

Table S1 Oligonucleotides used in McEwan et al

Oligonucleotide	Sequence 5'-3'	Purpose
MS400	CTGTCTGCTTACATAAACAGTAATACAAGGGGTGTTATGATTCCGGG GATCCGTCGACC	Recombineering of pRT504
MS401	GCTCTGCCAGTGTTACAACCAATTAACCAATTCTGATTATGTAGGCTG GAGCTGCTTC	Recombineering of pRT504
ARM4	GGATACACATGTACACGTACGCGGGTGCTTAC	Cloning of int into pEHISTEV forward
ARM5	GGCTTCTCGAGTGTCTCGCTACGCCGC	Cloning of int into pEHISTEV reverse
OARM4	GAC AAG CTG TAC GCC GAG TGT GGC GCC GTC ATG	C336A forward
OARM5	CAT GAC GGC GCC ACA CTC GGC GTA CAG CTT GTC	C336A reverse
OARM6	GAC AAG CTG TAC TGC GAG GCT GGC GCC GTC ATG	C368A forward
OARM7	CAT GAC GGC GCC AGC CTC GCA GTA CAG CTT GTC	C368A reverse
OARM8	AGT GTG GCG CCG TCG CGA CTT CGA AGC	M372A forward
OARM9	GCT TCG AAG TCG CGA CGG CGC CAC ACT	M372A reverse
OARM10	TCA TGA CTG CGA AGC GCG GGG AAG AAT CG	S374A forward
OARM11	CG ATT CTT CCC CGC GCT TCG CAG TCA TGA	S374A reverse
OARM12	ACT TCG AAG CGC GCG GAA GAA TCG	G337A forward
OARM13	CGA TTC TTC CGC GCG CTT CGA AGT	G337A reverse
OARM14	ACT TCG AAG CGC CTG GAA GAA TCG	G377L forward
OARM15	CGA TTC TTC CAG GCG CTT CGA AGT	G377L reverse
OARM16	CGA TCA AGG ACT CTG CCC GCT GCC GTC G	Y385A forward
OARM17	C GAC GGC AGC GGG CAG AGT CCT TGA TCG	Y385A reverse
OARM18	CGA TCA AGG ACT CTT TCC GCT GCC GTC G	Y385F forward
OARM19	C GAC GGC AGC GGA AAG AGT CCT TGA TCG	Y385F reverse
OARM20	CGA TCA AGG ACT CTT TGC GCT GCC GTC G	Y385L forward
OARM21	C GAC GGC AGC GCA AAG AGT CCT TGA TCG	Y385L reverse
OARM22	ACT CTT ACC GCG CCC GTC GCC GGA AG	C387A forward
OARM23	CT TCC GGC GAC GGG CGC GGT AAG AGT	C387A reverse
OARM24	AGC ACG AAG GCA CGG CCA ACG TCA GC	C405A forward
OARM25	GC TGA CGT TGG CCG TGC CTT CGT GCT	C405A reverse
OARM26	CATGACTTCGAAGCGCGACGAAGAATCGATCAAG	G377D forward
OARM27	CTTGATCGATTCTTCGTCGCGCTTCGAAGTCATG	G377D reverse
OARM28	CATGACTTCGAAGCGCTTCGAAGAATCGATCAAG	G377F forward
OARM29	CTTGATCGATTCTTCCTTGCGCTTCGAAGTCATG	G377F reverse
OARM30	CATGACTTCGAAGCGCAAGGAAGAATCGATCAAG	G377K forward
OARM31	CTTGATCGATTCTTCCTTGCGCTTCGAAGTCATG	G377K reverse
OARM32	GTGTGGCGCCGTCATCACTTCGAAGCGCGGG	M372I forward
OARM33	CCCGCGCTTCGAAGTGATGACGGCGCCACAC	M372I reverse
OARM34	GGCGCCGTCATGACTCTGAAGCGCGGGGAAGAATC	S374L forward
OARM35	GATTCTTCCCCGCGCTTCAGAGTCATGACGGCGCC	S374L reverse
OARM36	CATGGACAAGCTGTACGCGGAGGCCGGCGCCGTCATGACTTC	C366A, C368A for
OARM37	GAAGTCATGACGGCGCCGCTCCGCGTACAGCTTGCCATG	C366A, C368A rev
OARM57	GTC ATG ACT TCG GCG CGC GGG GAA GAA TCG	K375A-forward
OARM58	CCT TGA TCG ATT CTT CCC CGC GCG CCG AAG TC	K375A-reverse
OARM59	CAT GAC TTC GAA GGC CGG CGA AGA ATC GAT C	R376A-forward
OARM60	CTT GAT CGA TTC TTC GCC GGC CTT CGA AGT C	R376A-reverse
OARM61	CTT CGA AGC GCG GCG CCG AAT CGA TCA AGG	E378A-forward
OARM62	GAG TCC TTG ATC GAT TCG GCG CCG CGC TTC	E378A-reverse
OARM63	GAA GCG CGG GGA AGC TAG CAT CAA GGA CTC	E379A-forward
OARM64	GTA AGA GTC CTT GAT GCT AGC TTC CCC GCG	E379A-reverse
OARM65	GCG CGG GGA AGA AGC GAT CAA GGA CTC TTA C	S380A-forward
OARM66	GCG GTA AGA GTC CTT GAT CGC TTC TTC CCC	S380A-reverse

