Step-by-step manual for APEX Pipeline in OT-2

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Getting Started

This manual provides all the necessary information to set up and start using the APEX pipeline in OT-2. In E. coli, a basic protein expression workflow usually consists of 4 steps outlined below, therefore, we designed APEX in a modular fashion to mimic this structure, by implementing 4 self-containing modules describing an OT-2 protocol for each step.

Protocol 1 Heat-shock transformation to deliver DNA to the cell.

Protocol 2 Antibiotic selection on agar plates to select colonies carrying the DNA payload.

Protocol 3 Colony sampling of transformed cells.

Protocol 4 Induction of protein expression.

APEX requires a JSON file to configure the OT-2 hardware settings and a CSV file containing detailed liquid handling instructions for a specific experiment. These files provide the necessary data for setting up and executing laboratory protocols. These input files are then integrated into a master Python script, such that it can be directly imported into the robot through the Opentrons app. Simultaneously, APEX generates a PDF document with instriuctions for the protocol along visual representations of the labware layout, serving as a reference for users to set up their experiments. The pipeline is managed using Nextflow, a workflow manager that simplifies the running of complex computational pipelines. APEX is also fully containerized using Docker. This ensures that all software dependencies are neatly packaged, allowing the pipeline to be deployed and run consistently across any computational environment without the need for additional software installations.

System Requirements

- **Hardware:** Opentrons OT-2
- **Software:** Nextflow

Installing Java

Ensure that Java is installed and up-to-date on your system: Java 8 or later (Check `https://www.java.com/en/download/` for installation instructions). This is essential for running Nextflow.

Installing Nextflow

Open a terminal and execute the following command to download and install Nextflow:

```
curl -s https://get.nextflow.io | bash
```

Move the Nextflow executable to a directory in your PATH for easier execution, for example:

```
sudo mv nextflow /usr/local/bin
```

Verify the installation by checking the version:

```
nextflow -version
```

Downloading and Running the Pipeline

The APEX pipeline is available on GitHub at `http://github.com/stracquadaniolab/apex-nf`. Use the following commands to download the pipeline:

```
    nextflow pull stracquadaniolab/apex-nf
```

Alternatively, you can directly use the GitHub URL:

```
    nextflow pull http://github.com/stracquadaniolab/apex-nf
```

The project is downloaded to the follwoing local directory:

```
    $HOME/.nextflow/assets/stracquadaniolab/apex-nf
```

Use the following commands to run the full pipeline:

```
    nextflow run apex-nf
```

To run a specific protocol from the pipeline, for example Protocol 1, run the following command:

```
    nextflow run apex-nf -entry PROTOCOL_1
```

To review project details, check where it was downlaoded or check for updates, use the command:

```
    nextflow info apex-nf
```

Configuration and Setup

The pipeline is located in the ”assets” folder which is organised as following:

data Contains JSON and CSV files for protocol configurations and experimental data respectively.

instructions Contains R markdown files detailing the experiment setup instructions.

labware Contains JSON files detailing all supported labware. Here you can also add any custom labware definitions (for more information click here).

protocols Contains Python template files for each specific protocol.

results Contains the output files of the pipeline, that is ready-to-use python protocols and PDF instructions.

Input files

After downloading the necessary software, setting up your experiment with APEX is straightforward. Create a JSON and a CSV file that contain all the details for your experiment. The structure and content of these files are critical as they dictate the setup and execution of the experiment.
Once you have created these files, place them in the ”data” folder within the APEX system directory. Ensure that the files are named according to the following conventions:

- **JSON File**: Name your JSON file as protocol-*-config.json, where * represents the ID number of the protocol. For example, the JSON file for a transformation experiment would be named protocol-1-config.json.
- **CSV File**: Similarly, name your CSV file as protocol-*-data.csv. For the transformation example, this would be protocol-1-data.csv.

To aid in file creation, examples of both file types can be found at the provided at `http://github.com/stracquadaniolab/APEX/testdata`. These examples can serve as templates to ensure that your files are formatted correctly and contain all necessary information.

Understanding JSON Configuration Files

The JSON (JavaScript Object Notation) configuration file is used in the pipeline for setting up the Opentrons. This file includes the details about the hardware, such as types of pipettes available and layout of the Opentrons deck, specifying where each piece of labware should be placed.

