

# Aneuploidy screening

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

MultiVysion PGT (Abbott)	MultiVysion PB (Abbott)
13 Spectrum Red	13 Spectrum Red
18 Spectrum Aqua	♦ 16 Spectrum Aqua
♦ 21 Spectrum Green	18 Spectrum Blue
♦ X Spectrum Blue	♦ 21 Spectrum Green
♦ Y Spectrum Gold	♦ 22 Spectrum Gold
	◆ CEPX/Y
	♦ CEP15



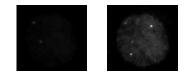
# MultiVysion, CEP probes (Abbott)

#### ♦ Analyte-specific reagents

- Produced under good manufacturing practice guidelines
- Extensive preclinical validation is required
  - \* Specificity
  - Sensitivity
- Probe's parameters (signal size, intensity, pattern, polymorphism)

#### Evaluation of signals (MultiVysion PB)

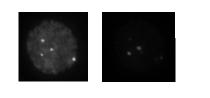
- chr.13 (red fluorochrome)
  - bright specific signals weak in gold filter
  - background signals
    - moderate signals also in gold filter
      suboptimal fixation



#### Evaluation of signals (MultiVysion PB)

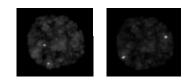
◆ chr.16, 18 (aqua, blue)

- both probes visible in both filters
- chr.18 gives brighter signal in blue filter
- overlapping signals source of errors



### Evaluation of signals (MultiVysion PB)

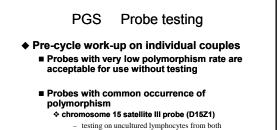
- ♦ differentiation between specific signals and background
  - specific signals are washed out during denaturation in the 2<sup>nd</sup> FISH



# 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

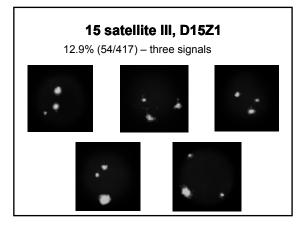
New lots of reagents and probes must be tested and compared to the previous lot equivalency

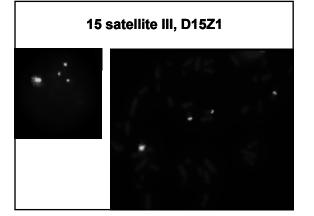




- testing on male partner's uncultured lymphocytes

Part		Desc	ription		- it	ı	ot	Expiration Date		
32-181015	CEP 1	CEP 15 (satellite III) SpectrumAqua					401941		12-15-2008	
QP / Revis	ion	Test Date		Probe Lot		CEP Hybr		idization Lot		
32-111000-3	00B	1-17-200	07	40133	3	400734				
austine Dete fe		ells, 0 dots	# cells,	1 dot	# cells,	2 dots	# cells, 3	dots	≇ cells, ≥3 dol	
Counting Data fo 00 nuclei		ells, 0 dots 0.00	# cells, 0,4		# cells, 199.		# cells, 3 0,00		# cells, >3 dol 0,00	
0 nuclei CEP 15 (sat li 5Z1) of human erphase nuclei e general popul infromere region s stage of the c	Proc ) DNA pr chromosi and on m ation, CEI no fone ci all cycle, (	0.00 duct Des obe hybridi ome 15. Th etaphase c P 15 may a hromosome DNA conde	cription zes to the hromoso iso fluore a 14 hom nsation,	67 n – Qua be satellite ized proto omes. Du esce with nologue ( and relat	ality D e III reg be fluore to a p i moder bands	eclar ion (ba isces v olymo ate to 14p11.	0.00 ation ind region with bright rphism pri bright inte 1-q11.1).	15p1 inten esent nsity Depe	0.00 1.2, locus sity both in in 10-15% of at the nding upon	
	Prod I) DNA pr chromosi and on m ation, CEI n of one c all cycle, f sionally ap	0.00 duct Des obe hybridi ome 15. Th etaphase c P 15 may a hromosome DNA conde ppear diffus	cription zes to the hybridi hromoso iso fluore a 14 hom nsation, ise or spill	n – Qua e sateliti ized protomes. Du esce with nologue ( and relat t.	ality D e III reg be fluore to a p i moder bands tive dist	eclar ion (ba isces v olymo ate to l 4p11. ances	ation and region with bright rphism pro bright inte 1-q11.1). between	15p1 inten esent Depe chrom	0.00 1.2, locus sity both in in 10-15% of at the ading upon atids, probe	

