Data Article

PCR data and comparative performance of *Bacteroidales* microbial source tracking genetic markers

Pornjira Somnark a, Natcha Chyerochanab, Akechai Kongprajugb, Skorn Mongkolsuk b,c,d, Kwanrawee Sirikanchanab,d,*

a Applied Biological Sciences, Chulabhorn Graduate Institute, Chulabhorn Royal Academy, Lak Si, Bangkok 10210 Thailand
b Research Laboratory of Biotechnology, Chulabhorn Research Institute, Lak Si, Bangkok 10210 Thailand
c Department of Biotechnology, Faculty of Science, Mahidol University, Bangkok 10400 Thailand
d Center of Excellence on Environmental Health and Toxicology (EHT), Ministry of Education, Bangkok 10210 Thailand

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**A B S T R A C T**

We reported modified endpoint PCR results analyzed by universal and human-, swine-, and cattle-specific *Bacteroidales* gene markers with human sewage and animal fecal samples (i.e., swine, cattle, chicken, goat, sheep, buffalo, and duck) from Tha Chin and Chao Phraya watersheds. Annealing locations of PCR primers were illustrated by maps of 16s rRNA *Bacteroidales* genes. We also summarized previously published work on the performance of the PCR assays. For further discussion of the data presented here, please refer to Somnark et al., Performance evaluation of *Bacteroidales* genetic markers for human and animal microbial source tracking in tropical agricultural watersheds, Environ. Pollut. 236 (2018) 100–110.

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* Corresponding author at: Research Laboratory of Biotechnology, Chulabhorn Research Institute, Lak Si, Bangkok, Thailand.
E-mail address: kwanrawee@cri.or.th (K. Sirikanchana).
### Specifications Table

| Subject area       | Biology                        |
|--------------------|--------------------------------|
| More specific subject area | Applied microbiology           |
| Type of data       | Tables and figures             |
| How data were acquired | PCR instrument (Mastercycler Pro thermocycler, Eppendorf), and literature review |
| Data format        | Analyzed                       |
| Experimental factors | Composite fecal and sewage samples were collected, and DNA extraction was performed |
| Experimental features | PCR primers originally designed as endpoint and quantitative PCR were used in the modified endpoint PCR assays. |
| Data source location | Samples were collected from Tha Chin (Chai Nat, Suphan Buri, Nakhon Pathom, and Samut Sakhon provinces) and Chao Phraya (Phra Nakhon Si Ayutthaya, Pathum Thani, and Bangkok provinces) watersheds, located in the central part of Thailand. |
| Data accessibility | Data are with this article      |

### Value of the data

- PCR results of *Bacteroidales*-modified endpoint PCR markers could be compared with microbial source tracking (MST) studies in other geographic areas for further development of region-specific MST methods.
- *Bacteroidales* PCR primer maps could offer an insight into annealing regions of primers for further design of new primers or evaluating currently available primers with their performance.
- A summary of PCR assays that are originally designed and adopted to other regions could serve as a database for comparing the MST method performance in different geographical areas.

### 1. Data

We performed endpoint PCR assays modified from published methods originally in PCR and qPCR platforms. PCR results of ten good-performing modified endpoint PCR assays against human sewage and animal fecal samples from Tha Chin and Chao Phraya watersheds are shown (Table 1). There were six modified endpoint PCR assays that demonstrated potentially low sensitivity or specificity during the process of testing against a limited number of samples and therefore were not further tested with total samples (Table 2). We also compiled sensitivity and specificity data of previously published *Bacteroidales* genetic markers from both studies that originally designed the assays and studies that adopted the designed assays to be used in another geographic location (Table 3). To provide further insight into PCR performance, we mapped PCR primers to 16s rRNA gene of human-, swine-, and cattle-associated *Bacteroidales* (Figs. 1 and 2). Amplified PCR products with universal and human-, swine-, and cattle-specific *Bacteroidales* PCR assays were presented (Fig. 3).

