Electronic Supplementary Material (ESI) for Analyst.

Supporting Information for

Rapid Quantification of microRNA-375 through One-Pot Primer-Generating Rolling Circle Amplification

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1. Supporting Figures

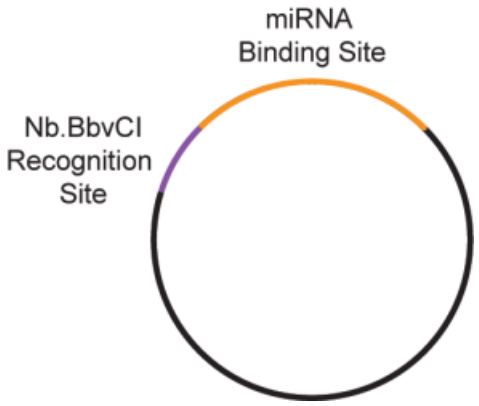

Figure S1. Circular template design. The pg-RCA circular template is illustrated with a miRNA binding site indicated in yellow and the Nb.BbvCI recognition site indicated in purple.

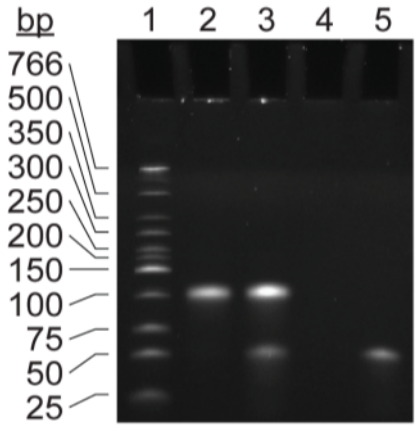

Figure S2. Characterization of circular template synthesis. Image of 10% polyacrylamide gel shows low molecular weight DNA ladder (lane 1), circularized template with Exonuclease I digestion (lane 2), circularized template without digestion (lane 3), linear template with Exonuclease I digestion (lane 4), and linear template without digestion (lane 5).

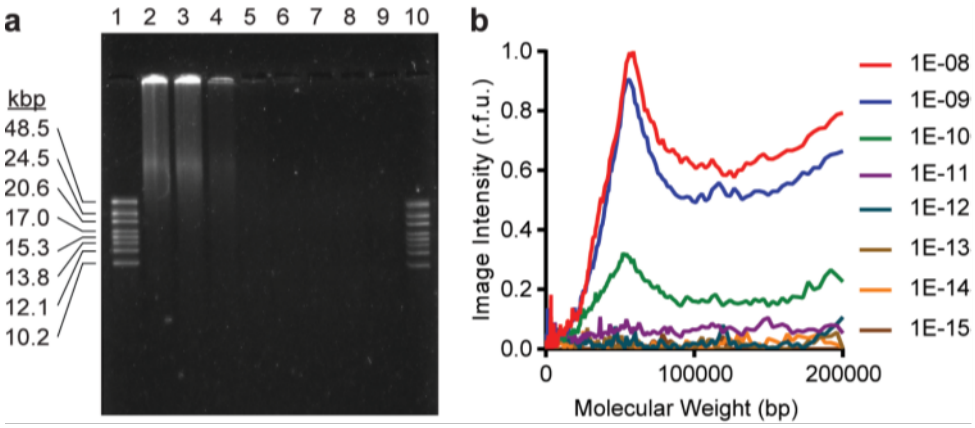

Figure S3. Agarose gel electrophoresis analysis of linear rolling circle amplification products. (a) Image of gel, showing lanes containing (1,10) molecular weight DNA ladder or (2–9) RCA reactions containing miR-375 at concentrations of (2) 10,000 pM, (3) 1,000 pM, (4) 100 pM, (5) 10 pM, (6) 1 pM, (7) 0.1 pM, (8) 0.01 pM, and (9) 0 pM. **(b)** Corresponding intensities for each lane containing miR-375. Legend values are shown in molar concentrations.

