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Opentrons Pipeline: PCR Preparation

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ABSTRACT

This protocol is a general automated pipeline to prepare mastermix at the plate level in a sterile way. The protocol volumes can be edited, as can sample numbers, to change the scale of the operation and optimise it to fit any end-point PCR.

This protocol was developed and optimised for the following:

- Platform: Opentrons OT-2 automated pipetting robot
- Tested with software version 6.3.1
- GoTaq G2 Hot Start Green Master Mix **Promega Catalog #M742B**
- Tips Used: 1 box and 1 column of 20uL filtered Opentrons Tips
- Number of samples: 91 samples and 5 controls in a 96 well plate

Important: In an ideal situation, there would be two OT-2 robots involved in this process: the first to prepare sterile reagents and make and aliquot the mastermix, and the second to add the template. In the absence of this facility, we have developed a means of keeping each ingredient on the deck singly, rather than having all ingredients open on the deck at once. So please take note of the protocol pauses in built to replace each ingredient with the next. In this way, no two ingredients are open inside the OT2 hood at the same time.

Python File Version 2.2.

Last Modified: Sep 25, 2023

PROTOCOL integer ID: 88226

Keywords: Opentrons, PCR, Mastermix, automated, insitulabs, OT2

GUIDELINES

Step 1: Please note we use a particular Taq but you can alter this to suit your needs. You can also edit your PCR mix recipe by following the template in this protocol.

Step 5: 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

Step 10: Take note of the two possible ways of loading gDNA. A skirted plate will clip into the deck, and a non-skirted plate or strip tubes will need support (and the labware definition file attached to this protocol).

Step 12: Note that we have specific wells in which we load controls, and for this you will need to remove the tips from the box so no template is added accidentally. We then load our controls later in a PCR hood.

MATERIALS

2 X Opentrons 20µL Filter Tips
1 X Opentrons Thermocycler Module - GEN2*
1 X Nest skirted PCR Plate
1 X 96-well Opentrons Aluminum Block
1 X P20 Gen 2 Multichannel Pipette
1 X P20 Gen 2 Single Pipette (optional)
1 X Nonskirted PCR plate (optional, see step 10)
2 X plate seals
1 X 0.2mL PCR strip tubes
1 x 1.5 mL microcentrifuge tube

PROTOCOL MATERIALS

GoTaq G2 Hot Start Green Master Mix **Promega Catalog #M742B**

Abstract, Step 1

10% Bleach **Contributed by users** Before starting, Step 14

Distilled Water **Contributed by users** Before starting, Step 14

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

Nuclease-Free Water- 25mL **Promega Catalog #MC1191** Step 1

SAFETY WARNINGS

Use PPE to handle all samples and liquids. Make sure to open ALL ingredients outside the OT2 deck, and place the plates in the deck only after any aerosols have dispersed.

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.

Materials and reagents

1 Reagents List

GoTaq G2 Hot Start Green Master Mix **Promega Catalog #M742B**

Primers Forward and Reverse [M] 10 micromolar (µM) or specific for each primer.

Nuclease-Free Water- 25mL **Promega Catalog #MC1191**

2 **Materials List**

2 X Opentrons 20µL Filter Tips
1 X Opentrons Thermocycler Module - GEN2*
1 X Nest skirted PCR Plate
1 X 96-well Opentrons Aluminum Block
1 X P20 Gen 2 Multichannel Pipette
1 X P20 Gen 2 Single Pipette (optional)
1 X Nonskirted PCR plate (optional, see step 10)
2 X plate seals
1 X 0.2mL PCR strip tubes
1 x 1.5 mL microcentrifuge tube

Note

The thermocycler module is not a critical component of this protocol, but removing it would require the user to make some small modifications to the protocol and labware definitions indicating the location of the destination plate, and the plate type. You can prepare the plate and remove it and run it on any protocol.

Items to prepare in advance

3 Prepare your Master Mix following your specific protocol.

	A	B	C	D	E
		start [ ]	desired [ ]	Volume (uL) per reaction	Per 96 reactions
	GoTaq G2 Hot Start Green Master Mix	2X	1X	6.25	600
	Forward Primer	Specific for each primer	Specific for each primer	Specific for each primer	
	Reverse Primer	Specific for each primer	Specific for each primer	Specific for each primer	

	A	B	C	D	E
	Nuclease-Free Water			to reach 10.5	
	Mastermix volume per reaction			10.5	
	Template			2	
	Final volume			12.5	

An example of a mastermix recipe.

