Supplementary Material

A Hairpin DNA-Based Piezoelectric E-Nose: Exploring the Performances of Heptamer Loops for the Detection of Volatile Organic Compounds

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A	Alcohols	Aldehydes	Esters	Hydrocarbons	Ketones
	ssDNA pentamer binding score (Kcal/mol)				
Max	-2.39	-1.55	-1.58	-1.63	-1.24
Min	-4.78	-3.89	-4.00	-4.43	-3.16
Median	-3.74	-2.87	-2.88	-3.09	-2.21
Average	-3.75	-2.91	-2.92	-3.11	-2.24
	ssDNA hexamer binding score (Kcal/mol)				
Max	-2.47	-1.64	-1.68	-1.62	-1.22
Min	-5.55	-4.69	-4.77	-5.35	-3.96
Median	-4.12	-3.21	-3.23	-3.45	-2.45
Average	-4.07	-3.17	-3.19	-3.40	-2.42
	ssDNA heptamer binding score (Kcal/mol)				
Max	-2.44	-1.83	-1.84	-1.84	-1.18
Min	-6.59	-5.34	-5.24	-5.99	-4.25
Median	-4.36	-3.41	-3.41	-3.71	-2.62
Average	-4.34	-3.41	-3.41	-3.68	-2.62

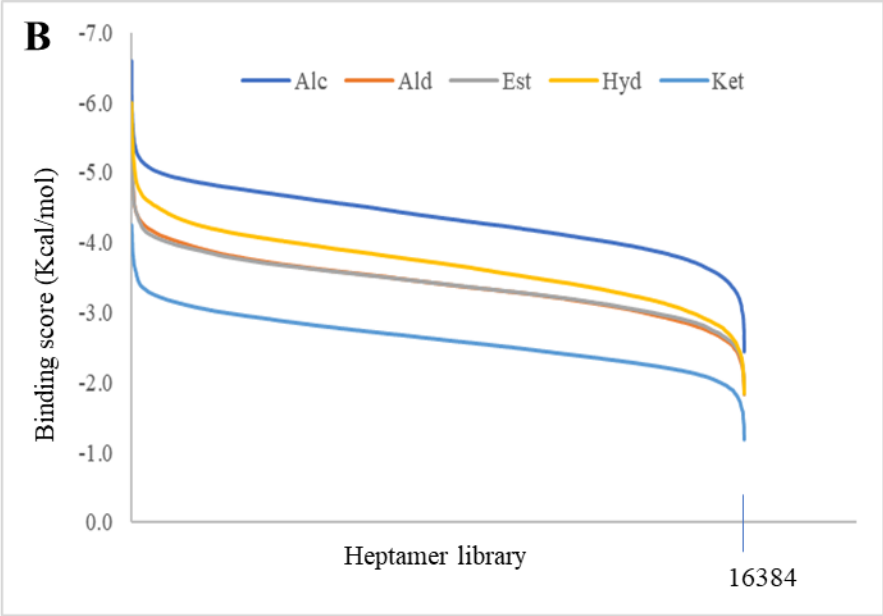

Figure S1. (**A**) Statistical summary (maximum, minimum, median and average) of the binding score calculated using ssDNA pentamer, hexamer (Mascini et al. 2019) and heptamer libraries toward the five chemical classes tested. The score values were calculated using chemgauss4 scoring function, thus lower values represented higher ssDNA–ligand affinity. (**B**) The binding score trend of the ssDNA heptamer library for the five chemical classes tested. The data were sorted in ascending order of score, thus not necessarily a correspondence must exist between the positions of the ssDNA in each curve.

