

The Reinke Lab Update



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Outline

- **Summary of ChIP-seq progress**
 - Completed datasets
 - Datasets in the pipeline
 - Worm pellets ready for ChIP
 - Factors that failed
 - ChIP'd factors for RNA-seq
 - DCC topics for discussion
- **Update on RNAseq project**
 - Backcrossed deletion mutants
 - Deletion strains waiting for backcross
 - RNA isolated for sequencing
 - RNA isolation in progress and backcrossed strains waiting for RNA isolation

Sequencing data for 136 new datasets

Bridge	0 Y1Q1	0 Y1Q2	10 Y1Q3	22 Y1Q4	17 Y2Q1	11 Y2Q2	21 Y2Q3	Y2Q4	15 Y3Q1	4 Y3Q2	3 Pending	2
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	OP558_LAG-1_LE	
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	OP476_UNC86_LE	
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	OP68_TTX-3_LE	
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_Y53C1_2C.1_LE			
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				
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				
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				
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				
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				
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				
JK1107_RPC-1_YA				YL529_LET-607_YA	YL557_WAGO-9_YA	OP496_ZTF-18_YA	OP252_DAO-5_L4	OP385_F55B11.4_YA				
LW1254_SMA-9C2_L2				OP480_NHR-71_L1	YL563_REC-8_YA		OP502_ALY-2_EE	OP544_ZIP-5_LE				
OP470_ZC204.2_L4				OP516_CEH-32_L4	OP529_F10B5.3_L4		RW10702_HLH-6_LE	OP539_NHR-85_L1				
OP358_CEU-2_L4				ZM8745_DAF-16_L4	OP522_DSC-1_L1		OP195_RBR-2_L4	OP543_SDZ-38_YA				
OP372_K09A11.1_LE				OP515_NHR-20_L1	OP383_F49E8.2_L4		RW10316_DIE-1_YA	TH184_HMG-11_L3				
OP398_DVE-1_LE				OP514_SPR-4_YA	OP520_REF-2_LE		OP78_FKH-6_LE					
OP398_DVE-1_L4				OP471_F13H6.1_LE	OP111_ELT-4_LE		RW10325_MES-4_YA					
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_BLMF-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
- 7 completed factors for year 3
- In total, data for 123 new TFs

OP514_SPR-4 has a rearrangement
OP558_LAG-1 is ZTF-16

Pending factors

	Factor	Strain	Stage	Notes
	F57C9.4	OP550	YA	Repeated IP, Library pending
	GEI-17	OP572	YA	Library pending
	SAEG-1	OP580	YA	Library pending
*	LIN-11	OP62	LE	Library pending
*	UNC-86	OP476	LE	Out for sequencing
*	TTX-3	OP68	LE	Out for sequencing

* Asterisk indicates factors for RNAseq project

Pellets ready for ChIP (10)

	Factor	Strain	Stage
	ZIM-3	OP581	YA
	SEA-2	OP586	YA
	MADF-2	OP587	LE
	GEI-8	OP589	L4
	ZIP-4	OP590	L1
	SOX-4	OP517	LE
*	CEH-88	OP593	L4
	FKH-3	OP594	YA
*	CEH-9	OP202	LE
	F52B5.7	OP606	YA

Strains awaiting growth/collection (15)

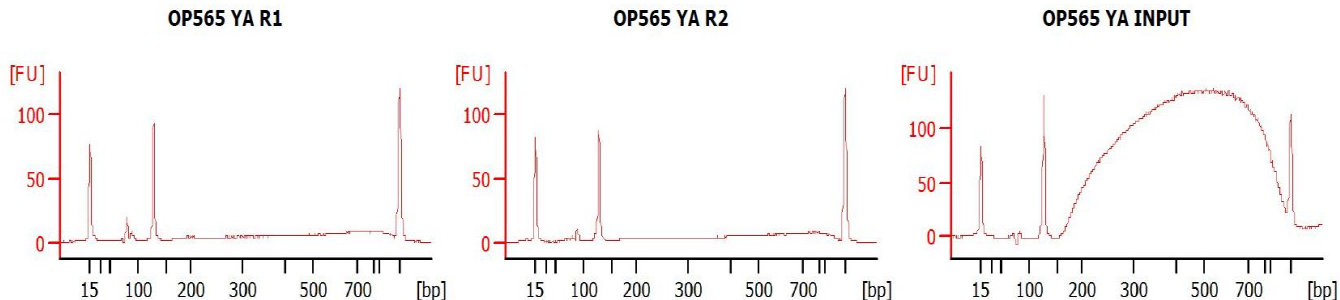
	Factor	Strain	Stage
	T26A5.8	OP598	YA
	HLH-4	OP599	L1
	LPD-2	OP601	L1
	F13C5.2	OP605	EE
	Y55F3AM.14	OP608	L2
	CHD-7	OP609	YA
	SUP-37	OP611	L1
	ETS-9	OP613	L4
	ZTF-3	OP614	YA
*	Y22D7AL.16	OP615	LE
	TBX-9	OP617	EE
	CEH-36	OP620	EE
*	GMEB-2	OP622	LE
	CCCH-3	OP627	L4
	C28G1.4	OP630	YA

