Primers were made to amplify the V6 region of the bacterial rRNA gene. The are written 5' to 3' as is usual and the primers were:

Left-side primers (EXAMPLES):

CCATCTCATCCCTGCGTGTCTCCGACTCAGtatcgCWACGCGARGAACCTTACC V6LT1
CCATCTCATCCCTGCGTGTCTCCGACTCAGtagacCWACGCGARGAACCTTACC V6LT2
CCATCTCATCCCTGCGTGTCTCCGACTCAGtgcatCWACGCGARGAACCTTACC V6LT3
CCATCTCATCCCTGCGTGTCTCCGACTCAGatgagCWACGCGARGAACCTTACC V6LT4
CCATCTCATCCCTGCGTGTCTCCGACTCAGacagtCWACGCGARGAACCTTACC V6LT5
CCATCTCATCCCTGCGTGTCTCCGACTCAGagatgCWACGCGARGAACCTTACC V6LT6
CCATCTCATCCCTGCGTGTCTCCGACTCAGctcacCWACGCGARGAACCTTACC V6LT7
CCATCTCATCCCTGCGTGTCTCCGACTCAGctgtaCWACGCGARGAACCTTACC V6LT8
CCATCTCATCCCTGCGTGTCTCCGACTCAGcgtgaCWACGCGARGAACCTTACC V6LT9
CCATCTCATCCCTGCGTGTCTCCGACTCAGcgactCWACGCGARGAACCTTACC V6LT10
CCATCTCATCCCTGCGTGTCTCCGACTCAGaactcCWACGCGARGAACCTTACC V6LT11
CCATCTCATCCCTGCGTGTCTCCGACTCAGcctatCWACGCGARGAACCTTACC V6LT12

The first part of the primer, in upper case is the Ion Torrent adapter sequence, and is identical across all left primers. The second part, in lowercase is the sequence tag that is used to identify each individual amplified product. This allows a mixture of PCR products to be identified by their unique sequence tag. The final part is the sequence complementary to the constant region on the left side of the V6 region. Standard nucleotide ase nomenclature is followed.

Right-side primer

CCACTACGCCTCCGCTTTCCTCTCTATGGGCAGTCGGTGATACRACACGAGCTGACGAC V6RT1

The first 41 nucleotides are the Ion Torrent right adapter sequence, the last 18 are complementary to the right side of the V6 rRNA region. No sequence tags are attached.

Standard PCR protocol for Ion Torrent V6 primers:

Primers have been diluted to 3.2 pMol/ul (3.2 ul of 200 pMol/ul stock into 200 ul H_2O)

Standard reaction volume of 40 ul

50 ul mineral oil
1.5 ul DNA (0.5ul to 2ul is OK. Aim for 1ng of template input)
10 ul P1
10 ul P2

Heat to 90ºC
(NOTE if not using mineral oil: mix template, primers, and mastermix and place reaction on heated 95ºC block for 2 min to activate Taq)

Add 20 ul colorless 2X GO Taq master mix (Promega)

Cycle x25 at 95ºC, 55ºC, 72ºC for 1 minute each..

At the end of the run, let the mixture cool to room temperature and place at 4 degrees.

Reid lab: Master mix in in yellow box in freezer #2. Yellow-capped tube. The run is labeled “Ion” on the thermocycler.

Quantitation and purification:

5 ul of each sample is taken out and mixed with 195 ul of QuBit broad-range fluorometric compound. After 2-10 minutes of incubation, the samples were read in a QuBit fluorometer and compared to the broad-range standard.

The fluorometric reading was taken to indicate the amount of double-stranded DNA in the sample, and was used to make an approximately equal concentration mixture of the amplified PCR products, where the largest volume available was approximately 10 ul, and more concentrated samples were added in proportionally lower amounts.

The PCR mixtures were purified away from contaminating primer sequence by using the Promega Wizard PCR purification kit.

Samples were further purified by running them on an agarose gel and extracting the about 200 bp band with a Pip-n-Prep matching using the widest possible gate. The exact center of the gate was the center of mass of the band. This corresponds to removing a set of bands between 175 and 225 bp inclusive.

Notes:
GoTaq colorless hot start mastermix:
http://www.promega.com/resources/protocols/product-information-sheets/g/gotaq-hot-start-colorless-master-mix-protocol/