Jan 03, 2025

Bacterial SYBR-Cell Staining and Counting with the Attune Flow Cytometer

Forked from a deleted protocol

DOI
https://dx.doi.org/10.17504/protocols.io.3byl4w3nzvo5/v1

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Protocol status: Working
We use this protocol and it's working

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Protocol Integer ID: 117417

Keywords: bacterial sybr, counting with the attune flow cytometer, cell staining, sybr cell, attune flow cytometer, cell

Abstract

SYBR Cell staining in MICO lab

Prep

1 Clean culture hood with 70% ethanol and use UV light to sterilize the hood (about 15 minutes)

2 Pre-heat mini-incubator to 37ºC.

3 Take foil-wrapped diluted SYBR stock out from fridge door and allow it to come to room temperature (about 5-10 minutes), while keeping it protected from light.

4 After warmed, vortex the SYBR stock and make sure it is well mixed

5 Take out samples for the day (in fridge, labeled with station, depth, date, and "BACT") and gently vortex each sample.

Note: cruise 3 has BACT 1 and 2 so that we have enough volume for the imaging flow cyto as well

Staining

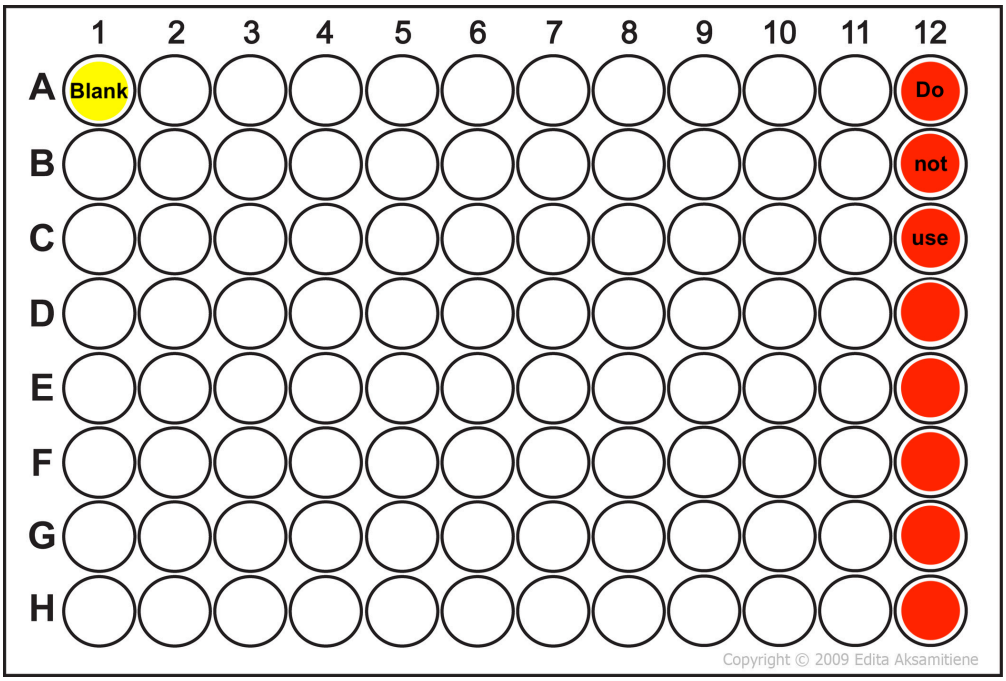
6 put a fresh 96-well plate, mixed samples, and mixed SYBR stock into the cleaned culture hood

7 Add 1 (one) µl of SYBR stock to each well you intend to use in the assay. Make sure to add the 1 µl to the bottom center of the well so that you can see it.

