Supplemental Figures NAR-01052-2005 Rose et al.

Table S1

EGFP Sequences

```
5′ caagctgaccctgaagttcatctgcaccaccggcaa               EGFP Site-1

5′        ACCCUGAAGUUCAUCUGCACC                      EGFPS1 21/21
3′      ACUGGGACUUCAAGUAGACGU

5′        ACCCUGAAGUUCAUCUGCACCA                     EGFPS1 R 22/22
3′      ACUGGGACUUCAAGUAGACGUG

5′       GACCCUGAAGUUCAUCUGCACC                      EGFPS1 L 22/22
3′     GACUGGGACUUCAAGUAGACGU

5′       pACCCUGAAGUUCAUCUGCACCACcg                  EGFPS1 R 25D/27
3′      ACUGGGACUUCAAGUAGACGUGGUGGC

5′  AAGCUGACCCUGAAGUUCAUCUGCACC                      EGFPS1 L 27/25D
3′  ttCGACUGGGACUUCAAGUAGACGUp


5′ ccatgaagcagcacgacttcttcaagtccgccatgc               EGFP Site-2

5′      AAGCAGCACGACUUCUUCAAGUCCGCC                  EGFPS2 R 27/27
3′      UUCGUCGUGCUGAAGAAGUUCAGGCGG

5′      aagCAGCACGACUUCUUCAAGUCCGCC                  EGFPS2 R 27(5′D)/27
3′      UUCGUCGUGCUGAAGAAGUUCAGGCGG

5′      AAGCAGCACGACUUCUUCAAGUCCgcc                  EGFPS2 R 27(3′D)/27
3′      UUCGUCGUGCUGAAGAAGUUCAGGCGG

5′      AAGCAGCACGACUUCUUCAAGUCCGCC                  EGFPS2 R 27/27(3′D)
3′      ttcGUCGUGCUGAAGAAGUUCAGGCGG

5′      AAGCAGCACGACUUCUUCAAGUCCGCC                  EGFPS2 R 27/27(5′D)
3′      UUCGUCGUGCUGAAGAAGUUCAGGcgg

5′      AAGCAGCACGACUUCUUCAAGUCCGCC                  EGFPS2 R 27/25
3′      UUCGUCGUGCUGAAGAAGUUCAGGC

5′      AAGCAGCACGACUUCUUCAAGUCCGCC                  EGFPS2 R 27/25(3′D)
3′      ttcGUCGUGCUGAAGAAGUUCAGGC

5′        GCAGCACGACUUCUUCAAGUCCGCC                  EGFPS2 R 25/27
3′      UUCGUCGUGCUGAAGAAGUUCAGGCGG

5′       pGCAGCACGACUUCUUCAAGUCCGcc                  EGFPS2 R 25D/27
3′      UUCGUCGUGCUGAAGAAGUUCAGGCGG

5′  CAUGAAGCAGCACGACUUCUUCAAGUC                      EGFPS2 L 27/25D
3′  gtACUUCGUCGUGCUGAAGAAGUUCp

5′        GCAGCACGACUUCUUCAAGUC                      EGFPS2 21/21
3′      UUCGUCGUGCUGAAGAAGUUC

5′        GCAGCACGACUUCUUCAAGUCC                     EGFPS2 R 22/22
3′      UUCGUCGUGCUGAAGAAGUUCA

5′       AGCAGCACGACUUCUUCAAGUC                      EGFPS2 L 22/22
3′     CUUCGUCGUGCUGAAGAAGUUC
```

