Electronic Supplementary Material (ESI) for Chemical Science.

Supplementary Information

Element Coding Based Accurate Evaluation of CRISPR/Cas9 Initial Cleavage

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Table S1. Oligonucleotides sequence

Primer	Sequence	Modification
ntDNA	ATATATTAGCTCATCGGGAGAACACACAC**TTTTTT**ATGGAGTCGAGCTCG	
ntDNA-1	ATATATTAGCTCATCGGGAGAACACACAC**TTTTT/iCHCHdT/**ATGGAGTCGAGCTCG	Int CHCH-dT
ntDNA-2	ATATATTAGCTCATCGGGAGAACACACAC**TTTT/iCHCHdT/T**ATGGAGTCGAGCTCG	Int CHCH-dT
ntDNA-3	ATATATTAGCTCATCGGGAGAACACACAC**TTT/iCHCHdT/TT**ATGGAGTCGAGCTCG	Int CHCH-dT
ntDNA-4	ATATATTAGCTCATCGGGAGAACACACAC**TT/iCHCHdT/TTT**ATGGAGTCGAGCTCG	Int CHCH-dT
ntDNA-5	ATATATTAGCTCATCGGGAGAACACACAC**T/iCHCHdT/TTTT**ATGGAGTCGAGCTCG	Int CHCH-dT
ntDNA-6	ATATATTAGCTCATCGGGAGAACACACAC**/iCHCHdT/TTTTT**ATGGAGTCGAGCTCG	Int CHCH-dT
biotin-tDNA-SH	CTCGACTCCAT**AAAAAAA**GTGTGTGTTCTCCCGATGAGCTAATATAT	3`SH C6 5`Biotin
sgRNA	GGAGAACACACACUUUUUUAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUU	
Primer 1 (T7 promoter contained)	TAATACGACTCACTATAGGGGAGAACACACACTTTTTTAGTTTTAGAGCTAGAAATAGC	
Primer 2 (Reverse)	AAGCACCGACTCGGTGCCTCTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC	
M-1	GGAGAACACACACUUUUUUCG… …	
M-2	GGAGAACACACACUUUUUCAG… …	
M-3	GGAGAACACACACUUUUCUAG… …	
M-4	GGAGAACACACACUUUCUUAG… …	
M-5	GGAGAACACACACUUCUUUAG… …	
M-6	GGAGAACACACACUCUUUUAG… …	
M-7	GGAGAACACACACCUUUUUAG… …	
M-8	GGAGAACACACAAUUUUUUAG… …	
M-9	GGAGAACACACCCUUUUUUAG… …	
M-10	GGAGAACACAAACUUUUUUAG… …	

M-11	GGAGAACACCCACUUUUUUAG… …
M-12	GGAGAACAAACACUUUUUUAG… …
M-13	GGAGAACCCACACUUUUUUAG… …
M-14	GGAGAAAACACACUUUUUUAG… …
M-15	GGAGACCACACACUUUUUUAG… …
M-16	GGAGCACACACACUUUUUUAG… …
M-17	GGACAACACACACUUUUUUAG… …
M-18	GGCGAACACACACUUUUUUAG… …
M-19	GCAGAACACACACUUUUUUAG… …
19'-1	GCAGAACACACACUUUUUUAG… …
19'-2	GCCGAACACACACUUUUUUAG… …
19'-3	GCCCAACACACACUUUUUUAG… …
19'-4	GCCCCACACACACUUUUUUAG… …
18'-1	GGCGAACACACACUUUUUUAG… …
18'-2	GGCCAACACACACUUUUUUAG… …
18'-3	GGCCCACACACACUUUUUUAG… …
18'-4	GGCCCCCACACACUUUUUUAG… …
17'-1	GGACAACACACACUUUUUUAG… …
17'-2	GGACCACACACACUUUUUUAG… …
17'-3	GGACCCCACACACUUUUUUAG… …
17'-4	GGACCCAACACACUUUUUUAG… …

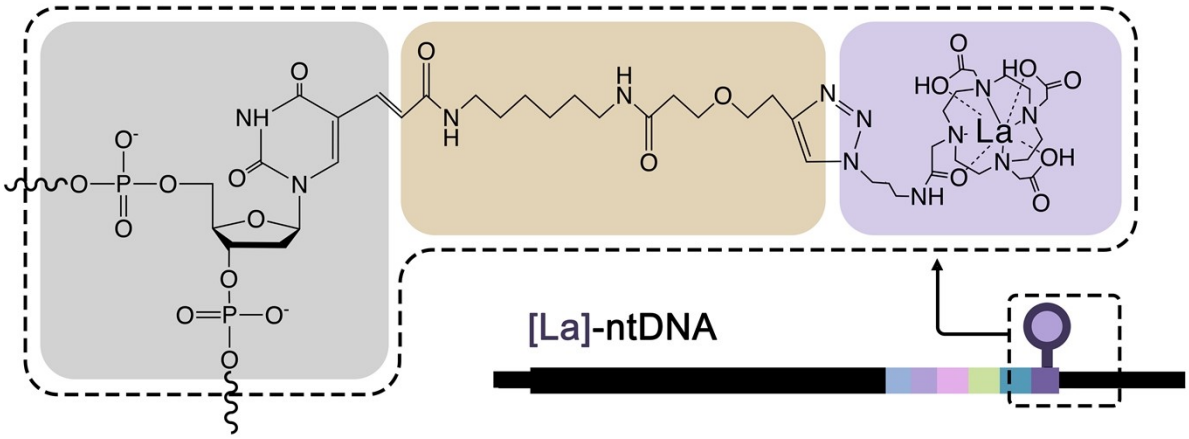
Table S2. Operating conditions of ICPMS

Parameters	Values
La (m/z)	138.906
Ce (m/z)	139.905
Pr (m/z)	140.907
Eu (m/z)	152.929
Tb (m/z)	158.925
Ho (m/z)	164.93
Tm (m/z)	168.934
ICP RF Power (W)	1300
Plasma Gas Flow (L/min)	18
Auxiliary Gas Flow (L/min)	1.20
Nebulizer Gas Flow (L/min)	0.94
Deflector Voltage (V)	11.75
Pulse Stage Voltage (V)	1050
Analog Stage Voltage (V)	1825
Sample Uptake Rate (mL/min)	0.25
Resolution	2060
Dwell Time (μs)	50
Detecting Time (s)	10

Figure S1. Isotope abundance and interferences of selected elements

Element	Isotope	Mass	Abundance	Interferences
La	La 138	137.907	0.090	138Ba, 138Ce
	La 139	**138.906**	**99.910**	
Ce	Ce 136	135.907	0.185	136Ba, 136Xe
	Ce 138	137.906	0.251	137Ba, 138La
	Ce 140	**139.905**	**88.450**	
	Ce 142	141.909	11.114	142Nd
Pr	**Pr 141**	**140.907**	**100.000**	
Eu	Eu 151	150.92	47.810	135BaO
	Eu 153	**152.929**	**52.190**	137BaO
Tb	**Tb 159**	**158.925**	**100.000**	143NdO
Ho	**Ho 165**	**164.93**	**100.000**	149SmO
Tm	**Tm169**	**168.934**	**100.000**	153EuO

Figure S2. Chemical structure of [La]-ntDNA.



