

Affinity purification of plasmid DNA by temperature-triggered precipitation

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Published online 17 May 2007; doi:10.1038/nprot.2007.171

This protocol presents a new method to purify plasmid DNA using temperature-triggered precipitation. The principle is based on the specific DNA-binding affinity of a bacterial metalloregulatory (MerR) protein to its cognate DNA sequence and the temperature responsiveness of elastin-like protein (ELP). A bifunctional ELP-MerR fusion protein is created to enable the precipitation of plasmid DNA, designed to contain the MerR recognition sequence, by a simple temperature trigger. The protocol covers all stages of the process from the design of ELP-MerR fusion proteins and MerR-binding plasmids, to the isolation of plasmid DNA from *Escherichia coli* cultures after boiling lysis, the subsequent temperature-triggered precipitation of plasmid DNA-fusion protein complexes and final elution of plasmid DNA by mild heating. This protocol is well suited to laboratory research-scale applications, producing plasmid DNA of better purity and similar yield as one of the most commonly used laboratory methods, standard alkaline lysis (known as the midprep procedure). The protocol takes approximately 30 min to obtain pure plasmid DNA from cell cultures using the temperature-triggered precipitation method.

INTRODUCTION

The completion of Human Genome Project has accelerated the development of nucleic acid-based therapy as an effective genetic disease-related treatment¹. To date, more than 1,000 different gene therapy clinical trials for the treatment of different diseases are in progress worldwide (<http://www.wiley.co.uk/genetherapy/clinical/>). The success of gene therapy is largely dependent on development of the gene delivery vector, which is generally divided into two categories: viral and nonviral². Although high efficiency of delivery is easily achieved by the use of viral vectors, safety concerns are inevitable owing to their toxicity and immunogenicity. Recently, nonviral vectors based on plasmid delivery have emerged as a safer alternative. Even though the gene transfection efficiency has been improved by different physical and chemical methods³, high doses of therapeutic plasmid DNA are still required in clinical trials. This need eventually will mandate the manufacturing of large amounts of plasmid DNA at the industrial scale if gene therapy prevails.

By exploiting the physiochemical properties of nucleic acids, nonspecific capture chromatography systems have been suggested for large-scale purification of plasmid DNA, including anion-exchange⁴, size-exclusion⁵, hydrophobic⁶ and reversed-phase chromatography⁷. However, the lack of specificity results in contamination with structurally related molecules like RNA or fragmented genomic DNA. These impurities not only decrease the capacity of the chromatography process, but also require further purification steps—usually additional chromatographic steps—that are expensive and difficult to scale up⁸.

Affinity-based plasmid DNA purification methods are particularly attractive in minimizing undesirable impurities because of the specificity between the ligand and the plasmid DNA. One representative technique is based on the formation of a triple helix between an oligonucleotide covalently linked to a chromatographic matrix and the complementary sequence present on the plasmid DNA to be purified⁹. The limitations of this method include the relatively slow helix formation and the need for high ionic strength and low pH². These limitations can be resolved by using protein-

mediated isolation of plasmid DNA that relies on the specific interaction between a DNA sequence and the corresponding binding protein. Target plasmids containing a specific recognition sequence have been captured by the use of zinc-finger-glutathione *S*-transferase fusion protein¹⁰, LacI repressor¹¹ and a 64-mer synthetic peptide representing the DNA-binding domain of LacI repressor¹². In all cases, purified proteins are immobilized onto a solid support and the binding efficiency can be greatly affected due to steric hindrance by random orientations and environment-induced denaturation.

Affinity precipitation is a new purification method developed in recent years that allows receptors and ligands to interact under homogeneous conditions, as opposed to heterogeneous conditions in chromatography. The receptor–ligand complex formed is recovered by a simple thermal precipitation. ELPs are artificial peptides consisting of repeating VPGVG that have been shown to undergo a reversible phase transition upon environmental stimuli from water-soluble forms into hydrophobic aggregates, facilitating recovery by precipitation¹³. Advances in genetic engineering have made it possible to fuse different protein partners to ELP while preserving the phase transition and the partners' functionalities^{14–16}. The use of ELP fusion proteins for affinity precipitation has been demonstrated during the purification of a wide range of ELP-fusion proteins, His-tagged proteins and antibodies^{14–17}.

