Inventory of Supplemental Information

1) Table S1, related to Figure 2A. Sequences of the wild-type and evolved ligase enzymes. The secondary structure of these molecules is depicted in Figure 2A, highlighting mutations that occur in representative clone #15.

2) Figure S1, related to Figure 3A. Amplification profiles of the wild-type and each of the three classes of evolved enzymes. Figure 3A presents similar data, focusing on representative clone #15.

3) Figure S2, related to Figure 3B. Activity of the wild-type and evolved enzymes in the presence of various concentrations of Mg^{2+} and various aminoglycosides. Figure 3B presents analogous data for various concentrations of neomycin.

Supplemental Information

Table S1, related to Figure 2A. Sequence alignment of the wild-type ligase enzyme and 20 cloned individuals obtained following compartmentalized evolution in the presence of neomycin.

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                10        20        30        40        50        60        70
  5′ .........|.........|.........|.........|.........|.........|.........| 3′
wt   GGAAGAACACACUAUAGUGACCCAGGAAAAGACAAAUCUGCCCUUAGAGCUUGAGAACAUCUUCGGAUGC

 1*  A......U...U.....................................................
 2   A......U...U.....................................................
 3   A......U...U.....................................................
 4   A..........U.....................................................
 5   A......U...U.....................................A...............
 6   A......U...U.....................................................

 7*  A......U...U.....................................................
 8   A......U...U.............A.......................................
 9   A......U...U.....................................................
10   A..........U.....................................................
11   A..........U.....................................................
12   A..........U.............................A.......................
13   A..........U.............................A...A...................
14   A......U...U.....................................................A.....

15*  A..........U.....................................A...............
16   A......U...U.....................................................A.......
17   A......U...U.............A.......................................
18   A..........U.............................A.......................
19   A..........U.....................................................
20   A..........U.....................................A.................A.......


                80        90       100       110       120       130
  5′ .........|.........|.........|.........|.........|.........|......... 3′
wt   ACGGGAGGCAGCUCGCGAUGGAAGUAACGGACCCAGCGUUCUCAACAGUGUUCACAGAACCUUAAUGC

 1*  ..................................................A.................G
 2   ..................................................A.................G
 3   ..................................................A..............G..G
 4   ..................................................A.....C........G..G
 5   ..A.............................A.............A.................G
 6   ............................A...................A.................G

 7*  ........................GCC.G.................A.................G
 8   ........................GCC.G.................A.................G
 9   ........................GCC.G.................A......--.........G
10   ........................GCC.G.....................................G
11   ...A..................A...GCC.G.....................................G
12   .......U..................UGCC.G.....................A.U..........G
13   .......U..................GCC.G...A.............A.................G
14   ........................G.CA.U...A.............A.................G

15*  ........U..................GCC.A.U.U..................A............G
16   ........................GCC.A.U.U.............A.................G
17   ........U..............A...GCC.A.U.U.............A....A..........C.G
18   ........U..................GCC.A.U.U..................A............G
19   ........U..................GCC.A.U.U..................A...-........G
20   ........U...........A......GCC.A.U.U..................A...U........G
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Residues identical to the wild-type sequence are indicated by a dot; deletions are indicated by a dash. Asterisks denote representative clones from each sequence class that were chosen for more detailed analysis. These classes were distinguished by the presence of zero (clones 1–6), four (clones 7–14), or six (clones 15-20) mutations within a portion of the P7 stem-loop (underlined for the wild-type). Clone 15 is the variant that was chosen for formal kinetic studies.

Figure S1, related to Figure 3A. Amplification profiles comparing the wild-type enzyme and representatives from each of the three evolved sequence classes, measured in either (A) the absence or (B) the presence of 100 μM neomycin. The yield of full-length cDNA was determined at various times for the wild-type (squares), clone 1 (diamonds), clone 7 (triangles), and clone 15 (circles) enzymes (see Table S1). Reaction conditions were as described in the legend to Figure 3.

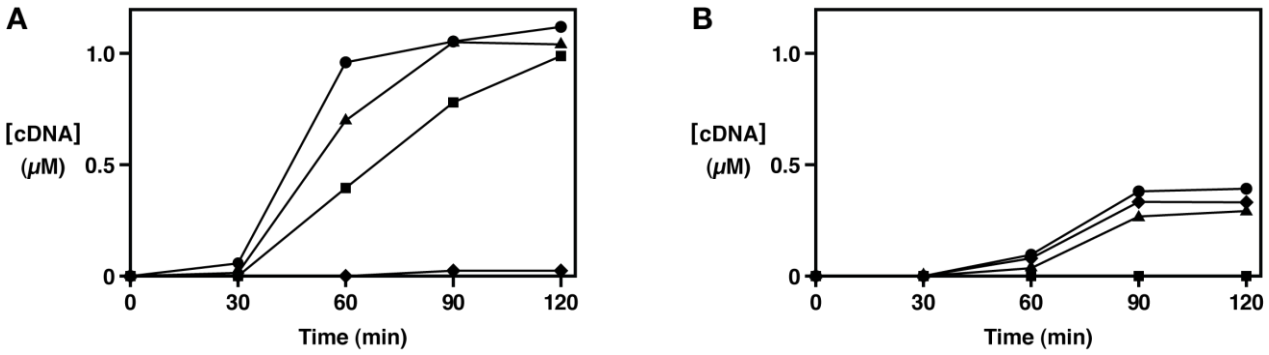
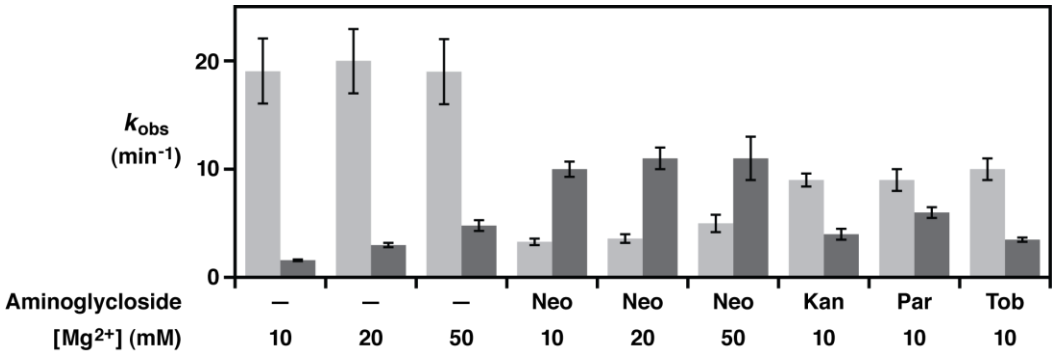


Figure S2, related to Figure 3B. Catalytic activity of the wild-type (light gray) and evolved (dark gray) enzymes in the presence of various concentrations of Mg^{2+} and in either the absence or presence of various aminoglycosides. Reaction conditions: 30 nM enzyme, 2.5 μM substrate, 0 or 100 μM aminoglycoside (Neo, neomycin; Kan, kanamycin; Par, paromomycin; Tob, tobramycin), 10–50 mM $MgCl_2$, and 50 mM KCl at pH 7.5 and 37 °C.


20
10
0
k_{obs}
(min^{-1})
Aminoglycoside
[Mg^{2+}] (mM)
—
10
—
20
—
50
Neo
10
Neo
20
Neo
50
Kan
10
Par
10
Tob
10