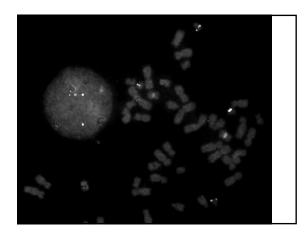


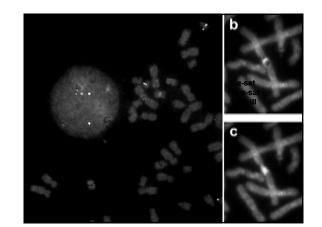


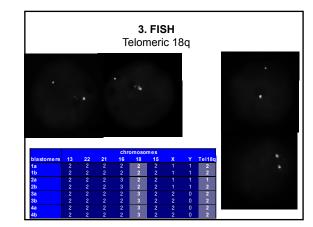
Probes with very low polymorphism rate 16 satellite II, D16Z3 18 α-satellite, D18Z1 X α-satellite, DXZ1 Y α-satellite, DYZ3

- 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

# MultiVysion PB 18 α-satellite, D18Z1 blastomere 13 22 21 16 18 15 X Y 1a 2 2 2 2 2 16 18 15 X Y 1b 2 2 2 2 2 1 1 2b 2 2 2 2 1 1 2b 2 2 2 2 1 1 3b 2 2 2 3 2 2 0 4b 2 2 2 2 3 2 2 0



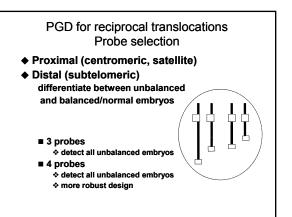


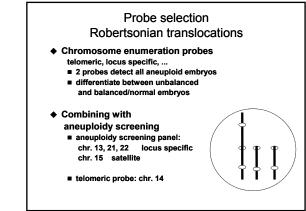


In cases of suspected rare polymorphisms in PGD, besides reanalysis of blastomeres using telomeric probes, we recommend the contemporaneous testing of polymorphism type on parents' lymphocytes if the slides

from the pre-cycle work-up are available.







# Manufacturers

- ♦ Abbott (Vysis) (ASR)
  ♦ CytoCell (IVD)
- ◆ Kreatech Diagnostics (IVD)
- It is possible to combine probes from different manufacturers
- fluorochromes
  - green

  - red (Cytocell, red filter)
     red (Kreatech, Abbott, gold filter)
  - blue

able 1 : Chr	Probe Specificatio Catalogue	Lecas	Chromosome	DNA		rightness	
	Number *	Locus	Region	Clam	Probe b		
					Red/ Green	Blue	
1.	LPEOIGR	DIZI	lq12	satellite III	3		
2	LPE 02G/R	D2Z2	2p11.1-q11.1	a-satellite	3		Manufacturers
3	LPE 03G/R	DJZI	3#11.1-#11.1	o-satellite	3		
4	LPE 04G/R	D4ZI	4011-011	o-satellite	2		Abbott (Vysis) AS
15/19	LPE 05G R	D1Z7 D5Z2	1p11.1-q11 Sp11.1-q11.1	e-satellite	3 2		← CytoCell IVI
6	LPE ING R.BL	D19Z3 D6Z1	19p11-q11 fp11.1-q11.1	a-saultine	3		Kreatech Diagnostics IVI
7	LPE 07G R.BL	D721	7p11.1-q11.1	a-satellite	3	2	
8	LPE ORG R	D872	Rp11.1-q11.1	a-satellite	3		
9	LPEONGR	DNZ3	9612	satellite III	3		•
10	LPE 10G-9, 9L	D1021	Hpll.I-gil.I	o-satellite	3	2	
	LPETIGR	DUZI	TIPILI-911.1	a satellite	-		
12	LPE LIGH						
		D1223	12p11.1-q11.1	o-satellite	3		
13/21	LPE DG R	D13Z1 D21Z1	13p11.1-q11 21p11.1-q11.1	in satellite	2.3		α-sat
14/22	LPE 14G/R	D14Z1 D22Z1	14p11.1-q11.1 22p11.1-q11.1	u-satellite	3		4 54
15	LPE ISG/R/BL	D15Z4	15p11.1-q11.1	m-satellite	)	2	
16	LPE ING R	D1622	Hp11.1-q11.1	a-satellite	1-2		
17	LPE ITG R.BL	D1721	17p11.1-q11.1	a satelline	3	3	
18	LPE ING RIBL	DIKZI	18p11.1-q11.1	a-satelline	3	3	
29	LPE 20G R	D20Z1	20p11.1-q11.1	o-satellite	1-2		
х	LPE ONG WIEL	DXZ1	Xp11.1-q11.1	o-satellite	2	3	
Y	LPEOYoGR	DYZ3	Yp11.1-q11.1	a satellite	2.3		α-sat
Y	LPE 0YqG/R/BL	DVZI	Ye12	satellite III	2.3	2	

