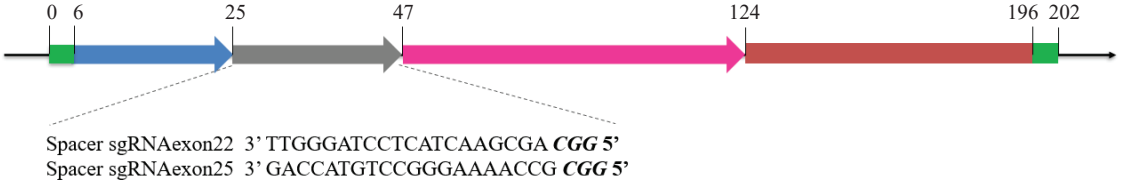
Supplementary 1.

Primer sequences and characteristics used for PCR amplification of *ERBB2* domains, exons, and sgRNA construct.

Name	Strand	Sequence (5' - 3')	Annealing Temp. (°C)	GC (%)	Fragment Length (bp)
ERBB2 Kinase Domain	Forward	TACACGATGCGGAGACTGCTG	58.8	57.14	967
	Reverse	AGCGGTAGAAGGTGCTGTCC	58.3	60.0	
ERBB2 Exon 22	Forward	ACTTTATTGTGGAGGCAGC	52.0	47.37	523
	Reverse	TGTAGACTGTGCCAAAAGC	52.4	47.37	
ERBB2 Exon 25	Forward	GACTCCCGCAAACCTAGACT	55.4	55	933
	Reverse	GGAAGCACCCATGTAGACCT	54.5	55	
sgRNA Cassette	Forward	GAATTCTAATACGACTCACT	46.5	35	202
	Reverse	GAATTCGAGAGCGTTCACC	53.6	52.6	

Supplementary 2.

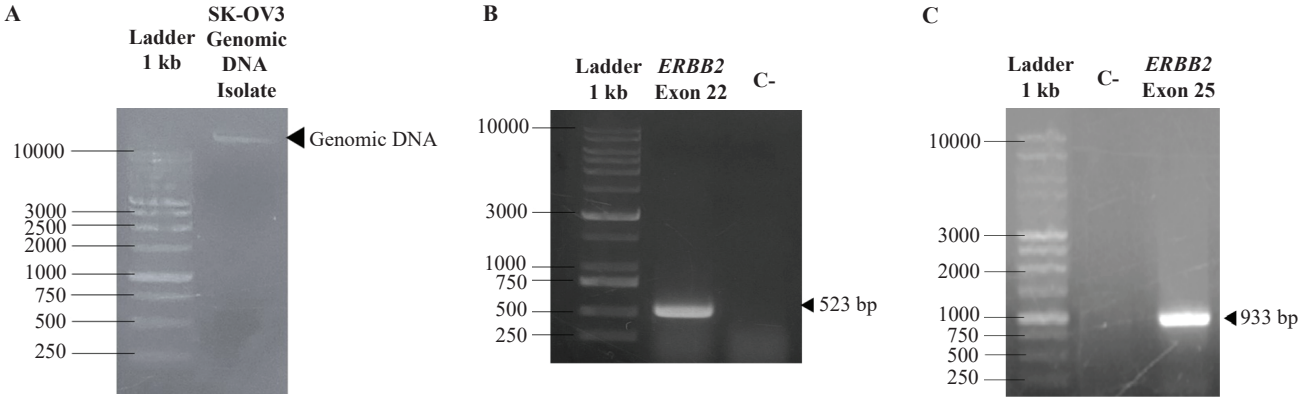
Schematic representation of the sgRNA expression cassette.



Schematic representation of the sgRNA expression cassette; the green region represents the EcoRI restriction site, the blue region represents the T7 promoter, the gray region represents the spacer, the pink region represents the Cas9 scaffold, and the red region represents the rrnB T1 terminator. The numbers indicate the nucleotide positions along the sgRNA cassette.

