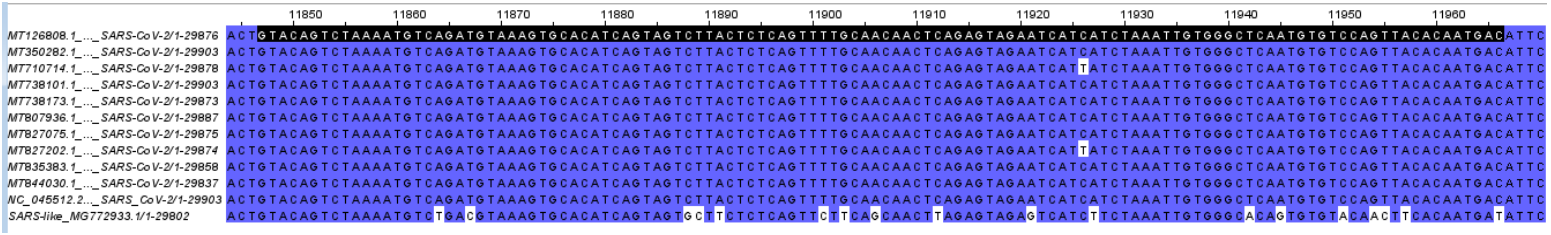
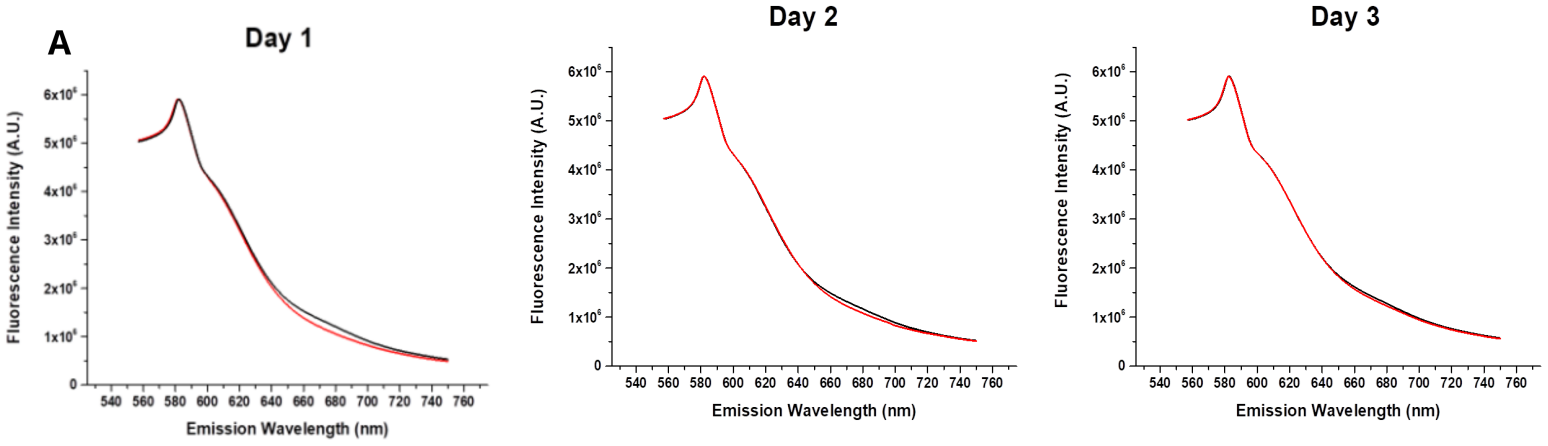
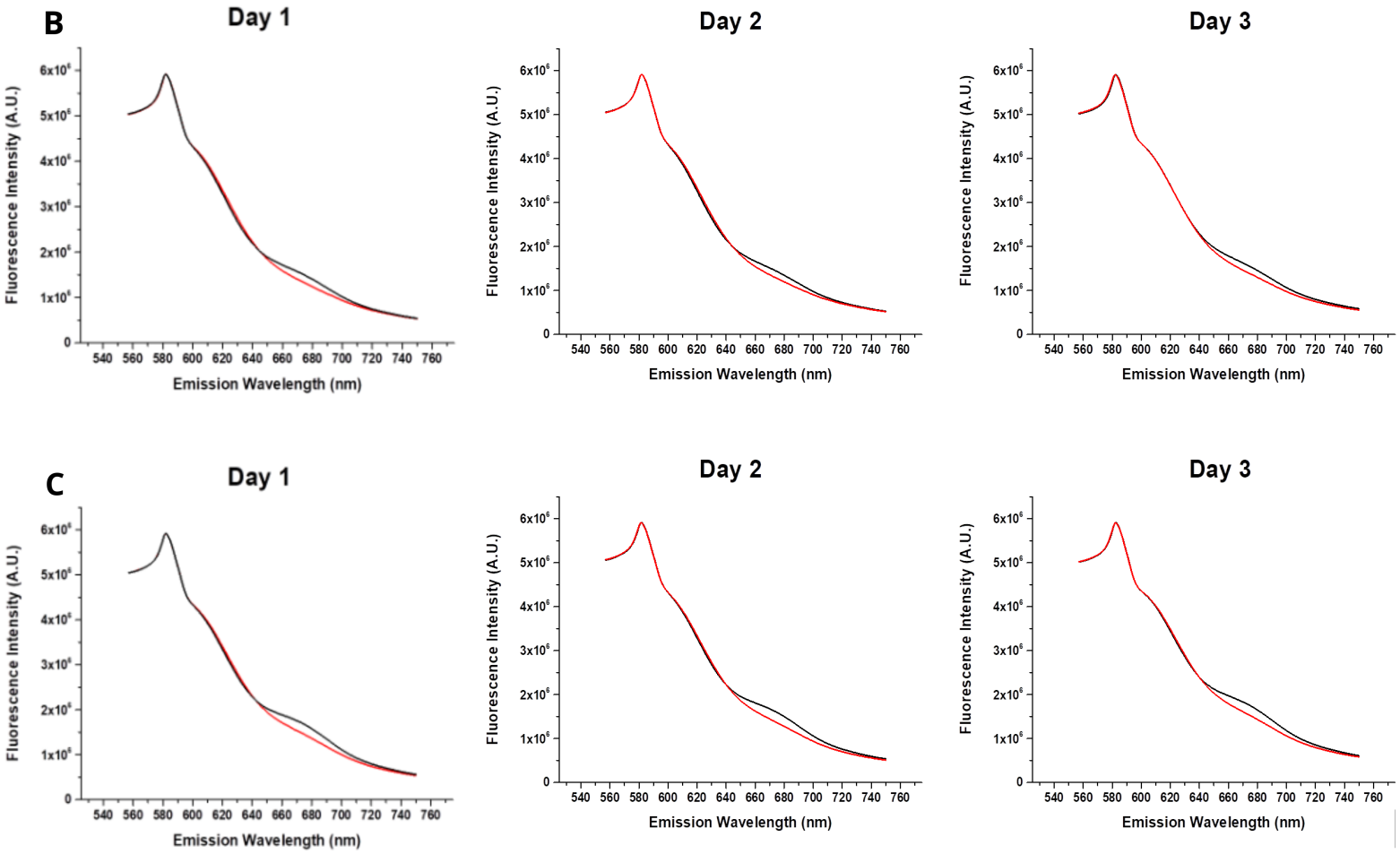
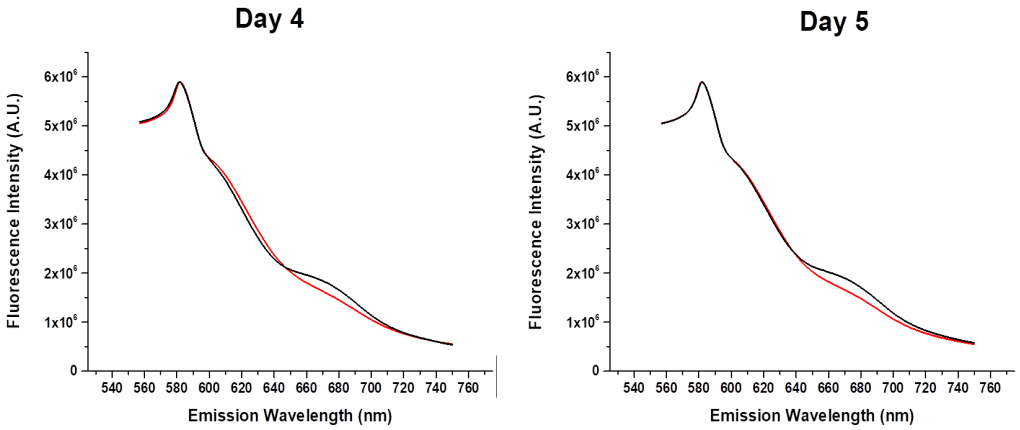
Supplementary figures



