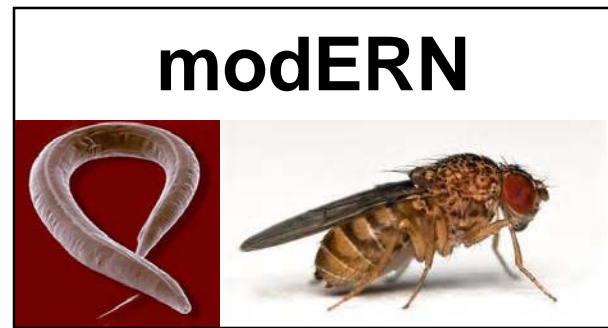


Reinke Lab Update



Michelle Kudron and Mei Han

Dec 8th, 2016

Outline

- Summary of ChIP-seq progress
 - Completed and pending datasets
 - Further optimization of chromatin shearing
 - Worm pellets ready for ChIP/growth
 - Specificity of the GoatV GFP Ab
 - N2 GoatV Mock IP subtraction examples
 - ChIP'd factors for RNA-seq
- Summary of RNA-seq progress
 - Backcrossed deletion mutants
 - Backcross in progress
 - RNA isolated for sequencing and backcrossed strains waiting for RNA isolation

Sequencing data for 177 new datasets

| 33 Bridge | 0 Y1Q1 | 0 Y1Q2 | 10 Y1Q3 | 22 Y1Q4 | 17 Y2Q1 | 11 Y2Q2 | 20 Y2Q3 | 15 Y2Q4 | 4 Y3Q1 | 6 Y3Q2 | 0 Y3Q3 | 16 Y3Q4 | 14 Y4Q1 | 9 Y4Q2 |
|-------------------|-----------|-----------|-------------------|------------------|------------------|---------------------|------------------|-------------------|--------------------|-----------------|-----------|---------------------|------------------|-------------------------|
| YL482_C06A8.2_YA | | | CS152_SMA-3_L2 | OP418_DMD-4_LE | YL487_SNPC-4_YA | OP399_F22D6.2_YA | OP525_C04F5.9_L1 | OP536_IRX-1_L1 | OP56_ELT-2_L1 | OP566_HLH-15_LE | | OP560_POP-1_EE | OP202_CEH-9_LE | N2_FLAG_L4 |
| YL479_EFL-1_YA | | | AGK154 UNC-130_L4 | OP312_LIR-3_L4 | YL551_SNPC-4_YA | OP63_HLH-8_LE | OP484_NHR-90_YA | OP537_MXL-1_YA | OP56_ELT-2_L3 | OP578_UNC-3_LE | | OP659_UNC-120_ME | OP673_CEH-24_LE | OR3349_HIF-1_L4_hypoxia |
| YL485_F08F3.9_YA | | | AGK541 UNC-130_YA | OP471_F13H6.1_L2 | OP521_SPR-1_L4 | OP201_PQM-1_LE | OP533_CEH-18_LE | OP538_HLH-12_YA | OP56_ELT-2_LE | OP562_AHR-1_LE | | OP573_HND-1_EE | OP652_FKH-8_L1 | XIL99_VAB-15_sL1 |
| YL497_T02C12.2_YA | | | OP509_ETS-4_L3 | OP511_ZTF-11_L1 | OP311_TBX-7_LE | OP154_PAG-3_LE | OP534_ZFP-2_L4 | OP210_CEH-90_L1 | OP443_Y53C12C.1_LE | OP558_ZTF-16_LE | | OP606_F52B5.7_YA | OP646_MEL-28_YA | OP553_CEH-79_yAd |
| YL478_EFL-1_YA | | | OP510_F37D6.2_L4 | OP508_MADF-10_YA | OP485_LIN-40_YA | OP164_FAX-1_LE | OP487_ETS-7_EE | OP493_NPAX-4_LE | | OP476_UNC-86_LE | | OP636_TBX-9_YA | OP656_HMBX-1_EE | OP658_F10E7.11_yAd |
| YL507_DPL-1_YA | | | OP388_LIM-6_LE | OP460_NHR-80_YA | OP517_SOX-4_L1 | OP528_SNU-23_L1 | OR3350_HIF-1_L4 | OP541_COG-1_ME | | OP68_TTX-3_LE | | OP598_T26A5.8_YA | OP640_TRA-4_EE | OP552_C34B4.2_L1 |
| OP462_RNT-1_L1 | | | OP512_SWSN-7_L1 | OP478_T07F8.4_YA | OP523_HLH-4_L1 | OP532_Y116A8C.19_L1 | OP489_NHR-232_LE | YL581_REC-8_YA | | | | OP615_Y22D7AL.16_LE | OP662_B0261.1_YA | OP696_M03D4.4_ME |
| OP401_LSY-27_YA | | | OP159_TBX-2_LE | OP513_C08G9.2_LE | OP524_CEH-34_LE | B0035.1_XTL1186_YA | OP476_UNC-86_L1 | YL576_HIM-1_L4/YA | | | | OP609_CHD-7_YA | OP677_CHE-1_LE | OP563_CEBP-1_yAd |
| OP474_ZK185.1_YA | | | OP159_TBX-2_L1 | OP159 (L3) | OP55_MEC-3_LE | OP315_CEH-14_LE | QP0661_XND-1_YA | YL577_HIM-1_YA | | | | OP622_GMEB-2_LE | N2_GFP_EM | OP550_F57C9.4_yAd |
| OP248_MES-2_L4 | | | OP506_XBP-1_L1 | GOU883_EGL-13_L1 | OP518_NHR-48_L4 | OP323_CEH-2_LE | OP494_ZTF-16_LE | OP100_FKH-4_L1 | | | | OP631_CEH-48_LE | OP605_F13C5.2_EE | OP688_REPO-1_yAd |
| JK1107_RPC-1_YA | | | | YL529_LET-607_YA | YL557_WAGO-9_YA | OP496_ZTF-18_YA | OP252_DAO-5_L4 | OP385_F55B11.4_YA | | | | OP620_CEH-36_EE | N2_GFP_yAd | |
| LW1254_SMA-9C2_L2 | | | | OP480_NHR-71_L1 | YL563_REC-8_YA | | OP502_ALY-1_EE | OP544_ZIP-5_LE | | | | OP594_FKH-3_YA | N2_FLAG_EM | |
| OP470_ZC204.2_L4 | | | | OP516_CEH-32_L4 | OP529_F10B5.3_L4 | | RW10702_HLH-6_LE | OP539_NHR-85_L1 | | | | OP587_MADF-2_LE | OP565_ZTF-26_YA | |
| OP358_CEY-2_L4 | | | | ZM8745_DAF-16_L4 | OP522_DSC-1_L1 | | OP195_RBR-2_L4 | OP543_SDZ-38_YA | | | | OP568_LSY-12_L3 | OP579_DPFF-1_YA | |
| OP372_K09A11.1_LE | | | | OP515_NHR-20_L1 | OP383_F49E8.2_L4 | | RW10316_DIE-1_YA | TH184_HMG-11_L3 | | | | OP642_B0310.2_EE | OP638_ATF-7_YA | |
| OP398_DVE-1_LE | | | | OP514_SPR-4_YA | OP520_REF-2_LE | | OP78_FKH-6_LE | | | | | OP645_MLS-2_ME | OP581_ZIM-3_YA | |
| OP398_DVE-1_L4 | | | | OP471_F13H6.1_LE | OP111_ELT-4_LE | | RW10325_MES-4_YA | | | | | | OP517_SOX-4_LE | |
| OP429_F23B12.7_YA | | | | OP370_CEH-31_LE | | | OP488_SYD-9_LE | | | | | | | |
| OP433_HLH-30_L4 | | | | OP463_NHR-102_L4 | | | OP173_UNC-42_LE | | | | | | | |
| OP433_HLH-30_LE | | | | OP462_RNT-1_LE | | | OP465_NHR-179_YA | | | | | | | |
| OP391_MED-1_ME | | | | OP481_NHR-47_L1 | | | OP92_SDC-2_LE | | | | | | | |
| OP404_NFYA-1_YA | | | | OP483_NHR-90_L1 | | | | | | | | | | |
| OP404_NFYA-1_L3 | | | | ZM7247_DAF-16_L4 | | | | | | | | | | |
| OP404_NFYA-1_EMB | | | | | | | | | | | | | | |
| OP109_BLMP-1_L2 | | | | | | | | | | | | | | |
| OP354_ELT-1_EMB | | | | | | | | | | | | | | |
| OP383_F49E8.2_YA | | | | | | | | | | | | | | |
| OP33_NHR-25_L4 | | | | | | | | | | | | | | |
| OP405_ODD-2_LE | | | | | | | | | | | | | | |
| OP154_PAG-3_L1 | | | | | | | | | | | | | | |
| OP312_LIR-3_L1 | | | | | | | | | | | | | | |
| OP477_NHR-43_L3 | | | | | | | | | | | | | | |
| N2_RPC-1_YA | | | | | | | | | | | | | | |

- 33 completed datasets for Bridge year (24 factors that missed the data freeze were sequenced at Stanford)
- 32 completed datasets for year 1
- 64 completed factors for year 2
- 25 completed factors for year 3
- 23 so far for year 4, quarters 1 and 2
- In total, data for 163 new TFs

OP514_SPR-4 has a rearrangement
 OP558_LAG-1 is ZTF-16
 OP494 is ZTF-16 and LAG-1
 OP677_BAR-1 is CHE-1

