Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

Microfluidic screening system based on boron-doped diamond electrodes and dielectrophoretic sorting for directed evolution of NAD(P)-dependent oxidoreductases

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Contents

Supplementary Table S1 The oligonucleotide sequences used in this study

Primer	Sequence
T7-F	5'-TAATACGACTCACTATAGGG-3'
T7-R	5'-GCTAGTTATTGCTCAGCGG-3'
T7-F2	5'-TAATACGACTCAACTATAGGGAGACCACAAC-3'
T7-R2	5'-GCTAGTTATTGCTCAGCGGTGGC-3'
Insert-F	5'-ACCGCGAACAGATTGGAGGTGCGGAAAAAGTGTCGTTTGA-3'
Insert-R	5'-AGCAGCCGGATCCTCGAGTCACTCGAGGGCGGCCGGAGCGT-3'
Vector-F	5'-ACCTCCAATCTGTTCGCGGTG-3'
Vector-R	5'-TGACTCGAGGATCCGGCTGCT-3'

Supplementary Table S2 The data corresponding to Figure 3 for peak currents of plugs at various concentrations (0, 1, 10, 20 and 50 μM) of NADH.

	0 μM	1 μM	10 μM	20 μM	50 μM
Mean	4.13	7.17	22.9	51.5	98.3
SD	2.08	3.38	4.75	3.71	12.0
Number of samples	1036	652	606	490	383

Supplementary Table S3 The data corresponding to Figure 4 for peak currents of plugs containing microbeads on which the wild-type ADH (WT) or randomly mutated ADH (Mn^{2+} concentration: 0.1, 0.3 or 0.5 mM) was immobilized.

	WT	0.1 mM	0.3 mM	0.5 mM
Mean	68.0	48.4	56.8	52.9
SD	16.0	10.8	9.9	36.8
Number of samples	208	182	327	141

Supplementary Table S4 The data corresponding to Figure 6 for relative peak currents of plugs containing microbeads on which the wild-type IDH (WT) or randomly mutated IDH library before (Mut) and after the first or second round of screening (1st and 2nd) was immobilized.

	WT	Mut (R0)	1st (R1)	2nd (R2)
Mean	1.0	0.92	0.98	1.0
SD	0.038	0.019	0.038	0.019
Number of samples	493	534	837	439

a

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         10        20        30        40        50        60        70        80        90
CATATGAAAGCAGCCGTGGTAGAACAGTTTAAAGAGCCGCTGAAAATCAAAGAAGTGGAGAAACCGACGATCTCGTATGGCGAAGTTTTA
    M  K  A  A  V  V  E  Q  F  K  E  P  L  K  I  K  E  V  E  K  P  T  I  S  Y  G  E  V  L

        100       110       120       130       140       150       160       170       180
GTGCGCATCAAAGCATGCGGTGTATGCCATACCGATCTTCACGCAGCTCATGGGGATTGGCCGGTGAAACCAAAACTGCCGTTAATTCCA
 V  R  I  K  A  C  G  V  C  H  T  D  L  H  A  A  H  G  D  W  P  V  K  P  K  L  P  L  I  P

        190       200       210       220       230       240       250       260       270
GGACACGAAGGTGTGGGCATCGTGGAAGAAGTTGGTCCTGGGGTTACGCACCTGAAAGTTGGCGATCGCGTCGGTATTCCTTGGCTGTAT
 G  H  E  G  V  G  I  V  E  E  V  G  P  G  V  T  H  L  K  V  G  D  R  V  G  I  P  W  L  Y

        280       290       300       310       320       330       340       350       360
TCAGCATGTGGCCATTGCGACTATTGCCTGTCTGGTCAGGAAACGTTATGCGAACACCAGAAGAATGCAGGCTATTCCGTGGATGGTGGT
 S  A  C  G  H  C  D  Y  C  L  S  G  Q  E  T  L  C  E  H  Q  K  N  A  G  Y  S  V  D  G  G

