Supporting Information

Super-resolution geometric barcoding for multiplexed miRNA profiling

Weidong Xu,[a,b,c] Peng Yin,*[a,b] and Mingjie Dai*[a,b]

[a] Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA 02115, USA.

[b] Department of Systems Biology, Harvard Medical School, Boston, MA 02115, USA.

[c] Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA 02138, USA.

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1. Methods

1.1 Materials and buffers

All DNA and RNA oligonucleotides were purchased from Integrated DNA Technologies (IDT). 3′ amino-modified DNA oligonucleotides ordered from IDT were used for in-house production of fluorophore-labeled imager strands. Cy3b NHS ester (catalog number PA63101) and size-exclusion columns prepacked with Sephadex G-25 (NAP-5, catalog number 45-000-151) were purchased from GE Healthcare Life Sciences. Streptavidin (catalog number S-888) was purchased from Invitrogen. Polyoxyethylenesorbitan monolaurate (Tween 20, catalog number P9416), biotinylated bovine serum albumin (BSA-biotin, catalog number A8549), Protocatechuate 3,4-Dioxygenase (PCD, catalog number P8279), Protocatechuic acid (PCA, catalog number 37580) and 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, catalog number 238813) were purchased from Sigma-Aldrich. Glass slides and coverslips were purchased from VWR. The M13mp18 scaffold was purchased from New England BioLabs. Freeze 'N Squeeze columns were purchased from Bio-Rad. Acetonitrile (ACN, HPLC grade, catalog number A998), triethylamine acetate (TEAA, 2 M, catalog number 400613), TBE buffer (10 ×, catalog number B52), SybrSafe DNA gel stain (catalog number S33102), biotin (Pierce biotin, catalog number 29129), NaCl (5 M, catalog number AM9760G), $MgCl_2$ (1 M, catalog number AM9530G), Tris-HCl (1 M, pH 8.0, catalog number AM9855G), and ethylenediaminetetraacetic acid (EDTA, 0.5 M, pH 8.0, catalog number AM9260G) were purchased from Thermo Fisher Scientific. Agarose (SeaKem LE agarose) was purchased from Lonza. XTerra MS C18 column (catalog number 186000602) was purchased from Waters. The miRNeasy mini kit (catalog number 217004) was purchased from Qiagen.

The following buffers were used for sample preparation and imaging: HPLC buffer A (0.1 M TEAA, 5% ACN), HPLC buffer B (0.1 M TEAA, 50% ACN), DNA origami folding buffer (12.5 mM MgCl2, 1 × TE buffer), DNA-PAINT imaging buffer A (10 mM Tris-HCl, 100 mM NaCl, 0.2% (v/v) Tween 20, pH 8.0), imaging buffer B (10 mM Tris-HCl, 15 mM $MgCl_2$, 1 mM EDTA, 0.2% (v/v) Tween 20, pH 8.0) and buffer TP (1 × buffer B, 10 nM PCD, 2.5 mM PCA, 1 mM Trolox).

1.2 Fluorescence microscopy set-up

Fluorescence imaging was carried out on an inverted Nikon Eclipse Ti microscope (Nikon Instruments) with the Perfect Focus System, applying an objective-type TIRF configuration using a Nikon TIRF illuminator with an oil-immersion objective (CFI Apo TIRF 100×, numerical aperture (NA) 1.49). Laser excitation with a 561 nm laser (200 mW, Coherent Sapphire) was passed through a clean-up filter (ZET561/10, Chroma Technology) and coupled into the microscope using a beam splitter (ZT488rdc/ZT561rdc/ZT640rdc, Chroma Technology). Fluorescence light was spectrally filtered with emission filter (ET60050m, Chroma Technology). Super-resolution movies were recorded with an electron multiplying charge-coupled device (EMCCD, used without EM gain option) camera (iXon X3 DU-897, Andor Technologies).

1.3 Preparation of Cy3b-labeled oligonucleotides (DNA-PAINT imager strands)

Conjugation reaction solution was prepared by mixing 10 µL 3′ amino-modified DNA oligonucleotide solution (1 mM) with 1 µL $NaHCO_3$ buffer (1 M $NaHCO_3$, pH 8.0) and 2 µL Cy3b NHS ester (in DMSO at 10 mg/mL), followed by incubation in dark on shaker for 2h at room temperature. The reaction solution was then run through the size-exclusion column (GE NAP-5); the first peak was collected and lyophilized overnight. The lyophilized sample was resuspended in 0.1 M TEAA and was HPLC purified (Agilent 1200 Semi Prep HPLC) through a C18 2.5 µm column (XTerra MS C18) with HPLC pumping rate at 1 mL/min and linear buffer gradient from 100% HPLC buffer A to 50%:50% HPLC buffer A and B over 30 min; the purification product from the sample peak where absorption at 260 nm and 559 nm can both be seen was collected. The sample was lyophilized overnight and resuspended in ultrapure water to 1 µM. The prepared imager strands were stored at -20 °C until use.

1.4 DNA origami design and self-assembly

DNA origami nanostructures were adapted from 20 nm square grid structures used in our previous work. DNA origami nanostructures were designed with the caDNAno software, and

were based on a twist-corrected variant of the rectangular structure (see Tables S2-5 for the sequence details). Eight staple strands were biotin-modified for surface fixation. Four staple strands were extended with DNA-PAINT docking sequence (P1) with two thymine bases spacers as boundary markers. Eight staple strands were extended with anchor sequences for the immobilization of miRNA targets. See Figure S for details.

The DNA origami nanostructures were self-assembled in a one-pot annealing reaction with 50 μL total volume containing 10 nM scaffold strands (M13mp18), 100 nM unmodified staple strands, 600 nM biotin-modified strands and 1 μM strands with DNA-PAINT or anchor strand extensions in DNA origami folding buffer. The solution was annealed with a thermal ramp cooling from 90 °C to 25 °C over the course of 72 h. The samples were purified by agarose gel electrophoresis (2% agarose, 0.5 × TBE, 10 mM MgCl2, 0.5 × SybrSafe pre-stain) at 4.5 V/cm for 1.5 h. For purification, the gel bands were cut, crushed and filled into a Freeze 'N Squeeze column and spun for 10 min at 800g at 4 °C.

1.5 Flow chamber sample preparation and DNA-PAINT super-resolution imaging

Sample preparation was performed in custom-constructed flow chambers between a piece of coverslip and a glass slide. DNA origami nanostructures were fixed on the surface via a biotin-streptavidin-biotin bridge by serially flowing BSA-biotin (1.0 mg/mL in buffer A, 2 min incubation followed by rinse with buffer A), streptavidin (0.5 mg/mL in buffer A, 2 min incubation followed by rinse with buffer A and buffer B), biotin-labeled DNA origami nanostructures (in buffer B, 10 min incubation followed by rinse with buffer B), and biotin (5 μg/mL in buffer B, 2 min incubation followed by rinse with buffer B). The DNA origami concentration was calibrated for different batches to make sure that similar numbers of DNA origami nanostructures are obtained in the final super-resolution images. For the blinking kinetics characterization, the flow chamber was then filled with 1 μM miRNA target and incubated for 10 min; the flow chamber was finally filled with an imaging buffer (an appropriate concentration of Cy3b-labeled imager strand for the corresponding miRNA target in buffer TP) for imaging. For the multiplexed assays, the flow chamber was filled with 3 nM P1 imager strand for pre-imaging of boundary markers; the flow chamber was then filled with miRNA samples (following exchange-PAINT protocol) and incubated for 90 min; the flow chamber was finally

filled with the imaging buffer containing imager strands for boundary markers and all miRNA targets at appropriate concentrations (see Table S7 for imager strand concentrations) for the final super-resolution imaging of all available docking sites.

DNA-PAINT super-resolution movies were captured with 5 Hz camera frame rate (200 ms per frame) for all images. The laser intensity was 0.3 kW/cm^2. For the blinking kinetics characterization, 15,000 frames were recorded. For the pre-incubation images of boundary markers, 20,000 frames were recorded. For the post-incubation images in the multiplexed assays, 30,000 frames were recorded.

1.6 Super-resolution data processing and image analysis

DNA-PAINT super-resolution movies were processed with custom-written MATLAB software we previously developed.[1] Briefly, super-resolution images were processed in 3 steps: (i) single-molecule spot detection and localization, (ii) drift correction, (iii) super-resolution rendering and analysis.

Quantification for the super-resolution images to obtain the number of miRNA molecules bound to the DNA nanostructures was performed in the following steps. First, DNA origami nanostructures with intact boundary markers were identified in the pre-incubation image, and the positions of the boundary marker sites were recorded. Next, the recorded boundary marker positions are mapped to the post-incubation super-resolution image, and used to align the nanostructures in the case any translational or rotational movement happened incidentally between the two images. The positions of each miRNA docking sites were then calculated from the positions of boundary markers. Finally, the total number of detected miRNA molecules on all of the docking sites of "qualified" nanostructure grids were recorded and used to calculate the final binding fraction. Here we defined "qualified" origami grids as those that are not distorted (i.e. rectangular in shape, with measured inter-marker distance as expected), and did not show significant movement between the pre- and post-incubation super-resolution images, and only used those nanostructures for the quantification, in order to ensure high detection specificity and reduce any background count caused by potential misalignment of DNA nanostructure grids.

1.7 Preparation of total RNA extraction from HeLa cells

We extracted total RNA (>18 nucleotides) from HeLa cells using the Qiagen miRNeasy mini kit, which combines phenol/guanidine-based lysis of samples and silica-membrane-based purification of total RNA. The HeLa cell pellet was prepared by trypsinizing and collecting confluent cells grown in a 100 mm dish, followed by centrifugation at 400 g for 5 min and aspiration of the supernatant. The sample was disrupted and homogenized in 700 μL QIAzol Lysis Reagent by incubating at room temperature for 5 min. The sample was mixed with 140 μL chloroform, followed by incubation at room temperature for 3 min and centrifugation for 15 min at 12,000g at 4 °C. The upper aqueous phase was collected and mixed with 525 μL 100% ethanol. The sample was collected into an RNeasy Mini column by centrifugation at 11,000g for 15 s at room temperature. The column was then washed with 700 μL buffer RWT once and 500 μL buffer RPE twice by centrifugation at 11,000g for 15 s respectively. The sample was eluted with 50 μL RNase-free water by centrifugation at 11,000g for 1 min. The total RNA concentration in the eluted sample was 400 ng/μL with $A_{260}/A_{280} \approx 2$. The total RNA was diluted to a final concentration of 40 ng/μL in buffer B for the multiplexed assay.

2. Supporting figures

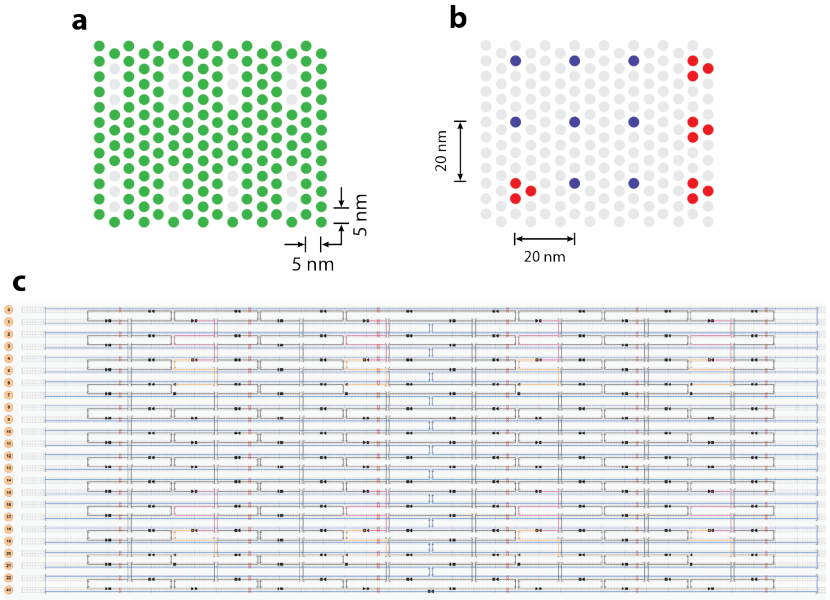

Figure S1. DNA origami nanostructures design schematics and strand diagram. (a) Schematic design diagram for the unmodified origami rectangle, where each staple is represented by a dot. Green dots represent staple strands that can be extended with DNA-PAINT docking strands or functionalized as miRNA anchor strands; grey dots represent staple strands that cannot be extended for DNA-PAINT imaging or functionalized as anchor strands. (b) Schematic design diagram for the 20 nm spacing "nano-array" used in this work. Blue dots represent anchor strands; red dots represent boundary markers. (c) Detailed strand diagram for the unmodified origami rectangle, showing twist-corrected DNA origami design. Red crosses show positions of deleted bases; thin blue lines represent circular scaffold strand; black lines represent unmodified staple strands; orange and crimson lines represent strands with biotin extension for surface fixation, and strands with modified wiring pattern to accommodate those orange ones.

