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Ultra High Purification of Oligonucleotides-DNA and siRNA

Abstract

Elchrom Scientific AG[1] offers a unique process for ultra high purification of oligonucleotides (oligos). It is based on Elchrom Scientific patented technology covering hydrogels [2] and electroelution system[3]. This versatile system is suitable for purification of DNA and RNA molecules with or without modifications in the size range of 15-150 nt/bp.

Protocol

Separation. The separation of oligos is performed on Elchrom Scientific Oligo Purification Hydrogels (OPGs)*1 (Fig. 1). The electrophoresis is performed in ORIGINS by Elchrom™ *2*3 at 55°C with buffer circulation, 120V (10V/cm) and 4.5 min pump delay. The running buffer is 30mM TAE*4. siRNAs*11 are resolved on OPG-SF gels at 55°C, 150V for 85 min.

Crude oligos from synthesis scale of 200 nmoles or less are dissolved in approximately 100 µl oligo loading buffer (30% sucrose in 30mM TAE). The oligos are loaded on preheated OPG-S4, -S6 or –S8 gel and run for N+10 minutes (N is the length of the oligo in nt).

Recovery. For recovery of oligos from the OPG, Elchrom Scientific electroelution apparatus (Eluter) is used.

After electrophoresis the desired oligo is cut out of the OPG under UV-shadowing *5. The Eluter is filled with 30mM TAE running buffer and Elchrom electroelution buffer. The gel slice with the oligo is placed into the eluter and oligo is recovered during 30 minutes at 200V.

Desalting. After recovery the eluted oligos are desalted on SPE columns, preferable on Oasis® HLB Vac RC 60mg from Waters. The columns are first equilibrated with 1ml acetonitrile (100%), 1ml acetonitrile (80%) and 2 times 1ml SPE buffer from Elchrom. Afterwards the recovered oligos are loaded on the column and washed two times with 1ml SPE buffer. The elution from the column is done by 500µl 80% acetonitrile and 500µl 100% acetonitrile.

Quality Control. The quality control of the purified oligos (>50nt) is done on Elchrom Scientific Spreadex EL 300 gels*6*7. The purified oligos are run next to the corresponding crude oligos. (Fig. 2). Crude oligos (CO) are diluted with 1x Elchrom Sample Loading Buffer (SLB 5xconc. is provided with the gels) in the ratio 2:400. The purified oligos are diluted 2:20 with 1xSLB. 5 µl of diluted samples are loaded per well. The electrophoresis is performed in ORIGINS by Elchrom™ at 55°C with buffer circulation, 120V for 55 minutes and 4.5 min pump delay. The running buffer is 30mM TAE. QC of oligos <50nt is done on SF gels *8. at 55°C, 150V for 85 min.

After the run the gel is detached from the plastic backing by a nylon string provided with the gels, stained for 30 minutes with SYBR Gold or SYBR Green II and destained in destaining solution *9.

Applications

1 Gene synthesis. Elchrom purified oligos lead to genes with up to 38% less errors*10.
2 siRNA. Elchrom purified RNA leads to reliable silencing results *11.
3 TaqMan probes. Elchrom purified double labeled fluorescent probes are clean of single labeled probes which lead to reliable real time PCR results.

References

1 www.elchrom.com
2. Patent # 5,840,877
3. Patent # 7,025,864

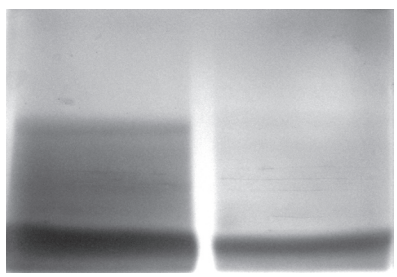

Fig 1. Separation of two crude oligos on Elchrom Scientific OPG.

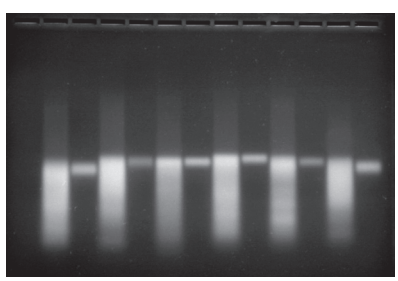

Fig 2. QC of purified oligos on Elchrom Spreadex EL300 gel.

For the remainder of this protocol, (*1-*11) please see www.biotechniques.com/protocol.