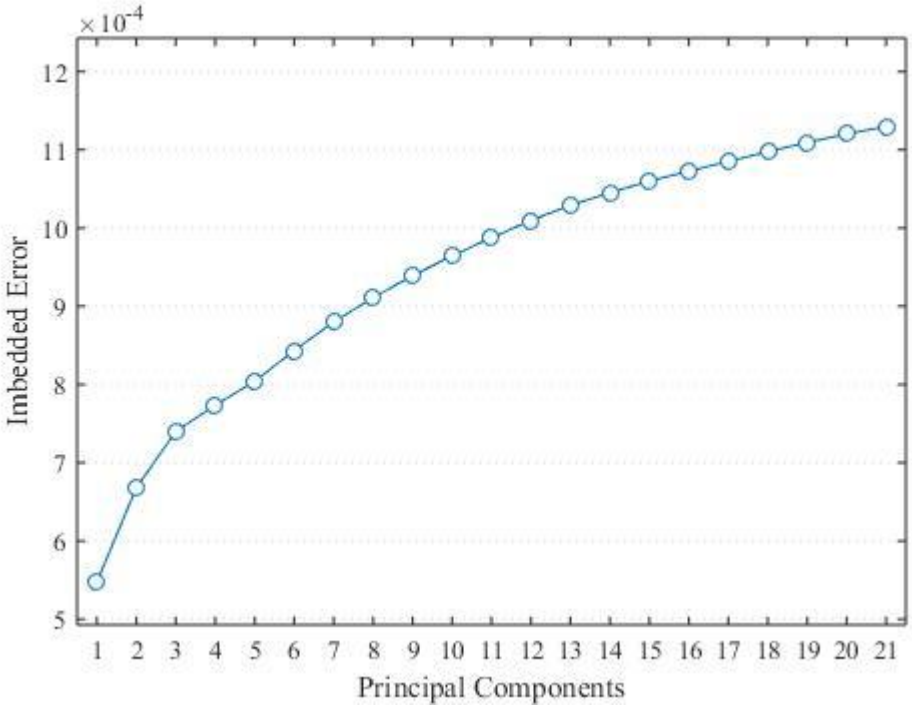

Figure S2. The imbedded Error calculated from the "Optimal number of Principal Components" toolbox [23] obtained from the matrix of 16384 rows (the ssDNA heptamer library) and 55 columns (the 50 VOCs plus the average of the five chemical classes).

