



TheOnyx

Semi-automated SNP genotyping set up for large sample size epidemiologic studies

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Background

Molecular epidemiology aims to elucidate the molecular pathways involved in disease etiology and progression. Large, well-characterized cohort studies with associated biobanks provide primary working material for molecular epidemiology. This type of investigation is the basis for identifying susceptible population groups as well as novel preventive and therapeutic targets for common multi-factorial disorders.

A primary concern of publicly-funded research projects is the efficient use of limited funding resources with a high output of relevant scientific publications on research that is not necessarily associated or referred to pharmacologic, therapeutic or clinical interest, but to public and environmental health aspects.

Aim

Our goal was to establish a versatile multi-user approach for reliable and robust SNP detection characterized by an increased degree of automation, large number sample handling, low error rate, limited amount and costs of consumables as well as standard infrastructure requirements.

Material and Methods

We dispose of a genetic biobank of 6300 probands. Each proband is characterized by anthropomorphic measurements, health state, clinical history, allergic state, lifestyle factors, and environmental exposure to air pollutants from two time-points over a time period of 11 years (base line examination and follow up). As genetic marker, we chose functionally well-characterized single nucleotide polymorphisms (SNPs) in genes known to cause or modify allergic response and pulmonary function.

After DNA extraction, all DNA sample tubes were barcode labeled. DNA samples were stored in single tubes with screw caps in order to minimize DNA contamination and to allow genetic analysis of a specific subpopulation (e.g. all smokers)

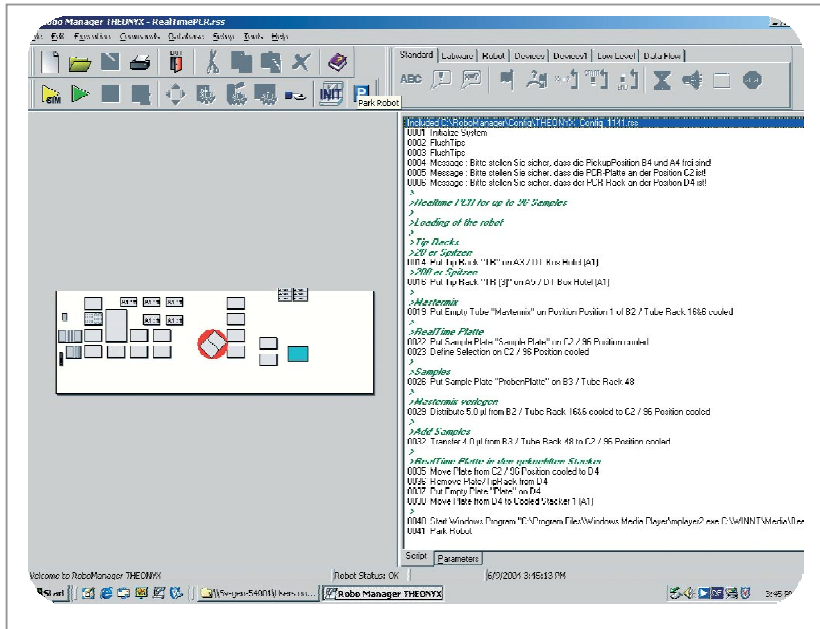


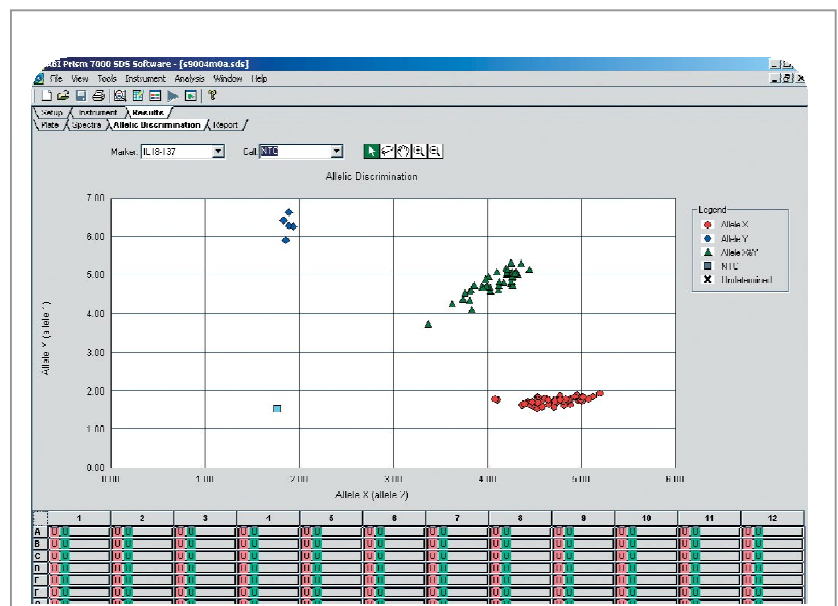
Figure 1:

Workflow details:
Set up of pipetting reaction using TheOnyx (script)

The SNP genotyping method of choice was 5' nuclease real time PCR (TaqMan) assay using fluorescently labeled allele-specific MGB probes and ABI Prism 7000 sequence detection system (both purchased from Applied Biosystems).

Figure 2:

End point detection of one 96-well plate by ABI prism 7000 SDS



The set up of the TaqMan SNP genotyping assay was performed using the TheOnyx liquid handling station. In the first step, 6 μ l of PCR master mix, containing SNP specific TaqMan probes and primers, was distributed in a barcode-labeled, optical 96-well plate. Subsequently, 4 μ l of diluted DNA was added to each well. After pipetting, the prepared plate was transferred by the rotating gripper tool to the 4°C stacker integrated on TheOnyx. After manual sealing, up to four reaction plates were simultaneously transferred to a tetra block thermocycler (MJ Research). Cycling conditions as well as PCR set up and fluorescent end-point detection were performed according to the manufacturer's instructions.