Gray part: thymine mononucleotide

Orange part: alkyne modification

Purple part: N_3-DOTA-[La]

Figure S3. The ESI-MS results of N_3-DOTA.

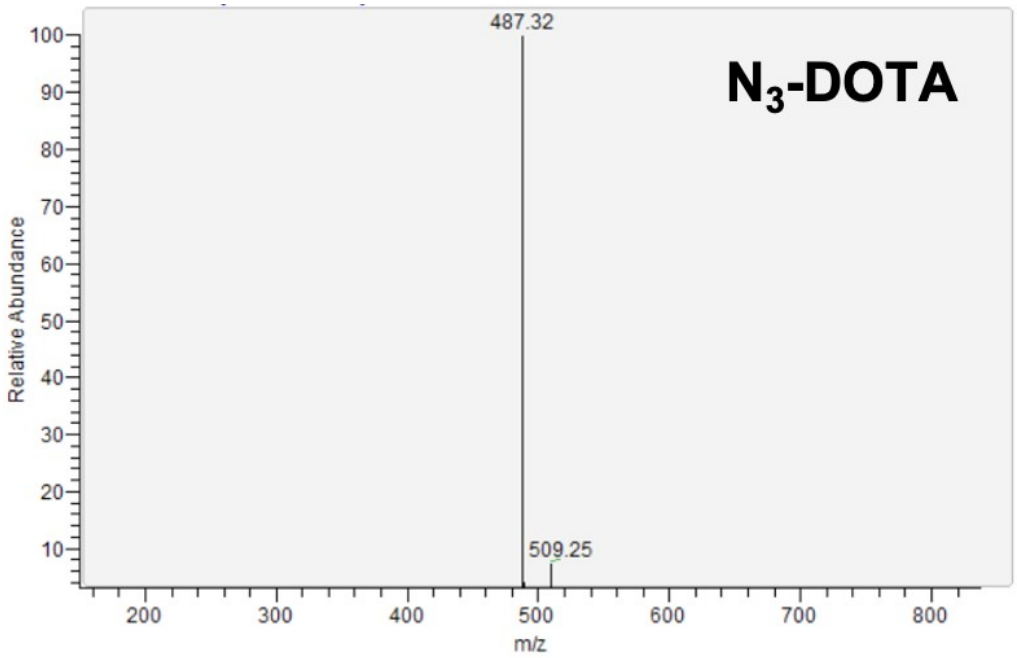


Figure S4. The ESI-MS results of six N_3-DOTA-[Ln].

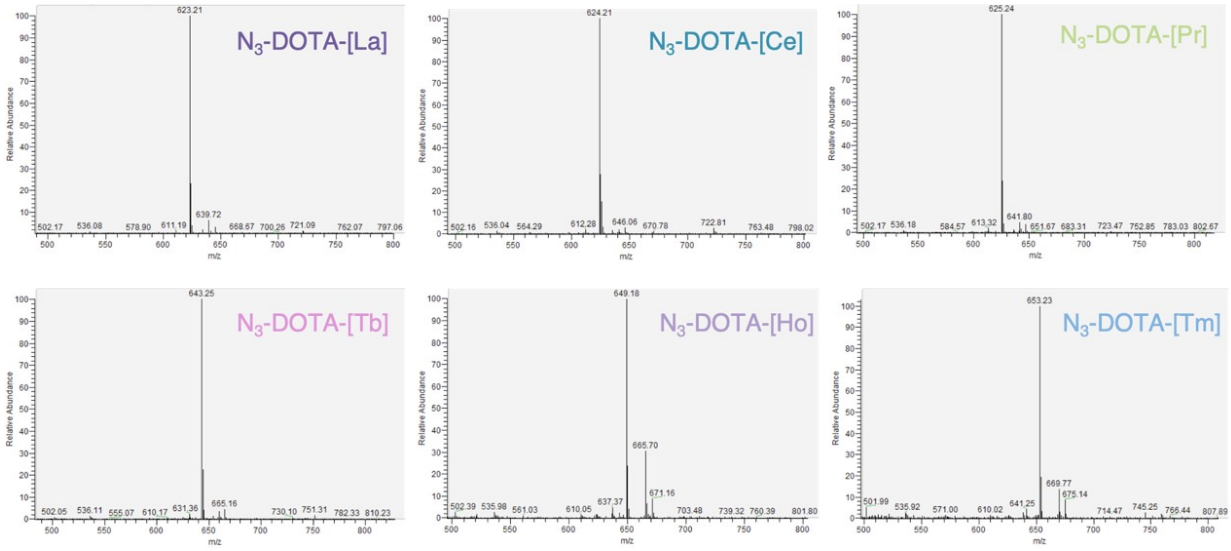


Figure S5. The MADI-TOF-MS characterization of original ssDNA (blue), DOTA modified ssDNA (green) and final lanthanide labeled ssDNA (yellow), respectively.

