Directed Evolution of Recombinase Specificity by Split Gene Reassembly

Charles A. Gersbach, Thomas Gaj, Russell M. Gordley, and Carlos F. Barbas, III*
The Skaggs Institute for Chemical Biology and the Departments of Chemistry and Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California, 92037

Supplementary Material

Table S1: Primer Sequences

5' XbaI pBla:	5'-CACCACTCTAGAACCCCTATTTGTTTATTTTTC-3'
3' KpnI AmpR:	5'-CACCACGGTACCTTACCAATGCTTAATCAGTG-3'
5' AmpR mid-Spe-Hind:	5'-ACTAGTCACCACCACAAGCTTACTTACTCTAGCTTCCCGGCAAC-3'
3' AmpR mid-Spe-Hind:	5'-TAAGCTTGTGGTGGTGACTAGTTAGTTCGCCAGTTAATAGTTTG-3'
5'-XbaI C4-20G-GFP:	5'-TTAATTAAGAGTCTAGAGCGGGAGGCGTGTCCAAAACCATGGTTTACAGCACGCCTCCCGCAGATCTAGGAGGAATTTAAAATGAG-3'
3'-HindIII C4-20G-GFP:	5'-ACTGACCTAGAGAAGCTTGCGGGAGGCGTGCTGTAAACCATGGTTTTGGACACGCCTCCCGCCTGCAGTTATTTGTACAGTTCATC-3'
3'-HindIII C4-20T-GFP:	5'-ACTGACCTAGAGAAGCTTGCGGGAGGCGTGCGAAATATTATAAATTATCACACGCCTCCCGCCTGCAGTTATTTGTACAGTTCATC-3'
5'-XbaI C4-20E-GFP:	5'-TTAATTAAGAGTCTAGAGCGGGAGGCGTGGTGAGCACCATGGAGCTGGCCACGCCTCCCGCAGATCTAGGAGGAATTTAAAATGAG-3'
3'-HindIII C4-20E-GFP:	5'-ACTGACCTAGAGAAGCTTGCGGGAGGCGTGGCCAGCTCCATGGTGCTCACCACGCCTCCCGCCTGCAGTTATTTGTACAGTTCATC-3'
ResGin-cat fo1 prim1:	5'-ACCACGATGACTGACCTAGAGCTCAGGAGGAATTTAAAATGCTGATTGGCTATGTAAGGG-3'
3'-ZF SS-AXEX prim2:	5'-CAGTATCACCTCGAGGAATTCTCTAGAGGCGCGCCTTATTGGCCGGCCTGGCCACTAG-3'

Supporting Information Figure S1: Sequences of Evolved Gin Variants on GE Substrate

