Transcription of Hammerhead Ribozyme and Spinach Aptamer to Measure with Fluorescence

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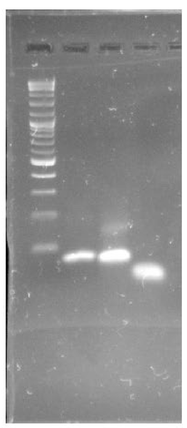
ABSTRACT

In our investigations, we utilize in vitro transcription from a DNA template, typically initiated from a promoter site such as TATA, to generate the specific RNA substrates required for detailed kinetic studies of hammerhead ribozyme activi-ty. Concurrently, our research explores the folding of the Baby Spinach aptamer.Hammerhead ribozymes, small self-cleaving RNA molecules, hold significant promise in genetic therapy due to their intrinsic catalytic RNA-cleaving activity. A comprehensive understanding of their enzymatic mechanism is crucial for optimizing therapeutic applications and elucidating the intricate RNA cleavage process. These ribozymes achieve site-specific cleavage of target RNA sequences, making them valuable tools for precise gene regulation and innova-tive antiviral strategies.The Baby Spinach aptamer is a synthetically engineered RNA molecule that transforms RNA visualization in living cells. This aptamer functions as a genetically encodable, fluorogenic tag for RNA, analogous to Green Fluorescent Protein (GFP) for proteins. Its unique functionality arises from its specific interaction with the non-fluorescent ligand, 3,5-difluoro-4-hydroxybenzylidene imidazolinone (DFHBI). Upon folding into its defined three-dimensional conformation, which critically involves the formation of a G4 quadruplex—a four-stranded, guanine-rich nucleic acid structure—the Aptamer creates a precise binding pocket. This interaction significantly enhances DFHBI's quantum yield, inducing bright green fluorescence.

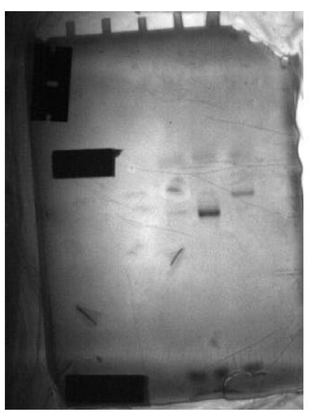
After transcription, Urea-PAGE purification and quantification by absorbance, the concentrations were 1.62μM, 3.97μM and 9.47μM for the Hammerhead Ribozyme, Hammerhead Substrate and the Baby Spinach Aptamer respectively. The concentrations were too low for a ribozyme activity. However, a single melting temperature denaturation of the Baby Spinach Aptamer-DFHBI by fluorescence was performed, leading to a Tm of 48.0ºC.The next steps would involve hammerhead kinetics as well as more baby spinach melting denaturation using chemi-cal compounds that could potentially (de)stabilize the G-Quadrupolex structure.



Conditions

1.0 mM MgCl2
10X Taq Buffer
0.4 mM dNTP
2.0 μM Primers (Foward)
2.0 μM Primers (Reverse)
Taq DNA Polymerase

