Non-Invasive Prenatal Diagnosis for Aneuploidies: what's new?

Sinuhe Hahn, University Women's Hospital Basel

Prenatal diagosis – invasive

Non-Invasive Prenatal Diagnosis: What do we need?

System needs to be:

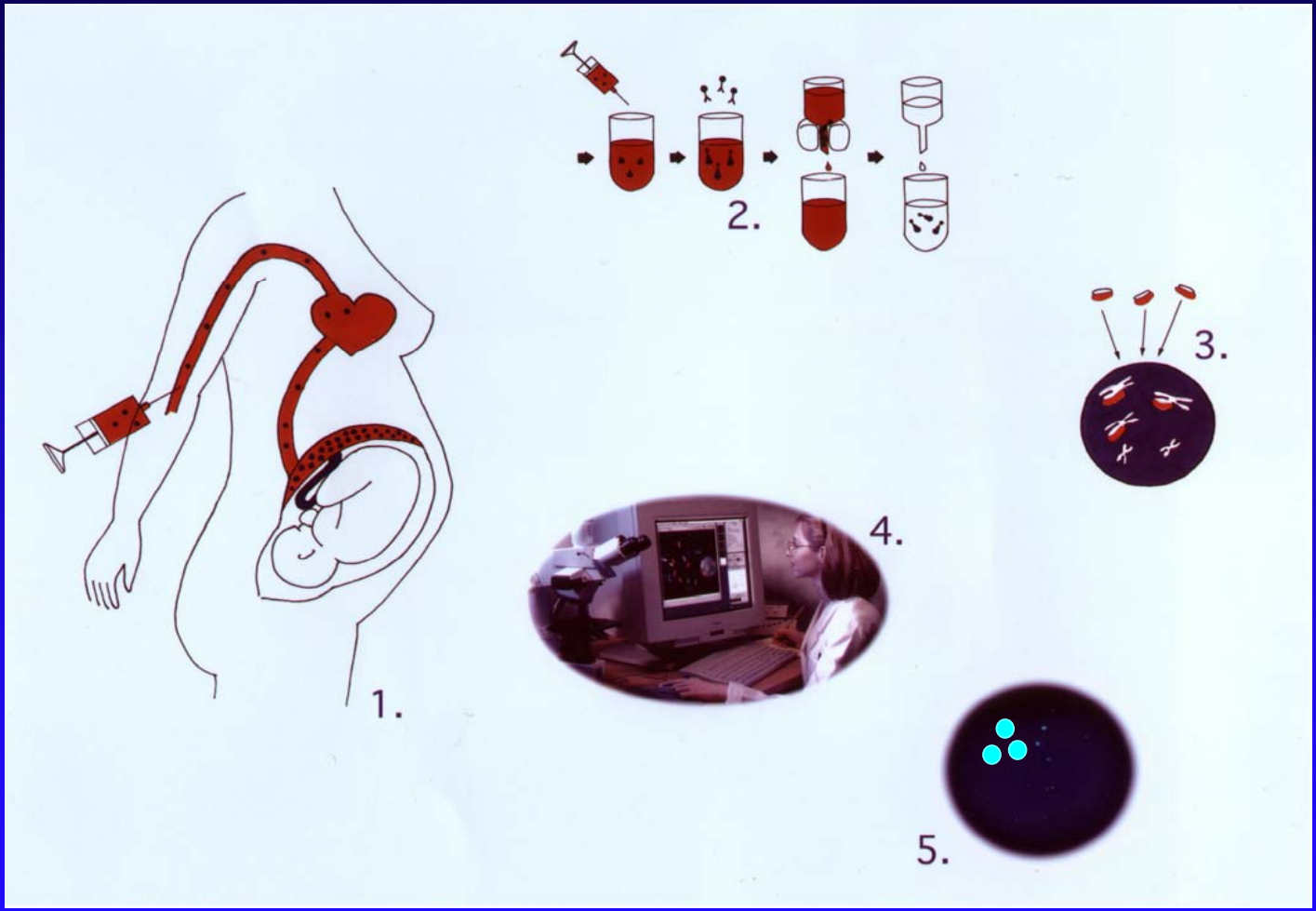
Simple & Automatable

Robust & High-throughput

Off-site - samples shipped

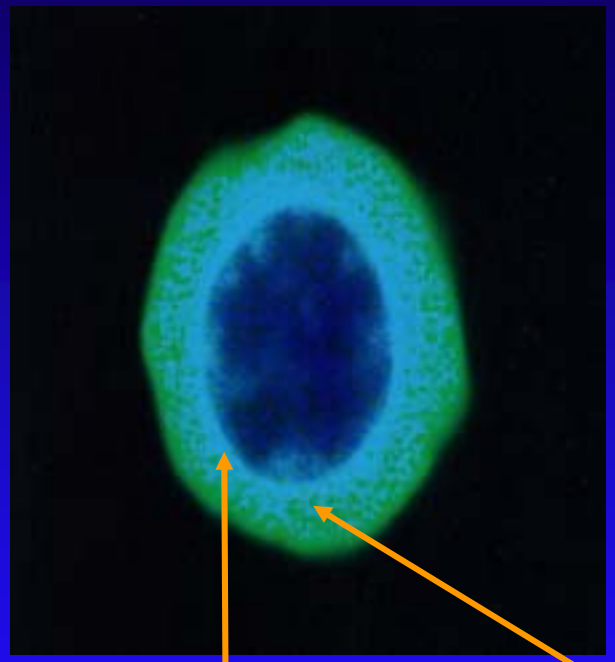
Quite a challenge!

Risk free prenatal diagnosis using fetal cells



1st attempt – 1979 Herzenberg, Bianchi, Schröder…..!

Erythroblast : preferred target cell



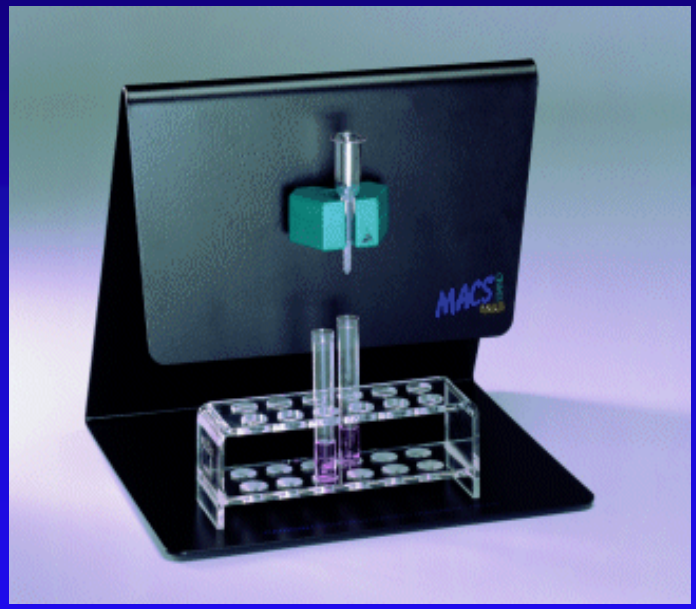
- Short life span
- Abundant in fetal blood
- Markers for enrichment and identification
- No genetic mosaicism

Anti HbF stain

Nucleus (DAPI) stain

NIH NIFTY Study 1994 - 2002

- 3000 cases – detect fetal aneuploidy.
- Full karyotype – gold standard
- Comparison of FACS and MACS



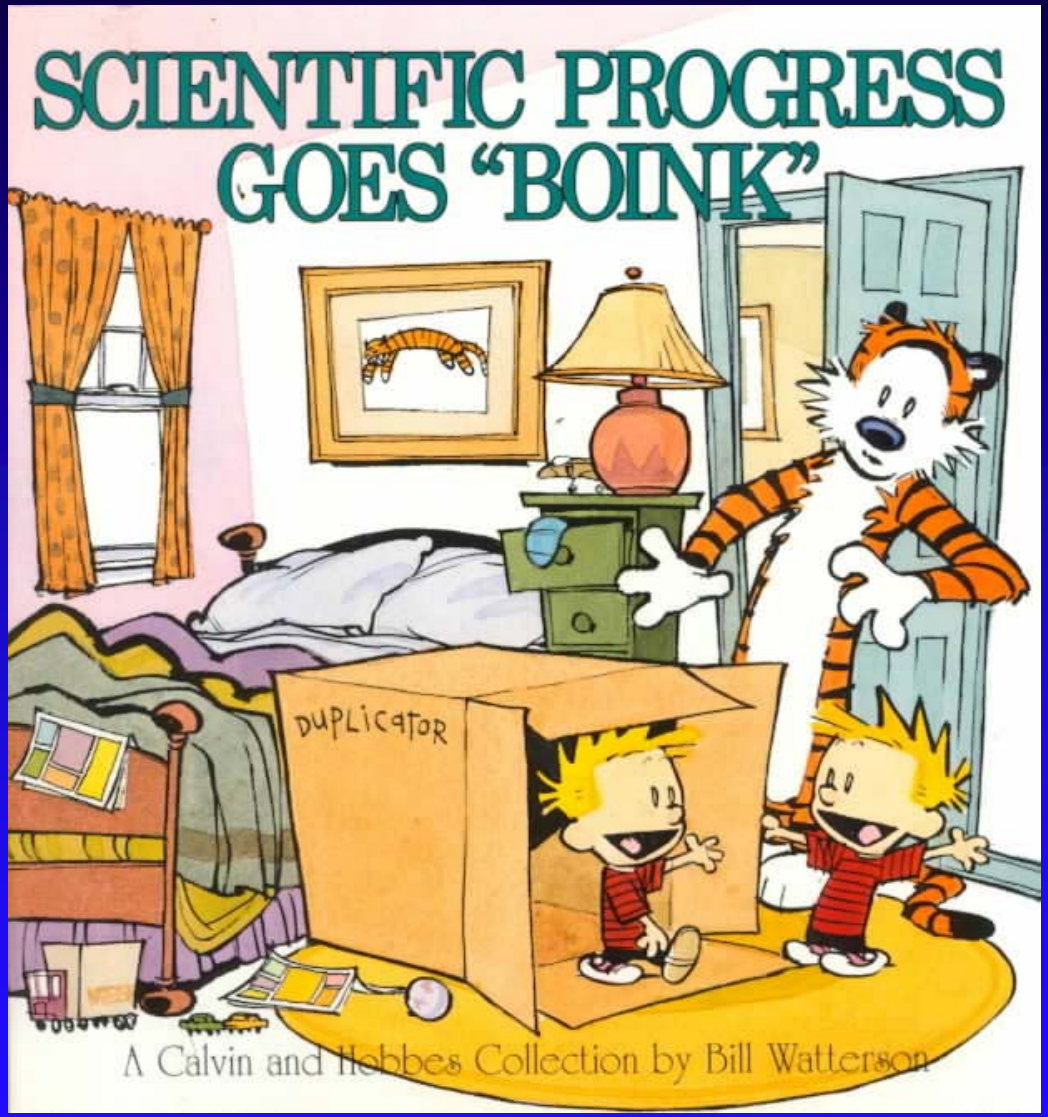
Sensitivity < 50%

Fetal cells: What do we need and what should we offer?

