

Supplementary table 1

Crypt Status	Frequency	1x [CA]₃₀	8x [CA]₃₀	20x [CA]₃₀
WPC	1680 per 10 ⁵ crypts	1 per 60 crypts	1 per 8 crypts	1 per 3 crypts
PPC	458 per 10 ⁵ crypts	1 per 218 crypts	1 per 27 crypts	1 per 12 crypts

Supplementary table 1. Estimates of the number of WPCs and PPCs observed in multiplex groups assaying different numbers of [CA]₃₀ genomic loci. The estimates are adapted from Kozar *et al* (2013) for the colon of a 300-day old mouse. The estimates account for autosomal loci being biallelic but only include the rate for in-frame mutations that would lead to YFP expression.

Supplementary table 2

Chrom	Position	Start	End	Type	Distance to nearest gene (bp)	Nearest gene
2	27425494	27425428	274254988	Intergenic	23820	BC050122
2	120629647	120629581	120629641	4th intron	0	Ttbk2
4	136517634	136517568	136517628	Intergenic	30605	Epha8
6	42765004	42764938	42764998	Intergenic	2534	Olfra450
8	33340003	33339937	33339997	Intergenic	77570	AK143160
8	67422965	67422899	67422959	Intergenic	48307	Tmem192
8	27334305	27334239	27334299	Intergenic	60968	Thap1
9	83283124	83283058	83283118	Intergenic	5664	Lca5
9	69438700	69438634	69438694	Intergenic	99322	Anxa2
10	20517916	20517849	20517909	Intergenic	73347	Pde7b
15	75067395	75067328	75067388	Intergenic	20213	BC025446
16	42959583	42959516	42959576	2nd intron	0	Zbtb20
19	45540170	45540103	45540163	4th intron	0	Btrc
X	139503016	139502950	139503010	Intergenic	31136	Rgag1

Supplementary table 2. A summary of each microsatellite locus used in the analysis of both wild-type and Msh2-deficient crypts. The positions relate to that seen in the mm9 reference genome.

Supplementary table 3

Parameter tested	Conditions trialed	Optimal result
Polymerase	NEB Q5, NEB Phusion, NEB Phusion HotStart, NEB Taq, NEB Vent, Kapa HiFi, TwistDx TwistAmp Basic, BioHelix IsoAmpII Universal tHDA	NEB Phusion HotStart
Mg2+:dNTP ratio	Mg2+: 1.5mM, 2.0mM, 2.5mM, 3.0mM	2.5mM Mg2+ : 1.0mM dNTP
	dNTP: 0.1mM, 0.2mM, 0.5mM, 1.0mM, 1.5mM	
Buffer concentration	1x, 1.5x, 2x, 2.5x	1x
Primer pool concentration (8 primers)	4μM, 2μM, 1μM, 0.5μM, 0.3μM	2μM (0.25μM per primer pair)
Annealing temperature	66.2°C, 67.9°C, 69.2°C, 70.1°C, 70.8°C, 71.0°C	66.2°C
Polymerase amount	0.2U, 0.4U, 0.6U, 0.8U, 1.0U	0.8U
Additional glycerol	0%, 1%, 2%, 5%, 7.5%, 10%	0%
Betaine additive	0M, 0.5M, 1.0M, 1.5M, 2.0M	0M

Supplementary table 3. Table summarizing the parameters that were optimized for the multiplex PCR amplification. The above conditions were tested in the order presented using a multiplex group containing 8 primer pairs. The final multiplex PCR conditions could simultaneously amplify up to 14 amplicons from a single crypt equivalent containing 1ng murine genomic DNA (equivalent to approximately 500 genomes copies) and subsequently from a single murine crypt.

Supplementary table 4

Locus	Lengths isolated
a4_1365	[CA] ₂₇ , [CA] ₂₈ , [CA] ₂₉ , [CA] ₃₀
s9_8328	[CA] ₃₀ , [CA] ₃₁ , [CA] ₃₃

Supplementary table 4. [CA] microsatellite lengths isolated from plasmids containing two native murine loci. Two genomic loci were isolated and mutated before expansion in competent bacterium. The different lengths of microsatellite isolated in each locus, as confirmed by Sanger sequencing, are summarized.