4 Dispense Master Mix in all 8 wells of a 0.2mL PCR strip tube equally.
Total volume per strip tube well = MasterMix total volume divided by 8

5 Import the lab ware file to your Opentrons app: denville_96_aluminumblock_200ul.json

Load python file to Opentrons app:
Automated Opentrons Pipeline for PCR Preparation V2.2 ISL.py

Attach a P20 Gen 2 Multichannel Pipette to the right pipette mount.

(Optional) Attach a P20 Gen 2 Single Pipette to the left pipette mount. Note this is not used in this protocol, but should you want to do less than a multiple of 8, you could edit the script to use this pipette to load those samples, potentially.

5.1 **Arrange the OT-2 deck as follows for 96 samples in a plate:**

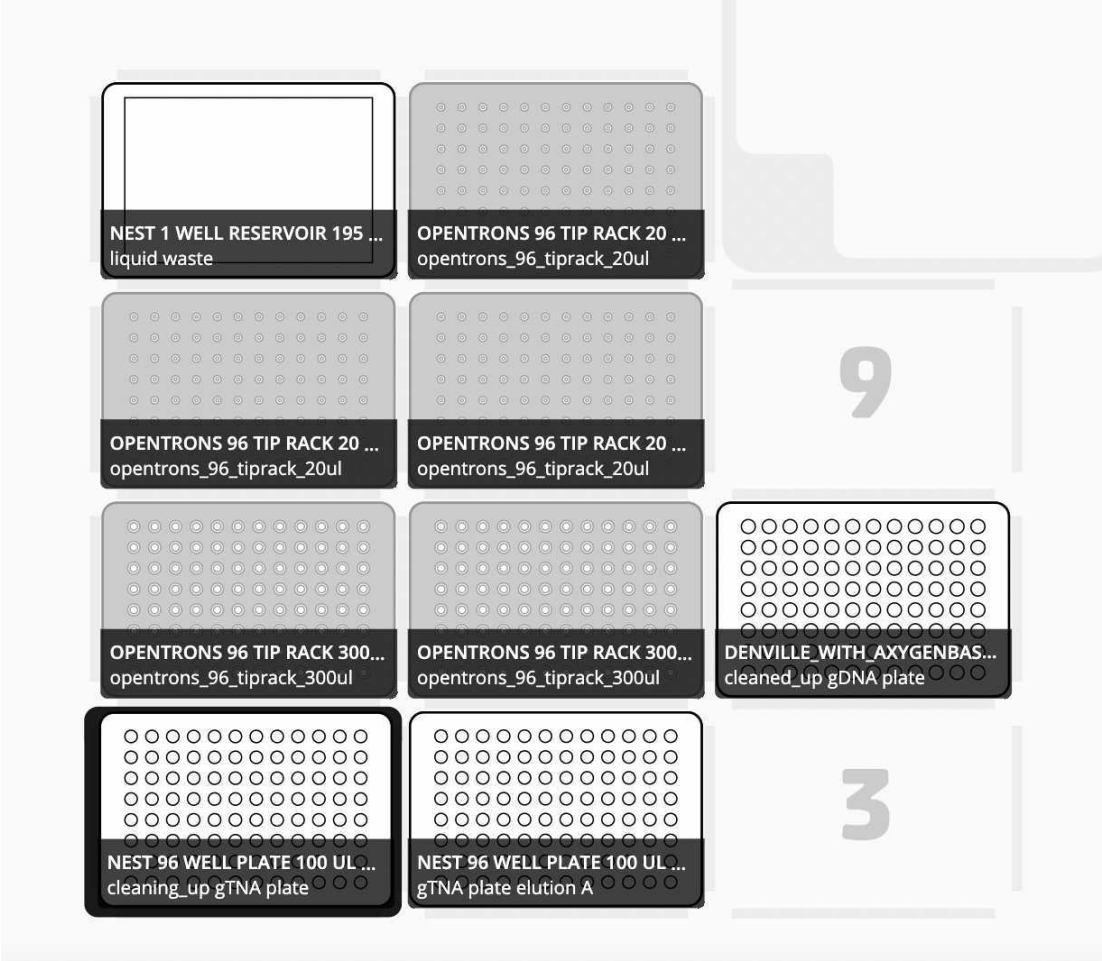
Slot 1: 96-well Aluminum Block with 200uL PCR strip tube with prepared mastermix