Quantum leap enrichment:
microfluidics, CHIPs

Optimized analysis:
Full karyotype, Arrays

Procedure will be complex and expensive


SCIENTIFIC PROGRESS GOES "BOINK"
DUPLICATOR
A Calvin and Hobbes Collection by Bill Watterson

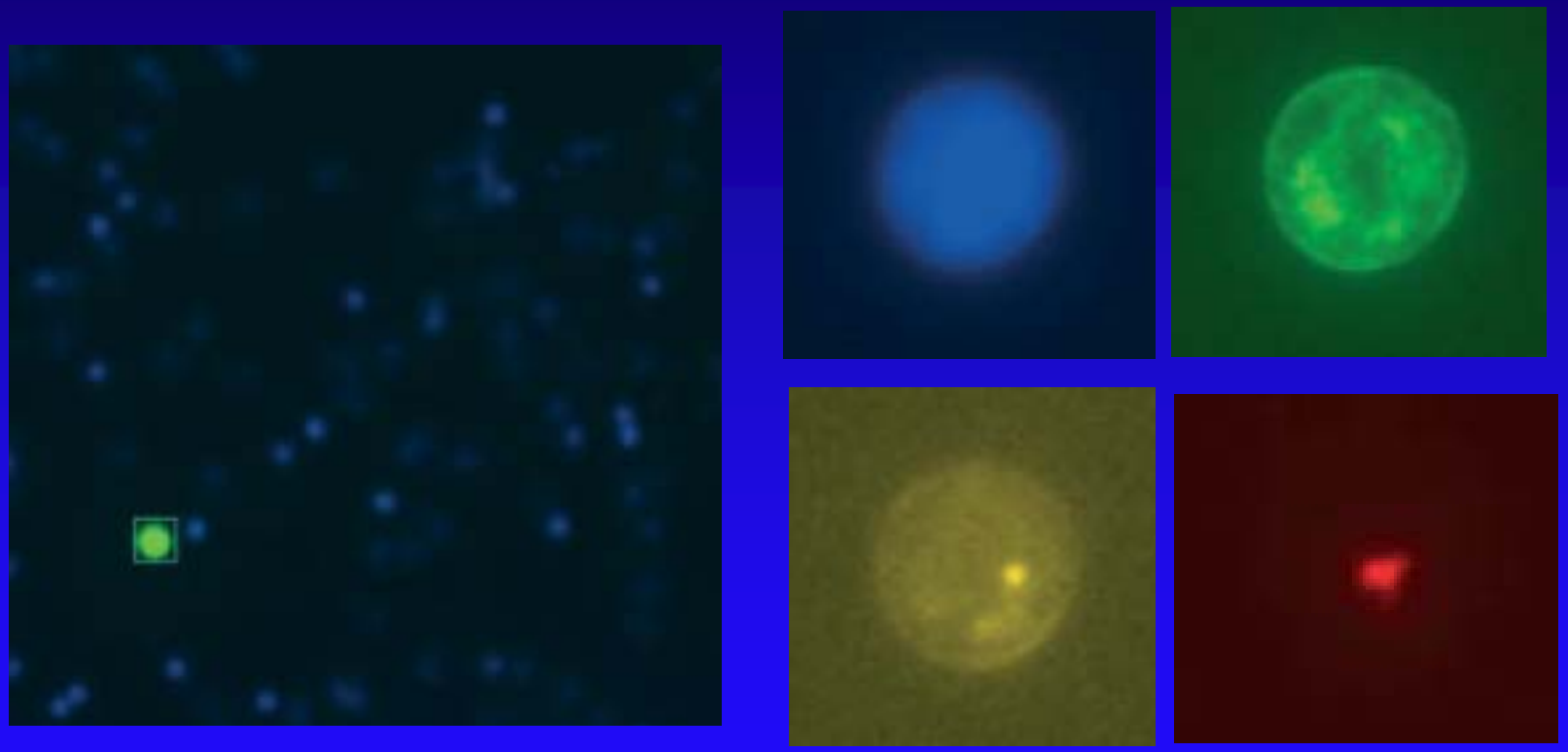
What are others already trying?
Ikyonysis Inc., USA

Minimal enrichment – scan for targets
User-friendly interface
High-throughput design
175 slide loading capacity
24/7 walk-away operation
Rapid servicing and field repairs

Good start – diagnostic lab in mind!

Only allows for FISH analysis!!!

Ikonisys – Approach



Seppo et al., PD 2008

Ikonisys – 1st Results

Analysis of whole blood samples

Table 1—Analysis of maternal whole blood samples for fetal cells based on scanning for the presence of nuclei containing a Y-chromosome

Cases			Whole Blood		
Trimester	Number	Fetal Sex	XY Cells/Case	Nuclei Analyzed/Case	XY Cells/10^6
1st	11	Male	3 (1–10)	3.73×10^6	0.8
	2	Female	0	3.80×10^6	—
2nd	18	Male	2 (0–9)	4.78×10^6	0.4

Seppo et al., PD 2008

Ikonisys – 1st Results

Analysis of ficol enriched samples

Table 2—Analysis of gradient enriched maternal PBS for fetal cells based on scanning for the presence of nuclei containing a Y-chromosome

Cases			Gradient Enriched Blood		
Trimester	Number	Fetal Sex	XY Cells/Case	Nuclei Analyzed/ Case	XY Cells/10^6
1st	11	Male	8 (1–20)	3.92×10^6	2.1
	2	Female	0	4.80×10^6	—
2nd	18	Male	7 (0–17)	3.07×10^6	2.3
	2	Female	1 (0–1)	2.35×10^6	0.4

Seppo et al., PD 2008

Ikonisys – Conclusions

Fetal cells appear to be present in all blood samples (93%)

Fetal cells can be found reliably by automated scanning

> 3 million events per case

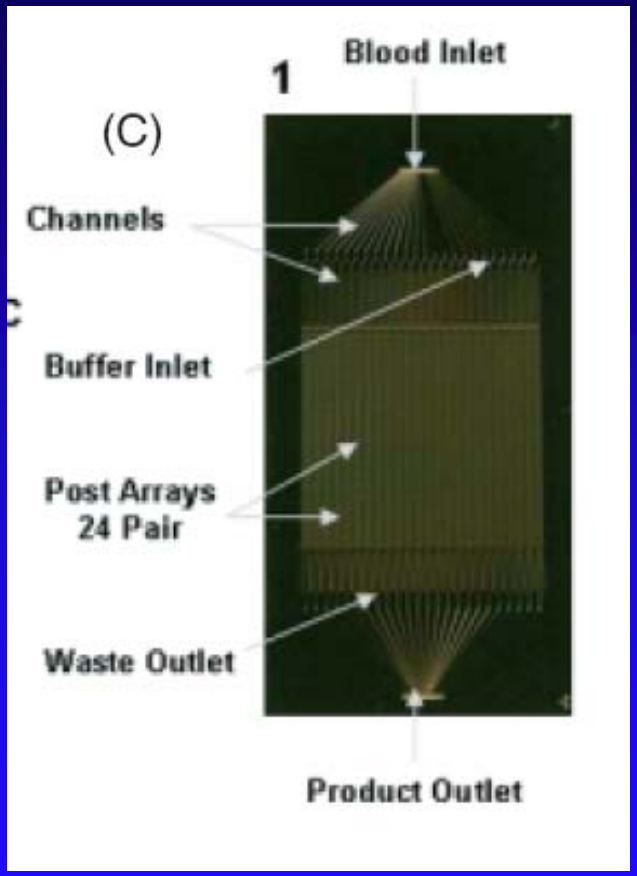
<1 fetal cell / 1 million maternal cells

