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Development of DNA isolation and purification method with automation by using Silica coated Magnetic Nanoparticles

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ABSTRACT

Development of DNA isolation and purification method with automation by using Silica coated Magnetic Nanoparticles. To develop a protocol to extract and purify the DNA, with automation using silica-coated magnetic particles, of two gram-negative bacteria E. coli DH5α for small volume culture (1ml) and large volume culture (5ml). Cell Lysis: Here all the analysis has been done on E. coli DH5α, gram negative bacteria. Washing: The second step comes in purifying the nucleic acids is the washing. Other than proteins, there are many kinds of impurities present with nucleic acids and these impurities need to be washed. Elution: The final step is the elution step. In this step, finally the DNA is extracted from the silica coated MNPs and hence purified.

Keywords— *Silica Coated Magnetic Nanoparticles*

1. OBJECTIVES

To develop a protocol to extract and purify the DNA, with automation using silica-coated magnetic particles, of two gram-negative bacteria E.coli DH5α for small volume culture (1ml) and large volume culture (5ml). To optimize the following parameters in the DNA extraction protocol.

(a) Selection of lysis buffer
(b) MNPs concentration
(c) Washing buffer volume
(d) Washing buffer timing
(e) Elution buffer volume
(f) Elution buffer pH

2. MATERIALS AND METHODS

2.1 DNA Extraction Process and Reagents

2.1.1 Cell Lysis: Here all the analysis has been done on *E. coli* DH5α, gram negative bacteria. We developed its own lysis buffer, which lyse the gram negative bacterial cells in just 5 minutes. The lysis buffer composition are 100µl of 1%SDS, 100µl of 50mM Tris (pH 7.5) and 200µl of 10mM EDTA, and 3M Guanidinium hydrochloride.

2.1.2 Washing: The second step comes in purifying the nucleic acids is the washing. Other than proteins, there are many kinds of impurities present with nucleic acids and these impurities need to be washed. These impurities can be organic compounds as well as inorganic compounds. The detergents, cell debris, salts, digested proteins, other fragments, etc. all these constitutes the impurities. So, for removing these impurities we are using 70% ethanol as our washing buffer. DNA is washed with 70% ethanol to remove some (or ideally all) of the salt from the pellet. If water was used as the wash then DNA would dissolve again and if 100% ethanol was used the salt would not wash off because sodium salts are poorly soluble in ethanol, because precipitation in 100% ethanol cause removal of all water molecule from DNA and Complete Dehydration, which make them not soluble, So we give 70% ethanol wash to let it retain some water molecule when make it soluble.

2.1.3 Elution: The final step is the elution step. In this step, finally the DNA is extracted from the silica coated MNPs and hence purified. The choice of the elution buffer completely depends upon the interaction of DNA with the elution buffer reagents. So, for the elution buffer we are using 10mM Tris-HCl, 0.5mM EDTA (pH 9.0).TE is a commonly used buffer solution in molecular biology, especially in procedures involving DNA or RNA. "TE" is derived from its components: Tris, a common pH buffer, and EDTA, a molecule that chelates cations like $Mg^{2+}$and Ca^{2+}The purpose of TE buffer is to solubilize DNA, while protecting it from

degradation. The pH is usually adjusted to 8.0 for both DNA and RNA. The respective DNA and RNA nucleases are completely inactive at pH 8.0 and can safely be used for storage of both DNA and RNA. EDTA further inactivates nucleases, by binding to metal cations required by these enzymes.

