Using the in-Silico PCR tool in the UCSC Genome Browser

In this tutorial, we will explore the UCSC Genome Browser's In-Silico PCR tool. This tool allows you to predict the sequence and size of products that would result from a polymerase chain reaction experiment using primers you may wish to use. You can see that there's a link to the tool directly on the main page, at genome.ucsc.edu.

Let's go to the Genome Browser and "Reset all user settings" because to use the in-silico PCR tool we must first have some primers to input into the tool, and we will navigate to the Genome Browser to find them.

[0:40 Set the Genome Browser to hg19 defaults]

Let's go to the hg19 assembly, as that's still the most commonly used human assembly, and use the "`go`" button to navigate directly to the Genome Browser graphic. Below the graphic, find the "`hide all`" button to turn off the numerous data tracks visible and set the "UCSC Genes" track to "`pack`."

[1:08 Navigate to a gene (CYP2D6) to obtain DNA for primers]

Let's navigate to the CYP2D6 gene, choose it from the drop down suggestion menu, and hit the "`go`" button to navigate there. You see we are on chromosome 22. The reason for choosing this gene is that there is a very obvious differentially spliced exon in the middle of the gene, which allows us to use the In-Silico PCR tool in both of its modes, which are essentially using either DNA or spliced RNA as template so that you can see the results of the experiment both ways.

[1:46 Get DNA with exons annotated]

So, let's zoom into the three exons with the differentially expressed exon in the middle of the screen, and go to the "View... DNA" pulldown in the bluebar menu to extract some DNA to use as primers.

"`Extended case/color options`" at the bottom of the screen allow us to use the UCSC Genes track to decorate the DNA output and make it more obvious where the exons are. Let's select "`lowercase`"

for the default so that we can toggle the case to capitals where the UCSC Genes track indicates the presence of the exons. We'll use 255 in the Blue box in the RGB color selector so that the capital letters are blue and much more obvious. Then let's "`submit`".

[2:40 Obtain primers for PCR from exons]

You can see here that we have three blue exons in capital letters and we obtain our first primer by copying from the top, the 5' end of the sequence, of the upper strand of this exon. We will get a chunk of DNA large enough to serve as a primer and paste it into a text file so that we can use it twice in our demonstration. Now we'll grab a chunk of DNA from the other end of this region, from the third exon, and save it also to a text file. Then let's return to the Genome Browser, and select "In-Silico PCR" from the "Tools" drop down menu in the bluebar at the top.

[3:25 Use primers to predict amplicon size from genome assembly]

We will paste the reverse primer that's still in our copy buffer into the "reverse primer" box, and we will flip the orientation of the reverse primer so that the 3' ends of our two primers are facing each other. We're essentially taking the second primer and using it on the lower strand, or reverse strand, of the DNA template.

Going now to the text file, we will grab our forward primer, and paste it into the "forward primer" box in the In-Silico PCR tool. Notice that the "target" is set to the default, "genome assembly," which is essentially using DNA as a template, with any introns included. Now hit "`Submit`".

[4:12 Observe amplified DNA in Genome Browser]

You'll notice in the results that we actually have two amplicons predicted by the tool. One of them is 1085 and the other is 1108 base pairs. Let's click into the top one, the 1085 bp amplicon, and we'll notice that we are on the genome on chromosome 22 but we are at CYP2D7P1, a pseudo gene that apparently closely resembles the 2D6 gene where we got our primers. In fact, you'll notice that there's a 1 base pair mismatch annotated here by a red tick mark, near the left end of this annotation.

Let's zoom out by a factor of 10X, and then another factor of 10X and we'll observe that there's a second amplicon to the left of this one that represents the CYP2D6 gene that we began with. In fact, the gene name is still highlighted from our having typed it into the position box when we navigated to this region of the genome. You'll see that this illustrates one of the primary purposes of having the isPCR tool and one of the features of the tool: It represents an opportunity to confirm that your primers amplify uniquely in the genome and will not amplify at a location elsewhere in the genome that might not have been known or might not have been expected when the primers were originally chosen.

[5:32 Use primers to predict amplicon size from RNA template]

So let's go back to the isPCR tool, and reset the target to "`UCSC Genes`", which is the In-Silico equivalent of having spliced mRNA as a template, and we will paste the forward primer, still in our copy buffer, into the forward primer box. Then we'll go back to our text page, grab the reverse primer, paste the reverse primer into its box, and notice that the flip-reverse-primer checkbox is still selected. Then hit "`submit`."

Now on this results page you'll notice that we have multiple results. We did expect to find at least the CYP2D6 and the CYP2D7 pseudogene because of our earlier result with these primers, and we'll also notice that we have multiple hits for each of these. Notice also that the CYP2D6 amplicons are 315 bp and 468 bp in length.

[6:36 Observe RNA results in Genome Browser]

Let's click into the upper CYP2D6 amplicon, navigate to the Genome Browser, and see that we actually have three annotations of two different types, representing the three isoforms of the gene, each as a separate amplification product. One includes the intervening exon and the other two do not. By zooming out by a factor of 10X, we see that the CYP2D7 pseudo gene actually has four predicted amplicons using the primers that we selected, corresponding to the four isoforms of this gene.

So that takes you through our tour of the isPCR tool in the UCSC Genome Browser, showing you the two different modes of operation, one using DNA and one using spliced RNA as the target. Note that the UCSC genes option, which is essentially the RNA amplification mode, is only available for human and mouse assemblies.

Thanks for watching and thanks for using the UCSC Genome Browser.