Composition
Graph Ruled

Gabe's Notebook

MS2 -

protein coat ~ 180 coat protein subunits

Coat protein subunits - A, B, C

The FG loop interactions play a complex role in MS2 particle assembly and stability

→ mutations in the FG loop → failed to form particles

→ some form - but phage w/ modified gene were not viable

A covalent coat protein dimer (CCPD) can be formed by [illegible] a coat protein gene behind the [illegible] [illegible] in the [illegible] codon

→ protein [illegible] [illegible] [illegible] [illegible]

~~Homology~~ Inserted [illegible] - [illegible] the CCPD @ 78-75 a.a. → 54 a.a.

β-hairpin - at the N-terminal set - [illegible] A & B

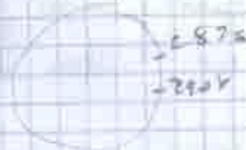
Introduce restriction sites into the gene with primers ~~[illegible]~~ amplifying gene with the restriction site

MS2 - β hairpin in coat protein @ A 1380 T & T 1383 C

$(5x)(L) = (.5x)\,1000$

$\frac{500}{5} = 100$

unit - amount DNA digest 1 hr



1 ug DNA plasmid

1-10 units enzyme to 1 ug

660 daltons × 5,206 bp

343 5,960 grams/mole

51 × 660   33,660 grams/mole

Ligation

DNA Fragments	1	
cloning vector		2 uL (10ng/uL)
10x ligation buffer		1 uL
Ligase (T4 DNA)?		1 uL (400 U/uL)
H_2O		
		10 uL

control – no DNA insert + with known blunt-ended insert

→ removed 5' phosphate from all RE. Phosph. – Purified.

• phosphatase treatment of plasmid digest – prevent plasmid: plasmid

– RE digest
+ Phosphatase
– Purified all the RE and phosphatase. (DNA) Purification

Ligase requires a 5' phosphate + 3' hydroxyl – on digested Fragment

Insert : Vector Ratio

minimum 3:1 to 10:1

plasmid DNA can be used directly from ligation rxns.

Note

but if more than 1 ng of DNA in ligation rxn
or if ligation DNA is from
a low gelling/melting temp. agarose —
use to dilute the ligation mix.

TBE

Making 10x Buffer

	10x	5x TBE Buffer
Trizma Base	108 g	54 g
Disodium EDTA	7.5 g	3.75 g
Boric Acid	55 g	27.5 g
dd H_2O to	1L	to 1L

Making 1% Agarose Gel (500 ml)

1) 500 ml 0.5x TBE Buffer
2) 5g agarose powder
3) Heat up in microwave
4) 25 µL EtBr

$(5x)(L) = (500ml)(.5x)$

$L = \frac{250}{5} = 50 ml$

V 90 L.I

M Kang Notes:

recommend to purify oligos longer than 50nt
by gel electrophoresis.
↳ found not beneficial

Annealing Oligo Protocol

1 µg of each oligo

mix in Rxn buffer (50mM NaCl, 10 mM tris-HCl, 10 mM $MgCl_2$, 1mM DTT, pH 7.9)

- heat to 95°C – 5min
- cool to Room Temp for 30 min

Subsequent ligation Rxn

1 µL of the annealed oligonucleotides

50-100 ng of BbsI-linearized AAV vector plasmid

Standard rxn conditions & ligation enzyme (T4 DNA ligase)

ShRNA AAV vector plasmids are selected & grown in
DH10B bacteria, using ampicillin-containing LB medium

this is a really small pencil
this is a [illegible] pencil
[illegible]

JS AAV - RSVc GFP-U6 Restriction Digest

with BbsI

	(-)	Exp 1	Exp 2	(+)	(-)
JS AAV-RSVc GFP-U6	1 µL	1 µL	10 µL		
pcDNA 3.1 (+)				1.5 µL	.9 µL
10x Buffer #2	2 µL	2 µL	2 µL	2 µL	2 µL
BbsI	—	2 µL	2 µL	1 µL	—
ddH$_2$O	17 µL	15 µL	6 µL	15.5	17 µL
	20 µL	20 µL	20 µL		20 µL

plasmid = .1 µg / µL

20 µL Rxn.

[BbsI] 5 units/µL

incubate @ 37°C for ≥ 60 min.

Need 50-100 ng of digested linearized ds AAV plasmid for ligation Rxn.

Add phosphatase to remove 5' (P) ?? ask Jennifer or Andy.

- check digest on gel.
- Purify the Plasmid DNA (same as PCR purification)

I ♡ GABE

5:37pm

Ligation (Insert : Plasmid) 20 µL rxn.

	#1 3:1	#2 5:1	#7 (−)	#3 10:1	#4 20:1	#5 50:1	[illegible]
SHRNA Insert (diluted)	0.195 µL / 1.95 µL (1:10)	.245 µL / 2.45 µL (1:10)	—	.495 µL / 4.95 µL (1:10)	.95 µL	2.35 µL	[illegible]
dsAAV-RSVeGFP -U6 (diluted)	1.11 µL (100 ng)	1.11 µL (100 ng)	1.11 µL	1.11 µL (100 ng)	1.11 µL	1.11 µL	[illegible]
10x ligase buffer DP (5/16/08)	2 µL	2 µL	2 µL	2 µL	2 µL	2 µL	[illegible]
T4 DNA ligase	1 µL (new/ [illegible])	1 µL	1 µL	1 µL	1 µL	1 µL	[illegible]
ddH₂O	13.94 µL	13.44	15.89	10.94 µL	14.94	13.44	[illegible]

@ Room temp for 10 min.

Note: after ligation you have to transform it !!

Transformation
Competent cell's (100 µL cells)
60 µL

need 3 µL of DNA ligations (no more than 5%)

1 µL

1 µL of 1B

Making 500 mL of annealing buffer

1M NaCl, 0.5 M EDTA, 1M Tris 7.5 pH 8.0

1,000 mL

NaCl $(50\ mM)(500\ mL) = (1000\ mM)(x)$ — 50 mL

$\frac{25000}{1000} = 25\ mL$

EDTA $(1\ mM)(500\ mL) = (500\ mM)(x)$ — 2 mL

$\frac{500}{500} = 1\ mL$

Tris $(10\ mM)(500\ mL) = (1000\ mM)\ x$ — 10 mL

$\frac{5000}{1000} = 5\ mL$

dH_2O — 938 mL

Annealing Oligonucleotides Protocol 11/8/2011

oligo #1	0.3127 mL (1 ug)
oligo #2	0.3127 mL (1 ug)

3.1 λ oligo A (1:10 d)

3.1 λ oligo B (1:10 d)

20 λ 5x annealing Buffer

73.8 λ H_2O

100 mL

- added Mineral Oil - 60 μL
  - Heated @ 95°C in PCR plate for 5 min
  - Left them to cool down in boiling water to room temp. for 40-60 min.

8/21/09

The pcDNA control did not show up in the gels.

- Might have loaded too little on the gel or we might have not loaded anything at all.

- Need to do the pcDNA positive (+) and negative (-) control again.

Run Gel again 8/21/09

- ladder
- AAV Expt
- AAV (-) control (untransfected)

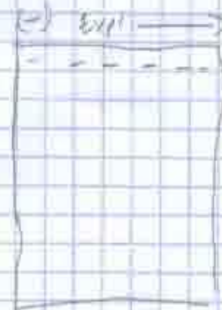
Came out good! See prev.

2 µL ladder 1000x → 2.0x

Gel for Gel isolation

1 tube	5x tubes
1.5 µL (-) AAV	3.5 µL Exp1
1.7 µL dye	1.7 µL dye
14.8 µL dH_2O	9.8 µL dH_2O

15 µL tubes.



Fuse 55

fuse 55 MC 1061

Tetracycline resistance ??

MC1061 - ~~[illegible]~~ uninfectible female.

using the DMSO - already pre ~~[illegible]~~ chemically competent.

make LB tet plates.

500 mL dH_2O
+ 17.5 g LB Agar

auto clave @ Slow for 20 min.

Rapid Gel Isolation Protocol

#1 .5768 g $\left(\frac{100\ \mu L}{.1\ g}\right)$ = 576.8 µL

.3164 g $\left(\frac{100\ \mu L}{.1\ g}\right)$ 316.4 µL

GeneClean turbo salt solution

(100 µL) for .1 g of gel !!

•) incubate @ 55°C for 5 min.

•) transfer < 600 µl @ time to column. cent. for 5 seconds. discard water

•) add 500 µL of GeneClean Turbo Wash to filter
centrifuge for 5 seconds
discard wash

centrifuge for 4 min.

1) New collection tube !! transfer cartridge.
add 30 µL GeneClean Turbo Elution.
incubate @ room temp for 5 min.

•) centrifuge for 1 minute and cap catch tube.

Standard transformation
protocol for the DH5α

Pre-warm plates to 37°C before starting

Single Tube Aliquots

1) Add 1-5 µL plasmid DNA to a thawed tube cells on ice.

Mix gently for a few seconds

Try to keep the volume of DNA less than 5% of total

2) Incubate on ice for 2-5 minutes (max 60 min)

3) Spread 50-100 µL onto a pre-warmed culture plate. Incubate the plate at the appropriate temp (37°C) for the colonies to grow.

2 ul dNA | 1 uL dNA
1.66 ul dye | 1.66 ul dye
11.33 dH2O | ~~[illegible]~~ 12.33 dH2O

(10x)(L) = (1x)(15) + L)

9x = 15

(375) = (500 mL) 1x

375x 15 mg/ml Tet stock
40 γ/ml plates

50ml / 375x = 0.133 ml = 133 ul / 50 ml LB Agar

133 ul

ug/ml

NEW ENGLAND BioLabs Inc. 133 ul / 50 ml agar

Introducing Crimson™ Taq

= 1.33.

10.66 uL

5/25/09

Redoing ligation with fresh RE digest of dsAAV-RSVe-GFP w/ BbsI and fresh oligo annealed 34KVA.

5/25/09

Annealing the oligos

100 λ final volume

3.2 λ oligo A (1:10d) ≈ 1 ug
3.2 λ oligo B (1:10d) ≈ 1 ug

20 λ 5X annealing buffer

73.6 λ ddH₂O

---

100 λ

Put in boiling water @ 3:08 pm
end 4:08 pm

8/25/09

[illegible] columns, [illegible] whole [illegible].

