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Characterization of Circulating HIV-1 Subtypes in the Central African Republic: Implications for Molecular Surveillance and Therapeutic Management

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Abstract

HIV-1 genetic diversity influences disease progression, antiretroviral therapy response, and diagnostic performance. In the Central African Republic (CAR), HIV-1 group M predominates, but detailed data on circulating subtypes and their clinical impact remain limited. This study aimed to characterize HIV-1 subtypes in CAR and assess their association with patients' immunovirological profiles.

A cross-sectional descriptive and analytical study was conducted in 2025 at the National Laboratory of Clinical Biology and Public Health in Bangui. Ninety adult HIV-1–infected patients were included. Viral RNA was extracted from plasma and amplified by RT-PCR targeting the gag p24 region. Subtyping was performed using restriction fragment length polymorphism (RFLP) analysis with HaeIII, MspI, and AluI enzymes. Agarose gel images were optimized using AI (Topaz Gigapixel AI) to enhance band clarity. Clinical and biological data were analyzed using Epi Info software, with univariate analysis and multivariate logistic regression evaluating associations between subtypes and immunovirological parameters.

Among the 90 patients (62% women; median age 34 years), the median CD4 count was 312 cells/mm³ and the median viral load was 63,500 copies/mL. Subtype distribution was: CRF02_AG (53.5%), A (17.8%), C (14.4%), and D (14.4%). CRF02_AG showed a tendency toward higher viral load and lower CD4 counts, though these associations were not statistically significant after adjustment. A viral load > 100,000 copies/mL was the only independent predictor of severe immunosuppression (CD4 < 200 cells/mm³; adjusted OR = 3.62; p = 0.019).

CRF02_AG predominates among HIV-1–infected patients in CAR, while subtypes A, C, and D co-circulate at lower frequencies. High viral load is the main determinant of severe immunosuppression, regardless of subtype. These findings support continuous molecular surveillance, optimization of treatment strategies, and reinforcement of public health interventions. The combined use of PCR-RFLP and AI-assisted gel imaging offers a reliable and feasible method for subtype monitoring in resource-limited settings.

Introduction

The human immunodeficiency virus (HIV) is an enveloped RNA retrovirus belonging to the family *Retroviridae* and the genus *Lentivirus*. It is characterized by its ability to preferentially infect CD4 + T lymphocytes, leading to progressive destruction of these cells and gradual immunodeficiency (1)(2). This immunosuppression predisposes infected individuals to opportunistic infections and certain malignancies, defining the clinical stage of acquired immunodeficiency syndrome (AIDS) in the absence of effective antiretroviral therapy (3).

Taxonomically, HIV is divided into two major types: HIV-1 and HIV-2. HIV-1 accounts for nearly all infections worldwide and exhibits substantial genetic diversity, including several groups (M, N, O, and P) and numerous subtypes and circulating recombinant forms (CRFs) (4)(5). Group M, responsible for the

global pandemic, comprises at least nine subtypes (A–K, excluding obsolete designations) and multiple complex recombinant forms, among which CRF01_AE and CRF02_AG are the most widespread (6)(7). In contrast, HIV-2 is less transmissible, largely confined to West Africa, and associated with slower disease progression (8)(9).

Globally, HIV epidemiology remains a major public health challenge. According to recent data from the World Health Organization (WHO), approximately 39 million people were living with HIV in 2024, with over one million new infections annually (9). Despite significant progress in expanding access to antiretroviral therapy and preventive interventions, the epidemic remains particularly severe in sub-Saharan Africa, where nearly two-thirds of people living with HIV reside (10). In this region, heterosexual transmission predominates, and social and economic inequalities, along with barriers to healthcare access, exacerbate population vulnerability (11).

HIV-1 genetic diversity is especially pronounced in Central Africa, considered a historical epicenter of viral diversification (12). In many countries of this region, recombinant forms such as CRF02_AG are frequently detected, alongside subtypes A, C, and D at varying frequencies (13)(14). This diversity has important implications for epidemiological surveillance, antiretroviral drug susceptibility, and diagnostic test performance (15).

