PCR Multiplex

Multiplex Ligation-dependent Probe Amplification(MLPA)

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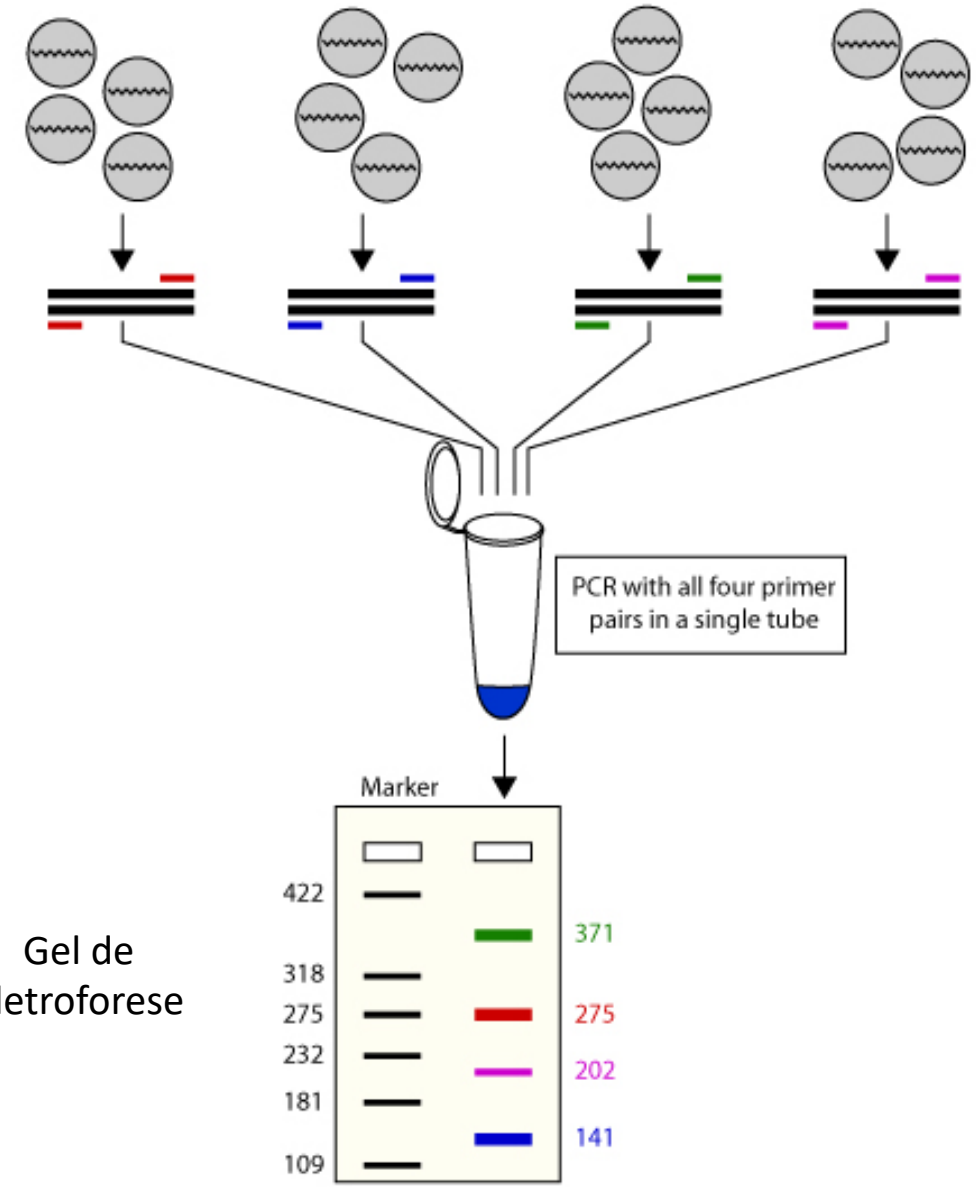
PCR MULTIPLEX



Gel de Eletroforese

VANTAGENS

- Amplificações de vários segmentos de DNA numa única reação
- Poupança de tempo
- Poupança de recursos
- Menor probabilidade de erro exprimental

DESVANTAGENS

- Incompatibilidades da **temperatura de annealing** dos primers escolhidos
- **Ligação não-específica** dos primers

PRIMEIRO ARTIGO CIENTÍFICO REFERENTE AO PCR MULTIPLEX

Volume 16 Number 23 1988 Nucleic Acids Research

Deletion screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification

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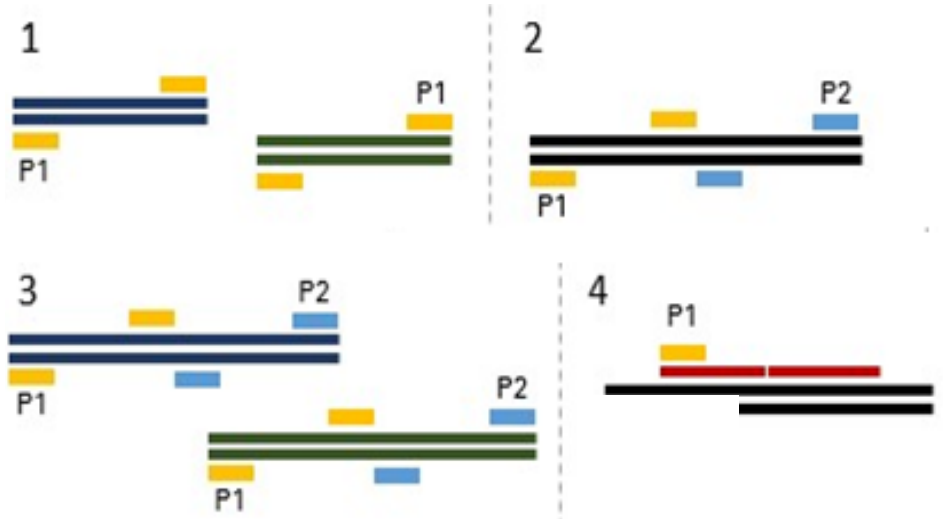
Received August 24, 1988; Revised and Accepted October 28, 1988 Accession nos X13045–X13048 (incl.)

