Dolan DNA Learning Center
Cold Spring Harbor Laboratory

Recipes for Reagents and Stock Solutions

Funded by NSF

Recipes for Reagents and Stock Solutions

The success of the laboratories depends on the use of high-quality reagents. Follow the recipes with care and pay attention to cleanliness. Use a clean spatula for each ingredient or carefully pour each ingredient from its bottle.

I. DNA Isolation

4.4 M Ammonium Acetate (NH_4OAc)
Edward's Buffer
0.5 M Ethylene Diamine Tetraacetic Acid (EDTA)
Phenol:chloroform (2:1; volume:volume)
5 mg/ml RNase A (Pancreatic RNase)
4 M Sodium Chloride (NaCl)
10% Sodium Dodecyl Sulfate (SDS)
1 M Tris (pH 8.0)
Tris/EDTA (TE) Buffer
Tris/EDTA Buffer with RNase A (TER)
Urea Extraction Buffer

II. Polymerase Chain Reaction

1% Cresol Red Dye
Cresol Red Loading Dye
KOD Hot Start DNA Polymerase
Primer/Loading Dye Mix
Primer Sequences

III. Agarose Gel Electrophoresis

1.0 or 2.0% Agarose
1 µg/ml Ethidium Bromide Staining Solution
0.2% Methylene Blue Stock Solution
0.025% Methylene Blue Staining Solution
pBR322/*Bst*NI Size Markers (0.1 µg/µl)
100 bp or 1 kb DNA Ladder (0.125 µg/µl)
20X Tris/Borate/EDTA (TBE) Electrophoresis Buffer
1X Tris/Borate/EDTA (TBE) Electrophoresis Buffer

Notes on Buffers

1. Typically, solid reagents are dissolved in a volume of deionized or distilled water equivalent to 70-80% of the finished volume of buffer. This leaves room for the addition of acid or base to adjust the pH. Then, water is added to bring the solution up to the final volume.
2. Buffers typically are used as 1X or 10X solutions. Buffers are diluted when mixed with other reagents to produce a working concentration of 1X.
3. The commercial enzymes used for these laboratories all come from the suppliers with appropriate buffers. These should be used unless noted otherwise.
4. Storage temperatures of 4°C and -20°C refer to normal refrigerator and freezer (non-frost free) temperatures, respectively.

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I. DNA Isolation

4.4 M Ammonium Acetate (NH_4OAc)

Makes approximately 200 ml.
Store at room temperature (indefinitely).

CAUTION: Avoid inhaling acetic acid or ammonium hydroxide; wear goggles and work in a fume hood!

Mix in a 500-ml beaker in a fume hood:
- 50.5 ml glacial acetic acid
- 105 ml of deionized or distilled water
- 45 ml of ammonium hydroxide (add slowly)

Edward's Buffer

Makes 50 ml.
Store at room temperature (indefinitely).

Mix in a 50-ml tube:
- 32.5 ml of deionized or distilled water
- 10 ml of 1 M Tris pH 8.0
- 2.5 ml of 5 M NaCl
- 2.5 ml of 0.5 M EDTA
- 2.5 ml of 10% SDS

0.5 M Ethylene Diamine Tetraacetic Acid (EDTA) (pH 8.0)

Makes 100 ml.
Store at room temperature (indefinitely).

1. Add 18.6 g EDTA (disodium salt, m.w. 372.24) to 80 ml deionized or distilled water.
2. Adjust to pH by slowly adding approximately 2.2 g of sodium hydroxide pellets (m.w. 40.00). (If a pH meter is not available, adding 2.2 g of NaOH pellets will make a solution of approximately pH 8.0).
3. Mix vigorously with a magnetic stirrer or by hand. EDTA will only dissolve after pH has reached 8.0 or higher.

NOTE: Use only the disodium salt of EDTA.

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Phenol: chloroform (2:1; volume:volume)

Makes 60 ml.
Store at room temperature (indefinitely).

CAUTION: Avoid inhaling phenol or chloroform; wear goggles and work in a fume hood! Avoid skin contact with phenol as it can cause severe burns.

