Sample ID: Banana Slug (BS) Date: 9/28/19
Mate-Pair 2x125 bp - Eve's Sample

Sample Information

Lab: Fourmand
Concentration: 255 ng/uL
Amount/ Volume used for preparing Library : 30µg / 118µL

Shear the DNA

Insert Size	Shearing Method	Hydroshear Speed Code/ Covaris S2 Protocol
4-5 kb	Hydroshear	Standard - Assembly SC 15, 5 cycles, 150 µL

1. If you are using the **Covaris™ S2 System**:

Component	Volume (µL)	Sample
99% Glycerol	100	
10 to 20 µg DNA	X	
Nuclease-free water	Up to 500	
Total	500	

2. If using the **HydroShear®**: - Some DNA does not get sheared (Common results for hydroshear)

Component	Tube 1	Tube 2
DNA (ug)	15	15
DNA (µL)	59	59
Nuclease Free Water (µL)	66	66
Total	125	125

Purify the sheared DNA using the QIAquick Gel Extraction Kit (QIAquick spin columns have a 10-µg capacity) ➔
This clean up removes Unsheared Genomic DNA

Component	Volume (µL)
Sample	
Buffer QG (3X)	
Isopropanol (1X)	
Final Sample	
Per Column	
Volume per column (times)	
Elution	

not done

Product	Lot. N°
Kit	
Buffer QG	
Buffer PE	
Buffer EB	
Column	

Nanodrop:_____ng/µL – x_____ µL= _____ng (total yield) _____µg (total yield)

- This step removes Unsheared DNA (Genomic DNA), so you don't waste end-repair reagents in the next step

Repair the DNA Ends

Components	Volume (µL)	Sample
Sheared DNA	x	217
10X End-It Buffer	30	40
End-It ATP (10 mM)	30	40
End-It dNTPs (2.5 mM)	30	40
End-It Enzyme Mix	10	12
Nuclease-free water	Variable	51
Total	300	400

Lot. N°
-
E85-81001
E85-81001
E85-81001
E85-81001
0903130
-

Incubate the mixture at room temperature for 30 minutes.

Sample ID: BS Date: 9/28/9

rify the DNA using the QIAquick Gel Extraction Kit.

Component	Volume (µL)
Sample	400
Buffer QG (3X)	1200
Isopropanol (1X)	400
Final Sample	2000
Per Column 3	666
Volume per column (1 times)	666
Elution 2×30 = 60×3	180

Product	Lot. N°
Kit	
Buffer QG	
Buffer PE	
Buffer EB	
Column	

Nanodrop: 83.73 ng/µL – x 180 µL= 15,071 ng (total yield) 15 µg (total yield)

Methylation of the Genomic EcoP15I Sites

Component	Volume (µL)	Sample
Sheared, end-repaired DNA	x	180
10X NEBuffer 3	25	25
100X BSA	2.5	2.5
EcoP15I Enzyme (10 U/µL)	x	15
S-adenosylmethionine (32 mM)	3	3
Nuclease-free water	Variable	26.5
Total	250	250

Lot. N°
–
0020801
0207
0030901
0740905
0903130
–

100 U – 1 µg
150 U – 15 µg

10 U – 1 µL
150 U – x
x = 15 µL

Incubate the methylation reaction mixture at 37 °C for 2 hours or overnight overnight (3:30 pm)

Note: Use a final concentration of at least 360 µM S-adenosylmethionine and 10 U of EcoP15I enzyme per 1 µg of end-repaired DNA. Adjust the final volume to 250 µL with nuclease-free water. The above reaction is set up for approximately 20 to 25 µg of DNA derived from 30 µg of starting input DNA. If the amount of starting input material is >30 µg, the reaction composition (based on the amount of enzyme needed) must be modified. Typically the final reaction volume is at least 10 times the enzyme volume.

Purify the methylated DNA using the QIAquick Gel Extraction Kit. – 9/29/9

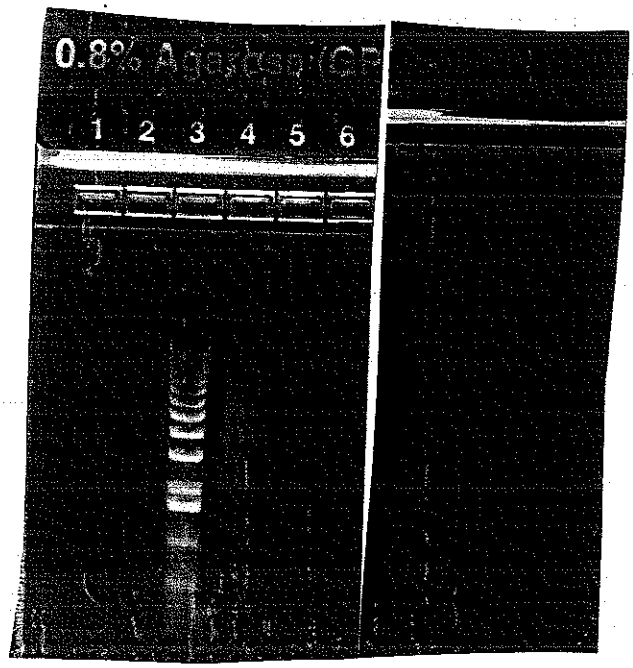
Component	Volume (µL)
Sample	250
Buffer QG (3X)	750
Isopropanol (1X)	250
Final Sample	1250
Per Column 2	625
Volume per column (1 times)	625
Elution 2×32 = 64×2	128

Product	Lot. N°
Kit	
Buffer QG	
Buffer PE	
Buffer EB	
Column	

Nanodrop: 97.01 ng/µL – x 128 µL= 12,417.28 ng (total yield) 12.4 µg (total yield)

Ran E-Gel 0.8%. After Methylation = used = 2.6µL = 252 ng for checking shearing profile.

re


1 2 3 4 5 6

Sample ID: B5 Date: 9/29/09

ation of the EcoP15I CAP Adaptors to the Methylated DNA

Calculate how many pmoles of EcoP15I CAP adaptors (ds) are needed, first calculate the picomoles of the insert DNA based on its size as follows: targeted multiple sizes, but used 2-3 kb for calculations.

(1µg DNA) X ($\frac{10^6 \text{ pg}}{\mu g}$) X ($\frac{1 \text{pmol}}{660 \text{pg}}$) X ($\frac{1}{2500 \text{ insert size in bp}}$) = 0.61 pmoles/µg DNA

(12.4 #µg DNA used) * (0.61 pmoles/µg DNA) = 7.52 # pmoles DNA in sample
(# 7.52 pmoles DNA in sample) * (100) = 752 # pmoles EcoP15I CAP adaptors needed
(# 752 pmoles adaptors needed) / (50 pmoles/µL EcoP15I CAP adaptors reagent) = 15 # µL EcoP15I CAP adaptor

Component	Volume (µL)	Sample	Lot. N°
EcoP15I CAP Adaptor (ds) (50 pmole/µL)	X	15.	0803006
2X NEB Quick Ligase buffer	150	150.	0010812
NEB Quick Ligase	7.8	7.8	1080906
DNA	Y	123	—
Nuclease-free water	Variable	4.2	0903130
Total	300	300	—

Incubate at room temperature for 10 minutes.

Note: If a larger reaction volume is required to incorporate all of the methylated DNA, scale up the Quick Ligase and Quick Ligase buffer. Add 1 µL of Quick Ligase per 40 µL of reaction volume. Add 1 µL of 2X NEB Quick Ligase buffer per 2 µL of reaction volume.

Purify the DNA using the QIAquick Gel Extraction Kit.

