MOLECULAR ECOLOGY RESOURCES

**Supplemental Information for:**

# Lysis-Hi-C as a method to study polymicrobial communities and eDNA

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## Table of Contents:

MOLECULAR ECOLOGY
RESOURCES

**Supplementary Table 1:** Sample names for Hi-C and lysis-Hi-C treatments for cells ("CNL", CSL", "CLL" comprising 3-species artificial polymicrobial mixtures or 'communities' including *Escherichia coli* str. K-12 substr. MG1655, *Staphylococcus aureus* subsp. aureus str. Newman, *Pseudomonas aeruginosa* PA14) and mouse wound biofilm communities ("MS", "MML", "MLL" seeded with *Staphylococcus aureus* SA31 and *Pseudomonas aeruginosa* PAO1).

| Name | Source Material | Treatment or Lysis Method |
|---|---|---|
| CNL | artificial polymicrobial community | No lysis |
| CSL | artificial polymicrobial community | Short lysis (1 hr Igepal) |
| CLL | artificial polymicrobial community | Long lysis (18 hr Igepal) |
| MS | mouse wound biofilm | No lysis (scoring/sonication) |
| MML | mouse wound biofilm | Mechanical disruption (TissueLyser) |
| MLL | mouse wound biofilm | Mechanical+long lysis (TissueLyser & 18 hr Igepal) |

MOLECULAR ECOLOGY
RESOURCES

**Supplementary Table 2:** Reference chromosome and plasmid names, sizes, and GenBank (NCBI) accession numbers using in mapping Hi-C reads.

| Name Abbrev. | Reference Species or Plasmid Name | Chromosome/ Plasmid Size (bp) | NCBI Accession |
|---|---|---|---|
| EC | *Escherichia coli* str. K-12 substr. MG1655, complete genome | 4,641,652 | NC_000913 |
| SA | *Staphylococcus aureus* subsp. aureus str. Newman DNA, complete genome | 2,878,897 | NC_009641 |
| PA<br>(P1 & P4) | *Pseudomonas aeruginosa* PAO1, complete genome<br>*Pseudomonas aeruginosa* UCBPP-PA14, complete genome | 6,264,404<br>6,537,648 | NC_002516<br>NC_008463 |
| pUCP18 | *Escherichia-Pseudomonas* shuttle vector with beta-lactamase (bla) and LacZ alpha peptide (lacZ alpha) genes, complete sequence | 4,557 | U07164.1 |
| pG527 | IncP-1-type plasmid, common naturally in Proteobacteria which can mobilize elements into Gram-positive bacteria (Sen et al. 2013) | 80,762 | JX469830 |

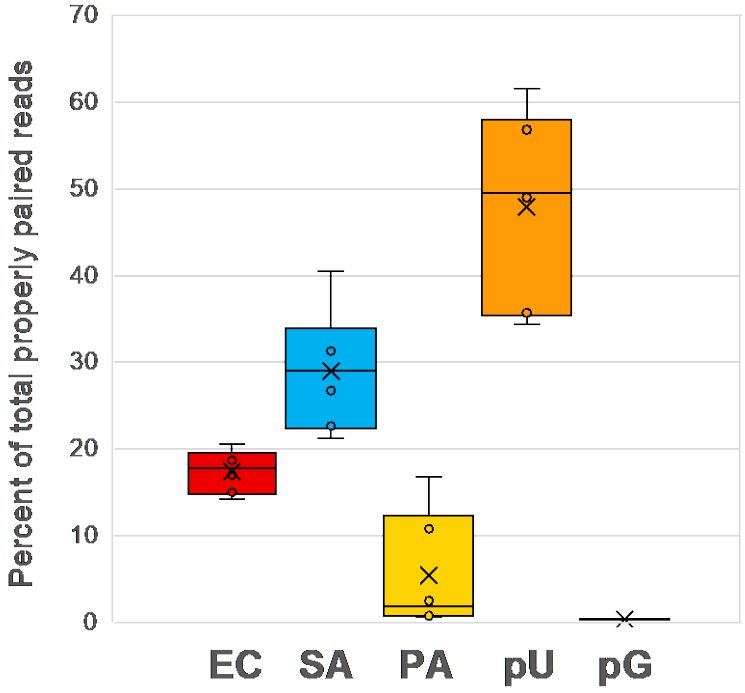
MOLECULAR ECOLOGY
RESOURCES

**Supplementary Table 3:** Number of raw Illumina reads per sample, mapped reads per sample, and reads mapped as pairs to each target microbe or plasmid with percent of total mapped reads mapping to targets, and average fold sequencing coverage per each target. CNL = cells no lysis, CSL = cells short lysis, CLL = cells long lysis, MS = mouse sonicated, MML = mouse mechanical lysis, MLL = mouse long lysis, EC = *Escherichia coli*, SA = *Staphylococcus aureus*, PA = *Pseudomonas aeruginosa*, pUCP18 and pG527 are plasmids.