Restriction digest
ds AAV-RSVe-GFP-UB

	(−)	(E)	(+) RDNA	(−) RDNA
ds AAV-RSVe GFP-UB	1 uL	10 uL	—	—
buffer #2	2 uL	2 uL	2 uL	2 uL
BSA	—	2 uL	2 uL	—
RDNA plasmid	—	—	4.5 uL	.9 uL
d H_2O	17 uL	6 uL	11.5 uL	17 uL

20 ml Rxn+ with BASE

new batch of buffer #2 open @ 8/25/09

Incubate @ 37°C for 60 mins
start 1:35 pm
end 2:35 pm

Check digest with a Gel
Run Gel to isolate

Purification

Plate Results
for Edy on 8/24/09

[illegible] 55 plate
[illegible] LB [illegible] TET

Good - [illegible] small [illegible] colonies ~ 100

Grow the

[illegible]

LB + Amp

3:1 ~ ✗ 2

10:1 ~ ✗ 3

20:1 ~ ✗ 2

50:1 ~ 1

each tube ~ 3 mL
of LB & Amp.

Amp [100 mg/L]

need 60 mL of Amp for 30 mLs of the LB

[200 mg/mL]

30 mLs 60 mL

(200 mg)(L) = (30)(L) [illegible]

3 mls each need ~ 30 mls

60 mL

$50 mg/L (L) = (30 mL)(1x)$

- Note You can store the LB colonies plate for about a month
You can store the LB liquid cells for about 1-2 days

Protocol for Miniprep

- Inoculate desired [illegible] in ≈ 3 mL of LB with amp.
  want amp to be ≈ [100 mg/L]
  stock is 500x.
  so 60 μL to 30 mL of LB.
  Incubate in shaker for 16-18 hours
  started around 3:00 pm.
  @ 240 RPM

FUSE 5S Growing

In 3 mL of LB plus tet
pick 2-3 colonies.
want Tet to be same [ ] as plates.
so need ≈ 10 mL LB + 26.667 μL of tet.

Incubate @ 37° 16-18 hrs
Start @ 3:49 pm.

@ 240 RPM

Next day - Centrifuge cells 7800 rpm for 3 min, to get a pellet.
used both 1.5 tubes and transfer the amount one by one.

- Note: when tube is foggy, it's a good sign that your cells grow!

8/25/08

Rapid Gel isolation

#1 = .4843 g $\left(\frac{100\ \mu L}{.1\ g}\right)$ = 483.3 μL

#2 = .5658 g $\left(\frac{100\ \mu L}{.1\ g}\right)$ = 565.7 μL

#3 .3818 $\left(\frac{100\ \mu L}{.1\ g}\right)$ = 381.8 μL

of Gene Clean turbo see [illegible]

1) Incubate @ 55°C for 5 min

2) transfer 600 μL to column - centri for 5 seconds.

<u>MiniPrep</u> Protocol 8/26/09

1) Resuspend pelleted bacterial cells in 250 µL Buffer P1

- Need to add RNase A to buffer P1
- No cell clumps should be visible after resuspension.
  ↳ pipet up & down
- If LyseBlue reagent has been added to P1 make sure it's well shaken.

2) Add 250 µL Buffer P2 & mix thoroughly by gently inverting tube 4-6 times. (close bottle immediately)

- Mix gently. Don't vortex - until it becomes slightly clear and viscous.
- Don't allow cells to lyse for more than 5 min.
- Should turn blue if LyseBlue was added to P1

3) Add 350 µL Buffer N3 & mix immediately & thoroughly by inverting the tube 4-6 times

- If LyseBlue was used, mix until it's colorless. Suspension should be colorless.

4) Centrifuge for 10 mins @ 13,000 rpm

- compact white pellet will form

8/24

5) Apply the supernatant from step 4 into the QIAprep spin column by decanting or pipetting. (750 µL)
don't get greedy
start from top → bottom

6) Centrifuge for 30-60s (13,000 rpm). Discard the flow through.

7) Wash the QIAprep spin by adding 0.5 mL Buffer PB and centrifuging for 30-60s. Discard the flow through.
(Not needed for DH5α)

8) Wash QIAprep spin column by adding 0.75 mL Buffer PE & cent. for 30-60's. (13,000 rpm)
(Need to add Ethanol!)

9) Discard the flow through & cent. for an additional 1 min. to remove residual wash buffer

10) place the QIAprep column in a clean 1.5 mL tube. To elute DNA, add 50 µL Buffer EB (10mM Tris-Cl...) or water to the center of each QIAprep spin column, let it stand for 1min. and centrifuge for 1min.

Note: Lyse Blue To Buffer P1
1:1000 dilution

8/26/09

Note. Accidently added twice as much of EB buffer (elution) to the 20:1 #2 (AAV [cells/ligation]) miniprep.

Running gel for minipreps. -

1 μL of each (except 20:1 tube 2 μL)

15 ul rxns

7 μl dH2O

1.5 dye.

12.5 dH2O

15 μL

ladder	Fuse #1	Fuse #2	Fuse #3	3:1 #1	3:1 #2	10:1 #1	10:1 #3	20:1 #2	50:1 #1
				120ng	120ng	100ng	100ng	60ng	60ng

5/27/09 Checking FUSE55 and AAV plasmid

with ~ digestions

FUSE55 [illegible]

- going to quantify it again (free 55 miniprep) with

[illegible] [illegible]

[illegible]

Ab[illegible]

	FUSE55		
	#1	#2	#3
	5 µl	5 µl	5 µl
	1.5 dye	1.5 dye	1.5 dye
	8.5 µl dH₂O	8.5 µl dH₂O	8.5 µl dH₂O
	15 µl	15 µl	15 µl

pAAV-shRNA digestion

with NdeI and NheI to check for shRNA

insert

should get a 305 bp band for insert

+ 165 bp band for no insert

160

200

Total [illegible]

	3:1 #1 (122 ng/µl)		3:1 #2 (122 ng/µl)		10:1 #1 (120 ng/µl)		10:1 #3 (120 ng/µl)		20:1 #2 (60 ng/µl)		50:1 #2 (60 ng/µl)	
	(-)	Exp	(-)	Exp	(-)	Exp	(-)	Exp	(-)	Exp	(-)	Exp
plasmid	1 µl	8.1	1 µl	8.1	1 µl	5 µl 12	1 µl	5 µl 16	1 µl	3 µl 16.35	1 µl	3 µl 16.33
Buffer #4	2 µl	2 µl	2 µl	2 µl	2 µl	2 µl	2 µl	2 µl	2 µl	2 µl	2 µl	2 µl
NdeI	-	1 µl	-	1 µl	-	1 µl	-	1 µl	-	1 µl	-	1 µl
NheI	-	1 µl	-	1 µl	-	1 µl	-	1 µl	-	1 µl	-	1 µl
H₂O	17 µl		17 µl		17 µl		17 µl		17 µl		17 µl	

8/27/09

Note – thrown away the LB amp and LB tet ...

pWPI – plasmid

150 ng/uL

	(+)	(+) (3ul plasmid)	(– pWPI)
2ul ...	2ul	2ul	1ul
2ul 11 ...	2ul	2ul	2ul
NdeI	–	0.1 ... ul	–
NheI	1.22 ul	–	–
dH_2O	15.78	16.876	17 ul

Gus SS digestion w/ NsiI → 7,000 → 3,000 (...)

(...)

positive control plasmid = ...

Gus SS

40ul reactions

start @ 5:32 pm @ 37°C

end 6:32 pm

	#1 100 ng (–)	#1 (E)	#2 100 ng (–)	#2 (E)	#3 200 ng (–)	#3 (E)	Positive (+)	pWPI (– positive)
Gus SS plasmid	4ul (...)	38ul (40...)	4ul (...)	38ul (...)	6ul (...)	34ul (...)	1ul	1ul
NsiI	–	2ul	–	2ul	–	3.4ul	1ul	–
Buffer #3	2ul	4ul	2ul	4ul	2ul	4ul	2ul	2ul
dH_2O	14 ul	...	14 ul	...	12 ul	2ul	16ul	17ul

(70% — 80%) Maxiprep

Note 20% glycerol be use a little more

Since glycerol is very dense, ex. [illegible]

we need 2.75 ml of glycerol and [illegible]

↑ take sample ~ [illegible] ↗ [illegible]

Save the glycerol for future use.

Store @ -80°c

↓

Then centrifuge the whole 500 ml

in the jumbo centrifuge.

8/27/09 fosmid 55 (Miniprep digestion (v))

$400\mu l$ = 4.44 dye

$200\mu l$ = 2.22 dye

μl = 1.5 dye
(15)
~~10~~ 12.5 dH_2O

	#1	#2	#3	pv	pUPP
1 kb ladder	(-) Ep	(-) Ep	(-) Ep	(v)	(+)

8/28/09

Yesterday data I believe fosmid 55 insert but gel was

inconclusive because the gel was poorly made (well where torn)

- grow more of fosmid 55 #1 & #2 for miniprep
  10ml of each (from previous miniprep tubes)
  5ml of LB with Tet (12.5 μg/ml)
  @ ~~2:30~~ 7:00 pm 37°C

Today – 1pm – ~~start~~ stopped incubation of fosmid 55 #1 & 2
incubated for 18 hours

Goals: do miniprep and digest to see if I got
insert fosmid 55

- this then ready to go ahead with gel growth.

8/28/09 **FUSE 55 Quantification**

of Minipep 8/27/09

Using the lambda ladder and 5µL of each minipep on a 1% Agarose gel

Fuse55 minipep (15 µL samples)

	#1	#2	#3.1	#3.2	#3.3	#3.4
DNA Plasmid	5µL	5µL	5µL	5µL	5µL	5µl
10X dye	1.5 µL	1.5 µL	1.5µL	1.5µL	1.5 µl	1.5 µl
ddH₂O	8.5 µl	8.5 µl	8.5 µl	8.5 µl	8.5 µl	8.5 µl
	→ 5ng/µl	2.5ng/µl	5ng/µl	2.5ng/µl	[illegible]ng/µl	5ng/µl
	~~200~~ ng → 100ng 100ng	~~100ng~~ 50ng	200ng	100ng ↓ 100ng	100ng ↓	200 ng → 200 ng

8/31/09 fuse55 digestion w/ NsiI and NdeI done on 7/1/09

fuse55 w/ NsiI

Positive controls w/ pwP1 plasmid = 150 ng/ul

	#1 (-)	#1 Exp	#2 (-)	#2 Exp	#3.1 (-)	#3.1 Exp	(-pwP1)	(+) pwP1
plasmid	4 ul ([illegible])	16 ul (110 ng)	8 ul (10 ng)	36 ul (90 ng)	4 ul (10 ng)	20 ul (100 ng)	1 uL	1 uL
Buffer #3 (10x)	2 uL	5 uL	2 uL	4.5 uL	2 uL	4 ul	2 uL	2 uL
NsiI (10x)	—	.18 uL 1.8 uL	—	.15 uL 1.5 uL	—	.2 uL 2 uL	—	.3 uL 3 uL (1:10 d)
dH_2O	14 uL	20.7	12 uL	2.7 uL	14 uL	19 ul	17 uL	14 uL

Expected sizes: 7317 bp, 1684 bp.

