

Biotage[®] PhyPrep

User Manual



Biotage® PhyPrep

User Manual

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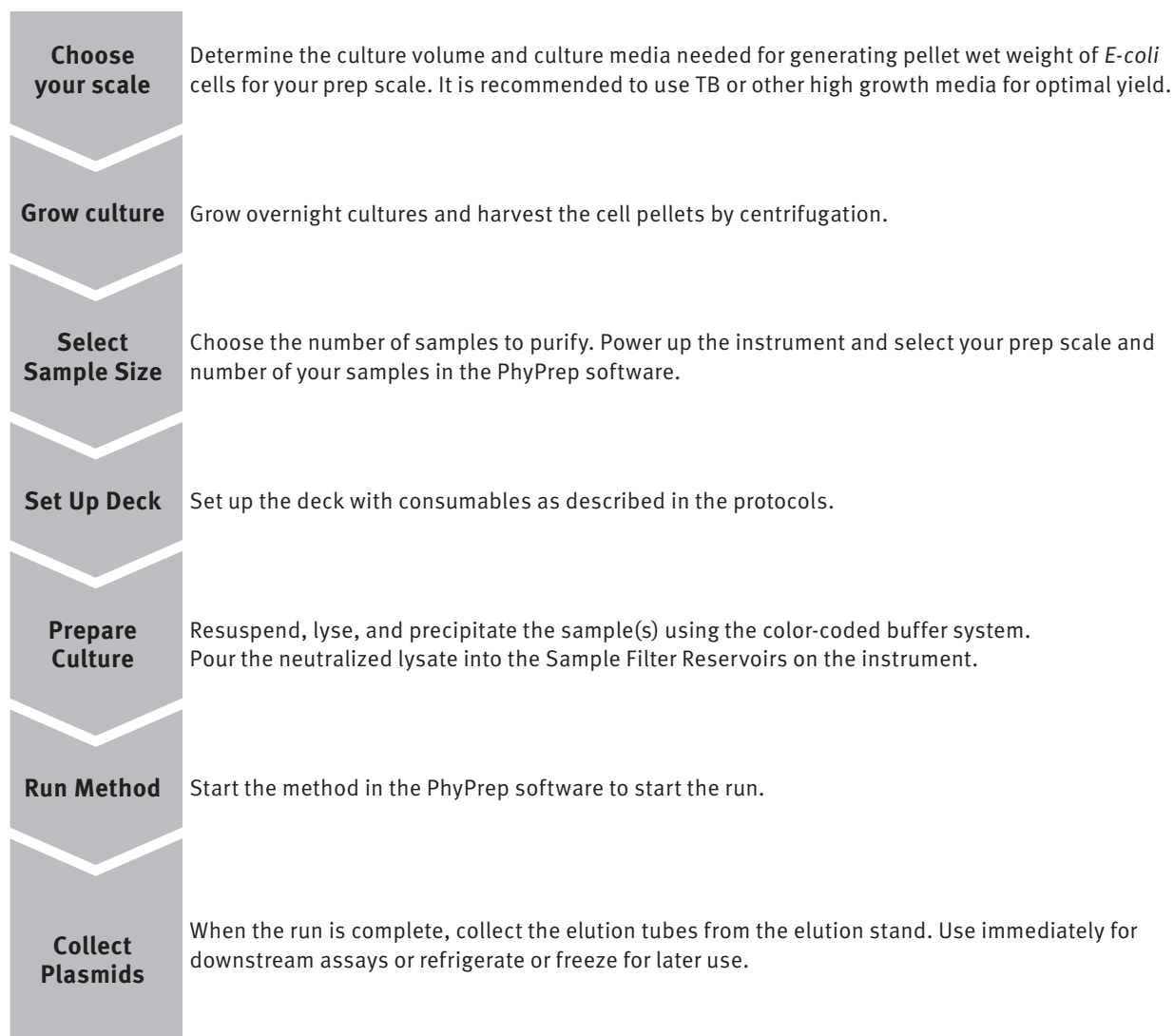
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System Overview

The Biotage® PhyPrep system is the only all-in-one instrument for automated endotoxin-free plasmid purification in maxi, mega, and giga scales. This system offers a variety of user friendly protocols for endotoxin-free plasmid purification. It is developed and supported by scientists and engineers to provide consistent high-quality endotoxin-free plasmids for transient transfection.

Prep Scale	Number of Sample Preps	Run Time (Hour/Minutes)	Culture Volume Per Sample	Pellet Wet Weight Per Sample	Optimal Pellet Wet weight	Plasmid Mass	Elution Volume
Maxiprep	1	42 m	150–250 mL	3–5 g	3 g	Up to 1 mg	5 mL
	2	42 m					
	3	1 h 10 m					
	4	1 h 10 m					
Megaprep	1	1 h 16 m	350–500 mL	6–8 g	7 g	Up to 5 mg	18 mL
	2	1 h 16 m					
	3	2 h 22 m					
	4	2 h 22 m					
Gigaprep	1	2 h 31 m	600–750 mL	14–16 g	15 g	Up to 10 mg	28 mL
	2	2 h 34 m					
	3	4 h 57 m					
	4	4 h 58 m					

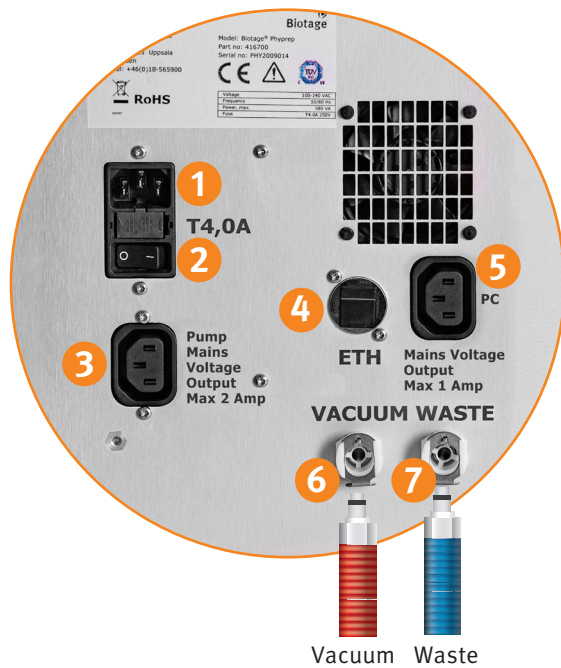
Quickstart Guide



Hardware & Connections

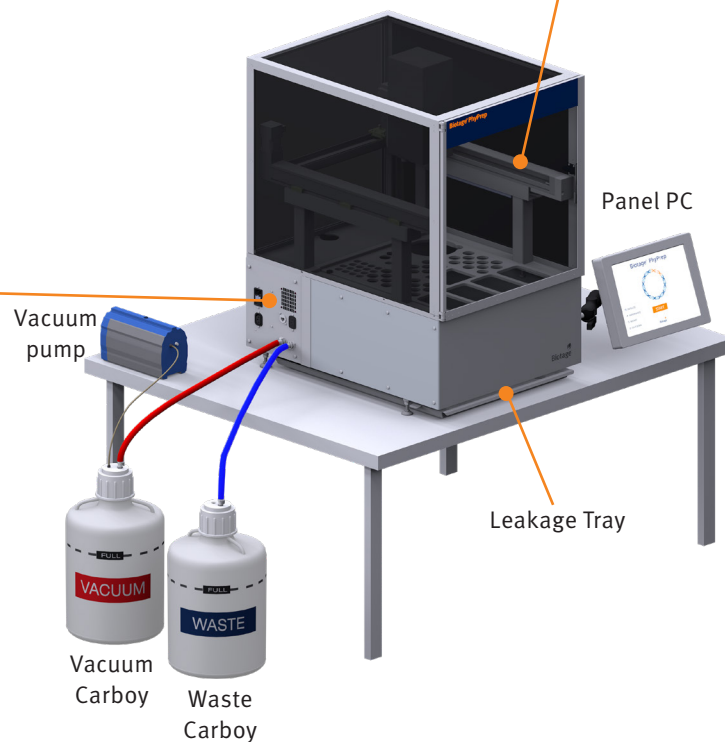
Instrument Side Panel

1. Power connector
2. Power switch
3. Vacuum power connector
4. Ethernet port
5. PC power connector
6. Vacuum port and red tubing
7. Waste port and blue tubing



Emergency Stop

Open the door for emergency stop.



Parts



Instrument Safety

Emergency Stop

The PhyPrep door works as an emergency stop. If the door is opened at any time during system operation, the instrument will stop operating immediately, and the current method will be aborted. It is not possible to resume the stopped method again after PhyPrep door is opened.

After an emergency stop, if the pipetting head has transfer tips or Våxel columns attached, eject these by using the “Initialize” button in the software. There should not be any columns attached to the pipetting head before starting a new run.

Important note

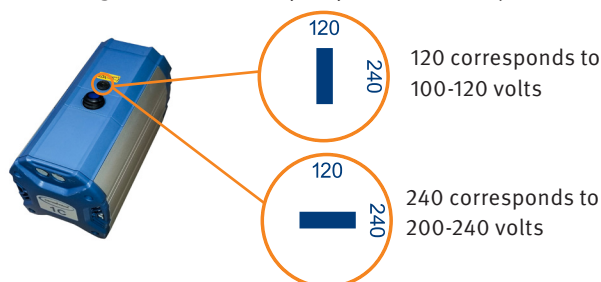
- » Never try to take off attached transfer tips or Våxel columns manually as you may harm your hand.

The STOP Button

If the STOP button is pressed during a method run, you are asked to confirm that the method should be stopped. If you confirm, the system will stop, and the current method will be aborted. It is not possible to resume the method after pressing STOP.

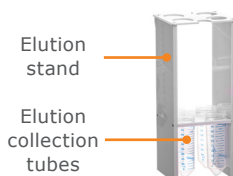
Vacuum Pump Voltage

Ensure the voltage on the vacuum pump is set correctly.



Elution Tube Stand

For each sample being purified, be sure to place a 50 mL pyrogen-free elution tube in the **bottom** level of the elution tube stand.



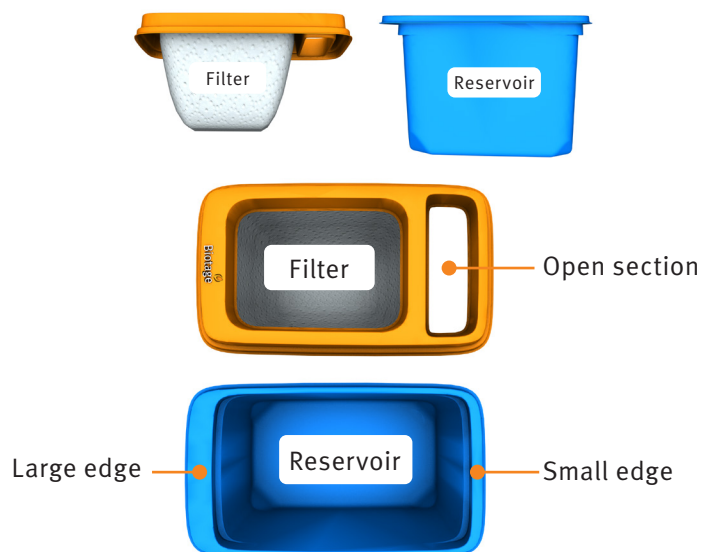
Instrument Cleaning

Do not spray alcohol directly onto the instrument robotics parts. Doing so will lead to damages to your PhyPrep. Ensure paper towels and or other materials are not placed in the vacuum station.

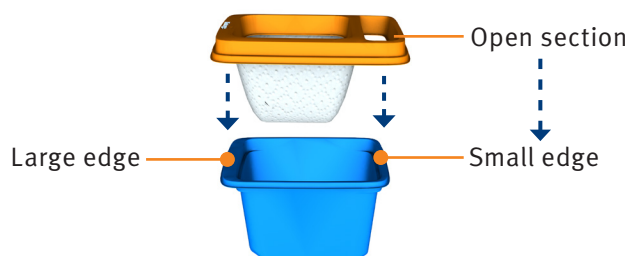
Sample Filter Reservoir

Assembly

Assemble the Sample Filter Reservoirs with the correct orientation before placing them on the deck. The reservoir consists of a filter top and a reservoir bottom. The filter lid has an open section and the reservoir has a large edge and a small edge for orientation.

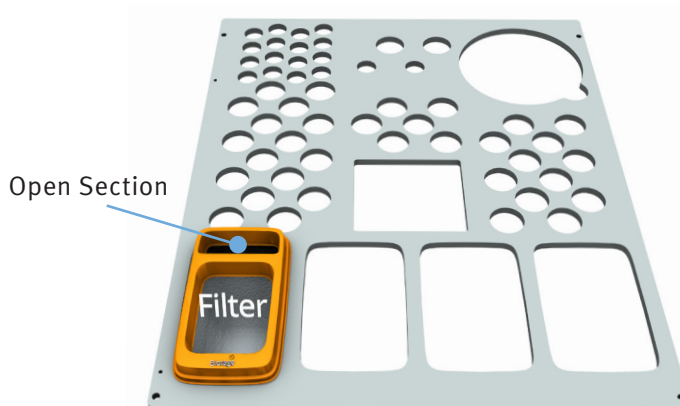


To assemble the Sample Filter Reservoir, place the open section of the filter top over the small edge of the reservoir bottom.



Placement on deck













Place the Sample Filter Reservoir on the deck with the filter section closest to the front of the instrument.















Biotage® PhyPrep Plasmid Kit Endotoxin-Free

Kit Contents













Maxiprep kit DPM-16-05-72-KIT

710-0001	900-0078	900-0081	900-0084	900-0091	900-0093	900-0087	900-0089	700-0030	DPM-16-05-72-RNA	417458SP	DPM-00-25-00
16 x	16 x	16 x	16 x	1 x	16 x	1 x	1 x	16 x	16 x	50 x	50 x
											
Växel Maxiprep Column	Resuspension Buffer	Lysis Buffer	Precipitation Buffer	Wash Buffer	Elution Buffer	Endotoxin Removal Buffer	Equilibration Buffer	Sample Filter Reservoir	RNase A Tubes	PhyPrep Tube	20 mL Transfer Tip

Megaprep kit DPM-16-10-72-KIT

710-0002	900-0079	900-0082	900-0085	900-0092	900-0094	900-0087	900-0089	700-0030	DPM-16-10-72-RNA	417458SP	DPM-00-25-00
16 x	16 x	16 x	16 x	1 x	16 x	1 x	1 x	16 x	16 x	100 x	50 x
											
Växel Megaprep Column	Resuspension Buffer	Lysis Buffer	Precipitation Buffer	Wash Buffer	Elution Buffer	Endotoxin Removal Buffer	Equilibration Buffer	Sample Filter Reservoir	RNase A Tubes	PhyPrep Tube	20 mL Transfer Tip

Gigaprep kit DPM-08-20-72-KIT

710-0003	900-0080	900-0083	900-0086	900-0092	900-0094	900-0088	900-0090	700-0030	DPM-08-20-72-RNA	417458SP	DPM-00-25-00
8 x	8 x	8 x	8 x	1 x	8 x	1 x	1 x	8 x	8 x	50 x	25 x
											
Växel Gigaprep Column	Resuspension Buffer	Lysis Buffer	Precipitation Buffer	Wash Buffer	Elution Buffer	Endotoxin Removal Buffer	Equilibration Buffer	Sample Filter Reservoir	RNase A Tubes	PhyPrep Tube	20 mL Transfer Tip

Instrument Deck

The Biotage PhyPrep has specific deck layouts for all of the pre-programmed methods.

Section 1: Transfer tips

Tips are placed in the upper left corner.

Section 2: Vacuum and waste station

Section 3: Wash Buffer Reservoir

The Wash Buffer Reservoir is located in the upper right corner. It should be removed from the deck prior to filling it with wash buffer to minimize the risk of spilling. Empty remaining volume after each run and clean with 70% alcohol.

Section 4: PhyPrep Tubes and Vaxel columns

This section contains space for 2 Vaxel columns and 10 PhyPrep Tubes.

Section 5: Supplemental buffer section

The supplemental buffer section contains 4 rows of PhyPrep Tube spots. Each row contains space for 2 tubes.

