Cell measurement protocol

Description:

Prior to performing the cell measurements you should perform all three of the calibration measurements. Please do not proceed unless you have completed the three calibration protocols. Completion of the calibrations will ensure that you understand the measurement process and that you can take the cell measurements under the same conditions. For the sake of consistency and reproducibility, we are requiring all teams to use E. coli K-12 DH5-alpha. If you do not have access to this strain, you can request streaks of the transformed devices from another team near you, and this can count as a collaboration as long as it is appropriately documented on both teams' wikis. If you are absolutely unable to obtain the DH5-alpha strain, you may still participate in the InterLab study by contacting the Measurement Committee (measurement at igem dot org) to discuss your situation.

For all of these cell measurements, you must use the same plates and volumes that you used in your calibration protocol. You must also use the same settings (e.g., filters or excitation and emission wavelengths) that you used in your calibration measurements. If you do not use the same plates, volumes, and settings, the measurements will not be valid.

Protocol Materials:

- *E. coli* DH5 alpha (https://identifiers.org/pubchem.substance:24901740)
- LB Broth+chloramphenicol (https://identifiers.org/pubchem.substance:24901740)
- chloramphenicol (https://identifiers.org/pubchem.substance:24901740)
- Negative control (https://identifiers.org/SBO:0000251)
- Positive control (https://identifiers.org/SBO:0000251)
- Test Device 1 (https://identifiers.org/SBO:0000251)
- Test Device 2 (https://identifiers.org/SBO:0000251)
- Test Device 3 (https://identifiers.org/SBO:0000251)
- Test Device 4 (https://identifiers.org/SBO:0000251)
- Test Device 5 (https://identifiers.org/SBO:0000251)
- Test Device 6 (https://identifiers.org/SBO:0000251)

Protocol Inputs:

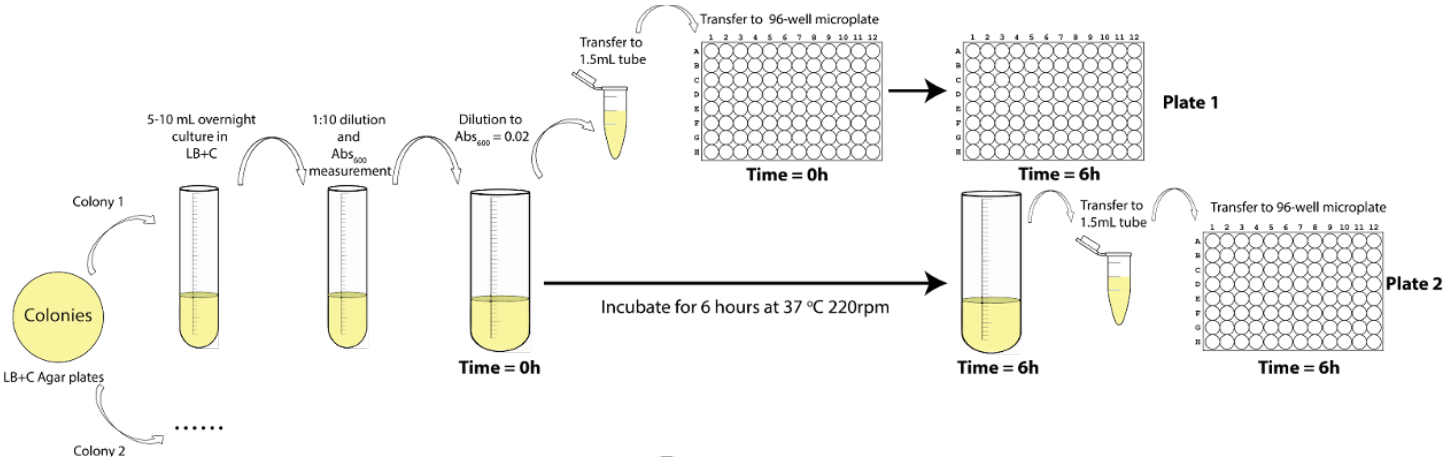
Protocol Outputs:

- `baseline absorbance of culture (day 2) measurements of cultures (0 hr timepoint)`
- `0 hr absorbance timepoint measurements of plate 1`
- `0 hr absorbance timepoint measurements of plate 1`
- `0 hr absorbance timepoint measurements of plate 1`
- `0 hr fluorescence timepoint measurements of plate 1`
- `0 hr fluorescence timepoint measurements of plate 1`
- `0 hr fluorescence timepoint measurements of plate 1`
- `2 hr absorbance timepoint measurements of plate 1`
- `2 hr fluorescence timepoint measurements of plate 1`
- `2 hr absorbance timepoint measurements of plate4`
- `2 hr fluorescence timepoint measurements of plate4`
- `4 hr absorbance timepoint measurements of plate 2`
- `4 hr fluorescence timepoint measurements of plate 2`
- `4 hr absorbance timepoint measurements of plate5`
- `4 hr fluorescence timepoint measurements of plate5`
- `6 hr absorbance timepoint measurements of plate 3`
- `6 hr fluorescence timepoint measurements of plate 3`
- `6 hr absorbance timepoint measurements of plate6`
- `6 hr fluorescence timepoint measurements of plate6`

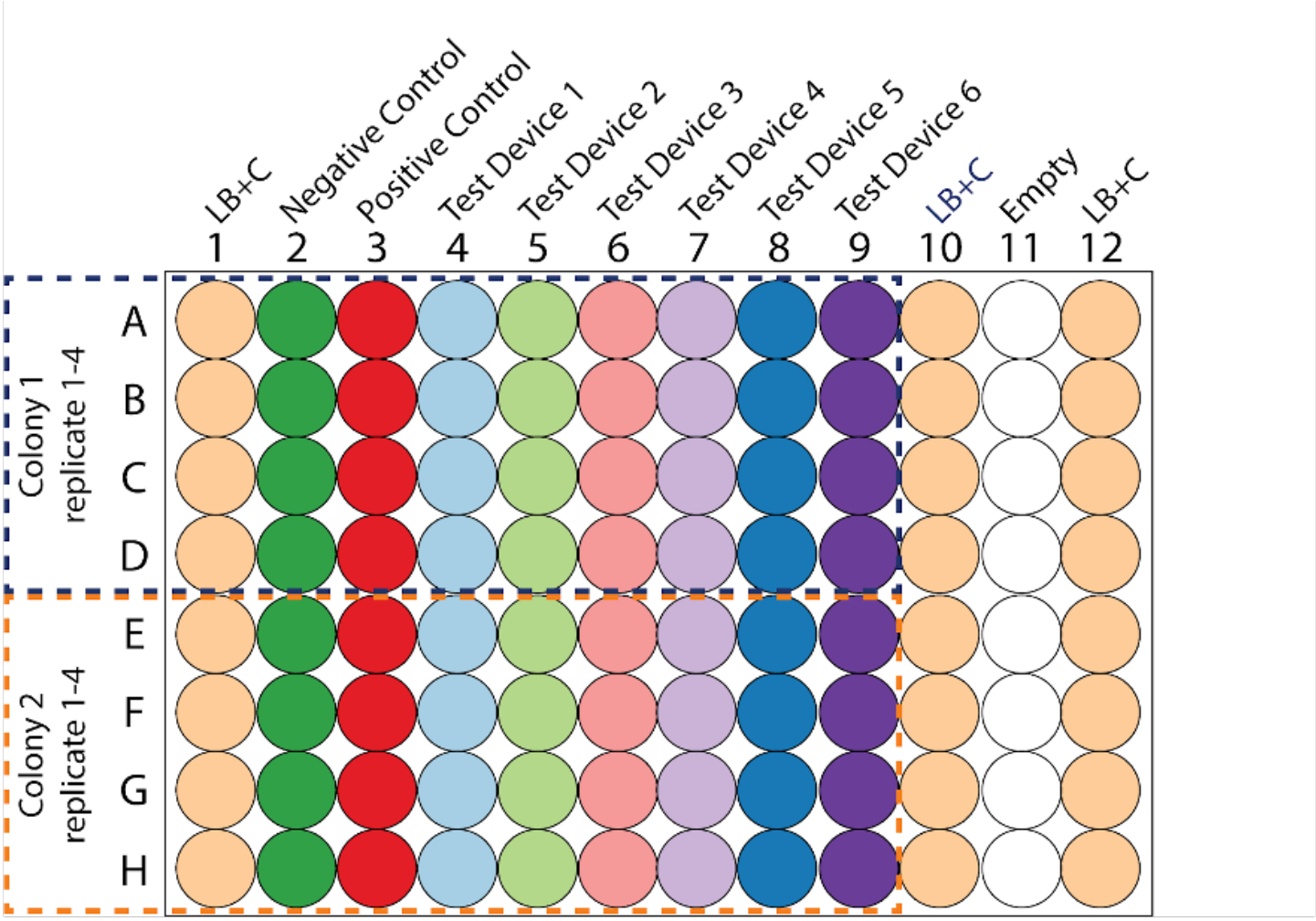
Steps

1. Transform `Negative control` DNA into *`E. coli`* `DH5 alpha` and plate transformants on LB Broth+chloramphenicol. Repeat for the remaining transformant DNA: `Positive control`, `Test Device 1`, `Test Device 2`, `Test Device 3`, `Test Device 4`, `Test Device 5`, and `Test Device 6`.
2. Provision 16 x culture tubes to contain `culture (day 1)`

