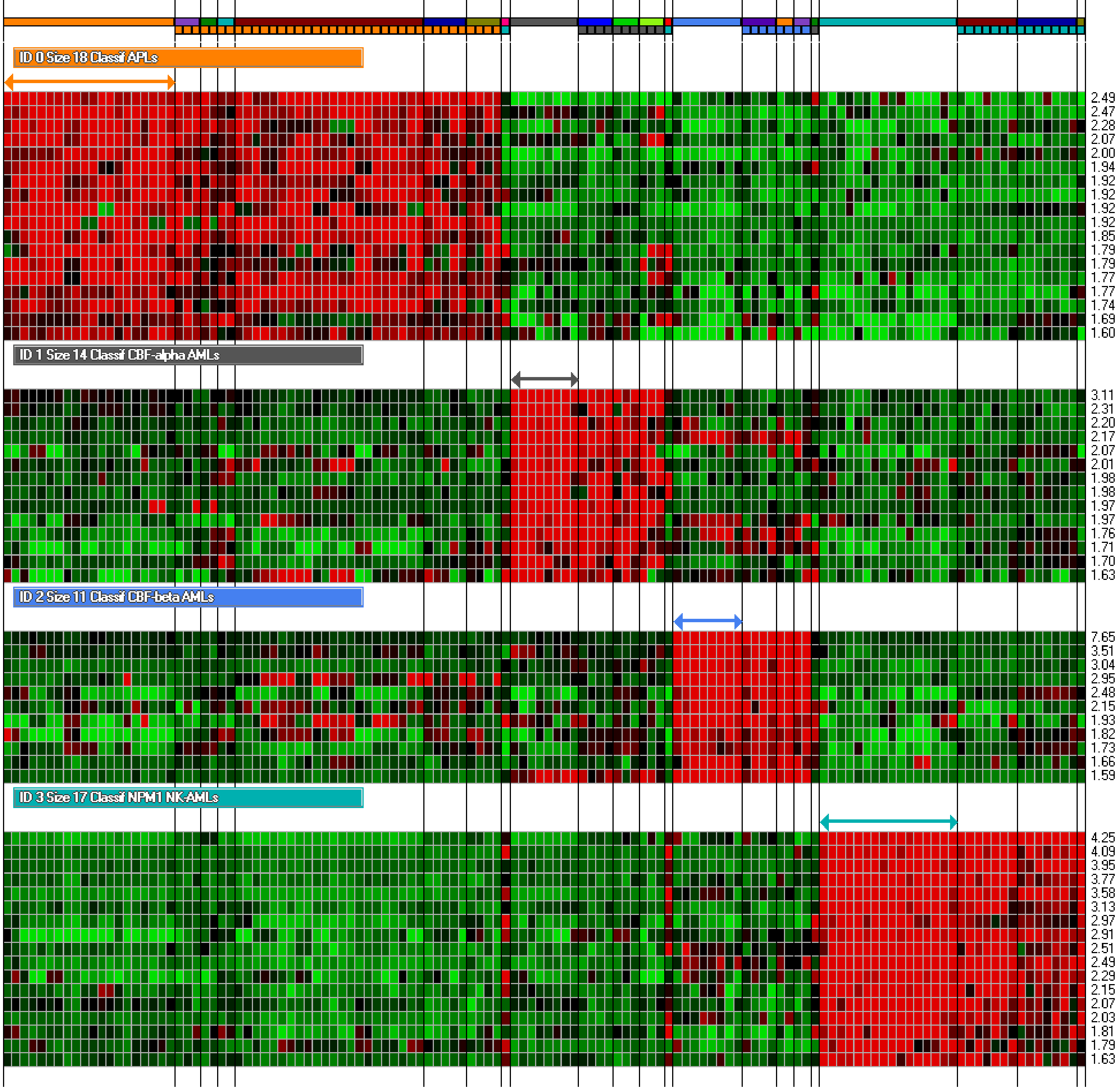


Fine
Down to
20% blasts
For
CBFB AMLs

1 error
14% blasts
relapse



Fine
Down to
23% blasts
For
NPM1+
NK-AMLs



Fine
Down to
20% blasts
For
M3-variants

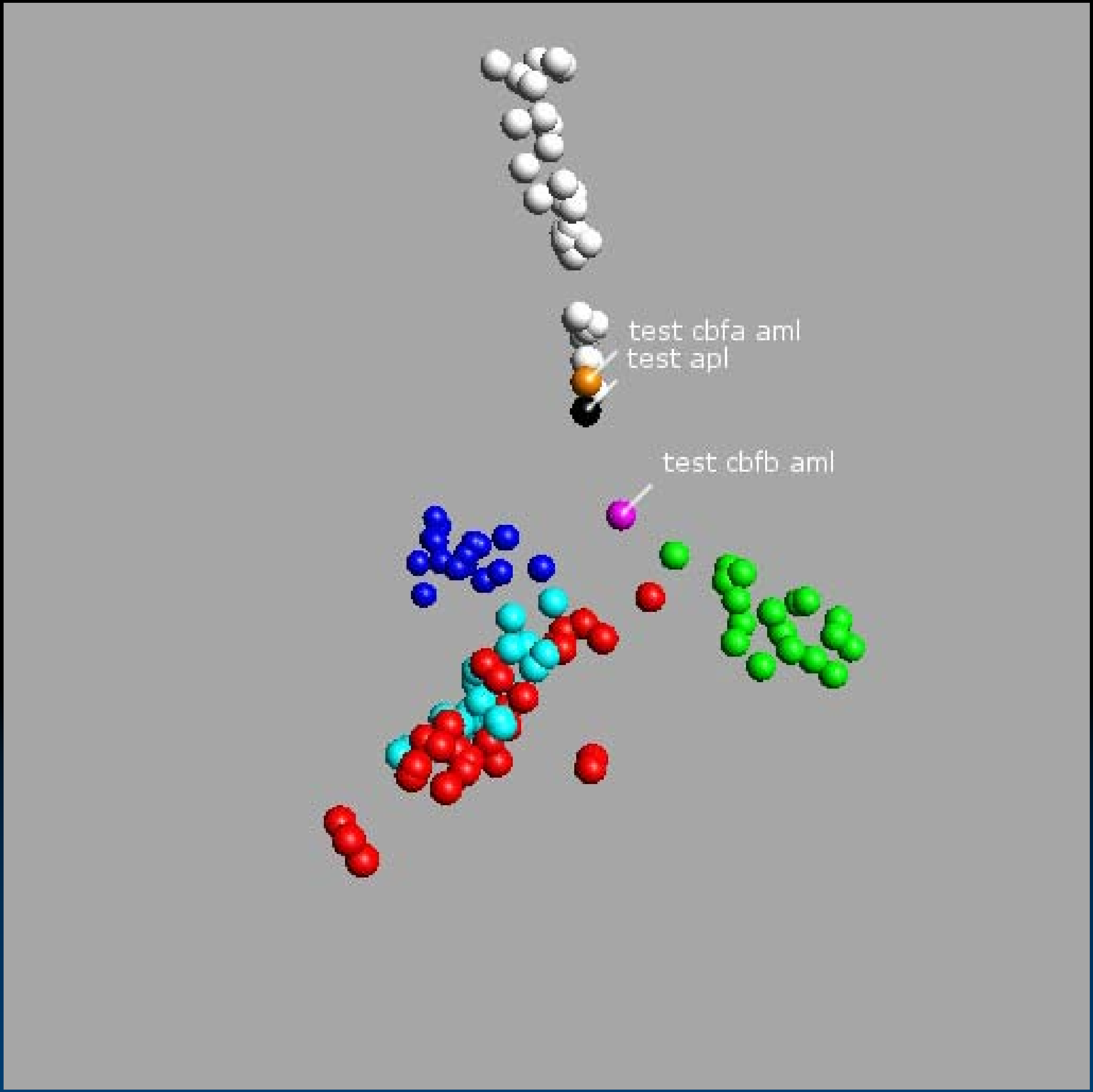