Completed factors for year 4, quarters 1 and 2 (23)

| | Factor | Strain name | Stage |
|---|----------|-------------|-------|
| * | CEH-9 | OP202 | LE |
| * | CEH-24 | OP673 | LE |
| | FKH-8 | OP652 | L1 |
| | MEL-28 | OP646 | yAd |
| | HMBX-1 | OP656 | EE |
| | TRA-4 | OP640 | EE |
| | B0261.1 | OP662 | yAd |
| | CHE-1 | OP677 | LE |
| | F13C5.2 | OP605 | EE |
| | ZTF-26 | OP565 | yAd |
| | DPFF-1 | OP579 | yAd |
| | ATF-7 | OP638 | yAd |
| | ZIM-3 | OP581 | yAd |
| * | SOX-4 | OP517 | LE |
| | HIF-1 | OR3349 | L4 |
| | VAB-15 | XIL99 | sL1 |
| | CEH-79 | OP553 | yAd |
| | F10E7.11 | OP658 | yAd |
| | C34B4.2 | OP552 | L1 |
| * | M03D4.4 | OP696 | ME |
| | CEBP-1 | OP563 | yAd |
| | F57C9.4 | OP550 | yAd |
| | REPO-1 | OP688 | yAd |

Asterisk indicates factors for the RNA-seq project

Pending factors (16)

| | Factor | Strain name | Stage | Status |
|---|-----------------|--------------------|--------------|--|
| | ZTF-3 | OP614 | yAd | Awaiting sequencing |
| * | LIN-11 | OP62 | LE | Awaiting sequencing |
| | ZTF-27 | OP705 | yAd | Awaiting sequencing |
| | ATHP-1 | OP685 | yAd | Awaiting sequencing |
| | SPTF-1 | OP707 | EM | Awaiting sequencing |
| | CEH-1 | OP571 | L1 | Failed again, given up on this one for now |
| | N2_FLAG | N2 | yAd | Repeated, failed again, process new pellets |
| | HAM-2 | OP639 | EM | Repeated, failed again, needs regrowth |
| | ATTF-2 | OP657 | EE | Repeated, failed again, needs regrowth |
| | BED-3 | OP651 | EM | Repeated, failed again, needs regrowth |
| | HND-1 | OP573 | yAd | No enrichment, regrow |
| | CEH-89 | OP556 | yAd | Failed, needs to be repeated |
| | C33H5.17 | OP570 | L4 | Failed again, despite regrowth and douncing |
| | CCCH-3 | OP627 | yAd | Failed again, despite regrowth and douncing |
| | GEI-17 | OP572 | yAd | One rep concentration too low despite douncing |
| | SAEG-1 | OP580 | yAd | One rep failed despite douncing |

Asterisk indicates factors for the RNA-seq project

Addition of douncing step to ChIP protocol improves DNA yield and library success rate

- Resuspend frozen pellet in .75 mLs of FA buffer and transfer to 2 mL dounce tube
- Dounce with small pestle 2 times, 15 plunges each, turning pestle a quarter turn each time, icing for one minute between each of the 15 plunges
- Dounce with large pestle 4 times, 15 plunges each, turning pestle a quarter turn each time, icing for one minute between each of the 15 plunges
- Transfer to conical, spin down, and bring volume to 1.5 mLs and then continue with sonication

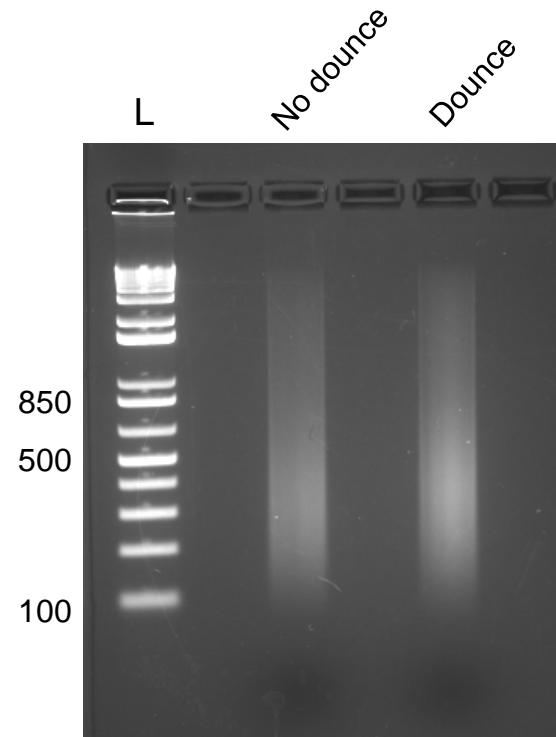
Lane 1: Kb plus ladder

Lane 3: OP638_ATF-7_yAd

No dounce

Lane 5: OP638_ATF-7_yAd

Dounce



OP638_ATF-7_yAd No dounce IP

sample failed library/sequencing.

Repeated with another pellet that was dounced and the library and sequencing passed

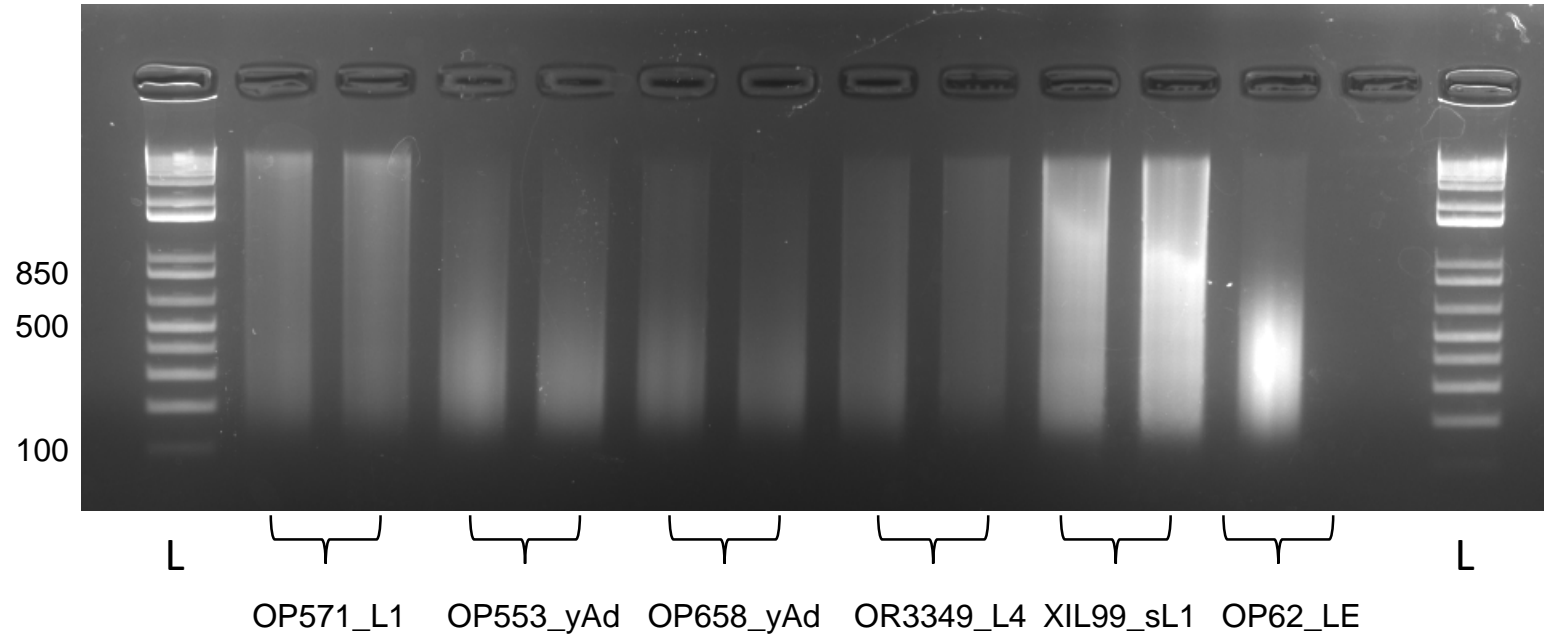
IP library success rates:

Failure rate of IP libraries before increasing amplitude = 62% (36/58)

Failure after increasing amplitude and adding douncing = 20.4% (11/54). Last call it was 12.5% (2/16).

Further optimization of chromatin shearing

- Currently optimizing conditions on new sonicator



- Test fixation step
 - Starved L1s and embryos have a lot more chromatin. The formaldehyde may not be penetrating the worm effectively.
 - Fix after douncing worms

Are we able to get more chromatin from worms if we fix after douncing?

Fix before douncing (current protocol)

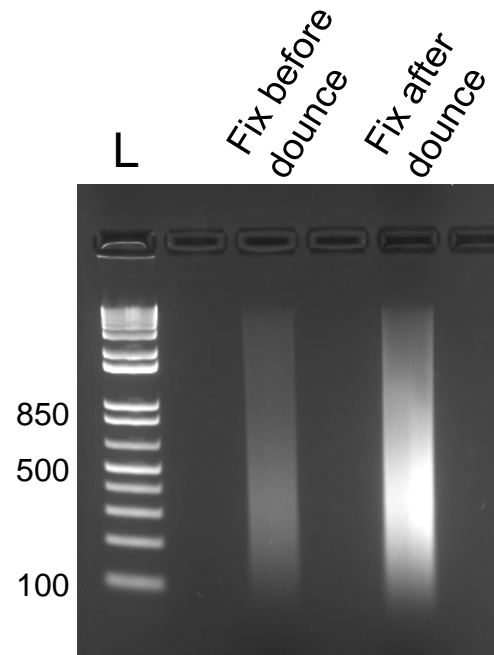
Modified from Ercan et al., 2007

- Collect worms
- 2% formaldehyde 28 min, RT
- Flash freeze and store at -80 degrees
- Thaw and dounce (30 strokes “A”, 60 “B”)
 - Sonicate

