

**Supplemental Information for
Saturation mutagenesis genome engineering of infective Φ X174 bacteriophage via
unamplified oligo pools and golden gate assembly**

Matthew S. Faber¹, James T. Van Leuven^{2,3}, Martina M. Ederer³, Yesol Sapozhnikov³,
Zoë L. Wilson³, Holly A. Wichman^{2,3}, Timothy A. Whitehead^{4,5}, Craig R. Miller^{2,3*}

Affiliations: ¹Dept. Biochemistry and Molecular Biology, Michigan State University,
East Lansing MI, 48824; ²Institute for Modeling Collaboration and Innovation,
University of Idaho, Moscow ID, 83844; ³Dept. of Biological Sciences, University of
Idaho, Moscow ID, 83844; ⁴Dept. of Chemical & Biological Engineering; University of
Colorado, Boulder, CO 80303; ⁵Dept. of Chemical Engineering & Materials Science;
Michigan State University, East Lansing MI, 48824

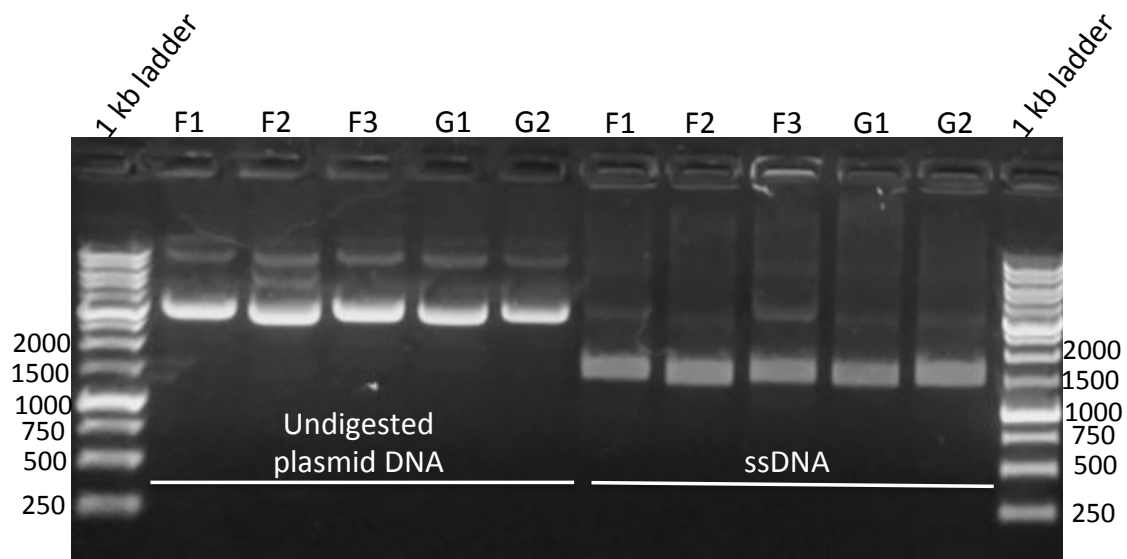
*Correspondence to:

jvanleuven@uidaho.edu

Department of Biological Sciences

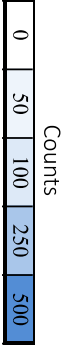
875 Perimeter MS 3051

Moscow, Idaho 83844-3051



Supplemental Figure S1. Introduction of nicking sites into shuttle vectors containing viral genes. Verification of the introduction of the BbvCI nicking site into the shuttle vector containing the viral genes is shown by the template prep step in the nicking mutagenesis protocol (generation of ssDNA). Samples were run on a 1% agarose gel with SYBR™ Safe DNA gel stain (Invitrogen) added before casting, the ladder used is the 1 kb DNA ladder from GoldBio.

F2 Tile 1 Heatmap of Counts		0	50	100	250	500																																																					
A	175	178	160	207	20	55	96	25	16	75	94	32	161	22	78	268	153	50	261	209	30	16	45	13	43	31	48	20	121	53	80	50	234	54	48	240	157	267	77	83	161	89	50	74	173	277	534	484	301	678	441	201	168	200	455	98	73	52	
B	215	147	199	42	203	64	24	106	21	23	-	125	25	130	115	36	222	128	46	259	88	49	10	32	15	31	-	2	25	84	48	90	81	170	62	89	150	-	122	68	57	28	113	70	69	200	257	410	453	319	473	428	166	234	187	256	89	95	67
C	201	244	267	188	203	23	77	69	18	20	49	104	61	137	48	87	307	146	78	823	115	78	19	57	1	26	45	4	47	88	31	92	86	225	130	113	192	153	47	108	66	62	136	46	97	33	306	440	566	421	615	407	161	183	163	301	113	103	68
D	178	216	144	77	142	71	97	85	254	60	62	86	62	170	82	67	270	159	72	257	107	93	14	55	20	32	7	50	69	551	120	138	194	75	75	160	199	129	409	85	31	201	57	125	232	287	398	660	369	318	228	227	1471	172	440	108	108	108	
E	162	238	272	74	141	75	82	-	-	-	59	105	38	307	54	62	252	169	28	282	106	40	35	45	7	32	36	4	41	62	45	105	108	194	75	75	160	199	129	409	85	31	201	57	125	232	287	398	660	369	318	228	227	1471	172	440	108	108	108
F	178	186	159	56	206	19	46	132	6	13	30	130	31	191	35	76	253	151	62	312	113	46	21	75	50	35	36	-	23	73	47	121	80	151	96	86	147	215	167	95	126	25	128	63	71	227	343	332	492	334	515	376	227	183	233	-	-	7467	
G	230	242	221	100	173	45	-	110	46	42	62	121	302	127	36	156	329	-	85	363	127	41	32	39	44	17	38	2	85	78	23	304	74	240	157	113	167	153	175	75	67	22	100	76	-	241	262	526	598	385	537	477	389	162	308	342	-	-	7467
H	163	217	156	55	149	39	112	86	36	29	50	106	38	162	48	54	255	254	67	279	247	42	10	111	17	26	26	13	40	51	24	122	88	177	89	87	148	154	363	75	55	25	112	30	146	220	277	-	368	343	649	411	-	185	238	398	248	275	87
I	206	212	224	50	311	28	52	-	19	20	46	133	37	180	40	131	291	152	71	344	-	65	8	-	23	10	26	17	19	64	25	210	86	135	103	135	160	209	184	58	76	107	130	90	111	-	275	473	485	311	642	311	201	317	207	478	108	112	80
J	187	242	270	50	206	19	73	127	27	28	42	137	52	199	36	102	304	160	104	242	139	75	28	77	59	28	17	143	27	63	45	91	96	133	103	135	160	209	184	58	76	107	130	90	111	-	275	473	485	311	642	311	201	317	207	478	108	112	80