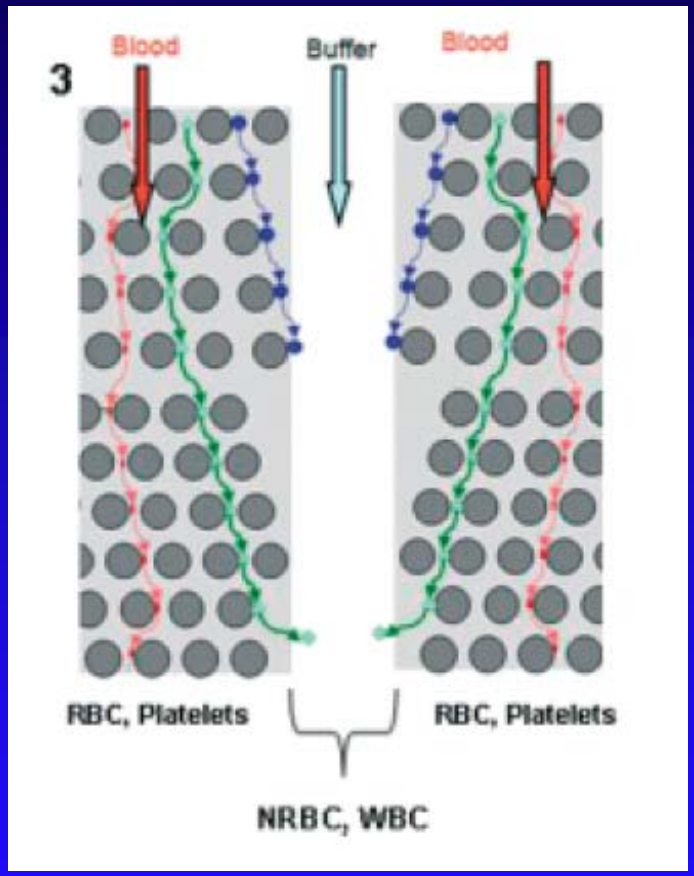
Ficol gradient leads to a 3-5 fold increase (8 cells / case)

Questions: Time taken / case

% of False Positives

Artemis - Microfluidics





Advantage: No Ficoll gradient – reduced loss of target cells (30-50%)

Huang et al., PD 2008

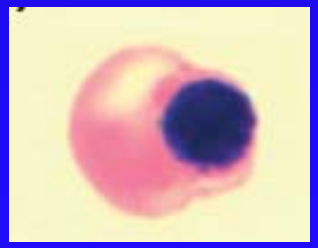
Microfluidics - Results

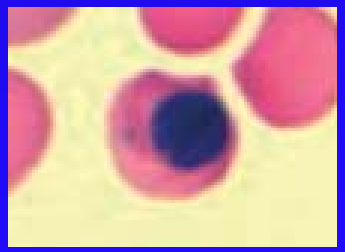
Table 1—Enrichment performance of microfluidic device

	NRBC	RBC	WBC	Platelet
Whole blood	100%	100%	100%	100%
After enrichment	Unknown	0.01%	0.01–0.10%	0%
Number of cells/mL remaining	1–275	5 000 000	8 000–80 000	0

NRBC, nucleated red blood cell; RBC, red blood cell; WBC, white blood cell.

Effectively clear sample of 99.9% RBCs – big achievement!!!
Appear to have good recovery of NRBCs





Huang et al., PD 2008

Microfluidics – Recovery

Process	Gestational age	NRBC/mL
Microfluidics	9-22 wks	38 cells/mL
Lectin	11-40 wks	16
MACS	11-40	2

Significant improvement in recovery!!!

Microfluidics - Conclusions

- Good depletion of RBCs
- Excellent recovery of NRBCs
- No indication of whether they are fetal or maternal!
- Speed of process / number of samples which can be processed?
- How does it compare to other systems:

i. Singapore Nanos system

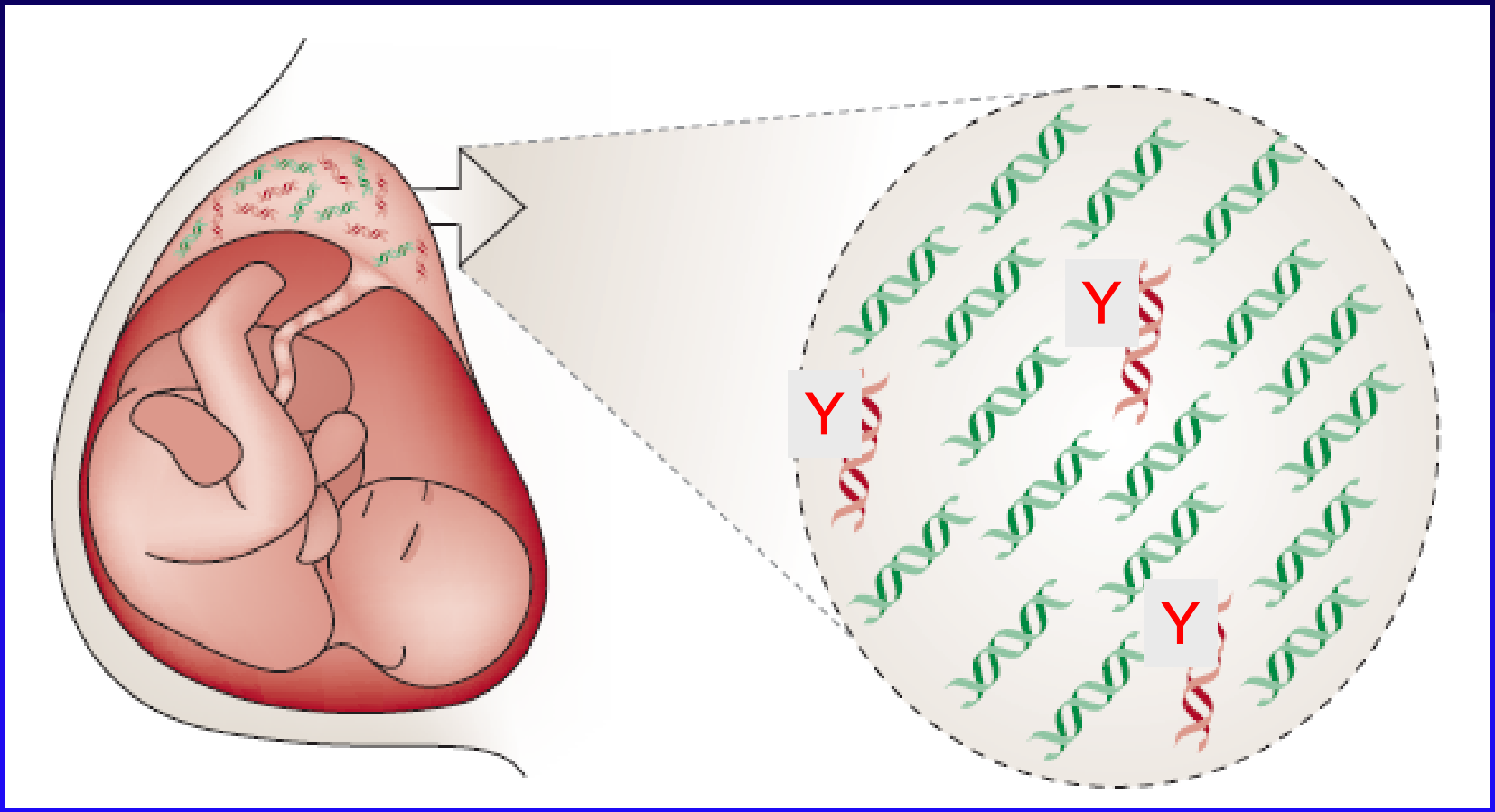
ii. Silicon Biosystems CHIP

Fetal cells - Conclusions

- Fetal cells ain't dead yet!
- Significant improvements in automated detection and enrichment procedures

Questions:
What about analysis – ADO / FISH?
Why focus solely on NRBCs?

What is the competition doing?



Fetal cf-DNA detected via Y chromosome

Lo YM et al., Lancet 1997

Cell free fetal DNA

- Cell free fetal DNA is almost exclusively of trophoblast origin
- Total (maternal) cell free DNA is largely of hemopoietic origin
- Cell free fetal DNA concentration <10%
- Cell free DNA has a short half-life = 15min