| Sample Name | Raw Illumina Reads | Total Mapped (%) | Mapped to EC (%) **[coverage]** | Mapped to SA (%) **[coverage]** | Mapped to PA (%) **[coverage]** | Mapped to pUCP18 (%) **[coverage]** | Mapped to pG527 (%) **[coverage]** |
|---|---|---|---|---|---|---|---|
| CNL | 48,324,248 | 36,451,655 (75.4) | 6,278,002 (17.2) **[78.8]** | 6,910,620 (19.0) **[118.3]** | 793,306 (2.2) **[6.502]** | 18,541,852 (50.9) **[235,702]** | 104,668 (0.287) **[74.9]** |
| CSL | 131,594,986 | 116,661,926 (88.7) | 22,827,642 (19.6) **[289.4]** | 29,829,790 (25.6) **[591.7]** | 18,818,704 (16.1) **[168.6]** | 39,916,130 (34.2) **[516,374]** | 423,350 (0.363) **[306.7]** |
| CLL | 114,287,676 | 107,215,039 (93.8) | 14,650,800 (13.7) **[189.9]** | 22,203,516 (20.7) **[441.0]** | 564,600 (0.527) **[4.86]** | 60,349,246 (56.3) **[787,750]** | 296,940 (0.277) **[219.7]** |
| MS | 172,963,348 | 898,672 (0.520) | 119,766 (13.3) **[1.48]** | 251,342 (28.0) **[4.58]** | 90,918 (10.1) **[0.790]** | 290,232 (32.3) **[3,617]** | 2,062 (0.229) **[1.45]** |
| MML | 255,541,910 | 2,047,183 (0.801) | 97,524 (4.76) **[1.19]** | 163,438 (7.98) **[3.10]** | 3,788 (0.185) **[0.298]** | 255,882 (12.5) **[3,149]** | 1,658 (0.081) **[1.18]** |
| MLL | 172,963,348 | 898,672 (1.88) | 147,080 (4.72) **[1.80]** | 274,598 (8.81) **[5.20]** | 9,668 (0.310) **[0.782]** | 434,730 (13.9) **[5,413]** | 2,652 (0.085) **[1.85]** |

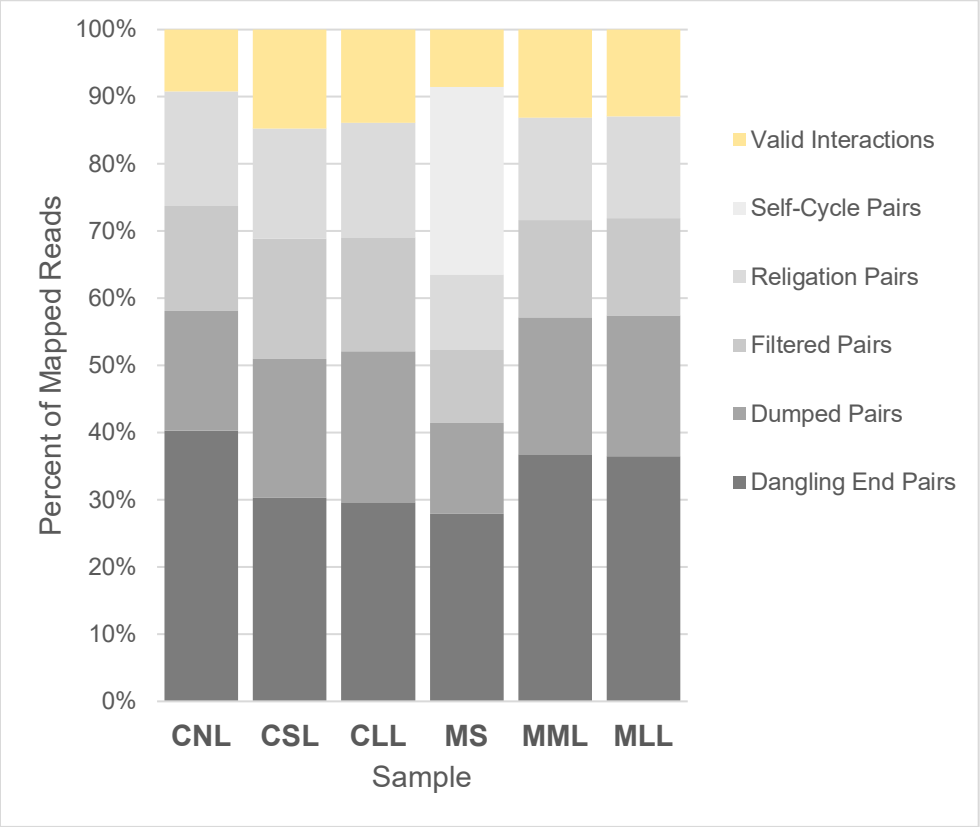
MOLECULAR ECOLOGY
RESOURCES

**Supplementary Figure 1:** Percent of reads mapped to each target microbe or plasmid, normalized across samples: CNL = cells no lysis, CSL = cells short lysis, CLL = cells long lysis, MS = mouse sonicated, MML = mouse mechanical lysis, MLL = mouse long lysis, EC = *Escherichia coli*, SA = *Staphylococcus aureus*, PA = *Pseudomonas aeruginosa*, pU = pUCP18 plasmid, pG = pG527 plasmid.