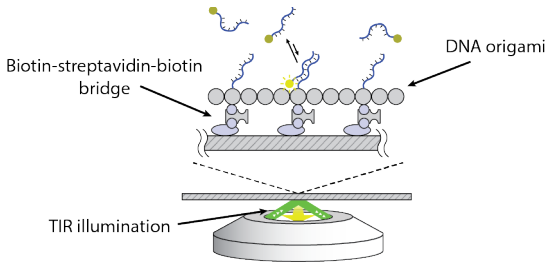

Figure S2. DNA-PAINT super-resolution experiment setup with DNA origami samples, illustrated in cross-section view. DNA origami nanostructures are attached to glass slide surface via BSA-biotin, streptavidin, biotin bridge, and illuminated under objective total internal reflection (TIR) setup.

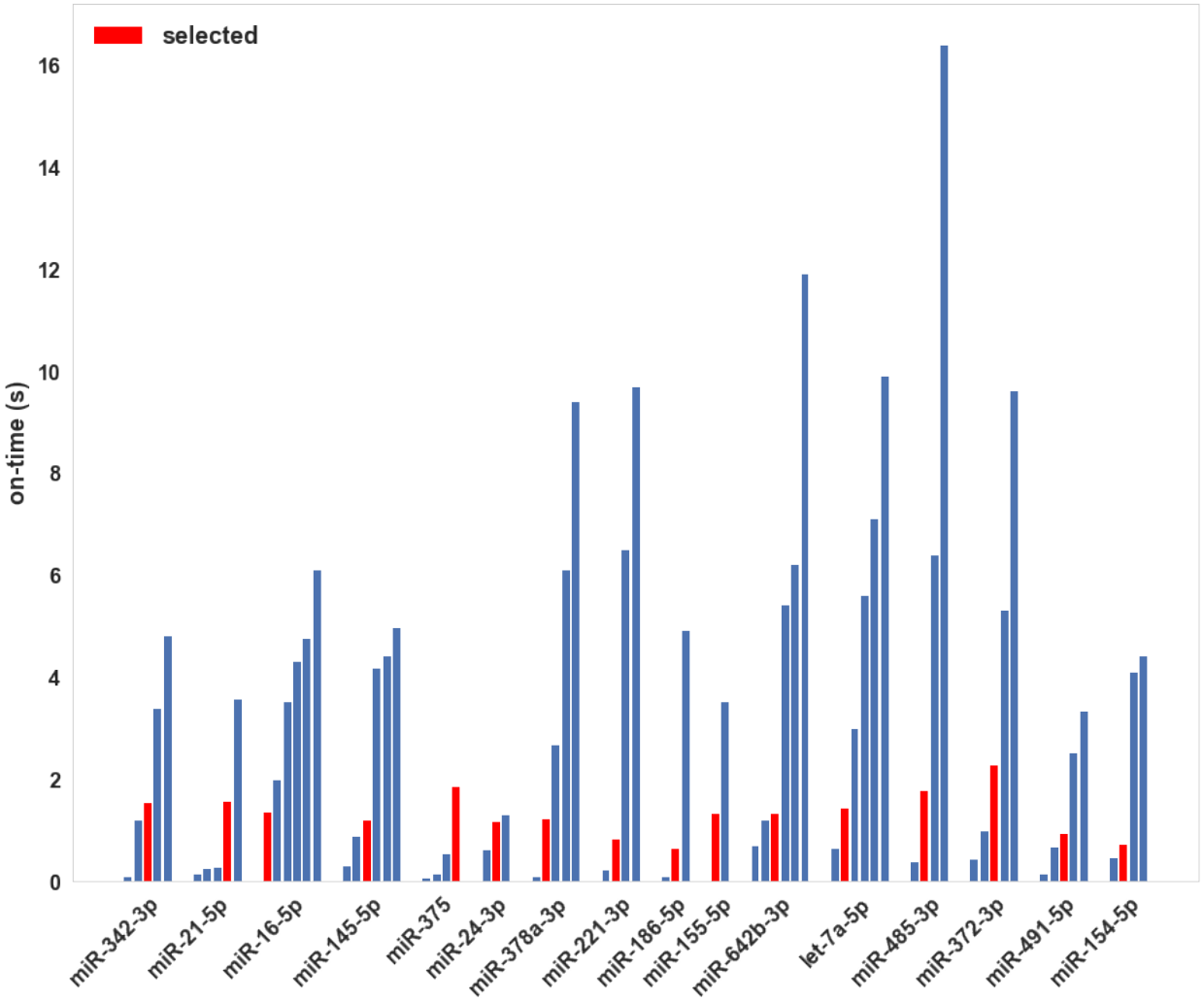

Figure S3. Blinking kinetics for 16 miRNA targets with different combinations of anchor strands and imager strands. Each bar from left to right corresponds to a row in Table S4 from top to bottom.

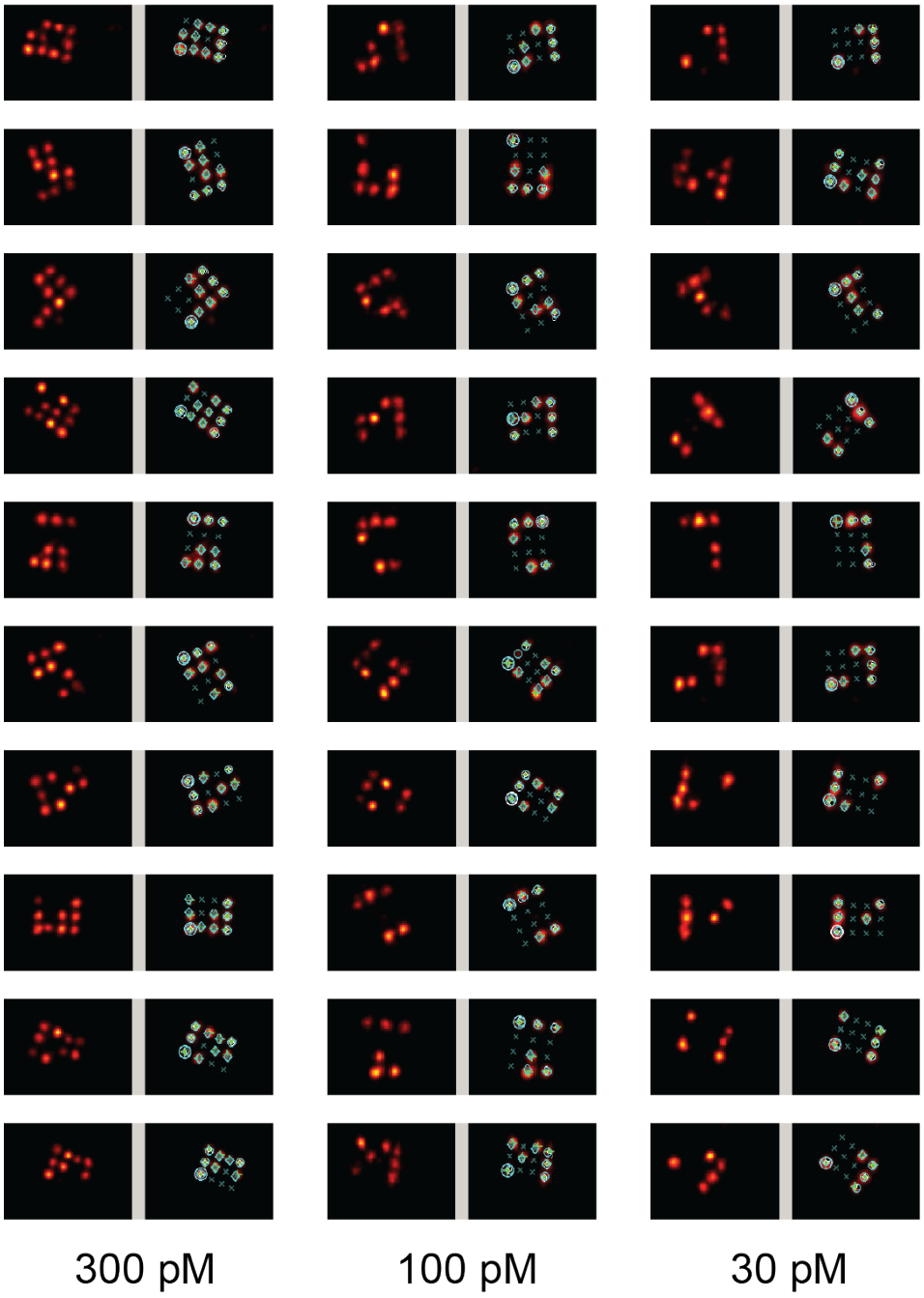

Figure S4-1. Part 1 of representative processed super-resolution images and fitting results for various miRNA concentrations in Figure 3b (miRNA incubation time 90 min).

