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RESEARCH ARTICLE

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Development and validation of a flexible DNA extraction (PAN) method for liquid biopsy of multiple sample types

Xiao-Xia Chen[1] | Wei Chen[2] | Yi-Long Liu[1] | Can-Xiang Lin[3] | Mengmeng Li[1] | Wen-Jie Chen[1] | Shang-Hang Xie[1,4] | Dong-Feng Lin[1] | Su-Mei Cao[1,4]

[1]Department of Cancer Prevention Research, Sun Yat-sen University Cancer Center, Guangzhou, China

[2]Department of Pathology, The Seventh Affiliated Hospital, Sun Yat-sen University, Shenzhen, China

[3]Department of Plastic Surgery, The First Affiliated Hospital of Jinan University, Guangzhou, China

[4]State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, and Guangdong Key Laboratory of Nasopharyngeal Carcinoma Diagnosis and Therapy, Sun Yat-Sen University Cancer Center, Guangzhou, China

Correspondence
Su-Mei Cao, Department of Epidemiology, Cancer Prevention Center, State Key Laboratory of Oncology in Southern China, Sun Yat-sen University Cancer Center, 651 Dongfeng East Road, Guangzhou 510060, China.
Email: caosm@sysucc.org.cn

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Abstract

Background: Liquid biopsy is gaining increasing popularity in cancer screening and diagnosis. However, there is no relatively mature DNA isolation method or commercial kit available that is compatible with different LB sample types. This study developed a PAN-sample DNA isolation method (PAN method) for liquid biopsy samples.

Methods: The PAN method has two key steps, including biosample-specific pretreatments for various LB sample types and high concentration guanidine thiocyanate buffer for lysis and denaturation procedure. Subsequently, the performance of PAN method was validated by a series of molecular analyses.

Results: The PAN method was used to isolate DNA from multiple sample types related to LB, including plasma, serum, saliva, nasopharyngeal swab, and stool. All purified DNA products showed good quality and high quantity. Comparison of KRAS mutation analysis using DNA purified using PAN method versus QIAamp methods showed similar efficiency. Epstein-Barr virus DNA was detected via Q-PCR using DNA purified from serum, plasma, nasopharyngeal swab, and saliva samples collected from nasopharyngeal carcinoma patients. Similarly, methylation sequencing of swab and saliva samples revealed good coverage of target region and high methylation of HLA-DPB1 gene. Finally, 16S rDNA gene sequencing of saliva, swab, and stool samples successfully defines the relative abundance of microbial communities.

Conclusions: This study developed and validated a PAN-sample DNA isolation method that can be used for different LB samples, which can be applied to molecular epidemiological research and other areas.

KEYWORDS
DNA extraction method, liquid biopsy, next-generation sequencing (NGS), Q-PCR, sanger sequencing (SS)

1 | INTRODUCTION

Clinical samples for nucleotide analysis can be generally classified into tissue biopsy (TB) and liquid biopsy (LB). TB samples, including bulk tumor tissue, endoscopic, and needle biopsy tissue, are considered gold standards for the diagnosis of cancers. However, TB is usually invasive and may not reflect the heterogeneity of tumors.[1] LB refers to the analysis of circulating tumor cells (CTCs), cell-free

circulating nucleic acids (cfDNA), exosomes, microvesicle, and platelets, etc, of which biosamples are involved include serum, plasma, swab, saliva, urine, and stool.[1,2] Compared with TB, LB is enjoying an increasing popularity for its less invasiveness, real-time detection, amenability to repeated sampling,[3-7] and its potential for early cancer diagnosis.[8] Although the promise of LB is high, there are still many challenges in both DNA extraction and analysis due to the complex characteristics of input DNA with low concentration, high fragment,[1] and the high background signals.[9] Especially, the various types of biofluids leading to a series of corresponding kits for DNA extraction make it even more complex.

DNA extraction is one of the most basic and routine techniques in the research and diagnostic labs that is performed with commercial kit most of the times nowadays. The success of molecular analyses, such as quantitative polymerase chain reaction (Q-PCR), next-generation sequencing (NGS), and sanger sequencing (SS), relies on high-quality DNA. Thus, it is very important to choose DNA-extracting methods that can yield both good quality and high quantity DNA.[9,10]

The first step in DNA isolation is usually cell lysing, which can be broadly classified into two categories. One is chemical-based methods, mainly involving the use of enzymes such as lysozyme[11-16] and proteinase K,[12,15-27] NaOH,[28-30] and detergents like sodium dodecyl sulfate (SDS),[11,12,15-17,21-24,26,31,32] cetyltrimethylammonium bromide (CTAB),[11,17,21,33,34] and guanidine thiocyanate.[13,35-37] Other is physical-based methods including grinding,[11-13,21,35,38] heating,[12,13,18,20,22,27] sonication,[39,40] liquid homogenization,[41] and freezing/thawing.[11] The combination of these two methods may help to lyse cell more efficiently. The second key step is the purification of DNA from cell lysing. Many different ways, such as alcohol precipitation,[42,43] binding by magnetic beads,[42,44,45] silica particles,[30,44] and the silicon column,[12,13,45,46] are applied.

The purification of cfDNA follows the same basic principles as general DNA isolation. Many different commercial kits are available for cfDNA purification from serum or plasma. These kits usually use magnetic beads or silica column to enrich cfDNA. Some studies suggest that magnetic beads give higher yield of cfDNA than the silica column, due to more efficient absorption of a broader range of DNA fragments (length ≥20 bp). Others argued that although silica column is only efficient for larger DNA fragments (for example, length ≥150 bp), it is enough to cover DNA fragments that are most useful for disease diagnosis.[2,47-49] For other liquid biopsy samples like microvesicle, urea, and stool, there are few options of commercial kits available.

