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1.0 Purpose

This SOP details the procedures, methods, material selections, and equipment required to perform highly reproducible agarose gel electrophoresis (AGE) separation and gel staining for a variety of nucleic acid forms. The subsequent recording and analysis of the gel image is performed per **SOP 22120, *Digital Gel Imaging Using the Kodak 400 Image System,*** or other appropriate document.

2.0 Scope

This SOP contains general methods to perform agarose gel electrophoretic separation and staining based detection of a variety nucleic acids, including but not limited to, ssDNA, oligonucleotides (oligos), dsDNA, supercoiled plasmid (sc)DNA, open circular plasmid (o)DNA, linear plasmid (l)DNA, restriction endonuclease fragments, mtDNA, gDNA, mRNA, rRNA, tRNA, PCR amplicons, and DNA/RNA hybrids. The procedures in this SOP refer to the AGE analysis of nucleic acid sizes ranging from 10 bp to 50Kbp and include both non-denaturing (primarily for DNA work) and denaturing (primarily for RNA work) AGE methods. The methods contained in this SOP are designed to provide the highest level of gel and image reproducibility with a minimum of gel artifacts. This SOP also provides for the “environmentally friendly” disposal of potentially mutagenic staining solutions and stained gels in excess of current OSHA requirements. This SOP refers to, but does not include, specific information required for the capture of gel images (refer to **SOP 22120, *Digital Gel Imaging Using the Kodak 400 Image System***) nor does this SOP include specific product or project information.

3.0 Authority and Responsibility

3.1 The Director, Process Analytics/Quality Control (PA/QC) has the authority to define this procedure.

3.2 PA is responsible for training laboratory personnel and documenting this training to Biopharmaceutical Quality Assurance (BQA).

3.3 PA/QC personnel are responsible for the performance of this procedure.

3.4 PA/QC is responsible for reviewing the data and documentation of the results of this procedure.

3.5 BQA is responsible for quality oversight of this procedure.

4.0 Reagents and Materials

- 5X TBE “AccuGene” Buffer (20L carboy), BioWhittaker BDP PN 50836
- 10X TAE “AccuGene” Buffer (4L), BioWhittaker BDP PN 50841
- 10X MOPS “AccuGene” Buffer (1L), BioWhittaker BDP PN 50876
- Ultrapure Agarose, Invitrogen BDP PN 10175
- SeaKem Gold Agarose (125g), BioWhittaker BDP PN 50150
- SeaKem LE Agarose (500g), BioWhittaker BDP PN 50004
- SeaKem GTG Agarose (500g), BioWhittaker BDP PN 50074
- NuSieve 3:1 Agarose (125g), BioWhittaker BDP PN 50090
- MetaPhor Agarose (125g), BioWhittaker BDP PN 50180
- 10X BlueJuice™ gel loading buffer, LTI/Invitrogen BDP PN 30177
- Pipette tips as required
- 1.5 mL Micro-centrifuge tubes as required

Assorted DNA or RNA nucleic acid standards, markers, and ladders as required.

- Invitrogen 500 bp ladder BDP PN 30217
- Invitrogen 250 bp ladder BDP PN 30209
- Invitrogen 50 bp ladder BDP PN 30210
- Invitrogen 1Kb Plus ladder BDP PN 30441 or 30433
- Invitrogen High Molecular Weight ladder BDP PN 30216
- Invitrogen Low Mass ladder BDP PN 30220
- Invitrogen Supercoiled DNA ladder BDP PN 30219
- Invitrogen 10 bp ladder BDP PN 30740
- Invitrogen Oligo sizing marker BDP PN 30739
- Fermentas Mass Ruler Mix BDP PN 30743
- Other appropriate nucleic acid standards or markers as required.

Assorted Nucleic Acid Dyes and Stains for detection in gels as required.

- Invitrogen Ethidium Bromide BDP PN 10166
- Invitrogen SYBR Green BDP PN 30202
- Invitrogen SYBR Gold BDP PN 30203

Other appropriate nucleic acid stains and dyes as required.

