Polar Body Analysis with Array-CGH

- a Clinical Pilot Study

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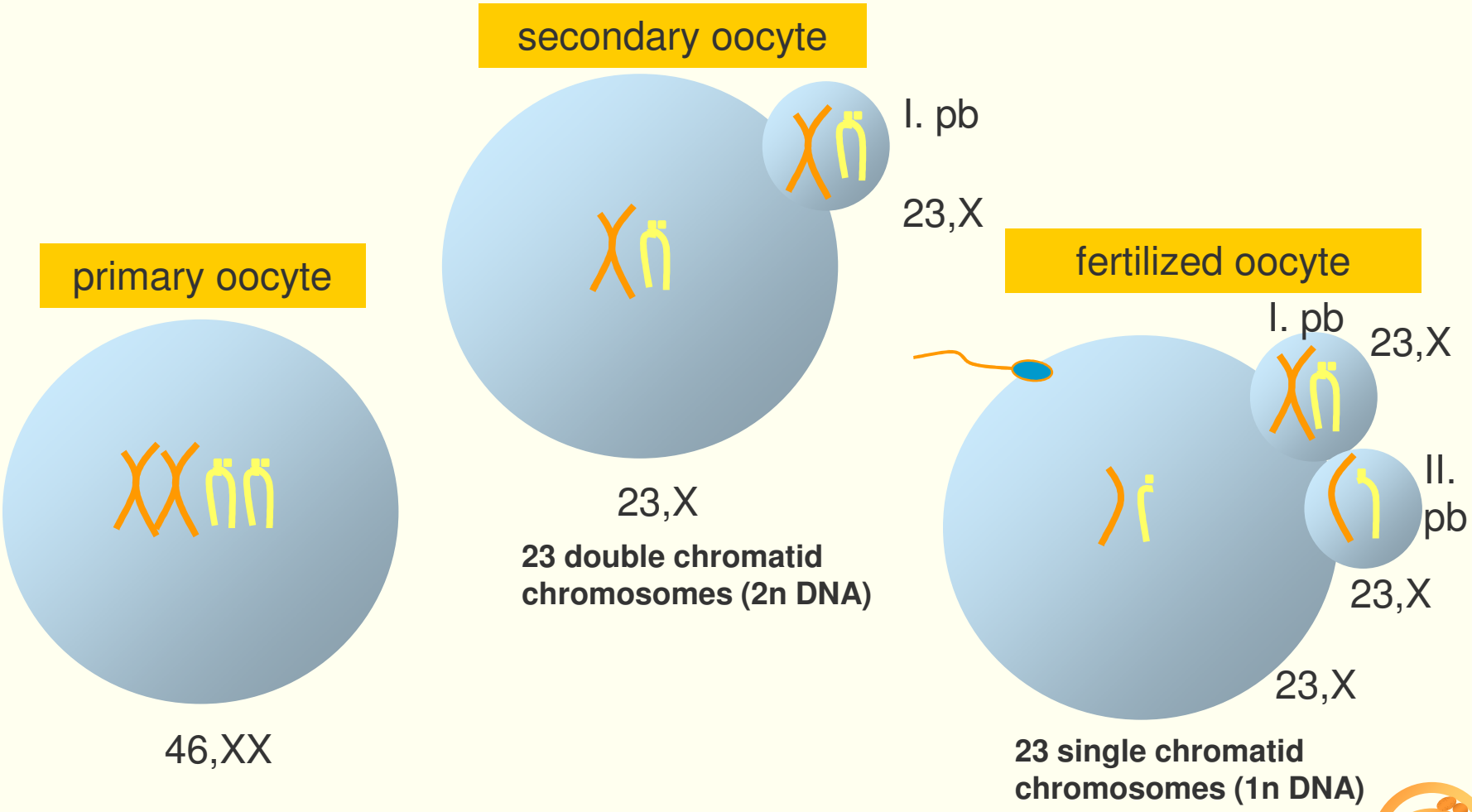
Aim of the Study

- analyse gain or loss of all 23 chromosomes
- analyse partial gain or loss of chromosomes
- restrictions of the German Embryo Protection Act
  - strict time frame
  - no analysis on blastomeres

I. and II. Meiosis of Oocytes



Study Design

1. Step - Validation

- Evaluation of polar body and corresponding oocyte
  for gain or loss of chromosomes (numeric aberration)
- 10 oocyte-/polar body pairs
  with required clinical information
  freshly retrieved and frozen polar bodies/oocytes

2. Step – clinical pilot study

- to confirm the time schedule
- to test feasibility of the procedure
- to test outcome and reliability of the diagnostic tool
- polar body analysis for 10 patients

3. Step – clinical study

- to compare pregnancy rates
- to evaluate certain aneuploidies
- 50 -100 patients, compared to a indication and age matched control group

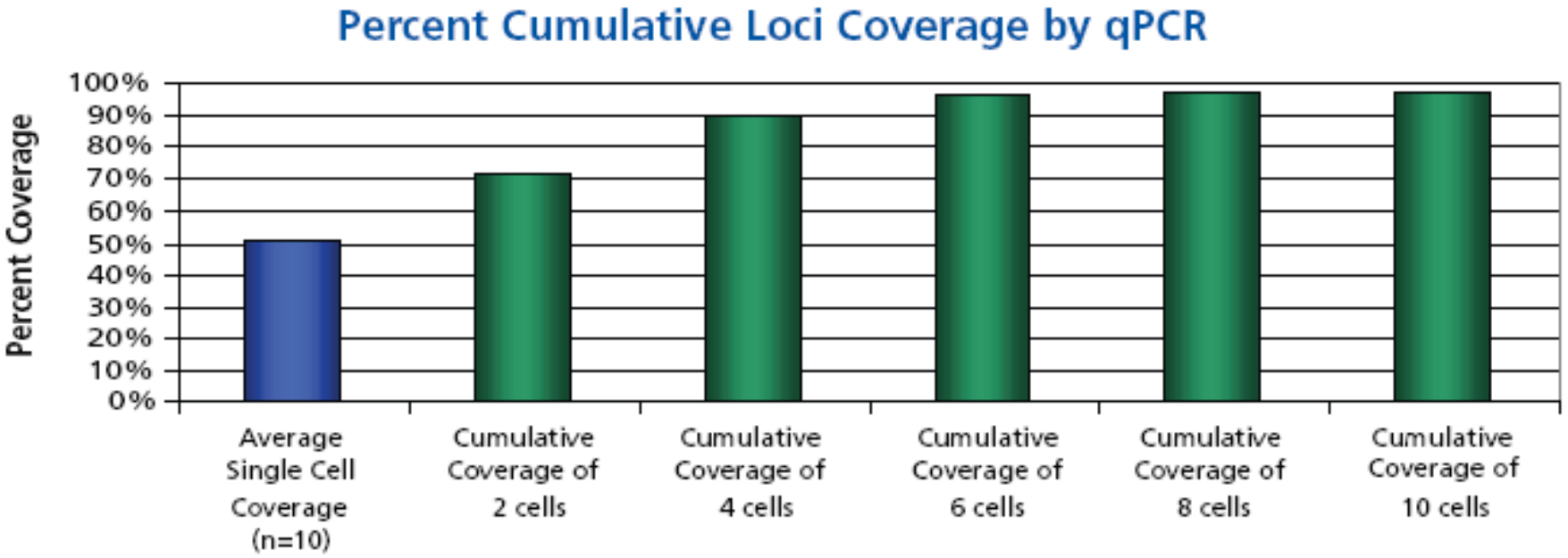
Amplification Methods

- - linker adapter PCR - the target DNA is digested with an appropriate restriction enzyme and then each end is ligated to an adapter. These known adapter sequences are used to uniformly amplify each of the many DNA fragments representing the original sample.

