Performing Cycle Sequencing using BDT v3.1
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This protocol continues from **Preparing Samples for Sequencing**, but allows for one user to mix the whole reaction, rather than two (primer+DNA and BDT separately).

Listed below is my recommendation for the reaction, which is different from ABI's recommended mixture. Changes include addition of Sequencing Buffer, and increased primer concentration. If you want to alter this reaction cocktail, I highly recommend that you first read the ABI protocol that accompanies BDTv3.1: maintaining buffer concentration in the ready reaction mixture is very important.

Please refer to old protocol **Performing Cycle Sequencing using BDTv3.0** for instructions on sample arrangement and other concerns (salt concentrations, pipette use and disposables use).

This cocktail should be mixed only as needed. It is advised that you aliquot your BDT ahead, and do not subject it to repeated freeze/thawing, or extended light exposure. Sequencing buffer should also be aliquoted ahead but stored at 4˚C.

Cocktail per sample:
2µl BDTv3.1 (ABI, cat# 4337455)
1µl Sequencing Buffer (comes with BDT)
1µl pcr water
2µl primer @ 4µM (yields 8pmol/rxn)
4µl template (pcr product)
10µl rxn total

1. Mix cocktail (without template) in separate tube. Keep on ice until ready to use, and dispense 6µl/well.

2. Add template. Mix well contents with pipette, but do not introduce bubbles.

3. Cover plate with sealing tape (sealing very well), and place immediately in thermocycler. Run under protocol 'abicyclo': set thermal ramp speed to 1˚C/s for all steps. Cycle the three steps below 26 times.

(96˚C, 10s
55˚, 5s
60˚, 4min), 26x;
4˚C forever.

4. Remove plate promptly to either 4˚ or –20˚C. Do not let sit overnight in thermocycler, as block temperature will increase and samples will possibly evaporate.

To continue, see protocol **EDTA/EtOH precipitation of Cycle-Sequenced Products or EDTA/NaOAc/EtOH precipitation of Cycle-Sequenced Products.**