



(43) International Publication Date
1 October 2015 (01.10.2015)

(10) International Publication Number
WO 2015/148909 A1

(51) International Patent Classification:
C12Q 1/68 (2006.01)

(21) International Application Number:
PCT/US2015/022963

(22) International Filing Date:
27 March 2015 (27.03.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/971,938 28 March 2014 (28.03.2014) US

(71) Applicant: **MAYO FOUNDATION FOR MEDICAL
EDUCATION AND RESEARCH** [US/US]; 200 First
Street S.W., Rochester, Minnesota 55905 (US).

(72) Inventors: **MARIANI, Andrea**; 3707 13th Avenue S.W.,
Rochester, Minnesota 55902 (US). **CHIA, Nicholas**; 912
7th Avenue S.W., Rochester, Minnesota 55902 (US).
WALTHER-ANTONIO, Marina R.; 5411 Weatherstone
Circle N.W., Rochester, Minnesota 55901 (US).

(74) Agent: **FINN III, J. Patrick**; Fish & Richardson P.C., PO
Box 1022, Minneapolis, Minnesota 55440-1022 (US).

(81) Designated States (*unless otherwise indicated, for every
kind of national protection available*): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,
BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,
DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR,
KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG,
MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM,
PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC,
SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,
TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every
kind of regional protection available*): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ,
TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU,
TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE,
DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU,
LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK,
SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: METHODS AND MATERIALS FOR TREATING ENDOMETRIAL CANCER

(57) Abstract: This document provides methods and materials for treating endometrial cancer. For example, methods and materials for identifying a female mammal as having endometrial cancer and surgically removing at least the uterus of the female are provided.



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METHODS AND MATERIALS FOR TREATING ENDOMETRIAL CANCER

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Serial No. 61/971,938 filed
5 March 28, 2015. This disclosure of the prior application is considered part of (and is
incorporated by reference in) the disclosure of this application.

BACKGROUND

1. Technical Field

10 This document relates to methods and materials involved in treating endometrial
cancer. For example, this document provides methods and materials for identifying a
female mammal as having endometrial cancer and surgically removing at least the uterus
of the female.

15 2. Background Information

Endometrial cancer is the most common gynecologic cancer currently afflicting
an estimated 600,000 women in the U.S. alone, with 49,000 new cases identified and
8,000 deaths in 2013. Genetic risk factors, such as hereditary non-polyposis colorectal
cancer, explain less than 5% of all endometrial cancer cases (Lancaster *et al.*, *Gynecol.*
20 *Oncol.*, 107(2):159-162 (2007) and Kwon *et al.*, *J. Clin. Oncol.*, 29(16):2247-2252
(2011)). Multiple non-genetic risk factors for endometrial cancer exist. These risk
factors can be broadly categorized into those linked to hormonal changes, such as obesity
and hormone therapy, and those linked to chronic inflammation, such as diabetes and
aging (Smith *et al.*, *Cancer J. Clinicians*, 51(1):38-75 (2001)).

25 SUMMARY

This document provides methods and materials for treating endometrial cancer.
For example, this document provides methods and materials for identifying a female
mammal as having endometrial cancer and surgically removing at least the uterus of the
30 female.

As described herein, a female mammal can be identified as having endometrial

cancer at an early stage based at least in part on the presence of a *Porphyromonas* species in the upper reproductive tract and/or based at least in part on the presence of an elevated level of bacteria of a *Porphyromonas* species in the lower reproductive tract. In some cases, endometrial biopsies, dilatation and curettage procedures, transvaginal ultrasound examinations, or combinations thereof can be used to confirm that a female has endometrial cancer. Once identified as having endometrial cancer as described herein, at least the uterus of the female mammal can be surgically removed. For example, a female mammal identified as having endometrial cancer as described herein can be treated by performing a total hysterectomy (e.g., a vaginal hysterectomy, an abdominal hysterectomy, a total laparoscopic hysterectomy, a total hysterectomy with lateral or bilateral salpingo-oophorectomy, or a radical hysterectomy). In some cases, a female mammal identified as having endometrial cancer as described herein can be treated using radiation therapy (e.g., high-energy x-ray therapy), chemotherapy (e.g., paclitaxel, carboplatin, doxorubicin, and/or cisplatin therapy), or hormone therapy (e.g., tamoxifen, goserelin, leuprolide, exemestane, anastrozole, and/or letrozole therapy) in combination with or in place of surgery.

In general, one aspect of this document features a method for treating a female mammal having endometrial cancer. The method comprises, or consists essentially of, (a) identifying the mammal as having a *Porphyromonas* species within the upper reproductive tract of the mammal or as having an elevated level of the species within the lower reproductive tract of the mammal, wherein the species is *Porphyromonas somerae* or a species having 16S rRNA that is greater than 98 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*, and (b) removing at least the uterus of the mammal. The mammal can be a human. The method can comprise identifying the mammal as having the species within the upper reproductive tract of the mammal. The method can comprise identifying the mammal as having the elevated level of the species within the lower reproductive tract of the mammal. The *Porphyromonas* species can be *Porphyromonas somerae*. The *Porphyromonas* species can be the species having 16S rRNA that is greater than 98 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*. The *Porphyromonas* species can be the species having 16S rRNA that is greater than 98.5 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*.

In another aspect, this document features a method for identifying a female mammal as having endometrial cancer. The method comprises, or consists essentially of, (a) detecting the presence of a *Porphyromonas* species within the upper reproductive tract of the mammal or the presence of an elevated level of the species within the lower reproductive tract of the mammal, wherein the species is *Porphyromonas somerae* or a species having 16S rRNA that is greater than 98 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*, and (b) classifying the mammal as having endometrial cancer. The mammal can be a human. The method can comprise detecting the presence of the species within the upper reproductive tract of the mammal. The method can comprise detecting the presence of the elevated level of the species within the lower reproductive tract of the mammal. The *Porphyromonas* species can be *Porphyromonas somerae*. The *Porphyromonas* species can be a species having 16S rRNA that is greater than 98 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*. The *Porphyromonas* species can be a species having 16S rRNA that is greater than 98.5 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*.

In another aspect, this document features a method for identifying a female mammal as having endometrial cancer. The method comprises, or consists essentially of, (a) obtaining a sample from the upper reproductive tract of the mammal, (b) extracting nucleic acid from the sample, (c) contacting the extracted nucleic acid with a primer pair capable of amplifying nucleic acid of a *Porphyromonas* species to form a mixture, (d) performing a polymerase chain reaction with the mixture to obtain an amplification product, (e) analyzing the amplification product to detect the presence of nucleic acid of a *Porphyromonas* species, wherein the species is *Porphyromonas somerae* or a species having 16S rRNA that is greater than 98 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*, and (f) classifying the mammal as having endometrial cancer. The mammal can be a human. The *Porphyromonas* species can be *Porphyromonas somerae*. The *Porphyromonas* species can be a species having 16S rRNA that is greater than 98 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*. The *Porphyromonas* species can be a species having 16S rRNA that is greater than 98.5 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*.

In another aspect, this document features a method for identifying a female

mammal as having endometrial cancer. The method comprises, or consists essentially of, (a) obtaining a sample from the lower reproductive tract of the mammal, (b) extracting nucleic acid from the sample, (c) contacting the extracted nucleic acid with a primer pair capable of amplifying nucleic acid of a *Porphyromonas* species to form a mixture, (d)

5 performing a polymerase chain reaction with the mixture to obtain an amplification product, (e) analyzing the amplification product to detect an elevated level of nucleic acid of a *Porphyromonas* species, wherein the species is *Porphyromonas somerae* or a species having 16S rRNA that is greater than 98 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*, and (f) classifying the mammal as having endometrial cancer.

