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One-Step Cellular Micro-RNA Detection with Programmable DNA Nanoswitches

Molly F. Maclsaac

University at Albany, State University of New York

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One-step cellular micro-RNA detection with programmable DNA nanoswitches

An honors thesis presented to the
Department of Biological Sciences,
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Molly F. MacIsaac

Research Mentor: Arun Richard Chandrasekaran, Ph.D.
Research Advisor: Kenneth Halvorsen, Ph.D.
Second Reader: Gabriele Fuchs, Ph.D.

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Abstract

MicroRNAs play important roles in gene regulation. Additionally, differential expression of specific microRNAs have been correlated with a wide range of diseases. Sensitive and selective detection of microRNAs is thus important for enabling their use as biomarkers, drugs, or drug targets. Current detection techniques such as northern blotting and quantitative real-time PCR require skilled personnel and expensive equipment to execute complex and time consuming assays. Here we develop and validate a one-step, non-enzymatic microRNA detection assay using DNA nanoswitches programmed to recognize and bind a specific microRNA. Binding induces a loop in the structure, allowing the target microRNA to be unambiguously detected on a standard agarose gel. We demonstrate single nucleotide specificity and sub-attomole sensitivity for synthetic microRNAs in buffer. The utility of the technique is illustrated by biological detection from differentiating muscle cells, where we detect cellular microRNAs from nanogram-scale RNA extracts. Start to finish detection is possible in one hour without expensive equipment or reagents, making this assay a compelling alternative to qPCR and northern blotting.
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Introduction

Detection of molecules with specific sequences, such as DNA and RNA, is of growing importance in bio-medical and even forensics fields. Mature microRNAs (miRNAs, miRs) are short, non-coding RNA fragments that are typically 22 nucleotides in length. They play a large role in the post-transcriptional repression of gene expression [1]. MiRNAs can also serve as disease precursors, and therefore act as a diagnostic and prognostic biomarker for many diseases, including muscular dystrophy, and certain cancers [1-2]. Currently, miRNAs are also being investigated as possible therapeutic targets because of their significant involvement in disease pathology. As miRNAs continue to grow in importance, specific detection of these miRNAs is crucial. However, current detection methods such as Northern Blotting or Quantitative Real Time Polymerase Chain Reaction (qRT-PCR, qPCR) can be expensive, complex, or both [3-5].

Cellular microRNA can be difficult to detect. They typically comprise only 0.01% of total RNA [6], and can be found in low abundance, ranging from a few copies per cell to tens of thousands of copies per cell [7]. Furthermore, microRNAs often occur in families that differ by one or two nucleotides [8] making detection specificity of crucial importance. The complementary base pairing exhibited between DNA and RNA allows for a new method of microRNA detection that is both simple and inexpensive. The DNA nanoswitch is a platform that capitalizes on base pairing, and a topological change, to visual miRNAs in an agarose gel electrophoresis [9].

Linearized, single-stranded DNA acts as the backbone of the DNA nanoswitch. Complementary backbone oligonucleotides are added to the DNA scaffold to create the double-stranded duplex. In two regions along the backbone, overhanging DNA detectors are added. This state of the nanoswitch is referred to as the linear, “off” conformation. The overhanging regions
of these detectors are designed and synthesized to be complimentary to a specific target sequence, such as a specific miRNAs. With introduction of the complementary target miRNA, the nanoswitch is forced into the looped, “on” conformation, as both detectors hybridize to either side of the miRNA, displacing the middle section of the nanoswitch. These two distinct conformations can be easily separated and visualized with an agarose gel electrophoresis. Because of the topological change and molecular shape, the looped “on” state will migrate slower in a gel than the linear “off” state, providing a simple method to visualize the presence of specific miRNAs (Figure 1a, inset). Each microRNA that binds and loops a nanoswitch shifts the location of thousands of dye molecules that have intercalated into the nanoswitch’s backbone. This provides a type of signal amplification, with each detected microRNA moving thousands of dye molecules.

