

### Supplementary file 1. List of strains used in this study

| Strain number | Genotype                                                                 | Source                         | Use                      |
|---------------|--------------------------------------------------------------------------|--------------------------------|--------------------------|
| SK1           | MG1655                                                                   |                                | Cloning<br>Doubling time |
| SK47          | BW25993 <i>rplA::rplA-mEos2</i>                                          | (Sanamrad et al., 2014)        | Imaging                  |
| SK52          | MG1655 $\Delta$ <i>araFGH</i> <i>araE::P13-araE</i>                      | C. Jacobs-Wagner               | Cloning                  |
| SK72          | NCM3416 <i>rne::rne-mCherry</i> FRT- <i>cat</i> -FRT                     | (Strahl et al., 2015)          | Cloning                  |
| SK98          | MG1655 $\Delta$ <i>lacYA</i>                                             | (Kim et al., 2019)             | qRT-PCR<br>Cloning       |
| SK105         | MG1655 $\Delta$ <i>lacIZYA</i>                                           | CJW6643(Kim et al., 2019)      | Cloning                  |
| SK107         | MG1655 <i>rne::rne</i> ( $\Delta$ MTS)- <i>mCherry</i>                   | CJW5685(Thappeta et al., 2024) | Cloning                  |
| SK186         | MG1655 <i>rne::rne</i> (1-592)- <i>yfp</i> FRT- <i>kan</i> -FRT          | C. Jacobs-Wagner, JRH474       | Cloning                  |
| SK187         | MG1655 <i>rne::rne-mEos3.2</i> FRT- <i>kan</i> -FRT                      | C. Jacobs-Wagner, JRH475       | Imaging                  |
| SK213         | BW25113 <i>hupA::hupA-mcherry</i> FRT- <i>kan</i> -FRT                   | CJW5158 (Xiang et al., 2021)   | Cloning                  |
| SK249         | MG1655 <i>rne::rne</i> $\Delta$ MTS- <i>mEos3.2</i> FRT- <i>kan</i> -FRT | This study                     | Imaging                  |
| SK290         | MG1655 <i>rne::rne-mEOS3.2</i>                                           | This study                     | Cloning                  |
| SK292         | MG1655 <i>lacYA::lacY-mEos3.2</i> FRT- <i>kan</i> -FRT                   | This study                     | Imaging                  |
| SK304         | MG1655 <i>rne::rne-mEos3.2</i> , $\Delta$ <i>rhIB::FRT-kan</i> -FRT      | This study                     | Imaging                  |

|       |                                                                                       |                    |         |
|-------|---------------------------------------------------------------------------------------|--------------------|---------|
| SK308 | MG1655 <i>rne::rne-mEos3.2, Δpnp::FRT-kan-FRT</i>                                     | This study         | Imaging |
| SK360 | MG1655 <i>ΔaraFGH araE::P13-araE araBAD::rne-yfp-kan rne::rne-mcherry FRT-cat-FRT</i> | This study         | Cloning |
| SK364 | MG1655 <i>ΔaraFGH araE::P13-araE araBAD::rne-yfp rne::rne-mcherry</i>                 | This study         | Cloning |
| SK370 | MG1655 <i>Δ(lacYA) rne::rne(1-592)-yfp FRT-kan-FRT</i>                                | (Kim et al., 2024) | qRT-PCR |
| SK373 | MG1655 <i>rne::rne(1-529)-mEos3.2 FRT-kan-FRT</i>                                     | This study         | Imaging |
| SK374 | MG1655 <i>rne::rne(1-592)-mEos3.2 FRT-kan-FRT</i>                                     | This study         | Imaging |
| SK384 | MG1655 <i>rne::rne(1-592)-yfp</i>                                                     | This study         | Cloning |
| SK394 | MG1655 <i>ΔlacYA ΔaraFGH araE::P13-araE araBAD::rne-yfp rne::rne-mcherry</i>          | This study         | qRT-PCR |
| SK404 | MG1655 <i>rne::rne(1-564)-lacY-mEos3.2 FRT-kan-FRT</i>                                | This study         | Imaging |
| SK405 | MG1655 <i>Δ(lacYA) rne::rne(1-564)-lacY-mEos3.2 FRT-kan-FRT</i>                       | This study         | qRT-PCR |
| SK407 | MG1655 <i>lacZYA::lacZ-mEos3.2 FRT-kan-FRT</i>                                        | This study         | Imaging |
| SK411 | MG1655 <i>rne::rne-mEos3.2</i><br>pUC19-lacI-lacZonly (amp)                           | This study         | Imaging |
| SK424 | MG1655 <i>lacYA::lacY(1-73)-mEos3.2 FRT-kan-FRT</i>                                   | This study         | Imaging |
| SK425 | MG1655 <i>lacYA::lacY(1-192)-mEos3.2 FRT-kan-FRT</i>                                  | This study         | Imaging |
| SK455 | MG1655 <i>ΔlacIZYA</i><br>pUC19-lacI-plac-mEos3.2-MTS (amp)                           | This study         | Imaging |
| SK466 | MG1655 <i>rne::rne(1-564)-lacY(1-73)-rneCTD-</i>                                      | This study         | Imaging |

