Appendix

This appendix provides a list of the equipment and supplies necessary for running each laboratory. Because many of the experiments described use the same equipment and supplies these are listed only once. Recipes for the solutions are also given for each exercise. Each recipe is listed only once for the laboratory when it is first required.

DNA Purification

Equipment and supplies

1. 8HQ (8-hydroxyquinoline) free base (Sigma Co., no. H6878). Do not use hemisulfate salt of the 8HQ.

2. Sterilized 15 ml and 50 ml conical polypropylene centrifuge tubes (e.g. Corning, nos 25319-15 and 25330-50).

3. Corex 25 ml centrifuge tubes with Teflon-lined caps (Corex, no. 8446-25).

4. EDTA (ethylenediaminetetra-acetic acid) (0.5 M at pH 8.0) (Ambion Inc., no. 9261).

5. Glass hooks. Glass hooks are made from Pasteur pipettes in the following way. First, place the end of a pipette horizontally into a Bunsen flame and seal it. Next, holding the pipette at a 45° angle, insert 0.5 cm of the tip into the flame. The end of the pipette will slowly drop under gravity forming a hook.

6. Ten times PBS (pH 7.4) (Ambion, no. 9625). The pH of the PBS solution is critical for collecting cheek cells. Preparation of this solution from basic ingredients is not recommended.

7. Redistilled, water-saturated phenol (Ambion Inc., no. 9712). Water-saturated phenol is preferable to the crystalline form because it is easier and safer to prepare buffered phenol from it. Water-saturated phenol can be stored indefinitely in a tightly closed, dark bottle at –70°C.

8. Proteinase K (Ambion Inc., no. 2546). Ambion Inc. is a low-cost source

of proteinase K. The enzyme is supplied in storage buffer containing 50 percent glycerol at a concentration of 20 mg ml^{-1}. Proteinase K solution should be stored at –20°C. Proteinase K remains active for several years at –20°C.

9. DNase-free ribonuclease A (Sigma, no. R4642). RNase is supplied in storage buffer with 50 percent glycerol at a concentration of 10 mg ml^{-1}. The enzyme can be stored indefinitely in a –20°C freezer.

10. DNase-free ribonuclease T1 (Ambion Inc., no. 2280). The enzyme is supplied in a storage buffer with 50 percent glycerol at concentration of 2000 units ml^{-1} and can be stored indefinitely in a –20°C freezer.

11. Sarcosyl (*N*-lauroylsarcosine) sodium salt (Sigma, no. L 5125).

Solutions to prepare

1. CIA (chloroform : isoamyl alcohol). Mix 24 volumes of chloroform with 1 volume of isoamyl alcohol. Because chloroform is light sensitive and very volatile, the CIA solution should be stored in a brown glass bottle, preferably in a fume hood.

2. Dilution buffer: 20 mM Tris–HCl (pH 8.5) and 100 mM Na_2 EDTA (pH 8.0). Prepare as described for lysis buffer.

3. Lysis buffer: 20 mM Tris (pH 8.5), 100 mM Na_2 EDTA (pH 8.0), 120 mM NaCl, and 1.2 percent Sarcosyl. Add the appropriate amount of 1 M Tris–HCl stock solution and 0.5 M EDTA stock to the water. Check the pH of the lysis buffer and titrate it to pH 8.5 with concentrated NaOH if necessary. Add NaCl and sterilize by autoclaving for 20 minutes. Do not add Sarcosyl to the stock solution. It will be added after resuspension of the cells. Store at 4°C.

4. Phenol 8HQ. Water-saturated, twice-distilled phenol is equilibrated with an equal volume of 0.1 M sodium borate. Sodium borate should be used rather than the customary 0.1 M Tris solution because of its superior buffering capacity at pH 8.5, its low cost, its antioxidant properties, and its ability to remove oxidation products during the equilibration procedure. Mix an equal volume of a water-saturated phenol with 0.1 M sodium borate in a separation funnel. Shake until the solution turns milky. Wait for the phases to separate and then collect the bottom phenol phase. Add 8HQ to the phenol at a final concentration 0.1 percent (v/w). Phenol 8HQ can be stored in a dark bottle at 4°C for several weeks. Store at –70°C for long-term storage. The solution can be stored for several years at –70°C.

