

SUPPLEMENTARY METHODS

In-solution Capture and Multiplexed Resequencing of CFTR

To carry out the DNA capture, we designed through Roche's Customer Services a custom NimbleGen SeqCap EZ Choice Library to target the complete genomic sequence of *CFTR* (chr7:117120017-117308718, hg19). Baits were designed to cover the coding regions, the noncoding intronic sequences, and 10 kb of genomic sequence flanking each end of *CFTR*. Thus, the coordinates of the final target region were chr7:117110017-117318718 (hg19), which account for 208,701 bp. After masking repetitive DNA elements, the total targeted DNA represented 181,539 bp (87% of target bases covered) distributed in 171 individual regions, which were selected using the most stringent settings for probe design (uniqueness tested by Sequence Search and Alignment by Hashing Algorithm [SSAHA]) [1]. No probe redundancy was allowed in the final capture design. The BED file of probe sequences is available on request to the authors.

In this study we took advantage of the capabilities of the NimbleGen SeqCap EZ Choice Library (Roche) to enrich multiple DNAs (pools of 8 to 24 samples) in one single capture reaction. Thus, capture of *CFTR* was carried out following the instructions of the NimbleGen SeqCap EZ Library SR User's Guide v3.0, that is available for free download, to perform sequence capture from pooled libraries prepared with the TruSeq DNA Sample Preparation Kits (Illumina). Briefly, for each sample, one microgram of genomic DNA was sheared by sonication to generate double stranded DNA fragments of 200-300 bp with 3' and 5' overhangs on a Covaris S2 instrument (Covaris) in 52.5 µl of 1X low TE (10 mmol/L Tris/0.1 mmol/L EDTA) for 2 min using frequency sweeping mode with duty cycle, 10%; intensity, 5.0; bursts per second, 200 at

a temperature of 5.5°C to 6°C; and power, 23 W. After sonication, DNAs were subjected to three enzymatic steps: end repair, A-tailing, and ligation to Illumina paired-end indexed adapters. All purification steps between the enzymatic reactions were carried out with AMPure XP beads (Beckman Coulter). The adapter-ligated library was amplified by polymerase chain reaction (PCR) for seven cycles with the TS-PCR oligos 1 and 2. PCR products were cleaned using the QIAquick PCR Purification Kit (QIAGEN) and quantified by a DNA1000 chip on a Bioanalyzer 2100 instrument (Agilent Technologies). Then, the DNA libraries were multiplexed in pools of 8, 12, 16 and 24 samples, for a final combined mass of 1.1 µg, and the resulting library pools were hybridized to the custom design of complementary DNA biotinylated oligonucleotides described above. After 72 hours of hybridization at 47°C, the library-bait hybrids were purified by incubation with streptavidin-bound T1 Dynabeads (LifeTechnologies) and washed with increasing stringency to remove nonspecific binding. After capture, each library pool was amplified by PCR for 17 cycles with the TS-PCR oligos 1 and 2. After PCR amplification, the library pools were cleaned using QIAquick PCR Purification Kit (QIAGEN), and quantified by a high-sensitivity chip on a Bioanalyzer 2100 instrument (Agilent Technologies). Then, different combinations of pools of different sample sizes were arranged to multiplex a total of 24 enriched samples per sequencer lane (four lanes in total). Sequencing was performed with 2×100 bp paired-end reads and a 6-bp index read using SBS v3 chemistry on a HiSeq2000 (Illumina). The resulting fastq files were analyzed with an in-house developed pipeline described below.

Bioinformatics Analysis of DNA Variants

The authors involved in the analysis of the mutations performed the study blindly, i.e. they had no information about the *CFTR* pathogenic variants known to be present in

each sample. Image analyses, base calling and de-multiplexing on each lane of data were performed using Illumina's Sequencing Analysis Pipeline version 1.7.0 (Illumina). Reads were aligned to the human reference genome hg19 using the Burrows-Wheeler Aligner (bwa aln) version 0.5.9 [2] allowing for maximally six mismatches and one gap of up to 20bp. Alignments in SAM format were generated using bwa sampe, sorted by genome position and converted to BAM format using samtools version 0.1.16 [3]. We also performed local re-alignment around potential insertions/deletions and SNP clusters, base-quality recalibration and duplication marking using the GATK pipeline [4] and picard-tools (<http://picard.sourceforge.net>). The resulting alignments were used as input for three different variant prediction tools, namely GATK Unified Genotyper [5], samtools mpileup [3] and SHORE (<http://1001genomes.org>). The three independent SNV and InDel predictions were subsequently quality filtered using GATK VariantFiltration (parameters MQ < 30.0 || QUAL <25.0 || QD <4.0 || DP <5 || DP >2000|| GQ <15) and intersected using GATK CombineVariants. Only SNVs and InDels of up to 30 bp, called by at least two approaches, and found within 150 bp of the ends of the enriched targets, were considered for subsequent analysis. Functional annotation of high quality variants was performed using Annovar [6], providing a comparison of predicted variants to the National Center for Biotechnology Information (NCBI) SNP Database (dbSNP) build 132, the March 2010 pilot release of the 1000 Genomes project (www.1000genomes.org), conservation around variants based on phastCons [7], segmental duplication filter, gene annotation (exon/intron/UTR), amino-acid substitutions and splice variants based on UCSC Genome Browser [8] tracks as well as multiple estimates of the impact of amino acid substitution on the structure and function of proteins (tools: Sift [9], Polyphen2 [10], PhyloP [11] and MutationTaster [12]).

To identify large InDels and SVs we used Pindel [13], Conifer [14], and PeSV-Fisher (<http://gd.crg.eu/tools>), which is a module-based tool for the detection of deletions, gains, intra- and inter-chromosomal translocations, and inversions. This algorithm provides comprehensive information on co-localization of SVs in the genome. The tool uses methods based on paired-end mapping and read-depth analysis corrected for oligonucleotide probe coverage and local guanine and cytosine (GC) content for 100-bp windows. The compendium of modules of PeSV-Fisher toolkit include: definition of anomalous read-pairs, clustering procedure and breakpoint prediction, read depth analysis, definition and interpretation of SVs, and filtering SV calls (FinalCountDown).

To characterize the TG-T haplotypes of the samples we developed an in-house script that scans the raw sequences in the fastq files and counts the number of reads that contain each possible c.1210-34TG(11-13)T(5-9) haplotype.

Characterization of Newly Identified Structural Variants

Different set of primers were designed using Primer3 v0.4.0 (<http://frodo.wi.mit.edu>) to corroborate the breakpoints of two large structural variants characterized for the first time in this study; a novel deletion involving exon 22, and CFTR50kbdel, which is a previously known complex genomic rearrangement [15]. PCR amplifications were performed using 1X Takara *Taq* polymerase (Clontech Laboratories), and PCR products were separated in a 1% low melting agarose gel and were subjected to Sanger sequencing.

Deletion of exon 22. Forward 5'-tgcagatgtatgccaatgact-3' (chr7:117267046-117267066) and Reverse 3'-gcatttttcattttgc-5' (chr7:117269178-117269202) primers were used to amplify and sequence the 1899 bp deleted region in the affected CF sample.

CFTR50kbdel (legacy name). First, primers Fw-A1 5'-cttggcaataccagggttag-3' (chr7:117169863-117169882) and Rv-A1 3'-ctcctcccagaaggctgtta-5' (chr7:117182143-117182162) were used to amplify and sequence the 1600 bp PCR product, which led to the identification of the first deleted region (exons 4-8) comprising 10.601 kb between chr7:117169909-117180509 with a TATAT inserted sequence in the junction. Then, primers Rv-A1 3'-ctcctcccagaaggctgtta-5' (chr7:117182143-117182162) and Rv-B1 3'-aggacaatttggcaccactc-5' (chr7:117255073-117255092) were used to amplify a 1700 bp fragment. Again, by Sanger sequencing we were able to determine the 3' breakpoint of the deletion. In order to also corroborate the *CFTR* 5' region of this complex rearrangement, primers Fw-7.11 5'-cttgcattcagagccttggt-3' (chr7:117169599-117169620) and Fw-B4 5'-tcttgcacgtgaatgatgag-3' (chr7:117215595-117215615) were designed. Sanger sequencing of the resulting 1400 bp PCR product evidenced the complexity of this mutation (Fig. S1). To evaluate the effect of this complex rearrangement at the transcript level, we analyzed the cDNA sample from a CF carrier of this complex rearrangement by PCR using primers Fw-e3: 5'-gctggctcaaagaaaaatcc-3' (chr7:117149103-117149123) and Rv-e22: 5'-tctgttggcatgtcaatgaac-3' (chr7:117267602-117267622).

