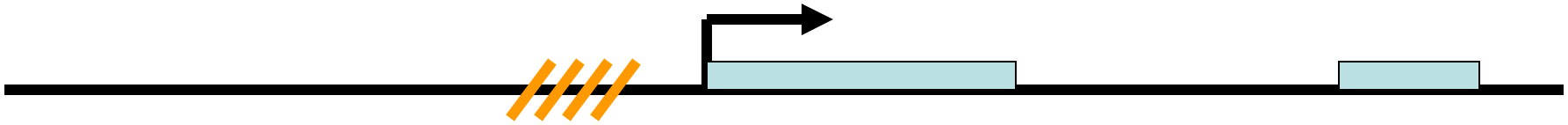
Elements	Presenter	Primary experimental data
Transcripts (mRNAs, ncRNAs, TSS, UTRs, miRNAs)	Celniker Lai	Tiling arrays, RNASeq, RT-PCR/RACE, Mass Spec, 3'UTR clone library, UAS-miRNA flies.
Enhancers, promoters, silencers, insulators	White	ChIP/chip, ChIP/seq, TF-tagged strains, anti-TF antibodies.
Chromatin marks	Pirrotta Ahmad	ChIP/chip and ChIP/seq of chromosome-associated proteins and nucleosome
DNA Replication origins	Orr-Weaver	ChIP/chip and ChIP/seq of essential initiator proteins, origin mapping and DNA copy number in differentiated tissues
Data Coordination Center	Suzi Lewis	www.modencode.org

A cis-regulatory map of the *Drosophila* genome

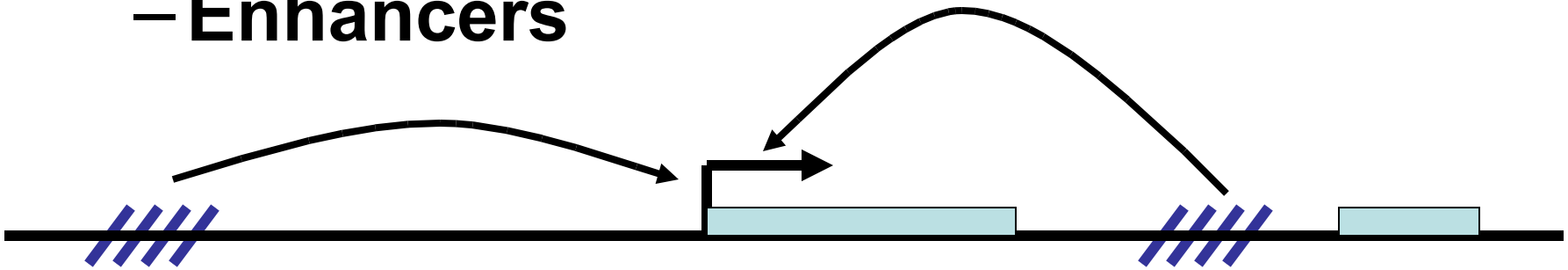
- **Data generation**
  - **Kevin White, U. Chicago (Antibody pipeline, ChIP chip pipeline)**
  - **Bing Ren, UCSD (Ab validation, ChIP chip pipeline)**
- **Computational identification of cis-regulatory sequences**
  - **Manolis Kellis, MIT (Comparative motif analysis, ChIP chip analysis)**
- **Validation**
  - **Jim Posakony, UCSD (Promoters/Enhancers)**
  - **Steve Russell, U. Cambridge (Insulators/Silencers)**
  - **Hugo Bellen, Baylor (element "necessity" validations)**

