Supplemental Material

Applied Microbiology and Biotechnology

CHO Cell Engineering via Targeted Integration of Circular miR-21 Decoy Using CRIS-PITCh/Bxb1 Hybrid System

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Table S1 Sequence of primers and stem loops.

No	Primer name	Function	5' to 3' sequence
1	5′junction F	5' junction amplification	GGACAGCACCCAGACTTACACC
2	5' junction R	5' junction amplification	CCTACCCGCTTCCATTGCTCAG
3	3' junction F	3' junction amplification	GCAACCTCCCCTTCTACGAGC
4	3' junction R	3' junction amplification	CATCGGGTAGATGAGCCCAC
5	Genomic F	Out-out amplification	GTCCAGGCTCCCATTTCTGACAC
6	Genomic R	Out-out amplification	CTTCTGCCTTCTGGTGGTGGAG
7	Sponge-Convergent F	Linear and circular structure of sponge amplification and qRT-PCR	AGTGCGGCCACCCTCAAGATC
8	Sponge-Convergent R	Linear and circular structure of sponge amplification and qRT-PCR	TTCAACACCGCGGCCACTTATG
9	Sponge-Divergent F	Circular structure of sponge amplification	CAGAACATAAGCTAGGCGTATGTGG
10	Sponge-Divergent R	Circular structure of sponge amplification	GTTCTGATGTTGATCTTGAGGGTGGC
11	Scramble-Convergent F	Linear and circular structure of scramble amplification and qRT-PCR	GTGCGGCCACCCTCAAGATAA
12	Scramble-Convergent R	Linear and circular structure of scramble amplification and qRT-PCR	TTCAACACCGCGGCCACTTATG

13	Scramble-Divergent F	Circular structure of scramble amplification	ACCACTAAATAACATGGGCCCACAT
14	Scramble-Divergent R	Circular structure of scramble amplification	TAGTGGTCGCATTATCTTGAGGGTGG
15	*TK*135 F	Amplification of *TK* for qRT-PCR	AGCAGAAAATGCCCACGCTA
16	*TK*135 R	Amplification of *TK* for qRT-PCR	AGTAAGTCATCGGCTCGGGT
17	*GFP* F	Amplification of *EGFP* for qRT-PCR	CAGAAGAACGGCATCAAGGT
18	*GFP* R	Amplification of *EGFP* for qRT-PCR	GTGCTCAGGTAGTGGTTGTC
19	*ACTB* F	Amplification of *ACTB* for qRT-PCR	GTCCTACCTGCCTTGACTACTT
20	*ACTB* R	Amplification of *ACTB* for qRT-PCR	TACGACCAGAGGCATACAGGG
21	miR-21 stem-loop	Synthesis of miR-21 cDNA	GTCGTATGCAGAGCAGGGTCCGAGGTATTCGCACTGGATACGACTCAACA
22	miR-21 F	Amplification of miR-21 for qRT-PCR	CCGGCCTAGCTTATCAGACTG
٢3	U6 stem-loop	Synthesis of U6 snoRNA cDNA	GTCGTATGCAGAGCAGGGTCCGAGGTATTCGCACTGGATACGACCGCTTC
٢4	U6 F	Amplification of U6 snoRNA for qRT-PCR	CTCGCTTCGGCAGCAC
٢5	Universal R	Amplification of miR-21 and U6 snoRNA for qRT-PCR	GTGCAGGGTCCGAGGT
٢6	*Trim33* F	Amplification of *Trim33* for qRT-PCR	TCTACCCCCACAGCCTACAAG

27	*Trim33* R	Amplification of *Trim33* for qRT-PCR	CGACTGCCAGTGCTAGATGTAC
28	*Pdcd4* F	Amplification of *Pdcd4* for qRT-PCR	GGTGGACGTGAAAGATCCCAAC
29	*Pdcd4* R	Amplification of *Pdcd4* for qRT-PCR	CCTCATTTGTGTCTCCGTGCTC
٣0	*Atp11b* F	Amplification of *Atp11b* for qRT-PCR	CTCTTGCACTCAGGGAGCAT
31	*Atp11b* R	Amplification of *Atp11b* for qRT-PCR	CCAACAGCCAATGTGATCGGT

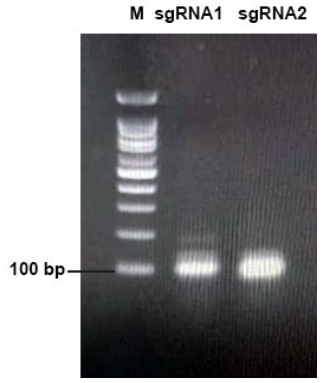


Fig. S1 2% agarose gel electrophoresis of the in vitro transcription product. sgRNA1 corresponds to the PITCh sgRNA, while sgRNA2 represents the genome-targeting sgRNA. The expected size for both sgRNAs is 100 bp. The M lane contains the 100 bp DNA marker.