        370       380       390       400       410       420       430       440       450
TATGCCGAGTACTGTCGTGCTGCGGCAGACTACGTGGTTAAGATTCCGGACAACTTGTCGTTTGAAGAAGCTGCCCCGATTTTCTGTGCT
 Y  A  E  Y  C  R  A  A  A  D  Y  V  V  K  I  P  D  N  L  S  F  E  E  A  A  P  I  F  C  A

        460       470       480       490       500       510       520       530       540
GGCGTCACAACCTACAAAGCGCTGAAAGTTACCGGTGCTAAACCAGGCGAATGGGTTGCGATTTATGGCATCGGTGGCTTAGGTCATGTT
 G  V  T  T  Y  K  A  L  K  V  T  G  A  K  P  G  E  W  V  A  I  Y  G  I  G  G  L  G  H  V

        550       560       570       580       590       600       610       620       630
GCGGTGCAATACGCGAAAGCCATGGGCCTGAATGTCGTGGCCGTGGACATTGGTGACGAGAAGCTGGAACTCGCCAAAGAACTCGGTGCA
 A  V  Q  Y  A  K  A  M  G  L  N  V  V  A  V  D  I  G  D  E  K  L  E  L  A  K  E  L  G  A

        640       650       660       670       680       690       700       710       720
GATCTGGTGGTCAATCCGCTGAAGGAAGATGCCGCGAAATTCATGAAAGAGAAAGTCGGAGGGGTGCATGCGGCTGTCGTAACTGCGGTA
 D  L  V  V  N  P  L  K  E  D  A  A  K  F  M  K  E  K  V  G  G  V  H  A  A  V  V  T  A  V

        730       740       750       760       770       780       790       800       810
AGTAAGCCGGCCTTTCAGAGCGCGTACAACAGCATTCGGCGTGGAGGCGCGTGTGTGCTGGTTGGGCTTCCGCCTGAAGAGATGCCCATT
 S  K  P  A  F  Q  S  A  Y  N  S  I  R  R  G  G  A  C  V  L  V  G  L  P  P  E  E  M  P  I

        820       830       840       850       860       870       880       890       900
CCGATTTTCGACACTGTGCTGAACGGGATCAAAATTATCGGAAGCATTGTAGGCACCCGCAAAGATCTGCAGGAAGCGCTCCAATTTGCC
 P  I  F  D  T  V  L  N  G  I  K  I  I  G  S  I  V  G  T  R  K  D  L  Q  E  A  L  Q  F  A

        910       920       930       940       950       960       970       980       990
GCGGAAGGCAAAGTCAAGACCATCATTGAAGTTCAACCCCTTGAGAAGATCAACGAAGTCTTTGATCGCATGTTGAAAGGCCAGATTAAT
 A  E  G  K  V  K  T  I  I  E  V  Q  P  L  E  K  I  N  E  V  F  D  R  M  L  K  G  Q  I  N

       1000      1010      1020      1030      1040      1050      1060      1070      1080
GGCCGTGTCGTACTGACATTGGAGGATAAAGGATCCGCGGCCGTTTACCCATACGATGTTCCTGACTATGCGGGCTATCCCTATGACGTC
 G  R  V  V  L  T  L  E  D  K  G  S  A  A  V  Y  P  Y  D  V  P  D  Y  A  G  Y  P  Y  D  V

       1090      1100      1110      1120      1130      1140
CCGGACTATGCAGGGTCCTATCCCTATGACGTTCCAGATTACGCTCCGGCCGCCCTCGAG
 P  D  Y  A  G  S  Y  P  Y  D  V  P  D  Y  A  P  A  A  L  E
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Supplementary Fig. S1 The DNA and amino acid sequences of dehydrogenases used in this study. (a) Alcohol dehydrogenase (ADH) from *Geobacillus stearothermophilus*. (b) Isocitrate dehydrogenase (IDH) from *Streptococcus mutans*. The codon usage is optimized for expression in *Escherichia coli*. NdeI, BamHI and XhoI recognition sites are indicated in the boxes. Underline indicates 3xHA-tag.