Section 6: PhyPrep Tubes and Vaxel columns

This section contains space for 2 Biotage Vaxel columns and 10 PhyPrep Tubes.

Section 7: Elution Tube Stand

The Elution Tube Stand is located near the front center. For each sample being purified, a 50 mL pyrogen-free elution tube must be placed in the **bottom** level of the elution stand. Before collecting the purified sample, carefully remove the Vaxel columns from the Elution Tube Stand. There may be a residual drop attached to the bottom of each tip. Lightly tap the drop onto the elution tube you would like to collect.

Section 8: Sample Filter Reservoirs

The Sample Filter Reservoirs are located in the frontmost portion of the deck. For each sample being purified, one sample reservoir should be used. Place the Filter Reservoirs in the correct orientation with the filter closest to the front of the deck.

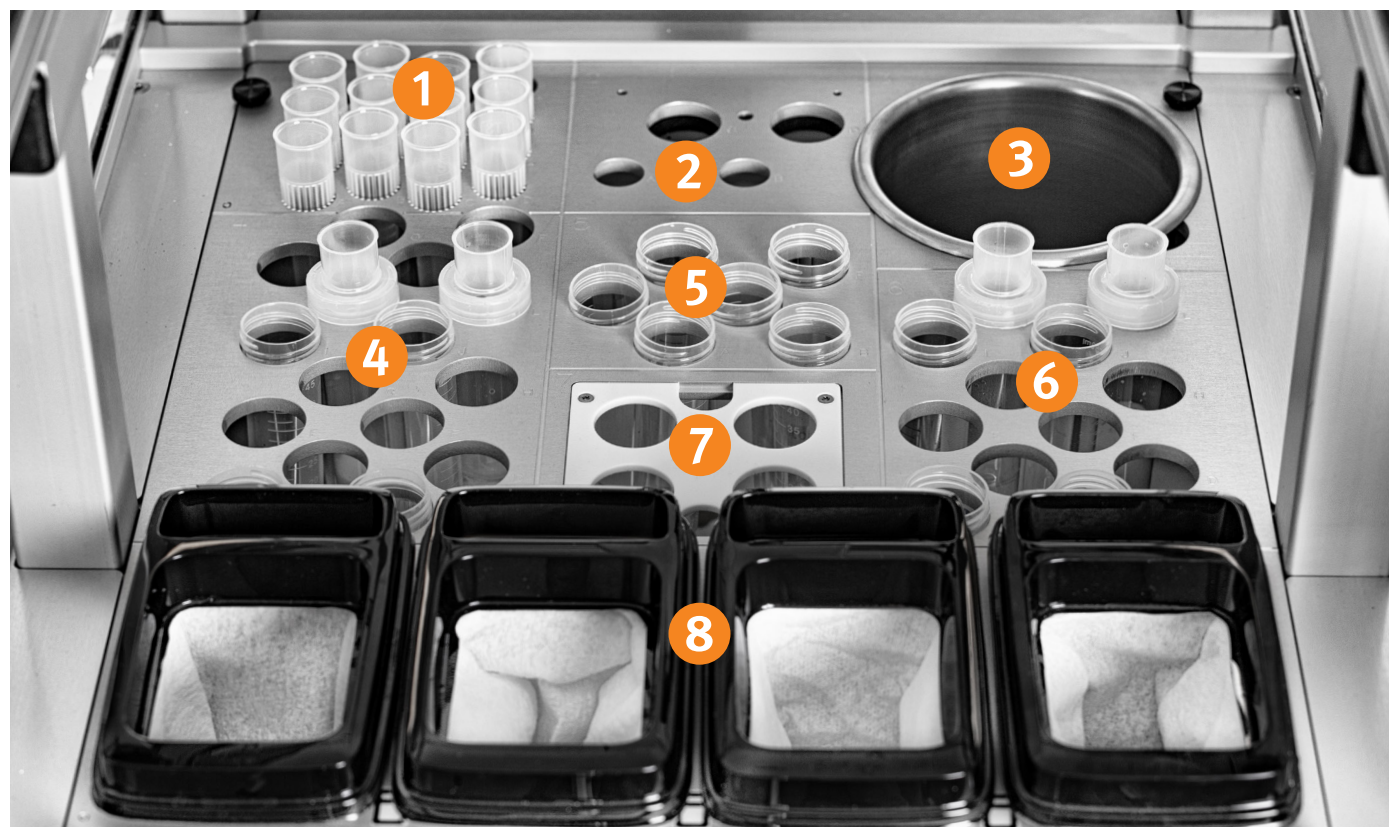
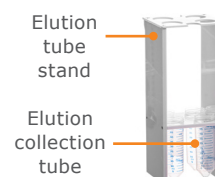
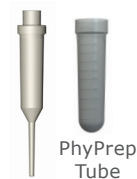


Figure 1. The PhyPrep deck layout 1) Transfer tubes 2) Vacuum station 3) Wash Buffer Reservoir 4) Biotage Vaxel columns and PhyPrep Tubes 5) Supplemental buffers 6) Biotage Vaxel columns and PhyPrep Tubes 7) Elution Tube Stand 8) Sample Filter Reservoirs

Before You Begin

Buffer Preparation

- » Add the provided lyophilized RNase A to **Resuspension Buffer** before use. Once RNase A is added to **Resuspension Buffer**, the solution should be stored at 2–8°C if not immediately used.
- » Check **Lysis Buffer** for precipitation due to low storage temperature. In the case of precipitation, dissolve by warming bottle(s) in water bath at 30–37°C for 10–20 minutes and mix by inversion.
- » Store these buffers at 4°C overnight before use for optimal yield:
 - » **Resuspension Buffer**
 - » **Precipitation Buffer**
 - » **Endotoxin Removal Buffer**
 - » **Equilibration Buffer**
- » To maintain the sterility of the equilibration buffer, endotoxin removal buffer, and wash buffer when aliquoting, use the fume hood and a flame. With the flame and fume hood on, pass the buffer container lid and neck through the flame and open the bottle while hovering near the flame.
When pouring out the specified amount of buffer, pour the buffer from the bulk bottle into the specified container for the deck while hovering near the flame. After aliquoting, flame through the cap and opening of the bottle before closing.

Preparing Resuspension Buffer

You can add the RNase A to **Resuspension Buffer** when you receive your kit. Once RNase A has been added to **Resuspension Buffer**, store at 2–8°C.

Recommended Culture Conditions for Optimal Results

The Biotage PhyPrep's robust instrumentation and chemistry allows for dense sample processing without the worry for clogging or contamination. Because of the instrument's flexibility and scale, Biotage recommends growing denser cultures compared to traditional manual preps.

Please follow the outlined recommendations for optimizing culture growth:

Growth Medias

The use of rich medias has the highest impact on cell growth density.

We recommend the following:

- » Terrific Broth (TB)
- » Thomson Plasmid+®
- » Agencourt Ale

Ratio of Media to Flask Size

Use a 1:5 ratio of media to flask size. The volume of media should not exceed 20% of the flask volume. This is to allow proper aeration of the culture during incubation and maximize cell division.

Incubator Temperature and Shaking Frequency

We recommend incubating at 37°C and 350 RPM. Larger cultures require faster shaking speeds to properly aerate the whole volume of cells. Certain plasmids (for example pUC19) will increase in copy number when grown at slightly higher temperatures.

Starter and Overnight Culture Times

A smaller culture grown for 7–8 hours to inoculate the overnight culture at a dilution of 1:1000 is recommended. In rich media, cultures may grow slightly longer for about 16 hours overnight.

Growth Flask and Air Permeable Seal

Cell density in cultures can be further optimized using Thomson flasks and seals.

- » Thomson Optimal Growth Flasks
- » Thomson AirOtop™ Enhanced Seals

Harvest Cells

- » 3–5 g pellet wet weight is needed for Maxiprep, achieved by growing 150–250 mL of an overnight bacterial culture.
- » 6–8 g pellet wet weight is needed for Megaprep, achieved by growing 350–500 mL of an overnight bacterial culture.
- » 14–16 g pellet wet weight is needed for Gigaprep, achieved by growing 600–750 mL of an overnight bacterial culture.
- » Centrifuge at $\geq 3500 \times g$ for 30 minutes. Discard supernatant.
- » The PhyPrep deck can be set up while waiting for cells to pellet. See appropriate deck layout per desired method for further instructions.

Sample Preparation Guide

All steps are carried out at room temperature. Please review the section “Before You Begin” on page 9 prior to proceeding with sample preparation.

1. Resuspend Cells

- » Add contents of **RNase A** tube to the **RESUSPENSION BUFFER**. Uncap the centrifuge bottle and add the entire contents of **RESUSPENSION BUFFER**. To aid in pellet resuspension, agitation beads are included in **RESUSPENSION BUFFER**. Recap the centrifuge bottle and shake vigorously until the bacterial pellet is completely resuspended.

Important reminder » Use 1 Resuspension Buffer bottle per sample. Verify that RNase A is added to Resuspension Buffer.

Important notes » Incomplete resuspension can result in poor recovery of plasmid DNA.

2. Lyse Cells

- » Uncap the centrifuge bottle and add the entire contents of **LYSIS BUFFER**. Recap the centrifuge bottle and mix by inverting gently 20–30 times. To aid in the lysis step, a blue indicator dye is present in **LYSIS BUFFER**. Ensure the solution turns completely blue. If nonblue portions are observed after 30 inversions, continue mixing gently. Let the lysis step occur for at least 3 minutes. Do not let the lysis step exceed 5 minutes.

Important reminder » Use 1 Lysis Buffer bottle per sample. Verify that Lysis Buffer has not precipitated.

Important notes

- » Do not shake or vortex during this step. Harsh mixing will shear genomic DNA and may contaminate the final recovered plasmid DNA.
- » Do not allow lysis to proceed longer than 5 minutes. Prolonged alkaline lysis may permanently denature the supercoiled plasmid DNA and may render it unsuitable for use in downstream applications.
- » If the buffer contents have precipitated due to low temperatures, warm bottle to 30–37°C for 10–20 min and shake to dissolve potential precipitation.

3. Precipitate

- » Uncap the centrifuge bottle and add the entire contents of **PRECIPITATION BUFFER**. Recap the centrifuge bottle and mix by inverting 20–30 times. A white precipitate containing cell debris, proteins, lipids, SDS, and chromosomal DNA will form. Upon complete neutralization, the solution will turn from blue to clear. If blue portions are observed after 30 inversions, continue mixing gently.

Important reminder Use 1 Precipitation Buffer bottle per sample.

Important notes

- » Mixing during this step should be gentle but thorough. Although it is important to completely mix the solution to ensure complete precipitation of cellular debris, do not shake or vortex during this step. Harsh mixing can cause break up of precipitated debris and may contaminate the final recovered plasmid DNA.
- » For increased yield, use cold Precipitation Buffer, Equilibration Buffer, Endotoxin Removal Buffer, and Resuspension Buffer that have been stored at 4°C.

4. Filter

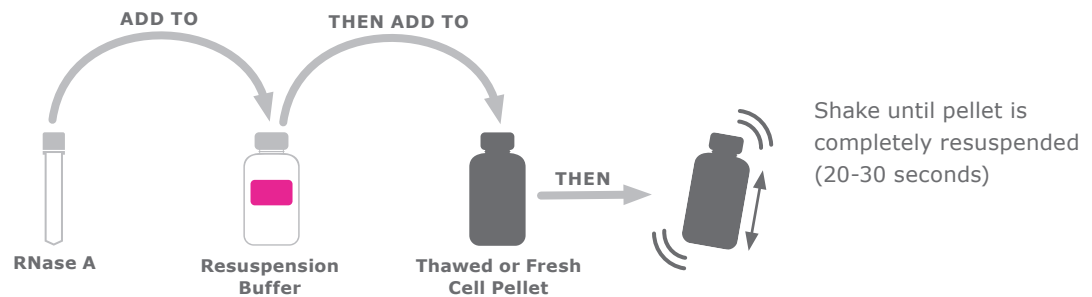
- » Place Sample Filter Reservoir on the PhyPrep deck. Then slowly pour entire contents of the centrifuge bottle into the middle of the Sample Filter Reservoir to allow the supernatant to go through the filter first. This will prevent the precipitated particulates clogging the filter.

Sample Preparation Guide

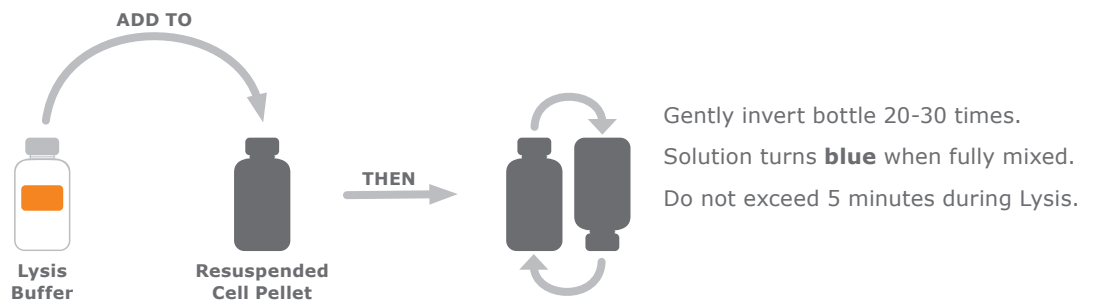
Required Kit



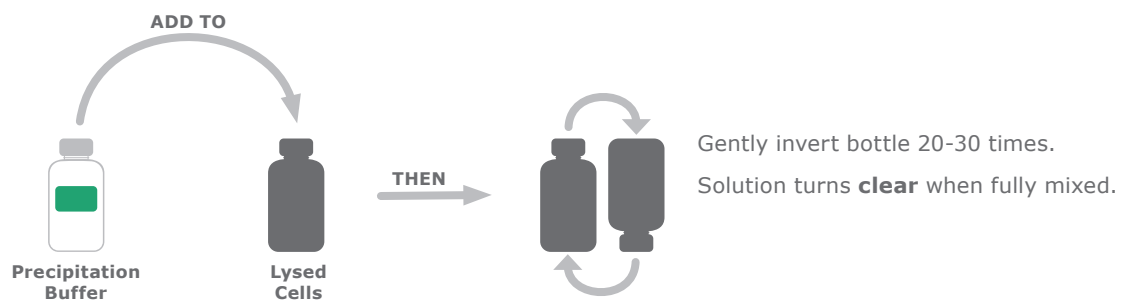
1. Resuspend Cells



2. Lyse Cells



3. Precipitate



4. Filter



Maxiprep 1 Sample Method










This protocol is designed for the preparation of up to 1 mg of high-copy plasmid DNA per sample using the Biotage PhyPrep Maxiprep kit. While setting up the deck, place the appropriate consumables in the corresponding numbered sections of the deck.

Recommended cell wet weight

- » 3-5 g per sample from culture volume range of 150–250 mL

Important notes

- » Ensure correct volume of Wash Buffer is added to the Wash Buffer Reservoir in Section 3. Ensure correct volumes of Equilibration Buffer and Endotoxin Removal Buffer are added to PhyPrep Tubes in sections 4 and 5.

Required Parts	20 mL Transfer Tip	Växel Maxiprep Column	Wash Buffer	Elution Buffer	Endotoxin Removal Buffer	Equilibration Buffer	50 mL Pyrogen-free Elution Tube	Sample Filter Reservoir	PhyPrep Tube
									
Quantity	3	1	1	1	1	1	1	1	3
Part #	DPM-00-25-00	710-0001	900-0091	900-0093	900-0087	900-0089	DPM-00-10-01	700-0030	417458SP

Section

1

- » Place 3 x Transfer Tips in A, E, and I.



Section

3

- » Add 130 mL of **WASH BUFFER** to the Wash Buffer Reservoir.



Section

4

- » Place 1 x PhyPrep Tube into A.
- » Place 1 x PhyPrep Tube into I.
- » Add 25 mL of **EQUILIBRATION BUFFER** to this tube.
- » Place 1 x Biotage® Växel Maxiprep column into K.



Section

5

- » Uncap and place 1 x **ELUTION BUFFER** tube into C.
- » Place 1 x PhyPrep Tube into E.
- » Add 20 mL of **ENDOTOXIN REMOVAL BUFFER** to this tube.



