Online Data Supplement

Multiple Mechanisms Influence Regulation of the *CFTR* Gene Promoter

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Supplementary Table 1

Oligonucleotides used in PCR for cloning into pGL3B or generation of expression vectors for alternative 5' exons.

Primer	Sequence	Location on AC000111/ AF325415/U20418
CFTR1aHindIIIBglII R	ATGAGATCTAAGCTTCGCCCAGCACCAGGCCCATC	19052
CFTR -1a XhoKpn F	AGG GTA CCC TCG AGG GAT AGA CAA GGA AC	18864
CFTR 1a Hind IIIBgl IIR	ATGAGATCTAAGCTTCGCCCAGCACCAGGCCCATC	19052
18017 Kpn F	AGG GTA CCG AGT TCA ATC ACA TGT CTG G	18017
18993 Hind III BglII R	ATGAGATCTAAGCTTT ATTAGTTTCAGGTTTAGGTGAGTG	18993
245 end BglII R	ATAGATCTCTGGGCTCAAGCTCCTAATG	18981
CFTR-1a 18951 F	TAC GTG TCC TAA GAT TTC TGT GCC A	18951
CFTR Promoter-2kbMluI F	ACGCGTAAATATTTGGAGTCTCCTCCAGC	17805
-800HindIII KpnII For	AGGGTACCAACTAATAAAGCTTGGTTCTTTTCTCCGACACG	19004
-632 Promoter Kpn For	AGG GTA CCG AAC CCG ACT AGG ATC ATC GGG AA	19169
0 Primer BglII Rev	ATAGATCTGGTCTCTCGGGCGCTGGGGTCCCTGCTAG	19801
Ov1aSF	Atttaaactcggttgtgcccttgcttttacaacag	1154-1223
Ov1aSR	Gtctgacaattccaagcgctgtctgtatcctttcttcaaaattggtgtggtccagctgactcacggaaac	1322-1336/183-237

Supplementary Table 2: Oligonucleotides used for site directed mutagenesis.

Site directed mutagenesis primer	Sequence
1A ATG/GTG DIR	GAC TGT CGC CCA CCT GCG GGG TGG GCC TGG TGC TGG GCG
1A ATG/GTG REV	CGCCCAGCACCAGGCCCACCCCGCAGGTGGGCGACAGTC
1A CTG/GTG DIR	CTA AAG AGA GGC CGC GAC TGT CGC CCA CGT GCG GGA TGG GCC TG
1A CTG/GTG REV	CAGGCCCATCCCGCACGTGGGCGACAGTCGCGGCCTCTCTTTAG
1A 2Valine GTG DIR	CTAAAGAGAGGCCGCGACTGTCGCCCACGTGCGGGGTGGGCCTGGTGCTG
1A 2Valine GTG Rev	CAGCACCAGGCCCACCCCGCACGTGGGCGACAGTCGCGGCCTCTCTTTAG
18818 A/G DIR (18841A/G)	TAAAACACTCCAAAGCCTTCCTTGAAAATGCGCACT
18853 A/G REV (18841A/G)	AGTGCGCATTTTCAAGGAAGGCTTTGGAGTGTTTTAG

Supplementary Table 3: Primers used for amplification after DNA bisulfite conversion.