b

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        10        20        30        40        50        60        70        80        90
CATATGGCGGAAAAAGTGTCGTTTGAAGAGGGTAAACTTCAAGTGCCGGATAAACCGGTTATACCGTACATTGAAGGTGATGGAGTTGGT
    M  A  E  K  V  S  F  E  E  G  K  L  Q  V  P  D  K  P  V  I  P  Y  I  E  G  D  G  V  G

       100       110       120       130       140       150       160       170       180
CAGGACATTTGGAAGAATGCGCAAATCGTCTTCGACAAAGCCATTGCCAAGGTGTATGGGGGTCACAAACAGGTGATCTGGCGTGAAGTT
 Q  D  I  W  K  N  A  Q  I  V  F  D  K  A  I  A  K  V  Y  G  G  H  K  Q  V  I  W  R  E  V

       190       200       210       220       230       240       250       260       270
CTTGCAGGCAAAAAGGCCTATAACGAAACGGGCAACTGGCTGCCGAATGAGACGCTGGAAATCATCAAAACCCACTTACTGGCGATAAAA
 L  A  G  K  K  A  Y  N  E  T  G  N  W  L  P  N  E  T  L  E  I  I  K  T  H  L  L  A  I  K

       280       290       300       310       320       330       340       350       360
GGGCCACTGGAAACTCCGGTTGGTGGGGGCATTCGTTCACTGAACGTAGCGTTACGTCAAGAACTGGACCTGTTTGCCTGCGTTCGACCA
 G  P  L  E  T  P  V  G  G  G  I  R  S  L  N  V  A  L  R  Q  E  L  D  L  F  A  C  V  R  P

       370       380       390       400       410       420       430       440       450
GTACGCTACTTCAAGGGTGTACCCTCTCCTCTGAAACATCCGGAAAAAACGGCTATTACGATTTTCCGGGAAAACACCGAGGACATTTAT
 V  R  Y  F  K  G  V  P  S  P  L  K  H  P  E  K  T  A  I  T  I  F  R  E  N  T  E  D  I  Y

       460       470       480       490       500       510       520       530       540
GCTGGCATTGAATGGAATGCGGGTACTGCGGAAGTGCAGAAAGTGATCAACTTTTTGCAGGATGATATGCAGGTAAAGAAGATCCGCTTT
 A  G  I  E  W  N  A  G  T  A  E  V  Q  K  V  I  N  F  L  Q  D  D  M  Q  V  K  K  I  R  F

       550       560       570       580       590       600       610       620       630
CCGAAAAGTTCCAGCATTGGCATTAAACCGATAAGCATCGAAGGGTCGCAACGCCTCATTAGAGCCGCAATTGAGTATGCACTGGCCAAT
 P  K  S  S  S  I  G  I  K  P  I  S  I  E  G  S  Q  R  L  I  R  A  A  I  E  Y  A  L  A  N

       640       650       660       670       680       690       700       710       720
AACCTGACCAAAGTGACCCTGGTCCATAAAGGAAACATCCAGAAGTTCACCGAAGGAGGCTTTCGCAAATGGGGGTATGAGTTGGCTAAA
 N  L  T  K  V  T  L  V  H  K  G  N  I  Q  K  F  T  E  G  G  F  R  K  W  G  Y  E  L  A  K

       730       740       750       760       770       780       790       800       810
CGCGAGTATGCTGCCGAACTTGCGAGTGGCCAGTTAGTGGTTGACGACATTATTGCGGATAACTTCCTCCAGCAGATTCTGCTGAAACCT
 R  E  Y  A  A  E  L  A  S  G  Q  L  V  V  D  D  I  I  A  D  N  F  L  Q  Q  I  L  L  K  P

