

# TALEColor Telomere Labeling by TALE-FPs in Fixed Cells (V11-11-2013)

This protocol was adapted from Ma H *et al*, PNAS, 2013. Additional details can be found in the paper.

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## A. Preparation of TALE-FP Probes by IVT

### Reagents:

- TALEColor plasmids for *in vitro* translation
- 1-Step Human Coupled *In Vitro* Translation Kit (Pierce, Cat. No. 88882)
- Steriled 80% Glycerol

<u>Reactions (20 ul):</u>	ul
HeLa Lysate	10
Accessory Proteins	2
Reaction Mix	4
TALEColor plasmid (250 ng/ul)	4
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Total	20

Incubate the reaction for 1-4 hours @ 30°C.

Add 20 ul (1 vol) of 80% Glycerol and stored the reactions at -20°C.

**Note 1:** Thaw HeLa Lysate, Accessory Proteins and Reaction Mix on ice, aliquot and quickly store at -80°C. These reagents should be freeze-thawed for no more than five times.

**Note 2:** Reaction could be scaled down to 10 ul for quick test or scaled up to 100 ul for storage.

**Note 3:** TALE-FP probes in glycerol are stable at -20°C for at least one month.

**Note 4:** TALEColor plasmids for telomere labeling in fixed cells are available at addgene.

## B. Labeling telomeres by TALE-FP probes

### Reagents:

• Methanol Fixative	ml
• PBS (4°C)	10
• Ice-cold methanol (-20°C)	90
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	100

- ProLong Gold Antifade Reagent with DAPI (Molecular Probes, Cat. No. P36935)

### Materials:

- 12-well plates
- Circular cover glass (#1, 18 mm Dia, Electron Microscopy Sciences, Cat. No 72229-01)
- Cover slides

### Procedures:

1. Cells grown on cover glass were rinsed by PBS and fixed in fresh methanol fixative for 10 min at -20°C;
2. Rinse cells with PBS for telomere labeling or could be stored at 4°C in PBS for at least three months;
3. Incubate cells with 2N HCl for 5 min;
4. Wash twice with PBS for 5 min ea. on shaker;
5. TALE-FP probe mix:

TALE-FP probe mix:	ul
TALE-FP reactions in Glycerol	2
PBS	18
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Each cover glass	20

Pipette probe mix directly onto parafilm and carefully place coverslip upside down and incubate for 30 min to 1 hour;
6. Wash twice with PBS for 5 min ea. on shaker;
7. Mount cover glass in Prolong Antifade Reagent and place samples in dark for O/N;
8. Capture images by fluorescence microscopy.