2013-2013 Aichi68 NP WT and N334H codon mutant libraries

Jesse Bloom

December-27-2012

I am going to retry making the codon library using mutagenesis primers for both the for and rev directions.
My primers all came at 100 uM. So I will make the following mixes, which will all be 100 uM total:

NP2-WT-for-mut-mix: Contains a total of 100 uM with equal amounts of WT-for-mut2 to WT-for-mut498. This is 40 ul each of all rows of Plate-NP2-1, Plate-NP2-2, Plate-NP2-3, Plate-NP2-4, Plate-NP2-5, and Plate-NP2-6 row A and row B columns 1-5.

NP2-N334H-for-mut-mix: Identical to NP2-WT-for-mut-mix, except WT-for-mutXXX is replaced with N334H-for-mutXXX for XXX = 328-333 and 335-339. This made just like WT-for-mut-mix, but excluding Plate-NP2-4 row D columns 3 to 8 and 10 to 12, and Plate-NP2-4 row E columns 1 and 2. Instead, we add Plate-NP2-6 row B columns 6 to 12 and Plate-NP2-6 row C columns 1 to 4.

NP2-WT-rev-mut-mix: Contains a total of 100 uM with equal amounts of WT-rev-mut2 to WT-rev-mut498. This is 40 ul each of all rows of Plate-NP2-7, Plate-NP2-8, Plate-NP2-9, Plate-NP2-10, Plate-NP2-11, and Plate-NP2-12 row A and row B columns 1-5.

NP2-N334H-rev-mut-mix: Identical to NP2-WT-rev-mut-mix, except WT-rev-mutXXX is replaced with N334H-for-mutXXX for XXX = 328-333 and 335-339. This made just like WT-rev-mut-mix, but excluding Plate-NP2-10 row D columns 3 to 8 and 10 to 12, and Plate-NP2-10 row E columns 1 and 2. Instead, we add Plate-NP2-12 row B columns 6 to 12 and Plate-NP2-12 row C columns 1 to 4.

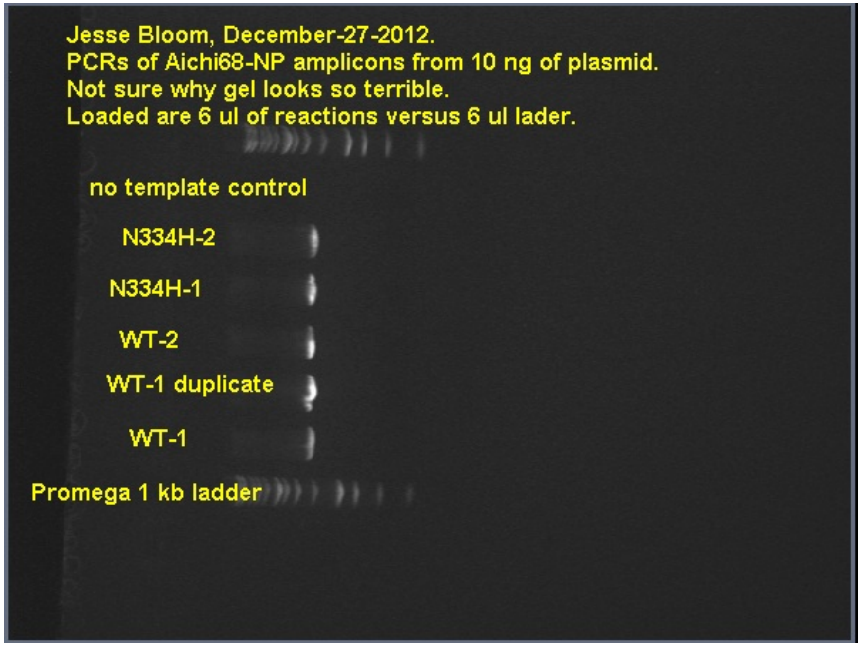
December-27-2012: amplification of DNA amplicons.
As templates, I will use two pHWAichi68-NP and two pHWAichi68-NP-N334H clones, all from April-28-2012 single colony preps. I will refer to these as WT-1, WT-2, N334H-1, and N334H-2. Diluted each of these plasmid stocks to 10 ng/ul.

For my PCRs, I will use the following primers:

```
>5'-BsmBI-Aichi68-NP: contains 6 nt tail, BsmBI cloning site, 60 C overlap with the 5' end of the
pHWAichi68-NP insert (which is the 3' end of the vRNA, includs U12). Does NOT extend into the coding
sequence.
CATGATcgtctcaggg agcaaaagcagggtagataatcactcacag

>3'-BsmBI-Aichi68-NP: contains 6 nt tail, BsmBI cloning site, 53 C overlap with the 3' end of the
pHWAichi68-NP insert through the stop codon (which is the 5' end of the vRNA, includes U13).
CATGATcgtctcGTATT agtagaaacaagggtatttttcttta
```

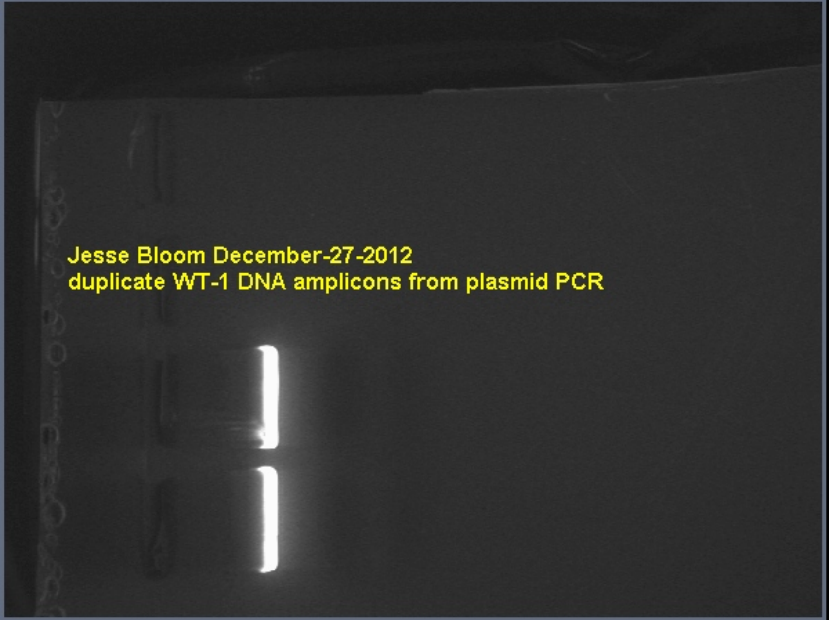
I will use the following 50 ul PCR reactions:
25 ul of KOD Master Mix
1 ul of 10 ng/ul plasmid template
1.5 ul of 10 uM 5'-BsmBI-Aichi68-NP
1.5 ul of 10 uM 3'-BsmBI-Aichi68-NP
21 ul of water
Ran two reactions with the WT-1 template, one reaction each for the WT-2, N334H-1, and N334H-2 templates, and one no-template control reaction.



I will use the following PCR program to make the amplicons.