Component	Volume (µL)
Sample	500
Buffer QG (3X)	900
Isopropanol (1X)	300
Final Sample	1500
Per Column 2	750
Volume per column (1 times)	750
Elution ~~3×33 = 64×2~~	128

2×18 = 30×2 = 60µl

Product	Lot. N°
Kit	
Buffer QG	
Buffer PE	
Buffer EB	
Column	

Nanodrop: ~~no~~ done ng/µL – x 128 µL= _____ng (total yield) _____µg (total yield)

Size Selection of DNA with a 0.8-1% Agarose Gel

Desired Insert Size	Percentage of Agarose Gel Needed	Sample
600 bp to 3000 bp	1.0%	
→ 3 kb to 6 kb	0.8%	—

0.48g - 60mL + Et Br (7µl) 1XTAE

60µl sample - 6µl 10x loading dye = 66µl
54µl sample - 5.4µl 10x = 59.4µl (19.8 µl p/well) = loaded all in 2 wells (repeated)

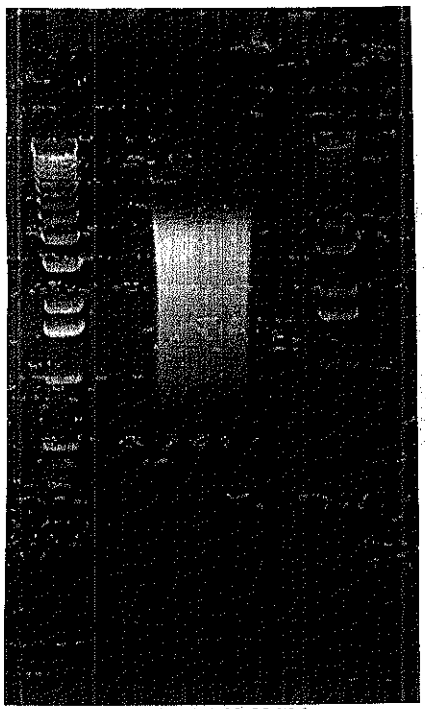
1Kb - 4µl + 20µl EB + 2.4µl 10x = 26.4µl ÷ 2 = 13.2 µl p/well

3-4

2-3

1-2

4-6 kb



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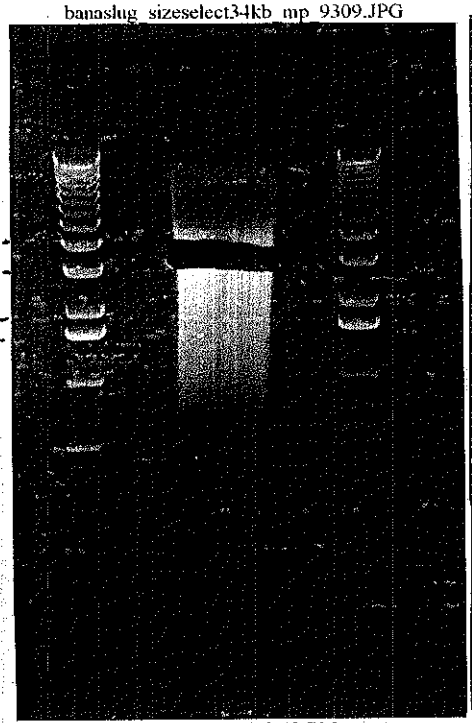
banaslug_sizeselect34kb_mp_9309.JPG



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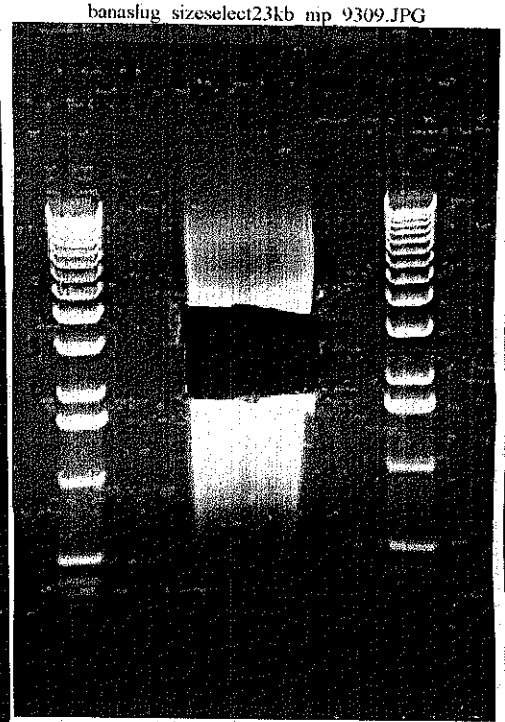

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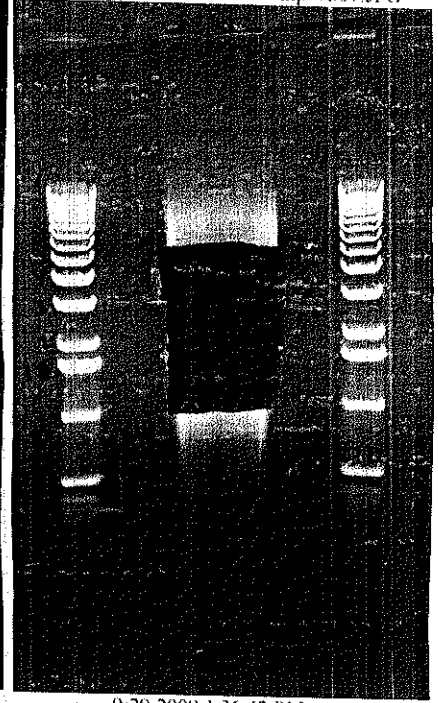

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Sample ID: BS Date: 9/29/9

Elute the DNA from the agarose with the QIAquick Gel Extraction kit

- Place each gel portion into a 15-mL conical polypropylene tube that is large enough to hold 3 times the volume of the gel. If the gel piece is large, slice it up before placing it into the tube.
- Tare the balance with an empty tube of the same kind (327 mg) 3-4 ICS Gel cut

→ Also processed other fragment sizes - check on the bacca

Component	Volume (µL)
Sample	327
Buffer QG (3X)	981
Isopropanol (1X)	327
Final Sample	1635
Per Column 1	1635
Volume per column (3 times)	550
Elution 2x32	64

Product	Lot. N°
Kit	Y278 Y577
Buffer QG	Y248054/
Buffer PE	Y248285 3
Buffer EB	Y248 2332
Column	130186139

Note: Don't forget to wash with extra 500 µl of buffer QG after loading the column with the sample

Nanodrop: 23.70 ng/µL – x 64 59 µL= 1398.3 ng (total yield) 1,4 µg (total yield)

Circularize the DNA 1/2 Akram / 1/2 Eve
29.5 w/ 29.5 w/

Using Table 2-2 below, determine the concentration of DNA needed to achieve intramolecular ligation of 95% of the sample based on the insert size of your library.

Table 2-2 Final concentration of DNA required for obtaining maximum circularization efficiency.

Insert Size	Final Concentration of DNA for Circularization
500 to 700 bp	4.3 ng/µL
700 to 1000 bp	3.75 ng/µL
→ 1 to 2 kb	2.74 ng/µL
→ 2 to 3 kb	2.1 ng/µL
→ 3 to 4 kb	1.8 ng/µL
→ 4 to 5 kb	1.6 ng/µL
5 to 6 kb	1.4 ng/µL

Product	Lot. N°
2X NEB Quick Ligase Buffer	0010812
Internal Adapter	0803006
NEB Quick Ligase	0980906

Based on the desired insert size, use the following table to prepare a 95% circularization reaction for every 1 µg of library. When using more than 1 µg of DNA for circularization, modify the reaction appropriately. For higher circularization efficiency, it is important to increase the amount of Quick Ligase enzyme in proportion to the volume (that is, 1 µL enzyme per 40 µL reaction volume):

494ng 624ng 699.15g 659ng

Components	500 to 700 bp	700 to 1000 bp	1 to 2 kb	2 to 3 kb	3 to 4 kb	4 to 5 kb	5 to 6kb	Sample
DNA	1 µg	1 µg	1 µg 30 w/	1 µg 29 w/	1 µg 29.5	1 µg 30.5	1 µg	
2X NEB Quick Ligase Buffer	117.5 µL	135 µL	182.5 µL	250 µL	280 µL	312.5 µL	360 µL	
Internal adaptor (ds) (2pmoles/ µL)	3.75 µL	2.84 µL	1.5 µL	0.9 µL	0.65 µL	0.5 µL	0.4 µL	
NEB Quick Ligase	6 µL	6.75 µL	9 µL	12.5 µL	14 µL	15.6 µL	18 µL	
Nuclease-Free water	Variable	Variable	Variable 142 w/	Variable 207.6	Variable 235.85	Variable 265.9	Variable	
Total	235 µL	270 µL	365 µL	500 µL	560 µL	625 µL	720 µL	

Note: Determine the quantity of nuclease-free water needed. Add the water to the sample, and then add the remaining reagents in order. Thoroughly mix all the components and finally add the enzyme to the reaction.

Incubate at room temperature for 10 minutes.

library ?