Shell script

```
my @qsub = ( "#!/bin/bash

#\$ -e $workdir/tmp/$sample
#\$ -o $workdir/tmp/$sample

#\$ -pe smp 4
#\$ -hard
#\$ -l h_vmem=5G

export TMPDIR=$workdir/tmp/$sample
export PATH=/users/GD/tools/annovar/annovar_2011Nov20/:$PATH

NAME=$name
READ1=$read1
READ2=$read2
OUTF=$outfolder/$name
EXOME=/users/xe/dtrujillano/Exomes/custom_capture/qgenomics
KNOWNINDEL=/users/GD/resource/human/hg19/databases/dbSNP/dbIndel132_20101103.vcf
BWA=/users/GD/tools/bwa/bwa-0.5.10/bwa
GATK=/users/GD/tools/GATK/GATK_src_1.4-15-gcd43f01/dist/GenomeAnalysisTK.jar
SAMTOOLS=/soft/bin
ANNOVAR=/users/GD/tools/annovar/annovar_2011Nov20
SHORE=/users/GD/tools/shore/shore
NGSBOX=/users/GD/tools/ngsbox
RSCRIPT=/soft/bin/Rscript

### Align reads with bwa
\$BWA aln -k 2 -i 5 -q -1 -t 2 -R 0 -n 6 -o 1 -e 20 -l 28 -f \$OUTF/\$NAME.r1.sai
\$REF \$READ1
\$BWA aln -k 2 -i 5 -q -1 -t 2 -R 0 -n 6 -o 1 -e 20 -l 28 -f \$OUTF/\$NAME.r2.sai
\$REF \$READ2

### Correct paired end files
\$BWA sampe -r \"@RG\tID:\$NAME\tSM:\$NAME\" -a 500 -o 100000 -n 10 -N 10 -f
\$OUTF/\$NAME.sam \$REF \$OUTF/\$NAME.r1.sai \$OUTF/\$NAME.r2.sai \$READ1 \$READ2

### Convert SAM to BAM
\$SAMTOOLS/samtools view -b -S -o \$OUTF/\$NAME.bam \$OUTF/\$NAME.sam

### Sort BAM file
\$SAMTOOLS/samtools sort \$OUTF/\$NAME.bam \$OUTF/\$NAME.sort

### Index sorted BAM file
\$SAMTOOLS/samtools index \$OUTF/\$NAME.sort.bam

### Local Re-alignment
java -Xmx4g -jar \$GATK -T RealignerTargetCreator -R \$REF -I
\$OUTF/\$NAME.sort.bam -o \$OUTF/\$NAME.intervals -known \$KNOWNINDEL --
minReadsAtLocus 6 --maxIntervalSize 200
java -Xmx4g -jar \$GATK -T IndelRealigner -R \$REF -I \$OUTF/\$NAME.sort.bam -
targetIntervals \$OUTF/\$NAME.intervals -o \$OUTF/\$NAME.realigned.bam -known
\$KNOWNINDEL --maxReadsForRealignment 10000 --consensusDeterminationModel USE_SW
```

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### Duplicate marking

java -Xmx4g -jar /users/GD/tools/picard/picard-tools-1.40/MarkDuplicates.jar
INPUT=\$OUTF/\$NAME.realigned.bam OUTPUT=\$OUTF/\$NAME.realigned.dm.bam
METRICS_FILE=\$OUTF/duplication_metrics.txt ASSUME_SORTED=true
VALIDATION_STRINGENCY=LENIENT
\$SAMTOOLS/samtools index \$OUTF/\$NAME.realigned.dm.bam

### Base quality recalibration

java -Xmx4g -jar \$GATK -T CountCovariates -nt 2 --default_platform illumina -cov
ReadGroupCovariate -cov QualityScoreCovariate -cov CycleCovariate -cov
DinucCovariate -recalFile \$OUTF/recal_data.csv -R \$REF -I
\$OUTF/\$NAME.realigned.dm.bam -knownSites \$KNOWNINDEL
java -Xmx4g -jar \$GATK -T TableRecalibration --default_platform illumina -R
\$REF -I \$OUTF/\$NAME.realigned.dm.bam -recalFile \$OUTF/recal_data.csv --out
\$OUTF/\$NAME.realigned.dm.recalibrated.bam

### Cleanup
rm \$OUTF/\$NAME.sam
rm \$OUTF/\$NAME.bam
rm \$OUTF/\$NAME.realigned.bam
rm \$OUTF/\$NAME.realigned.bai
rm \$OUTF/\$NAME.realigned.dm.bam
rm \$OUTF/\$NAME.realigned.dm.bai
rm \$OUTF/\$NAME.realigned.dm.bam.bai

### GATK: Call SNPs and Indels with the GATK Unified Genotyper

java -Xmx4g -jar \$GATK -T UnifiedGenotyper -nt 2 -R \$REF -I
\$OUTF/\$NAME.realigned.dm.recalibrated.bam -o \$OUTF/GATK.snps.raw.vcf -glm SNP
java -Xmx4g -jar \$GATK -T UnifiedGenotyper -nt 2 -R \$REF -I
\$OUTF/\$NAME.realigned.dm.recalibrated.bam -o \$OUTF/GATK.indel.raw.vcf -glm
INDEL

### MPILEUP: Call SNPs and Indels

\$SAMTOOLS/samtools mpileup -uf \$REF \$OUTF/\$NAME.realigned.dm.recalibrated.bam
| \$SAMTOOLS/bcftools view -bcg - > \$OUTF/MPILEUP.variant.raw.bcf
\$SAMTOOLS/bcftools view \$OUTF/MPILEUP.variant.raw.bcf | \$SAMTOOLS/vcftools.pl
varFilter -d5 -D$max_cov -W 20 > \$OUTF/MPILEUP.variant.raw.vcf
egrep \"INDEL|#\\" \$OUTF/MPILEUP.variant.raw.vcf > \$OUTF/MPILEUP.indel.raw.vcf
grep -v INDEL \$OUTF/MPILEUP.variant.raw.vcf > \$OUTF/MPILEUP.snps.raw.vcf

### SHORE: Prepare format map.list
mkdir \$OUTF/shore

\$SHORE convert --sort -r \$REF -n 6 -g 1 -e 20 -s Alignment2Maplist
\$OUTF/\$NAME.realigned.dm.recalibrated.bam \$OUTF/shore/map.list.gz

### SHORE: compute coverage plot in GFF format for browsers
\$SHORE coverage -m \$OUTF/shore/map.list.gz -o \$OUTF/shore/CoverageAnalysis

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### SHORE: Enrichment plots

grep enriched \$OUTF/shore/Count_SureSelect_plus150/meancov.txt | cut -f5 >
\$OUTF/shore/Count_SureSelect_plus150/exome_enriched.txt
grep depleted \$OUTF/shore/Count_SureSelect_plus150/meancov.txt | cut -f5 >
\$OUTF/shore/Count_SureSelect_plus150/exome_depleted.txt
grep enriched \$OUTF/shore/Count_SureSelect_plus150/readcount.txt | cut -f5 >
\$OUTF/shore/Count_SureSelect_plus150/exome_count_enriched.txt
grep depleted \$OUTF/shore/Count_SureSelect_plus150/readcount.txt | cut -f5 >
\$OUTF/shore/Count_SureSelect_plus150/exome_count_depleted.txt

### plot data

\$RSCRIPT \$NGSBOX/Statistics/R_examples/OCDpools_enrichment_stats.R
\$OUTF/shore/Count_SureSelect_plus150

### SHORE: Call SNPs and Indels
\$SHORE qVar -n \$NAME -f /users/GD/resource/human/hg19/shore/hg19.shore -o
\$OUTF/shore/Variants -i \$OUTF/shore/map.list.gz -s
/users/GD/tools/shore/scoring_matrices/scoring_matrix_het.txt -E
\$OUTF/shore/Count_SureSelect_plus150/meancov.txt -e -c 4 -d 4 -C \$max_cov -r 3 -
q 10 -Q 15 -a 0.25 -b 6 -y -v

### SHORE: Compute enrichment + plot CFTR
\$SHORE count -m \$OUTF/shore/map.list.gz -o \$OUTF/shore/CFTR -f
\$EXOME/CFTR_150.bed -H 1,1 -k

grep enriched \$OUTF/shore/CFTR/meancov.txt | cut -f5 >
\$OUTF/shore/CFTR/exome_enriched.txt
grep depleted \$OUTF/shore/CFTR/meancov.txt | cut -f5 >
\$OUTF/shore/CFTR/exome_depleted.txt
grep enriched \$OUTF/shore/CFTR/readcount.txt | cut -f5 >
\$OUTF/shore/CFTR/exome_count_enriched.txt
grep depleted \$OUTF/shore/CFTR/readcount.txt | cut -f5 >
\$OUTF/shore/CFTR/exome_count_depleted.txt

\$RSCRIPT \$NGSBOX/Statistics/R_examples/OCDpools_enrichment_stats.R
\$OUTF/shore/CFTR

### Clean up
rm -r \$OUTF/shore/Variants/ConsensusAnalysis/supplementary_data
gzip -9 \$OUTF/shore/Variants/ConsensusAnalysis/reference.shore

### Filter and compare SNP calls from 3 different pipelines
# Filtering
mkdir \$OUTF/SNP_Intersection

java -jar -Xmx4g \$GATK -T VariantFiltration -R \$REF -o
\$OUTF/SNP_Intersection/GATK.snps.filtered.vcf --variant \$OUTF/GATK.snps.raw.vcf
--mask \$OUTF/GATK.indel.raw.vcf --clusterWindowSize 10 --filterExpression \"MQ <
30.0 || QUAL < 25.0 || QD < 4.0 || HRun > 9\" --filterName CRG --
genotypeFilterExpression \"DP < 5 || DP > \$max_cov || GQ < 15\" --
genotypeFilterName CRGg

