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Dear Student,

In this issue of the journal we offer you three research articles authored by your fellow students of the Hostos Community College.

# Galileo Galilei: The Falling Bodies Experiment: Determination of Acceleration Due to Gravity

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Galileo Galilei stated that the acceleration of a free falling body is independent of its mass as well as that all objects fall with the same rate. This project aims at verifying Galileo’s prediction regarding free falling objects. We hypothesized that all objects fall at the same rate. In our experiment we used five objects with different masses and different shapes. We found that as the mass increases the rate of falling increases too. All objects travel the same distance and the object with the greatest mass took about 0.4398 s to fall, while the object with the smallest mass took about 0.4448 s to fall. Thus, we concluded that Galileo’s theory can be used only within a range of validity; he did not consider air resistance.

*Keywords—free fall, mass, velocity, acceleration, rate, air resistance.*

## INTRODUCTION

A Free falling object is an object that falls only under the influence of gravity (see Fig.1) [1]. Any object that is being acted upon only by the force of gravity is said to be in a state of free fall [1]. Galileo introduced the concept of free fall. His classic experiments led to the finding that all objects free fall at the same rate, regardless of their mass [2]. Galileo dropped balls of different mass from the Leaning Tower of Pisa to help support his ideas [2]. However, when the experiment finished Galileo was surprised with his results; he found that the heavy ball hit the ground first, but only because of air resistance [3].

Air resistance also called drag force is the opposition to the relative motion of an object through the air. Drag forces act opposite to the oncoming velocity flow [3]. One of the equations that describes the motion of a freely falling body is:  $y = v_0t - \frac{1}{2}gt^2$  [3]. When we choose the free falling body to be released from rest and we choose the coordinate of the object to be at the origin, the equation becomes:  $s = \frac{1}{2}gt^2$ , where “s” is the distance the ball travels, and “t” is the time that the ball takes to travel. In this experiment in order to find “g” the equation can be defined as:  $g = 2s/t^2$ .

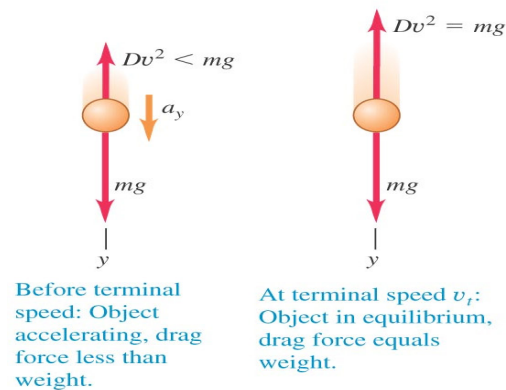
## OBJECTIVES

To determine acceleration due to gravity (g) using 5 different objects.

## HYPOTESIES

All objects fall at the same rate.

(a) Free-body diagrams for falling with air drag



(b) A skydiver falling at terminal speed



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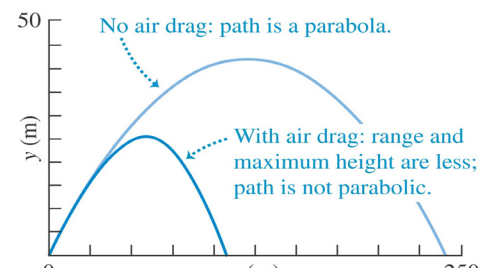


Figure 1. (a) Illustrate the changes resulting from air resistance, and (b) Examples of a falling body and projectile motion with and without air resistance [6].

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Figure 2. Photogate [5].

## RESULTS

Table 1.

Time Measurements and Acceleration due to Gravitational Force ( $g$ ) determination

$s = 1 \text{ m}$ ;  $\Delta s = 0.001 \text{ m}$ ;  $\Delta t = 0.1 \text{ ms}$ ;  $g_{\text{std}} = 9.802 \text{ m/s}^2$

Object	Mass (g)	$t_{\text{avg}}$ (s) ( $\text{m/s}^2$ )	$g_{\text{avg}}$ ( $\text{m/s}^2$ )	$\Delta g$	% error
Object 1	67.2060	0.4442	10.14	0.01	3.41
Object 2	28.4609	0.4452	10.09	0.01	2.94
Object 3	8.5908	0.4475	9.99	0.01	1.89
Object 4	8.3736	0.4474	9.99	0.01	1.94
Object 5	6.2471	0.4493	9.91	0.01	1.07

EXAMPLE OF CALCULATIONS:

## APPARATUS

- Free fall apparatus (see fig. 3).
- Balance.
- Five different objects: big ball, medium ball, small ball, small screw, big screw.
- Digital timer.
- Catcher.
- Photogate switch.
- Plumb.
- Electromagnet Launcher.
- Digital timer.

## EXPERIMENT AND DESIGN

- Set up the equipment (free fall apparatus).
- Make sure that the apparatus is balanced; the object that is thrown should pass through the photogate (see Fig.2).
- The photogate should be placed at 1m of distance from the maximum falling distance.
- Weigh the mass of the five objects, record the results.
- After the free fall apparatus is balanced, replace the plumb with the first object.
- Set the free fall switch on the digital timer to "RELEASE."
- The electromagnet will release the object.
- When the ball passes through the photogate, the timer will display the time.
- Record the time shown in the digital timer.
- Repeat the procedure 100 times for each object.
- Calculate the average time, and determine the value of  $g$ .



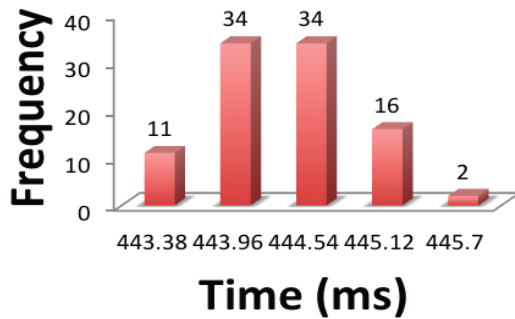
Figure 3. Free fall apparatus [4].

$$\%error_i = \frac{g_{avg,i} - g_{std}}{g_{std}} \times 100 \quad (3)$$

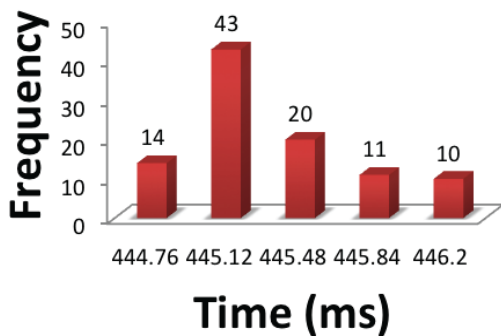
$$\%error_i = \frac{10.14 - 9.802}{9.802} \times 100 = 3.41\%$$

where  $g_{avg,i}$ ,  $\Delta g_i$ ,  $g_{std}$ , and  $\% error$  stand for average acceleration due to gravity, its uncertainty, and standard value in New York, and the percentage error, respectively.

