

## HSC70/HSP40 OVEREXPRESSION IN VIRAL VECTOR-PRODUCING SF9 CELLS IMPACTS AAV PRODUCTIVITY AND PRODUCT QUALITY

Juan Aponte-Ubillus, Drug Substance Technologies, BioMarin Pharmaceutical Inc  
Juan.aponte@bmrn.com

Jim Liu, Drug Substance Technologies, BioMarin Pharmaceutical Inc

Daniel Barajas, Drug Substance Technologies, BioMarin Pharmaceutical Inc

Joseph Peltier, Innovation and External Partnership, BioMarin Pharmaceutical Inc

Javier Femenia, Drug Substance Technologies, BioMarin Pharmaceutical Inc

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Cytosolic heat shock proteins such as HSC70 and HSP40 play an important role in protein folding and cell stress relief, making them potential targets for cell line engineering in production cell factories. We hypothesized that increasing amounts of these key chaperones in *Sf9* cells during AAV vector production might lead to improved recombinant vector productivity and/or product quality, conceivably by increasing the availability of active structural (VP1-3) and non-structural (small and large Rep) proteins and by ameliorating possible impacts of proteotoxic stress.

HSC70 and HSP40 overexpression in *Sf9* cells was provided by inclusion of expression cassettes in recombinant baculovirus (BV) already designed for AAV vector production, and results were compared against a condition overexpressing the innocuous GFP based on a similar cassette configuration. A preliminary evaluation using low and high BV multiplicity of infection suggested HSC70/HSP40 expression can lead to a modest improvement in vector genome titer; however, the extent of the impact is likely influenced by the cell culture production conditions. A follow-up study evaluated HSC70/HSP40 expression during the production of two AAV serotypes using different culture temperatures and feed supplements. Results suggested additional HSP40 expression led to increases in vector genome titer and in the percentage of genome-containing capsids. These purified capsids also showed slightly better transduction capacity as measured by an in-vitro assay. Moreover, additional HSC70 expression led to consistent reduction of DNA impurities normally present inside AAV capsids. In addition, these purified capsids showed a weak increase in transduction efficiency for one of the serotypes evaluated, that interestingly correlated with a reduction on AAV VP1 levels. Altogether, we concluded that HSC70 and HSP40 overexpression might be used as a strategy to improve AAV productivity and/or product quality in the insect cell platform.