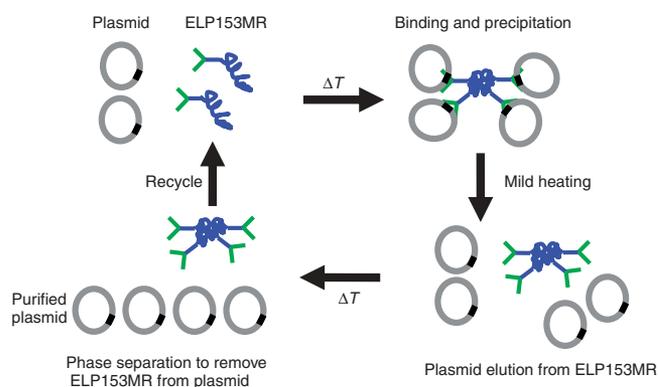
Our laboratory has recently developed a new concept of affinity purification of plasmid DNA based on temperature-triggered precipitation¹⁸ (see **Fig. 1**). The method is based on the interaction between a DNA-binding protein and its cognate DNA sequence presented on the target plasmid. Purified plasmids are recovered by co-precipitation with ELP-DNA-binding protein fusions, which undergo a reversible phase transition as the temperature increases. The T7 promoter-driven pET system is used to construct expression vectors to produce these fusion proteins. Optimum precipitation efficiency of the protein–DNA complexes is achieved by the addition of an inert (helper) ELP (see **Table 1**) as a co-aggregant, which facilitates the co-precipitation of the ELP–DNA complexes.

PROTOCOL

We have successfully demonstrated this principle for plasmid purification by exploiting the affinity of a bacterial metalloregulatory protein MerR (mercury-responsive transcriptional activator) towards its corresponding promoter sequence¹⁸, although other DNA-binding protein/promoter sequence pairs can be similarly employed¹⁹. The pBLUESCRIPT SK+ plasmid was used to initially demonstrate this method, and was modified to accommodate the MerR-binding region, comprising two 70-bp oligonucleotides, Pt-o1 and Pt-o2, that encode the Pt promoter region (−40 to +4) of the mer operon. Although the effect of plasmid size and sequence on the precipitation efficiency is still under investigation, we feel optimistic about the applicability of this system, thanks to the specificity of the MerR protein toward its cognate DNA sequence. Plasmid constructs containing the MerR-binding DNA sequence are separated from the cell lysate by binding to ELP-MerR fusion proteins (see **Table 1**) and undergoing temperature-triggered precipitation. Plasmids are released from the ELP-MerR fusion protein by mild heating, and are separated from the proteins by a further thermal precipitation. Using this method, more than 70% of plasmid DNA in cell lysate can be obtained with outstanding purity within half an hour, as no chromosomal DNA and protein contaminants are detectable by PCR or SDS-PAGE, respectively.

The protocol described here may potentially be optimized toward large-scale plasmid DNA purification, although technical limitations may be encountered during scale-up, including the usage of boiling lysis and centrifugation, which would require further optimization. This protocol is particularly suitable for

Figure 1 | Principle of the affinity purification of plasmid DNA based on temperature-triggered precipitation of ELP. An ELP153MR fusion protein, consisting of an ELP (represented in blue) and a bacterial metalloregulatory protein (MerR; represented in green), binds the plasmid of interest (represented by a gray circle) as the MerR protein binds to the MerR recognition sequence contained within the plasmid sequence (highlighted in black). Precipitation of the DNA-ELP-MerR aggregates is achieved by increasing the temperature to 37 °C. After centrifugation, the pellets are resuspended in a cold buffer and elution of plasmid DNA is achieved by mild heating of the solution. A second temperature-triggered phase separation is undertaken to remove the ELP from the eluted plasmid DNA. The ELP can be reused for next cycle. The figure was first published in ref. 13.



laboratory research-scale applications with comparable recovery and purity to other plasmid purification methods developed recently^{10–12}. For example, it can be used as a chromatography-free step to purify small amounts of plasmid DNA (10–300 µg) for phase-I and -II clinical trials². The cost associated with developing this protein system can be offset by the facts that (i) no chromatography is required in the protein purification process and (ii) fewer harsh chemicals are used in the protein production compared to other precipitation methods, such as polycation²⁰ and the use of polymer conjugates²¹. The use of DNA-binding proteins in plasmid DNA purification provides great flexibility for the future application of the system described here, as these proteins can potentially be engineered to bind to any DNA sequence of choice, by modifying any plasmid of interest to incorporate the target promoter region in a similar fashion.

