# Magnetic Trap Construction

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Protocol

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In recent years, techniques have been developed to study and manipulate single molecules of DNA and other biopolymers. In one such technique, the magnetic trap, a single DNA molecule is bound at one end to a glass surface and at the other to a magnetic microbead. Small magnets, whose position and rotation can be controlled, pull on and rotate the microbead. This provides a simple method to stretch and twist the molecule. The system allows one to apply and measure forces ranging from $10^{-3}$ to >100 picoNewtons (pN). In contrast to other techniques, the force measurement is absolute and does not require calibration of the sensor. This protocol describes a procedure for building and using a magnetic trap. It gives a method for constructing a microchamber suitable for magnetic tweezers studies, including antibody coating and passivation. It also describes a series of simple steps to achieve end-labeling of DNA anchoring fragments. One anchoring fragment is biotin-labeled and the other is labeled with digoxigenin. The anchoring fragments are then digested and ligated to a central DNA region containing the sequence of interest. The biotinylated DNA is adsorbed onto streptavidin-coated magnetic beads, and the DNA–bead mixture attaches specifically to the antidigoxigenin-coated surface of the microchamber.



## MATERIALS

**It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.**

**RECIPE: Please see the end of this article for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.**

### Reagents

Antidigoxigenin, polyclonal (100 µg/mL in phosphate-buffered saline; Roche)

Biotin-16-dUTP (1 mM; Roche)

Digoxigenin-11-dUTP (1 mM)

dNTPs (10 mM)

Ligase buffer (10×)

$Mg^{2+}$ (25 mM)

NaOH (5 M)

NEBuffer 4 (New England Biolabs)

Passivation buffer <R>

*Different protein systems may need different passivation buffers. The optimal passivation buffer for a particular application is best determined experimentally.*

Adapted from *Single-Molecule Techniques* (ed. Selvin and Ha). CSHL Press, Cold Spring Harbor, NY, USA, 2008.


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pBluescriptKS (250 ng/µL)

Phosphate-buffered saline (PBS)

Primer A (CTAAATTGTAAGCGTTAATATTTTGTTAAA) (100 µM)

Primer B (TATCTTTATAGTCCTGTCGGGTTTCGCCAC) (100 µM)

pSB 110 (100 ng/µL)

*This plasmid has restriction enzyme sites for XhoI, AatII, and Eco109I (gift of S. Bigot).*

Restriction enzymes: XhoI, AatII, Eco109I

Sigmacote (Sigma-Aldrich)

T4 DNA ligase (Fermentas)

*Taq* (New England Biolabs)

*Taq* buffer without $Mg^{2+}$ (10×)

Water, double-distilled ($ddH_2O$)

## Equipment

Beads, magnetic, streptavidin-coated, 1-µm diameter (Dynabeads; MyOne; Invitrogen)

Beads, polystyrene, nonmagnetic, 2-µm diameter (Polysciences) (optional; see Step 33)

*These beads are used as reporters of the microscope mechanical drift motion; see* ***Single-Molecule Studies Using Magnetic Traps*** (Lionnet et al. 2012).

Connectors, polypropylene (Fisher Bioblock or similar)

Coverslips, glass (24 × 60 mm; Menzel-Glaser)

Hot plate

Magnets (NdFeB cubic, 5 × 5 × 6 mm; VacuumSchmelze)

Microcentrifuge tubes

Microcon (YM-100 column; Millipore)

Microscope, inverted

Mylar film (50 µm thick)

PCR thermal cycler

Petri dish

Rotator

Syringe

Tape, double-sided (Duplocoll 375; Lohmann-Durabloc)

Tape, double-sided (60 µm thick; Duplocoll 3205; Lohmann-Durabloc)

Translation stage motor (PI C-150.PD; Polytec)

Translation stage, motorized (PI M-126.PD; Polytec)

Tygon tubing

## METHODS

### Digestion of the Central DNA Fragment

1. Combine the following ingredients:

   10 µL pSB110
   1 µL XhoI
   1 µL AatII

1 µL Eco109I
3 µL NEBuffer 4
36.5 µL $ddH_2O$

2. Incubate for 1 h at 37°C.
3. Incubate for 20 min at 65°C.

## Labeling of DNA Anchoring Fragments

*Perform labeling with digoxigenin and biotin separately, in parallel.*

4. Combine the following ingredients:

1 µL pBluescriptKS
1 µL Primer A
1 µL Primer B
1.5 µL dNTPs
2 µL $Mg^{2+}$
5 µL *Taq* buffer w/o $Mg^{2+}$
1 µL *Taq*
1.5 µL labeled dUTP (digoxigenin or biotin)
36.5 µL $ddH_2O$

5. Perform PCR as follows:

i. An initial denaturation step for 5 min at 94°C.
ii. Thirty cycles for 30 sec at 94°C, for 1 min at 54°C, and for 1 min at 72°C.
iii. A final incubation for 5 min at 72°C.