       820       830       840       850       860       870       880       890       900
GAGCGTTTTGATGTGGTGGCACTGACCAACCTTAACGGCGATTACGCCAGTGATGCGTTAGCTGCACAAGTTGGCGGTATCGGCATTTCA
 E  R  F  D  V  V  A  L  T  N  L  N  G  D  Y  A  S  D  A  L  A  A  Q  V  G  G  I  G  I  S

       910       920       930       940       950       960       970       980       990
CCAGGTGCGAATATCAATTACCAGACTGGCCATGCCATTTTCGAAGCGACACACGGAACCGCACCCGATATCGCAGGTCAAGATCTCGCG
 P  G  A  N  I  N  Y  Q  T  G  H  A  I  F  E  A  T  H  G  T  A  P  D  I  A  G  Q  D  L  A

      1000      1010      1020      1030      1040      1050      1060      1070      1080
AATCCTAGCTCCGTCTTGCTGTCGGGTTGTATGCTCTTTGACTATATCGGCTGGTCTAAAGTTAGCGATCTGATCATGAAAGCTGTCGAG
 N  P  S  S  V  L  L  S  G  C  M  L  F  D  Y  I  G  W  S  K  V  S  D  L  I  M  K  A  V  E

      1090      1100      1110      1120      1130      1140      1150      1160      1170
AAAGCAATTGCGAATGGCCAGGTGACAATCGATTTTGCCAAAGAACTAGGCGTCGAAGCGTTGACCACACGTCAGTTTAGCGAAGTCCTG
 K  A  I  A  N  G  Q  V  T  I  D  F  A  K  E  L  G  V  E  A  L  T  T  R  Q  F  S  E  V  L

      1180      1190      1200      1210      1220      1230      1240      1250      1260
CTGACGTACTTAGGATCCGCGGCCGTTTACCCATACGATGTTCCTGACTATGCGGGCTATCCCTATGACGTCCCGGACTATGCAGGGTCC
 L  T  Y  L  G  S  A  A  V  Y  P  Y  D  V  P  D  Y  A  G  Y  P  Y  D  V  P  D  Y  A  G  S