Current DNA extraction methods or commercial DNA extraction kits have some disadvantages. Firstly, some kits require toxic volatile components, such as phenol, chloroform, and SDS.[17,50-54] Secondly, many methods involve complicated and laborious procedures,[11,37,55-58] which are inconvenient. Thirdly, most commercial DNA extraction kits developed for a designated specimens[10] are not flexible enough to be compatible with other types of samples. Finally, the currently available DNA purification kits are usually suitable for certain sample volume, which is not expandable to larger sample volumes. Thus, an easier, flexible, and expandable DNA isolation method for LB is in great need.

This study developed a DNA isolation method for liquid biopsy that could be used for multiple sample types and input sample volume. In view of a pan-cancer diagnostic sensor that it is broadly applicable sensor for a range of human cancers,[59] this DNA isolation method was likewise named "PAN" method that can purify DNA efficiently with good quality and quantity, which is suitable for different molecular analyses, including SA, Q-PCR, SS, MS, and 16S rDNA sequencing. Therefore, the PAN method may be a good alternative to commercial kit and can be used for molecular epidemiological research.

2 | MATERIALS AND METHODS

2.1 | Sample collection

50 blood samples were collected from 44 healthy adult volunteers (non-NPC) from an ongoing trial of NPC screening in Sihui, China,[60-62] and 6 NPC patients from the biobank of Sun Yat-sen University Cancer Center (SYSUCC; Table S1). Half of the blood samples (22 non-NPC and 3 NPC patients in each group) were subjected to plasma and serum isolation, respectively. Similarly, 50 saliva and 50 nasopharyngeal swab samples were collected from 94 healthy adult volunteers (non-NPC) from another trial for NPC screening,[44-46] and 6 NPC patients from the Nasopharyngeal Department of SYSUCC (Tables S2, S3). There were equal number of non-NPC and NPC in the two groups. The 45 normal gastric tissues were collected from the Department of Gastrointestinal Surgery of the First Affiliated Hospital of Sun Yat-sen University (SYSUFAH), (Table S4). The stool samples came from two cohorts. One cohort consisted of 50 stool samples from non-CRC (colorectal cancer) patients (Table S5). The other cohort consisted of 71 specimens, including 50 stools from healthy participants and 21 stools consisting of 5 CRC patients and 16 healthy controls, which were obtained from SYSUFAH (Table S6). The detailed design of this study was depicted in the workflow in Figure 1. This study was approved by the institutional review board (IRB: ZDMB-2020–001) of the coordinator center, and all the participants provided written informed consent. (Trial registration number: NCT02586532).

2.2 | The protocol for PAN method

2.2.1 | Pre-treatment of samples before DNA extraction

The PAN method was optimized for processing 200 ~ 500 μl of input samples: For plasma and serum, 2 μl carrier RNA (GR101-06 Transgen Biotech China) was added into samples and intensively vortexed. The frozen nasopharyngeal swab was mixed with 750 μl store solution (final concentration:100 mM EDTA, 10 mM Tris-HCL, and 0.5 M