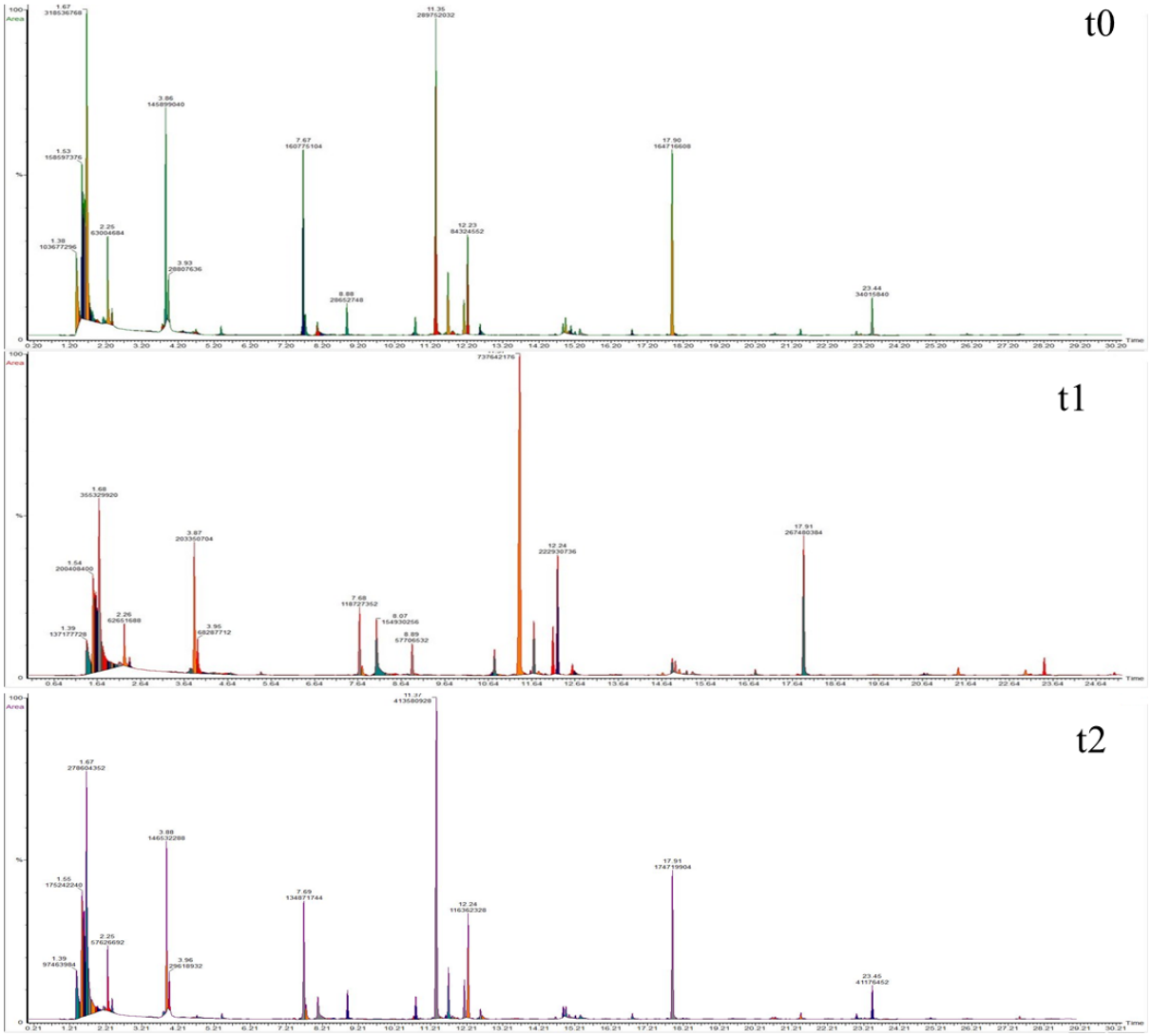

Figure S1. The three different chromatograms of beer fermentation for comparison among the aroma profiles. The chromatogram at time zero of fermentation (t0; raw beer), the chromatogram after 20 days of fermentation (t1), and the chromatogram after 40 days of fermentation (t2).

Table S1. The 50 volatile organic compounds (VOCs) belonging to five chemical classes (12 alcohols, 13 aldehydes, 17 esters, 5 hydrocarbons and 3 ketones) used as the targets to calculate the binding score values of the ssDNA heptamer library. Name, functional group, label, molecular weight, polar surface area and hydrophobicity (LogP) are also reported.

Name	Functional group	Label	MW	PSA	LogP
(1S,2R,5R)-2-isopropyl-5-methyl-cyclohexanol	Alcohol	A1	156	20	3.2
(2S)-propane-1,2-diol	Alcohol	A2	76	40	-1.34
(2Z)-3,7-dimethylocta-2,6-dien-1-ol	Alcohol	A3	154	20	3.28
(3R)-3,7-dimethylocta-1,6-dien-3-ol	Alcohol	A4	154	20	3.28
(3R,6Z)-3,7,11-trimethyldodeca-1,6,10-trien-3-ol	Alcohol	A5	222	20	4.39
(3S)-3,7-dimethyloct-6-en-1-ol	Alcohol	A6	156	20	2.75
(3S)-oct-1-en-3-ol	Alcohol	A7	128	20	2.11
(4S,4ar,8ar)-4,8a-dimethyldecalin-4a-ol	Alcohol	A8	182	20	3.11
3-methylbutan-1-ol	Alcohol	A9	88	20	1.16
Ethanol	Alcohol	A10	46	20	-0.16
Hex-3-en-1-ol	Alcohol	A11	100	20	0.75
Hexan-1-ol	Alcohol	A12	102	20	1.85
(2E,6Z)-nona-2,6-dienal	Aldehyde	D1	138	17	2.48
(2S)-2-methylbutanal	Aldehyde	D2	86	17	1.23
(2Z)-3,7-dimethylocta-2,6-dienal	Aldehyde	D3	156	17	2.87
(3S)-3,7-dimethyloct-6-enal	Aldehyde	D4	154	17	3.48
(E)-non-2-enal	Aldehyde	D5	140	17	3.17
(Z)-hex-3-enal	Aldehyde	D6	98	17	1.54
2-methylpropanal	Aldehyde	D7	72	17	1.2
3-methylbutanal	Aldehyde	D8	86	17	1.31
Acetaldehyde	Aldehyde	D9	44	17	-0.16
Decanal	Aldehyde	D10	156	17	3.97
Hexanal	Aldehyde	D11	100	17	1.76
Nonanal	Aldehyde	D12	142	17	3.38
Octanal	Aldehyde	D13	128	17	2.89
5-methylhexanoate	Ester	E1	158	26	2.37
Ethyl(2S)-2-methylbutanoate	Ester	E2	116	26	1.2
Ethyl3-methylbutanoate	Ester	E3	158	26	2.23
Ethyl acetate	Ester	E4	88	26	0.54
Ethyl butanoate	Ester	E5	116	26	1.39
Ethyl hexanoate	Ester	E6	144	26	2.82
Ethyl octanoate	Ester	E7	172	26	3.37
Ethyl propanoate	Ester	E8	102	26	1.21
Hexyl acetate	Ester	E9	114	26	2.83
Isopentyl acetate	Ester	E10	130	26	2.21
Methyl acetate	Ester	E11	74	26	0.18
Methyl butanoate	Ester	E12	102	26	1.24
Methyl methanoate	Ester	E13	61	26	0.42
Methyl propanoate	Ester	E14	88	26	0.71
Octyl acetate	Ester	E15	172	26	3.9
Pentyl butanoate	Ester	E16	158	26	2.51
Pentyl pentanoate	Ester	E17	172	26	2.91
(4S)-1,4-dimethylcyclohexene	Hydrocarbon	H1	110	0	4.23
(4S)-4-isopropenyl-1-methyl-cyclohexene	Hydrocarbon	H2	136	0	4.45
4-isopropylidene-1-methyl-cyclohexene	Hydrocarbon	H3	136	0	4.67
7-methyl-3-methylene-octa-1,6-diene	Hydrocarbon	H4	136	0	4.58
Hexane	Hydrocarbon	H5	86	0	3.55
(1S,4S)-1,7,7-trimethyl norbornane-2-one	Ketone	K1	152	17	2.20
Acetone	Ketone	K2	58	17	0.26
Butane-2,3-dione	Ketone	K3	86	34	-0.18