3. PROCEDURE

(a) *E.coli* DH5α culture was grown culture in the 100ml conical flask.
(b) 1ml culture from the flask was taken and poured it in the 1.5ml eppendorf tube.
(c) The cells were centrifuged at 6000rpm for 5 minutes.
(d) Pellet of cells was formed and the supernatant was removed.
(e) The pellet was dissolved in the 100µl of 3µl RNAse containing Tris-EDTA buffer.
(f) The cells were lysed using the SDS lysis buffer and 200µl of Guanidinium Hydrochloride was added.
(g) 2µl of silica coated MNPs was added to the lysis solution.
(h) The eppendorf tube was placed on the external magnet to attract the MNPs.
(i) The pellet of MNPs was kept and the supernatant removed.
(j) 70% Ethanol was used as a washing buffer and add it's in the 1.5ml eppendorf was added and hold there as it as for 5 seconds.
(k) Again the eppendorf tube was placed on the external magnet to attract the MNPs.
(l) Pellet of MNPs was formed at the bottom of the eppendorf tube and supernatant of the washing buffer was removed.
(m) Tris – EDTA with pH 9.0 was used as an elution buffer and its 300µl was added to the eppendorf tube and hold there as it is for almost 5 seconds.
(n) Again the eppendorf tube was placed over the external magnet to attract the MNPs.
(o) The supernatant was taken and poured in another 1.5ml eppendorf tube as now it contained the DNA and MNPs pellet was thrown.
(p) The DNA was analysed using Nanodrop Spectrophotometer and agarose gel electrophoresis.

4. EXPERIMENT 1

4.1 Aim

To extract the DNA from *E.coli DH5α* using silica coated magnetic particles and to determine the exact concentration of silica coated magnetic particles needed to extract the DNA from overnight grown 1ml of *E.coli DH5α* culture.
Surface: COOH, size: 250nm
Quantity: 10ml, 10mg/ml
Suspension in water
Storage: 2-8°C
Micromod

Table 1: Amount of *E.*coli genomic DNA extracted using different concentration of silica coated MNPs along with their respective absorbance values and purity analysis.

Silica coated MNPs	Concentration (ng/µl)	A260	A280	260/280	260/230
50µg/ml	49.0	0.999	0.588	1.70	0.25
100µg/ml	44.1	0.882	0.517	1.71	0.34
150µg/ml	44.4	0.888	0.481	1.85	0.28
200µg/ml	36.7	0.734	0.431	1.70	0.28
250µg/ml	30.4	0.607	0.334	1.82	0.24
300µg/ml	14.5	0.290	0.116	2.50	0.18
350µg/ml	30.2	0.604	0.287	2.11	0.22
400µg/ml	22.1	0.441	0.218	2.02	0.24
450µg/ml	24.1	0.481	0.251	1.92	0.26
Positive Control	29.0	0.579	0.303	1.91	1.16

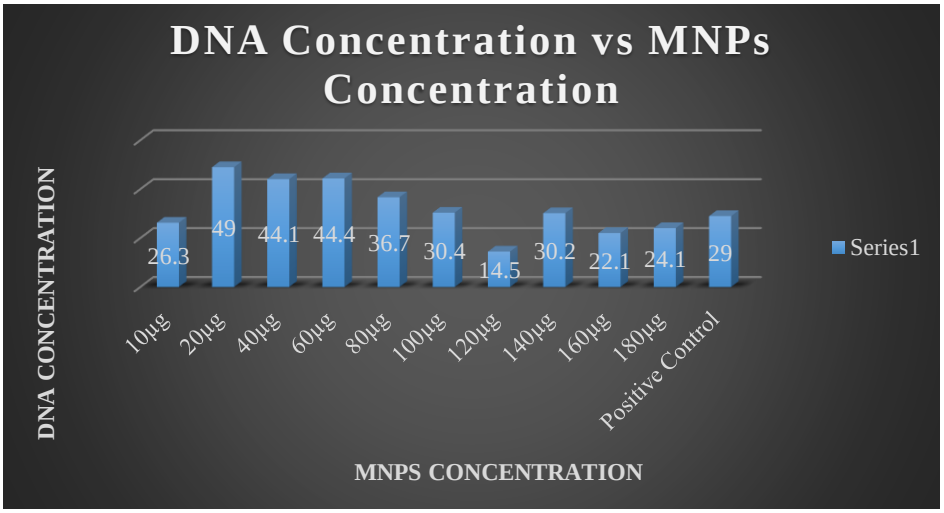

Fig. 1: Histogram showing the amount of DNA extracted from *E.coli* DH5α cells using different concentration of silica coated MNPs.

