Standard Operational Procedure

Opentrons OT2 Protocol - Template

Version 1.0.0

The following Standard Operational Procedure (SOP) outlines the procedure for working with the Opentrons OT2 Template protocol. The SOP contains the primary information regarding safety, startup, workflow, and FAQ. 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 a summary of Opentrons OT-2 guidelines and “Safety and Regulatory Compliance Information”, which can be examined for further details.

## Hardware

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 Alberdi Lab include automatic “set\_rail\_lights” to automatically switch lights on during runs and off when completed to indicate an active robot. The Opentrons offers “[Auto-stop](https://support.opentrons.com/s/article/Pause-a-protocol-when-the-robot-door-opens)” function to automatically pause work, however, this feature is not turned on for the AlberdiLab, as the auto pause is prone to cause error for “smart labware”, in particular the thermocycler unit, potentially resulting in automation runs crashing.

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 to switch off power to the unit while working with it. 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, turn 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 the 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 a decrease in temperature.

## Chemicals

At the AlberdiLab / within the KU, the 3 chemicals **Guanidine Thiocyanate**, **Iso(-2-)propanol**, and **Ethanol** are all associated with risk. As such please refer to the local guidelines and risk assessment for proper handling and actions of these chemicals during laboratory work.

# Manual Work and Preparation

# Opentrons Setup and Preparation

## DNA extraction protocol

### Materials & Reagents (not complete)

Materials

|  |  |  |
| --- | --- | --- |
| Amount | Item | Catalog number |
| 1 | thermoscientificnunc\_96\_wellplate\_1300ul |  |
| 12x  OR 1x  OR  1X | Generic pcr strip (In aluminium block)  OR LVLXSX200\_wellplate\_200ul  OR  biorad\_96\_wellplate\_200ul\_pcr |  |
| 1X | deepwellreservoir\_12channel\_21000ul |  |
| 8X | opentrons\_96\_filtertiprack\_200ul |  |

Reagents

|  |  |
| --- | --- |
| Spri Beads | - |
| Absolute Ethanol |  |
| EBT |  |
| Buffer C |  |
| Tris-EDTA (TE) buffer |  |

### 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: 48 (52.8) (required plus 10%) (µL) | Volume Tube |
| Beads - DNA fraction | 15 µl | 792 | 2 mL |
| Buffer C | 200 µL | 10560 | 15 mL\*\* |
| 80% EtOH - Final wash | 400 µl | 21120 | 25 mL |
| Elution buffer\*\* | 50 µL | 2640 | 5 mL |

1. 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.
2. Discard the clear supernatant.
3. 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.
4. Discard the supernatant.
5. Repeat steps 6 and 7.
6. 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 dead volume must be added to the reservoir in the automated protocol such that the bottom is covered.

### 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 the DNA plate and add it to the OT-2.
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 10ºC 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. Set the Thermo Mixer: 5 minutes, 25 C, 1500 rpm.
9. When prompted: Incubate DNA plate: 5 minutes at 25 ºC 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.
13. Measure the DNA (and/or RNA) concentration by Qubit HS Assay using 2 µL DNA extract.

## DNA Shearing and Library Preparation

### DNA shearing

Materials

|  |  |  |
| --- | --- | --- |
| Amount | Item | Catalog number |
| 12x  OR 1x  OR  1x | Generic pcr strip (In aluminium block)  OR LVLXSX200\_wellplate\_200ul  OR  biorad\_96\_wellplate\_200ul\_pcr |  |
| 1x | Covaris plate |  |
| 1x | Opentron Rack |  |
| 2x\* | eppendorf\_2ml\_safelock\_ |  |
| 1x | opentrons\_96\_filtertiprack\_200ul |  |
| 1x | opentrons\_96\_filtertiprack\_10ul |  |

\*Varies dependent on total number of samples

Reagents

|  |  |
| --- | --- |
| H2O (sterile, Nuclease Free) | - |

1. Upload qubit concentration, subsequent normalisation and adaptor values through a CSV file at <https://alberdilab-opentronsscripts.onrender.com/> [Template available] to create the library building files.
2. Transfer sterile dH2O for dilutions to 1x 2 mL tube for less than 72 samples or 2x ml tubes for 72 or more samples
3. Turn on OT-2. Clean with 5% Bleach and 70% ethanol. Add labware.
4. Control offsets and begin OT-2 run.
5. Once completed, seal the Covaris plate. Spin the plate to remove air bubbles and collect samples.
6. Run DNA fragmentation using a 96 Covaris, 103 S for 350 bp fragments.
7. Transfer to a new PCR plate for library building. Store at -20 if not continued straight away.

