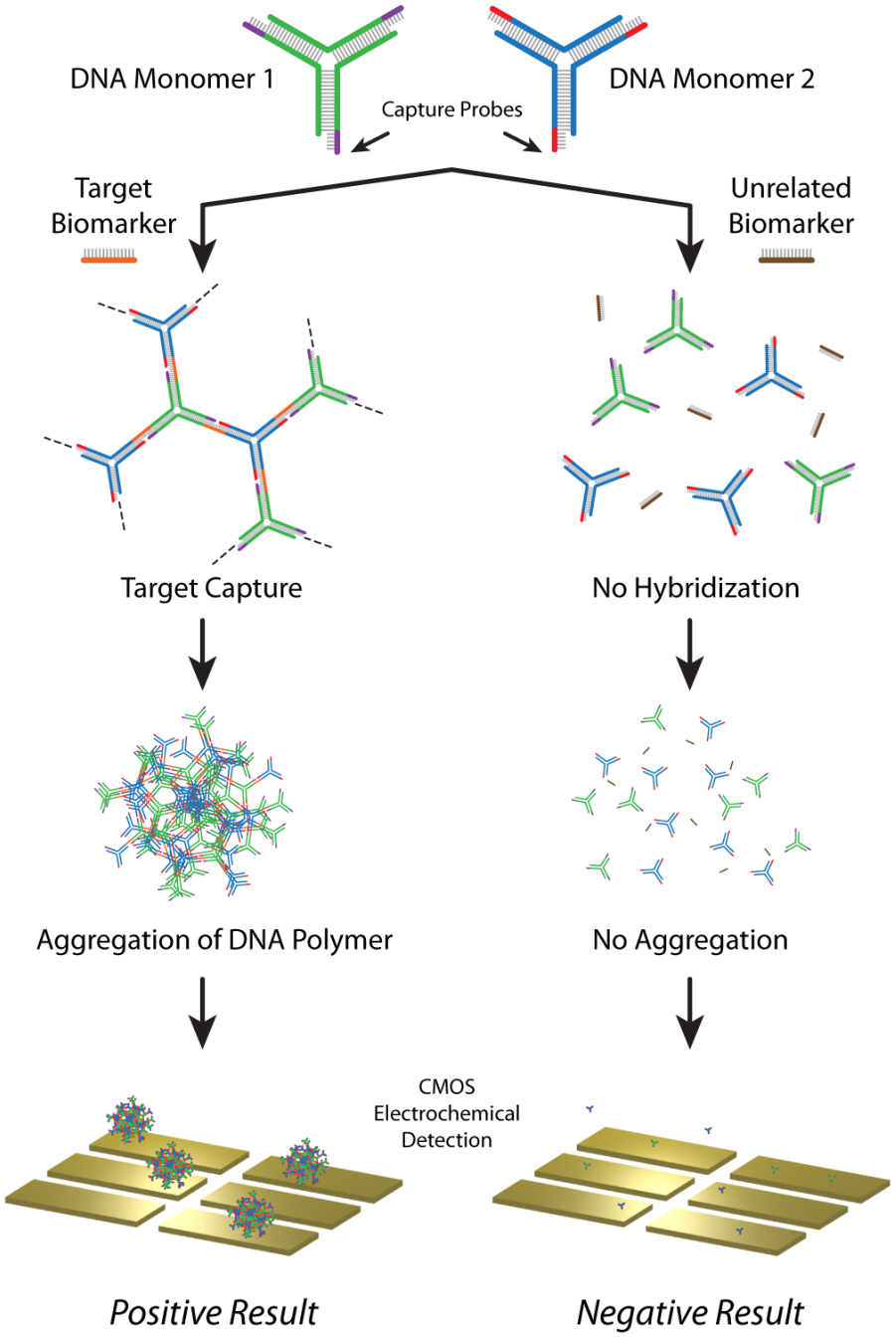


Figure 1: Overall scheme as proposed in 2011. DNA nanostructures are prepared with capture probes, which can recognize and bind to pathogen target biomarkers.

Recognition: In the presence of a pathogen biomarkers, these DNA nanostructures are linked together to form aggregates.

Amplification: The large size of these aggregates effectively amplifies the signal from the pathogen target biomarker. In the absence of pathogen target, the DNA nanostructures remain disassociated.

Detection: These aggregates can then be detected electrochemically.

