STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 70, 1, 2025 (p. 7-18)
doi:10.24193/subbbiol.2025.1.01

# Fumed silica-coated magnetic particles: DNA extraction application

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*Article history: Received 15 January 2025; Revised 03 March 2025; Accepted 11 March 2025; Available online 25 June 2025*



**Abstract.** Tetraethyl orthosilicate (TEOS) is widely used for preparing magnetic particles in DNA extraction kits, but it poses toxicity and safety concerns. To address this issue, we developed magnetic particles using fumed silica as a safer alternative to TEOS for magnetite particle preparation for DNA extraction. The method effectively extracts DNA from various biological matrices, including urine, serum, saliva, sputum, whole blood, and plasma, without detectable inhibitors. The extracted DNA is suitable for downstream molecular applications such as qPCR and sequencing. The proposed magnetic particles with fumed silica not only reduce chemical hazards but also provide a safer, efficient, and reliable method for routine DNA extraction in molecular biology laboratories.

**Keywords:** DNA extraction, fumed silica, magnetite

## Introduction

DNA extraction is a routine procedure that has become essential in various molecular laboratories. The demand for DNA extraction kits has risen due to the need for a straightforward, simple procedure that can handle a wide range of samples. Many available kits are based on spin-column and magnetic

nanoparticle technologies, which offer reliable and efficient solutions for DNA extraction. Spin-column kits utilize a silica membrane to bind DNA, while magnetic nanoparticle kits use magnetic particles to isolate DNA.

Unlike spin-column kits, those using magnetic nanoparticles require relatively lower energy, as they utilize magnetic force to separate DNA from impurities. Besides magnetic nanoparticles, the materials used include coating agents to prevent $Fe_3O_4$ from oxidizing into $Fe_2O_3$, which has weaker ferromagnetic properties (Dzeranov *et al.,* 2023). Tetraethyl Orthosilicate (TEOS) is the most commonly used coating agent (Fan *et al.,* 2019; Torres-Rodríguez *et al.,* 2019; Alghuthaymi, 2020; Nguyen *et al.,* 2022; Firoozeh *et al.,* 2023).

TEOS has irritating properties to the eyes and respiratory tract, as stated in its material safety data sheet. TEOS serves as a source of silica. Another source of silica that lacks these irritant properties is fumed silica. Fumed silica is produced from the hydrolysis of silicon tetrachloride by forced evaporation with combustion (Flörke *et al.,* 2012). Consequently, fumed silica could be a safer alternative to TEOS.

This study aims to test fumed silica-coated magnetic particles for DNA extraction from various matrices, including serum, urine, saliva, sputum, whole blood, and plasma, and to evaluate the suitability of the extracted DNA for sequencing procedures.

## Materials and methods

### *Material*

The materials used in this study included $FeSO_4$ and $FeCl_3$ as precursors; tetraethyl orthosilicate (TEOS), ammonia solution, NaOH, Tris, and EDTA from Merck (Darmstadt, Germany); fumed silica from Wacker (Nünchritz, Germany); Triton-X from Vivantis (Selangor, Malaysia); SDS from BASF (Hong Kong); PEG 6000 from Clariant (Gendorf, Germany); NaCl from Dominion Salt (Mount Maunganui, New Zealand); and isopropanol and ethanol from Bratachem (Solo, Indonesia).

The bacterial strain used for DNA extraction was *Mycobacterium tuberculosis* ATCC 25177 from Microbiologics (United States). The media used for bacterial growth were Lowenstein Jensen and Trypticase Soy Broth (TSB), both purchased from Himedia (Mumbai, India). Specimens were obtained from the Konimex Diagnostic Center repository (Solo, Indonesia).

### *Magnetite synthesis*

$FeSO_4$ and $FeCl_3$, with a molarity ratio of 1:2, were mixed in water. NaOH solution was added dropwise while stirring until the pH reached 8-10. The solution was then allowed to settle, and the supernatant was decanted. The

precipitate was washed twice with water. The washed precipitate was dried in the oven at 60-100°C for two hours. The dried magnetite was then coated with fumed silica using a previously defined protocol (Thangaraj *et al.,* 2019). In this protocol, TEOS was substituted with 20 µg/mg fumed silica per magnetite. The resulting particles are referred to as fumed silica-coated magnetic particles (FsMP).

