TaKaRa

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Novel approach enabling the simultaneous detection of SNPs and CNV for PGT-M and PGT-A using a single-tube assay

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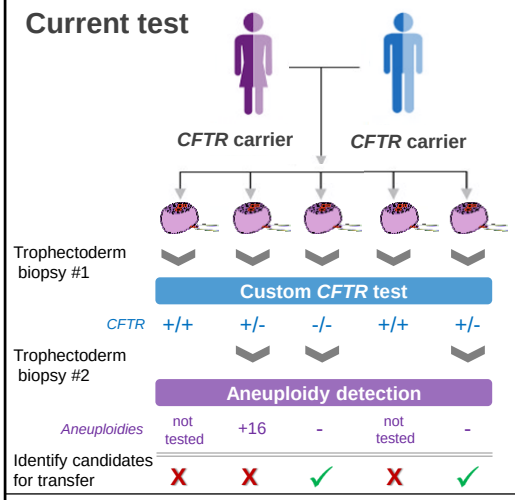
Takara Bio's all-in-one solution for PGT-A / PGT-M



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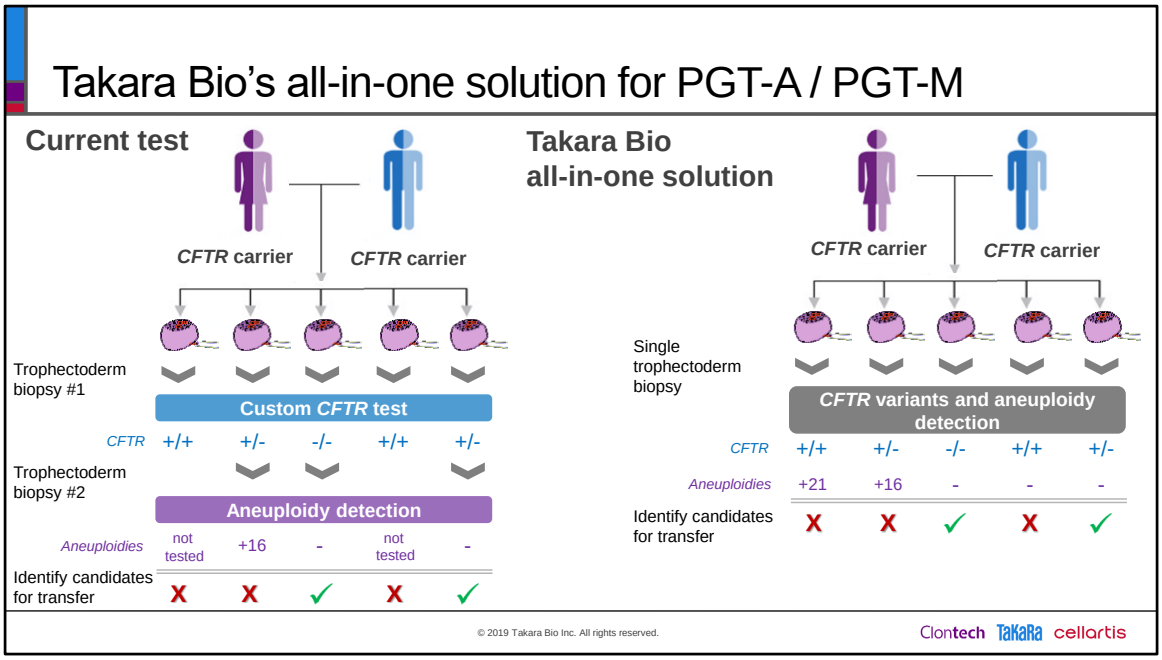
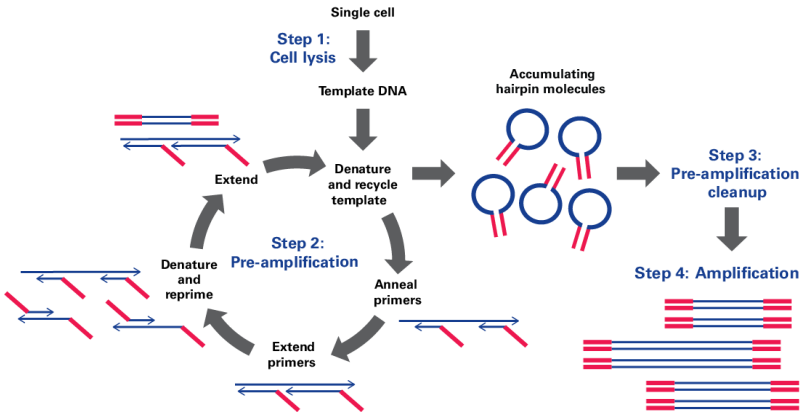
Often, couples wishing to use assisted reproductive technology do so because they are both carriers for a monogenic disease and would like to avoid passing the disease on to their offspring. One of the most common diseases amongst Caucasians is Cystic Fibrosis – *CFTR*. As shown here, the current standard of care is to first screen trophectoderm (TE) biopsies of the couples' embryos for *CFTR* using a mutation-specific panel. Embryos that are not affected with *CFTR* are then carried forward for a second biopsy to test for aneuploidy. Embryos that pass both tests are candidates for transfer. This two-step process is not only time consuming, but adds additional cost and requires subjecting the embryos to multiple manipulations. To address these concerns, Takara Bio has developed an all-in-one test built on our highly regarded PicoPLEX® technology for whole genome amplification.



