Electronic Supplementary Material (ESI) for Analyst.

Nucleic Acid Amplification Test (NAAT) Conducted in a Microfluidic Chip to Differentiate Between Various Ginseng Species

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Supporting Information

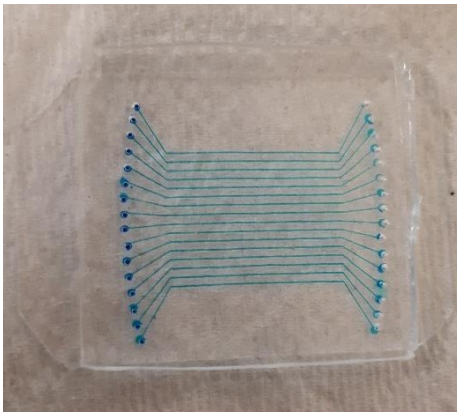

Figure S1: The microfluidic PDMS-glass chip with the PDMS slab sealed on a glass slide (50 mm × 75 mm) and filled with blue dyed solution for illustration.

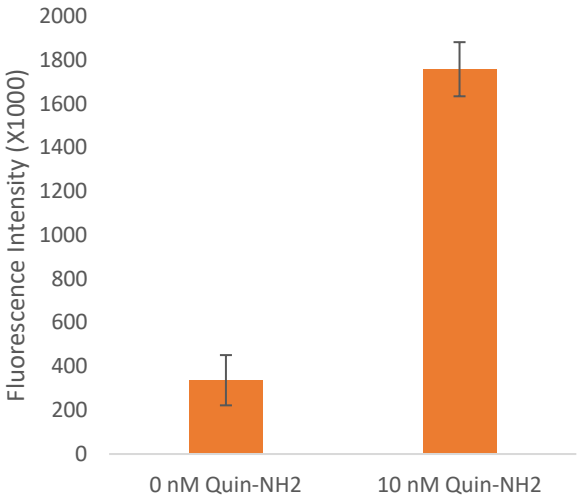

Figure S2: The effect of adding free-solution Gin-NH_2 or Quin-NH_2 in the ligation mixture. Quin-NH_2 (0 or 10 nM) were added to the ligation mixture, to be compared with controls containing no free-solution Quin-NH_2. The mixture was incubated for 4 h. AmG2 genomic templates (1.2 ng/µL), 100 CEU/µL NEB ligase and 10 mM Mg^{2+} were used for all ligation reactions.