BS 1-2 kb	BS 2-3 kb	BS 4-5 kb	BS 3-4 kb
0.509 g	0.379 g	0.293 g	0.327 g
2 columns	1 column	1 column	1 column
QG 1527 µl	1137	879 µl	981 µl
ISO 509 µl	379	293 µl	327 µl
Total 2545 µl	1895	1465 µl	1635 µl
÷ 2 columns	1 column	1 column	1 column
1272.5 µl/col.	1895 µl column	1465 µl	1635 µl
÷ 650 µl	÷ 650 µl	÷ 650µl/7yo	÷ 550µl
loaded 2x 650 each	loaded 3x 650 each	loaded 2x 740 µl	loaded 3X 550 µl
eluted 2x 17µl each	eluted 2x 32µl	eluted 2x32µl	eluted 2x 32µl
Total = 68 µl	Total = 64 µl	Total 64 µl	59 actually Total 64µl
16.48 ng/µl	21.53 ng/µl	21.61 ng/µl	29.70 ng/µl
1120.64 ng / 1,1 ng	1377.92 ng / 1,3.7ng 1,37ng	1383.04 ng / 1,3.8 ng	1398.3 ng / 1,4ng
1/2 Akram	1/2 Akram	1/2 Akram	1/2 Akram
60 % 2 = 30µl Akram	58 % 2 = 29 µl Akram	61 % 2 = 30.5 Akram	29.5 µl

Gel extraction kit Purification - After Circularization

	BS 1-2 KB	BS 2-3 KB	BS 3-4 KB	BS 4-5 KB
	1	1	1	1
QG	365 ul	500 ul	560 ul	625 ul
Isopropanol	1095 ul 365 ul	1500 ul 500 ul	1680 ul 560 ul	1875 ul 625 ul
Total	1825 ul	2500 ul	2800 ul	3125 ul
	700 ul	700 ul	700 ul	700 ul
	2.6 times	3.6 times	4 times	4.5 times
÷				
loaded column				
	1	1	1	1
Eluted	2x 32 ul	2x 32 ul	2x 32 ul	2x 32 ul
Total	60 ul	60 ul	60 ul	60 ul

Plasmid Safe

Component	BS 1-2 KB ul	2-3 KB ul	3-4 KB ul	4-5 KB ul
ATP	5	5	5	5
Plasmid safe Buffer	10	10	10	10
DNAse	0.3	0.3	0.3	0.3
DNA	60	60	60	60
Water	24.7	24.7	24.7	24.7
Total	100	100	100	100

Incubated 37°C for 40 minutes (Qiagen)

Qiagen Gel Extraction kit After Plasmid Safe

	1-2 kb	2-3 kb	3-4 kb	4-6 kb
DNA	100	100	100	100
QG	300	300	300	300
Isopropanol	100	100	150	100
	500	500	500	500 — 1 column
Total Eluted	2x 27ul /	2x 27ul /	2x 27ul /	2x 27ul = 54 ul /
	3.41 ng/ul x 50	3.58 ng/ul x 50	3.93 ng/ul x 50	3.22 ng/ul x 50
	170.5 ng	179 ng	196.5 ng	161 ng

196.5 ng → Continue sample prep.

Goal: Minimum 200 ng to continue sample preparation

Eco P15I Digestion

1-2 kb (1 tube)

$171\text{ ng DNA} \times \frac{10\text{ U}}{100} \times \frac{1}{10} =$

= 1.71 ul of Eco P15I

2-6 kb (3 tubes)

2-3 kb: $\frac{179 \times 5}{1000} =$ 0.895 ul enzyme

3-4 kb: $\frac{197 \times 5}{1000} =$ 0.985 ul enzyme

4-6 kb: $\frac{161 \times 5}{1000} =$ 0.805 ul enzyme

	1-2 kb ul	2-3 kb ul	3-4 kb ul	4-6 kb ul
	~~50~~ 48	~~50~~ 48	~~50~~ 48	~~50~~ 48
DNA	10	10	10	10
10x NEB 3	1	1	1	1
100x BSA	1	1	1	1
Sinefungin	20	20	20	20
10x ATP	1.71	0.89	0.985	0.805
Eco P15I	16.29	19.1	19.1	19.2
Water	100	100	100	100
Tot				

37°C / Overnight PCR Machine

Digest circularized DNA II (4 samples 1-2 kb, 2-3 kb, 3-4 kb, 4-6 kb)

Same Tube — PCR Machine

Eco 151 - Digested DNA	100
10mM Sinefungin	1
10X ATP	2
Eco 7151	0.5
	103.5

Incubated 37°C 1h, 65°C 20 minutes, ice 5 minutes.

End-Repair Klenow (4 samples)

Same Tube - PCR Machine

Eco Digested DNA	103.5
dNTP Mix	1.5
DNA Polymerase, Klenow	1
Total	106

Room Temperature - 30 minutes
Denatured 65°C - 20 minutes
Ice - 5 minutes.

Streptavidin Binding Buffer

Tris-HCl, pH 7.5 500mM

1M Tris. $V_1 = 0.5(500\,mM).100$
$V = 50\,\mu l$ Tris (1M) / 50µl Nuclease free water

		Lot No.
0.5M Tris -	20 ml	- 118218
5M Sodium chloride -	400 ml	- 0710003
0.5M EDTA	- 2 ml	- 0805005
Nuclease free water	- 578 ml/1000 ml	

Bind Library to MyOne T1 Streptavidin Beads

1x BSA

	Lot N°
100x BSA = 20 µl	- 0207
Nuclease free water = 1980 µl	
2000 µl	

Beads Cat N° = 656.02
Lot N° = G22404

1x Quick Ligase Buffer

	Lot N°:
2x Quick Ligase (NEB) = 1200 µl	- 0010903
Nuclease free water = 1200 µl	
2400 µl	

Ligate P1 and P2

1-2 kb

$1 \times \frac{10^6}{1} \times \frac{1}{660} \times \frac{1}{1536}$

$\frac{10^6}{1,013,760} = 0.98$ pmol/µg DNA

1 pmol/µg DNA

$0.17\,\mu g \times \frac{1}{1} \times 30 \times \frac{1}{50}$

= 0.1 µl P1 / 0.1 µl P2

0.2 µl P1
0.2 µl P2

2-3 kb

$\frac{10^6}{660 \times 2536} = 0.6$ pmol/µg DNA

$\frac{0.18\,\mu g \times 0.6 \times 30}{50}$

0.064 µl adapter needed.

3-4 kb

$\frac{10^6}{660 \times 3536} = 0.4$

$\frac{0.2\,\mu g \times 0.42 \times 30}{50}$

0.05 µl adapter

4-6 kb

$\frac{10^6}{660 \times 4536} = 0.$

$\frac{0.16 \times 0.33 \times 30}{50}$ (0.03 µl)

0.2 µl P1 / P2 all reactions

Ligate P1/P2 Adaptors

4 reactions

	µl	Lot No.
DNA Bead-complex	97.5	0806011
P1 adaptor	0.2	P1-0806011
P2 adaptor	0.2	P2-0806010
Quick ligase	2.5	98/1108
	100 µl	Ligase from Robot kit.

Wash DNA-bound beads

		Lot No.
10X NEB 2	60 x 4 = 240	1207
Water	540 x 4 = 2160	

Nick-translation (4 samples)

		Lot No.
DNA Bead Complex	96 µl	
dNTP Mix	2 µl	
DNA Polymerase I	2 µl	86
	100 µl	

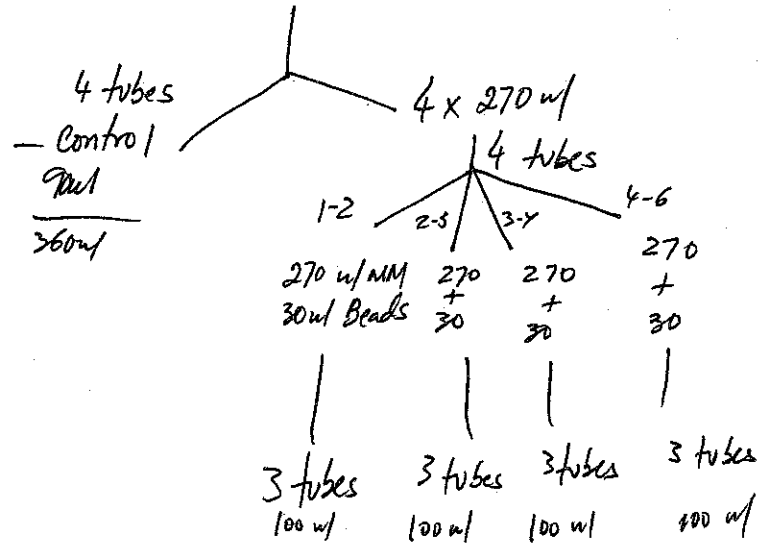
Left Beads in 30µl EB at 4°C. (over weekend)

