SYNTHESIZED MULTIPLE CONTROL DNA SEQUENCES OPTIMIZE GENOMIC TEST QUALITY CONTROL.

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Principle

Highly reliable molecular genetic analysis requires simultaneously analyzing controls for all possible tested sequences. Given total genomic template DNA from a single individual from any species or infectious organism to be tested or DNA clones with all sequences to be assayed, primers can be selected and used to PCR amplify any previously reported sequence. Synthesized products can be spliced together by PCR amplification and cloned into vectors using restriction sites synthesized in flanking primer sequences.

Synthesized control genetic disease sequences can provide standards for any reported mutation or polymorphism no matter how rare or unavailable.

Synthesized control infectious disease sequences can also be amplified by PCR primers selected from total genomic sequences that are noninfectious. Multiple noninfectious sequences can be ligated, cloned, and distributed with recommended PCR primer sequences for widespread distribution without regard for security.

Proof of Principle

We synthesized 30 homozygous mutations and 3 homozygous cystic fibrosis variants on fragments >400 bp in length. These included:

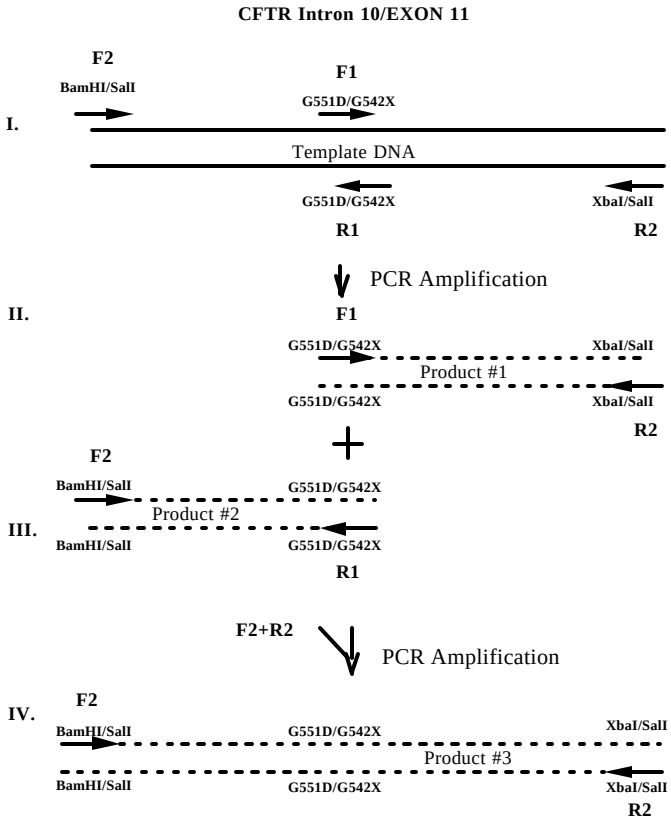
(1) All 25 mutations recommended in the core cystic fibrosis panel on 17 fragments including four cystic fibrosis mutations previously unavailable through any source (2184delA, 1078delT, 1898+1G->A, and I148T),

(2) Seven mutations on two different exonic fragments that were spliced together and cloned into pUC19.

