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Brief communication

# Saliva is a reliable and practical source of germline DNA for genome-wide studies in chronic lymphocytic leukemia

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ABSTRACT

High-throughput genomics requires tumor DNA matched to germline DNA, that cannot be easily obtained in the context of leukemia. Using chronic lymphocytic leukemia as a model, saliva DNA was frequently devoid of tumor DNA also during overt disease, and passed quality controls for SNP-array (77/102, 75.4%) and next generation sequencing (71/102, 69.6%). Compared to saliva, urine provides germline DNA of similar quality but in lower amounts. Saliva DNA was successfully run on SNP 6.0 arrays, and passed quality control call rate thresholds. On these bases, saliva represents a useful source of germline DNA for high-throughput genomic studies of hematologic neoplasia.



## 1. Introduction

Availability of patients' germline DNA has become mandatory in the era of high throughput genomics, such as SNP array and next generation sequencing [1,2]. In fact, identification of tumor-specific somatic mutations requires calling of sequence variants in both tumor and matched germline DNA in order to filter out the vast number of private or unknown germline polymorphisms that, otherwise, could be erroneously scored as tumor-specific mutations [1,2].

In solid cancers, peripheral blood (PB) is commonly utilized as a source of germline DNA [1]. Conversely, in leukemic disorders, PB cells provide a suitable source of germline DNA only when the disease is in profound complete remission (CR) [1]. Consequently, in leukemic patients who do not achieve CR because of refractoriness or because they are not candidate to treatment, difficulties in obtaining germline DNA might hamper identification of tumor-specific genetic abnormalities. In chronic lymphocytic leukemia (CLL), for example, outside the setting of a profound CR, PB fractions containing granulocytes or T-cells are often contaminated by neoplastic cells that might introduce tumor DNA in the control samples [1,2]. Based on these assumptions, alternative sources of germline DNA should be sought in leukemic disorders.

Skin biopsies have been recently proposed as an alternative source of germline DNA in hematologic malignancies [1]. However, patients' compliance to this invasive procedure may be suboptimal, and surgical trauma might cause leukemic cell contamination of the biopsy. In contrast, saliva and urine can be easily collected without invasive procedures, although their value in genome-wide investigations of hematologic neoplasia has not been formally established.

This study assessed the adequacy of saliva and urine as germline DNA sources for genome-wide studies. CLL was chosen as a model since: (i) most cases are left untreated at diagnosis; and (ii) treatment rarely achieves a profound CR [3], thus preventing the achievement of pure germline DNA in most patients.

## 2. Materials and methods

### 2.1. Patients and samples

One ml of saliva was collected before a meal from 82 CLL with overt disease (median B-lymphocyte count $14.4 \times 10^9$/L; range 5.0–$151.1 \times 10^9$/L) and from 20 CLL in CR. A spot urine sample (50 ml) was collected from 30/82 CLL with overt disease (median B-lymphocyte count $20.0 \times 10^9$/L; range 5.0–$94.6 \times 10^9$/L) and from 5/20 CLL in CR. Paired blood samples (10 ml) were also collected. PB granulocytes were separated from mononuclear cells by Ficoll-Paque density

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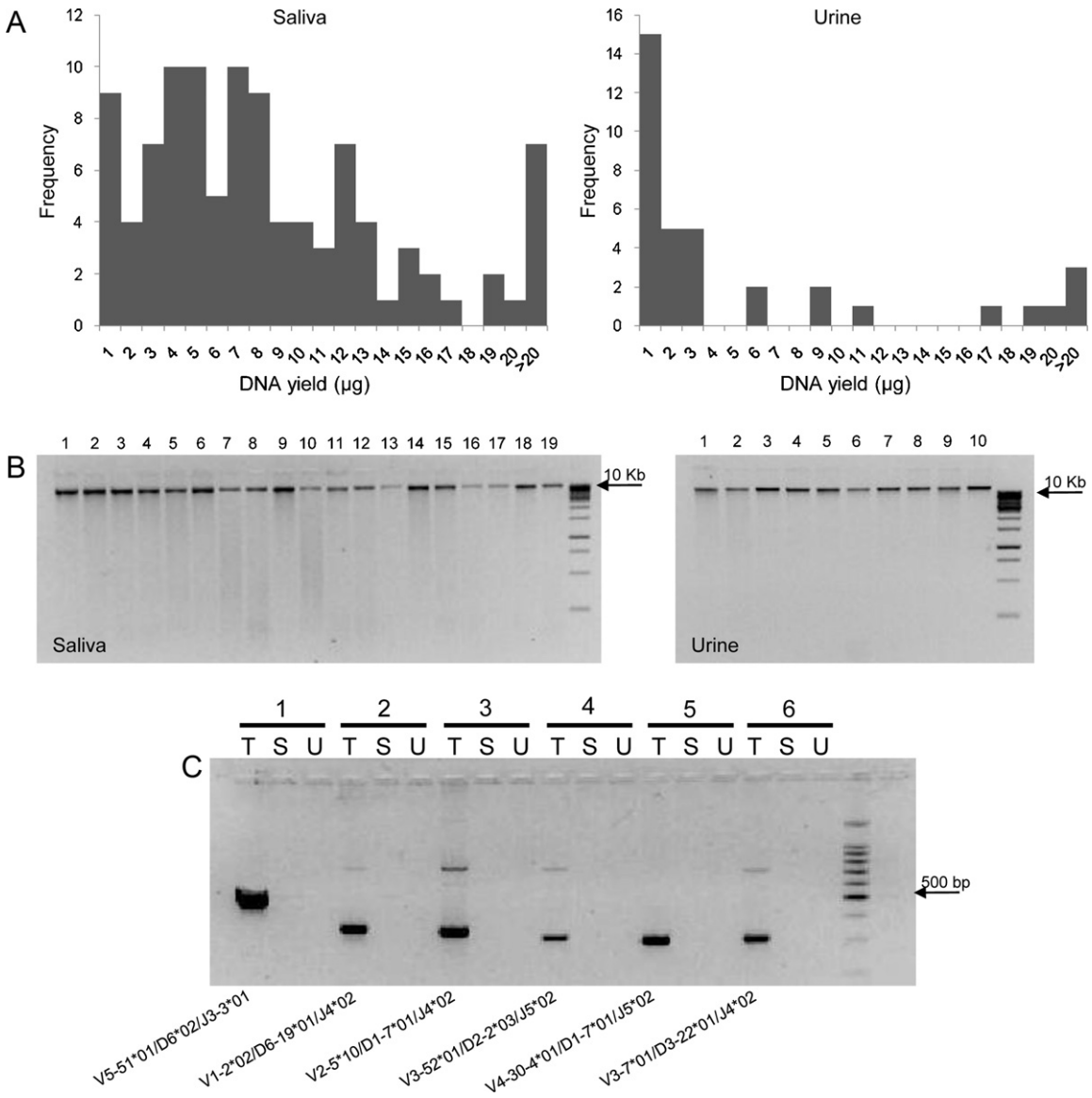