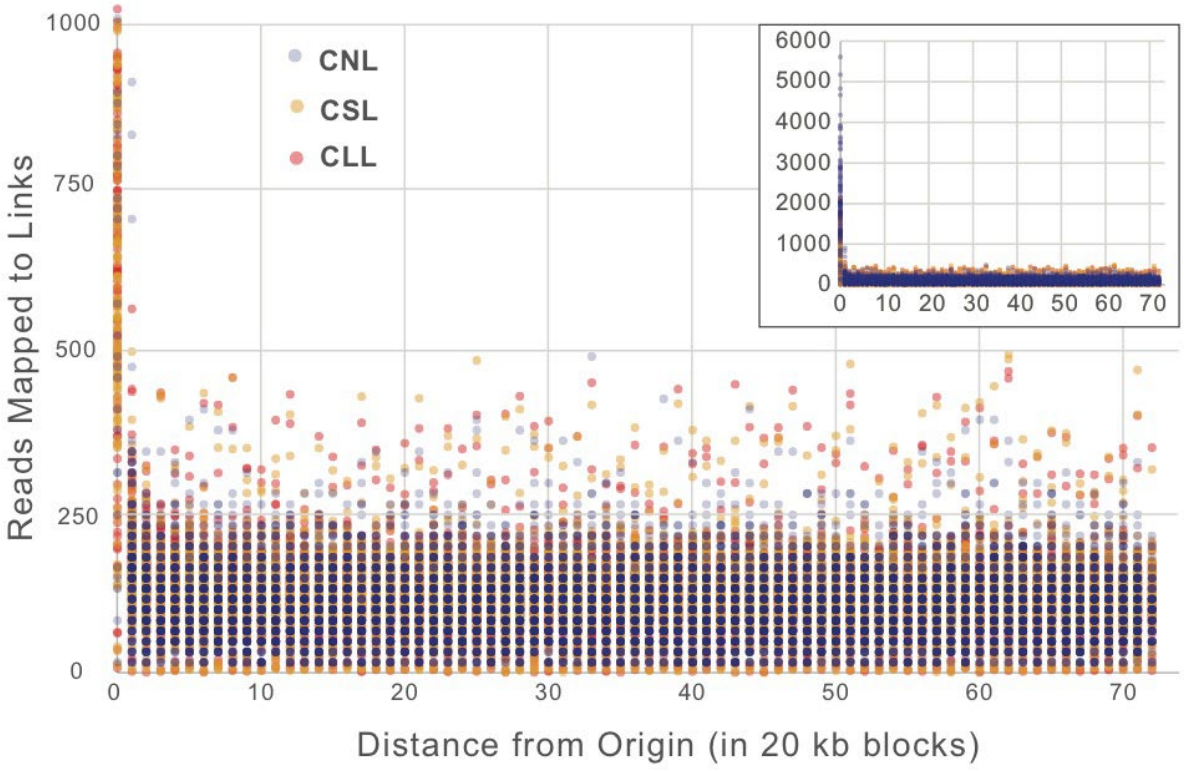
MOLECULAR ECOLOGY
RESOURCES

**Supplementary Figure 2:** Hi-C interaction types for all mapped reads from HiC-Pro. CNL = cells no lysis, CSL = cells short lysis, CLL = cells long lysis, MS = mouse sonicated, MML = mouse mechanical lysis, MLL = mouse long lysis.



MOLECULAR ECOLOGY
RESOURCES

**Supplementary Figure 3:** Read counts of Hi-C links within *Staphylococcus aureus* (i.e., self-links) measured from origin of replication, normalizing read counts between treatments. CNL = cells no lysis, CSL = cells short lysis, CLL = cells long lysis. Inset shows the whole plot, whereas the main plot is displayed with the vertical axis truncated to better-visualize read density differences for distances >20 kb.



