XXVII Congresso Italiano della Fibrosi Cistica
XVII Congresso Nazionale della Società Italiana per lo studio della Fibrosi Cistica

Detection and functional characterization of an ALU insertion in the CFTR gene

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UNIVERSITA' DEGLI STUDI DI NAPOLI

FEDERICO II

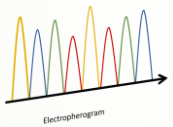
Cystic Fibrosis molecular diagnosis at C.E.I.N.G.E.

1st level

Frequent mutations (Reverse Dot Blot)

2nd level

Single Nucleotide Variations (Sanger sequencing)

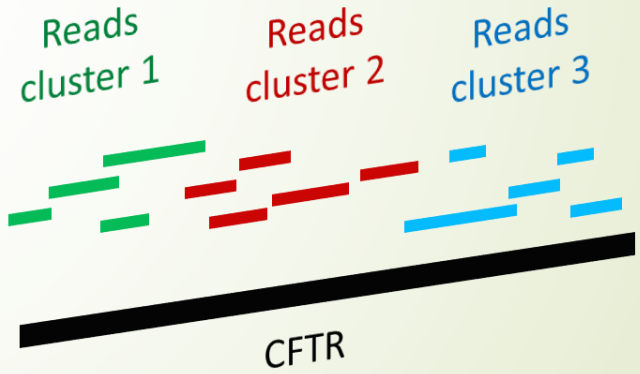


3rd level

Macro-deletions (Reverse Dot Blot)

The *CFTR* genetic testing strategy was classically performed step by step.

Today, the NGS analysis, based on the complete sequencing of the *CFTR* exons and regulatory regions, allows to detect both single-nucleotide variation (SNV) and copy number variation (CNV).



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NGS Technologies

Pros

- Cost effective
- Time saving
- Easy-to-use commercial kits
- Capability to process a large number of samples in parallel

Cons

- Short read lengths
- Need of sufficient coverage
- Big amount of data
- Time-consuming interpretation of data

The analysis of data is a real challenge and requires a multidisciplinary comparison to have a correct interpretation of the results.

