nature portfolio

https://doi.org/10.1038/s41596-023-00819-6

Supplementary information

Temporally resolved transcriptional recording in E. coli DNA using a Retro-Cascorder

In the format provided by the authors and unedited

Supplementary Information

Temporally resolved transcriptional recordings into *E. coli* DNA using a Retro-Cascorder

Authors: Sierra K. Lear[1,2,#], Santiago C. Lopez[1,2,#], Alejandro González-Delgado[1], Santi Bhattarai-Kline[1,3], Seth L. Shipman[1,4,5]

[1]Gladstone Institute of Data Science and Biotechnology, San Francisco, CA, USA

[2]Graduate Program in Bioengineering, University of California, San Francisco and Berkeley, CA, USA

[3]UCLA-Caltech Medical Scientist Training Program, David Geffen School of Medicine, University of California, Los Angeles, CA, USA

[4]Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, CA, USA

[5]Chan Zuckerberg Biohub, San Francisco, CA

[#]These authors contributed equally

Correspondence to: seth.shipman@gladstone.ucsf.edu

This PDF file includes:

Supplementary Methods
Supplemental Materials

Reagents
Sera-Mag bead preparation and testing

- Sera-Mag™ Magnetic SpeedBeads, carboxylated, 1 um, 3 EDAC/PA5 (GE Healthcare Life Sciences #65152105050250) !CAUTION contains 0.05% sodium azide, which is toxic; avoid contact with skin or eyes.
- HCl solution,1.0 N (Sigma-Aldrich, cat. no. H9892-100ML) !CAUTION HCl is corrosive and an irritant; avoid contact with skin and eyes. When handling HCl, wear gloves and eye protection.
- Tris base (1M, add 6.057 g in 50 mL UltraPure™ Distilled Water, sterile-filtered; Thermo Fisher Scientific, cat. no. BP152-500)
- NaCl (5M, add 14.610 g in 50 mL UltraPure™ Distilled Water; Thermo Fisher Scientific, cat. no. S271-3)
- Disodium-EDTA (0.1M, add 1.816 g in 50 mL UltraPure™ Distilled Water; J.T. Baker, cat. no. 6381-92-6) !CAUTION Disodium-EDTA is toxic if swallowed and is an irritant to skin and eyes. When handling disodium-EDTA, wear gloves and eye protection.
- Tween 20, non-ionic, aqueous solution, 10% (w/v) (Sigma-Aldrich, cat. no. 11332465001)
- PEG 8000 (Sigma-Aldrich, cat. no. 89510-1KG-F)

Equipment

- LP Vortex Mixer Thermo Fisher Scientific, cat. no. 88880017)
- Magnetic rack (MagRack 6) for 1.5 mL microcentrifuge tubes bead magnet (GE Healthcare Life Sciences, cat. no. 26980)

Reagent Setup

50%(w/v) PEG 8000
Add 12.5 g PEG 8000 in 14 mL UltraPure™ Distilled Water. Shake to dissolve and let incubate on benchtop at room temperature for at least 1 hour until all bubbles dissipate. Add distilled water until solution reaches a total volume of 25 mL. Mix well. Store at 4°C for up to 1 year.

Tween-TE DNA Binding Buffer
Mix 48.564 mL UltraPure™ Distilled Water, 0.5 mL 1M Tris base, 0.5 mL 0.1M disodium-EDTA, 0.25 mL 10% (v/v) Tween 20, and 0.186 mL 1N HCl. Make fresh, right before use.

Nucleic acid incomplete binding buffer
Mix 25 mL 5M NaCl, 3.582 mL UltraPure™ Distilled Water, 0.5 mL 1M Tris base, 0.5 mL 0.1M Disodium-EDTA, and 0.168 mL 1N HCl.
CRITICAL Prepare nucleic acid incomplete binding buffer fresh during bead washing steps (see Supplementary Method step 8).

Supplemental Protocol

Preparation of CRISPR arrays for deep sequencing: Preparation and cleaning of Sera-Mag beads for DNA clean-up

● Timing 2 h, 1.5 h hands-on

CRITICAL This supplementary protocol prepares Sera-Mag beads for DNA clean-up, which will be used in steps 41-48 of the main protocol. To save time, users should also consider purchasing AMPure XP beads instead, which can be immediately used without any additional preparation or clean-up. AMPure XP beads, however, are very expensive, so we offer this alternative protocol to reduce the cost of performing the protocol.

1. *Clean Sera-Mag beads.* This step cleans Sera-Mag beads and stores them in a nucleic acid binding buffer used during PCR clean-up. Once an aliquot of beads has been cleaned and moved into binding buffer, it can be reused for several months if properly stored. Invert the bottle containing Sera-Mag beads and pipette the solution up and down several times to resuspend beads well.
2. Transfer 1 mL of resuspended beads to a 1.5 mL microcentrifuge tube.
3. Place the microcentrifuge tube on magnet until beads migrate near the magnet and the solution is clear (~30 s).
4. Remove the supernatant.
5. Add 1 mL of DNA buffer (see TE-Tween DNA Buffer in Reagent Setup) to the bead pellet and close the microcentrifuge tube.
6. Remove the microcentrifuge tube from the magnet and resuspend beads by vortexing for at least 15 s. Following mixing, the solution should appear cloudy and homogenous. Spin down the liquid using a microcentrifuge.
7. Repeat Supplementary Method steps 3-6 twice more for a total of 3 washes.
8. Prepare 29.75 mL of freshly made nucleic acid incomplete binding buffer (see Reagent Setup) in a 50 mL conical tube.
9. Remove supernatant from beads and immediately add 1 mL of incomplete binding buffer into the microcentrifuge tube while still on the magnet.
10. Remove the microcentrifuge tube from the magnet and resuspend beads by vortexing for at least 15 s. If liquid is stuck onto the sides, briefly spin down the microcentrifuge tube in a microcentrifuge but be careful not to also pellet the beads.
11. Transfer the 1 mL of beads in the incomplete binding buffer to the conical tube containing the rest of the incomplete binding buffer. Cap the tube and vortex for at least 30 s until beads are well mixed into the entire buffer.
12. Using a 25 mL serological pipette, add 20 mL of 50% (w/v) PEG stock (see Reagent setup) to the conical tube. Dispense slowly to allow the viscous liquid to slide down the inside walls of the pipette to ensure an accurate volume of 50% PEG is added.
13. Add 0.25 mL 10% (w/v) Tween 20.
14. Cap the tube and mix solution through inversion gently until the color of the solution appears homogenous. This solution contains prepared and cleaned beads in complete binding solution that can be immediately used for DNA clean-up. To distinguish between Sera-Mag beads that still need to be prepared and those in complete binding solution that are ready to be used for DNA clean-up, these prepared beads will be referred to as SPRI beads.

