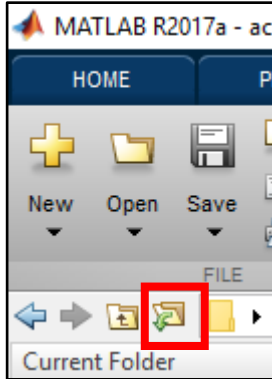


How to design H-probes with  
the new probe design program.

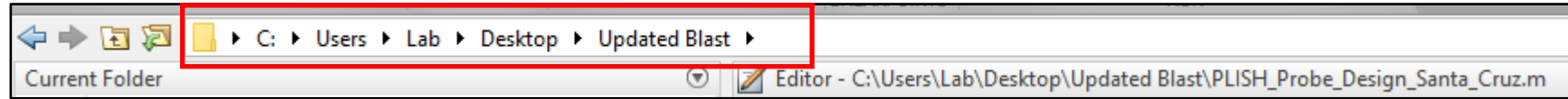
1. Open Matlab:



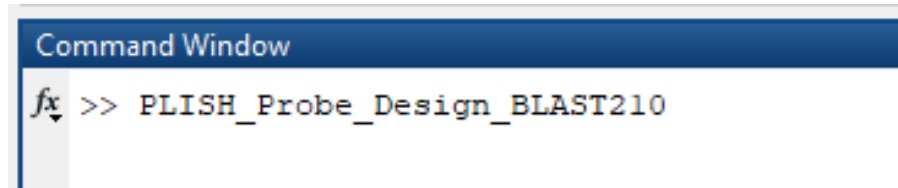
2. Use this button



to make sure MATLAB is in the following working directory:

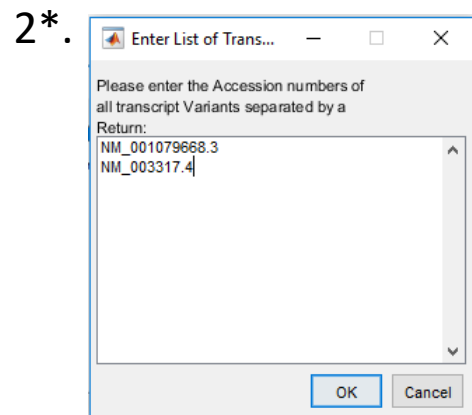
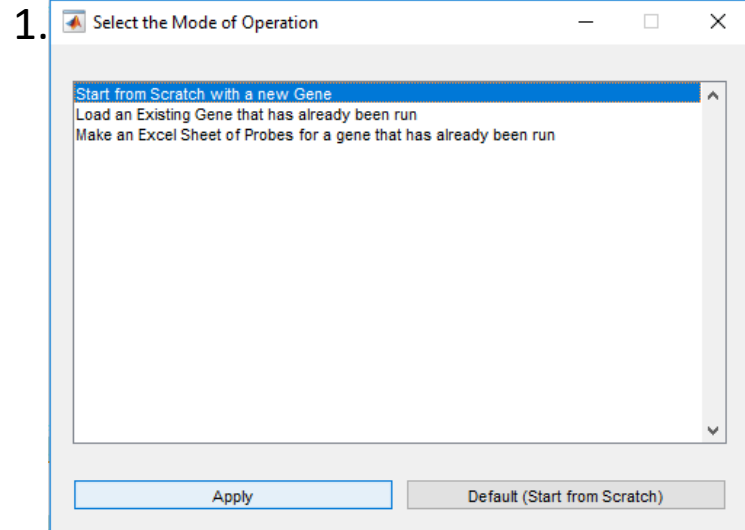


