Electronic Supplementary Information

An efficient and recyclable electroeluter: From momemade to modular design for potential mass production

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Materials.

All chemicals were of analytical or biochemical grade and were used without further purification. The chemically synthesized oligonucleotide, Universal UNIQ-10 Column DNA Purification Kit, Taq PCR Master Mix, SanPrep Column PCR Product Purification Kit, 4S GelRed Nucleic Acid Stain, Bovine Serum Albumin (BSA), 6×loading buffer, SDS-PAGE preparation kit, glycogen, Common Stain Buffer, and linear acrylamide solution (LPA, 5 mg/mL) were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Dialysis membrane (5 kD molecular weight cut off) was purchased from Spectrum Labs (Asheville, NC, USA). G-Capsule electroelution device was purchased from G-Biosciences, a registered trademark of Geno Technololgy, Inc. (St. Louis, USA). Kits of ssDNA high-sensitivity quantification and dsDNA broad range quantification were purchased from Hangzhou Allsheng Instruments Co., Ltd. (Hangzhou, China). The water used throughout all experiments was purified through a Milli-Q Biocell System.

Instruments.

The concentrations of all DNA solutions were determined using a Nano-500 micro-spectrophotometer (Hangzhou AllSheng Instruments Co., Ltd., Hangzhou, China), with an added module to measure fluorescence. Gel electrophoresis was performed with a JY600C Universal Power Supply and associated JY-SPCT Horizontal Electrophoresis Cell as well as JY-SCZ2+ Mini-Protean Vertical Electrophoresis Cell (Beijing JUNYI Electrophoresis Co., Ltd., Beijing, China). After electrophoresis, the gel was digitally imaged using an SH-Advance 523 Multi-functional Imaging System (Shenhua Science Technology Co., Ltd., Hangzhou, China). Polymerase Chain Reactions (PCR) were performed on TC1000-G thermal cycler (DLAB Scientific Co., Ltd., Beijing, China). High-speed centrifugation was performed on a Refrigerated Centrifuge 5424 R system (Eppendorf China Ltd., Beijing, China). Fluorescent emission spectra were recorded on an RF-6000 spectrofluorometer (Shimadzu (China) Co., Ltd., Beijing, China).

Denaturing Polyacrylamide Gel Electrophoresis.

Polyacrylamide gels (3%) were prepared with 10×TBE or Tris-Glycine (1 mL), 19:1 acrylamide/N,N'-methylenebisacrylamide (1.5 mL, 30%), urea (4.2 g), and water (8 mL). Then ammonium persulfate (APS) (30 μL, 10%) and tetramethylethylenediamine (TEMED) (10 μL) were added and allowed to polymerize at least 30 minutes before use. The gel was run at a constant voltage of 100 V in 1×TBE or Tris-Glycine as a running buffer.

Agarose Gel Electrophoresis

Agarose gels (1%) were prepared with 1×TAE or TBE (40 mL) and agarose (0.4 g) in a 250 ml flask, the mixture was heated to boil and kept simmering until agarose is completely dissolved. After slowing cooling down to around 60 °C, 4 μL GelRed was added to the gel solution and mixed up. Then the solution was poured into a gel tray with the good comb in place, which is further placed in the dark to solidify. The gel was run at a constant voltage of

