

Moderní laboratorní přístupy v cytogenetice člověka

DNA čipy a metoda MLPA

Jan Smetana a kol.

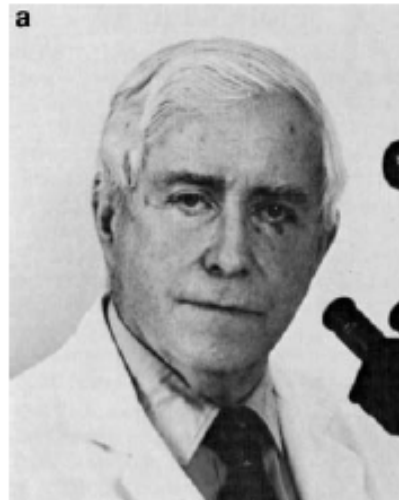
Ústav experimentální biologie PŘF MU v Brně
Odd. lékařské genetiky FN Brno



1956 - spočítání lidských chromozomů



Joe Hin Tjio (1919 - 2001)



Albert Levan (1905 -1998)

Tjio, T.H., Levan, A.:
The chromosome number of man.
Hereditas 42:1, 1956



Āk m v jdře kade sv tln bunky
23 pr chromozom,
celkem m tedy 46 chromozom

In situ hybridization

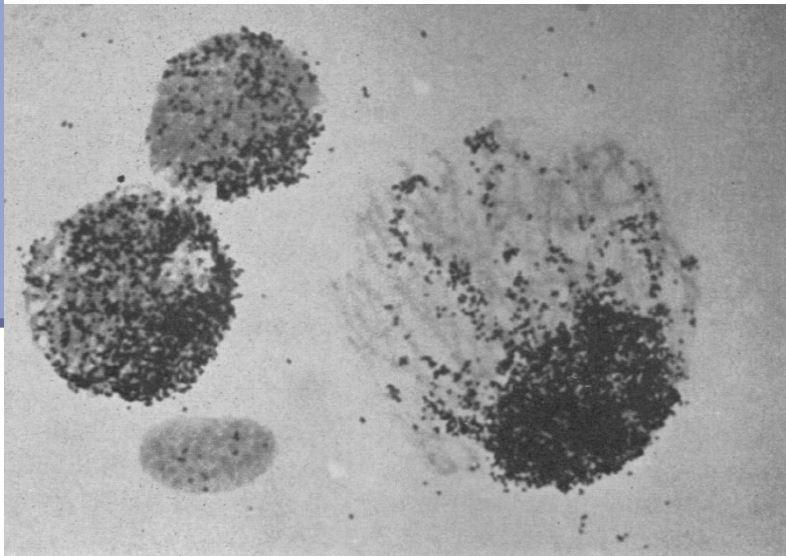
FORMATION AND DETECTION OF RNA-DNA HYBRID MOLECULES IN CYTOLOGICAL PREPARATIONS*

BY JOSEPH G. GALL AND MARY LOU PARDUE

KLINE BIOLOGY TOWER, YALE UNIVERSITY

Communicated by Norman H. Giles, March 27, 1969

Abstract.—A technique is described for forming molecular hybrids between RNA in solution and the DNA of intact cytological preparations. Cells in a conventional tissue squash are immobilized under a thin layer of agar. Next they are treated with alkali to denature the DNA and then incubated with tritium-labeled RNA. The hybrids are detected by autoradiography. The technique is illustrated by the hybridization of ribosomal RNA to the amplified ribosomal genes in oocytes of the toad *Xenopus*. A low level of gene amplification was also detected in premeiotic nuclei (oogonia).



472

* Bergman, J. W., and Maan, S. S., *Fourth Int. Wheat Genet. Symp.*, Missouri, 329-335 (1973).
† Davis, J., *Ann. N.Y. Acad. Sci.*, 121, 404-427 (1964).
‡ Davidson, E. H., and Britten, R. J., *Q. Rev. Biol.*, 48, 565-606 (1973).
§ Schwartz, D., *Genetics*, 67, 411-425 (1970).

High resolution detection of DNA-RNA hybrids *in situ* by indirect immunofluorescence

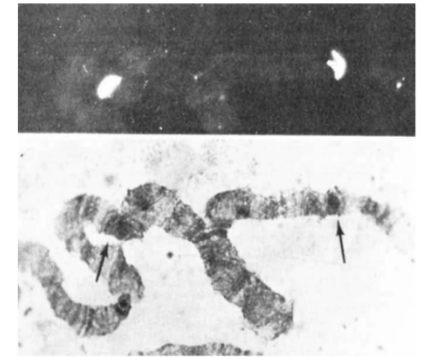
We describe here a new method for the detection of RNA-DNA hybrids in cytological preparations with which we have revealed the locations of hybrid molecules on polytene chromosomes. The critical reagent is an antiserum raised in rabbits against poly(rA)-poly(dT) complexed with methylated bovine serum albumin, originally described by Stollar¹. The specificity and resolving power of the indirect immunofluorescence procedure are demonstrated using *in situ* hybridisation of 5S rRNA (ribosomal RNA) to polytene chromosomes of *Drosophila melanogaster* as a model system. The method has significant advantages over the autoradiographic procedures²⁻⁵ used so far.

The procedure for visualising the *in situ* hybrids follows Alfageme *et al.*⁶ It consists of exposing the cytological preparation to the rabbit anti-hybrid antiserum, then to anti-rabbit IgG prepared in goat and tagged with rhodamine, followed by examination in a fluorescence microscope (see legend to Fig. 1). Our test objects were polytene chromosomes of *Drosophila melanogaster* (giant phenotype) to which 5S rRNA had been

Nature Vol. 265 February 3 1977

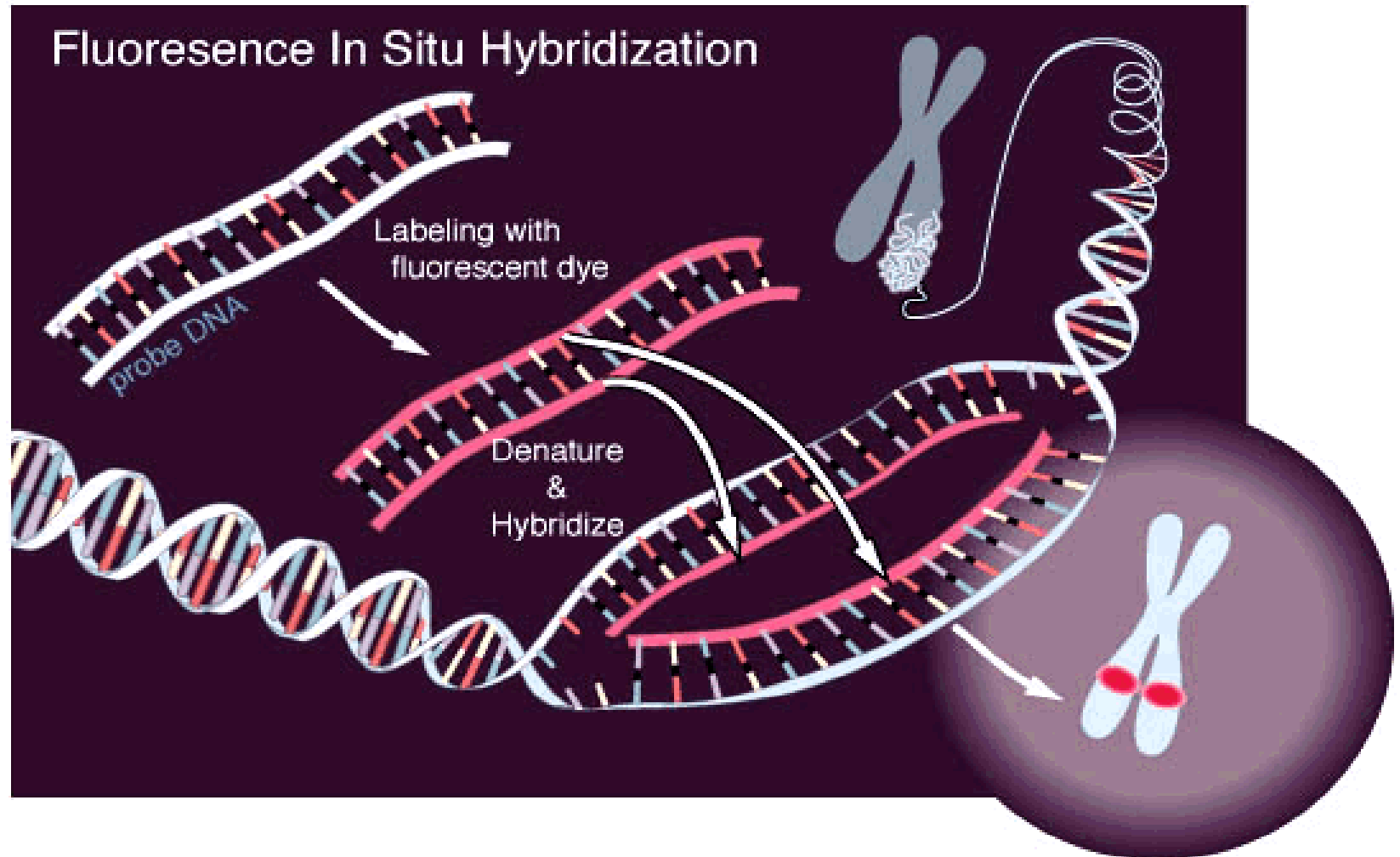
serum, the hybrid was the only reactive polynucleotide class, even in assays with undiluted serum.

There is a variable amount of fluorescence in cytoplasmic components, the origin of which is not yet known. Experiments are projected to attempt to block it while leaving the activity against hybrid nucleic acids intact. Occasional pale fluorescence observed in nucleoli is attributed to contamination of the 5S rRNA probe with fragments of 18S and 28S nucleolar rRNA,



Rudkin GT, Stollar BD. High resolution detection of DNA-RNA hybrids *in situ* by indirect immunofluorescence. Nature. 1977 Feb 3;265(5593):472-3.

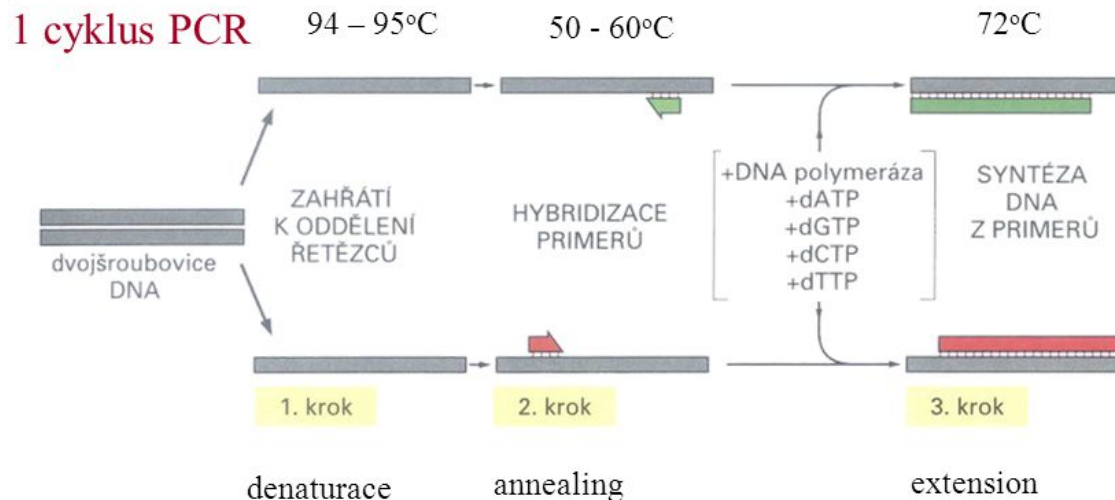
80 léta – fluorescenční *in situ* hybridizace



80 léta – objev PCR reakce /K. Mulvis

- Vytvoření (amplifikace) identických kopií určitého úseku DNA za specifických podmínek (Termocyklery)

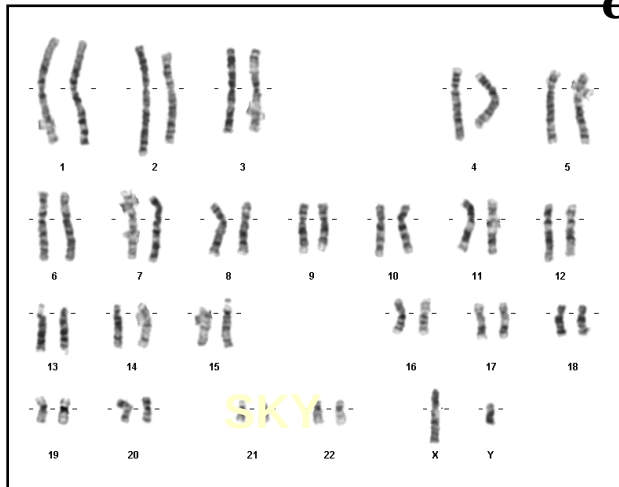
Polymerázová řetězová reakce (PCR)



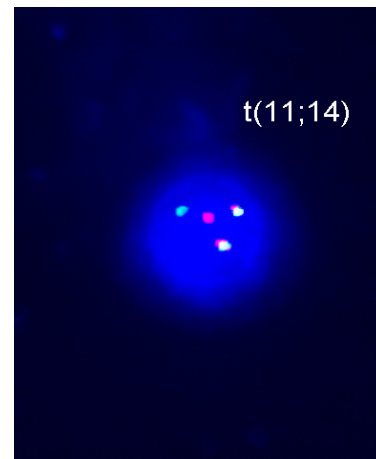
Při 30 cyklech - amplifikace více než 10^9 kopií úseku DNA

Možnosti vyšetření karyotypu člověka

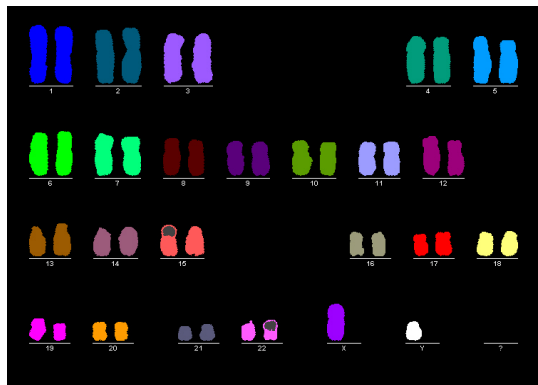
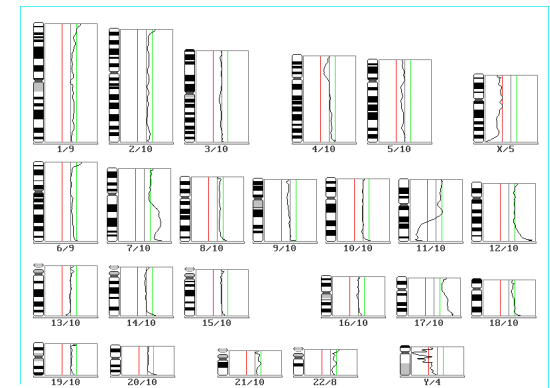
■ Metafázní cytogenetika



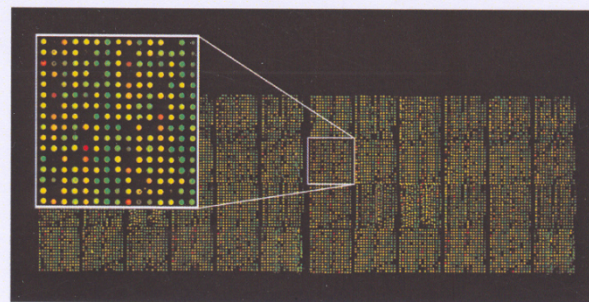
● Interfázní cytogenetika



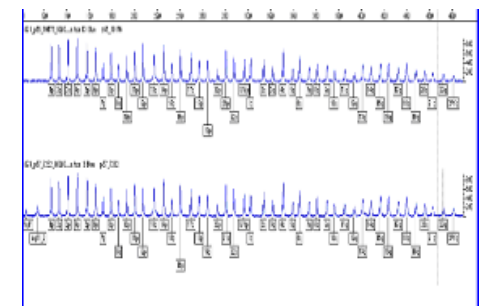
● Cytogenetika založená na izolaci DNA



Mikročipové technologie



MLPA



Molekulární karyotypování – 1000x citlivější než klasická cytogenetika

		Resolution	Coverage
a Cytogenetics	Karyotyping	> 10 Mb	Complete
	SKY	> 2 Mb	Complete
	Traditional CGH	> 2 Mb (cytoband)	Complete
	FISH (interphase)	≥ 20 Kb	Probe Specific
	FISH (metaphase)	≥ 100 Kb	Probe Specific
b aCGH	BAC	100 Kb (Spectral Genomics - 2 Mb)	Complete
	cDNA	2 Kb	Genes Only
	Oligo (60-mer)	0.06 Kb	Complete

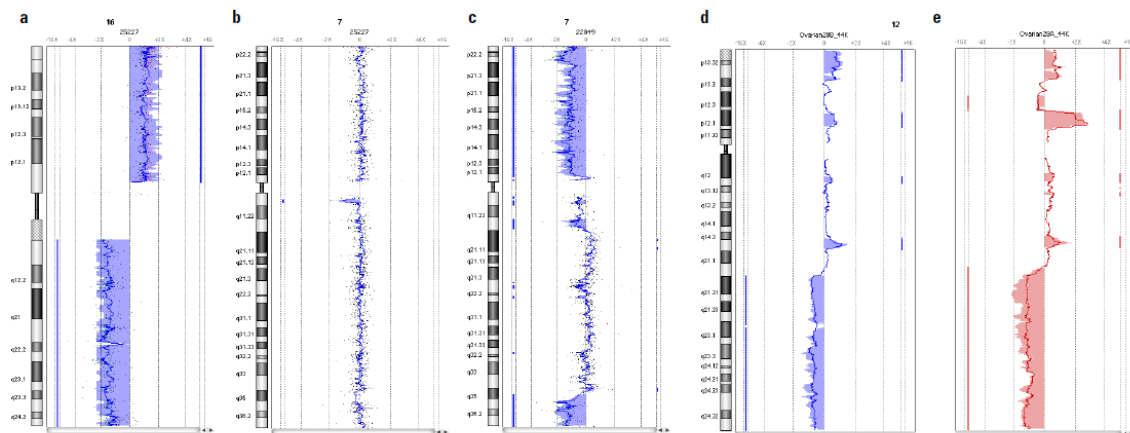


Figure 7. Examples of various chromosomal aberrations in different tumor samples. (a) Gain of p-arm and loss of q-arm on Chromosome 16; (b) single focal deletion on Chromosome 7 and (c) a mix of alterations on the same chromosome from two patients diagnosed with Acute Myeloid Leukemia. Aberrations on Chromosome 12 of ovarian cancer samples obtained from the same patient before (d) and after (e) chemotherapy treatment.

Komparativní genomová hybridizace (CGH)

Comparative Genomic Hybridization for Molecular Cytogenetic Analysis of Solid Tumors

Anne Kallioniemi,* Olli-P. Kallioniemi, Damir Sudar,
Denis Rutovitz, Joe W. Gray, Fred Waldman, Dan Pinkel

Comparative genomic hybridization produces a map of DNA sequence copy number as a function of chromosomal location throughout the entire genome. Differentially labeled test DNA and normal reference DNA are hybridized simultaneously to normal chromosome spreads. The hybridization is detected with two different fluorochromes. Regions of gain or loss of DNA sequences, such as deletions, duplications, or amplifications, are seen as changes in the ratio of the intensities of the two fluorochromes along the target chromosomes. Analysis of tumor cell lines and primary bladder tumors identified 16 different regions of amplification, many in loci not previously known to be amplified.

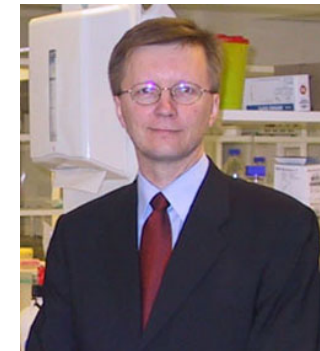
The discovery of genetic changes involved in the development of solid tumors has proven difficult. Karyotyping is impeded by the low number of high-quality metaphase spreads and the complex nature of chromosomal changes (1). Molecular genetic studies of isolated tumor DNA have been more successful and have been used to detect

common regions of allelic loss, mutation, or amplification (2, 3). However, such molecular methods are highly focused; they target one specific gene or chromosome region at a time and leave the majority of the genome unexamined.

We have developed a molecular cytogenetic method, comparative genomic hybridization (CGH), that is capable of detecting and mapping relative DNA sequence copy number between genomes. A copy number karyotype can be generated for a tumor by the comparison of DNAs from malignant and normal cells, thereby identifying regions of gain or loss of DNA. In this application of CGH, biotinylated

A. Kallioniemi, O.-P. Kallioniemi, D. Sudar, J. W. Gray, F. Waldman, D. Pinkel, Division of Molecular Cytometry, Department of Laboratory Medicine, University of California at San Francisco, San Francisco, CA 94143. D. Rutovitz, Medical Research Council Human Genetics Unit, Western General Hospital, Edinburgh EH4 2XU, United Kingdom.