3. Type the following after the command prompt:



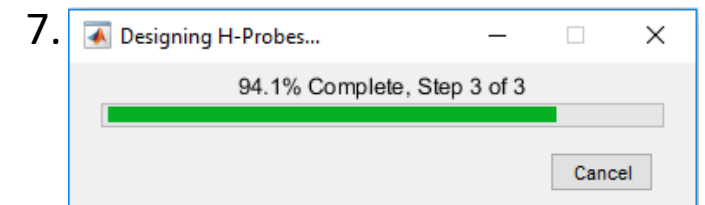
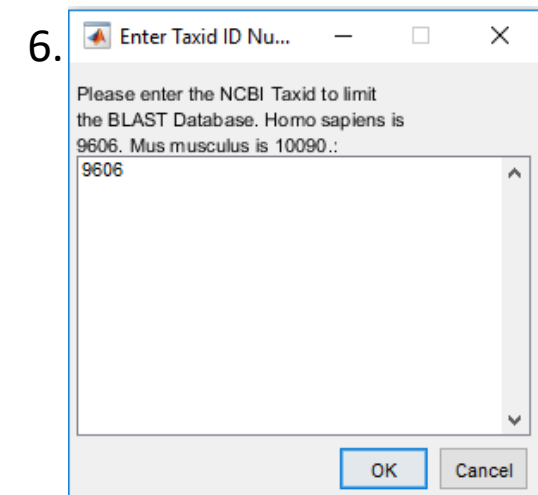
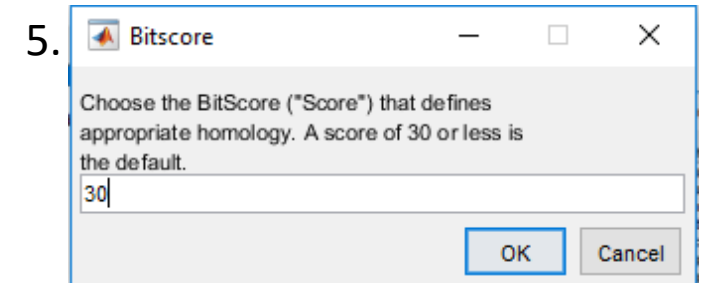
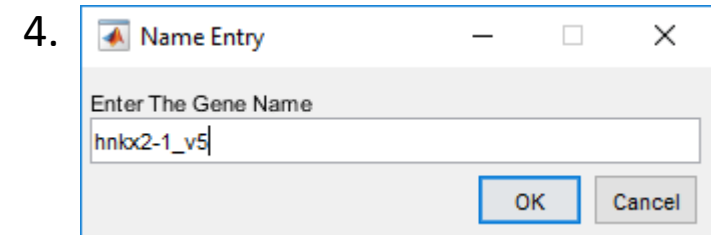
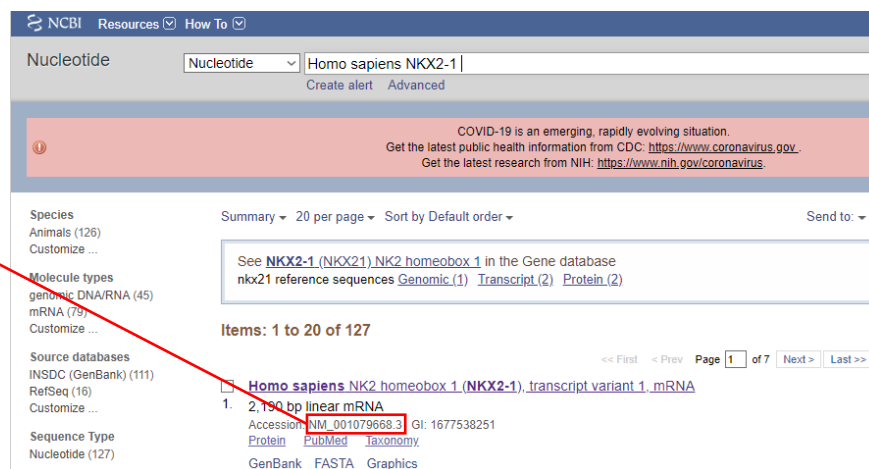
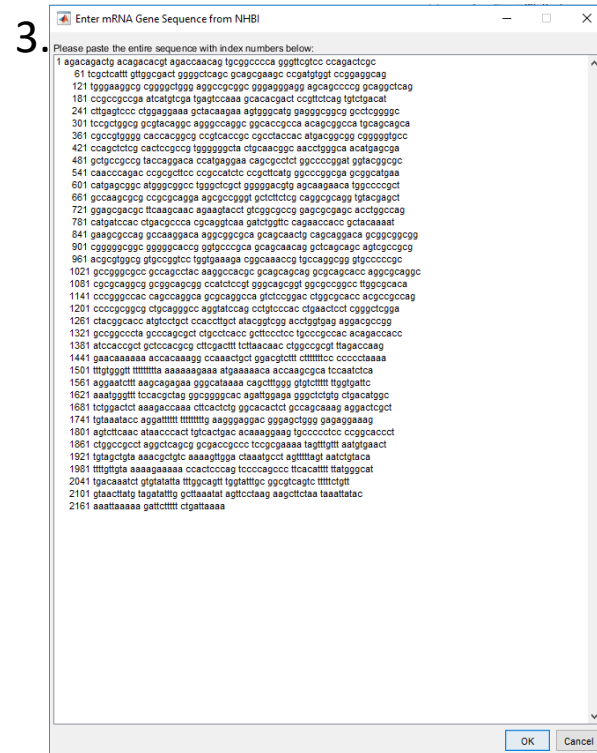
4. Press Enter.

# Workflow:



\* You have to manually look up the accession number in “NCBI nucleotide” for all the transcript variants. At the least, just put in the ID for the mRNA you are interested in.

<https://www.ncbi.nlm.nih.gov/nucleotide/>

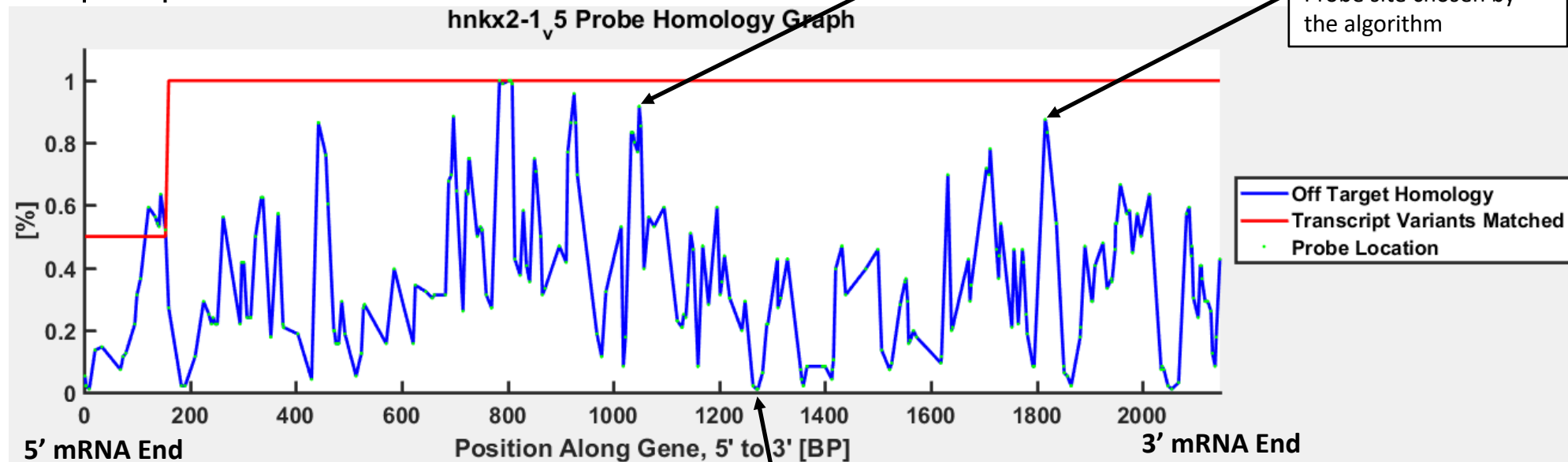


# Graphical Output:

Probes designed in this region of the submitted sequence will only target ½ of all possible transcript variants.