Mix in a 50-ml tube:
40 ml of phenol
20 ml of chloroform

5 mg/ml RNase A (Pancreatic RNase)

Makes 20 ml.
Store at –20°C (indefinitely).

CAUTION: Avoid inhaling acetic acid; wear goggles and work in a fume hood!

1. Dissolve 100 mg of RNase A in 20 ml of 0.05% glacial acetic acid, and transfer to a 50-ml conical tube.
2. Place the tube in a boiling-water bath for 15 minutes.
3. Cool the solution, and neutralize by adding 120 µl of 1 M Tris (pH 8.0).
4. Dispense 1-ml aliquots in 1.5-ml tubes.

NOTES:
- Use only RNase A from bovine pancreas.
- Dissolving RNase in the acetic acid prevents subsequent precipitation of the RNase. The solution can be prepared by simply dissolving RNase in deionized or distilled water; however, the RNase will occasionally precipitate from the solution and activity will be lost.

4 M Sodium Chloride (NaCl)

Makes 500 ml.
Store at room temperature (indefinitely).

1. Dissolve 116.9 g of NaCl (m.w. 58.44) in 250 ml of deionized or distilled water.
2. Add deionized or distilled water to make a total volume of 500 ml of solution.

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10% Sodium Dodecyl Sulfate (SDS)

Makes 100 ml.
Store at room temperature (indefinitely).

CAUTION: Avoid inhaling SDS powder; wear mask over nose and mouth!

1. Dissolve 10 g electrophoresis-grade SDS (m.w. 288.37) in 80 ml deionized water.
2. Add deionized or distilled water to make 100 ml total solution.

NOTE: SDS is the same as sodium lauryl sulfate.

1 M Tris (pH 8.0)

Makes 100 ml.
Store at room temperature (indefinitely).

CAUTION: Avoid inhaling Tris powder; wear mask over nose and mouth!

1. Dissolve 12.1 g Tris base (m.w. 121.10) in 70 ml deionized or distilled water.
2. Adjust pH by slowly adding concentrated hydrochloric acid (HCl); monitor with a pH meter.
3. Add deionized or distilled water to make 100 ml total solution.

NOTES:
- A yellow-colored solution indicates poor-quality Tris. Discard, and obtain from a different source.
- Many types of electrodes do not accurately measure the pH of Tris solutions; check with manufacturer to obtain a suitable one.
- The pH of Tris solutions is temperature dependent; make pH measurements at room temperature.

Tris/EDTA (TE) Buffer

Makes 100 ml.
Store at room temperature (indefinitely).

Mix in a 200-ml beaker:
99 ml of deionized or distilled water
1 ml of 1 M Tris pH 8.0
200 µl of 0.5 M EDTA

Tris/EDTA Buffer with RNase A (TER)

Makes 5 ml.
Make fresh each time.

Mix:
100 µl 5 mg/ml RNase A
4.9 ml TE buffer

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Urea Extraction Buffer

Makes 1000 ml.
Store at room temperature (indefinitely).

Mix in a 2000-ml beaker:
- 420 g of urea
- 87.5 ml of 4 M NaCl
- 50 ml of 1 M Tris pH 8.0
- 0.5 M EDTA
- 10 g n-lauryl sarcosine

Add deionized or distilled water to make a total volume of 1000 ml of solution.

II. Polymerase Chain Reaction

1% Cresol Red Dye

Makes 50 ml.
Store at room temperature (indefinitely).

Mix in a 50-ml tube:
- 500 mg cresol red dye
- 50 ml of distilled water

Cresol Red Loading Dye

Makes 50 ml.
Store at –20°C (indefinitely).

1. Dissolve 17 g of sucrose in 49 ml of distilled water in a 50-ml tube.
2. Add 1 ml of 1% cresol red dye and mix well.

KOD Hot Start DNA Polymerase and Buffer

KOD Hot Start DNA polymerase is purchased from Novagen (catalog number 71086-3). The polymerase is supplied with 10X buffer, a 10X dNTP mix (2 mM each), and 25 mM $MgSO_4$.