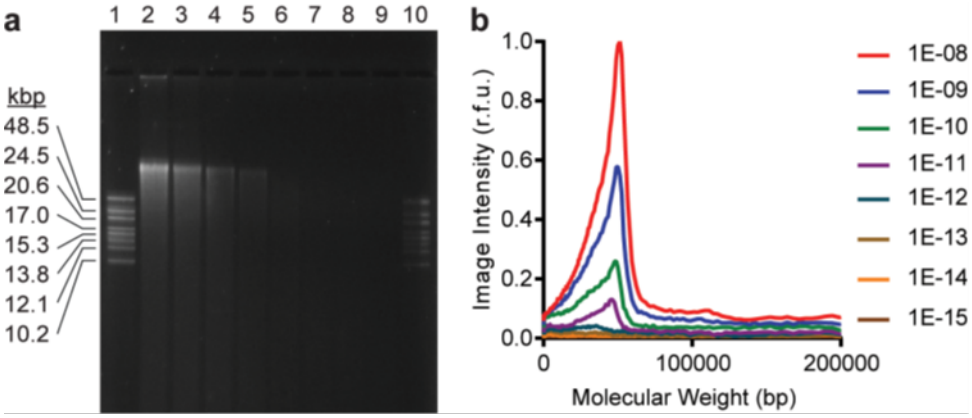

Figure S4. Agarose gel electrophoresis analysis of primer generating rolling circle amplification products. (a) Image of gel, showing lanes containing (1,10) molecular weight DNA ladder or (2–9) RCA reactions containing miR-375 at concentrations of (2) 10,000 pM, (3) 1,000 pM, (4) 100 pM, (5) 10 pM, (6) 1 pM, (7) 0.1 pM, (8) 0.01 pM, and (9) 0 pM. **(b)** Corresponding intensities for each lane containing miR-375. Legend values are shown in molar concentrations.