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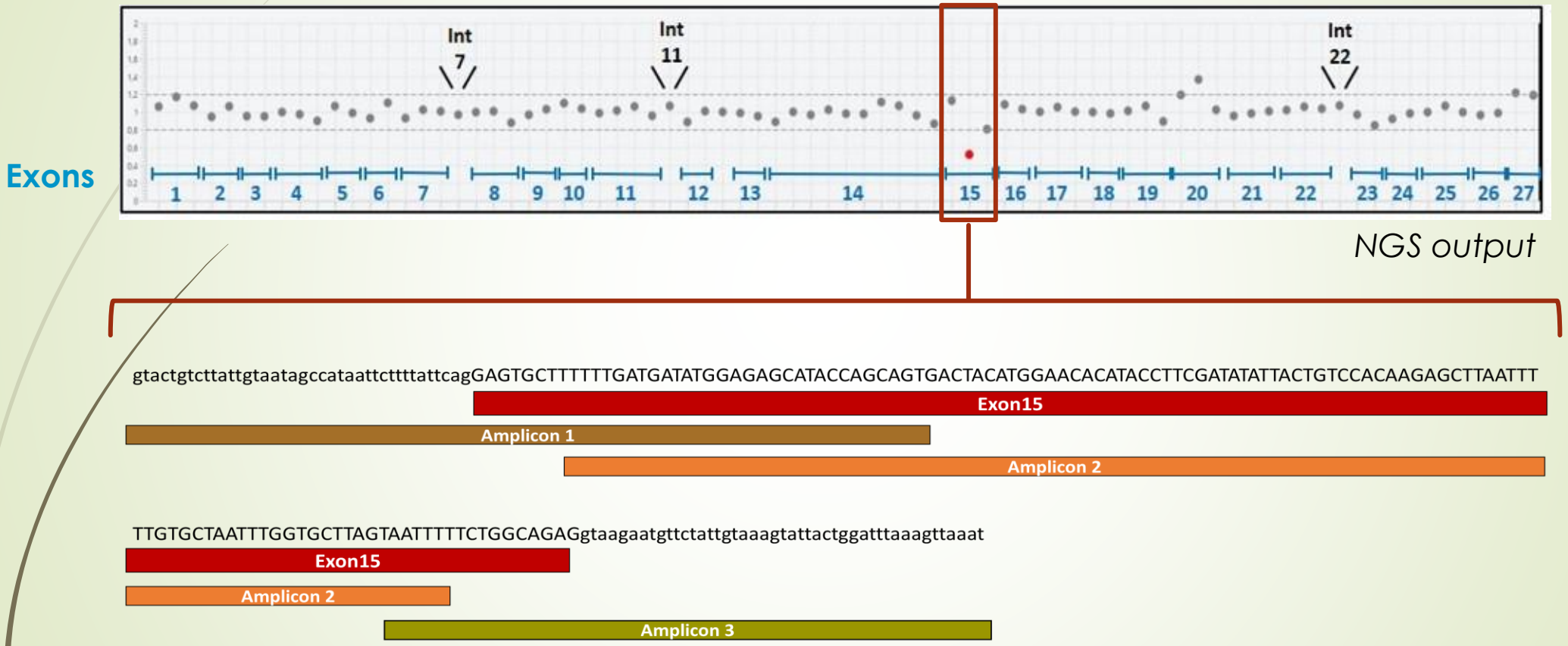
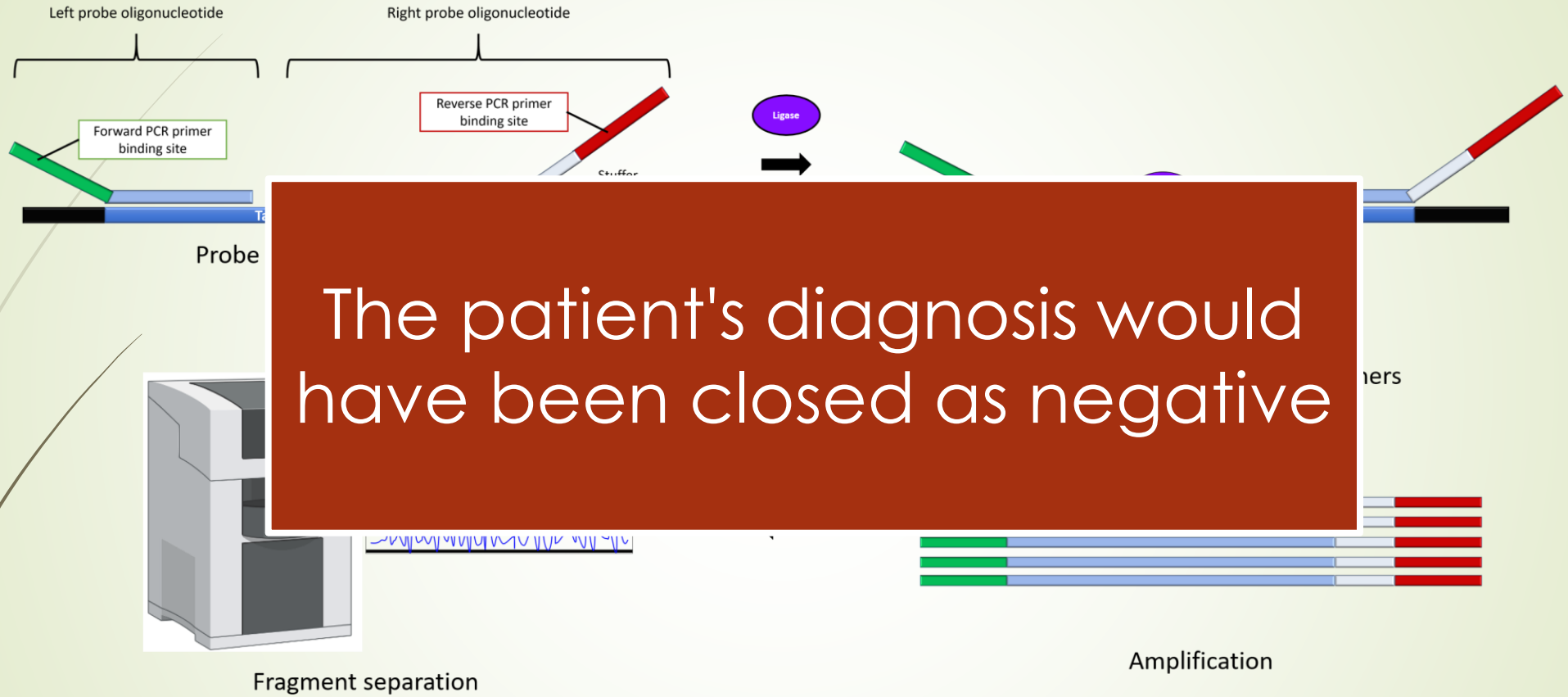
Case Report

Step 1. NGS



The software showed a *score warning* at the level of the amplicon 2 of exon 15, thus suggesting a possible deletion.