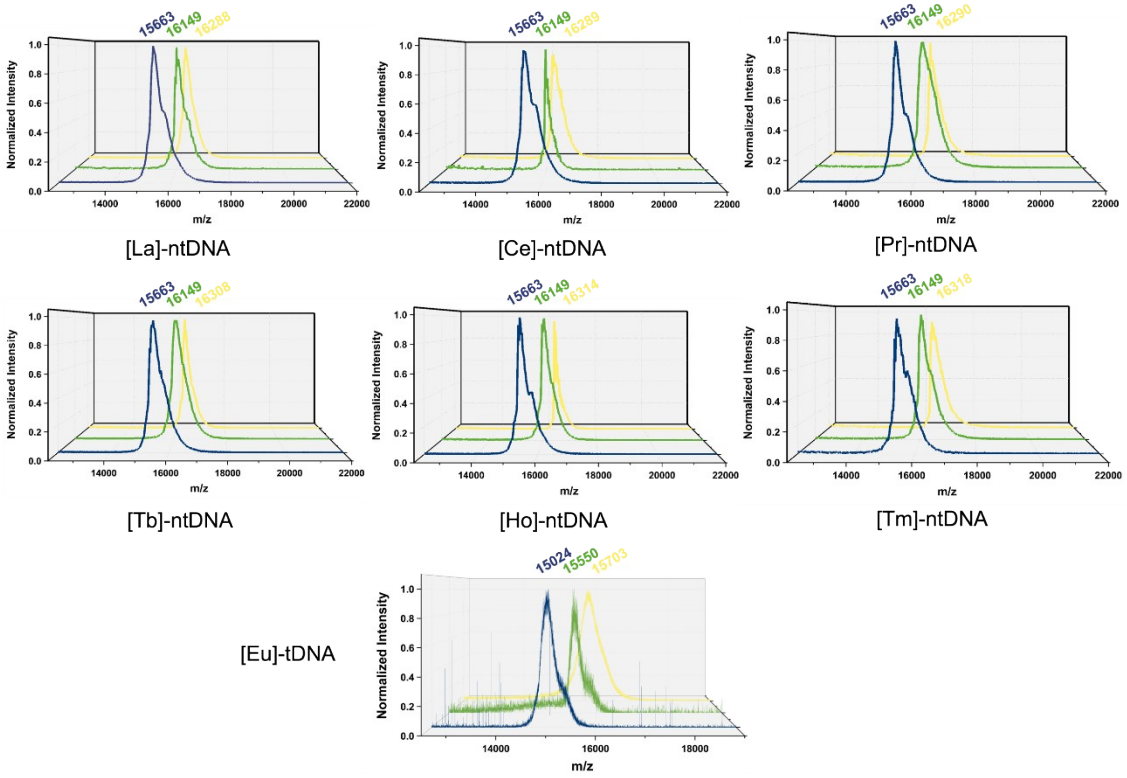
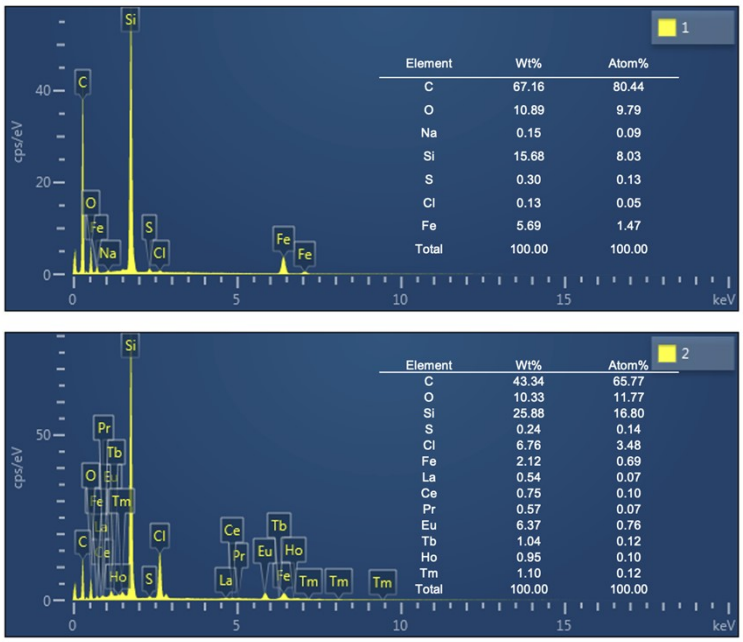
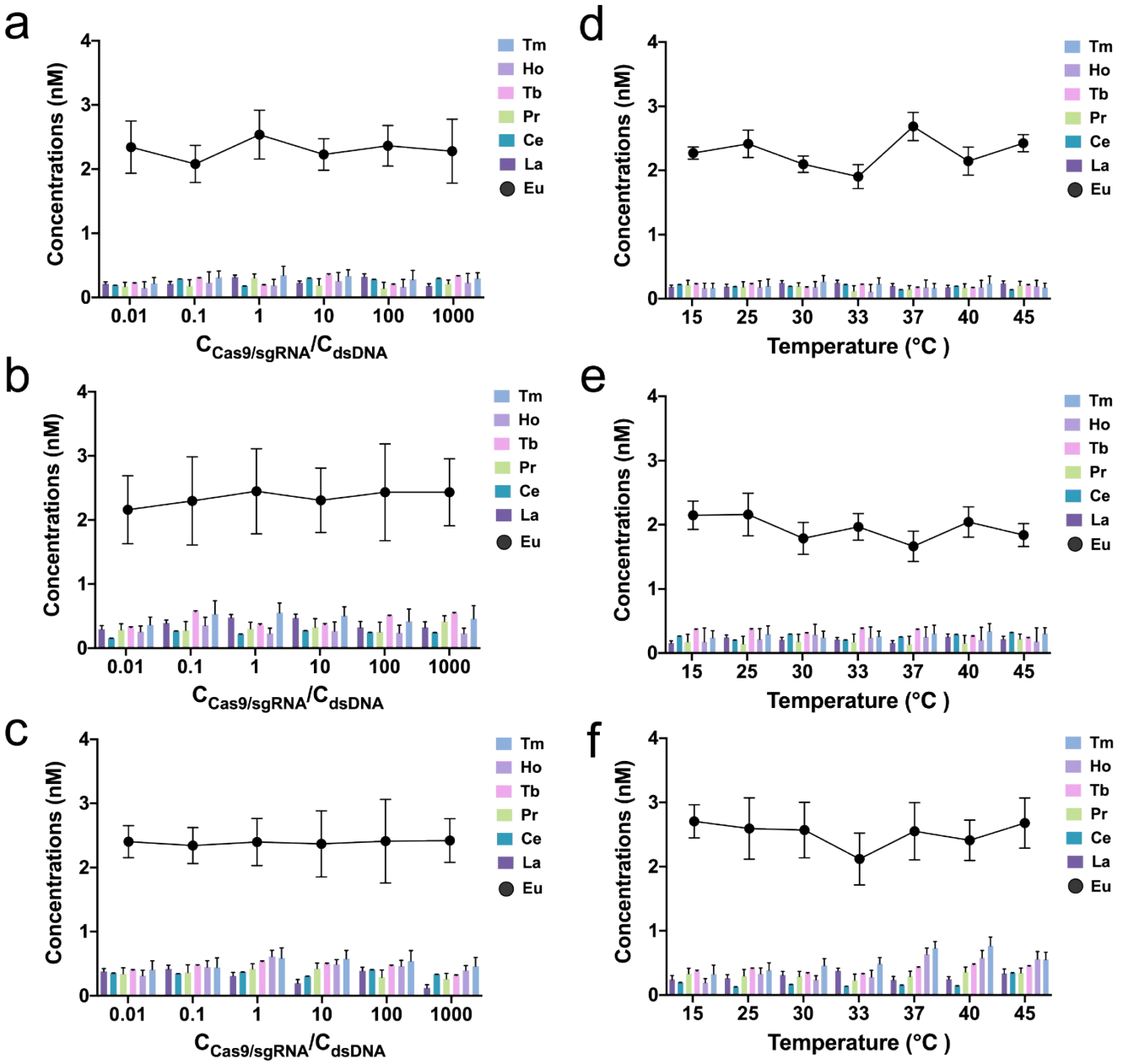


Figure S6. The EDS result of EC-CRISPR probes captured by SA-MBs.



