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ORIGINAL ARTICLE

An affordable plasmid miniprep suitable for proficient microinjection in *Caenorhabditis elegans*

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Abstract
A variety of techniques, including CRISPR-Cas9 genome editing, have been developed to produce genetically modified cell lines and animal models. In many cases, the success of the genome-editing techniques is dependent on the quality of the introduced DNA. However, the preparation of high-quality plasmids required for small-scale microinjection has not been explored. Here, we compared various types of plasmid preparation methods for their microinjection proficiency and developed an efficient and affordable plasmid mini preparation method suitable for *Caenorhabditis elegans* microinjection. By combining the advantages of Triton X-114 and column-based mini preparation (hence, we named it TXC), the new TXC method was affordable, efficient, and equivalent to expensive plasmid midiprep method based on microinjection efficiency. Besides, TXC was compatible with general molecular biology grade reactions and worked proficiently for different types of plasmids.

Keywords CRISPR-Cas9 · Genome editing · Microinjection · Plasmid miniprep

Introduction

Microinjection has been widely used in *Caenorhabditis elegans* research, including extrachromosomal gene expression, MosSCI, CRISPR-Cas, and RNAi. Independent reports have found that the quality of nucleic acids is essential for microinjection. There are multiple methods for obtaining high-quality DNA for microinjection, including a cesium chloride gradient or silica spin column purification. The development of plasmid preparation kits utilizing silica spin columns reduced the cost and time spent on plasmid DNA purification.

Commercial midiprep kits produce a higher level of expression efficiency compared to miniprep and provide sufficient expression with plasmids used for genome editing (Norris et al. 2015; Kim et al. 2018; Kim and Colaiacovo 2019). However, midiprep kits are expensive, and require a significant amount of time and effort due to the larger volume and more costly column. Miniprep, on the other hand, is affordable and swift. However, regular miniprep is not suitable for microinjection and genome editing due to a quality issue, although some miniprep kits claimed that their purity is sufficient for microinjection (for examples, Monarch Plasmid Miniprep and Endofree Tiangen).

In addition to the commercial kits, some protocols incorporated various agents such as PEG, LiCl2, CTAB, and Triton X-114 for the high-quality purification (Lis 1980; Gustincich et al. 1991; Lander et al. 2002). These agents helped the removal of genomic DNA, RNA, proteins, or endotoxins presented in the bacterial lysates. Here, we hypothesized that these agents might enhance the transgenic efficiency of the miniprep method. We compared multiple plasmid purification methods for microinjection using *C. elegans* as a model system. We found that miniprep kits, including commercial kits claiming high-quality transfection, were not as efficient as midiprep for microinjection.

Furthermore, we invented a simple and efficient method of plasmid extraction that yielded plasmid DNA with sufficient purity and quantity for microinjection in the *C. elegans* germline. By combining the advantages of Triton X-114 and column-based mini preparation (hence, we named it TXC), the new TXC method provided a better quality plasmid DNA sufficient for microinjection. The TXC method was

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affordable, efficient, and equivalent to expensive plasmid midiprep method based on microinjection efficiency.

Methods

Strains and maintenance

The Bristol N2 strain was used for all experiments. Animals were grown at 20 °C on nematode growth medium (NGM) plates seeded with *E. coli* strain OP50 as previously described (Brenner 1974), unless otherwise noted.

DNA preparation and microinjection condition

pCFJ90, pCFJ104 (Pmyo-2::RFP and Pmyo-3::RFP vectors from Addgene), and BHK709 (pBlue-T vector containing 91 bp *dpy-10* genomic DNA) were purified from *E.coli* host as mentioned in the text. The microinjection was prepared at the final concentrations of plasmids 5 ng/μL for both pCFJ90 and pCFJ104, and injected into young adult N2 animals as previously described (Kim and Colaiacovo 2019). Since the microinjection efficiency may vary among individuals, microinjection was mainly performed by one person under the blinded condition to avoid unintentional bias. Of note, higher concentrations of these plasmids were known for toxicity. Injected animals were transferred to new NGM plates and grown at 25 °C. 3 and 4 days after injection, we monitored F1 worms expressing fluorescence signals.

Data availability

pCFJ90, pCFJ104, and their DNA sequences are available for distribution from Addgene (addgene.org). *C.elegans* N2 worms are available from CGC (http://cgc.umn.edu).

mCherry expression assay

pCFJ90 plasmid purified with various methods including control/canonical alkaline lysis, Qiagen midiprep, NEB Monarch miniprep, Tiangen miniprep, cTAB, TX, TXC, TX + NEB column, TX + NEB column + Spin X, TX + Tiangen column + Spin X, and Tiangen Endofree. To minimize variations during microinjection, temperature (20-24 °C) and humidity (40-55%) were maintained. Prepared plasmids were injected into the germline of P0 hermaphrodite worms. ~ 15 P0 worms were injected for each microinjection (Table 1). Either one (~ 2/3) or both arms (~ 1/3) of the gonad were injected. Three and four days after injection, the number of mCherry(red) expressing F1 progenies was counted under the fluorescent microscope (Nikon P-DSL32). We performed two-to-five independent microinjections from two independent plasmid preparations.

