The implementation of Alu insertion polymorphism as a genetic marker for forensic investigation in a Jordanian population sample

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Abstract

Alu element is one of the most abundant short interspersed nuclear elements (SINE) in the human genome with great forensic potential. The current study focused on the analysis of the utility of Alu insertion polymorphism in forensic DNA typing in a Jordanian population sample (central Jordan). Alu insertions in seven different genetic loci were amplified using Polymerase Chain Reaction (PCR). The frequencies of the Alu insertions were 0.345 for ACE, 0.441 for TPA25, 0.291 for PV92, 0.845 for APO, 0.468 for FXIIIB, 0.727 for HS3.23 and 0.527 for B65. APO and FXIIIB Alu insertions were the most dominant alleles, while PV92 Alu insertion was the least frequent. Combined Power of Discrimination (PD) for those seven loci was 0.9982, whereas the combined Power of Exclusion (PE) was 0.4987. Combining the seven Alu insertion/deletion data along with the Jordanian combined STR data should give an exceptional resolution power between Jordanian individuals.

Keywords: Forensic science, Alu polymorphism, Allelic frequency, Jordanians

1. Introduction

The implementation of DNA fingerprinting of different DNA sequences such as variable number tandem repeats (VNTR), short tandem repeats (STRs) and single-nucleotide polymorphisms (SNPs) in criminal situations has tremendously advanced the field of forensic investigation. STRs increased the capability of solving questionable situations through their extremely high power of identification or discrimination and paternity testing due to their polymorphic nature (Butler, 2002; and Alonso et al., 2018). Though STRs capabilities are still undisputed, many investigators in recent years have studied other polymorphic DNA sequences. For example, the dimorphic Arthrobacter luteus restriction endonuclease (Alu) mobile elements are studied for potential use in population genetic studies and forensic investigations in order to increase the certainty of juridical perceptiveness (Gill, 2001; Frudakis et al., 2003; Petkovski et al., 2003; and Terreros et al., 2009).
The Alu elements are approximately 300 bp in length and constitute one of the most plentiful short interspersed nuclear elements (SINE) with more than one million copies accounting for more than 10% of the mass of the human genome (Lander et al., 2001; Deininger and Roy-Engel, 2002; Batzar et al., 2002; and Ray et al., 2007). They are mostly fixed which means that at a particular locus all individuals are homozygous for Alu insertion (Ray et al., 2007). Furthermore, it has been estimated that they can insert themselves in the human genome at a rate of 100 to 200 copies per million years via a process known as retro insertion (Batzar et al., 1994). Yet, no mechanism for complete Alu element removal after insertion is known (Roy-Engel et al., 2002).

Some of these insertions are still polymorphic for presence or absence at a specific location due to recent transposition in the human genome, and the absence of the insertion is considered as the ancestral state and presumably, the gain of the Alu element is the mutational change (Stoneking et al., 1997). The ability of the Alu elements to determine the ancestral state and the direction of mutational changes renders them as unique markers in population genetic studies. In this context, Alu polymorphism is widely considered as a tool in evolutionary, population’s ethnicity and origin, and genetic diversity studies among populations (Batzar et al., 1996; Roy-Engel et al., 2001; Tripathi et al., 2017; Bahri et al., 2011; and Zanetti et al., 2014). It has been proved that it is a useful tool for inference of human geographic origins (Ray et al., 2007). In addition, some of these elements are present in large frequencies in some populations and are identified as “population specific alleles” so they can be used as a differential marker between two well-defined populations (Shriver et al., 1997).

Many investigators have reported the potential use of Alu elements in forensic identification such as paternity testing. Chadli and coworkers (2009) demonstrated the forensic importance of seven loci which have the insertion or deletion of an Alu fragment in south Morocco. Studies on the German, Japanese, Taiwanese and other populations showed that Alu polymorphisms provide a useful tool in forensic DNA analysis where Alu repeats might be unique to an individual and thus they can provide information on human population relationships (Wallace et al., 1991; Batzar et al., 1996; Schneider et al., 1996; Hsieh et al., 2002; and Asari et al., 2012).

