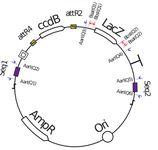
MAV1212



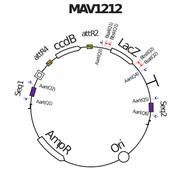
This is the starting multi-assembly vector (MAV) we had synthesized. It contains att sites for Gateway assembly with promoter-gene combinations, Gibson sequences for assembling transcription units and three sets of Golden Gate enzyme recognition sites. BbsI is used for assembling miRNA target sites from annealed ultramers into the plasmid, BsaI is used for donating miRNA target sites to new plasmids, and AarI is used for repositionalizing the Gibson or Golden Gate overhangs to a combination that is more useful for a given construction.

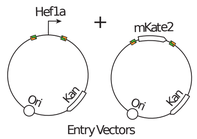
In the assemblies shown here, we repositionalize the low sensor MAV to have Q1-Q3 BsaI overhangs while retaining the BbsI overhangs and the Gibson sequences. We also repositionalize the high sensor MAV to have Q3-Q4 BsaI overhangs and Seq2-Seq3 Gibson overhangs while retaining the BbsI overhangs. We should also consider assembling miRNA target sites that donate with Q4-Q2 BsaI overhangs, though the most useful promoter-gene combination besides the low and high sensor here is yet to be determined.

The three dif erent overhang combinations for single miRNA target sites that we propose here can then be used to assemble any 3-input miRNA sensor (eg. Hef1a-mKate2-Q1-miR 1 target-Q3-miR 2 target-Q4-miR 1 target-Q2).

Low Sensor

MAV1212

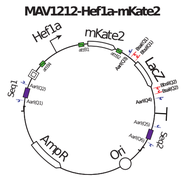


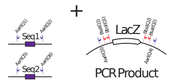


Entry Vectors

Gateway

MAV1212-Hef1a-mKate2



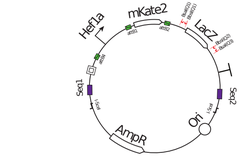


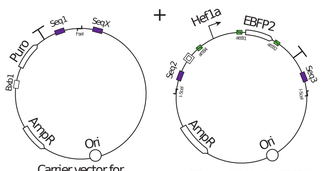
Annealed Oligos

PCR Product

Golden Gate (AarI)

MAV1213-Hef1a-mKate2





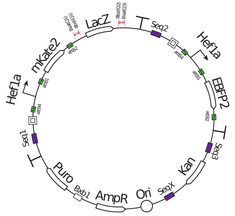
Carrier vector for Bxb1 integration

pZDonor23-Hef1a-EBFP2

PCR Product

Gibson (I-SceI, FseI linearized)

Integrative Low Sensor Backbone



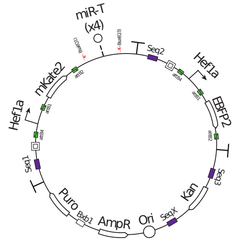
miR-T (x4)

Annealed Ultramers (400 variants)

Golden Gate (BbsI)

Perform on chip

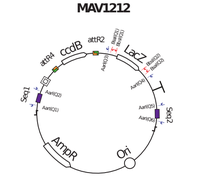
All Integrative Low Sensors

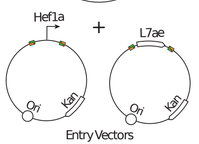


Donates miRNA target sites with Q1, Q3 overhangs using BsaI

High Sensor

MAV1212

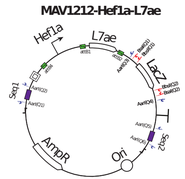


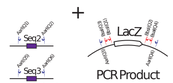


Entry Vectors

Gateway

MAV1212-Hef1a-L7ae



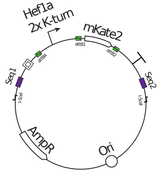


Annealed Oligos

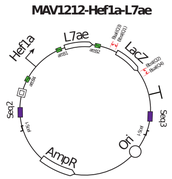
PCR Product

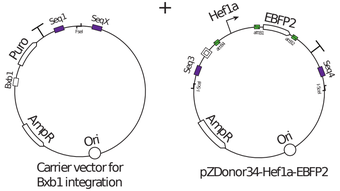
Golden Gate (AarI)

pZDonor12-Hef1a 2x K-turn-mKate2



MAV1212-Hef1a-L7ae





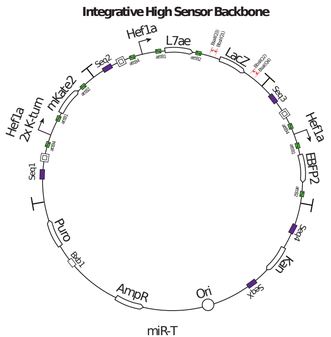
Carrier vector for Bxb1 integration

pZDonor34-Hef1a-EBFP2

PCR Product

Gibson (I-SceI, FseI linearized)

Integrative High Sensor Backbone



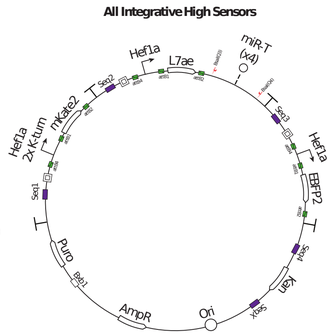
miR-T (x4)

Annealed Ultramers (400 variants)

Golden Gate (BbsI)

Perform on chip

All Integrative High Sensors



Donates miRNA target sites with Q3, Q4 overhangs using BsaI

? Sensor

Gateway

Golden Gate (AarI)

Gibson (I-SceI, FseI linearized)

Golden Gate (BbsI)

Perform on chip

Some other single sensors (Cas9 guide RNA?)

Donates miRNA target sites with Q4, Q2 overhangs using BsaI