Protocol

Efficient Genomic DNA Extraction from Low Target Concentration Bacterial Cultures Using SCODA DNA Extraction Technology

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INTRODUCTION

Methods for the extraction of nucleic acids are straightforward in instances where there is ample nucleic acid mass in the sample and contamination is minimal. However, applications in areas such as metagenomics, life science research, clinical research, and forensics, that are limited by smaller amounts of starting materials or more dilute samples, require sample preparation methods that are more efficient at extracting nucleic acids. Synchronous coefficient of drag alteration (SCODA) is a novel electrophoretic nucleic acid purification technology that has been tested successfully with both highly contaminated and dilute samples and is a promising candidate for new sample preparation challenges. In this article, as an example of SCODA's performance with limited sample material, we outline a genomic DNA (gDNA) extraction protocol from low abundance cultures of *Escherichia coli* DH10B. This method is equally well suited to high biomass samples.

RELATED INFORMATION

This protocol is based on methods described in Broemeling et al. (2008), Marziali et al. (2005), and Pel et al. (2009).

MATERIALS

CAUTIONS AND RECIPES: Please see Appendices for appropriate handling of materials marked with <**!**>, and recipes for reagents marked with <**R**>.

Reagents

Agarose, SeaKem LE (Lonza)

E. coli DH10B cultures, streaked

<**R**>LB (Luria-Bertani) liquid medium

This medium is available commercially.

<**R**>SCODA bacterial lysis (BL) buffer (Krsek and Wellington 1999)

<**!**>Lysozyme (50 mg/mL in nuclease-free H_2O)

<**!**>Proteinase K (20 mg/mL in nuclease-free H_2O)

<**!**>RNase A (100 mg/mL in nuclease-free H_2O)

<**R**>TBE buffer

Dilute the TBE to 0.25X and 0.05X working solutions.

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Cite as: Cold Spring Harb Protoc; 2010; doi:10.1101/pdb.prot5506

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Equipment

Culture tubes
Ice
Incubator, shaking, preset to 37°C
Microcentrifuge
Micropipettors and tips
SCODA 1.4 Development System (Boreal Genomics)
Spectrophotometer
Syringe with 21-gauge needle
Tubes, microcentrifuge, 1.5-mL
Water bath or heating block preset to 37°C

METHOD

Lysis/Pretreatment

1. Prepare an overnight culture of *E. coli* DH10B:
   i. Inoculate 2 mL of LB liquid medium with a single colony of *E. coli* DH10B.
   ii. Incubate with agitation at 225 rpm for 16 h at 37°C.
   The final OD_{600} should be ~2.5-3.0.
   iii. Place the culture on ice.
2. Prepare a 1:1000 dilution of the overnight culture in LB liquid medium by performing three 1:10 serial dilutions.
3. Aliquot 0.4 mL of the 1:1000 diluted culture into a 1.5-mL microcentrifuge tube.
   The 1:1000 diluted culture is used as an equivalent to 400 nL of culture.
4. Centrifuge the bacteria at 5000*g* for 5 min. Carefully remove and discard the supernatant.
5. Resuspend the pellet in 0.4 mL of SCODA BL buffer. Add 4 µL of RNase A, 4 µL of lysozyme, and 4 µL of Proteinase K.
   The lysozyme may precipitate upon addition but will clear during incubation.
6. Incubate the suspension for 20 min at 37°C.
   The solution will be highly viscous at this point.
7. Gently solubilize and shear the DNA by passing the lysate 10 times through a 21-gauge needle attached to a syringe.
   This step is required to shear any megabase-sized DNA fragments that would be slow to focus in SCODA, as well as to break up any remaining large DNA/protein complexes.
8. Rinse the syringe barrel and needle twice with 300 µL of 0.05X TBE. Combine the solution from the rinses with the sheared lysate for a total volume of ~1 mL.
9. Dilute the sample to 5 mL with H_2O.

SCODA

Perform Steps 10-16 with the SCODA 1.4 Development System, in conjunction with the manufacturer's instructions.

10. Prepare a 4-mm thick SCODA gel of 1% SeaKem agarose in 0.25X TBE and a 50-µL diffusive extraction well.
11. Set up the SCODA 1.4 System as described by the manufacturer.
12. Load the 5-mL sample from Step 9 into the SCODA sample chamber, and fill the remaining buffer reservoirs with 0.25X TBE buffer.

13. Set up and run the SCODA Injection program block at 90 V for 30 min.

    See Troubleshooting.

14. After injection, remove the sample and barrier gel from the sample chamber and replace it with 5 mL of 0.25X TBE.

15. Set up and run the Concentration/Wash and Final Concentration program blocks on the SCODA 1.4 Development System with the following parameters:

    i. Concentration/Wash:

SCODA Field	50 V/cm
SCODA Cycle Period	4 sec
Duration	2 h
Electrode Bias	A: 5 V, B: 2.5 V, C: 0 V, D: 2.5 V

    ii. Final Concentration:

SCODA Field	50 V/cm
SCODA Cycle Period	4 sec
Duration	2 h

16. After the SCODA concentration blocks have completed, remove the purified DNA in 50µL of buffer from the center of the extraction well.

TROUBLESHOOTING

Problem: If the voltage across the gel during injection is higher than 30 V, the DNA in the sample may not inject efficiently into the SCODA gel, leading to poor recovery.

[Step 13]

Solution: This is an indication that the salinity of the sample is too high relative to the gel. To solve this problem, resuspend the pellet in Step 5 in 0.2 mL of SCODA BL buffer rather than 0.4 mL, which effectively reduces the conductivity of the sample.