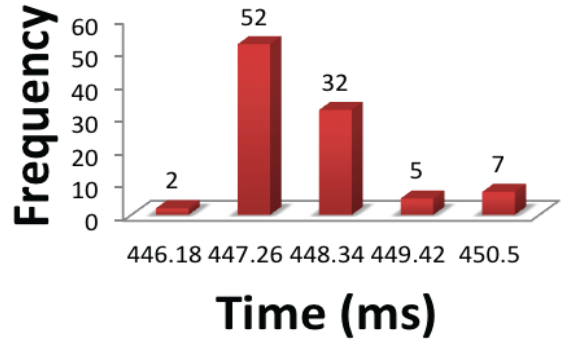
**Histogram for Time (Object 1)**



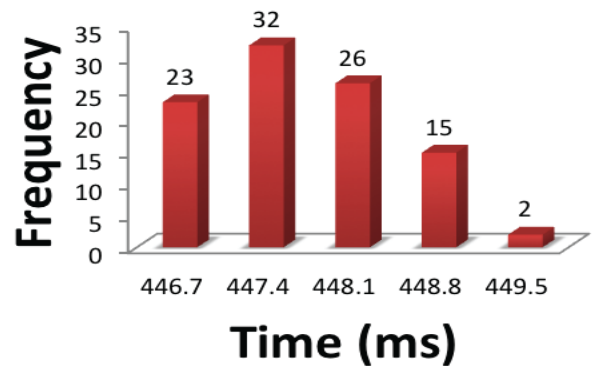
**Histogram for Time (Object 2)**



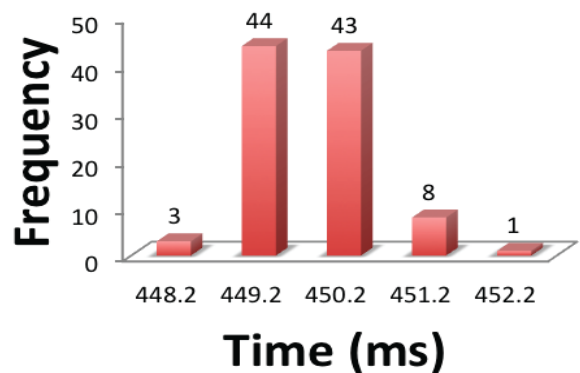
**Histogram for Time (Object 3)**



**Histogram for Time (Object 4)**



**Histogram for Time (Object 5)**



**Figure 4.** Time's histograms for objects 1 to 5.

## **DISCUSSION**

According to our hypothesis different objects will fall at the same rate, independently of its masses and shape. However, our results showed something different. As we were performing the lab we used five objects with different shapes and masses to test our hypothesis. After we recorded one hundred measurements of the time that each object took to travel one meter, we calculated the average value of the acceleration of each object using equation number 1. Our results do not support our hypothesis (see Table 1). Our results show that as the mass and the shape of an object change, its acceleration (g) will also change. For example, our experiment acceleration for the object with the largest mass was 10.14 m/s<sup>2</sup> while the object with the smallest mass had an experimental acceleration of 9.91 m/s<sup>2</sup> (see table 1). In addition, the standard acceleration for New York is 9.802 m/s<sup>2</sup>; the object that has the experimental acceleration closest to this value is the object with the smallest mass. According to our results shown on Table 1, as the mass of the object increased and the shape of the object changed our percentage errors also increased. Our percentage errors change as the mass and the shape of the object change because of air resistance. Any free falling object that is not within a surrounding vacuum will be exposed to air resistance.

## **CONCLUSION**

We concluded that the rate of a free falling body will always depend on the mass and shape of the body because of air resistance. Our calculations differ from our hypothesis; however, in order to have results that agree with our hypothesis we must complete our experiment in a vacuum.

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## Physics experience

# A Roller Coaster Experience

## How Much Height to Loop the Loop and Conservation of Mechanical Energy

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The law of conservation of energy states that “in a given process, the kinetic energy, potential energy, and internal energy of a system may all change, but the sum of those changes is always zero.” This statement says that energy is neither created nor destroyed. Energy is transformed from one type to another. This experiment attempts to simulate a frictionless environment in which all of the potential energy of a system will be converted into kinetic energy, which is a combination of translational and rotational energy. This task will be accomplished by predicting the initial drop height of the steel ball and comparing it to the actual height required for the ball to successfully pass through the loop. The actual height was experimentally determined to be roughly 1.5 times greater than the theoretical calculation. As a result it was concluded that the mechanical energy of the system was not conserved; an average of 65% of the initial potential was dissipated.

### Introduction

The goal of this experiment is to determine how much of a system’s initial potential energy will be converted into kinetic energy. Potential energy ( $U$ ) is energy associated with an objects position. It is not dependent on the path that it followed, only on the difference between the final and initial position. Another important aspect of potential energy is that it is a shared property. “The potential energy increases if the earth stays fixed and the body moves upward (away from the earth), it also increases if the body stays fixed and the earth moves away from it (1). Kinetic energy ( $K$ ) is the energy of an object associated with its velocity ( $v$ ), or how fast its displacement is changing relative to time. Kinetic energy is a result of the total work done on an object by all forces. In physics, work ( $W$ ) is defined as a force ( $F$ ) exerted on an object during its total displacement ( $s$ )<sup>1</sup>. Since displacement and force are both vector quantities work can be stated using the following relationship relationship:

$$W = F \cdot s \quad (\text{Eq. 1})$$

Since this is a scalar product we can also state that work is equivalent to:

$$W = Fs \cos \theta \quad (\text{Eq. 2})$$

Where  $\Theta$  is the angle relative to the direction of an object’s displacement. As previously stated, potential energy is the energy associated with an objects position only; since gravity is a constant downward force the work will be positive if an object is moving downward and the work will be negative if the object is moving upward (depending on the chosen reference co-ordinate system). Potential energy can be stated mathematically as the work done by gravity over a certain distance:

$$\Delta U_g = W_g = (mg(y_2 - y_1)) \cos(180^\circ) \quad (\text{Eq. 3})$$

$$\Delta U_g = W_g = -(mgy_2 - mgy_1) \quad (\text{Eq. 4})$$

$$\Delta U_g = W_g = -(U_{2g} - U_{1g}) \quad (\text{Eq. 5})$$

$$\Delta U_g = W_g = U_{1g} - U_{2g} \quad (\text{Eq. 6})$$

Where  $m$  is the mass of the object;  $y_2$  and  $y_1$  are position 2 and position 1, respectively.

