Comparative Analysis of some DNA extraction kits used for Molecular Analysis of Iron Ore samples

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Citation: Ekpa E et al. (2016), Comparative Analysis of some DNA extraction kits used for Molecular Analysis of Iron Ore samples. Int J Biotech & Bioeng. 2:2, 58-63.DOI: 10.25141/2475-3432-2016-2.0066

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Received: September 13, 2016; Accepted: October 07, 2016; Published: October 30, 2016

Abstract:
Bioleaching simply refers to the microbial-catalyzed process of conversion of insoluble metals into their soluble forms. As an applied biotechnology, it represents an extremely interesting field of research where genomic and proteomic techniques can be used in terms of knowledge development, but more in terms of process design, control, and optimization. Good quality DNA is a prerequisite for all experiments requiring DNA manipulation. DNA isolation is often the most fundamental and crucial in the entire process. As a result of different DNA isolation kits constantly becoming available, it has become imperative to compare their efficacies. This study compared some popular isolation kits used on bioleaching microorganisms from an Iranian Iron mine with that of Zymo research kit used on Agbaja Iron stones of Nigeria. The Iron mine of Agbaja in kogi State possess similar characteristics with those of the Iranian Iron mine-hence the choice of the comparative protocols. Modified CTAB method (Sigma) and kits of the QIAmp Investigator (Qiagen) and Accuprep Genomic DNA (Bioneer) were used to compare to that of ZR Genomic DNA (Zymo Research) used in this work. Spectrophotometric results showed that the highest concentration of DNA from 100µl of bioleaching bacteria culture was obtained by Qiagen kit (165.50μg/μl) followed by Zymo kit (50.40μg/μl), Bioneer kit (34.33μg/μl) and CTAB (23.65μg/μl). The Bioneer test took a total of 40 minutes to isolate bacterial DNA, followed by Zymo kit, 45 minutes, Qiagen, 50 minutes, and the CTAB 60minutes. These preliminary findings may serve as future baseline information in helping laboratories/Researchers make appropriate decisions in process optimization for isolating quality DNA especially from bioleaching microorganisms.

Keywords: Bioleaching, DNA Isolation, Iron Mine, Extraction Kits, Agbaja, Nigeria

Introduction:
The employment of microorganisms for metal recovery from low-grade ores, mineral concentrates, and secondary materials, has developed into a successful and expanding area of biotechnology (Appia-Ayme et al. 2006). In association with this interest, microbial communities of extreme acidiphilic prokaryotes from bioleaching environments have long been the subject of active research; however, the components and interactions within these microbial communities’ remains poorly understood. Recent acquisition of genomic data directly from organisms living in naturally extreme environments in combination with genome sequencing projects of individual species provides a novel opportunity for prediction and exploration of the metabolic details that control both individual microorganisms and microorganism communities (Parro et al. 2007; Ram et al. 2005; Huggler et al. 2005). Acidophilic prokaryotes involved in metal recovery from sulfide minerals include members of the Bacteria and Archaea domains. Three species of chemolithotrophic bacteria are mainly involved: Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans and Leptospirillum sp., all of which obtain energy primarily from iron and/or sulphur oxidation (Justice et al; 2014 ). A. ferrooxidans is capable of oxidizing reduced sulfur compounds and Fe2+ ions to form sulfate and Fe3+, respectively.
A. thiooxidans can only oxidize reduced sulfur compounds such as thiosulfate, tetrathionate, metal sulfides and elemental sulfur to form sulfate. Leptospirillum sp. is solely capable of oxidizing Fe^{2+} ions to form Fe^{3+}. These autotrophic microorganisms utilize the energy and reducing power derived from iron and or sulfur oxidation for several metabolic processes, including CO2 fixation and acquisition of several sources of nitrogen (Pandey and Pandey, 2011). In both Acidithiobacillus species, CO2 fixation occurs via the Calvin-Benson-Bassham cycle whereas Leptospirillum sp. grows autotrophically; however the molecular mechanisms involved in carbon fixation remain obscure. In acidic bioleaching environments, dissolved inorganic carbon can reach levels below atmospheric concentrations average. Therefore, it is not surprising that CO2 concentrating mechanisms have been identified in autotrophic prokaryotes present in such environments (Kenedy, 2015).

