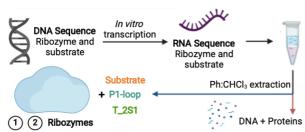
Materials and Methods RNA World and the Origin of life

1. RNA Production



General scheme of RNA production for P1-Loop, T_2S1, substrate and ribozymes sequences

In vitro replication of main sequences of sunY and *Azoarcus* mutants were carried out in an Eppendorf Tube, using the following protocol in quantities and the later the PCR program used.

All sequences were preserved at -20°C before use.

			Stock solut	ion	Unit	V (μl)		Final conc.	Unit
	Ribozyme	e template	1		ng/µL	10		0,2	ng/μL
	Bu	ffer	5		х	10		1	х
	Forward	d primer	10		μΜ	3,75		0,75	μΜ
ſ	Reverse	e primer	10		μΜ	3,75		0,75	μΜ
ſ	dNTI	P Mix	10		mM	1		0,2	mM
ſ	Polymeras	e (Phusion)	2		U/µL	0,5		0,02	U/µL
ľ	Wa	iter	-		-	21		50	
	PCR pr				Total	50			
			ogram:						
		Ste	ер 1	98 °	C	30 sec			
		Ste	ep 2	98 °C 10 sec Denat		enaturation			
	Ste		ер 3	59 °	'C	30 sec	Ar	nnealing	
		Ste	ep 4	72 °	C	30 sec	El	ongation	
	Ste		ep 5	Go t	o step 2	30 times	1		
		Ste	ep 6	72 °	C	3 min			
I		Ste Ste Ste Ste	2 2 2 2 2 2 2 3 2 2 4 2 5	98 ° 59 ° 72 ° Go t	C C C co step 2	10 sec 30 sec 30 sec 30 times	Ar	nnealing	

After PCR, 2µL of each PCR product + 1µl stopping buffer were used for the agarose gel analysis. 25 mL of 2% gel were prepared by mixing 1 tablet of TopVision Agarose Tablets, containing 0,5 g agarose, and 25 mL TAE buffer 1X. Solution was heated in a microwave until it appeared clean. The gel was pre-run 10 minutes using the same buffer, at 12 V/cm gel was left 25 min. The purpose of analysis was to determine if the PCR cycles had been successful in replicating the sequence.

Only then RNA production can begin with the usage of HiScribeTM T7 High Yield RNA Synthesis Kit which uses the T7 RNA Polymerase. Protocol was followed as indicated by the fabricant each reaction was carried out in 20 μ L. After the reaction purification was needed. It comprised several steps:

Phenol-chlorophorm extraction in acid medium:

Resuspension of the IVT solution with water (nuclease free 100µl) and transfer the solution into a 1,5 mL Eppendorf tube, under the hood 100 µL of phenol-chloroform pH 4 were add, vortex a few minutes, then centrifuge 4 minutes at 11 000 rpm, during the centrifugation, add 0.1 volume of 3M NaAc (sodium acetate) in new tubes at the end of the centrifugation, gently pipette the upper aqueous phase (containing RNAs) into the tubes containing NaAc add 2.5 volumes of cold 100% ethanol (stored at -20°C) and mix by up and down (the solution should become cloudy, RNAs precipitate quickly) let the RNAs precipitate at -20°C for 1h or overnight, centrifuge for 1h at 4°C 13 000 rpm, remove the supernatant, a white pellet should be visible. Wash the pellet with 150 µL of cold 70% ethanol (stored at -20°C) very gently (without dissolving the pellet) then centrifuge briefly and remove the supernatant. Repeat the wash a second time removing as much ethanol as possible cover the tube with parafilm (to prevent the pellet from escaping) and pierce some holes with a syringe dry the pellet in the concentrator under vacuum for a few minutes resuspend the pellet with 40 µL of water (pipette well until the pellet is dissolved)

DNAse I Treatment

We begin with 5 μ l of DNase I buffer (10X) and add 5 μ L of enzyme and incubate 15 min at 37°C immediately add 50 μ l of stopping buffer. The sample can be stored at -20°C or can be deposited directly on extraction gel RNA extraction on acrylamide (PAGE) gel. Run the 8% acrylamide (PAGE) gel and preheat the gel (24W, 15 minutes). Denature the samples by heat treatment in thermocycler (5 minutes at 75°C) migration is done until the bottom marker is at the bottom of the gel (about 3 hours, 24W). Remove the gel, cut the top right edge to make a marker, and place it between 2 plastic films, place the gel on a silicon plate, illuminate at 254 nm and mark the outline with a marker, cut the gel with a razor blade. Place the gel pieces in the sample tubes, crush with a 1mL cone the gel on the wall of the tube and add 0.5 mL of 0.3M NaAc. Incubation in Thermomix for 5 hours at 26°C, finally centrifugation at 450-500 rpm.

RNA purification by EtOH precipitation

Transfer the contents of the tubes (taking care not to over-pipette the gel ends) into COSTAR columns and centrifuge for 4 minutes at 11 000 rpm (-add 1µl of glycogen if necessary). Add 2.5 volumes of 100% EtOH cooled and incubate at -20°C for 1 hour. Centrifuge at 13 000 rpm at 4°C for 1 hour. Wash the pellets with 150µL of cold 70% EtOH. Evaporate the ethanol (under vacuum or hood), resuspend in 20µl of water and measure the concentration with the nanodrop.

2. Catalytic Assays

All catalytic assays were performed in the same fashion manner. According to the variability of experimental conditions several catalytic assays could be run with the same controls. All sequences were introduced leaving at the end the ribozyme when the start point was taken immediately. All Eppendorf tubes were kept in a thermostat at 40 °C for minutes. Only for the actual assay samples (2 μ L) were taken at 0, 2, 4, 7 and 24 hours for SunY and 0, 0,5, 1, 2, 4, 7 and 24 hours for Azoarcus. For controls only initial and final points were acquired. These samples were left in 6 μ L stopping buffer (10 mM EDTA, 80% formamide, 5% bromephenol blue, 5% xylene cyanol). Before loading of the samples to the gel denaturation by temperature was done in thermocycler at 80°C for 5 minutes.