Start 4 pm

fuse55 w/ NdeI

Positive controls w/ pwP1 plasmid = 150 ng/ul

	#1 (-)	#1 Exp	#2 (-)	#2 Exp	#3.4 (-)	#3.4 Exp	(-) pwP1	(+) pwP1
plasmid	4 ul (10 ng)	18 uL (90 ng)	4 uL (10 ng)	16 uL (90 ng)	4 ul (10 ng)	[illegible] (90 ng)	1 uL	1 uL
Buffer #4 (10x)	2 ul	5 uL	2 uL	[illegible] uL	2 uL	5 uL	2 uL	2 uL
NdeI (20x)	—	.09 uL .9 uL	—	.09 uL .9 uL	—	.19 uL 1.9 uL	—	.15 uL 1.5 uL (1:10 d)
dH_2O	14 uL	24.1	14 uL	19.6 uL	14 uL	5.5 uL	17 uL	15.5 uL

Expected band sizes = 4,300, 3150, 1281, 675

8/31/09

Strep Avidin – Ibeu Zaun Box well 9 P
transformation (J36 846)
in DH5α cells

100 uL of DH5α cells

3 uL of DNA

plate on Amp LB plates, incubate @ 37°C

incubated @ 6.10 pm @ 37°C

Sat, 9/1/09 – many colonies 1,000's !!

Going to pick 8 for colonies to grow
overnight. on LB + Amp
start @ 5 pm

9/2/09 – nice cloudy tubes – good – for the Strep Avidin
incubation tubes.

– doing miniprep of them 9/2/09.

vol. dry

9/2/04

- did Minipep of the Strep Avidin.

- Ran gel to check Fuse 55 w/ Ndel digest

  - Seems that Ndel did not cut but it did cut controls. — DNA might be the culprit?

- Running gel of Fuse 55 w/ NSI

- Running gel to quantify the Strep Tag Mini preps — running 1 ul of each sample

Strep Avidin

		#1	#2	#3
$\frac{20}{9}$ =	2.22 dye	50 ng/ul	100 ng/ul	↑ 122 ng/ul

$\frac{95}{9}$ = 5 ul dye.

Tomorrow - do glycerol sample of the yeast adhered cells.

9/3/09

Digest to do to the original d3 AAH (5206 bp)

redo the NheI w/ (+) controls
to try buffer #2 and #4.

- do double digest with NheI and XhoI ~ should digest ~40 bp band.

- do single digest SmaI and KpnI to check RFP's
integrity

SmaI need to be incubated @ 25°C !!

Expected bands

with KpnI

- 3,681 band
- 153 band
- 1371

SmaI

9,495
720
It [illegible] [illegible] be able to see it

XhoI

[illegible] [illegible]
[illegible]

pQDNA

+4

20

[illegible] [illegible]
[illegible]
[illegible]

9/3/07 Single digest of ds AAV (original)

with Sma I ~~and~~ ~~[illegible]~~

Sma I - incubated @ 25°C

pcDNA = 110 ng/uL

	(-) ds AAV	Exp Sma I	(-) pcDNA	(+) pcDNA
plasmid	1 uL	2 uL	1 uL	1 uL
Buffer 4	2 uL	2 uL	2 uL	2 uL
Sma I	—	.02 uL [.4 uL (8U)]	—	.011 [.22 uL (11U)]
dH_2O	17 uL	15.6 uL	17 uL	16.89 uL

~~[illegible]~~
20 uL Rxns

Expected band sizes

ds AAV	pcDNA
4,495	~~[illegible]~~
720	~~[illegible]~~
11 (won't be able to see)	~~[illegible]~~
	98
	22

incubated @ 25°C for 1 hrs

start: 3:23 pm
end 4:24 pm.

9/3/06

Double digest with NdeI and XhoI
dsAAV (original).

Positive controls

	(−) dsAAV	Exp NdeI	Exp XhoI	double	(−) PwPI	NdeI PwPI	XhoI PwPI
Add dsAAV	1 µL	1 µL	1 µL	10 µL	1 µL	1 µL	1 µL
Buffer #4	2 µL	2 µL	2 µL	2 µL	2 µL	2 µL	2 µL
NdeI	—	.1 µL [1 µL (1:10)]	—	1 µL	—	.15 µL [1.5 µL (1:10)]	—
XhoI	—	—	.05 µL [.5 µL (1:10)]	.5 µL	—	—	.075 µL [.75 µL (1:10)]
dH2O	17 µL	16 µL	16.5 µL	6.5 µL	17 µL	15.5 µL	16.25 µL
BSA	1 µL	1 µL	1 µL	1 µL	1 µL	1 µL	1 µL
20 µL rxn's							

double digest expect a ~110 bp band
on 2% gel.

XhoI PwPI - expect = 9,300
2,400
400

Run on a 2.5% gel!!

Incubate @ 37°C for 1hr.

9/3/09

Single digest of sc43AAV (original) w/ KpnI

	(−) sc43AAV	Exp KpnI	(−) pUPI	(+) pUPI
Plasmid	1uL	3uL	1uL	1.5 uL
Buffer #1	2uL	2uL	2uL	2uL
BSA	.2uL	.2uL	.2uL	.2uL
KpnI	—	.3 uL 3uL (1:10)	—	.225 2.25 uL (1:10)
mH2O	16 uL	17 uL	16 uL	13.25 uL

20uL Rxns

Expected bands

sc43AAV	pUPI
5,682	10,500
158 (bug)	1,600 (to ug)
13.71	

incubated @ 37°C for 1hr

9/4/09 Today - Check dsRED plasmid IFR's again w/ Sma I - this time w/ a little more and hope to see the 700 bp band

- do - double digest of the RFP minipreps with NdeI and XhoI

<u>Small digest dsRED</u> (9/9/09)

	(-) dsRED	Small dsRED	(-) pcDNA	(+) pcDNA
Plasmid	1uL	~~10~~ uL	1uL	1uL
* Buffer # 4	2uL	5uL	2uL	2uL
Sma I	—	.1uL 1uL (1:20d)	—	.1uL .25uL (1:20 d)
dH_2O	17uL	34uL	17uL	16.75uL

start 1:39 pm
end 2:39 pm

9/4/05 Silencing α and β

where transform in DH5α
cells

5 ul cells for each & 50 LB for each plate

- were plated @ 4:30 pm

incubated @ 37°C.

9/8/05 - To do list

* Transform 3 colonies of each plate (silencing α & β plasmids) in LB
* Run gel of dsRNA miniprep double digest in a 2.5% gel
* do Miniprep of the fuse 55

<u>Silencing α and β</u>

- 3 colonies of each plate
in 3 ml of LB and Amp

29.5 ml

(30 ml) L = (1x) 30 ... = 1

L = 30 / 500 = 42 ... Amp

incubated @ 37°C for ... 1:30 pm

Note: - [illegible] gel if [illegible] ... [illegible]

- did Miniprep of fuse 55 9/8/05 -> 20 ...

- Run double digest of dsRAV miniprep ... 9/8/05

... [illegible]

[illegible] | 7AL

[illegible]/[illegible]

Things to do today

- Minipreps of [illegible] α and β

- double digest of [illegible] shRNA minipreps again
  [illegible] [illegible] 1.5 of [illegible] original plasmid
  and [illegible] the [illegible]
  25–30 [illegible] this time

- [illegible] of [illegible] 53 miniprep w/ [illegible] and [illegible]

dsAAV shRNA double digest [illegible] - 1% gel

	1	2	3	4	5	6	7	8	9	10
	3.1 [illegible]	3.1 [illegible]	3.1 [illegible]	5.1 [illegible]	6.1 [illegible]	5.1 [illegible]	10.1 [illegible]	20.1 [illegible]	20.1 [illegible]	original dsAAV
plasmid	17.5 µL	20µL	5.5µL	10µL	21µL	10µL	9.2µL	10µL	10µL	15µL
buffer #4 [illegible]	2.5µL	2.5µL	2.5µL	2.5µL	2.5µL	2.5µL	2.5µL	2.5µL	2.5µL	2.5µL
BSA	.25µL	.25µL	.25µL	.25µL	.25µL	.25µL	.25µL	.25µL	.25µL	2.5µL (1:10x)
NheI	1µL	1µL	1µL	1µL	1µL	1µL	1µL	1µL	1µL	1.5µL
XhoI	.5µL	.5µL	.5µL	.5µL	.5µL	.5µL	.5µL	.5µL	.5µL	.75µL
H2O	3.25µL	.75µL	11.15µL	5.75µL	X	5.75µL	11.15µL	5.75µL	6.75µL	.25µL

Master mix (10 [illegible]) = BSA 2.5µL
Buffer #4 25µL
NheI 10µL
XhoI 5µL

4.25µL for each tube
except [illegible] original dsAAV

Controls

Original dsAAV

#	(+) dsAAV	PhoI dsAA	NdeI dsAA
plasmid	1uL	1uL	1uL
BSA	2uL (1:10)	2uL (1:10)	—
Buffer #4	2uL	2uL	2uL
NdeI	X	X	1uL (1:10d)
PhoI	X	.5uL (1:10d)	X
dH2O	15uL	14.5uL	17uL

Start @ 3:10pm @ 37°C

end 4:10pm

Gel wells on 4% Agarose gel .5xTBE, 1 F/ul EtBr

+dsAAV
+ 3:1 #1
+ 3:1 #2
+ 3:1 #6
+ 6:1 #1
+ 3:1 #3
+ 1:1 #3
+ 30:1 #1
+ 30:1 #3

11/13/...