The environmental consequences of mining and quarrying are vast, for example habitat erosion, soil contamination and water contamination are some of the direct disadvantages. Due to an increase in consumerism, heightened dependence on technology, and an overall world population growth, more and more minerals and metals need to be extracted from the earth’s surface. Even though the recycling industry is growing, it cannot meet these growing demands for minerals. Furthermore, with numerous accessible mining sites already exploited, the human race is in a constant struggle to find feasible mining methods.

One way to mitigate the environmental consequences of mining and quarrying is through the use of bioleaching microbes. These “rock munching microbes” such as the Acidithiobacillus and Leptospirillum bacterium, can clean up abandoned mine sites in a more environmentally friendly manner (Hettich, 2005). Kogi State of Nigeria is blessed with iron ore reserve, the main ones been Itakpe and Agbaja (about 2 billion tonnes of unexplored iron ore reserve). The economic gains that will arise from exploitation are very enormous that is in terms of employment generation and rapid industrialization.

Though the environmental aspect of these bacteria in bioleaching is very good, the efficiency of this process needs further studies, hence, the need to investigate the molecular basis of this challenge. The molecular characteristics of these isolated organisms may become the fulcrum for future cloning activities. However, all these begin with a carefully planned method for extracting DNA especially with the use of affordable and readily available kits. Good quality DNA is a prerequisite for all experiments of DNA manipulation.

The isolation and purification of DNA from cells is one of the most common procedures in contemporary molecular biology and embodies a transition from cell biology to molecular biology (in vivo to in vitro). The main aim of this work therefore, is to compare the efficiency of some already documented DNA isolation kits with those of Zymo research practically applied in this work. This is with a view to investigating the comparative advantage or otherwise of the Zymo Kits for rapid DNA analysis of some bioleaching microorganisms.

**Materials and Method:**

**Sample Collection:**

Ore samples were collected from various drilled holes sites in Agbaja Iron Ore mine of Kogi state, Nigeria using sterile polystyrene bags and special geological forks. Samples were maintained on ice and immediately taken to the Laboratory for further work.

**Bacterial growth and Culture Conditions:**

The bacteria species used in this study were those previously isolated and identified in the preliminary stage of this research. They included Acidithiobacillus and Leptospirillum species. The bacteria were cultured in a modified 9k medium containing (per litre): KH2PO4 (0.4 g), CaCl2.2H2O (0.2 g), MgSO4.7H2O (0.4 g), (NH4)2SO4 (0.4 g), FeSO4.7H2O (33.3 g), pH: 1.5-2 in 15g of nutrient Agar at 30°C and 180 rpm. The last bacteria were grown in LB medium at 37°C, Ore samples were treated with H2SO4 (0.5 N) for three weeks, then, crushed into small particles (0.2-0.5 μm in diameter). Cultures in 9k medium were incubated for 1 week until growth was observed microscopically or until a chemical change occurred in the medium compared with un-inoculated control. In cultures the growth was accompanied by a characteristics ferric precipitation and orange colouring of medium. Solid media were gelled with 0.6% (w/v) agarose.

**DNA Extraction:**

ZR fungal/Bacterial DNA MiniprepTM kit (Zymo Research) was used for the extraction of DNA from bacteria isolated from Agbaja Iron ore samples of Kogi state. The efficiency of the kit was then compared with those of Accurep kit from Biosensor (catalogue Number 6540-400), modified CTAB method, and QIAamp DNA Investigator Kit (Qiagen) recorded by some literature elsewhere (Sepalin et al. 2008) so as to look at the merit or demerit of our chosen protocol. DNA extraction procedures were performed by following the manufacturer’s instructions. Extracted samples were stored at-20°C for further analysis.

**AccuPrep Genomic DNA Extraction Kit (Bioneer), (as described by Sepalin et al. 2010):**

The AccuPrep Genomic DNA Extraction Kit was purchased from Bioneer Corporation. The procedure was as follows: 20μl of Proteinase K was added to a 1.5 ml micro centrifuge tube followed by 100μl of bacterial cell culture and 200μl of Binding buffer. The sample was mixed immediately by vortex mixer. After 10 minute incubation at 60°C, 100μl of Isopropanol was added to the sample.
DOI: 10.25141/2475-3432-2016-2.0066

