

Table 3. Libraries and screening conditions

Library	1 <sup>*</sup>	2	3	4	5	6	7	8	9 <sup>†</sup>	10	11
Library size <sup>‡</sup>	$1 \times 10^7$	$1 \times 10^7$	$5 \times 10^6$	$2 \times 10^7$	$6 \times 10^7$	$5 \times 10^7$	$6 \times 10^7$	$5 \times 10^7$	$1 \times 10^8$	$1 \times 10^8$	$1 \times 10^8$
Leu ( $\mu\text{M}$ ) <sup>§</sup>	45	30, 15 <sup>¶</sup>	7.5, 0 <sup>¶</sup>	0	0	0	0	0	0	0	0
No. of sorting	2	2	2	2	2	2	2	3 <sup>  </sup>	2	2	3 <sup>  </sup>

<sup>\*</sup>Measured mutation rates in libraries 1 and 9 were 2.4 and 1.9 mutations per gene, respectively.

<sup>†</sup>Clone 8.3.3 was used for library generation.

<sup>‡</sup>Library size was determined by counting colonies after spreading on agar plates.

<sup>§</sup>Leu concentration in medium during protein expression.

<sup>¶</sup>Leu concentration for the first and second sortings, respectively.

<sup>||</sup>After sorting twice, the genes for the GFP mutants were transferred into fresh pQE-80L plasmids.