... ng

Plasmid

Buffer #

NdeI

dH2O

plasmid

buffer #4

NdeI

dH2O

9/13/04

- To do
  - check fuse 55 miniprep
  - check the Sfixden α and Silvian β with digestion [illegible] [illegible] fine
  - [illegible] testing the shRNA-dsRNA gel again

[illegible]

Fuse 55 miniprep — done by [illegible] & [illegible]

digest w/ NsiI

	(-) fuse 55	fuse 55 E/P	- PuPE	(+) PuPE
Plasmid	1.5 uL (30 ng)	10 uL (200 ng)	1 uL	1 uL
Buffer # #3	2 uL	2 uL	2 uL	2 uL
NsiI	—	.4 uL / 4 uL (1:10d)	—	.3 uL / 3 uL (1:10d)
ddH₂O	16.5 uL	4 uL	17 uL	14 uL

incubate @ 37°c for 1 hour

digest w/ NheI

	(-) fuse 55	fuse E/P	- PuPE	(+) PuPE
plasmid	1.5 uL	17.5 uL	1 uL	1 uL
Buffer #4	2 uL	2.3 uL	2 uL	2 uL
NheI	—	.35 uL / 3.5 uL (1:10d)	—	.15 uL / 1.5 uL (1:10d)
ddH₂O	16.5	—	17 uL	15.5

incubate @ 37°c for 1 hour

[illegible] of [illegible] and [illegible]

1 ul 10 ng for PCR

20 – 25 cycles.

Dilute the oligos – 20

10 micro molar

5 ul → 95 ul of water

4 ul of Taq polymerase

run the 5 ul of the PCR then on the gel.

4x [illegible] BCB/

Protocol – on the computer.

Notes: add 3 drops of [illegible] gel using

the PCR.

9/12/09 Gel Quantification of Selection α, β

α #1-3 : 57 ng/μL   on a 1% agarose gel.

β #1-3 : 32 ng/μL

Order of PCR tubes 9/12/09

	Control	Exp
Plasmid dilut[illegible]	C 1	1
3:1 #1	C 2	2
3:1 #2	C 3	3
3:1 #3	C 4	4
5:1 #1	C 5	5
5:1 #2	C 6	6
5:1 #3	C 7	7
10:1 #1	C 8	8
10:1 #3	C 9	9
[illegible]	C 10	10
20:1 #3	C 11	11
50:1 #1	C 12	12
50:1 #3	C 13	13
100:1 #2	C 14	14
100:1 #3	C 15	15

Order of [illegible] gel wells for [illegible] mapping
single digest w/ NsiI and NdeI

(-) NdeI (+) NsiI (-) PvuI (+) PvuI   (-) NdeI (+) NdeI (-) PvuI (+) PvuI

9/14/09

To do: - run gel of PCR [illegible]
- digest & [illegible] α and β

PCR - [illegible] gel - [illegible] gel.

[illegible] Sal [illegible]

[illegible] gel - [illegible] gel - [illegible]

[illegible]
the [illegible]

- [illegible]

[illegible] $\left(\frac{[illegible]}{[illegible]}\right) = 3$ $\left(\frac{[illegible]}{[illegible]}\right) = .[illegible]$ [illegible] = [illegible]

[illegible] $\left(\frac{[illegible]}{[illegible]}\right)$ $\left(\frac{[illegible]}{[illegible]}\right) = .85$ [illegible]

	[illegible]
[illegible]	10 [illegible]
[illegible] ([illegible])	10 [illegible]
[illegible] ([illegible])	7.5 [illegible]

[illegible] 2.75 [illegible]
[illegible]
[illegible]

4/14/04

Restriction digest of Silencer α & β minipreps w/ EcoRV

Silencer α = 59 ng/µL

Silencer α w/ EcoRV

pcDNA = 110 ng/µL

	Silencer α						Positive controls	
	(-) #1	Exp #1	(-) #2	Exp #2	(-) #3	Exp #3	(-) pcDNA	(+) pcDNA
plasmid	[illegible]	2.7µL (150ng)	1µL	2.7µL (150ng)	1µL	2.7µL (150ng)	1µL	1µL
Buffer #2	1µL	1µL	1µL	1µL	1µL	1µL	1µL	1µL
BSA	1µL (1:10 dil)	1µL (1:10 dil)	1µL (1:10 dil)	1µL (1:10 dil)	1µL (1:10 dil)	1µL (1:10 dil)	1µL (1:10 dil)	1µL (1:10 dil)
[illegible]	—	.25µL (1:10 dil)	—	.75µL (1:10 dil)	—	.75µL (1:10 dil)	—	.55µL (1:10 dil)
dH_2O	7µL	4.55	7µL	4.55	7µL	4.55	7µL	6.45µL

Incubate @ 37° for 1 hr

Expected bands 5.3K and 1.7K

Silencer β w/ EcoRV

Expected bands 5.5K, 707, 1.1K

	#1 (-)	Exp 1	(-) #2	Exp #2	(-) #3	Exp #3	(-) pcDNA	(+) pcDNA
plasmid	[illegible]	4.1µL (150ng)	2µL	4.1µL (150ng)	2µL	4.1µL (150ng)	1µL	1µL
Buffer #2	1µL	1µL	1µL	1µL	1µL	1µL	1µL	1µL
BSA	1µL (1:10 dil)	1µL (1:10 dil)	1µL (1:10 dil)	1µL (1:10 dil)	1µL (1:10 dil)	1µL (1:10 dil)	1µL (1:10 dil)	1µL (1:10 dil)
EcoRV	—	.75µL (1:10 dil)	—	.75µL (1:10 dil)	—	.75µL (1:10 dil)	—	.5µL (1:10 dil)
dH_2O	6µL	3.15µL	6µL	3.15µL	6µL	3.15µL	7µL	

9/15/09

- Things to do

- miniprep [illegible] 25AAA-shuttle

- PCR [illegible] 25AAA-shuttle

✓ - digest [illegible] [illegible] & [illegible] with EcoRV

✓ - PCR using 2° AAAV plate

- AAAV plate got a lawn - very small colonies - too much plated? - needed to probably diluted it

- started culturing @ 2:15 pm

Silicon α & β [illegible] [illegible] [illegible] order

1. [illegible]
#1 (-)
#1 Exp
#2 (-)
#2 Exp
#3 (+)
#3 Exp

} α

#1 (-)
#1 Exp
#2 (-)
#2 Exp
#3 (-)
#3 Exp
(-) [illegible]
(+) NAc I
(+) NAc I

} β

Silicon α [illegible]		(-)
[illegible]	4 uL	4 uL
BSA (1:10)	9 uL	9 uL
NAc I (1:10)	3 uL	
NMe I (1:10)	6 uL	
dH₂O	17.2 uL	26 uL

7.5 uL to each [illegible]

9 uL to each [illegible]

Silicon β master [illegible] 9 [illegible]		(-)
[illegible]	6 uL	6 uL
BSA (1:10)	6 uL	6 uL
NAc I (1:10)	6 uL	
NMe I	12 uL	
dH₂O	5.2 uL	9 uL

5.6 uL to each tube

13 uL to [illegible]

9/15/09

Selection of #1 [illegible] w/ NdeI and NheI

	(-)#1	#1 Exp	(-)#3	#3 Exp	#5 (-)	#5 Exp	(-) [illegible] DNA	NheI (A)	NheI (B)
plasmid	1µL	2.7µL	1µL	2.7µL	1µL	2.7µL	1µL	1µL	1µL
Buffer 2	1µL	1µL	1µL	1µL	1µL	1µL	1µL	1µL	1µL
BSA (10x) ([illegible])	1µL	1µL	1µL	1µL	1µL	1µL	1µL	1µL	1µL
NdeI ([illegible])	—	.75µL	—	.75µL	—	.75µL	—	.55µL	—
NheI ([illegible])	—	1.5µL	—	1.5µL	—	1.5µL	—	—	1.1µL
dH2O	7µL	3.05µL	7µL	3.05µL	7µL	3.05µL	7µL	6.45µL	5.9µL

incubate @ 37°C for 1 hour

expect 5.5K and 1.7Kb bands. start 4:41 pm

[illegible]

Selection B # [illegible] w/ NdeI and NheI

No good [illegible]

	(-)#1	Exp #1	(-)#2	Exp #2	(-)#5	#5 Exp	(-) [illegible] DNA	NdeI	NheI
plasmid	2µL	9.4µL	2µL	9.7µL	2µL	9.7µL	1µL	1µL	1µL
Buffer 2	1.5µL	1.5µL	1.5µL	1.5µL	1.5µL	1.5µL	1µL	1µL	1µL
BSA (10x)	1.5µL	1.5µL	1.5µL	1.5µL	1.5µL	1.5µL	1µL	1µL	1µL
NdeI (10U/µL)	—	1.5µL	—	1.5µL	—	1.5µL	—	.55µL	—
NheI ([illegible])	—	.3µL	—	.3µL	—	.3µL	—	—	1.1µL
dH2O	10µL	.8µL	10µL	.8µL	10µL	.8µL	7µL	6.45µL	5.9µL

[illegible]

9/19/[illegible]

- Table the [illegible]
- Digest of the [illegible] & [illegible] again at different [illegible]
- [illegible] [illegible] [illegible]
- Check that I get [illegible] [illegible]

[illegible] #3 for [illegible]

– [illegible] –

#1 50:1 ~ 120 mg

50:1 #3 120 mg

[illegible] #1 [illegible] 100 mg

[illegible] 9/[illegible]/[illegible]

#1 – 100 mg
#2 – 110 mg
#3 – 110 mg

[illegible] good

Digestion with [illegible] → [illegible] the [illegible] and [illegible]

[illegible] [illegible] [illegible] with [illegible]

9/17/08 Checking [illegible] minipreps

with [illegible] I and KpnI

KpnI digest of [illegible] minipreps.

