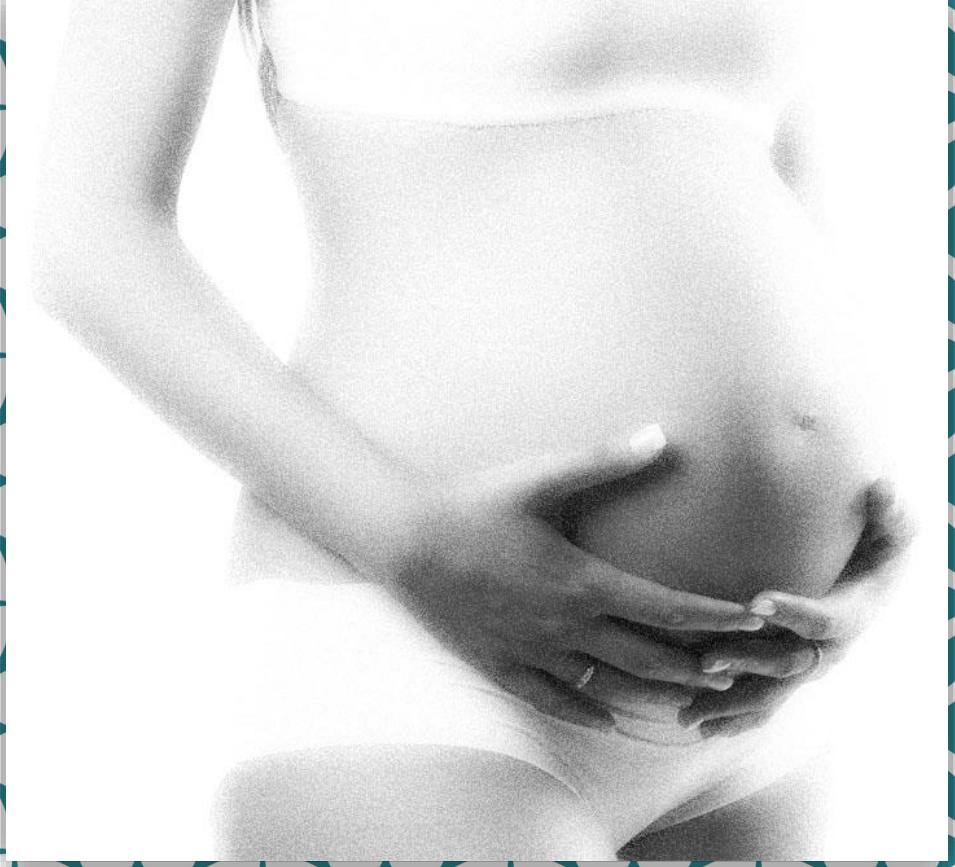

TOWARDS AFFORDABLE NIPT - LOW COVERAGE WHOLE GENOME RESEQUENCING BASED NIPT ON SMALL BENCHTOP NGS SYSTEMS

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ANEUPLOIDIES NON-INVASIVELY

1997 – cffDNA discovery

2005 – First next generation sequencing system was introduced

- Digital molecule counting possible

2009 – Proof of concept that next generation sequencing works for trisomies

2011 – High sample number studies confirm performance, first tests become available

2012 – Non Invasive Prenatal aneuploidy Test (NIPT) suitable alternative for screening of high risk pregnancies (ACOG Opinion)

2015 – ISPD committee for chromosome abnormality screening statement – NIPT should replace standard screening schemes for assesing the risk of T21, T18 and T13 pregnancies

NIPT SCREENING – WHERE IS THE PROBLEM?