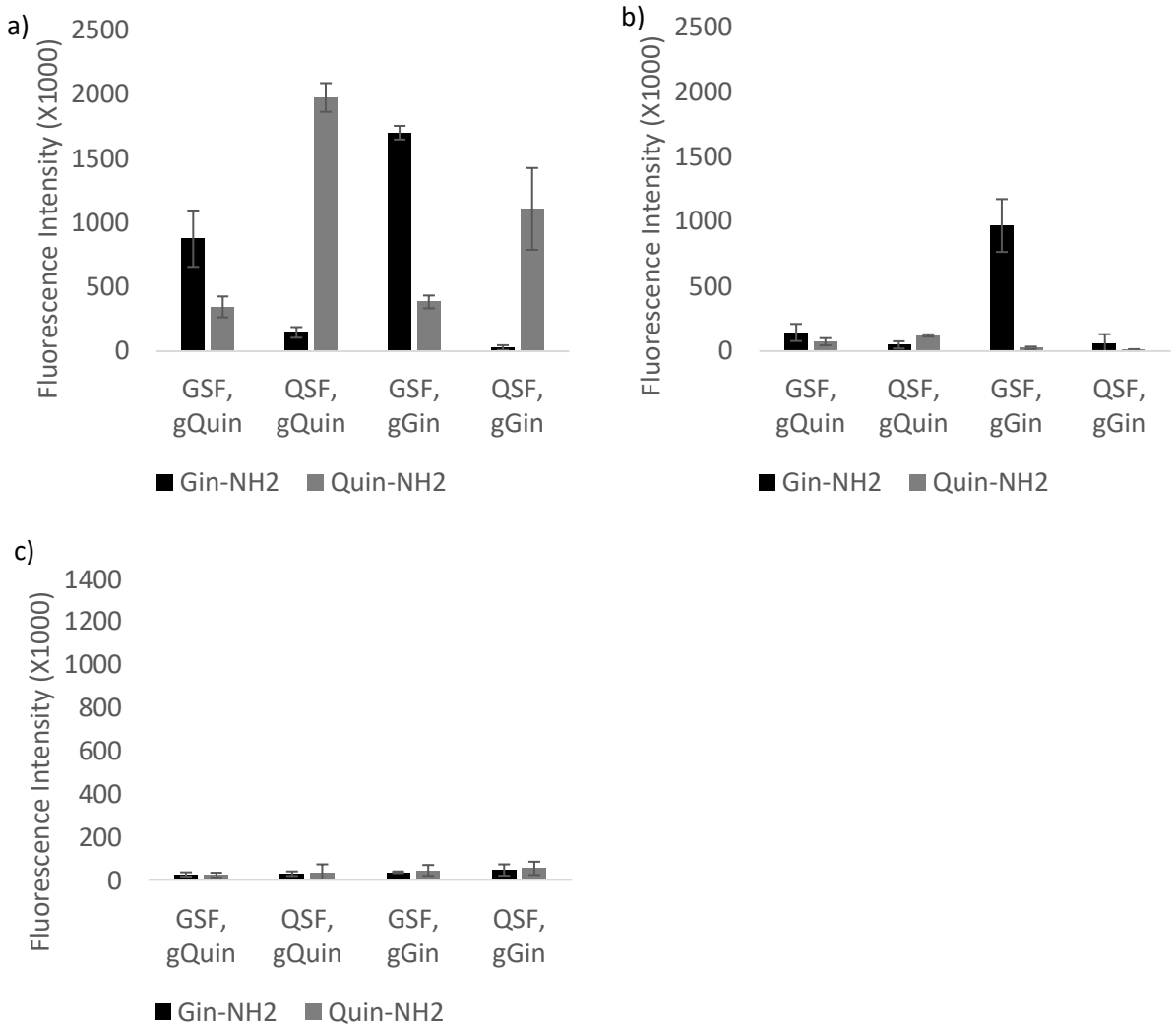

Figure S3: Effect of NEB Ligase Concentration in LIDA. Signal intensities obtained in LIDA using a) 100 CEU/µL, b) 75 CEU/µL and c) 50 CEU/µL concentrations of NEB ligase.

