



Conference Abstract

Advice to consider when developing a CRISPR-Cas assay for single species detection using eDNA

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Abstract

Development of simple and rapid techniques to monitor species of conservation importance is vital to further the capabilities of environmental DNA. Conventional methods for eDNA detection pose a logistical challenge for on-site monitoring due to the need for high temperatures and thermal cycling. To circumvent this, we recently adapted an isothermal CRISPR-Cas based detection assay for single-species assessment of *Salmo salar* as a route to a simple, cost-effective biosensor device (Williams et al., 2019).

CRISPR-Cas for detection (rather than genome editing) was first developed for clinical diagnostic applications. The variety of Cas nucleases allow detection of either RNA or DNA with attomolar sensitivity (Chen et al., 2018; Gootenberg et al., 2017). This detection approach is versatile and has recently been adopted for the detection of SARS-CoV-2 (Broughton et al., 2020). The CRISPR-Cas detection system consists of two main elements; a guide RNA specific to the target and an effector Cas12a nuclease. The Cas12a nuclease will only cleave at the target site when a specific protospacer adjacent motif (PAM) is present downstream. The requirement to recognise two separate sequences supports a highly specific recognition system that can distinguish closely related species. However, although its use is expanding rapidly for the detection of pathogens, it is yet to be fully embraced for eDNA detection. The RPA-CRISPR-Cas methodology we have developed utilises the isothermal recombinase polymerase amplification and CRISPR-Cas12a detection, leading to four unique sequence recognition elements, which require

stringent design and in-lab testing to ensure assay specificity. Development of our published *S. salar* CRISPR-Cas assay (Williams et al., 2019), and subsequent assays for *Salmo trutta* and *Salvelinus alpinus*, highlighted critical steps to consider and pitfalls to avoid when designing such isothermal assays.

1) Only the target sequence should contain the required PAM site.

- In version 1 of our assay, both *S. salar* and *S. trutta* contained the PAM site; we were unable to distinguish them.

2) An RPA primer screen is essential.

- Multiple forward and reverse primers are screened up-/down-stream of the gRNA binding region to select the optimum primer pair.

3) Specificity tests should be carried out on tissue from the target species and other species present in the sampling environment.

- *In silico* design is not sufficient to ensure assay specificity.

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