Here’s an example JSON configuration file that outlines a simple setup for an Opentrons robot. For each protocol within the pipeline, an example JSON template will be provided under in this manual, with each parameter explained.

```
{
    "pipette_name":"p20_multi_gen2",
    "source_plate_name": " armadillo_96_wellplate_200ul_pcr_full_skirt",
    "msource_plate_slot":4
}
```

To create a JSON file, use a simple text editor like Notepad (Windows) or TextEdit (Mac, set to plain text mode). Type your JSON data and save the file with a '.json' extension by selecting 'Save As' from the File menu. Move the saved file to the "data" folder within the APEX project directory.

Understanding CSV Data Files

CSV (Comma-Separated Values) files are used to provide detailed data for operations like liquid transfers within the protocol. These files allow the user to specify the volume of liquid to be transferred, the source and destination wells among others. This allows users to easily modify experimental parameters without altering the underlying code.

Example of a CSV file:

```
source_well,destination_well,volume
A1,A2,50,
B1,C2,50,
```

To create a CSV file, use either a basic text editor or a spreadsheet program like Microsoft Excel. Enter data, ensuring each field is separated by a comma without spaces. Save the file with a '.csv' extension, selecting 'CSV (Comma delimited) (*.csv)' if using a spreadsheet program. Move the saved file to the "data" folder within the APEX project directory.

Output files

After your run the pipeline, all the outputs will be saved in the folder "results". Output files are named according to the following conventions:

- **Python protocol**: protocol-*.py, where * represents the ID number of the protocol. For example, the Python protocol for a transformation experiment would be named protocol-1.py.
- **PDF instrcutions**: protocol-*.pdf. For the transformation example, this would be protocol-1.pdf.

Protocol 1: Heat shock Transformations

Design

Use the JSON (protocol-1-config.json) and CSV (protocol-1-config.csv) files to create a protocol tailored for heat shock transfromations. Define parameters as described below in 1 and 2.

Parameter	Description	Example
right_pipette_name	model of the right pipette	"p20_multi_gen2"
right_pipette_tiprack_name	right pipette's tip racks	"opentrons_96_tiprack_20ul"
right_pipette_tiprack_slot	deck slot(s) of the right pipette's tip racks	[2, 3]
left_pipette_name	model of the left pipette	"p300_multi_gen2"
left_pipette_tiprack_name	left pipette's tip racks	"opentrons_96_tiprack_20ul"
left_pipette_tiprack_slot	deck slot(s) of the right pipette's tip racks	[6, 9]
dna_plate_name	labware containing DNA	"armadillo_96_wellplate_200ul_pcr_full_skirt"
dna_plate_slot	deck slot of dna_plate_name	1
cells_plate_name	labware containing competent cells	"armadillo_96_wellplate_200ul_pcr_full_skirt"
cells_plate_slot	deck slot of cells_plate_name	1
media_plate_name	labware containing media	"armadillo_96_wellplate_200ul_pcr_full_skirt"
media_plate_slot	deck slot of media_plate_name	1
destination_plate_name	labware where transformations occur (compatible with thermocycler if thermocycler used)	"armadillo_96_wellplate_200ul_pcr_full_skirt"
destination_plate_slot	deck slot for destination_plate_name (use "thermocycler" is using OT-2 thermocycler, otherwise deck number)	"thermocycler"
pre_shock_incubation_temp	pre heat shock cooling incubation temperature (°C)	4
pre_shock_incubation_time	pre heat shock cooling incubation duration (minutes)	20
heat_shock_temp	heat shock temperature (°C)	42
heat_shock_time	heat shock duration (seconds)	30
post_shock_incubation_temp	post heat shock incubation temperature (°C)	4
post_shock_incubation_time	post heat shock cooling incubation duration (minutes)	2
recovery_temp	recovery incubation temperature (°C)	37
recovery_time	recovery incubation duration (minutes)	60

Table 1: JSON file configuration parameters for Protocol 1

Preparation

Prepare the required labware including plasmid DNA, competent *E. coli* cells, and recovery media. Ensure to add extra volume to each well to allow for precise volume aspiration by the robot. It is advisable to include a positive control plasmid that carries the appropriate antibiotic resistance marker, as well as a negative control (no DNA). Keep the cells chilled on ice until instructed by the