### 2. Experimental design, materials and methods

#### 2.1. Sample collection and DNA extraction

Raw human sewage and non-human fecal samples were collected from Tha Chin and Chao Phraya watersheds. One composite fecal sample was prepared by mixing fresh feces of at least 20 individuals. Samples were transported on ice to the laboratory. DNA extraction of composite fecal samples and 0.22-μm-pore-size mixed cellulose ester membrane (Merck Millipore, Billerica, MA, USA) after 50-
Table 1
Positive PCR results of modified endpoint PCR markers showing good performance with samples from Tha Chin and Chao Phraya watersheds.

| Host   | Assay name                  | Tha Chin watershed | Chao Phraya watershed |
|--------|-----------------------------|---------------------|-----------------------|
|        |                             | Human (19)          | Swine (20)            |
|        |                             | Cattle (20)         | Chicken (19)          |
|        |                             | Goat (7)            | Sheep (5)             |
|        |                             | Buffalo (5)         | Duck (5)              |
| Universal | BacUni EP                   | 19                  | 20                    |
|         | GenBac3 EP                  | 19                  | 20                    |
|         | Bac32F/Bac708R              | 15                  | 20                    |
| Human  | BacHum EP                   | 18                  | 17                    |
|        | HF183F/BFDrev EP            | 16                  | 4                     |
|        | Modified HF183F/Bac708R     | 18                  | 4                     |
| Swine  | Pig-2-Bac EP               | 0                   | 20                    |
|        |                             | 0                   | 0                     |
| Cattle | Bac2                        | 0                   | 0                     |
|        | Bac3                        | 0                   | 0                     |
|        | Cow-Bac2 EP                 | 0                   | 1                     |

| Host   | Assay name                  | Tha Chin watershed | Chao Phraya watershed |
|--------|-----------------------------|---------------------|-----------------------|
|        |                             | Human (9)           | Swine (8)             |
|        |                             | Cattle (5)          | Chicken (2)           |
|        |                             | Goat (3)            | Buffalo (1)           |
| Universal | BacUni EP                   | 9                   | 8                     |
|         | GenBac3 EP                  | 9                   | 8                     |
|         | Bac32F/Bac708R              | 9                   | 8                     |
| Human  | BacHum EP                   | 9                   | 7                     |
|        | HF183F/BFDrev EP            | 9                   | 0                     |
|        | Modified HF183F/Bac708R     | 9                   | 0                     |
| Swine  | Pig-2-Bac EP               | 0                   | 8                     |
|        |                             | 0                   | 0                     |
| Cattle | Bac2                        | 0                   | 5                     |
|        | Bac3                        | 0                   | 5                     |
|        | Cow-Bac2 EP                 | 0                   | 5                     |
100 mL human sewage filtration was performed with a ZR Fecal DNA MiniPrep kit (Zymo Research, Irvine, CA, USA). DNA concentrations were measured using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

2.2. **PCR method modification and performance criteria**

PCR primers targeting universal and human-, swine-, and cattle-specific fecal markers were selected from both endpoint and quantitative PCR platforms (Table 4). A 10-μL PCR is composed of 0.5 μL each of 10 μM forward primers and 10 μM reverse primers, 1-μL of DNA template (corresponding to 0.2, 2.0 or 20 ng total DNA), 5 μL of DreamTaq PCR Master Mix (2×; Thermo Fisher Scientific, Waltham, MA, USA), and sterile water. The reaction was processed in a Mastercycler Pro thermocycler (Eppendorf, Hamburg, Germany). PCR cycling conditions were modified as follows: initial denaturation at 95 °C for 3 min; 30 cycles of a denaturation step at 95 °C for 30 s, an annealing step at varying temperature and time (Table 4), and an elongation step at 72 °C for 30 s; and a final extension at 72 °C for 10 min. PCR products were visualized with a Gel Doc XR system (BIO-RAD, Hercules, CA, USA). PCRs were run in duplicate. No-template controls and extraction blanks were included for quality control. Performance criteria including sensitivity, specificity, and accuracy were calculated as TP/(TP+FN), TN/(TN+FP), and (TP+TN)/(TP+FP+TN+FN), respectively, where TP, FN, TN, and FP, are true positive, false negative, true negative, and false positive, respectively.