Probes designed at “Peaks” have the highest amount of normalized (Range 0-1) Off-target homology and are suboptimal choices.

Each green dot is an H-Probe site chosen by the algorithm



Probes designed in “Valleys” have the lowest amount of normalized (Range 0-1) Off-target homology and are good choices.

# Text output:

This is the gene name typed in earlier.

FASTA File of most promising Probe Sites based on a Composite Score:

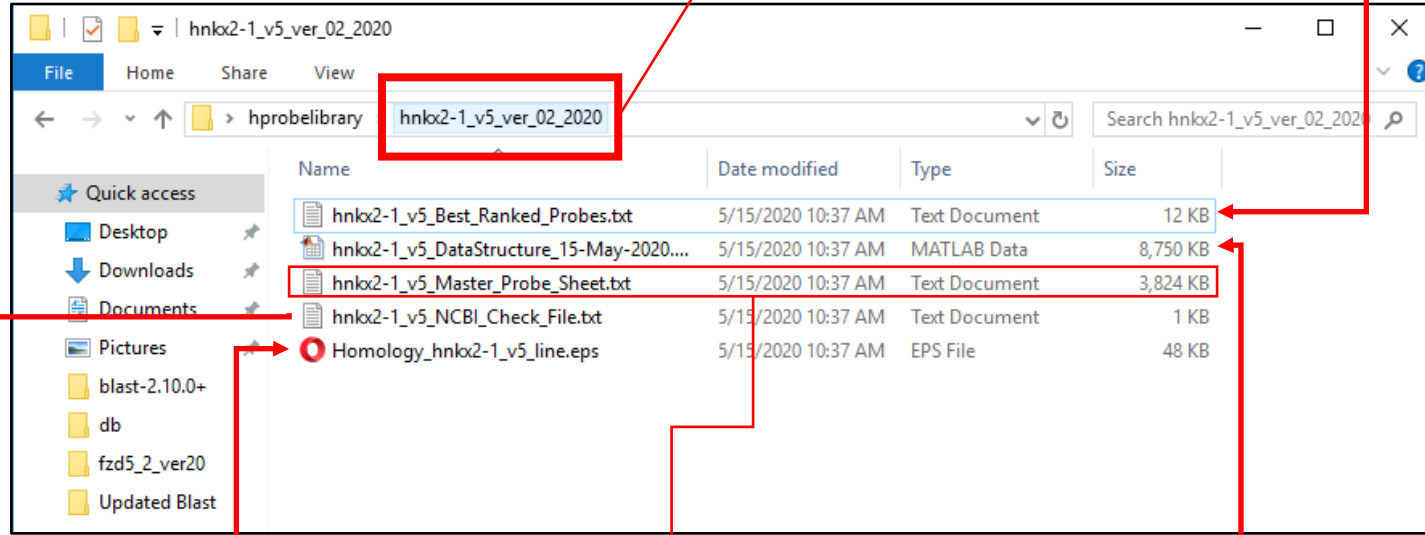


Figure Shown on Last Slide

**All Data is Contained Here**  
(open this in Opera or other Browser) – See Next Slide

All Matlab Data Generated By the Script is saved in this structure

Second FASTA File of all Probe Sites chosen by algorithm on basis fo off target hits alone:

PLISH Probe Generator  
Program "BLAST210" Output: Probes Ranked by Composite Hit Score

Location mRNA Sequence

```
>4
cagactgacagacacgttagaccaacagtcggccccaggg
>315
acaggcaggccaggcggcaccgccaacagcgccatgca
>353
cagcagcacccgtggggcaccacggcgcgtcaccgccc
>367
ggggcaccacggcgcgtcaccgcccctaccacatgacg
>377
ggcgcgcgtcaccgcccctaccacatgacggcgggggg
>443
gggggctactgcaacggcaacctgggcaacatgagcgagc
>473
atgagcgagctgcccgtaccaggacacatgaggaaca
>481
gctgcgcgcgtaccaggacacatgaggaacagcgctct
>524
cccggatggtagggccaacccagaccgcgttccccg
>530
tggtagggcgaaccagaccgcgttccccgccatct
>728
cgcttcaagcaacagaagtacctgtcggcgcggagcgcg
>752
tcggcggcgagcgcgagcacctggccagcatgatccacc
>770
cacctggcgagcatgatccctgacgccacgcagggtca
>806
gtcaagatctgggttcagaaccaccgctacaaatgaagc
```

PLISH Probe Generator  
Program "BLAST210" Output

Location mRNA Sequence

```
0 Off Target Hits
Location
1 Off Target Hits
>1271
atgtcctgtccacctgtctataggtcggaacctggtgag
>1273
gtcctgtctccacctgtctataggtcggaacctggtgagag
>2055
atattatttggcagtttggatttttggcgtcagctctttt
>10
gacagacacgttagaccaacagtcggccccagggttcgtc
Location
2 Off Target Hits
>1264
cggcaccatgtcctgtccacctgtctataggtcggaacc
>1359
cctgcccgcacacagaccacatccaccgctgtccacg
>1865
ccgctaggctcagcggcgaccgcccctccgaaaatagt
>1264
cggcaccatgtcctgtccacctgtctataggtcggaacc
>1359
cctgcccgcacacagaccacatccaccgctgtccacg
>1865
ccgctaggctcagcggcgaccgcccctccgaaaatagt
>184
ccgccaatcatgtcagtgagtcgaagcacacgactccg
>191
atcatgtcagtgagtcgaagcacacgactccgttctcag
>2048
tctgtgtatatttggcagtttggatttttggcgtcga
>4
cagactgacagacacgttagaccaacagtcggccccaggg
>4
cagactgacagacacgttagaccaacagtcggccccaggg
Location
3 Off Target Hits
```

