

### Methylation analysis D4Z4 (LUMC, Leiden)

This method is used to determine the methylation at the FseI site in the most proximal D4Z4 unit. We experienced that some liquid DNA samples may contain impurities (proteins or salts) that might inhibit the FseI digestion. To overcome these problems the DNA is column purified prior to the FseI digestion. Prior to the purification the DNA samples are double digested by BglII/EcoRI (in large volume and overnight) to obtain fragments < 50 kb (required for most purification methods). After the purification step the samples are digested by FseI for >4 hours.

We got good results using the purification columns obtained from Machery nagel (nucleospin extract II, cat nr. 740609250).

DO NOT use the High Pure PCR Cleanup Micro Kit from Roche (cat. Nr. 04 983 912 001), after DNA purification with this kit, the yield is lower than 30%, and if you use the binding enhancer the purified DNA cannot be treated anymore with FseI!!

#### Liquid DNA

BglII (10U/uL)	2 uL
EcoRI (10U/uL)	2 uL
buffer H 10x	10 uL
spermidine 0.1M	3,3 uL
<u>water</u>	<u>42,7 uL</u>
total (mix)	60 uL

<u>water + 2 ug DNA</u>	<u>40 uL</u>
Total	100 uL

#### Plugs DNA

BglII (10U/uL)	5 uL
EcoRI	3 uL
buffer O 10x	15 uL
spermidine 0.1M	5 uL
DTT 0.1M	1,5 uL
<u>water</u>	<u>122,5 uL</u>
Total	150 uL

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Perform DNA purification by Machery Nagel kit (nucleospin extract II) according to the manufactures and elute DNA with 30 ul elution buffer (yield 90%). For plugs; check DNA concentration after extraction and adjust to 2,0 ug prior to FseI digestion

DNA	30 uL
NEB4 10x	4 uL
BSA 100x	0,4 uL
FseI (2U/ul)	3 uL
<u>Water</u>	<u>2,6 uL</u>
Total	40 uL

Note: FseI is very instable and needs to be stored at -80 C (at -20 C the activity drops dramatically). FseI is not recommended for digest over 1 hour.

After hybridization with probe P13E-11, subsequent washings 10' with 2xSSC+0.1%SDS, 10' with 1xSSC+0.1%SDS and 10' with 0.3xSSC+0.1%SDS, a methylated band at 4061 bp and a unmethylated band at 3387 bp can be seen. Methylation level is calculated by the ratio between these two bands.