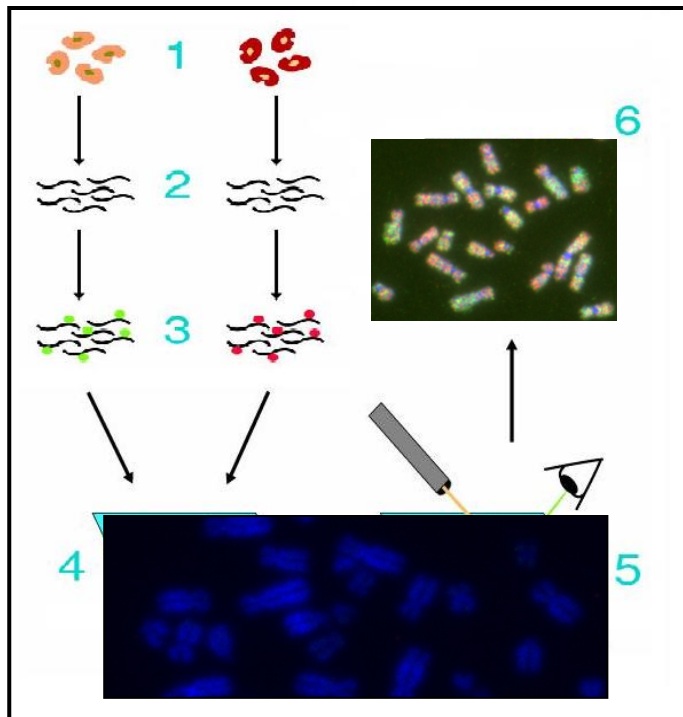
*To whom correspondence should be addressed.



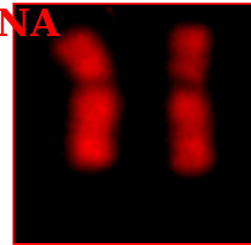
Science, 1992

Komparativní genomová hybridizace (CGH)

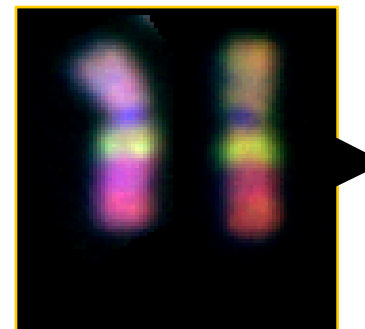
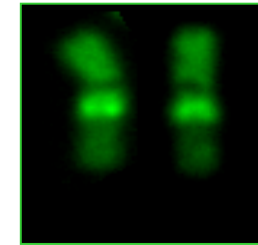
- umožňuje během jedné hybridizační reakce stanovit ztráty (delece) či zisky (duplikace, amplifikace) sekvencí DNA v celém genomu
- rozlišovací schopnost – **10 Mb**, klonální zastoupení – **50 %**
- nevyžaduje mitotickou aktivitu zkoumaných buněk



Referenční DNA

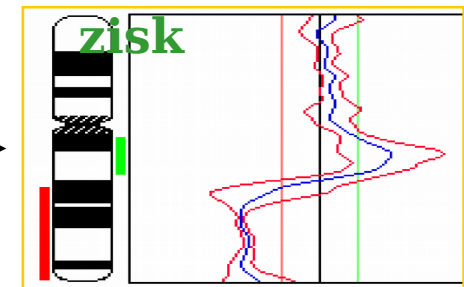


Testovaná DNA



zelená -

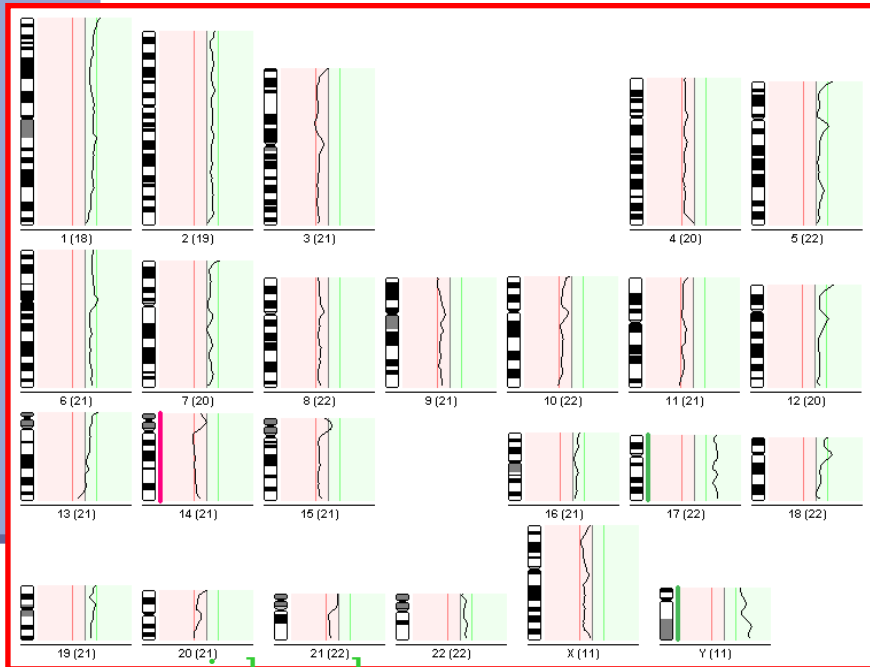
zisk



červená -

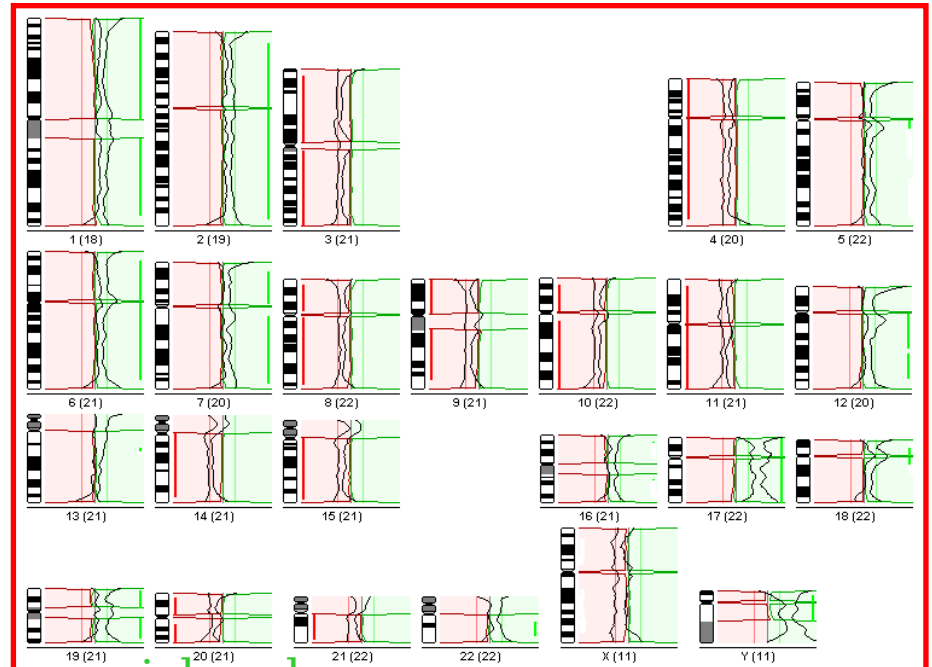
Výsledek vyšetření pacienta s neuroblastomem pomocí CGH a HR-CGH - profily

CGH



rev ish enh
(17,Y)
rev ish dim
(14)

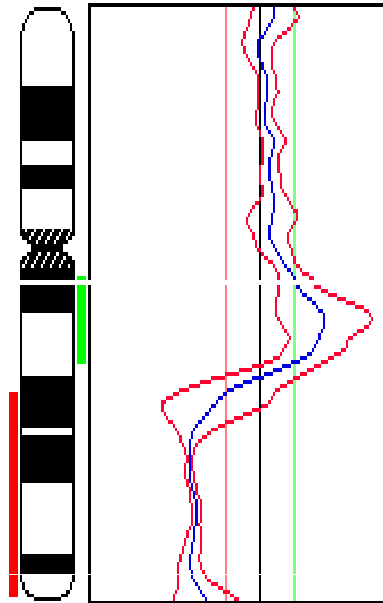
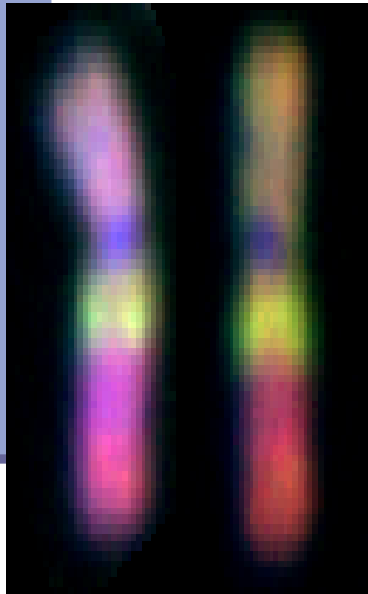
HR-CGH



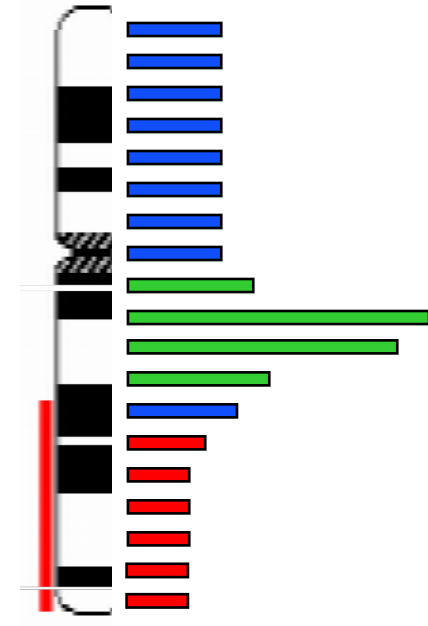
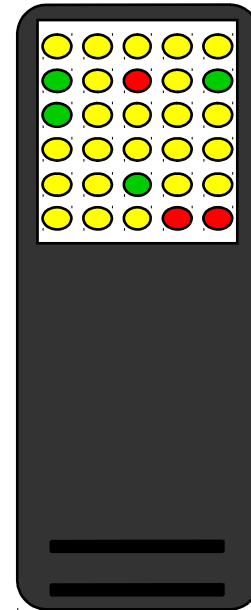
rev ish enh
(1,2,6,7,12q,17,19,22,Y)
rev ish dim
(3,4,8,9,10,11,14,15,20,21)

Technika array-CGH aneb rozdíl je jen v podkladě...

CGH



Array-CGH



Solinas-Toldo et al., Genes, Chromosomes, Cancer (1997): Matrix - CGH
nahrazení chromozomů separovanými klony - BAC, PAC, c-DNA klony,
oligonukleotidy ~ 35 kb

Typy DNA čipů

Zdroje cílových DNA

Diagnostics

Genome
Resolution

Genome
Coverage



>10Mb

100%

G-Banding

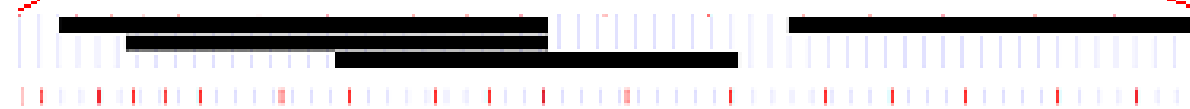
Discovery



>175Kb

96%

BAC arrays (175Kb)



>10Kb

<1%

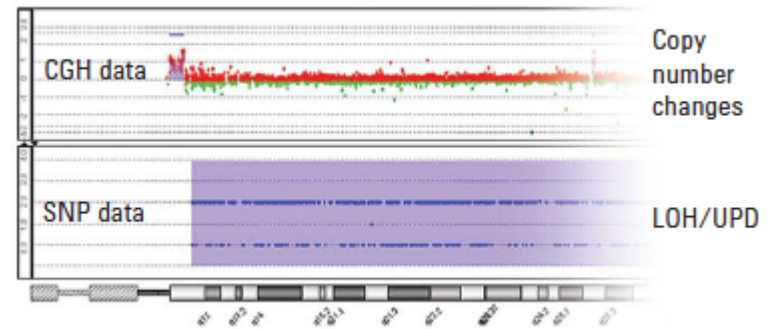
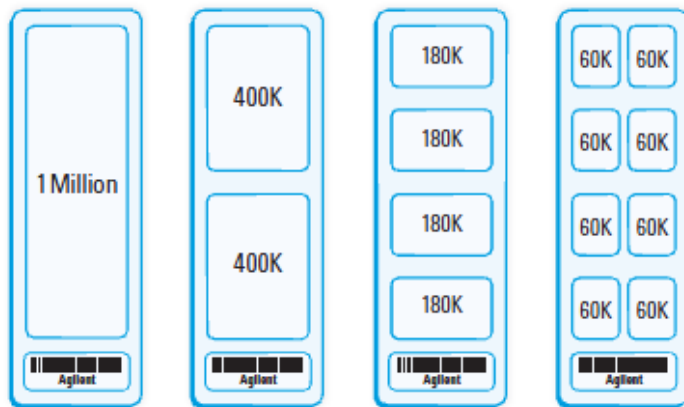
Oligonucleotide arrays (25 – 85mer)

Agilent Technologies CGH microarrays

- Fotolitotrofické spotování – technologie Inkjet Printing technology
- Postup používaný při mikrovýrobě pro selektivní opracování částí tenkých vrstev nebo materiálu podložky
- Pro přenos geometrických obrazců (motivů) do chemicky aktivované vrstvy nanesené na podložce se používá světlo

<http://www.youtube.com/watch?v=eSr5CxAdiww>

Formáty DNA čipů Agilent



Format	Part Number	Median CGH probe spacing	Area of Focus	LOH/UPD resolution
2x400K	G4842A	7kb	Exons	~ 5-10Mb
4x180K	G4890A	25kb	ISCA* regions	~ 5-10Mb

Array CGH: The Complete Process

Step 1

Patient DNA Control DNA

Step 2

Step 3

Step 4

HYBRIDIZATION

Equal hybridization DNA dosage loss DNA dosage gain

Steps 1-3 Patient and control DNA are labeled with fluorescent dyes and applied to the microarray.

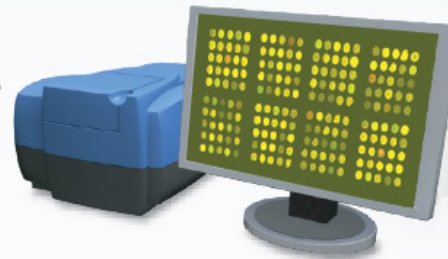
Step 4 Patient and control DNA compete to attach, or hybridize, to the microarray.

Step 5 The microarray scanner measures fluorescent signal intensity.

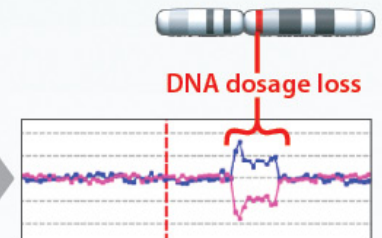
Step 6 Computer software gathers the data and generates a plot.

Step 5

Step 6



COMPUTER SOFTWARE



DATA PLOT
(Chromosome 7)

Příprava DNA čipů pro techniku array-CGH

1. Target loading



2. Assemble hybrid. chamber



3. Incubation



4. Wash



5. Scanner



Kontrola kvality (QC metrics)

- **DLRSpread**

- odchylka od průměru intenzity signálu v jednom spotu
- výše „šumu (noise)“ na vzorek (DLRs < 0,3)

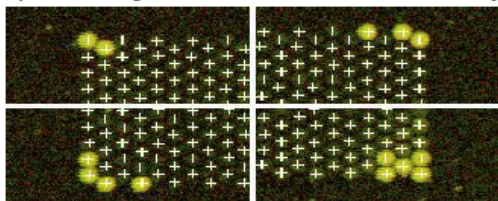
- **Signal Intensity** – pro každý fluochrom (Cy3 > 250, Cy5 > 350)

- **Background noise** – intenzita nespecifických signálů pozadí (< 10)

- **Signal to Noise Ratio** – vzdálenost hodnoty signálu od hodnoty pozadí
= s jakou jistotou je SW schopen odečíst intenzitu spotu od pozadí

Date	Monday, March 09, 2015 - 15:32	Sample(red/green)	2.9.2.4
User Name	admin	FE Version	
Image	252983024981_S01 [1_1]	BG Method	Detrend on (NegC)
Protocol	CytoCGH_0209_4x_Mar14 (Read Only)	Multiplicative Detrend	True
Grid	029830_20111015	Dye Norm	Linear
Saturation Value	779183 (r), 778309 (g)		
DyeNorm List		NA	
No of Probes in DyeNorm List		NA	

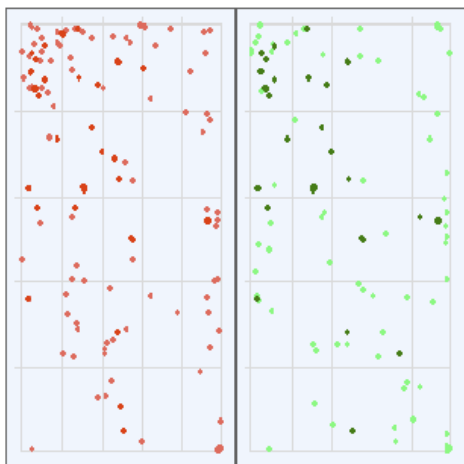
Spot Finding of the Four Corners of the Array



Grid Normal

Outlier Numbers with Spatial Distribution

1064 rows x 170 columns



• Red FeaturePopulation • Red Feature NonUniform
• Green FeaturePopulation • Green Feature NonUniform

Feature	Red	Green	Any	%Outlier
Non Uniform	62	49	68	0.04
Population	100	83	158	0.09

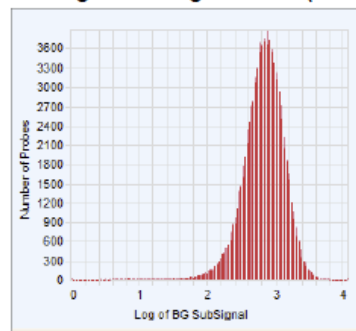
Evaluation Metrics for CytoCGH_QCMT_4x_Mar14

Excellent (11) ; Good (2)

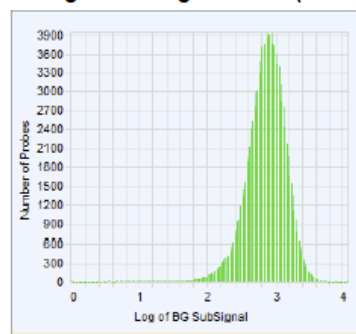
Metric Name	Value	Excellent	Good	Evaluate
IsGoodGrid	1.00	>1		NA
AnyColorPrctFeatNonU...	0.04	<1	1 to 5	>5
DerivativeLR_Spread	0.17	<0.20	0.20 to 0.30	>0.30
gRepro	0.07	0 to 0.10	0.10 to 0.20	<0 or >0.20
g_BGNoise	4.07	<10	10 to 20	>20
g_Signal2Noise	157.72	>100	50 to 100	<50
g_SignalIntensity	641.42	>400	200 to 400	<200
rRepro	0.10	0 to 0.10	0.10 to 0.20	<0 or >0.20
r_BGNoise	4.27	<10	10 to 20	>20
r_Signal2Noise	137.92	>100	50 to 100	<50
r_SignalIntensity	589.18	>400	200 to 400	<200
RestrictionControl	0.94		0.80 to 1	<0.80 or >1
LogRatioImbalance	-0.14	-0.26 to 0.26	(-0.75 to -0.2...	<-0.75 or >0.75