5.0 Equipment

- Biometra (formerly LTI) Horizon 11 x 14 cm Gel Apparatus PN 11068012
- Biometra (formerly LTI) Horizon 20 x 25 cm Gel Apparatus PN 21069018
- Biometra Gel Apparatus Power Cords PN 11099041
- Biometra Gel Casting Dams (11 x 14 cm) PN 11068053
- Biometra Gel Casting Dams (20 x 25 cm) PN 21069059
- Biometra Gel Apparatus acrylic UVT gel tray (11 x 14 cm) PN 11084019
- Biometra Gel Apparatus acrylic UVT gel tray (20 x 25 cm) PN 31006026
- Biometra (formerly LTI) Assorted Delrin Gel Combs as required.
  - 14 tooth, 2 mm thick PN 31081029
  - 14 tooth, 1 mm thick PN 31081011
  - 10 tooth, 2 mm thick PN 1195108
  - 10 tooth, 1 mm thick PN 11951068
  - 20 tooth, 1 mm thick PN 11951076
  - 20 tooth, 2 mm thick PN 11951092
  - 12 tooth, 1 mm thick (multi-channel) PN 11951175
  - 24 tooth, 1 mm thick (multi-channel PN 11951159
  - Preparative comb, 2 mm thick PN 61010021
  - 15 tooth, 1 mm thick (20 x 25 gel) PN 11953072
  - 30 tooth, 1 mm thick (20 x 25 gel) PN 11951043
  - 21 tooth, 1 mm thick (20 x 25 gel, multi-channel) PN 11951183
  - 42 tooth, 1 mm thick (20 x 25 gel, multi-channel) PN 11951167
  - Preparative comb, 3 mm thick (20 x 25 gel) PN 41086018
- VWR Model 500 AccuPower Power Supply PN 13272-264
- VWR Low Flow Peristaltic Buffer Re-circulation Pump PN 54856-070
- Daigger High Flow Rate Peristaltic Waste Filtration Pump PN LX20715N
- VWR Gel Staining Trays or Staining Boxes PN 62686-283
- Micro-Balance or Analytical Balance (0.5 – 5 g range measurements).
- Various TYGON vinyl tubing and plastic connectors as required for pumps
- Bubble Level for Gel Apparatus prior to gel pouring
- 65°C Water Bath with thermometer
- 500 mL Erlenmeyer Flasks
- Graduated Thermometer for gel solution temperature
- 1L or 5L Graduated Cylinders

- Microwave Oven
- Gel Scoops (plastic or acrylic only)
- Electronic Alarm/Timer
- Single or Multi-channel Pipettes as required
- Micro-centrifuge tube Vortexer
- Micro-centrifuge tube Centrifuge
- NEN/Kodak 440CF Gel Imaging Station (refer to SOP 22120, Digital Gel Imaging Using the Kodak 400 Image System)

5.1 For previously developed QC assays, follow the assay profile and/or product-specific SOP for the detailed gel electrophoresis set-up requirements. Information contained in sections 6.2 through 6.7 of this SOP may not be necessary.

5.2 Selecting the correct Molecular Weight Standard (MWS) ladders and markers.

5.2.1 Determine each test sample's chemical base structure (DNA or RNA), form (single or double stranded), topology (linear and open-circle, or supercoiled) and the expected size range of the test samples (from 10 bp up to ~50 Kb).

5.2.2 Independent gels will be required for samples of differing chemical identity or form (DNA vs. RNA and ssDNA vs. dsDNA).

5.2.3 Choose the standard ladder(s) or marker(s) that have similar chemical base structure, form, and topology, and that contain bands which bracket the expected test sample band size(s). Use of more than one standard on a gel is encouraged, especially if the expected size range is speculative or if a broad range (greater than a factor of 10 from the smallest to the largest band) is expected for a sample.

5.2.4 Many gels will contain several samples with differing topology or sizes. Separate standard markers or ladders are required for each type of quantized sample to be run on a gel. For example, separate ladders are required for supercoiled plasmids and restriction cut (linearized) plasmids.

5.2.5 If at all possible, a sample control lot or a prior lot sample should be run as an additional control on the gel. The control or prior lot sample(s) should be treated similarly as the test sample(s) to verify the test sample identity.

5.3 Electrophoresis Running and Gel Buffer Selection

5.3.1 Use 0.5X or 1.0X (final concentration) TBE buffer for all non-preparative gel assays where the maximum nucleic acid size is < 12 Kbp.

5.3.2 Use 1.0X (final concentration) TBE buffer for all non-preparative gel assays where the maximum nucleic acid size is expected to be > 12 Kbp and long migration distances (> 8 cm) or high voltage conditions (> 8 V/cm) are to be used.

5.3.3 Use 1.0X (final concentration) TAE buffer for all preparative gel-based purification methods and for assays requiring resolution of bands > 12 Kbp in size.

5.3.4 Use 1.0X (final concentration) MOPS based buffer for all denaturing gel applications, such as assays requiring RNA, oligonucleotide, and ssDNA electrophoretic separation. See required buffer formulas below.

- MOPS Formaldehyde + Formamide loading, Formaldehyde gel, and running buffer formulas for denaturing gel electrophoresis
- MOPS Formaldehyde running buffer (1X) – Use BioWhittaker 10X MOPS solution PN 50876 and dilute to 1X with purified or WFI water
- MOPS Formaldehyde gel buffer (1X), for a 1L solution use:

  178.6 mL 37% Formaldehyde stock solution (2.2M [final])

  100 mL 10X MOPS and 721.4 mL purified or WFI water
- MOPS Formaldehyde sample loading buffer (1X), 20 applications of solution:

  20 μL 10X MOPS.

