DNA extraction from Chorionic Villus Samples

Extraction protocols are offered by MRC-Holland as a service only. MRC-Holland has limited experience with DNA and RNA extraction and the current protocols are based on feedback from MLPA users. Unfortunately, MRC-Holland cannot guarantee that good results will be obtained by following these protocols. If you have any feedback on these protocols, be it positive or negative, please contact us at info@mlpa.com.

MRC-Holland, Customer Support

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1 DNA EXTRACTION FOLLOWING PROTEINASE K TREATMENT OF WHOLE VILLI

The following is taken from the methods section of the aforementioned article:

DNA from at least two chorionic villi was extracted using a QIAamp DNA Mini Kit (Qiagen) following the protocol "Isolation of DNA from soft tissues using the TissueLyser and QIAamp DNA Mini Kit" (Qiagen, Westburg bv, The Netherlands). In this procedure, incubation at 56 °C with proteinase K results in lysis of the villi prior to DNA extraction. Proteinase K has a specific activity and degrades tissue to facilitate the purification of the DNA. Finally, DNA was eluted in 50 µl AE buffer (10 mM Tris-HCl, 0.5 mM EDTA, pH 9.0).

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2 DNA EXTRACTION FOLLOWING ENZYMATIC DISSOCIATION OF VILLI

The following is taken from the methods section of the aforementioned article, which is a modification of the method described by Mann1:

Digestion of cleaned villi with collagenase (800 units/ml; 37°C, 30 min), was followed by trypsin digestion (0.5% trypsin/EDTA, 37°C, 30 min). After collagenase digestion, the suspension was separated from the remaining villi and transferred to a tube containing 4 ml PBS and 10% FCS to stop the digestion. After centrifugation (1200 rpm, 5 min) the supernatant was removed and the cell pellet was resuspended in 300 µl PBS (fraction C). After digestion of the remaining villi with trypsin, 4 ml PBS + 10% FCS were added to stop the reaction. After centrifugation (1200 rpm, 5 min) the supernatant was removed and the cell pellet was resuspended in 300 µl PBS (fraction M). Finally, 100 µl of fraction C and 100 µl of fraction M were mixed (1:1) (fraction T) and used for DNA isolation. DNA extractions were performed by incubation of the cell population with proteinase K at 56°C. DNA was purified using a QIAamp DNA Mini Kit (Qiagen) following the protocol "Blood and body fluid spin protocol". Finally, DNA was eluted in 50 µl AE buffer (10 mM Tris-HCl, 0.5 mM EDTA, pH 9.0).

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