| Fecal origin | Assay name | No. of positive samples/no. of samples tested | Sensitivity | Specificity | Accuracy |
|--------------|------------|---------------------------------------------|-------------|------------|----------|
| Human        | Swine      | PF163F/Bac708R 0/0                            | 1.00        | 0.77       | 0.83     |
|              | Swine      | CowM2 EP 0/0                                  | 1.00        | 0.00       | 0.54     |
|              | Swine      | BacCow EP 0/0                                 | 1.00        | 0.00       | 0.54     |
|              | Swine      | CF193F/Bac708R 0/0                            | 1.00        | 0.00       | 0.54     |
|              | Swine      | CF128F/Bac708R 0/0                            | 1.00        | 0.05       | 0.54     |
|              | Cattle     | BoBac EP 0/0                                  | 1.00        | 0.05       | 0.54     |

* Limited number of animal samples tested for certain assays due to potentially low sensitivity or specificity.
| Host source | Assay name | Platform | Geographical region | Original/Adopted | Sensitivity (n)a | Specificity (n) | Non-target hosts | Reference |
|-------------|------------|----------|---------------------|------------------|-----------------|-----------------|------------------|-----------|
| Universal BacUni | qPCR | California, USA | Original | 1.00 (n=73) | NAb | Human*, cow, horse, dog, cat, seagull, WWTPd (primary influent) | [1] |
| PCR | Tha Chin watershed, Thailand | Adopted | 1.00 (n=100, composite) | NA | Swine, cattle, chicken, goat, sheep, buffalo, duck, sewage | [2] |
| PCR | Chao Phraya watershed, Thailand | Adopted | 0.96 (n=28, composite) | NA | Swine, cattle, chicken, goat, buffalo, sewage | [2] |
| Universal GenBac3 | qPCR | Louisiana, Michigan, Mississippi, USA | Original | NA | NA | Surface water sample | [3] |
| PCR | Tha Chin watershed, Thailand | Adopted | 1.00 (n=100, composite) | NA | Swine, cattle, chicken, goat, sheep, buffalo, duck, sewage | [2] |
| Chao Phraya watershed, Thailand | Adopted | 1.00 (n=28, composite) | NA | Swine, cattle, chicken, goat, buffalo, sewage | [2] |
| Universal Bac32F/ Bac708R | PCR | Oregon, USA | Original | 1.00 (n=30) | NA | Human, cow | [4] |
| PCR | Southeast Queensland, Australia | Adopted | 1.00 (n=186) | NA | Cattle, pig, sheep, goat, horse, chicken, dog, duck, pelican, kangaroo, WWTP | [5] (one base pair mismatch for Bac32F primer) |
| PCR | Wisconsin, USA | Adopted | 1.00 (n=89) | NA | Cow, WWTP | [6] |
| PCR | Missouri, USA | Adopted | 0.89 (n=286) | NA | Human, sewage, dog, beef cattle, dairy cattle, chicken, turkey, horse, swine, goose | [7] |
| PCR | Brittany and Normandy, France | Adopted | 0.96 (n=136) | NA | Pig, cow, sheep, chicken, wild bird | [8] |
| Saskatchewan, Canada | Adopted | 1.00 (n=273) | NA | Human, WWTP, cow, pig, chicken, goose, moose, deer, caribou, bison, goat | [9] |
| PCR | Illinois, Nebraska, Ohio, Texas, Delaware, and West Virginia, USA | Adopted | 0.78 (n=222) | NA | Cattle, human, chicken, raccoon, horse, pig, pig manure pit, pig waste lagoon | [10] |
