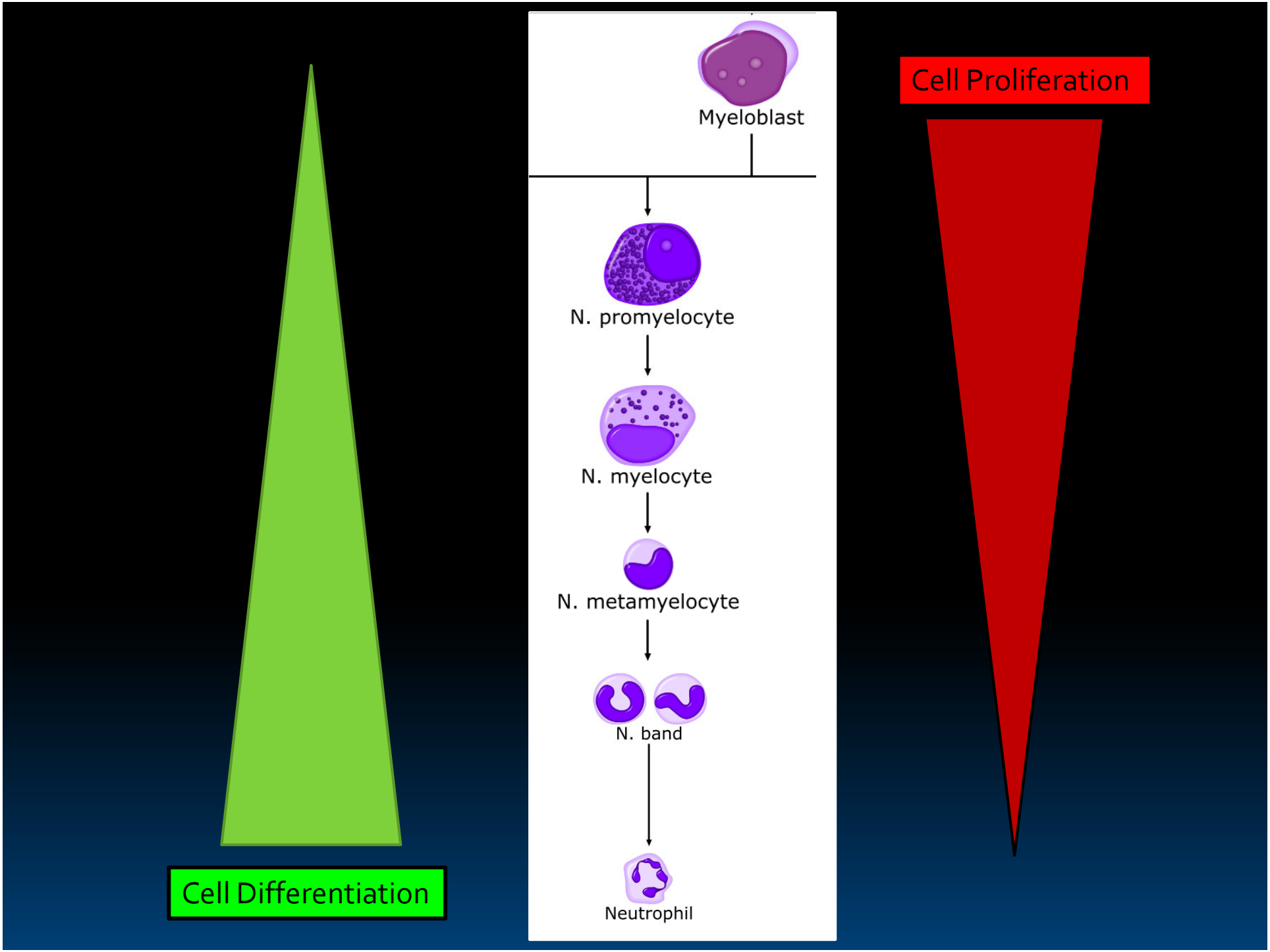
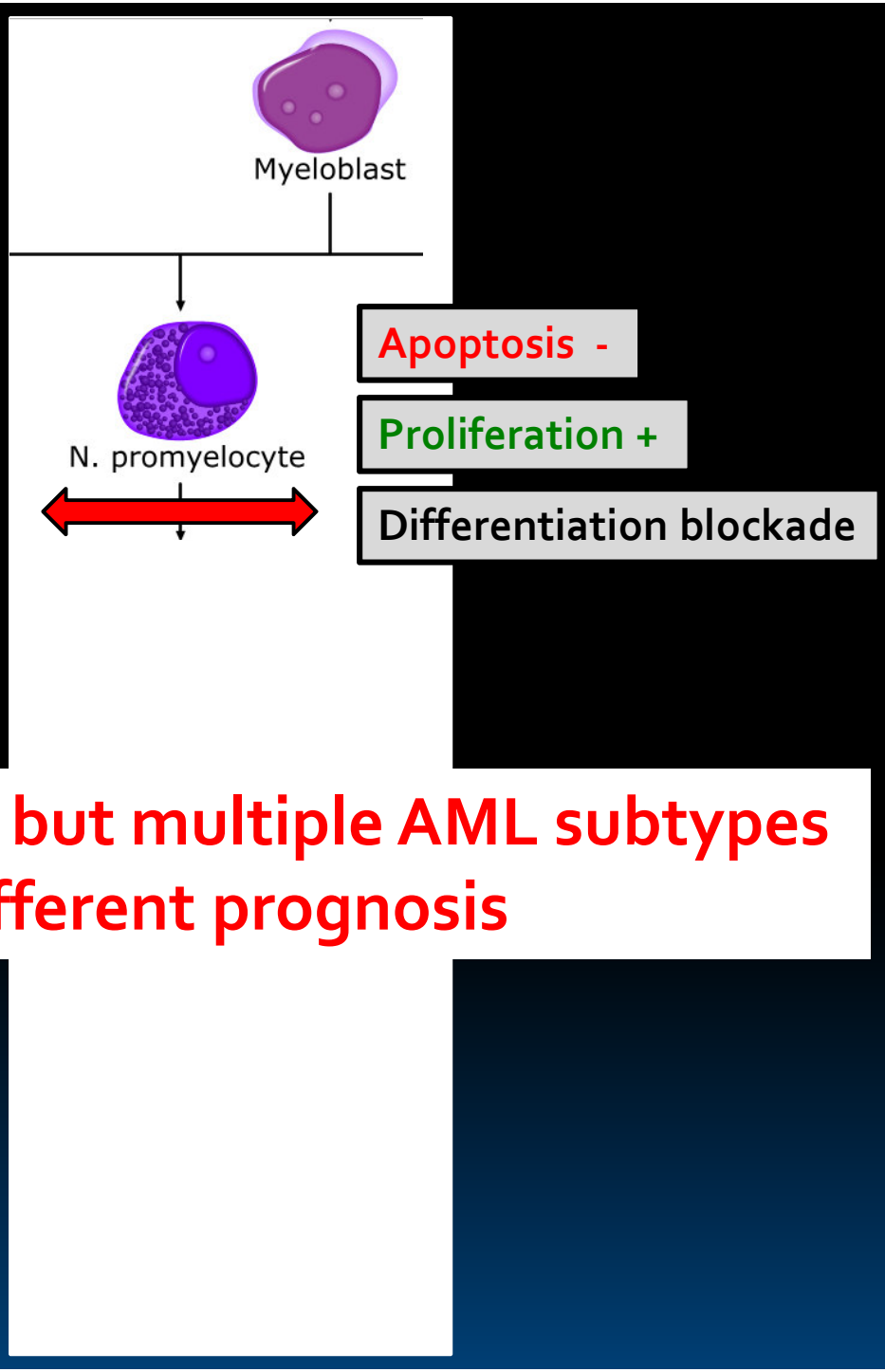
CAN WE USE MICROARRAY-BASED GENE EXPRESSION PROFILING IN A ROUTINE WORKFLOW FOR THE DIAGNOSTIC / PROGNOSTIC OF EVERYDAY PATIENTS WITH ACUTE MYELOID LEUKAEMIA?

Dr Philippe Guardiola (Phguardiol@aol.com)
Plateforme SNP, Transcriptome & Epigénomique
Service des Maladies du Sang
Centre Hospitalier Universitaire
Angers - France


Cell Proliferation
Myeloblast
N. promyelocyte
N. myelocyte
N. metamyelocyte
N. band
Neutrophil
Cell Differentiation

In AMLs…

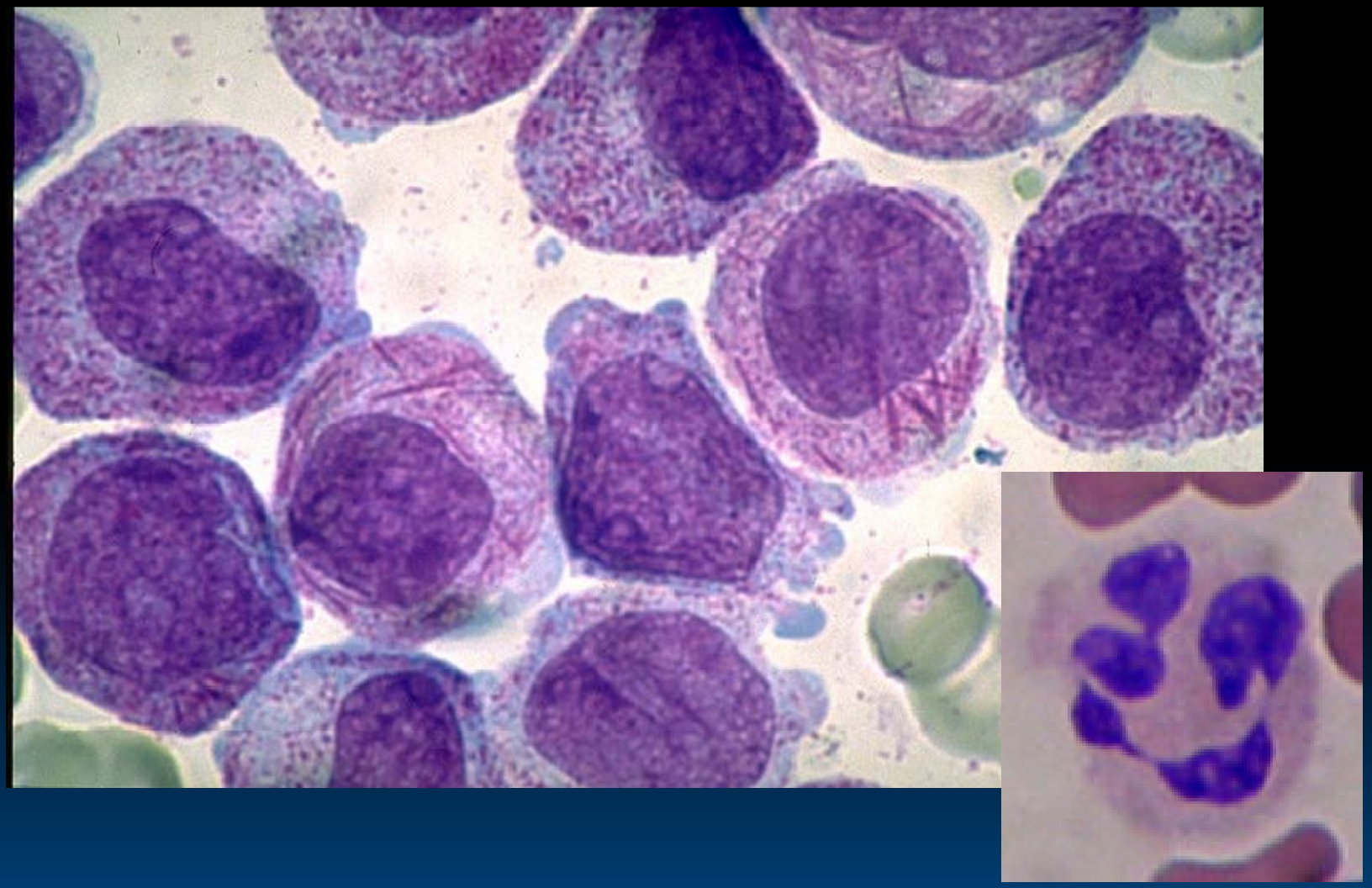


Not one AML type but multiple AML subtypes with different prognosis

Cytological Abnormalities

FAB Classification: M0 to M7

M3 : promyelocytes + Auer rods



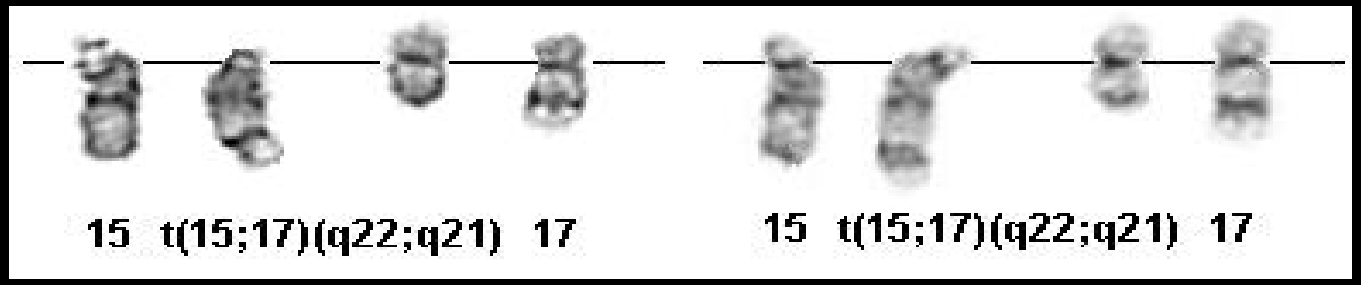
Cytological Abnormalities

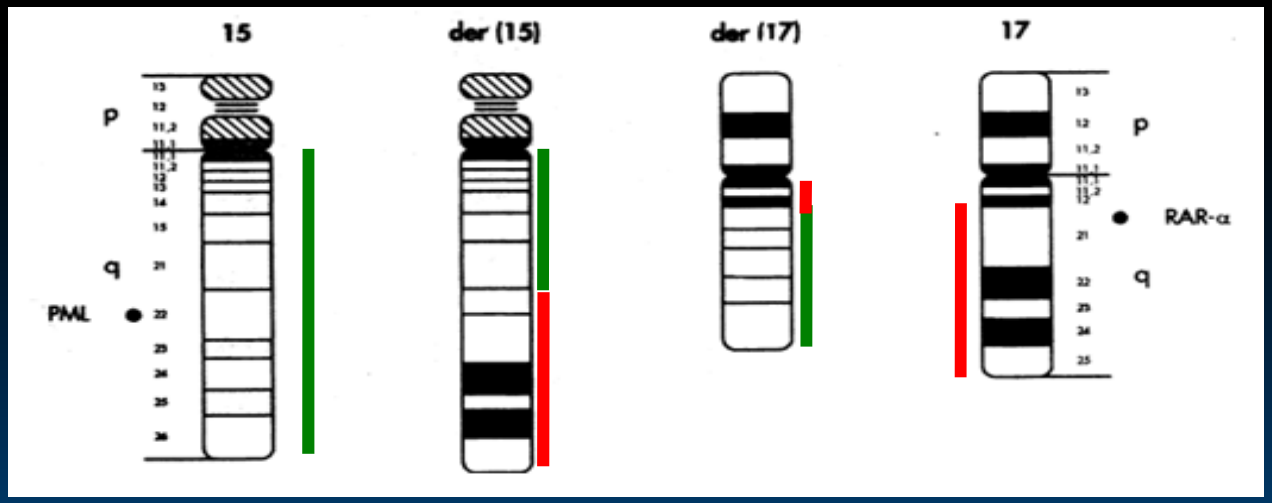
FAB Classification: **M3 promyelocytes + Auer rods**