Task	Status/Deadline
Objective 1: Apply our DNA nanobarcode system (as shown in Figure 1) to detect a panel of common plant pathogens with high sensitivity and specificity.	
Design and validate probe sequences	Completed
Prepare DNA nanostructures with capture probes	Completed
Demonstrate detection of model viruses under ideal conditions	Completed
*Evaluate sensitivity & specificity of assay	Cancelled
Objective 2: Integrate the sample preparation and signal readout modules into the platform and evaluate the robustness of our technology by testing with real plant tissue samples.	
Demonstrate extraction and detection of model viruses from infected plant tissue	March 2013
Demonstrate detection of fluorescently-labeled microbeads in portable format	Cancelled
**Demonstrate enzymatic amplification of realistic pathogen samples (Figure XXX)	July 2013
Evaluate the accuracy and robustness of test under real world operating conditions	Sept 2013

Figure 2: Research progress and timeline.
* In 2012 we decided it was unnecessary to continue testing with synthetic targets; instead we moved on to work with realistic samples.
**New task (not in original proposal). This replaces the task above, which was ruled out after initial testing as shown in Figure 5. Enzymatic amplification will enable great increases in sensitivity and feasibility with realistic sample types. Some success with this approach has already been demonstrated; see Figure 11.

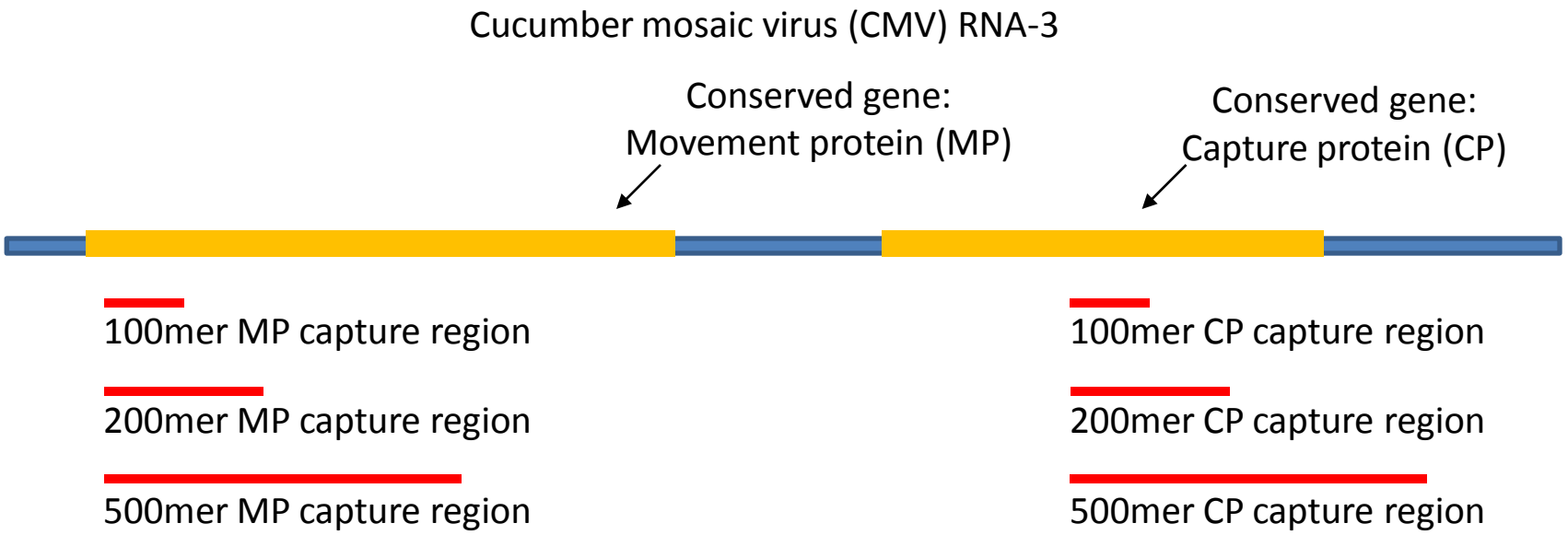
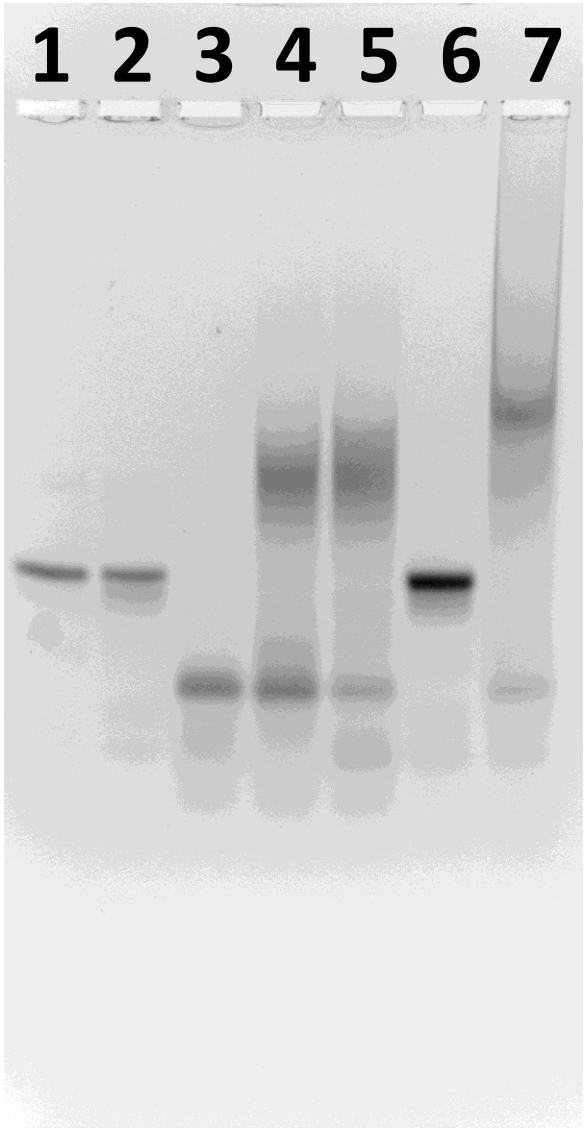
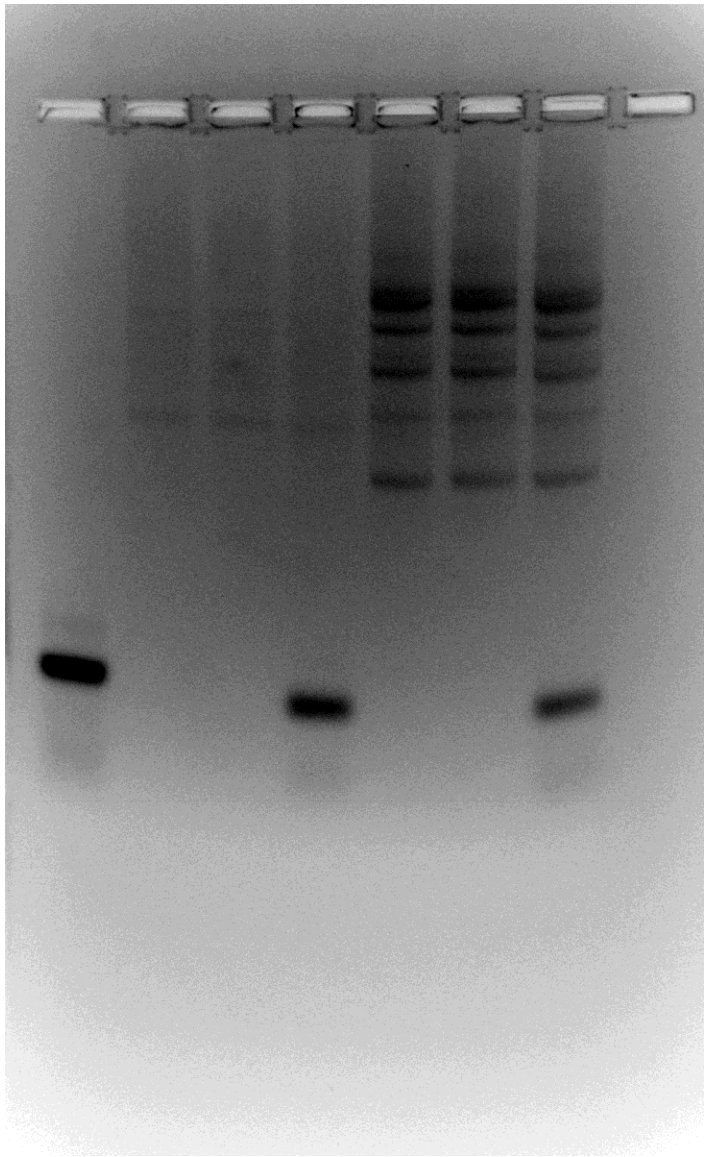