As shown on the right, this method tests directly for specific mutations – in this case, *CFTR* – and CNV in a single-reaction assay.

PicoPLEX® technology: enabling even coverage of the genome



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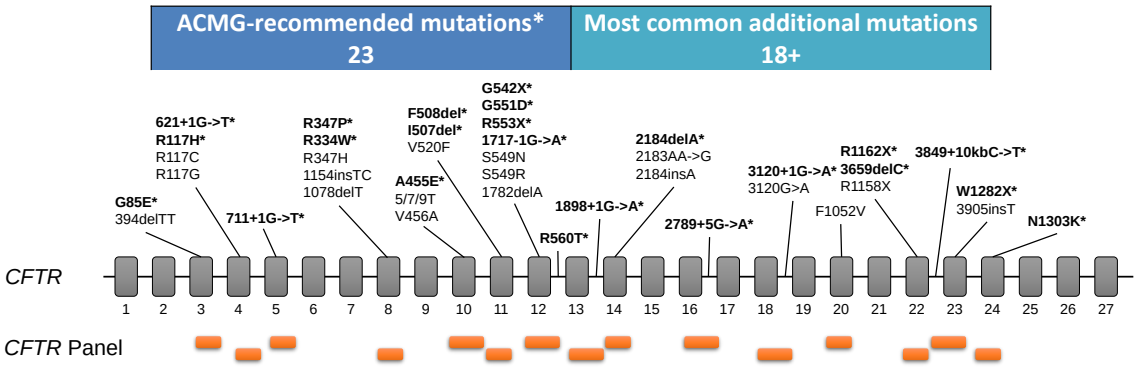
Our test is based on our patented PicoPLEX technology, which uses an initial quasi-linear preamplification step to ensure even coverage of the genome, critical to accurate calling of CNVs. We have now extended this chemistry to also enable targeted amplification at high depth to enable SNP detection.

ACMG-recommended *CFTR* mutations and more



*ACMG recommended mutations. *Genet. Med.* **6,** 387–91 (2004).

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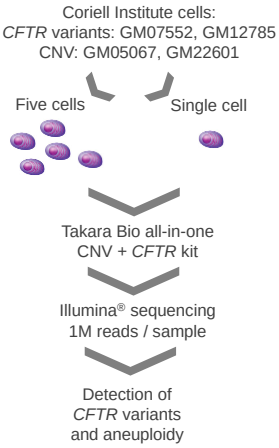
As a proof of principle, we have initially developed this method as a test for *CFTR* using a panel that covers all of the 23 ACMG-recommended *CFTR* mutations, which will detect ~88% of carriers in non-Hispanic Caucasians. 18 additional mutations are also covered. A full list of mutations is provided at the end of this presentation.

