**Supplementary File 1A | Manually curated QS synthases and receptors.**

|  |  |  |  |
| --- | --- | --- | --- |
| QS system name | Bacterium | Cognate signal | GenBank (I/R) |
| LasI/Ra | *Pseudomonas aeruginosa* PAO1 | 3OC12-HSL | AAG04821.1/ AAG04819.1 |
| RhlI/R | *P. aeruginosa* PAO1 | C4-HSL | AAG06864.1/ AAG06865.1 |
| PfvI/R | *Pseudomonas fuscovaginae* UPB0736 | 3OC10/ 3OC12-HSL | CAQ15950.2/ CAQ15948.2 |
| PfsI/R | *P. fuscovaginae* UPB0736 | C10/ C12-HSL | CBI67625.1/ CBI67623.1 |
| CepI/R | *Burkholderia vietnamiensis* G4 | C8-HSL | ABO58211.1/ ABO58209.1 |
| BviI/R | *B. vietnamiensis* G4 | C10-HSL | ABK32009.1/ ABK32010.1 |
| BtaI/R1 | *Burkholderia thailandensis* E264 | C8-HSL | ABC35524.1/ ABC34804.1 |
| BtaI/R2 | *B. thailandensis* E264 | 3OHC10-HSL | ABC34067.1/ ABC34774.1 |
| BtaI/R3 | *B. thailandensis* E264 | 3OHC8-HSL | AIP27810.1/ AIP27980.1 |
| CepI/R | *Burkholderia cenocepacia* J2315 | C8-HSL | CAR55728.1/ CAR55726.1 |
| AbaI/R | *Acinetobacter baumannii* ATCC17978 | 3OHC12-HSL | AKQ28471.1/ AKQ28469.1 |
| CviI/R | *Chromobacterium violaceum* ATCC 12472 | 3OHC10-HSL | AAQ61751.2/ AAQ61750.2 |
| CviI/R | *Chromobacterium violaceum* ATCC 31532 | C6-HSL | PLV42917.1/ ADC79709.1 |
| LuxI/R | *Vibrio fischeri* ES114 | 3OC6-HSL | AAW87994.1/ AAW87995.1 |
| LuxI/Ra | *Vibrio fischeri* MJ11 | 3OC6-HSL | ACH64323.1/ ACH63788.1 |
| ExpI/R | *Pectobacterium parmentieri* SCC3193 | 3OC8-HSL | AFI92653.1/ AFI92652.1 |
| BjaI/R | *Bradyrhizobium japonicum* USDA110 | Isovaleryl-HSL | BAC46328.1/ BAC46327.1 |
| MupI/R | *Pseudomonas fluorescens* NCIMB 10586 | 3OC10-HSL | AAK28505.1/ AAK28504.1 |
| PpuI/R | *Pseudomonas putida* IsoF | 3OC10/3OC12-HSL | AAM75411.1/ AAM75413.1 |
| TraI/R | *Agrobacterium tumefaciens* C-58 | 3OC8-HSL | AAK91000.1/ AAK91098.1 |
| MbaI/R | *Methylobacter tundripaludum* 21/22 | 3OHC10-HSL | WP\_150113271.1/ WP\_006890625.1b |
| YruI/R | *Yersinia ruckeri* ATCC 29473 | 3OC8-HSL | KGA49182.1/ KGA49159.1 |
| GtaI/R | *Rhodobacter capsulatus* SB1003 | C16-HSL | ADE84094.1/ ADE84093.1 |
| AhyI/R | *Aeromonas hydrophila* ML09-119 | C4-HSL | AGM42354.1/ AGM42355.1 |

a Covariation analyses were mapped onto LasI/R from *P. aeruginosa* PAO1 and LuxI/R from *V. fischeri* MJ11.

b The accession number provided is for the NCBI Reference Sequence.

