Supplementary Methods

Methods of Genotyping

Genotyping was performed in the Mayo Clinic Genomics Shared Resource laboratory. The SNPstream is an automated multiplexed system that can process 12 SNPs in each well of an arrayed 384-well plate by utilizing multiplexed PCR in conjunction with tagged-array, multiplexed, single-base primer extension (SBE) technology. Multiplex PCR and SBE were carried out using AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA) based on the SNPstream protocol. Primer sets for PCR and tagged SBE reactions were designed using the web-based design site http://www.autoprimer.com provided by Beckman Coulter. After PCR in 12-plex reactions, the amplicons were treated with the SBE Clean-up reagent (USB Corp, Cleveland, Ohio) designed specifically for the SNPstream platform to degrade unincorporated PCR primers and deoxynucleotide triphosphates. The tagged extension primers were extended with single TAMRA- or Bodipy-fluorescein-labeled nucleotide terminator reactions and then hybridized to the complementary oligonucleotides arrayed on the SNPware 384-well tag array plates. Each of the SNP primers in the multiplex had a unique 5´-tagged sequence that was spatially resolved by hybridization to the tag array plate. Following hybridization, tag array plates were imaged using the SNPstream Imager (Beckman Coulter, Fullerton, CA). The raw image data was collected and analyzed automatically through SNPstream Software Suite (Beckman Coulter, Fullerton, CA). The genotype calls were made based on the relative fluorescent intensities of each spot and were reviewed by graphical cluster and Hardy-Weinberg equilibrium (HWE) analysis before uploading to the SNPstream database. Individual sample genotype data were

generated on the basis of the relative fluorescent intensities for each SNP and computer processed for graphical review.

Quality control was implemented in multiple steps: (1) All DNA samples were assessed for quality prior to running any high throughput analysis. Verification of optical density (OD) was conducted using a pico green assay. (2) In each of the genotype assays, controls were used in the PCR amplification in duplicate and were run through the system, both within and between runs, to ensure genotyping accuracy. (3) A CEPH DNA trio was included in each 96 well plate (Coriell Institute), each in duplicate. Estimates of reproducibility were calculated periodically during the genotyping process. (4) Two to six no DNA template wells were on each plate as negative controls. (5) Duplicates of 2% of the samples were included in the plate design. (6) A robotic workstation was used to plate out the DNA for each assay (each plate of DNA has a bar code) to eliminate sample mix-up. Control spots were found in each tag-array well, for each of the possible genotypes (XX, XY, YY and negative). Analysis of genotyping data were carried out using SNPstream GENOMELab software.

The ΔF508 deletion was determined by PCR amplification using fluorescent primers tagged with 6-FAM (Applied Biosystems, Foster City, CA). All amplifications were performed in 10ul of PCR reaction mix containing 10ng of template DNA, 10 pmol of each primer (Forward: 5'-TCAGTTTTCCTGGATTATGCC-3' Reverse: 5'-TAAATAACCATTGAGGACGTTTGTC-3'), 0.2 mM of each dNTP, 1.5 mM $MgCI_2$, and 0.25 U of the TaqGold polymerase (Applied Biosystems, Foster City, CA). Polymerase chain reaction condition included initial denaturation at 95°C for12 minutes, 35 cycles with denaturation at 95°C for 35 seconds, annealing at 58°C for 35 seconds,

extension at 72°C for 1 minute, and a final extension at 72°C for 10 minutes. 1ul of 20X diluted amplicons were subjected to electrophoresis on an ABI-3730 DNA sequencer (Applied Biosystems, Foster City, CA) and analyzed using GeneMapper software.

Figure 1.