Supplementary 3.

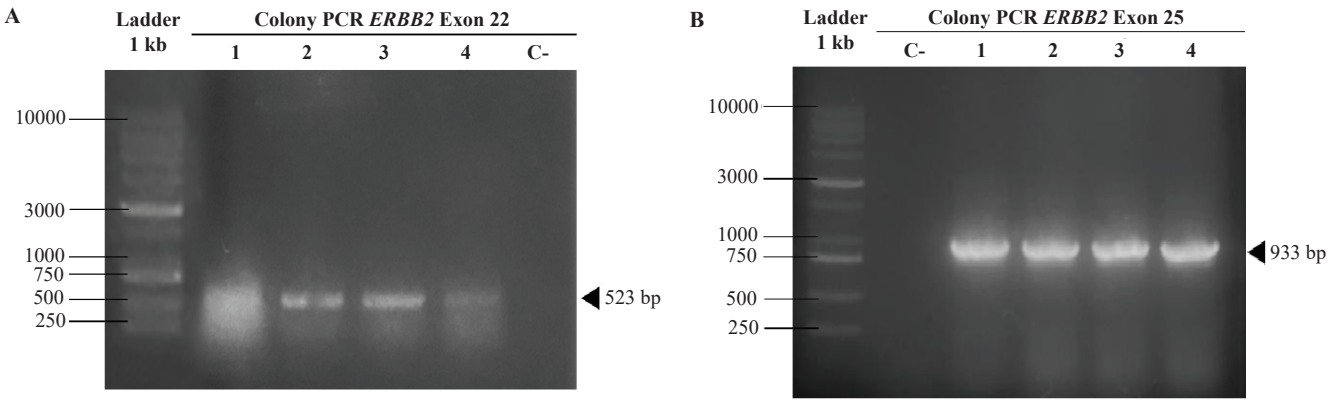
Target gene amplification.



Agarose gel electrophoresis (1% agar ose in 1× TAE buffer) of *ERBB2* gene amplification from SK-OV3 cells. A: extracted genomic DNA from SK-OV3 cells; B: amplified *ERBB2* exon 22, indicated a single band at 523 bp; C: PCR amplification of *ERBB2* exon 25, indicated a single band at 933 bp.

Supplementary 4.