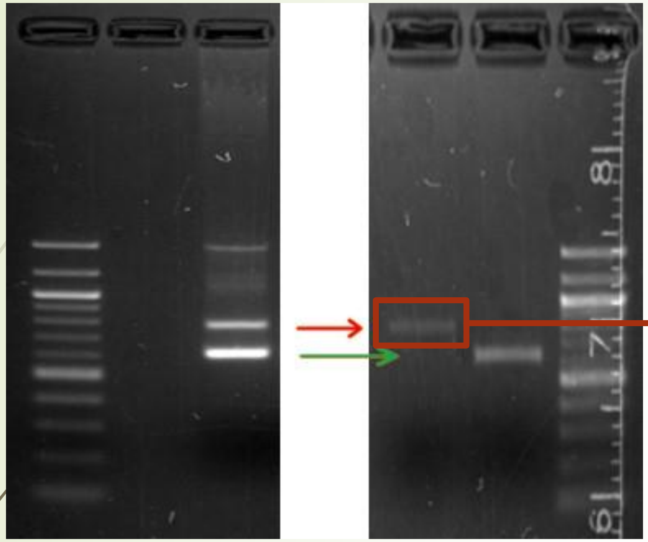
Step 2. MLPA

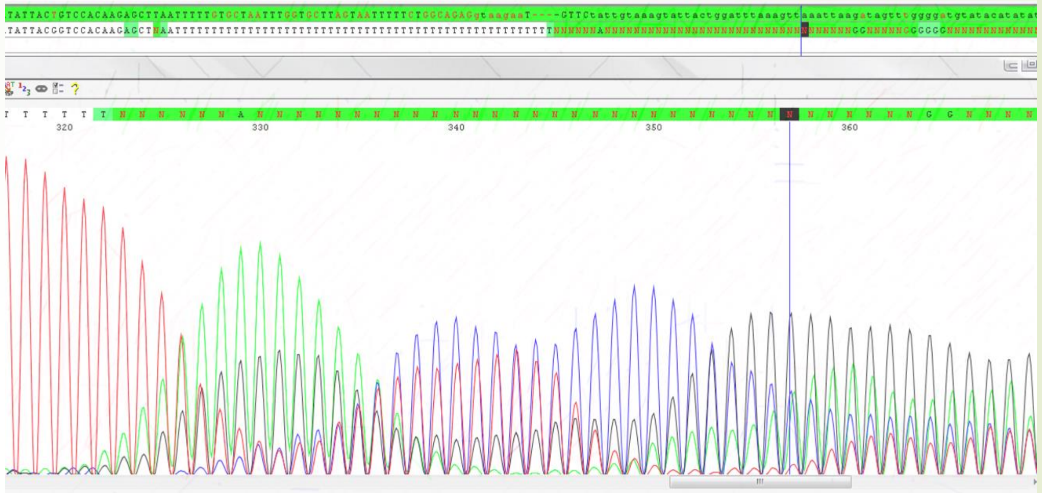


The patient's diagnosis would have been closed as negative

MLPA analysis did not show any deletion at the level of exon 15 of the *CFTR* gene.

Step 3. Sanger and Nanopore sequencing





gtactgtcttattgtaatagccataattcttttattcagGAGTGCTTTTTTGATGATATGGAGAGCATACCAGCAGTGACTACATGGAACACATACCTTCGATATATTACTGTCCAC**AAGAGCTTAA**