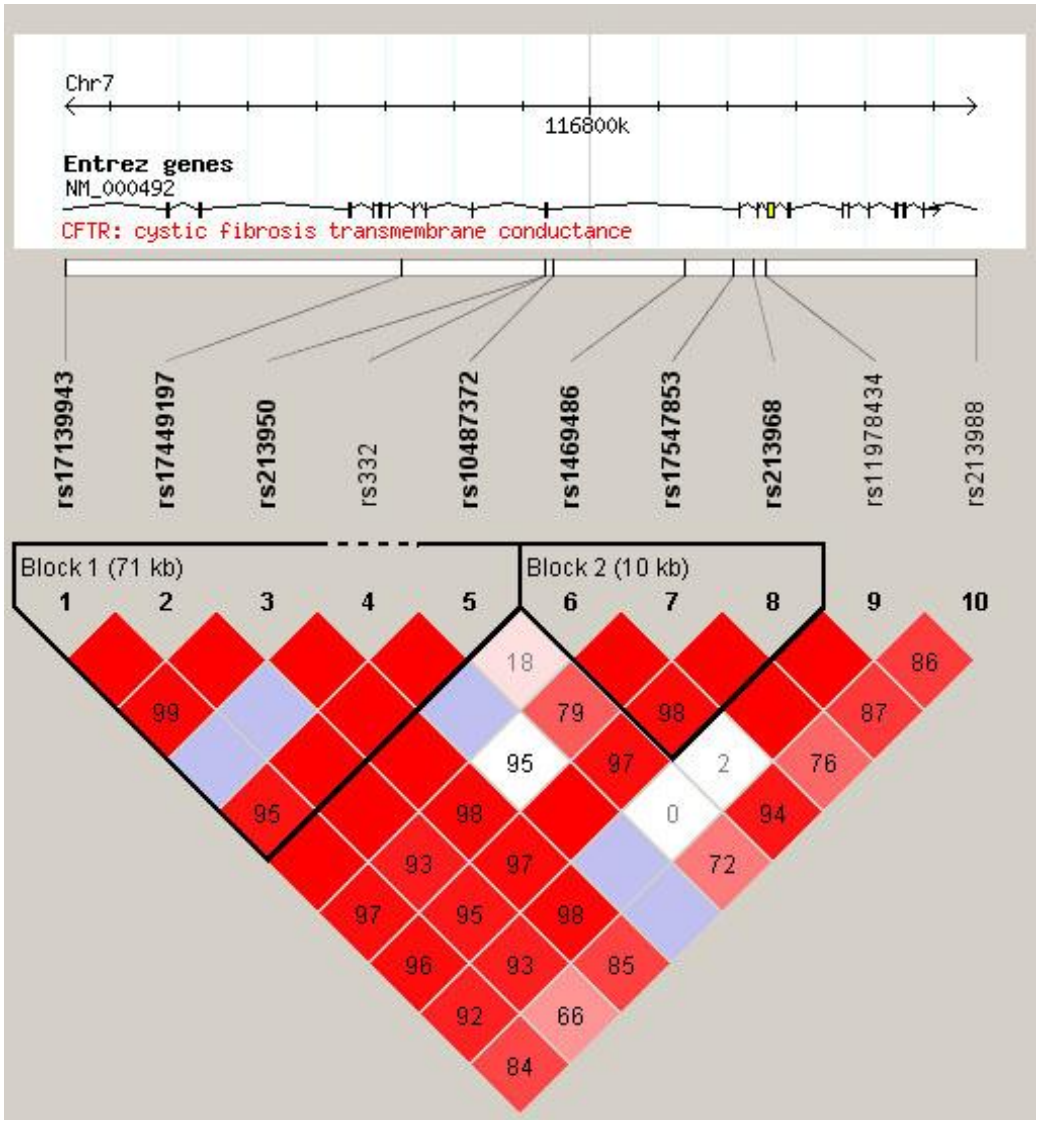
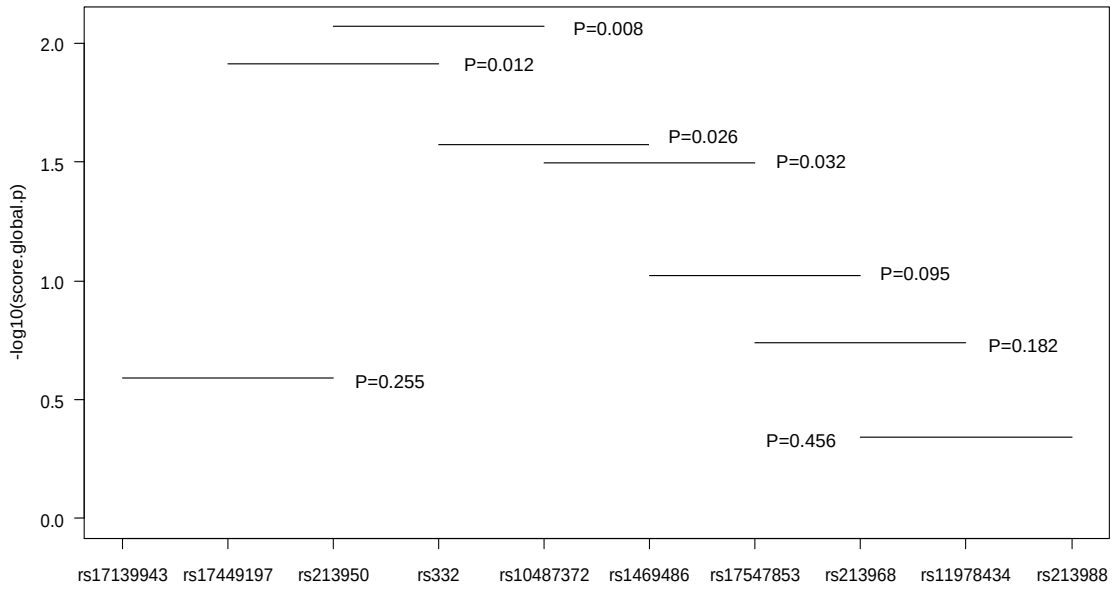

