96 Well Gel Extraction with Nucleo Spin Kit

using the Nucleo Spin Multi96 Extract Kit in combination with gel extraction buffer NT1 (MachereyNagel)
other equipment needed:
- QIAvac unit or similar
- Centrifuge capable of spinning 96 well plates (we used a Heraeus 3SR)
- plastics: a 2.2 ml 96 deep well block (square wells with conical base), 96 micro well plates

- Excise the DNA fragments* from the agarose gel and place each slice in appropriate well of a 2.2 ml 96 deep well block
- Add 4,5 volumes of NT1 (prewarmed to 55°C) to one volume of gel
  This equates to approximately 1 ml of NT1 to each well
- Close block with adhesive film and heat block at 55 °C in a bacterial shaker, shaking until gel slices are completely dissolved (30 minutes); pipetting up and down after the 30 min incubation time may help dissolving
- Place yellow-white column plate on top of the QIAvac apparatus containing the white waste tray
- Load the dissolved samples to the columns using the multipipette
- Suck the liquid from the columns through to the waste tray
  (keep pressure less than 400 mbar)
- Wash column plate with 500 µl NT3, suck through
- Wash column plate with 900 µl NT3, suck through
- Seal column plate and place it into 96 micro well plate, spin for 10 min, 4800 rpm (to get rid of NT3 completely);
- Discard (or keep for later re-use) micro well plate; remove seal and dry column plate 5 min at 55°C
- 75 µl buffer NE to each well of column plate and let stand for 2 minutes. Seal column plate and elute the DNA into a fresh 96 micro well plate by spinning for 3 min, 4800 rpm

* if excising DNA fragments obtained after ligating PCR fragments to inverted repeats: you will observe 2 bands on the gel: one of the expected size of 2XPCR fragment and one of the size of 1XPCR fragment; this is OK, just cut out the upper band