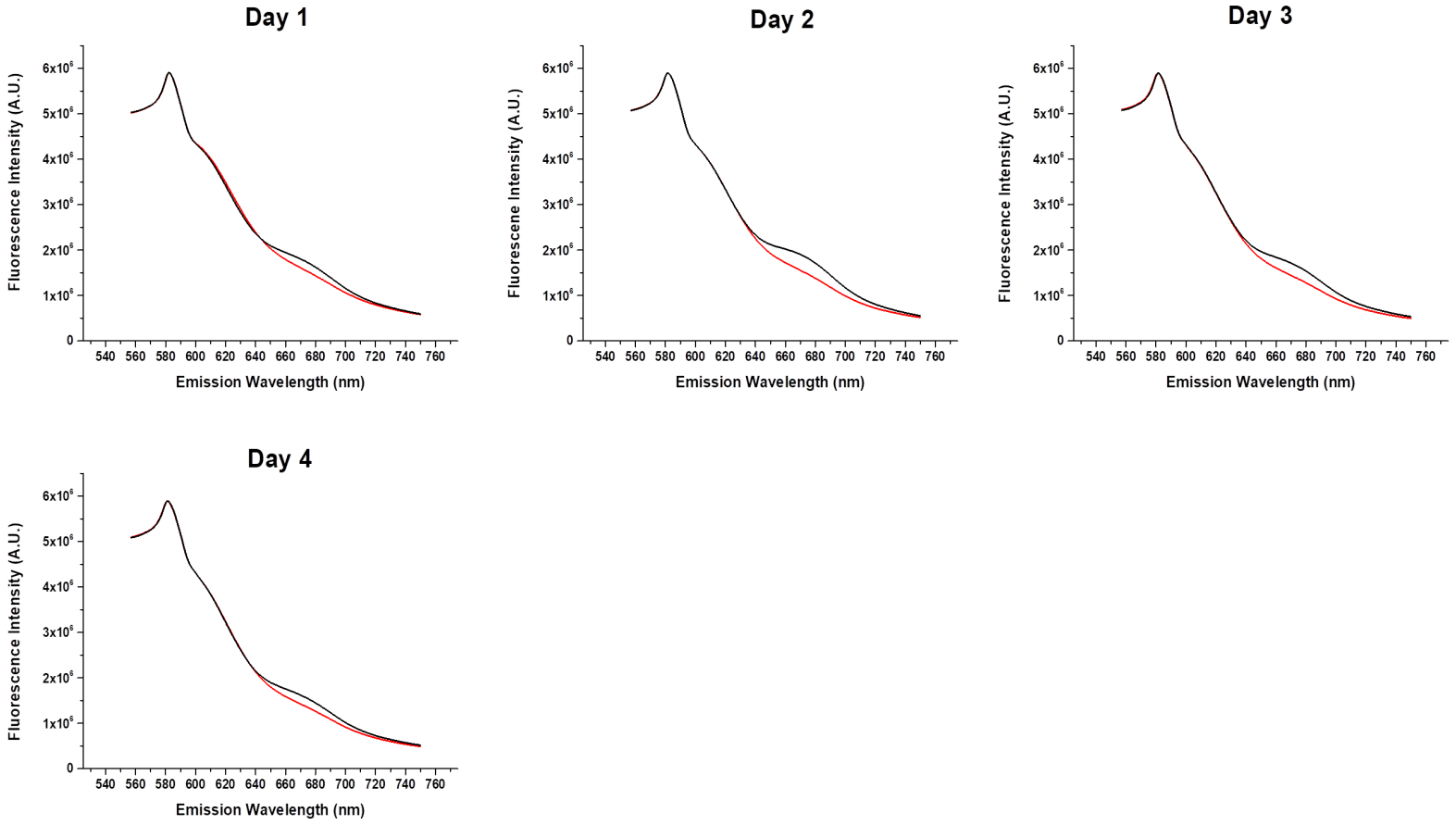
Supplementary Figure 1: Alignment of 10 different variants of SARS-CoV-2 and SARS-like genomes using the MAFFT open access software. Black line indicates the sequence used as a template for ribozyme synthesis as well as their cleavage site and white dots indicate SNP.



