Tutorial RASE: Real-time PCR Annotation of Splicing Event
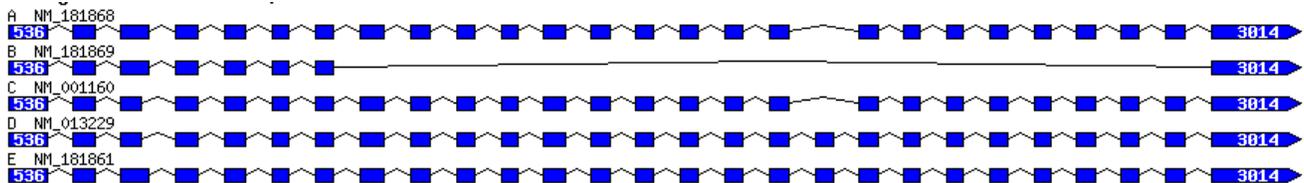
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version 1.0

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Prior to use RASE

Step 1: Search for your gene of interest and identify the event you want to design primers on. For the tutotrial we will use the APAF1 gene using the NCBI RefSeq version 2 annotation. Here is the annotation for the APAF1 gene:

Step 2: Find the event of interest in the gene annotation and extract the UCS, AIR and DCS sequences. The algorithm designs primers within the sequence provided by user.
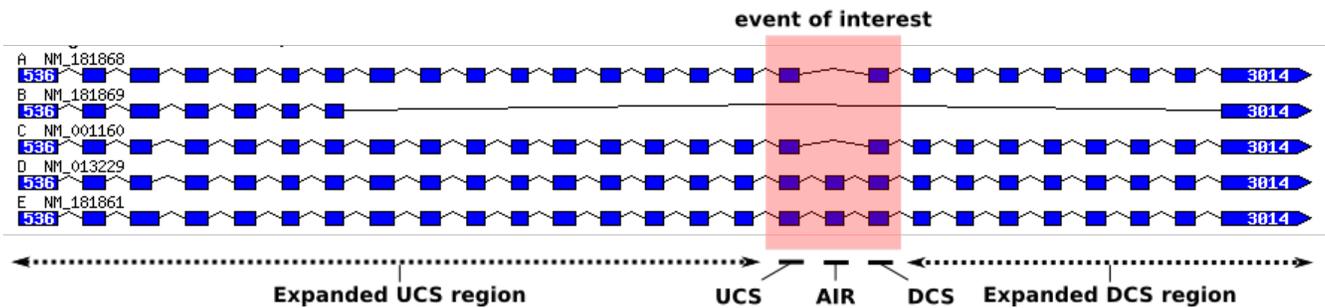
The UCS and DCS can be expanded using exons common to both splicing isoform. For improved results, paste as much cDNA sequence (we recommend at least 40 to 300 nt for UCS and DCS sequences). The following schema shows you how to expand your UCS / DCS sequences given differents cases.
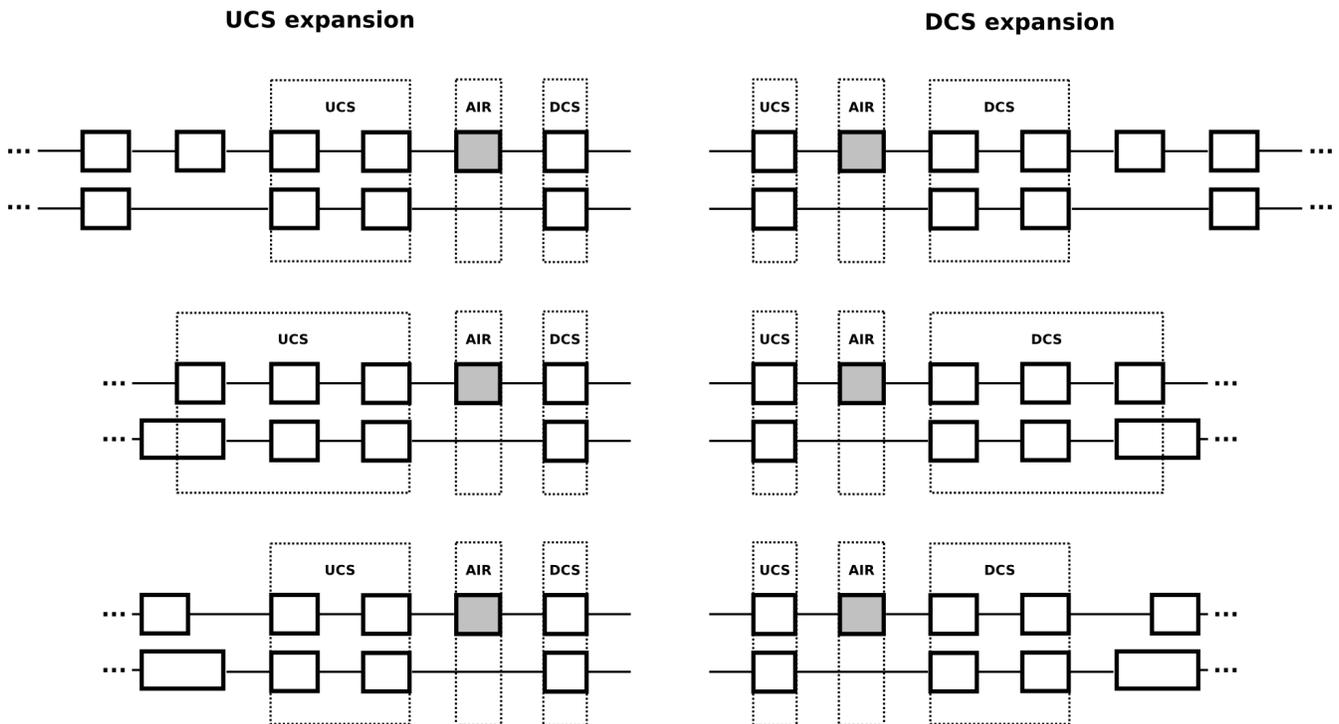
For the example event above, the sequences are the following:

