

APPENDICES

APPENDIX A

SHARED BIOSYNTHETIC GENE CLUSTERS PRESENCE IN ALL STRAINS

Table S1 BGCs annotated from antiSMASH found in wild-type and all evolved strains.

Type	Average Size	Most similar known cluster
NI-siderophore	31,146	
NRPS	43,980	detoxin S1*
ectoine	10,404	ectoine ⁺
NI-siderophore	25,270	desferrioxamin B/desferrioxamine E ⁺
NI-siderophore	29,799	
terpene	21,031	
terpene	18,379	hopene [#]
T3PKS	35,833	flaviolin/1,3,6,8-tetrahydroxynaphthalene ⁺
NRP-metallophore,NRPS	63,409	coelibactin ⁺
lanthipeptide-class-i	24,786	
hgLE-KS,T1PKS	32,896	
T1PKS,lanthipeptide-class-v	53,706	cacaoidin*
T1PKS,butyrolactone	31,815	4-hexadecanoyl-3-hydroxy-2-(hydroxymethyl)-2H-furan-5-one [#]
T2PKS,azole-containing-RiPP	50,802	cinerubin B ⁺
lassopeptide	15,874	
NRPS,NAPAA	34,095	
T2PKS	15,603	spore pigment [#]
NI-siderophore	25,572	
terpene	6,492	geosmin ⁺