Cis regulatory Element types

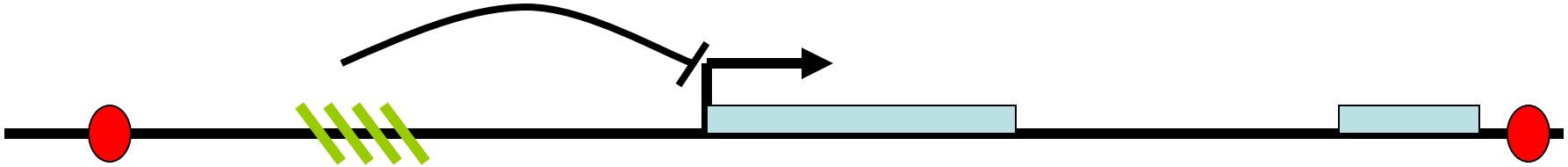
- Promoters



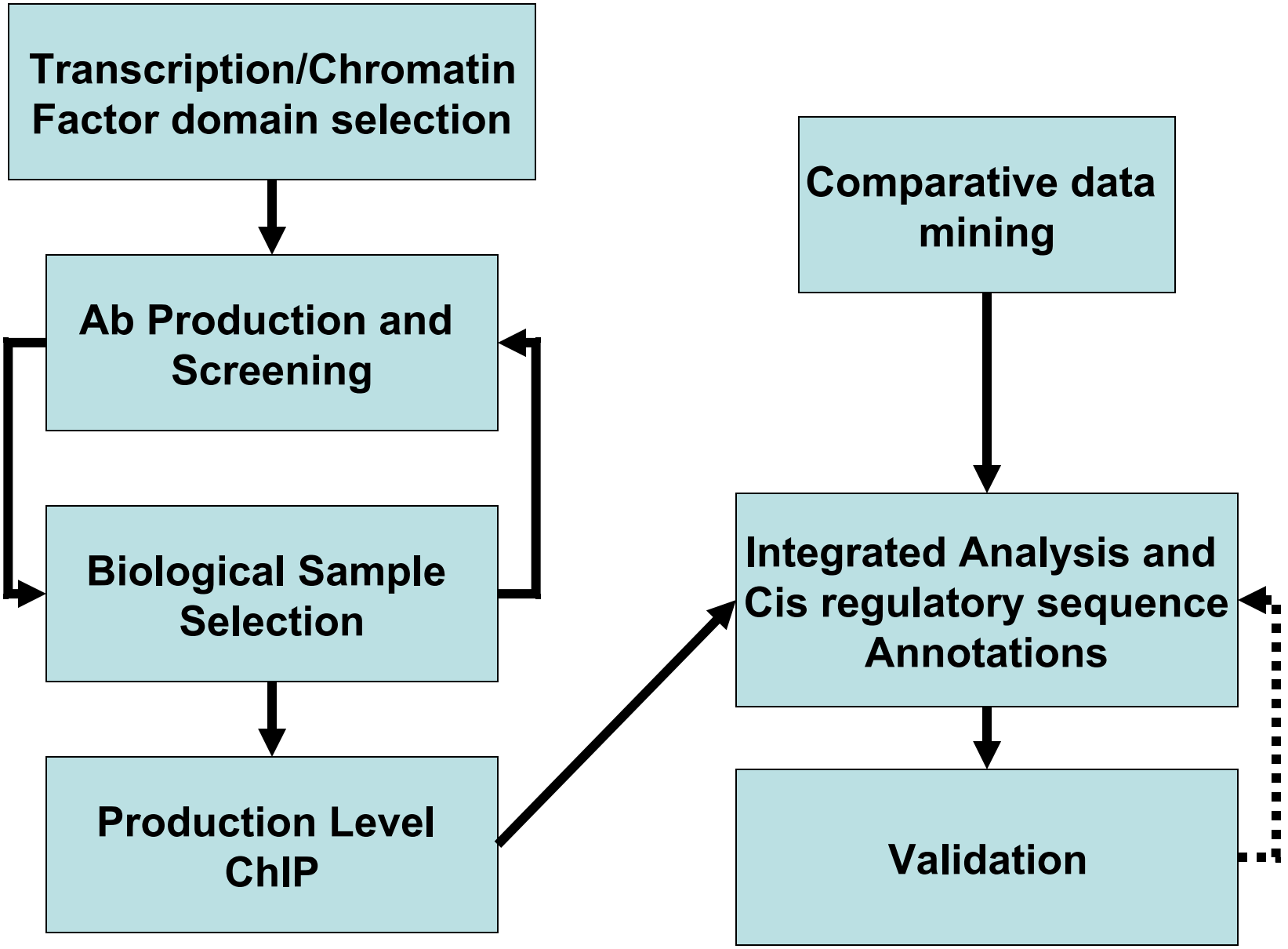
- Enhancers



- Silencers and Insulators



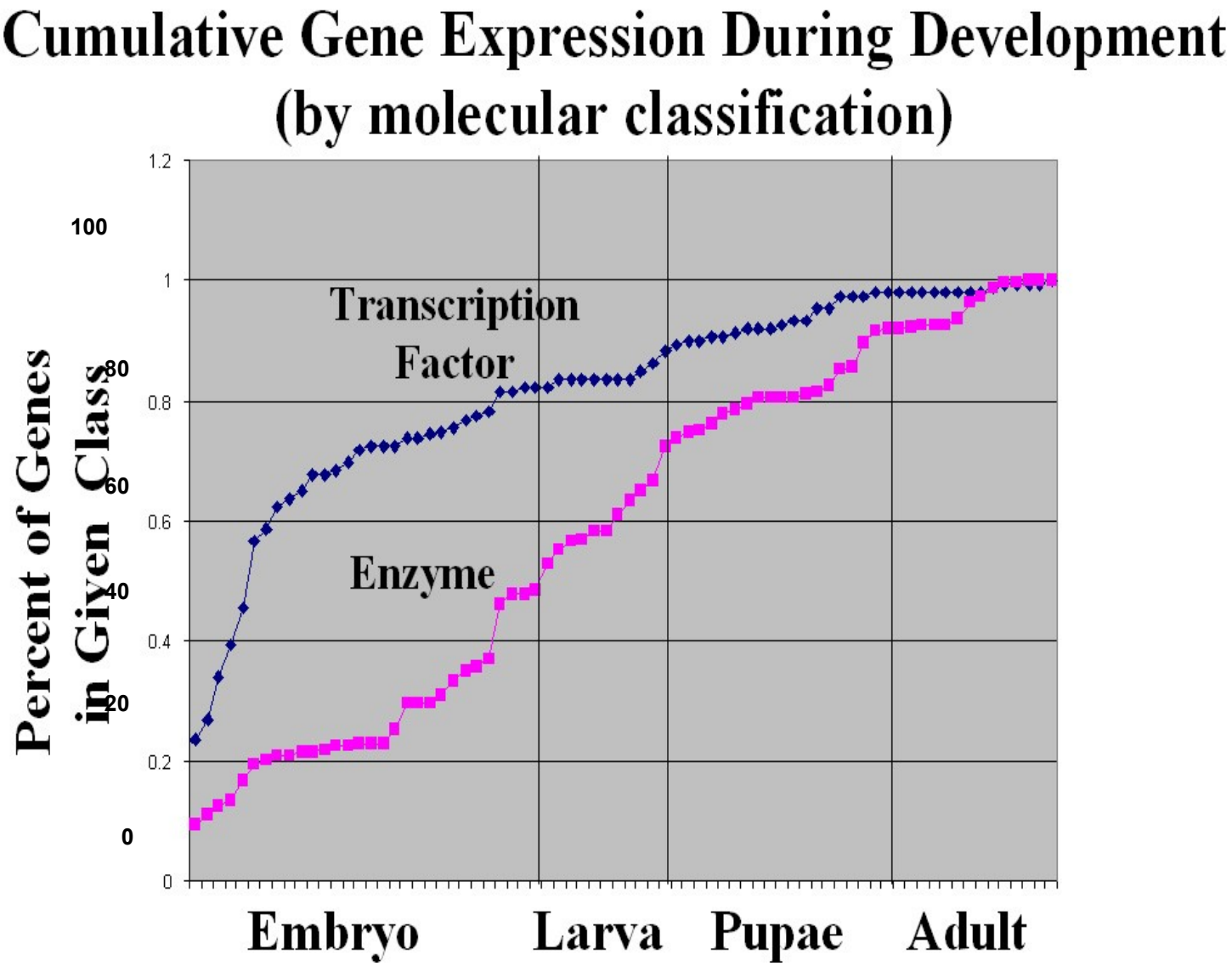
A Cis-Regulatory Annotation Pipeline



Target Proteins to define each type of Cis regulatory sequence

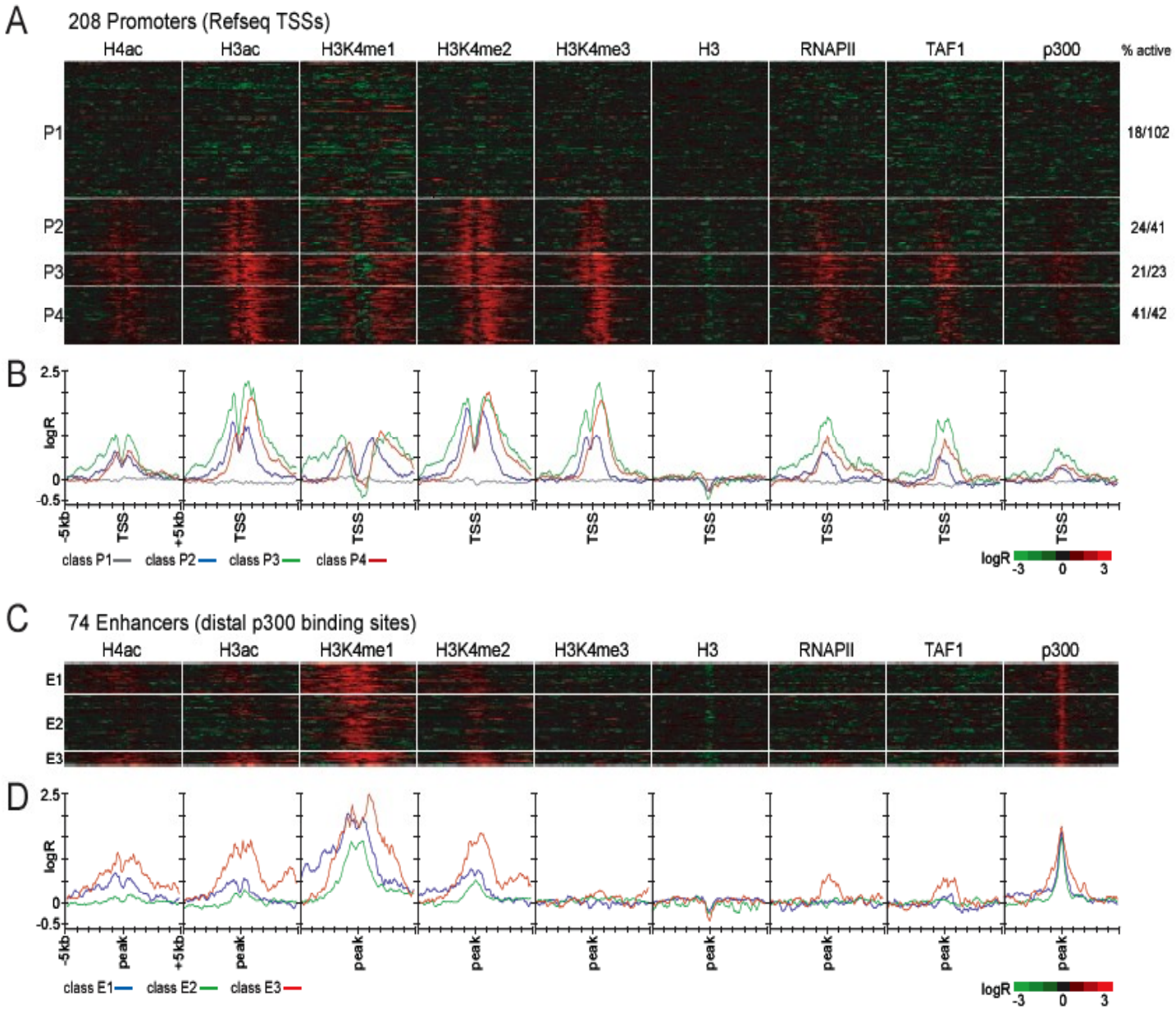
- **Promoters**
  - Basal transcriptional machinery (e.g. Pol II)
  - Histone state (e.g. H3K4 tri-methylation)
- **Enhancers**
  - Site-specific TFs (target >350)
  - Histone state (e.g. H3K4 mono-methylation)
  - Co-factors (e.g. p300)
- **Silencers and Insulators**
  - Silencers – (e.g. PcG)
  - Insulators - (e.g. Su(Hw), Mod(mdg4))

What samples to ChIP?