| PCR | Puerto Rico, USA | Adopted | 0.89 (n=356) | NA | Cow, goat, horse, swine, monkey, fish, pigeon, chicken, guinea fowl, duck, turkey, swan, WWTP | [11] |
| PCR | Tha Chin watershed, Thailand | Adopted | 0.96 (n=100, composite) | NA | Swine, cattle, chicken, goat, sheep, buffalo, duck, sewage | [2] |
| Chao Phraya watershed, Thailand | Adopted | 1.00 (n=28, composite) | NA | Swine, cattle, chicken, goat, buffalo, sewage | [2] |
| Species | Location | qPCR Type | Sample Type | Sensitivity | Specificity | Species Detected |
|---------|----------|-----------|-------------|-------------|-------------|------------------|
| Human | California, USA | BacHum qPCR | Original | 0.67 (n = 18); 1.00 (n = 14, sewage) | 0.98 (n = 41) | Cow, horse, dog, cat, seagull |
| PCR | Southeast Queensland, Australia | Adopted | 1.00 (n = 50, WWTP) | 0.96 (n = 136) | Cattle, pig, sheep, goat, horse, chicken, dog, duck, pelican, kangaroo |
| PCR | Tha Chin watershed, Thailand | Adopted | 0.95 (n = 19, sewage) | 0.54 (n = 81, composite) | Swine, cattle, chicken, goat, sheep, buffalo, duck |
| Chao Phraya watershed, Thailand | Adopted | 1.00 (n = 9, sewage) | 0.53 (n = 19, composite) | Swine, cattle, chicken, goat, buffalo |
| Human | Michigan, Minnesota, Colorado, South Dakota, Wyoming, Hawaii, Virginia, Ohio, Florida, North Carolina, and New York, USA | HF183/ BFDrev qPCR | Original | 1.00 (n = 14, WWTP) | 0.60 (n = 5, composite) | Cow, pig, chicken, dog, cat |
| PCR | Tha Chin watershed, Thailand | Adopted | 0.84 (n = 19, sewage) | 0.77 (n = 81, composite) | Swine, cattle, chicken, goat, sheep, buffalo, duck |
| Chao Phraya watershed, Thailand | Adopted | 1.00 (n = 9, sewage) | 1.00 (n = 19, composite) | Swine, cattle, chicken, goat, buffalo |
| Human | Oregon, USA | HF183/ Bac708R qPCR | Original | 0.85 (n = 13); 1.00 (n = 3, WWTP) | 1.00 (n = 46) | Cow, deer, elk, cat, dog, duck, pig, gull, goat, llama, sheep |
| PCR | Southeast Queensland, Australia | Adopted | 1.00 (n = 52, WWTP) | 1.00 (n = 155) | Duck, kangaroos, cattle, horse, dog, chicken, pig, pelican, goat, deer, wild birds, sheep |
| PCR | Spain | Adopted | 0.50 (n = 40, WWTP) | 0.71 (n = 73) | Poultry, pig, cow |
| PCR | Southeast Queensland, Australia | Adopted | 1.00 (n = 59, WWTP); 0.80 (n = 20) | 0.95 (n = 214) | Bird, camel, cattle, chicken, dog, duck, horse, kangaroo, pig, possum |
| PCR | Brittany and Normandy, France | Adopted | 0.98 (n = 44); 0.75 (n = 16, sewage WWTP) | 0.99 (n = 86) | Pig, cow, sheep, chicken, wild bird |
| PCR | Puerto Rico, USA | Adopted | 1.00 (n = 16, sewage WWTP) | 1.00 (n = 340) | Cow, goat, horse, swine, monkey, fish, pigeon, chicken, guinea fowl, duck, turkey, swan |
| PCR | Wisconsin, USA | Adopted | 1.00 (n = 14, WWTP) | 1.00 (n = 75) | Cow |
| PCR | Saskatchewan, Canada | Adopted | 1.00 (n = 8, WWTP); 0.94 (n = 54) | 1.00 (n = 211) | Cow, pig, chicken, goose, moose, deer, caribou, bison, goat |
| PCR | Tha Chin watershed, Thailand | Adopted | 0.95 (n = 19, sewage) | 0.70 (n = 81, composite) | Swine, cattle, chicken, goat, sheep, buffalo, duck |
Table 3 (continued)