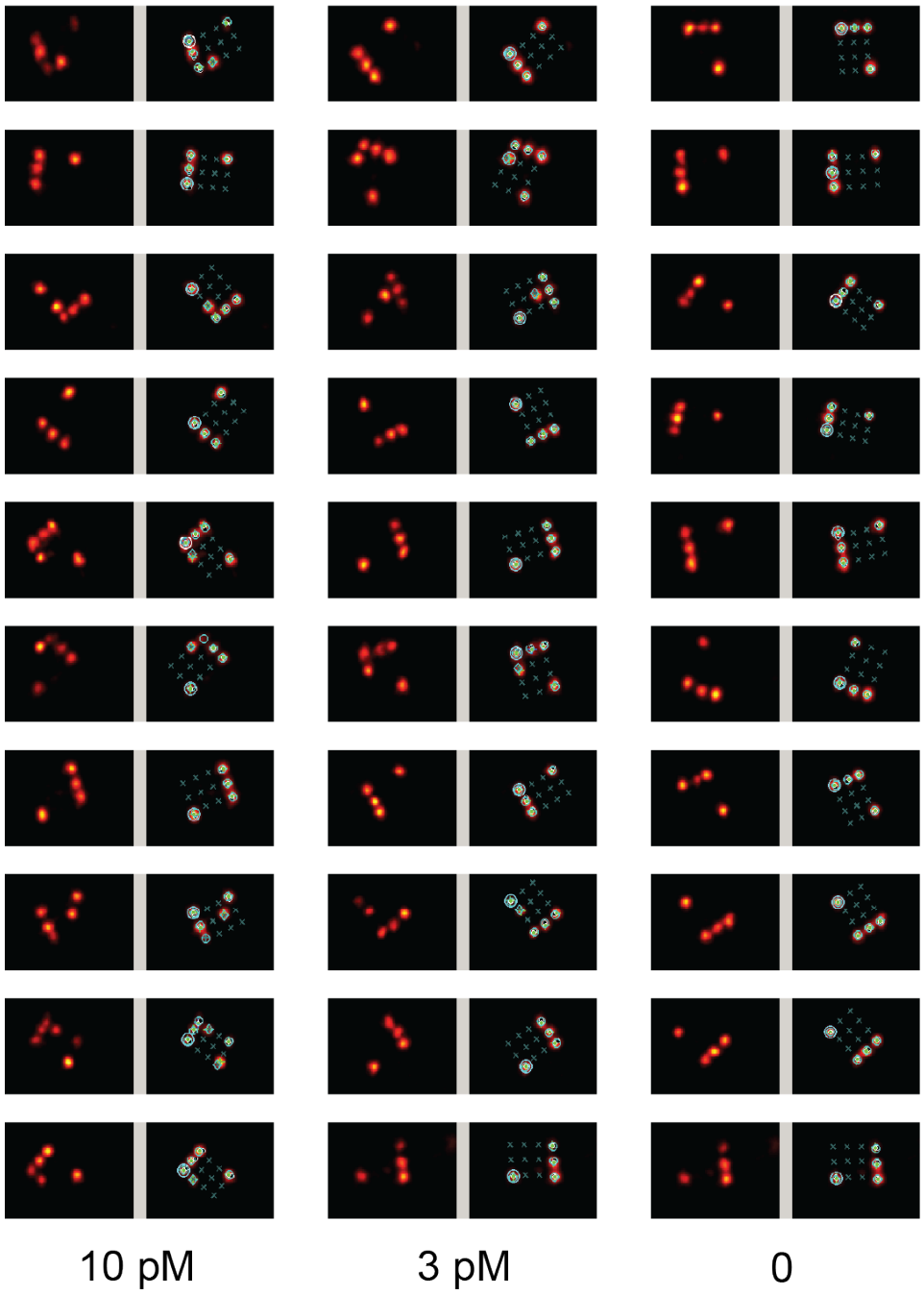

Figure S4-2. Part 2 of representative processed super-resolution images and fitting results for various miRNA concentrations in Figure 3b (miRNA incubation time 90 min).

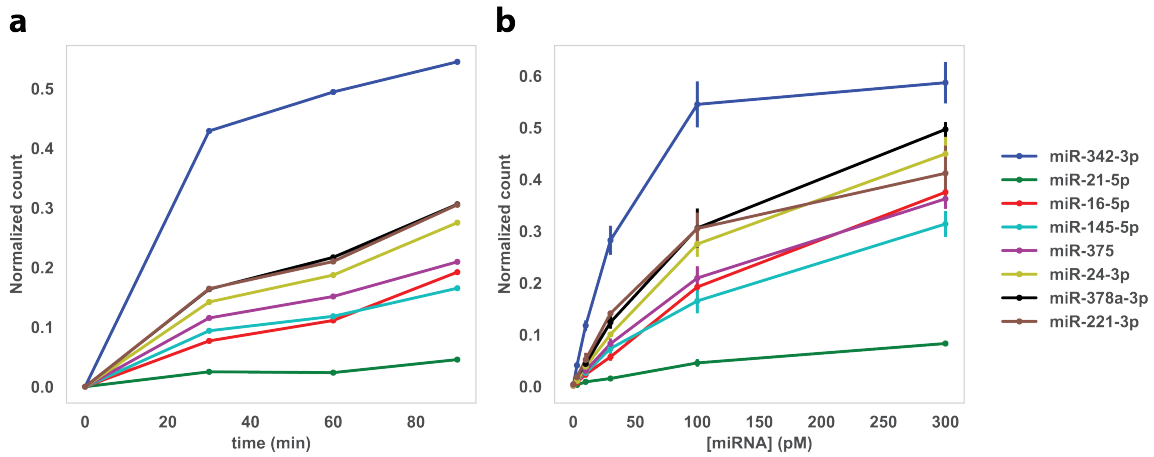

Figure S5. Quantification of immobilized miRNAs in the multiplexed assay. Normalized count is calculated by the total count of points at each anchor site in the processed super-resolution image normalized by the total number of valid origamis. (a) Normalized counts for 8 miRNA targets at 100 pM under different lengths of miRNA incubation time. (b) Normalized counts for 8 miRNA targets incubated for 90 min with different concentrations. Error bars represent the standard deviations from 3 independent experiments.

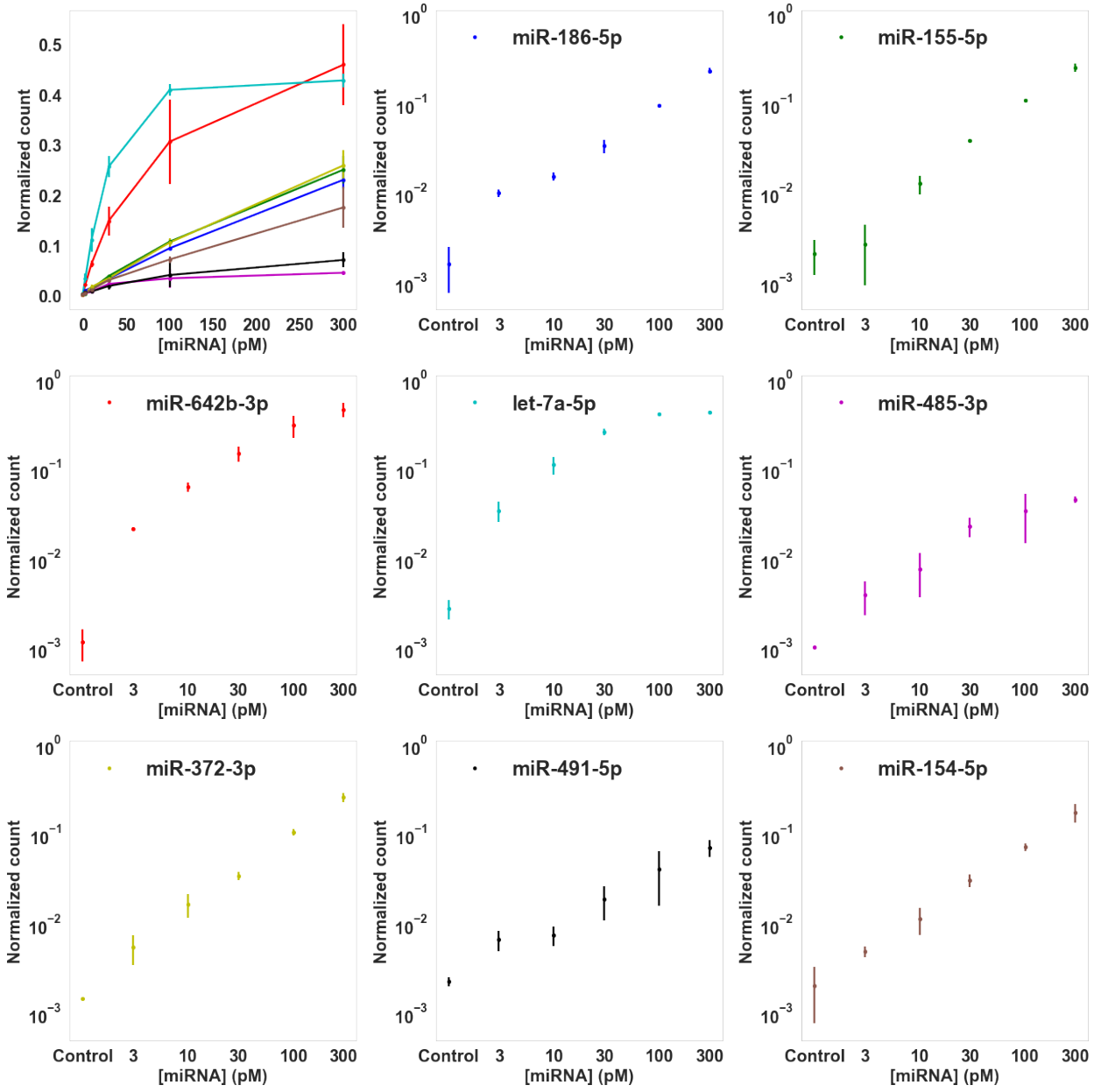

Figure S6. Calibration curves with varying target concentrations for the second group of 8-plex assays. Error bars represent the standard deviations from 3 independent experiments. Theoretical limit of detection (LoD) was shown in Table S8.

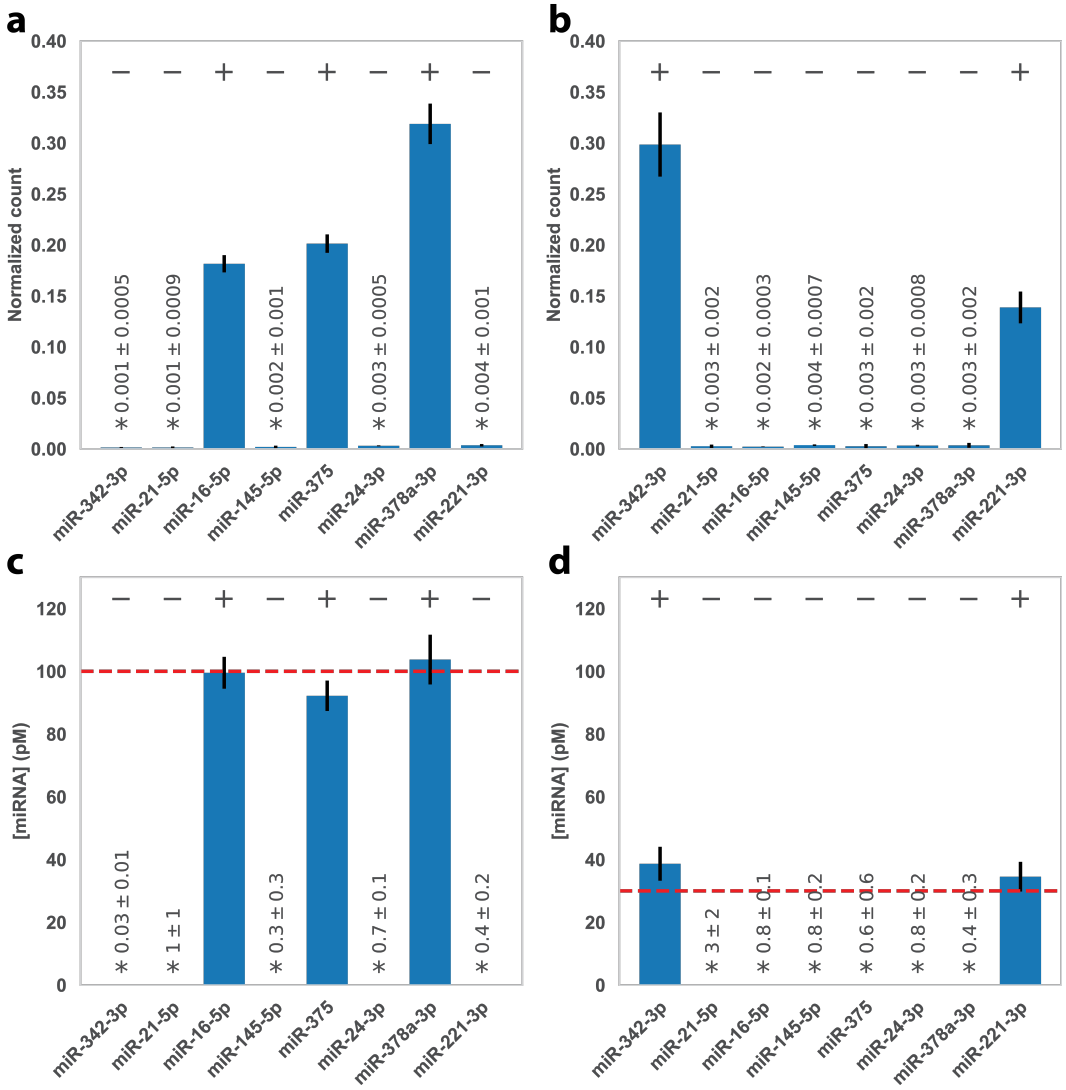

Figure S7. Normalized counts for multiplexed detection and quantification of miRNA targets in Figure 4a and 4b. (a, b) Normalized counts for multiplexed detection of different combination of miRNA targets at 100 pM (a) and 30 pM (b). (c, d) The fitted values of miRNA concentrations correspond to a and b. Error bars represent the standard deviation from 3 independent experiments. Plus/minus sign indicates the presence/absence of target miRNAs in samples. Stars indicate normalized counts are lower than limit of detection, where the values of mean and standard deviation of measured normalized counts (a, b) or the values of fitted concentrations (c, d) are shown above.

