miRNA ~ gene -> transcribed into short RNA -> processed into 21-nucleotide RNA (=siRNA) -> loaded onto Dicer -| target

Making miRNA:

- constitutive:
    - DNA ---(Pol2) --> preRNA
    - DNA ---(Pol3) --> ncRNA
    - For Pol3, use U6 promoter (a constitutive promoter that makes non-coding RNAs)
- inducible

Do we need to introduce a copy of BACE1 into cells? Find out!

Check that miRNA works in mammalian cells:

Constitutive:
eYFP

- Two plasmids:
    - U6 + miRNA against eYFP
    - hEF1a + eYFP
- Transfections
    - eYFP plasmid alone -> Yellow
    - eYFP plasmid + miRNA plasmid -> No yellow

BACE1

- Transfections
    - U6 + miRNA against BACE1 -> BACE1 downregulated
    - U6 + miRNA against eYFP -> BACE1 levels normal
    - No transfection -> ?

Inducible: introns
Check that introns work: eYFP

- Two plasmids:
    - 1: hEF1a + [mKate (left) -- miRNA (against eYFP) -- mKate (right)]
    - 2: hEF1a + eYFP
- Transfections
    - 1 -> red
    - 2 -> yellow
    - 1 + 2 -> red + less yellow

Check that can be induced: eYFP

- Plasmids:
    - TRE-T + [mKate (left) -- miRNA (against eYFP) -- mKate (right)]
        - CAG + rTTA3

        - rTTA3 + Dox induces expression -> add different levels of Dox
        - linear relationship between [mKate] and log[Dox]
    - hEF1a + eYFP
- Logic
    - mKate is a measurement of miRNA expression: [mKate] = [miRNA]
    - Measure output through eYFP expression (yellow)
    - Graph [output] vs. [miRNA] to characterize system
    - How much input do I need to get output that I can measure?