Primer	*Sequence and genomic coordinates*
Promoter region 1 (25 CpGs)	**AC000111:116907024 -116907613**
CFTR pr R1- EF	5’ - TGT TAA TTG GAT TTA AAG AGA GG - 3’
CFTR pr R1- ER	5’ - CTT AAA ATT CTA ACT TTT CAA TTA C - 3’
CFTR pr R1 - IF	5’ - GAG GGA GGT TGG GAG TTA G - 3’
CFTR pr R1- IR	5’ - TCT CTA TTC AAT CAA CTT CAA TTC - 3’
Promoter region 2 (20 CpGs)	**AC000111:116906568-116906923**
CFTR pr R2- EF	5’ - GAT AGA TAA GGA ATA TAT TTT GGG – 3’
CFTR pr R2- ER	5’ - CCC AAC ACC AAA CCC ATC C - 3’
CFTR pr R2 - IF	5’- AAG ATT TTT GTG TTA TTT TTG GAG -3’
CFTR pr R2- IR	5’ - ACC TCT CTT TAA ATC CAA TTA AC -3’
Promoter region 3 (3 CpGs)	**AC000111:116906434-116906567**
CFTR pr gR2-R3- EF	5’ - GGT TAG TTT ATA TTG TTT TTT GTT A - 3’
CFTR pr gR2-R3- ER	5’ - CAA TAT AAA TCT AAT ACA TTT ACC - 3’
CFTR pr gR2-R3 - IF	5’ - AGG ATA GAT AAG GAA TAT ATT TTG G - 3’
CFTR pr gR2-R3- IR	5’ - TTT CAA ATT TAA ATA AAT AAA CTC C - 3’
Promoter region 4 (10 CpGs)	**AC000111:116906034-116906433**
CFTR pr R3- EF	5’- GGG TGA TTA TAA GTT AAT AAT AAG - 3’
CFTR pr R3- ER	5’ - ATT TCA AAT TTA AAT AAA TAA AAT CC - 3’
CFTR pr R3 - IF	5’ - TAT AAG ATT TTT GTT TTA GAT GTG - 3’
CFTR pr R3- IR	5’ - CTT ATC TAT CCT TTT TAA CCC - 3’

EF/ER:external primers; IF/IR: internal primers

Supplementary Figures:

Figure S1. In the absence of exon 1, CFTR initiation occurs at methionines in exon 3 and 4. Products of *in* vitro transcription/translation reactions separated on 6% SDS/PAGE, gels dried and exposed to autoradiographic film. 1, Full length CFTR in plasmid pCMV963C (arrow A); 2 and 3, CFTR exons 2-24 in pSP73; 2, wild-type (generates proteins at B and C), 3, mutations in all four methionines in exons 3 and 4 (M82V/M150V/M152V/M156V) abolishes translation initiation (arrow B). Mutation of individual methionines or combinations of methionines does not remove protein B suggesting that initiation can occur at any site (not shown).

Figure S2. Ovine 5' exons Ov1aS and Ov1aL cause inefficient *in vitro* transcription and translation from human exons 2-24. Products of *in* vitro transcription/translation reactions separated on 6% SDS/PAGE, gels dried and exposed to autoradiographic film. 1, Ov1aS/2-24; 2, Ov1aL/2-24; 3, -1a/1a/2-24; 4, full length CFTR in plasmid pCMV963C (arrow A); 5, CFTR exons 2-24 in pSP73 (arrow B).

Figure S3. Luciferase reporter gene assays using CFTR promoter fragments constructs. The bar chart shows the luciferase activities for each construct relative to pGL3B245 (CFTR basal promoter construct =1) in Beas2B cells. Luciferase activities

were normalized for transfection efficiency by co-transfection with pCMV/βgal. Each bar is the average of at least three transfection experiments, with each sample assayed in triplicate and standard deviations are shown. Stars indicate statistical significance of a comparison between pGL3B245 values and those of the other construct, $p<0.05$ *, $p<0.01$ **. For construct details see Fig. 3A.

Figure S4. DNA methylation analyses by sodium bisulfite sequencing of the CFTR 5' region in other samples. The figure depicts representative examples of four regions sequenced after sodium bisulfite treatment for MCF7, skin fibroblasts, Beas2B, and Calu. (*Top*) Scaled map of the 5' region of the CFTR gene. The bold arrow represents the ATG and numbers in each sequenced region represent the first and last CpG dinucleotide. Small arrows represent scores for putative promoters analyzed *in silico*. (*Bottom*) Each circle represents a CpG dinucleotide. Between eight and twelve clones or alleles were sequenced for each region of these samples. A total of 58 CpGs in all regions were analyzed by this method. Unmethylated: open circle, Methylated; black circle. Percentages of DNA methylation are indicated on the right of the panel.