Figure 3: Sequences used as model pathogen for testing. Genomic RNA sequence from cucumber mosaic virus (CMV) was selected as a model pathogen nucleic acid sequence. Total length of sequence is 2216 b, from Fny strain (GenBank accession number NC_001440.1). RNA-3 includes the conserved regions for movement protein (MP) and coat protein (CP). We tested various lengths of this sequence including: (1) entire RNA-3 sequence; (2) 500mer capture region; (3) 200mer capture region; (4) 100mer capture region.



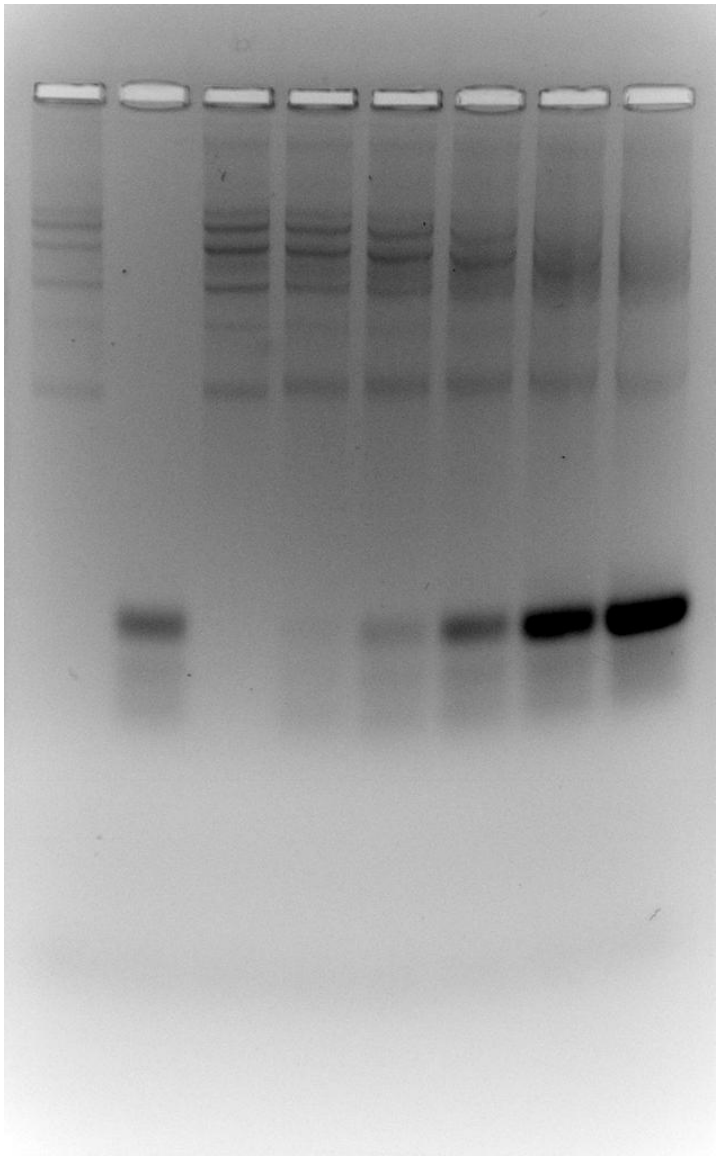
Lane	Sample	Schematic representation
1	YDNA 1	
2	YDNA 2	
3	Target	
4	YDNA 1 + Target	
5	YDNA 2 + Target	
6	YDNA 1 + YDNA 2	
7	YDNA 1 + YDNA 2 + Target (aggregate)	

Figure 4: Preliminary demonstration of aggregation using 100mer synthetic DNA targets. The long smear in Lane 7 indicates aggregation, which occurs only in the presence of target pathogen DNA sequence. Gel electrophoresis demonstrates that DNA nanostructures recognize and bind to pathogen DNA as expected.