10 The mammal can be a human. The *Porphyromonas* species can be *Porphyromonas somerae*. The *Porphyromonas* species can be a species having 16S rRNA that is greater than 98 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*. The *Porphyromonas* species can be a species having 16S rRNA that is greater than 98.5 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*.

15 In another aspect, this document features a method for treating a female mammal having endometrial cancer or likely to develop endometrial cancer. The method comprises, or consists essentially of, (a) identifying the mammal as having a *Porphyromonas* species within the upper reproductive tract of the mammal or as having an elevated level of the species within the lower reproductive tract of the mammal,

20 wherein the species is *Porphyromonas somerae* or a species having 16S rRNA that is greater than 98 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*, and (b) administering an antibiotic to the mammal to reduce the number of the species within the mammal. The mammal can be a human. The method can comprise identifying the mammal as having the species within the upper reproductive tract of the mammal. The

25 method can comprise identifying the mammal as having the elevated level of the species within the lower reproductive tract of the mammal. The *Porphyromonas* species can be *Porphyromonas somerae*. The *Porphyromonas* species can be the species having 16S rRNA that is greater than 98 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*. The *Porphyromonas* species can be the species having 16S rRNA that is

30 greater than 98.5 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*. The antibiotic can be benzylpenicillin, tetracycline, amoxicillin, ampicillin, ticarcillin,

piperacillin, cephalothin, cefuroxime, cefotaxime, ceftazidime, imipenem erythromycin, cefamandole, cephaloridine, oleandomycin, metronidazole, spiramycin, or clindamycin.

In another aspect, this document features a method for identifying a female mammal as having endometrial cancer. The method comprises, or consists essentially of, (a) contacting a sample from the mammal with a FISH probe during a FISH technique to detect the presence of a *Porphyromonas* species within the upper reproductive tract of the mammal or the presence of an elevated level of the species within the lower reproductive tract of the mammal, wherein the species is *Porphyromonas somerae* or a species having 16S rRNA that is greater than 98 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*, and (b) classifying the mammal as having endometrial cancer. The mammal can be a human. The method can comprise detecting the presence of the species within the upper reproductive tract of the mammal. The method can comprise detecting the presence of the elevated level of the species within the lower reproductive tract of the mammal. The *Porphyromonas* species can be *Porphyromonas somerae*. The *Porphyromonas* species can be the species having 16S rRNA that is greater than 98 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*. The *Porphyromonas* species can be the species having 16S rRNA that is greater than 98.5 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*. The FISH probe can comprise the sequence of SEQ ID NO:8 or SEQ ID NO:9.

In another aspect, this document features a method for identifying a female mammal as having endometrial cancer. The method comprises, or consists essentially of, (a) contacting a sample from the mammal with a FISH probe during a FISH technique to detect the presence of a *Porphyromonas* species within the upper reproductive tract of the mammal or the presence of an elevated level of the species within the lower reproductive tract of the mammal, wherein the species is *Porphyromonas somerae* or a species having 16S rRNA that is greater than 98 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*, (b) contacting a sample from the mammal with PCR primers during an amplification reaction to detect the presence of the *Porphyromonas* species within the upper reproductive tract of the mammal or the presence of an elevated level of the species within the lower reproductive tract of the mammal, and (b) classifying the mammal as having endometrial cancer. The mammal can be a human. The method can

comprise detecting the presence of the species within the upper reproductive tract of the mammal in the step (a) and the step (b). The method can comprise detecting the presence of the elevated level of the species within the lower reproductive tract of the mammal in the step (a) and the step (b). The *Porphyromonas* species can be *Porphyromonas somerae*. The *Porphyromonas* species can be the species having 16S rRNA that is greater than 98 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*. The *Porphyromonas* species can be the species having 16S rRNA that is greater than 98.5 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*. The FISH probe can comprise the sequence of SEQ ID NO:8 or SEQ ID NO:9. One of the PCR primers can comprise the sequence of SEQ ID NO:1 or SEQ ID NO:5.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF THE DRAWINGS

Figure 1 is an ordination plot using principle coordinates analysis (PCA) to identify the relative clustering and differences between the uterine, vaginal, and cervical microbiomes of pre-menopausal (top elliptical region) and postmenopausal (larger elliptical region on right side of graph) women. The primary separation between these microbiomes is not driven by menopausal status. PCA with 95% Confidence Region.

Figure 2 is a bar graph presenting the microbiome at the phylum level by abundance in the indicated samples. Statistical differences between the cancer and

benign microbiomes in the upper and lower reproductive tracts was calculated using permanova with 999 permutations and resulted in pseudo-F=0.007 and pseudo-F=0.008, respectively.

Figure 3 shows the relative abundance of *Porphyromonas* sp. in cancer and benign cases in both upper and lower reproductive tracts. Statistical testing using the Kruskal-Wallis test with 1000 permutations results in high significance for cancer detection: Uterus Cancer vs. Benign: p-value: 8.55E-7 (FDR: 6.42E-4; Bonferroni: 6.42E-4). Cervix/Vagina Cancer vs. Benign: p-value: 2.02E-6 (FDR: 1.52E-3; Bonferroni: 1.52E-3).

Figure 4 is a maximum likelihood phylogenetic tree of V3-V5 16S rDNA region. ^aRecovered from children with atopic dermatitis (Kong *et al.*, *Genome Research*, 22(5), 850-859 (2012)); ^bRecovered from buffaloes with postpartum endometritis; ^cRecovered from Holstein dairy cows with postpartum metritis (Peng *et al.*, *Folia microbiologica*, 58(6):593-600 (2013)). Produced with FASTTREE.

DETAILED DESCRIPTION

This document provides methods and materials for treating endometrial cancer. For example, this document provides methods and materials for identifying a female mammal as having endometrial cancer and surgically removing at least the uterus of the female. As described herein, female mammals can be identified as having endometrial cancer at an early stage based at least in part on the presence of a *Porphyromonas* species in the upper reproductive tract and/or based at least in part on the presence of an elevated level of bacteria of a *Porphyromonas* species in the lower reproductive tract. The *Porphyromonas* species can be *Porphyromonas somerae* or a species having 16S rRNA that is greater than 98 percent identical (e.g., greater than 98 percent identical, greater than 98.5 percent identical, greater than 99 percent identical, or greater than 99.5 percent identical) to a 16 rRNA sequence of *Porphyromonas somerae*. A *Porphyromonas somerae* strain designated WAL 6690 is available from the ATCC[®] (ATCC[®] deposit number: BAA-1230[™]). See, also, Summanen *et al.*, *J. Clin. Microbiol.*, 43(9):4455 (2005)).

A reproductive tract sample can be an upper or lower reproductive tract sample.

For example, an upper reproductive tract sample can be a sample obtained from a uterine swab, scrape, or biopsy. In some cases, an upper reproductive tract sample can be an endometrial, ovarian, fallopian tube, or peritoneal wash sample. A lower reproductive tract sample can be a sample obtained from a vaginal and/or cervical swab and scrape.

As described herein, detecting the presence of a *Porphyromonas* species within an upper reproductive tract sample can indicate that the female mammal has endometrial cancer, while detecting the presence of an elevated level of a *Porphyromonas* species within a lower reproductive tract sample can indicate that the female mammal has endometrial cancer. The term “elevated level” as used herein with respect to a level of a *Porphyromonas* species within a reproductive tract sample refers to any level that is greater than a reference level of a *Porphyromonas* species. The term “reference level” as used herein with respect to the level of a *Porphyromonas* species refers to the level of a *Porphyromonas* species typically observed in comparable reproductive tract samples from healthy female mammals without endometrial cancer. For example, a reference level of a *Porphyromonas* species can be the average number of *Porphyromonas* species present in reproductive tract samples obtained from a random sampling of 50 female humans free of endometrial cancer. In some cases, an elevated level of *Porphyromonas* species can be a level that is at least 10, 25, 50, 100, or 200 percent greater than a reference level of *Porphyromonas* species. In some cases, detecting the presence of an elevated level of a *Porphyromonas* species within an upper reproductive tract sample can indicate that the female mammal has endometrial cancer. In some cases, endometrial biopsies, dilatation and curettage procedures, transvaginal ultrasound examinations, or combinations thereof can be used to confirm that a female has endometrial cancer.

