Agilent Plasmid Topology Kit

Quick Guide

For Research Use Only. Not for use in diagnostic procedures.

The Agilent Plasmid Topology Kit (5191-6627) analyzes all three plasmid isoforms (supercoiled, linear, and open circular) along with detection of other plasmid forms such as concatemers. It is designed to determine plasmid DNA purity and size supercoiled plasmid DNA between 2,000–10,000 bp. Applications include quality control and sizing of supercoiled plasmid DNA. This Quick Guide is intended for use with the Agilent 5200 and 5300 Fragment Analyzer systems* only. This kit is validated only for 12-cap and 48-cap arrays.

Specifications

Analytical Specifications	Plasmid Topology Kit
DNA Sizing Range	2,000 bp–10,000 bp
Recommended Concentration Range	0.1-1.0 ng/µL Final in well DNA Concentration in pDNA Dilution Buffer
Physical Specifications [1]	
Total Electrophoresis Run Time	33 cm: 45 minutes
Samples Per Run	12 or 48; depending on instrument type[1]
Sample Volume Required	Variable, depends on input concentration (24 µL minimum final sample volume required)
Guaranteed Shelf Life	4 months

* Instrument with updated Vent Valve (#M5301A) is required to run this assay.

[1] Not validated for 96-capillary array.

Important Notice - Kit Specific Instructions

This manual contains instructions specific to the Plasmid Topology Kit, 5191-6627. Although this kit shares some components with other Fragment Analyzer kits, certain components, procedures, settings and usage guidelines outlined here are unique and must be followed exactly to ensure accurate results and optimal performance.

Kit Components - 275 Sample Kit (#5191-6627) - *Refer to product label for proper storage conditions*

Kit Component Number	Part Number (Re-order Number)	Description	Quantity Per Kit
5191-6666*		Plasmid Topology Kit, 4°C	
	5191-6660+	Genomic DNA Separation Gel, 240 mL	1
	5191-6661+	BF-25 Blank Solution, 8 mL	1
5191-6667*		Plasmid Topology Kit, FR	
	5191-6664+	4x pDNA Dilution Buffer, 4.5 mL	1
	5191-6662+	SYBR™ Gold Dye**, 30 µL	1
	5191-6663+	pDNA Ladder, 0.8 mL	1
	5191-6665+	pDNA Marker, 3 mL	1
5191-6614*		Qualitative DNA, 500, RT	
	FS-SMO15	Mineral Oil Dropper Bottle, 15 mL	1
	DNF-475-0050	5x Capillary Conditioning Solution, 50 mL	1

* not orderable
+ currently not available as a la carte items

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Important Notice

Altering any reagents and/or use of unapproved or non-recommended reagents may materially alter the performance of the instrument such that the instrument no longer performs to Agilent specifications. Any work performed by Agilent to bring the instrument back into compliance with Agilent specifications will be performed at the customer's expense.

Additional Material Required to run the Plasmid Topology Kit (not supplied)

Part	Part Number
Fragment Analyzer vent valve upgrade kit*	M5301A

* Required only once per instrument

Instrument	Compatible Arrays	Part Number
5200 Fragment Analyzer	FA 12-Capillary Array Short	A2300-1250-3355
5300 Fragment Analyzer	FA 48-Capillary Array Short	A2300-4850-3355

Software

- Fragment Analyzer controller software version 5.1.0 or newer
- ProSize data analysis software version 6.0.1 or newer

Reagents

- Capillary Storage solution (GP-440-0100)

Additional equipment required (not supplied)