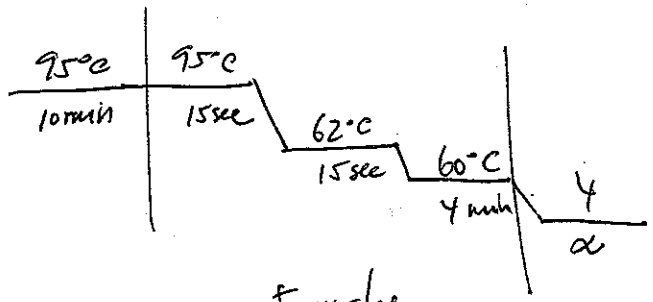
16°C - 30 minutes

	Lot No.
1x Bead Wash	0805007
1x Bind Wash	0809008

PCR Amplification 10/9/9

		x 4 Samples
Platinum PCR Super Mix	= 200	= 800
Library Primer 1	= 8	= 32
Library Prime 2	= 8	= 32
Cloned Pfu	= 1	= 4
Water	= 143	= 572
Total	360	1440





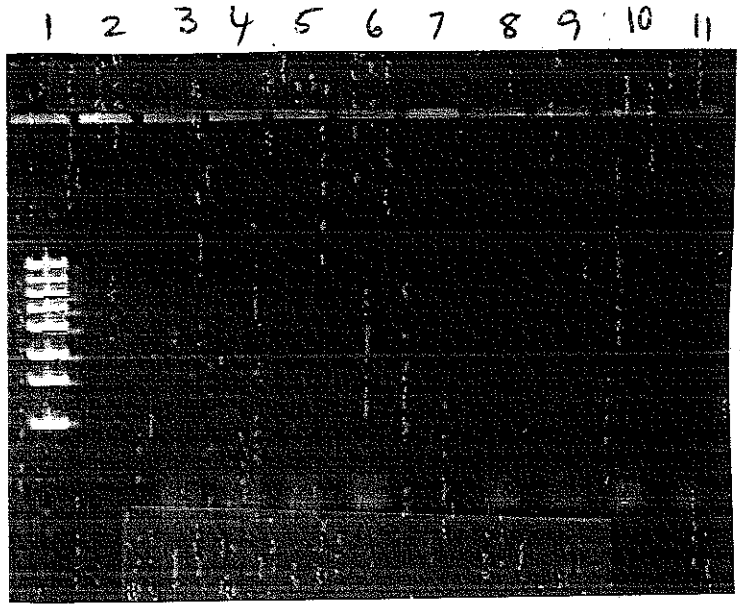
PCR 9700 - Gold Block

5 cycles

⇓

Lonza Gel ⇒ Loaded 4ul of PCR product from each sample + Controls (-)

Lonza - 5 cycles - No Amplification was observed (10/9/19)



1 - 100 - 4Kb ladder
2 - Blank
3 - 1-2 Kb
4 - 2-3 Kb
5 - 3-4 Kb
6 - 4-6 Kb
7 - Blank
8 - Control - 1-2 Kb
9 - Control - 2-3 Kb
10 - Control - 3-4 Kb
11 - Control - 4-6 Kb

PCR - 5 More cycles

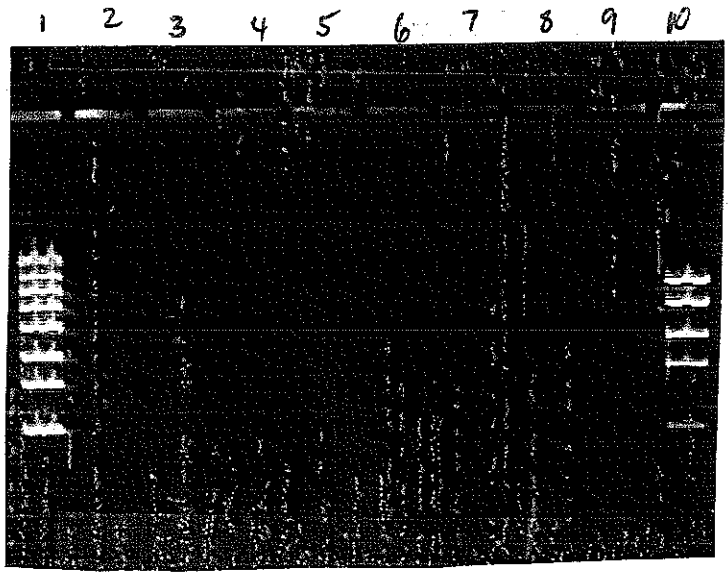
95°C 15 sec → 62°C 15 sec → 60°C 4 min. → 4 ∞

5 cycles

PCR 9700 - Gold Block

Total 10 cycles

Lonza Gel



1 - 100 - 4Kb ladder
2 - 1-2 Kb - Positive Band
3 - 2-3 Kb - Positive Band
4 - 3-4 Kb - Positive Band
5 - 4-6 Kb - No Band
6 - 1-2 Kb - Control
7 - 2-3 Kb - Control
8 - 3-4 Kb - Control
9 - 4-6 Kb - Control
10 - Quant ladder

4-6 Kb - Mate Pair

⇓

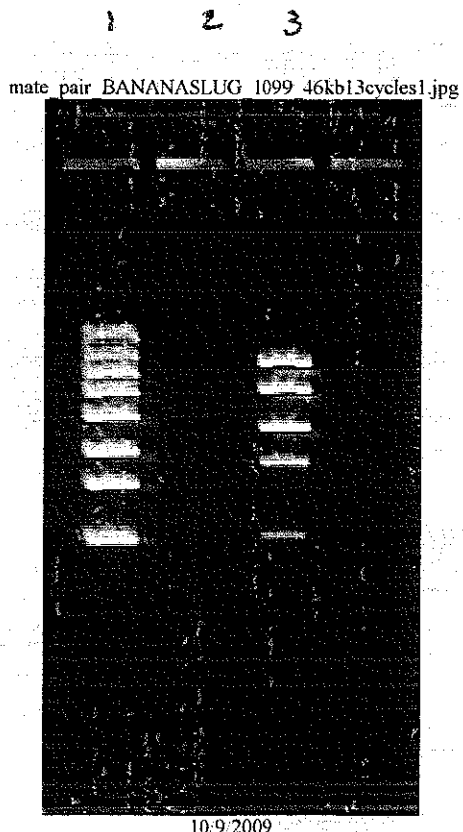
PCR - 3 more cycles ⇒ Total 13 cycles

95°C
15sec
62°C
15sec
60°C
4min
4°C
∞
3 cycles

PCR 9700 - Gold Block

⇓

Lonza Gel



1 - 100-4Kb ladder
2 - 4-6 Kb - Positive Band
3 - Quant ladder

All samples were purified according to Mod Solid.

Samples were magnetic beads separated – Removed Beads

↓

Supernatant was purified with Qiagen gel Extraction kit

↓

All samples were considered as containing 292 ul

	ul
Sample	292
QG	876
Isopropanol	292
	1460
÷	730 ul

loaded 730 µl per column / 2 columns each sample

1-2 kb	2-3 kb	3-4 kb	4-6 kb
2 columns	2 columns	2 columns	2 columns
730 ul each	730 ul each	730 ul each	730 ul each
Eluted each 2x15 ul = 30	Eluted 2x15 ul = 30	Eluted 2x15 ul = 30	Eluted 2x15 ul = 30
60 ul	60 ul	60 ul	60 ul

2 columns samples were kept at -20°C.

↓

4% Gel – Remove Self ligated Adaptors

Banana Slug - Mate-Pair 9/23/9

Shearing Conditions for obtaining 5-6 kb fragments

Instrument: Hydroshear
Program: Large-Assembly 5-6 kb

① Sc 16
cycles 20
volume 150 µl

② SOLiD Sc 16
cycles 25
volume 150 µl

DNA - 1µg of DNA sheared - 2 tubes (each 1µg)
Banana slug DNA extracted 9/22/9 - 250 ng/µl
4µl of DNA = 1µg

Tube 1 = 4µl Banana slug DNA
12µl Nuclease free water (NFW)
↓
Condition ① - 20 cycles

Tube 2 = 4µl Banana slug
12µl NFW
↓
Condition ② - 25 cycles

After Shearing DNA was concentrated → purification using Qiaquick gel extraction kit.