...\_Master\_Probe\_Sheet.txt Output:

hnxk2-1\_v5\_Master\_Probe\_Sheet.txt 5/15/2020 10:37 AM Text Document 3,824 KB

PLISH Probe Generator  
Main Output File  
Adam M. Andruska, 10/2018

Gene = hnxk2-1\_v5  
Bitscore Cutoff = 30  
Transcript Variants Considered = nm\_001079668.3  
nm\_003317.4

FASTA Sequence IDs and mRNA Sequences For NCBI Blast:

```
>hnxk2-1_v5-1-4
cagactgacagacagctagaccaacagtggcgccacagg
>hnxk2-1_v5-1-315
acaggcaggcgccaggcgccaccgccaacagcgccatgca
>hnxk2-1_v5-1-353
cagcagcagcgccgtggggcaccacggcgccgtaccgcgc
>hnxk2-1_v5-1-367
ggggcaccacggcgccgtaccgcccgtaccacatgacg
>hnxk2-1_v5-1-377
ggcgccgtcaccgcccctaccacatgacggcgccggggg
>hnxk2-1_v5-1-443
gggggctactgcaacggcaacctggggcaacatgagcgagc
>hnxk2-1_v5-1-473
atgagcgagctgcccgcgtaccaggacacatgaggaaca
>hnxk2-1_v5-1-481
gctgcgcgctgaccaggacacatgaggaacagcgccctt
>hnxk2-1_v5-1-524
cccggatggtgacggcgcaaacacagacccgcgttccccg
>hnxk2-1_v5-1-530
tggtagcggcgccaacacagacccgcgttccccgccatct
>hnxk2-1_v5-1-728
cgcttcaagcaacagaagtacctgtggcgccggagcgcg
>hnxk2-1_v5-1-752
tcggcgccggagcgcgagcacctggccagcatgatccacc
>hnxk2-1_v5-1-770
cacctggcgacatgatccacctgacgcccacgacgaggtca
>hnxk2-1_v5-1-806
gtcaagatctgggttcgaacacaccgctacaaaatgaagc
```

1. The FASTA File portion can be copied and directly pasted into NCBI Blast to double check homology.

3. Below the blast report, the reverse compliment halves of the mRNA sequence have been conjugated to corresponding bridge sequences for all variable bridges (and PLISH 1.0 circles). Tm, ΔG, Hairpins, and Dimers are also shown for each individual H-probe.

ID: >hnxk2-1\_v5-1-315  
Rank 2  
BP 315  
mRNA Sequence acaggcaggcgccaggcgccaccgccaacagcgccatgca  
BLAST Score 21  
%Transcript Variants Targeted 100.0%

Hit #: 1  
Target: NM\_001079668.3 Homo sapiens NK2 homeobox 1 (NKX2-1), transcript variant 1, mRNA  
Strand: Plus/Plus  
Score: 73.4000  
Expect: 6.00000e-13  
# BP Matching: 40  
% BP Matching: 100.00  
Query: 1 - ACAGGCAGGGCAGGCGGACCCGCCAACAGCGGCCATGCA - 40  
Subject: 315 - ACAGGCAGGGCAGGCGGACCCGCCAACAGCGGCCATGCA - 354

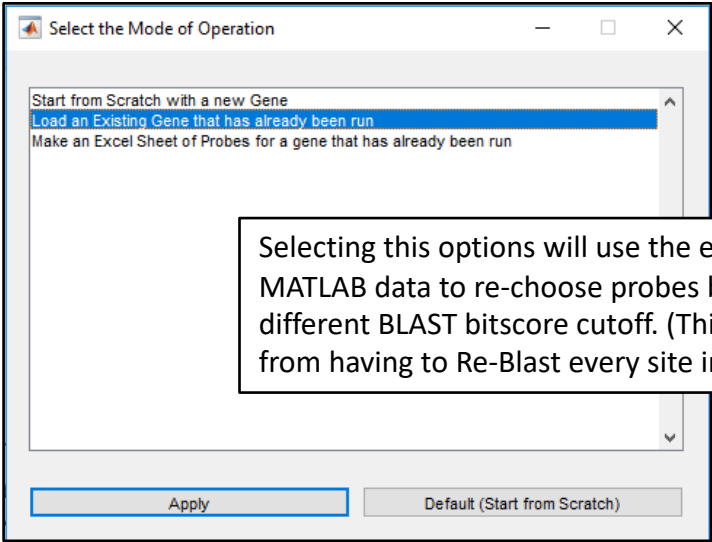
Hit #: 2  
Target: NM\_003317.4 Homo sapiens NK2 homeobox 1 (NKX2-1), transcript variant 2, mRNA  
Strand: Plus/Plus  
Score: 73.4000  
Expect: 6.00000e-13  
# BP Matching: 40  
% BP Matching: 100.00  
Query: 1 - ACAGGCAGGGCAGGCGGACCCGCCAACAGCGGCCATGCA - 40  
Subject: 321 - ACAGGCAGGGCAGGCGGACCCGCCAACAGCGGCCATGCA - 360

Hit #: 3  
Target: NM\_032193.4 Homo sapiens ribonuclease H2 subunit C (RNASEH2C), mRNA  
Strand: Plus/Minus  
Score: 33.7000  
Expect: 5.60000e-01  
# BP Matching: 18  
% BP Matching: 100.00  
Query: 9 - GGCCAGGCGGCACCGCCA - 26  
Subject: 275 - GGCCAGGCGGCACCGCCA - 258

