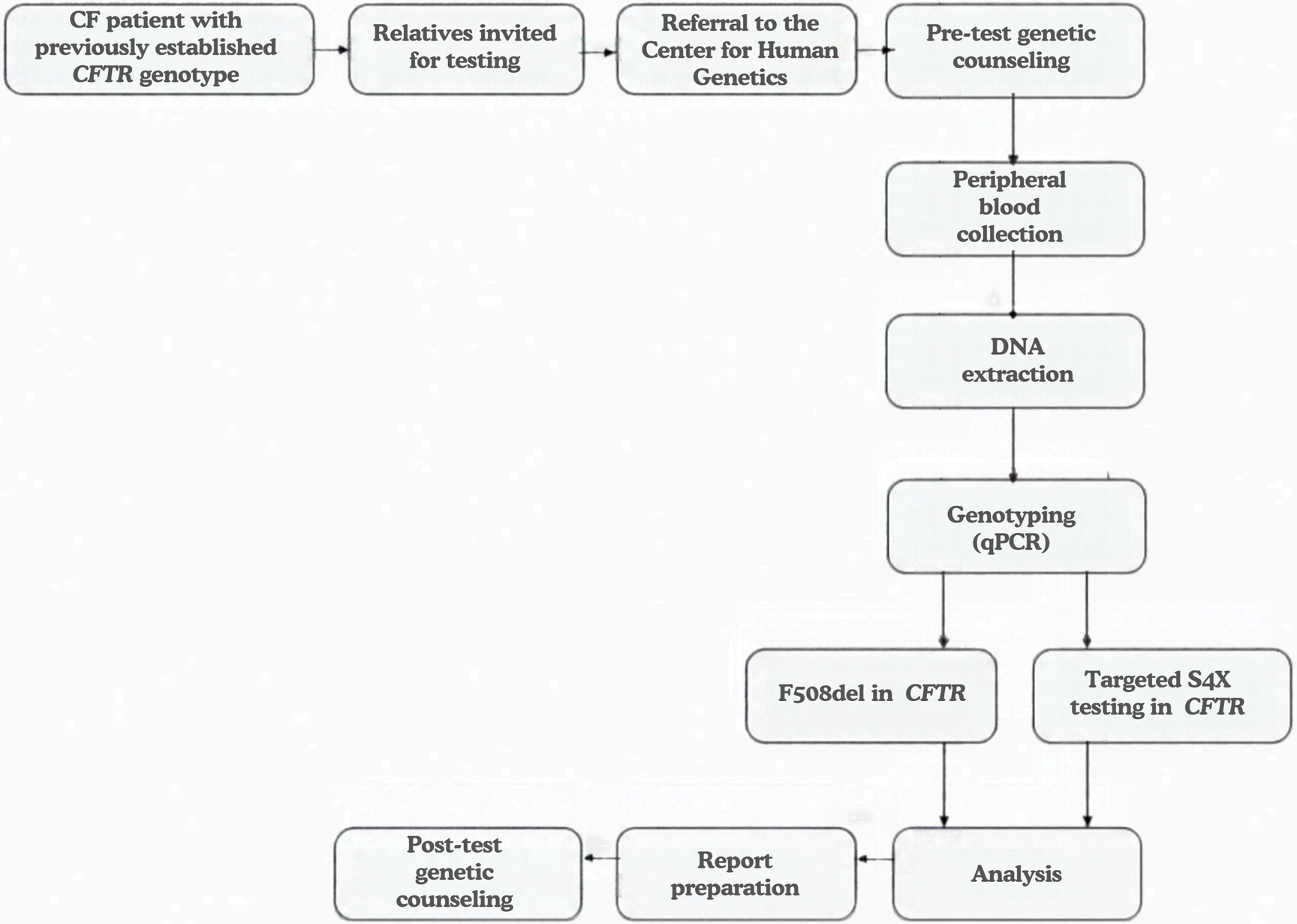
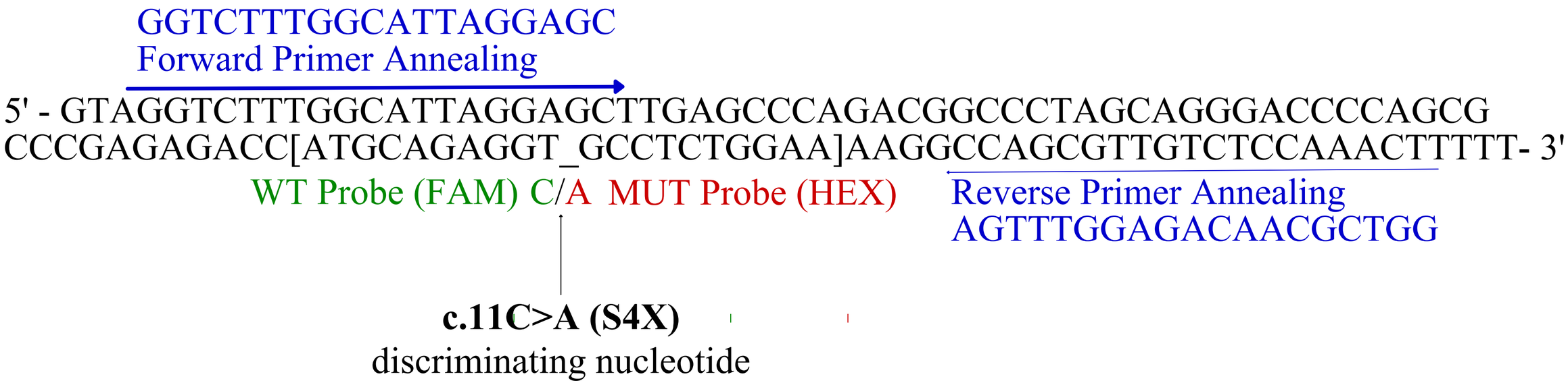
Supplementary Figure S1. Workflow of cascade genetic screening in families affected by cystic fibrosis.