Cytogenetic Abnormalities

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

Qualitatives: **t(15;17)**, inv(16), t(8;21), …





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), …

Molecular Abnormalities

PML-RARA CBFB-MYH11 AML1-ETO

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), …

Molecular Abnormalities

PML-RARA	CBFB-MYH11	AML1-ETO
FLT3-ITD/TKD	MLL-PTD	NPM1
CEBPA	KIT	RAS
JAK2	WT1	RUNX1

Cytological Abnormalities

FAB Classification: **M3 promyelocytes + Auer rods**

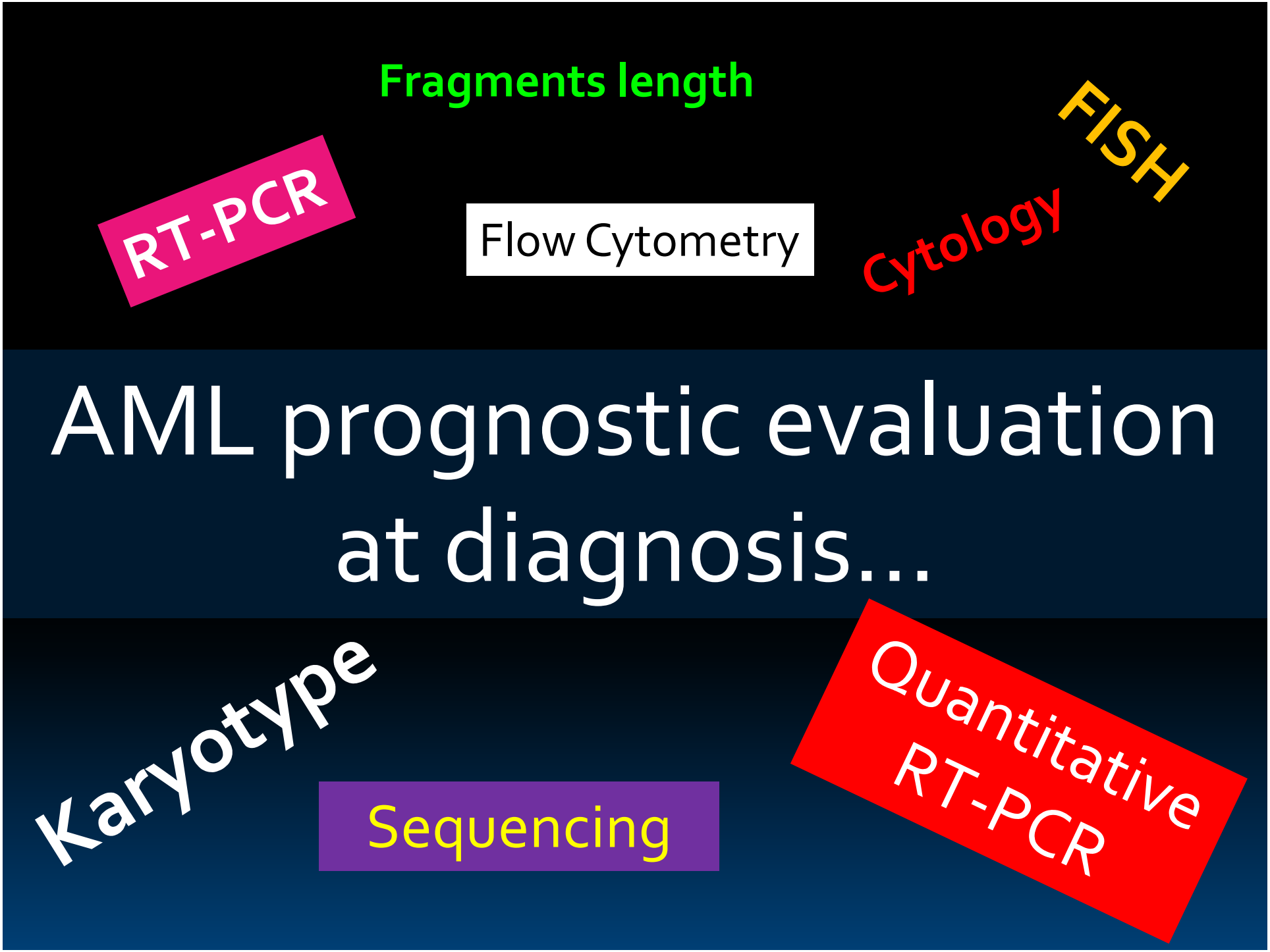
Cytogenetic Abnormalities

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

Qualitatives: **t(15;17)**, inv(16), t(8;21), …

Molecular Abnormalities

PML-RARA	CBFB-MYH11	AML1-ETO
FLT3-ITD/TKD	MLL-PTD	NPM1
CEBPA	KIT	RAS
JAK2	WT1	RUNX1
EVI-1	ERG	FLT3
BAALC	MN1	PRAME…


Fragments length
FISH
RT-PCR
Flow Cytometry
Cytology
AML prognostic evaluation at diagnosis…
Karyotype
Quantitative RT-PCR
Sequencing

HOW EFFICIENT ARE WE TO DIAGNOSE AMLS WITH FAVORABLE-RISK CYTOGENETICS USING GEX PROFILING ?

The issue…

- Most reported studies used AML samples containing at least 60% of leukemic blasts for class prediction analyses… usually > 80% blasts
- Definition of acute leukemia: Blasts ≥ 20%
- How do classifiers behave with everyday « real life » samples :
  - **With low blast contents: 20% to 60% ?**
  - **With poor quality control criteria ?**

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

AMLs with Favorable-risk cytogenetics

- **APLs**
  - Reciprocal balanced translocation t(15;17)
  - Fusion gene PML-RARA
- **CBFA-AMLs**
  - Reciprocal balanced translocation t(8;21)
  - Fusion gene RUNX1-RUNX1T1 / AML1-ETO
- **CBFB-AMLs**
  - Inversion inv(16) or translocation t(16;16)
  - Fusion gene CBFB-MYH11

Class Prediction Analysis

- **APLs vs. CBFA-AMLs vs. CBFB-AMLs vs. NK-AMLs**

  Angers & Reims University Hospitals

  - **APLs (n=40 samples from 20 patients)**
  - **CBFA-AMLs (n=27 samples from 11 patients)**
  - **CBFB-AMLs (n=40 from 24 patients)**
  - **NK-AMLs (n=58 samples from 31 patients)**
  - **Normal Bone Marrows (N=18 samples from 9 patients)**

  Total: 95 patients – 183 samples

- **Data from GEO (Affymetrix)**
  - **Verhaak et al - Haematologica 2009**

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

Methods

- **Genome Studio 2010.1 - Gene Expression Module 1.6.0**
  - Processing of the signal (Probe Level Analysis)
  - Invariant Set Normalization
- **ArrayMiner 5.3.3 (Optimal Design - Belgium)**
  - Class prediction analysis based on Grouping Genetic Algorithms
  - Log-transformed data
  - Filter of the data
    - Threshold maximum: 50,000
    - Fold change > 1.50
    - Absolute change > 150
- **Omics Explorer 2.1 (Qlucore - Sweden)**
  - 3-D dynamic PCA

BUILDING OF THE CLASSIFIERS
TRAINING SET (BLASTS ≥ 60%)

APLs	20 samples from 14 patients
CBFA-AMLs	12 samples from 6 patients
CBFB-AMLs	11 from 10 patients
NK-AMLs	29 samples from 28 patients
Normal BM	18 samples (9 healthy donors + 9 pools)

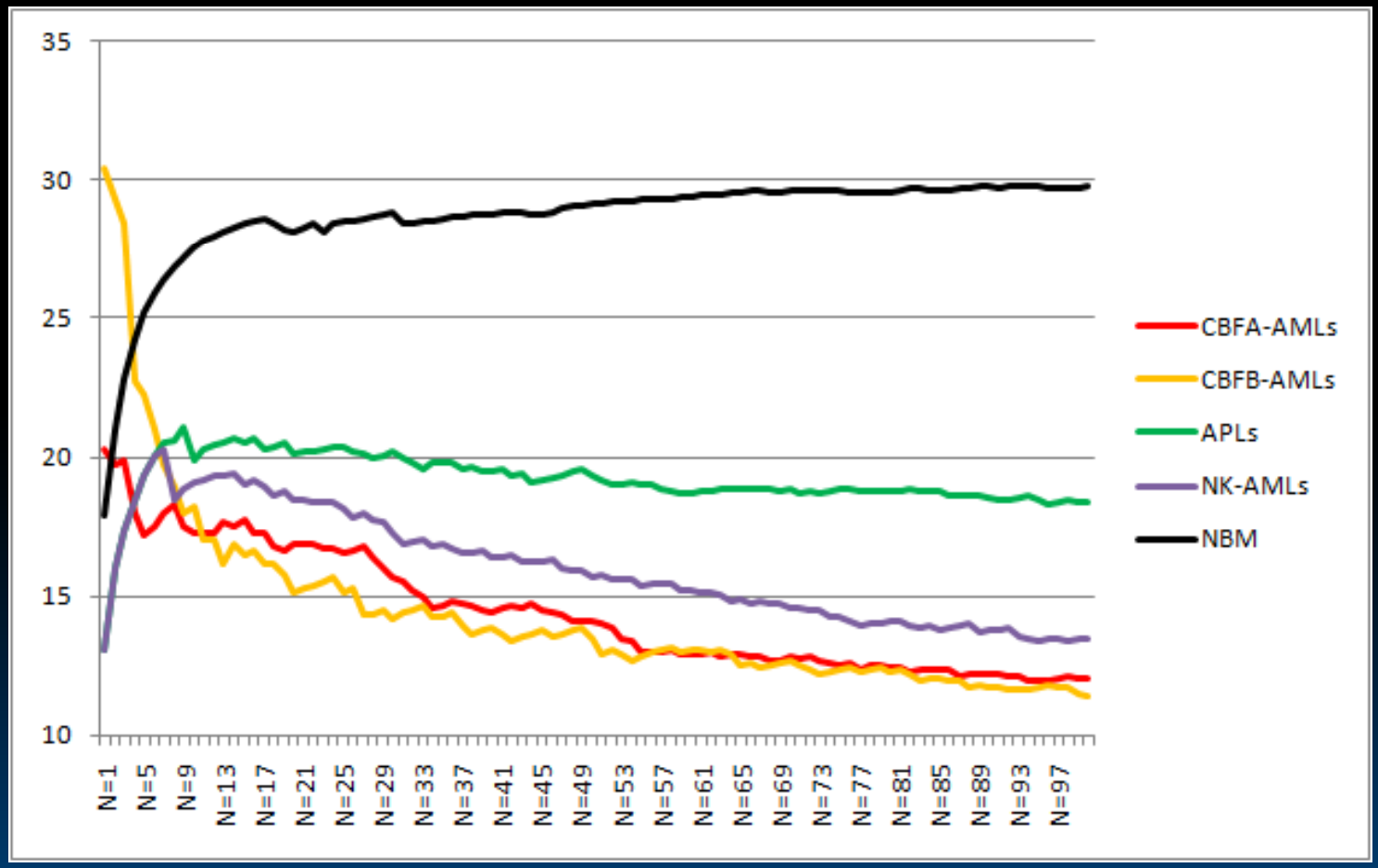
Training Set: 58+9 patients – 90 samples

How many markers per class ?

With the Training Set samples,
the best fitness of the model
was achieved
with 14 markers per class
Error rate, 0%

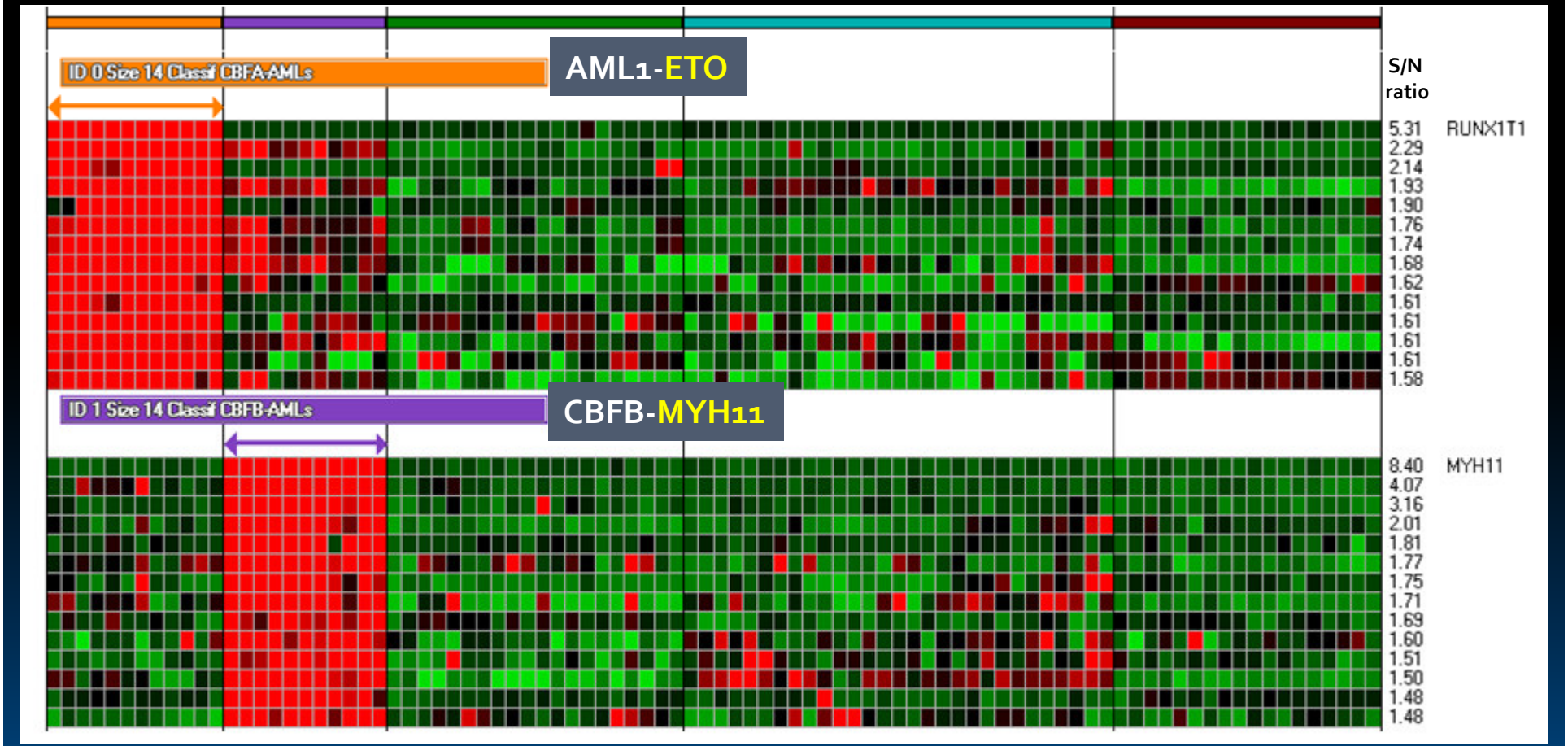
Median confidence level of sample assignment per class

from 1 to 100 markers per class



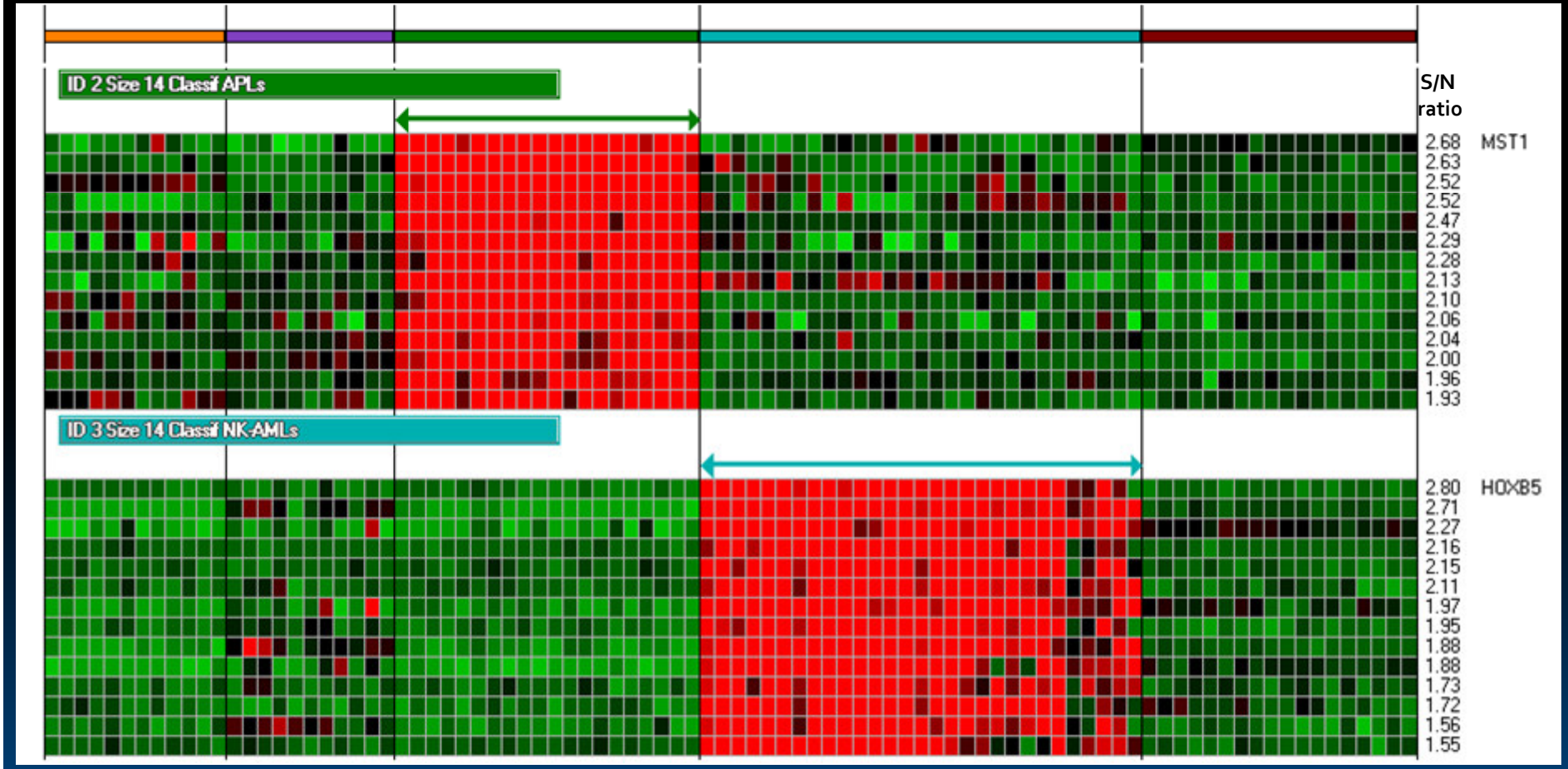
14-markers classifiers
CBFA and CBFB-AMLs

