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Title: Colonization with Murine *pks+* *Escherichia coli* Under Non-Inflammatory Conditions

Authors and Affiliations:

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Author Questionnaire

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

- 3. Filming location:** Will the filming need to take place in multiple locations? **No**

- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 22

Number of Shots: 47

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

The answers are edited in accordance with the journal guidelines

INTRODUCTION:

~~**REQUIRED:** Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Claire McCartney:** This protocol describing *pks+* *E. coli* colonization and quantification in mice allows for the study of host-microbiota interactions involving a key pathobiont implicated in colorectal cancer development.

- 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~**Describe the research gap or limitation in existing methods and how this protocol addresses them.**~~

- 1.2. **Claire McCartney:** In comparison to other methods that utilize genetically or chemically-induced models of colon inflammation, this protocol describes colonization under non-inflammatory conditions.

- 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

~~**What does this protocol allow researchers to study or measure?**~~

- 1.3. **Claire McCartney:** This protocol describes several methods for the validation of *pks+* *E. coli* colonization and quantification of *pks+* *E. coli* growth by bacterial culturing and PCR amplification.

- 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~**Are there any additional methods or analyses that can be performed following this procedure?**~~

- 1.4. **Claire McCartney:** This method can be combined with one of several colorectal cancer models, allowing for the assessment of the *pks+* *E. coli*-associated mutagenesis in colon tissue.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Claire McCartney:** This method can be used to study microbiota-targeting methods to limit *pks+* *E. coli* expansion and mutagenic potential in healthy hosts.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Institutional Animal Care Committee of the Centre de recherche du Centre hospitalier de l'Université de Montréal

Protocol

2. Antibiotic Treatment of the Mouse and Preparation of *E. coli* NC101 Inoculum

Demonstrator: Claire McCartney

- 2.1. To begin, add ampicillin, colistin, and streptomycin to a container [1] and then pour sterile drinking water to dilute the antibiotics [2-TXT].
 - 2.1.1. WIDE: Talent adding the antibiotics to the container containing water. **TXT: Ampicillin: 1 mg/mL; Colistin: 1 mg/mL; Streptomycin: 5 mg/mL**
 - 2.1.2. Talent adding sterile drinking water to the antibiotic container.
- 2.2. Let the animal access the antibiotic-containing drinking water for 3 days [1].
 - 2.2.1. Talent placing the antibiotic drinking water bottle into the animal cage.
- 2.3. Then, remove the antibiotic-containing drinking water [1] and restore access to sterile drinking water for a 24-hour washout period before the gavage [2].
 - 2.3.1. Talent removing the antibiotic water bottle from the animal cage.
 - 2.3.2. Talent placing a bottle containing sterile drinking water onto the cage.
- 2.4. Meanwhile, using an inoculating loop, pick a single colony of *Escherichia coli* NC101 from the Luria–Bertani agar and inoculate it in 5 milliliters of sterile Luria–Bertani broth [1]. Incubate the culture overnight at 37 degrees Celsius in a benchtop incubation shaker at 150 revolutions per minute [2].
 - 2.4.1. Talent picking a single bacterial colony from agar plate and inoculating a culture tube containing 5 milliliters of sterile Luria–Bertani broth.
 - 2.4.2. Talent placing the inoculated culture tube into a benchtop incubation shaker.
- 2.5. Then, centrifuge the culture tube at 1500 *g* for 5 minutes at room temperature [1]. Remove and discard the supernatant while avoiding disturbance of the bacterial pellet [2]. The bacterial pellet appears as an off-white deposit at the base of the tube [3].
 - 2.5.1. Talent placing the culture tube into a centrifuge.
 - 2.5.2. Talent carefully aspirating and discarding the supernatant from the culture tube.

2.5.3. Close-up shot of the off-white bacterial pellet at the base of the tube.

2.6. Resuspend the bacterial pellet in 5 milliliters of 0.9 percent sterile saline [1-TXT].

2.6.1. Talent resuspending the bacterial pellet in the tube with 5 milliliters of sterile saline by pipetting. **TXT: Centrifuge again and wash with 0.9% saline**

2.7. After the second wash, measure the optical density of the suspension at 600 nanometers using a cell density meter [1]. Adjust the optical density at 600 nanometers to 0.625, corresponding to approximately 1×10^8 colony-forming units per mouse, using sterile saline [2].