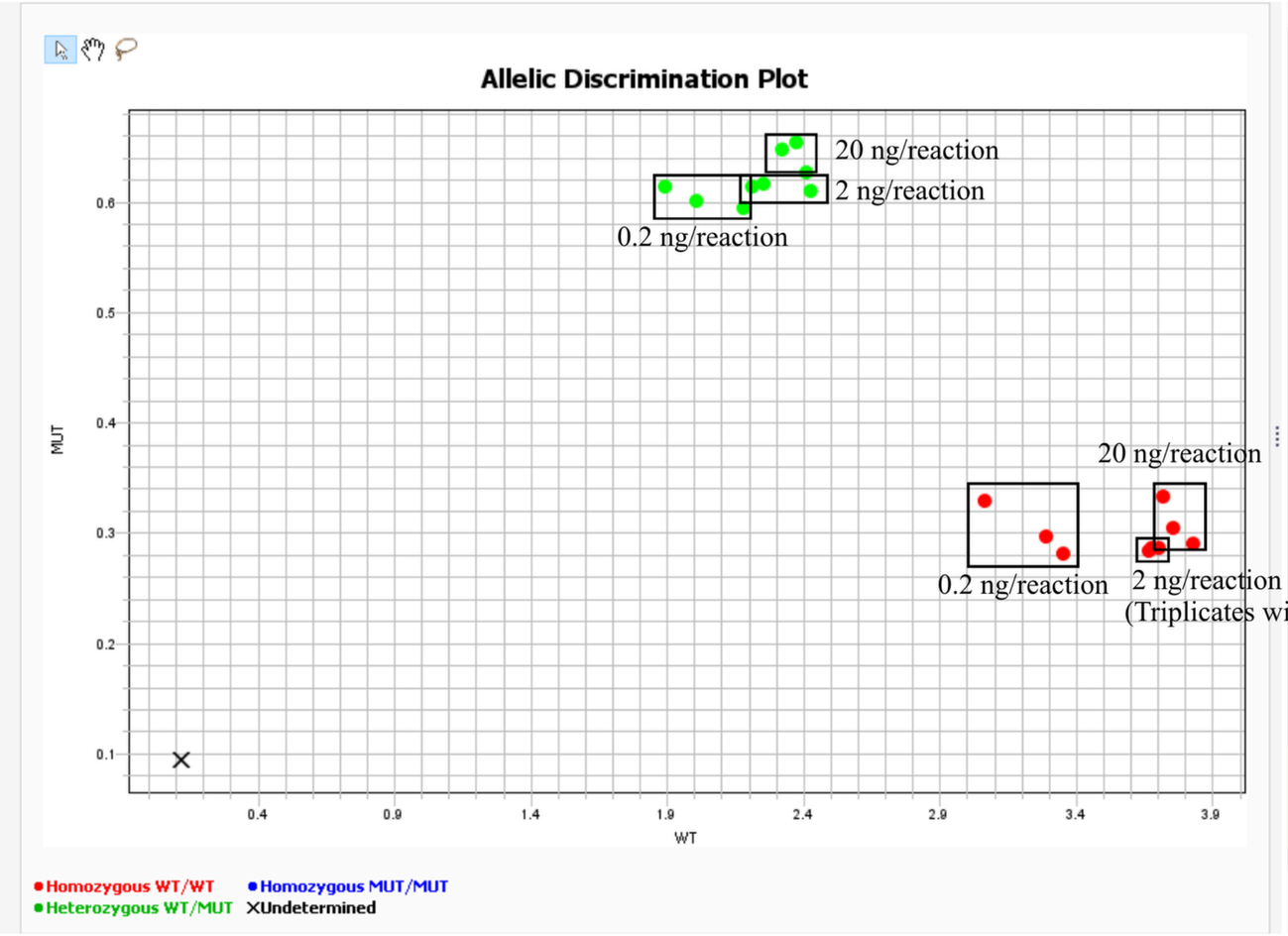


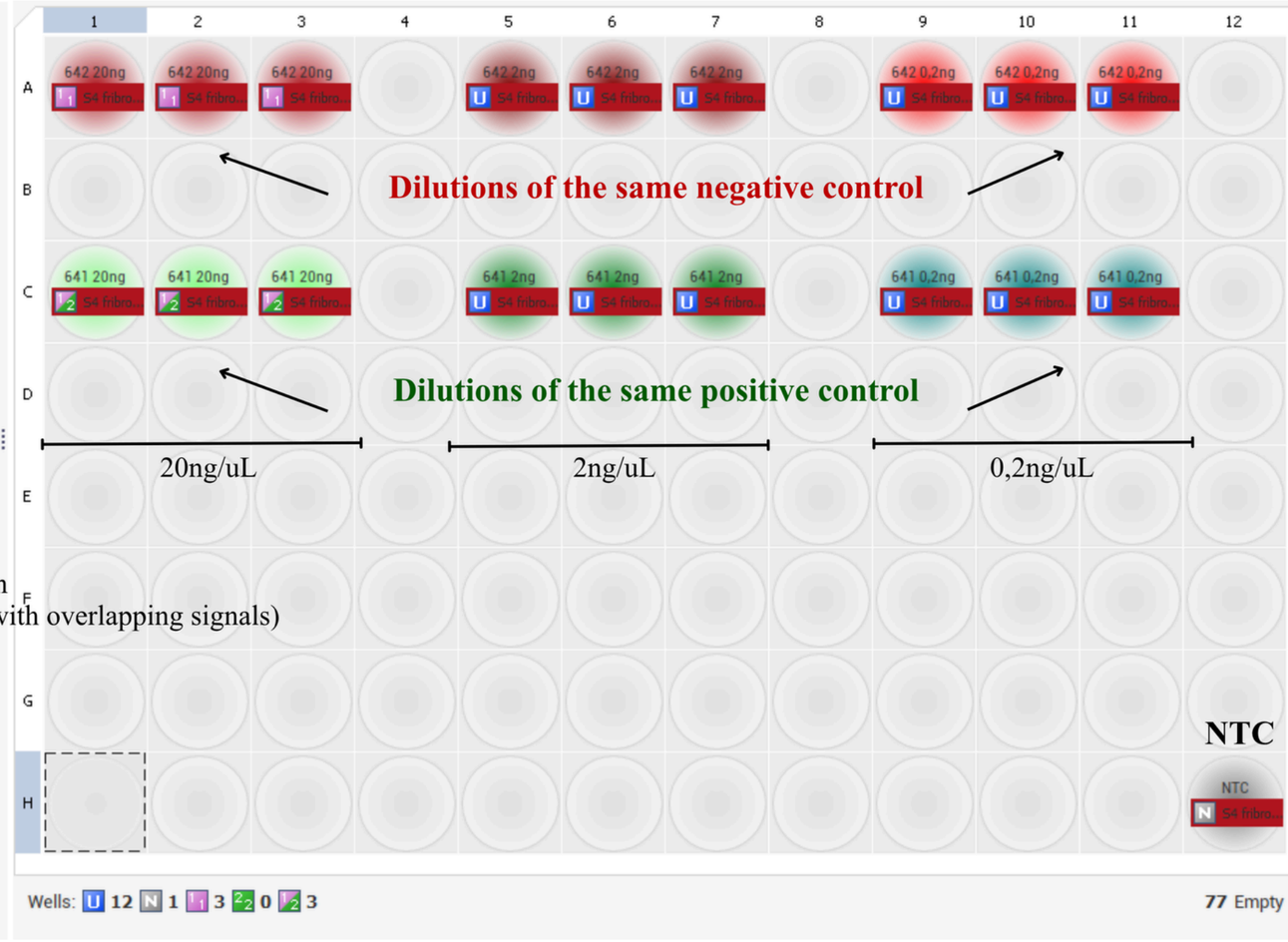
Supplementary Figure S2. Primer and probe binding sites for the *CFTR* S4X variant.



Schematic representation of primer and allele-specific probe binding sites, highlighting the c.11C>A discriminating nucleotide.

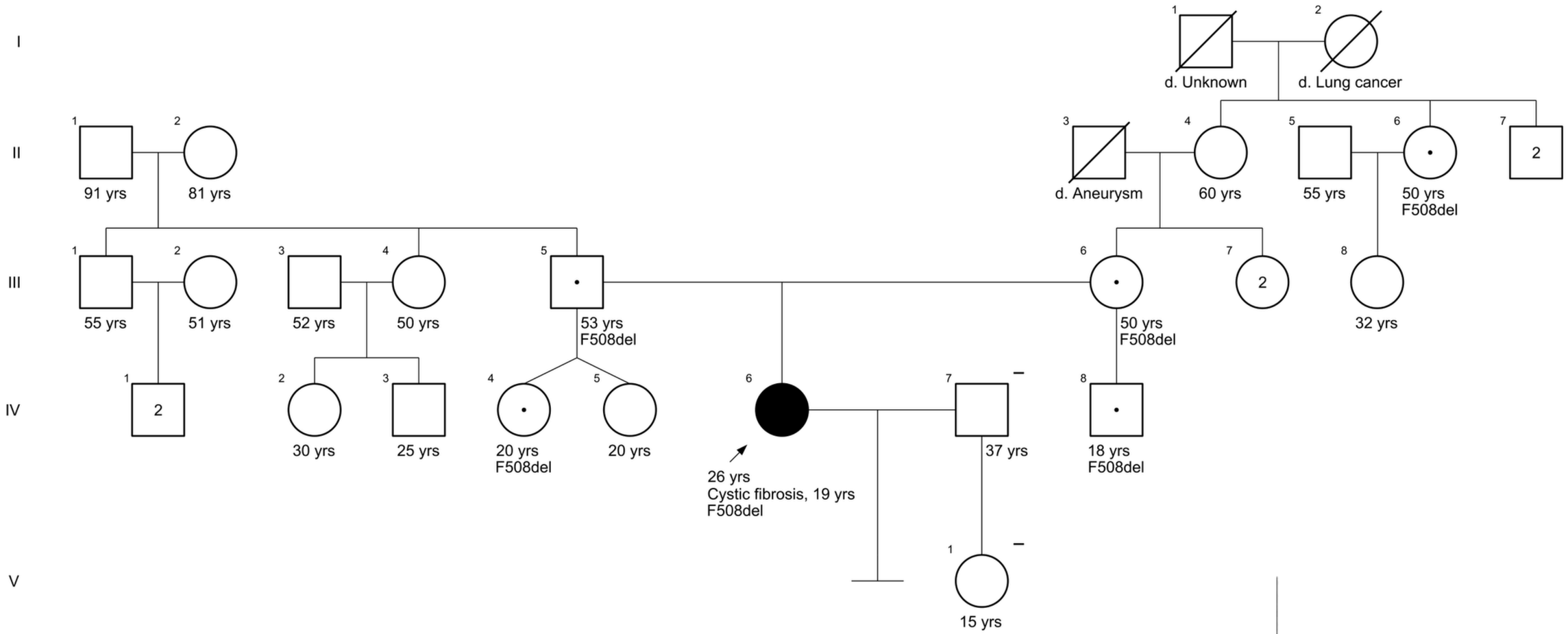
Supplementary Figure S3. Analytical sensitivity of the custom qPCR assay for the *CFTR* S4X variant.



