Multiplexed quantification of protein panels by nanopore sequencing of reporter oligonucleotides

Adapting a sandwich enzyme-linked immunosorbent assay (ELISA) to give a DNA-based readout enables an accurate PCR-free and multiplexed method of protein quantification

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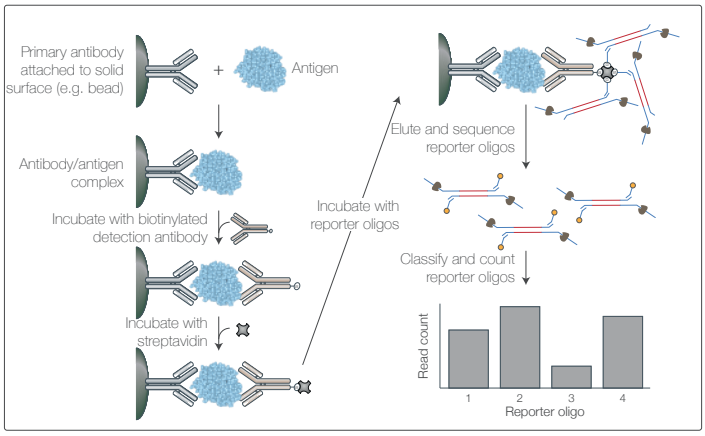

Fig. 1 Workflow diagram for nanopore protein quantification

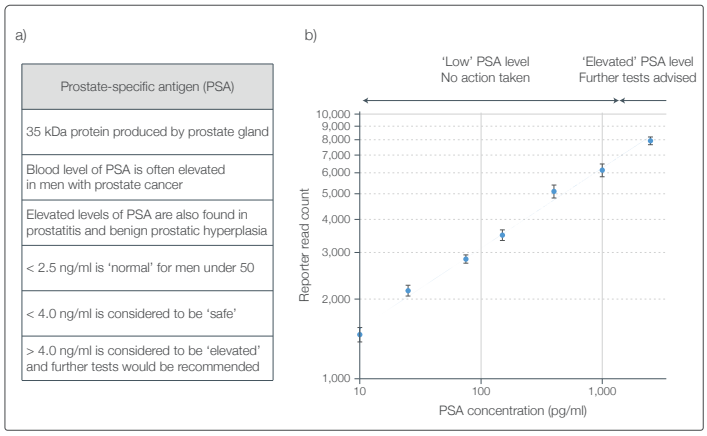

Fig. 2 a) PSA b) Sandwich ELISA sequencing assay showing quantitative results

PCR-free workflow for ELISA-based protein quantification nanopore assay

The sandwich ELISA is a sensitive, specific and robust method for the quantification of proteins present in a biological sample. A primary antibody is immobilised on a solid surface, and when an antigen is present, this binds to the primary antibody. A secondary antibody then binds to the antigen. In our adapted version of the protocol this secondary antibody has a biotin group attached to the free end of one heavy chain. Following antibody/antigen complex formation, streptavidin is added. Streptavidin is capable of binding four biotin groups, meaning that when a streptavidin molecule binds to a heavy chain, an average of three binding sites are still available. We add reporter oligonucleotides to these available binding sites (Fig. 1).

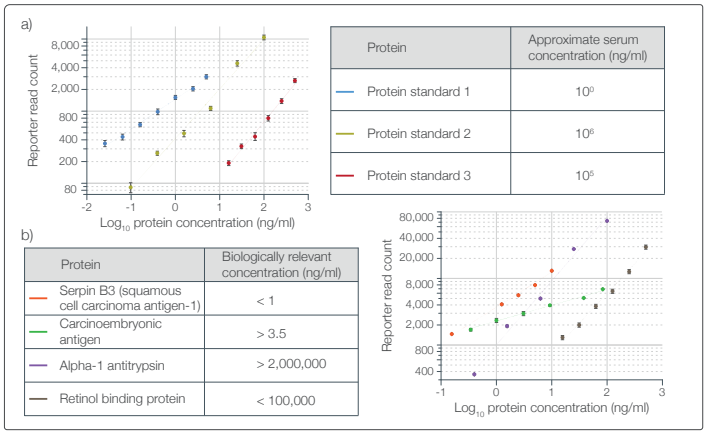

Fig. 3 Multiplexed protein quantification a) 3 protein standards b) lung cancer biomarker panel

Multiplexed quantification of proteins by nanopore sequencing

It is typically necessary to quantify panels of two or more 'biomarker' proteins in a single assay to measure disease progress. To see if this would be possible, we designed assays for several proteins. Antibody capture was performed separately for each protein over a range of concentrations, and antibody-protein conjugates were labelled with reporter oligonucleotides, using a different reporter for each protein, and a different barcode for each protein concentration. The eluted reporters were pooled and sequenced together on one flowcell. Fig. 3a shows titration curves for three protein standards and Fig. 3b shows results for a lung cancer biomarker panel. The sensitivity of each assay exceeds that required to measure these proteins at their physiological concentrations.

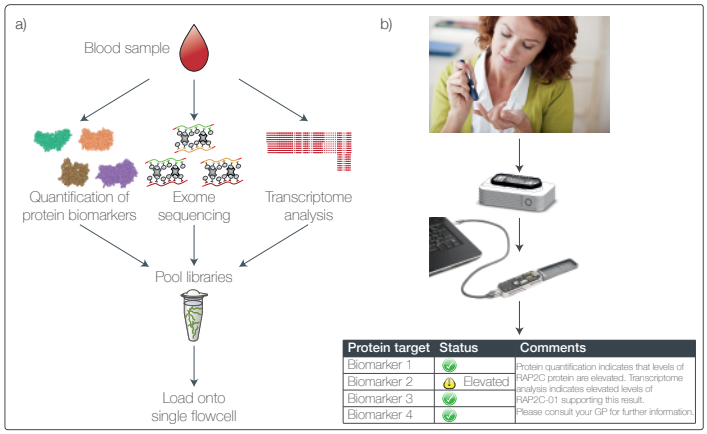

Fig. 4 Combined testing of biomarker proteins, transcriptome and exome on single flowcell

Quantitative uniplex modified sandwich ELISA protocol demonstrated on PSA

To demonstrate the effectiveness of this approach in quantifying a protein, we developed an assay for PSA, a protein which is frequently described as a biomarker for prostate cancer. Blood PSA concentrations greater than 4 ng/ml are considered to be 'elevated' and warrant further tests (Fig. 2a). We took a range of PSA concentrations in duplicate, both above and below 4 ng/ml, and performed the method outlined in Fig. 1, using reporter strands which were barcoded differently for each PSA concentration. All eluted strands were then pooled and sequenced together on a single flowcell, and read counts for each reporter oligo were reported. We obtained a good correlation between reporter read count and PSA concentration (Fig. 2b).

Combined nanopore testing of exome, transcriptome and biomarkers

Although for the experiments presented here we only multiplexed at the level of sequencing, pre-assembling the secondary antibody/reporter oligonucleotides will enable several proteins to be assayed in a single reaction. In addition, this will allow us to balance the reporter read count for all the different proteins in a panel, even though they may occur at vastly different concentrations in serum. The relatively low number of reporter reads needed for sensitive protein quantification means that a large biomarker panel could be quantified on a single flowcell while that same flowcell is used to sequence that person's exome and transcriptome simultaneously (Fig. 4a). This could be used to provide a comprehensive assessment of the status of a person's health, and when combined with VolTRAX, could be performed outside of the laboratory (Fig. 4b).