RP-HPLC Purification of Oligonucleotides

Overview

The following protocol should be followed for the preparation of oligonucleotides prior to submission for analysis using the Mass Spectrometry Facility Biological Service run by Elisabete Pires. Please submit samples with a completed sample submission form which can be found on the MS Facility website.

The Tom Brown group are experienced in using this protocol for oligonucleotides sample preparation and have kindly offered their assistance and use of their equipment if required. Please contact Dr Afaf El-Sagheer (afaf.el-sagheer@chem.ox.ac.uk).

If you have any further questions or queries regarding how to complete this protocol, please contact Elisabete Pires (elisabete.pires@chem.ox.ac.uk).

Protocol

1. Oligonucleotides which have been purchased in a purified form or have already been purified by RP-HPLC can be submitted directly for analysis.

2. All other oligonucleotides, including those which have been used in a reaction sequence, or have been modified chemically, **MUST** be RP-HPLC purified before submission as described below.

Purification

RP-HPLC purification: Oligonucleotides must be dissolved in water and/or desalted to remove buffer/small molecule contaminants. This also ensures the pH of the sample is above 4 and below 8 (required column pH tolerances). Samples should then be injected (max. 1.2 mL) on a Gilson HPLC system with ACE 10 C8 column (250 mm x 10 mm, i.d.; ACE-132-2510) or equivalent system with a gradient of acetonitrile in triethylammonium bicarbonate buffer (0 to 50% acetonitrile over 20 min, flow rate 4 mL/min). The elution buffer should be: buffer A (0.1 M triethylammonium bicarbonate, pH 7.5), buffer B (0.1 M triethylammonium bicarbonate, pH 7.5, mixed with 50% acetonitrile). The gradient must be decided based on the oligonucleotide and the modifications used with higher buffer B acetonitrile percentages used when necessary for more hydrophobic modifications.
The detection wavelength for the oligonucleotides varies from 260 to 298 nm depending upon the oligonucleotide amount. The precise wavelength can be determined by taking a full absorbance spectrum of the diluted sample, correcting the absorbance for dilution and determining the wavelength at which the absorbance is ~1.
The desired fractions from HPLC should be freeze-dried and re-suspended in water.
For other HPLC buffers, such as TEAA (triethyl ammonium acetate) or AA (ammonium acetate), the desired HPLC fraction **MUST** be desalted using a NAP column (GE healthcare) or spin column before submitting it for analysis.

Sample Preparation

Samples should be dissolved in **water**, and **20 ul** of a **20 uM** solution provided. This volume requires inset vials (e.g. Thermo Scientific **C4000-11**). Thermo Scientific **C5000-54A** lids should be used. The injection volume will be 1-2 uL.