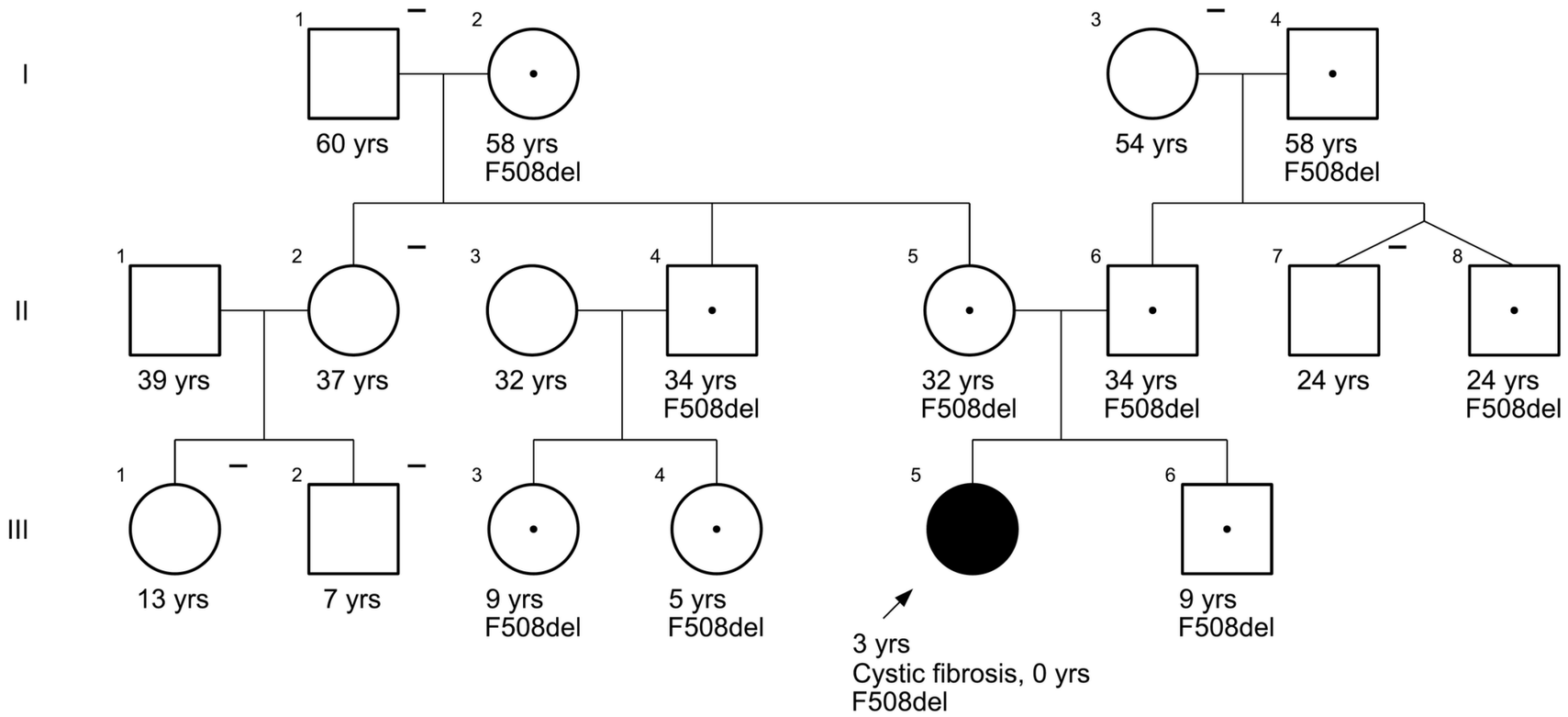


Allelic discrimination plot (left) and qPCR plate layout (right) showing wild-type and heterozygous controls tested