In the Central African Republic (CAR), the HIV epidemic has remained generalized since its first descriptions in the 1980s. UNAIDS and national population surveys report a significant adult prevalence, with a higher burden among women and vulnerable populations (16)(17). However, despite this substantial public health burden, detailed data on the molecular diversity of circulating HIV-1 subtypes in the country remain limited.

A comprehensive understanding of HIV-1 genetic diversity represents a major public health priority. Detailed analysis of circulating variants strengthens molecular surveillance and enables more accurate monitoring of epidemiological dynamics. It also supports the adaptation of therapeutic strategies by considering virological characteristics that may influence antiretroviral response. In CAR, HIV-1 group M remains predominant, while rare forms such as HIV-1 group O and HIV-2 are detected at very low frequencies (approximately 0.05% each), requiring continuous molecular monitoring to prevent diagnostic misclassification (18).

While previous studies have primarily identified the rare presence of HIV-1 group O and HIV-2 in CAR, the present study provides a distinct contribution by characterizing in detail the distribution of major HIV-1 subtypes (CRF02_AG, A, C, and D) and evaluating their association with immunovirological parameters. These findings offer directly applicable data for clinical monitoring and optimization of therapeutic strategies. Furthermore, knowledge of subtype distribution facilitates improved prevention strategies by enhancing diagnostic performance and guiding targeted interventions. Finally, molecular characterization enables anticipation of the emergence and spread of drug resistance mutations, ensuring more effective and sustainable patient management (19)(20)(21).

Research Question

What is the distribution of HIV-1 subtypes among infected patients in the Central African Republic, and how are these subtypes associated with patients' clinical and immunological characteristics?

The objective of this study was to determine the distribution of circulating HIV-1 subtypes in the Central African Republic using PCR-RFLP and to evaluate their association with immunovirological parameters.

Materials and Methods

Study Design and Setting

A cross-sectional descriptive and analytical study was conducted between January and December 2025 at the National Laboratory of Clinical Biology and Public Health in Bangui, Central African Republic. The target population consisted of HIV-1–infected patients receiving follow-up care in CAR.

Study Population and Inclusion Criteria

The study enrolled adult patients living with HIV-1 who were recruited from several HIV care centers across the Central African Republic. Eligibility was restricted to individuals aged 18 years or older with a confirmed diagnosis of HIV-1 infection. All participants provided written informed consent prior to enrollment. In addition, inclusion required the availability of an adequate plasma sample suitable for subsequent PCR analysis.

Patients were excluded if they had been receiving antiretroviral therapy for less than six months, in order to avoid potential bias related to early treatment effects on viral dynamics. Individuals with degraded or insufficient plasma samples were also excluded to ensure the reliability and quality of molecular analyses.

Sampling

A consecutive non-probability sampling method was used. All eligible HIV-1–infected adults followed during the study period (January–December 2025) were enrolled until a total of 90 participants was reached. Sample size was determined based on operational feasibility and reagent availability.

Data Collection

Sociodemographic and clinical data were collected using structured questionnaires and medical records, including age, sex, marital status, CD4 T-cell count (cells/mm³), and plasma viral load (copies/mL).

Biological Sample Collection

For each participant, 5 mL of venous blood was collected in EDTA tubes. Samples were transported at + 4°C to the central laboratory within four hours. Plasma was separated and stored at − 80°C until viral RNA extraction.

Viral RNA Extraction

Viral RNA was extracted from 200 µL of plasma using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. Extracted RNA was stored at − 80°C until amplification.

RT-PCR Amplification

The p24 region of the HIV-1 *gag* gene was specifically targeted for amplification using a one-step RT-PCR approach with designated forward and reverse primers. The primer pair—Gag-F (ATG GGT GCG AGG AGA GAG) and Gag-R (TGC CTT CCT GGC TGC TCC)—was designed to generate an expected amplicon of 297 base pairs.