Parameter	Description	Example
dna_id	identifier for the DNA sample	pUC19
dna_well	well location of the DNA in dna_plate_name (specify ”NA” when adding no DNA)	A1
dna_volume	volume of DNA used for transformation (specify 0 when adding no DNA) (µL)	1
cells_id	identifier for the competent cells	DH5a
cells_well	well location of the competent cells on the cells_plate_name from JSON file	A2
cells_volume	volume of competent cells to be transformed (µL).	10
media_id	identifier for the recovery media	SOC
media_well	well location of the media_plate_name	A3
media_volume	volume of media used for recovery (µL)	50
destination_well	well location of transformation on the destination_plate_name	A1

Table 2: CSV file configuration data for Protocol 1

robot interface to place the labware on the robot deck. If the same labware is to be used for multiple reagents, simply repeat the labware name and deck slot in the configuration JSON file.

Example Execution

Step 1: APEX automatically selects which pipettes to use for each transfer, based on available pipettes and required volumes.

Step 2: APEX pre-cools the thermocycler to $4\,^{\circ}\mathrm{C}$. Once the temperature is reached, the thermocycler opens and pauses to prompt the user to insert the destination plate into the thermocycler and places the labware containing competent cells/DNA/media onto the deck.

Step 3: APEX gently resuspends the cells once and distributes $10\,\mu\mathrm{L}$ into the destination plate.

Step 4: APEX transfers $1\,\mu\mathrm{L}$ DNA into the corresponding wells, mixing gently once. After a blowout, the pipette descends again to add any droplet left at the tip to the well.

Step 5: The thermocycler then closes and incubates the cells at $4\,^{\circ}\mathrm{C}$ for 30 minutes, followed by a heat shock at $42\,^{\circ}\mathrm{C}$ for 30 seconds, and then a further incubation at $4\,^{\circ}\mathrm{C}$ for 2 minutes.

Step 6: After reopening, the robot adds $50\,\mu\mathrm{L}$ per well of SOC media, resuspending the transformed cells twice in the destination plate.

Step 7: Finally, the thermocycler heats up to $37\,^{\circ}\mathrm{C}$ and incubates the cells for 60 minutes.

Protocol 2: Colony selection

Design

Use the JSON (protocol-2-config.json) and CSV (protocol-2-config.csv) files to create a protocol tailored for colony selection via selective agar plate spotting. Define parameters as described below in 3 and 4.

Parameter	Description	Example
pipette_name	model of the pipette	"p20_multi_gen2"
pipette_mount	side of the robot the pipette is mounted on	"left" or "right"
tiprack_name	pipette's tip racks	"opentrons_96_tiprack_20ul"
tiprack_slots	deck slot(s) of the pipette's tip racks	[5, 6]
source_plate_name	labware containing the source material, i.e., transformed cells.	"armadillo_96_wellplate_200ul_pcr_full_skirt"
source_plate_slot	deck slot of source_plate_name (if using the OT-2 thermocycler, specify "thermocycler", otherwise the deck slot)	1
agar_plate_name	labware for the agar plate	"nunc96grid_96_wellplate_10ul"
agar_plate_slot	deck slot(s) of agar_plate_name	1
agar_plate_area	the base area of agar_plate_name well (mm^2)	9469.2
empty_agar_plate_weight	the weight of the empty agar_plate_name without agar (g)	[38.92]
agar_plate_weight	the weight of the agar_plate_name with agar (g)	[68.92]
agar_density	agar density (g cm^{-3})	0.911
spotting_volume	volume to be spotted (µL)	5
additional_volume	additional volume that is added to the spotting volume but not dispensed (µL)	1
spotting_height	additional height relative to the agar surface for spotting (at value 0 the pipette tip will be right at the height of the agar; a negative value will lower the spotting height) (mm)	0.5

Table 3: JSON file configuration parameters for Protocol 2 (prtocol-2-config.json)

Parameter	Description	Example
id	identifier of the sample	pUC19-DH5a
agar_plate_location	deck slot(s) of agar_plate_name	1
source_well	well location on source_plate_name with transformed cells to be aspired	A1
destination_well	well location on agar_plate_name where the transformed cells are spotted	A1
spotting_volume	volume of cells to be spotted	5

Table 4: CSV file configuration data for Protocol 2 (prtocol-2-data.csv)

Preparation

Prepare LB agar plates containing the appropriate selection markers and allow them to solidify. We recommend pouring the agar at approximately $50\,^{\circ}\mathrm{C}$ on a leveled bench to ensure consistent distribution. After pouring, allow the agar to solidify at room temperature for 15 minutes before transferring the plates to a flow hood for drying. Drying the plates for approximately 40 minutes in the flow hood is essential to prevent excessive diffusion of the spotted transformed cells. Record the weights of the empty plate(s) before puring agar and the weights of these plates with agar, ensuring that the order of the list is preserved.