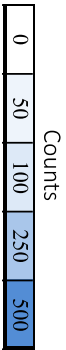
Supplementary Figure S3: F2 tile 1 heatmap of mutational frequency represented by number of sequencing read counts “counts”.

Location	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	
Mutation	F	O	T	F	I	S	R	H	N	S	N	F	E	S	D	K	L	V	T	S	V	T	P	A	S	A	P	V	L	O	T	P	K	A	T	S	S	T	Y	F	D	S	L	T	V	N	A	G	N				
...	29	89	4	73	45	35	38	23	23	96	50	71	140	82	67	34	35	80	110	29	86	102	149	574	319	575	412	132	97	403	137	416	424	317	267	165	209	132	137	45	212	272	334	115	100	83	7	20	24	32	26	35	
F	30	6	-	104	108	30	21	28	64	36	-	-	87	69	10	39	195	129	34	110	84	128	464	310	721	334	108	52	390	214	267	439	384	202	178	165	101	39	35	169	220	-	142	125	177	12	697	21	39	42	49		
W	35	78	8	144	48	28	58	120	92	42	99	195	31	61	37	109	108	115	35	83	102	128	456	367	635	442	114	64	345	132	261	380	275	162	245	120	189	60	72	151	209	379	208	268	163	29	34	30	38	28	20		
Y	73	40	11	179	72	62	20	129	57	60	59	132	172	133	144	16	43	100	95	34	136	94	118	461	380	758	402	96	71	351	148	269	440	398	226	208	149	107	74	73	144	-	495	242	98	70	1	32	103	36	25	75	
P	18	102	32	69	31	109	86	43	25	201	59	82	109	136	72	20	341	128	286	131	119	98	159	-	355	663	515	147	-	199	207	243	235	141	112	143	213	290	117	100	87	135	29	11	68	21	29						
M	44	85	16	75	79	27	26	25	47	43	90	90	132	55	80	65	398	99	117	61	84	115	126	471	281	605	577	89	52	339	205	305	517	360	222	204	256	162	46	204	118	231	337	133	212	178	464	19	23	31	39	99	
I	73	30	54	163	-	31	20	23	87	46	103	128	213	64	73	12	55	312	145	105	60	182	493	320	630	348	82	78	440	196	308	537	348	207	214	206	103	51	52	142	251	438	169	249	58	13	773	96	45	22	102		
L	101	165	16	178	68	37	88	120	24	132	39	327	209	63	61	30	-	112	-	54	92	127	132	599	361	658	587	147	103	369	-	493	493	424	189	190	202	300	125	92	-	207	415	107	106	-	12	90	17	21	22	33	
V	92	47	9	107	101	31	26	16	30	65	47	116	141	71	148	38	272	-	134	57	73	-	134	477	476	620	502	279	56	-	155	307	300	300	184	338	130	161	62	114	151	181	421	403	207	203	14	-	14	343	497	24	
A	23	69	63	140	38	63	58	17	28	99	65	87	183	84	116	29	129	210	97	134	77	183	244	539	-	615	381	-	53	407	233	319	493	371	183	-	406	303	92	206	150	188	440	333	160	183	448	100	23	-	65	26	
G	33	44	23	103	50	40	95	15	124	75	89	128	179	63	181	25	73	197	135	38	76	121	109	409	395	557	415	160	67	418	135	367	457	321	197	336	126	125	93	50	91	255	394	383	293	96	8	197	15	233	-	27	
C	30	38	13	199	38	55	199	18	39	95	108	153	168	89	76	13	134	119	108	71	109	106	105	514	435	667	366	151	68	329	134	325	454	317	229	265	150	173	63	56	124	358	558	199	223	10	8	29	17	43	622	39	
S	80	43	36	134	66	-	126	36	33	-	224	162	214	-	37	19	33	100	183	162	-	117	206	553	457	-	-	156	84	411	229	345	589	397	280	353	366	-	171	153	-	151	160	271	174	106	74	-	33	72	127	22	65
T	32	25	-	77	80	61	38	25	40	152	1824	88	118	87	54	10	37	106	156	-	115	95	-	697	448	767	457	692	140	299	158	377	-	398	275	242	-	171	153	-	151	160	271	174	106	74	-	33	72	127	22	65	
N	43	52	14	68	81	18	52	55	-	60	-	93	141	79	128	48	24	100	151	66	94	112	157	534	325	676	419	90	59	339	154	294	470	356	350	172	213	111	80	65	102	223	385	205	187	61	15	31	-	37	27	-	
Q	41	-	7	68	31	23	76	70	38	92	74	116	132	54	113	40	274	112	126	38	52	151	131	492	318	609	548	138	77	312	199	-	450	331	271	183	175	160	53	73	150	175	324	179	199	136	4	28	29	22	34	44	
D	42	42	24	109	52	38	41	26	91	112	85	149	207	111	-	14	47	237	116	39	101	184	143	459	523	587	495	75	96	439	158	304	453	368	269	437	186	97	59	56	137	278	428	-	181	57	6	163	139	828	143	128	
E	31	57	19	122	44	33	33	11	29	134	65	92	170	62	214	116	26	166	131	47	80	105	126	659	355	655	343	149	50	354	167	319	534	311	337	302	259	101	87	70	174	208	447	337	154	72	8	64	33	44	29	44	
H	50	100	26	86	55	41	280	-	49	92	55	96	146	98	134	10	172	112	220	63	122	91	142	558	380	725	333	118	154	345	181	409	539	441	286	274	191	122	55	86	102	300	397	247	152	63	6	33	52	38	23	75	
K	13	33	15	75	33	14	22	12	48	51	114	85	140	56	67	-	31	82	90	53	74	83	163	545	304	683	336	93	66	344	160	361	580	296	-	169	347	102	56	79	149	199	297	123	155	48	303	29	1175	28	44	645	
R	34	188	16	94	35	37	-	99	35	75	101	94	144	52	84	52	299	104	224	54	72	111	116	531	412	646	403	137	113	356	145	542	536	404	260	336	183	136	55	90	126	216	403	156	201	84	199	23	26	69	86	34	



