Procedure & Checklist - Using the Sage Science™ Pippin Pulse Electrophoresis Power Supply System

Before You Begin

This procedure can be used to evaluate genomic DNA (gDNA) to ensure that it is of high quality and high molecular weight prior to being used in library construction. Quality analysis of the library (during construction) is also important when targeting larger insert sizes (>20 kb) and determining the effectiveness of shearing strategy. Finally, it can help determine the appropriate size selection protocol to use.

Overview

Pulsed-field gel electrophoresis is a strategy for resolving large fragments of DNA for analysis. When running a typical direct-current agarose gel, fragments above 10 - 15 kb can migrate in a non-predictable manner (not related to their size). Pulsed-field gels work by shuttling DNA back and forth in the gel, effectively slowing down large DNA fragments that might otherwise run at the same rate as smaller ones. By switching the direction of the electric field in a gel, DNA will change its direction of migration. Since smaller molecules can change direction faster than larger molecules, more differentiated separation can be achieved by rapidly switching, or pulsing, the electric field.

A single-pulse duration typically separates a relatively narrow range of DNA sizes. For best results, it is necessary to use a range of pulse times. This is accomplished by using a “ramp” (progressively increasing the forward and/or reverse intervals from a lower limit to an upper limit). For more detailed information, please refer to the Pippin Pulse User Manual.

Note: Pre-programmed protocols, available in the Pippin Pulse software, are based on using the recommended gel box systems and a 12 cm x 14 cm gel. Protocols may need to be optimized if using a gel box system and gel of another size.

Materials Needed

Item	Vendor	Part Number
Electrophoresis Reagents		
10X KBB Electrophoresis Buffer	Sage Science	KB1001
SeaKem Gold Agarose	Lonza	50152
DNA Markers		
CHEF DNA Size Standard 5kb	BioRad	170 - 3624
CHEF DNA Size Standard 8-48 kb	BioRad	170 - 3707
Staining/Imaging		
FlashGel 5X Loading Dye	Lonza	50462
1X Elution Buffer	Pacific Biosciences	100-159-800
SYBR Safe DNA Gel Stain or	Life Technologies	S33102
Gel Red	Biotum	41003
Equipment		
Pippin Pulse Power Supply	Sage Science	PP10200
Galileo Biosciences RapidCast Complete Mini-Gel System	Sage Science	PGB0001
Kimble 500 ml Bottle	Cole-Parmer	170-4417
KS 260 Basic Shaker	IKA	0002980201

Prepare and Run the Gel

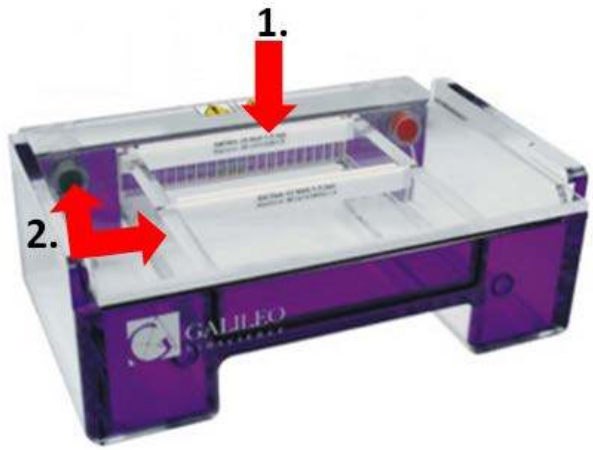
The following procedure is for 12 cm x 14 cm gel tray. The required volume of melted agarose depends on the size of the gel tray.

1. **Prepare 2 liters of 0.5X KBB Electrophoresis Buffer**.

   a. In a 1 Liter graduated cylinder, add 50 mL of 10X KBB Electrophoresis Buffer to 950 mL of deionized water. Repeat for a total of 2 liters.

2. **Prepare a 0.75% Agarose Gel**.

   a. On an analytical balance, carefully measure out 1.113 g of Lonza SeaKem Gold Agarose.
   b. Aliquot 150 mL of 0.5X KBB Electrophoresis Buffer into a 500 mL Kimble bottle.
   c. Pour the 1.113 g of Lonza SeaKem Gold Agarose to the 150 mL of 0.5X KBB Electrophoresis Buffer. Mix the solution while adding the agarose to prevent clumping.
   d. Place bottle in a microwave and heat on high for 1 – 2 minutes or until powder is fully dissolved. **Caution:** Bottle and content will be hot. Handle with care.
   e. Set the IKA Shaker to 50 rpm. Place the bottle on the shaker and shake gently for 10 minutes for solution to cool off. Note: After 10 minutes of shaking, the bottle should be cool to the touch. However, further cooling may be required. Do not let the solution cool too long because it will solidify in the bottle.
   f. Place the gasketed gel tray into the gel tray platform of the buffer chamber so that the gasketed ends are pressing against the walls of the buffer chamber.