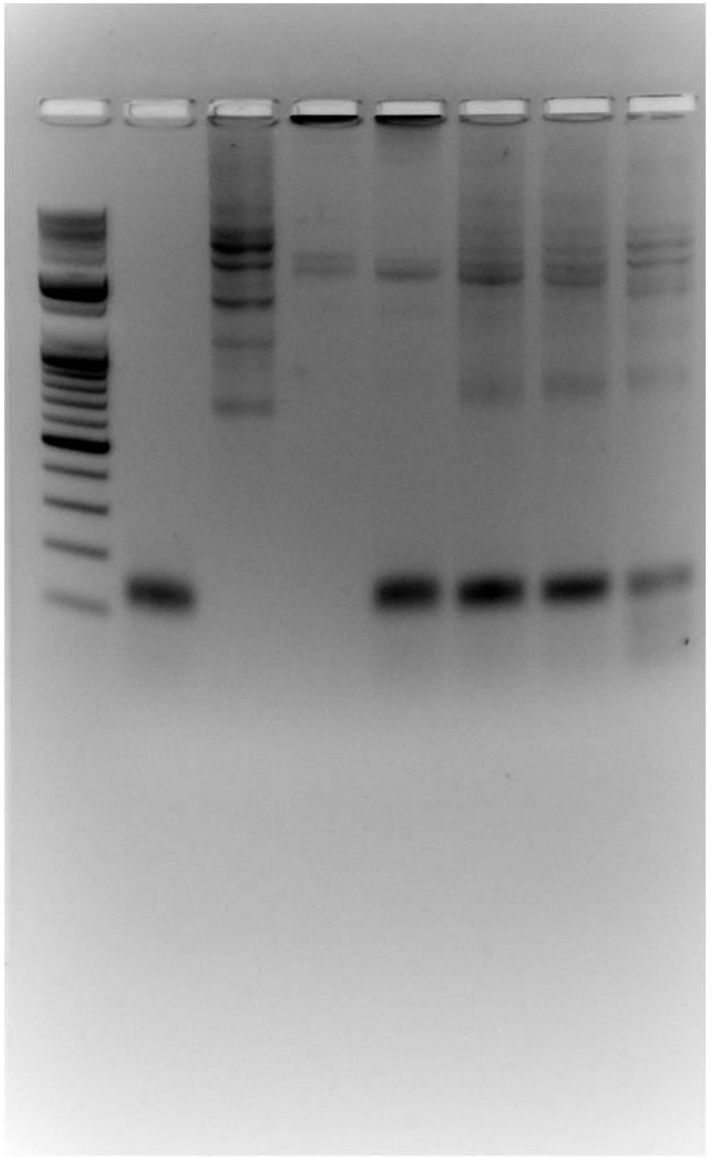
Lane	Sample
1	YDNA alone
2	RNA-1 alone - fresh
3	RNA-1 alone - after incubation
4	RNA-1 + YDNA (all probes)
5	RNA-3 alone - fresh
6	RNA-3 alone - after incubation
7	RNA-3 + YDNA (all probes)

Figure 5: Initial test of 2000 b CMV genomic RNA-3 target (GenBank accession number NC_001440.1). YDNA was mixed with RNA-3 target and incubated at room temperature for one hour. Electrophoresis results indicated no clear aggregation occurred under any conditions. RNA-1 target, also derived from CMV, was also included as a negative control.



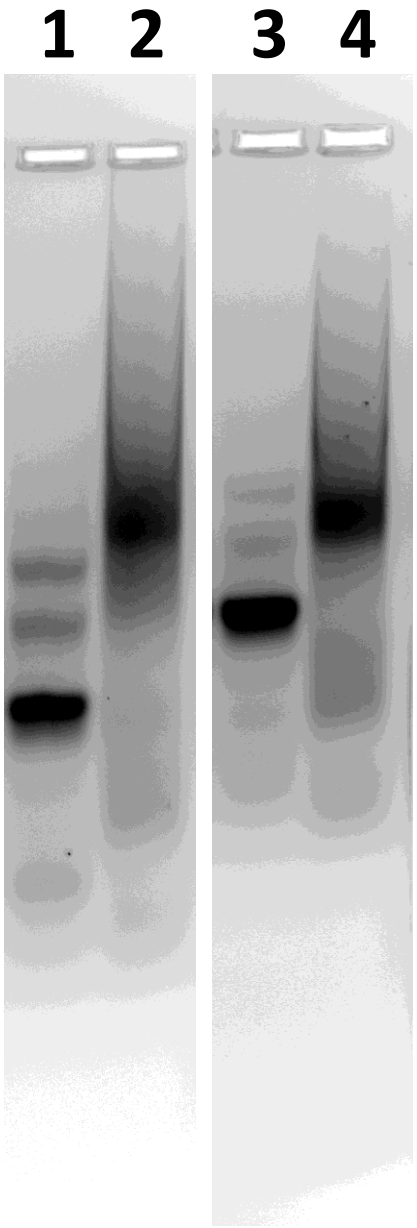
Lane	Sample
1	RNA-3 alone – fresh
2	YDNA only
3	RNA-3 alone - after incubation
4	RNA:YDNA 4:1 - after incubation
5	RNA:YDNA 2:1 - after incubation
6	RNA:YDNA 1:1 - after incubation
7	RNA:YDNA 1:2 - after incubation
8	RNA:YDNA 1:4 - after incubation