	#1(-)	#1 B/g	#[illegible](-)	#2 B/g	#3(-)	#3 B/g	[illegible] (-)	[illegible]
plasmid	1uL	3uL (super)	1uL	3uL	1uL	3uL	1uL	1.5uL
Buffer #1	1uL	1uL	1uL	1uL	1uL	1uL	1uL	1uL
BSA (1:10)	1uL	1uL	1uL	1uL	1uL	1uL	1uL	1uL
KpnI (10u)	—	.4uL	—	.4uL	—	.4uL	—	.225 uL [illegible]
dH2O	7uL	4.6uL	7uL	4.6uL	7uL	4.6uL	7uL	4.[illegible] uL

Master KpnI mix
4.4x

Buffer - 4uL
BSA (1:10) - 4uL
KpnI - 1.6uL
dH2O - 18.4uL

add 2uL to each tube

Master mix
4.4x

Buffer 1 - 4uL
BSA (1:10) - 4uL
dH2O - 28

add 9uL to each tube

incubated @ 37°C
@ 3:45 pm
and 4:45 pm

9/17/09

SmaI digest of [illegible]-EGFP-[illegible]

	#1(-)	#1 Bg	#2(-)	#2 Bg	#3(-)	#3 Bg	(-) pcDNA	(+) SmaI
plasmid	1uL	10uL	1uL	10uL	1uL	10uL	1uL	1uL
buffer #4 (9/17/09)	1.5uL	1.5uL	1.5uL	1.5uL	1.5uL	1.5uL	1.5uL	1.5uL
SmaI ([illegible])	—	1uL	—	1uL	—	1uL	—	1.22uL (1:20 dil)
dH2O	12.5uL	2.5uL	12.5uL	2.5uL	12.5uL	2.5uL	12.5uL	12.28uL

digest 1ug of DNA

incubated @ 25°C
@ 3:15 pm
end 4:30 pm

Master mix SmaI

	4x
buffer #4	6uL
SmaI ([illegible])	4uL
dH2O	10uL

add 5uL to each tube

(-) master mix

buffer #4	6uL
dH2O	50uL

add 14uL to each tube

Silverstain α & β digest w/

NheI and XhoI

9/19/04 – did not have time to finish silverstain digestion until Friday at [illegible]

β exposed	α exposed
5,315	1175
1,450	5,315
	309
6765	6753

I only used 150μl of the [illegible]

maybe [illegible]

β

	[illegible]	[illegible]
buffer #2	4μl	4μl
BSA (10x)	4μl	4μl
NheI	2μl	—
XhoI	1μl	—
dH_2O	19.5	44 μl

↳ add 3.3 ul ↳ add 15ul

α

	[illegible]	[illegible]
buffer #2	3μl	3μl
BSA (10x)	3μl	3μl
NheI	6 μl	—
XhoI	7.5 μl	—
dH_2O	—	6 μl

[illegible] (date)

[illegible]

[illegible]

[illegible]

	(-) #1	#1 Exp	(-) #2	#2 Exp	(-) #3	#3 Exp	(-) pcDNA	(+) NheI	XbaI (+)
plasmid	2uL	7.5 (321 ng)	2uL	7.5 uL (321 ng)	2uL	7.5uL (321 ng)	1uL	1uL	1uL
buffer #2	1uL	1uL	1uL	1uL	1uL	1uL	1uL	1uL	1uL
BSA ([illegible])	1uL	1uL	1uL	1uL	1uL	1uL	1uL	1uL	1uL
NheI	—	.5uL	—	.5uL	—	.5uL	—	.5uL ([illegible])	—
XbaI	—	.25uL	—	.25uL	—	.25uL	—	—	.1uL ([illegible])
dH2O	6uL	4.75uL	6uL	4.75uL	6uL	4.75uL	7uL	[illegible]	[illegible]

[illegible] @ [illegible] pm

[illegible]

[illegible]

[illegible]

[illegible]

	(-) #1	#1 Exp	#2 (-)	#2 Exp	#3 (-)	#3 Exp	(-) pcDNA	(+) NheI	XbaI (+)
plasmid	1uL	14uL	1uL	14uL	1uL	14uL			
buffer #2	2uL	2uL	2uL	2uL	2uL	2uL			
BSA ([illegible])	2uL	2uL	2uL	2uL	2uL	2uL			
NheI	—	1.6	—	1.6	—	1.6			
XbaI	—	.8uL	—	.8uL	—	.8uL			
dH2O	15uL	—	15uL	—	15uL	—			

[illegible] 13 [illegible]

5 mg to [illegible] [illegible] [illegible] 36 [illegible]

1.5 mg

3:1 1 - 122 mg — 15.7 mL
3:1 2 - 120 mg — 15.9 mL
10:1 - 1 100 mg — 15 mL ✓
10:1 - 3 - 100 mg — 11 mL
3:1 - 2 60 mg — 28 mL
50:1 - 1 60 mg — 25 mL

} 8/26/09 [illegible]

3:1 - 2 - 50 mg — 7 mL
3:1 - 3 - 122 mg — 14.3 mL ✓
3:1 - 1 - 100 mg — 15 mL ✓
10:1 - 2 ? — n/a
5:1 - 3 100 mg — 15 mL
10:1 - 2 133 mg — 15.7 mL ✓
50:1 - 1 100 mg — 15 mL ✓
100:1 #3 100 mg — 15 mL
50:1 #2 100 mg — 15 mL
50:1 - 2 [illegible] ? — n/a ✓
50:1 3 100 mg — 15 mL
9/15/09 → 100:1 - 2 100 mg — 15 mL
100:1 - 2 133 mg — 15.7 mL
100:1 - 3 133 mg — 15.8 mL ✓

Started @ 4:06 pm [illegible] [illegible] [illegible]

End 6:06 pm

9/19/09

- did [illegible] multigraph [illegible] [illegible] [illegible]-shirt

gels

20:1 #1
5:1 #1
100:1 #3
50:1 #2

after freeze thaw
in glycerol @ -80°C

place in the 2009 [illegible] box.

9/23/09 - doing miniprep of the [illegible]-shirt

- made some more [illegible] [illegible] [illegible] [illegible] tubes
for the multigraph on Sunday.

- Need to [illegible] [illegible]-shirt with [illegible]
to check [illegible]'s.

100:1 – 1 mg – 5.1 uL

20:1 – 500 mg 5 uL

5:1 100:1 – 60 mg 3 uL of [illegible]

50:1 – 11.3 mg [illegible] –>

[illegible]

2nd control

	(-) 5:1	BCP 5:1	(-) 20:1	BCP 20:1	(-) 50:1	BCP 50:1	(-) 100:1	BP 100:1	(-) PcDAg	
pound	8 uL	7 uL (6 mg)	8 uL ~~[illegible]~~	5 uL 5 mg	2 uL	12.5 uL (8 mg)	1 uL	9.1 uL ([illegible] mg)	1 uL	1 uL
[illegible] #4	1.5	2.5 uL	1.5	1.5 uL	1.5	1.5 uL	1.3 uL	1.5 uL	6.5 uL	7.5 uL
										(1137 uL)
CuS (1:1 [illegible])	—	.60 uL	—	50 uL	—	.80 uL	—	1 uL	—	.27 uL
H_2O	10.5	1.9	12.3	13 uL	10.5	.14 uL	12.5	7.4	12.5 uL	12.75 uL

~~[illegible]~~ Start @ 5:16 pm

end 8:16 pm

9/22/04

Running gel of the Step I digests of the [illegible] on [illegible]

checking for [illegible] integrity of the [illegible] plasmid.

$\mathrm{[illegible]} = 1:[illegible]$

[illegible] = 1:[illegible]

Today - [illegible] [illegible] [illegible] [illegible] and [illegible] α and β in glycerol for [illegible] [illegible].

3096 - 6119

1:3 [illegible]

[illegible] 55 5687574.70 [illegible] = 1.7582 $\times 10^{-5}$ moles

70 mg = 5.275 $\times 10^{-6}$ moles

[illegible] 1673361.77 [illegible]

2316.58 / 5[illegible]

[illegible]

need 100mg of [illegible] 5.[illegible] $\times 10^{-5}$ moles

new [illegible] of [illegible] = 1.583 $\times 10^{-5}$ moles

need 580.52 mg of [illegible] for 1:3:1 [illegible].

[illegible] 300 mg

9/23/04

Repeat

Fncss midprep 20 ng/uL

Fnc 55 with Msp A1 I — then add phosphatase

	(−) Fnc55	Exp Fnc 55 #1	Exp Fnc 55 #2	(−) pcDNA 3.1	pcDNA 3.1 MspA1 I
Fncss	3 uL	(250 ng) 12.5 uL	(250 ng) 12.5 uL	1 uL	1 uL (110 ng)
Buffer # 4	2 uL	2 uL	2 uL	2 uL	2 uL
BSA (1:10)	2 uL	2 uL	2 uL	2 uL	2 uL
MspA1 I (1U/uL)	—	2.5 uL	2.5 uL	—	1.1 uL
dH2O	13 uL	1 uL	1 uL	15 uL	13.9

start 5:07 pm
end 8:1? pm

9/25/04
start 12:07 pm
end @ 2:18 pm

28 hrs

9/25/04 — 9/10/04 ...

incubated @ 37° for 1 hr

... with PMEI ... MS ...

	(−) ...	1003 ...	(−) pcDNA 3.1	pcDNA 3.1 PMEI
...	1 uL	(200 ng) 5.7 uL	1 uL	1 uL (110 ng)
buffer #?	1 uL	2 uL	2 uL	2 uL
PMEI	—	.7 uL	—	1 uL
dH2O	17 uL	11.6 uL	17 uL	15.9 uL

... 8% gel

start 5:0? pm
end 6:3? pm

Antarctic phosphatase and Shrimp Phosphatase

9/25/08 [illegible]

	84p tube 55 #1	84p tube 55 #2
PKB [illegible]	20 uL	20 uL
Dephos Buffer (10X)→1(1X)	2.33 uL	2.37 uL of Antarctic Phosphatase
alkaline Shrimp	1 uL (1U)	~~[illegible]~~
Antarctic phosphatase	—	.3 uL —

heat for 15 min @ 37°C

→ @ 37°C 60 min

run digest on a 0.5% gel w/ uncut DNA

purify and gel ~~[illegible]~~ [illegible]

expected band ~ 4.2 kb

Expected bands for the [illegible] cut with PvuII

a [2715 bp] and 1513 bp

want the 2715 bp

[illegible] on a .8% gel

9/23/07 Sunday

Shrimp - start @ 1:15 pm

Start the
Antarctic @ 1:55 pm

1.5 grams of Agarose dissolved in
300 mL of .5% gel.

[illegible] gel

Ladder [illegible] Antarctic 1 2 Shrimp 1 2

Gel isolation

SAP

#1 .4028 #2 .4780

$g\left(\frac{100\ \mu L}{.1\ g}\right)$

402.5 μL 478.0 μL

Antarctic

#1 .3980 #2 .3045

$.7886\ g\left(\frac{100\ \mu L}{.1\ g}\right)$

398.0 μL 304.5 μL

1) incubate @ 55°C for 5 min

7/23/09 ΔSAW-ShRd1 and Fine 5T ligation. (Blunt end ligation)

3:1 Ligation

	(-) SAP	SAP treat	(-) Autolig	Autolig. Expt
Fine 5T digested	100 ng 1.5 uL	100 ng [1.5 uL]	100 ng 1.5 uL	100 ng [1.5 uL]
ΔSAW-ShRd1 digested	—	886.53 ng [5.31 uL]	—	886.53 ng [5.31 uL]
10x Ligase Buffer	2.5 uL	2.5 uL	2.5 uL	2.5 uL
T4 DNA Ligase	1 uL (400 U)	1 uL (400 U)	1 uL (400 U)	1 uL (400 U)
ddH2O	6.5 uL	1.19 uL	6.5 uL	1.19 uL

@ 9pm

Incubate @ room temp (25°C)

Overnight ligation.