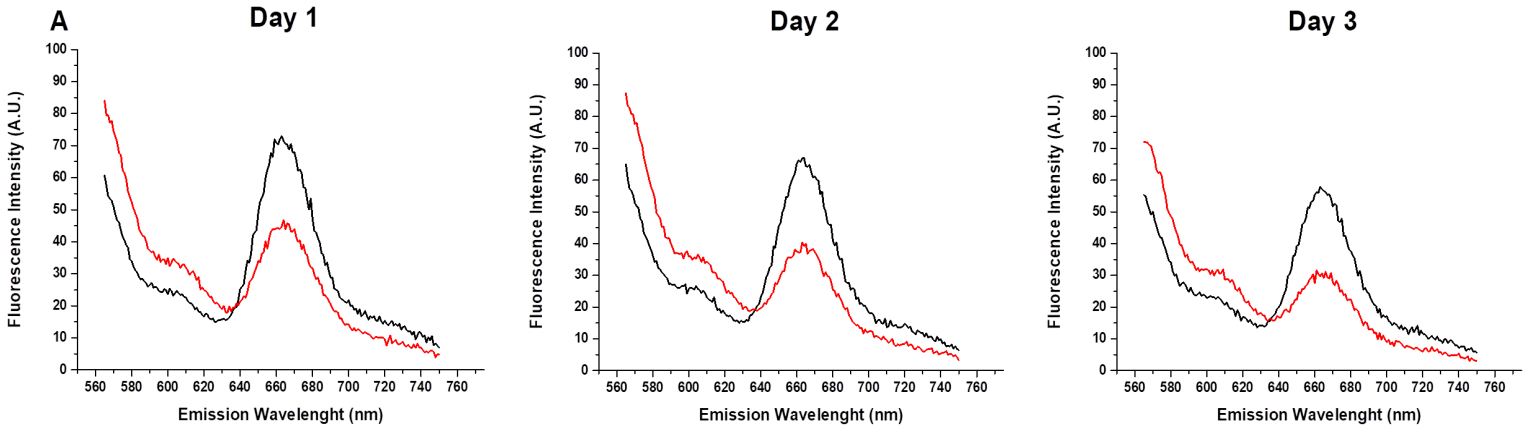

B
Day 1
Day 2
Day 3
Fluorescence Intensity (A.U.)
Emission Wavelength (nm)
C
Day 1
Day 2
Day 3
Fluorescence Intensity (A.U.)
Emission Wavelength (nm)