Routinely carried out on high throughput sequencers = HIGH INFRASTRUCTURE COSTS

Most samples analyzed in two places – California and China = HIGH LOGISTICS COSTS

TEST PRICES HIGH

Limited use

SCREENING THE POPULATION

Very high sensitivity and specificity

Currently by far the most expensive scheme

The price cost should be under 300 EUR

(Evans et al. - **Cell-free fetal DNA screening in the USA: a cost analysis of screening strategies,** Ultrasound Obstet Gynecol., 2015)

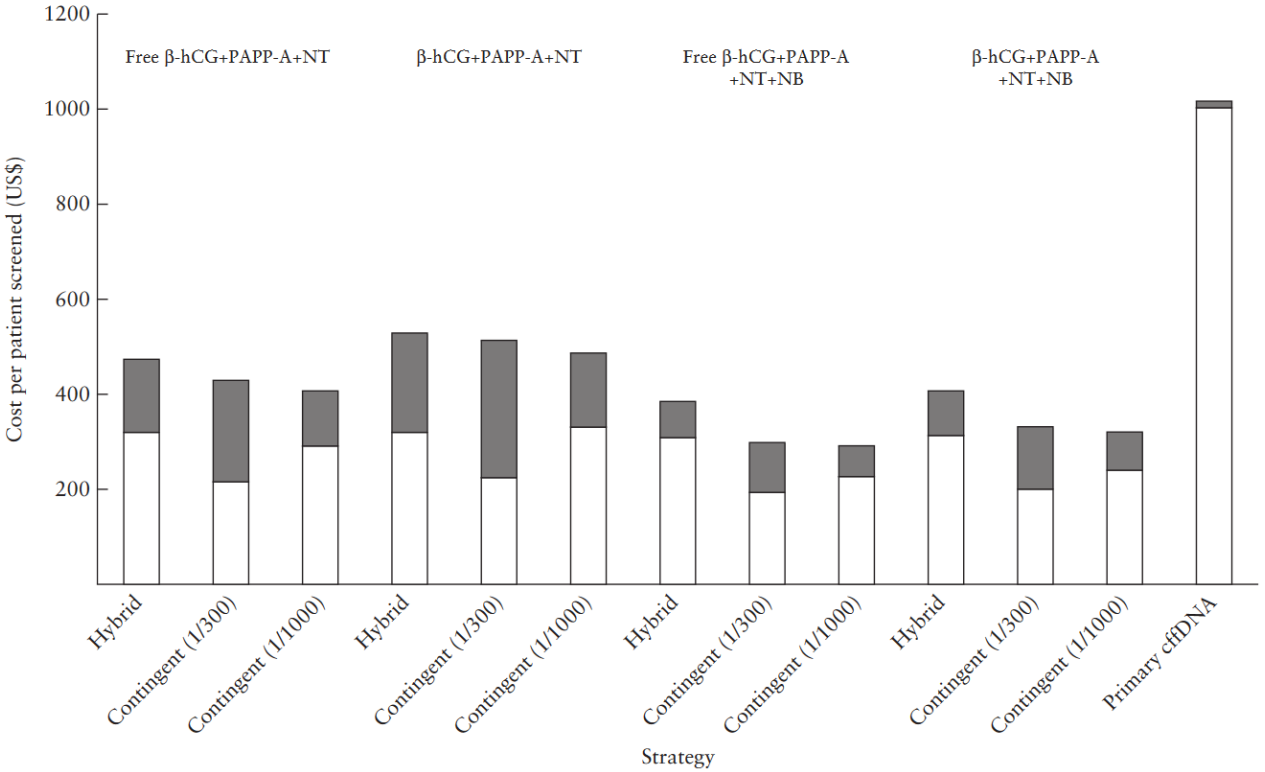


Figure 2 Total cost of implementation of each cell-free fetal DNA (cffDNA) screening strategy, including clinical and laboratory costs (□) and cost of care for missed cases (■). Numbers in parentheses are risk cut-off.

