# INFLUENCE OF CULTIVATION TEMPERATURE ON pDNA PRODUCTION BY *E. coli* DH5α

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*Key words: pDNA, temperature, thermo-induction*

**Introduction.** Recent progress of plasmid DNA (pDNA) biopharmaceuticals in clinical trials, foresees a high demand of pDNA in the short-term (1). Meeting such demand in an economically feasible basis, is mandatory for its successful introduction in the market, which relies greatly on increasing pDNA yields in *E. coli*. An attractive and effective strategy to accomplish such goal is thermo-induction. It consists on a simple temperature up-shift, commonly from 30 to 42 °C, and is based on an increase of replication rates of pUC-derived plasmids when subjected to temperatures higher than 30 °C (2). However, increasing cultivation temperature above 37 °C have deleterious effects in *E. coli* growth. Thus, it is important to identify the best growth and production temperatures in recombinant *E. coli* in order to define a thermo-induction pDNA production process.

In this work, the effect of cultivation temperature on growth and pDNA production performance of *E. coli* DH5α transformed with a mumps virus vaccine model plasmid (pHN) containing a pUC replication origin (3), was evaluated.

**Methods.** Shake flask cultivations of *E. coli* DH5α pHN were carried out at constant temperatures of 30, 35, 37, 40, 42 and 45 °C. Cells were grown in mineral media with 5 g/L glucose, pH of 7.2 and shaken at 200 rpm. Biomass concentration was estimated from 600 nm optical density measurements. Glucose and acetate were determined with enzymatic and HPLC methods, respectively (4). pDNA was isolated using Qiagen´s miniprep kit and quantified by UV-spectrophotometry. pDNA topologies were separated by agarose gel electrophoresis, and the content of supercoiled pDNA (SCF) was determined by image densitometry. All conditions were evaluated by duplicate.

**Results.** Increasing cultivation temperature resulted in a progressive reduction of biomass on glucose yield ($Y_{xs}$), whereas specific growth rate ($\mu$) reached a plateau between 35 and 40 °C, possibly due to balance between augmented enzymatic activities and pDNA metabolic burden; at temperatures above 40 °C $\mu$ decreased (Fig1a). Glucose specific consumption rate ($q_s$) increased between 30 and 40 °C. Acetate concentration increased above 37 °C, more importantly at temperatures above 42 °C (Fig1 b), where drastic reductions in $Y_{xs}$, $\mu$, Specific pDNA yield ($Y_{px}$), and productivity ($q_p$) ocurred. The highest pDNA concentration was attained at 37 °C. SCF was not importantly affected by temperature and was close to 80%. (Fig1 c). $Y_{px}$ and $q_p$ increased up to 5 times when increasing the temperature from 30 to 42 °C.

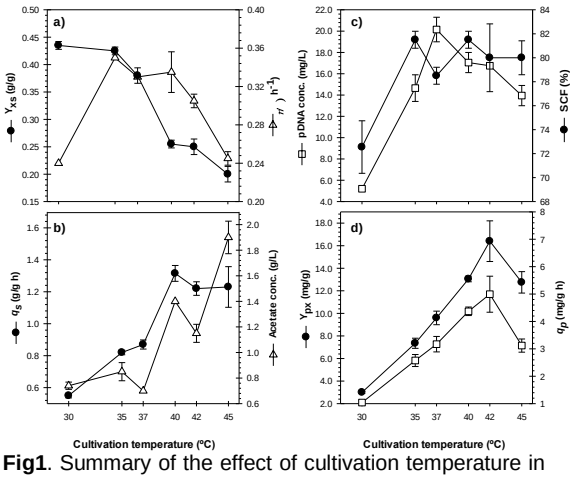


**Fig1**. Summary of the effect of cultivation temperature in (a) $Y_{xs}$, $\mu$, (b) $q_s$, acetate concentration, (c) pDNA concentration, SCF, (d)$Y_{px}$, and $q_p$.

**Conclusions.** Based on $Y_{xs}$ and $\mu$, the best growth temperature for *E. coli* DH5α pHN was 35 °C, whereas 42 °C was the best temperature for supercoiled pDNA production according to $Y_{px}$, $q_p$, and SCF.

**Acknowledgements**. This project was funded by CONACyT grant 183911, DGAPA grant IN 203212 and PROMEP grant 10828.

**References.**

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