in triplicate at three DNA inputs (20, 2, and 0.2 ng per reaction).

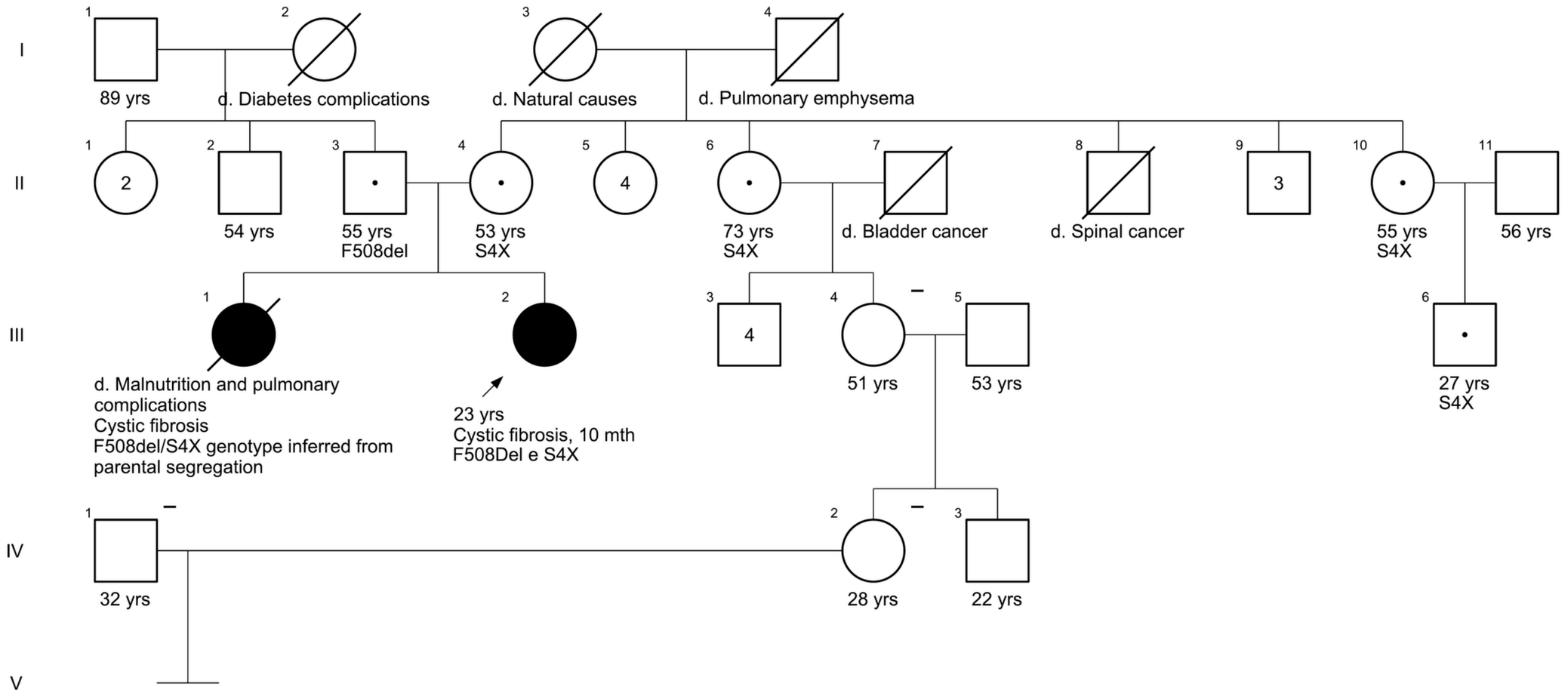
Supplementary Figure S4A. Pedigree of Proband 1



