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Histone Methodology Development Report

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Aims

Histone protein modifications are an important epigenetic marker. When isolating histones from samples it is therefore essential that post translation modifications such as acetylation, methylation and phosphorylation are maintained. Isolation methods may influence the state of these modifications. The aim of this element of the work package was to develop protocols that would enable histone analysis from new collections of blood samples as well as stored lymphoblastoid cell lines (LCLs). Evaluation involved 2 optimisation stages

1. Optimisation of methodology for isolating histones from samples
2. Optimisation of use of two-dimensional liquid chromatography-tandem mass spectrometry (2D LC-MS/MS) to characterise histone modifications (Tian et al, 2012).

Methodologies

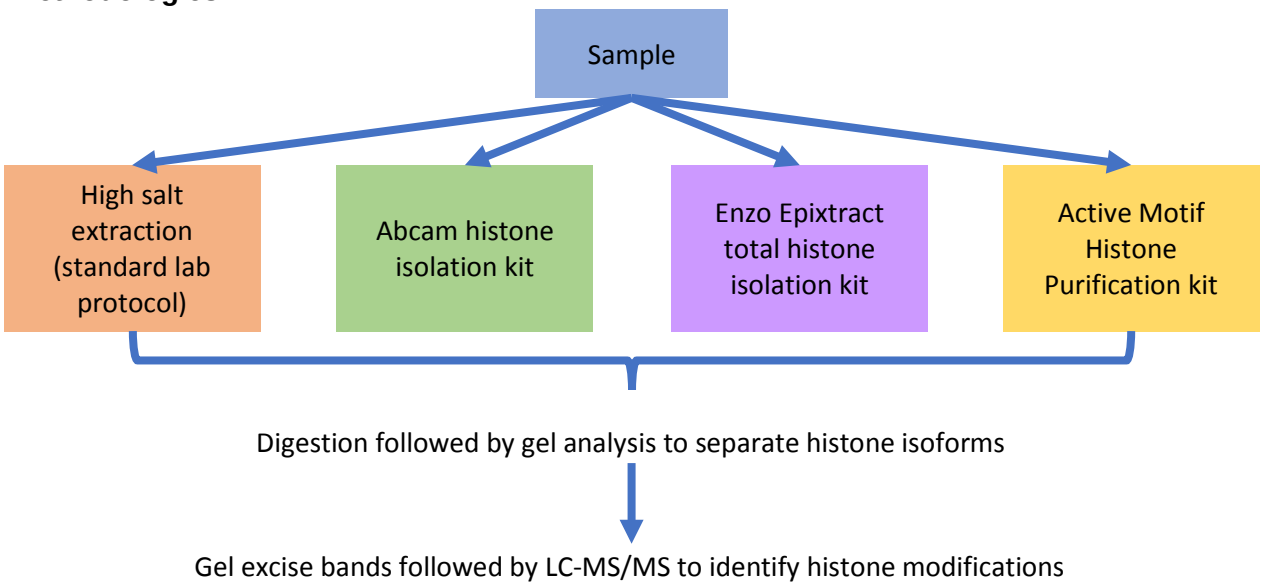

Figure 1: Workflow to assess histone isolation methods on post translational modification

The workflow to identify whether isolation method affects histone post translational modifications is depicted in figure 1. 4 methods were piloted to extract histones from LCLs (Table 1), 3 of these were commercial kits and 1 was a protocol based on the method published by Shechter (2007).

	METHOD			
	High Salt extraction (based on Shechter)	Abcam histone isolation kit[a]	Enzo Epixtract total histone isolation kit[b]	Active Motif Histone Purification kit[c]
Time taken for isolation	2 days	60-90 mins	60 mins	2 days (includes two overnight incubations)
Use of column	Non-column based	Non-column based	Non-column based	Column based
Recommended starting material	10^7 cells	10^5 cells (recommended minimum) or more than10^6 cells	10^5 cells (recommended minimum) or more than10^6 cells	10^8 cells
Yield expected	Highly variable	0.4 mg per 10^7 cells	0.4 mg per 10^7 cells	0.4-0.5 mg total core histones

Table 1: Methods used to extract histones from LCLs

There are 5 families of histones; H2A, H2B, H3 and H4 are known as core histones and H1 the linker histone. All are important for epigenetic research so the extracts were examined to determine if all 5 histone families could be detected.

To visualise if histone extraction was successful samples were separated on an acrylamide gel. This part of the procedure caused a substantial amount of technical difficulty which had not been anticipated. Technical advice was sought from 2 companies that supply gel systems, Bio-Rad and Invitrogen as well as from the School of Chemistry, University of Bristol. Once the method had been optimised it was found that the Abcam and Enzo kits had the highest concentration of histones and were able to extract all 4 core histones as well as the linker H1 (table 2).

	METHOD			
	High Salt extraction (based on Shechter)	Abcam histone isolation kit[a]	Enzo Epixtract total histone isolation kit[b]	Active Motif Histone Purification kit[c]
H1 (linker)		X	X	
H2A (core)	X	X	X	X
H2B (core)	X	X	X	X
H3 (core)	X	X	X	X
H4 (core)	X	X	X	X

Table 2: Ability of methods to isolate histone types, X = successful extraction.

Due to the technical challenges of the histone separation the workflow could not progress to analysis of histone modifications owing to time constraints.

Outcome

The more successful histone extraction methods were found to be 2 commercial kits, Abcam and Enzo. Further work is required to ascertain if extracting histones with these methods alters the post translational modification state.

References

Enhanced top-down characterization of histone post-translational modifications, Tian *et al* Genome Biology 13:R86 (2012)

Extraction Purification and Analysis of Histones, Shechter *et al* Nature Protocols 2(6): 1445-1457 (2007)

[a] Histone Extraction Kit (ab113476) from Abcam plc (http://www.abcam.com)

[b] EpiXtract™ Total Histone Extraction Kit (ENZ-45014) (http://www.enzolifesciences.com)

[c] Histone Purification Kit (40025 from Active Motif (htEvatp://www.activemotif.com)