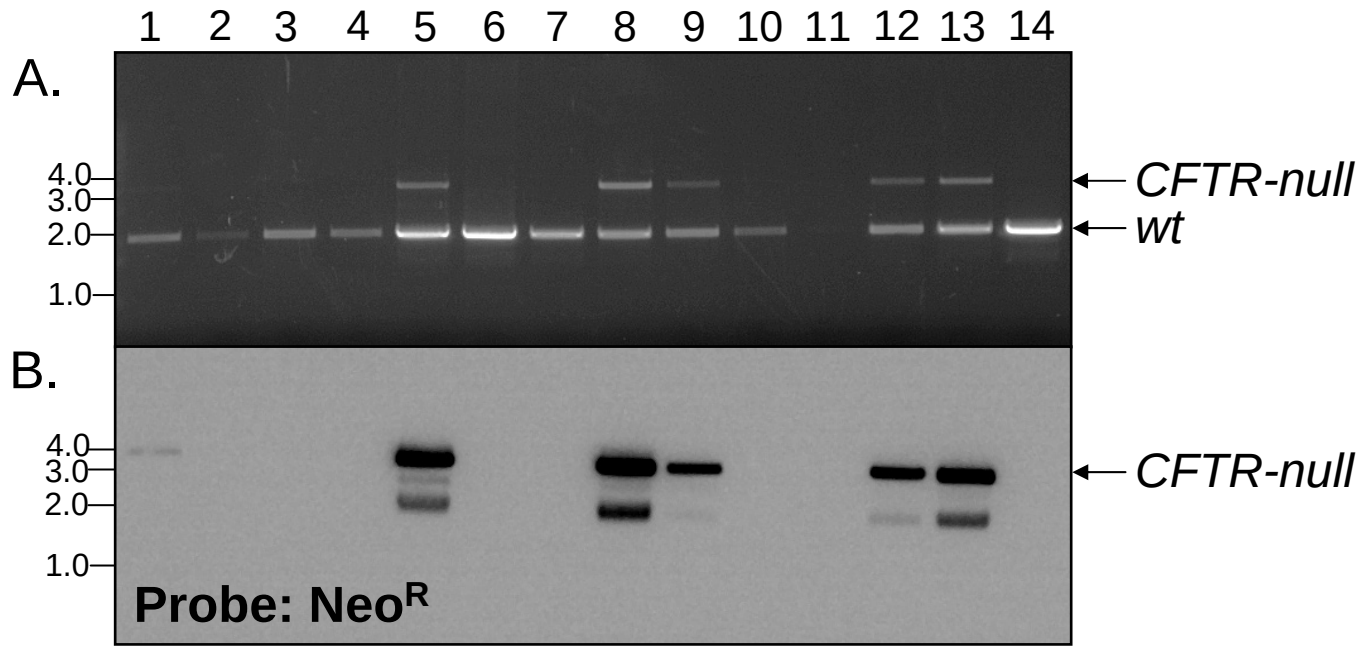
1 2 3 4 5 6 7 8 9 10 11 12 13 14
A.
4.0
3.0
2.0
1.0
CFTR-null
wt
B.
4.0
3.0
2.0
1.0
CFTR-null
Probe: Neo^R