Table 1 Transgenic mCherry expression efficiency for multiple types of plasmid preparation methods

Type	Mean number of transgenic mCherry + F1s per injected P0	Mean number of transgenic bright mCherry + F1s per injected P0	Number of independent microinjections (the total P0 numbers)
Control (Alkaline lysis)	0	0	3 (48)
Midi prep (Qiagen)	12.798	6.698	5 (72)
Mini prep (NEB)	5.759	2.193	4 (60)
Mini prep (Tiangen)	4.499	0.040	3 (45)
Endofree mini prep (Tiangen)	6.833	2.978	3 (45)
cTAB	0.540	0	2 (32)
Triton X-114	3.069	0.724	3 (44)
TXC	17.300	5.949	4 (58)
TX + column (NEB)	6.694	2.056	2 (30)
TX + column (NEB) + SpinX	6.34	2.100	2 (30)
TX + column (Tiangen) + SpinX	0	0	2 (30)
Control (pCFJ104)	0	N/A	2 (32)
TXC (pCFJ104)	8.885	N/A	2 (32)
Midi (Qiagen, pCFJ104)	9.105	N/A	2 (32)

The efficiency of the generation of transgenic animals was measured by the number of transgenic worms(mCherry +) generated per the number of worms injected. pCFJ90 plasmid purified with different types of methods was injected into P0 hermaphrodites, and the number of mCherry expressing F1 progenies was counted under the fluorescent microscope. Multiple independent microinjections were performed from 2 to 3 independent plasmid preparations. ~ 15 P0 worms were injected for each sample

Fluorescence microscopy

Transgenic animals were imaged with a Nikon Ti2E microscope and DSQi2 camera. Acquired images were collected and processed in Nikon NIS Elements.

Statistical analysis

Statistical comparisons between genotypes were performed using the two-tailed Mann–Whitney test, 95% confidence interval. Mean with SD values were calculated using Prism or Instat software.

Reagents for plasmid preparation

Plasmid midiprep Qiagen 12143
Plasmid miniprep kit Tiangen Tianprep DP103-03
Plasmid miniprep kit NEB Monarch T1010s
Plasmid Endofree Tiangen DP118-02
PCR and DNA cleanup kit NEB Monarch T1030s
Universal DNA purification kit Tiangen DP214-03
Spin X Costar CA membrane 0.22 µm 8160
Triton X-114 Solarbio T8210
P1: 50 mM Tris pH8.0 10 mM EDTA pH 8.0
P2: 200 mM NaOH, 1% SDS.
P3: 3 M Potassium acetate pH5.5 (adjust PH with glacial acetic acid).

TritonX-114 protocol

Incubate bacterial culture for ~ 18 h in a shaking incubator (~ 180 RPM). All centrifugation steps were carried out at ~ 13000 × *g*.

1. ~2 ml of bacterial culture was collected with centrifugation for 1 min. Pour off the supernatant.
2. Add 200 µl P1. Vortex to re-suspend the bacterial pellet.
3. Add 200 µl P2 and Invert ten times to mix the solutions. Incubate at 37 °C for 5 min.
4. Add 300 µl P3. Gently invert the tube ten times to ensure the solutions are completely mixed. A white precipitate will appear. Incubate on ice for 10 min.
5. Centrifuge the tube for 10 min. The white precipitate will form a pellet on the side of the tube. Pipette supernatant(~ 800ul) into a new 1.5 ml tube without disturbing the pellet.
6. Add 82 µl 10% Triton X-114 to final 1%. Solution will become cloudy. Invert tube 10–20 times.
7. Incubate samples on ice for 10 min. Incubate at 42 °C 10 min.
8. Centrifuge 10 min. After centrifugation, there will be two layers. Do not disturb the dark yellow bottom layer(~ 100 ul). Transfer the top light yellow colored to new Eppendorf tubes.
9. Add 0.7 × volume of Isopropanol (~ 560ul). And Invert tube ten times.
10. Centrifuge 10 min and dump supernatant.
11. Add 500 µl 70% Ethanol. Centrifuge 5 min and pipette out the supernatant
12. Air dry 5 min at 37 °C.
13. Re-suspend the pellet(DNA) in 30 µl elution 0.5× TE.

TXC protocol

Incubate bacterial culture for ~ 18 h in a shaking incubator (~ 180 RPM). All centrifugation steps were carried out at ~ 13,000 g.

