Jason Cham start 8/01/11

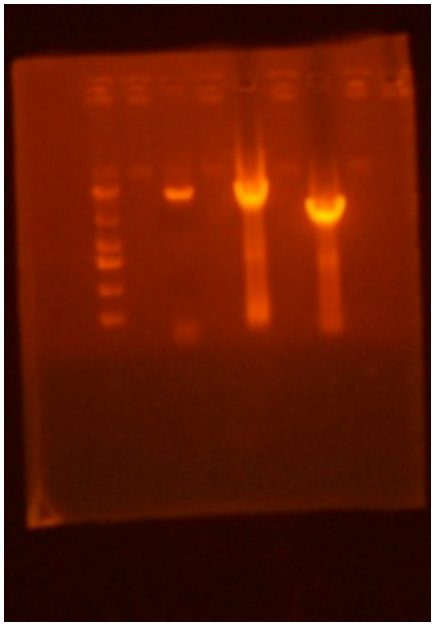
From AndersonLab wiki

Contents

Jcham 04:36, 01 August 2011 (PDT)

- Set up gold reaction for J3L (3), N1L (2), X1L (1), X1R(1), X2R(1)
- Set up EIPCR for X2L(1), N2L(2), N2R(2)

Jcham 04:00, 02 August 2011 (PDT)



1 Kb Plus DNA Ladder
0.7 µg/lane
0.9% agarose gel

```
Lane 1: X2L(1)
Lane 2: N2L(2)
Lane 3: N2R(2)
```

- gel purify X2L, N2L, N2R (elute with 15uL)
- set up gold reaction:

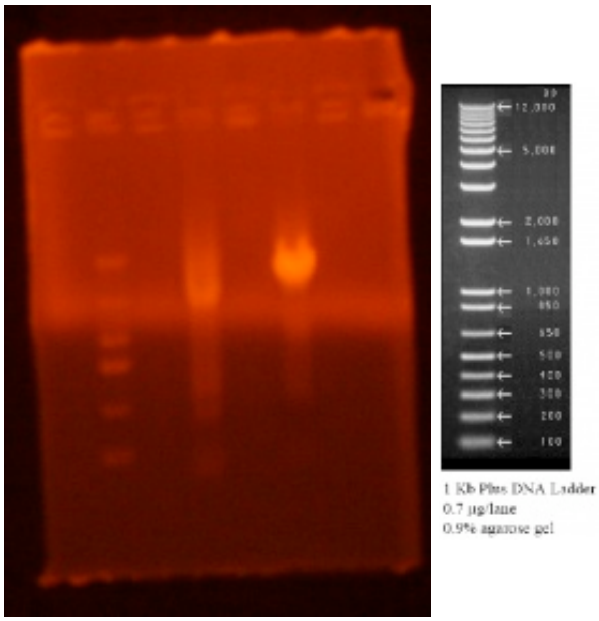
```
15uL DNA, 70 uL water, 10 uL ligase buffer, 1 uL dpn1, 2 uL bsaI, 2uL ligase
```

- zymo clean J3L, N1L, X1L, X1R, X2R (elute with 8 uL)
- electroporate J3L=5.6 , N1L=5.4 , X1L=5.6 , X1R=5.4 , X2R=5.4

Jcham 03:00, 03 August 2011 (PDT)

- N1L did not work. I need to redesign the oligos
- X1R and X1L worked. I scraped and minipreped them.
- sequence x1R and x1L

- Set up EIPCR round 2 for them.




```
lane 1: x1L eipcr3
lane 2: x1R eipcr3
```

- gel purify
- set up gold reaction(3) on x1L and x1R

- zymo clean X2L, N2L, N2R
- electroporate X2L=5.2 , N2L=5.4, N2R=5.4, j3L($)=5.4, x2R($)=5.2

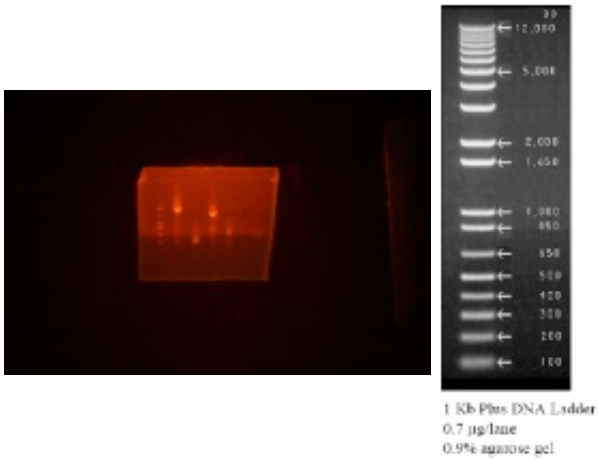
Jcham 05:00, 04 August 2011 (PDT)

- j3L($) and x2R($) did not work.

- Discovered that x2R oligos for eipcr1 is wrong.
- stop with x2 library

- zymo clean x1L and x1R
- electroporate x1L(3/4) and x1R($).

- set up eipcr jc3L(3/3), n2L(3/3), n2R(3/4), x2L(2/4), x2R(2/2)

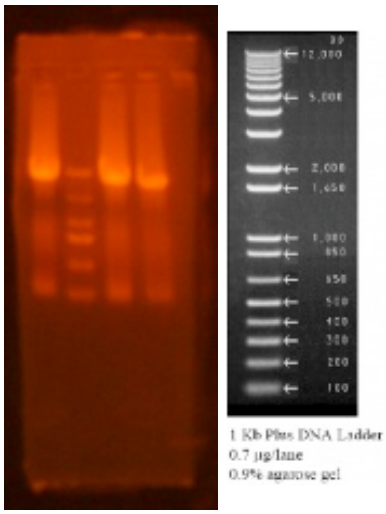



```
lane 1:J3L
lane 2:n2L
lane 3:n2R
lane 4:x2L
lane 5:x2R
```

- gel purify J3L and N2R.
- set up gold reaction for jc2L n2R. set up eipcr 3 for n2L again.

