AQ BIOTECH OY

DESCRIPTION OF THE PHAGE-BIOSENSOR DEVELOPMENT PROCESS

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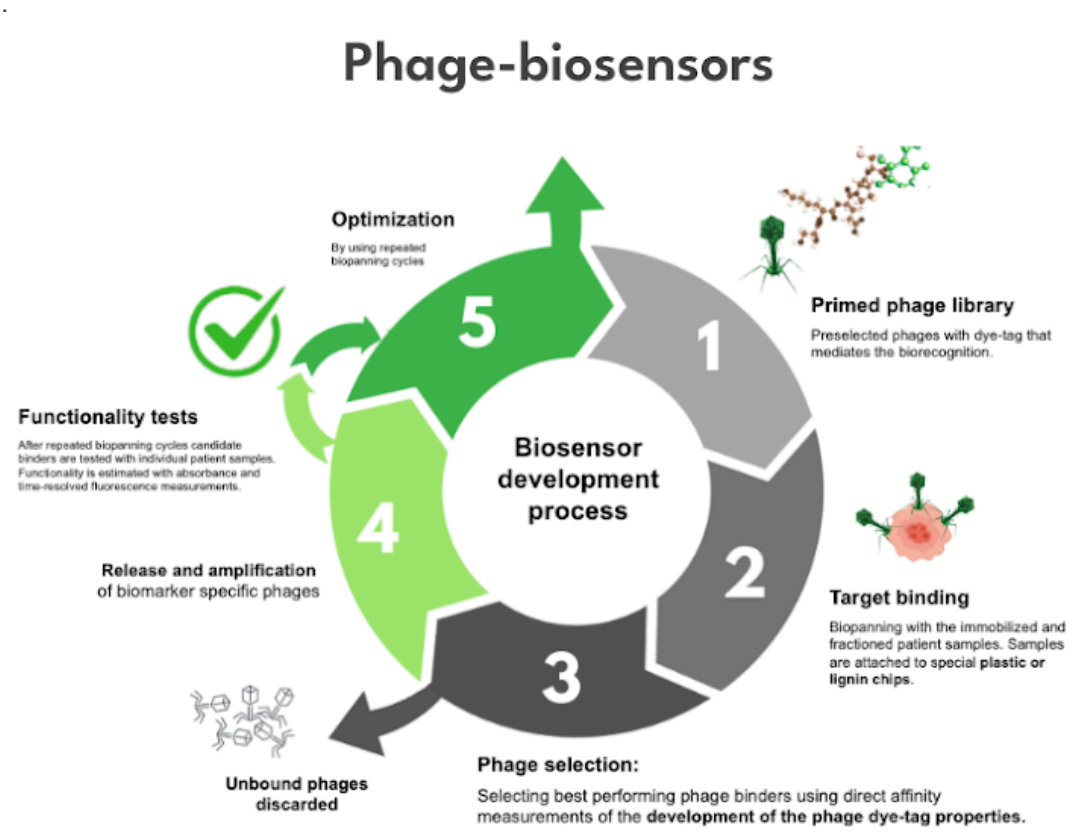

1. Primed phage library

AQ Biotech's phage biosensors are developed from proprietary, preselected bacteriophages that exhibit specific affinity for a reporter dye molecule (Figure 1). These so-called primed phages have been engineered to bind selectively to the dye. The phage-dye interaction enables detection by absorbance and time-resolved fluorescence (TRF), as phage-analyte interactions alone cannot be measured using these methods. In addition, primed phages serve as highly effective controls when assessing sample status. In other words, the primed phages give a reference point for the sample, to which the measurements from the disease-specific biosensors can be compared to.

AQ Biotech's phage-based biosensors are bifunctional, which means that they can bind both the reporter dye molecule and the targeted disease biomarkers. This bifunctionality is what enables accurate classification of patient samples.

2. Target binding

The biopanning process is where the phages gain their bifunctional status giving them the ability to bind to the target biomarker(s) in addition to the reporter dye. Biopanning is a method where phage clones are selected based on their affinity towards the target biomarker(s) through affinity selection. The biopanning process begins with immobilizing sample pools or individual samples onto a special plastic surface or lignin chips. The samples may consist of serum, urine, or saliva, which are easily obtained in an non-invasive manner. In some cases, the samples are fractioned prior to immobilization to allow for better separation of the target biomarkers.

After immobilizing the samples and washing the solid surface to remove unbound molecules, the primed phages are added. Phages with affinity for the target biomarkers then bind to the biomarkers immobilized to the surface.

3. Phage selection

Unbound phages are discarded from the solid surface using simple washing steps. This phage selection step discards the unwanted phages that are not able to identify the target biomolecules. After this the phages are grown in a strain of *E. coli*.

4. Release and amplification

After growing the phage binders in *E. coli*, the phages are released from their hosts by centrifugation. Growth efficiency is assessed with absorbance measurements, and phages showing poor growth are discarded. Performance of the potential phages are evaluated using sample pools. Phages that demonstrate the clearest and most consistent differentiation between positive and negative samples are selected for amplification. Best performing phages are amplified in additional biopanning cycles to enhance binding specificity. The biopanning is repeated 2-3 times before moving on to the functionality tests.

Counterselection is a negative selection method that can be done at this point to enhance specificity and get rid of non-specific binders.

5. Functionality tests

After 2-3 rounds of biopanning the best biosensor candidates have been selected, and are then tested on test set samples. The test set samples are simply individual patient samples that include known positive samples and known negative samples. This step is critical for enabling the phages to discriminate accurately between negative and positive samples across a range of disease-specific biomarker concentrations. In this functionality test assay, the real-time measurements of absorbance and TRF allows for biosensor evaluation after just one hour.

AQ Biotech's biosensor platform allows for a fast, real-time evaluation of phage performance. This method eliminates the need for any time-consuming washing steps, and gives the results about five times as fast as the commonly used ELISA method, which requires multiple washes and substrate additions. These advantages are explained by the fact that AQ Biotech phage-binders are not labelled but instead interact with a free reporter dye and fluorescent label. The liquid crystalline properties of the phages change in response to the presence or absence of the target biomarker. When the target biomarker is absent, the phages bind to the reporter dye and form an organized smectic phase. This ordered phase decreases absorbance, and the label remains unquenched, resulting in a stronger TRF signal. When the target biomarker is present, the phages instead interact with the biomarker, and their liquid crystalline behavior shifts from an organized smectic phase to a disordered isotropic phase. This leads to higher absorbance because the dye remains free in the sample. The free dye quenches the label, producing a lower TRF signal. This is the principle used in the AQ Biotech phage-based biosensors, where the change in liquid crystal behaviour demonstrates the presence of the target biomarker.

6. Optimization of phage biosensors

When functionality tests have resulted in promising biosensor candidates the best ones can be further optimized with mutagenesis methods if necessary.