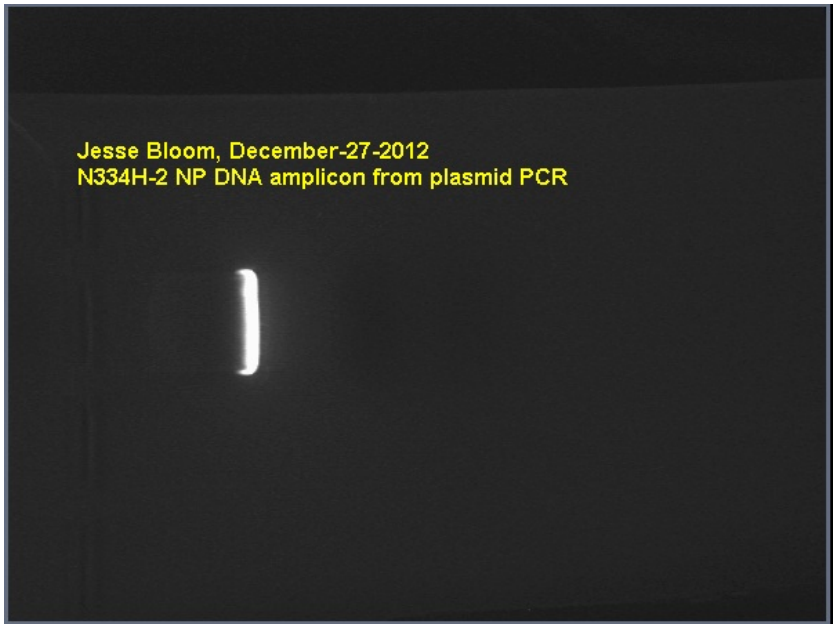
1. 95 C for 2:00
2. 95 C for :20
3. 70 C for :01
4. 50 C for :30, cooling to 50 C at 0.5 C/second
5. 70 C for :40
6. Goto 2, 19 times
7. 4 C forever

Then ran 5 ul of each reaction on a gel with 6 ul of Promega 1 kb ladder. The gel is shown at right – it is very poor quality, but makes clear that all of the reactions worked well. Then ran the remainder of each reaction on a separate clean gel in a cleaned rig with fresh buffer, using clean Saran Wrap and gloves to cut out the bands (gel images on next page). Purified the bands over Zymo Columns, eluting in 40 ul of EB. They were all between 40-50 ng/ul as assessed by NanoDrop, and had good 260/230 and 260/280 ratios as shown by the NanoDrop report on the page following the next. Stored these DNA amplicons at -20 C.

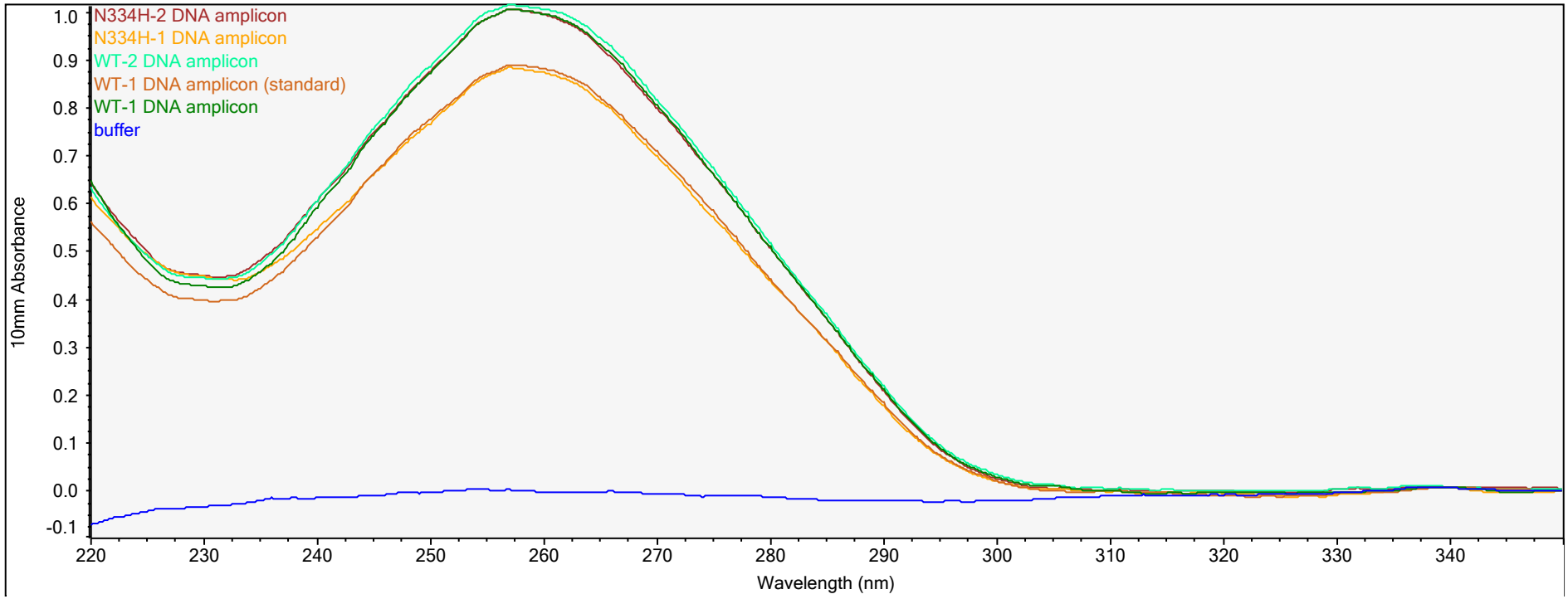

Jesse Bloom December-27-2012
duplicate WT-1 DNA amplicons from plasmid PCR


Jesse Bloom, December-27-2012
N334H-1 NP DNA amplicon from plasmid PCR


Jesse Bloom, December-27-2012
WT-2 NP DNA amplicon from plasmid PCR


Jesse Bloom, December-27-2012
N334H-2 NP DNA amplicon from plasmid PCR

Sample ID	Date and Time	Nucleic Acid Conc.	A260	A280	260/280	260/230
buffer	12/27/2012 1:56:06 PM	-0.5	-0.010	-0.021	0.49	0.25
WT-1 DNA amplicon	12/27/2012 1:56:37 PM	49.6	0.991	0.504	1.97	2.35
WT-1 DNA amplicon (standard)	12/27/2012 1:57:39 PM	43.9	0.877	0.438	2.00	2.24
WT-2 DNA amplicon	12/27/2012 1:58:21 PM	50.1	1.002	0.513	1.95	2.28
N334H-1 DNA amplicon	12/27/2012 1:59:17 PM	43.4	0.868	0.434	2.00	1.97
N334H-2 DNA amplicon	12/27/2012 2:00:02 PM	49.6	0.992	0.503	1.97	2.23



PCR for library assembly, January-22-2013, round 1

My templates will be the amplicons (WT-1, WT-2, N334H-1, N334H-2) described on the previous slides diluted to 3 ng/ul. Used the WT mut primer mixes for the WT templates, and the N334H mut primer mixes for the N334H templates.

for-frags

15 ul KOD Master Mix
2 ul of 4.5 uM 3'-BsmBI-Aichi68-NP
2 ul of WT-for-mut-mix diluted to 4.5 uM
4 ul of 3 ng/ul of amplicon template.
7 ul water

rev-frags

15 ul KOD Master Mix
2 ul of 4.5 uM 5'-BsmBI-Aichi68-NP
2 ul of WT-rev-mu-tmix diluted to 4.5 uM
4 ul of 3 ng/ul of amplicon template.
7 ul water

Ran each of these with the following PCR program (7 total PCR cycles)

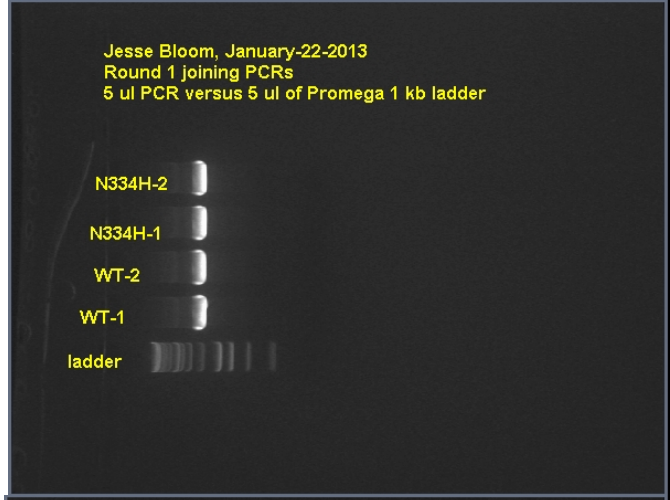
1. 95 C for 2:00
2. 95 C for :20
3. 70 C for :01
4. 50 C for :30, cooling to 50 C at 0.5 C/second
5. 70 C for :40
6. Goto 2, 6 times
7. 4 C forever