QG = 375 µl
Isopropanol = 125 µl
Total = 625 µl
1 column

Eluted = 8µl x 2 = 16µl

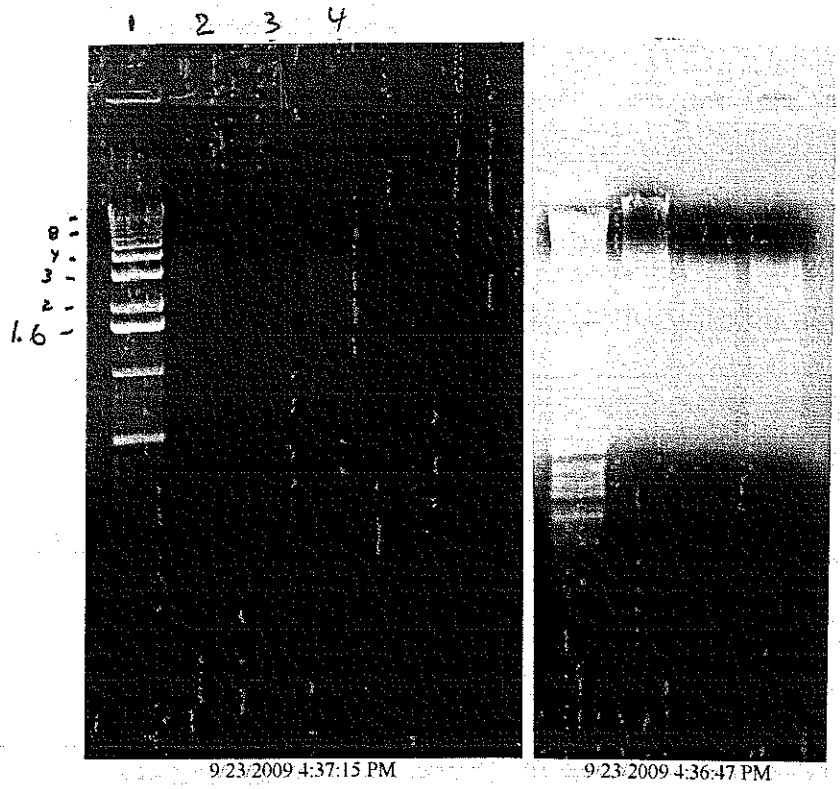
Genomic DNA = 300 ng

BsaI (Nanodrop) = 16.98 ng/ul x16 = 272 ng (lost = 72.8%) (started 1000 ng)

BsrI (Nanodrop) = 22.79 ng/ul x16 = 364 ng (lost = 63.6%) (started 1000 ng)

Gel – 0.8% 0.5X TBE (EtBr)

1) 1Kb
2) Genomic DNA
3) Banana Slug I (20 cycles)
4) Banana Slug II (25 cycles)



DNA seems concentrated between 3-4 Kb either for 20-25 cycles.

Banana Slug Shearing Conditions 9/24/19

Genomic DNA = 255 ng/ul

Used 8ul = 2ug

Mixed 8ul DNA with 117 ul H2O

⇓

125 ul (2 tubes)

1 — 150 ul, 16 speed code, 30 cycles

2 — 150 ul, 16 speed code, 40 cycles

Hydroshear

Large Assembly

1 → 890 ng (7.12 ng/ul)

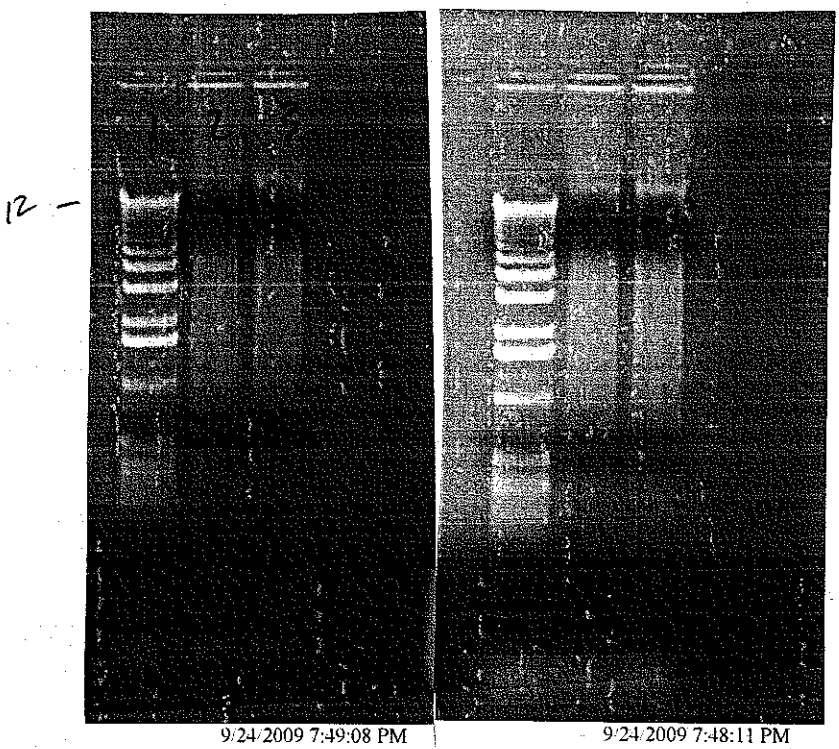
2 → 786 ng (6.28 ng/ul)

Nanodrop

Concentrated with speed vacuum – 1h

Gel – 0.8% 1X ~~TAE~~ + ~~ET~~ Br

R1: Out of 2ug we recovered about 800 ng after shearing (No Purification performed). So, we are loosing a lot of DNA during the shearing.



1) 1Kb ladder
2) 30 cycles
3) 40 cycles

L2: The Genomic DNA was not completely sheared. (The band above 12 Kb). We don't see this band anymore after purification with Qigen kit. (This result is expected for shearing with hydroshear).

Banana Slug - Shearing Conditions 9/24/9

Genomic DNA - 255 ng/ul

↓

Used 2ug = 8 ul of DNA

Mixed 8 ul of DNA with 117 ul of nuclease free water

2 tubes

1

2ug DNA

130 ul

16 speed code

25 cycles

Hydroshear conditions ⇒

Large Assembly

2

2ug DNA

150 ul

16 speed code

30 cycles

Qiagen Qiaquick Gel extraction kit

QG = 325 ul

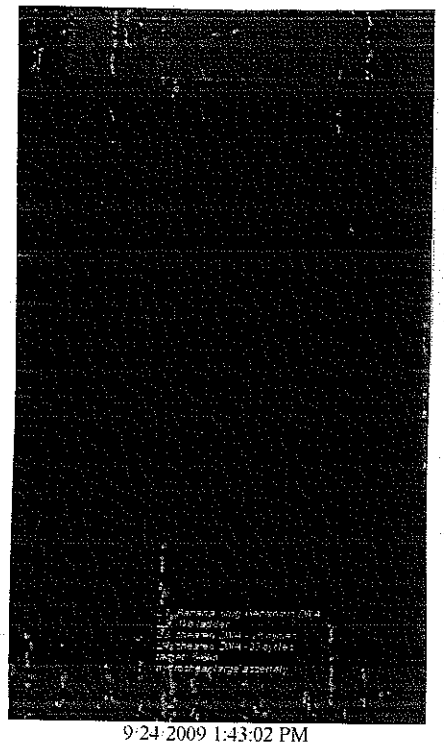
Isopropanol = 125 ul

Eluted = 2 x 9 ul = 18 ul

Qiagen Qiaquick Gel Extraction kit

Gel = 0.8 %. made with 1x TAE + Ethidium Bromide (7ul to 60 ml)

1) 1Kb ladder
2) Banana Slug Genomic DNA + 3.5 ul 6x loading dye
3) Shearing 1 - 25 cycles (16 ul sample + 3.5 ul 6x loading dye)
4) Shearing 2 - 30 cycles



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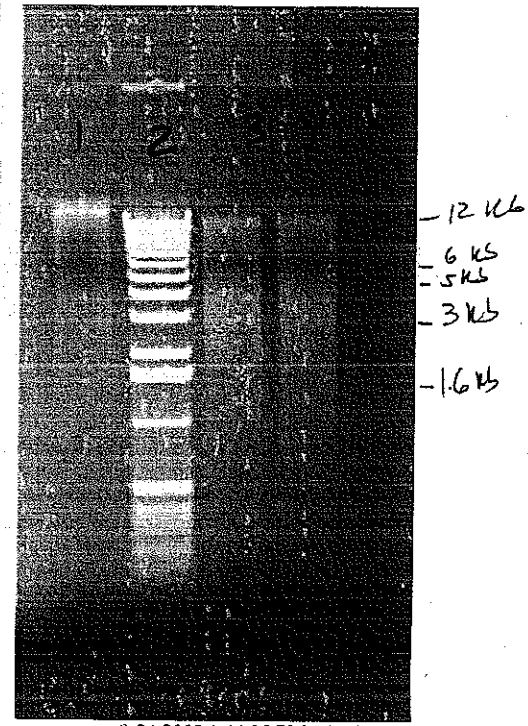

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1) Genomic DNA
2) 1 KB ladder
3) Banana slug 1 - 42.66 ng/ul x 18 ul = 768 ng (lost 62%)
4) Banana slug 2 - 44.85 ng/ul x 18 ul = 807 ng (lost 60%)

DNA seems concentrated between 3-4 KB
We might be able to collect between 11-12 KB
There is a slight difference between the amount of DNA we lose between 25 cycles and 30 cycles (30 cycles loses less DNA)
After Qiagen Purification we don't recover fragments above 12 KB. ~~[illegible]~~
What happens if we don't purify?