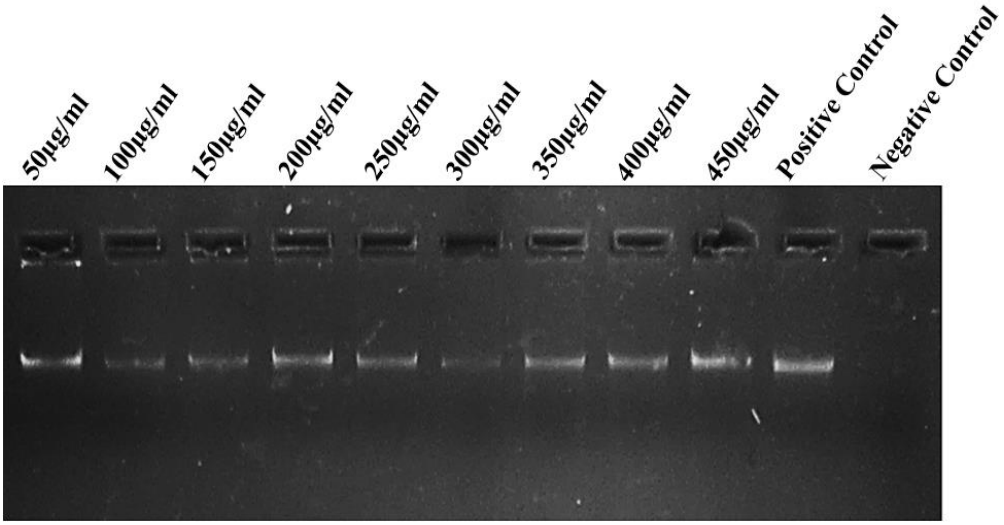

Fig. 2: Agarose gel electrophoresis analysis of genomic DNA extracted from *E.coli* DH5α cells at different concentrations of silica coated MNPs.

10µg - 180µg: Silica coated MNPs concentration.
Positive Control: *E.coli* DH5α genomic DNA purified using QiaAMP DNA mini kit.
Negative Control: Mixture of lysis Buffer and elution Buffer.

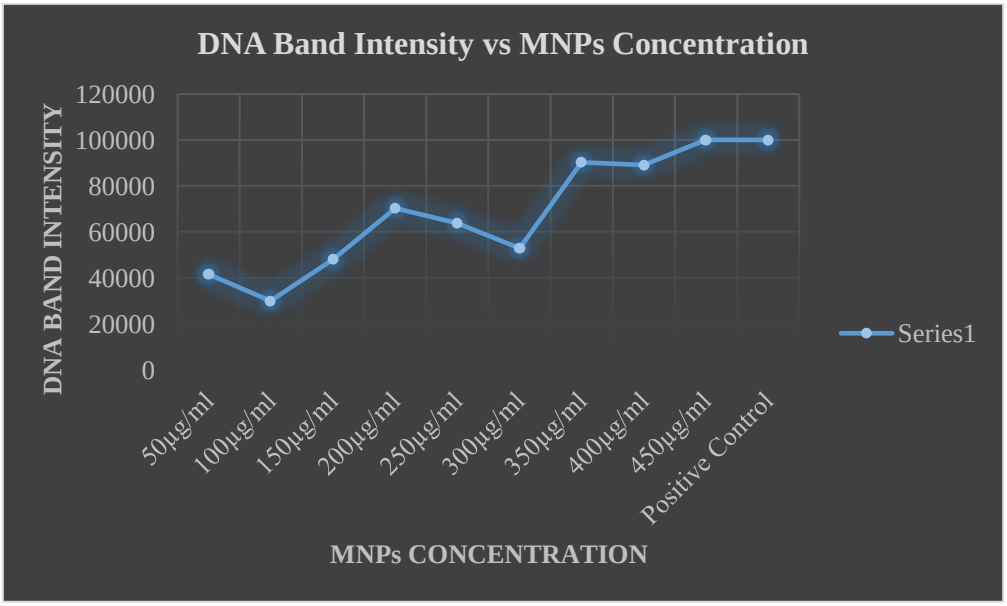

Fig. 3: DNA band intensity curve at different concentration of silica coated MNPs obtained through the Image software

5. EXPERIMENT 2

5.1 Aim

To extract the DNA from *E.coli* using silica coated magnetic particles and to determine the exact washing volume required for the washing buffer to wash out the unwanted contaminants from the DNA bound MNPs solution.

Table 2: Analysis of the amount of 70% Ethanol required as a washing buffer to remove all the contaminants and get the optimum purity of the DNA.