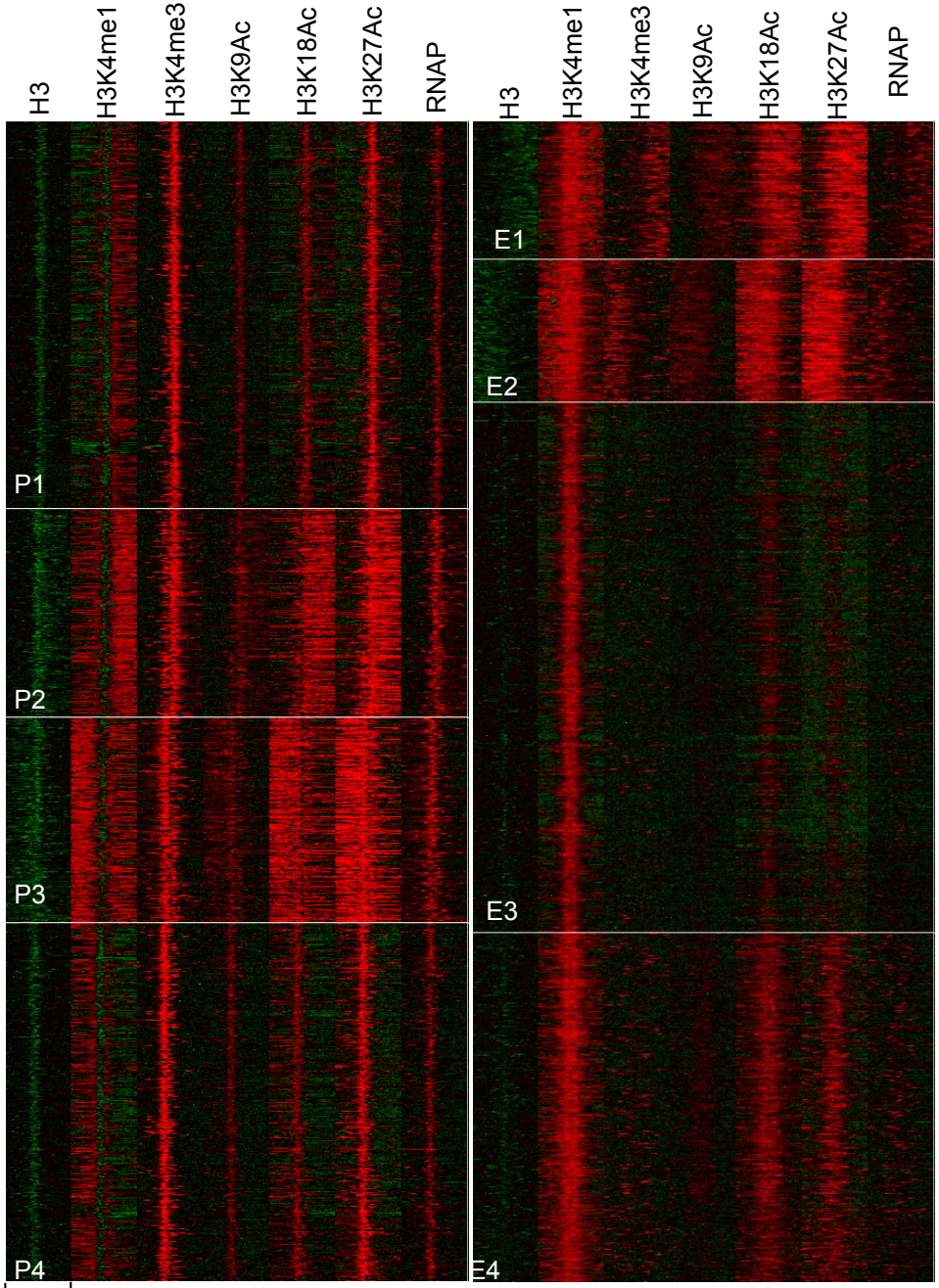


Data from Arbeitman et al. Science 2002

Preliminary data - Chromatin signatures (B Ren)



Heintzman et al. Nature Genetics 2007



Drosophila S2 Clusters

- Active Promoters (3997)
  - + H3K4me3
  - + H3K9Ac
  - + H3K18Ac
  - + H3K27Ac
  - + RNAP
  - - H3
  - - H3K4me1
- Enhancers (4242)
  - + H3K4me1*
  - + H3K18Ac
  - + H3K27Ac
  - - H3
  - - H3K4me3
  - - H3K9Ac
  - - RNAP

