Supplemental data: Preparation of DNA samples

The DNA samples were prepared using PCR conducted with a Light Cycler (Roche Diagnostics). The vector pGEX-4T3 plasmid (GE Healthcare) was used as the DNA template. The primers are listed as the follows:

TK4E-69-FW (5'-tac ggt gtt tcg aga att gc-3')
TK4E-69-RV (5'-cat gaa gtc agg atg ggt tac a-3')
and
TK4E-83-FW (5'-ata gca tgg cct ttg cag-3')
TK4E-83-RV (5'-cgc tac gtg act ggg tca t-3')

The real-time PCR followed the protocol recommended in the LightCycler® DNA Master SYBR Green I (Roche) documents. The detailed parameters are listed in Tables 1 & 2

Table 1: Reagents for real-time PCR

Reagents	Volume (μL)
Light Cycler FastSmart DNA Master	7.5
Forward primer (10 μM)	0.75
Reverse primer (10 μM)	0.75
H_2O	6.0
DNA template	5.0
Total volume	20.0

Table 2: Parameters for real-time PCR

Cycle No	Cycle step	Temperature (^{0}C)	Duration
30–40	Precondition	95	10 min
	Denaturation	95	10 s
	Annealing	60	30 s
	Extension	72	15 s

The first pair yielded a DNA with a T_m of 78 ^{0}C and 89 ^{0}C for the latter, as measured by the LightCycler at a scanning rate of 0.10 ^{0}C/s as a benchmark. (see Fig. S1 below):