Dot blot validation of antibodies used in the ENCODE project:

Broad Institute ChIP-Seq Group

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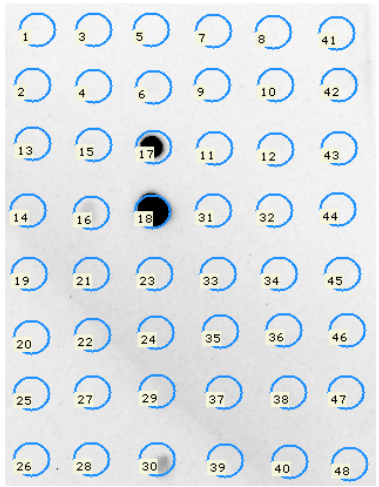
Protocol

- Dot Blot Fabrication:
  - Suitably modified peptides modeled on histone tails were obtained from Abcam.
  - Each peptide is added to Bio-Dot apparatus at 0.01 and 0.1 ug / 50uL total volume (BioRad)
  - Let sit on rocker 60 minutes.
  - Pull through with vacuum, wash once with 100uL TBS and twice with 200uL TBSTw.
  - Remove membrane from Bio-Dot and transfer to 10mL Pierce SuperBlock in tray.
  - Place on rocker for 30 minutes.
  - Dump off SuperBlock.
- Antibody hybridization and imaging:
  - Add Primary Antibody in 10mL total volume.
  - Place on rocker for 30 minutes.
  - Wash 3 x 5 minutes in TBSTw.
  - Add secondary antibody diluted in SuperBlock in 10mL total volume.
  - Place on rocker for 30 minutes.
  - Wash 3 x 15 minutes in TBSTw, 1 x 10 minutes in TBS.
  - Image using ECL and a FluorChem Peltier-cooled camera (Cell Biosciences)

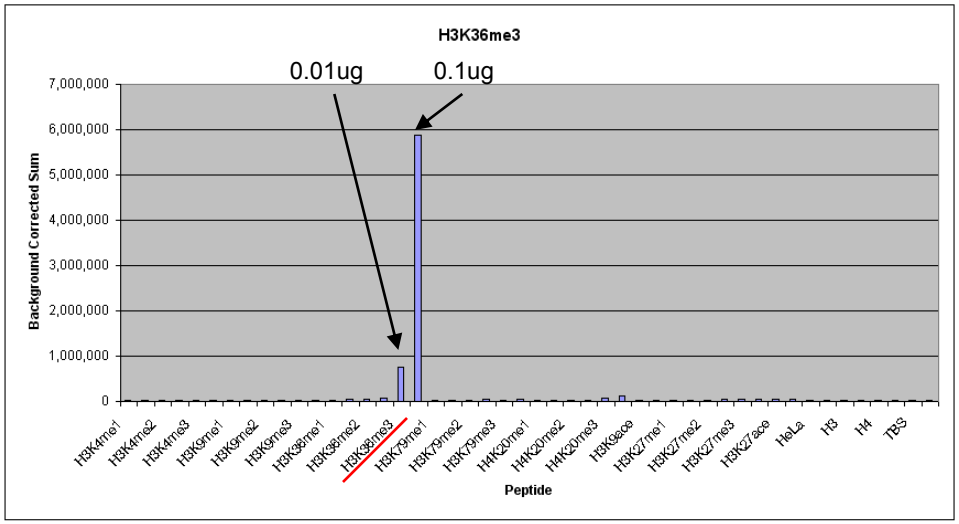
Antibody: Rabbit Polyclonal to Histone H3 (tri methyl K36)
Vendor: Abcam
Immunogen: Synthetic peptide conjugated to KLH derived from within residues 1 - 100 of Human Histone H3, tri methylated at K36.
Product Number: ab9050
Lot Number: 499302

- H3K36me3



Gel Setup

	1	2	3	4	5	6
A	K4m1 0.01ug	K4m2 0.01ug	K4m3 0.01ug	K9m1 0.01ug	K9m1 0.1ug	Hela 0.01ug
B	K4m1 0.1ug	K4m2 0.1ug	K4m3 0.1ug	K9m2 0.01ug	K9m2 0.1ug	Hela 0.1ug
C	K36m1 0.01ug	K36m2 0.01ug	K36m3 0.01ug	K9m3 0.01ug	K9m3 0.1ug	H3 0.01ug
D	K36m1 0.1ug	K36m2 0.1ug	K36m3 0.1ug	K9ace 0.01ug	K9ace 0.1ug	H3 0.1ug
E	K79m1 0.01ug	K79m2 0.01ug	K79m3 0.01ug	K27m1 0.01ug	K27m1 0.1ug	H4 0.01ug
F	K79m1 0.1ug	K79m2 0.1ug	K79m3 0.1ug	K27m2 0.01ug	K27m2 0.1ug	H4 0.1ug
G	K20m1 0.01ug	K20m2 0.01ug	K20m3 0.01ug	K27m3 0.01ug	K27m3 0.1ug	TBS
H	K20m1 0.1ug	K20m2 0.1ug	K20m3 0.1ug	K27ace 0.01ug	K27ace 0.1ug	TBS



Performed at Broad Institute

Test Date 4/13/2010
User MC

Primary Antibody H3K36me3
Vendor Abcam
Part # ab9050
Lot # 499302
Concentration 1 :1000
Incubation Time 60m

Secondary Antibody
Vendor USB #72560
Part # 72560
Lot # 123941
Concentration 1 :10,000
Incubation Time 30m

Develop Time 5m

Protocol
- Add each peptide into BioDot at above concentrations in 50uL total volume
- Let sit on rocker 60 minutes
- Pull through with vacuum, wash once with 100uL TBS and twice with 200uL TBSTw
- Remove membrane from BioDot and transfer to 10mL Pierce SuperBlock in tray
- Place on rocker for 30 minutes
- Dump off SuperBlock and add Primary Antibody in 10mL total volume
- Place on rocker for 60 minutes
- Wash 3 x 5 minutes in TBSTw
- Add secondary antibody diluted in SuperBlock in 10mL total volume
- Place on rocker for 30 minutes
- Wash 3 x 15 minutes in TBSTw, 1 x 10 minutes in TBS
- Develop in Fluorchem