2	Catalogue	Chang	Mariker	Accession	Man.	Com.		
Tube	Number	name		Numbers	plicoleal distance from telemete (kb)	hybridiariom & polymorphism		
12	UPTELPR-G	CEBIOR	CEBURNT?		<300		Manufacturers	
14	UPTRIQUG	1406(23	Lpd19	0(\$3794	80		Manufacturers	
14	LPTICPR-G	DAM/SG24	3ptc37	0252463				
24	LPTROB.G	DUHEROUT	2 ppd47	D252988	240	Polymorphism #2 Non-polymorphic#		
26.9%	LPTHOQSP R.G	(12)13	MINTERNOT 12	005447	240	Non-pelymorphic#	Abbott (Vysis)	ASR
19	LPTRIPEG.	Chill Beachille	April 25	0.054539				
34	LP195QR.G	19648	340,006	0151212			-CvtoCell	IVD
49	LPT64P8.G	36823	Apric 104	0453360	275.500			IVD
- 14	LPTHQR.G LPTHPR.G	0,000,000	April 1 Special		Unknown		Kina ata ah Diann aatiaa	11/15
2	LPTRICEG	2406(1)	590.09	0152947	7.01		Kreatech Diagnostics	IVD
14	LPTIMPR G	6(311)				-		
the l	LPTONORIG	579424	6404054	(m62432	290			
19	LPIETPR-G	10%at		4031341	-245			
34	LPT07QR/G	200845		-631349	- 3			
80	LPTIMPR.G	Children, S	Apparter	(ms211)	256	Ap with Ip & 3g		
7	LPT0HQR/G	485014	50011	(385)/425	170			
97 94	LPTINPE.G	40%n	9ps:00 9qs:03	[9952]48	400	74 with 16th &		
						Hp. Hp. NgYa		
194	LPTHPRG UPTHORG	30647	10px;143	D1053488 D1053480	326			
114	UPTHQUG UPTHPRG	1378.24 (annual17)	10pc04 11pc803	0110,7490	339 230 134	Tip with 17p		
114	LPT11QR-G	D(779G7	11que08	DUSIN	43	Tig with 12g (immutital)		
134	UPTISPRG	496A11	12ps/07		Unknown	12p with tip. 26p		
124	LPTING G	2216(18	12peix7	01262143	1.000	and the second second		
1.54	LPT15OR/G	16309	1340006	01751825	176			
144	LPT1#Q8/G	Data Salud 14	14(pc81)	01451429	200			
154	LPTINGEG	154P1		EX 75436	500			
140	DTIMPEG DTIMOLG	12184	14ptg/011	Distant	140			
	UPTINGEG	2406310	15pc048	01752194	44			
14	LEUNING	36/84	(Speil)	111 Tel. 100	-	17q with 1p.4q		
14	LPTINPR G	746218	Vib. KRMChell	D185532	229			
141	LPTINORG	01996-4149	15pcil:1	D1851390	290			
	UPT19PR.G	D1546C11			250.500			
194	LPT/HOR-G	#21243			Unknown			
24	UPT2IPEG	D/UR615.1	2lptlp10	00/6592	110			
Nu	LPTSORG LPT21QRG	\$1812 638C4	Shpel14	02(8)(775	175	Ny with to		
214	LPT21QEG	434034	21.get07	00261375	173	Thursday In		
			254401			23q with 3q computitials		
Xette	UPTXYPR/ G	3396320		DXY5129	148			
Nette	LPTX100	22586		600481553/ 03072751E	182			
	te "Expected Real	(8.21		DOCT/Sel	85			

#### 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
    - Interpretending of efficiency of 95% for individual probe
    - FISH efficiency is higher in blastomeres than in lymphocytes