Jcham 03:00, 05 August 2011 (PDT)

- miniprep from scrape x1L
- EIPCR 4, gel purify, gold x1L



- set up EIPCR N1L
- zymo gold reaction of J3L and N2R

- gel purify eipcr of N2L and set up gold reaction

- sequence x1R midi and x1L eipcr3

```
Plan for Tomorrow
1. zymo gold reaction of N2L($), X1L($)
2. scrape and miniprep N2R. Set up EIPCR of N2R($)
3. gel purify, set up gold EIPCR of N1L($)
```

Jcham 01:30, 06 August 2011 (PDT)

- scrape miniprep n2R. Set up eipcr n2R($)
- zymo gold reaction of n2L($) and x1L($)
- set up eipcr of x1L(3/4) because sequencing failed.

Tonight

- gel purify x1L and n2R, set up gold reaction of x1L(3/4) and n2R($)

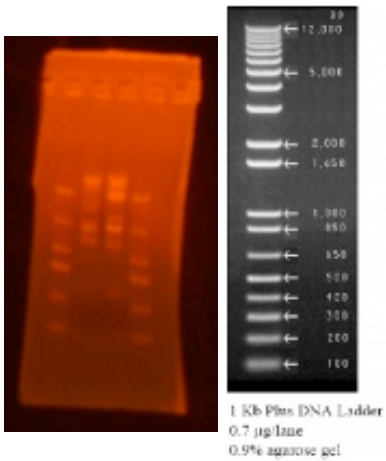
Jcham 04:00, 08 August 2011 (PDT)

- set up EIPCR for N1L($). It failed over the weekend. If it doesn't work this time, we'll have to drop this library.

- transform 1 uL of J3 and X1 into 50 uL of Bss52. Recover with 50 uL of 2yt for 1 hr.

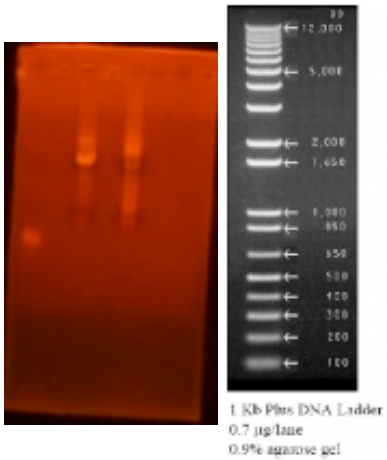
```
   -plate 1 uL add 50 uL water
   -plate 99 uL of the rest of the cells.
```

- run 1uL of J3 and X1 on a gel




```
lane 1: J3
lane 2: X1
```

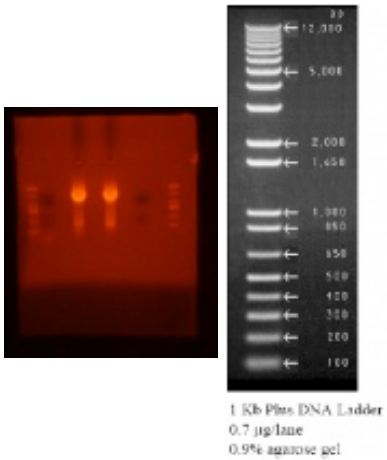
- gel purify N2R and set up gold of N2R



1 Kb Plus DNA Ladder
0.7 µg/lane
0.9% agarose gel

```
lane 1: N2R
lane 2: X1L
```

- miniprep x1L (3/4) from saturated cultures
- set up EIPCR and then gold of X1L($) and N2R($)



1 Kb Plus DNA Ladder
0.7 µg/lane
0.9% agarose gel

```
lane 1: x1L (fail)
lane 2: x1L
lane 3: n2R
lane 4: n1L (fail)
```

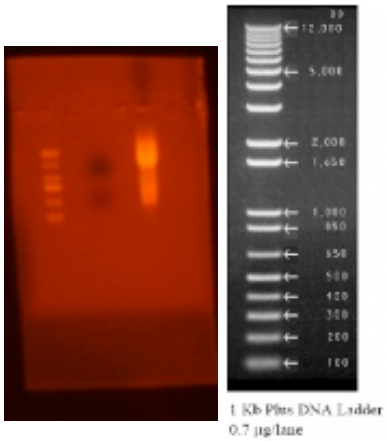
- sequence J3L, N2L, X1L midi and X1L eipcr3

Jcham 05:00, 09 August 2011 (PDT)

- zymo purify gold reaction of n2R$
- electroporate N2R$. recover into flasks.

- sequencing of J3L and N2L midi were good. X1L midi and X1L eipcr3 failed.
- set up sequencing for JC1 JC2 JC3 final libraries

- set up eipcr of N1L($) and X1L(3/4) again. N1L($) is running 6K60
- gel purify and set up gold reaction for N1L($) and X1L(3/4).



1 Kb Plus DNA Ladder
0.7 µg/lane
0.9% agarose gel

```
lane 1: n1L$ (Fail)
lane 2: x1L (3/4)
```

- check oligos for X1 and X2. They don't seem to work so I will check the amount of homology.

```
    redesigned/reordered x1L_F m3 and x1L_R m4
```

Jcham 05:00, 10 August 2011 (PDT)

- N1L$ EIPCR failed again.
- zymo gold reaction of X1L (3/4)
- electroporate and plate on Kan X1L(3/4)