Detection of genetically modified organisms using DNA microarrays

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With the increasing production of genetically modified organisms (GMOs), the quick detection system is required. Microarrays offer a suitable and time – saving method. Our aim is to develop DNA microarrays for detection of GMOs.

HOW WE PRODUCE OUR SLIDES

- Selected GMO sequences were cloned to plasmid vectors and these vectors were transformed to competent *Escherichia coli* cells
- Plasmids with GMO inserts were isolated and amplified with T3 and T7 primers, PCR amplicons were purified
- Plasmids with GMO inserts and PCR amplicons were concentrated with vacuum evaporater
- Final concentration of DNA probes was set up: **plasmid DNA c = 0.65 – 1.3 ug/ul**
  PCR amplicons c = 0.35 – 0.7 ug/ul
- Plasmids with GMO inserts and PCR amplicons were printed on amino-glass support

POST-PRINTING SLIDES HANDLING

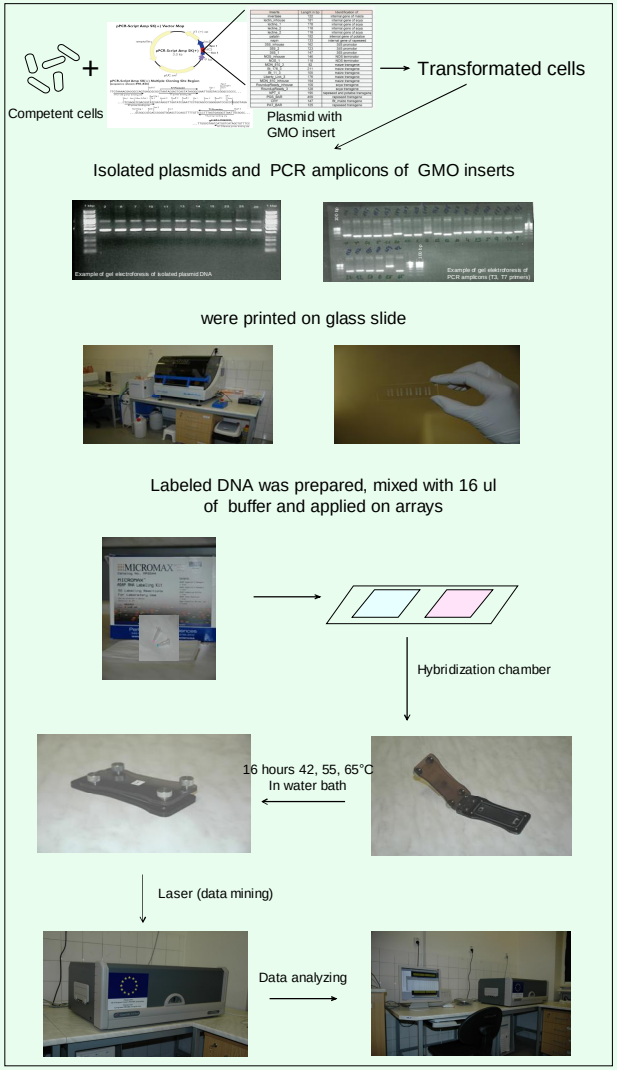
- Crosslinking
- Prehybridization: solution composition – 5x SSC, 0.1% SDS, 10 mg/ml BSA
  prehybridization condition - 42°C, 4 hours
- DNA denaturation in boiling H_2O for 30 sec and in cold 100% EtOH for 10 sec

DNA TARGET

- Two PCR amplicons, **lectin_inhouse and 35S_promoter**, were purified, concentrated and labeled with fluorescent dyes cyanin-5 and cyanin -3, respectively
- Labeled DNA was purified, precipitated, denaturated (95°C, 2-3 min) and together with hybridization buffer applied on the array

HYBRIDIZATION CONDITIONS

- Hybridization was carried out in hybridization chamber at 42°C, 55°C and 65°C for 16 hours
- Hybridization buffers – commercially produced – same salt and formamide composition, various in terms of hybridization kinetics and blocking reagent



RESULTS AND DISCUSION

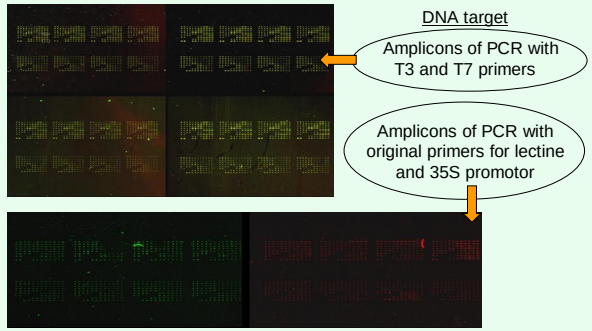
- The aim was to determine the specifity of our slides. Hybridization reactions were carried out under various conditions – temperature (42, 55, 65°C) and buffer composition.

Sample DNA used in hybridization:

1) Mixture of PCR amplicons of lectine and 35S promotor (used primers - T3, T7)

2) PCR amplicons of lectine and 35S promotor (used primers were original to lectine and 35S promotor sequences)

- Slides were scanned with GeneTAC™ UC4 scanner and pictures were compared visually
- We expected to see signals only in the spots belonging to lectine and 35S promotor (spots in white circles). But in every experiment all spot were visible. There were some differences in the intensity of spots. It seems, that DNA bounds nonspecifically. One of the reasons can be that DNA sequences for T3 and T7 primers are present in each PCR amplicon. That is why we tried to hybridize amplicons of PCR, where original primers for lectine and 35S promotor were used. But the results were almost the same, only intensities of spots decreased.



CONCLUSION

- We have to try other approaches in slides producing

- amplicons of PCR (with original primers) imobilized on the slide
- oligonucleotides imobilized on the slide
- new way of DNA labeling – in PCR reaction

- New hybridization conditions should be applied

- buffer composition, time of hybridization

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