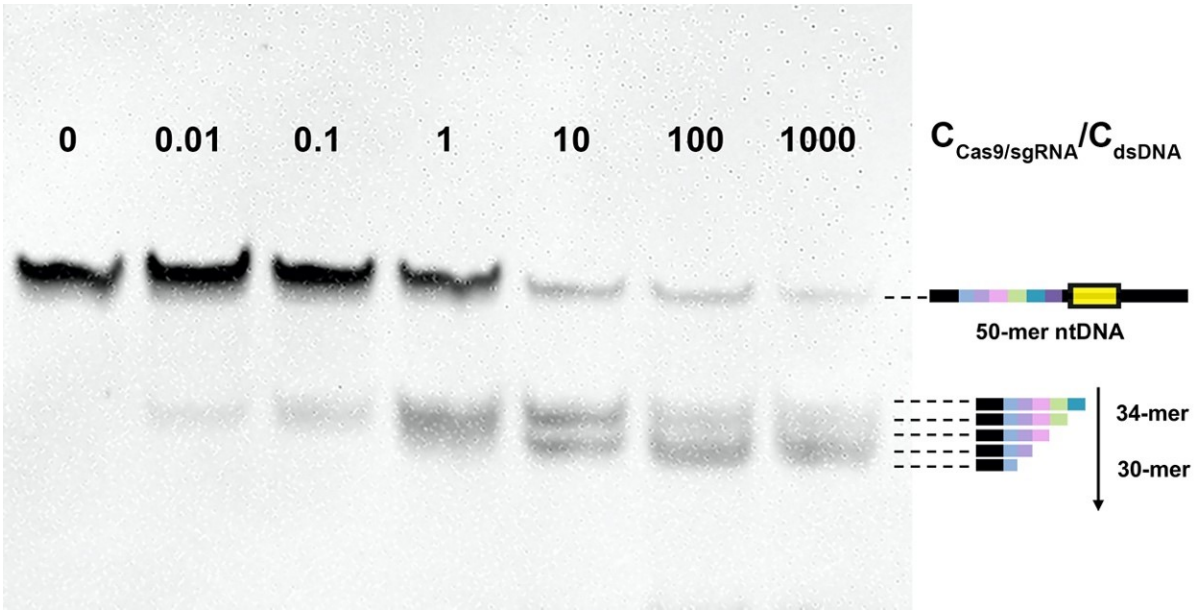
1. EDS spectrum of naked SA-MBs.
2. EDS spectrum of EC-CRISPR probes captured by SA-MBs.

Figure S7. Control experiment of Cas9/sgRNA concentrations and reaction temperature effect.



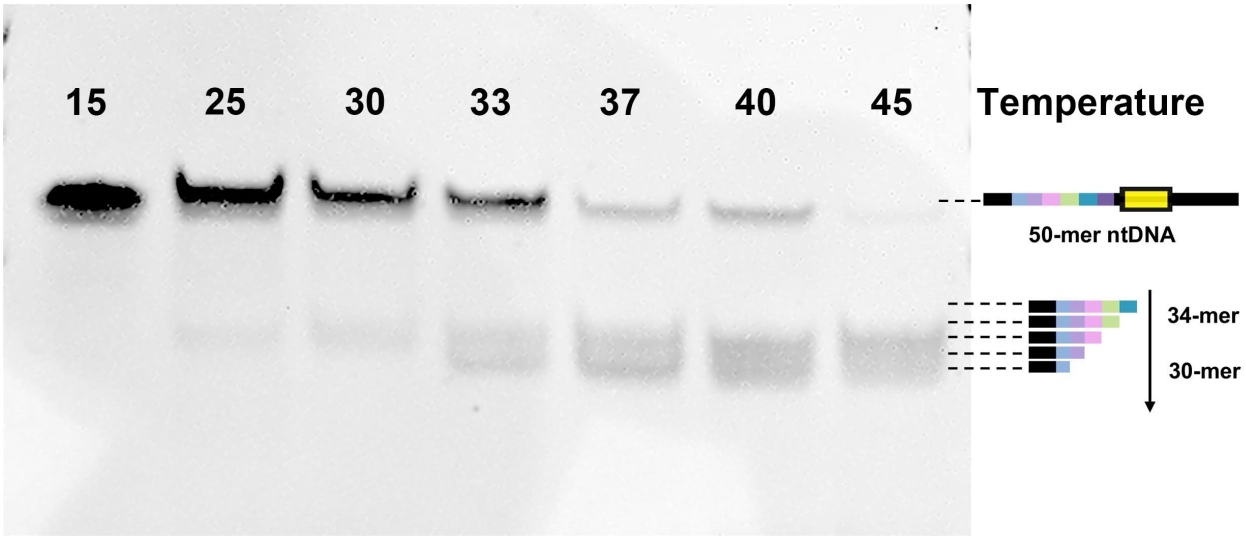
Control groups in Cas9/sgRNA concentration study: (a) Neither Cas9 nor sgRNA was introduced. (b) Only Cas9 protein was introduced. (c) Cas9 protein and non-specific sgRNA. Control groups in reaction temperature study: (d) Neither Cas9 nor sgRNA was introduced. (e) Only Cas9 protein was introduced. (f) Cas9 protein and non-specific sgRNA. All experimental conditions keep the same as Figure 3.

Figure S8. PAGE image of CRISPR/Cas9 cleavage products in varies Cas9/sgRNA concentrations.



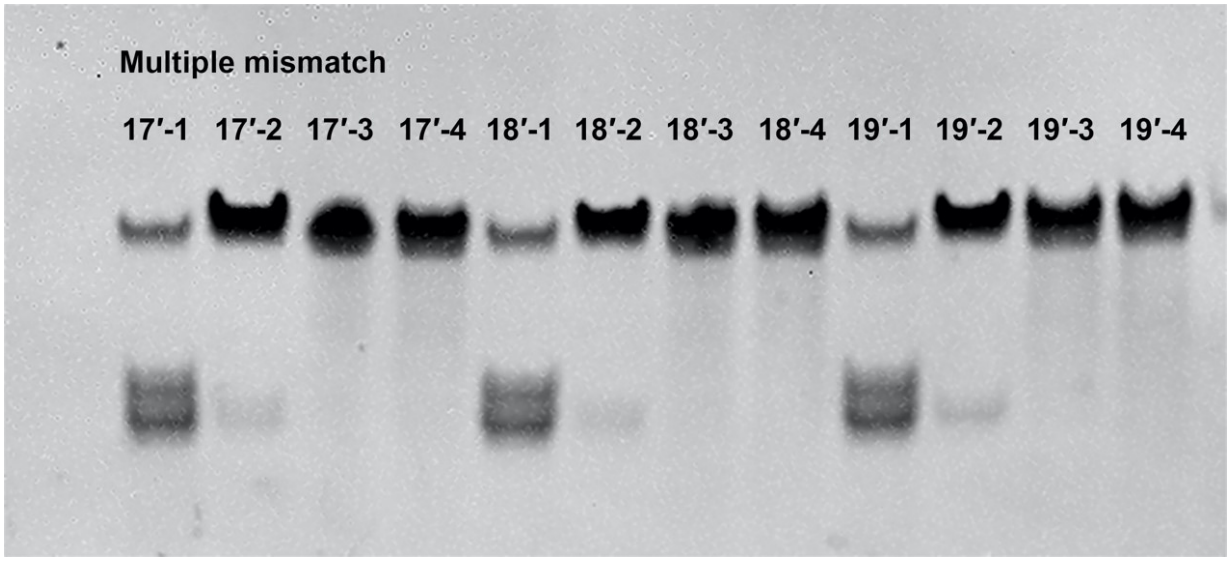
Reaction conditions: Cas9/sgRNA pre-incubated for 30 min (0.3, 3, 30, 300 nM, 3 μM and 30 μM), followed by introducing 30 nM mixed biotin-dsDNA substrate. Then incubated for 2h at 37 °C. 3'-terminal biotin labeled tDNA was removed by SA-MBs after heat to TM.