Sequenom Mass-Array Compact

Highly specific detection of point mutations and SNPS

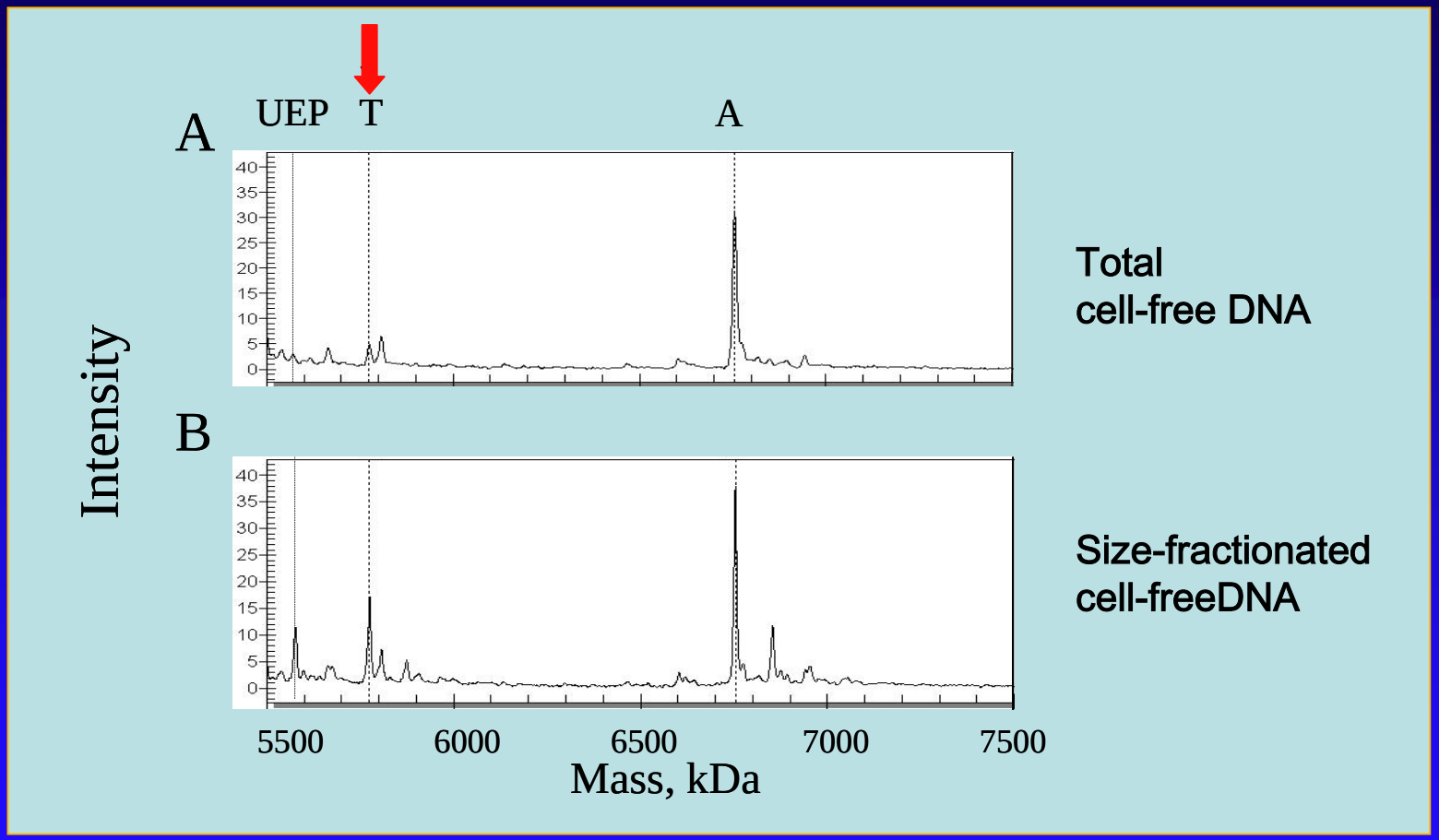
Real-time data acquisition analysis

Analysis of over 3000 samples/day

Automated data analysis

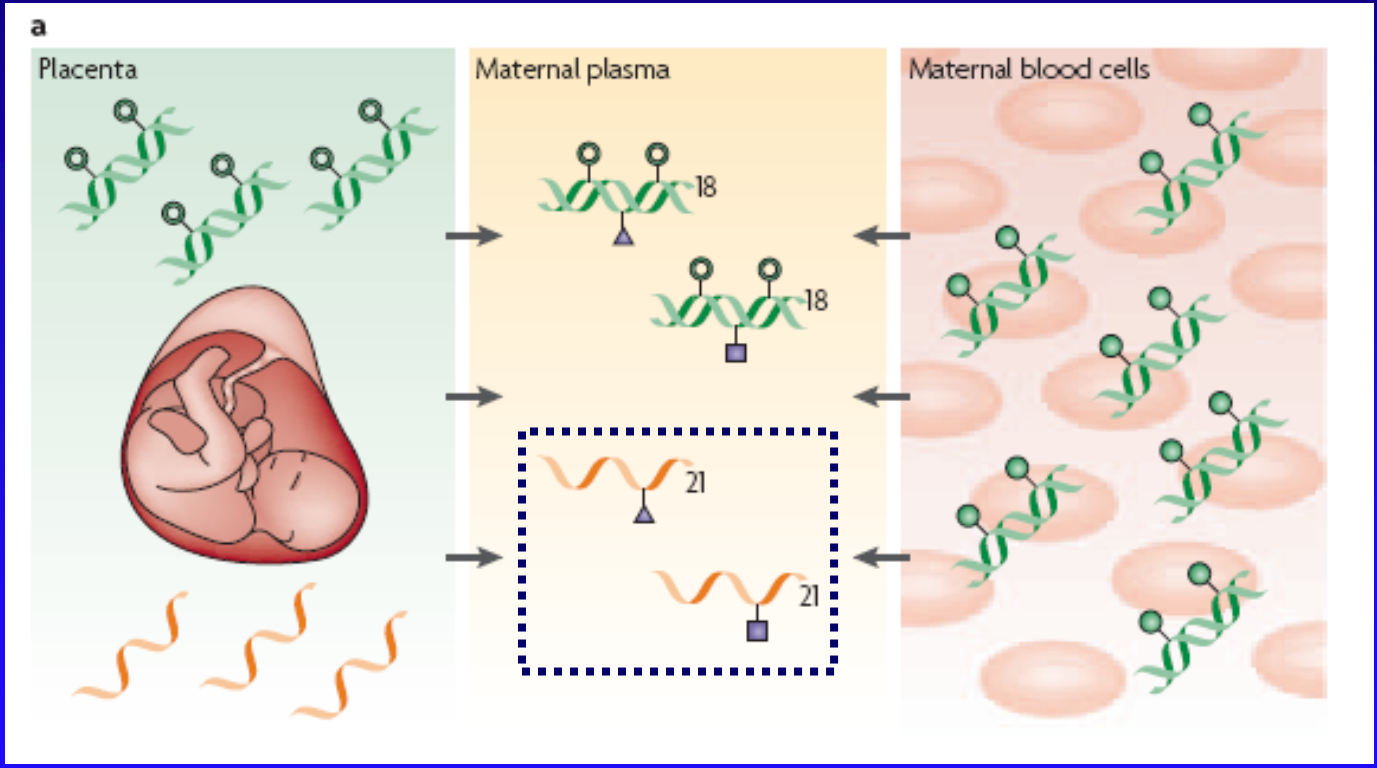
Cost: € 300K

Detection of thalassemia



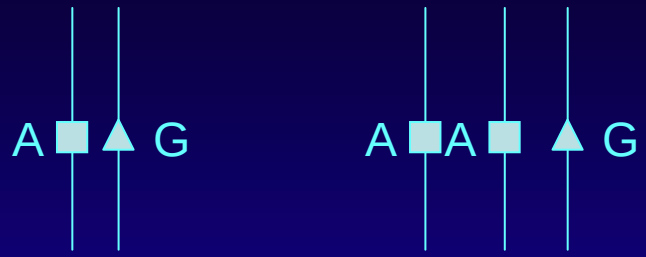
Limited to detection of paternal mutant allele!!

Cell-free placental mRNA

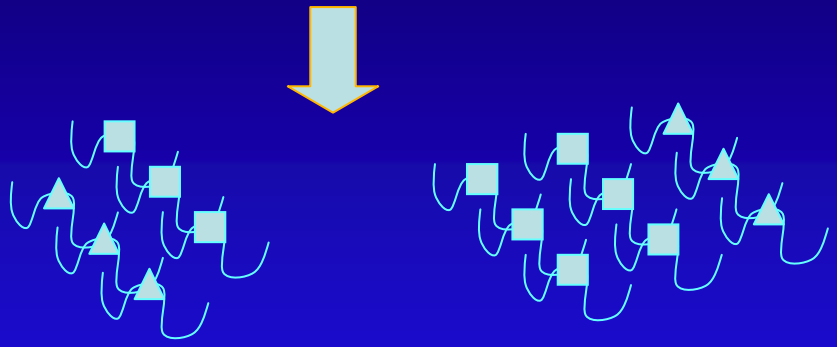


Advantage of placental mRNA

- Select fetal mRNA not expressed by mother – very similar to Y chromosome
- Select genes present on chr. 21
- Select loci in these genes which can be used for quantitative analysis e.g. SNP
- PLAC1 mRNA



Transcription and presence as cff-RNA



RT-PCR and MS Quantitation of SNP allelic ratios

mRNA and Tri 21

- 90% sensitivity, 3.5% FP
- Small study size (10 cases / 56 controls)
- Need fetus that is heterozygous for SNP locus i.e. almost 100 cases excluded
- Need more markers to make test robust
- Need additional genes on Chr 21
- Extend test for chromosomes 13 and 18

mRNA is difficult to ship and process

Sequenom

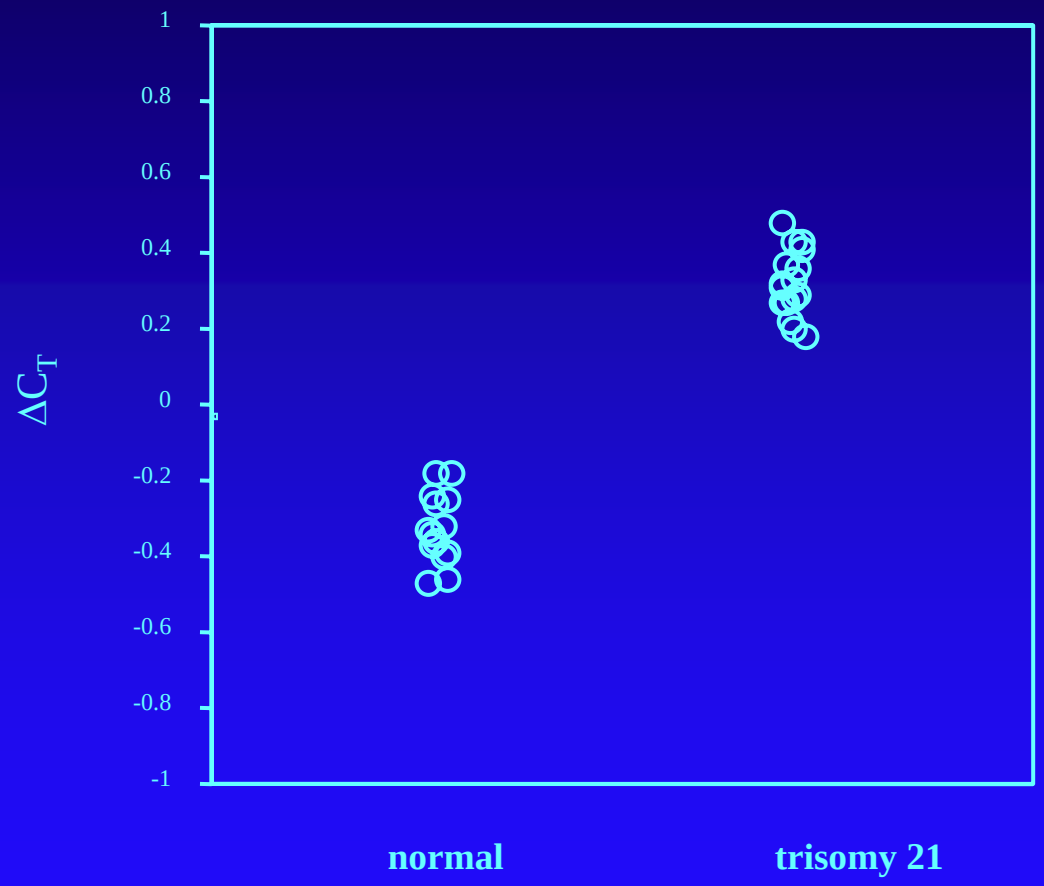
Press release 28 Jan 2009

- 459 samples
- 8/8 Downs correctly identified (1st Tr)
- 14/15 Downs correctly identified (2nd Tr)

Working on a DNA based test for 13, 18, 21.

