CALGARY TEAM

For our contribution we attempted to make competent cells using calcium that anyone could buy from the pharmacy. Below are a few notes and observations in bullets for clarity:

There were many types and blends of calcium supplements at the pharmacy. Our first choice was calcium-carbonate because from my understanding it seemed physiologically compatible.

- After trying this out we failed to make our cells competent. Upon further investigation we discovered that calcium-carbonate as very low solubility (this probably means it has less bioavailability in us too)
- No cells were successfully transformed using this form of calcium

Further reading leads us to pick calcium-citrate as our most viable option. It is physiologically compatible and has a relatively high solubility.

- Physiological compatibility was important because we didn't want anything that could potentially harm our cells
- We found that there was a lot of filler in the pills, so we had to crush many calcium-citrate pills to reach the desired concentration of calcium in solution
- Because of this, the solution was very cloudy and contained a lot of debris from the filler. This is the reason why we repeated the experiment with this type of calcium - the transformation, although successful, was at a very poor frequency and there was plenty of visible debris on the plates after spreading the transformed cells.
- In the second experiment we centrifuged the calcium-citrate solutions so that we could obtain a relatively clarified solution. The supernatant was used for treating our cells to make them competent

We performed our experiment with 4 different concentrations of calcium from the calcium-citrate tablets (5mM, 10mM, 50mM, 100mM). Our positive control used 50mM calcium chloride, because that is what we use for our competent *E. coli* in the lab.

Next are three photos of three different treatments of *E. coli* to produce competent cells. We tested competency by transforming with RFP (BBa_J04450), which yields red cells. As you can see, the positive control/standard (50mM calcium chloride) showed efficient transformation, which was replicated in the 50mM calcium-citrate treatment. For comparison, the 10mM calcium-citrate treatment yielded only a few colonies, though they were not red.

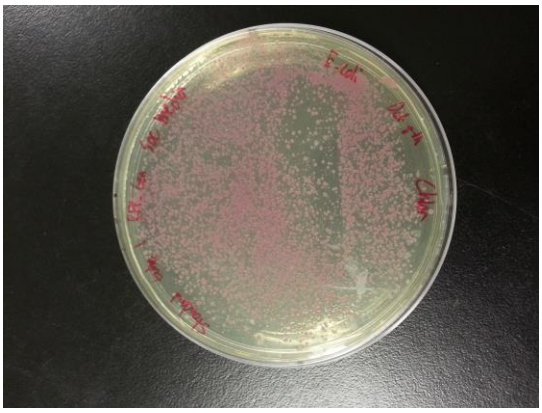

Figure 1 Positive control/standard (50mM calcium chloride)

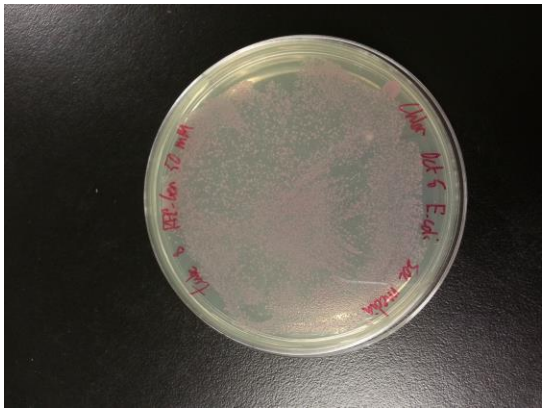

Figure 2 50mM calcium-citrate treatment

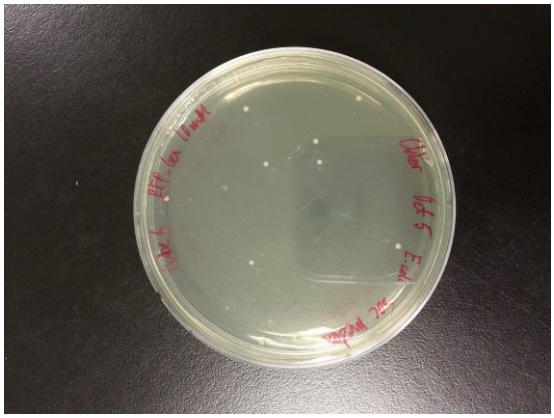

Figure 3 10mM calcium-citrate treatment

If we had more time we would repeat it again for more consistency. But based on the results from this experiment and from our previous attempt, we're confident that this method has potential. Ampicillin-resistance was used as a selection marker, the plasmids containing RFP also contained a gene for Amp-resistance. The lack of red colonies does not necessarily indicate a lack of transformation, since cells were still able to grow, chances are that they did not express the somewhat toxic RFP.

Unfortunately we cannot give a detailed outline of the price, because it follows our typical protocol almost exactly and we don't know the prices of all of our materials. We can however say that for the protocol outlined below, the amount of calcium-citrate tablets needed comes out to be about $0.21 CAD worth. So it's essentially the same cost as a typical lab protocol.

The one downside is the centrifuge... The good thing is that another team thought up a DIY centrifuge!

Below is our protocol for making competent cells.

1. Innoculate 5mL of LB using Top10 E. coli. Shake at 37C overnight
2. subculture 1mL of the overnight culture into 49mL of LB. Shake at 37C until OD600 = 0.4 to 0.6 (approx 2.5hrs)
3. Centrifuge the culture at 10000xg for 10 min at 4C. Discard supernatant
4. Resuspend pellet in 12.5mL of the cold calcium solution. Incubate on ice for 10 min
5. Repeat centrifugation. Discard supernatant
6. Resuspend pellet in 2mL of the cold calcium solution with 15% glycerol. Incubate on ice for 10min
7. Make 20x 100uL or 10x 200uL aliquots. Store at -80C