DISCUSSION

Pulsed field gel electrophoresis (PFGE) analysis of purified DNA indicates that the SCODA method typically yields DNA between 23 kb and 194 kb in length (see Fig. 1). Comparative DNA yields for this method versus phenol:chloroform extraction and a commercially available kit are summarized in Figure 2.

The significantly higher recovery of this method may be attributed to the fact that SCODA extraction and purification relies on the unique physical properties of the DNA molecules, as opposed to its binding interactions with a solid matrix or precipitation. The low surface area-to-volume ratio of the SCODA system reduces the loss of molecules to irreversible binding and dead volume. In addition, precipitation, which is inefficient at low molecule concentrations and prone to user error, is avoided. It should also be noted that SCODA is fundamentally different from other electrophoretic separation

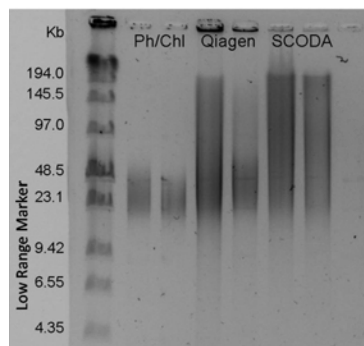

FIGURE 1. PFGE analysis of recovered DNA from phenol:chloroform, QIAGEN, and SCODA methods. QIAGEN (Genomic-tip 20/G kit) and SCODA methods show recovery of DNA between 23 kb and 194 kb, while phenol:chloroform extraction (Ph/Chl) yields DNA ranging between 23 and 48.5 kb in size. To visualize length distributions of recovered DNA, the protocol was performed with 400 µL of undiluted ovenight culture rather than the 400 nl equivalent. (Figure adapted from Pel et al. [2010] and reprinted with permission from Laboratory Focus Magazine © 2010.)

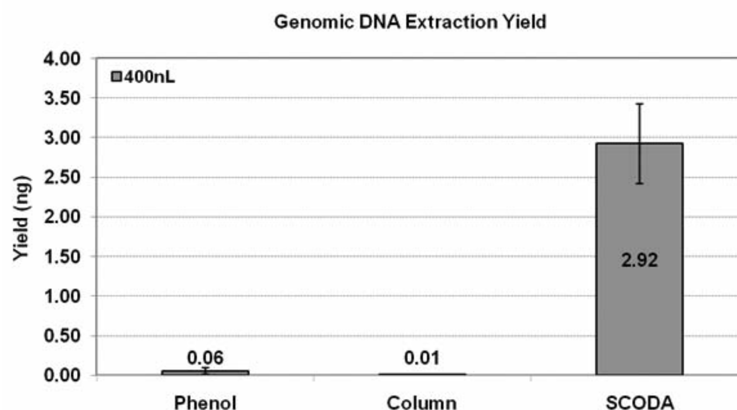

FIGURE 2. DNA yields from phenol:chloroform, QIAGEN, and SCODA methods. Comparison of gDNA yield from 400 nL equivalent of overnight *E. coli* DH10B cultures, prepared with phenol:chloroform, column, and SCODA methods, and assayed with real-time quantitative polymerase chain reaction (qPCR) using the beta-D-glucuronidase gene (*uidA*) (Shaban et al. 2008) as the target. SCODA yields are 50-fold greater than phenol extraction and 300-fold greater than a column-based method (QIAGEN Genomic-tip 20/G kit). Because the low amounts recovered precluded the use of A_{260}/A_{280} ratios as an index of purity, sample purity was inferred by assaying serial dilutions (1:10 and 1:100) of the recovered samples via real-time qPCR, and indicated that no inhibitors were present (data not shown). (Figure adapted from Pel et al. [2010] and reprinted with permission from Laboratory Focus Magazine © 2010.)

and sample preparation techniques (such as PFGE) because SCODA can concentrate all DNA molecules above ~300 bp into a buffer sample while simultaneously removing contaminants. Other electrophoretic mechanisms are unable to concentrate samples, and also do not provide a method for effective contaminant removal (Pel et al. 2009). This protocol highlights SCODA's strengths as a general method for recovering small amounts of DNA from contaminated samples (Broemeling et al. 2008; Pel et al. 2009).

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Appendix 1: Recipes

[NOTE: This print edition of *CSH Protocols* contains only recipes for reagents requiring multiple components or non-obvious critical steps. Recipes for reagents marked with the <**R**> symbol not listed below can be found online at http://www.cshprotocols.org/recipes.]

Agar plates (enriched with fruit juice)

MATERIALS

Reagents

32 g agar
16.6 g sucrose
300 mL fruit juice (apple or grape)
<**!**>Propionic acid
<**!**>Phosphoric acid

Equipment

Beaker
Graduated cylinder
Hot plate
Petri dishes (5 cm)
Pipettes
Scale

METHOD

1. Combine the first three ingredients. Add H_2O to 1 L.
2. Boil the ingredients while stirring on a hot plate at high setting (the solution will foam and rise; it may be necessary to take it off the heat and put it back a couple of times before fully dissolving the agar).
3. Cool to touch.
4. Prepare a mixture of propionic acid:phosphoric acid (9:1). Add 5 mL of this to the cooled mixture and stir.
5. Pour into 5-cm Petri dishes. Keep the lids off the plates for 10 min.
6. Cover and store the plates at 4°C.