Amplification reactions were carried out in a final volume of 25 µL, comprising 12.5 µL of One-Step RT-PCR Master Mix (QIAGEN) and primers at a final concentration of 0.4 µM each. Reverse transcription was first performed at 50°C for 30 minutes to synthesize complementary DNA, followed by an initial denaturation step at 95°C for 15 minutes to activate the polymerase and denature the template. The amplification process consisted of 35 cycles, each including denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute. A final extension step was conducted at 72°C for 10 minutes to ensure complete elongation of all PCR products.

The amplified products were subsequently analyzed by electrophoresis on a 2% agarose gel stained with ethidium bromide, allowing visualization of the expected 297 bp fragment under ultraviolet illumination.

Molecular Subtyping by RFLP

HIV-1 subtype determination was carried out using restriction fragment length polymorphism (RFLP) analysis of the amplified *gag* gene products. Three specific restriction enzymes were selected based on their recognition sites: **HaeIII** (GGCC), **MspI** (CCGG), and **AluI** (AGCT). These enzymes were chosen for their ability to generate distinct restriction patterns corresponding to different HIV-1 subtypes.

For each digestion reaction, 10 µL of the PCR product were incubated with 2 µL of the appropriate reaction buffer and 1 µL of the respective restriction enzyme. The mixture was incubated at 37°C for 3 hours to ensure complete enzymatic digestion of the amplified DNA fragments.

Following digestion, the resulting restriction fragments were separated by electrophoresis on a 3% agarose gel. The banding patterns obtained were then carefully analyzed and compared with established

reference restriction profiles in order to determine the corresponding HIV-1 subtype.

Electrophoresis Image Optimization

Agarose gel images were acquired using a standard UV documentation system. To enhance band clarity (contrast, sharpness, and noise reduction), AI-assisted digital processing was applied.

Topaz Gigapixel AI (Topaz Labs, USA) was used to improve image resolution and reduce background noise without altering the biological content of the images. Interpretation of RFLP profiles was performed on the original gels prior to digital optimization.

Quality Control

Positive controls (HIV-1 RNA of known subtype) and negative controls (sterile water) were included in each experimental series. All tests were performed in duplicate to ensure reproducibility.

Statistical Analysis

Data were entered and analyzed using Epi Info version 5. Quantitative variables were presented as medians and interquartile ranges (IQR), while qualitative variables were expressed as frequencies and percentages.

Associations between subtypes and clinical parameters were assessed using Chi-square or Fisher's exact tests, with calculation of odds ratios (OR) and 95% confidence intervals (CI). Significant variables were included in multivariate logistic regression models. Statistical significance was set at $p < 0.05$.

Ethical Considerations

This study was conducted in accordance with the principles of the Declaration of Helsinki and international guidelines for research involving human participants. The protocol was reviewed and approved by the Doctoral School of Science and Technology in the Central African Republic.

All participants were informed of the study objectives, procedures, benefits, and potential risks prior to enrollment. Written informed consent was obtained from each participant. Participants were informed of their right to withdraw at any time without affecting their medical care.

Confidentiality was strictly maintained. Each participant was assigned an anonymous code, and personal data were accessible only to authorized study personnel. Biological samples were handled according to Biosafety Level 2 (BSL-2) standards, and biological waste was disposed of in accordance with national laboratory regulations.

This ethical framework ensured the protection of participants' rights, safety, and dignity throughout the study.

Results

Sociodemographic Characteristics

A total of 90 HIV-1–infected patients were included in the study. Participants' ages ranged from 18 to 62 years, with a median age of 34 years. Women accounted for 62% of the sample (n = 56), while men represented 38% (n = 34). The majority of participants were single (41%), followed by married individuals (36%) and those with other marital statuses (23%).