CBFA AMLs | CBFB AMLs | APLs | NK-AMLs | Normal BM



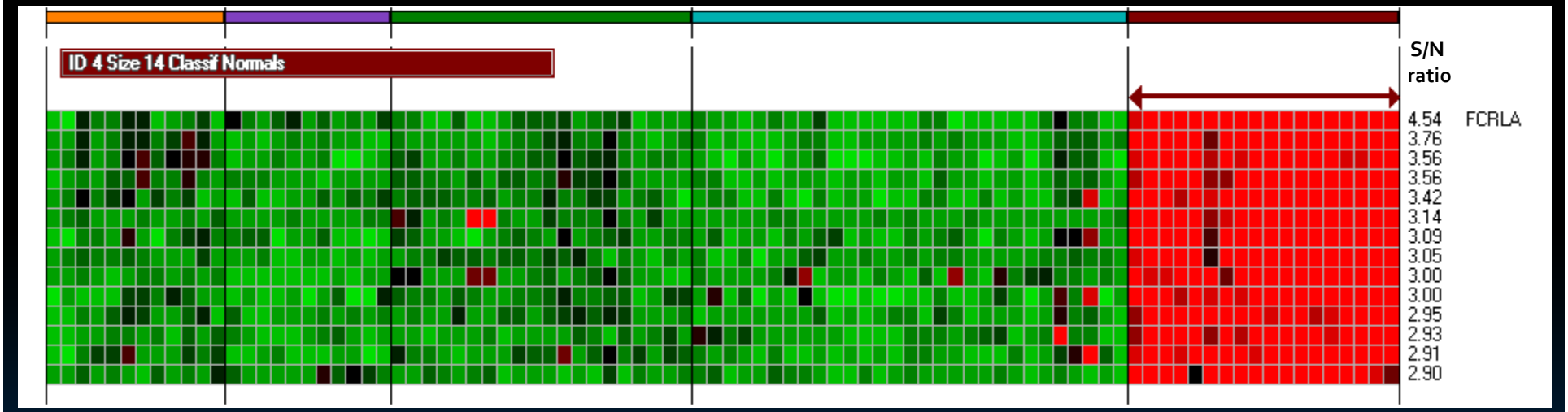
14-markers classifiers APLs and NK-AMLs

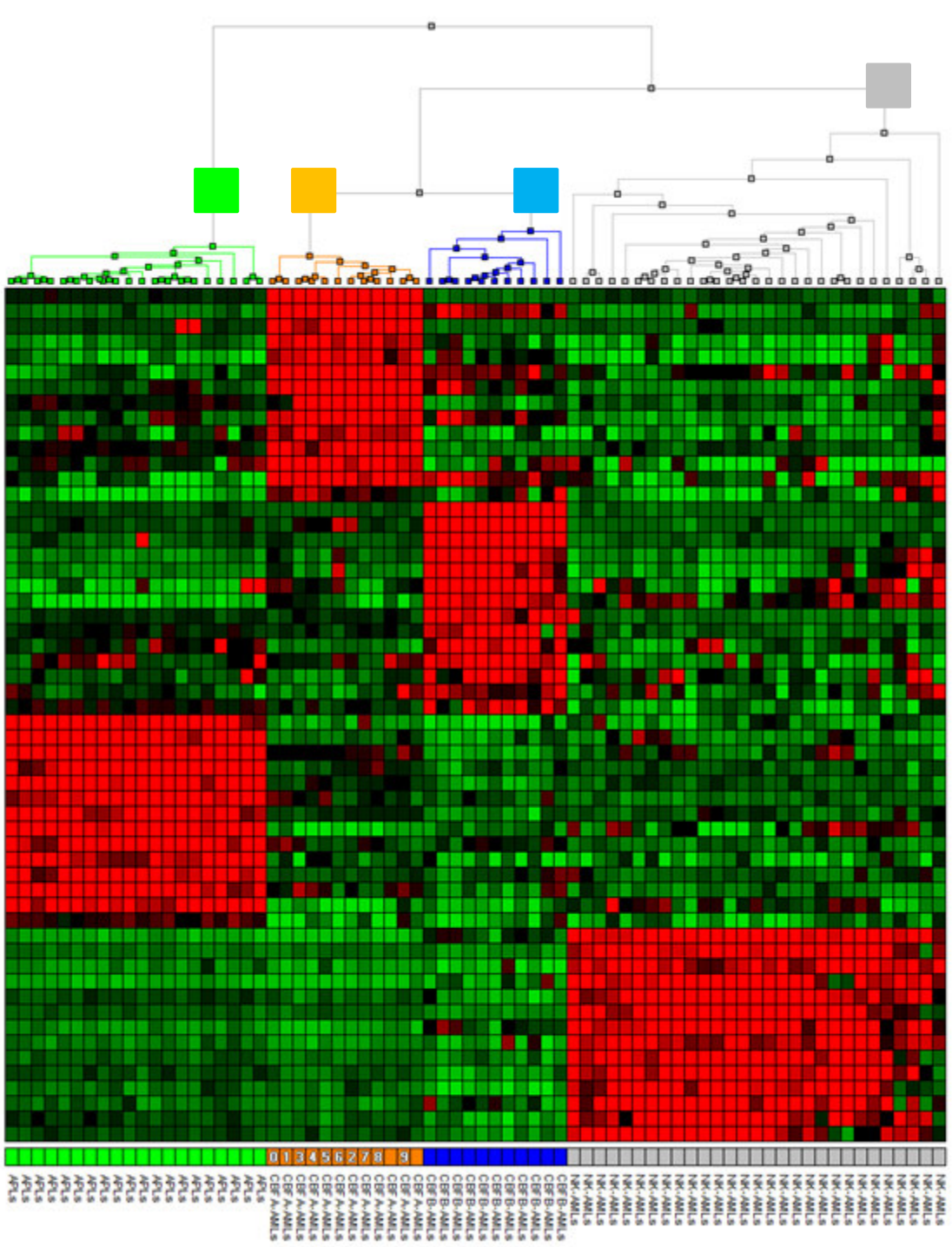
CBFA AMLs | CBFB AMLs | APLs | NK-AMLs | Normal BM



14-markers classifiers Normal Bone Marrows

CBFA AMLs | CBFB AMLs | APLs | NK-AMLs | Normal BM





CBFA-AMLs

CBFB-AMLs

APLs

NK-AMLs

CLASS PREDICTION ANALYSIS ON LOW LEUKEMIC BLAST LOAD SAMPLES - TEST SET #1

Samples with a low blast load: 5 - 59%

At diagnosis or at relapse - N=22 samples

Samples with high blast load but diluted at 50% and 75% **in a pool of 9 normal bone marrow samples - N=57 samples**

Start with 80 - 100% blasts → 20 - 25% blasts

Class Prediction - Results

Low Blast Load – APLs

Nr.	Experiment name	Class	Assigned Class	Blast%	Dilution
1	UPN77_PB_undiluted_Blasts 7%	APLs	APLs	7	No
2	UPN41_PB_undiluted_Blasts 11%	APLs	APLs	11	No
3	UPN41_PB_undiluted_dup_Blasts 11%	APLs	APLs	11	No
4	UPN2_PB_undiluted_Blasts 15%	APLs	APLs	15	No
5	UPN43_PB_dilution 50%_Blasts 27%	APLs	APLs	27	50%
6	UPN34_BM_dilution 50%_Blasts 33%	APLs	APLs	33	50%
7	UPN57_BM_dilution 50%_Blasts 39%	APLs	APLs	39	50%
8	UPN16_BM_dilution 50%_Blasts 40%	APLs	APLs	40	50%
9	UPN46_BM_dilution 50%_Blasts 43%	APLs	APLs	43	50%
10	UPN43_PB_dilution 75%_Blasts 14%	APLs	APLs	14	75%
11	UPN34_BM_dilution 75%_Blasts 16%	APLs	APLs	16	75%
12	UPN16_BM_dilution 75%_Blasts 20%	APLs	APLs	20	75%
13	UPN57_BM_dilution 75%_Blasts 20%	APLs	APLs	20	75%
14	UPN46_BM_dilution 75%_Blasts 22%	APLs	APLs	22	75%

Error Rate 0% - Lower limit: 7% blasts

Class Prediction - Results
Low Blast Load – CBFA-AMLs

Nr.	Experiment name	Class	Assigned Class	Blast%	Dilution
1	UPN84_PB_undiluted_Blasts 16%	CBFA-AMLs	CBFA-AMLs	16	No
2	UPN15_BM_undiluted_Blasts 21%	CBFA-AMLs	CBFA-AMLs	21	No
3	UPN20_BM_undiluted_Blasts 27%	CBFA-AMLs	CBFA-AMLs	27	No
4	UPN68_PB_undiluted_Blasts 31%	CBFA-AMLs	CBFA-AMLs	31	No
5	UPN54_BM_dilution 50%_Blasts 24%	CBFA-AMLs	CBFA-AMLs	24	50%
6	UPN61_BM_dilution 50%_Blasts 30%	CBFA-AMLs	CBFA-AMLs	30	50%
7	UPN44_BM_relapse_dilution 50%_Blasts 38%	CBFA-AMLs	CBFA-AMLs	38	50%
8	UPN61_PB_dilution 50%_Blasts 41%	CBFA-AMLs	CBFA-AMLs	41	50%
9	UPN24_PB_dilution 50%_Blasts 49%	CBFA-AMLs	CBFA-AMLs	49	50%
10	UPN61_BM_dilution 75%_Blasts 15%	CBFA-AMLs	CBFA-AMLs	15	75%
11	UPN44_BM_relapse_dilution 75%_Blasts 19%	CBFA-AMLs	CBFA-AMLs	19	75%
12	UPN61_PB_dilution 75%_Blasts 21%	CBFA-AMLs	CBFA-AMLs	21	75%
13	UPN24_PB_dilution 75%_Blasts 25%	CBFA-AMLs	CBFA-AMLs	25	75%

Error Rate 0% - Lower limit: 15% blasts

Class Prediction - Results

Low Blast Load – CBFB-AMLs

Nr.	Experiment name	Class	Assigned Class	Blast%	Dilution
1	UPN4_BM_relapse_undiluted_Blasts 5%	CBFB-AMLs	APLs	5	No
2	UPN30_PB_relapse_undiluted_Blasts 14%	CBFB-AMLs	CBFB-AMLs	14	No
3	UPN29_PB_undiluted_Blasts 23%	CBFB-AMLs	CBFB-AMLs	23	No
4	UPN59_PB_undiluted_Blasts 24%	CBFB-AMLs	CBFB-AMLs	24	No
5	UPN36_PB_undiluted_Blasts 31%	CBFB-AMLs	CBFB-AMLs	31	No
6	UPN78_PB_undiluted_Blasts 35%	CBFB-AMLs	CBFB-AMLs	35	No
7	UPN6_BM_undiluted_Blasts 35%	CBFB-AMLs	CBFB-AMLs	35	No
8	UPN30_BM_diagnosis_undiluted_Blasts 39%	CBFB-AMLs	CBFB-AMLs	39	No
9	UPN79_BM_undiluted_Blasts 46%	CBFB-AMLs	CBFB-AMLs	46	No
10	UPN25_BM_undiluted_Blasts 50%	CBFB-AMLs	CBFB-AMLs	50	No
11	UPN45_BM_undiluted_Blasts 56%	CBFB-AMLs	CBFB-AMLs	56	No
12	UPN30_BM_diagnosis_dilution 50%_Blasts 19%	CBFB-AMLs	CBFB-AMLs	19	50%
13	UPN45_BM_dilution 50%_Blasts 28%	CBFB-AMLs	CBFB-AMLs	28	50%
14	UPN45_BM_dilution 50%_Blasts 28%	CBFB-AMLs	CBFB-AMLs	28	50%
15	UPN7_BM_dilution 50%_Blasts 33%	CBFB-AMLs	CBFB-AMLs	33	50%
16	UPN40_BM_dilution 50%_Blasts 41%	CBFB-AMLs	CBFB-AMLs	41	50%
17	UPN38_BM_dilution 50%_Blasts 50%	CBFB-AMLs	CBFB-AMLs	50	50%
18	UPN30_BM_diagnosis_dilution 75%_Blasts 10%	CBFB-AMLs	CBFB-AMLs	10	75%
19	UPN45_BM_dilution 75%_Blasts 14%	CBFB-AMLs	CBFB-AMLs	14	75%
20	UPN7_BM_dilution 75%_Blasts 16%	CBFB-AMLs	CBFB-AMLs	16	75%
21	UPN40_BM_dilution 75%_Blasts 20%	CBFB-AMLs	CBFB-AMLs	20	75%
22	UPN38_BM_dilution 75%_Blasts 25%	CBFB-AMLs	CBFB-AMLs	25	75%
23	UPN40_PB_dilution 75%_Blasts 25%	CBFB-AMLs	CBFB-AMLs	25	75%

Error Rate 4% - Lower limit: 10% blasts

Class Prediction - Results

Low Blast Load – NK-AMLs

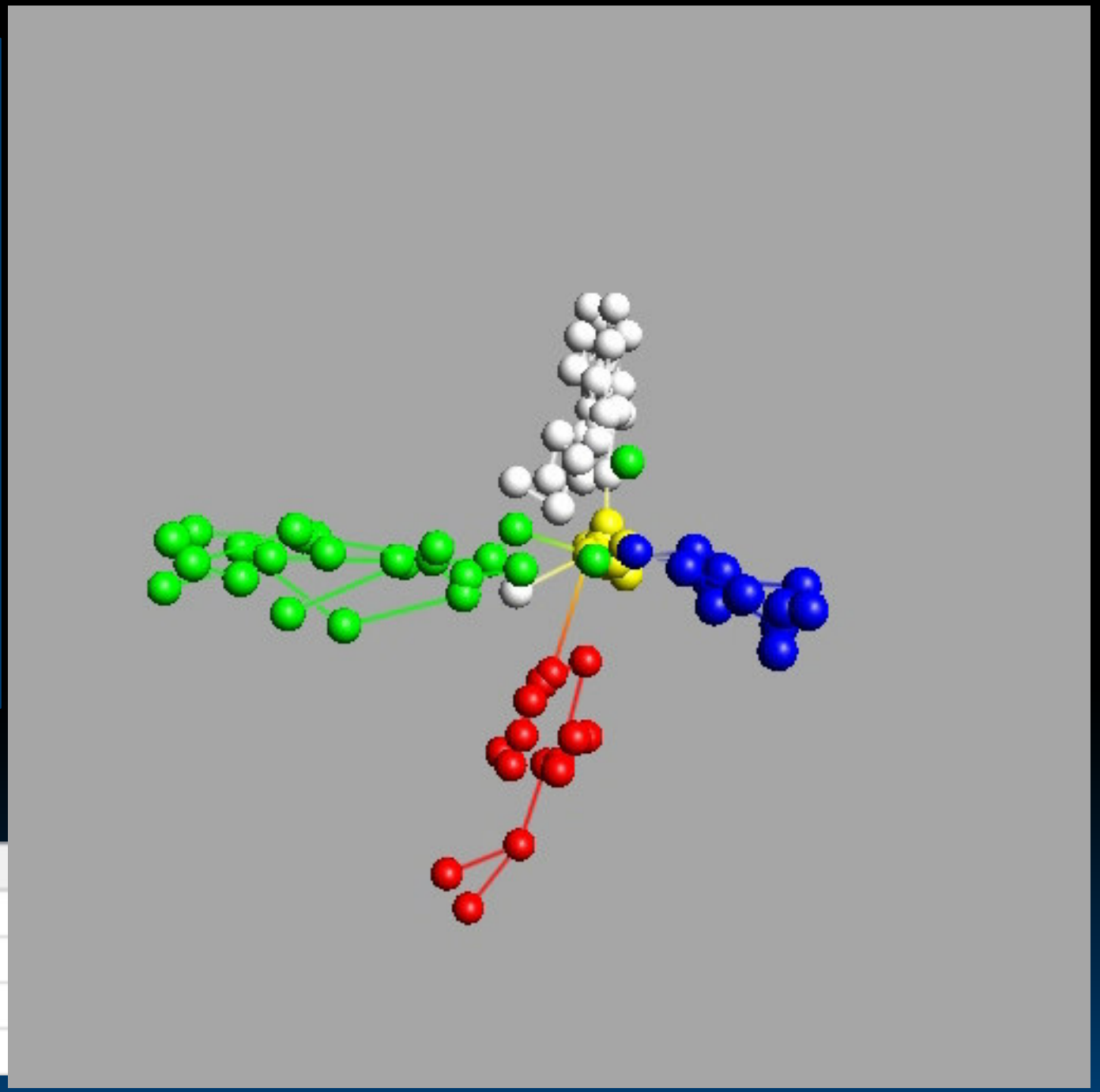
Nr.	Experiment name	Class	Assigned Class	Blast%	Dilution
1	UPN85_PB_undiluted_Blasts 35%	NK-AMLs	NK-AMLs	35	No
2	UPN13_BM_undiluted_Blasts 39%	NK-AMLs	NK-AMLs	39	No
3	UPN53_PB_undiluted_Blasts 50%	NK-AMLs	CBFA-AMLs	50	No
4	UPN8_BM_dilution 50%_Blasts 37%	NK-AMLs	NK-AMLs	37	50%
5	UPN31_BM_dilution 50%_Blasts 40%	NK-AMLs	NK-AMLs	40	50%
6	UPN80_BM_dilution 50%_Blasts 40%	NK-AMLs	NK-AMLs	40	50%
7	UPN66_BM_dilution 50%_Blasts 40%	NK-AMLs	NK-AMLs	40	50%
8	UPN18_BM_dilution 50%_Blasts 43%	NK-AMLs	NK-AMLs	43	50%
9	UPN1_PB_dilution 50%_Blasts 46%	NK-AMLs	NK-AMLs	46	50%
10	UPN58_BM_dilution 50%_Blasts 46%	NK-AMLs	NK-AMLs	46	50%
11	UPN70_PB_dilution 50%_Blasts 46%	NK-AMLs	NK-AMLs	46	50%
12	UPN37_PB_dilution 50%_Blasts 47%	NK-AMLs	NK-AMLs	47	50%
13	UPN65_PB_dilution 50%_Blasts 47%	NK-AMLs	NK-AMLs	47	50%
14	UPN86_BM_dilution 50%_Blasts 47%	NK-AMLs	NK-AMLs	47	50%
15	UPN5_BM_dilution 50%_Blasts 48%	NK-AMLs	NK-AMLs	48	50%
16	UPN64_BM_dilution 50%_Blasts 49%	NK-AMLs	NK-AMLs	49	50%
17	UPN8_BM_dilution 75%_Blasts 19%	NK-AMLs	NK-AMLs	19	75%
18	UPN31_BM_dilution 75%_Blasts 20%	NK-AMLs	NK-AMLs	20	75%
19	UPN80_BM_dilution 75%_Blasts 20%	NK-AMLs	NK-AMLs	20	75%
20	UPN66_BM_dilution 75%_Blasts 20%	NK-AMLs	NK-AMLs	20	75%
21	UPN18_BM_dilution 75%_Blasts 21%	NK-AMLs	NK-AMLs	21	75%
22	UPN58_BM_dilution 75%_Blasts 23%	NK-AMLs	NK-AMLs	23	75%
23	UPN1_PB_dilution 75%_Blasts 23%	NK-AMLs	NK-AMLs	23	75%
24	UPN65_PB_dilution 75%_Blasts 23%	NK-AMLs	NK-AMLs	23	75%
25	UPN70_PB_dilution 75%_Blasts 23%	NK-AMLs	NK-AMLs	23	75%
26	UPN37_PB_dilution 75%_Blasts 24%	NK-AMLs	NK-AMLs	24	75%
27	UPN5_BM_dilution 75%_Blasts 24%	NK-AMLs	NK-AMLs	24	75%
28	UPN86_BM_dilution 75%_Blasts 24%	NK-AMLs	NK-AMLs	24	75%
29	UPN64_BM_dilution 75%_Blasts 25%	NK-AMLs	NK-AMLs	25	75%

