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Mediator probes for electrochemical DNA detection: Universal electrode functionalization for specific detection of different targets

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A growing number of commercially available devices for electrochemical nucleic acid detection demonstrate the strength of the technology for sample-to-answer or point-of-care systems especially if multiplexed panels are required. However, to achieve high multiplexing degrees, electrodes usually need to be functionalized with capture probes, to which the target sequence specifically hybridizes. In current approaches, capture probe design and electrode array functionalization needs to be adapted for each new panel. Furthermore, single-stranded DNA is needed for hybridization, which usually requires an additional digestion step or asymmetric amplification method. To overcome these drawbacks, the mediator probe (MP) approach that was previously reported for fluorescent based PCR systems, was adapted for electrochemical detection. In order to reduce adaption efforts for different panels, a universal reporter (UR) is used as electrode-bound capture probe that features a generic sequence. Reaction specificity is maintained by introducing a bipartite MP in the amplification reaction. It consists of a target specific site labeled with an electroactive molecule and a mediator region that is complementary to the UR. Hybridization of the intact MP to the electrode-bound UR leads to a high signal. However, if the target is present during reaction, the mediator is cleaved and the electroactive molecule is not enriched on the electrode surface. The principle was demonstrated for four targets, using three different URs. PCR results were directly analyzed on functionalized screen-printed electrodes by square wave voltammetry (SWV) without further treatment. No unspecific signals were detected, if MPs do not match the URs. The sensitivity is comparable to fluorescence based real-time PCR, which was performed in parallel to proof results. An electrode array functionalized with URs has the potential to be used for various panels, without losing specificity. As the MP is single-stranded, endpoint hybridization detection is possible without additional digestion step.

Recent Publications

1. Nijhuis R H T, et al. (2017) Comparison of ePlex respiratory pathogen panel with laboratory-developed real-time PCR assays for detection of respiratory pathogens. J Clin. Microbiol. 56(11):1938-1945.
2. Kawai K, et al. (2017) Detection of bovine mastitis pathogens by loop-mediated isothermal amplification and an electrochemical DNA chip. J. Vet. Med. Sci. 79(12):1973-1977.
3. Pearce D M, et al. (2013) A new rapid molecular point-of-care assay for Trichomonas vaginalis: preliminary performance data. Sex. Transm. Infect. 89(6):495-497.
4. Faltin B, et al. (2012) Mediator probe PCR: a novel approach for detection of Real-Time PCR based on label-free primary probes and standardized secondary universal fluorogenic reporters. Clin. Chem. 58(11):3546-1556.
5. Wadle S, et al. (2016) Simplified development of multiplex real-time PCR through master mix augmented by universal fluorogenic reporters. Biotechniques 61(3):123-128.

Biography

Martin Trotter studied BSc in Medical Engineering and MSc in Microsystems Engineering. He currently focusses on electrochemical sensors for nucleic acid detection. This includes also related topics such as functionalization of electrode materials, amplification procedures – PCR as well as isothermal approaches, electrode miniaturization and their integration in microfluidic chips for low-cost production.

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