

Table S1. The construction of the vectors.

System	Cassette	Vector	Restriction sites	Reaction
Bivector system	pMD18T-CB (pMD18T-ZmCTA1 _{pro} ::BBM)	pHB-CB-NW (pHB-ZmCTA1 _{pro} ::BBM-NOS _{pro} ::WUS)	<i>EcoRI</i> <i>HindIII</i>	ligase
	pMD18T- NW (pMD18T-NOS _{pro} ::WUS)		<i>HindIII</i> <i>BamHI</i>	ligase
	pMD18T-CB (pMD18T-ZmCTA1 _{pro} ::BBM)	pHB-CB-AW (pHB-ZmCTA1 _{pro} ::BBM-Axig1 _{pro} ::WUS)	<i>EcoRI</i> <i>HindIII</i>	ligase
	pMD18T- NW (pMD18T- Axig1 _{pro} ::WUS)		<i>HindIII</i> <i>BamHI</i>	ligase
	pMD18T-CB (pMD18T-ZmPLTP _{pro} ::BBM)	pHB-PB-AW (pHB-ZmPLTP _{pro} ::BBM-Axig1 _{pro} ::WUS)	<i>EcoRI</i> <i>HindIII</i>	ligase
	pMD18T- NW (pMD18T- Axig1 _{pro} ::WUS)		<i>HindIII</i> <i>XbaI</i>	ligase
	GFP ORF	pCA-GFP (pCA-35S _{pro} ::GFP)	<i>PstI</i>	recombination
Linkage vectors	pMD18T-CB (pMD18T-ZmCTA1 _{pro} ::BBM)	pHB-CB-NW-GFP (pHB-ZmCTA1 _{pro} ::BBM-NOS _{pro} ::WUS-35S _{pro} ::GFP)	<i>EcoRI</i> <i>HindIII</i>	ligase
	pMD18T- NW (pMD18T-NOS _{pro} ::WUS)		<i>HindIII</i> <i>BamHI</i>	ligase
	pCA-GFP (pCA-35S _{pro} ::GFP)		<i>HindIII</i>	recombination
	pMD18T-CB (pMD18T-ZmCTA1 _{pro} ::BBM)	pHB-CB-AW-GFP (pHB-ZmCTA1 _{pro} ::BBM-Axig1 _{pro} ::WUS-35S _{pro} ::GFP)	<i>EcoRI</i> <i>HindIII</i>	ligase
	pMD18T- NW (pMD18T- Axig1 _{pro} ::WUS)		<i>HindIII</i> <i>BamHI</i>	ligase
	pCA-GFP (pCA-35S _{pro} ::GFP)		<i>HindIII</i>	recombination
	pMD18T-CB (pMD18T-ZmPLTP _{pro} ::BBM)	pHB-PB-AW (pHB-ZmPLTP _{pro} ::BBM-Axig1 _{pro} ::WUS-35S _{pro} ::GFP)	<i>EcoRI</i> <i>HindIII</i>	ligase
	pMD18T- NW (pMD18T- Axig1 _{pro} ::WUS)		<i>HindIII</i> <i>XbaI</i>	ligase
	pCA-GFP (pCA-35S _{pro} ::GFP)		<i>HindIII</i>	recombination