Section

7

- » Remove the Elution Tube Stand from the PhyPrep deck.
- » Uncap and place 1 x 50 mL pyrogen-free conical tubes into position A.
- » Elution collection tube should rest in the **bottom** portion of the elution stand.
- » Place the Elution Tube Stand back onto the PhyPrep deck.



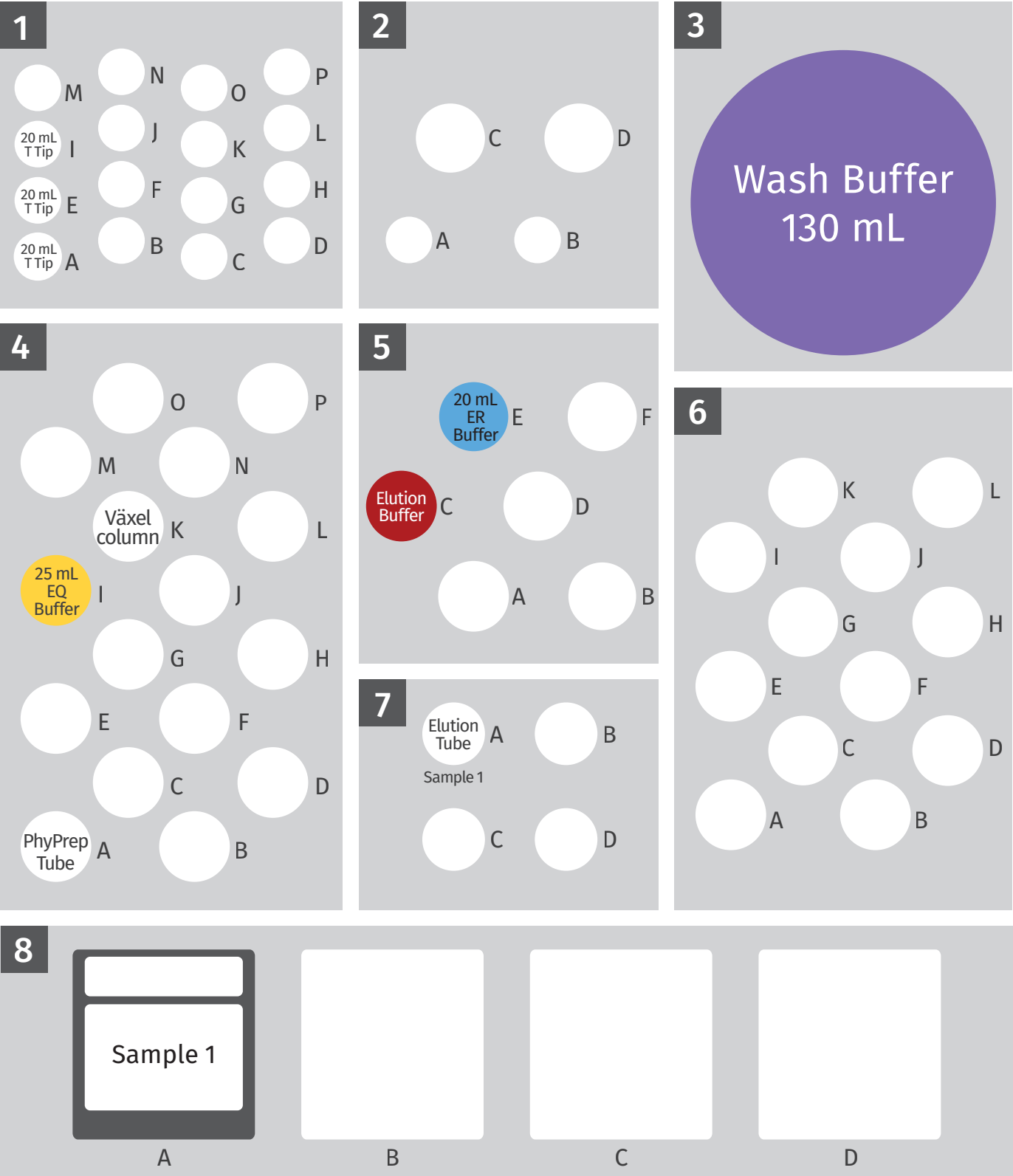
Section

8

- » Place 1 x Sample Filter Reservoir into A.
- » Ensure the filter part is towards the front of the deck.



Maxiprep 1 Sample Deck Layout



Maxiprep 2 Samples Method










This protocol is designed for the preparation of up to 1 mg of high-copy plasmid DNA per sample using the Biotage PhyPrep Maxiprep kit. While setting up the deck, place the appropriate consumables in the corresponding numbered sections of the deck.

Recommended cell wet weight

» 3–5 g per sample from culture volume range of 150–250 mL

Important notes

» Ensure correct volume of Wash Buffer is added to the Wash Buffer Reservoir in Section 3. Ensure correct volumes of Equilibration Buffer and Endotoxin Removal Buffer are added to PhyPrep Tubes in sections 4 and 5.

Required Parts	20 mL Transfer Tip	Växel Maxiprep Column	Wash Buffer	Elution Buffer	Endotoxin Removal Buffer	Equilibration Buffer	50 mL Pyrogen-free Elution Tube	Sample Filter Reservoir	PhyPrep Tube
									
Quantity	6	2	1	2	1	1	2	2	6
Part #	DPM-00-25-00	710-0001	900-0091	900-0093	900-0087	900-0089	DPM-00-10-01	700-0030	417458SP

Section

1

» Place 6 x Transfer Tips in A, C, E, G, I, and K.



Section

3

» Add 190 mL of **WASH BUFFER** to the Wash Buffer Reservoir.



Section

4

» Place 2 x PhyPrep Tubes into A and B.
 » Place 2 x PhyPrep Tubes into I and J.
 Add 25 mL of **EQUILIBRATION BUFFER** to these tubes.
 » Place 2 x Biotage® Växel Maxiprep columns into K and L.



Section

5

» Uncap and place 2 x **ELUTION BUFFER** tubes into C and D.
 » Place 2 x PhyPrep Tubes into E and F.
 Add 20 mL of **ENDOTOXIN REMOVAL BUFFER** to these tubes.



Section

7

» Remove the Elution Tube Stand from the PhyPrep deck.
 » Uncap and place 2 x 50 mL pyrogen-free conical tubes into positions A and B.
 » Elution collection tubes should rest in the **bottom** portion of the elution stand.
 » Place the Elution Tube Stand back onto the PhyPrep deck.



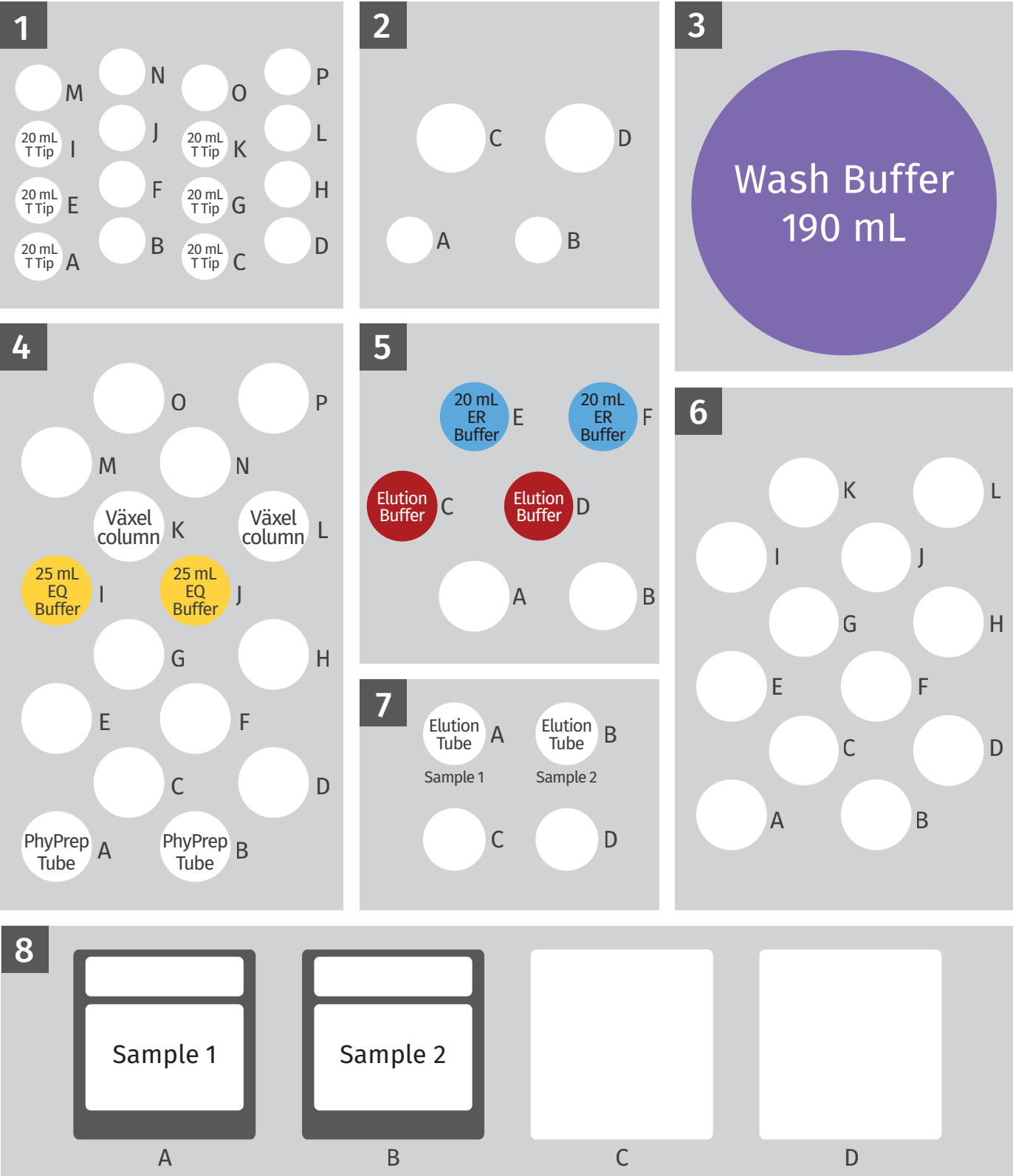
Section

8

» Place 2 x Sample Filter Reservoirs into A and B.
 » Ensure the filter part is towards the front of the deck.



Maxiprep 2 Samples Deck Layout



Maxiprep 3 Samples Method

This protocol is designed for the preparation of up to 1 mg of high-copy plasmid DNA per sample using the Biotage PhyPrep Maxiprep kit. While setting up the deck, place the appropriate consumables in the corresponding numbered sections of the deck.

Recommended cell wet weight

» 3–5 g per sample from culture volume range of 150–250 mL

Important notes

» Ensure correct volume of Wash Buffer is added to the Wash Buffer Reservoir in Section 3. Ensure correct volumes of Equilibration Buffer and Endotoxin Removal Buffer are added to PhyPrep Tubes in sections 4, 5, and 6.

Required Parts	20 mL Transfer Tip	Växel Maxiprep Column	Wash Buffer	Elution Buffer	Endotoxin Removal Buffer	Equilibration Buffer	50 mL pyrogen-free Elution Tube	Sample Filter Reservoir	PhyPrep Tube
Quantity	9	3	1	3	1	1	3	3	8
Part #	DPM-00-25-00	710-0001	900-0091	900-0093	900-0087	900-0089	DPM-00-10-01	700-0030	417458SP

Section

1

» Place 9 x Transfer Tips in A, B, C, E, F, G, I, J, and K.



Section

3

» Add 250 mL of **WASH BUFFER** to the Wash Buffer Reservoir.



Section

4

» Place 2 x PhyPrep Tubes into A and B.
 » Place 2 x PhyPrep Tubes into I and J.
 Add 25 mL of **EQUILIBRATION BUFFER** to these tubes.
 » Place 2 x Biotage® Växel Maxiprep columns into K and L.



Section

5

» Uncap and place 3 x **ELUTION BUFFER** tubes into A, C and D.
 » Place 2 x PhyPrep Tubes into E and F.
 Add **ENDOTOXIN REMOVAL BUFFER** as follows: 40 mL into E and 20 mL to F.



Section

6

» Place 1 x PhyPrep Tube into A.
 » Place 1 x PhyPrep Tube into I.
 Add 25 mL of **EQUILIBRATION BUFFER** to this tube.
 » Place 1 x Biotage® Växel Maxiprep column into K.



Section

7

» Remove the Elution Tube Stand from the PhyPrep deck.
 » Uncap and place 3 x 50 mL pyrogen-free conical tubes in position A, B, and C.
 » Elution collection tubes should rest in the **bottom** portion of the elution stand.
 » Place the Elution Tube Stand back onto the PhyPrep deck.



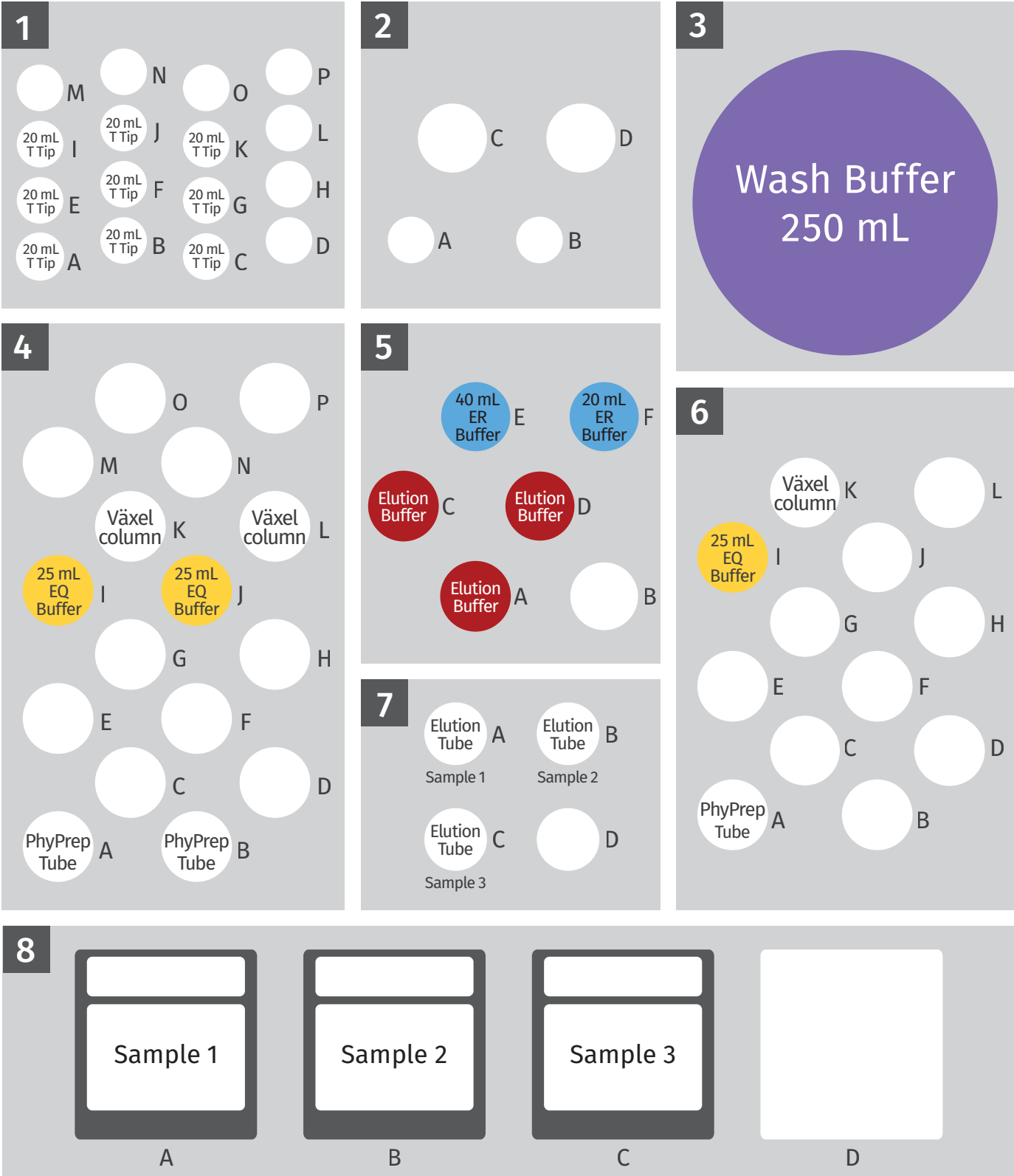
Section

8

» Place 3 x Sample Filter Reservoirs into A, B, and C.
 » Ensure the filter part is towards the front of the deck.



Maxiprep 3 Samples Deck Layout



Maxiprep 4 Samples Method










This protocol is designed for the preparation of up to 1 mg of high-copy plasmid DNA per sample using the Biotage PhyPrep Maxiprep kit. While setting up the deck, place the appropriate consumables in the corresponding numbered sections of the deck.

Recommended cell wet weight

» 3-5 g per sample from culture volume range of 150–250 mL

Important notes

» Ensure correct volume of Wash Buffer is added to the Wash Buffer Reservoir in Section 3. Ensure correct volumes of Equilibration Buffer and Endotoxin Removal Buffer are added to PhyPrep Tubes in sections 4, 5, and 6.

Required Parts	20 mL Transfer Tip	Växel Maxiprep Column	Wash Buffer	Elution Buffer	Endotoxin Removal Buffer	Equilibration Buffer	50 mL pyrogen-free Elution Tube	Sample Filter Reservoir	PhyPrep Tube
									
Quantity	12	4	1	4	1	1	4	4	10
Part #	DPM-00-25-00	710-0001	900-0091	900-0093	900-0087	900-0089	DPM-00-10-01	700-0030	417458SP

Section

1

» Place 12 x Transfer Tips in A, B, C, D, E, F, G, H, I, J, K, and L.



Section

3

» Add 310 mL of **WASH BUFFER** to the Wash Buffer Reservoir.



Section

4

» Place 2 x PhyPrep Tubes into A and B.
 » Place 2 x PhyPrep Tubes into I and J.
 Add 25 mL of **EQUILIBRATION BUFFER** to these tubes.
 » Place 2 x Biotage® Växel Maxiprep columns into K and L.



Section

5

» Uncap and place 4 x **ELUTION BUFFER** tubes into A, B, C and D.
 » Place 2 x PhyPrep Tubes into E and F. Add 40 mL of **ENDOTOXIN REMOVAL BUFFER** to these tubes.



Section

6

» Place 2 x PhyPrep Tubes into A and B.
 » Place 2 x PhyPrep Tubes into I and J.
 Add 25 mL of **EQUILIBRATION BUFFER** to these tubes.
 » Place 2 x Biotage® Växel Maxiprep columns into K and L.



Section

7

» Remove the Elution Tube Stand from the PhyPrep deck.
 » Uncap and place 4 x 50 mL pyrogen-free conical tubes in position A, B, C, and D.
 » Elution collection tubes should rest in the **bottom** portion of the elution stand.
 » Place the Elution Tube Stand back onto the PhyPrep deck.



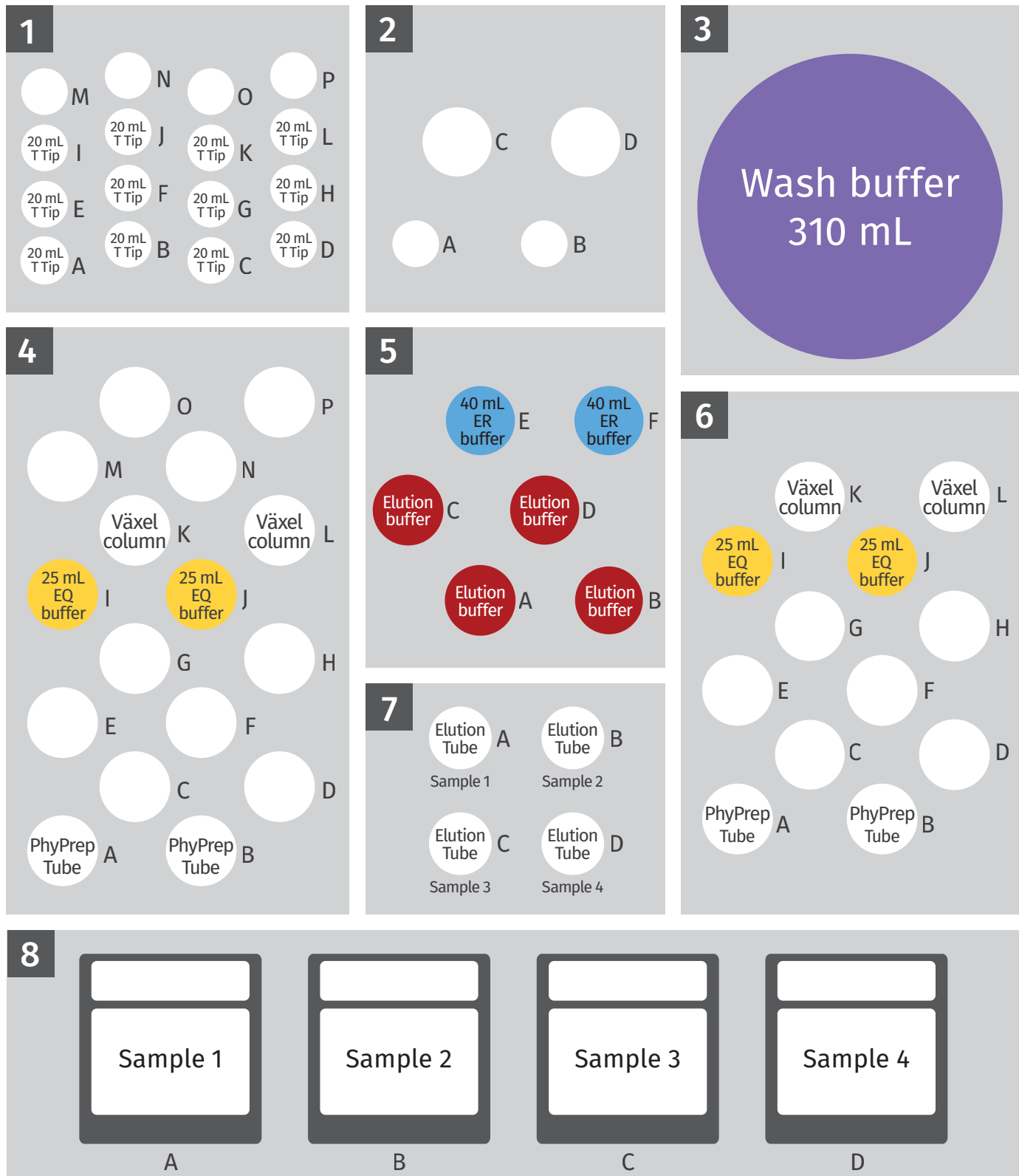
Section

8

» Place 4 x Sample Filter Reservoirs into A, B, C, and D.
 » Ensure the filter part is towards the front of the deck.



Maxiprep 4 Samples Deck Layout



Megaprep 1 Sample Method










This protocol is designed for the preparation of up to 5 mg of high-copy plasmid DNA per sample using the Biotage PhyPrep Megaprep kit. While setting up the deck, place the appropriate consumables in the corresponding numbered sections of the deck.

Recommended cell wet weight

- » 6-8 g per sample from culture volume range of 350–500 mL

Important notes

- » Ensure correct volume of Wash Buffer is added to the Wash Buffer Reservoir in Section 3. Ensure correct volumes of Equilibration Buffer and Endotoxin Removal Buffer are added to PhyPrep Tubes in sections 4 and 5.

Required Parts	20 mL Transfer Tip	Växel Megaprep Column	Wash Buffer	Elution Buffer	Endotoxin Removal Buffer	Equilibration Buffer	50 mL Pyrogen-free Elution Tube	Sample Filter Reservoir	PhyPrep Tube
									
Quantity	3	1	1	1	1	1	1	1	5
Part #	DPM-00-25-00	710-0002	900-0092	900-0094	900-0087	900-0089	DPM-00-10-01	700-0030	

Section

1

- » Place 3 x transfer tips in A, E, and I.



Section

3

- » Add 270 mL of **WASH BUFFER** to the Wash Buffer Reservoir.



Section

4

- » Place 3 x PhyPrep Tubes in A, C, and E.
- » Place 1 x PhyPrep Tube into I.
- » Add 30 mL of **EQUILIBRATION BUFFER** to this tube.
- » Place 1 x Biotage® Växel Megaprep column in K.



Section

5

- » Uncap and place 1 x **ELUTION BUFFER** tube into C.
- » Place 1 x PhyPrep Tube into E.
- » Add 20 mL of **ENDOTOXIN REMOVAL BUFFER** to this tube.



Section

7

- » Remove the Elution Tube Stand from the PhyPrep deck.
- » Uncap and place 1 x 50 mL pyrogen-free conical tube in position A.
- » Elution collection tubes should rest in the **bottom** portion of the elution stand.
- » Place the Elution Tube Stand back onto the PhyPrep deck.



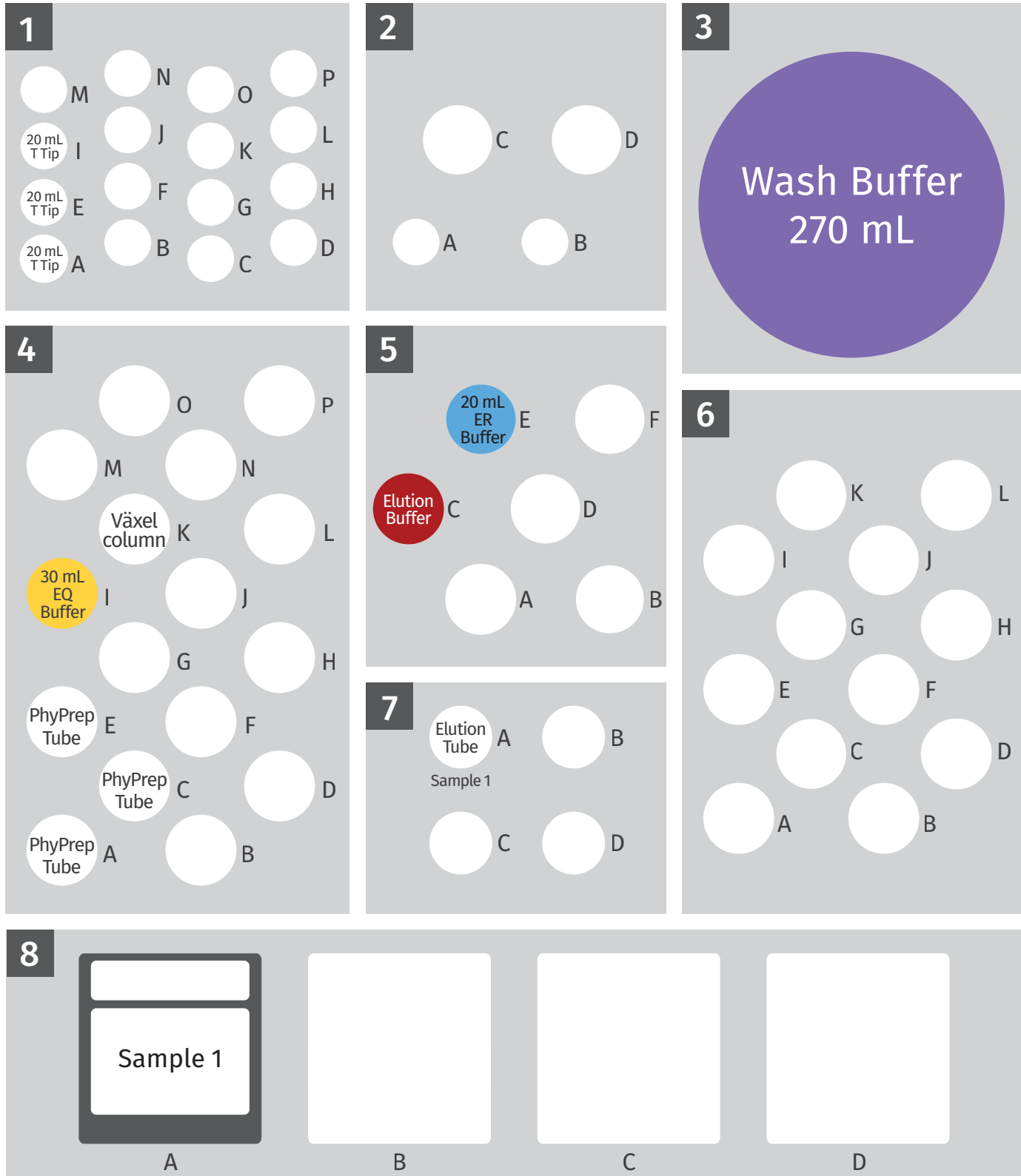
Section

8

- » Place 1 x Sample Filter Reservoir into A.
- » Ensure the filter part is towards the front of the deck.



Megaprep 1 Sample Deck Layout



Megaprep 2 Samples Method










This protocol is designed for the preparation of up to 5 mg of high-copy plasmid DNA per sample using the Biotage PhyPrep Megaprep kit. While setting up the deck, place the appropriate consumables in the corresponding numbered sections of the deck.

Recommended cell wet weight

» 6-8 g per sample from culture volume range of 350–500 mL

Important notes

» Ensure correct volume of Wash Buffer is added to the Wash Buffer Reservoir in Section 3. Ensure correct volumes of Equilibration Buffer and Endotoxin Removal Buffer are added to PhyPrep Tubes in sections 4 and 5.

Required Parts	20 mL Transfer Tip	Växel Megaprep Column	Wash Buffer	Elution Buffer	Endotoxin Removal Buffer	Equilibration Buffer	50 mL Pyrogen-free Elution Tube	Sample Filter Reservoir	PhyPrep Tube
									
Quantity	6	2	1	2	1	1	2	2	10
Part #	DPM-00-25-00	710-0002	900-0092	900-0094	900-0087	900-0089	DPM-00-10-01	700-0030	417458SP

Section

1

» Place 6 x Transfer Tips in A, C, E, G, I, and K.



Section

3

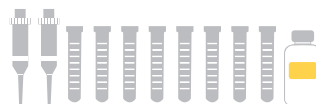
» Add 470 mL of **WASH BUFFER** to the Wash Buffer Reservoir.



Section

4

» Place 6 x PhyPrep Tubes into A, B, C, D, E, and F.
 » Place 2 x PhyPrep Tubes into I and J.
 Add 30 mL of **EQUILIBRATION BUFFER** to these tubes.
 » Place 2 x Biotage® Växel Megaprep columns into K and L.



Section

5

» Uncap and place 2 x **ELUTION BUFFER** tubes into C and D.
 » Place 2 x PhyPrep Tubes into E and F.
 Add 20 mL of **ENDOTOXIN REMOVAL BUFFER** to these tubes.



Section

7

» Remove the Elution Tube Stand from the PhyPrep deck.
 » Uncap and place 2 x 50 mL pyrogen-free conical tubes in position A and B.
 » Elution collection tubes should rest in the **bottom** portion of the elution stand.
 » Place the Elution Tube Stand back onto the PhyPrep deck.



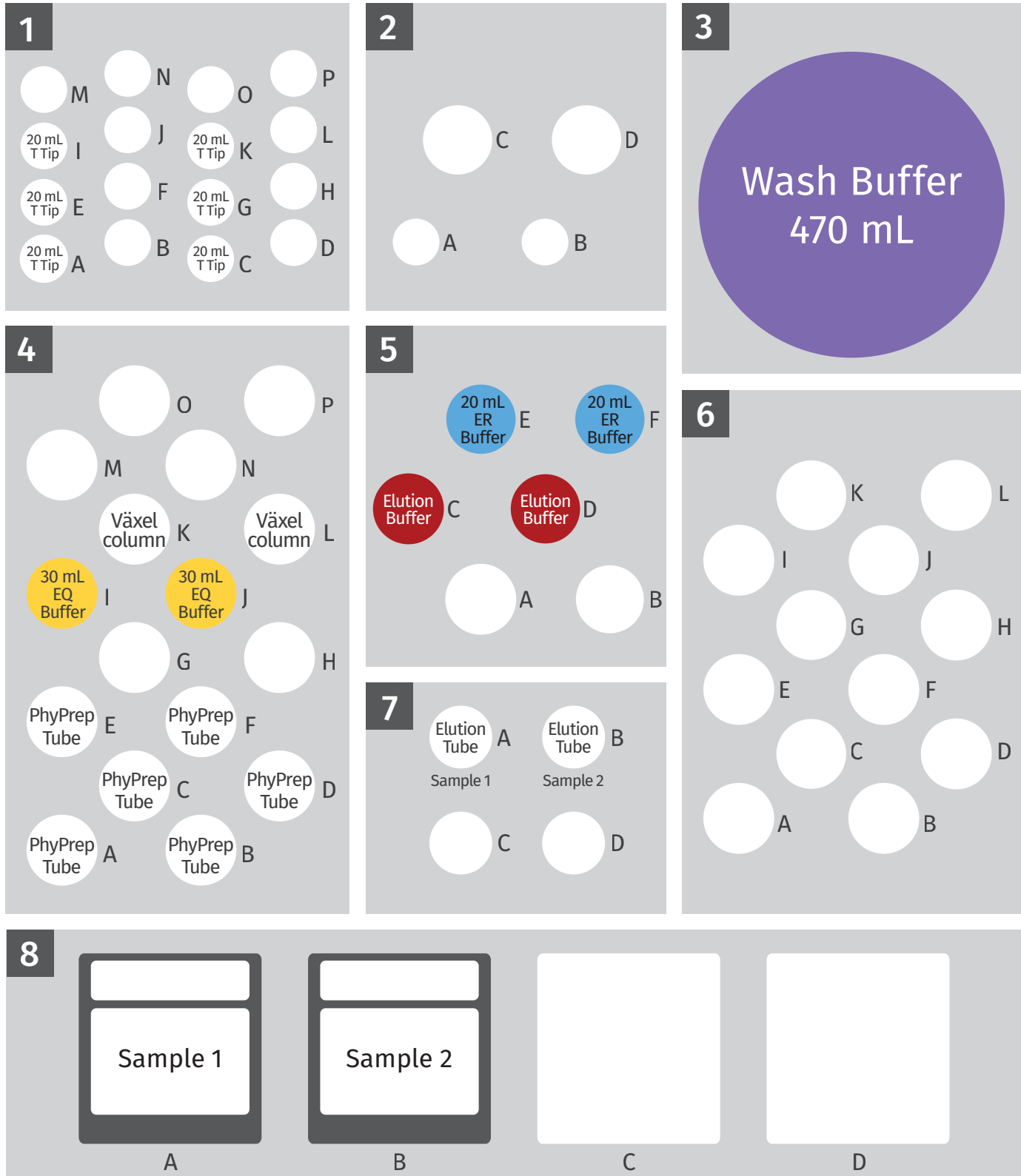
Section

8

» Place 2 x Sample Filter Reservoirs into A and B.
 » Ensure the filter part is towards the front of the deck.



Megaprep 2 Samples Deck Layout



Megaprep 3 Samples Method










This protocol is designed for the preparation of up to 5 mg of high-copy plasmid DNA per sample using the Biotage PhyPrep Megaprep kit. While setting up the deck, place the appropriate consumables in the corresponding numbered sections of the deck.

Recommended cell wet weight

» 6-8 g per sample from culture volume range of 350–500 mL

Important notes

» Ensure correct volume of Wash Buffer is added to the Wash Buffer Reservoir in Section 3. Ensure correct volumes of Equilibration Buffer and Endotoxin Removal Buffer are added to PhyPrep Tubes in sections 4, 5, and 6.

Required Parts	20 mL Transfer Tip	Växel Megaprep Column	Wash Buffer	Elution Buffer	Endotoxin Removal Buffer	Equilibration Buffer	50 mL Pyrogen-free Elution Tube	Sample Filter Reservoir	PhyPrep Tube
									
Quantity	9	3	1	3	1	1	3	3	14
Part #	DPM-00-25-00	710-0002	900-0092	900-0094	900-0087	900-0089	DPM-00-10-01	700-0030	417458SP

Section

1

» Place 9 x Transfer Tips in A, B, C, E, F, G, I, J, and K.



Section

3

» Add 670 mL of **WASH BUFFER** to the Wash Buffer Reservoir.



Section

4

» Place 6 x PhyPrep Tubes into A, B, C, D, E, and F.
 » Place 2 x PhyPrep Tubes into I and J.
 Add 30 mL of **EQUILIBRATION BUFFER** to these tubes.
 » Place 2 x Biotage® Växel Megaprep columns into K and L.



Section

5

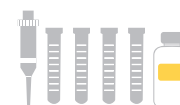
» Uncap and place 3 x **ELUTION BUFFER** tube into A, C and D.
 » Place 2 x PhyPrep Tubes into E and F.
 Add **ENDOTOXIN REMOVAL BUFFER** as follows: 40 mL into E and 20 mL to F.



Section

6

» Place 3 x PhyPrep Tubes into A, C, and E.
 » Place 1 x PhyPrep Tube into I.
 Add 30 mL of **EQUILIBRATION BUFFER** to this tube.
 » Place 1 x Biotage® Växel Megaprep columns into K.



Section

7

» Remove the Elution Tube Stand from the PhyPrep deck.
 » Uncap and place 3 x 50 mL pyrogen-free conical tubes in position A, B, and C.
 » Elution collection tubes should rest in the **bottom** portion of the elution stand.
 » Place the Elution Tube Stand back onto the PhyPrep deck.



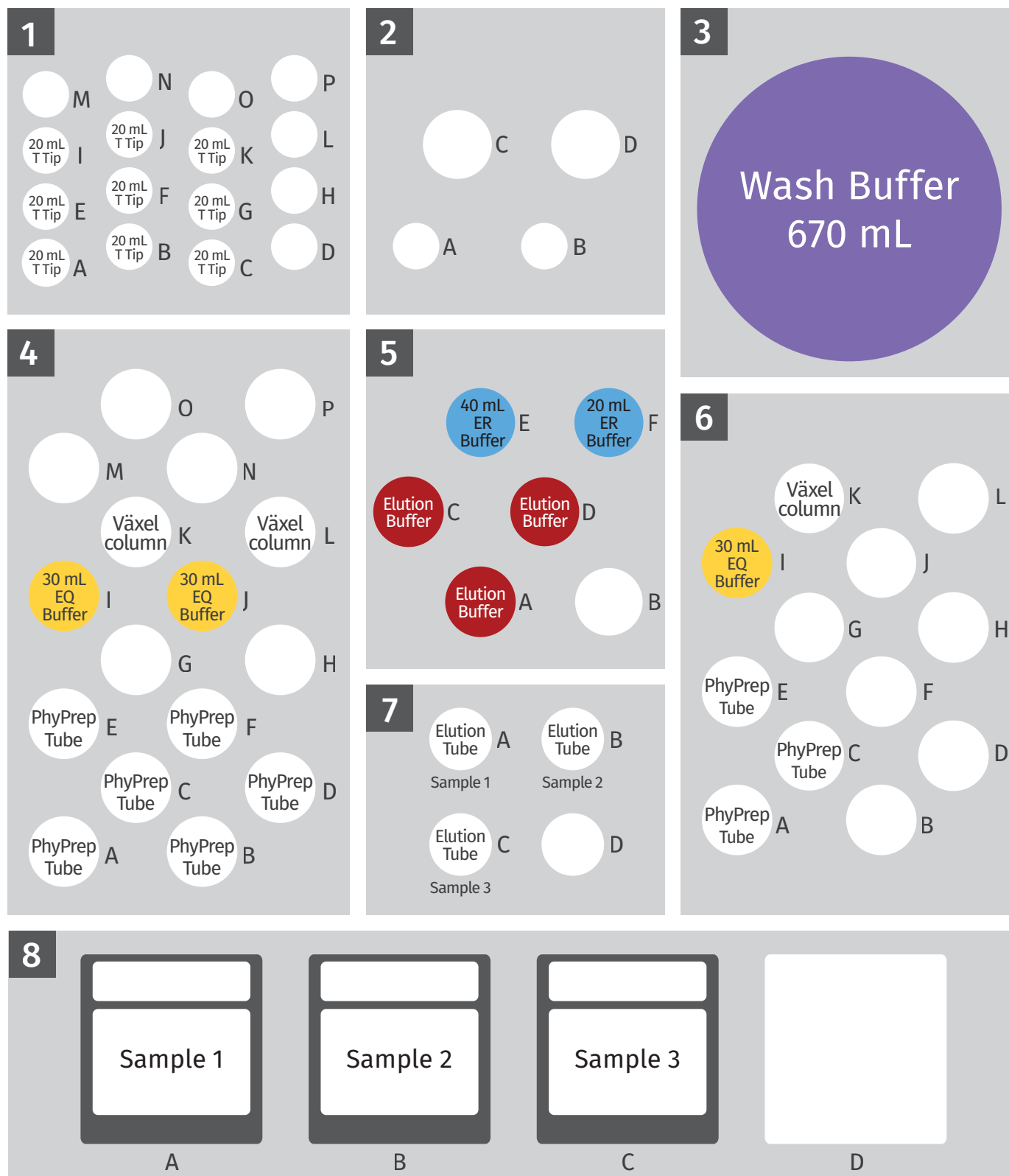
Section

8

» Place 3 x Sample Filter Reservoirs into A, B, and C.
 » Ensure the filter part is towards the front of the deck.



Megaprep 3 Samples Deck Layout



Megaprep 4 Samples Method










This protocol is designed for the preparation of up to 5 mg of high-copy plasmid DNA per sample using the Biotage PhyPrep Megaprep kit. While setting up the deck, place the appropriate consumables in the corresponding numbered sections of the deck.

Recommended cell wet weight

» 6-8 g per sample from culture volume range of 350–500 mL

Important notes

» Ensure correct volume of Wash Buffer is added to the Wash Buffer Reservoir in Section 3. Ensure correct volumes of Equilibration Buffer and Endotoxin Removal Buffer are added to PhyPrep Tubes in sections 4, 5, and 6.

Required Parts	20 mL Transfer Tip	Växel Megaprep Column	Wash Buffer	Elution Buffer	Endotoxin Removal Buffer	Equilibration Buffer	50 mL Pyrogen-free Elution Tube	Sample Filter Reservoir	PhyPrep Tube
									
Quantity	12	4	1	4	1	1	4	4	18
Part #	DPM-00-25-00	710-0002	900-0092	900-0094	900-0087	900-0089	DPM-00-10-01	700-0030	417458SP

Section

1

» Place 12 x Transfer Tips in A, B, C, D, E, F, G, H, I, J, K, and L.



Section

3

» Add 870 mL of **WASH BUFFER** to the Wash Buffer Reservoir.



Section

4

» Place 6 x PhyPrep Tubes into A, B, C, D, E, and F.
 » Place 2 x PhyPrep Tubes into I and J.
 Add 30 mL of **EQUILIBRATION BUFFER** to these tubes.
 » Place 2 x Biotage® Växel Megaprep columns into K and L.



Section

5

» Uncap and place 4 x **ELUTION BUFFER** tubes into A, B, C and D.
 » Place 2 x PhyPrep Tubes into E and F.
 Add 40 mL of **ENDOTOXIN REMOVAL BUFFER** to these tubes.



Section

6

» Place 6 x PhyPrep Tubes into A, B, C, D, E, and F.
 » Place 2 x PhyPrep Tubes into I and J.
 Add 30 mL of **EQUILIBRATION BUFFER** to these tubes.
 » Place 2 x Biotage® Växel Megaprep columns into K and L.



Section

7

» Remove the Elution Tube Stand from the PhyPrep deck.
 » Uncap and place 4 x 50 mL pyrogen-free conical tubes in position A, B, C, and D.
 » Elution collection tubes should rest in the **bottom** portion of the elution stand.
 » Place the Elution Tube Stand back onto the PhyPrep deck.



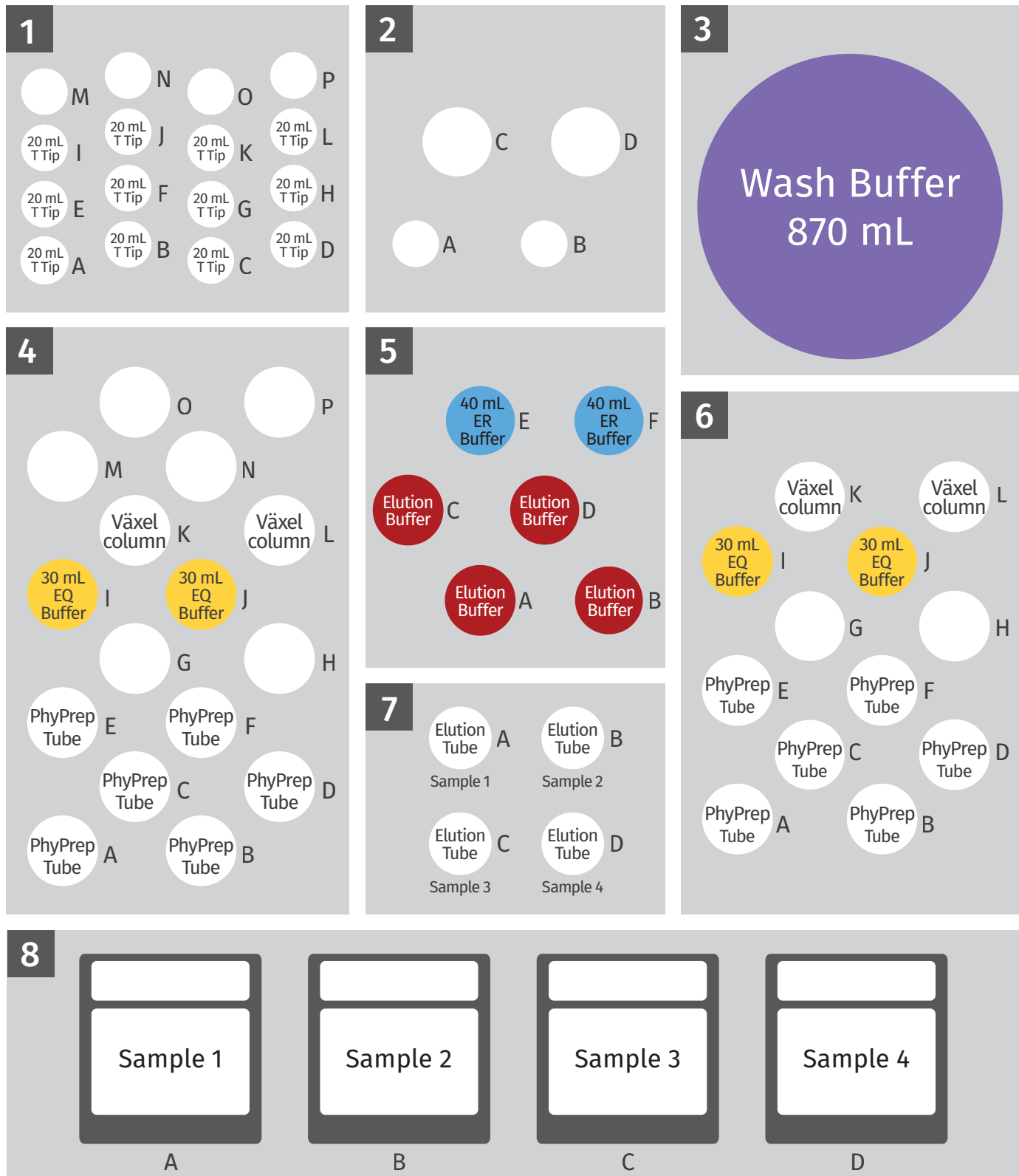
Section

8

» Place 4 x Sample Filter Reservoirs into A, B, C, and D.
 » Ensure the filter part is towards the front of the deck.



Megaprep 4 Samples Deck Layout



Gigaprep 1 Sample Method










This protocol is designed for the preparation of up to 10 mg of high-copy plasmid DNA per sample using the Biotage PhyPrep Gigaprep kit. While setting up the deck, place the appropriate consumables in the corresponding numbered sections of the deck.

Recommended cell wet weight

- » 14-16 g per sample from culture volume range of 600–750 mL

Important notes

- » Ensure correct volume of Wash Buffer is added to the Wash Buffer Reservoir in Section 3. Ensure correct volumes of Equilibration Buffer and Endotoxin Removal Buffer are added to PhyPrep Tubes in sections 4 and 5.

Required Parts	20 mL Transfer Tip	Växel Gigaprep Column	Wash Buffer	Elution Buffer	Endotoxin Removal Buffer	Equilibration Buffer	50 mL Pyrogen-free Elution Tube	Sample Filter Reservoir	PhyPrep Tube
									
Quantity	3	1	1	1	1	1	1	1	6
Part #	DPM-00-25-00	710-0003	900-0092	900-0094	900-0088	900-0090	DPM-00-10-01	700-0030	417458SP

Section

1

- » Place 3 x Transfer Tips in A, E, and I.



Section

3

- » Add 480 mL of **WASH BUFFER** to the Wash Buffer Reservoir.



Section

4

- » Place 4 x PhyPrep Tubes in A, C, E, and G.
- » Place 1 x PhyPrep Tube into I.
- » Add 40 mL of **EQUILIBRATION BUFFER** to this tube.
- » Place 1 x Biotage® Växel Gigaprep column in K.



Section

5

- » Uncap and place 1 x **ELUTION BUFFER** tube into C.
- » Place 1 x PhyPrep Tube into E.
- » Add 30 mL of **ENDOTOXIN REMOVAL BUFFER** to this tube.



Section

7

- » Remove the Elution Tube Stand from the PhyPrep deck.
- » Uncap and place 1 x 50 mL pyrogen-free conical tube in position A.
- » Elution collection tubes should rest in the **bottom** portion of the elution stand.
- » Place the Elution Tube Stand back onto the PhyPrep deck.



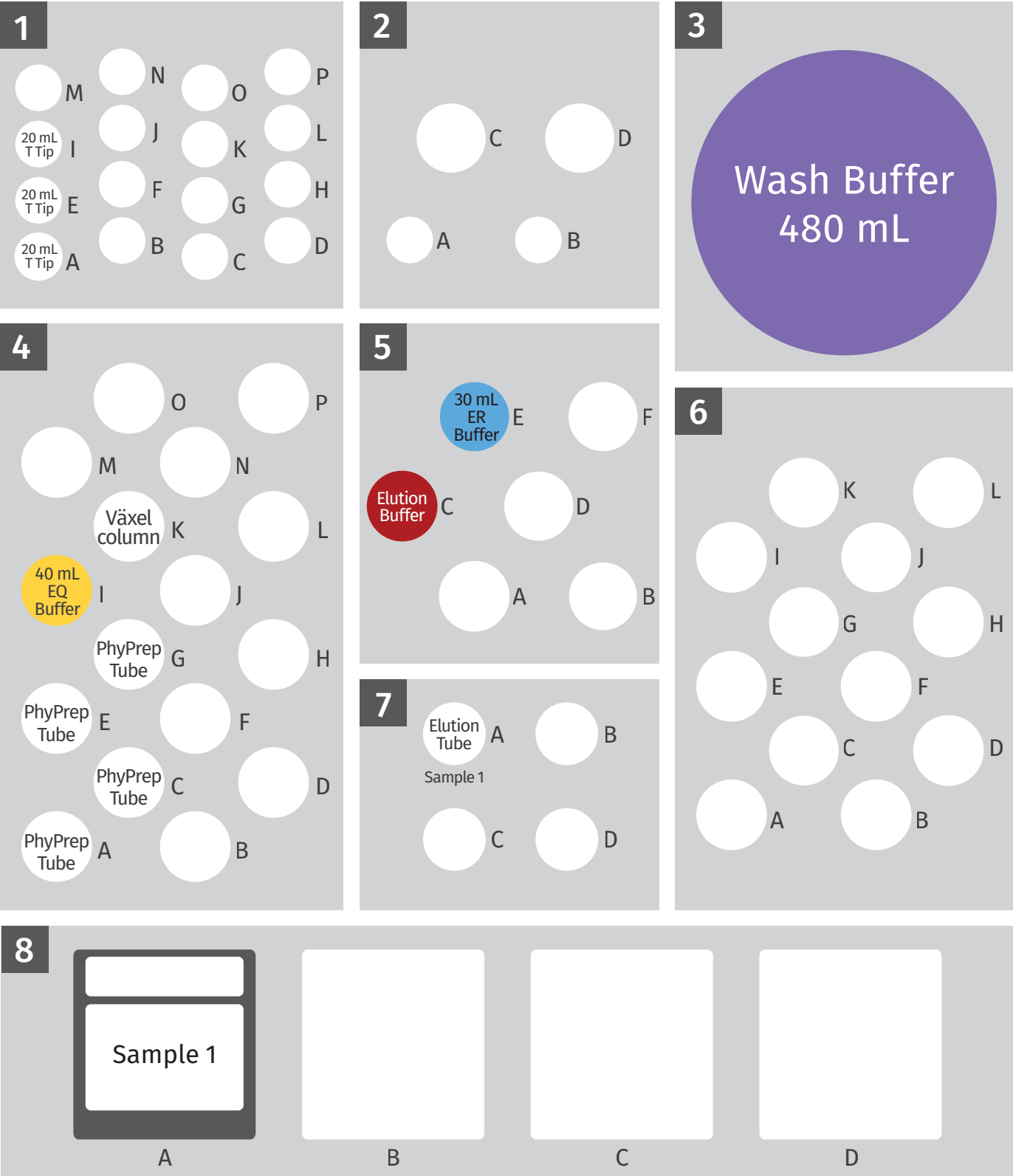
Section

8

- » Place 1 x Sample Filter Reservoir into A.
- » Ensure the filter part is towards the front of the deck.



Gigaprep 1 Sample Deck Layout



Gigaprep 2 Samples Method










This protocol is designed for the preparation of up to 10 mg of high-copy plasmid DNA per sample using the Biotage PhyPrep Gigaprep kit. While setting up the deck, place the appropriate consumables in the corresponding numbered sections of the deck.

Recommended cell wet weight

» 14-16 g per sample from culture volume range of 600–750 mL

Important notes

» Ensure correct volume of Wash Buffer is added to the Wash Buffer Reservoir in Section 3. Ensure correct volumes of Equilibration Buffer and Endotoxin Removal Buffer are added to PhyPrep Tubes in sections 4 and 5.

Required Parts	20 mL Transfer Tip	Växel Gigaprep Column	Wash Buffer	Elution Buffer	Endotoxin Removal Buffer	Equilibration Buffer	50 mL Pyrogen-free Elution Tube	Sample Filter Reservoir	PhyPrep Tube
									
Quantity	6	2	1	2	1	1	2	2	12
Part #	DPM-00-25-00	710-0003	900-0092	900-0094	900-0088	900-0090	DPM-00-10-01	700-0030	417458SP

Section

1

» Place 6 x Transfer Tips in A, C, E, G, I, and K.



Section

3

» Add 880 mL of **WASH BUFFER** to the Wash Buffer Reservoir.



Section

4

» Place 8 x PhyPrep Tubes into A, B, C, D, E, F, G, and H.
 » Place 2 x PhyPrep Tubes into I and J.
 Add 40 mL of **EQUILIBRATION BUFFER** to these tubes.
 » Place 2 x Biotage® Växel Gigaprep columns into K and L.



Section

5

» Uncap and place 2 x **ELUTION BUFFER** tubes into C and D.
 » Place 2 x PhyPrep Tubes into E and F.
 Add 30 mL of **ENDOTOXIN REMOVAL BUFFER** to these tubes.



Section

7

» Remove the Elution Tube Stand from the PhyPrep deck.
 » Uncap and place 2 x 50 mL pyrogen-free conical tubes in position A and B.
 » Elution collection tubes should rest in the **bottom** portion of the elution stand.
 » Place the Elution Tube Stand back onto the PhyPrep deck.



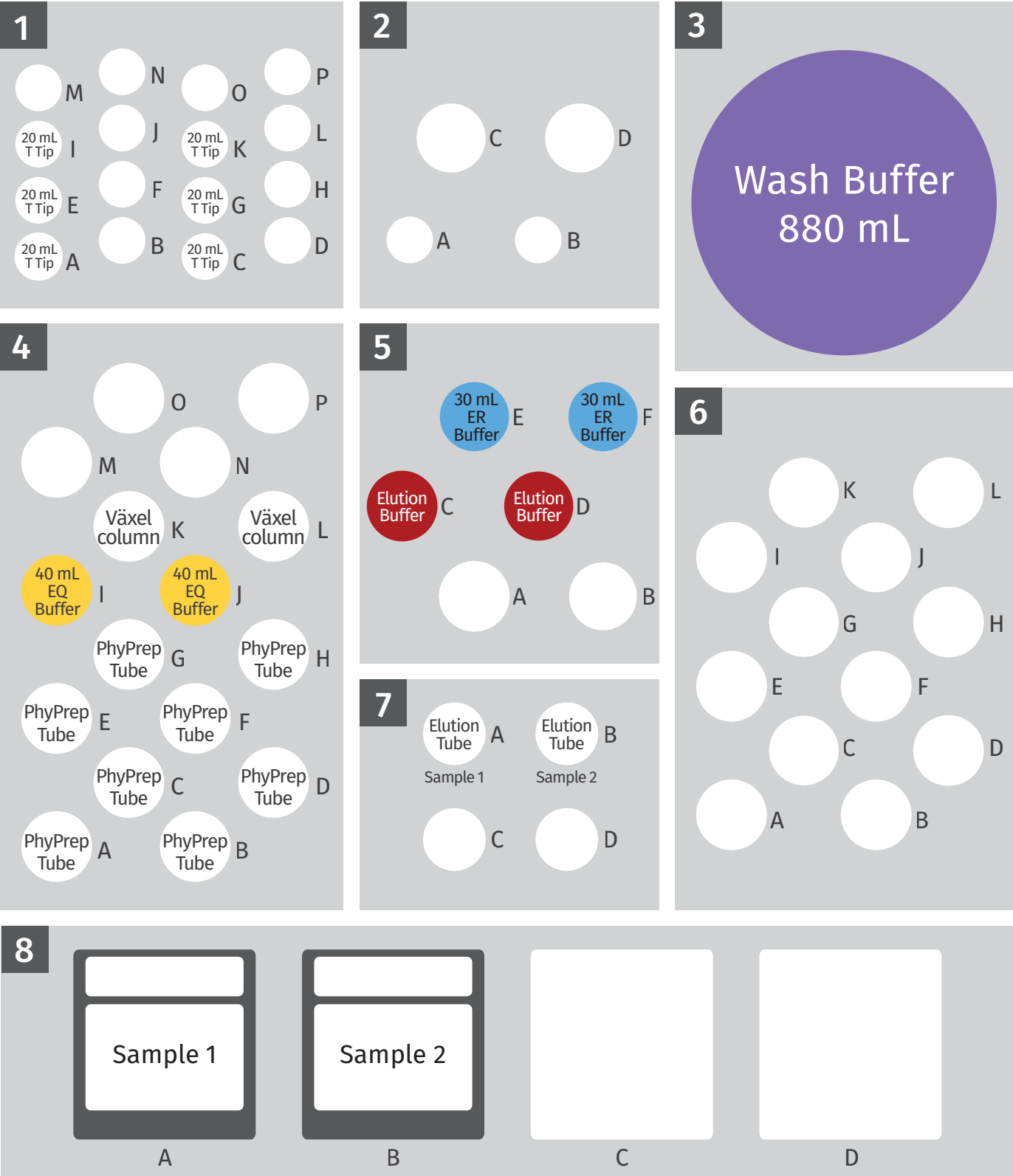
Section

8

» Place 2 x Sample Filter Reservoirs into A and B.
 » Ensure the filter part is towards the front of the deck.



Gigaprep 2 Samples Deck Layout



Gigaprep 3 Samples Method










This protocol is designed for the preparation of up to 10 mg of high-copy plasmid DNA per sample using the Biotage PhyPrep Gigaprep kit. While setting up the deck, place the appropriate consumables in the corresponding numbered sections of the deck.

Recommended cell wet weight

» 14-16 g per sample from culture volume range of 600–750 mL

Important notes

» Ensure correct volume of Wash Buffer is added to the Wash Buffer Reservoir in Section 3. Ensure correct volumes of Equilibration Buffer and Endotoxin Removal Buffer are added to PhyPrep Tubes in sections 4, 5, and 6.

Required Parts	20 mL Transfer Tip	Växel Gigaprep Column	Wash Buffer	Elution Buffer	Endotoxin Removal Buffer	Equilibration Buffer	50 mL Pyrogen-free Elution Tube	Sample Filter Reservoir	PhyPrep Tube
									
Quantity	9	3	1	3	1	1	3	3	17
Part #	DPM-00-25-00	710-0003	900-0092	900-0094	900-0088	900-0090	DPM-00-10-01	700-0030	417458SP

Section

1

» Place 9 x Transfer Tips in A, B, C, E, F, G, I, J, and K.



Section

3

» Add 1280 mL of **WASH BUFFER** to the Wash Buffer Reservoir.



Section

4

» Place 8 x PhyPrep Tubes into A, B, C, D, E, F, G, and H.
 » Place 2 x PhyPrep Tubes into I and J.
 Add 40 mL of **EQUILIBRATION BUFFER** to these tubes.
 » Place 2 x Biotage® Växel Gigaprep columns into K and L.



Section

5

» Uncap and place 3 x **ELUTION BUFFER** tubes into A, C, and D.
 » Place 2 x PhyPrep Tubes into E and F.
 Add **ENDOTOXIN REMOVAL BUFFER** as follows: 50 mL into E and 30 mL to F.



Section

6

» Place 4 x PhyPrep Tubes into A, C, E, and G.
 » Place 1 x PhyPrep Tube into I.
 Add 40 mL of **EQUILIBRATION BUFFER** to this tube.
 » Place 1 x Biotage® Växel Gigaprep column into K.



Section

7

» Remove the Elution Tube Stand from the PhyPrep deck.
 » Uncap and place 3 x 50 mL pyrogen-free conical tubes in position A, B, and C.
 » Elution collection tubes should rest in the **bottom** portion of the elution stand.
 » Place the Elution Tube Stand back onto the PhyPrep deck.



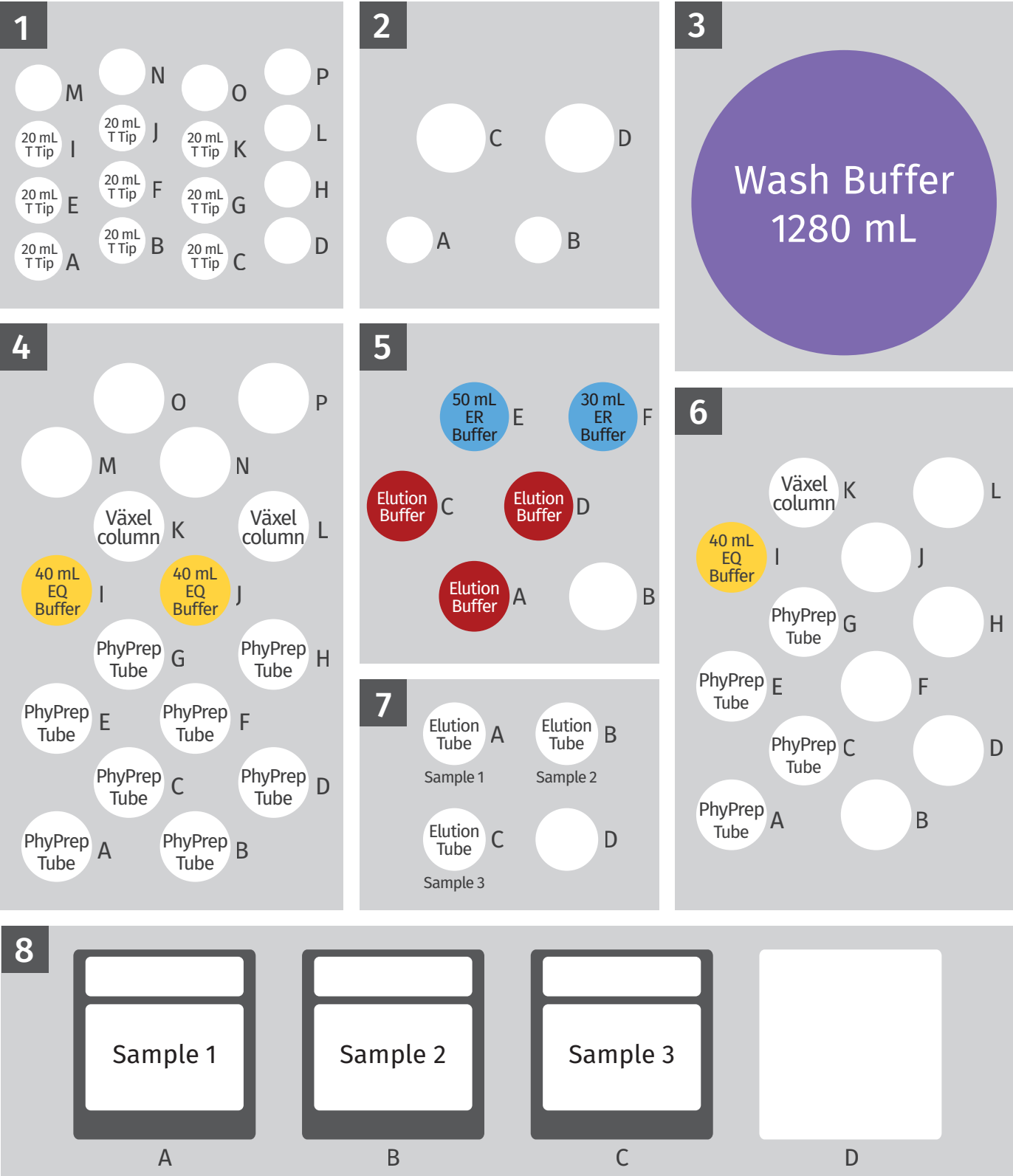
Section

8

» Place 3 x Sample Filter Reservoirs into A, B, and C.
 » Ensure the filter part is towards the front of the deck.



Gigaprep 3 Samples Deck Layout



Gigaprep 4 Samples Method










This protocol is designed for the preparation of up to 10 mg of high-copy plasmid DNA per sample using the Biotage PhyPrep Gigaprep kit. While setting up the deck, place the appropriate consumables in the corresponding numbered sections of the deck.

Recommended cell wet weight

» 14-16 g per sample from culture volume range of 600–750 mL

Important notes

» Ensure correct volume of Wash Buffer is added to the Wash Buffer Reservoir in Section 3. Ensure correct volumes of Equilibration Buffer and Endotoxin Removal Buffer are added to PhyPrep Tubes in sections 4, 5, and 6.

Required Parts	20 mL Transfer Tip	Växel Gigaprep Column	Wash Buffer	Elution Buffer	Endotoxin Removal Buffer	Equilibration Buffer	50 mL Pyrogen-free Elution Tube	Sample Filter Reservoir	PhyPrep Tube
									
Quantity	12	4	1	4	1	1	4	4	22
Part #	DPM-00-25-00	710-0003	900-0092	900-0094	900-0088	900-0090	DPM-00-10-01	700-0030	417458SP

Section

1

» Place 12 x Transfer Tips in A, B, C, D, E, F, G, H, I, J, K, and L.



Section

3

» Add 1680 mL of **WASH BUFFER** to the Wash Buffer Reservoir.



Section

4

» Place 8 x PhyPrep tubes into A, B, C, D, E, F, G, and H.
 » Place 2 x PhyPrep Tubes into I and J.
 Add 40 mL of **EQUILIBRATION BUFFER** to these tubes.
 » Place 2 x Biotage® Växel Gigaprep columns into K and L.



Section

5

» Uncap and place 4 x **ELUTION BUFFER** tubes into A, B, C, and D.
 » Place 2 x PhyPrep Tubes into E and F.
 Add 50 mL of **ENDOTOXIN REMOVAL BUFFER** to these tubes.



Section

6

» Place 8 x PhyPrep tubes into A, B, C, D, E, F, G, and H.
 » Place 2 x PhyPrep Tubes into I and J.
 Add 40 mL of **EQUILIBRATION BUFFER** to these tubes.
 » Place 2 x Biotage® Växel Gigaprep columns into K and L.



Section

7

» Remove the Elution Tube Stand from the PhyPrep deck.
 » Uncap and place 4 x 50 mL pyrogen-free conical tubes in position A, B, C, and D.
 » Elution collection tubes should rest in the **bottom** portion of the elution stand.
 » Place the Elution Tube Stand back onto the PhyPrep deck.



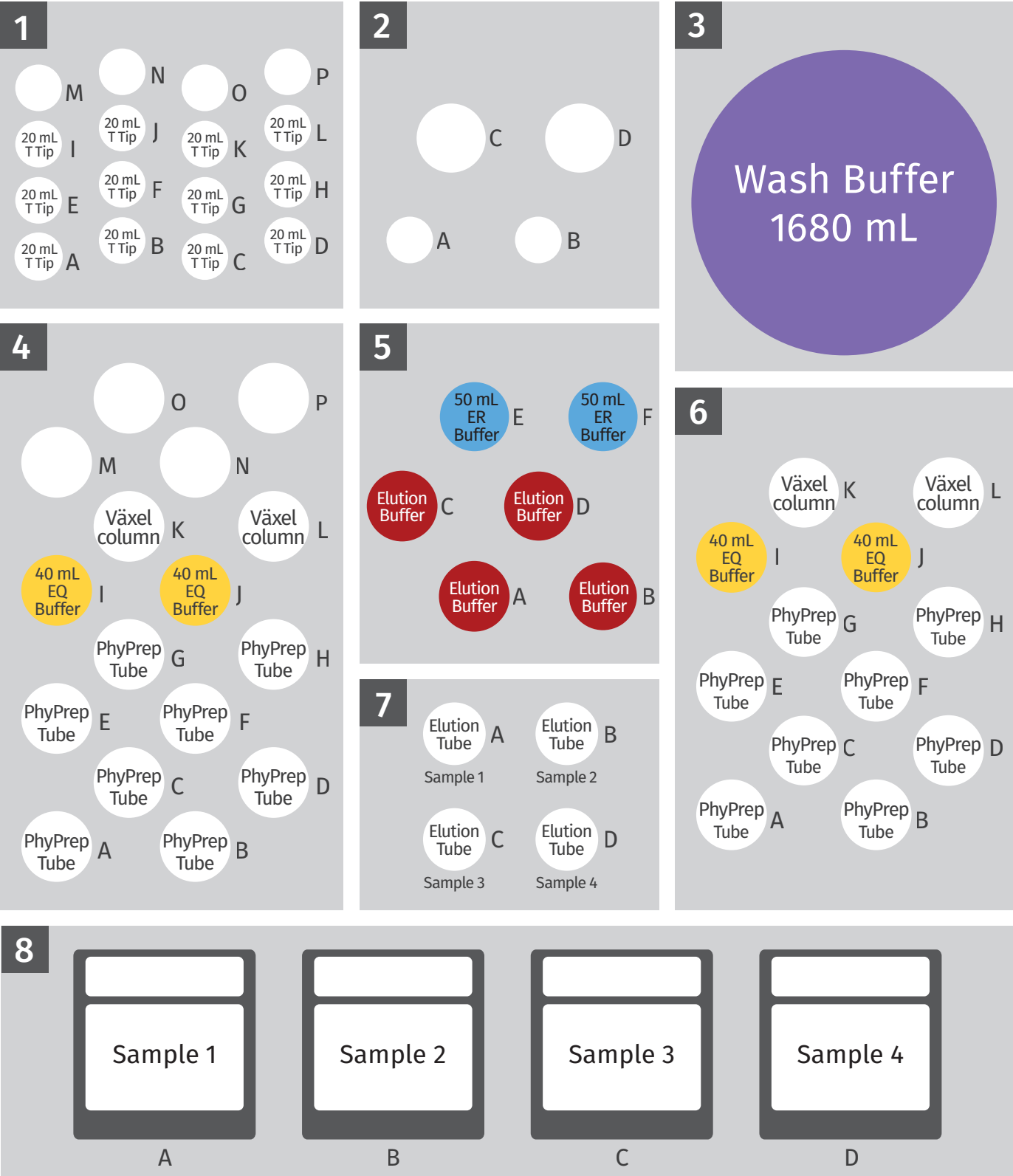
Section

8

» Place 4 x Sample Filter Reservoirs into A, B, C, and D.
 » Ensure the filter part is towards the front of the deck.

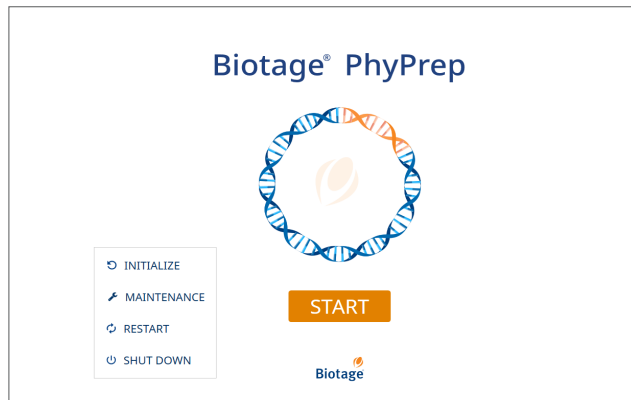


Gigaprep 4 Samples Deck Layout

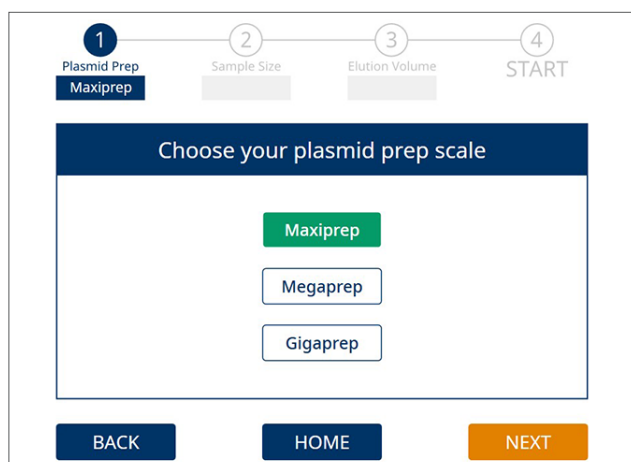


Software Overview

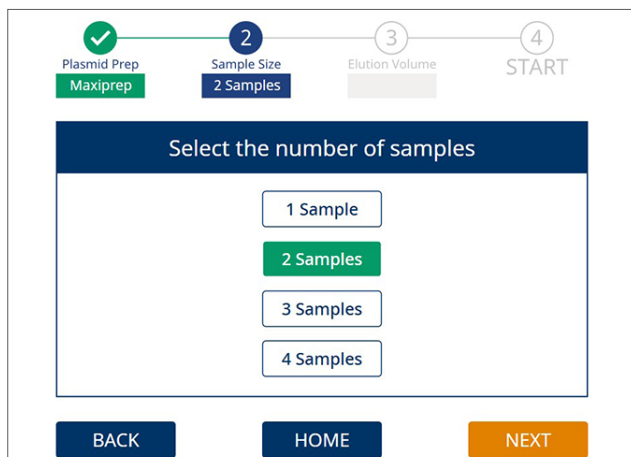
1. Press **START** to go to the prep scale selection screen.



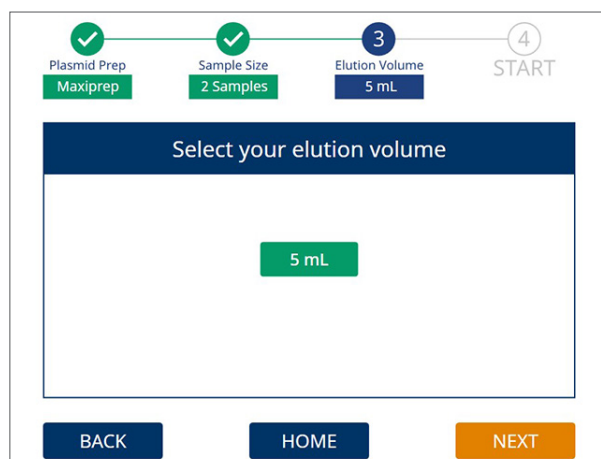
2. Select your desired plasmid prep scale. Then press **NEXT**.



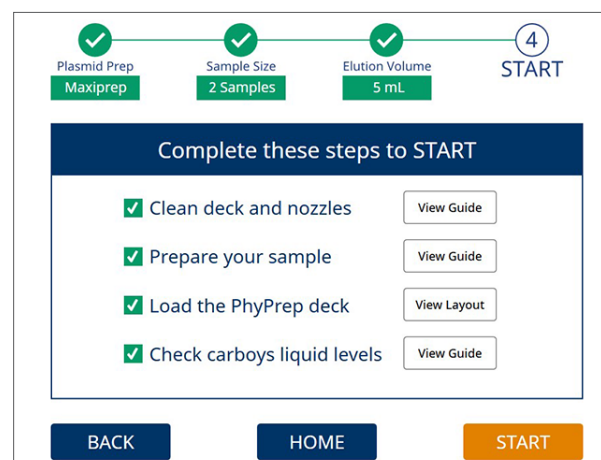
3. Select the number of samples. Then press **NEXT**.



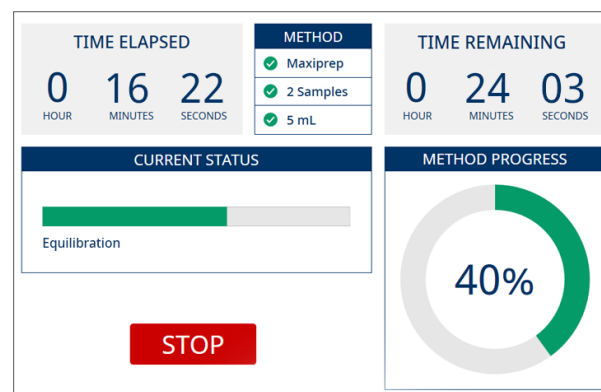
4. Select your elution volume. Then press **NEXT**.



5. Review the instructions for sample preparation and deck layout for your sample and check the carboys liquid levels to ensure they are not full. Check the boxes to confirm and press **START**.



6. Once the method is completed, press **QUIT**.



Recommended Alcohol Precipitation Protocols

The elution buffer is 50 mM Tris-HCL, 500 mM NaCl, pH 8.5. If downstream assays require desalting, we recommend the following alcohol precipitations.

Important Note

» To avoid contamination of plasmid DNA, it is recommended to use only certified endotoxin-free and pyrogen-free plasticware and endotoxin-free reagents.

Maxiprep

Total time: 24 minutes

1. Add 0.1 volume of 3M KoAc pH 5.3.
2. Vortex for 30 seconds.
3. Add 0.7 volume of isopropyl alcohol.
4. Vortex for 1 minute.
5. Centrifuge at $\geq 15,000 \times g$ for 5 minutes at 4°C.
6. Carefully decant supernatant and remove as much supernatant as possible.
Note: Do not disturb the pellet.
7. Wash pellet with 5 mL of 70% ethanol.
Note: Agitate the tube manually to dislodge the pellet from sides to wash completely. DO NOT VORTEX.
8. Centrifuge at $\geq 15,000 \times g$ for 3 minutes at 4°C.
9. Carefully decant the ethyl alcohol and remove as much as possible.
Note: Do not disturb the pellet.
10. Air dry at room temperature for 10 minutes.
11. Resuspend pellet in endotoxin-free and nuclease-free water.

Megaprep

Total time: 40 minutes

1. Add 0.1 volume of 3M KoAc pH 5.3.
2. Vortex for 30 seconds
3. Add 0.7 volume of isopropyl alcohol.
4. Vortex for 1 minute.
5. Incubate at room temperature for 15 minutes.
6. Centrifuge at $\geq 15,000 \times g$ for 6 minutes at 4°C.
7. Carefully decant supernatant and remove as much supernatant as possible.
Note: Do not disturb the pellet.
8. Wash pellet with 5 mL of 70% ethanol.
Note: Agitate the tube manually to dislodge the pellet from sides to wash completely. DO NOT VORTEX.
9. Centrifuge at $\geq 15,000 \times g$ for 3 minutes at 4°C.

10. Carefully decant the ethyl alcohol and remove as much as possible.

Note: Do not disturb the pellet.

