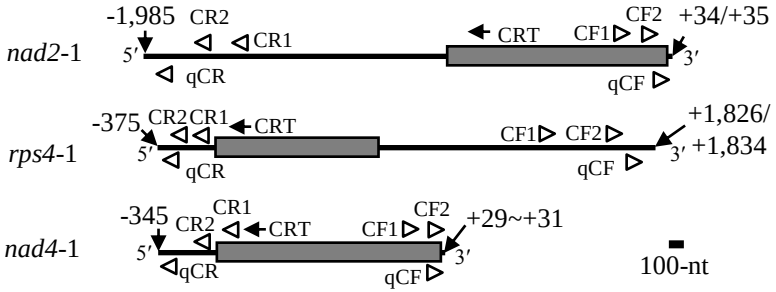

Figure S1: Position of primers for representative maize mitochondrial transcripts. CRT: reverse transcription primer; CF1, CF2, CR1, and CR2: divergent primers for PCR amplification; qCF and qCR: divergent primers for RT-qPCR. The transcript termini of maize *nad2*-1, *rps4*-1, and *nad4*-1 have been determined previously (Zhang *et al*., 2019). Positions of 5'- and 3'-ends relative to AUG (+1) and the last nucleotide of stop codon (-1) are shown. The coding regions and UTRs are indicated as gray boxes and bold lines, respectively.