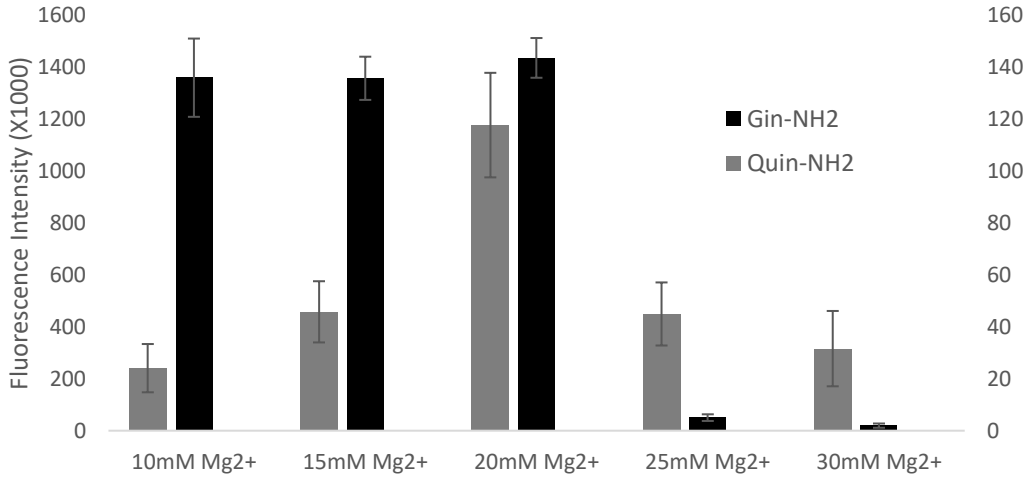

Figure S4: Effect of [Mg^{2+}] on LIDA. The ligation buffer already contains 10 mM Mg^{2+} (for 1x buffer concentration) and so additional Mg^{2+} was added to the buffer to result in the final concentrations of 15, 20, 25 and 30 mM. The black bars of signal on the Gin-NH_2 lane when GSF/gGin were used are measured using the left vertical axis and the grey bars of signal on the Quin-NH_2 lane when QSF/gQuin were used are measured using the right vertical axis.

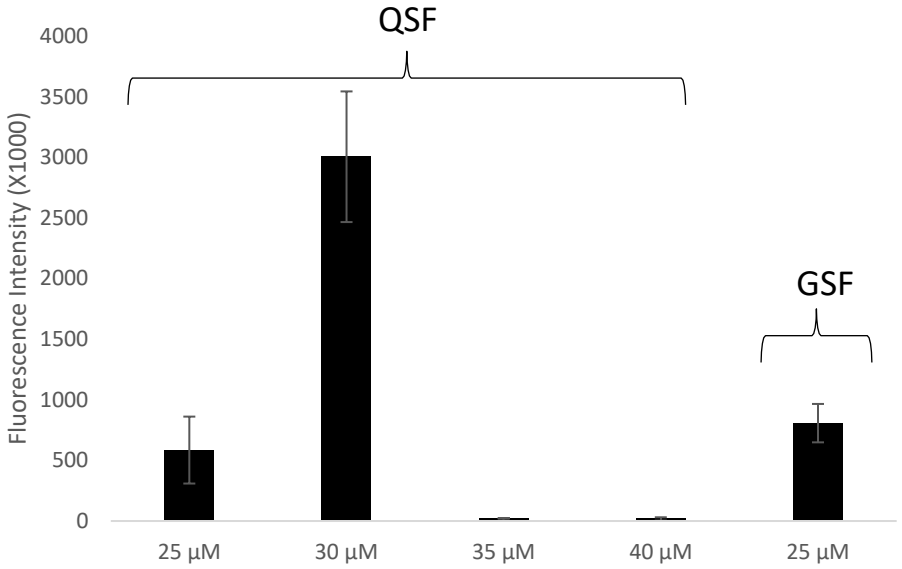

Figure S5: The effect of [QSF] on gQuin/Quin-NH_2 signals. gQuin (1.0 pg/µL) was used as the template; Quin-NH_2 was added; 100 CEU/µL ligase was used with 4 h incubation. The last bar pertains to 25 µM GSF used with 1.0 pg/µL gGin on Gin-NH_2 lanes.

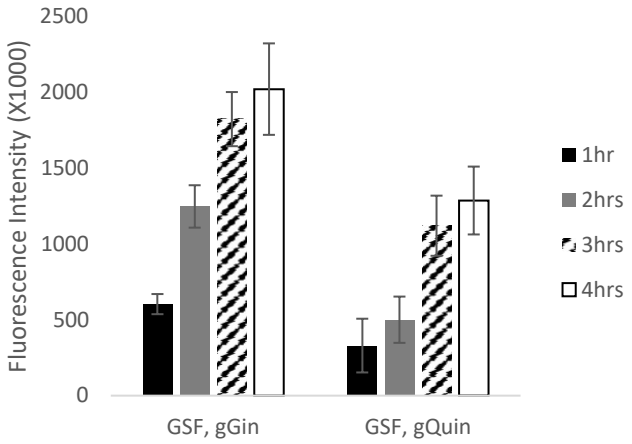

Figure S6: Optimization of LIDA reaction time for TF ligase. Signal intensities obtained in LIDA from different reaction times for GSF-containing lanes with either gGin or gQuin template. The ligation reactions contain Gin-NH_2 (10 nM), GSF (30 μM), Det (25 μM), Frag (25 μM), $MgCl_2$ (20 mM) and 1.25 U/μL TF ligase.