Supplementary Figure S7: G1 file 1 heatmap of mutational frequency represented by number of sequencing read counts “counts”.

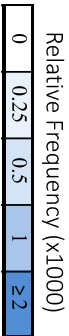
G2 heatmap of counts																																										
Location	Mutation	P	G	N	N	V	Y	Y	G	F	M	V	W	S	N	N	F	T	A	T	K	C	R	G	L	V	S	L	N	Q	V	I	K	E	I	I	C	L	Q	P	L	K
*		880	725	785	330	686	1018	769	660	607	348	82	241	504	163	839	422	599	645	651	706	390	844	360	1176	486	457	879	756	980	829	1454	576	458	232	172	174	240	164	252	522	
F	623	548	782	243	585	1000	767	771	-	372	147	182	477	156	-	378	444	713	421	607	311	627	311	1175	464	304	871	573	987	842	1018	448	683	525	154	183	112	72	293	386		
W	724	708	612	276	788	934	697	1100	592	508	86	-	362	135	890	391	579	700	519	713	281	645	327	841	601	445	850	908	845	691	1128	1060	383	215	524	135	278	135	203	467		
Y	733	604	895	380	720	-	793	668	554	425	109	215	493	175	984	439	563	679	551	665	385	665	332	1140	537	309	1121	661	1050	757	1218	408	536	426	202	140	120	152	261	367		
P	-	757	689	227	776	882	822	950	610	513	92	293	568	184	964	496	543	859	529	560	329	638	378	1078	715	702	936	1093	1073	751	1263	1015	442	256	118	326	361	-	292	520		
M	878	700	894	250	765	946	743	866	544	-	107	259	400	184	1001	390	525	823	627	692	352	703	365	1090	655	585	1110	890	1094	1022	1175	1039	834	566	164	213	291	158	255	527		
I	716	648	825	454	722	970	830	753	550	660	158	181	405	142	1072	544	594	594	554	561	344	658	278	1217	446	388	1147	663	1163	-	1406	468	-	-	146	248	122	66	323	485		
L	910	771	723	358	682	962	752	839	642	668	101	243	466	155	1009	529	538	751	467	608	352	789	-	1143	704	-	860	1192	960	834	1252	985	488	222	136	-	511	218	-	515		
V	776	664	743	243	-	803	-	1196	622	700	-	311	360	149	870	425	737	709	570	660	389	795	411	-	568	586	963	735	-	841	1177	1605	511	345	167	195	223	122	315	526		
A	988	765	740	316	956	931	838	1292	658	611	142	281	428	175	1054	547	-	846	537	676	331	666	270	1152	740	494	983	785	1136	911	1265	1648	482	266	201	160	267	177	201	538		
G	614	-	655	249	762	915	790	-	673	518	128	265	331	211	880	473	674	690	629	757	386	-	320	1132	550	369	901	881	1182	822	1265	1245	472	184	223	241	194	116	251	464		
C	839	1133	792	346	799	1032	797	944	673	550	100	337	451	217	942	517	573	794	546	-	513	649	396	1238	538	421	932	835	1067	903	1258	841	495	281	-	251	221	160	251	474		
S	811	1001	884	352	792	914	793	878	758	486	93	243	-	260	926	613	916	776	543	760	492	865	319	1324	-	362	1133	721	1140	931	1540	893	513	348	190	275	291	197	270	542		
T	915	892	882	1487	749	1093	794	854	609	469	74	208	530	248	1051	-	581	-	521	657	433	745	314	1234	532	297	1139	813	960	755	1333	608	474	249	146	197	144	166	249	474		
N	935	809	-	-	747	1160	841	816	683	384	96	133	480	-	1006	548	551	783	580	566	383	775	295	1170	522	334	-	931	1077	845	1661	603	501	285	139	252	136	166	249	474		
Q	1125	808	808	320	818	872	739	847	534	567	83	265	481	167	892	512	532	783	549	618	347	722	322	1086	693	684	1021	-	1061	831	1292	1229	381	258	138	301	-	233	309	494		
D	691	988	907	368	768	1105	958	1141	665	479	169	230	369	196	953	428	775	767	620	617	344	719	272	1236	559	328	1076	796	1233	865	1393	1195	568	411	174	160	186	104	256	460		
E	713	701	767	326	940	1009	836	1010	613	427	108	196	436	183	944	421	647	781	598	721	431	911	286	1191	514	330	1025	741	1072	878	1544	-	479	334	181	201	180	174	224	480		
H	905	718	776	393	795	995	866	815	586	479	130	161	481	176	1060	400	530	710	535	569	478	786	252	1198	445	520	1154	1023	1065	784	1097	477	621	316	111	292	419	179	368	413		
K	783	786	913	338	750	941	749	799	630	405	96	215	400	218	866	440	577	670	-	587	416	872	366	1093	511	283	1140	752	917	832	-	572	487	267	115	213	176	137	255	-		
R	848	907	842	380	809	883	807	859	668	459	110	216	502	223	969	540	655	783	501	813	-	838	277	1076	641	546	947	1033	1125	930	1429	839	508	260	213	504	353	200	388	498		



Supplementary Figure S9: G2 heatmap of mutational frequency represented by number of sequencing read counts “counts”.