Table S1. Inter-day variability for hpDNA based E-Nose. The frequency shift (ΔF in Hz) response of the hpDNA gas sensors with heptamer loops was obtained using 15 pure VOCs (5 alcohols, 3 aldehydes, 3 esters, 1 ketone, and 3 hydrocarbons) tested by using N2 as carrier gas directly in the measuring chamber. The standard deviation was calculated using three measurements taken in 3 different days.

VOCs	AATCAGC	CCCTGTC	CCGATTT	GCGAAGG	GTCCCTA	GTCCGTT
Ethanol	82 ± 9	23 ± 8	91 ± 5	18 ± 4	36 ± 6	11 ± 1
1-pentanol	21 ± 9	9 ± 4	49 ± 6	12 ± 8	19 ± 3	3 ± 1
1-hexanol	23 ± 8	5 ± 2	54 ± 6	7 ± 4	11 ± 1	3 ± 3
α-terpineol	25 ± 2	8 ± 4	62 ± 9	12 ± 12	19 ± 7	3 ± 1
Linalool	16 ± 9	6 ± 5	48 ± 3	7 ± 4	14 ± 8	18 ± 3
Hexanal	21 ± 7	20 ± 6	66 ± 3	14 ± 4	41 ± 2	6 ± 5
Octanal	22 ± 9	27 ± 3	64 ± 4	14 ± 5	47 ± 6	4 ± 2
Nonanal	44 ± 5	20 ± 3	72 ± 4	18 ± 5	47 ± 6	3 ± 2
Ethyl acetate	47 ± 9	40 ± 5	77 ± 8	29 ± 6	86 ± 3	7 ± 4
Ethyl-2-methylbutyrate	45 ± 6	31 ± 8	53 ± 9	29 ± 3	82 ± 4	14 ± 2
Ethyl octanoate	46 ± 8	37 ± 3	77 ± 6	28 ± 4	83 ± 7	7 ± 5
Butane-2,3-dione	36 ± 7	38 ± 3	94 ± 6	25 ± 5	73 ± 8	4 ± 9
β-caryophyllene	6 ± 2	14 ± 3	53 ± 4	14 ± 5	25 ± 2	2 ± 1
O-cymene	9 ± 2	15 ± 8	59 ± 6	15 ± 1	13 ± 7	4 ± 3
Terpinolene	12 ± 6	11 ± 8	60 ± 4	11 ± 9	8 ± 4	2 ± 2

Table S2. Relative concentrations of the 23 VOCs identified by SPME/GC-MS analysis in beer samples at time zero of fermentation (t0; raw beer), after 20 days of fermentation (t1) and after 40 days of fermentation (t2). Data were expressed as percentage of the total GC area. The VOCs were sorted in descending order of the average concentration. Av = average and CV = coefficient of variation of the five beer samples at three different fermentation times (t0, t1 and t2).

