Protocol for genetic dual selection

Materials:

- M9 minimal medium was supplemented with 0.1 % casamino acids (Sigma) and 0.8 % glycerol as carbon source. Where appropriate, M9 medium was supplemented with carbenicillin at 75 μg/ml （M9-amp）for plasmid maintenance.

Procedure:

1. Construction of riboswitch-tetA plasmid library:

1.1 Plasmid pLac-thiM#2-tetA-gfpuv was acquired from Prof. Yohei Yokobayashi. This plasmid expresses riboswitch-controlled tetA-GFP mRNA from the *E. coli* lac promoter, expresses β-lactamase for carbenicillin resistance, and contains a ColE1 origin of replication.

1.2 The wild type hammerhead was introduced by performing PCR using primers wthh-r and wthh-f (see Supplement information) using pLacthiMtetA as a template.

1.2.1 The PCR reaction was performed in total volume of 50μl using PrimeSTAR DNA polymerase (TAKARA) according to the manufacturer's protocol.

1.3 The adenine response aptamer was introduced by performing PCR using primers aahh-r and aahh-f (see Supplement information) using step 2.2 product as a template.

1.4 The PCR product was purified by column and phosphorylated by T4 polynucleotide kinase (TAKARA) and self-ligated using Ligation Kit (TAKARA) and immediately transformed into competent DH5αcells.

2 The transformed cells were plated on LB agar plates containing carbenicillin and incubated overnight at 37 °C. Pick up clones and culture in 96-deep-well plate at 250r.p.m.

3 Dual genetic selection:

3.1 Overnight cultures of DH5αcells transformed with the appropriate plasmids were diluted to a final OD600 of 0.005 in 0.5 ml fresh M9-amp with ligand, and cultured for 8 h at 250 r.p.m. to let the expression level of TetA stabilize.

3.2 The cells were further diluted to a final OD600 of 0.005 in 1 ml fresh M9-amp with ligand supplemented with an appropriate concentration of tetracycline and cultured for 24 h for positive selection.

3.3 The selected cells were washed once with M9-amp and further diluted to a final OD600 of 0.005 in 0.5 ml fresh M9-amp without ligand, and cultured for 8 h at 250 r.p.m. to let the expression level of TetA stabilize

3.4 The cells were further diluted to a final OD600 of 0.005 in 1 ml fresh M9-amp without ligand supplemented with an appropriate concentration of $NiCl_2$ and cultured for 24 h for negative selection.

3.5 The selected cells were washed twice with M9-amp and streak culture on LB agar plates and incubated overnight at 37 °C.

Supplement information:

wthh-f :

CGAAACGCGCTTCGGTGCGTCCTGGATTCCACGAGGAGGTACTAGATGCAAGTCGACCTTGCTGGA

wthh-r:
5’TCCTATTTGGGACTCATCAGCTGGATGTACCGAGGAGGUAAAGTTTGGTTGAATTCAATTGTTATCC
aahh-f:
TCTACCAAGAGCTTAAACTCTTGATNNNNNNTCCTGGATTCCACGAGGAGGT
aahh-r:
AACTCCCAAACCATATCATTAGGATTANNNNNNTTTCGTCCTATTTGGGACTCATC

Reference

Norihito Muranaka, Vandana Sharma, Yoko Nomura and Yohei Yokobayashi （2009）An efficient platform for genetic selection and screening of gene switches in Escherichia coli，Nucleic Acids Research，37：e39