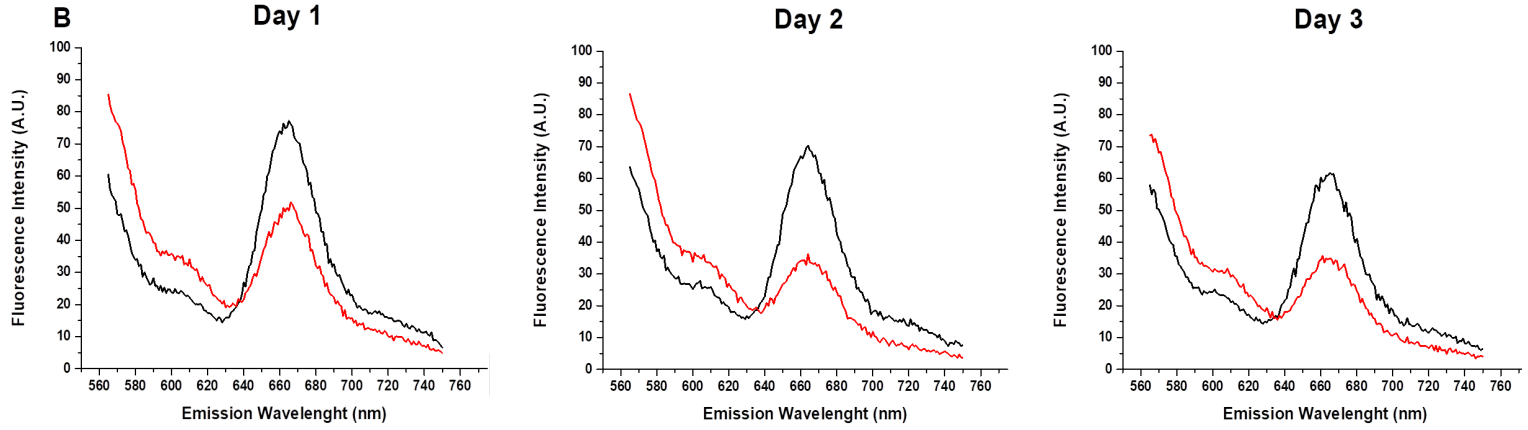


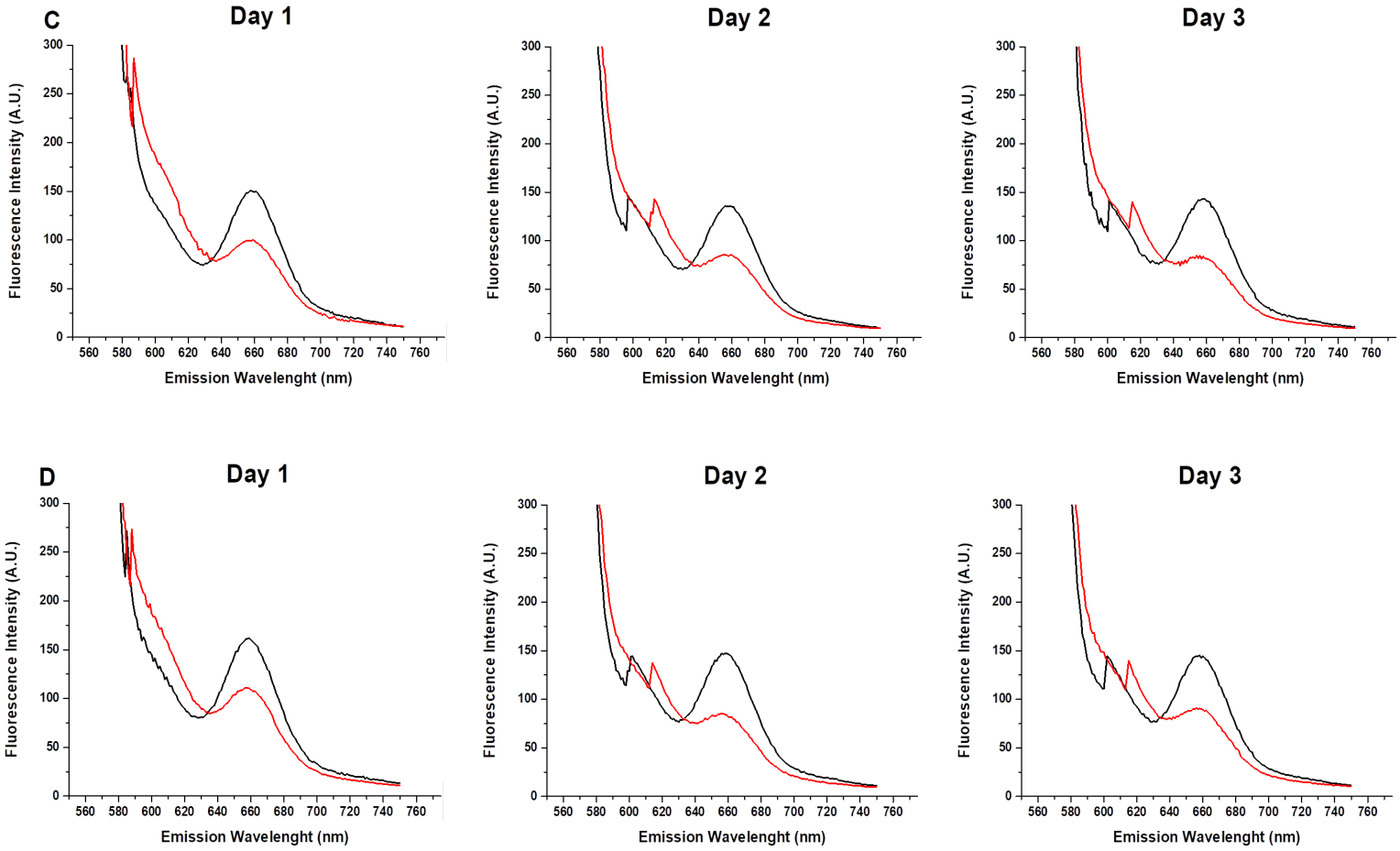
Supplementary Figure 2- Fluorescence intensity emission spectra of fluorescence resonance energy transfer (FRET-HCR) from H1 (600 nM) to H2 (600 nM) due to the hybridization chain reaction at 200 nM (**A**), 300 nM (**B**) and 600 nM (**C**) of the 18 bp initiator DNA molecule in 5X SSCT buffer, from three to five independent days (black lines). Negative control was performed with no initiator molecule (red lines). All the assays were performed with technical duplicates.



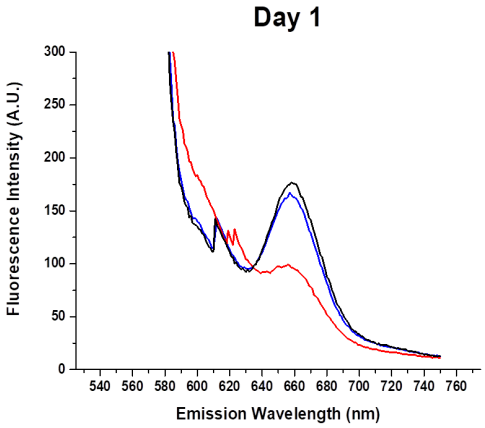
Supplementary Figure 3- Fluorescence intensity emission spectra of fluorescence resonance energy transfer (FRET-HCR) from H1 to H2 due to the hybridization chain reaction at 600 nM final concentration of H1, H2 and the 18 bp initiator DNA molecules in 5X SSC buffer, from four independent days (black lines). Red lines correspond to FRET-HCR assays performed with no initiator DNA molecule (negative control). All the assays were performed with technical duplicates.

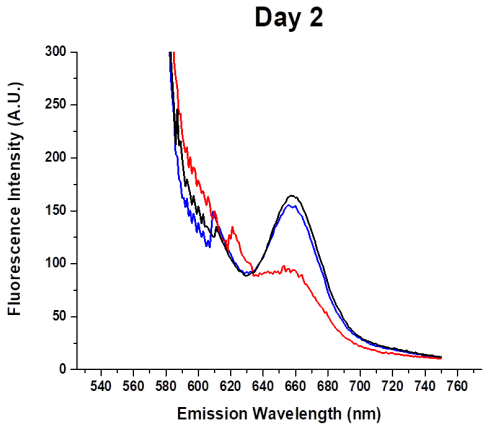

A
Day 1
Day 2
Day 3
Fluorescence Intensity (A.U.)
Emission Wavelenght (nm)
0
10
20
30
40
50
60
70
80
90
100
560
580
600
620
640
660
680
700
720
740
760

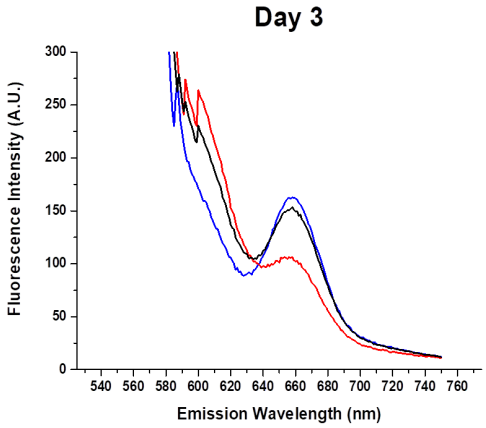

B
Day 1
Day 2
Day 3
Fluorescence Intensity (A.U.)
Emission Wavelenght (nm)
0
10
20
30
40
50
60
70
80
90
100
560
580
600
620
640
660
680
700
720
740
760

