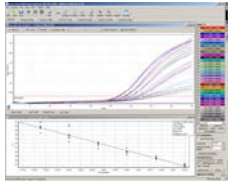


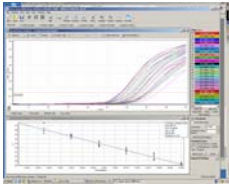
Allergen determination in food by multiplex qPCR

Veronika Dvorak, Franziska Zimmerli, Alda Breitenmoser, René Köppel
rene.koeppel@klzh.ch
Official Food Control Authority of the Canton of Zürich

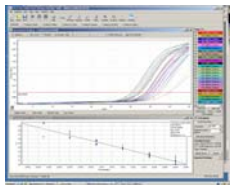
celery



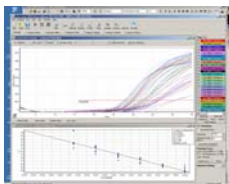
soja



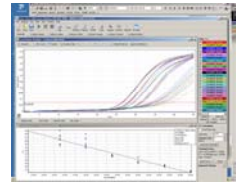
peanut



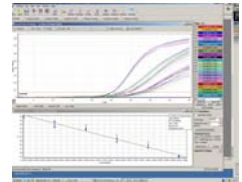
hazelnut



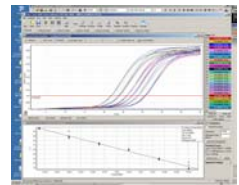
almonds



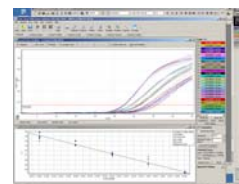
egg (chicken)



milk (beef)



sesame



Two to six percent of the human population exhibit allergic reactions due to the consumption of food containing allergens. In order to increase the well being and safety of these people, food samples are regularly examined for the presence of allergens and the compliance of declaration. Currently, there are 18 allergens listed in the food law of EU and Switzerland. In principle, food samples need to be analysed for all these allergens. Although only few food samples have to be tested for all possible combinations, still these analyses are very laborious and time-consuming. Therefore, multiplex PCR is a promising approach to produce the results in a more economic and fast way. Here, we present two tetraplex qPCR-systems. They simultaneously measure contents of peanut (Ara-h2-gen), hazelnut (Cor-a-1-gen), celery (Mannitol-dehydrogenase-gen), soy (Lectin-gen), egg (TGF- β 3-gen), milk (Cyt-B-gen), almond (Pru-A1-gen) and sesame (Oleasin-gen), respectively. DNA of the following organisms were isolated and tested for possible crossreactions in both multiplex PCR systems (AIIAII A and AIIAII B): 8 nuts (almonds, walnut, macadamia, cashew, pistachio, pecan, brazil nut, coconut) 10 legumes (white beans, lentils, kidney beans, mung beans, kidney beans, chickpeas, peas, runner beans, lupinus albus and lupinus august) 7 animals and their products (beef, chicken, egg-powder, whole egg-powder, pig, lamb, turkey, goat) 11 herbs (parsley, chive, nutmeg, onion, garlic, white pepper, cinnamon, aniseed, cloves, paprika, sesame) 6 fruits and vegetables (carrot, tomato, potato, apricots, peach, plum) rice and wheat. The only cross-reactivity above 1% emerged using peach and apricot as a template. These lead to a 10% signal in the hazelnut system and in case of apricot to a 1.2% signal in the almond-system.

These two tests exhibit a good accuracy and precision (see Table 1 and 2) in the range of 0.01% to 10%. In order to check for the applicability of these PCR-systems forty bakery products (e.g. cookies, cereals, mueslibars, chocolate chips) were analysed in parallel by ELISA and by PCR for the presence of almond and hazelnut. The comparison of these results revealed a qualitative accordance of 97% for hazelnut and 98% for almond. These two multiplex PCR-systems proved their applicability for routine analysis.

AIIAIIA	precision	accuracy
hazelnut	+/-42%	+/-14%
peanut	+/-41%	+/-11%
celery	+/-35%	+/-10%

AIIAII B	precision	accuracy
sesame	+/-24%	+/-19%
beef	+/-13%	+/-7%
almond	+/-20%	+/-18%