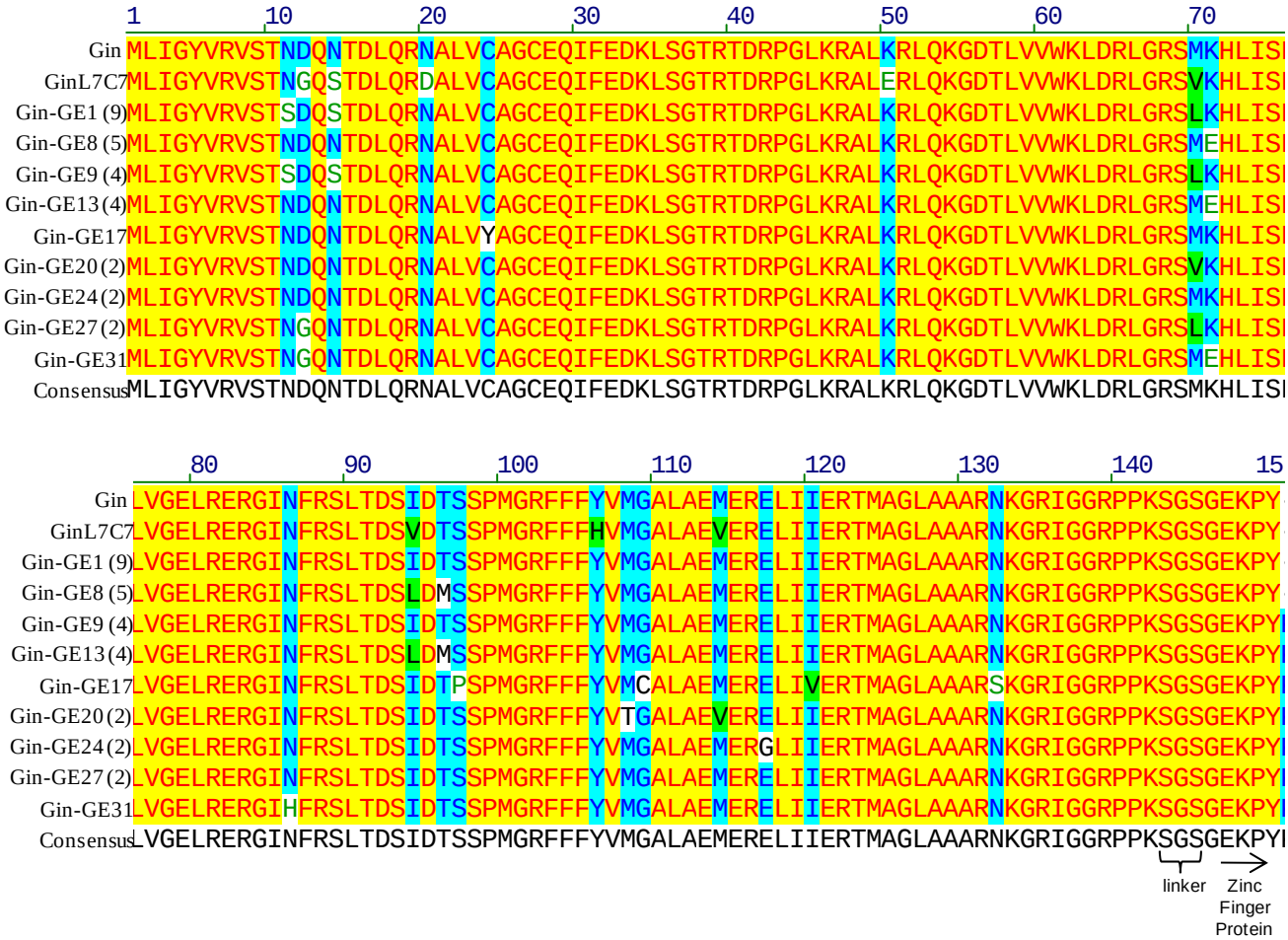

Figure S1. Individual sequences of isolated variants from round 3 of evolutions of the Gin catalytic domain on the GE substrate, as shown in Figure 4. The number in parentheses indicates the number of sequenced colonies in which that particular sequence was repeated. The activities of Gin-GE1 and Gin-GE8 were tested independently, as shown in Figure 5A. Several mutations were repeated and/or similar in chemistry and are highlighted in Figure 6. Additionally, many selected residues showed chemical homology to mutations selected in previous evolutions, such as those in the GinL7C7 variant (D12G, N14S, M70V/L, I94V/L, M114V) (10).

Supporting Information Figure S2: Sequences of Evolved GinL7C7 Variants on EE Substrate