OARM71	(P) ¹ GCGCCACACTCGCAGTACAGCTTGTCCATG	K375_R376_SDMREV
OARM79	(P)CGTCATGACTTCGCAACGCGGGGAAGAATC	K375Q
OARM80	(P)CGTCATGACTTCGCGGCGCGGGGAAGAATC	K375R
OARM81	(P)CGTCATGACTTCGGAACGCGGGGAAGAATC	K375E
OARM82	(P)CGTCATGACTTCGAAGAAGGGGAAGAATCGATC	R376K
OARM83	(P)CGTCATGACTTCGAAGGAAGGGGAAGAATCGATC	R376E
OARM84	(P)CGTCATGACTTCGAAGCAAGGGGAAGAATCGATC	R376Q
OARM85	(P)GAAGTCATGACGGCGCCACACTCGCAGTAC	S380_SDMREV
OARM86	(P)GAAGCGCGGGGAAGAAGACATCAAGGACTCTTAC	S380D
OARM87	(P)GAAGCGCGGGGAAGAAAACATCAAGGACTCTTAC	S380N
OARM88	(P)GAAGCGCGGGGAAGAAACGATCAAGGACTCTTAC	S380T

¹(P) = 5'phosphate

Table S2. Structural estimates for Int and derivatives from circular dichroism studies

Protein	NRMSD	Helix segment /100 residue	Strand segment /100 residue	Ave helix length / segment	Ave strand length / segment	Helix	Strand	Turns	Unordered	Total
Wt Int	0.031	3.399	4.144	8.492	4.823	0.265	0.218	0.228	0.288	0.999
hCTD	0.059	2.950	4.469	7.903	5.311	0.234	0.246	0.231	0.290	1.001
hInt	0.045	3.388	4.120	8.794	4.698	0.276	0.231	0.214	0.279	1
hIntK375A	0.039	3.964	3.400	8.974	4.125	0.343	0.172	0.210	0.275	1
hIntK375Q	0.026	3.888	3.419	8.936	4.316	0.338	0.194	0.200	0.268	1
hIntR376A	0.032	3.872	3.582	8.968	4.123	0.328	0.183	0.213	0.276	1
hIntK375E	0.029	3.877	3.590	9.109	4.230	0.337	0.185	0.205	0.272	0.999
hIntR376E	0.037	3.834	3.523	8.895	4.281	0.319	0.194	0.217	0.271	1.001
hIntS380A	0.040	3.777	3.781	9.012	4.156	0.319	0.188	0.211	0.282	1
hIntS380D	0.063	4.347	3.061	9.625	3.637	0.415	0.126	0.196	0.263	1
hIntS380N	0.036	3.941	3.452	8.944	4.229	0.327	0.188	0.214	0.270	0.999
hIntS380T	0.050	3.832	3.678	8.922	4.027	0.314	0.179	0.226	0.281	1