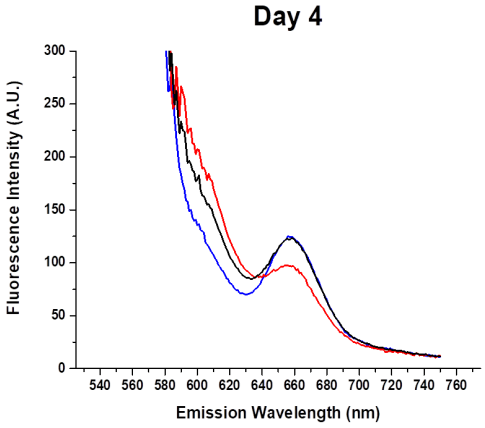


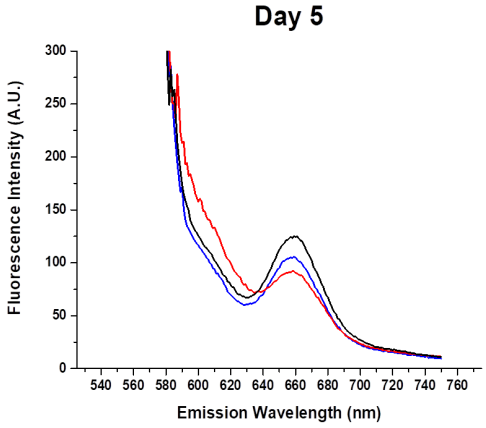
Supplementary Figure 4- Fluorescence emission intensity spectra from energy transfer between H1 (600 nM) and H2 (600 nM) due to the hybridization chain reaction at 6000 nM (**A** and **C**) and 600 nM (**B** and **D**) of the 18 bp initiator DNA in 5X SSC buffer, from three independent days (black lines). (**A**) and (**B**) fluorescence spectra obtained in Varioskan and (**C**) and (**D**) fluorescence spectra obtained in SpectraMax spectrofluorimeters. As negative control there was no initiator molecule (red line).

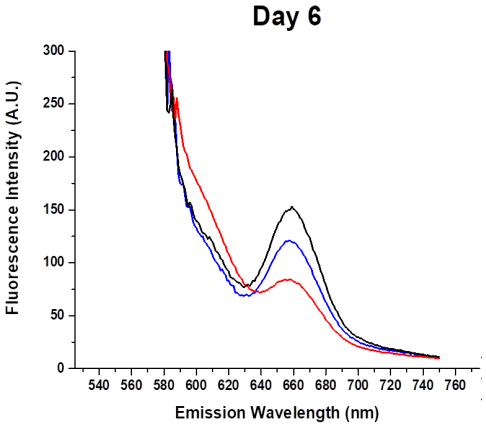

Day 1
Fluorescence Intensity (A.U.)
0
50
100
150
200
250
300
540 560 580 600 620 640 660 680 700 720 740 760
Emission Wavelength (nm)

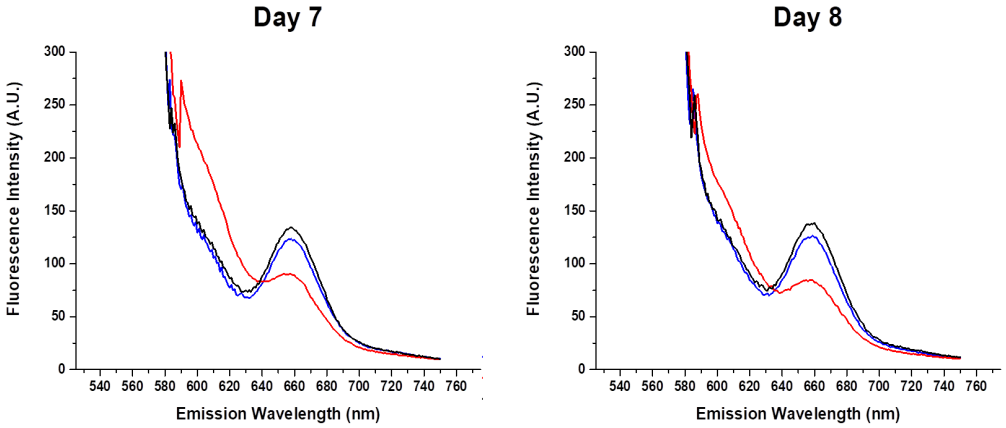

Day 2
Fluorescence Intensity (A.U.)
0
50
100
150
200
250
300
540 560 580 600 620 640 660 680 700 720 740 760
Emission Wavelength (nm)


Day 3
Fluorescence Intensity (A.U.)
0
50
100
150
200
250
300
540 560 580 600 620 640 660 680 700 720 740 760
Emission Wavelength (nm)

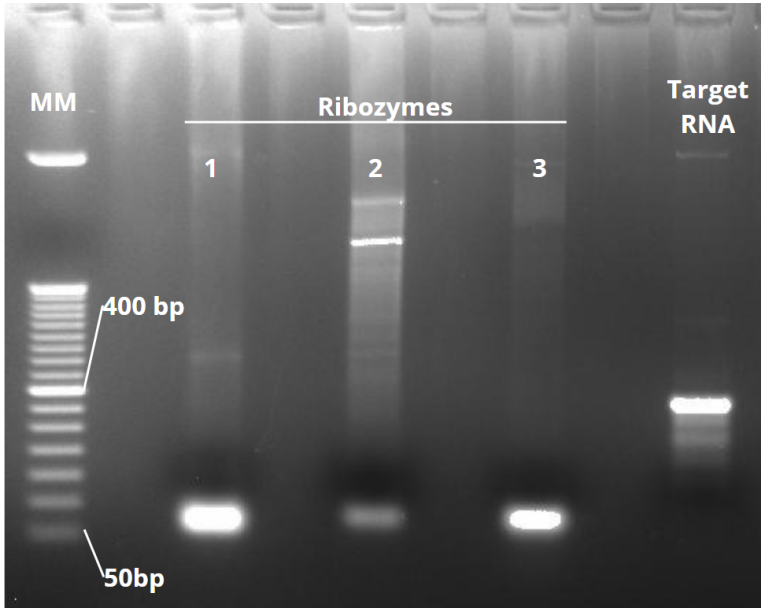

Day 4
Fluorescence Intensity (A.U.)
0
50
100
150
200
250
300
540 560 580 600 620 640 660 680 700 720 740 760
Emission Wavelength (nm)


Day 5
Fluorescence Intensity (A.U.)
0
50
100
150
200
250
300
540 560 580 600 620 640 660 680 700 720 740 760
Emission Wavelength (nm)

