

Table S1: *SynIII* minichunk plasmids

Minichunk Plasmids	<i>SynIII</i> minichunk synthetic coordinates	
	Start	Stop
pNA001	377	3360
pNA002	2604	5525
pNA003	4837	7822
pNA004	6991	9992
pNA005	9247	12190
pNA006	11448	14470
pNA007	13656	16704
#pNA008	15957	19975
#pNA009	19209	21034
pNA010	20272	23218
pNA011	22513	25491
pNA012	24688	27641
pNA013	26897	30022
pNA014	29219	32257
pNA015	31504	34450
pNA016	33703	36704
pNA017	35918	39144
pNA018	38130	41352
pNA019	40623	43599
pNA020	42851	45941
pNA021	45081	48168
pNA022	47407	50415
pNA023	49615	52303
#pNA024	51819	54436
pNA025	53759	56768
pNA026	56016	58624
pNA027	58251	60795
#pNA028	60142	63071
pNA029	62310	65291

pNA030	64463	67422
pNA031	66774	69332
pNA032	68583	71593
#pNA033	70780	73348
pNA034	73072	75912
pNA035	75308	78094
pNA036	77338	80282
pNA037	79534	82556
pNA038	81802	84692
pNA039	84030	87021
pNA040	86271	88862
pNA041	88358	91081
pNA042	90335	93322
pNA043	92544	95550
#pNA044	94785	97386
pNA045	96965	100330
pNA046	99617	101869
pNA047	101114	103366
pNA048	102521	104851
pNA049	104099	106207
pNA051	105585	107676
pNA051	107077	109234
pNA052	108564	112198
pNA053	111451	115169
pNA054	114417	118160
pNA055	117337	121133
pNA056	120381	122627
pNA057	121873	124110
pNA058	123360	125432
pNA059	124847	126655
pNA060	126332	129580
pNA061	128849	132517
pNA062	131872	133987

pNA063	133360	135600
pNA064	134713	136982
pNA065	136236	138468
pNA066	137710	141447
pNA067	140661	144345
pNA068	143666	147115
pNA069	146546	150079
pNA070	149331	151572
pNA071	150817	153206
pNA072	152457	156143
pNA073	155397	159091
pNA074	158343	162131
#pNA075	161901	165291
pNA076	164565	168240
pNA077	167496	171196
pNA078	170502	173840
pNA079	173106	176794
pNA080	176049	179720
#pNA081	178903	180524
*#pNA082	180019	182639
*pNA083	181876	184124
pNA084	183355	185579
pNA085	184832	187104
pNA086	186352	188602
pNA087	187846	190095
pNA088	189339	191568
pNA089	190781	194705
pNA090	193910	196318
pNA091	195480	197901
pNA092	197048	199408
pNA093	198616	201021
pNA094	200270	204163
pNA095	203398	207373

pNA096	206516	208891
pNA097	208094	210551
pNA098	209670	212039
pNA099	211283	213535
pNA100	212769	215016
pNA101	214272	216498
pNA102	215767	219487
pNA103	218743	220974
*pNA104	220241	222455
#pNA105	221702	225023
pNA106	224652	228367
pNA107	227721	231390
pNA108	230579	234346
pNA109	233601	235849
pNA110	235097	237304
pNA111	236559	238842
pNA112	238077	240245
pNA113	239574	243307
pNA114	242549	246264
pNA115	245504	247802
pNA116	247013	249330
pNA117	248530	250868
pNA118	250052	252364
*pNA119	251576	255452
#pNA120	255142	258615
pNA121	257818	260126
pNA122	259337	261686
pNA123	260896	264829
pNA124	264031	267913
pNA125	267124	269429
pNA126	268674	270998
pNA127	270219	272499

*Minichunks with pre-existing mutations: pNA082, 2324T deletion; pNA083, 467T deletion; pNA104, 1427T deletion; and pNA119, 825A deletion. The deletion numbers represent positions of mutations within the minichunks.

#Minichunks that were digested with appropriate restriction enzymes to remove the mutated regions from overlapping BBs, but leaving sufficient overlap for HR.