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QBiC QBiTs

NEWSLETTER OF RIKEN Quantitative Biology Center

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Talking With...

To recognize the 2012 Nobel Prize in Medicine, QBiC visited the Center for iPS cell Research and Application (CiRA), which is directed by of this year's winner Shinya Yamanaka. We spoke to two of its principle investigators, **Knut Woltjen** and **Akitsu Hotta**, who both came to Kyoto and joined CiRA in 2010 after being personally recruited by Prof. Yamanaka.

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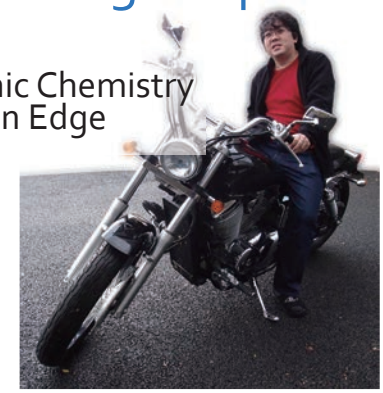

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Talking With . . .

A Canadian, a Japanese, and a stem cell walk into a bar...

Knut Woltjen and Akitsu Hotta talk about stem cells, their big discoveries in Toronto, and collaborations at QBiC

The difference between iPS cells and stem cells

[Knut]

iPS cells are stem cells. 'Stem cells' is a broad term meaning any cell that can do two things: self-renew and give rise to a daughter cell with a specific function. That self-renewal is very important. The epitome of the stem cell is the embryonic stem cell, which can self-renew and, under the right conditions, differentiate to give rise to cells from any part of the body. iPS cells are the embryonic stem cell equivalent. Functionally they are the same. However, their derivation differs in that embryonic stem cells come from the early embryo, whereas iPS cells are artificially created from somatic cells.

First time in Japan

[Knut]

It was during my Ph.D in 2001. I was working with one of our collaborators, Terohisa Susuki. My supervisor in Calgary, Derrick Rancourt, and Tehohisa were postdocs together. I didn't know anything about Japan. Couldn't speak the language or anything. I was here for just a couple of weeks and I loved it. People were great. I felt really comfortable. Living seemed really easy. Every modern convenience is here. It's just in Japanese.

Their discoveries during their post-doctoral work in Toronto

[Akitsu]

I was looking for postdocs somewhere outside Japan for a long time. I wanted to go abroad. My Ph.D. project was working with retroviral vectors in chick embryos. I wanted to know the molecular mechanism behind the transduction silencing of viral vectors. I was looking at papers that were doing mouse ES cell research using retroviral vectors and came up with papers from James Ellis' group who specializes with gene therapy using stem cells. *Before that, did you have experience with stem cells?* No, never. James' lab had been working with mouse ES cells extensively. My project was developing a reporter vector to specifically mark undifferentiated ES cells.

> *"We whipped it off in about a year. We had the paper published. It went really quick."*

I started the project in 2006. Soon after, Yamanaka published his iPS cell paper. End of 2007 we started our own iPS induction experiment independent of Yamanaka. At that time I was testing my own reporting vector. I wanted to see if it becomes active after induction of pluripotency. We could publish about the reporter vector to specifically mark the pluripotent program of iPS cells not only in mouse but also in humans.

[Knut]

When I first came to Toronto I was doing gene targeting, mostly tool development, working with recombinases. How we started the iPS cell work is kind of funny actually. I came in 2006 and we had [Yamanaka's] paper posted outside the postdoc office. And it just kind of sat there for a year and a half. We never thought to do it ourselves until one day Andras [Nagy] came back from a meeting at [the Wellcome Sanger Institute]. He was meeting with Pentao Liu who was working with *piggyBac* transposons. Andras got

Stem Cells at QBiC

Chikara Furusawa, with colleague Kunihiko Kaneko, has recently written a review to explain why some cells have only the potential to proliferate, whereas other cells, namely stem cells, have the potential to proliferate and differentiate. The article details how Waddington's epigenetic landscape can be extended to show that whereas a differentiated cell remains in a fixed energy well, stem cells can vacillate between many. In particular, the article introduces the reader to how stem cells have the paradoxical properties of remaining robust to perturbations, allowing them to remain in the proliferation state, and yet be adequately sensitive to signals that trigger a new cell type, thus taking the differentiation state. The review appears in an October issue of *Science*.

a hold of the transposons and asked, 'Why don't we try to use transposons to make iPS Cells'? He sketched it all out on a napkin and he brought it to the lab meeting. He was all excited. 'Who wants to try this'? Everyone was silent but I was like 'I want to do this. It sounds totally cool'. *Why didn't anyone else volunteer?* I don't know. Probably because it was more work. We whipped it off in about a year and had the paper published. It went really quick.