PCR on Agarose Gel

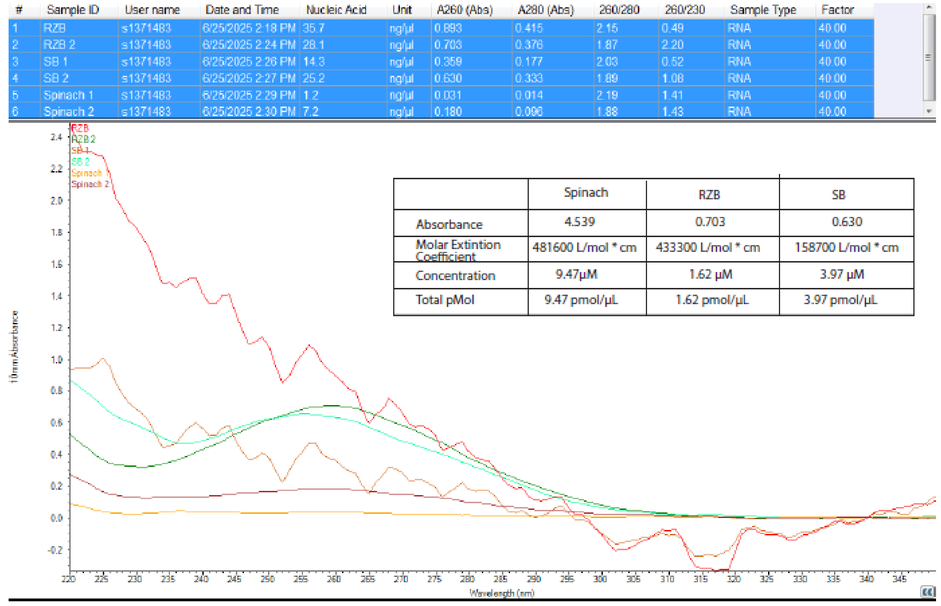


Conditions

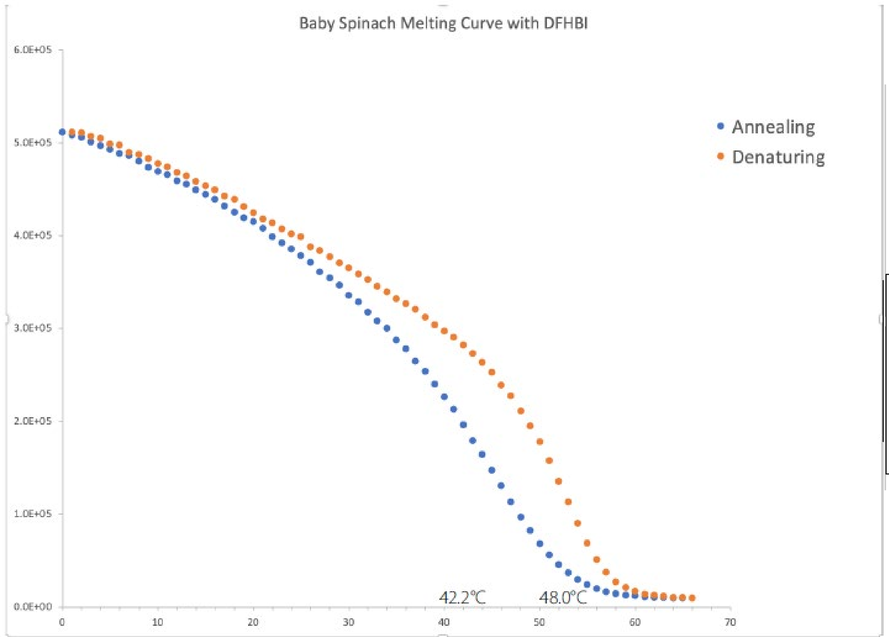
1.6X T7 RNA buffer
6.4 mM rNTP mix
4 mM DTT
2 μM 51-52 PCR DNA sample
YIPP
T7 RNA polymerase mix

TRX on PAGE Gel

#	Sample ID	User name	Date and Time	Nucleic Acid	Unit	A260 (Abs)	A280 (Abs)	260/280	260/230	Sample Type	Factor
1	RZB	s1371483	6/25/2025 2:18 PM	35.7	ng/μl	0.893	0.415	2.15	0.49	RNA	40.00
2	RZB 2	s1371483	6/25/2025 2:24 PM	28.1	ng/μl	0.703	0.376	1.87	2.20	RNA	40.00
3	SB 1	s1371483	6/25/2025 2:26 PM	14.3	ng/μl	0.359	0.177	2.03	0.52	RNA	40.00
4	SB 2	s1371483	6/25/2025 2:27 PM	25.2	ng/μl	0.630	0.333	1.89	1.08	RNA	40.00
5	Spinach 1	s1371483	6/25/2025 2:29 PM	1.2	ng/μl	0.031	0.014	2.19	1.41	RNA	40.00
6	Spinach 2	s1371483	6/25/2025 2:30 PM	7.2	ng/μl	0.180	0.096	1.88	1.43	RNA	40.00



	Spinach	RZB	SB
Absorbance	4.539	0.703	0.630
Molar Extintion Coefficient	481600 L/mol * cm	433300 L/mol * cm	158700 L/mol * cm
Concentration	9.47μM	1.62 μM	3.97 μM
Total pMol	9.47 pmol/μL	1.62 pmol/μL	3.97 pmol/μL



In Cuvvete

40.0 μM Tris HCl pH 7.5
125.0 μM KCl
2.0 μM $MgCl_2$
1μM Spinach RNA
100.0 mM DFHBI
1.25% DMSO

Fluorescence Conditions

Emission = 503 nm (bandwidth @ 2.0 nm)
Excitation = 466 nm (bandwidth @ 1.0 nm)
adjust bandwidth for fluorescents between 400,000-500,000
Open Temperature Scan
Make sure all three bokes are checked
Starting temp:
  Annealing: 2°C
  Denaturing: 65°C
Final temp:
  Annealing: 65°C
  Denaturing: 2°C
Dwell time = 0.200 second
Emission initial setting = 275,000 cps
Emission ideal initial setting: ~150,000-200,000 cps

Acknowledgements

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