*Watson M. S., Cutting G. R., Desnick R. J., *et al.* Cystic fibrosis population carrier screening: 2004 revision of American College of Medical Genetics mutation panel. *Genet. Med.* **6,** 387–391 (2004).

Optimal CNV and SNV detection in a single reaction



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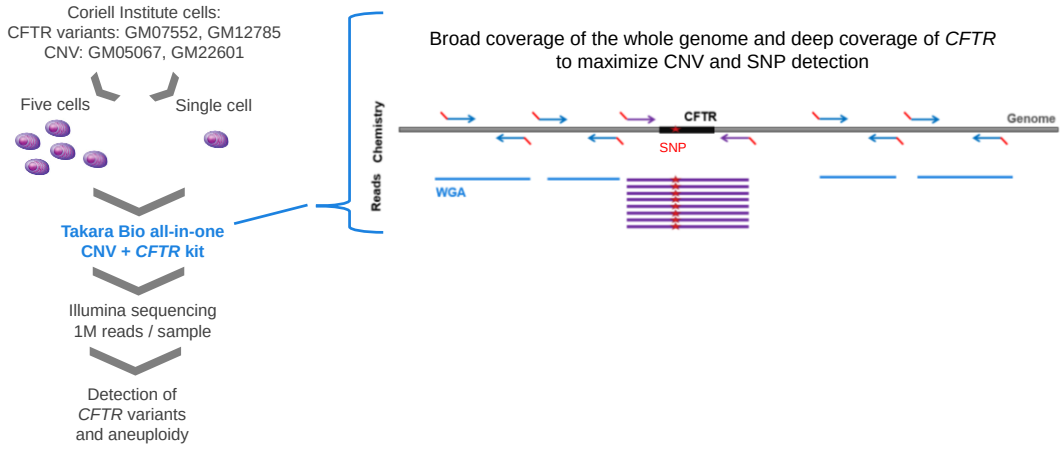
To detect aneuploidies and copy number variations, a shallow and even coverage of the genome is sufficient. However, to detect variants such as SNPs or small indels, a robust and deeper coverage of the region of interest is required. The number of reads allocated to the coverage of the whole genome (WG) and the targeted regions of *CFTR* was optimized to maximize both CNV and SNP detection by overall shallow sequencing.

To initially test the assay for SNP and CNV detection, we took cells from the Coriell Institute bearing either known *CFTR* mutations or known CNVs, sorted them into plates at either 5 cells or 1 cell per well, subjected them to our all-in-one PicoPLEX Gold *CFTR* technology, and sequenced the libraries on Illumina MiSeq® to obtain ~1M reads per library.