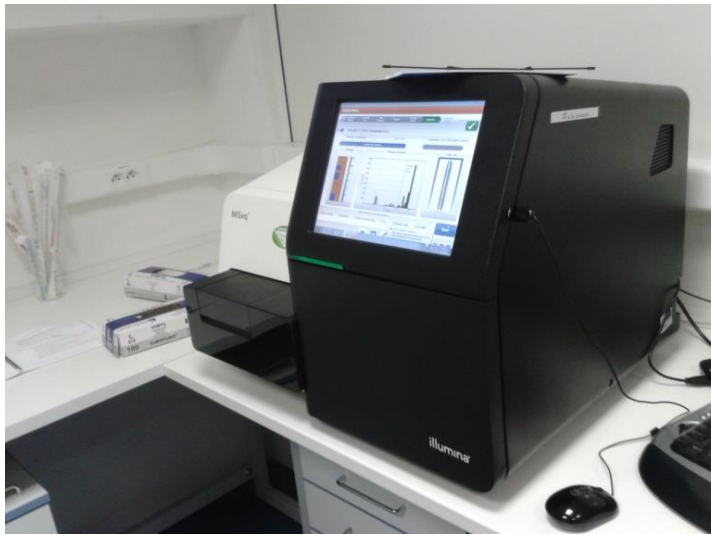
SMALL NGS SYSTEMS

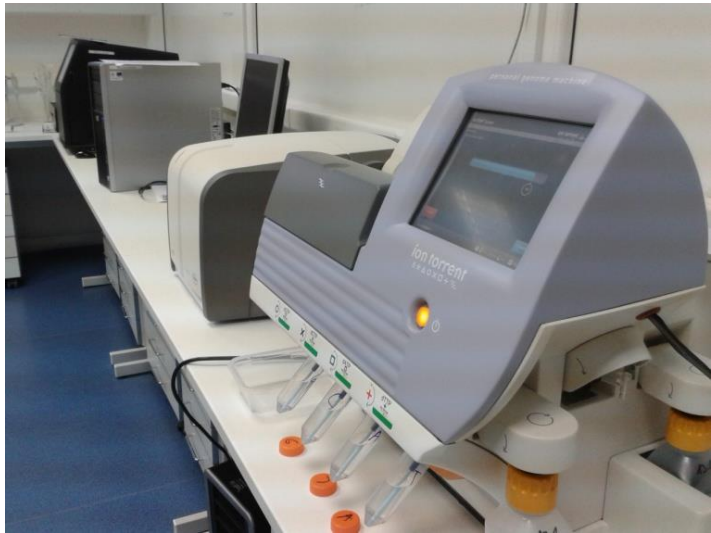
Advantage

- Cheapest NGS systems
- Easy to deploy
- Cheap and easy to run
- No need for extra computational resources
- Automation not essential

Drawback

- Lower read counts = lower multiplexing
- Higher cost per base





NIPT ON SMALL NGS
WHAT IS THE POINT?

Can be easily adopted in smaller general genetics lab

Closer lab = faster results (1-2 days turnaround)

- Where speed is a priority

Good for low numbers of samples (small countries of Europe)

Where sample number flexibility is a priority (1-10 samples in one batch)

In countries with legislative barrier for sending samples abroad

WHAT DID WE DO?

NIPT with whole genome approach

Two systems were used – Illumina MiSeq and Life Tech Ion Torrent PGM

2 x 100 cycle paired end reads used on MiSeq and 200 cycle sequencing on ion Torrent

*In silic*o size selection to virtually over-represent fetal fraction

Z-score determined by novel appoach using different statistical distribution model

Smaller sample set analyzed on two systems, due to costs larger sample set analyzed on Illumina Miseq

170 plasma samples were collected from pregnant women with amnio results available (12 gestation week or higher)

Only **130** were used in comparison of both systems (all samples were analyzed only on Illumina MiSeq system)

