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Light-scattering techniques as a tool for on-line monitoring of artificial gene assembly by thermostable DNA polymerases

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The artificial genes or synthetic DNA is highly demanded by the fast-growing field of synthetic biology [1]. The building of synthetic DNA is a multistage process that includes phosphoramidite synthesis, oligonucleotide preparation, enzymatic gene assembly, and quality control. The results of gene assembly are evaluated by gel electrophoresis and by Sanger sequencing. These procedures add additional efforts and time to the gene synthesis workflow. The ability for real-time monitoring of gene assembly was previously reported by an indirect method using real-time polymerase chain reaction [2]. Since gene assembly is accompanied by the formation of duplex DNA from smaller oligonucleotides we decided to test light-scattering techniques as a direct method for on-line gene assembly monitoring. Our results clearly confirm that oligonucleotide annealing and gene assembly process could be monitored by static/dynamic light-scattering (SLS/DLS) measurements. However, we should state that modern SLS/DLS equipment is not adapted for such tasks, mainly due to the inertia of heating and heat transfer. Therefore, it is necessary to conclude, that if there is an opportunity to overcome the stated problems by a special design of the sample compartment and measuring cell, light-scattering techniques may be considered an alternative method for the optimization of gene assembly methods.

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2. Ye, H.; Huang, M. C.; Li, M. H.; Ying, J. Y. Experimental analysis of gene assembly with TopDown one-step real-time gene synthesis. *Nucleic acids research* **2009**, *37*, e51.