

## Management of FISH probe testing

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## Aneuploidy screening

- ◆ at least 5 probes
- ◆ standard 8 probes (13, 15, 16, 18, 21, 22, X, Y)

**Efficiency of hybridization decreases with increasing number of probes in a given hybridization cocktail (despite overall good performance of individual probes).**

Efficiency of individual probes	Overall efficiency of 5 probes	Overall efficiency of 8 probes
0.99	0.96	0.93
0.95	0.81	0.68

## Aneuploidy screening

- at least 5 probes
- standard 8 probes (13, 15, 16, 18, 21, 22, X, Y)

### MultiVysion PGT (Abbott)

- ◆ 13 Spectrum Red
- ◆ 18 Spectrum Aqua
- ◆ 21 Spectrum Green
- ◆ X Spectrum Blue
- ◆ Y Spectrum Gold

### MultiVysion PB (Abbott)

- ◆ 13 Spectrum Red
- ◆ 16 Spectrum Aqua
- ◆ 18 Spectrum Blue
- ◆ 21 Spectrum Green
- ◆ 22 Spectrum Gold

- ◆ CEPX/Y
- ◆ CEP15

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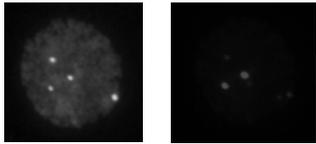
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Evaluation of signals (MultiVysion PB)

- ◆ **chr.16, 18 (aqua, blue)**
  - both probes visible in both filters
  - chr.18 gives brighter signal in blue filter
  - overlapping signals – source of errors



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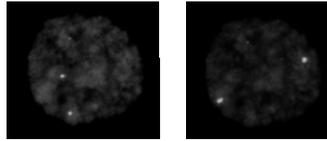
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Evaluation of signals (MultiVysion PB)

- ◆ **differentiation between specific signals and background**
  - specific signals are washed out during denaturation in the 2<sup>nd</sup> FISH



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Validation of ASR probe

- ◆ **Probe sensitivity**
  - Percentage of cells with expected signal pattern
- ◆ **Probe specificity**
  - Percentage of signals that hybridize to the correct locus
    - ❖ At least 200 distinct genomic targets from at least 5 control male individuals
    - ❖ Before introducing the probe to the laboratory

**New lots of reagents and probes must be tested and compared to the previous lot equivalency**

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## PGS Probe testing

### ◆ Pre-cycle work-up on individual couples

■ Probes with very low polymorphism rate are acceptable for use without testing

■ Probes with common occurrence of polymorphism

❖ chromosome 15 satellite III probe (D15Z1)

– testing on uncultured lymphocytes from both reproductive partners

❖ chromosome Y satellite III probe (DYZ1, Yq12)

– testing on male partner's uncultured lymphocytes

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6J5415  
32-181015

### Certificate of Analysis CEP 15 (satellite III) SpectrumAqua (32-181015)

#### Section 1: Lot Information and Component Traceability

Part	Description	Lot	Expiration Date
32-181015	CEP 15 (satellite III) SpectrumAqua	401941	12-15-2008

QP / Revision	Test Date	Probe Lot	CEP Hybridization Lot
32-111090-300B	1-17-2007	401333	400734

#### Section 2: Lot Testing Information

	# cells, 0 dots	# cells, 1 dot	# cells, 2 dots	# cells, 3 dots	# cells, >3 dots
Counting Data for 200 nuclei	0.00	0.67	199.33	0.00	0.00

#### Product Description – Quality Declaration

SA CEP 15 (sat III) DNA probe hybridizes to the satellite III region (band region 15p11.2, locus D15Z1) of human chromosome 15. The hybridized probe fluoresces with bright intensity both in interphase nuclei and on metaphase chromosomes. Due to a polymorphism present in 10-15% of the general population, CEP 15 may also fluoresce with moderate to bright intensity at the centromere region of one chromosome 14 homologue (bands 14p11.1-14p11.1). Depending upon the stage of the cell cycle, DNA condensation, and relative distances between chromatids, probe signals may occasionally appear diffuse or split.

Note: It is recommended that normal control specimens be incorporated into each CEP 15 assay.