4/29/0[illegible]

Todo - transform ligation rxn
into tet plates

1st make tet plates

for 500mL - need 132 mL of tet

pour 20-25 mL of tet/agar into each plate.

- Note ligation - 100 ng total DNA used!!!

used [illegible] @ [illegible] [illegible] DH5α

50 uL - for each - total 4 → 2.5 uL of ligation
incubate on ice for 5 min
add 200/250 uL of LB and incubate them
in shaker for an hour.

start @ 3:03 pm
end @ 4:03 pm

plates started @ 4:30 pm @ 37°C

(20 uL) = ([illegible] uL)(10 uL)

5 uL) 5 uL

(5 uL)

Stratagene - robocycler - gradient 40

PCR - set-mixture

gradient PCR   Sample PCRs

	35 linker Reagents
1uL	pcEP4-FL3-her I-Σ_{33} (1ng)
10uL	5x Phusion HF buffer 9/24/04
5uL	2mM dNTP's
2uL	5'pel 35 linker 5' (10uM)
2uL	5'pel 35 linker 3' (10uM)
29.5uL	dH2O
.5uL	Phusion DNA polymerase (9/24/04)

Her/Sulc step T_m #1

	Reagents
1uL	pcEF4-FL3-her 2-Σ_{33} (1ng)
10uL	5x Phusion HF buffer 9/24/04
5uL	2mM dNTP's
2uL	Her2/Sulc 5' (10uM)
2uL	Her2/Sulc inner 3' (10uM)
29.5uL	dH2O
.5uL	phusion DNA polymerase 9/24/04

#) control MH PCR - 115-aa mutant

	Reagents
1uL	PCR - 115-aa mutant
10uL	5x Phusion HF buffer (9/24/04)
5uL	2mM dNTPs
2uL	[illegible] 5'
2uL	[illegible] 3'
29.5uL	dH2O
.5uL	phusion DNA polymerase new/old (6/04/04) 9/24/04

master mix 5 rxn

50uL buffer
25uL dNTPs
147.5 dH2O

49.5 to each tube

9/25/14

- Got very ~~little~~ useful results for the fuse55-[illegible] plates

  1 colony per plate.

- Need to redo ligation, this time using a higher concentration ligase and less DNA !!

- ~~today~~ Need to cut more of fuse55 w/ [illegible]

  - reading protocol on 9/25/14.

  - had saved PCR products

    - [illegible] – 1.5 µL

    - 55 [illegible] – 2.8 µL

1.5% Agarose – 4.5 g for 300 mL

SAP dephosphorylation of fuse55-[illegible] started @ 2:52 pm
end 3:52 pm

Antarctic started @ [illegible] pm – 3:35 pm
end 3:40 pm

9/25/[illegible]

T4 Ligation – [illegible] SAP & Ant with [illegible]

1:3 Ligation (plasmid : Insert) | Overnight @ 16°C

	(−) SAP	SAP [illegible]	(−) [illegible]	[illegible] [illegible]
plasmid	50 ng 7.14 uL	30 ng (4.3 uL)	7?5 ng [illegible] 5 uL	50 ng (1.5 uL)
[illegible] insert DNA	—	26.6 ng 1.59 uL	—	26.6 ng 1.59 uL
Buffer T4 ligase	2 uL	2 uL	2 uL	2 uL
T4 DNA ligase (High Conc)	1 uL	1 uL	1 uL	1 uL
dH2O	9.86	11.11 uL	9 uL	[illegible] uL

x / 15 ng = 26.6 / 30 ng

15 ng + [illegible] 13.2

3:1 [illegible] 17 ng – [illegible] insert 15 ng = ~.89 uL

5:1 17 ng – 25 ng ~ 1.49 uL

9/26/07 PCR - strep-her2 outer oligos

✓

	Reagents 50 uL rxns
1 uL	PCR Amp-her2-inner oligo #1 (10 ng/uL)
10 uL	5x phusion HF Buffer (9/24/07)
5 uL	2 mM dNTPs
2 uL	Her2 / strep 5' ~~[illegible]~~ (10 uM)
2 uL	Her2 / strep 3' outer (10 uM)
29.5 uL	dH_2O
.5 uL	phusion DNA polymerase (9/24/07)

- Ligation - [illegible] - transformation stopped @ 7:30 pm

incubate @ 37 °C for 1 hour

before plating

added 250 uL of LB to each tube of 50 uL of cells

used 2.5 uL of DNA.

[illegible] – [illegible]

#1 .3917 [illegible]

#2 . ~~[illegible]~~ .2750

#3 .1999

#4 .5686

9/25/09 – [illegible]

– 2 [illegible] – [illegible] Antibody

– [illegible] (–) Antibody

– 3 [illegible] (–) SAP

– 0 [illegible] SAP [illegible]

9/28/09 – [illegible]

– check ~~[illegible]~~ [illegible]

–

[illegible] – [illegible]

#1 – .3310 g

#2 .3520 g

[illegible]

[illegible]

5 [illegible]

Check [illegible] of [illegible] in [illegible]

[illegible]

[illegible]

[illegible]	8.4 kb
[illegible]	[illegible] kb
[illegible]	[illegible] kb
[illegible]	11.7 kb
[illegible]	[illegible], 5.8 kb
[illegible]	7.1 kb, [illegible] kb

[illegible] Buffer [illegible] — [illegible] HI [illegible]

	[illegible] (−)	[illegible] Exp	[illegible] (−)	[illegible] Exp	SAP #2 (−)	SAP Exp	Ant 2 (−)	Ant 1 Exp	Ant 2 (−)	Ant 2 Exp	Ant 3 (−)	Ant 3 Exp	Ant 4 (−)	Ant 4 Exp	Ant 4 (−)	Ant 6 Exp
[illegible]	6mg	11mg	6mg	11mg	6mg	11mg	6mg	11mg	6mg	11mg	6mg	11mg	6mg	11mg	6mg	11mg
	1μL	2μL	5μL	6μL	5μL	15μL	5μL	10μL	5μL	10μL	2.5μL	5μL	5μL	10μL	5μL	10μL
Buffer #3	2μL	2μL	2μL	2μL	2μL	2μL	2μL	2μL	2μL	2μL	2μL	2μL	2μL	2μL	2μL	2μL
[illegible]	2μL	2μL	2μL	2μL	2μL	2μL	2μL	2μL	2μL	2μL	2μL	2μL	2μL	2μL	2μL	2μL
[illegible]	—	1.2	—	1.2	—	1.2	—	1.2	—	1.2	—	1.2	—	1.2	—	1.2
H_2O	13.6	12.8	13	5.8	11	4.8	11	4.8	11	1.8	13.5	4.8	11	9.8	11	4.8

	Ant 6 (−)	Ant 6 Exp
[illegible]	6mg / 12.5mL	12mg / 5μL
Buffer #3	2μL	2μL
[illegible]	2μL	2μL
[illegible]	—	1.2
H_2O	13.5	9.8

master mix	10 rxns	(−) [illegible]
Buffer #3	20μL	20μL
BSA (1:10)	20μL	20μL
Bam 12 (1:10)	12μL	
1.5 enzyme / 13.5 dH2O	6.2 μL [illegible]	4μL [illegible]

Started @ 5:[illegible] pm

8/25/09 - trying to do single digest with XhoI

to the Fire85-[illegible]-[illegible]

to check if control insert [illegible] [illegible], [illegible]

and was [illegible] filled

	(-) Sibb	Sibb Exp	(-) Fire85	Fire85 Exp	(-) SAP	SAP Exp	Ant 1 (-)	Ant 1 Exp	Ant 2 (-)	Ant 2 Exp	Ant 3 (-)	Ant 3 Exp	Ant 4 (-)	Ant 4 Exp	Ant 5 (-)	Ant 5 Exp
DNA	60ng 1uL	100ng .83	60ng 3uL	100ng 5uL	60ng 5uL	100ng 3.33	60ng 5uL	100ng 8.33	60ng 5uL	100ng 8.30	60ng 2.5uL	100ng 4.1	60ng 5uL	100ng 8.33	60ng 5uL	100ng 8.37
buffer #3	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
BSA (1:10)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
XhoI	—	1uL	—	1uL	—	1uL	—	1uL	—	1uL	—	1uL	—	1uL	—	1uL
H_2O	11uL	10.67	9uL	8uL	7uL	2.67	7	2.67	7	2.67	9.5	6.9	7	2.67	7uL	2.67

	Ant 6 (-)	Ant 6 Exp
DNA	60ng 2.5uL	100ng 4.1uL
buffer #3	1.5	1.5
BSA	1.5	1.5
XhoI	—	1uL
H_2O	9.5 uL	6.9 uL

Master mix 11 rxns

Buffer #3	16.5
BSA (1:10)	16.5
XhoI ([illegible])	11uL

4uL to each tube

Ant [illegible] (-)

Buffer #3	16.5
BSA 1:10	16.5

3uL to each tube

PCR setting

98°C → 2 min

98°C → 38 sec
58°C → 30 sec } 19x
72°C → 45 sec

72°C → 7 min

4°C → forever

PvuII – 1464 – 4579

9/30/04

~5687574.70 Da

- digest fires55 with BglII in NEB buffer 3 — 250

- digest Her2sdu-step-dy with BglII — 372 bp — 5603942.06 Da

- digest pUC19 as a control DNA with BglII

3.1

5 ug = 40.741 x 10^{-6} pmole

$\frac{x}{50\,ng} = \frac{13.73}{50\,ng} = 8.232$

need 7.6373 x 10^{-5} pmole of fires = 13.73 ng of

insert.

	(-) fires55	fires55 BglII	Her2 (-) step	Her2 step BglII	pUC19 (-)	pUC19 (+)
plasmid	100 ng (3 uL)	100 ng 1.5 uL	60 ng 1.5 uL	100 ng 2.5 uL	60 ng 1.2 uL	100 ng 2 uL
buffer #3 NEB	1.5 uL	1.5 uL	1.5 uL	1.5 uL	1.5 uL	1.5 uL
BglII (1 U/uL)	—	2 uL	—	2 uL	—	2 uL
dH2O	10.5 uL	6.5 uL	12 uL	9 uL	11.3 uL	9.5 uL

started @ 2:25 pm

Incubated @ 37°C

end 3:25 pm.

