Artificial microRNA design:

1. amiR sequence requirements:
- artificial microRNA has length 21
- Position 1 is U (this can also be engineered since a mismatch at position1 is tolerated)
- Position 10 is A
- Position 19 is G or C
- require 5' instability
- GC content should be about 50%

2. target requirements:
- No mismatch to positions 2 to 12 of microRNA
- no more than 3 mismatches to positions 13 to 21 of microRNA
- at least 75% of perfect match energy (<-30kcal/mole at least)
- no clusters of more than two mismatches in a row

genes you do NOT want to target:
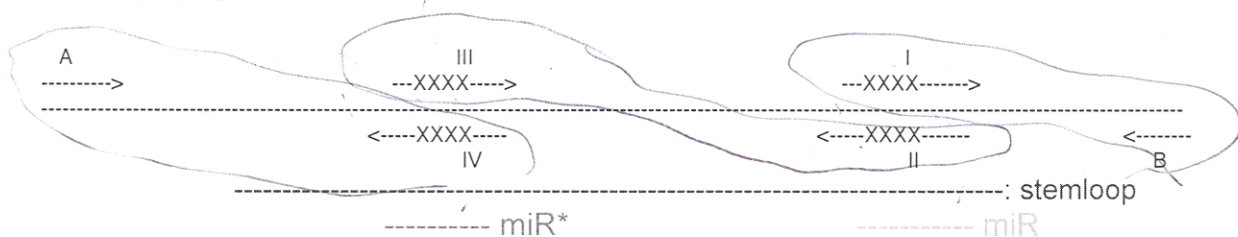- less than 65% of perfect match energy (always > -30kcal/mole)

All of 1. and 2. are automated.
Output: 4 oligo sequences (I to IV) to engineer the artificial microRNA into miR319a stemloop by site-directed mutagenesis.

3. Template: RS300 -> miR319a stemloop in pbluescript (SK).

4. cloning steps:

PCR scheme:

```
   A                          III                              I
---------->            ---XXXX----->              ---XXXX------->
-----------------------------------------------------------------------------------------      <--------
                      <-----XXXX-----              <-----XXXX-------                              B
                           IV                           II
       ----------------------------------------------------------------------------: stemloop
          ----------- miR*                              ------------ miR
```

Oligos A and B are outside outside of the MCS of pbluescript to generate bigger PCR products.

4368 A: 5' ctg CAA GGC GAT TAA GTT GGG TAA C 3'
4369 B: 5' gcg GAT AAC AAT TTC ACA CAG GAA ACA G 3'

Oligos I-IV:

I	II	III	IV
miR-sense	miR-anti	miR*sense	miR*anti