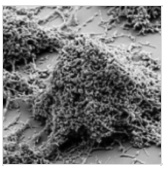

Sep 10, 2020

Mycoplasma

In 1 collection

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1 Works for me dx.doi.org/10.17504/protocols.io.8gphtvn

Neurodegeneration Method Development Community
Tech. support email: ndcn-help@chanzuckerberg.com


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ABSTRACT

Detection of mycoplasma utilizing the bulldog-bio kit. Steps include, gDNA isolation from cells, nanodrop, PCR, and running an agarose gel to detect mycoplasma within a cell culture line.

Image from https://cellculturedish.com/cell-culture-basics-mycoplasma-101-a-practical-guide-to-prevention-detection-and-elimination-of-mycoplasma-contamination/

DOI

dx.doi.org/10.17504/protocols.io.8gphtvn

PROTOCOL CITATION

Andrea Argouarch 2020. Mycoplasma. **protocols.io** https://dx.doi.org/10.17504/protocols.io.8gphtvn

COLLECTIONS

Dural Cell Isolation and Culturing - Collection

KEYWORDS

dura mater, dural cells, mycoplasma, bulldog

CREATED

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28911

PARENT PROTOCOLS

Part of collection
Dural Cell Isolation and Culturing - Collection

STEPS MATERIALS

NAME	CATALOG #	VENDOR

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Epicentre QuickExtract™ DNA Extraction Solution	QE09050	Epicentre
Trypsin-EDTA (0.05%), phenol red	25300062	Thermo Fisher
DPBS, no calcium, no magnesium	14190250	Thermo Fisher
e-Myco PLUS Mycoplasma PCR Detection Kit	25234	
Agarose	PBSA1705	
Ethidium Bromide (1% Solution/Molecular Biology)	BP130210	Fisher Scientific
Gel Loading Dye Blue (6X) - 4.0 ml	B7021S	New England Biolabs
GeneRuler 1 kb Plus DNA Ladder, ready-to-use	SM1333	Thermo Fisher

Observations

1 Culture should be in at least 3-6 days of subculture after split

2 During isolation and PCR, use clean gloves, spray down bench, and keep reagents and tubes cold
On ice

Isolation of gDNA

3 Isolate cells once confluent, at least 3-6 days after split

4 After trypsinization for freezing cells, rinse remaining cells in both flasks with 12 mls **12 mL** PBS

Trypsin-EDTA (0.05%), phenol red
by Thermo Fisher
Catalog #: 25300062

DPBS, no calcium, no magnesium
by Thermo Fisher
Catalog #: 14190250

5 Spin for 5 mins **00:05:00** at 100 rpm **1000 rpm** at 4C **4 °C**

6 Aspirate supernate

7 Place in ice bucket **On ice** on bench or store in -80C **-80 °C**

Citation: Andrea Argouarch (09/10/2020). Mycoplasma. https://dx.doi.org/10.17504/protocols.io.8gphtvn

8 Add 40 µl **40 µl** of Quick Extract to pellet

Epicentre QuickExtract™ DNA Extraction Solution
by Epicentre
Catalog #: QE09050

9 Pipette up and down and transfer to PCR tubes
a. Label PCR tube with line, date, and P for pellet

10 Extract gDNA with thermocycler program
a. Turn on Power button on the back of machine
b. Open lid with handle
c. Put PCR tubes in small slot and close lid
d. Select File, click Enter, select pcr chamber and start program
i. 65C **65 °C** for 15 mins **00:15:00**
ii. 98C **98 °C** for 10 mins **00:15:00**
iii. 4C **4 °C** to hold
e. Run time around ~30mins **00:30:00**
f. Click enter to exit after run
g. Turn off machine and place on ice **On ice**

11 Centrifuge PCR tubes for 5 mins **00:05:00** at max **13.4 rpm** and transfer SN to new 1.5 mls tubes labeled gDNA, line, and date **On ice**
a. Tiny pellet ok left in PCR tube

12 Nanodrop to determine gDNA (pre-PCR) concentration on ice **On ice**

13 Store at -20C **-20 °C** or run PCR

Nanodrop

14 Items to bring to nanodrop machine
a. p2 pipette, p2 tips, pen, kimwipes, ice bucket
b. Keep samples on ice **On ice**

15 Open the NanoDrop 1000 software and select nucleic acid from the main menu

Citation: Andrea Argouarch (09/10/2020). Mycoplasma. https://dx.doi.org/10.17504/protocols.io.8gphtvn