1. ~1.5 ml of bacterial culture was collected with centrifugation for 1 min.
2. Pour off the supernatant and add 200 µl B1 from plasmid miniprep (NEB Monarch mini prep). Vortex to re-suspend the bacterial pellet. Make sure there are no clumps left.
3. Add 200 µl B2 and Invert ten times to mix the solutions. Incubate at 37 °C for 5 min.
4. Add 300 µl B3. Gently invert the tube ten times to ensure that the solutions are thoroughly mixed. A white precipitate will appear. Incubate on ice for 10 min.
5. Centrifuge the tube for 10 min.
6. Transfer supernatant(~ 800 ul) to a new Eppendorf tube. Add 82 µl 10% Triton X-114 and Invert tube 10–20 times.
7. Incubate samples on Ice 10 min. Incubate at 42 °C 10 min.
8. Centrifuge the tube for 10 min.
9. There will be two layers after centrifugation. Do not disturb the dark yellow bottom layer. Transfer the top light yellow colored to the column and centrifuge for 1 min. Discard flow-through.
10. Re-insert column in the collection tube and add 200 µl of wash Buffer 1. Incubate 5 min at RT. Centrifuge for 1 min. Discard the flow-through.
11. Add 400 µl of Plasmid Wash Buffer 2 and centrifuge for 1 min. Discard the flow-through.
12. Centrifuge for 2–3 min and discard the flow-through to remove residual wash buffer.
13. Transfer the column to a new 1.5 ml Eppendorf tube.
14. Warm the DNA Elution Buffer to ~ 50 °C. Add 30 µl DNA Elution Buffer to the center of the column matrix. Wait for 1 min and then spin for 1 min to elute DNA.

Triton X-114 with DIY alkaline lysis solution and DNA purification column protocol

Incubate bacterial culture for ~ 18 h in a shaking incubator (~ 180 RPM). All centrifugation steps were carried out at ~ 13,000 × *g*.

1. ~2.5 ml of bacterial culture was collected with centrifugation for 1 min. Pour off the supernatant.
2. Add 400 µl P1. Vortex to re-suspend the bacterial pellet.
3. Add 400 µl P2 and Invert ten times to mix the solutions. Incubate at 37 °C for 5 min.
4. Add 600 µl P3 + 10 µl of 10 mg/ml RNase. Gently invert the tube 10–20 times to ensure the solutions are 5ompletely mixed.
5. Incubate 37 °C for 15 min.
6. Centrifuge 10 min at 4 °C. The white precipitate will form a pellet on the sidewall of the tube.
7. Pipette supernatant (~ 1300 µl) to a new 1.5 ml tube without disturbing the pellet.
8. Add 130 µl 10% Triton X-114 to final 1%. Solution will become cloudy. Invert tube 20 times.
9. Incubate samples on ice for 10 min and 42 °C for 10 min. Centrifuge 20,000 × *g* 10 min. Two layers will be visible after centrifugation. Transfer the top layer to a new 1.5 ml tube without disturbing the yellow bottom layer (~ 150 ul). Repeat this step.
10. Split supernatant in half (500 µl × 2 1.5 ml tubes) and add a 2 × volume of cold ethanol (1000 µl × 2 tubes). Store samples in − 80 °C freezer for 20 min to precipitate DNA.
11. Centrifuge 20,000 × *g* 10 min and discard the supernatant.
12. Add 1 ml 70% ethanol. After centrifuge for 3–5 min pipette out supernatant: repeat this step.
13. Air dry 5 min at 37 °C to dry residual ethanol.
14. Resuspend pellet in ~ 30 µl nuclease-free water.
15. Transfer DNA to the DNA purification column (NEB T1030s). Allow 2 min for binding.
16. Add wash solution and incubate for 5 min.
17. Warm the DNA Elution Buffer to ~ 50 °C. Add ~ 30 µl DNA Elution Buffer to the center of the column matrix. Wait for 1 min and then spin for 1 min to elute DNA.
18. (Optional) Transfer samples to the Spin X column. Centrifuge 5 min.

Results

To compare the efficiency of gene expression upon microinjection, a plasmid pCFJ90, which expressed GFP signal under the control of the *myo-2* promoter, was prepared with various plasmid preparation methods. We performed multiple independent microinjections with independently prepared DNA samples. After microinjection of pCFJ90 into the germline of *C. elegans*, pCFJ90 signal, found in either bright and even or dim and partial mCherry + , in the pharynx muscle was monitored as a reporter for the transgenic efficiency of each plasmid samples in microinjection (Fig. 1a). The number of mCherry(red) expressing F1 (per injected P0 worm) was counted under the fluorescent microscope.

Midiprep displayed a higher efficiency of expression than miniprep in both the number of mCherry and the number of bright mCherry

We found that plasmids prepared with Qiagen midiprep displayed a higher frequency of expression in the number of total mCherry (bright + dim) than the control plasmid prepared with canonical alkaline preparation method (12.798 vs 0, respectively, Fig. 1b and Table 1). Similarly, the mean number of bright mCherry expression was higher in Qiagen midiprep than the control alkaline lysis prep (6.698 vs 0). These data suggested that both the number of total mCherry and bright mCherry reflect the quality of plasmids for expression.