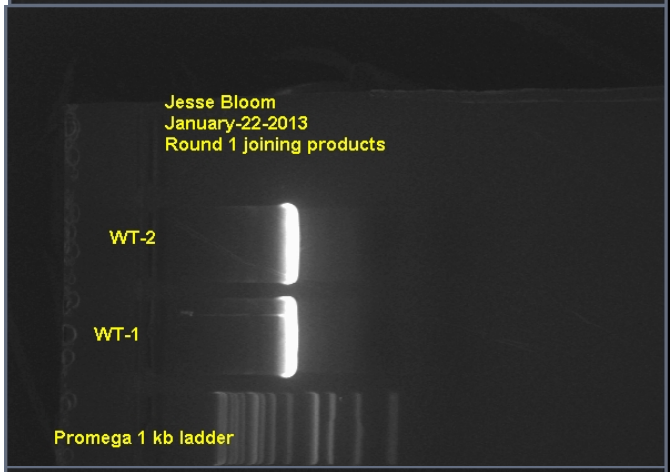
Then diluted these fragment reactions 1:4 by adding 90 ul of EB to the 30 ul reactions. Then set up the following reaction for each of the pairs of fragments

join

15 ul KOD Master Mix
4 ul of 1:4 diluted for-frags reaction
4 ul of 1:4 diluted rev-frags reaction
2 ul of 4.5 uM 5'-BsmBI-Aichi68-NP
2 ul of 4.5 uM 3'-BsmBI-Aichi68-NP
3 ul water

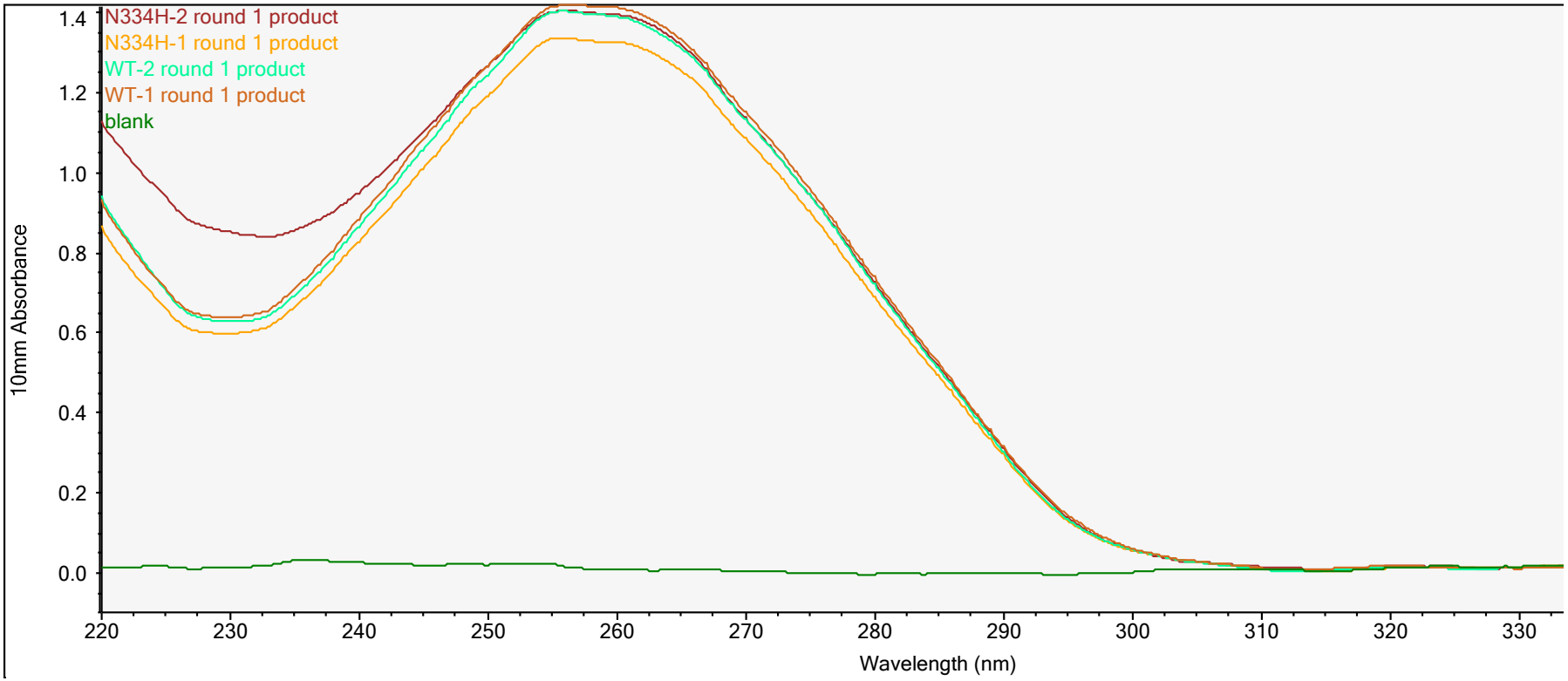
Then ran the same PCR program as above except used 20 rather than 7 cycles. Finally, ran the joined PCR products out on agarose gels. Added 6 ul of 6X loading buffer to the 30 ul reactions. Then on an analytical gel, ran 5 ul of this versus 5 ul of Promega 1 kb ladder. The analytical gel looked good – all bands are similar brightness. Then ran the WT and N334H bands on separate clean gels (cleaned rigs, fresh buffer, etc) and cleanly excised the bands (Saran wrap, fresh gloves, etc). Excised the bands, purified over Zymo columns eluting in 30 ul of EB. The NanoDrop report (shown on the next page) shows consistent pure yield for all samples.





January-23-2013, NanoDrop report for round 1 joining products

Sample ID	Date and Time	Nucleic Acid Conc.	A260	260/280	260/230
blank	1/23/2013 6:13:09 AM	-0.2	-0.003	0.23	-0.60
WT-1 round 1 product	1/23/2013 6:14:00 AM	70.3	1.407	1.92	2.23
WT-2 round 1 product	1/23/2013 6:14:54 AM	69.1	1.382	1.93	2.23
N334H-1 round 1 product	1/23/2013 6:15:53 AM	66.0	1.319	1.93	2.24
N334H-2 round 1 product	1/23/2013 6:16:47 AM	69.4	1.388	1.93	1.64



PCR for library assembly, January-23-2013, round 2

My templates for the second round will be the round 1 joined products NanoDropped on the previous slide, diluted to 3 ng/ul in EB. Made these dilutions for all four and used these as templates. Repeated the exact same PCR procedure:

for-frags

15 ul KOD Master Mix
2 ul of 4.5 uM 3'-BsmBI-Aichi68-NP
2 ul of WT-for-mut-mix diluted to 4.5 uM
4 ul of 3 ng/ul of 3 ng/ul round 1 template.
7 ul water

rev-frags

15 ul KOD Master Mix
2 ul of 4.5 uM 5'-BsmBI-Aichi68-NP
2 ul of WT-rev-mu-tmix diluted to 4.5 uM
4 ul of 3 ng/ul of 3 ng/ul round 1 template.
7 ul water

Ran each of these with the following PCR program (7 total PCR cycles)

