Study on factors affecting the performance of a CRISPR/Cas assisted new immunoassay: detection of salivary insulin as an example

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Supplementary information

Table S1. The sequences of ssDNA and ssRNA using in the system

Name	Oligo type	Sequence 5'-3'	Length (nt)	Modification
Triggering ssDNA 1	ssDNA	ACA CAA CCA CCC AAC ACA ACC AAC CCC	27	3'-biotin
Triggering ssDNA 2	ssDNA	GAA GAC ACC CTA CCA ACC CCC CCC ACC ACC	30	3'-biotin
Guiding RNA (gRNA) 1	ssRNA	UAA UUU CUA CUA AGU GUA GAU GGG GUU GGU UGU GUU GGG UGG	42	N/A
Guiding RNA (gRNA) 2	ssRNA	UAA UUU CUA CUA AGU GUA GAU GGG GGG GGU UGG UAG GGU GUC	42	N/A
ssDNA reporter	ssDNA	TTATT	5	5'-Texa Red; 3'-BHQ2

Table S2. The analytes used in the system

Name	Cat. No.	Company
Recombinant human insulin	I2643	Sigma Aldrich
Recombinant human IFN-γ	285-IF	R&D Systems
Recombinant human IP10	Ab9810	Abcam
Recombinant IL-6	206-IL	R&D Systems
Recombinant TNF-α	210-TA	R&D Systems
Recombinant IGF-1	291-G1	R&D Systems
Recombinant human proinsulin C-Peptide	NBP235211	Novus Biologicals

Table S3. Antibodies used in the system

	Name	Cat. No.	Company	Concentration (μg/mL)
Capture antibody	Rabbit polyclonal anti-human insulin (Pair #1)	Ab53591	Abcam	10
	Rabbit polyclonal anti-human human insulin antibody (Pair #2)	NBP1-87485	Novus Biologicals	10

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	Rabbit polyclonal anti-human insulin (biotin)	Ab53592	Abcam	10
Detection antibody IgG Antibodies	Mouse monoclonal anti-human insulin (Pair #1)	Ab6995	Abcam	10
	Mouse monoclonal anti-human human insulin antibody (Pair #2)	NBP100-73008	Novus Biologicals	10
Antibody for probe fabrication	Goat anti-mouse IgG	GTX77316	GeneTex	1000
	Goat anti-rabbit IgG	GTX77061	GeneTex	1000
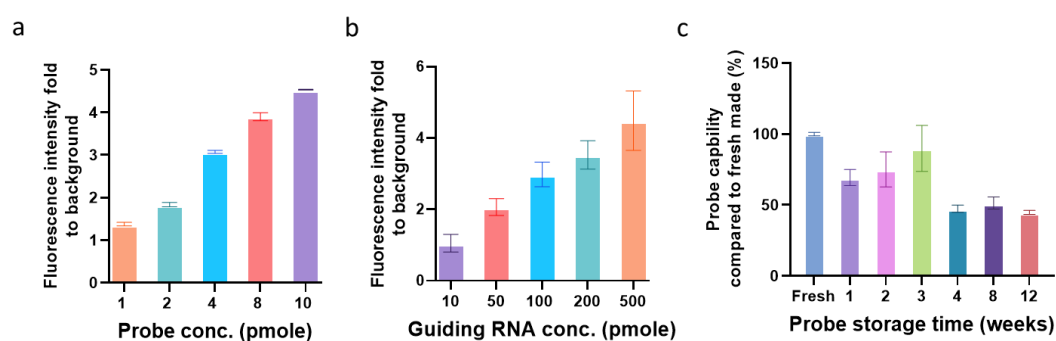
Fig. 1S Optimisation of the concentrations of anti-IgG-ssDNA probe (a), guiding RNA (b) and assessment of anti-IgG-ssDNA probe storage time (c).