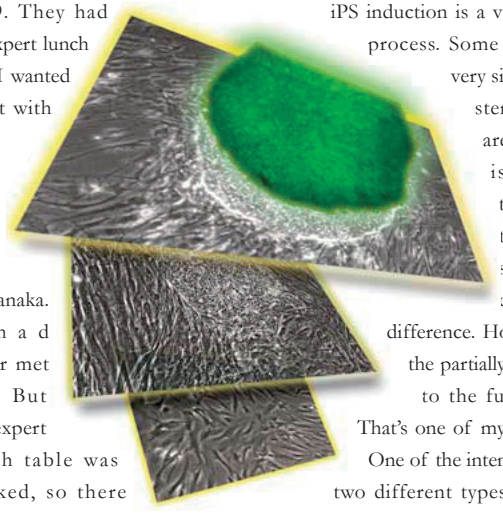
Their first encounter with Yamanaka

[Akitsu]

I had no connection to the stem cell community in Japan. I don't exactly remember when I met [Yamanaka]. James met with Shinya first. James had a chance to present my postdoc work in front of Yamanaka and at the end of the acknowledgement slide mentioned my name with my picture. Yamanaka asked James, 'If your post-doc is interested in coming back to Japan, please contact me.' I got an interview together with Knut.

[Knut]

I had always entertained the idea of coming back to Japan to do research. But I always figured getting a PI position would be tough as a foreigner. I was at the ISSCR meeting in Barcelona, 2009. They had an expert lunch and I wanted to sit with Yamanaka. I had never met him. But the expert lunch table was booked, so there was no way to get at his table. At the end of the lunch I introduced myself very primitively in Japanese. And he says to me in perfect English, 'Oh, you're Knut, I wanted to talk to you. We are setting up an institute and have openings for junior investigators. You should apply.' I came [to CiRA] for an interview at the same time as Akitsu and within a week I got the phone call, 'When can you be here'?



Akitsu's collaboration with QBiC

[Akitsu]

I met with Tomo [Watanabe] at the Sakigake program [a prestigious Japanese grant for young scientists]. We both got a grant at the same time in the iPS field. I am an iPS expert; he's a microscopy and measurement expert. My proposal is related to nuclear architecture by high-resolution microscopy, kind of related to imaging. I provide the cells and some materials to him, and he is going to do the imaging and measurement. That's the plan. But he's been busy building his own lab. Things are moving slow. We just provided our materials a month ago. *What's the goal of the collaboration?* The project aim is the iPS induction is a very heterogeneous process. Some iPS cells become very similar to embryonic stem cells, but some are not. My interest is by comparing these cells to see the molecular signature; determine the difference. How can we convert the partially programmed cells to the fully formed state? That's one of my research interests. One of the intentions is to measure two different types of iPS cells and know the differences between them.

> *"Yamanaka asked James, 'If your post-doc is interested in coming back to Japan, please contact me.'"*

Particularly in the context of the nuclear architecture, because reprogramming is all about epigenetic events. Knowing the difference is one goal, and once we know the difference probably we will try to convert the cell to the other state. I don't have a good way to measure it. I can do gene expression analysis, but I want to do something more fancy. His system is very attractive.

General thoughts on the field, science and Japan

[Knut]

Nobody knows what's going on still. We have somatic cells and iPS cells. We can use these iPS cells as embryonic stem cell equivalents. How they got to that point is a black box, but it's clearly a reproducible process. We know about epigenetic changes that result in gene expression changes. But the key changes that must occur are still very poorly defined.

North Americans tend to relate more to Europe than to Asia. Even though there is fantastic research going on in Japan. Even though major discoveries - iPS cells, Nanog - just within the stem cell field - Oct 4 - were all made in Japan. Medical science and biological science in Japan is really good. Really good! The really meaningful publications in a lot of biological subfields have Japanese researchers on them.