Spectrophotometer (Thermo Scientific). DNA quality was assessed 50μl of triethyl (TE) buffer. Yields and quality of the DNA isolated by centrifugations at 8,000 rpm for 1 minute each. The column was washed with 700μl W2 buffer and also with 50μl of triethyl (TE) buffer. Yields and quality of the DNA isolated by the three kits were measured by use of a Nano Drop ND-1000 Spectrophotometer (Thermo Scientific). DNA quality was assessed using the absorbance ratio at 260:280 nm. DNA and protein absorb differently at these wavelengths. An A260/A280 ≥ 1.8 is generally accepted as “pure” for DNA; a ratio of ≥ 2.0 indicates that the preparation is contaminated with RNA; a ratio of ≤ 1.8 indicates a possible contamination with proteins or other contaminants that absorb strongly at or near 280 nm. The quality of the isolated DNA samples were also analyzed by electrophoresis on 1.0% agarose (wt/vol) gels using Tris, Borate EDTA pH 8.3 buffer and ethidium bromide staining. Gel imaging was done by using a Fluor Chem Q Analyzer from Alpha Innotech Company. PCR then followed according to the manufacturers instruction.

Cetyltrimethyl ammonium bromide (CTAB) method (as described by Sepalin et al. 2008):

An equal volume of CTAB dilution precipitation buffer was added to the solution containing crude DNA from the bacterial culture of 100μl. The CTAB-nucleic acid salts were pelleted by microcentrifugation at 8000 rpm for 10 minutes and the precipitate was washed 2-3 times with 300ml of cold wash solution to remove any free CTAB. The CTAB-nucleic acid pellet was dissolved in 100ml salt dissolving solution and the DNA was precipitated with 2 volumes of 95% ethanol and microfuging for 10 minutes. The resulting DNA pellet was washed with cold 70% ethanol and resuspended in deionized water.

The crude DNA solution was made up to 0.5 ml with distilled water and an equal volume of phenol-chloroform-isoamylalcohol was added, inverted and microcentrifuged at 11000 rpm for 5 minutes. The aqueous phase was removed and added to an equal volume of chloroform-isoamylalcohol, inverted and again microcentrifuged for 5 minutes. The aqueous phase was removed and the DNA was precipitated from it with either an equal volume of propan-2-ol, or 2 volumes of ethanol and 0.1 volumes of sodium acetate and chilled at -20°C. The resulting DNA pellet was washed with cold 70% ethanol and re-suspended in deionized water and the resulting DNA from the extractions/purifications were visualised for quality on 1 % agarose gels. PCR was the performed as described by the same Author above.

QIAamp DNA Investigator Kit (as described by Sepalin et al. 2008, Saiyed et al. 2008):

The QIAamp DNA Investigator Kit was purchased from Qiagen Company. Isolation of DNA began with the addition of 10μl of proteinase K to 100μl liquid broth bacterial culture and 100μl Buffer AL. After 10 minutes of incubation at 56 °C, 50μl ethanol was added. The mixture was incubated for 3 minutes at room temperature and then transferred to a QIAamp MinElute column and centrifuged at 8,000 rpm for 1 minute. The column was washed with 500μl W1 buffer and also with 700μl W2 buffer by centrifugations at 8,000 rpm for 1 minute each. The column was washed with 700μl ethanol. The filter-bound DNA was eluted with 50μl of triethyl (TE) buffer. Yields and quality of the DNA isolated by the three kits were measured by use of a Nano Drop ND-1000 Spectrophotometer (Thermo Scientific). DNA quality was assessed using the absorbance ratio at 260:280 nm. DNA and protein absorb differently at these wavelengths. An A260/A280 ≥ 1.8 is generally accepted as “pure” for DNA; a ratio of ≥ 2.0 indicates that the preparation is contaminated with RNA; a ratio of ≤ 1.8 indicates a possible contamination with proteins or other contaminants that absorb strongly at or near 280 nm. The quality of the isolated DNA samples were also analyzed by electrophoresis on 1.0% agarose (wt/vol) gels using Tris, Borate EDTA pH 8.3 buffer and ethidium bromide staining. Gel imaging was done by using a Fluor Chem Q Analyzer from Alpha Innotech Company. PCR then followed according to the manufacturers instruction.