Since potential energy is the change of an object’s position, and kinetic energy is related to how fast the object changes position we can derive a relationship between the two; stating that the change in potential energy is equal to the change in kinetic energy, or vice versa:

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$$\Delta U_g = \Delta K \quad (Eq. 7)$$

$$U_{1,g} - U_{2,g} = K_2 - K_1 \quad (Eq. 8)$$

$$U_{1,g} + K_1 = K_2 + U_{2,g} \quad (Eq. 9)$$

This equation is only valid if gravity is the only force acting on an object. If all of the potential energy will be converted into kinetic energy the minimum required height to release the steel ball so that it will successfully travel through the loop can be predicted. Since a ball is spherical; there are two types of kinetic energy associated with a rolling object; translational kinetic energy ( $K_t$ ), which is the motion of a body through space:

$$(K_t) = \frac{1}{2}mv^2 \quad (Eq. 10)$$

and rotational kinetic energy ( $K_r$ ), which is the motion of a body through its axis:

$$K_r = \frac{1}{2}I\omega^2 \quad (Eq. 11)$$

Where  $I$  is the moment of inertia with respect to center of mass; and  $\omega$  is the rotational velocity. The moment of inertia for a solid sphere with respect to center of mass is  $I = \frac{2}{5}mr^2$ , where  $m$  is the mass, and  $r$  is the radius of the sphere. Rotational velocity can be related

$$v = r\omega \quad (Eq. 12)$$

Therefore;

$$\omega = \frac{v}{r} \quad (Eq. 13)$$

To determine the theoretical height necessary to successfully travel through the loop, the conservation of energy equation must be represented in terms of all the variables that have been provided. The conservation of energy equation is as follows:

$$U_1 + K_{t1} + K_{r1} = U_2 + K_{t2} + K_{r2} \quad (Eq. 14)$$

At point A the ball will be at rest, therefore, there is no kinetic energy. The potential energy at point B is twice the height of the radius (see Fig. 1); therefore,  $K_{t1}$ , and  $K_{r1}$  are 0; and  $U_2 = mg^2R$ .

$$U_1 = U_2 + K_{t2} + K_{r2} \quad (Eq. 15)$$

Newton's second law of motion which states that when "a net external force acts on a body, the body accelerates. The direction of acceleration is the same as the direction of the net force. The mass of the body times the acceleration of the body equals the net force vector" (1) (Young 117). In order for the object to successfully pass through the loop it must overcome the constant downward force of the gravity. This will happen when the normal force  $N$  is zero, and the magnitude of centripetal acceleration ( $a_y$ ) is equal to the magnitude of

Making the appropriate substitutions:

$$mgy_1 = mg2R + \frac{1}{2}mv^2 + \frac{1}{2}I\omega^2 \quad (Eq. 16)$$

Where  $R$  is the radius of the loop, and  $r$  is the radius of the ball. The moment of inertia with respect to center of mass  $I = \frac{2}{5}mr^2$ , and angular velocity  $\omega = \frac{v}{r}$ , thus:

$$mgy_1 = mg2R + \frac{1}{2}mv^2 + \frac{1}{2}\left(\frac{2}{5}mr^2\left(\frac{v}{r}\right)^2\right) \quad (Eq. 17)$$

After cancelling like terms:

$$gy_1 = g2R + \frac{1}{2}v^2 + \frac{1}{5}v^2 \quad (Eq. 18)$$

The theoretical height will be a function of  $R$  so a relationship between  $v$ , and  $R$  must be made. It can be obtained through a free-body diagram and Newton's second law of motion. Fig. 1 and Fig. 2. will provide a visual aid:

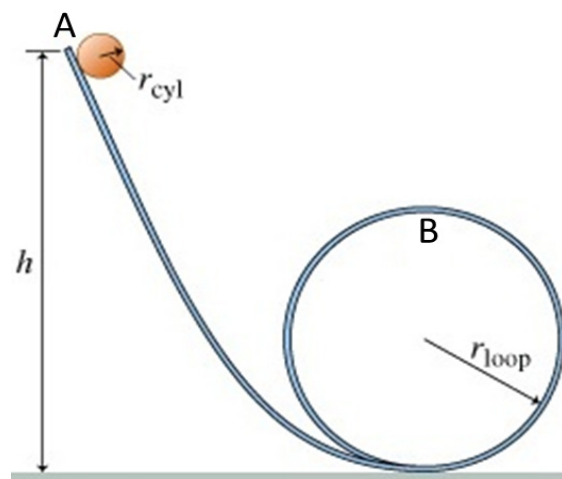


Figure 1 Visual aid for the experimental apparatus (3)

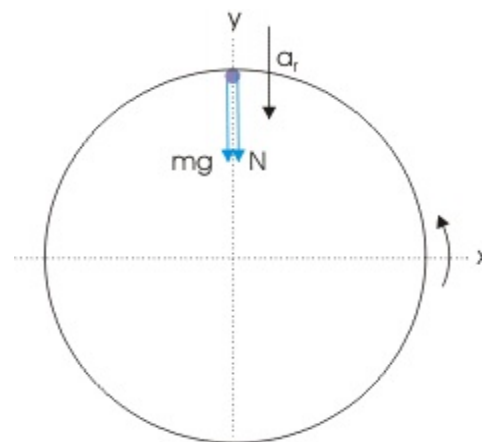


Figure 2 Free-body diagram of the steel ball at point B (3)

acceleration due to gravity ( $g$ ). At point B there are only forces acting in  $y$ -direction. Therefore Newton's second law can be expressed as:

$$\Sigma F_y = ma_y \tag{Eq.19}$$

There are only two forces acting on the body at point B, the normal force, and the force due to gravity ( $mg$ ); they are both pointing in the negative direction (according to the chosen reference system):

$$-N - mg = -ma_y \tag{Eq.20}$$

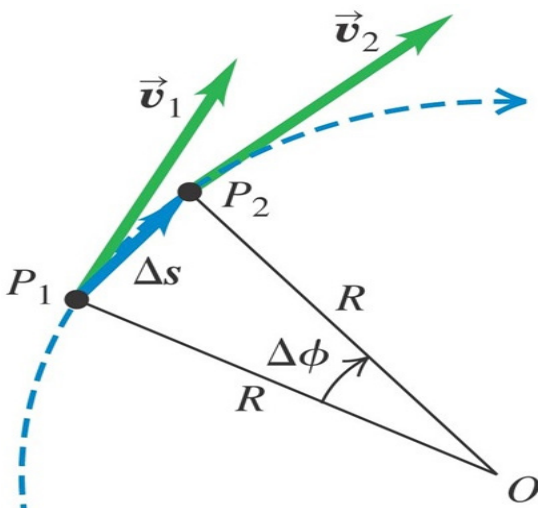
The normal force is zero:

