HoPo 2 Lab Notes Scans

HoPo2 (Homopolymer Experiment Rev 2)

Add the following components to 1.5-ml microcentrifuge tubes *on* ice.

	A	B	C
1	Table A	Amount	Final Concentration
2	dNTP	10 uL = 100%	100uM
3	oligonucleotide	Alan	
4	5X TdT Reaction buffer	10 uL	5X
5	autoclaved, distilled water		(total volume to 50uL)

Primer: 100% = 5 µL dilution

	A	B	C	D	E	F
1	Table B	Function	1	2	3	4 (Incubation Time)
2	A	Just Primers (No TdT)	20min	40min	60min	80min
3	B	Primers+50% Extra dNTPs+TdT	20min	40min	60min	80min
4	C	Primers+dNTPs+50% Extra TdT	20min	40min	60min	80min
5	D	Primers+dNTPs+TdT	20min	40min	60min	80min
6	E	Primers+50% Extra dNTPs+50% Extra TdT	20min	40min	60min	80min
7	F	200% Primers+dNTPs+TdT	20min	40min	60min	80min
8	G	200% Primers+50% Extra dNTPs+50% Extra TdT	20min	40min	60min	80min
9	H	200% Primers+dNTPs+TdT	20min	40min	60min	80min
10	I	200% Primers+50% Extra dNTPs+50% Extra TdT	20min	40min	60min	80min

35

5µL Primer

2.2µL Primer

10µL Primer

50% less Primer = 50% Primer

1. Add 1 µl TdT (15 units/µl). Mix by gentle pipetting. (Still on ice)
2. Incubate at 37ºC for given time, see above, table B. (35)
3. Stop the reaction by bringing the solution to 95ºC for 20 minutes.
4. Then, bring to 20ºC for 1 min for handling
5. Prepare Agarose (Use Cobalt Chloride as a buffer for Gel)
   a. To volume 80mL of H2O and Buffer, add 1.2g Agarose
   b. Heat in increments in microwave until fully dissolved
   c. Pour into gel casting tray
   d. Place well template (comb) in correct slot
   e. Let cool
   f. More info: http://www.edvotek.com/Electrophoresis_Guide.pdf
6. Stain Samples with Cybr Green (pre stain, added to gel. 8µL of 10000X)
7. Run Gel @135V for 20 minutes. (D was run for 40min)
8. Analyze Gel on Gel Viewer
9. Record (Pictures, notes)

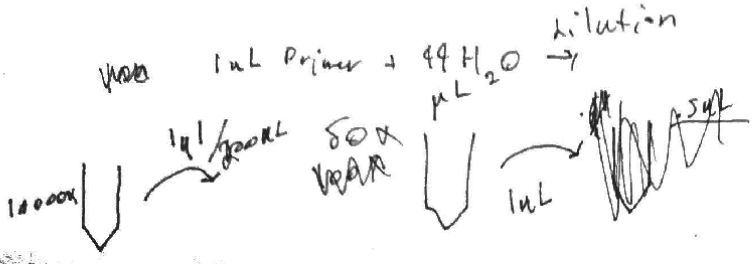
Alan Amount

An Alan amount is defined as follows:

> *Heads up - https://www.neb.com/protocols/1/01/01/a-typical-dna-tailing-reaction lists that you want 5pmols of DNA. That means you roughly should dilute 100x, and then add 5 microliters of that intermediate dilution. Math on this:*

> *The tube has 29.6 nanomoles of DNA suspended in 296 microliters of DNA. In each microliter, there is .1 nanomole of DNA. .1 nanomoles is 100 picomoles. Ergo, you want a twentieth of that.*

> *Your needs and concentrations might be different, so my math might not match yours. If your experiment didn't pan out due to too much DNA, try my suggestion. - Alan Tomusiak*



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.8

INb Ladder	A	B	C	D	E	F	G	H	I

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5	autoclaved, distilled water		(total volume to 50uL)

Amount water — Amount each substance — TdT

	A	B		C	D	E	F
1	Table B	Function		1	2	3	4 (Incubation Time)
2	A 10, 5, 7.5, 10	Just Primers (No TdT)	0	20min	40min	60min	80min
3	B 1, 5, 7.5, 10	Primers+50% Extra dNTPs+TdT	1	20min	40min	60min	80min
4	C 1.5, 5, 5, 10	Primers+dNTPs+50% Extra TdT	1.5	20min	40min	60min	80min
5	D 1, 5, 5, 10	Primers+dNTPs+TdT	1	20min	40min	60min	80min
6	E 1.5, 5, 7.5, 10	Primers+50% Extra dNTPs+50% Extra TdT	1.5	20min	40min	60min	80min
7	F 1, 2.5, 10, 10	50% Primers+dNTPs+TdT	1	20min	40min	60min	80min
8	G 1.5, 2.5, 15, 10	50% Primers+50% Extra dNTPs+50% Extra TdT	1.5	20min	40min	60min	80min
9	H 1, 10, 7.5, 10	200% Primers+dNTPs+TdT	1	20min	40min	60min	80min
10	I 1.5, 10, 10, 10	200% Primers+50% Extra dNTPs+50% Extra TdT	1.5	20min	40min	60min	80min

35 — 26.5 — 28.5 — 29 — 26 — 24.5 — 21 — 21.5 — 18.5

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5. Prepare Agarose (Use Cobalt Chloride as a buffer for Gel)
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1	Table B	Function	1	2	3	4 (Incubation Time)
2	A	Just Primers (No TdT)	20min	40min	60min	80min
3	B 150	Primers+50% Extra dNTPs+TdT	20min	40min	60min	80min
4	C 100	Primers+dNTPs+50% Extra TdT	20min	40min	60min	80min
5	D 100	Primers+dNTPs+TdT	20min	40min	60min	80min
6	E 150	Primers+50% Extra dNTPs+50% Extra TdT	20min	40min	60min	80min
7	F 100	50% Primers+dNTPs+TdT	20min	40min	60min	80min
8	G 150	50% Primers+50% Extra dNTPs+50% Extra TdT	20min	40min	60min	80min
9	H 100	200% Primers+dNTPs+TdT	20min	40min	60min	80min
10	I 150	200% Primers+50% Extra dNTPs+50% Extra TdT	20min	40min	60min	80min

start dTTPs

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