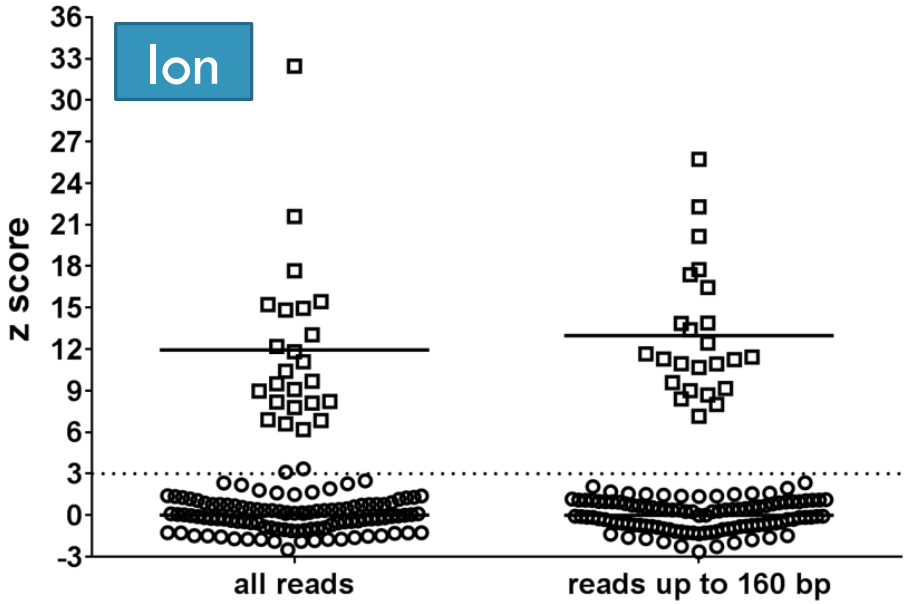
24 cases of T21, **4** cases of T18, only **2** cases of T13 (1 excluded due to sample processing error)

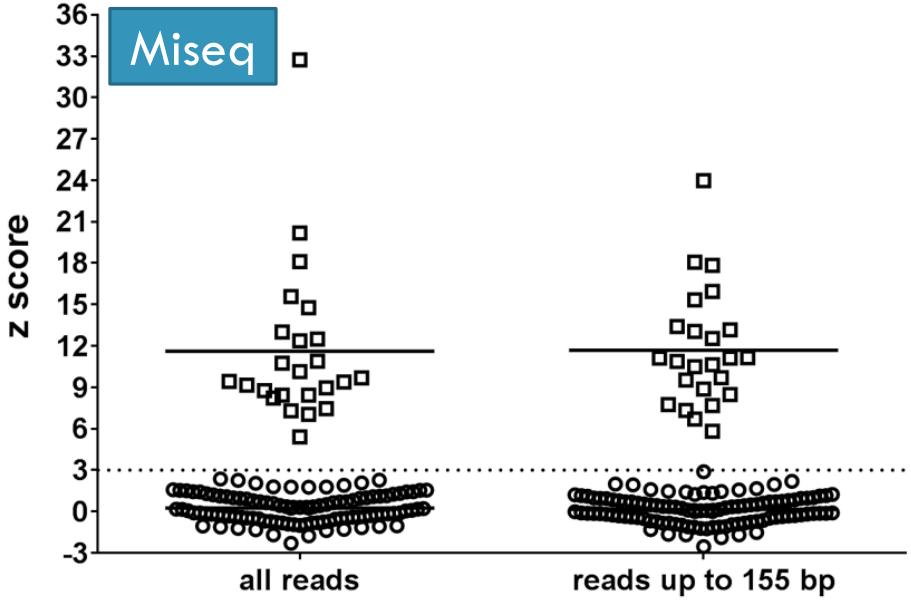
COMPARISON OF T21 RESULTS FOR BOTH

130 sample set run on both systems

Z-score calculation by Sehnert et al. 2011

	Ion Torrent PGM no size selection	Ion Torrent PGM *in silico* size selection	MiSeq no size selection	MiSeq *in silico* size selection
Sensitivity	100%	100%	100%	100%
Specificity	98.11%	100%	100%	100%



