Standard Operating Procedure (SOP) for Gel Electrophoresis with the E-Gel System

I. SCOPE AND PURPOSE

Agarose gel electrophoresis is a rapid technique used to resolve nucleic acids and to estimate their molecular weight. DNA molecules are negatively charged due to their phosphate backbone. During electrophoresis they migrate toward the positively charged electrode. Small DNA fragments migrate more rapidly in the gel matrix compared to large fragments due to size-dependent sieving effects of the gel matrix. As a result, DNA molecules are separated based on their size.

E-Gels are self-contained bufferless, pre-cast agarose gels designed to provide fast, convenient, and easy electrophoresis. Each E-gel contains agarose, electrodes, and ethidium bromide all packaged inside a dry, disposable, UV-transparent cassette eliminating the need to weigh, melt, and pour agarose and to dispose of liquid waste containing ethidium bromide. They offer excellent resolution of up to 96 samples at a time in as little as 12 minutes.

The E-Editor software allows for quick reconfiguration of digital E-Gel images for analysis and documentation. All gel photographs must be of sufficient resolution and quality (low background, clear signal, absence of bubbles) to permit accurate interpretation.

Any deviation from this Standard Operating Procedure will be noted in LabVantage and with an incident report; the number of the samples affected by the deviation will be noted as well.

This procedure establishes the process for performing gel electrophoresis of DNA samples using the E-Gel system by trained lab personnel. It is applicable to native, genomic DNA and is used to determine the overall integrity of genomic DNA following nucleic acid extraction.

II. PROCEDURE

A. Safety Procedures

1. Wear personal protective equipment (lab coat and gloves).
2. Ethidium bromide is a potential carcinogen and known mutagen (GHS WARNING! Mutagenic (Category 2)). Nitrile gloves and lab coat must be worn when handling anything that has come into contact with ethidium bromide. **Latex gloves will not provide protection**.
3. Discard gels containing ethidium bromide in the appropriate biohazard waste container.
4. This SOP involves the use of an ultraviolet (UV) light source. An overexposure to UV radiation can cause sunburn, some forms of skin cancer, and eye damage. Proper protective shielding should be used anytime UV light sources are in use.

B. Equipment and Materials

1. PPE (lab coat, gloves)
2. E-Base (Invitrogen, EB-M03, no substitution)

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3. 96-well plate (USA Scientific, 1402-9700)
4. E-Gel 48 1% agarose gels (Invitrogen, G8008-01)
5. Aerosol-barrier pipette tips
6. 10X Blue Juice (Invitrogen, 10816-015)
7. Nuclease-free water (Fisher, BP2484-50)
8. 0.1X Tris-EDTA (TE) Buffer (1 mM Tris, 0.1 mM EDTA; 100X Concentrate, Sigma, T9285)
9. UVP gel imaging system
10. Vortexer
11. Adhesive foil for 96 well plates (BioRad, PMSF1001)
12. E-Gel 1Kb Plus DNA Ladder (Invitrogen, 10488-090)
13. Centrifuge capable of holding 96-well plates

It is possible to substitute disposable materials and certain equipment from other vendors as long as they are equivalent to the item described above.

In the event that a reagent or disposable item either becomes contaminated or is suspected of being contaminated, it must be discarded.

C. Quality Control

1. The loading dye is colored and easily identifiable for sample loading and tracking. It includes a visual marker to indicate where the endpoint of electrophoresis occurred.
2. Ladder of known molecular weight is run in every row with extracted and normalized DNA to estimate the size range of DNA fragments.
3. All gel photographs must be labeled adequately so that each sample can be identified and cross-reference the LIMS system.
4. All new lots of reagents are tested in parallel with the one in current use before being put into use. Results are recorded in QC log.
5. Some common situations that may cause analytically inaccurate results would include, but are not limited to: the gel ran for too long, the gel is improperly inserted into the E-base, or the gel was not examined within 30 minutes after it was run.

D. Reagent Preparation

1. Preparing 0.1X TE Buffer from 100X Concentrate:

(1) To make a 50 mL aliquot: add 5 mL 100X TE to 45 mL sterile water.

(2) 0.1X TE can be stored at room temperature and expires one year from date prepared. The container should be labeled with the stock 100X TE log number, date prepared, date of expiration, applicable hazard warnings, preparer initials, and storage requirements.