Washing Volume	Concentration (ng/µl)	A260	A280	260/280	260/230
No Wash	130	2.60	1.003	2.59	0.18
50µl	37.0	0.740	0.297	2.49	0.20
100µl	40.1	0.801	0.321	2.5	0.17
200µl	37.6	0.752	0.298	2.52	0.17
300µl	70.0	1.40	0.618	2.26	0.47
400µl	22.1	0.443	0.175	2.52	0.33
500µl	8.2	0.131	0.026	5.04	0.14
600µl	12.0	0.239	0.075	3.19	0.16
800µl	10.5	0.209	0.075	2.79	0.17
1000µl	11.7	0.234	0.076	3.07	0.16
Positive Control	33.5	0.671	0.354	1.90	0.99

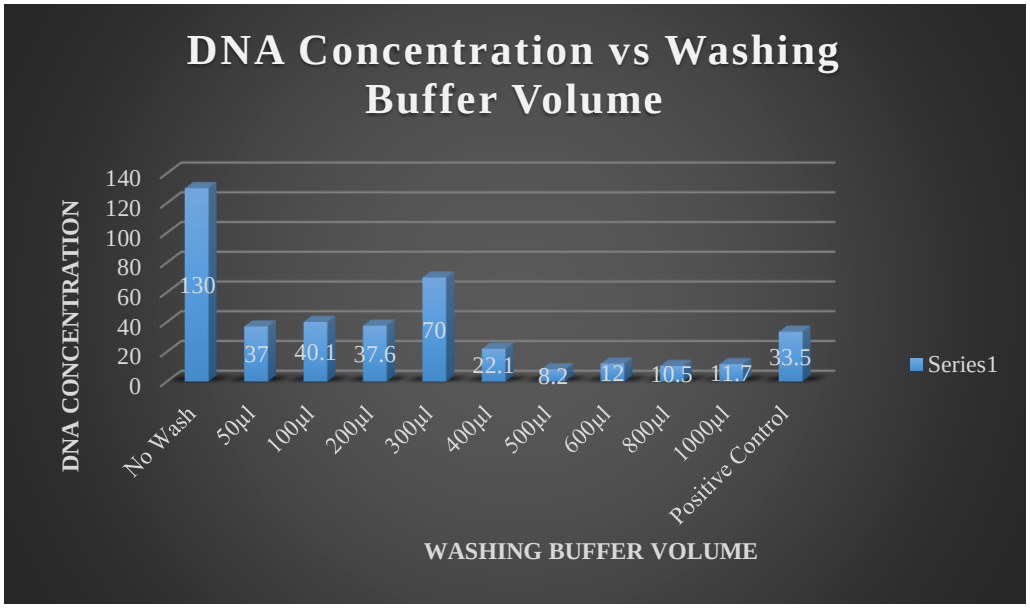

Fig. 4: Histogram showing the optimum volume of 70% ethanol required as the washing buffer to remove all the contaminants and purify the DNA.