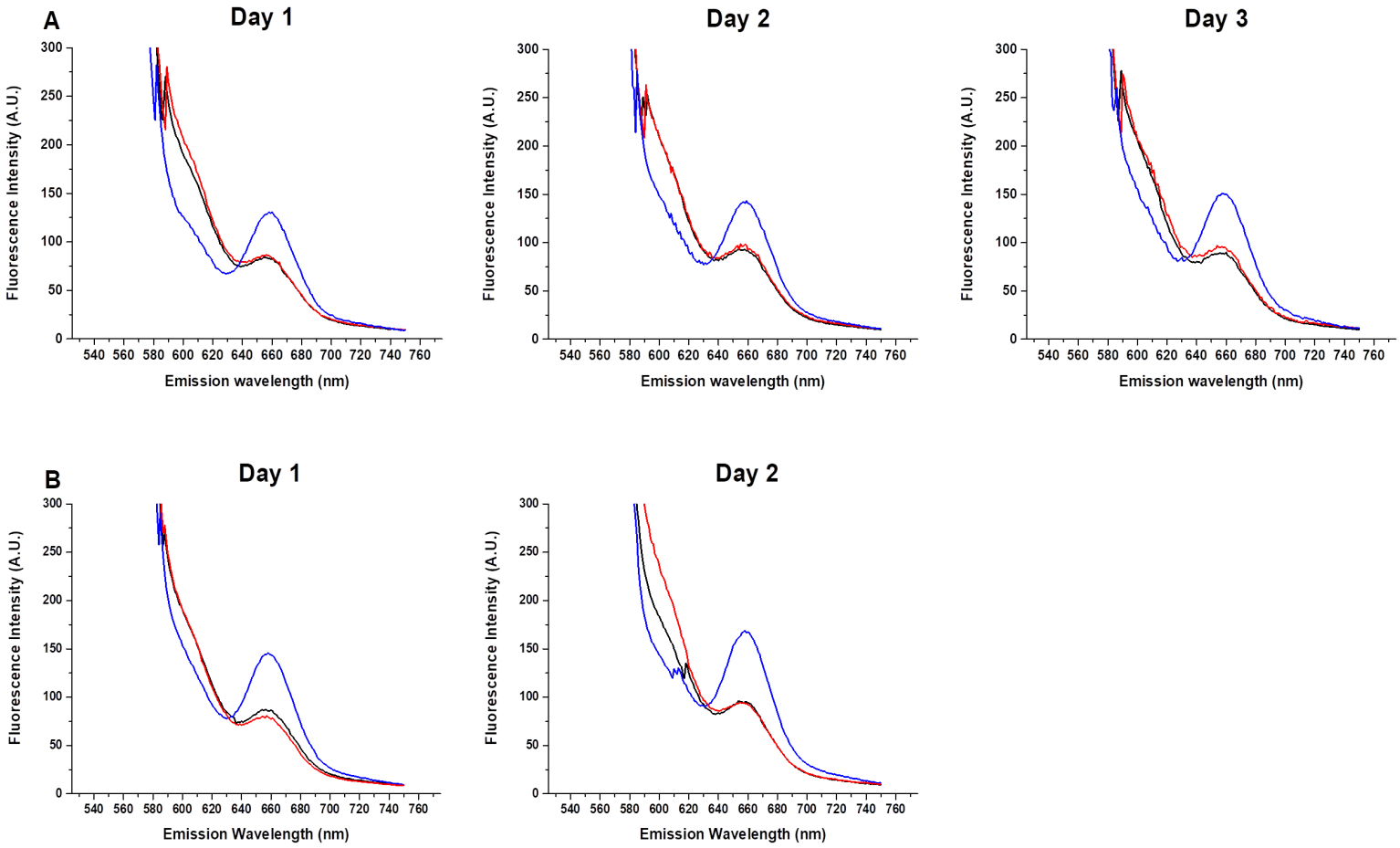

Day 6
Fluorescence Intensity (A.U.)
0
50
100
150
200
250
300
540 560 580 600 620 640 660 680 700 720 740 760
Emission Wavelength (nm)



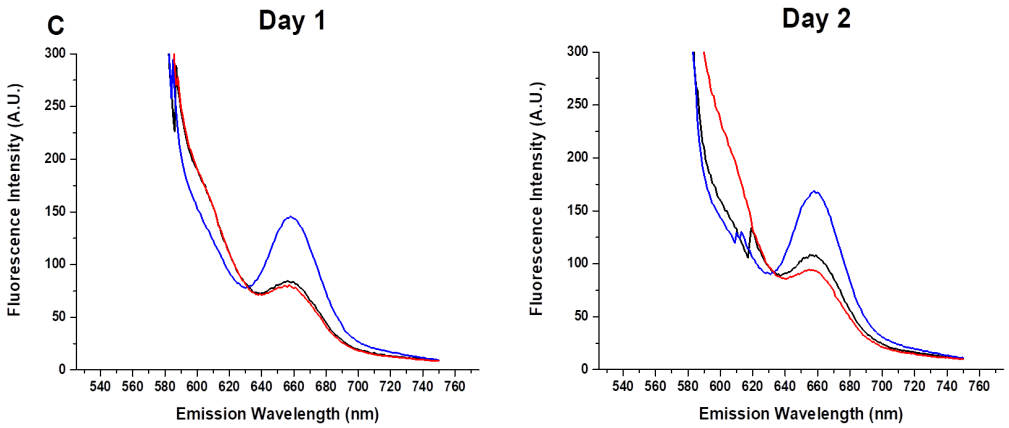
Supplementary Figure 5- Fluorescence emission intensity spectra from energy transfer between H1 and H2 due to the hybridization chain reaction at 600 nM of H1, H2 and the 18 bp initiator RNA molecule, also at 600 nM final concentration, in 5X SSC buffer from eight independent days (black lines). All the assays were performed with technical duplicates. Positive control consisting of 18 bp initiator DNA at the same concentration as the 18 bp initiator RNA molecule (600 nM) is indicated as the blue line. Negative control without an initiator molecule is indicated as the red line.



