Methylation PCR Protocol

Materials required:

- EZ DNA Methylation-Direct Kit (ZYMO RESEARCH)
- Dynazyme polymerase and Optimalized buffer for Dynazyme polymerase
- 100% ethanol
- DNase free water
- automatic pipettors (100 µl, 1000 µl)
- 200 µl PCR tubes
- PCR apparatus
- microfuge

I.) Bisulphite conversion with EZ DNA Methylation-Direct Kit (ZYMO RESEARCH)

➢ Preparation:

- **CT Conversion Reagent**: - powder reagent
  - 790 µl M-Solubilisation Buf.
  - 300 µl M-Dilution Buffer

  } Mix thoroughly by vortex and by hand shaking for 10 minutes.

Add 160 µl M-Reaction Buffer and shake it for another 1minute.

Storage: room temperature overnight/ 4 ºC for one week/ -20 ºC for a month.

- **M-Wash Buffer:** add 24 ml 100% ethanol to 6 ml M-Wash Buffer concentrate.

➢ Work flow:

a.) Conversion mix: - 20 µl DNA (50 pg – 2 µg, suggested amount: 1 µg)
- 130 µl CT Conversion Reagent

Mix well, centrifuge briefly

b.) PCR:
98 ºC 8 min
64 ºC 3.5 h
4 ºC 20 h (max.)

c.) Purifying converted DNA:

- assemble the filter (column+collection tube) and add 600 µl M-Binding Buffer
- add the sample and mix by inverting
- centrifuge at maximum speed for 30 s
- discard the flow-through
- add 100 µl M-Wash Buffer to the column
- centrifuge at maximum speed for 30 s
- add 200 µl M-Desulphonation Buffer to the column
- incubate at room temperature for 15-20 min

- centrifuge at maximum speed for 30 s

- add 200 µl M-Wash Buffer to the column
- centrifuge at maximum speed for 30 s } repeat once again
- place the filter in a 1.5 ml clean tube.

d.) Elution:

- add 10 µl M-Elution Buffer directly to the matrix
- centrifuge on maximum spin capacity for 30 s

Storage: under -20 ºC

II. Methylation PCR

- **PCR mix:**
  - 2.5 µl Optimalized Buffer for Dynazyme DNA polymerase
  - 0.5 µl 10 mM dNTP mix
  - 1.5 µl 12.5 pM primer (methylated, unmethylated allele-specific primers)
  - 0.25 µl enzyme (Dynazyme)
  - 15.25 µl DNase free water
  - 5 µl DNA

- **PCR settings:** 94 ºC 2 min

  94 ºC 30 sec
  60 ºC 30 sec } 45x
  72 ºC 30 sec

  72 ºC 10 min
  4 ºC hold temp.

III. Gel electrophoresis

- assemble a 3% agarose gel (3 g agarose in 100 ml 1 x TBE buffer) as described in "Agarose gel electrophoresis protocol for DNA"
- 1 x TBE puffer
- mix 10 µl DNA sample with 2 µl electrophoresis dye
- use 6 µl 100 bp Promega DNA ladder in the next well of the gel

The product is around 160 bp (160 bp the methylated product and 165 bp the unmethylated product).