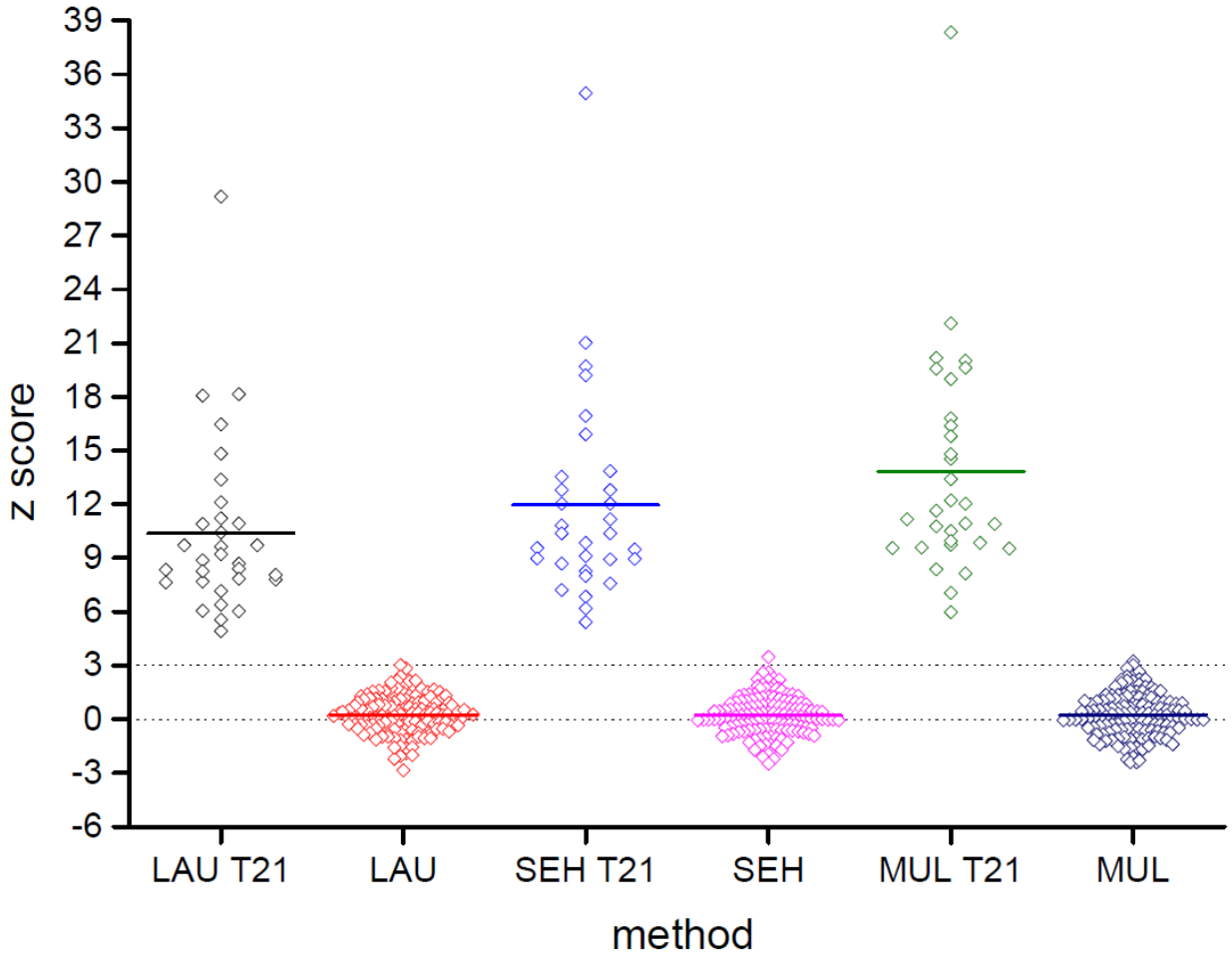


TRISOMY 21 ON MISEQ

All **170** samples analyzed only on Illumina MiSeq

31 cases of T21

1 false positive case was observed (sensitivity 100%, specificity 99.3%)