**Supplementary File 1B | Select previously reported data for LasR homologs with relevant amino acid substitutions**. The native signal for each receptor is indicated in parentheses.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| LasR residue | LasR  homolog | Mutation | Effect on receptor activity**a** | Reference |
| **L36** | LuxR  (3OC6-HSL) | L42Ab | Reduced sensitivity | (Koch et al., 2005) |
| L42S | Reduced sensitivity |
| TraRc  (3OC8-HSL) | A38V | Reduced activity | (Luo et al., 2003) |
| **G38** | QscR (3OC12-HSL) | G40F | Altered selectivity: impaired response to 3OC12-HSL, increased sensitivity to 3OC6-HSL | (Lintz et al., 2011) |
| **P57** | TraR | H54Y | Reduced activity | (Luo et al., 2003) |
| **R61** | LasR (3OC12-HSL) | R61M | Altered selectivity: impaired response to 3OC12-HSL, maintained response to C12-HSL | (Collins et al., 2006) |
| LasR | R61M | Reduced sensitivity | (Gerdt et al., 2015) |
| LuxR | R67M | Altered selectivity: impaired response to 3OC6-HSL, maintained response to C6-HSL | (Collins et al., 2006) |
| TraRc | Q58L | Altered selectivity: improved sensitivity to 3OC6-HSL | (Chai and Winans, 2004) |
| Q58F | Reduced activity |
| **T75** | LasR | T75V | Increased sensitivity | (Gerdt et al., 2015) |
| **V76** | QscR | V78F | Reduced activity | (Lintz et al., 2011) |
| **A127** | LasR | A127W | Altered selectivity: impaired response to 3OC12-HSL, maintained response to shorter AHLs | (McCready et al., 2019b) |
| LasR | A127F | Reduced sensitivity | (McCready et al., 2019a) |
| LuxR | M135I | Altered selectivity: impaired response to 3OC6-HSL; improved response to C8-HSL | (Collins et al., 2005) |
| M135V |
| LuxR | M135A | Reduced sensitivity | (Koch et al., 2005) |
| **S129** | LasR | S129A | Reduced sensitivity | (Gerdt et al., 2015; Manson et al., 2020) |
| LasR | S129C | Reduced sensitivity | (McCready et al., 2019b) |
| S129W | Reduced sensitivity |
| S129F | Reduced sensitivity |
| S129T | Reduced sensitivity |
| S129M | Reduced sensitivity |
| TraR | T129S | No change from wild-type | (Chai and Winans, 2004) |
| T129L | Reduced activity |
| T129I | Reduced activity |
| T129F | Reduced activity |
| T129A | Altered selectivity: impaired activity; equally sensitive to C8-HSL and 3OC8-HSL |
| T129V |
| **L130** | LasR | L130F | Altered selectivity: increased sensitivity to 3OC12-HSL and to other AHLs | (McCready et al., 2019b) |

a All studies measured the activity of the receptor in *Escherichia coli* unless otherwise noted.

b Numbering is according to the *Vibrio fischeri* ES114 sequence.

c This study was performed in *Agrobacterium tumefaciens*, using a *traR* expression plasmid.

**Supplementary File 1C | Select previously reported data for LasI homologs with relevant amino acid substitutions.** The native signal for each synthase is indicated in parentheses.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| LasI residue | LasI homolog | Mutation | Effect on synthase activity**a** | Reference |
| **S103** | RhlI  (C4-HSL) | S103E | Impaired activity | (Parsek et al., 1997) |
| EsaI  (3OC6-HSL) | S99A | Impaired activity | (Watson et al., 2002) |
| **T142** | LasI  (3OC12-HSL) | T142G | Impaired activity | (Gould et al., 2006) |
| T142A | Slightly altered selectivity |
| T142S | Slightly altered selectivity |
| EsaI  (3OC6-HSL) | T140A | Altered selectivity: Increased production of C6-HSL | (Gould et al., 2006; Watson et al., 2002) |
| EsaI | T140V | Impaired activity | (Watson et al., 2002) |
| **T144** | LasI | T144V | Impaired activity | (Gould et al., 2006) |
| **M152** | RhlI | F147Lb | Increased activity | (Kambam et al., 2009) |
| MesIc  (C6-HSL) | L153A | Altered selectivity: Increased C8-HSL production | (Dong et al., 2020) |
| L153F | Altered selectivity: Increased C4-HSL production |
| BjaIc  (isovaleryl-HSL) | F147Y | Altered selectivity: Increased C4-HSL production | (Dong et al., 2020) |

a All studies measured activity of the synthase in *Escherichia coli* unless otherwise noted.

b F147L was one of three amino acid substitutions in a synthase obtained by directed evolution.

c Substrate selectivity was studied using purified enzymes.