Figure S1

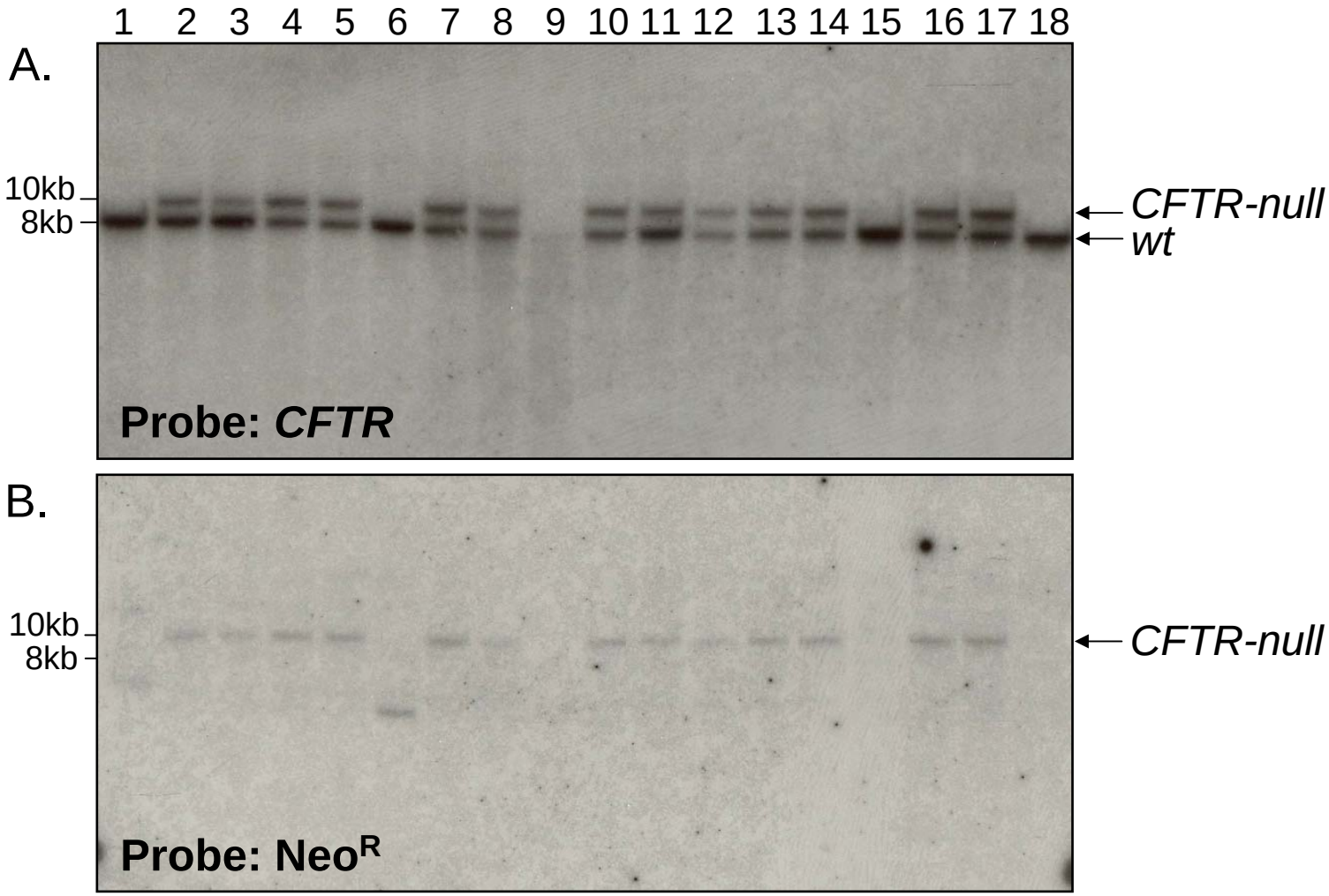
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
A.
10kb
8kb
CFTR-null
wt
Probe: CFTR
B.
10kb
8kb
CFTR-null
Probe: NeoR

Figure S2

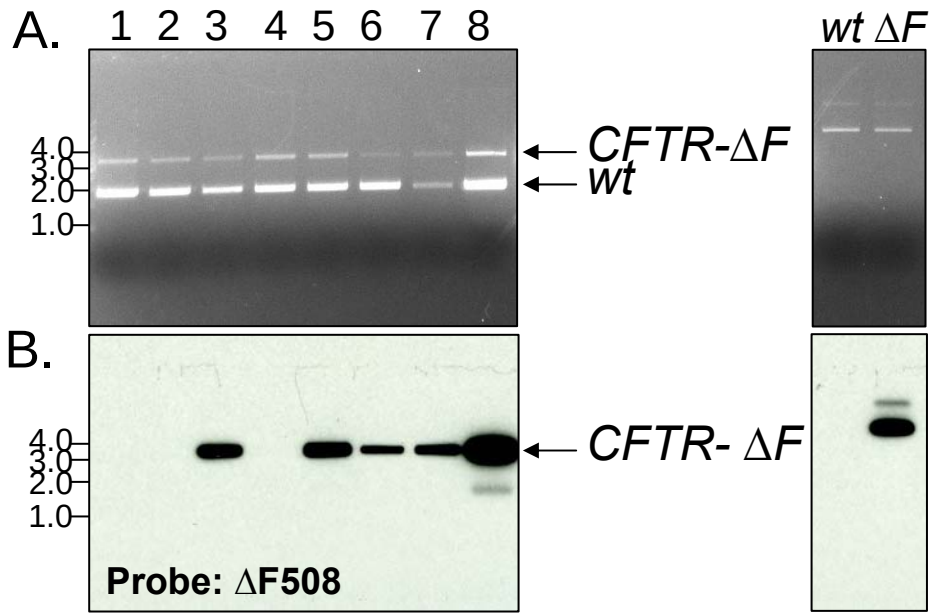
A.
1 2 3 4 5 6 7 8
wt ΔF
4.0
3.0
2.0
1.0
CFTR-ΔF
wt
B.
4.0
3.0
2.0
1.0
CFTR- ΔF
Probe: ΔF508