(3) Record reagent information into lab QC book and enter results once QC has passed.

E. Creating A Genomic Gel in LabVantage

1. Log into LabVantage
2. Under Storage, click on “File Samples.”

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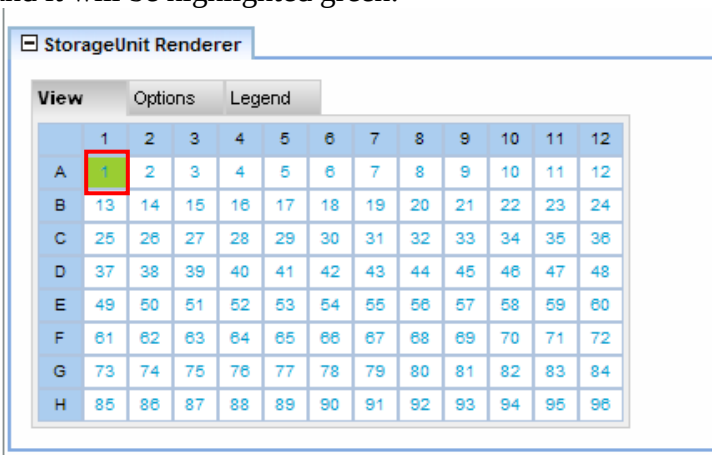
3. Click on magnifying glass. Click on "Sorted Box." Click on "MyBoxes."
4. Click on an empty Matrix Rack box.
5. Place the cursor on the spot that corresponds with where the first sample will be filed. Click on the spot and it will be highlighted green.



	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	3	4	5	6	7	8	9	10	11	12
B	13	14	15	16	17	18	19	20	21	22	23	24
C	25	26	27	28	29	30	31	32	33	34	35	36
D	37	38	39	40	41	42	43	44	45	46	47	48
E	49	50	51	52	53	54	55	56	57	58	59	60
F	61	62	63	64	65	66	67	68	69	70	71	72
G	73	74	75	76	77	78	79	80	81	82	83	84
H	85	86	87	88	89	90	91	92	93	94	95	96

6. Place the cursor on the TrackItem Management box. Now the barcode on the sample can be scanned to file it in LabVantage.

7. Click "Save."
8. Under Molecular Analyte, click on "Create Robot Rack."
9. Select a Matrix Rack, and click on "Create Gel."
10. Click on the magnifying glass to choose the Transfer Mode.
11. Click on T-00019 - PCR Plate to 48 Row Gel - Top Half.
12. Type in 1 for the Aliquot Volume (uL).
13. Click "OK."
14. Select the Gel, and click "Print Gel." Print the gel layout.
15. Under Molecular Analyte, click on "Create Robot Rack."
16. Select the Matrix Rack again, and click on "Create Gel."
17. Click on the magnifying glass to choose the Transfer Mode.
18. Click on T-00021 - PCR Plate to 48 lane gel bottom half.
19. Type in 1 for the Aliquot Volume (uL).
20. Click "OK."
21. Select the Gel, and click "Print Gel." Print the gel layout.

F. Prepare samples as follows for native DNA:

1. Add 17 µL nuclease-free water to each well of a 96-well plate that will receive sample.
2. Visually inspect that there is liquid in each well.
3. Add 2 µL of Blue Juice to each well.

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4. Add 1 µL of DNA (normalized to 0.165 µg/µL ± 0.015 µg/µL) to each well.
5. Visually inspect that there is dye in each well.
6. Cover the plate with an adhesive foil and hold down uniformly while vortexing until dye is evenly dispersed. Spin down the plate for 1 minute at 2200 rpm (room temperature).

G. Setting up the E-Gel

1. Plug the E-base into the wall and ensure that the display reads "EG." Push the "pwr/prg" button to change the program if required.
2. Remove the gel from the packaging and insert it into the E-base. If properly inserted, a red light illuminates and the display reads "20." **The electrophoresis run must be started within 15 minutes of opening the gel**.
3. Load samples individually, adding 15 µL total prepared sample per well. NOTE: Use caution to ensure each sample is placed into the correct well.
4. Load 15 µL of ladder in appropriate wells (one at each end of each row).
5. Load all remaining empty wells with 15 µL of water. The gel will not run properly without liquid in all empty wells.
6. Press the "pwr/prg" button to begin electrophoresis within 1 minute of loading samples. The red light changes to green to indicate the run is in progress.
7. Completion of electrophoresis is indicated by a flashing red light and beeping sound after 20 minutes. The gel should be analyzed within 30 minutes.
8. Remove the gel from the base and capture an image of the gel with the gel imaging system (see MGL-EQP-32 or MGL-EQP-36 for UVP instruction).

