

Massively Parallel Qubit DNA Quantification Using OpenTrons Version 3

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Abstract

This is a protocol for using the OpenTrons, an automated pipetting robot, to transfer Qubit solution and samples to use on the Qubit Fluorometer.

Here, we use OpenTrons to prepare 96 samples for qubit measurement. This protocol also saves time by requiring only one initial calibration for each container.

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Guidelines

Brief Overview:

Measurement of DNA can be done using two broad methods (Spectrophotometric or Fluorometric).

Qubit is a fluorometric method and uses a double stranded DNA specific dye. Therefore the sensitivity is higher and impurities like RNA or nucleotides in the sample will not affect the quantification as it would in spectrophotometric methods like a Nanodrop.

The Qubit is first calibrated using two standards. To make a measurement, 199ul of buffer +1ul of dye + 1ul of sample is added to a qubit tube, which is inserted into the instrument.

Here, we use OpenTrons to prepare 96 samples for qubit measurement. It also saves time by requiring only one initial calibration for each container.

Please see the flowchart of the protocol below.

Materials (per sample):

Qubit dsDNA HS Assay Kit (1)

Invitrogen catalog number: Q32851

Qubit Assay Tube (1)

Invitrogen catalog number: Q32856

Run Samples Placed in Strip Tubes

Instruments:

Qubit Fluorometer (1)

Invitrogen catalog number: Q32866

14ml Falcon Tube (1)

Alkali Scientific catalog number: CT5250

14ml Tube Rack (1)

96-well Qubit Tube Rack (1-2)

96-well Strip Tube Rack (1)

1000µl Tips

Fisher catalog number: 13-611-126

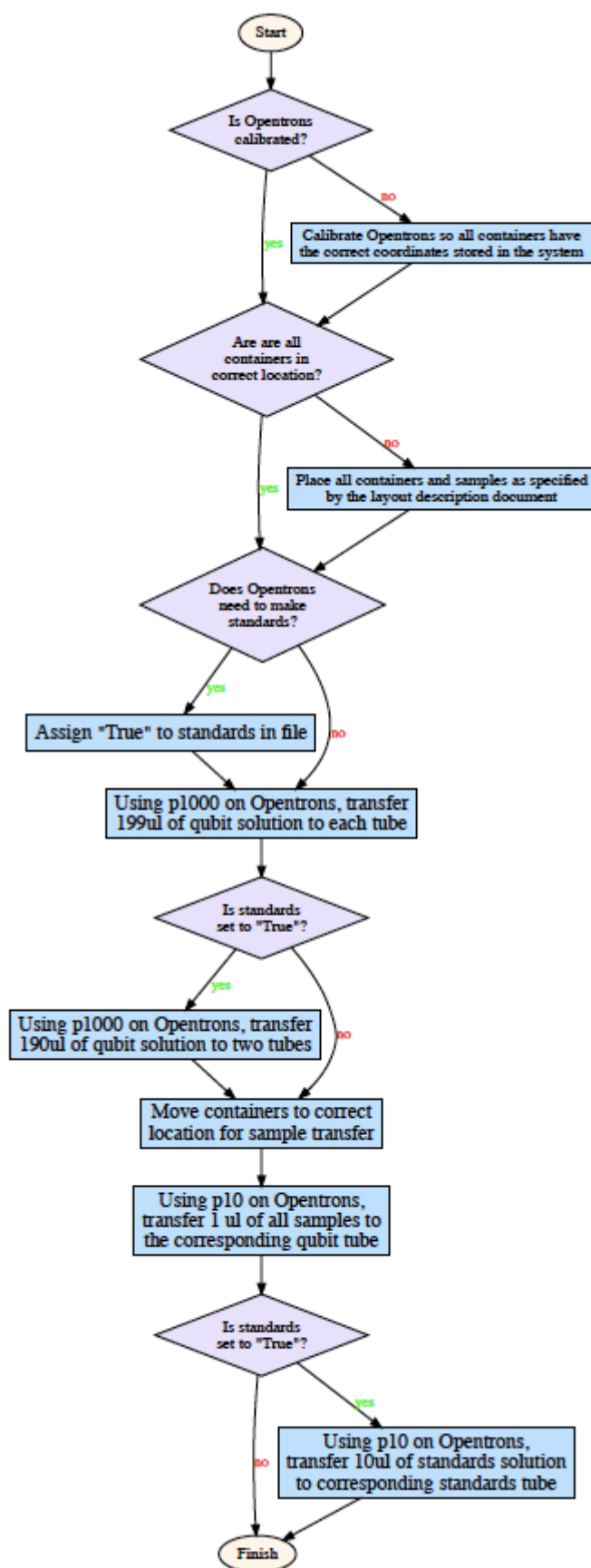
10µl Tips

Eppendorf catalog number: 022491504

OpenTrons Pro (1)