      1270      1280      1290      1300
TATCCCTATGACGTTCCAGATTACGCTCCGGCCGCCCTCGAG
 Y  P  Y  D  V  P  D  Y  A  P  A  A  L  E
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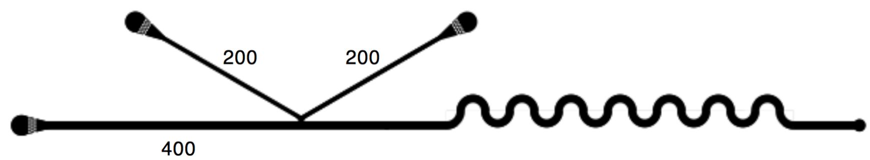
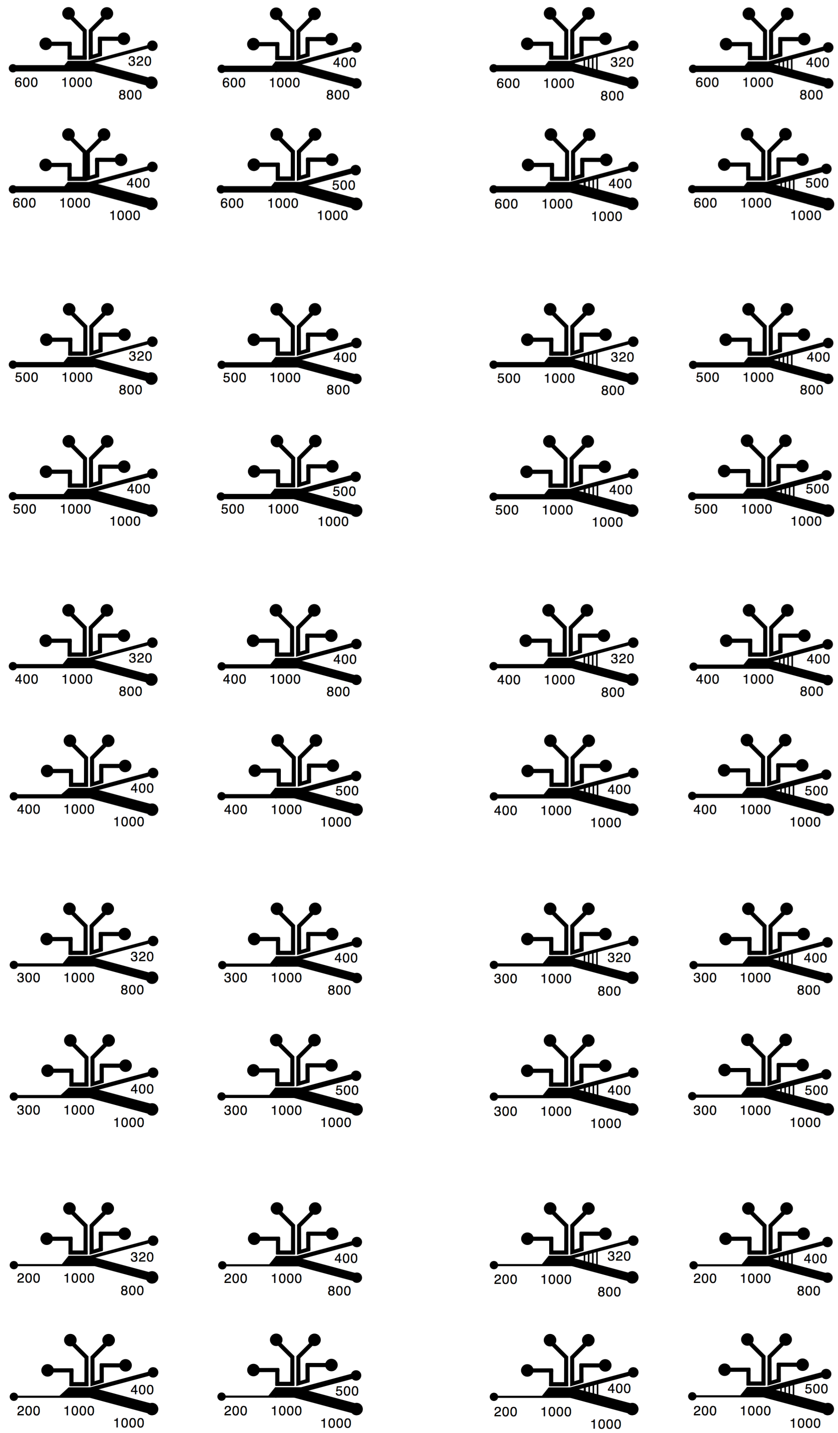
Supplementary Fig. S1 (*continued*)



