

SELEX NOTEBOOK

Date: 9/9/22

Friday

PCR CYCLE number :5

Nanodrop concentration of selex library(ss): **886.5 nanogram/microlitre**

Volume needed for 1 microgram of DNA template : **1.12 microlitres**

Forward and reverse primer aliquot: 10 micromolar concentration:
180 microlitres of nuclease-free water + 20 microlitre primer

Total reaction mixture volume: 25microlitre

10x buffer	2.5 microlitre
2.5miliomolar dNTPs	1.5 microlitre
10 micromolar Forward primer	1.25 microlitre
10 micromolar Reverse primer	1.25 microlitre
Taq polymerase	0.25 microlitre
DNA Library	1.12 microlitre
Total	25 microlitre

The above reaction mixture was made 4 times:

- a) Positive control(30X cycle): With template DNA
- b) Negative control(30X cycle): Without template DNA
- c) Positive control(10X cycle):With template DNA
- d) Negative control(10X cycle):Without template DNA

Values obtained after PCR Run for 1hr (30 cycles) and 22 minutes(10 cycles)

#	Sample Name *	ng/ μ L	A260/A280	A260/A230	A260	A280
1	Sample 1	887.9	1.45	0.73	17.76	12.24
2	Sample 2	644.6	1.05	0.37	12.89	12.30
3	Sample 3	899.5	1.34	0.63	17.99	13.40
4	Sample 4	811.4	0.56	0.24	16.23	28.77

Sample 1: Positive control (30 X)

Sample 2:Negative control(30X)

Sample 3:Positive control(10X)

Sample 4:Negative control(10x)

Notes to self:

- 1) Please note that the concentration is shown for 1 microlitre and the reaction volume is 25 microlitre. Each value gets multiplied by 25. Nanodrop for double stranded was done. PCR Amplification has taken place!
- 2) Purified version using protocol did not work

Agarose Gel preparation and loading:

Preparation: 3% gel

3% gel = 3 w/v= 3 grams of agarose in 100ml of TBE buffer(1X)

We used 50 ml; therefore agarose used= 1.5 grams.

And 50 ml of TBE buffer (1x)

ie total calculation= 1.5grams of agarose+ 50ml of 1X TBE buffer= 3%gel

0.5 microlitre- loading dye

Each sample was loaded as:

From left(Cathode black side) to right

Positive control(10 x cycle): 10 microlitre

Negative control (10 x cycle):10 microlitre

Positive control(30 x cycle):10 microlitre

Negative control(30 x cycle):10 microlitre

Selex library:10 microlitre

Ladder : 3 microlitre

Date: 14/09/22

Wednesday

Time: 00:08

Asymmetric PCR

(ALL VOLUMES IN MICROLITRES). Total mixture volume : 25 microlitre

Dividing 100 microlitre into 2 batches

Positive and negative

Positive control:

10 x buffer	2.5 microlitre
dNTPs 2.5mM	2
Taq polymerase	0.25
Forward primer	1.25(100microM stock)
Template	1
MQ water	19.25

Negative control:

10 x buffer	2.5 microlitre
dNTPs 2.5mM	2
Taq polymerase	0.25
Forward primer	1.25(100microM stock)
Template	nil
MQ water	18.25

Divide 10 microlitre stock into two batches:

Positive and negative

Positive control:

10 x buffer	2.5 microlitre
dNTPs 2.5mM	2
Taq polymerase	0.25
Forward primer	1.25(10microM stock)
Template	1
MQ water	19.25

Negative control:

10 x buffer	2.5 microlitre
dNTPs 2.5mM	2
Taq polymerase	0.25
Forward primer	1.25(10microM stock)
Template	0
MQ water	19.25

Divide 1 microlitre stock into two batches:

Positive and negative

Positive control:

10 x buffer	2.5 microlitre
dNTPs 2.5mM	2
Taq polymerase	0.25
Forward primer	1.25(1microM stock)
Template	1
MQ water	19.25

Negative control:

10 x buffer	2.5 microlitre
dNTPs 2.5mM	2
Taq polymerase	0.25
Forward primer	1.25(1microM stock)
Template	0
MQ water	19.25

Well loading:(6 microlitre(5 microlitre sample+ 1 microlitre of 6X loading dye)

100 positive:

100 negative

10 positive

10 negative

1 positive

1 negative

Common negative(Without template)

Ladder: 3 microlitre

SYNTHESIZING DNA LIBRARY (After-steps)

Overflow of our experiment:

79 bases=79*3 =4 hrs approx for synthesizing

Step 1: Fit the resin in the column. The resin provides a matrix for the synthesisation

Step 2: Add 750 ml of NH4OH and 750 ml (1:1 AMA)=TOTAL : 1.5 ml of AMA solution

Step 3: Incubate for 65 degree celsius for 30 min

Bring to room temperature or place in ice-bath

Step 4: Take the liq part and put in 2 ml Solution

Step 5: Go for speedvac. Time= infinity. Make sure to open the column caps

Step 6: Resususpend in 200 microlitre of water.

Step 7: Purify library by desalting

Step 8: Take nanodrop reading and run gel to verify .

Desalting procedure:

Step 1: Column Wash- Add 2 ml of 100%ACN to the columns (Number of times depend upon quantity and quality

Step 2: Vacuum pump and drain ACN

Step 3: Add 1 ml of 2M TEAA to the column

Step 4: Vacuum out the TEAA

Step 5: Add the library obtained after synthesis

Step 6: Now add 2 ml of 0.1 M TEAA and wash the library with it. Vacuum out the TEAA

Step 7: Give a water wash (2 ml)

Step 8: DNA elution with 0.5ml of 10% ACN and repeat the step for 2 times

Step 9: Collect the elute

Step 10: overnight Speedvac and suspend in 200 microlitre of DEPC water.

PCR after purification:

Date: September 29,2022

25 microlitre reaction

Protocol same

Cycles: 7 and 10

Nanodrop reading:

Negative 7 cycle: 551 ng/uL
Positive 7 cycle:3082.3 ng/uL
Negative 10 cycle:686.3 ng/uL
Positive 10 cycle:1263.8 ng/uL

8 PERCENT UREA PAGE:

Date: September 30,2022

Protocol:

40% polyacrylamide: 2.5 mL

10X TAE: 1ml

Miliq: 6.5 ml

APS: 90 uL

TEMED: 10uL

RUN TIME: 50 min

Voltage: 120 V

Asymmetric pcr

Attempt 2 after synthesis

Date: October 2,2022

2 positives

- a) Forward primer (10mM)- 1.25ul
- b) Forward

2 negatives