

Supporting Information

Table S1 Primers, probes and SD used in SD-Digital-PCR for T21 detection

Oligonucleotide	Sequences (5' to 3')
SD-Digital-PCR-F	GTTGTTCTGCAAAAAACCTTCGA
SD-Digital-PCR-R	CTTGCCAGAAATACTTCATTACCATAT
chr21-probe	FAM-TACCTCCATAATGAGTAAA-MGBNFQ
chr1-probe	VIC-CGTACCTCTGTAATGTGTAA-MGBNFQ
SD-chr21	GTTGTTCTGCAAAAAACCTTCGAGATATTGATGAAGTCTCATCTCTACTTCGTACCTCCATAATGA GTAAACAATATGGTAATGAAGTATTTCTGGCCAAG
SD-chr1	GTTGTTCTGCAAAAAACCTTCGAGATGTTGATGAAGTCTCATCTCTACTTCGTACCTCTGTAATGT GTAAACAATATGGTAATGAAGTATTTCTGGCCAAG

Table S2 The expected and observed ratios of chr21 / (chr1+chr21) in the case of 0%, 3%, 10%, 15%, 20%, 25%, 50%, 100% T21-fetal DNA samples

Sample	Expected ratio ($m = 0.5$)	Observed ratio	mean value \pm SD, n	p-value with 0%T21	95% CI Cut-off value (mean \pm 1.96 \times SD)
100% T21	0.5728	0.5737	0.5654 \pm 0.008, 3	0.0010	[0.5506,0.5801]
		0.5591			
		0.5633			
50% T21	0.5415	0.5404	0.5344 \pm 0.005, 3	0.0007	[0.5243, 0.5446]
		0.5316			
		0.5313			
25% T21	0.5223	0.5205	0.5197 \pm 0.003, 3	0.0004	[0.5134,0.5260]
		0.5162			
		0.5225			
20%T21	0.5181	0.5103	0.5159 \pm 0.005, 3	0.0032	[0.5063,0.5255]
		0.5194			
		0.5180			
15%T21	0.5138	0.5156	0.5112 \pm 0.004, 3	0.0026	[0.5036,0.5188]
		0.5096			
		0.5084			
10%T21	0.5093	0.5097	0.5073 \pm 0.003, 3	0.0029	[0.5020,0.5125]
		0.5077			
		0.5044			
3%T21	0.5029	0.4996	0.4972 \pm 0.002, 3	0.0614	[0.4927,0.5017]
		0.4970			
		0.4950			
0%T21	0.5000	0.4944	0.4911 \pm 0.003, 3	/	[0.4848, 0.4974]
		0.4909			
		0.4880			

Table S3 The ratio of chr21 / (chr1+chr21) from clinical samples

Sample	Sample type	ChrY (copies/ μ L)	Chr1 (copies/ μ L)	Fetal DNA%	Templates copies	m	Expected ratio	Measured ratio
1	Euploid 21	374.3	738.0	50.72%	3690	0.1845	0.5	0.5006
2	Euploid 21	380.0	791.5	48.01%	3957.5	0.1979	0.5	0.4892
3	Euploid 21	367.1	750.0	48.95%	3750	0.1875	0.5	0.4991
4	Euploid 21	325.1	700.2	46.43%	3501	0.1751	0.5	0.4925
5	Euploid 21	241.0	680.0	35.44%	3400	0.1700	0.5	0.4930
6	Euploid 21	329.6	840.0	39.24%	4200	0.2100	0.5	0.4968
7	Euploid 21	269.5	545.0	49.45%	2725	0.1363	0.5	0.4990
8	Euploid 21	238.5	670.0	35.60%	3350	0.1675	0.5	0.4961
9	Euploid 21	385.9	850.0	45.40%	4250	0.2125	0.5	0.4868
10	Euploid 21	/	339.4	/	1697	0.0849	/	0.4948
11	Euploid 21	/	156.6	/	783	0.0392	/	0.4955
12	Euploid 21	/	480.6	/	2403	0.1202	/	0.4973
13	Trisomy 21	237.0	598.5	39.60%	2992.5	0.1496	0.5415	0.5405
14	Trisomy 21	/	375.5	/	1877.5	0.0939	/	0.5667
15	Trisomy 21	/	71.3	/	356.5	0.0178	/	0.5523

The real-time PCR amplification was performed using Rotor-Gene Q Real-Time PCR (QIAGEN) in a total reaction volume of 20 μ L containing 2 \times SYBR[®] *Premix Ex Taq*[™] (TaKaRa), template and 200 μ M of each primers (chr1-F: 5'-TGCCATGCAACTACTGAAGAGGA, chr1-R: 5'-GTGACAAATGATGCAGCGACTA, chrY-F: 5'-CAGGCAGGACAGCTTAAAAG, chrY-F: 5'-ACTGTGGCAAAGTTGCTTTC). The reaction mixture was preheated at 95 °C for 3 mins, followed by 40 cycles of 15 secs at 95 °C, 60 secs at 60 °C, and a final extension step at 72 °C for 7minutes.