Rapid detection is also attainable. Binding of miRNA targets to the nanoswitch can occur in less than 30 minutes, and detection visualization in a gel can also be achieved in only 30 minutes, providing a miRNA detection assay that can be completed in less than one hour. Additionally, the cost of this detection is less than a penny per lane loaded [9]. This provides a simple, quick, and low cost alternative to the current miRNA detection methods.

Finally, the ability to order synthesized DNA detectors, with any sequence, allows the nanoswitch to be completely customizable for detection of a variety of nucleic acid targets, not just miRNAs, ranging in sequence, length, and type. With continued research, this nanoswitch-based technology could eventually be used in the medical field to detect specific disease biomarkers. The simplicity, robustness, and availability of the nanoswitch, make it an incredibly powerful bioanalytical tool that can be used a vast number of ways.
Materials and Methods

Design and oligonucleotide mixtures

To construct the nanoswitch, a mixture of complementary oligonucleotide (oligos) strands are added to, and hybridize to a linearized M13 scaffold, resulting in a double stranded duplex. Additionally, a set of 12 “variable” oligos is designed to bind to the nanoswitch at prospective detector regions along the backbone. Certain variable oligos are withheld from construction and replaced by detector strands resulting in nanoswitches of particular loop sizes. Oligonucleotides were purchased from Integrated DNA Technologies (IDT) with standard desalting.

Construction of the nanoswitches

To create the nanoswitch, a circular, single-stranded DNA scaffold (7249-nucleotide bacteriophage M13) was linearized with enzyme Btscl, which cleaves the DNA at a single site. Unique detectors are designed and synthesized to target a specific nucleic acid sequence. These detectors, as well as the complementary oligonucleotide strands are added in 10-fold excess to the linearized M13 scaffold. The scaffold-oligonucleotide-detector mixture was then subject to a temperature ramp of 90°C to 4°C at 1°C/min, annealing the strands, and creating double-stranded, linear DNA.

PEG purification

After hybridization of the nanoswitch, there is an excess of complementary oligonucleotides, and the mixture is purified for increased detection sensitivity. A 2-step 4%, 8K Polyethylene Glycol (PEG) precipitation can be used to remove most of the excess oligonucleotides. However, this method induces variability in purification, solely based on the nature of the purification.
Dilutions after purification would fluctuate due to this variability. This encouraged optimization of a different purification method: high performance liquid chromatography (HPLC).

**HPLC purification**

High performance liquid chromatography (HPLC) nanoswitch purification introduces a more repeatable, reliable, and accurate purification method. Flow rates, buffer quantities and column composition are unchanged for each purification, providing this stability. After HPLC purification, the nanoswitch was diluted in a 1XPBS and 4%, 8K PEG mixture.

**Synthetic target detection**

Concentration of the nanoswitch, and concentration of the synthetic nucleic acid target, and incubation time varied for each experiment. Target sequence length also varied, but was 22 nucleotides unless otherwise noted. Additionally, each detector strand was half of the length of the target sequence (11 and 11 nucleotides when detecting a 22 nucleotide sequence). When detecting double stranded DNA, the DNA was heated to 90°C for two minutes to denature the strands and create single-stranded DNA that is more likely to hybridize with the detectors. Nanoswitch-target mixtures were in incubated at room temperature or subject to a temperature ramp from 46°C to 4°C.

**Biological target detection**

To detect biological miRNAs, small RNA and total RNA extractions from mice myoblast cells were provided to us from our collaborators. Concentration of the nanoswitch, synthetic “spike-in” target sequence, and amount of small RNA and total RNA varied in each experiment. The
nanoswitch’s detectors were designed to be perfect complements to the target microRNA, with two overhangs of 11 nucleotides. Sample-nanoswitch incubation typically occurred on a 40°C to 25°C ramp. The incubation time varied per experiment.