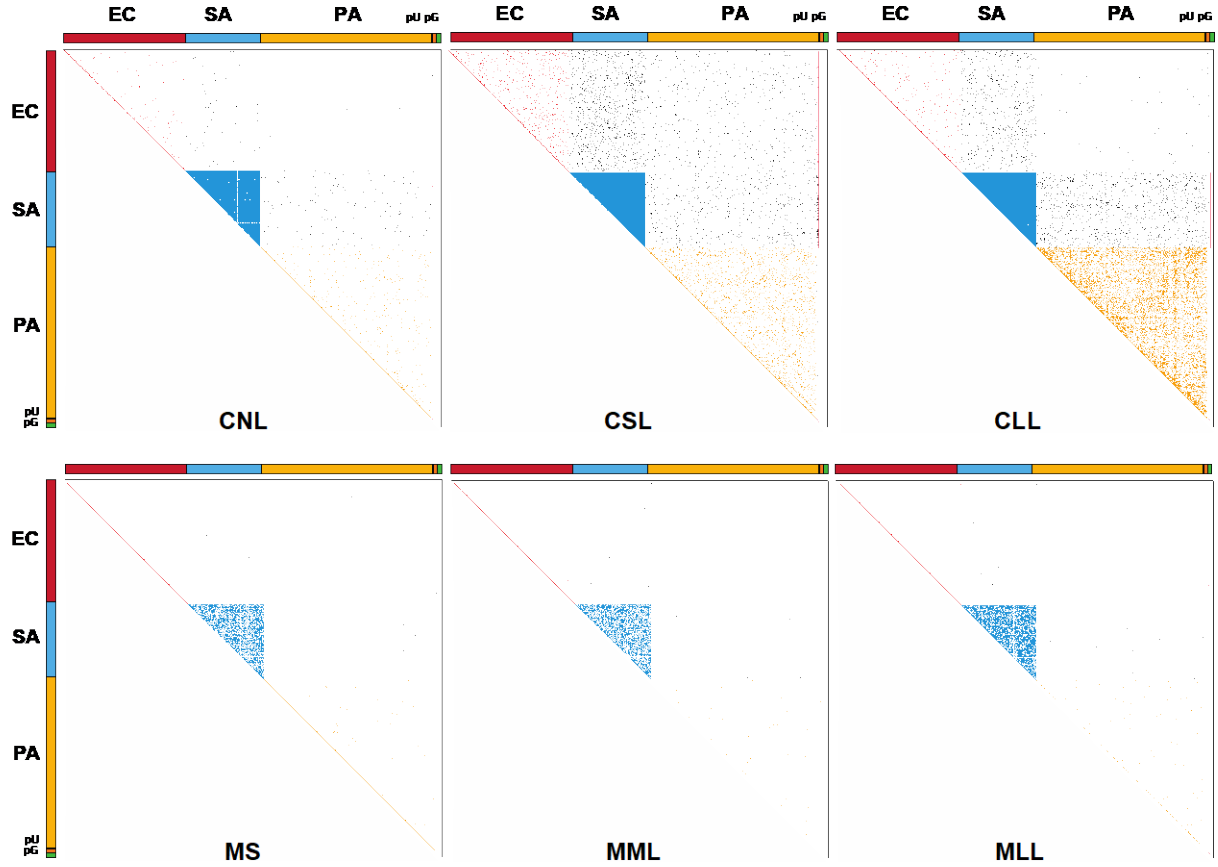
MOLECULAR ECOLOGY
RESOURCES

**Supplementary Table 4:** Statistical test results from two-proportion z-tests for Hi-C reads occurring within close (< 20 kb distance) regions within *Staphylococcus aureus*. CNL = cells no lysis, CSL = cells short lysis, CLL = cells long lysis, MS = mouse sonicated, MML = mouse mechanical lysis, MLL = mouse long lysis. Prop = overall sample proportion, SE = standard error, z = z-score.

| | CNLvCSL | CSLvCLL | CNLvCLL | MSvMML | MMLvMLL | MLLvMS |
|---|---|---|---|---|---|---|
| | | | | | | |
| prop= | 0.072003546 | 0.068739707 | 0.081195345 | 0.06629863 | 0.05643175 | 0.064928131 |
| SE= | 0.000851505 | 0.0003218 | 0.000908641 | 0.00359819 | 0.003111663 | 0.003200268 |
| z= | 122.00846 | -24.72002823 | -105.5817345 | 5.66664731 | -0.509372846 | -5.875969382 |
| P-value | 0.0000000 | 0.0000000 | 0.0000000 | 0.0000000 | 0.3052455 | 0.0000000 |

MOLECULAR ECOLOGY
RESOURCES

**Supplementary Figure 4:** Hi-C contact maps showing raw inter- and intra-species connections, drawn using HiTC. CNL = cells no lysis, CSL = cells short lysis, CLL = cells long lysis, MS = mouse sonicated, MML = mouse mechanical lysis, MLL = mouse long lysis, EC = *Escherichia coli*, SA = *Staphylococcus aureus*, PA = *Pseudomonas aeruginosa*, pU = pUCP18 plasmid, and pG = pG527 plasmid.