java -jar -Xmx4g \$GATK -T VariantFiltration -R \$REF -o
\$OUTF/SNP_Intersection/MPILEUP.snps.filtered.vcf --variant
\$OUTF/MPILEUP.snps.raw.vcf --mask \$OUTF/GATK.indel.raw.vcf --clusterWindowSize

```

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10 --filterExpression \"MQ < 30.0 || QUAL < 15.0 || DP < 5 || DP > $max_cov\" --
filterName CRG --genotypeFilterExpression \"DP < 5 || DP > $max_cov || GQ < 15\" --
--genotypeFilterName CRGg

perl \$NGSBOX/Parser/VCF/vcf_filter/vcf_filter.pl
\$OUTF/shore/Variants/ConsensusAnalysis/snp.vcf \$max_cov >
\$OUTF/SHORE.snps.raw.vcf

java -jar -Xmx4g \$GATK -T VariantFiltration -R \$REF -o
\$OUTF/SNP_Intersection/SHORE.snps.filtered.vcf --variant
\$OUTF/SHORE.snps.raw.vcf --mask \$OUTF/GATK.indel.raw.vcf --clusterWindowSize 10
--filterExpression \"QUAL < 20.0 || DP < 5 || DP > \$max_cov\" --filterName CRG

# greping
grep -v \"CRG\" \$OUTF/SNP_Intersection/GATK.snps.filtered.vcf | grep -v
\"SnpCluster\" > \$OUTF/SNP_Intersection/GATK.snps.filtered.cleaned.vcf
grep -v \"CRG\" \$OUTF/SNP_Intersection/MPILEUP.snps.filtered.vcf | grep -v
\"SnpCluster\" > \$OUTF/SNP_Intersection/MPILEUP.snps.filtered.cleaned.vcf
grep -v \"CRG\" \$OUTF/SNP_Intersection/SHORE.snps.filtered.vcf | grep -v
\"SnpCluster\" > \$OUTF/SNP_Intersection/SHORE.snps.filtered.cleaned.vcf

# Correct sample names in VFC files
sed -i -e \"s/FORMAT\t\$NAME/FORMAT\t\$NAME-GATK\t\""
\$OUTF/SNP_Intersection/GATK.snps.filtered.cleaned.vcf
sed -i -e \"s/FORMAT\t\$NAME/FORMAT\t\$NAME-MPILEUP\t\""
\$OUTF/SNP_Intersection/MPILEUP.snps.filtered.cleaned.vcf
sed -i -e \"s/FORMAT\t\$NAME/FORMAT\t\$NAME-SHORE\t\""
\$OUTF/SNP_Intersection/SHORE.snps.filtered.cleaned.vcf

# Intersecting
java -jar -Xmx4g \$GATK -T CombineVariants -R \$REF -genotypeMergeOptions
PRIORITIZE -V:SHORE \$OUTF/SNP_Intersection/SHORE.snps.filtered.cleaned.vcf -
V:GATK \$OUTF/SNP_Intersection/GATK.snps.filtered.cleaned.vcf -V:MPILEUP
\$OUTF/SNP_Intersection/MPILEUP.snps.filtered.cleaned.vcf -priority
GATK,MPILEUP,SHORE -o \$OUTF/SNP_Intersection/merged.vcf

# Evaluation
java -jar -Xmx4g \$GATK -T VariantEval -R \$REF --dbsnp \$KNOWNINDEL -select
'set==\"Intersection\"' -selectName Intersection -select 'set==\"SHORE\"' -
selectName SHORE -select 'set==\"MPILEUP\"' -selectName MPILEUP -select
iset==\"GATK\"' -selectName GATK -select 'set==\"GATK-MPILEUP\"' -selectName
GATK_MPILEUP -select 'set==\"GATK-SHORE\"' -selectName GATK_SHORE -select
iset==\"MPILEUP-SHORE\"' -selectName MPILEUP_SHORE -o
\$OUTF/SNP_Intersection/report.all.txt --eval \$OUTF/SNP_Intersection/merged.vcf
-l INFO

# Annotate Enrichment
perl \$NGSBOX/Parser/VCF/vcf_filter/vcf_filter_enriched.pl
\$EXOME/NimbleGen_Tiled_Regions_150.bed \$OUTF/SNP_Intersection/merged.vcf >
\$OUTF/SNP_Intersection/merged.all.vcf

# Evaluate calls on enriched regions
grep -v \"NOTENRICHED\" \$OUTF/SNP_Intersection/merged.all.vcf >
\$OUTF/SNP_Intersection/merged.enriched.vcf

java -jar -Xmx4g \$GATK -T VariantEval -R \$REF --dbsnp \$KNOWNINDEL -select
'set==\"Intersection\"' -selectName Intersection -select 'set==\"SHORE\"' -
selectName SHORE -select 'set==\"MPILEUP\"' -selectName MPILEUP -select
iset==\"GATK\"' -selectName GATK -select 'set==\"GATK-MPILEUP\"' -selectName
GATK_MPILEUP -select 'set==\"GATK-SHORE\"' -selectName GATK_SHORE -select
iset==\"MPILEUP-SHORE\"' -selectName MPILEUP_SHORE -o
\$OUTF/SNP_Intersection/report.enriched.txt --eval
\$OUTF/SNP_Intersection/merged.enriched.vcf -l INFO

```

```

### Filter and compare indel calls from 3 different pipelines
# Filtering
mkdir \$OUTF/Indel_Intersection

java -jar -Xmx4g \$GATK -T VariantFiltration -R \$REF -o
\$OUTF/Indel_Intersection/GATK.indel.filtered.vcf --variant
\$OUTF/GATK.indel.raw.vcf --filterExpression \"MQ < 30.0 || QUAL < 20.0 || MQ0 >
5 || QD < 4.0 || HRun > 9\" --filterName CRG --genotypeFilterExpression \"DP < 5
|| DP > \$max_cov || GQ < 15\" --genotypeFilterName CRGg

java -jar -Xmx4g \$GATK -T VariantFiltration -R \$REF -o
\$OUTF/Indel_Intersection/MPILEUP.indel.filtered.vcf --variant
\$OUTF/MPILEUP.indel.raw.vcf --filterExpression \"MQ < 30.0 || QUAL < 10.0 || DP
< 5 || DP > \$max_cov\" --filterName CRG --genotypeFilterExpression \"DP < 5 || DP
> \$max_cov || GQ < 15\" --genotypeFilterName CRGg

java -jar -Xmx4g \$GATK -T VariantFiltration -R \$REF -o
\$OUTF/Indel_Intersection/SHORE.indel.filtered.vcf --variant
\$OUTF/shore/Variants/ConsensusAnalysis/indels.vcf --filterExpression \"QUAL <
2.0 || DP < 4 || DP > \$max_cov || RE > 1.3\" --filterName CRG

# greping
grep -v \"CRG\" \$OUTF/Indel_Intersection/GATK.indel.filtered.vcf >
\$OUTF/Indel_Intersection/GATK.indel.filtered.cleaned.vcf
grep -v \"CRG\" \$OUTF/Indel_Intersection/MPILEUP.indel.filtered.vcf >
\$OUTF/Indel_Intersection/MPILEUP.indel.filtered.cleaned.vcf
grep -v \"CRG\" \$OUTF/Indel_Intersection/SHORE.indel.filtered.vcf | grep -v
\"SHOREFILTER\" > \$OUTF/Indel_Intersection/SHORE.indel.filtered.cleaned.vcf

# Correct sample names in VFC files
sed -i -e \"s/FORMAT\t\$NAME/FORMAT\t\$NAME-GATK/\" \
\$OUTF/Indel_Intersection/GATK.indel.filtered.cleaned.vcf
sed -i -e \"s/FORMAT\t\$NAME/FORMAT\t\$NAME-MPILEUP/\" \
\$OUTF/Indel_Intersection/MPILEUP.indel.filtered.cleaned.vcf
sed -i -e \"s/FORMAT\t\$NAME/FORMAT\t\$NAME-SHORE/\" \
\$OUTF/Indel_Intersection/SHORE.indel.filtered.cleaned.vcf

# Intersecting
java -jar -Xmx4g \$GATK -T CombineVariants -R \$REF -genotypeMergeOptions
PRIORITY -V:SHORE \$OUTF/Indel_Intersection/SHORE.indel.filtered.cleaned.vcf -
V:GATK \$OUTF/Indel_Intersection/GATK.indel.filtered.cleaned.vcf -V:MPILEUP
\$OUTF/Indel_Intersection/MPILEUP.indel.filtered.cleaned.vcf -priority
GATK,MPILEUP,SHORE -o \$OUTF/Indel_Intersection/merged.vcf

# Evaluation
java -jar -Xmx4g \$GATK -T VariantEval -R \$REF --dbsnp $KNOWNINDEL -select
'set==\"Intersection\"' -selectName Intersection -select 'set==\"SHORE\"' -
selectName SHORE -select 'set==\"MPILEUP\"' -selectName MPILEUP -select
'set==\"GATK\"' -selectName GATK -select 'set==\"GATK-MPILEUP\"' -selectName
GATK_MPILEUP -select 'set==\"GATK-SHORE\"' -selectName GATK_SHORE -select
'set==\"MPILEUP-SHORE\"' -selectName MPILEUP_SHORE -o
\$OUTF/Indel_Intersection/report.all.txt --eval
\$OUTF/Indel_Intersection/merged.vcf -l INFO

# Annotate Enrichment
perl \$NGSBOX/Parser/VCF/vcf_filter/vcf_filter_enriched.pl
\$EXOME/NimbleGen_Tiled_Regions_150.bed \$OUTF/Indel_Intersection/merged.vcf >
\$OUTF/Indel_Intersection/merged.all.vcf