  200 μL formamide (> 98% pure).

  70 μL 12.32M (37%) Formaldehyde.

  Use 17.5 μL of loading solution to every 2.5 μL of sample.

5.4 Determining Lane Loading Masses and Loading Volumes

Limits to loading mass in agarose gels are based on the gel percentage and the comb tooth **area (tooth width multiplied by the sample load height) in the well. The following chart will provide an upper limit for loaded mass based on a typical 4 mm high, Ethidium Bromide stained, 1% gel and a maximum 3.2 mm sample load height in the wells.**

GEL LOADING CHART – See gel parameters above

Comb Dimensions	Tooth Width (mm)	Max Sample Volume (μL)/Well	Max Load Mass/Well
14 tooth, 1 mm thick	4.7	15	250 ng
14 tooth, 2 mm thick	4.7	30	250 ng
10 tooth, 1 mm thick	7.9	25	420 ng
10 tooth, 2 mm thick	7.9	50	420 ng
20 tooth, 1 mm thick	3.8	12	200 ng
20 tooth, 2 mm thick	3.8	24	200 ng
12 tooth, 1 mm thick	7.2	20	385 ng
24 tooth, 2 mm thick	2.8	15	150 ng
15 tooth, 1 mm thick	9.5	30	510 ng
30 tooth, 1 mm thick	4.7	15	250 ng
21 tooth, 1 mm thick	7.2	20	385 ng
42 tooth, 1 mm thick	2.8	15	150 ng
H11 x 14 Preparative, 2 mm	92.0	600	5000 ng
H20 x 25 Preparative, 3 mm	165.0	1600	85500 ng

5.4.1 For SYBR Gold or Green stained gels, reduce the load mass per well by 5X for optimal resolution. This does not apply to preparative purification gels.

5.4.2 For Gels of varying agarose percentage (> or < 1%), divide the maximum load mass per well (for the 1% gel in the table above) by the desired gel percentage to obtain the corrected maximum load mass per well.

5.4.3 Poured gel heights less than 3 mm and greater than 5 mm are not recommended. If a gel pour height change is made, adjust the maximum sample load height accordingly to arrive at the correct sample volume and maximum mass load per well.

5.4.4 Adding more mass per well will result in overloading of the gel lane causing reduced linearity of quantitation, band spreading, reduced sizing accuracy, and with extreme overloading, excessive band smearing. Some protocols will require overloading gel lanes (in some cases excessively) in order to detect the presence of low mass or low copy number contaminants in a sample.

5.5 Designing the Gel-Loading Scheme

5.5.1 Determine the number and various types of samples that the test protocol requires on the gel(s).

5.5.2 Include the presence of any control or previous lot samples if available.

5.5.3 If possible, do not use the outside wells (lanes) of the gel since excessive band “smiling” usually occurs due to reduced electrophoretic mobility in the outer gel lanes.

5.5.4 Include the required or selected standards and markers. Markers and standards must bracket their respective test and control sample lanes in order to allow accurate sizing of the sample bands between the standards.

5.5.5 Determine the total number of lanes (comb wells) required for the assay based on the above requirements.

5.5.6 Fill out the Lane Designation Form for each lane prior to pouring the gel.

5.6 Selecting the ideal gel size, comb specifications, and sample volumes

5.6.1 Based on the required gel loading scheme and the lane designation form, determine if more than one gel is required to complete the assay, select the correct size gel (11 x 14 cm is recommended for most assays), and select the comb that provides the least number of unused wells (lanes) on the gel; not including the two empty outer lanes if feasible.

5.6.2 If the sample volumes are greater than the well volume permits, either use a thicker comb (2 mm vs. 1 mm) or reduce the load mass per well.

Note: Do not load the well with a greater volume than the amount indicated on (or calculated from) the loading chart. Overloaded wells will result in band doublets and render the assay invalid. Band doublets result from overloaded wells due to the upper portion of the gel experiencing a greater electrophoretic current than the interior region of the gel.

5.6.3 Record the gel comb used on the AGE Assay Form

Varying the percentage (w/v) of agarose of a gel will shift the region of optimal size resolution. Select the appropriate gel percentage based on the expected si**ze(s) of the test and control samples. If the expected band sizes of a sample (or multiple samples) differ by >15X, two gels of differing percentages or agarose types may be necessary. Alternatively, longer migration distances (> 10 cm) and run times may be used with some loss in resolution for smaller bands.**

5.6.4 Optimal size resolution (in bp) of selected agarose types and percentages (w/v) using the indicated running/gel buffer and a 7-10 cm indicator dye migration distance.

