

Supporting Information for:

Isothermal Discrimination of Single-Nucleotide
Polymorphisms via Real-Time Kinetic Desorption
and Label-Free Detection of DNA using Silicon
Photonic Microring Resonator Arrays

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Chemical and Biochemical Functionalization of Sensor Surfaces.

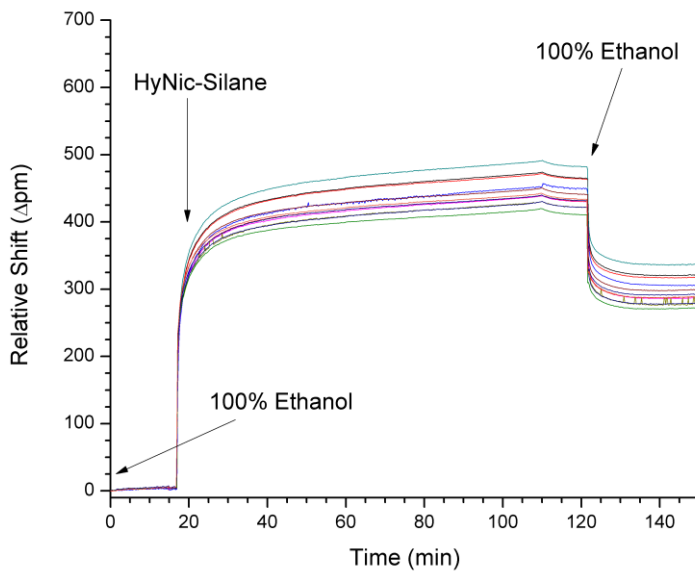


Figure S1. Addition of 1 mg/mL HyNic-Silane solution to 12 microring sensors. Each trace represents a different microring sensor. The net shift of \sim 300 pm after rinsing the surface with 100% ethanol indicates the covalent attachment of the HyNic Silane to the microring surfaces.

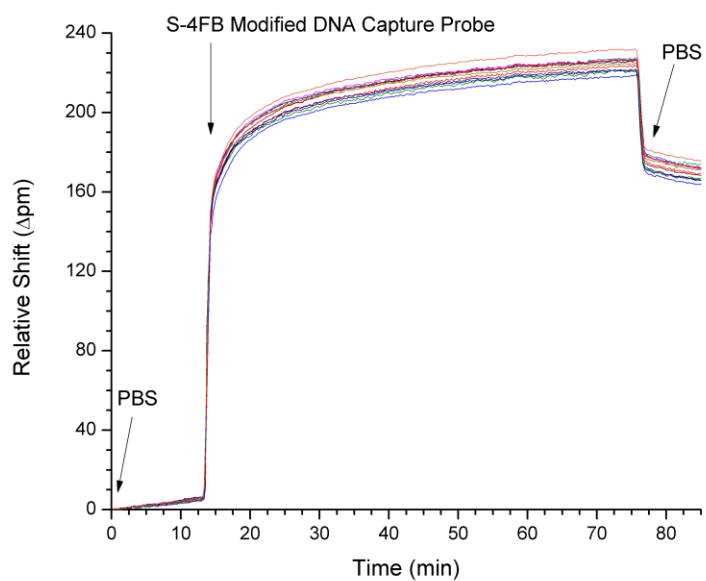


Figure S2. Addition of an S-4FB modified ssDNA capture probe to a set of 12 microrings functionalized with a HyNic-Silane layer.

DNA Detection and Surface Regeneration.

Table S1. Probe sequences utilized in DNA detection and repeated regeneration experiments (shown in Figure 1, Figure S3, and Figure S4).

Strand	Sequence
A	5'-NH ₂ - (CH ₂) ₁₂ - GGTAGTACAGCATATTGGAAAGTGTATAAGATT-3'
A'	3'-TTTCACATATTCTAA-5'
B	5'-NH ₂ - (CH ₂) ₁₂ - AGAATGCAGGGCCTCACGTTACCCTACCACATA-3'
B'	3'-AATGGGATGGTGTAT-5'

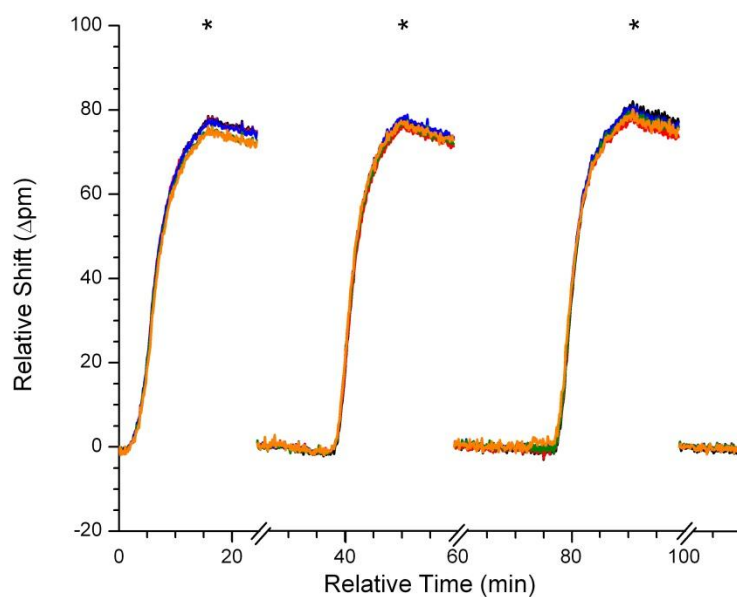


Figure S3. Repeated real time shifts in resonance wavelength from 5' ssDNA modified microrings responding to a 1 μ M complementary target sequence. The surface-bound duplexes were dissociated with 8 M urea to regenerate the surface prior to the next hybridization cycle. For clarity, the regeneration step has been omitted due to a large bulk refractive index shift that goes off scale. Asterisks indicate the time points at which the solution was switched back to pure buffer (no DNA).

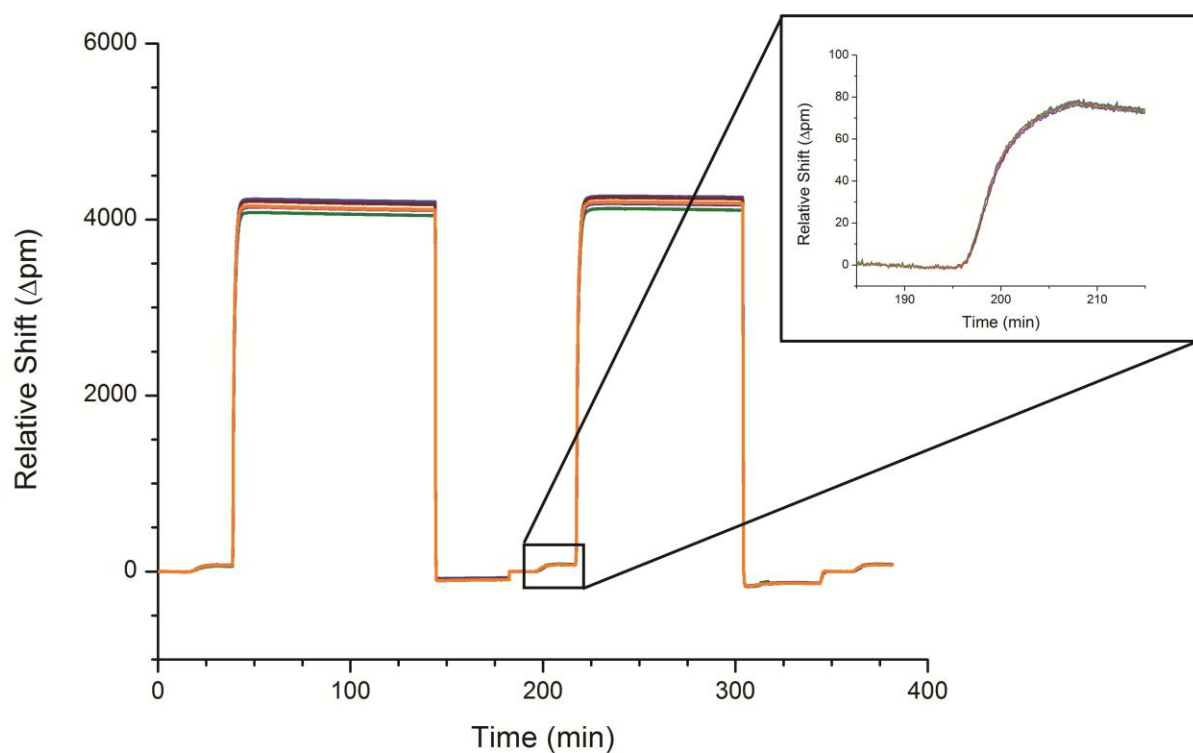


Figure S4. Repeated real time shifts in resonance wavelength from 5' ssDNA modified microrings responding to a 1 μM complementary target sequence. The surface-bound duplexes were dissociated with 8 M urea to regenerate the surface prior to the next hybridization cycle. In contrast to Figure S3, the regeneration steps with 8 M Urea (the ~ 4000 pm increases in signal) are included. The inset highlights an individual hybridization event.