MOLECULAR ECOLOGY
RESOURCES

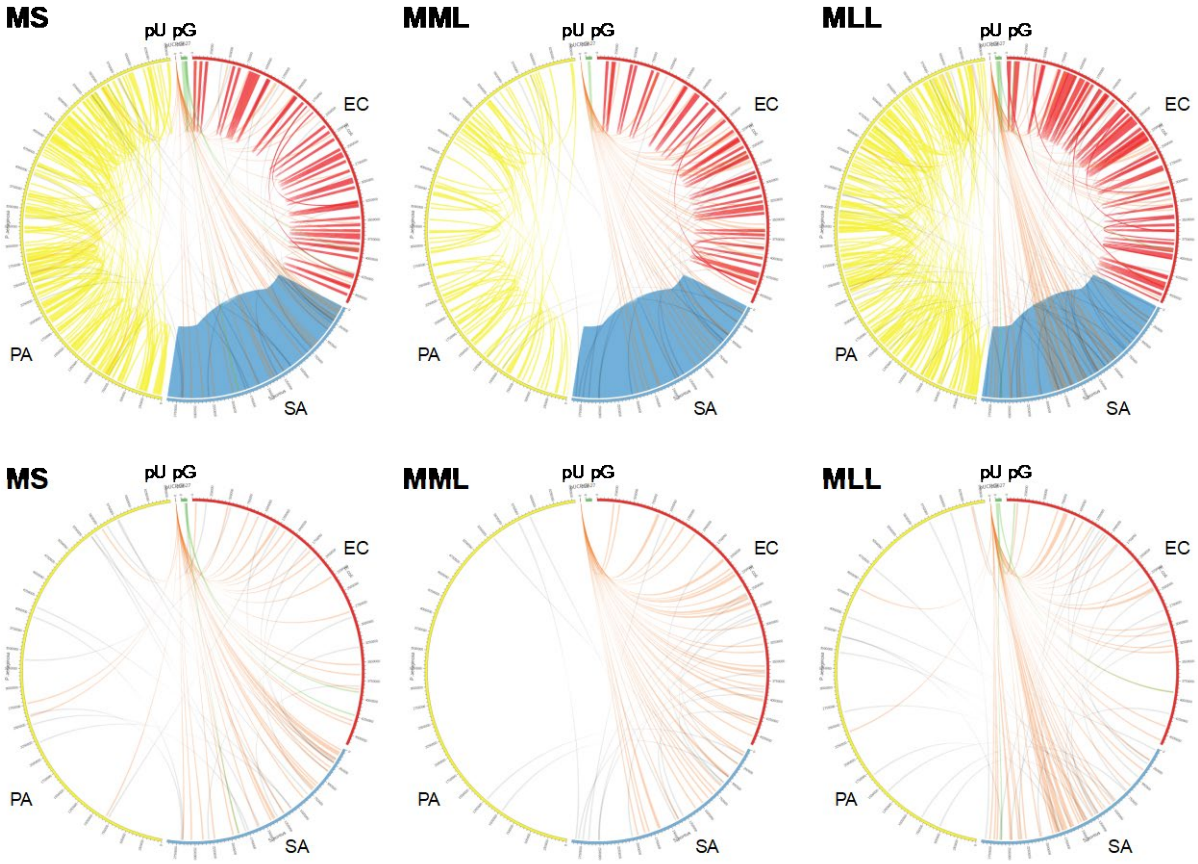
**Supplementary Table 5**: Statistical test results from two-proportion z-tests for Hi-C links and reads occurring between species or between chromosome and plasmid for cell polymicrobial 'communities' and mouse wound biofilms. CNL = cells no lysis, CSL = cells short lysis, CLL = cells long lysis, MS = mouse sonicated, MML = mouse mechanical lysis, MLL = mouse long lysis. Prop = overall sample proportion, SE = standard error, z = z-score.