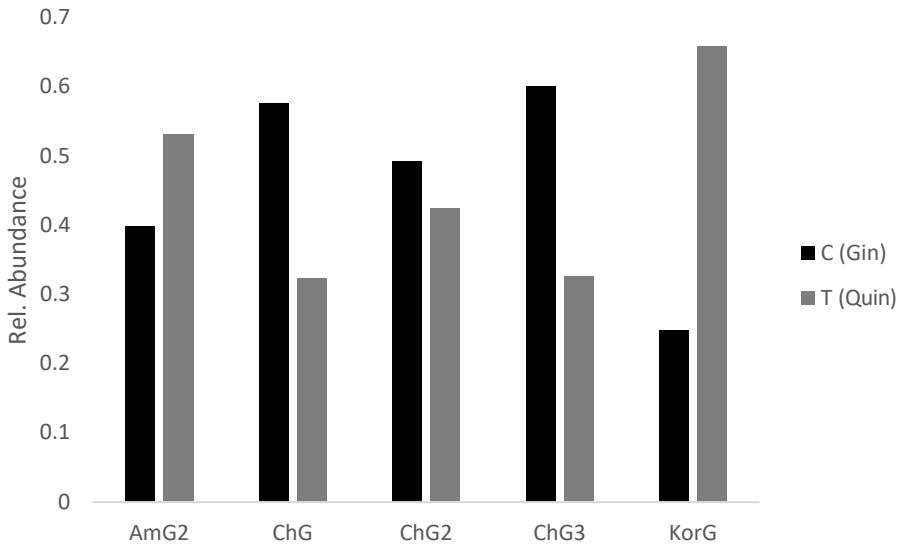

Figure S7: The relative abundances of N2 SNP site which is C (*P. ginseng*) or T (*P. quinquefolius*) in the five samples: AmG2, ChG, ChG2, ChG3 and KorG. BWA was used to align the sequences in the FASTQ files.

To decide on which SNP site to use, DINAMelt[42] was used to predict the Tm value of each of the DNA fragments used in the 2016 report (both perfectly complementary and 1 bp mismatch).[34] Next, similar length of DNA fragments corresponding to the N1 and N2 SNP sites in the DS gene were designed on DINAMelt,[42] see Table S1 for more details. The fragments corresponding to the N2 site had the most similar Tm values of the ligated product (green-colored entry #2 in Table S1c) to those used previously (entry #2 in Table S1a).[34] Even though the Tm values of three of the remaining four green coloured entries are higher for N2 than for the U of A sequences,[34] these N2 sequences are associated with the hybridization of the short fragments meaning that a higher Tm value is a good feature. On the other hand, the differences in Tm values between pm and mm are higher in N2 than in N1, indicating the higher differentiation capability of the former SNP site. Therefore, N2 was the locus chosen for the solid-phase LIDA reaction.

Table S1*:* Tm calculations used to decide on N2 instead of N1 for designing LIDA oligonucleotides. The table contains a list of all the duplexes that can form among the various DNA fragments and templates for the sequences used by Kausar *et al* at U of A (a),[32, 34] by the proposed ginseng N1 site (b) and by the proposed ginseng N2 site (c). "Temp" referred to the *P. ginseng* sense template that helped produce the ligated product of "CompSNP Frag pm" and "CompAbFrag"; "AbCompTemp" refers to this ligated product. "SNP Frag mm" and "CompSNP Frag mm" are similar sequences to "SNP Frag pm" and "CompSNP Frag pm", respectively containing a single mismatching nt each, and "CompTemp" is a perfectly complementary sequence to "Temp". "Ab" in any of the names indicates that the strand contains an abasic position. When the N2 site is used in case c, Temp and CompTemp are the 17 nt sense strand around N2 of *P. ginseng* and *P. quinquefolius*, respectively; CompSNP Frag pm is Gin-NH_2 and CompSNP Frag mm is Quin-NH_2; CompAbFrag is Det; SNP Frag mm is QSF and SNP Frag pm is GSF. Finally, Frag is the same in either the N1 or N2 site (see Table 2). The Tm values highlighted in green pertain to the most relevant duplexes in the amplification reaction. Since DINAMelt interprets the abasic positions as a conventional mismatching bp, the Tms of the "Temp-AbCompTemp" duplexes are overestimated[37] for all three loci.