Immunological and Virological Profile

The median CD4+ T-cell count was 312 cells/mm³ (IQR: 185–445), with 22 patients (24.4%) presenting severe immunosuppression (CD4 < 200 cells/mm³) and 46 patients (51.1%) having CD4 counts below 350 cells/mm³.

The median plasma viral load was 63,500 copies/mL (IQR: 25,000–118,000). Among the participants, 31% had a viral load >100,000 copies/mL.

Distribution of HIV-1 Subtypes

PCR Amplification (297 bp)

Amplification of the target fragment generated a single band at 297 bp for all positive samples as well as for the positive control, confirming successful PCR amplification.

No band was observed in the negative control, ruling out experimental contamination, as shown in **Figure 1.**

Enzymatic Digestion with *HaeIII*

Digestion with *HaeIII* allowed differentiation of HIV-1 subtypes according to specific restriction fragment patterns:

- **CRF02_AG**: fragments of 210 bp and 87 bp
- **Subtype A**: absence of digestion (undigested fragment at 297 bp)
- **Subtype C**: fragments of 180 bp and 117 bp
- **Subtype D**: fragments of 200 bp and 97 bp

The positive control showed the expected restriction profile, whereas the negative control showed no amplification, as illustrated in **Figure 2.**

Enzymatic digestion with MspI

Digestion with MspI confirmed the differentiation of HIV-1 subtypes:

- **CRF02_AG**: fragments of 165 bp and 132 bp

- **Subtype A**: fragments of 220 bp and 77 bp
- **Subtype C**: fragments of 150 bp and 147 bp
- **Subtype D**: fragments of 170 bp and 127 bp

These fragment profiles were consistent with the expected theoretical restriction patterns, as shown in **Figure 3.**

Enzymatic Digestion with *AluI*

Digestion with *AluI* revealed characteristic subtype-specific profiles:

- **CRF02_AG**: fragments of 190 bp and 107 bp
- **Subtype A**: fragments of 160 bp and 137 bp
- **Subtype C**: fragments of 120 bp, 90 bp, and 87 bp
- **Subtype D**: fragments of 210 bp and 87 bp

The combination of all three restriction enzymes allowed reliable discrimination of the studied subtypes, as shown in **Figure 4.**

Table I shows the distribution of HIV-1 subtypes determined by the PCR-RFLP technique among the 90 patients included in the study.

Table I: Distribution of HIV-1 Subtypes Identified

Here is the table translated into English and properly formatted:

Subtype	Number of Patients	Percentage
CRF02_AG	48	53.5%
A	16	17.8%
C	13	14.4%
D	13	14.4%
Total	**90**	**100%**

PCR-RFLP Analysis and Subtype Distribution

The analysis of the 90 samples by PCR-RFLP revealed a clear predominance of subtype **CRF02_AG**, accounting for **53.5%** of cases (n = 48). This high proportion indicates that this circulating recombinant form is the major strain in the study population.

Pure subtypes **A** (17.8%) and **C** (14.4%), as well as subtype **D** (14.4%), were also detected but at considerably lower frequencies. This distribution reflects a moderate genetic diversity of HIV-1,

characterized by the co-circulation of multiple subtypes.

Univariate Analytical Associations

Univariate analysis examined the relationships between subtype **CRF02_AG** and various sociodemographic, immunological, and virological parameters.

Associations between the CRF02_AG subtype and various sociodemographic, immunological, and virological parameters are summarized in Table II

Table II: Association between subtype CRF02_AG and different sociodemographic, immunological, and virological parameters

Variables	CRF02_AG (n=48)	Other Subtypes (n=42)	OR	95% CI	p-value
Female sex	28 (58.3%)	28 (66.7%)	0.76	0.32–1.80	0.54
Age ≥35 years	27 (56.3%)	18 (42.9%)	1.70	0.72–4.01	0.23
CD4 <350 cells/mm³	31 (64.6%)	19 (45.2%)	2.20	0.92–5.27	0.08
Viral load >100,000 copies/mL	21 (43.8%)	10 (23.8%)	2.57	0.98–6.76	0.05

Patients infected with **subtype CRF02_AG** tended to have **higher viral loads** and **lower CD4 counts**, although these associations did **not reach statistical significance**.