• Excellent • Good • Evaluate

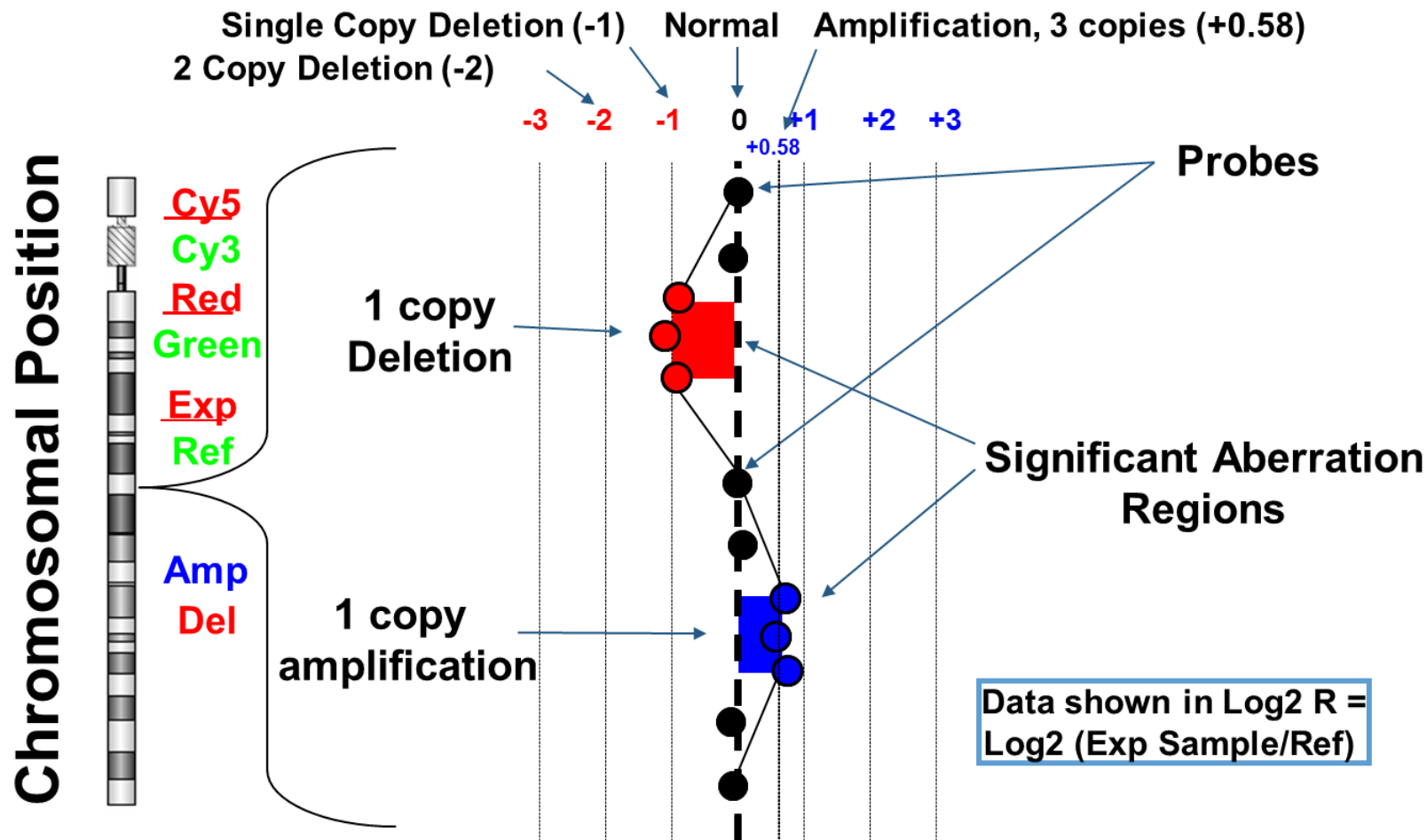
Histogram of Signals Plot (Red)



Histogram of Signals Plot (Green)



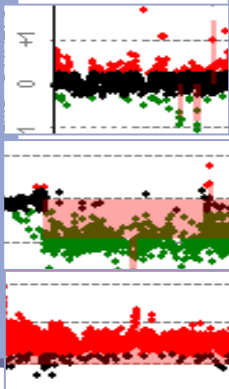
Princip hodnocení CGH dat



Princip hodnocení CGH dat

„matematika cgh“

Hodnotíme rozdíl mezi intenzitou fluo signálu mezi vzorkem DNA a referencí vyjádřený jako $\log_2 X (= \text{vzorek} / \text{reference})$. Data jsou lehce komprimovaná kvůli biologické variabilitě vzorku



Description	Average Sample CN	Ref CN	Ratio (S/R)	Ideal Log2(Ratio)	Actual data
Diploid	2	2	1	0	0
Deletion	1	2	0.5	-1	-0.9
Trisomy	3	2	1.5	+0.58	+0.53

Princip hodnocení CGH dat

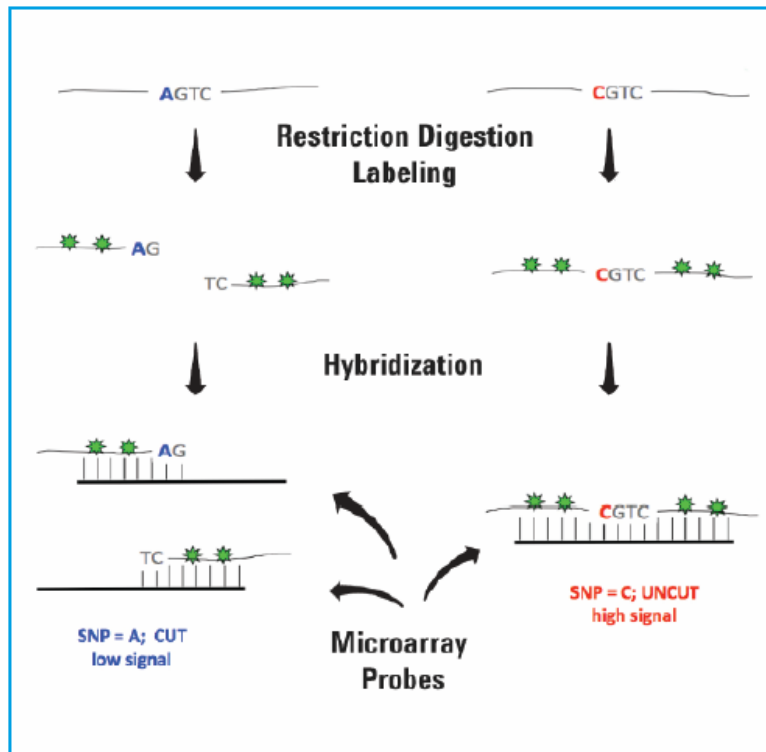
„matematika cgh“

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Diploid	2	2	1	0	0
Deletion	1	2	0.5	-1	-0.9
Trisomy	3	2	1.5	+0.58	+0.53
50% mosaic deletion	1.5	2	0.75	-0.41	-0.37
50% mosaic trisomy	2.5	2	1.25	+0.32	+0.29
20% mosaic deletion	1.8	2	0.9	-0.15	-0.13
20% mosaic trisomy	2.2	2	1.1	+0.14	0.12

Analýza oblastí UPD /LOH

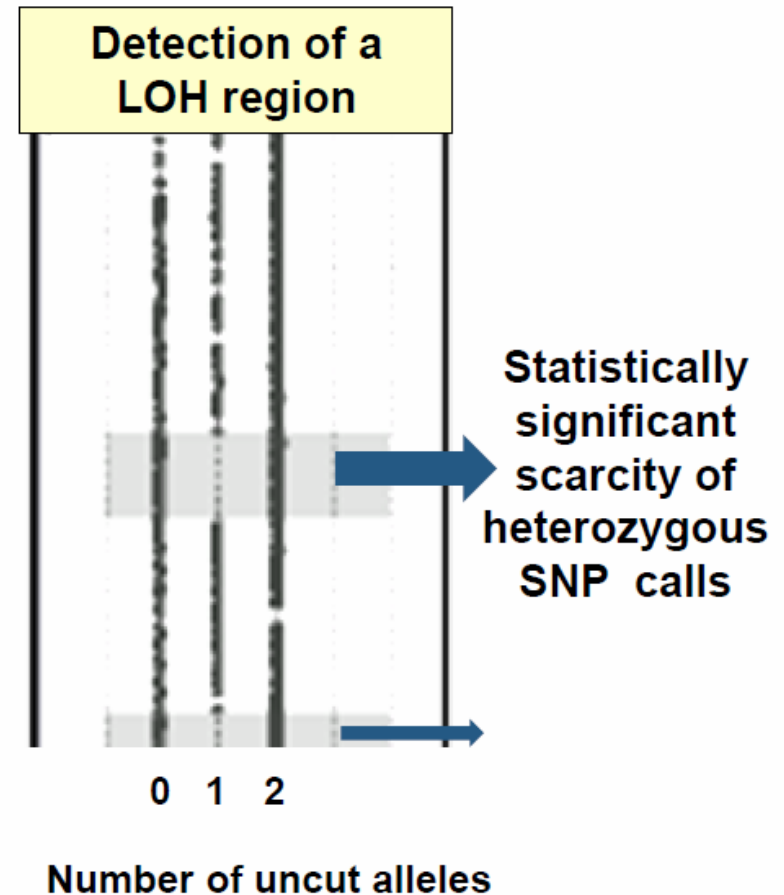
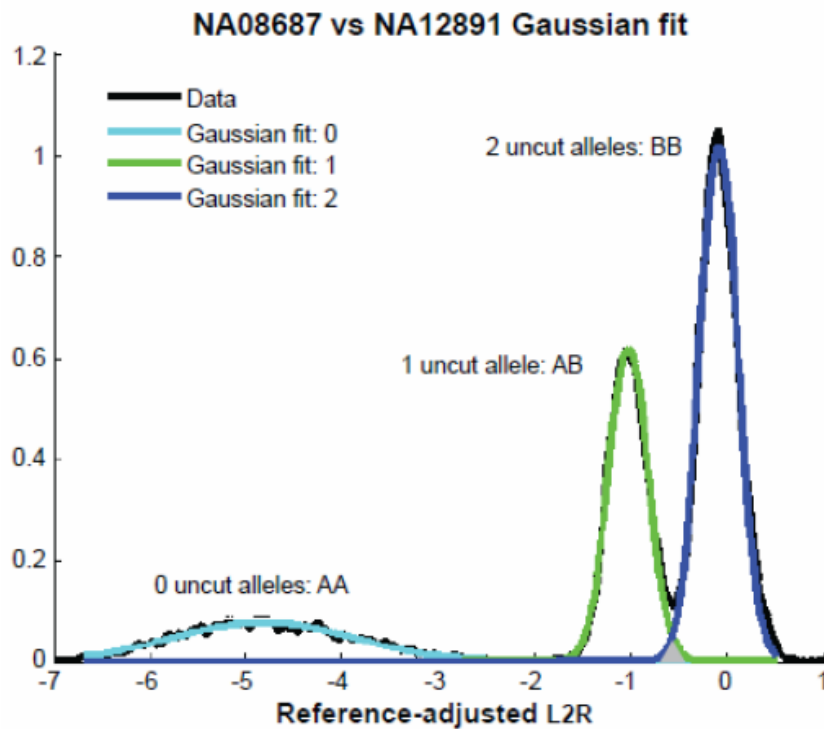
Measuring SNPs using restriction enzymes



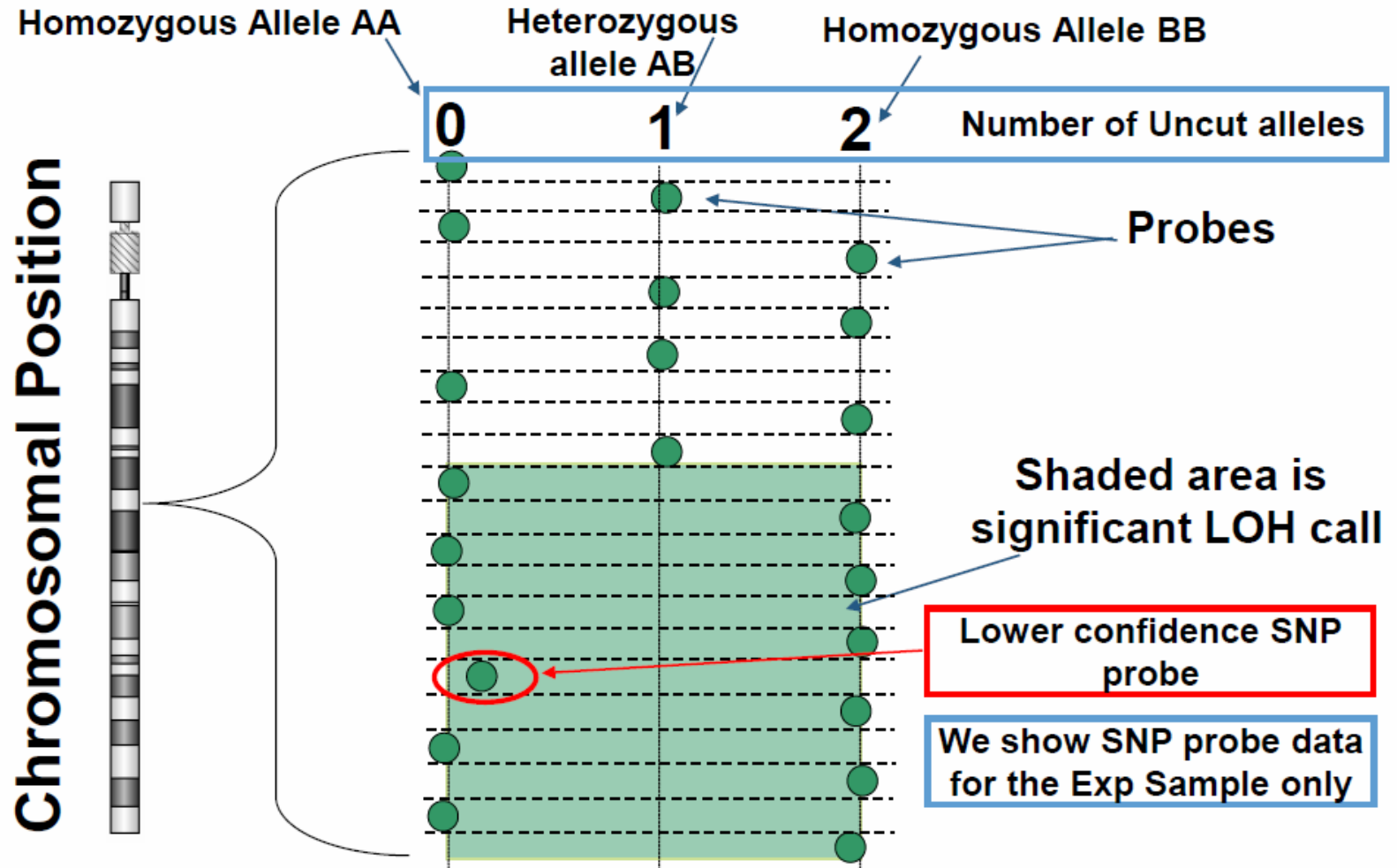
- A subset of probes on a CGH array measures SNPs in parallel to copy number, **on a single array**
- Genotype SNPs using **restriction digestion** (Alu/Rsa)
- Measure the copy number of one allele at each SNP site relative to a **known reference**
- Regions of LOH are located by finding genomic regions with a **statistically significant scarcity of heterozygous calls**
- **~5-10 Mb LOH/UPD resolution** across the entire genome

Analýza oblastí UPD /LOH

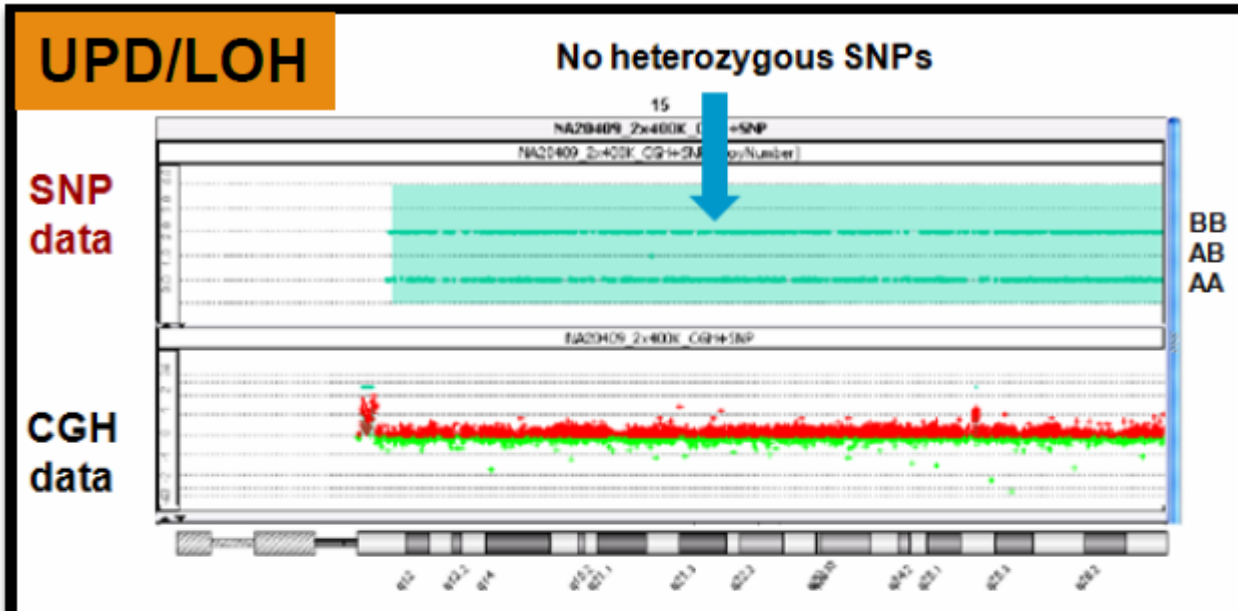
Distribution of the SNP Probe reference-corrected log2 ratios



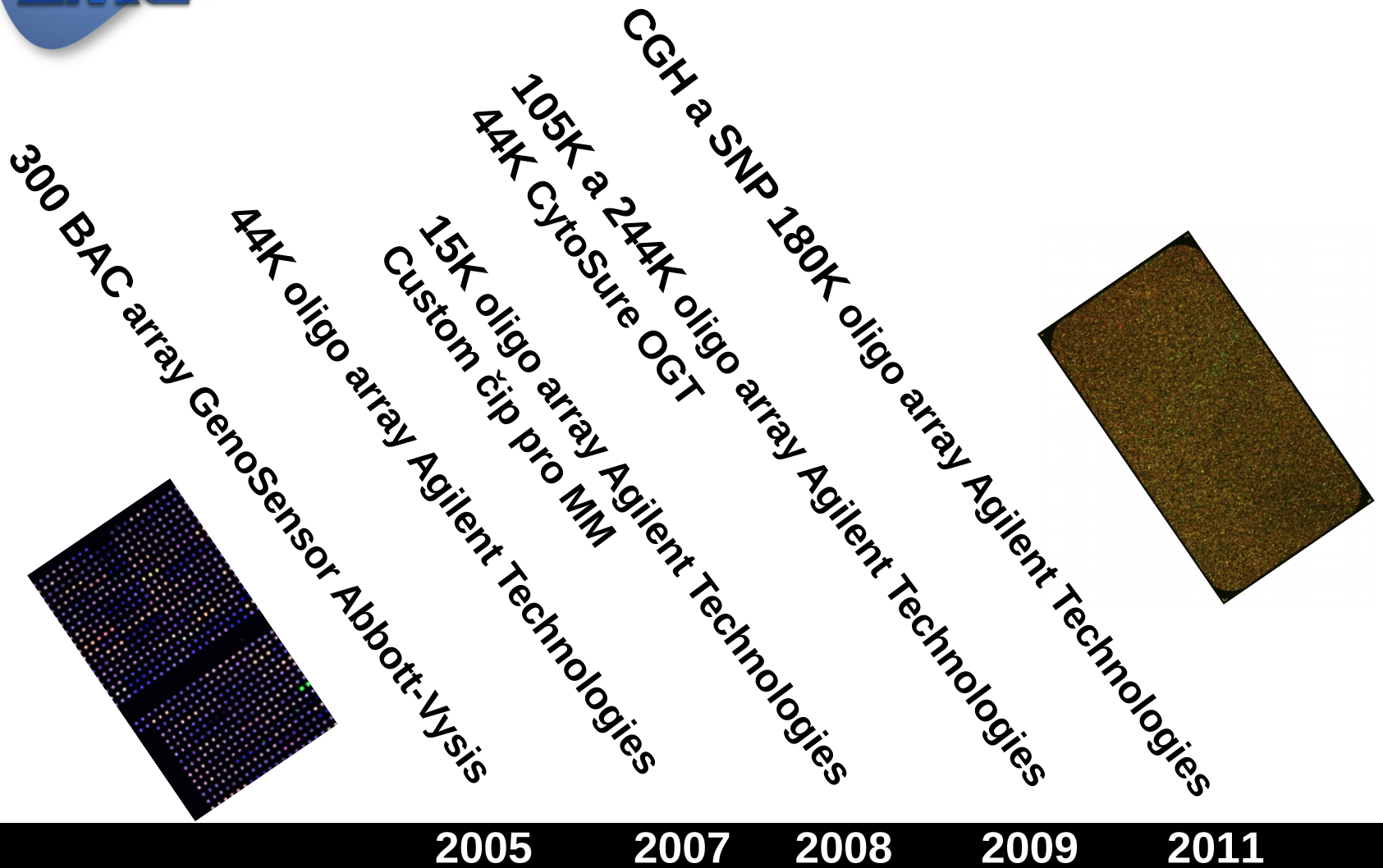
Analýza oblastí UPD /LOH



Analýza oblastí UPD /LOH



Historie array CGH v Laboratoři molekulární cytogenetiky



Využití array CGH v klinické diagnostice na OLG FN Brno



Klinická cytogenetika

Postnatální vyšetření pacientů OLG (pacienti s MR/VVV)

- Od roku 2007
- 44K Agilent Technologies
- 44K CytoSure OGT (Syndrome Plus)
- 105K, 244K Agilent Technologies
- 180 K Agilent Technologies

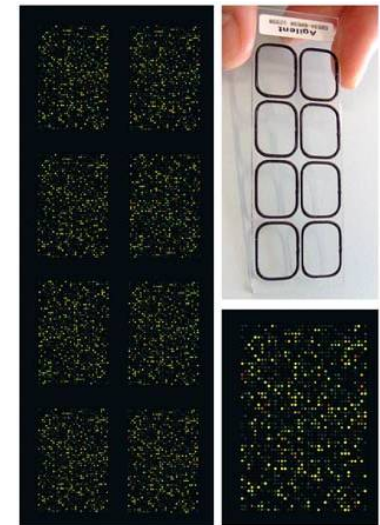
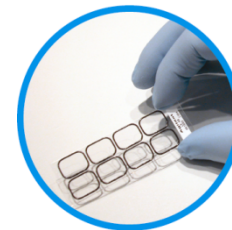
Nádorová cytogenetika