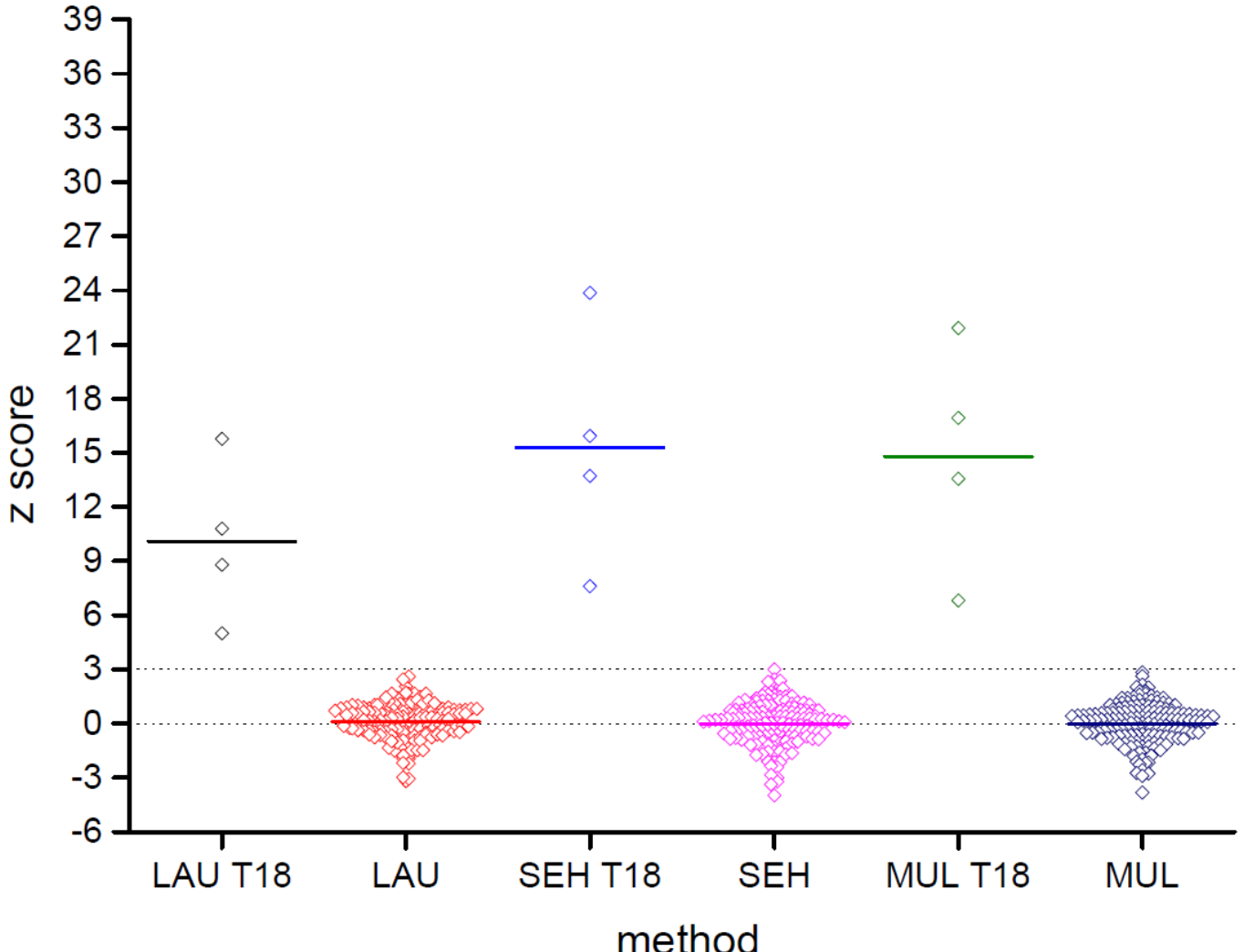


TRISOMY 18 ON MISEQ

All **170** samples

0 false positive

0 false negative



CONCLUSION

Small sequencers work for NIPT (we need a prospective study to confirm results)

Paper submitted

We continue only with the Illumina MiSeq due to reagent costs

Local labs can adopt these systems and significantly reduce time to patient results

Lower cost could lead to lower test prices and final implementation in routine screening

ACKNOWLEDGMENTS

Circulating fetal DNA research

- **Gabriel Minárik**

Budapest collaboration

- **Bálint Nagy**

MiSeq lab work

- **Michaela Hýblová**

Ion Torrent PGM Team

- Ema Nagyová
- Katarína Šoltys
- Gabriela Repiská

Clinical cooperation

- Rastislav Sisák
- Mária Geryková Bujalková

FUNDING

- REVOGENE project supported by the Research & Development Operational Programme funded by the ERDF