- 96-well PCR sample plates (*Refer to Appendix in Fragment Analyzer User Manual*)
- Multichannel pipettor and/or liquid handling device capable of dispensing 1–100 µL (for sample plates) and 1,000 µL (for inlet buffer plate)
- Pipette tips
- 96-well plate centrifuge
- Adhesive PCR plate seals
- Sub-micron filtered DI water system: for dilutions of Inlet Buffer and Capillary Conditioning Solution
- 96-deepwell 1 mL plate: inlet buffer and/or waste plate (Agilent #P60-20 or Fisher Scientific #12-566-120)
- Reagent reservoir 50 mL: for use in pipetting inlet buffer plates (VWR #89094-680, or similar)
- Conical centrifuge tubes for prepared separation gel + dye mixture and/or 1x Capillary Conditioning Solution
    - 50 mL for 5200 Fragment Analyzer system (BD Falcon #352070, Fisher Scientific #14-432-22 or VWR #21008-940)
    - 250 mL for 5300 and 5400 Fragment Analyzer systems (Corning #430776, Fisher Scientific #05-538-53 or VWR #21008-771)
- Vortexer
- UV Spectrophotometer

WARNING Working with Chemicals

- Refer to product safety data sheets for further information
- When working with the Fragment Analyzer kit components follow the appropriate safety procedures such as wearing personal protective equipment (PPE).

Essential Measurement Practices

Environmental conditions	• Ambient operating temperature: 19–25°C (66–77°F) • Keep reagents at room temperature during sample preparation.
Steps before sample preparation	• Allow reagents to equilibrate at room temperature for 30 min prior to use. • Spin tubes after thawing to ensure liquid is at the bottom of the tube.
Dilution Buffer preparation	• Dilute 4x pDNA Dilution Buffer prior to sample preparation to a working concentration of 0.3x ◦ For 10 mL 0.3x pDNA Dilution Buffer: 0.75 mL of 4x pDNA Dilution Buffer stock + 9.25 mL DI water • Prepared 0.3x pDNA Dilution Buffer can be stored at 4°C; consume within two weeks
Sample input concentration	• Starting sample concentration is recommended to be greater than 10 ng/µL measured by UV absorbance. • Dilute sample using 0.3x pDNA Dilution buffer to the recommended in-well concentration of 0.1 – 1 ng/µL. Mixing by pipetting is preferred to prevent plasmid degradation. • Recommended maximum signal should be below 12,000 RFU
Pipetting practice	• Pipette reagents against the side of the 96-well sample plate or sample tube. • Ensure no sample, dilution buffer or pDNA Marker solution remains within or on the outside of the tip • It is recommended to mix plasmid samples by pipetting up and down at least 3 times instead of vortexing, as too strong vortexing could degrade the plasmid integrity
Inlet Buffer preparation	• Dilute 4x DNA Inlet Buffer to working concentration of 1x ◦ For 10 mL 1x pDNA Inlet Buffer: 2.5 mL of 4x pDNA Inlet Buffer + 7.5 mL DI water (volume for one buffer row (A) for a 12-capillary array) ◦ For 40 mL 1x pDNA Inlet Buffer: 10 mL of 4x pDNA Inlet Buffer + 30 mL DI water (volume for four buffer rows (A-D) for a 48-capillary array) • Diluted 1x pDNA Inlet Buffer should be stored at 4°C and consumed within one month
Marker Plate preparation	• Bring ready-to-use pDNA Marker solution to room temperature before use. • Vortex and centrifuge pDNA Marker solution to mix. • Dispense 30 µL/well of pDNA Marker solution into wells of a separate sample plate (12-capillary; row A, 48-capillary array; rows A-D). • Cover the wells with 1 drop or 30 µL of mineral oil to allow reuse for 30+ injections or ~1 month. • Place plate in drawer "M". If not using it right away, cover and keep in the dark at

	4°C, warm to RT and centrifuge before running plate. • **The pDNA Marker solution is light and temperature sensitive**. When not stored on the instrument, the plate should be stored in the dark at 4°C.
Sample Plate Preparation	• Pipette 24 µL of each sample diluted with 0.3x pDNA Dilution Buffer to the concentration range specified. • Run the DNA Ladder in parallel with samples, pipette 24 µL pDNA Ladder solution (ready-to-use) directly into the specified ladder well of the sample plate or row to be analyzed. • Fill any unused wells within the sample row or plate with 24 µL of 0.3x pDNA Dilution Buffer. • Run samples immediately after preparation, or within a day with mineral oil overlay. If more than one run in planned, cover the samples with mineral oil to prevent sample evaporation.
Mixing and centrifugation recommendations	• Using a separate pipette tip set to a larger volume of 20 µL, mix the diluted sample and 0.3x pDNA Dilution Buffer by pipetting up and down 3-5 times. After mixing, centrifuge the plate to remove any air bubbles. • Run samples immediately after preparation, or within a day with mineral oil overlay. If not using right away, cover and keep at 4°C, warm to RT and centrifuge before running plate.

