**Introduction**

Metabolic acidosis occurs when the body has accumulated an excess of acid and lacks sufficient bicarbonate to effectively neutralize the effects of the acid. Metabolic acidosis can be a mild symptom associated with starvation, a high protein diet, or a gastrointestinal disorder such as constipation or diarrhea. Alternatively, it can indicate a more serious problem such as the lack of insulin or a defect in function of the liver, lung, heart, or kidney. Acidosis can also be caused by conditions such as chronic renal failure, liver failure, or severe diarrhea. Lactic acid build up in the blood due to heart failure, shock, or cancer may also induce metabolic acidosis.

Metabolic acidosis decreases the pH of the body by increasing the amount of acid and reducing the amount of bicarbonate. This can lead to a decrease in the activity of certain enzymes, which can affect the body's ability to maintain homeostasis. The pH of the blood is maintained within a narrow range, typically between 7.35 and 7.45, to ensure proper functioning of enzymes and other biological processes. Metabolic acidosis can be a result of a decrease in bicarbonate levels or an increase in acidity, which can be caused by various factors such as kidney disease, liver failure, or certain medications.

**Methods & Results**

**Cloning**

The pcDNA3.1-βG vector is designed for high-level, constitutive expression in a variety of mammalian cell lines. The vector contains a pH-responsive increase in glutamine metabolism, which can be modulated by the addition of 0.5 mg/ml of Dox or 50 ng/ml of tetracycline.

**Cell Selection**

The Tet-off system in the 8C cells contains the tTA protein that binds to the tet-responsive element (TRE) only in the absence of Dox. In high concentrations, Dox will bind to the tTA protein, inhibit it from binding to the TRE, and transcription ceases. In the absence of Dox, tTA will bind to the TRE and transcription occurs.

**Selection of Stable Cell Lines**

All cells were treated with 0.8 mg/ml of hygromycin. The cells that incorporated the plasmid DNA were resistant to the toxic effects of this drug, whereas cells that did not incorporate the plasmid underwent apoptosis after 7 days of selection. Surviving cell colonies were selectively removed with cloning rings. 24 clones were chosen, placed into 12 well plates and grown with 0.2 mg/ml G418, 0.8 mg/ml hygromycin and 50 mM doxycycline.

**Screening Clonal Lines for ζ-Crystallin**

A Western blot was performed to determine which clonal lines contained the ζ-crystallin construct. Clonal lines I-6, II-4, and II-7 were chosen for their ability to respond to Dox and were subjected to Dox treatment.

**Screening Clonal Lines for the Presence of β-Globin (βG)**

A Northern blot was performed to determine if the chosen clone contained the βG-globin cDNA. The chosen clone was then subjected to Dox treatment and screened with a βG-globin specific antibody.

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