Mass spectrometry-based absolute quantification reveals rhythmic variation of mouse circadian clock proteins

报告人：亓慧敏

技术和方法：

cell-free system（非细胞体系）：来源于细胞，而不具有完整的细胞结构，但包含了进行正常生物学反应所需的物质（如供能系统和酶反应体系等）组成的体系。

PURE system：consists of purified factors and enzymes for E. coli translation machinery, synthesized peptides are rarely challenged by protease degradation that usually occurs in cell-extract systems. Additionally, isotope scrambling or dilution is avoided without the need to adjust system components .

- 质谱法（MS）：用电场和磁场将运动的离子按其质荷比分离后进行检测的方法，可测出待测物的化合物组成。

- 液相色谱-质谱联用(LC-MS)：液相色谱与质谱的联用，优点非常显著，大大拓宽了分离范围。

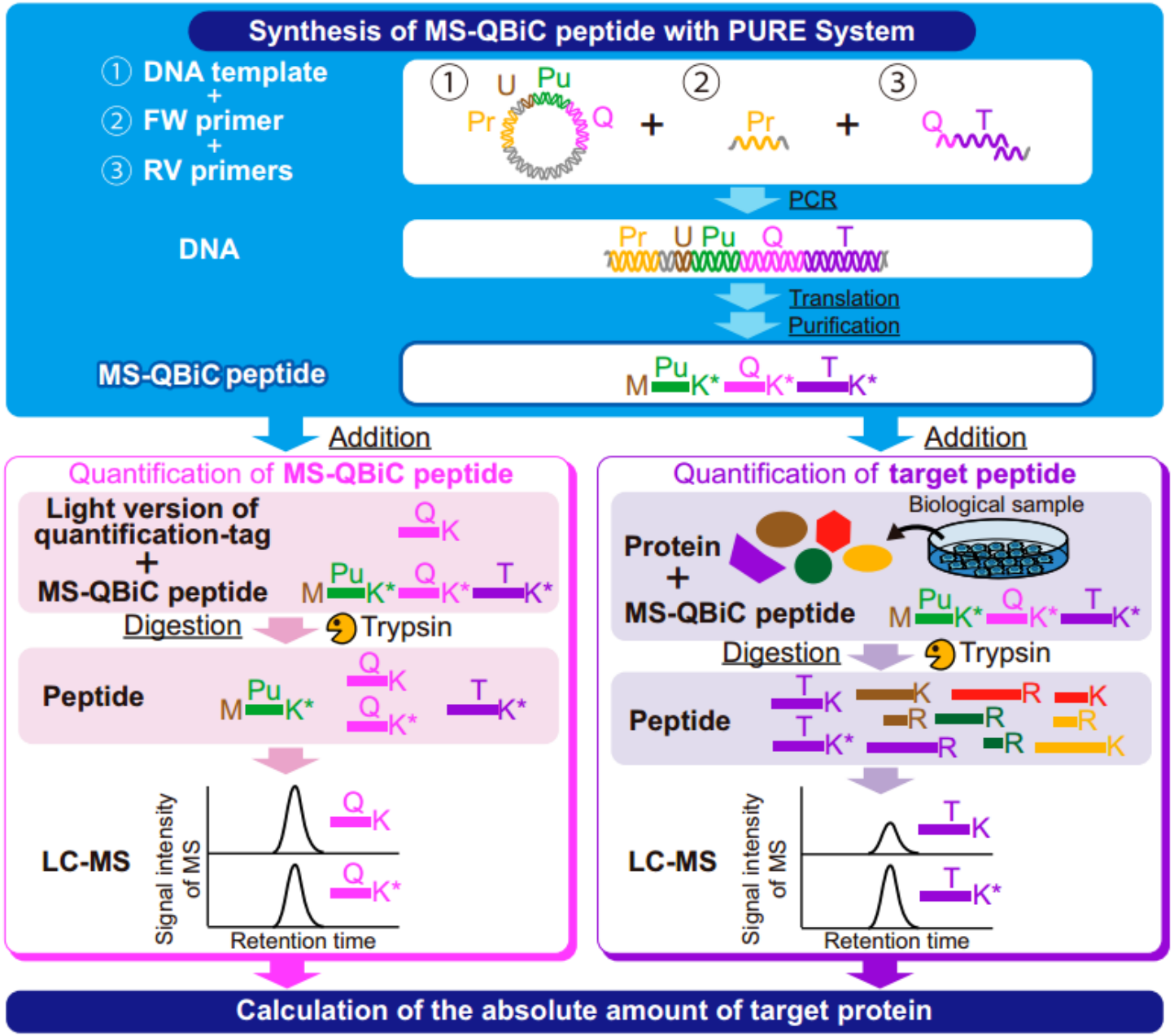
- MS-QBiC：将同位素标记的非细胞体系的产物进行质谱定量的方法。

- 离子交换色谱法（SCX）是利用离子交换原理和液相色谱技术的结合来测定溶液中阳离子和阴离子的一种分离分析方法。利用被分离组分与固定相之间发生离子交换的能力差异来实现分离。

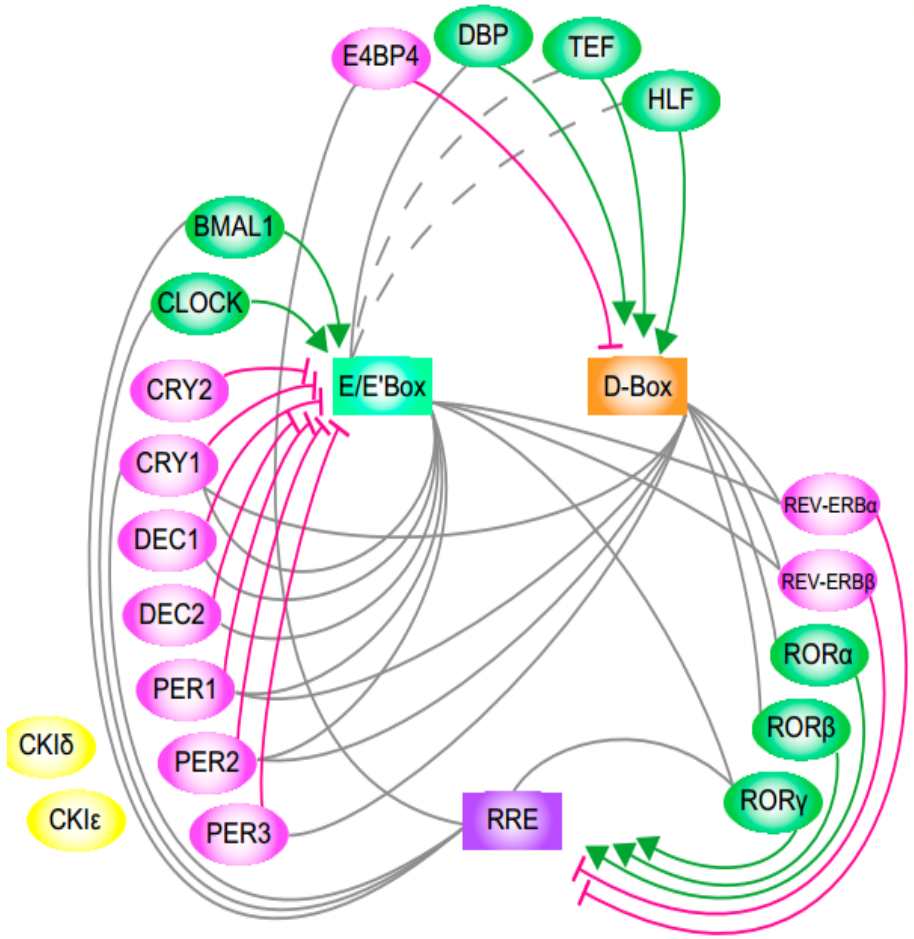
内标法是一种间接或相对的校准方法。在分析测定样品中某组分含量时，加入一种内标物质以校准和消除出于操作条件的波动而对分析结果产生的影响，以提高分析结果的准确度。