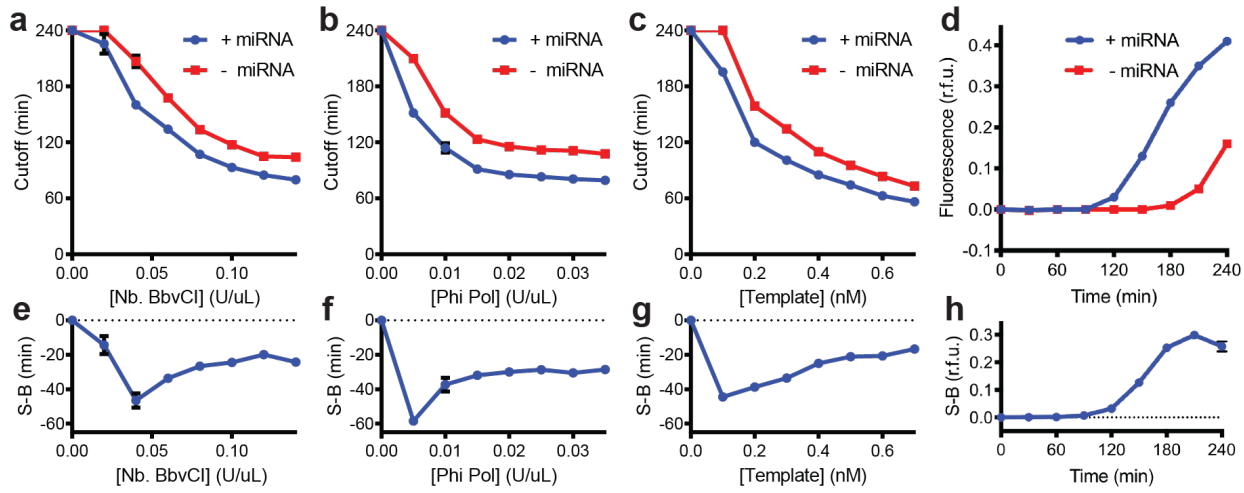

Figure S5. Optimization of pg-RCA reaction conditions. Reactions were performed with linearly incremented concentrations of **(a)** nickase (Nb.BbvCI) at 0.05 U μL^{-1} Φ29 DNA Polymerase and 0.4 nM template, **(b)** Φ29 DNA polymerase at 0.2 U μL^{-1} nickase and 0.4 nM template, and **(c)** circular DNA template at 0.05 U μL^{-1} Φ29 DNA polymerase and 0.2 U μL^{-1} nickase. All reactions were performed in the presence or absence of 1 pM miR-375. The time at which SYBR Gold fluorescence intensity reaches the threshold value is plotted for each reaction condition. **(d)** The impact of time on reaction fluorescence intensity in the presence or absence of 1 pM miR-375. Graphs below each experimental parameter show the difference between signal threshold time **(e,f,g)** or fluorescence intensity **(h)** for 1 pM samples (S) and background 0 pM samples (B) as S-B. All data points represent the average of 3 technical replicates with error bars indicating standard deviation between replicates.

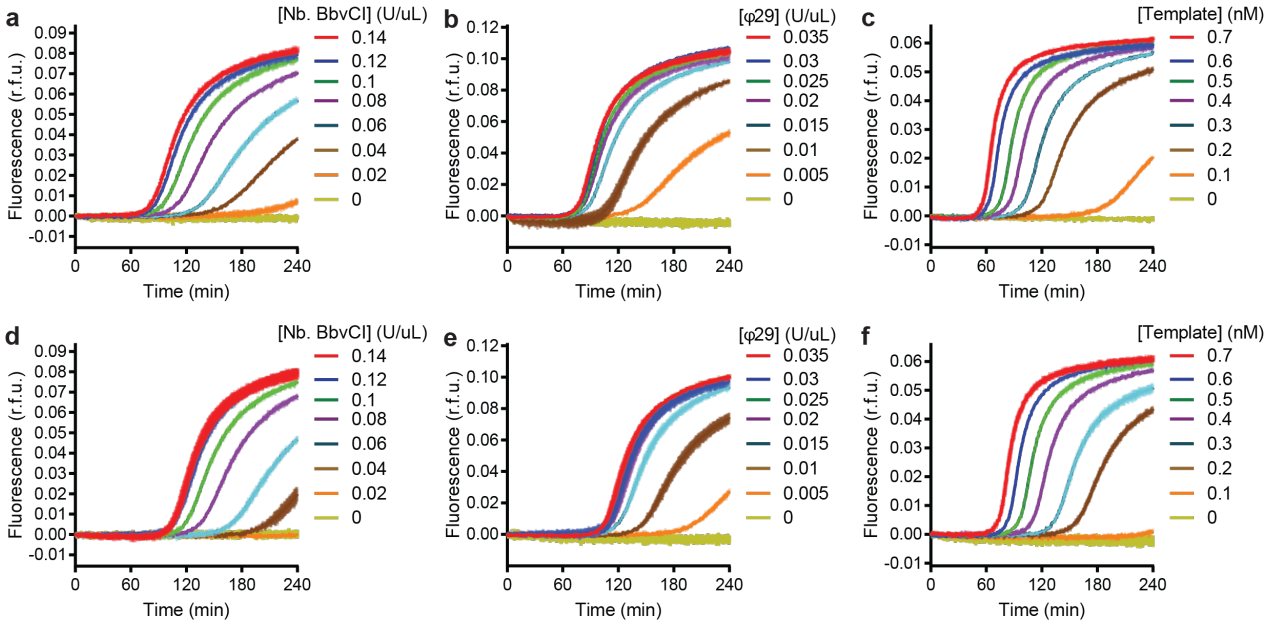

Figure S6. Raw data for optimization of pg-RCA reaction conditions. Time-traces of fluorescence intensities of reactions are shown for tuned concentrations of **(a,d)** Nb.BbvCI, **(b,e)** Φ29 DNA polymerase, and **(c,f)** circuclar DNA template, with all other reaction parameters held constant. Reactions were performed in the presence of 1 pM miRNA **(a-c)** or in the absence of miRNA **(d-f)**. All data represents the averages of 3 technical replicates with line width indicating the standard deviation between samples.

