7.25 Yuting Gao

In the preliminary work, we verified that eMuta T7 can function normally on low-copy plasmids and target plasmids have suitable mutation rate of target genes by double-transmutation eMuta T7 and PL12s-dual T7-STS plasmids. The verification process is as follows:

eMuta T7 (200 ng) and PL12s-dual T7-STS (300 ng) were added to receptive cell BW25113, incubated in an ice box for 20 min, heat shock at 42 °C for 45s, immediately resting on ice for 2 min, adding 600 μL non-resistant LB, and cultured at 37 °C for 1 h. Coated on C30+S50 plate and cultured in 37 °C constant temperature incubator for 12h. The growth status of the colony is as follows.

Select a single clone and transfer it to a 5ml LB medium supplemented with 30 mg/L chloramphenicol and 50 mg/L streptomycin. Incubate the culture in a shaking incubator at a constant temperature of 37°C for 12 hours. Subsequently, induce the system according to the following protocol.

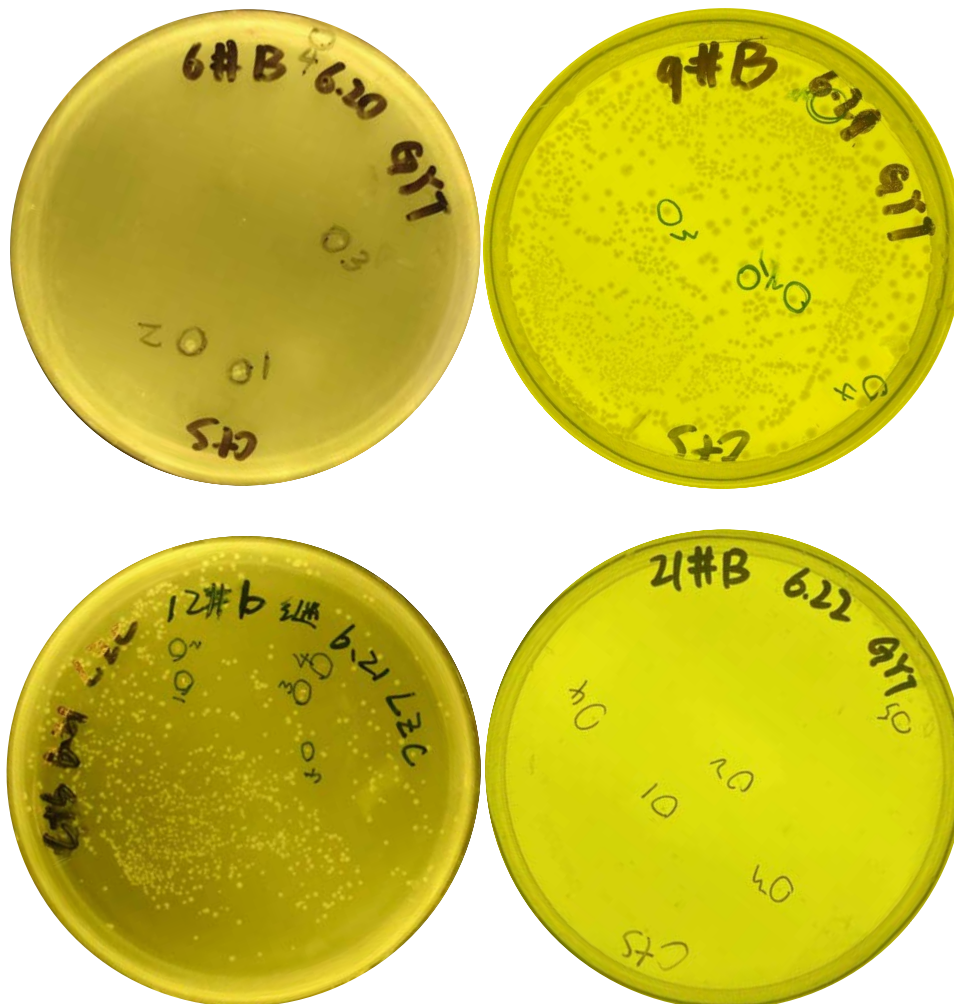
Induction system	
ZY medium	5mL
50 x 5052	100 μL
50×M	100 μL
1M MgSO4	10 μL
1000 x trace elements	10 μL
20% Ara	50 μL
IPTG	5 μL
30g/L Cmr	5 μL
50 g/L Str	5 μL
Bacterial solution	50 μL

The induction system should be placed in a constant temperature incubator set at 30°C for cultivation, with each round lasting for a duration of 4 hours.

