Development of cell-free toehold switch for hepatitis A virus type I on-site detection

Supplementary material

Table S1. Sequences of 10 pairs toehold switch sensors and corresponding target trigger RNAs

	Target sequence	Sensor sequence	Normalized ensemble defect (%)
S1	AAUGCAGGAAGCCAUAUAA AGAAUUGAGAUUAGAAG	GGGCUUCUAAUCUCAAUUCUUUAUAUGGCUUCCUGCAUUGG ACUUUAGAACAGAGGAGAUAAAGAUGAAUGCAGGAAGCAACC UGGCGGCAGCGCAAAAG	17.2
S2	GCAGGAAGCCAUAUAAAGA AUUGAGAUUAGAAGUUG	GGGCAACUUCUAAUCUCAAUUCUUUAUAUGGCUUCCUGCGG ACUUUAGAACAGAGGAGAUAAAGAUGGCAGGAAGCCACAACC UGGCGGCAGCGCAAAAG	17.5
S3	AUGCAGGAAGCCAUAUAAA GAAUUGAGAUUAGAAGU	GGGACUUCUAAUCUCAAUUCUUUAUAUGGCUUCCUGCAUGG ACUUUAGAACAGAGGAGAUAAAGAUGAUGCAGGAAGCGAACC UGGCGGCAGCGCAAAAG	17.6
S4	CAGGAAGCCAUAUAAAGAA UUGAGAUUAGAAGUUGG	GGGCCAACUUCUAAUCUCAAUUCUUUAUAUGGCUUCCUGGG ACUUUAGAACAGAGGAGAUAAAGAUGCAGGAAGCCAUCAACC UGGCGGCAGCGCAAAAG	17.9
S5	UGCAGGAAGCCAUAUAAAG AAUUGAGAUUAGAAGUU	GGGAACUUCUAAUCUCAAUUCUUUAUAUGGCUUCCUGCAGG ACUUUAGAACAGAGGAGAUAAAGAUGUGCAGGAAGCCUAACC UGGCGGCAGCGCAAAAG	18.9
S6	GAAUGCAGGAAGCCAUAUA AAGAAUUGAGAUUAGAA	GGGUUCUAAUCUCAAUUCUUUAUAUGGCUUCCUGCAUUCGG ACUUUAGAACAGAGGAGAUAAAGAUGGAAUGCAGGAACAACC UGGCGGCAGCGCAAAAG	17.8
S7	AGAAUGCAGGAAGCCAUAU	GGGUCUAAUCUCAAUUCUUUAUAUGGCUUCCUGCAUUCGG	18.6

	AAAGAAUUGAGAUUAGA	ACUUUAGAACAGAGGAGAUAAAGAUGAGAAUGCAGGAAAACC	
		UGGCGGCAGCGCAAAAG	
S8	AGGAAGCCAUAUAAAGAAU UGAGAUUAGAAGUUGGG	GGGCCCAACUUCUAAUCUCAAUUCUUUAUAUGGCUUCCUGG ACUUUAGAACAGAGGAGAUAAAGAUGAGGAAGCCAUACAACC UGGCGGCAGCGCAAAAG	18.2
S9	CAUAUAAAGAAUUGAGAUU AGAAGUUGGGAAACAAA	GGGUUUGUUUCCCAACUUCUAAUCUCAAUUCUUUAUAUGGG ACUUUAGAACAGAGGAGAUAAAGAUGCAUAUAAAGAAUAACC UGGCGGCAGCGCAAAAG	20.9
S10	CACUCUGAUGAAUAUUUG UCUUUUAGUUGCUAUUUG	GGGCAAAUAGCAACUAAAAGACAAAUAUUCAUCAGAGUGGGA CUUUAGAACAGAGGAGAUAAAGAUGCACUCUGAUGACAACCU GGCGGCAGCGCAAAAG	24.2

Fig S1. Partial trigger and switch candidate sequences.