| Host source | Assay name | Platform | Geographical region | Original/Adopted | Sensitivity (n)* | Specificity (n) | Non-target hosts | Reference |
|-------------|------------|----------|---------------------|------------------|-----------------|----------------|------------------|-----------|
| Swine       | PF163F/| PCR      | Chao Phraya watershed, Thailand | Adopted          | 1.00 (n=9, sewage) | 0.68 (n=19, composite) | Swine, cattle, chicken, goat, buffalo | [2]       |
|             | Bac708R   |          |                     |                  |                 |                |                  |           |
|             | Swine     | PCR      | Cincinnati, Ohio    | Original         | 1.00 (n=19)     | NA              | NA               | [17]      |
|             |           |          | Saskatchewan, Canada | Adopted          | 1.00 (n=50)     | 1.00 (n=223)   | Human, WWTP, cow, chicken, goose, moose, deer, caribou, bison, goat | [9]       |
|             |           | PCR      | Illinois, Nebraska, Ohio, Texas, Delaware, and West Virginia, USA | Adopted | 0.87 (n=97); 1.00 (n=6, slurry) | 0.77 (n=119) | Human, chicken, raccoon, horse | [10]      |
|             |           | PCR      | Puerto Rico, USA    | Adopted          | 1.00 (n=30)     | 0.75 (n=261)   | Human, cow, sheep, chicken, wild bird | [8]       |
|             |           | PCR      | Brittany and Normandy, France | Adopted       | 1.00 (n=25)     | 0.98 (n=105) | Cow, goat, horse, monkey, fish, pigeon, chicken, guinea fowl, duck, swan | [11]      |
|             |           | PCR      | Tha Chin watershed, Thailand | Adopted  | 1.00 (n=20, composite) | 0.77 (n=61, composite) | Cattle, chicken, goat, sheep, buffalo, duck | [2]       |
|             | Swine     | qPCR     | Brittany, France    | Original | 1.00 (n=25); 1.00 (n=23, slurry) | 1.00 (n=54) | Human, bovine, horse, sheep | [18]      |
|             |           | PCR      | Tha Chin watershed, Thailand | Adopted          | 1.00 (n=20, composite) | 0.98 (n=80, composite) | Human, horse, pig, gull, turkey | [12]      |
| Cattle      | CowM2     | qPCR     | West Virginia, Georgia, Wyoming, Delaware, Florida, and Ohio, USA | Original | 1.00 (n=60) | 1.00 (n=20, composite) | Cattle, chicken, goat, buffalo, sewage | [2]       |
|             |           | PCR      | Tha Chin watershed, Thailand | Adopted          | 0.86 (n=7, composite) | 0.00 (n=6, composite) | Swine, chicken, goat | [2]       |
| Cattle      | BacCow    | qPCR     | California, USA      | Original         | 1.00 (n=8)     | 0.95 (n=65)    | Human, horse, dog, cat, seagull, WWTP (primary effluent) | [1]       |
|             |           | PCR      | Tha Chin watershed, Thailand | Adopted          | 1.00 (n=7, composite) | 0.00 (n=6, composite) | Swine, chicken, goat | [2]       |
| Cattle      | CF193/    | PCR      | Oregon, USA          | Original         | 1.00 (n=19)     | 0.72 (n=43)    | Human, WWTP, deer, elk, cat, dog, duck, pig, gull, goat, llama, sheep | [13]      |
|             | Bac708R   |          |                      |                  |                 |                |                  |           |
|             | Swine     | PCR      | Wisconsin, USA       | Adopted          | 0.85 (n=75)     | NA              | NA               | [6]       |
|             |           | PCR      | Saskatchewan, Canada | Adopted          | 0.16 (n=32)     | NA              | NA               | [9]       |
| Country/Region | PCR Type | Original/Adopted | Animal Species |
|---------------|----------|-----------------|----------------|
| Spain, UK, Cyprus, France, and Sweden | PCR | Adopted | 0.00 (n = 19, ruminant) 0.99 (n = 94) WWTP, poultry, pig |
| USA | PCR | Adopted | 0.68 (n = 247) 1.00 (n = 175) Alpaca, pronghorn, elk, gazelle, giraffe, goat, mule deer, okapi, sheep, takin, tufted deer, moose, white-tailed deer, Canadian goose, cat, chicken, dog, duck, horse, human, pelican, pig, raccoons, sea gull, turkey |
| Tha Chin watershed, Thailand | PCR | Adopted | 0.00 (n = 7, composite) 1.00 (n = 6, composite) Swine, chicken, goat |
| Oregon, USA | Cattle CF128F/Bac708R PCR | Original | 1.00 (n = 19) 0.77 (n = 43) Human, WWTP, deer, elk, cat, dog, duck, pig, gull, goat, llama, sheep |
| Wisconsin, USA | PCR | Adopted | 1.00 (n = 75) 0.93 (n = 14) WWTP |
| Brittany and Normandy, France | PCR | Adopted | 1.00 (n = 32) 0.60 (n = 98) Human, pig, chicken, sheep, wild bird |
| Saskatchewan, Canada | PCR | Adopted | 0.96 (n = 51, cow); 0.62 (n = 222, cow); 0.93 (n = 152, ruminant = cow, deer, caribou, bison, moose, goat) Human, WWTP, pig, chicken, goose |