2.7.1. Talent placing the bacterial suspension into a spectrophotometer to measure optical density at 600 nanometers.

2.7.2. Talent diluting the suspension with saline to adjust OD600.

3. Oral Gavage of *E. coli* NC101 into the Mouse

Author's NOTE: Show 3.2 first and then 3.1.

The correct order of shots here should be Added shot (checking size), 3.2.1 (fitting needle syringe), 3.1.1 (mixing bacteria) and 3.2.1 (drawing bacteria to syringe)

3.1. Gently agitate the bacterial inoculum to ensure equal distribution of the bacteria [1]. Fill the syringe with 0.2 milliliters of the bacterial inoculum and remove any air bubbles [2].

3.1.1. Talent gently mixing the bacterial inoculum tube by hand.

3.2.2. Talent drawing 0.2 milliliters of bacterial inoculum into the syringe and removing air bubbles. **NOTE: 3.2.2 is move here after 3.1.1**

3.2. Select an appropriate gavage needle based on the size of the animal [1]. Attach the gavage needle to a 1-milliliter syringe [2].

Added shot: Talent checking the size of the gavage needle by aligning gavage needle next to the mouse from mouth to base of ribcage.

3.1.1. Talent fitting a gavage needle onto a 1 milliliter syringe.

3.2. Now, remove the mouse from the cage [1]. Scruff the mouse by grasping the skin just behind the ears to the shoulder blades using the thumb pad and the side of the index finger [2]. Secure the tail with the middle or ring finger of the same hand [3].

- 3.2.1. Talent removing a mouse from the cage.
- 3.2.2. Talent scruffing the mouse by grasping the skin behind the ears to the shoulder blades.
- 3.2.3. Talent securing the mouse tail with the middle or ring finger while maintaining the scruff.
- 3.3. Hold the mouse in an upright position [1]. Insert the gavage needle into the side of the mouth [2]. Glide the tip of the gavage needle along the roof of the mouth into the esophagus and stomach while gently pushing the mouse head back to extend the neck [3-TXT].
 - 3.3.1. Talent holding the scruffed mouse upright.
 - 3.3.2. Talent inserting the gavage needle into the side of the mouse mouth.
 - 3.3.3. Talent advancing the gavage needle along the roof of the mouth toward the esophagus while extending the mouse neck. **TXT: Remove the gavage needle if resistance is felt**
- 3.4. Slowly inject 0.2 milliliters of the bacterial inoculum [1] and withdraw the gavage needle following the same angle as insertion [2]. Monitor the mouse 5 minutes after gavage for signs of respiratory distress and discomfort [3-TXT].
 - 3.4.1. Talent depressing the syringe plunger to inject the bacterial inoculum.
 - 3.4.2. Talent withdrawing the gavage needle from the mouse mouth.

Added shot: Talent monitoring the mouse after gavage for signs of respiratory distress/discomfort. **TXT: Check the breathing pattern and activity of the mouse after gavage and before leaving the animal room**
- 3.5. Return the mouse to the cage [1] and place the animal on a diet supplemented with inulin or a no-fiber control diet [2]. **Authors: This point has been introduced to provide context to the results**
 - 3.5.1. Talent placing the mouse back into the cage.
 - 3.5.2. Talent placing the diet food pellets near the cage.
- 4. **Collection of Mouse Fecal Samples and Quantification of *E. coli* NC101 by CFU Enumeration**

- 4.1. Collect a fecal pellet from each mouse in a 1.5-milliliter microcentrifuge tube by scruffing the mouse as previously demonstrated [1]. Alternatively, place the mouse in a clean empty cage and wait up to 30 minutes for defecation to occur [2].
 - 4.1.1. Talent scruffing a mouse while holding a 1.5 milliliter microcentrifuge tube beneath the mouse to collect a fecal pellet.
 - 4.1.2. Talent placing the mouse into a clean empty cage to allow fecal pellet collection.
- 4.2. Snap-freeze the sample by placing the microcentrifuge tube in liquid nitrogen [1-TXT].
 - 4.2.1. Talent placing the microcentrifuge tube containing the fecal pellet into liquid nitrogen. **TXT: The sample can be frozen at -80 °C until analysis**
- 4.3. Next, weigh an empty 1.5-milliliter microcentrifuge tube [1].
 - 4.3.1. Talent placing an empty 1.5 milliliter microcentrifuge tube on a balance to record its weight.
- 4.4. Add approximately 20 milligrams of stool to the 1.5 milliliter microcentrifuge tube [1] and record the weight of the tube containing the stool sample [2].
 - 4.4.1. Talent placing approximately 20 milligrams of stool into the 1.5 milliliter microcentrifuge tube.
 - 4.4.2. ~~Talent weighing the microcentrifuge tube containing the stool sample on a balance.~~ **NOTE: delete**
- 4.5. Now, add 200 microliters of 0.9 percent sterile saline to the tube [1].
 - 4.5.1. Talent pipetting 200 microliters of 0.9 percent sterile saline into the microcentrifuge tube containing the stool sample.
- 4.6. After 10 to 15 minutes, vortex the tube for 5 minutes at maximum speed to produce a homogeneous suspension [1]. Small particles of undigested food material may remain visible [2].
 - 4.6.1. Talent placing the microcentrifuge tube into a vortex mixer.
 - 4.6.2. Close-up shot of the homogenized stool suspension in the tube showing small particles of undigested material.