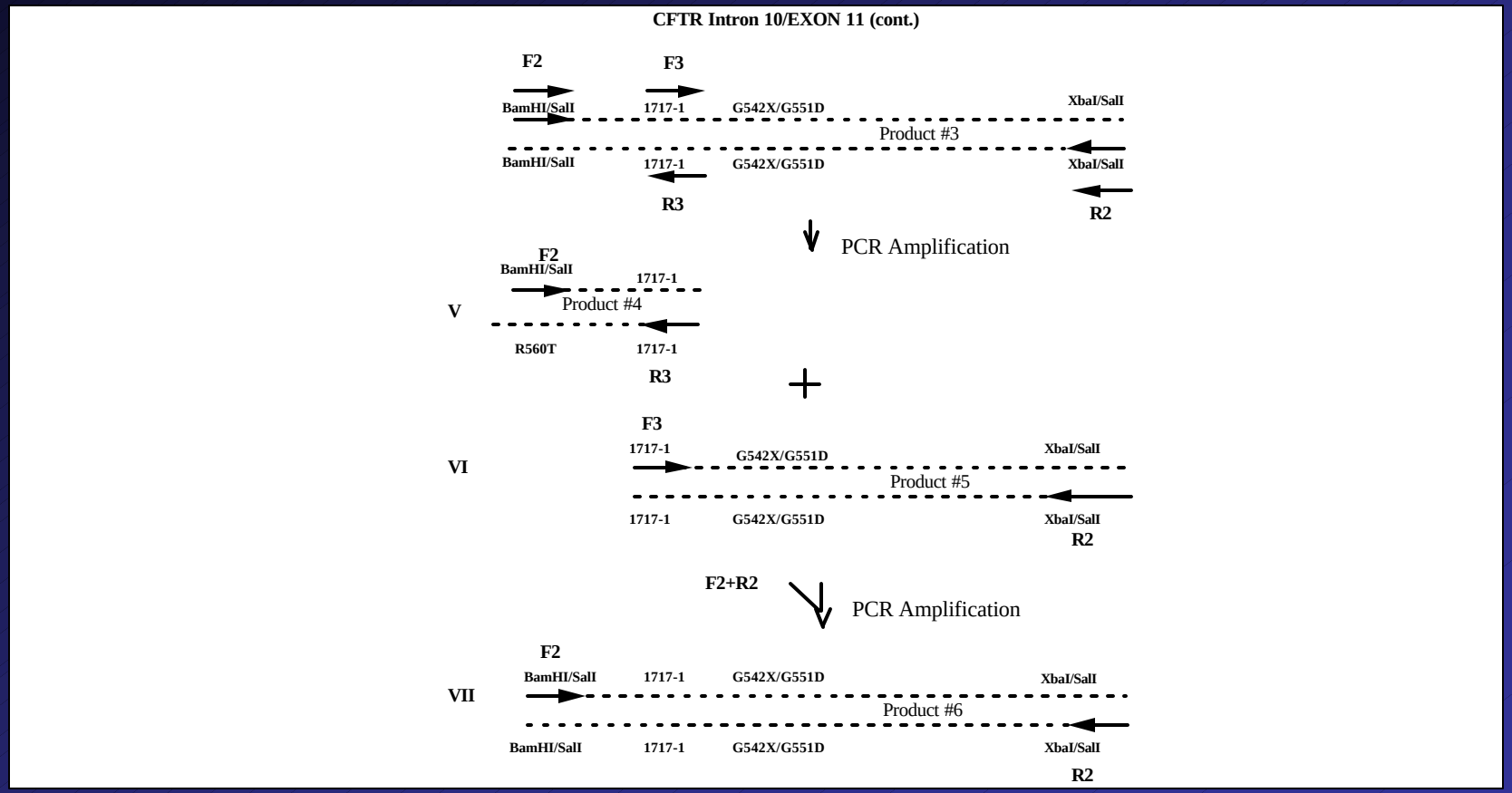
(3) All four recommended reflex test controls.

(4) Currently our laboratory amplifies 30 homozygous cystic fibrosis controls with 3 PCR multiplex reactions and analyzes the products on 2 Innogenetics test strips with each patient test run

