```
27uL CTC gel Extraction (26.5ng/uL)
6uL phosphorylated MULTIMER primer FWD
4uL T4 ligase
3.6 T4 ligase buffer
```

(5) PCR purification (primer removal) Used kit to do a PCR purification to remove excess primer.

```
Concentration: 19ng/uL
```

FINAL LIGATION
16C for 3hrs; 4C 9hrs; 50C 20 mins; 4C forever

----------	Ligation 1	Ligation 2	Control 1	Control 2
Vector (CTC + primer ligation;19ng/uL)	5.26	1.5	5.26	5.26
Insert	2.74 (500bp;30ng/uL)	(1000bp;7ng/uL)	none	none
Ligase Buffer	1	1	1	0
Ligase	1	1	1	0
H20	0	0	0	4.7

8/10/10

File:BottomgelJunkScreeningCTC.jpg File:TopGelJunkScreening.jpg

8/12/10

File:ColonyPCRJunkCTCCloning.jpg File:ColonyPCRJunkandKeyScreening.jpg

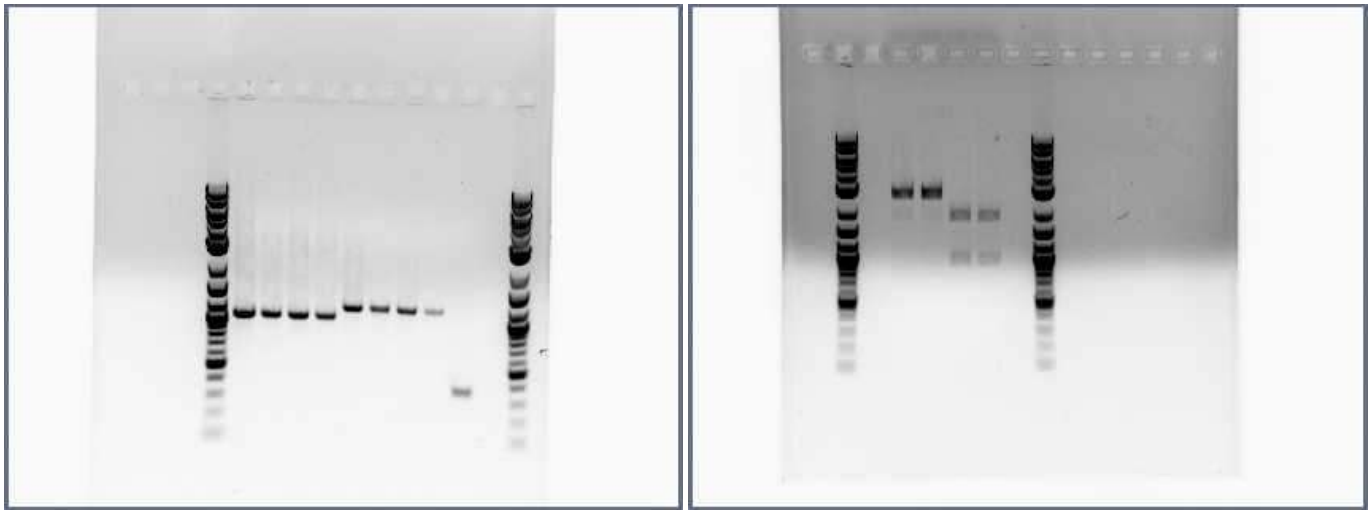
8/15/10

File:JunkScreeningLIGHT.jpgFile:JunkScreeningDARK.jpg

