It was shown by Dumoulin *et al.* (1998) that the use of Ca^{++} and Mg^{++}-free medium to de-compact the embryos did not have a detrimental effect on the development of the embryo. This is why most centres performing PGD at the cleavage stage incubate the embryos in Ca^{++} and Mg^{++}-free medium, either only before biopsy, or during the whole biopsy procedure (ESHRE PGD Consortium Steering Committee, 2002).

For the biopsy itself, several techniques have been developed and applied with changing success. Biopsy by flow displacement (where a flow of fluid is applied through one opening in the zona to blow out the blastomere through another hole) or extrusion (where pressure is applied to the embryo to push a blastomere through the opening in the ZP) are just mentioned for completeness. The most widely used method is described extensively in Joris *et al.* (2003). The embryo is held with a holding pipette (100 μm outer, 25-30 μm inner diameter) through the application of a slight negative pressure. A blunt biopsy pipette (inner diameter of 35-40 μm) is introduced through the hole in the ZP and the blastomere is gently aspirated. The blastomere is usually not aspirated completely into the pipette, but is "grabbed" partially and pulled through the hole in the ZP (Figure 1).

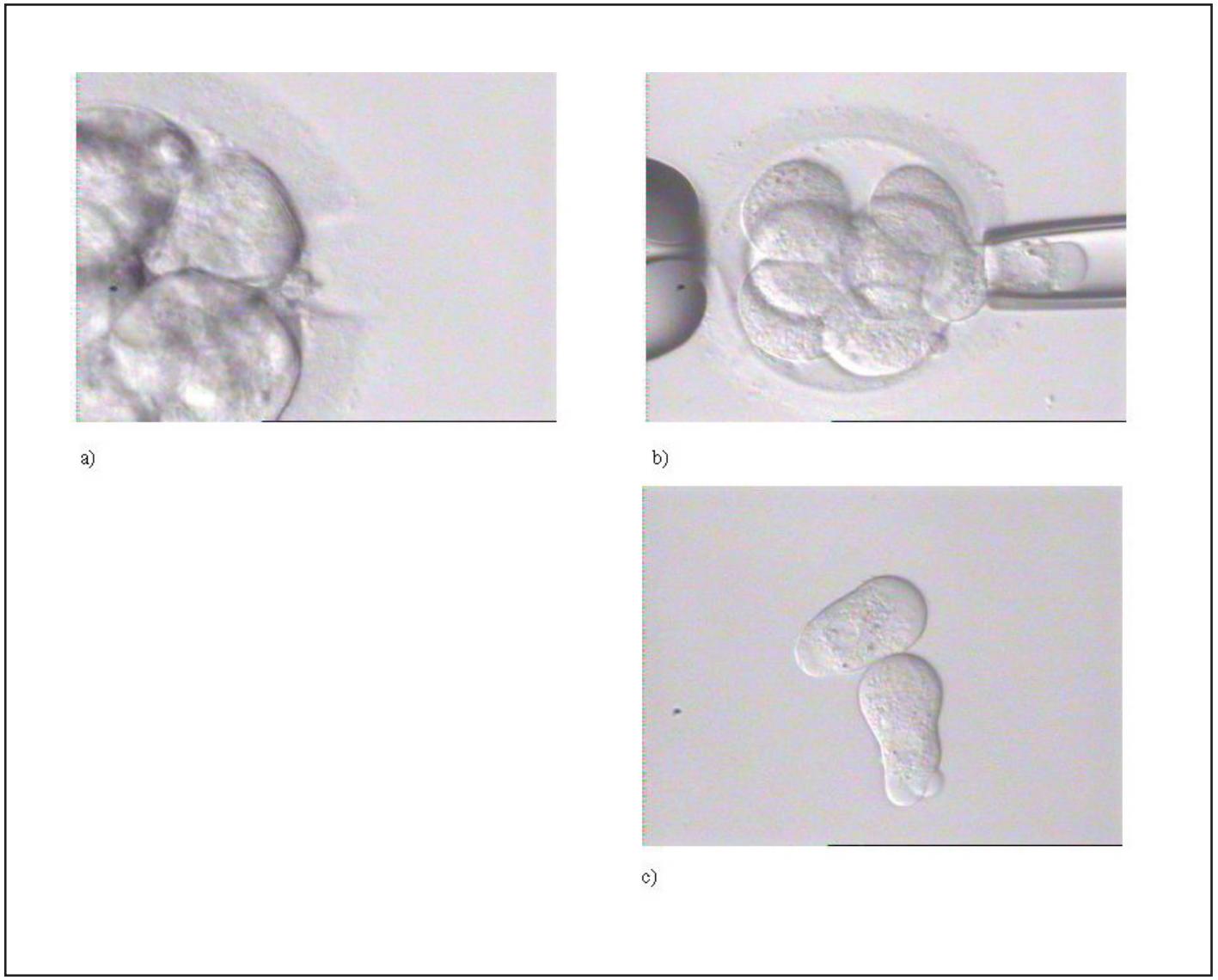

Fig. 1 – *Example of an embryo biopsy. Panel a) shows the hole in the zona pellucida after application of the laser for zona hatching. Panel b) shows how one blastomere is gently aspirated outside the embryo. Panel c) shows two biopsied blastomeres with a clear nucleus.*