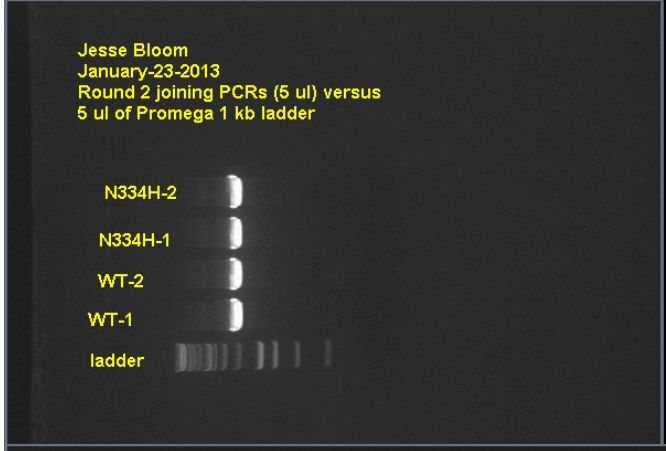
1. 95 C for 2:00
2. 95 C for :20
3. 70 C for :01
4. 50 C for :30, cooling to 50 C at 0.5 C/second
5. 70 C for :40
6. Goto 2, 6 times
7. 4 C forever

Then diluted these fragment reactions 1:4 by adding 90 ul of EB to the 30 ul reactions. Then set up the following reaction for each of the pairs of fragments

join

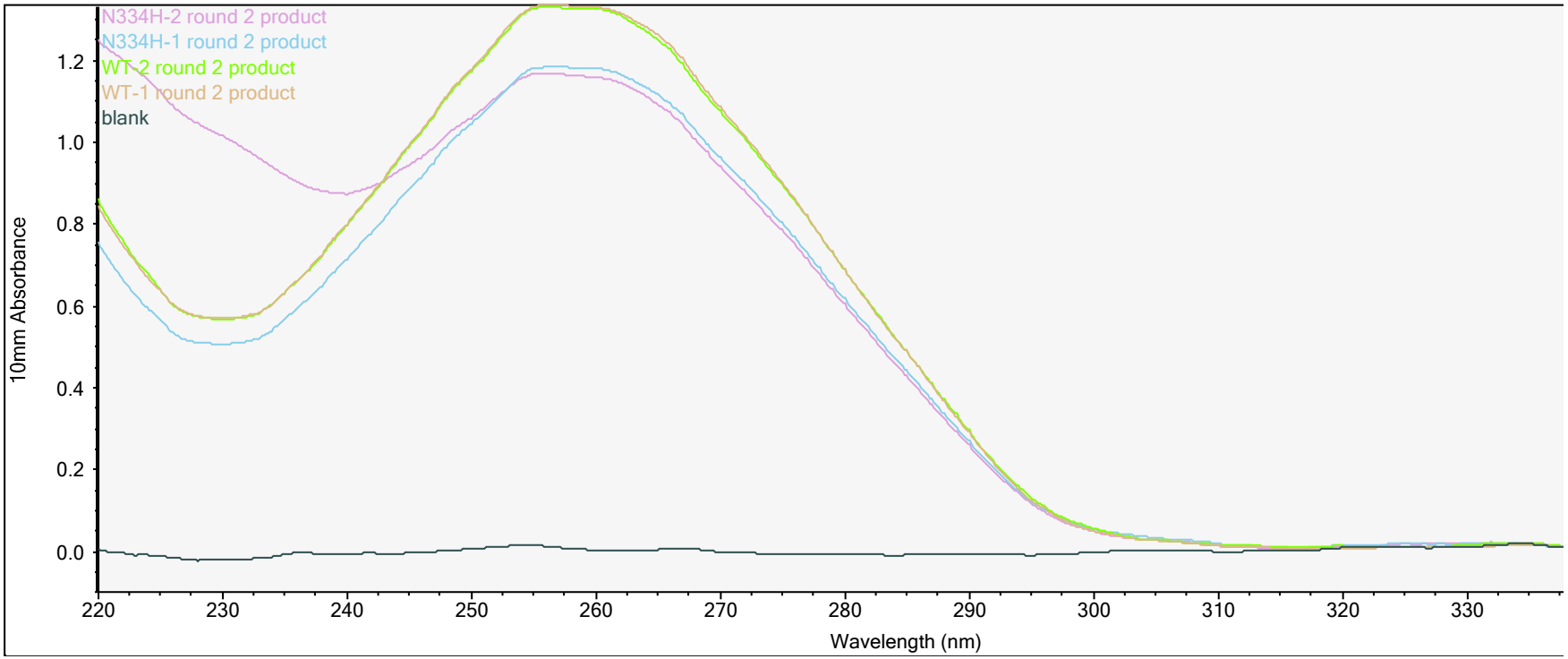
15 ul KOD Master Mix
4 ul of 1:4 diluted for-frags reaction
4 ul of 1:4 diluted rev-frags reaction
2 ul of 4.5 uM 5'-BsmBI-Aichi68-NP
2 ul of 4.5 uM 3'-BsmBI-Aichi68-NP
3 ul water

Then ran the same PCR program as above except used 20 rather than 7 cycles. Finally, ran the joined PCR products out on agarose gels. Added 6 ul of 6X loading buffer to the 30 ul reactions. Then on an analytical gel, ran 5 ul of this versus 5 ul of Promega 1 kb ladder. The analytical gel looked good – all bands are similar brightness. Then ran the WT and N334H bands on separate clean gels (cleaned rigs, fresh buffer, etc) and cleanly excised the bands (Saran wrap, fresh gloves, etc). Excised the bands, purified over Zymo columns eluting in 30 ul of EB. The NanoDrop report (shown on the next page) shows consistent yield for all samples.



January-23-2013, NanoDrop report for round 2 joining products.
The 230 signal was a bit high for the N334H-2 round 2 product, but it should be OK as I will purify it again after the BsmBI digest anyway.

Sample ID	Date and Time	Nucleic Acid Conc.	A260	260/280	260/230
blank	1/23/2013 9:13:00 AM	-0.3	-0.006	0.35	0.21
WT-1 round 2 product	1/23/2013 9:14:00 AM	66.2	1.323	1.94	2.35
WT-2 round 2 product	1/23/2013 9:14:35 AM	66.0	1.320	1.93	2.36
N334H-1 round 2 product	1/23/2013 9:15:12 AM	58.7	1.174	1.92	2.36
N334H-2 round 2 product	1/23/2013 9:15:48 AM	57.6	1.151	1.93	1.14



PCR for library assembly, January-23-2013, round 3

My templates for the second round will be the round 2 joined products NanoDropped on the previous slide, diluted to 3 ng/ul in EB. Made these dilutions for all four and used these as templates. Repeated the exact same PCR procedure:

for-frags

15 ul KOD Master Mix
2 ul of 4.5 uM 3'-BsmBI-Aichi68-NP
2 ul of WT-for-mut-mix diluted to 4.5 uM
4 ul of 3 ng/ul of 3 ng/ul round 1 template.
7 ul water

rev-frags

15 ul KOD Master Mix
2 ul of 4.5 uM 5'-BsmBI-Aichi68-NP
2 ul of WT-rev-mu-tmix diluted to 4.5 uM
4 ul of 3 ng/ul of 3 ng/ul round 1 template.
7 ul water

Ran each of these with the following PCR program (7 total PCR cycles)

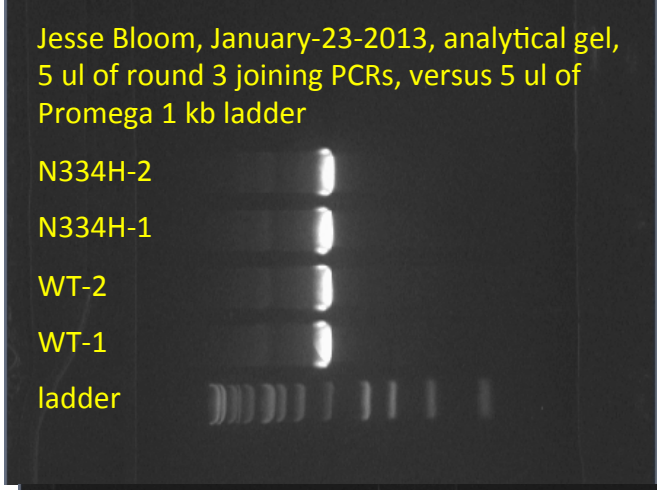
1. 95 C for 2:00
2. 95 C for :20
3. 70 C for :01
4. 50 C for :30, cooling to 50 C at 0.5 C/second
5. 70 C for :40
6. Goto 2, 6 times
7. 4 C forever