Figure S3

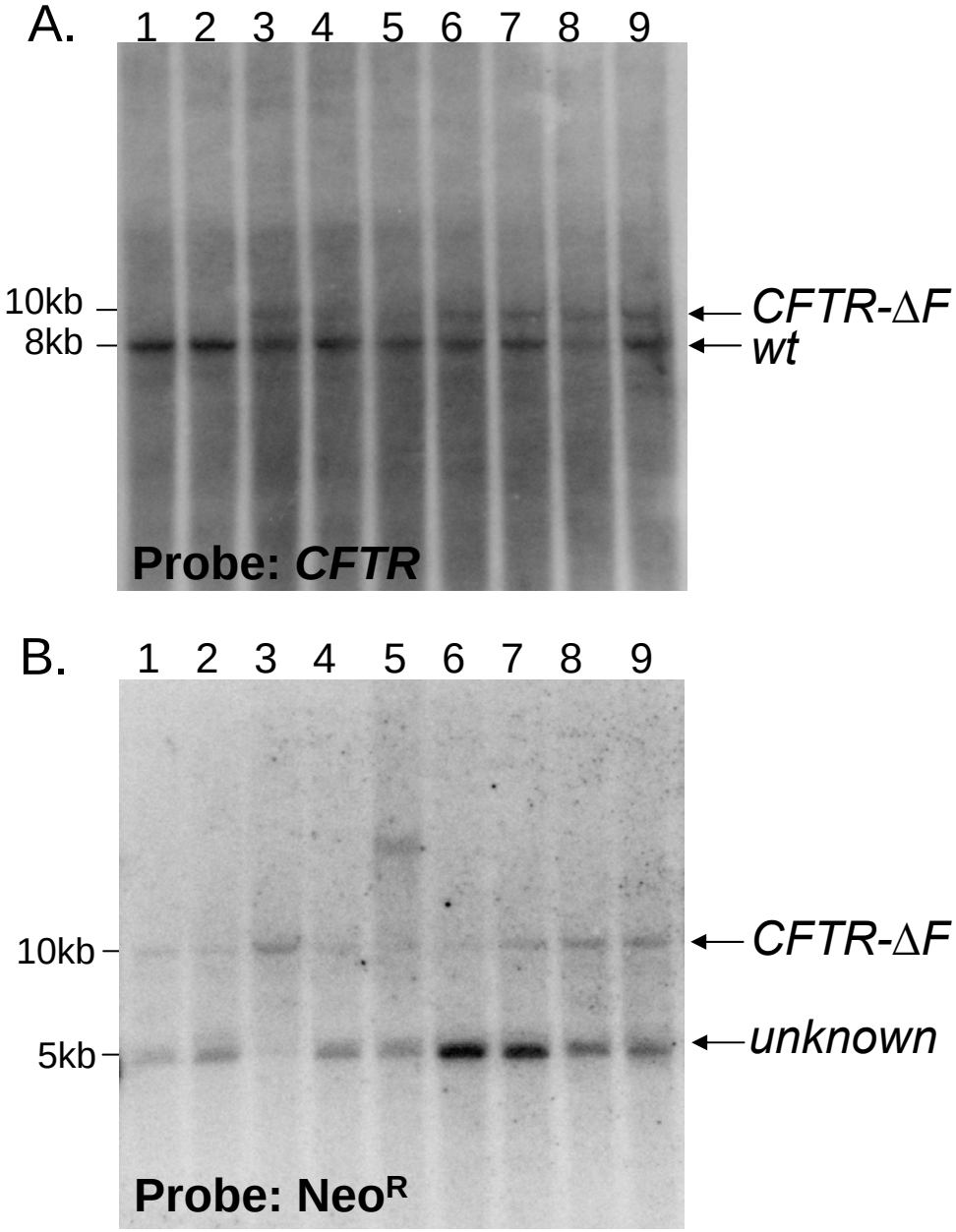
A.
1 2 3 4 5 6 7 8 9
10kb
8kb
CFTR-ΔF
wt
Probe: CFTR
B.
1 2 3 4 5 6 7 8 9
10kb
5kb
CFTR-ΔF
unknown
Probe: NeoR