● CBFB

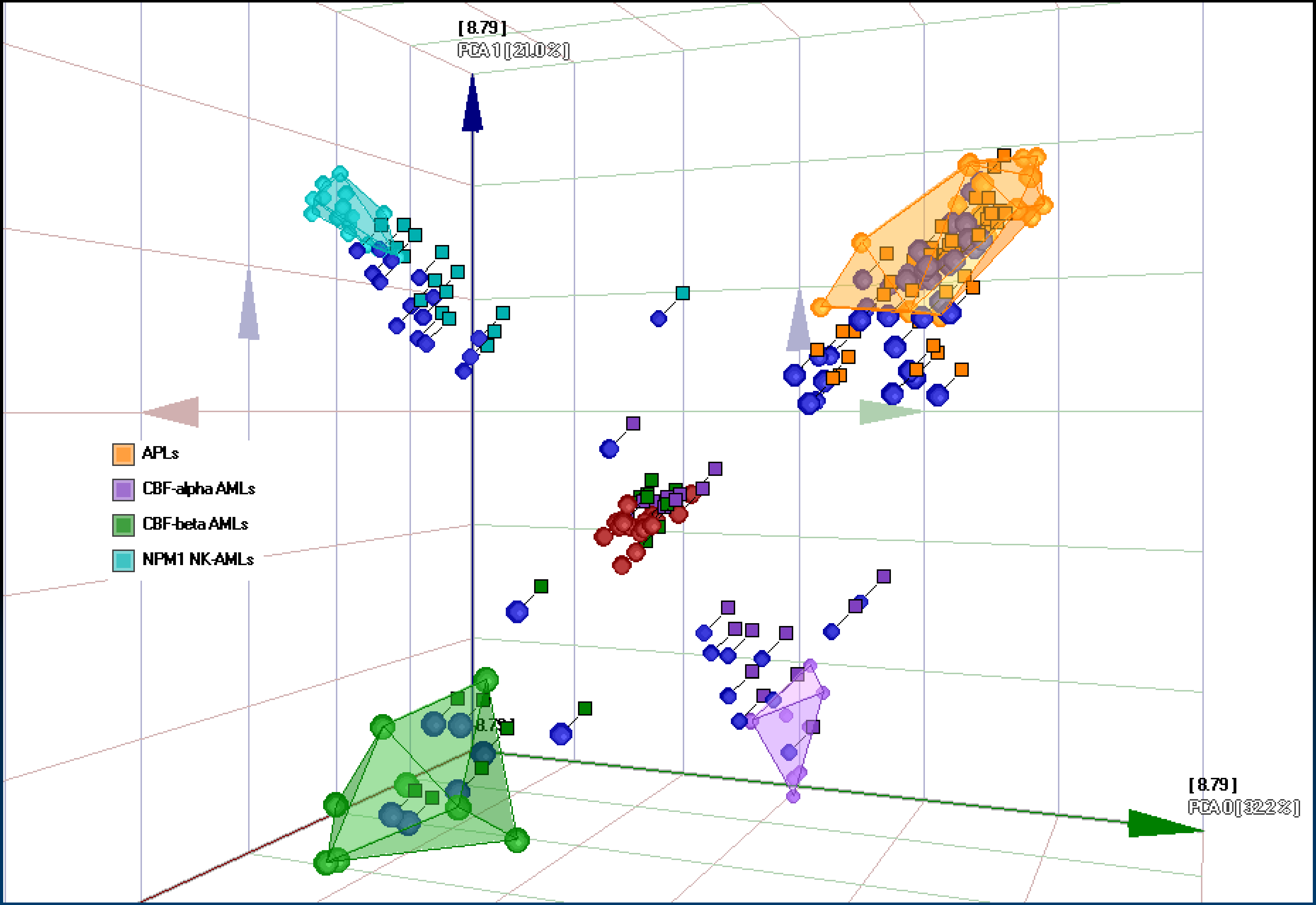
● APL

● CBFA

● NPM1 NK

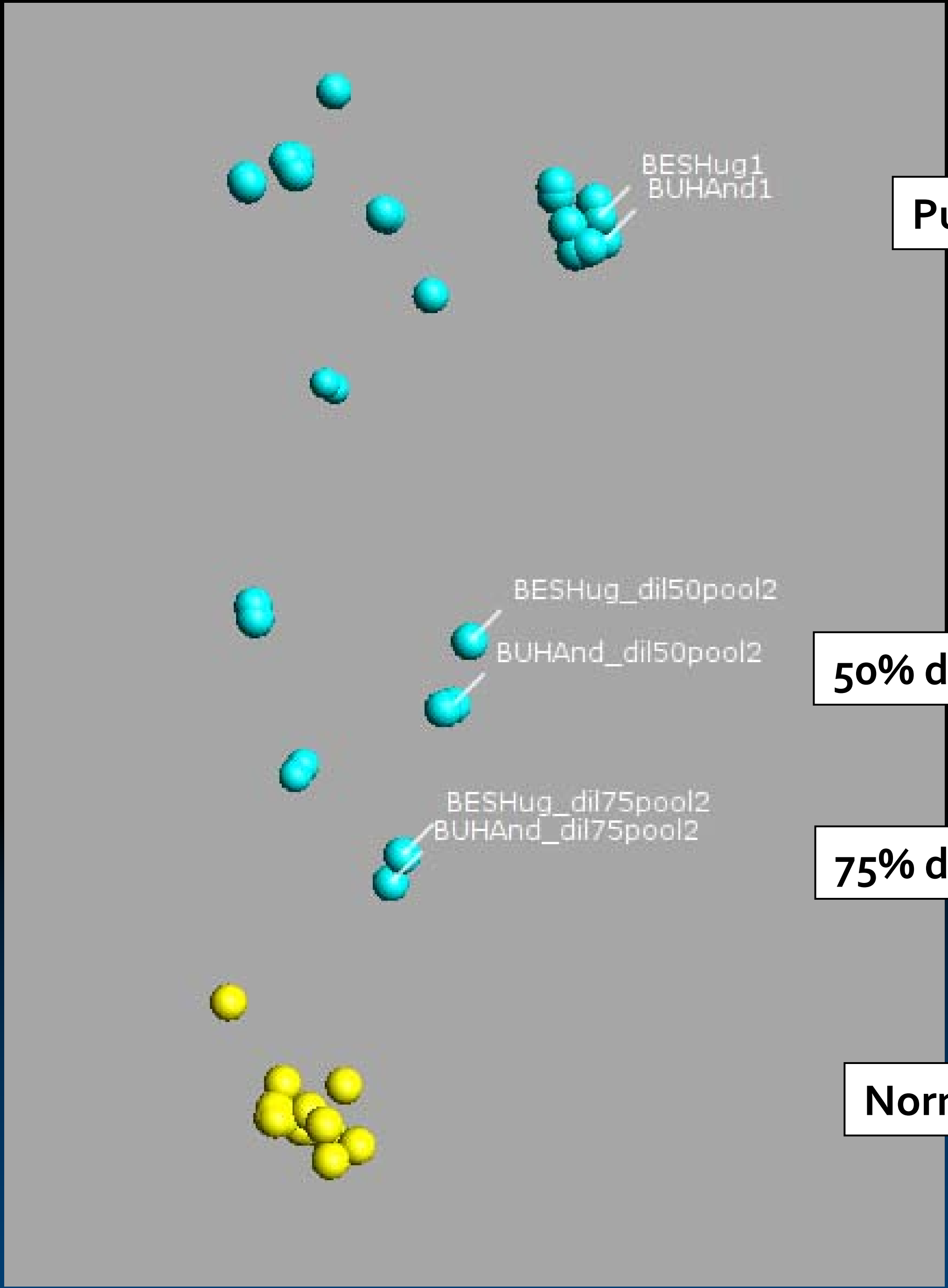
● M3-variants




[8.79]
PCA 1 [21.0%]
[8.79]
PCA 0 [32.2%]
8.79
APLs
CBF-alpha AMLs
CBF-beta AMLs
NPM1 NK-AMLs