Samples were analyzed in 18% PAGE (Polyacrylamide gel with 50% as denaturing agent). 40 mL of Polyacrylamide solution were prepared using 198 μ L 10% APC and 66 μ L TEMED as polymerization agents. 4 μ L of sample were introduced by lane. 4 μ L ladders of 1, 0,5 and 0,25 mM were used of molecular weights of 21, 25, 36, 46 and 55 bp for all cases except in P1-Loop-Azo ligation where 25 bp standard was replace by a 26 bp standard. Gel was pre-run for 10 min in TBE buffer 1X, at 25 V/cm gel was left around an hour. Visualization of the gel was done using SYBR Gold system with a Blue Tray.

Protocol for P1 Loop ligation (Azoarcus vs SunY experiment)

Catalytic assay						
Reagent	Stock sol.	Units	V(μl)	Final conc.		
Ribozyme	16	μΜ	0,3	0,5		
P1 Loop	100	μM	1,5	15		
S1_LL	100,0	μM	1,5	15		
MgCl2	240	mM	2,5	60		
Water	up to		4,2			
		Sum	10			

		Sum	10	
		1	tual	
	21	Loop + S1 Con	troi	
Reagent	Stock sol.	Units	V(µl)	Final conc.
Ribozyme	16	μΜ	0,0	0
P1 Loop	100	μΜ	1,2	15
S1_LL	100,0	μΜ	1,2	15
MgCl2	240	mM	2,0	60
Water	up to		3,6	

Sum

P1 Loop Control						
Reagent	Stock sol.	Units	V(µl)	Final conc.		
Ribozyme	16	μΜ	0,0	0		
P1 Loop	100	μΜ	1,2	15		
S1_LL	100,0	μΜ	0,0	0		
MgCl2	240	mM	2,0	60		
Water	up to		4,8			
		Sum	8			

Ribozyme Control						
Reagent	Stock sol.	Units	V(μl)	Final conc.		
Ribozyme	16	μΜ	0,3	0,5		
P1 Loop	100	μΜ	0,0	0		
S1_LL	100,0	μΜ	0,0	0		
MgCl2	240	mM	2,0	60		
Water	up to		5,8			
		Sum	8			

Ribozyme + P1 Loop Control					
Reagent	Stock sol.	Units	V(μl)	Final conc.	
Ribozyme	16	μM	0,3	0,5	
P1 Loop	100	μM	1,2	15	
S1_LL	100,0	μM	0,0	0	
MgCl2	240	mM	2,0	60	
Water	up to		4,6		
		Sum	8		

Ribozyme + S1 Contol					
Reagent	Stock sol.	Units	V(µl)	Final conc.	
Ribozyme	16	μM	0,3	0,5	
P1 Loop	100	μΜ	0,0	0	
S1_LL	100,0	μΜ	1,2	15	
MgCl2	240	mM	2,0	60	
Water	up to		4,6		
		Sum	8.0		

S1 Control					
Reagent	Stock sol.	Units	V(µl)	Final conc.	
Ribozyme	16	μM	0,0	0	
P1 Loop	100	μM	0,0	0	
S1_LL	100,0	μM	1,2	15	
MgCl2	240	mM	2,0	60	
Water	up to		4,8		
		Sum	8		

P1-Loop and T_2S1 analysis with A and B *Azoarcus* modifications were done in similar manner 10 μ L for catalytic assays and 8 μ L for controls. Only concentrations were modified. Assay **A-B-P1-Loop**: Substrates 15 μ M. Assay **A-B-P1-Loop**: Substrate S1 15 μ M + 2.5 μ M T_2S1.

Protocol for SunY Mg optimization in Water

MgCl2 50 mM						
Reagent	Stock sol.	Units	V(μl)	Final conc.		
SunY	16	μΜ	0,3	0,5		
T_2S_RNA	100	μΜ	0,5	5		
S1_LL	100,0	μΜ	1,5	15		
MgCl2	240	mM	2,1	50		
Water	up to		5,6			
		Sum	10			

MgCl2 100 mM						
Reagent	Stock sol.	Units	V(μl)	Final conc.		
SunY	16	μM	0,3	0,5		
T_2S_RNA	100	μΜ	0,5	5		
S1_LL	100,0	μΜ	1,5	15		
MgCl2	240	mM	4,2	100		
Water	up to		3,5			
		Sum	10			

T_2S + S1 Control						
Reagent	Stock sol.	Units	V(µl)	Final conc.		
SunY	16	μΜ	0,0	0		
T_2S_RNA	100	μΜ	0,4	5		
S1_LL	100,0	μΜ	1,2	15		
MgCl2	240	mM	3,3	100		
Water	up to		3,1			
		Sum	8			

T_2S Control						
Reagent	Stock sol.	Units	V(μl)	Final conc.		
SunY	16	μM	0,0	0		
T_2S_RNA	100	μM	0,4	5		
S1_LL	100,0	μM	0,0	0		
MgCl2	240	mM	3,3	100		
Water	up to		4,3			
		Sum	8			

		SunY Control		
Reagent	Stock sol.	Units	V(μl)	Final conc.
SunY	16	μM	0,3	0,5
T_2S_RNA	100	μM	0,0	0
S1_LL	100,0	μM	0,0	0
MgCl2	240	mM	3,3	100
Water	up to		4,4	
		Sum	8	

		MgCl2 75 mM		
Reagent	Stock sol.	Units	V(μl)	Final conc.
SunY	16	μM	0,3	0,5
T_2S_RNA	100	μM	0,5	5
S1_LL	100,0	μM	1,5	15
MgCl2	240	mM	3,1	75
Water	up to		4,6	
		Sum	10,0	

Sui	n Y + T_2S Con	trol	
Stock sol.	Units	V(μl)	Final conc.
16	μM	0,3	0,5
100	μM	0,4	5
100,0	μM	0,0	0
240	mM	3,3	100
up to		4,0	
	Sum	8	
	Stock sol. 16 100 100,0 240	Stock sol. Units 16 μM 100 μM 100,0 μM 240 mM up to	16 μM 0,3 100 μM 0,4 100,0 μM 0,0 240 mM 3,3 up to 4,0

Sun Y + S1 Contol Reagent Stock sol. V(µl) Final conc. Units 16 100 100,0 240 up to μM 0,3 0,0 SunY T_2S_RNA 0,5 0 15 100 μM μM ______S1_LL MgCl2 Water 1,2 mM 3,3 3,2 Sum 8,0

		S1 Control		
Reagent	Stock sol.	Units	V(μl)	Final conc.
SunY	16	μМ	0,0	0
T_2S_RNA	100	μM	0,0	0
S1_LL	100,0	μM	1,2	15
MgCl2	240	mM	3,3	100
Water	up to		3,5	
		Sum	8	

