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IS1236_1 IS1236_6	TGATCCTAGCCAAAATAATGGACAGTACTTCCCTCTAAAGATAACGTAACGTTAACTTTG TGGTCCTAGGCAAAATAATGGACACATGGTCCCTCTAAAGATAACGTAACGTTAACTTTG ** ***** **************************	60 60
IS1236_1 IS1236_6	GAGATCCAAGAAATGGCCAAACGTTTTAGTCCTGAATTTAAACAGC GAGATGTAGGGAAAGGCTATCTTGCGCAGCAGTGCTGCTCAAGTCCAGAATTTAAATAGC ***** * * ** *** * * * * * * * * * * *	106 120
IS1236_1 IS1236_6	AAGCAATTGATTATGCACTTTCAAACTCACACGAGCCTATAGCTACAATCGCCCAGAAAT AAGCAATTGATTATGCACTTTCAAACTCACACGAGCTGTAGCTGCAATCGCCCAGAAGT *********************************	166 180
IS1236_1 IS1236_6	TAGGTGTGGGTTATTCAACTTTAGACAAATGGATTCGTGAAGCCAATCCAGTGGGTTCAA TAGGTGTGGGGTTATTCAACATTAGATAAATCGATTCGTGAGGCTAATCCCACGGGTTCAA **********************************	226 240
IS1236_1 IS1236_6	GCAAACGTCAACTTTCACCAGAACAACAGCGGATCTTGGAATTAGAGAAGAAGTCAAAC GCAAACGCCAACTCTCGCCAGAACAACAGCGTATTCTCGATTTAGAAAAAGAAGTCAAAC ****** ***** ** ******************	286 300
IS1236_1 IS1236_6	AGCTCAGGGAAGCCAATGACATCTTAAAAAAAGCGCATGTGTACTTTCTGACAGATCATG AACTCAGGGAAGCCAATGATATTTTAAAAAAGGCGCATGTGTACTTCCTAACAGATCATG * ***********************************	346 360
IS1236_1 IS1236_6	CCAAGAAAAGTACACGGTAATTCAAGATCTGGATGTGAATGAA	406 420
IS1236_1 IS1236_6	CTGTAAATGCCTGGGTGTCAGCACTTCAGGCTATTATGCCTGGCGAAAACGCCAGACCAA CTGTAAATGCCTAGGTGTCAGCACTTCAGGCTATTATGCCTGGCGAAAACGCCAAGCCAA	466 480
IS1236_1 IS1236_6	TCTAGCGCAGAAATATAATGATTTAAAAGCCGTGTATTGGCAGCATCATGCACGATT TACAGCGCAGAAATACAATGATTGAAAATTTTGTATATTGGCAGCATCATCATGCGCGCGTT * *********** ****** ***** *** ** ******	523 540
IS1236_1 IS1236_6	GGGTGCACCTTCATTGGTACATGACATGCATGATTTAGGTTACAGCATGAGCGAACGAA	583 600
IS1236_1 IS1236_6	CGTTGGAAGAATGCTTAAAAAACTTGGTTTACGGAGCAAAATTGCACGTAAATACAAGCA GGTTGGAAGAAAGTTAAAAAAGCTCGGTTTACGCAGCAAGATTGCACGTAAATATAAGTA ********** * * ***** ** ******* *******	643 660
IS1236_1 IS1236_6	TACGACTGATTCAAACCATCGTTTGCCTACAGCGCCTAACTTGTTGGATCGTCAATTTAC TACGACTGATTCGAATCATCGTTTGCCGACGGCACCAAATTTATTAGATCGCCAGTTTAC ************ ** *********************	703 720
IS1236_1 IS1236_6	GGTCAATGAACCCAATAAGATCTGGACAACGGATATTACCTATATCCGTACTAAGCAAGG AGTCACTCGGCCTAACAAGGTTTGGACAACCGACATAACGTATATCCGAACTAAAGAAGA **** * ** ** ** ** *** * ******* ** **	763 780
IS1236_1 IS1236_6	TTGGCTGTATTTATGTGTGGATGCTGGATCTATTCAGCCGTCGTATTGTCGGTTGGCAAAC CTGGCTTTAATTATGTGTGGATGCTAGATTTATTTAGTCGTCGTCATTGTAGGTTGGCAAAC ***** ** *************************	823 840
IS1236_1 IS1236_6	CAGCCATCGAATAGACCGTCAATTGGTGTGTGTGTGATGCGTTTCATTATGCAATGGCTCGTCA TAGCCATCGAATAGACCGTGAATTGGTGTGTGTGATGCATTTAATTATGCAATAGCTCGTCA ************************************	883 900
IS1236_1 IS1236_6	GGGGTATCCAATGGGTGTCATGGTACATTCGGACCAAGGCTCACAGTACTGTAGTCGTGA AGGGTATCCAACGGGTATTATGGTGCATTCTGATCAAGGTCGTGAGTACTGTAGTCGTGA ********** **** ***** ***** ***** ******	943 960
IS1236_1 IS1236_6	TTTTAGGGCGCTATTATTGACGAATAACTGCGTTCAAAGTATGTCACGCAGCTTTAGACTGCTATTGTTGACGAATAACTGCCTTCCAAGCATGTCTAGACGACGACGCCTTGC	994 1020
IS1236_1 IS1236_6	GGGAAACTGTTGGGATAATGCAGTGACCGAAAGCTTCTTTCATACATTGAAAGGT GCAGTATACTGCTCATGGGATAATGCGGTGACGGAAAACTTCTTTCATACATTGAAAGGT * * **** * *************************	1049 1080
IS1236_1 IS1236_6	CATATGGTCCATGGCAGTGTGTTTGCCACTCGAAAAGAAGCGAATGCTGTCTTGTTTGAC CATGTGGTACACGGGAGTGTTTTACTACCCGAAAAGAAGCAAATGCTATCTTGTTTGAA *** **** ** ** *** **** *** * ** ******	1109 1140
IS1236_1 IS1236_6	TATATTGAGATTTATTACAATCGAATCAGACGACATTCTACAAACGGCTGGTTAAGCCCA TATATTGAAGTTTACTACAATCGAATTAGACGACATTCTGCAAATGATTGGTTAAGTCCT ******* **** *********** **********	1169 1200
IS1236_1 IS1236_6	GAAGCCTTTGAACAGAAATATTTCAAGAATTTAGAGGGATTTGTTGTCCACGATACTGTC GAGGCTTTTGAACTGAAATATTTTATAAATTTAGAGGGATCGGTTGTCCTCGATACTGTC ** ** ******* ******** * ************	1229 1260
IS1236_1 IS1236_6	TAGGATCA 1237 TAGGATCA 1268 *******	