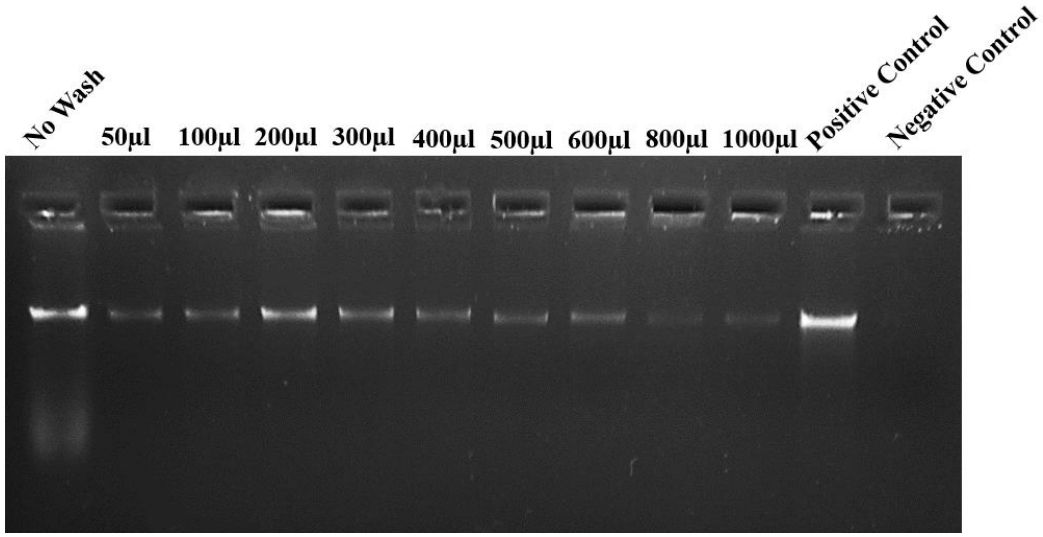

Fig. 5: Agarose gel electrophoresis analysis of genomic DNA extracted from *E.coli* DH5α cells at different amounts of volume of 70% Ethanol used as a washing buffer

50µl - 1000µl: Different volumes of 70% ethanol used to purify the DNA.
Positive Control: *E.coli* DH5α genomic DNA purified using QiaAMP DNA mini kit.
Negative Control: Mixture of lysis Buffer and elution Buffer.

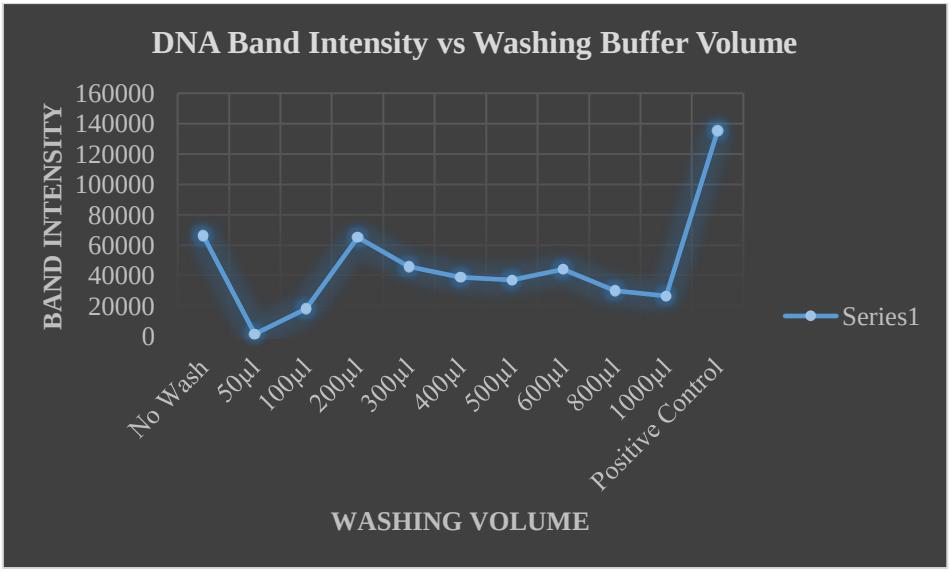

Fig. 6: DNA band intensity curve at different amount of volume of washing buffer obtained through the Imagej software.

6. EXPERIMENT 3

6.1 Aim

To extract the DNA from *E.coli* using silica coated magnetic particles and to optimize the washing timings required for the washing buffer to wash out all the unwanted contaminants and purify the DNA.

Table 3: Analysis of the washing treatment timings required to wash out all the unwanted contaminants and purify the DNA

Washing Timings	Concentration (ng/µl)	A260	A280	260/280	260/230
5seconds	54.1	1.081	0.481	2.25	0.18
10seconds	24.5	0.491	0.227	2.17	0.20
30seconds	24.8	0.496	0.217	2.28	0.20
1minute	27.7	0.553	0.263	2.11	0.22
10minutes	22.7	0.453	0.195	2.32	0.19
30minutes	19.4	0.366	0.177	2.07	0.22
1Hour	21.7	0.434	0.202	2.15	0.21
5Hours	9.3	0.186	0.087	2.15	0.25
10Hours	17.4	0.348	0.180	1.94	0.23
Positive Control	33.6	0.517	0.159	1.99	1.67