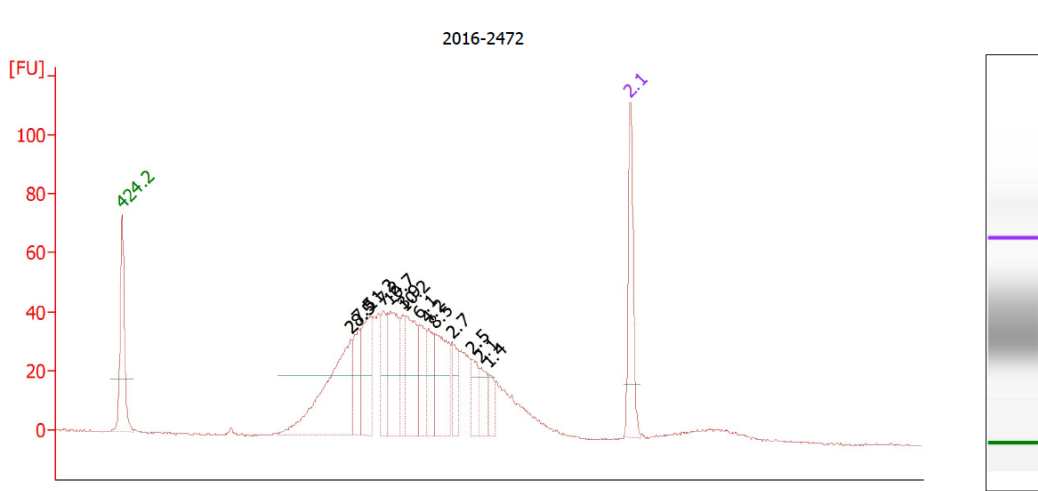
Fix after douncing

Modified from Ni et al., 2014

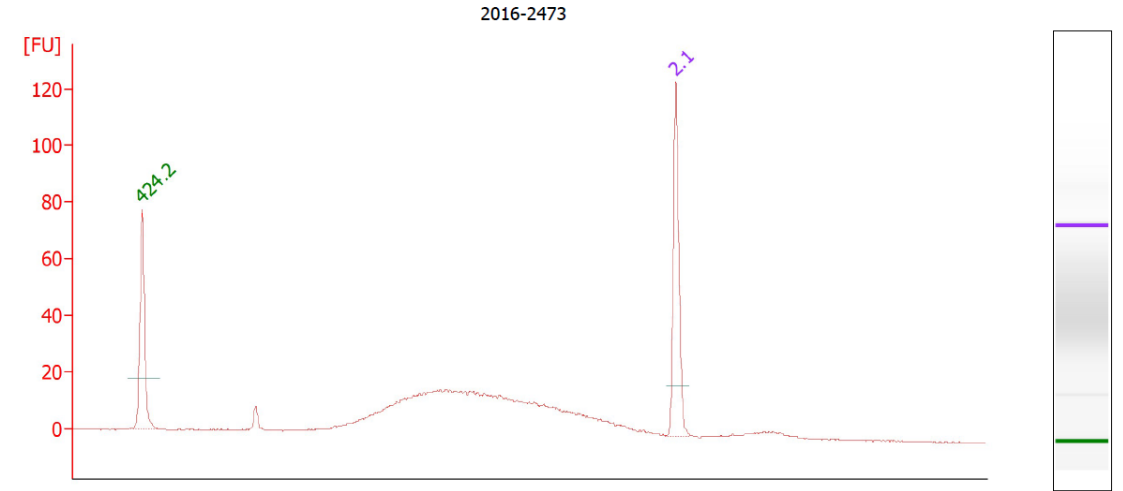
- Collect worms, flash freeze in liquid nitrogen
- Thaw and dounce (30 strokes “A”, 60 “B”)
- 2% formaldehyde 10 min, 4 degrees
 - Sonicate



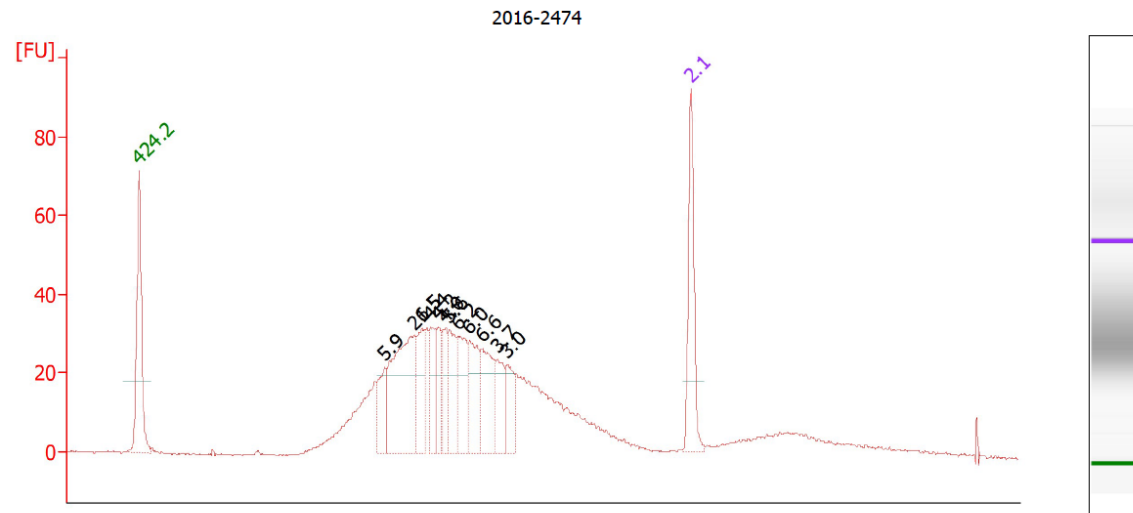
Library enrichment is vastly improved when the sample is fixed after douncing



IP_fixing after douncing



IP_fixing before douncing



Input_fixing after douncing

Factors ready for ChIP (25)

| | Factor | Strain name | Stage |
|---|------------|-------------|-------|
| | CEH-93 | OP554 | L1 |
| | NHR-111 | OP569 | L4 |
| | GEI-8 | OP589 | L4 |
| | ZIP-4 | OP590 | L1 |
| | CEH-88 | OP593 | L4 |
| | LPD-2 | OP649 | L1 |
| | Y55F3AM.14 | OP608 | L2 |
| | SUP-37 | OP611 | L1 |
| | C28G1.4 | OP630 | yAd |
| | HMG-4 | OP660 | EE |
| | MBL-1 | OP664 | L1 |
| | CEH-74 | OP680 | EE |
| | Y5F2A.4 | OP682 | L4 |
| * | PHA-2 | OP687 | LE |
| * | DMD-10 | OP689 | LE |
| | T26A8.4 | OP692 | L4 |
| | HLH-17 | OP643 | L1 |
| | MLS-1 | OP694 | L1 |
| | SPR-3 | OP703 | L4 |
| | B0336.7 | OP714 | yAd |
| | R151.8 | OP672 | L1 |
| | SDZ-12 | OP670 | L4 |
| | CEH-49 | OP699 | ME |
| | DRAP-1 | OP700 | ME |
| | ALR-1 | OP362 | L1 |

Asterisk indicates factors for the RNA-seq project

Factors awaiting growth and collection (18)

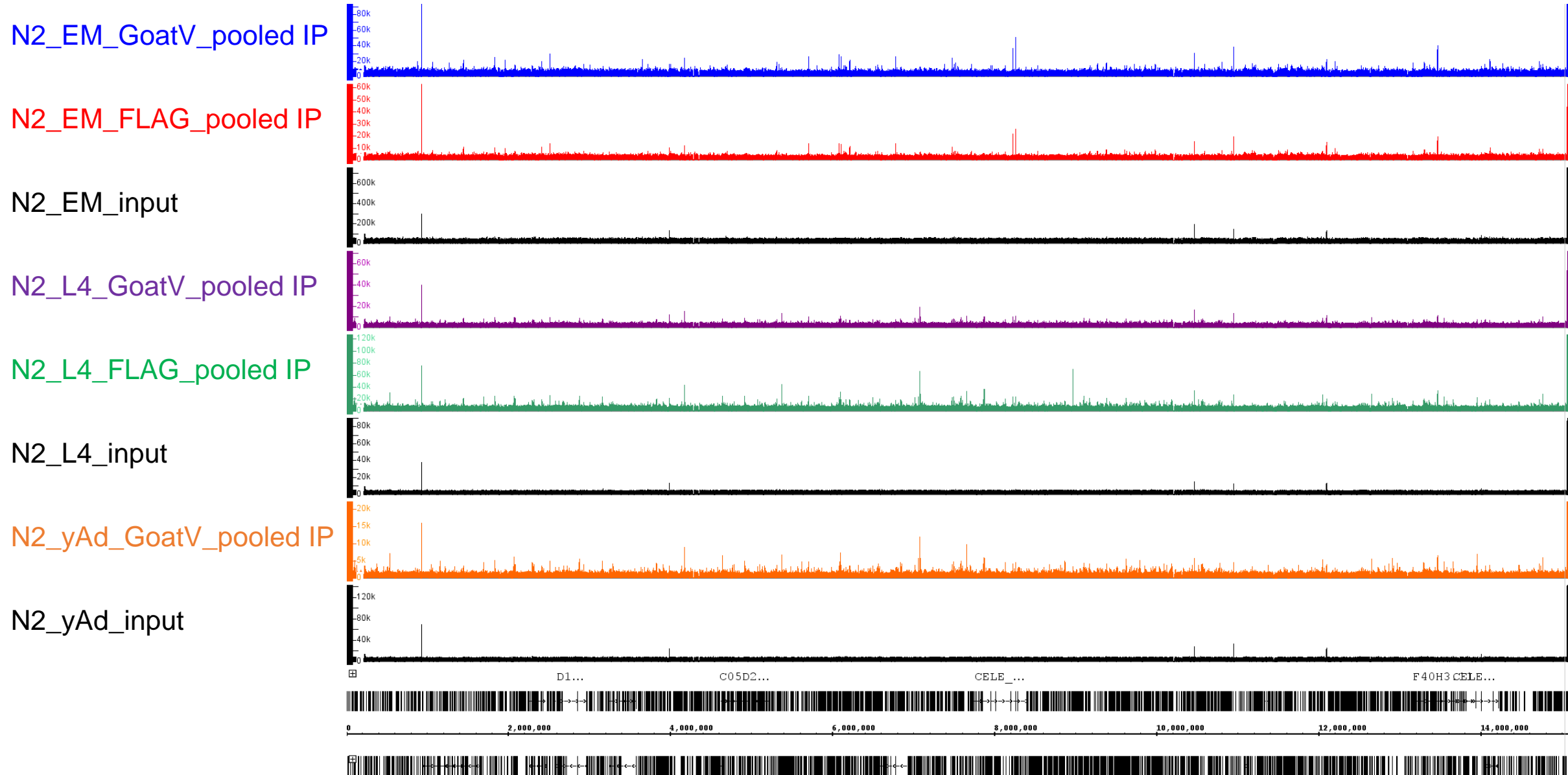
| Factor | Strain name | Stage |
|---------------|--------------------|--------------|
| SEM-2 | OP701 | EE |
| FLH-1 | OP702 | EE |
| TBX-8 | RW10470 | EE |
| C38D4.7 | OP708 | LE |
| T23F11.4 | OP709 | LE |
| NHR-190 | OP448 | yAd |
| PAX-3 | OP190 | L2 |
| CEH-40 | OP232 | yAd |
| LIR-2 | OP175 | yAd |
| ZTF-1 | OP207 | L2 |
| NHR-270 | OP208 | L1 |
| K12H6.12 | OP214 | yAd |
| F26F4.8 | OP716 | yAd |
| TAG-97 | OP719 | yAd |
| LSL-1 | OP720 | yAd |
| ATTF-5 | OP724 | L4 |
| SKNR-1 | OP726 | L4 |
| CEH-83 | OP727 | EE |