Chr7
116800k
Entrez genes
NM_000492
CFTR: cystic fibrosis transmembrane conductance
rs17139943
rs17449197
rs213950
rs332
rs10487372
rs1469486
rs17547853
rs213968
rs11978434
rs213988
Block 1 (71 kb)
Block 2 (10 kb)
1
2
3
4
5
6
7
8
9
10
99
95
18
79
98
95
97
2
86
87
76
98
0
94
93
97
72
97
95
98
96
93
85
92
66
84

Figure 2 A.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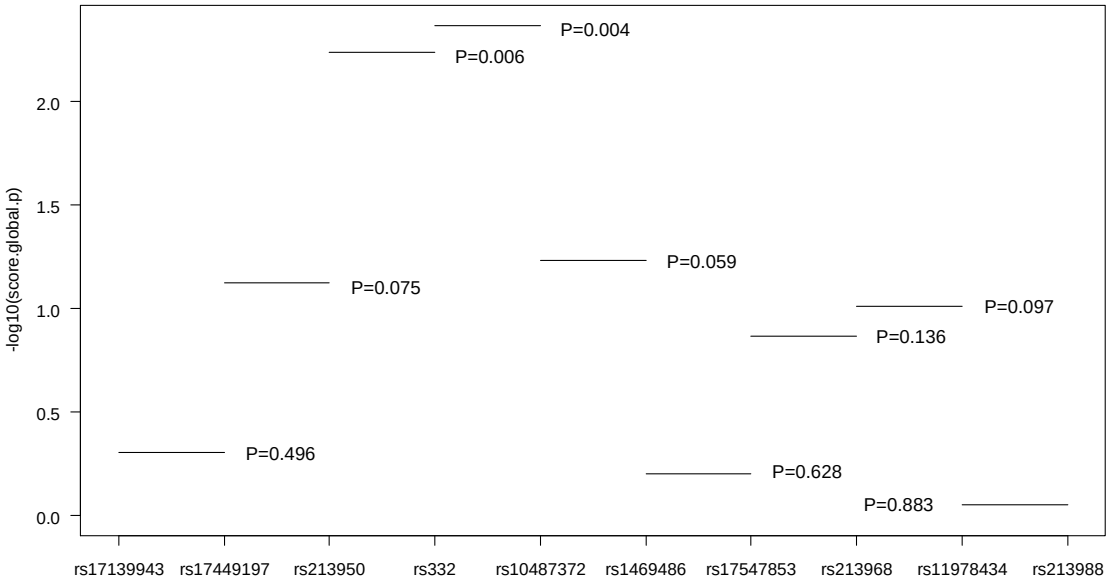
P=0.026
P=0.032
P=0.095
P=0.182
P=0.255
P=0.456
-log10(score.global.p)
2.0
1.5
1.0
0.5
0.0
rs17139943
rs17449197
rs213950
rs332
rs10487372
rs1469486
rs17547853
rs213968
rs11978434
rs213988