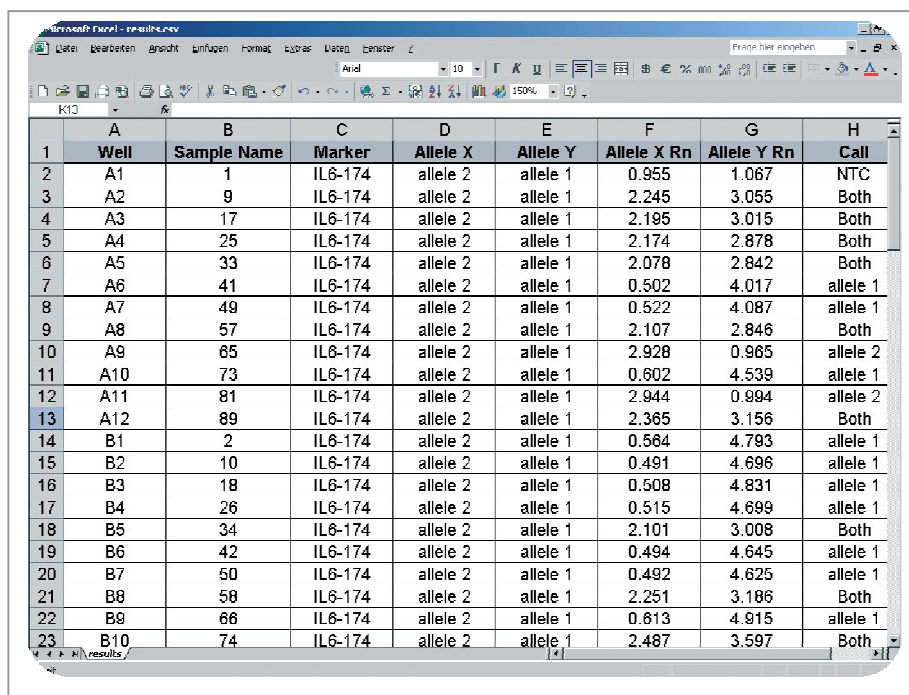
Results

Procedure steps as pipetting set up, thermocycling and plate-reading were dissociated in order to adapt to the needs of the multi-user platform and to the availability of the technical operator. Workflow can be discontinued by –20°C storage of the prepared PCR master mix or reaction plate either immediately after set up or after thermocycling. Thus, infrastructure capacities can be ideally exploited and used by more than one protocol at a time.

Time considerations of the working flow:		
Set up of pipetting reaction one 96-well plate:	25 min	380 DNA samples can be tested for one SNP in 4 hours.
Cycling of four 96-well plates:	1 h 30 min	
End-point detection of one 96-well plate:	5 min	

Error rate considerations:

The overall genotyping failure rate 128/12'366 (0.9%). Failures of genotyping were due to negative fluorescent signals. Repeated genotyping of 5% of random DNA samples in addition to the 128 genotyping failures allowed to i) rescue 116 DNA samples, ii) reproduce the genotyping results. THE SNP typing assay used is extremely specific since no false positive results occurred. Unsuccessful genotyping (12/12'366; <0.01%) was mainly due to insufficient DNA concentrations.



	A	B	C	D	E	F	G	H
	Well	Sample Name	Marker	Allele X	Allele Y	Allele X Rn	Allele Y Rn	Call
1	A1	1	IL6-174	allele 2	allele 1	0.955	1.067	NTC
2	A2	9	IL6-174	allele 2	allele 1	2.245	3.055	Both
3	A3	17	IL6-174	allele 2	allele 1	2.195	3.015	Both
4	A4	25	IL6-174	allele 2	allele 1	2.174	2.878	Both
5	A5	33	IL6-174	allele 2	allele 1	2.078	2.842	Both
6	A6	41	IL6-174	allele 2	allele 1	0.502	4.017	allele 1
7	A7	49	IL6-174	allele 2	allele 1	0.522	4.087	allele 1
8	A8	57	IL6-174	allele 2	allele 1	2.107	2.846	Both
9	A9	65	IL6-174	allele 2	allele 1	2.928	0.965	allele 2
10	A10	73	IL6-174	allele 2	allele 1	0.602	4.539	allele 1
11	A11	81	IL6-174	allele 2	allele 1	2.944	0.994	allele 2
12	A12	89	IL6-174	allele 2	allele 1	2.365	3.156	Both
13	B1	2	IL6-174	allele 2	allele 1	0.564	4.793	allele 1
14	B2	10	IL6-174	allele 2	allele 1	0.491	4.696	allele 1
15	B3	18	IL6-174	allele 2	allele 1	0.508	4.831	allele 1
16	B4	26	IL6-174	allele 2	allele 1	0.515	4.699	allele 1
17	B5	34	IL6-174	allele 2	allele 1	2.101	3.008	Both
18	B6	42	IL6-174	allele 2	allele 1	0.494	4.645	allele 1
19	B7	50	IL6-174	allele 2	allele 1	0.492	4.625	allele 1
20	B8	58	IL6-174	allele 2	allele 1	2.251	3.186	Both
21	B9	66	IL6-174	allele 2	allele 1	0.613	4.915	allele 1
22	B10	74	IL6-174	allele 2	allele 1	2.487	3.597	Both

Table1:

Transfer of DNA sample and genotyping information to excel and stata data files

Conclusions

The resulting semi-automated high-throughput set up proved effective and provided a flexible and adaptable resource to increase productivity. The use of the TheOnyx as a pipetting workstation was highly reliable for our SNP genotyping application since the volumes of PCR master mix and DNA sample were distributed accurately.

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