ZR fungal/Bacterial DNA MiniprepTm kit (Actually carried out in this work):

The ZR fungal/Bacterial DNA MiniprepTm kit was obtained from Zymo Research Company. The methodology used was described on the kit’s manual. About 100μg (100μl) of the cell isolate was scooped under the laminar flow hood, suspended in 750μl lysis buffer and lysed. It was then vortexed for about 5 minutes and transferred to the centrifuge to spin at 12,000 rpm for about 3 minutes. The supernatant was transferred into the Zymo-spin TM spin filter; it was then spin at 10,000 rpm for about 2 minutes. 1,200μl of Bacterial DNA binding buffer was added to the filtrate, thereafter, it was transferred to zymo spin IIC column and spin at 12,000 rpm for 2 minutes. Then, DNA pre-wash buffer was added to column and centrifuge at 12,000 rpm for 2 minutes. Also, fungal/bacterial DNA wash buffer was added and centrifuge at 12,000 rpm for about 2 minutes. The column was transferred into clean 1.5ml micro tube and 20-100μl of DNA elution was added to concentrate it, then it was spin at 12,000 rpm for about 1 minute to elute the DNA. Finally, electrophoresis was carried out on the DNA sample.

Determination of concentration of nucleic acids:

The concentration and purity of nucleic acids aqueous solution was determined by measuring absorbance at 260 nm and 280 nm in a quartz cuvette of 1 cm path length against distilled water blank in a Hewlett Packard 8453 spectrophotometer. The nucleic acid concentration was determined on the assumption that DNA and oligonucleotides (20μg/ml), RNA (40μg/ml) and double-stranded DNA (50μg/ml), (Sinnett et al. 1998). The purity of nucleic acid samples was investigated by determining the ratio of Abs260/Abs280. A ratio of >1.5:1 indicated relatively pure DNA with no contaminating proteins or polysaccharides.

Results and Discussion:

The three DNA Extraction Kits were used to isolate genomic DNA from an Iranian iron ore samples whose characteristics were similar to those of Agbaja Iron ore of Kogi state and the yields were measured by uv-vis spectroscopy (all results were then compared to those of Zymo Research kits which was actually carried out in this project). The results in Table 2 showed that the
Table 1: Characterization of the DNA extraction kits used in comparison with the Zymo Kit

<table>
<thead>
<tr>
<th>Method</th>
<th>Name of Kit</th>
<th>Vendor</th>
<th>Main Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Accuprep Extraction kit</td>
<td>Bioneer</td>
<td>Proteinase K digestion and DNA binding to glass fibre</td>
</tr>
<tr>
<td>2</td>
<td>CTAB (Modified manual method)</td>
<td>Sigma</td>
<td>Combination of different lysing Chemicals for DNA extraction</td>
</tr>
<tr>
<td>3</td>
<td>Q1Amp Extraction kit</td>
<td>Qiagen</td>
<td>Proteinase K digestion and DNA binding to silica gel</td>
</tr>
<tr>
<td>*4</td>
<td>Zymo fungal/bacterial miniprep Extraction kit</td>
<td>Zymo Research</td>
<td>Genomic DNA lysis and special Zymo beads bind DNA and water elution</td>
</tr>
</tbody>
</table>

*Actual method carried out in this work and used as a standard for comparing with the others Informations from the other methods were based on their manufacturer’s manual

Table 2: DNA yield for the different methods as compared to that of Zymo Research

<table>
<thead>
<tr>
<th>DNA extraction method</th>
<th>First Extraction (μg/μl)</th>
<th>Second Extraction (μg/μl)</th>
<th>Mean (X) (μg/μl)</th>
<th>StdDev (μg/μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuprep</td>
<td>34.43</td>
<td>36.55</td>
<td>35.49</td>
<td>1.5</td>
</tr>
<tr>
<td>CTAB</td>
<td>24.50</td>
<td>22.80</td>
<td>23.65</td>
<td>1.2</td>
</tr>
<tr>
<td>Q1Amp</td>
<td>150.35</td>
<td>180.65</td>
<td>165.50</td>
<td>21.43</td>
</tr>
<tr>
<td>*Zymo</td>
<td>48.68</td>
<td>52.12</td>
<td>50.40</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Accuprep DNA concentrations were 34.43μg/μl, and 36.55μg/μl, respectively. The average concentration was 35.49μg/μl. The average total DNA yield from a cell culture of 1x107cfu/ml from a starting cell culture of 6.4x105cfu/ml (Table 4) is 6.554μg. The results of uv-vis spectrophotometry for DNA isolated with the CTAB modified method are shown in Table 3. The concentrations of the two extractions of DNA from Table 2 were 24.50μg/μl, and 22.8μg/μl. The average concentration was 23.65μg/μl. The total DNA yield from a starting cell culture of 5.3x108 is 2.073μg. The results from uv-vis spectrophotometry of DNA isolated with the QIAamp DNA Investigator Kit are presented in Table 2. The results show that the concentrations of DNA isolated from cell culture samples were 150.35μg/μl, and 180.65μg/μl.