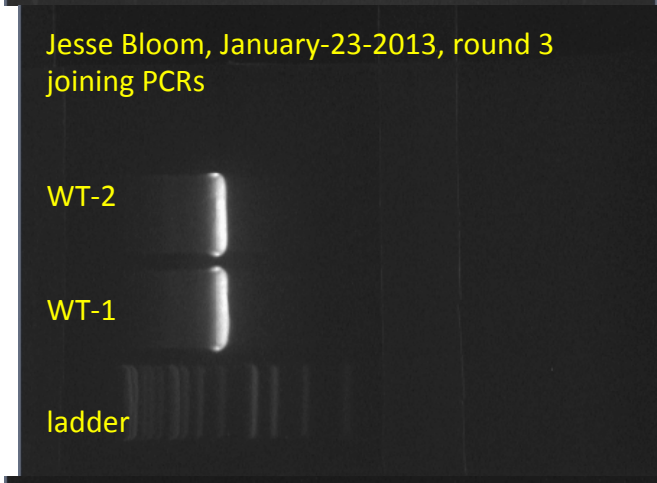
Then diluted these fragment reactions 1:4 by adding 90 ul of EB to the 30 ul reactions. Then set up the following reaction for each of the pairs of fragments

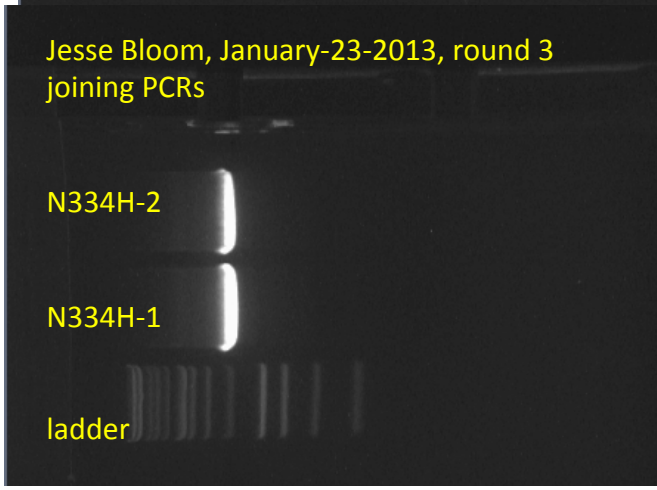
join

15 ul KOD Master Mix
4 ul of 1:4 diluted for-frags reaction
4 ul of 1:4 diluted rev-frags reaction
2 ul of 4.5 uM 5'-BsmBI-Aichi68-NP
2 ul of 4.5 uM 3'-BsmBI-Aichi68-NP
3 ul water

Then ran the same PCR program as above except used 20 rather than 7 cycles. Finally, ran the joined PCR products out on agarose gels. Added 6 ul of 6X loading buffer to the 30 ul reactions. Then on an analytical gel, ran 5 ul of this versus 5 ul of Promega 1 kb ladder. The analytical gel looked good – all bands are similar brightness. Then ran the WT and N334H bands on separate clean gels (cleaned rigs, fresh buffer, etc) and cleanly excised the bands (Saran wrap, fresh gloves, etc). Excised the bands, purified over Zymo columns eluting in 30 ul of EB.







BsmBI digests of round 2 and round 3 PCR products, January-23-2013

Set up BsmBI digests of my gel purified round 2 and round 3 PCR products. Reactions:

28 ul of purified DNA

5 ul NEB Buffer 3

1.5 ul BsmBI

13.5 ul water

50 ul total volume, incubated at 55 C for 2.5 hours followed by 80 C for 20 minutes and then hold at 4 C. The NanoDrop of these and the pHW2000 digest (described on the next page) are shown below. Set up ligations:

6 ul of 5X Invitrogen T4 DNA Ligase Buffer

1 ul Invitrogen T4 DNA Liagase (15224-041)

100 ng of pHW2000 digest (2.4 ul)

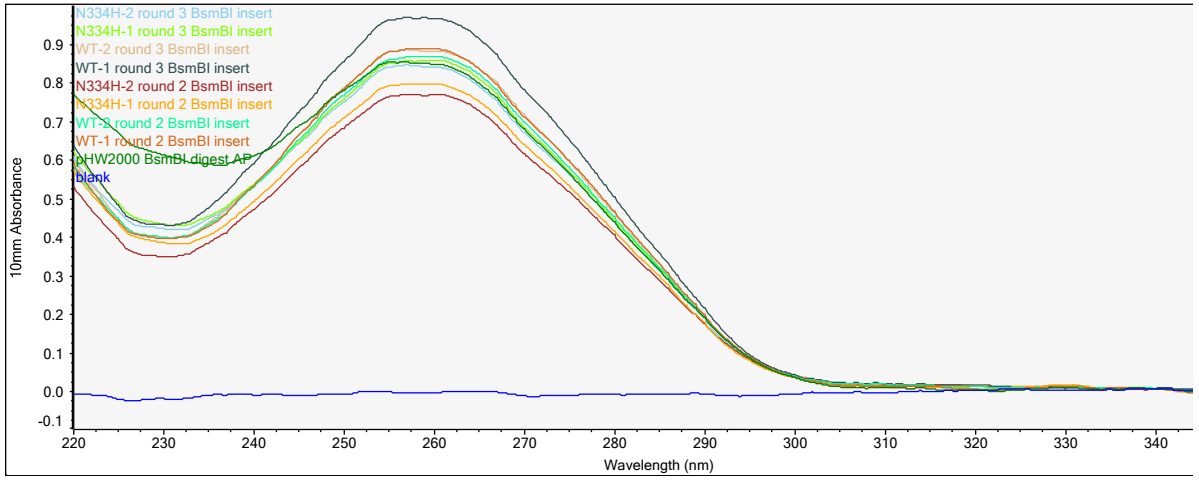
200 ng of insert (4.8 ul based on average concentration of various inserts, or 4.8 ul EB for no-insert control)

15.8 ul of water for 30 ul total volume

Incubated at room temperature.

After 30 minutes, transformed 1.5 ul of ligations into 20 ul Stellar Competent Cells and plated 20 ul of a 1:10 dilution on LB-AMP. Left the rest of the ligations at room temperature overnight.

Sample ID	Date and Time	Nucleic Acid Conc.	A260	260/280	260/230
blank	1/23/2013 5:25:23 PM	-0.6	-0.012	0.81	0.46
pHW2000 BsmBI digest AP	1/23/2013 5:26:07 PM	42.2	0.844	1.94	1.39
WT-1 round 2 BsmBI insert	1/23/2013 5:27:10 PM	44.1	0.883	1.91	2.27
WT-2 round 2 BsmBI insert	1/23/2013 5:28:15 PM	43.2	0.864	1.93	2.19
N334H-1 round 2 BsmBI insert	1/23/2013 5:29:12 PM	39.6	0.792	1.93	2.09
N334H-2 round 2 BsmBI insert	1/23/2013 5:30:11 PM	38.2	0.764	1.91	2.23
WT-1 round 3 BsmBI insert	1/23/2013 5:31:16 PM	48.1	0.962	1.92	2.26
WT-2 round 3 BsmBI insert	1/23/2013 5:32:10 PM	43.9	0.878	1.92	2.24
N334H-1 round 3 BsmBI insert	1/23/2013 5:33:08 PM	42.7	0.854	1.94	2.00
N334H-2 round 3 BsmBI insert	1/23/2013 5:34:18 PM	41.9	0.837	1.93	2.01



pHW2000 digest, January-23-2013

Set up a a BsmBI digest of pHW2000:

15 ul of 215 ng/ul pHW2000

5 ul NEB Buffer 3

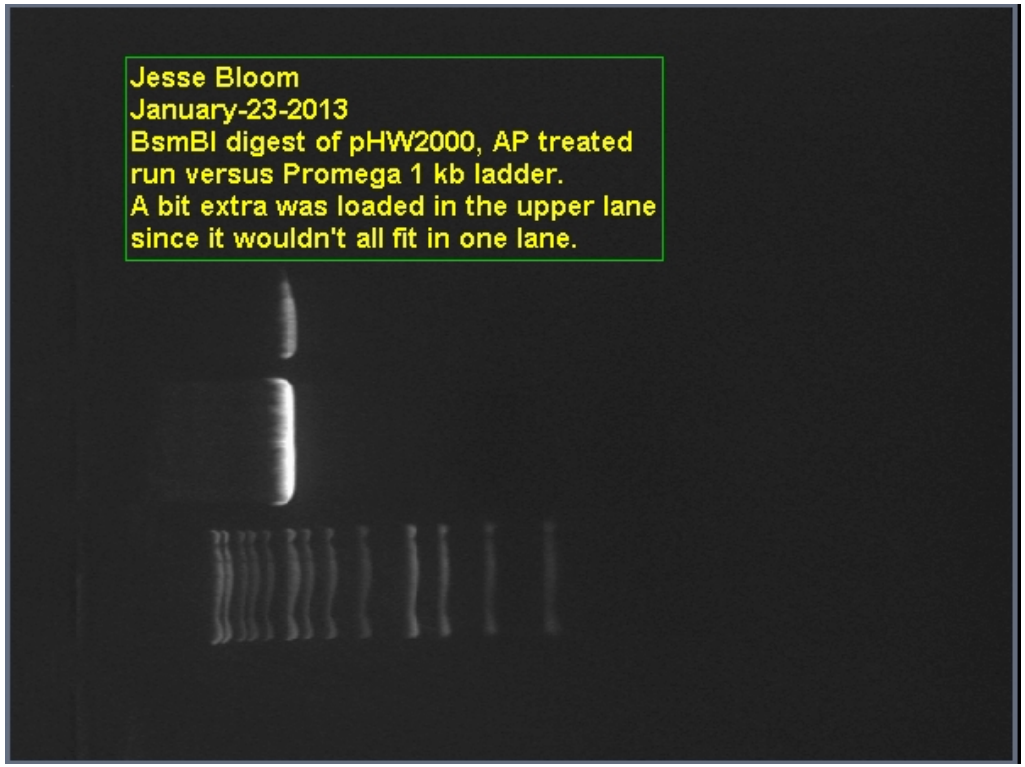
28.5 ul of water

1.5 ul of BsmBI

Incubated at 55 C for 2.5 hours, then 80 C for 20 minutes, then 4 C.

Then added 5.6 ul of 10X Antarctic Phosphatase Buffer and 1 ul of Antarctic Phosphatase. Incubated at 37 C for one hour followed by 65 C for 5 minutes, then 4 C.

Then ran out on a clean gel (cleaned gel rig, Saran wrap on cutting tray, etc). Purified over a Zymo column, eluting in 30 ul of EB. The NanoDrop analysis is on the previous page.



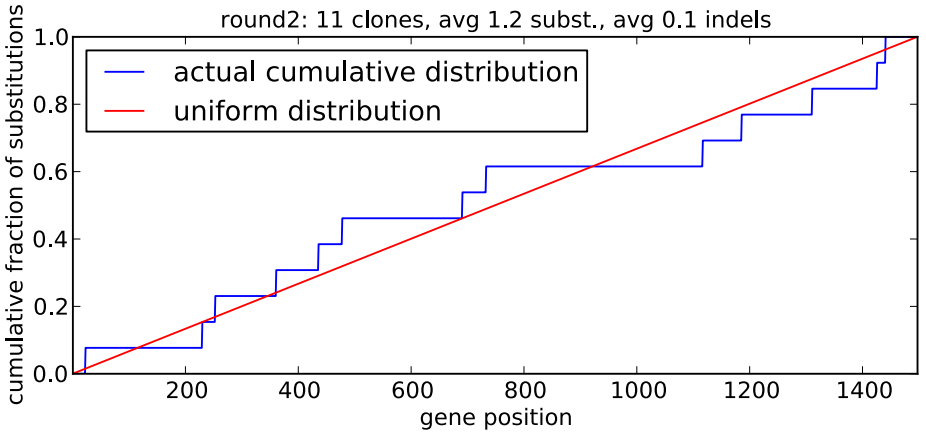
January-24-2013, picking of colonies for mini-preps

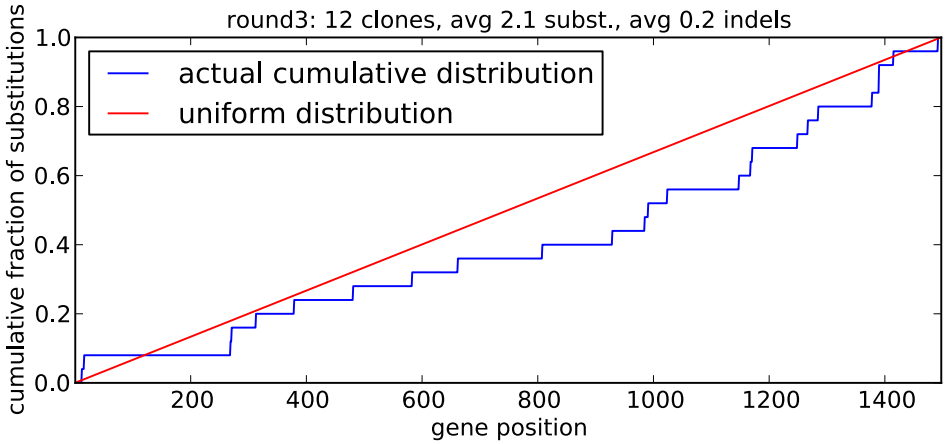
At 8:30 AM, looked at yesterday's transformations. All of the ligations had 150-200 colonies. The no-insert control had none. The positive control had >1000. Picked 3 cultures of each into LB-AMP, numbering as:

WT-1 round 2 : 1-3
WT-2 round 2: 4-6
N334H-1 round 2: 7-9
N334H-2 round 2: 10-12
WT-1 round 3: 13-15
WT-2 round 3 : 16 to 18
N334H-1 round 3: 19 to 21
N334H-2 round 3: 22 to 24

Submitted all of this cultures for sequencing with the following 2 primers:
>Aichi68-for
GCAGAGCTCTCTGGCTAACTAGAGAACC
>Aichi68-rev2
GGCTGATCAGCGAGCTCTAGCATTTAG

WT1round2-1: None
WT1round2-2: None
WT1round2-3: C361T, ACA436GTC
WT2round2-1: A1311T
WT2round2-2: AA230CG, GCG253CGC
WT2round2-3: ACT1186CGA
N334H1round2-1: ATG1441TCA
#8 N334H1round2-2: appears to lack insert
N334H1round2-3: GG23AC, delAA686, CA691AG
N334H2round2-1: AGT733TCG, GCT1117AGA, GT1426AG
N334H2round2-2: None
N334H2round2-3: GAT478CTA
WT1round3-1: delCT392, AG583CT
WT1round3-2: ATG991TCC, AA1249CG, delA1257, TT1390GA
WT1round3-3: AAA271CGG, TT1285AC, G1378T
WT2round3-1: GC1148AA
WT2round3-2: AC16CT, AG269GC, TTC1390AGA
WT2round3-3: CCC481AAG, AC1168GT
N334H1round3-1: GT808AC
N334H1round3-2: None
N334H1round3-3: GAT379TAA, G662T, CG1415GC
N334H2round3-1: ATG313CGT, GC929CA, GTG985TGA, AAT1492TTG
N334H2round3-2: A12C, A1171G, CCA1267GTG
N334H2round3-3: AGA1024CTG





