Table S1. - Strains and Plasmids

| Strain       | Genotype                                                                 | Source or Reference                                                                 |
|--------------|--------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| MG1655       | *Escherichia coli* K12 F' l' ilvG' rfb-50 rph-1                           | Obtained from Nadim Majdalani, Ph.D.                                                 |
| DJ480        | MG1655 ΔlacZYA                                                           | Obtained from Nadim Majdalani, Ph.D.                                                 |
| KMT41        | F' lacI378 lacZ-GCG proB' araBAD (lac-pro)XIII miaB::Tn10dCm              | TX3346 obtained from Malcolm Winkler, Ph.D. (Esberg et al. 1999)                      |
| KMT194       | DJ480 mini-λ::tet                                                        | Obtained from Nadim Majdalani, Ph.D.                                                 |
| KMT283       | MG1655 rssB::tet                                                         | AB003, Obtained from Alexander Bougdour, PhD (Bougdour et al. 2006)                   |
| KMT592       | DJ480 ΔmiaA2::zeo                                                        | This work, *E. coli* DJ480 mini-λ::tet induced + ΔmiaA2::zeo                         |
| KMT30000     | MG1655 ΔaraBAD, araC*, mal::lacF' 80- lacI::P_{BAD}-rpoS990-lacZ translational fusion | This work, PM1805 x P_{BAD}-rpoS990-lacZ PCR on M63-glycerol-sucrose XG               |
| KMT30003     | MG1655 ΔaraBAD, araC*, mal::lacF' 80- lacI::P_{BAD}-rpoS990-lacZ translational fusion rssB::tet | This work, KMT30000 x P1 (KMT283 - ΔrsB::tet)                                          |
| KMT30011A    | MG1655 ΔaraBAD, araC*, mal::lacF' 80- lacI::P_{BAD}-rpoS990-lacZ translational fusion rssB::tet ΔmiaA2::zeo | This work, KMT30003 x P1 (KMT592 - ΔmiaA2::zeo)                                        |
| KMT30029     | MG1655 ΔaraBAD, araC*, mal::lacF' 80- lacI::P_{BAD}-rpoS990-lacZ translational fusion pBR-pLac | This work, KMT30003 + pBR-pLac (TSS Transformation)                                   |
| KMT30030     | MG1655 ΔaraBAD, araC*, mal::lacF' 80- lacI::P_{BAD}-rpoS990-lacZ translational fusion rssB::tet pBR-pLac-leuX | This work, KMT30003 + pBR-pLac-leuX (TSS Transformation)                              |
| KMT30035     | MG1655 ΔaraBAD, araC*, mal::lacF' 80- lacI::P_{BAD}-rpoS990-lacZ translational fusion rssB::tet ΔmiaA2::zeo pBR-pLac | This work, KMT30011a + pBR-pLac (TSS Transformation)                                  |
| KMT30036     | MG1655 ΔaraBAD, araC*, mal::lacF' 80- lacI::P_{BAD}-rpoS990-lacZ translational fusion rssB::tet ΔmiaA2::zeo pBR-pLac-leuX | This work, KMT30011a + pBR-pLac-leuX (TSS Transformation)                             |
| KMT30054     | MG1655 ΔaraBAD, araC*, mal::lacF' f80- lacI::P_{BAD}-rpoS990-lacZ translational fusion rssB::tet miaB::Tn10dCm | KMT30003 x P1 (KMT41 - miaB::Tn10dCm)                                               |
| KMT33000     | MG1655 ΔaraBAD, araC*, mal::lacF' 80- lacI::P_{BAD}-rpoS990(lei*3)-lacZ translational fusion | This work, PM1805 x P_{BAD}-rpoS990(lei*3)-lacZ PCR on M63-glycerol-sucrose XG        |
| KMT33001     | MG1655 ΔaraBAD, araC*, mal::lacF' 80- lacI::P_{BAD}-rpoS990(lei*3)-lacZ translational fusion rssB::tet | This work, KMT33000 x P1 (KMT283 - ΔrsB::tet)                                         |
| KMT33002     | MG1655 ΔaraBAD, araC*, mal::lacF' 80- lacI::P_{BAD}-rpoS990(lei*3)-lacZ translational fusion | This work, KMT33001 x P1 (KMT592 - ΔmiaA2::zeo)                                        |
rssB::tet ΔmiaA::zeo