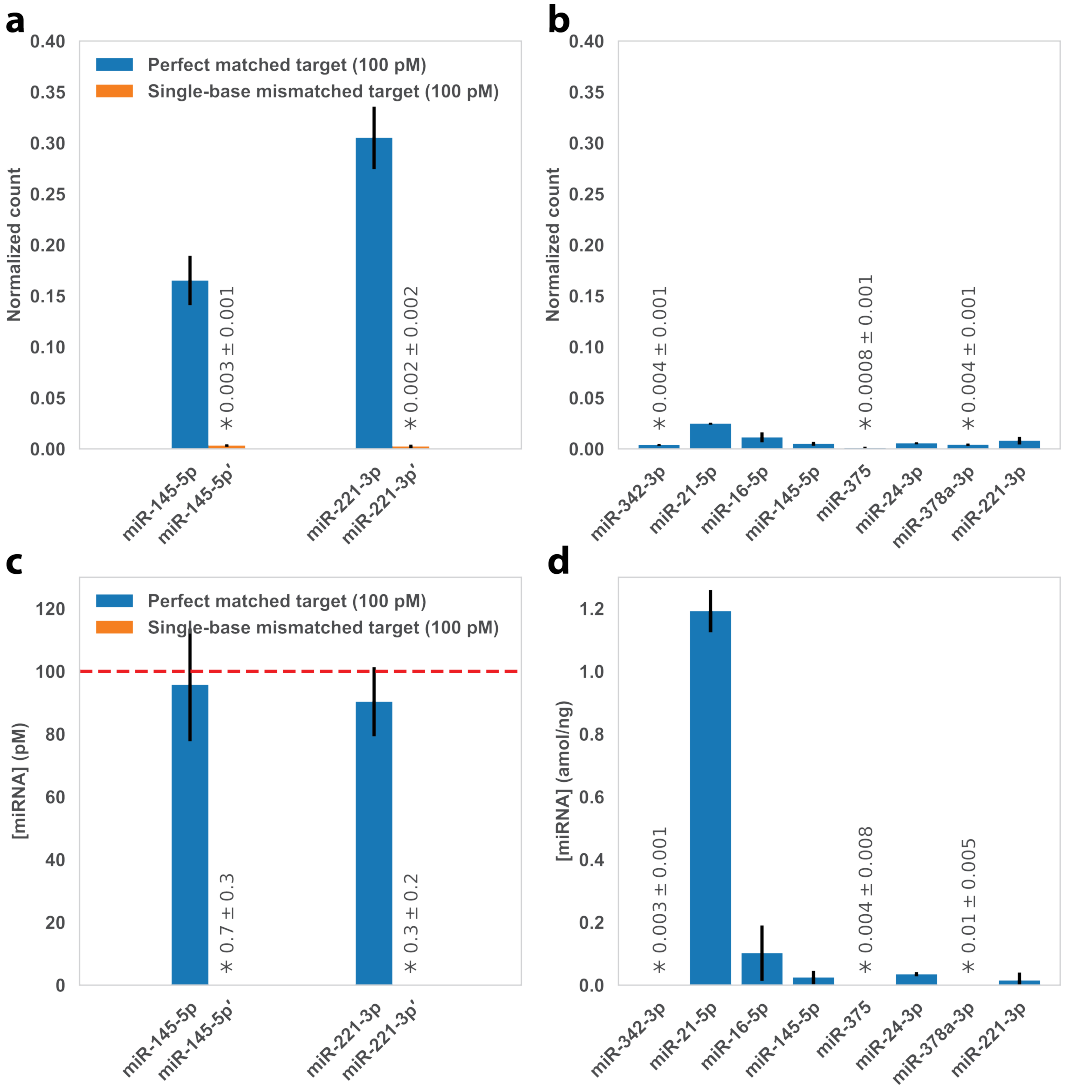

Figure S8. Normalized counts for multiplexed detection and quantification of miRNA targets in Figure 4c and 4d. (a) Normalized counts for 2 miRNA targets and their corresponding single-base mismatched targets. (b) Normalized counts for multiplexed detection of miRNA targets in total RNA samples extracted from HeLa cells. Error bars represent the standard deviation from 3 independent experiments. Plus/minus sign indicates the presence/absence of target miRNAs in samples. Stars indicate normalized counts are lower than limit of detection, where the values of mean and standard deviation of measured normalized counts (a, b) or the values of fitted concentrations (c, d) are shown above.

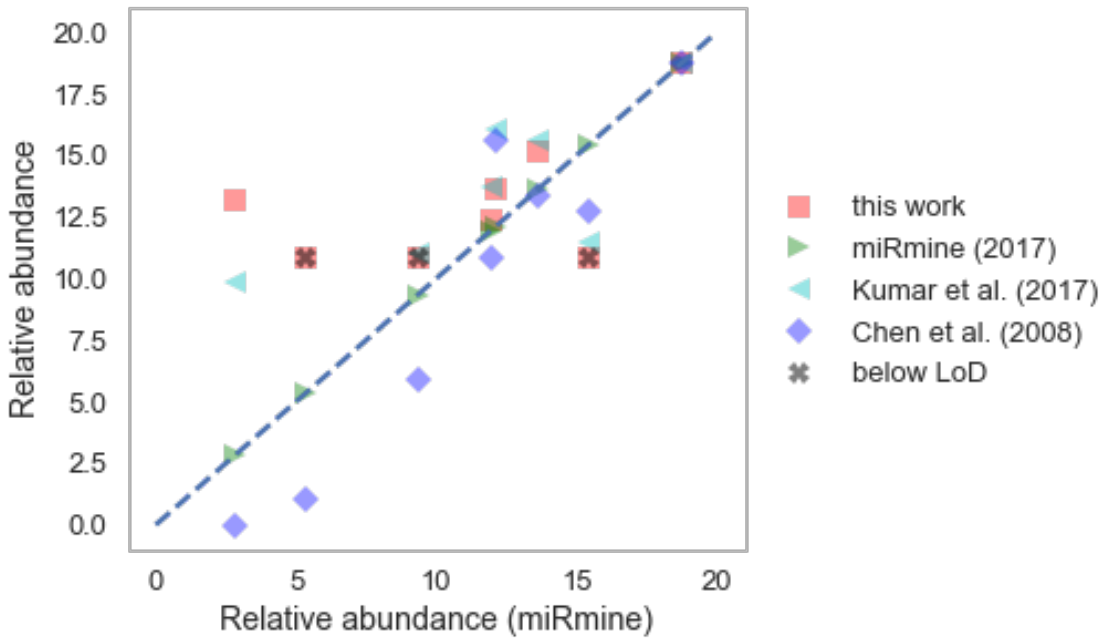

Figure S9. Comparison of measurements of first panel of miRNA targets with previous reports using RNA sequencing[2] and microarrays[3-4] based methods. Relative abundance is acquired by adding an offset to the logarithmically scaled results for each set of results so that the measurements for the most abundant miRNA species are aligned.

3. Supporting tables

Table S1. List of miRNA sequences.

ID	miRNA name	Sequence (5′ → 3′)
1	miR-342-3p	Phos–UCUCACACAGAAAUCGCACCCGU
2	miR-21-5p	Phos–UAGCUUAUCAGACUGAUGUUGA
3	miR-16-5p	Phos–UAGCAGCACGUAAAUAUUGGCG
4	miR-145-5p	Phos–GUCCAGUUUUCCCAGGAAUCCCU
5	miR-375	Phos–UUUGUUCGUUCGGCUCGCGUGA
6	miR-24-3p	Phos–UGGCUCAGUUCAGCAGGAACAG
7	miR-378a-3p	Phos–ACUGGACUUGGAGUCAGAAGGC
8	miR-221-3p	Phos–AGCUACAUUGUCUGCUGGGUUUC
9	miR-186-5p	Phos–CAAAGAAUUCUCCUUUUGGGCU
10	miR-155-5p	Phos–UUAAUGCUAAUCGUGAUAGGGGU
11	miR-642b-3p	Phos–AGACACAUUUGGAGAGGGACCC
12	let-7a-5p	Phos–UGAGGUAGUAGGUUGUAUAGUU
13	miR-485-3p	Phos–GUCAUACACGGCUCUCCUCUCU
14	miR-372-3p	Phos–AAAGUGCUGCGACAUUUGAGCGU
15	miR-491-5p	Phos–AGUGGGGAACCCUUCCAUGAGG
16	miR-154-5p	Phos–UAGGUUAUCCGUGUUGCCUUCG
	Single-base mismatched miR-145-5p	Phos–GUCCACUUUUCCCAGGAAUCCCU
	Single-base mismatched miR-221-3p	Phos–AGCUAGAUUGUCUGCUGGGUUUC

Table S2. List of staple sequences for self-assembly of the rectangular DNA origami nanostructure.

Strand ID	Sequence (5′ → 3′)	Notes
0[111]1[95]	TAAATGAATTTTCTGTATGGGATTAATTTCTT	Structure staple
0[143]1[127]	TCTAAAGTTTTGTCGTCTTTCCAGCCGACAA	Structure staple
0[175]0[144]	TCCACAGACAGCCCTCATAGTTAGCGTAACGA	Structure staple
0[207]1[191]	TCACCAGTACAAACTACAACGCCTAGTACCAG	Structure staple
0[239]1[223]	AGGAACCCATGTACCGTAACACTTGATATAA	Structure staple
0[271]1[255]	CCACCCTCATTTTCAGGGATAGCAACCGTACT	Structure staple
0[47]1[31]	AGAAAGGAACAACTAAAGGAATTCAAAAAAA	Structure staple
0[79]1[63]	ACAACTTTCAACAGTTTCAGCGGATGTATCGG	Structure staple
1[160]2[144]	TTAGGATTGGCTGAGACTCCTCAATAACCGAT	Structure staple
1[224]3[223]	GTATAGCAAACAGTTAATGCCCAATCCTCA	Structure staple
1[96]3[95]	AAACAGCTTTTTGCGGGATCGTCAACACTAAA	Structure staple
2[111]0[112]	AAGGCCGCTGATACCGATAGTTGCGACGTTAG	Structure staple
2[143]1[159]	ATATTCGGAACCATCGCCCACGCAGAGAAGGA	Structure staple
2[175]0[176]	TATTAAGAAGCGGGGTTTTGCTCGTAGCAT	Structure staple
2[207]0[208]	TTTCGGAAGTGCCGTCGAGAGGGTGAGTTTCG	Structure staple
2[239]0[240]	GCCCGTATCCGGAATAGGTGTATCAGCCCAAT	Structure staple
2[271]0[272]	GTTTTAACTTAGTACCGCCACCCAGAGCCA	Structure staple
2[47]0[48]	ACGGCTACAAAAGGAGCCTTTAATGTGAGAAT	Structure staple
2[79]0[80]	CAGCGAAACTTGCTTTCGAGGTGTTGCTAA	Structure staple
3[160]4[144]	TTGACAGGCCACCACCAGAGCCGCGATTTGTA	Structure staple
3[224]5[223]	TTAAAGCCAGAGCCGCCACCCTCGACAGAA	Structure staple
3[32]5[31]	AATACGTTTGAAAGAGGACAGACTGACCTT	Structure staple
3[96]5[95]	ACACTCATCCATGTTACTTAGCCGAAAGCTGC	Structure staple
4[143]3[159]	TCATCGCCAACAAAGTACAACGGACGCCAGCA	Structure staple
4[207]2[208]	CCACCCTCTATTCACAAACAAATACCTGCCTA	Structure staple
4[271]2[272]	AAATCACCTTCCAGTAAGCGTCAGTAATAA	Structure staple
4[79]2[80]	GCGCAGACAAGAGGCAAAAGAATCCCTCAG	Structure staple
5[160]6[144]	GCAAGGCCTCACCAGTAGCACCATGGGCTTGA	Structure staple
5[224]7[223]	TCAAGTTTCATTAAAGGTGAATATAAAAGA	Structure staple
5[32]7[31]	CATCAAGTAAAACGAACTAACGAGTTGAGA	Structure staple
5[96]7[95]	TCATTCAGATGCGATTTTAAGAACAGGCATAG	Structure staple
6[111]4[112]	ATTACCTTTGAATAAGGCTTGCCCAAATCCGC	Structure staple
6[143]5[159]	GATGGTTTGAACGAGTAGTAAATTTACCATTA	Structure staple
6[175]4[176]	CAGCAAAAGGAAACGTCACCAATGAGCCGC	Structure staple
6[207]4[208]	TCACCGACGCACCGTAATCAGTAGCAGAACCG	Structure staple
6[239]4[240]	GAAATTATTGCCTTTAGCGTCAGACCGGAACC	Structure staple
6[271]4[272]	ACCGATTGTCGGCATTTTCGGTCATAATCA	Structure staple

