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Addendum:

Methodological examples for the application of the Polymerase chain reaction

A) Characterization of oncogenes

Detection of mutations at codon 61 of the c-Ha-ras gene in small precancerous liver lesions of the C3H mouse

A1

R Bauer-Hofmann, A Buchmann, F Klimek, M Schwarz

Isolation and direct sequencing of PCR-cDNA fragments from tissue biopsies H Klocker, F Kaspar, J Eberle, G Bartsch	A4
Differential PCR: Loss of the β 1-interferon gene in chronic myelogenous leukemia (CML) and acute lymphoblastic leukemia (ALL) A Neubauer, C Schmidt, B Neubauer, W Siegert, D Huhn, E Liu	A11
B) Detection of infectious agents	
Long-term persistence of <i>Borrelia burgdorferi</i> in neuroborreliosis detected by polymerase chain reaction S Bamborschke, A Kaufhold, A Podbielski, B Melzer, A Porr, B Rehse-Küpper	A15
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The application of polymerase chain reaction for studying the phylogeny of bacteria G Köhler, W Ludwig, KH Schleifer	A56

Site-directed mutagenesis facilitated by PCR

O Landt, U Hahn

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Ectopic transcription in the analysis of human genetic disease

J Reiss

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Appendix III: DNA sequencing chromatograms

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1.1. PCR: A cyclic, exponential, in-vitro amplification process

The polymerase chain reaction (PCR) permits the selective in vitro amplification of a particular DNA region by mimicking the phenomena of in vivo DNA replication. The following reaction components are required: single-stranded DNA template, primers (oligonucleotide sequences complementary to the ends of a defined sequence of DNA template), deoxynucleotide triphosphates (dNTPs) and a DNA

polymerase. The commonly used reaction buffers in PCR contain Mg²⁺, monovalent cations and some co-solvents. The co-solvents may help to stabilize the enzyme, influence the enzyme processivity and/or DNA melting temperature (T_m). The introduction of a heat stable DNA polymerase (Saiki 1988, Mullis 1985) brought significant improvements in PCR and automation became a typical feature of PCR methods. Most of the DNA polymerases used in PCR are heat stable and can withstand temperatures up to 95-97°C. The polymerase chain reaction itself requires three

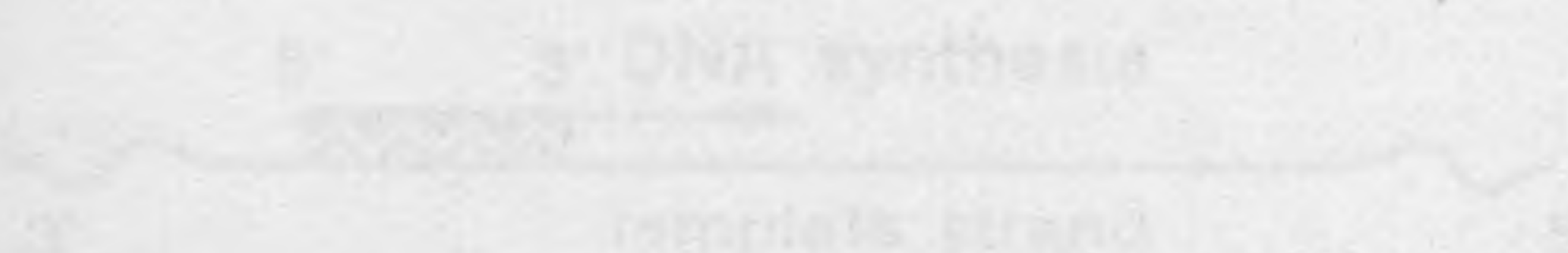
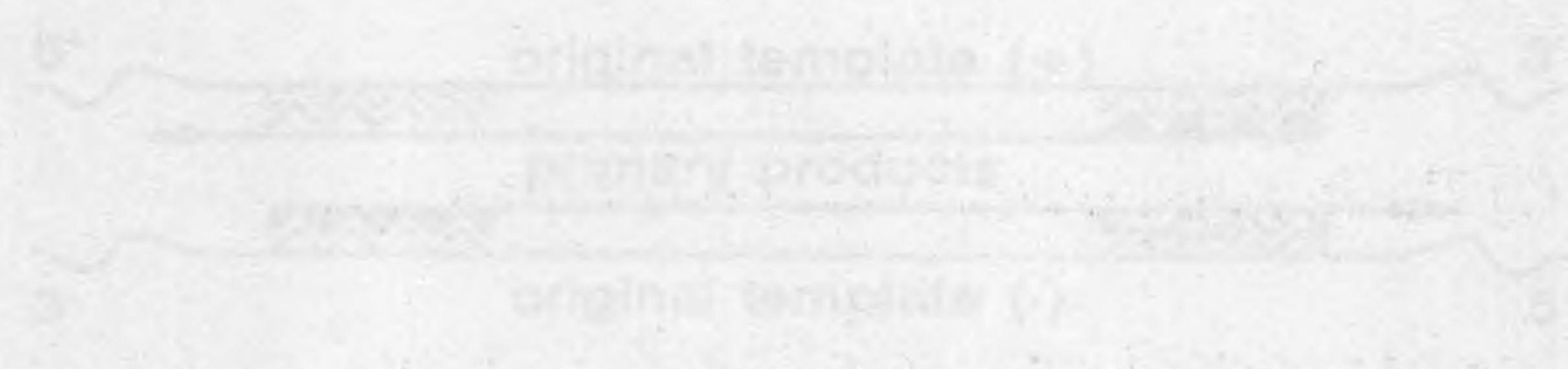


Figure 1.1: Enzymatic synthesis of a DNA strand starting at the free 3'-OH terminus of the primer. The newly synthesized strand will be complementary to the template. For symbols, see legend from figure 1.2

polymerase enzyme. A new DNA strand complementary to the desired template can then be enzymatically synthesized under appropriate conditions (see figure 1.1). The various reaction components for PCR are readily available. Single-stranded DNA template is easily generated by heat-denaturing (melting) double stranded DNA. Synthetic oligonucleotide primers can either be synthesized in one's own laboratory or



primer 1	primer 2	
TTTTT	AAAA	primers in active extension
AAAA	TTTTT	primers annealing sites
TTTTT	AAAA	primer sequences

Figure 1.2: First DNA denaturation/cycle. DNA synthesis is started at two different priming sites on the two original template strands. Through the pairing of the primer annealing sites, each primer product posses again a newly synthesized primer annealing site. The newly synthesized strands from the primary products do not have a distinct length.