### *FsMP regeneration test*

FsMP was used to extract DNA using previously defined protocol (Tjoa *et al.,* 2025) from *M. tuberculosis* culture. The purity factor was measured using a nanophotometer at 260/280 nm. The DNA was also amplified using a quantitative Polymerase Chain Reaction (qPCR) CFP10 gene amplification kit from Konimex Diagnostic Center (Solo, Indonesia) using MicPCR machine to obtain the Cycle threshold (Ct) value. The used FsMP was washed with 500 µl elution buffer and reused for DNA extraction. DNA extraction using the same FsMP were repeated five times with three replications. The purity factors and Ct values from the repeated steps were compared.

### *Matrices suitability test*

FsMP was used to extract DNA from *M. tuberculosis* culture spiked into urine, serum, saliva, sputum, whole blood, and plasma. The extracted DNA was diluted into three consecutive dilutions. Each dilution was then amplified using a qPCR CFP10 gene amplification kit to obtain Ct values. The Ct values and dilutions were plotted to create a regression equation, which was used to determine the PCR efficiency value. The ΔCt value was calculated by comparing the actual Ct result with the Ct value predicted by the regression equation. These values were used to assess inhibition in the PCR process.

### *Sequencing*

The amplicon or PCR product, from *M. tuberculosis* obtained in the PCR step using qPCR CFP10 gene amplification kit from Konimex Diagnostic Center (Solo, Indonesia) using MicPCR machine underwent sequencing using the Sanger method, specifically the single-pass, two-reaction approach. The sequencing results were aligned with the *M. tuberculosis* reference genome and the nucleotide database using BLAST from NCBI

## Results and Discussion

FsMP was successfully synthesized and evaluated for its potential use in repeated DNA extraction. The regeneration test was conducted to assess its

reusability and efficiency across multiple DNA extraction cycles, providing insight into the material's stability and consistent performance. The test was performed using one loop of *M. tuberculosis* in 1 ml of TSB media, with DNA extraction carried out five times in three independent repetitions. The extracted DNA was assessed for purity based on the A260/280 ratio and its suitability for PCR amplification. The results are presented in Fig. 1.

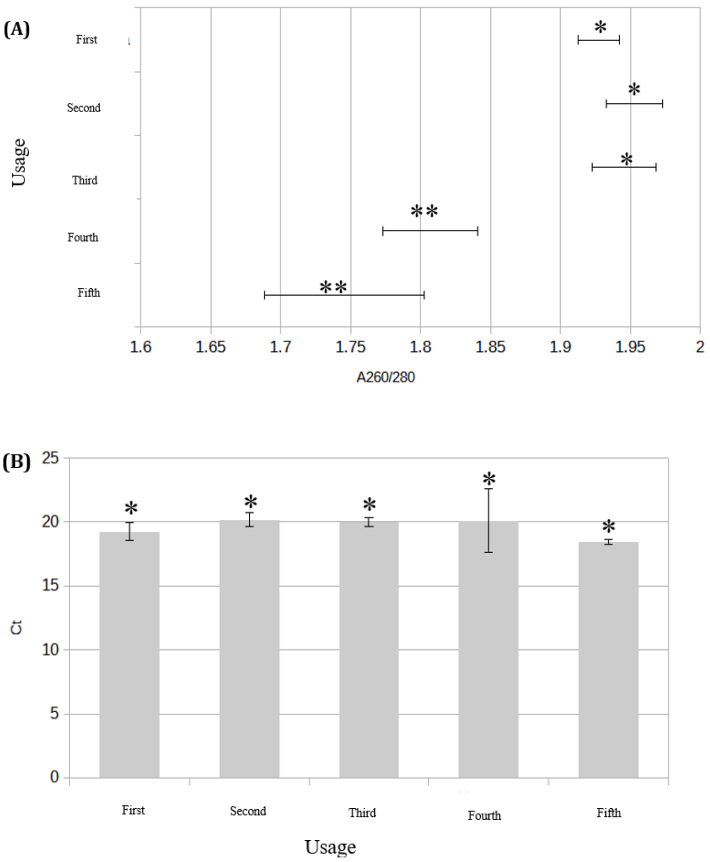



**Figure 1.** (A) A260/280 comparison and (B) Ct value comparison from extracted DNA using FsMP in 5 times repeatance. Notation (*) showed same group based on post-hoc analysis using Tukey with α value: 0.05.

