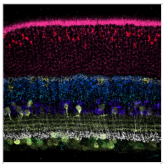

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# 4: User-friendly protocol: Cost-efficient Primer Exchange Reaction (PER) concatemerization (SABER-FISH)

In 1 collection

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

Human Cell Atlas Method Development Community

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ABSTRACT

This protocol explains the cost-efficient Primer Exchange Reaction (PER) concatemerization.

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

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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

PER, Primer Exchange Reaction, concatemerization



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PARENT PROTOCOLS

Part of collection

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

MATERIALS

| NAME | CATALOG # | VENDOR |
|---|---|---|
| Magnesium Sulfate (MgSO4) Solution - 6.0 ml | B1003S | New England Biolabs |
| UltraPure™ DNase/RNase-Free Distilled Water | 10977023 | Thermo Fisher |
| E-Gel™ EX Agarose Gels, 1% | G402001 | Thermo Fisher |
| 10x PBS | | |
| Deoxynucleotide (dNTP) Solution Set | N0446S | New England Biolabs |
| Clean.G hairpin (1µM) | | |
| Bst DNA Polymerase (large fragment) | BPL-300 | MCLAB |
| Hairpin (5µM) | | |
| Probe oligo pool (10µM) | | |
| SYBR Gold dye | | |
| PB Buffer | | |
| MinElute PCR Purification Kit | 28004 | Qiagen |

MATERIALS TEXT

Clean.G (*CCCCGAAAGTGGCCTCGGGCCTTTTGGCCCGAGGCCACTTTCG*) is ordered with standard desalting and diluted in $H_2O$.

*Note 1:* **dGTP nucleotides** are **excluded from the reaction** as a string of C's in the hairpin is used as a stop sequence. The Clean.G oligo incorporates contaminating dGTPs (see Fig. S6 from ref[30]). The dNTP mix is generated by ordering dNTP's in separate tubes and mixing A, C, T to a final concentration of [M]**6 Milimolar (mM)** each. Bst large fragment polymerase NEB (M0275L) can be used instead.

Bst DNA Polymerase Lg Frag - 8,000 units
by New England Biolabs
Catalog #: M0275L

*Note 2:* Hairpin final concentration may be adjusted depending on the desired probe length and properties of the specific hairpin. Extension rates can be quite variable depending on the specific hairpin. As a starting point try **0.5 Micromolar (µM)** final hairpin concentration for a 60 minute extension reaction. Hairpin concentration, extension time, and other reaction conditions can be adjusted to modify concatemer length (see Fig. 1b and Fig. 1a in the Nature Methods article).

*Note 3:* We recommend using **ddH2O** or **ideally molecular grade water** such as UltaPure DNase/RNase-free distilled water (Invitrogen #10977023), especially if you will be using the concatemers for RNA FISH.

UltraPure™ DNase/RNase-Free Distilled Water
by Thermo Fisher
Catalog #: 10977023

You can also use dense agarose gel (~1.25%) instead of 1% E-Gel EX agarose gels (Thermo Fisher G402001).

**Equipment:**
- Thermocycler
- nanodrop

SAFETY WARNINGS

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

BEFORE STARTING

Heat cycler to **37 °C** .

## PER mix and extension

1

Mix with the following components **On ice** :

**Add the polymerase last.**

| Component | Volume ( µL) |
|---|---|
| 10×PBS | 10 |
| 100mM MgSO4* (NEB) | 10 |
| dNTP mix* (A,C,T only 6mM each, NEB) | 5 |
| Clean.G (1µM) | 10 |
| Bst LF polymerase* (McLab) | 0.5 |
| $H_2O$ | 44.5 |

*Indicates component concentration can easily be varied to control reaction kinetics and therefore concatemer length, see comments below.

2 

Add mix to strip tube with **10 µl 5µM hairpin** and mix.

3 

Incubate for **00:15:00** at **37 °C** , then pause cycler.

4 

Remove tube from the cycler, add **10 µl 10µM probe oligos** and mix.

5 

Incubate at **37 °C** for desired extension time (see Note 2).

6 

Heat to **80 °C** **00:20:00** to inactivate the Bst polymerase.

7 Cool to **4 °C** .

## Check Extension lengths

8 To check the lengths of extensions, load **10 µl of reaction** with loading dye on a dense agarose gel (~ **1.25 %** ). You can also load samples into **1 % E-Gel EX agarose gels** (Thermo Fisher G402001).

*Note 5:* We recommend using primary probes extended to ~500-650 nt and branch probes extended to ~250-450 nt.

*Note 6:* Because there aren't G bases in the concatemer sequences, and they are highly single-stranded, intercalating dyes are not as effective as they are at staining other types of sequences (we recommend using the **Sybr Gold dye**). This means the bands may look faint on the gel but still be efficiently elongated, so

don’t be surprised if you need to turn up the contrast quite a bit.

## Purification of probes

9 While purification of probes is not usually required for cell applications, we have found it helpful **for SABER-FISH in tissue.**

10 

Purify probes by running **90 µl of reaction** (for 500 nt probe) over one MinElute PCR purification column (Qiagen #28004).
Add PB buffer to 7×the volume of reaction being purified.

11 

Elute in **25 µl ddH2O** .

12 

Determine concentration after purification by nanodrop using ssDNA setting.

13 Store probes at **-20 °C** .