

Supplementary Materials: Liquid Hybridization and Solid Phase Detection: A Highly Sensitive and Accurate Strategy for MicroRNA Detection in Plants and Animals

Fosheng Li, Lanju Mei, Cheng Zhan, Qiang Mao, Min Yao, Shenghua Wang, Lin Tang and Fang Chen

Table S1. Difference in detections between digoxigenin DIG/Biotin/fluorescein isothiocyanate (FITC) systems.

	DIG	Biotin	FITC [31]
Detection buffer (pH 9.5)	Yes (3–5 min)	No	No
Substrate for detection	CDP Star ^a	CDP Star	Luminol ^b
Enzyme used for crosslinking	Alkaline phosphatase	Alkaline phosphatase	Horseradish peroxidase
Incubation time for detection	5 min	5 min	5 min
Exposure time	5–20 min	5–20 min	5–20 min
Duration of luminescence	24–48 h	24–48 h	6 h
Supporting multiple exposure	Yes	Yes	Yes (adding other color labels)
Identification of single-base differences	Yes	Yes	Yes
Sensitivity	Dot blot 0.01 fmol Band blot 0.25 fmol	0.005 fmol 0.1 fmol	0.05 fmol 0.5 fmol
			0.01 pmol 0.1 pmol

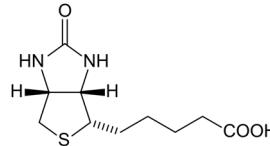
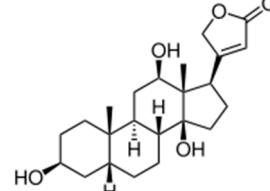
^a CDP Star: disodium 3-[4-methoxyspiro{1,2-dioxetane-3,2'-(5'-chloro)tricyclo[3.3.1.13,7]decen}-4-yl]phenyl phosphate; ^b Luminol: 5-amino-2,3-dihydro-1,4-phthalazinedione.

Table S2. MicroRNA (miRNA) detection from different organisms by liquid hybridization and solid phase detection (LSHPD) strategy.

miRNAs	Species (tissues)	Sequences (5'→3')	Results
Osa-miR156	Oryza sativa-stem	UGA CAG AAG AGA GUG AGC AC	 Biotin-Luminol  Biotin-CDP Star  DIG-CDP Star
Osa-miR156	Oryza sativa-roots	UGA CAG AAG AGA GUG AGC AC	 Biotin-Luminol  Biotin-CDP Star  DIG-CDP Star
Osa-miR156	Oryza sativa-leaves	UGA CAG AAG AGA GUG AGC AC	 Biotin-Luminol  Biotin-CDP Star  DIG-CDP Star
miR263	Oryza sativa-leaves	GUG GGU CUA AGG ACU AUA UUA ACC	 Biotin-CDP Star
miR557	Oryza sativa-leaves	AUU UGU UGU AUU AGG GAA UGU CUC	 Biotin-CDP Star
miR1188	Oryza sativa-leaves	UGG AUG UGA CAU ACU CUA GUA	 Biotin-CDP Star
miR156	Jatropha curcas-leaves	UGA CAG AAG AGA GUG AGC AC	 Biotin-CDP Star
miR156	Arabidopsis thaliana-leaves	UGA CAG AAG AGA GUG AGC AC	 Biotin-CDP Star
let-7a	Homo sapiens-blood	UGA GGU AGU AGG UUG UAU AGU U	 Biotin-Luminol
let-7a	Mus musculus-muscle	UGA GGU AGU AGG UUG UAU AGU U	 Biotin-CDP Star
let-7a	Gallus domesticus-blood	UGA GGU AGU AGG UUG UAU AGU U	 Biotin-CDP Star

miR263 and miR557 are low-abundance intronic miRNAs and miR1188 is an abundant intronic miRNA in *Oryza sativa* [32].

Table S3. Some properties of FITC, biotin and digoxigenin.

Name	Molecular Formula	Molecular Weight	Chemical Structure
Biotin	C ₁₀ H ₁₆ N ₂ O ₃ S	244.31	
Digoxigenin (DIG)	C ₂₃ H ₃₄ O ₅	390.51	
Fluorescein isothiocyanate (FITC)	C ₂₁ H ₁₁ NO ₅ S	389.382	