3. Inoculate *E. coli* DH5 alpha+Negative control transformant into 5.0 milliliter of LB Broth+chloramphenicol in culture (day 1) and grow for 16.0 hour at 37.0 degree Celsius and 220.0 rpm. Repeat this procedure for the other inocula: *E. coli* DH5 alpha+Positive control transformant, *E. coli* DH5 alpha+Test Device 1 transformant, *E. coli* DH5 alpha+Test Device 2 transformant, *E. coli* DH5 alpha+Test Device 3 transformant, *E. coli* DH5 alpha+Test Device 4 transformant, *E. coli* DH5 alpha+Test Device 5 transformant, and *E. coli* DH5 alpha+Test Device 6 transformant. Inoculate 2 replicates for each transformant, for a total of 16 cultures.
4. Provision 16 x culture tubes to contain culture (day 2)
5. Dilute each of 16 culture (day 1) samples with LB Broth+chloramphenicol into the culture tube at a 1:10 ratio and final volume of 10.0 milliliter. Maintain at 4.0 degree Celsius while performing dilutions.
6. Provision 16 x 1.5 mL microfuge tubes to contain cultures (0 hr timepoint)
7. Hold cultures (0 hr timepoint) at 4.0 degree Celsius. This will prevent cell growth while transferring samples.
8. Transfer 1.0 milliliter of each of 16 culture (day 2) samples to 1.5 mL microfuge tube containers to contain a total of 16 cultures (0 hr timepoint) samples. Maintain at 4.0 degree Celsius during transfer.
9. Measure baseline absorbance of culture (day 2) of cultures (0 hr timepoint) at 600.0 nanometer.
10. Provision 16 x 50 ml conical tubes to contain back-diluted culture The conical tube should be opaque, amber-colored, or covered with foil.
11. Back-dilute each of 16 culture (day 2) samples to a target OD of 0.02 using LB Broth+chloramphenicol as diluent to a final volume of 40.0 milliliter. Maintain at 4.0 degree Celsius while performing dilutions.