$$-mg = -ma_y \tag{Eq.21}$$

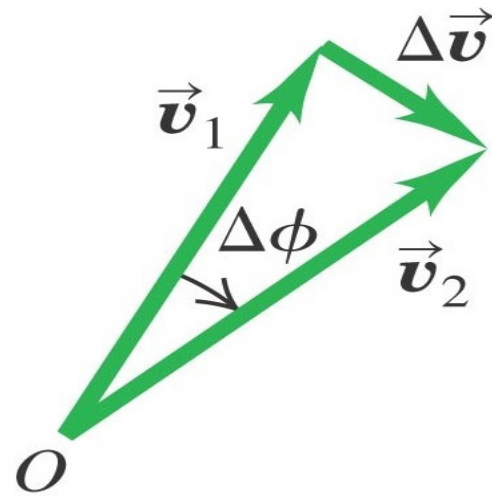
Canceling like terms:

$$a_y = g \tag{eq.22}$$

A relationship must be made between  $v$ ,  $R$ , and  $g$ . When a particle is moving along the curved path, there is always a non-zero component of acceleration pointing inward from the circle (1).



**Figure 3** The magnitude and direction of velocity along the curved path (1).



**Figure 4.** Change of velocity along the curved path (1).

Fig. 3 and Fig.4 show the motion of an object as it travels along a curved path. Translating the velocity vectors juxtapose, the resultant vector is the change of the velocity vectors. Translating these two vectors also created a similar triangle to the one in Fig. 3. It is clear that:

$$\frac{|\Delta v|}{v_1} = \frac{\Delta s}{R} \tag{Eq.23}$$

$$|\Delta v| = \left(\frac{v_1}{R}\right) \Delta s \tag{Eq.24}$$

Since the average acceleration is equivalent to:

$$a_{av} = \frac{|\Delta v|}{\Delta t} \tag{Eq.25}$$

Replacing  $\Delta v$  with the relationship derived in Eq. 23, results in

$$a_{av} = \frac{\Delta s}{\Delta t} \left(\frac{v_1}{R}\right) \tag{Eq.26}$$

The magnitude of the instantaneous acceleration is the limit as  $\Delta t$  tends to 0, where  $v_1$  and  $R$  are constants:

$$a = \left(\frac{v_1}{R}\right) \lim_{\Delta t \rightarrow 0} \frac{\Delta s}{\Delta t} \tag{Eq.27}$$

The limit as  $\Delta t$  tends to 0 of  $\frac{\Delta s}{\Delta t}$  is the velocity at point  $P_1$ :

$$a = \left(\frac{v_1}{R}\right) v_1 = \frac{v^2}{R} \tag{Eq.28}$$

placing Eq. 22 in Eq. 28:

$$g = \frac{v^2}{R} \tag{Eq.29}$$



solving for  $v^2$ :

$$gR = v^2 \quad (\text{Eq. 30})$$

placing Eq. 30 into Eq. 18:

$$gy_1 = g2R + \frac{1}{2}gR + \frac{1}{5}gR \quad (\text{Eq. 31})$$

cancelling like terms on both sides of the equation:

$$y_1 = 2R + \frac{1}{2}R + \frac{1}{5}R \quad (\text{Eq. 32})$$

and finally obtaining:

$$y_1 = \frac{27}{10}R \quad (\text{Eq. 33})$$

Eq. 33 shows that the minimum height required for the object to successfully pass through the loop is  $2.7R$ . The reason why this concept is so important is that it will help prove our hypothesis that all of the potential energy will be converted into kinetic energy. If all of the potential energy remains within the steel ball then a height of  $2.7R$  will result in a successful “loop-the-loop.”



**Figure 5.** “Loop-the-Loop” apparatus (5). This is similar apparatus. This is not the apparatus that was used.

## MATERIALS AND APPARATUS:

1. Pre-fabricated “Loop-the-Loop” (Fig. 5.)
2. Steel ball (spherical)
3. Digital timer with two photogates (Fig. 6.)
4. Meter stick
5. Vernier caliper
6. Measuring tape
7. 6 cm ruler

## Experimental Procedure:

### Procedure A:

A pre-fabricated construction of an inclined plane, covered with foam insulation (attempting to simulate a frictionless surface) that connects to a loop was set up. The same size loop was used for each of the trials. The radius was measured using a measuring tape and a 6 cm ruler. The steel ball was dropped from  $2.7R$  ( $29.16 \pm 0.01$  cm) along the inclined plane. The height was increased until the steel ball successfully traveled through the loop.



**Figure 6.** Photogate

### Procedure B:

To determine how much potential energy is converted into kinetic energy the ball was released from 60.5 cm for each trial, attempting to release it from the exact same position with zero initial velocity. At the end of the loop two photogates arranged to accurately measure the time it takes to travel 4.2cm. 500 trials were performed; two of which were erroneous. The average time of the 498 trials was used to calculate the average velocity; the average velocity was used to determine

the kinetic energy. The difference between the mechanical energy of the system at position A and B is the amount of mechanical that was dissipated from the system.

**Experimental Results:**

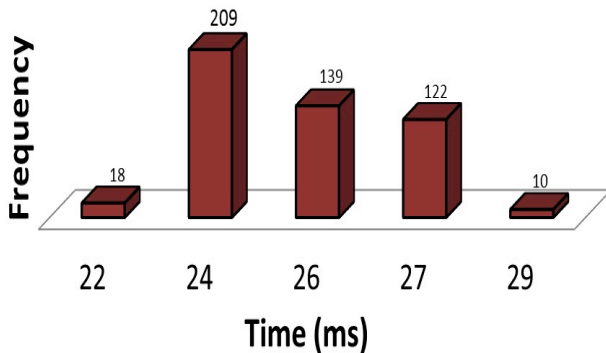
**OBJECTIVE:**

1. To determine the minimum drop height required for the steel ball to successfully pass through the loop.
2. To determine how much potential energy is converted into a marble's kinetic energy.

The theoretical height required for the steel ball to successfully pass through the loop was determined to be  $29.2 \pm 0.4$  cm which was calculated from Eq. 33 and the measured radius of  $10.8 \pm 0.2$  cm. The experimental height was measured at  $44.5 \pm 0.1$  cm.

