Creating with CRISPR

Gtf2i is gene which encodes the general transcription factor, TFII-I. TFII-I is ubiquitously expressed, but all its many functions are not known. It both positively and negatively regulates transcription and its isoforms appear to be differentially expressed throughout the body. Interestingly, Gtf2i is one of the genes deleted in Williams Syndrome, and has been connected to social phenotypes in mice, dogs, and humans.

Your lab wants to explore the functions of TFII-I in the hypothalamus, starting with a mouse knockout model. You have been tasked with creating the guides that will be used to make this model, and to ensure all main isoforms of Gtf2i are affected.

1. Briefly describe two general ways you could use guide RNAs (gRNA) with classic CRISPR/Cas9 to achieve a knockout. (What would you target, generally? How could your target change if you used 2 gRNAs? Resources provided at the bottom of the page.)

2. Which method would you use? Why?

3. Where in Gtf2i would you target? Why? (Be relatively specific: which intron(s), exon(s), etc.)

4. Using crispr.mit.edu (or another guide design site if specified in part b), design the guide RNAs you would use. The site below has the sequence for the full Gtfi2i transcript. http://useast.ensembl.org/Mus_musculus/Transcript/Exons?db=core;g=ENSMUSG00000060261;r=5:134237834-134314760;t=ENSMUST00000059042
   a. List the top five guides (or guide pairs).
   b. How does the MIT algorithm rank these (generally)? If you used an alternative, explain i) what you used, ii) why you chose it, and iii) how it works.

5. Succinctly describe the process of making a knockout mouse using your guide(s). Include the general method used and the specific molecular process taking place. Note, these methods would most likely be done at a facility after you provide the guides.

6. How would you test to validate the knockout?

7. You successfully make a knockout mouse! However, after genotyping, you realize your crosses are only producing wild type and heterozygous mice. What does this indicate?

8. Briefly describe how you could change your approach to study homozygous knockouts.

Resources:
Blog post: https://innovativegenomics.org/blog/how-to-make-a-guide-rna-for-cas9/
Primary lit: https://www.nature.com/articles/ncomms9083
Review (see 8 &9): https://www.hindawi.com/journals/sci/2017/8765154/
Addgene Reference: https://www.addgene.org/crispr/genomic-deletions-mammalian-cell-lines/