Cloning of pdsAAV2-miRNA constructs

1. Initial miRNA-expression vector:

- For the cloning of the initial miRNA constructs, we used the "BLOCK-iT™ Pol II miR RNAi Expression Vector Kits" (Invitrogen, Catalog nos. K4935-00, K4936-00, K4937-00, K4938-00): In short, single-stranded miRNA-oligos were selected using Invitrogen´s RNAi designer (www.invitrogen.com/rnai), annealed and inserted into the pcDNA6.2-GW/EmGFP-miR vector (Gateway®-adapted expression vector for the expression of miRNA in mammalian cells under control of Pol II promoters) according to manufacturer's protocol.

- It is recommended to choose several miRNA sequences for a particular target and to test for knockdown efficiency in vitro. Alternatively, one might consider already validated, pre-designed miRNA sequences if available.

2. Subcloning into pdsAAV vector

- The miRNA sequence is subcloned from the pcDNA6.2-GW/EmGFP-miR vector to the pdsAAV vector. We use by default SalI and BglII digestion.

- AAV serotype 2 vectors (Amp resistance):
  - ✓ original vector: pdsAAV-CMV-GFP (Wang et al., Gene Therapy 2003) provided by Z. Wang and X. Xiao via J. Kleinschmidt (DKFZ).
  - ✓ pdsAAV2-CMV-GFP-miRNA
  - ✓ pdsAAV2-LP1-GFPmut-miRNA (hepatocyte-specific artificial promoter; no GFP translation)

- Following ligation, SURE 2 Supercompetent cells (Stratagene) are transformed with pdsAAV constructs according to manufacturer´s protocol.

- Pick several clones for each constructs for Miniprep cultures. Send the constructs for sequencing of the miRNA using the EGFP-C1-F primer (e.g. LGC, included in standard primer collection). Check miRNA sequences for potential mutations.
  EGFP-C1-F: GAAGCGCGATCACATGGTC

- Perform a restriction test for intact inverted terminal repeats (ITRs; sequencing is difficult):
  ITR-restriction panel:

Enzyme	Fragments
Sma I	3762; 1262; 788
BssII/PvuI	1976; 1471; 1272; 945; 85
BssII/Pst I	3688; 1481; 495; 85
BssII/NotI	3688; 1485; 295; 196; 85
MscI	5329; 505