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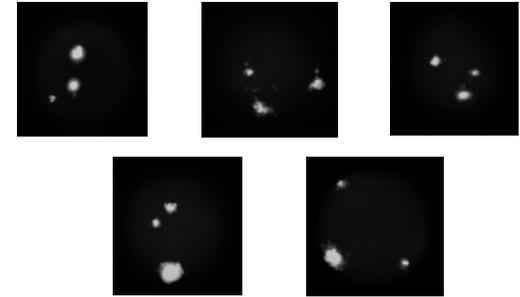
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## 15 satellite III, D15Z1

12.9% (54/417) – three signals




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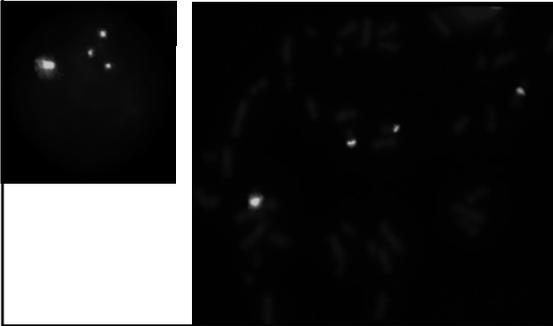
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### 15 satellite III, D15Z1




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### Probes with very low polymorphism rate

16 satellite II, D16Z3  
18 α-satellite, D18Z1  
X α-satellite, DXZ1  
Y α-satellite, DYZ3

- ◆ Variability in signal intensity
- ◆ Weak or even missing signal due to reduction in the copy number of alpha-satellite sequences
  - for all probes mentioned above
- ◆ Hybridization to the third nonspecific chromosome
  - X α-satellite to chromosome 19
  - 18 α-satellite to chromosome 22 and 9

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### MultiVysion PB 18 α-satellite, D18Z1

blastomere	chromosomes							
	13	22	21	16	18	15	X	Y
1a	2	2	2	2	2	2	1	1
1b	2	2	2	2	2	2	1	1
2a	2	2	2	3	2	2	1	1
2b	2	2	2	3	2	2	1	1
3a	2	2	2	2	3	2	2	0
3b	2	2	2	2	3	2	2	0
4a	2	2	2	2	3	2	2	0
4b	2	2	2	2	3	2	2	0

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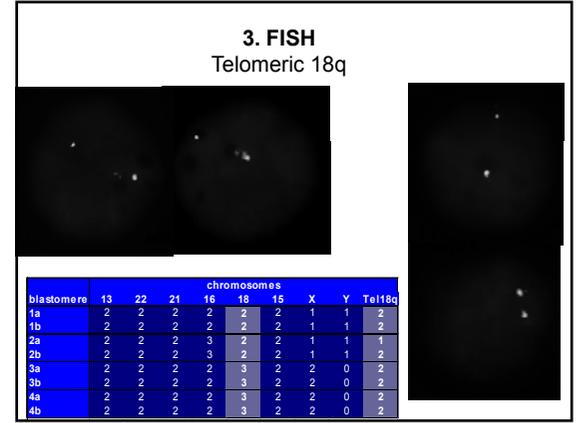
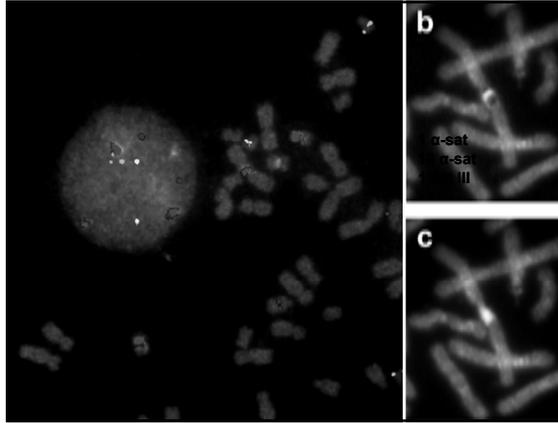
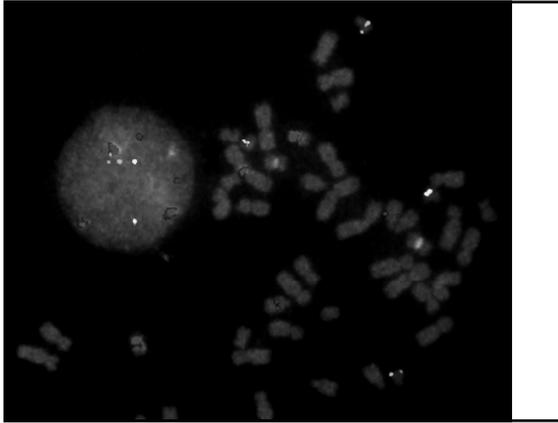
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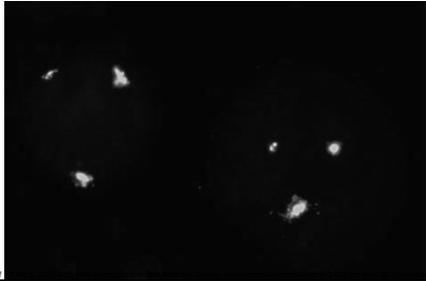
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In cases of suspected rare polymorphisms in PGD, besides reanalysis of blastomeres using telomeric probes, we recommend the contemporaneous testing of polymorphism type on parents' lymphocytes if the slides from the pre-cycle work-up are available.