Any appropriate method can be used to detect the presence or amount of a *Porphyromonas* species within a reproductive tract sample. For example, PCR-based assays designed to amplify nucleic acid of a *Porphyromonas* species can be used to detect the presence or amount of a *Porphyromonas* species within a reproductive tract sample. In some cases, rRNA nucleic acid can be amplified and sequenced to detect the presence or amount of a *Porphyromonas* species within a reproductive tract sample.

In some cases, a PCR-based technique can be performed to amplify nucleic acid of a *Porphyromonas* species. Once amplified, the nucleic acid can be sequenced to

confirm the presence of nucleic acid from a *Porphyromonas* species. In some cases, a pair of PCR primers or one or more probes specific for nucleic acid of a *Porphyromonas* species can be designed and used to detect the presence or amount of a *Porphyromonas* species within a sample with or without performing nucleic acid sequencing. For
5 example, a PCR assay that includes a PCR primer pair designed to amplify nucleic acid of a *Porphyromonas* species and not nucleic acid from other species can be used to detect the presence or amount of a *Porphyromonas* species within a sample.

In some cases, fluorescence *in situ* hybridization (FISH) can be performed to detect the presence or amount of a *Porphyromonas* species within a sample. For
10 example, FISH can be performed using one or more FISH probes designed to hybridize to nucleic acid of a *Porphyromonas* species. Such a FISH technique can be used to detect the presence or amount of a *Porphyromonas* species within a sample.

In some cases, a reproductive tract sample can be assessed using both a PCR-based technique and a FISH technique. In such cases, those samples where both the
15 PCR-based technique and the FISH technique revealed the presence of a *Porphyromonas* species can be identified or classified as containing a *Porphyromonas* species, while those samples where both the PCR-based technique and the FISH technique revealed the absence of a *Porphyromonas* species can be identified or classified as lacking a
20 *Porphyromonas* species. In those cases where only one of the two techniques revealed the presence of a *Porphyromonas* species, the sample can be re-assessed and/or further analysis can be performed (e.g., sequencing).

Once identified as having endometrial cancer based at least in part on the presence of a *Porphyromonas* species in the upper reproductive tract and/or an elevated level of a *Porphyromonas* species in the lower reproductive tract as described herein, at
25 least the uterus of the female mammal can be surgically removed. For example, a female mammal identified as having endometrial cancer as described herein can be treated by performing a total hysterectomy (e.g., a vaginal hysterectomy, an abdominal hysterectomy, a total laparoscopic hysterectomy, a total hysterectomy with lateral or bilateral salpingo-oophorectomy, or a radical hysterectomy). In some cases, a female
30 mammal identified as having endometrial cancer as described herein can be treated using radiation therapy (e.g., high-energy x-ray therapy), chemotherapy (e.g., paclitaxel,

carboplatin, doxorubicin, and/or cisplatin therapy), or hormone therapy (e.g., tamoxifen, goserelin, leuprolide, exemestane, anastrozole, and/or letrozole therapy) in combination with or in place of surgery.

In some cases, a female mammal having endometrial cancer and the presence of a *Porphyromonas* species in the upper reproductive tract and/or an elevated level of a *Porphyromonas* species in the lower reproductive tract can be treated with one or more agents (e.g., antibiotics) having the ability to kill *Porphyromonas* species. For example, a female mammal identified as having endometrial cancer as described herein can be treated by administering an antibiotic to the mammal under conditions wherein the number of *Porphyromonas* species bacteria present within the upper reproductive tract and/or lower reproductive tract is reduced.

In some cases, a female mammal having a likelihood of developing endometrial cancer and having a *Porphyromonas* species in the upper reproductive tract and/or an elevated level of a *Porphyromonas* species in the lower reproductive tract can be treated with one or more agents (e.g., antibiotics) having the ability to kill *Porphyromonas* species. For example, a female mammal identified as having a *Porphyromonas* species in the upper reproductive tract can be treated by administering an antibiotic to the mammal under conditions wherein the number of *Porphyromonas* species bacteria present within the upper reproductive tract is reduced. Examples of antibiotics that can be used to treat a female mammal as described herein include, without limitation, benzylpenicillin, tetracycline, amoxicillin, ampicillin, ticarcillin, piperacillin, cephalothin, cefuroxime, cefotaxime, cefoxitin, imipenem erythromycin, cefamandole, cephaloridine, oleandomycin, metronidazole, spiramycin, and clindamycin.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1 – Presence of Elevated Level of *Porphyromonas* Species in the Reproductive Tract Indicates the Presence of Endometrial Cancer

Subject Enrollment

16 human subjects were enrolled in this study. The inclusion criteria were the

following: 18 years of age or older; females undergoing hysterectomy by any standard surgical approach; and undergoing hysterectomy for benign disease, hyperplasia, or any stage of endometrial cancer. Patients with any of the following criteria were excluded from this study: women who were pregnant or nursing; women who took antibiotics
5 within two weeks preceding surgery; and women where the surgeon anticipates the use of a morcellator during the hysterectomy procedure due to the size of the uterus or for any other reason. Upon enrollment, the subjects were requested to fill out an optional questionnaire about sexual and reproductive health and history. The metadata from the questionnaires were stored at REDCap (Harris *et al.*, *J. Biomed. Inform.*, 42:377-81
10 (2009)).

Vaginal and Cervical Sample Collection

All subjects were requested not to douche with betadine on the surgery day or the day immediately preceding it. The vaginal and cervical swabs and scrapes were collected
15 by the surgeon with guidance on site by the research team immediately after the administration of anesthesia and immediately preceding the standard pre-surgical betadine douche. Both the vaginal and cervical swabs were performed with three sterile Dacron swabs each and placed in a sterile tube with 1 mL of Tris-EDTA (TE) buffer kept on dry ice until storage at -80°C. One of the vaginal swabs was used for immediate on-
20 site vaginal pH measurement with a Hydrion measuring pH tape. The scrapes were performed using sterilized (autoclaved at 121°C for 20 minutes) pap smear spatulas and placed in sterile tubes with TE buffer kept in dry ice until storage.

Uterine, Fallopian, and Ovarian Sample Collection

25 Once removed, the uterus, fallopian tubes, and ovaries were handed by the surgeon to the instrumentalist nurse who placed them inside a sterile transport bag and into a closed sterile container. The container was transported to the pathology lab (within the same clean area), where the organs were handed to a pathology assistant (PA) who processed them under sterile conditions. The grossing station where the specimen was
30 processed was sterilized by the research team, including all tools needed by the PA for handling. The PA used surgical gloves and mask when handling the specimen. The PA

performed a bilateral cut of the uterus and splayed it. If the uterus belonged to a non-cancer subject, then the research team advanced to the collection of the uterine swabs (Dacron) and scrapes (sterilized pap smear spatulas) and documentation (by placement of push pins in sampled locations and digital photograph). The PA then proceeded to the aseptic collection of samples needed for the diagnosis, and once complete, the research team collected the uterine, fallopian, and ovarian biopsies (approximately 4 mm of tissue was collected per biopsy by the use of a sterile tweezers, scalpels, and surgical ruler). If the uterus belonged to a cancer subject, the collection of the swabs and scrapes was performed after the complete diagnostic procedures so as to avoid delay in the cancer staging determination, which can impact in the progression of the surgery in the operating room. Each collected sample was placed in a sterile tube with 1 mL of TE buffer and kept on dry ice until storage at -80°C. A petri dish with LB was kept open on the grossing station during sample collection to detect any possible airborne contamination of the specimen. The LB was swabbed, and the swab was stored in a tube with 1 mL of TE and kept on dry ice until storage along with the other samples.