**Gel electrophoresis**

After the nanoswitches are incubated with the target sequence, they are then run in a 0.8% agarose gel. The agarose is dissolved in 0.5x Tris-borate EDTA (TBE) buffer. GelRed DNA stain intercalates into the backbone of the nanoswitch, and was used to visualize detection in the gel. Three different GelRed protocols were used. The first is referred to as a pre-staining, where the GelRed is dissolved with the agarose before casting. The second is referred to as post-staining, where the gel is cast, loaded, run, and then placed in a GelRed and buffer solution before imaging. Finally, the third protocol is referred to as “spiking-in”, where the GelRed is added to the samples before they are run in the gel. Before loading, samples were mixed with a Ficoll-based loading solution. Gel were run at either 4°C or room temperature for 90 minutes, unless noted otherwise. Gels were imaged with a Bio-Rad Gel Doc XR+ gel imager and then analyzed. Negative and positive controls in buffer were present in small RNA and total RNA experiments to determine the migration pattern of the linear and looped band.
Results

Magnesium effect on synthetic DNA and RNA detection

Before detection of biological samples could be attempted, optimization and characterization of synthetic DNA and RNA detection was studied. Let-7b microRNA is a well-studied microRNA, part of the Let-7 family, which was used as a model system. Synthetic let-7b microRNA, as well as DNA analogs of the let-7b sequence, was ordered (from IDT), and a 7b nanoswitch was designed and created to target this sequence. To determine the effect magnesium has on the detection of synthetic DNA and RNA, 100mM MgCl$_2$ was added to the DNA target and RNA target reaction mixtures. The DNA and RNA target concentration was 25nM, which is a typical experimental concentration for that yields optimal detection. Reaction mixtures were incubated at room temperature for two hours. Then the effect of adding Mg$^{2+}$ to the nanoswitch-target mixture was examined (Figure 2a). The gel bands, and computer analysis of the intensities show in this case, the addition of Mg$^{2+}$ increased the amount of looped nanoswitches in both the DNA and RNA samples, although not by significant amounts.

Time dependence of synthetic RNA detection

The detection time for let-7B DNA and RNA was also tested. This experiment was done without the addition of Mg$^{2+}$. Seven different time points were observed (0 minutes -160 minutes). It was previously known that target DNA could bind to a nanoswitch in as little as 10 minutes [10]. However, it was unknown how rapidly synthetic RNA could be detected using the nanoswitch. Here, distinct banding patterns are visible when the nanoswitch and target were incubated for only 8 minutes for both DNA and RNA, (Figure 2b). This is evidence that DNA nanoswitches are a very fast method for specific sequence detection of not only DNA but RNA as well.
**Target-Detector length variations**

The next set of experiments analyzed the ability of the nanoswitch to recognize target sequences of different lengths, with detectors of different lengths. 25nM RNA targets of 30 nucleotides (nt), 40nt, and 60nt lengths were added to nanoswitches that had 15nt, 20nt, and 30nt detector lengths (on each side). Nanoswitch-target mixtures were incubated at room temperature. These nanoswitches showed distinct conformational changes (Figure 2c). The looped “on” state was the most profound for detector lengths of 15nt. When the detector and target lengths increased in nucleotide number, the amount of looped switches decreased in number. However, detection was still possible. Schematic representation of the possible target-detector length combinations is shown (Figure 2d).

**RNA and DNA detection repeatability**

Repeatability of the nanoswitch was tested to assure consistency with results. MiR-7b DNA and RNA sequences were tested with their complementary nanoswitch at three different concentrations (Figure 2e). Each concentration was tested five times to determine standard deviations (Figure 2f). The coefficient variance (CV) ranged from 0.025 to 0.197. The CV was the smallest when the fraction of nanoswitches bound was higher. The CV when the target concentration was 25nM was 0.025 for DNA and 0.041 for RNA. When the target concentration was lowered to 2.5nM the CV was 0.049 and 0.041 for DNA and RNA respectively. Finally, when the target concentration was lowered to 0.25nM, the fraction of nanoswitches bound significantly decreased, and the CV was 0.197 and 0.130 for DNA and RNA respectively.
The CV was fairly consistent for DNA and RNA at 25nM, 2.5nM, and 0.25nM target concentrations, showing the binding capabilities of the nanoswitch are consistent and repeatable.

