Creation and Verification of HDAC4-GFP Lentiviral Construct

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Introduction

- Bones have two types of cells; osteoclasts which remove bone tissue and osteoblasts which generate new bone tissue.1,2
- The balance between osteoclast and osteoblast activity is crucial, because if there is more osteoclast activity then bone loss occurs resulting in diseases such as osteoporosis.3
- Osteoclast differentiation requires two factors: macrophage colony stimulating factor (M-CSF) and receptor activator of NF-kappaB ligand (RANKL).4

![Figure 1: Differentiation of osteoclast in bone marrow.](image)

- Besides factors that promote osteoclast differentiation, there are also negative factors such as histone deacetylases (HDACs) that inhibit osteoclast differentiation.3
- One mechanism by which HDACs are regulated is through their subcellular localization.1,5

Objectives

- Create a construct of HDAC4 whose expression is fused with a green fluorescent protein (GFP).
- HDAC4-GFP construct will be used to visualize HDAC4’s cellular location during osteoclast differentiation.

![Figure 2: Plasmid map of pLex-myc-HDAC4-GFP plasmid.](image)

Methods

Molecular cloning of HDAC4 was performed as described in Fig. 3. To verify the construct, a transfection was performed and the presence of GFP positive cells was determined using immunofluorescence microscopy. Additionally, polymerase chain reaction and western blot were performed to verify the presence and expression of HDAC4.

Results

- Sample nine and ten of HDAC4-GFP in Figure 3 shows three bands of the appropriate size as shown in Figure 2 indicating the presence of HDAC4.
- GFP pigments can be visualized in Figure 4 which support the presence of GFP being expressed from the clone.
- Additionally HDAC4 clone verified the presence of the appropriate size band by PCR as shown in Figure 5.
- Western blot provided evidence of HDAC4 as shown in Fig. 6.
- Thus, the results demonstrated that sample ten of HDAC4-GFP expresses HDAC4 fused to GFP.

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References