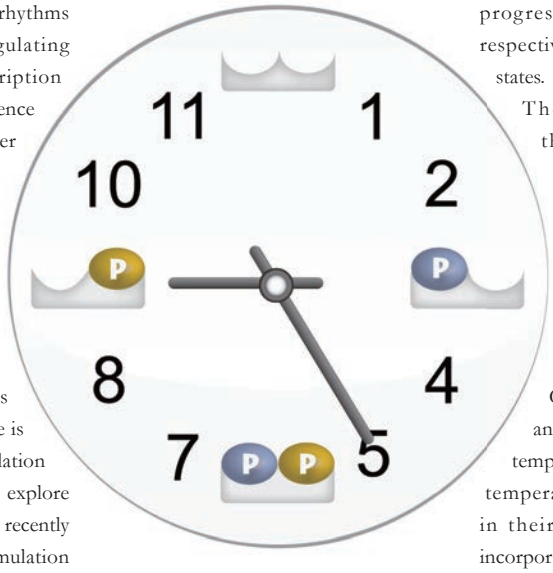
I am definitely a strong supporter of people moving into new environments. Not just from a research perspective, but also from a cultural or a geographical perspective. It helps build your own personality. It helps you learn new things. I would encourage any of my students or postdocs to do the same thing. You're never going to forget what you had done before. You're only going to learn new things and improve from the experience.

Paper Highlight

The Laboratory for Synthetic Biology has designed a model that suggests post-transcriptional regulation is sufficient for oscillating behavior

Much of our behaviour and physiology varies with the time of the day. Our sleep habits, our body temperature, even our hormone secretion cycle 24 hours. These circadian rhythms are a staple of life and their disruption can cause several disorders and syndromes. For years it had been generally thought that these rhythms are mostly governed by regulating gene expressions via transcription and translation. Growing evidence has shown, however, that other factors, like post-translational regulation, come into play. In fact, one type of post-translational regulation, phosphorylation, has been found sufficient for regulating the circadian clock, at least in cyanobacteria. Yet, because this is an only recent discovery, little is known about how phosphorylation controls circadian rhythms. To explore this matter, the Hiroki Ueda lab recently published in *Cell Reports* a simulation study first authored by QBiC's Koji Ode and Craig Jolley, a member of the Riken Center for Developmental Biology, that suggests a kinase, a phosphatase and a substrate with two phosphorylation sites are sufficient to create oscillatory behaviour. Their post-translational oscillator (PTO) requires no gene expression, and therefore gives insight on a new way to control circadian rhythms.

Craig explains how Hiroki Ueda approached him about the project. "He asked me in his own way. He suggested I might want to consider this, which means 'Drop everything and do this'". The authors began by considering the simplest condition possible for oscillations. Koji showed that oscillations can be achieved if a substrate has only two phosphorylaton sites and therefore four states: one when neither site is phosphorylated, one site is, the other site is, and both sites are (see image). Two phosphorylation



sites means 16 parameters: 8 reaction rates and 8 binding constants. Using reaction rates typical for MAP kinase, they implemented a clustering algorithm and found two clusters that showed unidirectionality and sequestration. Unidirectionality is almost mandatory for any circadian clock. Without a preferred direction, the system can reverse its path suddenly, meaning no steady period and therefore no steady oscillation. The key to achieving this property was the very fast reaction rates in one direction and slow ones in the other. Sequestration is crucial, because it provides a way for autonomous agents to communicate their state, in effect creating checkpoints. No communication means no synchrony, so that while cells may oscillate they do so independently of one another to create an incoherent system. Similar to the reaction rates, the binding constants were selected so that the states where neither or both sites are phosphorylated progress more slowly to the next respective states than the other two states.

The two also had to make their simulation robust to temperature. Although PTOs depend on reaction rates, which are inherently temperature dependent, circadian clocks are robust to temperature. Very recently, a temperature insensitive kinase, CKIε, has been reported. Koji and Craig, therefore, assumed a temperature insensitive kinase and temperature sensitive phosphatase in their model. The challenge to incorporating this additional constraint on the simulation had less to with the physics or biology than raw computation power. Consequently, the number of parameter sets searched to find the aforementioned clusters increased from millions to billions. Nevertheless, they successfully showed that their simple PTO is indeed temperature robust.

