SEP 25, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.dm6gp39njvzp/v1

Protocol Citation: Kristina Vsevolodova, Gideon Erkenswick, Mrinalini Watsa 2023. Opentrons Pipeline: DNA Extraction with the Omega Biotek Stool Kit . **protocols.io** https://dx.doi.org/10.17504/protocols.io.dm6gp39njvzp/v1

Protocol status: Working

Created: Sep 19, 2023

Opentrons Pipeline: DNA Extraction with the Omega Biotek Stool Kit

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Last Modified: Sep 25, 2023

PROTOCOL integer ID: 88037

Keywords: Opentrons, OT2, DNA extraction, Extraction, Omega Biotek, Stool , Stool extraction, insitulabs, automated

ABSTRACT

This protocol is an automated pipeline to extract a full 96-well plate of DNA from stool lysates prepared in two separate plates. Lysing of the stool can be performed as you wish, depending on species and their diet. The protocol itself begins after lysates have been created.

This protocol was developed and optimized for the following:
Platform: Opentrons OT-2 automated pipetting robot
Kit: Omega Biotek
Tips Used: 4 boxes (4 x 200uL Opentrons Filtered Tip boxes)
Recommended number of samples: 96

GUIDELINES

This protocol is recommended for a full 96-well plate. Do not use it for less than 12 columns. A version for variable sample numbers is forthcoming in the next version.

Things to consider:

Before beginning: Load your lysates into two plates, a 1 mL deep well with 300uL of lysate and a 200uL Plate with 200uL of lysate. This is because, during the COVID pandemic, locating 2mL deep well plates for small labs (i.e. to buy in numbers less than 10,000) was impossible to do. So, we made our protocol flexible to be able to use a larger amount of lysate (total of 500uL) without the 2mL deepwell plate.

Step 4: The 50mL Falcon tubes are recommended as means of sterile transport of accurate reagent amounts between the lab and the OT-2. They can be replaced with a different brand of tube or reservoir.

Step 6: Import the labware file BEFORE you import your protocol or it will give an error. This protocol has been validated against Opentrons software app version 6.2.1

Step 7.2: In the newer software versions for the OT-2, calibration is not required before every run. In some cases the user will not be asked to calibrate the machine, and they should not have to.

MATERIALS

- 5x Nest 50mL Falcon Tubes
- 4x Opentrons 200µL Filter Tips
- 1x VWR 96 Deep Well Plates 1mL
- 2x NEST 1-Well Reservoirs, 195 mL
- 2x NEST 12-Well Reservoirs, 15 mL
- 1x 96-Well PCR Plate Non-skirt, 200µl
- 3x Nest skirted PCR Plate
- 2x Aluminium Seals
- 6x 2mL Tubes
- 100-1000µL pipette
- 1000µL pipette tips
- Incubator or water bath that can reach 70°C or more

PROTOCOL MATERIALS

SPM Buffer **Omega Biotek Catalog #SPM-300** In 5 steps

Qubit® dsDNA HS Assay Kit **Thermo Fisher Scientific Catalog #Q32854** Step 1

Qubit® 3.0 Fluorometer **Thermo Fisher Scientific Catalog #Q33216** Step 1

Quantus(TM) Fluorometer **Promega Catalog #E6150** Step 1

70% Alcohol **Contributed by users** In Before starting and 2 steps

VHB Buffer **Omega Biotek Catalog #VHB-440** In 3 steps

100% Ethanol **Contributed by users** In 5 steps

Mag-Bind Particles CH **Omega Biotek Catalog #MBPCH- 50** In 9 steps

Mag-Bind Stool DNA 96 Kit **Omega Biotek Catalog #M4016-01** Step 1

Quantus(TM) NGS Starter Package **Promega Catalog #E5150** Step 1

Quant-iT™ PicoGreen® dsDNA Assay Kit **Life Technologies Catalog #P11496** Step 1

XP2 Binding Buffer **Omega Biotek Catalog #PDR040** In 3 steps

Elution Buffer **Omega Biotek Catalog #PDR048** In 7 steps

Nuclease-Free Water, 150ml **Promega Catalog #P1195** Step 1

10% Bleach **Contributed by users** Before starting, Step 8

Distilled Water **Contributed by users** Before starting, Step 8

BEFORE START INSTRUCTIONS

Clean the OT2 deck and walls with:

10% Bleach **Contributed by users** 1 rinse

Distilled Water **Contributed by users** 1 rinse

70% Alcohol **Contributed by users** 2 rinses

Note

Avoid wetting electronic parts.