Important Notice

A Method F flush or a Method G flush is required when switching between kit types. Refer to "Poor performance or late migration" corrective actions in the Troubleshooting section below for more details.

Gel Preparation*

Vortex and centrifuge dye vial prior to opening the vial to reduce risk of leaking. Ensure the gel + dye is mixed without generating bubbles, gently invert tube 5-10 times.

Number of Samples	SYBR™ Gold Dye Volume (µL)	Separation Gel Volume (mL)
12	0.8	10
24	1.2	15
48	2.0	25
96	4	50
192	8	100

Conditioning Solution

The provided 5x Conditioning Solution must be diluted to 1x using submicron DI water prior to use. Invert to mix.

Number of Samples	Volume of 1x Conditioning Solution
12	10
24	15
48	25
96	50
192	80

* Gel and dye volumes are unique to this kit

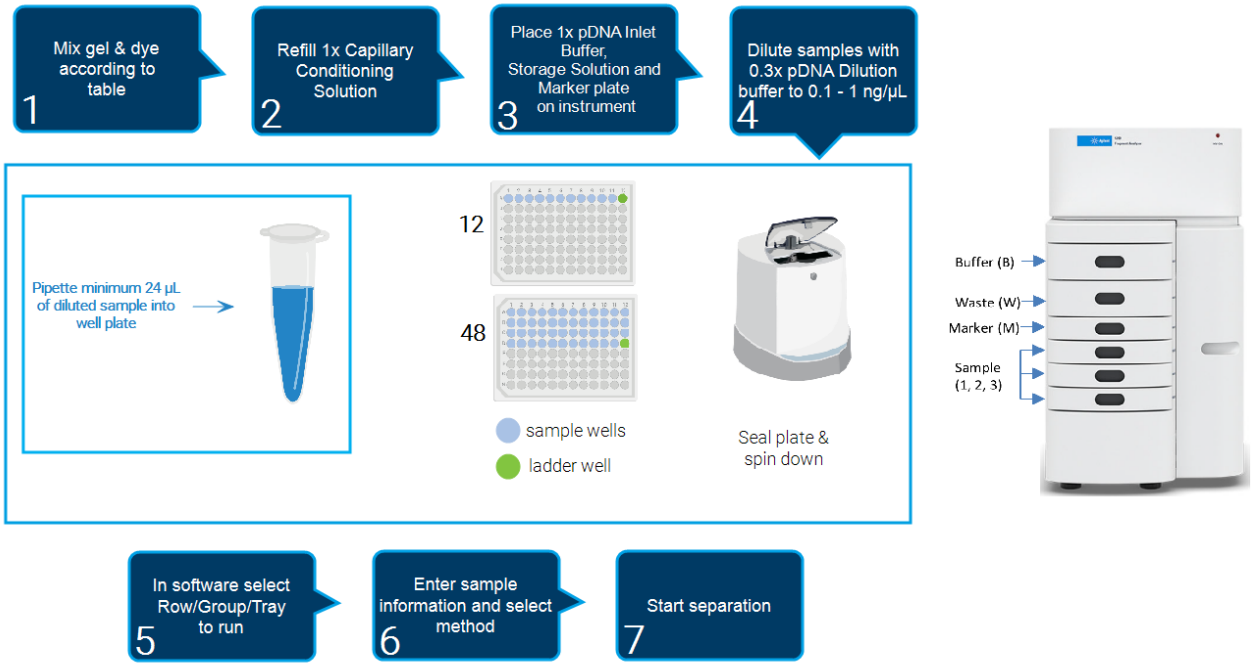
Agilent Plasmid Topology Kit Operating Procedure