Vyšetření pacientů KDO (neuroblastom)

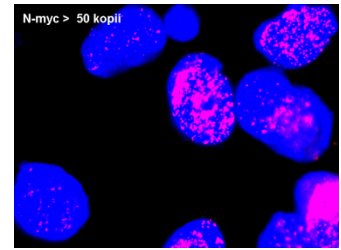
- Od roku 2009
- 44K Agilent Technologies

Vyšetření pacientů s mnohočetným myelomem

- Od roku 2009
- 44K Agilent Technologies



Neuroblastom



- pevný dětský nádor sympatického nervového systému
- 5–10 případů /10⁶ dětí/rok
- 10 % rakoviny v dětském věku; 50 % do 2 let; 90 % do 6 let
- klinická stádia (1,2 / 3,4; 4S) (International Staging System for Neuroblastoma)

G.M.Brodeur et al., 1995: Molecular Basis for Heterogeneity in Human Neuroblastomas

Charakteristika	TYP I (LR)	Typ II (IMR)	TYP III (HR)
N-myc	normální	normální	amplifikováno
1p36 LOH	ne	±ano	obvykle ano
Ploidie	hyper2n / 3n	2n / 4n	2n / 4n
Věk*	obvykle < 1rok	obvykle = 1 rok	obvykle 1-5 let
Stadium ^o	obvykle 1, 2, 4s	obvykle 3, 4	obvykle 3, 4
Pravděpodobnost přežití 3 roky	95%	25-50%	< 5%

* v době stanovení diagnózy
• International Staging System for Neuroblastoma

Význam array CGH u dětských pacientů s neuroblastomem

VOLUME 27 · NUMBER 7 · MARCH 1 2009

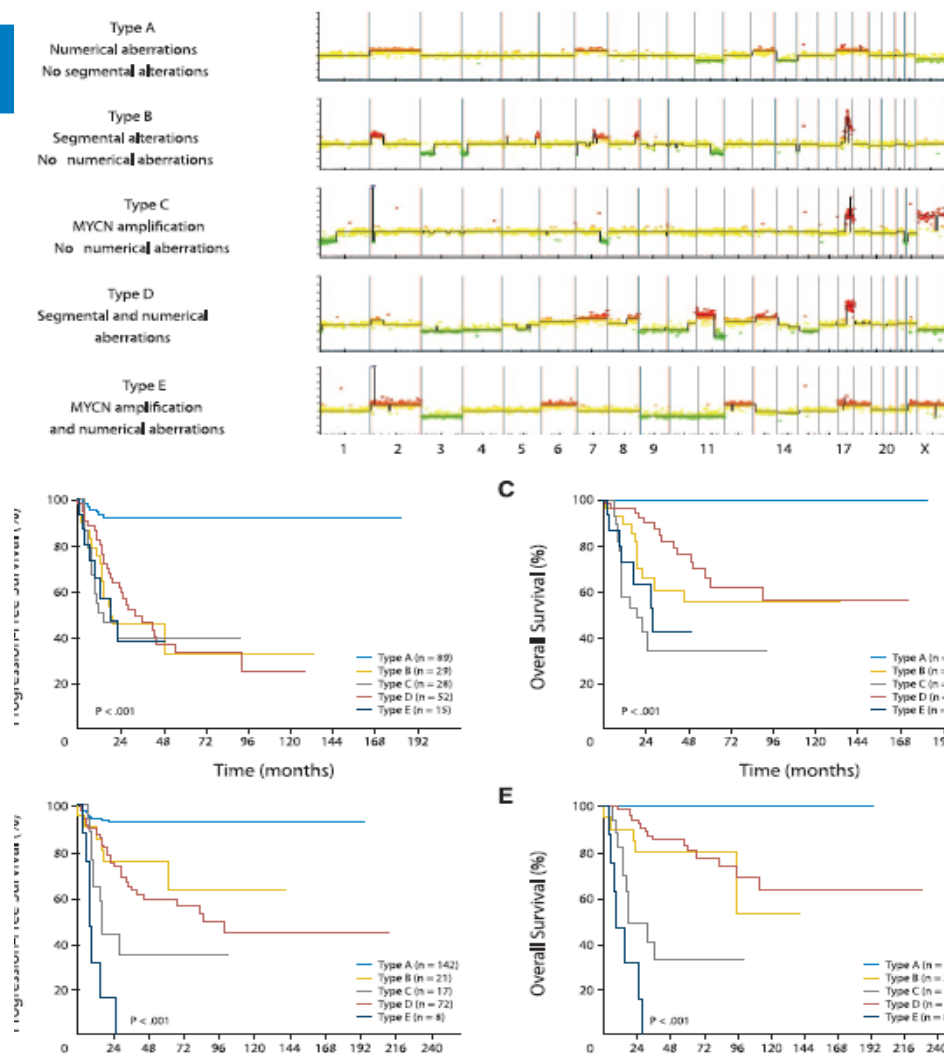
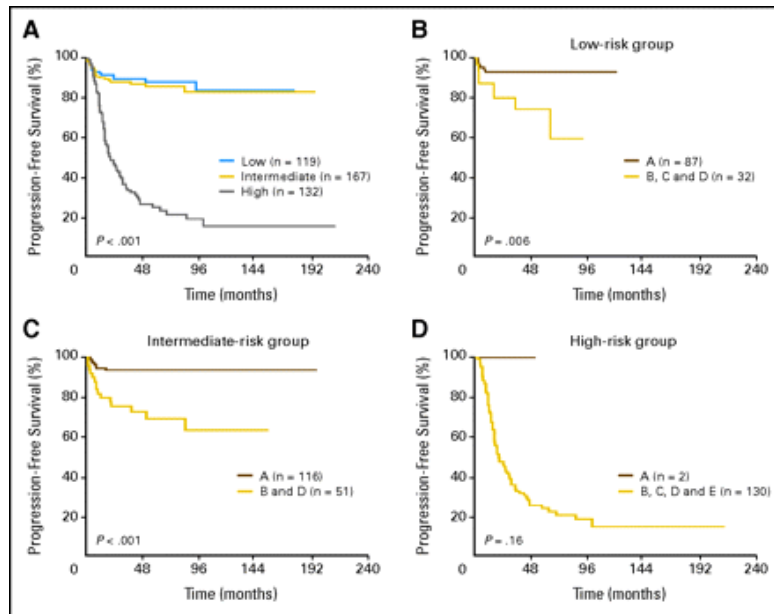
JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

From L'Institut National de la Santé et de la Recherche Médicale (INSERM) U830, Laboratoire de Génétique et Biologie des Cancers; Institut Curie, Département de Pédiatrie; Service de Biostatistiques, Unité de Génétique Somatique, and Unité de Cytogénétique; Hôpital Robert Debré, Service de Pathologie, Paris; Institut Gustave Roussy, Département de Biologie et de Pathologie Moléculaire; Service de Pathologie Médicale; Département de Pédiatrie, Villejuif; Centre Hospitalier

Overall Genomic Pattern Is a Predictor of Outcome in Neuroblastoma

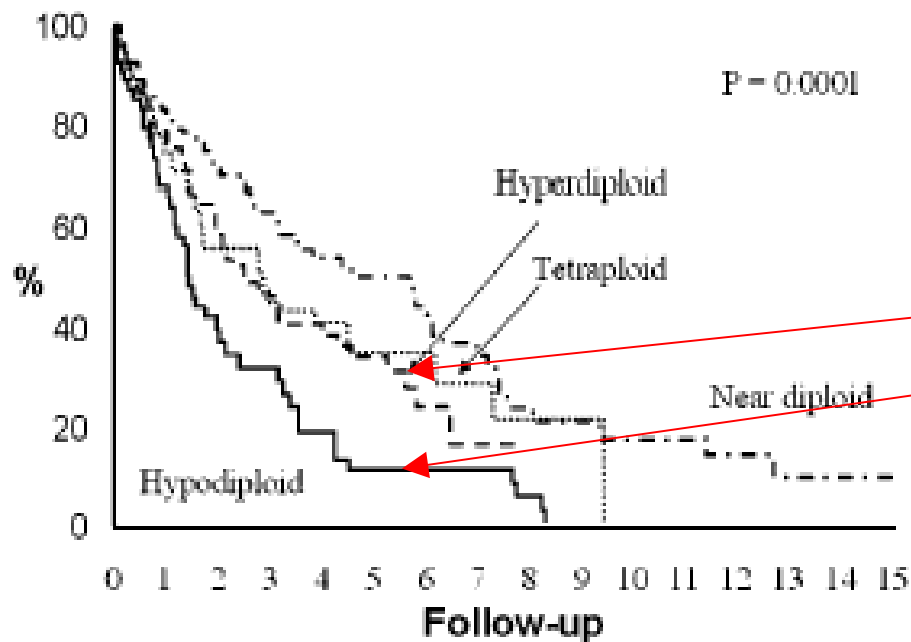
Isabelle Janoueix-Lerosey, Gudrun Schleiermacher, Evi Michels, Véronique Mosseri, Agnès Ribeiro, Delphine Lequin, Joëlle Vermeulen, Jérôme Couturier, Michel Peuchmaur, Alexander Valent,



Mnohočetný myelom (MM)

- hematoomkologické onemocnění, **jehož podstatou je maligní transformace B-lymfocytů** na maligní plazmocyty - akumulace plazmocytů buněk v kostní dřeni
- **klinické projevy**: destrukce kostí, anemie, porucha protilátkové imunity, myelomová nefropatie ...
- Postihuje zejména starší osoby, v současné době nevyлéčitelný x udržitelnost
- **Výrazně geneticky heterogenní – aCGH screening + FISH (IgH)**

Array CGH u pacientů s MM



Status	5 Year Survival (%)
Pseudodiploid	49.9
Near-tetraploid	34.6
Hyperdiploid	33.5
Hypodiploid	10

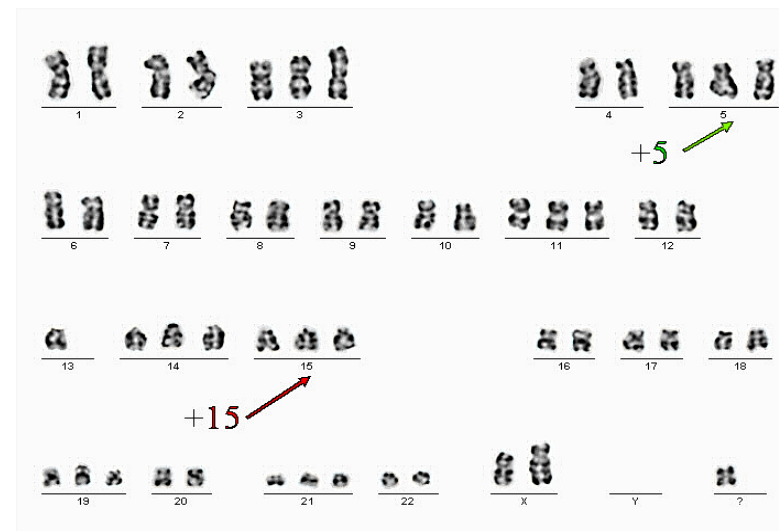
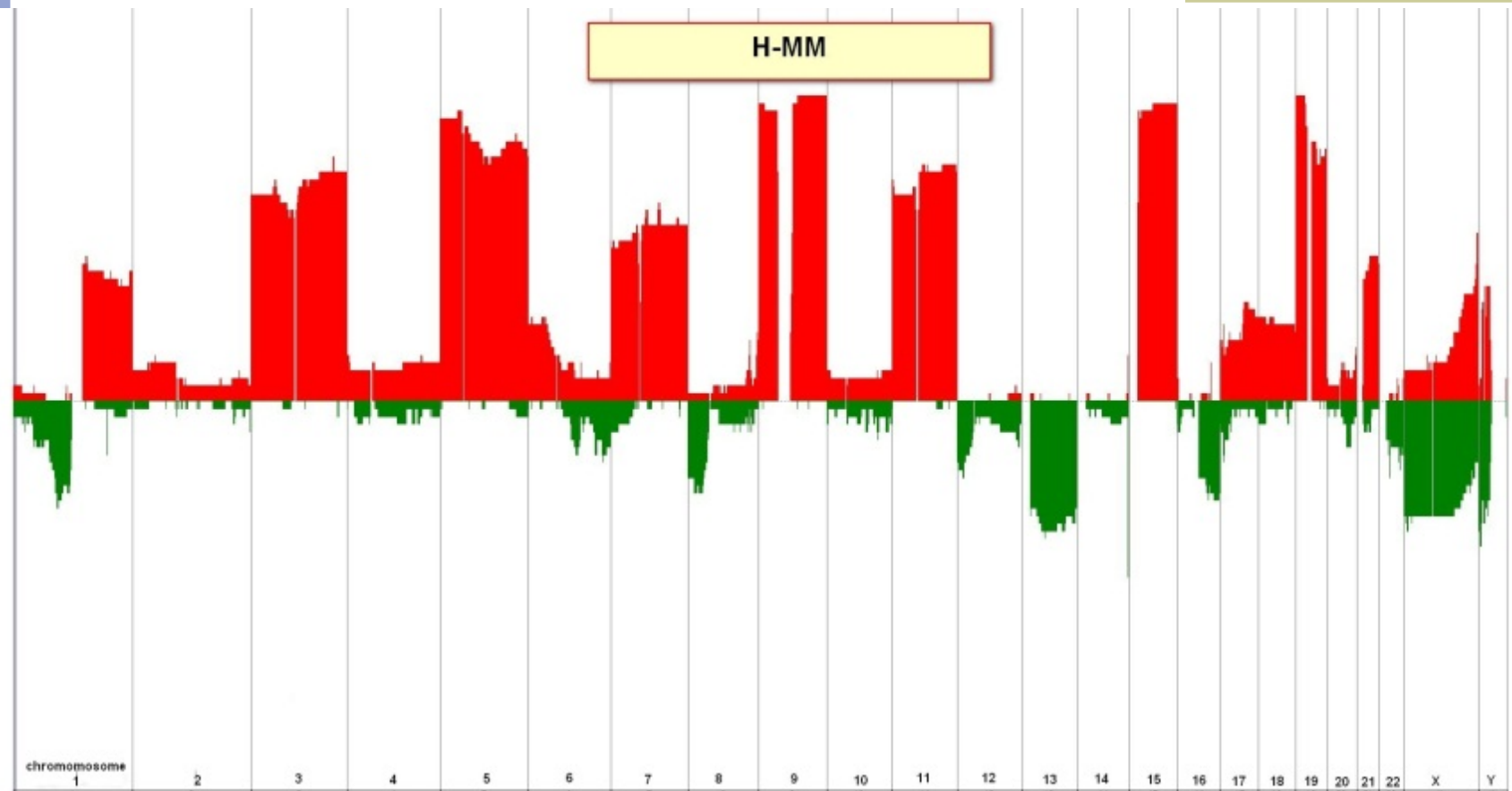


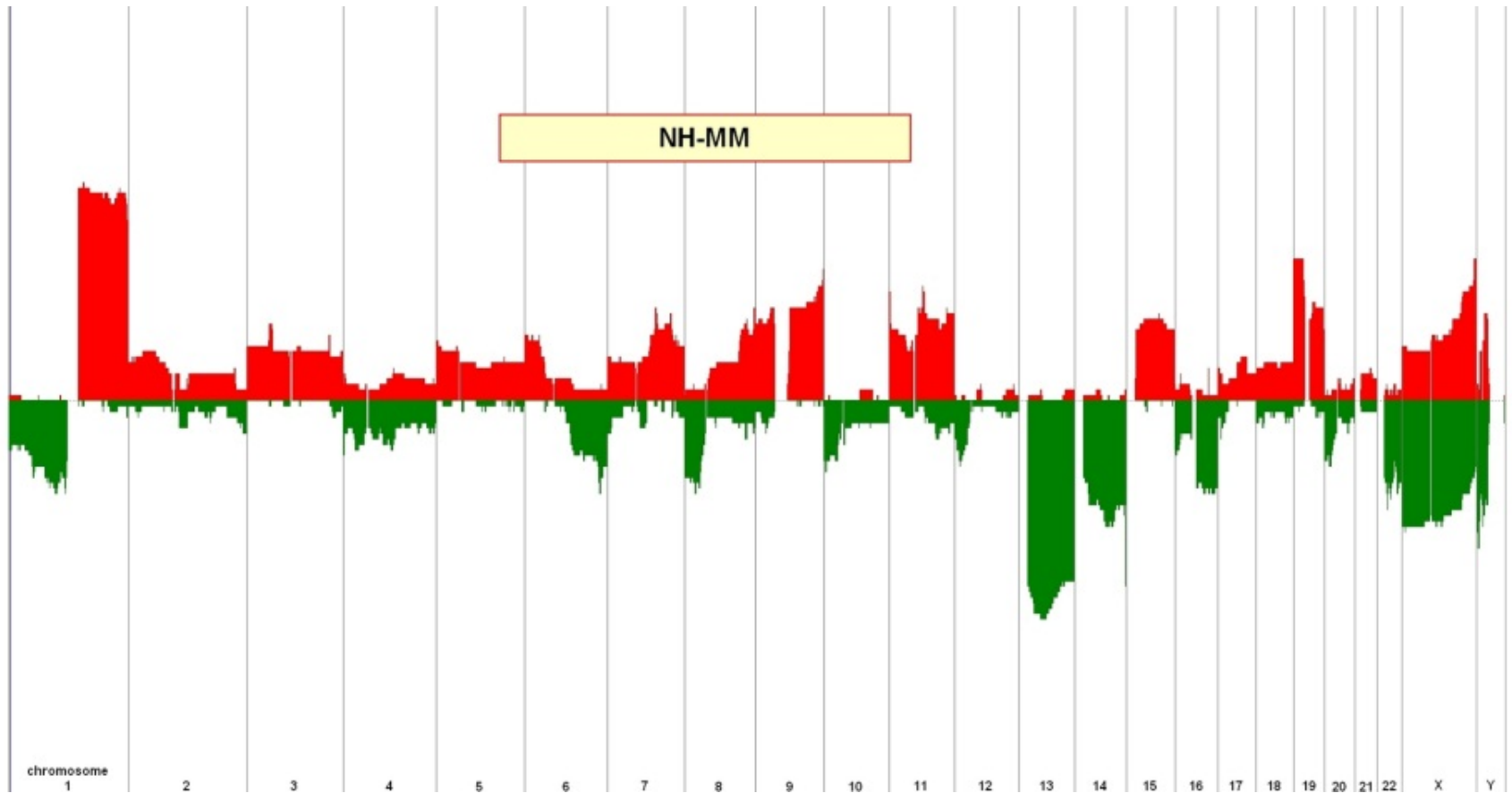
Figure 6 Overall survival of patients according to ploidy status. Kaplan-Meier survival analysis of patients according to ploidy category. The survival since diagnosis time is presented in the x-axis in years and the *P* value is the univariate log-rank probability. The chromosomal abnormality in question is always represented by the dotted line.