KMT33009 MG1655 ΔaraBAD, araC+, mal:.lacf $^\phi$80- lacf: $^{_P}_{BAD}$rpoS990(leu*3)-lacZ translational fusion rssB::tet pBR-pLac
KMT33010 MG1655 ΔaraBAD, araC+, mal:.lacf $^\phi$80- lacf: $^{_P}_{BAD}$rpoS990(leu*3)-lacZ translational fusion rssB::tet pBR-pLac-leuX
KMT33011 MG1655 ΔaraBAD, araC+, mal:.lacf $^\phi$80- lacf: $^{_P}_{BAD}$rpoS990(leu*3)-lacZ translational fusion rssB::tet ΔmiaA::zeo pBR-pLac
KMT33012 MG1655 ΔaraBAD, araC+, mal:.lacf $^\phi$80- lacf: $^{_P}_{BAD}$rpoS990(leu*3)-lacZ translational fusion rssB::tet ΔmiaA::zeo pBR-pLac-leuX
KMT36000 MG1655 ΔaraBAD, araC+, mal:.lacf $^\phi$80- lacf: $^{_P}_{BAD}$rpoS990(leu*1)-lacZ translational fusion

This work, KMT33001 + pBR-pLac (TSS Transformation)
This work, KMT33001 + pBR-pLac-leuX (TSS Transformation)
This work, KMT33002 + pBR-pLac (TSS Transformation)
This work, KMT33002 + pBR-pLac-leuX (TSS Transformation)
This work, PM1805 x $^{_P}_{BAD}$rpoS990(leu*1)-lacZ PCR on M63-glycerol-sucrose XG
This work, KMT36000 x P1 (KMT283 - ΔrssB::tet)
This work, KMT36003 x P1 P1 (KMT592 - ΔmiaA2::zeo)
This work, KMT37000 x P1 (KMT283 - ΔrssB::tet)
This work, KMT37002 x P1 P1 (KMT592 - ΔmiaA2::zeo)
This work, KMT37002 x P1 P1 (KMT592 - ΔmiaA2::zeo)
This work, PM1800 x $^{_P}_{BAD}$ira258-lacZ PCR on M63-glycerol-sucrose XG
This work, KMT42000 x P1 (KMT592 - ΔmiaA2::zeo)
This work, KMT42000 + pBR-pLac (TSS Transformation)
This work, KMT42000 + pBR-pLac-leuX (TSS Transformation)
This work, KMT42000 + pBR-pLac-leuZ (TSS Transformation)
This work, KMT42000 x (KMT41 - miaB::Tn10dCm)
This work, PM1800 x $^{_P}_{BAD}$ira258(leu*1)-lacZ PCR on
KMT43002 MG1655 ΔaraBAD, araC*, mal::lacF *80- lacI::PBAD-iraP258(leu*1)-lacZ translational fusion
ΔmiaA::zeo
This work, KMT42000 x P1 (KMT592 - ΔmiaA2::zeo)

KMT44000 MG1655 ΔaraBAD, araC*, mal::lacF *80- lacI::PBAD-iraP258(leu*2)-lacZ translational fusion
This work, PM1800 PBAD-ira258(leu*2)-lacZ PCR on M63-glycerol-sucrose XG

KMT44002 MG1655 ΔaraBAD, araC*, mal::lacF *80- lacI::PBAD-iraP258(leu*2)-lacZ translational fusion
ΔmiaA::zeo
This work, KMT44000 x P1 (KMT592 - ΔmiaA2::zeo)

KMT45002 MG1655 ΔaraBAD, araC*, mal::lacF *80- lacI::PBAD-iraP258(leu*3)-lacZ translational fusion
ΔmiaA::zeo
This work, KMT45000 x P1 (KMT592 - ΔmiaA2::zeo)

KMT45003 MG1655 ΔaraBAD, araC*, mal::lacF *80- lacI::PBAD-iraP258(leu*3)-lacZ translational fusion pBR-pLac
This work, KMT45000 + pBR-pLac

KMT45004 MG1655 ΔaraBAD, araC*, mal::lacF *80- lacI::PBAD-iraP258(leu*3)-lacZ translational fusion pBR-pLac-leuX
This work, KMT45000 + pBR-pLac-leuX