**Fig. 1.** Quality controls of genomic DNA from saliva and urine. Panel A: yields of genomic DNA from saliva and urine samples. Panel B: molecular weight estimation in representative genomic DNA samples of saliva ($n = 19$) and urine ($n = 10$) by 0.8% agarose gel electrophoresis. High molecular weight standards are shown for comparison. In all samples, a band corresponding to high molecular weight DNA is observed. Panel C: PCR analysis of patient-specific IGHV-D-J rearrangement in genomic DNA from tumor (T), saliva (S) and urine (U). In all samples shown in the figure, a band of IGHV-D-J rearrangemnent can be readily observed in tumor samples, but not in matched samples of saliva and urine.

gradient centrifugation (GE Healthcare, Milan, Italy). Genomic DNA was purified by cell lysis followed by digestion with proteinase K, "salting out" extraction, and ethanol precipitation. Patients provided informed consent in accordance with local IRB requirements and Declaration of Helsinki.

### 2.2. DNA quality control

UV spectrum readings (Nanodrop 2000C, Thermo Scientific, Wilmington, DE) were used to determine DNA purity and concentration. DNA molecular weight was estimated by 0.8% agarose gel electrophoresis. Contamination by tumor DNA was assessed by PCR analysis of tumor-specific markers, i.e. IGHV-D-J rearrangements. By dilution experiments, the sensitivity of this strategy was $10^{-3}$. Each PCR product visualized by agarose gel electrophoresis was sequenced by ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems) and aligned to ImMunoGeneTics sequence directory (http://imgt.cines.fr/) in order to characterize the IGHV-D-J rearrangement [4]. The yield of human DNA in saliva and urine was estimated using the Quantifiler Human DNA Quantification kit (Applied Biosystems). Efficiency in PCR amplification of DNA from saliva, urine, and granulocytes was monitored by real time amplification of the *KRT1* control gene. Samples were processed using the Affymetrix Human Mapping GeneChip 6.0 arrays (Affymetrix, Santa Clara, CA, USA) as previously described [5]. Call rates were calculated using Affymetrix Genotyping Console 4.0 with the Birdseed algorithm.

## 3. Results and discussion

Overall, the median yield of DNA extracted from 1 ml of saliva was 7.4 μg (range 0.3–176.2 μg) (Fig. 1A). Purity of saliva DNA was acceptable (median $OD_{260/280}$: 1.8; range: 1.6–2.0). DNA from saliva was of high molecular weight in 99/102 (97.0%) cases (Fig. 1B). Because buccal DNA may contain non-human DNA from oral microflora or from food remnants that may affect large-scale genotyping [6], we quantified the representation of human DNA. The median percentage of human DNA in saliva DNA was 93% (range 70–100%). Contamination of saliva DNA by tumor DNA was restricted to 13/102 (12.7%) cases, including 12/82 (14.6%) CLL with overt disease and 1/20 (5.0%) CLL in CR (Fig. 1C). DNA from saliva was amplifiable for the *KRT1* control gene in all cases as efficiently (median $C_T$: 24.0; range 20.6–26.8) as DNA from matched PB granulocytes (median $C_T$: 24.4; range 22.0–28.3) (Fig. 2).

For comparative purposes, contamination by tumor DNA was also assessed in matched PB granulocyte DNA. PB granulocyte DNA