Normalized ensemble defect: 17.2 %

trigger_strand AAUGCAGGAAGCCAUAUAAAGAAUUGAGAUUAGAAG

switch_strand GGGCUUCUAAUCUCAAUUCUUUAUAUGGCUUCCUGCAUUGGACUUUAGAACAGAGGAGAU AAAGAUGAAUGCAGGAAGCAACCUGGCGGCAGCGCAAAAG

To Analysis

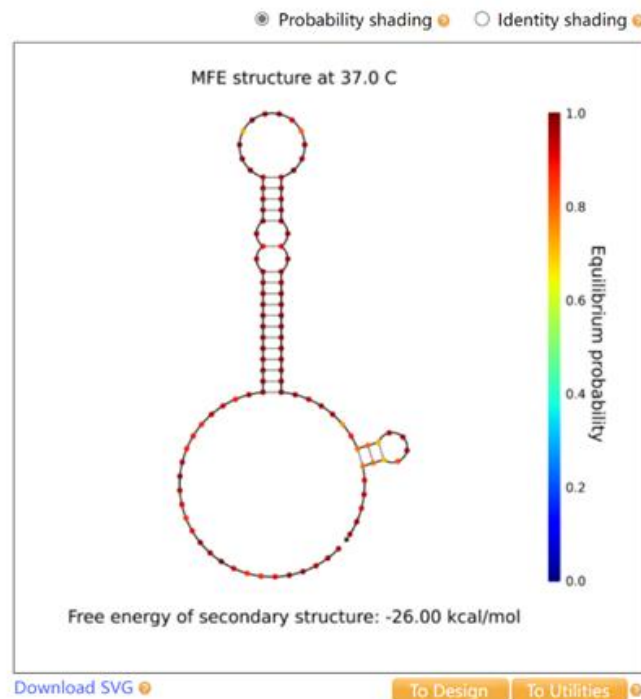**Fig S2. Probability shading structure after analysis of a sequence.**

Probability shading   Identity shading

MFE structure at 37.0 C

Free energy of secondary structure: -26.00 kcal/mol

Equilibrium probability

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Table S2. Sequences of different genotypes of HAV for experiments

	sequence	identities
HAV-I	cagtataaaaaagagagaaattttggcttgtgaaaacagtcccttaattttcctagggggtggaagtacttcatttg acaattcctcctgagcatacttgagtctttgtttcccaacttctaatctcaattctttatatggcttcctgcattctat gtgactctcaaatcttttgtcctcctctgatctaggatcatccactgatgactccaagtctccagctgcaattctgc tcatcattgattcagtggataacatggcatttgaattcaatggagctctgggaaaataaaactctgattgttctg tgacagacaaatagcaactaaaagacaaatattcatcagagtg	91%
HAV-II	cagtataaaacaaagaaattttgcctgtgaaaacagacctttcattttcctaggaggtggaagcacttcatttg acaattcttcctgggcatactttagtctctgcttcccaacctcaagtcttaattctttgtatggttttctgctctcaat atgactttcaaatctcctatcttcttctgatctgggatcatccactgaagattccaaatctccagctgcaattctgc tcatcatagtttcagtggacagcatagcattagagttcaagggggcccttggaaaataaaactctgactgctca gtaacagatagataacagctaaaagacaaatattcatcagaatg	91.69%
AV-III	tgtaaaataaggagattttgcttgtgaaaacacacccttaattttacgaggaggaggcaacacttcatttgac aattcttcctgagcatatttaagtctttgcttacctacctccaatcttaactctttataaggtttcctcttttcaatat gactttcaaatttacgatcctcctcagatcgggatcatcaactgaggattcaagatcaccaagagcaattctat ccatcattgtttctgatgacatcatagcattggtattcaaaggtgctctaggaaaataaaactcagactgttcag tcacagacaagtaacagctaaaagacaaatattcatctgagtg	90.39%
HAV-VII	cagtataaaacaaagaaattttgcctgtgaaaacagacctttcattttcctaggaggtggaagcacttcatttg acaattcttcctgggcatactttagtctctgcttcccaacctcaagtcttaattctttgtatggttttctgctctcaat atgactttcaaatctcctatcttcttctgatctgggatcatccactgaagattccaaatctccagctgcaattctgc tcatcatagtttcagtggacagcatagcattagagttcaagggggcccttggaaaataaaactctgactgctca gtaacagatagataacagctaaaagacaaatattcatcagaatg	92.46%

