Low Budget iGEM Project

Title: Cheap and easy DNA extraction from Saccharomyces cerevisiae and Escherichia coli: two of the most used model organisms for iGEM work

Introduction

One of the most widely used procedures in biological fields such as biochemistry, molecular biology, biomedicine and biotechnology is DNA extraction. The theory behind this method can be summarized as follows: Lysate of the cells, removal of contaminants (such as proteins) and precipitation in alcohol. These steps are omnipresent for extraction of DNA from any cell, but an additional consideration must be met when pursuing high yields and quality for your extract: a buffer and a chelating agent. Regarding the buffer, numerous protocols have been made where cheaper buffers are used, but rarely has the role of the most common chelating agent, EDTA, been defied and/or replaced. In consequence, in this work we focus on describing and testing a known cheap protocol for DNA extraction from S. cerevisiae, and then trying to apply it for E. coli, while at the same time proposing a novel natural and cheap reactive to accomplish the chelating need.

Theoretical framework

General

In the first step for DNA extraction, lysis, a detergent or soap is usually used. It basically interrupts the interactions in the phospholipid bilayer of the membrane, therefore letting intracellular molecules out. In the conventional protocol, SDS, a detergent that is more-or-less expensive, is employed to accomplish this feat (plus NaOH). In our work, we replaced SDS by a supermarket dishwashing soap, called *Axion*, that has a similar pH and chemical components rich in OH groups, which we theorize will work in a similar manner. (Marshall University, 2012)

In the second step, proteins are precipitated using potassium acetate, by specific amino acid interaction and denaturalization. Even though it is not expensive, in this work, another even cheaper salt, table salt (NaCl) will be evaluated as a decontamination agent. (Marshall University, 2012)

In the third step, ethanol precipitates the DNA pellet by creating a less ionic environment that interrupts the association of ions with the phosphate backbone groups. This step will be left the same, since EtOH is already cheap and difficult to replace. (Marshall University, 2012)

Main proposal: Why choose garlic and grape juice in order to replace EDTA

EDTA serves as a chelating agent, which is a compound that incorporates a mineral ion or cation into a complex ring structure, therefore sequestering it and preventing it from performing its function in the system where it resides. This is a necessary requirement when extracting DNA, since when lysed, several nucleases that employ metal anions as cofactors can act and degrade the target plasmid. Several studies have shown that garlic as well as malic acid (mainly found in grapes and apples) have strong chelating capacities, and many therapies for metal detox using these components have been created (Flora & Pachauri, 2010; Sears, 2013).

Garlics have sulphur-rich molecules, and studies have revealed how these compounds have high affinity for metals such as lead and cadmium. Malic acid, on the other hand, can sequester many metals such as Pb, Cd, Zn and Cu (Ding, Song, Feng, & Guo, 2014). We deduce that since EDTA can sequester both toxic and nontoxic metals (cofactors), then garlic and malic acid have also high probabilities of being able to accomplish this feat.

Procedure

Protocol 1

1. Make a mix adding 20 g of yeast to 40 ml of distilled water.

2. Mix well until it is homogeneous.

3. Add 0.1 g of salt and 5 drops of lemon (you have to previously take out the seeds and to be careful of not letting pulp into the mix). Mix well.

4. Take 20 ml of the mix and centrifuge at 7000 rpm for 10 minutes.

5. Get rid of the supernatant.

6. Add 10ml of distilled water, 0.1 g of salt, 1 ml of ethylic alcohol 70% and 1 drop of detergent.

7. Vortex 5 minutes.

8. Add 0.5 g of salt.

9. Vortex 1 minute.

10. Vortex during 12 min at 7000 rpm

11. Recover the supernatant and keep it in another tube.

12. Add 3 times its volume in ethylic alcohol 70%.

13. Mix by inversion.

14. Recover 1 ml in a 1.5 ml tube.

15. Vortex 5 min at 14000 rpm.

16. Get rid of supernatant.

17. Let dry for 10 min.

18. Add 50 µl of free-nuclease water.

19. Store de DNA at 4ºC.

Protocol 2

1. Centrifuge the starter culture (This will be carried out from two different starter cultures, to diminish random errors).

2. Decant the supernatant and resuspend the pellet in about 3mL of LB media

3. Separate into three different Eppendorf tubes to make tests 2, 3 and 4.

4. Centrifuge 14,000 rpm / 1 minute

5. Decant the supernatant

6. Add 200uL of Solution I.

7. Use a pipette to resuspend the pellet

8. Incubate 5min at room temperature 10. Add 200uL of Solution II

11. Mix gently by inversion 6 times.

12. Incubate 5 min at room temperature

13. Add 200uL of solution III 3M.

14. Mix gently by inversion 6 times.

15. Incubate 5 min on ice

16. Centrifuge 14,000 rpm for 10 min

17. Transfer the supernatant to a new tube (around 500uL)

18. Add 2 volumes cool of 100% EtOH (around 1mL)

19. Incubate at -20°C for 10 min (can be from 10 min to 2 hours)

20. Centrifuge 14,000 rpm for 10 min

21. Decant the supernatant

22. Add 200uL of cool 70% EtOH

23. Use a pipette to resuspend the pellet

24. Centrifuge 14,000 rpm for 5 min

25. Decant the supernatant

26. Dry pellet at 37 ° C for 5 min

27. Add 30uL of ddH2O and resuspend pellet

28. Measure DNA concentration in nanodrop

29. Run electrophoresis to observe DNA

Testing and results

Test number	Description	Reagents	Results: DNA concentration and purity (Mean of both starter cultures) (Appendix A)
1 (S. cerevisiae)	(Proof of concept)	Protocol 1	DNA concentration = 4101.43 ng/L DNA/Protein ratio= 1.75 DNA/Salt ratio = 1.5
2 (E.coli)	Control (normal)	Protocol 2 Solution I. (50 mM TRIS pH 8.0, 10 mM EDTA) Solution II (200 mM NaOH, 1% w/v SDS) Solution III (3 M potassium acetate, pH 5.5)	DNA concentration = 1179.89 ng/L DNA/Protein ratio= 1.965 DNA/Salt ratio = 2.46
3 (E.coli)	Normal protocol using garlic and grape juice instead of EDTA	Protocol 2 Solution I. (50 mM TRIS pH 8.0, concentrated garlic and grape juice) Solution II (200 mM NaOH, 1% w/v SDS) Solution III (3 M potassium acetate)	DNA concentration = 1053.09 ng/L DNA/Protein ratio= 1.94 DNA/Salt ratio = 2.345
4 (E.coli)	Novel modified protocol	Protocol 2 Solution I. (25 mM TRIS pH 8.0*, concentrated garlic and grape juice) Solution II (Axion Detergent) Solution III (3 M NaCl)	DNA concentration = 636.6 ng/L DNA/Protein ratio= 1.54 DNA/Salt ratio = 1.01

Cost comparison (Ethanol is used in all tests, so it is omitted) (It is assumed that approximately equal quantities of each analogue reagent are used)

Article (1kg or 1L)	**Price (American Dollars)**	**Test 1**	**Test 2**	**Test 3**	**Test 4**
TRIS	109.20		*	*	*/2
EDTA	74.25		*		
SDS	113.01		*	*	
NaOH	2.5		*	*	
Lemon	1	*			
Garlic	6.5			*	*
Grape juice (Assuming 1kg grape = 1L grape juice)	4	*		*	*
Table Salt (NaCl)	0.6	*			*
Axion Detergent	1	*			*
TOTAL per kg/L	**-**	**6.6 dollars**	**298.96 dollars**	**235.21 dollars**	**66.7 dollars**

Future perspectives:

Possible improvements

1.- Make a negative control for comparison and further evaluation of the efficacy of garlic/grape concentrate versus EDTA as a chelating agent. To do this, we would have to carry out the complete same protocol but without including EDTA.

2.- Even though TRIS buffer is inexpensive, we consider that it can be possible to look for another buffer with a similar pH that is even more inexpensive.

3.- We consider that due to the properties of the meat tenderizer, it can be used as a substitute of potassium acetate in solution III.

4.- It is possible to extract the malic acid from the grape juice with the help of a solvent, which could make the solution more efficient.

5.- Due to the inefficiency of the NaCl in precipitating the proteins, we consider that a acetic acid was needed. For this, we propose the option of making a solution III with NaCl and vinegar. Also, just a bigger concentration of NaCl can be used.

6.- Since results were not of high purity, a possible improvement could be the use of purifying columns to obtain pure DNA afterwards.

Other proposals for low budget molecular biology procedures

Other good ideas that emerged in the iGEM Mty team to lower the cost of some procedures and further innovate. One of them is the development of an alternative electroporation system, which could save approximately 3000 dollars (cost of electroporation equipment). This can be done relatively easy by using an AA 1.5V battery as a DC source, which would then be converted to a high voltage alternate current, say, 450 V. Finally a specific designed voltage multiplier can further convert the voltage up to a certain desired value, which in this case would be 2500 V. A small device called microcontroller can set the duration of the pulse, and the system would be connected with aluminum electrodes to allow insertion of the cuvette. It would be able to give a personalized efficient electric shock, and transform the cells.