- gel isolate the [illegible] and strep-Tag.

fox55 [illegible] w/ BglII gel isolation

0.3381 g/mL?

gel quantification - Hov2/ ~ 5 ng/uL 3 uL
fox55 ~ [illegible] ng - 5 uL

Ligation - fox55 - strep/Hov2

3:1 (insert : plasmid)

	(-)	Ligation
plasmid fox55	[illegible] ng 5 uL	30.[illegible] ng 13.75
strep/[illegible] insert	[illegible]	8.232 13.[illegible] ng — 3.08 uL
buffer T4 DNA ligase	2.5 uL	2.5 uL
T4 DNA ligase	1 uL	1 uL
dH_2O	13.5 uL	—

started @ 6:47 pm
@ 25° C

10/5/02

- doing minipreps of the [illegible] (1-6)

the [illegible] spin done for me

[illegible] miniprep
Digest E_g I I and NAE

convert - Puc19

E_g I I — [illegible] E_g I I = 9192 bp, 19 bp

[illegible] = 9192 bp, 51 bp

NAE — [illegible] = 9,300 bp, 2100 bp, 1100 bp, 695 bp

[illegible] = 9,300 bp, [illegible] bp, [illegible] bp, 695 bp

	1 (-)	1 (+)	2 (-)	2 (+)	3 (-)	3 (+)	4 (-)	4 (+)	5 (-)	5 (+)	6 (-)	(6) ±	Puc19 (-)	Puc19 (+)
plasmid	[illegible] 1.5 ul	30 ng 8 ul	30 ng 3.5 ul	30 ng 13 ul	30 ng 2.5	30 ng 2.5	30 ng 2.5	30 ng 2.5	30 ng 2.5	30 ng 2.5	30 ng 2.5	30 ng 2.5	100 ng [illegible]	[illegible] 2 ul
Buffer [illegible]	[illegible]	3 ul	2 ul	3 ul	2 ul	3 ul	2 ul	3 ul	2 ul	3 ul	2 ul	3 ul	2 ul	2 ul
[illegible]														
E_g I I	—	.4 ul	—	.3 ul	—	.5 ul	—	.4 ul	—	.4 ul	—	.4 ul	—	0.4 ul
H_2O	16.5	11.4	15.5	1.4	15.5	1.4	15.5	1.4	15.5	1.4	15.5	1.4	17 ul	

started @ 4:33 pm @ 37°C

end @ 5:48 pm

10/6/09 fur35-Hers and pSilder

double digest w/ XhoI and SpeI

on pCDNA3.1 Zeo as positive control

— hand to cmate's pSilder PCR #2 —

	fur35/hers #1	fur37/hers Exp	pSilder Exp	pCDNA3.1 (—)	pCDNA3.1 SpeI	pCDNA3.1 XhoI
plasmid	80 ng	200 ng	~~100 ng~~ 50 ng	100 ng	100 ng	100 ng
	1.8 uL	10 uL	15 uL	1 uL	1 uL	1 uL
Buffer #2	2 uL	2 uL	2 uL	2 uL	2 uL	2 uL
BSA (10X)	2 uL	2 uL	2 uL	2 uL	2 uL	2 uL
SpeI	—	.2 uL (1 uL)	.15 uL 1.5 uL	—	.1 uL	—
XhoI	—	.2 uL (1 uL)	.15 uL 1.5 uL	—	—	.1 uL
dH_2O	14.2	2 uL	—	15 uL	13.9	13.9

incubate @ 37°C start: 3:42 pm

end @ 4:52 pm

1.4 ug/uL

1400

(1400) (X?)

= (100 ng) [illegible]

= 57.5 uL

C-H55 Hd$_{\mathrm{II}}$ = 6175062.11 Da — 57.5 $\left(\frac{14-2}{6175062}\right)$ = 5.17 × 10^{-6}

G1(lane) — 54490.41 Da

[illegible] 1.552 × 10^{-5} [illegible]
of [illegible] for 5.1 = .931 [illegible]

[illegible] — [illegible] in 5ul

Radom [illegible] PCR

	[illegible] Reagents
1ul	p[illegible] – [illegible] (10uM)
10ul	5x [illegible] HF [illegible] 4/24/0[illegible]
5ul	2 mM dNTP's
2ul	[illegible] 5' (10uM)
2ul	[illegible] 3' (10uM)
29.5ul	dH$_2$O
.5ul	phusion DNA polymerase (4/24/0[illegible])

gel [illegible]

#1 – 0.3711

#2 – 0.1728

#3 – 0.1287

10/[illegible]/[illegible]

Gox55-[illegible] and [illegible] SpeI and PstI digest.

	(−) [illegible]	BCP ([illegible])	Gs [illegible]	[illegible] 3.1	(+) SpeI	(+) [illegible]
plasmid	6 ug (13 uL)	250 ng (12.5 uL)	100 ng (15.6 uL)	100 ng 1 uL	100 ng 1 uL	100 ng 1 uL
buffer	2 uL	2 uL	2 uL	2 uL	2 uL	2 uL
BSA (10X)	2 uL	2 uL	2 uL	2 uL	2 uL	2 uL
SpeI 1:10	—	.25 uL 2.5 uL	.1 uL 1 uL	—	.1 uL 1 uL	—
PstI 1:10	—	.25 uL 2.5 uL	.1 uL 1 uL	—	—	.1 uL 1.1 uL
H_2O	14.2	—	—	15 uL	13.9 uL	13.9 uL

Start 5:51 pm @ 37°C

Gox-[illegible]
gel extraction .2214 g.

10/13/09

Sm! digest - Gibson and fuse5-Her

gel quantification

fuse5-Her - 8ug in 5uL

Gibson - 8ug in 5uL

Ligation
fuse5-Her with gibson inserts

	fuse5-Her no insert	fuse5-Her gibson insert
plasmid fuse5-Her	2x1b 3.5ng 19 uL	35ng 21.47 uL
Insert gibson	—	1ng 1uL ~1.1ng
T4 ligase buffer	2.5uL	2.6uL
T4 ligase 05/07	1uL	1uL
ddH2O	3.5	—

3:1 (insert : plasmid)

@ 25°C

start @ 1:32pm

end 1:54pm

Speed down for 1 hour

in 250uL of LB

before plating

plasmid

buffer # 4
digestion

BSA
(1:100)

ddH2O

Enzyme

plasmid

buffer # 2
ligation

insert

ddH2O

ddH2O

10/12/07

Spl dilut - Gibson and Fac55-Her

95° [illegible]

Fac55-Her - 9mg in 5mL

Gibson - 8mg in 5mL

Ligation
Fac55-Her with Gibson insert

3:1 (insert : plasmid)

	Fac55-Her no insert	Fac55-Her Gibson insert
plasmid Fac55-Her	~~2x[illegible]~~ 35mg	35ng
	19 uL ~~21.80~~ uL	(21.47 uL)
Insert Gibson	—	1ug (1uL) ~ 1.1ug
Ligation buffer	2.5uL	2.6 uL
T4 Ligase 05/07	1uL	1uL
dH2O	3.5	—

@ 25°C

Start @ 1:32pm

end 1:52pm

Slow down for 1 hour

to 250uL of LB

before plating

10/3/06

Luxss - Mlu ϕ1 digestion

diAAV-short1 (9/30/06) 50:1 #2) - PvuII digest

Luxss - Mlu ABC digest

pcDNA 3.1 = 1lμg/μL

	(-) luxss	sample #1 luxss	#1 lux58	(-) PCDNA 3.1	pcDNA 3.1 Mlu ABC
[illegible]	600ng 3μL	700ng (35μL)	700ng (35μL)	1μL	1μL
buffer #4 [illegible]	2μL	4.5μL	4.5μL	2μL	2μL
BSA [illegible]	2μL	4.5μL	4.5μL	2μL	2μL
Mlu ABC	—	.7μL	.7μL	—	.110μL ((1μL 10μL)) 1:10
H_2O	13μL	.3μL	.3μL	15μL	13.9μL

Started @ 3:25 pm @ 37°C

end 7:44pm

diAAV-short1 - digest with PvuII

	(-) diAAV	50:1 #2 diAAV-short1	3.1 (-) PCDNA	pcDNA 3.1 PvuII
[illegible]	1μL	1μg (16.667μL)	1μL	1μL
buffer #2 [illegible]	2μL	2μL	2μL	2μL
PvuII	—	1μL	—	.1μL (1μL 10μL)
H_2O	17μL	.33μL	17μL	15.45μL

10/12/[illegible]

Activity of SAP

fuse 55 - digested w/ AhpAEt

	fuse 55 SAP	fuse 55 Antarc
fuse [illegible]	46 [illegible]	46 [illegible]
Dephos Buffer	5.16 µL	5.2 µL
phosphatase	SAP .5 µL	Ant 1 µL

↳ @ 37°C for 1 hr (SAP)

↳ @ 37°C ↳ 30 min (Ant)

gel order

ladder	pcDNA (–)	pcDNA (+)	(+) fuse 50	Ant fuse 55	SAP fuse 55
–	–	–	–	– – –	– – –

gel

10/13/04

Ligation of

[illegible] -- pBABe-Shock

	SAP & Am (-) no insert	SAP 3:1	Am 3:1	SAP 5:1	Am 5:1
plasmid [illegible]	30ng 3uL	17ng 1.7uL	17ng 1.7uL	17ng 1.7uL	17ng 1.7uL
insert 9/23/04	—	15ng (.84uL)	15ng (.84uL)	25ng ~~1.5uL~~ [illegible]	25ng 1.5uL
Buffer T4 ligase	1.8uL	1.5uL	1.8uL	2.3uL ~~[illegible]~~	1.8uL
T4 DNA ligase	1uL	1uL	1uL	1uL	1uL
dH2O	9.5	9.41	9.41	~~[illegible]~~	9.36[illegible]

New Batch 1.5ng/uL

10/15/05

- did [illegible] 2°

[illegible] - [illegible]

#1 [illegible]

#3 [illegible]

- [illegible] [illegible]

& [illegible]

[illegible]