Protocol for SunY Mg optimization in EPPS 30 mM

Volume	10	μι	MgCl2	0	mM
name	stock	unit	vol(µl)	final conc.	unit
SunY	9	μM	0,6	0,5	μΜ
T-25	100	μM	0,5	5	μΜ
S1_LL	100,0	μM	1,5	15	μΜ
MgCl2	240	mM	0,0	0	mM
Buffer	200	mM	1,5	30	mM
Water	up to		5,9		
		Sum	10,0		

Volume	10	μί	MgCl2	50	mM
name	stock	unit	vol(µl)	final conc.	unit
SunY	9	μM	0,6	0,5	μM
T-25	100	μM	0,5	5	μM
S1_LL	100,0	μΜ	1,5	15	μΜ
MgCl2	240	mM	2,1	50	mM
Buffer	200	mM	1,5	30	mM
Water	up to		3,9		
		Sum	10,0		

Volume	10	μί	MgCl2	100	mM
name	stock	unit	vol(µl)	final conc.	unit
SunY	9	μΜ	0,6	0,5	μΜ
T-2S	100	μΜ	0,5	5	μΜ
S1_LL	100,0	μM	1,5	15	μΜ
MgCl2	240	mM	4,2	100	mM
Buffer	200	mM	1,5	30	mM
Water	up to		1,8		
		Sum	10,0		

Volume	10	μί	MgCl2	25	mM
name	stock	unit	vol(µl)	final conc.	unit
SunY	9	μM	0,6	0,5	μΜ
T-25	100	μΜ	0,5	5	μΜ
S1_LL	100,0	μM	1,5	15	μΜ
MgCl2	240	mM	1,0	25	mM
Buffer	200	mM	1,5	30	mM
Water	up to		4,9		
		Sum	10,0		

Volume	10	μί	MgCl2	75	mM
name	stock	unit	vol(µl)	final conc.	unit
SunY	9	μM	0,6	0,5	μΜ
T-25	100	μM	0,5	5	μΜ
S1_LL	100,0	μM	1,5	15	μΜ
MgCl2	240	mM	3,1	75	mM
Buffer	200	mM	1,5	30	mM
Water	up to		2,8		
		Sum	10,0		

Volume	8	μί	MgCl2	100	mM	Volume	8	μL	MgCl2	100	mM
name	stock	unit	vol(µl)	final conc.	unit	name	stock	unit	vol(µl)	final conc.	u
SunY	9	μM	0,4	0,5	μM	SunY	9	μM	0,4	0,5	μ
T-25	100	μМ	0,4	5	μM	T-25	100	μM	0,0	0	μ
S1_LL	100,0	μМ	0,0	0	μM	S1_LL	100,0	μM	1,2	15	μ
MgCl2	240	mM	3,3	100	mM	MgCl2	240	mM	3,3	100	m
Buffer	200	mM	1,5	30	mM	Buffer	200	mM	1,5	30	m
Water	up to		2,3			Water	up to		1,5		
		Sum	8,0	Sun	(+ P1			Sum	8,0	Sun Y	+ S1
Volume	8	μί	MgCl2	100	mM	Volume	8	μL	MgCl2	100	mM
name	stock	unit	vol(µl)	final conc.	unit	name	stock	unit	vol(µl)	final conc.	ur
SunY	9	μM	0,0	0	μM	SunY	9	μΜ	0,4	0,5	μ
T-25	100	μM	0,4	5	μM	T-25	100	μM	0,0	0	μ
S1_LL	100,0	μМ	1,2	15	μM	S1_LL	100,0	μM	0,0	0	μ
MgCl2	240	mM	3,3	100	mM	MgCl2	240	mM	3,3	100	m
Buffer	200	mM	1,5	30	mM	Buffer	200	mM	1,5	30	m
Water	up to		1,6			Water	up to		2,7		
		Sum	8,0	S1 -	+ P1			Sum	8,0	Su	ηY
Volume	8	uL	MgCl2	100	mM	Volume	8	uL	MgCl2	100	mM
name	stock	unit	vol(µl)	final conc.	unit	name	stock	unit	vol(µl)	final conc.	ur
SunY	9	μΜ	0,0	0	μM	SunY	9	μΜ	0,0	0	μ
T-25	100	μM	0,4	5	μM	T-25	100	μM	0,0	0	μ
S1_LL	100,0	μM	0,0	0	μM	S1_LL	100,0	μM	1,2	15	μ
MgCl2	240	mM	3,3	100	mM	MgCl2	240	mM	3,3	100	m
Buffer	200	mM	1,5	30	mM	Buffer	200	mM	1,5	30	m
Water	up to		2,8			Water	up to		2,0		
		Sum	8.0		1			Sum	8,0	s	1

