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PROJECT FINAL REPORT

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[2] The home page of the website should contain the generic European flag and the FP7 logo which are available in electronic format at the Europa website (logo of the European flag: http://europa.eu/abc/symbols/emblem/index_en.htm ; logo of the 7th FP: http://ec.europa.eu/research/fp7/index_en.cfm?pg=logos). The area of activity of the project should also be mentioned.

4.1 Final publishable summary report

1. Executive summary


Quantitative pathway analysis of natural variation in complex disease signaling in *C. elegans* (PANACEA).

EU-HEALTH, Systems Biology, Integrated Project, 7th Framework Programme, Period: 2009-2013.

Complex human diseases arise from the interaction between many different genes and the environment and are the main causes of death in Europe. In contrast to the successful identification of genes underlying rare monogenic diseases, studying the genetic basis of common complex diseases has been more challenging. Evidence is mounting to suggest that the genetic background or genotype (i.e. the genetic make-up) has a profound impact on a wide variety of complex disease phenotypes and signaling pathways in humans. But so far, the genetic mechanisms underlying these modifiers are unknown and are difficult to study in humans.

Here we investigated the nematode worm *Caenorhabditis elegans*, which is an important model species, for the identification of genes underlying complex diseases in humans. We took advantage of the natural genetic variation present in different populations and used this to identify key genes which were associated with cancer genetic pathways. By developing specific techniques for knocking down genes in different worm populations we identified novel regulators of cancer pathways. We used this information to mathematically identify, model and predict the development of the worm based on the cancer genetic pathways. This has resulted in a collection of key cancer regulators which have been further investigated for human homologs. A special data base and analysis system has been introduced to facilitate the comparison and investigations into genetic variation underlying complex disease pathways in *C. elegans* and humans.

With regard to public dissemination we have increased our efforts to bring the complex human disease model *C. elegans* to the larger public by participating in the Micro-Zoo. The Micro-Zoo is a bacterial and microorganism zoo in the well-known Artis Zoo in Amsterdam that is expected to open its doors end of 2013. Hands-on experiments can be performed by the public and live worms can be observed on large TV screens. This will bring the worm and its unique research possibilities to the attention of about 1.5 million people per year.

2. A summary description of project context and objectives.

Complex human diseases arise from the interaction between many different genes and the environment and are the main causes of death in Europe. Up to 60% of the adult population dies from complex diseases such as cancer and diseases of the circulatory system (WHO, 2003). In contrast to the successful identification of genes underlying rare monogenic diseases, studying the genetic basis of common complex diseases has been more challenging (Botstein and Risch, 2003; Lowe et al. 2007).

Evidence is mounting to suggest that the genetic background or genotype (i.e. the genetic make-up of all alleles at all loci) has a profound impact on a wide variety of complex disease phenotypes. For instance Freeman et al. (2006) showed that the genetic background is likely to influence the onset and progression of tumorigenesis. Kristensen et al. (2006) reported different genotypes to be associated with somatic gene expression data in human breast tumors and Seitz et al. (2006) found that the genetic background of different cancer cell lines influenced tumor development. But also in many other complex diseases ranging from urinary stone formation, to autoimmune disorders and retinal degeneration it was found that the genetic background acts as an important modifier of disease pathways (Salido et al., 2006; Tsuchiya et al. 2006; Thompson et al. 2005). The genetic mechanisms underlying these modifiers are unknown and within PANACEA we address the following **two key questions**:

i) <u>how</u> the genetic background affects complex disease signaling pathways, and

ii) whether we can <u>predict</u> the effect of the genetic background on these pathways.

The European Commission spearheads research programmes which are designed to lead to a better understanding of the genetic variation of complex human diseases. Although many genes have been linked to complex diseases, fundamental research is still needed into why these genes are important, what their role is in the disease and how genetic variation contributes to disease phenotypes.

Strategic objectives and activities

To address the two key questions we have identified the following objectives:

Genomics

1. **Presentation of a set of genes affecting gene expression networks in *C. elegans* which are associated with the cancer signaling pathways: the EGF/RAS/MAPK pathway, the Notch/LIN-12 pathway and the Wnt pathway.**

Proteomics

2. **Presentation of a set of protein-protein interactions in *C. elegans* underlying natural genetic variation in protein networks of the three signaling pathways: the EGF/RAS/MAPK pathway, the Notch/LIN-12 pathway and the Wnt pathway.**

Cellular development

3. **Presentation of a set of vulval development and apoptosis phenotypes (important readouts for disease pathways) across a *C. elegans* recombinant population**

Vulva development modeling

4. **A predictive model for the vulva development based on the data sets from objective 1, 2, and 3.**

Data base and comparative analysis

5. **A data base of the outcomes of objective 1, 2, and 3 and a comparative data analysis across other models species, including human.**

The objectives integrate a wide variety of biological data and development and application of system approaches to understand and model biological processes at different levels of biological organization. The project focuses on collecting, analyzing and applying quantitative data to enable systems biology approaches addressing basic biological processes relevant to health. To reach these objectives the following activities were carried out:

- perform high-throughput gene expression profiling and proteomics in a *C. elegans* population of 200 recombinant inbred lines (RILs) obtained from a cross between the genetically divergent strains Bristol N2 and CB4856.

- measure a range of molecular, and cellular phenotypes related to vulva development and developmental apoptosis in the RILs.

- perform quantitative trait loci (QTL) analysis on the gene expression, proteomics and phenotypic data for detecting candidate genes.

- perform high-throughput RNAi screening to validate the function of candidate genes in the RILs.

- develop a quantitative model for predicting the effect of wild type polymorphisms on vulva development based on the genomic, proteomic and cellular QTL and RNAi data.

- set up a data base of natural genetic variation in cancer signaling pathways and comparison across other model species

The PANACEA systems biology approach is unique

The PANACEA project is unique in its approach toward understanding the mechanisms underlying complex human diseases. The project generates and analyzes large scale data sets at various organisation levels ranging from the molecular genetic, genomic and proteomic level to cells and tissues. As a *proof of concept,* part of the project comprises the use of mathematical modeling to explain and explore the predictability of the impact of genetic variation on the complex development of the nematode vulva system. For the first time EU-wide groups mined and modeled the wide range of empirical data from “genes to cells” in an important developmental system, the worm *C. elegans*. Molecular and quantitative geneticists worked together with developmental biologists and bioinformaticians in order to connect the dots from genome, to proteome and cellular development. The project builds on the most recent discoveries of gene expression networks and systems biology models. This provides a firm basis and excellent timing of PANACEA which puts new approaches for human disease on the forefront of biological and human health research. As such PANACEA strengthens European research groups to be world leaders in systems biology approaches which will further open up disease prevention and treatment for the European people.

Our project focuses on gathering qualitative and quantitative data associated with human cancers at the genomic and proteomic level at different levels of system complexity. It increases our knowledge on the influence of genetic variation on these pathways; valuable information which cannot be gathered from human studies because of ethical and statistical reasons. The outcomes provides detailed insight into the effect of natural alleles and proteins and the homology across mammals, including humans.

Within the PANACEA project we gathered global gene expression profiles together with protein-protein interactions at a proteomic scale and molecular genetic and cellular data. These high-throughput data collections were be catalogued, analyzed and translated to

nematode vulva development and developmental apoptosis, both representative for cancer formation in humans.

The PANACEA project brings together expertises from different disciplines, i.e. molecular biologists, developmental biologists, quantitative geneticists and bio-informaticians. Together we have joined forces to assess the influence of natural genetic variation on pathways and processes from the gene to individual level.

3. A description of the main S&T results/foregrounds

Complex human diseases including cancer and neurodegenerative diseases arise from interactions between many different genes and the environment. Evidence is mounting that suggests that genetic background has a profound impact on a wide variety of complex disease phenotypes. For instance, different genotypes are associated with somatic gene expression data in human breast tumors (Kristensen et al. 2006). However, the exact genetic mechanisms underlying these background modifiers in humans are largely unknown.

The mouse has been used extensively as a quantitative genetic model to study pathways associated with human diseases and disorders (Flint et al. 2005). These studies focus on the detection of modifier genes that are capable of altering the phenotype of a mutant gene. A clear example of a modifier gene was reported for tubby hearing 1 gene (moth-1), a gene in which mutations lead to hearing loss (Ikeda et al. 2002). These researchers positionally cloned a QTL, the modifier of moth-1, and found that wildtype alleles from three different strains protect moth-1 (a SNP in a natural allele) mice from hearing loss. These polymorphisms change the binding efficiency of MTAP1A (microtubule-associated protein 1a) to postsynaptic density molecule 95 (PSD95), a core component in the structure of synapses. This finding indicates that the observed polymorphisms are functionally important. Using a similar approach, QTLs associated with hematopoietic stem cell (HSC) numbers in a mouse RIL population were mapped (Liang et al. 2007). Using a combination of fine mapping with an NIL approach, gene expression and protein analysis, they identified Latexin (Lxn), whose expression inversely correlates with HSC number. The differential transcription of Lxn was associated with the allelic differences among the RILs. They identified SNP clusters upstream of the Lxn transcriptional start site; at least two were associated with potential binding sites for stem cell regulatory transcription factors. Thus, promoter polymorphisms between the two parental alleles might affect Lxn gene expression and consequently influence the number of HSCs.