Non-Complementary Sequence Specificity.

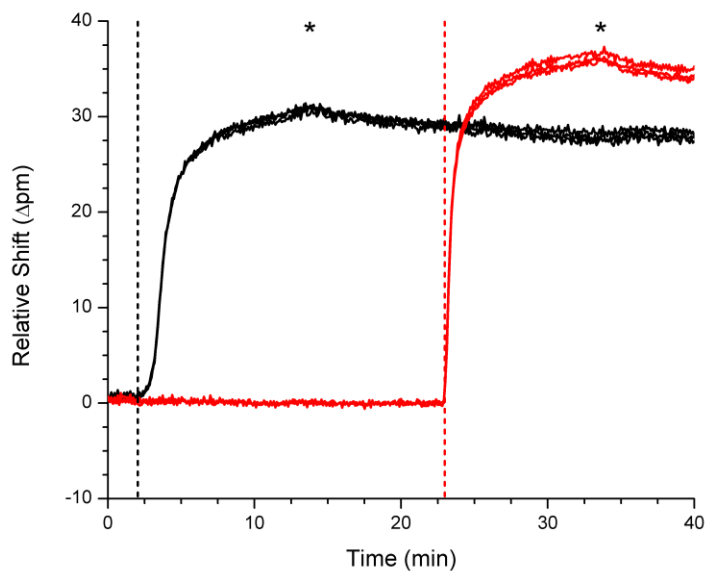


Figure S5. Sequence specific response of two sets of microrings (3 per set) functionalized with DNA capture probes A (black) and B (red). Dotted lines indicate the addition of 1 μ M of target DNA probes, A' and B'. Asterisks indicate the time points at which the solution was switched back to pure buffer (no DNA).

Detection of Single Nucleotide Polymorphisms.

Table S2. Sequences for the DNA capture and target sequences (perfectly complementary A' and three SNPs) utilized in the single-plexed SNP assay (as seen in Figure 2). Highlighted bases in the target probes indicate the presence of a SNP.

Strand	Sequence
A	5'-NH ₂ – (CH ₂) ₁₂ – GGTAGTACAGCATATTGGAAAGTGTATAAGATT-3'
A'	3'-TTTCACATATTCTAA-5'
A' SNP: T to A	3'-TTTCACA <u>A</u> ATTCTAA-5'
A' SNP: T to C	3'-TTTCACA <u>C</u> ATTCTAA-5'
A' SNP: T to G	3'-TTTCACA <u>G</u> ATTCTAA-5'

Multiplexed Detection and Identification of Single Nucleotide Polymorphisms.

Table S3. Sequences for low G-C Content DNA probes (used in Figure 3, Figure 4, Figure S6, and Figure S8). X and Y = A, T, C, or G.

Strand	Sequence
Capture Probe	5'-NH ₂ – (CH ₂) ₁₂ – GGTAGTACAGCATATTGGAAAGTGT <u>X</u> TAAGAAT-3'
Target Probe	3'-TTTCACAY <u>Y</u> ATTCTAA-5'

Table S4. Sequences for high G-C Content DNA probes (used in Figure S7 and Figure S10). X and Y = A, T, C, or G.

Strand	Sequence
Capture Probe	5'-NH ₂ – (CH ₂) ₁₂ – ATTAAAAAATAATTATAGCTTGATG <u>X</u> TCTGTTG-3'
Target Probe	3'-GAACTAC <u>Y</u> AGACAAC-5'

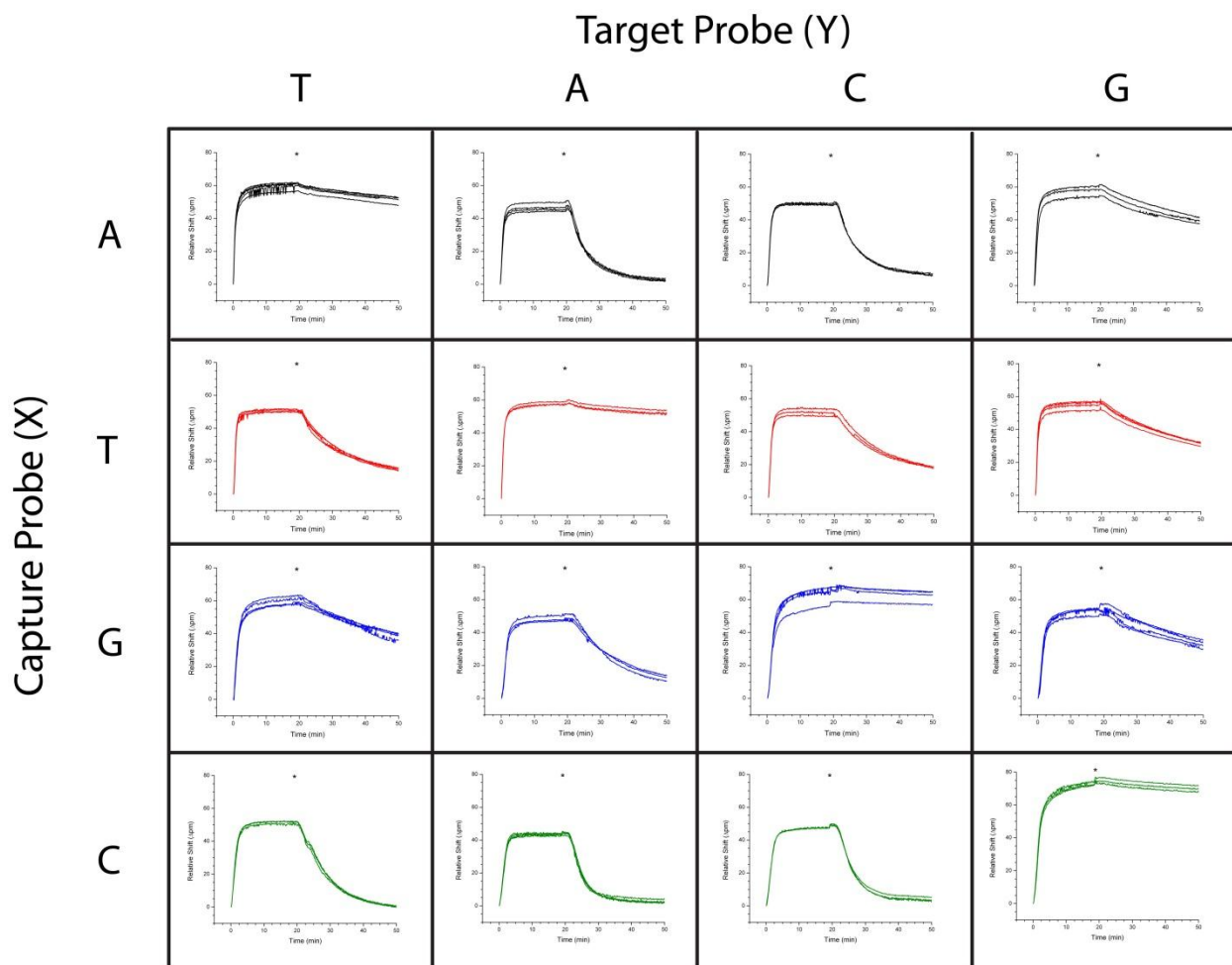


Figure S6. Multiplexed SNP assay containing the hybridization and desorption responses (non-normalized) for each of the low G-C content DNA probes (described in Table S3). While it is difficult to distinguish the perfectly complementary and SNP mismatch pairs based on their hybridization response, the clear difference in desorption responses allows for identification of the target. The perfectly complementary duplexes, seen along the diagonal from the top left-to-bottom right, have the lowest desorption rates in their respective columns.