8/17/10

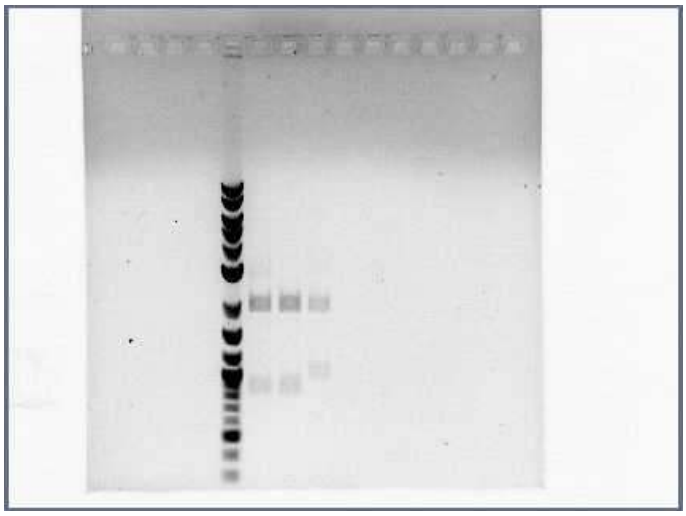
File:K19CTCHTCgelExt8172010.jpg

8/17/10



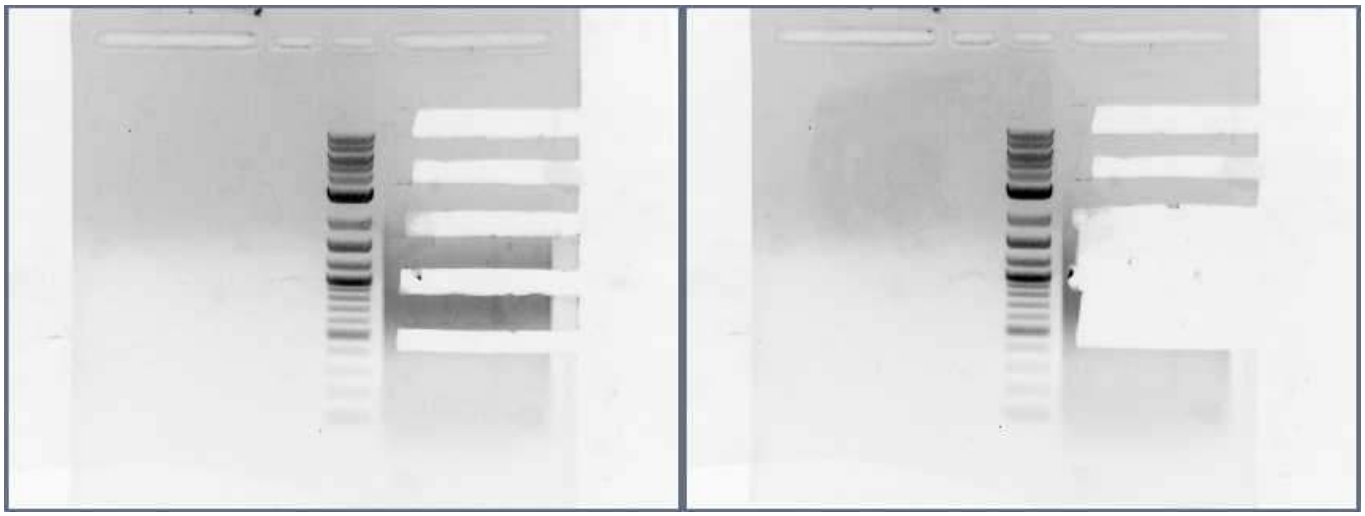
The tubes were mixed up. Lanes 1 and 2 are screen A single cut and lanes 3 and 4 are screen F double cut.

8/19/10



A single, A double, F single. I have no idea why all three look double cut???

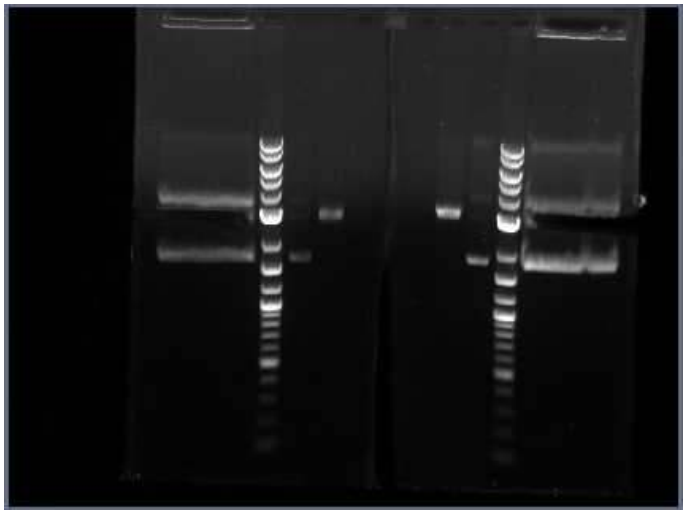
8/21/10

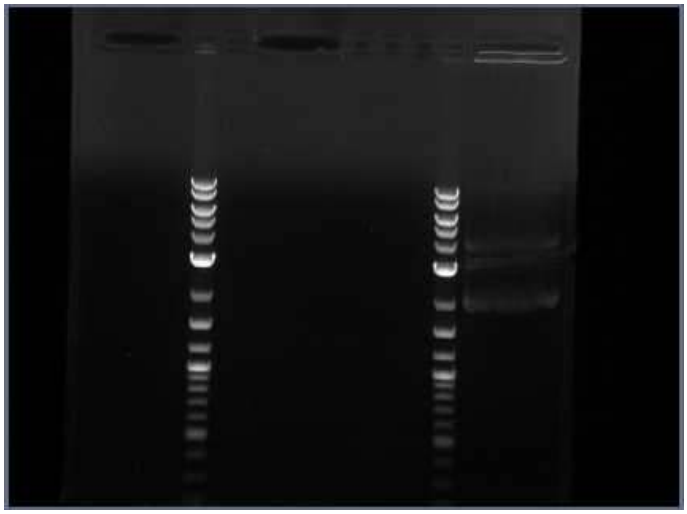


Above: multimer reaction, 4ul Ligase, 25:27 Fwd:Rev, (45 mins kInase before ligation), quenched at 3.5 hours with reverse primer, then overnight ligation

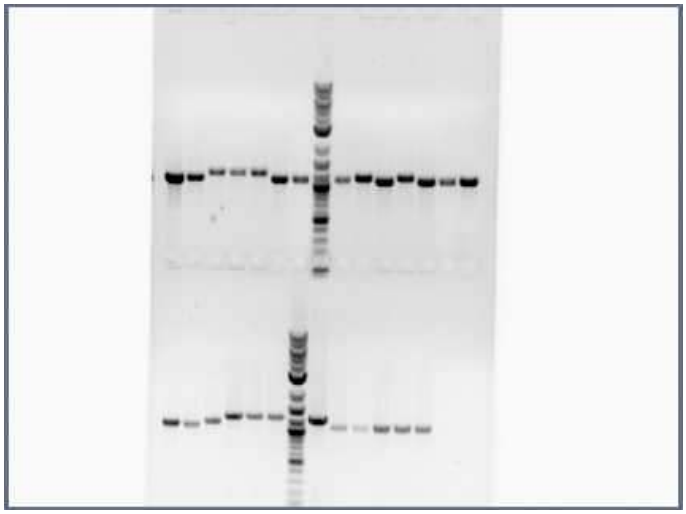
8/23/10

8/24/10





8/25/10



C+, C11,C12,C13,C14<C15,C151,Ladder,C152,C153,C251,C252,C253,C254,C255
C31,C32,C33,L+,L151,L152,Ladder,H+,H151,H152,H153,H154,H155