● M3-variants

● Normals

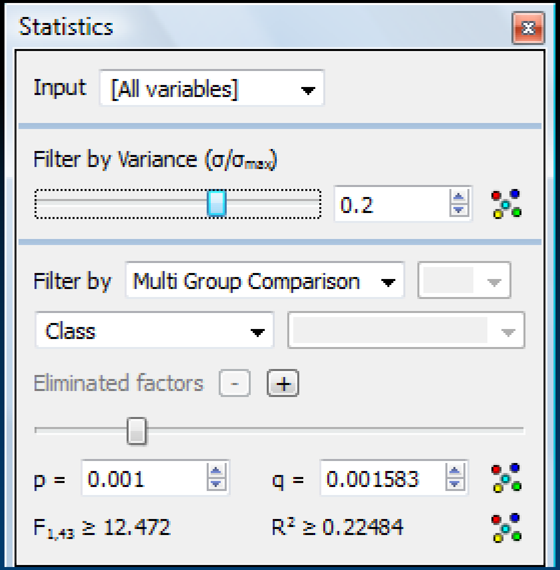


Pure

50% diluted

75% diluted

Normals


Statistics

Input [All variables]

Filter by Variance (σ/σ_{max}) 0.2

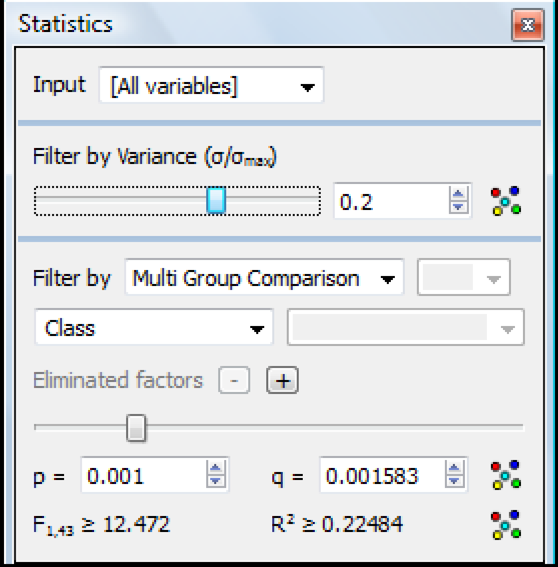
Filter by Multi Group Comparison

Class

Eliminated factors - +

p = 0.001 q = 0.001583

$F_{1,43} \geq 12.472$ $R^2 \geq 0.22484$



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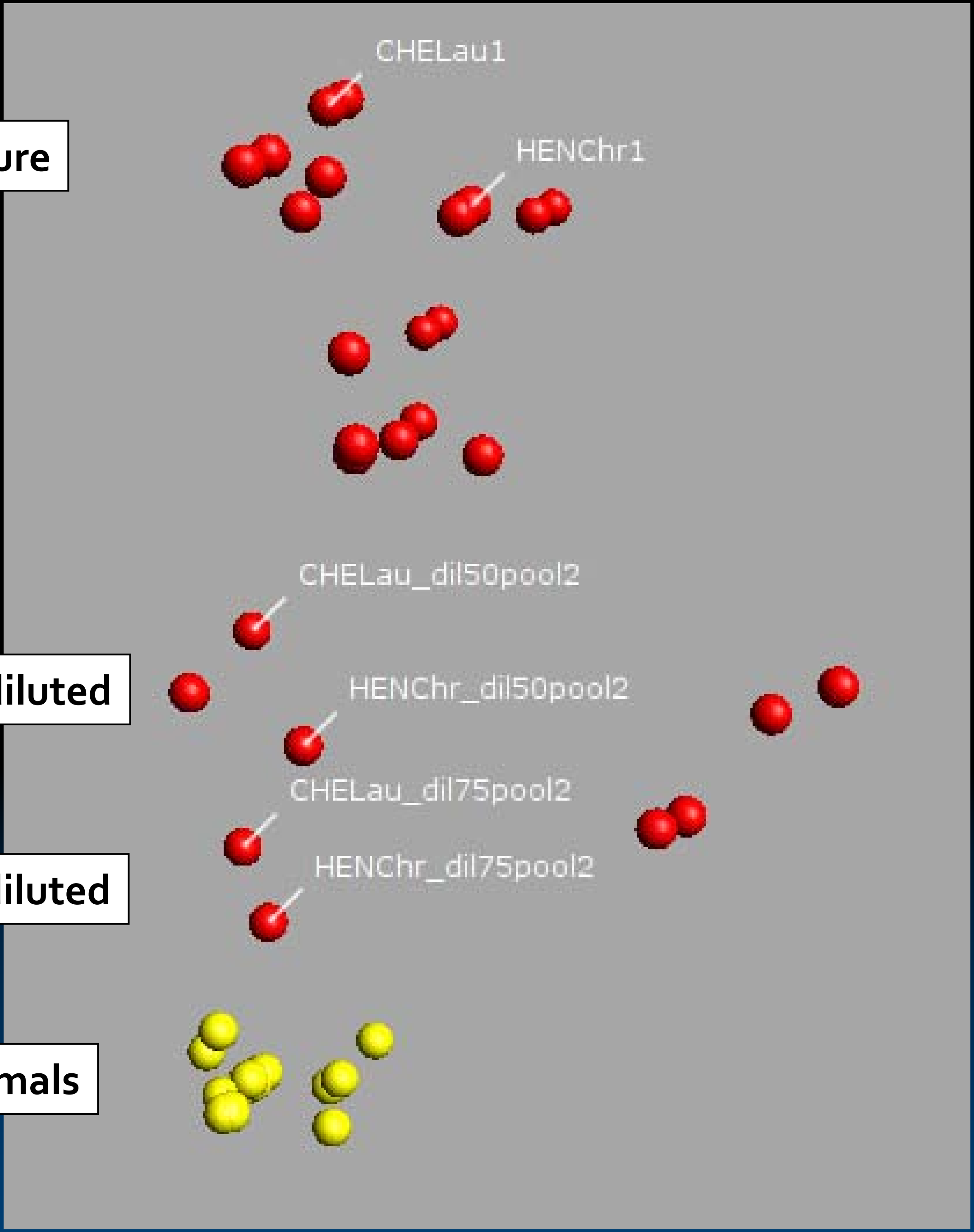