hnRNPH Sequences

5′ ttgttgaacttgaatcagaagatgaagtcaaattggc 3′ hnRNPH Sites 1 & 3

5′ AACUUGAAUCAGAAGAUGAAG H1 21/21
3′ ACUUGAACUUAGUCUUCUACU

5′ pAACUUGAAUCAGAAGAUGAAGUC**aa** H1 R 25D/27
3′ ACUUGAACUUAGUCUUCUACUUCAGUU

5′ pAACUUGAAUCAGAAGAUGAAGUC**aa** H1 R 25D/27D
3′ **ac**UUGAACUUAGUCUUCUACUUCAGUU

5′ UUGUUGAACUUGAAUCAGAAGAUGAAG H1 L 27/25D
5′ **aa**CAACUUGAACUUAGUCUUCUACUp

5′ CUUGAAUCAGAAGAUGAAGUC H3 21/21
3′ UUGAACUUAGUCUUCUACUUC

5′ pCUUGAAUCAGAAGAUGAAGUCAA**at** H3 R 25D/26
3′ UGAACUUAGUCUUCUACUUCAGUUUA

5′ pCUUGAAUCAGAAGAUGAAGUCAA**at** H3 R 25D/27
3′ UUGAACUUAGUCUUCUACUUCAGUUUA

5′ pCUUGAAUCAGAAGAUGAAGUCAA**at** H3 R 25D/27D
3′ **tt**GAACUUAGUCUUCUACUUCAGUUUA

5′ pCUUGAAUCAGAAGAUGAAGUCAA**at** H3 R 25D/28
3′ CUUGAACUUAGUCUUCUACUUCAGUUUA

5′ pCUUGAAUCAGAAGAUGAAGUCAA**at** H3 R 25D/29
3′ ACUUGAACUUAGUCUUCUACUUCAGUUUA

5′ GUUGAACUUGAAUCAGAAGAUGAAGUC H3 L 27/25D
3′ **ca**ACUUGAACUUAGUCUUCUACUUCp

hnRNPH Site 3, 3’-overhang variant sequences

5′ ttgttgaacttgaatcagaagatgaagtcaaattggc 3′ hnRNPH Site 3

5′ pCUUGAAUCAGAAGAUGAAGUCAA**at** 3′UU
3′ UUGAACUUAGUCUUCUACUUCAGUUUA

5′ pCUUGAAUCAGAAGAUGAAGUCAA**at** 3′UA
3′ UAGAACUUAGUCUUCUACUUCAGUUUA

5′ pCUUGAAUCAGAAGAUGAAGUCAA**at** 3′UC
3′ UCGAACUUAGUCUUCUACUUCAGUUUA

5′ pCUUGAAUCAGAAGAUGAAGUCAA**at** 3′UG
3′ UGGAACUUAGUCUUCUACUUCAGUUUA

5′ pCUUGAAUCAGAAGAUGAAGUCAA**at** 3′AU
3′ AUGAACUUAGUCUUCUACUUCAGUUUA

5′ pCUUGAAUCAGAAGAUGAAGUCAA**at** 3′AA
3′ AAGAACUUAGUCUUCUACUUCAGUUUA

5′ pCUUGAAUCAGAAGAUGAAGUCAA**at** 3′AC
3′ ACGAACUUAGUCUUCUACUUCAGUUUA

5′ pCUUGAAUCAGAAGAUGAAGUCAA**at** 3′AG
3′ AGGAACUUAGUCUUCUACUUCAGUUUA

5′ pCUUGAAUCAGAAGAUGAAGUCAA**at** 3′CU
3′ CUGAACUUAGUCUUCUACUUCAGUUUA

5′ pCUUGAAUCAGAAGAUGAAGUCAA**at** 3′CA
3′ CAGAACUUAGUCUUCUACUUCAGUUUA

5′ pCUUGAAUCAGAAGAUGAAGUCAA**at** 3′CC
3′ CCGAACUUAGUCUUCUACUUCAGUUUA

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5’        pCUUGAAUCAGAAGAUGAAGUCAAat                3’CG
3’       CGGAACUUAGUCUUCUACUUCAGUUUA

5’        pCUUGAAUCAGAAGAUGAAGUCAAat                3’GU
3’       GUGAACUUAGUCUUCUACUUCAGUUUA

5’        pCUUGAAUCAGAAGAUGAAGUCAAat                3’GA
3’       GAGAACUUAGUCUUCUACUUCAGUUUA

5’        pCUUGAAUCAGAAGAUGAAGUCAAat                3’GC
3’       GCGAACUUAGUCUUCUACUUCAGUUUA

5’        pCUUGAAUCAGAAGAUGAAGUCAAat                3’GG
3’       GGGAACUUAGUCUUCUACUUCAGUUUA
```

Luciferase Sequences