UCS:

CTTTGGGATGCGACATCAGCAAATGAGAGGAAAAGCATTAATGTGAAACAGTTCTTCCTA
AATTTGGAGGACCCTCAAGAGGATATGGAAGTGATAGTGAAGTGTTGTTCGTGGTCTGCT
GATGGTGCAAGGATAATGGTGGCAGCAAAAAATAAAATCTTT

AIR:

CTTTTTGACATTCATACTAGTGGCCTATTGGGAGAAATCCACACGGGCCATCACAGCACCA
TCCAGTACTGTGACTTCTCCCCACAAAACCATTTGGCAGTGGTTGCTTTGTCCCAGTACTG
TGTAGAG

DCS:
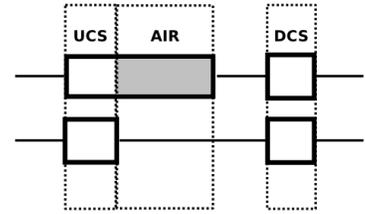
TTGTGGAATACAGACTCACGTTCAAAGGTGGCTGATTGCAGAGGACATTTAAGTTGGGTT
CATGGTGTGATGTTTTCTCCTGATGGATCATCATTTTTGACATCTTCTGATGACCAGACAAT
CAGG

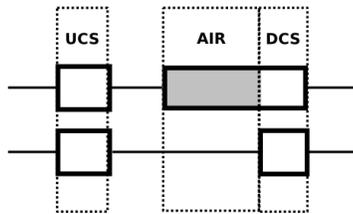The following schema explains how to identify the UCS, AIR and DCS from different types of splicing event.