* low, # medium, + high similarity confidence

APPENDIX B

ADDITIONAL BGC-RELATED ORTHOLOG CLUSTERS

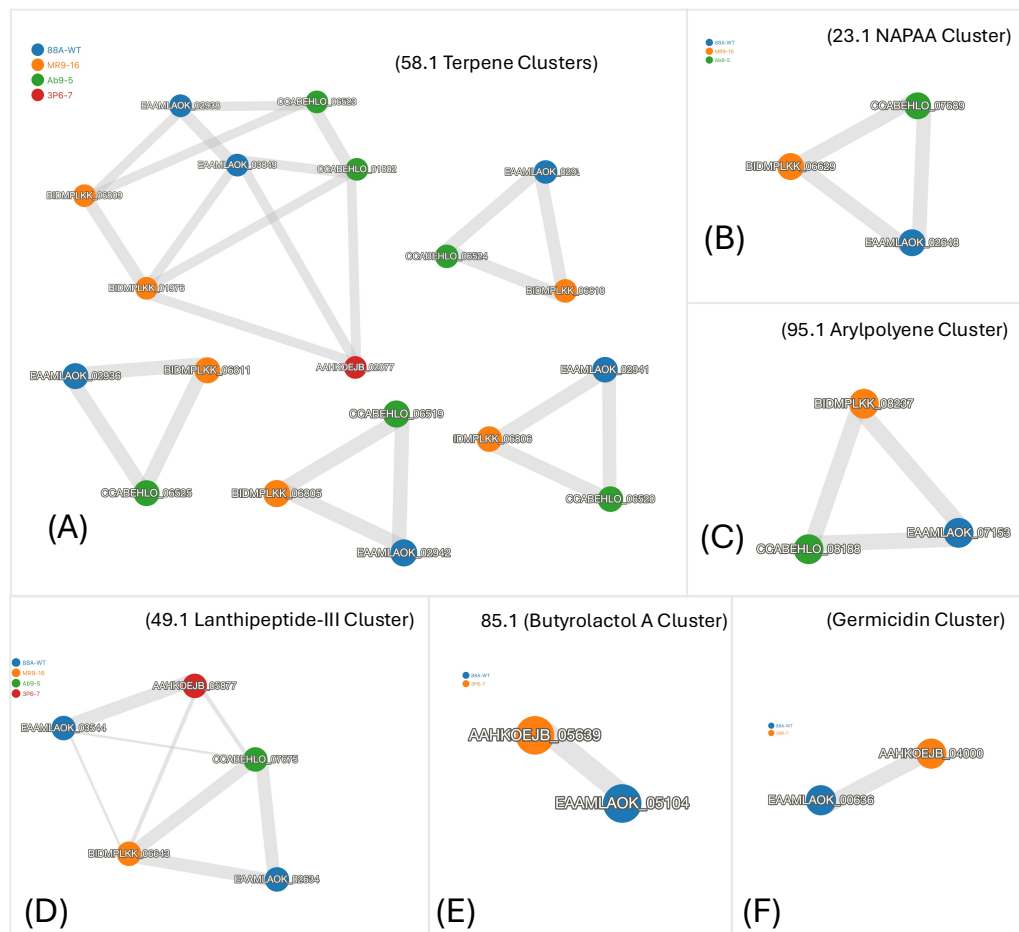


Figure S1 Orthologous cluster visualization of biosynthetic gene clusters.

(A, B, and C) shared between wild-type and non-producing strain (SSUT88A^{3P6-7}). (A) Terpene clusters, (B) Non-alpha poly-amino acids like e-Polylysine cluster, and (C) Arylpolyene cluster.

(D, E, and F) shared between wild-type and non-producing strain (SSUT88A^{3P6-7}). (D) Lanthipeptide class III cluster and (E) Butyrolactol A cluster, and (F) Germicidin cluster.

APPENDIX C

CRITERIA FOR HIGH-CONFIDENCE MUTATION SELECTION

To filter biologically meaningful deletions from initial Breseq predictions, multiple exclusion criteria were applied. The following figures illustrate common reasons for excluding contigs from downstream interpretation. These examples collectively informed the final selection of representative deletions used in the main analysis.



Figure S2 Breseq-predicted mutations with no biological relevancy, which were deprioritized from analysis. In this case, mutations at contigs 101, position 25,213 that cause base substitution from C to T were present in all strains and were categorized as biologically irrelevant.

