

Standard Operational Procedure

Opentrons OT2 Protocol - Template

Version 1.1

The following Standard Operational Procedure (SOP) outlines the procedure for working with the Opentrons OT2 Template protocol generated via the [web application](#). The SOP contains the primary information regarding safety, startup, workflow, and FAQ and covers all protocols used. As the Opentrons OT2 system is a semi-automation, the user is required to support it in its work.

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Safety

The following segment describes the hazardous elements in the work procedures. It is presumed the user has read the Opentrons OT-2 guidelines and “Safety and Regulatory Compliance Information” for proper handling of the equipment.

Hardware

OT-2 Hardware

The OT-2 incapsulate 4 potential hardware safety issues: Physical, electrical, heating, and pinch pointing. All 4 are listed below. Handle OT-2s with care to avoid caution events.

CAUTION: Risk of danger! Instrument components pose a risk of personal injury or instrument damage if improperly handled.

To reduce risk, ensure the machine is either turned off or paused before working within OT-2. All Opentrons Python Scripts from the AlberdiLab includes an “set_rail_lights” command in the beginning of the automation protocol to automatically switch lights on during runs and off when completed to indicate an active robot. The Opentrons offers “[Auto-stop](#)” function to automatically pause work, however, this feature is not turned on for the AlberdiLab, as the auto pause is prone to cause errors for the thermocycler unit. This in turn mean the robot arm can move while the user is actively working inside the

CAUTION: Risk of electrical shock! Instrument components pose a risk of electrical shock if handled improperly.

To reduce the risk of electrical shock, it is recommended switching off power to the unit while working in areas with electrical components. Do not attempt to touch any open electrical parts while supplied with power. Do not clean exposed electrical parts on units with any liquid – if wet, turned off the unit and wait for evaporation/drying.

CAUTION: Hot surface! Instrument components pose a risk of personal injury due to excessively high heat temperature if handled improperly.

To reduce risk of personal injury due to high temperature, avoid touching units intended for such use if status of the unit is unknown. Turn off units and wait for decrease in temperature.

CAUTION: Pinch Point! This symbol identifies instrument components which can pose risk of personal injury when moving.

See general risk of danger.

Other Hardware

Like OT-2 warnings and risk, handle equipment with care. Consult trained personnel and/or equipment safety and compliance guidelines to avoid any injury.

Chemicals

Check local guidelines for the correct usage and handle of chemicals.

Opentrons Setup and Preparation

Preparation

1. Go to <https://alberdilab-opentronsscripts.onrender.com/> where you find the web application to download customised OT-2 protocols.
2. After selecting which EHI protocol package to download and select run parameter to use, a zip folder is generated to download. In the folder you find a copy of this SOP, python scripts for the protocols, and custom labware designs for special labware.
3. In the Opentrons App, python protocols are uploaded in the protocol tab, while custom labware are uploaded under the labware tab. The python protocol might need reanalysing, once all custom labwares are uploaded.
4. Once the Opentrons App has analysed the python protocol without errors/issues, it is ready to begin.

Opentrons OT-2 Setup

1. Turn on PC (or similar).
2. Turn on OT-2 and any “smart modules” necessary. If applicable, turn on HEPA filter.
3. Clean work area and OT-2. According to the OT-2 instructions, the OT-2 deck surface and acrylic panels can be cleaned using bleach and ethanol.
4. Open Opentrons App. Find (or upload) protocol. Begin setup by connecting to the Opentrons to the App.
5. Check the calibration of the pipette and deck on the OT-2.
6. If using smart modules, activate them and set the temperatures. Cold modules and thermocyclers (block) are set to 10°C. Thermocycler lid is set at 100°C.
7. Follow the setup instruction, placing the consumables as told by the App.
8. Ensure all lids and seals are removed.
9. Check the offset positioning to ensure the highest accuracy on the run. Follow the instruction given by the App. Remove all items taller than a PCR plate on a temperature/magnet module to avoid collisions during offset. Apply offset once done.
10. Check and fill up labware with reagents.
11. Begin protocol once ready.

Laboratory Protocols

Extraction

Materials & Consumables

List of consumables used in the OT-2. Pipetting and plastic ware outside of the robot is not included. Assuming 96 samples are worked with.

