Lyophilization of Cas13a on paper

Aim of the Experiment

In this experiment, the reaction mixture for Cas13a-dependent detection of RNA (described in protocol ”plate reader experiments”) is freeze-dried on paper to present a storable version of this assay.

Materials

Preparation of Paper:

- Freeze-dryer
- Heat plate (magnetic stirrer, RH basic, IKA, Germany)
- Fluorescence-detector ”Lightbringer” (designed by our team)
- Nuclease-free glass fiber filter paper (glass microfiber paper 934-AH RTU, Whatman, GE healthcare, Germany)
- Nuclease-free bovine serum albumine (BSA, 5%) (VWR life sciences, Germany)
- nuclease-free H_2O (nf H_2O, Sigma Aldrich, USA)

Cas13a reaction mixture without target RNA:

- Cas13a Lbu protein (from the species *Leptotrichia Buccalis*)
- Lbu-specific and target-specific crRNA (DNA template for *in vitro* transcription: Biomers)
- Target-RNA (extracted from cell lysate or *in vitro* transcribed)
- Murine RNase inhibitor (NEB, Germany)
- RNase A (IDT, Germany)
- RNase processing buffer (10x, 200 mM HEPES [pH 6.8], 500 mM KCl, 50 mM $MgCl_2$, 50 % glycerol)
- nuclease-free H_2O (nf H_2O, Sigma Aldrich, USA)

Table 1: Cas13a mix final concentrations

Concentration	**Chemicals**
300 nM	Cas13a
50 nM	crRNA
1,5 U	RNase inhibitor
1x	RNase processing buffer

Procedure

- Block filter paper (1 cm^2 per reaction) with BSA over night followed by thorough rinsing with nuclease-free water and dry on a heat plate at 80 °C for 20 min.
- Add 15 µlCas13a reaction mix without target RNA onto the blocked paper.
- Immediately flash-freeze at -80 °C (alternatively: liquid nitrogen).
- Place the loaded paper in the freeze-dryer and lyophilize at -60 °C for at least 4 h.
- To perform an assay with this freeze-dried mixture, variable amounts of target-RNA-containing solution can be added and measured by the fluorescence detector.