Note

Keep the Aluminum Block cold until the protocol starts.
This slot is used for placing the master mix, and then later the genomic DNA template in a Nest skirted PCR Plate

Slot 3: Opentrons 20µL Filter Tips
Slot 6: Opentrons 20µL Filter Tips

Slot 7: Opentrons Thermocycler Module - GEN2 with an empty Nest skirted PCR Plate



Placement of Labware and Tips in the OT2 Deck for transferring samples and Master Mix.

5.2 Calibrate the deck in the Opentrons app and follow instructions on the app.

OT2 PCR script definitions

6 **Master Mix**
PCR Master Mix

Position: Slot 1**,** 1 0.2mL PCR strip tube in 96-well Aluminum Block
Name in the Deck: MM
Labware name in the script protocol: MM_and_samples_plate
Variable name in the script: MMix

7 **gDNA samples**
Template DNA

Position: Slot 1**,** 96-well Aluminum Block with Nest skirted PCR Plate
Name in the Deck: gDNA cleaned samples
Labware name in the protocol: MM_and_samples_plate
Variable name in the script: template

8 **PCR samples**
Destination for template + Master Mix

Position: Slot 7**,** Opentrons Thermocycler Module - GEN2 with Nest skirted PCR Plate empty
Name in the Deck: PCR plate
Labware name in the script protocol: final_PCR_plate
Variable name in the script: pcr_samples

9 **Protocol variables definition**
The following variables can be edited within the python script in a text editor program to fit your specific PCR setup needs, by changing the **"MM_vol"** and **"template_vol"** definitions in the third line of the script.

The default protocol is written to use 2 µL of Template + 10.5 µL of Master Mix to reach 12.5 µL as final volume.

Standard protocol variable definitions:
"sample_number" indicates the number of samples that you will process: **96**
"MM_vol" indicates the volume of Master Mix per well: **10.5**
"template_vol" indicates the template volume added to each well: **2**

Note

It is better if sample_number is a multiple of 8 given that the OT-2 uses a multichannel pipettes for transferring samples

OT2 Protocol: Transferring mastermix and samples

10 **Transferring Master Mix to final_PCR_plate**

- ***Place the PCR strip tube (already containing the MasterMix) in the first column of the 96-well Aluminum Block***

10.5 µL of Master Mix is transferred from the PCR strip tube in the aluminum block in Slot 1 to the PCR plate in the Opentrons Thermocycler Module - GEN2 in Slot 7

Note: Only the first column of **tips** in the Slot 6 rack is used for dispensing Master Mix to all columns *of PCR plate*.

- Once this step is finished the robot will PAUSE. During this PROTOCOL PAUSE, carefully remove the PCR strip tube of MasterMix and now replace it with the plate of gDNA.

Note

You have two options here:

1. Either load your gDNA in a skirted NEST 100uL plate, which will clip into Slot 1, after you remove the aluminum block. If you do, you don't need the additional labware definition we have provided, and can simply edit the script to give the definition of the NEST plate (available on the

OR

2. Leave the aluminum block in, and add
a) a set of strip tubes (no caps) with gDNA, OR
b) a non-skirted plate with gDNA.

Both products linked above will fit the labware definition provided in this protocol.

Then, resume the run to continue with Step 11.

11 Transferring gDNA samples to final_PCR_plate

2 µL of template are transferred from MM_and_samples_plate in Slot 1 to final_PCR_plate in Opentrons Thermocycler Module - GEN2

Tips in Slot 3 are used for this step

Note

IMPORTANT NOTE: Take out tips in Slot 3 for established positions for controls:
Negative controls: Positions G3, B10
Positive controls: Positions B3, G10
Sample control: D6

Note

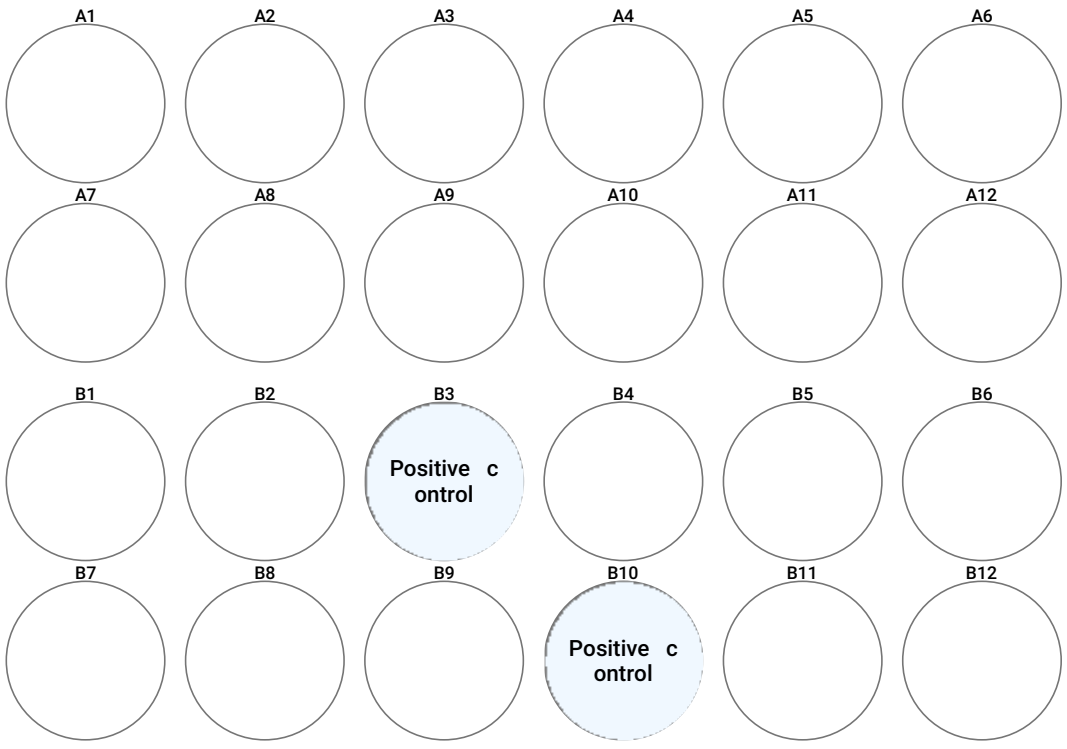
Samples are mixed in this step before and after transferring.

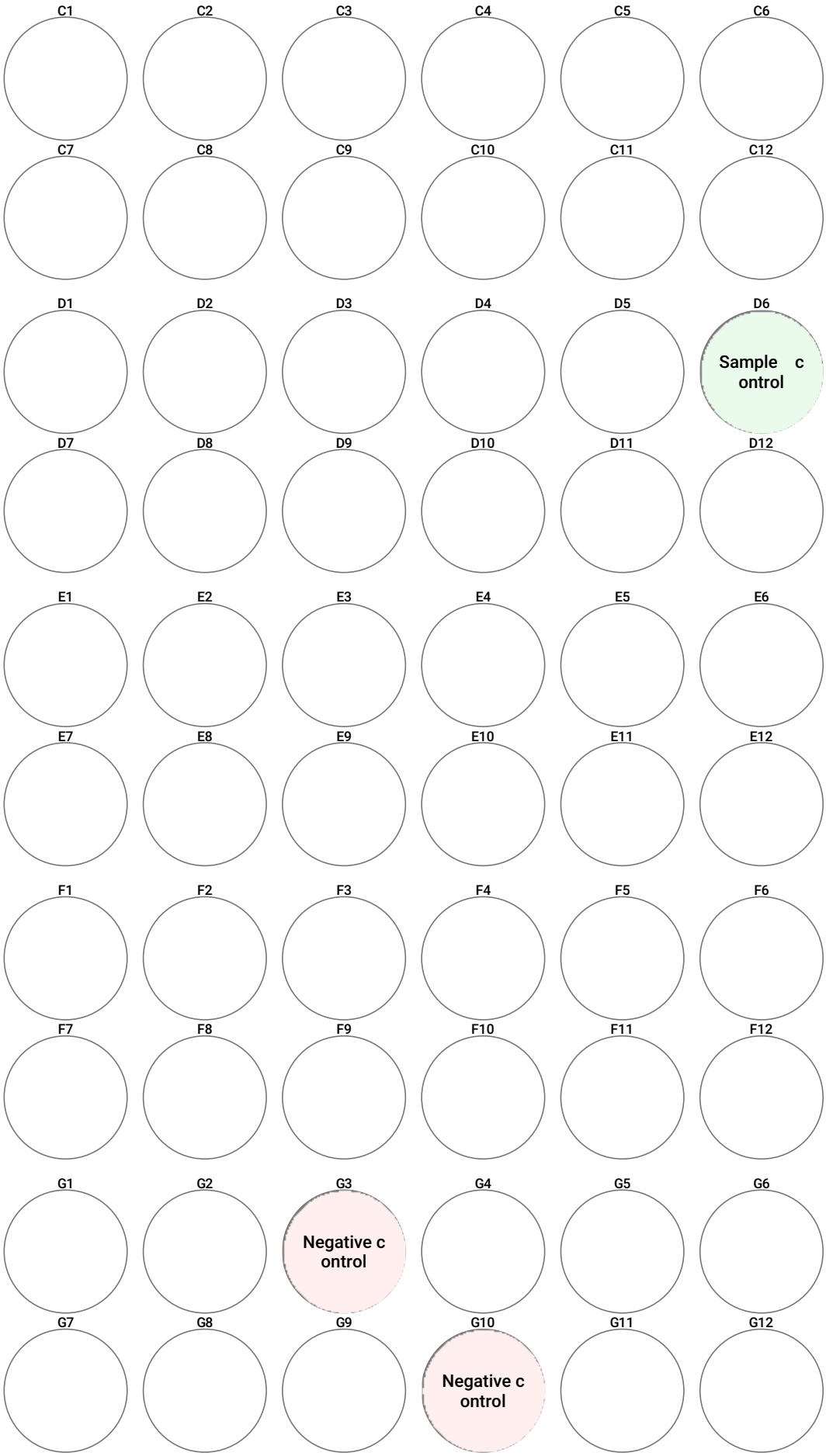
Note: Once finished, cover the plate with a seal to move it into the PCR cabinet to add the controls.

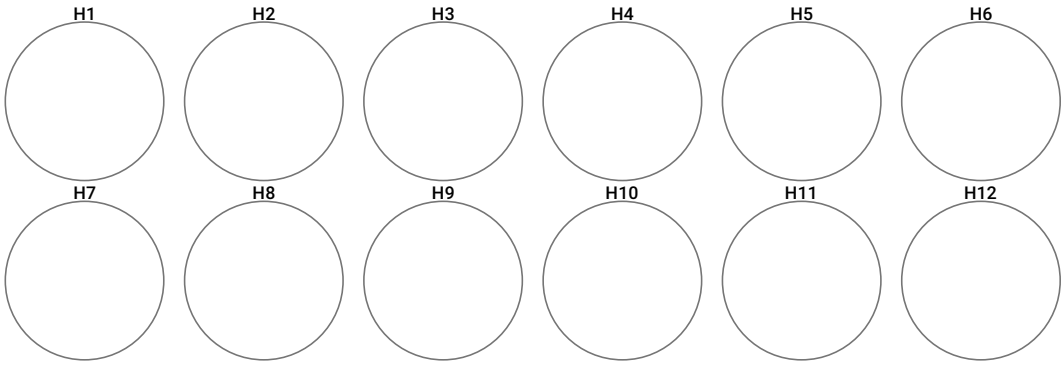
Manually adding controls

12 Adding controls

Add the controls in a sterile PCR cabinet, in the following pre-determined spaces.




C1
C2
C3
C4
C5
C6
C7
C8
C9
C10
C11
C12
D1
D2
D3
D4
D5
D6
Sample c ontrol
D7
D8
D9
D10
D11
D12
E1
E2
E3
E4
E5
E6
E7
E8
E9
E10
E11
E12
F1
F2
F3
F4
F5
F6
F7
F8
F9
F10
F11
F12
G1
G2
G3
Negative c ontrol
G4
G5
G6
G7
G8
G9
G10
Negative c ontrol
G11
G12



13 The PCR plate is now ready to be loaded into a thermal cycler.

CLEANUP

14 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.

15 Clean OT2 module with:

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

Note

Avoid wetting electronic parts.

16 Air dry OT2 robot and modules.