```

```

# Evaluate calls on enriched regions
grep -v \"NOTENRICHED\" \$OUTF/Indel_Intersection/merged.all.vcf >
\$OUTF/Indel_Intersection/merged.enriched.vcf

java -jar -Xmx4g \$GATK -T VariantEval -R \$REF --dbsnp \$KNOWNINDEL -select
'set==\"Intersection\"' -selectName Intersection -select 'set==\"SHORE\"' -
selectName SHORE -select 'set==\"MPILEUP\"' -selectName MPILEUP -select
iset==\"GATK\"' -selectName GATK -select 'set==\"GATK-MPILEUP\"' -selectName
GATK_MPILEUP -select 'set==\"GATK-SHORE\"' -selectName GATK_SHORE -select
iset==\"MPILEUP-SHORE\"' -selectName MPILEUP_SHORE -o
\$OUTF/Indel_Intersection/report.enriched.txt --eval
\$OUTF/Indel_Intersection/merged.enriched.vcf -l INFO

### Annotate SNPs with ANNOVAR: Union, any tool predicts SNP
mkdir \$OUTF/SNP_Intersection/AnnovarUnion
\$ANNOVAR/convert2annovar.pl -includeinfo -format vcf4
\$OUTF/SNP_Intersection/merged.all.vcf >
\$OUTF/SNP_Intersection/AnnovarUnion/snps.ann
\$ANNOVAR/custom_summarize_annovar.pl --buildver hg19 --outfile
\$OUTF/SNP_Intersection/AnnovarUnion/sum
\$OUTF/SNP_Intersection/AnnovarUnion/snps.ann --ver1000g 1000g2011may
\$ANNOVAR/hg19/

### Annotate Indels with ANNOVAR: Union, indels predicted by any tool
mkdir \$OUTF/Indel_Intersection/AnnovarUnion
\$ANNOVAR/convert2annovar.pl -includeinfo -format vcf4
\$OUTF/Indel_Intersection/merged.all.vcf >
\$OUTF/Indel_Intersection/AnnovarUnion/indels.ann
\$ANNOVAR/custom_summarize_annovar.pl --buildver hg19 --outfile
\$OUTF/Indel_Intersection/AnnovarUnion/sum
\$OUTF/Indel_Intersection/AnnovarUnion/indels.ann --ver1000g 1000g2011may
\$ANNOVAR/hg19/

### Clean up
rm \$OUTF/MPILEUP.variant.raw.bcf

\n"):

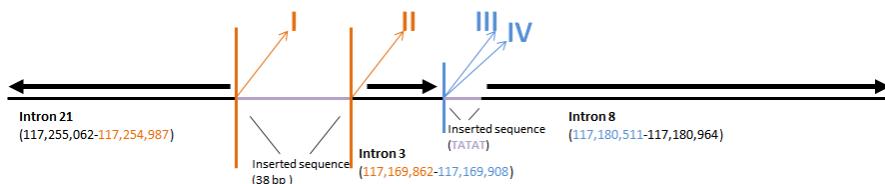
print OUT @qsub;
close OUT;

```

A)

PCR primers: Rv-A1 (intron 9) and Rv-B1 (intron 21)

Sanger sequencing using Rv-B1

**B)**

PCR primers: Fw-7.11 (intron 3) and Fw-B4 (intron 11)

Sanger sequencing using primer Fw-7.11

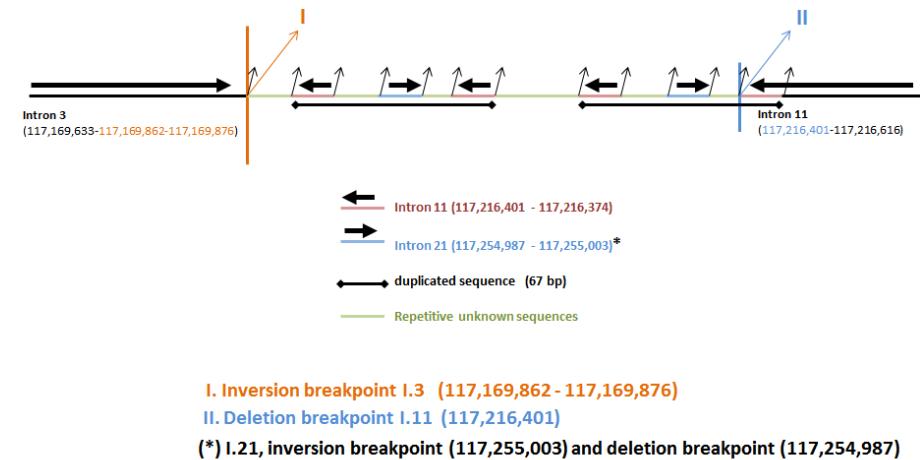


Fig. S1. Diagram showing the position of the primers used for the validation of the breakpoints of CFTR50kbdel. (A) Regions amplified with PCR primers Rv-A1 and Rv-B1. (B) Regions amplified with PCR primers Fw-7.11 and Fw-B4.

Table S1. Sequencing quality control parameters, coverage and detected variants by targeted resequencing of the *CFTR* gene by sample

Sequencing	Sample	1FQ	2FQ	3FQ	4FQ	5FQ	6FQ	7FQ
Coverage	QC-passed reads	17.255.934	19.355.484	17.214.012	15.075.448	22.476.580	18.270.438	21.955.770
	% Mapped	97,27	97,20	97,03	97,04	97,02	97,22	97,16
	% Properly paired	96,22	96,07	95,98	95,94	95,87	96,05	96,04
Coverage	Mean coverage (X)	375	451	377	315	503	398	484
	Mean coverage extended 150 bp (X)	324	390	325	269	436	342	420
	Max coverage (X)	1188	1263	1137	1087	1455	1309	1489
	% Enrichment	58,23	59,65	58,29	57,40	59,03	58,43	58,45
	% target bases covered = 0X	0,102	0,077	0,007	0,052	0,000	0,000	0,000
	% target bases covered >= 1X	99,90	99,92	99,99	99,95	100,00	100,00	100,00
	% target bases covered >= 5X	99,81	99,84	99,83	99,75	99,87	99,92	99,85
	% target bases covered >= 10X	99,74	99,79	99,78	99,65	99,80	99,79	99,78
	% target bases covered >= 20X	99,61	99,70	99,64	99,33	99,70	99,62	99,69
	% target bases covered >= 50X	98,78	99,19	98,79	97,66	99,22	98,77	99,17
Variants	% target bases covered >= 100X	95,64	97,43	96,01	92,74	97,71	96,05	97,41
	SNVs	205	130	141	82	131	135	45
	Novel SNVs	2	4	9	6	1	6	4
	Exonic SNVs	3	1	3	2	2	2	1
	Missense, nonsense and splice site SNPs	1	2	1	2	2	2	2
	SNVs in CFTR database	3	2	3	2	2	2	2
	InDels	48	24	36	20	24	26	13
	Novel InDels	32	16	23	13	16	18	7
	Frameshift and non-Frameshift InDels	2	1	1	0	0	1	0
	InDels annotated in CFTR database	2	1	1	0	0	1	0

8FQ	9FQ	10FQ	11FQ	12FQ	14FQ	15FQ	16FQ	17FQ	18FQ	19FQ
21.895.400	17.133.058	19.670.588	23.977.106	13.535.418	13.022.662	17.755.884	19.176.270	16.841.600	7.730.556	9.645.318
97,11	96,40	96,18	95,83	95,12	96,71	96,53	97,08	96,98	95,92	95,34
96,04	95,15	94,72	94,00	93,42	95,56	95,52	96,12	95,93	94,42	93,60
499	375	407	482	260	236	361	391	356	99	114
430	357	418	225	206	317	344	310	328	87	99
1503	1284	1382	2074	1100	973	1225	1299	1463	408	496
58,84	57,50	56,93	56,19	55,20	57,25	57,30	58,22	58,36	44,35	42,79
0,004	0,000	0,000	0,000	0,051	0,003	0,000	0,028	0,000	0,094	0,065
100,00	100,00	100,00	100,00	99,95	100,00	100,00	99,97	100,00	99,91	99,94
99,84	99,96	99,96	99,82	99,82	99,79	99,92	99,82	99,81	99,55	99,58
99,79	99,84	99,82	99,76	99,70	99,72	99,80	99,79	99,77	98,89	98,98
99,71	99,74	99,71	99,64	99,23	99,26	99,70	99,72	99,65	96,70	97,15
99,29	99,21	99,18	98,92	96,37	96,58	99,11	99,16	98,54	81,12	85,12
97,72	96,62	96,64	96,71	86,86	85,47	96,41	96,64	95,02	42,05	51,91
126	125	204	76	17	41	154	22	70	63	120
7	3	8	4	3	4	6	9	6	3	1
3	4	2	3	0	0	3	0	1	1	5
1	2	1	2	0	0	2	0	0	0	2
3	4	3	3	0	0	3	0	1	1	5
40	36	58	29	10	19	46	10	21	22	38
26	22	39	21	8	13	28	6	14	15	29
0	0	0	0	1	1	0	0	1	1	0
0	0	0	0	1	1	0	0	1	1	0

20FQ	21FQ	22FQ	23FQ	24FQ	25FQ	26FQ	27FQ	29FQ	30FQ	31FQ
9.823.268	6.484.528	7.110.480	7.678.796	9.785.126	9.332.386	10.205.974	8.380.250	7.333.946	7.660.972	8.108.704
94,43	95,25	94,88	94,71	91,10	95,22	93,67	95,05	93,63	95,13	96,55
92,69	93,60	92,94	92,38	87,21	93,23	91,56	93,11	91,79	93,29	95,25
113	77	85	89	93	114	106	105	107	105	151
98	68	74	78	82	99	93	90	92	92	130
472	310	370	427	583	445	430	401	364	380	640
42,44	42,88	43,10	42,97	39,31	44,08	40,16	43,96	47,30	46,17	55,51
0,167	0,183	0,099	0,055	0,157	0,100	0,127	0,175	0,164	0,074	0,151
99,83	99,82	99,90	99,95	99,84	99,90	99,87	99,82	99,84	99,93	99,85
99,52	99,31	99,32	99,16	98,91	99,53	99,52	99,45	99,46	99,61	99,46
98,95	98,22	98,00	97,89	97,46	98,88	98,80	98,78	98,79	98,95	98,79
96,97	94,12	94,50	94,42	92,78	97,12	96,64	96,66	96,94	97,08	97,15
83,53	68,31	72,49	73,02	70,89	84,62	82,36	82,21	83,76	83,64	88,72
50,32	26,15	32,60	35,57	37,83	52,14	46,71	46,00	48,91	47,49	65,60
122	127	201	67	116	157	130	16	208	129	173
6	1	1	2	5	1	2	3	2	5	2
4	2	3	4	7	2	2	1	4	2	3
2	2	2	3	4	2	2	0	3	3	2
4	2	4	4	7	2	2	1	5	3	3
35	27	36	22	30	29	23	12	42	25	33
22	18	24	14	21	15	14	9	29	17	20
0	1	1	0	0	2	0	1	0	0	0
0	1	1	0	0	2	0	1	0	0	0