MATERIALS

REAGENTS

- Luria Broth (BD, cat. no. 244610)
- Terrific Broth (BD, cat. no. 243820)
- *Escherichia coli* strain BLR-DE3 (Novagen, cat. no. 69053)
- Expression vector pET-38b(+) (Novagen)
- PBLUESCRIPT SK+ (Stratagene, cat. no. 212207)
- Kanamycin sulfate (Fisher, cat. no. BP906-5)
- Ampicillin sodium salt (FisherBiotech, cat. no. BP1760-5)
- 2-Mercaptoethanol (Bio-Rad, cat. no. 161-0710) **! CAUTION** Harmful if swallowed. Avoid direct contact with skin.
- Lysozyme (EMD, cat. no. 4403)
- Tris (Bio-Rad, cat. no. 161-0715)
- NaCl (Fisher, cat. no. S671-500)
- HCl (Fisher, cat. no. A142-212)
- EDTA pH 8.0 (Sigma, cat. no. 03690-100ML)
- Triton X-100 (Sigma, cat. no. T9284-1L)

- Ethidium bromide 1% solution (w/v) (FisherBiotech, cat. no. BP1302-10) **! CAUTION** Avoid direct contact with skin.
- Acetic acid (Fisher, cat. no. S70048)
- Agarose (EMD, cat. no. 121852)
- Silver staining kit (Bio-Rad, cat. no. 161-0443)
- Coomassie blue G-250 (Bio-Rad, cat. no. 20279)
- *SalI* (New England Biolabs, cat. no. R0138S)
- *NotI* (New England Biolabs, cat. no. R0189S)
- *ScaI* (New England Biolabs, cat. no. R0156S)
- T4 ligase (New England Biolabs, cat. no. M0202S)
- *Taq* DNA polymerase (New England Biolabs, cat. no. M0273S)
- Pt-o1 (5′-tcgactgcagccttggcgcttgactccgtacatgagtcaggaagtaaggtta-cgctatccttggctgcag-3′; IDT Inc.)
- Pt-o2 (5′-tcgactgcagccaaggatagcgttaaccttactccgtactcatgtacggagt-caagcgccaaggctgcag-3′; IDT Inc.)
- arsf0 (5′-gcaccgggtgcatgtcattctgttacc; IDT Inc.)

TABLE 1 | ELP proteins used for purification.

Name	Protein sequence	Amino acids	Da
ELP153MR	MGP(GVGVP) ₁₅₃ -GVGPGT-linker ^a -MerR	933	80,111
ELP153H6	MGP(GVGVP) ₁₅₃ -GVGPGT-GM-H ₆	782	64,434

^alinker = GGGSAGGAGSGGG. The amino acids of the protein sequence are represented by standard single-letter code. MerR refers to the bacterial metalloregulatory protein (mercury-responsive transcriptional activator). ELP153MR is the ELP-MerR fusion protein used to bind to the plasmid of interest, whereas ELP153H6 is the inert helper protein. The table was first published in ref. 18.

- arsb (5'-cctctgcagttaactgcaaatgtctt; IDT Inc.)
- Zymoclean Gel DNA Recovery Kit (Zymo Research, cat. no. D4001)
- JM109 Competent Cells (Stratagene, cat. no. 200235)

EQUIPMENT

- Beckman J2-HS centrifuge (Beckman Coulter Inc.)
- Beckman Coulter UV-Vis DU 640 Spectrophotometer (Beckman Coulter Inc.)
- Eppendorf benchtop centrifuge 5415 R (Eppendorf North America Inc., cat. no. 022621408)
- Horizontal electrophoresis apparatus (Bio-Rad Inc., cat. no. 165-4112)
- Vertical electrophoresis apparatus (Bio-Rad Inc., cat. no. 170-4401)
- Thermal cycler/PCR machine (MJ Research, PTC-200)

