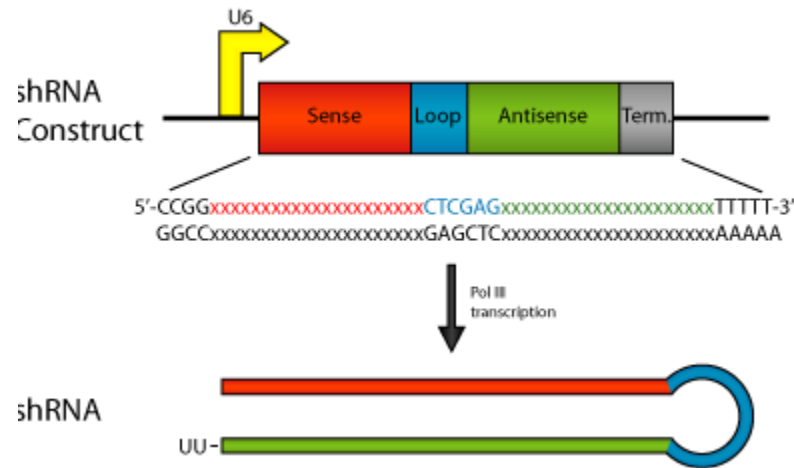


# How to design and build up a shRNA?

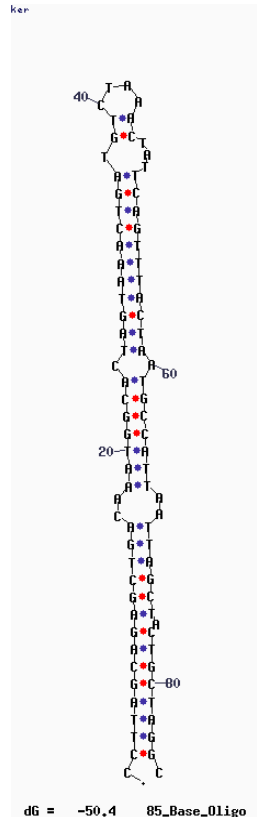


# Human *hcr* gene comprising miR-122

TGGAGGTGAAGTTAACACCTTCGTGGCTACAGAGTTTCCTTAGCAGAGCTGTGGAGTGTGACAA  
TGGTGTGTTGTGTCTAAACTATCAAACGCCATTATCACACTAAATAGCTACTGCTAGGCAATCCTTC  
CCTCGATAAATGTCTTGGCATCGTTTGCTT

Green: guide strand

Red: passenger strand





# 4 oligos need to be ordered:

- **hcrfor** (GAGTC **ACCGGT** TGGAGGTGAAGTTAACACCTTCGTG)  
(AgeI)
- **hcrrev** (CTCGA **GTCGAC** AAGCAAACGATGCCAAGACATTTATCG )  
(Sall)
- **Gene for** (**ACTC** TGTCTAAACTAT **GAGTACATTAACAATTCCTAT**  
TAGCTACTGCTAGGC)  
**green: 4 nucleotides of 3' guide strand**  
**red: passenger strand**
- **Gene rev** (**ACTC** ATAGTTTAGACA **GAGTACATTAATCAATTCCATT**  
CAGCTCTGCTAAGG)  
**green: 4 inverted nucleotides of 5' passenger strand**  
**red: inverted guide strand**

# PCR to „build up“ the shRNA

- **1st round:** template: hcr [miRsAg];  
Primer: hcrfor and Gene R  
+ Primer: hcrrev and Gene F
- **2nd round:** 2 products= template;  
Primers: hcrfor and hcrrev
- programm saved: shRNA