Some commercial miniprep kits claimed quality high enough for transfection. We tried three different miniprep kits for microinjections. Of note, we have also considered the Genejet miniprep (catalog no K0502) for microinjection. However, its manual only recommends maxiprep (catalog no K0861) for transfection; therefore, we drop out the miniprep for our assay. In short, none of them was comparable to midiprep in either the number of totals or bright mCherry transgenic efficiency. For the total number of mCherry expression assay, regular plasmid prep kits displayed 2.8-fold lower efficiency compared to Qiagen midiprep (4.499 vs 12.798, $p = 0.0358$ for Tiangen miniprep, Table 1 and Fig. 1b). Interestingly, two transfection compatible plasmid kits were also lower than the midiprep by ~ 2-fold (5.759 vs 12.798, $p = 0.0195$ for miniprep NEB and 6.833 vs 12.798, $p = 0.0358$ for Endofree).

Similarly, we found a significant reduction in the bright mCherry expression. 2–3-fold decrease was found in the transfection compatible kits compared to Qiagen midi (2.193 vs 6.698, $p = 0.0159$ for NEB miniprep and 2.978 vs 6.698, $p = 0.0357$ for Endofree kit). The reduction was

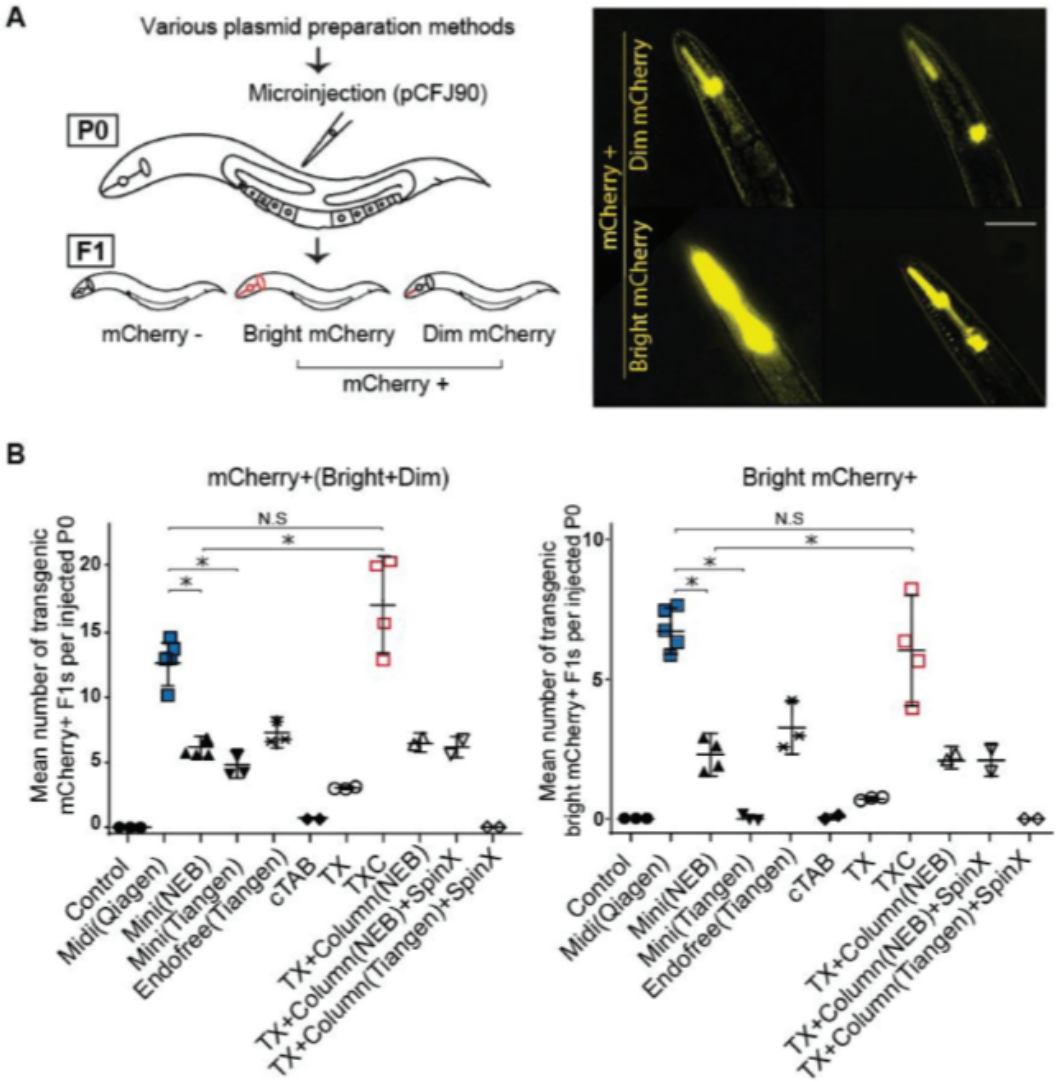


Fig. 1 mCherry expression assay: TXC method produced a high efficiency of mCherry expression. **a** Left, a schematic illustration of the mCherry expression assay. pCFJ90 plasmid purified by different methods was injected into P0 hermaphrodite worms and the number of mCherry(red) expressing F1 progenies (per injected worm) was counted under the fluorescent microscope. ~ 15 P0 worms were injected for each sample. The number of mCherry + (bright or dim) and mCherry- was scored. Right, representative images of mCherry expressing worms. mCherry expression was either dim and partial or bright and even. Bar = 50 µm. **b** Quantitation of the efficiency of the generation of transgenic animals (mCherry) with various types of plasmid purification methods. TXC method produced a high efficiency of mCherry expression comparable to midiprep in either the total number of mCherry (bright + dim, left panel) or bright mCherry (right panel). Each plot represented a single microinjection for ~ 15 P0 worms. The efficiency of the generation of transgenic animals was measured by the number of transgenic worms generated per the number of worms injected. Multiple independent microinjections were performed from 2–3 independent plasmid preparations. Mean with SD values were plotted. Asterisks (*) indicated statistical significance compared to control by the Mann–Whitney *U* test (95% CI). N.S indicated that two values were not statistically significant

even higher with the regular miniprep kit by 167-fold (0.04 vs 6.698, $p = 0.0357$ for Tiangen miniprep).