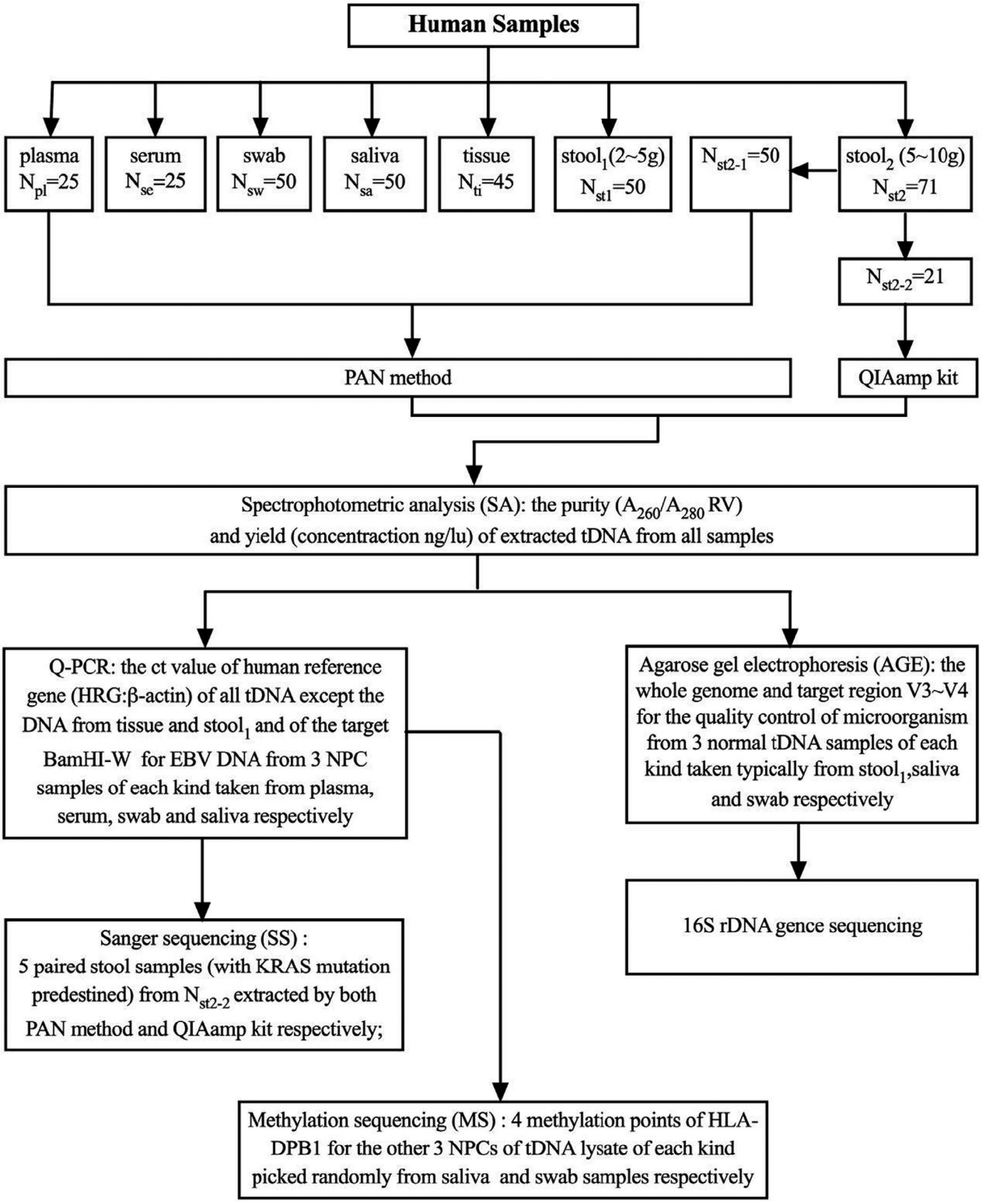

FIGURE 1 Experimental Workflow

NaCl), which yielded around 500 μl output. The fresh swab was submerged directly into the equivalent volume of the lysis buffer (see the following sections). The swab was not removed until the end of lysis and denaturation process. Then, the mixture was vortexed thoroughly at high speed for at least 1 min. Similarly, about 500 μl saliva preserved in store solution was applied to DNA extraction.

For tissue, 750 μl lysis buffer was added into approximately 0.2 g sample size, and then, the mixture was thoroughly grinded.

The input quantity of stool samples varied based on the aim of the study. For human gene analysis, 5 ~ 10 g stool was mixed with 5 ~ 10 ml store solution. For microbial gene analysis, 0.5~2 g stool was mixed with 0.5 ~ 2 ml store solution. The stool was then intensely vortexed to obtain the homogeneous supernatant. Next, the mixture was centrifuged (5418R, Eppendorf, Germany) at full speed (20,000 g or 14,000 rpm) for more than 1 min to pellet stool particles. The supernatant was collected for downstream purification. (Table 1). It should be noticed that a larger amount of starting material will increase the likelihood of purifying DNA from low-titer sources samples. Similarly, when purifying DNA from plasma, serum, more than 1ml input supernatant is needed for DNA purification. This will yield enough DNA for detection of low-frequency gene mutation, or methylation analysis.

2.2.2 | Lysis and denaturation

After pretreatment of samples, equal volume of 6 M guanidine thiocyanate buffer (GT, CG5961, Xiangbo Bio-Tech) was added into cell lysate. Then, the mixture was thoroughly homogenized by vortexing for more than 1 min and centrifuged immediately. The supernatant was transferred into a new tube and incubate (TS100, Ruicheng Instrument Bio-Tech) at 70℃ for 15 min. For purification of microorganism DNA, denaturation at 85℃ for 20 min was recommended, as some bacteria and parasites have rigid cell wall.[13] After the incubation step, equal volume of ethanol (96%~100%) was added to the lysate and mixed by vortexing gently to precipitate DNA (Table 1).

2.2.3 | Purification and Recovery tDNA using silica spin column

First, 650 μl lysate from the lysis and denaturation step was applied to silica spin column (NP20-A, Jiayan Bio-Tech, China) without moistening the rim. Then, the cap was closed securely to avoid aerosol formation and the column was centrifuged at full speed for 1 min. The filtrate in the collection tube was discarded. This step was repeated several times until all lysate passed through the silica spin column. Next, the silica spin column was carefully opened and 750 μl of 75% ethanol buffer was added, and the column was centrifuged at full speed for 1 min. Then, the silica spin column was placed in a new 2 ml collection tube. The ethanol washing step was repeated once again. Afterward, the mini column was placed into a clean 2 ml collection tube and centrifuged at full speed for 5 min to dry the membrane completely. Last, the mini column was transferred into a new 1.5 ml microcentrifuge tube. 100 μl elution buffer (10 mM Tris-HCl, pH8.0~8.5) was added to the column (30ul elution buffer for plasma and serum samples). The column was incubated for 2 min at room temperature and centrifuged at full speed for 3~5 min to elute tDNA.

2.3 | The validation measures for tDNA

2.3.1 | Concentration and purity determination for assessment

DNA quality was assessed by spectrophotometric assay using Multiskan GO spectrophotometer (Thermo Scientific, Germany). Absorbance was measured at wavelengths of A_{260} and A_{280} nm, respectively. The absorbance quotient (A_{260}/ A_{280}) provides an estimate of DNA purity. An absorbance quotient value of 1.8< ratio (R) <2.0 was considered to be good and purified DNA.[22]

2.3.2 | Q-PCR detections for assessment

The human reference gene of ß-actin was qualified by cycle threshold (Ct) value of tDNA. Samples with Ct value of ß-actin <35 were considered to have enough human genomic DNA and can be used for downstream analysis. The BamHI-W locus of EBV DNA was quantified for EBV testing. The Ct value of BamHI-W <35 was considered EBV positive. DNA was quantified using Q-PCR platform (CFX96, Bio-Rad, USA) employing conventional TaqMan Probe method.

2.3.3 | KRAS mutation detection in stool samples

For detection of KRAS mutation in stool samples, the exon 12 of KRAS was amplified by PCR. Then, PCR product was analyzed by 1% AEG to ensure specific amplification. The PCR product was purified with AmPure XP purification system (Agencourt, Beckman Coulter). The purified PCR product was sequenced with both forward and reverse primers and finally analyzed with ABI Genetic Analyser 3500×.

2.3.4 | Methylation sequencing (MS) and 16S rDNA gene sequencing

For methylation sequencing, DNA was treated with bisulfite (Qiagen). Primers were designed to amplify four different typical hypermethylated sites between chr6:33043762 and chr6:33048809 of HLA-DPB1 gene (hg19 coordinate). Two-step PCR strategy was used for library preparation (Morgene Bio-Tech Company) using a total of 100 ng DNA. The prepared libraries were sequenced on HiSeqXTen PE150 (Illumina). For 16S rDNA gene sequencing, the V3-V4 hypervariable region of the bacteria was amplified with 2×HiFi HotStart Ready Mix (KAPA, United States) using a total of 50 ng DNA. Sequencing was performed on HiSeq 2500 (Illumina).