Protocol for SunY Mg optimization in Tris-HCl 30 mM

Volume	10	μL	MgCl2	0	mM		Volume	10	μL	MgCl2	25	mM
name	stock	unit	vol(µl)	final conc.	unit	n	name	stock	unit	vol(µl)	final conc.	unit
SunY	9	μM	0,6	0,5	μM	S	SunY	9	μΜ	0,6	0,5	μΜ
T-2S	100	μM	0,5	5	μM	1	T-2S	100	μΜ	0,5	5	μM
S1_LL	100,0	μM	1,5	15	μM	s	S1_LL	100,0	μM	1,5	15	μM
MgCl2	240	mM	0,0	0	mM	N	MgCl2	240	mM	1,0	25	mM
Buffer	200	mM	1,5	30	mM	В	Buffer	200	mM	1,5	30	mM
Water	up to		5,9			v	Water	up to		4,9		
		Sum	10,0						Sum	10,0		
				1								
Volume	10	μL	MgCl2	50	mM		Volume	10	μL	MgCl2	75	mM
name	stock	unit	vol(µl)	final conc.	unit	n	name	stock	unit	vol(µl)	final conc.	unit
SunY	9	μM	0,6	0,5	μM	s	SunY	9	μM	0,6	0,5	μM
T-25	100	μM	0,5	5	μM		T-25	100	μΜ	0,5	5	μM
S1_LL	100,0	μM	1,5	15	μM	s	S1_LL	100,0	μM	1,5	15	μM
MgCl2	240	mM	2,1	50	mM		MgCl2	240	mM	3,1	75	mM
Buffer	200	mM	1,5	30	mM		Buffer	200	mM	1,5	30	mM
Water	up to		3,9				Water	up to		2,8		
		Sum	10,0						Sum	10,0		
				,							,	
Volume	10	μL	MgCl2	100	mM							
name	stock	unit	vol(µl)	final conc.	unit							
SunY	9	μM	0,6	0,5	μM							
T-25	100	μM	0,5	5	μM							
S1_LL	100,0	μM	1,5	15	μM							
			4,2	100	mM							
	240											
MgCl2	240 200	mM mM										
MgCl2 Buffer	200	mM	1,5	30	mM							
MgCl2		mM	1,5 1,8									
MgCl2 Buffer	200		1,5									
MgCl2 Buffer Water	200 up to	mM Sum	1,5 1,8 10,0	30	mM	CONTROLS						
MgCl2 Buffer Water Volume	200 up to	mΜ Sum	1,5 1,8 10,0 MgCl2	30	mM		Volume		μι	MgCl2		mM
MgCl2 Buffer Water Volume name	200 up to 8 stock	mM Sum μL unit	1,5 1,8 10,0 ΜgCl2 vol(μl)	30 100 final conc.	mM mM unit	r	name	stock	unit	vol(µl)	final conc.	unit
MgCl2 Buffer Water Volume name SunY	200 up to 8 stock 9	mM Sum μL μM	1,5 1,8 10,0 ΜgCl2 vol(μl) 0,4	30 100 final conc. 0,5	mM mM μM	r	name SunY	stock 9	unit μM	vol(µl) 0,4	final conc. 0,5	unit μM
MgCl2 Buffer Water Volume name SunY T-2S	200 up to 8 stock 9 100	mM Sum μL μM μM	1,5 1,8 10,0 MgCl2 vol(μl) 0,4 0,4	30 100 final conc. 0,5 5	mM mM μM μM	r	name SunY T-2S	stock 9 100	unit μM μM	vol(μl) 0,4 0,0	final conc. 0,5 0	unit μM μM
MgCl2 Buffer Water Volume name SunY T-2S S1_LL	200 up to 8 stock 9 100 100,0	mM Sum μL μM μM	1,5 1,8 10,0 MgCl2 vol(µl) 0,4 0,4 0,0	30 100 final conc. 0,5 5 0	mM mM μM μM		name SunY T-2S S1_LL	9 100 100,0	unit μM μM μM	vol(μl) 0,4 0,0 1,2	final conc. 0,5 0 15	unit μM μM
MgCl2 Buffer Water Volume name SunY T-2S S1_LL MgCl2	200 up to 8 stock 9 100 100,0 240	mM Sum μL μM μM μM mM	1,5 1,8 10,0 MgCl2 vol(µl) 0,4 0,4 0,0 3,3	30 100 final conc. 0,5 5 0 100	mM unit μM μM μM mM	r S N	name SunY T-2S S1_LL MgCl2	stock 9 100 100,0 240	unit μM μM μM mM	vol(μl) 0,4 0,0 1,2 3,3	final conc. 0,5 0 15 100	unit μM μM μM mM
MgCl2 Buffer Water Volume name SunY T-2S S1_LL MgCl2 Buffer	200 up to 8 stock 9 100 100,0 240 200	mM Sum μL μM μM	1,5 1,8 10,0 MgCl2 vol(µ) 0,4 0,4 0,0 3,3 1,5	30 100 final conc. 0,5 5 0	mM mM μM μM	r S N B	name SunY T-2S S1_LL MgCl2 Buffer	stock 9 100 100,0 240 200	unit μM μM μM	vol(µl) 0,4 0,0 1,2 3,3 1,5	final conc. 0,5 0 15	unit μM μM
MgCl2 Buffer Water Volume name SunY T-2S S1_LL MgCl2	200 up to 8 stock 9 100 100,0 240	mM Sum μL μM μM μM μM μM μM	1,5 1,8 10,0 MgCl2 vol(µ) 0,4 0,4 0,0 3,3 1,5 2,3	30 100 final conc. 0,5 5 0 100 30	mM unit μM μM μM mM mM	r S N B	name SunY T-2S S1_LL MgCl2	stock 9 100 100,0 240	unit μM μM mM mM	vol(µl) 0,4 0,0 1,2 3,3 1,5 1,5	final conc. 0,5 0 15 100 30	unit μM μM mM mM
MgCl2 Buffer Water Volume name SunY T-2S S1_LL MgCl2 Buffer	200 up to 8 stock 9 100 100,0 240 200	mM Sum μL μM μM μM mM	1,5 1,8 10,0 MgCl2 vol(µ) 0,4 0,4 0,0 3,3 1,5	30 100 final conc. 0,5 5 0 100 30	mM unit μM μM μM mM	r S N B	name SunY T-2S S1_LL MgCl2 Buffer	stock 9 100 100,0 240 200	unit μM μM μM mM	vol(µl) 0,4 0,0 1,2 3,3 1,5	final conc. 0,5 0 15 100	unit μM μM mM mM
