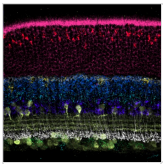

Aug 21, 2020

2: User-friendly protocol: Oligo ordering and preparation (SABER-FISH)

In 1 collection

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1 *Works for me* This protocol is published without a DOI.

Human Cell Atlas Method Development Community

Jocelynkishi

ABSTRACT

This protocol is about oligo ordering and preparation.

This protocol is part of the SABER-FISH collection.

EXTERNAL LINK

http://saber.fish/

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Kishi, J.Y., Lapan, S.W., Beliveau, B.J. et al. SABER amplifies FISH: enhanced multiplexed imaging of RNA and DNA in cells and tissues. Nat Methods 16, 533–544 (2019). https://doi.org/10.1038/s41592-019-0404-0

ATTACHMENTS

SABER amplifies FISH_enhanced multiplexed imaging of RNA and DNA in cells and tissues.pdf

PROTOCOL CITATION

Jocelyn Y. Kishi, Sylvain W. Lapan, Brian J Beliveau, Emma R. West, Allen Zhu, Hiroshi M. Sasaki, Sinem Saka, Yu Wang, Constance L Cepko, Peng Yin 2020. 2: User-friendly protocol: Oligo ordering and preparation (SABER-FISH). **protocols.io** https://protocols.io/view/2-user-friendly-protocol-oligo-ordering-and-prepar-bh9gj93w

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

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EXTERNAL LINK

http://saber.fish/

COLLECTIONS

SABER-FISH – Signal amplification for multiplexed fluorescence in situ hybridization assays

KEYWORDS

olio, probe oligos, ordering, preparation

CREATED

Jul 06, 2020

LAST MODIFIED

Aug 21, 2020

OWNERSHIP HISTORY

Jul 06, 2020 Julia Rossmanith protocols.io

Aug 19, 2020 Jocelynkishi

PROTOCOL INTEGER ID

38920

PARENT PROTOCOLS

Part of collection
SABER-FISH – Signal amplification for multiplexed fluorescence in situ hybridization assays

MATERIALS TEXT

Probe oligos are ordered from **IDT** in a 96 well format with standard desalting. Cost is significantly reduced by ordering plates at **10 nanomole** synthesis scale.

Additional ordering specs are:

- Resuspended in IDTE **pH 7.5**
- v bottom plate
- normalized in nanomoles

Individual wells from the plate can be pooled by multichannel pipetting equal volumes from all wells into a trough. IDTE **pH 7.5** is used for dilutions of probe primers down to **[M] 10 Micromolar (µM)** .

PER[30] **hairpins** typically function well if synthesized with *standard desalting* and a polyT of 7 T's on the 3' end of the hairpin (unless the primer sequence ends in a A bases, in which case the 3' tail should be designed to not hybridize to the concatemer sequence). They can be resuspended in **IDTE** (**pH 7.5**) and stored as **[M] 100 Micromolar (µM)** stocks. Dilutions of hairpin down to **[M] 5 Micromolar (µM)** for extension are also done in **IDTE**. Certain hairpins, however, require the addition of an **Inverted dT (InvdT)** at the 3' end and *HPLC purification* (see Step 1).

Fluorescent oligos are ordered with a **5' fluorescent adduct** and require *HPLC purification*. Yield is variable and cost is significantly reduced by ordering in bulk. These are resuspended in **ddH2O** or **IDTE** to **[M] 100 Micromolar (µM)** for storage, or as **[M] 10 Micromolar (µM)** dilutions (diluted in ddH2O or IDTE). A 3' InvdT on fluorescent oligo is not essential. A full list of primers, hairpins, and branches are available in **Supplementary Table 1.xlsx** (available on the Nature Methods website).

SAFETY WARNINGS

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

1 To generate a catalytic (telomerase-like) hairpin corresponding to the primers sequence (RC = reverse complement):

A(primer sequence)GGGCCTTTTGGCCC(RC of primer sequence)T(RC of primer sequence)/3InvdT/

For example, given the primer 27 sequence of CATCATCAT, the catalytic hairpin h.27.27 sequence would be:
*A**CATCATCAT**GGGCCTTTTGGCCC**ATGATGATGTATGATGATG**/3InvdT/*

2 The */3InvdT/* can be replaced with *TTTTTTT* for most hairpins to save cost. We have found empirically however, that some hairpins (including those for primers 25, 32, and 41 seem to require the InvdT modification).

3 To change the primer appended to a probe set, a hairpin for primer switching ('re-mapping') can be used (Fig. S1a-c). Remapping hairpin is introduced in the same reaction as the catalytic hairpin, and the concentration is flexible (**[M]0.05 Micromolar (µM)** - **[M]0.25 Micromolar (µM)**). Reactions involving primer switching may require additional time for extension to equivalent lengths.