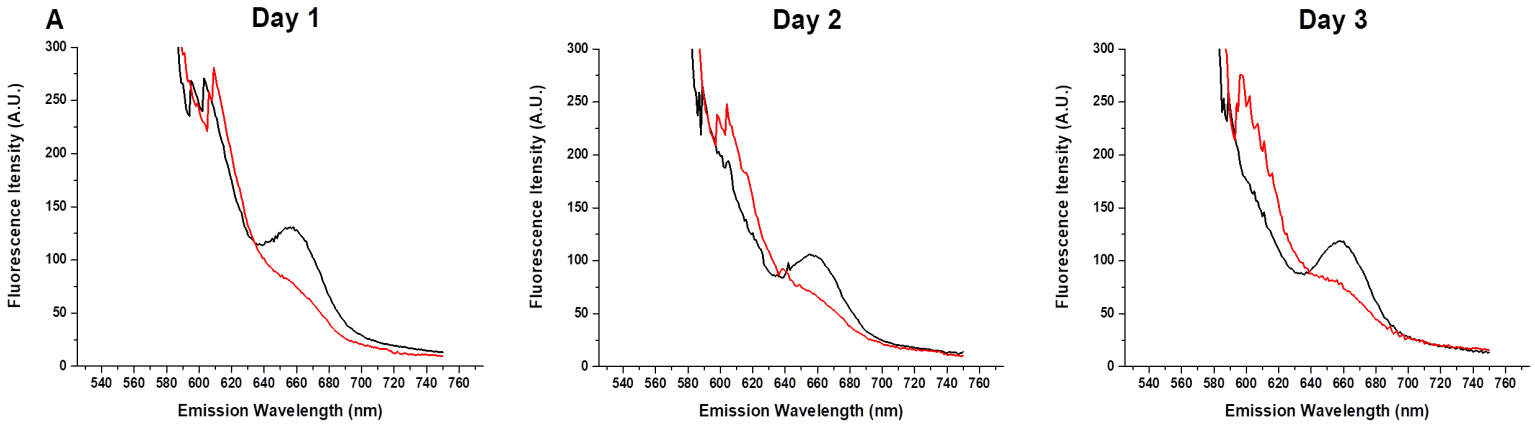
Supplementary Figure 6: Target RNA and ribozymes transcribed in vitro in 2% agarose gel electrophoresis . The target RNA is approximately 400 nt and the ribozymes are approximately 60 nt. Invitrogen 50 bp DNA Ladder molecular marker.


A
Day 1
Day 2
Day 3
Fluorescence Intensity (A.U.)
Emission wavelength (nm)
0
50
100
150
200
250
300
540 560 580 600 620 640 660 680 700 720 740 760

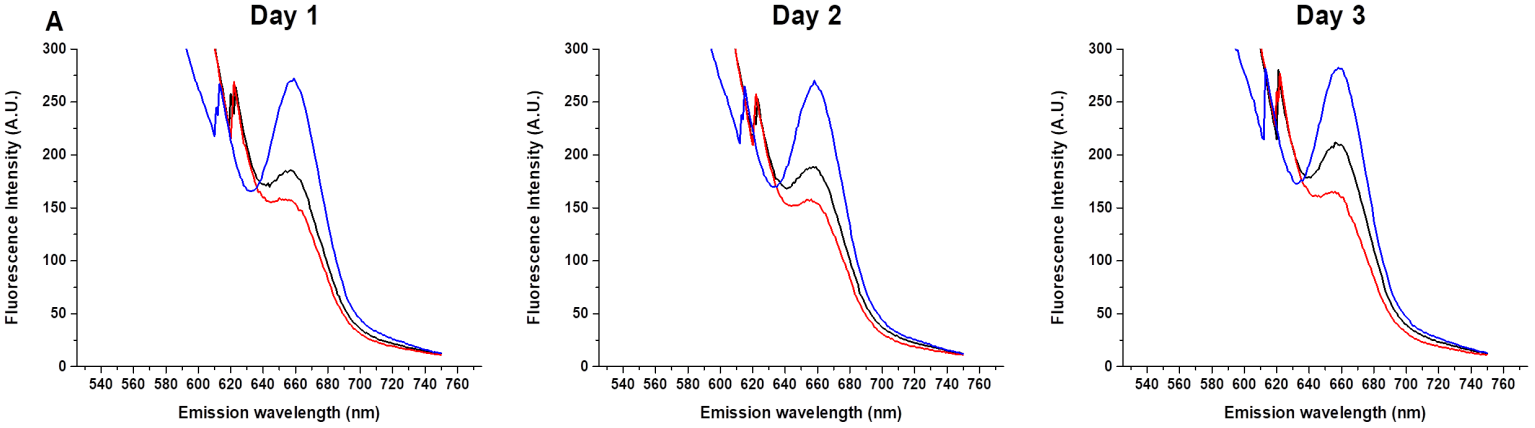
B
Day 1
Day 2
Fluorescence Intensity (A.U.)
Emission Wavelength (nm)
0
50
100
150
200
250
300
540 560 580 600 620 640 660 680 700 720 740 760



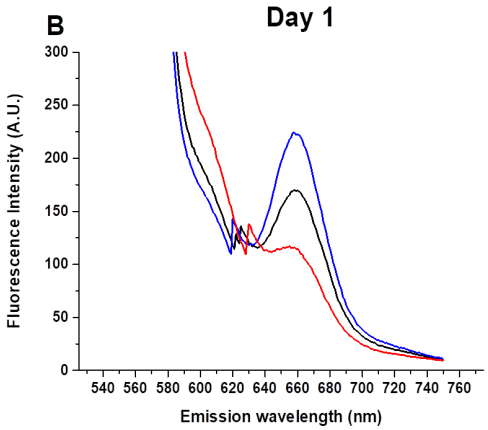
Supplementary Figure 7- Fluorescence emission intensity spectra from energy transfer between H1 and H2 due to the hybridization chain reaction at 600 nM of H1, H2 and target RNA or ribozyme cleaved products in a 2:1:1 ratio in 5X SSC buffer. All the assays were performed with technical duplicates. FRET-HCR assay coupled with intact 400 bp target RNA (**A**), cleaved products of ribozyme 1(**B**), and cleaved products of ribozyme 3 (**C**). The **A** assay was performed on three independent days and **B** and **C** assays were performed on two independent days. Blue line represents the positive control, 18 bp initiator RNA molecule, the red line represents the negative control, with no target molecule, and the black line, the FRET-HCR assay followed by the ribozyme enzymatic cleavage or performed with the 400 bp intact RNA target molecule.

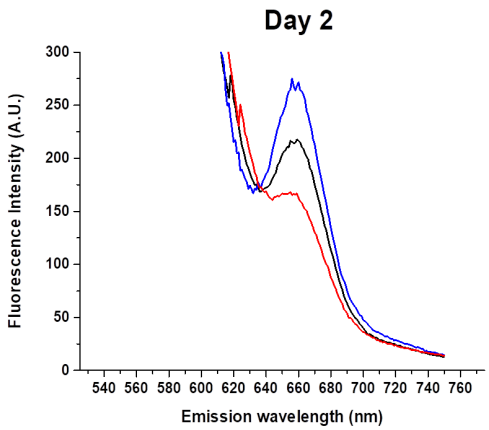


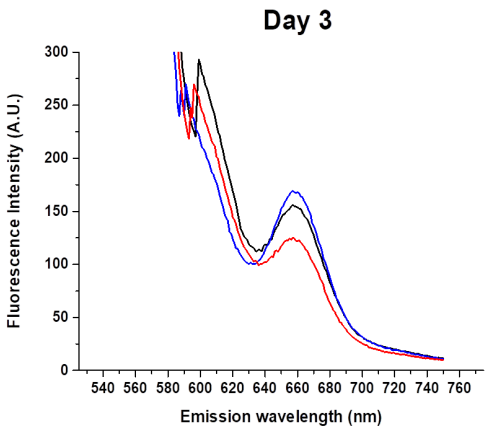
Supplementary Figure 8- Fluorescence emission intensity spectra from energy transfer between H1 and H2 due to the hybridization chain reaction at 600 nM of the second set of H1, H2 and its 18 bp initiator RNA molecule, also at 600 nM final concentration, in 5X SSC buffer from three independent days shown separately and as an average (black lines). All the assays were performed with technical duplicates. Negative control without an initiator molecule is indicated as the red line.