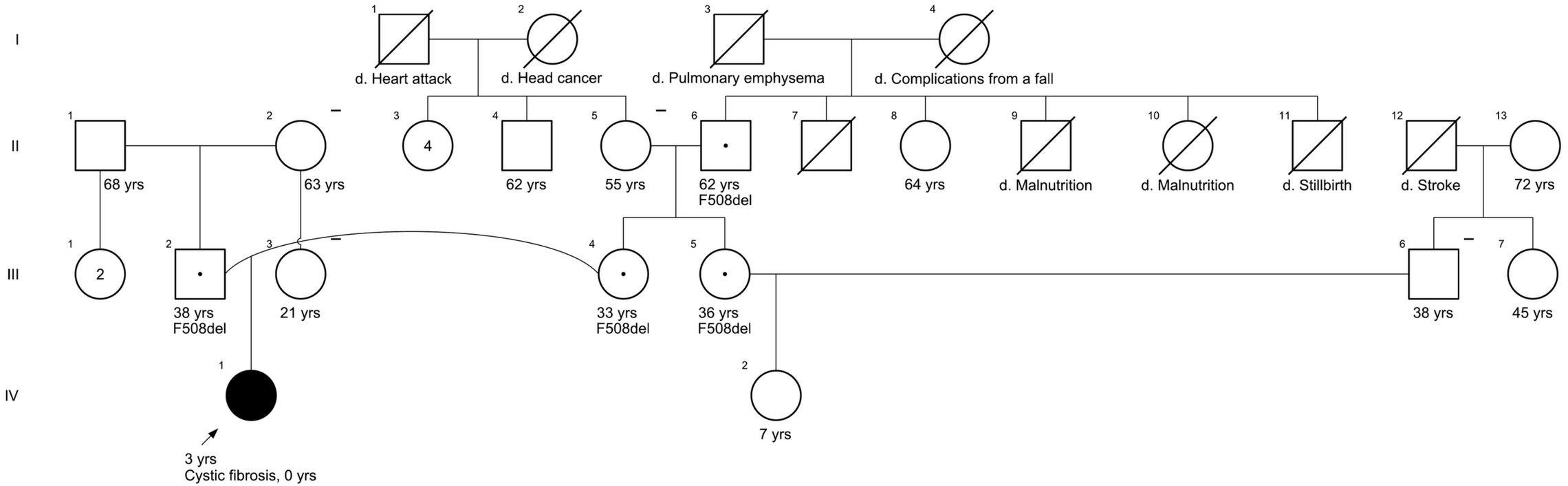
Supplementary Figure S4B. Pedigree of Proband 2



Supplementary Figure S4C. Pedigree of Proband 3



Supplementary Figure S4D. Pedigree of Proband 4



Individuals

Male Female

Affected

Unaffected

Genetic test result

Carrier of a *CFTR* pathogenic variant

Tested negative for the investigated *CFTR* variant(s)