Exon15

TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGAGACGGAGTCTCGCTCTGTCGCCCAGGCCGGACTGCGGACTGCAGTGGCGCAATCTCGGCTCA

Exon15

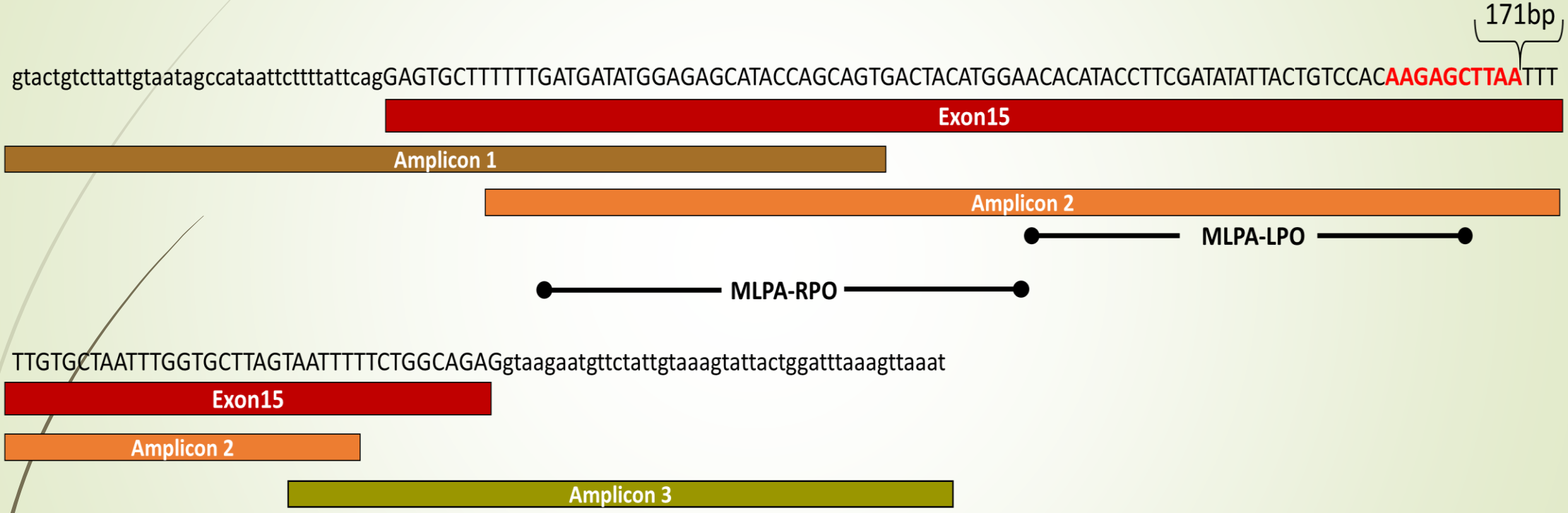
Insertion

*CTGCAAGCTCCGCCTCCCGGGTTCACGCCATTCTCCTGCCTCAG**AAGAGCTTAA***TTTTTGTGCTAATTTGGTGCTTAGTAATTTTTCTGGCAGAGgtaagaatgttctattgtaaagtattact

Exon15

A deep analysis by Sanger and Nanopore sequencing showed an insertion of 171bp in the exon15 of *CFTR*.

Why we couldn't see any insertion?



The insertion falls downstream of the target binding site of the MLPA-probes.

Step 4. Blast Analysis

Homo sapiens AluYb9 repeat region; and disrupted pyruvate kinase L/R (PKLR) gene, exon 6

Sequence ID: MG677548.1 Length: 633 Number of Matches: 1

Range 1: 426 to 586 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
292 bits(158)	3e-75	160/161(99%)	0/161(0%)	Plus/Minus

```
Query  1    tttttttttttttttttttttttttttttttttttttttttttttttttttttttGAGACGG  60
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  586  TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGAGACGG  527

Query  61   AGTCTCGCTCTGTCGCCCAGGCCGGACTGCGGACTGCAGTGGCGCAATCTCGGCTCACTG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  526  AGTCTCGCTCTGTCGCCCAGGCCGGACTGCGGACTGCAGTGGCGCAATCTCGGCTCACTG  467

Query  121  CAAGCTCCGCCTCCCGGGTTCACGCCATTCTCCTGCCTCAG  161
            |||||||||| ||||||||||||||||||||||||||||||
Sbjct  466  CAAGCTCCGCTTCCCGGGTTCACGCCATTCTCCTGCCTCAG  426
```

The BLAST analysis revealed the homology of the insertion found in *CFTR* with the AluYb9 element, previously found in *PKLR* gene.

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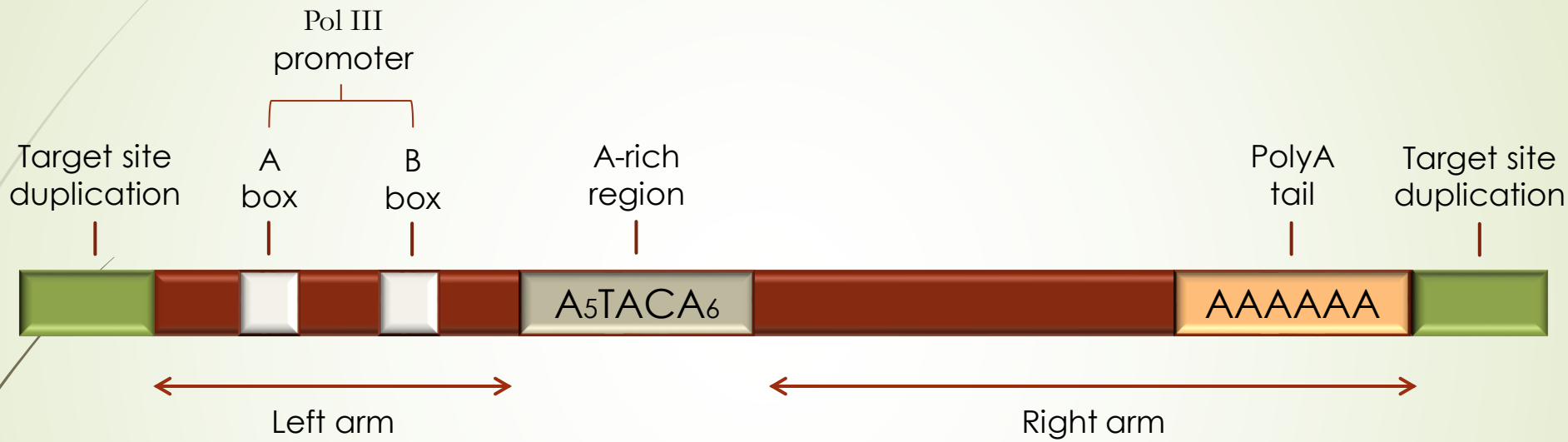
Alu elements



Alu elements are short interspersed elements (SINEs) of approximately 300 nucleotides in length. Despite their being genetically functionless, recent findings suggest that Alu elements may affect gene structures, protein sequences, splicing motifs and expression patterns.