Mg(12 Buffer Water Volume name SunY T-25 S1_LL Mg(12 Buffer Water	200 up to 8 stock 9 100 100,0 240 200 up to	mM Sum μL μM μM μM mM mM Sum	1,5 1,8 10,0 MgCl2 vol(µ1) 0,4 0,4 0,0 3,3 1,5 2,3 8,0	30 t 100 final conc. 0,5 5 0 100 30 Sun	mM unit μM μM μM mM mM Y + P1	r S N B	name SunY T-2S S1_LL MgCl2 Buffer Water	stock 9 100 240 200 up to	unit μM μM mM mM Sum	vol(µl) 0,4 0,0 1,2 3,3 1,5 1,5 8,0	final conc. 0,5 0 15 100 30 Sun Y	unit μΜ μΜ mM mM
Mg(12 Buffer Water Volume name SunY T-25 S1_LL Mg(12 Buffer Water Volume	200 up to 8 stock 9 100 100,0 240 200 up to 8	mM Sum μL μM μM μM mM mM Sum	1,5 1,8 10,0 MgCl2 vol(µ) 0,4 0,4 0,0 3,3 1,5 2,3 8,0 MgCl2	30 100 final conc. 0,5 5 0 100 30 Sun 100	mM unit μM μM mM mM Y + P1 mM	s S N B V	Name SunY T-2S S1_LL MgCl2 Buffer Water Volume	stock 9 100 240 200 up to 8	unit μM μM mM mM Sum	vol(µl) 0,4 0,0 1,2 3,3 1,5 1,5 1,5 8,0 MgCl2	final conc. 0,5 0 15 100 30 Sun Y 100	unit μΜ μΜ mM mM '+ S1
MgCi2 Buffer Water Volume name SunY T-25 S1_LL MgCi2 Buffer Water Volume name	200 up to 8 stock 9 100 100,0 240 200 up to 8 stock	mM Sum μL μM μM mM mM Sum Sum	1,5 1,8 10,0 Vol(µl) 0,4 0,4 0,0 3,3 1,5 2,3 8,0 MgCl2 vol(µl)	30 final conc. 0,5 0 100 30 Sun final conc.	mM unit μM μM mM mM Y+P1 vnit	r S N B V	name SunY T-2S S1_LL MgCl2 Buffer Water Volume name	stock 9 100,0 240 200 up to 8 stock	unit μM μM mM mM Sum	vol(µl) 0,4 0,0 1,2 3,3 1,5 1,5 1,5 8,0 MgCl2 vol(µl)	final conc. 0,5 0 15 100 30 Sun Y 100 final conc.	unit μM μM mM mM '+ S1 mM unit
Mg(12 Buffer Water Volume name SunY T-2S S1_LL Mg(12 Buffer Water Volume name SunY	200 up to 8 stock 9 100 240 200 up to 8 stock 9	mM Sum unit unit uM uM mM mM Sum unit unit unit unit	1,5 1,8 10,0 MgCi2 vol(µi) 0,4 0,4 0,0 3,3 1,5 2,3 8,0 MgCi2 vol(µi) 0,0	30 final conc. 0,5 5 0 100 30 Sun final conc. 0	mM unit μM μM mM mM Y + P1 Y + P1 unit μM	s s b v	name SunY T-2S S1_LL MgCl2 Buffer Water Water Volume name SunY	stock 9 100,0 240 200 up to 8 stock 9	unit μM μM mM mM Sum <u>μL</u> μM	vol(µl) 0,4 0,0 1,2 3,3 1,5 1,5 1,5 8,0 MgCl2 vol(µl) 0,4	final conc. 0,5 0 15 100 30 Sun Y 100 final conc. 0,5	unit μM μM mM mM '+ S1 mM unit μM
MgCl2 Buffer Water Volume name SunY T-25 S1_LL MgCl2 Buffer Water Volume name SunY T-25	200 up to 8 stock 9 100 100,0 240 200 up to 8 stock 9 9 100	mM Sum μL μM μM μM mM Sum Sum	1,5 1,8 10,0 MgCl2 vol(µi) 0,4 0,4 0,4 0,4 0,4 0,4 0,4 0,4 0,5 1,5 2,3 8,0 MgCl2 vol(µi) 0,0 0,4 0,4 0,0 0,4 0,0 0,0 0,0 0,0 0,0	30 100 100 100 30 30 30 30 30 30 30 30 30	mM unit μM μM μM mM mM wm mM unit μM	S N B V	name SunY T-2S S1_LL MgCl2 Buffer Water Volume name SunY T-2S	stock 9 100,0 240 200 up to 8 stock 9 100	unit μM μM mM mM Sum μL μM μM	vol(μ) 0,4 0,0 1,2 3,3 1,5 1,5 8,0 Mgcl2 vol(μ) 0,0 0,0	final conc. 0,5 0 15 100 30 Sun Y 100 100 0 100 conc. 0,5 0	unit μM μM mM mM '+ S1 mM unit μM μM
MgCl2 Buffer Water Name SunY T-25 S1_LL Buffer Water Volume name SunY T-25 S1_LL	200 up to 8 stock 9 100 100,0 240 200 up to 8 stock 9 100 100,0	mM Sum µL µM µM µM mM mM Sum µL unit µM µM µM µM	1,5 1,8 1,8 0,4 0,4 0,4 0,4 0,4 0,4 0,4 0,4 0,4 0,4	30 100 100 100 30 100 30 100 100	mM unit µM µM µM mM mM Y + P1 v + P1 unit µM µM µM µM	S S N B V V	name SunY T-2S S1_LL MgCl2 Buffer Water Volume name SunY T-2S S1_LL	stock 9 100 240 200 up to 8 stock 9 100 100,0	unit μM μM mM mM Sum μL μM μM μM	vol(µ) 0,4 0,0 1,2 3,3 1,5 1,5 1,5 8,0 MgCl2 vol(µl) 0,4 0,0 0,0	final conc. 0,5 0 15 100 30 Sun Y 100 final conc. 0,5 0 0 0	unit μM μM mM mM '+ S1
MgCl2 Buffer Water Volume name SunY T-25 S1_LL MgCl2 Buffer Water Volume name SunY T-25	200 up to 8 stock 9 100,0 240 200 up to 8 stock 9 1000 100,0 240	mM Sum μL μM μM μM mM Sum Sum	1,5 1,8 10,0 10,0 10,0 10,0 1,8 1,8 1,5 2,3 8,0 1,5 2,3 8,0 Mgcl2 vol(µi) 0,0 0,0 0,0 0,4 1,2 3,3	30 100 100 100 30 30 30 30 30 30 30 30 30	mM unit μM μM μM mM mM wm mM unit μM		name SunY T-25 S1_LL MgCl2 Buffer Water Volume name SunY T-25 S1_LL MgCl2	stock 9 100,0 240 200 up to 8 stock 9 100	unit μM μM mM mM Sum μL μM μM	vol(µ) 0,4 0,0 1,2 3,3 1,5 1,5 8,0 MgCl2 vol(µl) 0,4 0,0 0,0 3,3	final conc. 0,5 0 15 100 30 Sun Y 100 100 0 100 conc. 0,5 0	unit μΜ μΜ mM mM '+ S1 mM unit μM μM
