

Table S1. Oligonucleotides used in this study

Primers

Primers for the SIBA artificial system

SB-F30	TGCCAATCCAACAAGAAGGCGT <u>ACTCGACC</u>
SB-F35	CAATTTGCCAATCCAACAAGAAGGCGT <u>ACTCGACC</u>
SB-F21	AACAAGAAGGCGT <u>ACTCGACC</u>
SB-F21 NHOM1	TGCCAATCCAACAAGAAGGCG
SB-F18	CAAGAAGGCGT <u>ACTCGAC</u>
SB-F16	CAAGAAGGCGT <u>ACTCG</u>
SB-F17	TGATGTGT <u>ACTGAGATC</u>
SB-F14	AACAAGAAGGCGTA
SB-R30	GCAATGAGCTCAGTTGATGTGT <u>ACTGAGATC</u>
SB-R35	ACCATGCAATGAGCTCAGTTGATGTGT <u>ACTGAGATC</u>
SB-R21	CAGTTGATGTGT <u>ACTGAGATC</u>
SB-R21 NHOM1	ATGAGCTCAGTTGATGTGTAC
SB-R21 NHOM2	GCAATGAGCTCAGTTGATGTG
SB-R18	TTGATGTGT <u>ACTGAGATC</u>
SB-R16	TTGATGTGT <u>ACTGAGA</u>
SB-R14	TGTGT <u>ACTGAGATC</u>

Primers used for the *Salmonella* system

SM-F18 CTGGCGATATTGGTGTTT

SM-R16 ACCGCAGGAAACGTTG

Regions homologous to the IO are underlined.

Templates

Template used for the SIBA artificial system

SB-TEMPLATE LONG

CAATTTGCCAATCCAACAAGAAGGCGTACTCGACCTGATACACGTTATCGTCCATACGGATTCGGG
ATCTCAGTACACATCAACTGAGCTCATTGCATGGT

SB-TEMPLATE

AACAAGAAGGCGTACTCGACCTGATACACGTTATCGTCCATACGGATTCGGGATCTCAGTACACAT
CAACTG

Non-homologous template used for the SIBA artificial system

SB NHOM Template

TACTGGCGATATTGGTGTTTTTATGGCGTCCTTCTACAAAGACAGAATCCTCTCTTTTTCAACGTTTC
CTGCGGTA

Templates (harboring point mutations) used for the SIBA artificial system specificity test

Point mutations located in regions homologous to the IO 2'-O-methyl RNA region

SB-MET1 TEMPLATE

AACAAGAAGGCGTACTCGACCTGATACACGTTATCGTCCATACGGATT**C**GGATCTCAGTACACAT
CAACTG

SB-MET2 TEMPLATE

AACAAGAAGGCGTACTCGACCTGATACACGTTATCGTCCATACGGATT**CC**GATCTCAGTACACAT
CAACTG

SB-MET3 TEMPLATE

AACAAGAAGGCGTACTCGACCTGATACACGTTATCGTCCATACGGATT**GCC**GATCTCAGTACACAT
CAACTG

SB-MET4 TEMPLATE

AACAAGAAGGCGTACTCGACCTGATACACGTTATCGTCCATACGGAT**TGCC**GATCTCAGTACACAT
CAACTG

Point mutations located in regions homologous to the IO-DNA region

SB-DNA1 TEMPLATE

AACAAGAAGGCGTACTCGACCT**C**ATACACGTTATCGTCCATACGGATT**C**GGATCTCAGTAC
ACATCAACTG

SB-DNA2 TEMPLATE

AACAAGAAGGCGTACTCGACCT**C**ATACACGTTATCGTCC**T**TACGGATT**C**GGATCTCAGTAC
ACATCAACTG

SB-DNA3 TEMPLATE

AACAAGAAGGCGTACTCGACCT**C**ATACAC**C**TTATCGTCC**T**TACGGATT**C**GGATCTCAGTAC
ACATCAACTG

SB-DNA4 TEMPLATE

AACAAGAAGGCGTACTCGACCT**C**ATACAC**C**TT**A**CGTCC**T**TACGGATT**C**GGATCTCAGTAC
ACATCAACTG

Point mutations are underlined and in bold.