Alu in the *CFTR* gene

Detection of two *Alu* insertions in the *CFTR* gene

Jian-Min Chen [a,b,*], Emmanuelle Masson [a,c], Milan Macek Jr. [d,e], Odile Raguénès [a,f], Tereza Piskackova [d,e], Brigitte Fercot [a,f], Libor Fila [e,g], David N. Cooper [h], Marie-Pierre Audrézet [a,f], Claude Férec [a,b,c,f]

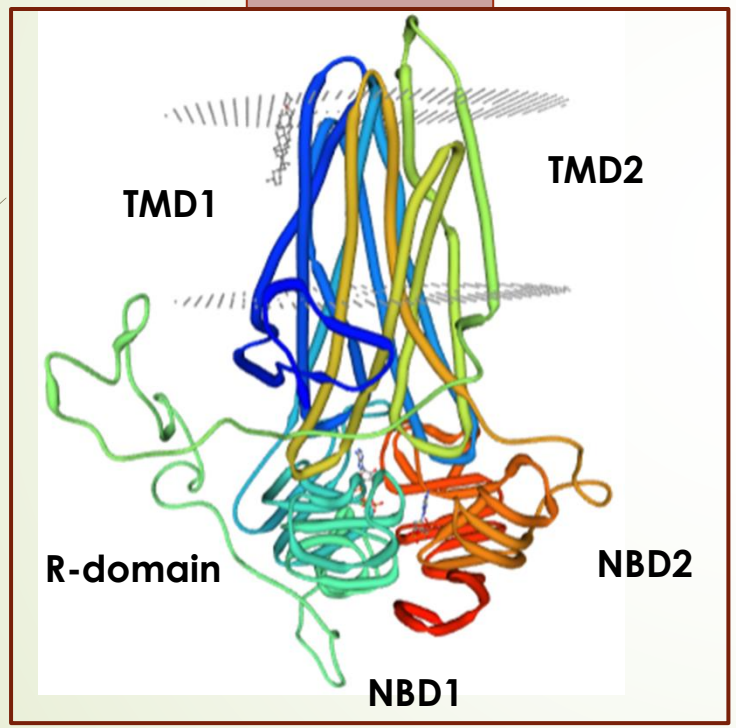
Published in 2008

	Integration site	Alu sub-family	Lenght	Lenght of Poly(A) tail	Orientation of insertion	Size of target site duplication
#1	Exon 18	Y	103bp	57bp	Antisense	18bp
#2	Exon 20	Ya5	337bp	56bp	Sense	19bp
#3	**Exon 15**	**Yb9**	**171bp**	**53bp**	**Antisense**	**10bp**

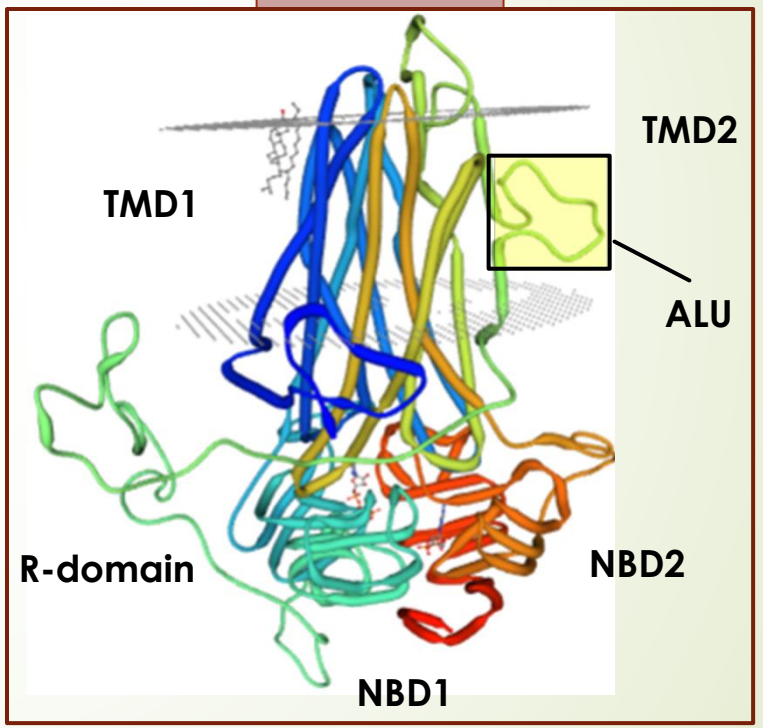
We identified the 3rd Alu insertion in the *CFTR* gene.

