Preparation of BMP Conditioned Medium (BMP-CM) Using AdBMPs in HCT116

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1. Plate HCT116 (a human colon cancer line; highly infectable by adenovirus) in T-25 flask with 7ml McCoy's 5A Medium containing 10% FCS; Grow the plated cells at 37C CO_2 incubator overnight **(e.g., ~12 to 15 hours, but not >24 hours) or for 2 hr to 4 hr prior to adenovirus infection**.
2. Add adenoviruses expressing BMPs (volume based on virus titers; typically, use 2-5ul virus stock at ~10^{12} pfu/ml for each T-25 flask). **It is important that cell confluence should be about 50% to 70% at the time of infection**.
3. Incubate for 4 to 12 hrs at 37^{o}C (**Note: Most viral infection should be done within the first couple of hours. However, overnight infection could maximize the gene transduction efficiency. So, longer incubation is desired if your viral titer is low. Conversely, shorter incubation is equally effective if high titers or more viruses are used. In fact, this is a preferred approach for some applications, in which gene expression needs to be synchronized!**).
4. Wash remaining/non-infecting adenovirus away with McCoy's 5A Medium 1 to 2 times.
5. Confirm infection efficiency (i.e., GFP signal) under fluorescence microscope at ~15 to 24 hours after infection (**Note: An ideal infection should exhibit 50% to 70% GFP positivity. If <30% positive, BMP production may be low, while >90% positive could lead to significant cytotoxicity and hence lower BMP production**).
6. Add **7 to 10 ml** of fresh McCoy's 5A medium or DMEM containing with 0.5% FCS for additional 24 to 36 hrs.
7. Harvest BMP containing medium (you can centrifuge at 4C briefly to remove any cell debris). The prepared BMP-CM can be used freshly or stored at 4^{0}C for 1-2 weeks (**Note: You can check the cells under fluorescence microscope at 24 hours after BMP-CM treatment to determine whether the conditioned medium is contaminated with any AdBMP virus**).
8. **NOTE: This protocol can be scaled up for BMP-CM production in T-75 flask if a larger quantity of CM is desired**.