

BigDye® Terminator Cycle sequencing protocol with PCR template (take effect from 2010/14/06)

The following protocol uses **1/16** of the original BigDye ® mix and is suitable for the versions v1.1/v3.1. It has been adapted to the Beckman Coulter cleanSEQ chemistry, which is used to remove contaminants prior the sequencing run since 2010/14/06. Please be sure to use this tested protocol, otherwise there is the risk that signal intensities are too high!

In general, higher DNA quantities give higher signal intensities. Higher DNA quantities may also give shorter read lengths and top-heavy data. Do not use more than 10ng/100bp!

	Volume in µl
BigDye® terminator v1.1/v3.1	0,25
5 x BigDye sequencing buffer	2,25
Primer 10 µM	0,25
PCR template	2-10ng per 100bp
TOTAL VOLUME	10,0

A dilution of the reaction mixture to 20µl is no longer needed and wanted! Also take notice of the protocol for self made sequencing buffer.

Program: 10 s 96°C
5 s 55°C x 32 Cycles
4 min 60°C

BigDye v1.1 or v3.1 ?

V3.1. was designed to

- work better for GT-rich templates
- produce longer reads
- give better signal distribution for heterozygous SNPs

On the other hand

- most templates are not GT-rich
- the CCG uses short capillaries which are not optimized for long reads
- you nevertheless find heterozygous SNPs with v1.1
- v.1.1 is better in base calling adjacent to the primer
- Some times we observe better sequence data when dealing with the 1.1 version

Source: <http://portal.ccg.uni-koeln.de/ccg/service/sequencing>
Contact: ccg-seqservice@uni-koeln.de