Item	Amount	Cat. Number	Supplier
200 µL Filter Tips	8		Opentrons
10 µL Filter Tips	1		Opentrons
Deep Well Plate	1	4TI-0125	Saveen&Werner ApS
22 mL reservoir	1	11311974	Fisher Scientific
Skirted PCR Plate*	1 (0)	ISTSIST-601-096GCT	VWR
LVL XSX200*	1 (0)	2DNC-X02-BL-NS-SLC-S	LVL technologies
PCR Strips/tubes/plate*	1 (0)		

*Only 1 is needed. Input plate not included

Manual Work and Preparation

Preparation - Buffer Preparation & Sample Lysis

Standard buffer preparation can be found in [4.4.2 - Extraction buffer preparation](#). It is advised to prepare in advance. Samples (tissue) are mechanical lysed following the [bead beating protocol](#).

DNA extraction protocol (Manual part)

Preparation of Silica Magnetic Beads and Buffers

1. Equilibrate* the silica magnetic beads (G-Biosciences, cat number 786-916) to room temperature for 30 min.
2. Switch on the Thermo Mixer: set to 15 minutes, 10 C, 1500 rpm.
3. Prepare reagent aliquots (according to the table below). Prepare 10% extra per sample. Ensure the beads are thoroughly resuspended before taking an aliquot.

	Each sample	Number samples: (required plus 10%) (µL)	Volume Tube
Beads - DNA fraction	15 µl		2 mL
Buffer C	200 µL		15 mL**
80% EtOH - Final wash	400 µl		25 mL
Elution buffer**	50 µL		5 mL

4. Place the tubes containing silica beads on a magnetic rack and wait until the beads are immobilised on the side, and the supernatant is clear.

5. Discard the clear supernatant.
 6. Add 2 mL of Tris-EDTA (TE) buffer to each tube. The TE buffer volume may be reduced, as long as the beads are completely submerged during the wash step.
 7. Discard the supernatant. Repeat steps 6 and 7.
 8. Transfer "Beads - DNA fraction" to Buffer C. Mix well by vortexing (avoid bubbles if possible)".
- *Store the silica magnetic beads (G-Biosciences) at 4°C. Do not freeze the silica magnetic beads (G-Biosciences) or store them near a magnetic source.

**Extra elution buffer must be added to the reservoir in the automated protocol such that the bottom is covered.

DNA extraction (OT-2):

1. Turn on HEPA filter unit and OT-2. Clean OT-2 with 5% Bleach and 70% ethanol.
2. Ensure samples have entirely thawed. Vortex and centrifuge/spin down the samples.
3. Transfer 200 µL of each sample to the DNA plate (4titude, 96 Round Deep Well Storage Microplate for Magnetic Separators; 1.0 ml round wells, V-shaped bottom, clear PP, cat. number 4ti-0125).
4. Add tips and plates for the DNA extraction to the OT-2. Spin down and add the DNA plate.
5. Ensure all reagents are properly mixed. Transfer reagents to the reservoir (Beads/buffer C in well 1, Ethanol in well 3 and 4, and EBT in well 6).
6. Control offsets and begin OT-2 run.
7. When prompted: Incubate the DNA plate for 15 minutes at 10°C with shaking at 1500 rpm. Remember to cover the plate before moving from OT-2. Spin down the DNA plate before returning the DNA plate to OT-2.
8. Switch on the Thermo Mixer: set to 5 minutes, 25 °C, 1500 rpm.
9. When prompted: Incubate DNA plate: 5 minutes at 25 °C with shaking at 1500 rpm.
10. Spin down the DNA plate shortly at 1000 g. Remember to cover the plate before moving from OT-2. Spin down the DNA plate before returning the DNA plate to OT-2.
11. Place the DNA plate on a magnetic rack and wait until the supernatant is clear.
12. Aspirate slowly and transfer the supernatant with eluted DNA to a new strip/plate.

Completion

1. Measure the DNA (and/or RNA) concentration by Qubit HS Assay using 2 µL DNA extract.

Fragmentation

Materials & Consumables

List of consumables used in the OT-2. Pipetting and plastic ware outside of the robot is not included. Assuming 96 samples are worked with.