100 V in 1×TAE or TBE as a running buffer. Densitometric quantification of DNA bands was performed with ImageJ software.

DNA Strand Sequence

Associated DNA sequence (5'-3')	
CDR1as-201	(From 1 to 201 bp) GGTTTCCGATGGCACCTGTGTCAAGGTCTTCCAACAACTCCGGGTCTTCCAGCGACTTCAAGTCTTC CAATAATCTCAAGGTCTTCCAGATAATCCTGAGCTTCCAGAAAATCCACATCTTCCAGACAATCCA TGTCTTCCGGACAATCCATGTCTTCCAAGAAGCTCCAAGTCTTCCAGTAAATCAAGTCTTCCAGCAA A
CDR1as-486	(From 1 to 486 bp) GGTTTCCGATGGCACCTGTGTCAAGGTCTTCCAACAACTCCGGGTCTTCCAGCGACTTCAAGTCTTC CAATAATCTCAAGGTCTTCCAGATAATCCTGAGCTTCCAGAAAATCCACATCTTCCAGACAATCCA TGTCTTCCGGACAATCCATGTCTTCCAAGAAGCTCCAAGTCTTCCAGTAAATCAAGTCTTCCAGCAA ATCCAGTCTTCCAGCAATTACTGGTCTTCCACCAAATCCAGATCTTCCAGGAAAATCCACGTCTTCC AGGAAATCCATGTCTTCCAATAATTTCAAGGTCTTCCATCAAATACAGATCTTCCAGCTAATCCATG TCTTCCAGAAAAATCTGTGTCTTCCACCAAATCCAAGTCTTCCAGTAAATCTAGTTCTTCCAGAAAA ATCTAGATCTTCCAGTCAATCAGTGTCTTCCAGAAAGAAATCCAGGTCTTCCAGTCAATCAGTGTCT TCCAGAAAGAAATCCAGG
CDR1as	(From 1 to 1485 bp) GGTTTCCGATGGCACCTGTGTCAAGGTCTTCCAACAACTCCGGGTCTTCCAGCGACTTCAAGTCTTC CAATAATCTCAAGGTCTTCCAGATAATCCTGAGCTTCCAGAAAATCCACATCTTCCAGACAATCCA TGTCTTCCGGACAATCCATGTCTTCCAAGAAGCTCCAAGTCTTCCAGTAAATCAAGTCTTCCAGCAA ATCCAGTCTTCCAGCAATTACTGGTCTTCCACCAAATCCAGATCTTCCAGGAAAATCCACGTCTTCC AGGAAATCCATGTCTTCCAATAATTTCAAGGTCTTCCATCAAATACAGATCTTCCAGCTAATCCATG TCTTCCAGAAAAATCTGTGTCTTCCACCAAATCCAAGTCTTCCAGTAAATCTAGTTCTTCCAGAAAA ATCTAGATCTTCCAGTCAATCAGTGTCTTCCAGAAAGAAATCCAGGTCTTCCAGTCAATCAGTGTCT TCCAGAAAGAAATCCAGGTCTTCCAGTCAGTCAGTGTCTTCCAGAAAAATCTACGTCTTCCACCAA ATCCAGGTCTTCCAGTCAATCCACATCTTCCGGAAAAAATCCAGGTCTTCCAGCCAATATATGTCTT CCTGAAGATCCACGTCTTCCAGAAAATCCATGTCTTCCAGAAAATCCATGTCTTCCAGTAACCTCCC AGTCTTCCAGAAAATCCACGTCTTCCCAACAATCCAAGTCTTCCGGATAATTTGGGTCTTCCTGAAA ATCTACGTCTTCCAAAAAAGCCATGTCTTCCAGAAAATCCACATCTTCCAATGGCCTCCAGGTCTTC CAGACTATCCATGTCTTCCAGAAAATCCTTGTCTTCCCTTAAATCTATAGCTTCCAAAAAATCCGGG TCTTCCAGGAAATCCGTGTCTTCCAGCAAGTCCACGTCTTCCAACAAAGCCATGTCTTCCAGACTAT CCATGTCTTCCAGAAAATCCTTGTCTTCCCTCAAATCCATAGCTTCCGAAAAATCCAGGTCTTCCAG GAAATCCGTGTCTTCCAGCAAATCCACGTCTTCCAACAAAGCCATGTCTTCCATCAAATTAATGTCT TCCAGCCTACTTGTGTCTTCCAACAAAGGTACGTCTTCCAACAAAGGTACGTCTTCCAACAAAGGT ATGTCTTCCAACAAAGGTACGTCTTCCAGAAAATCCACGTCTTCCAACCAAGCCATGTCTTCCAGA AAATCCACGTCTTCCAGAAAATATATGTCTTCCAACTAAGCTACGTCTTCCAACAAATCCATGTCTT CCTATATCTCCAGGTCTTCCAGCATCTCCAGGGCTTCCAGCATCTGCTCGTCTTCCAACATCTCCAC GTCTTCCAGCATCTCTGTGTCTTCCAGCATCTTCATGTCTTCCAACAACTACCCAGTCTTCCATCAAC TGGCTCAATATCCATGTCTTCCAACGTCTCCAGTGTGCTGATCTTCTGACATTCAGGTCTTCCAGTG TCTGCAATATCCAG
Primer-Frw	GGTTTC CGATG GCACC TGTGT CAAG
201 bp Primer-Rew	TTTGC TGGAA GACTT GATTT ACTGG
486 bp Primer-Rew	CCTGG ATTTC TTTCT GGAAG ACAC
1485 bp Primer-Rew	CTGGA TATTG CAGAC ACTGG AAGAC
Associated assitant sequence for ssDNA separation from dsDNA (5'-3', sequence number indicates where it is located in CDR1as targeting the complementary sequence)	
S1 (15-64)	CCTGT GTCAA GGTCT TCCAA CAACT CCGGG TCTTC CAGCG ACTTC AAGTC
S2 (83-132)	CTTCC AGATA ATCCT GAGCT TCCAG AAAAT CCACA TCTTC CAGAC AATCC
S3 (149-198)	CCATG TCTTC CAAGA AGCTC CAAGT CTTCC AGTAA ATCAA GTCTT CCAGC
S4 (230-279)	CACCA AATCC AGATC TTCCA GGAAA ATCCA CGTCT TCCAG GAAAT CCATG
S5 (280-339)	ATTTC AAGGT CTTCC ATCAA ATACA GATCT TCCAG CTAAT CCATG TCTTC
S6 (408-457)	ATCTT CCAGT CAATC AGTGT CTTCC AGAAA GAAAT CCAGG TCTTC CAGTC

Methods

General Recipe for (Asymmetric) Polymerase Chain Reaction (PCR)

General Recipe for PCR			
Component	Amount		
Taq mix (2×)	3−12.5 μL		
Primer-Frw	0.2 μM (5 μL 1 μM stock solution)		
Primer-Rew	0.2 μM (5 μL 1 μM stock solution)		
Template	0.5 ng 1486 bp dsDNA template (or 125 ng DNA extracted from cells for 1486 bp dsDNA preparation)		
dd H_2O	To final 25 μL		
General Thermal Cycle Procedure for PCR Amplification			
	Temperature	Time	Cycle
Denaturation	95°C	5 min	
Denaturation	94°C	30 s	35 cycles
Annealing	64°C	30 s	
Extension	72°C	2 min	
Final Extension	72°C	5 min	
Ending	4°C	∞	

Heat Denature of DNA

1. The aqueous solution of DNA is heated to beyond 95°C for 5–10 min in 500 μL polystyrene tube.
2. Immediately immerse into an ice bath for about 5 min to cool down.

Electroelution Procedure

1. Desired DNA can be purified through electrophoretic separation, and the target DNA band is sliced off with a blade razor and collected in a tube (Note: Sliced gel band should be subjected to electroelution as soon as possible. Or it could be kept in the fridge at 4°C for a couple of days before electroelution. It's not suggested to freeze the gel, otherwise the gel will become too soft to handle when defrosting it and there will be some solution leaking out from the gel, which also contains some target molecules);
2. The sliced gel is placed in the indicated room of homemade (Figure 1) or module-assembled electroeluter (Figure 4 and Supplementary Section 6) ;
3. Place the electroelution device in a horizontal electrophoresis cell and add elution buffer required (Supplementary Section 6, and for homemade electroeluter, elution buffer should be enough to immerse the gel holding tube.)
4. For module-assembled electroeluter, electroelution time can be estimated according to molecular weight and electrophoresis voltage used based on Figure 6. An extra electroelution of about 25 minutes could guarantee the completion of electroelution without affecting the recovery yield.
   With module-assembled electroeluter, the electroelution time and voltage used for CDR1as-201, CDR1as-486, and CDR1as are about 20 minutes (6 V/cm), 30 minutes (6.5 V/cm), and 40 minutes (7 V/cm), which are all checked for completion under fluorescence image system and subjected to increased electroelution time to guarantee the recovery yield because the 3D model product isn't crystal enough for clear monitoring. For BSA protein, electroelution was run at 20 V/cm for about 25 minutes, which is also over electroelution because it's invisible and otherwise can't be eluted out if stained with Common Stain Buffer. The band position of BSA protein in gel is