- - modified DOP PCR (WGA-Sigma) - DOP-PCR uses Taq polymerase and semi-degenerate oligonucleotides that bind at a low annealing temperature at approximately one million sites in the human genome. The first cycle is followed by a PCR, allowing only for the amplification of the fragments that were tagged in the first step.

- - MDA-PCR (multiple displacement-PCR) (Qiagen–Kit) - The procedure is a non PCR based procedure that involves the binding of random hexamers to denatured DNA followed by strand displacement synthesis at a constant temperature using the enzyme Phi29 DNA polymerase.

Whole Genome Amplification



Vassar-Nieto, et. al. Sigma-Aldrich

Procedure Time

Conventional CGH (total more than 76h)	
Amplification	4:00
Nick-Translation	2:15
Precipitation	**12:00**
Preparation	3:00
Hybridisation	**48:00**
Wash	2:30
Analysis *	**5:00**

Modified CGH (total about 26h)	
Amplification	4:00
Nick-Translation	2:15
Precipitation	1:30
Preparation	1:30
Hybridisation	12:00
Wash	0:30
Analysis *	**5:00**

Array-CGH (total about 20h)	
Amplification	4:00
Preparation	4:00
Hybridisation	10:00
Wash	1:00
Analysis *	1:00

* To analyse pb of 5 oocytes

Finding the best match ...

Amount of tested DNA	250 ng (High background)	1 µg	2 µg	3 µg (artefacts)
Kind of reference DNA	Amplified	not amplified		
Amount of reference DNA	250 ng	1 µg	2 µg	3 µg (artefacts)
Hybridisation time	7 :00	9:00	11:00	15:00
Resolution	1 MB		600 clones	

Constitutional Chip® - Perkin Elmer

BAC clones covering the whole genome

- > 600 clones printed in triplicate
- Two arrays on same slide (for dye swap)

⇨ Targeted detection of 41 disorders, all trisomies and 41 subtelomeric regions

Pilot Study - Design

2. Step – clinical pilot study

- 10 patients
- 5 oocytes max per patient
- pb 1 and 2 sequentially biopsied and immediately frozen
- amplification of the polar bodies
- scoring of 2 PN oocytes
- further procedure on only the 5 best (most promising) 2PN stages

Pilot Study - Patients

Inclusion:

- 35 - 40 years: mean age 37.3 years
- 2 - 4 unsuccessful cycles of assisted reproduction (ART)
- at least 8 mature oocytes after stimulation

Exclusion:

- malignant disease in one partner
- immunological disorder in female partner
- diabetes mellitus
- other chronic disease
- BMI more than 30
- endometriosis
- chromosomal abnormality
- uterine abnormality
- TESE

Pilot Study - Schedule

		Regensburg	Zentrum für Polkörperdiagnostik
day	**time**		
0	8:00	Oocyte retrieval	
0	11:00	ICSI – oocyte culture	
0	13:00	1. PB biopsy on max. 10 MII oocytes – immediate freezing	
0	20:00	2. PB biopsy on max. 10 MII oocytes – immediate freezing	
1	7:00	Send all individually frozen PB Evaluation PN status Discard all morphologically abnormal oocytes	
1	8:00	Culture of max 5 fertilized oocytes	Arrival of individually frozen PB Amplification (WGA) of max 5 PB according to information of regular 2 PN status (8h)
1	16:00		Array - CGH Procedure (16h)
2	8:00		Array - CGH Analysis and Report

Pilot study - Clinical Results

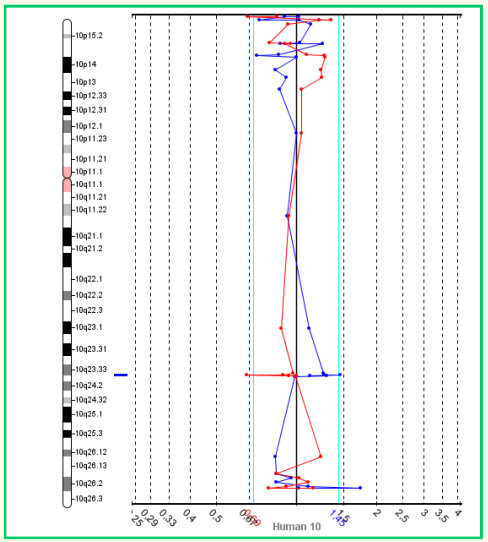
Pat	age	oocytes	PN-stage	Pb analysed	Pb aneuploid	Pb euploid	Transfer	biochem. pregnancy	Ongoing pregnancy
FEL	40	10	6	5	3	2	2	+	n
SPS	35	22	16	5	3	2	2	-	n
TEA	40	9	6	5	3	2	2	+	n
GAG	36	15	9	5	4	1	0	-	-
DÜK	35	15	9	5	3	2	2	+	n
REM	35	15	9	5	2	3	2	+	n
WER	38	5	2	2	1	1	1	-	n
STU	40	17	8	5	3	2	2	-	n
YAY	40	8	6	5	2	3	3	-	n
MAS	35	6	3	3	2	1	1	-	n
Mean %	**37,4**	**12,2**	**7,4**		**58%**	**42%**	**1,7**		