Pure

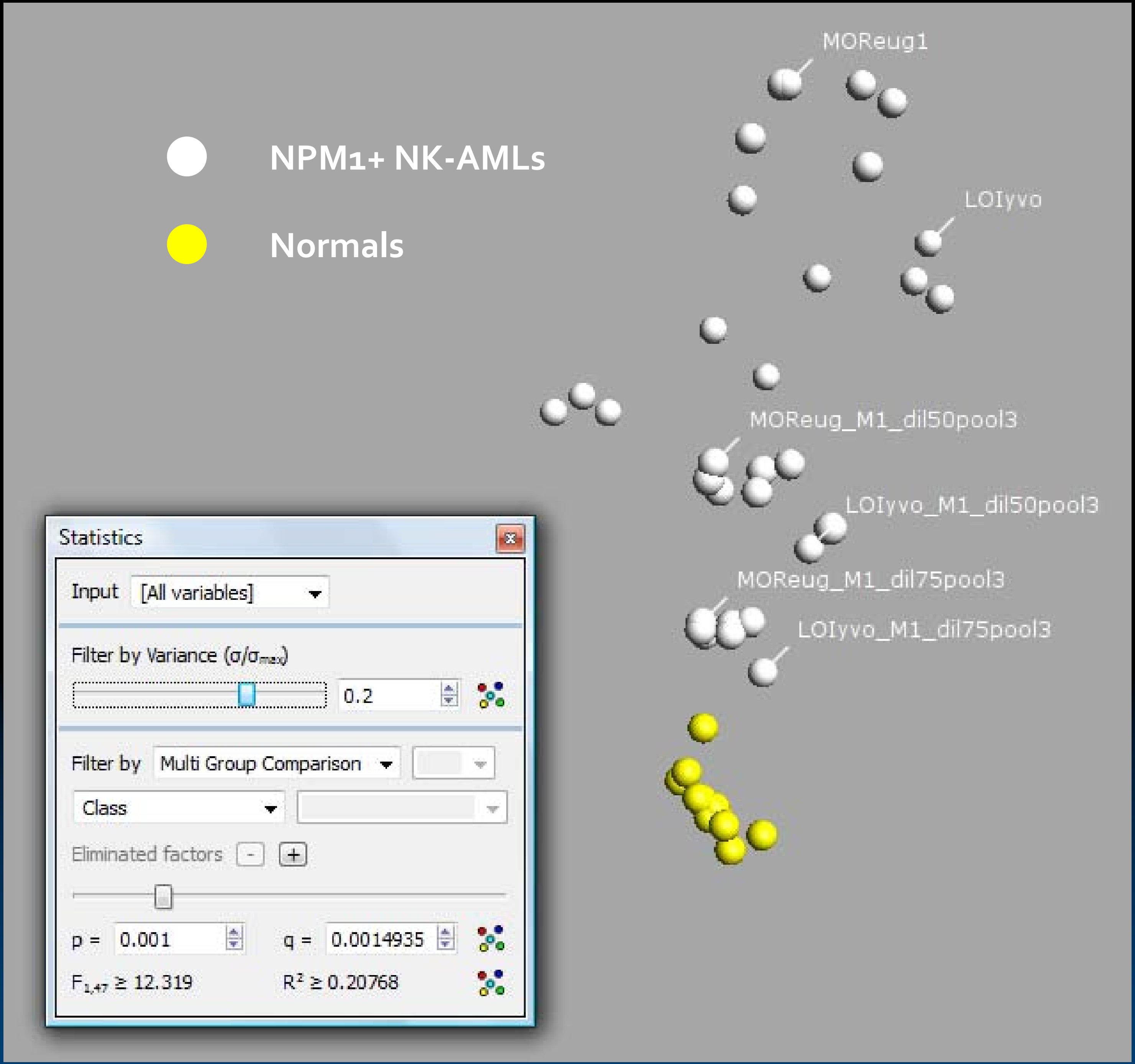
50% diluted

75% diluted

Normals

🔴 **APLs**

🟡 **Normals**



○ NPM1+ NK-AMLs

● Normals

Input [All variables]