Step 5. *In silico* prediction

CFTR WT

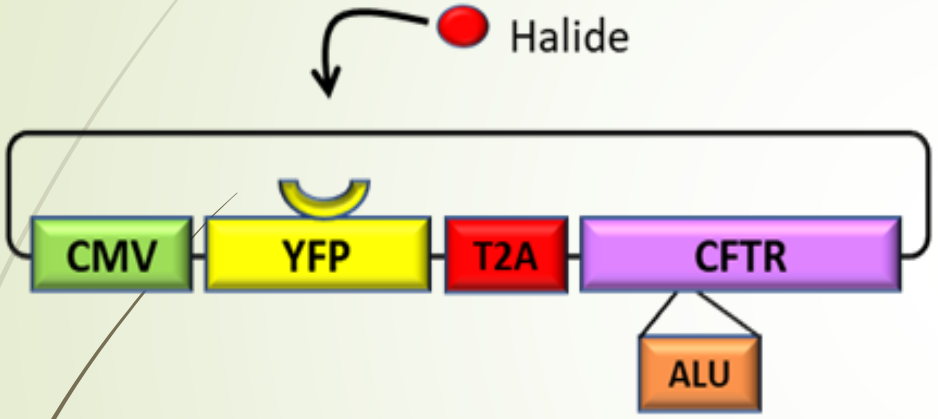


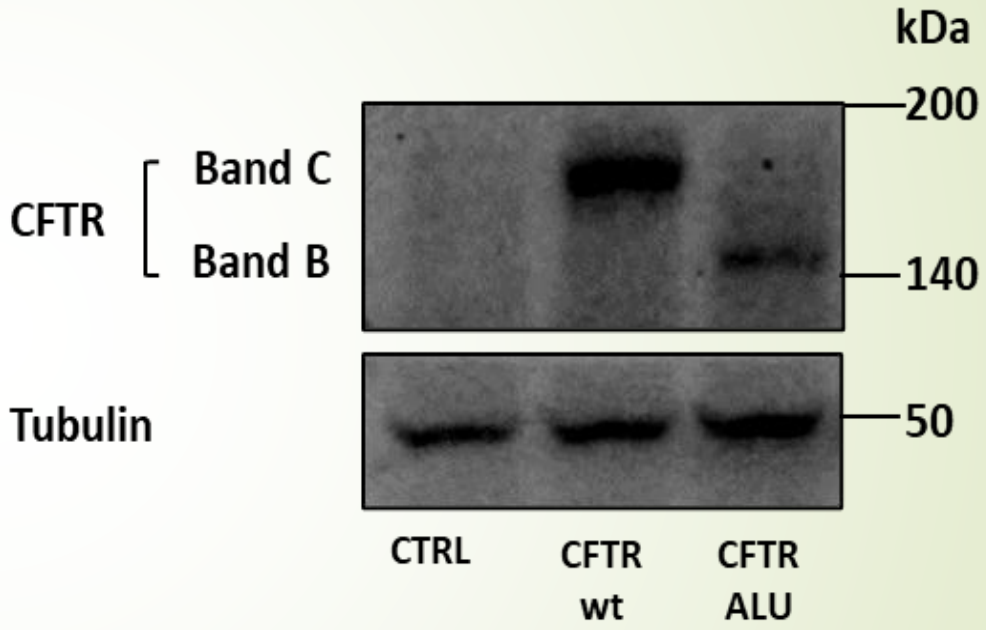
CFTR ALU



Protein structure modelling performed by Swiss-model.