In the current study, we focused on amplifying Alu insertion on seven different loci including Angiotensin Converting Enzyme (ACE), Tissue Plasminogen Activator (TPA25), A polipoprotein (APO), Improved Coagulation Factor XIIIB (FIIIXB), Alu Insertion PV92 (PV92), Human Specific 3.23 (HS3.23), and HLA-B65 (B65). The variation in allele and genotype frequencies was studied in a Jordanian population sample residing in central Jordan to test the potential use of these loci as genetic markers for identity testing.

2. Materials and Methods

2.1. Sample collection and DNA isolation

Blood samples were collected in EDTA tubes from 110 unrelated Jordanian individuals residing in central Jordan, including 55 men and 55 women. Informed consent was obtained from each individual, and the study was approved by the Institute Review Board (IRB) of Hashemite University, which conforms to the World Medical Association Declaration of Helsinki. DNA was isolated using the Wizard® Genomic DNA Purification Kit (Promega, Madison, USA). DNA samples were quantified using spectrophometry (Biowave II, Biochrom, UK) and stored at −80 °C until further use.

2.2. DNA amplification using PCR

Alu fragment on seven loci including ACE, TPA25, PV92, APO, FXIIIB, HS3.23, and B65 were amplified using the Polymerase Chain Reaction (PCR). Fourteen specific primers for the flanking Alu insertion sequence (Table 1) were used for site amplifications (Chadli et al., 2009). The PCR amplification was carried out using

| Locus / Position | Forward primer | Reverse primer | Annealing temperature | Product size (bp) |
|------------------|----------------|----------------|-----------------------|------------------|
| ACE17q23         | CTGGAGACCACTCCCA TCTTTTCT | GATGTTGCCA TCAATTCGTCAGAT | 58 °C | 480-191 |
| TPA258p11.2      | GTAAGAGTTCGGTAAC AGGACAGCT | CCCACCCCTAGGAG AACTTCTCTTTT | 58 °C | 400-110 |
100 ng DNA, 1.5 mM MgCl$_2$, 0.2 mM dNTP, 1 unit of Taq polymerase (Bio Basic, Canada) and 0.24 pM of each primer (GeneWiz, USA). The PCR protocol involved 35 cycles of repeated denaturation of DNA template at 94 °C for 1 min, annealing of primers for 2 min as described previously (Chadli et al., 2009), and DNA synthesis at 72 °C for 2 min. The electrophoresed PCR products were visualized on 2% agarose gel stained with RedSafe™ Nucleic Acid Staining Solution (iNtRON Biotechnology DR, USA) under UV.

3. Statistical Analysis

Allelic and genotypic frequencies were calculated according to the counting method. Observed (H$_O$) and Expected (H$_E$) Heterozygosity, Power of Identity (P$_I$), Power of Discrimination (P$_D$), Power of Exclusion (P$_E$), Polymorphic Information Content (PIC), Combined or Joined P$_I$, P$_D$ and P$_E$ were calculated according to the formulas described previously (Brenner and Morris, 1990; Evett and Weir, 1998; and Chesnokov and Artemyeva, 2015). The observed genotypes and alleles frequencies were compared with those expected in order to verify the Hardy-Weinberg Equilibrium (HWE). The chi-square test and Fisher’s exact test were performed for the polymorphism frequency. Statistical analysis for allele interpopulation analysis was performed using Statistica software, StatSoft Inc, Tulsa, OK, USA (version 10). A value of $p < 0.05$ was considered statistically significant.

![Figure 1: Representative PCR amplification products of the seven Alu DNA genetic loci from unrelated Jordanian blood samples](image)

**Note:** Lanes 1, 5, 9, 13, 17, 21, and 25: 100 bp ladder. The rest of the lanes represents Alu DNA polymorphism for the seven loci with their respective molecular size in base pair.
4. Results and Discussion

Alu DNA fragment exhibits a dimorphic nature in which its polymorphism depends on its stable integration or absence at a certain locus (Sawada et al., 1985; Sawada and Schmid, 1986; Bailey and Shen, 1993), and the rare loss of such insertions (Edwards and Gibbs, 1992). It has been speculated that the Alu elements have a copy number of over a million per genome (Edwards and Gibbs, 1992) with potential impacts in human evolution and forensics. In the current study, Alu insertions in seven loci (including ACE, TPA25, PV92, APO, FXIIIIB, H53.23 and B65) were analyzed for their prospective forensic implications in a Jordanian population sample (Figure 1).