Testing the specificity of the GoatV GFP Antibody

- Perform ChIP using GoatV and FLAG Abs on N2 worms in 3 different stages:
 - Embryo (EM)
 - Late larval (L4)
 - Young adult (yAd)
- Perform ChIP with FLAG Ab on a factor that has previously passed using GoatV to test the overlap of GoatV and FLAG peaks
 - OP609_CHD-7_yAd

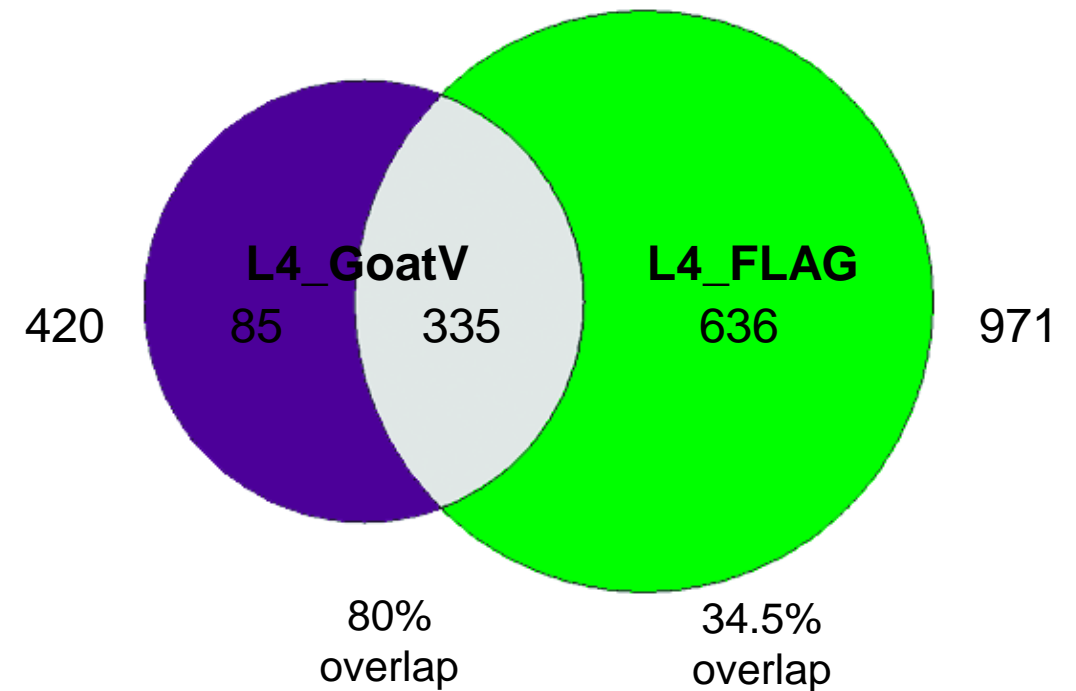
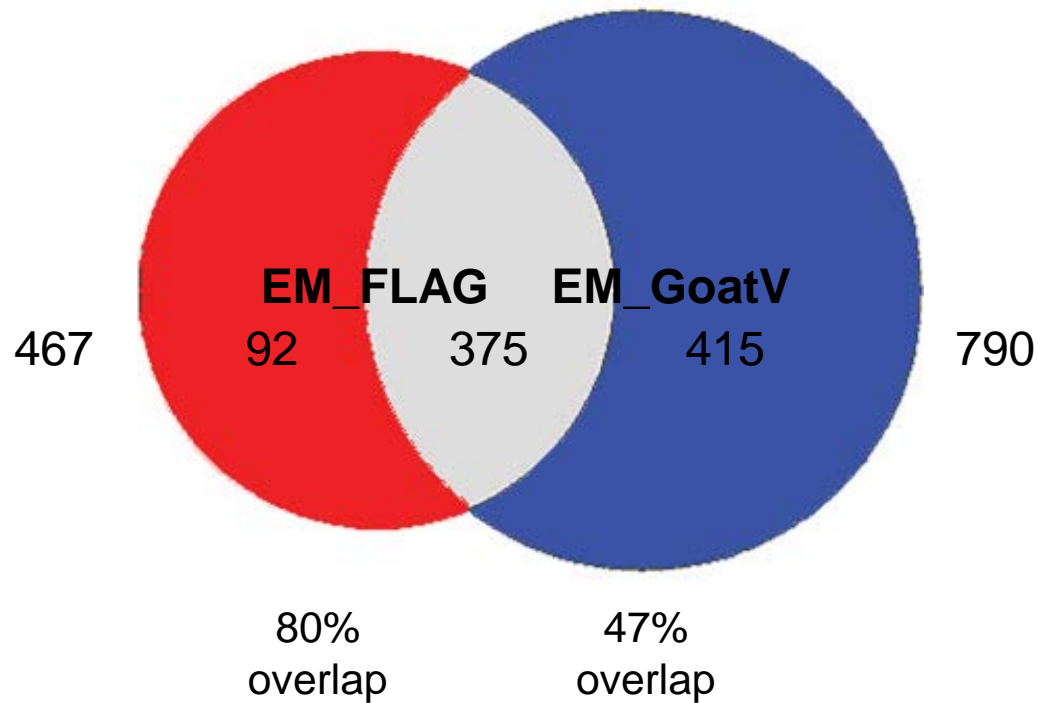
| Factor | Stage | GoatV GFP Antibody | FLAG Antibody |
|---------------|--------------|---------------------------------------|----------------------|
| N2 | EM | Sequencing complete | Sequencing complete |
| | L4 | Sequencing complete | Sequencing complete |
| | yAd | Sequencing complete | Failed |
| CHD-7 | yAd | 2 replicates completed for comparison | Sequencing complete |

Non-specific binding of Flag and GoatV GFP antibodies in N2



Comparison of embryo, L4 and young adult N2 ChIP-seq with GoatV GFP and Flag antibodies

- N2_EM_GoatV = 811 peaks, IDR = 1.57
- N2_EM_Flag = 482 peaks, IDR = .95
- N2_L4_GoatV = 448, IDR = .84
- N2_L4_Flag= 987, IDR = .80
- N2_yAd_GoatV = 598, IDR = .97



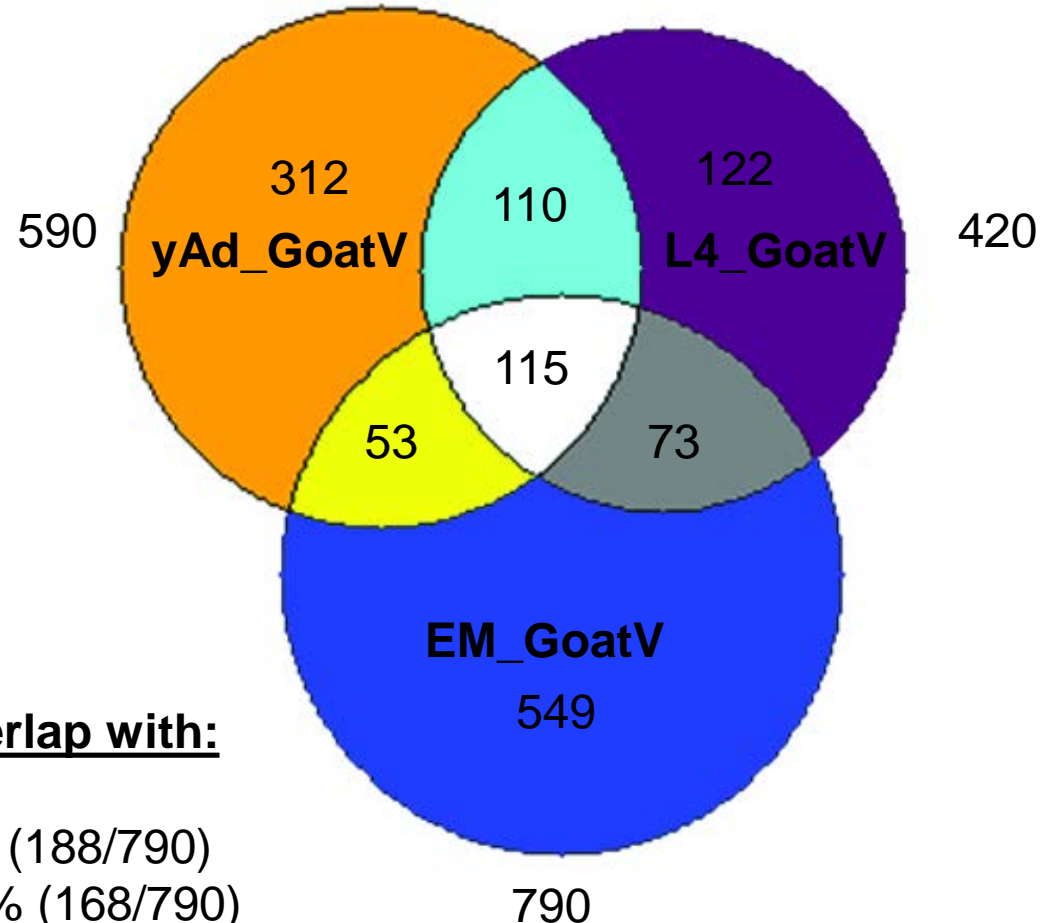
Numbers provided by Alec

Venn diagrams: <http://jura.wi.mit.edu/cgi-bin/bioc/tools/venn.cgi>

GoatV GFP Ab binds mainly to different sites in the embryo, L4 and young adult stages

yAd GoatV overlap with:

L4_GoatV 38% (225/590)
EM_GoatV 28% (168/590)



L4 GoatV overlap with:

EM_GoatV 45% (188/420)
yAd_GoatV 54% (225/420)

EM GoatV overlap with:

L4_GoatV 24% (188/790)
yAd_GoatV 21% (168/790)

Need to perform IPs in the other stages: L1, L2, L3 and other embryonic stages

Numbers provided by Alec

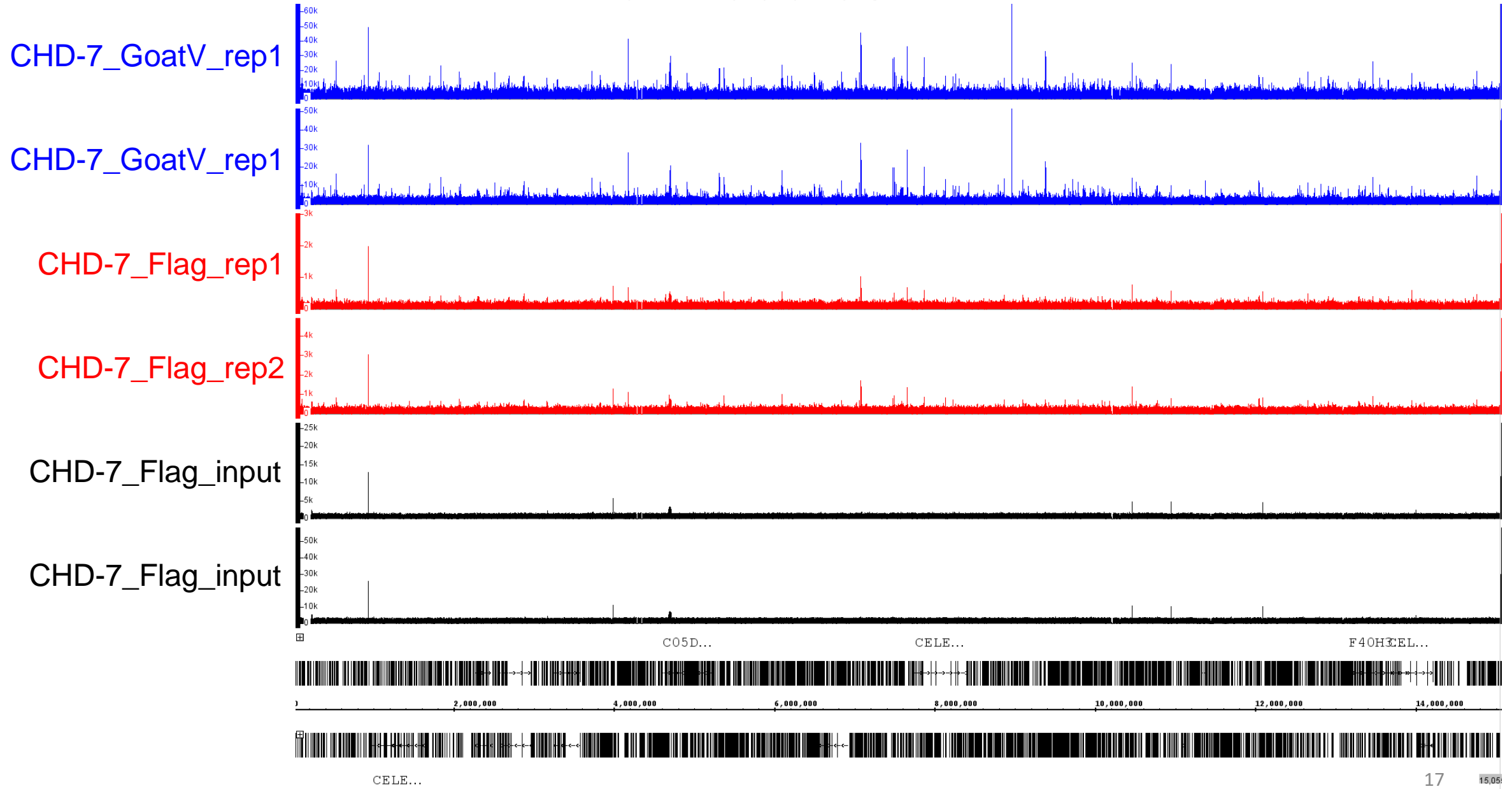
Venn diagrams: <http://jura.wi.mit.edu/cgi-bin/bioc/tools/venn.cgi>

How many of these non-specific sites are HOT?

- N2_EM_GoatV = 73% are HOT (576/790)
- N2_EM_Flag = 91% are HOT (424/467)
- N2_L4_GoatV = 84% are HOT (351/420)
- N2_L4_Flag = 73% are HOT (711/971)
- N2_yAd_GoatV = 75% are HOT (441/590)

GoatV GFP and Flag antibodies are mainly binding to HOT sites

CHD-7_yAd binding using GoatV GFP and Flag antibodies



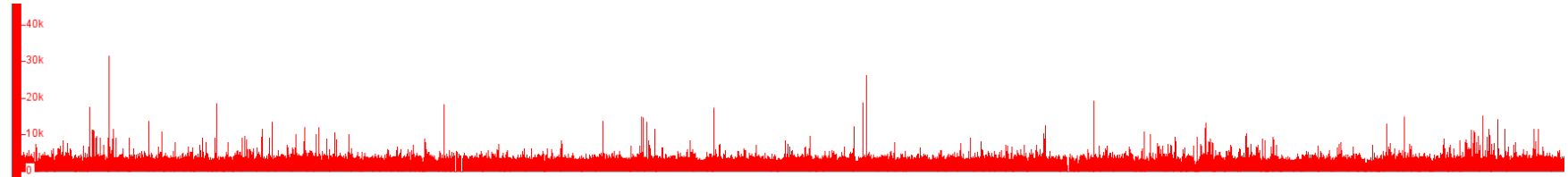
CHD-7 binding using GoatV GFP and Flag antibodies

- **Stats of Flag and GoatV datasets**
 - OP609_CHD-7_yAd_Flag = 148 peaks, IDR .98
 - OP609_CHD-7_yAd_GoatV = 1843 peaks, IDR 1.26
- **Comparison of Flag and GoatV datasets**
 - 23 unique to the FLAG dataset.
 - 1678 are unique to the GoatV dataset
 - 121 peaks are common between the two datasets.
- **Overlap with HOT sites**
 - 122/144 FLAG peaks are HOT sites, 85%.
 - 113/121 common peaks are HOT sites, 94%.
 - 1497/1800 GFP peaks are HOT sites, 83%.

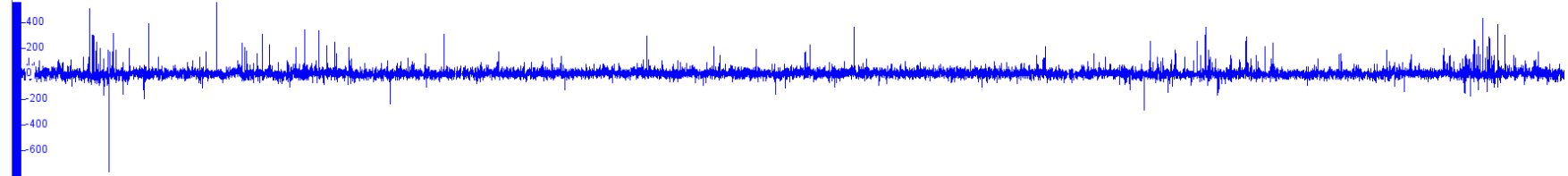
Flag IP did not work very well, used same lysate as used for GoatV IP

