Supplementary Information

PNA Microprobe for Label-Free Detection of Expanded Trinucleotide Repeats

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Table S1. Single-stranded and double-stranded repeat sequences.

Repeat type	Sequence	#Repeats
PNA-CTG-6	HS-C_6H_{12}-CTG CTG CTG CTG CTG CTG	6
ssCAG-6	5′-CAG GAG CAG CAG CAG CAG -3′	6
ssCAG-10	5′-CAG GAG CAG CAG CAG CAG CAG CAG CAG CAG-3′	10
ssCAG-15	5′-CAG CAG GAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG-3′	15
ssCAG-20	5′-CAG CAG GAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG-3’	20

Extraction of Total RNA from Neural Stem Cells (NSCs). For RNA extraction of prepatterned activin A treated human NSCs, total RNA was isolated according to the manufacturer's instructions using the ISOLATE II RNA Mini Kit (Bioline, BIO-52072), and eluted in 50 µl of RNase-free water and store at -80°C. The quantity and quality of RNA were assessed by measuring the optical density (OD) at 260 nm and 280 nm using a NanoDrop® ND-1000 Spectrophotometer (Thermo Fisher, MA, USA). The RNA quality of the sample was measured by monitoring the absorbance at A260/280 and A260/230 ratios. The extraction of CAG containing total RNA has been previously reported.[1-3] The HD+ RNA had 72CAG/19CAG repeats, i.e. there is one allele with 72CAG and the other one with 19CAG. While the control sample (HD-) was generated from a genetic correction of HD72 by decreasing the CAG length to a

normal repeat of 21CAG. Therefore, the control sample (HD-) had 21CAG/19CAG repeats which leads to a normal phenotype. The extracted RNA was stored at -80 ^{o}C. For sample preparation, the RNA was thawed in cool environment and then diluted to working solutions of 10 ng·μL^{-1} and 1 ng·μL^{-1} in the working buffer of 100 μM Tris containing 20 mM $MgCl_2$ and 200 mM NaCl (pH = 8.5).

HD CAG PCR. The HD- (21CAG) and HD+ (72CAG) repeats were confirmed by performing PCR in the NSC cells. The HD- and HD+ NSC cells were purified with DNeasy Blood and Tissue Kit (Cat. No. 69504). The HD PCR mix is composed of 10 ng·μL^{-1} of sample DNA (1 μL), GoTaq Green Master Mix (10 μL), DMSO (0.5 μL), 10 μM CAG-1 Forward primer 1μL, 10 μM HU3R Reverse Primer 1ul, Nuclease free water 6.5 μL (20 μL total). CAG-1 (ATGAAGGCCTTCGAGTCCCTCAAGTCCTTC) and HU3R (GGCGGCTGAGGAAGCTGAGGA). The PCR program used the following parameters: 96 ^{o}C, 10 min-> 95 ^{o}C, 30 sec|65 ^{o}C, 30 sec|72 ^{o}C, 1 min 30 sec (35 cycles) -> 72^{o}C, 10 min -> 16 ^{o}C, infinite. 2% Agarose gel (2 g PCR agar in 100 mL 1XTAE buffer+ 1 μl Gelred) was used for electrophoresis and 10 μl nuclease free water was added to 10 μl PCR mix and loaded for gel run for 20-25min, 100V with a 100 bp PCR marker. The image was taken using Bio-Rad imager with Gelred protocol.

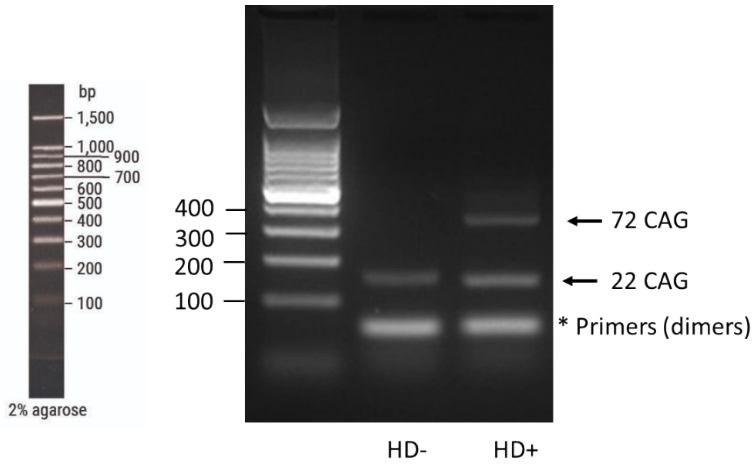

Figure S1. PCR of the CAG region of HD- (21CAG) and HD+ (72CAG). The expected product of 146 base pair was obtained for the HD- (21CAG). The expected products of around ~150 basepair and ~300 basepair were found for the HD+ (72CAG) DNA sample. The size of the molecular weight marker is in base pair for the x-axis.

Table S2. EIS extracted parameters of Figure 1. The average values with standard error of modified Randle's equivalent circuit elements. The errors were calculated for N ≥ 4 separate measurements.