Data analysis was performed by comparing the A260/280 ratios and Ct values from each repetition. The DNA purity values obtained from repeated FsMP extractions consistently fell within the range of 1.6 to 2.0, indicating acceptable purity. From the third repetition onward, the purity values slightly

differed from the initial use, though they remained within the acceptable range. The Ct value represents the relative concentration of DNA obtained based on the number of PCR amplification cycles required for detection. The Ct values remained consistent across all extractions, indicating that the DNA concentration did not vary significantly even after five consecutive uses of this particular preparation of FsMP.

A matrices suitability test was conducted using matrices spiked with *M. tuberculosis* culture at a 1:1 ratio. The extracted DNA was subsequently amplified using qPCR. The resulting qPCR amplicon was visualized on an electrophoresis gel, as shown in Fig. 2, revealing a DNA band of approximately 120 bp in length. This indicates successful amplification. Furthermore, the DNA extracted from the tested matrices did not introduce any inhibitors that could interfere with the PCR reaction.

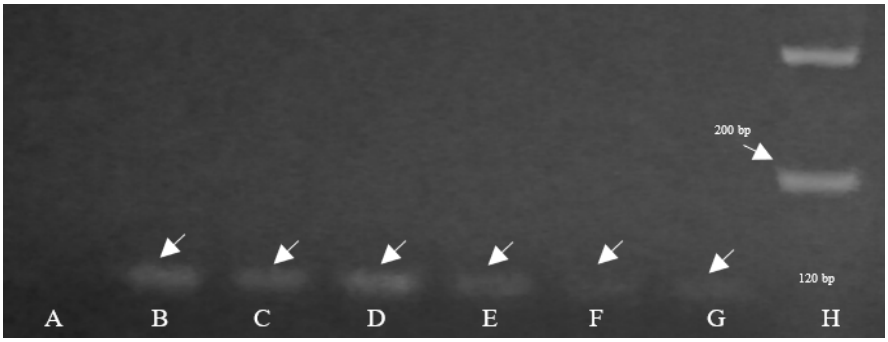



**Figure 2.** Amplicon visualization with electrophoresis. Amplicon size around 120 bp. (A) Negative. (B) Culture. (C) In urine. (D) In serum. (E) In saliva. (F) In whole blood. (G) In sputum. (H) Ladder.

Experiments using various types of specimens were also conducted to evaluate the efficiency of qPCR. qPCR efficiency reflects the capacity of all components in the reaction to amplify DNA. Ideally, each PCR cycle results in the duplication of DNA, producing two copies from a single DNA template, which corresponds to an efficiency value of 100%. The method for calculating DNA efficiency is presented in the following formula (Nybo, 2018).

$$PCR\ efficiency\ (\%) = 100\ x\ \left(10^{\left(\frac{-1}{a}\right)} - 1\right)$$

$a = slope.$
$y = ax + b$
$y = Ct$
$x = Log\ (concentration)$

PCR efficiency testing was performed on DNA extracts prepared in three dilutions. The results are presented in Fig. 3, which includes a normalized fluorescence versus cycle graph, an efficiency calculation plot, and a ΔCt comparison between the observed and calculated Ct values. PCR efficiency values approaching 100% (75% - 110%) with $R^2$ values ≥ 0.98 indicate that the PCR reactions are running efficiently, unaffected by inhibitors (which can lower efficiency) or activators (which can inflate efficiency beyond optimal levels) (Booth *et al.,* 2010).