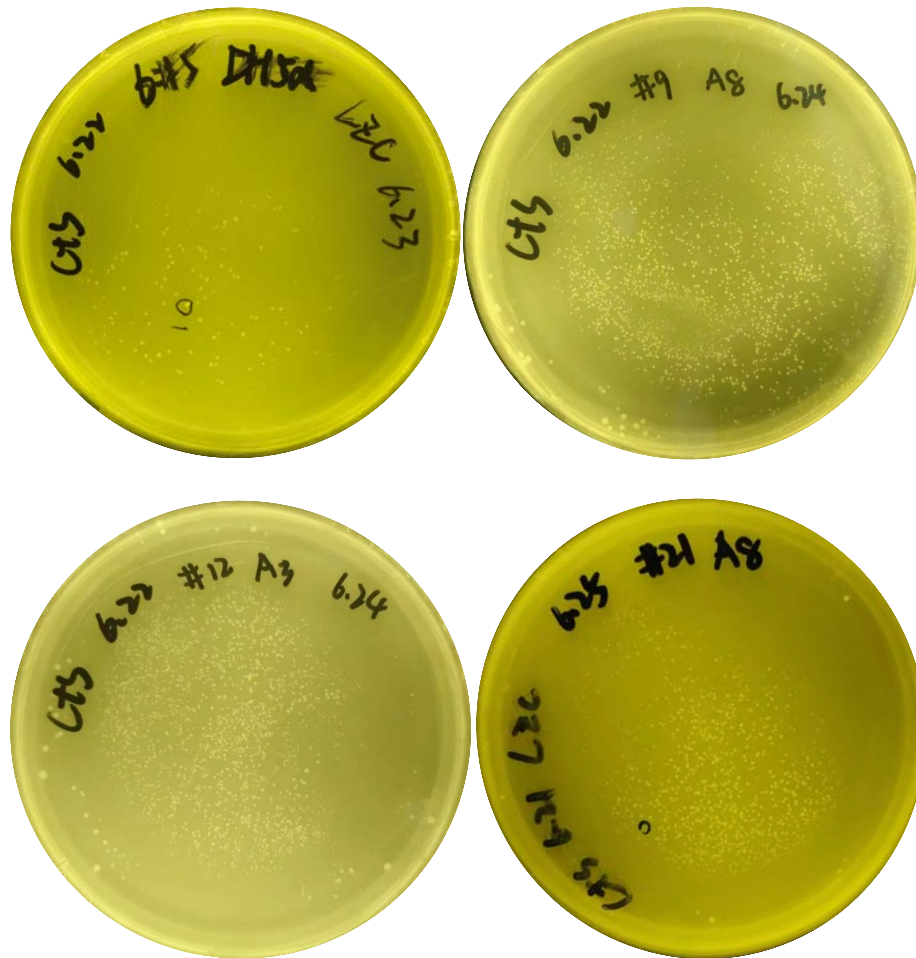
Supplement nutrients, inducers and antibiotics for #6, #12, #18 and #24. Suck the 200μL inducer before supplementing. The refill system is as follows.

Induction system	
50 x 5052	100 μL
50×M	100 μL
1M MgSO4	10 μL
1000 x trace elements	10 μL
20% Ara	50 μL
IPTG	5 μL
30 g/L Cmr	5 μL
50 g/L Str	5 μL

For #6, #9, #12, #21, the bacterial solution was absorbed and coated in C30+S50 petri dish, and the plate was placed in a constant temperature incubator at 37°C for 12 hours. The colony growth was as follows.



Select a single clone and transfer it to a 5ml LB medium supplemented with 30 mg/L chloramphenicol and 50 mg/L streptomycin. Incubate the culture in a shaking incubator at a constant temperature of 37°C for 16 hours. The plasmid was extracted, transformed (200 ng) into DH5α, and coated in C30+S50 petri dish. The growth of colonies #6, #9, #12 and #21 after transformation was as follows.