```
5’ acgccctggttcctggaacaattgcttttacagatg             Luciferase Site-1

5’  CGCCCUGGUUCCUGGAACAAUUGCUUU                     Luc-1 L 27/25D
3’  gcGGGACCAAGGACCUUGUUAACGAp

5’       pGGUUCCUGGAACAAUUGCUUUUAca                 Luc-1 R 25D/27
3’      GACCAAGGACCUUGUUAACGAAAAUGU


5’ gacatcacttacgctgagtacttcgaaatgtccgttc            Luciferase Site-2

5’  ACAUCACUUACGCUGAGUACUUCGAAA                     Luc-2 L 27/25D
3’  tgUAGUGAAUGCGACUCAUGAAGCUp

5’       pCUUACGCUGAGUACUUCGAAAUGtc                 Luc-2 R 25D/27
3’      GUGAAUGCGACUCAUGAAGCUUUACAG


5’ ttgcgcccgcgaacgacatttataatgaacgtgaat             Luciferase Site-3

5’  UGCGCCCGCGAACGACAUUUAUAAUGA                     Luc-3 L 27/25D
3’  acGCGGGCGCUUGCUGUAAAUAUUAp

5’       pCGCGAACGACAUUUAUAAUGAACgt                 Luc-3 R 25D/27
3’      GGGCGCUUGCUGUAAAUAUUACUUGCA


5’ cgattttgtgccagagtccttcgatagggacaagac             Luciferase Site-4

5’  GAUUUUGUGCCAGAGUCCUUCGAUAGG                     Luc-4 L 27/25D
3’  ctAAAACACGGUCUCAGGAAGCUAUp

5’       pGUGCCAGAGUCCUUCGAUAGGGAca                 Luc-4 R 25D/27
3’      AACACGGUCUCAGGAAGCUAUCCCUGU
```

La Sequences

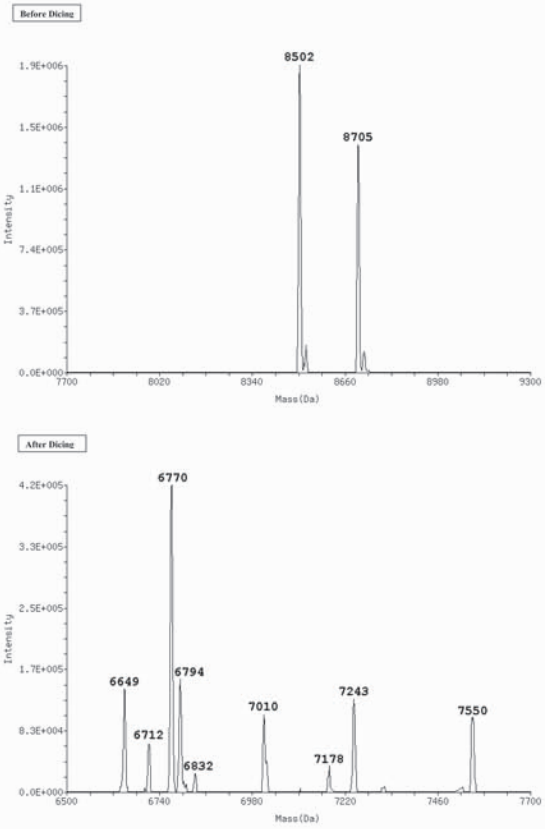
```
5’ gataaaactggatgaaggctgggtacctttggag               La Site-1