Results of the univariate analysis of factors associated with severe immunosuppression are presented in Table III.

Table III presents the results of the univariate analysis of factors associated with **severe immunosuppression**, defined as a **CD4 count below 200 cells/mm³**.

Table III: Univariate Analysis of Factors Associated with Severe Immunosuppression

Variables	CRF02_AG (n=48)	Other Subtypes (n=42)	OR	95% CI	p-value
Female sex	28 (58.3%)	28 (66.7%)	0.76	0.32–1.80	0.54
Age ≥35 years	27 (56.3%)	18 (42.9%)	1.70	0.72–4.01	0.23
CD4 <350 cells/mm³	31 (64.6%)	19 (45.2%)	2.20	0.92–5.27	0.08
Viral load >100,000 copies/mL	21 (43.8%)	10 (23.8%)	2.57	0.98–6.76	0.05

After multivariate logistic regression, a plasma viral load >100,000 copies/mL was the **only independent factor** associated with severe immunosuppression (aOR = 3.62; 95% CI: 1.23–10.64; p = 0.019). The CRF02_AG subtype was **not significantly associated** with severe immunodeficiency (aOR = 2.11; p = 0.16).

The results indicate that the CRF02_AG recombinant subtype is the **most prevalent** among HIV-1-infected patients in the Central African Republic (CAR). Although this subtype tends to be associated with higher viral loads and lower CD4 counts, **only high viral load remains an independent factor** for severe immunosuppression. These observations reflect the genetic diversity of HIV in Central Africa and highlight the importance of **immunovirological monitoring** to optimize antiretroviral therapy.

Discussion

This study demonstrates a clear predominance of the recombinant form CRF02_AG (53.5%) among HIV-1–infected patients in the Central African Republic (CAR). This distribution is consistent with molecular epidemiology data reported across West and Central Africa. In Ghana, Agyeman et al. (2020) identified CRF02_AG as the dominant subtype, accounting for 54% of treatment-naïve cases, a proportion remarkably similar to that observed in our cohort (22). Likewise, in Cameroon, Tongo et al. (2019) reported CRF02_AG at 48%, in the context of substantial viral diversity including subtype A and several unique recombinant forms (23). Although our findings are broadly comparable, the slightly lower diversity observed in our study may be explained by methodological differences, as we used PCR-RFLP rather than full-genome sequencing, which provides greater resolution for detecting complex recombinants.

In contrast, data from the Democratic Republic of Congo reported by Makuwa et al. (2021) revealed greater genetic heterogeneity, with subtypes A and C predominating and CRF02_AG representing approximately 30% of circulating strains (24). This suggests a more complex molecular dynamic in that setting compared to CAR.

Regarding subtype C (14.4%), our findings differ markedly from those reported in Botswana by Novitsky et al. (2018), where subtype C accounted for more than 95% of infections, reflecting strong geographic clustering and regional specificity of HIV-1 subtypes (25). For subtype D (14.4%), the frequency observed in our study is comparable to that reported in Uganda by Ssemwanga et al. (2020), who found a prevalence of 17% (26). However, in our cohort, subtype D was not independently associated with severe immunosuppression.

Univariate analysis in our study suggested a trend toward higher viral load among patients infected with CRF02_AG. Nevertheless, this association did not remain statistically significant after multivariate adjustment. Similar observations were reported in Uganda by Kiwanuka et al. (2019) (27). Furthermore, a European multicenter cohort study conducted in Switzerland by Kouyos et al. (2017) demonstrated that apparent differences in disease progression between non-B subtypes lost statistical significance after adjustment for confounding factors (28).