One clone was selected from each plate and transferred to 5ml LB+ 30 mg/L Chl+50 mg/L Str system. The incubator was expanded and shaken at 37°C for 16 h. The plasmid was extracted and the STS gene was sequenced. The results showed that the base mutation rate of STS was suitable and the mutation direction was correct. Thus, the effectiveness of eMuta T7 plasmid in base mismatch against low copy plasmids was verified.

Therefore, the eMuta T7 in vivo continuous mutation tool was applied to the high-copy target plasmid gY9s-dual T7-HMAS-Bio 177. eMuta T7 (200 ng) and gY9s-dual T7-HMAS-Bio 177 (300 ng) were added to 100 μL receptive cells BW25113 and BWΔCD, incubated in an ice box for 20 min, heat shock at 42 °C for 45s, and immediately placed on ice for 2 min. Add 600μL non-resistant LB, culture at 37 °C for 1 h, coated on C30+S50 plate, and culture in 37 °C constant temperature incubator for 12h. eMuta T7+gY9s-dual T7-HMAS-Bio 177/BW25113 Colony growth is as follows.

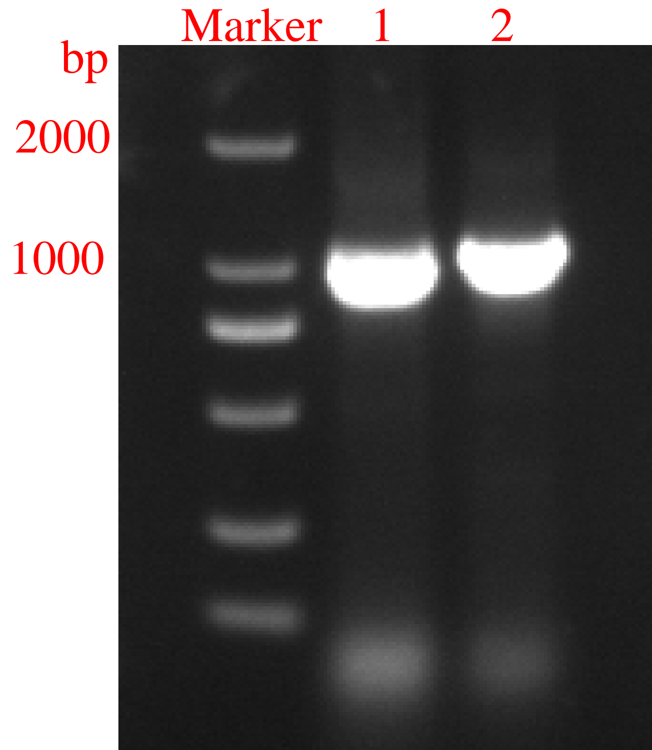
eMuta T7+gY9s-dual T7-HMAS-Bio 177/BWΔCD Colony growth is as follows.

7.26 Zichang Lu

Colony PCR was performed on eMuta T7+gY9s-dual T7-HMAS-Bio 177/BW25113 and eMuta T7+gY9s-dual T7-HMAS-Bio 177/BWΔCD to detect transformation. The PCR system is as follows.

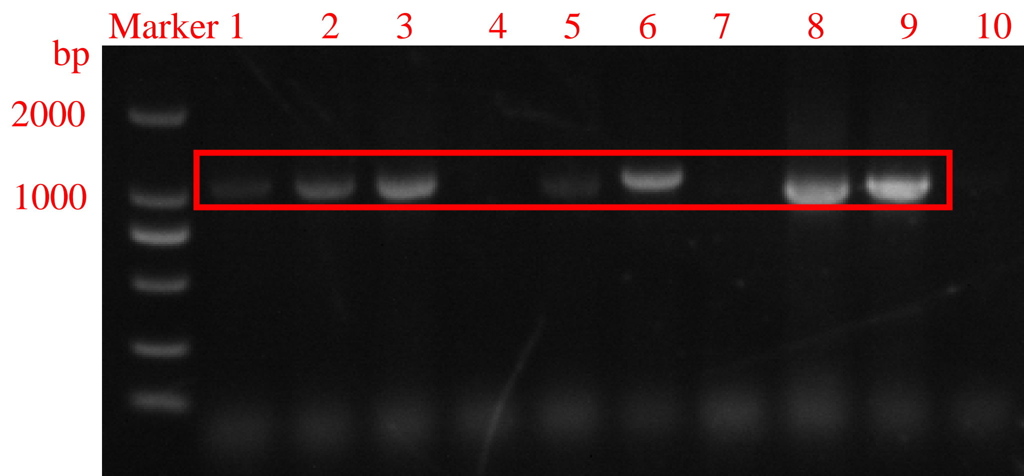
PCR system (10 μL)	
AM-gg-F=0709	0.4 μL
AM-R=0709	0.4 μL
2×Mix	5μL
DDW	4.2μL

eMuta T7+gY9s-dual T7-HMAS-Bio 177/BW25113 Agarose gel electrophoresis is as follows.