6[79]4[80]	TTATACCACCAAATCAACGTAACGAACGAG	Structure staple
7[120]9[127]	CGTTTACCAGACGACAAAGAAGTTTTGCCATAATTCGA	Structure staple
7[160]8[144]	TTATTACGAAGAACTGGCATGATTGCGAGAGG	Structure staple
7[184]9[191]	CGTAGAAAATACATACCGAGGAAACGCAATAAGAAGCGCA	Structure staple
7[224]9[223]	AACGCAAAGATAGCCGAACAAACCCTGAAC	Structure staple
7[248]9[255]	GTTTATTTTGTCACAATCTTACCGAAGCCCTTTAATATCA	Structure staple
7[32]9[31]	TTTAGGACAAATGCTTTAAACAATCAGGTC	Structure staple
7[56]9[63]	ATGCAGATACATAACGGGAATCGTCATAAATAAAGCAAAG	Structure staple
7[96]9[95]	TAAGAGCAAATGTTTAGACTGGATAGGAAGCC	Structure staple
8[111]6[112]	AATAGTAAACACTATCATAACCCTCATTGTGA	Structure staple
8[143]7[159]	CTTTTGCAGATAAAAACCAAAATAAAGACTCC	Structure staple
8[175]6[176]	ATACCCAACAGTATGTTAGCAAATTAGAGC	Structure staple
8[207]6[208]	AAGGAAACATAAAGGTGGCAACATTATCACCG	Structure staple
8[239]6[240]	AAGTAAGCAGACACCACGGAATAATATTGACG	Structure staple
8[271]6[272]	AATAGCTATCAATAGAAAATTCAACATTCA	Structure staple
8[47]6[48]	ATCCCCCTATACCACATTCAACTAGAAAAATC	Structure staple
8[79]6[80]	AATACTGCCCAAAAGGAATTACGTGGCTCA	Structure staple
9[128]11[127]	GCTTCAATCAGGATTAGAGAGTTATTTTCA	Structure staple
9[160]10[144]	AGAGAGAAAAAAATGAAAATAGCAAGCAAACT	Structure staple
9[192]11[191]	TTAGACGGCCAAATAAGAAACGATAGAAGGCT	Structure staple
9[224]11[223]	AAAGTCACAAAATAAACAGCCAGCGTTTTA	Structure staple
9[256]11[255]	GAGAGATAGAGCGTCTTTCCAGAGGTTTTGAA	Structure staple
9[64]11[63]	CGGATTGCAGAGCTTAATTGCTGAAACGAGTA	Structure staple
9[96]11[95]	CGAAAGACTTTGATAAGAGGTCATATTTCGCA	Structure staple
10[111]8[112]	TTGCTCCTTTCAAATATCGCGTTTGAGGGGGT	Structure staple
10[143]9[159]	CCAACAGGAGCGAACCAGACCGGAGCCTTTAC	Structure staple
10[175]8[176]	TTAACGTCTAACATAAAAACAGGTAACGGA	Structure staple
10[207]8[208]	ATCCCAATGAGAATTAACTGAACAGTTACCAG	Structure staple
10[239]8[240]	GCCAGTTAGAGGGTAATTGAGCGCTTTAAGAA	Structure staple
10[271]8[272]	ACGCTAACACCCACAAGAATTGAAAATAGC	Structure staple
10[47]8[48]	CTGTAGCTTGACTATTATAGTCAGTTCATTGA	Structure staple
10[79]8[80]	GATGGCTTATCAAAAAGATTAAGAGCGTCC	Structure staple
11[128]13[127]	TTTGGGGATAGTAGTAGCATTAAAAGGCCG	Structure staple
11[160]12[144]	CCAATAGCTCATCGTAGGAATCATGGCATCAA	Structure staple
11[192]13[191]	TATCCGGTCTCATCGAGAACAAGCGACAAAAG	Structure staple
11[224]13[223]	GCGAACCTCCAAGAACGGGTATGACAATAA	Structure staple
11[256]13[255]	GCCTTAAACCAATCAATAATCGGCACGCGCCT	Structure staple
11[32]13[31]	AACAGTTTTGTACCAAAAACATTTTATTTC	Structure staple
11[64]13[63]	GATTTAGTCAATAAAGCCTCAGAGAACCCTCA	Structure staple
11[96]13[95]	AATGGTCAACAGGCAAGGCAAAGAGTAATGTG	Structure staple
12[143]11[159]	TTCTACTACGCGAGCTGAAAAGGTTACCGCGC	Structure staple

12[207]10[208]	GTACCGCAATTCTAAGAACGCGAGTATTATTT	Structure staple
12[271]10[272]	TGTAGAAATCAAGATTAGTTGCTCTTACCA	Structure staple
12[79]10[80]	AAATTAAGTTGACCATTAGATACTTTTGCG	Structure staple
13[128]15[127]	GAGACAGCTAGCTGATAAATTAATTTTTGT	Structure staple
13[160]14[144]	GTAATAAGTTAGGCAGAGGCATTTATGATATT	Structure staple
13[192]15[191]	GTAAAGTAATCGCCATATTTAACAAAACTTTT	Structure staple
13[224]15[223]	ACAACATGCCAACGCTCAACAGTCTTCTGA	Structure staple
13[256]15[255]	GTTTATCAATATGCGTTATACAAACCGACCGT	Structure staple
13[32]15[31]	AACGCAAAATCGATGAACGGTACCGGTTGA	Structure staple
13[64]15[63]	TATATTTTGTCATTGCCTGAGAGTGGAAGATT	Structure staple
13[96]15[95]	TAGGTAAACTATTTTTGAGAGATCAAACGTTA	Structure staple
14[111]12[112]	GAGGGTAGGATTCAAAAGGGTGAGACATCCAA	Structure staple
14[143]13[159]	CAACCGTTTCAAATCACCATCAATTCGAGCCA	Structure staple
14[175]12[176]	CATGTAATAGAATATAAAGTACCAAGCCGT	Structure staple
14[207]12[208]	AATTGAGAATTCTGTCCAGACGACTAAACCAA	Structure staple
14[239]12[240]	AGTATAAAGTTCAGCTAATGCAGATGTCTTTC	Structure staple
14[271]12[272]	TTAGTATCACAATAGATAAGTCCACGAGCA	Structure staple
14[79]12[80]	GCTATCAGAAATGCAATGCCTGAATTAGCA	Structure staple
15[160]16[144]	ATCGCAAGTATGTAAATGCTGATGATAGGAAC	Structure staple
15[224]17[223]	CCTAAATCAAAATCATAGGTCTAAACAGTA	Structure staple
15[32]17[31]	TAATCAGCGGATTGACCGTAATCGTAACCG	Structure staple
15[96]17[95]	ATATTTTGGCTTTCATCAACATTATCCAGCCA	Structure staple
16[111]14[112]	TGTAGCCATTAAAATTCGCATTAAATGCCGGA	Structure staple
16[143]15[159]	GCCATCAAGCTCATTTTTTAACCACAAATCCA	Structure staple
16[175]14[176]	TATAACTAACAAAGAACGCGAGAACGCCAA	Structure staple
16[207]14[208]	ACCTTTTTATTTTAGTTAATTTCATAGGGCTT	Structure staple
16[239]14[240]	GAATTTATTTAATGGTTTGAAATATTCTTACC	Structure staple
16[271]14[272]	CTTAGATTTAAGGCGTTAAATAAAGCCTGT	Structure staple
16[47]14[48]	ACAAACGGAAAAGCCCCAAAAACACTGGAGCA	Structure staple
16[79]14[80]	GCGAGTAAAAATATTTAAATTGTTACAAAG	Structure staple
17[160]18[144]	AGAAAACAAAGAAGATGATGAAACAGGCTGCG	Structure staple
17[96]19[95]	GCTTTCCGATTACGCCAGCTGGCGGCTGTTTC	Structure staple
18[111]16[112]	TCTTCGCTGCACCGCTTCTGGTGCGGCCTTCC	Structure staple
18[143]17[159]	CAACTGTTGCGCCATTCGCCATTCAAACATCA	Structure staple
18[175]16[176]	CTGAGCAAAAATTAATTACATTTTGGGTTA	Structure staple
18[207]16[208]	CGCGCAGATTACCTTTTTTAATGGGAGAGACT	Structure staple
18[239]16[240]	CCTGATTGCAATATATGTGAGTGATCAATAGT	Structure staple
18[271]16[272]	CTTTTACAAAATCGTCGCTATTAGCGATAG	Structure staple
18[47]16[48]	CCAGGGTTGCCAGTTTGAGGGGACCCGTGGGA	Structure staple
18[79]16[80]	GATGTGCTTCAGGAAGATCGCACAATGTGA	Structure staple
19[160]20[144]	GCAATTCACATATTCCTGATTATCAAAGTGTA	Structure staple

