Binding score:

50% of **Tm score** + 25 % of the **Junction score** + 25% of the **CDS score**

Tm calculation: The melting temperature (Tm) of a DNA sequence was calculated using the nearest-neighbor model.

Tm score: based on the Tm from the arm with the highest Tm: $\frac{(Tm_{Arm\ with\ \max(Tm)} - 40)}{22}$

Junction score: 1 if the probe does NOT span a junction, 0 otherwise

CDS score: % of the probe within a CDS

Final score: (50 x Tm score) + (25 x Junction score) + (25 x CDS score)

Specificity score

The target sequences were aligned to the corresponding genome using both full-length (32-nt) and 16-nt trimmed sequences. For each probe, all possible 16-nt subsequences (i.e., positions 1–16, 2–17, ..., 17–32) were independently aligned. All alignments were performed using STAR alignment v2.7.2a (https://github.com/alexdobin/STAR) and convert to SAM using samtools v1.9 (https://github.com/samtools/samtools). The 32nt alignment provide the information for genes different to the intended target with sequences that are 100% reverse-complementary to the probe arms (column *Perfect Match Genes* in CSV output) or with have a single mismatch (column *One Mismatch Genes* in CSV output). To find other genes with more mismatches, alignments using 16-nt trimmed sequences were performed. When several consecutive windows matched the same gene, the length of the exact match was extended accordingly (column *Longest exact match* in CSV output). A total of 4 scores were calculated whether the probe matches exon, intron, in the sense or antisense orientations. The penalty was calculated using the table underneath and the final score was calculated as:

$$100 - \min\left(\begin{array}{c}\left(\left(\sum_{i=1}^{n}\min\left(\sum_{j=1}^{m} Penalty\ exon\ sense_{ij}, \text{Max penalty } exon\ sense_i\right)\right)_{exon\ sense}\\ +\left(\sum_{i=1}^{n}\min\left(\sum_{j=1}^{m} Penalty\ exon\ antisense_{ij}, \text{Max penalty } exon\ antisense_i\right)\right)_{exon\ antisense} +\\ \left(\sum_{i=1}^{n}\min\left(\sum_{j=1}^{m} Penalty\ intron\ sense_{ij}, \text{Max penalty } intron\ sense_i\right)\right)_{intron\ sense} +\\ \left(\sum_{i=1}^{n}\min\left(\sum_{j=1}^{m} Penalty\ intron\ sense_{ij}, \text{Max penalty } intron\ antisense_i\right)\right)_{intron\ antisense},\\ \max\left(Max\ Penalty\ Exon\ Sense, Max\ Penalty\ Exon\ Antisense, Max\ Penalty\ Intron\ Sense, Max\ Penalty\ Intron\ Antisense\right)\end{array}\right)$$

i = Length of exact match

j= Number of genes for specific length of exact match

Specificity table for exon sense:

Length of exact match	32	31	30	29	28	27	26	5	24	23	22	21	20	19	18	17	16
Penalty	100	99	98	96	94	91	88	85	80	65	53	40	30	15	5.5	2.6	0.5
Max penalty	100	99	99	97	95	93	90	87	84	79	64	52	39	29	14.5	5.3	2.5

Specificity table for exon antisense:

Length of exact match	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17	16
Penalty	100	91	81	71	61	51	41	36	31	26	21	16	11	5.1	2.1	1.1	0.2
Max penalty	100	99	90	80	70	60	50	40	35	30	25	20	15	10	5	2	1

specificity table for intron sense:

Length of exact match	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17	16
Penalty	100	90	80	70	60	50	44	42.5	40	32.5	26.5	20	15	7.5	2.75	1.3	0.25
Max penalty	100	99	89	79	69	59	49	43	41	39	32	25	19	14	7.4	2.7	1.2

specificity table for intron antisense:

Length of exact match	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17	16
Penalty	100	90	80	60	40	25	20	15	12	10	8	5	3	2	1	0.5	0.1
Max penalty	100	99	89	79	59	39	24	19	14	11.5	9.5	7.5	4.9	2.9	1.9	0.9	0.49

Exon probe generation

The entire cDNA transcript was scanned using a 32-nt sliding window. A sequence was retained only if it satisfied the following criteria:

1) The melting temperature (Tm) of both the 5′ arm and the 3′ arm is > 45°C.
2) The absolute difference in Tm between the 5′ arm and the 3′ arm is < 9°C.
3) The GC content of the 32-nt window is between 45% and 65%.
4) The junction does not contain the dinucleotides 'GC', 'GG', or 'CG'.

Only sequences meeting all these criteria were retained. Sequences were ranked by binding score, and in cases of overlap, only the sequence with the highest binding score was retained.

Exon Junction probe generation

Only the region around the exon junction were scanned with 32-nt sliding window. Scanning starts -20nt before the junction and ends +20nt after the junction. A sequence was retained only if it satisfied the following criteria:

1) The melting temperature (Tm) of both the 5′ arm and the 3′ arm is > 41°C.
2) The absolute difference in Tm between the 5′ arm and the 3′ arm is < 9°C.
3) The GC content of the 32-nt window is between 45% and 65%.
4) The junction does not contain the dinucleotides 'GC', 'GG', or 'CG'.

Only sequences meeting all these criteria were retained. Sequences were ranked by binding score, and in cases of overlap, only the sequence with the highest binding score was retained.

Intron probe generation

Each intron sequence was individually scanned using a 32-nt sliding window. A sequence was retained only if it satisfied the following criteria:

1) The melting temperature (Tm) of both the 5′ arm and the 3′ arm is > 45°C.
2) The absolute difference in Tm between the 5′ arm and the 3′ arm is < 9°C.
3) The GC content of the 32-nt window is between 45% and 65%.
4) The junction does not contain the dinucleotides 'GC', 'GG', or 'CG'.

Only sequences meeting all these criteria were retained. Sequences were ranked by binding score, and in cases of overlap, only the sequence with the highest binding score was retained.