The strips in lane 1 and 2 are as expected. The bacteria corresponding to lane 1 were transferred to 5ml LB+ 30 mg/L Chl+50 m/L Str system and shaken at 37℃ for 12 hours.

eMuta T7+gY9s-dual T7-HMAS-Bio 177/BWΔCD Agarose gel electrophoresis is as follows.



The strip sizes of the samples in lanes 1, 2, 3, 5, 6, 8 and 9 are as expected. The monoclonal strains corresponding to lane 3 were incubated in 5ml LB(C30+S50) and cultured in a 37℃ shaker for 12 h.

7.27 Mengjin Zhang

Induction of eMuta T7+gY9s-dual T7-HMAS-Bio 177/BW25113 and eMuta T7+gY9s-dual T7-HMAS-Bio 177/BWΔCD. The induction system is as follows.

Induction system	
ZY medium	5mL
50 x 5052	100 µL
50×M	100 µL
1M MgSO4	10 µL
1000 x trace elements	10 µL
20% Ara	50 µL
IPTG	5 µL
30 g/LCmr	5 µL
50 g/LStr	5 µL
Bacterial solution	50 µL

Then the induction system was cultured in a constant temperature incubator at 30°C, once every 4 hours.

7.28 Zichang Lu
Supplement #6 with nutrients, antibiotics, and inducers. Suck the 200µL inducer first. The refill system is as follows.

Induction system	
50 x 5052	100 µL
50×M	100 µL
1M MgSO4	10 µL
1000 x trace elements	10 µL
20% Ara	50 µL
IPTG	5 µL
30 g/L Cmr	5 µL
50 g/L Str	5 µL

7.29 Yuting Gao
Supplement #12 with nutrients, antibiotics and inducers. Start by sucking the 200µL inducer. The refill system is as follows.

Induction system	
50 x 5052	100 µL
50×M	100 µL
1M MgSO4	10 µL
1000 x trace elements	10 µL
20% Ara	50 µL
IPTG	5 µL
30 g/L Cmr	5 µL
50 g/L Str	5 µL

7.30 Zichang Lu
Supplement #18 with nutrients, antibiotics, and inducers. Suck the 200µL inducer first. The refill system is as follows.

Induction system	
50 x 5052	100 μL
50×M	100 μL
1M MgSO4	10 μL
1000 x trace elements	10 μL
20% Ara	50 μL
IPTG	5 μL
30 g/L Cmr	5 μL
50 g/L Str	5 μL

Absorb #21 bacterial solution, smear it in C30+S50 petri dish, and place the plate in 37℃ constant temperature incubator for 12 hours.

7.31 Yuting Gao

Supplement #24 with nutrients, antibiotics, and inducers. Start by sucking the 200μL inducer. The refill system is as follows.

Induction system	
50 x 5052	100 μL
50×M	100 μL
1M MgSO4	10 μL
1000 x trace elements	10 μL
20% Ara	50 μL
IPTG	5 μL
30 g/L Cmr	5 μL
50 g/L Str	5 μL

Absorb #21 bacterial solution, smear it in C30+S50 petri dish, and place the plate in 37℃ constant temperature incubator for 12 hours.
Colony #21 transferred to BW25113 receptive cells grew as follows.

The bacteria were incubated in 5ml LB(C30+S50) and cultured in a shaking bed at 37℃ for 12 hours.

8.1 Mengjin Zhang

The #30 bacterial solution was absorbed and coated in C30+S50 petri dish, and the plate was placed in 37℃ constant temperature incubator for 12 hours. Colony #30 transferred to BW25113 receptive cells grew as follows.

Colony growth of #30 cells transferred to BWΔCD receptive cells is as follows.

The bacteria were incubated in 5ml LB(C30+S50) and cultured in a shaking bed at 37℃ for 12 hours.