理想地说，内标物应当是一个能得到纯样的已知化合物，这样它能以准确、已知的量加到样品中去，它应当和被分析的样品组分有基本相同或尽可能一致的物理化学性质、色谱行为和响应特征，最好是被分析物质的一个同系物。当然，在色谱分析条什下，内标物必须能与样品中各组分充分分离。

MS-QBiC workflow

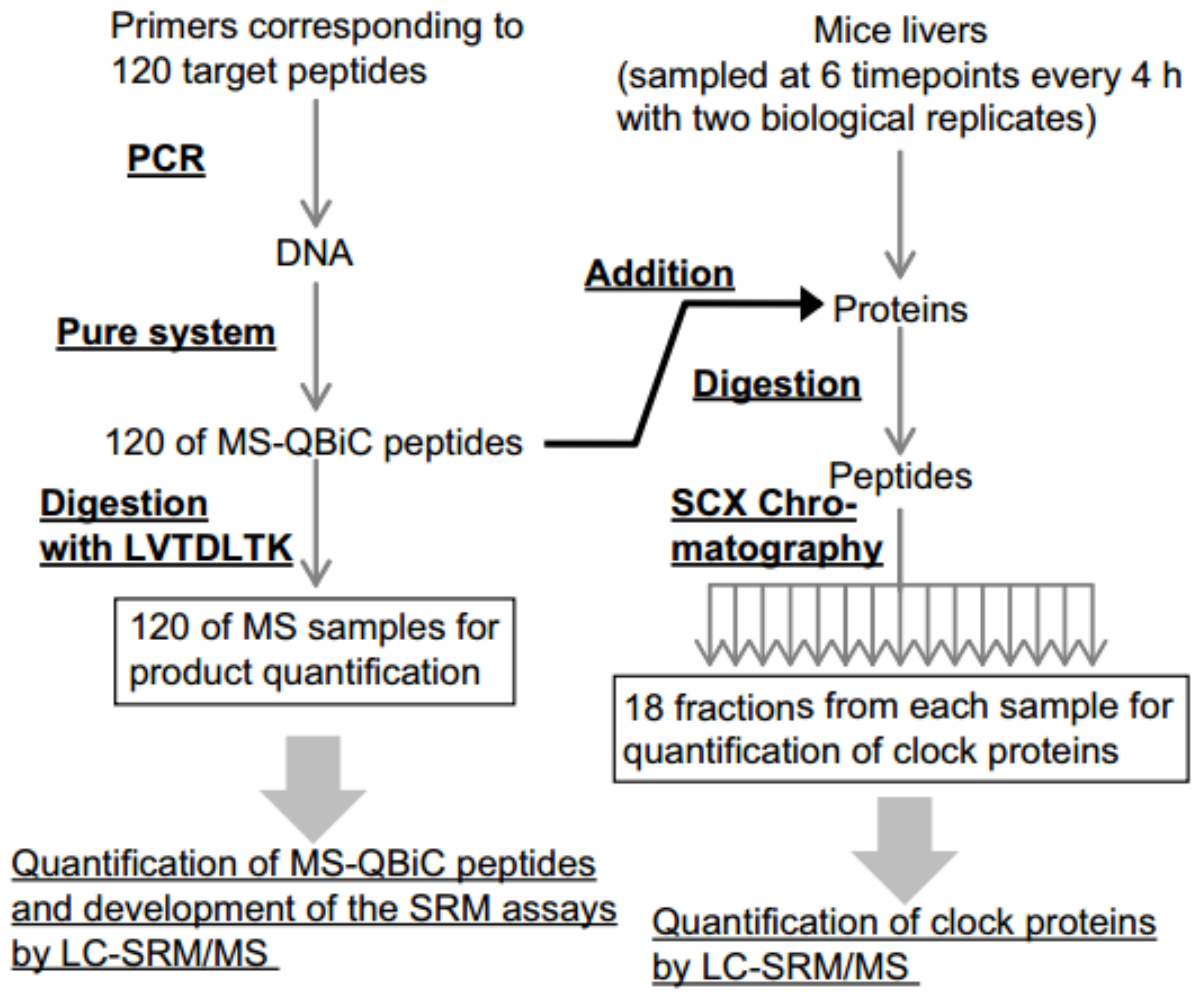


The transcriptional circuit of the mouse circadian clock



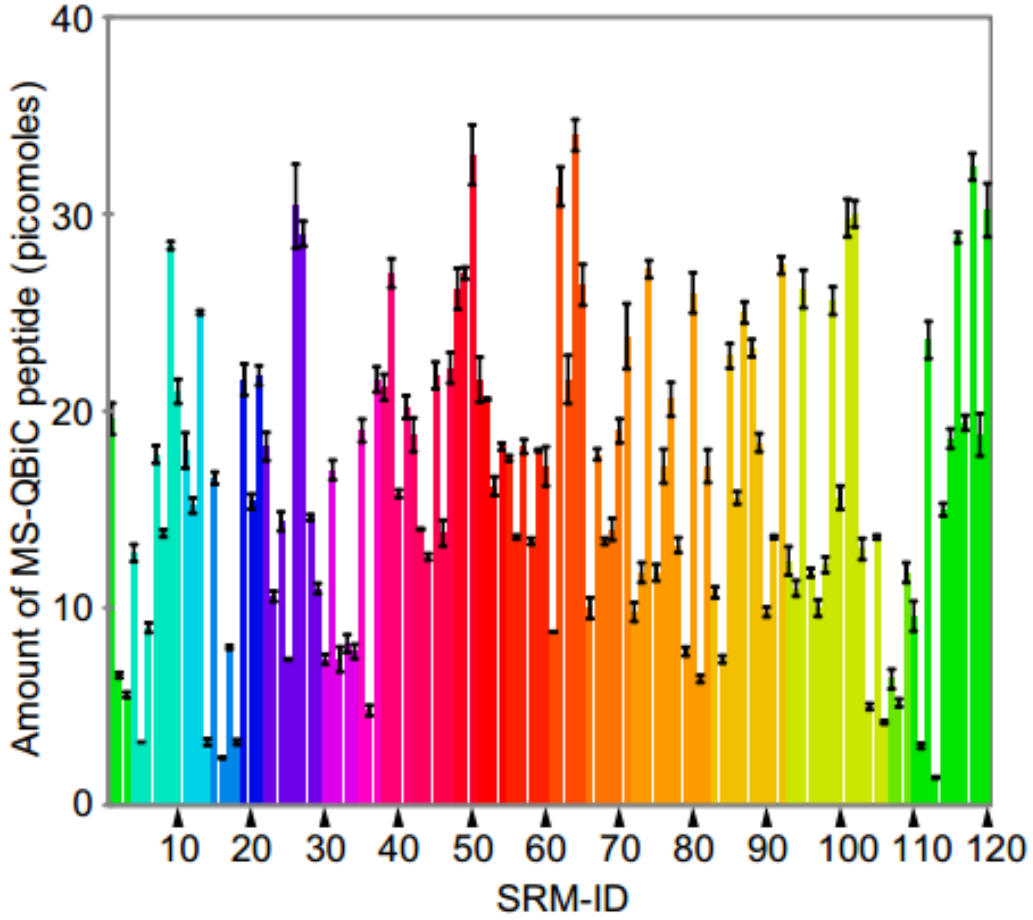
Target protein	Target peptide (Detected peptide)
BHLHE40 (DEC1)	3 (2)
BHLHE41 (DEC2)	8 (0)
BHLHE40/41 (DEC1/2)*	3 (1)
BMAL1 (ARNTL)	4 (3)
CLOCK	3 (3)
CRY1	8 (4)
CRY2	5 (2)
CKIδ	3 (3)
CKIε	4 (3)
CKIδ/ε*	3 (3)
DBP	3 (2)
TEF	3 (2)
DBP / TEF*	1 (1)
HLF	3 (0)
NFIL3 (E4BP4)	6 (2)
NR1D1 (REV-ERBα)	5 (3)
NR1D2 (REV-ERBβ)	5 (3)
NR1D1/2 (REV-ERBα/β)*	1 (0)
PER1	12 (3)
PER2	10 (3)
PER3	14 (3)
RORα	3 (0)
RORβ	8 (0)
RORα/β*	1 (0)
RORγ	2 (1)
Total	120 (47)

experimental workflow :