2.4 | Statistical analysis

The statistical analysis was performed with GraphPad Prism 8 (GraphPad Software Incorporate). The consistency between

TABLE 1 Application characteristics of PAN method in this study

					PAN method			
Samples	**Cases**	**Storage**	**Pre-treatment**	**Sample Size**	**Primary Components**	**Incubated Temperature and Time**	**Elution Volume**	**Molecular Analysis**
Plasma	25	−80°C	2 μl carrier RNA added	200 μl	6 M GT buffer, 96 ~100% ethanol, 75% ethanol and elution buffer	70°C, 15 min	30 μl	SA, Q-PCR
Serum	25							
Tissue	45		0.2 g tissue sample thoroughly grinded in lysis buffer and the supernatant acquired by centrifugation	500 μl			100ul	SA (reference)
Swab	50		1 swab thoroughly mixed with 750 μl store solution, intensively vortexed, and maintained after the incubation process completed					SA, Q-PCR, NGS
Saliva	50	room temperature	1ml saliva thoroughly mixed with 1 ml store solution and the supernatant acquired by centrifugation					
Stool_ß-actin	71		5~10 g stool preserved in 5ml~10ml store solution intensively vortexed and the supernatant acquired by centrifugation					SA, Q-PCR, SS
Stool_16s	50		0.5 ~ 2 g stool preserved in 0.5 ml~2ml store solution intensively vortexed and the supernatant acquired by centrifugation	200 μl		85°C, 20 min		SA, Q-PCR, AGE, NGS

different methylation sites of HLA-DPB1 was analyzed with Pearson correlation. The difference the efficiency of tDNA between PAN and QIAamp extraction methods was compared by paired t test. $p < 0.05$ was considered as statistically significant.

3 | RESULTS

3.1 | Development of a versatile DNA extraction method

This study reported a PAN-sample DNA isolation method. The overview of this study was depicted in Figure 1. Different liquid biopsy (LB) samples, including plasma, serum, saliva, nasopharyngeal swab, and stool, were collected in large cohort of epidemiological studies (Table 1 and Table S1–S6). For DNA purification, LB samples were firstly pretreated. Next, samples were subjected to high concentration guanidine thiocyanate buffer treatment for lysis and denaturation. The released DNA was then isolated using silica columns. In the next step, the performance of PAN method was validated by a series of molecular analyses.

3.2 | Quantitative and qualitative analysis of extracted DNA from different samples

To test the validity of this method, various human samples, including plasma, serum, saliva, nasopharyngeal swab and stool, were used for DNA isolation. The purity of isolated DNA was assessed by measuring the absorbance at 260 and 280 nm wavelengths (Table S1–S6). All of the isolated DNA samples had A260/A280 ratio between 1.81 ± 0.04–2.00 ± 0.03 (Figure 2A), which was indicative of high DNA purity.[12] The concentration of extracted DNA ranged from 55.18 ± 24.16 to 497.50 ± 286.80 ng/μl (Figure 2B). The quantity of DNA was evaluated by Q-PCR of β-actin gene. The Ct value was in the range of 21.77 ~ 31.81, which is indicative of good quality of input DNA (Figure 2C, Table 2).

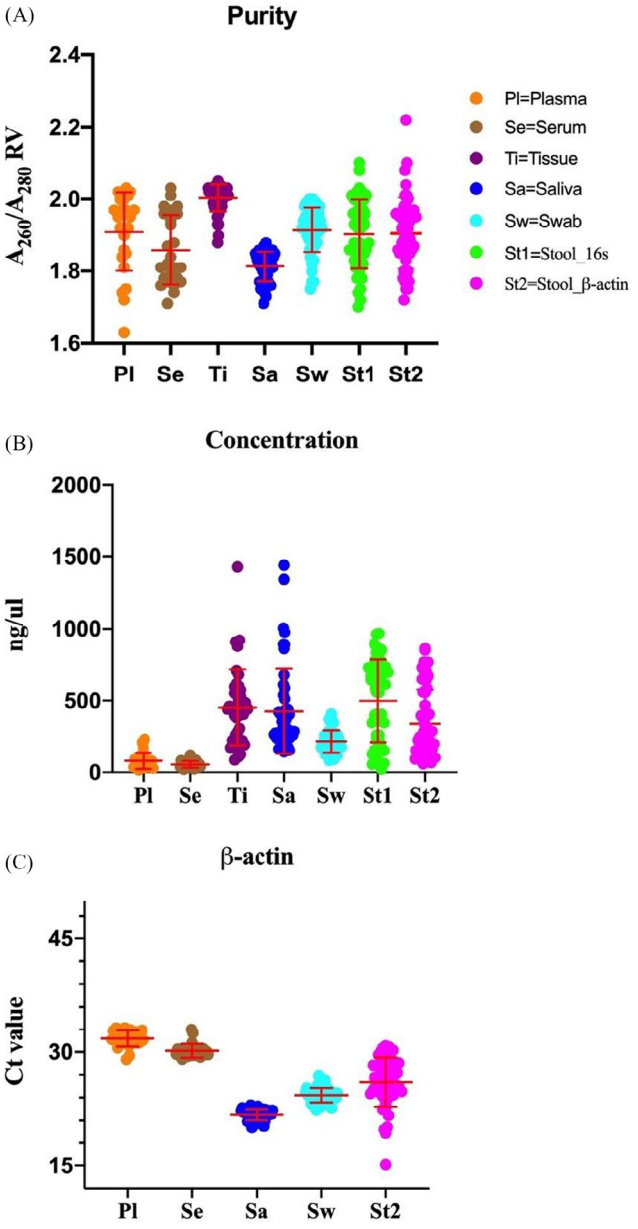

FIGURE 2 Stripchart showing the quantitative and qualitative analysis of DNA isolated from various kinds of samples

3.3 | Detection of human KRAS mutation from stool samples

Stool samples from healthy participants or CRC patients were both used for PAN method DNA isolation, of which 21 stool samples consisting of 16 healthy participants and 5 CRC patients were also applied by QIAamp method, simultaneously. DNA concentration and purity, as well as Q-PCR for ß-actin, displayed good tDNA quality and no significant difference between two methods (Table S6, Table 3, and Figure 3). Then, 5 paired stool DNAs from CRC patients (with KRAS 12 exon mutation confirmed by NGS) were subjected to KRAS mutation analysis by SS. Consequently, KRAS mutations were detected in all samples, suggesting the high sensitivity and excellent coherence of both methods (Figure 4).