Table S3. Estimated molecular weights of hInt derivatives in free solution

Int variant	Apparent Mol. weight (kDa)	Ratio: Apparent from SEC/calculated monomeric mass
Int	167.2	2.3
hINT	130.1	1.8
hIntS374A	nd ¹	nd
hIntS374L	156.1	2.2
hIntK375A	151.2	2.1
hIntK375Q	146.0	2.0
hIntK375E	135.3	1.9
hIntR376A	161.6	2.2
hIntR376E	123.7	1.7
hIntR376P	nd	nd
hIntG377L	nd	nd
hIntG377A	nd	nd
hIntG377D	159.4	2.2
hIntG377F	143.1	2.0
hIntG377K	147.1	2.0
hIntE378A	138.1	1.9
hIntE379A	142.2	2.0
hIntS380A	144.4	2.0
hIntS380D	145.4	2.0
hIntS380N	159.7	2.2
hIntS380T	nd	nd
hIntI381M	nd	nd

¹nd = not done

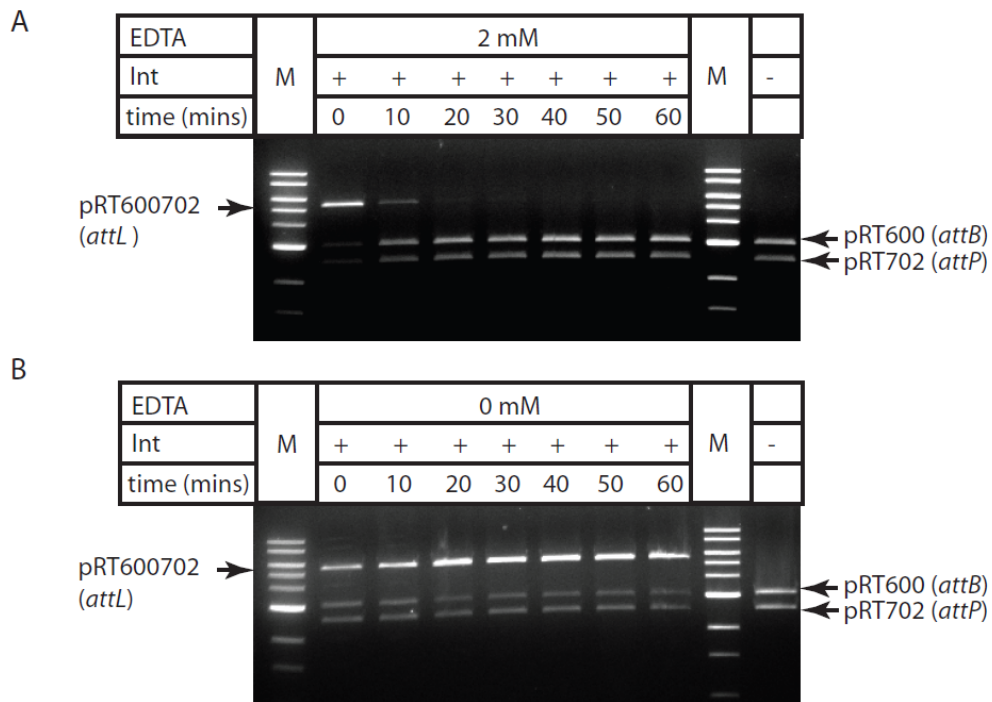


Figure S1. Time course of EDTA inhibition on integration by ϕ C31 Int. Panel A. Substrates pRT600 and pRT702, containing *attB* and *attP* respectively, were added after Int had been pre-incubated with EDTA for the indicated times and the recombination allowed to proceed for a further incubation (1 hr, 30°C). Recombination products were then detected by restriction digest of the assay mixture with HindIII. Appearance of the 5.3 kbp band is indicative of integration activity in which pRT600 and pRT702 combine to generate a cointegrate, pRT600702. Panel B. In the absence of EDTA Integrase remains active. Preincubation of Int with 2 mM EDTA for 20 minutes is sufficient to inhibit integration activity .