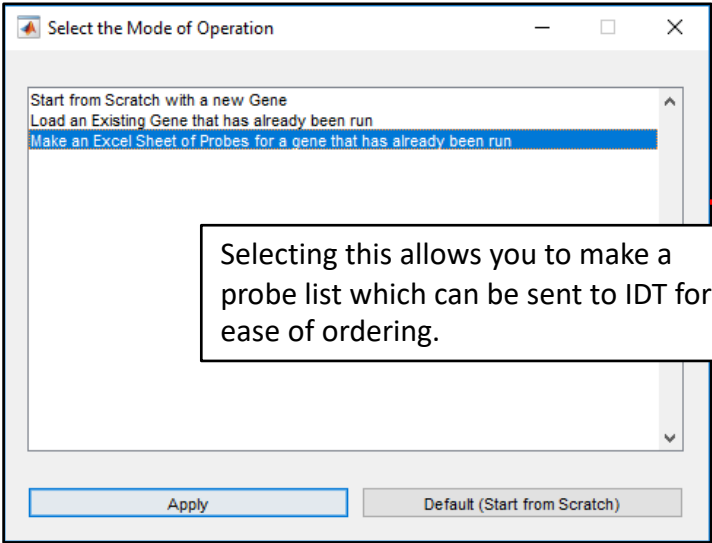
2. Each probe-site has it's associated BLAST report printed below it (one can use Ctrl-F to search for the ID in the box above) as well as the % of transcript variants it targets