Interpretation: Based on these results, the round-3 libraries look to have a a more appropriate mutation rate. I will therefore move forward with these round-3 libraries. To get more sequence data, picked 5 more colonies of each of the types of round-3, numbering as:

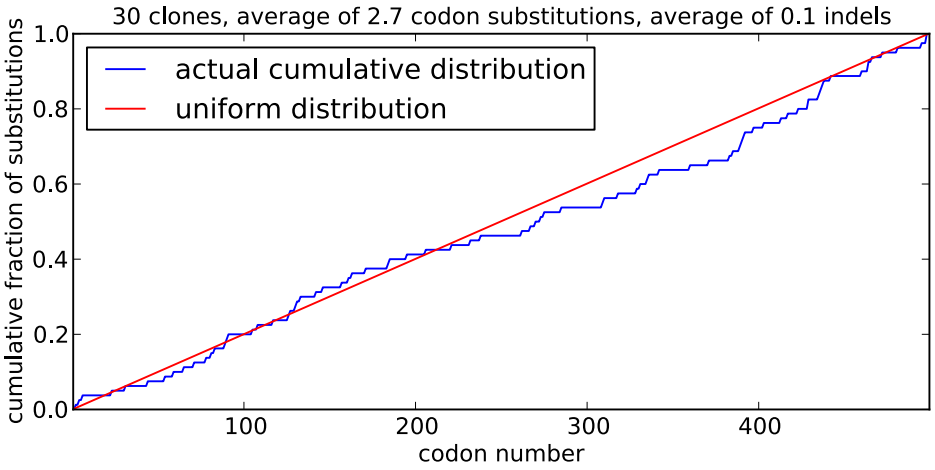
WT-1 round 3: 25-29
WT-2 round 3: 30 to 34
N334H-1 round 3: 35 to 39
N334H-2 round 3: 40 to 44

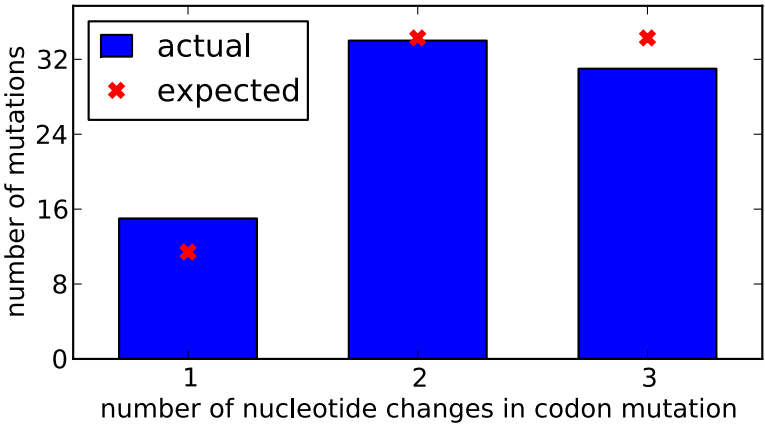
In the afternoon, mini-prepped these and submitted for sequencing as before.

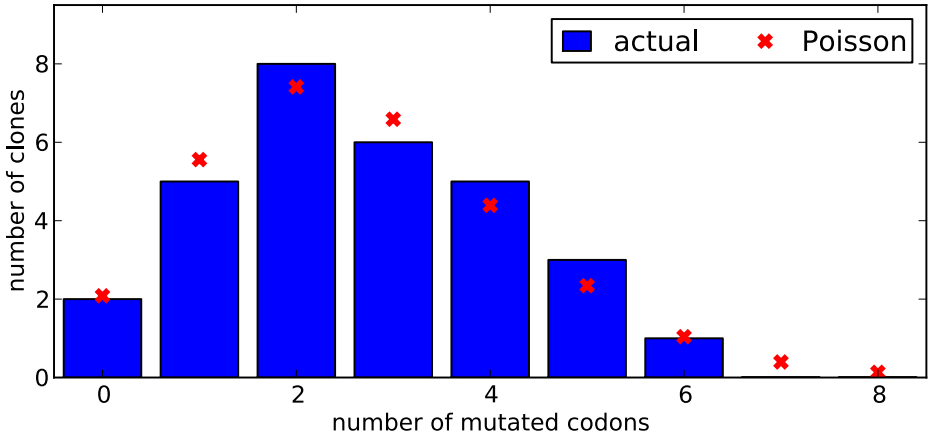
Comprehensive results for sequencing round3 cultures, January-26-2013.
I had picked five additional cultures each of four round3 libraries and sequenced. Below are the merged results from this and the previous day's sequencing:

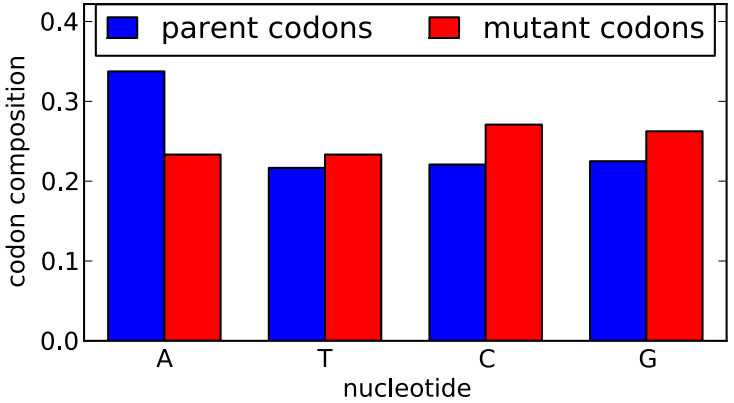
Sequencing from Jan-25-2013 for clones 1 to 3 for each.
Sequencing from Jan-25-2013 for clones 4 to 8 for each.
WT1round3-1: delCT392, AG583CT
WT1round3-2: ATG991TCC, AA1249CG, delA1257, TT1390GA
WT1round3-3: AAA271CGG, TT1285AC, G1378T
WT1round3-4: AA175GC, CTC1396GAA
WT1round3-5: TAT232GTA, ACA388TAC, CT1007AC, AAT1492GCA
WT1round3-6: AAC925GGG, GGA1303TCG
WT1round3-7: None
WT1round3-8: GGA553ATC, A696C, TG713AT, CA1208GT
#
WT2round3-1: GC1148AA
WT2round3-2: AC16CT, AG269GC, TTC1390AGA
WT2round3-3: CCC481AAG, AC1168GT
WT2round3-4: TTT211GTA, C322G
#WT2round3-5: mixed template
WT2round3-6: AAA817TTT, AGA1306CTC, delA1311
WT2round3-7: AG92CC, ACA436GTC, ATG1441GAA
WT2round3-8: C397T, TA854CT, CCG952ACA, A1314T, GAG1483CCC
#
N334H1round3-1: GT808AC
N334H1round3-2: None
N334H1round3-3: GAT379TAA, G662T, CG1415GC
N334H1round3-4: A1078G
N334H1round3-5: T825C, AT1111TA, AAT1189GCG, ACA1309CCT
N334H1round3-6: G241A, AAA550TGG
N334H1round3-7: TCT1237CCG, TC1286GG
N334H1round3-8: TG130AC, AG349GA, G391C, AT487TA, AG799CA
#
N334H2round3-1: ATG313CGT, GC929CA, GTG985TGA, AAT1492TTG
N334H2round3-2: A12C, A1171G, CCA1267GTG
N334H2round3-3: AGA1024CTG
N334H2round3-4: G6C, GG160TT, CC265TT, GGT376AAC, C512T, TCT1003GCC
#N334H2round3-5: no insert
N334H2round3-6: CCC247TTT, TCT784GAG
N334H2round3-7: ACT67GTG, AGA193CGT, T616A, TAC1153GTA, AGT1174CAC
N334H2round3-8: AT425GC, CC470AT, AGA1165GTC, GCA1324CAG