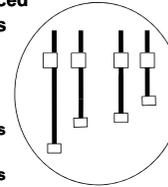
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### PGD for reciprocal translocations Probe selection

- ◆ **Proximal (centromeric, satellite)**
- ◆ **Distal (subtelomeric)**  
differentiate between unbalanced and balanced/normal embryos

- **3 probes**
  - ❖ detect all unbalanced embryos
- **4 probes**
  - ❖ detect all unbalanced embryos
  - ❖ more robust design

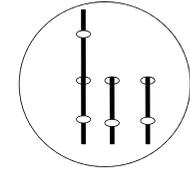


### Probe selection Robertsonian translocations

- ◆ **Chromosome enumeration probes**  
telomeric, locus specific, ...
  - 2 probes detect all aneuploid embryos
  - differentiate between unbalanced and balanced/normal embryos

- ◆ **Combining with aneuploidy screening**
  - aneuploidy screening panel:  
chr. 13, 21, 22 locus specific  
chr. 15 satellite

- telomeric probe: chr. 14




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Probe testing  
PGD for translocations

- ◆ **It is necessary to assess each probe combination prior to clinical treatment (probe validation)**
  - **on metaphase chromosomes**
    - ❖ informativeness (ability to detect unbalanced rearrangements)
  - **on interphase cells**
    - ❖ quantitative assessment of the assay and qualitative assessment of FISH signal intensity and discreteness
    - ❖ efficiency of 95% for individual probe
    - ❖ FISH efficiency is higher in blastomeres than in lymphocytes

Testing probes on metaphase chromosomes

t(1;2)(q32;q11)

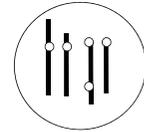
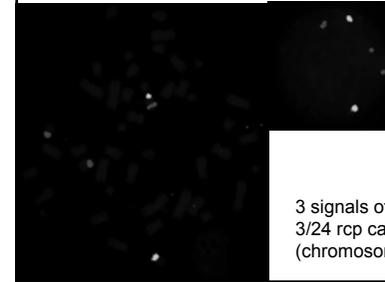
1class.sat 2α-sat

TEL1q TEL14q



t(10;15)(p12;q26)

10 α-sat 15 sat III



3 signals of centromeric probe  
3/24 rcp cases in our lab  
(chromosomes 6, 10, 11)

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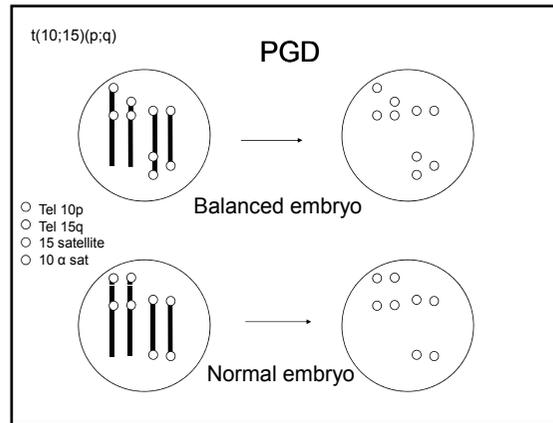
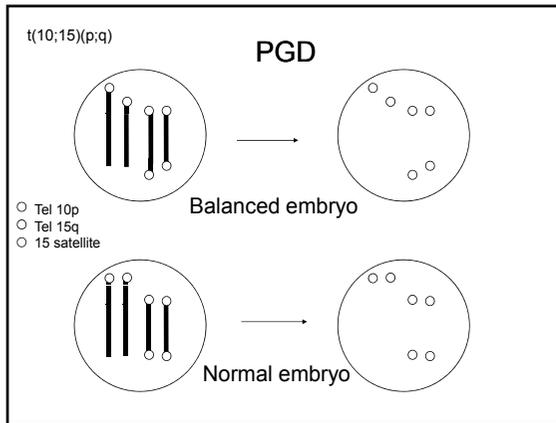
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Repromeda 

Genprogress 

**Thank you for your attention**

  
Veterinary Research Institute

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