Taken together, these results suggested that transfection compatible miniprep kits were not as efficient as a midiprep, although they might be better alternatives than regular plasmid kits for *C. elegans* microinjection. These results are consistent with the previously observed idea among researchers that midiprep kit provides a better quality DNA for microinjection than miniprep kits (Kim and Colaiacovo 2019).

Triton X-114 treatment enhanced the mCherry expression level

We tried various plasmid purification strategies to enhance the efficiency of mCherry expression. cTAB is known for its effectiveness in precipitating plasmid DNA (Mello and Fire 1995; Lander et al. 2002). Consistent with these reports, we found that cTAB treatment enhanced the level of mCherry expression (0.54 and 0 for cTAB compared to 0 and 0 for control alkaline lysis, respectively); however, the efficiency was not comparable to mini or midi kits in our hands in either the number of totals or bright mCherry worms.

In addition to cTAB, Triton X-114 has been shown to remove endotoxin from plasmids and to enhance gene expression in transfection experiments (Bordier 1981; Ma et al. 2012; Teodorowicz et al. 2017). We tested whether Triton X-114 treatment enhances the expression of arrays upon microinjection in the *C. elegans* germline. In brief, after alkaline lysis of *E. coli* harboring plasmid, Triton X-114 was added to remove endotoxin, followed by precipitation of plasmid DNA (Fig. 2; see technical details in the Materials and methods). Plasmid extracted with Triton X-114 displayed a higher expression level compared to the control plasmid extracted without Triton X-114 treatment in either total (bright or dim, 3.069 for Triton X-114, and 0 for control) or bright mCherry worms (0.724 for Triton X-114 and 0 for control; P value was not available since the control's SD is zero.).

TXC (Triton X-114 + column) enhanced the mCherry expression to the level of midiprep

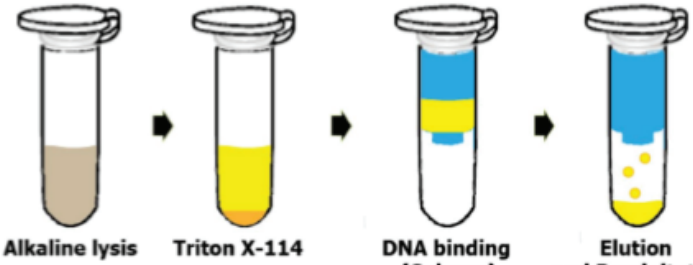
Since both Triton X-114 and NEB miniprep increased mCherry expressing transgenic animals, we combined the two procedures in a single plasmid miniprep purification. This approach modified column-based alkaline lysis miniprep kit to incorporate the Triton X-114 treatment; hence, we named it TXC (Triton X-114 + Column). Surprisingly, TXC purification enhanced the mCherry expression significantly. The level of total transgenic mCherry worms was higher than NEB miniprep or Triton X-114 treatment by 3–5.6-fold significantly (17.3 for TXC; $p = 0.0286$ for NEB mini compared to TXC and $p = 0.0286$ for Triton X-114 compared to TXC). Likewise, the level of transgenic bright mCherry worms was higher by 2.8–8.2 fold significantly (5.949 for TXC, for NEB mini compared to TXC and $p = 0.0286$ for Triton X-114 compared to TXC).