| Spain | PCR | Adopted | 0.26 (n = 19, ruminant) 1.00 (n = 95) WWTP, poultry, pig |
| USA | PCR | Adopted | 0.85 (n = 247) 0.76 (n = 175) Alpaca, pronghorn, elk, gazelle, giraffe, goat, mule deer, okapi, sheep, takin, tufted deer, moose, white-tailed deer, Canadian goose, cat, chicken, dog, duck, horse, human, pelican, pig, raccoons, sea gull, turkey |
| Puerto Rico, USA | PCR | Adopted | 0.64 (n = 66) 0.90 (n = 290) Goat, horse, swine, monkey, fish, pigeon, chicken, guinea fowl, duck, turkey, swan, WWTP |
| Tha Chin watershed, Thailand | PCR | Adopted | 1.00 (n = 7, composite) 0.00 (n = 6, composite) Swine, chicken, goat |
| USA | Cattle Bac2 PCR | Adopted | 0.54 (n = 148) 1.00 (n = 279) Bird, human, domestic, wildlife, pets, water by cattle Alpaca, pronghorn, elk, gazelle, giraffe, goat, mule deer, okapi, sheep, takin, tufted deer, moose, white-tailed deer, Canadian goose, cat, chicken, dog, duck, horse, human, pelican, pig, raccoons, sea gull, turkey |
| USA | PCR | Adopted | 0.54 (n = 247) 1.00 (n = 175) Alpaca, pronghorn, elk, gazelle, giraffe, goat, mule deer, okapi, sheep, takin, tufted deer, moose, white-tailed deer, Canadian goose, cat, chicken, dog, duck, horse, human, pelican, pig, raccoons, sea gull, turkey |
| Tha Chin watershed, Thailand | PCR | Adopted | 0.70 (n = 20, composite) 1.00 (n = 80, composite) Swine, chicken, goat, sheep, buffalo, duck, sewage |
| Thailand Chao Phraya watershed | PCR | Adopted | Swine, chicken, goat, buffalo, sewerage |
| Host source | Assay name | Platform | Geographical region | Original/Adopted | Sensitivity (n)* | Specificity (n) | Non-target hosts | Reference |
|-------------|------------|----------|---------------------|------------------|-----------------|----------------|-----------------|-----------|
| Cattle      | Bac3       | PCR      | USA                 | Original         | 1.00 (n=5, composite) | 1.00 (n=23, composite) | Human, sewage, bovine, chicken, black vulture, Canadian goose, peacock, pigeon, dog, cat, guinea pig, domestic goat, pig, sheep, horse, alpaca, llama, armadillo, bobcat, coyote, gray squirrel, rabbit, opossum, raccoon, whitetail deer, wild turkey, hedgehog, prairie dog Alpaca, pronghorn, elk, gazelle, giraffe, goat, mule deer, okapi, sheep, takin, tufted deer, moose, white-tailed deer, canadian goose, cat, chicken, dog, duck, horse, human, pelican, pig, raccoons, sea gull, turkey | [21] |
|             |            |          |                     | Adopted          | 0.91 (n=148)     | 0.99 (n=245)    |                 |           |
|             |            |          |                     |                  |                 |                |                 |           |
| Cattle      | Cow-Bac2   | qPCR     |                     | Original         | 1.00 (n=7)       | 1.00 (n=9)      | Human, pig      | [22] |
|             |            |          | Sapporo and Ebetsu Cities, Japan | Adopted | 1.00 (n=5, composite) | 1.00 (n=23, composite) | Swine chicken, goat, sheep, buffalo, duck, sewage | [2] |
|             |            |          |                     |                  |                 |                |                 |           |
| Cattle      | BoBac      | qPCR     | Tennessee, Pennsylvania, and Texas, USA | Adopted | 1.00 (n=11) | 0.87 (n=15) | Human, swine, canine, equine | [23] |
|             |            |          |                     |                  |                 |                |                 |           |
|             |            | PCR      | Chao Phraya watershed, Thailand | Adopted | 1.00 (n=5, composite) | 0.78 (n=23, composite) | Swine chicken, goat, buffalo, sewage | [2] |
|             |            |          |                     |                  |                 |                |                 |           |
|             |            | PCR      | Tha Chin watershed, Thailand | Adopted | 0.90 (n=20, composite) | 0.50 (n=21, composite) | Swine chicken, goat, sheep, buffalo, duck, sewage | [2] |
|             |            |          |                     |                  |                 |                |                 |           |
|             |            | PCR      | Chao Phraya watershed, Thailand | Adopted | 1.00 (n=5, composite) | 0.78 (n=23, composite) | Swine chicken, goat, buffalo, sewage | [2] |
|             |            |          |                     |                  |                 |                |                 |           |

