Supporting Information

Macdonald et al. 10.1073/pnas.1118715109

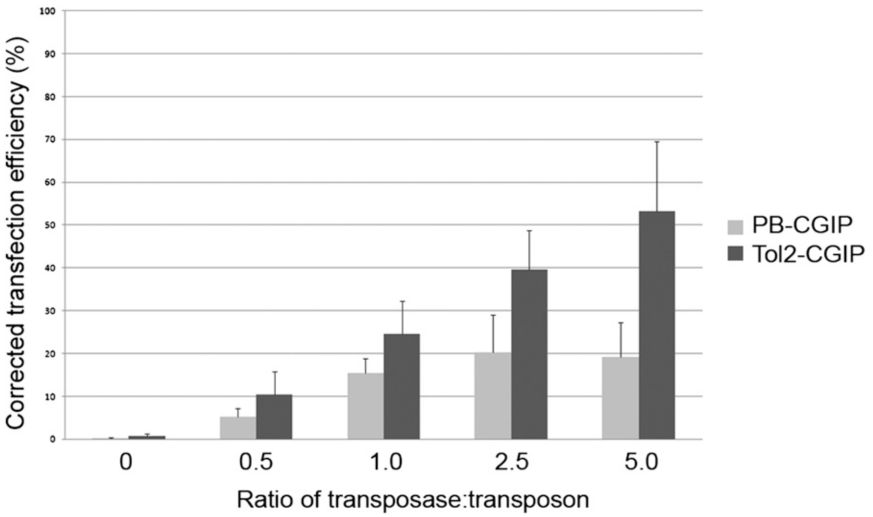

Fig. S1. Increased ratios of DNA transposase increase the stable transfection rates in DF-1 cells. DF-1 cells were transfected with 1.0 µg of a PB-CGIP or a Tol2-CGIP vector and increasing amounts (micrograms) of the appropriate DNA transposon and assayed 3 wk after transfection for GFP expression by flow cytometry. All transfection contained equal total amounts of plasmid DNA. Data represent three independent experiments. The stable transfection efficiencies are corrected for the initial rate of transfection. Error bars, SEM.

A Tol2-193 integration on chromosome 1

GGGTACATATTA**TAAATGAC**CAGAGGTG....CACCTCTG**TAAATGAC**CCTTTTCTCCTA

Chromosome 1genomic sequence

AGGCCAGCAATGCCAGGGTACATATTA**TAAATGAC**CCTTTTCTCCTACAGGCCCACAGACT

Tol2-T1 integration on chromosome 4

GCAGCCTGCATG**CAGAAGTG**CAGAGGTG....CACCTCTG**CAGAAGTG**CTTTCTAATGTT

Chromosome 4 genomic sequence

CCACAGCCTCTCTGGGCAGCCTGCATG**CAGAAGTG**CTTTCTAATGTTTAAATACAATCTT

Tol2-T2 integration on chromosome 21

CTGCACAGTGT**CACATAGG**CAGAGGTG....TACACCTCTG**CACATAGG**GTCCGTGGATG

Chromosome 21 genomic sequence

TTTCACAGCTGCAGCACTGCACAGTGT**CACATAGG**GTCCGTGGATGGAGCACTGCACCCCC

Tol2-T1 integration on chromosome 2

TTCGGTACAGA**AATAAACC**CAGAGGTG....TACACCTCTG**AATAAA**CCACAGGCAGGGC

Chromosome 2 genomic sequence

GAGCTTGTGAGAGACATTCGGTACAGA**AATAAACC**ACAGGCAGGGCTGGCACAACTCATCT

Tol2-11.10 integration on chromosome 9

ATTTCTTCTGT**GATCAGGC**CAGAGGTG....TACACCTCTG**GATCAGGC**AGATGTCTTCT

Chromosome 9 genomic sequence

CACAACCACTTGCCAATTTCTTCTGT**GATCAGGC**AGATGTCTTCTGTCTGGGACTT

B

Fig. S2. Tol2 integration sites in the genome of PGCs. (*A*) Five transposon–genomic junction fragments were identified on both sides of the Tol2 transposon, using inverse PCR. The PGC transfection identification number for each insert is given. Red, Tol2 ITRs; boldface type, 8-bp duplication. (*B*) The 8 bp flanking the integration sites for the Tol2 CGIP transposon were analyzed using WebLogo display (1) to generate a graphical representation of the nucleotide frequency at the integration site.