Example Execution

Step 1: APEX calculates the agar weight and determines the height of the agar. Adjust the spotting height parameter in the configuration JSON file to modify the height of spotting relative to the agar surface. Verify the precise plate well depth and agar height using the attached Jupyter notebook.

Step 2: APEX picks up a new tip for each transfer and resuspends the cells twice. To spot the same sample multiple times without changing tips, list the destination wells in the source field using the ”—” symbol. For instance, `A1|A2` directs APEX to dispense aliquots from the source well into both wells A1 and A2.

Step 3: APEX aspirates $5\,\mu\mathrm{L}$ of outgrowth followed by an additional $1\,\mu\mathrm{L}$. This approach is preferred over using a blowout to prevent sample dispersion and to achieve more precise volume dispensation. After dispensing, the protocol includes a 5-second delay to ensure smooth transfer of the sample.

Step 4: Allow the spotted plates to dry for a few minutes, or until the spots are visibly dry, before transferring them from the deck for incubation at $37\,^{\circ}\mathrm{C}$.

Protocol 3: Colony Sampling

Design

Use the the JSON (protocol-3-config.json) and CSV (protocol-3-data.csv) files to create a protocol tailored for colony sampling. Define parameters as described below in Table 5 and Table 6.

Parameter	Description	Example
right_pipette_name	model of the right pipette	"p20_single_gen2"
right_pipette_tiprack_name	right pipette's tipracks	"opentrons_96_tiprack_20ul"
right_pipette_tiprack_slot	deck slot(s) of right_pipette_tiprack_name	[5]
left_pipette_name	model of the left pipette	"p300_multi_gen2"
left_pipette_tiprack_name	left pipette's tipracks	"opentrons_96_tiprack_300ul"
left_pipette_tiprack_slot	deck slot(s) of left_pipette_tiprack_name	[6]
media_plate_name	labware containing media	"usascientific_96_wellplate_2.4ml_deep"
media_plate_slot	deck slot of media_plate_name	2
destination_plate_name	labware where cells will be propagated	"usascientific_96_wellplate_2.4ml_deep"
destination_plate_slot	deck slot of destination_plate_name	3
agar_plate_name	labware with agar containing colonies	"armadillo_96_wellplate_200ul_pcr_full_skirt"
agar_plate_slot	deck slot(s) of agar_plate_name labwares	[1]
agar_plate_area	the base area of agar_plate_name well (mm^2)	9469.2
empty_agar_plate_weight	the weight of the empty agar_plate_name without agar (g)	[38.92]
agar_plate_weight	the weight of the agar_plate_name with agar (g)	[68.92]
agar_density	agar density ($g\ cm^{-3}$)	0.911
agar_pierce_depth	additional depth relative to the agar surface for sampling (at value 0 the pipette tip will be right at the height of the agar; a negative value will pierce the agar) (mm)	-0.5
sampling_method	method used for sampling colonies from the agar plate	"spiral"
spiral_radius	radius of the spiral path used during sampling (mm)	2.5
spiral_points	number of discrete points along the spiral path	25
spiral_rotations	number of full rotations the pipette makes to complete the spiral path	3

Table 5: JSON configuration data details for Protocol 3 (protocol-3-config.json)

Parameter	Description	Example
colony_id	identifier of the colony	pUC19-BL21(DE3)
agar_plate_location	deck slot(s) of agar_plate_name	1
colony_well	well location of colony on agar_plate_name	A1
media_id	identifier of the media	LB-kan
media_well	well location on media_plate_name	A1
media_volume	volume of the media transferred from media_source_well to destination_well (µL)	500
destination_well	well location on destiantion_plate_name	A1

Table 6: CSV file configuration data for Protocol 3 (protocol-3-data.csv)

Preparation

Record the weights of the empty plate(s) and those containing agar with developed colonies. Update these values in the configuration JSON file, ensuring that the order of the list is preserved. Based on your experimental needs, decide on a colony sampling strategy. For high colony densities, you can use a single pierce method; for lower densities, consider using a spiral sampling method and adjust the parameters to design the appropriate spiral path. If preparing media plates, you can either prepare a plate containing media supplemented with antibiotics to be transferred into the destination plate or use a pre-distributed destination plate with media. If using a pre-distributed plate, ensure the names and slots on the media plate match those of the destination plate and set the media volume to ”0”.