The trouble with simulations, though, is that without any biological evidence, they are nothing but speculation on how nature works, not evidence. The next challenge then is to "find [a PTO] or make one". (10.1016/j.celrep.2012.09.006)

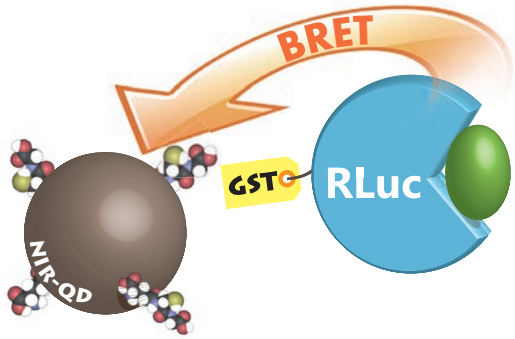
QBiC symposium

Last November, QBiC held its inaugural symposium, Toward Whole Cell Modeling. Over 300 people attended the three-day event in Kobe. Because this was QBiC's first formal opportunity to share its research to an international audience, both RIKEN President, Ryoji Noyori, and former Osaka University President and philanthropist, Tadamitsu Kishimoto, gave introductory remarks about the vision and future of the institute. Half of the 25 speakers represented foreign universities, institutes, and companies. Michael Sheetz, Director of the Mechanobiology Institute at Singapore (MBI), and Adrian Elcock, Professor at the University of Iowa, gave the keynote speeches. The two examined intracellular decision-making, but from different approaches – one experimental and one mathematical. On the last day, Group Leader Makoto Taiji gave a tour to all the speakers of the K computer, something to the delight, if not highlight of the many visiting computational biologists. There was also much discussion outside the talks about creating and sustaining long-term collaborative relationships, including some preliminary talks between Michael Sheetz and QBiC Director Toshio Yanagida about such a program between our respective institutes.

Briefs

BRET technology

Although fluorescence resonance energy transfer (FRET) is more common, bioluminescence resonance energy transfer (BRET) is fast becoming a popular technique for in vivo imaging due to its inherent advantages. BRET systems involve conjugating a luciferase (luc) with a quantum dot (QD). Although BRET may be preferred for certain imaging studies, the most common conjugation process, carbodiimide coupling, can be tedious. A report in *Chem Comm* from the Takashi Jin lab and first authored by Miyuki Hasegawa describes a new method that uses gluatathione-s-transferase (GST) and glutathione (GSH) to significantly simplify the conjugation. The authors take advantage of the affinity between GST and GSH to achieve easy purification of the luc-QD complex. Their BRET ratio, 1.5, was found to surpass that achieved with the carbodiimide method. As a final step, they tested the feasibility of their BRET system in mice, finding it shows great promise for in vivo imaging. The group is now considering proteins other than luc for their system. (10.1039/C2CC36870F)



Nucleosome Dynamics

Nucleosomes describe the structure of DNA-wrapped histones and are normally folded into 30 nm chromatin fibres. It was recently discovered, however, that this structure is not seen in mitotic chromosomes, suggesting instead that during mitosis nucleosomes take a polymer melt-like structure that allows them to be moving constantly in a tight region. In partnership with the Japan National Institute of Genetics and Riken ASI, the Koichi Takahashi lab has published in *Cell Reports* insights on how local dynamics of such nucleosomes regulate genome function. Kazunari Kaizu, who shared first authorship, contributed a Monte Carlo simulation to the comprehensive study that also applied fluorescence correlation spectroscopy and single molecule imaging. Nucleosomes are connected to one another by DNA linkers, which can act like springs limiting how far the nucleosomes can move, but giving them significant freedom inside that range. The result is a melt-like structure taken by the nucleosome that facilitates protein mobility and chromatin accessibility, and therefore could have large implications in fundamental DNA processes like repair, replication and recombination. (10.1016/j.celrep.2012.11.008)

Interesting People

Katsuhiko Matsumoto of the Hiroki Ueda lab, QBiC's own Easy Rider

Katsuhiko Matsumoto was sitting with his unopened bento to his side. He begins by speaking about how he first came to Hiroki Ueda's lab, starting with his undergraduate period at Nihon University in Fukushima. Fukushima still strikes thoughts of 2011's nuclear disaster, but Katsuhiko assures that he and all his friends had left well before then. At that time, he had already arrived at Kyoto University for his Ph.D. studies. "I was always interested in genome mutations", he says, which seems odd considering that he views himself an organic chemist. His relationship with the Ueda lab first formed midway during his Ph.D. studies when his mentor, Kazuki Tainaka, joined the group to make a chemistry synthesis room. From then on, Katsuhiko would travel from Kyoto to Kobe to attend monthly meetings, which eventually led to his first post-doctorate here last April.