1. Mix fresh gel and dye according to the volumes in the preparation table. Update solution level in controller software.
2. Refill 1x Capillary Conditioning Solution as needed. Update solution level in controller software.
3. Inspect and empty, if necessary, waste plate located in drawer "W".
4. Place a fresh 1x pDNA Inlet Buffer plate, 800 µL/well, in drawer "B". Replace Inlet Buffer daily. Enough for up to 8 injections, replace after 8 injections (or after 10h) if more runs are planned. Make sure to start the run sequence within 1 hour after pipetting the 800 µL/well 1x pDNA Inlet Buffer.
   - 5200–Row A
   - 5300–48 capillary, rows A-D

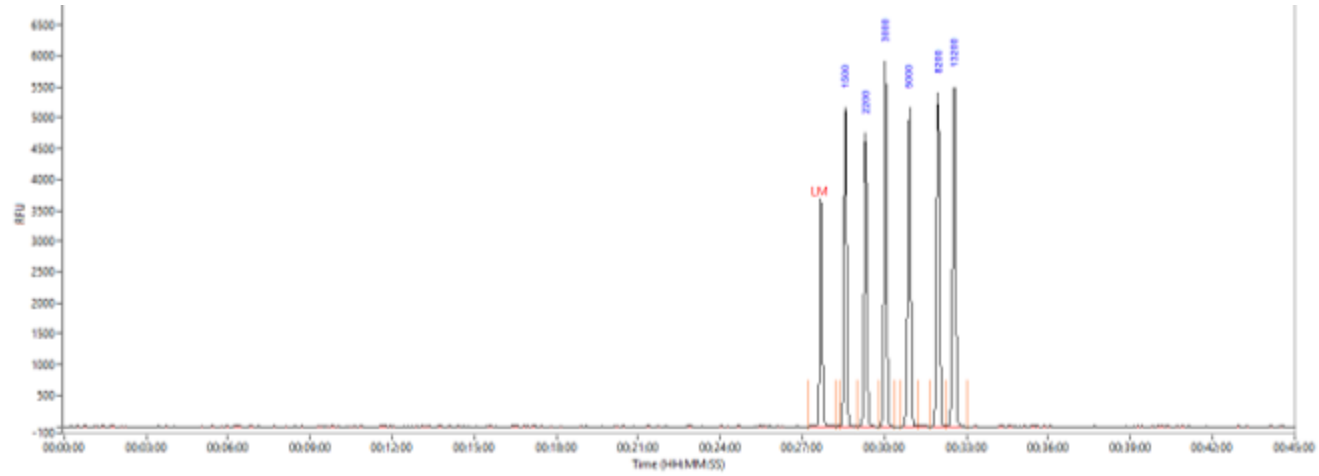
   Prepare Capillary Storage Solution plate. Replace storage solution every 2 weeks for optimal results.
   - 5200–Row H, 1 mL/well, drawer "B"
   - 5300–48 capillary, rows A-D, 100 µL/well, drawer 3

   Prepare Marker plate and place in drawer “M”, 30 µL/ well. Add 1 drop or ~30 µL of mineral oil to each well. The Marker plate should last for 30+ injections or ~1 month.
   - 5200–Any row (ensure the row matches the location in the software)
   - 5300–48 capillary, rows A-D or E-H (ensure rows match the location in the software)
5. Add samples to sample plate at a concentration of 0.1–1 ng/µL (prepared in 0.3x pDNA Dilution Buffer).
6. Add ready to use ladder in corresponding well (see sample plate image below), depending on capillary array used.
7. Select Row/Group/Tray to run. Enter sample ID and Tray ID, if desired.
8. Add to queue, from the dropdown select the Plasmid Topology Kit method:
   - 5191-6627-33-Plasmid Topology 10 kb

   Enter Tray Name, Folder Prefix and Notes, if desired
9. Add method to the queue by selecting “OK”, press play  to start the separation.