12. Provision 16 x 50 ml conical tubes to contain Tube 1 The conical tubes should be opaque, amber-colored, or covered with foil.
13. Provision 16 x 50 ml conical tubes to contain Tube 2 The conical tubes should be opaque, amber-colored, or covered with foil.
14. Provision 16 x 50 ml conical tubes to contain Tube 3 The conical tubes should be opaque, amber-colored, or covered with foil.
15. Transfer 1.0 milliliter of each of 16 back-diluted culture samples to 50 ml conical tube containers to contain a total of 16 Tube 1 samples. Maintain at 4.0 degree Celsius during transfer.
16. Transfer 1.0 milliliter of each of 16 back-diluted culture samples to 50 ml conical tube containers to contain a total of 16 Tube 2 samples. Maintain at 4.0 degree Celsius during transfer.
17. Transfer 1.0 milliliter of each of 16 back-diluted culture samples to 50 ml conical tube containers to contain a total of 16 Tube 3 samples. Maintain at 4.0 degree Celsius during transfer.
18. Provision a 96 well microplate to contain plate 1
19. Provision a 96 well microplate to contain plate 2
20. Provision a 96 well microplate to contain plate 3
21. Hold plate 1 at 4.0 degree Celsius.
22. Transfer 100.0 microliter of each Tube 1 sample to 96 well microplate plate 1 in the wells indicated in the plate layout. Maintain at 4.0 degree Celsius during transfer.
23. Transfer 100.0 microliter of LB Broth+chloramphenicol sample to wells A1:H1, A10:H10, A12:H12 of 96 well microplate plate 1. Maintain at 4.0 degree Celsius during transfer. These samples are blanks.
24. Transfer 100.0 microliter of each Tube 2 sample to 96 well microplate plate 2 in the wells indicated in the plate layout. Maintain at 4.0 degree Celsius during transfer.
25. Transfer 100.0 microliter of LB Broth+chloramphenicol sample to wells A1:H1, A10:H10, A12:H12 of 96 well microplate plate 2. Maintain at 4.0 degree Celsius during transfer. These samples are blanks.
26. Transfer 100.0 microliter of each Tube 3 sample to 96 well microplate plate 3 in the wells indicated in the plate layout. Maintain at 4.0 degree Celsius during transfer.
27. Transfer 100.0 microliter of LB Broth+chloramphenicol sample to wells A1:H1, A10:H10, A12:H12 of 96 well microplate plate 1. Maintain at 4.0 degree Celsius during transfer. These samples are blanks.



28. Cover `plate 1` samples in 96 well microplate with your choice of material to prevent evaporation.
29. Cover `plate 2` samples in 96 well microplate with your choice of material to prevent evaporation.
30. Cover `plate 3` samples in 96 well microplate with your choice of material to prevent evaporation.
31. Measure 0 hr absorbance timepoint of `plate 1` at 600.0 nanometer.
32. Measure 0 hr absorbance timepoint of `plate 1` at 600.0 nanometer.
33. Measure 0 hr absorbance timepoint of `plate 1` at 600.0 nanometer.
34. Measure 0 hr fluorescence timepoint of `plate 1` with excitation wavelength of 488.0 nanometer and emission filter of 530.0 nanometer and 30.0 nanometer bandpass
35. Measure 0 hr fluorescence timepoint of `plate 1` with excitation wavelength of 488.0 nanometer and emission filter of 530.0 nanometer and 30.0 nanometer bandpass
36. Measure 0 hr fluorescence timepoint of `plate 1` with excitation wavelength of 488.0 nanometer and emission filter of 530.0 nanometer and 30.0 nanometer bandpass
37. Incubate all `Tube 1` samples for 2.0 hour at 37.0 degree Celsius at 220.0.
38. Incubate all `plate 1` samples for 2.0 hour at 37.0 degree Celsius at 220.0.
39. Incubate all `Tube 2` samples for 4.0 hour at 37.0 degree Celsius at 220.0.
40. Incubate all `plate 2` samples for 4.0 hour at 37.0 degree Celsius at 220.0.
41. Incubate all `Tube 3` samples for 6.0 hour at 37.0 degree Celsius at 220.0.
42. Incubate all `plate 3` samples for 6.0 hour at 37.0 degree Celsius at 220.0.
43. Hold all `Tube 1` samples at 4.0 degree Celsius. This will inhibit cell growth during the subsequent pipetting steps.
44. Hold all `plate 1` samples at 4.0 degree Celsius. This will inhibit cell growth during the subsequent pipetting steps.
45. Provision a 96 well microplate to contain `plate4`
46. Transfer 100.0 microliter of each `Tube 1` sample to 96 well microplate `plate4` in the wells indicated in the plate layout. Maintain at 4.0 degree Celsius during transfer.
47. Transfer 100.0 microliter of `LB Broth+chloramphenicol` sample to wells A1:H1, A10:H10, A12:H12 of 96 well microplate `plate4`. Maintain at 4.0 degree Celsius during transfer. These samples are blanks.
48. Measure 2 hr absorbance timepoint of `plate 1` at 600.0 nanometer.