MgCl2 Buffer Water Nolume name SunY T-2S S_LL MgCl2 Buffer Water Volume name SunY T-2S S_LL MgCl2	200 up to 8 stock 9 1000 240 240 200 up to 8 stock 9 100 100,0 100,0 240 220	mM Sum μL μM μM μM mM mM Sum μL μM μM μM μM μM μM μM	1,5 1,8 10,0 0,4 0,4 0,4 0,4 0,4 0,4 0,4 0,4 0,4	30 100 final conc. 0,5 5 0 100 30 5 100 final conc. 0 5 100 100 5 100 100	mM μM μM μM μM γ+ P1 mM μM μM μM μM μM μM μM μM	s S N B V V S S N N B B B B B	name SunY T-2S S1_LL MgCl2 Buffer Water Volume name SunY T-2S S1_LL	stock 9 100 240 200 up to 8 stock 9 100,0 200 200 200 200 200 200 200 200	unit μM μM mM mM Sum μL μM μM μM μM μM	vol(µ) 0,4 0,0 1,2 3,3 1,5 1,5 8,0 MgCl2 vol(µ) 0,4 0,0 0,0 0,0 3,3 1,5	final conc. 0,5 0 15 100 30 Sun Y 100 final conc. 0,5 0 0,5 0 100	unit μM μM mM mM *+S1 ***S1 ***S1 *** *** *** *** **
MgCi2 Buffer Water Volume name SunY T-25 S1_LL MgCi2 Buffer Volume name SunY T-25 SunY T-25 S1_LL MgCi2 Buffer Buffer	200 up to 8 stock 9 100,0 240 200 up to 8 stock 9 1000 100,0 240	mM Sum μL μM μM μM mM mM Sum μL μM μM μM μM μM μM μM	1,5 1,8 10,0 10,0 10,0 10,0 1,8 1,8 1,5 2,3 8,0 1,5 2,3 8,0 Mgcl2 vol(µi) 0,0 0,0 0,0 0,4 1,2 3,3	30 1000 1001 30 100 30 100 100 30 100 10	mM μM μM μM μM γ+ P1 mM μM μM μM μM μM μM μM μM	s S N B V V S S N N B B B B B	name SunY T-25 S1_LL MgCl2 Buffer Water Volume name SunY T-25 S1_LL MgCl2 Buffer	stock 9 100 240 200 up to 8 stock 9 100 100,0 240	unit μM μM mM mM Sum μL μM μM μM μM μM	vol(µ) 0,4 0,0 1,2 3,3 1,5 1,5 8,0 MgCl2 vol(µl) 0,4 0,0 0,0 3,3	final conc. 0,5 0 15 100 30 Sun Y 100 final conc. 0,5 0 0,5 0 100	unit μM μM mM mM * \$1 mM unit μM μM μM mM mM
MgCi2 Buffer Water Volume name SunY T-25 S1_LL MgCi2 Buffer Volume name SunY T-25 SunY T-25 S1_LL MgCi2 Buffer Buffer	200 up to 8 stock 9 1000 240 240 200 up to 8 stock 9 100 100,0 100,0 240 220	mM Sum μL μM μM μM μM sum Sum μ L μM μM μM μM μM μM μM μM μM	1,5 1,8 10,0 vol(µ) 0,4 0,4 0,0 3,3 1,5 2,3 8,0 vol(µ) 0,0 0,0 0,4 1,2 3,3 1,5 1,6	30 1000 1001 30 100 30 100 100 30 100 10	mM unit μM μM μM mM mM y + P1 μM μM μM μM μM μM μM μM μM μM	s S N B V V S S N N B B B B B	name SunY T-25 S1_LL MgCl2 Buffer Water Volume name SunY T-25 S1_LL MgCl2 Buffer	stock 9 100 240 200 up to 8 stock 9 100 100,00 240 200,00,00 240 200,00,00 240 200 200	unit μM μM mM mM Sum Sum <u>μL</u> μM μM μM mM mM	vol(µ1) 0,4 0,0 1,2 3,3 1,5 1,5 8,0 MgCl2 vol(µ1) 0,4 0,0 0,0 0,0 3,3 1,5 2,7	Sun Y 0,5 0 15 100 30 Sun Y 100 final conc. 0,5 0 100 30	unit μM μM mM mM * \$1 mM unit μM μM μM mM mM
MgCi2 Buffer Water Volume name SunY T-25 S1_LL MgCi2 Buffer Volume name SunY T-25 SunY T-25 S1_LL MgCi2 Buffer Buffer	200 up to 8 stock 9 100 100,0 240 200 up to 8 stock 9 9 100,0 240 200 up to up	mM Sum μL μM μM μM μM sum Sum μ L μM μM μM μM μM μM μM μM μM	1,5 1,8 10,0 vol(µ) 0,4 0,4 0,0 3,3 1,5 2,3 8,0 vol(µ) 0,0 0,0 0,4 1,2 3,3 1,5 1,6	30 1000 100 30 100 30 100 5 100 5 100 30 30 5 15 100 30 5 15 100 30 5 15 100 30 5 100 5 100 100 100 100 100 1	mM unit μM μM μM mM mM y + P1 μM μM μM μM μM μM μM μM μM μM	s S N B V V S S N N B B B B B	name SunY T-25 S1_LL MgCl2 Buffer Water Volume name SunY T-25 S1_LL MgCl2 Buffer	stock 9 100,0 240 200 up to 8 stock 9 100 100,0 240 200 up to	unit μM μM mM mM Sum Sum <u>μL</u> μM μM μM mM mM	vol(µ1) 0,4 0,0 1,2 3,3 1,5 1,5 8,0 Vol(µ1) 0,4 0,0 0,0 0,0 0,0 0,0 3,3 1,5 2,7 8,0	final conc. 0,5 0 15 100 30 30 5un Yr 5un Yr 100 final conc. 0,5 0 0 0 0 100 30 20 5un Yr 5un	unit μM μM mM mM * \$1 mM unit μM μM μM mM mM
MgCl2 Buffer Water Volume name SunY T-25 S1_LL MgCl2 Buffer Volume name SunY T-25 S1_LL MgCl2 Buffer S1_LL MgCl2 Buffer Water	200 up to 8 stock 9 100 100,0 240 200 up to 8 stock 9 9 100,0 240 200 up to up	mM Sum unit µM µM µM mM mM mM µM µM µM µM µM Sum	1,5 1,8 10,0 Wol(µi) 0,4 0,4 0,0 0,0 3,3 1,5 2,3 8,0 Wg(2) Wol(µi) 0,0 0,0 0,4 1,2 3,3 1,5 5 1,6 8,0	30 1000 100 30 100 30 100 5 100 5 100 30 30 5 15 100 30 5 15 100 30 5 15 100 30 5 100 5 100 100 100 100 100 1	mM unit μM μM μM mM mM y+ P1 mM μM μM μM μM μM μM μM μM μM μ	r S N B V V V V	Name SunY T-25 S1_LL MgCl2 Buffer Water Volume name SunY T-25 S1_LL MgCl2 Buffer Water	stock 9 100,0 240 200 up to 8 stock 9 100 100,0 240 200 up to	unit µM µM µM mM Sum Sum	vol(µ1) 0,4 0,0 1,2 3,3 1,5 1,5 8,0 MgCl2 vol(µ1) 0,4 0,0 0,0 0,0 3,3 1,5 2,7	final conc. 0,5 0 15 100 30 30 5un Yr 5un Yr 100 final conc. 0,5 0 0 0 0 100 30 20 5un Yr 5un	սու արչ արչ արչ արչ արչ արչ արչ արչ
