DNA SPOTTING ON CX AND HCX SLIDES

Instructions for use

GENERAL INFORMATION

CX and HCX slides are coated with a 3D layer of amine reactive NHS activated carboxyl functionalities. Their reactivity results in high coupling yields but on the other hand makes the activated groups sensitive to hydrolysis. Therefore, the slide packs should be protected from humidity and opened right before spotting. Opened bags can be stored up to three weeks at or below -20 °C in a tightly closed receptacle over molecular sieve or P_2O_5.

To prevent condensation on the slide surface, which will result in surface inhomogeneity and loss of activity, the packaging should be allowed to equilibrate to room temperature before opening.

MATERIALS

- CX or HCX Slides.
- Slide orientation and handling: CX slides are activated on both sides. It is recommended not to spot on surfaces which are facing towards the wall of the transport box. The slides should be handled with tweezers. Avoid touching the surface.
- Spotting solution: 20–100 ng amino functionalized DNA/µL spotting buffer. Ensure that the DNA is of high purity and free from ammonia / amines as these will quench the activated coupling sites. In doubt consider purification (see below).
- Spotting buffer: 50 mM sodium hydrogen carbonate buffer, pH 9.
- Add 0.001–0.01 % Tween® 20 to adjust the spot diameter. Increasing Tween® 20 concentration will result in bigger spots.
- Quenching buffer: 1 M Ethanolamine* HCl, pH 8.5.
- Hybridization buffer: 4 x SSC, 0.1 % SDS.

Note: Lyophilized DNA is sometimes difficult to dissolve in diluted aqueous buffers. In addition, even purified oligos are frequently contaminated with ammonia or methylamine traces. Both issues can be addressed by dissolving the DNA in 200 mM Na_2SO_4 pH 7 and desalting this solution against spotting buffer.

Never use Tris or any other amine or alcohol containing buffers before the quenching step, as these will deactivate the reactive NHS esters on the slide surface!

SPOTTING PROTOCOL

1. 2–12 hrs before use take the slide package out of the freezer and let it reach room temperature.
2. Remove the slides from the container with tweezers or powder-free gloves. Don't touch or scratch the surface as this will adversely affect the immobilization process.
3. Place the slides in a spotter and spot oligos. Recommended humidity is 10–40 %. To minimize hydrolysis, longer spotting runs should be conducted at lower humidity.
4. Post printing: Let the spots dry and incubate at 60 % humidity for 2–4 hrs at 60 °C.
5. Hydrolyse remaining active groups 30 min with quenching buffer.
6. Incubate in hybridization buffer for 30 min.
7. Rinse thoroughly with dd water and centrifuge the slides dry (for example in a 50 mL Falcon tube). Alternatively the water can be removed with a compressed clean (oil-free) air or nitrogen jet. Don't let water droplets dry on the slide surface as this might cause surface inhomogeneity.

Spotted surfaces can be stored dry (over molecular sieve) at or below – 20 °C under nitrogen or argon in a dark place over several months. Before hybridisation rehydrate the surface at least 30 min with hybridisation buffer.

HYBRIDISATION

Several commonly used buffer systems were tested for hybridisation and gave equally good results. Generally 2–5x SSC works well. Depending on the length of the oligos up to 50 % formamide may be added to increase the specifity. As the surface is negatively charged, nonspecific interactions with the substrate do not occur and addition of blocking agents such as BSA or DNA is not recommended.

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