| LINKS | | CNLvCSL | CSLvCLL | CNLvCLL | MMLvMS | MSvMLL | MMLvMLL |
|---|---|---|---|---|---|---|---|
| ECtoOTHER | prop= | 0.61102312 | 0.65475598 | 0.51334142 | 0.04020979 | 0.045 | 0.04682274 |
| | SE= | 0.01854397 | 0.01144785 | 0.02039217 | 0.01642815 | 0.01694223 | 0.01729702 |
| | z= | -26.270901 | 4.81755565 | 21.1853919 | 0.22995509 | -0.754959 | 0.52106943 |
| | P-value | 0.0000000 | 0.0000007 | 0.0000000 | 0.4090633 | 0.2251368 | 0.3011592 |
| ECvsPLAS | prop= | 0.17666778 | 0.16248867 | 0.2349857 | 0.08955224 | 0.07488697 | 0.09952607 |
| | SE= | 0.01528687 | 0.01330618 | 0.01874374 | 0.02325998 | 0.02113202 | 0.02380827 |
| | z= | 8.89626892 | -5.3681136 | -3.4447239 | 1.85647694 | -1.0121836 | -0.9153176 |
| | P-value | 0.0000000 | 0.0000000 | 0.0002858 | 0.0316928 | 0.1557251 | 0.1800125 |
| SAtoOTHER | prop= | 0.16768256 | 0.26371844 | 0.17076212 | 0.00335452 | 0.00412163 | 0.00436335 |
| | SE= | 0.00479496 | 0.00523362 | 0.00482311 | 0.0010868 | 0.00113558 | 0.0011829 |
| | z= | -45.464189 | -0.9128919 | 46.1893673 | 0.46355779 | -1.6235154 | 1.13267146 |
| | P-value | 0.0000000 | 0.1806497 | 0.0000000 | 0.3214823 | 0.0522396 | 0.1286761 |
| SAtoPLAS | prop= | 0.01683197 | 0.01902927 | 0.01528249 | 0.00423355 | 0.00543647 | 0.00507373 |
| | SE= | 0.00177685 | 0.00187307 | 0.00169571 | 0.00121986 | 0.00130244 | 0.00127472 |
| | z= | -4.2524199 | 1.62410826 | 2.66190574 | -0.7076156 | -1.091573 | 1.79246863 |
| | P-value | 0.0000106 | 0.0521764 | 0.0038850 | 0.2395920 | 0.1375104 | 0.0365290 |
| PAtoOTHER | prop= | 0.28528559 | 0.17593182 | 0.12868546 | 0.03797468 | 0.04500703 | 0.08544304 |
| | SE= | 0.01203269 | 0.00497219 | 0.00846903 | 0.02322738 | 0.01649346 | 0.03631613 |
| | z= | -8.901836 | 36.3051308 | -8.6672211 | 3.17899263 | -3.19779 | -0.5809277 |
| | P-value | 0.0000000 | 0.0000000 | 0.0000000 | 0.0007389 | 0.0006924 | 0.2806446 |
| PAtoPLAS | prop= | 0.04273848 | 0.02125247 | 0.01311732 | 0.00931099 | 0.01164483 | 0.0170068 |
| | SE= | 0.00600572 | 0.00214474 | 0.00316378 | 0.01216357 | 0.00190023 | 0.01753547 |
| | z= | -4.0545417 | 16.2931055 | -3.3485574 | 0.43463717 | -4.955125 | 0.23547291 |
| | P-value | 0.0000251 | 0.0000000 | 0.0004062 | 0.3319129 | 0.0000004 | 0.4069209 |
| READS | | | | | | | |
| ECtoOTHER | prop= | 0.18454069 | 0.18036558 | 0.11547213 | 0.19047619 | 0.16981132 | 0.18709677 |
| | SE= | 0.00714417 | 0.0044074 | 0.00614415 | 0.07000013 | 0.0605689 | 0.06411942 |
| | z= | -22.751932 | 16.7537685 | 14.4369787 | 0.62691292 | -0.0162129 | -0.6690951 |
| | P-value | 0.0000000 | 0.0000000 | 0.0000000 | 0.2653582 | 0.4935323 | 0.2517174 |
| ECvsPLAS | prop= | 0.29106122 | 0.29089344 | 0.31164232 | 0.35849057 | 0.23821723 | 0.35051546 |
| | SE= | 0.00714579 | 0.004783 | 0.0075635 | 0.07618486 | 0.06379124 | 0.06913578 |
| | z= | 7.35328577 | -4.8387051 | -3.8872897 | 1.95014374 | -0.1710124 | -1.991188 |
| | P-value | 0.0000000 | 0.0000007 | 0.0000507 | 0.0255795 | 0.4321070 | 0.0232301 |
| SAtoOTHER | prop= | 0.00355569 | 0.00381789 | 0.00463931 | 0.00233617 | 0.00249517 | 0.00272016 |
| | SE= | 0.00023832 | 8.4398E-05 | 0.00027432 | 0.00074787 | 0.00069642 | 0.00074439 |
| | z= | 17.2520165 | -12.933004 | -11.008855 | 0.72644301 | -1.0193662 | 0.2238323 |
| | P-value | 0.0000000 | 0.0000000 | 0.0000000 | 0.2337836 | 0.1540146 | 0.4114439 |
| SAtoPLAS | prop= | 0.00489082 | 0.00557248 | 0.00661458 | 0.00323179 | 0.00380836 | 0.003543 |
| | SE= | 0.0002794 | 0.00010178 | 0.00032732 | 0.00087886 | 0.00085922 | 0.00084894 |
| | z= | 6.89141075 | -17.810124 | -0.3446286 | -0.7981231 | -0.4718005 | 1.30376921 |
| | P-value | 0.0000000 | 0.0000000 | 0.3651868 | 0.2123995 | 0.3185346 | 0.0961561 |
| PAtoOTHER | prop= | 0.135237 | 0.10910996 | 0.10087965 | 0.09130435 | 0.09815951 | 0.11946903 |
| | SE= | 0.0087842 | 0.00283398 | 0.00765951 | 0.04218133 | 0.03295983 | 0.04766335 |
| | z= | 9.54093336 | 12.2312146 | -15.467366 | 1.04996806 | -1.1900712 | -0.1062557 |
| | P-value | 0.0000000 | 0.0000000 | 0.0000000 | 0.1468664 | 0.1170092 | 0.4576898 |
| PAtoPLAS | prop= | 0.0339789 | 0.02295575 | 0.01547619 | 0.02336449 | 0.02649007 | 0.0245098 |
| | SE= | 0.0051509 | 0.0014278 | 0.00347639 | 0.02323138 | 0.00224014 | 0.02399971 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | z= | -1.0965889 | 13.6829188 | -3.9949629 | -0.3615647 | -0.7839837 | 0.42316678 |
| | P-value | 0.1364106 | 0.0000000 | 0.0000324 | 0.3588387 | 0.2165248 | 0.3360868 |