|       |                                                                                      |            |                    |
|-------|--------------------------------------------------------------------------------------|------------|--------------------|
|       | <i>mEos3.2 FRT-kan-FRT</i>                                                           |            |                    |
| SK467 | MG1655 <i>rne::rne(1-564)-lacY(1-192)-rneCTD-mEos3.2 FRT-kan-FRT</i>                 | This study | Imaging            |
| SK482 | MG1655 <i>rne::rne-venus hupA::hupA-mcherry FRT-kan-FRT</i>                          | This study | Imaging            |
| SK486 | MG1655 <i>rne::rne(1-592)-venus hupA::hupA-mcherry FRT-kan-FRT</i>                   | This study | Imaging            |
| SK505 | MG1655 $\Delta(lacYA)$ <i>rne::rne(1-564)-lacY(1-73)-rneCTD-mEos3.2 FRT-kan-FRT</i>  | This study | qRT-PCR            |
| SK506 | MG1655 $\Delta(lacYA)$ <i>rne::rne(1-564)-lacY(1-192)-rneCTD-mEos3.2 FRT-kan-FRT</i> | This study | qRT-PCR            |
| SK507 | MG1655 <i>rne::rne(1-564)-lacY(1-73)-mEos3.2 FRT-kan-FRT</i>                         | This study | Imaging            |
| SK508 | MG1655 $\Delta(lacYA)$ <i>rne::rne(1-564)-lacY(1-73)-mEos3.2 FRT-kan-FRT</i>         | This study | qRT-PCR            |
| SK512 | MG1655 <i>rne::rne-mEos3.2 hupA::hupA-mcherry kan</i>                                | This study | Imaging            |
| SK592 | MG1655 <i>rne::rne(1-564)-lacY(1-192)-mEos3.2 FRT-kan-FRT</i>                        | This study | Imaging            |
| SK593 | MG1655 $\Delta(lacYA)$ <i>rne::rne(1-564)-lacY(1-192)-mEos3.2 FRT-kan-FRT</i>        | This study | qRT-PCR            |
| SK594 | MG1655 $\Delta(lacYA)$ <i>rne::rne(1-592)-yfp</i>                                    | This study | Cloning            |
| SK595 | MG1655 $\Delta(lacYA)$ <i>rne::rne-mEos3.2 FRT-kan-FRT</i>                           | This study | qRT-PCR            |
| SK598 | MG1655 $\Delta(lacYA)$ <i>rne::rne(1-564)-lacY-rneCTD-mEos3.2 FRT-kan-FRT</i>        | This study | Imaging<br>qRT-PCR |
| SK741 | MG1655 $\Delta(lacYA)$ <i>rne::rne(1-564)-</i>                                       | This study | Imaging            |

|                                       |                                                                                                                |                                                                                                                                                               |                    |
|---------------------------------------|----------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------|
|                                       | MTS(F574AF575A)- <i>rne</i> CTD - <i>mEos3.2</i> FRT- <i>kan</i> -FRT                                          |                                                                                                                                                               | qRT-PCR            |
| SK742                                 | MG1655 $\Delta(lacYA)$ <i>rne::rne</i> (1-564)-MTS(F575E)- <i>rne</i> CTD- <i>mEos3.2</i> FRT- <i>kan</i> -FRT | This study                                                                                                                                                    | Imaging<br>qRT-PCR |
| SK743                                 | MG1655 $\Delta(lacYA)$ <i>rne::rne</i> (1-564)-MTS(F582E)- <i>rne</i> CTD- <i>mEos3.2</i> FRT- <i>kan</i> -FRT | This study                                                                                                                                                    | Imaging<br>qRT-PCR |
| SK748                                 | MG1655 $\Delta(lacYA)$ <i>rne::rne</i> (1-564)-MTS(F574AF575A)- <i>mEos3.2</i> FRT- <i>kan</i> -FRT            | This study                                                                                                                                                    | Imaging<br>qRT-PCR |
| SK749                                 | MG1655 $\Delta(lacYA)$ <i>rne::rne</i> (1-564)-MTS(F575E)- <i>mEos3.2</i> FRT- <i>kan</i> -FRT                 | This study                                                                                                                                                    | Imaging<br>qRT-PCR |
| SK750                                 | MG1655 $\Delta(lacYA)$ <i>rne::rne</i> (1-564)-MTS(F582E)- <i>mEos3.2</i> FRT- <i>kan</i> -FRT                 | This study                                                                                                                                                    | Imaging<br>qRT-PCR |
| <b>Plasmids</b>                       |                                                                                                                |                                                                                                                                                               |                    |
| pBAD18Kan                             |                                                                                                                | (Guzman et al., 1995)                                                                                                                                         | Cloning            |
| pET29b-H6_<br>Streptavidin_<br>_sfGFP |                                                                                                                | A gift from Mark Arbing<br>(Addgene plasmid # 124296;<br><a href="http://n2t.net/addgene:124296">http://n2t.net/addgene:124296</a> ;<br>RRID: Addgene_124296) | Cloning            |
| pKD13                                 |                                                                                                                | (Datsenko and Wanner, 2000)                                                                                                                                   | Cloning            |
| pUC19                                 |                                                                                                                |                                                                                                                                                               | Cloning            |
| SJK1606                               | pBAD18kan- <i>mEos3.2</i> -MTS                                                                                 | This study                                                                                                                                                    | Cloning            |
| SJK1689                               | pUC19-lacI-lacY2-CTD- <i>mEos3.2</i> -Kan                                                                      | This study                                                                                                                                                    | Cloning            |
| SJK1697                               | pUC19-lacI-lacY6-CTD- <i>mEos3.2</i> -frtKanfrt                                                                | This study                                                                                                                                                    | Cloning            |

|         |                                         |                            |         |
|---------|-----------------------------------------|----------------------------|---------|
| SJK1716 | pUC19-lacI-lacY12-CTD-mEos3.2-frtKanfrt | This study                 | Cloning |
| SK141   | pUC19-lacI-lacZonly                     | CJW6647 (Kim et al., 2019) | Cloning |
| SK189   | pBAD18 rne-yfp-kan                      | C. Jacobs-Wagner, JRH515   | Cloning |
| SK567   | pET29b-H6_Streptavidin_mEos3.2          | This study                 | Cloning |