Supplementary data for

Developing a Transformation-Independent and Unbiased qPCR Assay to Rapidly Evaluate the Determinants of DNA Assembly Efficiency

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Table S1

Primers used for quantification.

Primers [a]	Sequence 5′→3′
q-RL1-F	acggcgtcttgtgtggtcagag
q-RL1-R	gcatgttaacggcgttgctcgg
q-RL23-F	aacgggcaactgcgtgatgtga
q-RL23-R	tggtggtggcggaacggtactt
q-RL45-F	ccgcagctacgagccgttaaca
q-RL45-R	accgccgtcaccagagacagaa
q-RL67-F	agccgaatagcctctccaccca
q-RL67-R	ggctttcttgccgccaaggatc
q-GG-F	tacctgtccgccttttctcccttcg
q-GG-R	cctcgctctgctaatcctgttacca
q-GA1-F	cggttatggtgttcaatgctttgcg
q-GA1-R	acgtgtcttgtagttcccgtcatct
q-GA2-F	atgccgtcgttggaactgctttgat
q-GA2-R	ggcgtttgcgaatctctccatttgc
q-GA3-F	ggctggcttaactatgcggcatca
q-GA3-R	accctggcgttacccaacttaatcg
q-GA4-F	actttctgacgccgatgctgtttcc
q-GA4-R	tgccagtgccgatgcttcaatgac
q-GA5-F	gcgggatttcagcagcaactcagt
q-GA5-R	attaagcacgtcaacctccggcatc
q-GA6-F	ctgatgccaacgaagcggatgtag
q-GA6-R	tcactgtgctgctccaccagatc
q-Joint-F	acaatacgaatcggctgtcgcacg
q-Joint-R	ttcaccctcgccaacgcacttaaag
q-P1-F	tgccggaggcgtgatcaatgtc
q-P1-R	ccatgcggtctgctgcgagata
q-BP1-F	cagcaggacgcactgaccgaatt
q-BP1-R	tcttccgaagcgtaagtccacatgc
q-B-F	tcgacgccgcttcgttctacca
q-B-R	gcacaacaactggcgggcaaac
q-purple-F	acggcgtcttgtgtggtcagag
q-purple-R	gcatgttaacggcgttgctcgg

[a] Primers q-RL1-F to q-RL67-R were used to quantify the DNA fragments in restriction ligation. Primers q-GG-F and q-GG-R were used to quantify the DNA fragments in Golden Gate assembly. Primers q-GA1-F to q-GA6-R were used to quantify the DNA fragments in Gibson assembly. Primers q-Joint-F, q-Joint-R,

q-purple-F, and q-purple-R were used for the measurement of the fidelity of Gibson assembly. Primers q-P1-F to q-B-R were used to quantify the DNA fragments in multi-part assembly. Primers q-purple-F and q-purple-R were used to study the effects of terminal secondary structures on Gibson assembly. Primers q-RL1-F and q-purple-F were identical, and primers q-RL1-R and q-purple-R were also identical.

Table S2
Primers for the amplification of DNA fragments with secondary structures in the overlap regions.