Plasmid #21/BW25113 was extracted and transformed into DH5α, which was coated in C30+S50 petri dish. The colony growth after transformation was as follows.

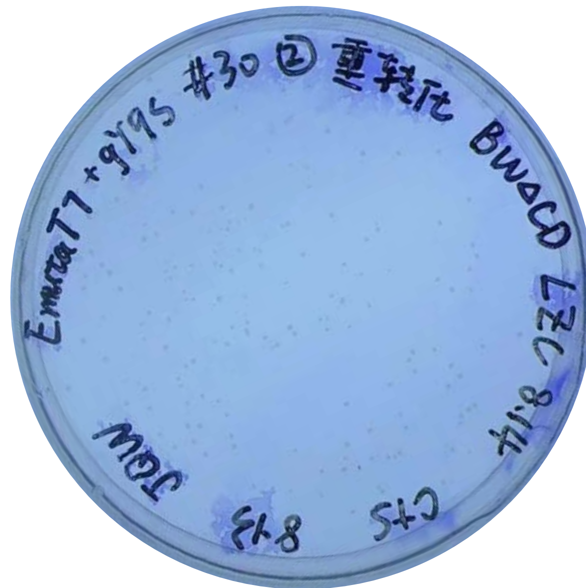
Cultured #21/DH5α in 5ml LB(C30+S50) in a shaking bed at 37°C for 12 hours. Plasmid sequencing was performed to detect HmaS gene mutation.

8.2 Yuting Gao

Extract plasmid #30/BW25113 and transform 200 ng plasmid into 100 μL DH5α, coated in C30+S50 petri dish. The colony growth after transformation was as follows.

The #30/DH5α was inoculated into 5ml LB(C30+S50) and cultured in a shaking bed at 37°C for 12 hours. Plasmid sequencing was performed to detect *hmaS* gene mutation.

Extract plasmid from #30/BW25113 and transform 200 ng of plasmid into 100 μL DH5α, then spread onto C30+S50 culture plates. The colony growth after transformation was as follows.



The #30/DH5α was inoculated into 5ml LB(C30+S50) and cultured in a shaking bed at 37℃ for 12 hours. Plasmid sequencing was performed to detect HmaS gene mutation.

8.13 Zichang Lu

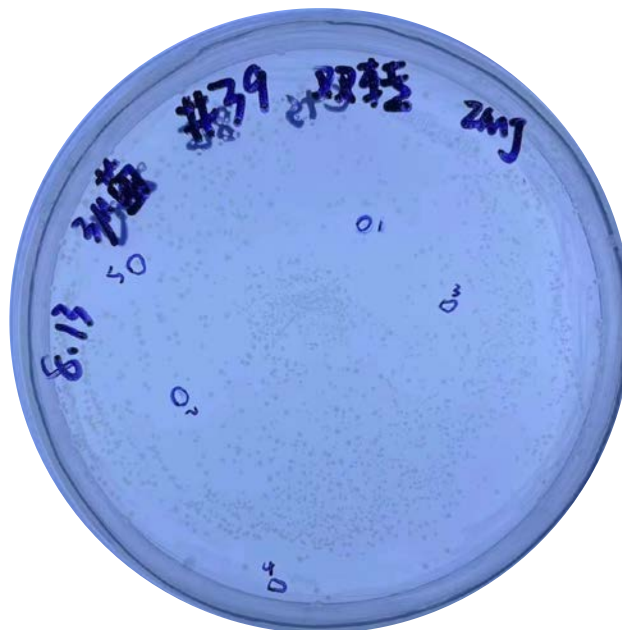
Supplement #36 with nutrients, antibiotics, and inducers. Start by sucking the 200μL inducer. The refill system is as follows.

Induction system	
50 x 5052	100 μL
50×M	100 μL
1M MgSO4	10 μL
1000 x trace elements	10 μL
20% Ara	50 μL
IPTG	5 μL
30 g/L Cmr	5 μL
50 g/L Str	5 μL

Absorb #36 bacterial solution, smear it in C30+S50 petri dish, and place the plate in 37℃ constant temperature incubator for 12 hours. Colony #36 transferred to BWΔCD receptive cells grew as follows.

The bacteria were incubated in 5ml LB(C30+S50) and cultured in a shaking bed at 37℃ for 12 hours.