- 4.7. Transfer 100 microliters of each suspension onto a MacConkey agar plate [1] and evenly spread the homogenate using an L-shape spreader [2].
 - 4.7.1. Talent pipetting 100 microliters of the stool suspension onto a MacConkey agar plate.
 - 4.7.2. Talent spreading the suspension across the agar surface using an L-shape spreader.
- 4.8. Incubate the plates overnight at 37 degrees Celsius [1].
 - 4.8.1. Talent placing the MacConkey agar plate into an incubator.
- 4.9. Finally, count the number of pink colonies on each plate [1]. Normalize the colony counts by the weight of each fecal pellet and the plated volume [2]. Extract genomic DNA from the fecal samples [3]. Quantify the *clb* (C-L-B) genes and *Enterobacteriaceae* abundance using quantitative PCR with gene-specific and family-specific primers [4].
 - 4.9.1. Close-up shot of pink colonies on a MacConkey agar plate.
 - 4.9.2. Talent recording colony counts in a book while looking at the plate.
 - 4.9.3. Talent adding NDA-extraction buffer to the fecal suspension.
 - 4.9.4. Talent placing a PCR tube with reaction mix in a qPCR machine.

Results

5. Results

5.1. The amplification and visualisation of the *clbA* (*C-L-B-A*) and *E. coli* 16S genes in feces at the endpoint by PCR and gel electrophoresis showed that [1], while both genes were visible in *E. coli* NC101-colonized mice [2], only the *E. coli* 16S gene was visible in control mice, confirming that *E. coli* NC101 was not present [3].

5.1.1. LAB MEDIA: Figure 2B.

5.1.2. LAB MEDIA: Figure 2B. *Video editor: Highlight the lanes "EcNC101"*

5.1.3. LAB MEDIA: Figure 2B. *Video editor: Highlight the "saline" lanes*

5.2. After 1 week, mice receiving the inulin-supplemented diet showed greater colony-forming unit recovery from fecal homogenates cultured on MacConkey agar compared to the no-fiber condition [1].

5.2.1. LAB MEDIA: Figure 2C. *Video editor: Highlight the image and data points for group labeled "INU" for "EcNC101"*.

5.3. After 1 week, mice receiving the inulin-supplemented diet showed increased levels of the colibactin P gene [1] and *Enterobacteriaceae* abundance compared to the no-fiber condition [2].