Galileo Rapidcast 1214
Position 1: Gasketed gel tray and comb position for gel pouring and solidifying
Position 2: Gasketed gel tray and wells of gel position in relation to the cathode

   g. Pour the solution into the gel tray with the 20-well comb in place. Note: Pour slowly to avoid bubbles which will disrupt the gel. Bubbles can be managed by pushing them toward the edges with a pipette tip.
   h. Let the gel sit at room temperature for 30 minutes until the gel is fully solidified.
   i. After 30 minutes carefully lift up the gasketed gel tray from the tray platform and rotate the gel 90º so that the wells are closest to the black (cathode) electrode.

3. Prepare samples.

a. Prepare up to 18 samples with 50 - 100 ng per well.

Component	Volume
Sample	*X* µL of 50 - 100 ng
Lonza FlashGel 5X Loading Dye	4 µL
1X Elution Buffer	Add to bring up to total volume
Total	**18 µL**

4. Prepare DNA Markers. We highly recommend using the following gel markers loaded on adjacent wells.

CHEF DNA Size Standard 5 kb

a. Prepare a 1:5 dilution of the CHEF DNA Size Standard. Add 1 µL of the CHEF DNA Size Standard + 4 µL 1X Elution Buffer.
b. Add 1 µL of the diluted CHEF DNA Size Standard + 12 µL 1X Elution Buffer + 5 µl Lonza FlashGel 5X Loading Dye for a total of 18 µL.

CHEF DNA Size Standard 8 kb - 48 kb

a. Prepare a 1:5 dilution of the CHEF DNA Size Standard. Add 1 µL of the CHEF DNA Size Standard + 4 µL 1X Elution Buffer.
b. Add 1 µL of the diluted CHEF DNA Size Standard + 12 µL 1X Elution Buffer+ 5 µl Lonza FlashGel 5X Loading Dye for a total of 18 µL.

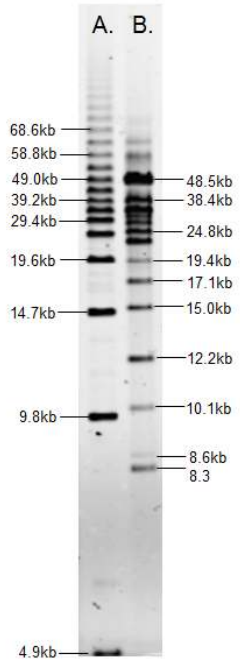


Fig 2. Bio-Rad DNA Markers on Pippin Pulse (5-80 kb, 95v) protocol for 16 hours

A. CHEF DNA Size Standard 5 kb consists of fragments from 4.9 kb to 98 kb.
B. CHEF DNA Size Standard 8-48 kb consists of 13 bands: 8.3 kb to 48.5 kb.

5. Create a New Protocol for the Pippin Pulse.

a. Click on the **Edit** button to open the Edit User Waveforms table and fill in with the following parameters:

Waveform Type: Create a new protocol name such as **5-80kb**, **95v**

V: 95, indicates the Electrophoresis voltage. **The Pippin Pulse Power Supply does not have a temperature regulation, therefore it is highly recommended to not exceed 100v.**

A: 150, Forward time at start of run in msec

B: 50, Reverse time at start of run in msec

C: 30, Increment added to A at each step in msec

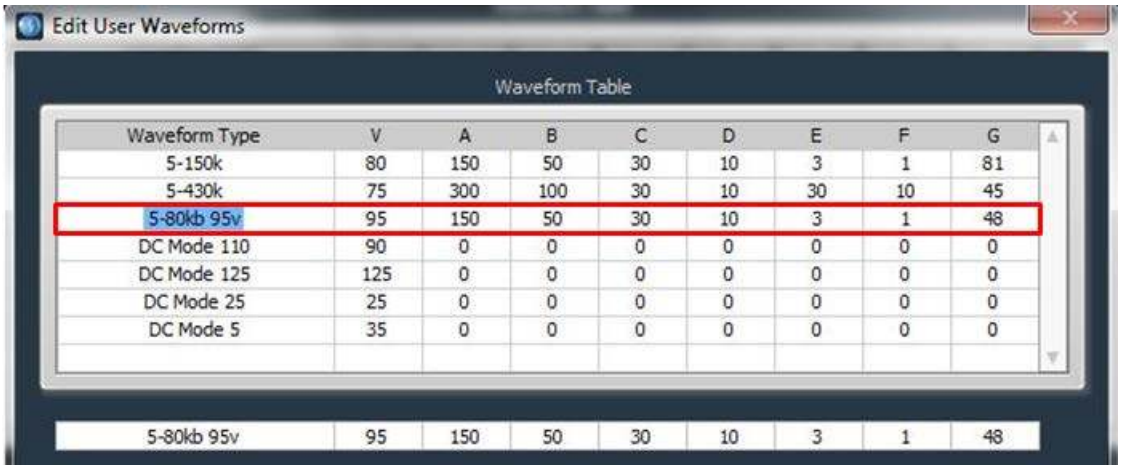
D: 10, Increment added to B at each step in msec