### BEST library preparation protocol (Manual)

Materials

|  |  |  |
| --- | --- | --- |
| Amount | Item | Catalog number |
| 12x  OR 1x  OR  1x | Generic pcr strip (In aluminium block)  OR LVLXSX200\_wellplate\_200ul  OR  biorad\_96\_wellplate\_200ul\_pcr |  |
| 1x | Covaris plate |  |
| 1x | Opentron Rack |  |
| 2x\* | eppendorf\_2ml\_safelock\_ |  |
| 1x | opentrons\_96\_filtertiprack\_200ul |  |
| 1x | opentrons\_96\_filtertiprack\_10ul |  |

\*Varies dependent on total number of samples

1. Prepare mastermixes for the end-repair, ligation, and fill-in (Gently Mix). Spin down mastermixes before use.
2. Thaw adaptors.
3. Setup OT-2. Upload script file including the CSV file to ensure the samples and their appropriate adaptor concentration are correctly aligned.
4. Begin Run.
5. When done: Store at -20° C if the magnetic beads purification is not performed on the same day.

#### End-repair reaction

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Reagent | Initial Conc. | Final Conc. | N reactions | |  |
|  |  |  | 1 |  |  |
| 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 |

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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **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 |
| ddH2O |  |  | 320 | µl |
| Total |  |  | 1000 | µl |

#### Ligation reaction

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Reagent** | **Initial Conc.** | **Final Conc.** | **N reactions** | |  |
|  |  |  | 1 |  |  |
| 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 |

Adaptors needed: 10 mM: 20 mM

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

#### Fill-in reaction

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Reagent | Initial Conc. | Final Conc. | N reactions | |  |
|  |  |  | 1 |  |  |
| 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 |
| ddH2O |  |  | 4,2 | 221.76 | µl |
| Total |  |  | 7,50 | 396 | µl |

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

## 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

1. Turn on HEPA filter unit and OT-2. Clean OT-2 with 5% Bleach and 70% ethanol. Add labware
2. Ensure the sample plate has entirely thawed. Vortex and centrifuge/spin down the samples. Add to the magnet module.
3. 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).
4. Control offsets and begin OT-2 run.
5. 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.
6. Prepare 1:20 dilution of samples for qPCR quality control in either a PCR plate or PCR strips.
7. Store in a freezer at -20° C until the next steps of library preps.

## QPCR

Materials

|  |  |  |
| --- | --- | --- |
| Amount | Item | Catalog number |
| 12x  OR 1x  OR  1x | Generic pcr strip (In aluminium block)  OR LVLXSX200\_wellplate\_200ul  OR  biorad\_96\_wellplate\_200ul\_pcr |  |
| 12x | 'bioplastics\_96\_aluminumblock\_100ul' |  |
| 1x | opentrons\_96\_aluminumblock\_generic\_pcr\_strip\_200ul |  |
| 1x | opentrons\_96\_filtertiprack\_200ul |  |
| 1x | opentrons\_96\_filtertiprack\_10ul |  |

\*Varies dependent on total number of samples

1. Create master mix on a cooling block

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Reagent | Initial Conc. | Final Conc. | N reactions | |  |
|  |  |  | 1 | 52.8 |  |
| 10x PCR Gold buffer | 10x | 1 | 2.5 | 132 | µl |
| MgCl2 Solution | 25 mM | 2.5 | 2.5 | 132 | µl |
| dNTPs Mix | 10 mM each | 0.08 | 0.2 | 10.56 | µl |
| Forward (F) Primer | 10 μM | 0.4 | 1 | 52.8 | µl |
| Reverse (R) Primer | 10 μM | 0.4 | 1 | 52.8 | µl |
| Sybr Green | - | - | 1 | 52.8 | µl |
| AmpliTaq GOLD DNA polym | 5U/μl | 2.5 | 0.5 | 26.4 | µl |
| H2O | - | - | 14.3 | 755.04 | µl |
| Total (W/O DNA) |  |  | 23 | 1214.4 | µl |
| DNA | - | - | 2 | - | µl |
| Total |  |  | 25 | - | µl |

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

\* Always include one negative control (QPCR water) and one positive control (POS)

3. Run qPCR

1. Vortex and spin down qPCR reactions
2. Set up qPCR program

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 | - |  |

1. Export and analyse results

## Index PCR Setup

Materials

|  |  |  |
| --- | --- | --- |
| Amount | Item | Catalog number |
| 12x  OR 1x  OR  1x | Generic pcr strip (In aluminium block)  OR LVLXSX200\_wellplate\_200ul  OR  biorad\_96\_wellplate\_200ul\_pcr |  |
| 1x | Covaris plate |  |
| 1x | Opentron Rack |  |
| 2x\* | eppendorf\_2ml\_safelock\_ |  |
| 1x | opentrons\_96\_filtertiprack\_200ul |  |
| 1x | opentrons\_96\_filtertiprack\_10ul |  |

\*Varies dependent on total number of samples

Reagents

|  |  |
| --- | --- |
| H2O | - |

# Version