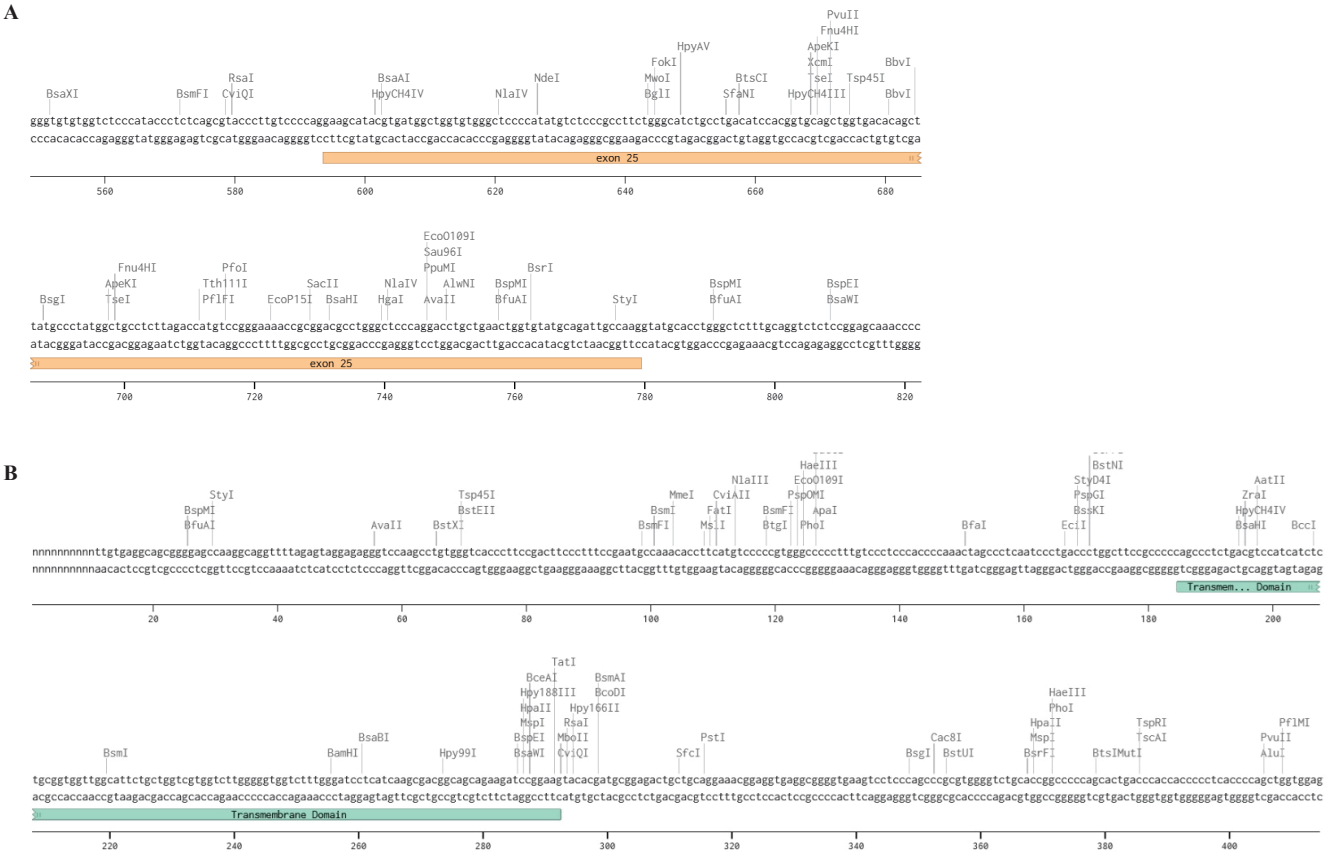
Colony PCR amplification of exon 22 and exon 25 fragments.



Agarose gel electrophoresis of colony PCR products targeting the *ERBB2* gene. A: amplified *ERBB2* exon 22 of 523 bp in colonies 2, 3, 8, and 9; B: amplified *ERBB2* exon 25 of 933 bp in colonies 1–4.

Supplementary 5.

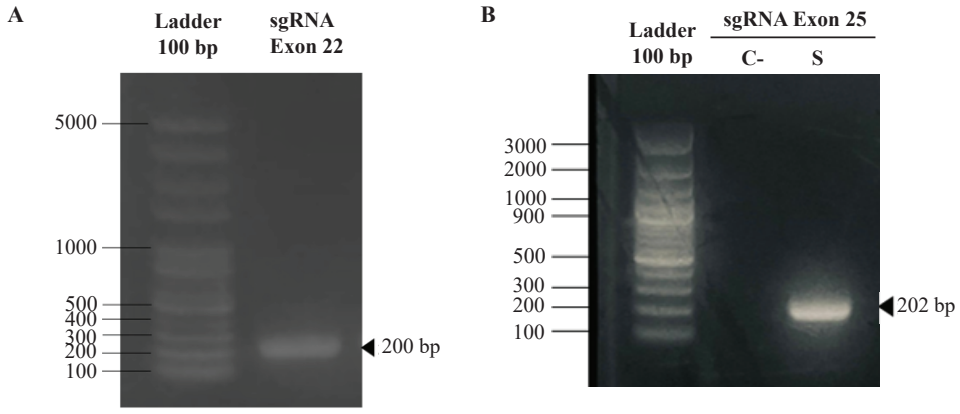
Results of Sanger sequencing of exon 22 and exon 25 fragments.