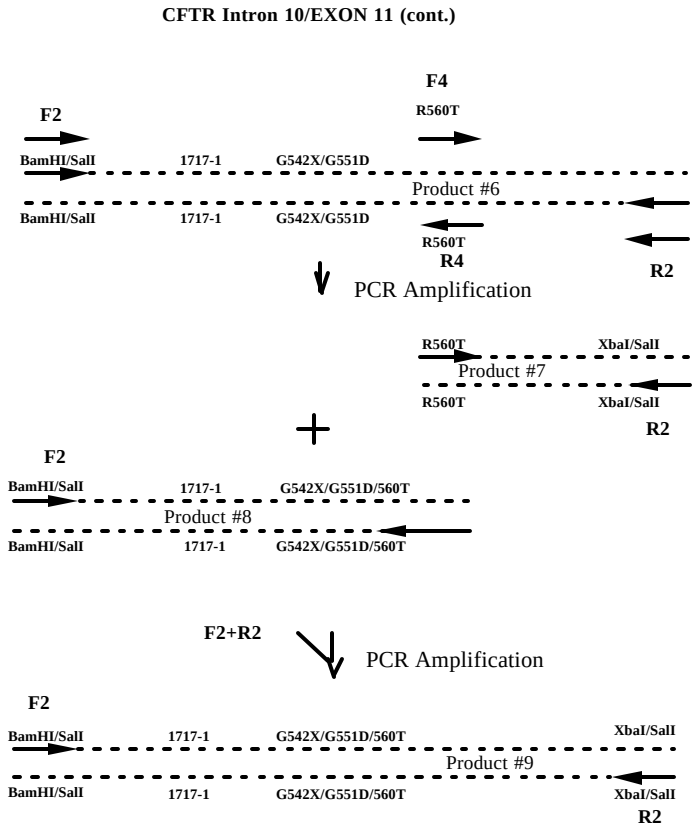
2 Homozygous Controls



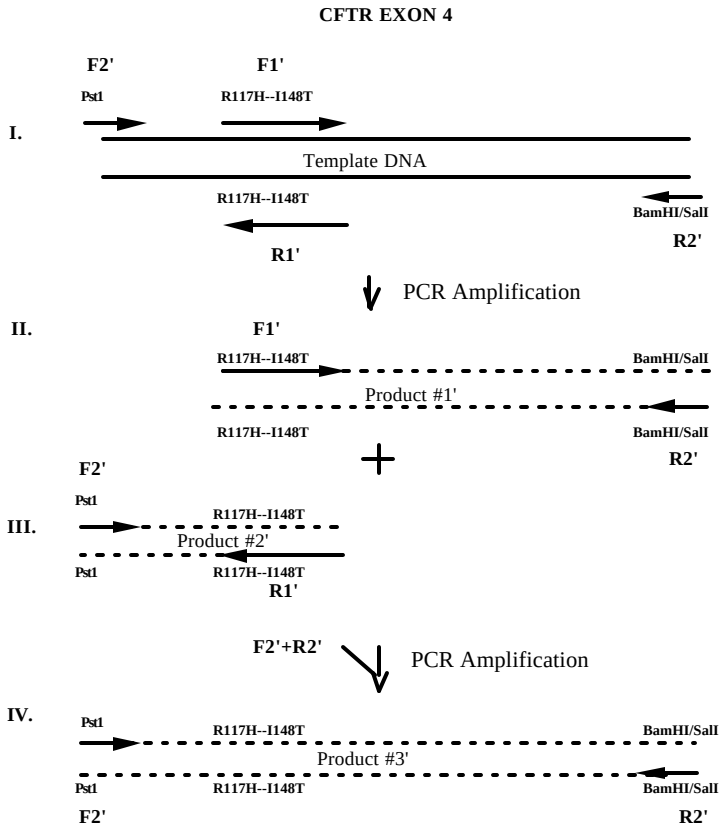
3 Homozygous Controls