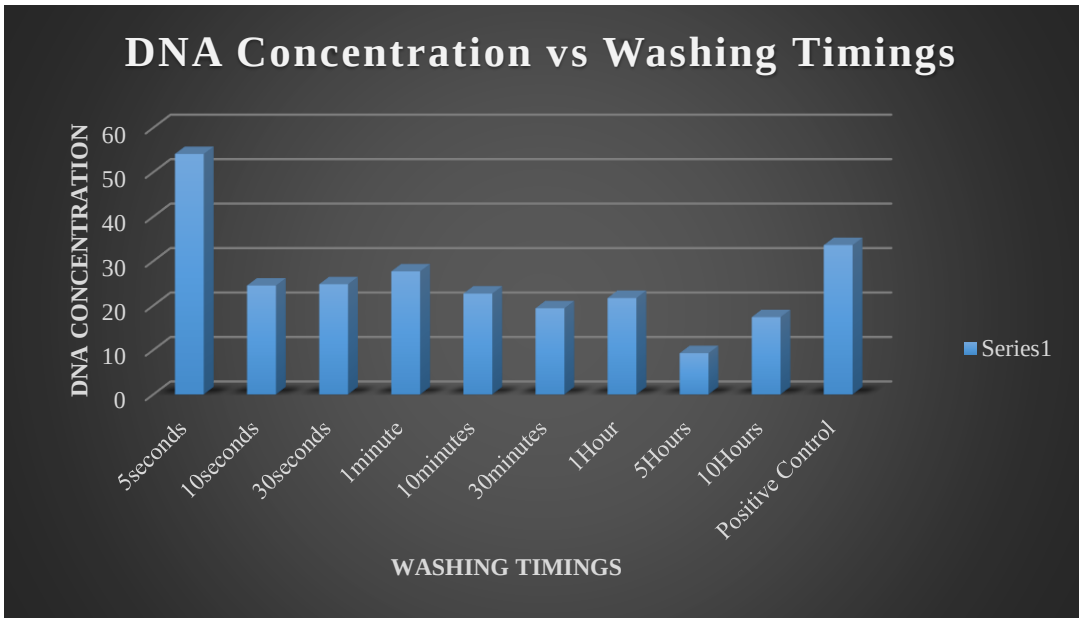

Fig. 7: Histogram showing the optimum washing timing required for the washing buffer to wash out all the contaminants and purify the DNA.

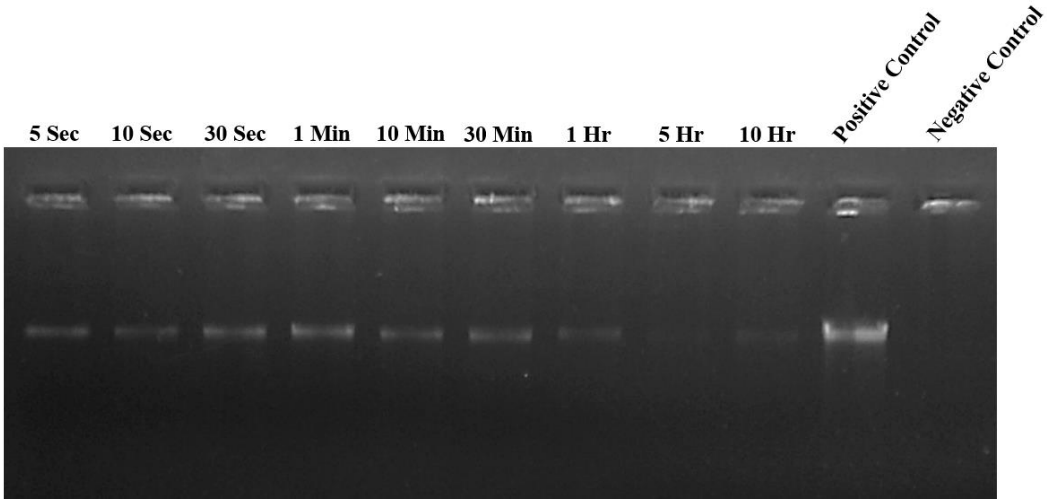

Fig. 8: Agarose gel electrophoresis analysis of genomic DNA extracted from *E.coli* DH5α cells when washing is done at different periods of incubation

50µl - 1000µl: Different volumes of 70% ethanol used to purify the DNA.
Positive Control: *E.coli* DH5α genomic DNA purified using QiaAMP DNA mini kit.
Negative Control: Mixture of lysis Buffer and elution Buffer.