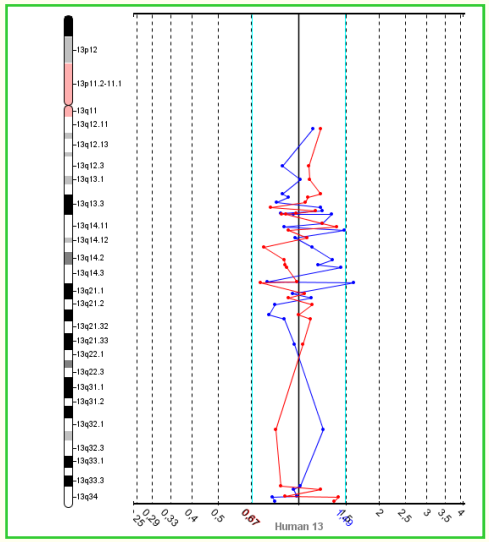
Pilot Study – Array - CGH Results

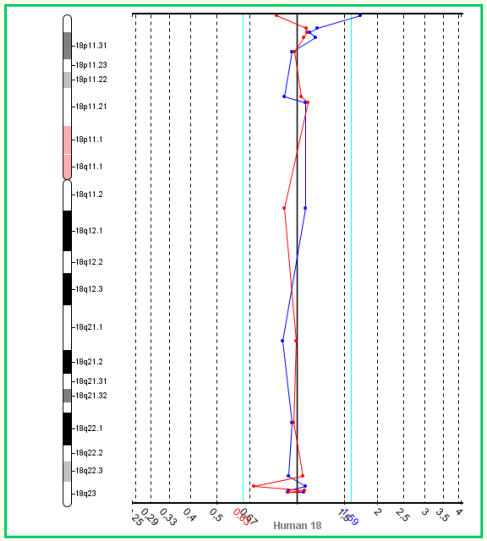
Pat	1	2	3	4	5
FEL	-1,-2	-1, -2	-2, -4, +11, -17	euploid	euploid
SPS	euploid	-1, -2	-1, -2, (+16)	(+5), (+20)	euploid
TEA	+13, -1	+8	euploid	-2	euploid
GAG	-2	euploid	-11	+16	-11
DÜK	-21	euploid	euploid	-16, +19, +22	-2, -9, +16
WER	euploid	+16, -21			
REM	euploid	euploid	+17, -20	-1, +7, +17	euploid
YAY	euploid	-15, +17	euploid	-11, -15, +16	euploid
STU	-1, -2	euploid	-2	euploid	-1, -2, -20
MAS	-2	-4, -11, +17	euploid		

