

**Table 2.** Sequence of the R8-11 ribozyme and of clones obtained at the start of continuous evolution (transfer 0) and after transfers 15, 50, 80, and 100

R8-11	AGA-GAAGGAAACUUCCUAAUAGUGAUUU-UGUGGU-UUU--UUGCGCAGUCUAAUCCUAAGGC-AAACGCUAUGGAUCAAUGGGUAGGA (CCA) UCCGUUCCUAGCA-GAC-UGC GCU-CC-----
0-1	.....G.....(N35).....A..U.....
0-2	.....-C.....U.....(N35).....U..U..C.....
0-3	.....G.....A.....-(N35).....C.....U.....
0-4	.....A.....G.....(N35).....C.....UA..C..
0-5	.....A..G.....(N35).....C.....U.....
0-6	.....A...A.....(N35).....U..U..C.....
0-7	.....A.....C.....(N35).....C.....U.....
0-8	.....G.....U.....(N35).....U..U..U.....
0-9	.....C.....(N35).....U..C.....U.....
0-10	.....-.....U.....(N35).....U.....U.....
0-11	.....C.....(N35).....U.....U.....
0-12	.....U-.....(N35).....C.A.....C..
15-1	.....A..U.....-.....-U.....A.....(N35).....U..U..C..
15-2	.....A..U.....AU.....(N35).....U..U..C..
15-3	.....A..U.....AA.....GG.....(N35)A.....-G.....A.....C..
15-4	.....A..U.....(N35)A.....U..C.....U..U..C..
15-5	.....A..U.....AUA.....(N35).....A..U..U..C..
15-6*	.....A..U.....(N35).....A..U..U..C..
15-7	.....A..U.....AU.....UGA.....U-.....(N35)A.....U..U..
15-8*	.....A..U.....UGA.....(N35)A.....U..U..C..
15-9	.....A..U.....C.....A.....(N35).....U..U..U..C..
15-10	.....A..U.....(N35).....A..U..U..C..
15-11	.....A..U.....C.....(N35).....AU.....C..
15-12	.....A..U.....C.....A.....U.....(N35).....U..U..C..
15-13	.....A..U.....U.....(N35).....A..G..U..U..C..
15-14	.....A..U.....U.....UGA.....(N35)A.....U..U..C..
15-15	.....A..U.....U.....C.....A.....(N35)A.....U..C..U..U..C..
15-16	.....A..U.....(N35).....U..U..C..
15-17	.....A..U.....C.....U.....(N35).....A..U..U..C..
15-18	.....A..U.....-.....U..A..U.....(N35)A.....U..C..U..U..C..
15-19	.....A..U.....-.....-U.....A.....(N35).....U..U..C..
15-20	.....A..U.....(N35).....U..C..U..U..C..
15-21	.....A..U.....AU.....U.....(N35).....U..U..C..

<b>R8-11</b>	AGA-GAAGGAAACUCCUAUUAGUGAUUU-UGUGGU-UUU--UUGCGCAGUCUCAAUCUAAGGC-AAACGCUAUGGAUCAAUGGGUAGGA (CCA) UCCGUUCCUAGCA-GAC-UGCGCU-CC---
50-1	...A..A....U..U.....A.....U.--.....G.....(N35).....U...U.U...AUAA
50-2	...A..A....U..U.....U...-.....U.--.....U.....U(N35).....U...U.U.U.U.AUAA
50-3	...A..A....U..U.....A.A...-.....U--.....A.....U(N35).....U..AU.U.U..AUAA
50-4	...A..A....U..U.....-.....U.--.....U.....U(N35).....U...U..U.G.U..AUAG
50-5	...A..A....U..U.....-.....U--.....A.....U(N35).....C.....U...U..U.G.U..AUAA
50-6	...A..A....U..U.....C.....U.--.....U.....U(N35).....U...U..U.U.U..AUAA
50-7	...A..A....U.C.U.....A.....U--.....A.....(N35).....U...U..AU.U.U..AUAA
50-8	...A..A....U..U.....-.....U.--.....U.....U(N35).....U...U..U.U.U..AUAA
50-9	...A..A....U..U.....-.....U.--.....U.....U(N35).....U...U..U.U.U..AUAA
50-10	...A..A....U..U.....-.....U.--.....U.....U(N35).....U...U..U.U.U..AUAA
80-1*	...A..A....U..U.....C.....A-.....U.--.....U.....U(N35).....U...U..U.U.UU.AUAA
80-2	...A..A....U..U.....C.....A-.....U.--.....U.....U(N35).....U...U..U.U.UU.AUAA
80-3	...A..A....U..U.....C.....A-.....U.--.....U.....U(N35).....U...U..U.U.UU.AUAA
80-4	...A..A....U..U.....C.....A-.....U.--.....U.....U(N35).....U...U..U.U.UU.AUAA
80-5	...A..A....U..U.....C.....A-.....U.--.....U.....U(N35).....U...U..U.U.UU.AUAA
80-7	...A..A....U..U.....C.....A-.....U.--.....U.....U(N35).....U...U..U.U.UU.AUAA
80-8	...A..A....U..U.....C.....A-.....U.--.....U.....U(N35).....U...U..U.U.UU.AUAA
80-9	...A..A....U..U.....C.....U..U.--.....A..U..G.....U(N35).....U...U..U.U.U..AUAA
80-10	...A..A....U..U.....C.....A-.....U.--.....U.....U(N35).....U...U..U.U.UU.AUAA
100-1*	...A..A....U..U.....C.....A-.....G..U.--.....U.....U(N35).....U...U.....AGUCGUAUUGGCAUAG
100-2	...A..A....U..U.....C.....A-..A.....--.....U.....U(N35).....U...U.....AGUCGUAUUGGCAUAG
100-3	...A..A....U..U.....C.....A-..A.....U.--.....U..G.....U(N35).....U...U.....AGUCGUAUUGGCAUAG
100-4	...A..A....U..U.....C.....G.A-.....G..G.--.....U.....U(N35).....U...U.....AGUCGUAUUGGCAUAG
100-5	...A..A....U..U.....C.....G.A-.....G..U.--.....U.....U(N35).....U...U.....AGUCGUAUUGGCAUAG
100-6	...A..A....U..U.....C.....A-..A.....--.....U.....U(N35).....U...U.....AGUCGUAUUGGCAUAG
100-7	...A..A....U..U.....C.....A-.....G..U.--.....U.....U(N35).....U...U.....AGUCGUAUUGGCAUAG
100-8	...A..A....U..U.....C.....A-.....G..U.--.....U.....U(N35).....U...U.....AGUCGUAUUGGCAUAG
100-9	...A..A....U..U.....C.....A-..A.....--.....U.....U(N35).....U...U.....AGUCGUAUUGGCAUAG
100-10	...A..A....U..U.....C.....A-.....G..U.--.....U.....U(N35).....U...U.....AGUCGUAUUGGCAUAG

The sequence of the 35-nt accessory domain (N35) is given in SI Table 1 and is not reproduced here. Residues identical to the R8-11 sequence are indicated by a dot; deletions are indicated by a dash. Asterisks indicate representative clones that were chosen for more detailed analysis. All of the clones obtained after transfer 100 contain a 7-nt insertion and two U→G changes (shaded gray) that confer complementarity to nucleotides of the substrate. Numbering of residues is based on the T80-1 ribozyme (see Fig. 2B); × indicates residues that are present in the R8-11 ribozyme but not in the T80-1 ribozyme.