Gnotobiotic Cockroach Gnotocol

Helen Dukes[1], Elizabeth Ottesen[1]
[1]University of Georgia

dx.doi.org/10.17504/protocols.io.t4ceqsw
Ottesen Lab

Helen Dukes

Working
Oct 01, 2018

ABSTRACT

Sterilization and post-hatch care of gnotobiotic *Periplaneta americana*, as adapted from Doll et. al. (see below)

Doll J, Trexler P, Reynolds L, Bernard G. 1963. The use of peracetic acid to obtain germfree invertebrate eggs for gnotobiotic studies. Am Midl Nat 69:231-239

PROTOCOL STATUS

Working
Reliable so far in producing cockroaches that live on LB agar with no visible growth! Might tweak the medium if nymphal development appears stunted.

MATERIALS TEXT

Laminar flow hood
5-mL eppendorf microcentrifuge tubes (4 per 3~5 egg cases)
0.1% SDS
0.1% peracetic acid
4-5 pairs of sterile forceps
1.5-mL eppendorf microcentrifuge tubes (2 per egg case)
Sterile water
LB agar slants (10g tryptone, 5g yeast extract, 10g NaCl, 20g agar; in 1000 mL water)
Secondary container (large enough to fit LB agar slants; we use plastic bug cages), sterilized (via bleach/ethanol/UV/autoclaving)
-- I just do bleach + ethanol + UV (20 min), since our containers are not autoclavable

Collect oothecae egg cases) from colony

1 Pulling egg cases directly off females' abdomens ensures the eggs have not been sitting in the litter a long time.
This also typically results in "cleaner" oothecae, with less excrement on the outside

Physically clean any visible/major excrement off of the outside of the oothecae

2 This can be done using kimwipes and water or SDS

Wash oothecae in 0.1% SDS

3 Place 3-5 egg cases in a 5-mL eppendorf with 1~2 mL 0.1% SDS
Vortex to wash
Transfer egg cases to new 5-mL tube and repeat above steps

Rinse oothecae

4 Transfer to new 5-mL eppendorf with 2~3 mL sterile water

Vortex to rinse
Transfer egg cases to new 5-mL tube and repeat above steps until there are no more suds

Prepare 0.1% peracetic acid for sterilization

5 *This must be done shortly prior to sterilization because the acid will become ineffective for sterilization when it breaks down into hydrogen peroxide and acetate*

Our peracetic acid comes as a 32% stock solution (stabile in the fridge)
0.1% peracetic acid = (10 uL stock) + (3.2 mL sterile water) ------ this is enough for up to 5 oothecae
Depending on how many eggs are being sterilized, this can be made in a 5-mL eppendorf, or a 15-mL conical tube for larger batches

Sterilize oothecae

6 Place washed/rinsed oothecae in 0.1% peracetic acid for 5 min, invert occasionally to ensure total surface sterilization.
At this point, transfer all materials to sterilized laminar flow hood, to prevent post-sterilization contamination.

Rinse oothecae (post-sterilization)

7 Using sterile (pre-autoclaved) forceps, pull out individual egg cases from acid bath, place each case in its own 1 mL of pre-aliquoted sterile water (in 1.5-mL eppendorf tubes), invert numerous times to rinse. (in laminar flow hood)
Transfer oothecae to new sterile water tubes for an additional rinse. (in laminar flow hood)

Incubate eggs @ 30 C until hatching

8 Using a new set of sterile forceps (different from those that retrieved the egg cases from the acid bath), transfer oothecae from rinse tubes to sterile LB agar slants (in laminar flow hood). Place slants in secondary container; this can now be removed from the laminar flow hood,
Incubate entire containment system at 30 C; oothecae should begin hatching ~4 weeks.
Check LB slants for microbial growth (especially in the first few days); remove and discard tubes with growth.

Post-hatch care

9 Newborns will eat LB agar, excreting it in small, translucent beads.
These young (nymphal) stages of the cockroach can be prone to dehydration; water by pipetting < 1 mL sterile water onto the agar while in laminar flow hood. Do this once a week.
Continue watching for unwanted growth on the agar.
Transfer nymphs to an Erlenmeyer flask (with LB agar at the bottom), when space becomes tight. This should also be done in a laminar flow hood.