Furthermore, the efficiency of transgenic expression with the TXC is equivalent to that of Qiagen midiprep (12.798 and 6.698 for Qiagen midiprep; $p = 0.1762$ for total mCherry and $p = 0.7302$ for bright mCherry), suggesting that TXC can be a substitute for midiprep.

Triton X-114 worked efficiently with DIY alkaline lysis solution and DNA purification column, however not as equivalent as TXC

Since the TXC methods adopts commercial NEB miniprep solutions and silica columns presented high efficiency of array expression, we further examined whether DIY(do-it-yourself) DNA purification solutions and columns can be alternatives for NEB miniprep kits. Regular DNA/PCR purification columns and DIY alkaline lysis solutions were adopted for plasmid preparation with Triton X-114 detergent. In brief, a bacterial pellet was lysed by an alkaline lysis method with DIY P1, P2, and P3 solutions. After the addition of Triton X-114, samples were further purified using DNA/PCR cleanup columns, followed by additional SpinX filtration to remove any remaining Triton X-114 (See "Materials and methods").

We found that DIY alkaline lysis solutions combined with a regular DNA purification column from NEB were not as efficient as TXC in either total or bright mCherry, although it is still comparable to NEB mini plasmid kit or Endofree kits (6.694 and 2.056 for TX + column(NEB)). Additional SpinX filtration process that removed residual Triton X-114



TXC	Alkaline lysis	Triton X-114	Column Purification	Precipitation
Triton X-114	Alkaline lysis	Triton X-114		Precipitation
Miniprep	Alkaline lysis		Column Purification	Precipitation

Fig. 2 Procedure for the TXC (Triton X-114 + Column) method compared to Triton X-114 or miniprep. Schematic illustration of purification for each step. In brief, bacteria-harboring the plasmid of interest was collected and underwent cell lysis. Triton-X114 treatment solubilized membrane and extracted LPS from the bacterial lysate. Finally, plasmid DNA was purified by silica column. Note that TXC incorporated Triton X-114 and silica column purification

did not improve the transgenic efficiency [6.34 and 2.100 for TX + column (NEB) + SpinX, (Teodorowicz et al. 2017)], suggesting that this protocol is indispensable for extra removal of Triton X-114. Interestingly, the same strategy from another supplier (PCR/DNA purification column from Tiangen) did not produce any bright or dim mCherry worms repeatedly [0, 0 for TX + column (Tiangen) + SpinX], suggesting that columns from different suppliers can have large effects on the efficiency for microinjection.

Taken together, Triton X-114 works efficiently together with DIY alkaline lysis solution and DNA purification column, however not equivalent to the midiprep or TXC in their transgenic expression efficiency.

TXC method worked proficiently for different types of plasmids

All our assays were based on mCherry expression under the control of the *myo-2* promoter plasmid (pCFJ90 plasmid) to assess plasmid gene expression in the same experimental background. However, the outcome of our assays might be biased if the plasmid (pCFJ90) does not represent regular plasmids. Therefore, we further tested whether TXC is an effective purification method for another expression marker. pCFJ104 prepared with TXC displayed much higher efficiency compared to control alkaline lysis and a similar efficiency as Qiagen midiprep (0, 8.885 and 9.105 for control, TXC, and midiprep, respectively. Table 1 and Supplementary Fig. 1). This result suggested that the TXC approach might be generally applicable for plasmid purification. Overall, the TXC miniprep method showed higher levels of transgenic mCherry expression, equivalent to Qiagen plasmid midiprep, which saves both the time and money required for microinjection groundwork.

TXC preparation procedure and features

It has been reported that the addition of Triton X-114 binds to lipopolysaccharides (LPS), which assists the removal of endotoxin in the sample (Bordier 1981). However, Triton X-114 has not been implemented together with a miniprep kit previously. TXC incorporated the addition of Triton X-114 early in the protocol after the neutralization (Fig. 2). After the addition of Triton X-114 to *E. coli* lysate, endotoxin containing layer was collected and discarded. Finally, plasmid DNA was further purified by silica column.

Although TXC improves expression efficiency, it does not require special equipment such as bulky high-speed centrifuge, large sample volume, or lengthy procedures as in the regular midiprep protocol (Table 2). Specifically, TXC requires a total of ~ 80 min (~ 30 min hands-on time) and ~ 1.5 ml volume of overnight grown bacterial culture for each preparation, which takes only about half of the time and costs ~ 7 times less compared to a regular midiprep kit. Therefore, TXC makes the entire microinjection workflow easier, especially when frequent microinjection is necessary.

The quantity of plasmid DNA from TXC methods is equivalent to that of regular miniprep kits. The average concentration of purified DNA was 34 ng/µl was measured by spectrophotometer, with the OD 260/280 in the range of 1.77–1.8 (Table 3). Of note, our protocol employed the right amount of RNase A to remove RNAs, which may interfere with quantifying the amount of DNA.