The #39 bacterial solution was absorbed and coated in C30+S50 petri dish, and the plate was placed in 37℃ constant temperature incubator for 12 hours. Colony #39 transferred to BWΔCD receptive cells grew as follows.



The bacteria were incubated in 5ml LB(C30+S50) and cultured in a shaking bed at 37℃ for 12 hours.

8.15 Yuting Gao

Extract plasmid #36/BWΔCD and transform 200 ng plasmid into 100 μL DH5α, coated in C30+S50 petri dish. The colony growth after transformation was as follows.

The #/DH5α was inoculated into 5ml LB(C30+S50) and cultured in a shaking bed at 37℃ for 12 hours.

8.20 Mengjin Zhang

After optimizing the gene pathway, the eMuta T7 plasmid and the gY9s-dual T7-Trrnb-HMAS (Scpa1)-Bio 177 plasmid were re-transformed.

eMuta T7 (200 ng) and gY9s-dual T7-Trrnb-HmaS (Scpa1)-Bio 177 (200 ng) were added to 100 μL receptive cell BW25113 and incubated in an ice box for 30 min and heat shock at 42 ℃ for 45s. Immediately after removal, the cells were placed on ice for 2min, 600 μL of non-resistant LB was added, cultured at 37 ℃ for 1 hour, coated on C30+S50 plate, and cultured at 37 ℃ in a constant temperature incubator for 12h.

8.21 Mengjin Zhang

eMuta T7+ gY9s-dual T7-TRRNB-HMAS (Scpa1)-Bio 177 /BW25113 Colony growth is as follows.

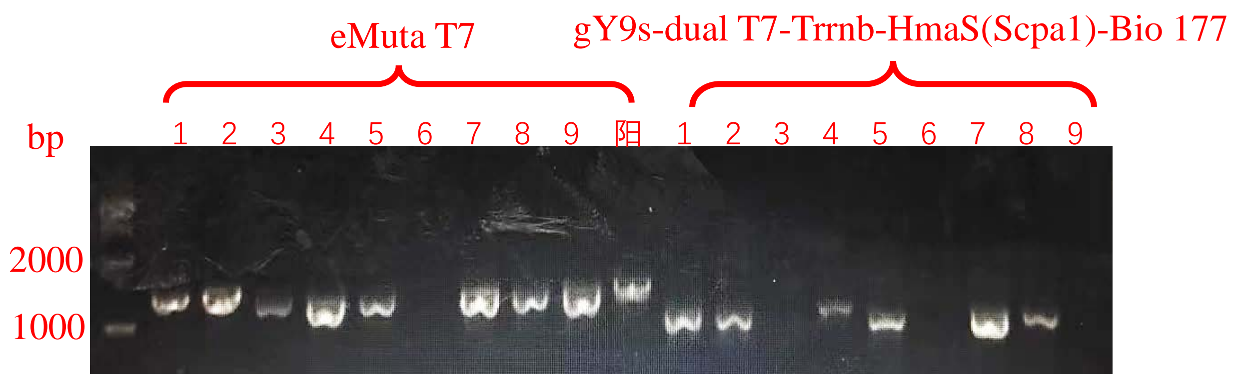
Two sets of colony PCR were performed on eMuta T7+ gY9s-dual T7-TRRNB-HMAS (Scpa1)-Bio 177 /BW25113. To detect the transformation of eMuta T7 plasmid and gY9s-dual T7-TrRNb-HMAS (Scpa1)-Bio 177 plasmid, respectively. The gY9s-dual T7-Trrnb-HmaS(Scpa1)-Bio 177 plasmid PCR system and procedure are as follows.

PCR system (10 μL)	
AM-gg-F=0709	0.4 μL =
AM-R=0709	0.4 μL =
2×Mix	5μL
DDW	4.2μL

The PCR system and procedure of eMuta T7 plasmid are as follows.

PCR system	
E-JP-F=0819	0.4 μL =
E-JP-R=0819	0.4 μL =
2×Mix	5μL
DDW	4.2μL

Agarose gel electrophoresis is as follows.



According to agarose gel electrophoresis, both the eMuta T7 plasmid and the gY9s-dual T7-Trrnb-HmaS (Scpa1)-Bio 177 plasmid corresponding to lane 1,2,4,5,7 and 8 were present. The bacteria corresponding to lane 1 were selected into 5ml LB(C30+S50) and cultured in a shaking bed at 37°C for 12 h.