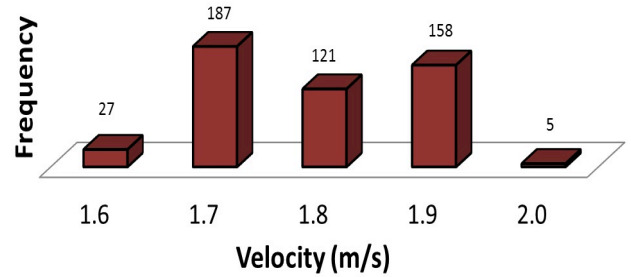
For the second procedure the steel ball was released from a height of  $60.5 \pm 0.1$  cm, it rolled down the inclined plane, through the loop, and then through two photogates separated by a distance of  $4.2 \pm 0.1$  cm. The average time of the 498 trials is  $25 \pm 2$  ms (milliseconds). The average calculated velocity is  $1.7 \pm 0.1$  m/s. Fig. 7 shows the frequency of the measured times for procedure B, Fig. 8 shows the frequency of the calculated velocities.

**Time Histogram**



**Figure 7.** Frequency of measured time for 498 trials.

**Velocity Histogram**



**Figure 8.** Frequency of calculated velocity for the 498 trials.

**Table 1:** Measurements and Calculations

Dimensions	Measurements/Calculations	Error Propagation /Sensitivity	Standard deviation
Minimum Height (cm)	44.5	$\pm 0.1$	N/A
Loop Diameter (cm)	21.6	$\pm 0.1$	N/A
Loop Radius (cm)	10.8	$\pm 0.2$	N/A
Mass of steel ball (g)	8.38	$\pm 0.01$	N/A
Time <sub>(avg)</sub> (ms)	25	$\pm 0.1$	$\pm 2$
Velocity <sub>(avg)</sub> (m/s)	1.7	$\pm 0.1$	$\pm 0.1$
$K_t$ (avg) (J)	0.012	$\pm 0.001$	$\pm 0.002$
$K_r$ (avg) (J)	0.0050	$\pm 0.0006$	$\pm 0.0006$
$U_{grav1}$ (J)	0.050	$\pm 0.002$	N/A
$E_{1(avg)}$ (J)	0.050	$\pm 0.002$	N/A
$E_{2(avg)}$ (J)	0.017	$\pm 0.002$	$\pm 0.002$
$\Delta E_{(avg)}$ (J)	-0.033	$\pm 0.002$	$\pm 0.002$

**Figure 9.** Overall results and measurements for trial data

See appendix A for error propagation calculations. J is the SI unit for Joules which represents energy.  $E_{1(avg)}$  is the mechanical energy of the system at position one;  $E_{2(avg)}$  is the mechanical energy of the system at position two;  $\Delta E_{(avg)}$  is the average difference of mechanical energy between position A and position B.

Sensitivity is used for the objects measured with a ruler or Vernier caliper. Standard deviation is used for time since it is a greater uncertainty for the sensitivity of the photogates. Error Propagation is used for the values calculated from time measurements since they are equal to the standard deviation.

$E_{1(avg)}$  is equivalent to  $U_{grav1}$  since there is only potential energy at position 1.  $E_{2(avg)}$  is the sum of translational and rotational energy at position 2.  $\Delta E_{(avg)}$  is the dissipated mechanical energy.

The subscript average indicates that it was calculated

**Discussion:**

The outcome of procedure A was not as hypothesized. The minimum height required for the ball to successfully pass through the loop is  $44.5 \pm 0.1$  cm, which is roughly 1.5 times greater than calculated.

The only explanation for this is that some of the potential energy was converted into energy other than that of the steel ball. Although the specific types of energy cannot be stated it can be speculated that the dissipated energy was converted to friction and vibrational energy. For example, a foam type of insulation was used as the surface for the marble to travel across; therefore some of it was lost to friction. The marble also generates sound while moving, so some of it is converted to sound, since sound is vibration it is another type of kinetic energy. The momentum of the ball also causes the track to wobble and vibrate, which means it is converted to kinetic energy of the track.

The energy dissipated from the system is due to the work of non-conservative forces ( $W_{nc}$ ). A non-conservative force is a force that dissipates energy from the system and that energy cannot be recovered by reversing the motion or in any other way (1) it is dissipated from the system permanently. A conservative force is “a force that offers a two-way conversion between kinetic and potential energy”<sup>1</sup> (Young 228). To determine the amount of mechanical energy dissipated from the system, the work of non-conservative forces must be considered. The Energy of the system at position A (see Fig. 1.) is represented by the following relationship:

$$E_{sys1} = U_1 + K_{t1} + K_{r1} \quad (Eq. 34)$$

The translational and rotational energy of the system at position 1 is zero. The energy of the system at position B (see Fig. 1.) is represented by the following relationship:

$$E_{sys2} = U_2 + K_{t2} + K_{r2} \quad (Eq. 35)$$

The potential energy at position B is zero. The work of non-conservative forces (which is represented by E in Table 1) can be represented by the following equation:

$$\Delta E_{sys} = E_{sys2} - E_{sys1} \quad (Eq. 36)$$

at position B, Eq. 36 can be represented as the following:

$$W_{nc} = K_{t2} + K_{r2} - U_1 \quad (Eq. 37)$$

Table 1 shows that there is a loss of mechanical energy, which is representative of the amount of energy that has been dissipated from the system. The slowest velocity of the experiment is  $1.4 \pm 0.1$  m/s; the fastest velocity is  $2.0 \pm 0.1$  m/s. The work of non-conservative forces ranges from  $-0.026 \pm 0.002$  J to  $0.037 \pm 0.002$  J, with an average of  $-0.032 \pm 0.002$ . The amount of energy that remained within the system ranges from 51.87% to 75.41%, with an average of 65.12%. Although we tried to minimize the external forces in the experiment 35% of the mechanical energy was dissipated from the system; therefore, the potential energy was not completely converted into kinetic energy of the steel ball. This was proven before procedure B began. If all of the potential energy was converted into kinetic energy then the minimum height would be  $2.7R$ . Since the ball was not able to travel through the loop from  $30 \pm 2$  cm, the potential energy at position A was converted into other types of energy; the amount of energy can be determined using Eq. 37. Since some of the potential energy of the marble is dissipated as heat, and other kinetic energy the minimum height must be greater than the height we calculated.

**Conclusion:**

Only 65% of the total mechanical energy was conserved which does not support our hypothesis that all of the potential energy will be converted into kinetic energy. This experiment can be improved by trying different surfaces on which the ball rolls; stabilizing the inclined plane and the loop to lower the translational motion of the track; using a magnet to release the ball from the exact same height on each attempt; or attempting to stabilize the ball as it rolls down the track so that less energy is converted into translational energy of the track.