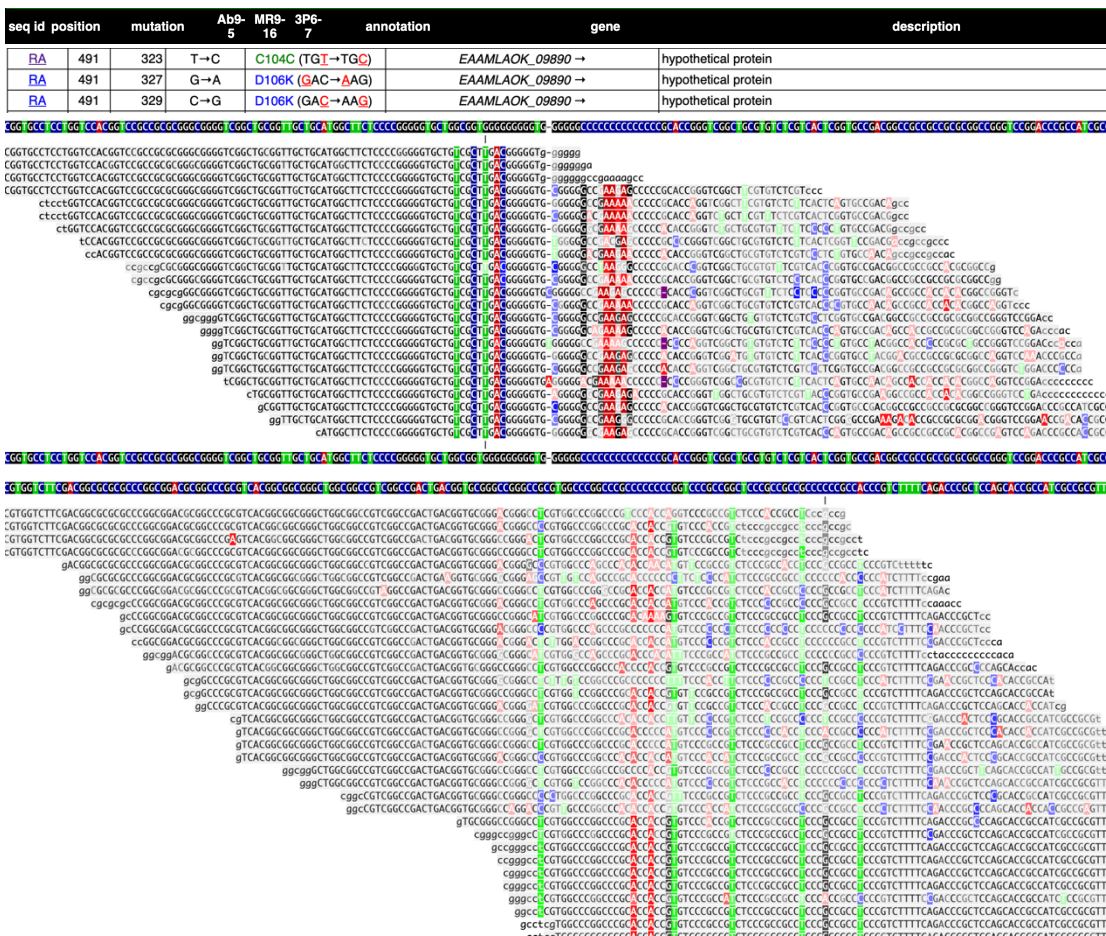


Figure S4 Example of poor overall mapping clarity. These contigs exhibit inconsistent read alignment, with frequent mismatches. Such noisy mapping profiles reduce confidence in structural variant calls and make it difficult to distinguish true deletions from artifacts. Contigs with this alignment pattern were excluded from downstream interpretation.

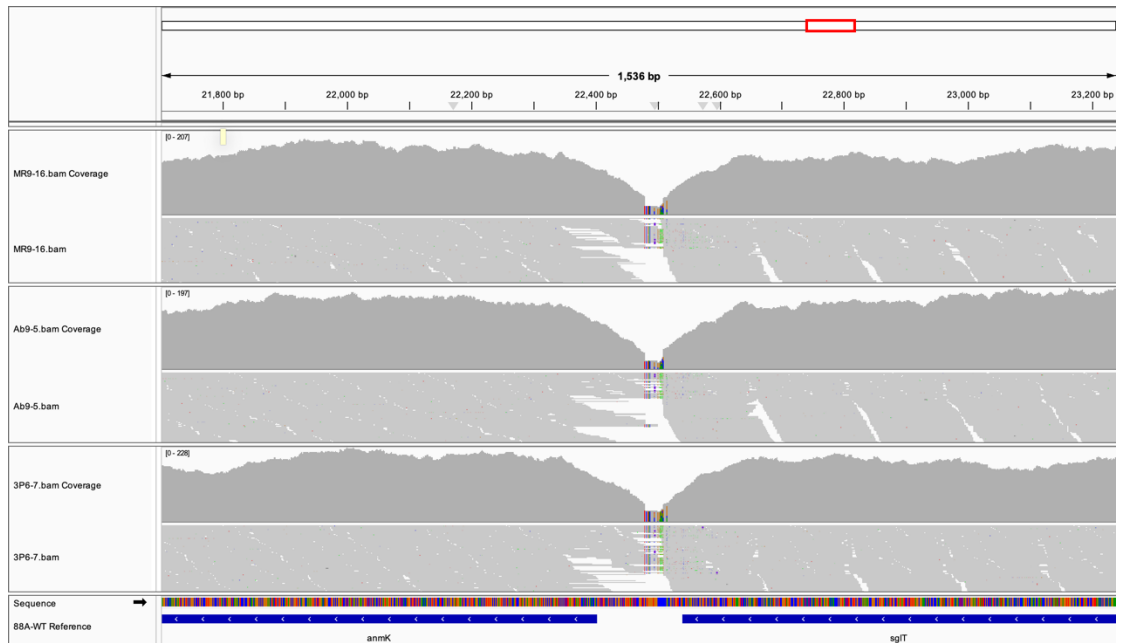


Figure S5 Outlying coverage drops restricted only to the mutation site. Unlike expected clean dropouts across the full contig, this pattern may represent mapping artifacts or sequencing bias rather than true biological deletion.