A few things to try when trying to get longer reads in sanger sequencing:

- -It is recommended that you have an excess of primers for longer reads, so try doubling that.
- -Maybe consider doubling the whole volume of the reaction to give it more physical space but not more than 20µl.
- -Also longer template may have trouble denaturing. So consider changing the first denaturing step of your program to 98^{o}C 5 minutes instead 96^{o}C for 1 minute.

  And in the cycles change the denaturing also to 98^{o}C and for 1 minute vs 30 seconds. Could also try adding 1µl of DMSO per 20µl reaction to help the template stay denatured. 1M final concentration of Betaine has also be used to great affect by many labs.
- -Can also try adding more cycles to amp up signal.

Recommended DNA template quantities for cycle sequencing BigDye Terminator v3.1

PCR product: 100 to 200 bp 200 to 500 bp

500 to 1000 bp 1000 to 2000 bp

>2000 bp

1 to 3 ng
3 to 10 ng
5 to 20 ng 10 to 40 ng 40 to 100 ng