**Supplementary File 1D | LasR amino acid substitutions evaluated in Figure 3.** Relative abundance is the frequency of a given amino acid at the indicated position across all LasR homologs.

|  |  |  |
| --- | --- | --- |
| **Residue in receptor** | **Amino acid** | **Relative abundance** |
| 38 | Gly\* | 0.482 |
| Leu | 0.095 |
| Val | 0.086 |
| Ala | 0.079 |
| 61 | Val | 0.231 |
| Gln | 0.160 |
| Leu | 0.128 |
| Met | 0.126 |
| Arg\* | 0.093 |
| 127 | Leu | 0.287 |
| Met | 0.174 |
| Ala\* | 0.102 |
| 129 | Ser\* | 0.536 |
| Thr | 0.273 |
| Ala | 0.092 |
| Asn | 0.049 |
| 130 | Leu\* | 0.359 |
| Phe | 0.253 |
| Ile | 0.176 |

\* Indicates identity in wild-type LasR.

**Supplementary File 1E | LasI amino acid substitutions evaluated in Figure 5 – figure supplement 1.** Relative abundance is the frequency of a given amino acid at the indicated position across all LasI homologs.

|  |  |  |
| --- | --- | --- |
| **Residue in synthase** | **Amino acid** | **Relative abundance** |
| 102 | Leu\* | 0.398 |
| Ser | 0.157 |
| Met | 0.156 |
| Ile | 0.094 |
| 142 | Gly | 0.379 |
| Thr\* | 0.314 |
| Ala | 0.154 |
| 145 | Ser | 0.231 |
| Thr\* | 0.199 |
| Pro | 0.170 |
| Asp | 0.135 |
| Ala | 0.009 |
| 157 | Trp | 0.271 |
| Val | 0.220 |
| Leu\* | 0.124 |

\* Indicates identity in wild-type LasI.

**Supplementary File 1F | Primers used in this study.**

|  |  |  |
| --- | --- | --- |
| Primer Name | Sequence | Description |
| lasI-pJN-F | TTGGGCTAGCATGATCGTACAAATTGG | Amplifies *lasI* adding homology to pJN105. *lasI* sequence underlined |
| lasI-pJN-R | TTGGAGCTCCTCATGAAACCGCCAGTC | Amplifies *lasI* adding homology to pJN105, including SacI site. *lasI* sequence underlined. |
| RBS-lasI-F | TTGGGCTAGCAAGGAGGAAGTGAAGATGATCGTACAAATTG | Amplifies *lasI*, including RBS, adding NheI site. *lasI* sequence underlined. |
| mupI-F | TTGGGCTAGCAAGGAGGAAGCCAGCATGAAATATCTAATAG | Amplifies *mupI*, adding RBS and NheI site. *mupI* sequence underlined. |
| mupI-R | TTGGAGCTCTCAAATAGCATTGACTGCGTCC | Amplifies *mupI*, adding SacI site. *mupI* sequence underlined. |
| mupI-pJN-F | TCCATACCCGTTTTTTTGGGCTAGCAAGGAGGAAGCCAG | Amplifies *mupI* PCR product produced by mupI-F & mupI-R, adding additional homology to pJN105 |
| mupI-pJN-R | CTCACTATAGGGCGAATTGGAGCTCCTCAAATAGCATTG | Amplifies *mupI* PCR product produced by mupI-F & mupI-R, adding additional homology to pJN105. *mupI* sequence underlined. |
| mupR-F | GCAAGGAGGAAGCCAGCATGCTTGAAGACATTCTGATG | Amplifies *mupR*, adding homology to pJN105. *mupR* sequence underlined. |
| mupR-R | GCGAATTGGAGCTCCTCAGGGCGTGACC | Amplifies *mupR*, adding homology to pJN105. *mupR* sequence underlined. |
| PmupI-F | GTACAAGCGCAGCAGCATGTAC | Amplifies *mupI* promoter from -300 relative to the translational start site |
| PmupI-R | ACGTGGTGATATGTTCTCGCGTTTG | Amplifies *mupI* promoter from +42 relative to the translational start site |
| PmupI-pPR-F | GCATGCCTGCAGGTCGACGTACAAGCGCAGCAGCATG | Amplifies *mupI* promoter, adding homology to pPROBE-GT. *mupI* promoter sequence underlined. |
| PmupI-pPR-R | CTCGGTACCCGGGGATCCACGTGGTGATATGTTCTCGC | Amplifies *mupI* promoter, adding homology to pPROBE-GT. *mupI* promoter sequence underlined. |