**Limit of detection for synthetic RNA**

To determine the nanoswitch’s limit of detection the synthetic let-7b microRNA was used. First, optimization of the sensitivity protocol was conducted. To accomplish low detection, a few procedures were followed. Even when using LoBind tubes, it is known that some of the sample sticks to the side of the tube. To avoid 7b RNA signal loss to due this phenomenon, the 7b RNA was diluted in “blocking oligos” which are random DNA sequences around 24nt in length that will coat the sides of the tube, allowing more 7b RNA to be available for detection. Additionally, when pipetting, tips are pre-coated with these blocking oligos for one minute prior to moving low-concentration (sub-nanomolar) RNA samples for the same concern that some RNA will stick to the inside of the tip, losing signal detection in the process. Furthermore, dilutions were performed in minimal steps (however, without exceeding 1000-fold dilutions) to again limit signal loss to tubes and pipette tips. Thinner gels were also cast to minimize background noise from the gel. Typically 25-50mL of 0.8% agarose was cast into mini-gel boxes, however to increase signal detection, this volume was decreased to 20mL. After these changes, sub-attomole detection of 7b RNA was achieved, correlating to a 130fM 7b RNA concentration (Figure 3a, gel inset). We report amount of material detected, rather than concentration, because our signal detection depends on amount, and not concentration. Quantification of the relative amounts or 7b RNA present is possible by integrating the area under the intensity profiles. This data is plotted to graphically represent the limit of detection curve (Figure 3a). Samples were incubated at room temperature for 16 hours.
Concentration-time dependence for RNA detection

At high concentrations (10-100pM) and long incubation times (16 hrs) the detection signal begins to saturate as shown in blue (Figure 3b). However, decreasing incubation time to 2 hours or 15 minutes as shown in green and orange respectively, bring in the dynamic range of this system, and eliminates this saturation. Shorter incubation time point can be chosen to differentiate between signals at higher concentrations. Additionally, detection of a 10pM samples can be achieved in just 15 minutes, shown by the data in orange.

Single-mismatch specificity

Biologically occurring microRNAs are often found in families that differ by only one or two nucleotides, so mismatch specificity is extremely important. Let-7b microRNA is part of the let-7 microRNA family, which varies by only one or two nucleotides. The let-7c sequence varies from the 7b sequence by one nucleotide and the let-7a sequence varies from the 7b sequence by two nucleotides (Figure 3c). The let-7b nanoswitch, whose detectors are complementary to the let-7b sequence, was used to detect the 7b, 7c, and 7a sequence. The fraction bound on the graph is normalized to let-7b detection. Introduction of one or two mismatch yields absolutely no signal. This is important, as off-target detection and single-mismatch specificity has been a challenge for other microRNA detection methods [11-12].
**Synthetic RNA detection in total RNA**

Optimization of the nanoswitch in a buffer solution has been the primary focus of the research thus far. As detection abilities expand toward biological samples, the nanoswitch must be optimized to function in an environment that mimics biological fluids. Detection of miRNA will occur in small RNA and total RNA extracts, and thus the nanoswitch must be fully operational within these environments.

Before detection of naturally occurring miRNA from biological extracts can be attempted, a full understanding of the nanoswitch’s behavior in total RNA must be achieved. To do so, commercially available total RNA extracts was used to mimic biological extract conditions. A synthetic target sequence, let-7b RNA, and its complementary nanoswitch, were introduced to the total RNA extract to optimize target detection. Immediately, significant loss of detection and poor banding patterns in the total RNA samples was seen (Figure 4a). Lanes 1 and 3 depict the linear (-) control and lanes 2 and 4 depict the nanoswitch looping in the presence of the let-7b RNA target sequence. A very significant loss of detection signal can be seen in lanes 3 and 4, and the bands lost their resolution and compaction.