Error Rate 3% - Lower limit: 19% blasts

3D-PCA
Test Set-1
Low blast load Samples

14-markers per classifiers

N=4 groups
56 markers



CLASS PREDICTION ANALYSIS ON POOR QUALITY SAMPLES - TEST SET #1

Samples with a RIN < 7.0 (RNA degradation) - N=2

Samples with a low amount of labeled cRNA - N=7
from 51 ng to 593 ng (should be 750 ng)

Samples with low RIN and low cRNA - N=1

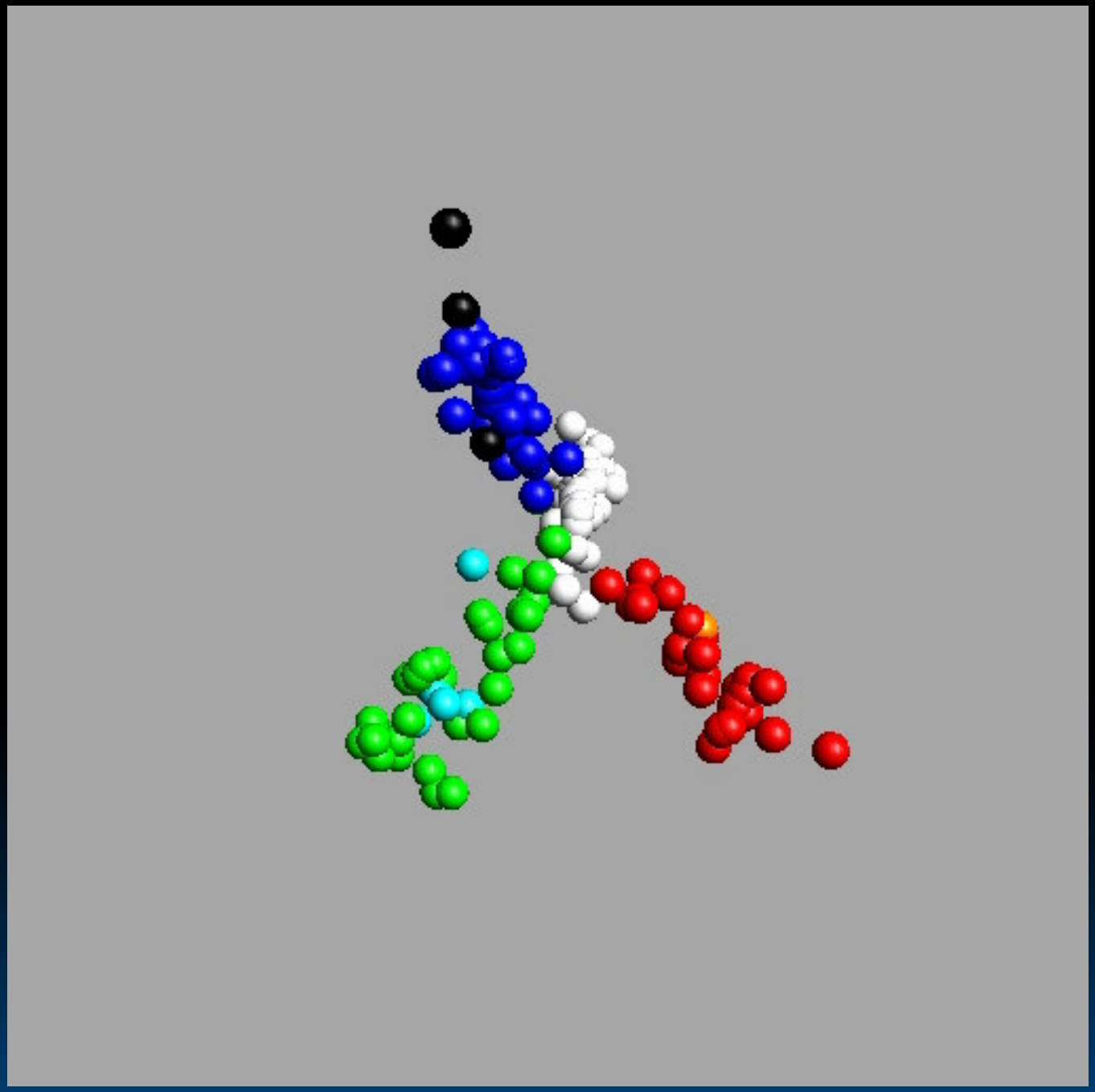
Samples with poor QC and low blast load - N=5

Nr.	Experiment name	Class	Assigned Class	Blast%	Dilution
1	UPN24_PB_QC low cRNA_Blasts 25%	CBFA-AMLs	CBFA-AMLs	25	No
2					
3					
4					

Nr.	Experiment name	Class	Assigned Class	Blast%	Dilution
1	UPN38_BM_QC low cRNA_Blasts %	CBFB-AMLs	CBFB-AMLs		No
2	UPN40_BM_QC low cRNA_Blasts %	CBFB-AMLs	CBFB-AMLs		No
3	UPN17_BM_QC low RIN low cRNA_Blasts 27%	CBFB-AMLs	CBFB-AMLs	27	No
4	UPN71_BM_QC low cRNA_Blasts 47%	CBFB-AMLs	CBFB-AMLs	47	No
5	UPN81_PB_QC low cRNA_Blasts 9%	CBFB-AMLs	CBFB-AMLs	9	No
6	UPN3_BM_QC low cRNA_Blasts 74%	CBFB-AMLs	CBFB-AMLs	74	No
7					
8					

Nr.	Experiment name	Class	Assigned Class	Blast%	Dilution
1	UPN51_PB_QC RIN low_Blasts 70%	APLs	APLs	70	No
2	UPN10_BM_QC cRNA low_Blasts 30%	APLs	APLs	30	No
3	UPN73_BM_QC RIN low_Blasts 64%	APLs	APLs	64	No
4					
5					
6					
7					
8					

Error rate, o%



3D-PCA Poor QC Samples 14-markers classifiers

APL	34/34
CBFA	25/25
CBFB	34/34
NK-AML	58/58
NORMALS	18/18
low QC APL	3/3
low QC CBFA	1/1
low QC CBFB	6/6

Nr.	Experiment name	Class	Assigned Class	Blast%	Dilution
4	UPN52_PB_atypical_undiluted_Blasts 27%	CBFA-AMLs		27	No

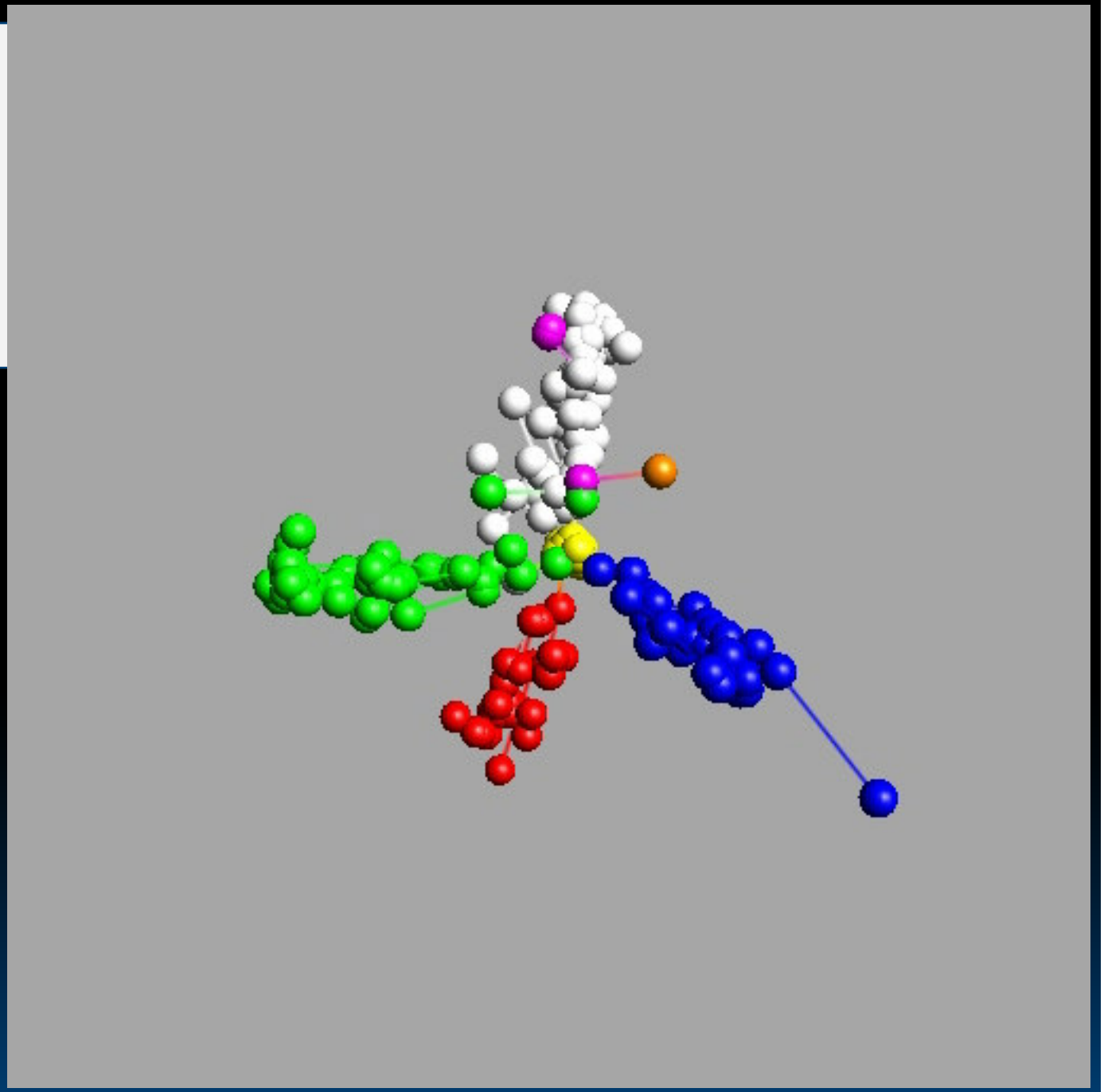
Nr.	Experiment name	Class	Assigned Class	Blast%	Dilution
6	UPN19_BM_atypical_Blasts 90%	APLs		90	No
7	UPN19_BM_atypical_dup_Blasts 90%	APLs		90	No
8	UPN32_PB_atypical_Blasts 70%	APLs		70	No

3D-PCA Atypical AMLs 14-markers classifiers

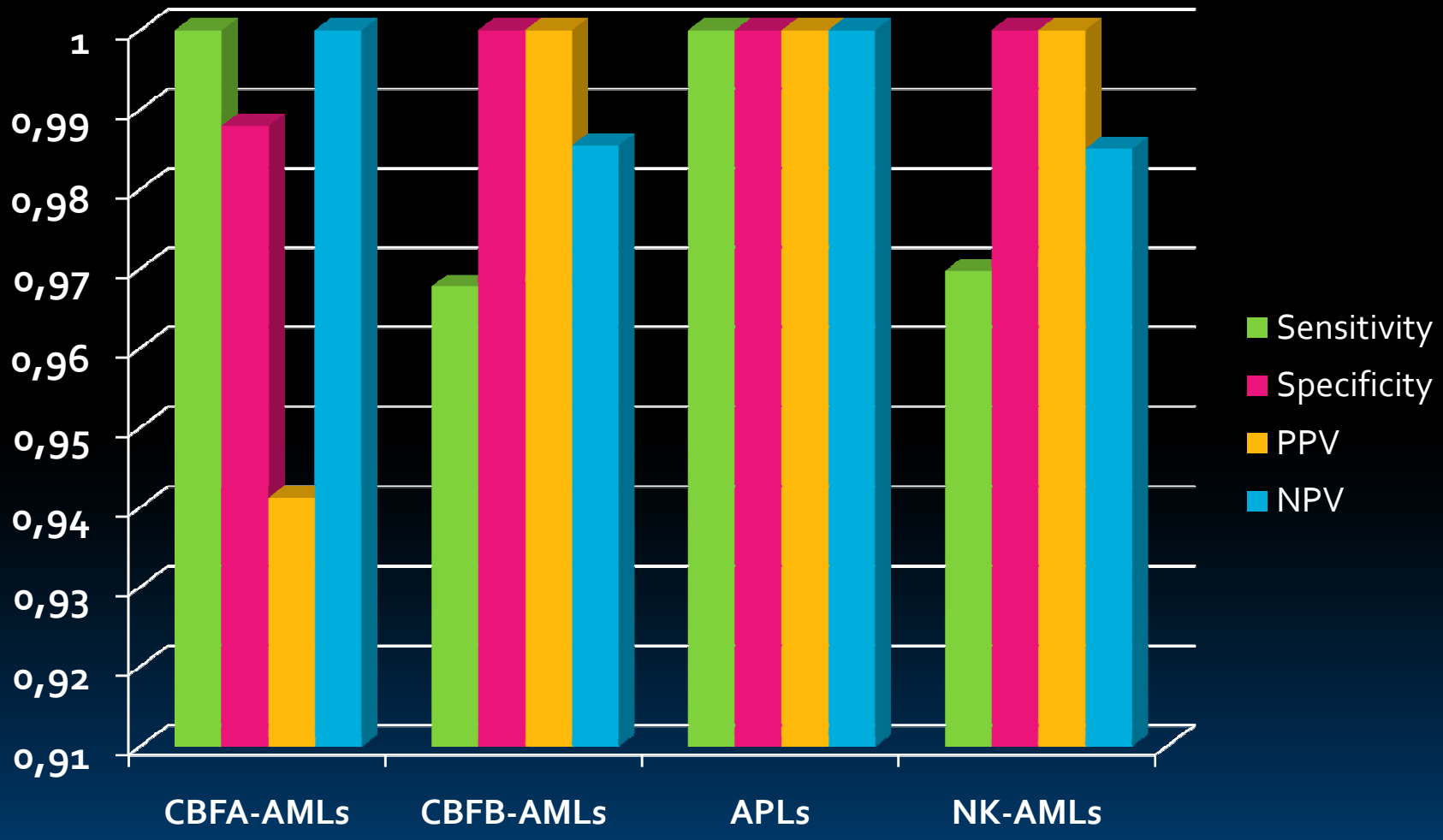
- APLs
- CBFA-AMLs
- CBFB-AMLs
- NK-AMLs
- Normals

Atypical AMLs

- APLs
- CBFA-AMLs



Class prediction accuracy
Test set #1



How do these 14-markers classifiers behave with independent samples processed with Affymetrix GeneChips ?