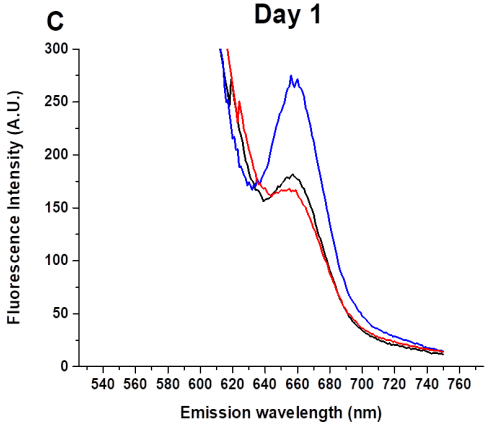
B

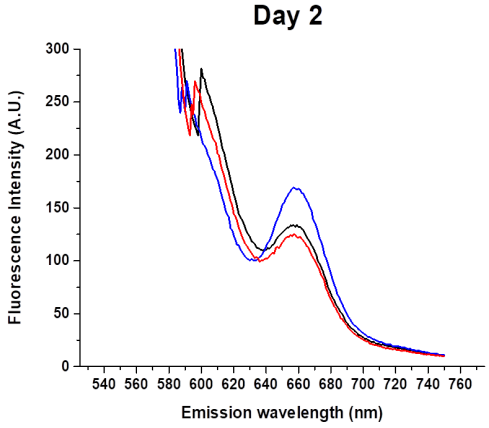

Day 1
Fluorescence Intensity (A.U.)
0
50
100
150
200
250
300
540 560 580 600 620 640 660 680 700 720 740 760
Emission wavelength (nm)

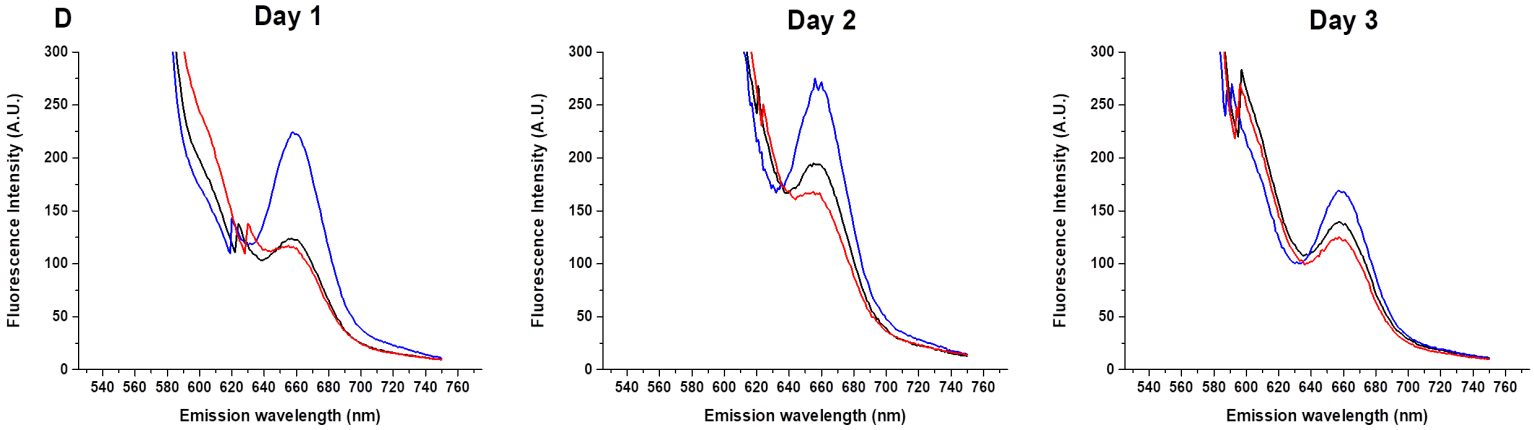

Day 2
Fluorescence Intensity (A.U.)
0
50
100
150
200
250
300
540 560 580 600 620 640 660 680 700 720 740 760
Emission wavelength (nm)


Day 3
Fluorescence Intensity (A.U.)
0
50
100
150
200
250
300
540 560 580 600 620 640 660 680 700 720 740 760
Emission wavelength (nm)

C


Day 1
Fluorescence Intensity (A.U.)
0
50
100
150
200
250
300
540 560 580 600 620 640 660 680 700 720 740 760
Emission wavelength (nm)


Day 2
Fluorescence Intensity (A.U.)
0
50
100
150
200
250
300
540 560 580 600 620 640 660 680 700 720 740 760
Emission wavelength (nm)



Supplementary Figure 9- Fluorescence emission intensity spectra from energy transfer between H1 and H2 due to the hybridization chain reaction at 600 nM of H1, H2 and target RNA or ribozyme cleaved products with ratio of 2:1:1 in 5X SSC buffer. All the assays were performed with technical duplicates. The assays **A**, **B** and **D** were performed on three independent days and assay **C** was performed on two independent days. Blue line represents the positive control, two sets of 18 bp initiator RNA molecules, the red line represents the negative control, with no target molecule, and the black line, the FRET-HCR assay followed by the ribozyme enzymatic cleavage or performed with the intact 400 bp RNA target molecule. These FRET-HCR assays were performed with 2 sets of H1 and H2 and the 400 bp intact RNA target **(A)**; the 2 sets of H1 and H2 and the cleavage product of ribozyme 1 **(B)**; the 2 sets of H1 and H2 and the cleaved product of ribozyme 3 **(C)**; the 2 sets of H1 and H2 and the cleaved products of ribozymes 1 and 3 **(D)**.