National Biobank of Korea: Quality control Programs of Collected-human Biospecimens

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Abstract

Personalized medicine is emerging as a main paradigm for risk prediction, pre-diagnosis, and effective prevention and treatment of disease. A large number of human biospecimens and their clinical data are essential resources for the success of personalized medicine as well as other biomedical research. The National Biobank of Korea (NBK) has collected well-annotated and high quality human biospecimens, and distributes them to the Korean biomedical scientists, through the Korea Biobank Project (KBP). The ultimate goal of NBK activities is to promote biomedical research and public health. As of December-2011, the NBK has collected various human biospecimens from 525,416 participants including 325,952 Korean populations and 199,464 patients. The purpose of this paper is to introduce the KBP and quality control programs for collection of human biospecimens with high quality of NBK.

1. Introduction

Worldwide many biobanks have collected, preserved, and distributed a large number of human biospecimens with high quality and their related information, which can be widely used for various studies on diagnosis, therapy, and prevention of diseases [1,2]. For example, UK Biobank has collected blood and urine samples from 500,000 individual participants aged 40–69 to study disease-associated lifestyle, environmental effects or genetic factors [3]. In Norway, the biobank for Norwegian mother and child cohort study (MoBa) has stored human biospecimens (such as whole blood, plasma, DNA and urine) of pregnant women, their partners and children collected at 50 hospitals [4]. In Japan, the Biobank Japan Project has been performed for collection of a large number of DNA and serum samples with clinical information [5].

The National Biobank of Korea (NBK) has collected various human biospecimens with high quality from population-based participants and disease-based participants through the Korea Biobank Project (KBP).
Human biospecimens from Korean populations have been collected by population-based cohort studies such as the Korean Genome and Epidemiology Study (KoGES), the National Survey of Health and Nutrition, and other government-supported survey projects. KoGES is a project with the aim of collecting human biospecimens (such as whole blood, serum, plasma, urine, and blood DNA) and health associated information from over 300,000 participants aged 40–69 to support the studies for the relationships between genetic background, environmental risk factors, and lifestyle and disease development in individuals and populations. Human biospecimens from disease-based participants have been collected through 17 regional biobanks of university hospitals in Korea. These collected human biospecimens have been distributed to internal scientists to promote biomedical studies and public health.

The objectives of this paper are to describe a summary of the KBP and introduce biobank activity of the NBK to collect a great many human biospecimens with high quality.

2. Activities of the National Biobank of Korea

2.1. Korea Biobank Project

The KBP was launched in 2008, which has been conducted by the NBK. The first phase of KBP aims to collect human biospecimens from 500,000 participants (including 300,000 population-based participants and 200,000 disease-based participants) with their epidemiological and clinical information until 2012. The NBK has collected and distributed a large number of human biospecimens from population-based participants and disease-based participants since 2001 and 2008, respectively. As of December-2011, the NBK has collected human biospecimens from 525,416 participants including 325,952 Korean populations and 199,464 patients. Population-based biospecimens included 2,078,544 (40%) serum, 1,953,270 (37%) plasma, 801,309 (15%) DNA, and 435,593 (8%) other biospecimens. Disease-based biospecimens contained 1,274,313 (50%) plasma, 568,041 (22%) serum, 222,872 (9%) tissue, 143,667 (6%) buffy coat, and 318,559 (13%) other biospecimens.

For KBP, the NBK has appointed and supported regional biobanks which collect disease-based human biospecimens. In 2008, the NBK appointed eight regional biobanks and increased up to 17 in 2012. Collected human biospecimens and their related information have been shared through the web-based Korea Biobank Network (KBN) system. This system is continuously developing to provide much more information for many scientists. The NBK has distributed collected human biospecimens to internal scientists to support various studies including translational research and personalized medicine. Especially, the NBK has supported the Korean Genome Analysis Project (KoGAP) which aims to identify genetic and environmental risk factors leading the development of the five common life-style-related diseases such as obesity, diabetes, hypertension, osteoporosis, and metabolic syndrome in a large number of Korean populations.

2.2. Quality control programs of human biospecimens

Quality of human biospecimens collected in NBK has been controlled by following guidelines. DNA samples must have the purity and integrity, and be adjusted to 500 ng of DNA per 1 μl. LCL samples must have a high viability and be not contaminated by microorganisms. In addition, all human biospecimens must be stored and transported on frozen condition. The NBK performs quality test of collected human biospecimens and then those with high quality are only stored in deep freezer (−80 °C) (DNA, whole blood, and urine) or liquid nitrogen tank (serum, plasma, lymphocyte, and LCL).