* Asterisk indicates factors for RNAseq project

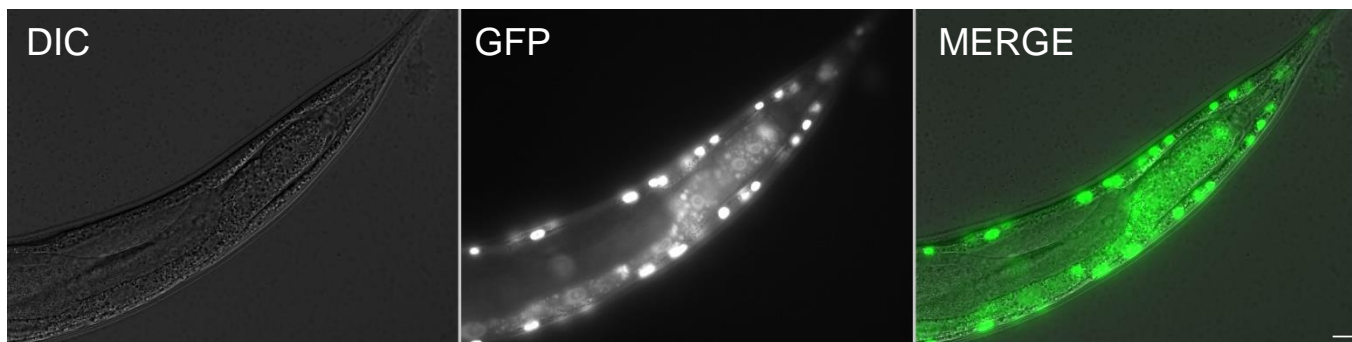
Factors that failed library preparation

Factor	Strain	Stage	Notes
OP563	CEBP-1	YA	
OP565	ZTF-26	YA	
OP568	LSY-12	L3/L4	
OP570	C33H5.17	L4	
OP579	DPFF-1	YA	
OP556	CEH-89	YA	Failed twice
OP553	CEH-79	YA	

Enrichment of OP565_ZTF-26 following library preparation



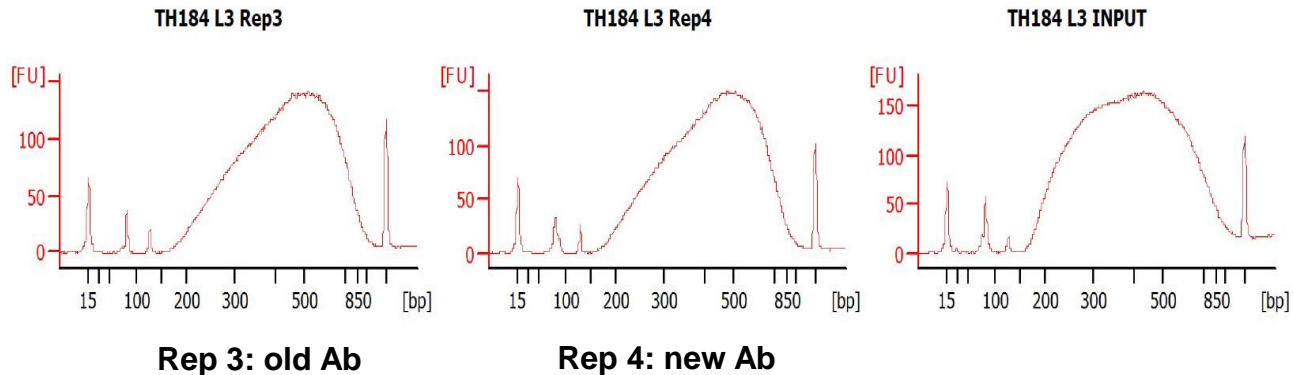
Input samples look good which rules out library issues