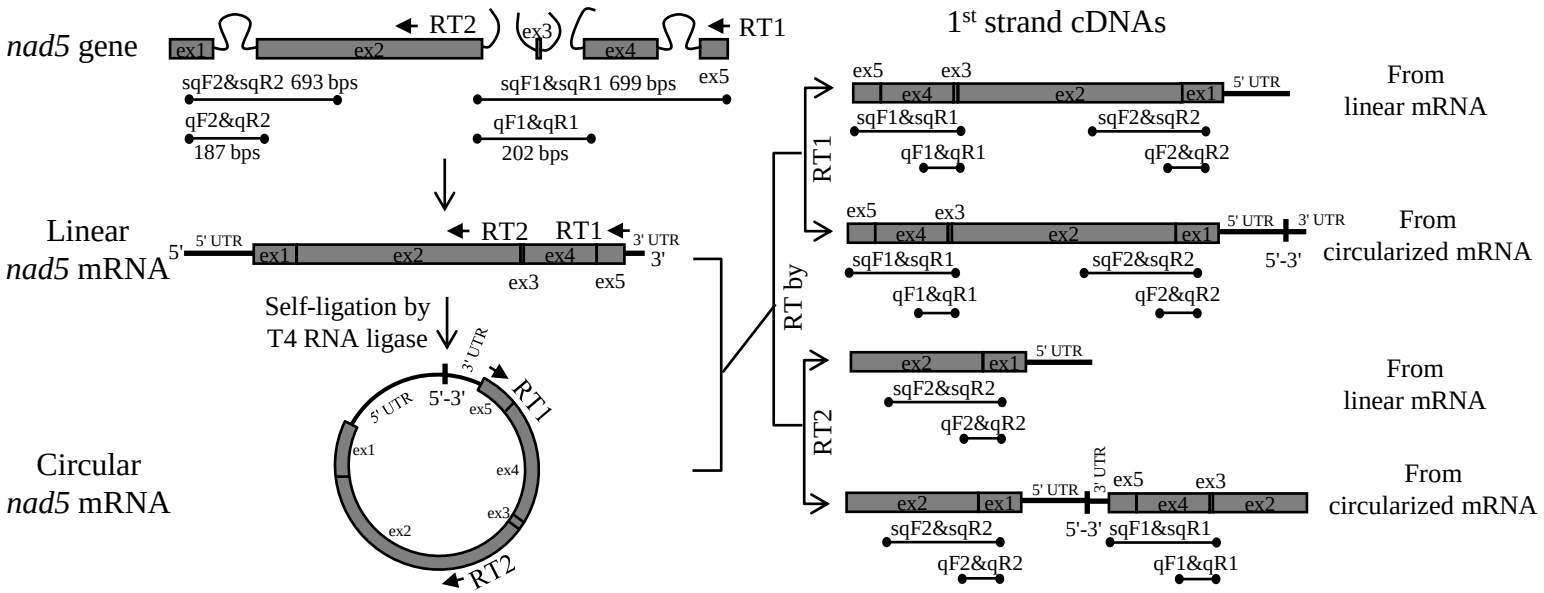

Figure S2: Principle to estimate circularization efficiency of *nad5* mRNA in maize. In a self-ligation reaction, only a fraction of mitochondrial RNAs is circularized. To calculate the ratio of circularized *nad5* mRNA, two gene-specific primers are used to synthesize the first strand cDNAs, i.e. *nad5*-RT1 and -RT2. In *nad5*-RT2 reverse transcription (RT) reaction, the PCR products amplified by sqF2&sqR2 (for RT-sqPCR) and qF2&qR2 (for RT-qPCR) are derived from both linear and circularized *nad5,* while the sqF1&sqR1 (for RT-sqPCR) and qF1&qR1 (for RT-qPCR) PCR products are derived from circularized *nad5* only; in *nad5*-RT1 reaction, all four pairs of PCR primers could amplify both forms of *nad5*. To calculate the ratio of circularized *nad5* mRNA, the two reactions are normalized by sqF2&sqR2 or qF2&qR2 PCR products. By comparing the abundance of sqF1&sqR1 or qF1&qR1 PCR products between the two RT reactions, the circularization efficiency of *nad5* mRNA is roughly estimated. ex: exon. Exons and introns are shown as gray boxes and curved lines, respectively. The positions of *nad5*-RT1 and -RT2 primers are indicated by arrows. Black dots indicate the positions of PCR primers, and the predicted size of the PCR products is shown.

Table S1: Primer information.

Primer name	Primer sequences (5'-3')	Use of Primer
cox2-CRT	TCATAGGTGTTGCTGCGTC	RT primers to prepare the cDNAs for normalization by *26S* rRNA and mapping of *cox2* transcript termini
26S-CRT	GAGGAATACTTAGGCTTAGAGG	
nad5-CRT	TCACTACGGTCAGGCTATC	
nad6-CRT	ATTGAACATCATAACCACGA	
nad7-CRT	GCTGAAGAATGAGCGTGTTC	
nad9-CRT	GATCGAAACTTGAACCCTTG	
cob-CRT	ACAACTCCGAGACACCAAAC	
cox1-CRT	TCCACGCATGTTGAAGATAG	
cox2-CF1	GTCGTTCAAATCTTACCTCCAT	cRT-PCR amplification of cox2-1 and -2
cox2-CF2	GCCTATCGTCGTAGAAGCAG	cRT-PCR amplification of cox2-1 and -2
cox2-CF3	CTGAAGCGGAAATGCA	cRT-PCR amplification of cox2-3 and -4
cox2-CR1	CAAAGAGCGATTGTGAGG	cRT-PCR amplification of cox2-1 and -2
cox2-CR2	AGCCAGGGTCCCATAAC	cRT-PCR amplification of cox2-3 and -4
cox2-qCF1	CTGAAGCGGAAATGCA	RT-qPCR amplification of cox2-1 to -3
cox2-qCF2	TTTAAGGCCGACCACTAC	RT-qPCR amplification of cox2-4
cox2-qCR1	GGGCTCGTCCTGTATCA	RT-qPCR amplification of cox2-1
cox2-qCR2	CTTTGTATCTGTGCTATTTCG	RT-qPCR amplification of cox2-2
cox2-qCR3	GGAGACTGAACACCGACAC	RT-qPCR amplification of cox2-3 and -4
26S-CF1	TCGCCGATGAAAGTGG	cRT-PCR amplification of *26S* mature rRNA
26S-CR1	CCAATCCACAACAAATCGA	
26S-qCF1	TGGTATGGAAGAACTGCTG	RT-qPCR amplification of *26S* mature rRNA
26S-qCR1	CAAAGAGCGCAGACTAGC	
cox2-probeF	GAATTCCAGCCATTACTATCAAAGC	To amplify the DNA fragment for preparation of *cox2* RNA probe
cox2-probeR	CCAATCCGCATAATCTTTC	
M13F	TGTAAAACGACGGCCAGT	Vector primers for colony PCR to screen positive clones containing the target inserts
M13R	CAGGAAACAGCTATGAC	
nad5-RT1	GTCCTGGCAAGCTCCTACA	To calculate the circularization efficiency of *nad5* mature mRNA by RT-sqPCR and RT-qPCR
nad5-RT2	CCGAACCCGCACTCAG	
nad5-sqF1	CATTCGGGCGAGACAG	
nad5-sqF2	GGATCTGAAGGAACCGCT	
nad5-sqR1	GTCCTGGCAAGCTCCTACA	
nad5-sqR2	GCCAACCTCCTGGAAAGAG	
nad5-qF1	CATTCGGGCGAGACAG	
nad5-qF2	GGATCTGAAGGAACCGCT	
nad5-qR1	GTTGGAGCAGCAAACTCG	
nad5-qR2	GATAGCCTGACCGTAGTGA	
nad1-RT1	GCCCCCTTCAGAAGAAACTT	To calculate the circularization efficiency of *nad1* mature mRNA by RT-sqPCR and RT-qPCR
nad1-RT2	CTCGAATTACAGGGACCTAC	
nad1-sqF1	GGTTATGTTCCTTATTCCTCGTC	
nad1-sqF2	GGCCCGATCATGAGTGAATA	
nad1-sqR1	GCCCCCTTCAGAAGAAACTT	
nad1-sqR2	ACCATTTGAGCTGCAGATCG	
nad1-qF1	GGTTATGTTCCTTATTCCTCGTC	
nad1-qF2	GCTACATTTATGTTAAGTCTGG	
nad1-qR1	GCCAACCTCCTGGAAAGAG	
nad1-qR2	ACCATTTGAGCTGCAGATCG	