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Simultaneous detection of miRNA and mRNA at the single-cell level in plant tissues

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We use this protocol and it's working

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Abstract

Detecting the simultaneous presence of a microRNA (miRNA) and a mRNA in a specific tissue can provide support for the prediction that the miRNA regulates the mRNA. We develop a method that uses sequence-specific miRNA-locked nucleic acid (LNA) and mRNA-LNA probes. Moreover, it augments the detection signal by rolling circle amplification, achieving a high signal-noise ratio at the single-cell level. Dot signals are counted for determining the expression levels of mRNA and miRNA molecules in specific cells. We show a high sequence specificity of our miRNA-LNA probe, revealing that it can discriminate single-base mismatches. Numerical quantification by our method is tested in transgenic rice lines with different gene expression levels.

1 miRNA hybridization

	A
	1. The slides with sections are taken out the freezer and equilibrated to RT for 40 mins.
	2. Permeabilized in 20 ug/ml proteinase k for proper duration
	3. Quickly wash in DEPC-PBS
	4. Quickly dehydrate the slides in EtOH (50, 70, 99 %) and then air dry
	5. Mount the secure seal reaction chambers onto the slides.
	6. Add 1x DEPC-PBS-tween 0.05 % (Wash buffer) into the chambers to keep the slides wet until RT reaction ready

2 Section permeabilization

	A	B	C
		Stock	Final
	Formamide	100%	50%
	SSC	20x%	5x
	tRNA	10 mg/ml	0.5 ug/ul
	Denhardt's	50x	1x
	LNA probe A	10 uM	2-3 pmole
	DEPC-H2O		

1. process

	A
	Hybridization under the predicted melting temperature of the probe for an hour
	Wash with 0.1X SSC three times at the temperature set in the Step 1

	A
	Wash with 2X SSC at RT once
	Wahs wtih the wash buffer (PBS, 0.05% Tween-20) once

3 mRNA cDNA synthesis

	Reagent	Stock	Final
	NEB Tag DNA ligase	40U/ul	0.5 U/ul
	Rnase H	5 U/ul	0.4 U/ul
	Ribolock Rnase inhibitor	40 U/ul	1 U/ul
	NEB Tag ligase buffer	10x	1x
	BSA	20 ug/ul	0.2 ug/ul
	KCl	1 M	0.05 M
	Formamide	100%	20%
	Pd_A	10 uM	0.1 uM
	Pd_B	10 uM	0.1 uM
	Pd_C	10 uM	0.1 uM
	DEPC-H2O		

Process

1. Add ligation mixture in chambers, seal with adhesive film
2. Incubate for 30 min at 37 C followed by 45 min at 48 C

3. Wash 2x, 1x DEPC-PBS-Tween 20, 0.05%

4 miRNA and mRNA padlock probe hybridization and ligation

	Reagent	Stock	Final
	NEB Tag DNA ligase	40U/ul	0.5 U/ul
	Rnase H	5 U/ul	0.4 U/ul
	Ribolock Rnase inhibitor	40 U/ul	1 U/ul
	NEB Tag ligase buffer	10x	1x
	BSA	20 ug/ul	0.2 ug/ul
	KCl	1 M	0.05 M
	Formamide	100%	20%
	Pd_A	10 uM	0.1 uM
	Pd_B	10 uM	0.1 uM
	Pd_C	10 uM	0.1 uM
	DEPC-H2O		

process

	A
	1. Add ligation mixture in chambers, seal with adhesive film
	2. Incubate for 30 min at 37 C followed by 45 min at 48 C
	3. Wash 2x, 1x DEPC-PBS-Tween 20, 0.05%

5 rolling circle amplification

	Reagent	Stock	Final
	Phi 29 polymerase	10 U/ul	1 U/ul
	Ribolock Rnase inhibitor	40 U/ul	1 U/ul
	10 X phi 29 buffer	10 x	1 x
	dNTP	10 mM	0.25 mM
	BSA	20 ug/ul	0.2 ug/ul
	Glycerol	50%	5%
	DEPC-H2O		

process

	A
	1. Add reaction mixture and seal chamber
	2. Incubate for over night at 37 C
	3. Wash 2x, DEPC-PBS-Tween 20, 0.05 %

6 detection oligo hybridization

	A	B	C
	Reagent	Stock	Final
	Hyb mixture	4 x	2 x
	Detection oligo 1-FITC	1 uM	0.1 uM

	A	B	C
	Detection oligo 2-Cy3	1 uM	0.1 uM
	Detection oligo 3-Cy5	1 uM	0.1 uM
	DEPC-H2O		

process

	1. Add reaction mixture
	2. Incubate for 30 min at 37 C
	3. Wash 2x, DEPC-PBS-Tween 20, 0.05 %
	4. Dehydrated by EtOH 50, 70, 99 %; then air dry.
	5. Mount cover slips