These mouse examples show that background effects can be mechanistically studied. This holds great promise for *C. elegans* because its transparent body and well-described human disease pathways, in combination with a defined cell lineage and completed genome sequence, allow detailed mechanistic studies of genetic background effects.

Project organisation

The PANACEA approach is summarized in Figure 1 and consists of 8 WPs. Briefly, WP 1 consists of rearing and curating a library of already existing inbred lines. WP 2 and 3 are comprised of global gene expression profiling and proteomics respectively, conducted on all RILs and (introgression lines) ILs. Within WP 4 and 5 we investigated various genetic pathways and the vulva developmental processes, respectively. WP 6 is the data analysis and storage package where all data are analyzed using eQTL and QTL mapping technologies. Candidate genes from the eQTL and QTL analysis were screened using RNAi knock-downs and subsequent phenotyping on selected RILs and ILs. WP 6 also comprises data storage and systems biology modeling facilities. In WP 6, all data are stored for further (meta)analysis in a specific PANACEA data base. Data generated during the project are accessible to all beneficiaries in pre-defined formats to optimize their use. The crosscutting management WP 7 secures the overall co-ordination of the project via the work of the Project Secretariat and a Project Management Team. WP 8 covers training of personnel, students and end-users during the whole project period in order to maintain the adequate skills available for the project. Dissemination of the results of the project had a high priority in PANACEA. PANACEA is a 4-year collaborative project where each WP has been running with varying activity levels during the whole project period.

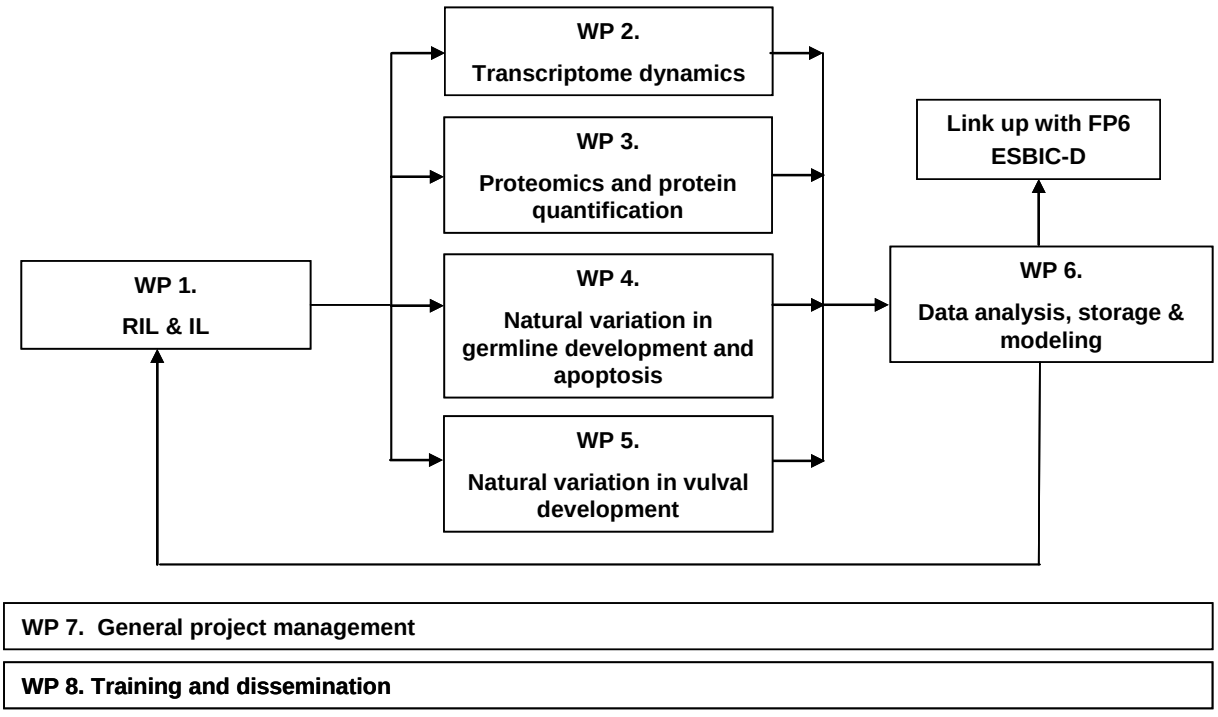

Figure 1. Pert diagram of the PANACEA project showing the WP structure.

We have developed 1000 RILs which are kept as frozen stocks. From these we have randomly taken 200 RILs. Within PANACEA these RILs were phenotyped for various gene functions, cellular characteristics and proteins across the selected pathways and processes. Next, all phenotypes were mapped using QTL or gene expression QTL (eQTL) approaches and analyzed for candidate genes or proteins. Fine mapping is facilitated by having, next to the RILs, a large set of introgression

lines (ILs). These data feed into an existing, but basic, model for vulval development which is further extended to our new findings. Together with the proteomic and pathway phenotyping data this allows for predictive modeling of vulval development which is a model system for human cancer development. All data are stored in a large data base, allowing for meta systems biology analysis.

Experimental results

S&T Work package-1: Providing the basic *C. elegans* stock material for the project experiments

The PANACEA project is centered around the genetic variation which exists in natural populations of *C. elegans*. To this end many strains were maintained and curated to provide the basic genetic material for all studies within the project. The strains were obtained from a cross between two of the most genetically divergent strains: the canonical strain Bristol N2 (originated from te UK) and the strain CB4856 (originated from Hawaii). Each recombinant strain is a genetic mosaic of the parents and harbors genome location from each parent. After crossing we obtained 200 of such recombinant inbred lines (RILs) (Figure 2). Each RIL was genotyped using single nucleotide polymorphic (SNP) markers and each RIL was assayed for the multiple phenotypes which are related to the three selected complex disease pathways (Ras, Notch, Wnt). The RIL library is a powerful resource because they do not change their genetic makeup, they can stored for a long time in the freezer and can easily be maintained for longer periods of time (many years). After storage, the worms can be thawed and used for subsequent research. The RILs were used for mapping phenotypic variation in complex disease pathway traits to particular loci (Quantitative Trait Loci, or QTL).

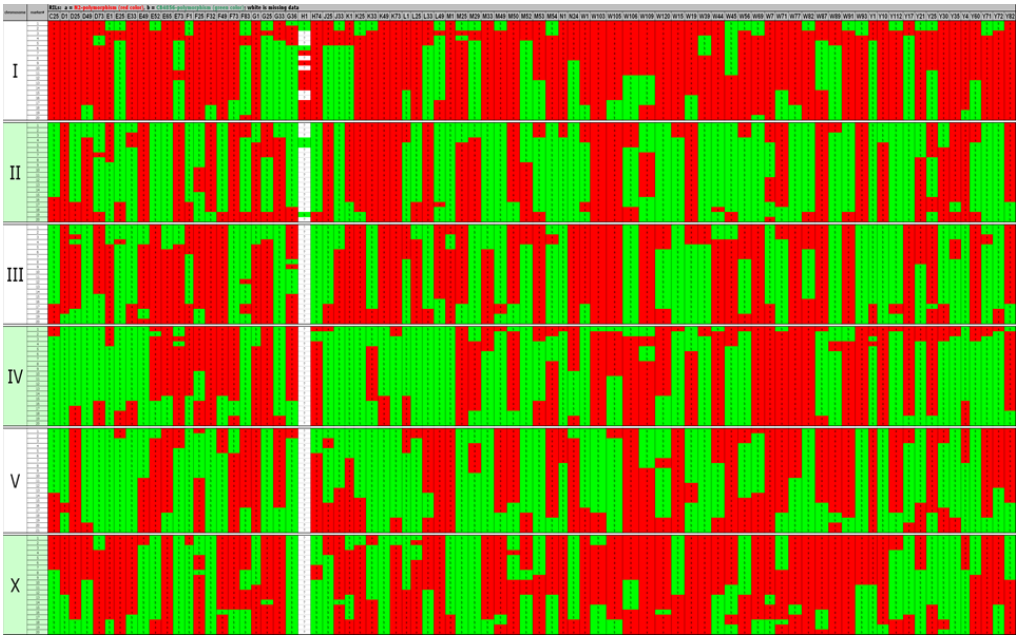

Figure 2. The developed 200 RILs derived from the parents N2 (in red) and CB4856 (in green). Vertically are shown the chromosomes I to X and horizontally the codes of al the 200 RILs. Together they present a genetic mosaic of the parental strains.