Invasion oligonucleotide (IO) sequences used in the SIBA artificial system

IO used for the artificial system

SB-IO

TTGTCCATAGACTG CTCGACCTGATACACGTTATCGTCCATACGGAT

mUmCmGmGmGmAmUmCmUmCmAmUmA /InvdT/

SB-IO NON-METH

TTGTCCATAGACTG CTCGACCTGATACACGTTATCGTCCATACGGAT TCGG TA /InvdT/

SB-IO-DNA

TTGTCCATAGACTG CTCGACCTGATACACGTTATCGTCCATACGGATTTCGGGATCTCA TA

/InvdT/

SB-IO DIFF-METH

TTGTCCATAGACTG CTCGACCTGATACACGTTATCGTCCATACGGAT

mAmGmCmCmCmUmCmAmGmAmGmUmA /InvdT/

The IO non-homologous region (5'-end) is underlined.

m = 2'-O-methyl RNA nucleotides.

/InvdT/ = inverted dT.

Non-homologous IO used for specificity evaluation

SB NHOM IO

TCCTCCTGTACCTT GTGTTTATGGGGTCGTTCTACATTGACAGAATCCT

mCmAmGmUmUmUmUmUmCmAmAmCmGmA /InvdT/

IO non-homologous region (5'-end) is underlined.

m = 2'-O-methyl RNA nucleotides.

/InvdT/ = inverted dT.

Invasion oligonucleotide (IO) sequence used for the *Salmonella* system**SM-IO**

TCCTCCTCTTCCTT TGTTTATGGGGTCGTTCTACATTGACAGAATC
 mCmUmCmAmGmUmUmUmUmUmCmAmAmCmGmA / InvdT /

The IO non-homologous region (5'-end) is underlined.

m = 2'-O-methyl RNA nucleotides.

/InvdT/ = inverted dT.

Oligonucleotides used in the strand exchange experiment

SX 16	GAGTACACATGAAGTG
SX 21	CTACTGAGTACACATGAAGTG
SX 25	AGCCCTACTGAGTACACATGAAGTG
SX 35	GCATACCGATAGCCCTACTGAGTACACATGAAGTG
SX 40	ATCGTGCATACCGATAGCCCTACTGAGTACACATGAAGTG
SX 49	ATAGACGTAATCGTGCATACCGATAGCCCTACTGAGTACACATGAAGTG

SX 16–49 were labeled (or not) with TAMRA at the 3'-end.

INVASION TEMPLATE

/FAM/CACTTCATGTGTACTCAGTAGGGCTATCGGTATGCACGATTACGTCTATG

Invasion oligonucleotide (IO) sequence used for the invasion strand exchange assay**IO-LONG**

TTGTCCATAGACTG CTGCACCTCATAGACGTAATCGTGCATACCGATAGCCCTACTGA
 GTACACATGA TA /InvdT/

IO-DNA

TTGTCCATAGACTG CTGCACCTCATAGACGTAATCGTGCATACCGATAGCCCTACTGATA

/InvdT/

IO-MET

TTGTCCATAGACTG CTGCACCTCATAGACGTAATCGTGCATACCGAT
AmGmCmCmCmTmAmCmTmGmAmTmA /InvdT/

IO DIFF-MET

TTGTCCATAGACTG CTGCACCTCATAGACGTAATCGTGCATACCGAT A
mCmAmUmGmAmUmGmAmAmGmCmU /InvdT/

The IO non-homologous region (5'-end) is underlined.

m = 2'-O-methyl RNA nucleotides.

/InvdT/ = inverted dT.

Duplex used for the invasion strand exchange assay

Duplex SHORT

AACAAGAAGGCGTACTGCACCTCATAGACGTAATCGTGCATACCGATAGCCCTACTGAGTACACAT
GAAGTG/TAMRA/
/FAM/CACTTCATGTGTACTCAGTAGGGCTATCGGTATGCACGATTACGTCTATGAGGTGCAGTAC
GCCTTCTTGTT

Duplex LONG

AACAAGAAGGCGTACTGCACCTCATAGACGTAATCGTGCATACCGATAGCCCTACTGAGTACACAT
GAAGTGAGTTCAGCAG/TAMRA/
/FAM/CTGCTGAACTCACTTCATGTGTACTCAGTAGGGCTATCGGTATGCACGATTACGTCTATGA
GGTGCAGTACGCCTTCTTGTT