Figure S11: Heatmap of relative frequencies for mutations in the F2T1 fragment. The table displays relative frequencies for 20 mutations (rows, labeled A through R) across 256 positions (columns, labeled A through Z).

Location	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
A	0.12	0.13	0.14	0.15	0.16	0.17	0.18	0.19	0.20	0.21	0.22	0.23	0.24	0.25	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.33	0.34	0.35	0.36	0.37
B	0.13	0.14	0.15	0.16	0.17	0.18	0.19	0.20	0.21	0.22	0.23	0.24	0.25	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38
C	0.14	0.15	0.16	0.17	0.18	0.19	0.20	0.21	0.22	0.23	0.24	0.25	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39
D	0.15	0.16	0.17	0.18	0.19	0.20	0.21	0.22	0.23	0.24	0.25	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40
E	0.16	0.17	0.18	0.19	0.20	0.21	0.22	0.23	0.24	0.25	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.41
F	0.17	0.18	0.19	0.20	0.21	0.22	0.23	0.24	0.25	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.41	0.42
G	0.18	0.19	0.20	0.21	0.22	0.23	0.24	0.25	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.43
H	0.19	0.20	0.21	0.22	0.23	0.24	0.25	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.43	0.44
I	0.20	0.21	0.22	0.23	0.24	0.25	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.43	0.44	0.45
J	0.21	0.22	0.23	0.24	0.25	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.43	0.44	0.45	0.46
K	0.22	0.23	0.24	0.25	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.43	0.44	0.45	0.46	0.47
L	0.23	0.24	0.25	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.43	0.44	0.45	0.46	0.47	0.48
M	0.24	0.25	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.43	0.44	0.45	0.46	0.47	0.48	0.49
N	0.25	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.43	0.44	0.45	0.46	0.47	0.48	0.49	0.50
O	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.43	0.44	0.45	0.46	0.47	0.48	0.49	0.50	0.51
P	0.27	0.28	0.29	0.30	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.43	0.44	0.45	0.46	0.47	0.48	0.49	0.50	0.51	0.52
Q	0.28	0.29	0.30	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.43	0.44	0.45	0.46	0.47	0.48	0.49	0.50	0.51	0.52	0.53
R	0.29	0.30	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.43	0.44	0.45	0.46	0.47	0.48	0.49	0.50	0.51	0.52	0.53	0.54
S	0.30	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.43	0.44	0.45	0.46	0.47	0.48	0.49	0.50	0.51	0.52	0.53	0.54	0.55
T	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.43	0.44	0.45	0.46	0.47	0.48	0.49	0.50	0.51	0.52	0.53	0.54	0.55	0.56
U	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.43	0.44	0.45	0.46	0.47	0.48	0.49	0.50	0.51	0.52	0.53	0.54	0.55	0.56	0.57
V	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.43	0.44	0.45	0.46	0.47	0.48	0.49	0.50	0.51	0.52	0.53	0.54	0.55	0.56	0.57	0.58
W	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.43	0.44	0.45	0.46	0.47	0.48	0.49	0.50	0.51	0.52	0.53	0.54	0.55	0.56	0.57	0.58	0.59
X	0.35	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.43	0.44	0.45	0.46	0.47	0.48	0.49	0.50	0.51	0.52	0.53	0.54	0.55	0.56	0.57	0.58	0.59	0.60
Y	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.43	0.44	0.45	0.46	0.47	0.48	0.49	0.50	0.51	0.52	0.53	0.54	0.55	0.56	0.57	0.58	0.59	0.60	0.61
Z	0.37	0.38	0.39	0.40	0.41	0.42	0.43	0.44	0.45	0.46	0.47	0.48	0.49	0.50	0.51	0.52	0.53	0.54	0.55	0.56	0.57	0.58	0.59	0.60	0.61	0.62



Supplementary Figure S11: Heatmap of relative frequencies for mutations in the F2T1 fragment.