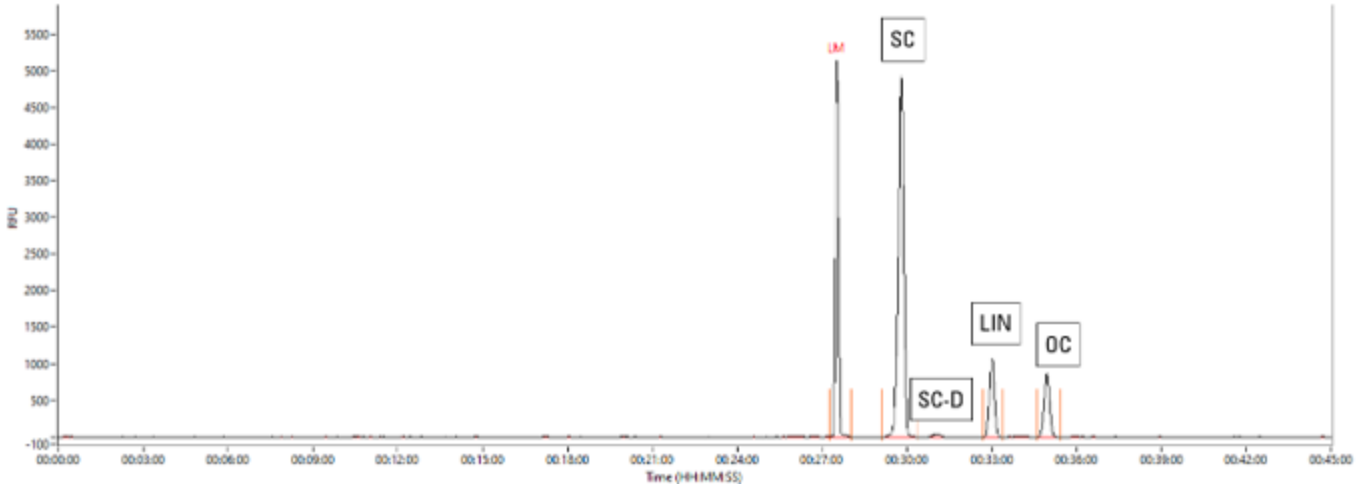


Plasmid DNA Ladder result



Representative pDNA Ladder results using the Fragment Analyzer system with the Plasmid Topology kit. Peaks annotated by size (bp). RFU values may differ between instruments.

Plasmid DNA example result



Example of plasmid DNA using the Fragment Analyzer system with the Plasmid Topology kit. RFU values, migration time and relative concentration of the topologies depend on input.

SC: Supercoiled form SC-D: Supercoiled-Dimer form LIN: Linear form OC: Open circular form

Troubleshooting

The following table lists several potential assay specific issues which may be encountered when using the Plasmid Topology Kit and suggested remedies. Contact Agilent technical support for any additional troubleshooting or maintenance questions.

Issue	Cause	Corrective Action
Peak signal is >> 60,000 RFU.	1 Input DNA sample concentration is too high. Ensure peak height does not exceed 12,000 RFU or total input concentration does not exceed recommended limits.	1 Further dilute input DNA sample concentration with pDNA Dilution buffer and repeat experiment.
No peak observed for DNA sample when expected. Lower Marker peaks observed.	1 Sample concentration too low and out of range. 2 Sample was not added to pDNA Dilution buffer or not mixed well.	1 Prepare more concentrated sample and repeat. 2 Verify sample was correctly added and mixed to sample well.
No sample peak or marker peak observed for individual sample.	1 Air trapped at the bottom of sample plate well or bubbles present in sample well. 2 Insufficient sample volume. A minimum of 20 µL is required. 3 Capillary is plugged.	1 Check sample plate wells for trapped air bubbles. Centrifuge plate. 2 Verify the proper volume of solution was added to sample well. 3 Check waste plate for liquid in the capillary well using a 96-deepwell plate. If no liquid is observed, follow the steps outlined in the System Manual for unclogging a capillary array.
Poor performance and/or poor resolution.	1 Fragment Analyzer system has no vent valve upgrade 2 Wrong inlet buffer used 3 Wrong inlet buffer volume 4 Incorrect intercalating dye 5 The Fragment Analyzer capillary array may require a NaOH conditioning flush.	1 Contact local sales representative and ask for a vent valve upgrade kit (M5301A). 2 Use 4x pDNA Inlet Buffer (5191-6661). 3 Make sure to use inlet buffer at 800 µL/well. 4 User SYBR™ Gold dye (5191-6662). 5 Perform a Method C flush followed by Method A (see Fragment Analyzer System Manual for flushing steps).
Open circular isoform relative concentration below expectation.	1 Open circular topology concentration is too high.	1 Keep concentration of open circular isoform below 0.3 ng/µL to ensure the signal remains within linear range. For example, if the expected relative