2.2.1. Quality control of DNA

Generally, spectrophotometric method has used for quantity and quality evaluation of nucleic acids including DNA and RNA. DNA and RNA have the maximum absorption at approximately 260 nm and protein absorbs strongly at near 280 nm. Contaminants such as EDTA, carbohydrates and phenol have absorption at near 230 nm. Therefore, A260/A280 and A260/A230 ratio show the purity degree of nucleic acids. DNA sample has been considered as pure if A260/A280 ratio is from 1.8 to 2.0 and A260/A230 ratio is over 2.0. However, DNA sample with lower A260/A230 ratio (1.7 ≤ A260/A230 ratios < 2.0) can be used for molecular biology experiments. Concentration of DNA (microgram/mL) is calculated as 50 × OD260 of the sample. Fluorochromes such as PicoGreen, Hoechst 33258, and SYBR Green I have been used to evaluate the quantity of double strand DNA selectively. Among them, DNA quantitation using PicoGreen which has an excitation maximum at 480 nm and an emission maximum at 520 nm is best sensitive and simple [6]. Limitation of DNA quantitation of PicoGreen assay is 0.25 ng of DNA per 150 μL of assay volume [6]. DNA integrity has generally been measured by two techniques including gel electrophoresis and PCR analysis [7,8]. In gel electrophoresis, DNA degradation is shown by double or smeared band compared with molecular weight marker of DNA. DNA quality can be also determined by yield of PCR amplicon.

In the NBK, quality of collected DNA has been tested with aliquot of each sample divided in clean bench. DNA integrity has been assessed through gel electrophoresis because can detect the tiny DNA degradation and measure the approximate quantity. DNA purity has...
been assessed through spectrophotometric method (using NanoDrop® machine) with advantage which can measure purity (A260/A280 ratio and A260/A230 ratio) and concentration (OD260) at the same time. Microbial (bacteria and mycoplasma) contamination has determined with each microbial specific primers (16S rRNA primers) using PCR method. In case of blood DNA, approximately ten percent which randomly selected in all collected blood DNA samples are used for test. Quality of LCL DNA is evaluated in all collected samples. DNA integrity is determined by the absence of a smeared or double band on gel electrophoresis and the standard of DNA purity is an A260/A230 ratio ≥ 1.8 and 1.8 ≤ A260/A280 ratio ≤ 2.0. PicoGreen analysis is performed when DNA degradation is observed on gel electrophoresis. Microbial contamination is determined by the presence of DNA band in gel electrophoresis of PCR amplicon.

2.2.2. Quality control of lymphoblastoid cell line

Lymphoblastoid cell lines (LCLs) are a research model which widely used in various studies. Quality control of LCLs is important for an exact experimental result. Generally, cell quality is assessed on viability, authentication, and microbial contamination. Cell viability shows proliferative capacity. Authentication test is performed to identify whether cross-contamination is or not, using morphology observation, karyotyping, DNA typing, or immunophenotyping. Contamination is or not, using morphology observation, karyotyping, DNA typing, or immunophenotyping. Cell viability shows proliferative capacity. Authentication test is performed to identify whether cross-contamination is or not, using morphology observation, karyotyping, DNA typing, or immunophenotyping. Cell viability shows proliferative capacity.

In the NBK, quality of LCLs has been assessed on bacterial and mycoplasma contamination and cell viability (number of live cells/number of total cells × 100) in approximately 10% randomly selected in all collected LCLs. A contamination test is carried out through macroscopy during cultivation and PCR experiment. In PCR experiments, DNA samples extracted from LCLs are amplified with each microbial specific primer (16S rRNA primers) and amplicon is detected on gel electrophoresis. The LCLs must be over 50% of cell viability and without contamination.

2.2.3. STR analysis

Short tandem repeats (STRs) are repeating DNA sequences with 2-6 bp length per a unit [14] that exist generally in eukaryotes (including human) and prokaryotes [15]. Most STRs exist on non-coding region and approximately 8% of STRs exist on coding region [16]. Analysis of STR has been used widely in various fields, including forensic identification, population-based genetic study, and identification of disease-associated genetic alteration.

In the NBK, STR analysis has been performed for DNA identification using commercial kit analyzing 15 STR loci (CSF1P0, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, FGA, THO1, TPOX, and vWA) and Amelogenin, the gender marker. STR data have been used as a yardstick of judgment to check whether the cross-contamination between samples was happened or not. In addition, these information have been used for comparison with sex information in donor’s epidemiological and clinical information. As of December-2011, STR analysis has been carried out with about 25,342 of blood DNA and LCL DNA.