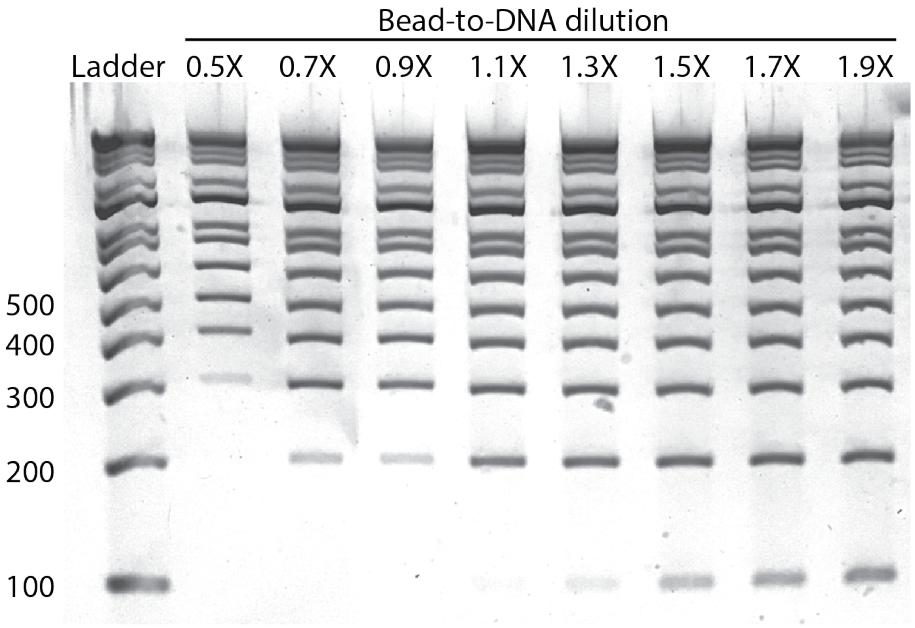
PAUSE POINT SPRI beads can be stored at 4°C for at least 1 year.
CRITICAL STEP Prior to each use, ensure solution has come to room temperature and is thoroughly mixed through inversion as beads will pellet at the bottom of the tube over time.

15. *Testing SPRI bead ratio for DNA clean-up.* This step determine what ratio of beads to DNA should be used to clean-up DNA without selecting for size. Mix 16 µL 1 kB+ DNA ladder with 144 µL water in a microcentrifuge tube. Pipette 20 µL of diluted ladder each into eight clean, new microcentrifuge tubes
16. Create dilution series of 8 different bead-to-ladder ratios in the microcentrifuge tubes each containing 20 µL diluted ladder as follows:

Microcentrifuge tube	Amount of beads per tube from step 14 of Supp. Method (µL)	Final bead-to-DNA dilution
1	10	0.5X
2	14	0.7X
3	18	0.9X
4	22	1.1X
5	26	1.3X
6	30	1.5X
7	34	1.7X
8	38	1.9X

17. Incubate the dilutions for 5 min at room temperature. Place all tubes on a magnet until the beads migrate and the solution clears (~1 min). Remove supernatant.
18. Add 200 µL fresh 70% ethanol to each tube and let incubate on the magnet until solution clears (~1 min). Remove supernatant.
19. Repeat Supplemental Method step 18 for an additional wash.
20. Let beads dry for <3 minutes.
    CRITICAL STEP DNA needs to dry to remove contaminant ethanol but allowing the beads to dry for too long will result in low recovery. The presence of cracks appearing in the beads is a sign that the DNA has been allowed to dry for too long.
21. Remove the plate from the magnet and resuspend the DNA with 25 µL water. Pipette up and down between 10-15 times to mix. Incubate at 5 minutes at room temperature.
22. Place the tubes back onto the magnet until the solution clears. Collect supernatant and move into new, fresh microcentrifuge tube.
23. Run the dilution series on a 2% agarose E-gel EX by adding 20 µL of each dilution per well. Also include the 1 kB+ ladder in the gel by adding 2 µL undiluted ladder and 18 µL water to the marker lane. Run gel for 10 minutes.
24. Determine the ideal ratio to use by selecting the smallest ratio that still retains most of the smaller size DNA band. An example gel of different dilutions ratios run along with a ladder is shown below (Supplemental Figure 1). Based on this gel, a bead-to-DNA ratio of 1.5X would be selected because it is the smallest ratio that nonetheless retains the 100-nucleotide long DNA band. Once a ratio has been determined for a given aliquot of beads, there is typically no need for re-testing.

CRITICAL STEP A typical bead-to-DNA ratio in our hands is between 1.3X – 2X.
? TROUBLESHOOTING



Supplementary Figure 1. Representative example of gel used to test bead-to-DNA ratios.