**Incubation temperature for total RNA detection**

The first step taken to improve the detection signal presented by the nanoswitch was to expose the switch to a slow decreasing temperature ramp that facilitated target sequence and detector binding. After many temperature and time variations, an optimal ramp protocol was determined that significantly increased the resolution of the detection signal. A temperature change from 40 to 25°C at 0.2°C/min elicited the most consistent, and successful detection signal. The difference between a room temperature incubation, and the thermocycler ramp
incubation can been seen (Figure 4b), with much more compact bands seen in lane 2. In addition, other parameters were investigated in order to obtain further increase in signal quality.

**Gel running conditions**

Next optimal gel conditions for target detection in total RNA extracts were explored. Running the gel electrophoresis assays at 4°C was tested, which was in contrast to the current protocol at the time, which was running the gel assay at room temperature. This also improved the detection signal, which can be visualized in lane 2 (Figure 4c). Additionally, the effects of varying voltage intensities and gel running times were investigated. It was found that a starting voltage of 20V, followed by 75V for 120 minutes improved our detection signal (Figure 4d). Five different conditions were tested, in which the samples were subject to the 20V condition for different lengths of time, and then all were increased to 75V to facilitate band separation. Lane 1, being exposed to the low voltage condition for the longest period of time, was the most condensed band. As the amount of time spent in the 20V condition decreased, so did the quality of the bands (as seen in lane 5, with no time spent at 20V).

**GelRed Effect on signal detection and band migration.**

The most drastic improvement however, was seen as the GelRed dye protocol was manipulated. GelRed is a DNA dye that intercalates into the backbone of the nanoswitch, producing the visual signal in the gel after imaging. Typical protocols follow either a pre-stain or a post-stain. When pre-staining, GelRed is dissolved with the agarose before casting. In post-staining, the gel is cast, loaded, run, and then placed in a GelRed and buffer solution before imaging. However, a third protocol was attempted in which GelRed was added to the samples before they are run thought
the gel. The drastic difference between pre-staining (lane 1) and spiking-in GelRed (lane 2) was observed (Figure 4d).

**Detection of miR-206 from small RNA**

To detect biological miRNAs, a well-studied model system was utilized. Our collaborating lab works with a mouse myoblast cell line, specifically C2C12. MicroRNA-206 (or miR-206) is known to be highly expressed in these cells [13], so this microRNA was used as the biological target [15-16]. Myoblast cells undergo differentiation with the addition of specific media (2% horse serum and growth factors). This differentiation induces a significant up-regulation of miR-206 [13-14]. Our collaborators used a TRIzol extraction to obtain small RNA and total RNA samples from the undifferentiated and differentiated cells (Figure 5a). They then provided us with these samples for the nanoswitch assay validation.

Only about 0.01% of totalRNA is comprised of miRNAs, and within the miRNA population, miR-206 is found [17]. Even with up-regulation, miR-206 is still a minuscule percentage of the totalRNA population. To attempt biological detection, the small RNA population was isolated from the totalRNA extractions. In theory, the small RNA should contain the highest concentration of miRNAs, and therefore contain the highest concentration of miR-206. Addition of the nanoswitch to 10ng of undifferentiated small RNAs yielded no detection. However, miR-206 detection was visualized in the differentiated small RNAs (figure 5b). This is consistent with the research showing up-regulation of miR-206 in differentiated muscle cells.
**Detection of miR-206 from total RNA**

Next, the nanoswitch’s capabilities in total RNA were tested. The same nanoswitch was added to 500ng of totalRNA extracted from undifferentiated and differentiated cells. Again, no miR-206 detection was visualized in the undifferentiated totalRNA; however, a very strong detection signal was seen from the differentiated totalRNA (figure 5c).

It was also shown that, using the differentiated samples; detection of miR-206 was possible in just 0.2ng of small RNA and 0.5ng of total RNA.