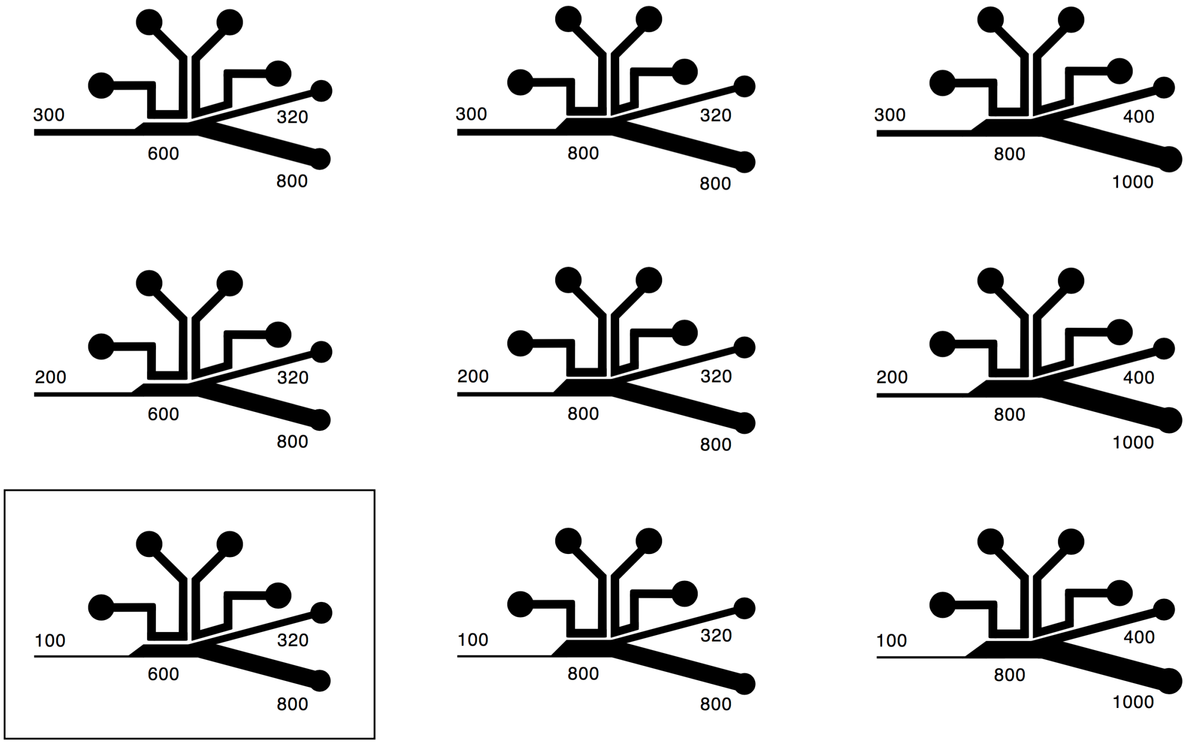
Supplementary Fig. S2 Masks for the fabrication of the encapsulating device.

a

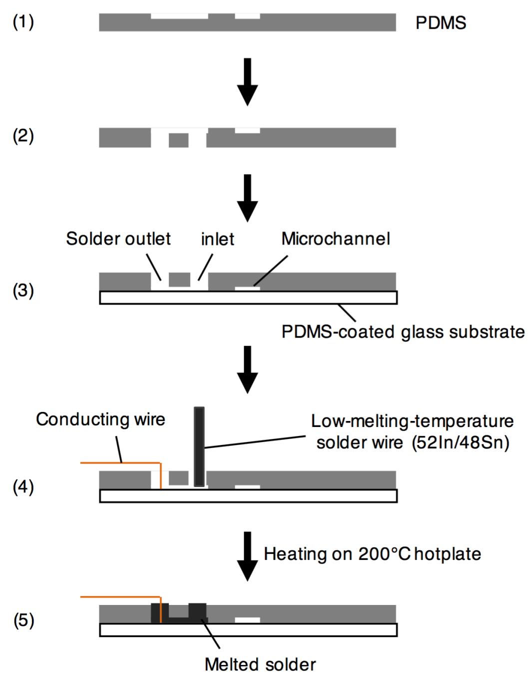


Supplementary Fig. S3 Masks for the fabrication of the droplets-sorting device.
(a) Forty patterns of devices with different channel widths [inlet, center, outlet (collect) and outlet (waste)] and with (right) or without (left) a shunt structure. (b) Nine patterns of devices. Box, optimized device.