32FQ	33FQ	34FQ	35FQ	38FQ	39FQ	40FQ	41FQ	42FQ	43FQ	44FQ
12.267.092	11.960.280	12.673.174	8.834.572	12.403.370	11.188.176	12.761.496	13.361.576	11.251.326	14.216.384	10.825.984
96,41	96,52	96,49	95,63	96,14	95,59	96,46	96,58	96,83	96,54	96,63
95,07	95,25	95,17	94,17	94,99	93,99	95,18	95,31	95,68	95,29	95,40
239	220	240	162	231	208	255	250	244	265	206
205	190	208	139	202	182	218	217	211	231	179
922	861	944	623	819	710	1810	964	794	1074	782
56,53	55,44	55,45	55,48	55,47	55,41	56,40	55,07	57,59	55,23	56,02
0,158	0,171	0,068	0,148	0,064	0,118	0,034	0,080	0,082	0,073	0,084
99,84	99,83	99,93	99,85	99,94	99,88	99,97	99,92	99,92	99,93	99,92
99,77	99,72	99,74	99,62	99,76	99,78	99,72	99,70	99,79	99,80	99,74
99,65	99,46	99,61	99,12	99,58	99,59	99,63	99,59	99,72	99,59	99,61
99,21	98,92	99,13	97,87	98,93	99,11	99,06	98,98	99,47	99,12	98,93
96,65	95,52	96,24	90,56	95,83	95,82	96,62	96,07	97,28	96,62	95,27
87,69	83,88	85,91	69,87	85,63	84,64	88,27	86,36	90,07	88,56	82,30
128	132	125	200	14	133	145	132	16	105	126
4	2	2	2	2	10	2	5	2	4	1
5	2	2	4	1	4	3	6	0	3	2
3	2	2	2	1	2	2	4	1	2	2
6	2	2	4	1	4	3	6	1	2	2
49	29	24	41	13	38	35	38	12	28	23
37	21	16	27	11	25	20	24	9	16	14
0	1	1	0	0	0	0	1	0	0	0
0	1	1	0	0	0	0	1	0	0	0

45FQ	46FQ	47FQ	48FQ	49FQ	50FQ	51FQ	52FQ	53FQ	54FQ	55FQ
6.637.998	8.190.110	8.038.462	8.284.024	9.080.058	8.159.248	7.664.812	9.987.790	10.079.020	8.358.090	8.962.716
93,46	96,74	96,96	96,83	96,50	96,55	96,90	96,13	96,64	96,70	96,54
91,05	95,48	95,80	95,68	95,09	95,17	95,71	94,70	95,43	95,38	95,24
141	155	157	160	183	167	150	216	205	167	176
119	134	136	138	157	144	130	187	179	144	152
459	610	568	554	661	624	531	643	737	553	594
57,16	55,14	55,64	55,33	56,38	56,62	56,09	57,72	56,36	56,52	56,49
0,048	0,113	0,161	0,131	0,094	0,118	0,050	0,034	0,044	0,078	0,077
99,95	99,89	99,84	99,87	99,91	99,88	99,95	99,97	99,96	99,92	99,92
99,68	99,74	99,71	99,75	99,75	99,77	99,72	99,80	99,82	99,75	99,75
99,21	99,47	99,47	99,51	99,58	99,62	99,51	99,68	99,68	99,55	99,65
98,10	98,57	98,61	98,81	98,90	99,00	98,58	99,41	99,42	98,90	99,18
90,82	93,01	93,85	93,89	95,50	94,75	93,47	96,86	96,95	94,55	95,39
67,29	71,05	72,47	73,27	79,77	76,81	71,04	88,04	86,83	75,95	78,97
158	130	130	126	197	124	73	139	190	152	131
4	1	0	1	4	4	3	4	4	5	10
3	3	2	2	6	4	3	3	4	1	5
3	2	2	2	3	2	3	1	3	2	3
4	3	2	2	6	4	3	3	5	2	5
33	25	23	26	56	44	25	39	48	28	37
24	16	15	18	46	32	17	27	33	20	23
0	1	1	1	0	0	0	1	0	1	0
0	1	1	1	0	0	0	1	0	1	0

56FQ	57FQ	58FQ	59FQ	60FQ	61FQ	62FQ	63FQ	64FQ	65FQ	66FQ
8.455.604	8.841.018	9.609.066	8.256.002	9.228.290	10.798.998	11.362.794	9.386.230	14.580.506	10.231.428	11.384.616
96,61	96,45	96,61	96,70	96,07	93,40	86,52	95,78	95,30	95,92	96,17
95,30	95,20	95,35	95,41	94,71	91,20	81,72	94,30	93,58	94,30	94,64
164	175	209	164	185	222	254	182	305	209	225
143	152	182	144	161	189	215	156	262	180	195
625	663	734	599	682	718	702	708	1005	2431	857
56,23	56,55	57,69	56,34	56,46	56,77	59,41	55,66	57,30	56,59	55,49
0,092	0,042	0,128	0,155	0,007	0,021	0,012	0,115	0,100	0,068	0,017
99,91	99,96	99,87	99,85	99,99	99,98	99,99	99,89	99,90	99,93	99,98
99,75	99,79	99,79	99,75	99,82	99,76	99,89	99,76	99,79	99,73	99,84
99,58	99,59	99,69	99,59	99,61	99,57	99,75	99,59	99,74	99,40	99,69
98,86	99,07	99,38	98,81	99,16	99,06	99,55	98,71	99,55	98,72	99,17
94,20	95,34	97,27	94,33	95,70	95,98	97,66	94,28	98,13	94,63	95,96
75,49	79,55	88,06	75,22	81,23	86,10	92,28	77,74	93,68	80,19	85,34
132	128	13	33	129	195	175	43	133	146	152
3	5	2	1	6	2	3	5	1	4	4
3	1	0	1	5	4	3	1	2	4	5
3	2	0	1	4	1	2	1	2	3	4
3	2	0	1	5	4	3	1	2	4	5
21	26	12	14	36	45	35	14	23	34	35
13	18	9	7	22	30	20	9	14	18	18
0	0	1	0	0	1	0	1	1	0	0
0	0	1	0	0	1	1	1	1	0	0

67FQ	68FQ	69FQ	70FQ	71FQ	72FQ	73FQ	74FQ	75FQ	76FQ	77FQ
15.272.850	10.516.996	10.842.778	8.913.236	4.497.442	8.974.522	14.370.752	3.974.106	9.772.282	12.316.454	12.962.294
95,23	96,05	95,80	92,32	95,23	94,85	93,58	93,64	95,39	92,43	87,14
93,25	94,66	94,28	89,66	93,11	92,95	91,09	91,42	93,62	89,37	83,54
283	211	199	204	89	187	289	79	198	253	292
245	184	168	170	77	161	247	68	170	216	251
1110	747	778	768	391	697	965	325	712	875	900
54,97	56,12	54,52	59,79	55,55	57,13	57,15	56,28	56,52	57,45	60,03
0,002	0,142	0,126	0,138	0,047	0,053	0,010	0,150	0,147	0,014	0,013
100,00	99,86	99,87	99,86	99,95	99,95	99,99	99,85	99,85	99,99	99,99
99,80	99,78	99,74	99,72	99,08	99,65	99,87	98,69	99,75	99,87	99,85
99,65	99,68	99,52	99,45	97,77	99,24	99,71	96,68	99,61	99,65	99,77
99,25	99,24	98,53	98,67	93,78	98,17	99,45	91,48	99,00	99,31	99,55
96,79	96,00	93,74	94,82	72,62	93,38	97,65	65,58	95,61	96,97	97,89
89,63	84,32	77,82	81,38	35,30	77,06	92,31	28,32	82,22	88,98	93,12
42	21	180	14	193	43	129	124	215	125	142
6	5	4	3	1	3	3	3	3	1	3
2	1	3	1	4	0	5	1	4	4	2
2	1	2	1	2	1	3	1	2	2	2
2	1	3	1	5	1	5	2	4	5	2
10	9	36	13	40	16	40	23	39	33	34
4	5	22	11	26	9	26	15	25	20	26
0	0	1	0	1	1	0	1	1	0	1
0	0	1	0	1	1	0	1	1	0	1