Position	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	
A	0.19	0.43	0.04	0.80	0.27	0.16	0.32	0.07	0.11	0.51	0.23	0.58	1.08	0.17	0.34	0.23	0.61	0.60	0.64	0.19	0.46	0.57	0.70	2.53	3.04	3.53	2.45	0.63	0.30	0.92	0.73	1.45	2.13	1.53	0.90	1.56	0.67	1.05	0.33	0.40	0.84	1.16	2.10	1.15	1.49	0.90	0.16	0.19	0.17	0.21	0.18	0.42
C	0.17	0.21	0.07	1.10	0.23	0.31	0.10	0.10	0.22	0.52	0.60	0.88	0.93	0.49	0.42	0.07	0.24	0.66	0.61	0.39	0.61	0.59	0.38	1.25	1.42	1.70	2.03	1.04	0.38	1.83	1.74	1.80	1.52	1.76	1.27	1.47	0.83	0.86	0.56	0.31	0.69	1.59	1.10	1.34	0.61	0.04	0.11	0.13	0.18	0.14	0.19	
G	0.17	0.21	0.07	1.10	0.23	0.31	0.10	0.10	0.22	0.52	0.60	0.88	0.93	0.49	0.42	0.07	0.24	0.66	0.61	0.39	0.61	0.59	0.38	1.25	1.42	1.70	2.03	1.04	0.38	1.83	1.74	1.80	1.52	1.76	1.27	1.47	0.83	0.86	0.56	0.31	0.69	1.59	1.10	1.34	0.61	0.04	0.11	0.13	0.18	0.14	0.19	
T	0.17	0.21	0.07	1.10	0.23	0.31	0.10	0.10	0.22	0.52	0.60	0.88	0.93	0.49	0.42	0.07	0.24	0.66	0.61	0.39	0.61	0.59	0.38	1.25	1.42	1.70	2.03	1.04	0.38	1.83	1.74	1.80	1.52	1.76	1.27	1.47	0.83	0.86	0.56	0.31	0.69	1.59	1.10	1.34	0.61	0.04	0.11	0.13	0.18	0.14	0.19	
U	0.17	0.21	0.07	1.10	0.23	0.31	0.10	0.10	0.22	0.52	0.60	0.88	0.93	0.49	0.42	0.07	0.24	0.66	0.61	0.39	0.61	0.59	0.38	1.25	1.42	1.70	2.03	1.04	0.38	1.83	1.74	1.80	1.52	1.76	1.27	1.47	0.83	0.86	0.56	0.31	0.69	1.59	1.10	1.34	0.61	0.04	0.11	0.13	0.18	0.14	0.19	
V	0.17	0.21	0.07	1.10	0.23	0.31	0.10	0.10	0.22	0.52	0.60	0.88	0.93	0.49	0.42	0.07	0.24	0.66	0.61	0.39	0.61	0.59	0.38	1.25	1.42	1.70	2.03	1.04	0.38	1.83	1.74	1.80	1.52	1.76	1.27	1.47	0.83	0.86	0.56	0.31	0.69	1.59	1.10	1.34	0.61	0.04	0.11	0.13	0.18	0.14	0.19	
W	0.17	0.21	0.07	1.10	0.23	0.31	0.10	0.10	0.22	0.52	0.60	0.88	0.93	0.49	0.42	0.07	0.24	0.66	0.61	0.39	0.61	0.59	0.38	1.25	1.42	1.70	2.03	1.04	0.38	1.83	1.74	1.80	1.52	1.76	1.27	1.47	0.83	0.86	0.56	0.31	0.69	1.59	1.10	1.34	0.61	0.04	0.11	0.13	0.18	0.14	0.19	
X	0.17	0.21	0.07	1.10	0.23	0.31	0.10	0.10	0.22	0.52	0.60	0.88	0.93	0.49	0.42	0.07	0.24	0.66	0.61	0.39	0.61	0.59	0.38	1.25	1.42	1.70	2.03	1.04	0.38	1.83	1.74	1.80	1.52	1.76	1.27	1.47	0.83	0.86	0.56	0.31	0.69	1.59	1.10	1.34	0.61	0.04	0.11	0.13	0.18	0.14	0.19	
Y	0.17	0.21	0.07	1.10	0.23	0.31	0.10	0.10	0.22	0.52	0.60	0.88	0.93	0.49	0.42	0.07	0.24	0.66	0.61	0.39	0.61	0.59	0.38	1.25	1.42	1.70	2.03	1.04	0.38	1.83	1.74	1.80	1.52	1.76	1.27	1.47	0.83	0.86	0.56	0.31	0.69	1.59	1.10	1.34	0.61	0.04	0.11	0.13	0.18	0.14	0.19	
Z	0.17	0.21	0.07	1.10	0.23	0.31	0.10	0.10	0.22	0.52	0.60	0.88	0.93	0.49	0.42	0.07	0.24	0.66	0.61	0.39	0.61	0.59	0.38	1.25	1.42	1.70	2.03	1.04	0.38	1.83	1.74	1.80	1.52	1.76	1.27	1.47	0.83	0.86	0.56	0.31	0.69	1.59	1.10	1.34	0.61	0.04	0.11	0.13	0.18	0.14	0.19	

Relative Frequency (x1000)

0 0.25 0.5 1 ≥2

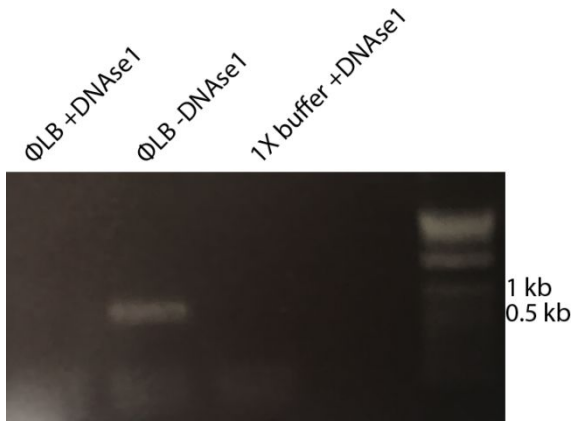
Supplementary Figure S15: Heatmap of relative frequencies for mutations in the G1T1 fragment.