E: 3, Increment added to C at each step in msec

F: 1, Increment added to D at each step in msec

G: 48, Number of steps per cycle

b. Click on **Add/Replace** to have the protocol added to the table. Then click on **Accept**.



The new protocol should show up in the Waveform Table.

6. Run the Pippin Pulse.

a. Add enough of the 0.5X KBB Electrophoresis Buffer to completely cover the gel but do not exceed the Fill Line on the gel box.

b. Load DNA samples and markers onto gel (18 µL per well).

c. Place the lid on top of the tray, ensuring the electrodes are connected to the power supply.

d. Ensure the Pippin Pulse is on by pressing the power button on the front panel.

e. Launch the Pippin Pulse v1.32 application by double-clicking the Pippin Pulse icon.

f. In the “Waveform Type” dialog box, select the “5-80kb 95v” protocol that was created. Note that the selected protocol will be highlighted in blue.

g. For the target duration, enter the desired run time (typically **14** to **16** hours). Note that the default value is **2** hours. Consider optimizing the run time based on the ambient lab temperature since the Pippin Pulse Electrophoresis System does not regulate the temperature in the gel chamber.

h. Click **START**.

7. Stain the gel.

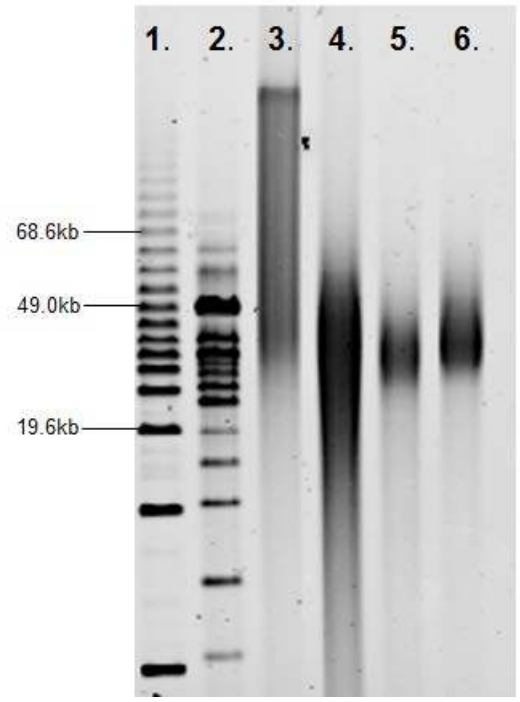
a. Make a 1X concentration of Gel Stain:
   - SYBR Safe DNA Gel Stain: 30 µL SYBR Safe DNA Gel Stain + 300 mL 0.5X KBB Electrophoresis Buffer **OR**
   - PAGE Gel Red: 30 µL of Gel Red Stain to 300 mL of deionized water

b. Cover the Pyrex dish with aluminum foil to prevent dye degradation.

c. Shake the gel and stain gently for 1 hour. Place the covered Pyrex dish on an IKA shaker and set shaker to 50 rpm.

8. Image the gel.

a. Image gel according to manufacturer's user guide.



1. Biorad CHEF 5 kb Ladder
2. Biorad CHEF 8-48 kb Ladder
3. Genomic DNA
4. 50 kb Shear using the Diagenode Megaruptor (MR)
5. 50 kb MR Shear – Size Selected at 20 kb using Blue Pippin system
6. 50 kb MR Shear – Size Selected at 30 kb using Blue Pippin system

Examples of gDNA quality, run on FIGE using a Pippin Pulse Power Supply.

(5-80kb protocol, 95v for 16 hours)

Revision History (Description)	Version	Date
Initial release. Converted from “Unsupported Protocol” with no changes	01	June 2018