Oncology 2025
The Cancer Control Challenge:
Better Understanding It to Better Face It

COMPARATIVE ANALYSIS OF *IN SILICO* PLATFORMS FOR PRIMER DESIGN IN THE DETECTION OF *ETV6::RUNX1* FUSION IN LEUKEMIA

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Introduction: Leukemia is a type of cancer frequently associated with the presence of gene fusions, which are essential for both the diagnosis and prognosis of the disease. The accurate detection of these fusions relies on the use of specific primers, making primer design a critical step in techniques such as PCR and sequencing. Properly designed primers ensure amplification specificity, preventing nonspecific results, which is crucial, particularly in cases where amplification has diagnostic relevance, such as in the *ETV6::RUNX1* fusion. Thus, selecting tools that combine specificity, efficiency and ease of use for this purpose can be decisive for the optimal construction of the assay. **Objectives:** To evaluate and compare *in silico* platforms used for primer design aimed at detecting gene fusions in oncohematology. **Methods:** This study was conducted entirely *in silico*. The platforms Primer-BLAST (NCBI), Primer3 and PrimerQuest (IDT) were selected, as they are widely utilized and recommended within the scientific community. For evaluation purposes, primers were designed for the *ETV6::RUNX1* fusion using junction region sequences obtained from NCBI. Based on these sequences, the following parameters were assessed: specificity (considering amplicon size, melting and annealing temperatures); formation of secondary structures (such as hairpins); presence of a G/C clamp at the primer ends (favoring stable binding); balanced base distribution (which influences temperatures and reduces secondary structure formation); and the availability of graphical visualization tools for primer positioning within gene regions. After design, all primers were subjected to BLAST analysis to confirm the reliability of the results. **Results:** In the comparative analysis, Primer-BLAST and PrimerQuest stood out, demonstrating superior performance across all evaluated parameters. Both platforms generated more than three primer pairs (forward and reverse), with intuitive and user-friendly interfaces. Their specificity criteria were well aligned, and the integration of hybridization tests ensured greater reliability in primer design. Additionally, the primers exhibited balanced base distribution and the presence of G/C clamps at the ends, indicating a well-structured algorithm to enhance primer performance. PrimerQuest further provided detailed exon sequence annotation, increasing design precision. Conversely, Primer3 demonstrated

inferior performance, featuring an outdated and less intuitive interface, limited user guidance, and generating only one primer pair, which showed poor base distribution and lacked G/C clamps. Furthermore, it did not provide melting and annealing temperatures nor define amplicon size. The absence of hybridization testing further compromised the reliability of the primers, thereby rendering Primer3 less suitable for the study of gene fusions such as *ETV6::RUNX1*. **Conclusion:** The analysis revealed significant limitations in Primer3, including poor intuitiveness and the absence of essential information necessary for proper primer construction. In contrast, Primer-BLAST and PrimerQuest demonstrated superior performance. PrimerQuest appeared more accessible for beginners, while Primer-BLAST exhibited higher efficacy in detecting gene fusions, such as *ETV6::RUNX1*, due to its precise visualization of the annealing site—a critical factor for the diagnosis and treatment of leukemia. Based on this analysis, the use of Primer-BLAST is recommended to ensure the effective construction of diagnostic assays for gene fusions in Acute Lymphoblastic Leukemia.

Keywords: Gene Fusion Detection; *ETV6::RUNX1* Fusion; Primer Design