

Enhanced lentiviral production through rational design of mammalian host cells

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Development of lentiviral-based therapeutics is hindered by high costs of cGMP manufacturing, a particular concern for *in vivo* delivery where high titres and volumes are typically required. The key cost component of viral-vector manufacturing is production titre, which is typically several log-orders lower for lentiviral vectors than non-enveloped vectors such as rAd or rAAV. Efforts to increase lentiviral production have largely focused on optimisation and scale-up of transient transfection-based processes. Conversely, little attention has been paid to the optimisation of the mammalian host cell, with nearly all reported processes relying on HEK293T derivatives. We hypothesised that HEK293T cells were not necessarily optimal for lentiviral production and have embarked on a rational design process to establish new cell lines with enhanced manufacturing properties. We identified 130 cellular factors active in the late phase of the lentiviral life cycle that are potentially relevant to lentiviral vector production. We evaluated the effect of siRNA knock-down of these factors (3 days production, 2e5 cells/well, 100nM siRNA/well in quadruplicate) on lentiviral vector production titre (3 days transduction, 5e4 cells/well). We identified 9 host factors, the knockdown of which significantly increased lentiviral production by 1.4 to 2.1-fold (threshold: >2 SD over control, $p < 0.05$, power >80%). CRISPR/Cas9-mediated genetic disruption of these host factors has yielded novel cell lines that support 2-fold greater lentiviral production following transient transfection of producer plasmids. We anticipate that combinatorial disruption will yield further lines with even greater ability to support higher production titres; offering reduced manufacturing costs and thus increasing the speed of clinical development.