Manual (30.05.2014)

Alkyne PCR

General procedure and considerations

This manual contains recommendations for PCRs using alkyne-modified nucleotides. These data may be used as a starting point for optimization of your particular Alkyne-PCR-procedures.

Preparation of DNA for Click

Generate DNA strands with reactive triple bonds by PCR through incorporation of C8-Alkyne dNTPs (200 µM, C8-Alkyne-dUTP, mi-N3002 and C8-Alkyne-dCTP, mi-N3001). This enables the attachment of fluorescent or non-fluorescent azides (Fluoresceine, Chromeo™, Eterneon™ and Tamra dyes, Dabcyl and Biotin, mi-C1001 to mi-C1015) by click chemistry – please see our mi-Click Chemistry manual and metabions homepage, Click chemistry section.
Alkyne-modified PCR products have to be purified prior to click reaction (e.g. using PCR purification kit, mi-PCR50/250).

Recommended polymerases for PCR using alkyne-modified nucleotides:
mi- Modimerase (mi-E9000, metabion), tested for amplicons up to 400 bp
alternatively: Pwo superyield (Roche), Deep vent exo- (NEB)
conventional *Taq* Polymerases cannot be used!

Substitution of dTTP or/ and dCTP
Standard nucleotides dTTP or/ and dCTP should be substituted 100 % in PCR by C8-Alkyne-dUTP and C8-Alkyne-dCTP respectively.
We recommend to use C8-Alkyne-dUTP for the incorporation of alkyne-modified nucleotides.
The incorporation of C8-Alkyne-dCTP is less efficient and seems to be influenced by the GC-content of the template. A low GC-content of the template (GC <50 %) is should be fine for the incorporation; at a higher GC-content (> 50 %) PCR might fail.

Suggested PCR conditions
Primer: 0,4 µM
dNTPs: 0,2 mM (each)
template: 1 ng - 10 ng
reaction volume: 25 µl and 50 µl
mi- Modimerase: 2,5 U
Deep vent exo-: 1 U
Pwo superyield: 2,5 U

PCR Program

for up to 300 bp:	*for up to about 2500 bp*:
1. 96 °C for 2:00	1. 99 °C for 2:00
2. 99 °C for 0:15	2. 99 °C for 0:45
3. 56 °C for 0:30 (-1 °C per cycle)	3. 58 °C for 0:30 (-1 °C per cycle)
4. 72 °C for 0:30 (2.- 4. for 9 times)	4. 72 °C for 5:00 (2.- 4. for 9 times)
5. 96 °C for 0:15	5. 99 °C for 0:45
6. 53 °C for 0:30 (your primer annealing temperature)	6. 53 °C for 0:30 (your primer annealing temperature)
7. 72 °C for 0:30 (5.- 7. for 30 times)	7. 72 °C for 5:00 (5.- 7. for 30 times)
8. 72 °C for 2:00	8. 72 °C for 10:00