Example Execution

Step 1: APEX automatically selects which pipettes to use for each transfer, based on available pipettes and required volumes.

Step 2: APEX distributes $500\,\mu L$ of LB media supplemented with appropriate antibiotics into the destination plate.

Step 3: For chosen spiral sampling method, APEX moves the pipette to the center of a pre-selected virtual well containing colonies and follows a spiral path to sample the colonies.

Step 4: APEX transfers the sampled cells into a designated well on the destination plate with media by mixing it twice.

Step 5: After sampling all chosen wells with colonies, the destination plate is moved for shaking incubation at $37\,^{\circ}C$.

Protocol 4: Protein Expression Induction

Design

Use the JSON (protocol-4-config.json) and CSV (protocol-4-data.csv) files to create a protocol tailored for protein expression induction. Define parameters as described below in Table 7 and Table 8.

Parameter	Description	Example
right_pipette_name	model of the right pipette	"p20_single_gen2"
right_pipette_tiprack_name	right pipette's tipracks	"opentrons_96_tiprack_20ul"
right_pipette_tiprack_slot	deck slot(s) of right_pipette_tiprack_name	[5]
left_pipette_name	model of the left pipette	"p300_multi_gen2"
left_pipette_tiprack_name	left pipette's tipracks	"opentrons_96_tiprack_300ul"
left_pipette_tiprack_slot	deck slot(s) of left_pipette_tiprack_name	[6]
media_plate_name	labware containing media	usascientific_96_wellplate_2.4 ml_deep
media_plate_slot	deck slot of media_plate_name	3
culture_plate_name	labware containing cultures	usascientific_96_wellplate_2.4 ml_deep
culture_plate_slot	deck slot of culture_plate_name	1
inducer_plate_name	labware containing chemcial inducer	armadillo_96_wellplate_200ul_p cr_full_skirt
inducer_plate_slot	deck slot of inducer_plate_name	3
destination_plate_name	labware were the the fresh culture will be porpagated	usascientific_96_wellplate_2.4 ml_deep
destination_plate_slot	slot deck of destination_plate_name	2

Table 7: JSON configuration data details for Protocol 4 (protocol-4-config.json)

Parameter	Description	Example
culture_id	identifier of the culture sample	pUC19-BL21(DE3)
culture_well	well location of culture_id on culture_plate_name	A1
culture_volume	volume of culture_id to be propagated in fresh media (µL)	5
media_id	identifier of media	fresh-LB
media_well	well location on media_plate_name	A1
media_volume	volume of media_id to be distibuted into destination_plate_name (µL)	500
inducer_id	identifier of chemcial inducer	0.1mM-IPTG
indcuer_well	well location on inducer_plate_name (specify "NA" when adding no inducer)	A1
inducer_volume	volume of indcuer_id to be transfered into destination_plate_name (µL) (specify 0 when adding no inducer)	5
destination_well	well location on destination_plate_name where cells will be propagated	A1

Table 8: CSV file configuration data for Protocol 4 (protocol-4-data.csv)

Preparation

Prepare a plate containing media with antibiotics to be distributed into the destination plate, or alternatively, prepare a pre-distributed destination plate with media. If using a pre-distributed plate, ensure that the names and slots on the media plate match those of the destination plate and set the media volume to ”0”. Prepare the inducing agent solution and distribute it into the inducer plate. Prepare the plate with overnight cultures.

Example Execution

Step 1: APEX automatically selects which pipettes to use for each transfer, based on available pipettes and required volumes.

Step 2: APEX distributes $500\,\mu$L of LB media supplemented with appropriate antibiotics into the destination plate.

Step 3: APEX aliquots $5\,\mu$L of overnight cultures using a new tip each time and inoculates the fresh media, mixing twice.

Step 4: The protocol pauses to allow for incubation: Incubate the plate with shaking at $37\,^{\circ}$C and grow culture to $OD_{600} = 0.6 - 0.8$

Step 5: Upon reaching the desired OD_{600}, resume the protocol. APEX adds $50\,\mu$L of IPTG to each culture to induce protein expression.

Step 6: Continue the incubation of the cultures with shaking at $18\,^{\circ}$C to facilitate protein production.