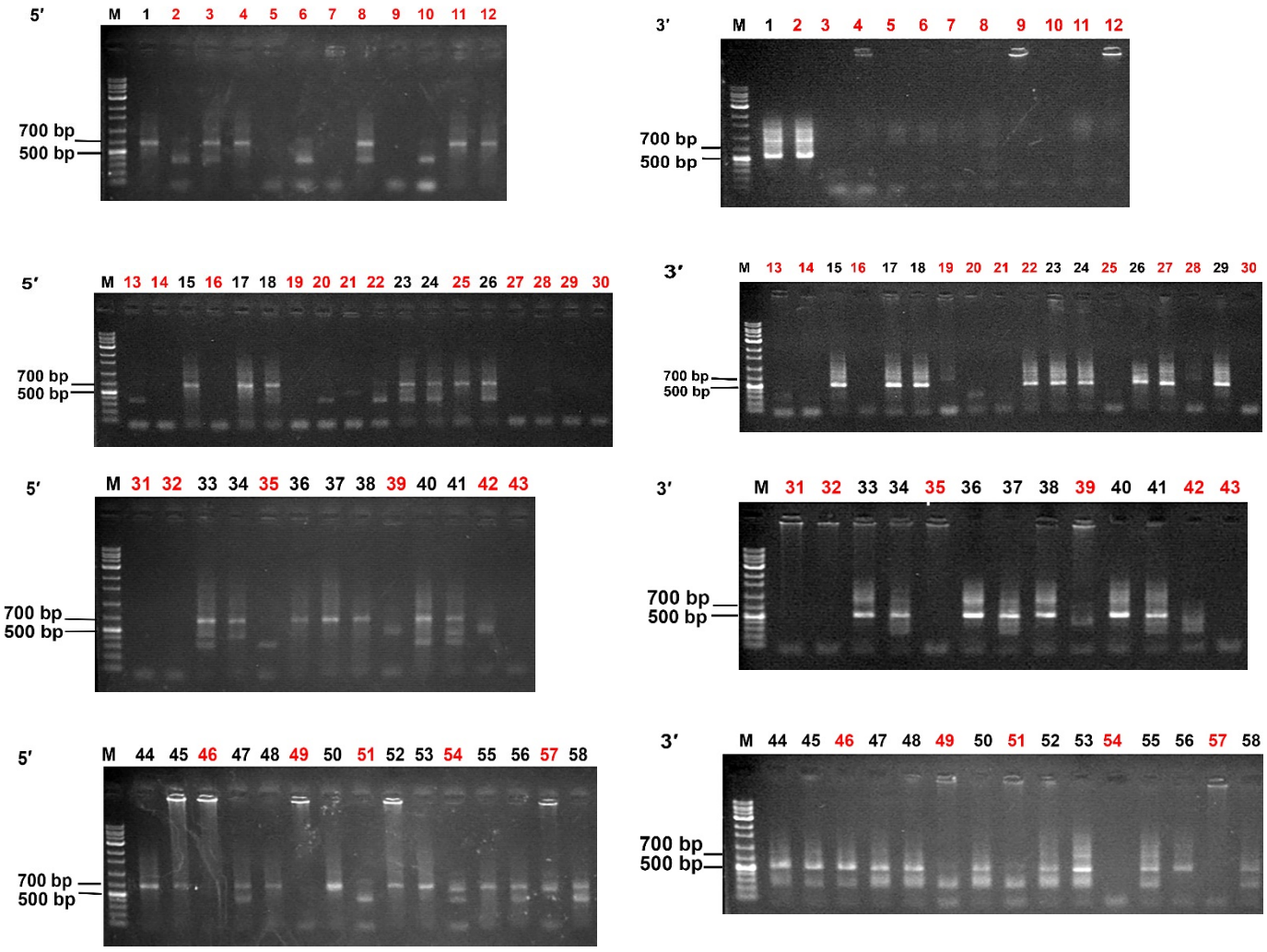


Fig. S2 The results of agarose gel electrophoresis for the 5′/3′ junction PCR of the landing pad on RNP based delivery system single clones. The expected PCR product sizes were 666 bp for the 5′ junction and 622 bp for the 3′ junction PCRs. Positive clones in both the 5′ and 3′ junction PCRs are indicated by black numbers and negative clones for either 5′ or 3′ junction PCRs are indicated by red numbers. The M lane corresponds to the 1 kb DNA marker.