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Primer/Loading Dye Mixes (For Use with Ready-to-Go PCR Beads™)

For *Arabidopsis Clf-2*, CAPS markers, *Bz* (Maize) analysis, and GMO testing

Makes enough for 50 reactions.
Store at –20°C for 1 year.

Mix in a 1.5-ml tube:

640 µl of distilled water
460 µl of Cresol Red Loading Dye
20 µl of 15 pmol/µl 5' primer
20 µl of 15 pmol/µl 3' primer

Primer Sequences

CLF genotyping:
primers for *CLF* (Wt)
5' *CLF1* 5'-TTAACCCGGACCCGCATTTGTTTCGG-3'
3' *CLF2* 5'-AGAGAAGCTCAAACAAGCCATCGA-3'
primers for (Mutant)
5' *CLF1* 5'-TTAACCCGGACCCGCATTTGTTTCGG-3'
3' *Ds* 5'-GTCGGCGTGCGGCTGGCGGCG-3'

CAPS mapping:
primers for *m235*-marker
m235-5' 5'-GAATCTGTTTCGCCTAACGC-3'
m235-3' 5'-AGTCCACAACAATTGCAGCC-3'
primers for *UFO*-marker
UFO-5' 5'-GTGGCGGTTCAGACGGAGAGG-3'
UFO-3' 5'-AAGGCATCATGACTGTGGTTTTTC-3'
primers for g4026-marker
g4026-5' 5'-GGGGTCAGTTACATTACTAGC-3'
g4026-3' 5'-GTACGGTTCTTCTTCCCTTA-3'
primers *H77224*-marker
H77224-5' 5'-GGATTTGGGGAAGAGGAAGTAA-3'
H77224-3' 5'-TCCTTAGCCTTGCTTTGATAGT-3'

Functional Genomics:
Example primers for control gene *At3g16240.1*:

At3g16240.1 primer1 5'- gctcgatccacctaggctCAAGCATCTTCACAGGTTTTGG-3'
At3g16240.1 primer2 5'- cacagctccacctccacctccaggccggccAAATCAGCAGAAGCAAGAGGA-3'
At3g16240.1 primer3 5'- tgctggtgctgctgcggccgctggggccGTTCCTCTTGCTTCTGCTGATTTC-3'
At3g16240.1 primer4 5'- cgtagcgagaccacaggaCCATCCATTAATTTGTCATTGTTGT-3'

Find primers for *genes* with unknown function at FTFLP (http://aztec.stanford.edu/gfp/index.html). Use *Target Selection* to **select a gene** from a "short" list of 4000 genes. Use *Search Database* to **search for primer sequences** for a locus. The course will tag the locus *AT1G08480*.

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Bz (Maize) analysis:
primers for *bz* (Wt)
bz-599 5'-CGAATGGCTGTTGCATTTCCAT-3'
bz-863R 5'-ACGGGACGCAGTTGGGCAGGA-3'
primers for *bz:Ac*
bz- 599 5'-CGAATGGCTGTTGCATTTCCAT-3'
Ac-132R 5'-TCTACCGTTTCCGTTTCCGTTT-3'

GMO testing:
primers for *35S*-promoter
35S-5' 5'-CCGACAGTGGTCCCAAAGATGGAC-3'
35S-3' 5'-ATATAGAGGAAGGGTCTTGCGAAGG-3'
primers for tubulin
Tub5 5'- GGGATCCACTTCATGCTTTCGTCC-3'
Tub3 5'- GGGAACCACATCACCACGGTACAT-3'

III. Agarose Gel Electrophoresis

1.0 % or 2.0% Agarose

Makes 200 ml.
Use fresh or store solidified agarose at room temperature (several weeks).

Prepare a 2% agarose solution by adding 2 g of agarose to 100 ml of 1X TBE in a 500 ml flask or beaker. Heat the flask or beaker in a boiling water bath (approximately 15 minutes) or in a microwave oven (approximately 4 minutes) until the agarose is completely dissolved. You should no longer see agarose particles floating in solution when the beaker is swirled. Allow the agarose to cool to approximately 60°C, and hold at this temperature in a hot water bath. Cover beaker or flask with aluminum foil, and skim any polymerized "skin" off the top of the solution before pouring.