neate the translocation breakpoints (Munné *et al.*, 1998, Munné *et al.* 2000). A more general approach was described by Conn *et al.* (1999), taking advantage of the availability of centromeric and subtelomeric probes for each chromosome. For Robertsonian translocations, a combination of one locus-specific probe centromeric to the breakpoint of one of the chromosomes involved and one on the telomere of the other chromosome involved, is applied. Three FISH probes are used for reciprocal translocations: two on the centromeric side of the breakpoints of the chromosomes involved, and a third one on one of the telomeres on the other side of the breakpoint (Figure 2). The two or three

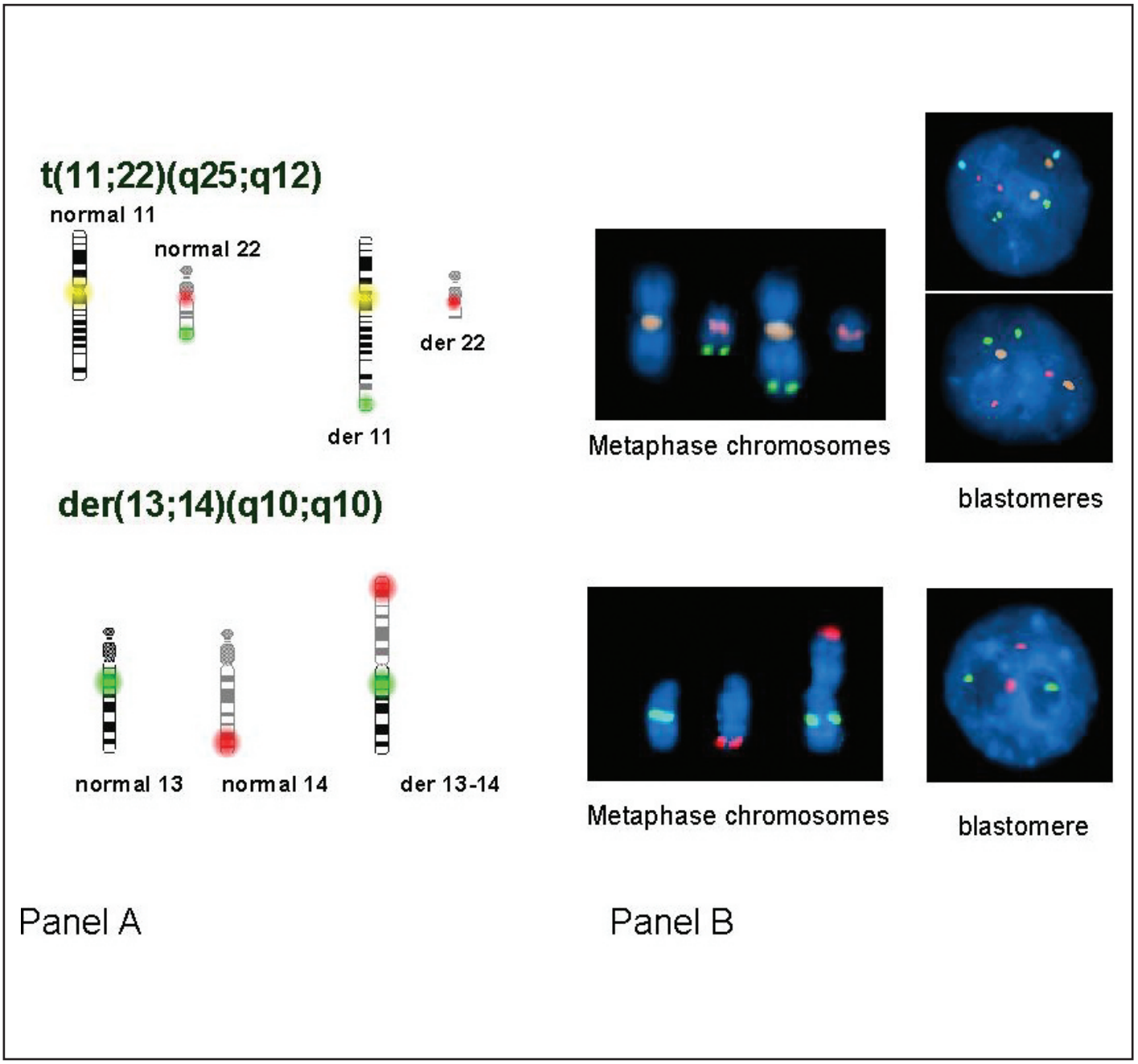

Fig. 2 – *An example of the probes chosen for a reciprocal translocation (t(11,22), panel a) and for a Robertsonian translocation (t(13;14), panel b). The results are shown schematically, on lymphocyte metaphase spreads, and on interphase spreads of blastomeres.*