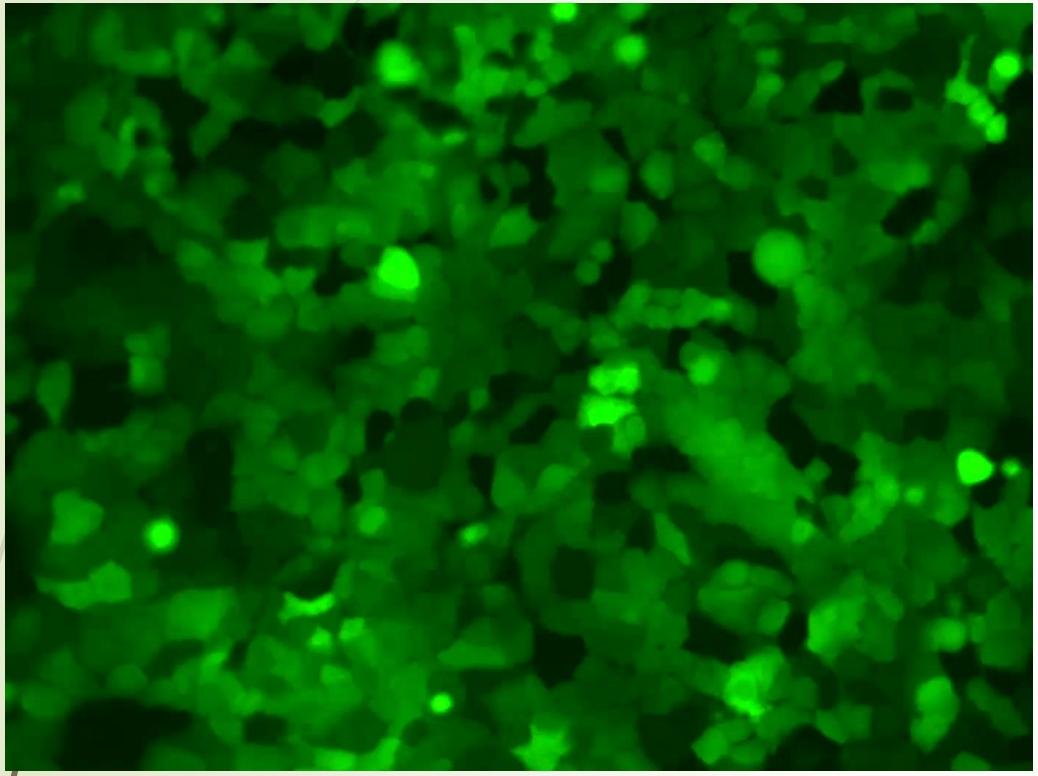
Step 6. Molecular characterization

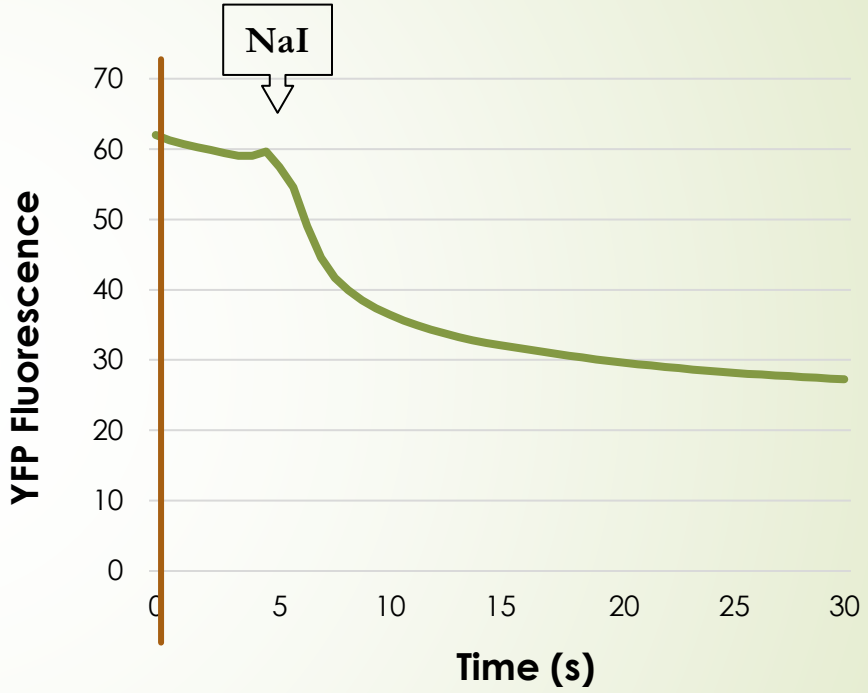




Western Blot analysis showed that only an immature form of CFTR channel could be found in the cells when the Alu insertion is present.