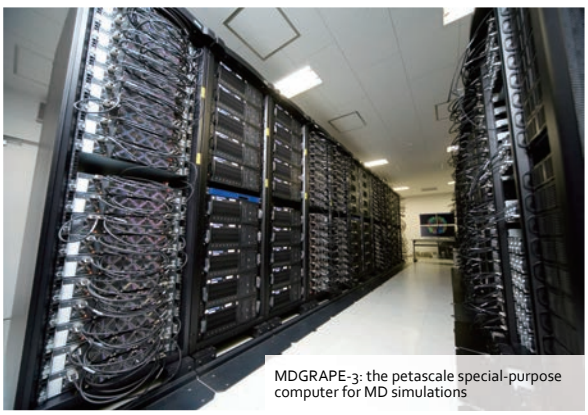
He talks about his research, the techniques that he uses and the clinical problems he hopes his methods will eventually solve. He speaks only of his work, not because he likes it, but because he spends almost all his working hours in the lab leaving him little else to discuss. His hands are in constant pantomime, but his elbows remain ensconced on the table. A long silence breaks out after he finishes. It's already been 30 minutes and there is nothing that would satisfy us calling Katsuhiko an "interesting person at QBiC". At this point he could step away from the table or open his lunch, but he sits staring as though there is something more he wants to add. Then, in a very timid voice he says, "I like motorcycling". And suddenly we have a winner! Turns out this organic chemist has his own Bruce Wayne/Batman complex: riding the train and wearing a white labcoat by day, but then dawning a black leather jacket and screeching through the streets at night (or at least the nights he is not sleeping at the lab). Motorcycling began after his graduation from high school. Uncharacteristic of most researchers, Katsuhiko did not immediately matriculate into university, instead spending three years drifting between part-time jobs at convenience stores, pizzerias and game centers that were "not interesting, because I couldn't play". That is not to say he did not like studying. Quite the contrary, he spent these years independently discovering his academic interests and concurrently preparing for entrance exams. It was also during this time he first started his two-wheel tour of the country. "Okinawa is my favorite, because of the beautiful sea". To get his bike to those southern islands, Katsuhiko took a ferry for two days, rode the roads for three, and then returned again by boat. He still has yet to ride either Kyushu or Hokkaido, which according to Katsuhiko is the most popular destination for bikers because of its size and scenery. His lone foreign bike ride was in Hawaii, where he rented a Harley. "I normally ride other bikes, but in America I had to go with the Harley ". Of all these trips, however, it was the one from Tottori to Fukushima, a 40-hour ride that Katsuhiko recalls most nostalgically. Katsuhiko normally camps when travelling overnight, as it offers a rare chance for social interactions while motorcycling. This time, though, he intended to catch a little snooze on park benches along the way just for a change of pace. Unfortunately, that left him extremely unprepared for the heavy rain. As a result, he just kept riding and riding until he arrived at home without any break. "It's difficult to explain why, but bikers are always battling nature. I didn't want to lose to the rain". He found himself improvising on the road, as 40-hour rides require a slightly different technique of concentration than that recommended by most driving schools. "I was asleep half of the time". Arriving at home, wet and tired, he found himself bustling around the house. After such a long, arduous ride, his mind would not allow him to sleep. "I was too excited".

> *"I normally ride other bikes, but in America I had to go with the Harley."*

Meet the QBiC Lab...

Makoto Taiji

The goal of the **Computational Molecular Design Research Group** led by Makoto Taiji is to predict the structure of drugs, peptides, and proteins by computer simulations, especially by molecular dynamics (MD) simulations. One fundamental challenge is the different scales of analysis. Consider, atomic motions of a molecule occur on the order of femtoseconds, while protein folding or ligand binding occur on much longer timescales of milliseconds. The group is therefore using the K computer, a 10-PFLOPS supercomputer at RIKEN, and are developing MDGRAPE-4, the latest version of customized supercomputing hardware that has become an international standard for MD simulations. Although these technologies are available to scientists of all fields including fluid dynamics and astrophysics, a greater aim is to expand and enhance the application of high-performance computing into the life sciences, with special interest in drug design.



MDGRAPE-3: the petascale special-purpose computer for MD simulations

Awards

Junya Yamagishi, a Ph.D. student in the Taiji lab, was awarded top prize last September at the 21st annual Meeting of the Japanese Society for Histocompatibility and Immunogenetics for his talk on prediction models for the interaction between major histocompatibility complexes (MHC) and the bovine leukemia virus (BLV). BLV has a relatively long peptide, which complicates most mathematical models. Junya's model not only overcomes this problem, but also considers the contribution of water, an important but commonly ignored factor in protein-protein interactions. His collaborator, Yoko Aida of the Riken Advanced Science Institute, was able to confirm Junya's predictions experimentally.

