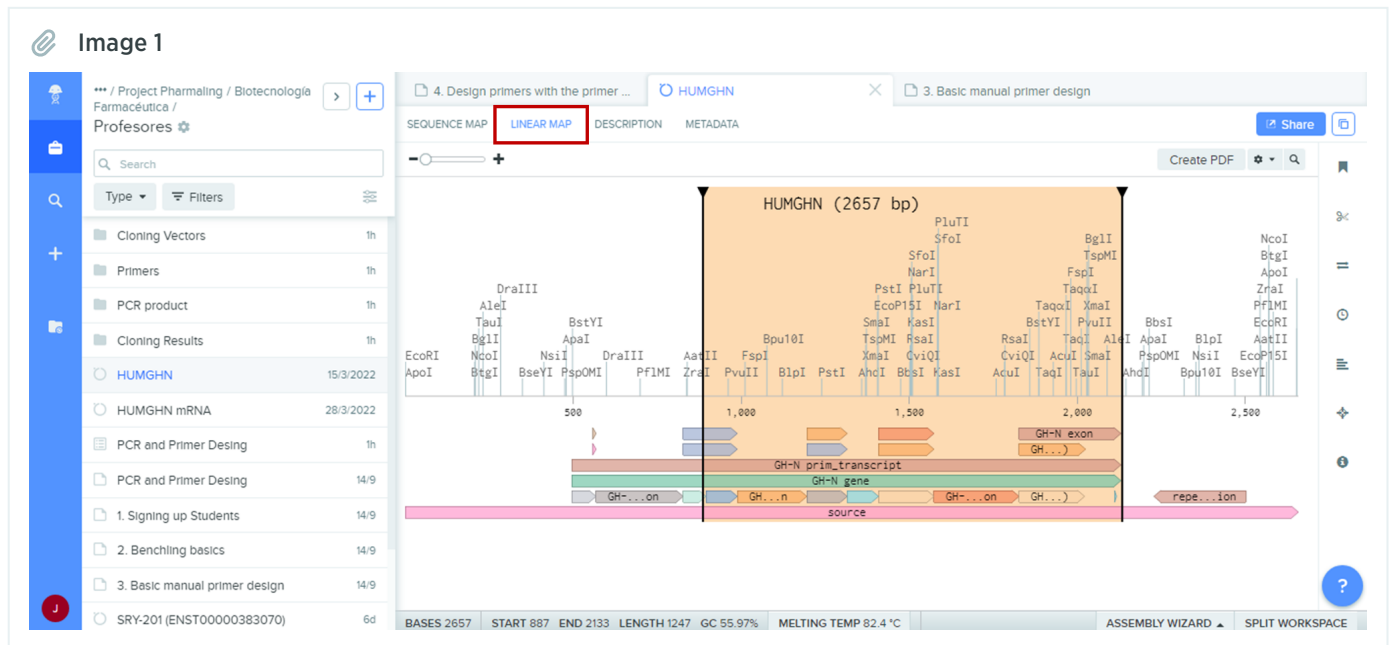


3. Design primers with the primer wizard

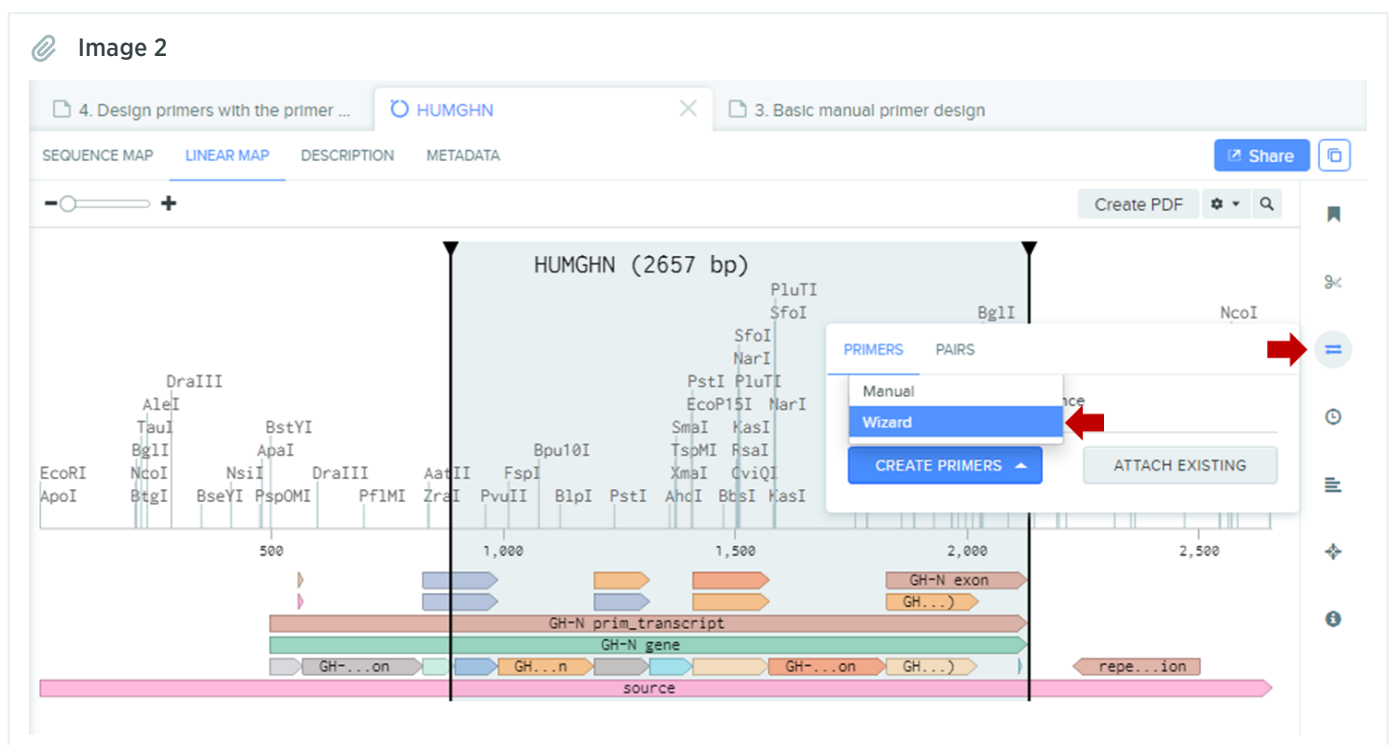
MIÉRCOLES, 21/9/2022

This doc will explain how to create and modify primers using Benchling primer wizard.