Figure 2 B.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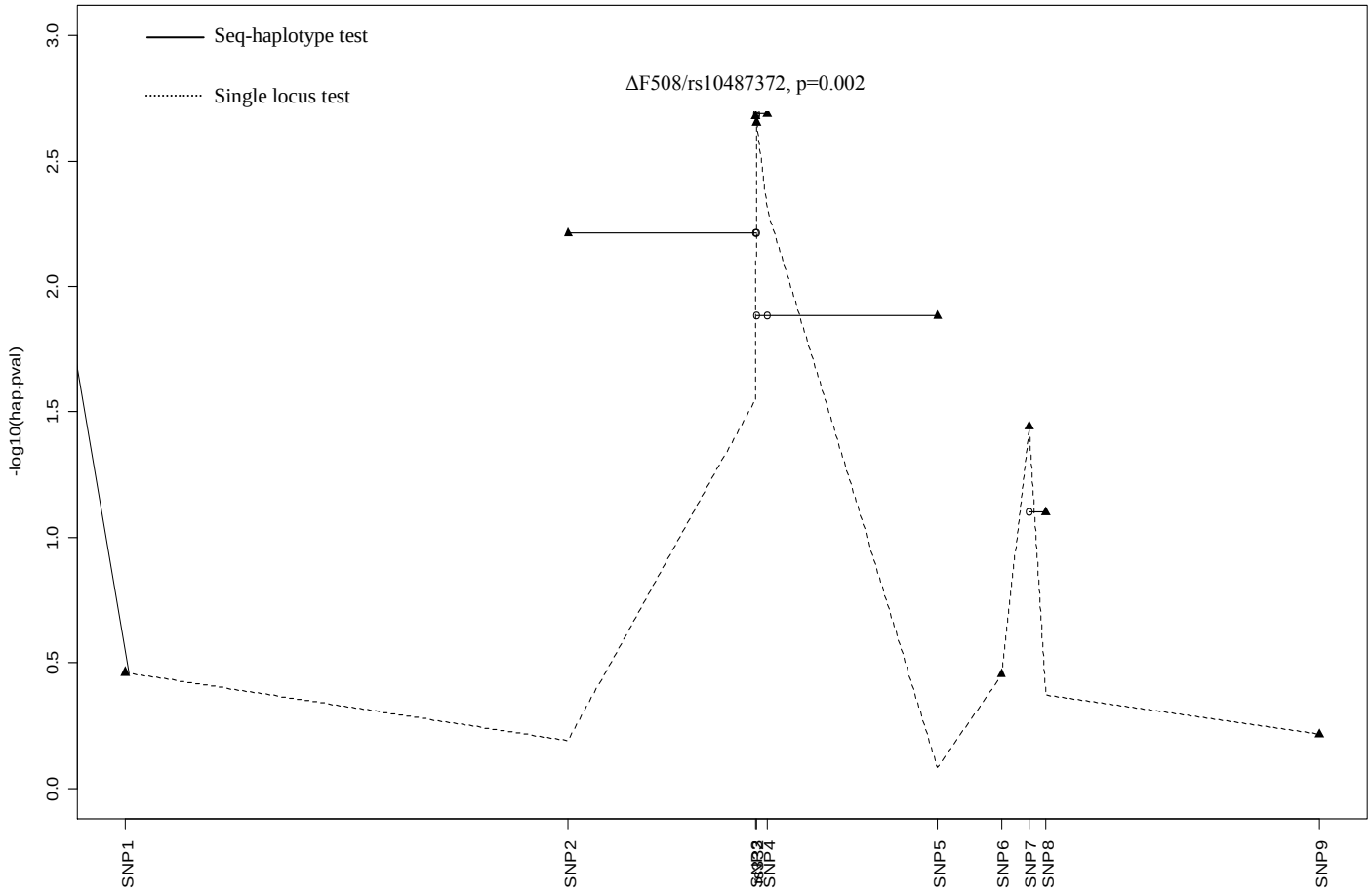
P=0.006
P=0.059
P=0.075
P=0.097
P=0.136
P=0.496
P=0.628
P=0.883
-log10(score.global.p)
2.0
1.5
1.0
0.5
0.0
rs17139943
rs17449197
rs213950
rs332
rs10487372
rs1469486
rs17547853
rs213968
rs11978434
rs213988

Figure 3.


Seq-haplotype test
Single locus test
ΔF508/rs10487372, p=0.002
-log10(hap.pval)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
SNP1
SNP2
SNP3
SNP4
SNP5
SNP6
SNP7
SNP8
SNP9