Size Range (bp)	Agarose Type	TBE Optimal Gel % (w/v)	MOPS/TAE Optimal Gel % (w/v)
100-3000	SeaKem LE/GTG or LTI Ultrapure	1.75	2.00
200-4000	SeaKem LE/GTG or LTI Ultrapure	1.25	1.50
300-7000	SeaKem LE/GTG or LTI Ultrapure	1.00	1.20
400-8000	SeaKem LE/GTG or LTI Ultrapure	0.85	1.00
800-10000	SeaKem LE/GTG or LTI Ultrapure	0.70	0.80
1000-23000	SeaKem LE/GTG or LTI Ultrapure	0.50	0.60
5000-50000	SeaKem Gold and LTI Ultrapure	N/A	0.30
10-100	NuSieve 3:1	5.0	6.0
100-500 NuSieve 3:1		3.0	4.0
500-1000	NuSieve 3:1	2.0	3.0
<80	MetaPhor	5.0	N/A
20-130	MetaPhor	4.0	5.0
50-250	MetaPhor	3.0	4.0
100-600	MetaPhor	2.0	3.0
150-800	MetaPhor	1.8	2.0

Other high purity agaroses can be substituted for the Cambrex SeaKem LE/GTG brands and Invitrogen/LTI Ultrapure Agarose.

5.7 Preparing the Running and Gel Buffer

5.7.1 Required gel solution volumes – refer to the following chart to determine the volume of gel solution required for the application:

Gel Size (cm)	Gel Thickness (mm)	Volume of Gel to be Poured	Volume of Gel to Make
11 x 14	3	47 mL	100 mL
11 x 14	4	63 mL	100 mL
11 x 14	5	78 mL	100 mL
20 x 25	4	202 mL	300 mL
20 x 25	5	252 mL	300 mL
20 x 25	6	302 mL	400 mL

Note: These volumes include a normal solution loss of ~1 mL for 11 x 14 cm gels and 2 mL for 20 x 25 cm gels.

5.7.2 Determining the volume of running and gel buffer required

5.7.2.1 Refer to the **following chart to determine the volume range of** running buffer required for electrophoresis.

Gel Size (cm)	Minimum Volume (mL)	Maximum Volume (mL)
11 x 14	800	1000
20 x 25	1600	2000

5.7.2.2 Add the “maximum running buffer volume” and the “gel solution volume to make” together to determine the minimum amount of diluted TBE or TAE buffer (not MOPS buffers since the gel and running buffers are different) to be made for the assay.

5.7.2.3 Dilute the desired gel and running buffer to the final concentration of use indicated in section 6.3 with purified water (distilled R.O. water or Millipore water, nuclease free, or bottled WFI), in a graduated cylinder.

5.7.2.4 Thoroughly mix the diluted buffer by covering the graduated cylinder with parafilm and inverting 3X or by magnetic stirring for a minimum of 3 minutes.

5.7.3 Record the running buffer, volume made, and concentration on the AGE Assay Form .

5.8 Preparing the Gel Apparatus, Gel Tray, Comb(s), and Peristaltic Pump

Note: Use of a peristaltic pump is optional for 11 X 14 cm gels.

5.8.1 Ensure the gel apparatus is level in both horizontal directions by placing a bubble level in the middle of the apparatus’ center “island.” If the apparatus is not level, the apparatus feet can be adjusted in height by rotating them.

5.8.2 Assemble the gel apparatus (box) by inserting a clean acrylic UVT gel tray on top of the center "island." Each UVT tray has two sets of cutouts for locating two gel combs. For QC assays, never use the centrally located comb position, always use the tray end location only. Ensure that the end comb cutouts are facing toward the black (negative) electrode end of the apparatus.

5.8.3 Insert the black gel casting dams on either end of the UVT tray. The dams' interior edges should be vertically straight when inserted properly. Ensure the dams make a tight seal against the UVT tray ends.

5.8.4 Insert the previously selected or required gel comb in to the UVT tray's end comb cutouts. The comb teeth should be as close to the negative electrode as possible. Ensure that the comb is fully seated and that none of the teeth are touching the UVT tray. Do not pour the gel if the comb teeth contact the UVT tray, replace the tray if necessary (since it is probably warped).

Note: Pouring excessively hot agarose solutions (> 70°C) on to the UVT tray will cause it to warp in the center and force the comb teeth to come into contact with the tray ruining the gel. Never pour gel solutions hotter than > 70°C.

5.8.5 Insert the colored power cords into a vertical connection set on the power supply, match red to red (positive) and black to black (negative). Do not attach the connectors to the gel apparatus until after loading.

5.8.6 Connect the TYGON buffer re-circulation tubing into each reservoir quick-connect located at the rear of the apparatus. If necessary, connect the free tubing ends (without a connector) on the peristaltic pump tubing. The direction of buffer circulation is not critical.

