geneLEAD and geneTYPIST:
Two new fully automated sample to answer systems

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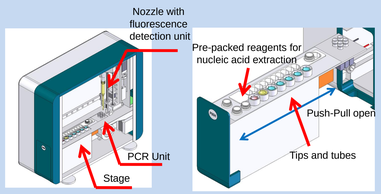
Introduction

Nucleic acid amplification tests (NATs) are powerful tools for the molecular diagnostic laboratory, such as virus detection, mRNA profiling, SNPs (single nucleotide polymorphism) typing and detection of somatic cell mutation. However, the manual operation of NATs are time consuming and require technical experience, with the workflow including several processes (extraction of nucleic acids, amplification, detection and analysis of data). Moreover, NATs should be processed by skilled laboratory technicians in regulated laboratory environments for assuring the quality of NATs results. These requirements seem to be limiting factors for further expansion of NATs in molecular diagnostic laboratory testing, especially for point-of-care (POC) and STAT testing. To eliminate these difficulties, easy to use, fully-automated and -integrated instruments are necessary.

We have developed two platforms which are fully-automated and integrated instruments for NATs. They are the 'geneLEAD' and 'geneTYPIST'. These instruments can process all steps for NATs, including extraction of DNA/RNA from various samples, reaction set up, amplification of target gene and detection/analysis of the products. These instruments use Magtration technology for nucleic acid extraction and incorporate different methods for analysis of the amplified products. Gene-LEAD can measure fluorescence signals from amplified products and can be used for endpoint or real time monitoring of amplified products. Gene-TYPIST uses a capillary bead array ('BIST' technology) for multiplex SNPs typing (up to 9 genes). We will present data obtained from these instruments for detection of mRNA in simple and multiplex SNPs typing.

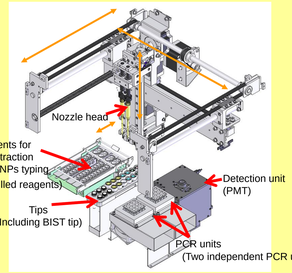
geneLEAD



Fully-automated real time PCR Can be used for virus/bacteria detection andSNPs typing

1. One nozzle with fluorescence detection unit
2. Use pre-packed reagents
3. Run turn-around time : 1.5 hour
4. Size D: cm, H: cm, W: cm
5. Use time-proven Magtration and PCR technologies

geneTYPIST



Fully-automated SNPs typing Using BIST technology

1. Two nozzle (X-Y-Z moving)
2. Use pre-packed reagents
3. Use BIST technology for SNPs typing
4. Up to 9 SNPs typing using one tip
5. Run turn-around time : 1.5 hour
6. Size ; D: 40 cm, H: 40 cm, W: 40 cm

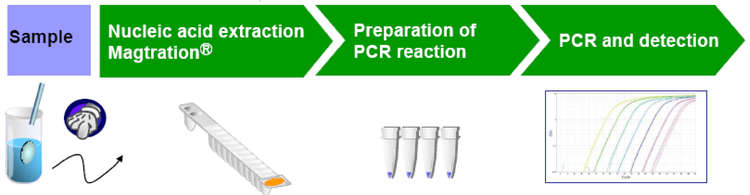


Figure 1. Process of fully-automated PCR with geneLEAD
Fully-automated workflow incorporating real time-PCR by geneLEAD. geneLEAD performs extraction of DNA or RNA (30-45 min depending on sample type), reaction set up, Real-Time PCR and analysis (about 30-40 min)

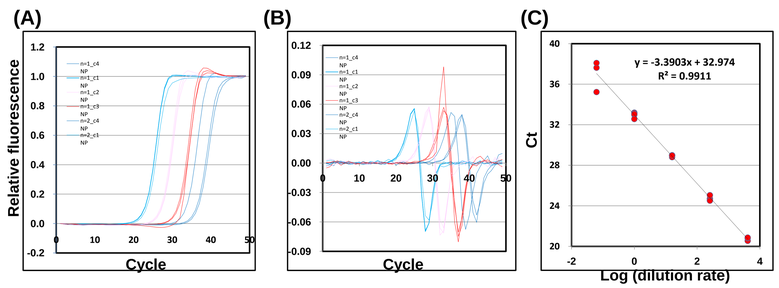


Figure 2. Performance of real time PCR unit of geneLEAD
Performance tests using the Real-Time PCR unit of geneLEAD were examined. Human genomic DNA extracted from whole blood was used in the real time PCR assay. The GAPDH gene was the PCR target. Four different concentrations of DNA samples were used for real-time PCR assay. **(A)** Amplification curve and **(B)** Second derivative plot of amplification curve for calculating Ct value. **(C)** Calculated Ct values showed that the PCR reactions was done by ideal condition.

