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Title: Medium-throughput Column DNA Extraction Using 24-well Racks

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

☒ Correct

☐ Incorrect

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **06/22/2026**

When you are ready to submit your video files, please contact our Content Manager, [Utkarsh Khare](#).

Current Protocol Length

Number of Steps: 15

Number of Shots: 44

Interviews

INTRODUCTION:

REQUIRED: ~~Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Tatyana Kalinina:** Our research focuses on developing 3D-printed racks for parallel spin-column DNA and RNA extraction, bridging manual and automated methods.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Tatyana Kalinina:** This protocol addresses limitations of labor-intensive manual spin-column methods by enabling simultaneous processing of up to 116 DNA or RNA samples.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Tatyana Kalinina:** This protocol allows researchers to obtain high-quality plasmid DNA for downstream applications, including cloning, sequencing, and PCR analysis.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Tatyana Kalinina:** The most important consideration when performing this protocol is preventing cross-contamination by maintaining watertight, non-overfilled 3D-printed reservoirs.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.5. **Tatyana Kalinina:** Additionally, following this procedure, these racks can be used for any column-based nucleic acid extraction analysis.

- 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Protocol

2. Preparation of 3D-printed Equipment

Demonstrator: Olesya Lygun

NOTE: SCREEN timestamps for protocol were added at the postshoot stage. Please contact the postshoot note integrator (Sri Lakshmi Priya) for queries regarding lab media.

- 2.1. To begin, use a fused-filament 3D printer with a 0.4-millimeter nozzle to print the rack [1]. Use generic polylactide or PLA (*P-L-A*), polyethylene terephthalate glycol or PETG (*P-E-T-G*), or acrylonitrile butadiene styrene or ABS (*A-B-S*) filament with 100 percent infill [2]. Set the layer height to 0.1 millimeter and enable surface smoothing [3]. Adjust the print settings to ensure watertightness and mechanical stability [4].
 - 2.1.1. WIDE: Talent standing near the fused filament 3D printer and preparing it with a 0.4 mm nozzle.
 - 2.1.2. Close shot of the filament spool as the talent loads generic PLA, PETG, or ABS filament for printing.
 - 2.1.3. SCREEN: 2.1.3 (ironing).png
 - 2.1.4. SCREEN: 2.1.4.png
- 2.2. Test the racks for waterproofness before using them for the first time [1]. Fill the printed reservoir with 70 percent ethanol to test it before use [2]. Centrifuge for progressively increasing durations to confirm the absence of leakage or structural damage [3-TXT].
 - 2.2.1. Talent adding water into racks and checking it.
 - 2.2.2. Talent filling the printed reservoir with 70 percent ethanol.
 - 2.2.3. Talent placing the reservoir into the centrifuge. **TXT: 1 min → 2 min → 5 min → 10 min → 30 min**
- 2.3. After successful testing using glue, join the Base Reservoir components together to form a sealed unit [1].
 - 2.3.1. Talent joining the Base Reservoir components to form a sealed unit.

3. Preparation of Bacterial Culture and Cell Lysate

Demonstrator: Maria Drozhzhina

- 3.1. Inoculate *Escherichia coli* or *E. coli* (*E-Coli*) transformants into 3 to 5 milliliters of lysogeny broth or LB (*L-B*) medium containing appropriate antibiotics [1]. Incubate the culture for 12 to 16 hours at 37 degrees Celsius with shaking [2].

- 3.1.1. Talent inoculating E. coli transformants into a tube containing lysogeny broth medium.
 - 3.1.2. Talent placing the culture tubes in an incubator shaker set to 37 degrees Celsius.
 - 3.2. Centrifuge at 2000 g for 10 minutes to harvest the cells [1]. Discard the supernatant [2].
 - 3.2.1. Talent placing the culture tubes in the centrifuge.
 - 3.2.2. Talent discarding the supernatant.
 - 3.3. Add 250 microliters of resuspension buffer to the pellet [1] and resuspend the pellet completely by vortexing or pipetting [2].
 - 3.3.1. Talent adding resuspension buffer to the cell pellet.
 - 3.3.2. Talent resuspending the pellet by vortexing or pipetting until the suspension is uniform.
 - 3.4. Add 250 microliters of lysis buffer [1] and mix gently by inverting the tube 4 to 6 times or by rocking the 24-well plate [2].
 - 3.4.1. Talent adding lysis buffer to the resuspended pellet.
 - 3.4.2. Talent gently inverting the tube 4 to 6 times to mix.
 - 3.5. Add 350 microliters of neutralization buffer [1] and mix it [2]. Observe the formation of a white precipitate to confirm successful neutralization [3].
 - 3.5.1. Talent adding neutralization buffer to the lysate.
 - 3.5.2. Talent inverting the tube to mix.
 - 3.5.3. Close shot of the tube showing the white precipitate after neutralization.