APLs	n= 25 samples
CBFA-AMLs	n= 34 samples
CBFB-AMLs	n= 34 samples
NK-AMLs	n= 152 samples

Test Set #2: 245 independant samples

Verhaak et al study (GEO access # GSE6891)

Affymetrix Human Genome U133 Plus 2.0 GeneChips

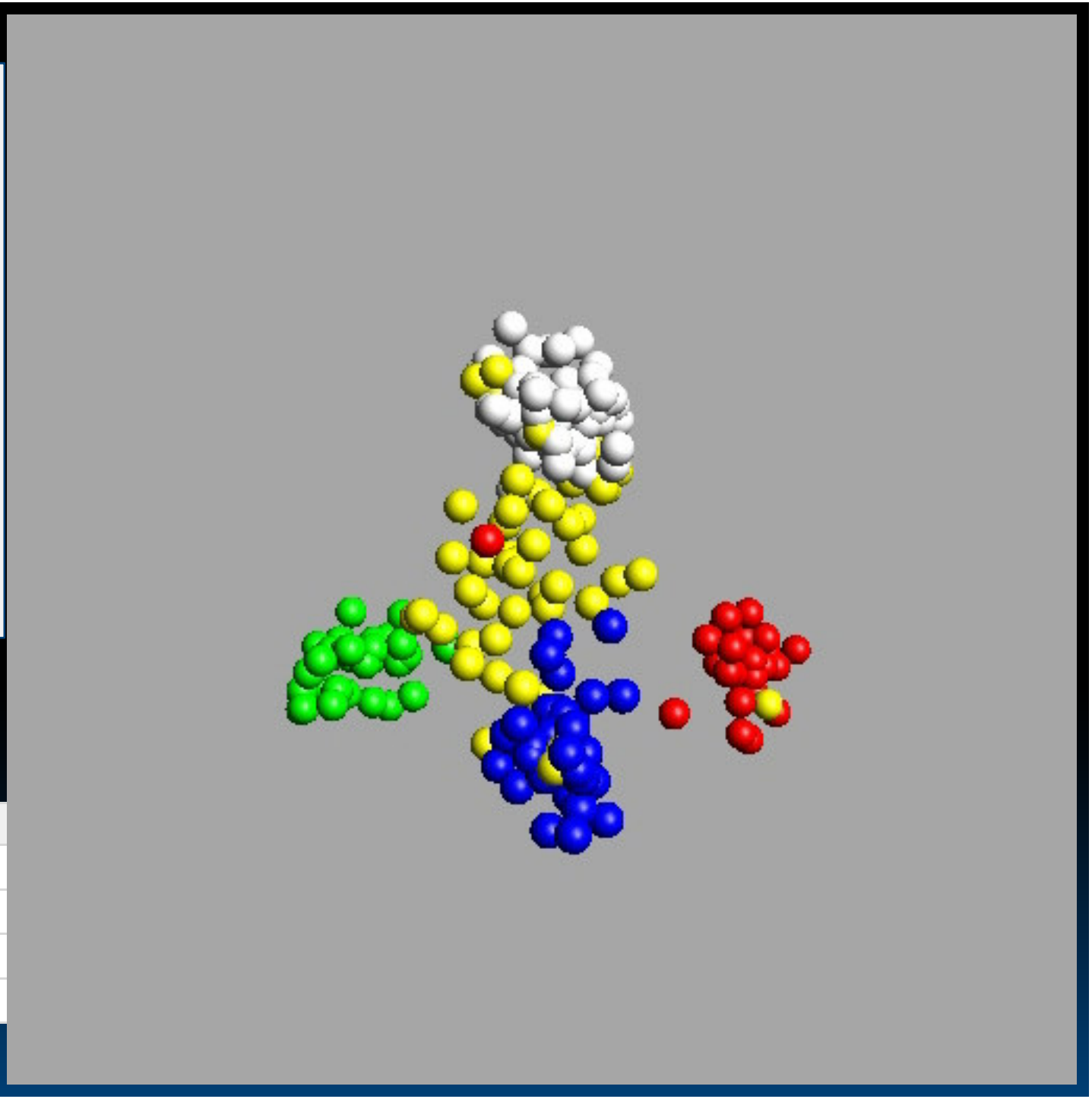
Illumina and Affymetrix probes

- Illumina HT-12 BeadChips
  - 56 probes (14 probes per class, n=4 classes)
- Affymetrix U133 A Plus 2.0 GeneChip
  - Corresponding to 103 probesets
  - Post-filtering, n=86 probesets
  - Two markers not present on Affymetrix GC
    - Hs.191591 and LOC440030

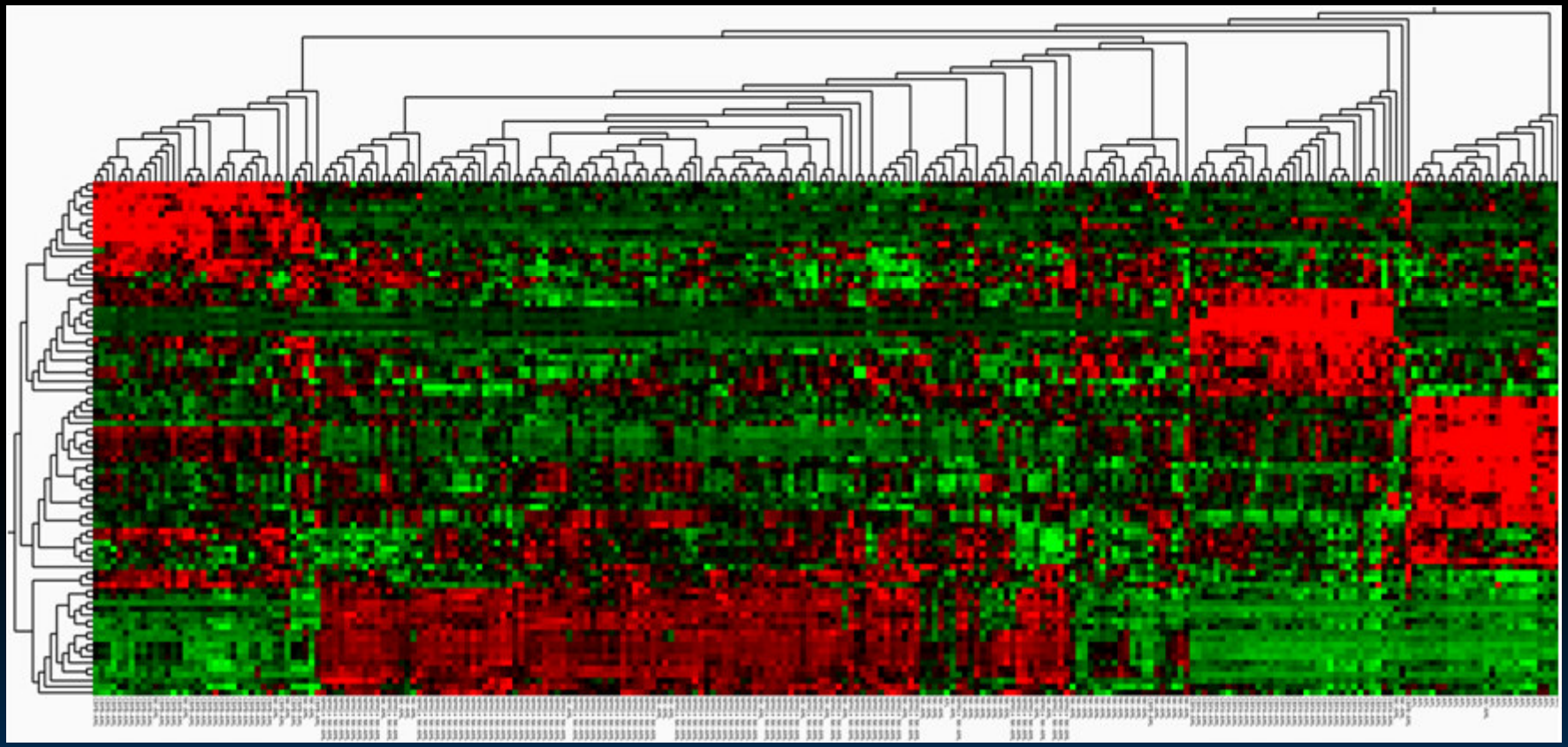
3D-PCA
Test Set-2
Affymetrix

14-markers
per classifiers

N=4 groups
86 markers



Unsupervised Analysis
Hierarchical clustering
Test Set #2



CBFB-AMLs
+ 6 NK-AMLs

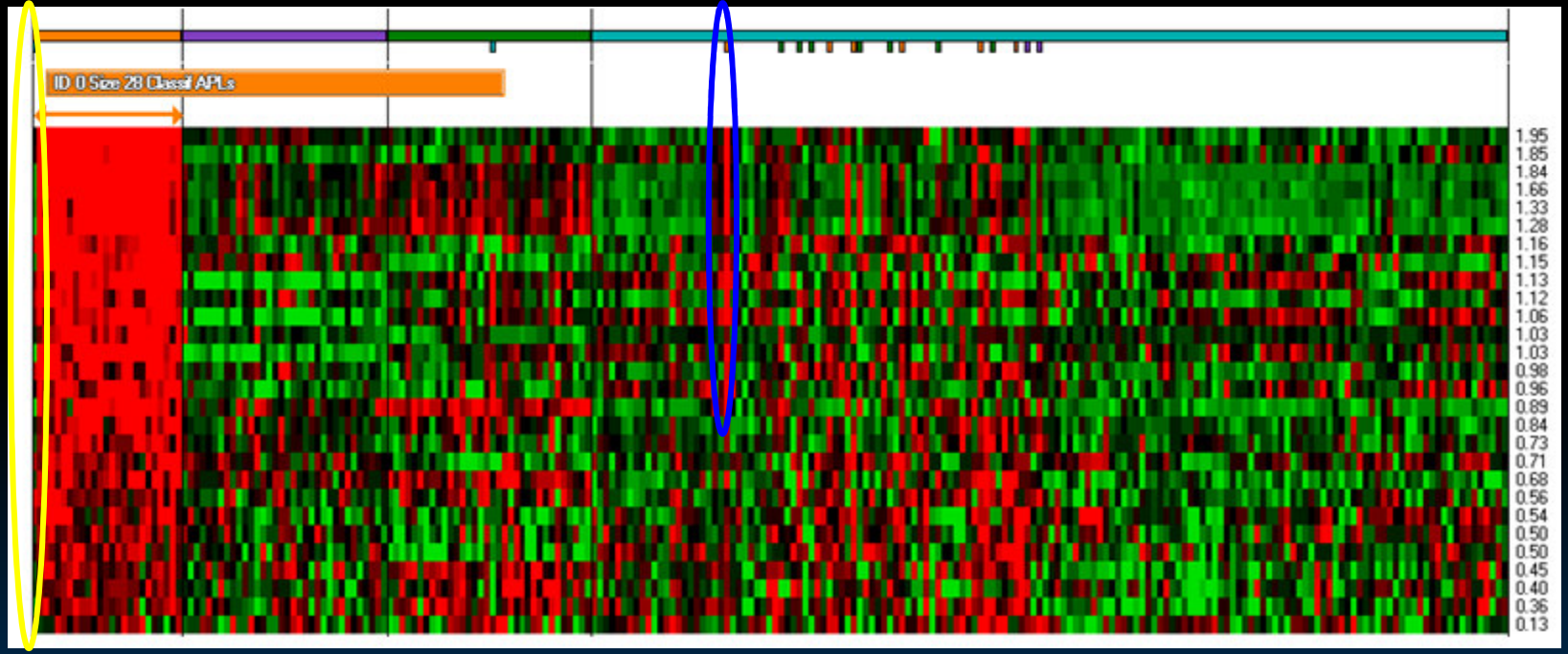
NK-AMLs
+ 1 CBFB-AML

CBFA-AMLs
+ 1 NK-AML

APLs
+ 1 NK-AML

Class Prediction – APLs Test Set #2

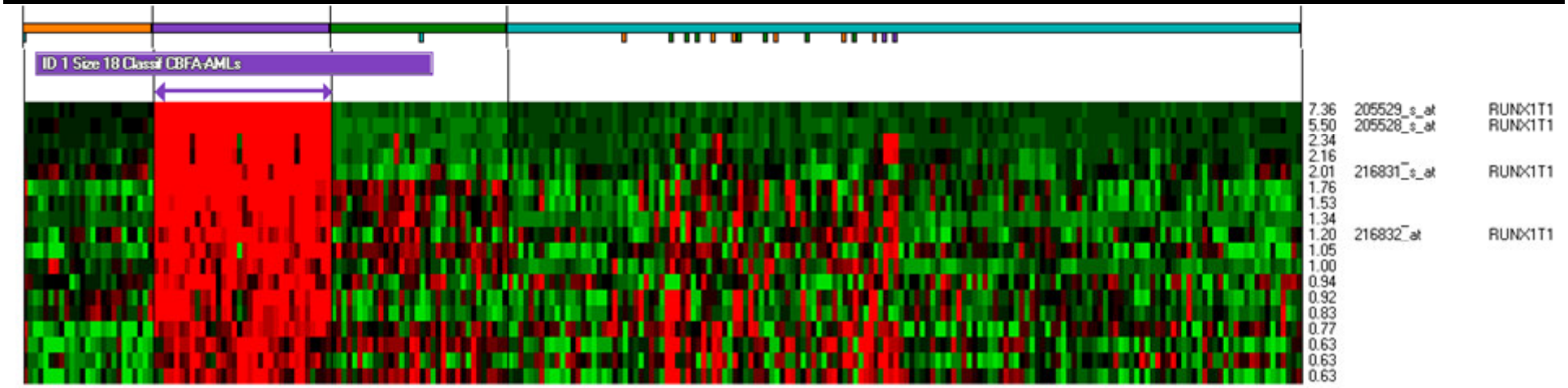
APLs CBFA AMLs CBFB AMLs NK-AMLs



15 misclassified NK-AMLs
1 misclassified CBFB-AML
1 misclassified APL

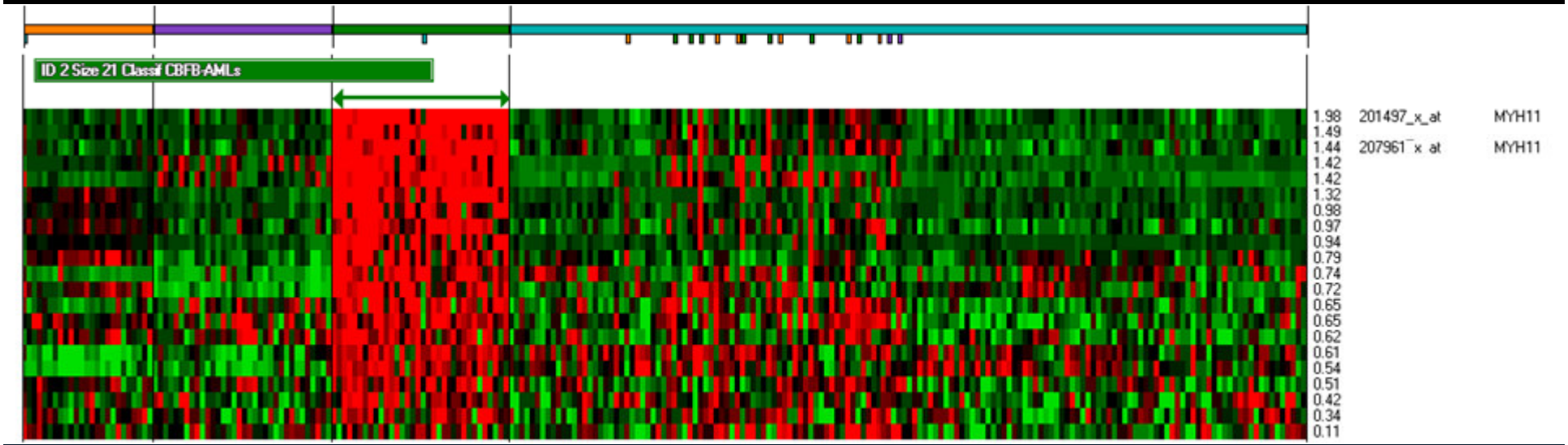
Class Prediction – CBFA-AMLs Test Set #2