Array Results 1

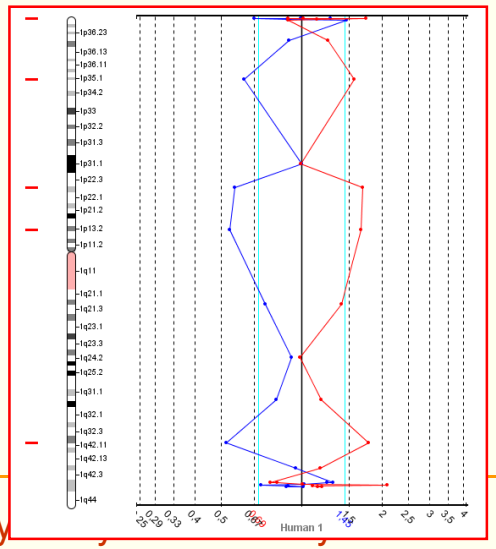
normal

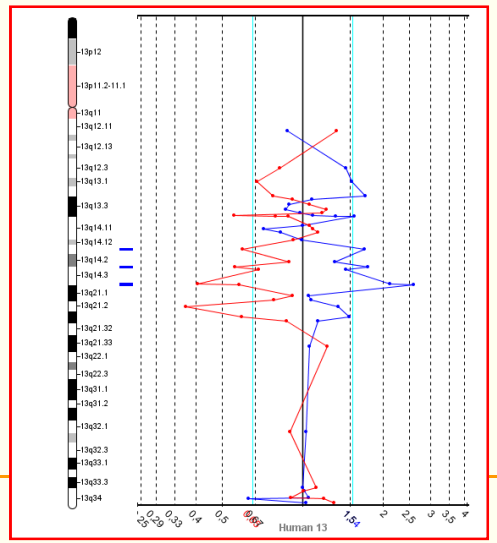


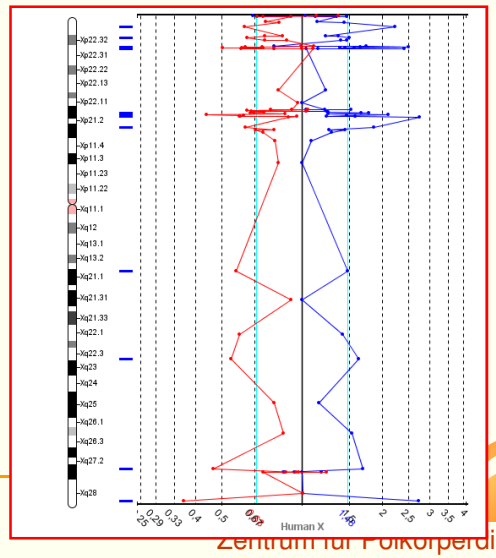




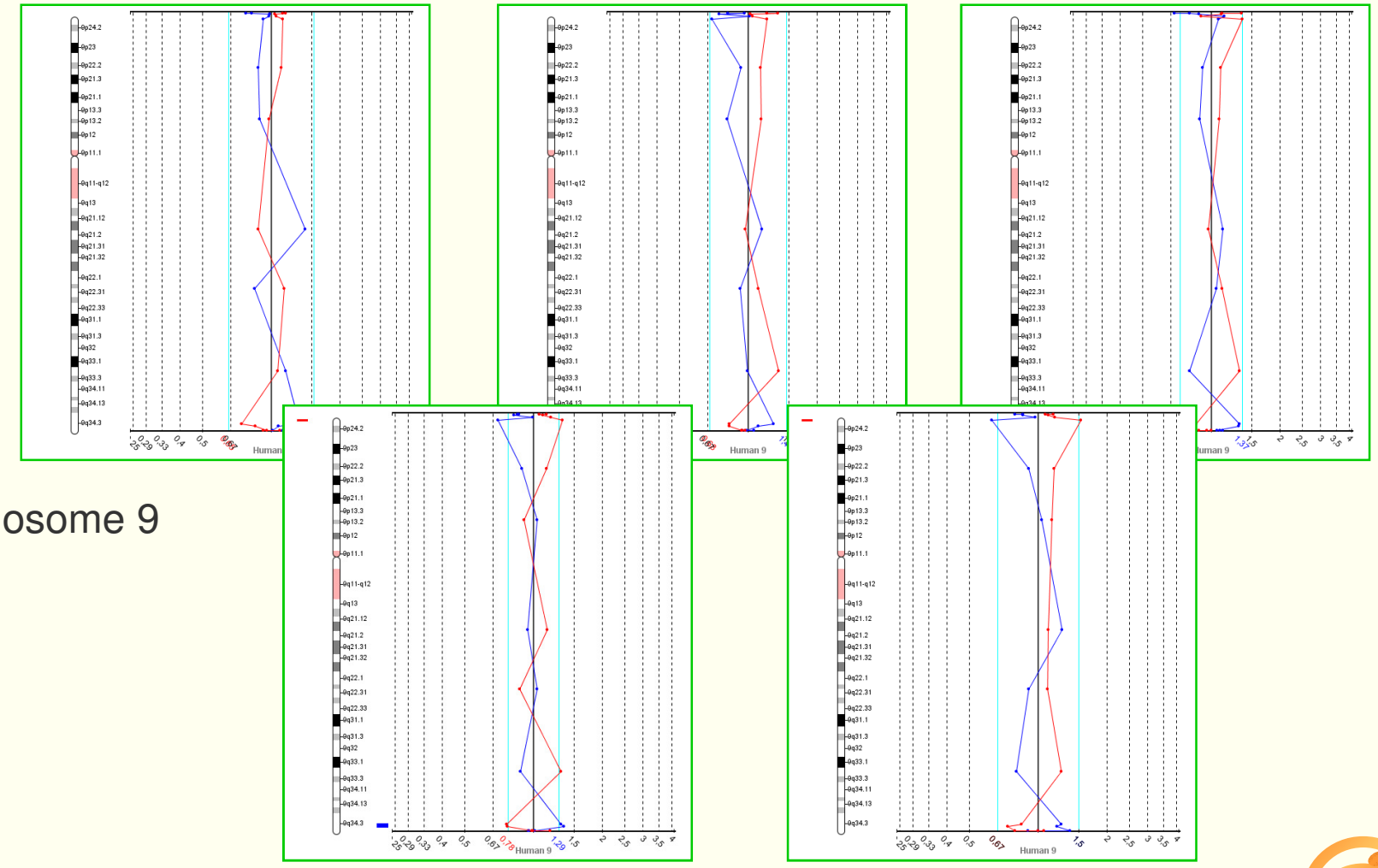
abnormal





