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Action Spectra protocol for Opentrons OT-1 liquid handling robot

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Protocol status: Working

We use this protocol and it's working

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
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))
```

```

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))
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()
```