Optimal CNV and SNV detection in a single reaction



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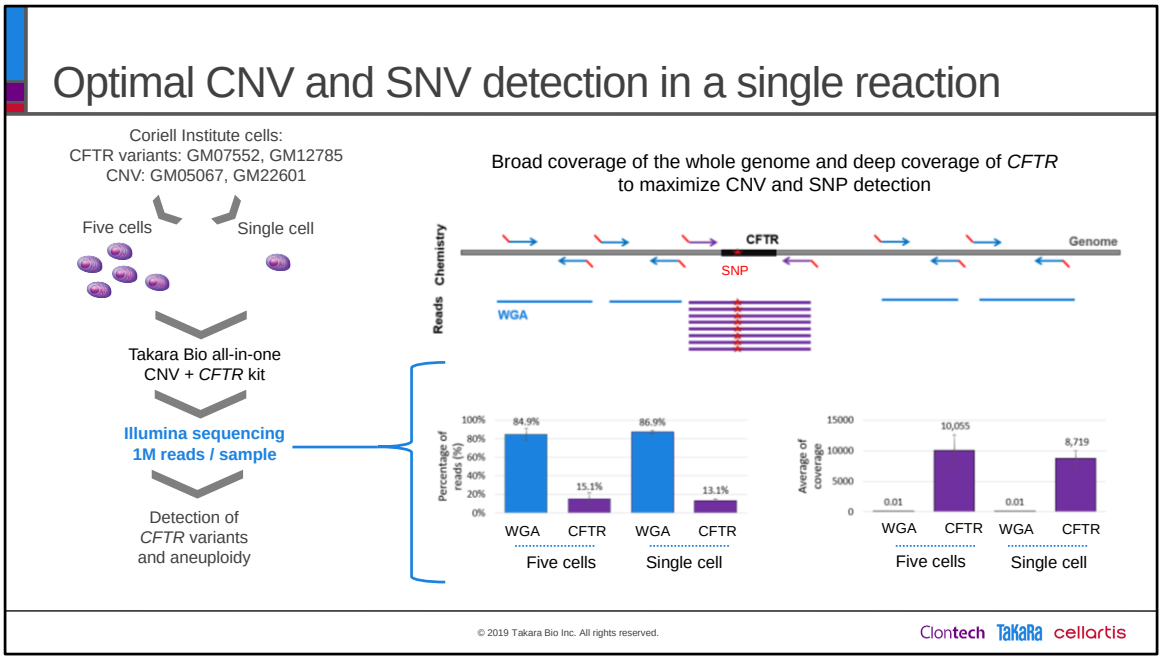
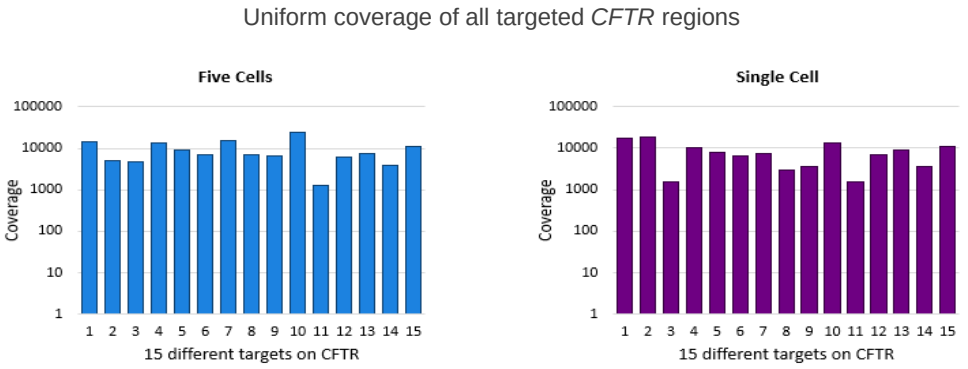
This provides even, low-depth coverage of the genome and high-depth coverage of targeted regions, so that SNPs and CNVs can be called accurately with just 1 million reads per sample.



As shown here, most reads align to the genome outside the targeted region; however, whereas the depth of coverage across the genome is low, the depth at the targeted regions is high. The percentage of reads allocated to whole genome (WG) and *CFTR* regions is shown in the graph on the left. Average coverage of the whole genome and *CFTR* regions is shown in the graph on the right.