3.4 | Quantification of EBV DNA from serum, plasma, saliva, and swab samples

To test whether our method could be used for EBV analysis, 3 NPC patients and 3non-NPC controls of each type sample randomly selected, including plasma, serum, saliva, and nasopharyngeal swab, were used respectively (Table S1–S3). The quality control of all DNAs by qPCR analysis of ß-actin showed good results (Figure 5A). The EBV loadings of plasma and serum samples from NPC patients were 28.95 ± 1.116 and 26.95 ± 1.369, respectively, which was significantly less than that of saliva and swab samples from NPC patients (20.07 ± 3.587 and 20.55 ± 3.340, respectively). This indicated that there were lower levels of EBV Cell-Free Circulating DNA (cfDNA) in plasma and serum than saliva and swab samples (Figure 5B). By

TABLE 2 Quantitative and qualitative analysis of DNA isolated from various human samples using PAN method

Sample types	Cases	A_{260}/A_{280} RV	tDNA CV(ng/ul)	β-actin_Ct
Plasma	25	1.90 ± 0.11	81.08 ± 57.91	31.81 ± 1.17
Serum	25	1.86 ± 0.10	55.18 ± 24.16	30.150.90
Tissue	45	2.00 ± 0.03	453.28 ± 263.57	–
Saliva	50	1.81 ± 0.04	426.99 ± 295.39	21.77 ± 0.71
Swab	50	1.91 ± 0.06	218.29 ± 77.38	24.29 ± 0.97
Stool_16s	50	1.90 ± 0.10	497.50 ± 286.80	–
Stool_ß-actin	71	1.90 ± 0.10	340.20 ± 236.94	26.03 ± 3.22

TABLE 3 Comparison between PAN method and QIAamp in purifying tDNA from stool samples

Methods	Cases	A_{260}/A_{280} RV	Pv1	tDNA_CV	Pv2	ß-actin_Ct	Pv3
PAN	21	1.90 ± 0.07	0.511	352.78 ± 264.67	0.106	22.81 ± 4.60	0.184
QIAamp	21	1.88 ± 0.07		234.27 ± 187.63		23.70 ± 4.24	

contrast, EBV was undetectable in samples from non-NPC participants (Table S1–Table S3).

3.5 | The extracted DNA from saliva and swab is amenable to methylation analysis

We next tested the methylation status of human leukocyte antigen (HLA) gene, which was used as a marker for NPC diagnosis.[63,64] Six tDNAs from NPC saliva and swab samples (3 each) were used for MS. The methylation of HLA-DPB1 gene was successfully evaluated by MS. High coverage and sequencing depth were observed for 4 different targeted loci of the HLA-DPB1 gene that aligned to the human genome assembly Hg19 showed high methylation status in NPC (Figure 5C–F), but extremely low in negative controls (data not provided). Among all samples, the methylation frequency on average of HLA-DPB1 in 4 sites was up to 55.8% (Table S7). These data suggested that our PAN method could generate good DNA yield and purity, which was suitable for MS analysis.

3.6 | The extracted DNA from stool can be used for microbial analysis using 16S rDNA gene sequencing

Similarly, nine tDNAs from stool, saliva, and swab samples (3 for each sample types from different healthy donors) were used for 16S rDNA gene sequencing analysis to detect the microbial communities. 1% AGE analysis indicated high integrity tDNA and specificity of 500bp amplicons of V3~V4 region (Figure 6A). On average, we got 77,234 (67,008 to 83,817) raw reads of good quality in each sample. After trimming and filtering, data were subjected to OTU analysis (defined based on 99% for microbial communities), which gave 24,342 (11,609 ~ 48,474) reads, (Table S8 and Figure 6B). The relative frequency of top 20 genera in microorganism was identified as Corynebacterium, Streptococcus, and Prevotella. In particular, saliva samples seemed to have more diversity of microbial community