Goat V mock IP subtractions on “good” TFs in embryos

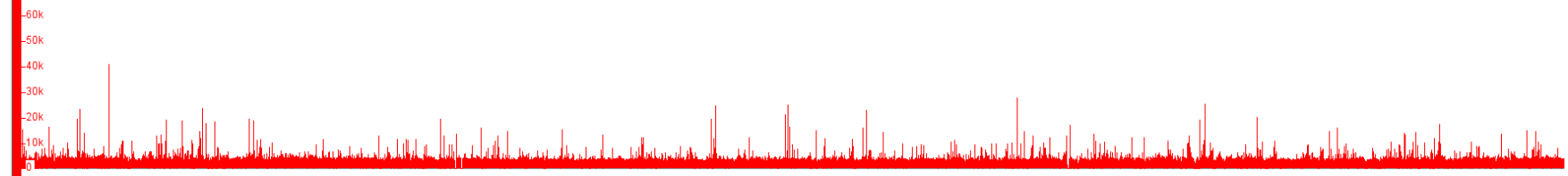
OP620_CEH-36_pooled IPs



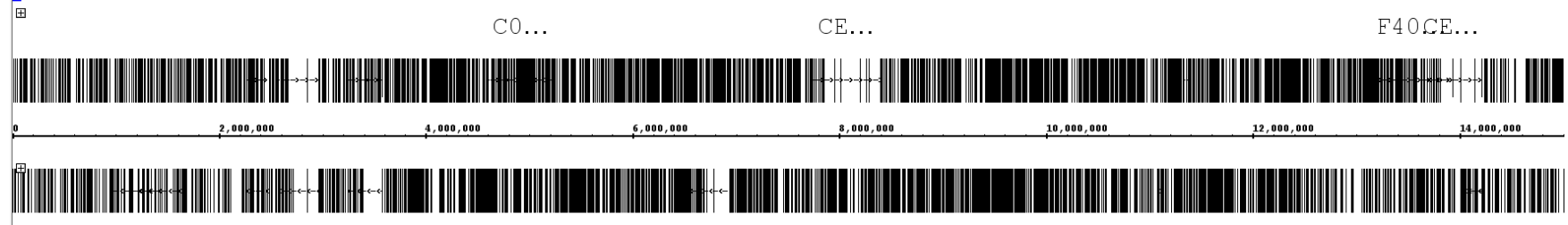
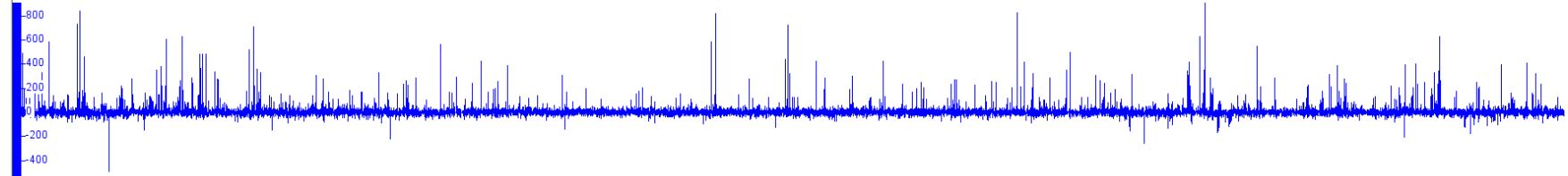
OP620_CEH-36_pooled IPs
w/ N2 GoatV subtracted



OP631_CEH-48_pooled IPs



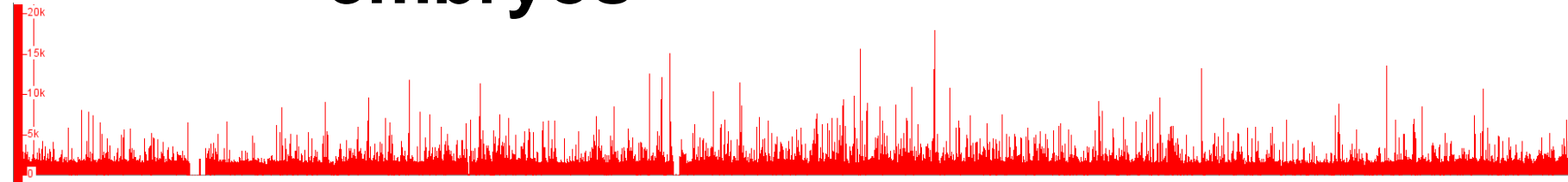
OP631_CEH-48_pooled IPs
w/ N2 GoatV subtracted



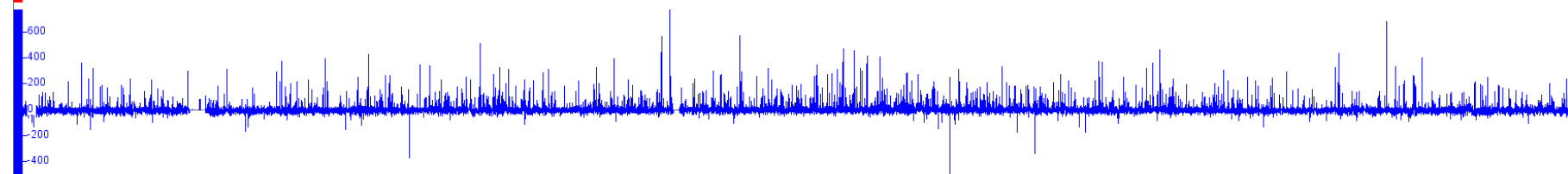
Most of the peaks are retained

Goat V mock IP subtractions on “good” TFs in embryos

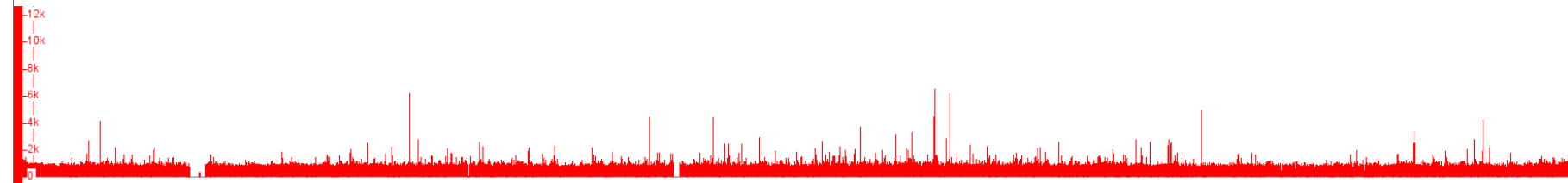
OP56_ELT-2_pooled IPs



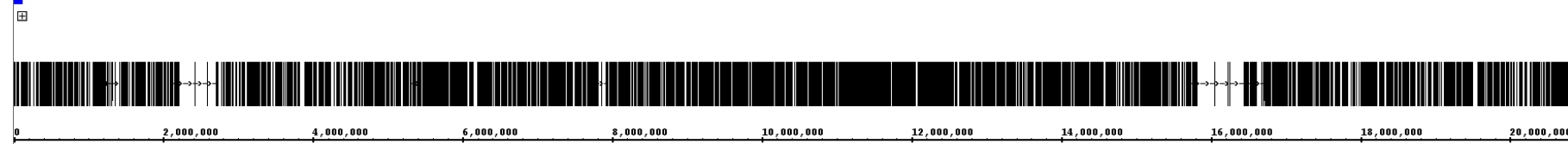
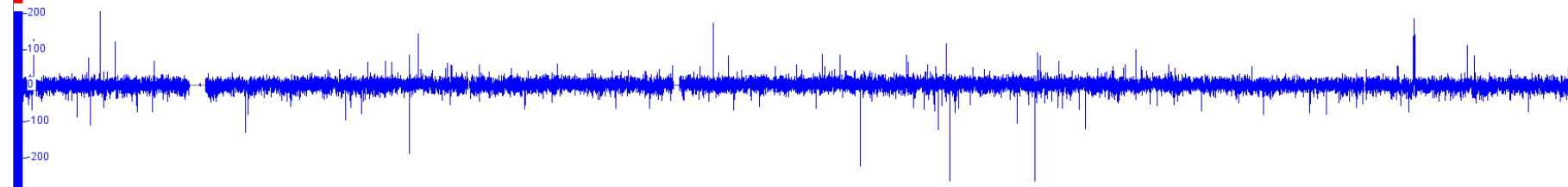
OP56_ELT-2_pooled IPs w/
N2 GoatV subtracted



OP578_UNC-3_pooled IPs



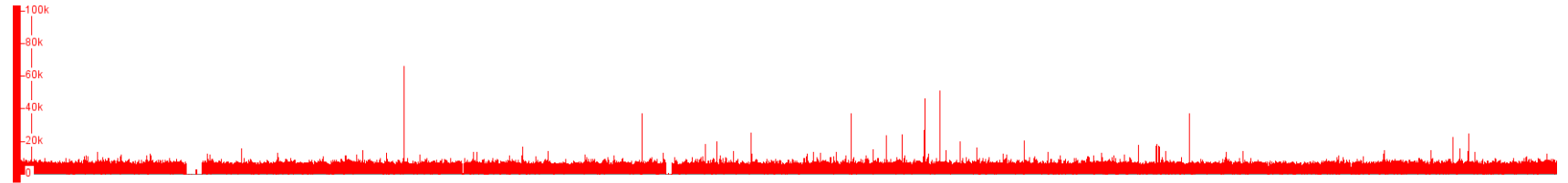
OP578_UNC-3_pooled IPs w/
N2 GoatV subtracted



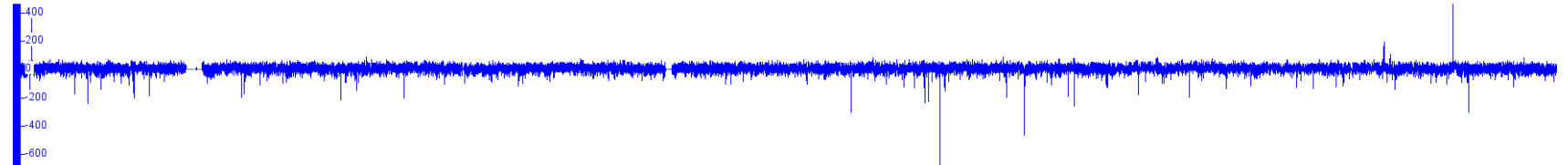
Most of the peaks are retained in OP56_ELT-2, OP578_UNC-3 is more of a moderate TF, where more peaks are lost