Importantly, the independent association observed between viral load greater than 100,000 copies/mL and severe immunosuppression in our study is consistent with longitudinal data from the United States reported by Mellors et al. (2018), which confirmed plasma viral load as the principal predictor of progression to AIDS, independent of viral subtype (29).

From a methodological perspective, Yamaguchi et al. (2021) in Japan demonstrated that PCR-RFLP shows good concordance with Sanger sequencing for the identification of major subtypes but has limited capacity to detect complex recombinant forms (30). This aligns with the limitations of our study and supports the need for more advanced sequencing approaches in future molecular surveillance efforts in CAR.

Conclusion

This study confirms that the recombinant CRF02_AG predominates among HIV-1-infected patients in CAR, while subtypes A, C, and D also co-circulate, highlighting significant genetic diversity. High viral load remains the main independent risk factor for severe immunosuppression (CD4 < 200 cells/mm³), underscoring the importance of **regular clinical and virological follow-up**.

Although CRF02_AG tends to associate with higher viral loads, this was not statistically significant in our cohort, reflecting the complex influence of subtypes on clinical progression. These findings provide a foundation for **continuous molecular surveillance** and the development of targeted **public health strategies** for prevention, treatment, and patient management. Future studies using **next-generation sequencing** will further elucidate the dynamics of recombinant forms and their impact on disease evolution.

Declarations

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Author Contribution

Author Contributions Statement B.D.H.L.M. conceived and designed the study, performed the laboratory analyses, analyzed the data, and wrote the first draft of the manuscript. O.G. contributed to data interpretation and manuscript revision. E.L.Y. supervised the research and critically revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

Data Availability

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request due to ethical and privacy restrictions.