Fig S3. Validation of NASBA amplification efficiency by agarose gel electrophoresis and concentration determination
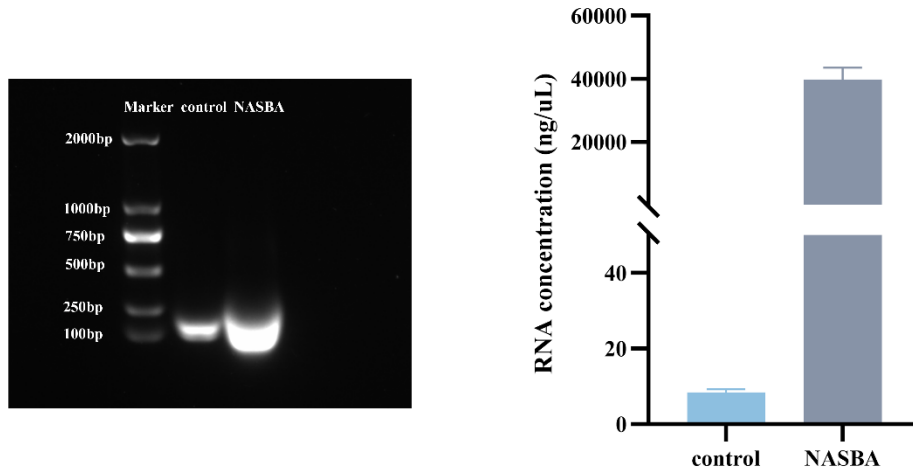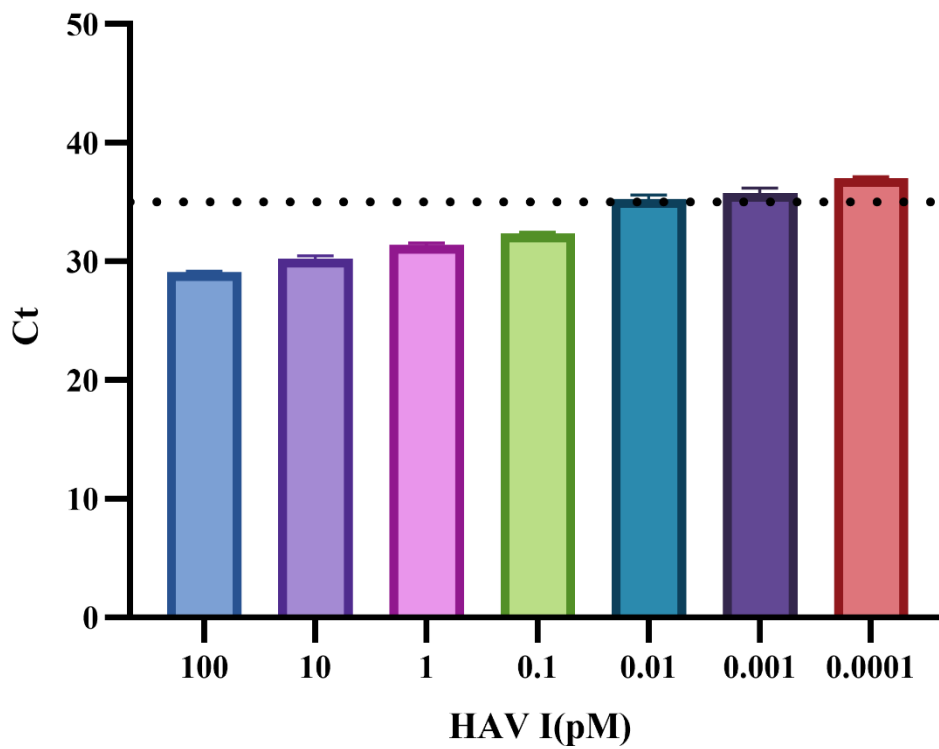

Fig S4. Results of the HAV I detection sensitivity with RT-PCR

Fig S5. Curves of fluorescence.