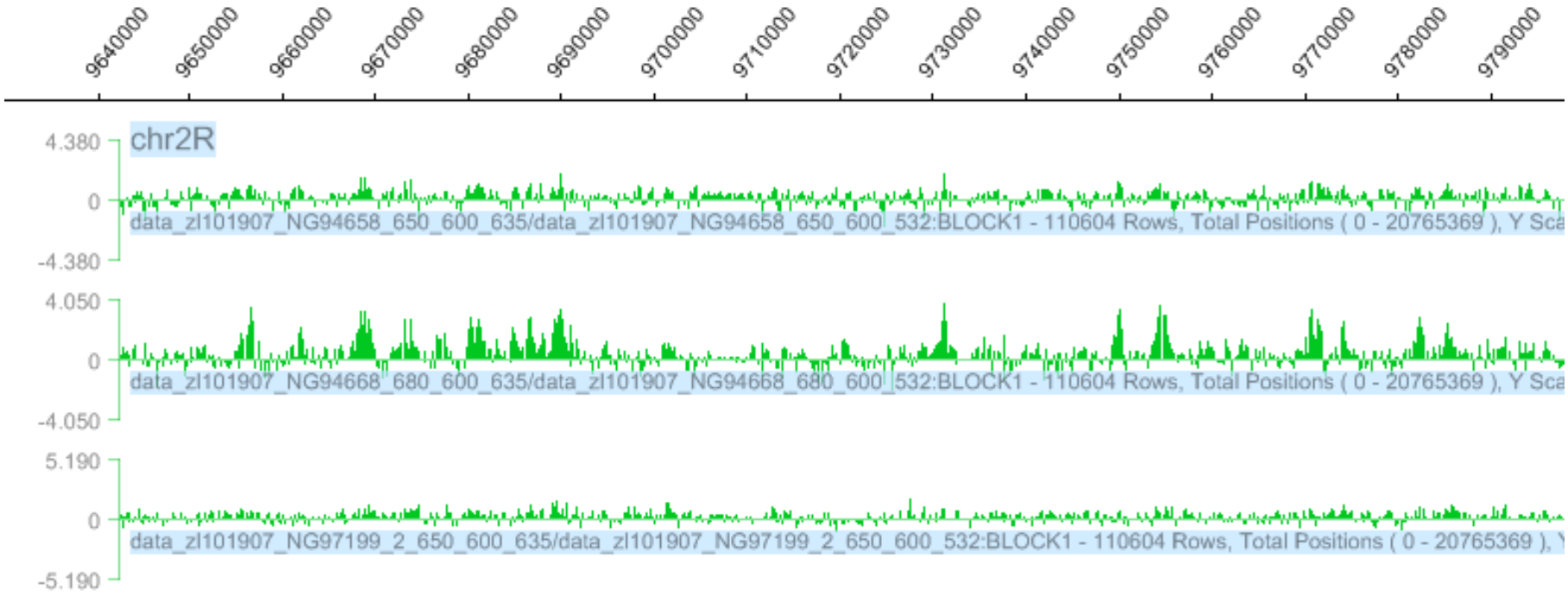
Sample Collections Aug-Nov 2007

Stages	*Amt. of Bio. Mat. (g)*	*Chromatin Equivalent*
0-4h	**0.994**	3300ug
4-8h	**0.159**	530ug
12-16h	**0.762**	2540ug
16-20h	**0.681**	2270ug
20-24h	**1.077**	3590ug
L1	**1.098**	3660ug
L2	**0.880**	2930ug
L3	**1.890**	6300ug
Pupae	**1.090**	3630ug
Female Adults	**0.757**	2520ug
Male Adults	**0.460**	1530ug

Histone Modifications
H3K4me1
H3K4me3
H3K9Ac
H3K9me3
H3K27Ac
H3K27me3
(PolII)

RNA Extractions (for the moment homogenized in TRIzol)

Date	*Sample*	*Amt of Bio. Material*
7.25.07	**Pupae**	**110.8mg**
9.10.07	**L3**	**135mg/98mg**
9.20.07	**0-4h**	**195mg/94mg**
9.21.07	**4-8H**	**329mg**
10.10.07	**20-24h**	**55mg**
10.11.07	**16-20h**	**110mg**