Sample Processing

Once thawed, the swab and scrape samples were vortexed to bring the collected material into solution. The biopsy samples were mashed by the use of sterile pestles. The swab and scrape samples were centrifuged for 10 minutes at 10,000g to collect the bacterial cells, and the supernatant was discarded. All genomic DNA extractions were performed by using the MoBio PowerSoil Kit (MoBio Laboratories, Inc., Carlsbad, CA); however, instead of vortexing, MP FastPrep (MP Biomedicals, Solon, OH) was used for 60 seconds at 6.0 m/s to obtain a more effective and rapid lysis of the cells. The incubation period was for a minimum of 30 minutes and a maximum of overnight. After extraction, the DNA content was measured using High Sensitivity Qubit (Life Technologies Corporation, Carlsbad, CA). The V3-V5 region of the 16S rDNA was then amplified through a polymerase chain reaction (PCR) as follows: 25 µL of Kapa HiFi (Kapa Biosystems, Woburn, MA), 1.5 µL (10 µM) forward primer, 1.5 µL (10 µM) reverse primer, 50 ng of DNA with the remaining volume being added by molecular grade water (up to a final volume of 50 µL per reaction). The forward primer was the

universal primer 357F (5'-GTCCTACGGGAGGCAGCAG-3'; SEQ ID NO:1) with the added construct on the 5' end of the 5' Illumina Adapter (5'-AATGATACGGCGAC-CACCGAGATCTACAC-3'; SEQ ID NO:2) + Forward Primer Pad (5'-TATGGT-AATT-3'; SEQ ID NO:3) to a total sequence: 5'-AATGATACGGCGACCAACGA-
 5 GATCTACACTATGGTAATTGTCCTACGGGAGGCAGCAG-3' (SEQ ID NO:4), and the universal bacterial reverse primer was 926R (5'-CCGTCAATTCMTTTRAGT-3'; SEQ ID NO:5) with an added construct on the 5' end of the reverse complement of 3' Illumina adapter (5'-CAAGCAGAAGACGGCATAACGAGATGCCGCATTCGAT-3'; SEQ ID NO:6) + Barcode (12 base pairs) to a total sequence: 5'-CAAGCAGAAG-
 10 ACGGCATAACGAGATGCCGCATTCGATXXXXXXXXXXXXCCGTCAATTCMTTTRAGT-3' (SEQ ID NO:7). The barcode introduced in the reverse primer construct was unique to each sample, functioning as a genetic ID for sequencing. The PCR cycle was the following: 95°C for 3 minutes, 98°C for 20 seconds, 70°C for 15 seconds, 72°C for 15 seconds; cycle repeated for 34 times and 72°C for 5 minutes. The products of the
 15 amplification were verified by TapeStation D1K Tape (2200 TapeStation Instrument, Agilent Technologies, Santa Clara, CA) to be free of contamination and the expected amplification size (approximately 700 base pairs). If the amplification was unsuccessful, the parameters of the reaction or cycle were adjusted in repeated attempts. In some cases (mostly biopsy samples), the amplification was not successful even after repeated
 20 attempts. The reduced number of microorganisms present in the upper reproductive tract was likely to justify this result and attests for the success of the sterile collection of the samples. Upon verification, the PCR products were purified using Agencourt AMPure (Beckman Coulter, Brea, CA). After purification, the concentrations were measured using Qubit High Sensitivity. The 16S sequencing was performed by the MGF (Medical
 25 Genome facility at Mayo Clinic, Rochester) using a high-throughput next-generation Illumina MiSeq (San Diego, CA) sequencing platform.

Sequence Analysis

Sequence reads were aligned with an alignment tool that merges paired end reads
 30 into a single multiple alignment and obtains taxa calls (Sipos *et al.*, *PLoS One*, 5:e15220 (2010)). IM-TORNADO then clustered sequences into operational taxonomic units

(OTUs) using AbundantOTU+ (Ye, *Proceedings IEEE Int. Conf. Bioinformatics Biomed*, 2010:153-7 (2011)).

Sequencing Outcome

A total of 16,366,472 sequence reads (17,657 to 828,181 reads per sample) were obtained (mean of $199,591 \pm 190,153$ reads) after quality control. Further processing for visualization was performed using QIIME (Caporaso *et al.*, *Nat. Methods*, 7:335-6 (2010)) and METAGENassist (Arndt *et al.*, *Nucleic Acids Research*, 40(Web Server issue):W88-95 (2012)).

Results

Results were obtained from 16 subjects: six with a benign diagnosis (including two with a diagnosis of menorrhagia, a risk factor for the development of endometrial cancer) and ten with biopsy proven endometrial cancer (Grade I-III). Ethnicity and age were not targeted, with all the enrolled subjects being Caucasian and a mean age of 59 ± 9 (51 ± 9 years old for the benign subjects, and 67 ± 8 years old for the cancer subjects). The benign cohort had 40% post-menopausal women, while the cancer cohort had 80% post-menopausal women. All subjects were asked to fill an optional gynecologic health and sexual history questionnaire. Of the benign subjects, 60% had previously undergone a gynecologic surgery (tubal ligation being the most common) with only 33% of the cancer subjects having had any previous gynecologic surgery. Benign subjects self-reported an average of 5 ± 3 sexual partners, while cancer subjects reported 1 ± 0 . The majority (63%) of the subjects self-reported incontinence (60% for benign subjects, and 67% for cancer subjects).

The effect of menopausal status on the microbial populations was examined in the uterus. Menopausal status was self-reported and confirmed by the measurement of the vaginal pH at the time of sample collection. All self-reported post-menopausal patients had a vaginal pH >4.5 . Of the 16 enrolled patients, 11 were post-menopausal. Menopausal status significantly impacted the microbiome in both the lower and upper reproductive tract (Figure 1). A particular microorganism, with 90% sequence similarity to *Barnesiella intestinihominis* was found significantly higher in postmenopausal women

throughout the reproductive tract. A variety of other microorganisms, commonly associated with the gastrointestinal tract were also detected at significantly different proportions between the two groups of women (Table 1).

- 5 Table 1. Significant differences found between the frequencies of the OTUs (Operational Taxonomic Unites) in postmenopausal women when compared to premenopausal women. Generated by Kruskal-Wallis analysis with 1,000 permutations through QIIME.

Uterus					
OTU	p-value	Bonferroni Correction	Post-Menopause Mean	Pre-Menopause Mean	Taxonomy
20	2.58E-06	0.001938339	6280	0	Bacteroidetes; Barnesiella; B.intestinihominis (90% identity)
469	2.58E-06	0.001938339	510	0	Firmicutes; Clostridium; C. sp. (99% identity)
16	5.06E-06	0.003798545	12319	62	Bacteroidetes; Barnesiella; B. intestinihominis (90% identity)
41	7.03E-06	0.005280118	0.3	1045	Proteobacteria; Pseudomonas; P. aeruginosa (100% identity)
36	1.06E-05	0.007964362	1532	0	Firmicutes; Oscillospira; O. guilliermondii (97% identity)
24	1.06E-05	0.007964362	2653	0	Bacteroidetes; Barnesiella; B. intestinihominis (89% identity)
11	1.10E-05	0.008225203	8268	84	Bacteroidetes; Bacteroides; B. acidifaciens (100% identity)
Lower Reproductive Tract					
OTU	p-value	Bonferroni Correction	Post-Menopause Mean	Pre-Menopause Mean	Taxonomy
16	6.31E-06	0.004740987	4228	12	Bacteroidetes; Barnesiella; B. intestinihominis (90% identity)
90	9.23E-06	0.00693405	0	145	Firmicutes; Lactobacillus; L. delbrueckii (100% identity)

The samples collected from non-cancer (6 subjects) and cancer (10 subjects) participants were analyzed and compared (Figure 2). A decreased abundance of Proteobacteria and an increased abundance of Bacteroidetes and Actinobacteria were found in the samples from the uterine cavity of cancer subjects when compared to samples collected from non-cancer subjects (Figure 2). This trend also was observed in the samples collected from the lower reproductive tract (vagina and cervix).