MOLECULAR ECOLOGY
RESOURCES

**Supplementary Figure 5**: Hi-C link positions within and between cells and plasmids for mouse wound biofilm communities, drawn in Circos. Upper row shows plots for all links (not normalized); lower row shows only interspecies or chromosome-to-plasmid links, normalized to the same number of links between samples. MS = mouse sonicated, MML = mouse mechanical lysis, MLL = mouse long lysis, EC = *Escherichia coli*, SA = *Staphylococcus aureus*, PA = *Pseudomonas aeruginosa*, pU = pUCP18 plasmid, pG = pG527 plasmid.



MOLECULAR ECOLOGY
RESOURCES

**Supplementary Materials:** Detailed Lysis-Hi-C Protocol

*Chemical Lysis*

1. To chemically lyse cells adhered on side of 1.5 mL tube or biofilms placed in 1.5 mL tube, soak surface with Igepal lysis buffer (5 mM Tris-HCl, 10 mM NaCl, and 0.02% Igepal CA-630 detergent) (e.g., use 40 µl of Igepal buffer for ~1 $cm^2$ surface area). Leave buffer on samples for designated lysis time(s) (e.g., 1 hr, 18 hr).

*Formaldehyde Crosslinking & Quenching*

2. Add sufficient 1% formaldehyde to each sample surface to fully soak tissue (e.g., 40 µl for ~1 $cm^2$ surface area) and let permeate for 20 min at room temperature.
3. Add sufficient 2.5 M glycine to soak each sample (e.g., slightly more volume than used in formaldehyde step, or 50 µl for ~1 $cm^2$ surface area) and place on ice for 10 minutes to quench crosslinking.

*Precipitate & Pellet Cells and eDNA*

4. Add 2.5 x volume of 100% ethanol (molecular grade ethanol should be used for all steps in this protocol) and 1/10 volume of 3 M sodium acetate (made in sequencing grade water; all solutions should use sequencing grade DNase-free water) to each tube (e.g., 500 µl per 1 $cm^2$ x 1 mm sample) and place at -20°C at least 3 hr to precipitate free eDNA.
5. After DNA precipitation, centrifuge for 30 min at 4°C and 14,000 g. Remove supernatant to wash off residual buffers and salts. Then wash pellet 3 times by adding 1 mL of 80 % ethanol, centrifuging pellet at 4°C and 14,000 g for 5 min, then removing and discarding the supernatant.

*Lyse Remaining Cells while Retaining Crosslinked eDNA*

6. Resuspend the pellet in 500 µl TBS-T and transfer to a 2 mL bead beating tube (Qiagen Pathogen Lysis S).
7. Add 1.3 µl of 20% SDS and vortex for 5 min at maximum speed (VWR Vortex Genie). (Proteinase K digestion is omitted as it may affect proteins bound with DNA in crosslinks).
8. Briefly centrifuge at maximum g then transfer lysate to a new tube.
9. Precipitate with 2.5x vol of 100% ethanol and 1/10 vol of 3M sodium acetate and place at -20°C at least 3 hr to precipitate free eDNA.
10. Centrifuge at 4°C and 17,000 g for 4 min. Discard supernatant to remove buffers and salts.

*Modified Dovetail™ Hi-C Library Kit Protocol:*

*Restriction Enzyme Digest*

11. Add to pellet in sample tube: 50 µl Restriction Digest Buffer, 1 µl Restriction Digest Enzyme Mix, 1 µl 50% TritonX-100

12. Vortex briefly, quick spin, and incubate for 1 hr at 37°C in an agitating thermal mixer.

*End Fill-In*

13. Precipitate sample (to preserve eDNA) with 2.5x vol of 100% ethanol and 1/10 vol of 3M sodium acetate and place at -20°C at least 3 hrs to precipitate free eDNA.
14. Centrifuge at 4°C and 17,000 g for 4 min. Discard supernatant to remove buffers and salts.
15. Add to sample tube: 50 µl End Fill-in Buffer, 1 µl End Fill-in Enzyme Mix, 1 µl 50% TritonX-100.
16. Fully resuspend the pellet and incubate for 30 min at 25°C in an agitating thermal mixer.

*Intra-Crosslink Cluster DNA End Ligation*

17. Precipitate sample (to preserve eDNA) with 2.5x vol of 100% ethanol and 1/10 vol of 3M sodium acetate and place at -20°C at least 3 hr to precipitate free eDNA.
18. Centrifuge at 4°C and 17,000 g for 4 min. Discard supernatant to remove buffers and salts.
19. Add to sample tube: 250 µl Intra-Aggregate Ligation Buffer, 1 µl Intra-Aggregate Ligation Enzyme Mix.
20. Fully resuspend the pellet and incubate for 1 hr at 16°C in an agitating thermal mixer.