Deep and robust coverage of *CFTR* regions for accurate detection of variants



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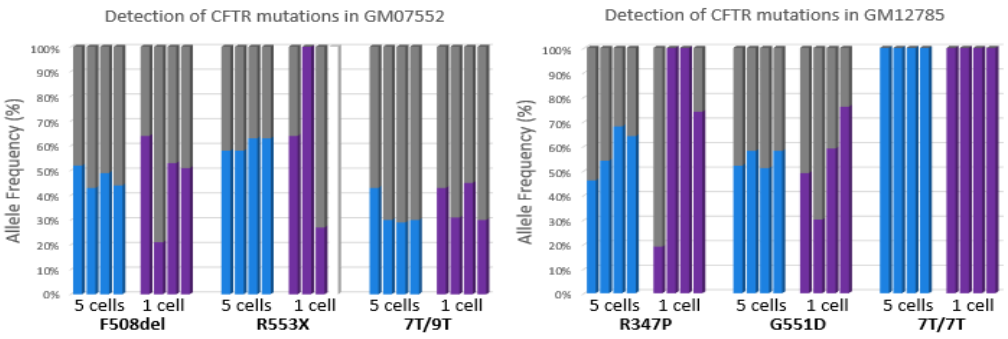
Coriell GM07552 and GM12785 cells were sorted as five- or single-cell samples then processed using PicoPLEX Gold *CFTR* technology. The uniformity of coverage between the 15 targeted regions of *CFTR* was assessed using five (n = 8) and single (n = 8) sorted cells. Here, we can see the coverage for each of the 15 targeted *CFTR* regions in both five-cell and single-cell samples. Coverage is very even across the 15 targeted regions in both cases, and the minimum coverage depth is 1,000 reads.

Deep and robust coverage of *CFTR* regions for accurate detection of variants

Highly reproducible and concordant detection of expected variants



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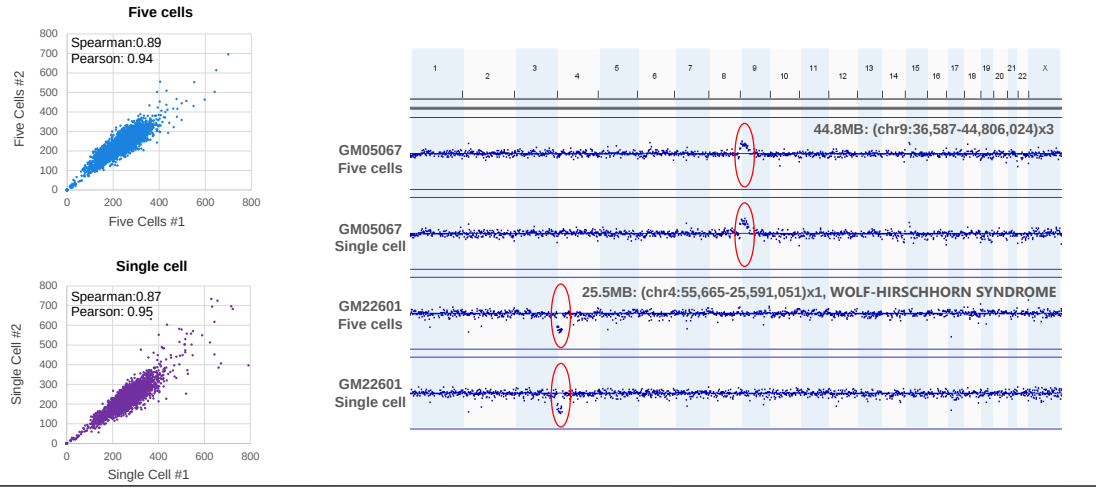
As shown here, this even coverage leads to highly accurate calling of expected variants. For 5-cell samples, the expected heterozygous variants were identified correctly in all cases at an allele frequency between 0.2 and 0.8. When single cells were used, heterozygous variants were called reliably in 16 out of 20 assays. Three allele dropouts (R347P and R553X) and one coverage dropout (white bar, R553X) were observed.

Uniform coverage of the whole genome for precise detection of copy number variations