AP buffer for *Aedes*

Reagent	Quantity (for 50 mL)	Final concentration
<**R**>$MgCl_2$ (1 M)	250 µL	5 mM
NaCl (1 M)	5 mL	100 mM
<**R**>Tris-Cl (1 M, pH 9.5)	5 mL	100 mM
Tween 20	50 µL	0.1%
H_2O	to 50 mL	

Prepare just before use. Store for no more than 3 h.

ATV solution (0.05%)

Reagent	Amount to add (for 1 L)
NaCl	8 g
<**!**>KCl	0.4 g
$NaHCO_3$	0.58 g
Glucose	1.1 g
<**!**>EDTA	0.2 g
<**!**>Trypsin (2.5%)	20 mL

Dissolve the reagents in 500 mL of H_2O. Adjust to a final volume of 1 L with H_2O. Use HCl to adjust the pH to 7.4. Incubate the solution at 37°C for at least 1 h to activate the trypsin. Sterilize the solution through a 0.22-µm filter, prepare 50-mL aliquots, and store them at –20°C.

Culture medium (AAV)

Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich)
10% fetal bovine serum (research grade; EU approved; e.g., Thermo Scientific HyClone)
50 units/mL penicillin (e.g., Cambrex)
<**!**>50 µg/mL streptomycin (e.g., Cambrex)

DAB + Ni

Reagent	Quantity (for 1 mL)	Final concentration
<**R**>DAB solution (0.3 mg/mL)	1 mL	0.3 mg/`mL
<**!**>$NiCl_2$•$6H_2O$ (8%)	8 µL	0.064%

Make an 8% stock solution of $NiCl_2$ in H_2O; this can be stored indefinitely at room temperature. Prepare DAB + Ni by thawing a 1-mL aliquot of 0.3 mg/mL DAB solution and adding 8 µL of 8% $NiCl_2$ stock solution just before use. Mix by inverting the tube several times. Do not store DAB + Ni because the nickel eventually precipitates as nickel phosphate.

DAB solution

Reagent	Quantity (for 33 mL)	Final concentration
<**R**>PBS(P) (1X)	33 mL	1X
Tween 20	16.5 µL	0.05%
<**!**>DAB (3,3′-Diaminobenzidine) (Sigma-Aldrich D5905)	10 mg	0.3 mg/mL

Add one 10-mg DAB tablet to a 50-mL tube containing 33 mL of PBS(P) and 16.5 µL of Tween 20. Rock gently in the dark for ~30 min. Filter through a 0.22-µm filter to remove particulate matter. Store aliquots at –70°C or in a nondefrosting –20°C freezer. Use aliquots immediately after thawing.

Detergent solution

Reagent	Quantity (for 50 mL)	Final concentration
<**R**>EDTA (0.5 M, pH 8.0)	100 µL	1 mM
NaCl (1 M)	7.5 mL	150 mM
<**!**>SDS (sodium dodecyl sulfate; 10%)	5 mL	1.0%
<**R**>Tris-Cl (0.5 M, pH 7.5)	5 mL	50 mM
Tween 20	250 µL	0.5%
H_2O	to 50 mL	

Store at room temperature.

DFCS culture medium

1X Dulbecco's minimal essential medium
2 mM L-glutamine
100 IU/mL penicillin
<!>100 mg/mL streptomycin
10% fetal calf serum (decomplemented)

EDTA

<!>EDTA (ethylenediamenetetraacetic acid)
<!>NaOH
To prepare EDTA at 0.5 M (pH 8.0): Add 186.1 g of disodium EDTA•$2H_2O$ to 800 mL of H_2O. Stir vigorously on a magnetic stirrer. Adjust the pH to 8.0 with NaOH (~20 g of NaOH pellets). Dispense into aliquots and sterilize by autoclaving. The disodium salt of EDTA will not go into solution until the pH of the solution is adjusted to ~8.0 by the addition of NaOH.

Glycerol solutions (50% and 70%)

Glycerol (ultrapure; Invitrogen)
<R>PBS(P) (1X)
Prepare 50% and 70% glycerol solutions by mixing the appropriate volumes of ultrapure glycerol with 1X PBS(P). Use pH paper to ensure a pH of ~7.4. Acidic glycerol will cause rapid fading of DAB reaction products.

Histone demethylase dialysis buffer

<!>Aprotinin (1 mg/mL)
<!>DTT (dithiothreitol; 1 mM)
Glycerol (10%)
<!>HEPES-KOH (40 mM, pH 7.9)
KCl (50 mM)
<!>Leupeptin (1 mg/mL)
Pepstatin A (1 mg/mL)
<!>PMSF (phenylmethylsulfonyl fluoride; 0.2 mM)
<R>For Type II reactions, add 0.2 mM EDTA.

Histone demethylase reaction buffer (type I)

α-ketoglutarate (1 mM)
Ascorbate (2 mM)
<!>Ammonium ferrous sulfate ($Fe[NH_4]_2[SO_4]_2$; 7-700 µM)
<!>HEPES-KOH (50 mM, pH 8.0)

Histone demethylase reaction buffer (type II)

Glycine (100 mM, pH 8.0)
KCl (50 mM)

Histone storage buffer

Glycerol (10%)
<**!**>HEPES-KOH (10 mM, pH 7.5)
KCl (10 mM)
<**!**>PMSF (phenylmethylsulfonyl fluoride; 0.2 mM)
<**R**>For Type II reactions, add 1 mM EDTA.