Supplementary table 5

Mouse ID	Age (days)	Locus	Number of crypts tested	PPC (%)	WPC (%)
16-4988	76	a2_2742	144	0.00	0.00
16-4988	76	s16_4295	144	0.00	0.69
16-4988	76	a4_1365	144	0.00	0.00
16-4988	76	s2_1206	144	0.00	0.00
16-4988	76	s9_8328	144	0.00	0.00
16-4988	76	a6_4276	144	0.00	0.69
16-4988	76	s8_2733	144	0.00	0.00
16-4988	76	s9_6943	144	0.00	0.00
16-20751	179	a2_2742	144	0.00	0.00
16-20751	179	s16_4295	144	0.00	0.00
16-20751	179	a4_1365	144	2.78	0.00
16-20751	179	s2_1206	144	0.00	0.00
16-20751	179	s9_8328	144	0.00	0.00
16-20751	179	a6_4276	144	1.39	0.00
16-20751	179	a10_2051	144	0.00	0.00
16-20751	179	s8_2733	144	0.00	0.00
16-20751	179	s9_6943	144	0.00	0.00
16-20751	179	a19_4554	144	0.00	0.00
16-20752	189	a2_2742	136	0.00	0.00
16-20752	189	s16_4295	136	0.00	0.00
16-20752	189	a4_1365	136	2.21	0.00
16-20752	189	s2_1206	136	0.00	0.00
16-20752	189	s9_8328	136	0.00	0.00
16-20752	189	a6_4276	136	0.00	0.00
16-20752	189	a10_2051	136	0.00	0.00
16-20752	189	s8_2733	136	0.00	0.00
16-20752	189	s9_6943	136	2.21	0.74
16-20752	189	a19_4554	136	0.00	0.74
14-11319	697	a2_2742	144	0.00	0.00
14-11319	697	s16_4295	144	0.00	0.00
14-11319	697	a4_1365	144	0.69	0.69
14-11319	697	s2_1206	144	0.00	0.00
14-11319	697	s8_3334	144	0.00	2.08
14-11319	697	s9_8328	144	0.00	2.08
14-11319	697	a6_4276	144	0.00	2.08
14-11319	697	s9_6943	144	4.86	1.39
14-11319	697	a19_4554	144	0.00	0.69

14-5007	735	a2_2742	176	0.00	0.57
14-5007	735	s16_4295	175	0.00	0.57
14-5007	735	a4_1365	176	1.14	0.57
14-5007	735	s2_1206	176	0.00	1.14
14-5007	735	s8_3334	176	0.00	2.27
14-5007	735	s9_8328	176	1.70	5.68
14-5007	735	a6_4276	176	0.00	1.70
14-5007	735	a10_2051	86	0.00	0.00
14-5007	735	s15_7506	85	0.00	1.18
14-5007	735	s8_2733	176	0.00	0.57
14-5007	735	s9_6943	176	5.11	0.57
14-5007	735	a19_4554	176	0.00	0.57

Supplementary table 5. A summary of all crypts sequenced in wild-type mice and the percentage of mutant crypts observed in each.