8.22 Mengjin Zhang

Induction of BW25113 transferred into eMuta T7 and gY9s-dual T7-TRRNB-HMAS (Scpa1)-Bio 177 plasmids. The induction system was as follows.

Induction system	
ZY medium	5mL
50 x 5052	100 μL
50×M	100 μL
1M MgSO4	10 μL
1000 x trace elements	10 μL
20% Ara	50 μL
IPTG	5 μL
30 g/L Cmr	5 μL
50 g/L Str	5 μL
Bacterial solution	50 μL

Then the induction system was cultured in a constant temperature incubator at 30°C, once every 4 hours.

8.23 Yuting Gao

Supplement #6 with nutrients, antibiotics, and inducers. Suck the 285μL inducer first. The refill system is as follows.

Induction system	
50 x 5052	100 μL
50×M	100 μL
1M MgSO4	10 μL
1000 x trace elements	10 μL
20% Ara	50 μL
IPTG	5 μL
30 g/L Cmr	5 μL
50 g/L Str	5 μL

Supplement #9 with nutrients, antibiotics, and inducers. Suck the 285μL inducer first. The refill system is as follows.

Induction system	
50 x 5052	100 μL
50×M	100 μL
1M MgSO4	10 μL
1000 x trace elements	10 μL
20% Ara	50 μL
IPTG	5 μL
30 g/L Cmr	5 μL
50 g/L Str	5 μL

8.24 Yuting Gao

Supplement #12 with nutrients, antibiotics, and inducers. Suck the 285μL inducer first. The refill system is as follows.

Induction system	
50 x 5052	100 μL
50×M	100 μL
1M MgSO4	10 μL
1000 x trace elements	10 μL
20% Ara	50 μL
IPTG	5 μL
30 g/L Cmr	5 μL
50 g/L Str	5 μL

Supplement #15 with nutrients, antibiotics, and inducers. Suck the 285μL inducer first. The refill system is as follows.

Induction system	
50 x 5052	100 μL
50×M	100 μL
1M MgSO4	10 μL
1000 x trace elements	10 μL
20% Ara	50 μL
IPTG	5 μL
30 g/L Cmr	5 μL
50 g/L Str	5 μL

8.25 Mengjin Zhang

Supplement #18 with nutrients, antibiotics, and inducers. Suck the 285μL inducer first. The refill system is as follows.

Induction system	
50 x 5052	100 μL
50×M	100 μL
1M MgSO4	10 μL
Trace elements	10 μL
Ara	50 μL
IPTG	5 μL
Cmr	5 μL
Str	5 μL

Supplement #21 with nutrients, antibiotics, and inducers. Suck the 285μL inducer first. The refill system is as follows.

Induction system	
50 x 5052	100 μL
50×M	100 μL
1M MgSO4	10 μL
1000 x trace elements	10 μL
20% Ara	50 μL
IPTG	5 μL
30 g/L Cmr	5 μL
50 g/L Str	5 μL

Absorb #21 bacterial solution, smear it in C30+S50 petri dish, and place the plate in 37°C constant temperature incubator for 12 hours. The colony growth of #21/BW25113 was as follows.

The bacteria were incubated in 5ml LB(C30+S50) and cultured in a shaking bed at 37°C for 12 hours.

8.26 Yuting Gao

Supplement #24 with nutrients, antibiotics, and inducers. Suck the 285μL inducer first. The refill system is as follows.

Induction system	
50 x 5052	100 μL
50×M	100 μL
1M MgSO4	10 μL
1000 x trace elements	10 μL
20% Ara	50 μL
IPTG	5 μL
30 g/L Cmr	5 μL
50 g/L Str	5 μL

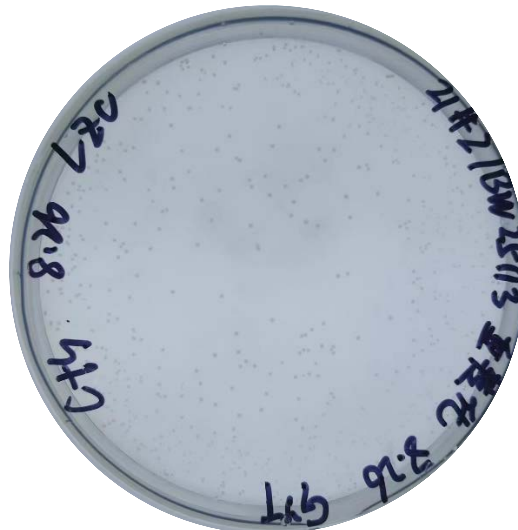
Supplement #27 with nutrients, antibiotics, and inducers. Suck the 285 μL inducer first. The refill system is as follows.

