Primer Designing for AKT1 gene using Primer-BLAST

1. Get the Exon and Intronic Sequences from NCBI

- **Search AKT1 Gene**: Find the **AKT1 gene** sequence on **NCBI** (e.g., **RefSeq ID: NG_012188.1**).
- **Select Exon Sequence**: Choose the specific exon region you want to design primers for.
- **Add Introns**: Include introns at the **5'** and **3' ends** to ensure accurate amplification and avoid mismatch.

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2. Input Sequence into Primer-BLAST

- **Go to Primer-BLAST**: Access the Primer-BLAST Tool.
- **Enter Sequence**: Paste the full sequence (including exon and intron regions) into the "Enter target sequence" box.
- **Select Organism**: Choose **Homo sapiens** for the AKT1 gene.

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3. Set Primer Parameters in Primer-BLAST

- **Primer Size**: Set primer length to **18-25 bp**.
- **Amplicon Size**: Set the amplicon size to **100-300 bp** for standard PCR.
- **Melting Temperature (Tm)**: Set Tm to **58-60°C** to optimize primer binding.
- **GC Content**: Choose primers with **40-60% GC content**.

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4. Primer Specificity Settings

- **Primer Database**: Select **nr** (Non-Redundant) or **nt** (Nucleotide) database.
- **Product Size**: Ensure the product size falls within the desired range (**100-300 bp**).
- **Check for Non-Specific Binding**: Ensure the "Allow Primer3 to evaluate the potential for non-specific amplification" option is checked.

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5. Generate Primers

- **Click "Get Primers"**: Primer-BLAST will generate forward and reverse primer pairs based on your sequence.

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6. Result Interpretation

Once Primer-BLAST generates the primer pairs, here's how to interpret the results:

- **Primer Sequences**: You will receive both **forward** and **reverse** primer sequences. Ensure they match your desired gene region, and that the **amplicon size** falls within the expected range (e.g., **100-300 bp**).
- **Amplicon Size**: Check the **amplicon size** (product size) reported in the result. Ensure it matches your expected target (e.g., **150-250 bp** for efficient PCR amplification).
- **Tm (Melting Temperature)**: Primer-BLAST will display the **Tm** values for both primers. The Tm of both primers should be within **±2°C** of each other for efficient binding during PCR. For example, if your forward primer has a Tm of **59°C**, the reverse primer should be between **57°C and 61°C**.
- **GC Content**: Ensure the **GC content** of both primers is within the optimal range of **40-60%**. This helps in creating stable primer-template bindings.
- **Specificity Report**:
    - **No Non-Specific Binding**: Ensure the primers are specific to your target region and do not bind to other genomic sequences. Primer-BLAST will provide a **specificity check** and show if any primer binds to unintended regions. If non-specific binding is detected, you may need to adjust the primer design (e.g., extend or reduce the region of amplification).
- **Secondary Structures**: Primer-BLAST will flag any **hairpins** or **primer dimers**. A **hairpin** is a secondary structure where a primer binds to itself, while **primer dimers** occur when the two primers bind to each other. Both can reduce PCR efficiency. Ensure neither structure is present for both forward and reverse primers.
- **Self-Dimer and Cross-Dimer Check**: Ensure that primers do not form **self-dimers** or **cross-dimers** (binding with each other). If dimerization occurs, adjust the primer sequences by changing the primer length, GC content, or sequence composition.