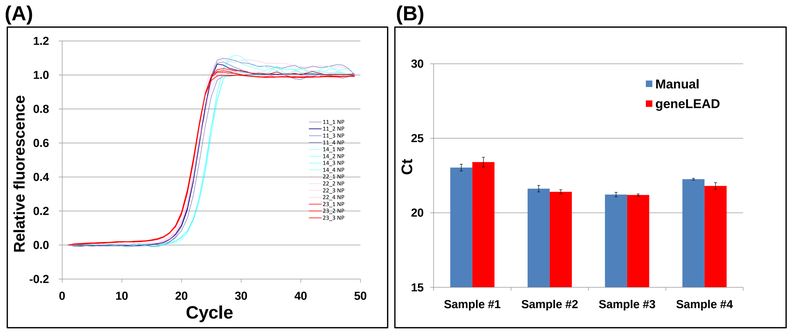


Figure 3. Nucleic acid extraction to detection by geneLEAD
Human whole blood samples (four different whole blood samples, #1 to #4) were used for fully-automated real time PCR assay. **(A)** Amplification curve of real time PCR. Extracted DNA samples were amplified with GAPDH primers. **(B)** Ct values were calculated from real time PCR results obtained by geneLEAD and compared to a manual extraction method (spin column purification) followed by real time PCR.

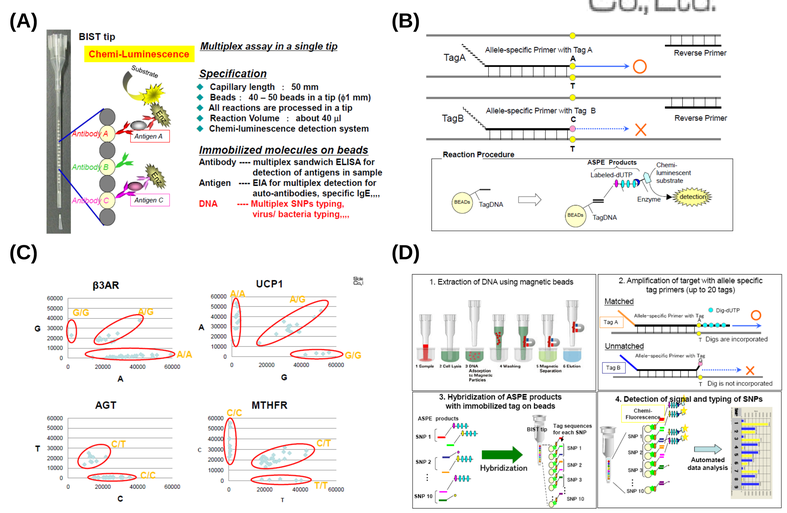
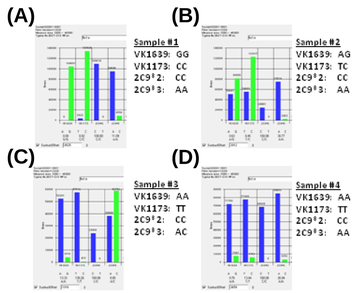


Figure 4. Principle of SNPs typing by BIST technology
(A) Specification of BIST system. BIST tip contains a bead array in its capillary. The beads can be coated with antibody, antigen and nucleic acid, and used for multiplex detection of targeted molecules within a single BIST tip. **(B)** Principle of allele specific primer extension PCR method used for SNPs typing by BIST. **(C)** Two hundred sixteen human DNA samples were subjected for SNPs typing assay incorporating 4 genes (beta-3AR, UCP1, AGT and MTHFR genes). SNPs typing results by BIST tip were shown in figure and correlate with SNPs typing results obtained by sequencing. **(D)** Workflow of fully-automated SNPs typing by geneTYPIST. geneTYPIST performs DNA extraction, ASPE-PCR, hybridization of PCR products with probes on beads, detection of signals and analysis of result. Time to result of SNPs typing with geneTYPIST is about 1.5 to 2 hour. This time is shorter than that of manual method (about 6 hours).



(E)

Sample #	VK1639 A/G	VK1173 T/C	2C9*2 C/T	2C9*3 A/C
Sample #1	GG	CC	CC	AA
Sample #2	AG	TC	CC	AA
Sample #3	AA	TT	CC	AC
Sample #4	AA	TT	CC	AA

Figure 5. Fully-automated SNPs typing by geneTYPIST
(A) to **(D)** Human whole blood samples (four different whole blood samples, #1 to #4) were used for fully-automated SNPs typing of VKORC1, CYP2C9*2 and CYP2C9*3 SNPs. SNPs typing results by geneTYPIST correspond with SNPs typing results by DNA sequencing (showed in (E)).

Summary

We have developed the 'geneLEAD' and 'geneTYPIST' platforms, which are fully-automated and integrated instruments for NATs. Preliminary results obtained from these two fully-automated platforms are comparable with results obtained by established manual methods.

These results showed that the integration of the Ease-of-Use workflow and robust platform design, show great promise for rapid and accurate NATs in the molecular diagnostic laboratory.