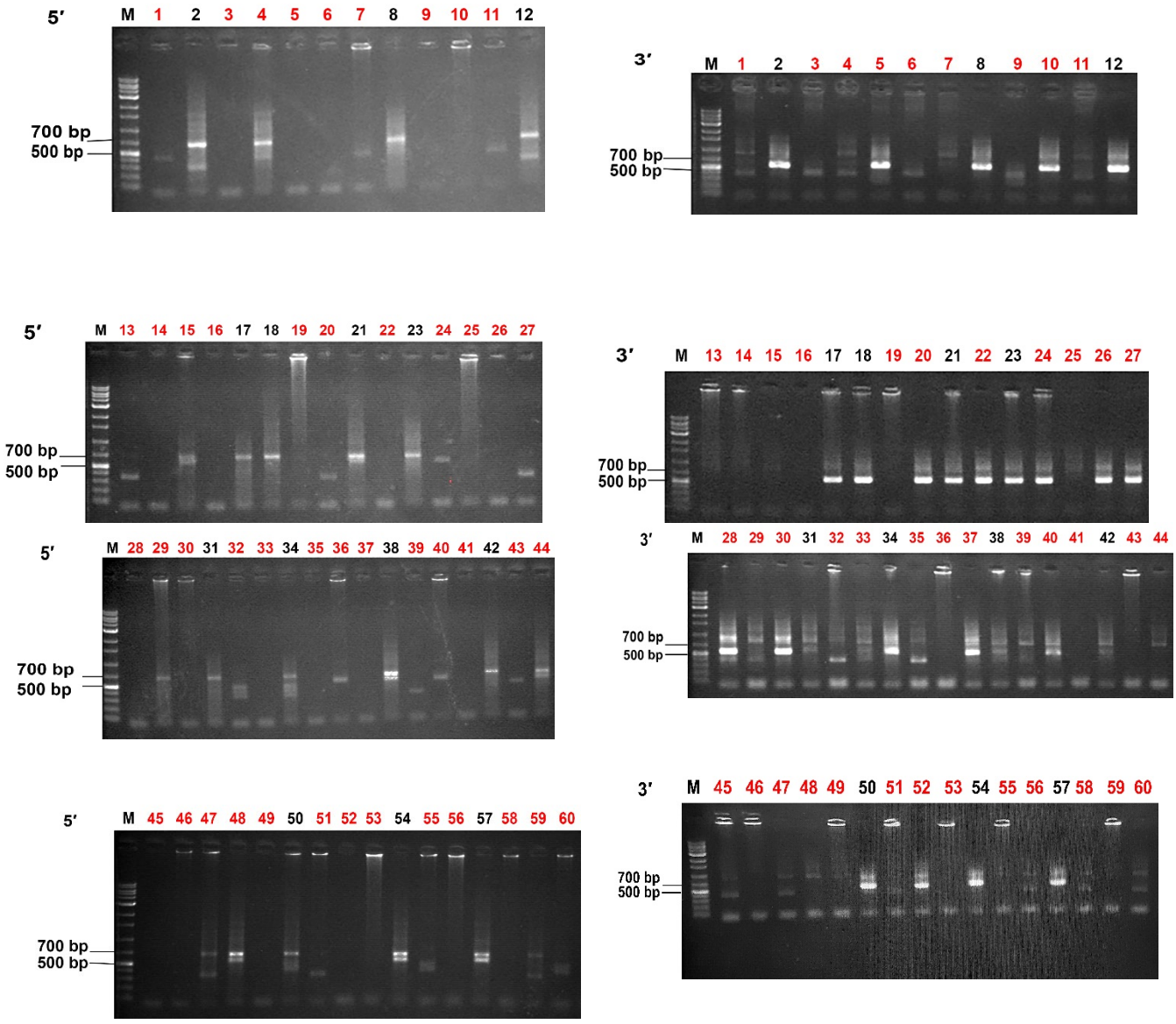


Fig. S3 The results of agarose gel electrophoresis for the 5′/3′ junction PCR of the landing pad on plasmid-based delivery system single clones. The expected PCR product sizes were 666 bp for the 5′ junction and 622 bp for the 3′ junction PCRs. Positive clones in both the 5′ and 3′ junction PCRs are indicated by black numbers and negative clones for either the 5′ or 3′ junction PCRs are indicated by red numbers. The M lane corresponds to the 1 kb DNA marker.