predicted with control wells, which are stained. And the other wells are for the recovery experiments. We are lucky here to have BSA position the same as the Bromophenol blue, which is one component of the loading buffer. For protein electroelution of interest, it is suggested to balance the charge before electroelution if it has to be stained, or compared to control as indicated in BSA electroelution.
For homemade electroeluter, it is difficult to predict the precise electrlelution time due to the resistance inherent in the trimmed tube. Yet, the material is crystal enough to check the completion of the electroelution process under the fluorescence image system. Here, the electroelution time and voltage used for CDR1as-201, CDR1as-486, and CDR1as are about 30 minutes (8 V/cm), 40 minutes (10 V/cm), and 40 minutes (12 V/cm), respectively.

5. For other associated information in detail, please see Supplementary Section 6.

Protocol for DNA Precipitation

1. Add 1/9 volume of 3 M NaOAc (pH 5.5) to ssDNA solution, and then add LPA (4 µL, 5 mg/mL) or Glycogen (25 µL, 2 mg/mL).
2. Add 1 volume of isopropanol, and shake vigorously for 5−10 seconds.
3. Place the tube in −20°C refrigerate for about 3 hours or overnight.
4. Spin at 10,000−13,000 rpm for 15 minutes at 4 °C, and then remove the supernanant by pipetting (remove as much supernatant as possible without disturbing the pellet).
5. Wash the pellet with ice-cold 70% ethanol (500 µL). Mix by gently inverting the tube. Spin at 10,000 rpm for 5 min and then remove the supernatant.
6. (Optional) Wash the pellet again.
7. Dry the pellet by placing the tube open in the air and on its side for about 10 minutes.
8. Resuspend the pellet in the desired buffer.

Supplementary Section 1

Complexity in CDR1as ssDNA Sequence

The structure of CDR1as ssDNA sequence is highly complicated. There are eight segments of "AGGTCTTCCAG" and "CCAGAAAAATC", respectively (Figure S1-1).

When it is narrowed to 20 nt length, there are three patterns of repeat with >90% similarity in sequence (Figure S1-2). There are more than 45 repeats with repeat over repeat somewhere, which makes it more complicated and may account for the great difficulty in all three asymmetric PCR systems. Actually, in CDR1as dsDNA case alone, it is difficult to prepare in commercial order due to repeats in sequence. Although we made great efforts to screen PCR conditions for CDR1as dsDNA, there is always more or less smear due to the highly complicated structure (Figure S1-3).

Sequencing Verification of CDR1as dsDNA Prepared by PCR

PCR reactions were performed as described in Methods, and PCR products were purified and sequenced by Sangon Biotech Co., Ltd. (Shanghai, China) with the exact same set of primers. Because the sequencing is performed with forward and reverse primers in the respective directions, resulting in the sequencing at both termini and their consensus sequence overlapping in the middle part (Fig. S1-4, without primer sequences aligned). However, care has to be taken to correct trace sequences where peaks are misdefined caused by sequencing errors (insertions, deletions, or mismatches), and to delete meaningless peaks in chaos especially when sequencing length is beyond 1000 bp.

Prepare CDR1as-201, CDR1as-486, and CDR1as by PCR

PCR reactions were performed to accumulate dsDNA at three different lengths: 201 bp, 486 bp and 1485 bp. All three dsDNA were characterized with agarose gel electrophoresis first (Figure S1-5), and then were scaled up in batches. The desired dsDNA was separated by agarose gel electrophoresis and the responding band of gel was collected. SanPrep kit was used to extract molecules from sliced gel for purified dsDNA substance and purity was checked with A260/A280 optical adsorption ratio at about 1.8. The prepared dsDNA substance was used to obtain the recovery yield of homemade as well as module-assembled prototype electroeluter.

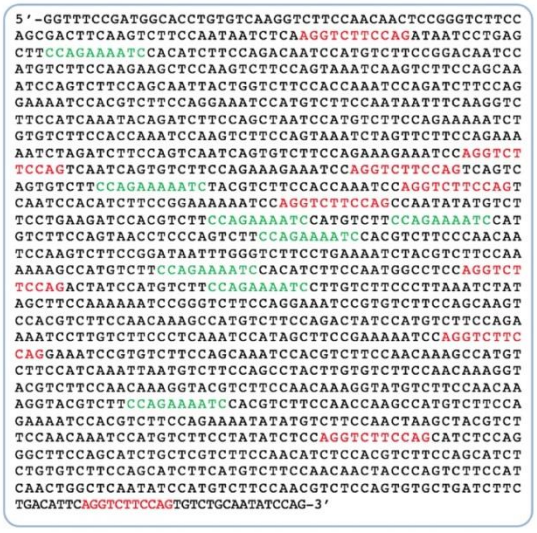

Figure S1-1 Two kinds of repeat sequences in CDR1as sequence are brightened in red and green color, respectively. "Red" sequence is AGGTCTTCCAG, and "Green" sequence is CCAGAAAATC.

