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Biomedical Engineering

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Labelling amine-modified DNA with DBCO-PEG$_4$-NHS ester

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1 Prepare reaction buffers

- Prepare a PBS-buffer with pH 7.2
- Dissolve 1 mg DBCO-PEG_4-NHS Ester in 19.2 µL DMSO (80 mM)
- Dissolve amine labelled primer in the PBS (pH 7.2) to a concentration of 4 mM

2 Reaction

Volumes may need to be adjusted, but keep the ratios the same (NH_2-labbeled oligo:DBCO-PEG_4-NHS ester = 1:20 and PBS:DMSO = 1:1)

- Add 5 µL of 4 mM stock solution oligo (final concentration in reaction: 0.5 mM)
- Add 15 µL PBS
- Add 15 µL DMSO
- 5 µL DBCO-PEG_4-NHS ester (final concentration: 10 mM)
- Incubate this mixture for 2 hours at room temperature in a shaker at 350 rpm whilst keeping the mixture in the dark (cover in aluminum foil)

3 Purifying the product

- Add 5M NaCl to reaction solution in 1:10 (5M NaCl : Reaction solution) ratio.
- Add cold (-30°C) absolute ethanol in 3:1 (ethanol : Reaction solution) ratio.
- Mix well and incubate in -30°C for 60 minutes
- At this point it is a good idea to pre-chill the table top centrifuge to 4°C
- The solution should appear turbid after this point
- Centrifuge the solation at 14,000 rpm for 15 minutes in a table top centrifuge set to 4°C to form a DNA pellet
- Remove supernatant as the DNA is in the pellet
- Wash pellet 3 times with 75% EtOH by carefully pipetting 100 µL EtOH on the DNA and removing it again with the pipette.
- The product can now be resuspended in your preferred solvent to arrive at the required concentration or kept as a dry pellet to be dissolved later

4 Measuring yield and concentration

- Before measuring any samples, be sure to ‘blank’ the spectrophotometer using the solution the DNA is resuspended in, but with no DNA added. 'Blanking' measures the background inherent to the machine and your solvent.
- If using a NanoDrop to measure your samples, place 2 µL of DNA DBCO conjugate reaction mixture onto the pedestal.
- Close the lid and click measure, be sure to write down absorbance at 260 nm in the 10 mm path.
- The concentration can be calculated using Lambert-Beer and the extinction coefficient of the DNA oligo given by the manufacturer, with $c = \frac{A_{260}}{\varepsilon}$