Construction of M-MuLV reverse transcriptase fusions

RT

The M-MuLV reverse transcriptase nucleotide sequence was amplified using RT-F1/RT-R1 primers (Supplementary Table) with NheI and NotI restriction sites, allowing the in-frame ligation into the pET23b vector (Novagen, USA). PCR was carried out using as template M-MuLV cDNA. The resultant 1.7-kbp DNA fragment and pET23b vector were digested with NdeI and NotI (SibEnzyme, Russia), ligated, and transformed into *E. coli* XL1-Blue cells according to the standard protocols (26). The fidelity of the resulting recombinant plasmid named pET-RT was confirmed by sequence analysis using primers pET-F and pET-R (Supplementary Table).

RT mut

The plasmid pUC-RT contained partial coding sequence M-MuLV reverse transcriptase with mutations D200N, T330P, L139P was constructed by Shanghai RealGene Bio-tech, Inc (China). Plasmid pUC-RT was digested with KpnI/SalI, and DNA fragment coding mutated M-MuLV RT was eluted from agarose gel, ligated with pET-RT (KpnI/SalI), and transformed into *E. coli* XL1-Blue cells according to the standard protocols. The resulting plasmid was named pET-RT-mut.

DBD-RT fusion

Pab-DBD and partial M-MuLV-RT nucleotide sequences were amplified using DBD-F1/DBD-R1 and RT–F2/RT-R2 primers (Supplementary Table), respectively. PCR was carried out using previously constructed pET-DBD (27), containing the nucleotide sequence of the DBD of ATP-dependent DNA ligase from *Pyrococcus abyssi*, and pET-RT. Resultant PCR fragments were digested with BamHI, followed by ligation according to the standard protocols. The fusion DNA fragment was eluted from agarose gel, digested with NdeI/KpnI, and ligated with pET-RT (NdeI/KpnI). The resultant plasmid was named pET-DBD-RT.

RT-DBD fusion

The Pab-DBD and partial M-MuLV-RT nucleotide sequences were amplified using DBD-F2/DBD-R2 and RT-F3/RT-R3 primers (Supplementary Table), respectively. PCR was carried out using previously constructed pET-DBD, and pET-Gss. Resultant PCR fragments were fused via PCR with RT-F3/DBD-R2 primers. The fusion DNA fragment was eluted from agarose gel, digested with SalI/NotI, and ligated with pET-RT (SalI/NotI). The resultant plasmid was named pET-RT-DBD.

Sto-RT fusion

The Sto7d and partial M-MuLV-RT nucleotide sequences were amplified using Sto-F1/Sto-R1 and RT-F2/RT-R2 primers (Supplementary Table), and the resulting DNA fragments were fused via PCR with Sto-F1/RT-R2 primers. PCR was carried out using previously constructed pET-Sto-Gss (24), containing the mutated nucleotide sequence of the Sto7d from *Sulfolobus tokodaii,* and pET-RT. Resultant PCR fragments were fused

via PCR with Sto-F1/RT-R2 primers. The fusion DNA fragment was eluted from agarose gel, digested with NheI/KpnI, and ligated with pET-RT vector (NheI/SalI). The resultant plasmid was named pET-Sto-RT.

RT-Sto fusion

The Sto7d and partial M-MuLV-RT nucleotide sequences were amplified using Sto-F2/Sto-R2 and RT-F3/RT-R4 primers (Supplementary Table). PCR was carried out using previously constructed pET-Sto-Gss, and pET-RT. Resultant PCR fragments were fused via PCR with RT-F3/Sto-R2 primers. The fusion DNA fragment was eluted from agarose gel, digested with SalI/NotI, and ligated with pET-RT vector (SalI/NotI). The resultant plasmid was named pET-RT-Sto.

RT-Sto mut fusion

The Sto7d and partial M-MuLV-RT nucleotide sequences were amplified using Sto-F2/Sto-R2 and RT-F3/RT-R4 primers (Supplementary Table). PCR was carried out using previously constructed pET-Sto-Gss, and pET-RT mut. Resultant PCR fragments were fused via PCR with RT-F3/Sto-R2 primers. The fusion DNA fragment was eluted from agarose gel, digested with SalI/NotI, and ligated with pET-RT vector (SalI/NotI). The resultant plasmid was named pET-RT-Sto-mut.

Supplementary table. Primers for cloning of the chimeric RTs.

Name	**5′-sequence-3′**	**Restriction site**
RT-F1	TATG**GCTAGC**CTAAATATAGAAGATGAGCATCGGC	NheI
RT-R1	GAGT**GCGGCCGC**ATCAAGGCAGTTGTGTTGC	NotI
DBD-F1	TCATG**CATATG**AGGTACATAGAGCTGGCCCA	NdeI
DBD-R1	ATTC**GGATCC**CTTTATTGGCTTACCAATCTGAATT	BamHI
RT-F2	ATTC**GGATCC**ctaaatatagaagatgagcatcggc	BamHI
RT-R2	GATGATGGTACCAGTATTCCCTGGTCC	KpnI
DBD-F2	ATTCAGATTGGTAAGCCAATAAAGAGGTACATAGAGCTGGCCCA	
DBD-R2	GATGAT**GCGGCCGC**ATTAGCTAATCCATCATTACCCTCA	NotI
RT-F3	CTCTTT**GTCGAC**GAGAAGCA	SalI
RT-R3	CTTTATTGGCTTACCAATCTGAATATCAAGGCAGTTGTGTTGC	
Sto-F1	GTCTC**GCTAGC**ATGGTAACAGTAAAGTTCAAGTATAA	NheI
Sto-R1	GCCGATGCTCATCTTCTATATTTAGACCGCCACCGCCTTTCTTTCCAGATTTTTCTAACATTT	
Sto-F2	GGTACCGGCGGTGGCGGTGTAACAGTAAAGTTCAAGTATAA	
Sto-R2	GATGAT**GCGGCCGC**TTTTTCTAACATTTGTAGTAATTCTT	NotI
RT-R4	ACCGCCACCGCCGGTACCATCAAGGCAGTTGTGTTGC	
pET-F	CCTATAGTGAGTCGTATTAATTTC	
pET-R	CAACTCAGCTTCCTTTCGG	