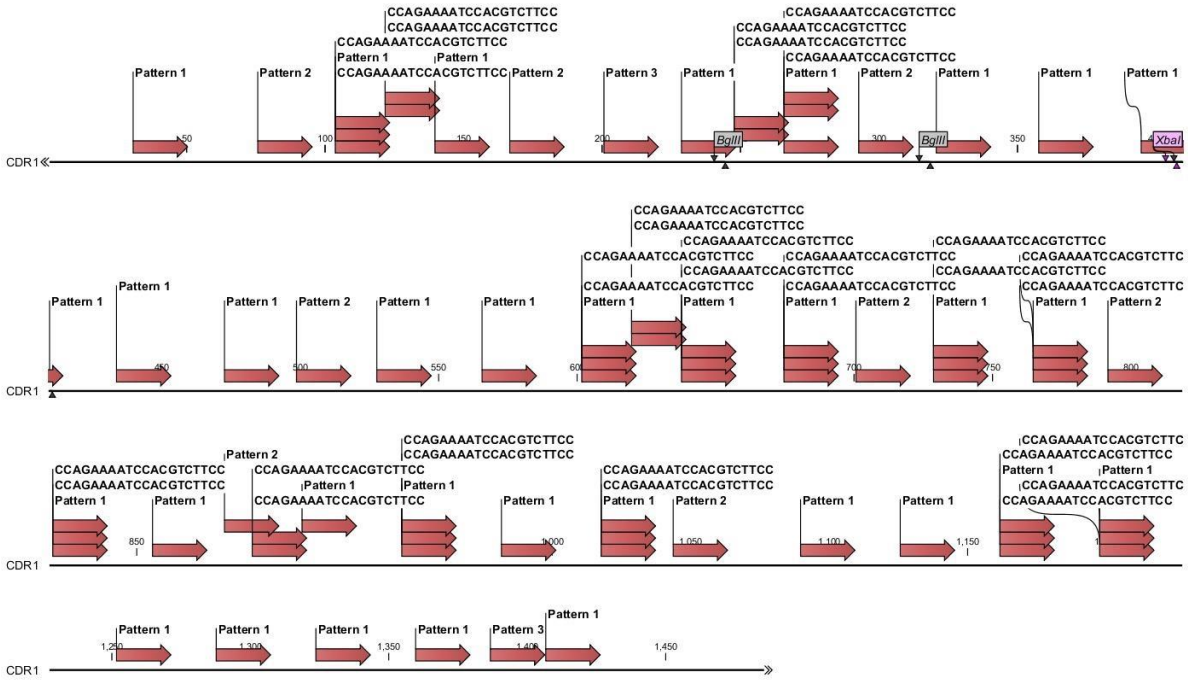

Figure S1-2 Three patterns of 20 nt repeats in CDR1as sequence, and the illustrated repeat sequences have more than 90% similarity.

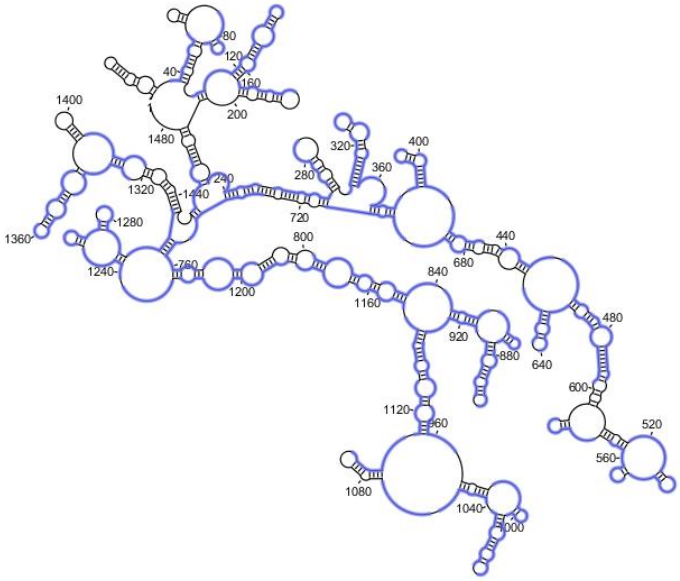

Figure S1-3 The highly complicated secondary structure of CDR1as ssDNA sequence predicted by RNAstructure software. Pattern 1 repeats are highlighted in blue.