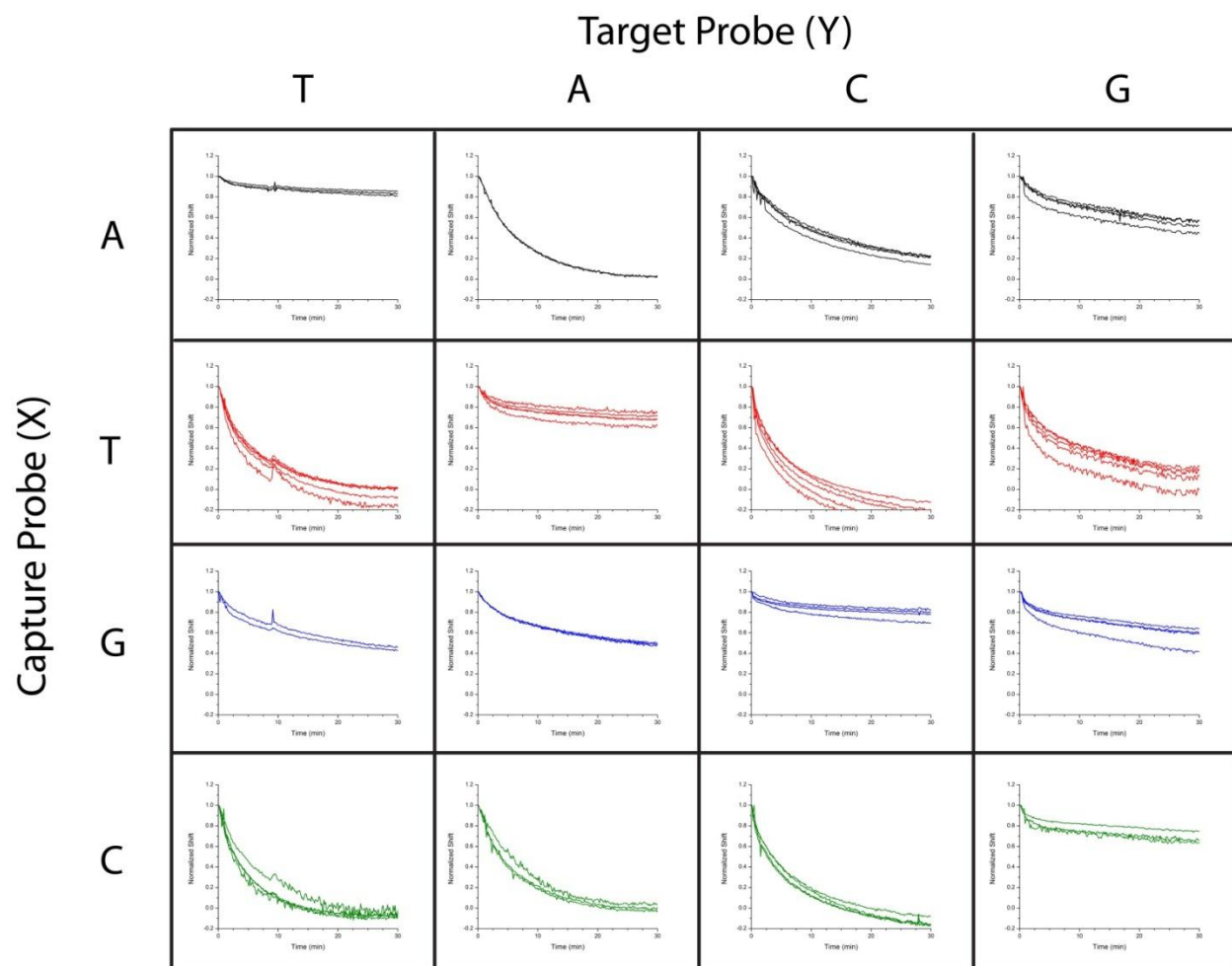


Figure S7. Normalized desorption response for each of the possible high G-C capture and target probe combinations in the multiplexed SNP assay (described in Table S4). In all cases, the desorption rate is slowest for the perfectly complementary duplexes, as observed from the top left-to-bottom right diagonal.

Determination of DNA Melting Temperatures.

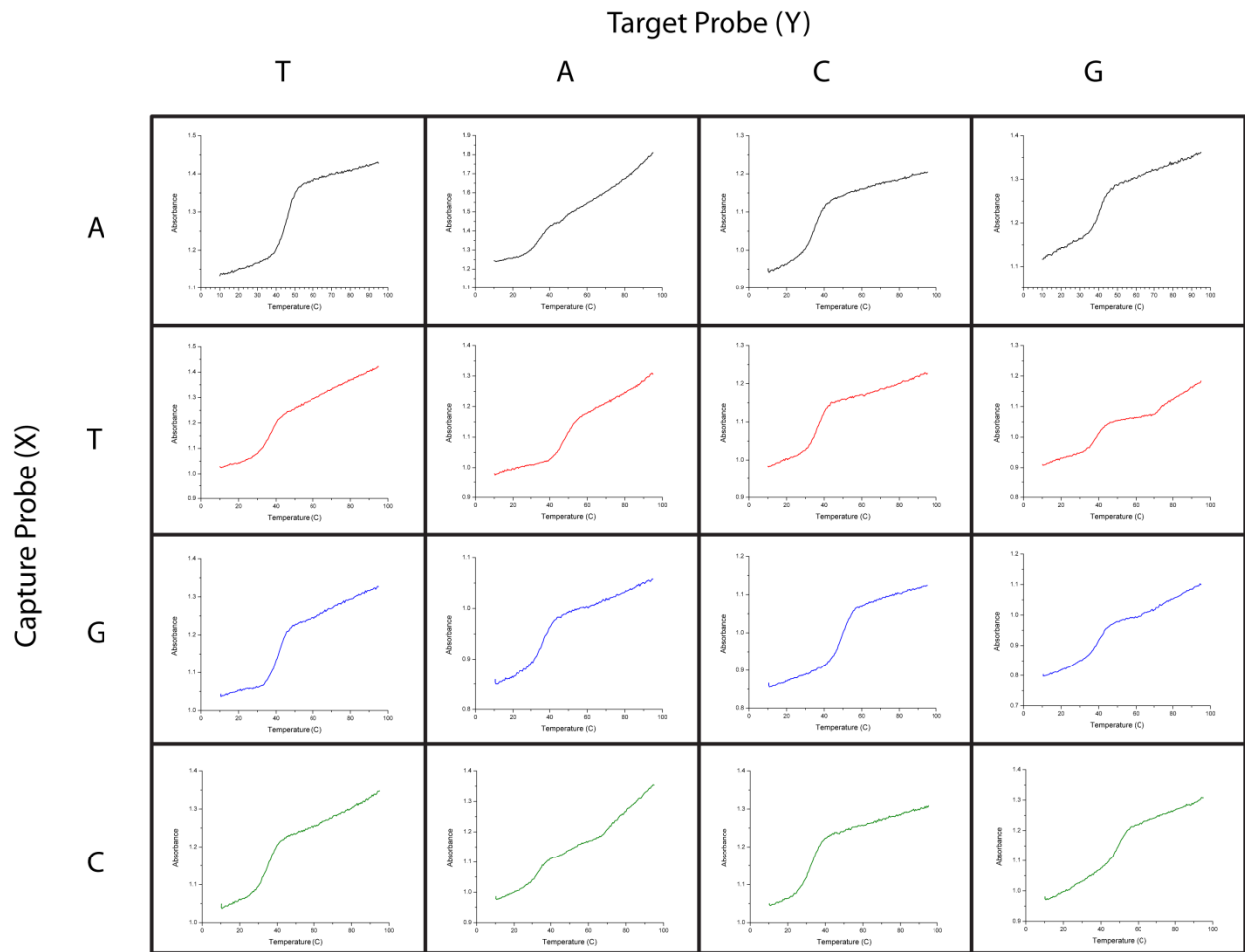


Figure S8. Temperature vs. absorbance curves for each of the low G-C content capture and target probe combinations (listed in Table S3).