TXC purified plasmids were compatible with general molecular biology grade reactions

The quality of TXC products is satisfactory for microinjection based on the level of transgenic mCherry worms (Fig. 1 and Table 1). We further investigated whether TXC purified plasmid was compatible with general molecular biology grade reactions. Similar to mini or midiprep samples, both Triton X-114 and TXC prepared plasmids gave readouts of > 900 base pairs by Sanger DNA sequencing, without any modification of the protocol (Fig. 3a). Extracted plasmids are predominantly supercoiled and some portion of open-circular form based on gel electrophoresis. The proportion of the structures is similar to that of Qiagen Midiprep based on band intensity. For TXC, 86, 3, 11% and for Qiagen Midiprep, 88, 2, 10% of supercoil, linear, and open/relaxed circular form, respectively, from three independent preparations. We further tested if TXC

Table 2 Comparison of commercial plasmid methods with the TXC

Type	Supplier	Catalog No.	Culture (ml)	Elution vol. (µl)	Time (min)	Cost/sample (USD)
Miniprep	Qiagen	27104	1.5-3	50	~30	1.9
	NEB	T1010s	1.5-3	30	~30	1.7
	TXC	N/A	1.5-3	30	~80 (30 m hands-on)	1.72
Midiprep	Qiagen	12143	25-100	N/A	~150	11.9

TXC requires longer times than regular miniprep kits due to the additional Triton X-114 step. However, it is ~ 70 min shorter and cost ~ 7 times less than a regular midiprep procedure. Values from commercial kits are from the manufacture's manuals

N/A Not applicable

Table 3 Quantity and O.D. 260/280 values of plasmid preparation methods

Type	Average amount of DNA (μg)	Average of OD 260/280
Control (Alkaline lysis)	950	1.99
Midi prep (Qiagen)	45	1.88
Mini prep (NEB)	75.5	1.85
Mini prep (Tiangen)	28.5	1.95
Endofree mini prep (Tiangen)	223.5	2.02
cTAB	21.7	2.10
Triton X-114	1246	1.99
TXC	34	1.78
TXC + Spin X	43	1.87
TX + column (NEB)	39	1.8
TX + column (NEB) + Spin X	33.25	1.82
TX + Column (Tiangen) + Spin X	27.3	1.9
Control (pCFJ104)	98	2.00
TXC (pCFJ104)	92.5	1.85
Midi (pCFJ104, Qiagen)	162.1	1.83

Each method was performed with at least two independent preparations. The amount of DNA and O.D (260/280) values were measured by spectrophotometer (Eppendorf Biospectrometer). Plasmid DNA was confirmed based on based on gel electrophoresis

plasmids are compatible with restriction enzyme digestion. Two different plasmids obtained by TXC preparation were used for *Eco*RI and *Hin*dIII digestion and gave the expected products, suggesting that the TXC method provides sufficient purity for molecular biology grade reactions (Fig. 3b).

Discussion

Plasmid preparation is a fundamental method for molecular biology. Notably, the recent development of genome editing brought attention to higher purity of the plasmid preparation method, since the quality of plasmid is indispensable for proficient genome modification. However, high-quality plasmid DNA preparation such as plasmid midiprep kits entails high value and substantial labor. Although regular miniprep is quick and affordable, it does not yield high competence for microinjection.

Therefore, we investigated various plasmid miniprep procedures to produce high-quality plasmid for microinjection to avoid the cost and effort required for commercial midiprep kit. Interestingly, miniprep kits, which claimed high purity sufficient for transgenic efficiency, were not equivalent to midiprep based on our assays of mCherry transgenic expression. On the other hand, by combining the advantages of Triton X-114 and column-based mini preparation, the TXC plasmid isolation strategy provides expression efficiency comparable to commercial plasmid midiprep kit (Qiagen), with significantly reduced time and cost (~ 70 min less and ~ 7 × less cost compared to midiprep, Table 2).

Combination of Triton X-114 and silica column improved efficiency

Bacterial lipopolysaccharides (LPS) present in the lysate can induce inflammation in the host cell, which would hamper the genome-editing process (Tobias et al. 1988; Cotten et al. 1994; Aballay et al. 2003). Triton X-114 can solubilize