H. Gel Imaging

1. Turn on the UVP.
2. Turn the UV Trans switch on. The transilluminator switch at the bottom also needs switched on. Place the gel in the UVP and close the door.
3. View the gel in LIVE mode.
4. Press the Time Stamp button to add the UVP gel number to the image.
5. Press the SNAP button to take a picture.
6. Press the PRINT button to print the gel.
7. Press the Save button to save the gel image on the USB drive.
8. Transfer the gel image file to the BCR/Gels/UVP Images folder.
9. From LabVantage, copy the Gel Report (created in step A14 above) into Excel. Paste into Excel; be sure to select Paste Options to "Use Text Import Wizard." Click Next twice. Click Finish. The samples from the gel file will now be in the correct format to paste into the gel template.
10. Highlight samples 1 through 24. Copy. Paste using the "Transpose" option. Now the sample ID's will be listed from left to right. Copy this transposed version of samples.
11. Open the Power Point file "Template for Labeling Gels." This template has a top and bottom gel to represent the physical gel that was run.

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12. On the top gel paste the first 24 samples copied from Excel. Under the Home tab, under Paragraph in Excel click on "Text Direction." Choose the option to "Rotate all text 270°."
13. Go back to Excel. Highlight samples 25-48. Copy. Paste using the "Transpose" option. Now the sample will be listed from left to right. Now copy this transposed version of samples.
14. Paste this set of samples on the bottom gel of the template. Under the Home tab, under Paragraph in Excel click on "Text Direction." Choose the option to "Rotate all text 270°."
15. Now insert the gel image twice. Open the gel image saved on the USB and crop the gel image to display the top gel and align it with the appropriate labels. Crop the gel image again, to display the bottom gel and align it with the appropriate labels.
16. Any lanes that do not have a sample should be labeled as "Blank."
17. Save the published file as "Genomic Gel ### UVP### MM-DD-YY." The genomic gel number is taken from LabVantage, the UVP is from the time stamp that is displayed on the gel image, and the date is the date the gel was run.
18. Print a copy of the labeled gel and staple the gel image from the UVP.

I. Interpretation:

1. Review gel to determine the overall qualitative level of sample integrity.
   a. **High:** A single high molecular weight band or a small smear of genomic DNA is present with all or most of the band/smear above 2,000 bp.
   b. **Medium:** A smear of genomic DNA is present with most of the smear lying between 500 and 2,000 bp.
   c. **Low:** A smear of genomic DNA is present with most of the smear below 500 bp.
   d. **Indeterminate:** If the sample's concentration is so low that it limits the visibility of any possible band being present.
2. After scoring sample integrity, update Gel Status in LabVantage using the 4 integrity categories listed above.
3. Place the printed gel file/gel image in the Genomic Gels binder.

III. REFERENCES

A. *E-Base Electrophoresis Device User Guide (Invitrogen)*

B. BCR-REF-001, "BCR Acronym List"

IV. COMPREHENSIVE REVISION HISTORY

A. Changes made in Version 4, Effective Date 4/27/2016

1. Made title not all capitalized
2. Safety - added GHS warning for ethidium bromide; noted that UV can also cause eye damage.
3. EQP and Materials: added PPE, 0.1X TE, and plate centrifuge.
4. Reagent Prep - added details for making 0.1X TBE
5. F. 6. added instruction to spin plate down after mixing.
6. G.3. Added note to use caution/add sample to correct well.

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7. G.8. added EQP SOP numbers for UVP's.
8. Removed details about "graphics editing software" (this is not required - per change request).
9. Gel Interpretation Section: re-written; now describes High, Medium, Low, and Indeterminate categories for DNA bands on gels.

B. Changes made in Version 3, Effective Date 09/02/2014

1. New format used
2. Updated LabVantage screenshot
3. Removed Tris-EDTA from materials
4. Updated volume of water added when preparing the samples

C. Version 2, Effective Date 10/2/2012

Signatures

Approved By: **Signature on file** **Date:** **Date on file**

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