1. Ethnicity of samples – Chinese, Asian?
2. Does mRNA approach not work for 13, 18?

Taqman detection of Down's syndrome: compare locus on chr.21 vs chr. 18



Will not work on cell-free fetal DNA due to preponderance of maternal sequences!

Zimmermann et al., Clin Chem 2002

Digital PCR – back to the future?

Correspondence

Detection of Aneuploidy with Digital Polymerase Chain Reaction

H. Christina Fan and Stephen R. Quake*

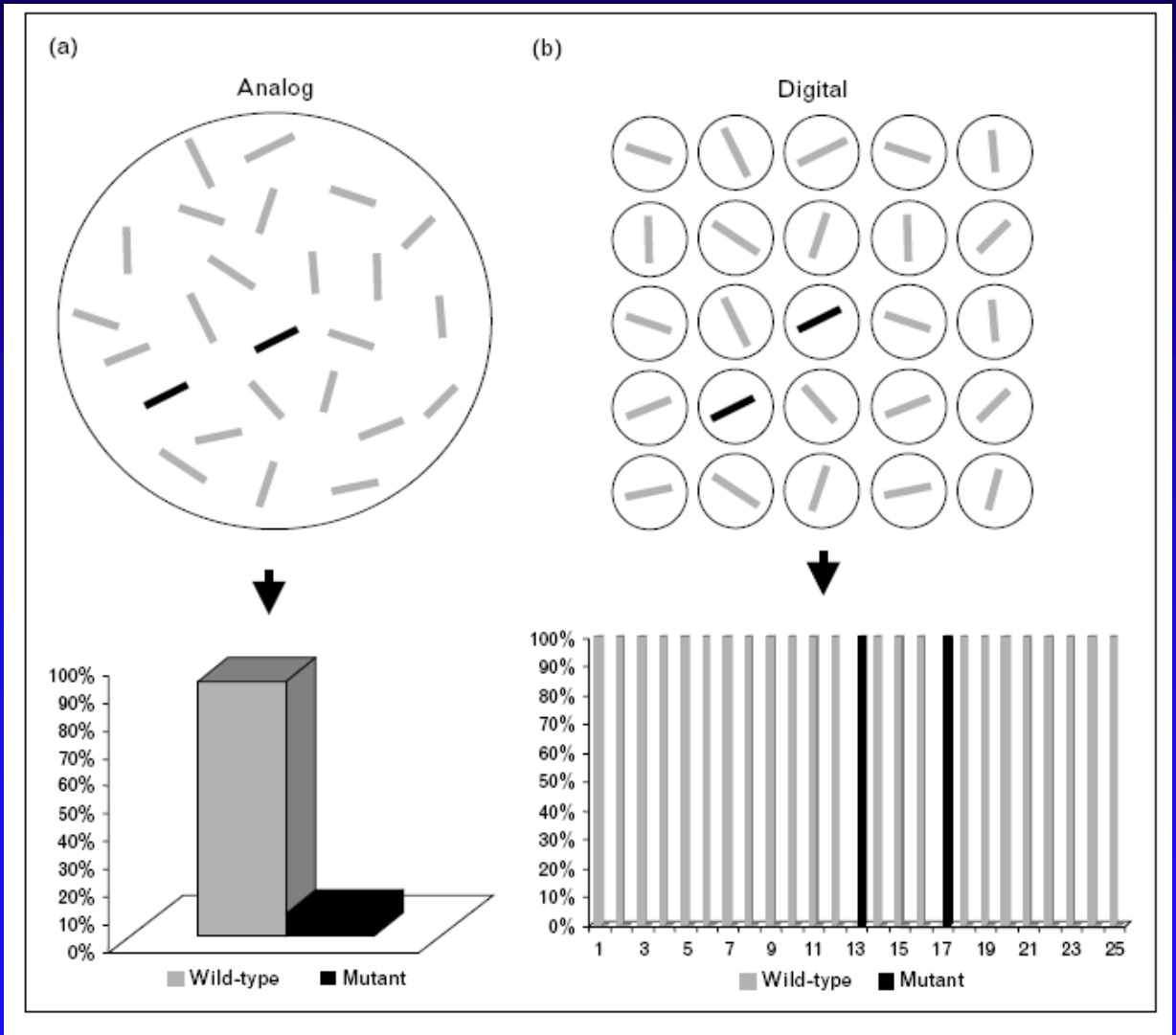
Department of Bioengineering, Stanford University and Howard Hughes Medical Institute, Stanford, California 94305

Digital PCR for the molecular detection of fetal chromosomal aneuploidy

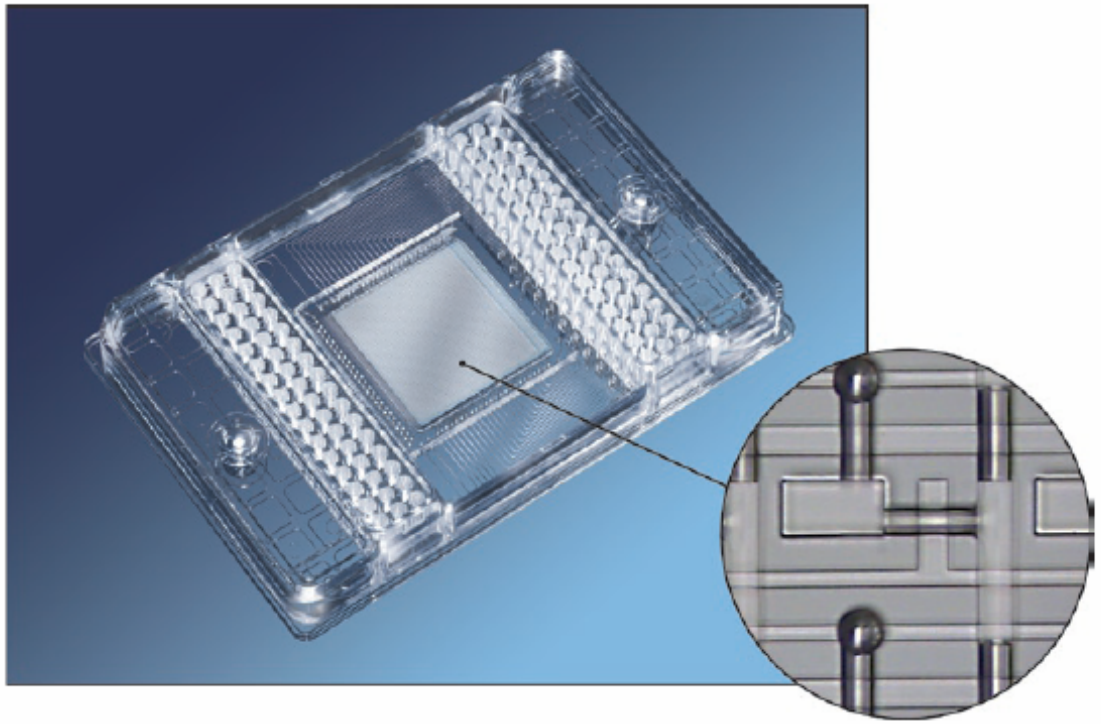
Y. M. Dennis Lo*[†‡], Fiona M. F. Lun[†], K. C. Allen Chan[†], Nancy B. Y. Tsui[†], Ka C. Chong[§], Tze K. Lau[¶], Tak Y. Leung[¶], Benny C. Y. Zee[§], Charles R. Cantor[‡‖], and Rossa W. K. Chiu*[†‡]

Explored by Vogelstein & Kinzler in 1999

Digital PCR: absolute values, not relative (analogue)