Debes-Marun CS et al. "Chromosome abnormalities clustering and its implication for pathogenesis and prognosis in myeloma". *Leukemia* (2003), 17:427- 436

Hyperdiploidie u MM

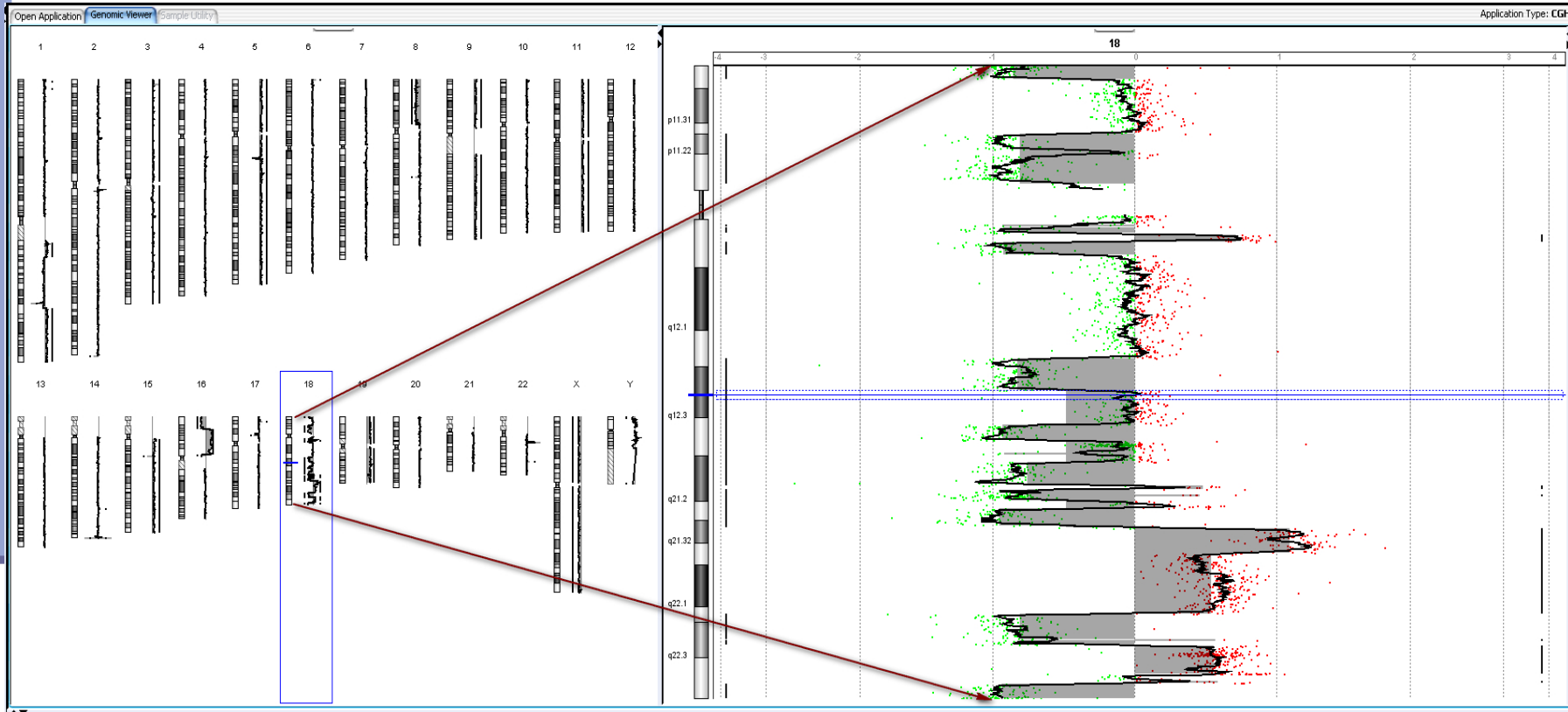


Non-hyperdiploidní MM



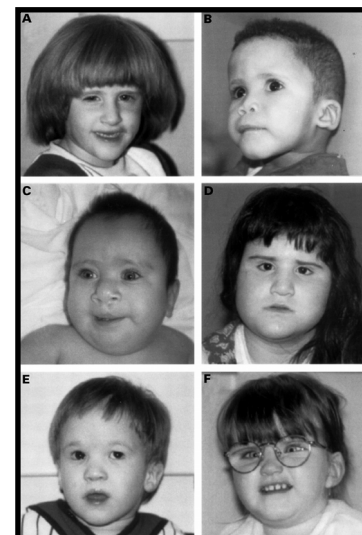
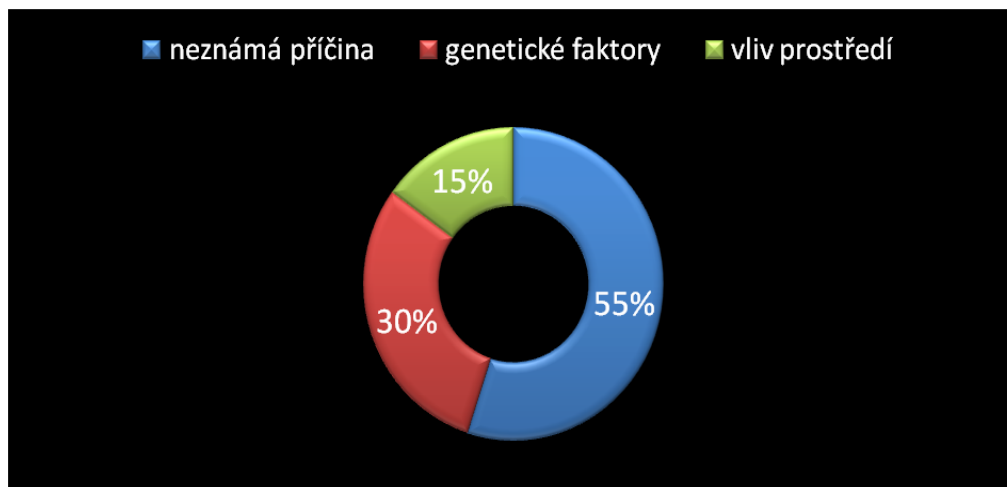
Význam array CGH u pacientů s MM

Hyperdiploidní MM + chromotripsis 18



Mentální retardace (MR): příčiny

Výskyt MR u 2-3 % populace



17- 20% pacientů s idiopatickou PMR
kauzální nebalancované aberace
celogenomový screening pomocí array-CGH →



Častý nález CNV - mikroleceí/mikroduplikací v genomu - mohou být postiženy různé chromozomy

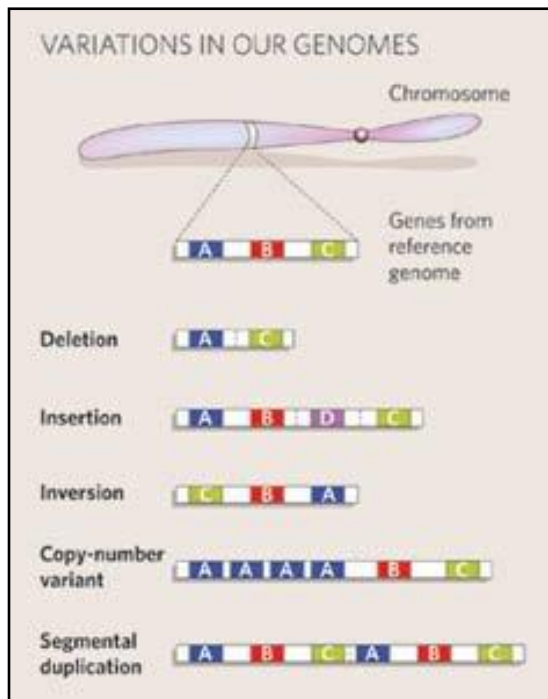
Consensus Statement: Chromosomal Microarray Is a First-Tier Clinical Diagnostic Test for Individuals with Developmental Disabilities or Congenital Anomalies

David T. Miller,^{1,*} Margaret P. Adam,^{2,3} Swaroop Aradhya,⁴ Leslie G. Biesecker,⁵ Arthur R. Brothman,⁶ Nigel P. Carter,⁷ Deanna M. Church,⁸ John A. Crolla,⁹ Evan E. Eichler,¹⁰ Charles J. Epstein,¹¹ W. Andrew Faucett,² Lars Feuk,¹² Jan M. Friedman,¹³ Ada Hamosh,¹⁴ Laird Jackson,¹⁵ Erin B. Kaminsky,² Klaas Kok,¹⁶ Ian D. Krantz,¹⁷ Robert M. Kuhn,¹⁸ Charles Lee,¹⁹ James M. Ostell,⁸ Carla Rosenberg,²⁰ Stephen W. Scherer,²¹ Nancy B. Spinner,¹⁷ Dimitri J. Stavropoulos,²² James H. Tepperberg,²³ Erik C. Thorland,²⁴ Joris R. Vermeesch,²⁵ Darrel J. Waggoner,²⁶ Michael S. Watson,²⁷ Christa Lese Martin,² and David H. Ledbetter^{2,*}

Chromosomal microarray (CMA) is increasingly utilized for genetic testing of individuals with unexplained developmental delay/intellectual disability (DD/ID), autism spectrum disorders (ASD), or multiple congenital anomalies (MCA). Performing CMA and G-banded karyotyping on every patient substantially increases the total cost of genetic testing. The International Standard Cytogenomic Array (ISCA) Consortium held two international workshops and conducted a literature review of 33 studies, including 21,698 patients tested by CMA. We provide an evidence-based summary of clinical cytogenetic testing comparing CMA to G-banded karyotyping with respect to technical advantages and limitations, diagnostic yield for various types of chromosomal aberrations, and issues that affect test interpretation. CMA offers a much higher diagnostic yield (15%–20%) for genetic testing of individuals with unexplained DD/ID, ASD, or MCA than a G-banded karyotype (~3%, excluding Down syndrome and other recognizable chromosomal syndromes), primarily because of its higher sensitivity for submicroscopic deletions and duplications. Truly balanced rearrangements and low-level mosaicism are generally not detectable by arrays, but these are relatively infrequent causes of abnormal phenotypes in this population (<1%). Available evidence strongly supports the use of CMA in place of G-banded karyotyping as the first-tier cytogenetic diagnostic test for patients with DD/ID, ASD, or MCA. G-banded karyotype analysis should be reserved for patients with obvious chromosomal syndromes (e.g., Down syndrome), a family history of chromosomal rearrangement, or a history of multiple miscarriages.

Array-CGH a variabilita v počtu kopií (CNVs)

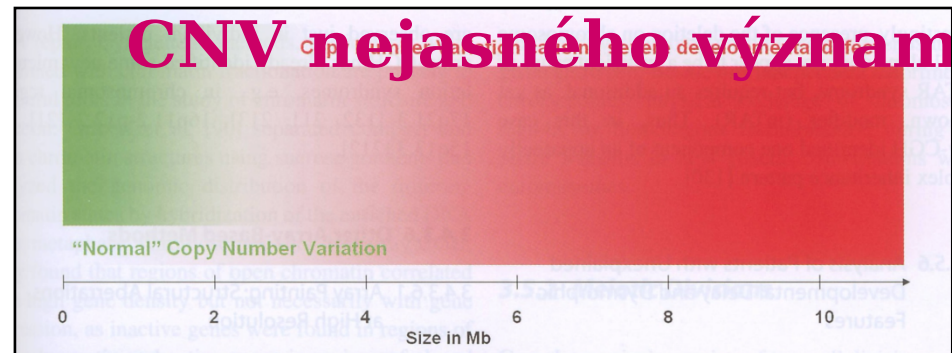
CNVs – segmenty DNA větší než 1 kb přítomné ve variabilním počtu kopií v porovnání s referenčním genomem



- doposud detekováno **29 133 CNV**
- 12 % lidského genomu obsahuje CNV
- 0,12 – 7,3 % rozdíly v CNV mezi jedinci
- 41 % všech CNV pokrývá geny

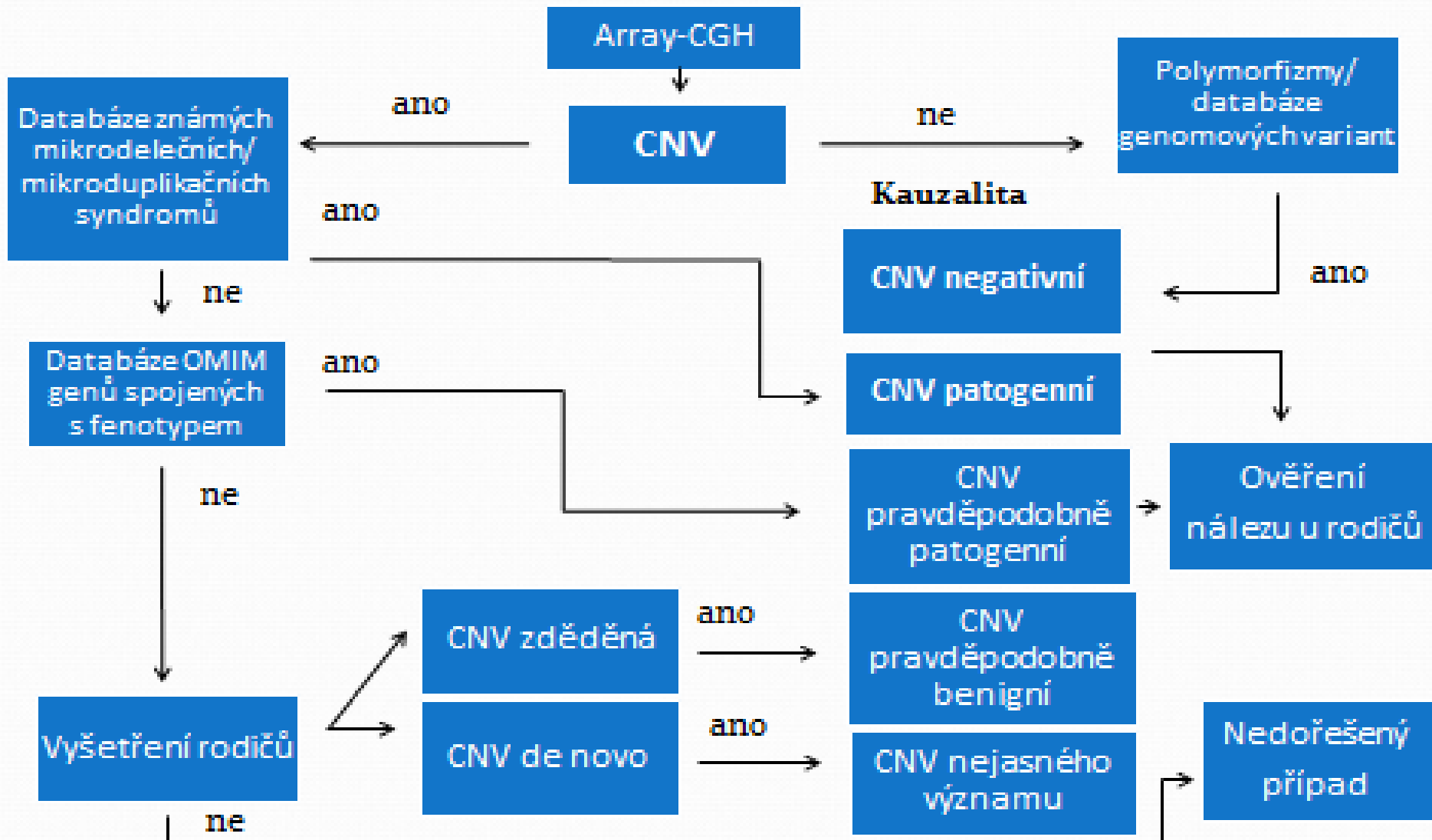
CNV patogenní x benigní

CNV nejasného významu



Algoritmus klinické interpretace CNAs u pacientů s PMR vyšetřených pomocí array-CGH pro rok 2015

Gijsbers et al. 2009



Array-CGH u pacientů s MR - korelace genotypu s fenotypem

Databáze jsou důležité pro:

- klinickou interpretaci výsledků array-CGH
- identifikaci kauzálních regionů pro nové chromozomové syndromy
- možnost vyhledat stejnou či podobnou aberaci a srovnat fenotypové projevy
- možnost rozlišení benigních CNVs
- odhad prognózy dle typu a funkce genů nacházejících se v sledované oblasti

Array-CGH u pacientů s MR - korelace genotypu s fenotypem

Využití web. databází k interpretaci výsledků

■ **DECIPHER:**

■ **ECARUCA:**

■ **GENOGLYPHIX:**

■ **CARTAGENIA BENCH:**



cartagenia

All tomorrow's genomes

Syndrom AMME

Alportův syndrom s mentální retardací, mid-face hypoplazií a eliptocytosou.

Vyřešení neobjasněné kazuistiky z r. 2005



Vranová, V., Hladílková, E., Hořínová, V., Kuglík, P.: Chromosome Xq22.3 gene deletion syndrome (ATS-MR) in a Czech family: additional characterization of the clinical and molecular aspects.

O.M. nar. 2005

- Na JIP v Brně 3 měsíce:
- Dg.: Sepsa, respirační selhání, neonatální hepatitida nejasné etiologie, zdvojení ledv. pánevičky vlevo, hyperechogenita ledvin, DAP s plic. hypertenzí a chlopenní insuficiencí, aorto-pulmonální kolaterály
- **Genetické vyšetření: stigmatizovaný novorozenec – plochý obličej, hypertelorismus, epicanthy, vpáčený široký kořen nosu, dyspl. nízko posazené ušní boltce, krátký hrudník, mikropenis, kryptorchismus, opičí rýha**
- karyotyp 46,XY
- CGH negativní



Strýc R.V. nar. 1988

sy Wiedemann-Rautenrausch

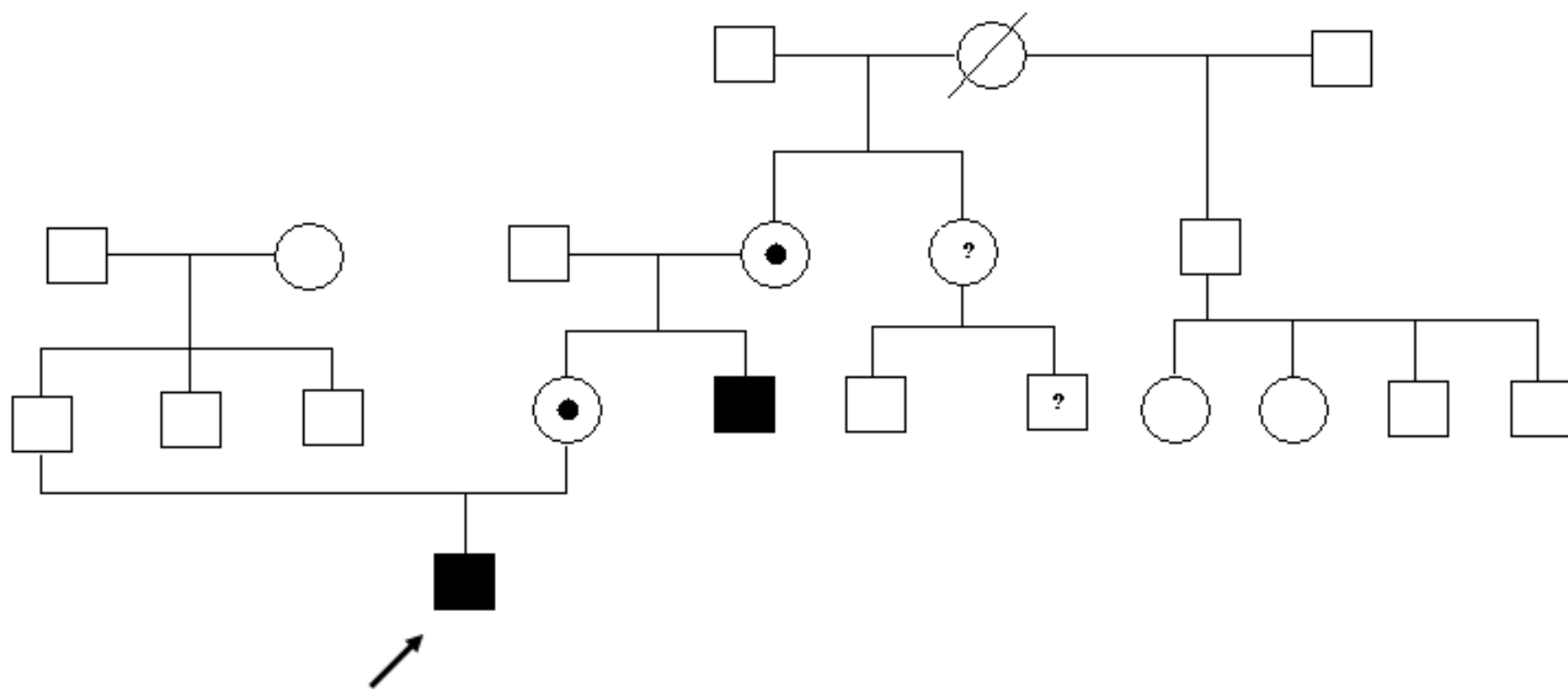
- Ze II. gravidity, nekomplikované, porod ve 39. t.g., s.z., p.v. 2900/49cm, hosp. v DN po porodu s dg. stigmatizace, glykosurie, hyperbilirubinemie, plicní hypertenze, neprospívání.
- II. hospitalizace ve VÚPs dg. stigmatizace, hepatopatie, snížená funkce levé ledviny, PMR.
- Vyšetřen na OLG: **plochý obličej, hypertelorismus, epicanthy, hypoplasie nosních kostí, kapří ústa, opičí rýhy, náznak srůstů 2-3. prstů DK, mikropenis, kryptorchismus.**
- Karyotyp 46,XY