Banana Slug

9/25/19

Hydroshear

Tested Standard Assembly – Conditions for obtaining 4-5 kb
Standard Assembly – speed code 15 - 5 cycles - 125 µl.

Tested a few samples and conditions.

1) Used Human DNA as control for shearing condition – 2ug each sample
   1.1) Sheared human DNA using large assembly for testing conditions to obtain fragments between 5-6 kb
   sc 16, 25 cycles, 150 µl. (2µg)
   1.2) Sheared human DNA using standard assembly for obtaining
   1.2.1) 4-5 kb – sc 15, 5 cycles, 150 µl (SOLiD Manual) (2µg)
   1.2.2) above 5 kb – sc 19, 5 cycles, 150 µl (Hydroshear manual) (2µg)

2) Used Banana Slug DNA – 2µg each tube
   Tested Standard Assembly for obtaining:
   2.1) 4.5 kb - sc 15, 5 cycles, 150 µl (SOLiD manual) - 2µg
   2.2) above 5 kb - sc 19, 5 cycles, 150 µl (Hydroshear manual) - 2µg

DNA – 2µg (8 µl – Slug / Human)
+ 117 µl H_2O

Total = 125 µl

Washing conditions: 2 washings with solution I
2 " solution II
3 " 4 solution III (Water)

Quantitate DNA after shearing

x125

SA { BS sc15 5 cycles - 12.71 ng/ul = 1588.75
SA { BC sc19 5 cycles - 12.03 ng/ul = 1503.75
LA - Human sc16 25 cycles LA 12.36 ng/ul = 1663,75
SA { Human sc15 5 cycles - 13.31 ng/ul = 1663.75
SA { Human sc19 5 cycles - 12.73 ng/ul = 1591,25

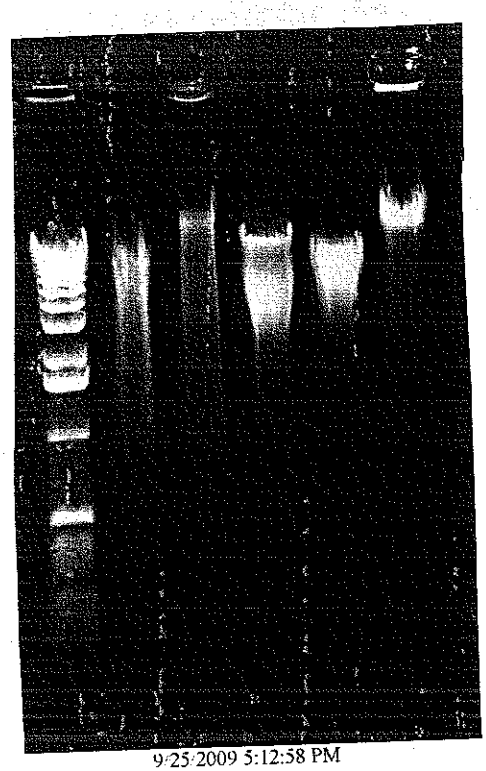
↓

After Quantitation - Speed Vacumeed sample for 30 minutes (which was too much)

↓

Run Gel - 0.8% 0.5X TBE + ~~ET~~Br

1) BS sc15 5 cycles SA
2) BS sc19 5 cycles SA
3) Human sc15 5 cycles SA
4) Human sc19 5 cycles SA
5) Human sc16 25 cycles LA



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Gel Purify Library 10/16/09

4% Agarose Gel(LE) in 1x TAE

150 ml Gel – $\frac{150 \times 4}{100}$ = 6g of Agarose in 1x TAE

1-2 Kbs = 60µl + 6µl 10x Loading dye
2-3 Kbs = 60µl + 6 µl "
3-4 Kbs = 60µl + 6µl "
4-6 Kbs = 60µl + 6µl "

loaded 3 wells each with 20µl of sample dye mixture

2µl Tracmit 25 bp ladder + 2µl loading dye + 16µl EB

Band 154-156 bp

25bp
3µl ladder
10µl EB

Gel 1
1st Well

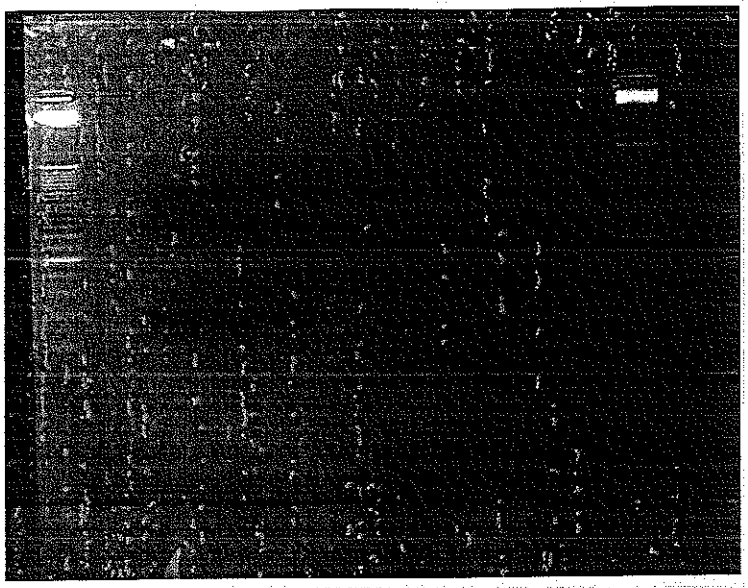
25 bp | 1-2 1-2 1-2 | 2-3 2-3 2-3 | 25 bp | |

Gel 2
1st well

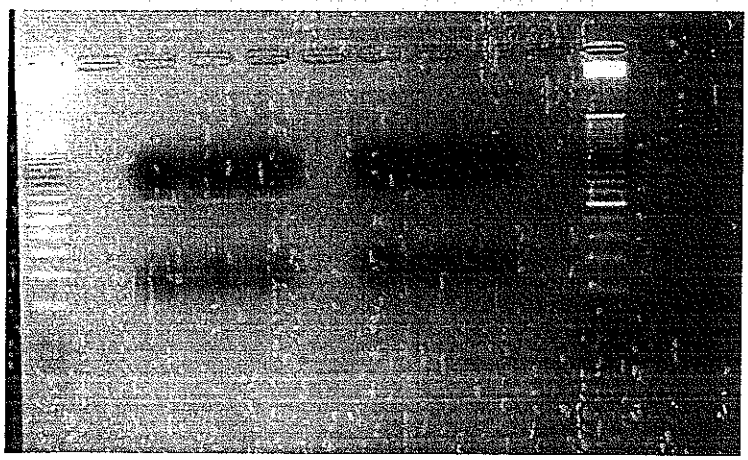
| 25 bp | 3-4 3-4 3-4 || 4-6 4-6 4-6 || 25 bp

Gel 1

banaslug_finalsizesel_mp_10169_2.JPG



1- 25 bp ladder
2- Blank
3, 4, 5 > 1-2 KB
Exposure 545 ms
6 - Blank
7, 8, 9 > 2-3 KB
10 - Blank
11 - 25 bp
12 - Blank