5.3.1. LAB MEDIA: Figure 2D. *Video editor: Highlight the group labeled "INU - EcNC101"*

5.3.2. LAB MEDIA: Figure 2E. *Video editor: Highlight the group labeled "INU EcNC101"*

1. Ampicillin

Pronunciation link: <https://www.merriam-webster.com/dictionary/ampicillin>

IPA: /,æm.pə'sɪl.ɪn/

Phonetic Spelling: am·puh·sil·in

2. Colistin

Pronunciation link: <https://www.merriam-webster.com/dictionary/colistin>

- IPA: /kə'lis.tɪn/
Phonetic Spelling: kuh·lis·tin
3. Streptomycin
Pronunciation link: <https://www.merriam-webster.com/dictionary/streptomycin>
IPA: /,strep.tə'maɪ.sɪn/
Phonetic Spelling: strep·tuh·my·sin
 4. Gavage
Pronunciation link: <https://www.merriam-webster.com/dictionary/gavage>
IPA: /gə'vɑːʒ/
Phonetic Spelling: guh·vaazh
 5. Inoculating
Pronunciation link: <https://www.merriam-webster.com/dictionary/inoculate>
IPA: /ɪ'noː.kjə.leɪt/
Phonetic Spelling: ih·naa·kyuh·layt
 6. Escherichia coli
Pronunciation link: <https://www.merriam-webster.com/dictionary/Escherichia%20coli>
IPA: /,ɛʃ.ə'riːk.i.ə'koʊ.laɪ/
Phonetic Spelling: esh·uh·rik·ee·uh koh·ly
 7. Incubate
Pronunciation link: <https://www.merriam-webster.com/dictionary/incubate>
IPA: /'ɪn.kjə.bert/
Phonetic Spelling: in·kyuh·bayt
 8. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /'sen.trə'fjuːdʒ/
Phonetic Spelling: sen·truh·fyooj
 9. Supernatant
Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>
IPA: /,suː.pər'nei.tənt/
Phonetic Spelling: soo·per·nay·tuhtnt
 10. Pellet
Pronunciation link: <https://www.merriam-webster.com/dictionary/pellet>
IPA: /'pel.ɪt/
Phonetic Spelling: pel·it
 11. Resuspend
Pronunciation link: <https://www.merriam-webster.com/dictionary/resuspend>
IPA: /,riː.sə'spend/
Phonetic Spelling: ree·suh·spend
 12. Saline
Pronunciation link: <https://www.merriam-webster.com/dictionary/saline>
IPA: /'seɪ.laɪn/
Phonetic Spelling: say·line
 13. Optical
Pronunciation link: <https://www.merriam-webster.com/dictionary/optical>

- IPA: /'ɑ:p.tɪ.kəl/
Phonetic Spelling: op·tuh·kuhl
14. Spectrophotometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/spectrophotometer>
IPA: /,spek.troʊ.fə'tɑ:.mə.tər/
Phonetic Spelling: spek·troh·fuh·taa·muh·ter
15. Inoculum
Pronunciation link: <https://www.merriam-webster.com/dictionary/inoculum>
IPA: /ɪ'no:.kjə.ləm/
Phonetic Spelling: ih·naa·kyuh·luhm
16. Esophagus
Pronunciation link: <https://www.merriam-webster.com/dictionary/esophagus>
IPA: /ɪ'sɑ:.fə.gəs/
Phonetic Spelling: ih·saa·fuh·guhs
17. Microcentrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/microcentrifuge>
IPA: /,maɪ.kroʊ'sen.trə,fju:dʒ/
Phonetic Spelling: my·kroh·sen·truh·fyooj
18. Vortex
Pronunciation link: <https://www.merriam-webster.com/dictionary/vortex>
IPA: /'vɔ:r.teks/
Phonetic Spelling: vor·teks
19. Homogenate
Pronunciation link: <https://www.merriam-webster.com/dictionary/homogenate>
IPA: /hə'mɑ:.dʒə'neɪt/
Phonetic Spelling: huh·maa·juh·nayt
20. MacConkey
Pronunciation link: No confirmed link found
IPA: /mə'kɑ:ŋ.ki/
Phonetic Spelling: muh·kong·kee
21. Incubator
Pronunciation link: <https://www.merriam-webster.com/dictionary/incubator>
IPA: /'ɪn.kjə'beɪ.tər/
Phonetic Spelling: in·kyuh·bay·ter
22. Enterobacteriaceae
Pronunciation link: No confirmed link found
IPA: /,ɛn.tə.roʊ.bæk'tɪr.i'eɪ.sɪ.i:/
Phonetic Spelling: en·tuh·roh·bak·teer·ee·ay·see·ee
23. Quantitative
Pronunciation link: <https://www.merriam-webster.com/dictionary/quantitative>
IPA: /'kwɑ:n.tə'teɪ.tɪv/
Phonetic Spelling: kwan·tuh·tay·tiv
24. PCR (Polymerase Chain Reaction)
Pronunciation link: <https://www.merriam-webster.com/dictionary/PCR>

IPA: /ˌpiː.sɪˈɑːr/

Phonetic Spelling: pee·see·ar

25. Electrophoresis

Pronunciation link: <https://www.merriam-webster.com/dictionary/electrophoresis>

IPA: /ɪˌlek.troʊ.fəˈriː.sɪs/

Phonetic Spelling: ih·lek·troh·fuh·ree·sis

26. Colibactin

Pronunciation link: No confirmed link found

IPA: /ˌkoʊ.lɪˈbæk.tɪn/

Phonetic Spelling: koh·lih·bak·tin