5.8.7 Set the peristaltic pump rheostat to the speed indicated on the pump housing and based on the gel size (11 x 14 cm or 20 x 25 cm). Do not start the pump until after the gel is loaded and electrophoresis has commenced for 5 minutes.

5.9 Preparing the Agarose Solution

5.9.1 Make the desired gel percentage solution by first determining the mass of powdered agarose required (See Attachment 1).

5.9.2 Multiply the desired gel percentage by the "gel volume to make" (from chart 6.8.1) divided by 100 mL = g of agarose required.

5.9.3 Weigh out the calculated amount of agarose with an analytical balance and add the measured agarose to a 500 mL (11 x 14 cm gel) or 1L (20 x 25 cm gel) Erlenmeyer flask.

5.9.4 Measure out the "gel volume to make" of diluted running buffer (or gel buffer for MOPS gels) from chart 6.8.1 and pour the buffer into the 500 mL Erlenmeyer flask containing the agarose.

5.9.5 Weigh and record the mass of the agarose solution, including the Erlenmeyer flask.

5.9.6 Mix the buffer-agarose solution by swirling and place in a microwave oven that allows viewing the oven contents.

5.9.7 Heat the agarose solution on high power just until the agarose dissolves (~60 - 120 seconds, with high percentage solutions taking >2 minutes) and begins to boil. **Do not let the solution boil over! If the solution boils over the flask mouth, discard the solution and begin section 6.10 again; reduce the length of heating.** Visually ensure that all of the agarose has gone into solution before continuing. For high percentage solutions (>2%), two or three microwave heating sessions may be required with swirl mixing in between each heat step to ensure the agarose has completely dissolved.

5.9.8 Remove the flask with the dissolved agarose after 15 seconds and reweigh.

Caution: The flask will be hot, remove the flask using tongs or thermal gloves, do not use your hands! Agitating the flask may result in boil over if not left for 15 seconds in the microwave after heating.

5.9.9 Add purified or WFI water back to the agarose solution until the mass originally recorded in section 6.10.5 is reached. Immediately mix the solution by swirling. This step is critical to ensure that the desired gel percentage is actually used, since water evaporation effectively increases the agarose percentage.

5.9.10 Insert a rod thermometer into the agarose solution to monitor the temperature.

5.9.11 Cover the Erlenmeyer flask mouth (thermometer inserted) with parafilm or plastic wrap and place in a pre-heated 65°C water bath.

5.9.12 Monitor the solution temperature until 70°C is reached, remove the flask from the water bath and pour the required “agarose gel pour volume” obtained from chart 6.8.1 into a small plastic (NOT glass or Pyrex) graduated cylinder. Immediately pour the agarose solution per section 6.11.

5.10 Gel Pouring Methods and Procedures

5.10.1 Only pour the amount of gel solution required for the desired gel height – see section 6.10.12. Ideally, only this amount should be in the graduated cylinder.

5.10.2 The optimal gel solution is between 65°C and 70°C for pouring, do not pour a gel solution outside this temperature range. Low solution temperature will result in premature solidification of the gel prior to completing the pour, while a high gel temperature will result in the UVT tray warping (especially with 20 x 25 cm gels) and dislodging the gel comb. If either of these events occurs, DO NOT continue the pour - clean the apparatus and re-make the gel solution.

5.10.3 The pour should be rapid (< 4 seconds) but not create any air bubbles in the gel. Initially pour the gel solution near the center of the comb and ensure that all the teeth are surrounded by the solution (no air bubbles), continue the pour in to center of the tray until complete.

5.10.4 While the solution remains liquid, small air bubbles on the gel surface can be removed by using a pipette tip.

5.10.5 Ensure that gel solution is not leaking past the casting dams and that the gel comb is fully immersed in solution. Ensure that the comb is still fully seated in the UVT tray cutouts and that the comb teeth do not have air bubbles below them. If necessary, the comb can be removed and reseated in the solution within the first 10 seconds of a pour.

5.10.6 Close the gel apparatus lid and wait a minimum of 45 minutes for gel polymerization prior to adding running buffer. Do not leave a gel polymerize for longer than 4 hours without adding running buffer.

5.10.7 While the gel is polymerizing, prepare the samples as indicated in section 7.0.

5.10.8 Record the gel type, thickness, volume poured, percentage, pouring temperature, and polymerization time on the AGE Assay Form.

6.0 Procedures for Sample Preparation and Loading

6.1 Selection and Preparation of Loading Dye Solutions

Most gels commonly will use Bromophenol Blue (BPB) and/or Xylene Cyanol (XC) as the indicator dye(s) for electrophoretic migration. LTI BlueJuice™ only uses Bromophenol Blue while the MOPS and MBI Loading solutions utilize both dyes. Only use the minimum amount of dye necessary to monitor a run since the dyes themselves can co-migrate with a band of interest and interfere with band quantitation. Refer to the following chart for selecting the appropriate dye(s) for the expected minimum band size.