HL-3 saline

Reagent	Quantity (for 1 L)	Final concentration
NaCl	4.09 g	70 mM
<**!**>KCl	0.37 g	5 mM
<**!**>$MgCl_2$•$6H_2O$	4.07 g	20 mM
$NaHCO_3$	0.84 g	10 mM
Sucrose	39.4 g	115 mM
Trehalose	1.89 g	5 mM
HEPES	1.19 g	5 mM

Dissolve the reagents in H_2O. Bring the pH to 7.2 and adjust the final volume to 1 L with H_2O.
<**!**>Add $CaCl_2$ from a 1 M stock solution to give the desired concentration of Ca^{2+} (normal saline has 2 mM Ca^{2+}).[a]

[a]For information on Ca^{2+} concentrations, see Stewart et al. 1994 (Stewart BA, Atwood HL, Renger JJ, Wang J, Wu C-F. 1994. *Drosophila* neuromuscular preparations in haemolymph-like physiological salines. *J Comp Physiol (A)* **175:** 179-191); Broadie 2000 (Broadie KS. 2000. Electrophysiological approaches to the neuromusculature. In Drosophila *protocols* [ed. W Sullivan et al.], pp. 273-295. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY).

HMT reaction buffer

<**!**>*S*-Adenosyl-L-[methyl-^{3}H] methionine (0.03 mCi/mL; PerkinElmer)
<**!**>DTT (dithiothreitol; 0.5 mM)
<**R**>EDTA (4 mM)
<**!**>PMSF (phenylmethylsulfonyl fluoride; 1 mM)
<**R**>Tris-Cl (20 mM, pH 8.0)

Hyb

Reagent	Quantity (for 50 mL)	Final concentration
<**!**>Formamide, deionized	25 mL	50%
<**!**>Heparin	2.5 mg	50 µg/mL
<**R**>SSC (20X)	12.5 mL	5X
Tween 20	50 µL	0.1%

Adjust the pH to 5.0 using HCl. Adjust the final volume to 50 mL with sterile H_2O. Store at –20°C.

Hyb-DNA-SDS

Reagent	Quantity (for 15 mL)	Final concentration
<R>Hyb	14.85 mL	
Salmon sperm DNA, sonicated (10 mg/mL; Invitrogen 15632-011)	150 µL	0.1 mg/mL

Store at –20°C.

<!>Prior to use, warm the solution and add SDS (sodium dodecyl sulfate) to a final concentration of 0.3%.

Juice plates

MATERIALS

Reagents

- 105 g agar
- 100 g sucrose
- <!>6 g methyl paraben (Tegosept)
- 1 L apple juice

Equipment

- Autoclave
- Flasks (one 6-L, one 2-L, five 1-L)
- Plates
- Stir plate and stir bar
- Stoppers for 1-L flasks

METHOD

1. In a 6-L flask, stir 105 g of agar into 3 L of H_2O.
2. In a 2-L flask, stir 100 g of sucrose and 6 g of methyl paraben into 1 L of apple juice and shake well.
3. Autoclave these flasks separately for 45 min. Sterilize five 1-L flasks by autoclaving (these flasks will be used for pouring the plates).
4. Slowly pour the sucrose/apple juice solution into the agar solution, add a stir bar, and stir for ~15 min (use settings of stir speed 9 and heat 6).
5. Pour ~800 mL of the combined solution into each of the autoclaved 1-L flasks. Stopper each flask so a skin does not form on the solution and take care not to agitate them to avoid the formation of bubbles. Once cooled slightly, pour the agar solution into the plates.

LB (Luria-Bertani) liquid medium

Reagent	Amount to add
H_2O	950 mL
Tryptone	10 g
NaCl	10 g
Yeast extract	5 g

Combine the reagents and shake until the solutes have dissolved. Adjust the pH to 7.0 with 5 N NaOH (~0.2 mL). Adjust the final volume of the solution to 1 L with H_2O. Sterilize by autoclaving for 20 min at 15 psi (1.05 kg/cm^2) on liquid cycle.

<R>For solid medium, see the recipe entitled “Media containing agar or agarose.”

Live yeast paste

Fill a vial (e.g., a scintillation vial) to about one-quarter with live baker's yeast. Add H_2O while stirring with a wooden stick until the consistency of peanut butter is attained. Keep refrigerated (otherwise the yeast will rise and spill out of the vial).

$MgCl_2$ (Magnesium chloride)

To prepare 1 L of 1 M $MgCl_2$, dissolve 203.3 g of $MgCl_2 \bullet 6H_2O$ in 800 mL H_2O. Adjust the volume to 1 L using H_2O. Dispense into aliquots and sterilize by autoclaving.

Note: $MgCl_2$ is extremely hygroscopic. Buy small bottles and do not store opened bottles for long periods of time. Once the crystals become saturated with H_2O, dispose of the chemical properly.

NASH reagent

<**!**>2,4-pentanedione (0.2%)
<**!**>Acetic acid (0.1 M)
Ammonium acetate (3.89 M)

Normal goat serum (NGS)

Heat-inactivate the serum for 30 min at 56°C. Filter through a 0.22-µm filter while still warm. Aliquot into sterile tubes and store the aliquots at –20°C. Once thawed, aliquots are stable for several months at 4°C.