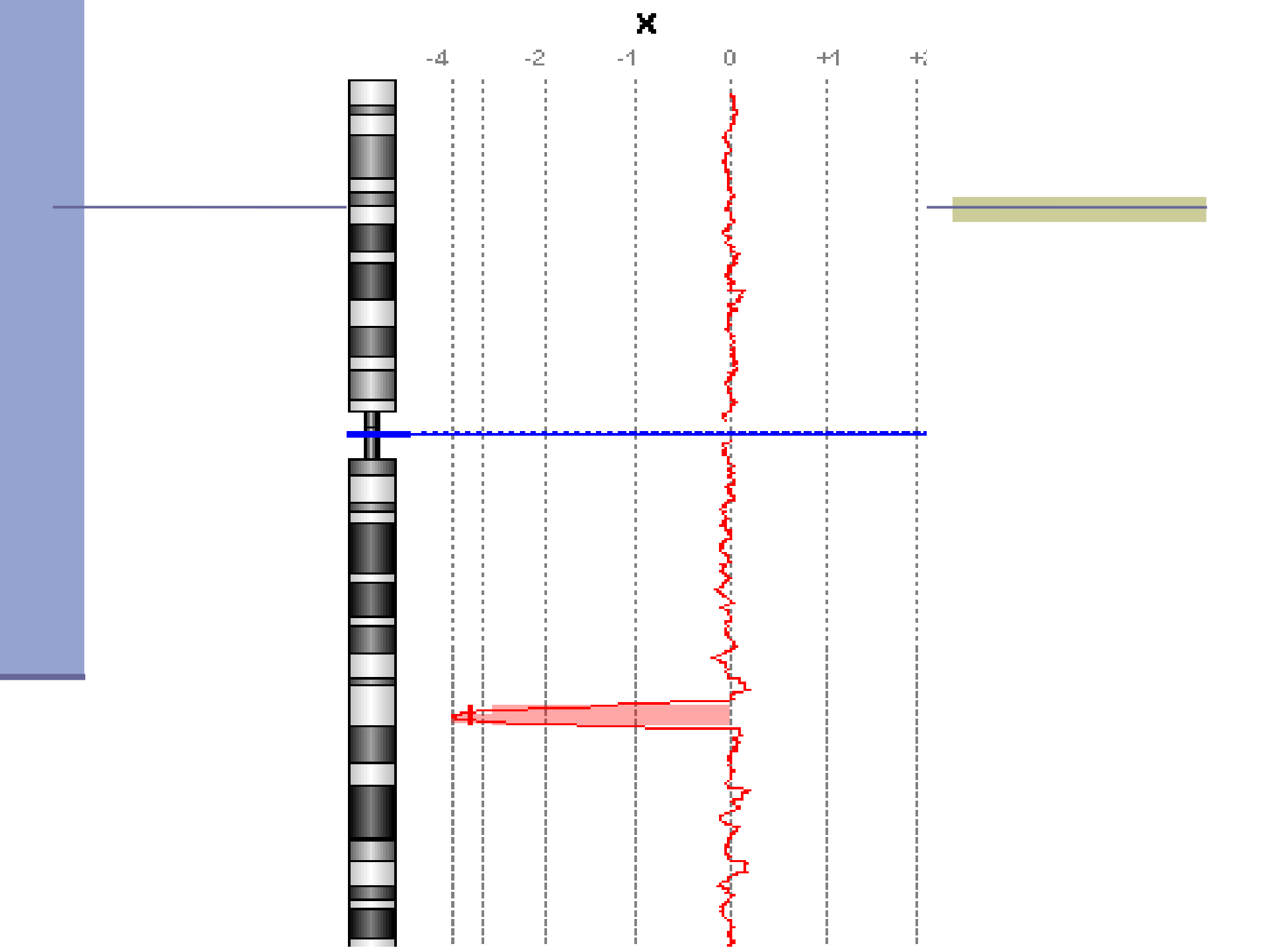
Strýc v roce 2006

Strýc 1988

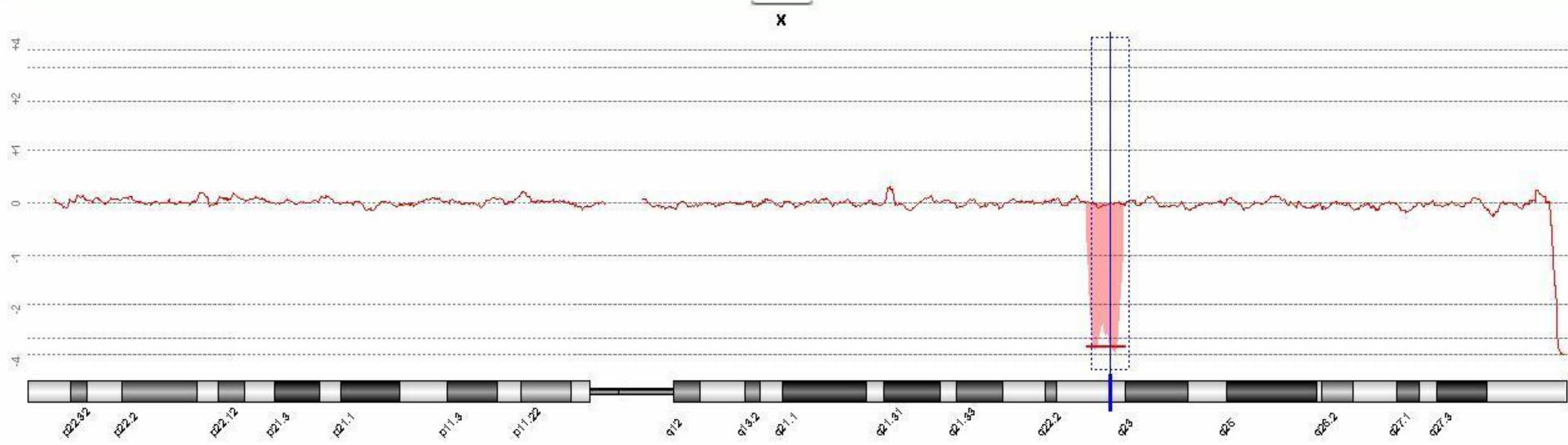
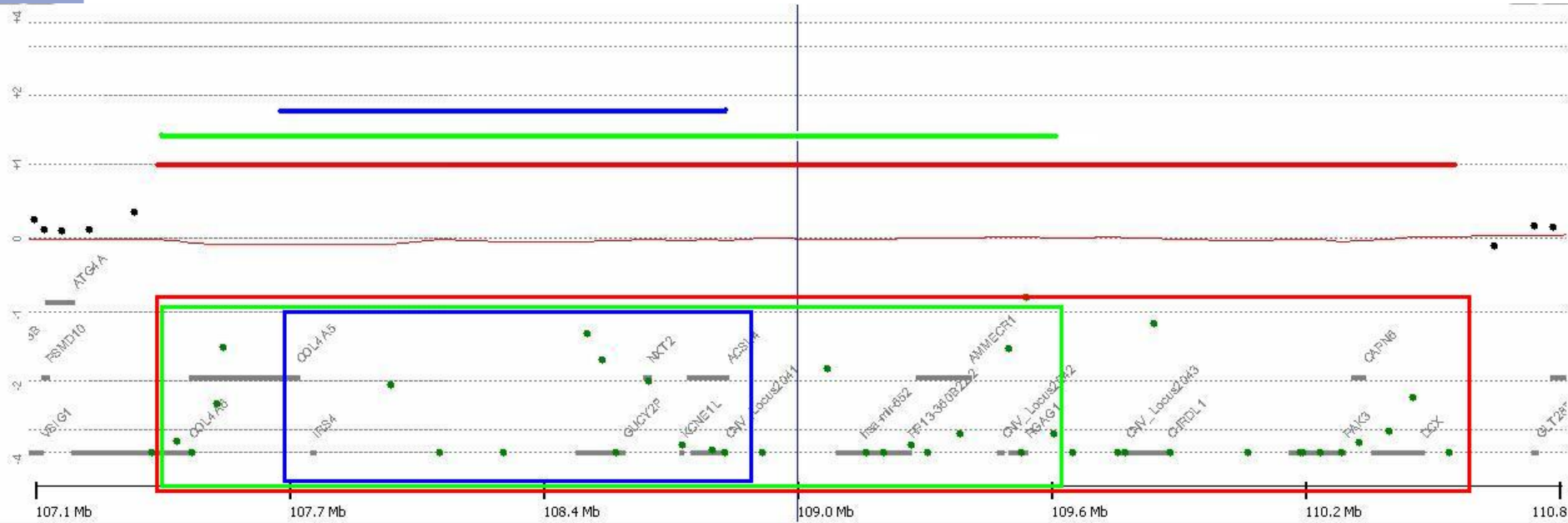


Naše rodina





Velikost delecije – naši pacienti



Alport syndrome with Mental retardation, Midface hypoplasia and Erythrocytosis

- 1. publikace 1998 J.Johnsson, F.Vitelli, Piccini M. a spol.: Delece COL4A5 genu
- Do r. 2008 popsané 2 rodiny
- Xq22.3
- COL4A5 a FACL4 geny
- Pojmenovali gen AMMECR1

Konsangvinita – CGH+SNP arrays

)-OLG-AMB=(kasikkat)
ka1 AMBULANTNÍ KARTA

ČERVENOBRADA PATRIK

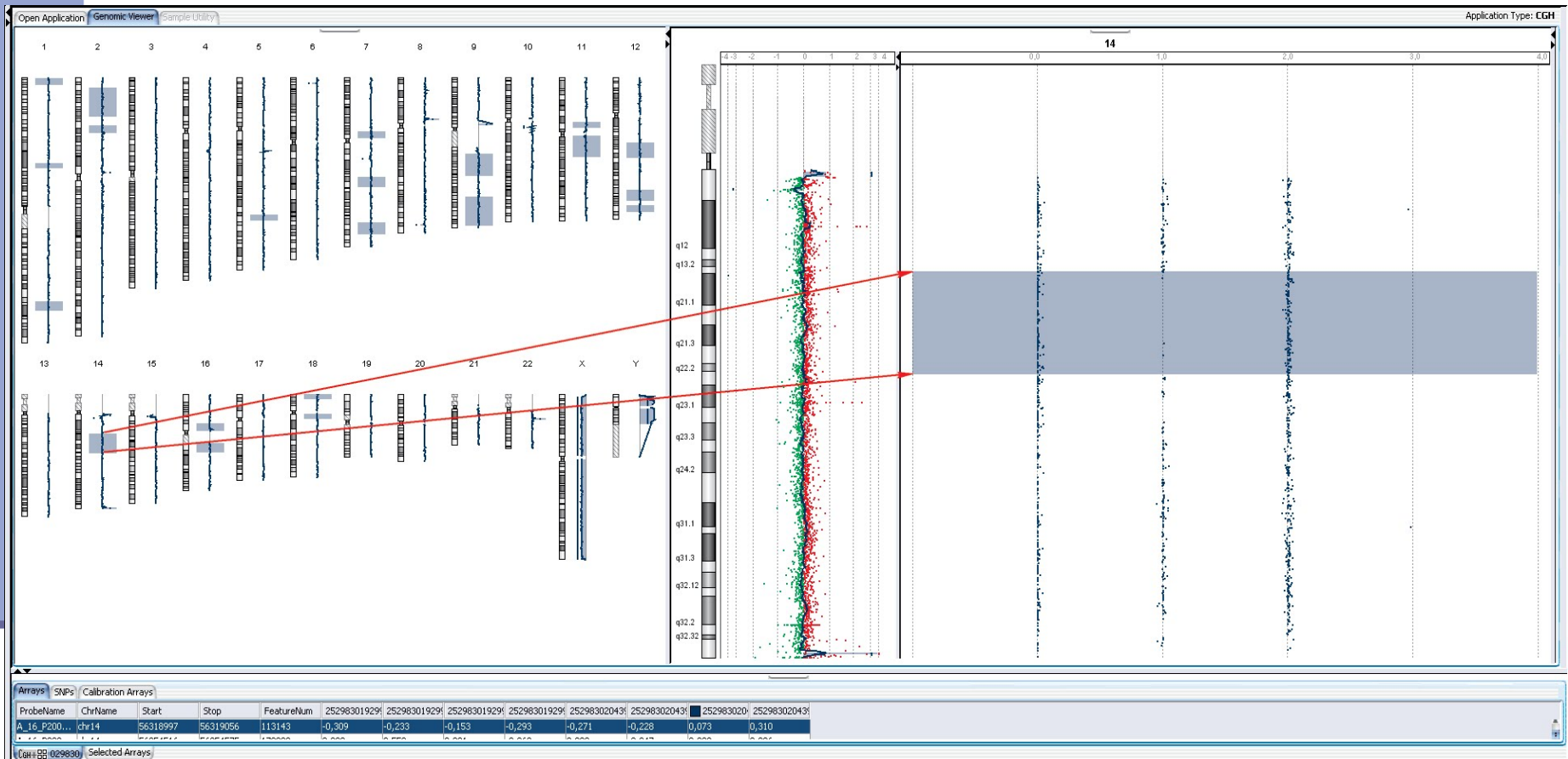
(1 / 1)

Chlapec z 2.chravidity.Poroed v tešrmínu SZ,PH 2600g,48cm.Poporodní adaptace dobrá.AS 10-10-10.Kříšen nebyl.Postupná těžká mentální retardace,zjištěna Dandy Walkwer varianta dle MRI z r.2009.sekundární epilepsie-susp.Lenoc Gastautův sy.
Mladší sestra Vanesa vyšetřována v r.2006 na OLG se stejným postížením (těžká PM retardace s praktickou zástavou vývoje,sekund.epilepsie. Syndromologicky nebyla zařazena,velmi pravděpodobně se u dětí jedná o AR metabolickou poruchu-onemocnění.

Odběr - karyotyp
- DNA zatím banka

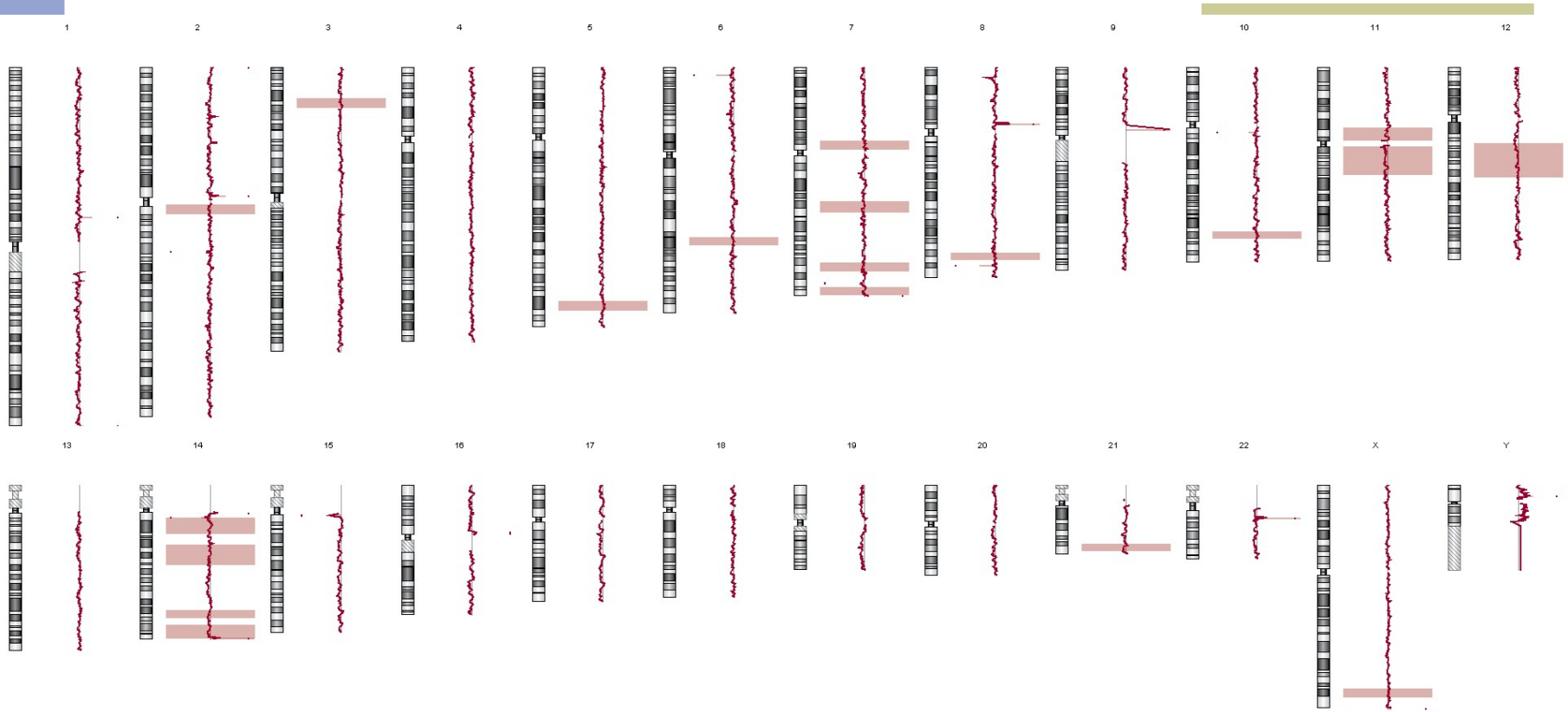
~END~

Konsangvinita – CGH+SNP arrays

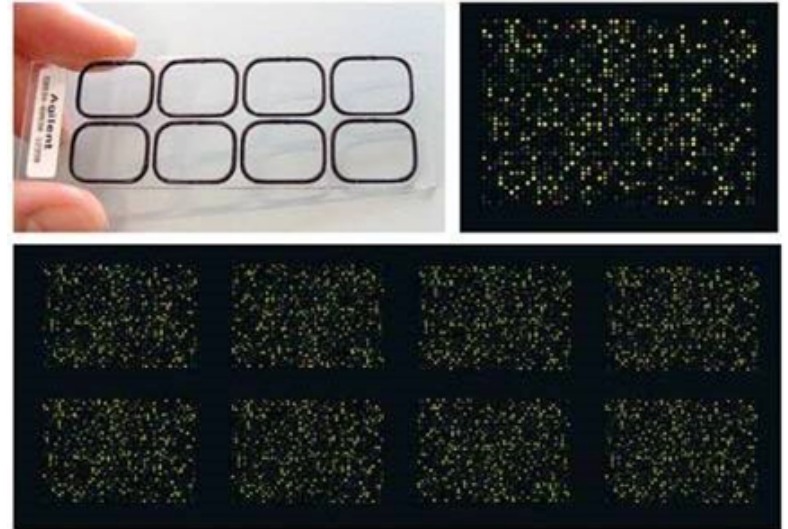


■ Parciální UPD > 10 Mbp na chr 1pq, 2p,5q, 7pq, 11q, 12q, 14q, 16q

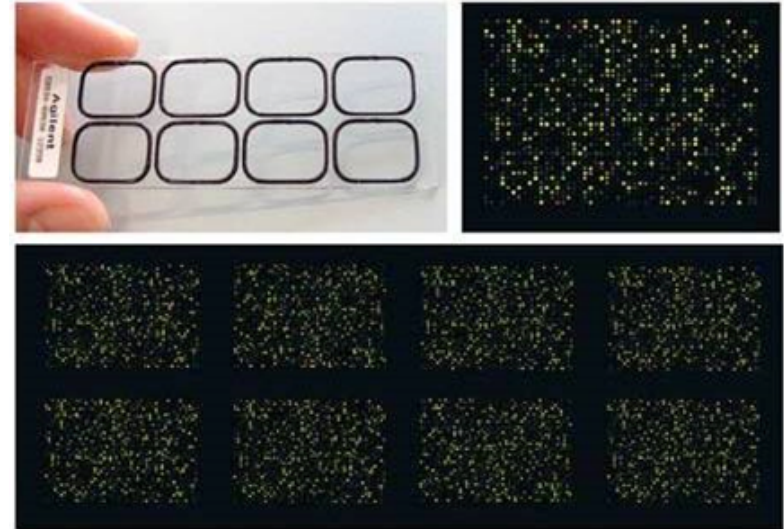
Konsangvinita – CGH+SNP arrays



Preimplantační genetický screening pomocí array-CGH

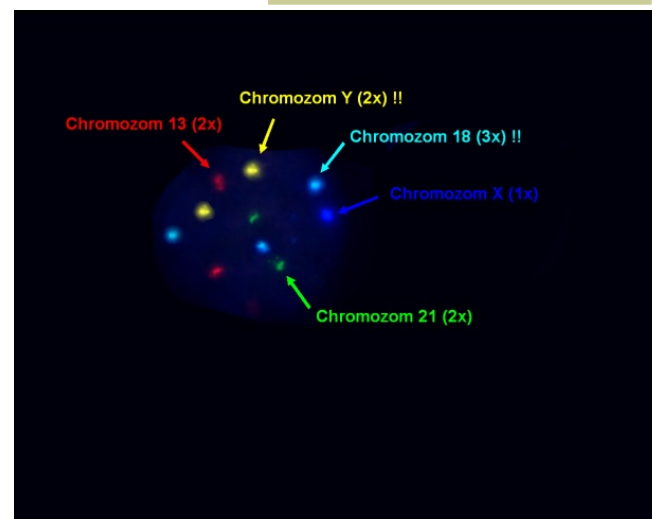


Preimplantační genetický screening pomocí array-CGH



Preimplantační genetická analýza „Single cell genomics“ pomocí oligo aCGH

- Využití technik single cell amplifikace (Rubicon) – z 5 -15 pg DNA amplifikovat 1,5 – 2 ug gDNA
- Potřeba ideální poměr cena / rychlost analýzy / objem a reprodukovatelnost dat
- Agilent 8x60k - rámci jednoho experimentu detekce aneuploidií, strukturních CNAs (> 1,5 Mbp), i nebalancovaných translokací



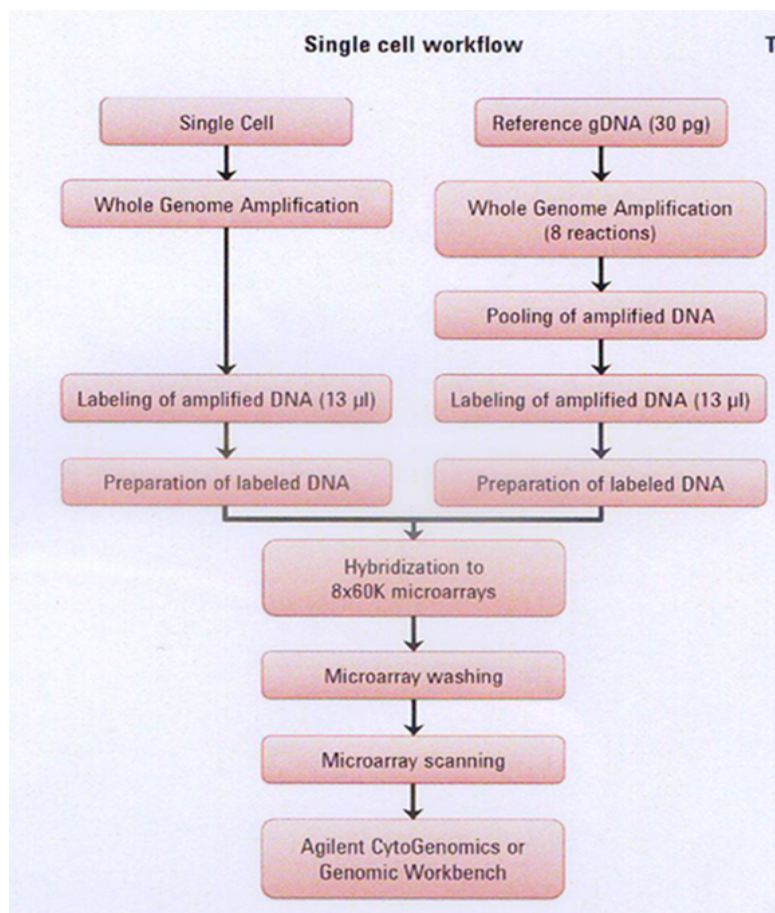
SurePrint G3 Human CGH Microarray Kit, 8x60K