Electrophoretic mobility (relative to dsDNA bp) of common indicator dyes in gels

Gel Type	% Agarose	TAE/MOPS buffers (bp)		TBE buffers (bp)	
		BPB	XC	BPB	XC
SeaKem or LTI Ultrapure	0.50	1650	11000	1350	1200
SeaKem or LTI Ultrapure	0.75	1000	10200	720	9200
SeaKem or LTI Ultrapure	1.00	500	6100	400	4100
SeaKem or LTI Ultrapure	1.25	370	3560	260	2500
SeaKem or LTI Ultrapure	1.50	300	2800	200	1800
SeaKem or LTI Ultrapure	1.75	200	1800	110	1100
SeaKem Gold or LTI Ultrapure	0.30	3550	24800	N/A	N/A
NuSieve 3:1	2.00	220	1800	100	1250
NuSieve 3:1	3.00	80	650	40	500
NuSieve 3:1	5.00	30	200	4	90
MetaPhor	1.80	85	575	45	350
MetaPhor	2.00	70	480	40	310
MetaPhor	3.00	40	200	35	140
MetaPhor	4.00	35	120	30	85
MetaPhor	5.00	30	85	15	60

6.1.1 Determine the maximum sample or standard load size and calculate the amount of the selected loading dye necessary to achieve a final 1X loading solution.

For example: If two sample volumes to be run are 5 μL and 8 μL, make the final load volume 10 μL for both samples. The volume difference will be made up with the loading solution and water (or running buffer). 1 μL of the 10X BlueJuice™ loading buffer will be required for each sample to obtain a 1X final loading buffer concentration. The remaining 4 μL and 1 μL, respectively, should be made up with water or running buffer.

6.1.2 Record the final sample volume and indicate the marker dye(s) used on the Line Designation Form, AGE Assay Form.

6.2 Preparing the Samples

6.2.1 Make each sample per section 7.1.2 in individually labeled micro-centrifuge tubes. The label should indicate the well number it is to be loaded into. Lanes are numbered Left to Right from the top of the gel. Remember - the first and last lanes (wells) are not typically used.

6.2.2 Mix the completed samples [sample or standard + loading buffer + water or running buffer, if necessary] by bump vortexing 3 times and spinning down in a micro-centrifuge for 10-30 seconds.

6.3 Sample Pre-heating

6.3.1 Transfer the sample and standard centrifuge tubes to a 65°C ± 3°C water bath for 10 minutes (± 1 minute) immediately prior to loading in order to reduce weak intermolecular and intramolecular interactions.

6.3.2 Samples must be reheated at 65°C ± 3°C for 10 minutes (± 1 minute) if the gel cannot be loaded when initially expected.

6.3.3 Record the sample heating time and temperature on the AGE Assay Form.

6.3.4 While the samples are being heated, continue with Section 7.4.

6.4 Addition of the Running Buffer and Comb Removal

6.4.1 Once the gel is polymerized, open the apparatus lid, and add the desired amount of diluted (0.5X or 1X) running buffer to the apparatus reservoirs. Do not pour the buffer directly on the gel.

6.4.2 The running buffer should cover the gel to a depth of 3-5 mm.

6.4.3 Slowly remove the comb by pulling it straight up. If necessary, a slight rocking motion can be used to initially free the comb from the gel.

6.4.4 For combs with teeth <3 mm wide, it may be necessary to flush the wells with running buffer using light pressure to dislodge small pieces of free agarose and air bubbles from the interior of the wells prior to loading.

6.4.5 Remove any air bubbles from beneath the UVT tray prior to loading the gel by re-seating the UVT tray. Be careful not to dislodge or move the gel off the UVT tray.

6.5 Gel Loading Methods

6.5.1 Load the samples and standards in the wells according to the loading scheme.

6.5.2 Place the pipette tip in the middle of the well and below the well surface before expelling the sample into the well. Ensure that the tip does not pierce the well bottom!

6.5.3 The pipette tip can be steadied using two hands while loading, one hand to stabilize the pipette shaft and the other to operate the plunger.

6.5.4 Load all lanes (wells) of the gel within 10-15 minutes, excessive loading times allow for sample diffusion. Dye diffusion will occur within 1-2 minutes and is not a significant concern.

6.5.5 Once loading is complete, gently close the apparatus lid and insert the electrophoresis power leads into the appropriate color-coded receptacles.

7.0 Procedures for Running Agarose Gels

7.1 Selecting Electrophoretic Voltage and Current Parameters

7.1.1 Horizontal agarose gels should be run within specified voltage gradients and voltage ranges according the chart below.