REAGENT SET UP

- Tris-HCl (1 M)** 121.1 g of Tris base in 1 liter deionized water. Adjust the pH by adding concentrated HCl.
- STET** 10 mM Tris-HCl pH 8.0, 0.1 M NaCl, 1 mM EDTA pH 8.0 and 5% (v/v) Triton X-100.
- 0.9% (w/v) NaCl** 9 g of NaCl in 1 liter deionized water.
- TB7.4M** 50 mM Tris-HCl buffer pH 7.4 with 50 mM 2-mercaptoethanol.
- TB7.4** 50 mM Tris-HCl buffer pH 7.4.
- Lysozyme solution** 10 mg ml⁻¹ of lysozyme in 10 mM Tris, pH 8 buffer.
- TB7.4S** 50 mM Tris and 150 mM NaCl, pH 7.4.
- 50× Tris-acetic acid-EDTA** 242 g Tris base, 57.1 ml acetic acid, 100 ml of 0.5 M EDTA, pH 8.5.

PROCEDURE

Production of ELP-MerR fusion protein (ELP153MR) and helper protein, ELP153H6

1| Inoculate *E. coli* BLR-DE3 cells containing the expression vector pETELP153MR (see ref. 18) into 250 ml terrific broth media supplemented with 30 μg ml⁻¹ kanamycin, and *E. coli* BLR-DE3 cells containing the expression vector pETELP153H6 (see ref. 15) into separate 250 ml terrific broth media supplemented with 30 μg ml⁻¹ kanamycin (see **Table 1**).

▲ **CRITICAL STEP** The protein ELP153MR contains the sequence coding for the bacterial metalloregulatory MerR with the cognate DNA binding capability toward its specific promoter sequence that is included in the plasmid to be purified. ELP153H6 is an inert protein that can serve as a control protein for specificity experiments.

▲ **CRITICAL STEP** The T7 promoter-driven pET system is used to construct the expression vectors, which contain ELP and MerR genes. The ArsR gene, which exists ubiquitously in the *E. coli* genome DNA, is also constructed using the pET vector to provide a positive control during the later PCR test for contaminating chromosomal DNA.

▲ **CRITICAL STEP** *E. coli* strain BLR-DE3 is used as the host cell in order to obtain a high yield of protein expression, and initial OD should be ~0.1.

▲ **CRITICAL STEP** The following protein purification procedures are identical for both the ELP-MerR fusion protein (ELP153MR) and the helper protein (ELP153H6), except for the buffers used (see below).

2| Incubate at 300 r.p.m. for 48 h at 30 °C without addition of IPTG.

3| To purify ELP153MR and ELP153H6 proteins, harvest 48-h cultures by centrifugation for 10 min at 3,440g at 4 °C and discard the supernatant.

4| Wash the recovered cell pellet with 250 ml cold 0.9% (w/v) NaCl solution and centrifuge for 10 min at 3,440g at 4 °C.

5| Discard the supernatant and resuspend the cell pellets in 40 ml cold TB7.4M buffer for ELP153MR or TB7.4 buffer for ELP153H6.

6| Sonicate the cell suspension at mid-power (5 s pulse and 5 s off for 15 min) on ice and centrifuge for 30 min at 30,000g at 4 °C to remove the cell debris.

7| Purify ELP153MR or ELP153H6 proteins from the cell extract (the supernatant from Step 6) by three cycles of reversible temperature transition, as follows: for each cycle, add NaCl to the sample to a final concentration of 1 M and heat the sample to 30 °C for 10 min to induce phase transition of the ELPs. Centrifuge the sample for 15 min at 30,000g at 30 °C, remove the supernatant and dissolve the pellet containing the precipitated ELP in ice-cold TB7.4M or TB7.4 buffer for the purification of ELP153MR or ELP153H6 proteins, respectively. Next, centrifuge the cold protein solution for 15 min at 30,000g at 4 °C and transfer the supernatant to a new tube.

8| Verify the purity of the proteins by SDS-PAGE²¹ followed by silver staining, according to the manufacturer's instructions.

▲ **CRITICAL STEP** Up to 800 mg liter⁻¹ of ELP can be produced and purified. The amount of protein loaded can be calculated depending on the final volume of protein solutions. A total of 1 μg of protein sample is sufficient for silver staining. A single band of the purified ELP should be expected at the desired size in the SDS-PAGE gel. More cycles of phase transition can be employed if impurities are observed in the final product.

9| To determine the protein concentration, dilute the protein solution 10:1 (v/v) in TB7.4M or TB7.4 buffer for ELP153MR or ELP153H6 proteins, respectively. Measure the absorbance at 215 nm using a spectrophotometer. Adjust the dilution if the reading is out of the range of 0.1–1. The final concentration of the protein is calculated using the extinction coefficient (69.87 (μg/ml)⁻¹ cm⁻¹) based on previous calibration.