5’       pCUGGAUGAAGGCUGGGUACCUUUgg                 La R 25D/27
3’      UUGACCUACUUCCGACCCAUGGAAACC

5’  AUAAAACUGGAUGAAGGCUGGGUACUU                     La L 27/25D
3’  taUUUUGACCUACUUCCGACCCAUGp
```

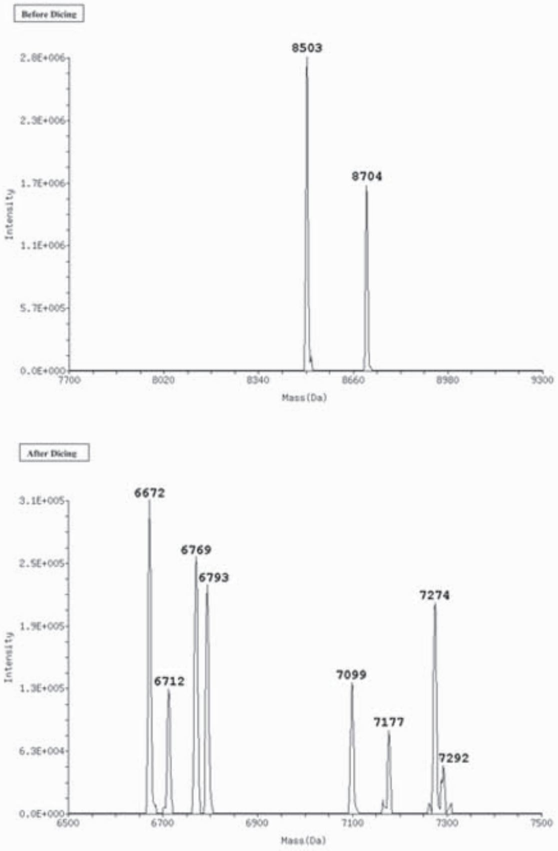
Table S1. RNA duplex sequences employed in this study. Sense strand target sequences are shown and RNA duplexes are aligned beneath in duplex form with S strand top (5’ → 3’) and AS strand bottom (3’ → 5’). RNA bases are upper case, DNA bases are lower case bold, and “p” represents 5’-phosphate.

A

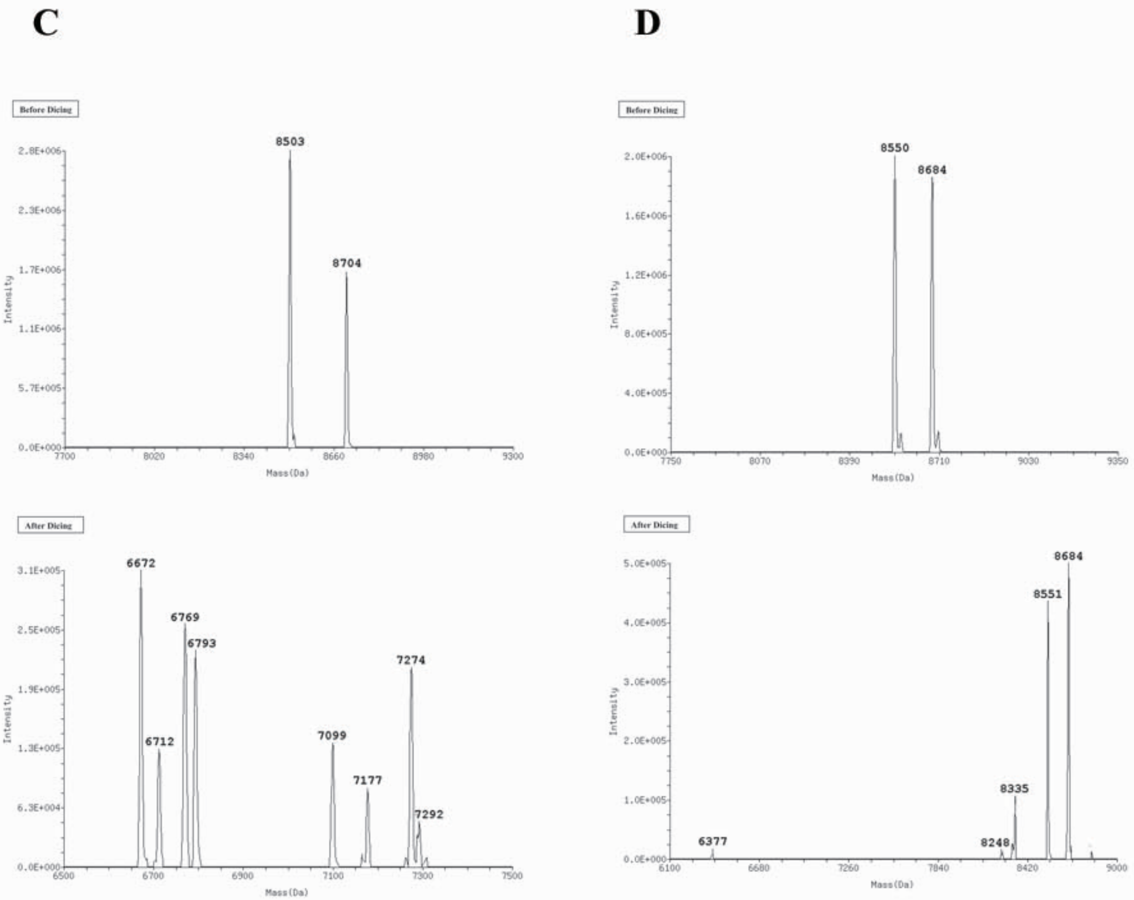


Sequence: EGFPS2 R 27(5’D)/27	Mol Wt	Length
5’ **aag**CAGCACGACUUCUUCAAGUCCGCC	8503	27
3’ UUCGUCGUGCUGAAGAAGUUCAGGCGG	8705	27
5’ pCACGACUUCUUCAAGUCCGCC	6648	21
3’ UCGUGCUGAAGAAGUUCAGGCp	6832	21
3’ UCGUGCUGAAGAAGUUCAGGCGp	7177	22
3’ GUCGUGCUGAAGAAGUUCAGGCp	7177	22
5’ pAGCACGACUUCUUCAAGUCCG	6712	21
3’ CGUCGUGCUGAAGAAGUUCAGp	6832	21
5’ **aag**CAGCACGACUUCUUCAAGUC	7242	23
3’ UUCGUCGUGCUGAAGAAGUUCp	6770	21
5’ **aag**CAGCACGACUUCUUCAAGUCC	7547	24