OP565_ZTF-26 expresses well

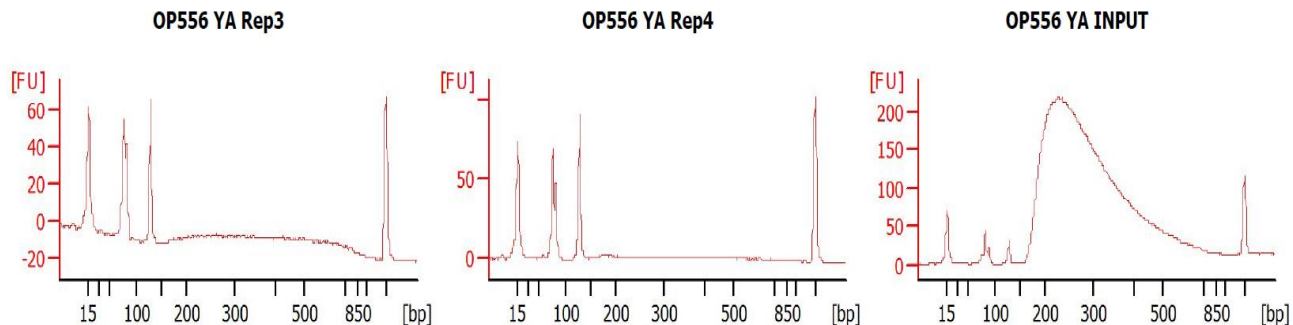
Failures are not due to an antibody issue

Repeated TH184 (HMG-11) that previously worked using old and new Ab aliquot



Conclusion: Our antibody aliquots received in September of 2014 are still working

Repeated OP556 (CEH-89) that failed using new Ab aliquot



Conclusion: OP556 failed again. All ChIP and library reagents are working. Issue is likely with the worm sample (fixation or sonication).

Troubleshooting ChIP issue

- Bought new formaldehyde
- Sonicator not working as well since Aug 2014 (lower protein concentrations). We purchased a new tip in April 2015 but it did not improve protein concentrations significantly. Despite this, there were no ChIP issues until now.
- We will increase the amplitude on the machine from 10 to 12 in order to improve sonication
- We will test new sonication conditions on OP556_CEH-89 frozen worm pellets

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

TFs with completed ChIP-seq data (32)

Deletion strain available (23)

CEH-30
FKH-10
MAB-5
PAX-1
LIM-6
RNT-1
MEC-3
ELT-4
TBX-7
CEH-34
CEH-14
CEH-2
FAX-1
PQM-1
PAG-3
UNC-42
HLH-6
CEH-18
FKH-6
SYD-9
ZIP-5
HLH-15
UNC-3

RNAi clone available (9)

BLMP-1
CEH-22
CEH-26
CES-1
DVE-1
DMD-4
HLH-8
SDC-2
COG-1

Waiting for library/sequencing (3)

Deletion strain available

UNC-86
TTX-3
LIN-11

Waiting for IP/Growth (4)

Deletion strain available

SOX-4
CEH-9
Y22D7AL.16
GMEB-2

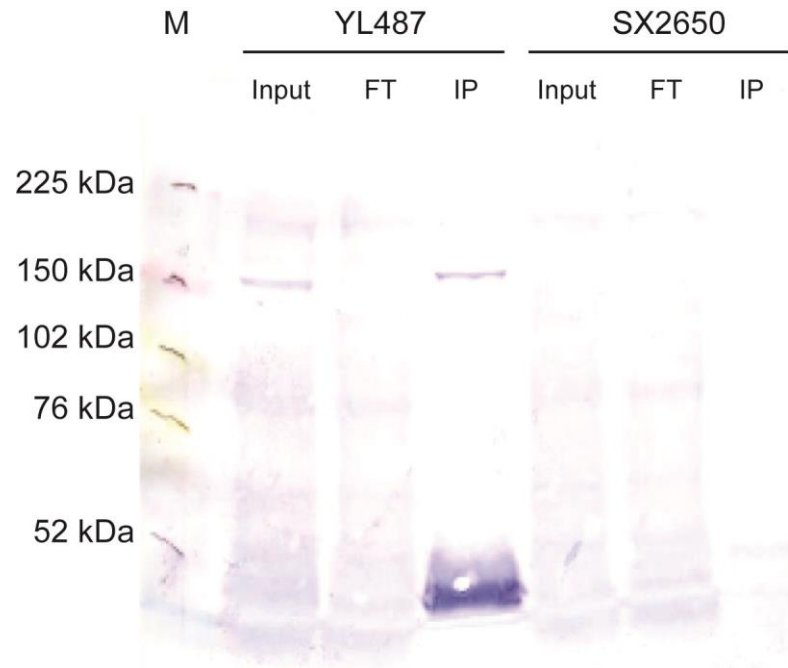
DCC issues for discussion

- DCC wants us to comply with the current quality standards of ENCODE
 - library complexity
 - IDR
 - Read depth
- These standards can't be used with worm and fly due to smaller genome size
- We are working to adapt these standards to our model systems

Antibody compliance is complete in worm for GoatV

Snyder lab performed an IP-mass spec using GoatV Ab which serves as a primary characterization

We performed an IP with GoatV Ab and then blotted with FLAG Ab



ChIP assays were conducted as previously described (Zhong et al., 2010). Young adults were collected from YL487 (snpc-4(tm4568) I; unc-119(ed3) III; wgl-179 [snpc-4:TY1 EGFP 3XFLAG; unc-119]) and SX2650 (mjSi74(pmx-5::mCherry::PRDE-1_STOP::par-5 3'UTR) I) strains. The blot specifically recognizes a single species of the expected size of SNPC-4 of 143 kDa. As expected, no band is detected in the IP for SX2650.