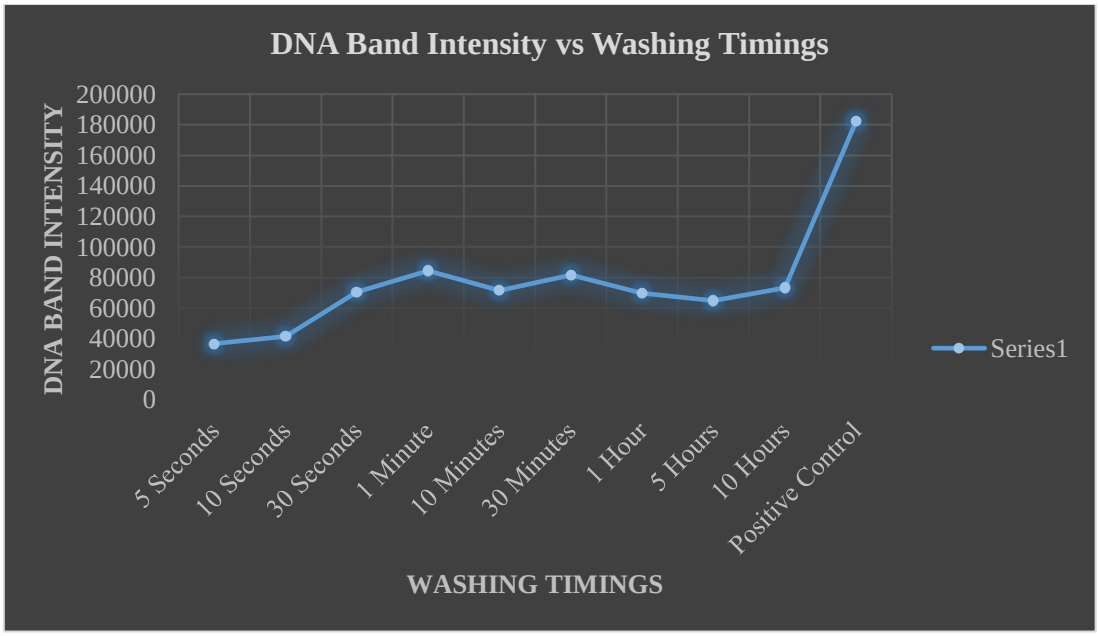

Fig. 9: DNA band intensity curve at different incubation periods of washing treatment obtained through the Imagej software.

7. EXPERIMENT 4

7.1 Aim

To extract the DNA from *E.coli* using silica coated magnetic particles and to determine the exact elution volume required for the buffer to elute the DNA completely bound MNPs solution.

Table 4: Analysis of the amount of elution buffer volume required as a washing buffer to remove all the contaminants and get the optimum purity of the DNA

Elution Volume	Concentration (ng/µl)	A260	A280	260/280	260/230
50µl	77.0	1.540	0.750	2.05	0.21
100µl	39.5	0.791	0.584	1.35	0.19
200µl	44.8	0.896	0.580	1.55	0.21
300µl	39.4	0.788	0.437	1.80	23.5
400µl	23.5	0.465	0.256	1.82	0.20
500µl	20.4	0.407	0.221	1.84	0.22
600µl	19.2	0.385	0.204	1.89	0.20
800µl	10.6	0.212	0.130	1.64	0.22
1000µl	8.3	0.166	0.101	1.65	.25
Positive Control	33.6	0.672	0.335	2.01	1.57