3’ UUCGUCGUGCUGAAGAAGUUCAp	7099	22
3’ UCGUCGUGCUGAAGAAGUUCAp	6793	21

B



Sequence: EGFPS2 R 27(3’D)/27	Mol Wt	Length
5’ AAGCAGCACGACUUCUUCAAGUCC**gcc**	8503	27
3’ UUCGUCGUGCUGAAGAAGUUCAGGCGG	8705	27
5’ pAGCACGACUUCUUCAAGUCC**gcc**	7274	23
3’ CGUCGUGCUGAAGAAGUUCAGGp	7177	22
5’ pCAGCACGACUUCUUCAAGUCC	6672	21
3’ UCGUCGUGCUGAAGAAGUUCAp	6793	21
5’ pGCAGCACGACUUCUUCAAGUC	6712	21
3’ UUCGUCGUGCUGAAGAAGUUCp	6770	21
3’ UUCGUCGUGCUGAAGAAGUUCAp	7099	22



Sequence: EGFPS2 R 27(3'D)/27	Mol Wt	Length
5' AAGCAGCACGACUUCUUCAAGUCC**gcc**	8503	27
3' UUCGUCGUGCUGAAGAAGUUCAGGCGG	8705	27
5' pAGCACGACUUCUUCAAGUCC**gcc**	7274	23
3' CGUCGUGCUGAAGAAGUUCAGGp	7177	22
5' pCAGCACGACUUCUUCAAGUCC	6672	21
3' UCGUCGUGCUGAAGAAGUUCAp	6793	21
5' pGCAGCACGACUUCUUCAAGUC	6712	21
3' UUCGUCGUGCUGAAGAAGUUCp	6770	21
3' UUCGUCGUGCUGAAGAAGUUCAp	7099	22

Sequence: EGFPS2 R 27/27(3'D)	Mol Wt	Length
5' AAGCAGCACGACUUCUUCAAGUCCGCC	8551	27
3' **ttc**GUCGUGCUGAAGAAGUUCAGGCGG	8685	27

No diced products were observed in the 21-23 base range.