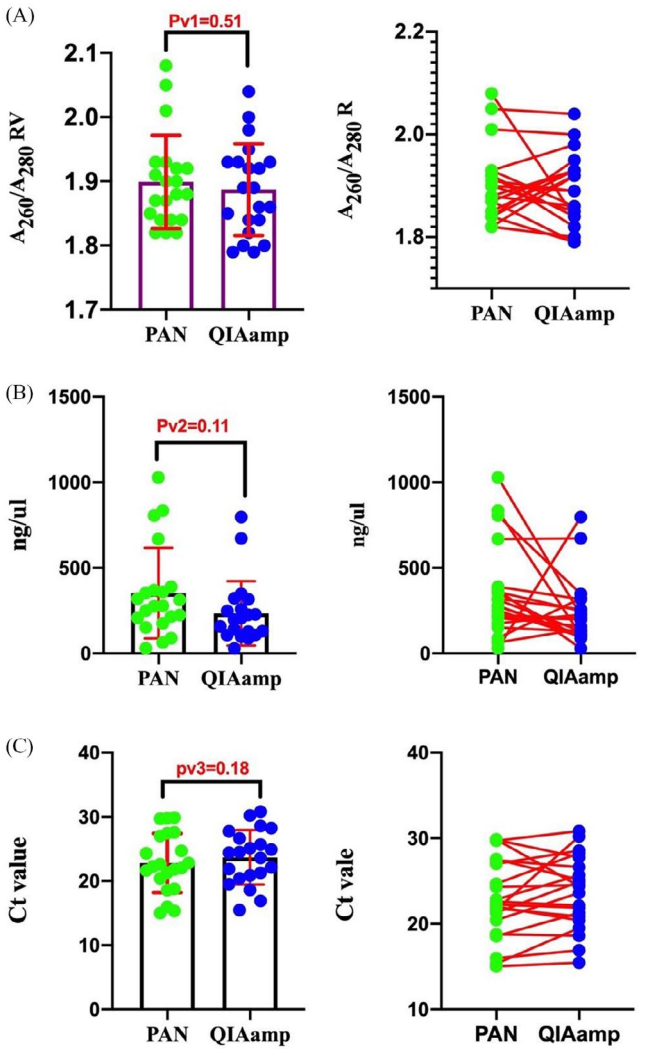

FIGURE 3 Stripchart comparing the DNA extracted using PAN method and QIAamp kit

(Figure 6C), which was consistent with previous observations.[12,20] These data demonstrated that our PAN method could provide high tDNA for 16S rDNA gene sequencing.

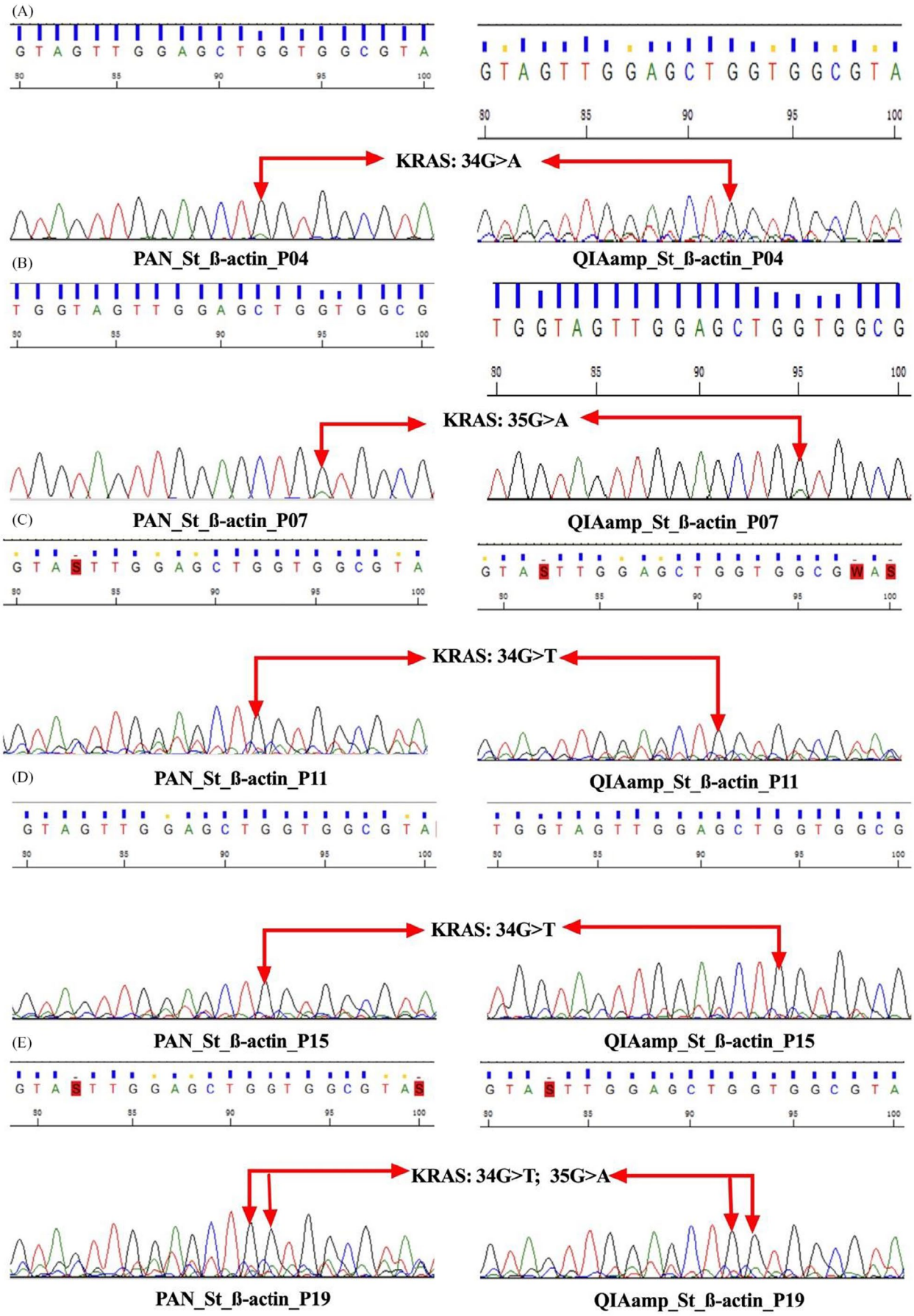

FIGURE 4 Sanger sequencing detecting KRAS mutation in paired stool samples

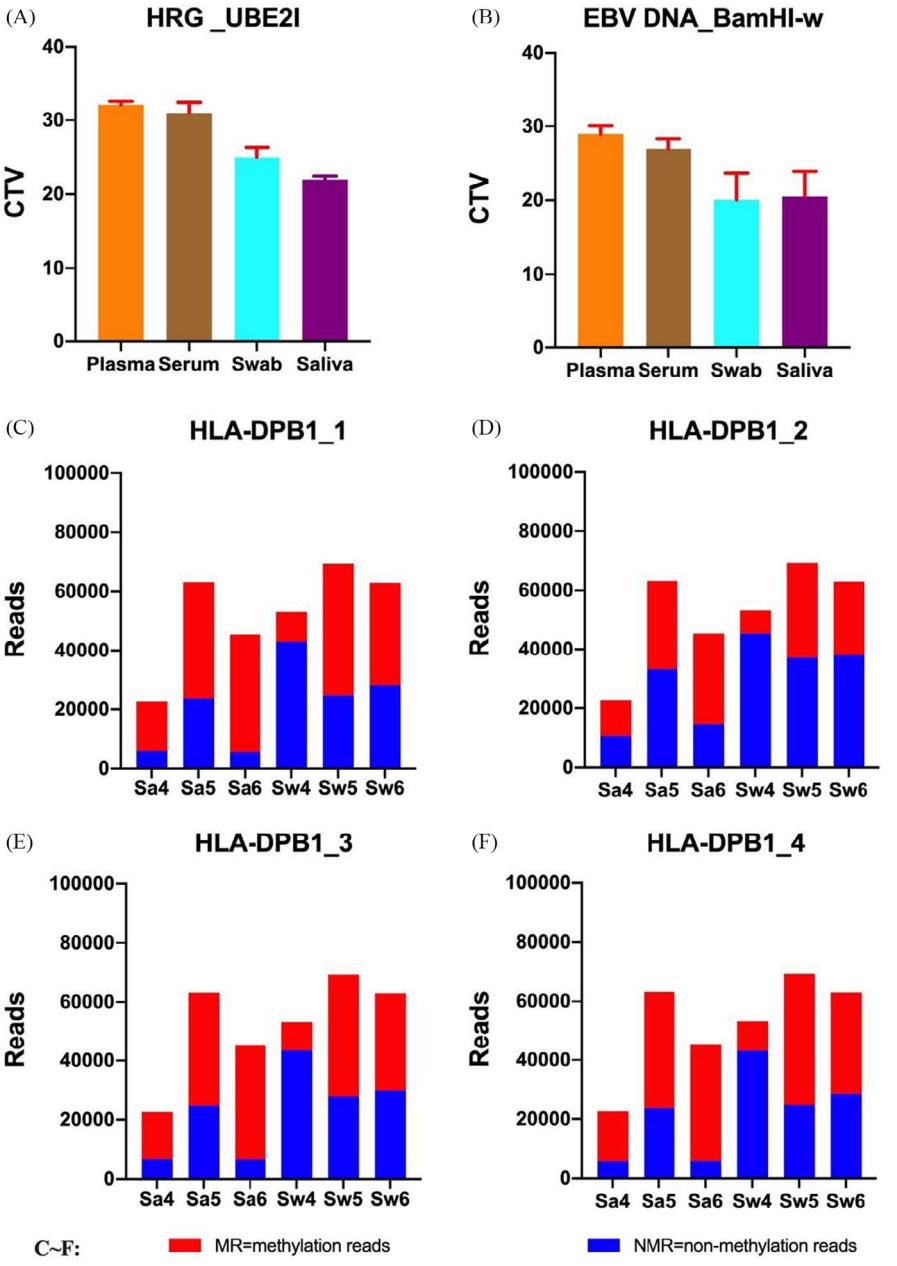

FIGURE 5 Barplot showing Q-PCR results for EBV DNA and MS reads count for HLA-DPB1

4 | DISCUSSION

To screen for the most valuable biomarkers, researchers usually need to collect different types of samples simultaneously from the same subject in large cohort studies. The purification of DNA from large quantity and diverse types of samples is challenging. In this study, we developed a flexible PAN method that can be used for liquid biopsy of multiple sample types, including plasma, serum, saliva, nasopharyngeal swab, and stool. Using a series of molecular analyses as validation exhibited above, the new method showed satisfactory performance with excellent concentration and purity of tDNA quality.

DNA extraction is a classic and one of the most basic assays in the laboratory. Extraction of DNA from LB samples is challenging because of low concentration and degradation of DNA. Currently, many different methods and commercial kits are available on the market for isolation DNA from LB samples, for example, chelex-based extraction,[65] selective capture of ctDNA on magnetic beads,[48] triamine-modified silica particles,[49] etc. Commercial DNA extraction kits like QIAamp Blood DNA Mini Kit, the MagNA Pure LC Instrument (Roche Diagnostics), or NucliSens silica-based DNA extraction (BioMerieux)[66] are available for the circulating cell-free DNA (cfDNA) extraction.[1,2,7] For other less common samples like urine, stool, saliva, nasopharyngeal swab, few options are targetedly available. In addition, switching between different kits is also inconvenient and costly.

However, those kits are often specialized for different purposes, and cross usages are not recommended. This may create troubles when multiple types of samples are needed for DNA extraction. To solve this problem, we have developed a PAN method that can be

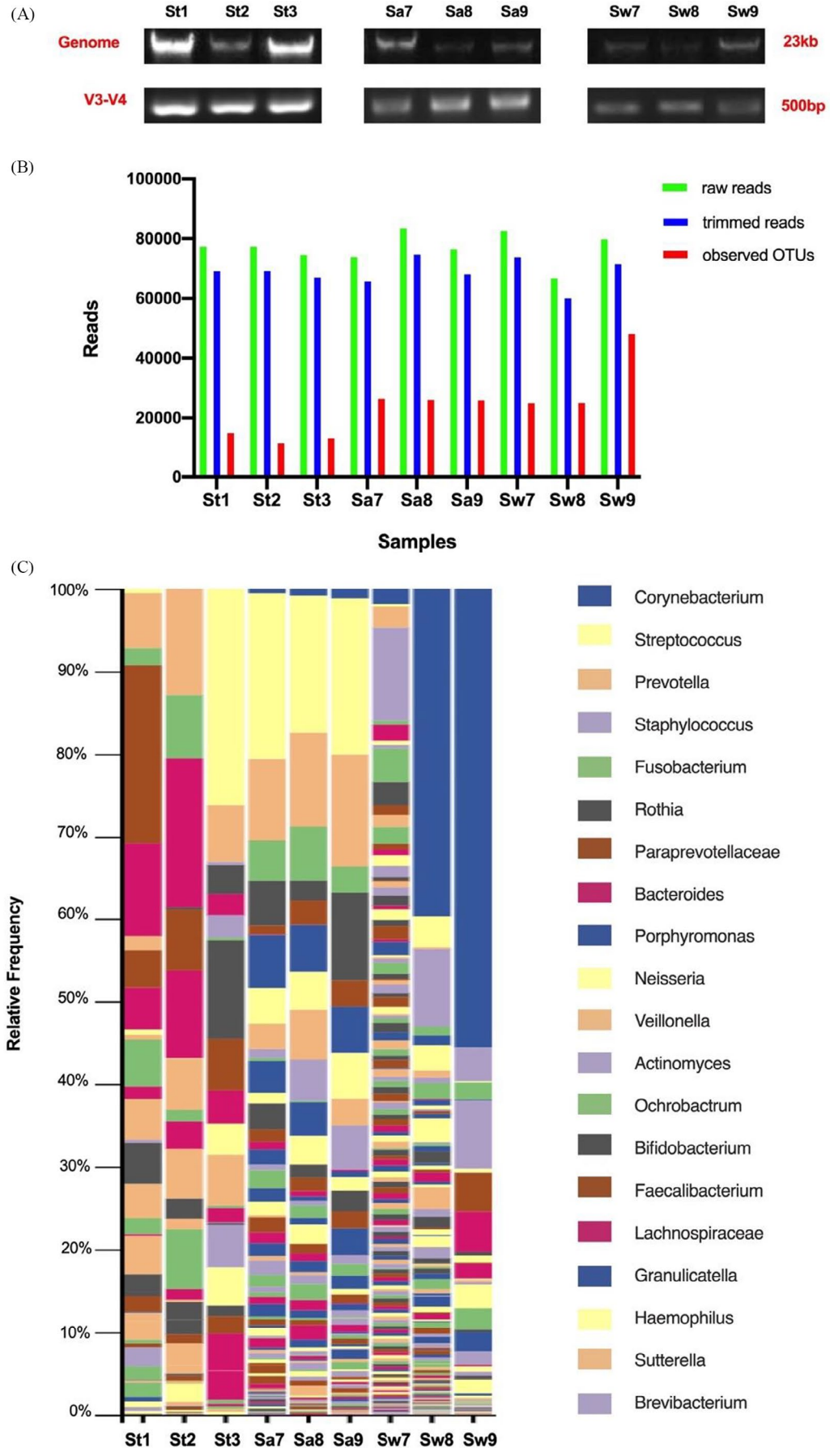

FIGURE 6 Microbial taxa summary plots of different samples analyzed by 16S rDNA gene sequencing

applied to various sample type, while maintaining a high quantity and good quality of DNA products. This method required simple chemical treatment combining with heating to intensively lyse cells, removing DNA-damaging substances and PCR inhibitors, which is extensively tested with different type of samples. Some minor modifications should be done when working with different samples, the detail recommendations as follow.

Given the low amount and high fragmentation of cfDNA from blood plasma or serum, we recommended 2 μl carrier RNA per 200ul input of plasma or serum (with ratio of 1:100) be added into the sample to maximize tDNA output. We also suggested that a larger starting volume up to 3.5ml of blood is used. For the pretreatment process, target enrichment with equal volume of concentrated reagent like PEG 6000 could be applied. In addition, increasing the amounts of GT and carrier RNA might be used to ensure sufficient cfDNA output.[67-70] The similar strategy could also be used for saliva and other type of samples.

For rare applications like swab and brushing samples, researchers reported using commercial kits like QIAamp DNA Mini Kit[71] and Qiagen DNeasy Blood & Tissue Kit (Qiagen)[72] for DNA extraction. However, those kits are not optimized for such samples, and the protocol used by the researchers is not clearly described.[6,71] Other researchers proposed a few physical treatments such as ultrasonication or removal of swab using tweezers. Those measures may lead to DNA loss or potential cross-contamination. In our method, the swab was maintained in lysis buffer to eliminate the potential disadvantages mentioned above.

For stool samples, many laboratories developed methods and commercial DNA extraction kits available. Those methods usually required a comprehensive pretreatment procedure like mechanical treatment using Mini BeadBeater, TissueLyser.[73] The chemicals used in these procedures may degrade DNA or inhibit downstream enzymatic reactions. For example, the QIAamp DNA Stool Mini Kit needed InhibitEX Tablets. In our method, the stool sample was intensely vortexed to get the thorough homogenization. Then, the supernatant was mixed with the equal volume of 6 M GT and incubated at 85°C for 20 min, which could break the rigid cell wall of bacteria and parasites.[13] On the contrary for human genome, incubation at 70°C for 15 min was adequate to generate enough tDNA for downstream molecular analysis.

To sum up, compared with existing assays, there are several advantages of our PAN method: Firstly, it is more flexible. With few modifications, the PAN method can be used for a diversity of specimens and can obtain high-quality DNA products suitable for PCR and other PCR-based reactions. Secondly, it is expandable. The PAN method is suitable for different amounts of input samples rather than relatively fixed quantity of input samples required by traditional methods. Lastly, the PAN method is simpler and more affordable, which is especially suitable for large-scale cohort studies involving large amount of different LB samples.

Nevertheless, several limitations of our study should be noted. Firstly, the guanidine thiocyanate buffer used in PAN assay is a little toxic. There may be some alternative safer and more user-friendly lysis buffer, which could achieve equivalent efficiency. Secondly, when dealing with samples mixed with solid impurities, the silica membrane could be blocked that needs prolonged centrifugation. Finally, the current PAN assay is not compatible with the automated DNA extraction equipment.

5 | CONCLUSION

We developed a PAN-sample DNA isolation method which is versatile, simple, and affordable. It may serve as a good alternative to the commercial kit for isolation DNA from multiple types of LB samples and can be potentially applied to molecular epidemiological research in cancer and other purpose.

CONFLICT OF INTEREST

None.

DATA AVAILABILITY STATEMENT

All data underlying the results are available as part of the article, and no additional source data are required.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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