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Figures

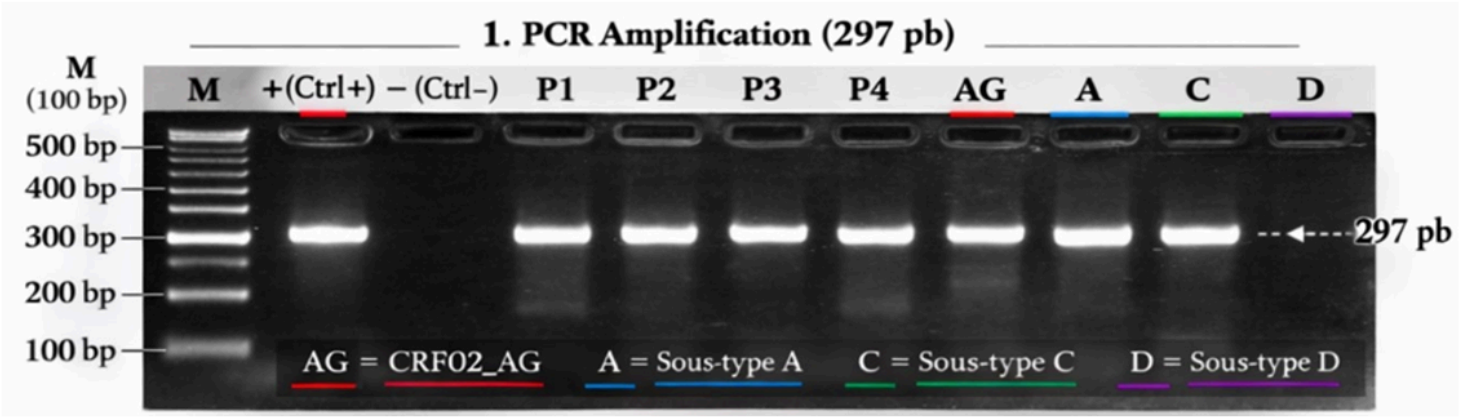


Figure 1

PCR Amplificatio

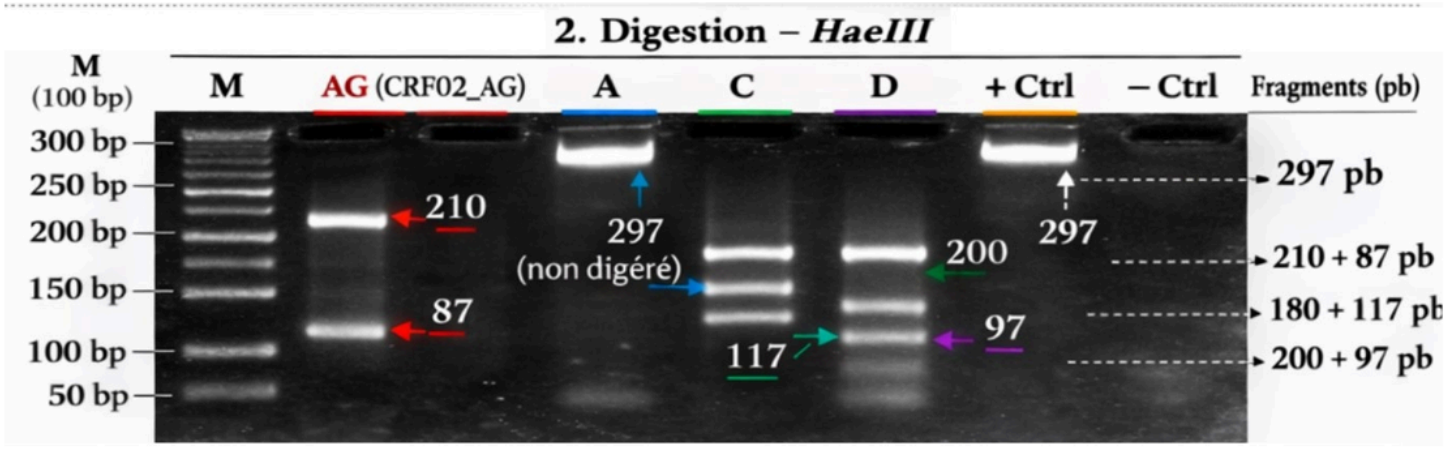


Figure 2

Digestion-HaeIII