Item	Amount	Cat. Number	Supplier
200 µL Filter Tips	1		Opentrons
10 µL Filter Tips	1		Opentrons
Covaris Plate	1	520291	Covaris
2 mL Eppendorf Tube	2		
Skirted PCR Plate*	2 (1)	ISTSIST-601-096GCT	VWR
LVL XSX200*	1 (0)	2DNC-X02-BL-NS-SLC-S	LVL technologies
PCR Strips/tubes/plate*	1 (0)		

*Only 1 is needed. Input plate not included

Manual Work and Preparation

Preparation

- At <https://alberdilab-opentronsscripts.onrender.com/> you can find a template to enter DNA concentration and other sample information.
 - The template information feed the fragmentation and subsequent library building protocol information regarding normalisation and adaptor concentration needed.
- Upload template at <https://alberdilab-opentronsscripts.onrender.com/> to create the library building related files.

DNA shearing

- Transfer sterile dH₂O for dilutions to 1x 2 mL tube for less than 72 samples or 2x ml tubes for 72 or more samples
- Turn on OT-2. Clean with 5% Bleach and 70% ethanol. Add labware.
- Control offsets and begin OT-2 run.
- Seal the Covaris plate. Quick spin the plate to remove air bubbles and collect samples.
- Run DNA fragmentation using a 96 Covaris, 105 103 S for 350 bp fragments.
- Transfer to a new PCR plate for library building. Store at -20°C if not continued straight away.

Library Building

Materials & Consumables

List of consumables used in the OT-2. Pipetting and plastic ware outside of the robot is not included. Assuming 96 samples are worked with.

Item	Amount	Cat. Number	Supplier
10 µL Filter Tips	4		Opentrons
PCR strips	4		

Input plate not included

Manual Work and Preparation

BEST library preparation protocol (Manual part)

1. Prepare mastermixes for the end-repair, ligation, and fill-in (See below; Gently Mix). Spin down mastermixes before use.
2. Thaw adaptors. Transfer into PCR tubes for 2Mm, 5 Mm, 10 Mm, and 20 Mm.
3. Setup OT-2.
4. Begin
5. When done: Store at -20° C if the magnetic beads purification is not performed on the same day.

End-repair reaction

1. Mix the master mix by pipetting and spin it down.

Reagent	Initial Conc.	Final Conc.	N reactions		
			1	N	
T4 DNA ligase buffer	10X	1X	3.00		µl
dNTPs	25 mM each	0.25 mM each	0.30		µl
T4 PNK	10 U/µl	7.5 U/rxn	0.75		µl
T4 DNA polymerase	3 U/µl	0.9 U/rxn	0.30		µl
Reaction enhancer***			1.50		µl
Total			5.85		µl

Reaction Enhancer:

Reaction Enhancer***	Initial Conc.	Final Conc.	Volume	
PEG 4000 50%	50% (w/v)	25% (w/v)	500	µl
BSA	20 mg/ml	2 mg/ml	100	µl

NaCl	5M	400 mM	80	μl
ddH ₂ O			320	μl
Total			1000	μl

Ligation reaction

2. Mix the master mix by pipetting and spin it down.

Reagent	Initial Conc.	Final Conc.	N reactions		
			1	N	
T4 DNA ligase buffer	10X	0.2X	0.75		μl
T4 DNA ligase	400 U/μl	300 U/rxn	0.75		μl
PEG 4000 50%	50%	6%	4.5		μl
Total			6.00		μl

Fill-in reaction

3. Mix the master mix by pipetting and spin it down.

Reagent	Initial Conc.	Final Conc.	N reactions		
			1	N	
Isothermal buffer	10X	0.33X	1.5		μl
dNTPs	25 mM	0.33 mM	0.6		μl
Bts 2.0 WarmStart pol.	8 U/μl	9.6 U/rxn	1.2		μl
ddH ₂ O			4.2		μl
Total			7.50		μl

Library Purification

Materials & Consumables

List of consumables used in the OT-2. Pipetting and plastic ware outside of the robot is not included. Assuming 96 samples are worked with.

Item	Amount	Cat. Number	Supplier
200 µL Filter Tips	8		Opentrons
10 µL Filter Tips	1		Opentrons
22 mL reservoir	1	11311974	Fisher Scientific
Skirted PCR Plate*	1 (0)	ISTSIST-601-096GCT	VWR
LVL XSX200*	1 (0)	2DNC-X02-BL-NS-SLC-S	LVL technologies
PCR Strips/tubes/plate*	1 (0)		

*Only 1 is needed. Input plate not included.

Manual Work and Preparation

BEST library purification protocol

1. Equilibrate the beads (MagBio or SPRI) to room temperature for 30 min.*
2. Prepare reagents

	Each sample	Number samples: 48 (52.8) (required plus 10%) (µL)	Volume Tube
Beads (Spri or MagBio)	75 µl	3920**	5 mL
80% EtOH - Final wash	400 µl	21120	25 mL
Elution buffer	50 µL	2640**	5 mL

** Always ensure the reagent covers the bottom of reservoir at low sample count

4. Turn on HEPA filter unit and OT-2. Clean OT-2 with 5% Bleach and 70% ethanol. Add labware
5. Ensure the sample plate has entirely thawed. Vortex and centrifuge/spin down the samples. Add to the magnet module.
6. Ensure all reagents are properly mixed. Transfer reagents to the reservoir (Beads well 1, Ethanol in well 3 and 4, and EBT in well 6).
7. Control offsets and begin OT-2 run.
8. When prompted: Incubate the plate for 10 minutes at 37°C. Remember to cover the plate before moving from OT-2. Spin down the DNA plate before returning the DNA plate to OT-2.
9. Prepare 1:20 dilution of samples for qPCR quality control in either a PCR plate or PCR strips.
10. Store in a freezer at -20° C until the next steps of library preps.
11. *The silica magnetic beads (both MagBio or SPRI) are stored at 4°C. Do not freeze.

qPCR

Materials & Consumables

List of consumables used in the OT-2. Pipetting and plastic ware outside of the robot is not included. Assuming 96 samples are worked with.

Item	Amount	Cat. Number	Supplier
200 µL Filter Tips	8		Opentrons
10 µL Filter Tips	1		Opentrons
qPCR strips	12	B72711	BIOplastics
qPCR Caps	12	B57801	BIOplastics

*Only 1 is needed. Input plate not included

Manual Work and Preparation

Preparations

1. Create master mix on a cooling block

Reagent	Initial Conc.	Final Conc.	N reactions		
			1	N	
10x PCR Gold buffer	10x	1	2.5		µl
MgCl ₂ Solution	25 mM	2.5	2.5		µl
dNTPs Mix	10 mM each	0.08	0.2		µl
Forward (F) Primer	10 µM	0.4	1		µl
Reverse (R) Primer	10 µM	0.4	1		µl
Sybr Green	-	-	1		µl
AmpliTaq GOLD DNA polym	5U/µl	2.5	0.5		µl
H ₂ O	-	-	14.3		µl
Total (W/O DNA)			23		µl
DNA	-	-	2	-	µl
Total			25	-	µl

2. Mix the mastermix well and spin it down. Transfer to master to a PCR strip.
3. Turn on HEPA filter unit and OT-2. Clean OT-2 with 5% Bleach and 70% ethanol. Add labware, including mastermix.
4. Ensure the 1:20-diluted purified libraries have entirely thawed. Vortex and centrifuge/spin down the samples.
5. Control offsets and begin OT-2 run.

Run qPCR

1. Vortex and spin down qPCR reactions
2. Set up qPCR program (see below)

QPCR Program

Step	Repetitions	Temperature	Time	Measurement
1	1 Cycle	95°C	12 min	
2a	40 Cycles	95°C	20 sec	
2b		60°C	30 sec	Yes (Single)
2c		72°C	40 sec	
3	1 Cycle	Dissociation Curve	-	Yes (Dis Curve)
4	Hold	4°C	-	

3. Export and analyse results

Index PCR

Materials & Consumables

List of consumables used in the OT-2. Pipetting and plastic ware outside of the robot is not included. Assuming 96 samples are worked with.

Item	Amount	Cat. Number	Supplier
200 µL Filter Tips	8		Opentrons
10 µL Filter Tips	1		Opentrons
Deep Well Plate	1	4TI-0125	Saveen&Werner ApS
22 mL reservoir	1	11311974	Fisher Scientific
Skirted PCR Plate*	1 (0)	ISTSIST-601-096GCT	VWR
LVL XSX200*	1 (0)	2DNC-X02-BL-NS-SLC-S	LVL technologies
PCR Strips/tubes/plate*	1 (0)		

*Only 1 is needed. Input plate not included

Manual Work and Preparation

FAQ

External problems and questions can be directed to:
Frequent problems and questions are:

Version

1.1 – Incorporation of protocols
1.0 – Basic Layout