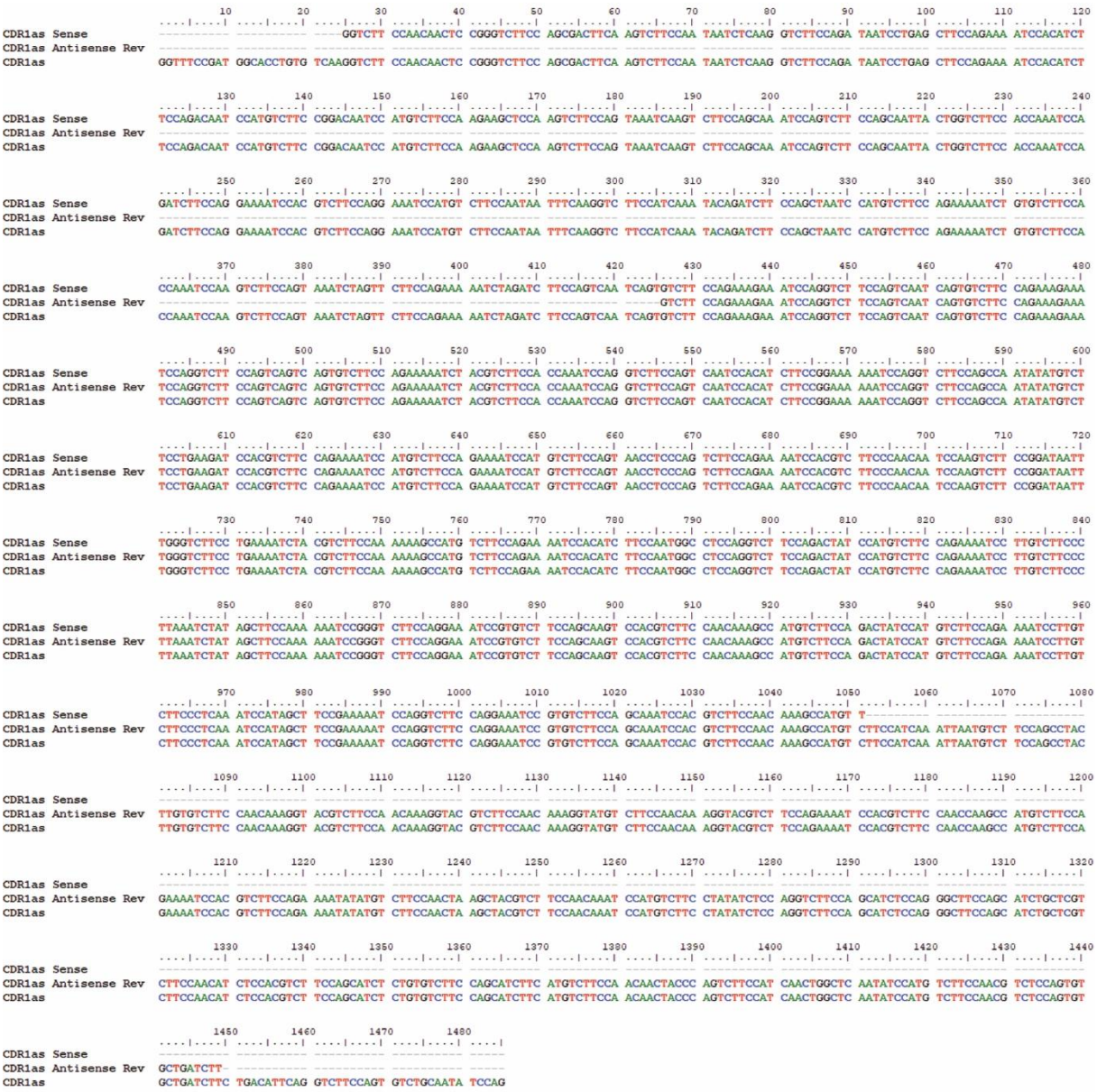

Figure S1-4 Sequence alignment to determine the correct CDR1as sequence obtained by PCR reactions from HEK293 genomic DNA. CDR1as is the reference sequence, CDR1as sense is the correct sequence from raw sequencing data with forward primer, and CDR1as antisense rev is the complementary reverse sequence of that gathered from raw sequencing data with the reverse primer. Neither of the primer sequences is included here.

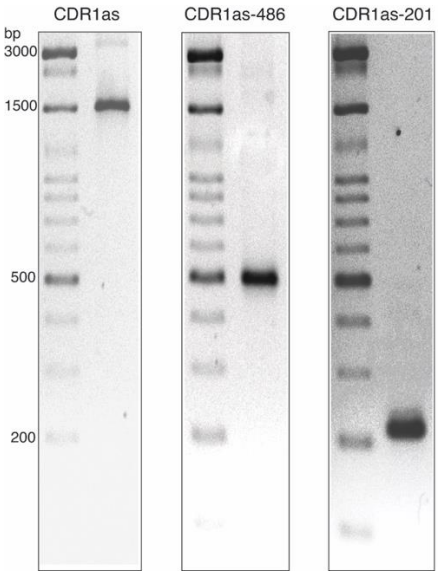

Figure S1-5 Characterization of CDR1as, CDR1as-486, and CDR1as-201 dsDNA with agarose gel electrophoresis.

Supplementary Section 2

Prepare 486 nt long ssDNA from CDR1as-486 dsDNA

We have designed one set of six assistant short strands to kinetically separate 486 nt long ssDNA from CDR1as-486 dsDNA. The location of six helper strands in 486 nt long ssDNA is shown in Figure S2-1. Their binding sites on the antisense sequence as well as its secondary structure are shown in Figure S2-2. CDR1as-486 dsDNA is mixed with six short strands (S1−S6) at high equivalents and then subjected to heat denature at 95 °C for about 10 minutes, and then immediately cooled on ice. This process allows the long dsDNA to denature to both long ssDNA strands and also allows the short assistant strands to hybridize with the complementary long ssDNA strands. This facilitates the following agarose gel electrophoresis to separate the desired long ssDNA, which could be sliced off and recovered with the developed homemade electroeluter or module-assembled model product.

The result is characterized with agarose gel as shown in Figure S2-3. Long ssDNA could be precipitated for precise quantification and further calculating efficiency.

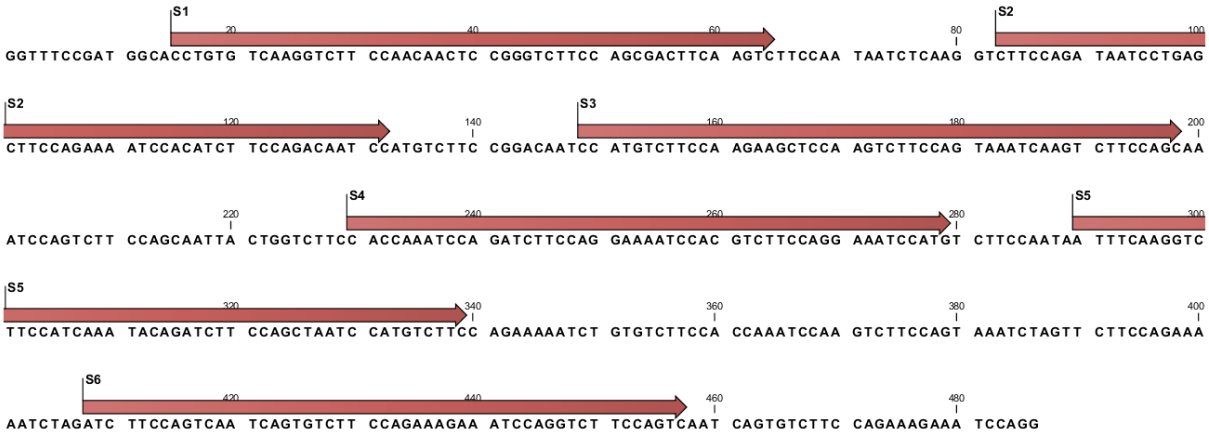

Figure S2-1 CDR1as-486 ssDNA sequence to prepare and six short helper strands for kinetic separation from CDR1as-486 dsDNA. Red arrows demonstrate where the helper strands S1, S2, S3, S4, S5, and S6 hybridize to the complementary antisense ssDNA sequence.