Figure S1. ESI-MS analysis of in vitro dicing reactions. Mass spectra of duplexes are shown before (top) and after (middle) digestion with recombinant human Dicer. Sequence of the substrate RNA duplex is provide with detected cleavage products along with calculated molecular weight and strand length (bottom). RNA bases are upper case, DNA bases are lower case bold, and "p" represents 5'-phosphate. A) blunt duplex EGFPS2 R 27(5'D)/27; B) blunt duplex EGFPS2 R 27(3'D)/27; C) blunt duplex EGFPS2 R 27/27(5'D); D) blunt duplex EGFPS2 R 27/27(3'D).

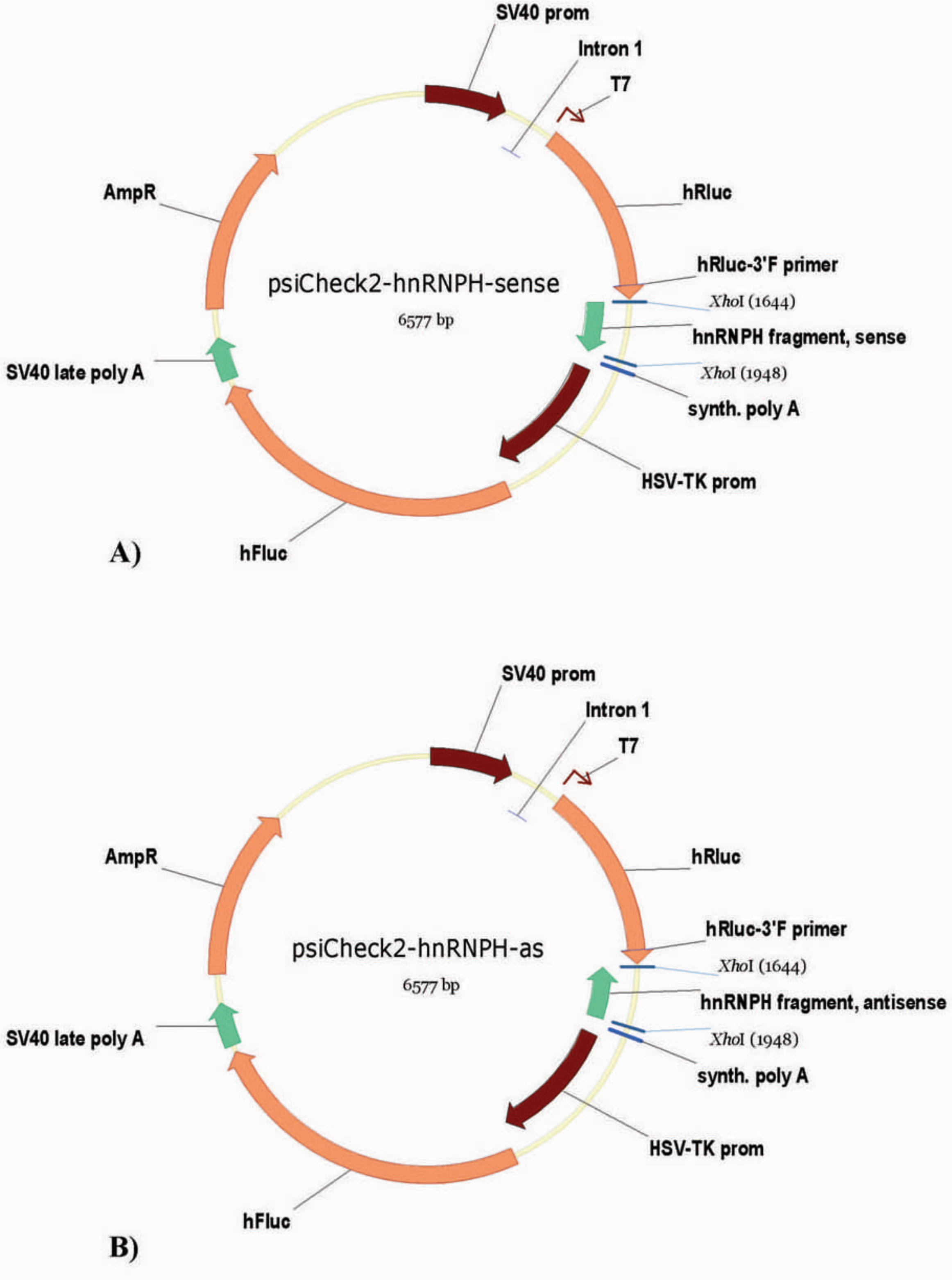
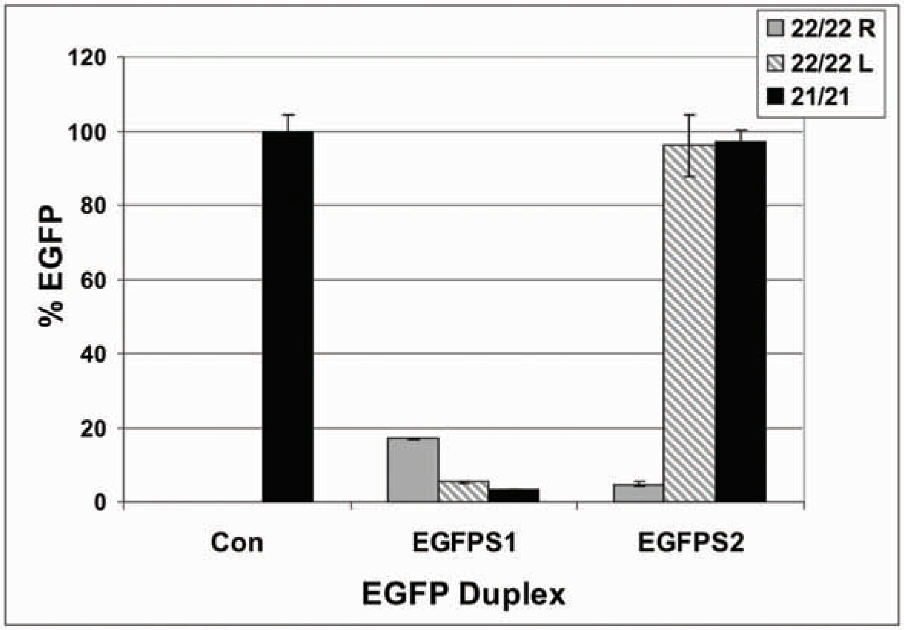


Figure S2. Map of hnRNPH "S" and "AS" targeting vectors. A fragment of the hnRNPH gene was cloned into the 3'-UTR of the Renilla luciferase gene in vector psiCheck-2™. Relative locations of the internal control firefly luciferase coding region, Renilla luciferase coding regions with inserted hnRNPH sequences in A) sense and B) antisense orientations are shown.