19[224]21[223]	CTACCATAGTTTGAGTAACATTTAAAATAT	Structure staple
19[32]21[31]	GTCGACTTCGGCCAACGCGCGGGGTTTTTC	Structure staple
19[96]21[95]	CTGTGTGATTGCGTTGCGCTCACTAGAGTTGC	Structure staple
20[143]19[159]	AAGCCTGGTACGAGCCGGAAGCATAGATGATG	Structure staple
20[207]18[208]	GCGGAACATCTGAATAATGGAAGGTACAAAAT	Structure staple
20[271]18[272]	CTCGTATTAGAAATTGCGTAGATACAGTAC	Structure staple
20[79]18[80]	TTCCAGTCGTAATCATGGTCATAAAAGGGG	Structure staple
21[120]23[127]	CCCAGCAGGCGAAAAATCCCTTATAAATCAAGCCGGCG	Structure staple
21[160]22[144]	TCAATATCGAACCTCAAATATCAATTCCGAAA	Structure staple
21[184]23[191]	TCAACAGTTGAAAGGAGCAAATGAAAAATCTAGAGATAGA	Structure staple
21[224]23[223]	CTTTAGGGCCTGCAACAGTGCCAATACGTG	Structure staple
21[248]23[255]	AGATTAGAGCCGTCAAAAAACAGAGGTGAGGCCTATTAGT	Structure staple
21[32]23[31]	TTTTCACTCAAAGGGCGAAAAACCATCACC	Structure staple
21[56]23[63]	AGCTGATTGCCCTTCAGAGTCCACTATTAAAGGGTGCCGT	Structure staple
21[96]23[95]	AGCAAGCGTAGGGTTGAGTGTTGTAGGGAGCC	Structure staple
22[111]20[112]	GCCCGAGAGTCCACGCTGGTTTGCAGCTAACT	Structure staple
22[143]21[159]	TCGGCAAATCCTGTTTGATGGTGGACCCTCAA	Structure staple
22[175]20[176]	ACCTTGCTTGGTCAGTTGGCAAAGAGCGGA	Structure staple
22[207]20[208]	AGCCAGCAATTGAGGAAGGTTATCATCATTTT	Structure staple
22[271]20[272]	CAGAAGATTAGATAATACATTTGTCGACAA	Structure staple
22[79]20[80]	TGGAACAACCGCCTGGCCCTGAGGCCCGCT	Structure staple
23[128]23[159]	AACGTGGCGAGAAAGGAAGGGAAACCAGTAA	Structure staple
23[160]22[176]	TAAAAGGGACATTCTGGCCAACAAAGCATC	Structure staple
23[192]22[208]	ACCCTTCTGACCTGAAAGCGTAAGACGCTGAG	Structure staple
23[224]22[240]	GCACAGACAATATTTTTGAATGGGGTCAGTA	Structure staple
23[256]22[272]	CTTTAATGCGCGAACTGATAGCCCCACCAG	Structure staple
23[32]22[48]	CAAATCAAGTTTTTTGGGGTCGAAACGTGGA	Structure staple
23[64]22[80]	AAAGCACTAAATCGGAACCCTAATCCAGTT	Structure staple
23[96]22[112]	CCCGATTTAGAGCTTGACGGGGAAAAAGAATA	Structure staple
1[128]4[128]	TGACAACTCGCTGAGGCTTGCATTATACCAAGCGCGATGATAAA	Biotin helper strand
1[192]4[192]	GCGGATAACCTATTATTCTGAAACAGACGATTGGCCTTGAAGAGCCAC	Biotin helper strand
1[256]4[256]	CAGGAGGTGGGGTCAGTGCCTTGAGTCTCTGAATTTACCGGGAACCAG	Biotin helper strand
1[64]4[64]	TTTATCAGGACAGCATCGGAACGACACCAACCTAAAACGAGGTCAATC	Biotin helper strand
15[128]18[128]	TAAATCAAAATAATTCGCGTCTCGGAAACCAGGCAAAGGGAAGG	Biotin helper strand
15[192]18[192]	TCAAATATAACCTCCGGCTTAGGTAACAATTTCATTTGAAGGCGAATT	Biotin helper strand
15[256]18[256]	GTGATAAAAAGACGCTGAGAAGAGATAACCTTGCTTCTGTTCGGGAGA	Biotin helper strand
15[64]18[64]	GTATAAGCCAACCCGTCGGATTCTGACGACAGTATCGGCCGCAAGGCG	Biotin helper strand
4[127]6[120]	Biotin-TTTTTTGTGTCGTGACGAGAAACACCAAATTTCAACTTTAAT	Biotin labeled strand
4[191]6[184]	Biotin-TTTTCACCCTCAGAAACCATCGATAGCATTGAGCCATTTGGGAA	Biotin labeled strand
4[255]6[248]	Biotin-TTTTAGCCACCACTGTAGCGCGTTTTCAAGGGAGGGAAGGTAAA	Biotin labeled strand
4[63]6[56]	Biotin-TTTTATAAGGGAACCGGATATTCATTACGTCAGGACGTTGGGAA	Biotin labeled strand

18[127]20[120]	Biotin-TTTTGCGATCGGCAATTCCACACAACAGGTGCCTAATGAGTG	Biotin labeled strand
18[191]20[184]	Biotin-TTTTATTCATTTTTGTTTGGATTATACTAAGAAACCACCAGAAG	Biotin labeled strand
18[255]20[248]	Biotin-TTTTAACAATAACGTAAAACAGAAATAAAAATCCTTTGCCCGAA	Biotin labeled strand
18[63]20[56]	Biotin-TTTTATTAAGTTTACCGAGCTCGAATTCGGGAAACCTGTCGTGC	Biotin labeled strand
1[32]3[31]	AGGCTCCAGAGGCTTTGAGGACACGGGTAATTATACATCTA	Boundary marker
4[47]2[48]	GACCAACTAATGCCACTACGAAGGGGGTAGCATTATACATCTA	Boundary marker
6[47]4[48]	TACGTTAAAGTAATCTTGACAAGAACCGAACTTTATACATCTA	Boundary marker
9[32]11[31]	TTTACCCCAACATGTTTTAAATTTCCATATTTATACATCTA	Boundary marker
12[47]10[48]	TAAATCGGGATTCCCAATTCTGCGATATAATGTTATACATCTA	Boundary marker
14[47]12[48]	AACAAGAGGGATAAAAATTTTTAGCATAAAGCTTATACATCTA	Boundary marker
17[224]19[223]	CATAAATCTTTGAATACCAAGTGTTAGAACTTATACATCTA	Boundary marker
17[32]19[31]	TGCATCTTTCCCAGTCACGACGGCCTGCAGTTATACATCTA	Boundary marker
20[239]18[240]	ATTTTAAAATCAAAATTATTTGCACGGATTCGTTATACATCTA	Boundary marker
20[47]18[48]	TTAATGAACTAGAGGATCCCCGGGGGGTAACGTTATACATCTA	Boundary marker
22[239]20[240]	TTAACACCAGCACTAACAACTAATCGTTATTATTATACATCTA	Boundary marker
22[47]20[48]	CTCCAACGCAGTGAGACGGGCAACCAGCTGCATTATACATCTA	Boundary marker
4[111]2[112]	GACCTGCTCTTTGACCCCCAGCGAGGGAGTTA	Anchor site 1
4[175]2[176]	CACCAGAAAGGTTGAGGCAGGTCATGAAAG	Anchor site 2
4[239]2[240]	GCCTCCCTCAGAATGGAAAGCGCAGTAACAGT	Anchor site 3
12[111]10[112]	TAAATCATATAACCTGTTTAGCTAACCTTTAA	Anchor site 4
12[175]10[176]	TTTTATTTAAGCAAATCAGATATTTTTTGT	Anchor site 5
12[239]10[240]	CTTATCATTCCCGACTTGCGGGAGCCTAATTT	Anchor site 6
20[111]18[112]	CACATTAAAATTGTTATCCGCTCATGCGGGCC	Anchor site 7
20[175]18[176]	ATTATCATTCAATATAATCCTGACAATTAC	Anchor site 8

Table S3. Sequence for M13mp18 phage single-stranded DNA scaffold.

TTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTT
ACGGCACCTCGACCCCAAAAAACTTGATTTGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGA
CGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGGCTATTCTTTTGATTTA
TAAGGGATTTTGCCGATTTCGGAACCACCATCAAACAGGATTTTCGCCTGCTGGGGCAAACCAGCGTGGACCGCTTGCTGCAACTC
TCTCAGGGCCAGGCGGTGAAGGGCAATCAGCTGTTGCCCGTCTCACTGGTGAAAAGAAAAACCACCCTGGCGCCCAATACGCAAAC
CGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCA
ATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGG
ATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGG
CATGCAAGCTTGGCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACA
TCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGC
GCTTTGCCTGGTTTCCGGCACCAGAAGCGGTGCCGGAAAGCTGGCTGGAGTGCGATCTTCCTGAGGCCGATACTGTCGTCGTCCCC
TCAAACTGGCAGATGCACGGTTACGATGCGCCCATCTACACCAACGTGACCTATCCCATTACGGTCAATCCGCCGTTTGTTCCCAC
GGAGAATCCGACGGGTTGTTACTCGCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGCGAATTATTTTTGATG
GCGTTCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAATGCGAATTTTAACAAAATATTAACGTTTACAATTTAAATAT
TTGCTTATACAATCTTCCTGTTTTTGGGGCTTTTCTGATTATCAACCGGGGTACATATGATTGACATGCTAGTTTTACGATTACCG
TTCATCGATTCTCTTGTTTGCTCCAGACTCTCAGGCAATGACCTGATAGCCTTTGTAGATCTCTCAAAAATAGCTACCCTCTCCGG
CATTAATTTATCAGCTAGAACGGTTGAATATCATATTGATGGTGATTTGACTGTCTCCGGCCTTTCTCACCCTTTTGAATCTTTAC
CTACACATTACTCAGGCATTGCATTTAAAATATATGAGGGTTCTAAAAATTTTTATCCTTGCGTTGAAATAAAGGCTTCTCCCGCA
AAAGTATTACAGGGTCATAATGTTTTTGGTACAACCGATTTAGCTTTATGCTCTGAGGCTTTATTGCTTAATTTTGCTAATTCTTT
GCCTTGCCTGTATGATTTATTGGATGTTAATGCTACTACTATTAGTAGAATTGATGCCACCTTTTCAGCTCGCGCCCCAAATGAAA
ATATAGCTAAACAGGTTATTGACCATTTGCGAAATGTATCTAATGGTCAAACTAAATCTACTCGTTCGCAGAATTGGGAATCAACT
GTTATATGGAATGAAACTTCCAGACACCGTACTTTAGTTGCATATTTAAAACATGTTGAGCTACAGCATTATATTCAGCAATTAAG
CTCTAAGCCATCCGCAAAAATGACCTCTTATCAAAAGGAGCAATTAAAGGTACTCTCTAATCCTGACCTGTTGGAGTTTGCTTCCG
GTCTGGTTCGCTTTGAAGCTCGAATTAAAACGCGATATTTGAAGTCTTTCGGGCTTCCTCTTAATCTTTTTGATGCAATCCGCTTT
GCTTCTGACTATAATAGTCAGGGTAAAGACCTGATTTTTGATTTATGGTCATTCTCGTTTTCTGAACTGTTTAAAGCATTTGAGGG
GGATTCAATGAATATTTATGACGATTCCGCAGTATTGGACGCTATCCAGTCTAAACATTTTACTATTACCCCCTCTGGCAAAACTT
CTTTTGCAAAAGCCTCTCGCTATTTTGGTTTTTATCGTCGTCTGGTAAACGAGGGTTATGATAGTGTTGCTCTTACTATGCCTCGT
AATTCCTTTTGGCGTTATGTATCTGCATTAGTTGAATGTGGTATTCCTAAATCTCAACTGATGAATCTTTCTACCTGTAATAATGT
TGTTCCGTTAGTTCGTTTTATTAACGTAGATTTTTCTTCCCAACGTCCTGACTGGTATAATGAGCCAGTTCTTAAAATCGCATAAG
GTAATTCACAATGATTAAAGTTGAAATTAAACCATCTCAAGCCCAATTTACTACTCGTTCTGGTGTTTCTCGTCAGGGCAAGCCTT
ATTCACTGAATGAGCAGCTTTGTTACGTTGATTTGGGTAATGAATATCCGGTTCTTGTCAAGATTACTCTTGATGAAGGTCAGCCA
GCCTATGCGCCTGGTCTGTACACCGTTCATCTGTCCTCTTTCAAAGTTGGTCAGTTCGGTTCCCTTATGATTGACCGTCTGCGCCT
CGTTCCGGCTAAGTAACATGGAGCAGGTCGCGGATTTCGACACAATTTATCAGGCGATGATACAAATCTCCGTTGTACTTTGTTTC
GCGCTTGGTATAATCGCTGGGGGTCAAAGATGAGTGTTTTAGTGTATTCTTTTGCCTCTTTCGTTTTAGGTTGGTGCCTTCGTAGT
GGCATTACGTATTTTACCCGTTTAATGGAAACTTCCTCATGAAAAAGTCTTTAGTCCTCAAAGCCTCTGTAGCCGTTGCTACCCTC
GTTCCGATGCTGTCTTTCGCTGCTGAGGGTGACGATCCCGCAAAAGCGGCCTTTAACTCCCTGCAAGCCTCAGCGACCGAATATAT
CGGTTATGCGTGGGCGATGGTTGTTGTCATTGTCGGCGCAACTATCGGTATCAAGCTGTTTAAGAAATTCACCTCGAAAGCAAGCT
GATAAACCGATACAATTAAAGGCTCCTTTTGGAGCCTTTTTTTTGGAGATTTTCAACGTGAAAAAATTATTATTCGCAATTCCTTT
AGTTGTTCCTTTCTATTCTCACTCCGCTGAAACTGTTGAAAGTTGTTTAGCAAAATCCCATACAGAAAATTCATTTACTAACGTCT
GGAAAGACGACAAAACTTTAGATCGTTACGCTAACTATGAGGGCTGTCTGTGGAATGCTACAGGCGTTGTAGTTTGTACTGGTGAC
GAAACTCAGTGTTACGGTACATGGGTTCCTATTGGGCTTGCTATCCCTGAAAATGAGGGTGGTGGCTCTGAGGGTGGCGGTTCTGA
GGGTGGCGGTTCTGAGGGTGGCGGTACTAAACCTCCTGAGTACGGTGATACACCTATTCCGGGCTATACTTATATCAACCCTCTCG
ACGGCACTTATCCGCCTGGTACTGAGCAAAACCCCGCTAATCCTAATCCTTCTCTTGAGGAGTCTCAGCCTCTTAATACTTTCATG
TTTCAGAATAATAGGTTCCGAAATAGGCAGGGGGCATTAACTGTTTATACGGGCACTGTTACTCAAGGCACTGACCCCGTTAAAAC
TTATTACCAGTACACTCCTGTATCATCAAAAGCCATGTATGACGCTTACTGGAACGGTAAATTCAGAGACTGCGCTTTCCATTCTG
GCTTTAATGAGGATTTATTTGTTTGTGAATATCAAGGCCAATCGTCTGACCTGCCTCAACCTCCTGTCAATGCTGGCGGCGGCTCT
GGTGGTGGTTCTGGTGGCGGCTCTGAGGGTGGTGGCTCTGAGGGTGGCGGTTCTGAGGGTGGCGGCTCTGAGGGAGGCGGTTCCGG
TGGTGGCTCTGGTTCCGGTGATTTTGATTATGAAAAGATGGCAAACGCTAATAAGGGGGCTATGACCGAAAATGCCGATGAAAACG
CGCTACAGTCTGACGCTAAAGGCAAACTTGATTCTGTCGCTACTGATTACGGTGCTGCTATCGATGGTTTCATTGGTGACGTTTCC
GGCCTTGCTAATGGTAATGGTGCTACTGGTGATTTTGCTGGCTCTAATTCCCAAATGGCTCAAGTCGGTGACGGTGATAATTCACC
TTTAATGAATAATTTCCGTCAATATTTACCTTCCCTCCCTCAATCGGTTGAATGTCGCCCTTTTGTCTTTGGCGCTGGTAAACCAT
ATGAATTTTCTATTGATTGTGACAAAATAAACTTATTCCGTGGTGTCTTTGCGTTTCTTTTATATGTTGCCACCTTTATGTATGTA
TTTTCTACGTTTGCTAACATACTGCGTAATAAGGAGTCTTAATCATGCCAGTTCTTTTGGGTATTCCGTTATTATTGCGTTTCCTC
GGTTTCCTTCTGGTAACTTTGTTCGGCTATCTGCTTACTTTTCTTAAAAAGGGCTTCGGTAAGATAGCTATTGCTATTTCATTGTT
TCTTGCTCTTATTATTGGGCTTAACTCAATTCTTGTGGGTTATCTCTCTGATATTAGCGCTCAATTACCCTCTGACTTTGTTCAGG
GTGTTCAGTTAATTCTCCCGTCTAATGCGCTTCCCTGTTTTTATGTTATTCTCTCTGTAAAGGCTGCTATTTTCATTTTTGACGTT
AAACAAAAAATCGTTTCTTATTTGGATTGGGATAAATAATATGGCTGTTTATTTTGTAACTGGCAAATTAGGCTCTGGAAAGACGC
TCGTTAGCGTTGGTAAGATTCAGGATAAAATTGTAGCTGGGTGCAAAATAGCAACTAATCTTGATTTAAGGCTTCAAAACCTCCCG
CAAGTCGGGAGGTTCGCTAAAACGCCTCGCGTTCTTAGAATACCGGATAAGCCTTCTATATCTGATTTGCTTGCTATTGGGCGCGG
TAATGATTCCTACGATGAAAATAAAAACGGCTTGCTTGTTCTCGATGAGTGCGGTACTTGGTTTAATACCCGTTCTTGGAATGATA
AGGAAAGACAGCCGATTATTGATTGGTTTCTACATGCTCGTAAATTAGGATGGGATATTATTTTTCTTGTTCAGGACTTATCTATT
GTTGATAAACAGGCGCGTTCTGCATTAGCTGAACATGTTGTTTATTGTCGTCGTCTGGACAGAATTACTTTACCTTTTGTCGGTAC

TTTATATTCTCTTATTACTGGCTCGAAAATGCCTCTGCCTAAATTACATGTTGGCGTTGTTAAATATGGCGATTCTCAATTAAGCC
CTACTGTTGAGCGTTGGCTTTATACTGGTAAGAATTTGTATAACGCATATGATACTAAACAGGCTTTTTCTAGTAATTATGATTCC
GGTGTTTATTCTTATTTAACGCCTTATTTATCACACGGTCGGTATTTCAAACCATTAAATTTAGGTCAGAAGATGAAATTAACTAA
AATATATTTGAAAAAGTTTTCTCGCGTTCTTTGTCTTGCGATTGGATTTGCATCAGCATTTACATATAGTTATATAACCCAACCTA
AGCCGGAGGTTAAAAAGGTAGTCTCTCAGACCTATGATTTTGATAAATTCACTATTGACTCTTCTCAGCGTCTTAATCTAAGCTAT
CGCTATGTTTTCAAGGATTCTAAGGGAAAATTAATTAATAGCGACGATTTACAGAAGCAAGGTTATTCACTCACATATATTGATTT
ATGTACTGTTTCCATTAAAAAAGGTAATTCAAATGAAATTGTTAAATGTAATTAATTTTGTTTTCTTGATGTTTGTTTCATCATCT
TCTTTTGCTCAGGTAATTGAAATGAATAATTCGCCTCTGCGCGATTTTGTAACTTGGTATTCAAAGCAATCAGGCGAATCCGTTAT
TGTTTCTCCCGATGTAAAAGGTACTGTTACTGTATATTCATCTGACGTTAAACCTGAAAATCTACGCAATTTCTTTATTTCTGTTT
TACGTGCAAATAATTTTGATATGGTAGGTTCTAACCCTTCCATTATTCAGAAGTATAATCCAAACAATCAGGATTATATTGATGAA
TTGCCATCATCTGATAATCAGGAATATGATGATAATTCCGCTCCTTCTGGTGGTTTCTTTGTTCCGCAAAATGATAATGTTACTCA
AACTTTTAAAATTAATAACGTTCGGGCAAAGGATTTAATACGAGTTGTCGAATTGTTTGTAAAGTCTAATACTTCTAAATCCTCAA
ATGTATTATCTATTGACGGCTCTAATCTATTAGTTGTTAGTGCTCCTAAAGATATTTTAGATAACCTTCCTCAATTCCTTTCAACT
GTTGATTTGCCAACTGACCAGATATTGATTGAGGGTTTGATATTTGAGGTTCAGCAAGGTGATGCTTTAGATTTTTCATTTGCTGC
TGGCTCTCAGCGTGGCACTGTTGCAGGCGGTGTTAATACTGACCGCCTCACCTCTGTTTTATCTTCTGCTGGTGGTTCGTTCGGTA
TTTTTAATGGCGATGTTTTAGGGCTATCAGTTCGCGCATTAAAGACTAATAGCCATTCAAAAATATTGTCTGTGCCACGTATTCTT
ACGCTTTCAGGTCAGAAGGGTTCTATCTCTGTTGGCCAGAATGTCCCTTTTATTACTGGTCGTGTGACTGGTGAATCTGCCAATGT
AAATAATCCATTTCAGACGATTGAGCGTCAAAATGTAGGTATTTCCATGAGCGTTTTTCCTGTTGCAATGGCTGGCGGTAATATTG
TTCTGGATATTACCAGCAAGGCCGATAGTTTGAGTTCTTCTACTCAGGCAAGTGATGTTATTACTAATCAAAGAAGTATTGCTACA
ACGGTTAATTTGCGTGATGGACAGACTCTTTTACTCGGTGGCCTCACTGATTATAAAAACACTTCTCAGGATTCTGGCGTACCGTT
CCTGTCTAAAATCCCTTTAATCGGCCTCCTGTTTAGCTCCCGCTCTGATTCTAACGAGGAAAGCACGTTATACGTGCTCGTCAAAG
CAACCATAGTACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGC
CCTAGCGCCCGCTCCTTTCGCTTTC

Table S4. Anchor strands and imager strands for characterizing blinking kinetics. Each row from top to bottom corresponds to a bar in Figure S3 from left to right. Highlighted rows represent anchor strands and imager strands used for the multiplexed assays. Sequences are in Table S5 and Table S6.

miRNA ID	miRNA name	Anchor strand ID	Imager strand ID
1	miR-342-3p	as1-15nt	is1-7nt
1	miR-342-3p	as1-16nt	is1-6ntL
1	**miR-342-3p**	**as1-16nt**	**is1-7nt**
1	miR-342-3p	as1-14nt	is1-7ntL
1	miR-342-3p	as1-14nt	is1-8nt
2	miR-21-5p	as2-13nt	is2-8nt
2	miR-21-5p	as2-15nt	is2-7nt
2	miR-21-5p	as2-14nt	is2-6ntL2
2	**miR-21-5p**	**as2-14nt**	**is2-7ntL**
2	miR-21-5p	as2-14nt	is2-8nt
3	**miR-16-5p**	**as3-16nt**	**is3-5ntL**
3	miR-16-5p	as3-13nt	is3-6ntL
3	miR-16-5p	as3-16nt	is3-6nt
3	miR-16-5p	as3-14nt	is3-6ntL
3	miR-16-5p	as3-13nt	is3-7nt
3	miR-16-5p	as3-15nt	is3-5ntL2
4	miR-145-5p	as4-14nt	is4-7ntL
4	miR-145-5p	as4-14nt	is4-6nt
4	**miR-145-5p**	**as4-15nt**	**is4-6ntL2**
4	miR-145-5p	as4-16nt	is4-6nt
4	miR-145-5p	as4-15nt	is4-7ntL
4	miR-145-5p	as4-14nt	is4-7nt
5	miR-375	as5-15nt	is5-7nt
5	miR-375	as5-13nt	is5-8nt
5	miR-375	as5-14nt	is5-6ntL2
5	**miR-375**	**as5-14nt**	**is5-7ntL**
6	miR-24-3p	as6-14nt	is6-6ntL
6	**miR-24-3p**	**as6-14nt**	**is6-7nt**
6	miR-24-3p	as6-13nt	is6-7nt
7	miR-378a-3p	as7-13nt	is7-6nt
7	**miR-378a-3p**	**as7-17nt**	**is7-5nt**
7	miR-378a-3p	as7-13nt	is7-6ntL
7	miR-378a-3p	as7-16nt	is7-6nt
7	miR-378a-3p	as7-13nt	is7-7nt