Feature	Specification
Agilent Product Number	G4450A
Design ID	021924
Format	8 x 60K
Arrays/Slide	8
Slides/Kit	3
Distinct Biological Features	55,077
Replicated Biological Features (X5)	1,000
Internal Quality Control Features	3,886
Gene List Annotations Design Files Probe Sequences	Visit Agilent's eArray application
Composition	Content sourced from - UCSC hg18 (NCBI Build 36), March 2006
Probe Spacing	41 KB overall median probe spacing (33 KB in Refseq genes)
Manufacturing	Agilent 60-mer SurePrint technology



Array-CGH – standardní validovaný protokol DNA čip 8x60K – 8 vzorků 5. denní embrya - odběr - vitřifikace

Obecné schéma: *pokud jsou vzorky ráno v připraveny v laboratoři, druhý den odpoledne jsou k dispozici výsledky*

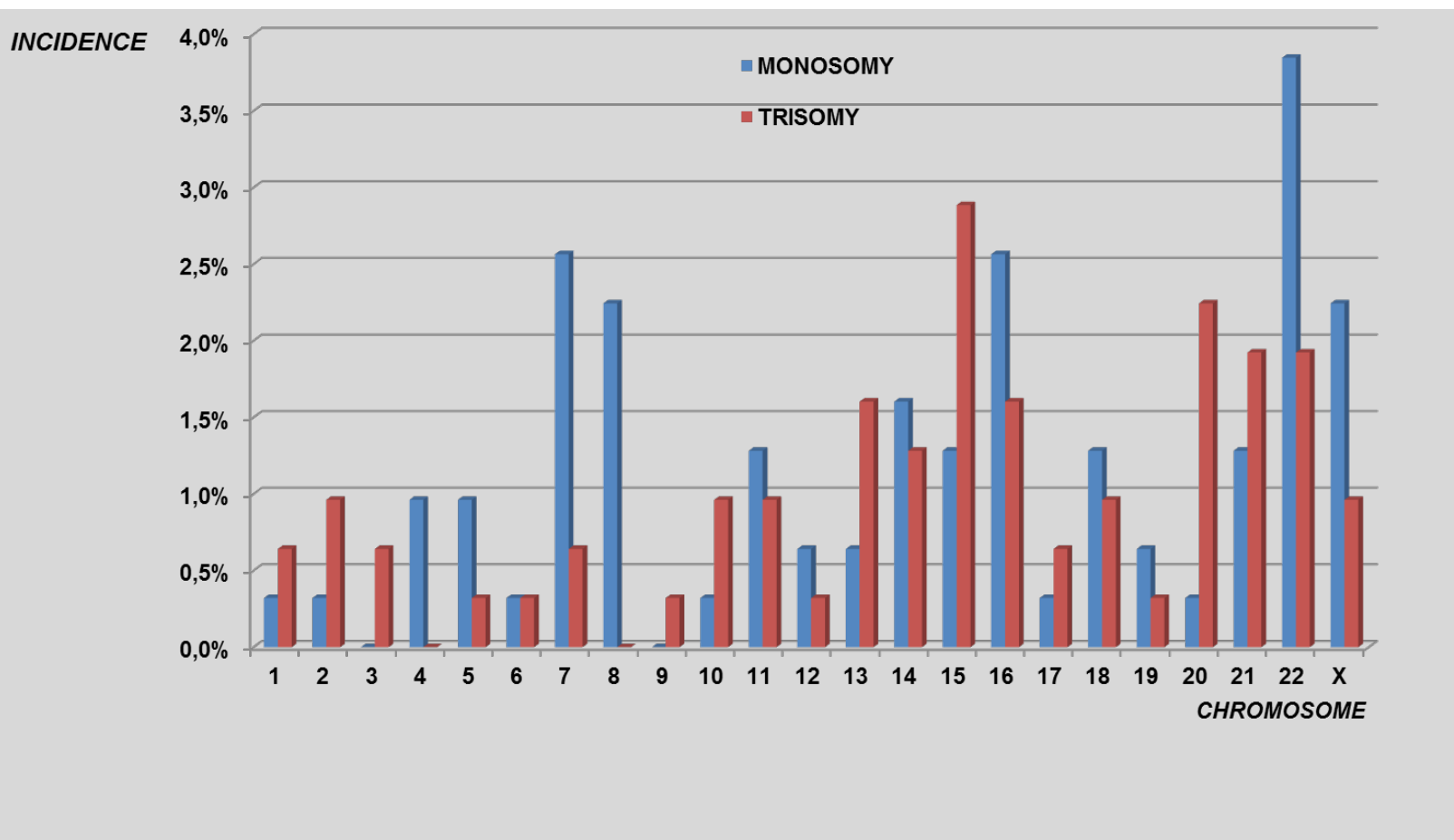


Časová kalkulace

Příjem vzorku	Den 1
Amplifikace a přečištění vzorku	4 hodiny (den 1)
Array-CGH protokol (značení, příprava čipu)	5 hodin (den 1)
Hybridizace	20 hodin
Odmývání, skenování	3 hodiny (den 2)
Softwarová analýza (kontrolní čtení výsledků)	4 hodiny (den 2)
Celková doba do vydání výsledku (protokol)	36 hodin

PGS shrnutí array-CGH za 2013-2014

- nejčastější **monozomie**: chromozom **22, 7, 16, 8**
- nejčastější **trizomie**: chromozom **15, 20, 21, 22, 13, 16**

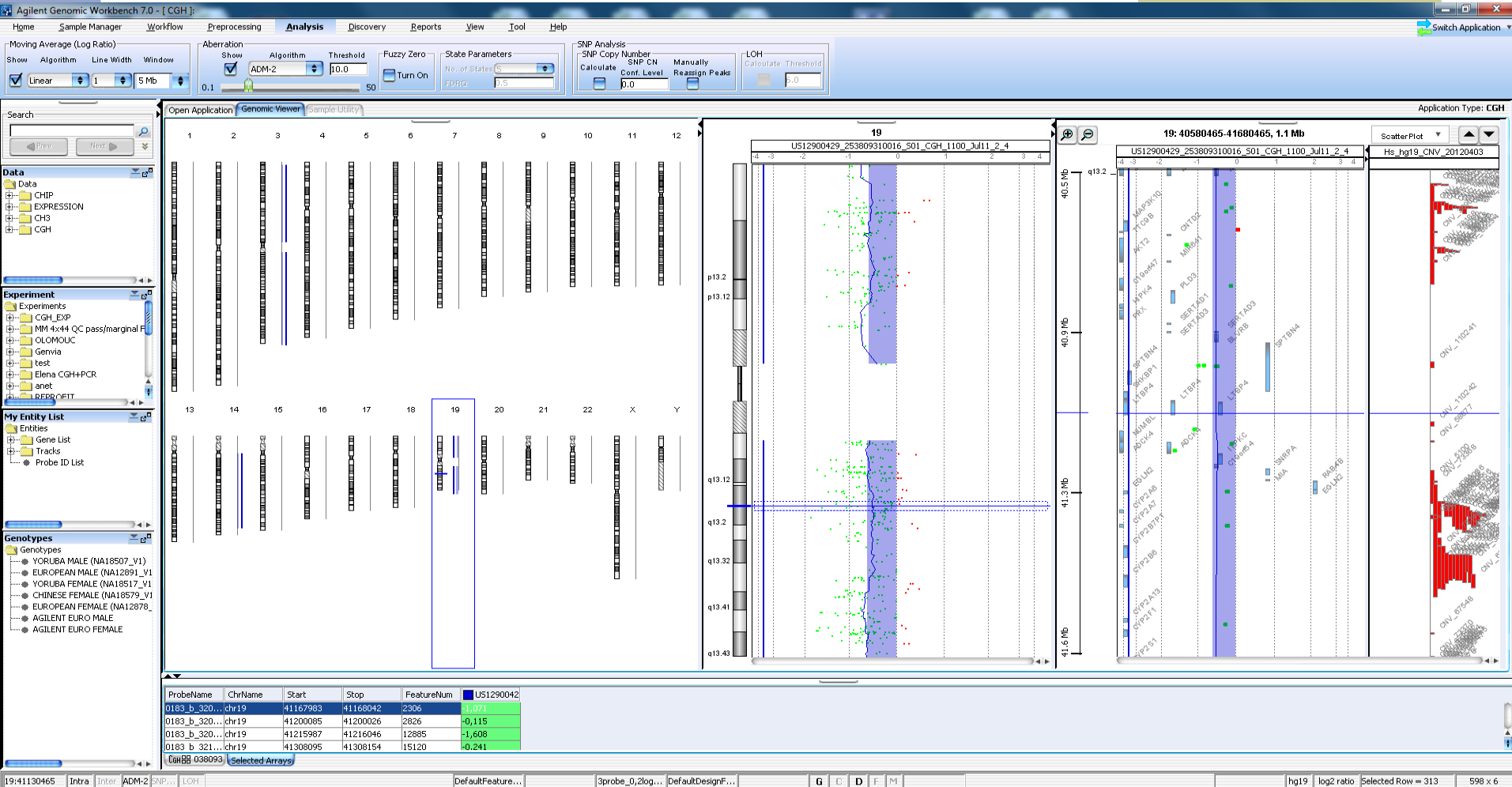


PGS shrnutí array-CGH za 2013-2014

Chromosome	Region	Gain	Loss	Size (Mbp)	PEM /DEM
1	1q21.1 - 1q42.2	+		84,7	PEM
2	2p25.3 - 2p21		+	44,4	PEM
2	2q12 - 2q37.3		+	136,2	PEM
3	3q11.1 - 3q29	+		104,1	PEM
5	5q23 - 5q35		+	56,9	PEM
5	5q21.1 - 5q35	+		79,5	PEM
5	5p13.3 - 5q35.3	+		149,5	PEM
7	7q33.3 - 7q37.3		+	35,2	PEM
7	7p21.3 - 7p12.1		+	49,1	DEM
8	8q24.21 - 8q24.3	+		23,5	PEM
8	8p23.2 - 8p11.22	+		43,3	PEM
8	8q24.22-q24.3		+	130,5	PEM
8	8p23 - 8p11.23		+	37,5	PEM
9	9p23-p21.3		+	15,1	PEM
9	9q21.1 - 9q34.3		+	69,8	PEM
9	9q21.11q34.3	+		69,5	PEM
12	12q13.2 - 12q24.32	+		71,3	PEM
13	13q21.32 - q22.2		+	8,4	PEM
13	13q21.33-13q34	+		43,6	DEM
13	13q12.11 - 13q33.1	+		102,5	PEM
14	14q21-14q32	+		57,2	PEM
14	14q22.1 - 14q32.3		+	53,5	PEM
14	14q24.3 - 14q32.33		+	31,6	PEM
16	16p13.3- 16p11.1		+	34,8	PEM
17	17p13.3 - 17p11.2	+		24,4	PEM
18	18q12.1 - 18q23		+	53,4	PEM
X	Xq21 - Xq28	+		36,2	PEM

- Mitotické poruchy
= STRUKRURNÍ CNAs
- cca10% případů
- medián velikosti **26,2 Mbp**
- 2 případy nebalancované translokace

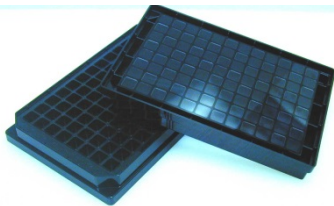
Preimplantační genetická analýza „Single cell genomics“ pomocí oligo aCGH



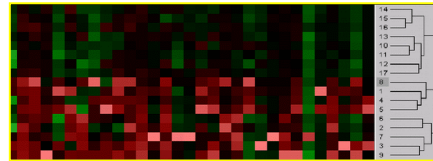
Genomový profil z buněk trofoektodermu 5 denního embrya. Nalezené aberace: **+3,+14,-19.**

Mikročipy Affymetrics

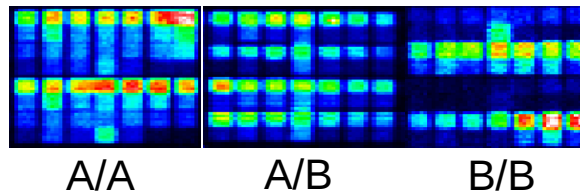
Multiple Formats



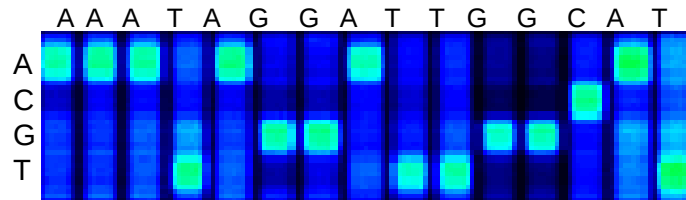
Multiple Questions



Expression Variability
(mRNA Analysis)



Sequence Variability
(DNA Analysis)



Resequencing

Multiple Apps

Basic Research

Pre-Clinical
Toxicology

Quality Control

Clinical Trials

Diagnosis

Prognosis

Rx Choice

Array-CGH vs. SNP mikročipy

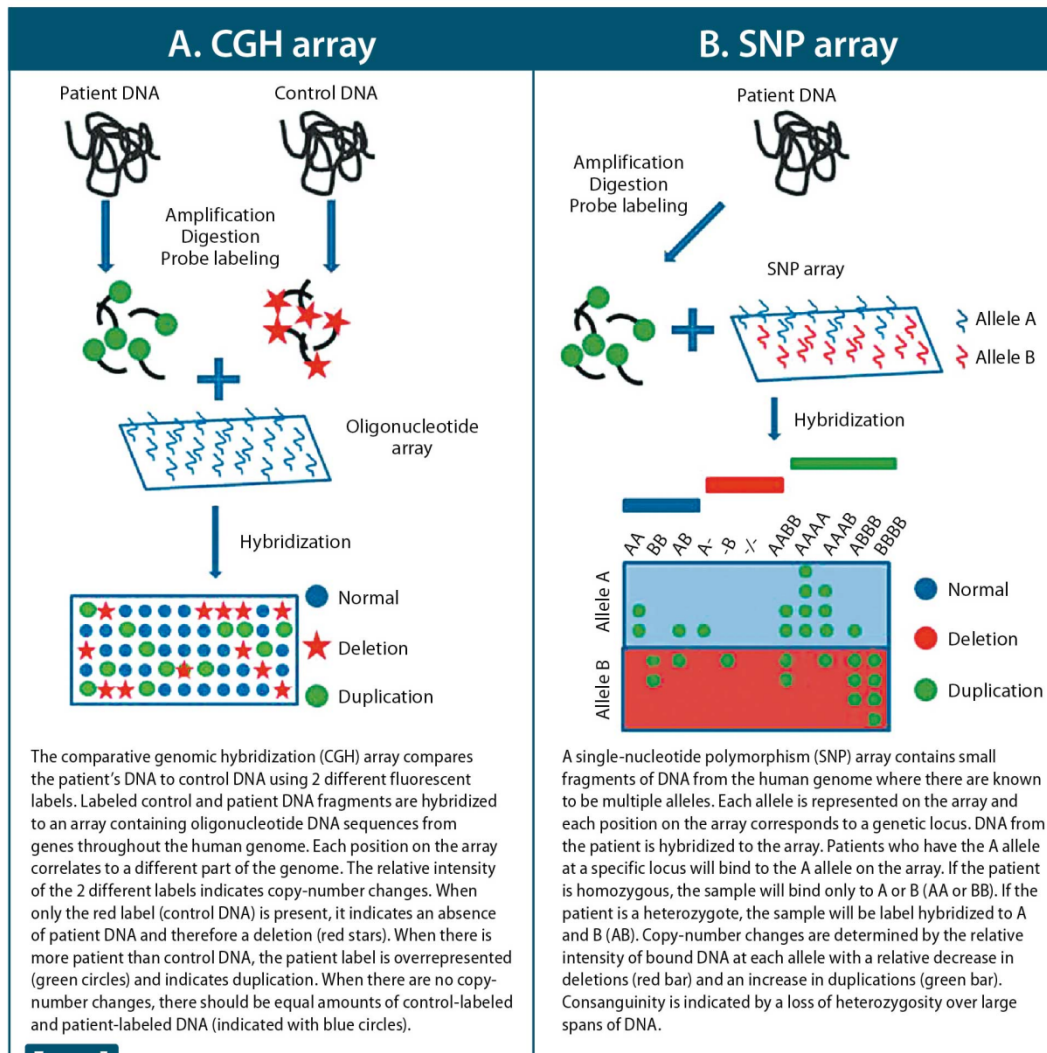
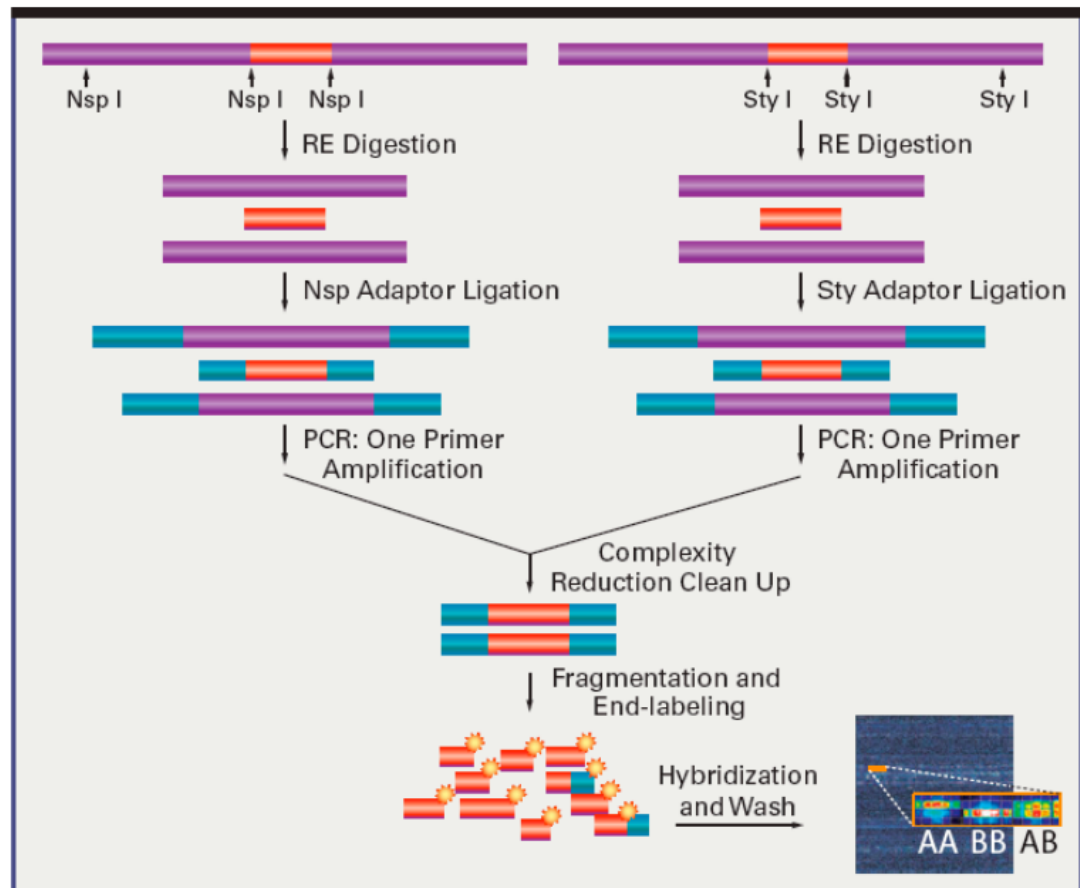
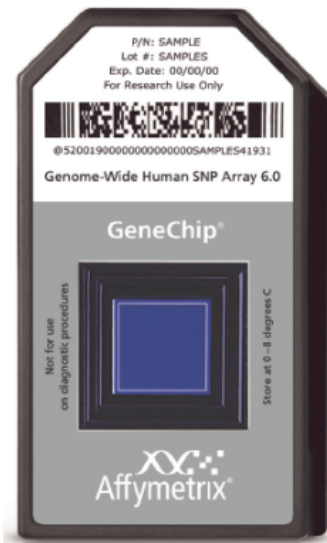