Sanger sequencing results of *ERBB2* target fragments: exon 22 (A) and exon 25 (B), where the highlighted regions denote exon sequences that match the corresponding *ERBB2* reference sequence, confirming the absence of mutations within the targeted regions.

Supplementary 6.

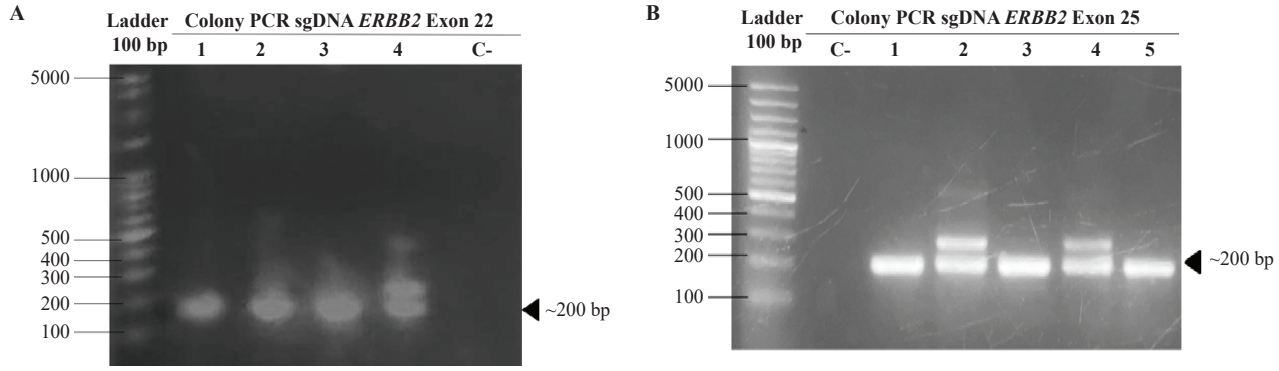
Amplification of synthesized sgRNA.



Agarose gel electrophoresis of amplified sgRNA synthesis products. A: sgRNA targeting *ERBB2* exon 22; B: sgRNA targeting *ERBB2* exon 25, both yielding single bands of approximately 200 bp. A 100 bp DNA ladder was used as a molecular size marker.

Supplementary 7.

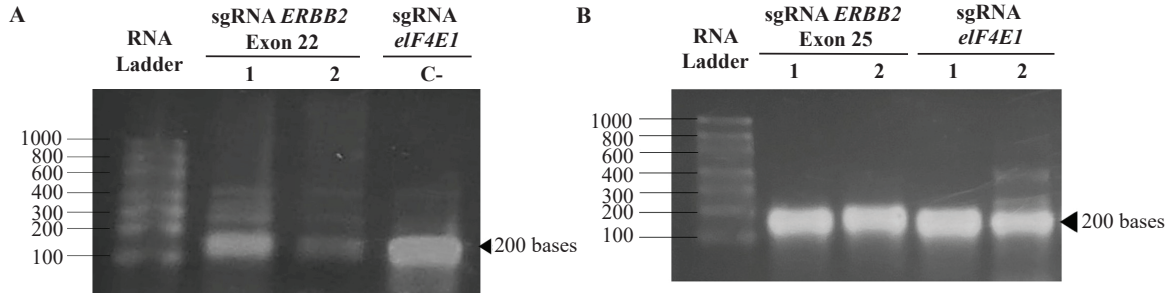
Colony PCR amplification of sgRNA exon 22 and exon 25 fragments.



Agarose gel electrophoresis of colony PCR products for sgRNA constructs. A: colony PCR of sgRNA targeting *ERBB2* exon 22, showing a single band of approximately 200 bp in colonies 1, 2, and 3; B: colony PCR of sgRNA targeting *ERBB2* exon 25, showing a single band of approximately 200 bp in colonies 1, 3, and 5. A DNA ladder was used as a molecular size marker.

Supplementary 8.

