

FIG S2. Transposition and 3-bp flanking repeats: different models of duplication predict the same chromosomal configuration in ACN1039-derived mutants. The bottom line of each panel represents the chromosome of an ACN1039-derived Ben⁺ amplification mutant. Two models are illustrated for the formation of the mutant (corresponding to the models in Fig. 3B and 3C). In panel A (and Fig. 3C), an IS1236 element transposes on one copy of the chromosome and then undergoes unequal homologous recombination with a sister chromosome. In panel B (and Fig. 3B), duplication involves illegitimate recombination without transposition. The lowercase typeface (cac) indicates the sequence that was identified immediately upstream of the IS element in the duplication junction of mutant ACN1223. In panel A, this sequence corresponds the 3-bp transposition target that typically becomes duplicated during the transposition of IS1236 (red text). The absence of direct repeats flanking IS1236_3 (blue text) may reflect sequence divergence that occurred after an ancient insertion. In the model shown in panel B, this lowercase sequence corresponds to the sequence downstream of the *cat* genes that undergoes illegitimate recombination with the end of IS1236_3. As shown by the bottom line of both panels, the resulting chromosomal sequence of the mutant (ACN1223) is the same whether it is generated by homologous recombination between two IS elements (dashed line, panel A) or by illegitimate recombination (thin solid line, panel B). Thus, efforts to identify 3-bp flanking repeats are unable to distinguish between different models of duplication (A and B).