NOTES:
- Samples of agarose powder can be preweighed and stored in capped test tubes until ready for use.
- Solidified agarose can be stored at room temperature and then remelted over a boiling-water bath (15-20 minutes) or in a microwave oven (3-5 minutes per beaker) prior to use. Always loosen cap when remelting agarose in a bottle.
- When remelting agarose evaporation will cause the concentration to increase. If necessary, compensate by adding back a small volume of water.

1 μg/ml Ethidium Bromide Staining Solution

Makes 500 ml.
Store in dark at room temperature (indefinitely).

1. Add 100 μl of 5 mg/ml ethidium bromide to 500 ml deionized or distilled water.
2. Store in unbreakable bottles (preferably opaque). Label bottles "CAUTION: Ethidium Bromide. Mutagen and cancer-suspect agent. Wear rubber gloves when handling."

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NOTE: Ethidium bromide is light sensitive; store in dark container or wrap container in aluminum foil.

0.2% Methylene Blue Stock Solution

CAUTION: Ethidium bromide is a mutagen by the Ames microsome assay and a suspected carcinogen. Wear rubber gloves when preparing and using ethidium bromide solutions.

Makes 100 ml.
Store at room temperature (indefinitely).

1. Dissolve 0.2 g methylene blue-trihydrate (m.w. 373.9) in 100 ml of deionized or distilled water.

0.025% Methylene Blue Staining Solution

Makes 500 ml.
Store at room temperature (indefinitely).

1. Add 62.5 ml of 0.2% methelyene blue stock solution to 437.5 ml of deionized or distilled water.

pBR322/*Bst*NI Size Markers (0.1 μg/μl)

Makes 100 μl.
Store at –20°C for 1 year.

Either buy pBR322 pre-cut with restriction enzyme *Bst*NI from New England Biolabs (#N3031) or prepare it according to the following protocol:

1. Add 1 μl of a solution of 10 μg/μl pBR322 to 75 μl deionized or deionized or distilled water.
2. Add 10 μl 10x buffer (provided by supplier of the enzyme).
3. Add 5 μl *Bst*NI restriction enzyme, and incubate at 60°C for 60 min.
4. Electrophorese 5 μl (plus 1 μl loading dye) in a 1-2% agarose gel to check for complete digestion. Exactly 5 bands should be visible, corresponding to 1,857 bp, 1,058 bp, 929 bp, 383, and 121 bp. Any additional bands indicate incomplete digestion; add additional enzyme and incubate again at 60°C.

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100 bp or 1 kb DNA Ladder (0.125 μg/μl)

Makes 100 μl.
Store at –20°C for 1 year.

Obtain the stock solution through New England Biolabs (#N3231S). The sold concentration is 0.5 μg/μl and should be kept at –20°C. You can dilute a small amount of the stock at a time following this procedure:

1. Add 50 μl of deionized or distilled water to a 1.5-ml tube.
2. Add 25 μl of Cresol Red Loading Dye.
3. Add 25 μl of 100-bp or 1-kb DNA Ladder stock.

20X Tris/Borate/EDTA (TBE) Electrophoresis Buffer

Makes 1 liter.
Store at room temperature (indefinitely).

1. Add the following dry ingredients to 500 ml of deionized or distilled water in a 2-liter flask.
   1 g of NaOH (m.w. 40.00)
   216 g of Tris base (m.w. 121.10)
   110 g of boric acid (m.w. 61.83)
   14.8 g of EDTA (disodium salt, m.w. 372.24)
2. Stir to dissolve, preferably using a magnetic stir bar.
3. Add deionized or distilled water to bring total solution to 1 liter.

NOTE: If stored 20X TBE comes out of solution, place the flask in a water bath (37°C to 42°C) and stir occasionally until all solid matter goes back into solution.

1X Tris/Borate/EDTA (TBE) Electrophoresis Buffer

Makes 10 liters.
Store at room temperature (indefinitely).

1. Into a spigoted carboy, add 9 liters deionized or distilled water to 1 liter of 10X TBE electrophoresis buffer.
2. Stir to mix.

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