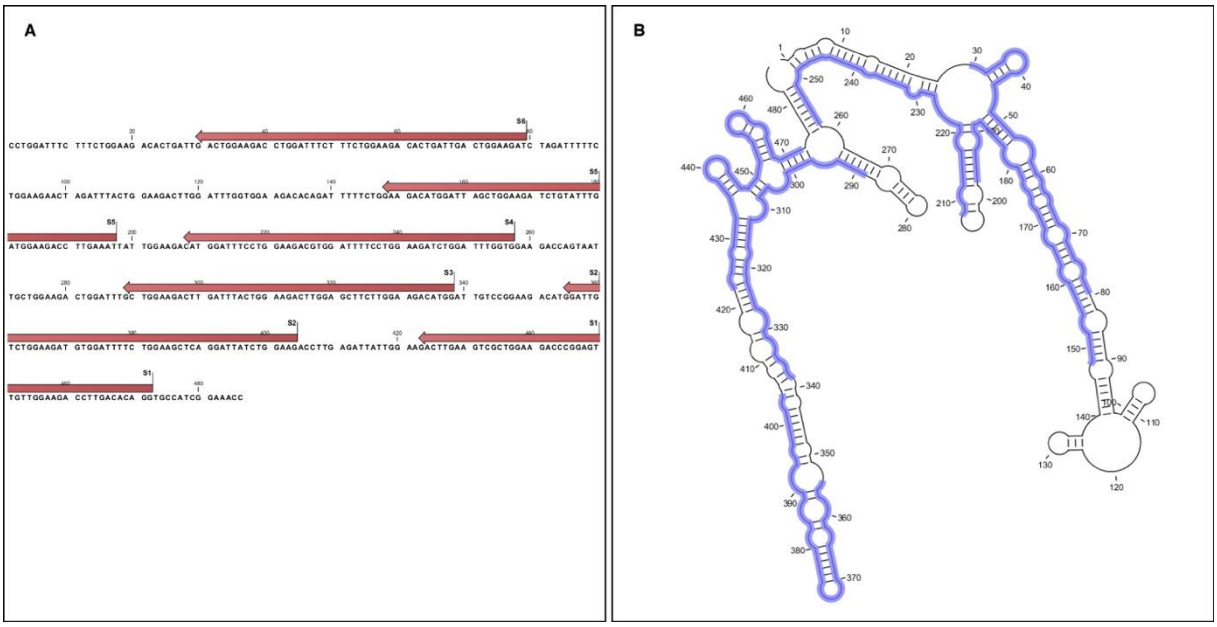

Figure S2-2 (A) CDR1as-486 antisense ssDNA sequence with hybridization sites for helper strand S1, S2, S3, S4, S5, and S6, indicated in red arrows. (B) The secondary structure of CDR1as-486 antisense sequence predicted with RNAstructure and highlighted are targets for S1, S2, S4, S5, and S6.

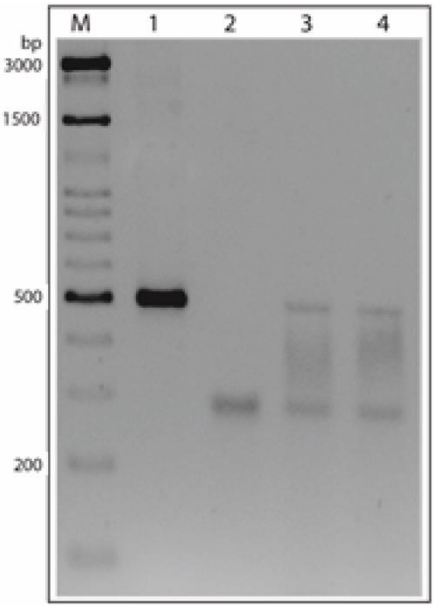

Figure S2-3 Electrophoresis characterization of kinetically separating 486 nt long ssDNA from its precursor CDR1as-486 dsDNA. Lane 1: CDR1as-486; Lane 2: denatured CDR1as-486 dsDNA precursor, including both complementary strands; Lane 3 and 4: denature of CDR1as-486 dsDNA in the presence of S1−S6 short helper strands.

Supplementary Section 3

DNA concentration determination with the fluorescence method

Kits of ssDNA high-sensitivity quantification and dsDNA broad range quantification are used on a Nano-500 microspectrophotometer to quantify respective ssDNA and dsDNA with fluorescence. All measurements were performed according to the manuals with the following constructed concentration regression curve (Figure S3-1).

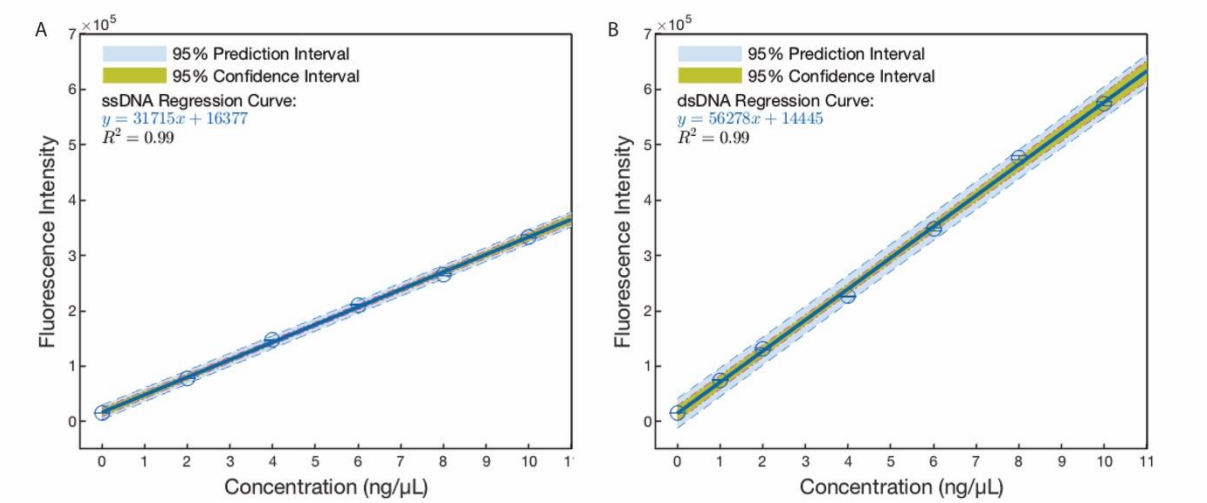

Figure S3-1 Concentration regression curve for (A) dsDNA and (B) ssDNA concentration determination with fluorescence.