Paraformaldehyde for *Drosophila* (4%)

MATERIALS

Reagents

<**!**>NaOH (one pellet; ~0.2 g)
<**!**>Paraformaldehyde powder (8 g)
<**R**>PB (0.1 M phosphate buffer pH 7.2)
Sodium phosphate dibasic stock (0.5 M)
Sodium phosphate monobasic stock (0.5 M)

Equipment

Beakers (100-mL, 250-mL)
Fume hood
Hot plate
pH paper
Stir rod

METHOD

1. Place the paraformaldehyde powder and NaOH pellet in a 250-mL beaker.
2. Prepare 100 mL of 0.1 M sodium phosphate dibasic: Use 20 mL of 0.5 M sodium phosphate dibasic stock and bring to 100 mL with H_2O. Add this to the beaker from Step 1.
3. Stir while heating on a hot plate under the fume hood until all the paraformaldehyde powder is dissolved. Do not boil. Turn off the heat.
4. Prepare 100 mL of 0.1 M sodium phosphate monobasic: Use 20 mL of 0.5 M sodium phosphate monobasic stock and bring to 100 mL with H_2O.
5. Slowly add 0.1 M sodium phosphate monobasic from Step 4 to the paraformaldehyde solution from Step 3 until the pH is 7.2 (check with pH paper).
6. Measure the volume of 0.1 M sodium phosphate monobasic left unused. Add this volume of PB to the fixative to give a total fixative volume of 200 mL.
7. Store at 4°C and discard after 5 d.

PB (0.1 M phosphate buffer pH 7.2)

MATERIALS

Reagents

Sodium phosphate dibasic
Sodium phosphate monobasic (anhydrous)

Equipment

Beakers
Graduated cylinders
Hot plate (optional; see Step 1 note)
Scale
Spatulas
Stir plate
Weigh boats

METHOD

1. Prepare sodium phosphate dibasic stock (0.5 M) by dissolving 35.5 g of sodium phosphate dibasic in a final volume of 500 mL of H_2O.
Some crystallization will occur when the solution is stored at 4°C. Warm on a hot plate and stir until the crystals dissolve.
2. Prepare sodium phosphate monobasic stock (0.5 M) by dissolving 30 g of anhydrous sodium phosphate monobasic in a final volume of 500 mL of H_2O.
3. Prepare 0.1 M sodium phosphate dibasic: Put 80 mL of sodium phosphate dibasic stock (0.5 M) from Step 1 in a beaker and add H_2O to give a final volume of 400 mL.
4. Prepare 0.1 M sodium phosphate monobasic: Put 30 mL of sodium phosphate monobasic stock (0.5 M) from Step 2 in a beaker and add H_2O to give a final volume of 150 mL.
5. Bring the 0.1 M sodium phosphate dibasic solution from Step 3 to pH 7.2 by adding as much as needed of the 0.1 M sodium phosphate monobasic solution from Step 4.
The resulting solution is 0.1 M phosphate buffer pH 7.2.

PBS (10X stock; pH 7.0)

Reagent	Amount to add (for 1L)	Final (10X) concentration
NaCl	74 g	1.27 M
$Na_2HPO_4 \bullet 7H_2O$	18.78 g	70 mM
$NaH_2PO_4 \bullet H_2O$	4.15 g	30 mM

Dissolve the components in <1 L of H_2O. Adjust pH to 7.0 with NaOH if necessary. Adjust the volume to 1 L with H_2O.

PBS(P)

Reagent	Quantity (for 1 L)	Final concentration (10X)
NaH_2PO_4	2.56 g	18.6 mM
Na_2HPO_4	11.94 g	84.1 mM
NaCl	102.2 g	1.75 M

Adjust the pH to 7.4 using NaOH or HCl as necessary. This recipe produces a 10X stock solution; prepare 1X PBS(P) by diluting with H_2O. Both 1X and 10X PBS(P) can be kept indefinitely at room temperature.

PBT for *Drosophila*

<**R**>PB (0.1 M phosphate buffer pH 7.2)
<**!**>0.2% (v/v) Triton X-100

PEM Buffer

0.1 M PIPES (pH 6.95)
2 mM EGTA
<**!**>1 mM $MgSO_4$

PEM-F

Reagent	Volume (for 10 mL)
<**!**>Formaldehyde (37%; Fisher F79-500)	1 mL
<**R**>PEM buffer[a]	9 mL

Prepare PEM-F fresh just before use.

[a] Adjust the pH of the PEM buffer to 7.0 with concentrated HCl. Filter through a 0.22-μm filter. Store PEM buffer for up to 1 yr at 4°C.

Phosphate-buffered saline (PBS)

Reagent	Amount to add (for 1X solution)	Final concentration (1X)	Amount to add (for 10X stock)	Final concentration (10X)
NaCl	8 g	137 mM	80 g	1.37 M
<**!**>KCl	0.2 g	2.7 mM	2 g	27 mM
Na_2HPO_4	1.44 g	10 mM	14.4 g	100 mM
KH_2PO_4	0.24 g	1.8 mM	2.4 g	18 mM
If necessary, PBS may be supplemented with the following:				
<**!**>$CaCl_2$•$2H_2O$	0.133 g	1 mM	1.33 g	10 mM
<**!**>$MgCl_2$•$6H_2O$	0.10 g	0.5 mM	1.0 g	5 mM

<**!**>PBS can be made as a 1X solution or as a 10X stock. To prepare 1 L of either 1X or 10X PBS, dissolve the reagents listed above in 800 mL of H_2O. Adjust the pH to 7.4 (or 7.2, if required) with HCl, and then add H_2O to 1 L. Dispense the solution into aliquots and sterilize them by autoclaving for 20 min at 15 psi (1.05 kg/cm^2) on liquid cycle or by filter sterilization. Store PBS at room temperature.

PT

Reagent	Quantity (for 1 L)	Final concentration
<**R**>PBS(P) (10X)	100 mL	1X
<**!**>Triton X-100 (100%)	1 mL	0.1%

Store at 4°C or room temperature.