Filter by Variance (σ/σ_{max}) 0.2

Filter by Multi Group Comparison

Class

Eliminated factors

$p = 0.001$ $q = 0.0014935$

$F_{1,47} \geq 12.319$ $R^2 \geq 0.20768$

Pure

50% diluted

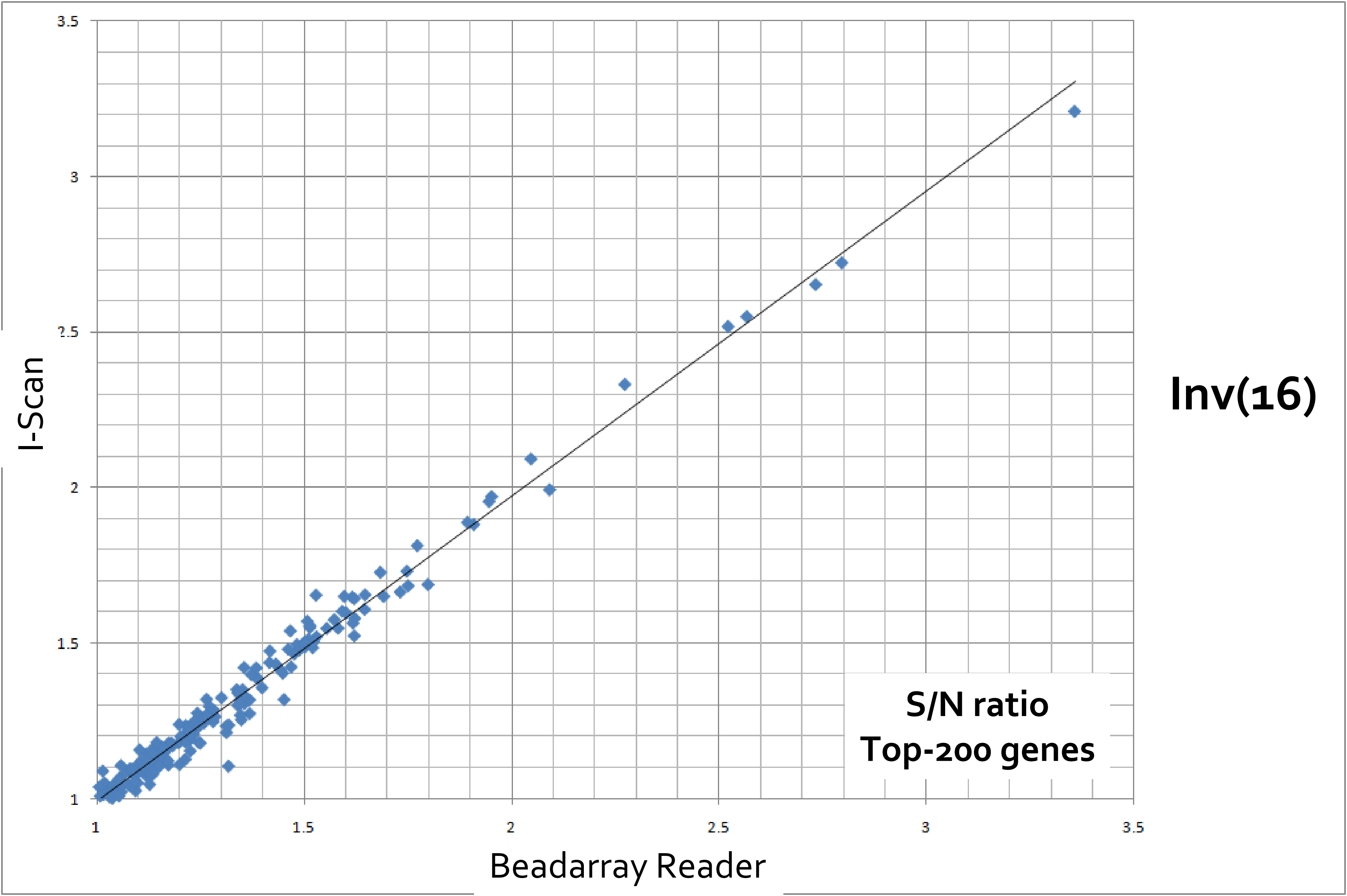
75% diluted

Normals

Gene Expression Profiling

BEADARRAY READER VS. I-SCAN

Beadarray Reader versus I-Scan

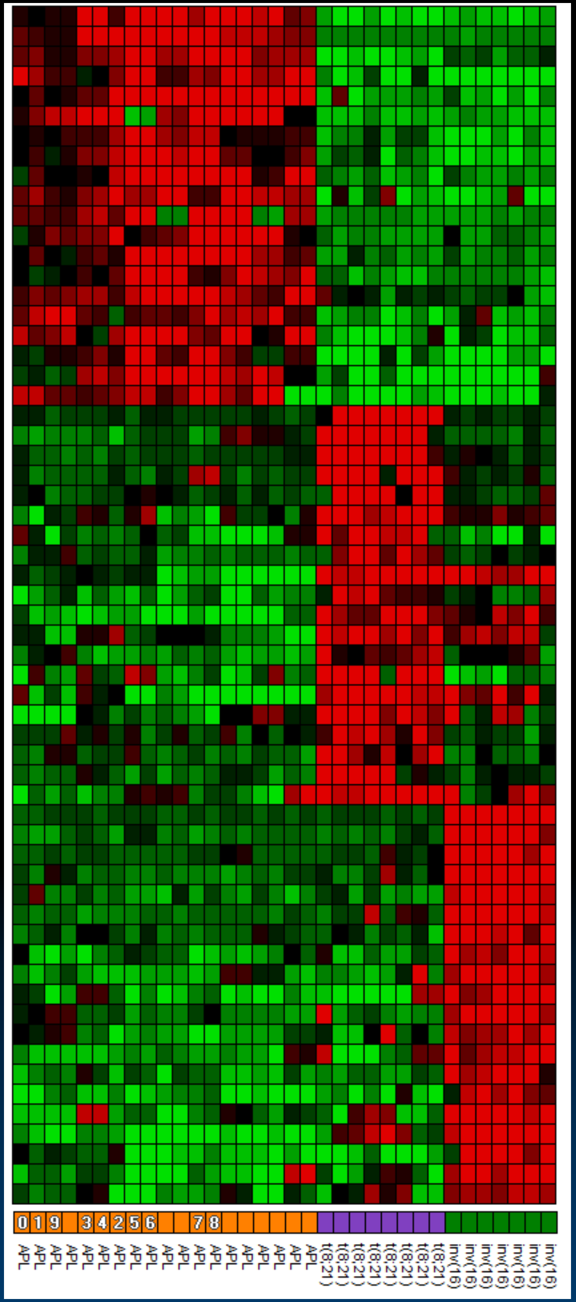


Combined Analysis - 2

GENE PROMOTER METHYLATION & GENE EXPRESSION PROFILING

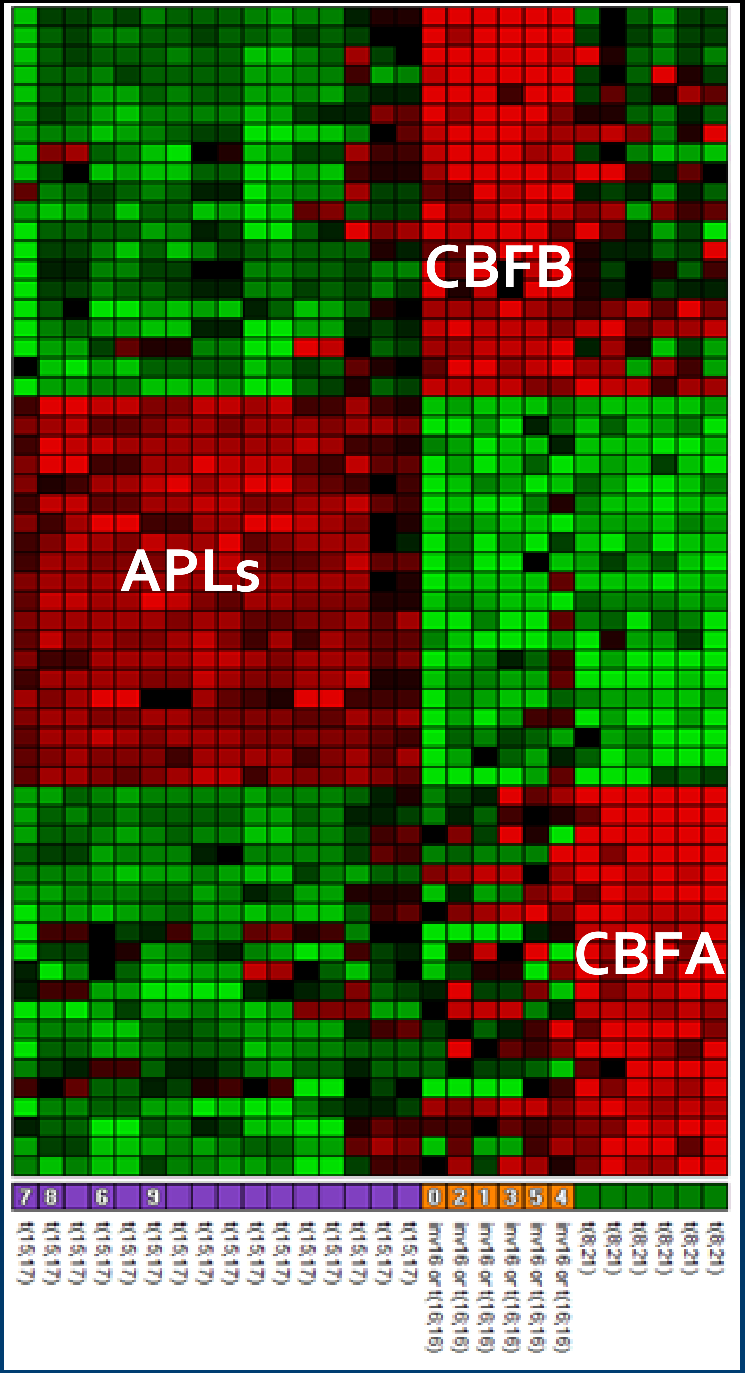
Methylation : HumanMethylation-27

- Targets 27,578 CpG sites for 14,495 genes
- Content based on the NCBI CCDS database (Genome Build 36)
  - More than 1,000 cancer-related genes
  - Promoter regions of more than 110 microRNA genes
- Starting amount: 500 ng of ds-DNA
- Bisulfite reaction per sample: 250 ng of ds-DNA