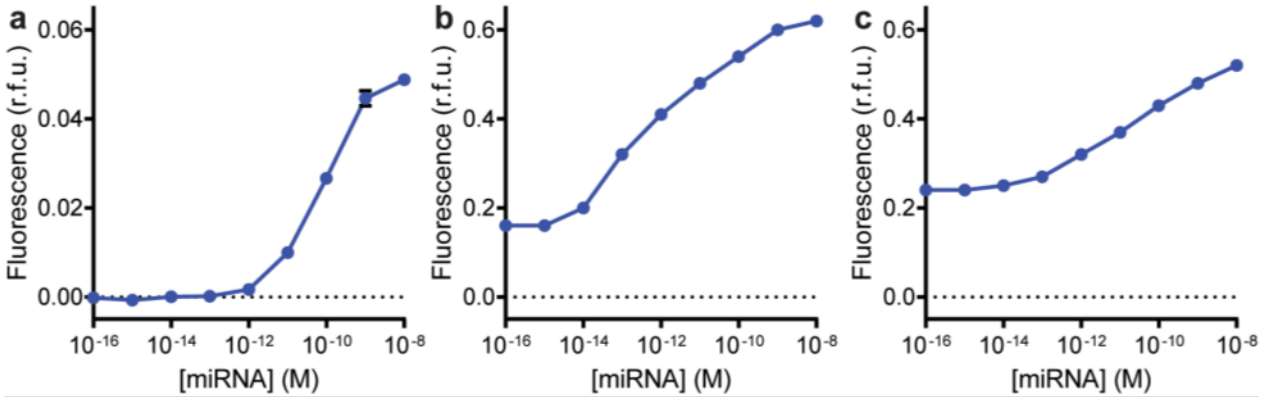

Figure S7. MicroRNA amplification reaction quantification using endpoint fluorescence intensity. (a) Linear RCA without nicking endonuclease Nb.BbvCI. **(b)** Primer-generating RCA performed with nicking endonuclease in sample buffer. **(c)** Primer-generating RCA performed in total RNA extract from human serum. Samples correspond to real-time reactions in **Figure 3a-c**. All data points represent the averages of 3 technical replicates with error bars indicating the standard deviation between samples.

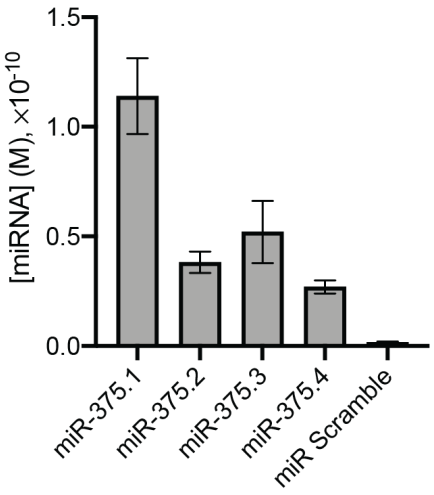

Figure S8. Characterization of pg-RCA sequence specificity. The four most prevalent miR-375 isoforms were analyzed at a concentration of 100 pM using pg-RCA. Effective concentration for each isoform was calculated using a standard curve generated for miR-375.1. Bar height and error bars represent the mean and standard deviation of 3 technical replicates, respectively. Sequences are shown in **Table S1**.

2. Supporting Tables

Table S1. Oligonucleotide sequences used in this work

Name	Sequence
pg-RCA template	/5Phos/GAA CGA ACA AAC CTC AGC AAA TAA TCA CAG CAA GCA AGG CTA CCA CAG GCA ATC CTC AGC AAA TAA TCT CAC GCG AGC C
miR-375 (miR-375.1)	rUrUrU rGrUrU rCrGrU rUrCrG rGrCrU rCrGrC rGrUrG rA
miR-375.2	rUrUrU rGrUrU rCrGrU rUrCrG rGrCrU rCrGrC rGrUrG
miR-375.3	rUrUrU rGrUrU rCrGrU rUrCrG rGrCrU rCrGrC rGrUrG rU
miR-375.4	rUrUrU rGrUrU rCrGrU rUrCrG rGrCrU rCrGrC rGrU
miR Scramble	rUrGrC rUrArA rGrGrU rCrCrG rUrGrU rCrCrA rUrArU rC

/5Phos/ indicates 5’ phosphate group modification.