Figure S9. PAGE image of CRISPR/Cas9 cleavage products in varies temperature.



Reaction conditions: 300 nM Cas9/sgRNA pre-incubated for 30 min, followed by introducing 30 nM mixed biotin-dsDNA substrate. Then incubated for 2h at 15, 25, 30, 33, 37, 40 and 45 °C. 3'-terminal biotin labeled tDNA was removed by SA-MBs after heat to TM.

Figure S10. PAGE image of CRISPR/Cas9 cleavage products with multiple mismatches.



Reaction conditions: 300 nM mismatch sgRNA containing mismatch base was pre-incubated with Cas9 for 30 min, followed by introducing 30 nM mixed bioting-dsDNA substrate. Then incubated for 2h at 37 °C. 3'-terminal biotin labeled tDNA was removed by SA-MBs after heat to TM.

Figure S11. [Ln] raw concentrations of different single mismatch site.

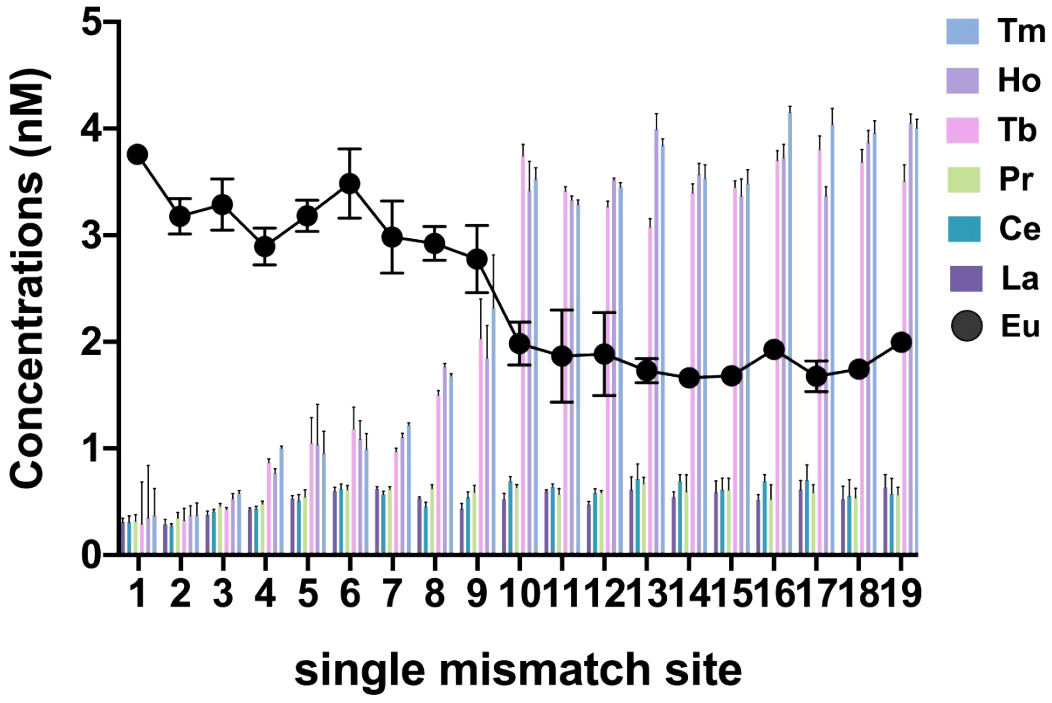


Figure S12. [Ln] raw concentrations of different multiple mismatch sites

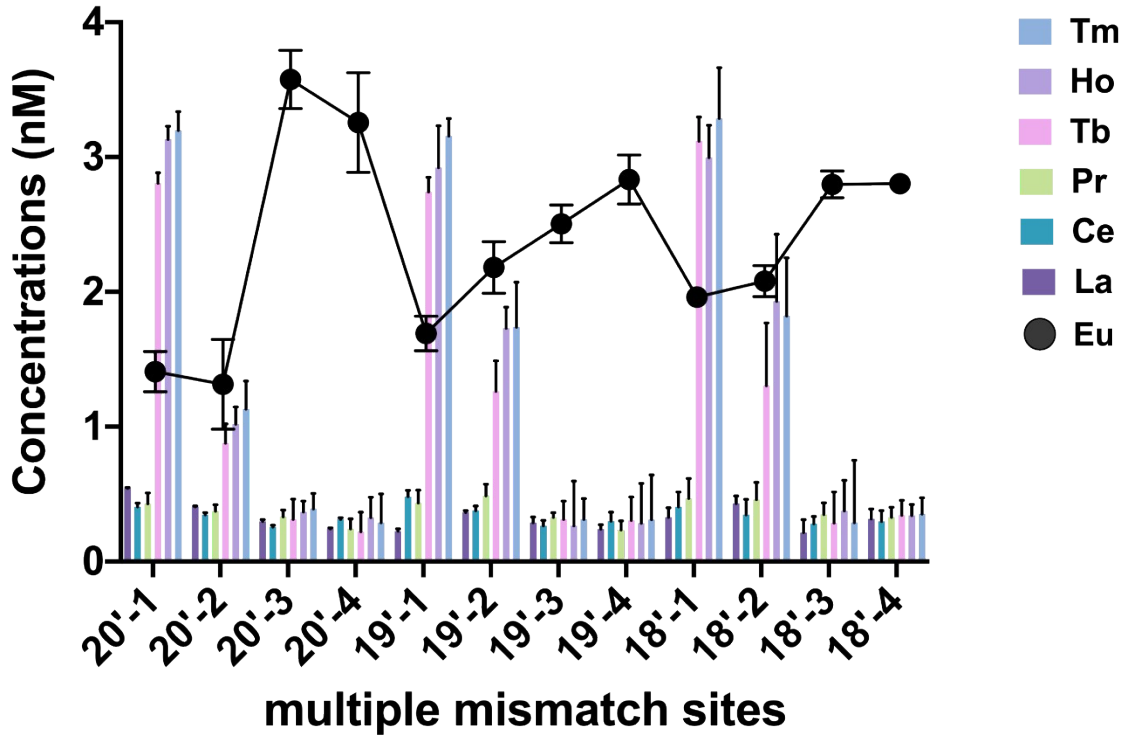


Figure S13. [Ln] raw concentrations of initial cleavage kinetic research.