Top: ChIP using 100ug adult female chromatin (30mg Bio. Material) and 10ug H3K4Me3 antibody, DNA was amplified by LM-PCR and labeled with the Nimblegen protocol (end labeled random priming).

Middle: Same as above except using 300ug adult female chromatin.

Bottom: ChIP using 100ug adult female chromatin (30mg Bio. Material) and 10ug H3K4Me3 antibody, DNA was amplified by WGA2 and labeled with random priming.

ChIP milestones

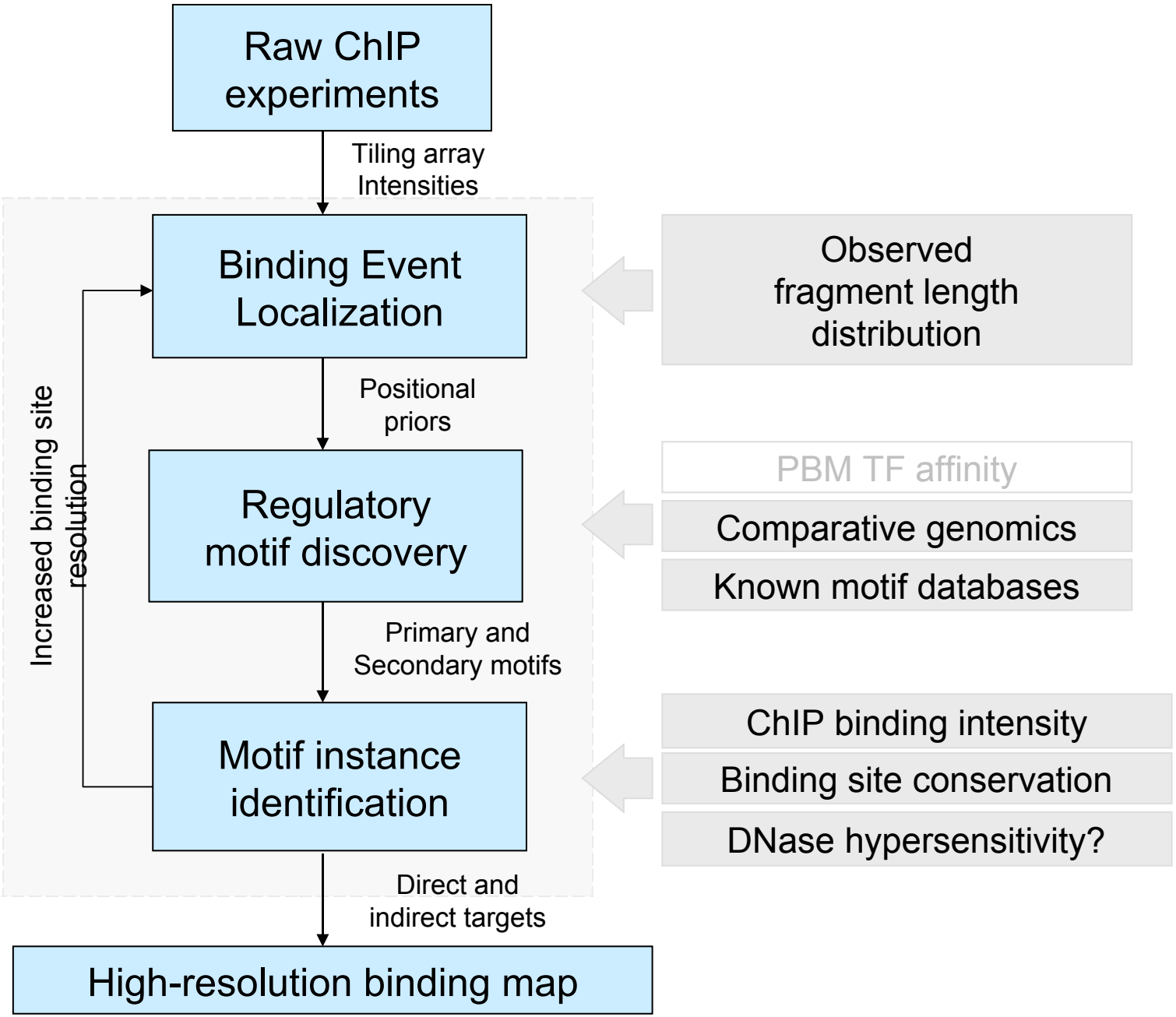
- **year 1 – Genome-wide scans with existing Abs to basal TF machinery and chromatin marks; 300 experiments total**
  - **Chromatin time course plus two Cell lines (S2, Kc) = 909 arrays (progress: 315 complete; 72-96 per week)**
- **year 2 – 1,000 total arrays (+700)**
- **year 3 – 2,000 total arrays (+1,000)**
- **year 4 – 3,500 total arrays (+1,500)**