**APPENDIX A**

**Error Propagation Calculations**

**Minimum height:**

$$h_0 = \frac{27}{10} R$$

$$\Delta h_0 = \partial f(R) = \frac{\partial h_0}{\partial R} \Delta R$$

$$\Delta h_0 = \frac{27}{10} \Delta R$$

**Velocity:**

$$x_1 - x_0 = v_{ox}t$$

$$\frac{x_1 - x_0}{t} = v_{ox}$$

$$v_{ox} = \frac{s}{t}$$

$$v_x = \frac{s}{t} = f(s, t)$$

$$\Delta v_x = dv_x = \partial f(s, t) = \left(\frac{\partial v_x}{\partial s}\right)_t \Delta s + \left(\frac{\partial v_x}{\partial t}\right)_s \Delta t$$

$$\Delta v_x = \frac{\Delta s}{t} + \frac{s\Delta t}{t^2}$$

**Translational kinetic energy:**

$$K_t = \frac{1}{2}mv_x^2 = f(m, v_x)$$

$$\Delta K_t = dK_t = \partial f(m, v_x) = \left(\frac{\partial K_t}{\partial m}\right)_{v_x} \Delta m + \left(\frac{\partial K_t}{\partial v_x}\right)_m \Delta v_x$$

$$\Delta K_t = \left(\frac{v_x^2}{2}\right) \Delta m + mv_x \Delta v_x$$

**Rotational kinetic energy:**

$$K_r = \frac{1}{2}I\omega^2$$

$$K_r = \frac{1}{2}\left(\frac{2}{5}mr^2\left(\frac{v_x}{r}\right)^2\right)$$

$$K_r = \frac{1}{5}mv_x^2$$

$$\Delta K_r = dK_r = \partial f(m, v_x) = \left(\frac{\partial K_r}{\partial m}\right)_{v_x} \Delta m + \left(\frac{\partial K_r}{\partial v_x}\right)_m \Delta v_x$$

$$\Delta K_t = \frac{v_x^2 \Delta m}{5} + \frac{2mv_x \Delta v_x}{5}$$

**Potential energy:**

g is a constant; therefore there the error propagation with respect to g is zero in this equation

$$U = mgh$$

$$\Delta U = \partial f(m, h) = \left(\frac{\partial U}{\partial m}\right)_h \Delta m + \left(\frac{\partial U}{\partial h}\right)_m \Delta h$$

$$\Delta U = gh\Delta m + gm\Delta h$$

**Work of non conservative forces:**

$$W_{nc} = K_{t2} + K_{r2} - U_1$$

$$W_{nc} = \frac{1}{2}mv^2 + \frac{1}{2}I\omega^2 - mgh$$

$$W_{nc} = \frac{1}{2}mv^2 + \frac{1}{2}\left(\frac{2}{5}mr^2\left(\frac{v_x}{r}\right)^2\right) - mgh$$

$$W_{nc} = \frac{1}{2}mv^2 + \frac{1}{5}mv_x^2 - mgh$$

$$W_{nc} = \frac{7}{10}mv_x^2 - mgh$$

$$\Delta W_{nc} = dW_{nc} = \partial f(m, v_x, h) = \left(\frac{\partial W_{nc}}{\partial m}\right)_{v_x, h} \Delta m + \left(\frac{\partial W_{nc}}{\partial v_x}\right)_{m, h} \Delta v_x + \left(\frac{\partial W_{nc}}{\partial h}\right)_{m, v_x} \Delta h$$

$$\Delta W_{nc} = \left(\frac{7v_x^2}{10} + gh\right) \Delta m + \left(\frac{7mv}{5}\right) \Delta v_x + mg\Delta h$$

**Calculations****Average value ( $\mu$ )**

$$\mu = \frac{1}{n} \sum_{i=1}^n x_i$$

where n is a number of trials and x is the value for each trial.

**Standard deviation ( $\sigma$ )**

$$\sigma = \sqrt{\frac{1}{n-1} \sum_{i=1}^n (x_i - \mu)^2}$$

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5. Digital Timer with Photogates. N.d. Photograph. [Http://www.bmelabandscience.com](http://www.bmelabandscience.com). Web. 17 May 2013. <[http://www.bmelabandscience.com/store/index.php?-main\\_page=product\\_info&products\\_id=177](http://www.bmelabandscience.com/store/index.php?-main_page=product_info&products_id=177)>.

# Isolation and Purification of CpCdc13A.

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Telomere-associated protein Cdc13 plays an important role in telomere maintenance. This work describes isolation and purification of one of the Cdc13 protein, Cdc13A, from yeast *Candida parapsilosis*.

## Introduction

Telomeres are located at the very end of eukaryotic chromosomes. Telomeres are complex structures composed of the repetitive chromosomal double-stranded and single-stranded DNA, RNA and many DNA associated proteins. Telomeres' functions are very important for maintaining a healthy cell. They protect the ends of chromosomes from degradation. If cells divide without telomeres, they would lose the end of their chromosomes and the genetic information they contain (1, 4). Cdc13 is a single-strand telomeric DNA binding protein, which plays a role in regulation of telomeres length (12). Recently, it was found that some *Candida* strains may carry variants of the Cdc13 protein, which were termed Cdc13A and Cdc13B (3). The main focus of this laboratory project was to express and purify recombinant Cdc13A protein from a fungi *Candida parapsilosis*. In the future, purified CpCdc13A preparation will be used to learn about DNA-binding properties of this protein

## Materials & Methods

### Bacterial Transformation

*E. coli* BL21 codon+ competent cells were thawed on ice. 45  $\mu$ l of competent cells were distributed into two tubes. 2  $\mu$ l of 50  $\mu$ g/ $\mu$ l pSMT3CpCdc13A and pSMT3 plasmids were distributed into each tube. Tubes were placed on ice for 30 minutes. After incubation on ice, tubes were placed into 42°C for 45 seconds and were further incubated on ice for two minutes for recovery. 450  $\mu$ l of LB medium was added to each tube, which were further incubated at 37°C water bath for one hour. Out of this transformation reaction, 50  $\mu$ l were placed on two LB plates supplemented with 50  $\mu$ g/ml of kanamycin.

### Protein Induction & Preparation of Protein Lysate

Three colonies of transformants obtained in the previous step (see above) were inoculated in 20 ml of LB medium supplemented with 50  $\mu$ g/ml kanamycin, and cultures were incubated at 37°C overnight. 15 ml of

of the overnight culture was inoculated into the 1L of LB medium supplemented with 50  $\mu$ g/ml kanamycin and was placed in the shaker at 37°C to grow until the optical density OD<sub>600</sub> of the culture reached 0.5. This process took two to three hours. The protein expression was induced by adding 200 mM IPTG up to the final concentration of 0.1 mM and 100% EtOH up to final concentration of 2%. Cultures were further incubated at 16°C overnight. Cultures were collected by centrifugation at 5000 rpm for 10 minutes. Pellet was re-suspended in 20 ml of buffer E (50 mM Tris-HCl, 250 mM NaCl, 10% glycerol) supplemented with protease inhibitors (1 mM PMSF, 1  $\mu$ g/ $\mu$ l pepstatin, 1  $\mu$ g/ $\mu$ l leupeptin). Cells were lysed by addition of lysozyme to the final concentration of 0.2 mg/ml and incubated on ice for 30 minutes. 10% Triton was added to the final concentration of 0.1% and cell suspensions were incubated on ice for 15 minutes. Cells were sonicated by using sonifier 250 set up at 80 % duty cycle for 10 minutes at 4°C. Sonicated cell suspensions were centrifuged at 32000 rpm for one hour at 4°C, in order to separate total protein from cells debris. Supernatant containing protein extract was collected, frozen in liquid nitrogen, and kept at -80°C until the next step.