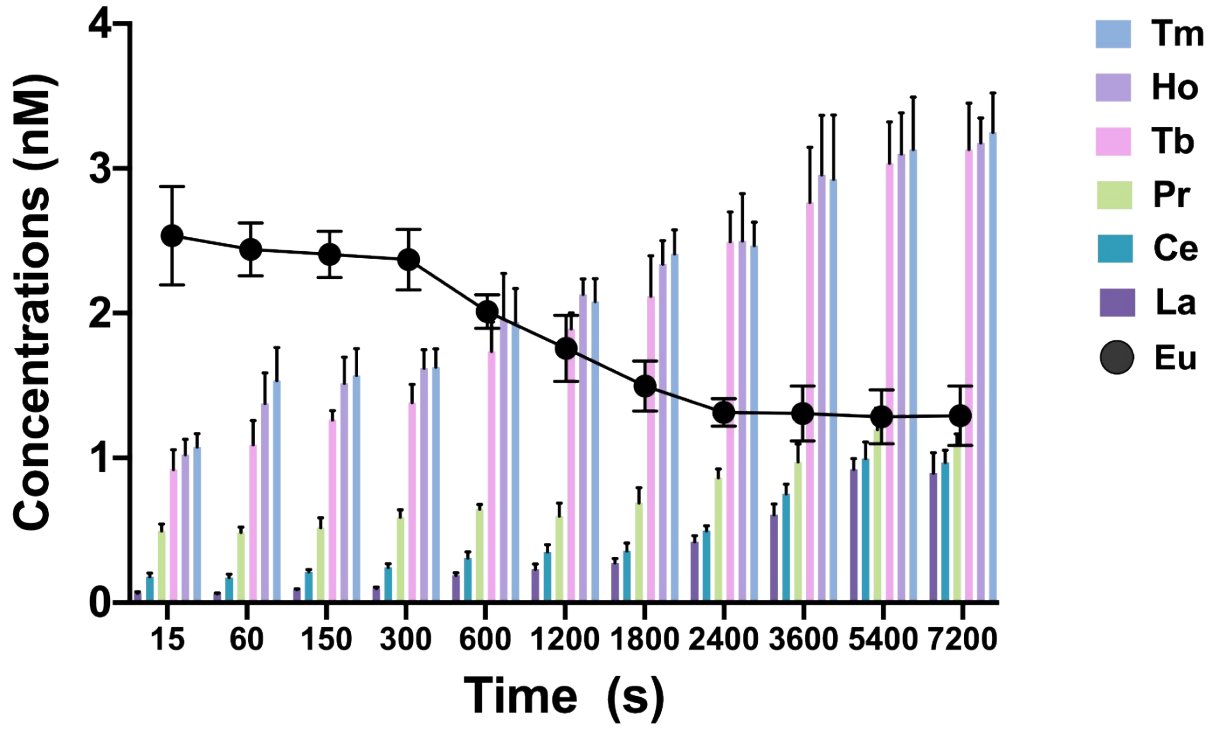


Figure S14. sgRNA sequence design route

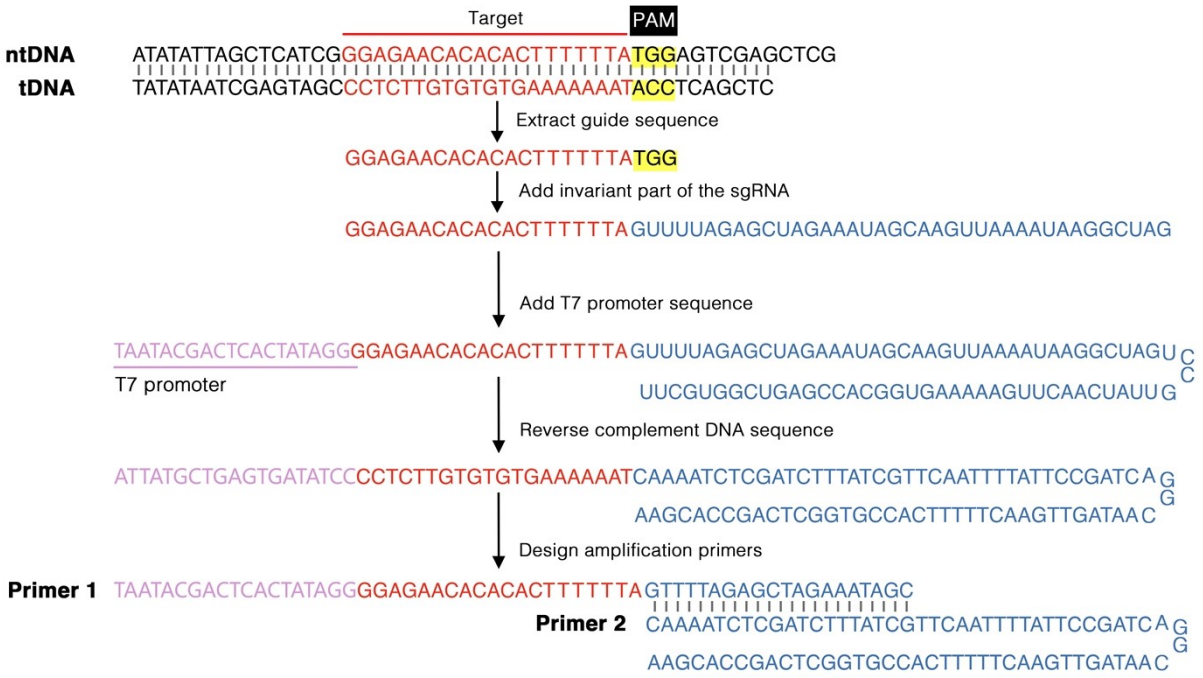
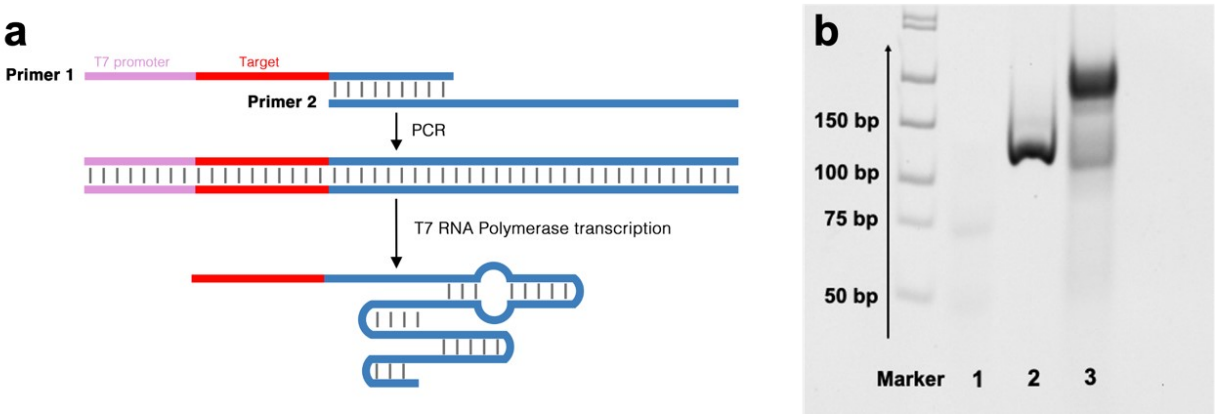


