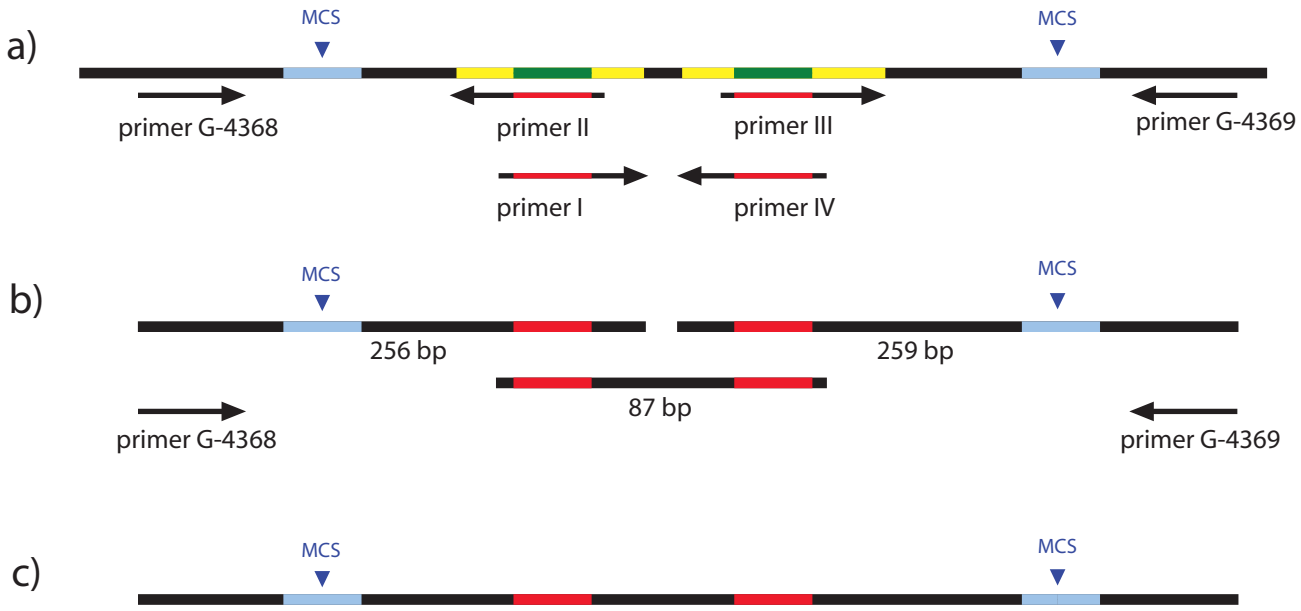
A)

