BacMam-Enabled Cellular Assays Measuring Histone Posttranslational Modifications

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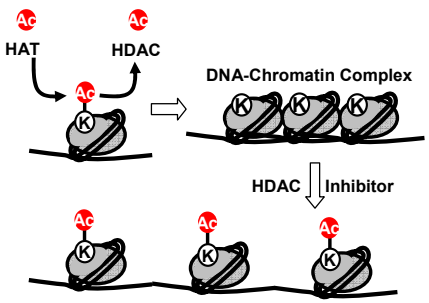
Introduction

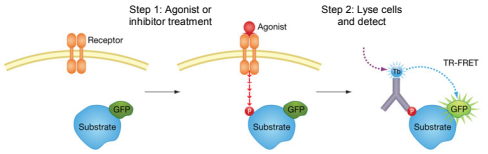
The eukaryotic nucleosome, composed of histones H2A, H2B, H3, and H4, regulates the structure of chromatin and consequently modulates gene transcription profiles in a concerted manner. Nucleosome function is directly regulated by a multitude of posttranslational modifications on amino-terminal tails of core histones, including acetylation, phosphorylation, methylation and ubiquitination. These modifications occur in a tightly regulated fashion, and affect numerous cellular processes including histone deposition, chromatin assembly, and chromosome condensation during both mitosis and meiosis. Therefore, a number of histone modifying enzymes have been identified as valuable targets for therapeutic intervention. The combination of Baculovirus-mediated gene delivery (BacMam) with LanthaScreen® cellular assay technology and measurements using the BioTek Synergy™ 4 Hybrid Multi-Mode Microplate Reader, enables a powerful platform for the analysis of target-specific posttranslational modifications of histones in the cell line of interest. Specifically, we have developed HTS-compatible cellular assays measuring acetylation of histone H3 at Lys9, phosphorylation of histone H3 at Ser10, and the ubiquitination status of histone H2B. These assays together with sensitive filter-based detection on the Synergy™ 4 represent a flexible method to measure the ability of compounds to affect histone posttranslational modifications in a HTS-compatible format.

Figure 1 - The Activity of HATs and HDACs Regulate Chromatin Structure and Transcription



Role of HDACs on chromatin structure and transcription. The balance between transcriptionally active and silent genetic loci is regulated by the activity of histone acetyltransferases (HATs) and histone deacetylases (HDACs). HDAC inhibitors such as Trichostatin A shift the equilibrium toward increased histone acetylation and increased transcription.

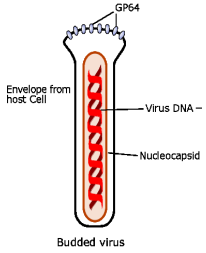
Figure 2 - LanthaScreen® Cellular Assay Schematic



Live Cells Expressing GFP-Histone H3 or GFP-H2B

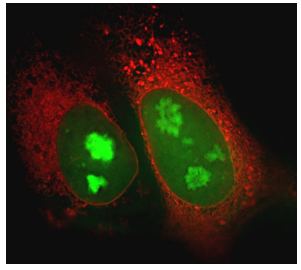
Description: LanthaScreen® cellular assays utilize genetic fusions of GFP with substrates known to be modified in a signal transduction pathway. GFP serves as a FRET acceptor for modification-specific Tb-labeled antibodies in a lysate-based immunoassay. Specific post-translational modifications are measured on a microplate reader as an increase in TR-FRET.

Figure 3 - Use of BacMam Delivery Method Enables Portable LanthaScreen® Cellular Assays



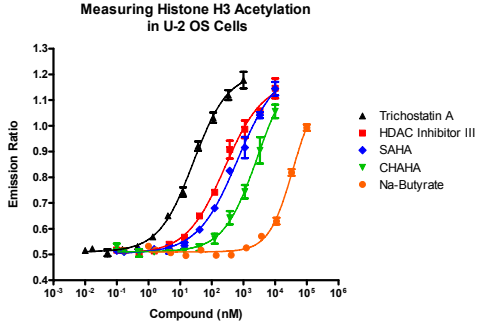
- **Baculovirus is an insect cell virus**
- **Non-replicating in mammalian cells**
- **Biosafety level 1**
- **High, CMV-driven expression**
- **Non-toxic to cells**
- **Easy to use; just add virus to cells**
- **Transient expression for 5 days**
- **Transduces hard-to-transfect cells**
  - **> Primary cells**
  - **> Stem cells**
- **Tolerates large inserts up to 38 kb**

Figure 4 - BacMam Enables Delivery of GFP-Histone Fusion Proteins in the Cell Line of Interest