KMT45005 MG1655 ΔaraBAD, araC*, mal::lacF *80- lacI::PBAD-iraP258(leu*3)-lacZ translational fusion pBR-pLac-leuZ
This work, KMT45000 + pBR-pLac-leuZ

KMT45006 MG1655 ΔaraBAD, araC*, mal::lacF *80- lacI::PBAD-iraP258(leu*3)-lacZ translational fusion ΔmiaA::zeo pBR-pLac
This work, KMT45002 + pBR-pLac

KMT45007 MG1655 ΔaraBAD, araC*, mal::lacF *80- lacI::PBAD-iraP258(leu*3)-lacZ translational fusion ΔmiaA::zeo pBR-pLac-leuX
This work, KMT45002 + pBR-pLac-leuX

KMT45008 MG1655 ΔaraBAD, araC*, mal::lacF *80- lacI::PBAD-iraP258(leu*3)-lacZ translational fusion ΔmiaA::zeo pBR-pLac-leuZ
This work, KMT45002 + pBR-pLac-leuZ

PM1805 MG1655 lacI::PBAD-cat-sacB::lacZ, ΔaraBAD, araC*, mal::lacF, mini-λ::tet *80- M63-glycerol-sucrose XG
Obtained from Nadim Majdalani, Ph.D.

Plasmids
pBR-pLac pBR322, AatII-P_lacO cloned SspI / AatII into, AmpR
(Guillier and Gottesman 2006)
pBR-pLac-leuX leuX (tRNA^leu^) gene cloned into AatII / EcoRI site of pBR-pLac, AmpR
This work
pBR-pLac-leuZ leuZ (tRNA^leu^) gene cloned into AatII / EcoRI site of pBR-pLac, AmpR
This work
Table S2 – Oligonucleotides

| Oligonucleotide number | Sequence | Function / use                                      |
|-----------------------|----------|---------------------------------------------------|
| KT1035                | CAATCTGGATTTACATCCGGCGATAAAAGCCTGAAAGCAGTGGTTCATAAAATTCAGTGGTACAATTAATCA | ΔmiaA2::zeo forward primer (complete ORF deletion) |
| KT1036                | CTGAAAAAATTGCACGAATACGTCATCTCAATTTGACACATTTGACCTCTCCCTCAGGCCA | ΔmiaA2::zeo forward primer (complete ORF deletion) |
| KT1102                | tgaGACGTCGCCCAGGATGTTGGAATCGGT | tRNA leuZ forward primer for cloning into pBR-pLac AatII |
| KT1103                | gggtaaGAAATTCTGTTGCTCCGGACGCACGGACTT | tRNA leuZ reverse primer for cloning into pBR-pLac EcoRI |
| KT1104                | tcGACGTCGCCGGAGTTGGCGAAATCGGT | tRNA leuX forward primer for cloning into pBR-pLac AatII |
| KT1105                | gggtaaGAAATTCTGTTGCTCCGGACGCACGGACTC | tRNA leuX reverse primer for cloning into pBR-pLac EcoRI |
| KT1115                | CATTGACAATTTGAGCGGATAACAGACT | pBR-pLac forward screening and sequencing primer |
| KT1123                | AcctgacgcttttttatgcaactctctactgtttcttccatTCACGCGGTAGGAGCCACCTT | Forward primer for amplification of P_{BAD-rpoS990-lacZ} AES |
| KT1124                | CGCCAGGGTTTCCCAGTCAGAGTGTTGTAACAGCGCGGCTCGCGGAACAGCGTTCTGA | Reverse primer for amplification and sequencing of lacZ fusions and gBlocks™ |
| KT1163                | CATTGACAATTTGAGCGGATAACAGACT | P_{BAD} forward amplification primer; can be used to amplify and sequence P_{BAD} (lacZ) fusion gBlocks™ |
| KT1204                | Acctgacgctttttatatgcaactctctactgtttcttccat | |