Future Finnish Collaborations?

This past October, Tsutomu Masujima gave the first invited talk at Nanoscience Days 2012 at the University of Jyväskylä, Finland, where he discussed his work on live single-cell nano-mass spectroscopy (SCNMS). Tsutomu was introduced to Jyväskylä last year when a delegation visited various Riken sites, including QBiC. At that time, Tsutomu spoke about his research and vision with Professor Aino Sallinen, President of the University, who then invited him onto Jyväskylä's Nanoscience Center's Advisory Council. Tsutomu is now preparing a Memorandum of Understanding to facilitate more interaction between the two centres. He has already invited one Jyväskylä professor to QBiC to learn his SCNMS technique and is in discussion with another on a grant from the Human Frontier Science Program.

Japan near top in *Science* publications

On Halloween, Science Senior Editor and Science Signaling Cofounder, Bryan Ray, came to the Kobe site of QBiC to discuss how papers are reviewed by Science. There were very little surprises in his recommendations, emphasizing that the research should ask important questions and seek to intersect different fields. Midway through the talk, Dr. Ray argued his dislike for impact factors and how easily they can be skewed. Much of the second half gave attention to prospective authors, who should, he argued, use their cover letter to articulate the significance of their work, and even be willing to contact editors directly. "You're not bothering us when you e-mail or call". His penultimate slide listed the number of Science publications in 2010 by nation, the most recent data he had available. At 42, Japan was one of only four nations to reach 30 publications that year.

The Boss' Birthday

In October, several members of QBiC celebrated Director Toshio Yanagida's birthday by joining him in Shiga. The day started at a pottery store, where everyone had the opportunity to get their hands dirty and make a piece of art.

After objective evaluation, all agreed the boss' stood above the rest. The group then had lunch at a nearby Japanese restaurant before heading to Miho Museum, known both for its domestic and foreign archaeological artifacts as well as its rustic surroundings. It is a place Yanagida has visited before, as many visiting scientists have asked to go.. Despite the cultural experience at Miho, for most participants the highlight of the day was at a complex that offered more contemporary culture – a new outlet mall. The day finished with nary a birthday cake, but the boss nevertheless went home very happy.

The newcomers to the QBiC

Me: Florent Seichepine
Lab: Team Frey
Hobbies: Rock Climbing, Hiking
Cheers:
HANSHIN TIGERS

Me: Tomotaka Komori
Lab:Team Yanagida
Hobbies: Fishing
Cheers:
HANSHIN TIGERS

Me: Hazuki Kotani
Lab:Team Furusawa
Hobbies: Anything with food
Cheers:
Hokkaido Nippon Ham Fighters

A Nobel Thought

"I wasn't disciplined. I did drop out of school for about three months."

Steven Chu
1997 Nobel Prize Winner Physics

THE CHOW DOWN

LAMB CHOPS WITH ORANGE

Ingredients (for 2)

4 Lamb chops (or spareribs)
15 g salt (for marinade)
60 g marmalade
3 g chili bean sauce (Dou ban jiang)
10 cc soy sauce
30 cc Cointreau (or brandy)
30 cc olive oil
1 piece garlic
1 onion
30 g ginger
Salt and pepper (for seasoning)
400 cc water
1 orange

1. Rub salt on lamb chops.
2. Mix marmalade, chili bean sauce, soy sauce, Cointreau, olive oil and grated garlic.
3. Rub (2) onto (1) and marinate for 30 min.
4. While waiting, remove and retain the orange peel. Cut 4 slices of the orange and put to the size. Take the juice of the remaining orange.
5. Chop onion and ginger. Lay them and (3) on a pot, add salt and pepper and water, and cook over low heat.
6. After 15 min, add 3/4 of the squeezed orange juice and half of the orange peel. Cook for 10 min or more.
7. Pick up the lamb chops and continue cooking the sauce for another 2-3 minutes to reduce its volume.
8. Return the lamb chops back into the pot and mix with the sauce and remaining orange juice.
9. Serve with orange slices (and turmeric rice).

Bon appetit!

Have a recipe you want to share, contact us at:
qbits@riken.jp

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