5. Twenty percent (w/v) Sarcosyl stock. Dissolve 40 g of Sarcosyl in 100 ml of double-distilled or deionized water. Adjust to 200 ml with double-distilled or deionized water. Sterilize by filtration through a 0.22 μm filter. Store at room temperature.

6. Sodium acetate (3 M). Sterilize the solution by autoclaving and store at 4°C.

7. TE buffer: 10 mM Tris–HCl (pH 7.5 or 8.0) and 1 mM Na_2 EDTA (pH 8.0). Sterilize by autoclaving and store at 4°C.

DNA Fingerprinting: Multi-locus Analysis

Equipment and supplies

1. Agarose powder SeaKem^{TM} LE (FMC BioProducts, no. 50001) or equivalent.
2. Anti-DIG (digoxigenin) alkaline phosphatase (750 u ml^{-1}) (Roche Molecular Biochemicals, no. 1093 274). The stock solution should not be frozen. Store at 4°C.
3. Capillary tubes (25 µl) (Idaho Technology, no. 1709).
4. CDP-Star solution (Tropix Co., no. MS100R). Store the solution in the dark at 4°C. CDP-Star is easily destroyed by ubiquitous alkaline phosphatase. Wear gloves and use sterilized tips when handling CDP-Star solution.
5. Dig Easy Hyb solution (Roche Molecular Biochemicals, no. 1603 558).
6. Dig Wash and Block Buffer Set (Roche Molecular Biochemicals, no. 1585 762). It is possible to prepare each reagent from basic ingredients, but the cost of it will be higher than the cost of the kit.
7. Ten times DIG-dUTP labeling mixture (Roche Molecular Biochemicals, no. 1 227 065).
8. DIG-labeled control DNA (Roche Molecular Bichemicals, no. 1093 657).
9. DNA Ladder (1 kb) (Life Technologies, no. 15615-016).
10. Ten times dNTP mix for an air cycler (Idaho Technology, no. 1774).
11. Ethidium bromide (Sigma Co., no. E 8751).
12. Ficoll 400 (Sigma Co., no. F 4375).
13. Gel electrophoresis apparatus (minimum 13 cm × 20 cm gel size) with power supply (e.g. Owl Scientific, no. A1). One per group.
14. *Hae*III restriction enzyme (NEB, no. 108S or equivalent).
15. Male and female human genomic DNA (Sigma, nos D-3160, D-3035, or equivalent).
16. Hybridization bottles (150 mm × 35 mm) (e.g. HyBaid Co., no. H9084 or equivalent).
17. A hybridization oven (e.g. HyBaid Co., no. H9320 or equivalent). The oven should be capable of rotation at variable speeds.
18. M13mp RF1 DNA (Amersham Pharmacia Biotech, no. 27-1547-01).
19. MagnaGraph nylon membrane (0.22 µ pore size) (Osmonics/MSI., no. NJTHY00010).
20. NBT solution (Roche Molecular Biochemicals, no. 1 383 213).
21. Ten times PCR (polymerase chain reaction) buffer mix for the air cycler (Idaho Technology, no. 1781).

22. A plastic bag sealer (Fisher Scientific, no. 01-812-13 or equivalent).
23. Plastic bags (e.g. Kapak Co., no. 402 or Roche Molecular Biochemicals, no. 1666 649).
24. Primers: M13 V F, GGTACATGGGTTCCTATT and M13 V R, CCCTTATTAGCGTTTGCCAT.
25. Pyrex glass dishes (two per group).
26. Rotary platform shakers.
27. A Stratalinker® ultraviolet (UV) oven (Stratagen Co., no. 400071 or equivalent). In order to cross-link DNA to the nylon membrane efficiently the UV source should be capable of delivering 120 mJ cm^{-2}. Excessive cross-linking will decrease the hybridization efficiency.
28. Taq DNA polymerase.
29. Whatman 3MM chromatography paper (Whatman Co., no. 3030917).
30. X-phosphate solution (Roche Molecular Biochemicals, no. 1 383 221).
31. X-ray film BioMax Light (Kodak, 8 × 10 in no. 178–8207 or equivalent).