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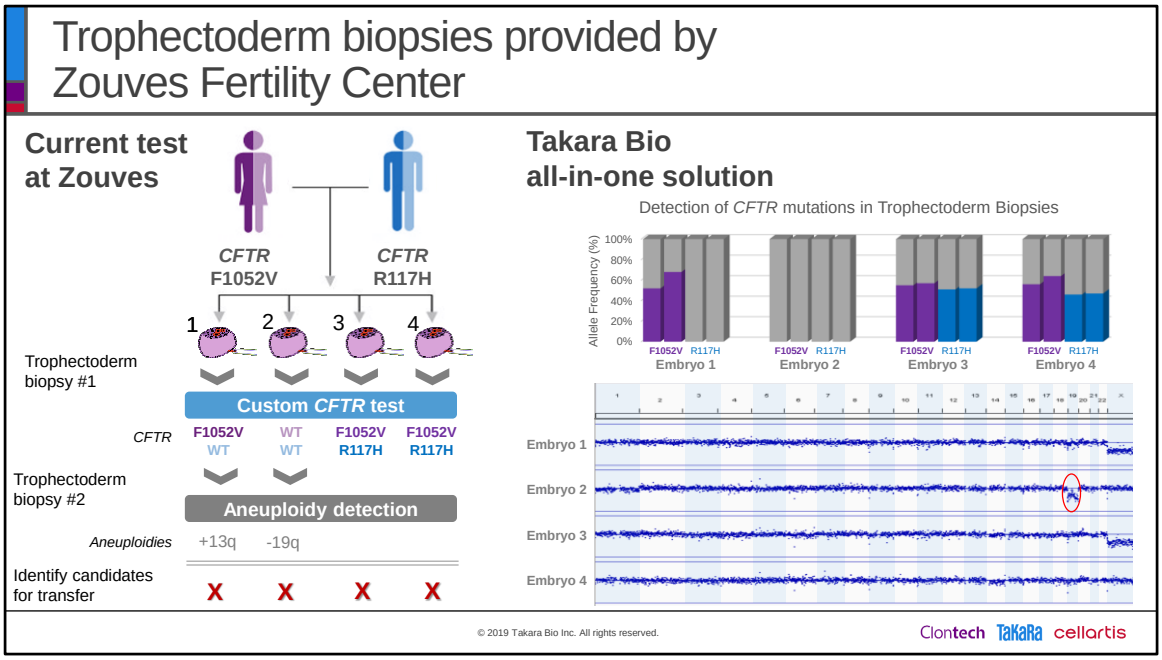
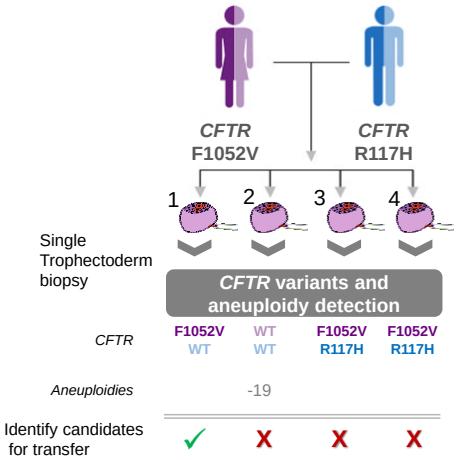
As expected from PicoPLEX technology, coverage of the genome is both even and highly reproducible. As shown on the left, both at 5-cell and single-cell levels, read counts in 1-Mb bins is highly reproducible. This enables highly sensitive detection of CNVs, as shown on the right. The top pair of tracks shows the detection of a partial trisomy on Chromosome 9 of approximately 45 Mb. The bottom panel shows the detection of a 25-Mb deletion on Chromosome 4. In each case, the CNV was detected in both 5-cell and single-cell samples. As mentioned, our method enables both targeted SNP detection and aneuploidy screening in a single-step assay.



To demonstrate the applicability of our assay to TE biopsies, we collaborated with Zouves Fertility center to reassess four embryos that they had previously called by traditional methods. Using our single-reaction assay, we were able to confirm the *CFTR* status of all four embryos, confirming that Embryos 3 and 4 were compound heterozygotes carrying *CFTR* mutations from both parents. Embryo 1 carried the mother's mutation only and Embryo 2 was wild type. The partial loss of Chromosome 19q in Embryo 2 was also confirmed. Embryos 3 and 4 had not previously been assessed for aneuploidy, since they were found to be affected with *CFTR*. Our assay showed that both embryos had a normal karyotype. In the case of Embryo 1, we did not detect the gain on 13q even though we repeated the assay twice. It is probable that this embryo was in fact mosaic and may therefore have been a candidate for transfer.

