***Mycoplasma hominis* Infections Transmitted through Amniotic Tissue Product** **Supplementary File A**

**Supplementary Method and Materials**:

Each isolate of *M. hominis* isolate was process using the method below:

Whole genome sequencing was performed using third generation sequencing technology, PacBio (Pacific Biosciences, Menlo Park, CA) RSII genome analyzer.

The 10kb - 20kb Template Preparation and Sequencing with Low Input DNA (PN 100-798-300-1) protocol was optimized by substituting processes from Very Low (10ng) Input 2kb Template Preparation and Sequencing with Carrier DNA protocol (PN: 100-800-200-01). See table below:

|  |  |  |
| --- | --- | --- |
| Task\Process Step | Protocol 1: 10 kb- 20kb Template Preparation and sequencing with Low (100 ng) Input DNA | Protocol 2:Very Low (10ng) Input 2kb Template Preparations and Sequencing with Carrier DNA |
| Insert size target: 10kb-20kb  | Insert size range to achieve 10kb-20kb fragment | 2kb insert size, not sufficient for task.  |
| Preparation for Carrier DNA | N\A | Procedure included instructions for preparation and care.  |
| Concentrate and Purify DNA using AMPure PB Beads | Excessive sample loss when performing 0.5X AMPure PB concentration and purification procedure. | Used procedure instructions to obtain the highest sample yield, 0.6X AMPure PB Beads |
| Purifiy SMRTbell Template- 2 step purification suing AMPure PB Beads | Used procedure instruction to obtain the highest sample yield, 0.45X AMPure PB Beads | Excessive sample loss when performing 0.6X AMPure PB purification procedure. |

**Note**: Highlighted cells indicate processed used from protocol to create the method used.

Covaris g-Tubes™ were used with an Eppendorf MiniSpin® centrifuge to shear gDNA into 20kb fragments. Pacific Biosciences SMRTbell™ Template Prep Kit 1.0 (catalog no: 100-259-100) was used to prepare 20kb fragments libraries using AMPure PB beads (catalog no: 100-262-900) for all DNA cleanup and purification at various stages in the workflow. The purified, exonuclease treated, non-adaptor ligated pBR322 plasmid (New England BioLabs Inc., catalog no. N3033S), was added to the target sample after ligase inactivation, prior to the exonuclease reaction. The DNA\Polymerase Binding Kit P6 v2 (catalog no: 100-372-700) was used during annealing and binding reactions. SureCycler™ 8800 Thermal Cycler from Agilent Technologies (catalog no: G8800A) was used for the all library preparation incubations, primer annealing and polymerase binding reactions. Non-standard loading concentration adjustments were made to the Binding Calculator (version: 2.3.1.1), provided by Pacific Biosciences, to calculate the sequencing primer annealing, polymerase binding and MagBead loading concentrations such that all library material available were loaded onto the sample plate for sequencing. The PacBio DNA Sequencing Kit 4.0 v2 (catalog no: 100-356-200), PacBio RSII SMRT Cells 8Pac v3 (catalog no: 100-171-800), and MagBead Kit v3 (catalog no: 100-133-600) were all used for sequencing.

Each 10kb-20kb sequencing reaction was performed using the P6 polymerase on the RSII with 2 (SMRT cells for each library) x 360 minute movies. MagBead OnceCellPerWell (OCPW) protocol as well as Stage Start was selected in RS Remote to run each well on the sample plate on a separate SMRT cell and to maximize insert read lengths.