Table S3 - P_{BAD-rpoS990-lacZ} translational fusion miaA^{+}/miaA^{-} activity ratio

| Strain | Time (min after induction) | 15 | 20 | 25 | 30 |
|--------|---------------------------|----|----|----|----|
| wild type |                           | 23 | 6.0 | 3.7 | 2.7 |
| leu^{+}1 |                           | 1.8 | 1.3 | 1.3 | 1.2 |
| leu^{+}2 |                           | 1.4 | 1.3 | 1.2 | 1.9 |
| leu^{+}3 |                           | 14 | 3.6 | 1.9 | 1.7 |
Table S4 - \( P_{\text{BAD-rpoS909-lacZ}} \) translational fusion activity ratio

| Strain           | Time (min after induction) |
|------------------|----------------------------|
|                  | 15 | 20 | 25 | 30 |
| wild type / leu*1| 1.5| 1.4| 1.3| 1.1|
| wild type / leu*2| 4.5| 3.5| 3.0| 2.4|
| wild type / leu*3| 1.4| 1.4| 1.3| 1.1|

Table S5 - \( P_{\text{BAD-iraP258-lacZ}} \) translational fusion \( miaA^+ / miaA^- \) activity ratio

| Strain     | Time (min after induction) |
|------------|----------------------------|
|            | 15 | 20 | 25 | 30 |
| wild type  | 15 | 4.7| 3.0| 2.8|
| \textit{leu}*1  | 1.8| 1.9| 2.0| 2.1|
| \textit{leu}*2  | 1.8| 1.8| 1.7| 1.7|
| \textit{leu}*3  | 3.2| 1.6| 1.3| 1.1|

Table S6 - \( P_{\text{BAD-iraP258-lacZ}} \) translational fusion \( miaA^+ / miaA^- \) activity ratio

| Strain           | Time (min after induction) |
|------------------|----------------------------|
|                  | 15 | 20 | 25 | 30 |
| wild type / leu*1| -1.8| -1.7| -1.7| -1.8|
| wild type / leu*2| 1.0 | 1.0 | 1.0 | 1.0|
| wild type / leu*3| 1.4 | 1.4 | 1.5 | 1.5|
Supplemental Figures

Figure S1. DNA sequence of \textit{P}_{\textit{BAD-rpoS990-lacZ}} translational fusion \textit{gBlock}™

AcctgacgcttttatgcaactctctactgtttctccatTCACGGGTAGGAGCCACCTTatgAGTCAGAATACGCTGAAAGTTCATGATTTAATGAAGATGCGGAATTTGATGAGAACGGAG
TTAGGTTTTGACGAAAAGGCCTTAAGTAACAGGAAACCCACTGATAACGATTAGCCAGGAGAAACACTGTTATCCAGGAGTCGAGTACGTTCCTCCTCGCCGCCGGAATGACAGTAGTACTGGCTGCTGTTGAAAAATTGCCCGCCGTTATGGCAATCGTGGTCTGGCGTTGACGCGACTCAGCTTTACCTTGGCCGAGGCCGTCGTTTTACAACGTCGTTGACTGGGAAAACCCTGGCGTTCCGATGGTCTGGACATCCTGGCCGATGAAAAAGAAATCGGCAACGTGGCACGATTCGGT

Figure S2. DNA sequence of \textit{P}_{\textit{BAD-rpoS990(leu*1)-lacZ}} translational fusion \textit{gBlock}™

AcctgacgcttttatgcaactctctactgtttctccatTCACGGGTAGGAGCCACCTTatgAGTCAGAATACGCTGAAAGTTCATGATTTAATGAAGATGCGGAATTTGATGAGAACGGAG
TTAGGTTTTGACGAAAAGGCCTTAAGTAACAGGAAACCCACTGATAACGATTAGCCAGGAGAAACACTGTTATCCAGGAGTCGAGTACGTTCCTCCTCGCCGCCGGAATGACAGTAGTACTGGCTGCTGTTGAAAAATTGCCCGCCGTTATGGCAATCGTGGTCTGGCGTTGACGCGACTCAGCTTTACCTTGGCCGAGGCCGTCGTTTTACAACGTCGTTGACTGGGAAAACCCTGGCGTTCCGATGGTCTGGACATCCTGGCCGATGAAAAAGAAATCGGCAACGTGGCACGATTCGGT
Figure S3. DNA sequence of PBAD-rps990(leu^2)-lacZ translational fusion gBlock™