Agarose Gel Type	Gel Apparatus	Optimal Voltage Gradient (V/cm)	Optimal Voltage Range (V)
SeaKem LE/GTG or LTI Ultrapure	11 x 14	2-8	47-188
SeaKem Gold (>12Kb) or LTI Ultrapure	11 x 14	1-2	23.5-47
NuSieve 3:1	11 x 14	4.5-5	106-118
MetaPhor	11 x 14	4.5-5	106-118
SeaKem LE/GTG or LTI Ultrapure	20 x 25	2-8	68-272
SeaKem Gold (>12Kb) or LTI Ultrapure	20 x 25	1-2	34-68
NuSieve 3:1	20 x 25	4.5-5	153-170
MetaPhor	20 x 25	4.5-5	153-170

In general, select lower voltages for larger (>5 Kb) expected band sizes and higher voltages for smaller (<1 Kb) band sizes. Buffer type is not critical, although long runs (or samples >12 Kbp in size) using TAE should use very low voltages. See SeaKem Gold on the above chart.

The cm distance is measured for the gradient calculations between the apparatus electrodes and is not based on the gel length. The 11 x 14 cm apparatus has an electrode separation of 23.5 cm. The 20 x 25 apparatus has an electrode separation distance of 34.0 cm.

7.1.2 Once the gel apparatus is connected to the power supply, turn on the power supply and select [Constant Voltage], use the [Select] button to view Volts on the digital display and use the rotary potentiometer to select the desired running Voltage.

7.1.3 Press the [Run/Stop] Button to start the electrophoretic run. Bubbles should appear on the apparatus' submerged electrodes.

7.2 Determining Gel Run Times and/or Optimal Run Distance

7.2.1 Once the correct agarose type and gel apparatus is chosen, run a gel until the band whose size is the "average" of the smallest and largest bands of interest will have migrated mid-way through the gel. For 11 x 14 cm gels, this is between 6-7 cm; for 20 x 25 cm gels, this is 12-14 cm.

7.2.2 Gauge the migration distance of the "average" band using the nearest loading dye front and the migration rate chart in 7.1.1.

7.3 Use of the Running Buffer Peristaltic Recirculation Pump (if used)

7.3.1 Once the gel has been running for 5-10 minutes, start the peristaltic pump by pressing the switch to [Forward]. Ensure that the flow rate is correctly set according to Section 6.9.7.

7.3.2 Air will purge from the line and buffer recirculation will start within ~2 minutes. Continue buffer recirculation until the run is complete.

7.3.3 Ensure that there are no buffer leaks from the recirculation fittings.

7.4 Stopping (and restarting) the Electrophoretic Run

7.4.1 To stop a completed run (desired migration distance is achieved), press the [Start/Stop] button on the power supply.

7.4.2 If at any time the run needs to be stopped for an emergency, to remove condensation from the apparatus lid, or for a test stain, simply press the [Start/ Stop] button on the power supply. Gels may be removed and returned to the running buffer and electrophoresis continued within 4-8 hours with minimal band diffusion in the gel.

7.4.3 Record the gel running time, voltage, and migration distance on the AGE Assay Form.

7.5 Disassembly of the Gel Apparatus, Cleaning, and Disposal of Buffers

7.5.1 Once the electrophoresis is complete and the gel transferred to the desired staining solution, start disassembling and cleaning the apparatus.

7.5.2 Stop the peristaltic pump, disconnect and drain the buffer recirculation lines in the sink. If using a MOPS/Formaldehyde buffer, drain into a Hazardous Chemical Waste receptacle.

7.5.3 Pour the remaining running TAE or TBE buffer in the apparatus in the sink. For MOPS/Formaldehyde buffer, pour into a Hazardous Chemical Waste receptacle.

7.5.4 Rinse the apparatus and recirculation lines with purified water, drain, and towel dry.

7.6 Cleaning the Gel Apparatus Accessories

7.6.1 Clean the UVT tray, gel casting dams, and combs with detergent and rinse with purified or WFI water, and air dry.

7.6.2 Ensure that any trace of dried agarose have been removed from the comb and UVT tray.

8.0 Procedures for Staining Agarose Gels

8.1 Selecting the Ideal Nucleic Acid Gel Stain

8.1.1 Use the following chart to determine the best stain for your agarose gel application.

Nucleic Acid Type	Optimal Staining Dye	Other Available Staining Dyes
ssDNA	SYBR Green II	SYBR Green I, Eithidium Bromide
Oligonucleotides	SYBR Green I	SYBR Green II, Eithidium Bromide
dsDNA (all forms)	SYBR Gold, Ethidium Bromide	SYBR Green I
PCR Amplicons	SYBR Gold, Ethidium Bromide	SYBR Green I
Restriction Fragments	SYBR Gold, Ethidium Bromide	SYBR Green I
RNA (all types)	SYBR Green II	Eithidium Bromide
DNA/RNA hybrids	SYBR Green II	SYBR Green I, Eithidium Bromide

See the Molecular Probes Handbook for more detailed information.