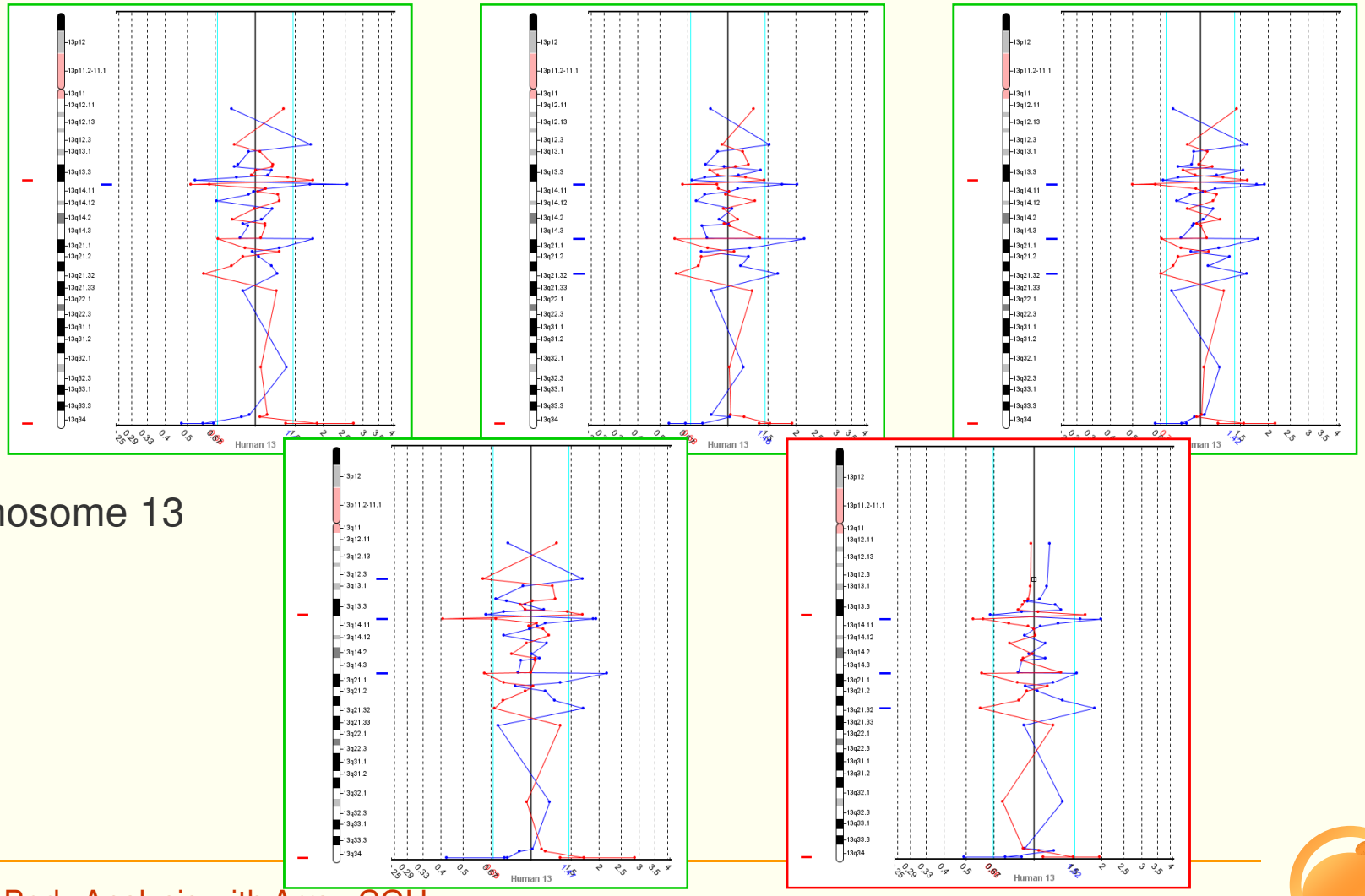


Array Results 2



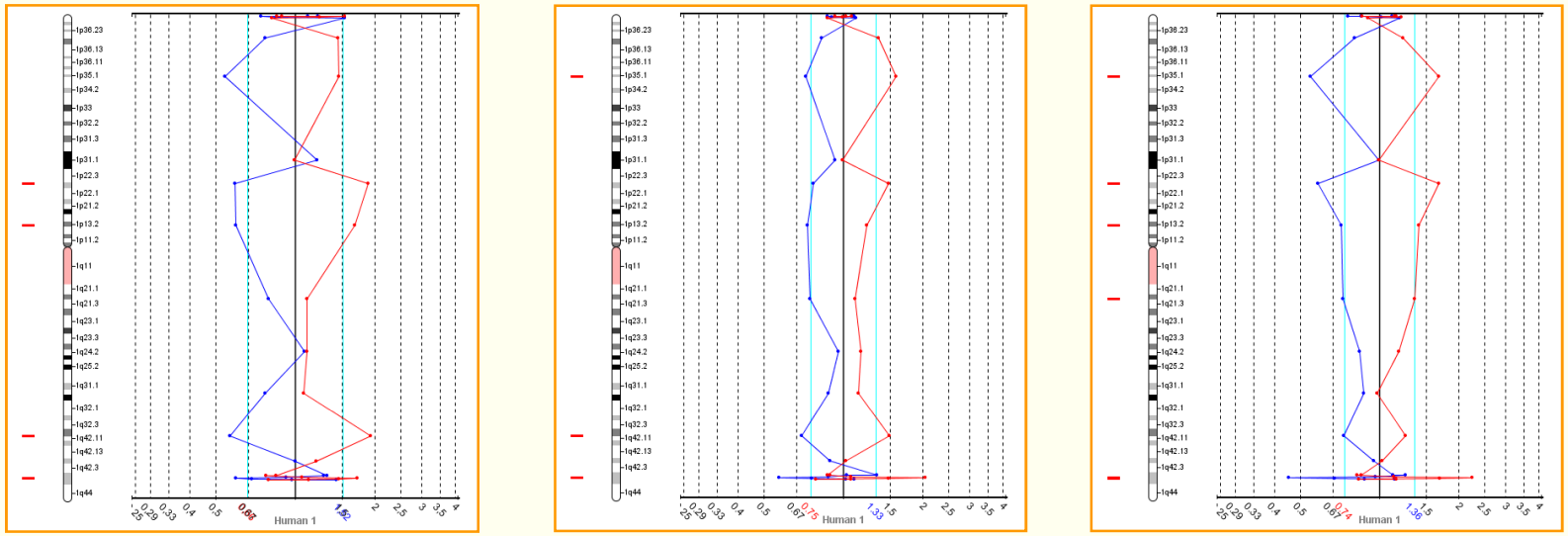
Chromosome 9

Array Results 3

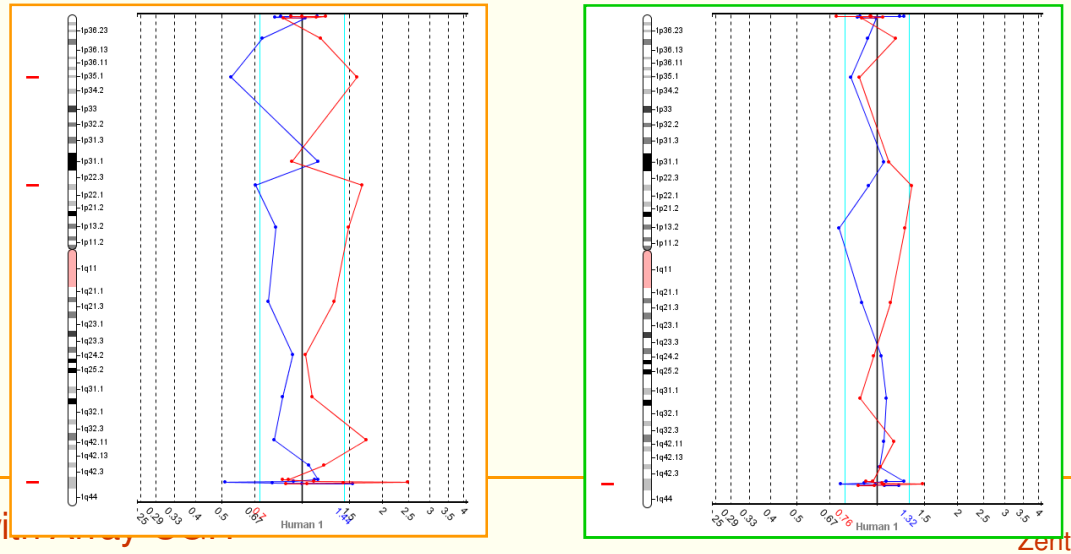


Chromosome 13

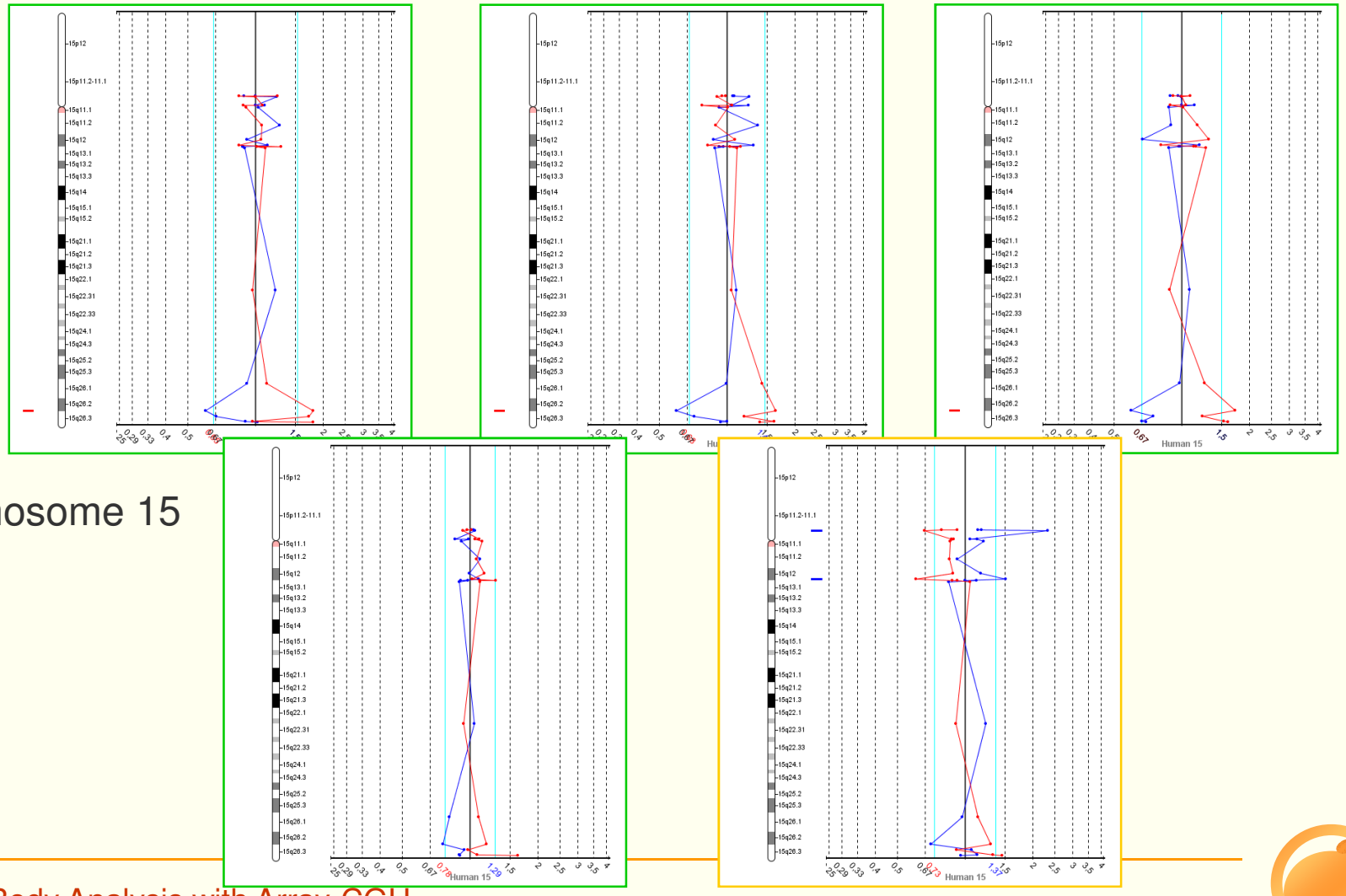