Figure S4

LEGENDS FOR SUPPLEMENTAL FIGURES

Figure S1. Screening results from *CFTR-null* targeted pig fetal fibroblasts. **A)** Example of PCR results. Primers amplified a 2.0 kb product from the wild-type allele and 3.7 kb product from the *CFTR-null* allele. Lanes 5, 8, 9, 12, and 13 are examples of PCR-positive clones. **B)** Southern blot of the PCR gel using a Neo^R-specific biotin-labeled oligonucleotide. This assay confirms that the 3.7kb product contains the Neo^R sequence. The weaker hybridization signal at 2.0 kb appears to be an artifact, with some of the targeted band co-migrating with the wild-type product. Note the differences in intensity of the two bands in panel A relative to panel B.

Figure S2. Genomic Southern blot of DNA from *CFTR-null* targeted pig fetal fibroblasts. **A)** *Bgl*II-digested genomic DNA was hybridized with a probe that detects pig *CFTR* downstream of the targeting vector boundary. *CFTR-null*-targeted allele yields a ~9.7 kb band and wild-type is ~7.9 kb. These blots also allowed us to identify wells containing monoclonal colonies and those containing more than one type of $G418^R$ colony. For example, wells 3 and 11 appeared to have a more intense signal in the wild-type band than the targeted band, indicating that those wells likely contained one targeted clone and one or more random integration events. **B)** The same digested DNAs were hybridized with a Neo^R-specific probe. The *CFTR-null*-targeted band is at ~9.7 kb. Note that the band in lane 6 likely represents a random integration event, and Lane 1 may have two random integration events. Wells 4, 5, 7, 8, 10, 12-14, 16 and 17 are examples of cells that may be ideal nuclear donors for generating a heterozygote animal

Figure S3. Screening results from *CFTR-ΔF508* targeted pig fetal fibroblasts. **A)** Example of PCR results. Primers amplified a 2.0 kb product from the wild-type allele and 3.7kb product from the *CFTR-ΔF508* allele. **B)** Southern blot of the PCR gel using a *ΔF508* allele-specific biotin-labeled oligonucleotide. This assay confirms some of the 3.7kb products contained the *ΔF508* mutation. Note that lanes 1, 2, and 4 contain clones that underwent homologous recombination but failed to carry the *ΔF508* mutation. On the right, wells contained either wild-type *CFTR* or *CFTR-ΔF508* plasmid DNA. This control is included to ensure that the assay Southern blot is specific to *ΔF508*.

Figure S4. Southern blot of amplified genomic DNA from *CFTR-ΔF508* targeted pig fetal fibroblasts. In contrast to our experience with the *CFTR-null* targeting, the *CFTR-ΔF508* targeted cells failed to proliferate after transfer to larger dishes. As a result, we were unable to obtain sufficient quantities of genomic DNA for a genomic Southern blot. Therefore, we used the relatively small amount of DNA for whole genome amplification. **A)** *Bgl*II-digested amplified genomic DNA was hybridized with a probe that detects pig *CFTR* downstream of the targeting vector boundary. The *CFTR-ΔF508*-targeted allele yields a ~9.7 kb band and the wild-type is ~7.9 kb. **B)** Digested DNAs from similar clones were hybridized with a Neo^R-specific probe. The *CFTR-ΔF508*-targeted band is at ~9.7 kb. Note that all lanes in this Southern blot contain an intense band at ~5 kb. This band was also present in non-infected fibroblast control DNA wells (not shown). This probe is possibly hybridizing to the endogenous PGK promoter sequence because the probe includes some PGK promoter sequence. Consistent with this, the Neo^R-probed blot

in Fig. 4A also contains a faint band at 5 kb in all samples if markedly overexposed (not shown).