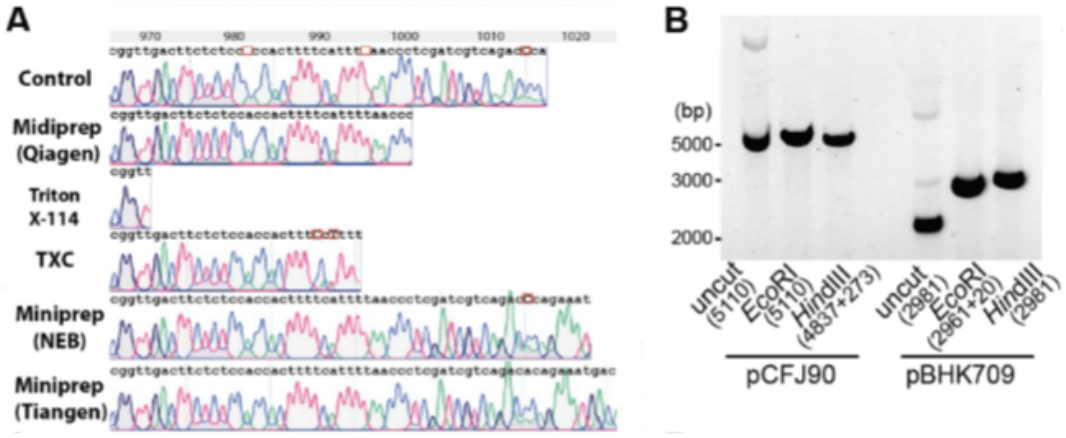


Fig. 3 TXC purified DNA was sufficient for Sanger sequencing and restriction enzyme digestion. **a** Representative Sanger sequencing result. All methods read over 900 bps before encountering signal degradation. Numbers indicate the distance from the first nucleotide. Red boxes indicate mismatches. Sanger sequencing was performed at least twice for each method. **b** Two TXC prepared plasmids (pCFJ90 and pBHK709) digested with *Eco*RI and *Hin*dIII displayed the expected size indicated in brackets. To avoid excessive digestion, 1 unit of restriction enzyme was used for 1 μg DNA in 20 min incubation. Of note, 1 unit of the enzyme could digest 2 μg of TXC DNA in 30 min

membranes and extract LPS from bacterial lysate (Cotten et al. 1994; Ma et al. 2012). With a minor modification, we adopted Triton X-114 to remove LPS from the plasmid extract. As expected, Triton X-114 enhanced the efficiency of plasmid gene expression. Surprisingly, a combination of Triton X-114 and commercial plasmid miniprep kits improves the efficiency to the level of midiprep.

How did TXC produce higher efficiency compared to Triton X-114 or miniprep? At temperatures below 20 °C, endotoxin containing Triton X-114 is miscible with aqueous solutions, while temperature above 20 °C two layers will be separated. Although Triton X-114 removes endotoxin by switching between two temperatures, the removal of Triton X-114 itself is equally important, since Triton X-114 was reported to be toxic in cells, as well (Aida and Pabst 1990). After removal of endotoxin and Triton X-114 by centrifugation, the subsequent silica column purification further removed residual endotoxin. This dual removal of endotoxin and Triton X-114 should contribute to the higher purity of plasmid.

Since the TXC method requires commercial columns from NEB, we tested whether DNA purification columns can replace the NEB plasmid purification kit used in the TXC method. Although the DNA/PCR purification column from NEB produced efficiency similar to the level of Endo-free or high-quality plasmid miniprep kits (NEB), mCherry microinjection efficiency was found less than that of TXC in either total or bright mCherry + worms [Fig. 1b and Table 1, for TX + column (NEB)]. Interestingly, an additional SpinX filtration procedure that removes residual Triton X-114 did not improve the efficiency, suggesting that the low efficiency was not due to the leftover Triton X-114 in the samples (Teodorowicz et al. 2017). In fact, numerous factors other than endotoxin and triton X-114 inhibit transgenic efficiency (Liu et al. 2013, Behringer et al. 2014). For example, ethanols, salts, proteins, alcohol, detergents, and solvents impede further cellular development. Any traces of these contaminants may inhibit transgenic efficiency, although endotoxin containing triton X-114 was absent in the purified DNA. Also, this explains the reason why measuring the level of endotoxin in the purified sample is not sufficient to estimate the level of the transgenic efficiency in the biological system.

Multiple commercially available reagents or kits claimed high efficiency for transfections. However, none of those has been investigated systematically to the best of authors' knowledge. We have investigated the efficiency of transgenic mCherry expression in *C. elegans* system. Although commercial kits claim to provide sample purity sufficient for transfection, their efficiencies were not comparable to a midiprep kit based on our assay. On the contrary, the TXC method showed quality DNA that matches the commercial midiprep kit. One drawback of TXC was that due to the additional purification steps, it did not produce a good yield of DNA compared with the conventional minipreps. However, this can be easily overcome by concentrating DNA, using a vacuum evaporator for example.

Taken together, TXC miniprep provided quality DNA that is suitable for Sanger sequencing and restriction enzyme digestion. Also, it provided an efficiency of transgenic expression level equivalent to Qiagen plasmid midiprep, suggesting that TXC miniprep might be a preferable method for microinjection in terms of cost and time consumption. By combining the advantages of Triton X-114 and column-based mini preparation, the new TXC method provided a better quality plasmid DNA sufficient for microinjection. The TXC method was affordable, efficient, and equivalent to expensive plasmid midiprep method.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest in the publication.

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