Solutions to prepare

1. Buffer A. Add 100 ml of buffer 1 (Roche Molecular Biochemicals Set) to 900 ml of sterilized distilled water. Store at 4°C.
2. Buffer B (blocking solution). Add 10 ml of blocking solution (Dig Wash and Block Buffer Set, Roche Molecular Biochemicals) to 80 ml of sterilized distilled water. Add 10 ml of maleic acid buffer (bottle 2, Dig Wash and Block Buffer Set, Roche Molecular Biochemicals). Always prepare freshly.
3. Buffer C (detection buffer): 0.1 M Tris–HCl (pH 9.5) and 0.1 M NaCl.
4. Denaturation solution: 0.5 N NaOH and 1.5 M NaCl. Prepare the solution using 10 N NaOH. The solution can be stored at room temperature for a few months. If a white precipitate forms, the solution should be discarded.
5. Depurination solution: 0.5 N HCl.
6. Ethidium bromide stock. (5 mg ml^{-1}): 100 mg ethidium bromide and 20 ml water. Dissolve the powder in the water by stirring under a chemical hood. Store at room temperature in a tightly closed, dark bottle.
7. Neutralization solution: 0.5 M Trisma base and 1.5 M NaCl. Add 60.5 g of Trisma base and 87.45 g of NaCl to 850 ml of deionized water. Dissolve the salts and adjust the pH to 7.5 with concentrated HCl. Fill with water to 1000 ml. Store at 4°C.
8. Ten percent SDS (sodium deodecyl sulfate) stock. Add 10 g of powder to 70 ml of distilled water and dissolve by slow stirring. Add water to a final volume of 100 ml and sterilize by filtration through a 0.45 μ filter. Do not autoclave. Store at room temperature.
9. Ten times SSC: 1.5 M NaCl and 0.15 M sodium citrate. Dissolve 87.5 g of NaCl and 44.1 g of sodium citrate in 850 ml of distilled or deionized water. Adjust the pH to 7.5 with 10 N NaOH and add water to 1,000 ml. Store at 4°C.

10. Standard DNA: 1 kb 100 µl Ladder DNA, 700 µl TE buffer, and 200 µl loading dye solution.
11. Stop solution (loading dye): 15 percent Ficoll 400, 5 M urea, 0.1 M sodium EDTA (pH 8.0), 0.01 percent bromophenol blue, and 0.01 percent xylene cyanol. Prepare at least 10 ml of the solution. Dissolve an appropriate amount of Ficoll powder in double-distilled or deionized water by stirring at 40–50°C. Add a stock solution of EDTA, powdered urea, and dyes and aliquot approximately 500 µl into microfuge tubes and store at –20°C.
12. Fifty times TAE (Tris–acetate EDTA) electrophoresis buffer: 2 M Trisma base, 1 M acetic acid, and 50 mM Na_2 EDTA (pH 8.0). Weigh 242 g of Trisma base and add to 800 ml of double-distilled or deionized water. Add 57.1 ml of glacial acetic acid and 100 ml of 0.5 M EDTA stock solution (pH 8.0). Dissolve the powder by continuous stirring for 30 minutes and add water to a final volume of 1 l. Do not autoclave. Store tightly closed at room temperature.
13. Washing solution II: two times SSC and 0.1 percent SDS.
14. Washing solution III: one times SSC and 0.1 percent SDS.