The Takara Bio advantage



Our assay:

- Reduces the number of trophectoderm biopsies
- Obtains higher accuracy in CNV and SNP detection
- Reduces processing time
- Eliminates need to outsource *CFTR* testing
- Reduces chance of mislabeling or specimen swapping
- Reduces cost

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Conclusions

- Takara Bio's all-in-one CNV + *CFTR* assay enables the detection of SNPs and CNVs in a single reaction.
- The number of sequencing reads allocated to both CNVs and SNPs was optimized to maximize the performance of the assay.
- When using five sorted cells, the detection of *CFTR* variants was 100% concordant. Small copy number variations of 25 Mb and 40 Mb were also identified.
- Both *CFTR* variants and aneuploidies could be detected from trophectoderm biopsies in a single reaction.

Special thanks to:
Manuel Viotti, Frank Barnes, Christo Zouves, Zouves Fertility Center


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ACMG-recommended *CFTR* mutations and more

ACMG-recommended mutations*		Most common additional mutations	
F508del	2789+5G->A	R347H	R1158X
G542X	R347P	5/7/9T	V520F
W1282X	711+1G->T	3120G>A	R117C
G551D	R334W	2184insA	S549R
621+1G->T	R560T	394delTT	F1052V
N1303K	R1162X	2183AA->G	V456A
R553X	3659delC	1154insTC	R117G
I507del	A455E	3905insT	1782delA
3849+10kbC->T	G85E	S549N	
3120G->A	2184delA	1078delT	
R117H	1898+1G->A		
1717-1G->A			

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ACMG recommended *CFTR* mutations and more

ACMG-recommended mutations*			Most common additional mutations		
cDNA nucleotide name	Protein name	Legacy name	cDNA nucleotide name	Protein name	Legacy name
c.1521_1523delCTT	p.Phe508del	F508del	c.1040G>A	p.Arg347His	R347H
c.1624G>T	p.Gly542X	G542X	c.1210−12[5/7/9]		5/7/9T
c.1652G>A	p.Gly551Asp	G551D	c.2988G>A		3120G>A
c.3909C>G	p.Asn1303Lys	N1303K	c.2052_2053insA	p.Gln685Thrfs	2184insA
c.350G>A	p.Arg117His	R117H	c.262_263delTT	p.Leu88Ilefs	394delTT
c.3846G>A	p.Trp1282X	W1282X	c.2051_2052delAAinsG	p.Lys684Serfs	2183AA>G
c.489+1G>T		621+1G>T	c.1021_1022dupTC	p.Phe342Hisfs	1154insTC
c.1657C>T	p.Arg553X	R553X	c.3773_3774insT	p.Leu1258Phefs	3905insT
c.1585-1G>A		1717-1G>A	c.1646G>A	p.Ser549Asn	S549N
c.3717+12191C>T		3849+10kbC>T	c.948delT	p.Phe316Leufs	1078delT
c.2657+5G>A		2789+5G>A	c.3472C>T	p.Arg1158X	R1158X
c.1519_1521delATC	p.Ile507del	I507del	c.1558G>T	p.Val520Phe	V520F
c.3484C>T	p.Arg1162X	R1162X	c.349C>T	p.Arg117Cys	R117C
c.254G>A	p.Gly85Glu	G85E	c.1645A>C	p.Ser549Arg	S549R
c.2052delA	p.Lys684Asnfs	2184delA	c.1647T>G	p.Ser549Arg	S549R
c.3528delC	p.Lys1177Serfs	3659delC	c.3154T>G	p.Phe1052Val	F1052V
c.1364C>A	p.Ala455Glu	A455E	c.1367T>C	p.Val456Ala	V456A
c.1000C>T	p.Arg334Trp	R334W	c.349C>G	p.Arg117Gly	R117G
c.1766+1G>A		1898+1G>A	c.1650delA	p.Gly551Valfs	1782delA
c.1679G>C	p.Arg560Thr	R560T			
c.579+1G>T		711+1G>T			
c.1040G>C	p.Arg347Pro	R347P			
c.2988+1G>A		3120+1G>A			

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