16 Clean the nanodrop pedestal with a kimwipe

17 Add 2 µl **2 µl** of nuclease free water to the center of the pedestal and gently close the lever arm

18 Click 'OK' to initialize the spectrophotometer

19 Select DNA from the dropdown menu

20 Clean the nanodrop pedestal with a kimwipe, add 2 µl **2 µl** of a blank sample, and click 'Blank'
a. Use quick extract solution as blank

21 Clean the nanodrop pedestal with a kimwipe, add 2 ul **2 µl** of sample and click 'Measure'

22 Record the concentration (ng/ul) of the sample on the tube and a table

23 Record the 260/280 ratio, and the 260/230 ratio

260/280 ratio should be above 1.8 (salt contamination)
260/230 ratio should be 1-2

24 Clean the nanodrop pedestal with a kimwipe in-between samples

25 Click exit

26 Leave kimwipe between the pedestal and the lever arm when finished

PCR Reaction

Citation: Andrea Argouarch (09/10/2020). Mycoplasma. https://dx.doi.org/10.17504/protocols.io.8gphtvn

27 Make a 20 µl **20 µl** of PCR reaction in provided eMyco Plasma PCR Tube **On ice**

e-Myco PLUS Mycoplasma PCR Detection Kit
Catalog #: 25234

a. QS to 20 µl **20 µl** with nuclease free water

b. Add 40 ng **40 ng** of sample

i. Can make working stock if concentration is too high

c. Include a negative control with just water (20 µl) **20 µl**

d. Include a positive control with provided positive control (1 µl) **1 µl**

28 Run PCR program

a. Turn on machine at the back

b. Tap to mix, place samples into thermocycler in small tube holes, and close lid

c. Run myco program

Initial Denature = 94C **94 °C** for 1 min **00:01:00**

ii. 35 Cycles

Denature = 94C **94 °C** for 30 secs **00:00:30**

Anneal = 58C **58 °C** 5 for 20 secs **00:00:20**

Extend = 72C **72 °C** for 1 min **00:01:00**

Final Extension = 72C **72 °C** for 5 mins **00:05:00**

Hold at 4C **4 °C**

d. Press enter to run program

g. Run time is around ~1.5hrs **01:30:00**

29 Can prep for agarose gel near the end of the PCR run

30 Store gDNA samples, at -20C **-20 °C**

31 After run, select enter, turn off machine, and place PCR product on ice **On ice**

32 Run gel or store at -20C **-20 °C**

Citation: Andrea Argouarch (09/10/2020). Mycoplasma. https://dx.doi.org/10.17504/protocols.io.8gphtvn

Agarose Gel

33 Wet rubber around gel chamber to help it slide into gel rig

34 Assemble with rubber on the black edges of the gel rig and place purple comb in the slot on the right

35 Make 2% Agarose in small beaker with screw cap for a mini rig size.

Agarose
Catalog #: PBSA1705

a.1.2 g **1.2 g** of agarose with 60 mls **60 mL** of 1X TAE (Stock is 20X TAE)

36 Microwave for 2 mins **00:02:00** with a loose cap and swirl every 30 seconds **00:00:30** until dissolved

37 When temperature is cool to the touch, add 1:10,000 of Etbr

Ethidium Bromide (1% Solution/Molecular Biology)
by Fisher Scientific
Catalog #: BP130210

Mutagen - may potentially cause carcinogenic or teratogenic effects

ie add 6 µl **6 µl** Etbr per 60 mls **60 mL** of agarose

38 Swirl to mix and add to chamber to set, allow for 10 mins **00:10:00** to cool in cold room **4 °C**

39 Prep samples for gel in PCR tubes on ice **On ice**

a. 5 µl **5 µl** of PCR product

b. 3 µl **3 µl** of Nuclease free water

c. 2.6 µl **2.6 µl** of 6X loading buffer (2X final concentration from a 6X stock, purple)

Citation: Andrea Argouarch (09/10/2020). Mycoplasma. https://dx.doi.org/10.17504/protocols.io.8gphtvn

Gel Loading Dye Blue (6X) - 4.0 ml
by New England Biolabs
Catalog #: B7021S

i.(6X)(a) = (2X)(8ul); a = 2.6ul

40 Rotate gel chamber when set, so the lanes are at the top of the chamber

41 Fill with 1X TAE until the top of the white knob in gel rig and remove comb slowly

42 Load 4 µl **4 µl** of 1kb O'gene plus ladder into first lane

GeneRuler 1 kb Plus DNA Ladder, ready-to-use
by Thermo Fisher
Catalog #: SM1333

43 Load 11 µl **11 µl** of each sample per well

44 Add lid, black at the top, red at the bottom, both at the right side

45 Run at 130V for 50 mins **00:50:00**

46 Store PCR product at -20C **-20 °C**

47 Dispose Etbr waste in proper container (liquid and solid)

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