Goat V mock IP subtractions on “bad” TFs in embryos

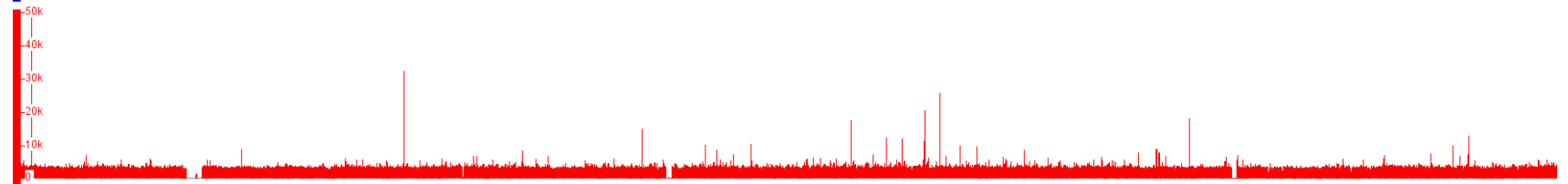
OP622_GMEB-2_pooled IPs



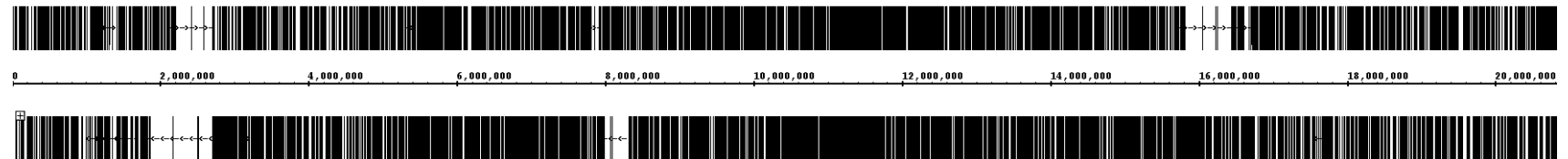
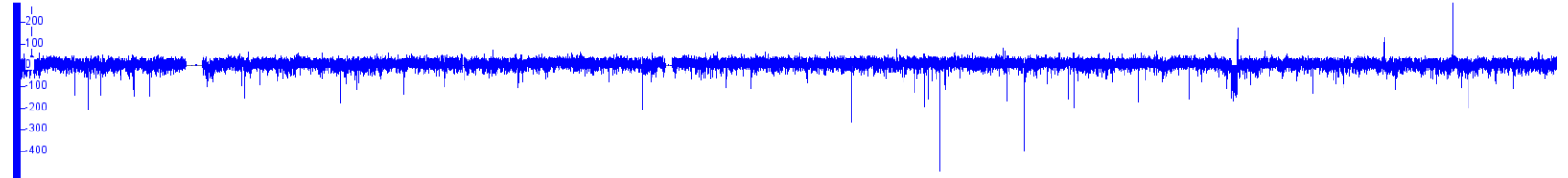
OP622_GMEB-2_pooled IPs
w/ N2 GoatV subtracted



OP615_Y22D7AL.16_pooled
IPs



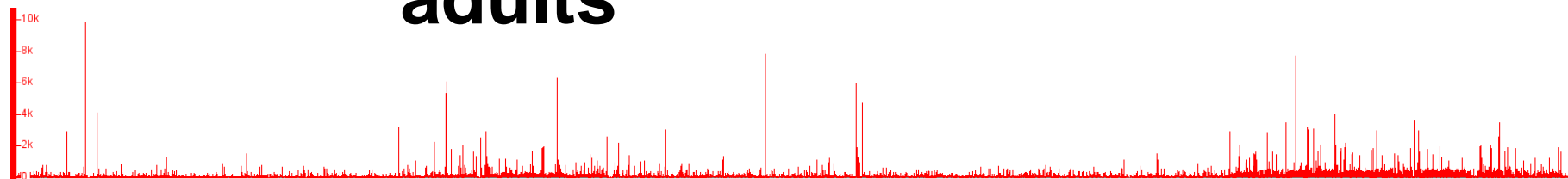
OP615_Y22D7AL.16_pooled
IPs w/ N2 GoatV subtracted



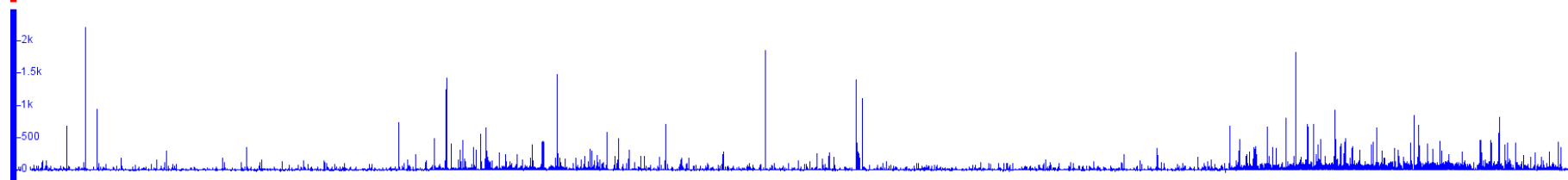
Most of the peaks are lost for these factors

Goat V mock IP subtractions on “good” TFs in young adults

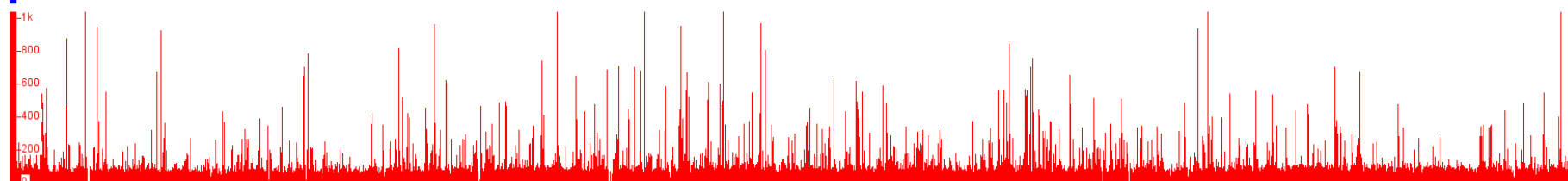
OP179_SNPC-4_pooled IPs



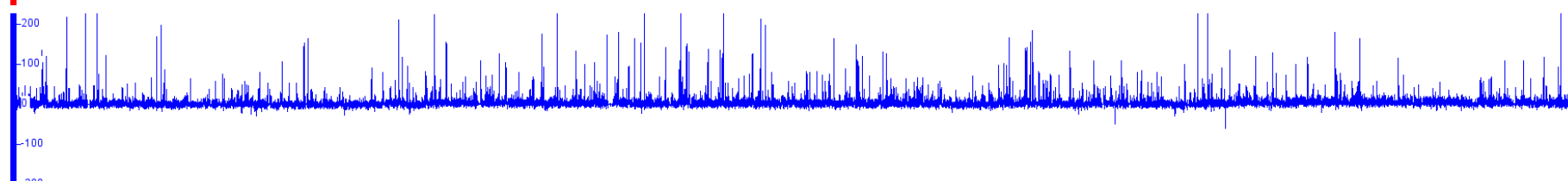
OP179_SNPC-4_pooled IPs
w/ N2 GoatV subtracted



OP383_F49E8.2_pooled IPs

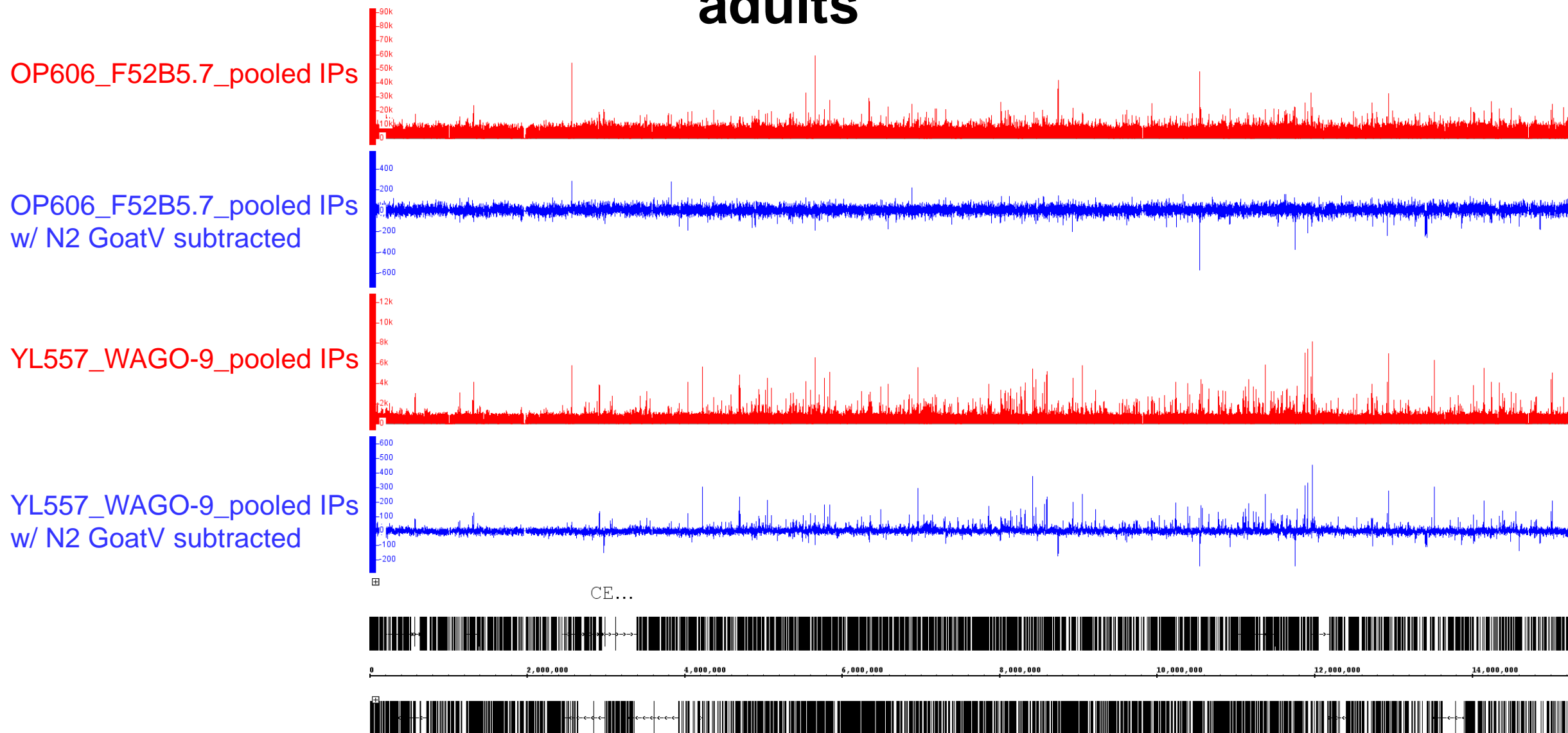


OP383_F49E8.2_pooled IPs
w/ N2 GoatV subtracted



Looks like all of the peaks are retained in OP179_SNPC-4, only a small number were lost in OP383_F49E8.2