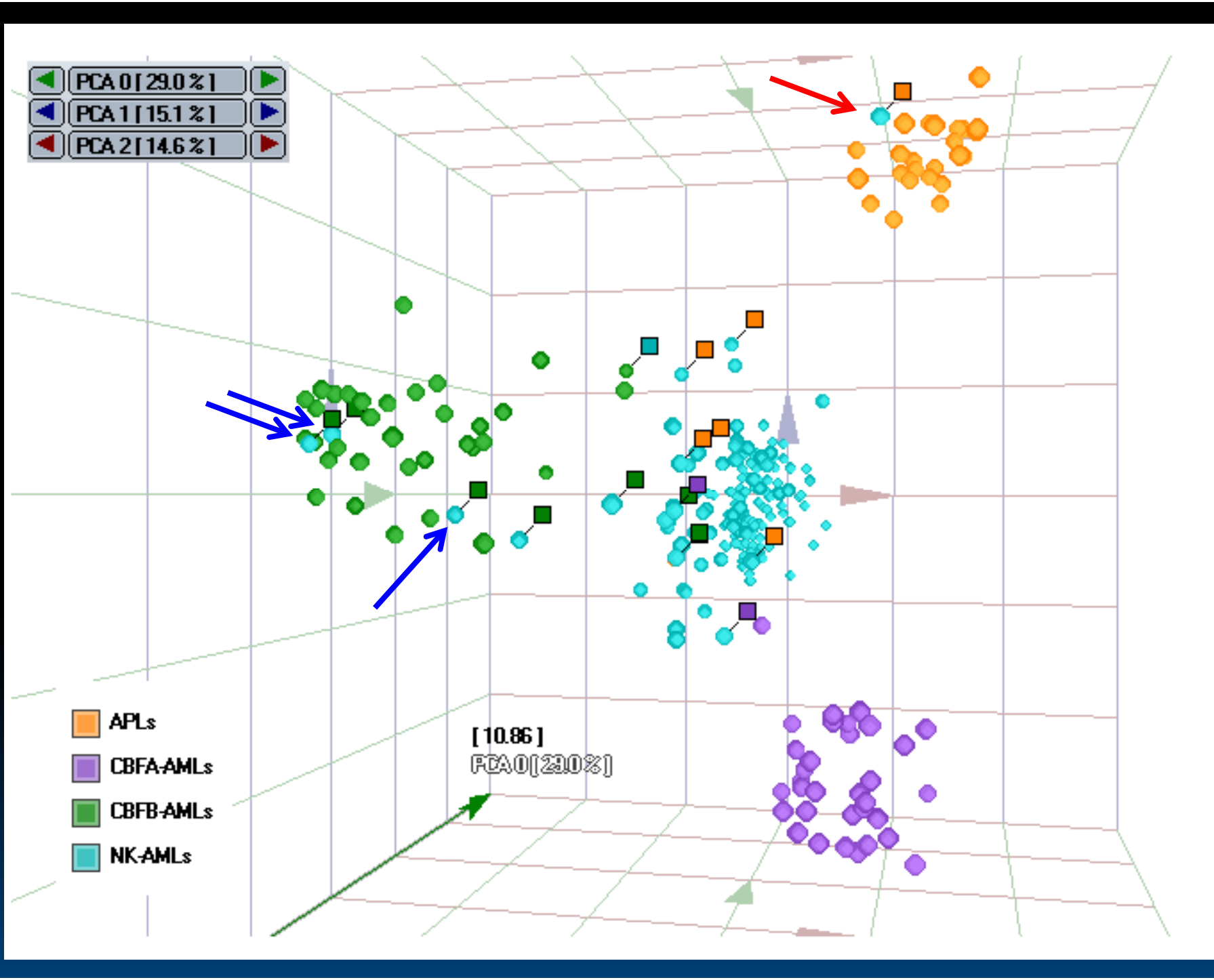
APLs | CBFA AMLs | CBFB AMLs | NK-AMLs



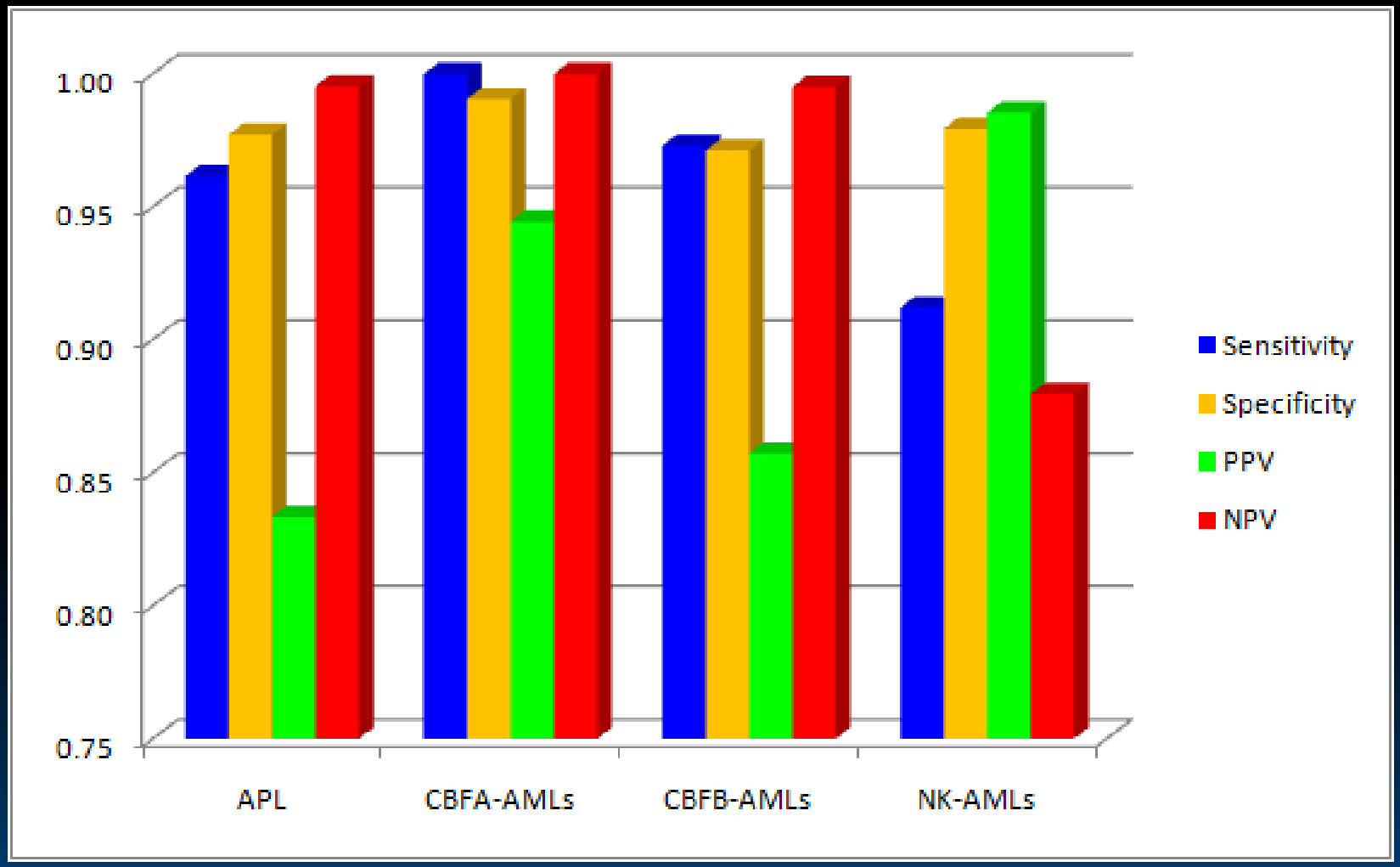
Class Prediction – CBFB-AMLs Test Set #2

APLs | CBFA AMLs | CBFB AMLs | NK-AMLs




PCA 0 [29.0 %]
PCA 1 [15.1 %]
PCA 2 [14.6 %]
APLs
CBFA-AMLs
CBFB-AMLs
NK-AMLs
[10.86]
PCA 0 [29.0 %]

Class prediction accuracy Test set #2

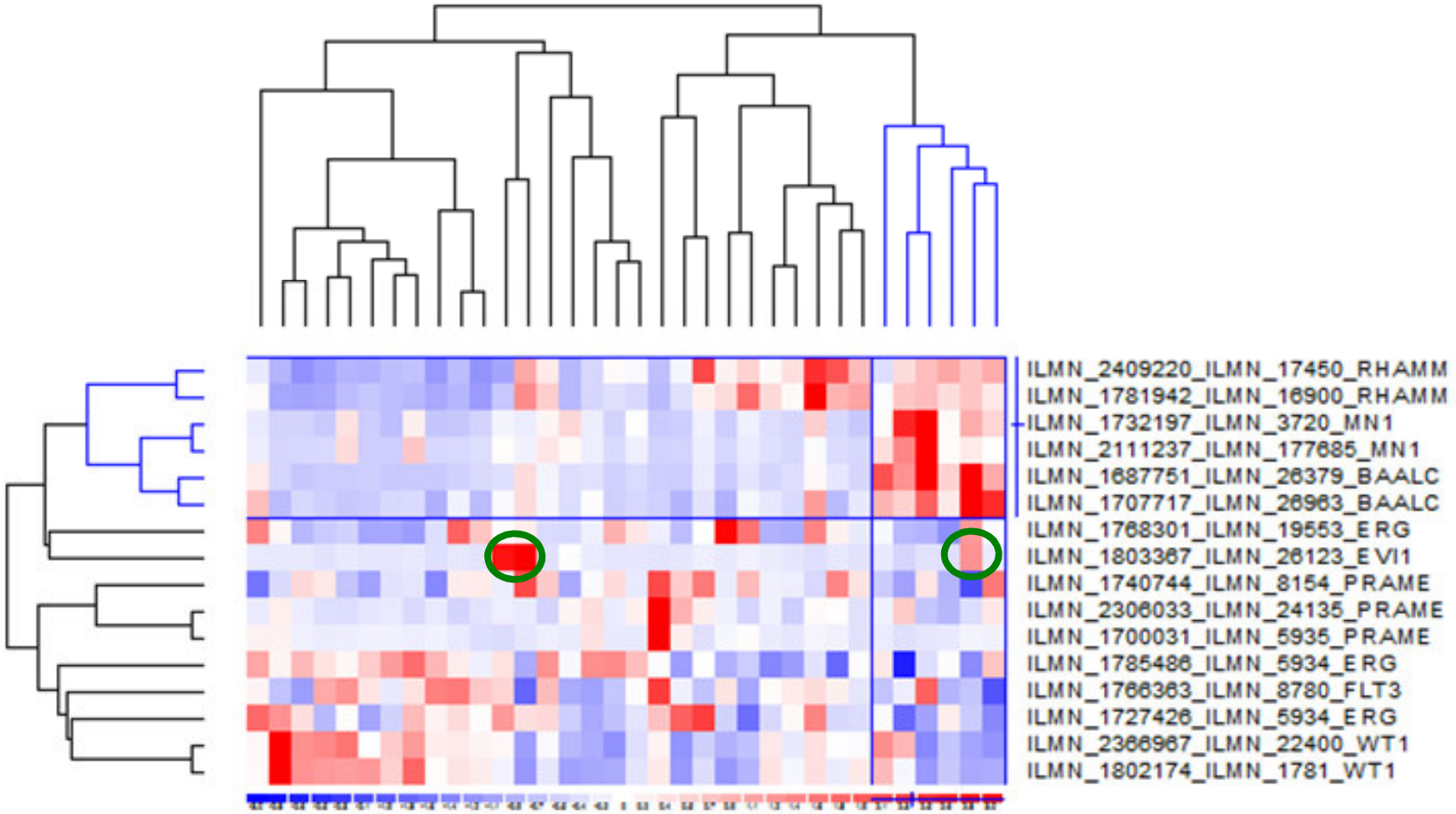


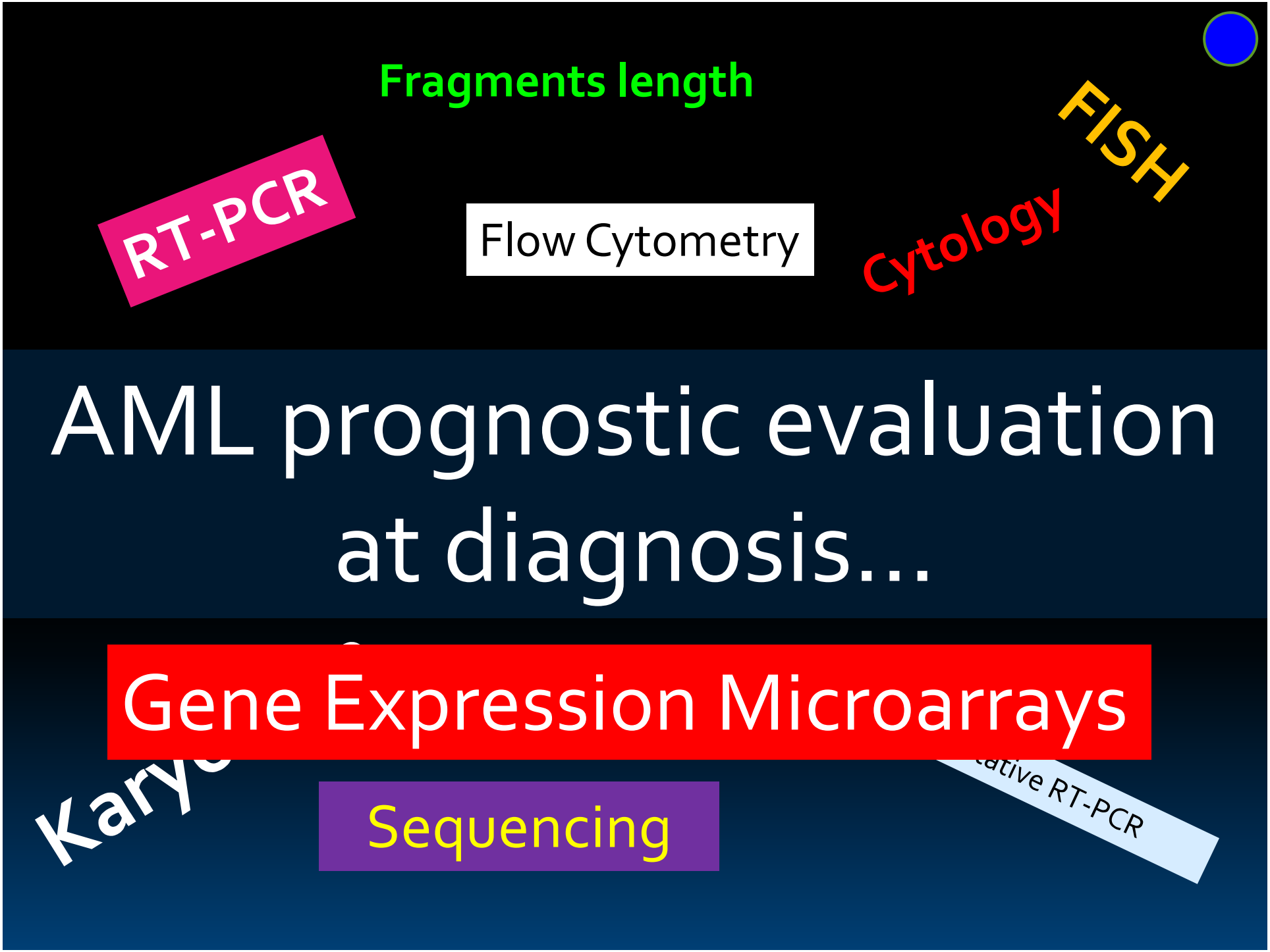
Conclusions

- Good sensitivities, specificities, NPV and PPV even below 20% of blasts.
- Can detect APLs of CBF-AMLs with normal karyotypes or karyotype failures on both Illumina and Affymetrix microarrays
- Poor quality samples / RNAs can be efficiently managed in class prediction analyses.
  - **Down to less than a tenth of the required amount of labeled cRNA for hybridization can still be fine !**
- NK-AMLs is an heterogeneous group of AMLs, in which HOX A and B genes are most of the time deregulated.
- CBFB-AMLs with a low MYH11 expression level can be misclassified. Not recommended to use a single-marker classifier.
- EVI1 expression in APLs could significantly modify the APL specific signature.

The expression level for multiple genes in AML can be used as Prognostic markers or Minimal Residual Disease markers

BAALC, ERG, EVI1, FLT3, MN1, PRAME, RHAMM, WT1…




Fragments length
RT-PCR
Flow Cytometry
Cytology
FISH
AML prognostic evaluation at diagnosis…
Gene Expression Microarrays
Sequencing
ative RT-PCR

Etude du Transcriptome – Leucémies Aigues

Nom du patient : BR Prénom du patient : Ge
Date de naissance : IEP :

Leucocytose: 5 G/L % Blastes Sanguins: 56% % Myélémie: 0%
FAB : M0 % Blastes Médullaires : 63%
Cytogénétique : t(4 ;17)

FLT3-ITD	wild type	FLT3 TKD D835	wild type
NPM1	wild type	CEBPA	not done
WT1	9%	FOXC1	31%

Autres marqueurs (cytométrie en flux)

CD34 + 98%	CD117 + 90%	CD33 + 56%
CD2 - 0%	CD5 - 0%	CD7 - 2%

Date du Prélèvement :
Type de cellules analysées :
Pourcentage de cellules d'intérêt dans l'échantillon :

Extraction ARN totaux réalisée au : Laboratoire d'Hématologie UF
Extraction ARN totaux par la technique : QIAGEN Mini kits
Lot #
Stockage : -80°C Laboratoire d'Hématologie UF Biologie Moléculaire
Qualification des ARN totaux par : puces Agilent RNA 6000 Nano
RIN : 9.2 Ratio 28S/18S :
Lot #
Quantification des ARN totaux sur : NanoDrop-1000
Quantité d'ARN total initiale utilisée : 200 ng
Kit de marquage / amplification / IVT utilisé : Illumina Total Prep Ambion
Lot #
Streptavidine utilisée :
Lot #
Type de puce utilisée : Illumina BeadChips Human HT-12 v3
Lot #
Examen réalisé du : au : par :

Analyse Biostatistique
Logiciel utilisé pour l'extraction du signal : Genome Studio 2010.1
Module Gene Expression Module 1.6.0
Normalisation utilisée : Invariant Rank
Soustraction du bruit de fond : No
Logiciel d'analyse supervisée utilisé : Array Miner - Class Marker Module
Version # 5.3.3.
Filtre : 50,000 – 0 – 1.5 – 150

Conclusions

Blastes exprimant les marqueurs pronostiques BAALC, EVI1 et MN1.

