# CHeartED

# News Bulletin
## March 2012

**'Heart development, although complex, is approachable at a network level.'**

Professor Richard P. Harvey is a pioneer in the investigation of the molecular mechanisms underlying cardiac development and specialized in genetic analysis in animal models and genetic screening in mouse and humans. His group plays an important role in workpackage 2b of the CHeartED project.

### The Harvey lab

Professor Richard P. Harvey is Deputy Director and Head of the Developmental Biology Division of Victor Chang Cardiac Research Institute, which is affiliated with the University of New South Wales in Sydney, Australia. One of his research lines focuses on networks of transcription factors that control genes involved in embryonic development. With their molecular techniques and systems biology approaches, his team aims to identify new genes and pathways specifically relevant to congenital heart defects, like outflow tract malformations and tetralogy of Fallot.

### The outflow tract

The outflow tract in the developing heart represents the structure that gives rise to the pulmonary and aortic trunks that are the conduits for propulsion of blood from the ventricles. Abnormalities during outflow tract development are a common and serious manifestation of congenital heart disease and include Tetralogy of Fallot. The CHeartED project aims at identifying genes

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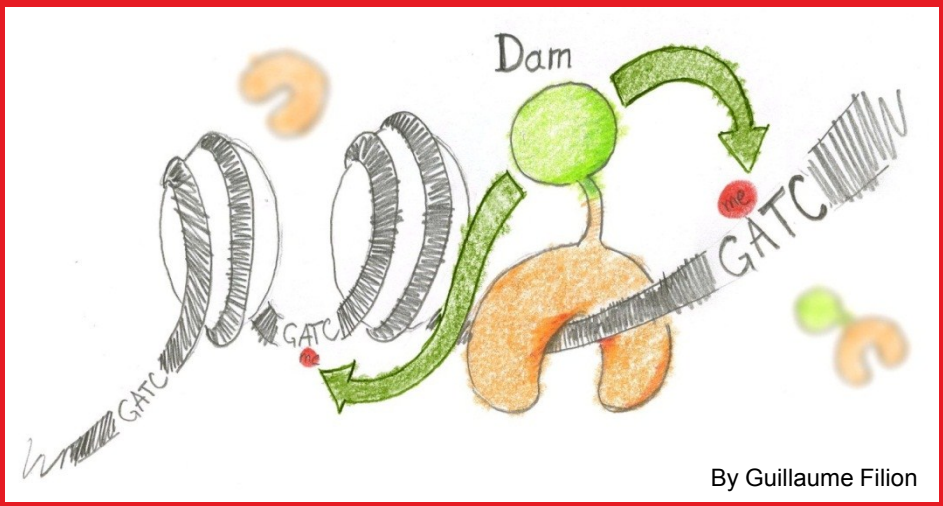
**Gene- Environment Interactions in Heart Development**

www.CHeartED.eu

COOPERATION




By Guillaume Filion

**DamID** (DNA adenine methyltransferase identification) is a technique that can be used to find sites in the DNA where proteins - like transcription factors - bind. The idea is that the protein of interest (orange) is fused to the protein DNA adenine methyltransferase (Dam; green) from the bacteria *Escherichia coli*. This Dam protein puts a methyl group (red) on de adenine in GATC sequences. Adenine methylation does not occur naturally in eukaryotes and therefore must have been caused by the fusion protein, implying the region is located near a binding site. This DNA region can subsequently be identified with a couple of assays that use restriction enzymes that specifically recognize methylated GATC sequences.

and pathways relevant for congenital heart diseases. Mirana Ramialison is one of the postdoctoral fellows in the Harvey lab: 'As the genetic basis of outflow tract defects remains only partly characterized, our work within Workpackage 2b is focused on deciphering the molecular networks underpinning heart formation that will also be implicated in outflow tract development in order to gain a better understanding of the origin of outflow tract anomalies.'

**Transcription factors**

The outflow tract originates from a particular heart precursor region named the second heart field. Several regulatory genes are known to be active in this field. These genes encode proteins like the GATA, T-box and NK2 homeodomain families of transcription factors. The transcription factors control the expression of other downstream genes. Nkx2-5 is one of these transcription factors that has been well studied genetically in the Harvey laboratory in the context of outflow tract malformations. Ramialison: 'We are seeking first to identify a comprehensive list of genes regulated by these transcription factors and to understand the logic of heart development at a network level. To achieve this, Dr. Romaric Bouveret in our laboratory has pioneered in mammalian cells the DamID technology. This technology was developed by Bas van Steensel (Netherlands Cancer Institute, Amsterdam, The Netherlands) in *Drosophila*, to identify the target genes of cardiac transcription factors in a systematic and unbiased manner.'

**Deciphering molecular networks**

'With a multi-disciplinary team composed of bioinformaticians (Tram Doan and Dr. Mirana Ramialison) as well as molecular biologists (Dr. Romaric Bouveret, Dr. Danielle de Jong and Dr. Nicole Schönrock), we have begun to decipher the complex genetic interactions that are taking place between these transcription factors at a genome-wide level,' Ramialison continues. 'Using DamID data for more then a dozen transcription factors and mutants, we have begun to characterize the strengths and limitations of the genome-wide approach and have reconstructed a preliminary network of

shared and unique targets. From the analysis of this cardiac gene regulatory network using bioinformatics tools thus far, we believe we have made two interesting discoveries.