*Crosslink Reversal*

21. Precipitate sample (to preserve eDNA) with 2.5x vol of 100% ethanol and 1/10 vol of 3M sodium acetate and place at -20°C for at least 3 hr to precipitate free eDNA.
22. Centrifuge at 4°C and 17,000 g for 4 min. Discard supernatant to remove buffers and salts.
23. Add to sample tube: 50 µl Crosslink Reversal Buffer, 1 µl Proteinase K.
24. Fully resuspend the pellet and incubate for 15 min at 55°C, followed by 45 min at 68°C, in an agitating thermal mixer.

*DNA Purification*

25. Clean DNA using the AMPure XP bead protocol following the manufacturer's directions, with a ratio of 1.8X volume beads to 1 volume sample. Ethanol washes should be performed with fresh 80% ethanol. Elute final DNA in 50 µl of sequencing grade water.
26. Quantify DNA using the Nanodrop or Qubit to be sure there is >100ng.

*Fragmentation*

27. Use a suitable sonicator as per manufacturer's directions. E.g., for the Diagenode Bioruptor® Pico Ultrasonicator, cool to 4˚C and transfer 200-1000 ng of sample DNA to a 0.1 ml Bioruptor tube.
28. Use TE Buffer pH 8.0 to bring the total volume in each Bioruptor tube to 50 µl.
29. Cool DNA in Bioruptor tubes for 10 min on ice.
30. Fragment 6 cycles of 30 sec ON/30 sec OFF.

31. Check the size distribution of 2 µl of the fragmented samples on Agilent TapeStation, aiming for fragments 250 - 450 bp. Re-fragment if significantly larger than this size samples for 3 more cycles of 30 sec ON/30 sec OFF.

*End Repair*

32. Place in a 0.2 ml PCR tube: 48 µl Fragmented Sample, 7 µl End Repair Buffer, 3 µl End Repair Enzyme Mix, 0.5 µl 250 mM DTT.
33. Briefly vortex and quick spin sample tube and incubate for 30 min at 20˚C, followed by 30 min at 65˚C in a thermal cycler. Hold at 12˚C.

*Adapter Ligation & USER Digest*

34. Add to the 0.2 ml tube containing 58.5 µl of end-repaired sample: 2.5 µl Adapters for Illumina 30 µl Ligation Enzyme Mix 1 µl Ligation Enhancer
35. Vortex and quick spin sample tube and incubate for 15 min at 20˚C. Hold at 12˚C.
36. Add 3 µl of USER Enzyme Mix to sample and pipet mix.
37. Incubate for 15 min at 37˚C. Hold at 12˚C.

*DNA Purification*

38. Add 100 µl resuspended AMPure XP Beads to the sample, and follow manufacturer's direction, eluting final DNA with 102 µl TE Buffer pH 8.0 in a new 1.5 ml tube.

*Ligation Capture*

39. For each sample, add 25 µl resuspended Streptavidin Beads to a new and empty 1.5 ml tube.
40. Place tube containing Streptavidin Beads on the magnet for 2-5 min. Pipet off and discard supernatant.
41. Wash the Streptavidin Beads twice with 200 µl TWB.
42. Add 100 µl 2X NTB to the Streptavidin Beads.
43. Transfer 100 µl of the sample (from step 38 above) to the tube containing the washed Streptavidin Beads and 100 µl of 2X NTB. This is now your sample tube.
44. Fully resuspend the Streptavidin Beads in the sample tube and incubate for 30 min at 25˚C in an agitating thermal mixer.

*Wash Sample on Streptavidin Beads*

45. Quick spin tube and place on magnet for 2-5 min. Pipet off and discard supernatant.
46. Wash the Streptavidin Beads once with 200 µl LWB.
47. Wash the Streptavidin Beads twice with 200 µl NWB.
48. Wash the Streptavidin Beads twice with 200 µl Wash Buffer.

*Index PCR*

49. After the last wash has been removed, add to the sample tube: 15 µl DNase and RNase free H2O, 25 µl HotStart PCR Ready Mix, 5 µl Universal PCR Primer, 5 µl Index Primer (Unique to each sample)

50. Transfer sample, including Streptavidin Beads, to a 0.2 ml PCR tube. Fully resuspend the beads.
51. Amplify sample in a thermal cycler as follows: 3 min at 98˚C, 11 cycles of : 20 sec at 98˚C, 30 sec at 65˚C, 30 sec at 72˚C, then 1 min at 72˚C. Hold at 12˚C.

*DNA Purification on AMPure XP Beads*

52. Place the 50 µl sample onto the magnetic stand for ~5 min, then move and keep the supernatant in a new tube. Discard the beads.
53. Add 100 µl resuspended AMPure XP Beads to the sample tube, mix well, and complete the AMPure purification following the manufacturer's protocol, eluting the final sample in 45 µl TE Buffer pH 8.0. This is the final lysis-Hi-C sequencing library.

*Check Final Library on the Agilent Tapestation*

54. Quantify 1 µl of the sequencing library to check that it is between 350-850 base pairs.