Primers [a]	Sequence 5′→3′
OL-I-F1	gtctgatggctggtctctgaGTCGCCCTTAATTGTGAGCG
OL-I-F2	gtatgatgcgtgcattatacGTCGCCCTTAATTGTGAGCG
OL-I-F3	ggaagctgcgtgcagccactGTCGCCCTTAATTGTGAGCG
OL-I-F4	gtctgatgcgtgaatcagatGTCGCCCTTAATTGTGAGCG
OL-I-F5	gtctgatgcgtgcatcaggcGTCGCCCTTAATTGTGAGCG
OL-I-F6	ggtggctgagtgcagcctcgGTCGCCCTTAATTGTGAGCG
OL-I-F7	gcctgatgcgtgcatcaggtGTCGCCCTTAATTGTGAGCG
OL-I-F8	gtcggatgcgtgcatccgatGTCGCCCTTAATTGTGAGCG
OL-I-F9	gtctgatgcgtgcatcagacGTCGCCCTTAATTGTGAGCG
OL-I-F10	gtcggatgcgtgcatccgacGTCGCCCTTAATTGTGAGCG
OL-b-R1	tcagagaccagccatcagacGGAAGAGCGCCCAATACGC
OL-b-R2	gtataatgcacgcatcatacGGAAGAGCGCCCAATACGC
OL-b-R3	agtggctgcacgcagcttccGGAAGAGCGCCCAATACGC
OL-b-R4	atctgattcacgcatcagacGGAAGAGCGCCCAATACGC
OL-b-R5	gcctgatgcacgcatcagacGGAAGAGCGCCCAATACGC
OL-b-R6	cgaggctgcactcagccaccGGAAGAGCGCCCAATACGC
OL-b-R7	acctgatgcacgcatcaggcGGAAGAGCGCCCAATACGC
OL-b-R8	atcggatgcacgcatccgacGGAAGAGCGCCCAATACGC
OL-b-R9	gtctgatgcacgcatcagacGGAAGAGCGCCCAATACGC
OL-b-R10	gtcggatgcacgcatccgacGGAAGAGCGCCCAATACGC
OL-I-R1	aagccagcgaccacccagccCCATGCCGAACTCAGAAGTG
OL-I-R11	gttcacgattcatcgtggacCCATGCCGAACTCAGAAGTG
OL-I-R12	gttcgcgattcatcgcggacCCATGCCGAACTCAGAAGTG
OL-b-F1	ggctgggtggtcgctggcttGCTTCCTCGCTCACTGACTCG
OL-b-F11	gtccacgatgaatcgtgaacGCTTCCTCGCTCACTGACTCG
OL-b-F12	gtccgcgatgaatcgcgaacGCTTCCTCGCTCACTGACTCG

[a] Small letters indicate overlap sequences. Primers OL-I-F4 and OL-b-R4 were also used for the amplification of the fragments used in the assembly OL11 and OL12. Primers OL-I-R1 and OL-b-F1 were used for the amplification of the fragments used in the assembly OL1–OL10.

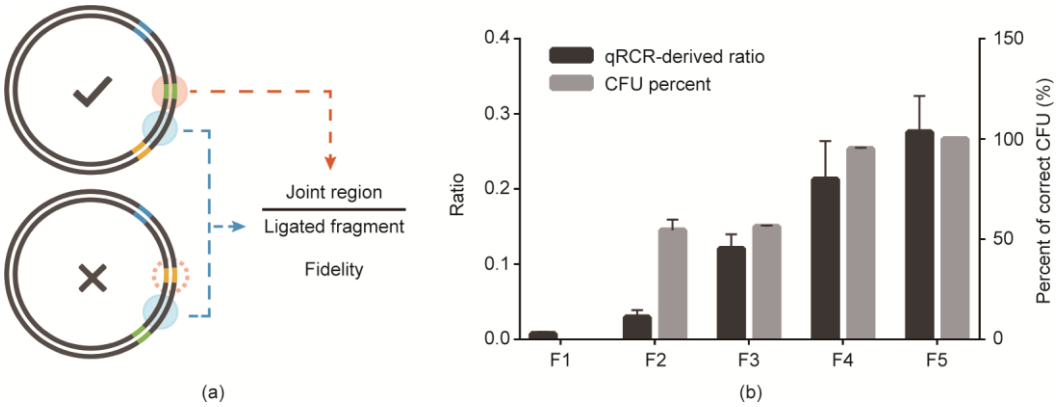

Fig. S1. (a) A modified qPCR assay for the measurement of the fidelity of DNA assembly. (b) The fidelity of five sets of Gibson assembly determined by both the CFU-based measurement and the alternative qPCR assay.