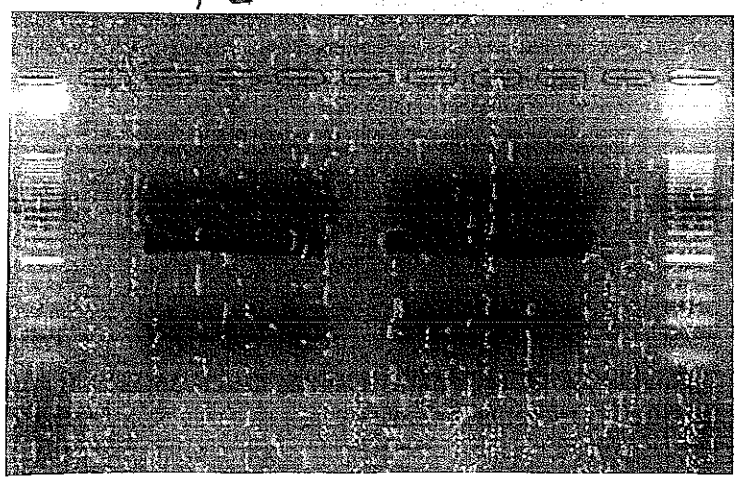
Exposure 1.9 s
Same picture as above

120 V 45 min
1X TAE

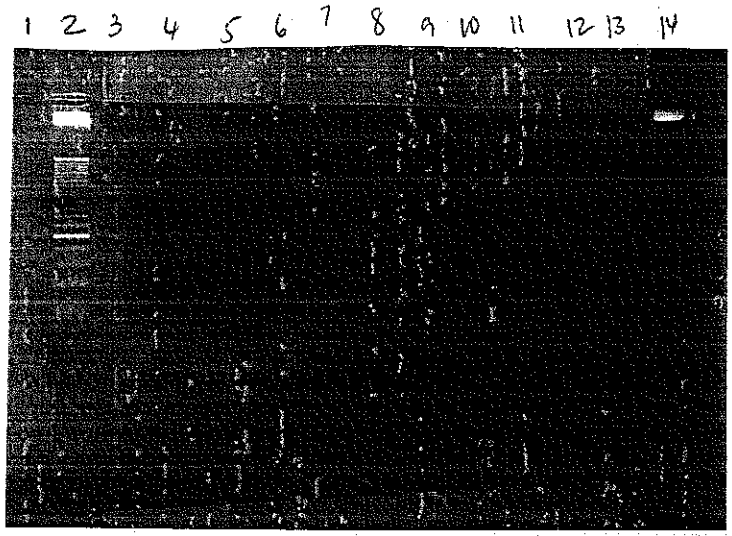
banaslug_afftfinalsizesel_mp_10169_2.JPG

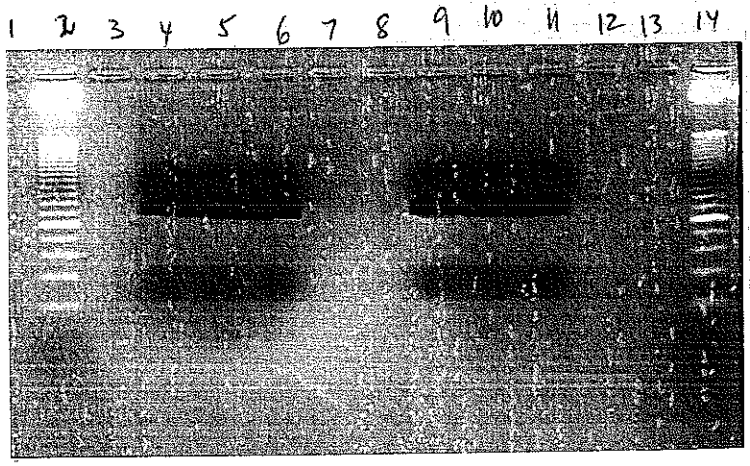
Gel

1-2 2-3 KB



Gel 2





1. Blank
2. 25bp ladder
3. Blank

4, 5, 6 > 3-4 Kb

7, 8 > Blank

9, 10, 11 > 4-6 Kb

12, 13 > Blank

14 - 25 bp ladder

120 V for 45 minutes
1X TAE

Qiagen Gel Purification 10/16/19

1-2 kb	2-3 kb	3-4 kb	4-6 kb
0.403 g x3	0.635 g	0.495 g	0.638 g
QG 1209 µl	1905 µl	1485 µl	1914 µl
Isoprop. 403 µl	635 µl	495 µl	638 µl
2015 µl	3175 µl	2475 µl	3190 µl
2 columns	2 columns	2 columns	2 columns
1007 µl p/column	1587/column	1237/column	1595/column
740	740	740	740
1.3x each column	2.1x each	1.7X each	2.15x each
2x 15µl each	2x 15µl each	2x 15µl each	2x 15µl each
Total= 60µl	60µl	60µl	60µl
-20°C	-20°C	-20°C	-20°C

Akram's Sample – BS Mate-Pair 2x25 bp
After Circularization / Plasmid Safe

Digestion – Eco P151

	1-2 kb	2-3 kb	3-4 kb	4-6 kb
	17.35 ng/ul (659.3 ng)	17.85 ng/ul (678.3 ng)	17.42 ng/ul (661.9 ng)	21.59 ng/ul (820.42 ng)
Eco	659 x 10/100 x 1/10	678 x 5/100 x 1/10	661 x 5/100 x 1/10	820 x 5/100 x 1/10
	= 6.59 ul	= 3.39 ul	= 3.3 ul	= 4.1 ul
DNA	38	38	38	38
NEB3	10	10	10	10
BSA	1	1	1	1
Sinefungin	1	1	1	1
SAM	20	20	20	20
Eco	6.6	3.4	3.3	4.1
Water	23.4	26.6	26.7	25.9
	100	100	100	100

37° C Overnight - PCR Machine

Digestion Part II – 4 samples

	ul
Digested DNA	100
10mM Sinefungin	1
10x ATP	2
Eco	0.5
	103.5

37°C – 1h / Denature 65°C 20 minutes – PCR Machine

End-Repair-Klenow – 4 samples

	w/
Eco DNA	103.5
dNTP Mix	1.5
Klenow	1
Total	106

Room Temperature 30 minutes
Denature 65°C for 20 minutes > PCR Machine

Streptavidin Binding Buffer

	w/
Tris-HCl pH 7.5 (500 mM)	20
Sodium chloride 5M	400
EDTA, 0.5 M	2
Water	578
	1000

Tris 500 mM
→ 100 µl
50 µl 1M Tris
50 µl H_2O

DNA = 106
Strep Buffer = 200
Nuclease free water = 94
400 w/

Dynabeads MyOne T1
Cat N: 656.02
Lot G22404

Quick ligase Buffer

	w/	x 4	
2x Quick ligase	300	=	1200
NFW	300	=	1200
	600		2400

NEBuilt
448 x 2 = 896
Quick ligation Buffer
E6010B-NP
Lot 1/0908

Akram's Sample

1-2 kb

$$1\mu g\ DNA \times \frac{10^6}{1} \times \frac{1}{660} \times \frac{1}{1536} = 1\ pmol/\mu l\ DNA$$

$$0.66\ \mu g \times \frac{1}{1} \times 30 \times \frac{1}{50} = 0.4\ \mu l\ P1/P2$$

2-3 kb

$$1\mu g\ DNA \times \frac{10^6}{1} \times \frac{1}{660} \times \frac{1}{2536} = \frac{10^6}{1673760} = 0.6\ pmol/\mu l\ DNA$$

$$0.68\ \mu g \times \frac{30 \times 0.6}{50} = 0.25\ \mu l\ P1/P2$$

3-4 kb

$$1\mu g\ DNA \times \frac{10^6}{1} \times \frac{1}{660} \times \frac{1}{3536} = \frac{10^6}{2333760} = 0.43\ pmol/\mu l\ DNA$$

$$0.66\mu g \times \frac{0.43}{1} \times 30 \times \frac{1}{50} = 0.17\mu l\ P1/P2$$

4-6 kb

$$1\mu g\ DNA \times \frac{10^6}{1} \times \frac{1}{660} \times \frac{1}{4536} = \frac{10^6}{2993760} = 0.33\ pmol/\mu l\ DNA$$

$$0.82\mu g \times \frac{0.33}{1} \times 30 \times \frac{1}{50} \approx 0.2\mu l\ P1/P2$$

1/2 LS DNA Complex 97.5
Quick ligase 2.5
P1 0.4 u/l
P2 0.4 u/l

3/4 LS DNA Complex
Quick ligase 2.5
P1 - 0.2 u/l
P2 - 0.2 u/l

Room Temperature for 15 minutes

2/3 LS DNA Complex 97.5
Quick ligase 2.5
P1 0.25 u/l
P2 0.25 u/l

4/6 LS DNA Complex 97.5
Quick ligase 2.5
P1 - 0.2 u/l
P2 - 0.2 u/l

Quick T4 DNA Ligase
E6090S-NP
Lot- 98/1108

NEB

		x 4	u/l	Lot No
10x NEB 2	= 60	=	240	1207
Water	= 540	=	2160	

Nick translate DNA

	u/l
DNA - bead Complex	= 96
100 mM DNTP mix, 25mM each	= 2
DNA Polymerase I	= 2
Total	= 100