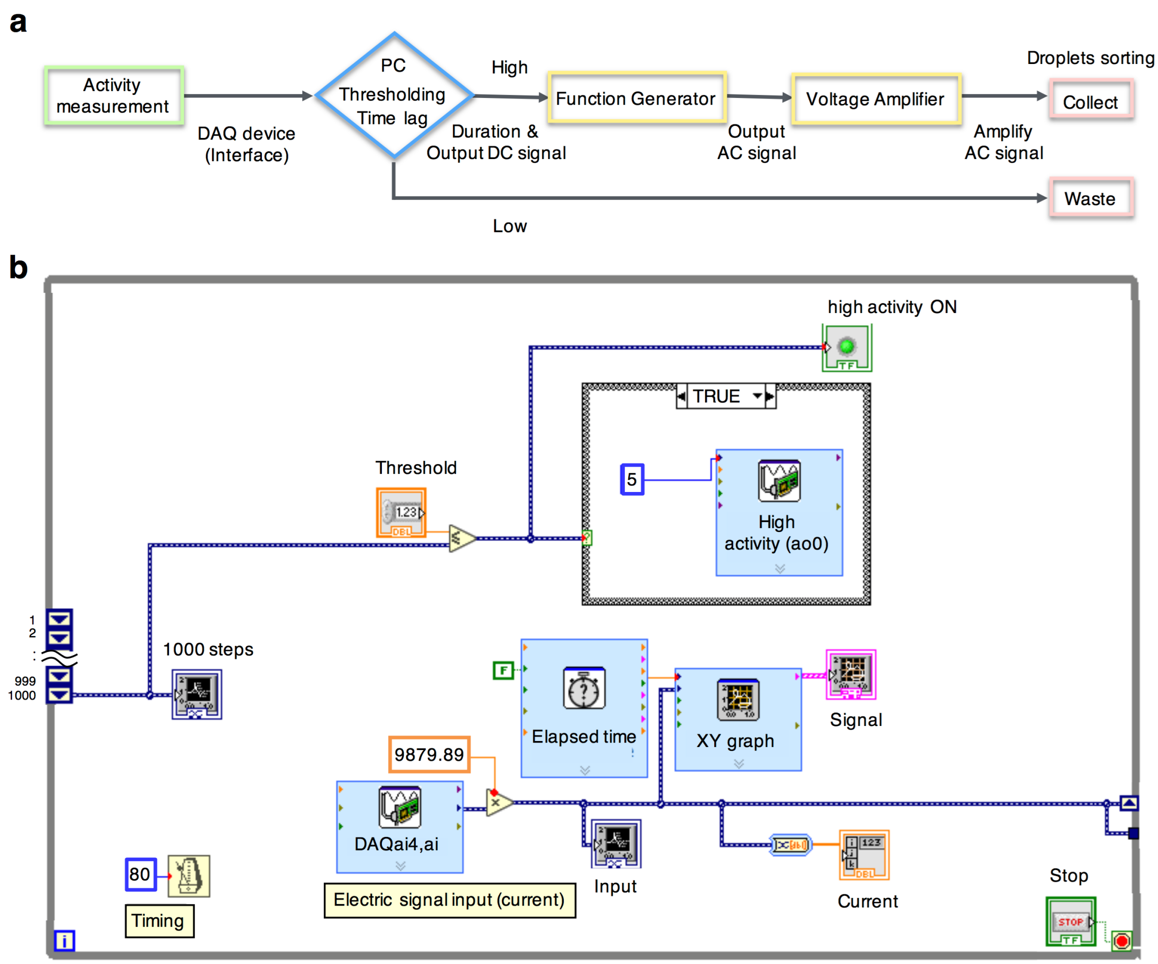
b



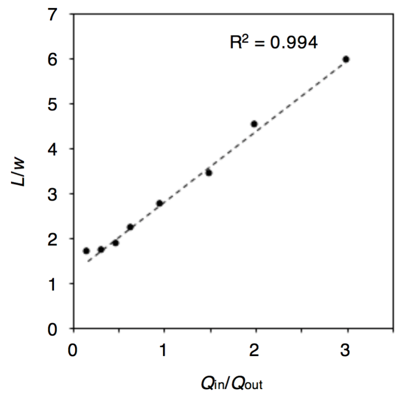
Supplementary Fig. S3 (*continued*)