The genotypic and allelic frequencies of these loci and the evaluation of the HWE in the sample population are shown in Table 2. The frequency of the Alu insertion were 0.345 for ACE, 0.441 for TPA25, 0.291 for PV92, 0.845 for APO, 0.468 for FXIIIIB, 0.727 for HS3.23 and 0.527 for B65. For APO and FXIIIIB, Alu insertion (Alu+) was the predominant allele with a frequency of 0.845 and 0.727 respectively, while the PV92 Alu insertion was the least with a frequency of 0.291. Chi-square test statistics values showed no significant departure from HWE for all of the seven loci. Results have also shown no significant difference in Alu insertion allelic distribution between Jordanian males and females (p > 0.05) (data not shown). Furthermore, no significant difference between observed and expected heterozygosity was also observed (p > 0.05).

The heterozygosity values (0.4181 and 0.4272 respectively) which indicates that the Jordanian population is variable in regards to these loci. On other hand, APO loci have showed the lower heterozygosity (0.2181).

The PD was calculated by locus and for all of the studied loci (Table 2). PD was 0.608 for ACE, 0.6598 for TPA25, 0.576 for PV92, 0.408 for APO, 0.652 for FXIIIIB, 0.562 for HS3.23 and 0.664 for B65. APO and FXIIIIB Alu insertions were respectively the most dominant alleles, while PV92 Alu insertion was the least frequent.

Table 2: Genotypic, allelic frequencies and forensic indices of the insertions and deletions of the Alu DNA fragment per seven genomic loci in 110 unrelated Jordanian individuals

| Forensic parameters | Alu insertion/deletion frequency per locus |
|---------------------|------------------------------------------|
|                     | ACE | TPA25 | PV92 | APO | FXIIIIB | H53.23 | B65 |
| Genotype            |     |       |      |     |         |        |     |
| Alu\(^+\)\(^+\)      | 0.1363 | 0.2818  | 0.1000 | 0.7363 | 0.2554 | 0.5454 | 0.3454 |
| Alu\(^+\)\(^-\)      | 0.4181 | 0.3181  | 0.3818 | 0.2181 | 0.4272 | 0.3636 | 0.3636 |
| Alu\(^-\)\(^-\)      | 0.4454 | 0.4000  | 0.5181 | 0.0454 | 0.3181 | 0.0909 | 0.2909 |
| Allele              |     |       |      |     |         |        |     |
| Alu\(^+\)           | 0.3450 | 0.4410  | 0.2910 | 0.8450 | 0.4680 | 0.7270 | 0.5270 |
| Alu\(^-\)           | 0.6550 | 0.5590  | 0.7090 | 0.1550 | 0.5320 | 0.2730 | 0.4730 |
| H\(_O\)             | 0.4181 | 0.3181  | 0.3818 | 0.2181 | 0.4272 | 0.3636 | 0.3636 |
| H\(_E\)             | 0.4520 | 0.4930  | 0.4126 | 0.2620 | 0.4980 | 0.3969 | 0.4985 |
| p-value             | 0.9601 | 0.8034  | 0.9617 | 0.9314 | 0.9199 | 0.9578 | 0.8475 |
| PIC                 | 0.4520 | 0.4930  | 0.4130 | 0.2620 | 0.4980 | 0.3970 | 0.4990 |
| P\(_I\)             | 0.3920 | 0.3402  | 0.424  | 0.5920 | 0.3480 | 0.3480 | 0.336 |
| P\(_D\)             | 0.6080 | 0.6598  | 0.5760 | 0.4080 | 0.6520 | 0.5620 | 0.6640 |
| P\(_E\)             | 0.1260 | 0.0710  | 0.1030 | 0.0350 | 0.1310 | 0.0940 | 0.0940 |
| CP\(_I\)            | 0.001714354 |
| CP\(_D\)            | 0.998285646 |
| CP\(_E\)            | 0.4987 |