2.2.4. Contamination assessment of liquid nitrogen tank

The liquid nitrogen tank can be contaminated by microorganisms when biospecimens have been stored for a long time. Indeed, other researchers investigated microbial contamination of embryos and semen samples stored for 6-35 years in 16 liquid nitrogen tanks. The results showed that different commensal or environmental microorganisms (32 bacteria and 1 fungi) was contaminated in liquid nitrogen, embryos, and semen samples [17]. Microbial contamination can be happened by ice accumulation in a liquid nitrogen tank [18]. This ice accumulation can be made by falling of ice forming in atmosphere when a liquid nitrogen tank opens. Ice formation on cold surface of tank, rack or sample box can also be a cause of microbial contamination in the liquid nitrogen tank.

The NBK has investigated microbial contamination of liquid nitrogen tanks using Bielanski’s method [17] every year since 2005 (data not shown).

2.2.5. Quality control of human biospecimens in regional biobanks

The NBK has recommended that regional biobanks should perform quality tests of human biospecimens collected from patients. The quality test is carried out as follows: For quality control of fresh frozen tissues, RNA samples are extracted from >3% of collected all tissues. Subsequently, the concentration of RNA samples are evaluated by spectrophotometric method and the integrity of these are assessed by gel electrophoresis and measurement of RNA integrity number.

2.2.6. Other activities

The quality of collected human biospecimens has managed by other various efforts of the NBK. For example, ISO 9001:2008 was implemented for the
collection, preservation, and distribution of human biospecimens (such as DNA, cell, serum, and urine) through the Korean Standard Association. Standard Operation Protocols (SOPs) were made for the collection, preservation and distribution of human biospecimens at first time in 2008 and annually edited to produce the best practice SOPs [19]. For systematic storage management, automated biospecimens management platform [20] was equipped in 2009 and 300,000 vials of DNA is storing at this platform with −80 °C condition.

2.3. Study for characteristics of human biospecimens

Many scientists in the NBK have been studying on various factors influencing the quality of human specimens and the effects of them on experimental data, to manufacture the best practice SOPs for human biospecimens collection with high quality. For examples, we investigated an influence of bacterial DNA contamination in blood DNA on SNP chip data. The result showed that there is no correlation [21]. We also are studying on the effect of the delay of plasma isolation from whole blood sample on biochemical expression (In preparation for publication). To make better use of LCLs in research, we have studied their characteristics. LCLs have been used in various research fields such as pharmacogenomics, human genetics, and immunology. However, there is some concern about the wide use of LCLs because genetic alterations can be occurred by EBV-transformation during generation or immortalization of LCLs. To identify expression phenotype change associated with immortalization of LCLs, we performed a continuous long-term culture with 20 LCLs strains [22] and then identified phenotype changes of genes [23] and miRNAs [24] during long-term cultivation of LCLs, through microarray approach. Fifty genes including NF-κB pathway-related genes (e.g. PTPN1 and HERC5) and carcinogenesis-related genes (e.g. XAF1, TCLA1, PTPN13, and CD38) commonly showed the different expression in most of all tested late-passage LCLs compared with early-passage LCLs. Nine miRNAs (miR-20b*, miR-28-5p, miR-99a, miR-125b, miR-151-3p, miR-151-9.1, miR-216a, miR-223*, and miR-1296) were differently expressed during long-term culture of LCLs. Copy numbers changes of DNA were investigated in early passage LCLs compared with primary B cells. The result revealed that DNA alterations were not observed in the most of tested regions [25]. We also identified that expression of STMNI, an oncogene, was increased in early passage LCLs compared to primary B cells [26].

3. Conclusion

The NBK has conducted the KBP with aim of human biospecimens collection from 300,000 population-based participants and 200,000 disease-based participants until 2012. Population-based human biospecimens have collected through government supported cohort studies. Disease-based human biospecimens have collected through 17 regional biobanks which appointed and supported by the NBK.

The collected human biospecimens have been managed by different quality control programs and their characteristics have been studied to increase the value of human biospecimens. Ultimately, these efforts of the NBK would contribute to identification of new scientific facts for promotion of health of many people.