MgCl2 Buffer Water Volume name SunY T-25 S_LL MgCl2 Buffer Volume name SunY T-25 S_LL MgCl2 Buffer Water MgCl2 Buffer Water Volume Volume	200 up to 8 stock 9 100 100,0 200 up to 200 up to 8 stock 9 100 100,0 200 up to 200 up to 8 8 stock 8 8 stock 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8	mM Sum μL μM μM mM mM sum μL unit μM mM mM sum sum μL Sum sum μL	1,5 1,8 10,0 MgCl2 vol(µi) 0,4 0,4 0,4 0,4 0,0 1,5 2,3 1,5 2,3 MgCl2 vol(µi) 0,0 0,4 0,0 0,4 0,0 0,4 0,0 0,0 0,0 0,0	30 1000 final conc. 0,5 5 0 100 30 5 100 100 5 15 15 100 30 30 5 15 100 30 30 5 100 100 5 100 100 100 100 1	mM unit μM μM μM mM mM v + P1 mM mM	r S N B V V S S N N S S V V	Name SunY T-2S S1_LL MgCl2 Buffer Water Volume name SunY T-2S S1_LL MgCl2 Buffer Water	stock 9 100,0 240 200 up to 8 stock 9 100 100,0 240 200 up to 8 8	unit µM µM µM mM mM Sum µL unit µM µM mM mM mM mM mM µM	vol(µ1) 0,4 0,0 1,2 3,3 1,5 1,5 8,0 MgCl2 vol(µ1) 0,4 0,4 0,0 0,0 0,0 0,0 3,3 1,5 2,7 8,0 MgCl2	final conc. 0,5 0 0,15 100 30 30 Sun Y 100 final conc. 0,5 0 0 100 30 30 Sun Y	unit μM μM mM mM * \$1 * \$1 μM μM μM μM mM mM mM mM
Mg(d2 Buffer Water Volume name SunY T-25 51_LL Mg(12 Buffer Water Volume name SunY T-25 SunY Water Volume name	200 up to 8 stock 9 100 100,0 240 200 up to 8 stock 9 100 100,0 240 200 up to 8 stock 8 stock	mM sum μL μM μM mM mM mM mM mM mM mM mM sum sum sum sum sum sum gut mM mM sum gut mt	1,5 1,8 10,0 vol(µ) 0,4 0,4 0,4 0,4 0,4 0,4 0,4 0,4 0,4 0,4	30 100 100 100 30 100 100 100 10	mM unit μM μM mM mM mM mM mM mM mM mM mM y+P1 mM mM mM mM mM mM mM mM mM		name SunY T-25 S1_LL MgCl2 Buffer Water Volume name SunY T-25 S1_LL MgCl2 Buffer Water	stock 9 100 100,0 240 200 up to 8 stock 9 100 100,0 240 200 up to 200 200 200 200 200 8 stock	unit µM µM mM mM Sum µL unit µM mM mM mM mM mM mM unit unit unit	vol(µ1) 0,4 0,0 1,2 3,3 1,5 1,5 8,0 MgCl2 vol(µ1) 0,0 0,0 0,0 0,0 0,0 0,0 1,5 8,0 MgCl2 vol(µ1) 0,0 0,0 0,0 0,0 0,0 0,0 0,0 0,	final conc. 0,5 0 15 100 30 30 final conc. 0,5 0 0 0 100 30 30 30 100 30 100 100 100 100 100 100 final conc. 100	unit µM µM mM mM * + S1 mM unit µM µM mM mM mM mM unit unit
Mgd2 Buffer Water Volume name SunY T-2S S1_LL Mgd2 Buffer Water Volume name SunY T-2S S1_LL Buffer Water Volume name SunY Volume SunY	200 up to 8 stock 9 100 100,0 240 200 up to 8 stock 9 100 100,0 240 200 00,0 100,0 240 200 up to 8 stock 9 100 100,0 240 200 200 200 200 200 200 20	mM Sum μL μM μM μM gamma mM mM mM gamma gamma	1,5 1,8 10,0 MgCl2 vol(µi) 0,4 0,4 0,4 0,4 0,4 0,4 0,4 0,4 0,0 3,3 1,5 2,3 8,0 MgCl2 vol(µi) 0,0 0,4 1,5 1,5 8,0 MgCl2 vol(µi) 0,0 0,4 0,4 0,4 0,4 0,4 0,4 0,4 0,4 0,4	30 1000 1000 1000 1000 100 100 10	mM unit μM μM μM mM mM Y + P1 mM unit μM mM mM mM mM mM	r S N V V V	Name SunY T-2S S1_LL MgCl2 Buffer Water Volume name S1_LL MgCl2 Buffer Water	stock 9 100 240 200 up to 8 stock 9 100 100,0 240 200 200 up to 8 stock 9 100,0 240 200 up to 8 stock 9	unit μM μM mM mM Sum μL unit μM mM μM μM μM μM μM μM μM unit μM	vol(µ) 0,4 0,0 1,2 3,3 1,5 1,5 1,5 1,5 1,5 1,5 1,5 1,5	final conc. 0,5 0 15 100 30 30 final conc. 0,5 0 0 0 100 30 30 Sur Y 100 30 100 100 30 Sur Sur Y 100 100 30 Sur Sur Y 100 100 0 0	unit μM μM mM mM unit μM mM nm mm nm mm nm nm mm nm mm mm mm mm unit unit μM