Supplementary Section 4

DNA Recovery Determination

The amount of DNA used to determine recovery yield is shown below. To calculate the recovery yield in every experiment, the concentrations of all single or double-stranded DNA stock solutions were measured in the beginning.

To demonstrate recyclability, we have also recycled the random 50 bp ssDNA since a large amount of ssDNA is needed in recycling the homemade electroeluter as many as 30 times continually. For 50 bp ssDNA substance, a certain amount of the starting substance was mixed with a trace amount of agarose gel solution in a tube when preparing gel to simulate the sliced gel. After electroelution with the use of homemade device, the volume of the final elute was recorded, and only the optical absorption method was used to determine its concentration. Accordingly, the recovery yield for 50 bp ssDNA was calculated by further taking the starting amount into account.

To determine dsDNA recovery yields precisely, agarose gel electrophoresis was performed for each dsDNA segment by loading a fixed amount of dsDNA, then the desired bands were sliced off and further proceeded with SanPrep kit, G-Capsule, and the constructed electroeluter, respectively, to draw target dsDNA out. Precipitation of dsDNA was further performed and the final precipitate was dissolved in a buffer at a certain volume before concentration determination. In determining dsDNA concentration, precipitation was also performed with the presence of only the same amount of precipitant for background deduction.

Because nucleic acid concentration determination belongs to trace analysis, in order to remove influence from buffer solution and precipitant as well as the instrumental stability, background deduction and control experiments were taken into account in calculating dsDNA recovery yields.

Amount of ssDNA or dsDNA in recovery determination

Amount of 50 bp ssDNA used to demonstrate the recyclability
(each batch of 50 bp ssDNA combination is indicated in rows)

50 bp ssDNA	Concentration (ng/μL)	Volume (μL)
1^{st} – 5^{th}	22.734/23.759/23.323/23.856/24.608	200
6^{th} – 10^{th}	17.990/17.984/17.76/17.513/23.148	200
11^{th} – 17^{th}	19.729/20.617/25.584/19.556/21.267/25.058/22.690	200
18^{th} – 23^{th}	31.755/34.444/36.308/33.523/34.686/36.236	200
24^{th} – 30^{th}	41.821/41.708/41.403/42.466/42.184/42.17/42.506	200

Amount of dsDNA used to determine recovery yield

	CDR1as-201	CDR1as-486	CDR1as
SanPrep Kit	50 μL×13.840 ng/ μL or 25 μL×35.688 ng/ μL	15 μL×59.668 ng/ μL	40 μL×8.581 ng/ μL
G-Capsule			
Homemade			
Module-assembled Prototype Model	100 μL×8.713 ng/ μL	100 μL×11.327 ng/ μL	100 μL×3.340 ng/ μL

Disscusion for dsDNA lost in recovery

The loss of dsDNA in recovery is probably caused by three reasons: i) maybe there is still a trace amount of substance left on agarose gel, which is invisible; ii) the adsorption on the surfaces of the device, membrane, and pipette tips may also compromise the recovery yields, and the monitoring of electroelution process under gel imaging system only could be possible for main product (Figure S3-1)), which shows that the adsorption on the membrane-holding part is trace and invisible after removal of the elute; iii) Although we performed control experiments of direct precipitation of starting substance for precise recovery calculation, there is still a possibility that uneven precipitation results could happen due to multi-step operation, including centrifugation, washing, and resuspension.

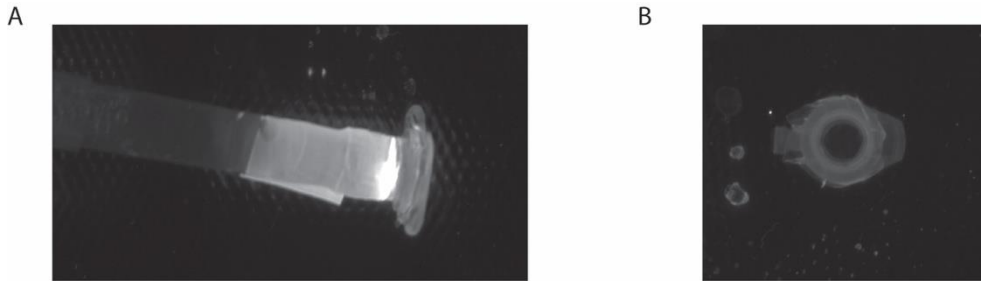

Figure S4-1 (A) Fluorescent image of homemade electroelution device after dsDNA elution. (B) Fluorescent image of the membrane-holding part after removal of dsDNA elute.