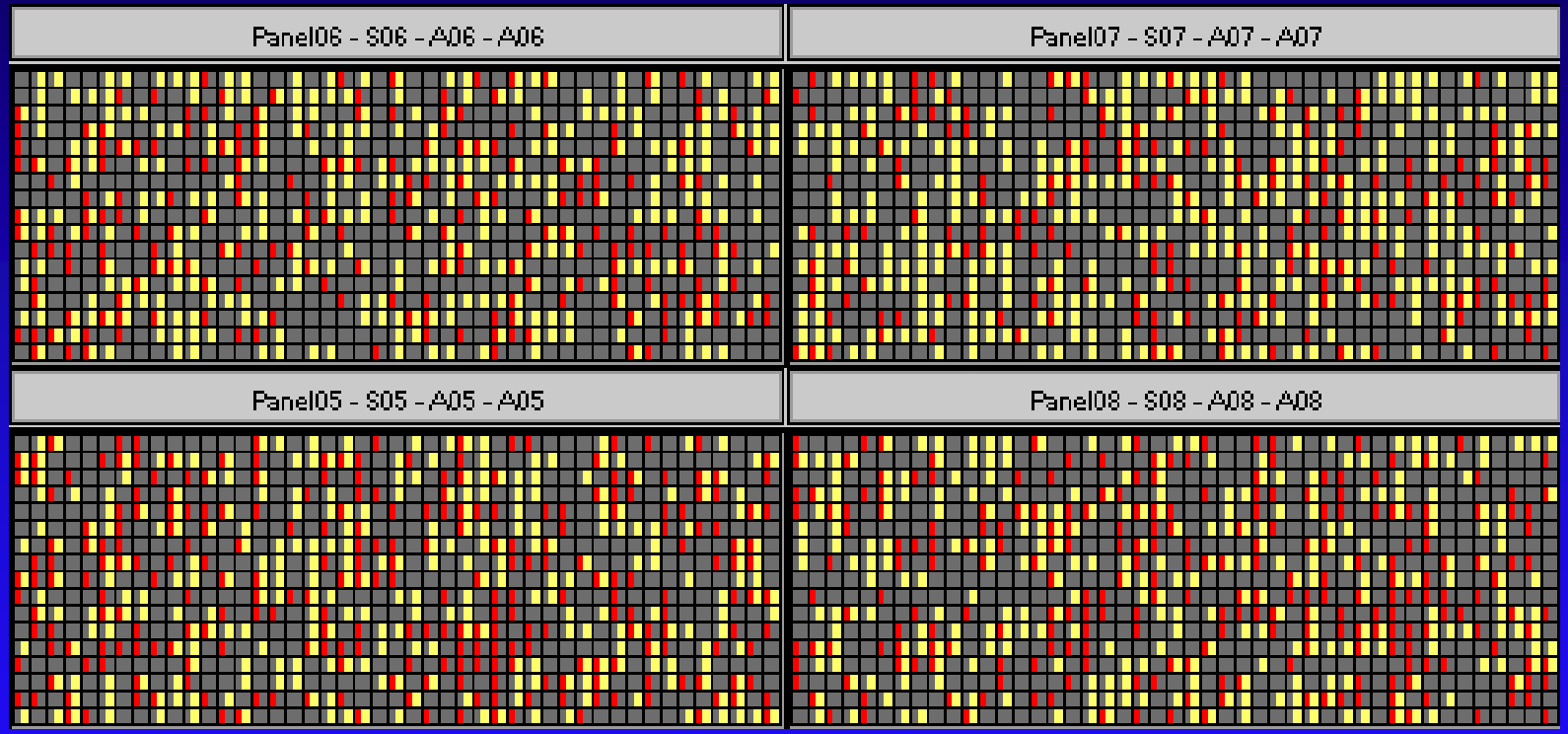


Fluidigm digital PCR device



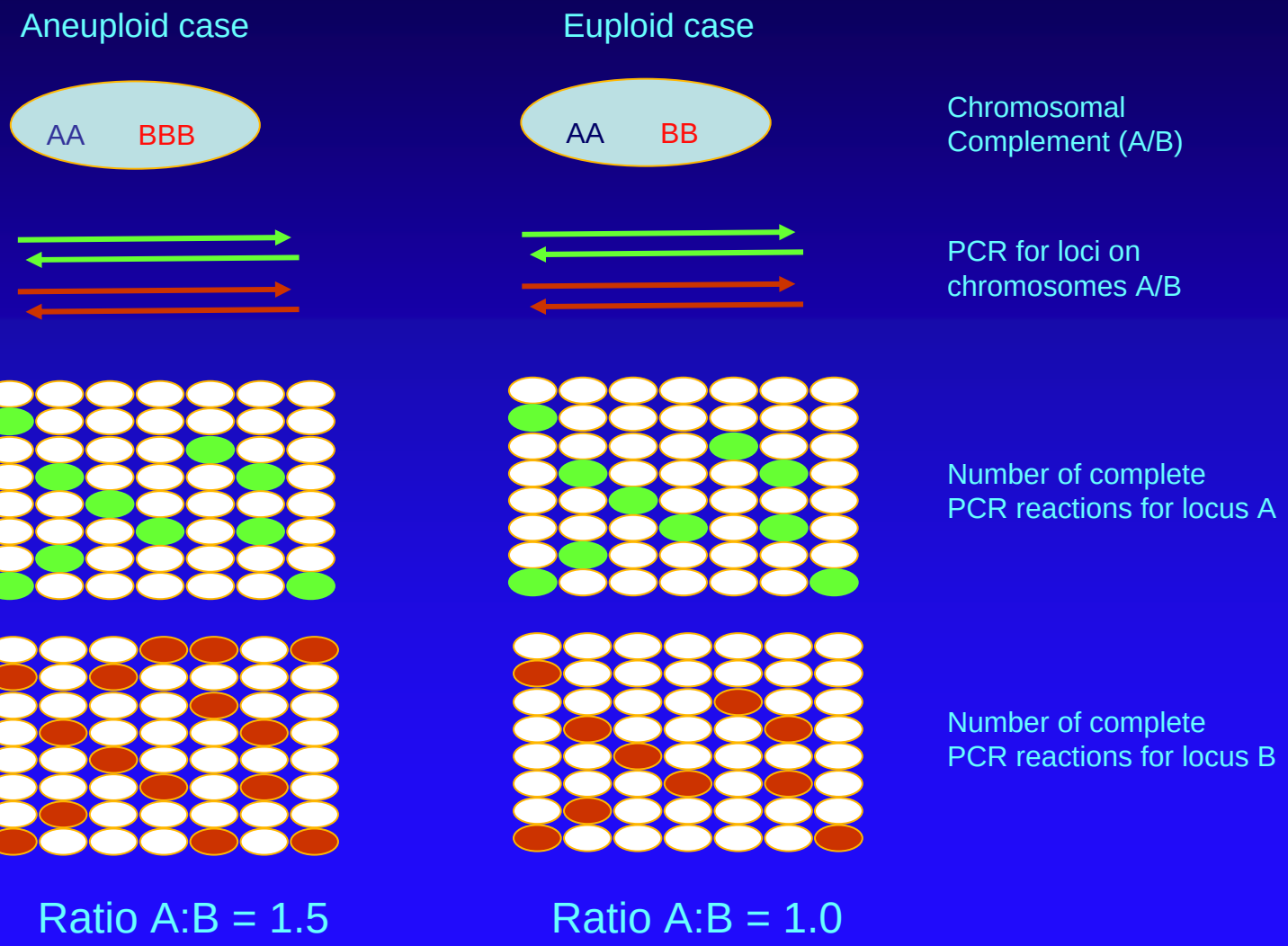
Fluidigm offers several array-based solutions for conventional and digital PCR. (Image courtesy of Fluidigm.)