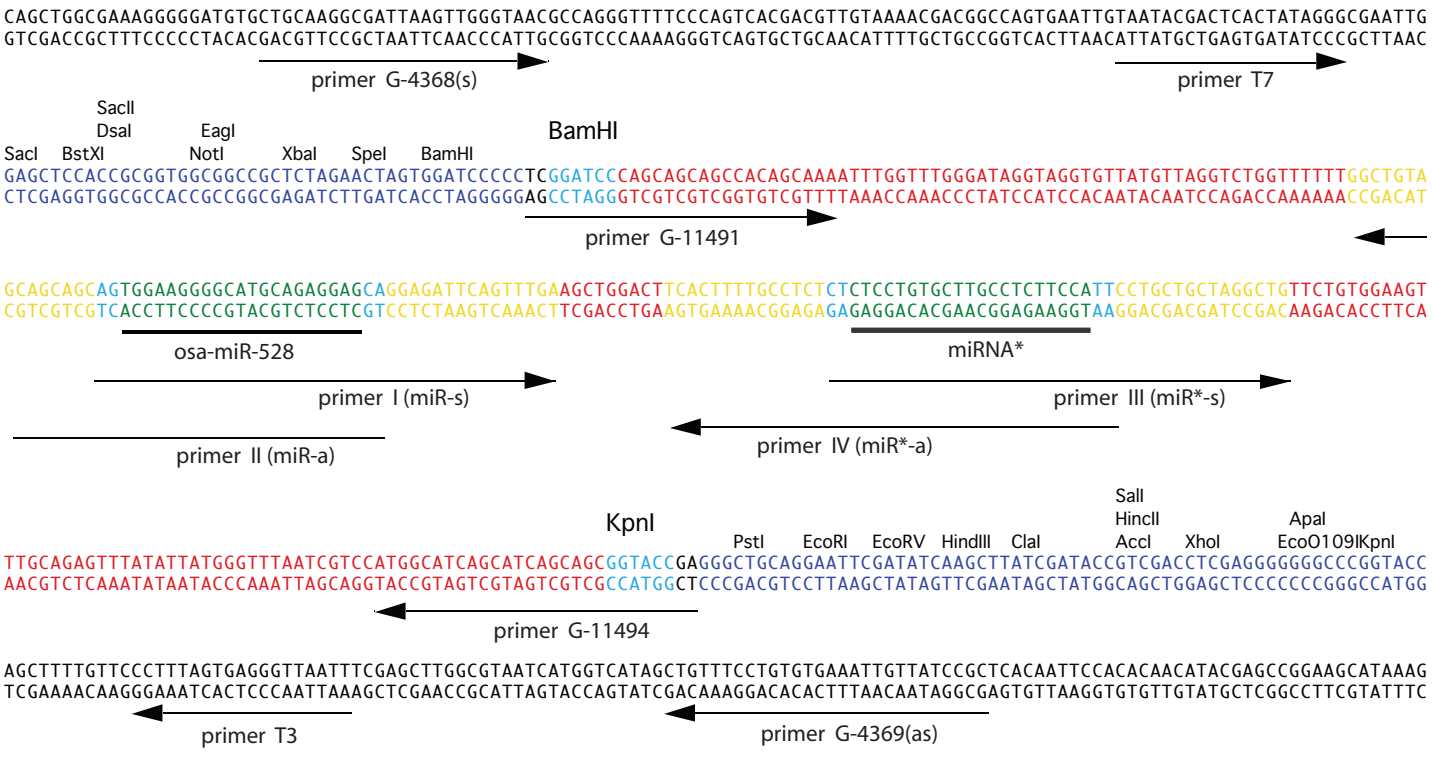
PCR scheme to produce artificial miRNA constructs from pNW55



a) The original miRNA 528 and miRNA* sequences of pNW55 (green) will be replaced by the artificial miRNA sequences (red) during the first PCRs. Sequences in pNW55 complementary to the primers are indicated in yellow and the multiple cloning sites in blue.
b) The 3 PCRs on pNW55 as template (G-4368 + II, I + IV, III + G-4369) result in 3 DNA fragments.
c) Fusion PCR on the 3 PCR products from b) with primers G-4368 + G-4369 results in one DNA fragment for subsequent cloning.

B)

pNW55 - osaMIR528 stemloop in pBluescript KS



PCR protocols for directed mutagenesis of pNW55[a].

Modification PCRs on template clone pNW55:		
Primer	Product size	PCR protocol
G-4368 + primer II	256 bp	95°C 2 min; 34 cycles of 95°C 30s, 55°C 30s, 72°C 30s; 72°C 7 min
Primer I + primer IV	87 bp	
Primer III + G-4369	259 bp	
Fusion PCR on a mixture of all PCR products of the Modification PCRs:		
Primer	Product size	PCR protocol
G-4368 + G-4369	554 bp	95°C 2 min; 34 cycles of 95°C 30s, 55°C 30s, 72°C 1min; 72°C 7 min

[a] All PCRs were performed with ProofStart ™ DNA Polymerase (Qiagen)

Universal primer sequences:

G-4368: `CTG CAA GGC GAT TAA GTT GGG TAA C`
G-4369: `GCG GAT AAC AAT TTC ACA CAG GAA ACA G`