1. Crooks GE, Hon G, Chandonia JM, Brenner SE (2004) WebLogo:A sequence logo generator. *Genome Res* 14:1188–1190.

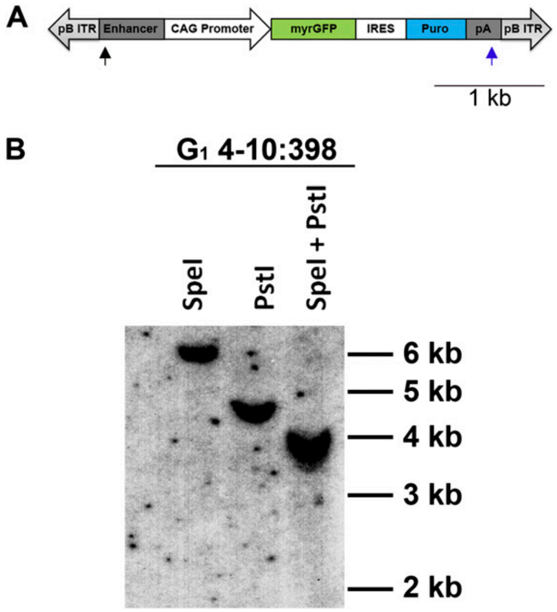

Fig. S3. Southern blot analysis of genomic DNA from a transgenic G_1 chicken. (*A*) Diagram of the piggyBac PB-CmyrGIP vector. The restriction sites for SpeI (black arrow) and PstI (blue arrow) are indicated. (*B*) Genomic DNA from the blood of a G_1 offspring (4-10:398) was analyzed for presence and copy number of the piggyBac transposon by digestion with SpeI or PstI to generate junction fragments and SpeI and PstI to release an internal transposon fragment. The Southern blot was hybridized with a DNA probe to the GFP sequence. The single bands indicate that a single integration event occurred.

