INTRODUCTION

Large-scale and reliable genotypes at individual level are required to understand the role of selective pressures on the evolution of highly polymorphic functional genes. Next-generation sequencing techniques allow for such large-scale sequencing, but post-processing treatment is still a challenge.

OBJECTIVES

→ We develop a methodology to obtain reliable genotypes after an optimized 454 sequencing and post-processing.
→ We apply this methodology to an extensive dataset.
→ We assess the quality of assigned genotypes through three different procedures.

454 SEQUENCING

Four loci of the major histocompatibility complex [1].

Well-optimized primers [1] and individual tags.

Moderate number of PCR cycles (24).

1,152 amplicons at each loci (4,608 amplicons) on 8 parts of a Roche® 454 FLX sequencing instrument.

→ Low amplicon coverage to improve the cost-efficiency.

454 POST-PROCESSING PROPOSED METHODOLOGY

<table>
<thead>
<tr>
<th>Processing description</th>
<th>UB reads (N amp)</th>
<th>UD reads (N amp)</th>
<th>DRB1 reads (N amp)</th>
<th>DRB2 reads (N amp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reads with individual tags and 3-bp loci markers</td>
<td>152,960 (1067)</td>
<td>113,862 (1069)</td>
<td>79,114 (966)</td>
<td>113,207 (1001)</td>
</tr>
<tr>
<td>Reads with &gt; 95% and &lt; 105% of locus sizes</td>
<td>72,214 (948)</td>
<td>59,642 (944)</td>
<td>49,212 (903)</td>
<td>64,110 (901)</td>
</tr>
<tr>
<td>Amplicons with &gt; 11 reads for a given and locus</td>
<td>71,611 (835)</td>
<td>58,911 (793)</td>
<td>48,291 (720)</td>
<td>63,254 (726)</td>
</tr>
<tr>
<td>Reads with within a given amplicon with &gt; 24% of allelic frequency with respect to the main read</td>
<td>70,839 (835)</td>
<td>58,481 (793)</td>
<td>47,667 (720)</td>
<td>62,682 (726)</td>
</tr>
</tbody>
</table>

→ High variability of coverage per amplicon decreased the number of retained reads and amplicons.
→ The optimization of 454 post-processing increased the number of retained reads and amplicons.
→ No artefacts, PCR chimeras or new alleles were found after 454 post-processing.

VALIDATION OF GENOTYPES

1,460 out of 3,074 obtained genotypes could be validated through intra-individual 454 sequencing repeatability (51 genotypes); 454 and Sanger sequencing repeatability (36 genotypes); and comparing the consistency of mother-father-offspring triads determined through paternity analysis (1,373 genotypes) [7].

RESULTS

High quality MHC genotypes were obtained at the four MHC sequenced loci for 895 genotyped individuals, with a mean of 3.43 ± 0.96 (± SD) genotypes per individual and an error rate estimated to 0.3%.

CONCLUSIONS

→ Methods applicable methods to any organism for which the number of loci is known.
→ Our results emphasize the potential of 454 sequencing and 454 post-processing to obtain large-scale highly reliable genotypes.
→ The use of the proposed methodology could improve research on the evolution of highly polymorphic functional genes.

REFERENCES


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