Sensor Layers	Solution Resistance	Constant Phase Equivalent		Charge Transfer Resistance	Warburg Impedance	Relative Error for R_{ct}
	R_s kΩ	CPE μF	n	R_{ct} kΩ	W kΩ	%RSD
Bare gold	0.05 (0.004)	5.5 (1.6)	0.90	1.2 (0.3)	0.008 (0.01) × 10^8	25.0
PNA	0.05 (0.002)	0.22 (0.03)	0.88	5.6 (0.7)	0.003 (0.004) × 10^{12}	12.5
MCH	0.05 (0.01)	0.23 (0.18)	0.80	17.9 (3.6)	0.002 (0.002) × 10^{12}	20.1
PNA-MCH	0.05 (0.001)	0.20 (0.05)	0.89	27.8 (0.5)	0.005 (0.006) × 10^9	1.7

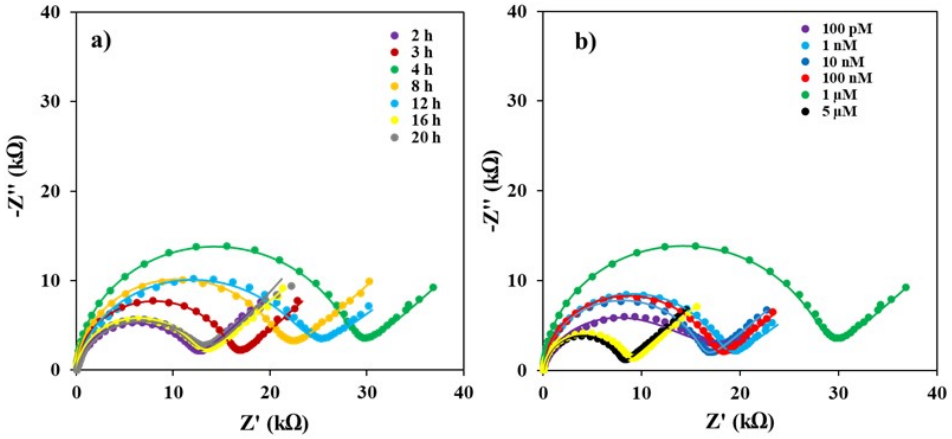

Figure S2. a) Nyquist plot of PNA probe (CTG-6) obtained at various incubation time. **b)** Nyquist plot of PNA probe (CTG-6) obtained at various concentration of the probe.

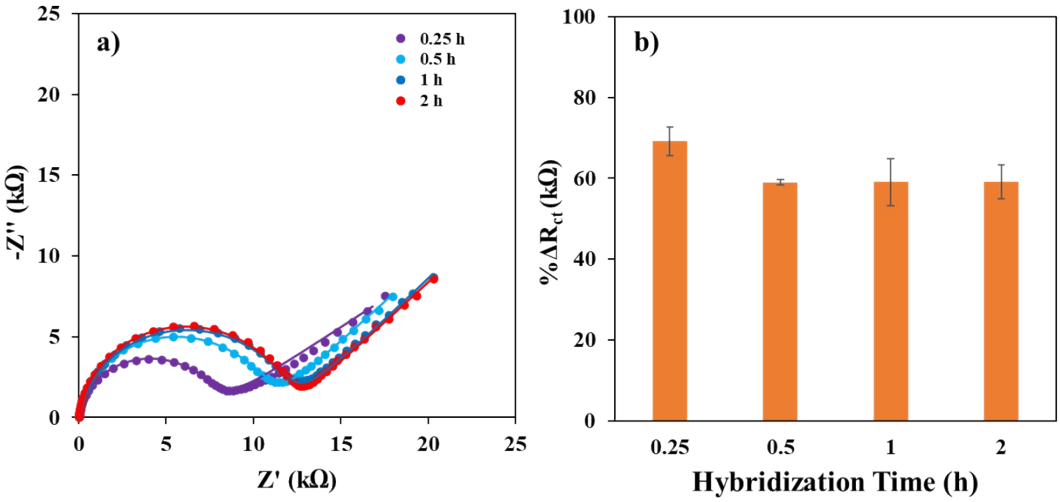

Figure S3. a) Nyquist plots of time of hybridization with the target (CAG-6). b) Percent change in R_{ct} after hybridization of 10 pM CAG-6 target. Error bar represents the standard deviation for $N \geq 4$.

Table S3. T-test to confirm significant difference between the absolute R_{ct} response of probe and the HD+ RNA sample

	PNA	HD+
#	R_{ct} (kΩ)	R_{ct} (kΩ)
1	27.3	23.3
2	27.7	23.3
3	28.2	23.9
4	30.5	25.3
Average	28.4	24.0
Standard Deviation	1.4	0.9
Variance	2.0	0.9
p-value, F-Test	0.511	> 0.05, Equal variance
p-value, one tail T-Test	0.00099	< 0.05, Significant difference

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