Site-specific TFs

Technical validations:

- **Replicate experiments**
- **Independent Antibodies (when available)**
- **qPCR/Solexa**

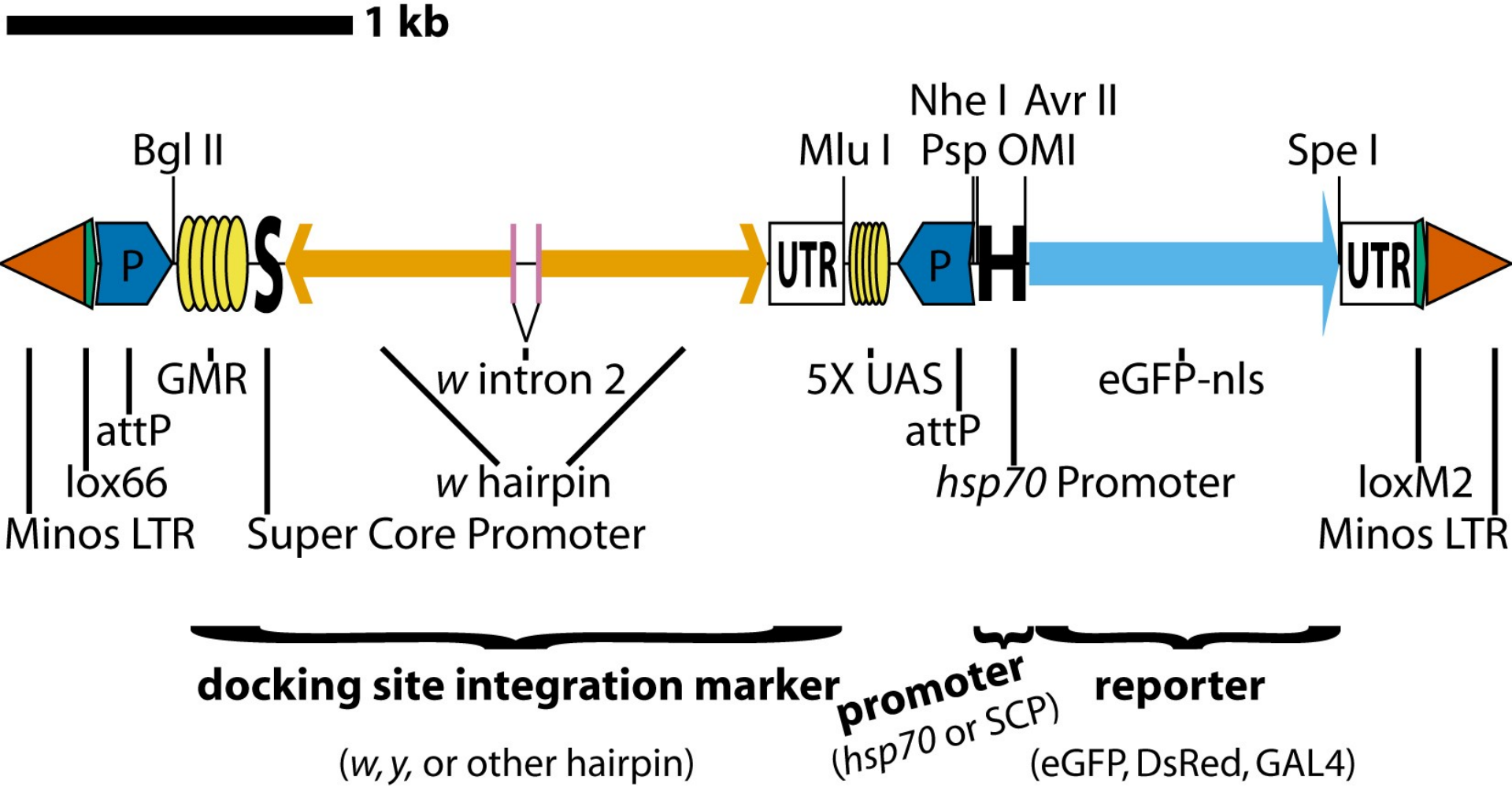
Regulatory motif discovery pipeline (M. Kellis)