**Detection of multiple cellular microRNAs**

To determine if the nanoswitch could detect other cellular miRNAs from the myoblast cell line, other highly expressed microRNAs were looked at. MiR-1a, miR-133-3p, miR-133-5p, in addition to miR-206, are known to be highly expressed in muscle cells [14]. To test the robustness of the nanoswitch, unique nanoswitches were designed switches for each additional miRNA. Addition of these four nanoswitches to the totalRNA extracted from the differentiated cells should produce the looped conformation, yielding detection of additional miRNAs. In fact, when these switches were added to 500ng of total RNA, detection was seen for each miRNA (figure 5e). However, miR-206 still produced the strongest signal, meaning miR-206 is most highly expressed in muscle cells, out of these four miRNAs.
Discussion

From the experiments conducted to optimize synthetic RNA detection, it was shown that the presence of Mg$^{2+}$ does in fact increase the amount of let-7b RNA and DNA detected as compared to samples that were not exposed to Mg$^{2+}$. The current protocol for cellular microRNA detection continues to use 100mM MgCl$_2$ in each sample. Additionally, it was also shown that detecting 25nM RNA with the nanoswitch can be achieved as quickly as detecting the same concentrations of DNA. This detection is achieved in just eight minutes. Producing a DNA nanoswitch that has a fast detection time for DNA and RNA is crucial to the nanoswitch’s future application in the medical and forensic fields. It was also demonstrated that shorter detector lengths (around 11-15nt) were optimal for signal detection. Finally, when experiments were performed in replicates at different concentrations for both DNA and RNA, low CV’s show that the binding capabilities of the nanoswitch are consistent and repeatable.

As experiments transitioned to optimize the functionality of the nanoswitch in a total RNA environment many challenges were faced. To study this, synthetic let-7b RNA was spiked into commercially available total RNA. Immediately, it was seen that signal detection was lost in this total RNA environment. Using a slow, decreasing temperature ramp, running gel-electrophoresis assay in a 4°C environment, and initially using a mild voltage condition of 20V, were three major changes that resulted in increased signal detection. However, the most important protocol change was altering when the GelRed DNA dye was added to the samples. The previous protocol used a pre-stain in which the GelRed was dissolved with the agarose before casting. GelRed is known to have stabilizing effects as it intercalates into the DNA backbone. However, adding GelRed to the sample, and not the gel, significant increased detection signal and clarity. When GelRed is present throughout the gel, as in a pre-stain, the
material that migrates more quickly (i.e. the saturated “junk” present at the bottom of each total RNA gel) will capture most of the dye molecules, leaving behind less stabilizing dye for the nanoswitch. This leads to the poor detection signal seen in many gels. However, the GelRed sample spike-in still allows for the stabilizing properties, while every DNA and RNA molecule has equal access to these dye molecules. This protocol significantly increased the detection yield of the nanoswitch and was used throughout biological microRNA experiments.

With these protocol changes, detection of biologically occurring miR-206 was possible. Detection of a highly expressed microRNA, miR-206 was shown in small RNA and total RNA samples. Given that the nanoswitch’s can detect as little as 0.2 amol (~100,000 copies), and that it detects miR-206 from just 0.2ng of small RNA, (which correlating to approximately 25 cells), it can be concluded that miR-206 is present in ~4000 copies per cell. Additionally, detection of other microRNAs such as miR-1a, miR-133-3p, miR-133-5p yielded signals that were 100-fold times lower, correlating with detection of microRNAs present in ~40 copies per cell.

In the future, the nanoswitch will continue to be optimized for detection in small RNA and total RNA samples. An assay comparison needs to be completed to understand the nanoswitch’s performance in comparison to current miRNA detection methods, such as Northern Blotting and qPCR. Eventually progression to detection from other biological sources, such as cell lysate or body fluids, will be investigated.