A particular *Porphyromonas* sp. having about 99% identity to *Porphyromonas somerae* was exclusively collected from the upper reproductive tract of cancer subjects (Figure 3). All sequenced samples (9 samples from 5 cancer subjects) from the upper reproductive tract of cancer subjects were positive for this microorganism, including samples collected from the Fallopian tubes and stool samples. By contrast, all sequenced samples (25 samples from 5 benign subjects) from the upper reproductive tract of non-cancer subjects were negative for this microorganism, including samples collected from the Fallopian tubes (including a cyst sample) and ovaries.

A phylogenetic analysis confirmed the close affiliation of the microorganism recovered with *Porphyromonas somerae* and other uncultured *Porphyromonas* species recovered from children with atopic dermatitis (Kong *et al.*, *Genome Research*, 22(5), 850-859 (2012)) and buffaloes with postpartum endometritis (Peng *et al.*, *Folia microbiologica*, 58(6):593-600 (2013)) (Figure 4). The phylogeny also revealed that the second closest cultured representative was *Porphyromonas levii* (96% sequence identity).

This *Porphyromonas* sp. microorganism also was found in significantly higher abundance (e.g., > 100 times greater abundance) in the lower reproductive tract of cancer subjects (25 samples, 9 subjects) than in non-cancer subjects (20 samples, 6 subjects) (Figure 3).

Example 2 – Treating Endometrial Cancer

An upper or lower reproductive tract sample is obtained from a female human, who is between about 25 and about 80 years old (e.g., between about 30 and about 65 years old, between about 35 and about 60 years old, or between about 40 and about 55 years old) and who lacks symptoms of endometrial cancer as observable from a routine physical examination. The obtained sample is examined for the presence of and/or

amount of a *Porphyromonas* species such as *Porphyromonas somerae* or a *Porphyromonas* species having greater than 98 percent identity (e.g., greater than 99 percent identity) to *Porphyromonas somerae*. In some cases, a PCR-based assay is performed to detect the presence of or amount of such a *Porphyromonas* species. If a

5 *Porphyromonas* species such as *Porphyromonas somerae* or a *Porphyromonas* species having greater than 98 percent identity (e.g., greater than 99 percent identity) to *Porphyromonas somerae* is detected in an upper reproductive tract sample or if an elevated level of such a *Porphyromonas* species is detected in a lower reproductive tract sample, then the female human is treated by performing a total hysterectomy (e.g., a

10 vaginal hysterectomy, an abdominal hysterectomy, a total laparoscopic hysterectomy, a total hysterectomy with lateral or bilateral salpingo-oophorectomy, or a radical hysterectomy). Optionally, an endometrial biopsy, dilatation and curettage procedure, transvaginal ultrasound examination, or a combination thereof is performed prior to the hysterectomy to confirm that the female human has endometrial cancer.

15 Example 3 – Using FISH to detect a *Porphyromonas* species within tissue samples

Frozen tissue sections and/or liquid culture sections are pretreated by allowing the sections to air dry for 30 minutes at room temperature. The sections are fixed in 4% paraformaldehyde for 15 minutes at room temperature and washed three times for 5

20 minutes each in PBS. The sections are incubated in 0.15% H₂O₂ in PBS for 30 minutes, and the slides are equilibrated in 4X SSC for 15 minutes.

Hybridization is performed in a humidity chamber. The humidity chamber is prepared with a hybridization box or tube with a formamide/water mixture. The sections are covered with hybridization buffer, and the slides are inserted into a preheated

25 humidity chamber. After incubating the slides for 20 minutes at 37°C, the buffer is removed, and a hybridization buffer mixed with a probe working solution (50 ng DNA/μL) in a ratio of 100:1 is applied to the sections. The probe is either 5'-CAG-CCAAGTCGCGTGAAGGA-3' (SEQ ID NO:8) or 5'-AGGATAGGTACGTGTACC-TATTAGA-3' (SEQ ID NO:9) with horseradish peroxidase (HRP) attached at the 5' end.

30 For each section, 50 μL is sufficient. The sections are incubated overnight at 37°C.

A washing step is performed by removing the hybridization buffer by dabbing the slide onto tissue. The slides are then dipped in the first washing buffer followed by a second washing buffer for 15 minutes at 37°C. The slides are incubated in 1X PBS (pH 8) under shaking conditions at room temperature for 15 minutes to 1 hour.

5 A humidity chamber is prepared by inserting the tissue to the bottom of 50 mL tubes and soaking them with water. A fresh solution of H₂O₂ (0.15% in PBS) is prepared and kept cool. An amplification buffer is mixed with the peroxide solution 100:1 and fluorescently labeled tyramide (1 mg dye/mL) and is kept in the dark.

10 The amplification mix is applied onto the sections, which are incubated at 37°C for 20 minutes in a humidity chamber. Excess liquid is removed and incubated in 1X PBS for 15 minutes at room temperature in the dark. The slides are washed with water and allowed to air dry before being counterstained with DAPI.

OTHER EMBODIMENTS

15 It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method for treating a female mammal having endometrial cancer, wherein said method comprises:

(a) identifying said mammal as having a *Porphyromonas* species within the upper reproductive tract of said mammal or as having an elevated level of said species within the lower reproductive tract of said mammal, wherein said species is *Porphyromonas somerae* or a species having 16S rRNA that is greater than 98 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*, and

(b) removing at least the uterus of said mammal.

2. The method of claim 1, wherein said mammal is a human.

3. The method of claim 1, wherein said method comprises identifying said mammal as having said species within the upper reproductive tract of said mammal.

4. The method of claim 1, wherein said method comprises identifying said mammal as having said elevated level of said species within the lower reproductive tract of said mammal.

5. The method of claim 1, wherein said *Porphyromonas* species is *Porphyromonas somerae*.

6. The method of claim 1, wherein said *Porphyromonas* species is said species having 16S rRNA that is greater than 98 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*.

7. The method of claim 1, wherein said *Porphyromonas* species is said species having 16S rRNA that is greater than 98.5 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*.

8. A method for identifying a female mammal as having endometrial cancer, wherein said method comprises:
- (a) detecting the presence of a *Porphyromonas* species within the upper reproductive tract of said mammal or the presence of an elevated level of said species within the lower reproductive tract of said mammal, wherein said species is *Porphyromonas somerae* or a species having 16S rRNA that is greater than 98 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*, and
 - (b) classifying said mammal as having endometrial cancer.
9. The method of claim 8, wherein said mammal is a human.
10. The method of claim 8, wherein said method comprises detecting the presence of said species within the upper reproductive tract of said mammal.
11. The method of claim 8, wherein said method comprises detecting the presence of said elevated level of said species within the lower reproductive tract of said mammal.
12. The method of claim 8, wherein said *Porphyromonas* species is *Porphyromonas somerae*.
13. The method of claim 8, wherein said *Porphyromonas* species is said species having 16S rRNA that is greater than 98 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*.
14. The method of claim 8, wherein said *Porphyromonas* species is said species having 16S rRNA that is greater than 98.5 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*.
15. A method for identifying a female mammal as having endometrial cancer, wherein said method comprises:

- (a) obtaining a sample from the upper reproductive tract of said mammal,
- (b) extracting nucleic acid from said sample,
- (c) contacting said extracted nucleic acid with a primer pair capable of amplifying nucleic acid of a *Porphyromonas* species to form a mixture,
- (d) performing a polymerase chain reaction with said mixture to obtain an amplification product,
- (e) analyzing said amplification product to detect the presence of nucleic acid of a *Porphyromonas* species, wherein said species is *Porphyromonas somerae* or a species having 16S rRNA that is greater than 98 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*, and
- (f) classifying said mammal as having endometrial cancer.

