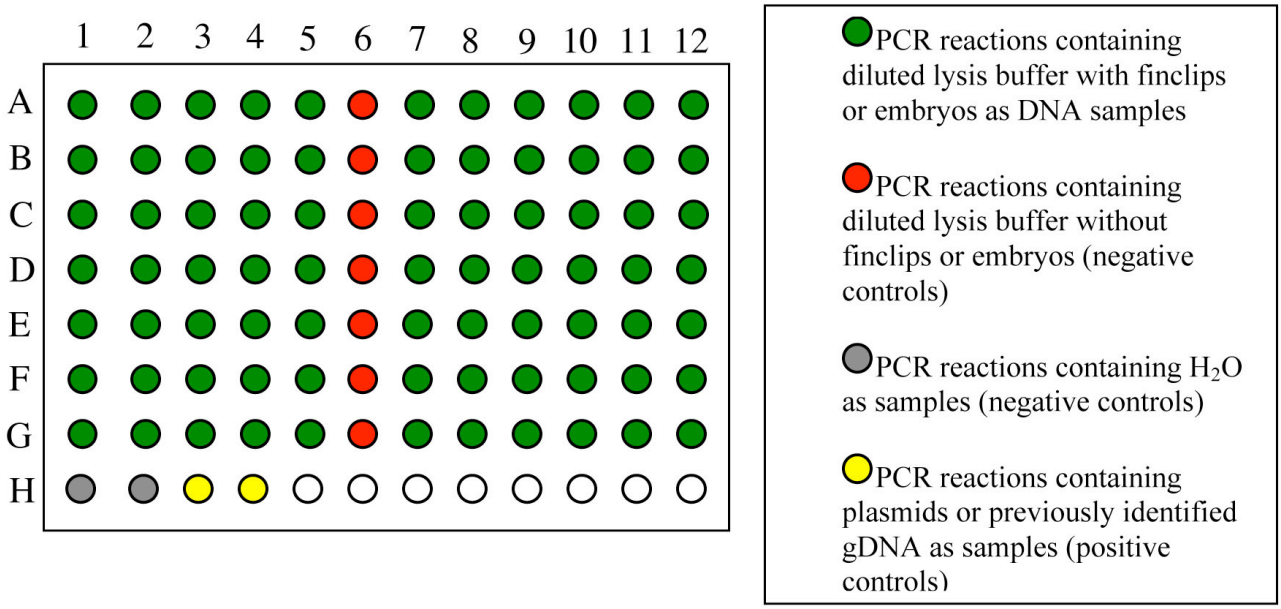
H. CONTROLS

Our greatest concern is to eliminate false positives. Because PCR is a very sensitive technique and even the slightest contamination may lead to false positives, we use several controls to monitor possible contamination. First, the wells in row 6 of all PCR plates are filled with lysis buffer, proteinase K and mineral oil, but contain no fin or other embryo material and should therefore produce no PCR amplification products. This serves as a control for the handling of the PCR plate between the time of the fin clip and the PCR reaction.

We use an additional negative control to monitor the PCR reaction. In this case, we add water instead of samples to designated wells containing the PCR master mix and primer sets. For positive controls, we use previously isolated genomic DNA samples. The setup of a typical PCR plate is shown below:



We use additional wild-type controls in RFLP and dCAPS genotyping assays to monitor restriction enzyme digestions. These controls are particularly important for assays in which wild-type DNA is cleaved as they provide crucial information with regard to whether digestions were carried out to completion. If digestions are not complete, partially digested wild-type samples may be mistakenly identified in these assays as heterozygous samples.

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