78FQ	79FQ	80FQ	81FQ	82FQ	83FQ	84FQ	85FQ	86FQ	87FQ	88FQ
11.402.816	13.845.360	10.442.674	9.996.370	13.482.678	13.020.756	22.079.312	12.873.084	15.079.064	9.773.980	11.544.038
91,20	90,14	92,99	93,06	93,22	92,12	90,27	91,21	85,60	93,21	91,61
87,46	86,47	90,38	90,81	90,43	89,52	86,59	87,57	80,68	90,55	88,40
228	315	242	242	279	257	332	276	326	209	261
196	272	207	208	237	220	278	235	279	178	224
894	998	784	813	1032	970	1585	1010	1072	793	957
57,46	60,00	59,81	60,28	58,17	56,40	50,53	58,44	59,54	58,15	59,05
0,066	0,061	0,015	0,145	0,057	0,060	0,121	0,026	0,009	0,066	0,023
99,93	99,94	99,99	99,85	99,94	99,94	99,88	99,97	99,99	99,93	99,98
99,73	99,83	99,84	99,76	99,73	99,75	99,66	99,83	99,87	99,73	99,80
99,48	99,74	99,71	99,69	99,58	99,59	99,43	99,65	99,77	99,47	99,61
98,79	99,56	99,28	99,37	98,99	99,00	98,71	99,13	99,48	98,57	99,07
95,18	98,03	97,08	97,13	96,31	96,26	95,36	96,63	97,76	94,27	96,14
83,15	94,15	89,47	90,85	88,65	87,33	86,94	89,19	93,57	80,78	88,11
19	124	150	131	87	136	21	127	24	26	131
4	4	8	4	5	2	7	6	5	7	5
0	3	2	6	3	2	1	4	1	4	1
0	3	2	4	2	1	1	2	2	4	1
0	4	2	6	3	2	2	5	2	4	1
9	32	29	38	24	23	7	35	9	10	23
7	20	17	24	16	15	5	21	7	7	15
0	0	0	0	1	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0

89FQ	90FQ	91FQ	92FQ	93FQ	94FQ	95FQ	96FQ	1FQ_bis	2FQ_bis	3FQ_bis
13.582.002	12.632.482	13.398.820	7.956.772	12.087.950	11.894.622	11.334.266	9.813.002	8.723.572	9.721.670	7.842.304
93,20	93,97	92,37	95,36	90,30	89,96	94,74	95,78	96,33	96,08	96,02
90,19	91,15	88,85	93,50	86,08	85,93	92,27	94,18	95,00	94,68	94,67
290	276	286	150	254	241	235	199	171	204	154
248	240	245	128	216	205	201	173	148	175	135
1088	1016	1045	664	997	1001	947	735	733	707	602
58,42	58,45	58,52	55,75	58,20	57,81	57,22	56,85	56,63	57,86	56,50
0,023	0,027	0,031	0,196	0,018	0,027	0,017	0,020	0,139	0,131	0,069
99,98	99,97	99,97	99,80	99,98	99,97	99,98	99,98	99,86	99,87	99,93
99,80	99,79	99,81	99,42	99,79	99,79	99,67	99,66	99,63	99,72	99,56
99,66	99,64	99,65	98,76	99,45	99,47	99,39	99,31	99,13	99,52	98,95
99,14	99,23	99,23	96,79	98,68	98,59	98,66	98,21	97,95	98,80	97,48
96,64	96,80	96,72	86,89	95,11	95,31	95,07	93,11	91,29	94,98	89,70
89,65	89,98	89,70	63,50	85,15	84,56	83,22	77,37	70,67	81,49	67,30
18	131	129	199	16	122	129	80	203	130	135
4	4	3	3	2	2	3	3	4	4	9
1	2	2	4	1	4	3	3	3	1	3
1	2	2	2	1	2	1	2	1	2	1
1	2	2	4	1	4	3	3	3	2	3
9	20	26	44	10	33	36	21	45	30	35
7	11	17	30	7	21	22	13	30	23	23
0	0	1	0	0	0	1	1	2	1	1
0	0	1	0	0	0	1	1	2	1	1

4FQ_bis

7.208.564

95,93

94,53

137

118

574

55,95

0,112

99,89

99,36

98,48

96,39

85,10

59,45

78

4

2

2

2

19

11

0

0

Table S2. Common *CFTR* polymorphisms identified in the 92 samples

Legacy name	HGVS name	hg19 position	nt change	dbSNP
-102T>A	c.-234T/A	117119915	T>A	-
125G>C	c.-8G>C	117120141	G>C	rs1800501
875+40A>G	c.743+40A>G	117175505	A>G	rs1800502
1001+11C>T	c.869+11C>T	117176738	C>T	rs1800503
1525-61A>G	c.1393-61A>G	117199457	A>G	rs34855237
1898+152T>A	c.1766+152T>A	117230645	T>A	rs4148711
3601-65C>A	c.3469-65C>A	117267511	C>A	rs213989
R74W	c.220C>T	117149143	C>T	rs115545701
R74Q	c.221G>A	117149144	G>A	-
I148T	c.443T>C	117171122	T>C	rs35516286
T351S	c.1052C>G	117180336	C>G	rs1800086
I506V	c.1516A>G	117199641	A>G	rs1800091
F508C	c.1523T>G	117199648	T>G	rs74571530
1540A>G (M470V)	c.1408A>G	117199533	G>A	rs213950
2694T>G (T854T)	c.2562T>G	117235055	T>G	rs1042077
2736A>G (V868V)	c.2604A>G	117235097	A>G	rs1800105
3030G>A (T966T)	c.2898G>A	117243826	G>A	rs1800109
3041-71G>C	c.2909-71G>C	117246657	G>C	rs34830471
I1027T	c.3080T>C	117250664	T>C	rs1800112
4002A>G (P1290P)	c.3870A>G	117282644	A>G	rs1800130
4029A>G (T1299T)	c.3897A>G	117292919	A>G	rs1800131
4404C>T (Y1424Y)	c.4272C>T	117306991	C>T	rs1800135
4521G>A (Q1463Q)	c.4389G>A	117307108	G>A	rs1800136
GATT repeat	-	117176569	GATT	rs67140043
TG repeat	c.1210-34[TG]	117188660	TG	rs3832534
TG repeat	-	117188661	TG	rs67408451
TG repeat	-	117188662	TG	-
1342-13G>T	c.1210-13G>T	117188682	G>T	rs10229820
3041-92G>A	c.2909-92G>A	117246636	G>A	rs35050470
3500-140A>G	c.3368-140A>G	117254527	A>G	rs213981
4269-139G>A	c.4137-139G>A	117305374	G>A	rs4727855
186-155G>C	c.54-155G>C	117144152	G>C	-
712-159G>A	c.580-159G>A	117175143	G>A	rs34159932
2751+106T>A	c.2619+106T>A	117235218	T>A	rs4148713
2751+85_2751+86delAT	-	117235197	AT	-

Table S3. Pathogenic *CFTR* mutations identified in the 92 samples

Sample	Phenotype	Allele	Region	HGVS Name	Legacy Name	total counts	% mutation counts
1	CF	allele 1	exon 11	c.1519_1521delATC	[delta]I507	330	50,61
		allele 2	exon 11	c.1521_1523delCTT	[delta]F508	334	38,62
1_bis	CF	allele 1	exon 11	c.1519_1521delATC	[delta]I507	146	58,21
		allele 2	exon 11	c.1521_1523delCTT	[delta]F508	149	36,24
2	CF	allele 1	exon 11	c.1521_1523delCTT	[delta]F508	464	39,22
		allele 2	intron 4	c.489+1G>T	621+ 1G- >T	510	49,02
2_bis	CF	allele 1	exon 11	c.1521_1523delCTT	[delta]F508	184	47,82
		allele 2	intron 4	c.489+1G>T	621+ 1G- >T	272	44,85
3	CFTR-RD	allele 1	exon 14	c.2051_2052delAAinsG	2183AA- >G	243	47,33
		allele 2	intron 9	c.1210-34TG(12)T(5)	5T-12TG	.	.
3_bis	CFTR-RD	allele 1	exon 14	c.2051_2052delAAinsG	2183AA- >G	73	56,16
		allele 2	intron 9	c.1210-34TG(12)T(5)	5T-12TG	.	.
4	CFTR-RD	allele 1	exon 22	c.3484C>T	R1162X	262	54,58
		allele 2	intron 9	c.1210-34TG(12)T(5)	5T-12TG	.	.
4_bis	CFTR-RD	allele 1	exon 22	c.3484C>T	R1162X	109	41,28
		allele 2	intron 9	c.1210-34TG(12)T(5)	5T-12TG	.	.
5	CF CARRIER	allele 1	exon 24	c.3909C>G	N1303K	378	50
6	CF	allele 1	exon 21	c.3454G>C	D1152H	417	47,48
		allele 2	exon 11	c.1521_1523delCTT	[delta]F508	339	35,1
7	CFTR-RD	allele 1	exon 19	c.2991G>C	L997F	612	50,98
		allele 2	intron 5	c.579+1G>T	711+ 1G- >T	301	42,19
8	CF CARRIER	allele 1	exon 14	c.1817_1900delAAATG GAACATTAAAGAAC TGACAAAATATTAATTT GCATGAAGGTAGCAGCT ATTTTATGGGACATTT CAGAACTCC	1949del84	.	.
9	CF CARRIER	allele 1	exon 8	c.1040G>A	R347H	429	51,28
10	CF CARRIER	allele 1	intron 11	c.1585-1G>A	1717- 1G- >A	316	42,09
11	CF CARRIER	allele 1	exon 4	c.350G>A	R117H	595	51,43
12	CF CARRIER	allele 1	exon 23	c.3773_3774insT	3905insT	293	36,52
14	CF CARRIER	allele 1	exon 22	c.3527delC:p.T1176fs	3659delC	219	40,18
15	CF CARRIER	allele 1a	exon 22	c.3484C>T	R1162X (in cis)	312	48,08
		allele 1b	intron 9	c.1210-34TG(13)T(5)	5T-13TG (in cis)	.	.
16	CF CARRIER	allele 1	intron 22	c.3717+12191C>T	3849+ 10kbC- >T	367	47,14
17	CF CARRIER	allele 1	exon 14	c.2471delT:p.I824fs	2603delT	383	48,04
18	CF CARRIER	allele 1	exon 14	c.1919_1920delTT:p.64 0_640del	2051delTT	47	48,94
19	CF	allele 1	exon 20	c.3196C>T	R1066C	133	95,49
		allele 2	exon 20	c.3196C>T	R1066C	133	95,49
20	CFTR-RD	allele 1	exon 11	c.1523T>G	F508C	92	52,17
21	CFTR-RD	allele 1	exon 11	c.1523T>G	F508C	49	63,27