Note: (\(^+\)): Homozygous insertion; (\(^-\)): Heterozygous insertion/deletion; (\(^-\)): Homozygous deletion; (\(^+\)): Alu insertion; (\(^-\)): Alu deletion; H\(_O\): Observed heterozygosity; H\(_E\): Expected heterozygosity; PIC: Polymorphic Information Content; P\(_I\): Power of Identity; P\(_D\): Power of Discrimination; P\(_E\): Power of Exclusion; CP\(_I\): Combined Power of Identity; CP\(_D\): Combined Power of Discrimination; and CP\(_E\): Combined Power of Exclusion.
insertion displayed the highest $P_D$ (0.664) with a $P_I$ value of 0.336, while the APO Alu insertion exhibited the lowest $P_D$ of 0.408. Therefore, APO is the least powerful locus and B65 is the most powerful loci among the seven loci studied. The combined $P_D$ for the seven loci included in this study was 0.998285646. $P_E$ ranged from 0.035 for APO and 0.131 for FXIIIB (Table 2), and the combined $P_E$ for the seven loci included in this study was 0.4987.

In addition, the discrepancy in Alu insertion frequency between Jordanians and other populations was analyzed (Table 3). The data showed a significant difference in Alu insertion allele frequency between Jordanians and other populations.

| Population            | TPA25 | PV92 | APO  | ACE  | FXIIIB | B65 | HS3.23 |
|-----------------------|-------|------|------|------|--------|-----|--------|
| Jordan (Current study)| 0.441 | 0.291| 0.845| 0.345| 0.468  | 0.527| 0.727  |
| African American      | 0.302 | 0.209| 0.57 | 0.488| 0.221  | -   | 0.99   |
| p-value               | **0.0419** | 0.1806 | **<0.0001** | **0.0403** | **0.0002** | -   | **<0.0001** |
| Afro-Caribbean        | 0.286 | 0.143| 0.5  | 0.524| 0.31   | -   | -      |
| p-value               | **0.0227** | 0.0111 | **<0.0001** | 0.0107 | 0.0219 | -   | -      |
| Alaska Natives        | 0.298 | 0.619| 0.917| 0.583| 0.917  | -   | -      |
| p-value               | **0.0362** | -   | **<0.0001** | 0.1159 | **0.0007** | -   | **<0.0001** |
| Armenians             | 0.43  | 0.013| 0.871| 0.477| 0.343  | 0.453| -      |
| p-value               | 0.8753 | **<0.0001** | 0.5984 | 0.0578 | 0.0718 | 0.2592| -      |
| Azerbaycan            | 0.513 | 0.382| 0.943| 0.216| 0.1    | 0.697| -      |
| p-value               | 0.3081 | 0.1733 | **0.0244** | **0.0423** | **<0.0001** | **0.0136** | -      |
| Benin                 | 0.463 | 0.225| 0.488| -    | -      | 0.763| -      |
| p-value               | **0.7546** | 0.2861 | -   | **<0.0001** | -   | -      | **0.0005** |
| Bretons               | 0.556 | 0.267| 0.9  | 0.478| 0.4    | -   | -      |
| p-value               | 0.1039 | 0.7051 | 0.2436 | 0.0560 | 0.3320 | -   | -      |
| Cambodian             | 0.5417 | 1 | 0.7917 | -    | -      | 0.4167| -      |
| p-value               | **0.1544** | -   | **<0.0001** | **0.3283** | -   | -      | **0.1182** |
| Cameron               | 0.343 | 0.343| 0.75 | -    | -      | 0.625| -      |
| p-value               | 0.1558 | 0.4294 | 0.0946 | -    | -      | 0.1608| -      |
| Cherkessians          | 0.386 | 0.167| 0.932| 0.39  | 0.439  | 0.651| -      |
| p-value               | 0.4297 | **0.0369** | 0.0506 | 0.5093 | 0.6804 | 0.0747| -      |
| Chinese               | 0.4412 | 0.8529 | 0.8824 | -    | -      | 0.4706| -      |
| p-value               | 0.9977 | **<0.0001** | 0.4408 | -    | -      | 0.4251| -      |
| Darginians            | 0.361 | 0.167| 0.864| 0.167| 0.143  | 0.321| -      |
| p-value               | 0.2484 | **0.0369** | 0.7032 | **0.0039** | **<0.0001** | **0.0032** | -      |
| Egyptian              | 0.513 | 0.125| 0.8  | -    | -      | 0.55 | -      |
| p-value               | 0.3081 | **0.0038** | 0.4050 | -    | -      | 0.7442| -      |
| European American     | 0.55  | 0.178| 0.944| 0.511| 0.467  | -   | -      |
Table 3 (Cont.)