6. Purify the PCR products with a Microcon YM-100 column according to the manufacturer's protocol.

## Digestion of Dig-Labeled Fragments

7. Combine the following ingredients:

10 µL Dig-labeled fragment (~1 kb at 50 ng/µL) (from Step 6)
2 µL XhoI
4 µL NEBuffer 4
4 µL $ddH_2O$

8. Incubate for 1 h at 37°C.
9. Incubate for 20 min at 65°C.

## Digestion of Biotin-Labeled Fragments

10. Combine the following ingredients:

10 µL biotin-labeled fragment (~1 kb at 50 ng/µL) (from Step 6)
2 µL AatII
4 µL NEBuffer 4
4 µL $ddH_2O$

11. Incubate for 1 h at 37°C.
12. Incubate for 20 min at 65°C.

## Ligation of Anchoring Fragments to Central DNA Fragment

13. Combine the following ingredients:

2 µL digested central fragment (~30 ng/µL) (from Step 3)
15 µL digested dig fragment (~20 ng/µL) (from Step 9)

15 µL digested biotin fragment (~20 ng/µL) (from Step 12)
10 µL 10× ligase buffer
4 µL T4 DNA ligase
52 µL $ddH_20$

14. Incubate for 2 h at 16°C.
15. Incubate for 20 min at 65°C.

## Treatment of Glass Coverslips

16. Rinse coverslips with 5 M NaOH. Let sit for ~1 min.
17. Rinse with $ddH_2O$.
18. Incubate on hot plate for 5 min at 125°C.
19. Add Sigmacote and incubate for 1–2 min.
20. Blow dry.
21. Incubate on hot plate for 30 min at 125°C.
22. Rinse with $ddH_2O$.

## Construction of Microchamber

*The dimensions of the resulting channel are 50 mm × 5 mm × 60 µm, giving a volume of 15 µL. See Figure 1B and* ***Single-Molecule Studies Using Magnetic Traps*** (Lionnet et al. 2012) for details.

23. Cut channel (50 × 5 mm) in 60-µm-thick double-sided tape.
24. Drill two holes in the 50-µm-thick Mylar sheet.

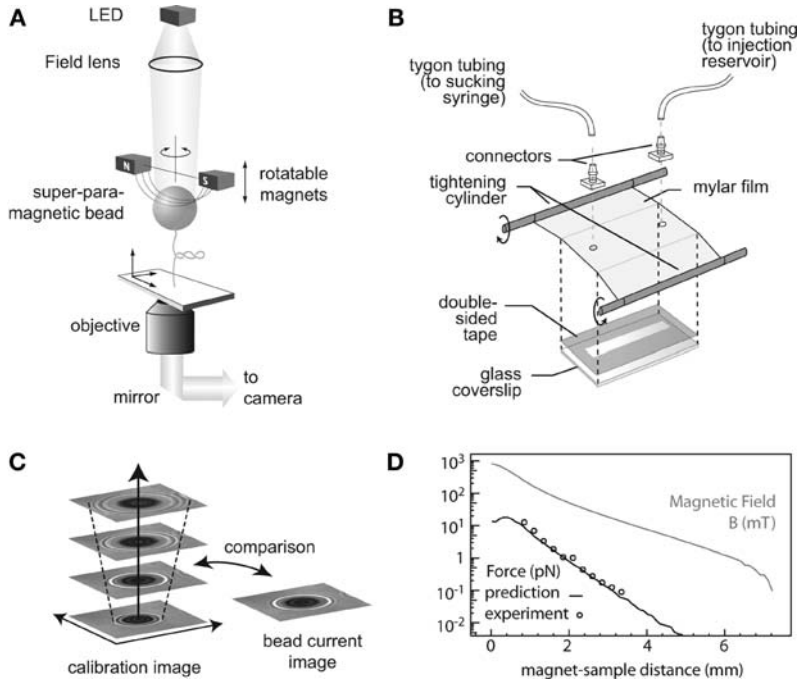


**FIGURE 1.** Experimental setup. (*A*) Schematic view of the setup (not to scale). (*B*) Design of the experimental microchamber. (*C*) Principle of the bead *z* position measurement. At each time, the current bead image is compared with the calibration image (see text). (*D*) Experimental measurement of the magnetic field (gray line) created by the magnet setup described in the text. Prediction of the resulting force generated on 1-µm-diameter beads (MyOne, Dynabeads) using manufacturer's magnetization data (black circles). Force actually measured using the method described in the text on a similar bead (black line).