		allele 2	exon 11	c.1521_1523delCTT	[delta]F508	59	32,2
22	CF	allele 1	exon 11	c.1521_1523delCTT	[delta]F508	81	43,21
		allele 2	intron 18	c.2988+1G>A	3120+ 1G- >A	29	37,93
23	CF	allele 1	exon 22	c.3472C>T	R1158X	69	43,48
		allele 2	exon 8	c.1040G>C	R347P	85	54,12
24	CF	allele 1	exon 14	c.2128A>T	K710X	75	68
		allele 2a	exon 13	c.1684G>A	V562I (in cis)	60	55
		allele 2b	exon 19	c.3017C>A	A1006E (in cis)	116	39,66
		allele 2c	intron 9	c.1210-34TG(11)T(5)	5T-11TG (in cis)	.	.
25	CF	allele 1	exon 11	c.1477_1478delCA:p.49_3_493del	1609delCA	105	49,52
		allele 2a	exon 11	c.1521_1523delCTT	[delta]F508 (in cis)	95	33,68
		allele 2b	exon 19	c.3080T>C	I1027T (in cis)	156	47,44
26	CF	allele 1	exon 6	c.695T>A	V232D	107	44,86
		allele 2	intron 12	c.1679+1.6kbA>G	1811+ 1.6kbA- >G	35	57,14
27	CF CARRIER	allele 1	exon 4	c.387delT:p.L129fs	519delT	151	45,7
29	CF	allele 1	exon 17	c.2668C>T	Q890X	151	43,05
		allele 2	intron 5	c.580-1G>T	712- 1G- >T	154	51,3
30	CF	allele 1	exon 20	c.3302T>A	M1101K	120	43,33
		allele 2	intron 4	c.489+1G>T	621+ 1G- >T	148	34,46
31	CFTR-RD	allele 1	exon 14	c.2260G>A	V754M	235	49,79
32	CF	allele 1	intron 16	c.2657+5G>A	2789+5G>A	537	49,53
		allele 2a	exon 13	c.1684G>A	V562I (in cis)	186	57,53
		allele 2b	exon 19	c.3017C>A	A1006E (in cis)	270	50
		allele 2c	intron 9	c.1210-34TG(11)T(5)	5T-11TG (in cis)	.	.
33	CF	allele 1	exon 11	c.1521_1523delCTT	[delta]F508	149	39,6
		allele 2	exon 13	c.1763A>T	E588V	141	48,23
34	CFTR-RD	allele 1	exon 11	c.1521_1523delCTT	[delta]F508	202	38,61
		allele 2	exon 7	c.772A>G	R258G	145	55,86
35	CF CARRIER	allele 1	exon 12	c.1652G>A	G551D	146	49,32
38	CF	allele 1	exon 23	c.3731G>A	G1244E	246	95,93
		allele 2	exon 23	c.3731G>A	G1244E	246	95,93
39	CF CARRIER	allele 1	exon 12	c.1673T>C	L558S	156	52,56
40	CFTR-RD	allele 1	exon 17	c.2758G>A	V920M	416	46,15
		allele 2	intron 9	c.1210-34TG(13)T(5)	5T-13TG	.	.
41	CFTR-RD	allele 1	exon 11	c.1545_1546delTA	1677delTA	181	53,04
		allele 2a	exon 10	c.1327G>T	D443Y (in cis)	200	32,5
		allele 2b	exon 13	c.1727G>C	G576A (in cis)	214	50,47
		allele 2c	exon 14	c.2002C>T	R668C (in cis)	153	49,02
42	CF CARRIER	allele 1	intron 3	c.273+1G>A	405+ 1G- >A	127	50,39
43	CFTR-RD	allele 1	exon 14	c.1934T>A	M645K	132	43,94
44	CF CARRIER	allele 1	exon 8	c.1094T>C	L365P	207	50,24
45	CF	allele 1	exon 9	c.1196C>A	A399D	91	54,95

		allele 2	intron 5	c.580-1G>T	712- 1G- >T	182	56,04
46	CF	allele 1	exon 4	c.476T>C	L159S	198	53,54
		allele 2	exon 11	c.1521_1523delCTT	[delta]F508	135	50,37
47	CF	allele 1	introns 22-23	c.3718-24_c.3873+601del781	CFTRdelete20	.	.
		allele 2a	exon 19	c.3080T>C	I1027T (in cis)	207	56,52
		allele 2b	exon 11	c.1521_1523delCTT	[delta]F508 (in cis)	137	39,42
48	CF	allele 1	exon 4	c.293A>G	Q98R	145	51,03
		allele 2	exon 11	c.1521_1523delCTT	[delta]F508	143	48,25
49	CFTR-RD	allele 1a	exon 15	c.2552G>T	R851L (in cis)	159	95,6
		allele 1b	exon 8	c.1052C>G	T351S (in cis)	187	98,4
		allele 2a	exon 15	c.2552G>T	R851L (in cis)	159	95,6
		allele 2b	exon 8	c.1052C>G	T351S (in cis)	187	98,4
50	CF	allele 1	exon 26	c.4143C>A	Y1381X	69	98,55
		allele 2	exon 26	c.4143C>A	Y1381X	69	98,55
51	CF	allele 1	exon 14	c.2017G>T	G673X	75	42,67
		allele 2	exon 20	c.3302T>A	M1101K	172	50,58
52	CF	allele 1	exon 17	c.2737_2738insG	2869insG	404	43,32
		allele 2	intron 19	c.3140-26A>G	3272- 26A- >G	199	51,26
53	CF CARRIER	allele 1	intron 22	c.3718-1G>A	3850- 1G- >A	254	42,91
54	CF	allele 1	exon 11	c.1521_1523delCTT	[delta]F508	132	40,91
		allele 2	intron 3	c.274-1G>A	406- 1G- >A	105	54,29
55	CFTR-RD	allele 1	exon 11	c.1516A>G	I506V (1648A/G)	157	51,59
		allele 2	exon 20	c.3275A>G	Y1092C	223	47,98
56	CF	allele 1	exon 12	c.1624G>T	G542X	134	50
		allele 2	exon 3	c.178G>A	E60K	99	56,57
57	CF CARRIER	allele 1	intron 22	c.3717+1G>A	3849+ 1G- >A	234	48,29
58	CF CARRIER	allele 1	promoter	c.-9_14del23	124del23bp	150	28
59	CF CARRIER	allele 1	exon 8	c.1076A>G	Q359R	163	40,49
60	CF	allele 1	exon 4	c.274G>A	E92K	150	54,67
		allele 2	exon 6	c.617T>G	L206W	245	53,88
61	CF CARRIER	allele 1	exon 7	c.805_806delAT	936delTA	164	39,02
		allele 2
62	CFTR-RD	allele 1a	exon 14	c.2260G>A	V754M (in cis)	225	50,22
		allele 1b	intron 2	c.164+2_164+3insT	296+ 3insT (in cis)	227	39,65
63	CF	allele 1	exon 1	c.44T>C	L15P	150	47,33
		allele 2	exon 14	c.2052_2053insA	2184insA	120	28,33
64	CF	allele 1	exon 13	c.1682C>A	A561E	220	53,64
		allele 2	exon 11	c.1521_1523delCTT	[delta]F508	266	40,23
65	CF CARRIER	allele 1	exon 12	c.1657C>T	R553X	191	49,21
66	CF	allele 1	exon 20	c.3266G>A	W1089X	296	48,99
		allele 2a	exon 14	c.1882G>C	G628R (in cis)	72	50
		allele 2b	exon 22	c.3705T>G	S1235R (in cis)	271	47,97

