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Protocol status: Working
We use this protocol and it's working on DNA extracted from blood, feces, and biopsies of a range of Neotropical vertebrates

Opentrons pipeline: gDNA bead cleanup

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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 x 200uL Opentrons Filtered Tip boxes and 3 x 20uL Opentrons Filtered Tip boxes)
- Number of samples: 96

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Keywords: gDNA clean up, DNA SPRI beads, Automated Opentrons pipeline, bead cleanup, opentrons, insitulabs

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

Solutions for Purifying DNA by solid-phase reversible immobilization (SPRI) lab-made **Contributed by users**

Step 1.1

Ethanol 70% [Note: freshly prepared] **Contributed by users** In 2 steps

Nuclease-free Water **Contributed by users** Step 1.1

Distilled Water **Contributed by users** Before starting, Step 6

gTNA **Contributed by users** Step 5.2

Ultrapure Distilled, Nuclease Free Water **Contributed by users** In 2 steps

Agencourt AmPure XP beads **Contributed by users Catalog #A63880** Step 1.1

70% Alcohol **Contributed by users** In Before starting and 3 steps

10% Bleach **Contributed by users** Before starting, Step 6

SAFETY WARNINGS

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

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 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 Single Channel Electronic Pipette	NAME
Pipette	TYPE
Opentrons	BRAND
P20S	SKU
https://shop.opentrons.com/single-channel-electronic-pipette-p20/	LINK

1.1 Reagents:

Prepare all reagents in advance:

1. Nuclease-free Water **Contributed by users**
2. Solutions for Purifying DNA by solid-phase reversible immobilization (SPRI) lab-made **Contributed by users**
3. 70% Alcohol **Contributed by users**, freshly prepared
4. Agencourt AmPure XP beads **Contributed by users 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 15uL. 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	15	1440	
	SPRI beads	36	3456	1.2x 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 1.2 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: 1.2 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

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_v4.3.py

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

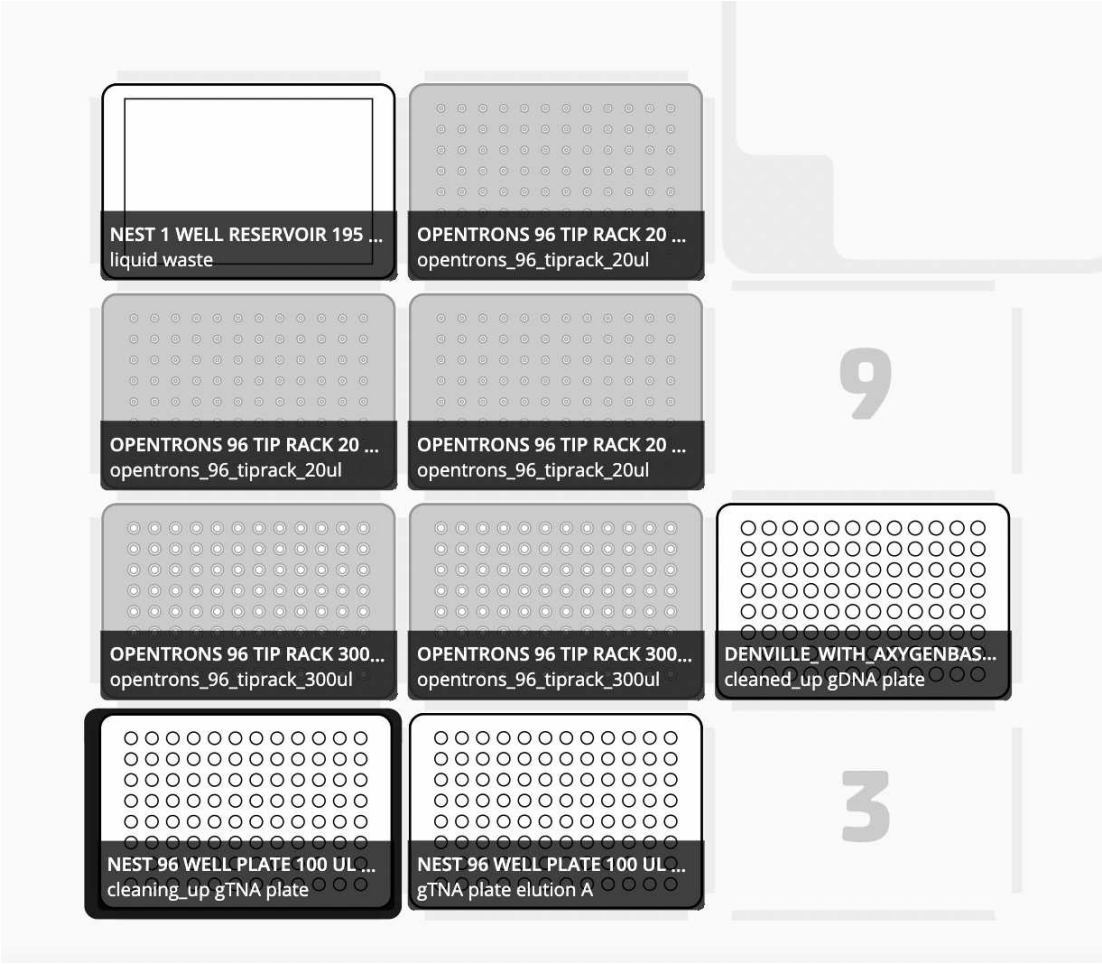
Slot 4: Opentrons 200µL Filter Tips

> 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 15 µL of gTNA sample + 15 µL of water to reach 30 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": **1.2** → 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

15 µL of Ultrapure Distilled, Nuclease Free Water **Contributed by users** 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

15 µL of gTNA **Contributed by users** 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

36 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] **Contributed by users** 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] **Contributed by users** are 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.

15 µL of Ultrapure Distilled, Nuclease Free Water **Contributed by users** 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

15 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 **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.

7 Clean OT2 module with:

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

Note

Avoid wetting electronic parts.

8 Air dry OT2 robot and modules.