### Protein Purification

Protein extract CpCdc13A was thawed on ice. Ni-NTA resin was equilibrated by washing it three times with buffer E (50mM Tris-HCl pH 7.5, 250 mM NaCl, and 10% glycerol pH 7.5). Protein extract was mixed with washed Ni-NTA beads and incubated at 4°C with rotation for 30 minutes, in order to induce the protein binding to resin. After that, the resin was washed sequentially with buffer E, buffer E supplemented with 25 mM imidazole, and buffer E supplemented with 100 mM imidazole. Eluate from the last wash was collected in three fraction of 1.4 ml each. CpCdc13A was eluted from the column with buffer E supplement with 300 mM imidazole and collected in 0.5 ml fractions.

### Preparation of Protein Gels

To separate individual proteins in a protein mix, 10% SDS-Polyacrylamide gels were used. For preparation

of SDS-acrylamide separating gel, 4 ml of water, 3.3 ml of 30% acrylamide mix (29:1), 2.5 ml 1.5 M TRIS pH 8.8, 0.1 ml 10% SDS, 0.1 ml 10% APS and 6  $\mu$ l of TEMED were mixed together and poured into prepared glass sandwich, (BioRad minigel apparatus) and allowed to polymerize for 30 minutes. The stacking gel was prepared by mixing 6.8 ml of water, 1.7 ml of 30% acrylamide mix (29:1), 1.25 ml 1M TRIS pH 6.8, 0.1 ml SDS, 0.1 ml 10% APS and 10  $\mu$ l of TEMED and poured on the top of the separating gel. After the comb was placed in the gel, the gel was allowed to polymerize for 45 minutes. Protein samples were prepared by mixing equal volume of protein and 2x Laemmli buffer (Bromophenol blue 1%, Dithiothreitol DTT 1 M, SDS 10%, Tris-Cl 1 M pH 6.8). After the proteins were loaded on a gel, electrical field was applied. The gel was run at 80 Volts for 30 minutes followed by 150 Volts for one hour and a half. Upon completion of electrophoresis, gels were stained with Coomassie blue dye for 30 minutes and de-stained in a mixture of 10% methanol/10% acetic acid overnight. Image of the stained gel was obtained by scanning the gel on Epson 200 scanner.

### Western Blot

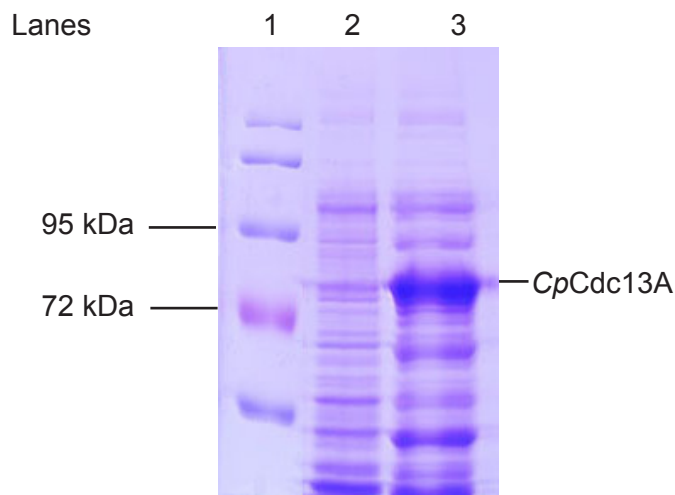
For the Western Blot assay, proteins were transferred from polyacrylamide gel onto the nitrocellulose membrane. This step was done by using electroblotting method, which used an electric current to pull proteins from the gel into the nitrocellulose membrane. The transfer sandwich was assembled by placing black side of the sandwich first, then the adding foam pad, the 3mm filter paper, the gel, the nitrocellulose membrane, the 3mm filter paper again, the foam pad and, lastly, the white plastic side. The blot sandwich was inserted into the transfer apparatus filled with transfer buffer, which was made by mixing 100 ml of 10 x transfer buffer, 700 ml of cold water, 200 ml methanol and 750  $\mu$ l 10% SDS. Transfer was conducted at 30 Volts at 4°C. After the transfer was completed, the nitrocellulose membrane was placed in 5 % BSA/ TTBS to block nonspecific protein binding and incubated for one hour at room temperature. Anti-His antibodies were diluted 1:2000 with 0.5% BSA-TTBS and applied to nitrocellulose membrane for one hour at room temperature. Blots were washed with 0.5% BSA-TTBS three times. Anti-rabbit alkaline phosphatase-conjugated antibodies were diluted 1:5000 with 0.5% BSA-TTBS, added to the membrane probed with anti-His antibodies and incubated at room temperature for 30 minutes. After completion of incubation, membranes were washed two times with 0.5 % BSA-TTBS and one time with 1X TBS. 66  $\mu$ l of NBT and 33  $\mu$ l of BCIP were mixed with

10 ml of alkaline phosphatase buffer (100 mM Tris pH 9.5, 10 mM NaCl, 5 mM MgCl<sub>2</sub>), added to the blots and incubated until color developed.

## Results

### Small Scale Protein Induction

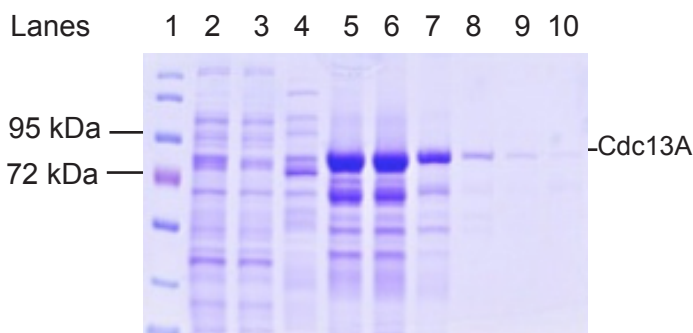
To verify induction of protein expression from the recombinant plasmid, the recombinant plasmid pSMT3Cp-Cdc13A and empty vector pSMT3 were transformed into BL21 codon+ competent cells. Transformant cells were inoculated into LB medium supplemented with 50  $\mu$ g/ml kanamycin, grown to an exponential phase, and protein expression was induced by addition of IPTG and Ethanol as described in the Materials and Methods section. To verify protein induction, 1 ml of each sample was collected. Cells were pelleted and boiled for five minutes in 2x Laemmli buffer to release cell content and centrifuged 15 seconds at 14000 rpm. After that, protein samples were loaded on a polyacrylamide gel and protein mixture in the sample was resolved by electrophoresis. In order to visualize protein bands, the gel was stained with Coomassie blue dye. In (Fig.1) it was easy to conclude that expression of CpCdc13A was induced.