PT + NGS

Reagent	Quantity (for 5 mL)
<**R**>PT	4.75 mL
<**R**>Normal goat serum (NGS)	0.25 mL

Store the solution at 4°C. PT + NGS will usually last for 2-3 wk. Discard if the solution looks cloudy.

PTX

Reagent	Amount to add (for 1L)	Final concentration
<R>PBS (10X stock; pH 7.0)	100 mL	1X
<!>Triton X-100 (10%, v/v)	10 mL	0.1% (v/v)
Add H_2O to give a final volume of 1 L.		

SCODA bacterial lysis (BL) buffer

<R>50 mM Tris-Cl
<R>50 mM EDTA (disodium salt)
<!>0.5% SDS
Adjust the pH of the solution to 7.5.

SDS loading buffer (5X)

<!>Bromophenol blue (0.25%)
<!>DTT (dithiothreitol; 0.5 M)
Glycerol (50%)
<!>SDS (sodium dodecyl sulfate; 10%)
<R>Tris-Cl (0.25 M, pH 6.8)

Sorensen's phosphate buffer (0.133 M, pH 7.2)

0.133 M Na_2HPO_4
0.133 M KH_2PO_4
Mix 71.5 mL of Na_2HPO_4 and 28.5 mL of KH_2PO_4 to obtain pH 7.2.

SSC

For a 20X solution: Dissolve 175.3 g of NaCl and 88.2 g of sodium citrate in 800 mL of H_2O. Adjust the pH to 7.0 with a few drops of a 14 N solution of HCl. Adjust the volume to 1 L with H_2O. Dispense into aliquots. Sterilize by autoclaving. The final concentrations of the ingredients are 3.0 M NaCl and 0.3 M sodium citrate.

Standard external saline

<!>2 mM $CaCl_2$
20 mM glucose
2.5 mM KCl
1 mM $MgCl_2$
125 mM NaCl
26 mM $NaHCO_3$
1.25 mM NaH_2PO_4
Aerate with 95% O_2 and 5% CO_2. The pH is ~7.3 at 21°C.

Standard pipette solution

10 mM HEPES
2.5 mM KCl
150 mM NaCl

TBE buffer

Prepare a 5X stock solution in 1 L of H_2O:
<**!**>54 g of Tris base
<**!**>27.5 g of boric acid
<**R**>20 mL of 0.5 M EDTA (pH 8.0)
The 0.5X working solution is 45 mM Tris-borate/1 mM EDTA.
TBE is usually made and stored as a 5X or 10X stock solution. The pH of the concentrated stock buffer should be ~8.3. Dilute the concentrated stock buffer just before use and make the gel solution and the electrophoresis buffer from the same concentrated stock solution. Some investigators prefer to use more concentrated stock solutions of TBE (10X as opposed to 5X). However, 5X stock solution is more stable because the solutes do not precipitate during storage. Passing the 5X or 10X buffer stocks through a 0.22-µm filter can prevent or delay formation of precipitates.

TE buffer

Reagent	Quantity (for 100 mL)	Final concentration
<**R**>EDTA (0.5 M, pH 8.0)	0.2 mL	1 mM
<**R**>Tris-Cl (1 M, pH 8.0)	1 mL	10 mM
H_2O	to 100 mL	

Tris-Cl

<**!**>Tris base
<**!**>HCl
To prepare a 1 M solution, dissolve 121.1 g of Tris base in 800 mL of H_2O. Adjust the pH to the desired value by adding concentrated HCl.

pH	HCl
7.4	70 mL
7.6	60 mL
8.0	42 mL

Allow the solution to cool to room temperature before making final adjustments to the pH. Adjust the volume of the solution to 1 L with H_2O. Dispense into aliquots and sterilize by autoclaving.

If the 1 M solution has a yellow color, discard it and obtain Tris of better quality. The pH of Tris solutions is temperature-dependent and decreases ~0.03 pH units for each 1°C increase in temperature. For example, a 0.05 M solution has pH values of 9.5, 8.9, and 8.6 at 5°C, 25°C, and 37°C, respectively.

Appendix 2: Cautions

[NOTE: For reagents marked with the <**!**> symbol not listed below, please consult the manufacturer's Material Safety Data Sheet for further information. Researchers using the procedures in these protocols contained in this issue of *CSH Protocols* do so at their own risk. Cold Spring Harbor Laboratory makes no representations or warranties with respect to the material set forth in these protocols and has no liability in connection with the use of these materials. Materials used in these protocols may be considered hazardous and should be used with caution.]

1-Pentanol is highly flammable in liquid and vapor forms and is toxic. It is harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses and use in a chemical fume hood. Keep away from heat, sparks, and open flame.

2,4-Pentanedione (Acetylacetone) is highly flammable in liquid and vapor forms. It is highly toxic as well as a teratogen and a mutagen, and is harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses and use in a chemical fume hood. Keep away from heat, sparks, and open flame. Do not breathe the vapor, mist, or gas.

α-Cyano-4-hydroxycinnamic acid (CHCA) may cause cardiac disturbances. Chronic effects may be delayed. It may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses.

Acetic acid (concentrated) must be handled with great care. It may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and goggles. Use in a chemical fume hood.

Acetone causes eye and skin irritation and is irritating to mucous membranes and upper respiratory tract. Do not breathe the vapors. It is also extremely flammable. Wear appropriate gloves and safety glasses.