8	miR-221-3p	as8-14nt	is8-7nt
8	**miR-221-3p**	**as8-16nt**	**is8-6ntL**
8	miR-221-3p	as8-14nt	is8-8nt
8	miR-221-3p	as8-16nt	is8-7nt
9	miR-186-5p	as9-16nt	is9-6nt
9	**miR-186-5p**	**as9-15nt**	**is9-7nt**
9	miR-186-5p	as9-14nt	is9-8nt
10	**miR-155-5p**	**as10-16nt**	**is10-7nt**
10	miR-155-5p	as10-15nt	is10-8nt
11	miR-642b-3p	as11-14nt	is11-7nt
11	miR-642b-3p	as11-13nt	is11-7ntL
11	**miR-642b-3p**	**as11-15nt**	**is11-6ntL**
11	miR-642b-3p	as11-13nt	is11-8nt
11	miR-642b-3p	as11-15nt	is11-7nt
11	miR-642b-3p	as11-14nt	is11-7ntL
12	let-7a-5p	as12-14nt	is12-7nt
12	**let-7a-5p**	**as12-17nt**	**is12-5nt**
12	let-7a-5p	as12-16nt	is12-5ntL
12	let-7a-5p	as12-16nt	is12-6nt
12	let-7a-5p	as12-15nt	is12-7nt
12	let-7a-5p	as12-15nt	is12-6ntL
13	miR-485-3p	as13-15nt	is13-6ntL
13	**miR-485-3p**	**as13-16nt**	**is13-6nt**
13	miR-485-3p	as13-14nt	is13-7nt
13	miR-485-3p	as13-15nt	is13-7nt
14	miR-372-3p	as14-17nt	is14-6nt
14	miR-372-3p	as14-15nt	is14-7nt
14	**miR-372-3p**	**as14-16nt**	**is14-5ntL2**
14	miR-372-3p	as14-16nt	is14-6ntL
14	miR-372-3p	as14-16nt	is14-7nt
15	miR-491-5p	as15-15nt	is15-5nt
15	miR-491-5p	as15-14nt	is15-5ntL2
15	**miR-491-5p**	**as15-15nt**	**is15-5ntL**
15	miR-491-5p	as15-17nt	is15-5nt
15	miR-491-5p	as15-15nt	is15-6nt
16	miR-154-5p	as16-15nt	is16-7nt
16	**miR-154-5p**	**as16-14nt**	**is16-6ntL2**
16	miR-154-5p	as16-14nt	is16-8nt
16	miR-154-5p	as16-14nt	is16-7ntL

Table S5. List of anchor strand sequences. Highlighted rows represent anchor strands used for the multiplexed assays.

Anchor strand ID	Sequences
as1-14nt	5′-Staple-ACGGGTGCGATTTC-3′
as1-15nt	5′-Staple-ACGGGTGCGATTTCT-3′
as1-16nt	**5′-Staple-ACGGGTGCGATTTCTG-3′**
as2-13nt	5′-Staple-TCAACATCAGTCT-3′
as2-14nt	**5′-Staple-TCAACATCAGTCTG-3′**
as2-15nt	5′-Staple-TCAACATCAGTCTGA-3′
as3-13nt	5′-Staple-CGCCAATATTTAC-3′
as3-14nt	5′-Staple-CGCCAATATTTACG-3′
as3-15nt	5′-Staple-CGCCAATATTTACGT-3′
as3-16nt	**5′-Staple-CGCCAATATTTACGTG-3′**
as4-14nt	5′-Staple-AGGGATTCCTGGGA-3′
as4-15nt	**5′-Staple-AGGGATTCCTGGGAA-3′**
as4-16nt	5′-Staple-AGGGATTCCTGGGAAA-3′
as5-13nt	5′-Staple-TCACGCGAGCCGA-3′
as5-14nt	**5′-Staple-TCACGCGAGCCGAA-3′**
as5-15nt	5′-Staple-TCACGCGAGCCGAAC-3′
as6-13nt	5′-Staple-CTGTTCCTGCTGA-3′
as6-14nt	**5′-Staple-CTGTTCCTGCTGAA-3′**
as7-13nt	5′-Staple-GCCTTCTGACTCC-3′
as7-16nt	5′-Staple-GCCTTCTGACTCCAAG-3′
as7-17nt	**5′-Staple-GCCTTCTGACTCCAAGT-3′**
as8-14nt	5′-Staple-GAAACCCAGCAGAC-3′
as8-16nt	**5′-Staple-GAAACCCAGCAGACAA-3′**
as9-14nt	5′-Staple-AGCCCAAAAGGAGA-3′
as9-15nt	**5′-Staple-AGCCCAAAAGGAGAA-3′**
as9-16nt	5′-Staple-AGCCCAAAAGGAGAAT-3′
as10-15nt	5′-Staple-ACCCCTATCACGATT-3′
as10-16nt	**5′-Staple-ACCCCTATCACGATTA-3′**
as11-13nt	5′-Staple-GGGTCCCTCTCCA-3′
as11-14nt	5′-Staple-GGGTCCCTCTCCAA-3′
as11-15nt	**5′-Staple-GGGTCCCTCTCCAAA-3′**
as12-14nt	5′-Staple-AACTATACAACCTA-3′
as12-15nt	5′-Staple-AACTATACAACCTAC-3′
as12-16nt	5′-Staple-AACTATACAACCTACT-3′
as12-17nt	**5′-Staple-AACTATACAACCTACTA-3′**
as13-14nt	5′-Staple-AGAGAGGAGAGCCG-3′
as13-15nt	5′-Staple-AGAGAGGAGAGCCGT-3′

as13-16nt	**5′-Staple-AGAGAGGAGAGCCGTG-3′**
as14-15nt	5′-Staple-ACGCTCAAATGTCGC-3′
as14-16nt	**5′-Staple-ACGCTCAAATGTCGCA-3′**
as14-17nt	5′-Staple-ACGCTCAAATGTCGCAG-3′
as15-14nt	5′-Staple-CCTCATGGAAGGGT-3′
as15-15nt	**5′-Staple-CCTCATGGAAGGGTT-3′**
as15-17nt	5′-Staple-CCTCATGGAAGGGTTCC-3′
as16-14nt	**5′-Staple-CGAAGGCAACACGG-3′**
as16-15nt	5′-Staple-CGAAGGCAACACGGA-3′

Table S6. List of fluorophore labeled imager strand sequences. Highlighted rows represent anchor strands used for the multiplexed assays.

Imager strand ID	Sequence (5′ → 3′)
P1	**CTAGATGTAT-Cy3b**
is1-6ntL	TGTGAG-Cy3b
is1-7nt	**TGTGAGA-Cy3b**
is1-7ntL	GTGTGAG-Cy3b
is1-8nt	GTGTGAGA-Cy3b
is2-6ntL2	ATAAGC-Cy3b
is2-7nt	TAAGCTA-Cy3b
is2-7ntL	**ATAAGCT-Cy3b**
is2-8nt	ATAAGCTA-Cy3b
is3-5ntL	**CTGCT-Cy3b**
is3-5ntL2	GCTGC-Cy3b
is3-6nt	CTGCTA-Cy3b
is3-6ntL	GCTGCT-Cy3b
is3-7nt	GCTGCTA-Cy3b
is4-6nt	CTGGAC-Cy3b
is4-6ntL2	**AACTGG-Cy3b**
is4-7nt	ACTGGAC-Cy3b
is4-7ntL	AACTGGA-Cy3b
is5-6ntL2	CGAACA-Cy3b
is5-7nt	GAACAAA-Cy3b
is5-7ntL	**CGAACAA-Cy3b**
is5-8nt	CGAACAAA-Cy3b
is6-6ntL	TGAGCC-Cy3b
is6-7nt	**TGAGCCA-Cy3b**
is7-5nt	**CCAGT-Cy3b**
is7-6nt	TCCAGT-Cy3b
is7-6ntL	GTCCAG-Cy3b
is7-7nt	GTCCAGT-Cy3b
is8-6ntL	**TGTAGC-Cy3b**
is8-7nt	TGTAGCT-Cy3b
is8-8nt	ATGTAGCT-Cy3b
is9-6nt	TCTTTG-Cy3b
is9-7nt	**TTCTTTG-Cy3b**
is9-8nt	ATTCTTTG-Cy3b
is10-7nt	**GCATTAA-Cy3b**
is10-8nt	AGCATTAA-Cy3b
is11-6ntL	**TGTGTC-Cy3b**

is11-7nt	TGTGTCT-Cy3b
is11-7ntL	ATGTGTC-Cy3b
is11-8nt	ATGTGTCT-Cy3b
is12-5nt	**CCTCA-Cy3b**
is12-5ntL	ACCTC-Cy3b
is12-6nt	ACCTCA-Cy3b
is12-6ntL	TACCTC-Cy3b
is12-7nt	TACCTCA-Cy3b
is13-6nt	**TATGAC-Cy3b**
is13-6ntL	GTATGA-Cy3b
is13-7nt	GTATGAC-Cy3b
is14-5ntL2	**GCACT-Cy3b**
is14-6nt	CACTTT-Cy3b
is14-6ntL	GCACTT-Cy3b
is14-7nt	GCACTTT-Cy3b
is15-5nt	CCACT-Cy3b
is15-5ntL	**CCCAC-Cy3b**
is15-5ntL2	CCCCA-Cy3b
is15-6nt	CCCACT-Cy3b
is16-6ntL2	**ATAACC-Cy3b**
is16-7nt	TAACCTA-Cy3b
is16-7ntL	ATAACCT-Cy3b
is16-8nt	ATAACCTA-Cy3b

Table S7. Concentrations of imager strands in the imaging buffer for the final super-resolution imaging in the multiplexed assays. Imager strands shown here ensure similar blinking duty cycle for each docking site.

miRNA ID	miRNA name	Imager strand ID	Concentration (nM)
		P1	2.00
1	miR-342-3p	is1-7nt	1.04
2	miR-21-5p	is2-7ntL	0.82
3	miR-16-5p	is3-5ntL	0.37
4	miR-145-5p	is4-6ntL2	1.10
5	miR-375	is5-7ntL	0.57
6	miR-24-3p	is6-7nt	1.05
7	miR-378a-3p	is7-5nt	0.53
8	miR-221-3p	is8-6ntL	1.49
9	miR-186-5p	is9-7nt	2.29
10	miR-155-5p	is10-7nt	0.92
11	miR-642b-3p	is11-6ntL	1.10
12	let-7a-5p	is12-5nt	0.35
13	miR-485-3p	is13-6nt	0.90
14	miR-372-3p	is14-5ntL2	0.51
15	miR-491-5p	is15-5ntL	0.85
16	miR-154-5p	is16-6ntL2	1.17

Table S8. Theoretical limit of detection (LoD) for miRNA targets in the multiplexed assays. The theoretical LoD for each miRNA was estimated by the mean of the fitted values for background signals (controls) plus 3 times the standard deviation of the fitted values, i.e.,

$$LoD = c_{control} + 3\sigma_{control},$$

where $c_{control}$ is the fitted value for controls, and $\sigma_{control}$ is the standard deviation.

miRNA ID	miRNA name	Theoretical LoD (pM)
1	miR-342-3p	0.5
2	miR-21-5p	7
3	miR-16-5p	3
4	miR-145-5p	1
5	miR-375	1
6	miR-24-3p	1
7	miR-378a-3p	1
8	miR-221-3p	1
9	miR-186-5p	0.9
10	miR-155-5p	5
11	miR-642b-3p	0.1
12	let-7a-5p	0.1
13	miR-485-3p	4
14	miR-372-3p	3
15	miR-491-5p	1
16	miR-154-5p	4

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