G2 heatmap of counts		136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175			
Location	P	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175			
Mutation	G	1.28	1.13	1.01	0.50	1.21	2.06	1.58	1.59	-	0.77	0.30	0.37	0.98	0.52	-	0.78	0.91	1.47	0.87	1.23	1.33	1.34	1.45	0.80	1.74	0.74	2.42	1.00	0.94	1.81	1.56	2.02	1.71	3.00	1.19	0.94	0.48	0.35	0.36	0.49	0.34	0.52	1.08
	N	1.49	1.46	1.26	0.57	1.62	1.92	1.44	2.27	1.22	1.05	0.18	-	0.73	0.28	-	0.81	1.19	1.44	1.40	1.14	1.37	0.79	1.33	0.67	1.75	1.24	0.92	1.75	1.87	1.74	1.42	2.52	2.18	0.79	0.44	1.08	0.32	0.38	0.23	0.51	0.60	0.80	
	V	1.51	1.24	1.84	0.78	1.48	-	1.65	1.38	1.14	0.88	0.22	0.44	1.02	0.56	2.03	0.90	1.16	1.40	1.14	1.37	0.79	1.31	0.67	2.25	1.11	0.64	2.31	1.36	2.16	1.56	2.51	0.84	1.10	0.88	0.42	0.29	0.25	0.31	0.54	0.76	0.85		
	P	-	1.56	1.42	0.57	1.60	1.82	1.60	1.95	1.53	1.78	1.12	-	0.22	0.53	0.82	0.38	2.06	0.80	1.08	1.20	1.29	1.43	0.73	1.45	0.57	2.51	0.92	0.80	2.26	1.37	2.40	2.14	1.72	1.17	0.34	0.44	0.60	0.33	0.53	1.09	0.60	1.07	
	M	1.81	1.44	1.84	0.52	1.58	1.95	1.69	1.96	1.26	1.06	0.19	0.60	1.17	0.38	1.99	1.02	1.12	1.22	1.22	1.14	1.16	0.71	1.36	0.57	2.51	0.92	0.80	2.26	1.37	2.40	2.14	1.72	1.17	0.34	0.44	0.60	0.33	0.53	1.09	0.60	1.07		
	I	1.47	1.33	1.70	0.94	1.49	2.00	1.71	1.55	1.13	1.26	0.33	0.37	0.83	0.29	2.21	1.12	1.22	1.22	1.14	1.16	0.71	1.36	0.57	2.51	0.92	0.80	2.26	1.37	2.40	2.14	1.72	1.17	0.34	0.44	0.60	0.33	0.53	1.09	0.60	1.07			
	L	1.87	1.59	1.49	0.74	1.40	1.98	1.55	1.73	1.32	1.38	0.21	0.50	0.96	0.32	2.08	1.09	1.11	1.55	0.96	1.25	0.73	1.63	-	2.35	1.45	-	1.77	2.46	1.98	1.72	2.58	2.03	1.01	0.46	0.28	-	1.05	0.45	-	1.06			
	V	1.60	1.37	1.53	0.50	-	1.65	-	2.46	1.28	1.44	-	0.64	0.74	0.31	1.79	0.88	1.32	1.24	1.11	1.39	0.68	1.37	0.56	2.37	1.52	1.02	2.03	1.62	2.34	1.88	2.61	3.39	0.99	0.53	0.41	0.33	0.55	0.56	0.41	1.11			
	A	2.04	1.57	1.52	0.65	1.97	1.92	1.73	2.66	1.36	1.26	0.29	0.58	0.88	0.36	2.17	1.13	-	1.74	1.11	1.59	0.68	1.37	0.56	2.37	1.52	1.02	2.03	1.62	2.34	1.88	2.61	3.39	0.99	0.53	0.41	0.33	0.55	0.56	0.41	1.11			
	G	1.26	-	1.35	0.51	1.57	1.88	1.63	-	1.39	1.07	0.26	0.55	0.68	0.43	1.81	0.97	1.39	1.42	1.30	1.56	0.80	-	0.66	2.33	1.13	0.76	1.86	1.81	2.43	1.69	2.61	2.56	0.97	0.38	0.46	0.50	0.40	0.24	0.53	0.96			
	C	1.73	2.33	1.63	0.71	1.65	2.13	1.64	1.94	-	1.39	1.13	0.21	0.69	0.93	0.45	1.94	1.07	1.18	1.64	1.12	-	1.06	1.34	0.82	2.25	-	0.75	2.33	1.49	2.35	1.92	3.17	1.84	1.06	0.72	0.39	0.57	0.60	0.41	0.56	1.12		
	S	1.67	3.06	1.82	0.73	1.63	1.88	1.63	1.81	1.56	1.00	0.19	0.50	-	0.54	1.91	1.26	1.89	1.60	1.12	1.57	1.01	1.78	0.66	2.23	-	0.75	2.33	1.49	2.35	1.92	3.17	1.84	1.06	0.72	0.39	0.57	0.60	0.41	0.56	1.12			
	T	2.09	1.84	1.82	3.06	1.54	2.25	1.64	1.76	1.44	0.97	0.13	0.43	1.09	0.51	2.17	-	1.20	-	1.07	1.31	0.89	1.53	0.65	2.54	1.10	0.61	2.35	1.67	1.98	1.56	2.75	1.25	0.98	0.51	0.30	0.41	0.30	0.34	0.53	0.97			
	N	1.93	1.67	-	-	1.54	2.39	1.75	1.68	1.41	0.79	0.20	0.27	0.99	-	2.07	1.13	1.14	1.61	1.19	1.17	0.79	1.60	0.61	2.24	1.08	0.69	-	1.92	2.22	1.74	3.42	1.24	1.03	0.59	0.29	0.52	0.28	0.22	0.51	0.98			
	Q	2.52	1.66	1.66	0.66	1.69	1.80	1.52	1.74	1.10	1.17	0.17	0.35	0.99	0.34	1.84	1.05	1.10	1.62	1.13	1.27	0.71	1.49	0.66	2.24	1.48	1.41	2.10	-	2.19	1.71	2.66	2.55	0.78	0.53	0.28	0.62	-	0.48	0.64	1.02			
	D	1.42	2.04	1.58	0.76	1.58	2.28	1.97	2.25	1.37	0.99	0.35	0.47	0.76	0.40	1.96	0.88	1.60	1.58	1.28	1.27	0.71	1.48	0.56	2.25	1.15	0.68	2.22	1.64	2.54	1.78	2.87	2.46	1.17	0.85	0.36	0.33	0.38	0.28	0.56	0.99	0.95		
	E	1.47	1.44	1.60	0.81	1.94	2.08	1.72	2.08	1.26	0.88	0.22	0.40	0.90	0.38	1.94	0.87	1.33	1.61	1.23	1.49	0.89	1.88	0.59	2.45	1.06	0.68	2.11	1.53	2.21	1.81	3.18	-	0.99	0.69	0.37	0.41	0.37	0.56	0.46	0.99			
	H	1.86	1.48	1.60	0.81	1.64	2.05	1.78	1.68	1.21	0.99	0.27	0.33	0.99	0.36	2.18	0.82	1.09	1.46	1.10	1.17	0.98	1.62	0.52	2.47	0.92	1.07	2.38	2.11	2.19	1.62	2.26	0.98	1.28	0.65	0.23	0.60	0.86	0.57	0.76	0.85			
	K	1.61	1.62	1.88	0.70	1.55	1.94	1.54	1.65	1.30	0.83	0.20	0.44	0.82	0.45	1.78	0.91	1.19	1.38	-	1.21	0.86	1.80	0.75	2.25	1.05	0.58	2.35	1.55	1.89	1.71	-	1.18	1.00	0.53	0.24	0.44	0.36	0.28	0.53	-			
	R	1.75	1.87	1.73	0.78	1.67	1.82	1.66	1.77	1.38	0.95	0.23	0.44	1.03	0.46	2.00	1.11	1.35	1.61	1.03	1.67	-	1.73	0.57	2.22	1.32	1.12	1.95	2.13	2.32	1.92	2.94	-	1.18	1.00	0.54	0.44	0.44	0.73	0.41	0.80	1.03		