Conclusion

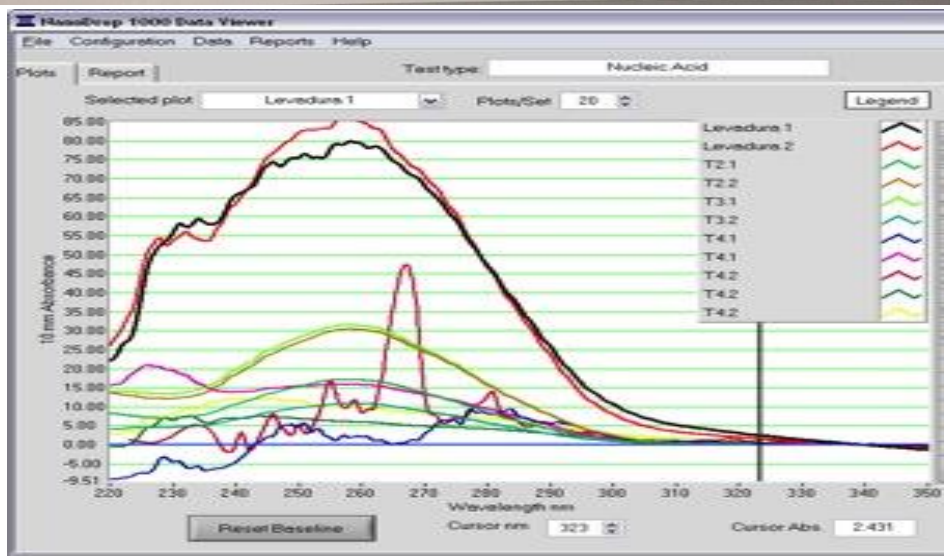
During this project, we realized that although standardized protocols are widely used for its proved efficiency, costs can be high, and several alternatives can be developed to obtain slightly deficient results but with the advantage of saving important quantities of economic resources. In many iGEM teams, obtaining sponsors and funding the reagents and the experimentation can sometimes be a problem. With the use of protocol from test 1, 292.36 dollars are saved. From test 3, 63.5 dollars can be saved from each kg or L used. Using solutions from test 4, 232.26 dollars are saved. It is easy to see that these alternatives have to be considered and further evaluated to incorporate them as a whole or parts of it as routine iGEM procedures, to contribute to making protocols more accessible and feasible for low resources laboratories.

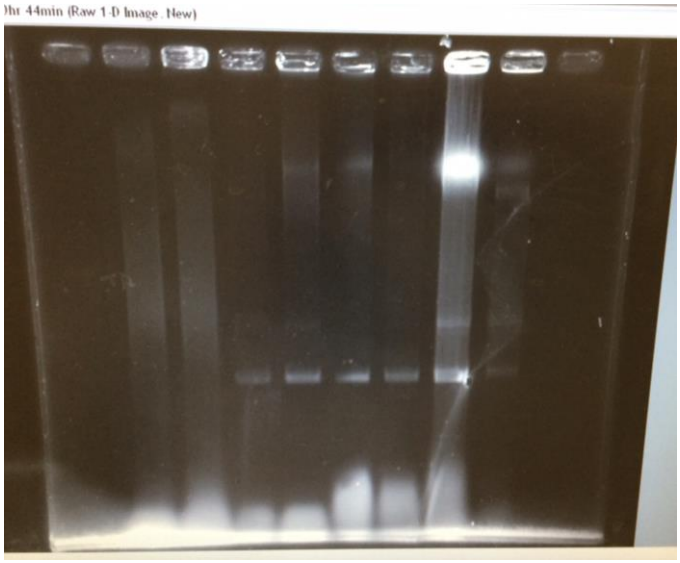
Therefore, with the use of this type of protocols, eventually a considerable profit can be obtained, and so it is of high importance to support these competitions and to invest time searching for new and cheaper ways to continue working on this beautiful discipline called synthetic biology.

Appendix A (Quantification of DNA, and electrophoresis images)

ort | Low Budget iGEM | Test Type | Nucleic Acid | Date/time | 01/10/2014 08:31 p.m. | Page #

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
evadura 1	Default	01/10/2014	08:13 p.m.	3961.26	79.225	47.102	1.68	1.42
evadura 2	Default	01/10/2014	08:15 p.m.	4241.60	84.832	46.689	1.82	1.58
T2.1	Default	01/10/2014	08:16 p.m.	849.55	16.991	8.466	2.01	2.43
T2.2	Default	01/10/2014	08:18 p.m.	1510.22	30.204	15.704	1.92	2.49
T3.1	Default	01/10/2014	08:19 p.m.	1564.67	31.293	15.890	1.97	2.33
T3.2	Default	01/10/2014	08:20 p.m.	541.49	10.830	5.664	1.91	2.36
[redacted]								
T4.1	Default	01/10/2014	08:23 p.m.	787.36	15.747	9.353	1.68	0.81
[redacted]								
T4.2	Default	01/10/2014	08:27 p.m.	485.91	9.718	6.951	1.40	1.21





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