4. Preparation of Lysate Using Titanium Dioxide Co-precipitation

Demonstrator: Maria Drozhzhina

- 4.1. Add lysis buffer into a tube containing titanium dioxide powder at a 2 to 1 volume-to-mass ratio [1]. Add 250 microliters of the titanium dioxide suspension to the lysate [2] and mix it by inverting the tube [3]. Add 350 microliters of neutralization buffer [4] and mix thoroughly [5].
 - 4.1.1. Talent adding lysis buffer into a tube containing titanium dioxide powder.
 - 4.1.2. Talent adding the titanium dioxide suspension to the lysate.
 - 4.1.3. Talent inverting the tube to mix the suspension with the lysate.
 - 4.1.4. Talent adding neutralization buffer to the mixture.
 - 4.1.5. Talent inverting the tube to mix the contents.
- 4.2. Centrifuge at 2000 g for 10 minutes [1]. Observe the cell pellet completely precipitated in the presence of titanium dioxide and the supernatant appearing fully

transparent [2]. In the absence of titanium dioxide, note the presence of residual sediment in the supernatant [3]. Collect the supernatant without disturbing the precipitate [4] and transfer it to the binding column [5].

- 4.2.1. Talent placing the tubes into the centrifuge.
- 4.2.2. Close-up of precipitated cell pellet and transparent supernatant.
- 4.2.3. Close-up of residual sediment in the supernatant.
- 4.2.4. Talent collecting the supernatant while leaving the precipitate undisturbed.
- 4.2.5. Talent transferring the supernatant to the binding column.

5. Assembly of the Filtration Unit for Lysate Clearance and DNA Binding

Demonstrator: Tatyana Kalinina

- 5.1. Place the deoxyribonucleic acid or DNA (*D-N-A*) binding columns into the column holder layer of the rack [1]. Position the column lids into the designated slots to secure them during centrifugation [2]. Place the clarification or filter columns into the filter column layer directly above the binding columns [3].
 - 5.1.1. Talent placing the DNA-binding columns into the column holder layer of the rack.
 - 5.1.2. Talent positioning the column lids into the designated slots on the rack.
 - 5.1.3. Talent placing the filter columns into the filter column layer above the binding columns.
- 5.2. Transfer the prepared neutralized lysate into the filtration columns [1]. Place the assembled rack into a swinging-bucket plate rotor [2]. Centrifuge at 2000 g for 1 minute at room temperature [3]. After removing the rack from the centrifuge, remove and discard the filter column layer [4].
 - 5.2.1. Talent transferring the neutralized lysate into the filtration columns.
 - 5.2.2. Talent placing the assembled rack into a swinging-bucket plate rotor.
 - 5.2.3. Talent placing the rotor into the centrifuge.
 - 5.2.4. Talent removing the filter column layer from the rack and discarding it.

6. Washing and Elution of DNA

Demonstrator: Tatyana Kalinina

- 6.1. Discard the flow-through from the reservoir into a waste container [1]. Reassemble the rack with the binding columns [2]. Add 500 to 700 microliters of wash buffer to each binding column [3]. Centrifuge at 2000 g for 1 minute [4]. Discard the flow-through from the reservoir and repeat the washing step as previously demonstrated [5].
 - 6.1.1. Talent pouring the flow through from the reservoir into a waste container.
 - 6.1.2. Talent reassembling the rack with the binding columns.

- 6.1.3. Talent adding wash buffer to each binding column.
- 6.1.4. Talent placing the rack in the centrifuge.
- 6.1.5. Talent discarding the flow through from the reservoir.

- 6.2. Remove the binding columns from the rack [1] and transfer them to 2-milliliter collection tubes [2]. Centrifuge at 10,000 g or higher for 1 minute to dry the membrane [3]. Transfer the columns to clean 1.5-milliliter tubes [4].
 - 6.2.1. Talent removing the binding columns from the rack
 - 6.2.2. Talent placing the binding columns into 2-milliliter collection tubes.
 - 6.2.3. Talent placing the collection tubes in the centrifuge.
 - 6.2.4. Talent transferring the dried columns into clean 1.5 milliliter tubes.

- 6.3. Add 50 microliters of elution buffer or water directly to the membrane and incubate the columns for 1 minute at room temperature [1]. Centrifuge at 10,000 g or higher for 1 minute to elute the DNA [2].
 - 6.3.1. Talent adding elution buffer or water directly onto the membrane of each column and keeping it aside for incubation.
 - 6.3.2. Talent placing the columns into the centrifuge.