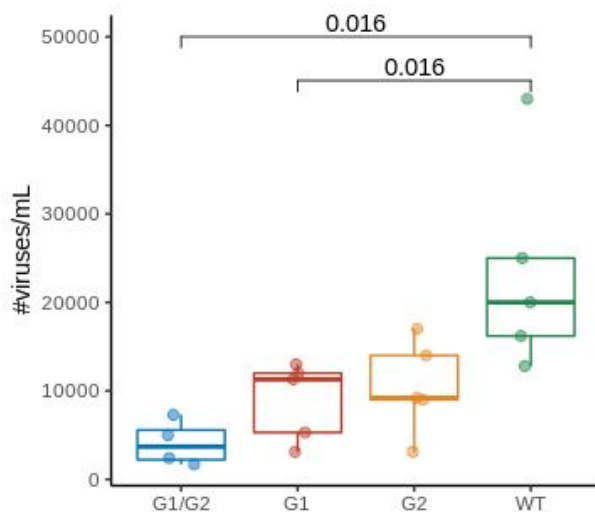
Supplementary Figure S17: Heatmap of relative frequencies for mutations in the G2 fragment.

Supplementary Figure S18: Agarose gel image showing effectiveness of DNase1 in Φ LB. 25 cycles of PCR were performed after a 1-hour 37°C incubation of WT ligation mix with DNase1. Primer sequences for phix_2375F and phix_2949R were 5'-ACACTGACGACATGGTTCTACAAtctgcttaggagtttaac-3' and 5'-TACGGTAGCAGAGACTTGGTCTgcaccaaacataaatcacc-3'. These primers include annealing sequences for Illumina 2nd round barcoding primers.



Supplemental Figure S19. Consistency between transformation replicates.

Virus titers for four replicate transformations were compared by Anova (global p.adj = 0.006) and pairwise tests with adjusted p-values (Wilcoxon) shown on the plot. These experiments were done using 1 uL of transformation mix, as arcing occasionally occurs with 4 uL. The G1/G2 construct is the result of ligating together the G1 and G2 libraries.



Supplemental table S1. Primers for incorporating BbvCI nicking sites into the pCR2.1-topo shuttle vector. Blue text is the overlap region with the shuttle vector, red is the KpnI site, green is the BbvCI site.

Nick_incorporation_fwd
GCTCTACGGGTACCGCTGAGGGAGCTCGGATCCACTAGTAACG
Nick_incorporation_rvs
GCTCTAACGGGTACCAAGCTTGGCGTAATCATGGTC

Supplemental table S2. Inner and outer primers for PCR reactions for Illumina sequencing. Red indicates overhang regions for attaching Illumina adapter primers (inner PCR primers) or overhangs for attaching to inner PCR product (outer PCR primers), black is the overlap region in the gene or the barcode, blue is the Illumina adapter.

Inner PCR Primers	Sequence (5' to 3')
Fragment F1 Fwd	gttcagagtctacagtcgacgaacccttactgaggataaatt
Fragment F2 Tile 1 Fwd	gttcagagtctacagtcgacgatcactcactatagggcgaa
Fragment F2 Tile 2 Fwd	gttcagagtctacagtcgacgatcagcttaatacaagatgatgct
Fragment F3 Tile 1 Fwd	gttcagagtctacagtcgacgatcgtctctctgggca
Fragment F3 Tile 2 Fwd	gttcagagtctacagtcgacgatcgaaggatgtttccgt
Fragment G1 Tile 1 Fwd	gttcagagtctacagtcgacgatcgaattggccctctag
Fragment G1 Tile 2 Fwd	gttcagagtctacagtcgacgatcggtaatgctgtaatgg
Fragment G2 Fwd	gttcagagtctacagtcgacgatcggccctctagatgca
Fragment F1 Rvs	ccttggcaccgagaattccattcgtctcacagtcgg
Fragment F2 Tile 1 Rvs	ccttggcaccgagaattccacaacggaaaccataacg
sdFragment F2 Tile 2 Rvs	ccttggcaccgagaattccattcgtctcccagag
Fragment F3 Tile 1 Rvs	ccttggcaccgagaattccattcttagacgaatcaccaga
Fragment F3 Tile 2 Rvs	ccttggcaccgagaattccatcacactcaatctttatca
Fragment G1 Tile 1 Rvs	ccttggcaccgagaattccatgcaatgaagaaaacca
Fragment G1 Tile 2 Rvs	ccttggcaccgagaattccacttcgtctccgtacg
Fragment G2 Rvs	ccttggcaccgagaattccattgaccgcctcca
Illumina outer primer adapter	aatgatacggcgaccaccgagatctacacgttcagagtctacagtcgga
Illumina outer PCR adapters and barcodes	
RPI31 (Fragment F1)	caagcagaagacggcatacagatATCGTGgtgactggagttccttggcaccgagaattcca
RPI15 (Fragment F2 Tile 1)	caagcagaagacggcatacagatTGACATgtgactggagttccttggcaccgagaattcca
RPI16 (Fragment F2 Tile 2)	caagcagaagacggcatacagatGGACGGgtgactggagttccttggcaccgagaattcca
RPI17 (Fragment F3 Tile 1)	caagcagaagacggcatacagatCTCTACgtgactggagttccttggcaccgagaattcca
RPI18 (Fragment F3 Tile 2)	caagcagaagacggcatacagatGCGGACgtgactggagttccttggcaccgagaattcca
RPI19 (Fragment G1 Tile 1)	caagcagaagacggcatacagatTTTCACgtgactggagttccttggcaccgagaattcca
RPI20 (Fragment G1 Tile 2)	caagcagaagacggcatacagatGGCCACgtgactggagttccttggcaccgagaattcca
RPI21 (Fragment G2)	caagcagaagacggcatacagatCGAAACgtgactggagttccttggcaccgagaattcca