* Total number of samples being tested.

** Not applicable.

† Human individual fecal sample.

‡ Influent of municipal wastewater treatment plant, unless stated otherwise.

§ Influent of wastewater treatment system in buildings or septic tanks.
Fig. 1. Primer map targeting the 16S rRNA gene of human- and swine-associated Bacteroidales. All primers were BLASTed against the NCBI database. The representative sequences from human feces (Accession no. AB242143.1 [24]) and swine feces (AB506329.1 [25]) were selected to align with specific primers. Human-specific, swine-specific and universal Bacteroidales primers are indicated in dotted, dashed and solid arrows, respectively.

Fig. 2. Primer map targeting the 16S rRNA gene of cattle-associated Bacteroidales. All primers were BLASTed against the NCBI database. The representative sequences (Accession nos. Q821871.1 [26], KRS14419.1, LCD28711.1, and LC028829.1) were selected to align with specific primers.
Fig. 3. PCR results showing amplification products for universal and human-, swine-, and cattle-specific Bacteroidales markers (see [2] for related information).
### Table 4
Primer sequences and PCR cycling conditions.

| Host                     | Assay name   | Primer name | Primer sequence (5′ - 3′)                                   | Annealing temperature (°C) | Annealing time (s) | Original platform | Reference |
|--------------------------|--------------|-------------|-------------------------------------------------------------|----------------------------|--------------------|-------------------|-----------|
| Universal                | BacUni EP    | BacUni-520f | CGT-TAT-CCG-GAT-ATA-TTG-GGT-TTA                            | 60.0                       | 30                 | qPCR              | [1]       |
|                          |              | BacUni-690r | CAA-TCG-TTG-TTC-CTG-ATA-TCT-A                             |                            |                    |                   |           |
| GenBac3 EP               | GenBac3F     |             | GGG-GTT-CTG-AGA-GGA-AGG-T                                  | 60.0                       | 30                 | qPCR              | [3]       |
|                          | GenBac3R     |             | CGG-TCA-TCC-AGC-CTG-ATA-TCT-CT                            |                            |                    |                   |           |
|                        | Bac32F/Bac708R| Bac32F      | AAC-GCT-AGC-TAC-AGG-CT                                      | 53.7                       | 60                 | PCR               | [4,27]    |
|                        |              | Bac708R     | CAA-TCG-GAG-TTC-TTC-CT                                      |                            |                    |                   |           |
| Human sewage            | BacHum EP    | BacHum-160f | TGA-GTT-CAC-ATG-TGG-TCA-GTA                                | 60.0                       | 30                 | qPCR              | [1]       |
|                          |              | BacHum-241r | CTG-TAC-CCC-GCC-TTC-GTA-TAT-CT                             |                            |                    |                   |           |
| HF183/BFDrev EP          | HF183        |             | ATC-AGT-CTG-GAT-CTG-CA                                     | 60.0                       | 30                 | qPCR              | [12]      |
|                        | BFDrev       |             | CTG-AGG-CTG-GAT-GAC-GT-CT                                   |                            |                    |                   |           |
|                        | Modified     | HF183F      | ATC-AGT-CTG-GAT-CTG-CT                                      | 55.3                       | 60                 | PCR               | [13,27]   |
|                        | HF183F/Bac708R| Bac708R    | CAA-TGG-TAC-GTG-CTG-CT                                      |                            |                    |                   |           |