67	CF	allele 1	exon 9	c.1209G>A	1341G- >A	165	43,03
		allele 2	exon 3	c.254G>A	G85E	148	44,59
68	CFTR-RD	allele 1	exon 19	c.3041A>G	Y1014C	239	39,33
69	CF	allele 1	introns 1-3	c.54-5940_273+10250del21kb	CFTRdele2,3	.	.
		allele 2a	exon 19	c.3080T>C	I1027T (in cis)	292	51,71
		allele 2b	exon 11	c.1521_1523delCTT	[delta]F508 (in cis)	205	48,29
70	CF CARRIER	allele 1	Prom - intron 3	c.1-6186_273+507dup35741	CFTRdupProm-3	.	.
71	CF	allele 1	intron 5	c.579+3A>T	711+3A>T	50	64
		allele 2a	exon 19	c.3080T>C	I1027T (in cis)	122	36,07
		allele 2b	exon 11	c.1521_1523delCTT	[delta]F508 (in cis)	94	41,49
72	CF	allele 1	exon 11	c.1477_1478delCA	1609delCA	167	34,13
		allele 2	intron 6	c.743+1G>A	875+ 1G- >A	135	47,41
73	CF	allele 1	exon 17	c.2668C>T	Q890X	395	37,47
		allele 2	exon 19	c.3083T>G	M1028R	333	50,75
74	CF	allele 1	exon 3	c.262_263delTT	394delTT	41	29,27
		allele 2	intron 13	c.1766+3A>C	1898+3A>C	50	52
75	CF	allele 1	exon 15	c.2551C>T	R851X	169	48,52
		allele 2	exon 11	c.1521_1523delCTT	[delta]F508	176	38,07
76	CFTR-RD	allele 1	intron 16	c.2657+5G>A	2789+5G>A	539	50,46
		allele 2a	exon 18	c.2930C>T	S977F (in cis)	112	43,75
		allele 2b	intron 9	c.1210-34TG(12)T(5)	5T-12TG (in cis)	.	.
77	CF	allele 1	exon 14	c.2125C>T	R709X	136	50,74
		allele 2	exon 11	c.1521_1523delCTT	[delta]F508	266	44,74
78	CFTR-RD	allele 1	exons 25-27	c.3964-3890_Stop+3143del9454 insTAAC	CFTRdele22-24	.	.
		allele 2	intron 9	c.1210-34TG(12)T(5)	5T-12TG	.	.
79	CF	allele 1	exon 23	c.3731G>A	G1244E	378	41,01
		allele 2	intron 7	c.870-2A>G	1002- 2A>G	209	54,07
80	CF	allele 1	exon 12	c.1647T>G	S549R	226	47,79
		allele 2
81	CFTR-RD	allele 1	exon 19	c.2991G>C	L997F	287	43,21
		allele 2a	exon 23	c.3846G>A	W1282X (in cis)	209	43,06
		allele 2b	exon 3	c.221G>A	R74Q (in cis)	157	49,68
82	CF	allele 1	exon 14	c.2051_2052delAAinsG	2183AA- >G	109	50,46
		allele 2	exon 1	c.4C>T	Q2X	265	47,17
83	CF CARRIER	allele 1	exons 4-8, 12-21	c.[274-1091_3468+236inv85141ins38; 274-1044_1116+111del10602insTATAT; 1585-11392_3468+219del385	CFTR50kbdel	.	.

				85]			
84	CF	allele 1	intron 18	c.2989-1G>A	3121- 1G- >A	323	97,52
		allele 2	intron 18	c.2989-1G>A	3121- 1G- >A	323	97,52
85	CF	allele 1	exon 3	c.254G>T	G85V	165	42,42
		allele 2a	intron 9	c.1210-34TG(12)T(5)	5T-12TG (in cis)	.	.
		allele 2b	intron 15	c.2619+3A>G	2751+3A>G (in cis)	236	41,95
86	CF	allele 1	exon 8	c.1000C>T	R334W	400	49,25
		allele 2	intron 12	c.1680-1G>A	1812- 1G- >A	240	59,58
87	CF	allele 1	exon 14	c.1826A>G	H609R	60	53,33
		allele 2a	exon 23	c.3808G>A	D1270N (in cis)	238	47,9
		allele 2b	exon 3	c.220C>T	R74W (in cis)	132	46,21
		allele 2c	exon 6	c.601G>A	V201M (in cis)	216	44,91
88	CF CARRIER	allele 1	exons 19-21	c.2989-2634_3468+1508del8600	CFTRdele17a-18	.	.
89	CF CARRIER	allele 1	exon 20	c.3299A>C	Q1100P	349	47,56
90	CFTR-RD	allele 1	exon 3	c.224G>A	R75Q	170	49,41
91	CF	allele 1	exon 6	c.613C>T	P205S	294	52,04
		allele 2	exon 11	c.1521_1523delCTT	[delta]F508	249	35,34
92	CF	allele 1	exon 6	c.595C>T	H199Y	165	55,76
		allele 2	intron 19	c.3140-26A>G	3272- 26A- >G	127	51,97
93	CF	allele 1	exon 20	c.3274T>C	Y1092H	361	43,49
		allele 2	exon 22	c.3469-420_3717+1230del1899	.	.	.
94	CFTR-RD	allele 1	exon 11	c.1584G>A	1716G/A	159	50,94
95	CF CARRIER	allele 1	exon 14	c.2215delG:p.V739fs	2347delG	331	46,22
96	CFTR-RD	allele 1	exon 8	c.948delT	1078delT	266	49,62
		allele 2a	exon 20	c.3222T>A	F1074L (in cis)	230	46,52
		allele 2b	intron 9	c.1210-34TG(12)T(5)	5T-12TG (in cis)	.	.

Table S4. CFTR intron 9 c.1210-34TG(11-13)T(5-9) Haplotypes

Sample	T-TG Haplotype	Haplotype Counts
1FQ	T7-TG10/T9-TG10	33/23
1FQ_bis	T7-TG10/T9-TG10	10/9
2FQ	T9-TG10	59
2FQ_bis	T9-TG10	20
3FQ	T7-TG10/T5-TG12	29/28
3FQ_bis	T5-TG12/T7-TG10	10/9
4FQ	T5-TG12/T7-TG10	27/20
4FQ_bis	T5-TG12/T7-TG10	12/6
5FQ	T7-TG11/T9-TG10	31/27
6FQ	T7-TG11/T9-TG10	27/25
7FQ	T7-TG11/T7-TG10	50/7
8FQ	T7-TG11/T7-TG10	31/2
9FQ	T7-TG10/T7-TG11	27/21
10FQ	T7-TG10/T7-TG12	31/26
11FQ	T7-TG10/T7-TG11	34/22
12FQ	T7-TG11/T7-TG10	23/2
14FQ	T7-TG11/T7-TG10	39/2
15FQ	T7-TG12/T5-TG13	25/19
16FQ	T7-TG12/T7-TG11	28/27
17FQ	T7-TG11/T7-TG10	59/2
18FQ	T7-TG11	9
19FQ	T7-TG10	23
20FQ	T7-TG11/T7-TG10	8/1
21FQ	T9-TG10/T7-TG12	5/4
22FQ	T7-TG11/T9-TG10	6/5
23FQ	T7-TG10/T7-TG11	4/1
24FQ	T5-TG11/T7-TG11	7/2
25FQ	T9-TG10/T7-TG11	10/7
26FQ	T7-TG11/T9-TG10	7/6
27FQ	T7-TG11/T7-TG12	6/5
29FQ	T7-TG10/T9-TG10	6/6
30FQ	T9-TG10/T7-TG11	8/4
31FQ	T9-TG10/T7-TG11	12/11
32FQ	T5-TG11/T7-TG10	19/15
33FQ	T9-TG10/T7-TG11	33/1
34FQ	T7-TG11/T9-TG10	17/15
35FQ	T7-TG10/T9-TG10	12/10
38FQ	T7-TG11/T7-TG10	26/4
39FQ	T7-TG10/T7-TG11	23/16
40FQ	T5-TG13/T7-TG11	18/18
41FQ	T7-TG11/T7-TG10	21/14
42FQ	T7-TG11/T7-TG10	32/2
43FQ	T7-TG10/T7-TG11	30/16

44FQ	T9-TG10/T7-TG11	19/18
45FQ	T7-TG10/T9-TG10	18/1
46FQ	T7-TG12/T9-TG10	11/5
47FQ	T7-TG11/T9-TG10	12/12
48FQ	T9-TG10/T7-TG11	13/6
49FQ	T7-TG11/T9-TG10	19/2
50FQ	T7-TG10	21
51FQ	T7-TG11/T7-TG10	12/6
52FQ	T7-TG10/T7-TG11	17/14
53FQ	T7-TG10/T9-TG10	24/13
54FQ	T7-TG10/T9-TG10	17/7
55FQ	T7-TG10/T7-TG11	13/12
56FQ	T9-TG10/T7-TG11	11/9
57FQ	T9-TG10/T7-TG11	11/10
58FQ	T7-TG11	23
59FQ	T7-TG11/T7-TG10	25/3
60FQ	T7-TG11/T7-TG10	6/1
61FQ	T7-TG11/T7-TG11	12/4
62FQ	T9-TG10/T7-TG11	17/12
63FQ	T7-TG11/T7-TG10	17/1
64FQ	T7-TG11/T9-TG10	22/22
65FQ	T7-TG11/T7-TG12	14/14
66FQ	T7-TG11/T7-TG12	12/6
67FQ	T7-TG11/T7-TG10	35/1
68FQ	T7-TG11/T7-TG12	12/3
69FQ	T9-TG10/T7-TG11	19/8
70FQ	T7-TG11	17
71FQ	T7-TG10/T9-TG10	3/3
72FQ	T7-TG11	19
73FQ	T7-TG10/T7-TG11	16/14
74FQ	T7-TG11/T9-TG10	8/3
75FQ	T9-TG10	16
76FQ	T7-TG10/T5-TG12	16/11
77FQ	T7-TG10/T9-TG10	17/3
78FQ	T5-TG12/T7-TG11	13/6
79FQ	T7-TG12/T7-TG11	24/21
80FQ	T7-TG11/T7-TG10	36/1
81FQ	T7-TG10/T9-TG10	19/15
82FQ	T7-TG10/T7-TG11	12/8
83FQ	T7-TG11/T9-TG10	17/12
84FQ	T7-TG11/T5-TG12	26/1
85FQ	T7-TG10/T5-TG12	19/15
86FQ	T7-TG11/T7-TG10	26/2
87FQ	T7-TG11/T7-TG10	18/2
88FQ	T7-TG11/T9-TG10	16/16

89FQ	T7-TG11/T9-TG10	20/1
90FQ	T9-TG10/T7-TG11	15/11
91FQ	T9-TG10/T7-TG11	12/8
92FQ	T9-TG10/T7-TG10	9/8
93FQ	T7-TG12/T7-TG11	19/18
94FQ	T7-TG11/T7-TG10	12/9
95FQ	T7-TG10/T7-TG11	14/10
96FQ	T7-TG11/T5-TG11	16/10

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