# Action Spectra protocol for Opentrons OT-1 liquid handling robot

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Feb 07, 2021
1 Works for me
dx.doi.org/10.17504/protocols.io.br6vm9e6

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DOI
dx.doi.org/10.17504/protocols.io.br6vm9e6

PROTOCOL CITATION
andrei.herdean 2021. Action Spectra protocol for Opentrons OT-1 liquid handling robot . **protocols.io** https://dx.doi.org/10.17504/protocols.io.br6vm9e6



CREATED
Feb 07, 2021

LAST MODIFIED
Feb 07, 2021

PROTOCOL INTEGER ID
47029

1 OT-1 setup:

A3 - box with 1 ml tips
B3 - 48 well plate 1
D3 - 48 well plate 2
C1 - 48 well plate 3

48 plate 1 setup:
C8 - oxygen optode
D8 - temperature sensor
A7, B7, C7, D7, E7, F7 - blank media
F4, F5, F6 - ddH2O
A8, B8, E8, F8 - empty
All other wells have 1 ml of algae culture

48 plate 2 setup:
All wells empty, except:
F2 - temperature sensor
F1 - oxygen optode

48 well plate 3 setup:
All wells empty, except:
C8 - oxygen optodes*
B8, D8 - temperature sensors
* a fiber optic cable connected to the OL490 is positioned in front of well C8

2 OT-1 protocol:

```
from opentrons import robot, containers, instruments
robot.home()
#containers to load
plateC1 = containers.load('48-well-plate', 'C1')
change_light = containers.load('6-well-plate', 'A1')
plateD3 = containers.load('48-well-plate', 'D3')
plateB3 = containers.load('48-well-plate', 'B3')
tiprack_1 = containers.load('tiprack-1000ul', 'A3')
#optode well definition
dark_optode = 'F1'
light_optode = 'C8'

#pipette definition
p1000 = instruments.Pipette(
  axis="b",
  max_volume=1000,
  tip_racks=[tiprack_1])

#how much additional distance to raise the tip
robot.arc_height = 20

#starting tip position
p1000.reset()
p1000.start_at_tip(tiprack_1['A1'])

#Test trigger
p1000.touch_tip(change_light.wells('A1'),radius=1.7) # trigger light
p1000.touch_tip(change_light.wells('A1'),radius=1.7) # trigger light
p1000.touch_tip(change_light.wells('A1'),radius=1.7) # trigger light
p1000.touch_tip(change_light.wells('A1'),radius=1.7) # trigger light

#START
#transfer blank samples (water to optodes)
p1000.pick_up_tip()
p1000.aspirate(1000, plateB3.wells('A7'))
p1000.dispense(1000, plateB3.wells(light_optode))
p1000.blow_out()

p1000.aspirate(1000, plateB3.wells('B7'))
p1000.dispense(1000, plateC1.wells(light_optode))
p1000.blow_out()

p1000.aspirate(1000, plateB3.wells('C7'))
p1000.dispense(1000, plateD3.wells(dark_optode))
p1000.blow_out()
p1000.delay(seconds=600)

#pre-loop
#step 1 - empty All light optode
```



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```
p1000.mix(3, 1000, plateB3.wells(light_optode))
p1000.aspirate(1000, plateB3.wells(light_optode))
p1000.dispense(1000, plateB3.wells('A7'))
p1000.blow_out()
#step 2 - sample1 in All light optode
p1000.mix(3, 1000, plateB3.wells('A1'))
p1000.aspirate(1000, plateB3.wells('A1'))
p1000.dispense(1000, plateB3.wells(light_optode))
p1000.blow_out()
p1000.delay(seconds=600)

#step 3 - empty OL490
p1000.mix(3, 1000, plateC1.wells(light_optode))
p1000.aspirate(1000, plateC1.wells(light_optode))
p1000.dispense(1000, plateB3.wells('B7'))
p1000.blow_out()
p1000.touch_tip(change_light.wells('A1'),radius=1.7) # trigger light

#step 4 - sample in OL 490
p1000.mix(3, 1000, plateB3.wells(light_optode))
p1000.aspirate(1000, plateB3.wells(light_optode))
p1000.dispense(1000, plateC1.wells(light_optode))
p1000.blow_out()
#Step 5 - sample 2 in All light optode
p1000.mix(3, 1000, plateB3.wells('A2'))
p1000.aspirate(1000, plateB3.wells('A2'))
p1000.dispense(1000, plateB3.wells(light_optode))
p1000.blow_out()
p1000.delay(seconds=600)

#Step 6 - empty Dark optode
p1000.mix(3, 1000, plateD3.wells(dark_optode))
p1000.aspirate(1000, plateD3.wells(dark_optode))
p1000.dispense(1000, plateB3.wells('C7'))
p1000.blow_out()
#Step 7 - Sample 1 in dark optode
p1000.mix(3, 1000, plateC1.wells(light_optode))
p1000.aspirate(1000, plateC1.wells(light_optode))
p1000.dispense(1000, plateD3.wells(dark_optode))
p1000.blow_out()
p1000.touch_tip(change_light.wells('A1'),radius=1.7) # trigger light