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25. Sandwich double-sided tape between glass coverslip and Mylar film.
26. Attach two polypropylene connectors to the Mylar using double-sided tape (Duplocoll 375).
27. Attach two Tygon tubes to connectors.
28. Attach one Tygon tube to syringe. (This is used to withdraw liquid from the chamber.)
29. Attach one Tygon tube to an open reservoir. (This is used to allow the injection of solutions into the chamber.)
30. Stretch Mylar film between two metal cylinders fixed to a translation stage. (This allows (*x*, *y*) positioning.)

## Antibody Treatment and Passivation of Microchamber

31. Add 50 µL of antidig to the microchamber.
32. Incubate in a humidified chamber (e.g., Petri dish with wet tissue) overnight at 37°C.
33. To bind nonmagnetic beads to the surface, proceed to Step 34. Otherwise, proceed to Step 40.

## Fixation of Nonmagnetic Beads

*Depending on the antibody/passivation combination used, a different protocol for binding nonmagnetic beads to the surface might be needed. In some cases, nonspecific binding of magnetic beads to the surface might be sufficient to provide the desired amount of fixed beads.*

34. Rinse chamber with $ddH_2O$.
35. Inject 10 µL of undiluted polystyrene bead solution into the reservoir connected to the chamber.
36. Use the syringe to draw the bead solution into the microchamber. Keep the reservoir full by adding the desired buffer.
37. When the beads are distributed evenly along the length of the chamber, stop the flow.
38. Allow the polystyrene beads to sediment. Incubate for ~1 min. (The exact incubation time may vary from one chamber to another.)
39. When the desired amount of beads is bound to the surface, aspirate excess buffer with the syringe.
40. Rinse with passivation buffer. (This also rinses away unbound nonmagnetic beads.)
41. Incubate with passivation buffer in humidified chamber for 2–4 h.

    *Microchambers can be stored at 4°C until use.*

## Anchoring of DNA to Magnetic Beads

42. Wash 10 µL of magnetic beads (10 mg/mL) in 200 µL of passivation buffer.
43. Resuspend beads in 10 µL of passivation buffer.
44. Deposit a 1-µL drop of DNA construct at the bottom of a microcentrifuge tube.
45. Deposit the 10 µL of beads onto the drop of DNA. Flick tube to mix.
46. Place on rotator for 5 min to keep beads from sedimenting.
47. Dilute the reaction by gently depositing 40 µL of passivation buffer onto the DNA-bead solution.
48. Resuspend the beads to homogeneity by tipping the tube upside-down (without causing the liquid to drop) or spinning the tube between thumb and forefinger.

    *Bead–DNA complexes can be stored a few days before use. Store on a rotator to keep beads from sedimenting.*



### Anchoring the DNA–Bead Mixture in the Microchamber

49. Place the magnets as far from the flow cell as possible (~25 mm).
50. Inject 5 µL of the bead–DNA mixture (from Step 48) into the reservoir connected to the chamber.
51. Use the syringe to draw the bead–DNA solution into the microchamber. Keep the reservoir full by adding the desired buffer.
52. When the beads are distributed evenly along the length of the chamber, stop the flow.
53. Allow magnetic beads to sediment. Incubate for 5 min.
54. Rinse with desired buffer.

*At this point, the samples are ready for observation and experimental manipulation with the inverted microscope/magnetic tweezers assembly.*

*See Troubleshooting.*

## TROUBLESHOOTING

***Problem (Step 54):*** DNA–magnetic bead complexes interact nonspecifically with the surface of the microchamber.

***Solution:*** Add small amounts (e.g., ~0.1% w/v) of bovine serum albumin (BSA) and Pluronic F127 in the desired buffer when anchoring the DNA-coated beads to the microchamber. For background information on magnetic traps, see **Single-Molecule Studies Using Magnetic Traps** (Lionnet et al. 2012).

## RELATED INFORMATION

The magnetic trap setup described for this protocol (microscope, magnets, translation stage, etc.) is similar to a commercially available version (PicoTwist; see www.picotwist.com). For another example of a specific protocol, see Revyakin et al. (2003).



## RECIPE

### *Passivation Buffer*

10 mg/mL bovine serum albumin (BSA; Promega)
1 mM EDTA
10 mM phosphate buffer (pH 8.0)
10 mg/mL Pluronic F127 surfactant (Sigma-Aldrich)
10 mM sodium azide

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## REFERENCES

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