| Population | TPA25 | PV92 | APO | ACE | FXIII B | B65 | HS3.23 |
|------------|-------|------|-----|-----|---------|-----|--------|
|            |       |      |     |     |         |     |        |
| p-value    | 0.1232| 0.0593 | 0.0227 | 0.0177 | 0.9887 |     |        |
| Finnish    | 0.4444 | 0.1563 | 0.9737 | -    | 0.4211 | -   |        |
| p-value    | 0.9614 | 0.0223 | 0.0015 | -    | 0.1337 | -   |        |
| French     | 0.557 | 0.227 | 0.989 | 0.477 | 0.42 | 0.625 | - |
| p-value    | 0.1099 | 0.3016 | 0.0002 | 0.0578 | 0.4945 | 0.1608 | - |
| French Acadians | 0.433 | 0.178 | 0.922 | 0.511 | 0.478 | - | - |
| p-value    | 0.9092 | 0.0593 | 0.0897 | 0.0177 | 0.8874 | - | - |
| Georgians  | 0.493 | 0.25 | 0.934 | 0.354 | 0.61 | 0.727 | - |
| p-value    | 0.4611 | 0.5140 | 0.0447 | 0.8938 | 0.0440 | 0.0035 | - |
| Greek Cypriots | 0.53 | 0.25 | 0.95 | 0.39 | 0.616 | - | - |
| p-value    | 0.2080 | 0.5140 | 0.0144 | 0.5093 | 0.0357 | - | - |
| Greenland Natives | 0.333 | 0.607 | 0.940 | 0.548 | 0.786 | - | - |
| p-value    | 0.1169 | <0.0001 | 0.0301 | 0.0039 | <0.0001 | - | - |
| Hispanic   | 0.625 | 0.523 | 0.92 | 0.545 | 0.705 | - | 0.74 |
| p-value    | 0.0091 | 0.0008 | 0.0996 | 0.0044 | 0.0007 | - | 0.8353 |
| Indian     | 0.5957 | 0.4662 | 0.8148 | - | - | 0.4596 | - |
| p-value    | 0.0286 | 0.0106 | 0.5698 | - | - | 0.3405 | - |
| Ingushians | 0.224 | 0.129 | 0.941 | 0.34 | - | 0.21 | - |
| p-value    | 0.0011 | 0.0049 | 0.0281 | 0.9406 | - | <0.0001 | - |
| Japanese   | 0.5 | 0.8571 | 0.8438 | - | - | 0.4118 | - |
| p-value    | 0.4032 | <0.0001 | 0.9813 | - | - | 0.1024 | - |
| Kenyan     | 0.363 | 0.45 | 0.613 | - | - | 0.625 | - |
| p-value    | 0.2606 | 0.0199 | 0.0002 | - | - | 0.1608 | - |
| Malays     | 0.25 | 0.5 | 0.8333 | - | - | 0.5 | - |
| p-value    | 0.0045 | 0.0025 | 0.8218 | - | - | 0.7025 | - |
| Moroccan   | 0.4550 | 0.1961 | 0.6974 | 0.1760 | 0.2670 | 0.3727 | 0.8038 |
| p-value    | 0.8422 | 0.1180 | 0.0130 | 0.0065 | 0.0032 | 0.0283 | 0.2000 |
| Nigerians  | 0.409 | 0.091 | 0.5 | 0.273 | 0.083 | - | - |
| p-value    | 0.6471 | 0.0003 | <0.0001 | 0.2706 | <0.0001 | - | - |
| Northern Europe | 0.5522 | 0.2537 | 0.9697 | - | - | 0.6186 | - |
| p-value    | 0.1158 | 0.5535 | 0.0024 | - | - | 0.1904 | - |
| Polish     | 0.75 | 0.1250 | 0.95 | - | - | 0.35 | - |
Table 3 (Cont.)