FIGURE 1

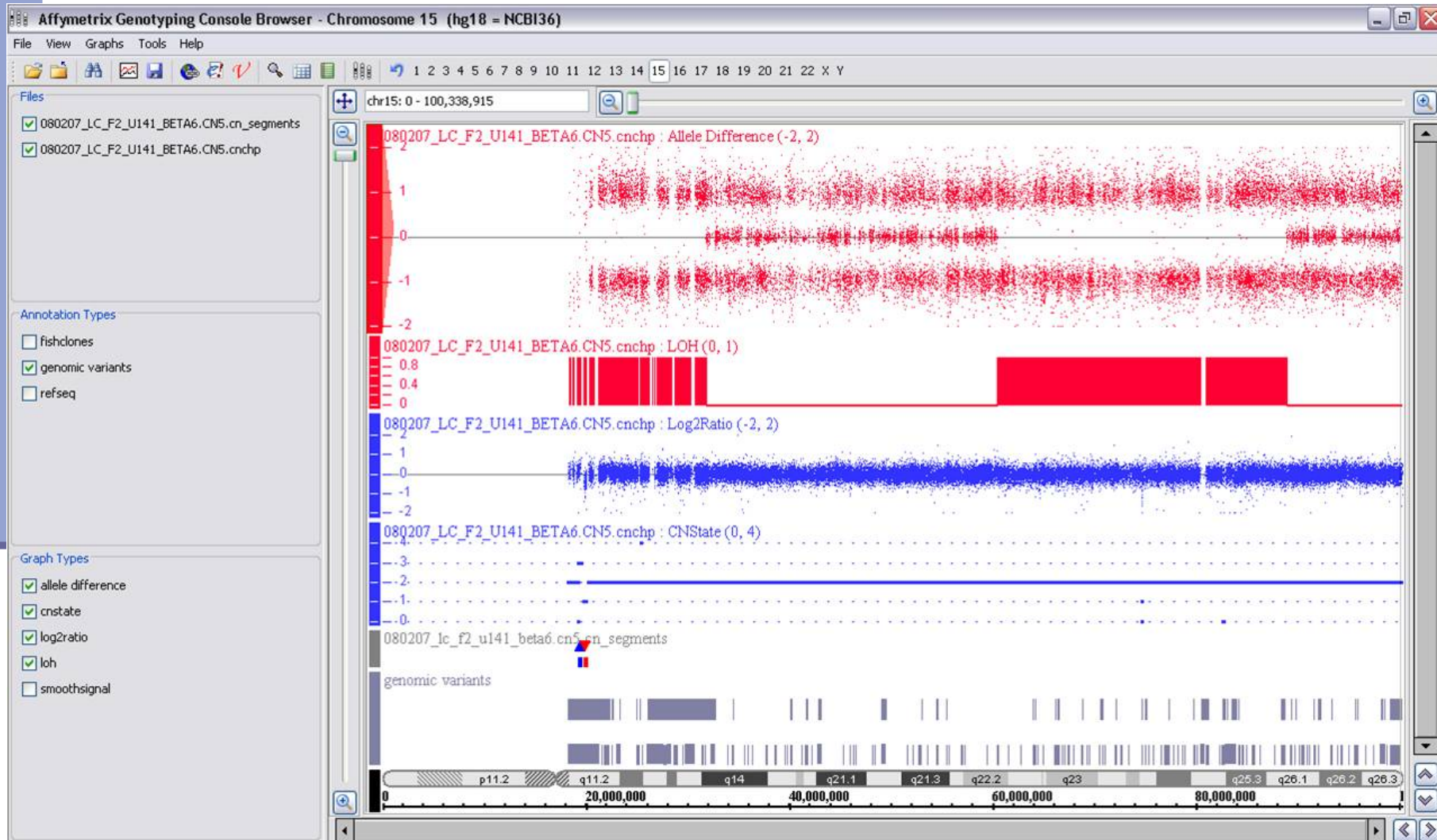
Affymetrix SNP mikročipy 6.0

Affymetrix SNP microarrays 5.0 / 6.0 Arrays (one single: Nsp+Sty)

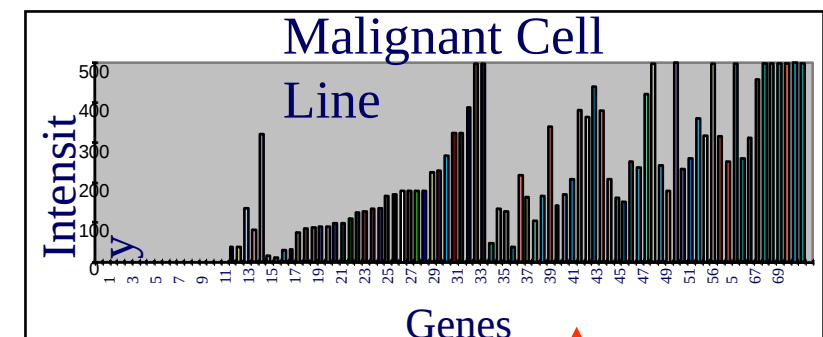
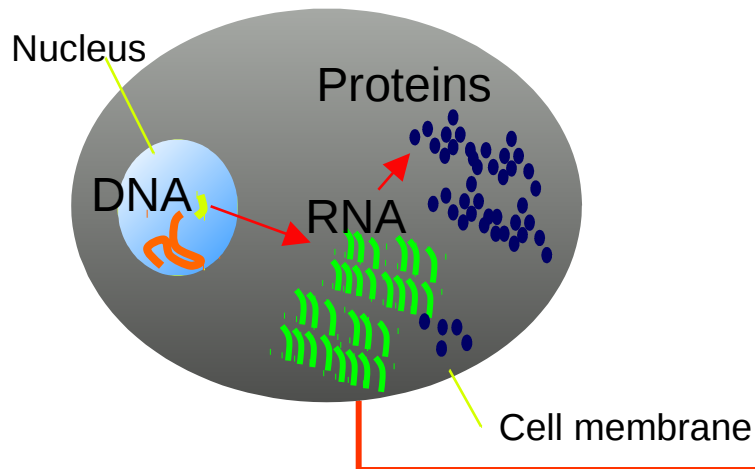
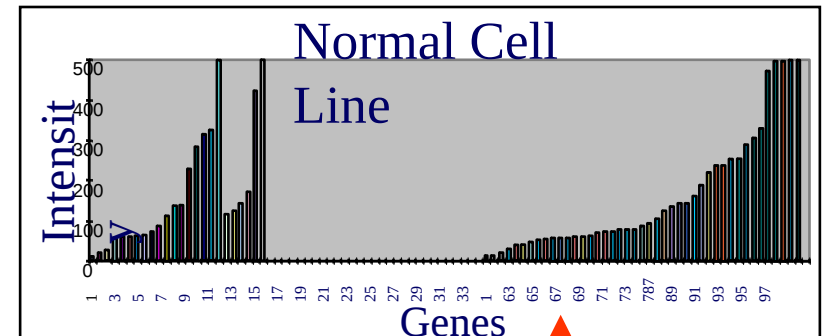
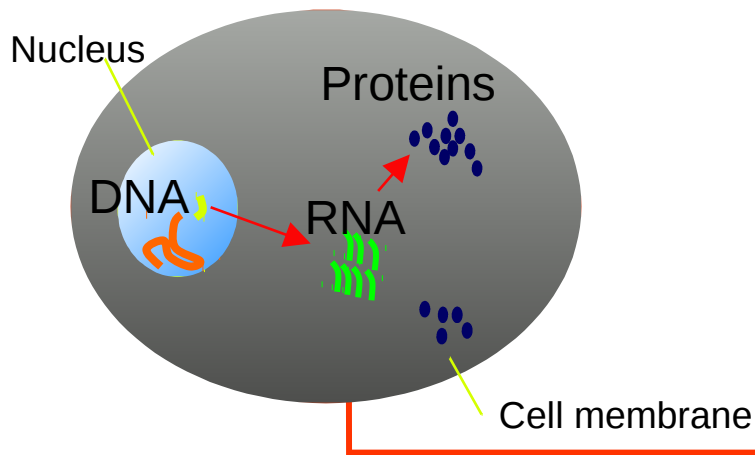
Genome-wide
SNP Assay
overview



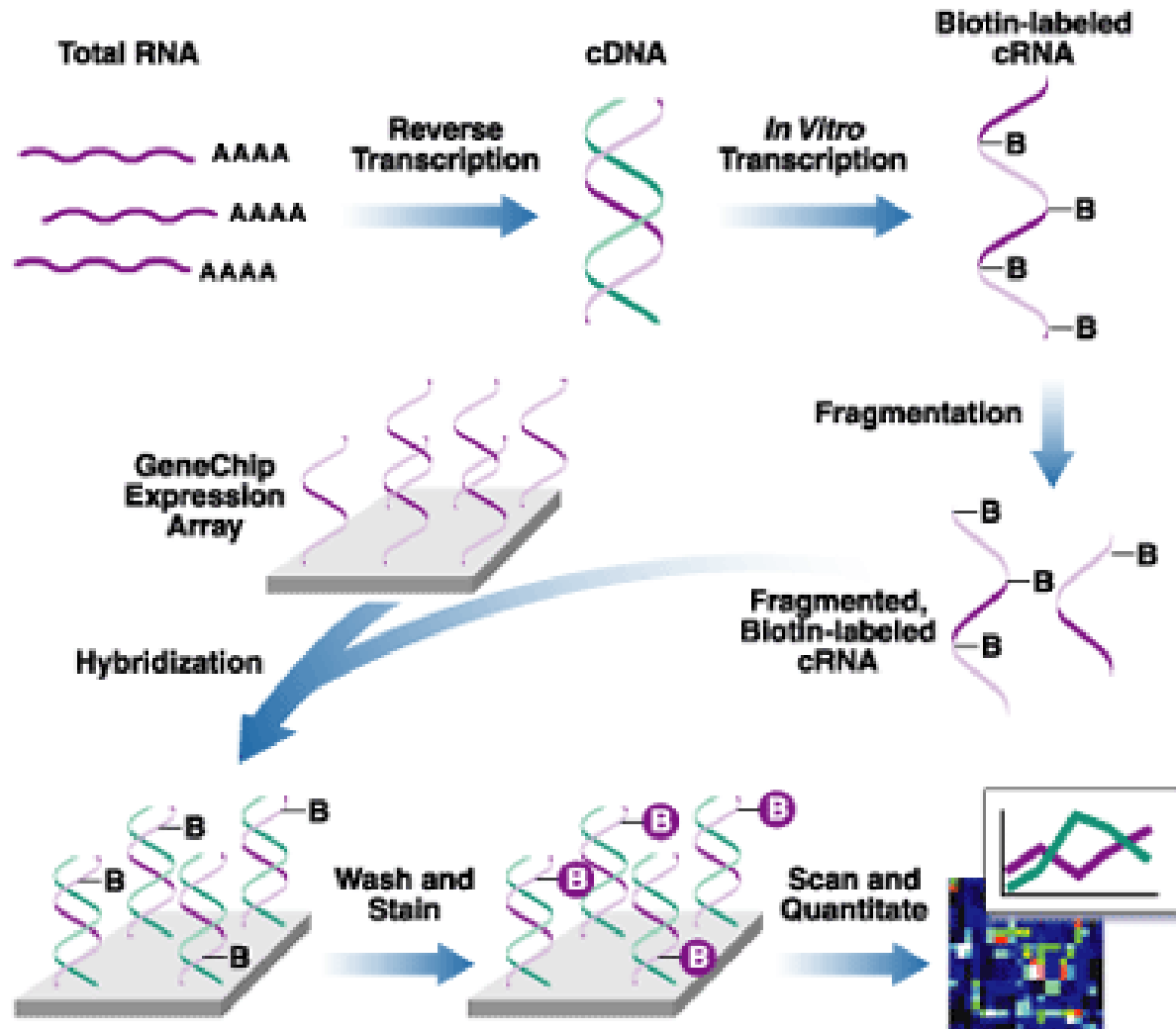
Affymetrix SNP mikročipy 6.0



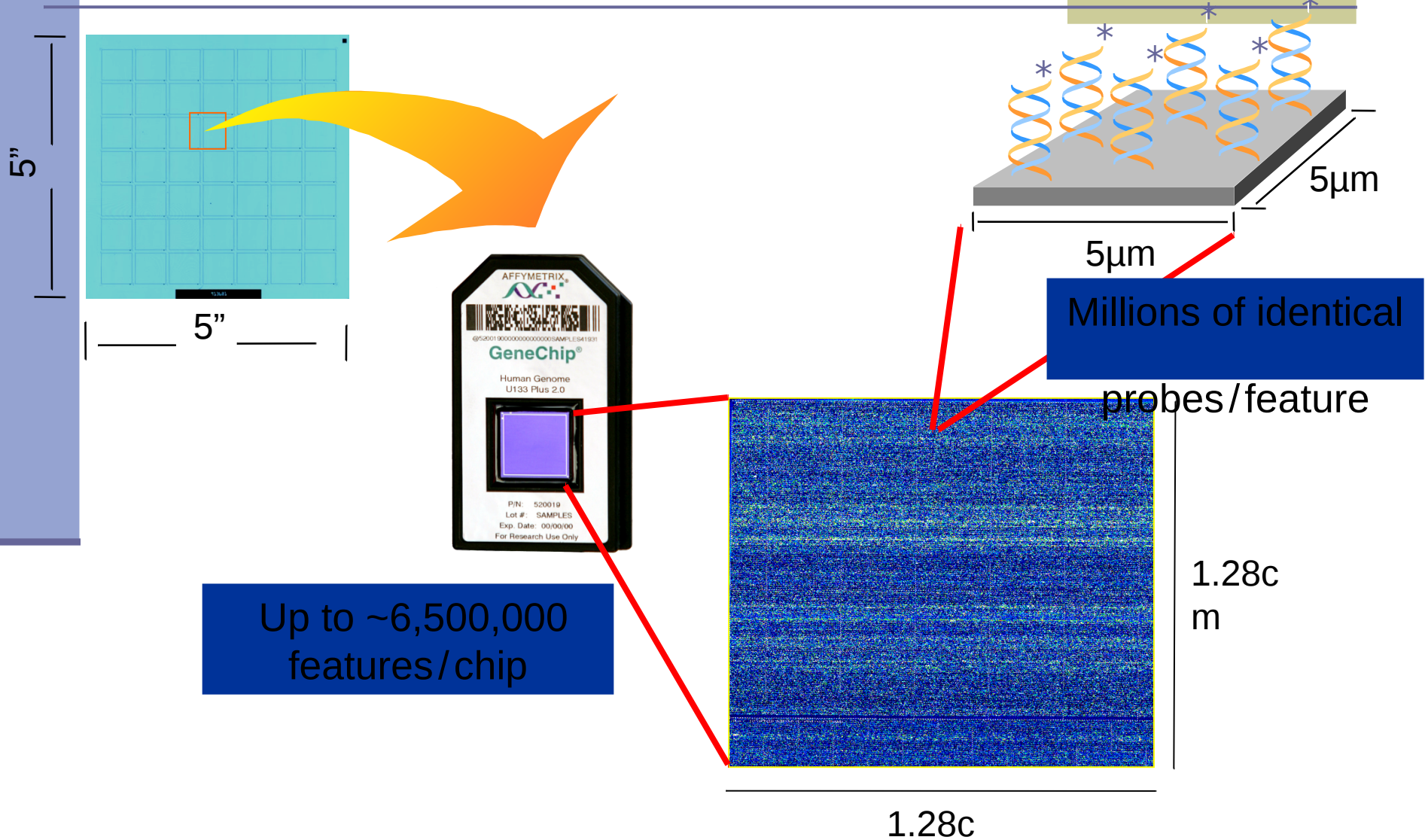
Genová exprese = množství RNA produkované aktivitou genu



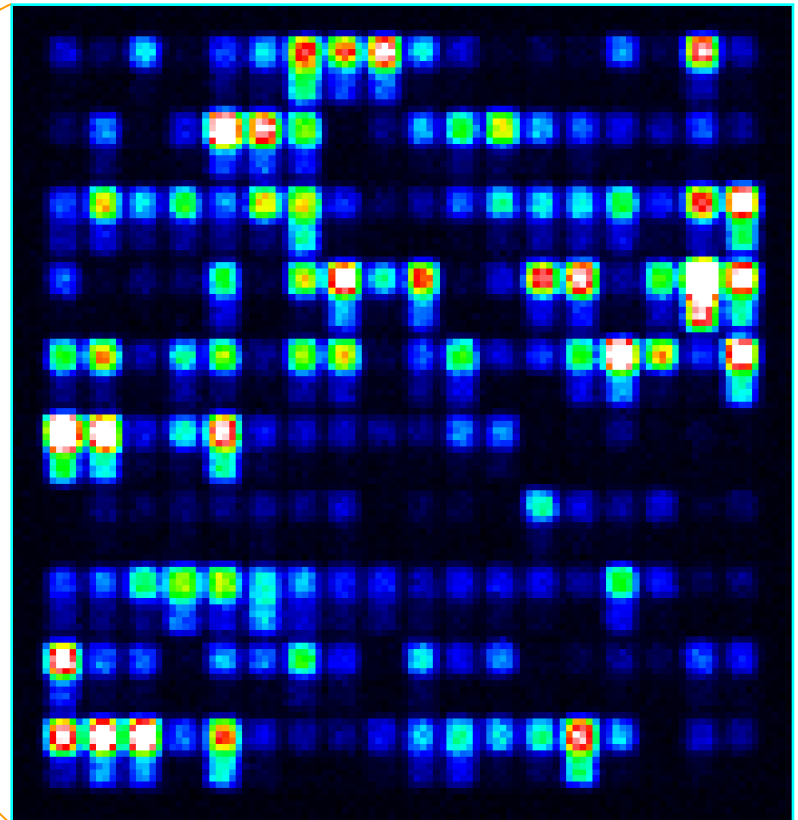
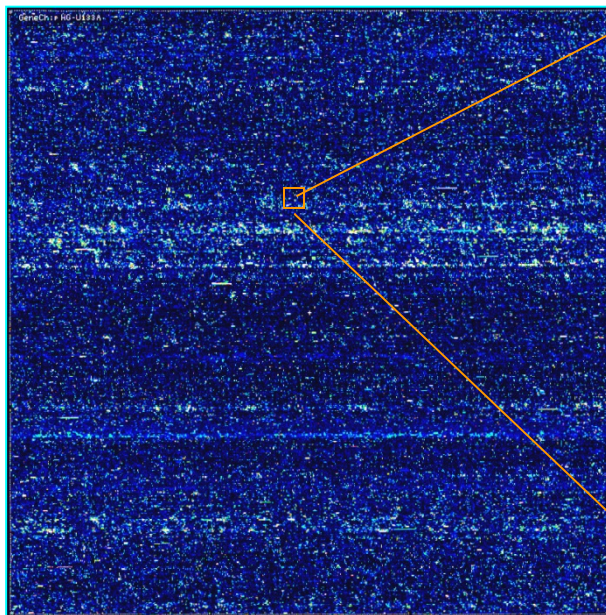
Expresní mikročipy Affymetrics



Struktura Affy GeneGenechip



Struktura Affy GeneGenechip



Využití GeneChip technologie v praxi

Food testing



Livestock diagnostics or grading

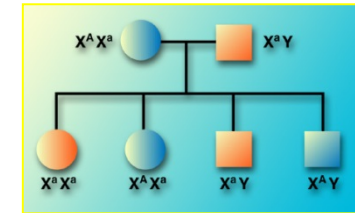
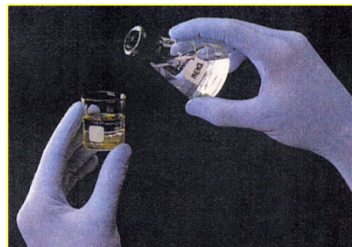


Environmental testing



Agricultural biotech

Basic Research



Identity testing

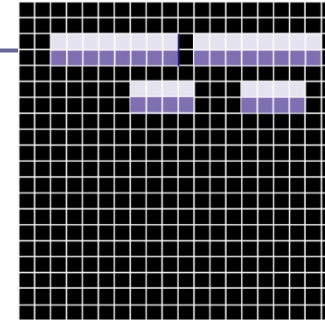
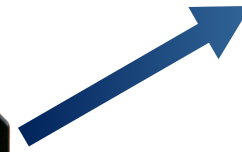
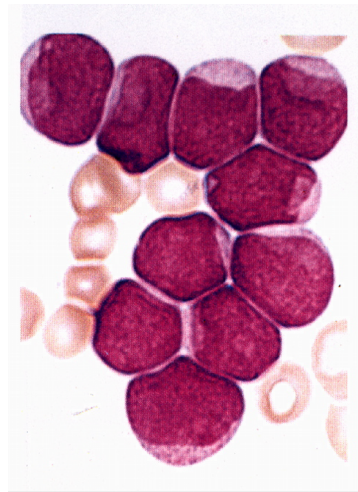


Individualized medicine

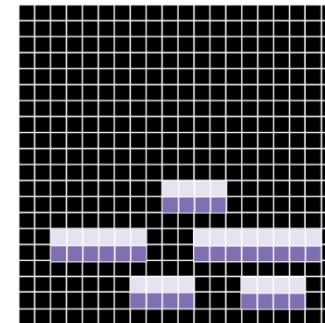
Human diagnostics



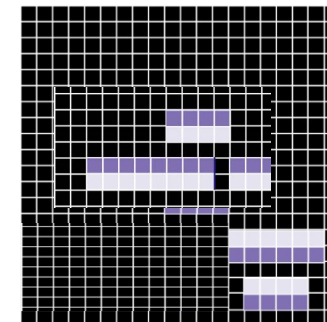
Klasifikace leukémií pomocí expresních mikročipů GeneChip



ALL



AML



MLL

Který typ leukémie ?

Golub, T.R., et al. Science 286: 531-537, 1999; Armstrong, S.A., et al. Nature Genetics 30: 41- 47, 2002



Děkuji za pozornost