Goat V mock IP subtractions on “bad” TFs in young adults



Looks like most of the peaks are lost in OP606_F52B5.7, only a small number were lost in YL557_WAGO-9 ChrII

Summary of embryo, L4 and young adult N2 ChIP-seq results with GoatV GFP and Flag antibodies

- Looks like GoatV GFP Ab binds to different sites in the embryo, L4 and young adult stages
 - need to perform the IPs in other stages: L1, L2, L3 and other embryonic stages
- GoatV GFP and Flag binding sites largely overlap with HOT sites
- Is the antibody binding to regions of open chromatin?
- Are these peaks background peaks that we would see in actual data?
- Should these non-specific peaks or HOT sites be subtracted from the data?
 - For the “bad” TFs examined, most peaks disappear
 - For the “good” TFs examined, the data looks cleaner, many of the peaks are retained

Summary of ChIP'd TFs for TF(RNAi/del)/RNA-seq

TFs with completed ChIP-seq data (44)

Deletion strain available (37)

CEH-30 UNC-86
FKH-10 TTX-3
MAB-5 BLMP-1
PAX-1 CES-1
LIM-6 DVE-1
RNT-1 CEH-31
MEC-3 C08G9.2
ELT-4 CEH-24
TBX-7 GMEB-2
CEH-34 CEH-48
CEH-14 CEH-9
CEH-2 Y22D7AL.16
FAX-1 CEH-24
PQM-1 SOX-4
PAG-3 M03D4.4
UNC-42
HLH-6
CEH-18
FKH-6
ZIP-5
HLH-15
UNC-3

RNAi clone available (7)

CEH-22
CEH-26
DMD-4
HLH-8
SDC-2
COG-1
SYD-9

Waiting for sequencing (1)

Deletion strain available

LIN-11

Waiting for IP/Growth (4)

Deletion strain available

PHA-2
DMD-10
HAM-2

RNAi clone available

MLS-1

Outline

- Summary of RNA-seq progress
 - Backcrossed deletion mutants
 - Backcross in progress
 - RNA isolated for sequencing and backcrossed strains waiting for RNA isolation

4X Backcrossed mutant strains with VC2010

| Gene Name | Deletion Strain | Strain Available | GFP Strain | Embryo Chip | Backcross |
|------------|--------------------|------------------|---------------|---------------|-----------|
| ceh-30 | ceh-30(n4289) X. | MT13544 | OP120 | Yes | 4X |
| mab-5 | mab-5(gk670) III. | VC1477 | OP19/26/27 | Yes | 4X |
| ceh-2 | ceh-2(ch4) I. | TB200 | OP323 | Yes | 4X |
| mec-3 | mec-3(gk1126) IV. | VC2396 | OP55 | Yes | 4X |
| tbx-7 | tbx-7(gk1033) III. | VC1976 | OP311 | Yes | 4X |
| fkx-10 | fkx-10(ok733) I. | RB884 | OP337/DCC4025 | Yes | 4X |
| pqm-1 | pqm-1(ok485) II. | RB711 | OP201 | Yes | 4X |
| rnt-1 | rnt-1(ok351) I. | VC200 | OP462 | Yes | 4X |
| pag-3 | pag-3(ok488) X. | VC369 | OP154 | Yes | 4X |
| ceh-31 | ceh-31 X. | tm239 | OP370 | Yes | 4X |
| ceh-18 | ceh-18 X. | tm6181 | OP533 | Yes | 4X |
| ceh-14 | ceh-14(ch3) X. | TB528 | OP315 | Yes | 4X |
| lim-6 | lim-6(nr2073) X. | OH110 | OP388 | Yes | 4X |
| elt-4 | elt-4(ca16) X. | JM124 | OP111 | Yes | 4X |
| fax-1 | fax-1(ok624) X. | RB812 | OP164 | Yes | 4X |
| C08G9.2 | C08G9.2 IV. | tm4339 | OP513 | Yes | 4X |
| blmp-1 | blmp-1 I. | tm548 | OP109 | Yes | 4X |
| dva-1 | dva-1 X. | tm4803 | OP398 | Yes | 4X |
| ces-1 | ces-1 I. | tm1036 | OP174 | Yes | 4X |
| fkx-6 | fkx-6 II. | tm439 | OP78 | Yes | 4X |
| zip-5 | zip-5(gk646)_V. | VC1392 | OP544 | Yes | 4X |
| unc-42 | unc-42 V. | tm5335 | OP173 | Yes | 4X |
| lin-11 | lin-11 I. | tm5323 | OP62 | Yes | 4X |
| hlh-15 | hlh-15 X. | tm1824 | OP566 | Yes | 4X |
| ttx-3 | ttx-3 X. | tm268 | OP68 | Yes | 4X |
| unc-86 | unc-86 III. | tm6459 | OP476 | Yes | 4X |
| unc-3 | unc-3 X. | tm4776 | OP578 | Yes | 4X |
| ceh-9 | ceh-9 I. | tm2747 | OP202 | Yes | 4X |
| sox-4 | sox-4(gk700) X. | VC1544 | OP517 | Yes | 4X |
| eyg-1 | eyg-1(gk851) II. | VC1800 | OP443 | Yes | 4X |
| ceh-24 | ceh-24(cc539) V. | PD4588 | OP673 | Yes | 4X |
| ham-2 | ham-2 X. | tm5501 | OP639 | Yes | 4X |
| Y22D7AL.16 | Y22D7AL.16 III. | tm4265 | OP615 | Yes | 4X |
| gmeb-2 | gmeb-2 X. | tm6823 | OP622 | Yes | 4X |
| M03D4.4 | M03D4.4 IV | tm559 | OP696 | Yes | 2X |
| C52B9.2 | C52B9.2 X. | tm413 | No | No | 2X |
| hlh-19 | hlh-19 X. | tm3105 | No | No | 2X |
| F55B11.4 | F55B11.4 IV. | tm4294 | OP385 | No expression | 4X |
| npax-1 | npax-1 II. | tm1367 | OP626 | Dauer express | 2X |
| dac-1 | dac-1(gk211) III. | VC392 | OP188/189 | Not available | 4X |
| ZK337.2 | ZK337.2 X. | tm706 | OP955 | No expression | 4X |
| unc-55 | unc-55 I. | tm3355 | DCC4035 | Not available | 4X |
| ceh-33 | ceh-33 V. | tm244 | OP575 | Cytoplasmic | 4X |
| gei-3 | gei-3 X. | tm4380 | OP180 | No expression | 4X |
| unc-98 | unc-98 X. | tm601 | OP85 | Cytoplasmic | 4X |
| C34D1.1 | dmd-10(gk1131) V. | VC2341 | OP689 | Dauer express | 2X |

41 Deletion strains with 4X backcrossed, 34 of them have been chipped.

5 Deletion strains with 2X backcrossed, 1 of them has been chipped.

Backcross in progress

| Gene Name | Deletion Strains | Strains Available | GFP Strains | Embryos Chip | Backcross |
|-----------|------------------|-------------------|-------------|--------------|-----------|
| M03D4.4 | M03D4.4 IV. | tm559 | OP696 | Yes | 2X |

31 RNA for 6-timepoints shipped to UW for sequencing

| Y2Q3 | Y2Q4 | Y3Q1 | Y3Q2 | Y3Q3 | Y3Q4 | Y4Q1 |
|----------------|--------------|---------------|----------------|---------------------|---------------|---------------|
| VC1477_mab-5 | TB200_ceh-2 | RB812_fax-1 | tm4339_C08G9.2 | tm1824_hlh-15 | tm268_ttx-3 | VC1544_sox-4 |
| MT13544_ceh-30 | VC2396_mec-3 | tm548_blimp-1 | tm6181_ceh-18 | tm4776_unc-3 | tm6459_unc-86 | VC1800_eyg-1 |
| | RB884_fkh-10 | tm1036_ces-1 | VC369_pag-3 | tm439_fkh-6(Repeat) | | tm2747_ceh-9 |
| | VC1976_tbx-7 | tm439_fkh-6 | tm239_ceh-31 | | | PD4588_ceh-24 |
| | RB711_pqm-1 | | TB528_ceh-14 | | | |
| | VC200_rnt-1 | | JM124_elt-4 | | | |
| | | | tm4803_elt-4 | | | |
| | | | OH110_lim-6 | | | |
| | | | tm5323_lin-11 | | | |
| | | | VC1392_zip-5 | | | |
| | | | tm5335_unc-42 | | | |

Backcrossed strains waiting for RNA isolation

| Gene Name | Strains Available | GFP Strains | Embryo Chip | Backcross | RNA Isolation |
|------------|-------------------|-------------|-------------|-----------|----------------|
| gmeb-2 | gmeb-2 X. | tm6823 | OP622 | 4X | Ready to send |
| ham-2 | ham-2 X. | tm5501 | OP639 | 4X | Need to repeat |
| Y22D7AL.16 | Y22D7AL.16 III. | tm4265 | OP615 | 4X | Waiting |