■ **PAUSE POINT** Purified ELP153MR protein must be stored at 4 °C in TB7.4M buffer to prevent oxidation. Helper protein ELP153H6 can be stored at 4 °C in TB7.4 buffer. Prolonged period of storage over 6 months can be achieved by storing both ELPs at –20 °C. Frequent freezing or thawing is not recommended.

PROTOCOL

Target plasmid construct

10| To create a plasmid containing the MerR-binding region, assemble Pt-o1 and Pt-o2 oligonucleotides at a concentration of 2 μM by denaturing them for 1 min at 95 °C and annealing at RT (room temperature, 25 °C).

11| Linearize 0.5 μg of the plasmid of interest by digesting with an appropriate restriction enzyme; incubate the plasmid with 10 U of the restriction enzyme overnight in a final volume of 50 μl of corresponding buffer solution at 37 °C.

▲ CRITICAL STEP We have tested the protocol using the plasmid PBLUESCRIPT SK+ and digested with 10 U of *Sa*II in a 50 μl total volume. Any plasmid of interest can be easily modified to incorporate the Pt promoter region in a similar fashion. However, the effects of size and sequence on the purification efficiency are still under investigation.

12| Load 50 μl digestion product to 0.8% (w/v) agarose gel. Run the gel electrophoresis in 1 \times Tris-acetic acid-EDTA (TAE) buffer at 90 V for 30 min.

13| Isolate the linearized plasmid by Zymoclean Gel DNA Recovery Kit, according to the manufacturer's protocol. Obtain the final plasmid product in 16 μl deionized water.

14| Incubate 2–3 μl of the linearized plasmid with 6 μl of 2 μM assembled oligonucleotides in the presence of 1 μl T4 ligase with accompanying enzyme buffer overnight at 16 °C. The resultant plasmid is known as pBLU-Pt with the size of 3,028 bp.

15| To transform the plasmid into *E. coli* cells, incubate 5 μl of the ligation mixture or 1 μl control plasmid (pBLUESCRIPT SK+) with 100 μl *E. coli* JM109 heat-shock-competent cells on ice for 30 min. Heat the mixture for 90 s at 42 °C and incubate with 800 μl LB medium for 1 h at 37 °C. Plate 100 μl of the cell culture onto LB-agar plate supplemented with the appropriate antibiotic (ampicillin in the case of pBLU-Pt and pBLUESCRIPT SK+) and incubate at 37 °C overnight. Select colonies and inoculate into 2 ml LB media. Incubate at 37 °C overnight, and screen for colonies containing the correct sequence of pBLU-Pt by isolating the plasmid DNAs²² and checking the digestion pattern of the plasmid by running the digestion product with 0.8% agarose gel electrophoresis (90 V, 30 min, 1 \times TAE).

▲ CRITICAL STEP The enzymes used in the digestion to identify the correct colony will depend on the plasmid construct used. For pBLU-Pt, *Hinc*II enzyme can be used. Plasmid that cannot be digested by *Hinc*II has the correct insert.

Plasmid release by boiling lysis of cells

16| Inoculate cells harboring pBLU-Pt and the control vector pBLUESCRIPT into 5 ml LB broth with 100 $\mu\text{g ml}^{-1}$ ampicillin and incubate overnight at 37 °C.

17| Harvest the cells from 1 ml culture by centrifugation at 14,000g for 30 s at 4 °C.

18| Discard the supernatant and resuspend the pellet in 300 μl STET with 20 μl of lysozyme solution.

19| Boil the solution for 40 s and centrifuge at 14,000g for 10 min at RT. Transfer the supernatant to a fresh microcentrifuge tube.

Plasmid purification and precipitation by binding to ELP-MerR protein

20| Add 250 nM (20 $\mu\text{g ml}^{-1}$) of ELP153MR and 15.5 μM (1 mg ml^{-1}) of helper protein ELP153H6 to the 300 μl cell lysate (from Step 19) and make up to a total volume of 0.5 ml with TB7.4S.

21| Incubate the mixture for approximately 5 min at RT.

22| Heat the sample to 37 °C for 2 min to induce temperature-triggered precipitation of the ELP-MerR–DNA complex.