**Core of the network**

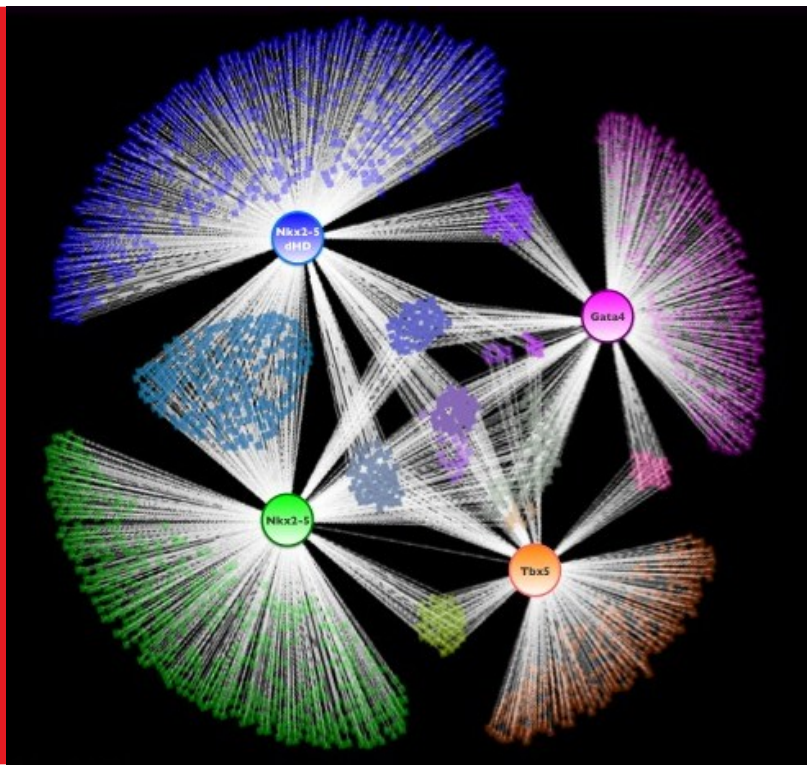
First, we have shown that the ELK transcription factors directly interact with Nkx2-5 and have predicted that gene targets of the ELK transcription factors, specifically Elk1 and Elk4, are directly embedded in the core of the heart regulatory network. To validate our findings, we took advantage of the amenable properties of the zebrafish model organism for rapidly assessing gene function. We discovered that the knockdown of Elk1/4 gene function led to heart specific defects, initially a surprise as ELK factors are rather ubiquitously expressed throughout development and have been implicated in the growth factor responses, particularly in smooth muscle.

**New set of interactions**

Second, we have discovered that when a transcription factor is mutated (such as in a disease context), not only do network interactions that occur in normal developmental processes collapse, but more importantly a new set of interactions are generated through a gained emphasis on interactions between the mutated transcription factor and other endogenous interacting cofactors. This latter discovery could challenge our current view of congenital heart disease pathology. Indeed it is widely accepted that congenital heart diseases arise because of mutations in “healthy” cardiac genes which consequently are not able to perform their normal function. Here we propose that in addition to this traditional view, mutated cardiac genes can also lead to disease by acquiring new (off-target) functions.’

**Invaluable resource**

‘This new area of network biology within the context of heart development is a very exciting field of research that will enhance our understanding of complex diseases such as Tetralogy of Fallot,’ Ramialison concludes. ‘We believe that cardiac gene regulatory network data generated by DamID will help researchers to identify key genes responsible for congenital heart diseases and therefore will be an invaluable resource for the CHeartED consortium and the larger scientific community working on heart development.’




Representation of a network of shared target genes: Nkx2-5 (green), Tbx5 (orange), Gata4 (pink) and Nkx2-5 lacking its DNA binding domain (blue).


Harvey Lab (from left to right, front to back): Gonzalo del Monte, Reena Singh, Amita Limaye, Mirana Ramialison, Grace Wei, Bernice Stewart, Nicole Schoenrock, Naisana S. Asli, Amirsalar Rashidianfar, Ivan Cheung, Mahdi Moradi Marjaneh, Romaric Bouveret, Richard Harvey, Henrik Reinhard.

## General remarks for CHeartED consortium

### Website

• We ask you to check the information on the website. If you have any adjustments, please notify the dissemination manager.

• Visit the open-access CHDwiki via the CHeartED website. The Wiki contains lots of information about genetic and environmental knowledge on heart development. We invite you to take a look at the website and actively help to enlarge the database.

### Dissemination

• To keep all consortium members informed, we ask the beneficiaries to announce CHeartED related meetings, courses, workshops etc. on the website.

• To share publications related to CHeartED with all consortium members, please upload all publications on the website.

To upload events or publications yourself, you have to login first. You may also ask the dissemination manager.

**Do not forget to mention EU support in all publications, courses, workshops etc. as follows:**

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## Contact:

**Coordinator:** Prof. dr. Antoon Moorman
tel: +31-(0)20-5664928 or 5664647
e-mail: A.F.Moorman@amc.uva.nl

**Project manager**: R. van der Gaag, PhD
e-mail: r.vandergaag@amc.uva.nl

**Dissemination manager:**
Nanna Claij, PhD
tel: +31-70-3155635
e-mail: n.claij@hartstichting.nl