```
5' caagctgaccctgaagttcatctgcaccaccggcaa  EGFP Site-1

5'        ACCCUGAAGUUCAUCUGCACC           EGFPS1 21/21
3'      ACUGGGACUUCAAGUAGACGU

5'        ACCCUGAAGUUCAUCUGCACCA          EGFPS1 R 22/22
3'      ACUGGGACUUCAAGUAGACGUG

5'       GACCCUGAAGUUCAUCUGCACC           EGFPS1 L 22/22
3'     GACUGGGACUUCAAGUAGACGU


5' ccatgaagcagcacgacttcttcaagtccgccatgc  EGFP Site-2

5'        GCAGCACGACUUCUUCAAGUC           EGFPS2 21/21
3'      UUCGUCGUGCUGAAGAAGUUC

5'        GCAGCACGACUUCUUCAAGUCC          EGFPS2 R 22/22
3'      UUCGUCGUGCUGAAGAAGUUCA

5'       AGCAGCACGACUUCUUCAAGUC           EGFPS2 L 22/22
3'     CUUCGUCGUGCUGAAGAAGUUC
```

Figure S3. Evaluation of 22mer products derived from Dicing "L" and "R" forms. Top panel: relative expression data are shown. Bottom panel: target sequences (S strand) are shown and transfected RNAi duplexes are aligned beneath in duplex form with S strand top (5' → 3') and AS strand bottom (3' → 5'). Relative EGFP fluorescence was measured following co-transfection of an EGFP expression plasmid with 1 nM concentration of the indicated duplexes.