Supplemental Table S3. Mutant library preparation summary. Summary table of the transformants required for sufficient library coverage, transformants obtained during comprehensive mutant library preparation by nicking scanning mutagenesis, and the fold excess of the number of transformants required for coverage.

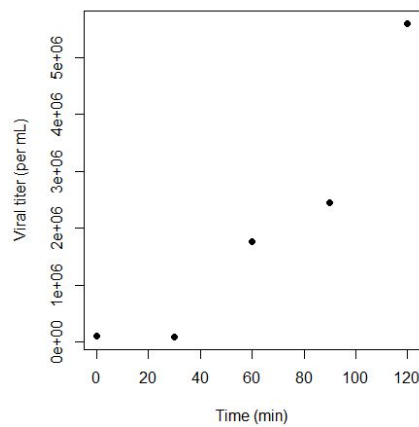
Gene mutated	F1	F2	F3	G1	G2
Number of Residues	95	144	182	132	40
Transformants obtained following NSM	620,000	890,000	370,000	620,000	640,000
Required transformants for 99.9% coverage of possible library	13781	20889	26401	19148	5803
Fold excess over amount required for coverage	45	43	14	32	110

Supplementary Table S4. Mutant plasmid library NGS statistics. Summary table of the libraries prior to viral genome assembly. Long fragments required separate amplicon sequencing reactions as shown.

Fragment	F1		F2		F3		G1		G2
Tile Number	1	1	2	1	2	1	2	1	
Residues	1-95	99-157	158-242	246-336	337-427	2-53	55-133	136-175	
Sequencing reads post quality filter	1163959	681935	1714460	800377	757177	747800	969275	813221	
Fold oversampling of codon combinations	441.1	499.8	851	364.8	345.1	632.8	517.6	821.9	
Percent of reads with:									
No nonsynonymous mutations	23.0%	63.3%	52.3%	54.0%	55.0%	68.3%	48.8%	25.2%	
One nonsynonymous mutation	52.6%	27.5%	36.3%	33.1%	32.1%	24.1%	39.8%	59.7%	
Multiple nonsynonymous mutation	24.4%	9.2%	11.3%	12.9%	12.8%	7.6%	11.4%	15.1%	
Coverage of possible single nonsynonymous mutations	100%	100%	99.8%	100%	99.9%	99.6%	100%	100%	

Supplemental Table S5. Viral growth rate during transformation cell recovery.

Viral titers immediately after transformation (0 min) and then for every 30 min after up to 2 hours. As the XL1-Blue cells are not susceptible, the increase in titer is the result of cell bursts, which releases viable phage. While the virus is replicating for the first 30 minutes, they are all held within a cell and will only make one plaque.



Time (min)	Dilution	#Plaques	Titer (viruses/mL)
0	1E-3	101	101,000
30	1E-3	90	90,000
60	1E-4	177	1,770,000
90	1E-4	245	2,450,000
120	1E-5	56	5,600,000

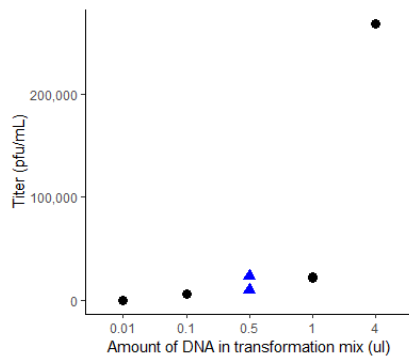
Supplemental Table S6. List of observed mutations in plaque sequencing.

Residues where more than one substitution was observed are bolded. Residues where mutations have been observed in previous studies (but not the exact substitution) are indicated*.

Fragment	Mutations
G1	L20T, S28I, Q33N, T34N , T34I , A37S, S46M , S46C , A51L, T64D, A69P,

	V75C, A83S, F87Q, C90Q, S97F, T102M, L103V, A106C* , A106Q* , T122G, V127N
G2	T151G, A152F, T153L, K154A, R156L , R156M
F1	H13M, D14H , D14Q , L15C, S16E, I29G*, M44Q, D45Q*, L52M, F68V, G79A, K84V*, D88M, N91T , N91Y , T93E , T93L , T93Y
F2	N134K, T145R, E146D, P149V, E177W, S181N, Q183P, T185S*, L196M, H204I*, T205D*, Y211H, S222E, N233R
F3	D254S, A296Y, N304H, G307S, A308Q , A308Q , G316A, L320T, K342V, Y354Q, E374C, S377Q, G378Y, Q393T*

Supplemental Table S7. Testing for the optimal amount of ligation mix to transform. Different amounts and dilutions of wildtype ligation mix were transformed into XL1-Blue cells to test for effects of DNA and salt concentration on transformation efficiency. The most transformants results from transforming 4 uL of ligated fragments.



Dilution	Amount in transformation	Plate dilution	#Plaques	Titer (viruses/mL)
0	4 uL	1E-3	268	268,000
0	1 uL	1E-3,1E-2	23, 220	22,500
10X	1 uL	1E-2	57	5,700
100X	1 uL	1E-1	0	0
2X	1 uL (clean)	1E-3,1E-2	23, 99	16,450