ID	Probe Name	Probe Sequence	Tm	ΔG	Hairpins (6 BP)	Dimers (6 BP)
VB1	HLC2-VB01-1-hnxk2-1_v5-315	AGGTCAGGAATACTACGTCGTTATGGTgcccgcctggccctgcctgt	Tm Left = 79.57	ΔG Left = -84.56	Hairpins Left = 3	Dimers Left = 0
	HRC2-VB01-1-hnxk2-1_v5-315	tcgatggccgcgtgttggcggTTATAGGTCAGATAGTATAGCCAGGTT	Tm Right = 77.58	ΔG Right = -82.18	Hairpins Right = 4	Dimers Right = 0
VB2	HLC2-VB02-1-hnxk2-1_v5-315	AGGTCAGGAATACTAGCTATTGATGGTgcgcctgcgcctgcctgt	Tm Left = 78.81	ΔG Left = -82.50	Hairpins Left = 4	Dimers Left = 1
	HRC2-VB02-1-hnxk2-1_v5-315	tcgatggccgcgtgttggcggTCTGTGTAGACGACTATAGCCAGGTT	Tm Right = 78.94	ΔG Right = -83.72	Hairpins Right = 3	Dimers Right = 1
VB3	HLC2-VB03-1-hnxk2-1_v5-315	AGGTCAGGAATACAGGTTGTAAATGGTgcgcctgcgcctgcctgt	Tm Left = 80.17	ΔG Left = -82.90	Hairpins Left = 7	Dimers Left = 0
	HRC2-VB03-1-hnxk2-1_v5-315	tcgatggccgcgtgttggcggTTATGATGAGTAATAGCCAGGTT	Tm Right = 76.31	ΔG Right = -80.43	Hairpins Right = 3	Dimers Right = 0
VB4	HLC2-VB04-1-hnxk2-1_v5-315	AGGTCAGGAATACGACTACGAGTACGAGTgcgcctgcgcctgcctgt	Tm Left = 80.48	ΔG Left = -83.30	Hairpins Left = 4	Dimers Left = 0
	HRC2-VB04-1-hnxk2-1_v5-315	tcgatggccgcgtgttggcggTCTGTGTGTAAATAGCCAGGTT	Tm Right = 78.20	ΔG Right = -83.20	Hairpins Right = 3	Dimers Right = 0
VB5	HLC2-VB05-1-hnxk2-1_v5-315	AGGTCAGGAATACGTGAGTGCACCGGtgccgcctgcgcctgcctgt	Tm Left = 83.64	ΔG Left = -89.22	Hairpins Left = 4	Dimers Left = 0
	HRC2-VB05-1-hnxk2-1_v5-315	tcgatggccgcgtgttggcggTACCCCTTGTGAGATATAGCCAGGTT	Tm Right = 79.14	ΔG Right = -84.16	Hairpins Right = 3	Dimers Right = 0
VB6	HLC2-VB06-1-hnxk2-1_v5-315	AGGTCAGGAATAAGACATGCTGACAGGTgcgcctgcgcctgcctgt	Tm Left = 81.09	ΔG Left = -84.92	Hairpins Left = 7	Dimers Left = 0
	HRC2-VB06-1-hnxk2-1_v5-315	tcgatggccgcgtgttggcggTCCACAAATATAGATATAGCCAGGTT	Tm Right = 76.31	ΔG Right = -80.43	Hairpins Right = 5	Dimers Right = 0
VB7	HLC2-VB07-1-hnxk2-1_v5-315	AGGTCAGGAATATTCATTTGGTACGGTgcgcctgcgcctgcctgt	Tm Left = 78.88	ΔG Left = -83.29	Hairpins Left = 4	Dimers Left = 1
	HRC2-VB07-1-hnxk2-1_v5-315	tcgatggccgcgtgttggcggTCCAGCATGGAAGATTATAGCCAGGTT	Tm Right = 79.63	ΔG Right = -84.86	Hairpins Right = 6	Dimers Right = 0
VB8	HLC2-VB08-1-hnxk2-1_v5-315	AGGTCAGGAATAGGACAAAGGTGCGTgcgcctgcgcctgcctgt	Tm Left = 81.83	ΔG Left = -86.79	Hairpins Left = 3	Dimers Left = 0
	HRC2-VB08-1-hnxk2-1_v5-315	tcgatggccgcgtgttggcggTCAGGCTAATCACATATAGCCAGGTT	Tm Right = 79.49	ΔG Right = -85.14	Hairpins Right = 5	Dimers Right = 1
VB9	HLC2-VB09-1-hnxk2-1_v5-315	AGGTCAGGAATACACTGGGACGAGGtgccgcctgcgcctgcctgt	Tm Left = 83.79	ΔG Left = -89.07	Hairpins Left = 6	Dimers Left = 0
	HRC2-VB09-1-hnxk2-1_v5-315	tcgatggccgcgtgttggcggTCAGAAAGAAAGAAATATAGCCAGGTT	Tm Right = 76.79	ΔG Right = -81.70	Hairpins Right = 3	Dimers Right = 1
VB10	HLC2-VB10-1-hnxk2-1_v5-315	AGGTCAGGAATAGTAACAAACCCATGGTgcgcctgcgcctgcctgt	Tm Left = 79.94	ΔG Left = -84.72	Hairpins Left = 3	Dimers Left = 1
	HRC2-VB10-1-hnxk2-1_v5-315	tcgatggccgcgtgttggcggTGCAGAGCGGAAATATAGCCAGGTT	Tm Right = 81.47	ΔG Right = -88.70	Hairpins Right = 3	Dimers Right = 0
VB11	HLC2-VB11-1-hnxk2-1_v5-315	AGGTCAGGAATAATCGAAGGAGTGGTgcgcctgcgcctgcctgt	Tm Left = 82.06	ΔG Left = -87.36	Hairpins Left = 5	Dimers Left = 1
	HRC2-VB11-1-hnxk2-1_v5-315	tcgatggccgcgtgttggcggTTAGGCTGCTTATCTATAGCCAGGTT	Tm Right = 79.36	ΔG Right = -84.59	Hairpins Right = 4	Dimers Right = 1
VB12	HLC2-VB12-1-hnxk2-1_v5-315	AGGTCAGGAATAGTCTCAGCTCCGAGGTgcgcctgcgcctgcctgt	Tm Left = 82.66	ΔG Left = -86.88	Hairpins Left = 3	Dimers Left = 0
	HRC2-VB12-1-hnxk2-1_v5-315	tcgatggccgcgtgttggcggTGGATTACTTCCAGTATAGCCAGGTT	Tm Right = 78.26	ΔG Right = -83.27	Hairpins Right = 5	Dimers Right = 0
VB13	HLC2-VB13-1-hnxk2-1_v5-315	AGGTCAGGAATATAGGCCGCAAAAGGTgcgcctgcgcctgcctgt	Tm Left = 80.07	ΔG Left = -85.90	Hairpins Left = 6	Dimers Left = 0
	HRC2-VB13-1-hnxk2-1_v5-315	tcgatggccgcgtgttggcggTTGTATACATAATATAGCCAGGTT	Tm Right = 74.05	ΔG Right = -78.38	Hairpins Right = 5	Dimers Right = 1
VB14	HLC2-VB14-1-hnxk2-1_v5-315	AGGTCAGGAATAGTGGCTTTGATAGGtgccgcctgcgcctgcctgt	Tm Left = 80.94	ΔG Left = -85.52	Hairpins Left = 3	Dimers Left = 0
	HRC2-VB14-1-hnxk2-1_v5-315	tcgatggccgcgtgttggcggTGAATGGACTAGCACTATAGCCAGGTT	Tm Right = 79.21	ΔG Right = -83.92	Hairpins Right = 3	Dimers Right = 1
VB15	HLC2-VB15-1-hnxk2-1_v5-315	AGGTCAGGAATACGGTGTCTGTTTGGTgcgcctgcgcctgcctgt	Tm Left = 81.06	ΔG Left = -87.29	Hairpins Left = 3	Dimers Left = 0
	HRC2-VB15-1-hnxk2-1_v5-315	tcgatggccgcgtgttggcggTCCGCTGTTCTACACTATAGCCAGGTT	Tm Right = 80.03	ΔG Right = -85.55	Hairpins Right = 3	Dimers Right = 1
VB16	HLC2-VB16-1-hnxk2-1_v5-315	AGGTCAGGAATAGACTACTCAAACTGGTgcgcctgcgcctgcctgt	Tm Left = 79.62	ΔG Left = -83.05	Hairpins Left = 3	Dimers Left = 0
	HRC2-VB16-1-hnxk2-1_v5-315	tcgatggccgcgtgttggcggTATGACACTTCAAGTATAGCCAGGTT	Tm Right = 78.22	ΔG Right = -82.82	Hairpins Right = 5	Dimers Right = 0
VB17	HLC2-VB17-1-hnxk2-1_v5-315	AGGTCAGGAATATGCTGTAATGATGGTgcgcctgcgcctgcctgt	Tm Left = 80.35	ΔG Left = -84.53	Hairpins Left = 1	Dimers Left = 1
	HRC2-VB17-1-hnxk2-1_v5-315	tcgatggccgcgtgttggcggTTAAGAGAACTTCTTATAGCCAGGTT	Tm Right = 76.32	ΔG Right = -81.30	Hairpins Right = 5	Dimers Right = 0
VB18	HLC2-VB18-1-hnxk2-1_v5-315	AGGTCAGGAATAAATTTAAGCAATGGTgcgcctgcgcctgcctgt	Tm Left = 78.43	ΔG Left = -83.66	Hairpins Left = 4	Dimers Left = 1
	HRC2-VB18-1-hnxk2-1_v5-315	tcgatggccgcgtgttggcggTAGCATATCTTCTATATAGCCAGGTT	Tm Right = 76.10	ΔG Right = -80.23	Hairpins Right = 3	Dimers Right = 0
VB19	HLC2-VB19-1-hnxk2-1_v5-315	AGGTCAGGAATAGACTTTCCGCGGGTgcgcctgcgcctgcctgt	Tm Left = 82.10	ΔG Left = -88.46	Hairpins Left = 6	Dimers Left = 1
	HRC2-VB19-1-hnxk2-1_v5-315	tcgatggccgcgtgttggcggTCTTTTGTAAACAAATATAGCCAGGTT	Tm Right = 75.73	ΔG Right = -81.33	Hairpins Right = 4	Dimers Right = 0
VB20	HLC2-VB20-1-hnxk2-1_v5-315	AGGTCAGGAATACTTCCGGAACAGGTgcgcctgcgcctgcctgt	Tm Left = 81.97	ΔG Left = -88.11	Hairpins Left = 6	Dimers Left = 1
	HRC2-VB20-1-hnxk2-1_v5-315	tcgatggccgcgtgttggcggTATAGTAACAGGACTATAGCCAGGTT	Tm Right = 77.58	ΔG Right = -82.18	Hairpins Right = 4	Dimers Right = 2
VB21	HLC2-VB21-1-hnxk2-1_v5-315	AGGTCAGGAATAAGGCGCCCTGCCGGTgcgcctgcgcctgcctgt	Tm Left = 84.91	ΔG Left = -91.81	Hairpins Left = 5	Dimers Left = 2
	HRC2-VB21-1-hnxk2-1_v5-315	tcgatggccgcgtgttggcggTGATCCCGACGATTTATAGCCAGGTT	Tm Right = 80.77	ΔG Right = -87.99	Hairpins Right = 6	Dimers Right = 0
VB22	HLC2-VB22-1-hnxk2-1_v5-315	AGGTCAGGAATAAGCAGCAAGTTGGTgcgcctgcgcctgcctgt	Tm Left = 81.14	ΔG Left = -86.85	Hairpins Left = 3	Dimers Left = 0
	HRC2-VB22-1-hnxk2-1_v5-315	tcgatggccgcgtgttggcggTAGAGGACGACGATATAGCCAGGTT	Tm Right = 80.96	ΔG Right = -87.53	Hairpins Right = 4	Dimers Right = 0
VB23	HLC2-VB23-1-hnxk2-1_v5-315	AGGTCAGGAATATGTACGGCGAGGGTgcgcctgcgcctgcctgt	Tm Left = 83.95	ΔG Left = -90.00	Hairpins Left = 8	Dimers Left = 2
	HRC2-VB23-1-hnxk2-1_v5-315	tcgatggccgcgtgttggcggTTTATACAGAGACTATAGCCAGGTT	Tm Right = 77.06	ΔG Right = -82.16	Hairpins Right = 4	Dimers Right = 0
VB24	HLC2-VB24-1-hnxk2-1_v5-315	AGGTCAGGAATACTGTACATACGGGGTgcgcctgcgcctgcctgt	Tm Left = 81.41	ΔG Left = -85.98	Hairpins Left = 3	Dimers Left = 1
	HRC2-VB24-1-hnxk2-1_v5-315	tcgatggccgcgtgttggcggTCCAACCGTGCCTATAGCCAGGTT	Tm Right = 82.04	ΔG Right = -89.38	Hairpins Right = 6	Dimers Right = 1