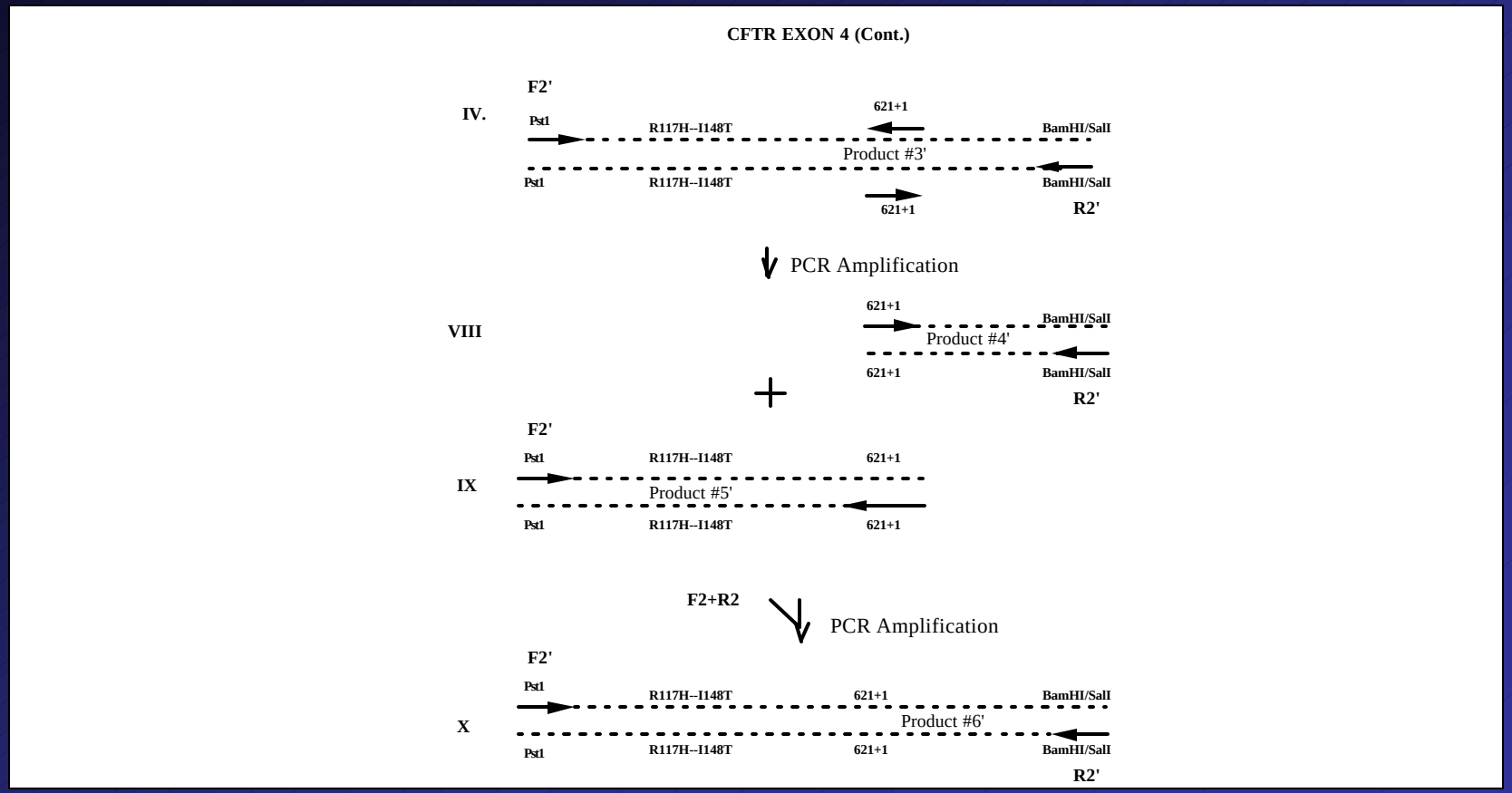
4 Homozygous Controls



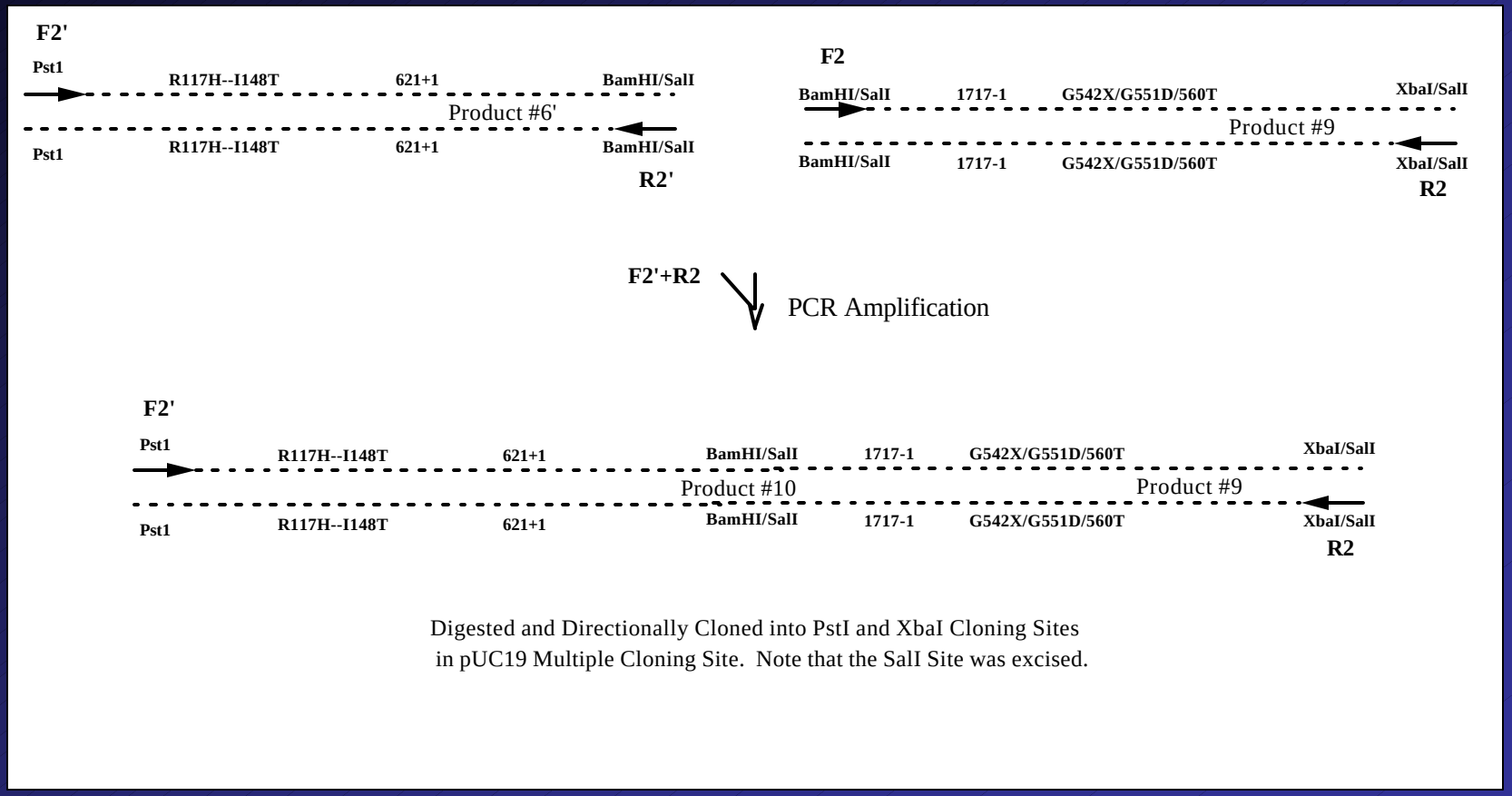
2 Homozygous Controls Fragment #2



3 Homozygous Controls Fragment #2

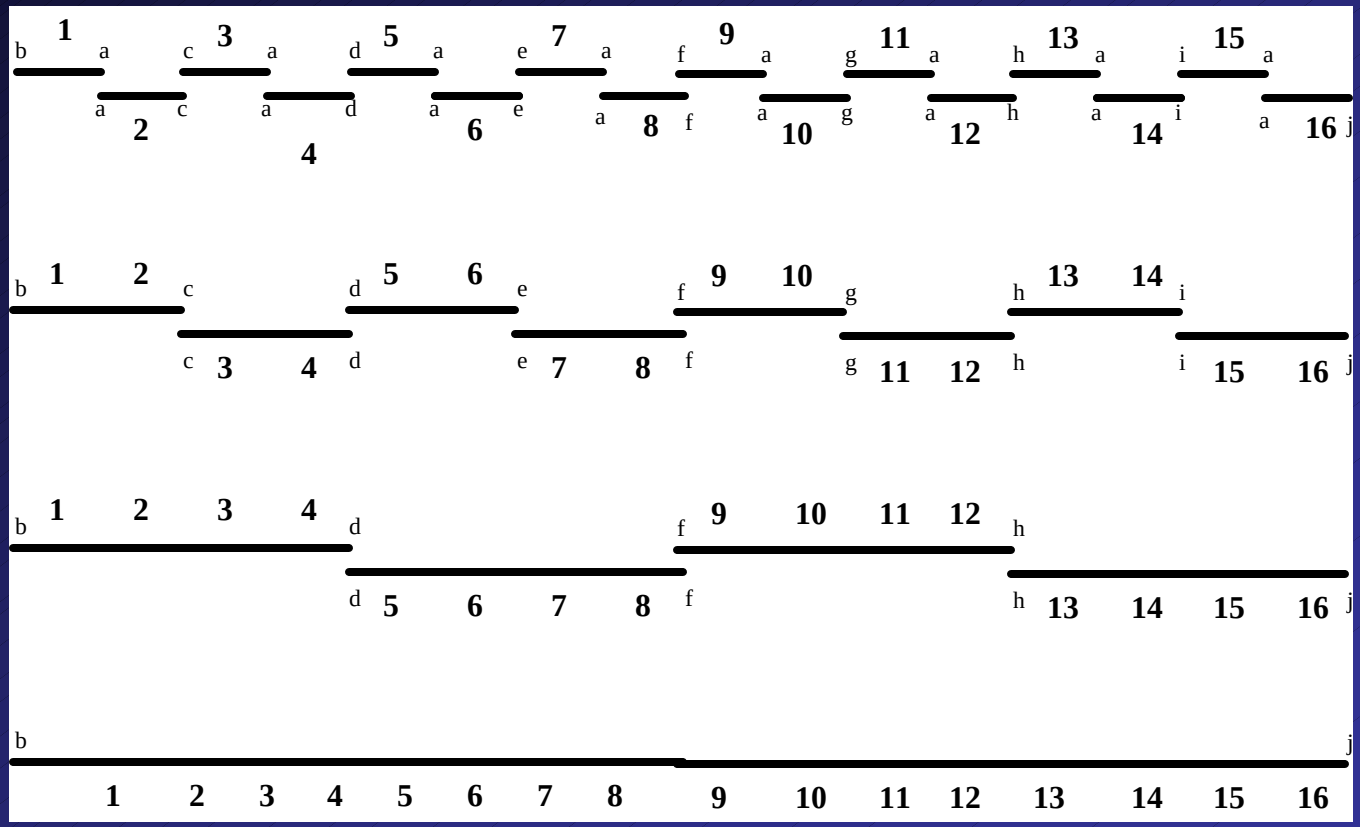


1 Fragment
7 Homozygous Controls

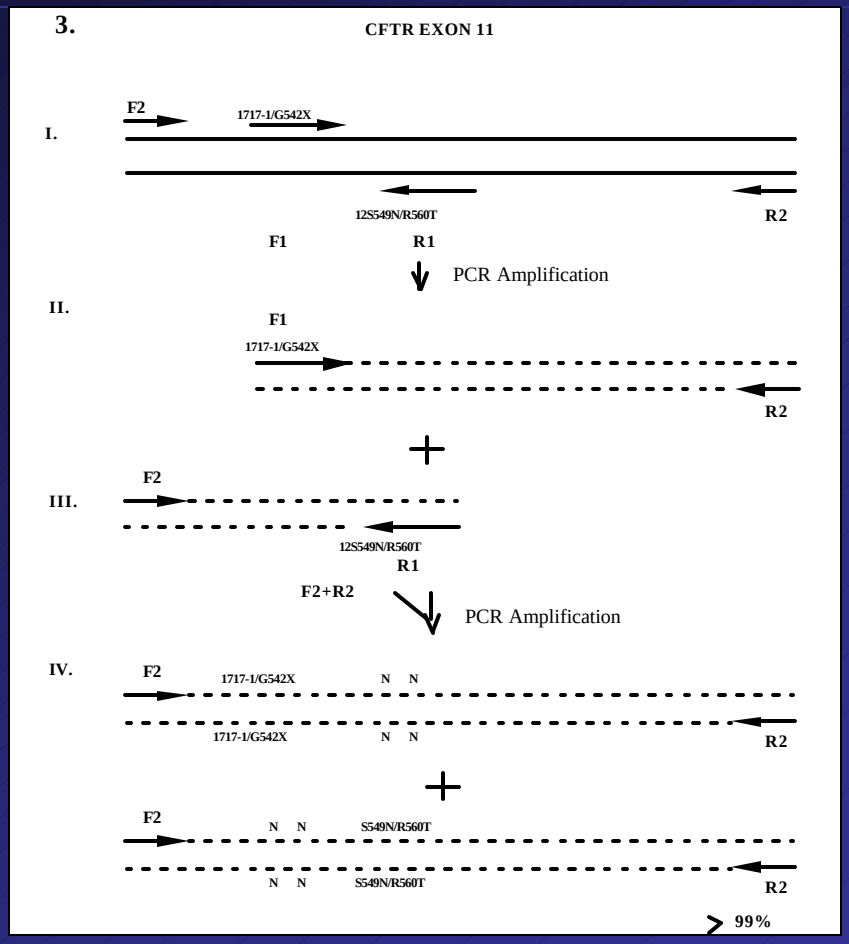


Digested and Directionally Cloned into PstI and XbaI Cloning Sites in pUC19 Multiple Cloning Site. Note that the SalI Site was excised.