Array Results 4



Chromosome 1

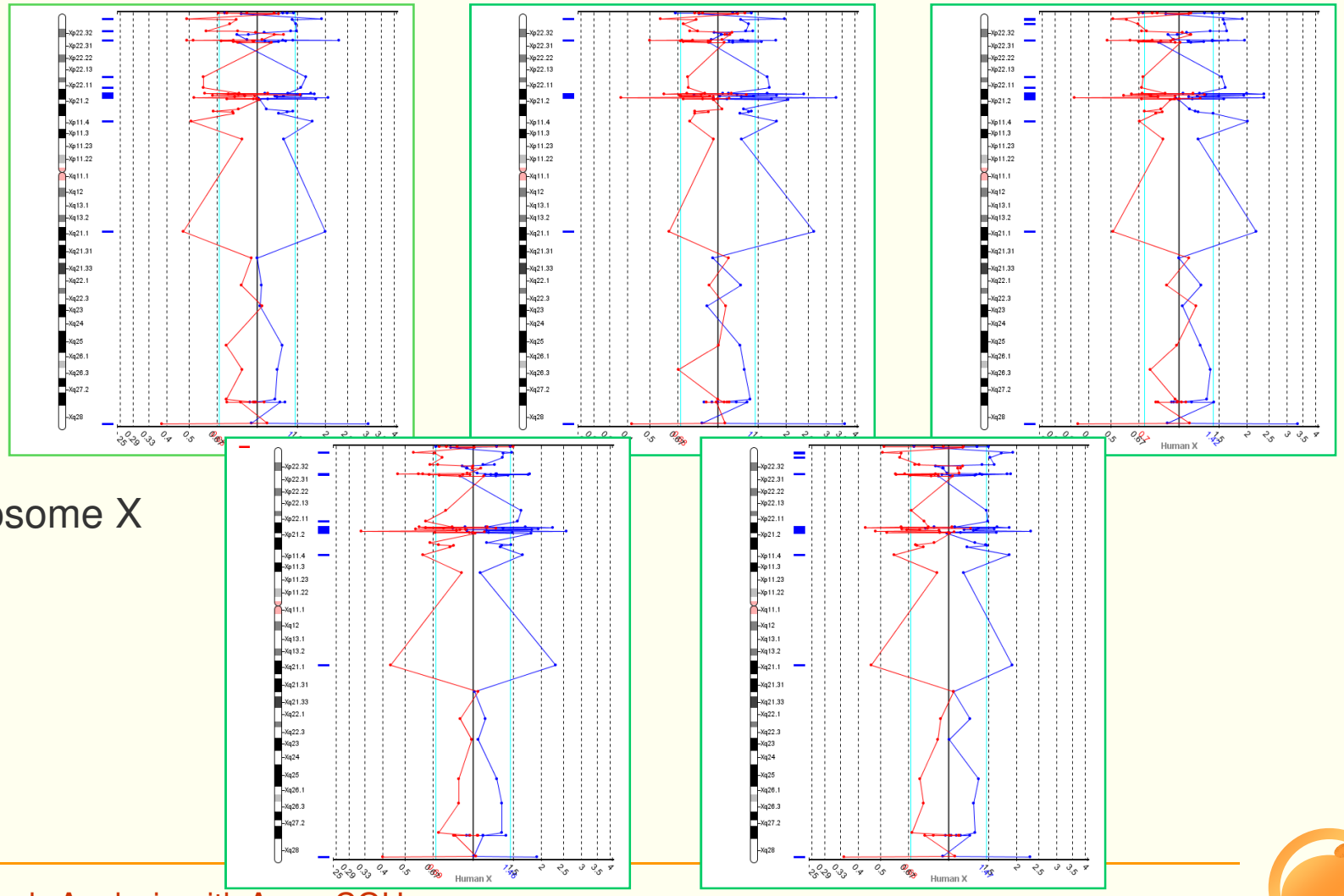


Array Results 5



Chromosome 15

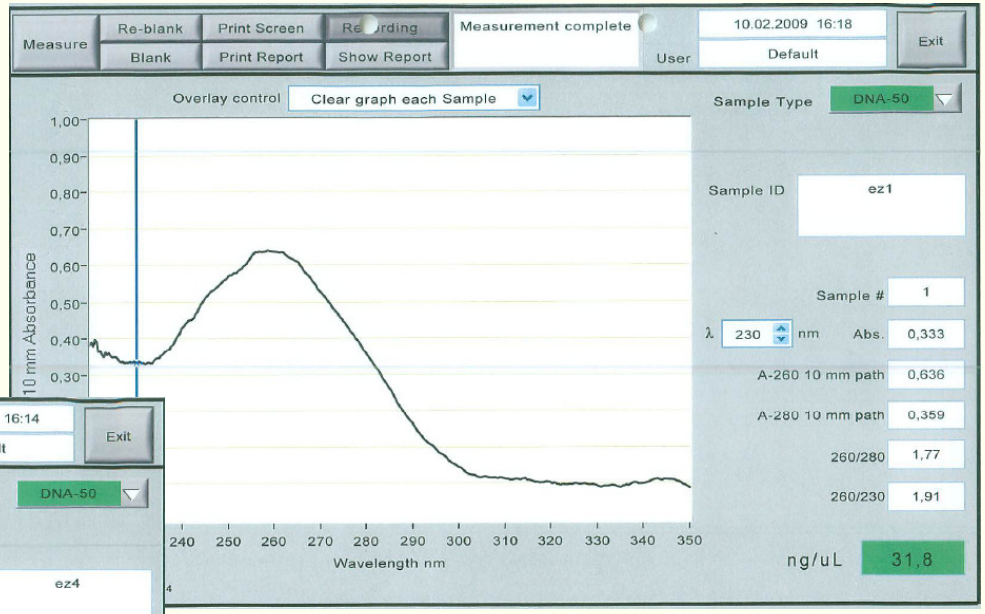
Array Results 6

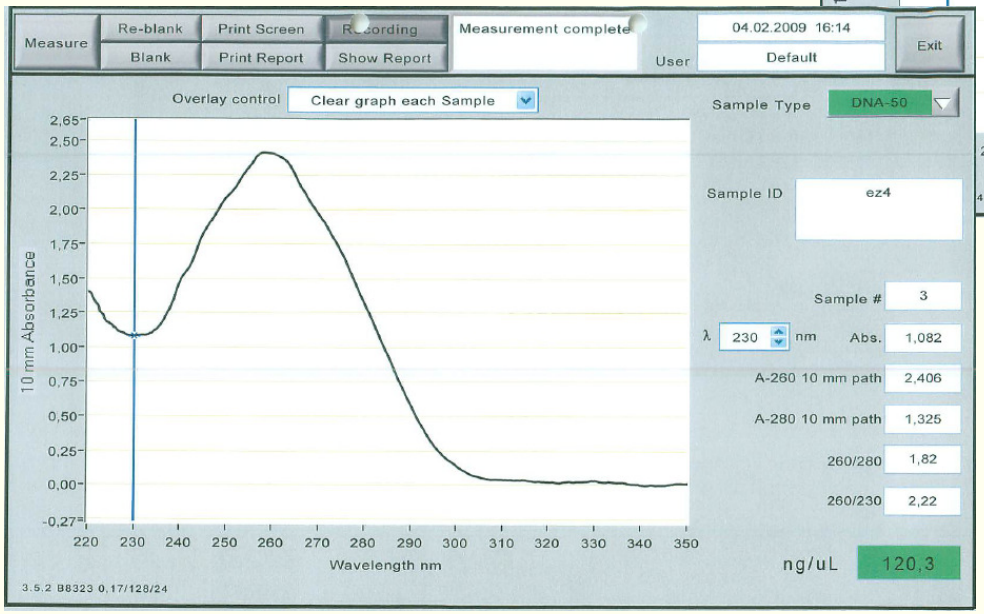