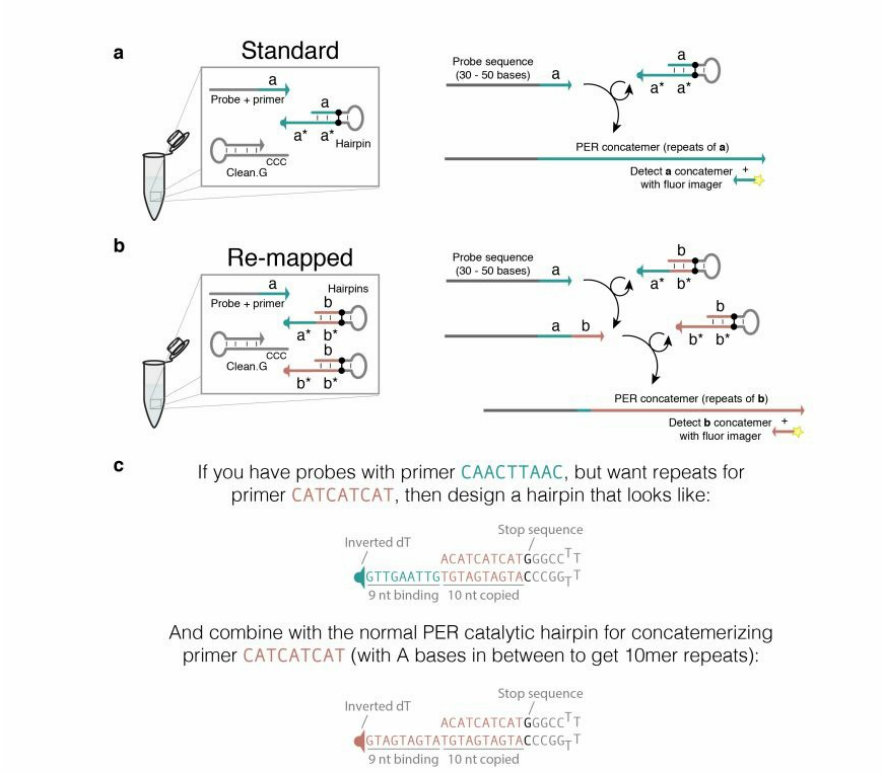


Figure S1: **Designing PER primer re-mapping reactions. a**, Standard PER setup, which uses one PER hairpin to concatemerize the **a** sequence. **b**, Re-mapping PER setup, which uses an additional re-mapping hairpin to swap one sequence for another (in this case, **b** for **a**). **c**, Design example for primer re-mapping primer *(A)CAACTTAAC* to repeats of sequence *(A)CATCATCAT*.

To generate a hairpin for primer switching:

A(new primer)GGGCCTTTTGGCCC(RC of new primer)T(RT of old primer sequence)/3InvdT/

A detailed schematic for designing such a hairpin can be seen in Fig. S1c. Here, as above, */3InvdT/* can generally be replaced with *TTTTTTT* for most hairpins to save cost.

4 Fluorescent oligos are designed as follows:

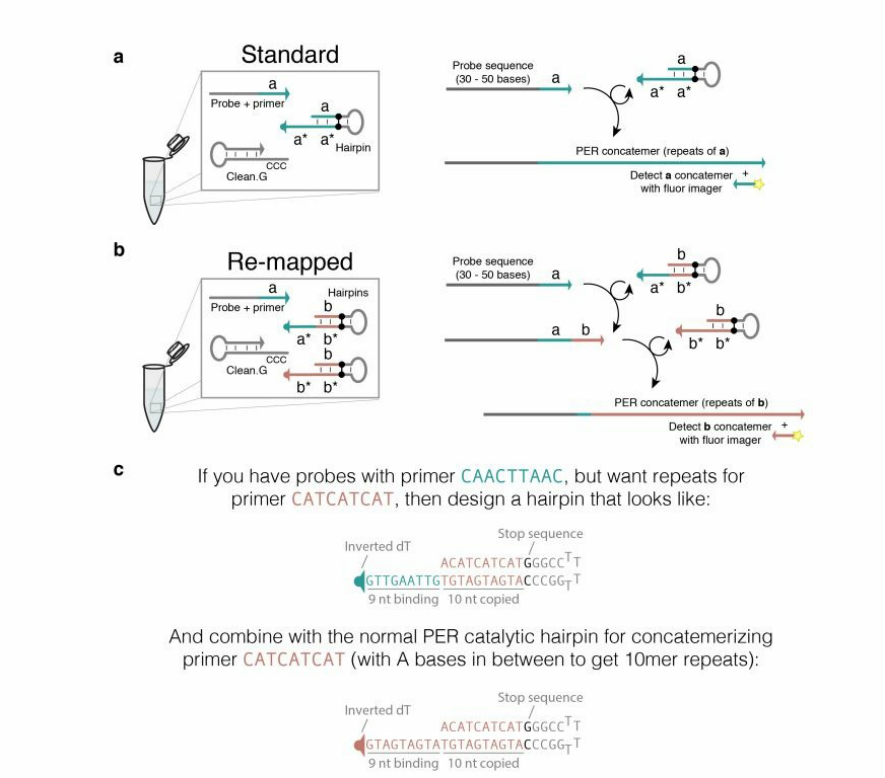
/5dye/TT(RC of primer sequence)T(RC of primer sequence)T

This represents a 20mer binding sequence with a 5' conjugated dye linked to the binding region by a TT' linker

PER primer re-mapping

5 Probe sets with one primer (e.g. primer sequence **a**) that have to be used in the same experiment with another set that has the same primer (**a**) can be re-mapped to alternate PER concatemer sequences using a two-hairpin reaction (Fig. S1a-b).

An example for designing re-mapping hairpins given a starting primer sequence and desired concatemer sequence is depicted in Fig. S1c. (The original 9 nt primer sequence is not predicted to hybridize to the complementary 20mer imager sequence at 37°C in 1×PBS, so one of the probe sets can usually be concatemerized with the original primer sequence. In this case it is good to have a control condition with just that probe set missing to verify no cross-talk between channels.)



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