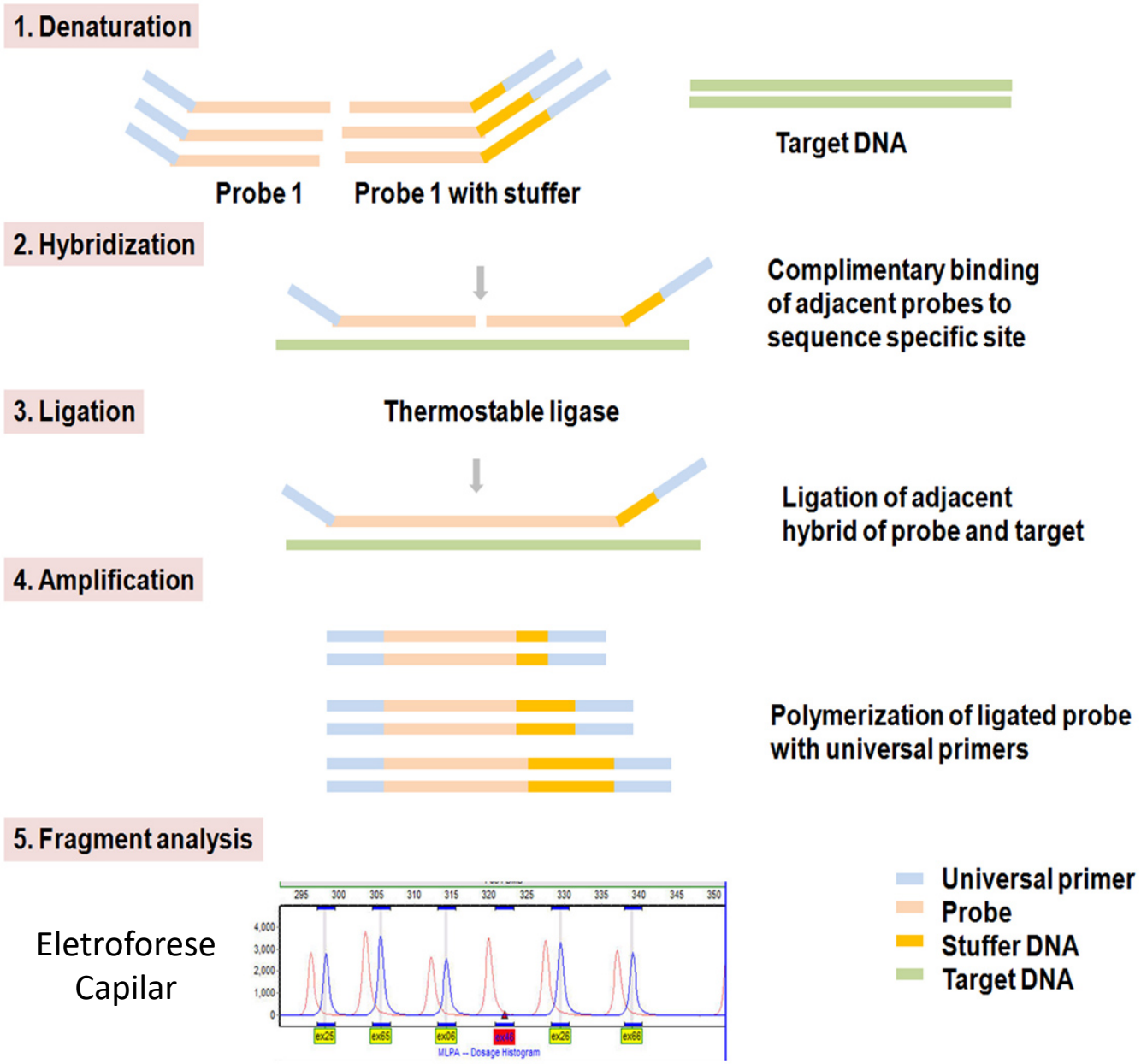
ABSTRACT

The application of recombinant DNA technology to prenatal diagnosis of many recessively inherited X-linked diseases is complicated by a high frequency of heterogenous, new mutations (1). Partial gene deletions account for more than 50% of Duchenne muscular dystrophy (DMD) lesions, and approximately one-third of all cases result from a new mutation (2-5). We report the isolation and DNA sequence of several deletion prone exons from the human DMD gene. We also describe a rapid method capable of detecting the majority of deletions in the DMD gene. This procedure utilizes simultaneous genomic DNA amplification of multiple widely separated sequences and should permit deletion scanning at any hemizygous locus. We demonstrate the application of this multiplex reaction for prenatal and postnatal diagnosis of DMD.



1. **Um** par de primers → **Múltiplos** Templates
2. **Múltiplos** pares de primers → **Um** Template
3. **Múltiplos** pares de primers → **Múltiplos** Templates
4. **Multiplex Ligation-Dependent Probe Amplification** (MLPA)

Multiplex Ligation-dependent Probe Amplification (MLPA)



Vantagens do MLPA em relação ao PCR Multiplex

- Possibilidade de **medições quantitativas**;
- Uso do **mesmo par de primers** para todos os amplicões;
- Sondas não-ligadas não são amplificadas e **evitam a ligação não-específica;**
- Possibilidade de **marcação fluorescente**.