Issue	Cause	Corrective Action
		concentration of open circular isoform is around 30%, then the total DNA concentration should stay below 1 ng/µL.
No peak integration in the marker region.	1 Inclusion region is set to > 900 bp.	1 If an impurity in the marker region is expected, consider removing the inclusion region parameter in ProSize. Run a blank to confirm the expected impurity is coming from the sample.
Poor performance or late migration.	1 Capillaries need cleaning.	1 When switching from the Plasmid Topology Kit (5191-6627) to other Fragment Analyzer kits (DNF-XXX) perform a Method G flush between runs. Example: Plasmid Topology Kit -> Method G -> DNF-471. (see appendix for the flushing protocol). When switching from a Fragment Analyzer kit (DNF-XXX) to the Plasmid Topology kit (5191-6627) perform a Method F flush between runs. Example: DNF-471 -> Method F -> Plasmid Topology kit (see appendix for flushing protocol).

Appendix:

Method F Plasmid Topology first run pre-clean protocol .mthdc serves as a pre-cleaning protocol designed to remove Non-Plasmid Topology reagents from the system. This flushing method is **required** when transitioning from all other Fragment Analyzer kits to the Plasmid Topology Kit, ensuring optimal performance with subsequent runs. It needs to be used only before the first Plasmid Topology method following other Fragment Analyzer kit use.

1. From the operations tab located on the main screen window, select Add to queue under the Capillary Array-Conditioning menu.
2. From the Select Conditioning Method window, select *Method F Plasmid Topology first run pre-clean protocol.mthdc* from the drop-down menu.
3. Select Edit to ensure that the method matches the parameters shown in Figure 1.

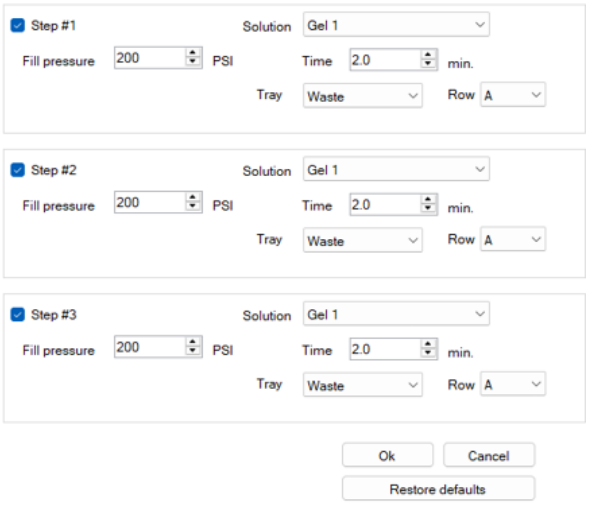


Figure 1 Conditioning Method: Method F Plasmid Topology first run pre-clean protocol.mthdc

4. Select OK.
5. Select OK again to add the method to the method queue.
6. Open the Fragment Analyzer systems' side compartment to replace the Gel 1 bottle with a bottle containing CE grade water. Minimum solution volume required to run Method F Flush: ≥ 50 mL for a 12-capillary array or 48-capillary array
7. From the Utilities menu, select Prime. Check the box next to Gel 1.
8. Select OK to prime the line.
9. Close the door to the instrument side compartment and select the start icon from the method queue to run the capillary conditioning method.
10. Once complete, load fresh pDNA Separation Gel onto gel line 1.
11. From the Utilities menu, select Prime. Check the box next to Gel 1.
12. Select OK to prime the line.