S&T Work package-2: Performing transcriptome experiments

To gain an understanding of the underlying genetic architecture of complex disease pathways (Ras, Notch and Wnt) in the model species *C. elegans* we measured and analyzed whole transcriptome profiles of the 200 recombinant inbred lines (RILs). We conducted these experiments in two different ways: first we analyzed the transcriptomes in the N2 x CB4856 RILs. Second we measured and analyzed the transcriptomes in a RIL population which was derived from a cross between a strain which carried a mutation in a selected gene which is part of the Ras, Notch or Wnt pathway (Figure 3). As an example we show here the generation of a RIL population derived from a cross between CB4856 and an N2 mutant *let-60*, a gene which is part of the RAS pathway.

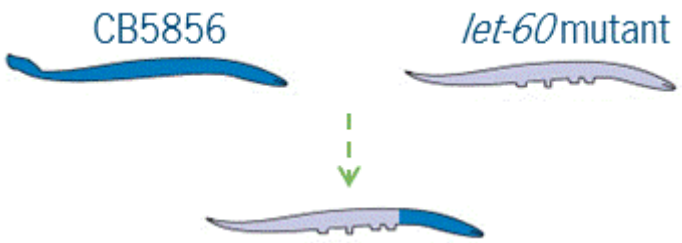

Figure 3. Generating RIL derived from a cross between the strains CB4856 and the *let-60* mutant.

The RILs derived in this way were called Mutant Introgressed Recombinant Inbred Lines (MIRILS). Figure 4 shows the genotypes for these MIRILS.

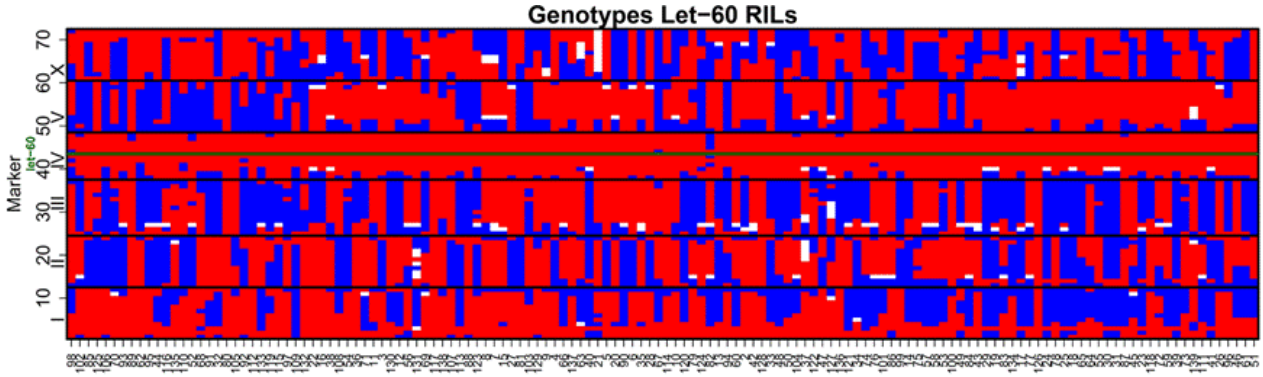

Figure 4. The developed MIRILs derived from the mutant *let-60* (in red) and CB4856 (in blue). Vertically are shown the chromosomes I to X and horizontally the codes of al the MIRILs. Together they present a genetic mosaic of the parental strains where the horizontal line on chromosome IV represents the *let-60* mutation.

These MIRILS provide the opportunity to investigate the hidden genetic variation of the Ras pathway. By perturbing the Ras pathway in the MIRILS we have been able to identify novel gene expression regulators which play a role in the genetic cancer pathway. This was investigated by analyzing the transcriptomes of each MIRIL and the subsequent identification of loci associated with gene expression variation, also called gene Expression Quantitative Trait Loci, or eQTL. Figure 5 shows the detected eQTL for the *let-60* RILS. In addition we measured the morphological phenotype of the *let-60* mutation in each MIRIL which is a reproductive aberration of the vulva formation in *C. elegans*. Figure 5 shows that a QTL of the reproductive aberration coincides with an

eQTL on chromosome II. This indicates that a regulatory locus on chromosome II plays an important role in the gene expression network underlying the *let-60* phenotype.

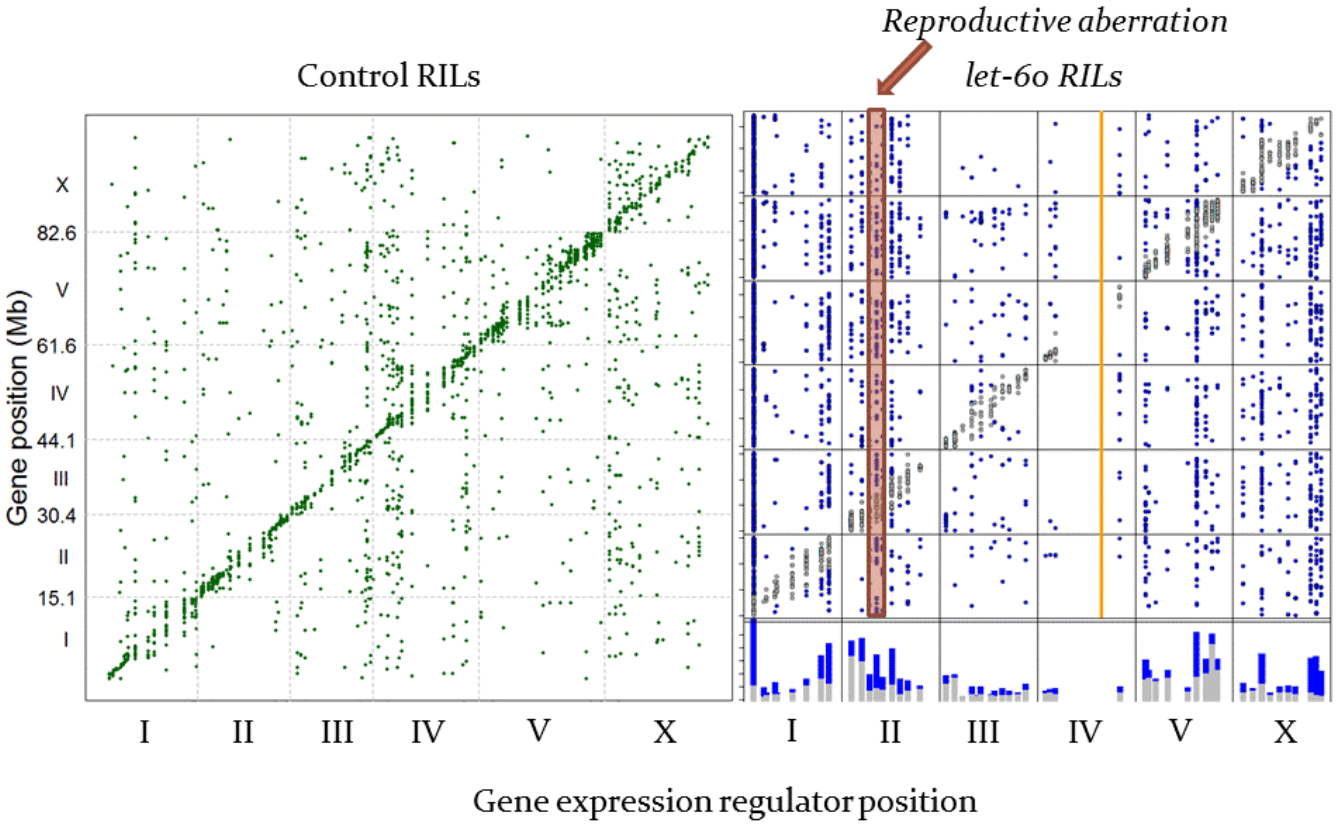

Figure 5. Gene expression regulators of the *let-60* network in the MIRILS. Each dot represents a significant eQTL where on chromosome II a strong *trans*-regulatory band of eQTL was detected which coincides with a strong QTL of the vulva development (red vertical band on the right panel of the figure).

To further provide insight into the detection of novel modifiers in the Wnt pathway we developed another MIRIL population. This population carries a *bar-1* mutation in each RIL and we phenotyped these using transcriptome analysis. We performed a preliminary analysis of the QTL of the wnt-pathway genes (Figure 6). We performed a preliminary analysis of the QTL of the Wnt-pathway genes. (Genes shown multiple times have multiple probes on the microarrays, mostly targeting splice variants or different parts of the transcripts).