Supplementary Section 5

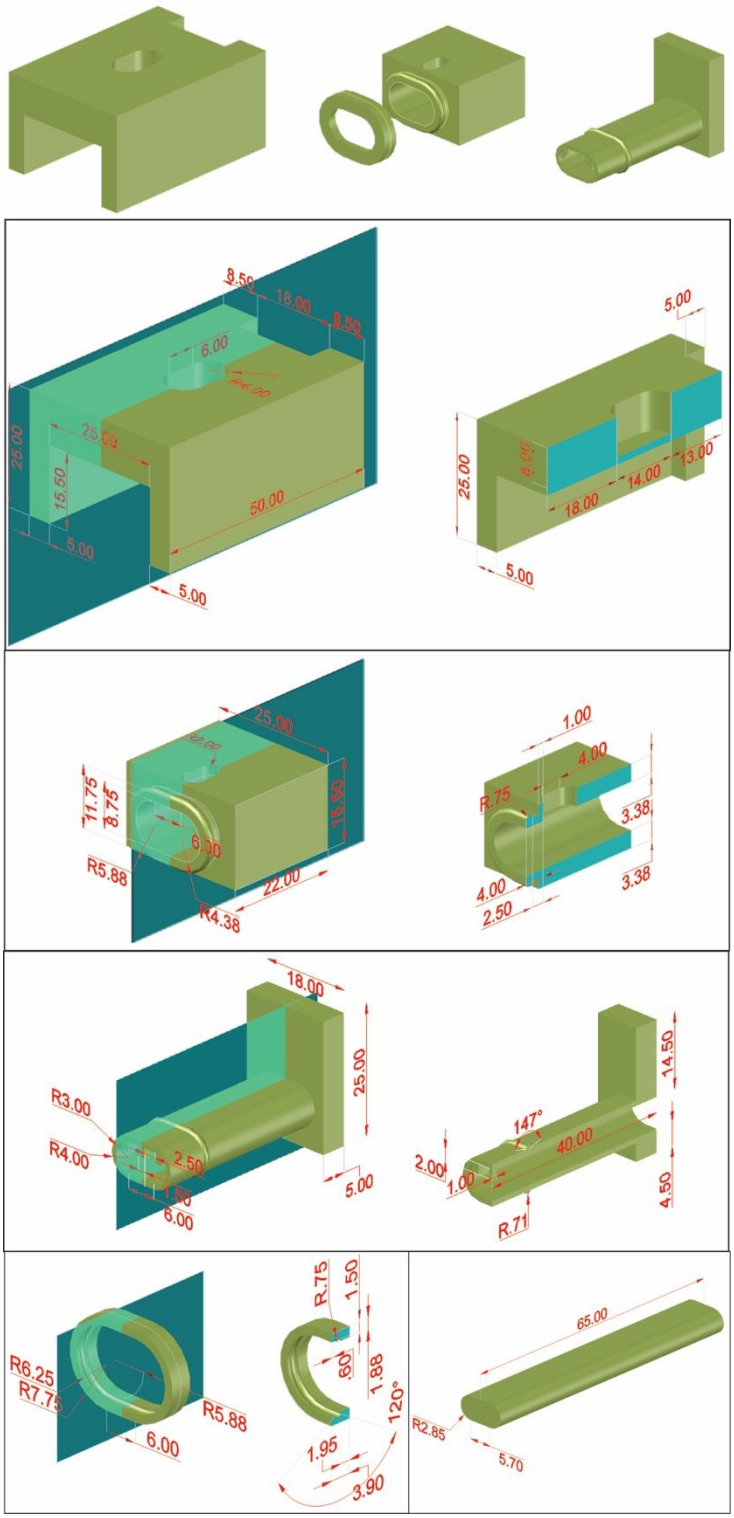

Figure S5-1 Detailed parameters based on sectional view for all parts associated with the module-assembled electroeluter. Unit: mm.

Supplementary Section 6

There are five basic steps with the use of a module-assembled prototype electroeluter:

Step 1: Fix the dialysis membrane;
Step 2: Fabricate block gel;
Step 3: Assemble the gel loading module and Dialysis membrane module;
Step 4: Run electroelution;
Step 5: Collect elution.

Notes for operating module-assembled prototype electroeluter shown in the video

1. To fabricate the block gel, it is recommended to use about 300 μL 2% agarose gel or 12% polyacrylamide gel accordingly (see step 2 of the video in the supporting information). In this step, the gel loading module is inserted into the top window of the anchor module. When put together, a piece of polyethylene film from any plastic glove is recommended to stick between the two modules (Figure S6-1), which facilitates the following separation or otherwise the vacuum tends to draw the block gel out when separated.

Figure S6-1 A little piece of polyethylene film is sticked between the gel loading module and the anchor module when put them together to make block gel.

Figure S6-2 A little piece of parafin film is applied to the gel loading module as demonstrated.

2. In the prototype model, parafim film should be used on the ring of gel loading module (see step 2 of the video in the supporting information and Figure S6-2), which makes it seamless when assembling it to the dialysis membrane module (see step 3 of the video). This is necessary for the prototype model, which could be eliminated when manafactued with injection modeling, which offers better precision than 3D printing.

3. It is recommended to use about 300 μL elution buffer for electroelution, which is injected with a pipette through the top window of dialysis membrane module (see step 3 of the video and Figure S6-3), before putting them further close.

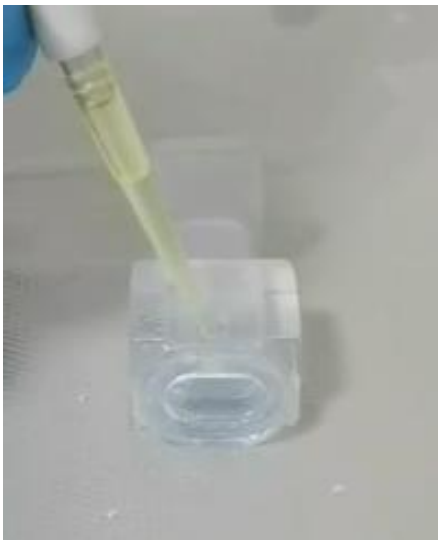

Figure S6-3 Elution buffer is added through the top window of the dialysis membrane module.

4. When adding the running buffer, the surface level is recommended to reach the top edge of the gel loading module when placing it in the horizontal electrophoresis system (see step 4 of the video in the supporting information).
5. When electroelution is completed, the extra running buffer in the gel loading module should be removed first with a pipette. Then hold them at a slight tilt angle and gently separate the gel loading module from the dialysis membrane module (see step 5 of the video in the supporting information and Figure S6-4).

Figure S6-4 Hold them at a slight tilt angle when gently separating them after electroelution is completed.

6. Pipette the elution on the dialysis membrane several times before collecting elution (see step 5 of the video in the supporting information).