Figure 6: Variation of YDNA:ssRNA ratio. YDNA concentration was increased with fixed ssRNA amount. YDNA was mixed with RNA-3 target and annealed from 65^{o}C. Electrophoresis results indicated no clear aggregation occurred under any conditions. Increasing YDNA amount led to ssRNA bands becoming less distinct. This may suggest that some non-specific interactions occurred between YDNA and ssRNA.



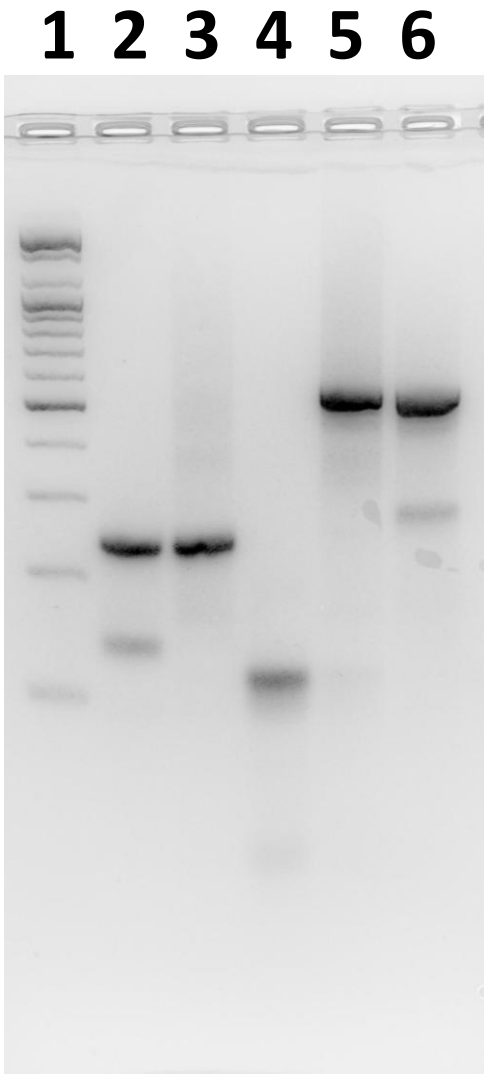
Lane	Sample
1	Ladder
2	YDNA only
3	RNA-3 alone – fresh
4	RNA-3 alone, 50 mM Mg^{2+}, after annealing
5	RNA-3 + YDNA,50 mM Mg^{2+}, after annealing
6	RNA-3 + YDNA, 25mM Mg^{2+}, after annealing
7	RNA-3 + YDNA, 12.5mM Mg^{2+}, after annealing
8	RNA-3 + YDNA, no Mg^{2+}, after annealing

Figure 7: Varying Mg2+ concentration. YDNA was mixed with RNA-3 target with the indicated amount of Mg^{2+} and annealed from 65^{o}C. Electrophoresis results showed no clear aggregation occurred under any conditions. 50 mM Mg^{2+} concentration led to ssRNA stuck in well (even without YDNA). 25 and 12.5 mM Mg^{2+} concentration led to some RNA bands smearing and spreading.