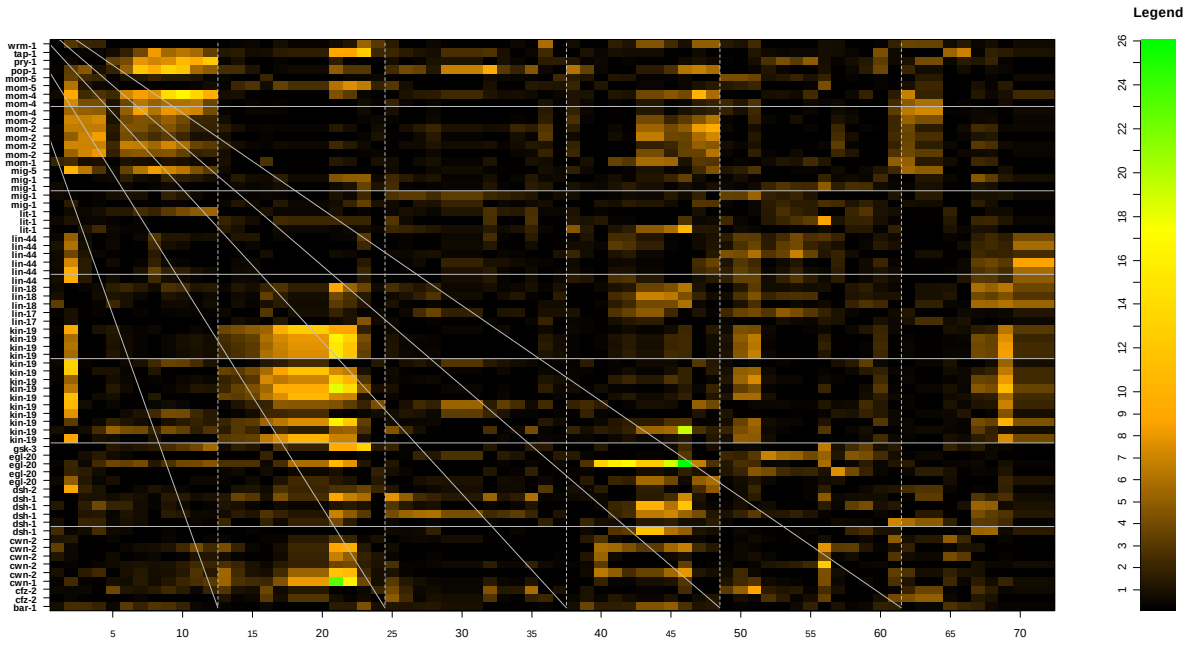

Figure 6. QTL results of the Wnt-pathway genes. On the y-axis are shown the genes in the Wnt pathway, the x-axis shows the whole genome length of *C. elegans* where the numbers designate the markers. Light-yellow to green areas designate the QTLs, loci affecting the Wnt gene transcripts.

Next to the MIRILS we developed a high throughput RNAi method that allows for rapidly comparing RNAi effects targeted against complex disease genes among diverse *C. elegans* genotypes. It is based on assessing the fitness of a population of worms by measuring the rate at which the bacterium *E. coli* (its standard food) is consumed, i.e. the feeding curve. Critically, we have demonstrated the analytical power of this method by using the feeding curves of worms treated with RNAi for quantitative trait loci (QTL) mapping. We tested a panel of 12 genes (*tag-214, mel-26, rab-5, pos-1, par-6, gld-1, let-502, lin-31, par-1, smo-1 and mpk-1*) on a recombinant inbred line population derived from a cross between N2 and CB4856. Using this method we have established a fast assay that improves the throughput of RNAi, that generates quantitative data, that is easy to implement in most laboratories, and importantly that enables QTL mapping using RNAi.

S&T Work package-3: Performing proteome experiments

In parallel with the transcriptome experiments we conducted proteomic experiments in which we investigated the genetic control of the proteome (almost all proteins in the worm) associated with the complex disease pathways Ras, Notch, and Wnt. There are two ways for quantitative proteome comparisons: targeted proteomics and unbiased shotgun proteomics. A few years ago – when the targeted proteomics method SRM/MRM (selected/multiple reaction monitoring) had been published – hopes were raised that SRM will also be applicable to phosphopeptides. But until today, SRM with phosphopeptides could not be established for high-throughput analyses. Therefore we had to replace this targeted part of the proteomics project with an unbiased shotgun approach for quantifying phosphopeptides in all the RILs. For the various pathways we focused on the relationship between gene expression differences across all RILs and the measured proteome quantification. Figure 7 shows the relation across all RILs for the two different parent alleles N2 and CB4856.

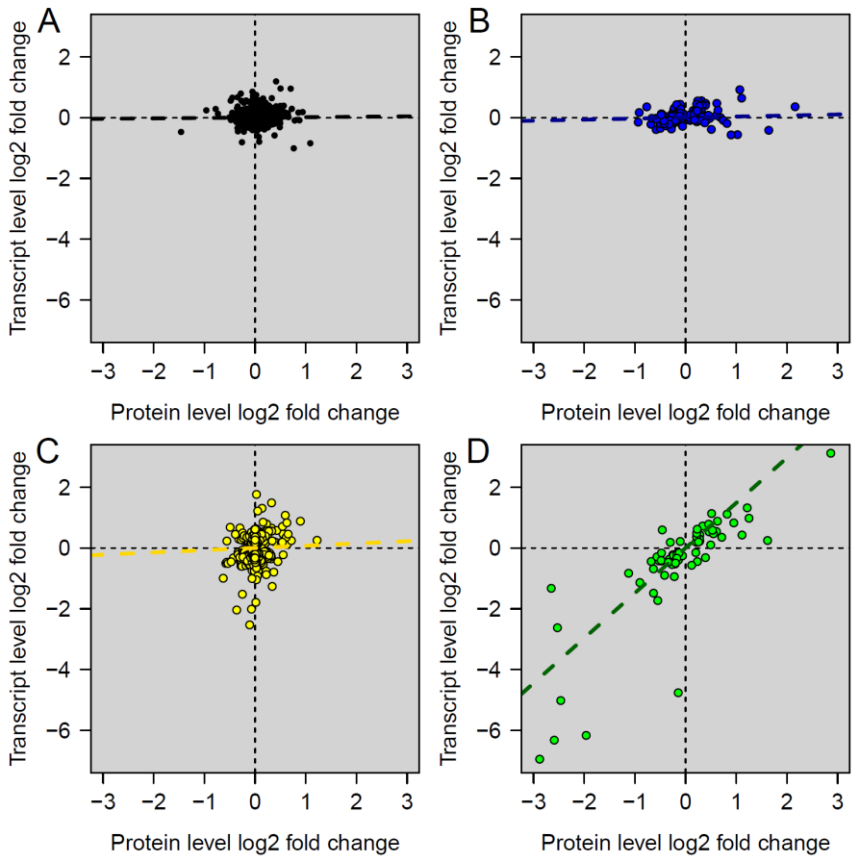

Figure 7. Relation between transcript and protein level differences between N2 and CB4856 alleles. On the x-axis log2 protein level fold chance, on the y-axis log2 transcript level fold change. Genes significantly different between N2 and CB4856, A) not on protein and not on transcript level, B) on protein level and not on transcript level, C) on transcript level not on protein level, D) on both levels.

S&T Work package-4: Investigating germline development

The germ line of the nematode *C. elegans* provides a paradigm to study essential developmental concepts like stem cell differentiation and apoptosis which are important features of complex disease process in humans. We created a computational model encompassing cell differentiation and apoptosis and the resulting movement of germ cells along the gonadal tube in *C. elegans* (Figure 8). We have used a technique based on molecular dynamics (MD) to model the physical movement of cells solely based on the force that arises from dividing cells. This novel way of using MD to drive the model enables calibration of simulation and experimental time. Based on this calibration, the analysis of our model shows that it is in accordance with experimental observations. In addition, the model provides insights into kinetics of molecular pathways within individual cells as well as into physical aspects like the cell density along the germ line and in local neighborhoods of individual germ cells. With regard to the predictive modeling, we have developed both a parental N2 and CB4856 germline development model using data on selected genes *lin-12, let-60, mpk-1* and *gld-1* in the germline development pathway. The CB4856 model of the germline predicts an increased cell density in the germline. Full transcriptome data of all RILs and their respective germline phenotypes have been conducted to verify the model outcome.