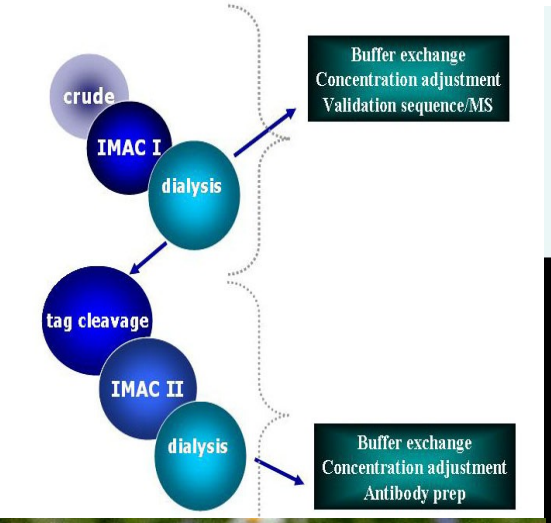
Validation milestones (J. Posakony)

- **year 1 – Establish criteria for choosing elements to validate, test and characterize in vivo reporter gene system, and train new personnel**
- **year 2 - Test 100 total elements each of enhancers and promoters (Posakony), 20 each of silencers and insulators (Russell)**
- **year 3 – Test 200 total elements each of enhancers and promoters, 40 each of silencers and insulators**
- **year 4 – Test 300 total elements each of enhancers and promoters, 60 each of silencers and insulators**

Reporter Construct Design



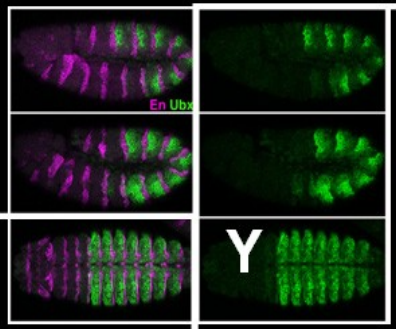
Protein/Ab Production



Phase I
Cell in situs
-staining?
-RNAi knockdown?

Phase II
Embryo in situs
- specific pattern?
- Correlation with RNA?

N



Phase III
Westerns
-predicted bands?
- correlation with RNA?
-RNAi knockdown?

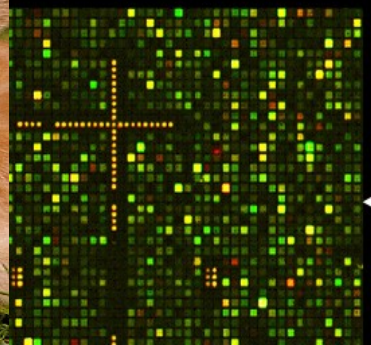
Y

N

Phase IV
Test array ChIP chip
-High signal to noise?
-Motif enrichment?

Y

N



Antibody production pipeline

- **Protein domains (~100a.a.) 35 antisera for 22 factors yielded 15 validated antibodies (43%) for 13 factors (59%)**
- **100 peptide based antibodies for 50 factors; 81 antibodies tested by microwestern (w/Rich Jones and Mark Ciaccio) 18/81 show specific bands in WB = 22%**
- **104 protein domains injected in December; 100 peptides representing 50 factors injected at beginning of Feb**
- **100+ domains have completed production phase, awaiting injection**

Antibody Production Milestones

Expectation that 20% of antibodies will be useful for ChIP

- **year 1 – produce 350 total antibodies**
  - **Progress (funding started 8/07): 339 injected with 100 more protein domains this month, 116 antisera received. Validated *in situ*/Western: 33/116 = 28%**
- **year 2 – 850 total antibodies produced (+500)**
- **year 3 – 1350 total antibodies produced (+500)**
- **year 4 – 1750 total antibodies produced (+400)**

Total proteins ~500 (2-3 domains each)
Total proteins that are ChIP validated ~350
(40-70% with multiple ChIP validated Abs)

Please help us prioritize Ab and ChIP experiments

- **Vote for your favorite transcription factor!!!**
  http://www.modencode.org/Vot

- **Send us your antibodies to test, validate and post ChIP data online!!! Nicolas Negre**
  nnegre@bsd.uchicago.edu