Results

7. Results

- 7.1. Total plasmid yield showed no significant difference between the conventional method and extraction using the rack configurations [1].
 - 7.1.1. LAB MEDIA: Figure 5. *Video editor: Emphasize the three bars labeled “Manual Extraction,” “Rack extraction,” and “Titanium dioxide”.*
- 7.2. Plasmid conformation was preserved, with similar distributions of relaxed, linearized, and supercoiled forms [1].
 - 7.2.1. LAB MEDIA: Figure 6. *Video editor: Highlight the band patterns under Standard Manual Extraction (column 2,3,4) and Rack Extraction (column 6,7,8).*
- 7.3. Dye distribution was confined to the waste area directly beneath the loaded columns [1], with no visible signal detected in adjacent wells or on neighboring column tips, indicating that the rack design reduces the likelihood of splashing and cross-column transfer [2].
 - 7.3.1. LAB MEDIA: Figure 7. *Video editor: Circle the wells with strong red dye.*
 - 7.3.2. LAB MEDIA: Figure 7. *Video editor: Circle the wells with a small center tip and have no red staining.*
- 7.4. Amplification was detected only in eluates from columns to which the amplicon had been added [1].
 - 7.4.1. LAB MEDIA: Figure 8. *Video editor: Highlight all the red curves that rise upward from cycle 20 and reach approximately 600 on the Y-axis by cycle 50*

Pronunciation guides:

1. Polylactide
 Pronunciation link: <https://www.merriam-webster.com/dictionary/polylactide>
 IPA: /ˌpɑːliˈlæk.təɪd/
 Phonetic Spelling: pah·lee·lak·tyde
2. Polyethylene
 Pronunciation link: <https://www.merriam-webster.com/dictionary/polyethylene>
 IPA: /ˌpɑːliˈeθ.əˌliːn/
 Phonetic Spelling: pah·lee·eth·uh·leen
3. Terephthalate
 Pronunciation link: <https://www.merriam-webster.com/dictionary/terephthalate>
 IPA: /təˈref.θəˌleɪt/
 Phonetic Spelling: tuh·ref·thuh·layt

4. Glycol
Pronunciation link: <https://www.merriam-webster.com/dictionary/glycol>
IPA: /ˈɡlaɪ,kɔ:l/
Phonetic Spelling: gly·kawl
5. Acrylonitrile
Pronunciation link: <https://www.merriam-webster.com/dictionary/acrylonitrile>
IPA: /ə,kɹɪl.əsˈnaɪ.trəl/
Phonetic Spelling: uh·kril·oh·nye·truhl
6. Butadiene
Pronunciation link: <https://www.merriam-webster.com/dictionary/butadiene>
IPA: /ˌbjuː.təˈdaɪ.i:n/
Phonetic Spelling: byoo·tuh·dye·een
7. Styrene
Pronunciation link: <https://www.merriam-webster.com/dictionary/styrene>
IPA: /ˈstaɪ,ri:n/
Phonetic Spelling: sty·reen
8. Filament
Pronunciation link: <https://www.merriam-webster.com/dictionary/filament>
IPA: /ˈfɪl.ə.mənt/
Phonetic Spelling: fil·uh·muhnt
9. Watertightness
Pronunciation link: <https://www.merriam-webster.com/dictionary/watertightness>
IPA: /ˈwɔː.təˌtaɪt.nəs/
Phonetic Spelling: waw·ter·tyte·nuhs
10. Ethanol
Pronunciation link: <https://www.merriam-webster.com/dictionary/ethanol>
IPA: /ˈeθ.əˌnɔ:l/
Phonetic Spelling: eth·uh·nawl
11. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /ˈsen.trəˌfjuːdʒ/
Phonetic Spelling: sen·truh·fyooj
12. Reservoir
Pronunciation link: <https://www.merriam-webster.com/dictionary/reservoir>
IPA: /ˈrez.əˌvwa:r/
Phonetic Spelling: rez·er·vwar
13. Escherichia
Pronunciation link: <https://www.merriam-webster.com/dictionary/Escherichia>
IPA: /ˌeʃ.əˈrɪk.i.ə/
Phonetic Spelling: esh·uh·rik·ee·uh
14. coli
Pronunciation link: <https://www.merriam-webster.com/dictionary/coli>
IPA: /ˈkoʊ.laɪ/
Phonetic Spelling: koh·lye
15. Transformants
Pronunciation link: <https://www.merriam-webster.com/dictionary/transformant>