Before starting

1 Ingredients

100% Ethanol **Contributed by users**

Nuclease-Free Water, 150ml **Promega Catalog #P1195**

Mag-Bind Stool DNA 96 Kit **Omega Biotek Catalog #M4016-01** (Contains everything for extraction including lysis reagents)

OR separate reagents (not including lysis reagents):

- SPM Buffer **Omega Biotek Catalog #SPM-300**
- VHB Buffer **Omega Biotek Catalog #VHB-440**
- XP2 Binding Buffer **Omega Biotek Catalog #PDR040**
- Mag-Bind Particles CH **Omega Biotek Catalog #MBPCH- 50**
- Elution Buffer **Omega Biotek Catalog #PDR048**

Note

For QC on individual level the
Qubit® dsDNA HS Assay Kit **Thermo Fisher Scientific Catalog #Q32854** on a
Qubit® 3.0 Fluorometer **Thermo Fisher Scientific Catalog #Q33216** can be used
OR the Quantus(TM) NGS Starter Package **Promega Catalog #E5150** on a
Quantus(TM) Fluorometer **Promega Catalog #E6150**

For QC on plate level use the
Quant-iT™ PicoGreen® dsDNA Assay Kit **Life Technologies Catalog #P11496**

1.1 For the 1x96 Kit

Dilute SPM Buffer with 70 mL 100% Ethanol **Contributed by users** per bottle and store at Room temperature.

Dilute VHB Buffer with 28 mL 100% Ethanol **Contributed by users** per bottle and store at Room temperature.

1.2 For the 4x96 Kit

Dilute SPM Buffer with 70 mL 100% Ethanol **Contributed by users** per bottle and store at Room temperature.

Dilute VHB Buffer with 112 mL 100% Ethanol **Contributed by users** per bottle and store at Room temperature.

2 Materials

5x Nest 50mL Falcon Tubes for holding accurate reagent amounts before starting protocol.
4x Opentrons 200µL Filter Tips
1x VWR 96 Deep Well Plates 1mL for 300µL of lysate.

2x NEST 1-Well Reservoirs, 195 mL for extraction waste collection during protocol.
2x NEST 12-Well Reservoirs, 15 mL for holding reagents during protocol.
1x 96-Well PCR Plate Non-skirt, 200µl for 200µL of lysate.
3x Nest skirted PCR Plate for holding non-skirt plate and for final elutions.
2x Aluminium Seals
6x 2mL Tubes
100-1000µL pipette
1000µL pipette tips
Incubator or water bath that can reach 70°C or more

2.1 Autoclave the NEST 1-Well Reservoirs, 195 mL and NEST 12-Well Reservoirs, 15 mL before use. These can be rinsed and autoclaved and reused and need not be purchased new for each extraction. Slight yellowing of product can occur, but we do not see that it affects final outcomes in any discernible way.

3 Opentrons Equipment List

Equipment	
OT-2	NAME
Liquid handler	TYPE
Opentrons	BRAND
OT-2	SKU

On the right pipette mount use the P300M

Equipment	
OT-2 8 Channel Electronic Pipette	NAME
Pipette	TYPE
Opentrons	BRAND
P300M	SKU
https://shop.opentrons.com/8-channel-electronic-pipette/	LINK

Magnetic Module to place in Slot 7

Equipment	
OT-2 Magnetic Module GEN2	NAME
Module	TYPE
Opentrons	BRAND
999-00098	SKU
https://shop.opentrons.com/magnetic-module-gen2/	LINK

Prepare reagents

4 After the reagents are properly diluted and materials are ready, prepare the following amounts:

	A	B	C	D
	Item Name	Amount per sample [uL]	Amount for 96 samples [uL]	Amount for 96 samples * 1.1 overage[uL]
	Mag-bind Bead Particles CH Round 1	10	960	1056
	XP2 Binding Buffer Round 1	300	28800	31680
	Mag-bind Bead Particles CH Round 2	10	960	1056
	XP2 Binding Buffer Round 2	300	28800	31680
	Wash 1: VHB Buffer	400	38400	42240
	Wash 2: SPM Buffer	400	38400	42240
	Wash 3: SPM Buffer	400	38400	42240
	Elution Buffer	100	9600	10560

4.1 Fill one Nest 50mL Falcon Tube with the amount of

XP2 Binding Buffer **Omega Biotek Catalog #PDR040**

Round 1 and

Mag-Bind Particles CH **Omega Biotek Catalog #MBPCH- 50** Round 1 required in the table, and mix well. Keep at Room temperature. Split between two tubes if needed.

4.2 Fill one Nest 50mL Falcon Tube with the amount of XP2 Binding Buffer **Omega Biotek Catalog #PDR040** Round 2 and Mag-Bind Particles CH **Omega Biotek Catalog #MBPCH- 50** Round 2 required in the table, and mix well. Keep at Room temperature. Split between two tubes if needed.

4.3 Fill one Nest 50mL Falcon Tube with the volume indicated in the table with wash 1: VHB Buffer **Omega Biotek Catalog #VHB-440**. Keep at Room temperature.

4.4 Fill one Nest 50mL Falcon Tube with the volume indicated in the table with wash 2: SPM Buffer **Omega Biotek Catalog #SPM-300**. Keep at Room temperature.

4.5 Fill one Nest 50mL Falcon Tube with the volume indicated in the table with wash 3: SPM Buffer **Omega Biotek Catalog #SPM-300**. Keep at Room temperature.

4.6 Distribute Elution Buffer **Omega Biotek Catalog #PDR048** between each 2mL tube evenly.

4.7 Set an incubator or water bath to 70 °C and heat Elution Buffer **Omega Biotek Catalog #PDR048** in the incubator to 70 °C.

OT-2 script definitions

5 Definition of samples and labware:

5.1 **Lysed Sample Plate 2 200uL**

Remainder of lysed samples that will be added to Slot 7 in second round of bead incubation.

Position: Slot 2, 96-Well PCR Plate Non-skirt, 200µl with 200uL of sample lysis on top of an empty Nest skirted PCR Plate (used as a base)
Name in the Deck: Lysis plate 2
Labware name in the protocol: denvillewithaxygenbase_96_wellplate_200ul
Sample name in the script: lysate

5.2 **Lysed Sample Plate 1 300uL**

Lysed samples that will undergo the first round of bead incubation.

Position: Slot 7, Opentrons Magnetic Module with VWR 96-Well Deep Well Plate full of 300uL of sample lysis on top
Name in the Deck: Lysis plate 1
Labware name in the protocol: vwr_96_wellplate_1000ul
Sample name in the script: magsamps

5.3 **Sample Elution 1**

Samples that have been eluted from the beads for the first round elution 1.

Position: Slot 3, Empty Nest skirted PCR Plate (to receive elution 1)
Name in the Deck: Sample Elution Plate 1
Labware name in the protocol: nest_96_wellplate_100ul_pcr_full_skirt
Sample name in the script: eluates

5.4 **Sample Elution 2**

Samples that have been eluted from the beads for the second round elution 2.

Position: Slot 2, 96-Well PCR Plate Non-skirt, 200µl with 200uL of sample lysis on top of an empty Nest skirted PCR Plate is replaced with new Nest skirted PCR Plate (to receive elution 2)
Name in the Deck: Sample Elution Plate 2
Labware name in the protocol: nest_96_wellplate_100ul_pcr_full_skirt
Sample name in the script: eluates2

Prepare the OT-2

6 Before loading your protocol, load the following labware files into your Opentrons app:

denville_96_axygenbase_200ul.json

This labware definition allows us to use a non-skirted plate in the Opentrons app by inserting it into a skirted plate, and also allows us to use a 200uL plate (where our skirted plates that clip in

are only 100uL. Feel free to replace with your own labware here).

vwr_96_wellplate_1000ul.json

This labware definition is for the 1mL deepwell plate from VWR. Note the rounded wells work well with the magnet.

Load this python file to the Opentrons app: OT2_Omegabiotekfecal_v4.0.py

6.1 Definition of Protocol Variables:

This protocol is written per column, best working for multiples of 8. Therefore, if you want to modify the sample number just open the script in a text editor program, and modify the following value in line 3 of the script:

"numSamps": 96 → Indicates the number of samples that you will process.

> **Note**
>
> This protocol is recommended for a full plate of 96 samples (12 columns). Any less columns are not optimal.

7 Arrange the OT-2 deck

7.1

Slot 1: NEST 12-Well Reservoirs, 15 mL with reagents preloaded in the following order:

	A	B	C	D	E	F	G	H	I	J	K	L
	Well 1	Well 2	Well 3	Well 4	Well 5	Well 6	Well 7	Well 8	Well 9	Well 10	Well 11	Well 12
	Wash 3: SPM Buffer	Wash 3: SPM Buffer	Wash 3: SPM Buffer	Wash 3: SPM Buffer	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	Elution Buffer (when prompted)

Slot 2: 96-Well PCR Plate Non-skirt, 200µl with 200uL of sample lysis on top of an empty Nest skirted PCR Plate

Slot 3: Empty Nest skirted PCR Plate (to receive elution 1)

Slot 4: NEST 12-Well Reservoirs, 15 mL with reagents preloaded in the following order:

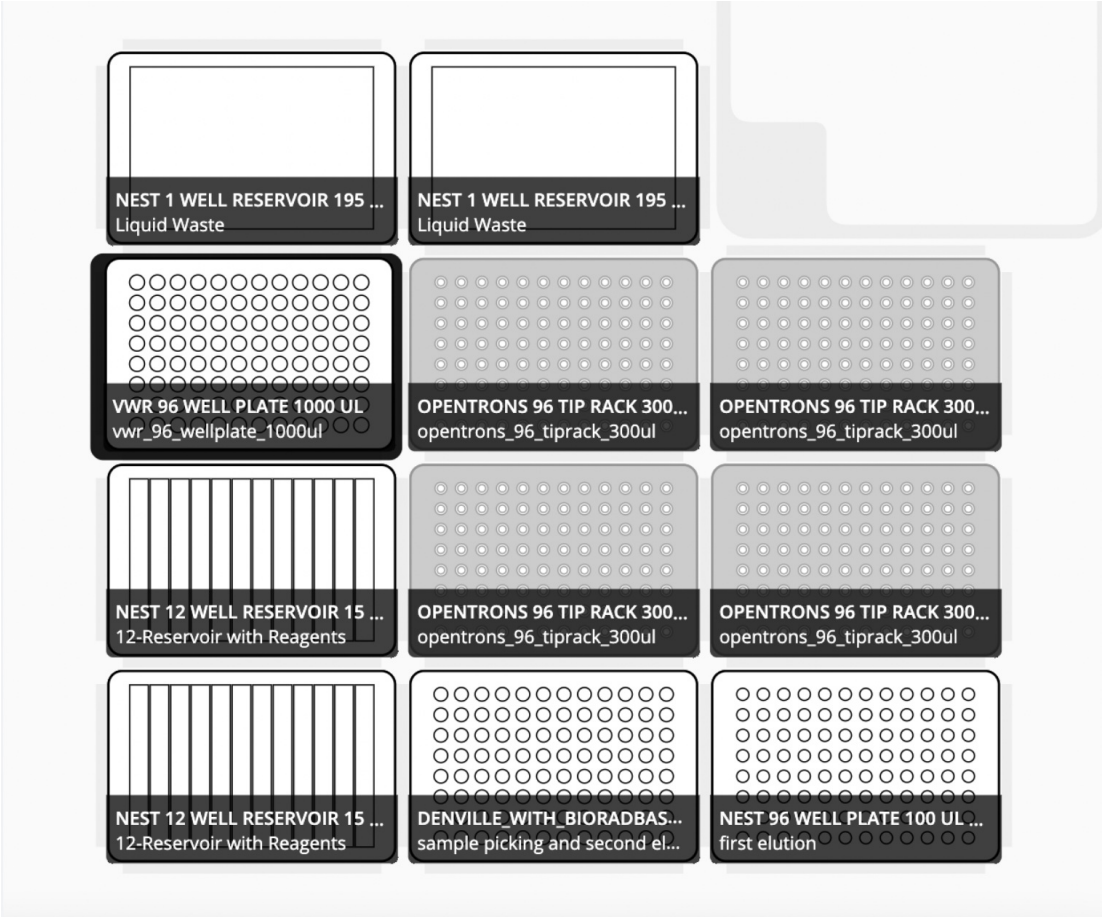
	A	B	C	D	E	F	G	H	I	J	K	L
	Well 1	Well 2	Well 3	Well 4	Well 5	Well 6	Well 7	Well 8	Well 9	Well 10	Well 11	Well 12
	XP2 Binding Buffer and Magbind Particles Round 1 then 2	XP2 Binding Buffer and Magbind Particles Round 1 then 2	XP2 Binding Buffer and Magbind Particles Round 1 then 2	EMPTY	Wash 1: VHB Buffer	Wash 1: VHB Buffer	Wash 1: VHB Buffer	Wash 1: VHB Buffer	Wash 2: SPM Buffer	Wash 2: SPM Buffer	Wash 2: SPM Buffer	Wash 2: SPM Buffer

Slot 5: Opentrons 200µL Filter Tips

Note

It is possible to use Opentrons 200µL Filter Tips or Opentrons 300 Tips (as in the image below). We usually use Opentrons 200µL Filter Tips to avoid cross contamination. The tips are in fact exactly the same dimensions, except that the P200F has a filter, while the P300 does not, and is therefore able to hold more liquid.

Slot 6: Opentrons 200µL Filter Tips
Slot 7: Opentrons Magnetic Module with VWR 96-Well Deep Well Plate full of 300uL of sample lysis on top
Slot 8: Opentrons 200µL Filter Tips
Slot 9: Opentrons 200µL Filter Tips
Slot 10: NEST 1-Well Reservoirs, 195 mL (for waste)
Slot 11: NEST 1-Well Reservoirs, 195 mL (for waste)



Placement of LABWARE and TIPS in the OT2 Deck used for the Omega Biotek Stool extraction protocol. These materials are for purifying 96 samples.

7.2 Calibrate the deck if needed. Follow the on screen instructions.

Run the OT-2 protocol

1h 9m

7.3 Mixing Buffer and Particles with Lysed Sample Plate 1

Column 2 of tips in **Slot 8** will align with column 1 in **Slot 7** to mix the sample by aspirating and dispensing 10 µL. The tips will then be returned to their original starting point. Each subsequent column of tips will continue on the same pattern until all of the samples are mixed.

7.4 Allowing beads to settle on the magnet

10m

The Opentrons Magnetic Module is engaged and incubates the mixed samples for

00:10:00 .

7.5 Removing the supernatant

The supernatant is removed in two steps very gently to avoid removing settled beads. Supernatant is discarded in the Liquid waste NEST 1-Well Reservoir, 195 mL in Slot 10. Column 2 of tips in **Slot 8** will align with column 1 in **Slot 7** to remove the supernatant and then will return the tips back to their original starting point. Each subsequent column of tips will continue on the same pattern until all of the samples have their supernatant removed.

7.6 Adding VHB Buffer to Lysed Sample Plate 1 for wash 1

Column 2 of tips in **Slot 5** will transfer VHB Buffer **Omega** **Biotek Catalog #VHB-440** from wells 5-8 in **Slot 4** in two 133 µL transfer steps to each sample in Lysed Sample Plate 1 in **Slot 7** without touching the lysates. The tips will then be dropped into the waste container.

7.7 Mixing VHB with Lysed Sample Plate 1

Column 2 of tips in **Slot 8** will align with column 1 in **Slot 7** to mix the sample by aspirating and dispensing 10 µL. The tips will then be returned to their original starting point. Each subsequent column of tips will continue on the same pattern until all of the samples are mixed.

7.8 Incubating VHB Buffer and lysate

4m

The Opentrons Magnetic Module is engaged and incubates the mixed samples for 00:04:00 .