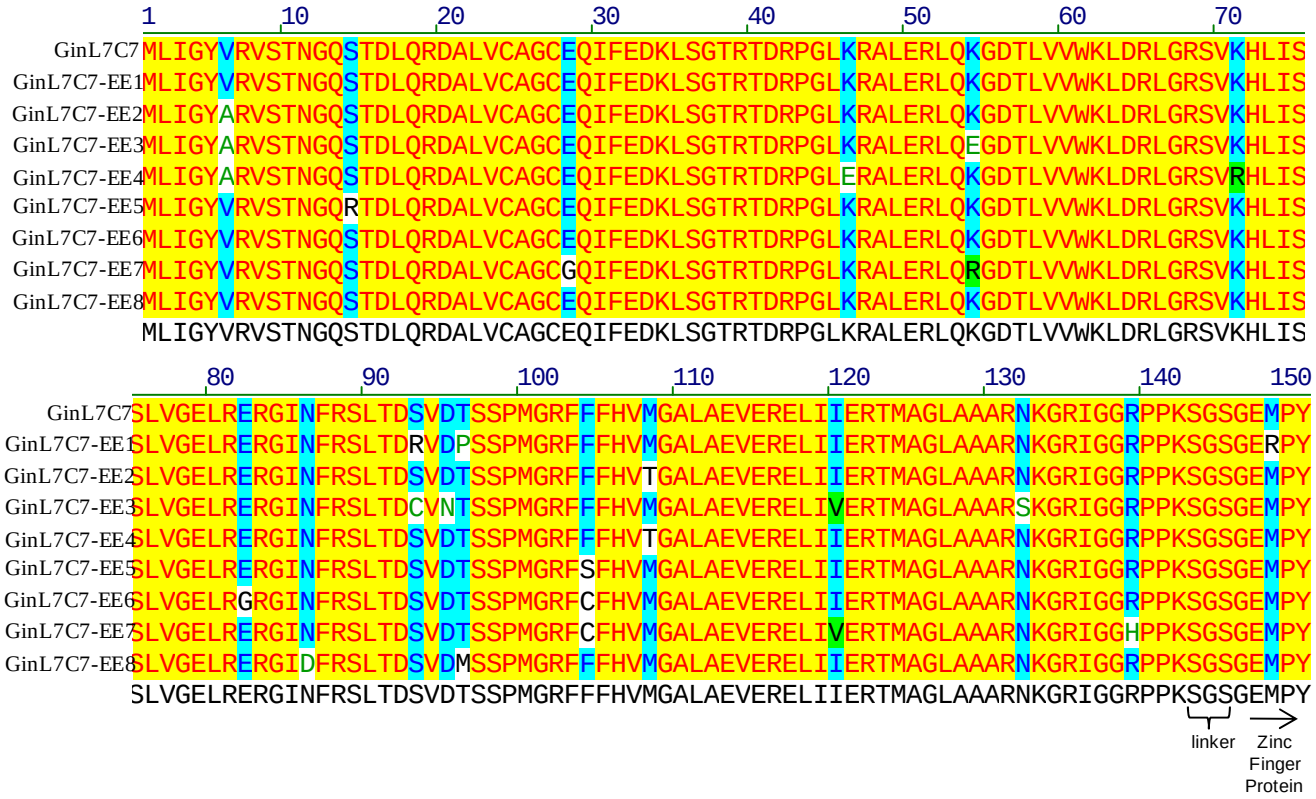

Figure S2. Individual sequences of isolated variants from round 3 of evolutions of the GinL7C7 catalytic domains on the EE substrate, as shown in Figure 4. All sequences were unique among the colonies that were sequenced. The activities of GinL7C7-EE2 and GinL7C7-EE3 were tested independently, as shown in Figure 5B. Several mutations were repeated and/or similar in chemistry and are highlighted in Figure 6.

Table S2: Summary of Mutations Conserved Among the Serine Recombinase Family

	Gin-GE total sequences	Gin-GE unique sequences	GinL7C7	GinL7C7-EE unique sequences
V6A	---	---	---	3/8
N11S	13/30	2/9	---	---
N14S	13/30	2/9	X	NA
N20D	---	---	X	NA
K50E	---	---	X	NA
M70L/V	17/30	4/9	X	NA
N86H	1/30	1/9	---	1/8 (N86D)
D95N	---	---	---	1/8
M114V	2/30	1/9	X	NA
I120V	1/30	1/9	---	2/8