23| To recover the ELP-MerR–DNA complex, centrifuge sample at 37 °C for 5 min at 2,300g in a benchtop microcentrifuge.

24| Discard the supernatant and dissolve the pellet in 50 μl fresh TB7.4S buffer on ice for 5 min. Pipette the pellet gently to help it dissolve.

▲ CRITICAL STEP Manipulation of plasmid concentration could be achieved by changing the volume of the buffer as long as no visible aggregates of protein–DNA complex are observed. As a control, vector pBLUESCRIPT without the MerR target sequence is used as an indicator of specificity and no removal of the control plasmid should be observed (in the supernatant fraction) by gel electrophoresis under the conditions mentioned above.

Plasmid DNA elution and characterization of DNA content

25| Elute the plasmid from the ELP-MerR–DNA complex by heating the solution at 60 °C for 10 min.

26| Centrifuge the solution at 37 °C for 2 min at 2,300g to separate the ELP aggregates and collect the supernatant into a fresh tube. To recycle the ELP-MerR fusion protein for reuse, resuspend ELP aggregates with ice-cold TB7.4M, preferably in the same volume added in Step 20.

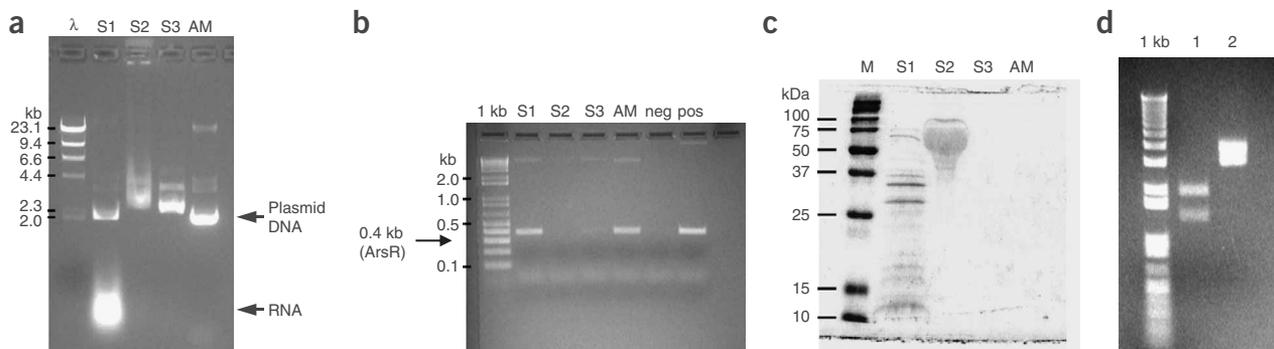


Figure 2 | Plasmid purification from cell lysate by temperature-triggered precipitation. **(a)** Agarose gel electrophoresis of purified plasmid samples (pBLU-Pt) for DNA quantification and detection of RNA contamination. **(b)** Detection of contaminating chromosomal DNA in purified plasmid samples by agarose gel electrophoresis of PCR-amplified *arsR* gene (expected size 400 bp), ubiquitously expressed in the *E. coli* genome. **(c)** Detection of contaminating protein in purified plasmid samples by SDS-PAGE, stained by Coomassie blue. **(d)** Restriction digest of purified plasmid samples by *ScaI* and *NotI* to detect the fragmentation of plasmid DNA pBLU-Pt. Two fragments, one at 1.78 kb and the other at 1.24 kb, are expected. Lane description: λ, *HindIII*-digested λ-DNA molecular weight marker (Promega); 1 kb, 1 kb Plus DNA Ladder (Invitrogen); M, Precision Protein Standards Broad Range (Bio-Rad); S1, crude cell extract; S2, purified plasmid-protein complex before plasmid elution; S3, final plasmid preparation; AM, pBLU-Pt plasmid purified by the standard alkaline miniprep method and diluted to final 100 μl by buffer TB7.4S, so that it could be compared with samples from the ELP-miniprep method; n, negative control in the PCR (water); p, positive control in the PCR 20 ng of plasmid pETR containing the *arsR* gene; 1, the digestion product of the eluted DNA; 2, undigested eluted DNA. Data were first published in ref. 18.