Splicing Amplified Fragments



4 Mutation Heterozygote



SUMMARY

- Synthesizing four unavailable cystic fibrosis controls in the recommended core test panel along with all 29 of the additional panel and reflex test mutations and polymorphisms with PCR primers demonstrates the ready ability to synthesize sequences for all applications in the most useful format.*

- APPLICATIONS
- 1. Infectious disease testing: spliced DNA sequences selected from conserved, noninfectious sites in infectious genomes.
- 2. Genetic testing: mutant and polymorphic controls.
- 3. Selected trinucleotide repeat length alleles for Huntington;s disease, other neuropathies, and other genes with simple sequence repeats (SSRs).
- 4. Identity and genealogy testing: polymorphic controls.

Applications

- 1. Multiple homozygous mutant sequences on the same single control DNA sequence.
- 2. Multiple heterozygous mutant sequences on different control DNA sequences.
- 3. Splicing multiple sequences together in any orientation from different locations with sufficient flanking regions for the test procedure.
- 4. Synthesizing multiplex controls for multiplex tests.
- 5. Cloning spliced fragments for ready maintenance and large scale distribution.

Advantages

- 1. Not limited by availability of rare organisms.
- 2. Uses multiplex controls in multiplex tests that streamlines quality control by minimizing the number of control assays required. This saves time and costly formatted test materials.
- 3. Facilitates production and distribution of large quantities by growing cloned spliced fragments.
- 4. Noninfectious fragments from disease organism genomes can be distributed without regard for safety and security.

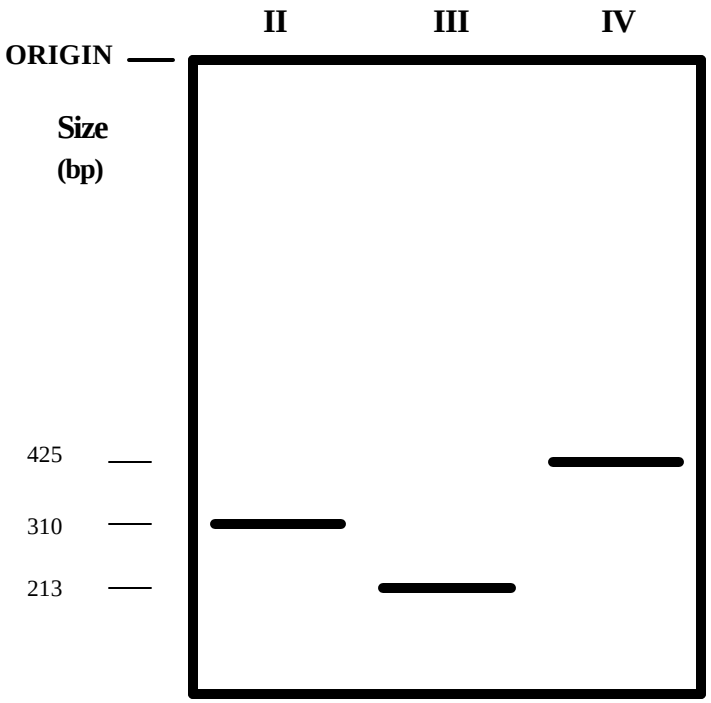
Marketability

(1) Akron Children's Hospital would prefer that a company skilled in synthesizing, validating, and distributing molecular genetic products would produce and market controls.
    (A) A company selling controls is unlikely to encounter FDA rules that prevent marketing complete commercial kits without prior approval as controls would not represent a complete kit but merely a single product that improves upon the quality control home brew assays and commercial test kits.
    (B) Market is broad:
        (1) All alleles commonly tested in dozens of human genetic disease loci. Typically obtaining and maintaining these controls is half of the effort in introducing any new genetic disease test in the Molecular Genetics laboratory.
        (2) Identity and parentage testing for humans with SNPs, and di- and tetranucleotide polymorphisms. Selected di- and tetranucleotide repeat numbers can be synthesized to provide the most optimal intratest comparison so that all laboratories could report the results to the same number of repeats.
        (3) Parentage testing for animals with economically important pedigrees like race horses, which are tested routinely in Australia.
        (4) Identifying the source of DNA from any animal, plant, bacteria, or virus.
        (5) Given the approval of the CDC, providing noninfectious multiplex sequence controls from infectious organisms such as anthrax distributed by terrorists, small pox which might be distributed by terrorists, HBV and HIV which are tested routinely in hospital laboratories, and the multiple new tests under development being added to infectious disease panels.
    (C) Low set up costs and minimal laboratory space required.
    (D) Opportune time to develop large market share given little apparent competition.
    (E) Anticipated selective patent licensing position.