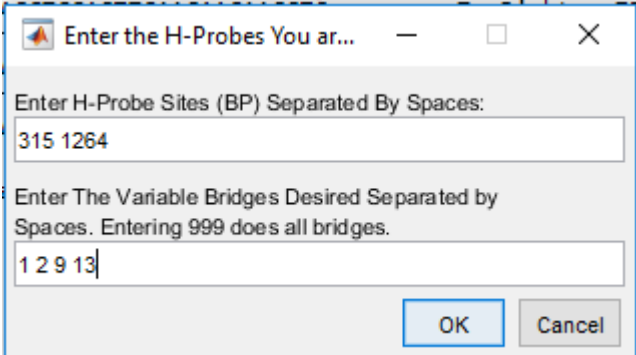
# Extra Options:



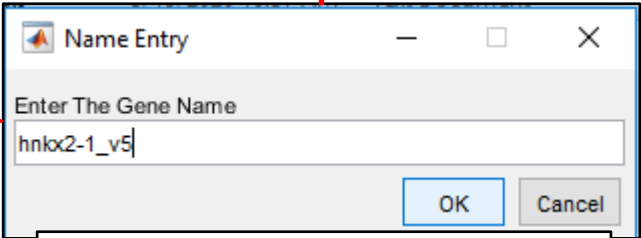
Selecting this options will use the existing MATLAB data to re-choose probes based on a different BLAST bitscore cutoff. (This saves you from having to Re-Blast every site in the gene).



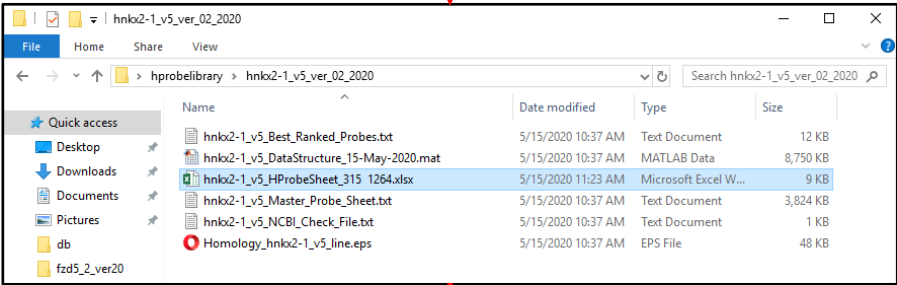
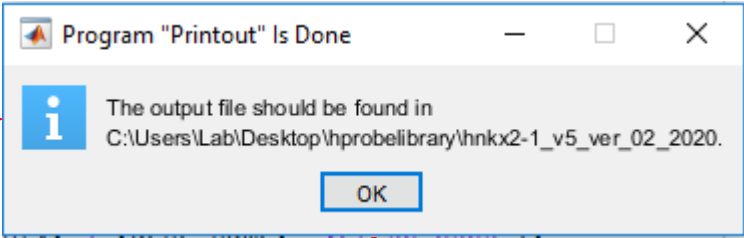
Selecting this allows you to make a probe list which can be sent to IDT for ease of ordering.