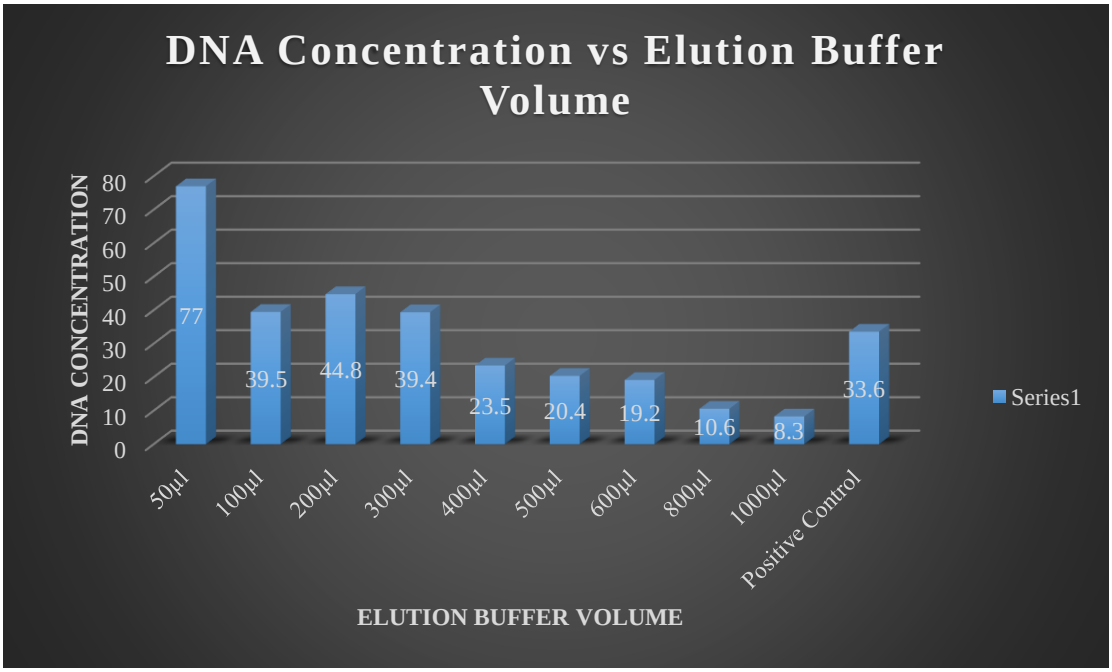

Fig. 10: Histogram showing the optimum volume of elution buffer required to obtain the sufficient amount of DNA concentration.

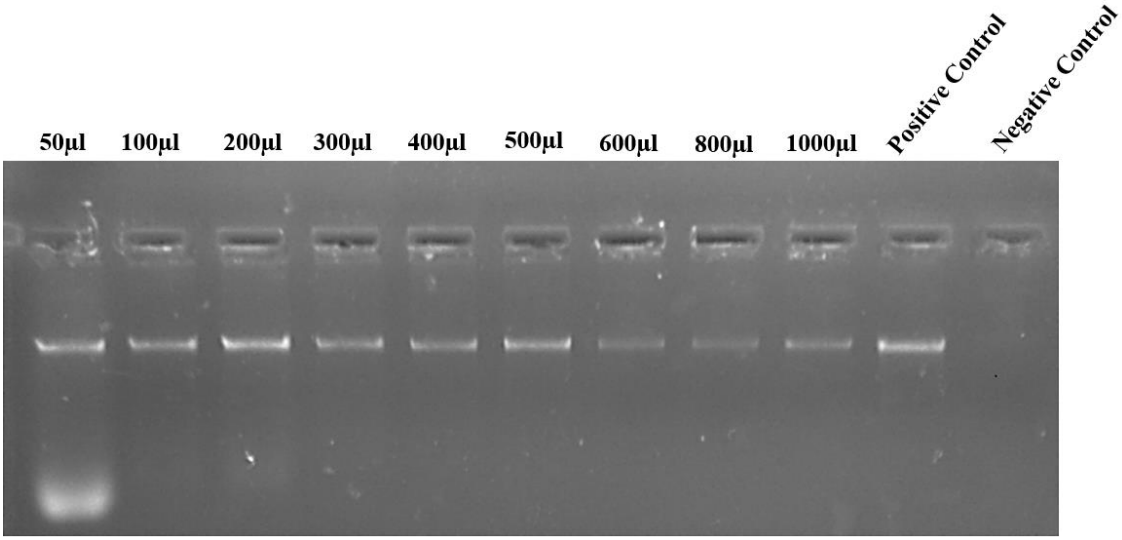

Fig. 11: Agarose gel electrophoresis analysis of genomic DNA extracted from *E.coli* DH5α cells at different amounts of volume of elution buffer.

50µl - 1000µl: Different volumes of 70% ethanol used to purify the DNA.
Positive Control: *E.coli* DH5α genomic DNA purified using QiaAMP DNA mini kit.
Negative Control: Mixture of lysis Buffer and elution Buffer.