Link to download graphviz code to generate this flowchart:

[Graphviz Dot File](#)



Materials

Qubit™ dsDNA HS Assay Kit [Q32851](#) by [Invitrogen - Thermo Fisher](#)

Qubit™ Assay Tubes [Q32856](#) by [Invitrogen - Thermo Fisher](#)

Qubit Fluorometer [Q32866](#) by [Invitrogen - Thermo Fisher](#)

14ml Polystyrene Cell Culture Tubes [CT5250](#) by [Alkali Scientific](#)

1000µL Basix™ Universal Pipette Tips [13-611-126](#) by [Fisher Scientific](#)

10µl Pipette Tips [022491504](#) by [Eppendorf](#)

OT-One Pro [View](#) by [Opentrons](#)

Protocol

Before Start

Step 1.

Before starting, please see the [Guidelines](#) for the flowchart and more information.

Set Up

Step 2.

Plug the OpenTrons into the usb port of the computer and turn on the robot (you should see a blue light).

Set Up

Step 3.

Open the OpenTrons App in Computer.

Set Up

Step 4.

Select the correct port in the drop down menu on the upper right hand corner of the app.

Set Up

Step 5.

Click 'Yes' when asked to Home the robot.

Set Up

Step 6.

Find the folder of the necessary protocol (i.e. documents/opentrons/qubit folder).

Set Up

Step 7.

Set up containers according to the layout_description and use the text description for the Transfer Qubit Solution page. See picture as reference.

Important things to note:

- When arranging qubit tubes, leave the first column blank and start on the second column, skipping a column in between every tip/tube (see picture). Skip a row in between every tube to leave room for open caps.
- If OpenTrons is needed to make standards, set two empty qubit tubes in a new row behind the last row of samples.

Transfer Solution to Qubit Tubes

A1: 1000ul tiprack

A2: Qubit tube container (WITH black spacer)- leave first well blank then skip a well for every tube placed. Skip a row in between wells to leave a space for caps.

A3: Trash container

B1: 15ml round bottom tube containing qubit solution (custom container)

B2: Overflow tube container for runs greater than 24 samples (Use black spacer)



Enter Run Details

Step 8.

Open the transfer_qubit_solution_1000.py and sample_to_qubit.py file in a text editor.

`cmd` **COMMAND**

```
sample_number = 4 #total number of samples
standard = False #assign True if robot is needed to make standards
aspirate_volumne = 1 #volume to aspirate
first_tip_row = 0 #row of first tip (first row is row 0, second row is row 1, etc.)
standard_volume = 10 #volume of standard solution to aspirate
stloc = 'A1' #row where standards are located

offset_d = (3, 3, -
.4) #due to bug that default offset is not (0, 0, 0) when run on the robot
gap = 2 #volume of air gap in ul, to minimize errors
```

```

start = 1 #if you need to start protocol in the middle, write sample number that you need to
start on (example: if you need to start at sample number 4, change to 4)

#make sure tip container has 4 tips per row, alternating a space in between with the first
column (A) empty
#if
#note that trash should be placed in slot D3

#allows file in speed_dir to control speed of the robot
speed_dir = '/Users/opentronsmac/Documents/opentrons/' #string of path to file that adjusts
speed
import sys
sys.path.insert(0, speed_dir)
import imp
import speed
imp.reload(speed)

from opentrons import containers, instruments

#containers
p10rack = containers.load(
    'tiprack-10ul', #container name in opentrons system
    'E1', #slot of container
    'tiprack', #name to remember calibrations
)

samples = containers.load(
    '96-PCR-tall',
    'D1',
    'sample_tubes',
)

tubes = containers.load(
    '96-deep-well',
    'D2',
    'd2qubit_tubes_spaced',
    # 'd2qubit_tubes',
)

tubes_overflow = containers.load(
    '96-deep-well',
    'E2',
    'e2qubit_tubes_spaced',
    # 'e2qubit_tubes',
)

standards = containers.load(
    '96-deep-well',
    'E3',
    'e3qubit_tubes',
)

trash = containers.load(
    'point',
    'D3',
    'trash',
)

#pipette
p10 = instruments.Pipette(