Case 1: single cassette exon
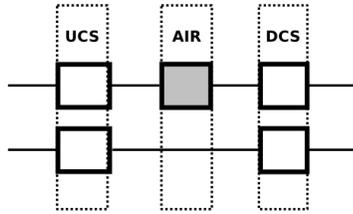
Case 2: alternative 5' splice site

Case 3: alternative 3' splice site

Case 4: intron retention
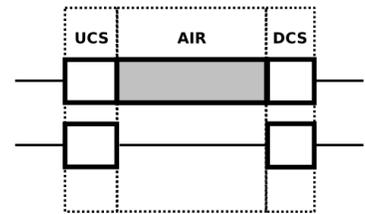
Now go to the RASE primer design website at http://designs.lgfus.ca

Step 3: Go to the RASE primer design web application. Input your gene symbol or accession and choose appropriate annotation. Please note that if you search for "AP", all genes starting with AP in their symbol or accession will be returned in the gene listing page.

RASE primer design

Search your gene

Gene name: apaf1

Annotation: NCBIGene 36.2

Submit Query   Reset

For a complete tutorial on how to use this application, click here.

Step 4: In the gene listing page, click on your gene.

Step 5: You will arrive at the RASE design parameters page. An example page for the APAF1 gene is shown below. Paste the UCS, AIR and DCS sequences previously identified and launch design.

RASE design parameters for: APAF1

Go back to gene search **here**

| **Detection** |

Exon information

Note: If you need help in identifying proper UCS, AIR and DCS sequences, click **here**.

* UCS:

CTTTGGGATGCGACATCAGCAAATGAGAGGAAAAGCATTAATGTGAAACAGTTCTTCCTAAATTTGGAGGACCCTCAAGAGGATATGGAAGTGATAGTGA
AGTGTTGTTCGTGGTCTGCTGATGGTGCAAGGATAATGGTGGCAGCAAAAAATAAAATCTTT

Paste the exact sequence of the UCS.

* AIR:

CTTTTTGACATTCATACTAGTGGCCTATTGGGAGAAATCCACACGGGCCATCACAGCACCATCCAGTACTGTGACTTCTCCCCACAAAACCATTTGGCAG
TGGTTGCTTTGTCCCAGTACTGTGTAGAG

Paste the exact sequence of the AIR.

* DCS:

TTGTGGAATACAGACTCACGTTCAAAGGTGGCTGATTGCAGAGGACATTTAAGTTGGGTTCATGGTGTGATGTTTTCTCCTGATGGATCATCATTTTTGA
CATCTTCTGATGACCAGACAATCAGG

Paste the exact sequence of the DCS.

Amplicon information

* Minimal length: 70

* Maximal length: 300

* Optimal length: 90

Design information

* Number of designs: 2

Please note that a typical run takes around 1 minute to complete.

Submit Query   Reset

Step 6: You will arive at the primer design results page. You will notice there are 2 tabs you can click on, one returns the short splice variant detection and the other go to the long variant detection. Once you have clicked on the tab, you can see each of the predictions with the primers mapped on the submitted DCS and UCS sequence. The forward primer is mapped in red and the reverse in green. Notice, the splice junction is marked with a slash character (marked in green on image below). In this example, you can see that the BSP is the reverse primer since its spans the splice junction.

RASE primer design results for the gene APAF1

Back to event design page.

| Short isoform | Long isoform |

Design 1

Primers details:

Characteristic	Forward	Reverse
Sequence	TTGTTCGTGGTCTGCTGATGGT	TGAACGTGAGTCTGTATTCCACAAAA
Tm	59.954	58.618
%GC	50.000	38.462
Penalty	2.045670	13.151270

Reaction details:
Amplicon size82
Amplicon Tm 74.0829
Pair penalty   15.1969

Note: The pair penalty score is provided by Primer3. It is calculated based on primers any (Self Complementarity) penalty, 3" (Self Complementarity) penalty

Offtargets details: None

Note: The offtarget blast database used is an inhouse spliced transcript sequences based on the AceView annotation.