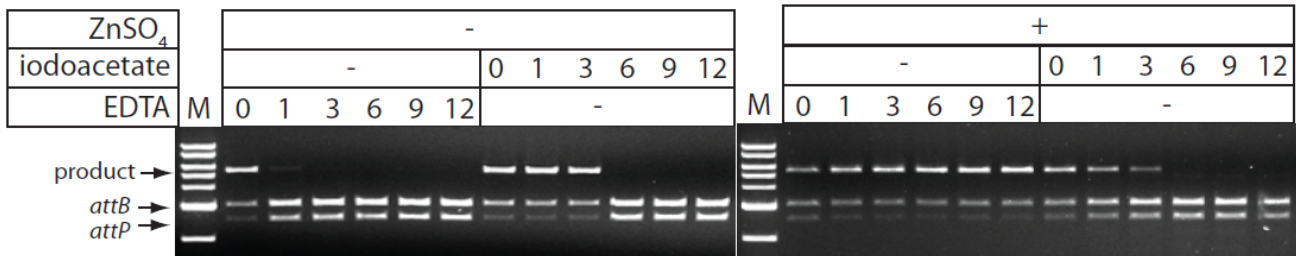
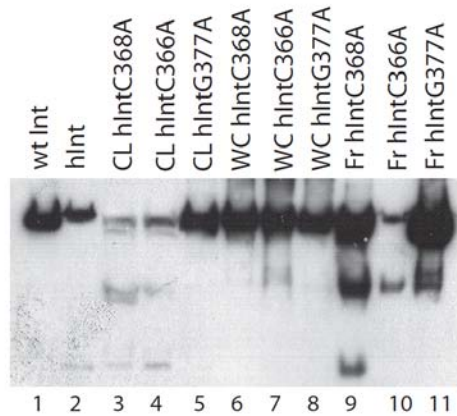


Figure S2. Inhibition by iodoacetate is not restored by addition of ZnSO₄. Int was preincubated with iodoacetate (1 hr, 30°C) and then either water or ZnSO₄ (final concentration 0.5mM) was added at the same time as the substrates for recombination, pRT600 (*attB*) and pRT702 (*attP*). Recombination was allowed to proceed for a further hour and the products then detected by restriction analysis with HindIII. The appearance of a 5.3 kbp band is indicative of integration activity in which pRT600 and pRT702 combined to generate a cointegrate, pRT600702. The data show that whilst Zn²⁺ ions can restore Int activity after treatment with EDTA, Zn²⁺ ions fail to restore activity to Int irreversibly modified with iodoacetate.

A. Int antibody



B. His-tag antibody

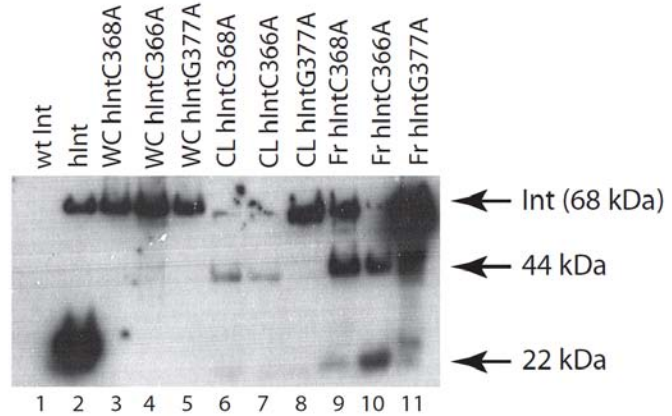


Figure S3. Breakdown of hIntC366A and hIntC368A. Western blots of whole cell extracts (WC) crude cell lysates (CL) or affinity column purified proteins (Fr) probed with either an antibody to Int (panel A) or an anti-His tag antibody (panel B). Whole cell extracts were prepared from BL21 DE3(pLysS) containing pADD008, pADD009 or pADD010 after IPTG induction and expressing hIntC368A, hIntC366A and hIntG377A respectively. Lanes 1 and 2 contain wild type Int and hInt respectively, purified as described in the main text. Arrows indicate bands containing full length Int and the major breakdown products at 44 and 22 kDa. hInt containing C368A and C366A substitutions have a high proportion of breakdown products after purification that cannot be avoided even after the addition of protease inhibitors.