Chromosome X

Amplification

low amount of DNA

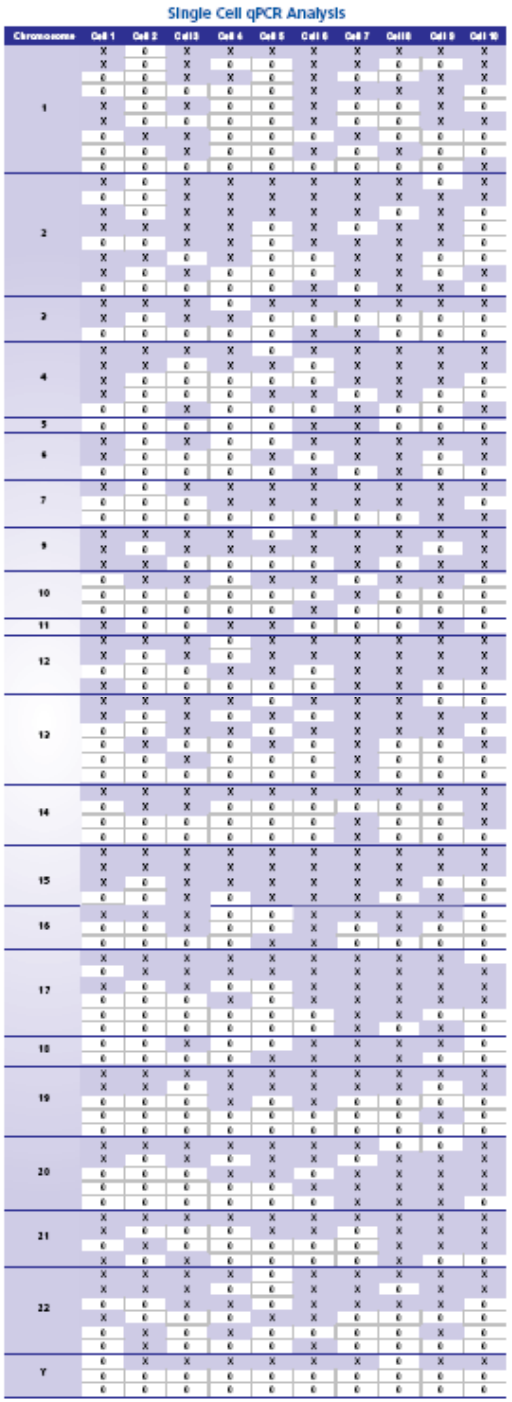




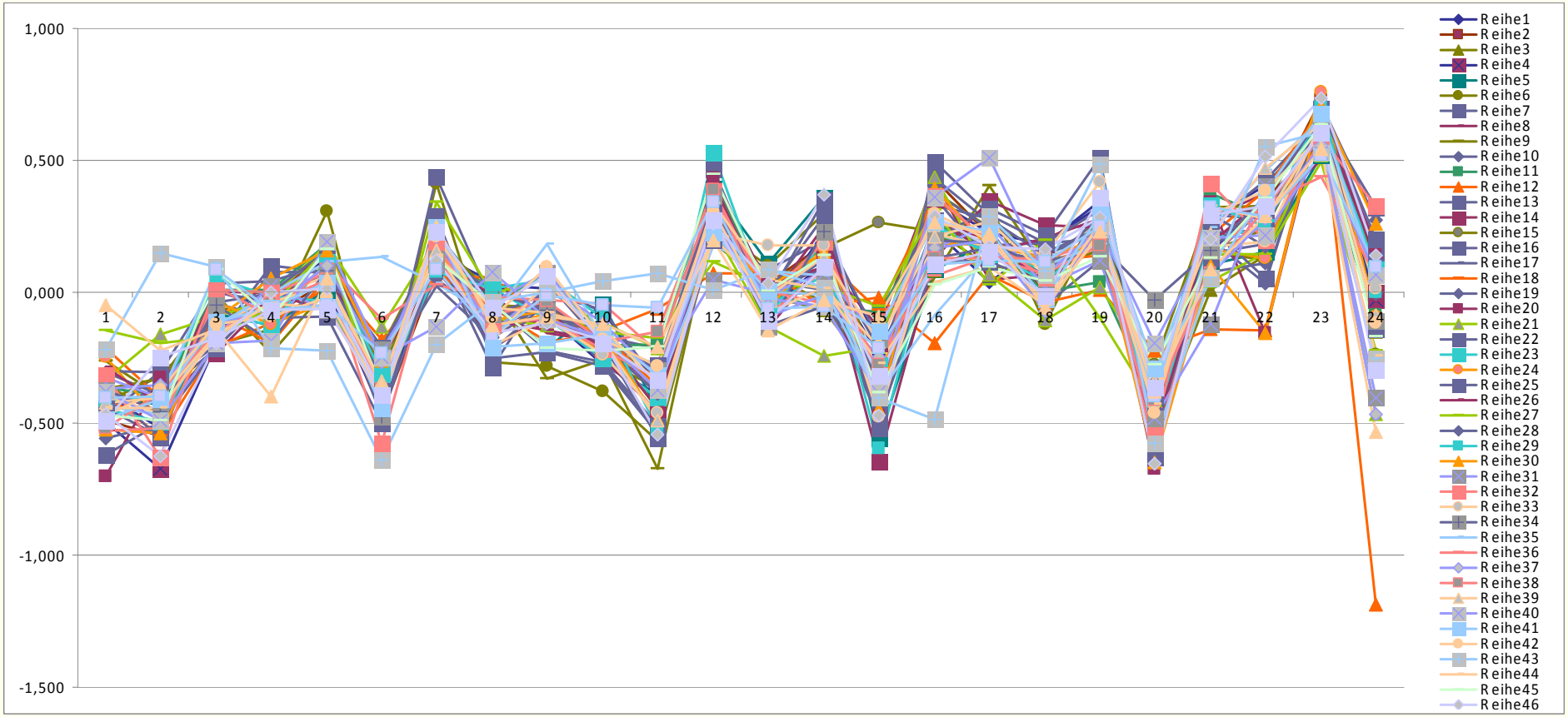
yield of DNA
in the majority of cases sufficient –
no information about quality

