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Probe Induced heteroduplex mobility assay (PRIMA)

PRIMA is a rapid and cost-efficient method to detect a single-nucleotide insertion/deletion. In experiments using genome-editing (e.g. CRISPR), screening and genotyping will be expedited. PRIMA (Probe-Induced HMA) is based on heteroduplex mobility assay (HMA), and is tested by electrophoresis machines as well as by gels.

Keywords	Genotyping, genome-editing, CRISPR, single nucleotide resolution
Inventors	Dr. Hiroyuki Kakui, Dr. Misako Yamazaki and Prof. Dr. Kentaro Shimizu
Reference	WO2021023896A1 Scientific manuscript submitted, currently under revision
Background	Genome-editing technology (ZFN/TALEN/CRISPR) typically induces a non-functional allele with a 1-bp insertion or deletion (indel) mutation. Many methods to detect a small base pair difference were developed (e.g. RFLP analysis, HMA, DNA melting analysis, T7 endonuclease I assay, etc.), but each method has pros and cons in terms of cost, time, and/or resolution. Heteroduplex mobility assay (HMA) is one of the cheapest/fastest methods for detecting small base pair differences but it does not provide 1bp resolution. Single nucleotide resolution with a handy method is also desired in different fields: forensic analyses, natural variation study in evolutionary biology, or agricultural and livestock breeding.
Invention	This invention “PRIMA” (Probe-Induced HMA) is developed based on the heteroduplex mobility assay (HMA). PRIMA uses a short mutated DNA as a “probe” and enables to distinguish single nucleotide differences. Traditional HMA needs two rounds of analysis to distinguish wild type homozygous, mutant homozygous or heterozygous genotypes, but PRIMA can distinguish three genotypes in a single run.
Fields of Use	Researcher using genome-editing or using single (or small) base pair variations in any species.
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Contact	*Unitectra, Technology Transfer University Zurich, Dr. Cornelia Fürstenberger, Scheuchzerstrasse 21, CH-8006 Zürich, +41 44 634 44 01, mail@unitectra.ch*