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References

1. Davey Smith G, Ebrahim S, Lewis S, et al. Genetic epidemiology and public health: hope, hype, and future prospects. Lancet 2005 Oct 22-28;366(9495):1484—98.
2. Patel AA, Gilbertson JR, Parwani AV, et al. An informatics model for tissue banks-lessons learned from the cooperative prostate cancer tissue resource. BMC Cancer 2006 May 5;6:120.
3. Elliott P, Peakman TC. The UK Biobank sample handling and storage protocol for the collection, processing and archiving of human blood and urine. Int J Epidemiol 2008 Apr;37(2):234—44.
4. Rønningen KS, Paltiel L, Meltzer HM, et al. The biobank of the Norwegian mother and child cohort study: a resource for the next 100 years. Eur J Epidemiol 2006;21(8):619—25.
5. Nakamura Y. The BioBank Japan Project. Clin Adv Hematol Oncol 2007 Sep;5(9):696—7.
6. Ahn SJ, Costa J, Emanuel JR. PicoGreen quantitation of DNA: effective evaluation of samples pre- or post-PCR. Nucleic Acids Res 1996 Jul 1;24(13):2623—5.
7. Feigelson HS, Rodríguez C, Robertson AS, et al. Determinants of DNA yield and quality from buccal cell samples collected with mouthwash. Cancer Epidemiol Biomarkers Prev 2001 Sep;10(9):1005—8.
8. Zayats T, Young TL, Mackey DA, et al. Quality of DNA extracted from mouthwashes. PLoS One 2009 Jul 7;4(7):e6165.
9. Cobo F, Stacey GN, Hunt C, et al. Microbiological control in stem cell banks: approaches to standardisation. Appl Microbiol Biotechnol 2005 Sep;68(4):456—66.
10. Halls N. Effects and causes of contamination in sterile manufacturing. In: Halls N, editor. Microbiological contamination control in pharmaceutical clean rooms. Boca Raton (FL): CRC Press; 2004. p. 1—22.
11. Owers KL, James E, Bannister GC. Source of bacterial shedding in laminar flow theatres. J Hosp Infect 2004 Nov;58(3):230—2.
12. Uphoff CC, Drexler HG. Detection of mycoplasma contamination. Methods Mol Biol 2005;290:13—23.
13. Ryan J. Understanding and managing cell culture contamination. Technical Bulletin. Corning Life Sciences; 2002.
14. Kimpton C, Gill P, D’Aloja E, et al. Report on the second EDNAP collaborative STR exercise. European DNA Profiling Group. Forensic Sci Int 1995 Jan 30;71(2):137—52.
15. Fan H, Chiu YJ. A brief review of short tandem repeat mutation. Genomics Proteomics Bioinformatics 2007 Feb;5(1):7—14.
16. Ellegren H. Heterogeneous mutation processes in human microsatellite DNA sequences. Nat Genet 2000 Apr;24(4):400—2.
17. Bielanski A, Bergeron H, Lau PC, et al. Microbial contamination of embryos and semen during long term banking in liquid nitrogen. Cryobiology 2003 Apr;46(2):146–52.

18. Morris GJ. The origin, ultrastructure, and microbiology of the sediment accumulating in liquid nitrogen storage vessels. Cryobiology 2005 Jun;50(3):231–8.

19. National Biobank of Korea. Standard Operating Procedures for Human Biospecimens; 2010.

20. Comley J. Automated biobanking—the next big step for biorepositories. Drug Discovery World Summer; 2007:49–70.

21. Lee JE, Hong EJ, Shim SM, et al. Bacterial contamination of blood DNA samples is associated with donor’s health condition. Biopreservation and Biobanking 2010 Sep 29;8(3):127–31.

22. Jeon JP, Nam HY, Shim SM, et al. Sustained viral activity of Epstein-Barr virus contributes to cellular immortalization of lymphoblastoid cell lines. Mol Cells 2009 Feb 28;27(2):143–8.

23. Lee JE, Nam HY, Shim SM, et al. Expression phenotype changes of EBV-transformed lymphoblastoid cell lines during long-term subculture and its clinical significance. Cell Prolif 2010 Aug;43(4):378–84.

24. Lee JE, Hong EJ, Nam HY, et al. MicroRNA signatures associated with immortalization of EBV-transformed lymphoblastoid cell lines and clinical traits. Cell Prolif 2011 Feb;44(1):59–66.

25. Jeon JP, Shim SM, Nam HY, et al. Copy number increase of 1p36.33 and mitochondrial genome amplification in Epstein-Barr virus-transformed lymphoblastoid cell lines. Cancer Genet Cytogenet 2007 Mar;173(2):122–30.

26. Baik SY, Yun HS, Lee HJ, et al. Identification of stathmin 1 expression induced by Epstein-Barr virus in human B lymphocytes. Cell Prolif 2007 Apr;40(2):268–81.