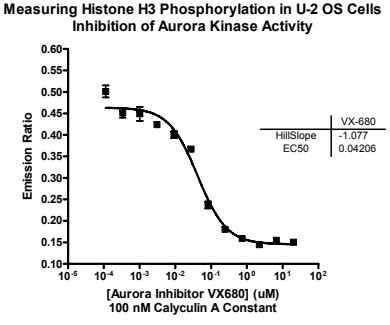
Left: HeLa cells were transduced with BacMam GFP-H3 and Invitrogen Organelle Lights™ ER-OFP. 24 hours post-transduction, cells were seeded on sterile coverslips and allowed to adhere overnight. Cells were then imaged on a DeltaVision fluorescence microscope using a 60X objective and a TRITC or GFP filter set. GFP-H3 is distinctly localized to the nucleus, indicative of proper functionality.

Figure 5 - BacMam-Enabled Assays Measuring Acetylation of Lysine 9 of GFP-Histone H3



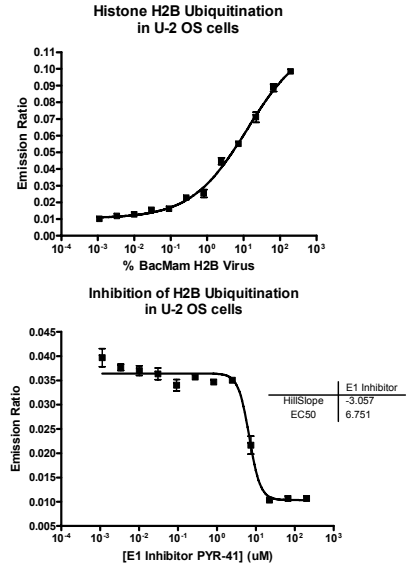
	Trichostatin A	HDAC Inh III	SAHA	CHAHA	Na-Butyrate
LogEC50	1.420	2.410	2.821	3.444	4.556
HillSlope	0.7944	0.7183	0.6813	0.8103	1.140
EC50	26.32	257.0	663.0	2778	35955

Figure 6 - Analysis of GFP-H3 Phospho-serine-10 Mediated by Aurora Kinase



Materials & Methods: **Figure 5:** U-2 OS cells were transduced by plating overnight in presence of BacMam GFP-H3 virus (directly into 384-well plates). 24 hours posttransduction, cells were stimulated with serial-diluted HDAC inhibitor and incubated for 3 hours. **Figure 6:** Cells were treated for 1 hour with serial diluted VX680 inhibitor prior to 1 hour treatment with calyculin A (phosphatase inhibitor). Cells were then aspirated and lysed by addition of lysis buffer containing LanthaScreen® Tb-anti-H3 [acetyl-lysine-9] antibody (top) or Tb-anti-H3 [pS10] antibody (bottom). Following a 2 hour equilibration at room temperature, TR-FRET signals were measured on a Synergy™ 4 Hybrid Multi-Mode Microplate Reader (BioTek Instruments, Winooski VT USA). LanthaScreen® Tb TR-FRET instrument setup: 340/30nm excitation filter, 495/10nm and 520/25nm emission filters, 400nm dichroic mirror, 100µsec delay, 200µsec integration and auto sensitivity selected. Error bars represent the average of 3 data points +/- S.E.

Figure 7 - Detection of Histone H2B Ubiquitination



Materials & Methods: Top: U-2 OS cells were transduced with serial-diluted BacMam GFP-H2B virus (directly into 384-well plates). Bottom: U-2 OS cells were transduced with a fixed concentration of BacMam GFP-H2B and immediately treated with E1 Inhibitor PYR-41, followed by 24 hour incubation. 24 hours posttransduction, cells were then aspirated and lysed by addition of lysis buffer containing LanthaScreen® Tb-anti-ubiquitin-FK2 antibody. Following a 2 hour equilibration at room temperature, TR-FRET signals were measured on a Synergy™ 4 Hybrid Multi-Mode Microplate Reader (BioTek Instruments, Winooski VT USA). LanthaScreen® Tb TR-FRET instrument setup: 340/30nm excitation filter, 495/10nm and 520/25nm emission filters, 400nm dichroic mirror, 100µsec delay, 200µsec integration and auto sensitivity selected. Error bars represent the average of 3 data points +/- S.E.

Results and Conclusions

- **The combination of BacMam delivery and LanthaScreen® technologies provides a HTS-compatible technology for measuring specific posttranslational modifications of histones.**
- **BacMam enables cellular assay development in the cell line of interest.**
- **A multitude of histone posttranslational modifications can be measured including phosphorylation, acetylation, and ubiquitination, demonstrating the flexibility of this technology.**

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