16. The method of claim 15, wherein said mammal is a human.

17. The method of claim 15, wherein said *Porphyromonas* species is *Porphyromonas somerae*.

18. The method of claim 15, wherein said *Porphyromonas* species is said species having 16S rRNA that is greater than 98 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*.

19. The method of claim 15, wherein said *Porphyromonas* species is said species having 16S rRNA that is greater than 98.5 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*.

20. A method for identifying a female mammal as having endometrial cancer, wherein said method comprises:

- (a) obtaining a sample from the lower reproductive tract of said mammal,
- (b) extracting nucleic acid from said sample,
- (c) contacting said extracted nucleic acid with a primer pair capable of amplifying nucleic acid of a *Porphyromonas* species to form a mixture,

- (d) performing a polymerase chain reaction with said mixture to obtain an amplification product,
- (e) analyzing said amplification product to detect an elevated level of nucleic acid of a *Porphyromonas* species, wherein said species is *Porphyromonas somerae* or a species having 16S rRNA that is greater than 98 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*, and
- (f) classifying said mammal as having endometrial cancer.

21. The method of claim 20, wherein said mammal is a human.

22. The method of claim 20, wherein said *Porphyromonas* species is *Porphyromonas somerae*.

23. The method of claim 20, wherein said *Porphyromonas* species is said species having 16S rRNA that is greater than 98 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*.

24. The method of claim 20, wherein said *Porphyromonas* species is said species having 16S rRNA that is greater than 98.5 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*.

25. A method for treating a female mammal having endometrial cancer or likely to develop endometrial cancer, wherein said method comprises:

- (a) identifying said mammal as having a *Porphyromonas* species within the upper reproductive tract of said mammal or as having an elevated level of said species within the lower reproductive tract of said mammal, wherein said species is *Porphyromonas somerae* or a species having 16S rRNA that is greater than 98 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*, and
- (b) administering an antibiotic to said mammal to reduce the number of said species within said mammal.

26. The method of claim 25, wherein said mammal is a human.
27. The method of claim 25, wherein said method comprises identifying said mammal as having said species within the upper reproductive tract of said mammal.
28. The method of claim 25, wherein said method comprises identifying said mammal as having said elevated level of said species within the lower reproductive tract of said mammal.
29. The method of claim 25, wherein said *Porphyromonas* species is *Porphyromonas somerae*.
30. The method of claim 25, wherein said *Porphyromonas* species is said species having 16S rRNA that is greater than 98 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*.
31. The method of claim 25, wherein said *Porphyromonas* species is said species having 16S rRNA that is greater than 98.5 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*.
32. The method of claim 25, wherein said antibiotic is benzylpenicillin, tetracycline, amoxicillin, ampicillin, ticarcillin, piperacillin, cephalothin, cefuroxime, cefotaxime, cefoxitin, imipenem erythromycin, cefamandole, cephaloridine, oleandomycin, metronidazole, spiramycin, or clindamycin.
33. A method for identifying a female mammal as having endometrial cancer, wherein said method comprises:
- (a) contacting a sample from said mammal with a FISH probe during a FISH technique to detect the presence of a *Porphyromonas* species within the upper reproductive tract of said mammal or the presence of an elevated level of said species within the lower reproductive tract of said mammal, wherein said species is

Porphyromonas somerae or a species having 16S rRNA that is greater than 98 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*, and

(b) classifying said mammal as having endometrial cancer.

34. The method of claim 33, wherein said mammal is a human.

35. The method of claim 33, wherein said method comprises detecting the presence of said species within the upper reproductive tract of said mammal.

36. The method of claim 33, wherein said method comprises detecting the presence of said elevated level of said species within the lower reproductive tract of said mammal.

37. The method of claim 33, wherein said *Porphyromonas* species is *Porphyromonas somerae*.

38. The method of claim 33, wherein said *Porphyromonas* species is said species having 16S rRNA that is greater than 98 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*.

39. The method of claim 33, wherein said *Porphyromonas* species is said species having 16S rRNA that is greater than 98.5 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*.

40. The method of claim 33, wherein said FISH probe comprises the sequence of SEQ ID NO:8 or SEQ ID NO:9.

41. A method for identifying a female mammal as having endometrial cancer, wherein said method comprises:

(a) contacting a sample from said mammal with a FISH probe during a FISH technique to detect the presence of a *Porphyromonas* species within the upper reproductive tract of said mammal or the presence of an elevated level of said species

within the lower reproductive tract of said mammal, wherein said species is *Porphyromonas somerae* or a species having 16S rRNA that is greater than 98 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*,

(b) contacting a sample from said mammal with PCR primers during an amplification reaction to detect the presence of said *Porphyromonas* species within the upper reproductive tract of said mammal or the presence of an elevated level of said species within the lower reproductive tract of said mammal, and

(b) classifying said mammal as having endometrial cancer.

42. The method of claim 41, wherein said mammal is a human.

43. The method of claim 41, wherein said method comprises detecting the presence of said species within the upper reproductive tract of said mammal in said step (a) and said step (b).

44. The method of claim 41, wherein said method comprises detecting the presence of said elevated level of said species within the lower reproductive tract of said mammal in said step (a) and said step (b).

45. The method of claim 41, wherein said *Porphyromonas* species is *Porphyromonas somerae*.

46. The method of claim 41, wherein said *Porphyromonas* species is said species having 16S rRNA that is greater than 98 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*.

47. The method of claim 41, wherein said *Porphyromonas* species is said species having 16S rRNA that is greater than 98.5 percent identical to a 16 rRNA sequence of *Porphyromonas somerae*.

48. The method of claim 41, wherein said FISH probe comprises the sequence of

SEQ ID NO:8 or SEQ ID NO:9.