The average concentration of the isolated DNA was 165.50μg/μl. The total DNA yield in a starting culture of 1.4x106 was 15.375μg. The results in Table 3 for DNA isolated using the Zymo Genomic DNA Extraction Kit showed A260/A280 nm ratios of 1.87 and 1.83 (Table 3).

Discussion:

Molecular methods have increasingly been used for the detection and quantification of diverse kinds of microbes. Moreover, the need for a rapid and easy molecular detection and quantification technique is imperative (Sinnet et al; 1998). An equally important step in nucleic-acid-based quantification of bacteria is sample processing preceding the actual assay, as has previously been mentioned in other literature. It was observed that the DNA obtained by QIAamp DNA Investigator Kit produced the highest concentration on an average of 165.50μg/μl, while the other two kits only yielded 50.40μg/μl (ZR Genomic DNA) and 35.49μg/μl (Accuprep) and the modified CTAB method yielded 23.65μg/μl from 100μl of the bacterial culture.

The significance of these results was seen in the large difference...
in the DNA concentration between ZR Genomic DNA kit and the other two kits along with the modified CTAB method. Measurement of DNA purity by using A260:A280 ratio showed an average DNA ratio of 1.82 for the DNA isolated with QIAmp DNA Investigator Kit, and a ratio of 1.85 for the DNA isolated with ZR Genomic DNA extraction kit. A ratio of 1.41 and 1.91 was obtained for the DNA isolated by the Accuprep DNA extraction kit and CTAB respectively which indicated the presence of contaminants that absorb strongly at or near 280nm. Based on this criterion QIAmp DNA Investigator Kit produced the highest purity DNA. Processing time varied considerably between the kits, ranging from 40 minutes for completion of the Accuprep Genomic DNA extraction kit to 60 minutes for the modified CTAB method (Table 4).

The results of this study confirm that DNA extraction is a key step in this process. Several strategies can be used for the extraction of DNA from microbial samples, such as enzymatic, chemical/thermal lysis, mechanical disruption of the cell wall by beads or sonication, or a combination of all of the above. A disadvantage of enzymatic lysis is that commercially available enzymes can be contaminated with microbial DNA. Highly sensitive and specific nucleic-acid-based methods for the detection of bacteria necessitate the use of DNA extraction reagents that are free from contaminating bacterial nucleic acids. In addition, the use of enzymes often requires special conditions and preservation requirements, such as refrigeration and buffer storage.

Chemical lysis often involves the use of aggressive and toxic chemicals, which is less desirable for on-site detection where laboratory safety conditions are absent (Butler, 2005). DNA extraction efficiency is limited by several factors (as shown in table 1). An important factor is cell concentration, which can be a limiting factor for the quantification of natural samples where cell concentrations will vary. At low concentrations, method sensitivity will play a role and at high concentrations, overloading of the method can reduce DNA extraction efficiency. All the merits of the extraction methods compared with that of Zymo used in this work are summarized in tables 1-4 in the result section. For Accuprep extraction method, problems can arise for certain harder-to-lyse species at concentrations above 107 cells ml−1. A second factor is the type of bacterial species that is being subjected to the extraction. For instance, the constitution of the bacterial cell wall may impede cell lysis and the liberation of DNA from the cell. A third factor may be the physiological state of the cells that are subjected to cell lysis, in which context, it can be noted that the cells used in the present study were of liquid cultures in stationary phase. Another factor that may influence the outcome of quantification is the size of the DNA fragments generated. Long fragment sizes generated by the DNA extraction method may result in incomplete denaturation during the thermal cycling of qRT-PCR, whilst, on the other hand, short fragment sizes may result in reduced amplification efficiency. The QIAamp® DNA Mini Kit was developed and commonly used for a variety of bacterial species. QIAamp® DNA Mini Kit(QIAGEN GmbH, Hilden, Germany), total DNA can be purified from a variety of biological, clinical and forensic specimens.

