様式19　別紙1

課題番号	LS127

先端研究助成基金助成金（最先端・次世代研究開発支援プログラム）
実施状況報告書（平成25年度）

本様式の内容は一般に公表されます

研究課題名	細胞分化に関与するノンコーディング RNA の全ゲノム解析
研究機関・ 部局・職名	独立行政法人理化学研究所　ライフサイエンス技術基盤研究センター トランスクリプトーム研究チーム　チームリーダー
氏名	カルニンチ　ピエロ

1.　当該年度の研究目的

For FY2013, we had planned:

(1) to complete the characterization of novel transcripts identified from our analyses, now published (*Fort A. et al., Nat. Genet., 2014: doi:10.1038/ng.2965*), where we described of a large set of novel transcripts named NASTs (Non-Annotated-Stem-Transcripts).

(2) to pursue the development of a new technology allowing the detection of specific interactions between ncRNA and chromatin loci. This tool is badly needed to understand the molecular mechanisms of ncRNA implicated in locus-specific chromatin modifications, part of global gene regulatory processes.

(3) to deepen our understanding of the mechanisms of action of some specific novel ncRNAs, in particular of NASTs associated with enhancer regions and retrotransposon elements.

2.　研究の実施状況

Lost of function attempts on NASTs were conducted using multiple types of reagents (*i.e.* short interfering RNAs, siRNA and lock nucleic acids antisense oligos, LNA) in order to obtain a reproducible knock-down of some NASTs candidates. Following a large experimental effort, screening a total of 77 NASTs with siRNA and another 30 with antisense LNAs, we have reported for four candidates, direct implication in the genetic regulation of the maintenance of pluripotency (Figure 1).

We are currently performing over-expression experiments of NAST candidates. 70 NASTs were cloned within and overexpressed in mouse embryonic stem cells to test for their ability to slow down passive differentiation process, following LIF removal. 10 of them lead to significant slow down of differentiation, when measuring Nanog expression. We are currently performing transcriptome wide analyses following overexpression of 6 of these 10 candidates.

We pursue the development of technology for the detection of RNA-chromatin interactions. We aim at finding optimal conditions for asymmetric ligation of RNA and DNA using home made linker. The goal being to obtain cDNA linked to fragmented chromatin pieces, which are isolated together only when ncRNAs are retained on the chromatin. These cDNA/genomic DNA chimeras are then analyzed with next-generation sequencing allowing the construction of a genome-wide map for RNA/chromatin interactions. Our efforts are currently focused on testing different crosslinking approaches as well as optimizing the linker sequences. We have collected preliminary

sequencing data confirming the actual production and detection of RNA/DNA chimeras.

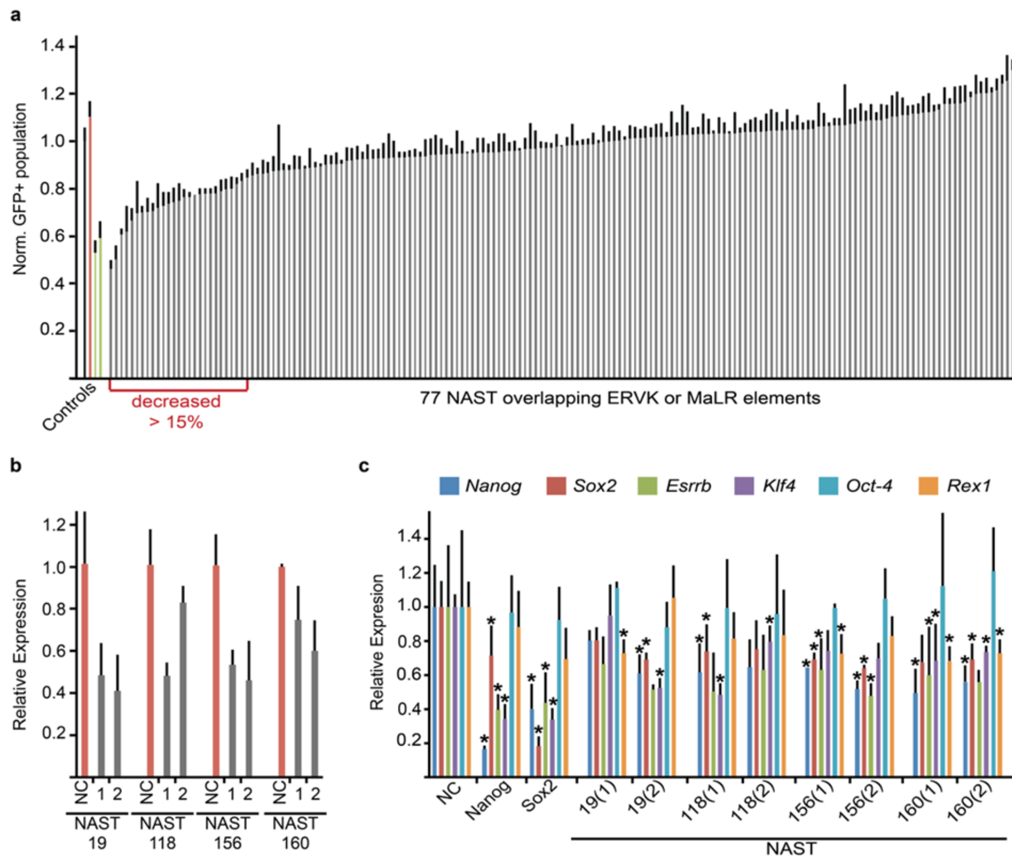

Figure 1: **a**. Normalized GFP positive population, adjusted to the mock control (black), quantified by flow cytometry analysis 48h after transient transfection of siRNA at 20nM. Scrambled siRNA (red), siRNAs targeting Nanog and Sox2 (green). **b**. Knock-down efficiencies measured by qRT-PCR, scrambled negative control (NC, red). **c**. qRT-PCR for stemness marker. Expression values were adjusted to the scrambled negative control (NC). n=3 independent experiments. Error-bars show S-D, P-values * < 0.05.

3.　研究発表等

雑誌論文 計 1 件	（掲載済み－査読有り）　計 1 件 Fort, A., Hashimoto, K., Yamada, D., Voineagu, I., Salimullah, M., Bonetti, A., Keya, C., Saxena, A., Bertin, N., Kratz, A., Noro, Y., Wong, C., De Hoon, M. J., Andersson, R., Sandelin, A., Suzuki, H., Wei, C., Koseki, H., c the FANTOM5, Hasegawa, Y., Forrest, A.R., <u>Carninci, P.</u> (2014) "Deep transcriptome profiling of mammalian stem cells supports a key regulatory role for retrotransposon in pluripotency maintenance," Nature Genetics, doi: 10.1038/ng.2965 (online). （掲載済み－査読無し）　計 0 件 （未掲載）　　　　計 0 件

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会議発表 計 9 件	専門家向け　計 9 件 1. Carninci, P. "The complexity of mammalian transcription," Invited talk at Genomics Core Facility, Guy's and St. Thomas' NHS Foundation Trust, London, United Kingdom, March 4, 2014, Genomics Core Facility, Guy's and St Thomas' NHS Foundation Trust. 2. Carninci, P. "The complexity of mammalian transcription," "Dahlem Colloquia in Molecular Genetics" seminar series, Max Planck Institute, Berlin, Germany, February 25, 2014, The Student Association of Max Planck Institute for Molecular Genetics. 3. Carninci, P. "Identification of novel long-non-coding RNAs that are important for maintenance of iPS," Small RNAs to Stem Cells & Epigenetic Reprogramming Asia-2013 Meeting, Sanjo Conference Hall, University of Tokyo Hongo Campus, Tokyo, Japan, November 25 to 26, 2013. GeneExpression Systems, Inc. 4. Carninci, P. "The complexity of mammalian transcription," Invited talk at Karolinska Institutete Karolinska Institutete, Stockholm, Sweden, November 19, 2013, Karolinska Institutete. 5. Carninci,P. "The complexity of mammalian transcription," Practical Workshop on High-Throughput Sequencing Data Analysis, Main campus and Seaside House at Okinawa Institute of Science and Technology, Okinawa, Japan, September 30 to October 4, 2013, Okinawa Institute of Science and Technology. 6. Carninci, P. "The complexity of mammalian transcription," 2013 RiboClub Annual Meeting, Hôtel Chéribourg, Quebec, Canada, September 23 to 25, 2013, RiboClub Society. 7. Carninci, P. "Complexity of mammalian Transcription…as a paradigm in decision making in science," BrainTrain workshop RIKEN Yokohama, Yokohama, Japan, September 16 to 20, 2013, BrainTrain and Center for Life Science Technologies, RIKEN. 8. Carninci, P. "The complexity of mammalian transcription," The Fukuoka International Symposium on Genomics & Epigenomics 2013 -Expanding Frontiers of Genomics Science, Kyushu University, Fukuoka, Japan, September 10, 2013, Kyushu University. 9. Carninci, P. "The complexity of mammalian transcription," Annual Gentics and Genomics BioConference Live (Webinar) Online, Yokohama, Japan, August 23, 2013, LabRoots, Inc. 一般向け　計 0 件
図　書 計 1 件	Harbers, Matthias and Carninci, Piero (2013) "Noncoding RNA: The Major Output of Gene Expression," Jane Wu (ed.), *Post-transcriptional Gene Regulation: RNA processing in eukaryotes*, Wiley-Blackwell, 181-2013. ISBN 978-3-527-32202-2
産業財産権 出願・取得状況 計 0 件	（取得済み）計 0 件 （出願中）計 0 件
Webページ （URL）	理化学研究所 ライフサイエンス技術基盤研究センター 機能性ゲノム解析部門 http://www.clst.riken.jp/activity/functionalgenomics.html
国民との科学・技術対話の実施状況	内　容: 横浜サイエンスフロンティア高等学校第5回文化祭への出展 （セントラル・ドグマについての3D 映画の放映、遺伝子やセントラル・ドグマについてのパネル展示） 実施日: 2013 年 9 月 21 日&22 日 場　所: 横浜サイエンスフロンティア高等学校
新聞・一般雑誌等掲載 計 1 件	読売新聞朝刊「学ぶ育むわかるサイエンス ヒトゲノム解読苦悩の 10 年」26 頁（2013 年 5 月 5 日）
その他	

様式１９　別紙１

4.　その他特記事項

様式19　別紙2

課題番号	LS127

実施状況報告書（平成２５年度）　助成金の執行状況

本様式の内容は一般に公表されます

１．助成金の受領状況（累計）　（単位：円）

	①交付決定額	②既受領額（前年度迄の累計）	③当該年度受領額	④(=①－②－③）未受領額	既返還額（前年度迄の累計）
直接経費	138,000,000	89,915,000	48,085,000	0	0
間接経費	41,400,000	26,974,500	14,425,500	0	0
合計	179,400,000	116,889,500	62,510,500	0	0

２．当該年度の収支状況　（単位：円）

	①前年度未執行額	②当該年度受領額	③当該年度受取利息等額（未収利息を除く）	④(=①＋②＋③）当該年度合計収入	⑤当該年度執行額	⑥（=④－⑤）当該年度未執行額	当該年度返還額
直接経費	923,337	48,085,000	0	49,008,337	49,006,484	1,853	0
間接経費	0	14,425,500	0	14,425,500	14,425,500	0	0
合計	923,337	62,510,500	0	63,433,837	63,431,984	1,853	0

３．当該年度の執行額内訳　（単位：円）

	金額	備考
物品費	35,212,638	シーケンス用キット、実験試薬、実験用消耗品等
旅費	503,575	海外出張費、国内旅費、赴任費
謝金・人件費等	13,099,647	職員人件費
その他	190,624	会議参加費、発送費
直接経費計	49,006,484	
間接経費計	14,425,500	
合計	63,431,984	

４．当該年度の主な購入物品（１品又は１組若しくは１式の価格が５０万円以上のもの）

物品名	仕様・型・性能等	数量	単価（単位：円）	金額（単位：円）	納入年月日	設置研究機関名
				0		
				0		
				0		