Fluidigm digital PCR device

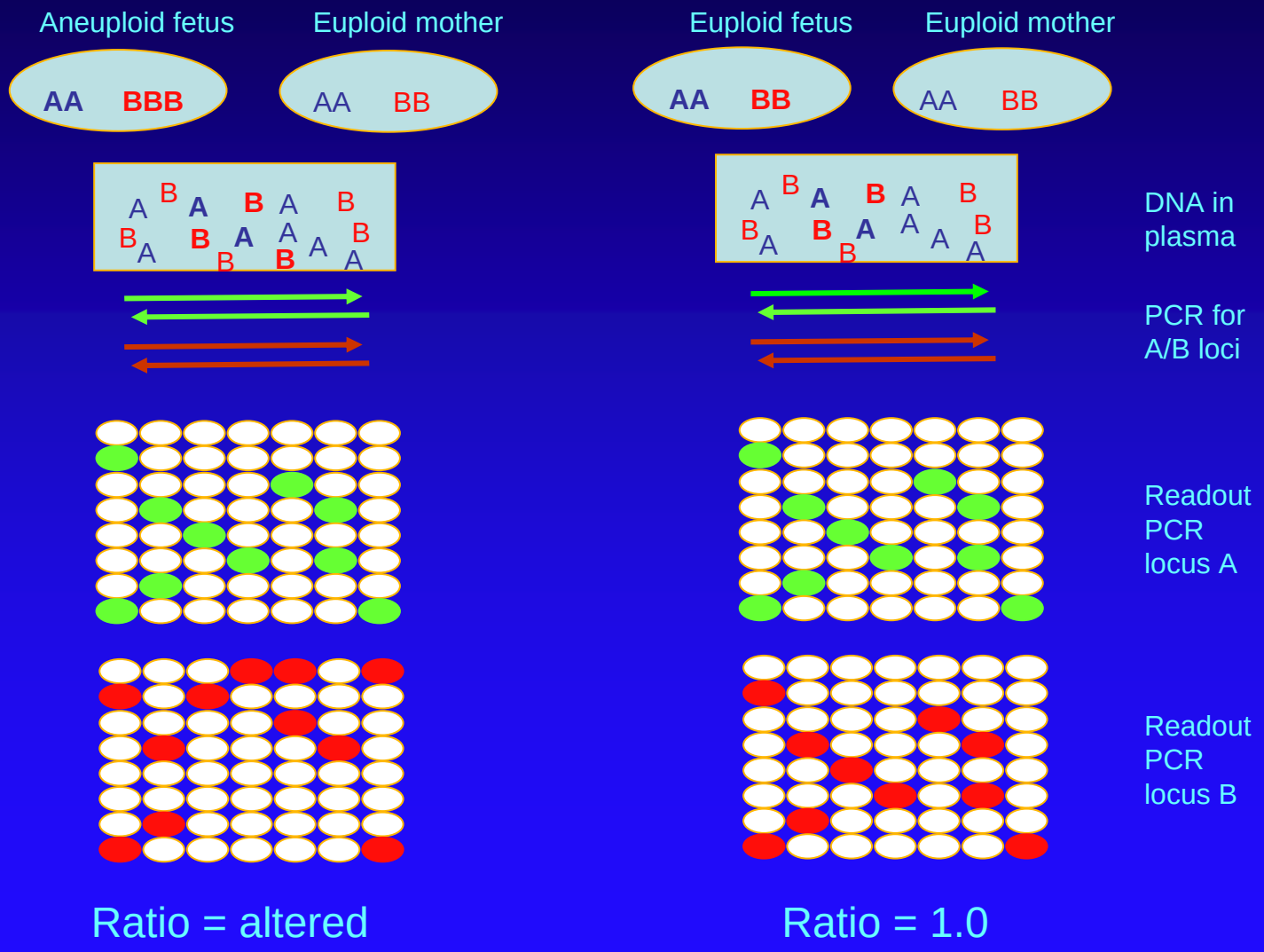


12765 individual reactions per chip

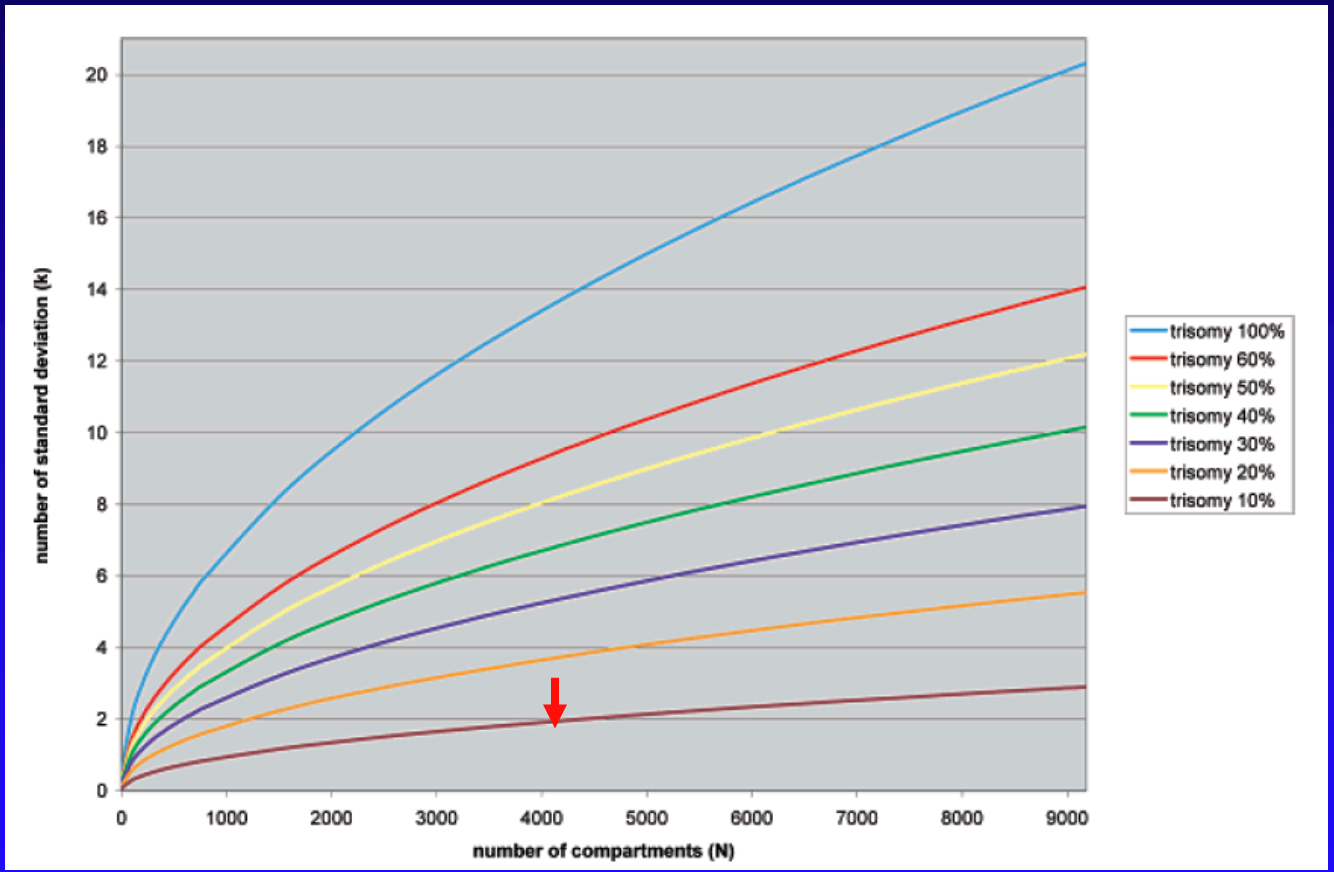
Fan and Quake: Digital PCR for Aneuploidy detection



Digital PCR detection of trisomy 21 via cell-free DNA in maternal plasma

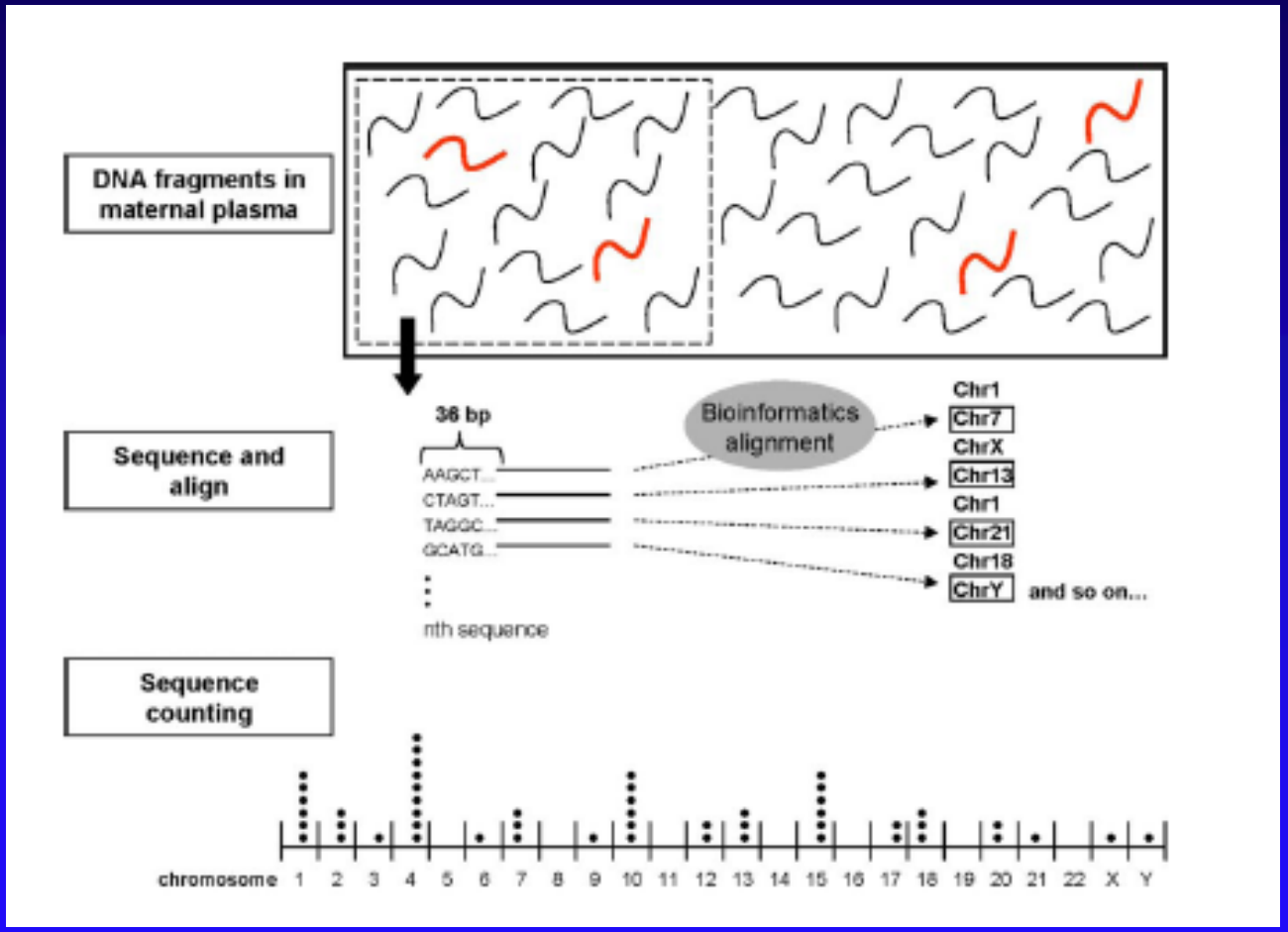


Fan and Quake: Dilution



Detect trisomy 21 at a concentration of 10% with 4000 events
Could be made to work on cell-free DNA in maternal plasma!