DNA Fingerprinting: Single-locus Analysis

Equipment and supplies

1. D2S44 probe (Promega, no. DK263A or equivalent).
2. MetaPhor™ agarose (FNC BioProducts, no. 501810).

Solutions to prepare

1. Hybridization solution: 0.5 m sodium phosphate (pH 7.2), 0.5 percent (v/v) Tween 20, and 1 percent casein (Hammerstein grade) or blocker casein in PBS (Pierce, no. 37528).
2. Twenty times SSC: 3 M NaCl and 0.3 M sodium citrate. Dissolve 175 g of NaCl and 88.2 g of sodium citrate in 900 ml of distilled or deionized water. Adjust the pH to 7.5 with 10 N NaOH and add water to 1,000 ml. Store at 4°C.
3. Stripping solution: 0.4 N NaOH and 0.1 percent SDS. The solution should be freshly prepared.
4. Ten times TBE (Tris–borate EDTA) electrophoresis buffer: 890 mM Tris base, 890 mM boric acid, and 20 mM EDTA. Dissolve the Tris and boric acid in deionized water and add the appropriate amount of 0.5 M EDTA (pH 8.0). Store at room temperature.
5. Ten times wash buffer I: 0.5 M sodium phosphate (pH 7.2) and 5 percent (v/v) Tween 20.

Equipment and supplies

1. Acetamide (Sigma Co., no. A 0500).
2. *Alu* primers: *Alu* F, CCTTCCACAGTGTATTGTGTC and *Alu* R, TAGAAATGTGTGGGACAGTTC.
3. Capillary tubes (10 µl) (Idaho Technology, no. 1705).
4. Low molecular weight DNA standard (BioMarker Low Bioventure Inc.).
5. Ten times high magnesium PCR buffer mix for an air cycler (Idaho Technology, no. 1781).
6. Ten times low magnesium PCR buffer mix for an air cycler (Idaho Technology, no. 1783).
7. TT Primers: TT F, TAATTGTTGGAGTCGCAAGCTGAAC and TT R, GCCTGAGTGACAGAGTGAGAACC.

Solutions to prepare

1. Fifty percent acetamide.
2. Low DNA standard: 150 µl (BioMarker Low), 70 µl TE buffer, and 40 µl BioTracker tracking dye. Store at –20°C.

DNA Sequencing

Equipment and supplies

1. An AeroMist disposable inhalator–nebulizer (Inhalation Plastic Inc., no. 4207).
2. Ampicillin, sodium salt (Sigma Co., no. A 9518).
3. ATP (100 mM) (Roche Molecular Biochemicals, no. 1 140 965 or equivalent).
4. DNA Sequencing Kit v2 (PE Biosystems, no. 4314417).
5. ElectroMax *Escherichia coli* cells DH 10 B (Life Technologies Inc., no. 318290015).
6. Electroporation cuvettes (0.1 mm gap) (Invitrogen Inc., no. P410-50).
7. Filtration cartridges (Edge Biosystem Inc., no. 42453 or equivalent).
8. Oak Ridge polypropylene 50 ml centrifuge tubes with caps (e.g. Nalgene® no. 21009).
9. PLG (Phase Lock Gel) I tubes (Eppendorf, no. 0032007953).
10. PCI (Phenol : CIA) mixture (Ambion, no. 9732).
11. Polypropylene culture tubes (Falcon, no. 2059 or equivalent). The

"Falcon 2059" tube of Becton Dickson Co. is the standard for transformation experiments. Other equivalent brands are acceptable, but batches of tubes are occasionally contaminated with surfactants that inhibit transformation.

12. pUC18 *Sma*I/BAP (Amersham Pharmacia Biotech, no. 27-4860-01).

13. Rapid DNA Ligation Kit (Roche Molecular Biochemicals, no. 1 635 379 or equivalent).

14. T4 DNA polymerase (NEB, no. 203S).

15. T4 polynucleotide kinase (NEB, no. 201S).

16. Transformation apparatus *E. coli* pulser (Bio-Rad, no. 3 165-2101 or equivalent).

17. Universal M13/pUC sequencing primer (NEB, no. 3 1211 or equivalent).

Solutions to prepare

1. Ammonium acetate (7.5 M). Dissolve 57.8 g of ammonium acetate in 60 ml of double-distilled or deionized water. Stir until the salt is fully dissolved. Do not heat to facilitate dissolving. Fill up to 100 ml and sterilize by filtration. Store tightly closed at 4°C. The solution can be stored for one to two months under these conditions. Long-term storage is possible at –70°C.