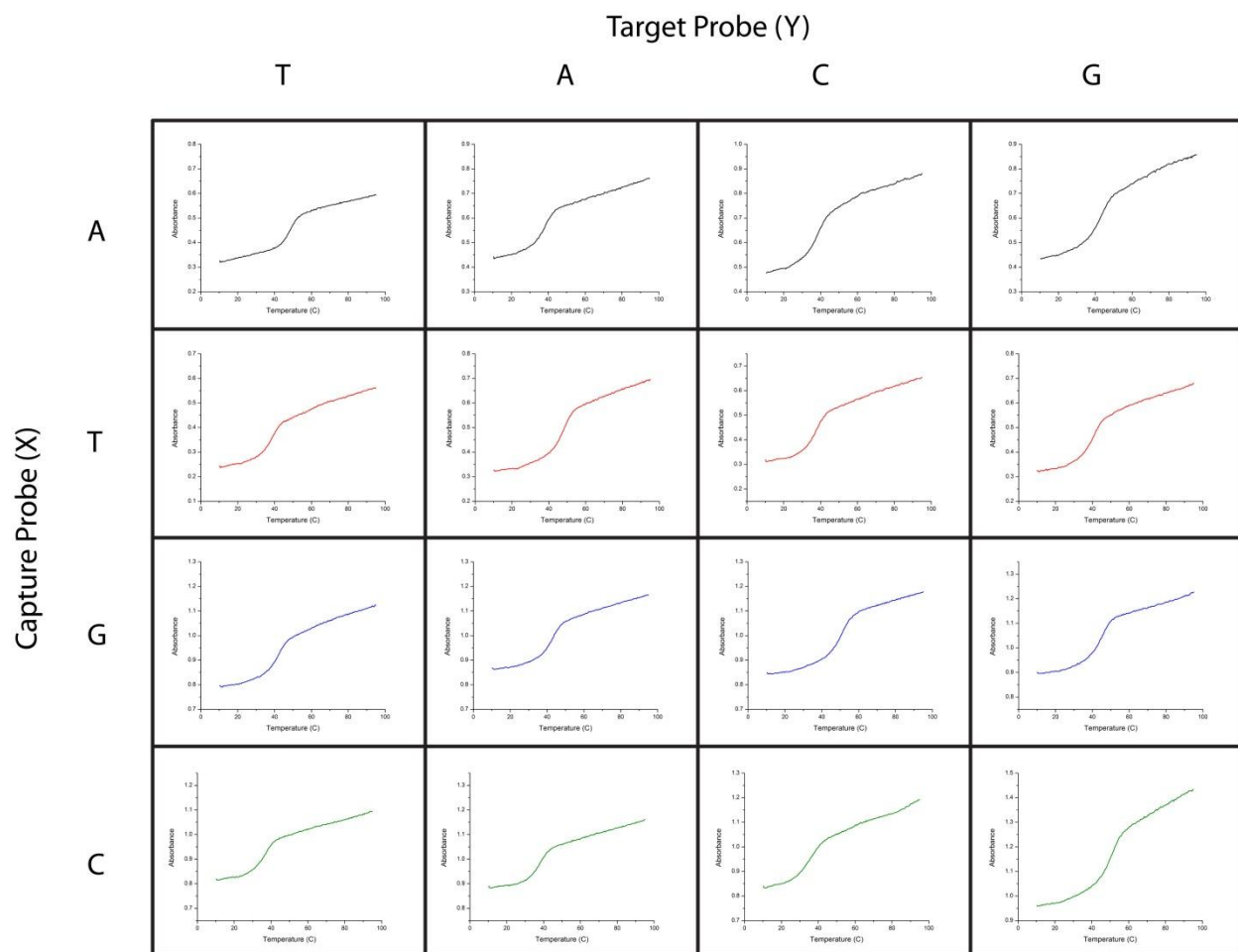


Figure S9. Temperature vs. absorbance curves for each of the high G-C content capture and target probe combinations (listed in Table S4).

Detection of SNP Heterozygotes.

Target Probe (Y)

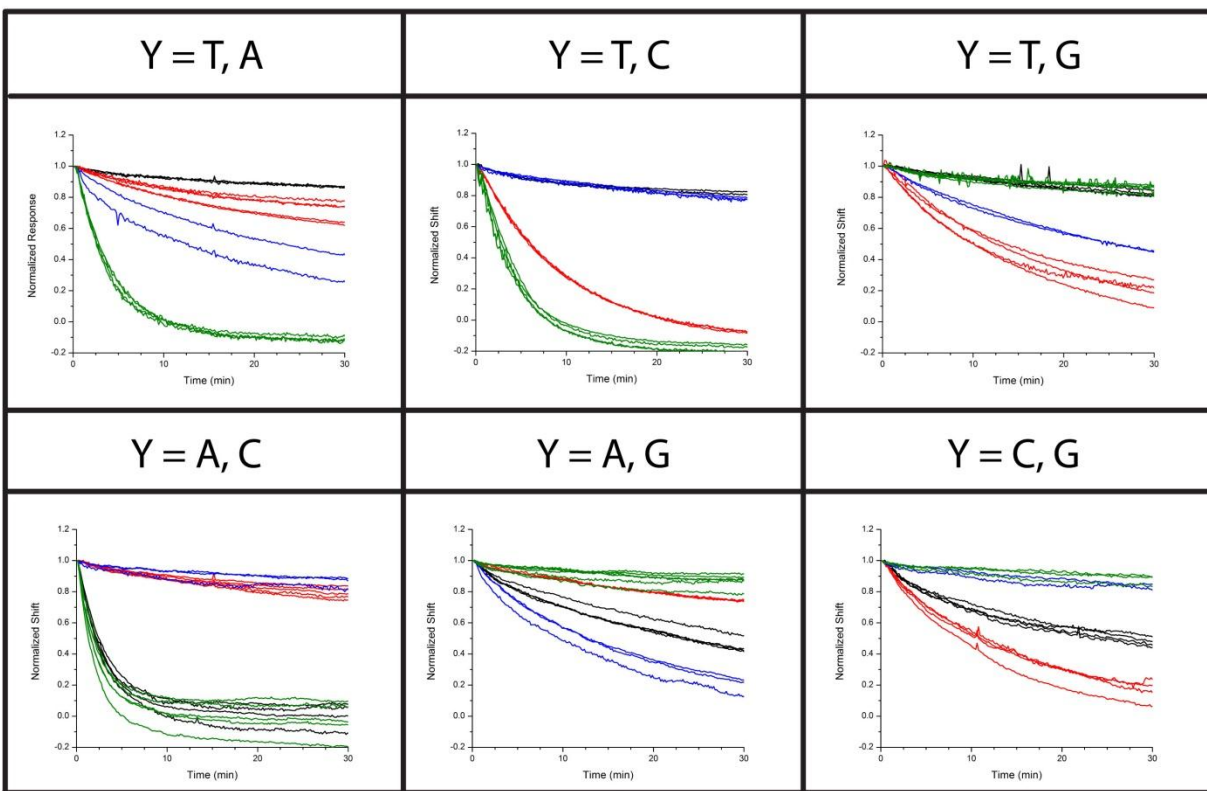


Figure S10. Normalized desorption response for each combination of target probes simulating a SNP heterozygote. The capture and targets probes are the same as those utilized in the low G-C content experiments (described in Table S3). The identity of the capture probe in each of the insets above is as follows: X = A (black), X = T (red), X = G (blue), and X = C (green). In each case, the two perfectly complementary target and capture probe combinations show the two lowest desorption rate in each of the plots.

Data Processing.

Table S5. Desorption Rates and Melting Temperature for A' (perfect complement) and three SNPs to the ssDNA capture probe A (shown in Figure 2).

Target Sequence	Desorption Rate (s⁻¹)	Standard Deviation (s⁻¹)	n	Desorption Rate Relative to A'	Melting Temperature (°C)
A' (perfect complement)	8.0901 x 10 ⁻⁵	3.924 x 10 ⁻⁶	5	1.00	45.65
A' SNP: T to A	2.3225 x 10 ⁻³	2.766 x 10 ⁻⁴	4	28.71	32.51
A' SNP: T to C	1.4733 x 10 ⁻³	6.51 x 10 ⁻⁵	3	18.21	35.09
A' SNP: T to G	2.1130 x 10 ⁻⁴	9.14 x 10 ⁻⁶	3	2.61	40.90

Table S6. Parameters used in fitting a 1:1 kinetic Langmuir Binding Isotherm from experiments used in generating the calibration curve for DNA detection (seen in Figure 1).

Concentration (nM)	A (pm)	B (min ⁻¹)	t ₀ (min)	AB (pm/min)	R ²	χ ²
1000	23.2179	0.0117	0.2938	16.2293	0.9550	2.5920
	22.1725	0.0161	0.2019	21.3521	0.9744	1.2027
	24.9700	0.0127	0.2486	19.0870	0.9704	1.8645
	23.0623	0.0142	0.2397	19.6491	0.9626	2.0316
	23.9485	0.0140	0.2191	20.0736	0.9693	1.7264
	23.5724	0.0144	0.2194	20.3524	0.9729	1.4762
	24.6268	0.0172	0.1866	25.3410	0.9753	1.3862
	22.7337	0.0164	0.1978	22.3290	0.9721	1.3685
	21.6393	0.0170	0.1935	22.0591	0.9733	1.1774
500	35.1112	0.0076	0.3846	16.0528	0.9150	10.3994
	30.2040	0.0050	0.3465	8.9706	0.9622	2.2036
	29.8253	0.0055	0.3478	9.8066	0.9630	2.3126
	28.5734	0.0061	0.2847	10.4922	0.9662	2.0348
	27.6611	0.0061	0.3265	10.0908	0.9624	2.1851
	9.4990	0.0210	0.2877	11.9459	0.1883	38.5315
	23.9500	0.0060	0.3221	8.6220	0.9614	1.6613
	27.3758	0.0063	0.3525	10.3316	0.9595	2.4211
	20.1431	0.0063	0.3672	7.6503	0.9592	1.3431
	20.2007	0.0072	0.3036	8.6903	0.9619	1.3128
250	21.4016	0.0031	0.2794	3.9422	0.9931	0.1995
	24.4589	0.0042	0.2869	6.1783	0.9920	0.3615
	21.0796	0.0032	0.3396	4.0093	0.9830	0.5038
	25.3695	0.0033	0.2722	5.0688	0.9955	0.1906
	27.1243	0.0034	0.3391	5.5659	0.9936	0.3247
	22.9953	0.0041	0.2187	5.6430	0.9853	0.5649
	23.2678	0.0038	0.1875	5.3051	0.9832	0.6318
	20.5398	0.0037	0.1498	4.5105	0.9898	0.2859
125	30.4027	0.0018	0.4528	3.2105	0.9782	0.8067
	29.8156	0.0020	0.3039	3.5779	0.9842	0.6260
	36.0050	0.0018	0.2909	3.7805	0.9825	0.8689
	26.5448	0.0024	0.3326	3.8065	0.9830	0.6370
	22.3475	0.0026	0.2758	3.4192	0.9861	0.3852

Table S7. Parameters used in linear fits for time resolved hybridization data from experiments used in generating the calibration curve for DNA detection (seen in Figure 1).