Mg(d2 Buffer Water Volume name SunY T-25 S1_LL S1_LL S1_LL Mg(cl2 Buffer Water Volume name SunY T-25 S1_LL Mg(cl2 Buffer Water Volume name SunY T-25	200 up to 9 100 100,0 240 200 up to 8 stock 9 100 100,0 200 200 200 200 200 200 200 200 9 100 8 stock 9 100 100,0 8 stock 8 9 100 100,0 10	mM Sum unit unit uM uM mM mM Sum unit unit unit Sum unit unit unit unit unit unit unit unit	1,5 1,8 10,0 MgCl2 vol(µi) 0,4 0,4 0,4 0,4 0,4 0,4 0,4 0,4 0,4 0,4	30 100 100 100 30 100 100 30 100 10	mM unit μM μM mM mM mM mM mM mM mM mM mM unit μM μM μM μM mM mM mM mM μM μM	r S N B V V V	name SunY T-25 S1_LL MgCl2 Buffer Water Volume name SunY T-25 S1_LL MgCl2 Buffer Water Volume name SunY T-25 SunY T-25	stock 9 100 200 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	unit μM μM mM Sum μL unit μM mM mM mM mM mM mM mM mM μM mM	vol(µ1) 0,4 0,0 1,2 3,3 1,5 1,5 8,0 MgCl2 vol(µ1) 0,4 0,0 0,0 0,0 3,3 1,5 2,7 8,0 MgCl2 2,7 8,0 MgCl2 2,7 8,0 MgCl2 2,7 8,0 MgCl2 0,0 0,0 0,0 0,0 0,0 0,0 0,0 0,0 0,0 0,	final conc. 0,5 0 15 100 30 30 final conc. 0,5 0 000 30 500 0 1000 30 Sun Y 0 0 100 30 Sun Y final conc. 0 0 0 0	unit μM μM μM mM mM mM μM μM μM mM mM mM mM mM μM μM μM
Mg(d2 Buffer Water Volume name SunY T-25 S1_LL Mg(d2 Buffer Water Volume name SunY T-25 S1_LL Mg(d2 Buffer Water Volume SunY T-25 S1_LL	200 up to 8 stock 9 100 200 up to 8 stock 9 100 200 240 200 240 200 up to 8 stock 9 100 8 stock 9 100 8 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100	mM Sum μL μM μM μM gamma Sum μL unit μM mM mM gamma Sum μL unit μM μM mM mM mM mM mM mM mM mM	1,5 1,8 10,0 MgCl2 vol(µi) 0,4 0,4 0,4 0,4 0,4 0,4 0,0 3,3 1,5 2,3 8,0 MgCl2 vol(µi) 0,0 0,0 0,4 1,2 3,3 1,5 1,6 8,0 MgCl2 vol(µi) 0,0 0,4 0,4 0,4 0,4 0,4 0,4 0,4 0,4 0,4	30 1000 1010 1010 1000 10	mM unit μM μM mM mM	r S S N V V S S S N N B V V V S S S S S S S S	Name SunY T-25 S1_LL MgCl2 Buffer Water Volume name SunY T-25 S1_LL MgCl2 Buffer Water Volume name SunY T-25 S1_LL Volume name SunY T-25 S1_LL	stock 9 100 100,0 240 200 up to 8 stock 9 100 100,0 240 200 up to 100,0 240 200 up to 8 stock 9 100 100,0 100 200 up to 100 100 100,0 100,0 100,0	unit µM µM mM sum Sum µL unit µM µM µM µM µM µM µM µM µM unit µM unit µM µM µM µM µM µM µM µM µM	vol(µ) 0,4 0,0 1,2 3,3 1,5 1,5 8,0 Mgct2 vol(µ) 0,4 0,0 0,0 0,0 0,0 3,3 1,5 2,7 8,0 Mgct2 vol(µ) 0,0 0,0 0,0 0,0 0,0 0,0 0,0 0,	final conc. 0,5 0 15 100 30 30 Sun Y 100 final conc. 0,5 0 0 0 100 30 Sun Y Infal conc. Infal conc. 0 0 15 5	սուլ μM μM mM mM mM v'+S1 mM μM μM mM mM mM mM mM mM mM μM mM
Mg(d2 Buffer Water Volume name SunY 7-25 S1_LL Mg(d2 Volume name SunY 7-25 S1_LL Mg(d2 Buffer Water Volume name SunY 7-25 S1_LL Mg(d2 Buffer Volume name SunY 7-25 S1_LL Mg(d2	200 up to 8 stock 9 100 100,0 240 200 up to 8 stock 9 100 100,0 240 200 up to 8 stock 9 100 240 200 up to 8 stock 9 100 100,0 240 200 100,0 240 200 100,0 240 200 100,0 240 200 100,0 240 200 100,0 240 200 100,0 240 200 100,0 240 200 240 24	mM Sum μL μM μM mM mM mM mM gunt μL unit μM μM gunt gunt mM mM gunt gunt gunt gunt gunt gunt mM mM mM mM	1,5 1,8 10,0 MgCl2 vol(µi) 0,4 0,4 0,4 0,4 0,0 3,3 1,5 2,3 8,0 MgCl2 vol(µi) 0,4 0,4 0,0 0,4 0,0 0,4 0,0 0,4 0,0 0,4 0,0 0,0	30 100 100 100 30 100 100 100 10	mM unit μM μM μM mM mM wf + P1 mM μM μM μM μM μM μM μM μM μM μ	r S N V V V V V V S S S S N B B S S S N B B B S S S N B B S S S N S S S N S S S S	Name SunY T-25 S1_LL MgCl2 Buffer Water Volume name SunY T-25 S1_LL MgCl2 Buffer Water Volume name SunY T-25 S1_LL MgCl2 S1_LL MgCl2	stock 9 100 100,0 240 200 up to 8 stock 9 100,0 240 240 200 up to 100,0 240 200 200 240 200 100,0 240 200 200 240 200 240	unit µM µM µM mM Sum µL unit µM µM µM µM µM µM mM wit µM mM mM mM mM mM mM mM	vol(µ) 0,4 0,0 1,2 3,3 1,5 1,5 8,0 MgCl2 vol(µ) 0,4 0,0 0,0 0,0 0,0 3,3 1,5 2,7 8,0 MgCl2 vol(µ) 0,4 0,0 0,0 1,2 1,5 1,5 1,5 1,5 1,5 1,5 1,5 1,5	final conc. 0,5 0 15 100 30 30 Sun Y 100 final conc. 0,5 0 0 0 100 Sun Y final conc. 0 100 Sun Y 100 final conc. 0 0 0 0 0 0 0 0 0 100 100	unit µM µM µM mM mM * \$1 * \$1 mM unit µM µM mM mM mM mM mM mM mM mM mM m

3. Image Processing

Processing was done in GelAnalyzer. Lanes were selected and use of the Ladder was used to determine the molecular weight of different fragments. Background estimation was done with rolling ball method and 5% pic fit. Deviation for error bars were done considering 10% of area outside of gaussian pic.