Induction system	
50 x 5052	100 μL
50×M	100 μL
1M MgSO4	10 μL
1000 x trace elements	10 μL
20% Ara	50 μL
IPTG	5 μL
30 g/L Cmr	5 μL
50 g/L Str	5 μL

Absorb #27 bacterial solution, smear it in C30+S50 petri dish, and place the plate in 37℃ constant temperature incubator for 12 hours. The colony growth of #27/ BW25113 was as follows.

The bacteria were incubated in 5ml LB(C30+S50) and cultured in a shaking bed at 37℃ for 12 hours.

Plasmid #21/ BW25113 was extracted, and 200 ng plasmid was transformed into 100 μL BW25113 and coated in C30+S50 petri dish. The colony growth after transformation was as follows.



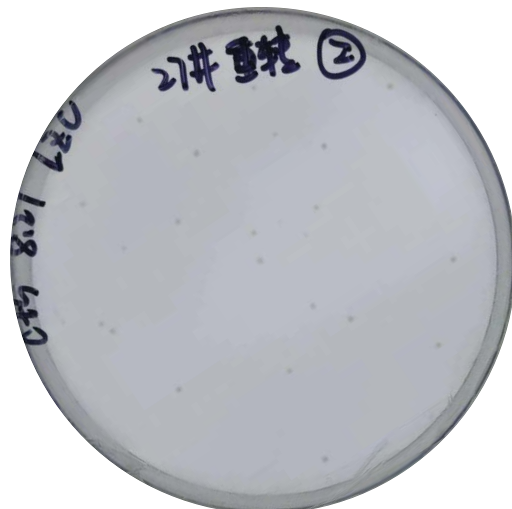
The bacteria were incubated in 5ml LB(C30+S50) and cultured in a shaking bed at 37℃ for 12 hours. Plasmids were extracted and sequenced.

8.27 Mengjin Zhang

The #30 bacterial solution was absorbed and coated in C30+S50 petri dish, and the plate was placed in 37℃ constant temperature incubator for 12 hours. The growth of #30/ BW25113 colonies was as follows.

The bacteria were incubated in 5ml LB(C30+S50) and cultured in a shaking bed at 37℃ for 12 hours.

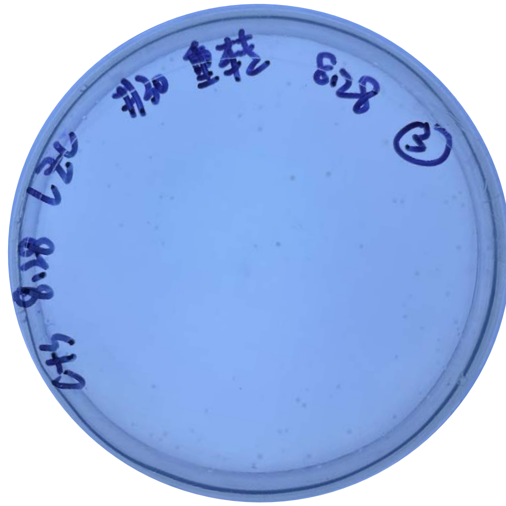
Plasmid #27/ BW25113 was extracted and 200 ng plasmid was transformed into BW25113 and coated in C30+S50 petri dish. The colony growth after transformation was as follows.



The bacteria were incubated in 5ml LB(C30+S50) and cultured in a shaking bed at 37℃ for 12 hours. Plasmids were extracted and sequenced.

8.28 Zichang Zhang

Extract plasmid #30/ BW25113 and transform 200 ng plasmid into 100 μL BW25113, coated in C30+S50 petri dish. The colony growth after transformation was as follows.



The bacteria were incubated in 5ml LB(C30+S50) and cultured in a shaking bed at 37℃ for 12 hours. Plasmids were extracted and sequenced.