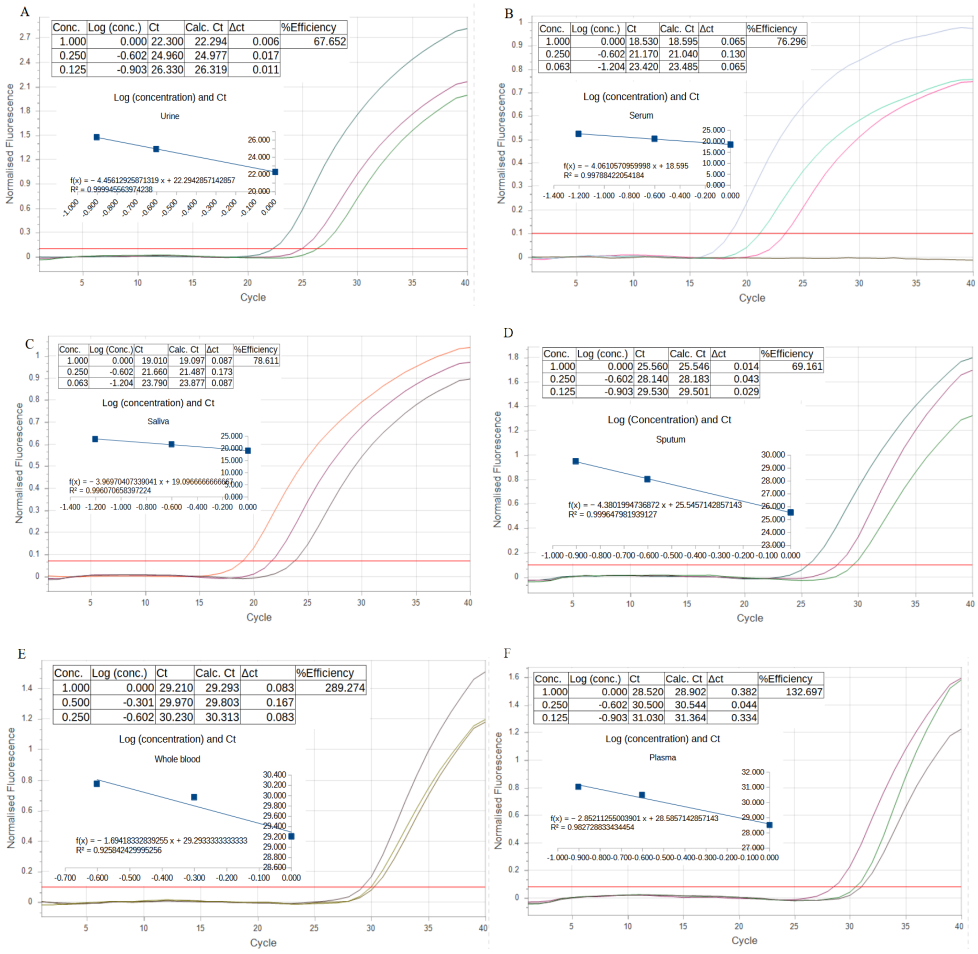



**Figure 3.** Normalised fluorescence and cycle graph, efficiency calculation graph, and ΔCt comparison of actual and calculated Ct for six matrices. (A) Urine. (B) Serum. (C) Saliva. (D) Sputum. (E) Whole blood. (F) Plasma.

The PCR efficiencies for *M. Tuberculosis* in urine, saliva, serum, sputum, whole blood, and plasma were 67.652% ($R^2$ = 1), 76.296% ($R^2$ = 0.998), 78.611% ($R^2$ = 0.996), 69.161% ($R^2$ = 1), 289.274% ($R^2$ = 0.926), and 132.697% ($R^2$ = 0.983), respectively. The PCR efficiencies ranged from 67.652% to 289.274%. Efficiency values for urine and sputum specimens were below 75%, indicating poor compatibility of the PCR process with the extracted DNA. To improve compatibility, an additional step to remove PCR inhibitors was necessary, such as the removal of urea and crystals from urine (Munch *et al.,* 2019) and biological factors from sputum (Reed *et al.,* 2016).