Figure S15. IVT procedure of sgRNA



a. IVT procedures, including primers PCR amplification and T7 transcription.

b. Gel electrophoresis characterization of (1) two primers, (2) product of PCR amplification and (s) sgRNA product.

Figure S16. calibration curves of label elements

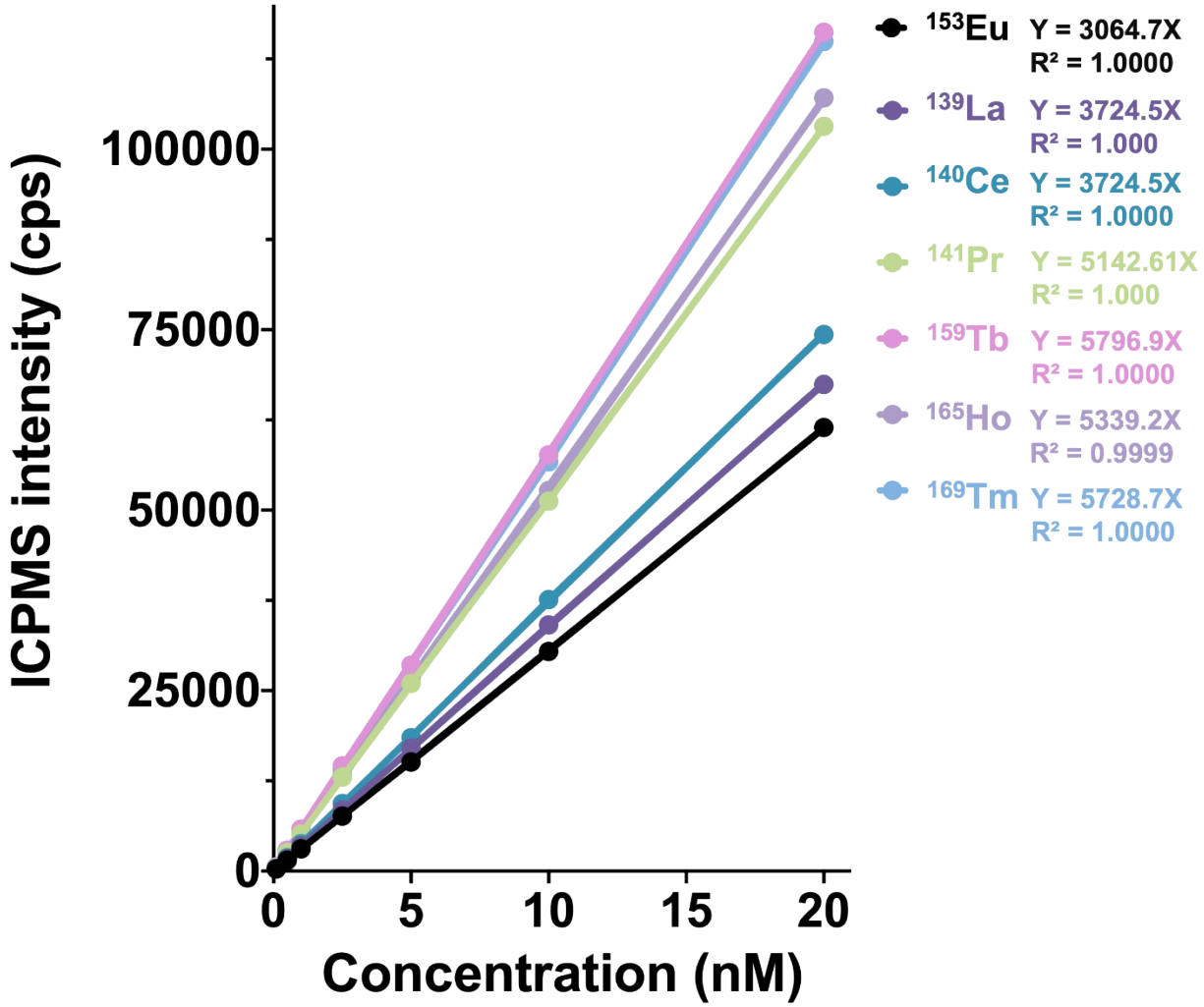


Figure S17. Raw ICPMS intensity of [Ln] in Cas9 concentration research

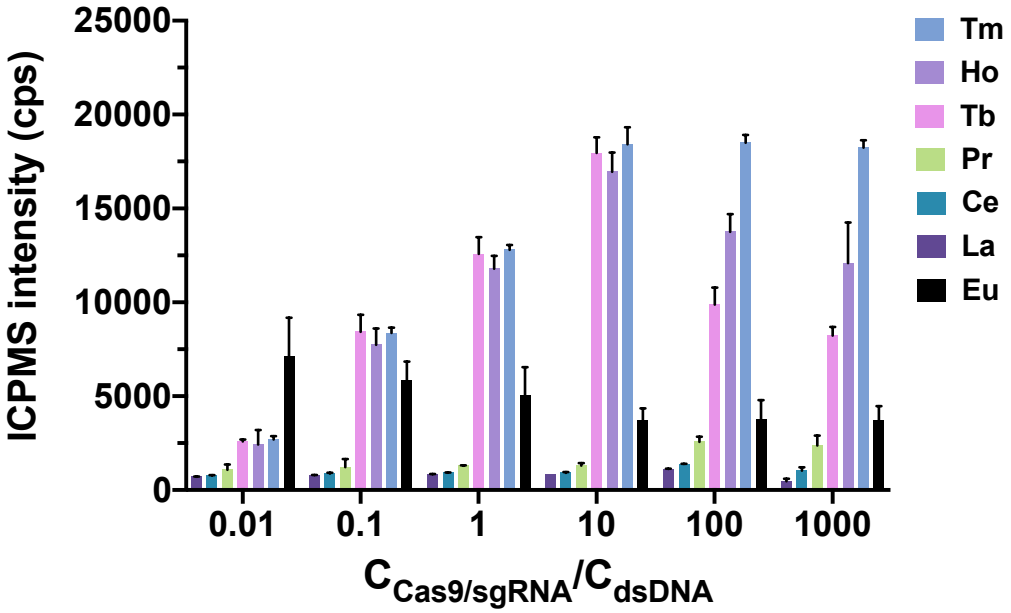