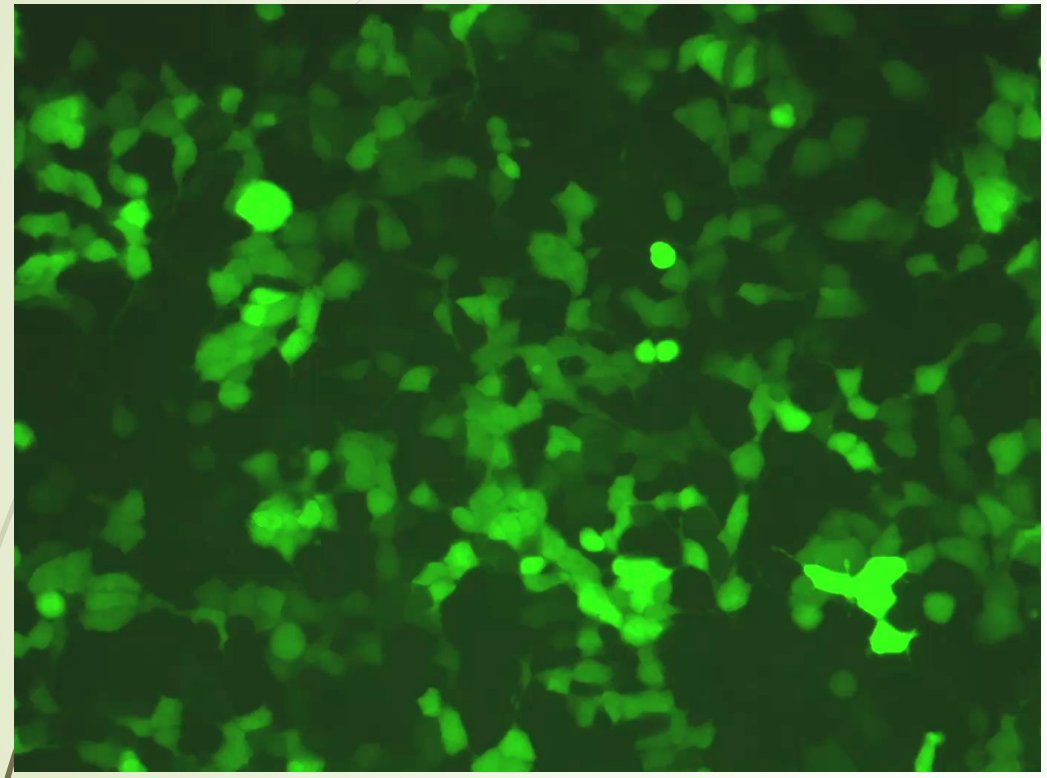
Step 7. Functional characterization

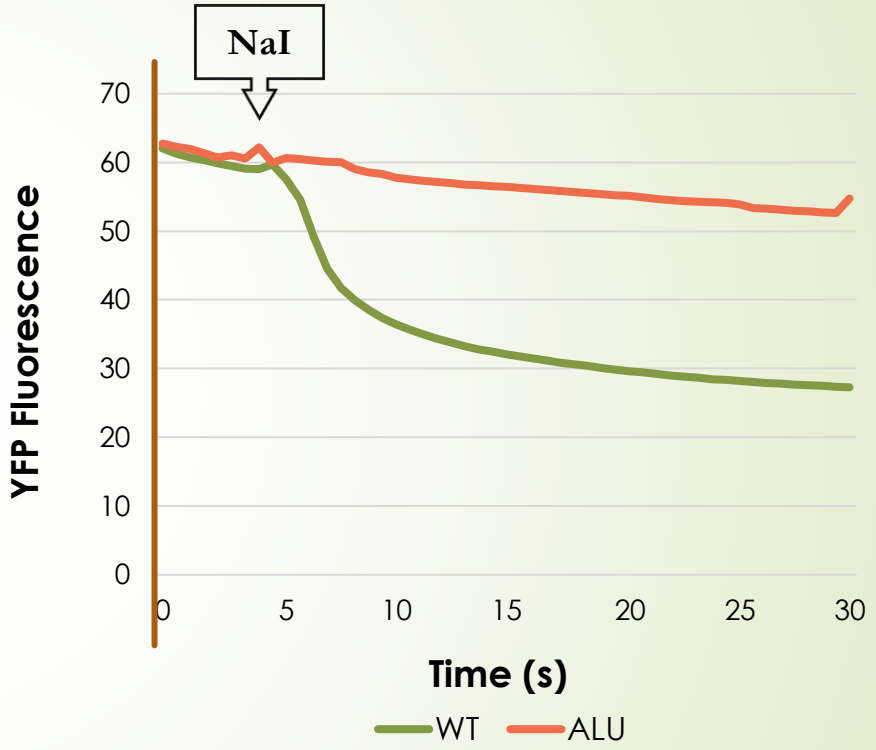




In the presence of high extracellular concentration of iodide (I^-), and only if CFTR is present at the membrane and gating, I^- can enter the cell and quench YFP fluorescence.

Step 7. Functional characterization





The functional analysis, based on halide-sensitive YFP assay, revealed that the CFTR-ALU protein is unable to function as chloride channel.

Conclusions

- Although NGS has represented a revolution in DNA sequencing, making it possible to process a large number of samples in parallel and detect the most common CF mutations in one reaction, the NGS results need to be carefully interpreted and corroborated by multidisciplinary approaches to have a correct genetic analysis.

- Functional studies play a fundamental role in the Cystic Fibrosis theratyping and personalized medicine.

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