IPA: /træns'fɔːr.mənts/

Phonetic Spelling: trans·for·muhnts

16. Lysogeny

Pronunciation link: <https://www.merriam-webster.com/dictionary/lysogeny>

IPA: /laɪ'sɑː.dʒə.ni/

Phonetic Spelling: lye·sah·juh·nee

17. Antibiotics

Pronunciation link: <https://www.merriam-webster.com/dictionary/antibiotic>

IPA: /,æn.taɪ.baɪ'ɑː.tɪk/

Phonetic Spelling: an·tye·bye·ah·tik

18. Supernatant

Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>

IPA: /,suː.pə'nei.tənt/

Phonetic Spelling: soo·per·nay·tuhnt

19. Resuspension

Pronunciation link: <https://www.merriam-webster.com/dictionary/resuspension>

IPA: /,riː.sə'spen.ʃən/

Phonetic Spelling: ree·suh·spen·shuhn

20. Vortexing

Pronunciation link: <https://www.merriam-webster.com/dictionary/vortex>

IPA: /'vɔːr.teks/

Phonetic Spelling: vor·teks·ing

21. Lysis

Pronunciation link: <https://www.merriam-webster.com/dictionary/lysis>

IPA: /'laɪ.sɪs/

Phonetic Spelling: lye·suhs

22. Neutralization

Pronunciation link: <https://www.merriam-webster.com/dictionary/neutralization>

IPA: /,nuː.trə.lə'zeɪ.ʃən/

Phonetic Spelling: noo·truh·luh·zay·shuhn

23. Lysate

Pronunciation link: <https://www.merriam-webster.com/dictionary/lysate>

IPA: /'laɪ.sert/

Phonetic Spelling: lye·sayt

24. Precipitate

Pronunciation link: <https://www.merriam-webster.com/dictionary/precipitate>

IPA: /prɪ'sɪp.ə.tət/ (noun), /prɪ'sɪp.ə.tet/ (verb)

Phonetic Spelling: prih·sip·uh·tuht; prih·sip·uh·tayt

25. Titanium

Pronunciation link: <https://www.merriam-webster.com/dictionary/titanium>

IPA: /taɪ'teɪ.ni.əm/

Phonetic Spelling: tye·tay·nee·uhm

26. Dioxide

Pronunciation link: <https://www.merriam-webster.com/dictionary/dioxide>

IPA: /daɪ'ɑːk.səɪd/

Phonetic Spelling: dye·ok·side

27. Co-precipitation
Pronunciation link: <https://www.merriam-webster.com/dictionary/coprecipitation>
IPA: /ˌkoʊ.prɪ.sɪp.əˈteɪ.ʃən/
Phonetic Spelling: koh-prih-sip-uh-tay-shuhn
28. Clarification
Pronunciation link: <https://www.merriam-webster.com/dictionary/clarification>
IPA: /ˌkler.ə.fəˈkeɪ.ʃən/
Phonetic Spelling: clair-uh-fuh-kay-shuhn
29. Deoxyribonucleic
Pronunciation link: <https://www.merriam-webster.com/dictionary/deoxyribonucleic%20acid>
IPA: /dɪ.ɑːk.si.raɪ.boʊ.nuːˈkliː.ɪk/
Phonetic Spelling: dee-ok-see-rye-boh-noo-klee-ik
30. Centrifugation
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifugation>
IPA: /ˌsen.trəˈfjuːˈgeɪ.ʃən/
Phonetic Spelling: sen-truh-fyoo-gay-shuhn
31. Rotor
Pronunciation link: <https://www.merriam-webster.com/dictionary/rotor>
IPA: /ˈroʊ.tər/
Phonetic Spelling: roh-ter
32. Elution
Pronunciation link: <https://www.merriam-webster.com/dictionary/elution>
IPA: /iˈluː.ʃən/
Phonetic Spelling: ee-loo-shuhn
33. Membrane
Pronunciation link: <https://www.merriam-webster.com/dictionary/membrane>
IPA: /ˈmem.breɪn/
Phonetic Spelling: mem-brayn
34. Plasmid
Pronunciation link: <https://www.merriam-webster.com/dictionary/plasmid>
IPA: /ˈplæz.mɪd/
Phonetic Spelling: plaz-mid
35. Conformation
Pronunciation link: <https://www.merriam-webster.com/dictionary/conformation>
IPA: /ˌkɑːn.fəˈmeɪ.ʃən/
Phonetic Spelling: kon-fer-may-shuhn
36. Linearized
Pronunciation link: <https://www.merriam-webster.com/dictionary/linearize>
IPA: /ˈlɪn.i.ə.raɪzd/
Phonetic Spelling: lin-ee-uh-ryezd
37. Supercoiled
Pronunciation link: <https://www.merriam-webster.com/dictionary/supercoil>
IPA: /ˌsuː.pəˈkɔɪld/
Phonetic Spelling: soo-per-koyld
38. Amplicon
Pronunciation link: <https://www.merriam-webster.com/dictionary/amplicon>

IPA: /'æm.plɪ,kɑ:n/

Phonetic Spelling: am·pli·kon

39. Eluates

Pronunciation link: <https://www.merriam-webster.com/dictionary/eluate>

IPA: /'el.ju:.et/

Phonetic Spelling: el·yoo·ayt