| Swine                   | PF163F/Bac708R| PF163F      | GCG-GAT-TAC-GTT-CTG-AGAT-A                                  | 52.4                       | 60                 | PCR               | [17,27]   |
|                        |              | Bac708R     | CAA-TCG-TTC-CTG-CTG-CT                                      |                            |                    |                   |           |
|                        | Pig-2-Bac EP | Pig-2-Bac41F| GCA-CTC-ATT-TAG-CTG-CT-AAA-CTT-GAT                         | 60.0                       | 30                 | qPCR              | [18]      |
|                        |              | Pig-2-Bac163Rm | ACC-CTC-ATT-CTG-ATG-CCA-CTG-C                              |                            |                    |                   |           |
| Cattle                  | CowM2 EP     | CowM2F      | CGG-CCA-ACT-CTG-CAT-CTG-CT                                  | 60.0                       | 30                 | qPCR              | [19]      |
|                        |              | CowM2R      | CCA-ACG-TTC-CTC-ATA-TGT-GA                                  |                            |                    |                   |           |
|                        | BacCow EP    | CF128F      | GGA-CCT-CTG-GTT-AGT-AGT-CT                                  | 60.0                       | 30                 | PCR               | [1]       |
|                        |              | CF193       | TAT-GAA-TGC-TGC-CTG-CTC                                     | 55.0                       | 30                 | PCR               | [13]      |
|                        | CF193/F/Bac708R| Bac708R    | CAA-TCG-GAG-TTC-CTG-CT                                      |                            |                    |                   |           |
|                        | Modified     | CF128F      | CCA-ACG-TTC-CTC-CTG-CT                                      | 62.0                       | 60                 | PCR               | [13,28]   |
|                        | CF128F/Bac708R| Bac708R     | CAA-TGG-TAC-GTG-CTG-CT                                      |                            |                    |                   |           |
|                        | Bac2         | Bac2F       | GCT-CTG-GTG-CTG-CAT-ATG-GAT-ATAT                           | 62.0                       | 30                 | PCR               | [21]      |
|                        |              | Bac2R       | ACA-AGC-CAG-TGT-ATAG-AGA-GAG-AGA                           |                            |                    |                   |           |
|                        | Bac3         | Bac3F       | CTA-ATG-AGA-AAT-GAA-TAT-CT-ATAT                           | 60.0                       | 30                 | PCR               | [21]      |
|                        |              | Bac3R       | GCA-CAC-CCA-ATG-AGT-AGA-GAG-AGA                           |                            |                    |                   |           |
| Cow-Bac2 EP             | qCS621F      |             | AAC-CAC-AGC-CTA-AGA-CA-CT                                  | 62.0                       | 30                 | SYBR qPCR         | [22]      |
|                        | qBac725R     |             | CAA-TGG-TAC-GTG-CTG-CTA-TCT-AGAT                           |                            |                    |                   |           |
| BoBac EP                | BoBac367f    |             | GAA-GAC-TGA-AGC-CCA-AGA-GTA                                  | 57.0                       | 30                 | qPCR              | [23]      |
|                        | BoBac467r    |             | GCT-TAT-TCA-TAC-GGT-ACA-TAC-AGA                             |                            |                    |                   |           |
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Author's statement

The authors declare that they have no competing interests.

Transparency document. Supporting information

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