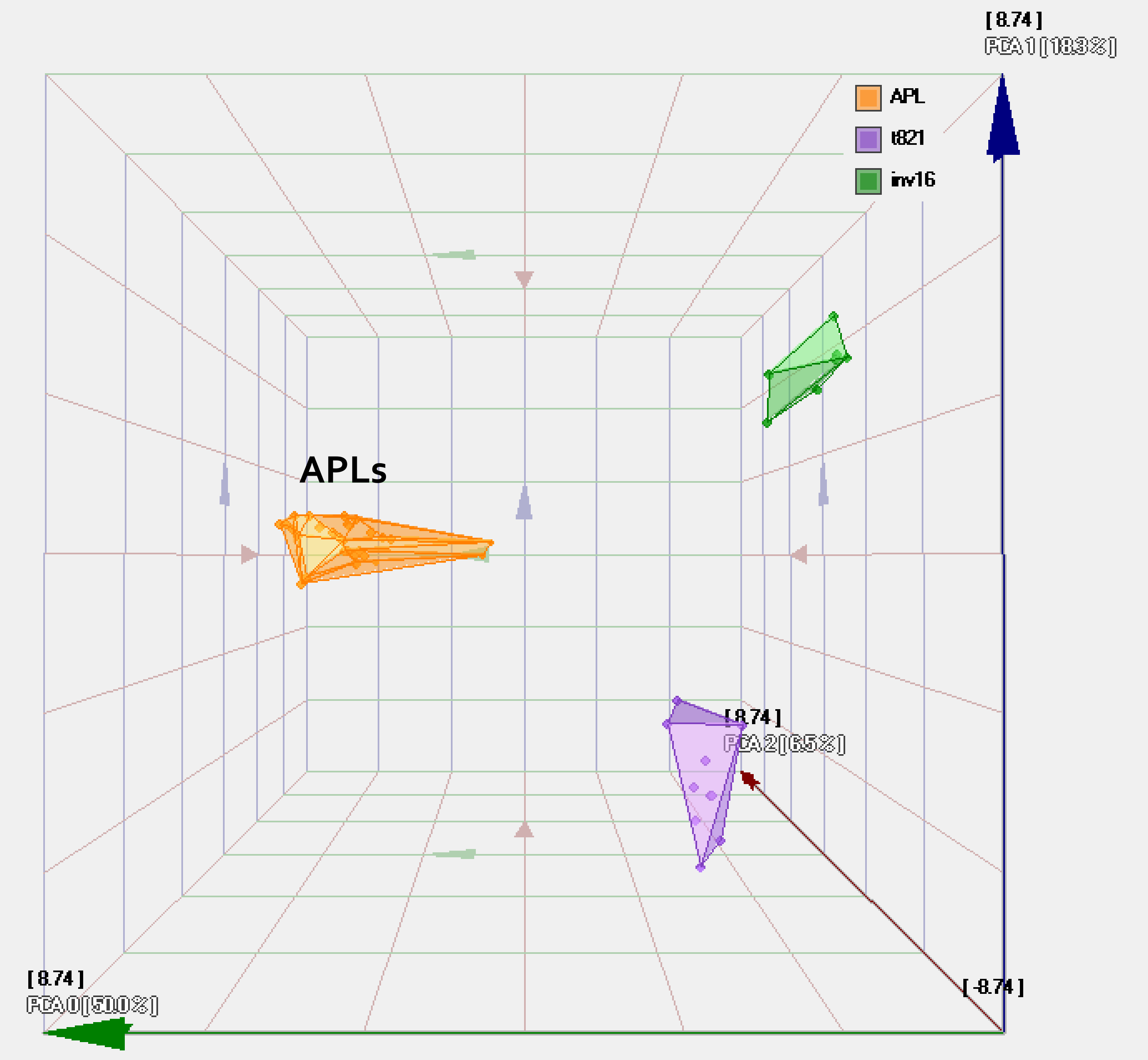


Gene Expression

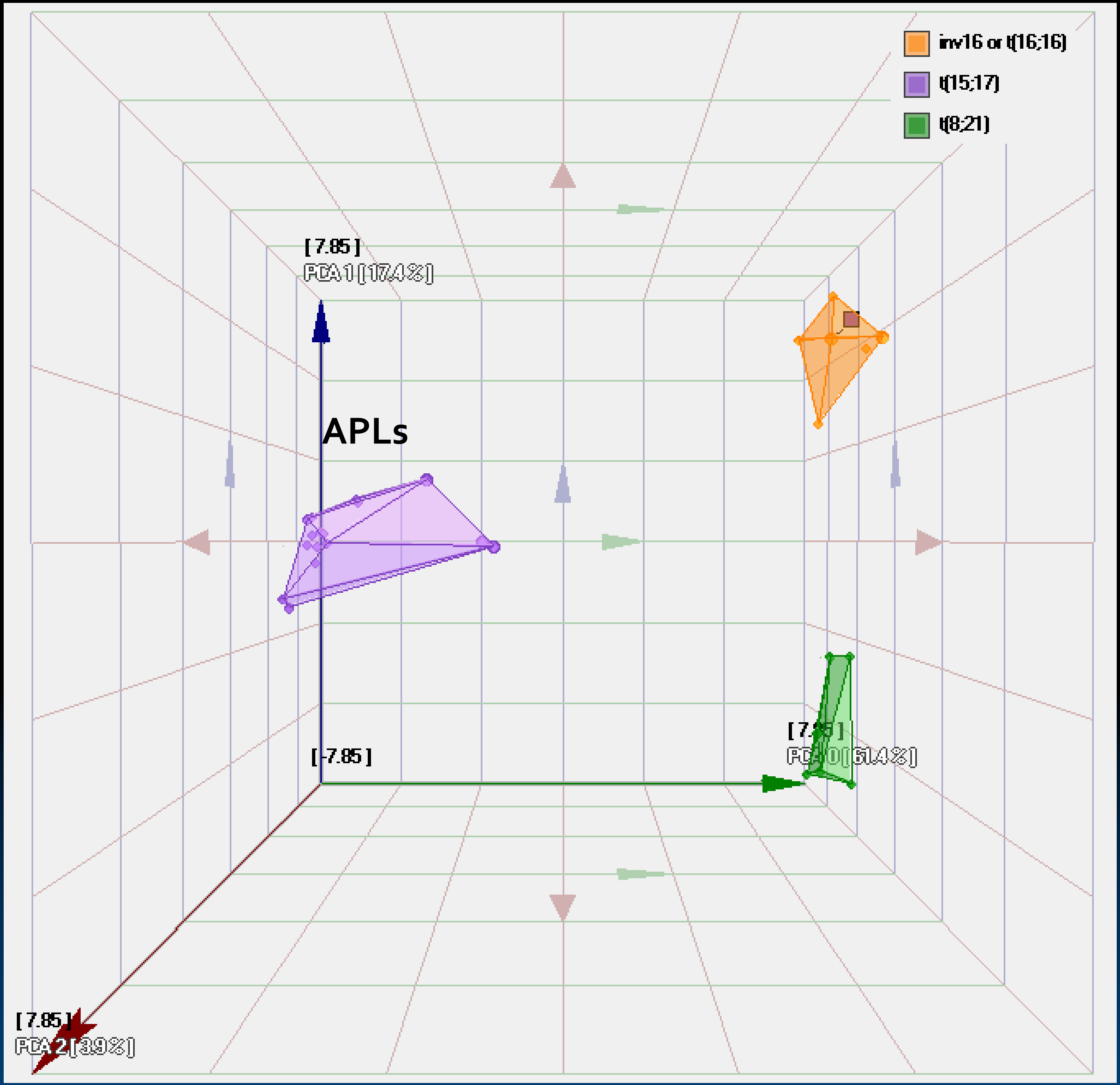
Gene Methylation



GEX
HT-12
Top-20

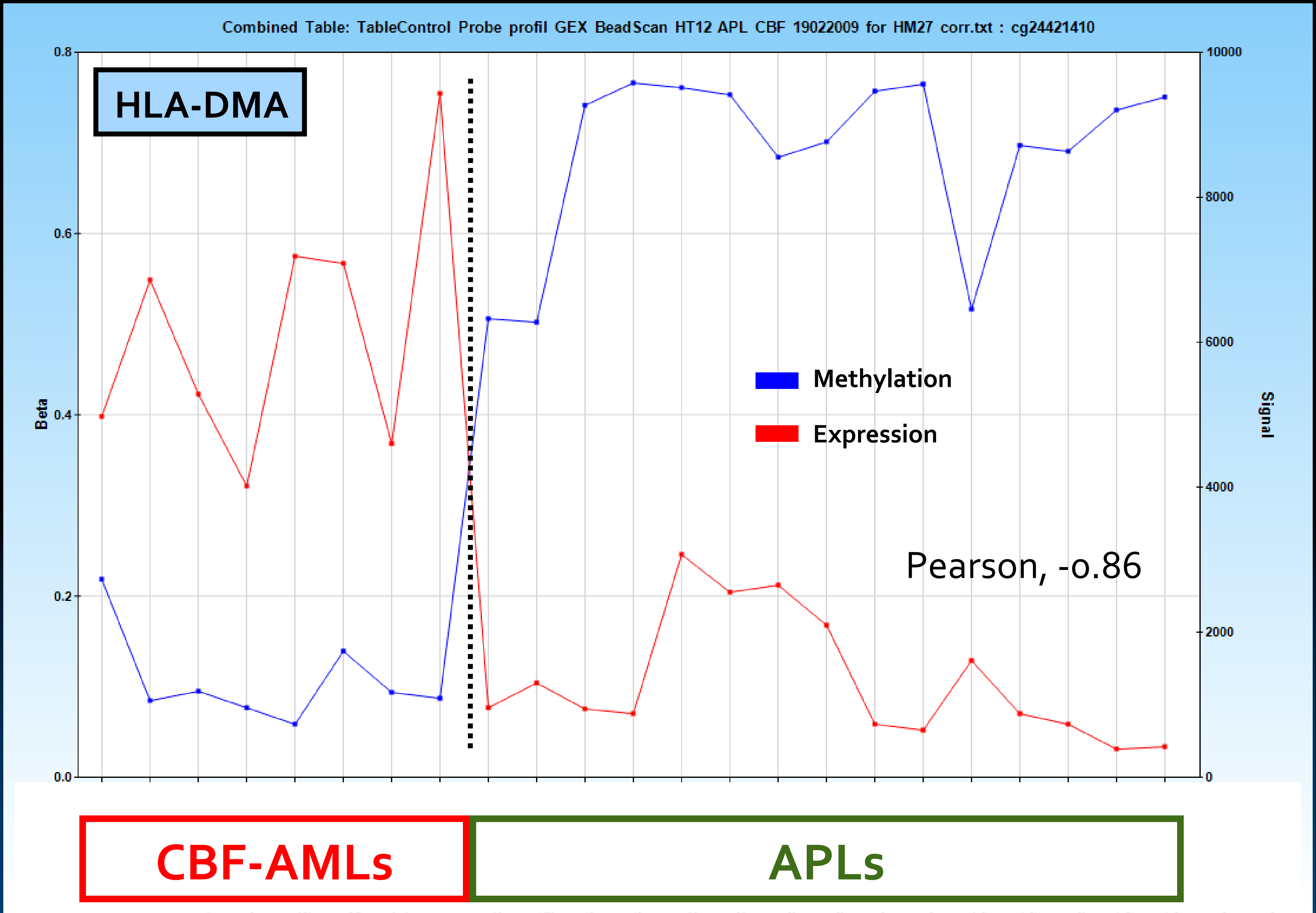


Methyl
HM-27
Top-20

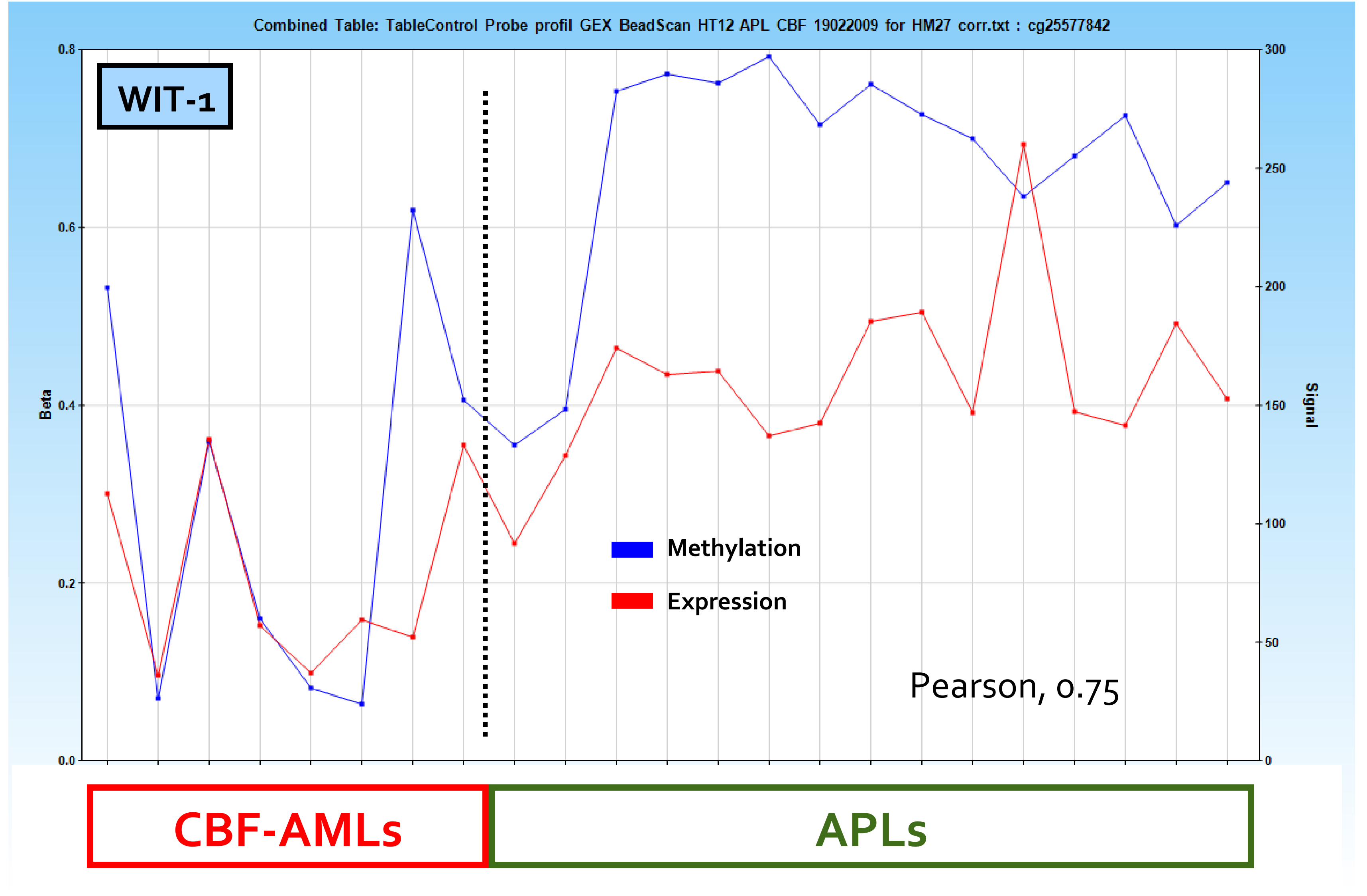


APLs

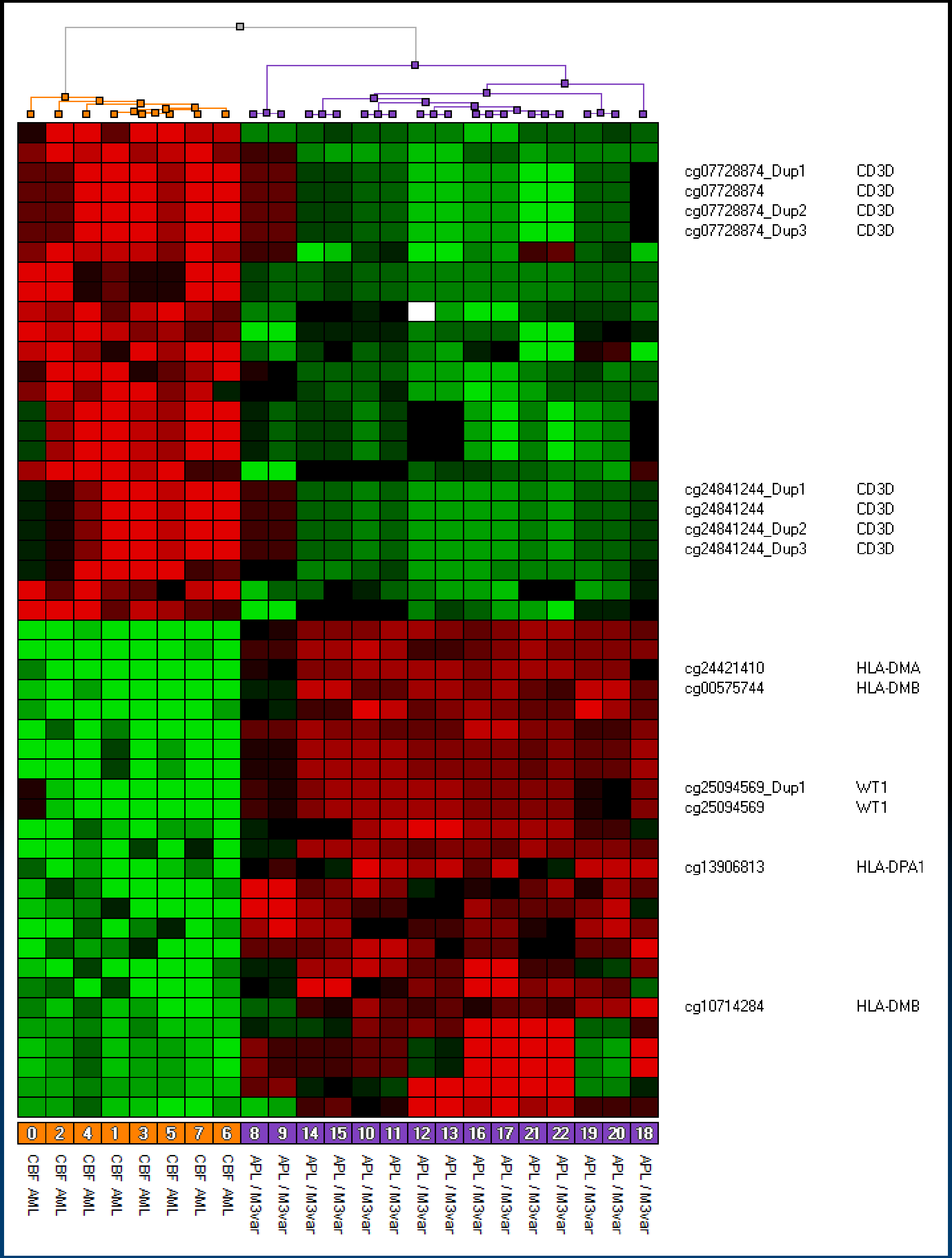
Methylation / Gene Expression Anti-Correlation



Methylation / Gene Expression Correlation



CBF AMLs versus APLs



Correlation ≥ 0.70
or
Anti-Correlation ≤ -0.70