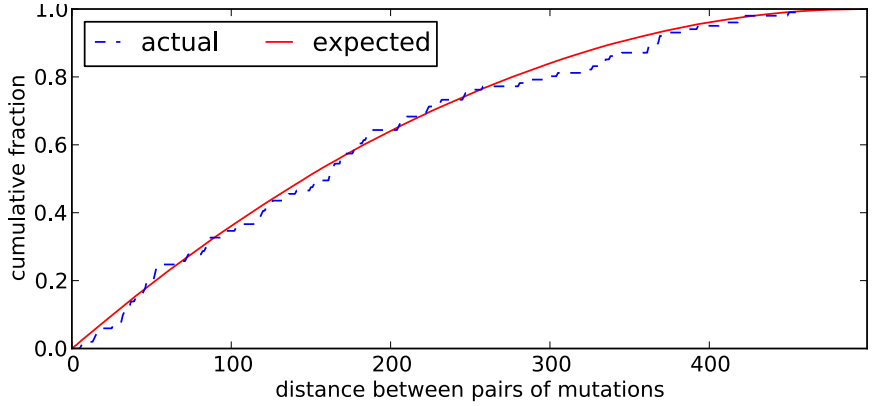
Results of comprehensive sequence analysis, January-27-2013. All of these quality control measures look good!









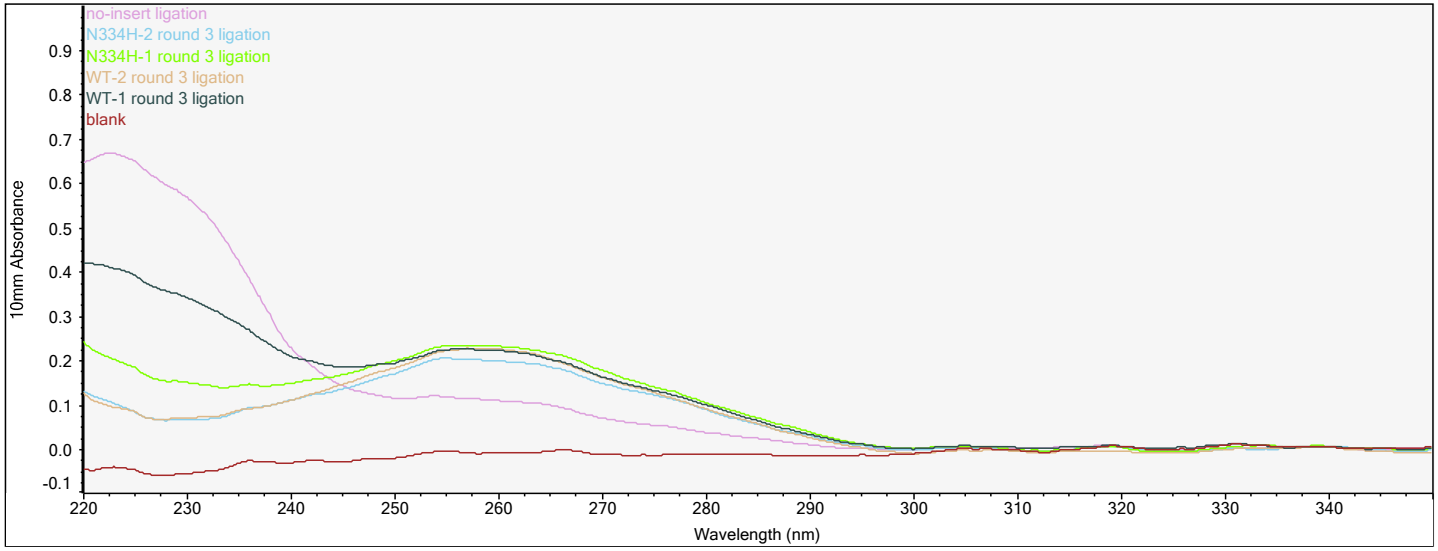


January-25-2013: high efficiency transformation of round 3 mutant library ligations.

I have decided to use my round 3 mutant libraries. My 30 ligations had sat for an additional day at room temperature before I moved them to 4 C. I now purified them over Zymo columns, eluting in 15 ul of EB. I NanoDropped these ligations as shown below. With complete DNA recovery, the ligations should be 20 ng/ul now. I find that they are around 10-11 ng/ul, indicating about 50% recovery. A few of them have somewhat higher than desirable absorbances at 230 nm, indicating some residual chemical contamination. However, since the DNA concentration is already low and I don't want to lose more, decided not to re-purify them. The only one with truly bad contamination is the no-insert control, which is obviously less important anyway – in fact, I don't even need to transform it this time since I got colony counts with my earlier lower efficiency January-23 chemically competent transformations.

For high-efficiency transformations, I will use Invitrogen DH10B T1 phage-resistant competent cells electroporated in 0.1 cm cuvettes at 2.0 kV (product # 12033-015). Aliquoted 2 ul of my column purified ligations into Eppendorf tubes on ice. Thawed a 100 ul aliquot of cells, and aliquoted 20 ul per tube. Then electroporated, and immediately added 240 ul of SOC. I then plated 200 ul of this, or 10 ul of a 1:200 dilution (5 ul into 995 ul of LB). This second plating represents a 1:4,000 dilution of the first plate. Repeated this whole procedure again, so that I have three plates and three dilution plates for each ligation. Finished at 5:30 PM.

Sample ID	Date and Time	Nucleic Acid Conc.	A260	260/280	260/230
blank	1/25/2013 11:36:06 AM	-0.8	-0.016	0.85	0.26
WT-1 round 3 ligation	1/25/2013 11:37:11 AM	10.8	0.217	2.32	0.64
WT-2 round 3 ligation	1/25/2013 11:38:21 AM	11.0	0.220	2.61	3.42
N334H-1 round 3 ligation	1/25/2013 11:39:20 AM	11.3	0.226	2.30	1.56
N334H-2 round 3 ligation	1/25/2013 11:40:20 AM	9.7	0.193	2.34	3.16
no-insert ligation	1/25/2013 11:41:07 AM	5.1	0.103	3.31	0.18



January-26-2013: high efficiency transformation of round 3 mutant library ligations, continued

At 11 AM, looked at yesterday's transformations. Here are the colony counts on the 1:4,000 dilution plates:

- WT-1: 180, 119, 213 colonies on the 3 plates
- WT-2: 183, 163, 208 colonies on the 3 plates
- N334H-1: 171, 219, 235 colonies on the 3 plates
- N334H-2: 99, 193, 203 colonies on the 3 plates
- Plasmid control: >1000 colonies on the one plate
- No-insert control: 4 colonies on the one plate

These counts indicate that all of the libraries have at least two million unique colonies on the three plates total. Used a glass spreader to scrape the colonies into 3 ml of liquid LB-AMP, which I then added to an addition 3 ml of liquid LB-AMP and grew for four hours at 37 C before mini-prepping, eluting in 70 ul EB. Did three mini-preps for each clone (one for each plate), and pooled together for a total of 210 ul of mutant library plasmid DNA. The mini-prep NanoDrop summaries are below.

Sample ID	Nucleic Acid Conc.	Date and Time	A260	260/280	260/230
blank	-0.2	1/26/2013 3:40:19 PM	-0.004	0.60	0.47
WT-1 mutant plasmid library	163.1	1/26/2013 3:43:35 PM	3.263	1.93	1.83
WT-2 mutant plasmid library	193.8	1/26/2013 3:44:20 PM	3.877	1.93	1.89
N334H-1 mutant plasmid library	132.3	1/26/2013 3:45:15 PM	2.646	1.95	1.73
N334H-2 mutant plasmid library	187.2	1/26/2013 3:46:03 PM	3.743	1.95	1.89