AcctgacgtttttttcgcaactcctctactgtttctccatTCACGGGTAGGAGCCACCTTatgAGTCAGAATACGCTGAAAGTTCATGATTAATGAAGATGCGGAATTTGATGAGAACGGAGTTGAGGTTTTTGACGAAAAGGCC

TTGAGTGTGGTTCAGAAAGGGCCTTAATGAGAAGAGGAGAGGACGTGTCGGAAGGGATCTGCGCCGCCTCTCTCCCGGCCGATCGAGAGTAACCTACCTCAACATACG

TAAGCTGGACCATGAACCAAGTGCGGAGGAGATCGCAGAGCAACTGGATAAGCCAGTTGATGACGTCAGCCGTATGCTTCGTCTTAACGAGCGCATTACCTCGGTAGACACCCCGCTGGGTGGTGATTCCGAAAAAGCG
Figure S4. DNA sequence of P_{BAD-rpoS990 (leu*3)-lacZ} translational fusion gBlock™

AcctgacgttttattcgaactctctactgttctccatTCACGGGTAGGAGCCACCTTatgAGTCAGAATACGCTGAAAGTTCATGAT

CTTCAGCCGACTGCCTCTGCGCCGGCGGATGATCGAGAGTAAC

Figure S5. DNA sequence of P_{BAD-iraP258-lacZ} translational fusion gBlock™

AcctgacgttttattcgaactctctactgttctccatTTGGATAAGGAAATACAGACatgAAAAATCTCATTGCTGAG

TTGTGACCGAGTTAGATTATCGTCACTGCAATGCTTCGCAATATGGCGCAAAATGACCAACAGCGG

CAGCATTCCTGACGACGATACGGAGCTGCTGCGCGATTACGTAAAGAAG

TTATTGAAAGCATCCTCGTCAGGCCGTCGTTTTACAACGTCGTGACTGGGAAACCCTGGCGTT
**Figure S6. DNA sequence of P_{BAD-iraP258(lei*1)}-lacZ translational fusion gBlock™**

AcctgacgcttttatcgcaacctctctactgtttctccatTTGGATAAGGAAATACAGACatgAAAAATCTCATTTGCTGAGTTTCTTTTTAAGGATGAGTTTCTTTTTTCAAGTACGGAGCTGCTGCGCGATTACGTAAAGAAGCTTTTGATAGCCCAAAAAGAAGAAGAGTCGAAAGAAC

**Figure S7. DNA sequence of P_{BAD-iraP258(lei*2)}-lacZ translational fusion gBlock™**

AcctgacgcttttatcgcaacctctctactgtttctccatTTGGATAAGGAAATACAGACatgAAAAATCTCATTTGCTGAGTTTCTTTTTAAGGATGAGTTTCTTTTTTCAAGTACGGAGCTGCTGCGCGATTACGTAAAGAAGCTTTTGATAGCCCAAAAAGAAGAAGAGTCGAAAGAAC

**Figure S8. DNA sequence of P_{BAD-iraP258(lei*3)}-lacZ translational fusion gBlock™**

AcctgacgcttttatcgcaacctctctactgtttctccatTTGGATAAGGAAATACAGACatgAAAAATCTCATTTGCTGAGTTTCTTTTTAAGGATGAGTTTCTTTTTTCAAGTACGGAGCTGCTGCGCGATTACGTAAAGAAGCTTTTGATAGCCCAAAAAGAAGAAGAGTCGAAAGAAC
Supplemental Figure Legend

**Figure S9. Translation RpoS and IraP in the absence of the ms\textsuperscript{2}A\textsubscript{37} tRNA modification.** (A) The *rssB\^miaB\textsuperscript{+} (KMT30003) and rssB\^miaB\textsuperscript{−} (KMT30054) P\textsubscript{BAD}\textsuperscript{rpoS990-lacZ} translational fusion strains were assayed for Specific β-galactosidase activity following arabinose induction. Each value represents the mean of at least three replicate experiments; the error bars represent the standard error of the mean (SEM). (B) The *miaB\textsuperscript{+} (KMT42000) and miaB\textsuperscript{−} (KMT42009) P\textsubscript{BAD}\textsuperscript{iraP258-lacZ} translational fusion strains were assayed for Specific β-galactosidase activity following arabinose induction. Each value represents the mean of at least three replicate experiments; the error bars represent the standard error of the mean (SEM).
References

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