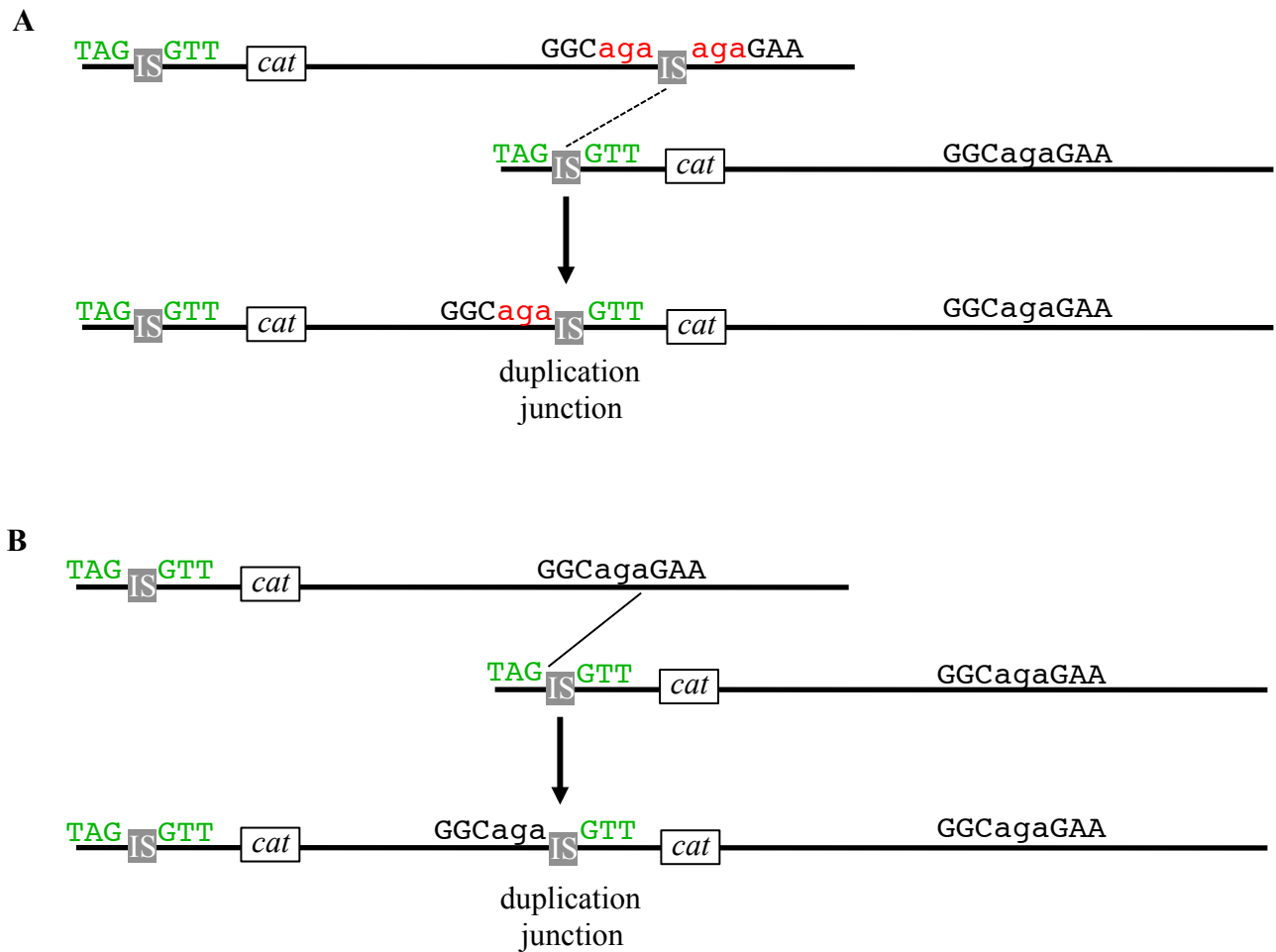


FIG S3. Transposition and 3-bp flanking repeats: different models of duplication predict the same chromosomal configuration in ACN1050-derived mutants. The bottom line of each panel represents the chromosome of an ACN1050-derived Ben⁺ amplification mutant. Two models are illustrated for the formation of the mutant (corresponding to the models in Fig. 3B and 3C). In panel A (and Fig. 3C), an IS1236 element transposes on one copy of the chromosome and then undergoes unequal homologous recombination with a sister chromosome. In panel B (and Fig. 3B), duplication involves illegitimate recombination without transposition. The lowercase typeface (aga) indicates the sequence that was identified immediately upstream of the IS element in the duplication junction of mutant ACN1056. In panel A, this sequence corresponds the 3-bp transposition target that typically becomes duplicated during the transposition of IS1236 (red text). The absence of direct repeats flanking IS1236_6 (green text) may reflect sequence divergence that occurred after an ancient insertion. In the model shown in panel B, this lowercase sequence corresponds to the sequence downstream of the *cat* genes that undergoes illegitimate recombination with the end of IS1236_6. As shown by the bottom line of both panels, the resulting chromosomal sequence of the mutant (ACN1056) is the same whether it is generated by homologous recombination between two IS elements (dashed line, panel A) or by illegitimate recombination (thin solid line, panel B). Thus, efforts to identify 3-bp flanking repeats are unable to distinguish between different models of duplication (A and B).

