Pea Aphid Microsatellite Multiplex

(7 Loci: AlA09M, AlB07M, AlB08M, AlB12M, ApF08M, ApH08M, ApH10M from Caillaud et al. 2004)

Material:

- **-** PCR plates/strips
- - ABI plates
- 1.5ml Tubes
- Réservoir
- Cold elements/ cool boy
- extra waste bag for HiDi waste

Chemical:

- Qiagen Master-Mix
- Primers
- HiDi: Suspected of damaging the unborn child, Suspected of causing cancer
  May cause damage to organs through prolonged or repeated exposure
  Work with lab coat and with nitrile gloves and the best would be to work under the hood.

PCR:

- Fill out *MP_Pea_aphid* excel sheet and pipette mastermix accordingly.
- Distribute 10 ul mastermix to each well of the PCR plate/strip, then add DNA (1 ul per well)
- Centrifuge everything down
- Let PCR run with PCR-program: *MP_pea_aphid.* If this is not saved on the machine yet, program the machine according to the MP_Pea_aphid excel sheet and save program in the Vorburger lab folder.

ABI:

- tell Esther or Katri in advance that you are going to prepare a ABI plate!!
- dilute PCR product 1:20 -> 1ul PCR + 19ul autoclaved MiliQ
- centrifuge plate quickly
- **Work now under the hood or put everything on a cold element and wear lab coat + nitrile gloves!!**
- put the ABI plate in a cool boy to cool down
- Prepare HiDi+Liz500 mix: - 9.9 ul Hidi *(Sample number + 10% sample number) + 0.1 ul Liz500*(Sample number + 10% sample number) Hidi waste goes in separate waste bag!
- Pipette in each well of an ABI plate 10ul of HiDi+Liz500 mix
- add 0.5 ul of diluted PCR product
- If one of the even numbered rows is empty, pipette 10ul of HiDi in each well of this row
- Fill out ABI data sheet and save it as a text and excel file under Q:\Querprojekte\MolGen\ABI new 2010\data sheets
- Put the plate in the fridge of the ABI room
- Put the waste bag under the hood and throw it away after one week
- Tell Esther or Katri that the plate is ready to let run