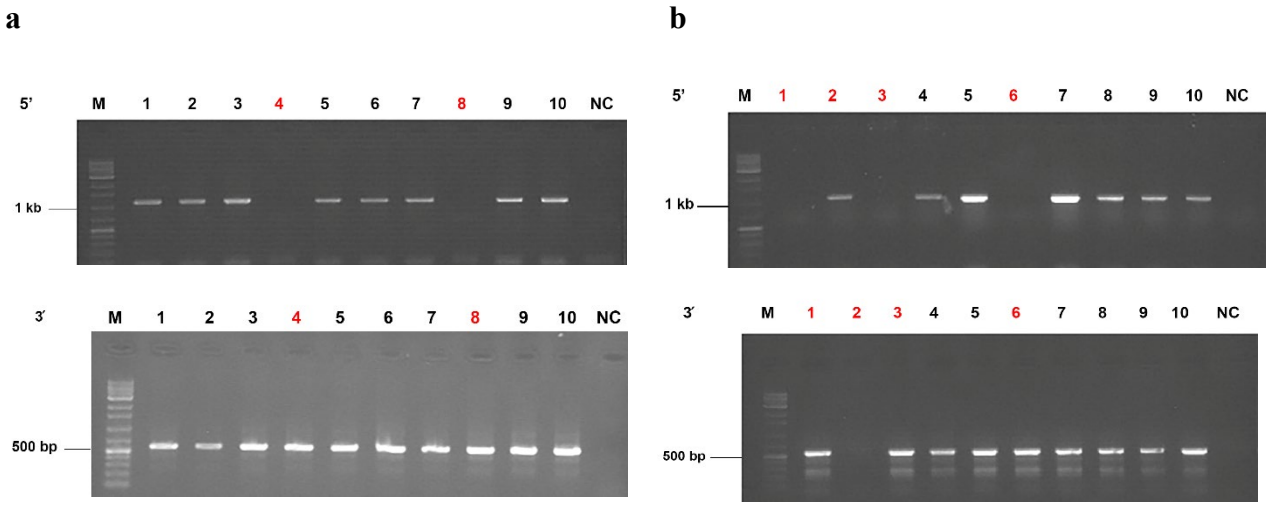
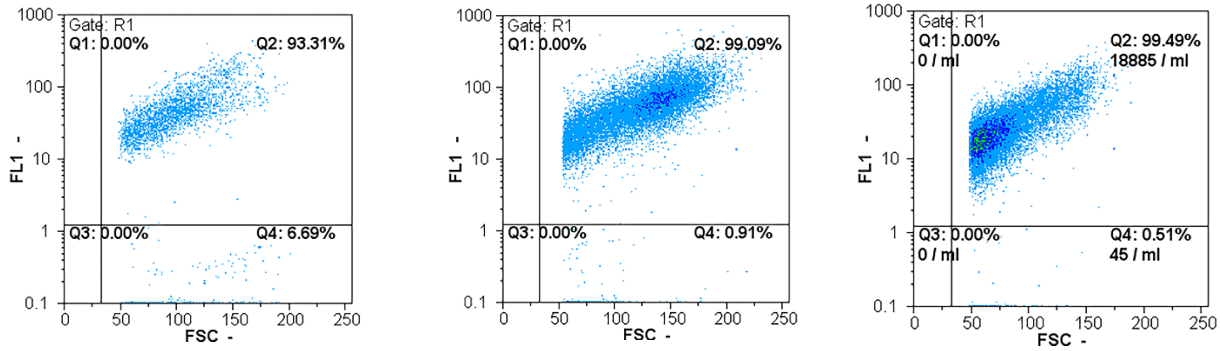


Fig. S4 The results of agarose gel electrophoresis for the 5′/3′ junction PCR on retargeted single clones. a Agarose gel electrophoresis for the 5′/3′ junction PCR on CM21D expressing single clones. **b** Agarose gel electrophoresis for the 5′/3′ junction PCR on CM21SD expressing single clones. The expected PCR product sizes were approximately 1360 bp for the 5′ junction and 646 bp for the 3′ junction PCR. Positive clones in both the 5′ and 3′ junction PCRs are indicated by black numbers and negative clones for either the 5′ or 3′ junction PCRs are indicated by red numbers. The M lane corresponds to the 1 kb DNA marker, while the NC lane represents the negative control.