Lane	Sample
1	RNA100mer target from CP + YDNA
2	DNA100mer target from CP + YDNA
3	RNA100mer target from MP + YDNA
4	DNA100mer target from MP + YDNA

Figure 8: Comparing 100mer RNA and 100mer DNA targets. Vary reaction components in hybridization mixture. Incubation at 37^{o}C for 1 hour. Mixture results in 3 bands: YDNA, transcript + 1 YDNA, transcript + 2 YDNA. Results in indicated that RNA gave poor detection results compared with DNA.



Lane	Sample
1	Ladder
2	200mer alone
3	200mer + YDNA (0.33 pmol of YDNA)
4	YDNA alone (2 pmol of YDNA)
5	500mer + YDNA (0.26 pmol of YDNA)
6	500mer alone

Figure 9: Aggregation using 200mer and 500mer nucleic acid. Targets here are single-stranded DNA targets from the CP region of CMV RNA-3. Incubated ssDNA samples with YDNA for 1 hr at 37 °C. Very weak smearing evident in samples containing YDNA. Lower band corresponds to ssDNA, upper band is dsDNA.

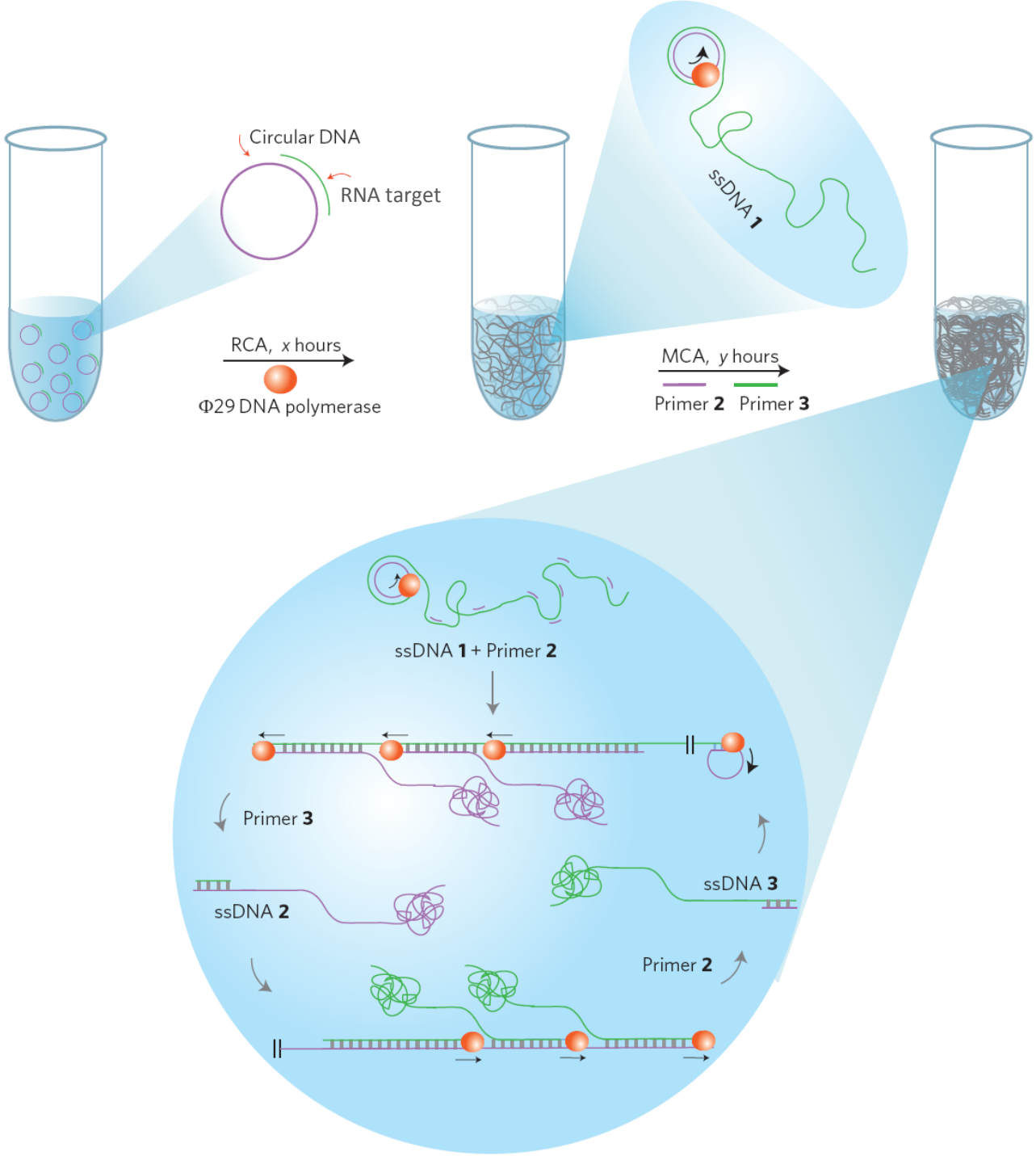
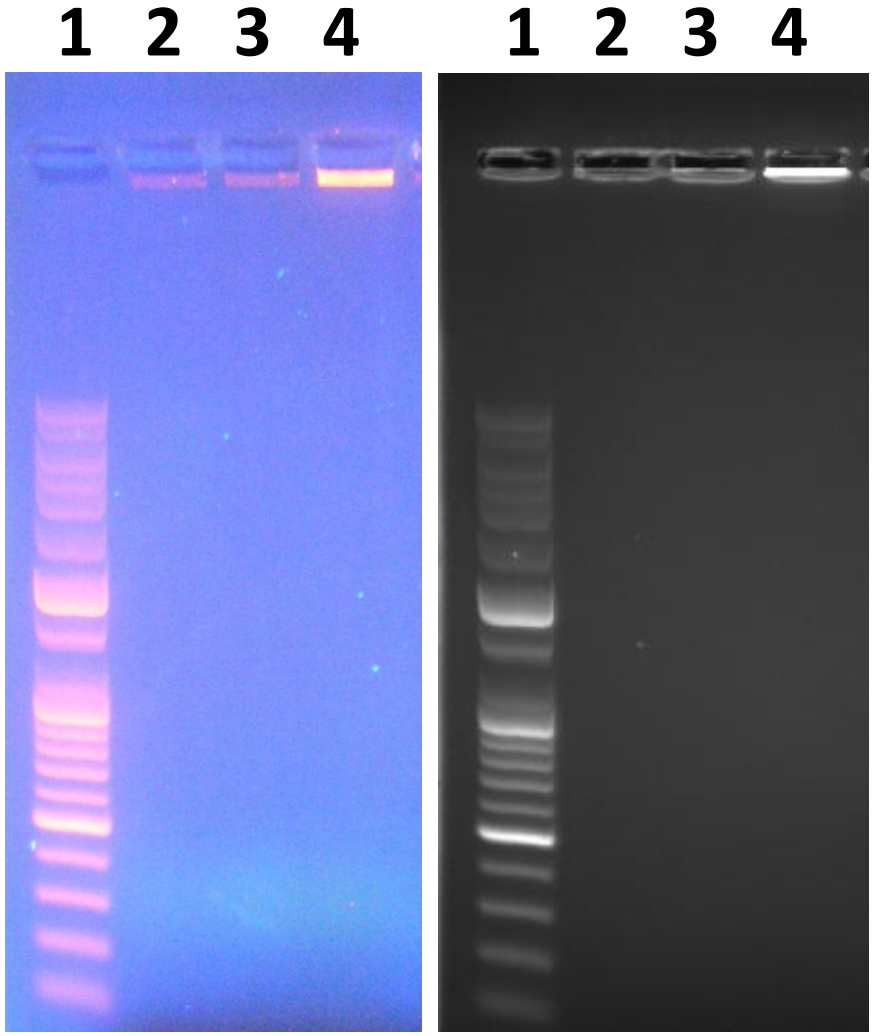


Figure 10: Schematic diagram of the stepwise approach for DNA hydrogel synthesis. The RNA target acts as a primer to rapidly amplify the amount of DNA product present in the sample. Upon addition of DNA polymerase enzyme, this RNA target is extended to produce a very long single-stranded DNA product. Additional primers are also added to the mixture further amplify the amount of DNA. The final result of this process is a very large amount of crosslinked DNA, which is produced only in the presence of the RNA target at the initial step.



Lane	Sample
1	Ladder
2	Negative control (no RNA)
3	Negative control (unrelated RNA)
4	Test sample

Figure 11: Isothermal amplification and aggregation of target RNA. Detection was carried out as described in Figure 10. Amount of target RNA for this preliminary test was 5 nanomolar concentration. Large DNA product stuck in well indicates positive result. Left image shows color photo, right image shows grayscale image, both under UV illumination.