Table S1. Primer Sequences		
Primer Name	Sequence	Strain
PCR analysis (Fig. 6 and Table 2)		
2783029_UP	CGCGCATCATCTACCGTACAGTCG	ACN1056, ACN1057, ACN1161, ACN1163
2783847_DN	GGCAAGTGTATAAGAAATCGTAGC	ACN1056, ACN1057, ACN1161, ACN1163
2781256_UP	GATCTGATCACCTCTCAAAGC	ACN1058
2782115_DN	GTAGAATGGGTTCGAGTCAGCAGATG	ACN1058
980893_UP	GGGATTGCAGATTTGGTTCGGG	ACN1040, ACN1140
981637_DN	CAAAGCGACGTTTCCATTGAGCCG	ACN1040, ACN1140
976931_UP	GAAGAGTGGCAACCGCGTGTAGAAG	ACN1101
977672_DN	CTTTGTGCCTTGCTGCTCATG	ACN1101
992475_UP	GGATTAGGATTGATATTGCTTGTGG	ACN1102, ACN1131
993492_DN	CATACCTGCGACTCTCCTTGGCG	ACN1102, ACN1131
2784210_UP	CAGGCCATGTGGTTCGCGCTG	ACN1105
2785055_DN	GTAGTAGGGTTTTGTTACTAGGATCTGTG	ACN1105
2762759_UP	GCATGCTGGTTTTGAAGTAAGGCC	ACN1106
2763624_DN	CTGAATTGTTGTACTTGAGTGTGG	ACN1106
970003_UP	GATGGCTGGATTCAATTTGATGGG	ACN1127
970819_DN	GGTCATAAAGCGTAACCCATTC	ACN1127
973132_UP	CCTTACGCAAATATCCGCAAG	ACN1128
974099_DN	GTCCTGCTCATACACCGTTGG	ACN1128
983351_UP	CACATATCGCAGCAGATAAATTG	ACN1130
984231_DN	CATGGGCTTCAAGTAAACGGGC	ACN1130
988993_UP	GGGAATATCTAATGCAAAGCGATG	ACN1141
989788_DN	GATGCCATCATTACCCAAGATACC	ACN1141
975336_UP	GACCATTACATACATTACTGCACGG	ACN1222
976218_DN	GCTTCACTGATATCGGAGGGTTG	ACN1222
971804_UP	TCCGCACCCACTACAACATCA	ACN1223
972916_DN	GGAACGCGCTGGCCTGCACATG	ACN1223
DIG-labeling for Southern hybridization (Fig. 7 and Table 3)		
2782954_UP	GCAGACAAACACTCGGTATGCGTTAC	ACN1056, ACN1057
2783350_DN	CTGCCAGCAGTAAATTAATTCATGG	ACN1056, ACN1057
2781264_UP	CACCTCTCAAAGCAAAGATCAATGG	ACN1058
2781669_DN	CTGTTGAGAGGGAATATCGTTAACTAAAC	ACN1058
977090_UP	CGGTGCAGGTCAGGCCGCGAG	ACN1101
977490_DN	CCAGTAAATTCGCCAAACGTTTCGC	ACN1101
Southern Size Standards (Fig. 7 and Table 3)		
2782954_UP	GCAGACAAACACTCGGTATGCGTTAC	ACN1056, ACN1057
2784779_DN	AGGCATTAATGAAGTCATTCCATAATG	ACN1056, ACN1057
2786049_DN	GAAAAAAAAACAATAGTGAATTTTGCGAATATGC	ACN1056, ACN1057
2787539_DN	TCACTGTTTCTACACCAGATTGAATTTTG	ACN1056
2787602_DN	CACCTTCGCCTGTAAATACATAATCAGC	ACN1057
2782750_UP	GCATGGTTATCCTCATAAAGTTTCGC	ACN1057
2781188_UP	CTATTGGTTATGTCCCAACTTACGAAG	ACN1058
2781264_UP	CACCTCTCAAAGCAAAGATCAATGG	ACN1058
2783861_DN	TGATTTACACGCGCGCAAGTGTATAAG	ACN1058
2786690_DN	AATAAATGTGAAGGGGAATCAGTTTTAATC	ACN1058
2785055_DN	GTAGTAGGGTTTTGTTACTAGGATCTGTG	ACN1058
966684_UP	TTCGGTAAAAATCTTGCCTTG	ACN1101
974759_UP	CAATATGCAATTGATAATGTCATG	ACN1101
975990_UP	GCAACATCCACAGGATTTAAACG	ACN1101
977490_DN	CCAGTAAATTCGCCAAACGTTTCGC	ACN1101

Table S2. Amplicon Analysis of Ben⁺ Mutants

Parent strain	GDA mutant	Amplicon size (kbp)	Amplicon Copy Number	Amount of amplified DNA (kbp) ^a	IS-mediated?
ACN1039	ACN1040	49	9	444	Yes
ACN1039	ACN1101	46	15	681	Yes
ACN1039	ACN1102	61	19	1138	Yes
ACN1039	ACN1127	39	31	1213	Yes
ACN1039	ACN1128	42	20	852	Yes
ACN1039	ACN1130	52	35	1826	Yes
ACN1039	ACN1131	61	27	1619	Yes
ACN1039	ACN1140	50	14	705	Yes
ACN1039	ACN1141	58	12	684	Yes
ACN1039	ACN1222	44	23	1012	Yes
ACN1039	ACN1223	41	2	82	Yes
ACN1050	ACN1056	69	7	507	Yes
ACN1050	ACN1057	69	9	595	Yes
ACN1050	ACN1058	67	8	545	Yes
ACN1050	ACN1105	70	6	402	Yes
ACN1050	ACN1106	49	10	473	Yes
ACN1050	ACN1161	69	26	1768	Yes
ACN1050	ACN1162	60	6	351	No
ACN1050	ACN1163	69	16	1105	Yes
ACN1050	ACN1164	78	16	1242	No
ACN1050	ACN1165	95	17	1606	No

^aAmount of amplified DNA is inferred from amplicon size and copy number. If the product of the listed amplicon size and its copy number differs from the listed amount of DNA, the discrepancy arises from rounding.