- do not use the furthest column to the right

8 add 99 (ninety-nine) µl of water to well 1A - this will be a blank

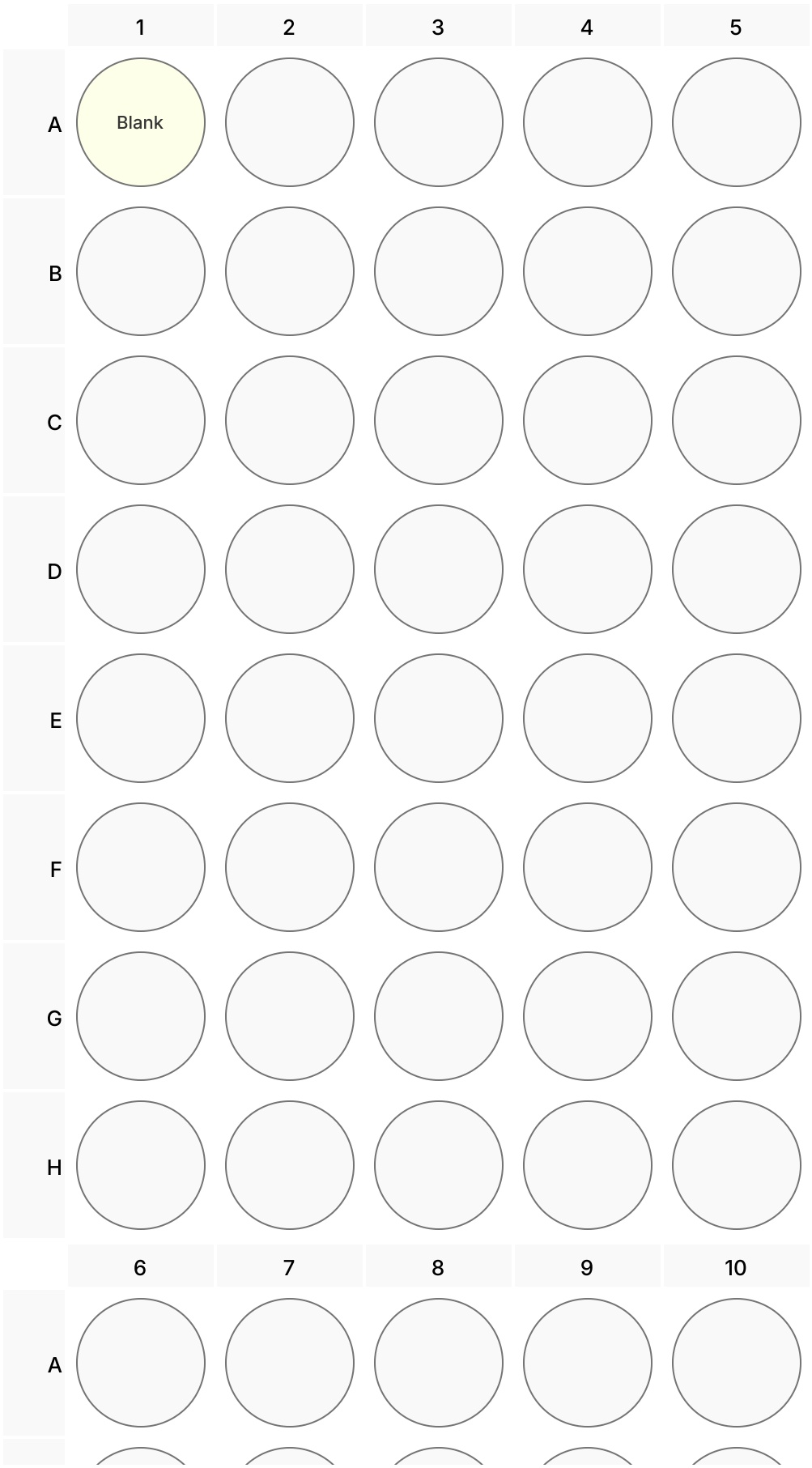
9 add 99 (ninety-nine) µl of sample to each of the other wells and mix 5-10x so that SYBR and samples are mixed.