Table S1. PCR Primers and Probes. All DNA sequences are 5'-3'. FAM: 6-carboxyfluorescein; NFQ: Non Fluorescent Quencher.

Supplementary Table S1.

Primer Name	Sequence (5'-3')
GC1F	TTTCTCTTCTGCCTATTTCCC
GC1R	AGAAAACACTGAAGGATGCCT
GC2F	GTTTCAAATAGTTACTCAGTTTGA
GC2R	CCTCCAACTGACACTAATCTTCTCA
GC3F	GTAGAGCTGTCAGAGAAGTAA
GC3R	AAGCCACAGAAGCATATGCAT
GC4F	AATCACTCTCAGGATGCACAT
GC5F	ATACTCAGAACAGGAAGTGCT
GC5R	ATAGCATAAGCTTCACTGTGC
GC6F	TGTCAGTAGAGAATTAGAGATTA
GC6R	GCACTACTCACCTACATCCA
GC7F	ACCTGGAAGTTGGAACACTCA
GC7R	GAAGACCCTTTACCTTCTTCTA
GC8F	CATCCAGCTGCAAACAACATT
GC8R	AATTATGCCAAACTCCATCTTAT
Ex10a5F	AGAATTTCATTCTGCTCTCAGT
G16-Neo5'F-XhoI	TCAGCTCGAGACCGCTTTGAGAGCAGGTTG
G16-Neo5'R-EcoRV	GATCGATATCCTAGATGATGTTTTCTTTAATGGTGC
G16-Neo3'F-BamHI	TAGTGGATCCTTGGTGTTTCCTATGATGAGTATA
G16-Neo3'R-HindIII	GACTAAGCTTGCCTACCAGAAACCTGCCT
dF-Neo 5'F-XhoI	TCAGCTCGAGTTTCCAAGGAATATACAACAGA
dF-Neo 5'R-EcoRV	GATCGATATCGTATGATTTAGGTAGTTTGAAGG
dF-Neo 3'F-BamHI	TAGTGGATCCATTTAACTTTTTATTCAATCAGTCT
dF-Neo 3'R-HindIII	GACTAAGCTTGCTCATTCTATGAAGGAGGTA

Biotin-labeled probes

Probe name	**Sequence (Biotin label is on 3' end)**
Neo^R probe	GCATCAGCCATGATGGATAC-Biotin
ΔF508 probe	AAAACATCATTGGTGTTTCC-Biotin

Quantitative RT-PCR primers and probes

Pig *CFTR* and *GAPDH* expression in fetal fibroblasts, nasal and rectal tissue (Figure 1) and *CFTR* expression in *CFTR* +/- pigs (figure 5A).

CFTR primers and probe

pCFTR-1819F (anneals within exon 18)

AGTGGGCTGTAAACTCCAGTATAGA

pCFTR-1819R (anneals withinin exon 19)

CCTTCTGCCGGCATATCAATAAACT

pCFTR-1819 probe (spans exon 18/19 junction) FAM-ATCGCATCAAGCTATCC-NFQ

GAPDH primers and probe

pGAPDH-TM-F	AAGCTCATTTCCTCGTACGACAAT
pGAPDH-TM-R	GGAGGCCATGTGGACCAT
pGAPDH-TM probe	FAM-TCCACCACCCTGTTGCT-NFQ

Pig *CFTR* and *ΔF508-CFTR* expression in *CFTR +/ΔF508* pigs (figure 5B). Primers are the same for both, probes are allele specific.

CFTR primers and probe

pCFTR-TM-F	TCATGCCGGGCACCATTAAA
pCFTR-TM-R	CGCTTTGATGACACTCCTGTATCTA
pCFTR-TM probe	FAM-ACACCAAAGATGATGTTTTC-NFQ
ΔF508 primers and probe	
delF-TM-Forward	TCATGCCGGGCACCATTAAA
delF-TM-Reverse	CGCTTTGATGACACTCCTGTATCTA
delF-TM-Probe	FAM-GAAACACCAATGATGTTTTC-NFQ