Acetonitrile is very volatile and extremely flammable. It is an irritant and a chemical asphyxiant that can exert its effects by inhalation, ingestion, or skin absorption. Treat cases of severe exposure as cyanide poisoning. Wear appropriate gloves and safety glasses. Use only in a chemical fume hood. Keep away from heat, sparks, and open flame.

Ammonium ferrous sulfate may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses.

Anesthetics Follow manufacturer's safety guidelines.

Animal treatment Procedures for the humane treatment of animals must be observed at all times. Consult your local animal facility for guidelines.

Aprotinin may be harmful by inhalation, ingestion, or skin absorption. It may also cause allergic reactions. Exposure may cause gastrointestinal effects, muscle pain, blood pressure changes, or bronchospasm. Wear appropriate gloves and safety glasses and use only in a chemical fume hood. Do not breathe the dust.

BCIP (5-Bromo-4-chloro-3-indolyl-phosphate) is an irritant and may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses. Do not breathe the dust.

Bleach (Sodium hypochlorite, NaOCl) is poisonous, can be explosive, and may react with organic solvents. It may be fatal by inhalation and is also harmful by ingestion and destructive to the skin. Wear appropriate gloves and safety glasses and use in a chemical fume hood to minimize exposure and odor.

Boric acid (H_3BO_3) may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and goggles.

Bromophenol blue may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses and use in a chemical fume hood.

$CaCl_2$ (Calcium chloride) is hygroscopic and may cause cardiac disturbances. It may be harmful by inhalation, ingestion, or skin absorption. Do not breathe the dust. Wear appropriate gloves and safety goggles.

Chloroform ($CHCl_3$) is irritating to the skin, eyes, mucous membranes, and respiratory tract. It is a carcinogen and may damage the liver and kidneys. It is also volatile. Avoid breathing the vapors. Wear appropriate gloves and safety glasses. Always use in a chemical fume hood.

Cyanoacrylate adhesives are harmful by inhalation, ingestion, and skin absorption. Immediate bonding of tissues can occur. Do not pull apart. Inhalation may cause lightheadedness. Wear appropriate gloves and safety glasses. Follow manufacturer's safety guidelines.

DAB (3,3′-Diaminobenzidine tetrahydrochloride) is a carcinogen. Handle with extreme care. Avoid breathing vapors.

Wear appropriate gloves and safety glasses and use in a chemical fume hood.

DEPC (Diethyl pyrocarbonate) is a potent protein denaturant and is a suspected carcinogen. Aim bottle away from you when opening it; internal pressure can lead to splattering. Wear appropriate gloves and lab coat. Use in a chemical fume hood.

DMSO (Dimethyl sulfoxide) may be harmful by inhalation or skin absorption. Wear appropriate gloves and safety glasses. Use in a chemical fume hood. DMSO is also combustible. Store in a tightly closed container. Keep away from heat, sparks, and open flame.

DTT (Dithiothreitol) is a strong reducing agent that emits a foul odor. It may be harmful by inhalation, ingestion, or skin absorption. When working with the solid form or highly concentrated stocks, wear appropriate gloves and safety glasses and use in a chemical fume hood.

Dyes Follow manufacturer's safety guidelines.

EDTA (Ethylenediamenetetraacetic acid) may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses. Severe overexposure can result in death.

Fixatives Follow manufacturer's safety guidelines.

Formaldehyde (HCOH) is highly toxic and volatile. It is also a carcinogen. It is readily absorbed through the skin and is irritating or destructive to the skin, eyes, mucous membranes, and upper respiratory tract. Avoid breathing the vapors. Wear appropriate gloves and safety glasses. Always use in a chemical fume hood. Keep away from heat, sparks, and open flame.

Formamide is teratogenic. The vapor is irritating to the eyes, skin, mucous membranes, and upper respiratory tract. It may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses. Always use in a chemical fume hood when working with concentrated solutions of formamide. Keep working solutions covered as much as possible.

HCl (Hydrochloric acid, Hydrochloride) is volatile and may be fatal if inhaled, ingested, or absorbed through the skin. It is extremely destructive to mucous membranes, upper respiratory tract, eyes, and skin. Wear appropriate gloves and safety glasses. Use with great care in a chemical fume hood. Wear goggles when handling large quantities.

Heparin is an irritant and may act as an anticoagulant subcutaneously or intravenously. It may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses.

Heptane may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses. It is extremely flammable. Keep away from heat, sparks, and open flame.

Hydrogen peroxide (H_2O_2) is corrosive, toxic, and extremely damaging to the skin. It may be harmful by inhalation, ingestion, and skin absorption. Wear appropriate gloves and safety glasses. Use only in a chemical fume hood.

Hygromycin B is highly toxic and may be fatal if inhaled, ingested, or absorbed through the skin. Wear appropriate gloves and safety goggles. Use only in a chemical fume hood. Do not breathe the dust.

Isoflurane is an irritant and may be harmful by inhalation, ingestion, or skin absorption. Chronic exposure may be harmful. Wear appropriate gloves and safety glasses.

Isopropanol (Isopropyl alcohol, 2-Propanol) is flammable and irritating. It may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses. Do not breathe the vapor. Keep away from heat, sparks, and open flame.

KCl (Potassium chloride) may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses.

Ketamine (Ketamine hydrochloride) is toxic and may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses.

KOH (Potassium hydroxide) is highly toxic and may be fatal if swallowed. It may be harmful by inhalation, ingestion, or skin absorption. Solutions are corrosive and can cause severe burns. It should be handled with great care. Wear appropriate gloves and safety goggles.