*Site-directed mutagenesis primer sequences are available upon request.*

**References**

Chai Y, Winans SC (2004) **Site-directed mutagenesis of a LuxR-type quorum-sensing transcription factor: Alteration of autoinducer specificity**. *Molecular Microbiology* **51**: 765-776.

Collins CH, Arnold FH, Leadbetter JR (2005) **Directed evolution of *Vibrio fischeri* LuxR for increased sensitivity to a broad spectrum of acyl-homoserine lactones**. *Molecular Microbiology* **55**: 712-723.

Collins CH, Leadbetter JR, Arnold FH (2006) **Dual selection enhances the signaling specificity of a variant of the quorum-sensing transcriptional activator LuxR**. *Nature Biotechnology* **24**: 708-712.

Dong S-H, Nhu-Lam M, Nagarajan R, Nair SK (2020) **Structure-guided biochemical analysis of quorum signal synthase specificities**. *ACS Chemical Biology* **15**: 1497-1504.

Gerdt JP, McInnis CE, Schell TL, Blackwell HE (2015) **Unraveling the contributions of hydrogen-bonding interactions to the activity of native and non-native ligands in the quorum-sensing receptor LasR**. *Organic & Biomolecular Chemistry* **13**: 1453-1462.

Gould TA, Herman J, Krank J, Murphy RC, Churchill MEA (2006) **Specificity of acyl-homoserine lactone synthases examined by mass spectrometry**. *Journal of Bacteriology* **188**: 773-783.

Kambam PKR, Eriksen DT, Lajoie J, Sayut DJ, Sun L (2009) **Altering the substrate specificity of RhlI by directed evolution**. *ChemBioChem* **10**: 553-558.

Koch B, Liljefors T, Persson T, Nielsen J, Kjelleberg S, Givskov M (2005) **The LuxR receptor: The sites of interaction with quorum-sensing signals and inhibitors**. *Microbiology* **151**: 3589-3602.

Lintz MJ, Oinuma K-I, Wysoczynski CL, Greenberg EP, Churchill MEA (2011) **Crystal structure of QscR, a *Pseudomonas aeruginosa* quorum sensing signal receptor**. *Proceedings of the National Academy of Sciences* **108**: 15763-15769.

Luo Z-Q, Smyth AJ, Gao P, Qin Y, Farrand SK (2003) **Mutational analysis of TraR: Correlating function with molecular structure of a quorum-sensing transcriptional activator**. *Journal of Biological Chemistry* **278**: 13173-13182.

Manson DE, O’Reilly MC, Nyffeler KE, Blackwell HE (2020) **Design, synthesis, and biochemical characterization of non-native antagonists of the *Pseudomonas aeruginosa* quorum sensing receptor LasR with nanomolar IC50 values**. *ACS Infectious Diseases* **6**: 649-661.

McCready AR, Paczkowski JE, Cong J-P, Bassler BL (2019a) **An autoinducer-independent RhlR quorum-sensing receptor enables analysis of RhlR regulation**. *PLoS Pathogens* **15**: e1007820.

McCready AR, Paczkowski JE, Henke BR, Bassler BL (2019b) **Structural determinants driving homoserine lactone ligand selection in the *Pseudomonas aeruginosa* LasR quorum-sensing receptor**. *Proceedings of the National Academy of Sciences* **116**: 245-254.

Parsek MR, Schaefer AL, Greenberg EP (1997) **Analysis of random and site-directed mutations in *rhlI*, a *Pseudomonas aeruginosa* gene encoding an acylhomoserine lactone synthase**. *Molecular Microbiology* **26**: 301-310.

Watson WT, Minogue TD, Val DL, von Bodman SB, Churchill MEA (2002) **Structural basis and specificity of acyl-homoserine lactone signal production in bacterial quorum sensing**. *Molecular Cell* **9**: 685-694.