**Figure 1.** Induction of CpCdc13A-small scale. Protein expression from pSMT3CpCDC13A was induced as described in Materials and Methods, and 1 ml of culture before and after induction was collected. Cells were pelleted by centrifugation, boiled with the 2 x Laemmli loading dye, centrifuged, and 25  $\mu$ l of supernatant was loaded on 10% SDS polyacrylamide gel. Proteins were resolved for 1 hour at 150 volts, and the gel was stained with Coomassie blue dye. Lane 1 - protein marker, lane 2 - uninduced culture, lane 3 - induced culture.

## Protein Purification

Protein extract was prepared as described above and incubated with Ni-NTA agarose resin for one hour at 4°C to promote binding of the tagged protein to Ni-NTA beads. The purpose of choosing Ni-NTA agarose to purify CpCdc13A from protein lysate was because Ni-NTA agarose beads have positive charge due to attached nickel ion. CpCdc13 protein was expressed with a negatively charged His-tag capable of binding to positively charged nickel ion. Ni-NTA and protein extract mix was loaded into a plastic 10 ml column by gravity and washed with five volumes of buffer E, then by buffer E supplemented with 25 mM Imidazole. Imidazole solution disrupted protein Ni-NTA binding. It was used in a concentration of imidazole below 100 mM to reduce non-specific protein - Ni-NTA binding, and in concentration 100 mM - 300 mM to disrupt specific protein binding. The only protein that bound specific to the column was CpCdc13A, so we expected that majority of CpCdc13A will be eluted with 100 mM - 300 mM imidazole solution. To assess the level of protein purification, samples of protein extract and different purification steps were resolved by electrophoresis in polyacrylamide gel (Figure 2).



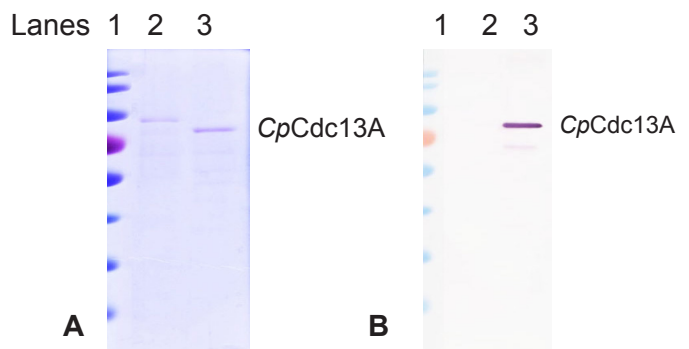
**Figure 2.** Results of CpCdc13A purification on Ni-NTA agarose column.

Protein extract was mixed with Ni-NTA resin, and CpCdc13A bound to the resin was eluted with imidazol as described in Materials and Methods.

Lane 1 - protein marker, lane 2 - protein extract, lane 3 - 3. Flow through, lane 4 - 25 mM Imidazole eluate, lane 5 -100 mM Imidazole eluate, fraction 1, lane 6 - 100 mM Imidazole eluate, fraction 2, lane 7 - 100 mM Imidazole eluate fraction 3, lane 8. 300 mM Imidazole eluate, fraction 1, lane 9 - 300 mM Imidazole eluate fraction 2, lane 10- 300 mM Imidazole eluate, fraction 3.

## Western Blot Assay

After the protein was purified, the next step was to verify that the purified protein was indeed CpCdc13A. To confirm that purified protein was CoCdc13A, the Western blot was performed. In this assay, we again have taken an advantage of the His-tag expressed as a part of the recombinant protein. Recombinant protein was the only one protein expressed in E coli cells that has a His-tag. Thus, if a protein has a His-tag, it can be confirmed that is CpCdc13A. Proteins were resolved by gel electrophoresis and transferred onto nitrocellulose membrane by electro-blotting as described in Materials and Methods. To prevent non-specific antibody the membrane was incubated in 5% BSA-TTBS solution for one hour at room temperature. Primary antibody was anti-His, which were expected to bind to the His-tag part of the recombinant protein. Since the primary antibodies were produced in rabbit, our secondary antibodies were anti-rabbit IgG antibodies conjugated with alkaline phosphatase enzyme. In this assay, the anti-His rabbit primary antibodies were expected to bind to the His-tag of the CpCdc13A immobilized on the nitrocellulose membrane. Secondary anti-rabbit-IgG antibodies were expected to bind to the primary antibodies. The enzyme alkaline phosphatase conjugated with secondary antibodies is capable of converting a substrate made of certain chemicals (NBT and BCIP) from colorless to slightly purple color. Since the alkaline phosphatase will be immobilized at the position of the His-tagged protein, that is where a change of the color was expected to take place. As can be seen on Figure 3 of the Western Blot assay, the protein band corresponding to CpCdc13A is visible clear enough for seeing the purple color of the band.



**Figure 3.** Western Blot Assay

A - Coomassie Blue staining of a protein gel before transfer to the nitrocellulose membrane. B - proteins immobilized on the nitrocellulose membrane were probed with anti-His antibodies.

Lane 1 - molecular weight marker, lane 2 - CpCdc13A, lane 3 - a protein without His-tag, lane 3 - CpCdc13



## Discussion

This project has demonstrated that it is possible to express *CpCdc13A* from the recombinant plasmid. Furthermore, this protein can be successfully purified using Ni-NTA agarose column. Once the recombinant proteins was purified, it can be used for analysis of its biochemical properties.

## Acknowledgement

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Front page photographs:

Morphology of M-Ras KO astrocytes. (A) Immunofluorescence analysis of WT (/\_/) and M-Ras KO (/\_/) astrocytes. Astrocytes are stained with a Cy3-conjugated anti-GFAP antibody (red), and the actin cytoskeleton at the cell periphery was detected with an antiphospho-ERM antibody (green). Two representative astrocytes are shown for each genotype. Magnification, X60.

Courtesy of Nelson Nunez-Rodriguez

Nuñez Rodriguez N., Lee I.N., Banno A., Qiao H.F., Qiao R.F., Yao Z., Hoang T., Kimmelman A.C., and A.M. Chan. 2006 *Mol Cell Biol.* 26:7145-54.

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