VOC	At0	Bt0	Ct0	Dt0	Et0	Av	CV (%)	At1	Bt1	Ct1	Dt1	Et1	Av	CV (%)	At2	Bt2	Ct2	Dt2	Et2	Av	CV (%)
(-)-β-Pinene	28.2	24.8	21.3	30.3	30.2	27.0	14.3	19.4	24.9	33.1	24.0	24.6	25.2	19.7	18.9	28.1	26.4	15.7	18.5	21.5	25.1
Methylhydrazine	16.0	13.0	19.2	25.2	14.5	17.6	27.6	17.3	13.7	14.9	15.7	15.2	15.4	8.6	20.5	13.8	18.2	19.0	14.9	17.3	16.4
Ethyl octanoate	7.9	8.5	8.9	8.1	9.1	8.5	6.0	9.8	11.8	14.4	12.0	13.7	12.3	14.7	10.8	14.6	6.9	11.5	10.5	10.8	25.3
3-Methylbutan-1-ol	4.0	7.1	8.0	11.7	7.2	7.6	36.2	8.3	8.0	7.8	8.7	9.7	8.5	8.8	9.4	8.4	9.0	9.8	9.3	9.2	5.5
Ethanol	7.0	6.1	7.8	6.8	7.3	7.0	8.8	8.7	3.6	9.4	7.3	11.6	8.1	36.6	5.0	7.5	9.9	7.3	11.9	8.3	31.4
3-Methylbutyl isobutyrate	3.0	2.6	6.1	7.3	8.1	5.4	46.2	2.1	2.7	6.2	2.8	2.7	3.3	49.8	1.9	2.6	1.1	1.9	2.6	2.0	31.7
Isopentyl acetate	5.1	4.1	4.2	4.7	4.7	4.5	9.3	7.2	8.0	7.2	8.5	8.4	7.9	8.1	10.4	11.4	7.2	9.7	9.1	9.5	16.5
Carbon dioxide	3.8	3.6	5.3	4.9	5.0	4.5	17.6	5.2	5.1	5.2	4.5	5.2	5.0	5.9	6.7	7.0	4.8	6.7	4.6	5.9	19.6
2-Methylbutyl isobutyrate	8.5	7.3	0.6	4.0	0.9	4.3	83.8	5.7	7.4	6.2	7.4	7.2	6.8	11.9	5.5	7.5	3.1	5.0	6.0	5.4	29.9
2-Methylbutyl propionate	2.2	1.0	2.4	5.9	6.9	3.7	69.5	2.0	1.0	2.1	0.9	2.0	1.6	37.2	3.0	3.0	2.0	3.0	3.0	2.8	16.5
Styrene	3.4	4.7	4.6	2.2	2.1	3.4	37.0	1.2	1.2	1.2	4.6	5.4	2.7	79.2	1.0	1.0	1.0	1.1	1.2	1.1	7.9
2-Methyl-1-butanol	1.9	7.7	1.6	2.3	2.5	3.2	78.8	1.7	1.6	1.6	0.4	0.4	1.1	61.8	1.9	1.9	1.9	2.0	1.9	1.9	2.2
Ethyl Acetate	3.3	2.6	3.4	3.9	2.3	3.1	21.1	3.2	3.0	3.1	3.0	3.7	3.2	9.5	2.6	6.7	4.0	3.9	4.1	4.2	35.4
Ethyl butanoate	0.4	3.0	0.2	6.0	4.3	2.8	89.9	0.3	0.4	0.3	0.3	0.2	0.3	26.5	2.0	1.6	0.4	0.6	0.6	1.0	69.2
Ethyl caproate	2.0	1.0	2.2	2.6	3.1	2.2	35.8	3.1	3.7	2.3	2.9	3.2	3.0	17.3	3.5	3.1	2.1	3.3	3.3	3.1	18.8
Isobutyl isobutyrate	2.4	1.9	1.7	1.9	1.8	1.9	14.2	1.6	2.0	1.8	2.2	1.1	1.7	25.2	1.9	1.9	1.1	1.5	1.9	1.6	21.7
Ethyl decanoate	1.6	0.9	0.9	1.6	3.0	1.6	54.1	2.9	2.9	2.8	2.6	2.8	2.8	3.6	2.2	4.2	0.1	2.3	1.7	2.1	69.5
3-Methylbutyl 2-methylbutanoate	0.3	0.2	1.3	1.3	1.7	1.0	68.0	0.2	0.2	0.5	0.3	1.1	0.5	80.5	1.0	1.0	0.2	1.0	1.0	0.8	40.4
2-Methylpropan-1-ol	0.9	0.9	0.7	0.9	0.6	0.8	15.8	0.9	0.6	0.7	0.0	0.2	0.5	76.1	1.3	1.3	1.3	1.3	1.3	1.3	0.0
Humulene	0.5	0.4	0.2	1.2	1.0	0.7	61.7	0.2	0.1	0.2	0.2	0.2	0.2	39.8	1.0	1.0	0.2	1.0	1.0	0.8	41.4
1-Pentyl isovalerate	0.2	0.2	1.0	0.6	1.2	0.6	70.8	0.2	0.6	0.9	1.0	0.2	0.6	71.3	2.0	2.0	2.0	2.0	2.0	2.0	0.0
2-Phenylethan-1-ol	0.8	0.5	1.0	0.1	0.2	0.5	79.4	0.5	0.5	2.2	0.5	2.9	1.3	85.5	2.0	2.0	2.0	2.0	2.0	2.0	0.0
3-(4-Methyl-3-pentenyl)-furan	0.7	0.7	0.1	0.3	0.3	0.4	61.7	0.0	0.3	0.2	0.3	0.3	0.2	53.6	2.0	2.0	1.7	2.0	2.0	1.9	7.9

