The 16s rRNA gene is often used to identify different species of bacteria. This is because the sequence is conserved enough that few changes can mean a lot but not conserved completely so there are some changes. This protocol will teach you how to amplify the DNA of a bacterial colony or streak from a plate.

Step 1:

- Setup your PCR reaction by adding:
  (For a 50uL PCR reaction)
  a. 0.5uL of 10uM 515F primer
  b. 0.5uL of 10uM 1492R primer
  c. 25uL 2x Master Mix or 10uL of 2x Master Mix
  d. Distilled water to 50uL (23uL for 2x Master Mix, 38uL for 5x Master Mix)
  e. Using an inoculation loop or pipette tip scrape a single colony or a tiny bit of bacteria and mix it into the tube. The amount of bacteria should be visible to the naked eye.

- Use the PCR protocol below

  1 x 95C 10 Minutes

  30 x
  95C 15s
  55C 1 minute
  68C 2 minute

  1x 68C 10 minutes

Step 2:

- Run Gel Electrophoresis on samples to determine success.
  a. If you have a gel electrophoresis unit run a gel and see if there is a band of DNA. This is so you know your PCR was successful.
    i. NOTE: If you do not see a band, try using more of your PCR reaction in the well. Normally 5uL is the standard amount. If you still do not see a band, the PCR amplification was not a success. Retry doubling the amount of bacteria and primers you used.
  b. Use your PCR purification kit to purify the rDNA that you amplified.
  c. Send your samples off to a company to sequence along with primers. I generally use the R primer for sequencing. You can submit multiple primers for the same sample just keep them in separate tubes and label all your samples! Usually, one will submit a (F)orward and a (R)everse primer.
  d. Genewiz is a great company to send your sequence to they will even purify the DNA so you can skip the above step **b**. https://www.genewiz.com/

- Once they receive the samples they will send you back two files for each sequencing run.

a. One is usually labeled <name of sample>.seq and <name of sample>.ab1
b. The .seq file is the one that contains the DNA sequence that they acquired from the run.
c. The .ab1 file contains a spectrogram.
d. To look at the ab1 file you need special software, FinchTV is probably one of the more popular ones (http://www.geospiza.com/Products/finchtv.shtml).

Step 3:

- In order to identify the bacteria that our sequence belongs to and so identify our bacteria of interest we need to compare our sequence(s) to the database at NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome)

- Where it says “Enter accession number(s), gi(s) or FASTA sequence(s)” paste your 16s rDNA sequence into that box.
    a. If the sequence in the file is really short (less than 30 bases) usually it means the sequencing failed.

- Where it says “organism” type in “bacteria”. Then go to the bottom and click the button that says “BLAST”.

- After 1-30 seconds your results should come up. It is often that the #1 hit is the genus of bacteria. The species can be a little more complicated as generally you won’t find a 100% match. If you also did a forward primer sequencing reaction BLAST that one also and compare the species. If both the forward and reverse primers match species there is a good chance that your bacteria belongs to that species or a very closely related one that has not been sequenced and identified yet!