Note: The SYBR stains are more sensitive, and have less general background fluorescence, as does Eithidium Bromide.

8.2 Preparation of the Gel Staining Solution

8.2.1 For 11 x 14 cm gels, make 400 mL of a 0.5-1X staining buffer solution. SYBR stains are sold as 10,000 X stock solutions, for Eithidium Bromide 1 X concentration is equivalent to 1.0 μg/mL (the EtBr stock solution is 10 mg/mL).

8.2.2 For 20 x 25 cm gels, make 800 mL of the 0.5-1X staining buffer.

8.2.3 Make the staining buffer dilution in a labeled, dated, opaque plastic container and mix by inversion 3X or magnetic stirring for 5 minutes. Do not expose the stains to unnecessary light, as this will cause degradation of the stain.

Caution: All nucleic acid stains are by nature mutagens and should be handled wearing gloves and a lab coat at all times! Of the stains referred to in this SOP, Eithidium Bromide has the highest mutagenicity (per Ames testing).

8.2.4 Store working gel staining solutions (0.5 - 1X solutions) at room temperature and protected from stray light. Working stain solutions are to be used only for 2 days maximum. Store stock (10,000X) staining solutions at the appropriate indicated temperature in the dark.

8.2.5 Record the stock lot and date of dilution of all stains made for QC assays on the AGE Assay Form.

8.3 Optimal Gel Staining Times

8.3.1 Product-specific SOP will contain the desired staining time (and stain concentration) to be used with the assay. If not or if the assay is a developmental run, refer to section 9.3.2.

8.3.2 Nucleic acid stains require from 30-90 minutes for complete staining, staining time is dependent on the gel percentage and gel thickness. For a typical 1%, 4 mm thick gel, 45-60 minutes is optimal.

8.3.3 Thicker gels (> 4 mm) and higher gel percentages (>1.2%) require longer staining times, 60-90 minutes.

8.3.4 Thinner (< 4 mm), lower percentage gels (< 0.8%) can utilize shorter staining times, 30 - 45 minutes.

Note: Excessive staining times with Eithidium Bromide can lead to excessive background fluorescence.

8.3.5 If necessary, gels may be de-stained in running buffer (or water if no further electrophoresis is to be performed) to reduce the level of undesired background staining intensity.

8.3.6 Place the gel into a staining tray and pour the working 0.5-1X stain solution over the gel. Ensure that the gel is completely covered and there are no air bubbles beneath the gel.

8.3.7 Cover the staining tray with aluminum foil and wait the indicated time before removing the gel for imaging. Use a plastic/acrylic gel scoop to move the stained gel to the Kodak Image Station platen.

8.3.8 Record the gel staining time on the AGE Assay Form.

8.3.9 Clean staining trays using purified or WFI water and towel dry.

8.4 Disposal of Gel Staining Solutions

8.4.1 Pour all stains into the Nucleic Acid Stain Hazardous Waste carboy using a funnel, if necessary.

8.4.2 Once the carboy is 25-50% full, turn on the peristaltic waste pump to strip all staining compounds from the solution via the in-line carbon filter. Dispose of all stripped waste liquids in the sink.

8.4.3 Date and replace the carbon filter every 6 months. Place used carbon filters in the Hazardous Solid Waste for Incineration.

9.0 Agarose Gel Imaging

9.1 Use of the Kodak 440C Gel Image Station – refer to **SOP 22120, *Digital Gel Imaging Using the Kodak 400 Image System***.

9.2 Other Methods of Gel Imaging

9.2.1 Digital capture of the gel image is recommended for all applications and is required for all QC assays.

9.2.2 In the event the Kodak Image Station is not available, the AP STORM Laser Imager can be used; this instrument is located in the BDP Product Development Group.

9.3 Disposal of Stained Gels (post-imaging)

9.3.1 Dispose of stained and unstained gels in the Hazardous Solid Waste for Incineration trash.

9.3.2 If necessary, gels may be kept at 2-8°C for up to 5 days for further use and for 4-8 hours at room temperature. Completely wrap all gels for storage in plastic wrap and place on solid support to prevent buffer evaporation and leakage.

10.0 References and Related Documents

10.1 **SOP 22120** *Digital Gel Imaging Using the Kodak 400 Image System*

10.2 **Form 22148-01** *AGE Assay*

10.3 **Form 22148-02** *Gel Lane Designation*

10.4 Cambrex Applications Catalog.

10.5 Invitrogen/Life Technologies Products and Reference Guide.

10.6 MBI Fermentas Molecular Biology Catalog and Product Application Guide.

10.7 *Molecular Probes Handbook of Fluorescent Probes and Research Chemicals, 6th Ed.*; Richard P. Haugland.

10.8 *Molecular Biology LabFax II: Gene Analysis*; T.A. Brown, Editor; Academic Press.

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