11. Air dry at room temperature for 10 minutes.
12. Resuspend pellet in endotoxin-free and nuclease-free water.

Gigaprep

Total time: 51 minutes

The final GigaPrep elution volume is generally 30 mL. With additions of KoAc and isopropyl alcohol, a 50 mL conical tube can process at maximum 27 mL of the eluted sample. If you would like to process the entirety of the Gigaprep sample, with the following protocol, it is recommended to use a 100 mL bottle instead of 50 mL tubes. The following protocol can be modified for a larger bottle by skipping step #1. Alternatively, the sample can be split into two 50 mL tubes.

1. If sample volume is ≥ 28 mL, transfer 27 mL of sample to a new sterile conical tube.
2. Add 0.1 volume of 3M KoAc pH 5.3.
3. Vortex for 30 seconds.
4. Add 0.7 volume of isopropyl alcohol.
5. Vortex for 1 minute.
6. Incubate at room temperature for 15 minutes.
7. Centrifuge at $\geq 15,000 \times g$ for 15 minutes at 4°C.
8. Carefully decant supernatant and remove as much supernatant as possible.
Note: Do not disturb the pellet.
9. Wash pellet with 5mL of 70% ethyl alcohol.
Note: Agitate the tube manually to dislodge the pellet from sides to wash completely. DO NOT VORTEX.
10. Centrifuge at $\geq 15,000 \times g$ for 5 minutes at 4°C.
11. Carefully decant the ethyl alcohol and remove as much as possible.
Note: Do not disturb the pellet.
12. Air dry at room temperature for 10 minutes.
13. Resuspend pellet in endotoxin-free and nuclease-free water.

Maintenance

Endotoxin Levels

Endotoxin levels are sensitive and can be easily influenced by a number of factors. Before each method run, it is recommended that the end user perform a sterilization procedure to ensure endotoxin free plasmid.

Cleaning the PhyPrep instrument

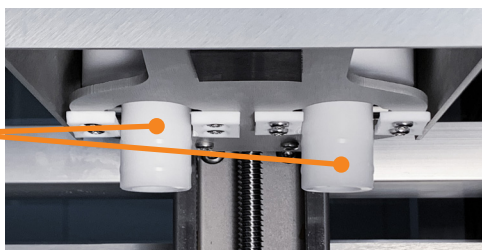
- » Spray and wipe down the deck, tray and elution stand with 70 – 80% alcohol spray. When spraying the deck with 70 – 80% alcohol, do not spray the z-motor with alcohol.

Spray and wipe down the deck



- » Wet a lint-free paper towel or kimwipe with 70 – 80% alcohol spray and wipe an exposed nozzle. Repeat with the other nozzle using another paper towel or kimwipe. When wiping the nozzle, do not let the towel or wipes touch and wet the robot components with alcohol.

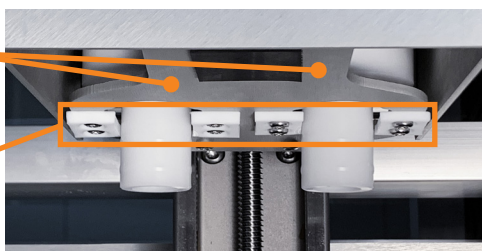
Spray and wipe down nozzles



- » Wet a paper towel or kimwipe with 70 – 80% alcohol spray and wipe the ejector and ejector pads.

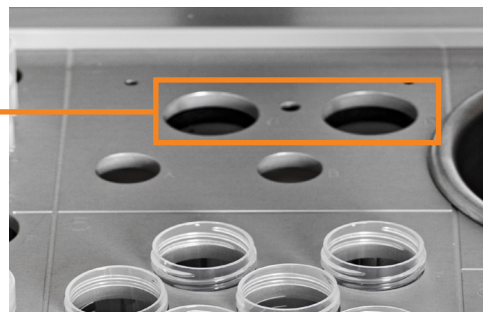
Wipe down ejector

Wipe down ejector pads



- » Spray approximately 5 mL of 70 – 80% alcohol into the vacuum drying station in a mist fashion to coat the entire inside of the vacuum position.

Spray alcohol in to the vacuum drying station



Using the endotoxin measuring instrument

- » When using and loading the FDA licensed cartridges, only touch the sides and/or the cartridge handle with clean gloves.
- » Maintain cleanliness of the endotoxin measuring instrument and the environment around it by using paper towels or kimwipes sprayed with 70 – 80% alcohol to wipe the surfaces.
- » Maintain cleanliness of the pipettes used for diluting and loading samples into the instrument by wiping the pipettes with kimwipes sprayed with 70 – 80% alcohol.
- » When diluting the plasmid elution, use endotoxin free or pyrogen free tips, water or LAL reagent, and tube. Using a new box of endotoxin free tips, fresh endotoxin free water or new bottle of LAL reagent, and tubes can reduce external endotoxin external contamination.
- » If the endotoxin measuring instrument is not in a clean room, maintaining a thermal updraft or placing the instrument inside a hood can lower the probability of endotoxins in the environment influencing the test.

Wash Buffer Reservoir

After each run, the Wash Buffer Reservoir should be wiped down with 70 – 80% reagent alcohol, dried, and clean before adding wash buffer into it.



Biotage recommends a yearly Preventative Maintenance to be performed by a Biotage field engineer. Consult your Biotage representative for more information.

Carboys Set Up and Care

A 20 L vacuum carboy and a 20 L waste carboy are included with the PhyPrep instrument. It is essential to empty the carboys once they reach 80% capacity.

Prep Type	20 L Waste Carboy	20 L Vacuum Carboy
Maxiprep	Not used	265
Megaprep	Not used	80
Gigaprep	130	40

Table 1. Number of samples completed to reach 80% fill capacity.

Carboys Contents Disposal

Dispose of carboys contents in compliance with EH&S and OSHA.

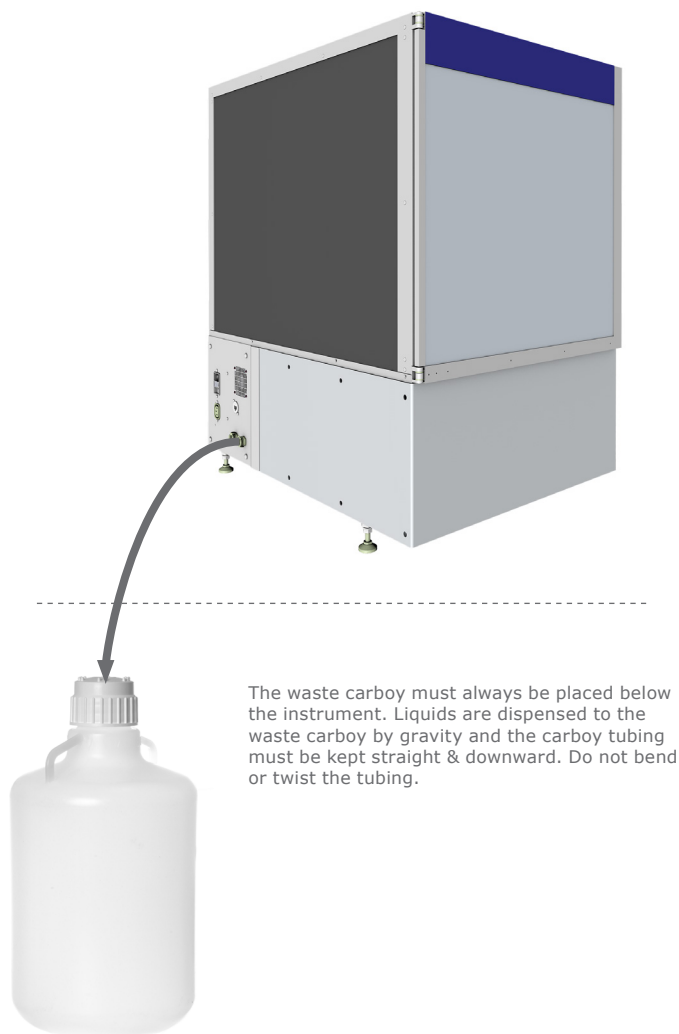
Waste Carboy Contents

80–90% *E. coli* cell lysate

0.1–1.0M SDS, sodium acetate

Vacuum Carboy Contents

0.1–1.0M NaCl



The waste carboy must always be placed below the instrument. Liquids are dispensed to the waste carboy by gravity and the carboy tubing must be kept straight & downward. Do not bend or twist the tubing.



Troubleshooting

This troubleshooting guide is designed to help solve most problems that may arise while using the Biotage PhyPrep. The scientists at Biotage are always happy to answer any questions you may have about the information or protocols in this manual. For questions not addressed here, please contact Biotage support to talk with a Biotage scientist.

Low DNA yield

Cause

Culture growth conditions

Explanations and actions

- » Poor aeration of culture
 - » *E. coli* cultures need sufficient oxygen to grow for a prolonged time. Use a culture volume to flask size ratio of 1:5 or less. For best aeration, use a baffled culture flask with a vented or gas permeable seal.
- » Insufficient or forgotten antibiotic
 - » Most antibiotics are light sensitive and degrade during long term storage at 2-8°C or through multiple freeze-thaw cycles. For growth of overnight cultures use fresh antibiotic at correct concentrations.
- » Plasmid did not propagate
 - » Bacteria culture may be too old. Check plasmid content in cleared lysate by running on a gel after performing an alcohol precipitation. Streak a fresh plate from a freezer stock. Inoculate antibiotic growth media with a freshly isolated bacterial colony from an overnight plate.

Low DNA yield

Cause

Cell density of overnight culture

Explanations and actions

- » The overnight culture density is too low
 - » Confirm that the cells were grown in optimal conditions.
 - » Increase the culture volume of the sample to be purified.
 - » Contact Biotage support for more information.
- » The overnight culture density is too high
 - » Do not exceed the recommended pellet wet weights.
 - » Contact Biotage support for more information.

Low DNA yield

Cause

Improper sample preparation

Explanations and actions

- » Incomplete cell resuspension
 - » Pelleted cells should be completely resuspended before proceeding with lysis. After adding **RESUSPENSION BUFFER** to the pelleted cells, shake beads until the pellet is dislodged from the bottom of the vessel. Shake for an extra 10-20 seconds to ensure full suspension of the bacteria.
- » Incomplete lysis
 - » Some bacteria cells are more resistant to lysis than others. Incubate suspended cells for 5 minutes after 30 slow inversions of the mixing bottle.
- » Precipitation of **LYSIS BUFFER**
 - » **LYSIS BUFFER** stored below 20°C will precipitate. Check **LYSIS BUFFER** for SDS precipitation before use. If necessary, incubate the bottle for several minutes in a warm water bath. Mix well after incubation to ensure SDS is dissolved.
- » Incomplete neutralization
 - » To completely precipitate SDS, chromosomal DNA, and other cellular debris after adding **PRECIPITATION BUFFER**, proper mixing is required. Incubating the lysate for 10-15 minutes after mixing **PRECIPITATION BUFFER** may help increase yield.
 - » Store **PRECIPITATION BUFFER** at 4° C for best results.
- » Improper mixing vessel
 - » Use a large enough mixing bottle to ensure full mixing of buffers. We recommend these bottle sizes:
 - 125 mL or larger bottle for Maxiprep
 - 250 mL or larger bottle for Megaprep
 - 500 mL or larger bottle for Gigaprep

Low DNA yield

Cause

Faulty wash buffer

Explanations and actions

- » **WASH BUFFER** should be capped when not in use to limit evaporation.

Low DNA quality

Cause

Improper sample preparation

Explanations and actions

- » Incomplete precipitation .
 - » Incomplete precipitation generates poor quality supernatant. Ensure that precipitation is complete by inverting the sample 30 times. Do not vigorously mix, this may cause additional contaminant release from the flocculent.
- » DNA is degraded.
 - » Make sure equipment is kept as sterile as possible to limit nuclease contamination. Do not lyse for more than 5 minutes.

Low DNA quality

Cause

Improper sample preparation

Explanations and actions

- » RNase A digestion was inefficient.
 - » Make sure to add RNase A to **RESUSPENSION BUFFER**. Store **RESUSPENSION BUFFER** at 2-8°C if RNase A has been added and is not immediately used.

Low DNA quality

Cause

Improper sample preparation

Explanations and actions

- » Overmixing of **LYSIS BUFFER** or **PRECIPITATION BUFFER**.
 - » Shearing of genomic DNA can occur during overmixing.
 - » Do not vortex the mixing bottle during these steps.

General Information

Consumables and Accessories

Only genuine Biotage consumables and accessories must be used with the system. To order consumables and accessories, visit our website www.biotage.com

Consumables

Part Number	Description	QTY
DPM-16-05-72-KIT	Biotage Plasmid MaxiPrep kit Endotoxin-Free (16 samples)	
	Biotage® Växel columns (Ion Exchange)	16
	Resuspension Buffer (60 mL bottles)	16
	Lysis Buffer (60 mL bottles)	16
	Precipitation Buffer (60 mL bottles)	16
	Elution Buffer (50 mL tube)	16
	Wash Buffer (4000 mL bottle)	1
	Equilibration Buffer (500 mL bottle)	1
	Endotoxin Removal Buffer (500 mL bottle)	1
	Sample Filter Reservoirs	16
	RNase A for Plasmid MaxiPrep kit	16
	25 x 20 mL transfer tips	50
	50 x PhyPrep Tubes	50
DPM-16-10-72-KIT	Biotage Plasmid MegaPrep kit Endotoxin-Free (16 samples)	
	Biotage® Växel columns (Ion Exchange)	16
	Resuspension Buffer (60 mL bottles)	16
	Lysis Buffer (125 mL bottles)	16
	Precipitation Buffer (125 mL bottles)	16
	Elution Buffer (50 mL tube)	16
	Wash Buffer (4000 mL bottle)	1
	Equilibration Buffer (500 mL bottle)	1
	Endotoxin Removal Buffer (500 mL bottle)	1
	Sample Filter Reservoirs	16
	RNase A for Plasmid MegaPrep kit	16
	20 mL Transfer Tips	50
	PhyPrep Tubes	100
DPM-08-20-72-KIT	Biotage Plasmid GigaPrep kit Endotoxin-Free (8 samples)	
	Biotage® Växel columns (Ion Exchange)	8
	Resuspension Buffer (125 mL bottles)	8
	Lysis Buffer (125 mL bottles)	8
	Precipitation Buffer (125 mL bottles)	8
	Elution Buffer (50 mL tube)	8
	Wash Buffer (4000 mL bottle)	1
	Equilibration Buffer (500 mL bottle)	1
	Endotoxin Removal Buffer (500 mL bottle)	1
	Sample Filter Reservoirs	8
	RNase A for Plasmid GigaPrep kit	8
	20 mL Transfer Tips	25
	PhyPrep Tubes	50

Accessories

Part Number	Description
417458SP	50 x PhyPrep Tubes for Biotage® PhyPrep
DPM-00-25-00	25 x 20 mL transfer tips for Biotage® PhyPrep
DPM-00-10-00	100 x 20 mL transfer tips for Biotage® PhyPrep
DPM-00-10-01	Sterile 100 x 20 mL transfer tips for Biotage® PhyPrep
DPM-00-20-00	50 mL conical tubes with caps, sterile, pyrogen-free
DPM-00-20-01	50 mL conical tubes WITHOUT caps, PhyPrep
700-0030	Biotage® PhyPrep Sample Filter Reservoir
DPM-16-05-72-RNA	RNase A for Plasmid MaxiPrep kit, Endotoxin-free (for 16 samples)
DPM-16-10-72-RNA	RNase A for Plasmid MegaPrep kit, Endotoxin-free (for 16 samples)
DPM-08-20-72-RNA	RNase A for Plasmid GigaPrep kit, Endotoxin-free (for 8 samples)

Notes

Your Complete Partner for Automated Protein and Plasmid Purification

Biotage is a worldwide supplier of instruments and accessories designed to facilitate the work of life science researchers. With our deep knowledge of the industry, academic contacts and in-house R&D teams, we can deliver the best solutions to your challenges. We take great pride in our flexibility and ability to meet our customer's individual needs. With strong foundations in analytical, organic and process chemistry, and biomolecules, we can offer the widest range of solutions available on the market.

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Literature Number: 417343-A

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