Table S3. Statistical significance of single VOCs detected in the beer samples at three fermentation times (t0, t1 and t2) by using analysis of variance (ANOVA) with the Tukey HSD (honestly significant difference) multiple comparison analysis. The criterion for statistical significance of differences was p-value<0.05 for all comparisons. The parameter F was used to sort the VOCs in descending order.

Compound	R^2	F	Pr > F	t0 mean	t1 mean	t2 mean	Significant
3-(4-Methyl-3-pentenyl)-furan	0.96	134.4	< 0.0001	1.93	0.41	0.21	Yes
Isopentyl acetate	0.84	31.5	< 0.0001	9.55	4.55	7.86	Yes
1-Pentyl isovalerate	0.82	26.9	< 0.0001	2.00	0.65	0.55	Yes
2-Methylpropan-1-ol	0.74	16.8	0.000	1.25	0.80	0.46	Yes
Humulene	0.49	5.8	0.017	0.84	0.68	0.19	Yes
2-Phenylethan-1-ol	0.48	5.6	0.019	2.00	0.52	1.34	Yes
Ethyl octanoate	0.46	5.0	0.026	10.85	8.52	12.34	Yes
3-Methylbutyl isobutyrate	0.44	4.7	0.032	2.03	5.42	3.32	Yes
Carbon dioxide	0.39	3.8	0.052	5.94	4.50	5.05	No
Ethyl butanoate	0.38	3.6	0.059	1.03	2.79	0.29	No
Styrene	0.37	3.5	0.063	1.06	3.39	2.70	No
Ethyl caproate	0.35	3.3	0.073	3.06	2.16	3.04	No
2-Methylbutan-1-ol	0.28	2.4	0.135	1.93	3.20	1.14	No
2-Methylbutyl propionate	0.28	2.3	0.140	2.78	3.69	1.59	No
Ethyl Acetate	0.26	2.2	0.158	4.23	3.08	3.21	No
Ethyl decanoate	0.25	2.0	0.182	2.11	1.58	2.81	No
(-)-β-Pinene	0.22	1.7	0.226	21.53	26.99	25.20	No
3-Methylbutyl 2-methylbutanoate	0.20	1.5	0.254	0.85	0.99	0.47	No
2-Methylbutyl isobutyrate	0.20	1.5	0.263	5.42	4.27	6.80	No
3-Methylbutan-1-ol	0.16	1.1	0.362	9.18	7.61	8.49	No
Isobutyl isobutyrate	0.14	0.9	0.418	1.65	1.95	1.73	No
Methylhydrazine	0.10	0.7	0.533	17.29	17.59	15.36	No
Ethanol	0.08	0.5	0.621	8.33	6.98	8.14	No