The concentrations of the anti-IgG-ssDNA probe and guiding RNA had been optimised. With increasing the concentration, the signal levels in CANi promoted. Applied 4 pmole of the probe and 100 pmole guiding RNA in the CANi, the signal levels reached more than 3 times as the blank control indicated 4 pmole and 100 pmole could be used in the future experiment. Additionally, anti-IgG-ssDNA probe storage time was assessed. Until 12 weeks after the probe was conjugated, the signal level could still keep 50 % as the freshly made probe.
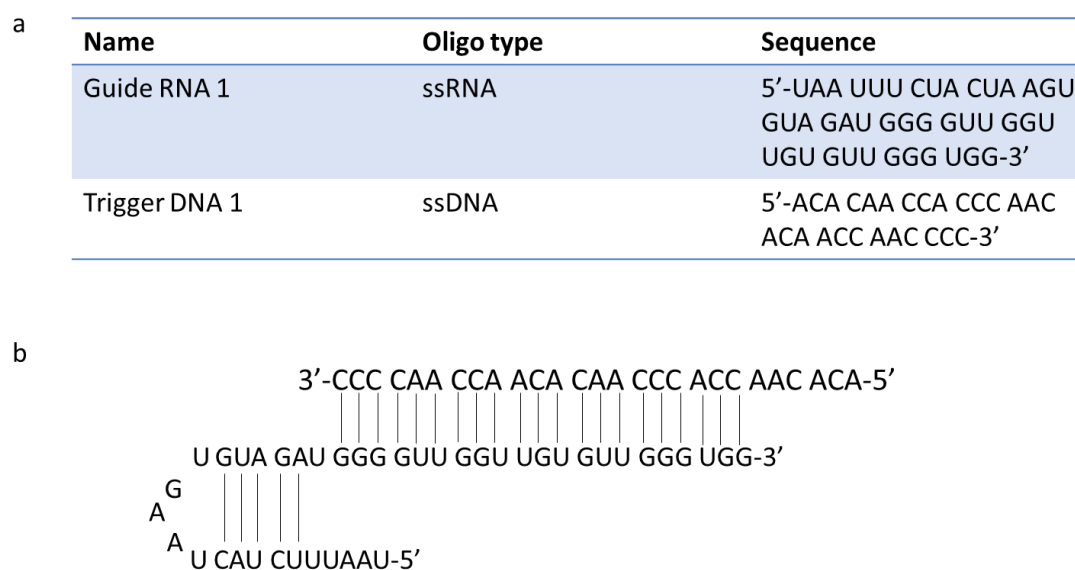
a

Name	Oligo type	Sequence
Guide RNA 1	ssRNA	5'-UAA UUU CUA CUA AGU GUA GAU GGG GUU GGU UGU GUU GGG UGG-3'
Trigger DNA 1	ssDNA	5'-ACA CAA CCA CCC AAC ACA ACC AAC CCC-3'

b

3'-CCC CAA CCA ACA CAA CCC ACC AAC ACA-5'
|||||||||||||||||||||||||||
U GUA GAU GGG GUU GGU UGU GUU GGG UGG-3'
 G |||||
A |||||
 A   U CAU CUUUAAU-5'

Fig. S2 The sequences (a) and secondary structures of guide RNA 1 and Trigger DNA 1 (b).

a

Name	Oligo type	Sequence
Guide RNA 2	ssRNA	5'-UAA UUU CUA CUA AGU GUA GAU GGG GGG GGU UGG UAG GGU GUC -3'
Trigger DNA 2	ssDNA	5'-GAA GAC ACC CTA CCA ACC CCC CCC ACC ACC-3'

b

5'-UAA UUU CUA CUA AGU GUA GAU GGG GGG GGU UGG UAG GGU GUC-3'
                                |||||||||||||||||||||||
                   CCC CCC CCA ACC ATC CCA CAG
              3'-CCA CCA   AAG-5'
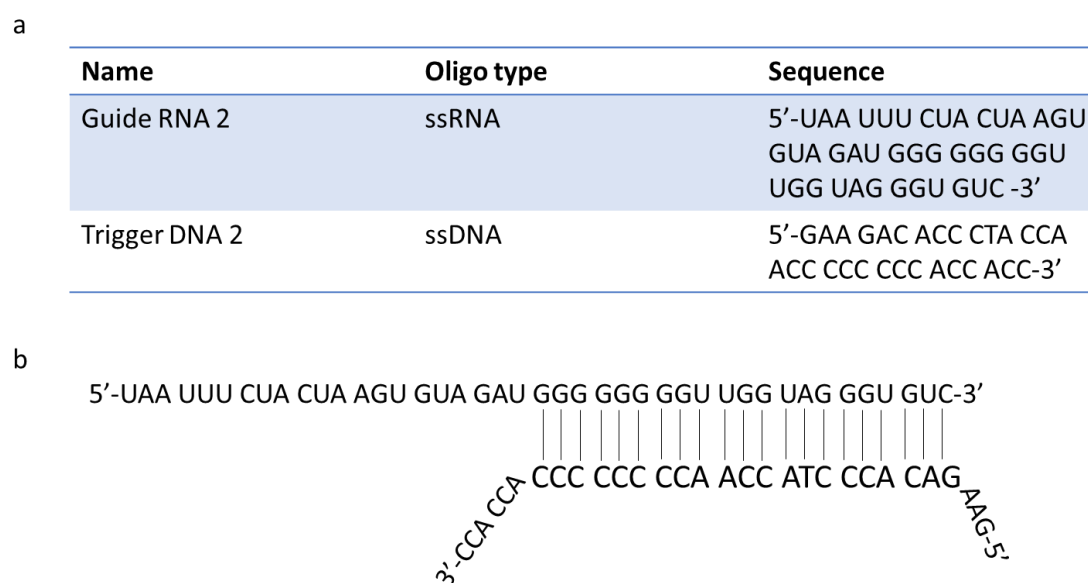
Fig. S3 The sequences (a) and secondary structures of guide RNA 2 and Trigger DNA 2 (b).