| Population       | TPA25 p-value | PV92 p-value | APO p-value | ACE p-value | FXIIB p-value | B65 p-value | HS3.23 p-value |
|------------------|---------------|--------------|-------------|-------------|---------------|-------------|----------------|
| Pygmies          | <0.0001       | 0.0038       | 0.0144      | -           | -             | 0.0117      | -              |
| p-value          | 0.0009        | 0.7812       | 0.3484      | 0.0516      | <0.0001       | -           | -              |
| Rwandan          | 0.275         | 0.413        | 0.913       | -           | -             | 0.675       | -              |
| p-value          | 0.0143        | 0.0709       | 0.1404      | -           | -             | 0.0326      | -              |
| Sudanese         | 0.363         | 0.4          | 0.7         | -           | -             | 0.75        | -              |
| p-value          | 0.2606        | 0.1051       | 0.0145      | -           | -             | 0.0010      | -              |
| Swiss            | 0.453         | 0.198        | 0.942       | 0.372       | 0.477         | -           | -              |
| p-value          | 0.8645        | 0.1260       | 0.0262      | 0.6905      | 0.8986        | -           | -              |
| Turkish Cypriots | 0.576         | 0.333        | 0.985       | 0.333       | 0.394         | -           | -              |
| p-value          | 0.0562        | 0.5215       | 0.0004      | 0.8577      | 0.2907        | -           | -              |
| Emirati          | 0.44          | 0.3          | 0.97        | 0.33        | 0.39          | 0.41        | -              |
| p-value          | 0.9886        | 0.8891       | 0.0023      | 0.8225      | 0.2651        | 0.0973      | -              |
| US Caucasian     | -             | -            | -           | -           | -             | 0.87        | -              |
| p-value          | -             | -            | -           | -           | -             | -           | 0.0117         |
| Vietnamese       | 0.2778        | 0.875        | 0.9444      | -           | -             | 0.4444      | -              |
| p-value          | 0.0162        | <0.0001      | 0.022       | -           | -             | 0.2426      | -              |

and Americans from different ethnicities, western Europeans, and Africans for A CE, FXIIB, HS3.23 and B65 (p \( \leq 0.05 \)) (Table 3) (Arcot et al., 1996; Batzer et al., 1996; Nasidze et al., 2001; Watkins et al., 2001; and Chadli et al., 2009). Furthermore, TPA25 locus showed a significant difference in Alu insertion between Jordanians and Americans and Asians populations including Indians, Vietnamese, and Malay (p \( \leq 0.05 \)), and Americans, Asians, and Europeans for APO and PV92 (p \( \leq 0.05 \)) (Arcot et al., 1996; Batzer et al., 1996; Nasidze et al., 2001; Watkins et al., 2001; Terreros et al., 2005; and Chadli et al., 2009). In general, the comparison between Alu insertion frequencies in Jordanian population and other populations [African, North and South Americans and Eastern European (African American, African Caribbean, Alaskan, Hispanic, Nigerian, Moroccan, Polish and Greenland] show a significant difference (Table 3) (Arcot et al., 1996; Batzer et al., 1996; Nasidze et al., 2001; Watkins et al., 2001; and Chadli et al., 2009) (p \( \leq 0.05 \). These genetic variances could be due to the large geographic distance between Jordanians and those populations (Terreros et al., 2009).

On the other hand, many other populations did not show a significant difference in the Alu insertion frequency (Armenians, Bretons, Cambodians, Cameroon, Cherkessians, Chinese, Egyptians, French Canadians, Japanese, Sudanese, Swiss, Turkish Cypriots, and Emirati) when compared to Jordanians (Table 3) (Batzer et al., 1996; Arcot et al., 1996; Watkins et al., 2001; Nasidze et al., 2001; Chadli et al., 2009; and Terreros et al., 2005) (p \( \geq 0.05 \)). This suggests that the similarity may be due to the occurrence of the Alu insertion event former to migration of modern human out of Africa 100,000 years ago, or in some cases, due to close geographic distance or the possible migratory action between these populations (Stringer and Andrews, 1988; Harpending et al., 1993; Cavalli-Sforza et al., 1994; Goldstein et al., 1995; Nei, 1995; Stoneking et al., 1997; and Terreros et al., 2009).

5. Conclusion

In the light of establishing an automated fluorescent based method for Alu genotyping (Asari et al., 2012), the researchers think that despite the small sample size included in the current study, the seven Alu insertion/
deletion data loci are applicable in forensic investigations and when combined with the Jordanian STR data (Yasin et al., 2005) an exceptional resolution power between Jordanian individuals is maintained.

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