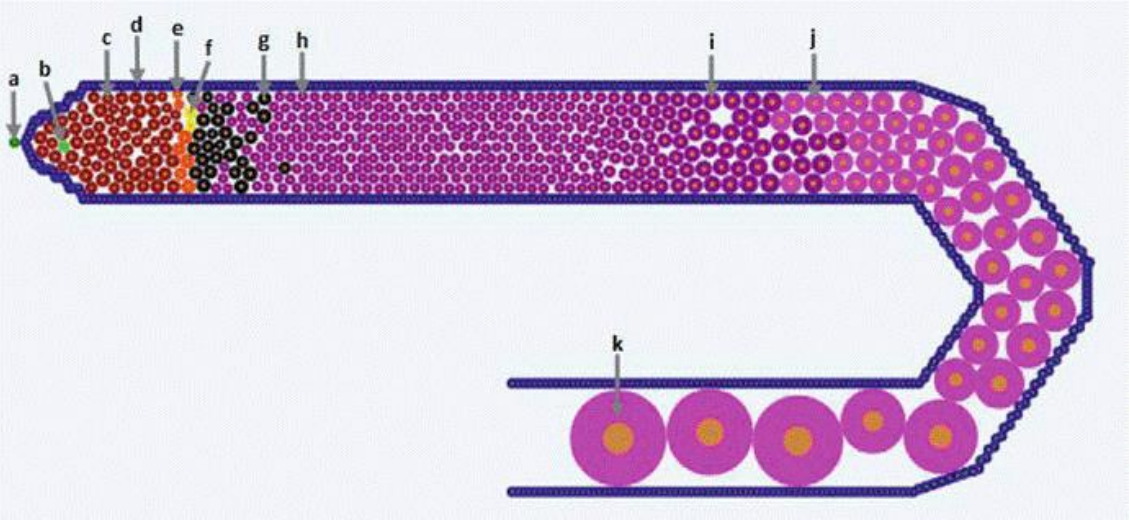

Figure 8. Snapshot of an execution of the germ line model: .a/ distal tip cell (dark green), .b/ marked cell (light green), .c/ mitotic cell with highest Notch level (dark red), .d / cells defining the border of the tube (blue), .e/ mitotic cell with Notch level between highest and 0.5 times the highest level (orange), .f / mitotic cell with Notch level between 0.5 times the highest level and 0 (yellow), .g/ mitotic cell with Notch level equal to 0 (black), .h/ meiotic cell (purple), .i / meiotic cell that has grown to about twice its original size (purple), .j / oocyte with Ras level above threshold (pink) and .k/ fully grown oocyte (pink) (from Beyer et al. 2012, Advances in Systems Biology, Advances in Experimental Medicine and Biology 736, Goryanin and A.B. Goryachev (eds.)).

S&T Work package-5: Investigating vulva development

With regard to the oncogenic RAS pathway we analyzed the *C. elegans* vulval development, which is a well-characterized system to study cell fate specification during organogenesis and disease formation. The detailed knowledge of the signaling pathways determining vulval precursor cell (VPC) fates permitted us to create a computational model based on the antagonistic interactions between the epidermal growth factor receptor (EGFR)/RAS/MAPK and the NOTCH pathways that specify the primary and secondary fates, respectively. A key notion of our model is called bounded asynchrony, which predicts that a limited degree of asynchrony in the progression of the VPCs is necessary to break their equivalence. While searching for a molecular mechanism underlying bounded asynchrony, we discovered that the termination of NOTCH signaling is tightly linked to cell-cycle progression. When single VPCs were arrested in the G1 phase, intracellular NOTCH failed to be degraded, resulting in a mixed primary/secondary cell fate. Moreover, the G1cyclins CYD-1 and CYE-1 stabilize NOTCH, while the G2 cyclin CYB-3 promotes NOTCH degradation. Our findings reveal a synchronization mechanism that coordinates NOTCH signaling with cell-cycle progression and thus permits the formation of a stable cell fate pattern. Through an iterative process of computational modeling, prediction, and experimentation, a molecular synchronization mechanism is revealed by which the cell-cycle regulates Notch signaling to allow the formation of a stable cell fate pattern (Figure 9).

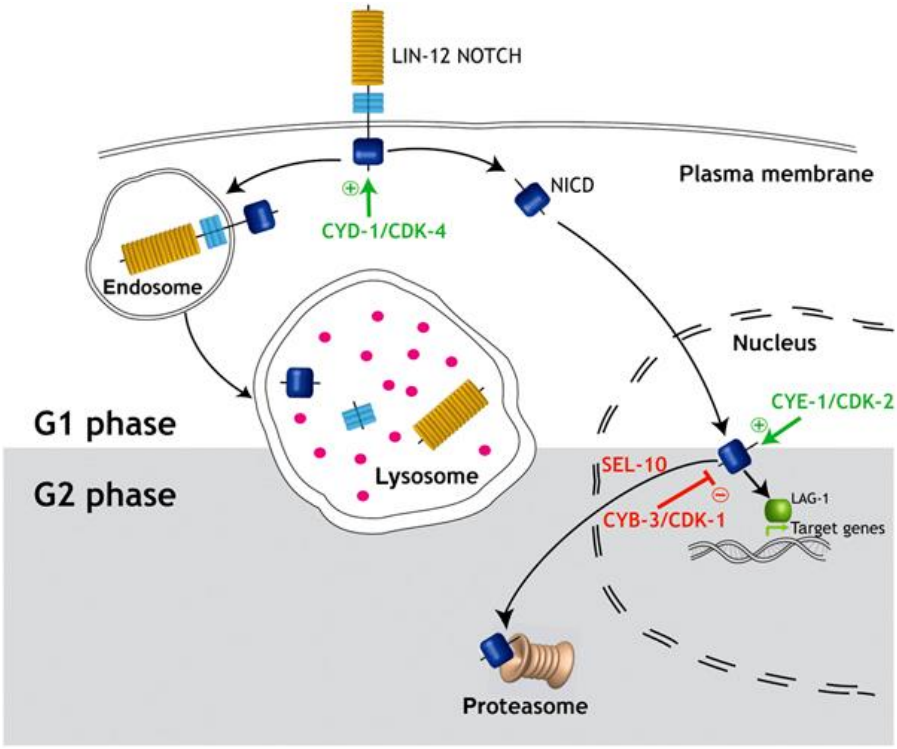

Figure 9. Model for cell-cycle regulation of LIN-12 NOTCH signaling. During the G1 phase of the cell cycle, LIN-12 NOTCH is cleaved upon binding to a DSL ligand, thereby releasing the NICD fragment that activates transcription of target genes. Alternatively, NOTCH can undergo endocytosis followed by lysosomal degradation. The activity of the G1-specific CDK-4/CYD-1 and CDK-2/CYE-1 complexes positively regulate LIN-12 NOTCH signaling in P5.p, P6.p, and P7.p by stabilizing full-length NOTCH at the apical plasma membrane and NICD in the nucleus, respectively. Degradation of NICD in P6.p occurs during the G2 phase, when activation of the G2-specific CDK-1/CYB-3 complex terminates NOTCH signaling by inducing ubiquitin-mediated proteasomal degradation of NICD (from Nusser-Stein et al. 2012, Cell-cycle regulation of NOTCH signaling during C. elegans vulval development, Molecular Systems Biology 8:618).

S&T Work package-6: Managing data and modeling data

We have finalized and published WormQTL (http://www.wormqtl.org), an easily accessible systems biology database enabling search, comparative analysis and meta-analysis of all data on variation in *Caenorhabditis spp*. Because *Caenorhabditis elegans* has become instrumental for molecular quantitative genetics and the systems biology of natural variation, these efforts have resulted in a valuable amount of phenotypic, high-throughput molecular and genotypic data across different developmental worm stages and environments in hundreds of *C. elegans* strains. WormQTL (Figure 10) provides a workbench of analysis tools for genotype–phenotype linkage and association mapping based on but not limited to R/qtl (http://www.rqtl.org). All data can be uploaded and downloaded using simple delimited text or Excel formats and are accessible via a public web user interface for biologists and R statistic and web service interfaces for bioinformaticians, based on open source workbench software. The candidate gene selection tools benefits from the most recent stable release of Wormbase (www.wormbase.org), the most widely used platform for worm biology.

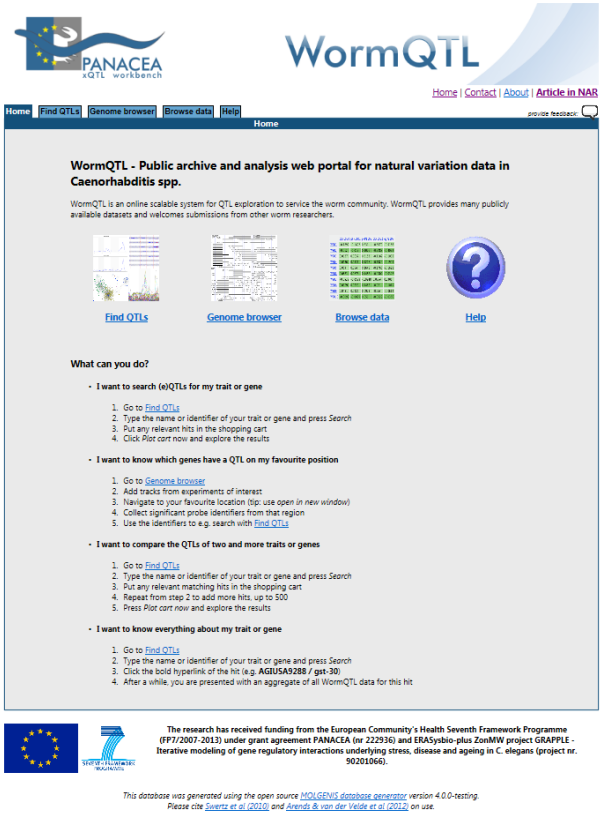

Figure 10. WormQTL, a versatile platform for data storage, analysis and investigations of natural; genetic variation in *C. elegans*.

4. The potential impact

Cancer is one of the most important causes of death and disease in Europe and is strongly related to both environmental and genetic factors. Knowledge of the genetic basis of cancer may lead to new insights into disease pathogenesis, the identification of novel drug targets, and ultimately contribute to human health. Family-based linkage analysis has been very successful in localizing causal variants for monogenic, rare Mendelian diseases. However, success has been rather limited for common diseases, where multiple loci are likely to act in concert and contribute only probabilistically.