The method uses chemical lysis by undisclosed lysis buffers containing chaotropic salt, enzymatic digestion by treatment with proteinase K and thermal lysis (Gullsby, 2008). The method uses a separate protocol for Gram-positive bacteria, with the additional use of enzymatic digestion by lysozyme or lysostaphin with Triton. DNA is bound to a silica-gel membrane in a spin-column, whilst PCR inhibitors are supposedly, not retained (Table 1). The bound

<table>
<thead>
<tr>
<th>Method</th>
<th>DNA conc(μg/μl)</th>
<th>DNA purity (std yield=1.8)</th>
<th>DNA yield per 1x10⁷cfu/ml</th>
<th>Time for assay (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuprep</td>
<td>35.49</td>
<td>1.41</td>
<td>6.554μg</td>
<td>40</td>
</tr>
<tr>
<td>CTAB</td>
<td>23.65</td>
<td>1.91</td>
<td>2.073 μg</td>
<td>60</td>
</tr>
<tr>
<td>QIAmp</td>
<td>165.50</td>
<td>1.82</td>
<td>15.375 µg</td>
<td>50</td>
</tr>
<tr>
<td>*Zymo</td>
<td>50.40</td>
<td>1.85</td>
<td>10.425 μg</td>
<td>45</td>
</tr>
</tbody>
</table>

Table 3: DNA purity as a ratio of A260/A280 for the methods as compared to that of Zymo

<table>
<thead>
<tr>
<th>Method</th>
<th>DNA conc(μg/μl)</th>
<th>DNA purity (std yield=1.8)</th>
<th>DNA yield per 1x10⁷cfu/ml</th>
<th>Time for assay (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuprep</td>
<td>1.42</td>
<td>1.40</td>
<td>1.81</td>
<td>StdDev 0.01</td>
</tr>
<tr>
<td>CTAB</td>
<td>1.93</td>
<td>1.88</td>
<td>1.91</td>
<td>StdDev 0.01</td>
</tr>
<tr>
<td>QIAmp</td>
<td>1.81</td>
<td>1.83</td>
<td>1.82</td>
<td>StdDev 0.04</td>
</tr>
<tr>
<td>*Zymo</td>
<td>1.87</td>
<td>1.83</td>
<td>1.85</td>
<td>StdDev 0.03</td>
</tr>
</tbody>
</table>

Table 4: Cost benefit of the Extraction kits compared to that of Zymo Research
DNA is then washed, eluted in buffer and can then be stored at −20°C. overloading of the column of the QIAamp® DNA Mini Kit may lead to significantly lower yields than expected, according to the manufacturer’s instructions.

The CTAB method was also developed for use with a broad spectrum of bacterial species. To our knowledge, the use of CTAB Elute for qualitative/quantitative bacterial estimation has been the most popular method till date. The main constituents and boiling procedure of the method have also been used for other species. However, as confirmed by our results, it must be taken into account that inter-species and even intra-species differences in DNA extraction efficiencies will exist, influencing the outcome of microbial quantification. In summary, most of the DNA extraction protocols described here have been found to be comparatively costly and time consuming and may lead to substantial loss of some DNA for molecular analysis.

However, they all have the following advantages: Average to Good yields ((Accuprep -CTAB-QIAmp and Zymo) of high quality genomic DNA, Circumvention of the use of liquid nitrogen for crushing of the bacterial biomass. Reduction in the extraction steps, Minimal requirement of chemicals and equipment needed for lysis/extraction and elimination of toxic and potentially hazardous substances. (Tables 1-4). High molecular weight DNA (>1.0kb, figure 2) resulted from the use of the Zymo method used in this work.

The ratio of the absorbance at 260nm and 280nm was found to be 1.85 indicating a good purity with very little smear on the DNA. The isolated DNA produced good banding patterns for Acidithiobacillus and Leptospirillum species indicating its good quality. The entire DNA extraction kit also worked well in releasing large quantities of DNA from the bacteria culture used (Table 4). The DNA isolated from the bacteria culture was well resolved on 1% agarose gel. This shows that the isolated DNA is free of polysaccharides and polyphenols which are known to inhibit tag DNA polymerase and restriction endonucleases. DNA eluted from the kits could ensure a very suitable DNA for amplification using PCR.

References: