miRNA Sensor Design & Target Selection

A few general notes:

The convention in recording sequences is to always write from the 5' terminus to the 3'-terminus. Ideally, both should be labeled. For example:

5'-TAG CTT ATC AGA CTG ATG TTGA-3'

For some miRNA species, it might not be immediately clear from the database(s) which sequences are the passenger strand or the active strand. Some pre-miRNA are able to be cleaved in multiple places to yield different, active, mature miRNA. In some miRNA species, both the passenger strand and active strand have been shown to have biological effects. You may have to search for additional papers that support which of multiple strands you should be actively sensing.

Resources:

- miRDB: http://mirdb.org/miRDB/index.html
- miRBase: http://www.mirbase.org
- rFam: http://rfam.xfam.org
- RNAfold: http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi
- UNAfold: http://unafold.rna.albany.edu

Target Selection Workflow:

- What miRNA are you interested in sensing?
- What cell lines is it expressed in?
- What are some references for its expression?
- Is it upregulated?
- What are references for it's upregulation?
- Is there differential expression in MCF7 relative to MDA-MB-231 cells?
- What are some mRNA targets that have been validated?
- What disease states is it known to effect, and how does it effect them (positively or negatively, pathways, mechanisms)?
- What is the sequence of the pre-miRNA?
- Do UNAfold and mfold predict similar folding of the pre-miRNA? Do they match the folds shown on mirbase?
- What mature miRNAs are formed from the pre-miRNA?
- Which mature miRNA is the "passenger strand", and which is the active strand?
- What is the sequence of the active, mature, miRNA target?

Sensor Design:

- What is the complementary sequence to your target miRNA? This is your sensing strand.
- What is the DNA analogue (T instead of U) of your target miRNA? This is your "target" strand. Later on, we will shorten it to create a "guide" strand.
- Using the DINAmelt Server (on UNAfold), what is the predicted melting temperature of the complement:analogue duplex at 1 µM and 100 mM Na^+?
- Is it stable at room temperature? Biological temperature?

For these experiments, prices dictate the use of a 5’-Fluorescein on the “sensing” strand (the complement to your miRNA). Accordingly, to be in close proximity, your guide strand should have either a Cy3 or Dabcyl at the 3’-terminus.

Start cutting off bases (1-2 at a time) from the 5’-end of your analogue to make potential guide strands. Run each sequence through DINAmelt hybridizing to your sensing strand. Save both the resulting melting temperatures (Tm) and the resulting melting curves. You’re looking for a point at which you’ve destabilized the guide strand: sensing strand duplex enough relative to the sensing strand: miRNA duplex enough to make a switch possible, but not by enough to make the resulting duplex fall apart at biological temperatures. A Tm in the mid-to-low 50s will likely be appropriate.

Next, you need to consider what kind of sensor you want to make. You can either make a “signal on” sensor where exposure to your target miRNA takes a quenched, dark sensor and “turns on” the fluorescence using a “dark quencher” at the 3’-terminus of your guide strand, or you can make a FRET sensor that switches from Cy3 (red) emission to fluorescein (green) emission on exposure to the target miRNA, by placing a Cy3 molecule at the 3’-terminus of your guide strand.

Each method has benefits and detractions. A FRET sensor allows you to track both the inactive sensor (by red fluorescence) and the active sensor (by green fluorescence), and gauge the relative switching. FRET is a lower efficiency process, however, and there’s usually a lower signal difference overall. A quenched sensor, on the other hand, provides very good signal change, but doesn’t allow you to track the inactive sensor at all.