Table S4. Frequency shift (ΔF in Hz) response of the hpDNA gas sensors array with pentamer-hexamer loops and the hpDNA gas sensors array of with heptamer loops for the 15 beer samples (3 replicates for each sample measurement) at the three fermentation times (t0, t1 and t2) and with an intraday RSD in the 15–25% range.

	AAGTA	CCCGA	TAAGT	ATAATC	CATCTG	AATCAGC	CCCTGTC	CCGATTT	GCGAAGG	GTCCCTA	GTCCGTT
	Frequency shift (ΔF in Hz)										
At0	108	40	73	160	178	670	415	462	31	39	32
At0	100	45	72	168	170	596	420	455	25	40	32
At0	115	35	73	165	175	680	416	460	29	36	26
Bt0	118	32	76	153	161	663	380	470	26	35	26
Bt0	120	31	78	156	161	665	385	452	25	36	25
Bt0	119	36	75	153	162	642	390	460	24	40	28
Ct0	115	41	78	157	176	700	405	468	25	33	23
Ct0	118	40	75	158	178	750	405	468	26	31	25
Ct0	115	35	76	156	165	652	401	460	24	29	28
Dt0	111	34	81	169	177	687	393	457	23	31	27
Dt0	111	35	80	170	180	690	400	465	22	30	28
Dt0	110	36	75	170	175	685	401	452	21	29	29
Et0	103	30	78	162	178	666	384	443	24	32	25
Et0	102	30	80	165	180	680	385	450	25	30	25
Et0	102	25	81	164	179	672	387	490	21	26	24
At1	112	52	92	169	185	675	508	481	25	43	26
At1	110	50	90	180	180	680	502	482	25	40	26
At1	105	51	100	170	175	674	504	470	24	35	24
Bt1	95	54	104	270	184	801	457	424	25	45	27
Bt1	96	56	102	278	180	802	456	432	25	49	26
Bt1	102	53	102	275	189	802	460	430	24	45	28
Ct1	103	64	102	288	189	785	472	451	35	47	40
Ct1	102	65	101	290	190	790	480	460	36	50	40
Ct1	101	63	103	291	180	796	490	452	35	51	45
Dt1	96	59	108	225	178	714	472	434	34	44	38
Dt1	98	60	101	223	180	715	480	435	36	45	39
Dt1	100	63	102	224	190	716	490	425	38	50	38
Et1	114	63	113	282	176	840	508	509	44	45	48
Et1	114	65	112	289	175	850	510	502	45	42	50
Et1	115	63	116	256	179	835	506	503	30	45	51
At2	120	46	92	181	173	610	361	477	36	45	38
At2	112	45	100	182	175	612	362	475	35	46	38
At2	125	43	95	183	174	610	365	480	36	48	37
Bt2	120	43	81	179	169	701	417	517	35	44	35
Bt2	120	45	82	180	170	701	415	520	35	45	35
Bt2	121	46	80	175	175	705	420	530	30	40	32
Ct2	112	39	88	171	159	670	380	507	31	40	31
Ct2	112	40	90	170	160	680	380	510	30	40	31
Ct2	113	41	91	175	168	675	381	512	35	41	32
Dt2	116	39	89	151	175	630	395	512	25	33	22
Dt2	115	40	90	150	175	640	380	514	25	33	22
Dt2	114	38	91	155	176	650	395	523	26	34	23
Et2	125	45	76	168	158	739	403	512	25	34	27
Et2	122	45	75	168	150	740	402	513	25	35	27
Et2	123	49	76	165	160	736	401	512	26	40	26