FIG S1 Sequence alignment of IS1236_1 and IS1236_6. IS1236_1 is identical to four other intact chromosomal copies of IS elements in which two partially overlapping genes, *orfA* (blue text) and *orfB* (underlined text), encode transposase proteins (17). Sequences that encode the residues of the catalytic DDE motif are in bold red font. In IS1236_6, mutations prevent production of full-length OrfA and OrfB proteins. Due to frameshift, the identical aligned GAA nucleotides corresponding to the glutamate (E) residue in the DDE motif do not encode this amino acid in IS1236_6.





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).

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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).

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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	GTAGAATGGGTCGAGTCAGCAGATG	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	CAGGCCATGTGGTCGCGCTG	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	GACCATTCATACATTACTGCACGG	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	CGGTGCAGGTCAGGCCGCAG	ACN1101						
977490_DN	CCAGTAAATTCGCCAAACGTTCGC	ACN1101						
Southern Size St	andards (Fig. 7 and Table 3)							
2782954_UP	GCAGACAAACACTCGGTATGCGTTAC	ACN1056, ACN1057						
2784779_DN	AGGCATTAAATGAAGTCATTCCTAAATG	ACN1056, ACN1057						
2786049_DN	GAAAAAAAACAATAGTGAATTTTGCGAATATGC	ACN1056, ACN1057						
2787539_DN	TCACTGTTTCTACACCAGATTGAATTTTG	ACN1056						
2787602_DN	CACCTTCGCCTGTAAATACATAATCAGC	ACN1057						
2782750_UP	GCATGGTTATCCTCATAAAGTTTCGC	ACN1057						
2781188_UP	CTATTGGTTATGTCCCAACTTACGAAG	ACN1058						
2781264_UP	CACCTCTCAAAGCAAAGATCAATGG	ACN1058						
2783861_DN	TGATTTACACGCGCGGCAAGTGTATAAG	ACN1058						
2786690_DN	AATAAATGTGAAGGGGAATCAGTTTTAATC	ACN1058						
2785055_DN	GTAGTAGGGTTTTGTTACTAGGATCTGTG	ACN1058						
966684_UP	TTCGGTAAAAATCTTGCCTTG	ACN1101						
974759_UP	CAATATGCAATTGATAATGTCATG	ACN1101						
975990_UP	GCAACATCCACAGGATTTAAACG	ACN1101						
977490_DN	CCAGTAAATTCGCCAAACGTTCGC	ACN1101						

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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

 Table S2.
 Amplicon Anaylsis of Ben+ Mutants

^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.