Testing genetic variants for association between cases and appropriate controls offers a more powerful approach to detect putative causal variants, but require large sample sizes to achieve adequate power. Complete ascertainment of genetic variation by resequencing is the only comprehensive approach to test all variants (both common and rare) directly for association. For the foreseeable future, routine resequencing in thousands of individuals will not be practical. The International HapMap Project provides genome-wide data in 269 individuals from four different population groups, and supports the selection of informative markers ("tag SNPs"). An important outstanding question is whether tag SNPs picked from HapMap will be transferable across independent disease samples for studying the genetic variation of the underlying mechanisms.

PANACEA has addressed this question by mobilising expertise and resources on a sufficient scale to give Europe a leading scientific position relating to studying genetic variation of disease signaling pathways. PANACEA provides fundamental knowledge to the European Environmental and Health Strategy (the SCALE initiative). In the long term it will help to combat cancer, which has been a long-standing European priority through the "Europe against Cancer" programme established in 1985 (cf. the proposal for a Council recommendation on cancer screening.

PANACEA helps to understand the effect of genetic background on cancer signaling pathways which are highly conserved across species including humans. The project provides increased insight and improvements to current knowledge in relation to complex genetic diseases. Especially, while fitting into the public health framework of European policy, the findings and data base contents of PANACEA contributes to improved understanding in support of future cancer prevention strategies.

This project yields insight into the natural variation of disease regulatory networks. The vulva developmental model facilitates the identification of multiple drug targets in cancers. The outcomes can be used for prioritizing those targets based on the extent of causal association to disease. Achieving this level of understanding of natural variation in oncogenic pathways opens possibilities to move away from the current "one drug fits all" paradigm and to deliver on the "personalized medicine" promise of getting the right drug to the right person (Schadt, 2006).

It is however still a long way toward application of our findings to treatment of human cancers. Our study provides potential novel target genes which can be studied for genetic variation in human populations. Because many genes in the vulva developmental pathway and apoptosis encode for highly conserved human homologs, association studies can be performed on the candidate genes our project have identified. These association studies may run in parallel with regular monitoring studies where DNA samples are taken from patients which are known to have developed carcinomas.

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Flint, J. et al. (2005) Strategies for mapping and cloning quantitative trait genes in rodents. Nat. Rev. Genet. 6, 271–286.

Freeman et al. (2006) Genetic background controls tumor development in Pten-deficient mice. Cancer Res. 66, 6492-6496.

Ikeda, A. et al. (2002) Microtubule-associated protein 1A is a modifier of tubby hearing (moth1). Nat. Genet. 30, 401–405.

Kristensen et al. (2006) Genetic variation in putative regulatory loci controlling gene expression in breast cancer. Proc. Natl. Acad. Sci. USA, 108, 7735–7740.

Liang, Y. et al. (2007) The quantitative trait gene latexin influences the size of the hematopoietic stem cell population in mice. Nat. Genet. 39, 178–188.

Lowe CE et al. (2007) Large-scale genetic fine mapping and genotype-phenotype associations implicate polymorphism in the IL2RA region in type 1 diabetes. Nat. Genet. doi:10.1038/ng2102.

Salido EC. et al. (2006) Alanine–glyoxylate aminotransferase-deficient mice, a model for primary hyperoxaluria that responds to adenoviral gene transfer. Proc. Natl. Acad. Sci. USA, 103, 18249–18254.

Schadt EE (2006) Association Studies: A genome-wide association approach to mapping the genetic determinants of the transcriptome in human populations. European Journal of Human Genetics 14, 891-893.

Seitz et al. (2006) Genetic background of different cancer cell lines influences the gene set involved in chromosome 8 mediated tumor suppression. Genes Chrom.Cancer 4, 612-627.

WHO, Atlas of Europe, 2003, 113 pages.

5. The address of the project public website: www.panaceaproject.eu

4.2 Use and dissemination of foreground

Section A

Management of Intellectual Property and Publications

Research results belongs to the institutions where the research was carried out, and the intellectual property rights were applied individually according to the rules of the employer under the European and national legislation. In cases of joint contribution, the ownership of intellectual property is shared in accordance with a notified agreement. The procedures for dissemination, protection and exploitation of the intellectual property were outlined in a Consortium Agreement and negotiated by the beneficiaries as part of the contract negotiation process. Before publication of research results, all draft publications have passed an initial review of its potential for patenting and intellectual property rights which might lead to commercial exploitation. This review was conducted by the respective WP leaders within 4 weeks after receipt of the manuscripts. The beneficiaries were responsible that all patentable data will result in an application for a patent, with shared intellectual ownership rights negotiated between the relevant beneficiaries on a case-by-case.

The responsibilities and roles of the different Beneficiaries were described in the Consortium Agreement (CA). Other aspects that are addressed formally in the CA are Intellectual Property Rights, Access Rights, Confidentiality and Liability and Indemnification and these are managed in accordance with the regulations in the EU contract.

At the moment of publication of the present report the PANACEA team has published 13 peer reviewed publications in international scientific journals. Up to 16 posters have been presented and 2 international workshops have been organized. Until now there are scientific papers which are still in progress and are being prepared for publications. We anticipate that at least 5 more papers will be published within the next 12 months.

Section B

This section should specify the exploitable foreground and provide the plans for exploitation. All these data can be public or confidential; the report must clearly mark non-publishable (confidential) parts that will be treated as such by the Commission. Information under Section B that is not marked as confidential **will be made available in the public domain** thus demonstrating the added-value and positive impact of the project on the European Union.

Section A (public)

This section includes two templates

- Template A1: List of all scientific (peer reviewed) publications relating to the foreground of the project.
- Template A2: List of all dissemination activities (publications, conferences, workshops, web sites/applications, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters).

These tables are cumulative, which means that they should always show all publications and activities from the beginning until after the end of the project. Updates are possible at any time.

TEMPLATE A1: LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES

NO.	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers[3] (if available)	Is/Will open access[4] provided to this publication?
1	*Cell-cycle regulation of NOTCH signaling during C. elegans vulval development*	*Fisher, J*	*Mol. Sys. Biol.*	*8:618*	*Nature Publ. Grp.*		*2012*	*1-14*	*10.1038/msb.2012.51*	*yes*
2	*WormQTL—public archive and analysis web portal for natural variation data in Caenorhabditis spp*	*Snoek, L.B.*	*Nucl. Ac. Res.*	*41, D738–D743*	*Oxford Univ. Press.*		*2013*	*738-743*	*10.1093/nar/gks1124*	*yes*
3	*WormQTHD - a comprehensive web database for linking human disease to natural variation data in C. elegans*	*Van der Velde, J.*	*Nucl. Ac. Res.*			*accepted*				*yes*

[3] A permanent identifier should be a persistent link to the published version full text if open access or abstract if article is pay per view) or to the final manuscript accepted for publication (link to article in repository).

[4] Open Access is defined as free of charge access for anyone via Internet. Please answer "yes" if the open access to the publication is already established and also if the embargo period for open access is not yet over but you intend to establish open access afterwards.

4	*A Dynamic Physical Model of Cell Migration, Differentiation and Apoptosis in Caenorhabditis elegans*	*Beyer, A.*	*Advances in Experimental Medicine and Biology*	*736:211-33*	*Springer*		*2012*	*211-233*	*10.1007/978-1-4419-7210-1_12*	*no*
5	*Big data, but are we ready?*	*Trelles, O*	*Nat. Rev. Genetic.*	*12, 224*	*Nature Publ. Grp.*		*2011*			*no*
6	*A fitness assay for comparing RNAi effects across multiple C. elegans genotypes*	*Elvin, M.*	*BMC Genomics*	*12:510*	*BMC*		*2011*		*10.1186/1471-2164-12-510*	*yes*
7	*xQTL workbench: a scalable web environment for multi-level QTL analysis*	*Arends, D.*	*Bioinformatics*	*28:1042-4*	*Oxford Univ. Press.*		*2012*		*10.1093/bioinformatics/bts049*	*yes*
8	*Aging Uncouples Heritability and Expression-QTL in Caenorhabditis elegans*	*Vinuela, A.*	*G3*	*2*	*Genet. Soc. America*		*2012*	*597-605*		*no*
9	*Genetic variation for stress-response hormesis in C. elegans lifespan*	*Rodriguez, M.*	*Exp. Gerontol.*	*47*	*Elsevier*		*2012*	*581-587*		*no*
10	*Global Genetic Robustness of the Alternative Splicing Machinery in Caenorhabditis elegans.*	*Li, Y.*	*Genetics*	*186*	*Genet. Soc. America*		*2010*	*405–410*	*10.1534/genetics.110.119677*	*no*
11	*Worms under stress: C. elegans stressresponse and its relevance to complexhuman disease and aging*	*Rodriguez, M.*	*Trends in Genetics*	*29*	*Cell press*		*2013*	*367-374*		*no*
12	*The MOLGENIS toolkit: rapid prototyping of biosoftware at the push of a button*	*Swertz, M.*	*BMC Bioinformatics*		*BMC*		*2010*	*11(Suppl 12): S12*	*10.1186/1471-2105-11-S12-S12*	*yes*
13	*Observ-OM and Observ-TAB: Universal Syntax Solutionsfor the Integration, Search, and Exchange of Phenotype AndGenotype Information*	*Swertz, M.*	*Human mutation*	*33*	*Wiley*		*2012*	*867–873*		*no*
14	*Predictive Modelling of Stem Cell Differentiation and Apoptosis in C. elegans*	*Beyer, A.*	*IPCAT*	*7223*			*2012*	*99–104*		*no*

TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES

Nº	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed
1	Posters	MICROSOFT RESEARCH LIMITED	New Insights into C. elegans Vulval Induction Using Computational Modeling	18/06/2010	Heidelberg	Scientific community (higher education, Research)		USA and Europe
2	Posters	THE UNIVERSITY OF MANCHESTER	A food consumption assay to enable super high throughput RNAi	18/06/2010	Heidelberg	Scientific community (higher education, Research)		USA and Europe
3	Presentations	MICROSOFT RESEARCH LIMITED	Cell cycle control of VPC fate specification by regulation of EGFR/RAS/MPK and NOTCH crosstalk	18/06/2010	Heidelberg	Scientific community (higher education, Research)		USA and Europe
4	Presentations	MICROSOFT RESEARCH LIMITED	Computational Modelling Providing New Insights into Signalling Crosstalk during C. elegans Vulval De	12/10/2010	Edinburgh	Scientific community (higher education, Research)		USA and Europe
5	Presentations	WAGENINGEN UNIVERSITEIT	Cryptic genetic variation in complex human disease and ageing pathways in C. elegans	09/11/2010	Wageningen	Scientific community (higher education, Research)		Netherlands
6	Presentations	WAGENINGEN UNIVERSITEIT	The next genomic leap in C. elegans: cryptic genetic variation	18/11/2010	Leiden	Scientific community (higher education, Research)		The Netherlands
7	Presentations	WAGENINGEN UNIVERSITEIT	Combining Genetic Variation with Targeted Knock-downs to Construct Gene Networks of	29/08/2011	Heidelberg	Scientific community (higher education, Research)		USA and Europe

Nº	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed
			Complex Human Di					
8	Posters	UNIVERSITAET ZUERICH	Quantitative pathway analysis of natural variation in complex disease signaling in C. elegans	05/09/2011	Geneva	Scientific community (higher education, Research)		Europe
9	Workshops	WAGENINGEN UNIVERSITEIT	WormQTL – Public archive and analysis web portal for C. elegans	05/04/2012	Cold Spring Harbor	Scientific community (higher education, Research)	50	USA, Europe
10	Posters	THE UNIVERSITY OF MANCHESTER	A fitness assay for comparing RNAi effects across C. elegans genotypes	05/04/2012	Cold Spring Harbor	Scientific community (higher education, Research)		USA and Europe
11	Posters	WAGENINGEN UNIVERSITEIT	“All” the sequence polymorphisms between Hawaii (CB4856) and	05/04/2012	Cold Spring Harbor	Scientific community (higher education, Research)		USA and Europe
12	Presentations	WAGENINGEN UNIVERSITEIT	Unveiling crpytic genetic varation in C. elegans	06/04/2012	Cold Sprinh Harbor	Scientific community (higher education, Research)		USA and Europe
13	Posters	WAGENINGEN UNIVERSITEIT	Combining natural genetic variation and vulval development mutants in Caenorhabditis elegans to unde	18/06/2012	Washington	Scientific community (higher education, Research)		US and Europe
14	Posters	WAGENINGEN UNIVERSITEIT	Combining genetic variation with targeted knock-downs/outs to understand complex human disease pathw	19/06/2012	Edinburgh	Scientific community (higher education, Research)		USA and Europe
15	Posters	UNIVERSITAET ZUERICH	Identification of polymorphic modifiers of ras and wnt signaling in C.	19/06/2012	Edinburgh	Scientific community (higher education, Research)		USA and Europe

Nº	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed
			elegans					
16	Workshops	WAGENINGEN UNIVERSITEIT	New tools and insights from mining genotype-phenotype relations across multiple genotypes of Caenorh	27/06/2013	Los Angeles	Scientific community (higher education, Research)	50	World
17	Presentations	WAGENINGEN UNIVERSITEIT	Uncovering genotype specific variation of Wnt signaling in C. elegans	29/06/2013	Los Angeles	Scientific community (higher education, Research)	1000	World
18	Presentations	UNIVERSITAET ZUERICH	Studying the effect of natural variation on protein abundance in C. elegans	29/06/2013	Los Angeles	Scientific community (higher education, Research)	1000	World
19	Presentations	WAGENINGEN UNIVERSITEIT	The sudden transcriptional switch to adulthood in L4 stage C. elegans	29/06/2013	Los Angeles	Scientific community (higher education, Research)	1000	World
20	Presentations	WAGENINGEN UNIVERSITEIT	Introducing WormQTL, a public archive and analysis web portal for natural variation data in Caenorha	29/06/2013	Los Angeles	Scientific community (higher education, Research)	1000	World

Section B (Confidential[5] or public: confidential information to be marked clearly)
Part B1

The applications for patents, trademarks, registered designs, etc. shall be listed according to the template B1 provided hereafter.

The list should, specify at least one unique identifier e.g. European Patent application reference. For patent applications, only if applicable, contributions to standards should be specified. This table is cumulative, which means that it should always show all applications from the beginning until after the end of the project.

TEMPLATE B1: LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS, ETC.					
Type of IP Rights[6]:	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Application reference(s) (e.g. EP123456)	Subject or title of application	Applicant (s) (as on the application)
-					

[5] Note to be confused with the "EU CONFIDENTIAL" classification for some security research projects.

[6] A drop down list allows choosing the type of IP rights: Patents, Trademarks, Registered designs, Utility models, Others.

Part B2
Please complete the table hereafter:

Type of Exploitable Foreground[7]	Description of exploitable foreground	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application[8]	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
	Ex: New supercond uctive Nb-Ti alloy			*MRI equipment*	*1. Medical 2. Industrial inspection*	*2008 2010*	*A materials patent is planned for 2006*	*Beneficiary X (owner) Beneficiary Y, Beneficiary Z, Poss. licensing to equipment manuf. ABC*
-								

In addition to the table, please provide a text to explain the exploitable foreground, in particular:

- Its purpose
- How the foreground might be exploited, when and by whom
- IPR exploitable measures taken or intended
- Further research necessary, if any
- Potential/expected impact (quantify where possible)

[19] A drop down list allows choosing the type of foreground: General advancement of knowledge, Commercial exploitation of R&D results, Exploitation of R&D results via standards, exploitation of results through EU policies, exploitation of results through (social) innovation.
[8] A drop down list allows choosing the type sector (NACE nomenclature) : http://ec.europa.eu/competition/mergers/cases/index/nace_all.html

4.3 Report on societal implications

Replies to the following questions will assist the Commission to obtain statistics and indicators on societal and socio-economic issues addressed by projects. The questions are arranged in a number of key themes. As well as producing certain statistics, the replies will also help identify those projects that have shown a real engagement with wider societal issues, and thereby identify interesting approaches to these issues and best practices. The replies for individual projects will not be made public.

A General Information *(completed automatically when **Grant Agreement number** is entered.*	
Grant Agreement Number:	222936
Title of Project:	Quantitative pathway analysis of natural variation in complex
Name and Title of Coordinator:	Jan E. Kammenga, Dr.