Expression de WT1 et de RHAMM peu intense.

Expression de FOXC1 peu intense.

Profil non-compatible avec une LAM-CBF (CBFA ou CBFB)

Profil non-compatible avec une LAM3 ou LAM3-variante

Profil non-compatible avec une LAM avec mutation du gène NPM1

Pas d'argument pour une trisomie 8

Au total, LAM de pronostic :

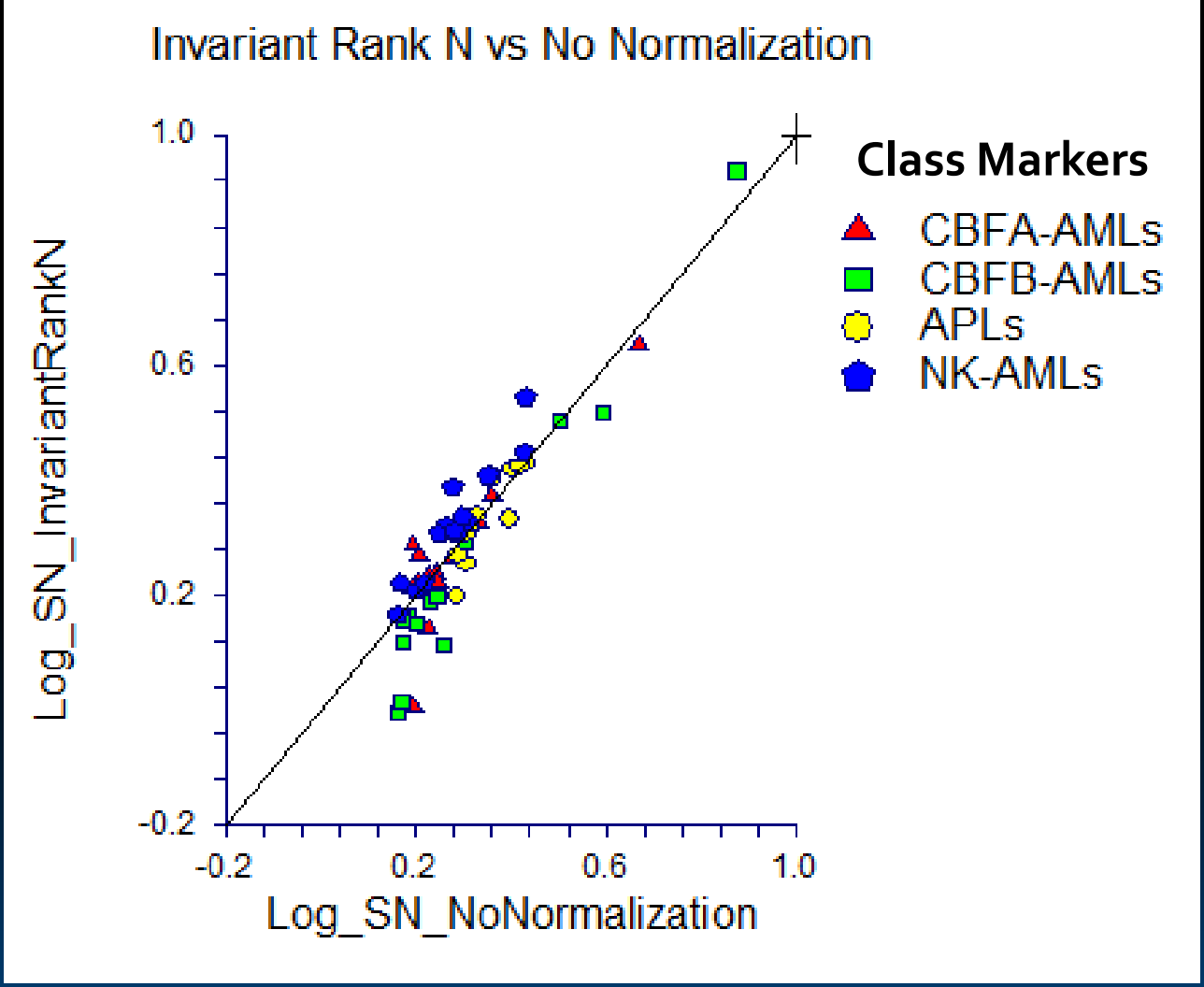
[ ] FAVORABLE [ ] INTERMEDIAIRE ■ DEFAVORABLE

Valide par : Dr Guardiola

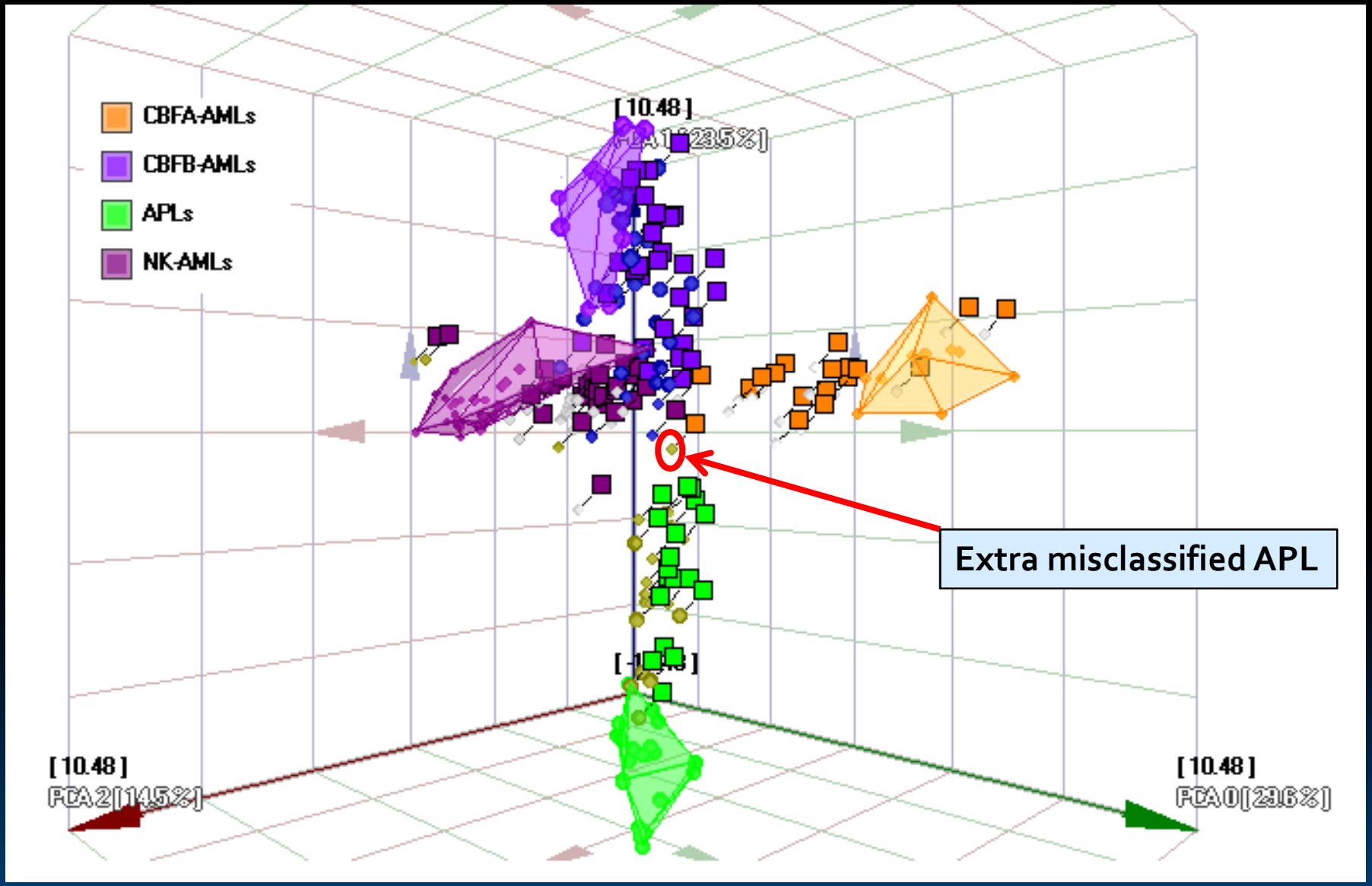
TO NORMALIZE OR NOT ?

EXTRAS & MAKING-OF

Impact of Normalization On the 14-markers classifiers



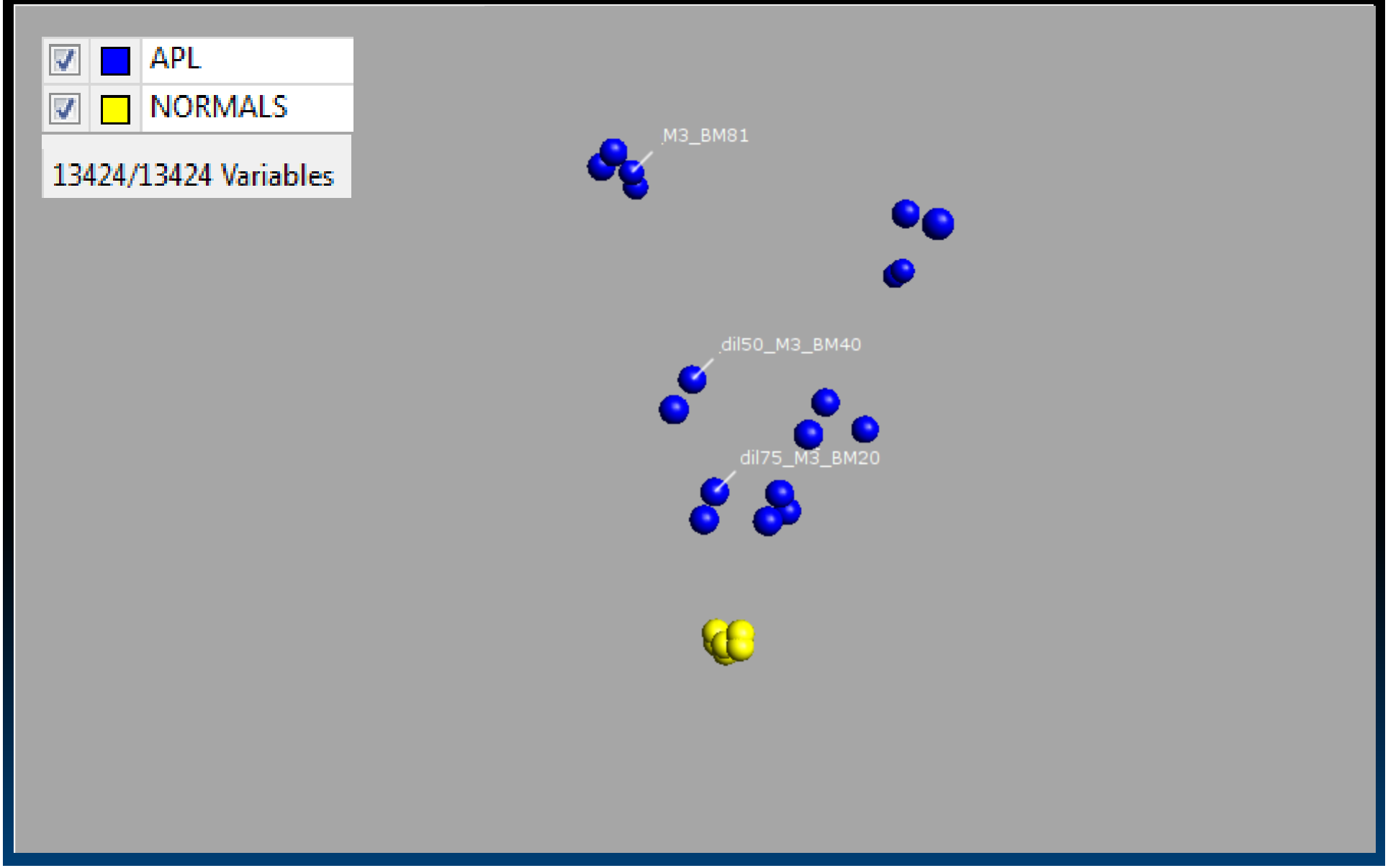
Impact of Normalization



DILUTION SERIES

EXTRAS & MAKING-OF

Dilution Series



REPRODUCIBILITY

EXTRAS & MAKING-OF 1

Reproducibility

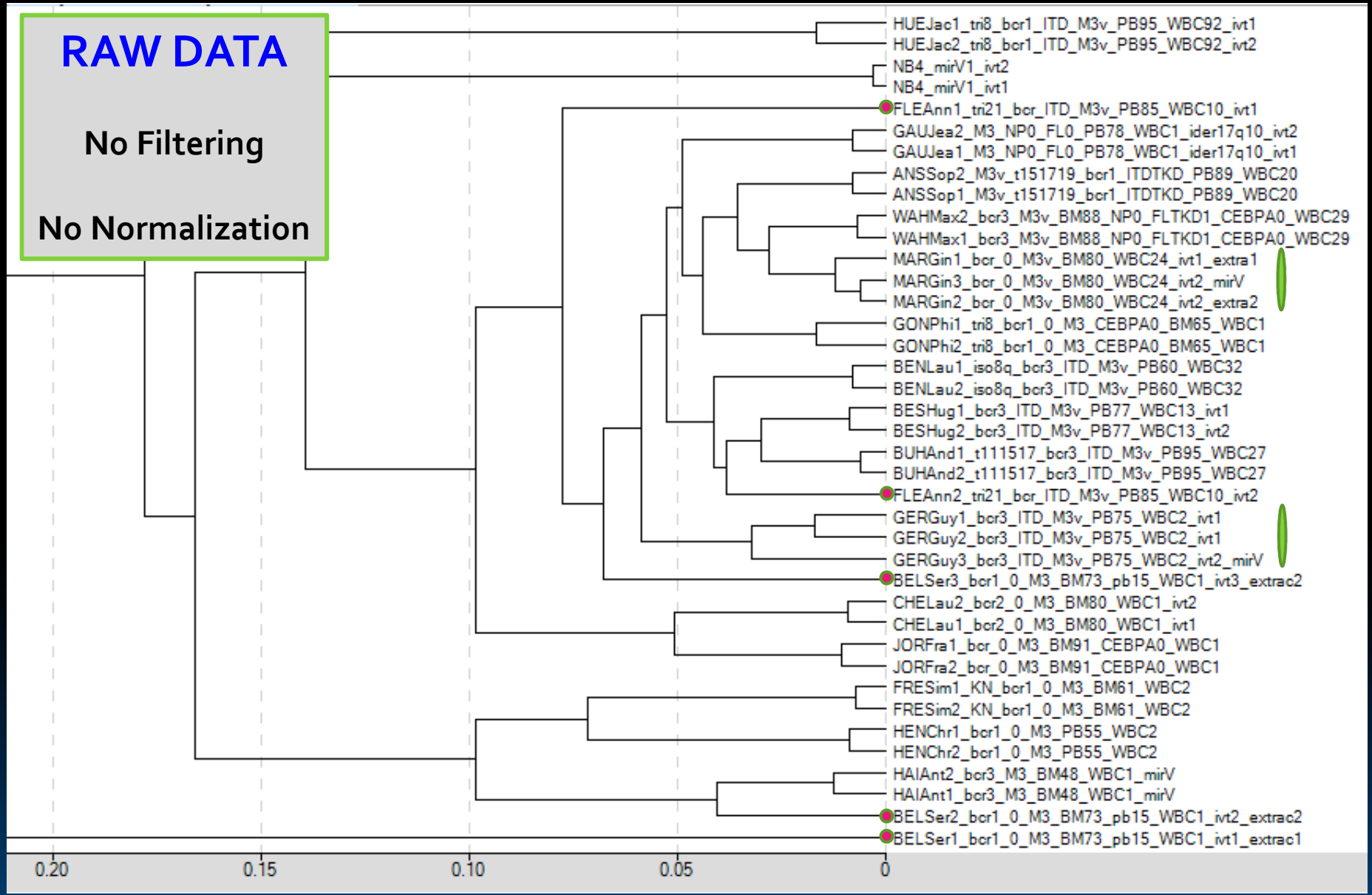
- Replicates (n=39 stripes)
  - **With different total RNA extraction kits**
    - **Duplicates RNEasy Mini kits QIAGEN (n=15 samples)**
    - **Triplicates QIAGEN + Mirvana kits AMBION (n=3 samples)**
  - **Duplicates on different HT-12 BeadChips**
  - **With different RNA extractions**
  - **With different batches of Illumina Total Prep Kits**
  - **At different time points**
  - **Performed by two different persons**
- 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