Table S1. Tol2 chromosomal integration sites

PGC experiment	Restriction enzyme	Insertion position in boldface type, transposon ITR	Insertion site	Chromosome	Gene name/ Ensembl ID/EST
5′ Tol2 integration mapping					
Tol2-T1	AluI	ATTCGGTACAGAAATAAACC**CAG**, 141,374,821	Intron	2	EXT1 (ENSGALT00000025965)
Tol2-T1	HaeIII	AAGTTCAAAGTTATATTAGC**CAG**, 96,237,040	Intron	3	EST BX269199
Tol2-T1	PsiI	GCAGCCTGCATGCAGAAGTG**CAG**, 22,273,235	Intron	4	PDGFc (ENSGALT00000015275)
Tol2-T2	AluI	ACTGCACAGTGTCACATAGG**CAG**, 783,547	Flanking	21	EST CO421607
Tol2-T2	AluI	ATTAGAGACCTCTTCCTGCC**CAG**, 29,873,784	Intron	4	EST BU218066
Tol2-T2	HaeIII	CGTCCTTGGGAGTTCGACTC**CAG**, 104,243,072	Flanking	1	EST CO761385
Tol2-193	AluI	CCCAAATCTGAGGAGACAGA**CAG**, 95,262,451	Intergenic[†]	2	
Tol2-193	AluI	GGGTACATATTATAAATGAC**CAG**, 179,487,746	Intron	1	EST DN851336
Tol2-193	AluI	AAGCCGATCATCAGTATCTC**CAG**, 51,020,236	Exon	3	ESR1 (ENSGALT00000021168)
Tol2-193	HaeIII	GCACAAGTCTCGCTCCCTGC**CAG**, 365,742	Intron	26	PTPN7 (ENSGALT00000000839)
Tol2-193	HaeIII	TGGTCCCTCATGGTCAGAGC**CAG**, 348,960	Intron	28	FBN2 (ENSGALT00000000433)
Tol2-11.10	AluI	AATTTCTTCTGTGATCAGGC**CAG**, 11,255,883	Intergenic*	9	
Tol2-11.10	HaeIII	TTCCCTCAGCCGCTTCCCAT**CAG**, 118,415,627	Intron	2	EST BU265869
Tol2-11.10	HaeIII	GCGTGGTATGCCCAATTCAC**CAG**, 115,800,237	Intergenic*	1	
Tol2-11.10	PsiI	TGATCCCTGGGGAACACCCC**CAG**, 73,330,982	Intergenic*	Z	EST DN928593
Tol2-11.10	PsiI	ACTCCAAATGCACAATAACG**CAG**, 63,936,395	Flanking	3	SERINC1 (ENSGALT00000023989)
Tol2-25.11	PsiI	TAGCTATAGAGAGCTATAGG**CAG**, 71,482,703	intergenic[†]	Z	
Tol2-25.11	PsiI	ATATTTTATCCTTACCTGCT**CAG**, 26,588,210	intron	Z	DOCK8 (ENSGALT00000038450)
Tol2-c8	AluI	TAATAGCCCATAGGATGCGG**CAG**, 10,380,102	Flanking	14	TMEM114 (ENSGALT00000011882)
Tol2-c8	HaeIII	AAAGGCATATGGCACTGCAT**CAG**, 65,834,417	Intron	2	RREBP1 EST BU405399
Tol2-c8.1	HaeIII	AGCCATCCCTAAGCTGCATG**CAG**, 646,187	Flanking	2	EST AJ454197
Tol2-c8.1	PsiI	AGAGTAAAAATTATTAGTCAC**CAG**, 2,010,484	Intron	26	ELK4 (ENSGALT00000000994)
Tol2-c8.2	HaeIII	AGAGCAGCCTCAGCTATTTC**CAG**, 24,173,347	Intron	7	TNS1 (ENSGALT00000038183)
Tol2-c8.2	HaeIII	ACGGAGGGTGAGGAAACGAG**CAG**	Intron	Un	SRCIN1 ENSGALT00000015804
Tol2-c8.3	PsiI	AGAGGGCGGGTTGGCATGGA**CAG**, 85,037,711	Intergenic*	3	
Tol2-c8.3	PsiI	CATATTAGCTCTATAATGGG**CAG**, 41,755,050	Intron	Z	Cdc14B (ENSGALT00000020613)
Tol2-c8.3	PsiI	TTACTGTGGTACTTTCCATC**CAG**, 14,496,226	Intron	14	IFT140 (ENSGALT00000015164)
Tol2-c8.3	PsiI	ACTGTTAGATCACACAGCAG**CAG**, 62,015,773	Intron	Z	LOC427324 (ENSGALT00000025177)
3′ Tol integration mapping					
Tol2-T2	AluI	**TCTG**GAGCCCCATAACATTTAGAG, 6,138,933	Flanking	20	EST AJ399365
Tol2-T2	HaeIII	**TCTG**CCAGCAGCTCAGGGTGCCCA, 130,611,961	Intron	2	TMEM67 (ENSGALT00000025689)
Tol2-T2	HindIII	**TCTG**CTATTATAAAAAAATAGACC	Flanking	Un	ALOXE3 (ENSGALT00000021689)
Tol2-193	AluI	**TCTG**TTCATTCTGTACCTGTGAT, 63,331,450	Flanking	4	EST DR418323
Tol2-193	HindIII	**TCTG**CCTGCTCGATGCCCCGCAGG, 3,943,565	Exon	26	DEF6 (ENSGALT00000004117)
Tol2-193	HindIII	**TCTG**TCAGTCACTCTCATCGTGGA, 65,222,883	Intron	3	MAN1A1 (ENSGALT00000037376)
Tol2-11.10	HaeIII	**TCTG**CAGGAGACCGCCGGCTGCCT, 4,548,348	Intron	7	IL28 (ENSGALT00000006043)
Tol2-11.10	HaeIII	**TCTG**CATTTCGAGATGCTGTAAAG, 27,384,165	Intron	7	GLI3 (ENSGALT00000018998)
Tol2-11.10	HindIII	**TCTG**GTGCTTTTGTTGTCCTGGGG, 3,833,736	Intron	28	mRNA BX929812
Tol2-11.10	HindIII	**TCTG**TGGAAGACATCAGGCAGAGT, 24,922,038	Flanking	5	C1QTNF4 (ENSGALT00000013115)
Tol2-16.11	HindIII	**TCTG**CCTCTGCGTTCAGGGTGCCA, 6,285,333	Flanking	17	USP20 (ENSGALT00000006585)
Tol2-16.11	HindIII	**TCTG**CCCTTGATATTTAGGGAAGC, 3,654,975	Intron	6	GLUD1 (ENSGALT00000003144)
Tol2-25.11	HaeIII	**TCTG**TCCGCAGCCATACTTACAGC, 17,654,237	Flanking	13	UQCRQ (ENSGALT00000011489
Tol2-25.11	HindIII	**TCTG**TTCATTTGCAGTATTCCTGGC, 626,147	Intron	22	EBF2 (ENSGALT00000000321)
Tol2-25.11	HindIII	**TCTG**GTGCCGGTGCAATGAATTTT, 19,486,141	Intergenic[†]	8	
Tol2-c8	HaeIII	**TCTG**ATCTACACCCACACAAACTA, 54,541,392	Intergenic*	5	
Tol2-c8	HindIII	**TCTG**GTGTCAGGGGAGGTTCAAG, 3,610,251	Flanking	2	mRNA CR524032
Tol2-c8.1	HaeIII	**TCTG**AGGGCAACGTGGGAATAGTG, 13,621,982	Flanking	1	EST BU423113
Tol2-c8.2	HaeIII	**TCTG**TGCTTTTGTAGCATTTGCGT	Intergenic*	Un	CN237546
Tol2-c8.2	HaeIII	**TCTG**GTGTGGTGTCTTAGCAGGTA, 52,906,872	Flanking	5	EST CN235218
Tol2-c8.2	HaeIII	**TCTG**TTTAGAAGCTATGAAGGTGC, 70,380,681	Intron	2	RNASEN (ENSGALT00000021065)
Tol2-c8.2	HaeIII	**TCTG**CTCTGAGCACAGAATGCCAC, 21,167,669	Intron	10	MAP2K5 (ENSGALT00000012883)

Tol2 transposition integration sites were mapped using inverse PCR from 10 independent transposition experiments, using two PGC lines. Un: virtual chromosome constructed from unanchored contigs. Flanking: 0.2–9 kb to nearest transcript.

*More than 9 kb to nearest transcript.

[†] More than 30 kb to nearest transcript.