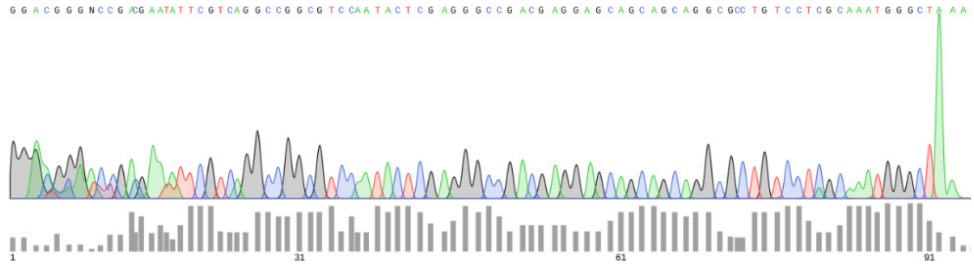
DNA extracted from whole blood and plasma exhibited efficiency values exceeding 110%, indicating that FsMP was ineffective in fully removing impurities that interfere with PCR. the excessive efficiency values likely result from PCR artifacts or altered reaction kinetics caused by residual contaminants. Potential interfering impurities in blood include heme, leukocytes, and anticoagulant compounds such as EDTA and heparin (Al-Soud and Rådström, 2001).

Plasma was included in the efficiency analysis to assess its impact on PCR performance. While separating plasma from red blood cells helps reduce interference by minimizing cellular components, plasma still contains residual contaminants such as such as immunoglobulin G, which can affect the PCR reaction (Al-Soud and Rådström, 2001). This persistent interference likely to contributes to the efficiency value exceeding 110%.

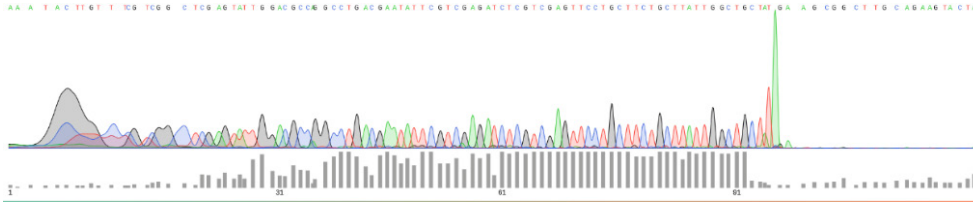
PCR efficiency is influenced by several factors beyond the quality of the DNA extract. It can be affected by the choice of primers, polymerase, and PCR parameters, as well as the compatibility of these components (Booth *et al.,* 2010; Louw *et al.,* 2011). DNA extracts can be considered free from inhibitors and activators if the ΔCt value is < 0.5 (Waiblinger and Grohmann, 2014).

The ΔCt value represents the difference between the actual Ct value and the Ct value predicted by the line equation. The ΔCt values for all specimens and their respective dilutions met the criteria for inhibitor-free DNA extracts. These results indicate that FsMP is capable of effectively extracting DNA from various specimens, including urine, serum, saliva, blood, sputum, and plasma.

PCR products were subsequently sequenced using the Sanger method, employing a single-pass DNA sequencing with two reactions. Two-direction sequencing was performed, using both forward and reverse primers. The sequencing results, presented in Fig. 4, show the CFP10 fragment gene chromatogram. The chromatogram reveals that at least one position in the forward sequence has an unreadable nucleotide, whereas all nucleotides in the reverse sequence are clearly readable. The differences observed between the forward and reverse sequences may be caused by sequence artifacts, PCR amplification inaccuracies, or DNA degradation during sample preparation (Al-Shuhaib & Hashim, 2023). However, the sequencing data remain reliable, as reads labeled with "N" account for less than 5% of the total reads (Crossley *et al*., 2020).



>Forward direction
GGACGGGNCCGACGAATATTCGTCAGGCCGGCGTCCAATACTCGAGGGCCGACGA
GGAGCAGCAGCAGGCGCCTGTCCTCGCAAATGGGCTAAAC



>Reverse direction
TCGTCGGCTCGAGTATTGGACGCCAGGCCTGACGAATATTCGTCGAGATCTCGTC
GAGTTCCTGCTTCTGCTTATTGGCTGCT

**Figure 4.** Chromatogram and FASTA format of the sequencing result for the CFP10 fragment gene.

Further analysis was performed using BLAST through the web services provided by NCBI (Zhang *et al.,* 2000). The reference genome used was NC_00962.3 from *Mycobacterium tuberculosis* H37Rv, a complete genome with a length of 4,411,532 bp. The BLAST results are presented in Fig. 5.