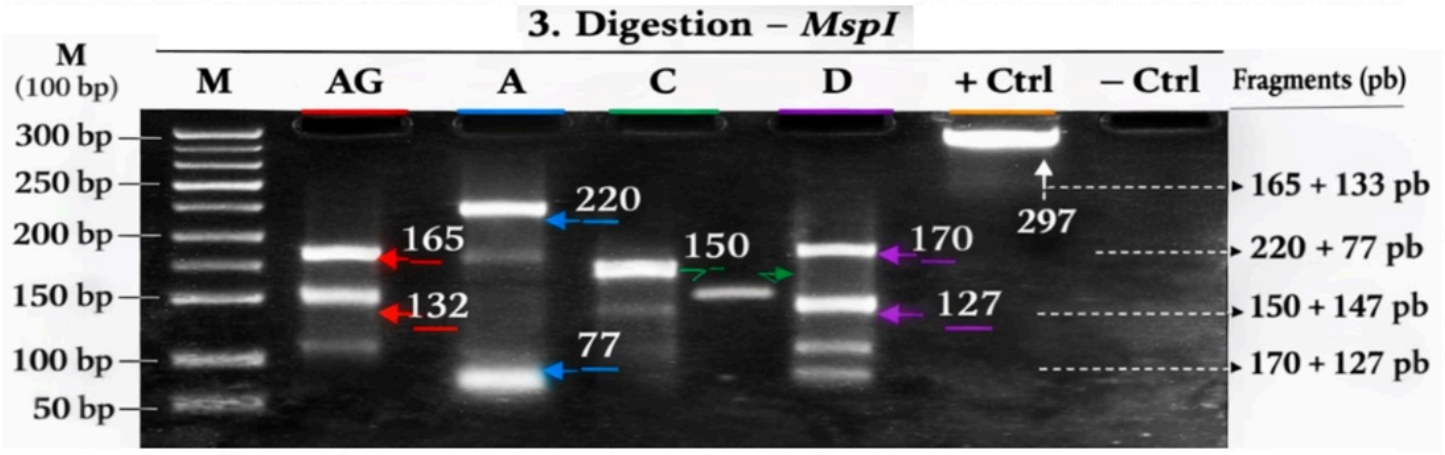


Figure 3

Digestion-MspI

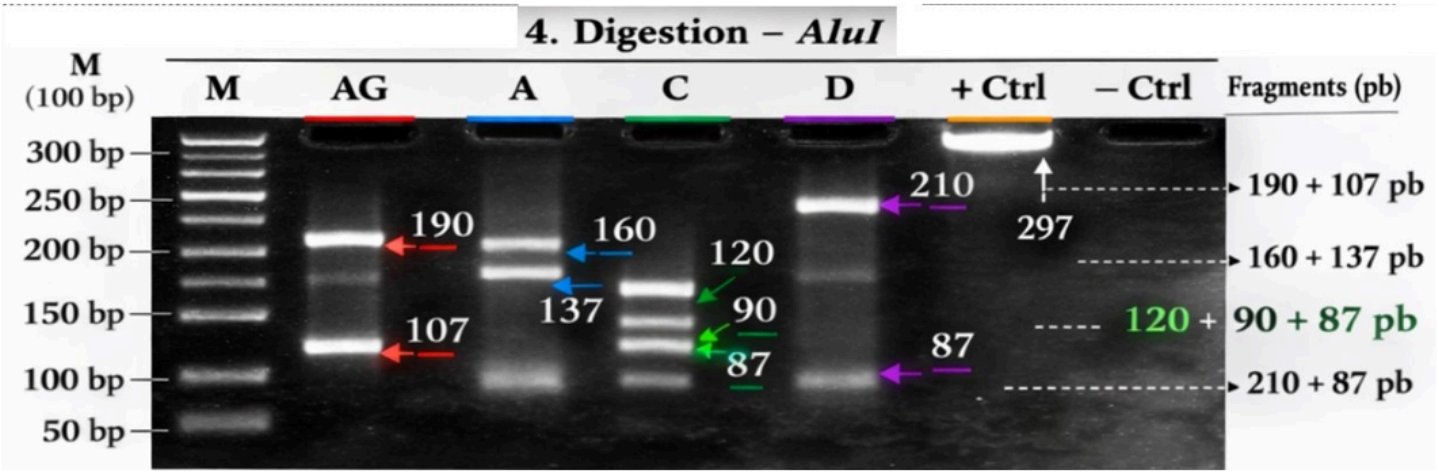


Figure 4

Digestion-AluI

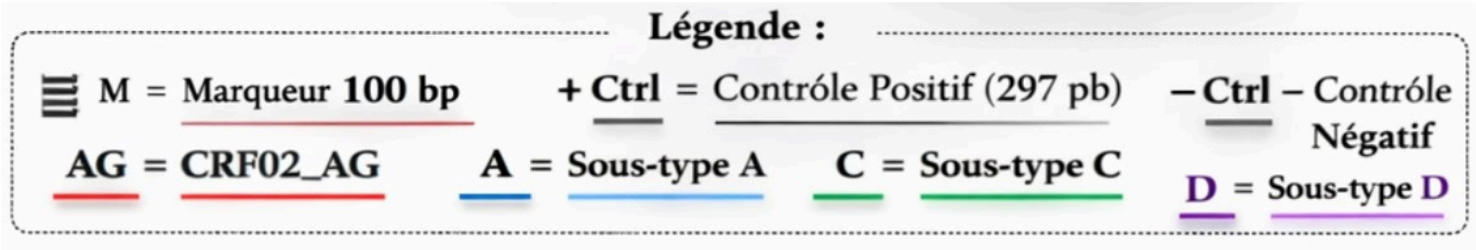


Figure 5

Legende