Enter the BP ID of the probes you want to use for this gene, as well as a list of variable bridges you want them designed in.




The name must match the one originally used exactly.



Next Slide



# Extra Options: Probe Ordering List

 hnkx2-1\_v5\_HProbeSheet\_315 1264.xlsx

5/15/2020 11:23 AM

Microsoft Excel W...

9 KB

hnkx2-1\_v5\_HProbeSheet\_315 1264.xlsx - Excel

FileHomeInsertPage LayoutFormulasDataReviewViewAcrobatTell me what you want to do...Sign inShare

CutCopyFormat Painter

Clipboard

Calibri11

**B***I*U

Font Color

Background Color

Font

Alignment

General

\$ % ' .0 0.00

Number

Conditional Formatting

Format as Table

NormalBadGoodNeutral

CalculationCheck CellExplanatory...Input

Styles

InsertDeleteFormat

Cells

AutoSumFillClear

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HLC2-VB01-1-hnkx2-1\_v5-315

	A	B	C	D	E	F	G	H
1	Count	Fluorophore	Common Circle	Probe Name	H-Probe Sequence	mRNA Target	Top Blast Result (Check)	
2	1	VB1	CCC2.1	HLC2-VB01-1-hnkx2-1_v5-315	AGGTCAGGAATACTTACGTCGTTATGGTgccgcctggccctgcctgt	acaggcagggccaggcggcaccgccaacagcgccatgca	NM_001079668.3 Homo sapiens NK2 homeobox 1 (NKX2-1), transcript variant 1, mRNA	
3				HRC2-VB01-1-hnkx2-1_v5-315	tgcatggccgctgttggcggTTATAGGTCGAGTAGTATAGCCAGGTT			
4	2	VB1	CCC2.1	HLC2-VB01-1-hnkx2-1_v5-1264	AGGTCAGGAATACTTACGTCGTTATGGTggagcaggacatggtgccg	cggcaccatgtcctgctccacctgtctatcggtcggacc	NM_001079668.3 Homo sapiens NK2 homeobox 1 (NKX2-1), transcript variant 1, mRNA	
5				HRC2-VB01-1-hnkx2-1_v5-1264	ggtccgaccgtatagcaaggTTATAGGTCGAGTAGTATAGCCAGGTT			
6								
7	1	VB2	CCC2.1	HLC2-VB02-1-hnkx2-1_v5-315	AGGTCAGGAATACTTAGCTATTGATGGTgccgcctggccctgcctgt	acaggcagggccaggcggcaccgccaacagcgccatgca	NM_001079668.3 Homo sapiens NK2 homeobox 1 (NKX2-1), transcript variant 1, mRNA	
8				HRC2-VB02-1-hnkx2-1_v5-315	tgcatggccgctgttggcggTTCTGTGTAGACGACTATAGCCAGGTT			
9	2	VB2	CCC2.1	HLC2-VB02-1-hnkx2-1_v5-1264	AGGTCAGGAATACTTAGCTATTGATGGTggagcaggacatggtgccg	cggcaccatgtcctgctccacctgtctatcggtcggacc	NM_001079668.3 Homo sapiens NK2 homeobox 1 (NKX2-1), transcript variant 1, mRNA	
10				HRC2-VB02-1-hnkx2-1_v5-1264	ggtccgaccgtatagcaaggTTCTGTGTAGACGACTATAGCCAGGTT			
11								
12	1	VB9	CCC2.1	HLC2-VB09-1-hnkx2-1_v5-315	AGGTCAGGAATACACTGGGCACGAGGtgccgcctggccctgcctgt	acaggcagggccaggcggcaccgccaacagcgccatgca	NM_001079668.3 Homo sapiens NK2 homeobox 1 (NKX2-1), transcript variant 1, mRNA	
13				HRC2-VB09-1-hnkx2-1_v5-315	tgcatggccgctgttggcggTCATGAAGAAAAAGAATATAGCCAGGTT			
14	2	VB9	CCC2.1	HLC2-VB09-1-hnkx2-1_v5-1264	AGGTCAGGAATACACTGGGCACGAGGtgagcaggacatggtgccg	cggcaccatgtcctgctccacctgtctatcggtcggacc	NM_001079668.3 Homo sapiens NK2 homeobox 1 (NKX2-1), transcript variant 1, mRNA	
15				HRC2-VB09-1-hnkx2-1_v5-1264	ggtccgaccgtatagcaaggTCATGAAGAAAAAGAATATAGCCAGGTT			
16								
17	1	VB13	CCC2.1	HLC2-VB13-1-hnkx2-1_v5-315	AGGTCAGGAATATAGGCCGAAAAAAGGtgccgcctggccctgcctgt	acaggcagggccaggcggcaccgccaacagcgccatgca	NM_001079668.3 Homo sapiens NK2 homeobox 1 (NKX2-1), transcript variant 1, mRNA	
18				HRC2-VB13-1-hnkx2-1_v5-315	tgcatggccgctgttggcggTTGTATACTATTAATATAGCCAGGTT			
19	2	VB13	CCC2.1	HLC2-VB13-1-hnkx2-1_v5-1264	AGGTCAGGAATATAGGCCGAAAAAAGTggagcaggacatggtgccg	cggcaccatgtcctgctccacctgtctatcggtcggacc	NM_001079668.3 Homo sapiens NK2 homeobox 1 (NKX2-1), transcript variant 1, mRNA	
20				HRC2-VB13-1-hnkx2-1_v5-1264	ggtccgaccgtatagcaaggTTGTATACTATTAATATAGCCAGGTT			
21								
22								

Variable  
Bridges  
Selected

Names and Full H-probe sequences for left and right probes.  
List may be copied and pasted into online ordering form

mRNA Site for probe set.  
For Double Checking.

Top blast result for this listed mRNA  
site. Again, double check.

Always double check the script output, the variable bridge sequence, and blast these sites by hand before ordering.