From the alignment results, both sequences showed a percentage identity (per ident) greater than 95%, with the largest gaps being 3%. These results indicate that the sequences produced from CFP10 gene fragment could be successfully read and identified as *Mycobacterium tuberculosis*, the organism from which the DNA was extracted. This finding is further supported by the BLAST results from the nucleotide database collection at NCBI, conducted on August 31, 2024, and presented in Fig. 6. Among the first 100 entries retrieved, 98 sequences were identified as *Mycobacterium tuberculosis* for both the forward and reverse sequences. The per ident values for the forward and reverse sequences were 98.74% and 96.74%, respectively.

(A)

Sequence ID: Query_4394065 Length: 95 Number of Matches: 1

**Range 1: 10 to 91** Graphics ▼ Next Match ▲ Previous Match

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 145 bits(78) | 3e-35 | 81/82(99%) | 1/82(1%) | Plus/Plus |

```
Query  4352491  CGACGAATATTCGTCAGGCCGGCGTCCAATACTCGAGGGCCGACGAGGAGCAGCAGCAGG  4352550
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  10       CGACGAATATTCGTCAGGCCGGCGTCCAATACTCGAGGGCCGACGAGGAGCAGCAGCAGG  69

Query  4352551  CG-CTGTCCTCGCAAATGGGCT  4352571
                || |||||||||||||||||||
Sbjct  70       CGCCTGTCCTCGCAAATGGGCT  91
```

Sequence ID: Query_4394064 Length: 83 Number of Matches: 1

(B)

**Range 1: 1 to 83** Graphics ▼ Next Match ▲ Previous Matc

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 137 bits(74) | 4e-33 | 82/85(96%) | 3/85(3%) | Plus/Minus |

```
Query  4352453  AGCAGCCAATAAGCAGAAGCAGGAACTCGACGAGATCTCGACGAATATTCGTCAGGCC-G  4352511
                |||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  83       AGCAGCCAATAAGCAGAAGCAGGAACTCGACGAGATCTCGACGAATATTCGTCAGGCCTG  24

Query  4352512  GCGTCCAATACTCGAGGGCCGACGA  4352536
                |||||||||||||||  |||||||
Sbjct  23       GCGTCCAATACTCGAG--CCGACGA  1
```

**Figure 5.** (A) CFP10 gene fragment forward direction sequence alignment. (B) CFP10 gene fragment reverse direction sequence alignment.

**(A)**

| Description | Scientific Name | Max Score | Total Score | Query Cover | E value | Per. Ident | Acc. Len | Accession |
|---|---|---|---|---|---|---|---|---|
| Mycobacterium tuberculosis strain 0B049XDR genome | Mycobacterium tuberculosis | 147 | 147 | 86% | 6e-31 | 98.78% | 4539433 | CP008971.1 |
| Mycobacterium orygis strain NIAB_BDWBCSHFL_1 chromosome, complete genome | Mycobacterium orygis | 145 | 145 | 86% | 2e-30 | 98.78% | 4347055 | CP138660.1 |
| Mutant Mycobacterium tuberculosis variant bovis isolate PHOPR KO3, partial genome | Mycobacterium tuberculosis variant bo… | 145 | 145 | 86% | 2e-30 | 98.78% | 4349904 | CP133601.1 |
| Mutant Mycobacterium tuberculosis variant bovis isolate PHOPR KO1, partial genome | Mycobacterium tuberculosis variant bo… | 145 | 145 | 86% | 2e-30 | 98.78% | 4349896 | CP133603.1 |
| Mycobacterium tuberculosis variant bovis strain AF2122/97 chromosome | Mycobacterium tuberculosis variant bo… | 145 | 145 | 86% | 2e-30 | 98.78% | 4349904 | CP133604.1 |
| Mutant Mycobacterium tuberculosis variant bovis isolate PHOPR KO2, partial genome | Mycobacterium tuberculosis variant bo… | 145 | 145 | 86% | 2e-30 | 98.78% | 4349904 | CP133602.1 |
| Mycobacterium tuberculosis strain AST-T7 chromosome, complete genome | Mycobacterium tuberculosis | 145 | 145 | 86% | 2e-30 | 98.78% | 4399274 | CP133045.1 |
| Mycobacterium tuberculosis strain AST-T3 chromosome, complete genome | Mycobacterium tuberculosis | 145 | 145 | 86% | 2e-30 | 98.78% | 4411248 | CP133041.1 |
| Mycobacterium tuberculosis strain AST-T4 chromosome, complete genome | Mycobacterium tuberculosis | 145 | 145 | 86% | 2e-30 | 98.78% | 4412499 | CP133042.1 |
| Mycobacterium tuberculosis strain AST-T6 chromosome, complete genome | Mycobacterium tuberculosis | 145 | 145 | 86% | 2e-30 | 98.78% | 4420361 | CP133044.1 |