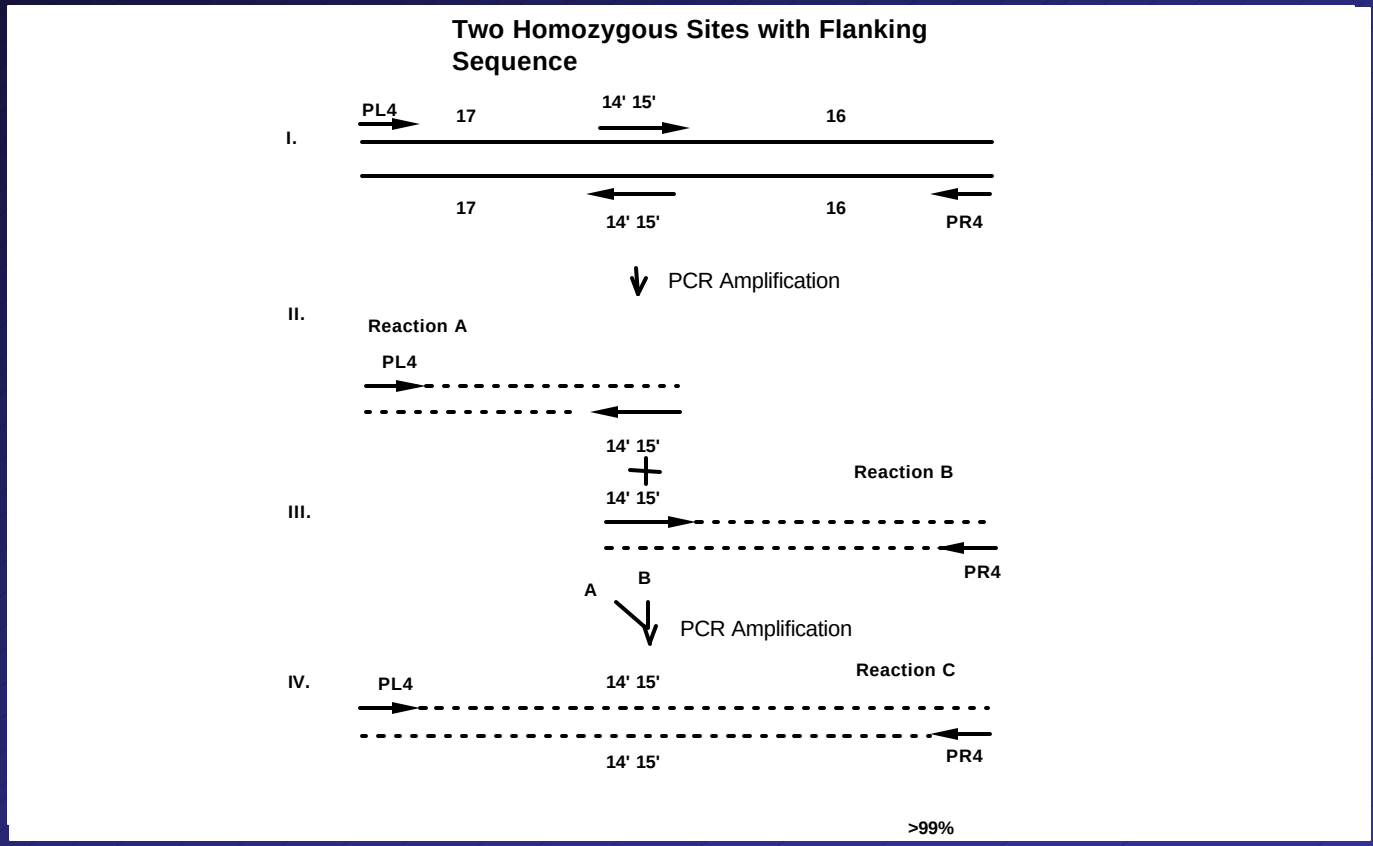
*Patent applied

Electrophoresis of Synthesized Fragments

3 cont. **CFTR EXON 11**
ELECTROPHORESIS



2 Mutation Homozygote



- 2 Homozygous Mutations