Figure S18. Raw ICPMS intensity of [Ln] in reaction temperature research

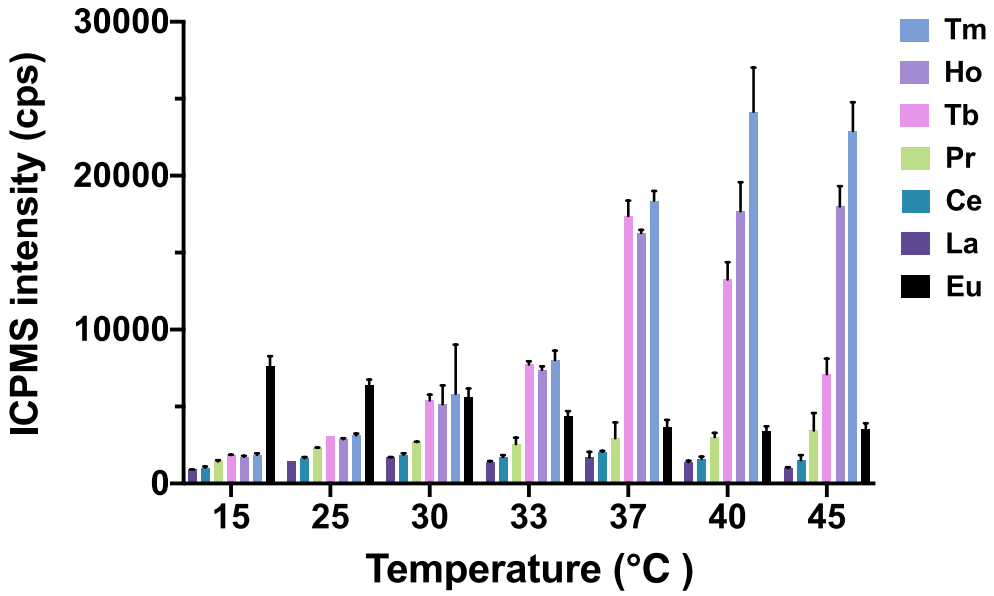


Figure S19. Raw ICPMS intensity of [Ln] in single mismatch research

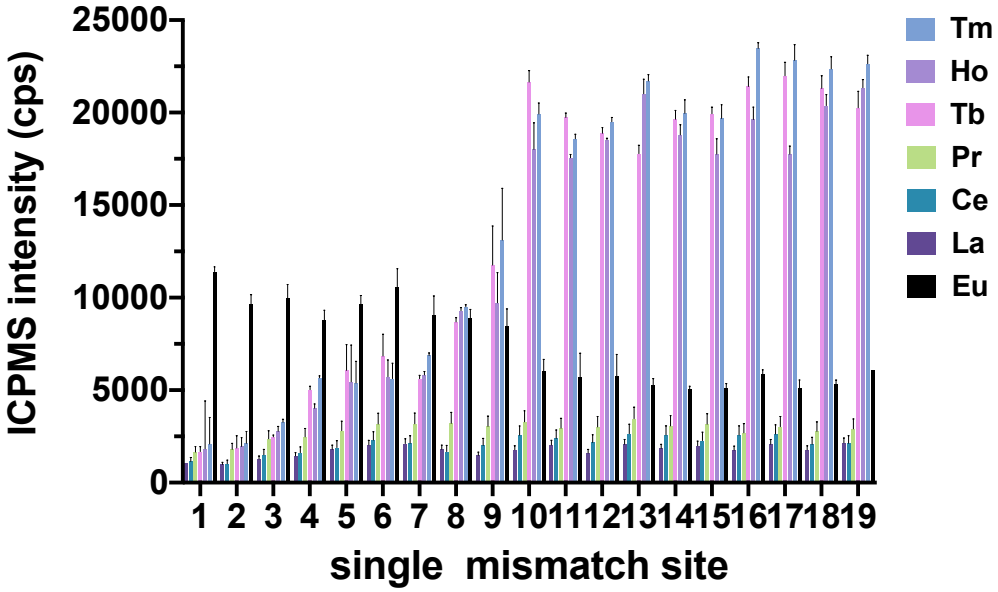


Figure S20. Raw ICPMS intensity of [Ln] in multiple mismatches research

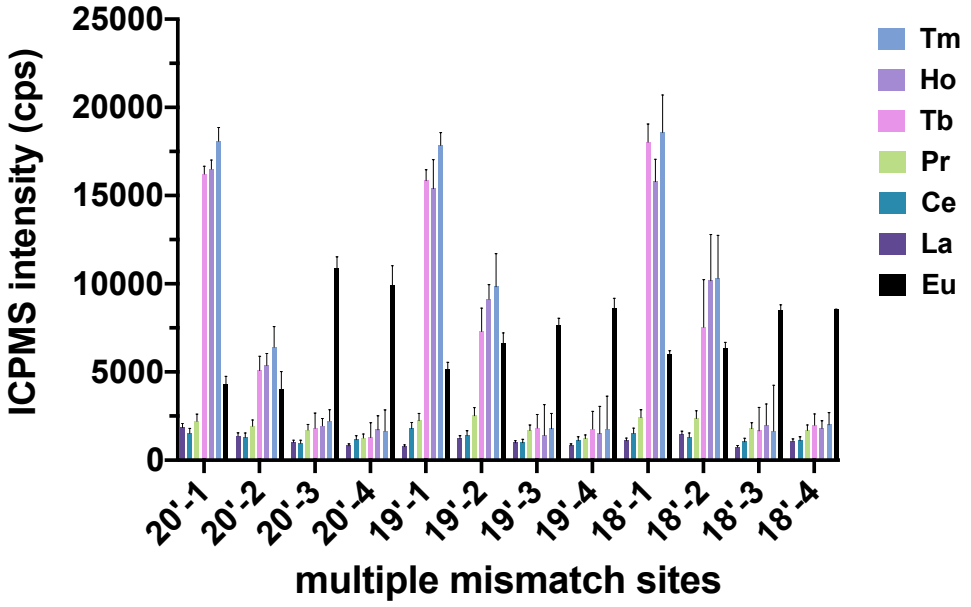


Figure S21. Raw ICPMS intensity of [Ln] in kinetic research