Leupeptin (or its hemisulfate) may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses and use in a chemical fume hood.

Lysozyme is caustic to mucous membranes. Wear appropriate gloves and safety glasses.

Magnesium sulfate ($MgSO_4$) presents chronic health hazards and affects the central nervous system and the gastrointestinal tract. It may be harmful by inhalation,

ingestion, or skin absorption. Wear appropriate gloves and safety glasses and use in a chemical fume hood.

$MgCl_2$ (Magnesium chloride) may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses and use in a chemical fume hood.

Methanol (MeOH, H_3COH) is poisonous and can cause blindness. It may be harmful by inhalation, ingestion, or skin absorption. Adequate ventilation is necessary to limit exposure to vapors. Avoid inhaling these vapors. Wear appropriate gloves and goggles. Use only in a chemical fume hood.

Methyl paraben (*p*-Hydroxymethylbenzoate, Methyl 4-hydroxybenzoate, Nipagin, Tegosept) is an irritant and may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses

Na_2HPO_4, See Sodium hydrogen phosphate.

NaOH (Sodium hydroxide) and solutions containing NaOH are highly toxic and caustic and should be handled with great care. Wear appropriate gloves and a face mask. All concentrated bases should be handled in a similar manner.

NBT (4-Nitro blue tetrazolium chloride) may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses.

$NiCl_2$ (Nickel chloride) is toxic and may be harmful by inhalation, ingestion, or skin absorption. Do not breathe the dust. Wear appropriate gloves and safety glasses.

Nitrogen (gaseous or liquid) may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses. Consult your local safety office for proper precautions.

Paraformaldehyde is highly toxic and may be fatal. It may be a carcinogen. It is readily absorbed through the skin and is extremely destructive to the skin, eyes, mucous membranes, and upper respiratory tract. Avoid breathing the dust or vapor. Wear appropriate gloves and safety glasses and use in a chemical fume hood. Keep away from heat, sparks, and open flame.

Phenol:chloroform Phenol is extremely toxic, highly corrosive, and can cause severe burns. It may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves, goggles, and protective clothing. Always use in a chemical fume hood. Rinse any areas of skin that come in contact with phenol with a large volume of water and wash with soap and water; do not use ethanol!

Chloroform ($CHCl_3$) is irritating to the skin, eyes, mucous membranes, and respiratory tract. It is a carcinogen and may damage the liver and kidneys. It is also volatile. Avoid breathing the vapors. Wear appropriate gloves and safety glasses. Always use in a chemical fume hood.

Phosphoric acid (H_3PO_4) is highly corrosive and is extremely destructive to the tissue of the mucous membranes and upper respiratory tract, eyes, and skin. It is harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses. Do not breathe the vapors.

PMSF (Phenylmethylsulfonyl fluoride, $C_7H_7FO_2S$, $C_6H_5CH_2SO_2F$) is a highly toxic cholinesterase inhibitor. It is extremely destructive to the mucous membranes of the respiratory tract, eyes, and skin. It may be fatal by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses. Always use in a chemical fume hood. In case of contact, immediately flush eyes or skin with copious amounts of water and discard contaminated clothing.

Propionic acid is highly corrosive and causes burns to any area of contact. It is flammable in both liquid and vapor forms and may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety goggles and use only with adequate ventilation. Keep away from heat, sparks, and open flame.

Proteinase K is an irritant and may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses.

Radioactive substances: When planning an experiment that involves the use of radioactivity, consider the physicochemical properties of the isotope (half-life, emission type, and energy), the chemical form of the radioactivity, its radioactive concentration (specific activity), total amount, and its chemical concentration. Order and use only as much as needed. Always wear appropriate gloves, lab coat, and safety goggles when handling radioactive material. X rays and γ rays are electromagnetic waves of very short wavelengths either generated by technical devices or emitted by radioactive materials. They might be emitted isotropically from the source or may be focused into a beam. Their potential dangers depend on the time period of exposure, the intensity experienced, and the wavelengths used. Be aware that appropriate shielding is usually made of lead or other similar material. The thickness of the shielding is determined by the energy(s) of the X rays or γ rays. Consult the local safety office for further guidance in the appropriate use and disposal of radioactive materials. Always monitor thoroughly after using radioisotopes. A convenient calculator to perform routine radioactivity calculations can be found at http://www.graphpad.com/calculators/radcalc.cfm.

RNase A is an irritant and may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses. Do not breathe the dust.

SDS (Sodium dodecyl sulfate) is toxic, an irritant, and poses a risk of severe damage to the eyes. It may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety goggles. Do not breathe the dust.

Streptomycin is toxic and a suspected carcinogen and mutagen. It may cause allergic reactions. It may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses.

Trichloroacetic acid (TCA) is highly caustic. Wear appropriate gloves and safety goggles.

Trifluoroacetic acid (TFA) may be harmful by inhalation, ingestion, or skin absorption. Concentrated acids must be handled with great care. Decomposition causes toxic fumes. Wear appropriate gloves and a face mask. Use in a chemical fume hood.

Tris base may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses.

Triton X-100 causes severe eye irritation and burns. It may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety goggles.

Trypsin may cause an allergic respiratory reaction. It may be harmful by inhalation, ingestion, or skin absorption. Do not breathe the dust. Wear appropriate gloves and safety goggles. Use with adequate ventilation.

Urethane is a mutagen and suspected carcinogen. It is also highly toxic and is readily absorbed through the skin. It may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses. Do not breathe the dust and use only in a chemical fume hood.

Xylazine may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses.