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ISL Opentrons pipeline: gDNA bead cleanup V.2

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We use this protocol and it's working

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Abstract

This protocol is an automated pipeline to clean a plate of extracted DNA using SPRI bead cleanup. It is functional for both. It is typically used to clean up a portion of extracted DNA which has suspected impurities capable of affecting downstream PCR or sequencing protocols. This is often a side effect of extraction by MagBeads with no spin-column based purifications. We have found this protocol to be effective on DNA extracted from blood, biopsy and feces in a wide range of Neotropical vertebrates.

This protocol was developed and optimised for the following:

- Platform: Opentrons OT-2 automated pipetting robot
- Kit: Ampure beads and home-brewed bead solutions
- Tips Used: 5 boxes (2 × 200uL Opentrons Filtered Tip boxes and 3 × 20uL Opentrons Filtered Tip boxes)
- Number of samples: 96

V2 - This is a new version of the original protocol modified as follows:

- We include an updated version of the protocol python file, which is optimized to work on version 6.3.1. Note Version 1 protocol's file is likely to glitch, so please use this file instead.
- To assist with implementing this new protocol in your lab, we also include a simplified version of the python file for a test water run in Section 2. The test reduces incubation times, and works for samples in multiples of 8, so you can test only 8 for a quicker version of the protocol.
- Error correction: OT-2 Single Channel Electronic Pipette P20 was changed to OT-2 8-Channel Electronic Pipette P20.
- Starting sample clean up volume was changed to 25uL from 15uL and all subsequent calculations and descriptions were changed to reflect this. Note: You can alter these volumes easily as well.
- Bead ratio was changed to 1.5X and all subsequent calculations and descriptions were changed to reflect this.Note: this can also be altered to match your specific use case.

Guidelines

There are few things to consider with this protocols:

Step 1.1: In our experience, DNA recovery following SPRI bead cleanups is higher when total volumes used for the incubation steps are higher. This, however, does cost more in terms of beads. If you can afford it in your protocol though, always augment your DNA needing cleanup to 100-200uL, and add beads in a 1.2 ratio to those volumes. Final elution can remain in a small volume.

Step 2: 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 6.3.1

Materials

- 2 **Opentrons 200µL Filter Tips**
- 3 **Opentrons 20µL Filter Tips**
- 1 **NEST 1-Well Reservoirs, 195 mL**
- 1 **NEST 12-Well Reservoirs, 15 mL**
- 1 **Nest skirted PCR Plate**
- 1 **96-Well PCR Plate Non-skirt, 200µl**
- 1 **Axygen™ PCR Tube Storage Rack**
- 1 **Polyester plate seal**
- 2 2mL microcentrifugue tubes

Protocol materials

70% Alcohol
10% Bleach
Distilled Water
70% Alcohol
Ethanol 70% [Note: freshly prepared]
10% Bleach
Distilled Water
70% Alcohol
70% Alcohol
10% Bleach
Distilled Water
70% Alcohol
Ultrapure Distilled, Nuclease Free Water
gTNA
Ethanol 70% [Note: freshly prepared]
Ultrapure Distilled, Nuclease Free Water
Agencourt AmPure XP beads **Catalog #**A63880
Nuclease-free Water
Solutions for Purifying DNA by solid-phase reversible immobilization (SPRI) lab-made
70% Alcohol

Troubleshooting

Safety warnings

Only the standard warnings apply - use PPE to ensure sterility. No ingredients used here are hazardous.

Before start

Clean the OT2 deck and walls with:

10% Bleach 1 rinse

Distilled Water 1 rinse

70% Alcohol 2 rinses

Note

Avoid wetting the electronic parts.

BEFORE STARTING

1 **Materials:**
Autoclave and UV the items you will use to ensure sterility. Some items can be autoclaved and reused as indicated below.

	A	B	C
	Item	#	Status
	Opentrons 200µL Filter Tips	2	NEW
	Opentrons 20µL Filter Tips	3	NEW
	NEST 1-Well Reservoirs, 195 mL	1	REUSED
	NEST 12-Well Reservoirs, 15 mL	1	REUSED
	Nest skirted PCR Plate	1	NEW
	96-Well PCR Plate Non-skirt, 200µl	1	NEW
	Axygen™ PCR Tube Storage Racks	1	NEW
	Polyester plate seal	1	NEW
	2ml microcentrifugue tubes	2	NEW

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

On the left pipette mount use the P20M

Equipment	
OT-2 8-Channel Electronic Pipette P20	NAME
Pipette	TYPE
Opentrons	BRAND
P20M	SKU
https://shop.opentrons.com/8-channel-electronic-pipette/	LINK

1.1 **Reagents:**

Prepare all reagents in advance:

1. Nuclease-free Water
2. Solutions for Purifying DNA by solid-phase reversible immobilization (SPRI) lab-made
3. 70% Alcohol, freshly prepared
4. Agencourt AmPure XP beads **Catalog #**A63880

Note

You can also make your own bead solution such as from **this protocol** on protocols.io

We begin with a cleanup sample volume of 25uL. This allows you to cleanup a small volume, but larger volumes are easily cleaned with the same protocol. Simply add water 1:1, and then proceed.

	A	B	C	D
	Ingredient	Amount per sample	Amount per 96 samples	Notes
	Water	25	2400	
	SPRI beads	36	7200	1.5x water+sample
	70% ethanol	250	24000	

Note

In our experience, DNA recovery following SPRI bead cleanups is higher when total volumes used for the incubation steps are higher. This, however, does cost more in terms of beads. If you can afford it in your protocol though, always augment your DNA needing cleanup to 100-200uL, and add beads in **a 1.5 ratio** to those volumes. Final elution can remain in a small volume.

1.2 Add water in a 1:1 ratio by volume of water to samples.

Note

*This reagent will be put in the **NEST 12-Well Reservoirs, 15 mL** in the position detailed in the Step 3 just before dispensing. It should be warmed to* 55 °C

1.3 Add beads in a 1:1.5 ratio (sample: beads) allowing for the fact that the sample contains both sample +water from step 2.1.

Note

*This reagent will be put in the **NEST 12-Well Reservoirs, 15 mL** in the position detailed in Step 3*

1.4 Each sample will be washed twice with freshly prepared Ethanol 70%.

Note

*This reagent will be put in the **NEST 12-Well Reservoirs, 15 mL** in the position detailed in the Step 3*

2 Before loading your protocol, load this labware file into your Opentrons app:
denville_96_axygenbase_200ul.json 25KB

This labware definition allows us to use a nonskirted plate in the Opentrons 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).

Load this python file to the Opentrons app: cleanup_gDNA_v4.4.py 12KB

To test the protocol before starting, there is a modified version of the python file with shortened incubation times and minimized sample total to input into the app if you wish to do so: cleanup_gDNA_v4.4.w.py 12KB

2.1 **Arrange the OT-2 deck**

Number of samples: 96

Slot 1: Opentrons Magnetic Module with Nest skirted PCR Plate empty (to receive cleaned DNA)
Slot 2: Nest skirted PCR Plate with TNA
Slot 3: 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	M
	Channel #	1	2	3	4	5	6	7	8	9	10	11	12
	Contents	SPRI BEADS				ALCOHOL	ALCOHOL			WATER			

Ingredients plan for a 12-well reservoir

Slot 4: 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.

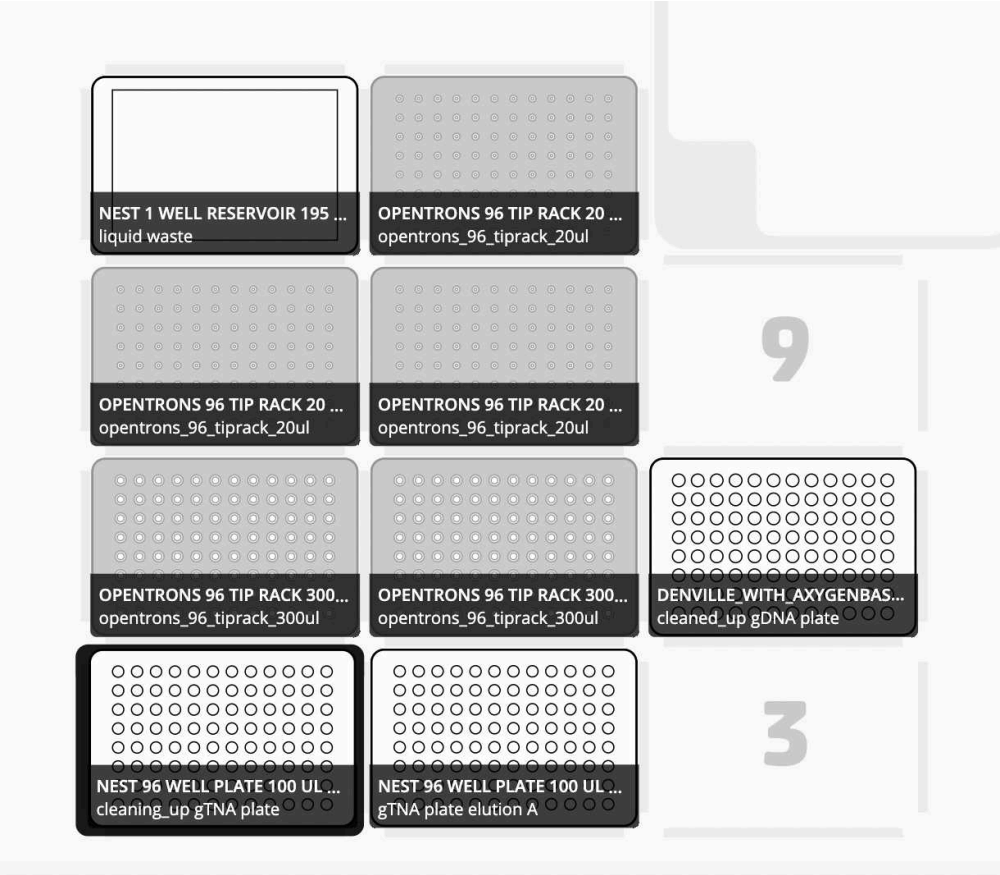
Slot 5: Opentrons 200µL Filter Tips
Slot 6: Axygen™ PCR Tube Storage Rack with 96-Well PCR Plate Non-skirt, 200µl

Note

Here, we use more affordable nonskirted plates over the NEST plates because they are a) cheaper and b) 200uL and able to hold more volume. By placing the plate inside the storage rack, we created a way for a nonskirted plate to be "clipped" into the deck. Together, they have a new labware definition titled: MISSING ADD LABWARE DEFINITION.

Slot 8: Opentrons 200µL Filter Tips
Slot 9: Opentrons 200µL Filter Tips
Slot 10: NEST 1-Well Reservoirs, 195 mL (for waste)

Slot 11: Opentrons 200µL Filter Tips



Placement of LABWARE and TIPS in the OT2 Deck used for the gDNA clean up protocol. These materials are for cleaning 96 samples.

2.2 Calibrate the deck. Follow the onscreen instructions.

OT2 SCRIPT DEFINITIONS

3 Definition of samples and labwares:

3.1 **gTNA samples**
Samples that will be cleaned and are in the elution plate A from previous TNA extraction.

Position: Slot 2**,** Nest skirted PCR Plate with TNA
Name in the Deck: gTNA plate elution A
Labware name in the protocol: gTNA_plate_A

Sample name in the script: gTNA_samples

3.2 **Samples to be cleaned**

Samples that are in the magnet to be cleaned.

Position: Slot 1**,** Nest skirted PCR Plate in Opentrons Magnetic Module
Name in the Deck: to_be_cleaned gTNA plate
Labware name in the script protocol: mag_plate
Sample name in the script: samples_to_be_cleaned

3.3 **Cleaned samples**

Samples that have been cleaned and will be eluted in the clean up plate.

Position: Slot 6**,** Axygen™ PCR Tube Storage Rack with 96-Well PCR Plate Non-skirt, 200µl
Name in the Deck: cleaned_up gDNA plate
Labware name in the protocol: clean_up_plate
Sample name in the script: cleaned_samples

4 **Protocol variables definition**

This protocol is written to use 25 µL of gTNA sample + 25 µL of water to reach 50 µL as final volume to start the clean up process for 96 samples.
Therefore, if you want to modify the volumes and sample number just open the script in a text editor program, search and modify the values in the third line of the script:

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

Note

It is better if it is a multiple of 8

"gTNA_volume": **15** → Volume of gDNA that will be cleaned.
"bead_ratio": **1.5** → Ratio of beads volume
"elution_buffer_volume": **15** → Volume of water for elution

Note

This is the same volume as gTNA cleaned.
You can also set it to be 1ul more than the desired volume to avoid losing beads

"incubation_time": **6** → Time in minutes for incubation of beads with the sample
"pelleting_time": **6** → Time with magnet engaged.
"drying_time": **5** → Time to the let alcohol evaporate

OT2 Clean up Protocol

2h 11m

5 Protocol

5.1 Transferring water to the gTNA plate

8m

25 µL of Ultrapure Distilled, Nuclease Free Water is transferred from Well 9 in the NEST 12-Well Reservoirs, 15 mL in Slot 3 to each column of a new Nest skirted PCR Plate placed in the Opentrons Magnetic Module in Slot 1.
The first column of **tips** in Slot 11 is used for dispensing water to all the columns.

5.2 Transferring gTNA to the gTNA plate

8m

25 µL of gTNA is transferred from the gTNA plate elution A in Slot 2 to a the **to_be_cleaned** gTNA plate in Slot 1.
Tips in Slot 11 are used for this step.

Note

Samples are mixed in this step before transferring, in a programmed mixing step.

5.3 Dispensing SPRI beads

8m

75 µL **of SPRI beads** are dispensed from Well 1 in the NEST 12-Well Reservoirs, 15 mL in Slot 3 to the **to_be_cleaned** gTNA plate in Slot 1.
The first column of **tips** in Slot 5 is used for dispensing SPRI beads to all the columns.

5.4 Mixing samples and beads

15m

Two mixing steps are defined in the script. The first column will be mixed, then the second and so on to the 12th column, then it will be repeated. The whole process is approximately 00:15:00 m long.

Tips in Slot 4 are used for this step. Each column of tips is used to mix each column of samples.

> Note
>
> Make sure samples are well mixed, samples should have an homogeneous color.

5.5 Allowing beads to settle on the magnet

6m

The Opentrons Magnetic Module is engaged for 00:06:00 m to allow beads settle.

5.6 Removing the supernatant

10m

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.
Tips in Slot 4 are used for this step. Each column of tips is used for one column of samples.

5.7 The first washing step

8m

100 µL of Ethanol 70% [Note: freshly prepared] is dispensed for washing beads from Well 5 in the NEST 12-Well Reservoirs, 15 mL in Slot 3 to the **to_be_cleaned** gTNA plate in Slot 1.
The second column of **tips** in Slot 5 is used for dispensing SPRI beads to all the columns.

5.8 Removing the 1st ethanol wash

10m

After an incubation of 00:00:30 s, the supernatant is removed in two steps very gently to avoid removing settled beads. Supernatant is discarded in the liquid waste NEST 1-Well Reservoirs, 195 mL in Slot 10.
Tips in Slot 4 are used for this step. Each column of tips is used for one column of samples.

5.9 The second washing step

8m

100 µL of Ethanol 70% [Note: freshly prepared] is dispensed for washing beads from Well 6 in the NEST 12-Well Reservoirs, 15 mL in Slot 3 to the **to_be_cleaned** gTNA plate in the Slot 1.
The second column of **tips** in Slot 5 is used for dispensing SPRI beads to all the columns.

5.10 Removing the 2nd ethanol wash

10m

After an incubation of 00:00:30 s, the supernatant is removed in two steps very gently to avoid removing settled beads. Supernatant is discarded in the Liquid waste **NEST 1-Well Reservoirs, 195 mL** in Slot 10.
Tips in Slot 4 are used for this step. Each column of tips is used for one column of samples.

5.11 Removing any remaining ethanol

8m

30 µL of remaining ethanol is removed very gently to avoid removing settled beads. Supernatant is discarded in Liquid waste **NEST 1-Well Reservoirs, 195 mL** in Slot 10.
Tips in Slot 4 are used for this step. Each column of tips is used for one column of samples.

Note

It is important to remove any residual ethanol before allowing beads to dry. Alcohol could prevent a good elution in the next step and inhibit further processes.

5.12 Drying the beads

5m

A pause of 00:05:00 m is set to allow beads to dry at Room temperature to evaporate remaining ethanol.

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.

5.13 Adding elution buffer or water

10m

Disengaged the **Opentrons Magnetic Module**.

19 µL of Ultrapure Distilled, Nuclease Free Water are transferred from the Well 9 in the **NEST 12-Well Reservoirs, 15 mL** in the Slot 3 to each column of the cleaning_up gTNA plate in the Slot 1. Samples are mixed after adding water.
Tips in the Slot 7 are used for this step. Each column of tips is used for each column of samples.

5.14 **Mixing the beads** 10m

This is the second mixing step of water and sample before elution. The first column will be mixed, then the second and so on to the 12th column
The whole process is approximately 00:10:00 m long.
Tips in the Slot 7 are used for this step.

5.15 **Binding beads to the magnet** 6m

The **Opentrons Magnetic Module** is engaged for 00:06:00 m to allow beads to pellet.

5.16 **Elution of final DNA** 8m

19 µL of cleaned gTNA are transferred from the to_be_cleaned gTNA plate in Slot 1 to the
cleaned_up gTNA plate in Slot 6.
Tips in the Slot 8 are used for this step. Each column of tips is used for each column of samples.

5.17 **Storage of cleaned gDNA**

Cover the cleaned_up gTNA plate with a plate seal and store at 4 °C for use or -20 °C for long term storage.

After finishing the protocol

6 Clean the OT2 deck and walls with:
10% Bleach 1 rinse
Distilled Water 1 rinse
70% Alcohol 2 rinses

Note

Avoid wetting any electronic parts.

7 Clean OT2 module with:
70% Alcohol 2 rinses

Note

Avoid wetting electronic parts.

8 Air dry OT2 robot and modules.

Protocol references

If you want to make your own beads, we recommend this protocol: **dx.doi.org/10.17504/protocols.io.bnz4mf8w**