27 | Analyze 1, 5 and 10 μl of the eluted DNA (supernatant from Step 26) amount by horizontal 0.8% agarose gel electrophoresis (90 V, 30 min, 1× TAE buffer) (see **Fig. 2a**). Perform quantification of DNA by a gel-documentation system (GelDoc 2000, Bio-Rad).

28 | Detect any contaminating chromosomal DNA by PCR amplification²² of the *E. coli* chromosomal gene *arsR*²³. Thermal cycling conditions are as follows: 10 min at 95 °C, followed by 30 cycles of 30 s at 95 °C, 45 s at 60 °C and 30 s at 72 °C. Load different volumes of PCR product (e.g., 1, 5 and 10 μl) to electrophoresis apparatus and visualize the band at 0.4 kb of the size of *arsR* gene. Make sure a negative control (water) and a positive control using 20 ng of plasmid pETR harboring the *ArsR* gene²⁴ are also used (see **Fig. 2b**).

▲ CRITICAL STEP The *arsR* gene ubiquitously exists in the *E. coli* genome DNA. Detection of chromosomal DNA can also be enabled by choosing another gene of interest. Detection limit is approximately 400 ng ml⁻¹.

29 | Detect protein contaminates by loading final elution product (a series of volumes is recommended) to a 12% SDS-PAGE gel and identify the protein by silver staining or Coomassie blue staining (see **Fig. 2c**). Perform quantification of proteins by the gel-documentation system aforementioned (GelDoc 2000, Bio-Rad).

30 | Digest 5 μl of the eluted DNA with *SacI* and *NotI* in 50 μl buffer solution at 37 °C for 3 h. Analyze by horizontal 1% (w/v) agarose gel electrophoresis (90 V, 30 min, 1× TAE) in the presence of 0.5 μg ml⁻¹ ethidium bromide to detect the fragmentation of plasmid DNA as well as inhibitory materials (see **Fig. 2d**).

? TROUBLESHOOTING

● TIMING

Steps 1–16: 48 h

Steps 17–26: ~30 min

Steps 27–30 (optional): 4 h

? TROUBLESHOOTING

Troubleshooting advice can be found in **Table 2**.

TABLE 2 | Troubleshooting table.

Problem	Possible reason	Solution
No removal of plasmid DNA in any condition	MerR protein in ELP153MR is not active: (i) oxidation of MerR (ii) degradation of ELP153MR	Change the TB7.4M buffer containing fresh 2-mercaptoethanol Use fresh purified protein for experiments
Nonspecific binding between ELP153MR and control vector	Excessive amount of EMP153MR	Lower the amount of protein to 12.5 μM or below
Incomplete precipitation of ELPs	Not enough inert protein ELP153H6	Add ELP153H6 to 15.5 μM or above

ANTICIPATED RESULTS

The described method allows the purification of plasmid DNA from cell lysate by two steps of temperature-triggered affinity precipitation: first precipitation to remove the protein–plasmid DNA complexes from bulk solution and second precipitation to remove the protein from eluted plasmid DNA. **Figure 2a** shows a typical purification product from *E. coli* (lane S3) in comparison to the plasmid DNA purified by the standard alkaline lysis “midiprep” method²¹ (lane AM). The discrepancy in size between plasmid DNA obtained by this protocol (lane S3) and the standard alkaline lysis method (lane AM) was likely due to the different relaxed forms of plasmid presented in the final preparation. Identification of which form required further study beyond the scope of this protocol. The purity of the product plasmid was further confirmed by PCR and SDS-PAGE, and no contaminating chromosomal DNA (see **Fig. 2b**), mRNAs (see **Fig. 2a**) and cellular proteins (see **Fig. 2c**) could be detected. The eluted plasmid DNA underwent restriction digestion by *SacI* and *NotI* and two bands at the expected size were obtained, indicating that no fragmentation of DNA occurred and DNA was free of inhibiting materials (see **Fig. 2d**). Even though the method described above successfully utilizes ELP-MerR protein, improved efficiency can be obtained by exploiting other DNA-binding proteins. One attractive approach to generalize the utility of this method to a broader class of target plasmids is to exploit the interaction between the replication origin and its binding protein. A single ELP containing this binding protein may be used for a wide range of plasmids containing the same replication origin.

ACKNOWLEDGMENTS This work was made possible by grants from NSF (BES329482 and CCF0330451) and US EPA (R82960601).

COMPETING INTERESTS STATEMENT The authors declare no competing financial interests.

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