Antibody compliance is in progress for Hyman GFP Ab

- Hyman antibody from old ModENCODE was used for several factors
- In order for these datasets to count towards the new ModERN project the Hyman Ab needs to comply with the new Ab standards
- Trying to seek exemption from these standards by providing two independent ChIP-seq comparisons
 1. EFL-1 native Ab compared to tagged EFL-1 Kudron et.al 2013
 2. ChIPseq data for SNPC-4 comparing GoatV and Hyman Abs

Update on RNA-seq

- Backcrossed deletion mutants
- Deletion strains waiting for backcross
- RNA isolated for sequencing
- RNA isolation in progress and backcrossed strains waiting for RNA isolation

4X Backcrossed mutant strains with VC2010

Gene Name	Deletion Strains	Strains Available	GFP Strains	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
fkh-10	fkh-10(ok733) I.	RB884	OP377/378	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	OP154/163	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	OP111/134	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
dve-1	dve-1 X.	tm4803	OP398	Yes	4X
ces-1	ces-1 I.	tm1036	OP174	Yes	4X
fkh-6	fkh-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
ceh-24	ceh-24(cc539) V.	PD4588	OP157/158	Hard to grow	4X
lin-11	lin-11 I.	tm5323	OP62/OP348	Sent to UC	4X
hlh-15	hlh-15 X.	tm1824	OP566	Sent to UC	4X
ttx-3	ttx-3 X.	tm268	OP68/69	Sent to UC	4X
unc-86	unc-86 III.	tm6459	OP476	Sent to UC	4X
ceh-9	ceh-9 I.	tm2747	OP202	Hard to collect	4X
unc-3	unc-3 X.	tm4776	OP578	Sent to UC	2X
F55B11.4	F55B11.4 IV.	tm4294	OP385	No expression	4X
npax-1	npax-1 II.	tm1367	OP626	No expression Dauer express	2X
dac-1	dac-1(gk211) III.	VC392	OP188/189	Not available	4X
ZK337.2	ZK337.2 X.	tm706	OP355	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
C52B9.2	C52B9.2 X.	tm413	No	No	2X
hlh-19	hlh-19 X.	tm3105	No	No	2X
ham-2	ham-2 X.	tm5501	No	No	2X
C34D1.1	dmd-10(gk1131) V.	VC2341	No	No	2X

35 Deletion strains with 4X backcrossed, 26 of them have been chipped.

6 Deletion strains with 2X backcrossed, 1 of them have been chipped.

Deletion strains waiting for backcross

Gene Name	Deletion Strains	Strains Available	GFP Strains	Embryos Chip	Backcross
unc-3	unc-3 X.	tm4776	OP578	Yes	2X
eyg-1	Y53C12C.1(gk851) II.	VC1800	OP443	Yes	Ordered
sox-4	C12D12.5(gk700) X.	VC1544	OP517	Yes	Ordered
Y22D7AL.16	Y22D7AL.16 III.	tm4265	OP615	In progress	Ordered
F53H4.5	gmeh-2 X.	tm6823	OP622	Not integrated	Ordered

20 RNA isolated for 6-timepoints for sequencing

Y2Q1	Y2Q2	Y2Q3	Y2Q4
VC1477_mab-5	TB200_ceh-2	RB812_fax-1	tm4339_C08G9.2
MT13544_ceh-30	VC2396_mec-3	tm548_blmp-1	tm6181_ceh-18
	RB884_fkh-10	tm1036_ces-1	VC369_pag-3
	VC1976_tbx-7	tm439_fkh-6	tm239_ceh-31
	RB711_pqm-1		TB528_ceh-14
	VC200_rnt-1		JM124_elt-4
			tm4803_elt-4
			OH110_lim-6

RNA isolation in progress and
backcrossed strains waiting for RNA isolation

Gene Name	Strains Available	GFP Strains	Embryo Chip	Backcross	RNA Isolation
VC2010	VC2010	NONE	NONE	WT	Ready for ship
lin-11	tm5323	OP62/OP348	Library prep	4X	Ready for ship
zip-5	VC1392	OP544	Yes	4X	In progress
unc-42	tm5335	OP173	Yes	4X	In progress
hlh-15	tm1824	OP566	Yes	4X	Not yet
ttx-3	tm268	OP68/69	Sequencing	4X	Not yet
unc-86	tm6459	OP476	Sequencing	4X	Not yet
unc-3	tm4776	OP578	Yes	2X	Not yet