2. One thousand times ampicillin: 500 mg ampicillin and distilled or deionized water. Add 500 mg of ampicillin to 5 ml of distilled water. Sterilize by filtration and store in small aliquots at –20°C.

3. ATP (0.5 mM). Dilute 5 mM ATP solution ten times. Add 2 µl of 5 mM ATP stock solution to 18 µl of sterile water. Prepare the solution freshly. Do not store.

4. ATP (5 mM). Prepare 100 µl of solution. Add 5 µl of stock ATP solution to 95 µl of sterile 5 mM Tris–HCl (pH 7.5). Store at –20°C.

5. Seventy percent ethanol. Add 25 ml of double-distilled or deionized water to 70 ml of 95 percent ethanol. Never use 100 percent ethanol because it contains an additive that can inhibit the activities of some enzymes. Store in a –20°C freezer.

6. IPTG (25 mg ml^{-1}): 2.5 percent IPTG. Dissolve 250 mg of IPTG in sterilized water. Store at –20°C.

7. LB agar amp plates: 1 percent Bacto Tryptone, 0.5 percent yeast extract, 0.5 percent NaCl, 1.5 percent Difco agar, and 100 µg ml^{-1} ampicillin. Add the first three ingredients to 1 l of distilled water in a 2 l Erlenmeyer flask. Stir to dissolve all the ingredients completely. Adjust the pH to 7.5 with 1 N NaOH. This will take approximately 4 ml of 1 N NaOH. Add the Difco agar and sterilize by autoclaving for 20 minutes. Cool the medium to 60–65°C and add 1 ml of ampicillin stock solution. Mix by swirling the flask and pour the plates. This will make 25–30 plates. The plates can be stored for two to three weeks at 4°C.

8. Ten times phosphate stock solution: 0.72 M KH_2PO_4 and 0.17 M K_2HPO_4. Dissolve in water and autoclave for 20 minutes. Store at 4°C.
9. Solution II (plasmid preparation): 0.2 N NaOH and 10 percent SDS. Prepare freshly before use.
10. Terrific broth medium (TB): 1.2 percent Bacto Tryptone, 2.4 percent yeast extract, 0.4 percent glycerol, and ten times phosphate stock solution. Mix the first three ingredients in 900 ml of deionized water and autoclave for 20 minutes to cool to room temperature and add 100 ml of phosphate stock solution. Store at 4°C.
11. X-gal (20 mg ml^{-1}): 2 percent X-gal and DMSO. Dissolve 200 mg of X-gal in 10 ml of DMSO. Store in the dark at –20°C. DMSO is used instead of the commonly used DMF (dimethylformamide) for X-gal preparation because DMF is very toxic.

Determination of Human Telomere Length

Equipment and supplies

1. Wizard Genomic Purification Kit (Promega, no. TM050).
2. *Hinf*I restriction endonuclease (NEB, no. 155S or equivalent).
3. *Rsa*I restriction endonuclease (NEB, no. 167S or equivalent).
4. Telo TAGGG Telomere Length Assay (Roche Molecular Biochemicals, no. 2 209 136).
5. TurboBlotters (one per group) (Midwest Scientific Co., no. 10-439-012).

RT-PCR of Human Genes

Equipment and supplies

1. RNaseZap™ solution (Ambion, no. 9780 or equivalent).
2. RNAwiz™ solution (Ambion, no. 9736 or equivalent).
3. RNAsecure™ resuspenstion solution (Ambion, no. 7010 or equivalent).
4. Formaldehyde load dye (Ambion, no. 3 8552).
5. Titan™ One Tube RT-PCR Kit (Roche Molecular Biochemicals, no. 1 939 823).
6. Human β-actin primers: forward, CCAAGGCCAACCGCGAGAA-GATGAC and reverse, AGGGTACATGGTGGTGCCGCCAGAC.

Index

Page numbers in *italics* refer to Figures; those in **bold** to Tables.