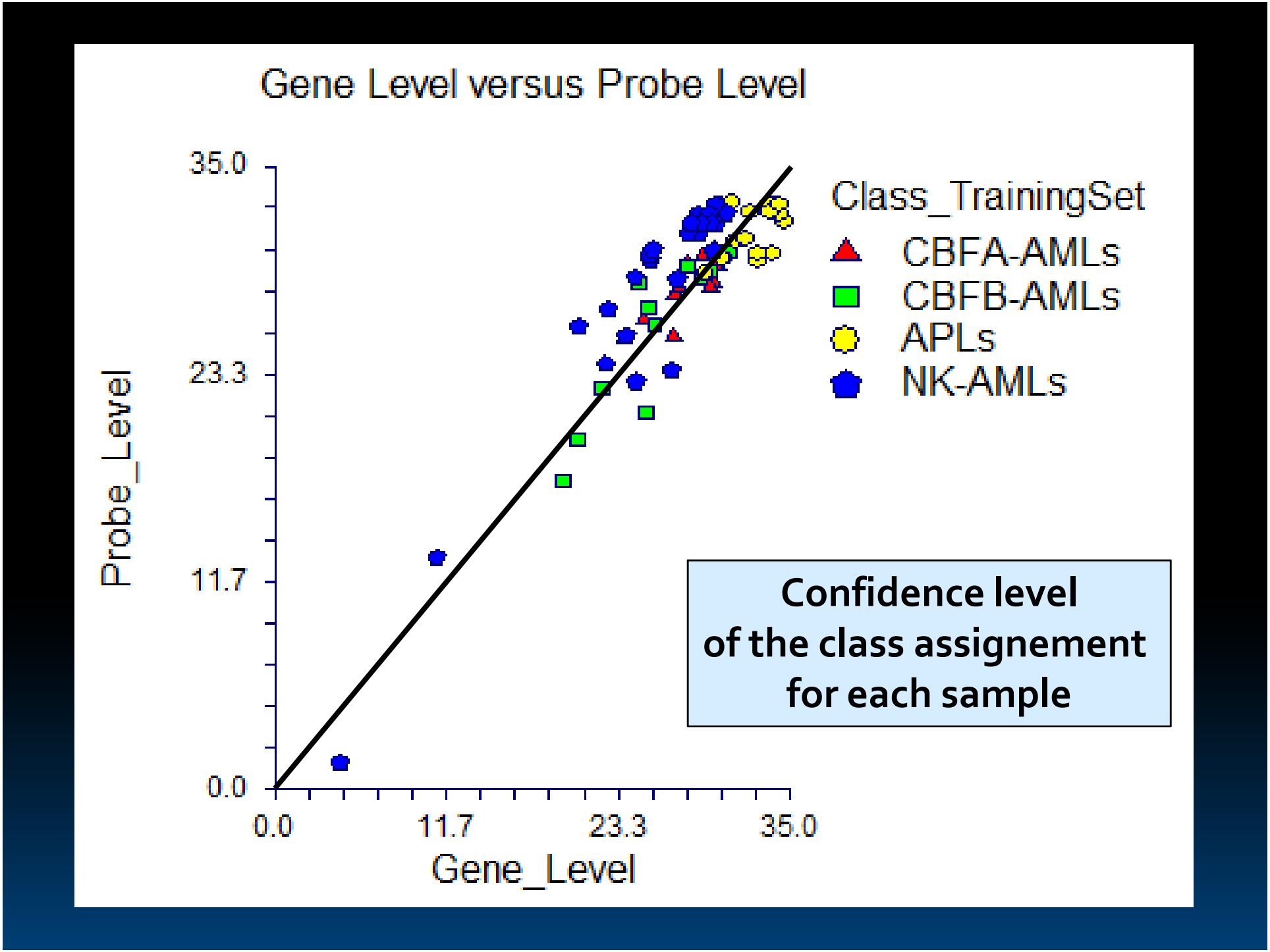
No Filtering

No Normalization



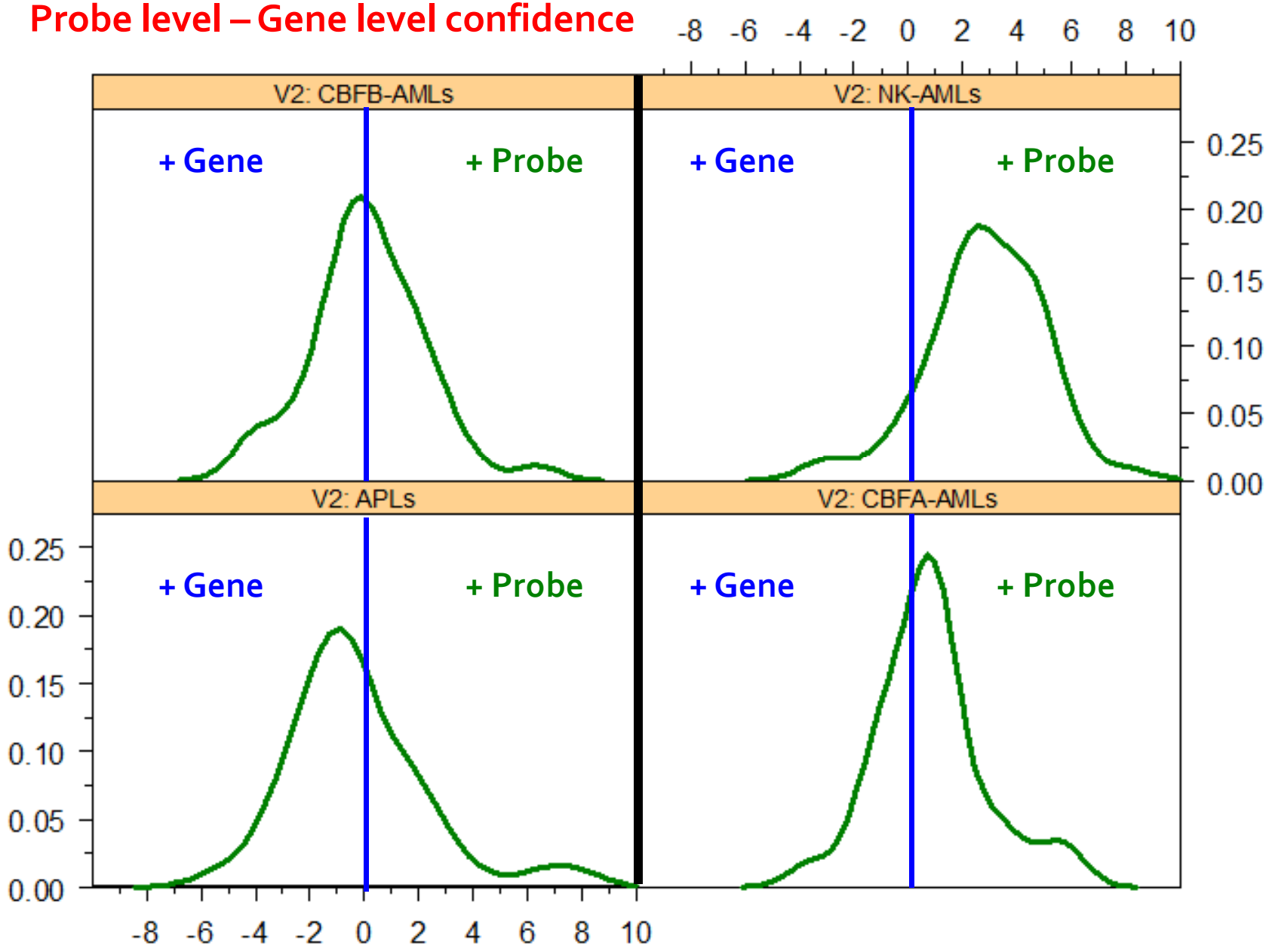
GENE OR PROBE LEVEL ANALYSES ?

EXTRAS & MAKING-OF


Gene Level versus Probe Level
35.0
23.3
11.7
0.0
Probe_Level
0.0
11.7
23.3
35.0
Gene_Level
Class_TrainingSet
CBFA-AMLs
CBFB-AMLs
APLs
NK-AMLs
Confidence level
of the class assignement
for each sample

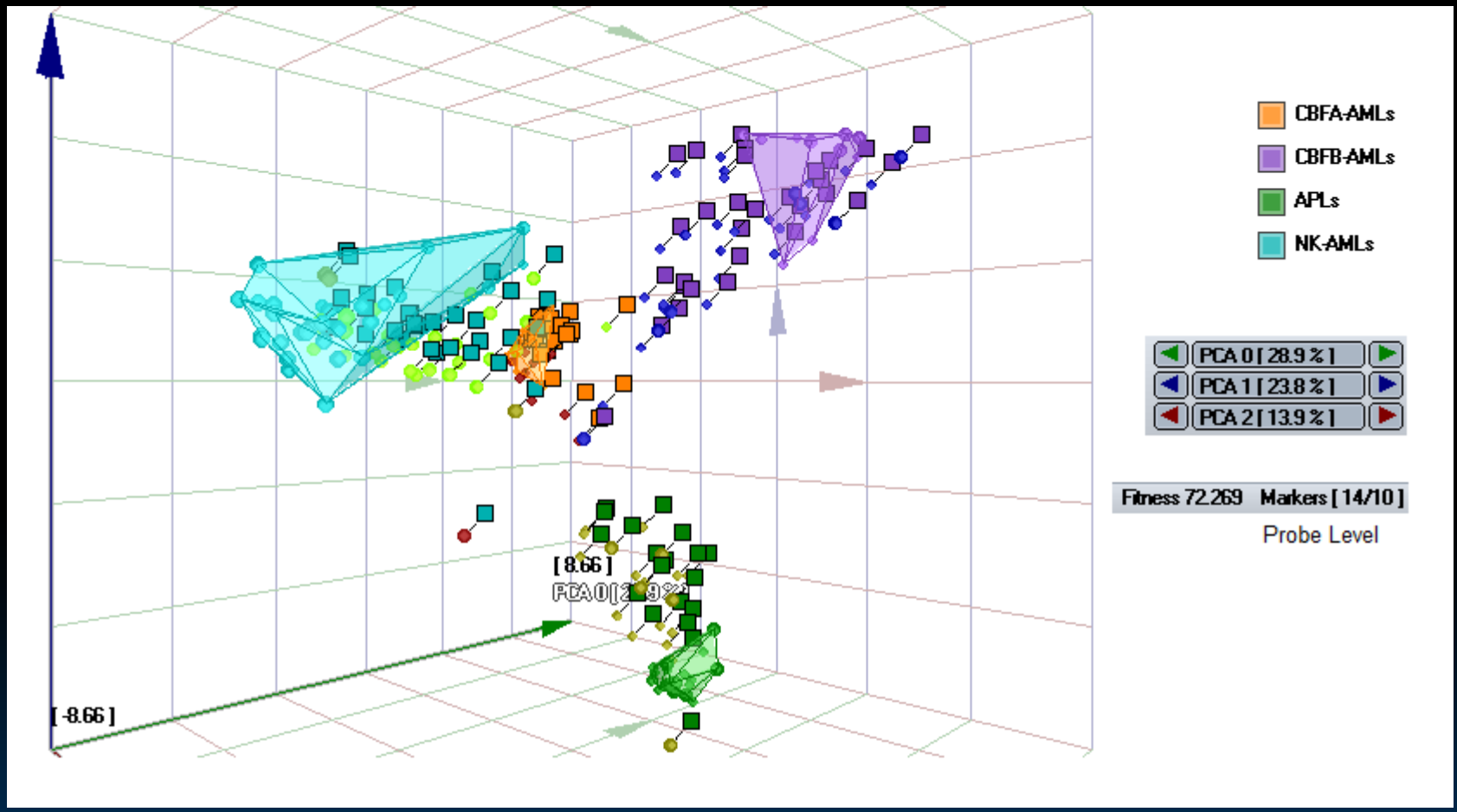
Difference in confidence level of the class assignement for each sample

Probe level – Gene level confidence



Probe or Gene Level Analysis

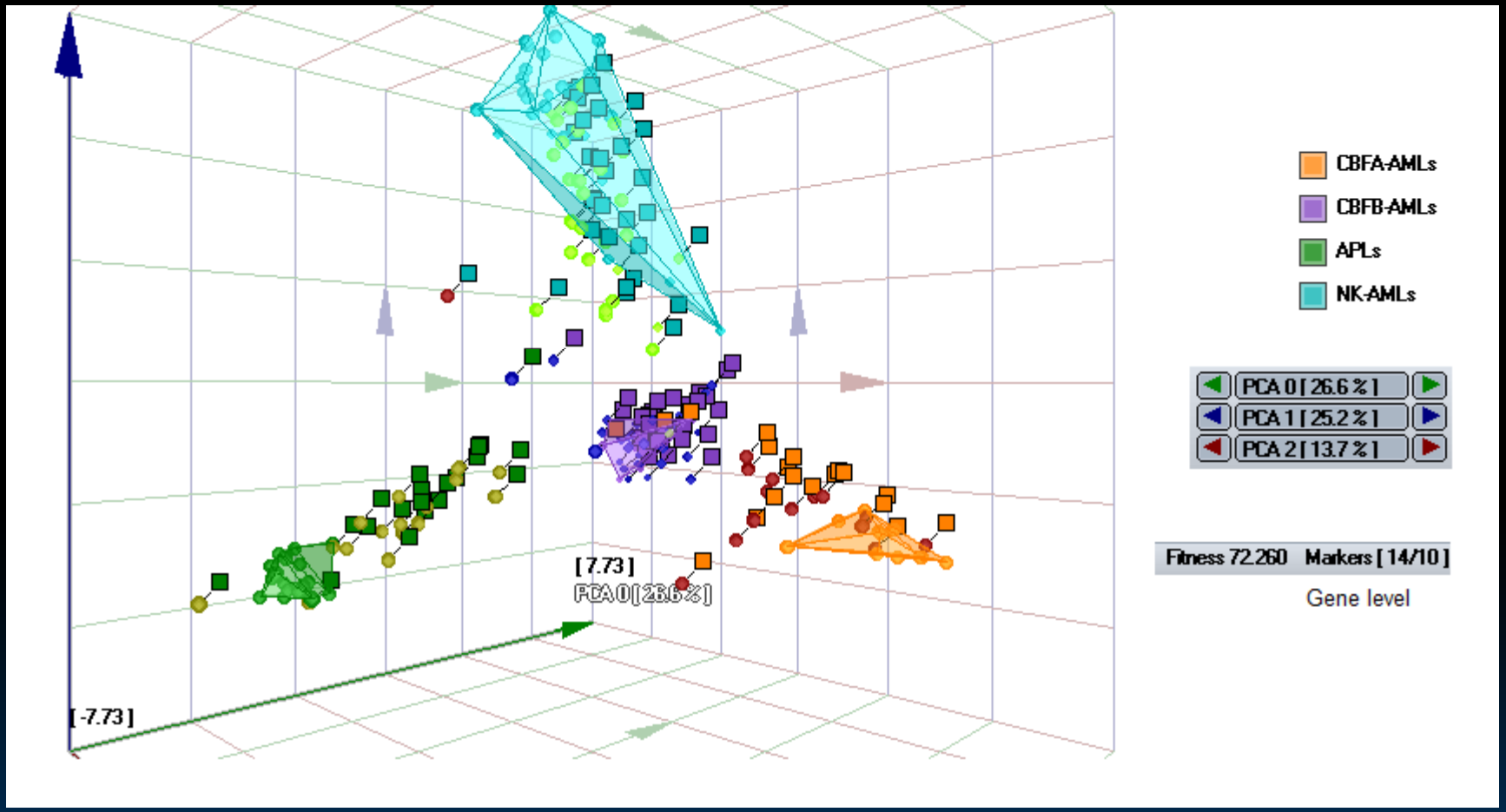
Probe Level Analysis with 14 Markers



Misclassified samples: CBFB-AML n=1 - NK-AML n=1 in Training Set -1

Probe or Gene Level Analysis

Gene Level Analysis with 14 Markers



Misclassified samples: CBFB-AML n=3 - NK-AML n=1 in Training Set-1

Conclusions

- Impact of the normalization used - if any - is low in the 20 to 100% blast content range.
  - One additional misclassified APL sample with 7% blasts.
  - Two =/= markers identified in two classes Invariant Rank versus No normalization for the top-14 class markers.
- Reproducibility is high
  - Up to one year between replicates.
  - Even when changing total RNA extraction kit…
- Probe level > Gene Level for class prediction analysis
- Useful to better characterize AMLs…?

Acknowledgments

- Diane Lambert
- Anne Coutolleau
- Dr Odile Blanchet (Laboratoire d'Hématologie)
- Pr Norbert Ifrah (Service des Maladies du Sang)
- Le Centre Hospitalier Universitaire d'Angers

- La Ligue contre le Cancer CD49-Maine et Loire
- L'Association Laurette Fugain
- Le Cancéropôle Grand-Ouest
- La Région Pays de la Loire

- Illumina France

IF YOU WANT TO TALK ABOUT GENOMICS

Join us on GenOMining LinkedIn Group…

Just contact me by email:
Phguardiol@aol.com