**(B)**

| Description | Scientific Name | Max Score | Total Score | Query Cover | E value | Per. Ident | Acc. Len | Accession |
|---|---|---|---|---|---|---|---|---|
| Mycobacterium orygis strain NIAB_BDWBCSHFL_1 chromosome, complete genome | Mycobacterium orygis | 137 | 137 | 100% | 3e-28 | 96.47% | 4347055 | CP138660.1 |
| Mycobacterium tuberculosis strain Beijing, complete genome | Mycobacterium tuberculosis | 137 | 137 | 100% | 3e-28 | 96.47% | 4378588 | CP011510.1 |
| Mycobacterium tuberculosis strain MTBCR170941 chromosome, complete genome | Mycobacterium tuberculosis | 137 | 137 | 100% | 3e-28 | 96.47% | 4396671 | CP104271.1 |
| Mycobacterium tuberculosis strain MTBCR170001 chromosome, complete genome | Mycobacterium tuberculosis | 137 | 137 | 100% | 3e-28 | 96.47% | 4424759 | CP097112.1 |
| Mycobacterium tuberculosis strain MTBCR170020 chromosome, complete genome | Mycobacterium tuberculosis | 137 | 137 | 100% | 3e-28 | 96.47% | 4442985 | CP097111.1 |
| Mycobacterium tuberculosis strain MTBCR170027 chromosome, complete genome | Mycobacterium tuberculosis | 137 | 137 | 100% | 3e-28 | 96.47% | 4456234 | CP097110.1 |
| Mycobacterium tuberculosis strain MTBCR170031 chromosome, complete genome | Mycobacterium tuberculosis | 137 | 137 | 100% | 3e-28 | 96.47% | 4417031 | CP097109.1 |
| Mycobacterium tuberculosis strain SCAID 252.0, complete genome | Mycobacterium tuberculosis | 137 | 137 | 100% | 3e-28 | 96.47% | 4439387 | CP016888.1 |
| Mutant Mycobacterium tuberculosis variant bovis isolate PHOPR KO3, partial genome | Mycobacterium tuberculosis variant bo… | 137 | 137 | 100% | 3e-28 | 96.47% | 4349904 | CP133601.1 |
| Mutant Mycobacterium tuberculosis variant bovis isolate PHOPR KO1, partial genome | Mycobacterium tuberculosis variant bo… | 137 | 137 | 100% | 3e-28 | 96.47% | 4349896 | CP133603.1 |

**Figure 6.** Ten first entries from BLAST in NCBI nucleotide collection. (A) Forward direction. (B) Reverse direction.

The sequencing analysis performed in this study utilized short-read sequencing. Overall, FsMP can be used to extract DNA suitable for downstream processes such as PCR and short-read sequencing.

For further exploration, the DNA extracted using FsMP can also be evaluated for its suitability in long-read sequencing, which may provide deeper insights and broaden its applications in genetic studies. Another recommendation is to compare different preparations of FsMP to assess batch-to-batch consistency in DNA extraction performance. This analysis will help determine the reproducibility and reliability of FsMP for downstream applications.

## Conclusions

In this study, we successfully prepared magnet particles which coated with fumed silica. Fumed silica is a safer option than TEOS as the coating agent. FsMP could be used in the DNA extraction for several type of biological samples such as serum, saliva, urine, sputum, whole blood, and plasma. Extracted DNA from FsMP could be used in the downstream process like PCR and short-read sequencing.

**Acknowledgements.** We would like to thank PT Konimex for the funds.

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