Concentration (nM)	Slope (pm/min)	Slope Error (pm/min)	Intercept (pm)	Intercept Error (pm)	R²
62.5	0.7074	0.0078	0.2764	0.0450	0.9873
	0.6438	0.0083	-0.3764	0.0477	0.9829
	0.5472	0.0069	-0.1487	0.0400	0.9833
	0.7194	0.0129	-0.3512	0.0748	0.9668
	0.6030	0.0092	-0.0949	0.0530	0.9761
	0.6138	0.0105	-0.2690	0.0609	0.9697
	0.7260	0.0063	-0.2625	0.0362	0.9922
	0.7944	0.0073	-0.0999	0.0423	0.9911
	0.7794	0.0076	-0.4657	0.0441	0.9899
	0.5232	0.0069	-0.1767	0.0397	0.9821
0.5874	0.0070	-0.6068	0.0406	0.9851	
31.3	0.1692	0.0058	0.0503	0.0335	0.8894
	0.1848	0.0061	0.1070	0.0353	0.8963
	0.3720	0.0088	-0.1940	0.0510	0.9436
	0.2202	0.0090	0.2629	0.0525	0.8469
	0.4818	0.0091	-0.3665	0.0528	0.9633
	0.3744	0.0085	-0.0463	0.0496	0.9472
	0.2214	0.0052	-0.0749	0.0299	0.9451
	0.1998	0.0114	-0.4766	0.0663	0.7399
	0.1692	0.0038	-0.2047	0.0222	0.8443
15.6	0.1272	0.0074	0.7174	0.0430	0.7374
	0.2766	0.0097	1.3964	0.0568	0.8838
	0.2874	0.0083	0.7696	0.0485	0.9187
	0.1350	0.0101	1.4250	0.0589	0.6264
	0.1524	0.0082	0.7544	0.0481	0.7626
	0.2484	0.0080	0.0174	0.0465	0.9015
	0.1212	0.0054	0.6109	0.0318	0.8225
	0.1302	0.0078	0.7056	0.0455	0.7240
	0.1614	0.0062	1.0438	0.0361	0.8650
	0.1092	0.0062	0.3297	0.0363	0.7431
0.1116	0.0054	0.2704	0.0317	0.7980	
3.91	0.1056	0.0050	0.0125	0.0289	0.8081
	0.1290	0.0059	0.1598	0.0341	0.8181
	0.1314	0.0079	-0.0096	0.0458	0.7211
	0.1092	0.0050	0.0502	0.0290	0.8165
	0.0487	0.0082	0.0478	0.0477	0.2417
	0.1770	0.0066	0.0157	0.0382	0.8710
	0.1200	0.0062	0.1171	0.0361	0.7768

	0.1062	0.0058	0.1918	0.0337	0.7567
1.95	0.0978	0.0069	-0.0149	0.0401	0.6493
	0.0918	0.0063	0.0353	0.0367	0.6615
	0.0852	0.0067	-0.0555	0.0387	0.6008
	0.1056	0.0101	-0.1722	0.0587	0.5016
	0.0930	0.0072	-0.2189	0.0419	0.6028
	0.0870	0.0065	0.1075	0.0379	0.6217
	0.0750	0.0064	-0.0333	0.0372	0.5566
	0.0972	0.0071	0.0097	0.0410	0.6348
0	0.0193	0.0085	-0.0822	0.0493	0.0377
	0.0519	0.0073	0.1256	0.0428	0.3120
	0.0774	0.0117	-0.3728	0.0679	0.2852
	-0.0077	0.0070	-0.1437	0.0409	0.0019
	-0.0190	0.0077	-0.1949	0.0449	0.0448
	0.0518	0.0131	-0.5543	0.0764	0.1191
	-0.0043	0.0055	-0.2833	0.0322	-0.0036

Table S8. Summary of Initial Slopes obtained from Tables S6-S7, and used in generating the calibration curve in Figure 1b.

Concentration (nM)	n	Mean Initial Slope (pm/min)	Standard Deviation of Mean (pm/min)
1000	9	20.7192	2.5167
500	10	10.2653	2.3644
250	8	5.0279	0.8066
125	5	3.5589	0.2244
62.5	11	0.6586	0.0921
31.25	10	0.2573	0.1243
15.63	11	0.1692	0.0677
3.91	8	0.1159	0.0357
1.95	8	0.0916	0.0093
0	7	0.0242	0.0367

Table S9. Parameters used in fitting the desorption rates for the single-plexed SNP experiment and low G/C content multiplexed SNP experiments (represented in Figure 2 and Figure 3, respectively). Note that the capture probe and target probes used in generating Figure 2 are the same as the capture probe X = A and the four target probes utilized in Figure 3.

Capture Probe (X)	Target Probe (Y)	A	Std. Error	k_d (s ⁻¹)	Std. Error (s ⁻¹)	χ^2	R ²	
A	T	59.9808	0.0374	8.0000×10^{-5}	6.25×10^{-7}	0.0690	0.9875	
		59.9044	0.0360	7.4433×10^{-5}	6.01×10^{-7}	0.0642	0.9866	
		59.2280	0.0353	8.2614×10^{-5}	5.98×10^{-7}	0.0613	0.9892	
		58.7008	0.0452	8.3785×10^{-5}	8.89×10^{-7}	0.0860	0.9802	
		55.5113	0.0254	8.3672×10^{-5}	4.60×10^{-7}	0.0318	0.9938	
	A	43.3651	0.4259	2.2100×10^{-3}	3.12×10^{-5}	2.4231	0.9787	
		43.3085	0.4161	2.0500×10^{-3}	2.84×10^{-5}	2.4792	0.9784	
		45.1291	0.3927	2.3300×10^{-3}	2.90×10^{-5}	1.9649	0.9840	
		50.3450	0.4168	2.7000×10^{-3}	3.20×10^{-5}	1.9214	0.9869	
	C	40.7366	0.5213	1.4700×10^{-3}	2.82×10^{-5}	5.2014	0.9481	
		41.1077	0.5890	1.4100×10^{-3}	3.06×10^{-5}	6.8542	0.9323	
		41.7201	0.4936	1.5400×10^{-3}	2.70×10^{-5}	4.5022	0.9574	
	G	52.6921	0.1210	2.0088×10^{-4}	2.42×10^{-6}	0.6421	0.9707	
		56.5411	0.1077	2.1794×10^{-4}	2.03×10^{-6}	0.5017	0.9824	
		60.0332	0.0667	2.1508×10^{-4}	1.18×10^{-6}	0.1926	0.9938	
	T	T	46.9323	0.1917	7.4303×10^{-4}	5.86×10^{-6}	1.0995	0.9885
			43.5835	0.3094	6.6835×10^{-4}	9.74×10^{-6}	3.0169	0.9599
			42.2102	0.2854	6.4301×10^{-4}	9.14×10^{-6}	2.6142	0.9620
			46.0296	0.2212	7.5725×10^{-4}	6.95×10^{-6}	1.4504	0.9845
A		58.1095	0.0425	5.1759×10^{-5}	7.17×10^{-7}	0.0922	0.9606	
		56.1172	0.0459	5.2053×10^{-5}	8.03×10^{-7}	0.1079	0.9516	

		55.4896	0.0678	5.0825×10^{-5}	1.20×10^{-6}	0.2349	0.8938	
	C	48.6860	0.2429	6.4053×10^{-4}	6.76×10^{-6}	1.8971	0.9788	
		44.5147	0.2029	5.8296×10^{-4}	5.97×10^{-6}	1.3795	0.9798	
		43.7841	0.2518	6.0019×10^{-4}	7.61×10^{-6}	2.0980	0.9692	
	G	54.9518	0.0935	3.2841×10^{-4}	1.92×10^{-6}	0.3454	0.9931	
		52.7017	0.1044	3.0340×10^{-4}	2.20×10^{-6}	0.4394	0.9893	
		49.5757	0.0915	3.0400×10^{-4}	2.06×10^{-6}	0.3378	0.9908	
		53.5543	0.1021	3.0722×10^{-4}	2.13×10^{-6}	0.4189	0.9903	
	G	T	60.5901	0.0461	2.6405×10^{-4}	8.31×10^{-7}	0.0872	0.9980
			56.5829	0.1351	2.1486×10^{-4}	2.55×10^{-6}	0.7820	0.9720
			62.2761	0.1746	3.3760×10^{-4}	3.19×10^{-6}	1.1809	0.9825
			58.0323	0.1076	2.2639×10^{-4}	1.99×10^{-6}	0.4912	0.9844
A		41.5170	0.1563	7.2476×10^{-4}	5.31×10^{-6}	0.7207	0.9901	
		45.6663	0.2045	1.0100×10^{-3}	7.50×10^{-6}	1.0195	0.9910	
		44.8127	0.2302	8.5350×10^{-4}	7.83×10^{-6}	1.4330	0.9851	
C		66.1746	0.0616	3.4314×10^{-5}	9.11×10^{-7}	0.1946	0.8712	
		67.6445	0.0507	3.0317×10^{-5}	7.31×10^{-7}	0.1321	0.8913	
		67.7671	0.0343	2.8791×10^{-5}	4.94×10^{-7}	0.0606	0.9420	
		58.6339	0.0219	1.7525×10^{-5}	3.62×10^{-7}	0.0249	0.9180	
G		47.6778	0.1161	2.7170×10^{-4}	2.67×10^{-6}	0.5579	0.9806	
		49.8770	0.2039	2.6792×10^{-4}	4.48×10^{-6}	1.7256	0.9454	
		53.0895	0.0708	2.3703×10^{-4}	1.44×10^{-6}	0.2130	0.9926	
		55.7501	0.1044	3.0160×10^{-4}	2.09×10^{-6}	0.4401	0.9903	
C		T	54.3834	0.2920	2.0600×10^{-3}	1.57×10^{-5}	1.1434	0.9943
			53.0226	0.3283	2.0900×10^{-3}	1.83×10^{-5}	1.4258	0.9926
			52.4628	0.1978	2.2100×10^{-3}	1.18×10^{-5}	0.4904	0.9973

	A	45.4177	0.4669	3.2700×10^{-3}	4.75×10^{-5}	1.8873	0.9820
		44.5762	0.6203	3.5900×10^{-3}	7.04×10^{-5}	3.0297	0.9677
		42.9259	0.5980	3.2500×10^{-3}	6.39×10^{-5}	3.1202	0.9657
		43.1804	0.9199	2.9000×10^{-3}	8.73×10^{-5}	8.3020	0.9100
	C	48.8279	0.7200	2.1600×10^{-3}	4.54×10^{-5}	6.8380	0.9499
		53.7713	0.5631	2.7700×10^{-3}	4.10×10^{-5}	3.2660	0.9796
		52.2116	0.5903	2.6400×10^{-3}	4.21×10^{-5}	3.7754	0.9751
	G	72.2300	0.0493	3.8751×10^{-5}	6.68×10^{-7}	0.1227	0.9417
		73.8707	0.0345	3.5626×10^{-5}	4.56×10^{-7}	0.0602	0.9670
		76.0808	0.0309	3.5879×10^{-5}	3.96×10^{-7}	0.0482	0.9753

Table S10. Summary of the desorption rates and melting temperatures the single-plexed SNP experiment and low G/C content multiplexed SNP experiments (represented in Figure 2 and Figure 3, respectively). Note that the capture probe and target probes used in generating Figure 2 are the same as the capture probe X = A and the four target probes utilized in Figure 3.

Capture (X)	Target (Y)	Average Desorption Rate (s ⁻¹)	Standard Deviation (s ⁻¹)	n	Melting Temperature (°C)	Desorption Rate Relative to Perfect Complement
A	T	8.0901 x 10 ⁻⁵	3.924 x 10 ⁻⁶	5	45.649	1.00
	A	2.3225 x 10 ⁻³	2.766 x 10 ⁻⁴	4	32.512	28.71
	C	1.4733 x 10 ⁻³	6.51 x 10 ⁻⁵	3	35.086	18.21
	G	2.1130 x 10 ⁻⁴	9.14 x 10 ⁻⁶	3	40.904	2.61
T	T	7.0291 x 10 ⁻⁴	5.581 x 10 ⁻⁵	4	35.948	13.63
	A	5.1546 x 10 ⁻⁵	6.41 x 10 ⁻⁷	3	47.107	1.00
	C	6.0789 x 10 ⁻⁴	2.955 x 10 ⁻⁵	3	36.640	11.79
	G	3.1076 x 10 ⁻⁴	1.188 x 10 ⁻⁵	4	39.319	6.02
G	T	2.6072 x 10 ⁻⁴	5.539 x 10 ⁻⁵	4	40.547	9.40
	A	8.6275 x 10 ⁻⁴	1.429 x 10 ⁻⁴	3	36.597	31.10
	C	2.7737 x 10 ⁻⁵	7.195 x 10 ⁻⁶	4	49.050	1.00
	G	2.6956 x 10 ⁻⁴	2.641 x 10 ⁻⁵	4	40.507	9.72
C	T	2.1200 x 10 ⁻³	7.937 x 10 ⁻⁵	3	34.724	57.68
	A	3.2525 x 10 ⁻³	2.819 x 10 ⁻⁴	4	32.815	88.50
	C	2.5233 x 10 ⁻³	3.213 x 10 ⁻⁴	3	32.832	68.66
	G	3.6752 x 10 ⁻⁵	1.736 x 10 ⁻⁶	3	49.001	1.00

Table S11. Parameters used in fitting the desorption rates for the high G/C content multiplexed SNP experiments (shown in Figure S7).

Capture Probe (X)	Target Probe (Y)	A	Std. Error	k_d (s ⁻¹)	Std. Error (s ⁻¹)	χ^2	R ²
A	T	36.5112	0.0843	6.9985×10^{-5}	2.287×10^{-6}	0.3211	0.8280
		45.2542	0.0905	6.4423×10^{-5}	1.975×10^{-6}	0.3721	0.8453
		34.5208	0.0812	8.5017×10^{-5}	2.347×10^{-6}	0.2944	0.8709
	A	21.6210	0.1605	2.2800×10^{-3}	2.39×10^{-5}	0.2921	0.9903
		23.6992	0.1849	2.3200×10^{-3}	2.56×10^{-5}	0.3811	0.9893
		19.8518	0.1652	2.4500×10^{-3}	2.87×10^{-5}	0.2881	0.9883
		18.6200	0.3270	3.1900×10^{-3}	7.85×10^{-5}	0.8643	0.9621
		16.4281	0.7016	4.5300×10^{-3}	2.702×10^{-4}	2.7675	0.8451
	C	27.7352	0.1425	4.0216×10^{-4}	6.04×10^{-6}	0.6967	0.9593
		29.8968	0.2201	4.1647×10^{-4}	8.72×10^{-6}	1.6428	0.9231
	G	25.9344	0.4676	4.0600×10^{-3}	1.024×10^{-4}	1.3764	0.9644
		19.0510	0.5036	4.2300×10^{-3}	1.566×10^{-4}	1.5281	0.9303
		26.3971	0.5436	4.6400×10^{-3}	1.336×10^{-4}	1.6175	0.9558
		29.6171	0.4470	2.5500×10^{-3}	5.43×10^{-5}	2.0233	0.9668
T	T	31.7730	0.1042	2.3100×10^{-3}	1.07×10^{-5}	0.1202	0.9981
		30.2386	0.1017	2.2300×10^{-3}	1.06×10^{-5}	0.1183	0.9980
	A	27.9358	0.1000	1.1670×10^{-4}	3.63×10^{-6}	0.4324	0.8416
		29.7215	0.1338	1.3201×10^{-4}	4.60×10^{-6}	0.7636	0.8083
		25.4645	0.1281	1.5149×10^{-4}	5.19×10^{-6}	0.6889	0.813
		24.9246	0.1302	1.5193×10^{-4}	5.39×10^{-6}	0.7111	0.8023
		21.7233	0.1674	1.9289×10^{-4}	8.12×10^{-6}	1.1354	0.7406
	C	26.0607	0.1595	3.8309×10^{-4}	7.13×10^{-6}	0.8816	0.9384

		30.1670	0.1726	3.4969×10^{-4}	6.54×10^{-6}	1.0608	0.93735	
		28.8019	0.1712	3.7037×10^{-4}	6.87×10^{-6}	1.0265	0.9384	
	G	25.9663	0.1418	2.6900×10^{-3}	2.06×10^{-5}	0.1908	0.9954	
	22.3761	0.1935	2.9600×10^{-3}	3.59×10^{-5}	0.3225	0.9895		
	34.2597	0.1742	2.1700×10^{-3}	1.56×10^{-5}	0.3576	0.9953		
G	T	28.6028	0.2136	8.6646×10^{-4}	1.148×10^{-5}	1.1121	0.9721	
		31.4324	0.2302	9.0151×10^{-4}	1.150×10^{-5}	1.2615	0.9745	
		28.2684	0.1602	8.5385×10^{-4}	8.65×10^{-6}	0.6312	0.9837	
		32.7684	0.3199	1.1000×10^{-3}	1.73×10^{-5}	2.1423	0.9648	
	A	20.5733	0.8378	3.9000×10^{-3}	2.225×10^{-4}	4.5556	0.8553	
		26.8440	0.6627	3.4600×10^{-3}	1.199×10^{-4}	3.2271	0.9342	
		20.1381	1.1911	4.7400×10^{-3}	3.914×10^{-4}	7.5126	0.7521	
		18.2169	1.8206	6.0400×10^{-3}	8.393×10^{-4}	13.5905	0.4975	
		14.2982	2.0124	7.8800×10^{-3}	1.5400×10^{-3}	12.4689	0.2228	
	C	38.0782	0.1015	8.2129×10^{-5}	2.659×10^{-6}	0.4570	0.8315	
		37.7280	0.0887	8.1424×10^{-5}	2.333×10^{-6}	0.3512	0.8626	
		23.7650	0.0826	1.4477×10^{-4}	3.56×10^{-6}	0.2878	0.8952	
		35.6760	0.0938	8.9138×10^{-5}	2.619×10^{-6}	0.3901	0.8564	
	G	26.2781	0.3912	2.8800×10^{-3}	6.02×10^{-5}	1.3614	0.9710	
		20.2104	0.7175	3.8500×10^{-3}	1.914×10^{-4}	3.3889	0.8828	
		19.9839	0.5931	3.3100×10^{-3}	1.380×10^{-4}	2.7059	0.9091	
		21.8720	0.7436	3.8400×10^{-3}	1.827×10^{-4}	3.6522	0.8911	
	C	T	31.2140	0.1611	2.9894×10^{-4}	4.95×10^{-6}	1.0918	0.9417
			31.6105	0.1812	2.5058×10^{-4}	5.33×10^{-6}	1.4474	0.9060
			32.2294	0.1513	2.5705×10^{-4}	4.38×10^{-6}	1.0018	0.9380
31.3304			0.2227	3.2632×10^{-4}	6.94×10^{-6}	2.0335	0.9075	

	A	18.1094	0.1812	9.5683×10^{-4}	1.520×10^{-5}	0.7978	0.9576
		24.5694	0.2769	8.3504×10^{-4}	1.571×10^{-5}	2.0418	0.9367
		17.8560	0.2268	9.4261×10^{-4}	1.910×10^{-5}	1.2630	0.9306
		15.6324	0.2030	1.2100×10^{-3}	2.35×10^{-5}	0.8368	0.9451
		12.4945	0.2381	2.6500×10^{-3}	7.1233×10^{-5}	0.5481	0.9438
	C	29.3149	0.1172	2.0788×10^{-4}	3.62×10^{-6}	0.6312	0.9354
		30.0669	0.1338	2.0151×10^{-4}	4.01×10^{-6}	0.8273	0.9167
		15.5254	0.1011	3.8019×10^{-4}	6.59×10^{-6}	0.3985	0.9377
		29.3023	0.1079	1.7628×10^{-4}	3.27×10^{-6}	0.5519	0.9270
	G	31.6423	0.0856	9.6440×10^{-5}	2.292×10^{-6}	0.3771	0.8852
		20.0153	0.0911	1.3193×10^{-4}	3.94×10^{-6}	0.4116	0.8297
		23.1016	0.1209	1.4635×10^{-4}	4.56×10^{-6}	0.7137	0.8174

Table S12. Summary of the desorption rates and melting temperatures for each of the high G/C content probe combinations (Figure S7).

Capture (X)	Target (Y)	Average Desorption Rate (s⁻¹)	Standard Deviation (s⁻¹)	n	Melting Temperature (°C)	Desorption Rate Relative to Perfect Complement
A	T	7.3142 x 10 ⁻⁵	1.0654 x 10 ⁻⁵	3	47.989	1.00
	A	2.9540 x 10 ⁻³	4.2622 x 10 ⁻⁴	5	37.473	40.39
	C	4.0931 x 10 ⁻⁴	1.0120 x 10 ⁻⁵	2	38.701	5.60
	G	3.8700 x 10 ⁻³	9.1305 x 10 ⁻⁴	4	41.719	52.91
T	T	2.2700 x 10 ⁻³	5.6569 x 10 ⁻⁵	2	37.923	15.23
	A	1.4900 x 10 ⁻⁴	2.8607 x 10 ⁻⁵	5	45.446	1.00
	C	3.6772 x 10 ⁻⁴	1.6857 x 10 ⁻⁵	3	36.840	2.47
	G	2.6067 x 10 ⁻³	4.0154 x 10 ⁻⁴	3	39.590	17.49
G	T	9.3045 x 10 ⁻⁴	1.1481 x 10 ⁻⁴	4	40.937	9.36
	A	5.2040 x 10 ⁻³	1.7901 x 10 ⁻³	5	41.542	52.37
	C	9.9364 x 10 ⁻⁵	3.0468 x 10 ⁻⁵	4	48.928	1.00
	G	3.4700 x 10 ⁻³	4.6726 x 10 ⁻⁴	4	43.435	34.92
C	T	2.8322 x 10 ⁻⁴	3.5847 x 10 ⁻⁵	4	35.896	2.27
	A	1.3189 x 10 ⁻³	7.5672 x 10 ⁻⁴	5	37.221	10.56
	C	2.4146 x 10 ⁻⁴	9.3486 x 10 ⁻⁵	4	35.220	1.93
	G	1.2491 x 10 ⁻⁴	2.5688 x 10 ⁻⁵	3	48.342	1.00

Table S13. Fitting parameters used in determining the desorption rates for SNPs with heterozygote alleles (seen in Figure S10).

Capture Probe (X)	Target Probes (Y)	A	Std. Error	k_d (s ⁻¹)	Std. Error (s ⁻¹)	χ^2	R ²
A	T, A	63.9413	0.0799	7.0754×10^{-5}	1.233×10^{-6}	0.2892	0.9441
		53.4259	0.0655	6.8792×10^{-5}	1.209×10^{-6}	0.1947	0.9433
		49.9496	0.0604	6.9896×10^{-5}	1.194×10^{-6}	0.1656	0.9462
T	T, A	44.1643	0.1117	1.6038×10^{-4}	2.61×10^{-6}	0.5231	0.9509
		56.6922	0.1479	1.3451×10^{-4}	2.66×10^{-6}	0.9372	0.9291
		60.9121	0.1470	1.7027×10^{-4}	2.50×10^{-6}	0.8979	0.9597
		48.6977	0.1189	2.6579×10^{-4}	2.66×10^{-6}	0.5424	0.9811
		43.0696	0.1092	2.4976×10^{-4}	2.74×10^{-6}	0.4638	0.9773
		30.1717	0.1661	7.5961×10^{-4}	7.93×10^{-6}	0.7293	0.9819
G	T, A	48.4226	0.1416	4.7571×10^{-4}	3.57×10^{-6}	0.6511	0.9898
		52.8133	1.3606	5.8900×10^{-3}	2.123×10^{-4}	7.9300	0.9379
C	T, A	48.7450	1.5777	5.3800×10^{-3}	2.440×10^{-4}	11.7338	0.9062
		49.9744	1.6757	5.3100×10^{-3}	2.497×10^{-4}	13.4059	0.9001
		50.9883	1.7474	5.9200×10^{-3}	2.839×10^{-4}	13.0069	0.8979
		57.0780	0.1329	7.4189×10^{-5}	1.866×10^{-6}	0.9782	0.8663
A	T, C	51.0464	0.1347	8.5330×10^{-5}	2.130×10^{-6}	0.9929	0.8680
		55.0719	0.8906	2.4800×10^{-3}	5.65×10^{-5}	8.3316	0.9604
T	T, C	51.0398	0.8500	2.5100×10^{-3}	5.89×10^{-5}	7.4890	0.9585
		47.3188	0.8931	2.5000×10^{-3}	6.65×10^{-5}	8.3078	0.9481
		52.5767	0.0838	1.1851×10^{-4}	1.313×10^{-6}	0.3712	0.9712
G	T, C	51.5859	0.0614	1.1308×10^{-4}	9.8×10^{-7}	0.2000	0.9823
		50.2226	0.0777	1.1919×10^{-4}	1.27×10^{-6}	0.3185	0.9732

C		44.9880	2.4379	5.6700×10^{-3}	4.292×10^{-4}	26.4151	0.7430
		50.6008	2.4961	5.8600×10^{-3}	4.041×10^{-4}	26.7102	0.7783
		39.2606	2.8259	6.6100×10^{-3}	6.628×10^{-4}	30.1786	0.6028
		42.9966	3.0527	6.2800×10^{-3}	6.218×10^{-4}	37.1735	0.6123
A	T, G	65.3973	0.0716	1.0899×10^{-4}	1.10×10^{-6}	0.2249	0.9806
		60.0055	0.0785	1.2014×10^{-4}	1.33×10^{-6}	0.2675	0.9770
		56.5098	0.0608	1.2027×10^{-4}	1.09×10^{-6}	0.1605	0.9844
		60.8153	0.1066	9.3088×10^{-5}	1.756×10^{-6}	0.5060	0.9357
T		50.8062	0.1886	7.6235×10^{-4}	5.38×10^{-6}	0.9417	0.9918
		43.4829	0.2937	9.6073×10^{-4}	1.101×10^{-5}	1.9980	0.9796
		50.0208	0.0792	9.5830×10^{-4}	2.58×10^{-6}	0.1454	0.9989
		48.5170	0.1079	1.2100×10^{-3}	4.2×10^{-6}	0.2301	0.9984
G		48.7209	0.1185	4.4762×10^{-4}	2.93×10^{-6}	0.4671	0.9922
		49.5111	0.0521	4.4952×10^{-4}	1.27×10^{-6}	0.0903	0.9986
		52.3797	0.1326	4.7920×10^{-4}	3.11×10^{-6}	0.5716	0.9926
C		53.0998	0.0917	1.0880×10^{-4}	1.74×10^{-6}	0.3688	0.9526
		40.5070	0.0785	7.1142×10^{-5}	1.921×10^{-6}	0.2798	0.8756
		56.0100	0.0893	6.5975×10^{-5}	1.576×10^{-6}	0.3635	0.9001
		60.3927	0.1142	6.9447×10^{-5}	1.871×10^{-6}	0.5920	0.8764
		58.8434	0.1964	7.5454×10^{-5}	3.313×10^{-6}	1.7423	0.7277
A	A, C	46.8757	1.4708	6.4000×10^{-3}	2.829×10^{-4}	8.7061	0.9128
		42.5288	0.7429	4.3800×10^{-3}	1.082×10^{-4}	3.2880	0.9555
		39.3734	0.8932	3.7700×10^{-3}	1.211×10^{-4}	5.5448	0.9163
		45.3326	0.2811	5.1800×10^{-3}	4.53×10^{-5}	0.3963	0.9952
T		46.1079	0.0832	1.1892×10^{-4}	1.83×10^{-6}	0.3007	0.9565
		55.3489	0.0908	9.2757×10^{-5}	1.639×10^{-6}	0.3663	0.9431

		62.2493	0.1092	1.1030×10^{-4}	1.77×10^{-6}	0.5219	0.9527
		52.2946	0.0741	1.6003×10^{-4}	1.46×10^{-6}	0.2303	0.9842
		47.0549	0.0879	1.4756×10^{-4}	1.92×10^{-6}	0.3272	0.9686
G		29.2136	0.0844	9.0766×10^{-5}	2.885×10^{-6}	0.3173	0.8358
		50.2907	0.0577	6.0684×10^{-5}	1.129×10^{-6}	0.1522	0.9374
		48.7898	0.0517	5.9871×10^{-5}	1.042×10^{-6}	0.1221	0.9449
C		36.1094	2.9464	1.1220×10^{-2}	1.280×10^{-3}	19.3269	0.5838
		23.1046	0.8353	3.8800×10^{-3}	1.988×10^{-4}	4.7003	0.7729
		37.7423	0.3068	7.2400×10^{-3}	8.28×10^{-5}	0.3333	0.9930
		34.5882	0.6143	7.2300×10^{-3}	1.804×10^{-4}	1.3389	0.9691
		28.4739	0.7676	4.9000×10^{-3}	1.865×10^{-4}	3.1294	0.8905
A	A, G	46.8060	0.1135	4.7501×10^{-4}	2.96×10^{-6}	0.4211	0.9929
		43.8659	0.0910	3.4670×10^{-4}	2.36×10^{-6}	0.2986	0.9914
		34.1311	0.1068	4.4851×10^{-4}	3.77×10^{-6}	0.3806	0.9871
		40.7340	0.1092	4.5968×10^{-4}	3.25×10^{-6}	0.3944	0.9909
T		43.8045	0.0581	1.5629×10^{-4}	1.37×10^{-6}	0.1424	0.9854
		46.5544	0.0571	1.6229×10^{-4}	1.27×10^{-6}	0.1370	0.9883
		51.7000	0.0733	1.6764×10^{-4}	1.47×10^{-6}	0.2248	0.9853
G		26.7882	0.0967	1.0800×10^{-3}	6.3×10^{-6}	0.2014	0.9951
		38.9356	0.0814	8.6504×10^{-4}	3.21×10^{-6}	0.1642	0.9978
		39.0394	0.1183	8.1733×10^{-4}	4.52×10^{-6}	0.3581	0.9950
C		31.5048	0.0831	1.2158×10^{-4}	2.67×10^{-6}	0.3002	0.9134
		50.5194	0.0630	6.6492×10^{-5}	1.230×10^{-6}	0.1809	0.9374
		33.8945	0.0411	4.1477×10^{-5}	1.182×10^{-6}	0.0787	0.8631
		44.3445	0.1151	6.1320×10^{-5}	2.556×10^{-6}	0.6076	0.7450
		45.1732	0.0450	5.4971×10^{-5}	9.77×10^{-7}	0.0931	0.9421

		42.1808	0.0519	7.2191×10^{-5}	1.218×10^{-6}	0.1224	0.9476
A	C, G	54.4163	0.2599	3.6747×10^{-4}	5.51×10^{-6}	2.3631	0.9594
		44.3384	0.2528	3.8975×10^{-4}	6.66×10^{-6}	2.1969	0.9479
		44.5219	0.2152	4.1516×10^{-4}	5.72×10^{-6}	1.5611	0.9658
		43.1168	0.2415	4.4798×10^{-4}	6.75×10^{-6}	1.9168	0.9595
T		47.1016	0.1899	9.5673×10^{-4}	6.55×10^{-6}	0.8278	0.9930
		54.5349	0.1672	9.8071×10^{-4}	5.05×10^{-6}	0.6315	0.9960
		47.2464	0.1232	1.4200×10^{-3}	5.5×10^{-6}	0.2634	0.9982
		45.2700	0.1710	9.9526×10^{-4}	6.28×10^{-6}	0.6549	0.9942
G		46.1395	0.1137	9.4366×10^{-5}	2.467×10^{-6}	0.5671	0.8834
		52.6328	0.0456	8.7428×10^{-5}	8.66×10^{-7}	0.0920	0.9816
C		57.8145	0.0501	4.3575×10^{-5}	8.48×10^{-7}	0.1155	0.9325
		34.8381	0.0302	5.7019×10^{-5}	8.52×10^{-7}	0.0413	0.9591
		51.3347	0.0727	8.7245×10^{-5}	1.413×10^{-6}	0.2332	0.9522

Table S14. Summary of the desorption rates for each of the capture and target probe combinations used in profiling SNPs with heterozygote alleles (shown in Figure S10).

Capture (X)	Targets (Y)	Average Desorption Rate (s⁻¹)	Standard Deviation (s⁻¹)	n
A	T, A	6.9814 x 10 ⁻⁵	9.84 x 10 ⁻⁷	3
T		1.9614 x 10 ⁻⁴	5.804 x 10 ⁻⁵	5
G		6.1766 x 10 ⁻⁴	2.0075 x 10 ⁻⁴	2
C		5.6250 x 10 ⁻³	3.248 x 10 ⁻⁴	4
A	T, C	7.9760 x 10 ⁻⁵	7.878 x 10 ⁻⁶	2
T		2.4967 x 10 ⁻³	1.53 x 10 ⁻⁵	3
G		1.1693 x 10 ⁻⁴	3.35 x 10 ⁻⁶	3
C		6.1050 x 10 ⁻³	4.223 x 10 ⁻⁴	4
A	T, G	1.1062 x 10 ⁻⁴	1.283 x 10 ⁻⁵	4
T		9.7284 x 10 ⁻⁴	1.8340 x 10 ⁻⁴	4
G		4.5878 x 10 ⁻⁴	1.771 x 10 ⁻⁵	3
C		7.8163 x 10 ⁻⁵	1.7462 x 10 ⁻⁵	5
A	A, C	4.9325 x 10 ⁻³	1.1360 x 10 ⁻³	4
T		1.2591 x 10 ⁻⁴	2.750 x 10 ⁻⁵	5
G		7.0441 x 10 ⁻⁵	1.7607 x 10 ⁻⁵	3
C		6.8940 x 10 ⁻³	2.8287 x 10 ⁻³	5
A	A, G	4.3248 x 10 ⁻⁴	5.821 x 10 ⁻⁵	4
T		1.6207 x 10 ⁻⁴	5.68 x 10 ⁻⁶	3
G		9.2079 x 10 ⁻⁴	1.56 x 10 ⁻⁶	3
C		6.9672 x 10 ⁻⁵	2.7538 x 10 ⁻⁵	6
A	C, G	4.0509 x 10 ⁻⁴	3.460 x 10 ⁻⁵	4
T		1.0882 x 10 ⁻³	2.218 x 10 ⁻⁴	4
G		9.0897 x 10 ⁻⁵	4.906 x 10 ⁻⁶	2
C		6.2613 x 10 ⁻⁵	2.2366 x 10 ⁻⁵	3

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