**SOP: Quality assurance for the isolation of *Campylobacter***

**Section 1: Provided substances**

1. Primers (if required).
2. Three mock infected chicken faeces and one negative control (A-D) in Preston broth. All faeces would be autoclaved for custom regulations purpose.
3. Six Eppendorf’s containing pure cultures in Preston broth with 0.16 % agar.

**Section 2: Isolating and identifying *Campylobacter* samples**

1. On arrival follow **OHPH Lab SOP 001 for samples A-D. Sample preparation and isolation protocol**
2. Invert each intestinal/faecal sample/Preston broth suspension 3-5 times to mix and place in an incubator at 37 ± 1 °C for 5 ± 1 h under microaerophilic conditions (i.e. Gas Jar with CampyGen) to allow for resuscitation of injured organisms. Transfer the broths to an incubator at 41.5 ± 1°C for a further 44 ± 4 h.
3. In case of poor growth, after plating bacteria, place poultry faeces back in incubator at 41.5 ± 1°C under microaerophilic conditions..
4. Run a gram stain and an oxidase test on potential *Campylobacter* bacteria, followed bya Hippurate to differentiate between *C. jejuni* or *C. coli*, as per protocol
5. Confirm identification using PCR conditions in section 2.

**Section 3: Testing transport media**

1. Plate either 12 μl or loop full of sample to either Preston agar (see OHPH Lab SOP 001 for preparation details) or Columbia Blood Agar base, prepared by adding 5 % lysed horse blood and Skirrow (SR0069E).
2. Incubate under microaerophilic conditions for 48 hours.
3. Inoculate 10 % glycerol with Brain heart Infusion broth (CM1135, Oxoid), and store in a -80 °C freezer.
4. Select 1 or 2 colonies worth of sample and test samples using **primer set 1** following PCR conditions in **section 4.**

**Section 4: PCR conditions for primer set 1 and 2**

**Lysing of bacteria for colony PCR**

Take 1 to 2 colonies. place onto 20 µl dH2O, of which 1 µl would be added to the PCR reaction.

**Preparation of primer**

1. Vortex lyophilised Primers for 20 seconds.
2. Spin down the primers for 5 minutes
3. Add dH2O to make to 100 µm each by following the recommended volume from the company sheet provided for the primers
4. Vortex and spin down
5. Store the primers at -20 °C when needed to be stored

**Primer set 1**

|  |  |  |  |
| --- | --- | --- | --- |
| **Name of Primer** | **Sequence (5’-3’)** | **Target Gene** | **Product Size (bp)** |
| CJF | ACTTCTTTATTGCTTGCTGC | *C. jejuni hipO* | 323 |
| CJR | GCCACAACAAGTAAAGAAGC | *C. jejuni hipO* | 323 |
| CCF | GTAAAACCAAAGCTTATCGTG | *C. coli glyA* | 126 |
| CCR | TCCAGCAATGTGTGCAATG | *C. coli glyA* | 126 |
| 23S rRNA F | TATACCGGTAAGGAGTGCTGGAG | *C. jejuni 23S rRNA* | 650 |
| 23S rRNA R | ATCAATTAACCTTCGAGCACCG | *C. jejuni 23S rRNA* | 650 |

**Primer preparation:**

* Use the main 100 µM primer stock and add volume as recommended below. This should give a final concentration of 10 μM CJF/CJR, 20 μM CCF/CCR and 4 μM of 23S rRNA F/23S rRNA R.
* So when we add 1 μl of this mix we will get 0.5 μM CJF/CJR, 1 μM CCF/CCR, 0.2 μM 23S rRNA F/23S rRNA R in 20 μl reaction, as recommended by article.
* Note: You can increase the volume of primer mix if PCR are being run i.e. to make 20 μl of primer mix multiply each volume by 2

NOTE\* Users can use a different approach to preparing the PCR mix if they wish

|  |  |  |  |
| --- | --- | --- | --- |
| **Name of Primer** | **Volume for 10 μl** | **Concentration (μM)** | **Final reaction concentration** |
| CJF | **1.0 μl**  | 10 μM | 0.5 μM |
| CJR | **1.0 μl** | 10 μM | 0.5 μM |
| CCF | **2.0 μl** | 20 μM | 1 μM |
| CCR | **2.0 μl** | 20 μM | 1 μM |
| 23S rRNA F | **0.4 μl** | 4 μM | 0.2 μM |
| 23S rRNA R | **0.4 μl** | 4 μM | 0.2 μM |
| dH2O | **3.2 μl** | - |  |

**PCR reaction preparation**

|  |  |  |
| --- | --- | --- |
| **Component** | **Reaction (μl)** | **Final concentration** |
| dH2O | 8 |  |
| Sample | 1 |  |
| Primer Mix | 1 |  |
| 2X Platinum SuperFi II Green PCR Master Mix | 10 | X1  |
| **Total reaction** | **20** |  |

**Cycling conditions**

**•** 95 °C for 6 minutes

• 30/35 cycles of

 ◦ 95°C for 30 seconds

 ◦ 59°C for 30 seconds

 ◦ 72°C for 30 seconds

• 72°C for 7 mins

Resolve PCR amplicons by gel electrophoresis using a 1.5% (w/v) agarose gel with a 100 bp DNA ladder and an appropriate stain.