WGA

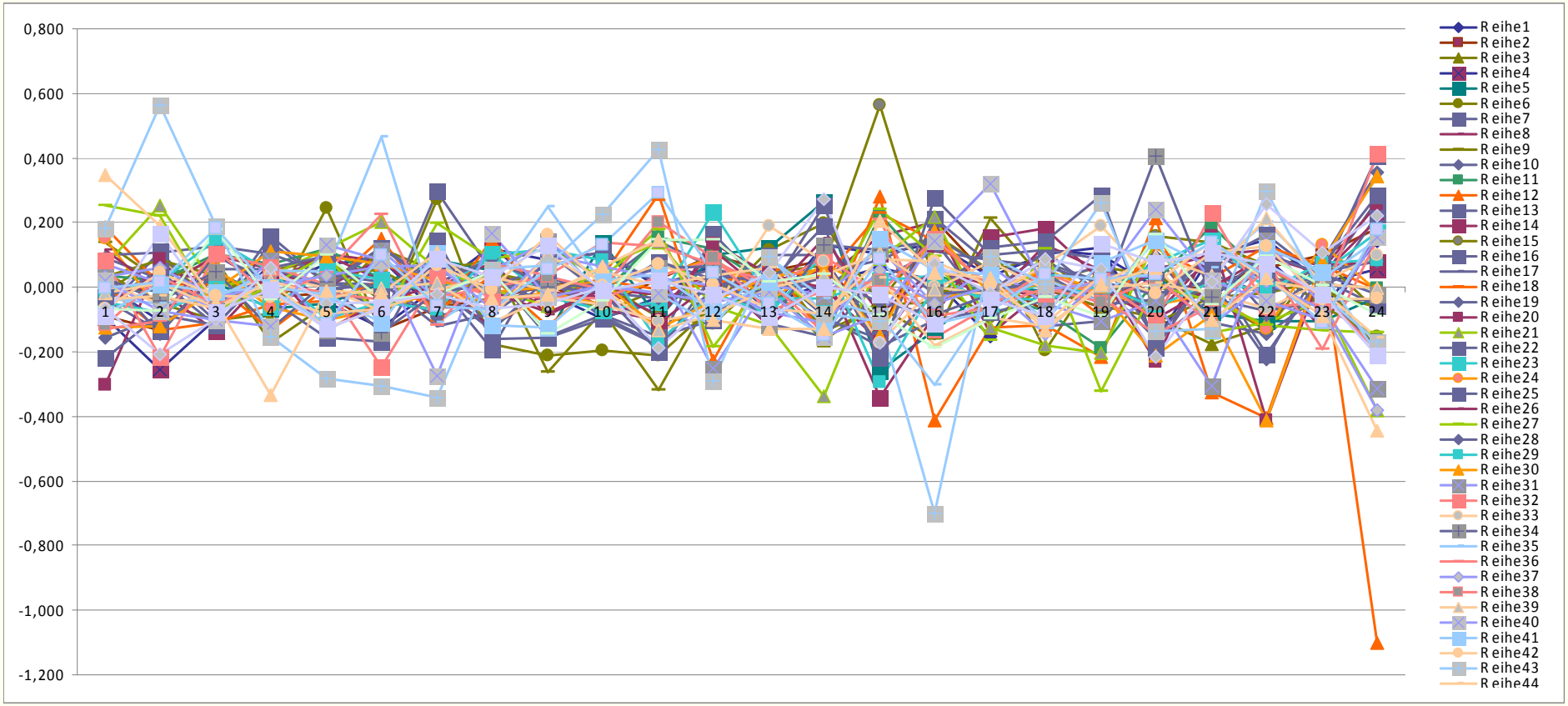
Frequency and distribution of amplified clones
out of one single cell with whole genome amplification



Cumulative Array Data 1



Cumulative Array Data 2



Pilot Study – Array CGH Results

pat	1	2	3	4	5
FEL	-1,-2	-1, -2	-2, -4, +11, -17, +17	euploid	euploid
SPS	Euploid, +20	-1, -2	-1, -2, (+16), euploid	(+5), (+20), euploid	euploid
TEA	+13, -1	+8, -16, -21, -22	euploid	-2, euploid	euploid
GAG	-2, euploid	euploid	-11, +15	+16, -14	-11, euploid
DÜK	-21	Euploid, +6, -16	euploid	-16, +19, +22, -6, -7, +11	-2, -9, +16
WER	Euploid,	+16, -21			
REM	euploid	euploid	+17, -20, euploid	-1, +7, +17	Euploid, -19
YAY	euploid	-15, +17	Euploid, -22	-11, -15, +16	Euploid, -22
STU	-1, -2	euploid	-2	euploid	-1, -2, -20, euploid
MAS	-2, euploid	-4, -11, +17	euploid		

Conclusions

- Polar body analysis for all 23 chromosomes with an Array-CGH approach under “optimal condition” seems to be feasible
- Restrictions in time frame can to be met
- Amount and quality of DNA following WGA still play the crucial role
  - freeze immediately after pb retrieval
- Adaptation of the Array
  - clones match to the amplicons
- The applicability and validation is now to be confirmed in a larger clinical study

in Cooperation with:

- **Gemeinschaftspraxis Prof. Seifert, PD Dr. Bals-Pratsch – Regensburg**
- **Ferring Arzneimittel**
- **Perkin Elmer**

Zentrum für Polkörperdiagnostik

www.zentrum-polkoerper.de