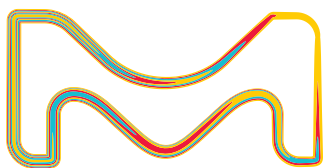


OligoArchitect™ Online

Designing Across Exon Splice Sites

1. Click on the **Dual-Labeled Probe** (or **SYBR® Green**) function at the top of OligoArchitect Online. Enter an Assay Name.
2. Populate the template sequence, e.g. Homo sapiens GAPDH mRNA, by entering the **Accession/GI number** (NM_002046.3) or copying/pasting the sequence.
3. If you have entered the accession number, click on **View Sequence** to display a list of SNPs and exons in a separate window. Identify the splice site between the most 3' pair of exons, e.g. position 1040 in the case of GAPDH. If you have copy/pasted the sequence, you will need to lookup the exon information via Entrez.
4. Under **Template Structure Search Parameters**, set the 'Search Range' to cover the region of the most 3' pair of exons, e.g. for GAPDH, positions 900 – 1261 should be acceptable. Click **Perform Template Structure Search**, and check the results to be certain that this region has acceptable secondary structures.
5. Under **Probe Parameters** and then under **Oligo Search Range**, set the **Amplicon Search Range** to cover the region in which the design will occur, e.g. for GAPDH, positions 900 – 1261 should be acceptable.
6. Enter the splice site (position 1040) into the **SNP Position** box, and the base at that position automatically populates the **Base** box. For GAPDH, position 1040 is a G. If the base is a G or C, try a different position nearby the splice site to get a T or A base, e.g. for GAPDH, position 1042 is a T. Enter an A base into the **Mutant Base** box and then click **Add SNP**.
7. Either use the default settings or manually adjust all categories of additional parameters, options, and conditions under Probe **Parameters** and then click **Search**.
8. The output of the design is two primers and two probes. Ignore the **Sense Mutant Dual-Labeled Probe**. Click **BLAST** for **Sense Primer**, **Anti-Sense Primer**, and **Sense Wild Dual-Labeled Probe** to be certain that there is no homology capable of interfering with the assay.
9. If the software does not provide an acceptable assay, e.g. there is an error message, then continuously repeat the process starting at number 3 above by working backwards toward the 5' end of the template checking each pair of exon splice sites until the software creates an assay without error.



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