Lot No

→ 86

16°C 30 minutes

1x Bead Wash Lot No - 0903013
1x Bind Wash Lot No - 0902006

Amplification – Aaram's libraries

	ul	x	4 samples
Platinum PCR Master Mix	200	=	800 ✓
Primer 1	8	=	32 ✓
Primer 2	8	=	32 ✓
Cloned Pfu	1	=	4 ✓
Nuclease free water	143	=	572 ✓
	360		1440

270 ul MM
30 ul 1-2 kb
↓
3 tubes
100 ul each
– C { 90 ul MM / 10 ul Water

270 ul MM
30 ul 2-3 kb
↓
3 tubes
– C 90 ul MM / 10 ul water

270 ul MM
30 ul 3-4 kb
↓
3 tubes
– C 90 ul MM / 10 ul water

270 ul MM
30 ul 4-6 kb
↓
3 tubes
– C 90 ul MM / 10 ul water

95°C	95°C	62°C	60°C	4°C
10 min	15 sec	15 sec	4 min	∞

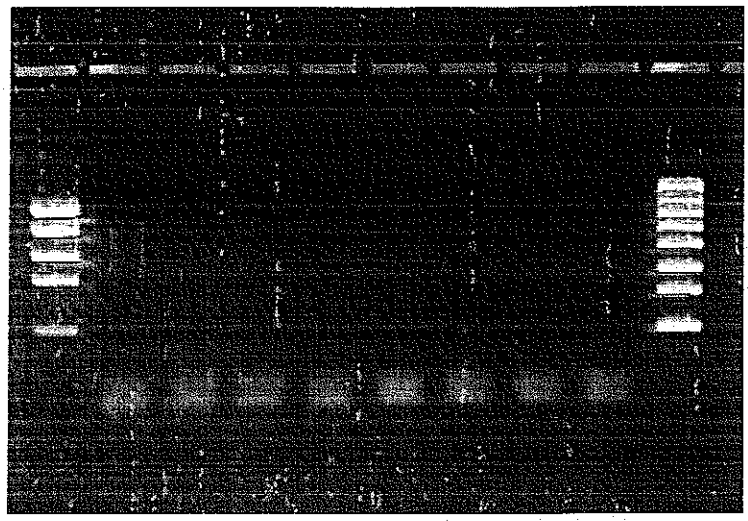
4 cycles

PCR 9700 – 60 lid
Block

↓

Lonza Gel

mate_pair_BANANASLUGakram_10129_4cycles.jpg



1- Quant ladder
2 - 1-2 Kb
3 - 2-3 Kb
4 - 3-4 Kb
5 - 4-6 Kb
6 - 1-2 Kb
7 - 2-3 Kb
8 - 3-4 Kb
9 - 4-6 Kb
} - Control
10 - 100 - 4 Kb ladder

⇓

No Amplification

10/13/2009

⇓

PCR

95°C
15 sec
62°C
15 sec
60°C
4 min
4
∞

2 more cycles

⇒ total 6 cycles
⇓
Lonza Gel

⇓
Repeat PCR cycles
3 more cycles

⇒ total 9 cycles
⇓
Lonza Gel

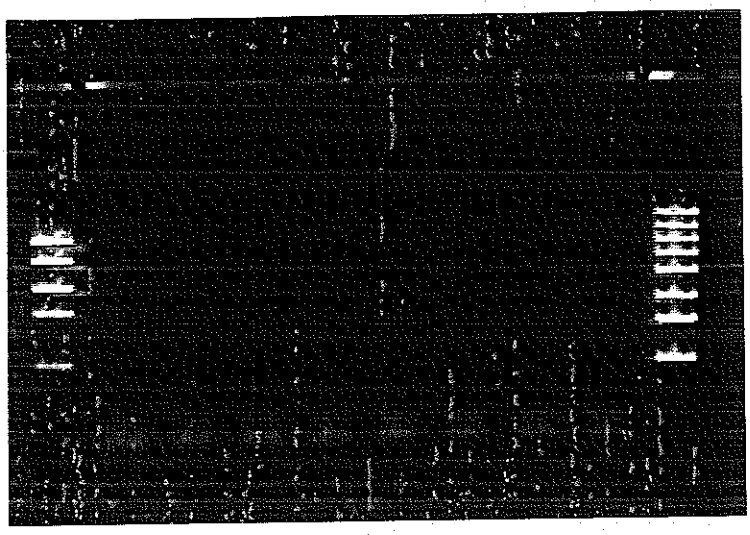
⇓
Repeat PCR cycles
4 more cycles

⇒ total 13 cycles
⇓
Lonza Gel

⇓
Repeat PCR cycles
7 more cycles

⇒ total 20 cycles
⇓
Lonza Gel

mate_pair_BANANASLUGakram_10129_6cycles.jpg



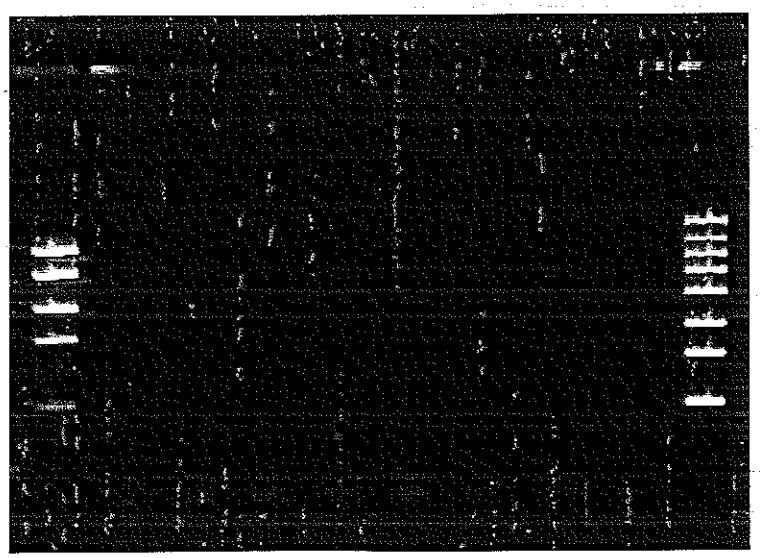
10/13/2009

Both Gels

1- Quant ladder
2- 1-2 Kb
3- 2-3 Kb
4- 3-4 Kb
5- 4-6 Kb
6- 1-2 Kb
7- 2-3 Kb
8- 3-4 Kb
9- 4-6 Kb
} - Control
10 - 100-4Kb ladder

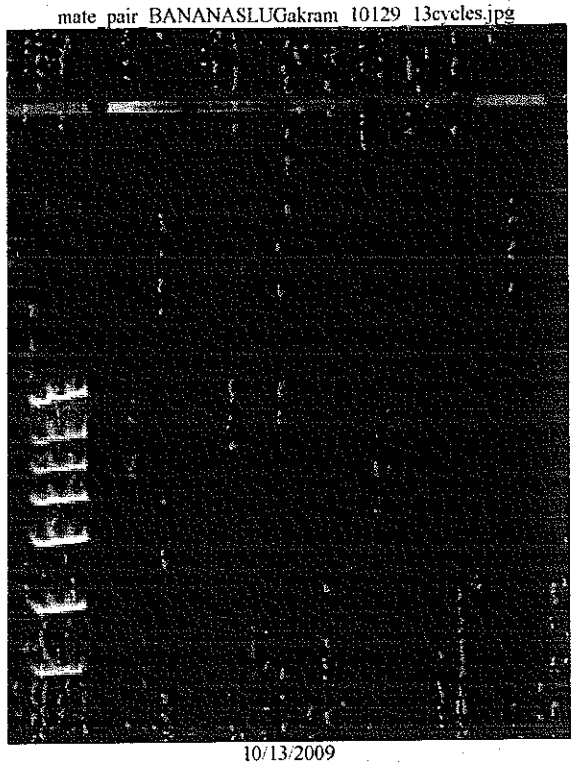
No Amplification

mate_pair_BANANASLUGakram_10129_9cycles.jpg



10/13/2009

mate_pair_BANANASLUGakram_10129_13cycles.jpg



10/13/2009

13 cycles

Checked only samples- No Controls

1- 100-4 KB ladder

2 - 1-2 KB
3- 2-3 KB
4- 3-4 KB
5- 4-6 KB
} No Amplification

mate_pair_BANANASLUGakram_10129_20cycles.jpg

10/13/2009

1- 100-4 KB ladder
2 - 1-2 Kb
3- 2-3 KB
4- 3-4 KB
5- 4-6 KB
} No Amplification

6- 1-2 KB
7- 2-3 KB
8- 3-4 KB
9- 4-6 KB
} -Control

⇓

Stopped Sample Prep.

⇓

Trouble shooting