#Step 8 - Sample 2 in OL490
p1000.mix(3, 1000, plateB3.wells(light_optode))
p1000.aspirate(1000, plateB3.wells(light_optode))
p1000.dispense(1000, plateC1.wells(light_optode))
p1000.blow_out()
#Step 9 - Sample 3 in All light optode
p1000.mix(3, 1000, plateB3.wells('A3'))
p1000.aspirate(1000, plateB3.wells('A3'))
p1000.dispense(1000, plateB3.wells(light_optode))
p1000.blow_out()
p1000.delay(seconds=600)

#Loop
def run_loop(sample_well1 = 'A4', sample_well2 = 'A1'): #00:00
  #Step 1 - empty DARK optode
  p1000.mix(3, 1000, plateD3.wells(dark_optode))
```



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```
    p1000.aspirate(1000, plateD3.wells(dark_optode))
    p1000.dispense(1000, plateB3.wells(sample_well2))
    p1000.blow_out()                    #00:54
    #Step 2 - OL490 to Dark
    p1000.mix(3, 1000, plateC1.wells(light_optode))    #01:22
    p1000.aspirate(1000, plateC1.wells(light_optode))   #01:24
    p1000.dispense(1000, plateD3.wells(dark_optode))    #01:31
    p1000.blow_out()                    #01:47
    p1000.touch_tip(change_light.wells('A1'),radius=1.7) # trigger light

    #Step 3 - ALL light to OL490
    p1000.mix(3, 1000, plateB3.wells(light_optode))
    p1000.aspirate(1000, plateB3.wells(light_optode))
    p1000.dispense(1000, plateC1.wells(light_optode))
    p1000.blow_out()                    #02:41
    #Step 4 - new sample in ALL light
    p1000.mix(3, 1000, plateB3.wells(sample_well1))
    p1000.aspirate(1000, plateB3.wells(sample_well1))
    p1000.dispense(1000, plateB3.wells(light_optode))
    p1000.blow_out()                    #11:26
    p1000.delay(seconds=600)

sample_wells = [name+number for name in 'ABCDEF' for number in '123456']

for i in range(3, len(sample_wells)):
    run_loop(sample_wells[i], sample_wells[i-3])

#Wash procedure
p1000.mix(3, 1000, plateB3.wells(light_optode))
p1000.aspirate(1000, plateB3.wells(light_optode))
p1000.dispense(1000, plateB3.wells('F4'))
p1000.blow_out()
p1000.return_tip()
p1000.pick_up_tip()

p1000.aspirate(1000, plateB3.wells('A7'))
p1000.dispense(1000, plateB3.wells(light_optode))
p1000.blow_out()
p1000.mix(3, 1000, plateB3.wells(light_optode))
p1000.aspirate(1000, plateB3.wells(light_optode))
p1000.dispense(1000, plateB3.wells('A7'))
p1000.blow_out()
p1000.return_tip()
p1000.pick_up_tip()

p1000.aspirate(1000, plateB3.wells('D7'))
p1000.dispense(1000, plateB3.wells(light_optode))
p1000.blow_out()

p1000.mix(3, 1000, plateC1.wells(light_optode))
p1000.aspirate(1000, plateC1.wells(light_optode))
p1000.dispense(1000, plateB3.wells('F5'))
p1000.blow_out()
p1000.return_tip()
p1000.pick_up_tip()

p1000.aspirate(1000, plateB3.wells('B7'))
p1000.dispense(1000, plateC1.wells(light_optode))
```



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```
p1000.blow_out()
p1000.mix(3, 1000, plateC1.wells(light_optode))
p1000.aspirate(1000, plateC1.wells(light_optode))
p1000.dispense(1000, plateB3.wells('B7'))
p1000.blow_out()
p1000.return_tip()
p1000.pick_up_tip()

p1000.aspirate(1000, plateB3.wells('E7'))
p1000.dispense(1000, plateC1.wells(light_optode))
p1000.blow_out()
#
p1000.mix(3, 1000, plateD3.wells(dark_optode))
p1000.aspirate(1000, plateD3.wells(dark_optode))
p1000.dispense(1000, plateB3.wells('F6'))
p1000.blow_out()
p1000.return_tip()
p1000.pick_up_tip()

p1000.aspirate(1000, plateB3.wells('C7'))
p1000.dispense(1000, plateD3.wells(dark_optode))
p1000.blow_out()
p1000.mix(3, 1000, plateD3.wells(dark_optode))
p1000.aspirate(1000, plateD3.wells(dark_optode))
p1000.dispense(1000, plateB3.wells('C7'))
p1000.blow_out()
p1000.return_tip()
p1000.pick_up_tip()

p1000.aspirate(1000, plateB3.wells('F7'))
p1000.dispense(1000, plateD3.wells(dark_optode))
p1000.blow_out()
p1000.delay(seconds=600)

p1000.aspirate(1000, plateB3.wells(light_optode))
p1000.dispense(1000, plateB3.wells('D7'))
p1000.blow_out()

p1000.aspirate(1000, plateC1.wells(light_optode))
p1000.dispense(1000, plateB3.wells('E7'))
p1000.blow_out()

p1000.aspirate(1000, plateD3.wells(dark_optode))
p1000.dispense(1000, plateB3.wells('F7'))
p1000.blow_out()

p1000.return_tip()
robot.home()
```



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