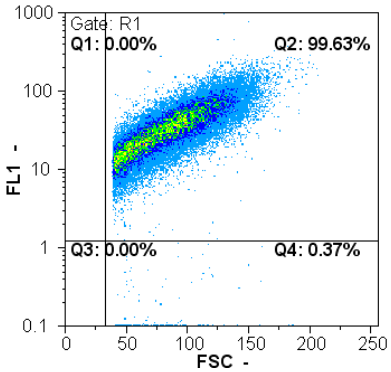
a



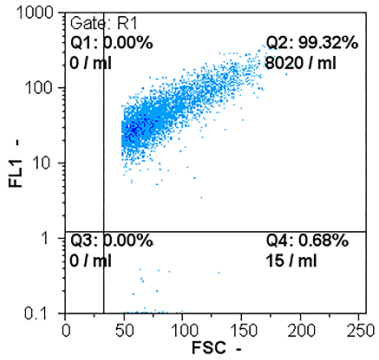
Clone 1

Clone 3

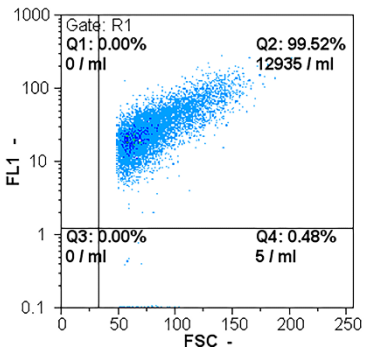
Clone 5



Clone 6

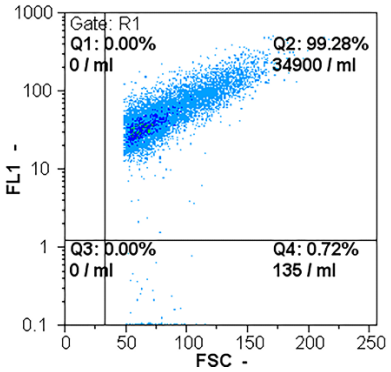


Clone 7

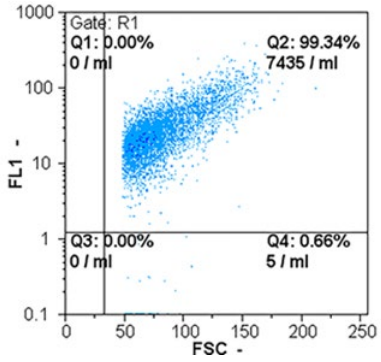


Clone 9

b



Clone 10



Clone 4

Fig. S5 EGFP expression analysis in retargeted single clones by flow cytometry. a Single clones retargeted with CM21D and expressed EGFP > 99% were selected for the next step. **b** A single clone retargeted with CM21SD, which also exhibited EGFP > 99%, was chosen next step.

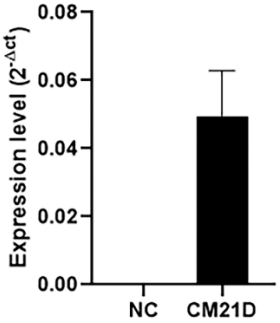
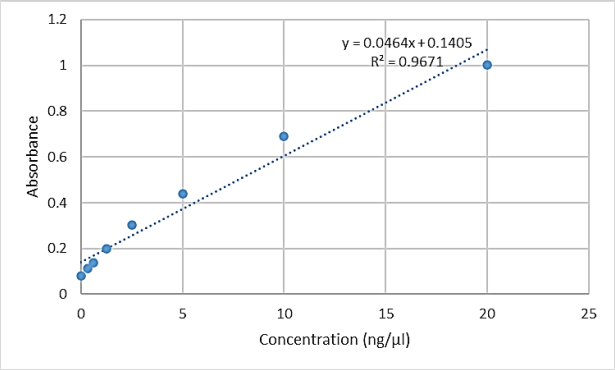


Fig. S6 Validating the expression of CM21D. Expression level of CM21D and CM21SD was measured using qRT-PCR (based on the formula $2^{(-\Delta Ct)}$). The error bars correspond to the standard deviations observed in technical replicates (n = 3).

a

Tube	Concentration (ng/µl)	Absorbance
1	20	1.004
2	10	0.69
3	5	0.4395
4	2.5	0.303
5	1.25	0.1975
6	0.625	0.1385
7	0.3125	0.1115



b

Test group		Control group	
OD	**Concentration (µg/ml)**	**OD**	**Concentration (µg/ml)**
0.721	6.143 µg/ml	0.452	3.263µg/ml
0.648	8.042 µg/ml	0.362	3.442µg/ml
0.5445	8.507 µg/ml	0.3493	4.326µg/ml

Fig. S7 Determination of hrsACE2 productivity using ELISA assay. a The standard curve is graphed with optical density (OD) plotted against the logarithm of protein concentration, as indicated in the table. **b** The concentration of hrsACE2 was determined in both the test and control groups. Three different dilutions were used: 1/500, 1/750, and 1/1000. Each dilution was tested in triplicate.