B Ethics	
1. Did your project undergo an Ethics Review (and/or Screening)? • If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final project reports? Special Reminder: the progress of compliance with the Ethics Review/Screening Requirements should be described in the Period/Final Project Reports under the Section 3.2.2 *'Work Progress and Achievements'*	***0Yes***
2. Please indicate whether your project involved any of the following issues (tick box) :	***NO***
RESEARCH ON HUMANS	
• Did the project involve children?	-
• Did the project involve patients?	-
• Did the project involve persons not able to give consent?	-
• Did the project involve adult healthy volunteers?	-
• Did the project involve Human genetic material?	-
• Did the project involve Human biological samples?	-
• Did the project involve Human data collection?	-
RESEARCH ON HUMAN EMBRYO/FOETUS	
• Did the project involve Human Embryos?	-
• Did the project involve Human Foetal Tissue / Cells?	-
• Did the project involve Human Embryonic Stem Cells (hESCs)?	-
• Did the project on human Embryonic Stem Cells involve cells in culture?	-
• Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?	-
PRIVACY	
• Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?	
• Did the project involve tracking the location or observation of people?	-
RESEARCH ON ANIMALS	
• Did the project involve research on animals?	yes
• Were those animals transgenic small laboratory animals?	no
• Were those animals transgenic farm animals?	no

• Were those animals cloned farm animals?	No
• Were those animals non-human primates?	No
RESEARCH INVOLVING DEVELOPING COUNTRIES	
• Did the project involve the use of local resources (genetic, animal, plant etc)?	-
• Was the project of benefit to local community (capacity building, access to healthcare, education etc)?	
DUAL USE	
• Research having direct military use	0 No
• Research having the potential for terrorist abuse	-

C Workforce Statistics

3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of Position	Number of Women	Number of Men
Scientific Coordinator	0	1
Work package leaders	1	2
Experienced researchers (i.e. PhD holders)	1	3
PhD Students	2	2
Other	1	2

4. How many additional researchers (in companies and universities) were recruited specifically for this project?	**6**
Of which, indicate the number of men:	2

D Gender Aspects

5.	**Did you carry out specific Gender Equality Actions under the project**?	X ○	Yes No

6. Which of the following actions did you carry out and how effective were they?

		Not at all effective				Very effective
❑	Design and implement an equal opportunity policy	○	○	X	○	○
❑	Set targets to achieve a gender balance in the workforce	○	○	X	○	○
❑	Organise conferences and workshops on gender	X	○	○	○	○
❑	Actions to improve work-life balance	○	○	X	○	○
○	Other:					

7. Was there a gender dimension associated with the research content – i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?

○ Yes- please specify

X No

E Synergies with Science Education

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

X Yes- please specify: Open days, teaching

○ No

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

○ Yes- please specify

X No

F Interdisciplinarity

10. Which disciplines (see list below) are involved in your project?

X Main discipline[9]:

○ Associated discipline[9]:

○ Associated discipline[9]:

G Engaging with Civil society and policy makers

11a	**Did your project engage with societal actors beyond the research community?** *(if 'No', go to Question 14)*	○ X	Yes No

11b If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?

○ No

○ Yes- in determining what research should be performed

○ Yes - in implementing the research

○ Yes, in communicating /disseminating / using the results of the project

[9] Insert number from list below (Frascati Manual).

11c In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?	○ ○	Yes No

12. Did you engage with government / public bodies or policy makers (including international organisations)

X	No
○	Yes- in framing the research agenda
○	Yes - in implementing the research agenda
○	Yes, in communicating /disseminating / using the results of the project

13a Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?

○	Yes – as a **primary** objective (please indicate areas below- multiple answers possible)
○	Yes – as a **secondary** objective (please indicate areas below - multiple answer possible)
X	No

13b If Yes, in which fields?

Agriculture		Energy		Human rights	
Audiovisual and Media		Enlargement		Information Society	
Budget		Enterprise		Institutional affairs	
Competition		Environment		Internal Market	
Consumers		External Relations		Justice, freedom and security	
Culture		External Trade		Public Health	
Customs		Fisheries and Maritime Affairs		Regional Policy	
Development Economic and Monetary Affairs		Food Safety		Research and Innovation	
		Foreign and Security Policy		Space	
Education, Training, Youth		Fraud		Taxation	
Employment and Social Affairs		Humanitarian aid		Transport	

13c If Yes, at which level?

- ○ Local / regional levels
- ○ National level
- ○ European level
- ○ International level

H Use and dissemination

14. How many Articles were published/accepted for publication in peer-reviewed journals?	**14**
To how many of these is open access[10] provided?	**6**
How many of these are published in open access journals?	**6**
How many of these are published in open repositories?	**0**
To how many of these is open access not provided?	
Please check all applicable reasons for not providing open access:	
X publisher's licensing agreement would not permit publishing in a repository ❑ no suitable repository available ❑ no suitable open access journal available ❑ no funds available to publish in an open access journal ❑ lack of time and resources ❑ lack of information on open access ❑ other[11]: ……	

15. How many new patent applications ('priority filings') have been made? *("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).*		**-**
16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	Trademark	
	Registered design	
	Other	
17. How many spin-off companies were created / are planned as a direct result of the project?		**-**
Indicate the approximate number of additional jobs in these companies:		

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:

X	Increase in employment, or	X	In small & medium-sized enterprises
❑	Safeguard employment, or	❑	In large companies
❑	Decrease in employment,	❑	None of the above / not relevant to the project
❑	Difficult to estimate / not possible to quantify		

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (*FTE = one person working fulltime for a year*) jobs:	*Indicate figure:* *8 FTE*

[10] Open Access is defined as free of charge access for anyone via Internet.
[11] For instance: classification for security project.

Difficult to estimate / not possible to quantify	❑

I Media and Communication to the general public

20. As part of the project, were any of the beneficiaries professionals in communication or media relations?

X Yes ○ No

21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?

○ Yes X No

22 Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?

X	Press Release	X	Coverage in specialist press
X	Media briefing	❑	Coverage in general (non-specialist) press
❑	TV coverage / report	X	Coverage in national press
X	Radio coverage / report	❑	Coverage in international press
❑	Brochures /posters / flyers	❑	Website for the general public / internet
❑	DVD /Film /Multimedia	❑	Event targeting general public (festival, conference, exhibition, science café)

23 In which languages are the information products for the general public produced?

X	Language of the coordinator	❑	English
❑	Other language(s)		

***Question F-10*:** Classification of Scientific Disciplines according to the Frascati Manual 2002 (Proposed Standard Practice for Surveys on Research and Experimental Development, OECD 2002):

FIELDS OF SCIENCE AND TECHNOLOGY

1. NATURAL SCIENCES

1.1 Mathematics and computer sciences [mathematics and other allied fields: computer sciences and other allied subjects (software development only; hardware development should be classified in the engineering fields)]

1.2 Physical sciences (astronomy and space sciences, physics and other allied subjects)

1.3 Chemical sciences (chemistry, other allied subjects)

1.4 Earth and related environmental sciences (geology, geophysics, mineralogy, physical geography and other geosciences, meteorology and other atmospheric sciences including climatic research, oceanography, vulcanology, palaeoecology, other allied sciences)

1.5 **Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences)**

2 ENGINEERING AND TECHNOLOGY

2.1 Civil engineering (architecture engineering, building science and engineering, construction engineering, municipal and structural engineering and other allied subjects)

2.2 Electrical engineering, electronics [electrical engineering, electronics, communication engineering and systems, computer engineering (hardware only) and other allied subjects]

2.3. Other engineering sciences (such as chemical, aeronautical and space, mechanical, metallurgical and materials engineering, and their specialised subdivisions; forest products; applied sciences such as

geodesy, industrial chemistry, etc.; the science and technology of food production; specialised technologies of interdisciplinary fields, e.g. systems analysis, metallurgy, mining, textile technology and other applied subjects)

3. MEDICAL SCIENCES

3.1 **Basic medicine (anatomy, cytology, physiology, genetics, pharmacy, pharmacology, toxicology, immunology and immunohaematology, clinical chemistry, clinical microbiology, pathology)**

3.2 Clinical medicine (anaesthesiology, paediatrics, obstetrics and gynaecology, internal medicine, surgery, dentistry, neurology, psychiatry, radiology, therapeutics, otorhinolaryngology, ophthalmology)

3.3 Health sciences (public health services, social medicine, hygiene, nursing, epidemiology)

4. AGRICULTURAL SCIENCES

4.1 Agriculture, forestry, fisheries and allied sciences (agronomy, animal husbandry, fisheries, forestry, horticulture, other allied subjects)

4.2 Veterinary medicine

5. SOCIAL SCIENCES

5.1 Psychology

5.2 Economics

5.3 Educational sciences (education and training and other allied subjects)

5.4 Other social sciences [anthropology (social and cultural) and ethnology, demography, geography (human, economic and social), town and country planning, management, law, linguistics, political sciences, sociology, organisation and methods, miscellaneous social sciences and interdisciplinary , methodological and historical S1T activities relating to subjects in this group. Physical anthropology, physical geography and psychophysiology should normally be classified with the natural sciences].

6. HUMANITIES

6.1 History (history, prehistory and history, together with auxiliary historical disciplines such as archaeology, numismatics, palaeography, genealogy, etc.)

6.2 Languages and literature (ancient and modern)

6.3 Other humanities [philosophy (including the history of science and technology) arts, history of art, art criticism, painting, sculpture, musicology, dramatic art excluding artistic "research" of any kind, religion, theology, other fields and subjects pertaining to the humanities, methodological, historical and other S1T activities relating to the subjects in this group]

2. FINAL REPORT ON THE DISTRIBUTION OF THE EUROPEAN UNION FINANCIAL CONTRIBUTION

This report shall be submitted to the Commission within 30 days after receipt of the final payment of the European Union financial contribution.

Report on the distribution of the European Union financial contribution between beneficiaries

THIS COULD NOT BE FILLED IN BY THIS DATE BECAUSE THE PAYMENTS HAVE NOT BEEN RECEIVED YET.

Name of beneficiary	Final amount of EU contribution per beneficiary in Euros
1.	
2.	
3	
4	
Total	