49. The method of claim 41, wherein one of said PCR primers comprises the sequence of SEQ ID NO:1 or SEQ ID NO:5.

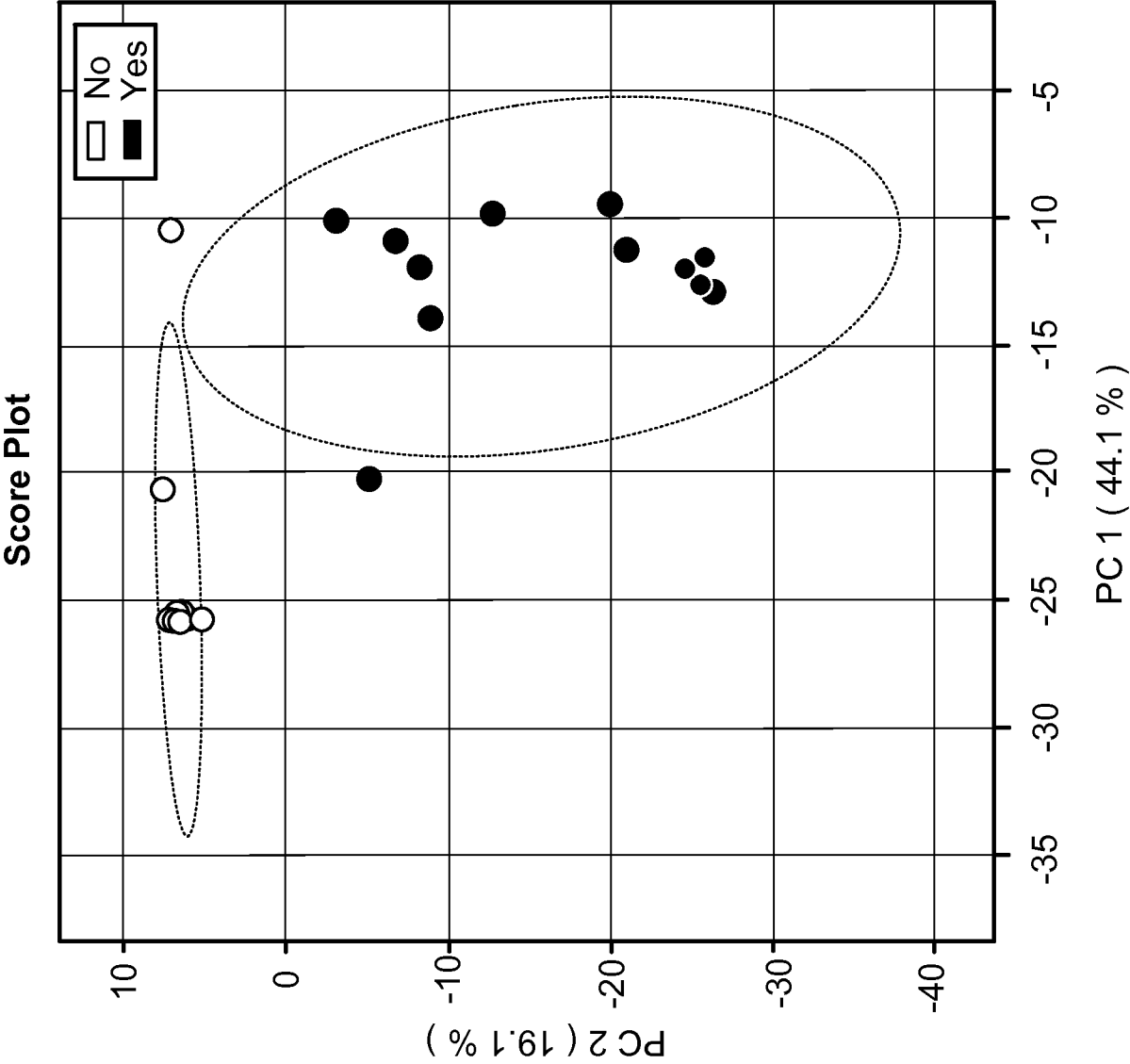


Figure 1

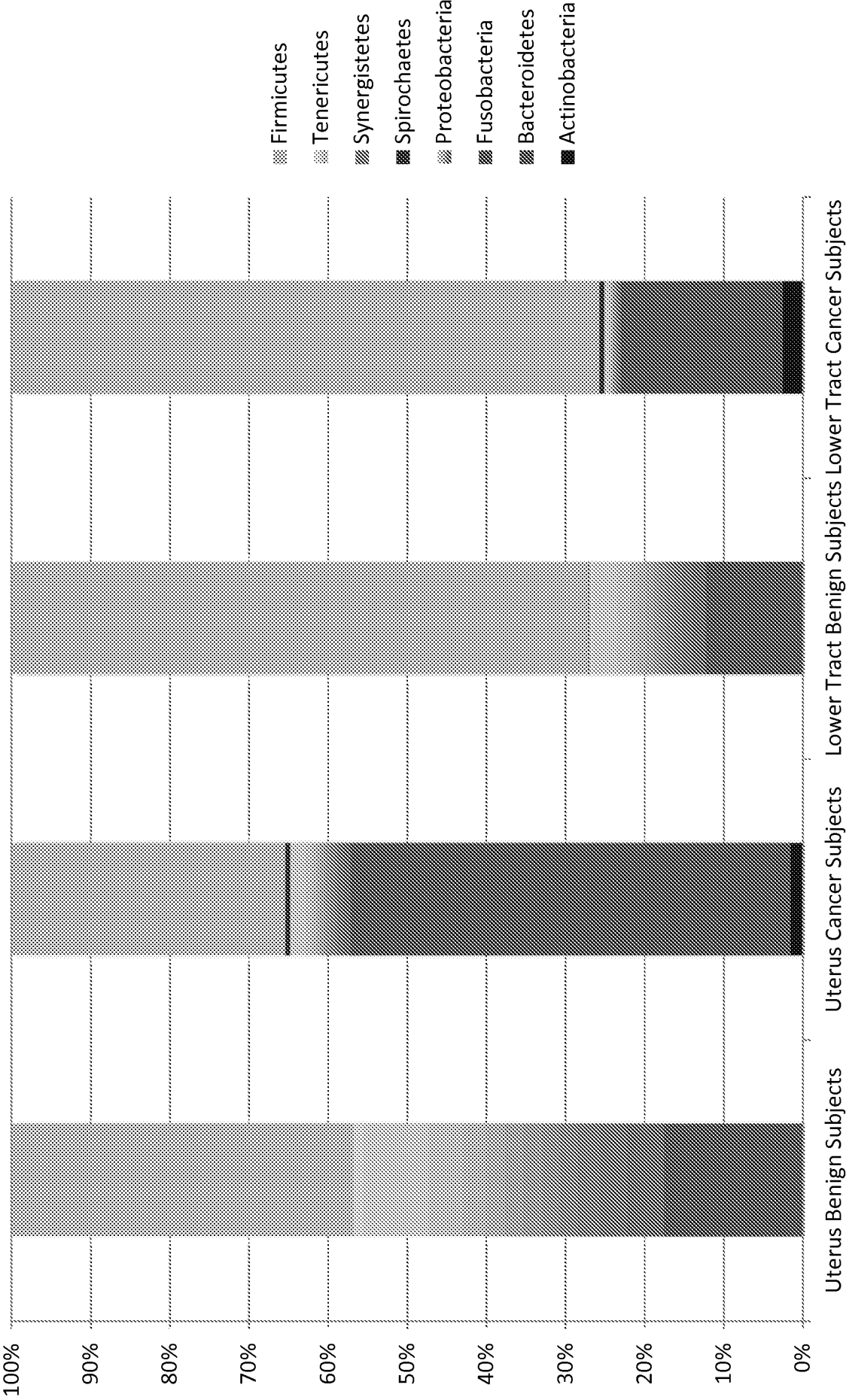


Figure 2

Porphyromonas sp. Figure 3

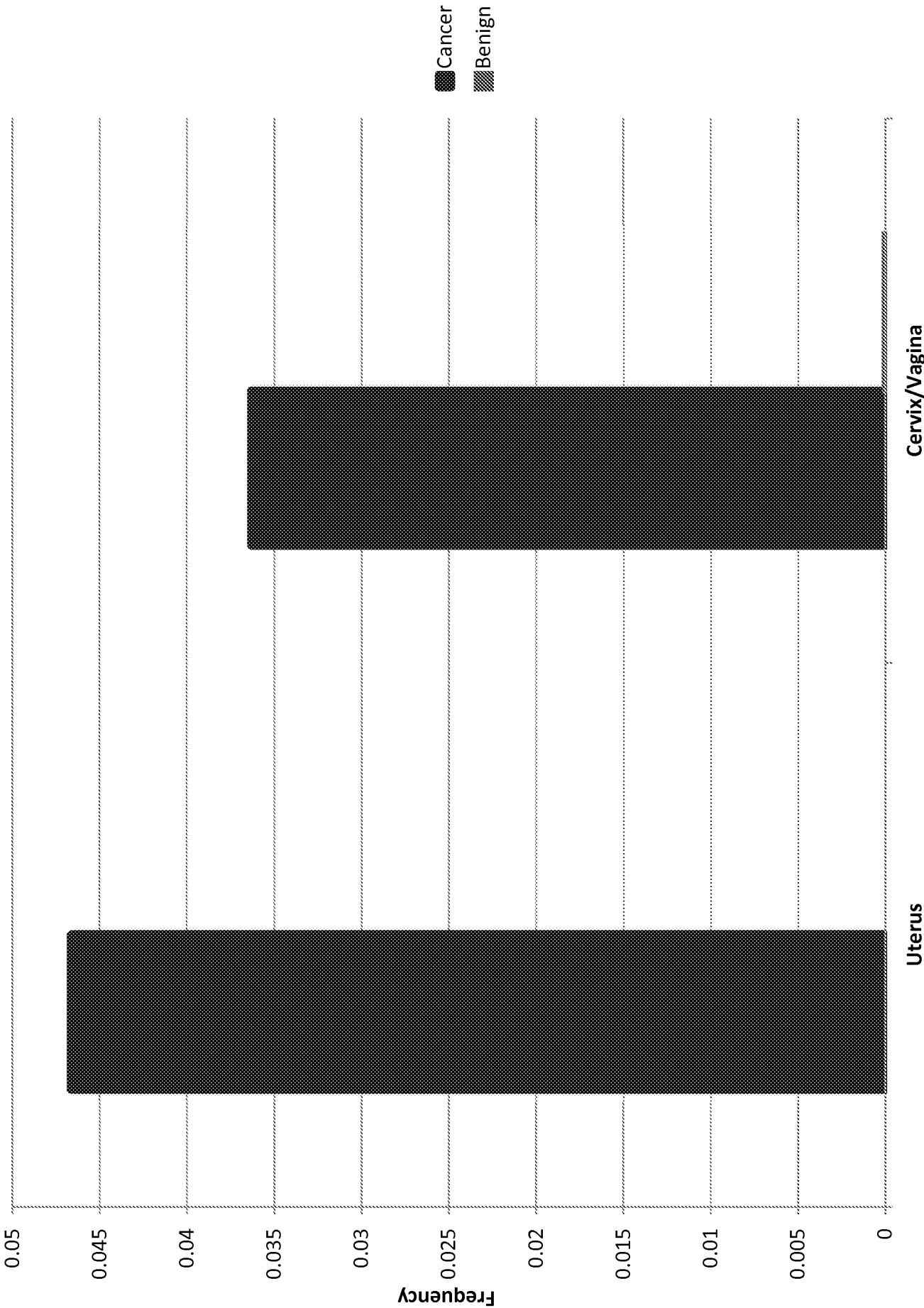
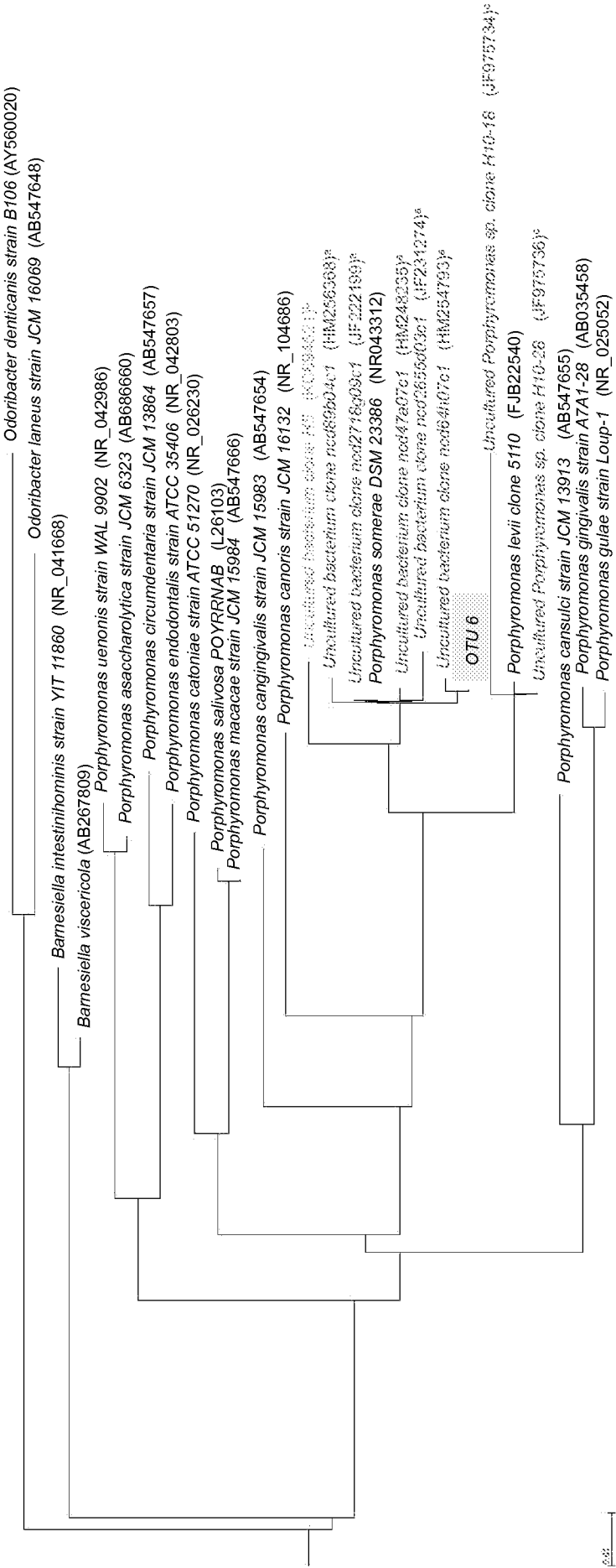


Figure 4



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 15/22963

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12Q 1/68 (2015.01)

CPC - C12Q 1/689

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): C12Q 1/68 (2015.01)

CPC: C12Q 1/689

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC: C12Q 1/6886, C12Q 1/04

(keyword limited; terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatBase, Google Patents, Google Scholar, Google Web, search terms: endometrial cancer, Porphyromonas, Porphyromonas somerae, 16S rRNA or 16 rRNA, FISH or fluorescence in situ hybridization, FISH probe, PCR or polymerase chain reaction, upper reproductive tract, lower reproductive tract, primer pair, female mammal, administering antibiotic, benzyltp