49. Measure 2 hr fluorescence timepoint of `plate 1` with excitation wavelength of 488.0 nanometer and emission filter of 530.0 nanometer and 30.0 nanometer bandpass
50. Measure 2 hr absorbance timepoint of `plate4` at 600.0 nanometer.
51. Measure 2 hr fluorescence timepoint of `plate4` with excitation wavelength of 488.0 nanometer and emission filter of 530.0 nanometer and 30.0 nanometer bandpass
52. Hold all `Tube 2` samples at 4.0 degree Celsius. This will inhibit cell growth during the subsequent pipetting steps.
53. Hold all `plate 2` samples at 4.0 degree Celsius. This will inhibit cell growth during the subsequent pipetting steps.
54. Provision a 96 well microplate to contain `plate5`
55. Transfer 100.0 microliter of each `Tube 2` sample to 96 well microplate `plate5` in the wells indicated in the plate layout. Maintain at 4.0 degree Celsius during transfer.
56. Transfer 100.0 microliter of `LB Broth+chloramphenicol` sample to wells A1:H1, A10:H10, A12:H12 of 96 well microplate `plate5`. Maintain at 4.0 degree Celsius during transfer. These samples are blanks.
57. Measure 4 hr absorbance timepoint of `plate 2` at 600.0 nanometer.
58. Measure 4 hr fluorescence timepoint of `plate 2` with excitation wavelength of 488.0 nanometer and emission filter of 530.0 nanometer and 30.0 nanometer bandpass
59. Measure 4 hr absorbance timepoint of `plate5` at 600.0 nanometer.
60. Measure 4 hr fluorescence timepoint of `plate5` with excitation wavelength of 488.0 nanometer and emission filter of 530.0 nanometer and 30.0 nanometer bandpass
61. Hold all `Tube 3` samples at 4.0 degree Celsius. This will inhibit cell growth during the subsequent pipetting steps.
62. Hold all `plate 3` samples at 4.0 degree Celsius. This will inhibit cell growth during the subsequent pipetting steps.
63. Provision a 96 well microplate to contain `plate6`
64. Transfer 100.0 microliter of each `Tube 3` sample to 96 well microplate `plate6` in the wells indicated in the plate layout. Maintain at 4.0 degree Celsius during transfer.
65. Transfer 100.0 microliter of `LB Broth+chloramphenicol` sample to wells A1:H1, A10:H10, A12:H12 of 96 well microplate `plate6`. Maintain at 4.0 degree Celsius during transfer. These samples are blanks.
66. Measure 6 hr absorbance timepoint of `plate 3` at 600.0 nanometer.
67. Measure 6 hr fluorescence timepoint of `plate 3` with excitation wavelength of 488.0 nanometer and emission filter of 530.0 nanometer and 30.0 nanometer bandpass
68. Measure 6 hr absorbance timepoint of `plate6` at 600.0 nanometer.
69. Measure 6 hr fluorescence timepoint of `plate6` with excitation wavelength of 488.0 nanometer and emission filter of 530.0 nanometer and 30.0 nanometer bandpass
70. Import data for `baseline absorbance of culture (day 2) measurements of cultures (0 hr timepoint),0 hr absorbance timepoint measurements of plate 1,0 hr absorbance timepoint measurements of plate 1,0 hr absorbance timepoint measurements of plate 1,0 hr fluorescence timepoint measurements of plate 1,0 hr fluorescence timepoint measurements of plate 1,0 hr fluorescence timepoint measurements of plate 1,2 hr absorbance timepoint measurements of plate 1,2 hr fluorescence timepoint measurements of plate 1,2 hr absorbance timepoint measurements of plate4,2 hr fluorescence timepoint measurements of plate4,4 hr absorbance timepoint measurements of plate 2,4 hr fluorescence timepoint measurements of plate 2,4 hr absorbance timepoint measurements of plate5,4 hr fluorescence timepoint measurements of plate5,6 hr absorbance timepoint measurements of plate 3,6 hr fluorescence timepoint measurements of plate 3,6 hr absorbance timepoint measurements of plate6,6 hr fluorescence timepoint measurements of plate6` into provided Excel file.

Timestamp: 2022-05-07 13:18:05.926239--- Protocol version: 1.0b