a) UofA Sequences	10mM Na, Mg, 37°C			
	ΔG (kcal/mol)	ΔH (kcal/mol)	ΔS (cal/molK)	Tm (°C)
Temp-CompTemp	-13.2	-129.6	-375.3	50.1
Temp-AbCompTemp	-12	-115.2	-332.8	48.3
Temp-Comp Frag	-6.4	-65.9	-191.9	29.8
Temp-CompAb Frag	-5.7	-59	-171.8	25.7
Temp-CompSNP Frag pm	-5.1	-55.2	-161.7	21.6
Temp-CompSNP Frag mm	-3.8	-44.9	-132.5	10.8
ComTemp-Frag	-5.3	-58.3	-170.9	23.5
ComTemp-SNP Frag pm	-5.8	-60.2	-175.4	26.3
AbComTemp-SNP Frag pm	-5.1	-55.2	-161.7	21.6
ComTemp- SNP Frag mm	-4.2	-47.8	-140.5	14.7
AbComTemp-SNP Frag mm	-4.2	-47.8	-140.5	14.7
b) Prop Ginseng Sequences (N1)	10mM Na, Mg, 37°C			
	ΔG (kcal/mol)	ΔH (kcal/mol)	ΔS (cal/molK)	Tm (°C)
Temp-CompTemp	-14.4	-128.8	-348.8	53.4
Temp-AbCompTemp	-12.7	-113	-323.5	50.5
Temp-Comp Frag	-6.2	-62.6	-181.7	28.8
Temp-CompAb Frag	-5.4	-54.7	-159.1	23
Temp-CompSNP Frag pm	-5.5	-51	-146.6	22.9
Temp-CompSNP Frag mm	-4.9	-43	-123	16.2
ComTemp-Frag	-5.1	-57.6	-169.2	22.6
ComTemp-SNP Frag pm	-6.4	-59.2	170.2	29.2
AbComTemp-SNP Frag pm	-5.5	-51	-146.6	22.9
ComTemp-SNP Frag mm	-4.9	-43	-123	16.2
AbComTemp-SNP Frag mm	-4.9	-43	-123	16.2
c) Prop Ginseng Sequences (N2)	10mM Na, Mg, 37°C			
	ΔG (kcal/mol)	ΔH (kcal/mol)	ΔS (cal/molK)	Tm (°C)
Temp-CompTemp	-14.5	-127.4	-364.1	53.7
Temp-AbCompTemp	-12	-110.8	-318.6	48.7
Temp-Comp Frag	-6.2	-50.3	-142.3	26.4
Temp-CompAb Frag	-5.1	-52.9	-154.2	20.9
Temp-CompSNP Frag pm	-5.8	-66.1	-194.5	27.1
Temp-CompSNP Frag mm	-4.1	-52.6	-156.4	15.8
ComTemp-Frag	-5.7	-54.8	-158.4	24.6
ComTemp-SNP Frag pm	-6.6	-68.2	-198.5	31.1
AbComTemp-SNP Frag pm	-5.8	-66.1	-194.5	27.1
ComTemp-SNP Frag mm	-4.1	-52.3	-155.5	15.6
AbComTemp-SNP Frag mm	-4.1	-52.3	-155.5	15.6