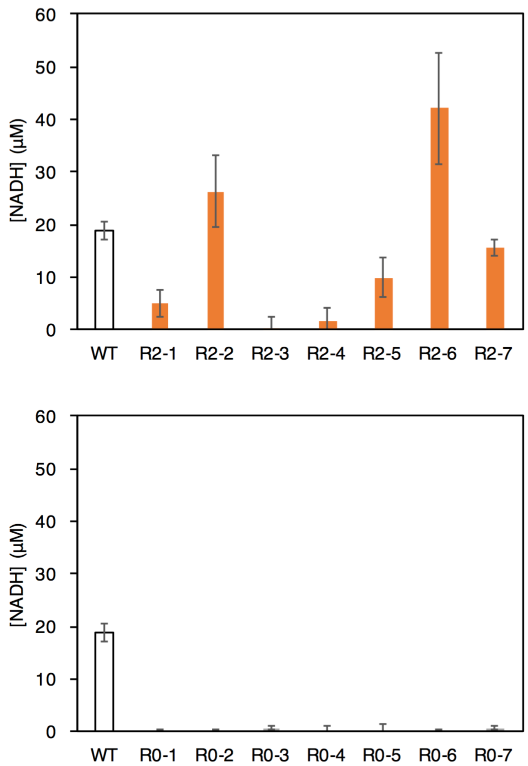
Supplementary Fig. S4 Fabrication method of the droplet-sorting device. (1) PDMS structured by using standard soft lithography. (2) Punched holes as the low-melting-temperature solder wire inlets and outlets. (3) The structured PDMS was sealed by oxygen plasma to a flat surface to enclose the channels. (4) The conducting wire was set at the outlet holes and the low-melting-temperature solder wire at the inlet hole on the 200°C hot plate. (5) After the solder wire melted, it was removed from the hot plate to harden.



Supplementary Fig. S5 Fabrication of data acquisition and control system. (a) A flow chart of our detection and sorting system. First, the continuous analog signals detected by the NAD(P)H-measuring device were converted to digital signals *via* a DAQ device. Then, each signal was compared with a threshold value and divided into high and low signals to the function generator by the LabVIEW program (b) on a PC. When only high signals were sent, the function generator output AC signal using burst oscillation. Then, signals were amplified by voltage amplifier. The amplified signals to the dielectrophoretic electrode controlled switching and duration. Finally, on the droplet-sorting device, droplets containing enzymes with higher activity were collected *via* the "collect channel." (b) A block diagram of the LabVIEW program.



Supplementary Fig. S6 Relationship between the plug size and flow rates in the encapsulating device. Dimensionless droplet length *(L/w)* was plotted as a function of ratios of the rates of flow of the dispersed and continuous phases (Q_{in}/Q_{out}) for the reference geometry (h = 65 μm, w = 400 μm).



Supplementary Fig. S7 Activity measurement of IDH clones by observing NADH absorbance.
Each seven clones were randomly picked from the library after two rounds of screening (top) and the initial library (bottom), respectively. These clones were translated by the PURE system and the activities of the products were measured by absorbance of NADH at 340 nm. Mean ± SD. N = 3. WT; wild-type. Mutants; R2-1 (V358A), R2-2 (I304V), R2-3 (P300L), R2-4 (R197C, Y420C), R2-5 (K12E, A36Q), R2-6 (K17R), R2-7 (M1T, S286G), R0-1 (frameshift), R0-2 (Q162H, V275M, K360N), R0-3 (A2E, Q13R, N104D, K131R), R0-4 (E24G, E153G, G157D), R0-5 (L251F, D254G, K268R, E270G, D343G, T368A, A431P), R0-6 (nonsense), R0-7 (frameshift).