Important Notice

Complete the gel priming procedure listed above in steps 10-12 before proceeding to the next run. Failure to do so may result in inconsistent data sets.

Method G Post Plasmid Topology system reset.mthdc is meant to help remove all Plasmid Topology reagents from the system prior to running other Fragment Analyzer kits. This flushing method is required when transitioning from the Plasmid Topology Kit 5191-6627 to all other Fragment Analyzer kits, ensuring optimal performance with any "DNF" method after use of the Plasmid Topology kit. It only needs to be used prior to the first "DNF" kit method following a Plasmid Topology kit method.

1. Open the Fragment Analyzer side compartment and replace Gel 1 bottle with CE grade water and Gel 2 bottle with a bottle containing 0.5 N NaOH. The minimum volume required to run the Method G flush: ≥ 50 mL for a 12-capillary array or 48-capillary array.
2. From the Utilities menu, select Prime. Check the boxes next to Gel 1 and Gel 2.
3. Select OK to prime the selected lines.
4. From the Operation tab located on the main screen menu, select Add to queue under the Capillary Array - Conditioning commands menu.
5. From the Select Conditioning Method window, select the *Method G: Post Plasmid Topology system reset.mthdc* from the drop-down menu.
6. Select Edit to ensure that the method matches the parameters seen in Figure 2.

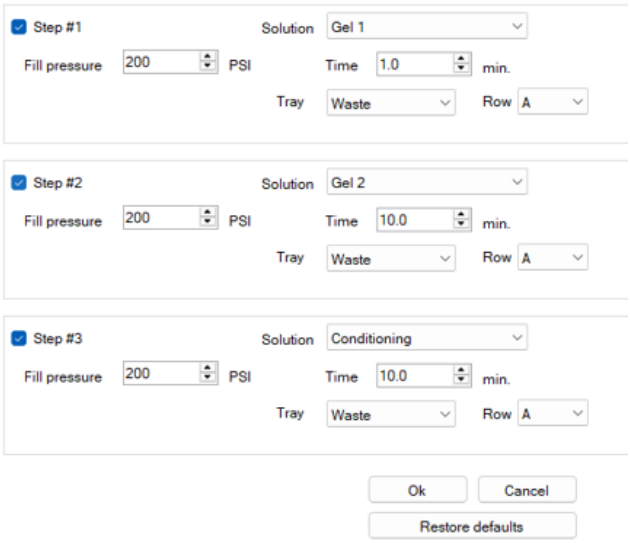


Figure 2 Conditioning Method: Method G Post Plasmid Topology system reset.methdc

7. Select OK.
8. Select OK again to add the method to the Method Queue.
9. Once the Post Plasmid Topology reset method is complete, open the waste drawer and empty the waste plate in the proper aqueous waste disposal area and return it to the waste drawer (second drawer from the top).
10. Load fresh DNF-XXX gel onto Gel line 1.
11. From the Utilities menu, select Prime. Check the box next to Gel 1.
12. Select OK to prime the line.

Important Notice

Complete the gel priming procedure listed above in steps 10-12 before proceeding to the next run. Failure to do so may result in inconsistent data sets.

WARNING 0.5 N NaOH is corrosive

Use extreme caution when handling, as exposure can cause severe eye and skin burns. Avoid contact with eyes, skin and clothing. Wear eye protection and impervious gloves. Clearly label containers to avoid accidental exposure.

Refer to the product material safety data sheets for all warnings and precautions before proceeding.

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Technical Support and Further Information

For technical support please visit www.agilent.com which offers useful information and support regarding the products and technology.

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D0149004 Edition 06/26

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