MiRNAs are a relatively recent discovery, and their importance in a number of biological processes is just being exploited. With scientific and diagnostic advancements, the detection of specific miRNAs will only gain importance. As microRNA detection become more pertinent, alternative detection methods that combat the complex, expensive, and time-consuming, pitfalls of the current methods will only grow in popularity. The nanoswitch is a very powerful
bioanalytical tool that allows for the detection of not only miRNAs, but many types of nucleic acid sequences. Detection with the nanoswitch is a simple, cost-effective, rapid, specific, and sensitive alternative that should certainly be capitalized on.
Figure 1. (a) The “off” conformation of the nanoswitch is a linear duplex formed with ssM13 scaffold (dark blue) and short complementary oligonucleotides (green). Programmable detector stands (orange) with overhangs specific to a complementary target sequence (red) are attached to the scaffold. The “on” conformation is observed when the target sequence binds with both detectors. Inset: gel electrophoresis where looped “on” state migrates slower than the linear “off” state. (b) Schematic depicting nanoswitch-based microRNA detection assay. Target microRNA is chosen, and detectors are designed to target that sequences. This nanoswitch is added to totalRNA extraction and gel electrophoresis produced detection signal.
Figure 2. (a) The effect Mg2+ has on synthetic 25nM 7b DNA and RNA detection. (-) denotes negative control containing only the nanoswitch. (b) 25nM DNA and RNA target detection with decreasing incubation time. (c) The effect target and detector lengths have on detection. 25nM DNA target lengths ranged from 30 to 60nts, and nanoswitch detector lengths ranged from 15 to 30nts. (d) Schematic depicting possible target-detector binding depending on length of each. (e) Repeatability measurements of let 7b DNA and RNA detection at three different concentrations. (f) Average fraction bound for these three concentrations of DNA and RNA, with each concentration tested 5 times.
**Figure 3.** (a) Limit of detection for synthetic RNA. The nanoswitch can detect sub-attomole levels of 7b RNA, correlating to femtomolar concentrations. (b) The dynamic range and tunable nature of this system is seen with varying incubation times and target concentrations. (c) Schematic depicting single-mismatch specificity, as the nanoswitch will not detect sequences with one or two mismatches. (d) Normalized fraction bound for zero, one, and two mismatches, with no signal seen for one or two mismatches.
Figure 4. (a) Agarose gel showing the effect of a 100ng total RNA introduction to a functional nanoswitch. (-) lanes function as negative control. (b) The effect of incubation temperature has on the quality of the detection signal from the nanoswitch. Temperature was decreased from 40°C to 25°C at 0.2°C/min in a thermocycler (lane 2). Lane 1 was incubated for the same time at room temperature. (c) The effect that gel running temperature has on the quality of the detection signal from the nanoswitch. Both lanes had identical experimental conditions, besides the gel temperature. (d) Agarose gel showing the effect a 20V condition has on the quality of the detection signal from the nanoswitch. The time spent in the 20V condition is indicated above each lane. After that time, the voltage was increased to 75V for 2 more hours for banding separation. (e) The effect a GelRed DNA dye spike in has on the quality of the detection signal from the nanoswitch.
Figure 5. (a) Schematic of totalRNA extractions and small RNA isolation from the C2C12 mouse myoblast cell line. (b) MiR-206 detection from 10ng of small RNA isolated from differentiated cells. Detection of miR-206 is not seen in the undifferentiated small RNA. Positive and negative buffer controls are utilized to determine the migration pattern of the linear and looped bands. (c) miR-206 detection from 500ng of total RNA extracted from differentiated myoblast cells. No miR-206 is seen in the undifferentiated totalRNA. Positive and negative buffer controls are utilized to determine the migration pattern of the linear and looped bands. Negative controls in undifferentiated and differentiated totalRNA ensure upper banding isn’t seen in lanes without the nanoswitch (d) Limit of detection of miR-206 in small RNA and total RNA extractions. (e) Detection of all four miRNAs from 500ng of totalRNA extracted from differentiated myoblast cells.
References