7.9 Removing the supernatant from the wash

The supernatant is removed in two steps very gently to avoid removing settled beads. Supernatant is discarded in the Liquid waste NEST 1-Well Reservoir, 195 mL in Slot 10. Column 2 of tips in **Slot 8** will align with column 1 in **Slot 7** to remove the supernatant and then will return the tips back to their original starting point. Each subsequent column of tips will continue on the same pattern until all of the samples have their supernatant removed.

7.10 Adding SPM Buffer to Lysed Sample Plate 1 for wash 2

Column 3 of tips in **Slot 5** will transfer SPM Buffer **Omega** **Biotek Catalog #SPM-300**

from wells 9-12 in **Slot 4** two 133 µL transfer steps to each sample in Lysed Sample Plate 1 in **Slot 7** without touching the lysates. The tips will then be dropped into the waste container.

7.11 Mixing SPM with Lysed Sample Plate 1

Column 2 of tips in **Slot 8** will align with column 1 in **Slot 7** to mix the sample by aspirating and dispensing 10 µL. The tips will then be returned to their original starting point. Each subsequent column of tips will continue on the same pattern until all of the samples are mixed.

7.12 Incubating VHB Buffer and lysate

4m

The Opentrons Magnetic Module is engaged and incubates the mixed samples for 00:04:00.

7.13 Removing the supernatant from the wash

The supernatant is removed in two steps very gently to avoid removing settled beads. Supernatant is discarded in the Liquid waste NEST 1-Well Reservoir, 195 mL in Slot 10. Column 2 of tips in **Slot 8** will align with column 1 in **Slot 7** to remove the supernatant and then will return the tips back to their original starting point. Each subsequent column of tips will continue on the same pattern until all of the samples have their supernatant removed.

7.14 Adding SPM Buffer to Lysed Sample Plate 1 for wash 3

Column 4 of tips in **Slot 5** will transfer SPM Buffer **Omega Biotek Catalog #SPM-300** from wells 1-3 in **Slot 1** in two 133 µL transfer steps to each sample in Lysed Sample Plate 1 in **Slot 7** without touching the lysates. The tips will then be dropped into the waste container.

7.15 Mixing SPM with Lysed Sample Plate 1

Column 2 of tips in **Slot 8** will align with column 1 in **Slot 7** to mix the sample by aspirating and dispensing 10 µL. The tips will then be returned to their original starting point. Each subsequent column of tips will continue on the same pattern until all of the samples are mixed.

7.16 Incubating SPM Buffer and lysate

4m

The Opentrons Magnetic Module is engaged and incubates the mixed samples for

00:04:00 .

7.17 Removing the supernatant from the wash

The supernatant is removed in two steps very gently to avoid removing settled beads. Supernatant is discarded in the Liquid waste NEST 1-Well Reservoir, 195 mL in Slot 11. Column 2 of tips in **Slot 8** will align with column 1 in **Slot 7** to remove the supernatant and then will return the tips back to their original starting point. Each subsequent column of tips will continue on the same pattern until all of the samples have their supernatant removed.

7.18 Allowing beads to air dry

1m

The Opentrons Magnetic Module is engaged for 00:01:00 to allow the Mag-Bind Particles CH **Omega Biotek Catalog #MBPCH- 50** to air dry.

7.19 Removing excess wash buffer

Column 2 of tips in **Slot 8** will align with column 1 in **Slot 7** to remove excess wash by aspirating 10 µL and dispensing into the Liquid waste NEST 1-Well Reservoir, 195 mL in Slot 11. The tips will then be returned to their original starting point. Each subsequent column of tips will continue on the same pattern until all of the wash buffer is removed.

> Note
>
> It is important to remove any residual wash buffer before allowing beads to dry as it contains alcohol. Alcohol could prevent a good elution in the next step and inhibit further processes.

7.20 Allowing beads to air dry

2m

The Opentrons Magnetic Module remains engaged for 00:02:00 to allow the Mag-Bind Particles CH **Omega Biotek Catalog #MBPCH- 50** to have a final air dry and then disengages.

> Note
>
> Do not let beads dry for too long to prevent cracking of the pellet.

Expected result

The color of beads will change from shining dark brown to light brown when dried.

7.21 Adding elution buffer to Lysed Sample Plate 1 for Elution 1

The user must remove the 2.0 mL tubes with Elution Buffer **Omega** **Biotek Catalog #PDR048** from the 70 °C incubator and pour 4 of these tubes into well 12 in **Slot 1**.

Column 5 of tips in **Slot 5** will transfer 60 µL of Elution Buffer **Omega** **Biotek Catalog #PDR048** from well 12 in **Slot 1** to column 1 in **Slot 7** and mix by aspirating and dispensing 40 µL. The tips will then be dispensed into the waste container. Each subsequent column of tips will continue on the same pattern until all of the samples are mixed with warmed elution buffer.

Note

If you own a Temperature module from Opentrons you can also use it to keep the elution buffer continually warm.

7.22 Incubating the beads with DNA in elution buffer

15m

The protocol is paused for 00:15:00 to allow for the Mag-Bind Particles CH **Omega** **Biotek Catalog #MBPCH- 50** to incubate the DNA in the elution buffer at Room temperature.

7.23 Allowing beads to settle on the magnet

2m

The Opentrons Magnetic Module is engaged for 00:02:00 to give the

Mag-Bind Particles CH **Omega Biotek Catalog #MBPCH- 50** time to settle on the magnet away from the elution.

7.24 Transferring each sample elution to Sample Elution Plate 1

Column 6 of tips in **Slot 5** will transfer 60 µL of each eluate to a new, clean Nest skirted PCR Plate in **Slot 3**. Each subsequent column of tips will continue on the same pattern until all of the sample eluates are transferred, extending into the tips in **Slot 9**. The tips will be returned to the tip boxes to be reused for Elution 2.

The Opentrons Magnetic Module is disengaged.

7.25 Adding elution buffer to Lysed Sample Plate 1 for Elution 2

The user must remove the 2.0 mL tubes with Elution Buffer **Omega Biotek Catalog #PDR048** from the 70 °C incubator and pour the remaining 2 tubes into well 12 in **Slot 1**. They must also replace the 96-Well PCR Plate Non-skirt, 200µl now empty of sample lysis on top of an empty Nest skirted PCR Plate in **Slot 2** with a new, clean Nest skirted PCR Plate to receive Elution 2

Column 5 of tips in **Slot 9** will transfer 40 µL of Elution Buffer **Omega Biotek Catalog #PDR048** from well 12 in **Slot 1** to column 1 in **Slot 7** and mix by aspirating and dispensing 20 µL. The tips will then be dispensed into the waste container. Each subsequent column of tips will continue on the same pattern until all of the samples are mixed with warmed buffer, extending into the tips in **Slot 6**.

7.26 Incubating the beads with DNA in elution buffer 15m

The protocol is paused for 00:15:00 to allow for the Mag-Bind Particles CH **Omega Biotek Catalog #MBPCH- 50** to incubate the DNA in the buffer at Room temperature.

7.27 Allowing beads to settle on the magnet 2m

The Opentrons Magnetic Module is engaged for 00:02:00 to give the

Mag-Bind Particles CH **Omega Biotek Catalog #MBPCH- 50** time to settle on the magnet away from the elution.

7.28 Transferring each sample elution to Sample Elution Plate 2

The same tips that were used for Elution 1 will transfer 40 µL of each eluate to a new, clean Nest skirted PCR Plate in **Slot 2**. Each subsequent column of tips will continue on the same pattern until all of the sample eluates are transferred. The tips will be dispensed into the waste container.

The Opentrons Magnetic Module is disengaged.

7.29 Storage of Sample Elution Plates 1 and 2

Cover the plates with an aluminium plate seal and store at 4 °C for use or -20 °C for long term storage.

7.30 QC of Sample Elution Plates 1 and 2

See QC Note in Step 1 for options.

After finishing the protocol

1h 9m

8 Clean the OT2 deck and walls with:

- 10% Bleach **Contributed by users** 1 rinse
- Distilled Water **Contributed by users** 1 rinse
- 70% Alcohol **Contributed by users** 2 rinses

> **Note**
>
> Avoid wetting any electronic parts.

9 Clean OT2 module with:

70% Alcohol **Contributed by users** 2 rinses

Note

Avoid wetting electronic parts.

10 Air dry OT2 robot and modules.