Supplementary table 6

Mouse ID	Post-induction (days)	Locus	Number of crypts tested	PPC (%)	WPC (%)
15-40981	28	a2_2742	143	7.69	2.10
15-40981	28	s16_4295	141	5.67	3.55
15-40981	28	a4_1365	141	12.06	9.93
15-40981	28	s2_1206	142	7.04	4.23
15-40981	28	s8_3334	142	7.04	4.23
15-40981	28	s9_8328	144	6.25	5.56
15-40981	28	a6_4276	144	5.56	8.33
15-40981	28	s15_7506	143	3.50	0.70
15-40981	28	s8_2733	142	4.23	2.82
15-40981	28	s9_6943	143	17.48	10.49
15-40981	28	a19_4554	144	4.17	2.78
16-38330	43	a2_2742	72	4.17	11.11
16-38330	43	s16_4295	72	4.17	11.11
16-38330	43	a4_1365	72	5.56	8.33
16-38330	43	s2_1206	72	4.17	8.33
16-38330	43	s8_3334	72	5.56	6.94
16-38330	43	s9_8328	72	12.50	4.17
16-38330	43	a6_4276	72	4.17	11.11
16-38330	43	s15_7506	72	2.78	11.11
16-38330	43	s8_2733	72	1.39	6.94
16-38330	43	s9_6943	72	5.56	6.94
16-38330	43	a19_4554	72	5.56	5.56
16-31579	59	a2_2742	72	4.17	18.06
16-31579	59	s16_4295	71	2.82	18.31
16-31579	59	a4_1365	70	8.57	21.43
16-31579	59	s2_1206	72	2.78	30.56
16-31579	59	s8_3334	72	2.78	13.89
16-31579	59	s9_8328	72	1.39	19.44
16-31579	59	a6_4276	71	5.63	22.54
16-31579	59	s15_7506	71	2.82	19.72
16-31579	59	s8_2733	72	6.94	13.89
16-31579	59	s9_6943	72	37.50	11.11
16-31579	59	a19_4554	72	8.33	25.00
15-37022	70	a2_2742	215	4.65	34.88
15-37022	70	s16_4295	215	4.65	28.84
15-37022	70	a4_1365	215	28.84	30.70
15-37022	70	s2_1206	216	6.02	37.04

15-37022	70	s8_3334	144	5.56	25.69
15-37022	70	s9_8328	216	24.54	35.19
15-37022	70	a6_4276	216	6.02	35.19
15-37022	70	s15_7506	215	8.84	19.53
15-37022	70	s8_2733	216	3.24	31.48
15-37022	70	s9_6943	216	11.11	47.22
15-37022	70	a19_4554	216	4.63	53.70
15-38571	102	a2_2742	96	12.50	43.75
15-38571	102	s16_4295	96	10.42	37.50
15-38571	102	a4_1365	96	12.50	39.58
15-38571	102	s2_1206	96	5.21	38.54
15-38571	102	s8_3334	96	9.38	36.46
15-38571	102	s9_8328	96	1.04	40.63
15-38571	102	a6_4276	96	3.13	41.67
15-38571	102	s15_7506	96	17.71	31.25
15-38571	102	s8_2733	96	6.25	45.83
15-38571	102	a19_4554	96	11.46	25.00
15-28159	121	a2_2742	192	6.25	29.69
15-28159	121	s16_4295	192	4.69	23.44
15-28159	121	a4_1365	192	7.81	31.77
15-28159	121	s2_1206	192	3.65	30.21
15-28159	121	s8_3334	192	7.29	26.04
15-28159	121	s9_8328	192	9.90	20.31
15-28159	121	a6_4276	192	4.69	25.00
15-28159	121	s15_7506	192	6.25	18.75
15-28159	121	s8_2733	189	2.65	28.57
15-28159	121	s9_6943	191	24.08	24.61
15-28159	121	a19_4554	192	3.13	22.92

Supplementary table 6. A summary of all crypts sequenced in Msh2 conditional knockout mice and the percentage of mutant crypts observed in each.

Supplementary table 7

Locus	Mutations per mitosis (95% C.I.)
s9_6943	0.0298 (0.0244 - 0.0365)
a19_4554	0.0286 (0.0237 – 0.034)
s2_1206	0.0286 (0.0239 – 0.0345)
a4_1365	0.0269 (0.0224 – 0.0325)
a2_2742	0.0264 (0.0219 – 0.0318)
a6_4276	0.0264 (0.0219 – 0.0318)
s8_2733	0.0241 (0.02 – 0.0293)
s9_8328	0.0222 (0.0185 – 0.0271)
s16_4295	0.0207 (0.017 – 0.0249)
s8_3334	0.0185 (0.0153 – 0.0227)
s15_7506	0.0145 (0.0118 – 0.0177)

Supplementary table 7. Summary of locus-specific microsatellite mutation rates at 11 loci in Msh2-deficient intestinal epithelium. Microsatellite mutation per mitosis rates were inferred from clone size dynamics in Msh2-deficient epithelium using the mathematical model described by Kozar et al (2013) and the values for functional stem cell number and stem cell replacement rate that they calculated. An approximately 2-fold range in mutation rate is observed across the loci studied.