Table S2. Primers used in this study

Purpose	Primer name	Sequence(5' to 3')
Vector Construction	GFP-f	ATGGTGAGCAAGGGCGAGGA
	GFP-r	TGTAATCGTACAGCTCGTCC
	AG-f	AAAGCAGCGATGGGAAGCTTATCTGTCACTTTATTGTGAA
	GP-r	TGTGGGGGATAGATAAGCTTATTTTCATACTCAACTACAA
	NG-f	CTCCGCTCATGATCAAGCTTATCTGTCACTTTATTGTGAA
	GC-r	TGTAATCGTACGCAAAGCTTATTTTCATACTCAACTACAA
	PS-Bbm-f	ACGGTCACATATGCGTCGACATGGCCACTGTGAACAACCTG
	CS-Bbm-f	GACACCTCAAATCAGTCGACATGGCCACTGTGAACAACCTG
	BS-Rbst-r	GATACGAACGAAAGGTGCGACTTAAGTGTCGTTCCAGACAC
	H-CTA1-f	AAGCTTTGCGTACGATTACATC
	CTA1-S-r	GTCGACTGATTTGAGGTGTCAC
	H-Nos-f	AAGCTTGATCATGAGCGGAGAATT
	Nos-S-r	GTCGACAGATCCGGTGCAGATTATT
	H-Axig1-f	AAGCTTCCCATCGCTGCTTTGTCT
	Axig1-S-r	GTCGACGGGATCACTTTCCCTTGAT
	H-PLTP-f	AAGCTTATCTATCCCCACAACCAC
	P-Sall-r	GTCGACGCATATGTGACCGTGTGTT
	Sm-Rbst-f	CCCGGGCTTTCGTTTCGTATCAT
	Rbst-E-r	CGGAATTCCATGTTGTCAATC
	Sa-Rbst-f	GTCGACCTTTCGTTTCGTATCAT
	Rbst-X-r	GCTCTAGACATGTTGTCAATCAA
	Sa-Rbst-f	GTCGACCTTTCGTTTCGTATCAT
	Rbst-B-r	GGATCCCATGTTGTCAATCAAT
Identifying Transgenic Corns	GFP-f	GGGCACAAGCTGGAGTACAA
	GFP-r	GAACGTTGTGCGAAACCGATGA
	WUS-f	AGATCGAGGGCAAGAACGTC
	WUS-r	CCGTCGTCGCCGCAG
	BBM-f	CAGGCCTGTACCACCCGT
	BBM-r	GCAGACACGACAGTCCCC
	Bar-f	GAAGTCCAGCTGCCAGAAAC
	Bar-r	GCACCATCGTCAACCACTAC
	HYP-f	CAAGACCTGCCTGAAACCGA
	HYP-r	ATTGCCGTCAACCAAGCTCT
Southern Blot	sGFP-f	CCTGAAGTTCATCTGCACCAC
	sGFP-r	CTTCTCGTTGGGGTCTTTGCT
Quantitative RT-PCR	qCTA1-f	AACCTCACTTGCTAGGCGAC
	qCTA1-r	TTTACTTTTCGCGAGGGTGC
	qGFP-f	GTACCCCGACCACATGAAG
	qGFP-r	TCTTGTAGTTGCCGTCGTCC
	Actin-f	TCCATCTTGGCATCTCTCAG
	Actin-r	GTACCCGCATCAGGCATCTG

Table S3. Summary data for Transformation Ratio of all trial groups.

Genotype	Design	Plasmid	Number of Immature Embryos	Number of T0 Plants	Transformation Ratio (%)
Zheng58	Three experimental groups for the bivector system test	pHB-NC	238	7	2.941176471
		pCA-GFP			
		pHB-AC	695	1	0.143884892
		pCA-GFP			
		pHB-AP	309	0	0
		pCA-GFP			
	Three experimental groups for the linkage vector test	pHB-NGC	302	0	0
		pHB-AGC	315	0	0
		pHB-AGP	305	0	0
	Control	pCA-GFP	319	0	0
Mo17	Three experimental groups for the bivector system test	pHB-NC	501	0	0
		pCA-GFP			
		pHB-AC	301	2	0.664451827
		pCA-GFP			
		pHB-AP	505	0	0
		pCA-GFP			
	Three experimental groups for the linkage vector test	pHB-NGC	309	0	0
		pHB-AGC	301	0	0
		pHB-AGP	509	8	1.571709234
	Control	pCA-GFP	189	0	0
Chang7-2	Three experimental groups for the bivector system test	pHB-NC	514	0	0
		pCA-GFP			
		pHB-AC	508	0	0
		pCA-GFP			
		pHB-AP	523	0	0
		pCA-GFP			
	Three experimental groups for the linkage vector test	pHB-NGC	126	0	0
		pHB-AGC	692	4	0.578034682
		pHB-AGP	200	0	0
	Control	pCA-GFP	202	0	0
B73	Three experimental groups for the bivector system test	pHB-NC	502	0	0
		pCA-GFP			
		pHB-AC	506	0	0
		pCA-GFP			
		pHB-AP	504	0	0
		pCA-GFP			
	Three experimental groups for the linkage vector test	pHB-NGC	306	0	0
		pHB-AGC	311	0	0
		pHB-AGP	309	0	0

	Control	pCA-GFP	301	0	0
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There are four inbred lines of Zheng58, Mo17, Chang7-2 and B73. Embryos were separated from the seeds of 14-16 day after Self-Pollination of inbred lines. Number of T0 plants is a quantity of successful transgenic corns. Transformation Ratio is calculated by number of T0 plants. [(number of T0 plants ÷ number of embryos) × 100].