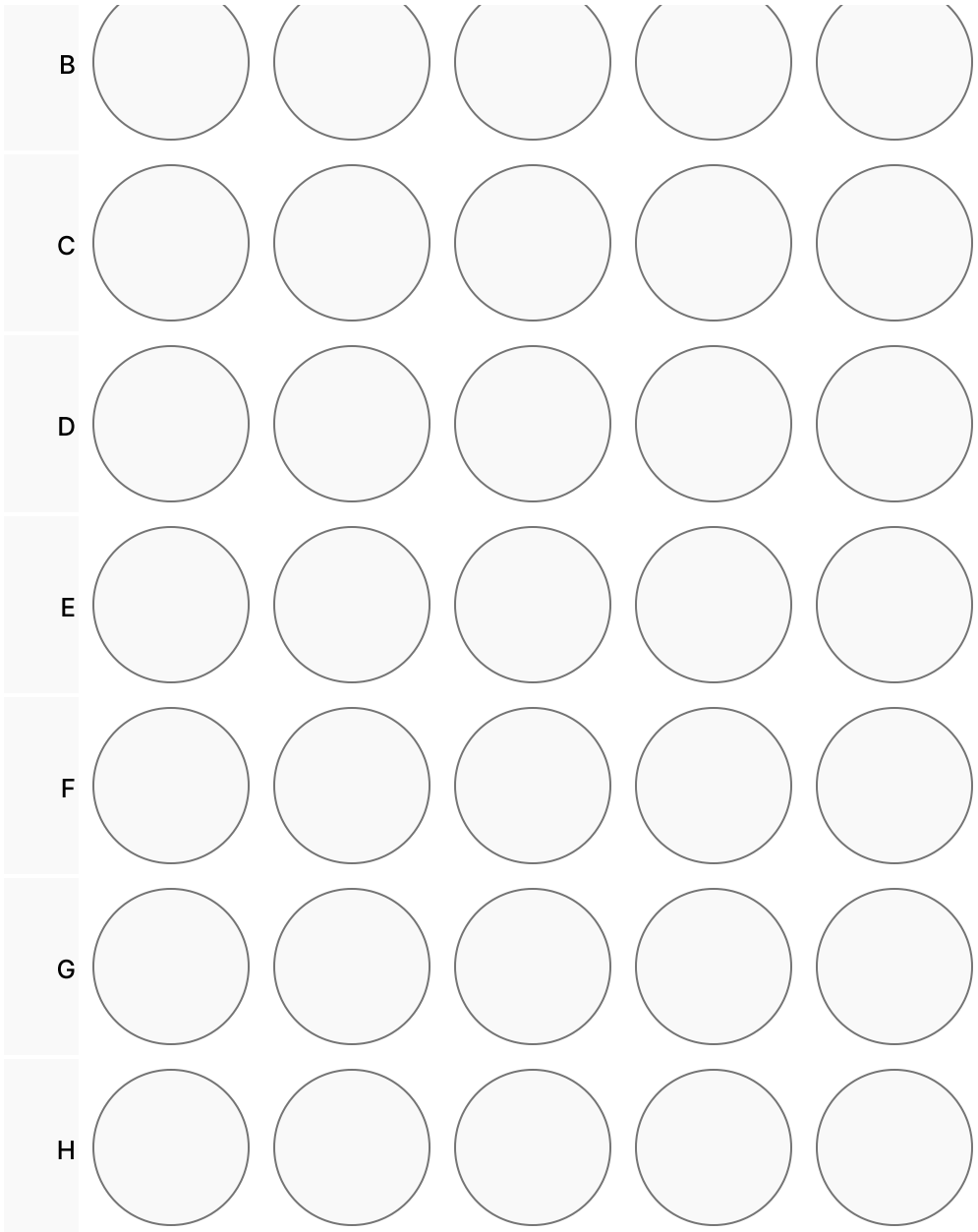


This can be printed and used to write in sample names

10 Record sample-well locations

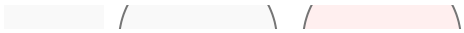
Either print template, use the postits, or record in protocols (makes sure you save run):


1
2
3
4
5
A
Blank
B
C
D
E
F
G
H
6
7
8
9
10
A


B
C
D
E
F
G
H
11
12
A
B
C

Incubate

11 put sample plate into the mini-incubator and incubate at 37ºC for 10 minutes



Edit Attune Template

12 Open proper experiment from template and edit sample names to match actual sample names

Run Experiment on Attune

13 use bacterial template