	(−) #1	3W4 #1	(−) #3	3W4 #3	(−) RDNA [illegible]	(+) PCDNA [illegible]
[illegible]	[illegible]	300 ng	[illegible]	300 / 12.5	1 ng	1 ng
	2.77 μL	16.667 μL	2.5 μL	[illegible]	1 μL	1 μL
[illegible]	2 μL	2.5 μL	2.6 μL	2.5 μL	2 μL	2 μL
BSA (1:10)	2 μL	2.5 μL	2 μL	2.5 μL	2 μL	2 μL
BstI (1:10)	—	.3 μL / 3 μL	—	.3 μL / 3 μL	—	.1 μL / [illegible]
dH_2O	13.23 μL	—	13.9 μL	4.5 μL	15 μL	13.[illegible]

Start @ 6:14 pm

End 7:14 pm

10/15/00

[illegible] 10/20/00

[illegible] w/ [illegible]

	(-) [illegible]	[illegible] #1	[illegible] #2	(-) [illegible] DNA	[illegible]
[illegible]	60 ng 3 uL	300 ng 15 uL	300 ng 15 uL	110 ng 1 uL	110 ng 1 uL
Buffer [illegible]	2 uL	2 uL	2 uL	2 uL	2 uL
BSA ([illegible])	2 uL	2 uL	2 uL	2 uL	2 uL
[illegible]	—	.3 uL	.3 uL	—	1.10 uL ([illegible]) [illegible]
dH_2O	13 uL	.7 uL	.7 uL	15 uL	13.5 uL

[illegible] [illegible]
3 [illegible]

[illegible] Buffer [illegible]
[illegible] BSA (1:10)
[illegible] uL [illegible]
[illegible] uL of dH_2O

[illegible]

Start @ 3:34 pm @ 37°C

[illegible] @ 11:54 am

Tuesday 10/20/09

scAAV-Shield digest w/ PvuII

	scAAV-Shield (–)	scAAV-Shield Exp #1	scAAV-Shield Exp #2	pcDNA (–)	pcDNA Exp. PvuII
plasmid	80 mg ~~1uL~~ ✓ ~~[illegible]~~	4 mg ~~[illegible]~~ 15uL	4 mg ~~[illegible]~~ 16uL	11 mg 1uL	11 mg 1uL
Buffer 2	2uL	2uL	2uL	2uL	2uL
PvuII	—	.4uL	.4uL	—	9.10 [illegible] (1:100) .4uL
dH_2O	17uL	2.6 ~~[illegible]~~ uL	2.6 ~~[illegible]~~ uL	17uL	15.9 uL

Master mix — 6uL buffer 2

1.2uL [illegible]

7.8 ~~[illegible]~~ uL of dH_2O

5uL
~~[illegible]~~ to each [illegible]

Started @ 3:41pm @ 37°C

Stopped @ 12:23 pm

10/15/04

10/20/04 – revising protocol.

digest five 5'-... SpeI + XhoI digest

	mini #85 (1) (10/18/04)	#2	#5	pcDNA 3.1 (-)	pcDNA SpeI	pcDNA XhoI
plasmid	60ng 5uL	150ng 15uL	180ng 15uL	1ug 1uL	1ug 1uL	1ug 1uL
Buffer #2 10x	2uL	2.5uL	2.5uL	2uL	2uL	2uL
BSA (1:100)	2uL	2.5uL	2.5uL	2uL	2uL	2uL
SpeI	—	.150uL 1.5uL	.18uL 1.8uL	—	.18uL	—
XhoI	—	.150uL 1.5uL	.18uL 1.8uL	—	—	.114uL
dH2O	11uL	1.4uL	1.9uL	15uL	13.9	13.9

started @ 5:11pm @ 37°C

10/21/04 Stored @ 11:33pm

12/19/[illegible]

– [illegible] – [illegible] – [illegible] [illegible]

with [illegible]

[illegible] [illegible]

[illegible] [illegible] & [illegible] – 7,624, 3,119, 1175

[illegible] [illegible] [illegible] – 5693, 3,114, 2,404

[illegible] [illegible] [illegible] – 8,100, 9,154

[illegible] [illegible] [illegible] 8,400, 3,119

[illegible] [illegible] – 6037, 3,119

#1 150 ng [illegible]

#7 [illegible] 80 ng [illegible]

[illegible] [illegible]

[illegible] 18 ml

[illegible] 5.7 ml

[illegible] 44.1

7.5 [illegible] [illegible]

[illegible]

[illegible]

[illegible] 7.5 ml

[illegible] 1.8 ml

[illegible] 5.7 ml

[illegible]

[illegible]

[illegible] #[illegible] 18 ml

[illegible] 135 ml

[illegible] [illegible] [illegible]

[illegible] – [illegible] w/ [illegible]

	(-) 3:1	(+) 3:1	1(-) 5:1	1(+) 5:1	2(-) 5:1	2(+) 5:1	3(-) 5:1	3(+) 5:1	#4(-) 5:1	#4(+) 5:1
[illegible]	3ul	12.5	3ul	12.5	3ul	12.5ul	3ul	12.5	3ul	12.5
Buffer #? [illegible]	2ul	2ul	2ul	2ul	2ul	2ul	2ul	2ul	2ul	2ul
[illegible]	—	.6ul	—	.6ul	—	.6ul	—	.6ul	—	.6ul
dH2O	15ul	4.9ul	15ul	4.9ul	15ul	4.9ul	15ul	4.9ul	15ul	4.9ul

	#5(-) 5:1	#5(+) 5:1	(-)#6 5:1	(+)#6 5:1	(-)#7 5:1	(+)#7 5:1	(-)#8 5:1	(+)#8 5:1	(-) pcDNA	(+)[illegible] pcDNA
[illegible]	3ul	10ul	3ul	10ul	3ul	12.5	3ul	12.5	1ul	1ul
Buffer #?	2ul	2.5	2ul	2.5	2ul	2ul	2ul	2ul	2.0ul	2.0ul
[illegible]	—	.6ul	—	.6ul	—	.6ul	—	.6ul	—	2.2ul ([illegible])
dH2O	15ul	1.9	15ul	1.9	15ul	4.9ul	15.6	4.9	17ul	14.8ul

Spin @ 1:47 pm @ 37°C

10/19/09

digesting [illegible] ~~[illegible]~~ maxiprep with mscl

	fur 55 (-)	2x fur 55 Exp	PCDNA31 (-)	PCDNA31 MSCL	10x fur 55 Exp
plasmid	6 mg 3 uL	1~~0~~0 mg 5.5	11 mg 1 uL	110 mg 1 uL	110 mg 5.5
Buffer #4 9/14/09	2	2	2	2	2
Mscl	—	.22	—	.22	.11
		2.2 uL		2.2 uL	1.1 uL
dH2O	15 uL	10.3	17 uL	14.8	11.4 uL

Started 10:52 am @ 37°C

End @ 11:53 AM

checking for [illegible] fur55 with xhoI.

	(-) fur55	Exp fur55	3:1 (-) 2	3:1 Exp	1 (-)	1 Exp	2 (-)	2 Exp	3 (-)	3 Exp	4 (-)	4 Exp	5 (-)	5 Exp
plasmid	60 mg 3 uL	100 mg 5 uL	60 mg 2.5	100 mg 4.2	60 mg 2.5	100 mg 4.2	60 mg 2.5	100 mg 4.2	60 mg 2.5	100 mg 4.2	60 mg 2.5	100 mg 4.2	60 mg 2.5	100 mg 4.2
Buffer 2	2 uL	2	2	2	2	2	2	2	2	2	2	2	2	2
BSA	2	2	2	2	2	2	2	2	2	2	2	2	2	2
xhoI	—	.1	—	.1	—	.1	—	.1	—	.1	—	.1	—	.1
dH2O	13 uL	10.9	13.5	11.7	13.5	11.7	13.5	11.7	13.5	11.7	13.5	11.7	13.5	11.7

	(−) 6	Exp 6	(−) 7	Exp 7	(−) 8	Exp 8	PcDNA (−)	PcDNA Xhol
prim	60ng 2.5	100ng 4.2	60ng 2.5	100ng 4.2	60ng 2.5	100ng 4.2	110ng 1uL	110ng 1uL
Buffer #2	2	2	2	2	2	2	2	2
[illegible]	2	2	2	2	2	2	2	2
Xhol	—	.1	—	.1	—	.1	—	.1 (10 U/uL)
H_2O	13.5	11.7	13.5	11.7	13.5	11.7	15	13.9

@ 18°

master mix –

	12 rxn	(−) rxn
Buffer #2	24uL	24uL
BSA (10x)	24uL	24uL
Xhol	1.2	—
dH_2O	190.4	162
add –	17.8	17.3 uL

10/17/0[illegible] checking expt 4-5-3

with SphI – XhoI 779, 1117

NheI – XhoI 1036, 850

SphI – NheI 1579, 287

SphI – XhoI

	(–) 1	Exp 1	(–) 2	Exp 2	pcDNA (–)	pcDNA NheI	pcDNA SphI	XhoI pcDNA
plasmid	1µL	1µL	1µL	1µL	1µL	1µL	1µL	1µL
Buffer #2	2	2	2	2	2	2	2	2
BSA	2	2	2	2	2	2	2	2
SphI	—	.2 1µL	—	.2	—	—	.22	—
XhoI	—	.1 1µL	—	.1	—	.11 NheI	—	.11
dH2O	15	8µL 13	15	8µL 13	15	13.9	13.9	13.9

1 = 1 miniprep

2 = miniprep #3

Started @ 3:41pm

NheI – XhoI

	(–) 1	Exp 1	(–) 2	Exp 2
plasmid	1µL	1µL	1µL	1µL
Buffer #2	2	2	2	2
BSA	2	2	2	2
NheI	—	.1	—	.1
XhoI	—	.1	—	.1
dH2O	15	8	15	8

SphI – NheI

	(–) 1	Exp 1	(–) 2	Exp 2
plasmid	1µL	1µL	1µL	1µL
Buffer #2	2	2	2	2
BSA	2	2	2	2
NheI	—	.1	—	.1
SphI	—	.2	—	.2
dH2O	15	8	15	8

10/20/09

- redo fuse55-digest with MspA1I
  dSARu-shank digest with PvuII
  fuse55-bext2 digest with SpeI & XhoI
  because they were lost in the gel isolation the previous time ~~bec~~ - error - the genecleen turbo wash bottle did not have ethanol

Using same protocols of 10/15/09

gel isolation
fuse55 digested w/ MspA1I

#1 .1997 g
#2 .1348
#3 .3155 g

dSARu-~~Sha~~ shank - digest with PvuII

#1 .2160
#2 .4402
#3 .1587
#4 .2798