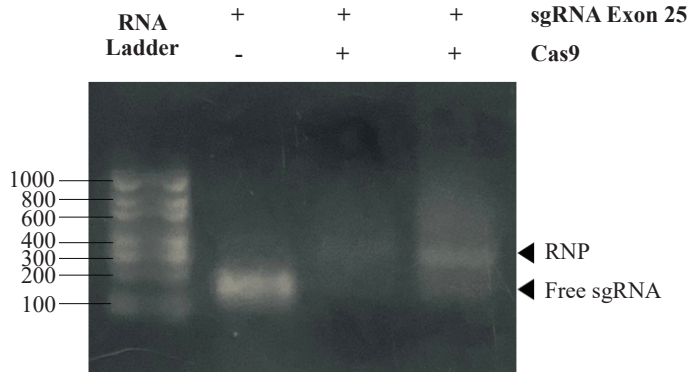
Results of sgRNA production by *in vitro* transcription.



Visualization of *in vitro*–transcribed sgRNA by agarose gel electrophoresis. A: sgRNA targeting *ERBB2* exon 22 and the positive control sgRNA300; b: sgRNA targeting *ERBB2* exon 25 and the positive control sgRNA196. The expected sgRNA product (~200 bp) is indicated.

Supplementary 9.

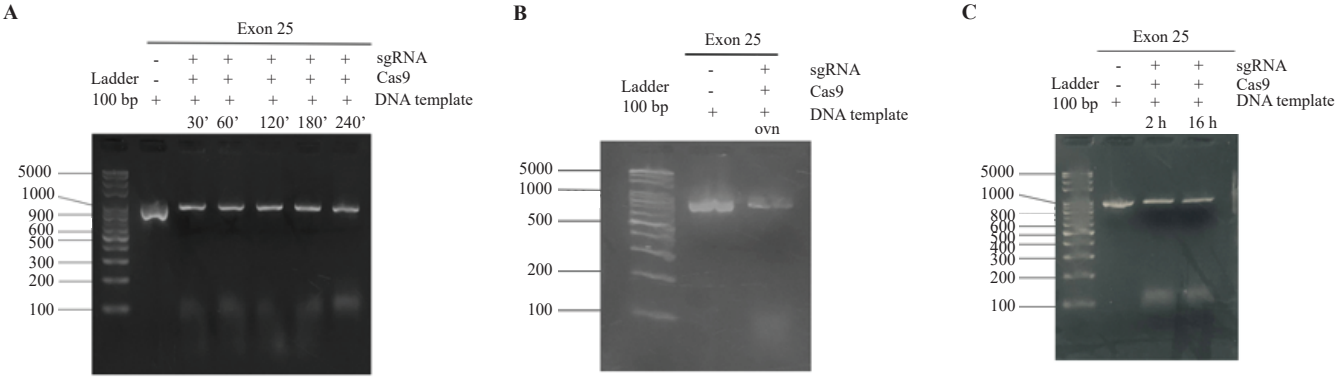
EMSA results of the sgRNA exon 25–Cas9 RNP complex.



Visualization of Cas9–sgRNARNP complex formation using Electrophoretic Mobility Shift Assay (EMSA) on a 2% agarose gel in 1× TBE buffer. Bands exhibiting reduced mobility indicate the formation of the RNP complex, whereas bands with higher mobility represent free sgRNA. An RNA ladder was used as a fragment size marker.

Supplementary 10.

Effect of incubation time variation on endonuclease activity of exon 25–targeting sgRNA.



Agarose gel electrophoresis analysis of *in vitro* CRISPR–Cas9 RNP endonuclease activity targeting *ERBB2* exon 25 under different incubation conditions. A: Incubation for 30, 60, 90, 120, 180, and 240 min at 37 °C; B: incubation for 1 h at 37 °C; C: incubation for 2 and 16 h at 21 °C. No detectable DNA cleavage fragments were observed under any of the tested conditions.