- 1. Open your sequence (sample) and click on the sequence Linear Map to have a look at it. Select the target sequence with the mouse clicking and dragging over the map (Image 1).



- 2. Click the Primer button on the right side bar. Select Create Primers > Wizard. (Image 2)



- 3. A new window will pop up where we can complete different parameters to generate the primers. Select **PCR** as the "task" for the primers. Click on "use selection" to add the selected sequence from step 1 as the target DNA. Complete the parameters for the primers like GC content, Tm or size. At the bottom of the window there is important and useful information to help you with details of the sequence like target length. (**Image 3**)

Image 3

4. Design primers with the primer ... HUMGHN 3. Basic manual primer design

SEQUENCE MAP LINEAR MAP PRIMER WIZARD x DESCRIPTION METADATA [Share](#)

Generate Primers

Task: PCR Tm Params Reset

Region

Target 887 2133 Use Selection Show Advanced

Primer

	Min	Opt	Max
✓ GC%	45	50	65
✓ Tm	55	62	65
✓ Size	18	22	26
3' GC Clamp	0		

BASES 2657 START 887 END 2133 LENGTH 1247 GC 55.97% MELTING TEMP 82.4 °C ASSEMBLY WIZARD SPLIT WORKSPACE

- 4. Scroll down to reach for more parameter and complete them, specially the **Amplicon size** window, the number of primers to be generated (**Results generation**) and, when need it, the **Forced Location** that will help with generating the primers on an specific position. (**Image 4**)

Image 4

4. Design primers with the primer ... HUMGHN 3. Basic manual primer design

SEQUENCE MAP LINEAR MAP PRIMER WIZARD DESCRIPTION METADATA

Generate Primers

Amplicon

	Min	Opt	Max
Size	1239	1239	1350

Result Generation

Results 5

Partial Design

Design Across Junctions

Force Location

	Start (5' End)	End (3' End)
Forward		
Reverse		

Our primer wizard is powered by Primer3. Visit the [manual](#) for more information on the different tasks and parameters. Primer3 does not support RNA

BASES 2657 START 887 END 2133 LENGTH 1247 GC 55.97% MELTING TEMP 82.4 °C ASSEMBLY WIZARD SPLIT WORKSPACE

- 5. Click on generate primers. A new result primer window will pop up where you can select a pair or many primers to be saved on your folder of choice. (Image 5). Before saving, it could be useful to see the primers location, see step 6.

Image 5

4. Design primers with the primer ... HUMGHN 3. Basic manual primer design

SEQUENCE MAP LINEAR MAP PRIMER WIZARD X PRIMER3 RESULTS X DESCRIPTION METADATA

Save Selected Primers Export as CSV T_m params Sort by Penalty

	Penalty	Direction	% GC	T _m °C	Location	Length	Product BP	Primer
<input checked="" type="checkbox"/>	0.873	<input checked="" type="checkbox"/> FWD	63.6%	62.9°	852-873	22	1349	5' cttttggcctgctctgctgccc 3' Edit
		<input checked="" type="checkbox"/> REV	59.1%	62.0°	2179-2200	22		5' ttgggcccttgctccatacca 3' Edit
<input checked="" type="checkbox"/>	1.357	<input checked="" type="checkbox"/> FWD	61.9%	62.3°	853-873	21	1348	5' ttttggcctgctctgctgccc 3' Edit
		<input checked="" type="checkbox"/> REV	59.1%	62.0°	2179-2200	22		5' ttgggcccttgctccatacca 3' Edit
<input checked="" type="checkbox"/>	1.418	<input checked="" type="checkbox"/> FWD	63.6%	63.4°	851-872	22	1350	5' gcttttggcctgctctgctgccc 3' Edit
		<input checked="" type="checkbox"/> REV	59.1%	62.0°	2179-2200	22		5' ttgggcccttgctccatacca 3' Edit
<input checked="" type="checkbox"/>	1.460	<input checked="" type="checkbox"/> FWD	63.6%	62.9°	852-873	22	1348	5' cttttggcctgctctgctgccc 3' Edit
		<input checked="" type="checkbox"/> REV	63.6%	62.6°	2178-2199	22		5' ttgggcccttgctccataccac 3' Edit
<input checked="" type="checkbox"/>	1.567	<input checked="" type="checkbox"/> FWD	63.6%	62.9°	852-873	22	1350	5' cttttggcctgctctgctgccc 3' Edit
		<input checked="" type="checkbox"/> REV	63.6%	61.3°	2180-2201	22		5' ctgggcccttgctccatacca 3' Edit

BASES 2657 START 887 END 2133 LENGTH 1247 GC 55.97% MELTING TEMP 82.4 °C ASSEMBLY WIZARD SPLIT WORKSPACE

- 6. It is useful to have a look to the location of the primers on the target sequence. This can be done by going back to the lineal map where now the primers will appear. (Image 6)

Image 6