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2011/0207622 A1 (WONG et al.) 25 August 2011 (25.08.2011) para [0010], [0014], [0028], [0062], [0119], [0121], [0128], [0161], [0162]	1-49
Y	VON GRUENIGEN et al. Bacteriology and Treatment of Malodorous Lower Reproductive Tract in Gynecologic Cancer Patients. Obstetrics & Gynecology. (July 2000), vol 96, no 1, pp 23-27. Especially pg 23, col 2, para 3, pg 24, col 1, para 2, col 2, para2, pg 25, col 2, para 3 pg 26, col 1 para 1 Tables 1, 2	1-49
Y	SUMMANEN et al. Porphyromonas somerae sp. nov., a Pathogen Isolated from Humans and Distinct from Porphyromonas levii. J. Clin. Microbiol. (September 2005), vol 43, no 9, pp 4455-4459. Especially pg 4455, col 1, para 1-2, pg 4457, col 1 para 1, pg 4458, col 1, para 2	1-49
Y	US 2007/0178495 A1 (FREDRICKS et al.) 02 August 2007 (02.08.2007) para [0099], [0100], [0129], [0187], [0191], SEQ ID NO: 65, Table 5	33-49
Y	US 2012/0058094 A1 (BLASER et al.) 08 March 2012 (08.03.2012) para [0011], [0244], SEQ ID NO: 13	49

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search

16 June 2015 (16.06.2015)

Date of mailing of the international search report

08 JUL 2015

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Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774