(Pearson or Spearman)

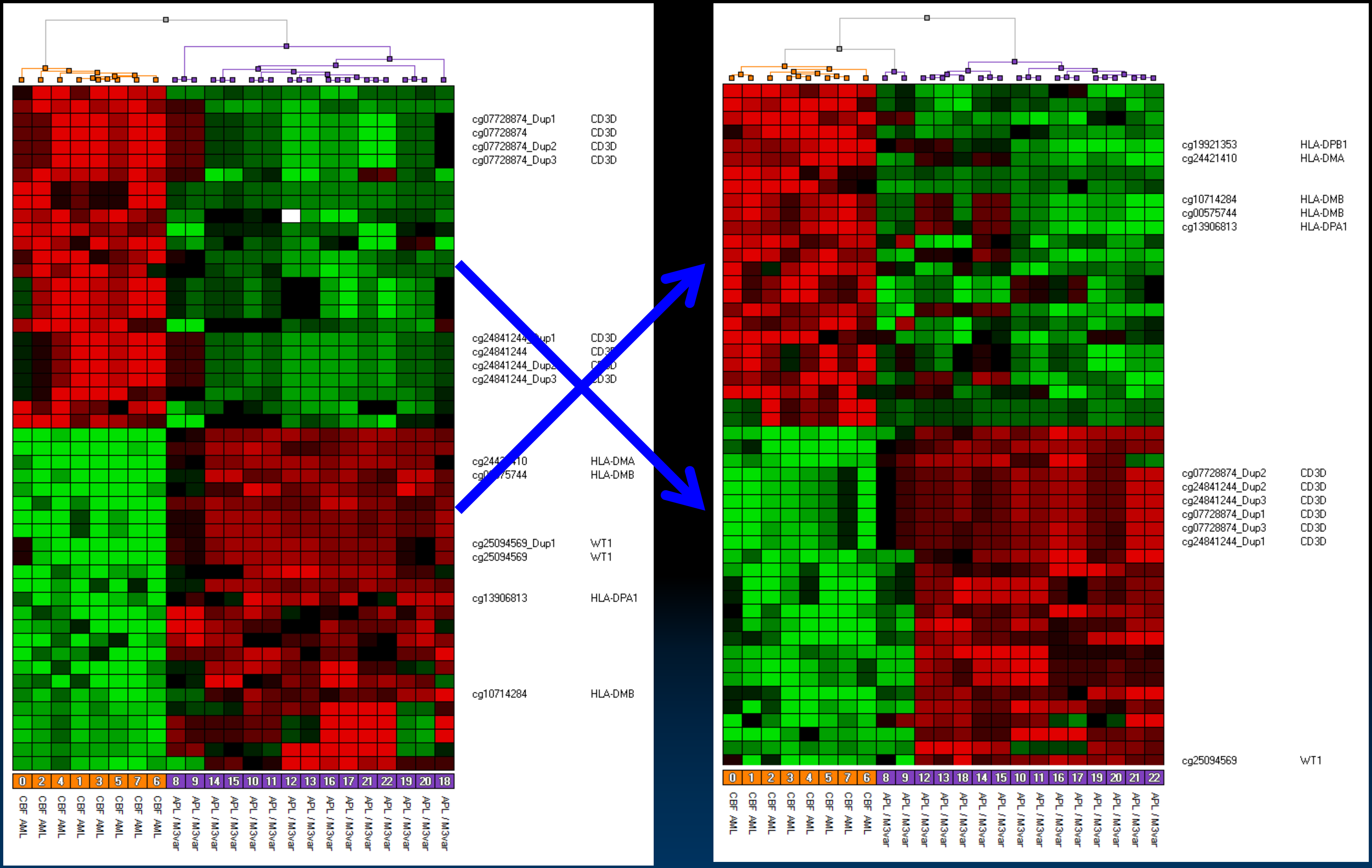
Between
Probes Expression HT12
And
Probes Methylation HM27

N=389

Methylation Data

Methylation Data

Expression Data



CBF | APLs

CBF | APLs

Conclusions

- Reproducibility, stability, sensitivity
  - Inter-technologies: Illumina & Affymetrix

- Efficient class prediction analysis even with low leukemic blast load

  → **Clinical application**

  Has already corrected some diagnoses

Perspectives

- Identify signatures for other AML subtypes

  → Clinical application

  → Identify new prognostic markers (survival analyses involving GEX)

- Combine GEX, methylation and miR data to
  - Improve class prediction analyses
  - **Guide therapeutic choices « a la carte »**

    → Demethylating agents

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- La Région Pays de la Loire
- L’Inter-Région Grand-Ouest

- Illumina France