RHO3 strain verification

1. Growth assay

Always grow RHO3 on LB medium supplemented with 400 μg/ml diaminopimelic acid (DAP). To check for the presence of Δ*asd* streak or patch single colonies on LB plates without DAP and LB plates with 400 μg/ml DAP. After overnight incubation at 37°C there should be no growth on the plate without DAP but growth on the plate with DAP.

2. PCR verification of presence of Δ*aph* and Δ*asd* in RHO3

Primers for *aphA*

P3522 5′-GCCAATCAGTGATGATGA
P3523 5′-ATGCGTGGTCCACTGTTG

Primers for *asd*

P3524 5′-TTCTACTTCTCAGCTTGG
P3525 5′-TCCACGGAATCAGGCTAC

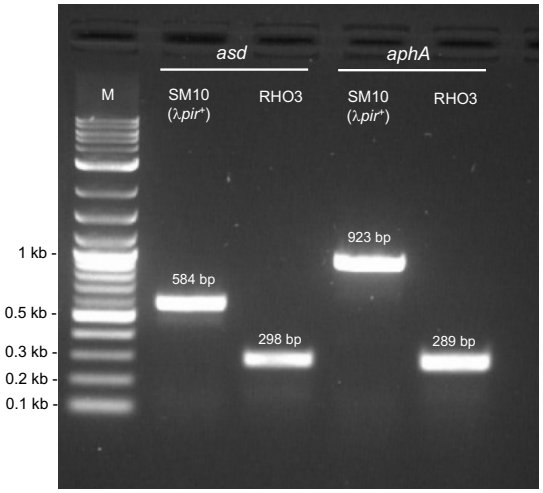
DNA template preparation

DNA templates were obtained by boiling. Briefly, a disposable 1 μl loop was used to scoop bacterial cells from a bacterial lawn grown overnight on LB agar plates (supplemented with 400 μg/ml DAP for RHO3). After suspension of the cells in 100 μl nuclease-free water, the suspension was boiled for 10 min in a heat block set at 110°C. Cell debris was pelleted by centrifugation at 12,000 rpm for 10 min. The supernatant was transferred to a clean microcentrifuge tube.

PCR reaction conditions

The 50 μl reaction PCR mixture consisted of 10 μl of 5x Q5 reaction buffer, 1 μl of 10 mM dNTPmix, 2.5 μl of 10 μM of each primer, 10 μl of 5x Q5 high GC enhancer, 22.5 μl water, 1 μl of boil prep DNA template and 0.5 μl of Q5 high-fidelity polymerase (New England Biolab, Ipswich, MA). PCR conditions were 98°C for 2 min; 30 cycles of 98°C for 30 s, 59°C for 30 s, 72°C for 30 s; followed by 72°C for 2 min.

Agarose gel



The *aphA* primers P3522 & P3523 amplify a 923 bp fragment from SM10(λ*pir*$^+$) DNA and a 289 bp fragment from RHO3 DNA. The latter fragment represents 179 bp from the *aphA* upstream and coding regions and a 110 bp *FRT* scar (see next page).

The *asd* primers P3524 & P3525 amplify a 584 bp internal to *asd* from SM10(λ*pir*$^+$) DNA and a 298 bp fragment from RHO3 DNA. The latter fragment represents 188 bp from the *asd* gene and a 110 bp *FRT* scar (see next page).

DNA sequences of RHO3 PCR fragments

***E. coli* RHO3 Δ*aphA*::*FRT* containing region (289 bp)**

```
        P3522
GCCAATCAGTGATGATGAAATGGTGAAAAGCGGACAGCATCAGTAACGAAAGTATCTTAGCGGGCATGAAAATGGCA
AATAACGGTCAAACATCGTGGCGTTGGGTACCGAGCTCGAATTGGGGATCTTGAAGTACCTATTCCGAAGTTCCTAT
TCTCTAGAAAGTATAGGAACTTCAGAGCGCTTTTGAAGCTAATTCGAGCTCGGTACCCGACGCATTGGCGGCGTTCA
   XbaI
TGCGCCGACTGCATGCGATCCCAGTGAGCGAATGCTCCTTCAACAGTGGACCACGCAT
                                                P3523
```

*Hinc*II half sites (yellow) flank the *FRT* scar (turquoise) with the *FRT* sequence (bold) and its resident XbaI site (underlined).

***E. coli* RHO3 Δ*asd*::*FRT* gene containing region (298 bp)**

```
        P3524
TTCTACTTCTCAGCTTGGCCAGGCTGCGCCGTCTTTTGGCGGAACCACTGGCACACTTCAGGATGCCTTTGATCTGG
AGGCGCTAAAGGCCCTCGATGGGTACCGAGCTCGAATTAGCTTCAAAAGCGCTCTGAAGTTCCTATACTTTCTAGAG
                                                                  XbaI
AATAGGAACTTCGGAATAGGTACTTCAAGATCCCCAATTCGAGCTCGGTACCCATCGAACGCAAAGTCACAACCTTA
ACCCGTAGCGGTGAGCTGCCGGTGGATAACTTTGGCGTGCCGCTGGCGGGTAGCCTGATTCCGTGGA
                                                  P3525
```

*Eco*RV half sites (yellow) flank the *FRT* scar (turquoise) with the *FRT* sequence (bold) and its resident XbaI site (underlined).