Primer mapped sequence:

```
CTTTGGGATGCGACATCAGCAAATGAGAGGAAAAGCATTAATGTGAAACAGTTCTTCCT
AAATTTGGAGGACCCTCAAGAGGATATGGAAGTGATAGTGAAGTGTTGTTCGTGGTCTG
CTGATGGTGCAAGGATAATGGTGGCAGCAAAAAATAAAATCTTT / TTGTGGAATACAGAC
TCACGTTCAAAGGTGGCTGATTGCAGAGGACATTTAAGTTGGGTTCATGGTGTGATGTT
TTCTCCTGATGGATCATCATTTTTGACATCTTCTGATGACCAGACAATCAGG
```

Design 2

Primers details:

Characteristic	Forward	Reverse
Sequence	TGTTCGTGGTCTGCTGATGGT	TGAACGTGAGTCTGTATTCCACAAAA
Tm	59.500	58.618
%GC	52.381	38.462
Penalty	2.690334	13.151270

Reaction details:
Amplicon size81
Amplicon Tm 74.1963
Pair penalty   15.8416

Note: The pair penalty score is provided by Primer3. It is calculated based on primers any (Self Complementarity) penalty, 3" (Self Complementarity) penalty

Offtargets details: None

Note: The offtarget blast database used is an inhouse spliced transcript sequences based on the AceView annotation.

Primer mapped sequence:

Step 7: Click on the long prediction tab, you can see each of the predictions as with the short tab. Again, the primers are mapped on the submitted DCS, AIR and UCS sequences and the forward primer is mapped in red and the reverse in green and the splice junctions are marked with a slash character. In this example, you can see that the long variant specific primer is the forward primer since it is inside the AIR sequence.

RASE primer design results for the gene APAF1

Back to event design page.

| Short isoform | Long isoform |

Prediction 1

Primers details:

Characteristic	Forward	Reverse
Sequence	TTTGGCAGTGGTTGCTTTGTCC	TCCTCTGCAATCAGCCACCTTT
TM	59.891	59.686
%GC	50.000	50.000
Penalty	2.108632	2.313568

Reaction details:

Amplicon size: 82

Amplicon TM: 77.0829

Pair penalty: 4.4222

Note: The pair penalty score is provided by Primer3. It is calculated based on primers any (Self Complementarity) penalty, 3" (Self C more information.

Offtargets details: None

Note: The offtarget blast database used is an inhouse spliced transcript sequences based on the AceView annotation.

Primer mapped sequence:

```
CTTTGGGATGCGACATCAGCAAATGAGAGGAAAAGCATTAATGTGAAACAGTTCTTCCT
AAATTTGGAGGACCCTCAAGAGGATATGGAAGTGATAGTGAAGTGTTGTTCGTGGTCTG
CTGATGGTGCAAGGATAATGGTGGCAGCAAAAAATAAAATCTTT / CTTTTTGACATTCAT
ACTAGTGGCCTATTGGGAGAAATCCACACGGGCCATCACAGCACCATCCAGTACTGTGA
CTTCTCCCCACAAAACCATTTGGCAGTGGTTGCTTTGTCCCAGTACTGTGTAGAG / TTGT
GGAATACAGACTCACGTTCAAAGGTGGCTGATTGCAGAGGACATTTAAGTTGGGTTCAT
GGTGTGATGTTTTCTCCTGATGGATCATCATTTTTGACATCTTCTGATGACCAGACAAT
CAGG
```

Prediction 2

Primers details:

Characteristic	Forward	Reverse
Sequence	AAATCCACACGGGCCATCACA	TCCTCTGCAATCAGCCACCTTT
TM	60.111	59.686
%GC	52.381	50.000
Penalty	2.301488	2.313568

Reaction details:

If you have questions or comments concerning this web application, do not hesitate to communicate with jean-francois.lucier@usherbrooke.ca