```

```

axis = 'a',
max_volume = 10,
min_volume = 0.5,
tip_racks = p10rack,
trash_container = trash,
channels = 8,
name = 'p10',
)

def tip_position(number, first = first_tip_row):
    """
    Converts current sample number to string of the
    corresponding row if there were 4 samples per row.
    """
    row = number // 4 + 1 + first
    return 'A' + str(row)

def sample_position(number):
    """
    Converts current sample number to a string of the corresponding row
    if there were 8 samples per row, plus a skip row in between samples.
    """
    row = (number // 8) * 2 + 1
    return 'A' + str(row)

def tube_position(number):
    """
    Converts current sample number to a string of the corresponding row
    if there were 4 samples per row, plus a skip row in between samples.
    Restarts when sample number is greater than 24.
    """
    row = 2 * (number // 4) + 1
    if row <= 12:
        return 'A' + str(row)
    else:
        return 'A' + str(row-12)

def offset_d(number, default=offset_d):
    """
    Converts current sample number to a tuple that alternates
    between an offset of 1 sample for the first half of the row,
    and the default offset for the second half of the rows.
    """
    remainder = number % 8
    if remainder <= 4:
        return (default[0] - 9, default[1], default[2])
    else:
        return default

def tube_container(number, original=tubes, overflow=tubes_overflow):
    """Given current sample number, will return the correct container for tubes."""
    if number <= 24:
        return original
    else:
        return overflow

for i in range(start, sample_number + 1, 4): #loop through as many times as there are samples
    p10.pick_up_tip(p10rack[tip_position(i)]) \
        .air_gap(gap) \

```



```

        .move_to((samples[sample_position(i)], offset_d(i))).aspirate(aspirate_volumne) \
        .dispense(aspirate_volumne+gap, (tube_container(i)[tube_position(i)].bottom(5))) \
        .drop_tip() #pick up tip, aspirate sample, dispense in qubit tube, drop tip

if standard: #if robot is needed to make standards
    while i % 4 != 0:
        i+=1 #starts pipette on a new row
        p10.pick_up_tip(p10rack[tip_position(sample_number+1)]) \
        .aspirate(standard_volume, standards[stloc]) \
        .dispense(standard_volume, (tube_container(i)[tube_position(i)].bottom(5))) \
        .drop_tip() #pick up tip, aspirate standard solution, dispense in qubit tube, drop t
ip
Sample to Qubit

```

Enter Run Details

Step 9.

Set the sample_number to the total number of samples in your run (not including standards), and the first tip to the location of the first tip in your tiprack (our p1000 tiprack formation ranges from [A-E][1-20] (i.e. A1, B4, E2), and p10 tiprack formation ranges from rows [0-11]).

Enter run details at the top for both files, do not change anything that is not in the top section.

Enter Run Details

Step 10.

If you would like OpenTrons to make standards, set standards equal to 'True', otherwise, set to 'False' (capitalize first letter).

Calibration

Step 11.

Save both edited text files.

Upload the transfer_qubit_solution_1000.py onto the OpenTrons app (ok to drag and drop).

Calibration

Step 12.

Check calibrations if you feel it is necessary (i.e. first time running protocol in the day, if you feel that calibrations may have shifted):

Click on the tiprack container and press 'Move to' for the pipette to move to the tiprack, see if pipette accurately fits the tip.

If tiprack calibration is accurate, the rest of the containers *usually* are correctly calibrated.

If calibration is off:

Z-axis is usually calibrated correctly, so move 20-40mm up, and slowly move back down the same amount changing X and Y to the correct location.

Press 'Save' when location is correct.

Check other containers:

Click on the other containers in the checklist while tip is on pipette, and click 'Move to'.

If calibration incorrect, calibrate just like how you calibrated the tiprack.

Repeat for all containers.

To drop tip:

Click on the tiprack container and press 'Move to'.

Raise the pipette 20mm up in the Z-axis and Click on the pipette in the checklist.

Find the Drop Tip section and click 'Move to'.

Qubit Solution Transfer

Step 13.

Click the 'Run' button to run the step.

When prompted to home, press OK.

This step uses the p1000 and transfers 199µl of qubit solution to each tube.

If standards is set to 'True', 190µl of qubit solution is transferred to the two tubes in the separate row in the end.

 AMOUNT

199 µl Additional info: Qubit Solution

 AMOUNT

190 µl Additional info: Qubit Solution

Set Up: Sample Transfer

Step 14.

If a different OpenTrons is needed for the next steps, home the current robot by pressing 'All' in the home section in the upper left corner of the OpenTrons App. Unplug the current robot and power off before repeating Steps 2-6 on the new robot to set up and power on the new robot.

 GOTO

If different robot needed for 10µl multichannel -> go to step #8

Set Up: Sample Transfer

Step 15.

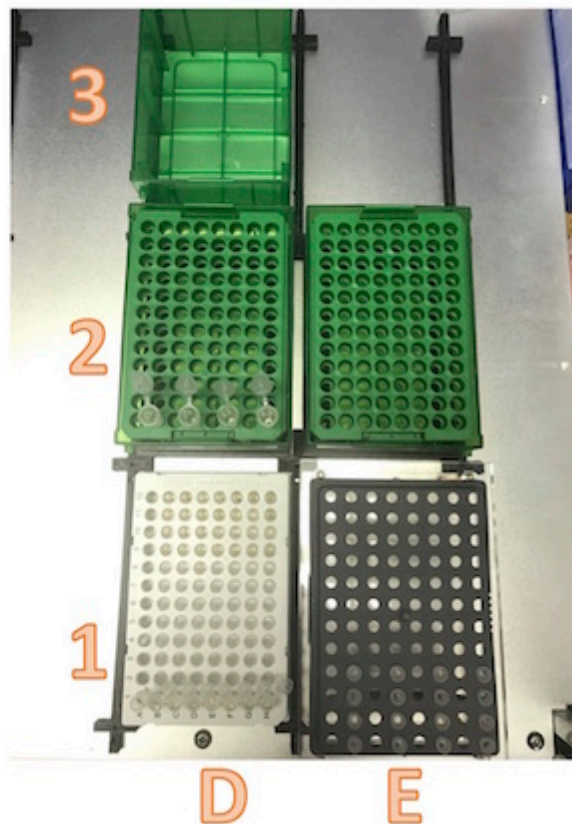
Set up containers according to the layout_description and use text description for the Transfer Sample to Qubit page. See picture as reference.

Important things to note

- When arranging tips , leave the first column blank and start on the second column, skipping a column in between every tip/tube (see picture)
- If OpenTrons is needed to make standards, place two extra tips in a new row behind the last row of samples, and set the container with the two standard solutions in slot E3 (see picture)

Transfer Samples to Qubit Tubes

- D1: Sample Container- Samples in PCR strips- skip row between samples to leave room for caps
- D2: Qubit tube container (WITH black spacer)- leave first well blank then skip a well for every tube placed. Skip a row in between wells to leave a space for caps. Has qubit solution from previous step.
- D3: Trash Container
- E1: 10ul tiprack- take out the tips in first, third, fifth, and seventh column
- E2: Overflow tube container for runs greater than 24 samples
- E3: (Not pictured) If standards needed, place two 0.5ml tubes containing the standards in slot B1 and D1 of green container



Calibration: Sample Transfer

Step 16.

Upload the sample_to_qubit.py onto the OpenTrons app (ok to drag and drop).

Repeat Step 12 for new containers to calibrate for p10.



GOTO

If p10 needs to be calibrated -> go to step #17

Transfer Samples to Qubit Tubes

Step 17.

Click the 'Run' button to run the step.

When prompted to home, press OK.

This step uses the p10 and transfers 1µl of a different sample to every tube.

If standards is set to 'True', the p10 transfers 10µl of the necessary standard solution to the standard tubes.

 AMOUNT

1 µl Additional info: individual samples

 AMOUNT

10 µl Additional info: standard soltuion

Finish

Step 18.

Home the current robot by pressing 'All' in the home section in the upper left corner of the OpenTrons App. Unplug the current robot and power off.