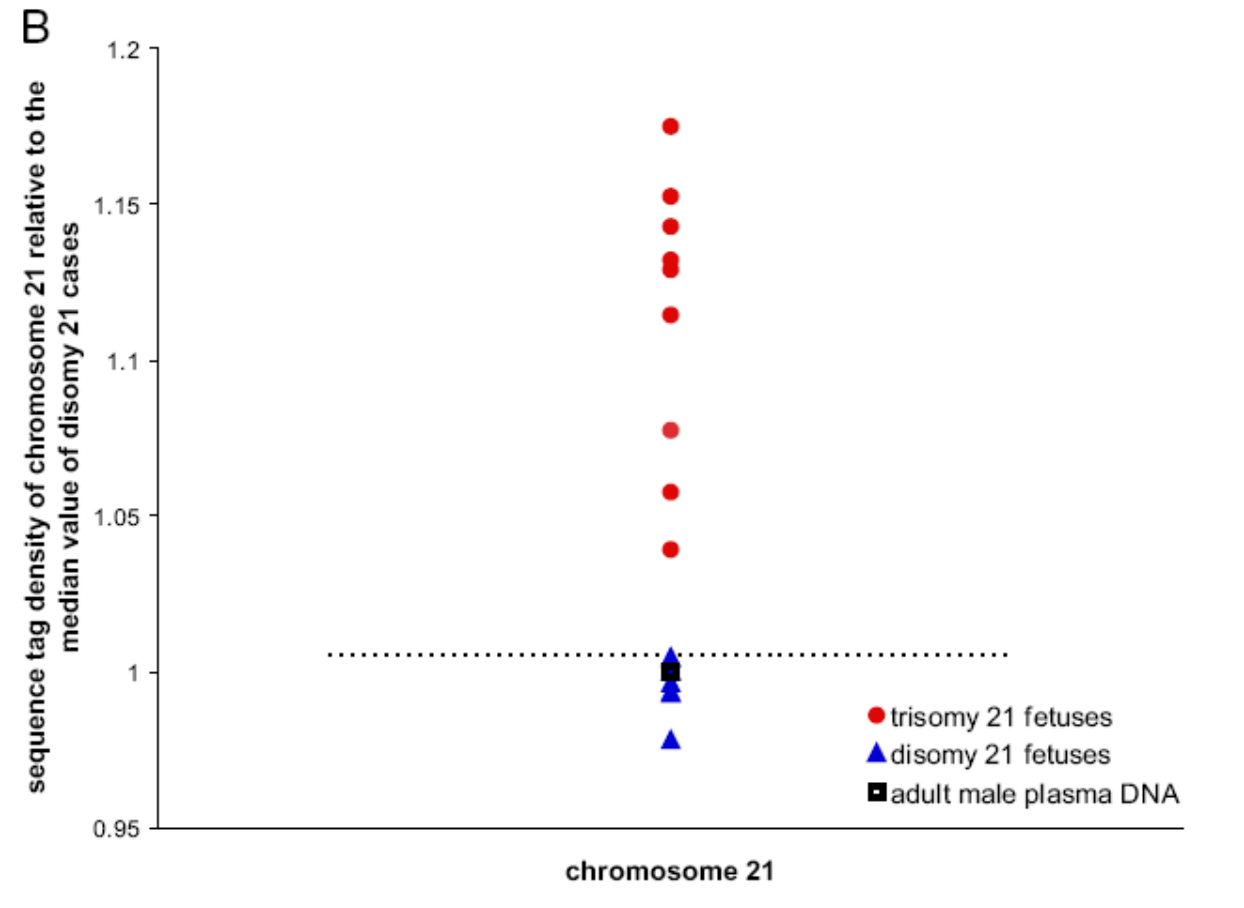
Shot-Gun Sequencing



Approximately 65 000 sequence tags for chromosome 21

Fan et al., /Chiu et al., PNAS 2008

Shot-Gun Sequencing



Fan / Quake PNAS 2008

Shot-Gun Sequencing

Fan/Quake: Examined samples from 18 pregnant women.
Correctly identified:
9 cases of Tri. 21 (Down syndrome)
2 cases of Tri.18 (Edward syndrome)
1 cases of Tri. 13 (Patau syndrome)
All 6 normal cases

Chiu/Lo/Cantor: Examined samples from 28 pregnant women.
Correctly identified:
14 cases of Tri. 21 (Down syndrome) / all normal cases

Samples taken post-invasive procedure in both studies

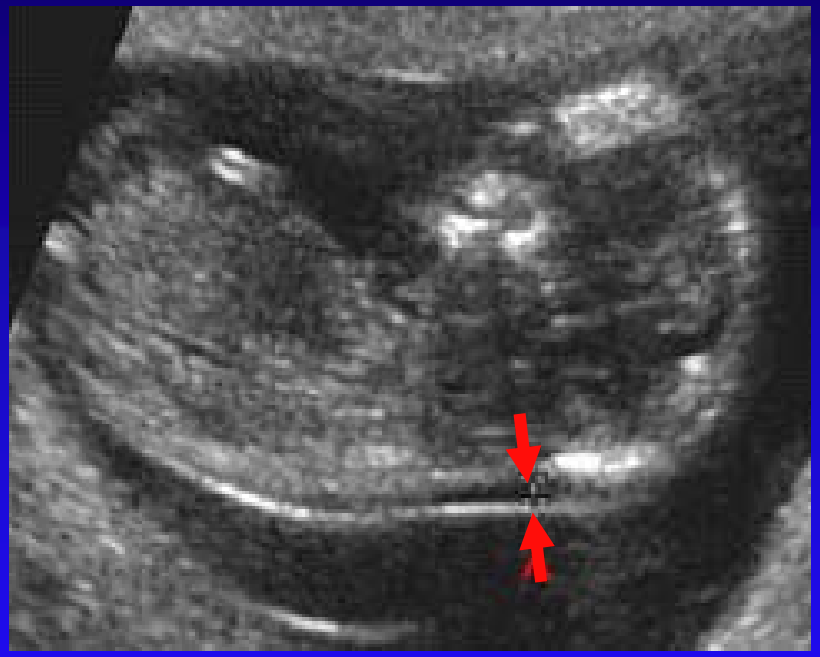
Shot-Gun Sequencing

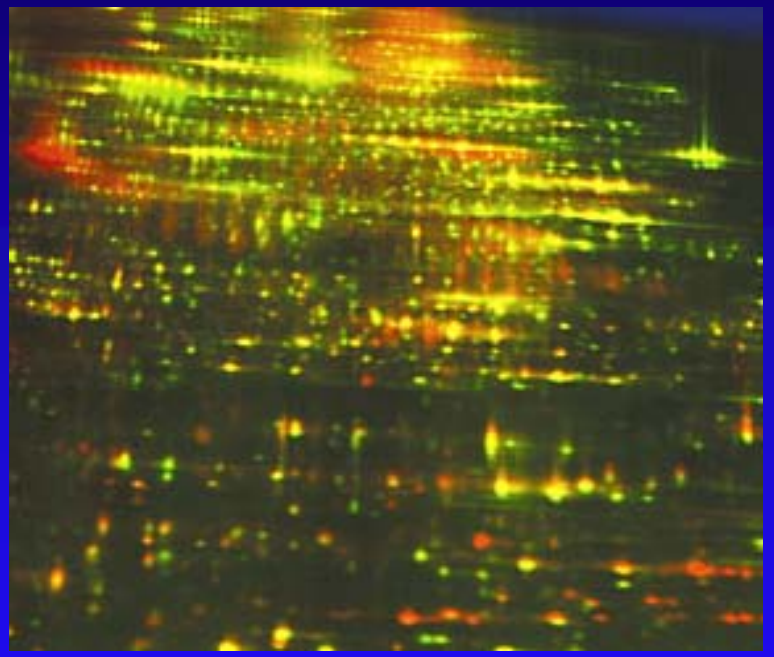
1. Preparation of gDNA library : 6 hours
2. Generation of clusters : 5 hours
3. Sequence clusters : 2.5 days
4. Data analysis : 1-2 days
5. $700/sample
6. 16 samples / week / Illumina instrument

Conclusions

- Powerful technique due to development of microfuidics / shot gun sequencing
- Tools and assays are expensive
- Useful for Mendelian disorders?
- Likely to large role in future analysis of cell-free DNA

What is the competition doing?

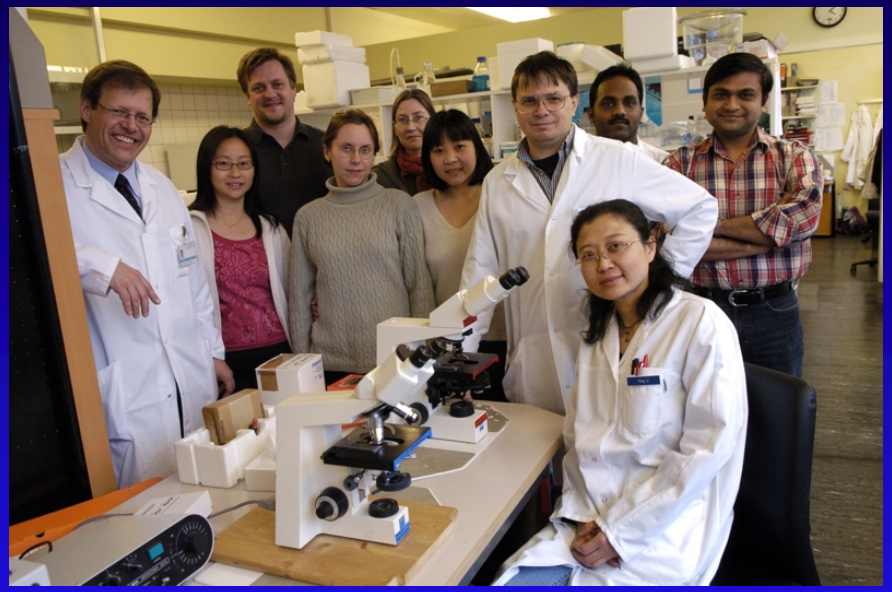




Proteomics – aneuploidy specific markers?

Is Sensitivity / Specificity > 95-98% achievable?

Thanks to:



C. Hahn

UFK, Basel:

W. Holzgreve, O.Lapaire,

I.Hösli, S. Tercanli,

Grants:

SNF, SAFE , Pregenesys

P. Hasler: Aarau

B. Huppertz: Graz

S. Gebhardt, R. Hillermann: Stellenbosch, RSA

E.Di Naro, A.Vitucci: Bari, Italy

M. Choolani, Singapore