Plasmid construction and viral infection

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Abstract

The plasmids were constructed using standard methods; all sequences were verified by appropriate restriction digestion and/or sequencing. Human full-length *IGFBP2* cDNA from ASCs fused to a M2-Flag tag was produced with a standard PCR protocol. This sequence (Flag-*IGFBP2*) was subcloned into the pQCXIN retroviral vector with AgeI and BglII restriction sites. For viral infections, MSCs were plated overnight, then infected with retroviruses in the presence of polybrene (6 µg/mL, Sigma-Aldrich, St. Louis, MO, USA) for 12 h. After 48 h, infected cells were selected with 600 µg/mL G418 for 10 days.

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Protocol

Plasmid construction

Step 1.

The plasmids were constructed using standard methods; all sequences were verified by appropriate restriction digestion and/or sequencing.

Plasmid construction

Step 2.

Human full-length *IGFBP2* cDNA from ASCs fused to a M2-Flag tag was produced with a standard PCR protocol.

Plasmid construction

Step 3.

This sequence (Flag-*IGFBP2*) was subcloned into the pQCXIN retroviral vector with AgeI and BglII restriction sites.

viral infections

Step 4.

MSCs were plated overnight, then infected with retroviruses in the presence of polybrene (6 µg/mL, Sigma-Aldrich, St. Louis, MO, USA) for 12 h.

viral infections

Step 5.

After 48 h, infected cells were selected with 600 µg/mL G418 for 10 days.