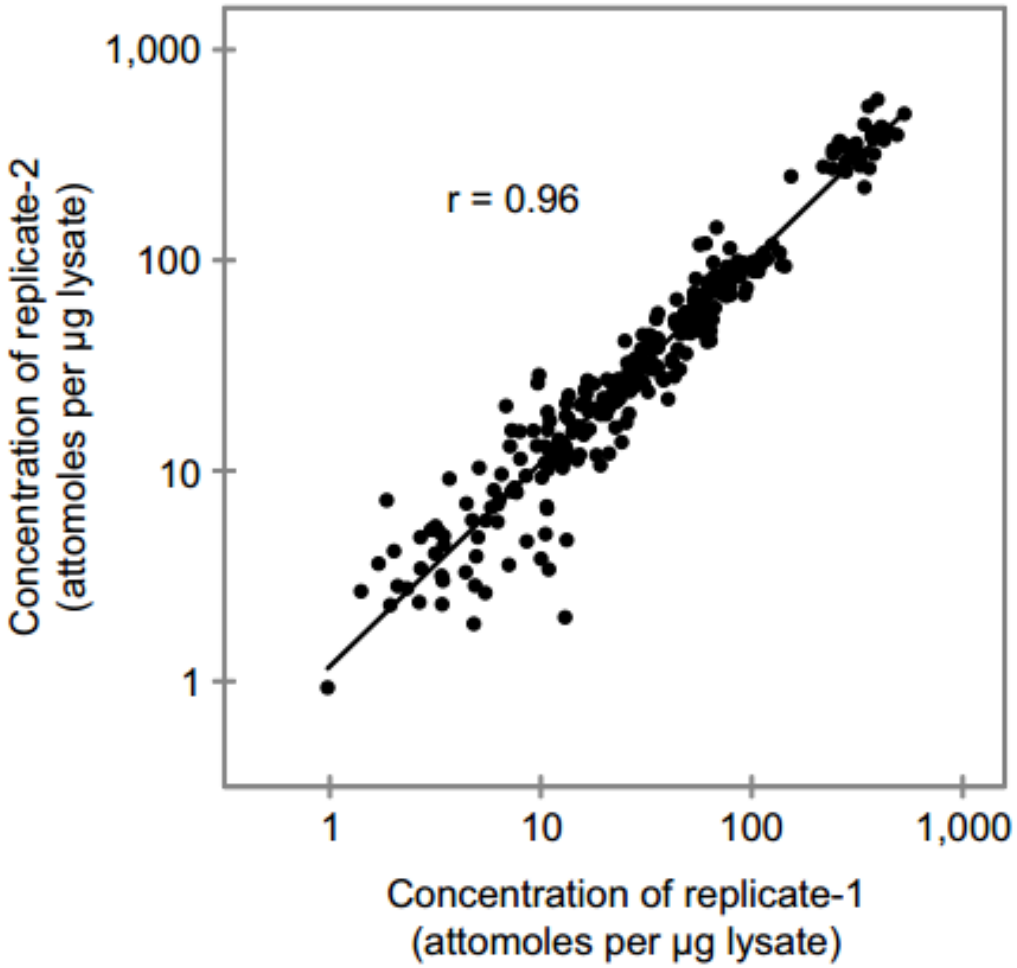


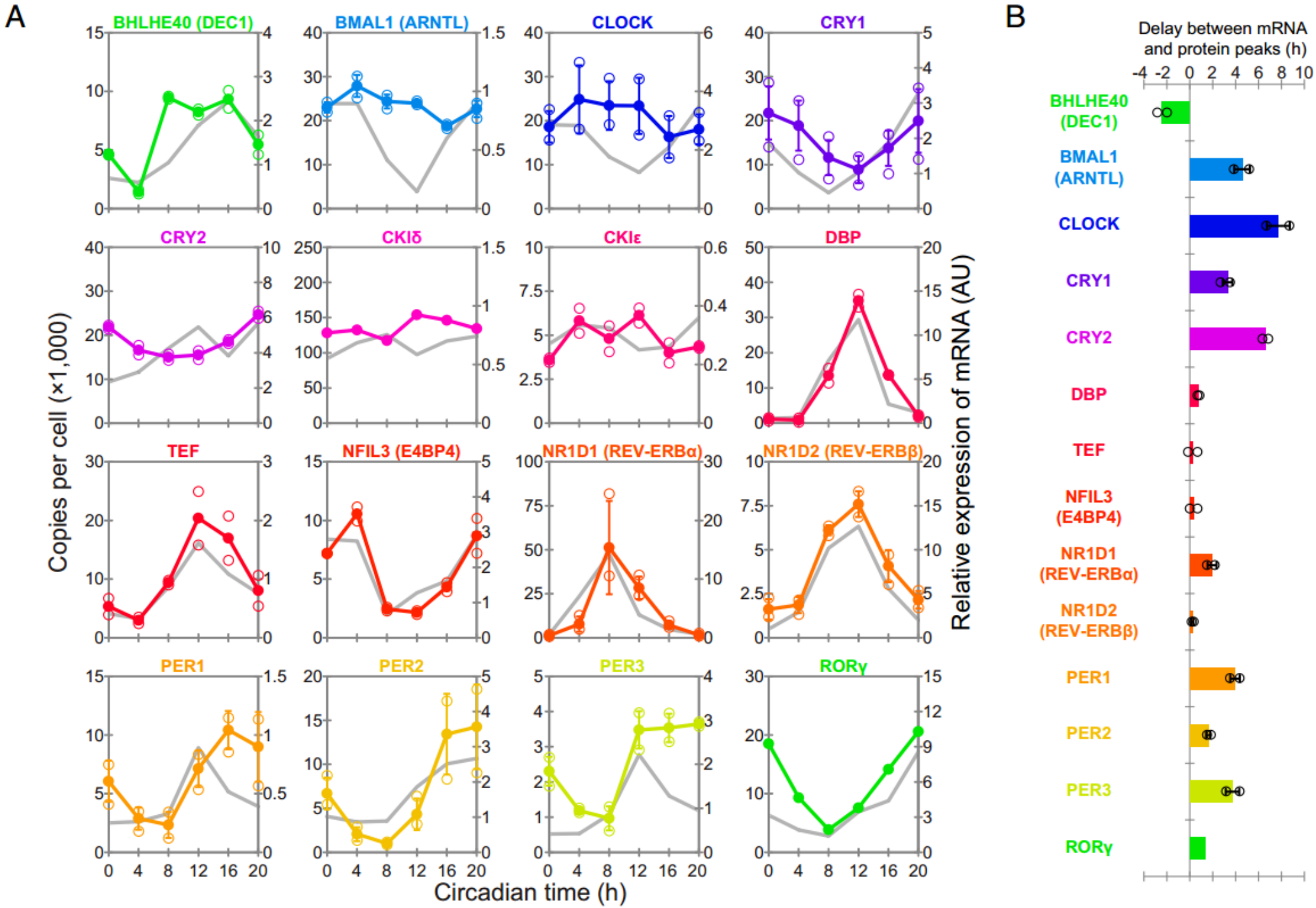
Target protein	Target peptide (Detected peptide)
BHLHE40 (DEC1)	3 (2)
BHLHE41 (DEC2)	8 (0)
BHLHE40/41 (DEC1/2)*	3 (1)
BMAL1 (ARNTL)	4 (3)
CLOCK	3 (3)
CRY1	8 (4)
CRY2	5 (2)
CKIδ	3 (3)
CKIε	4 (3)
CKIδ/ε*	3 (3)
DBP	3 (2)
TEF	3 (2)
DBP / TEF*	1 (1)
HLF	3 (0)
NFIL3 (E4BP4)	6 (2)
NR1D1 (REV-ERBα)	5 (3)
NR1D2 (REV-ERBβ)	5 (3)
NR1D1/2 (REV-ERBα/β)*	1 (0)
PER1	12 (3)
PER2	10 (3)
PER3	14 (3)
RORα	3 (0)
RORβ	8 (0)
RORα/β*	1 (0)
RORγ	2 (1)
Total	120 (47)

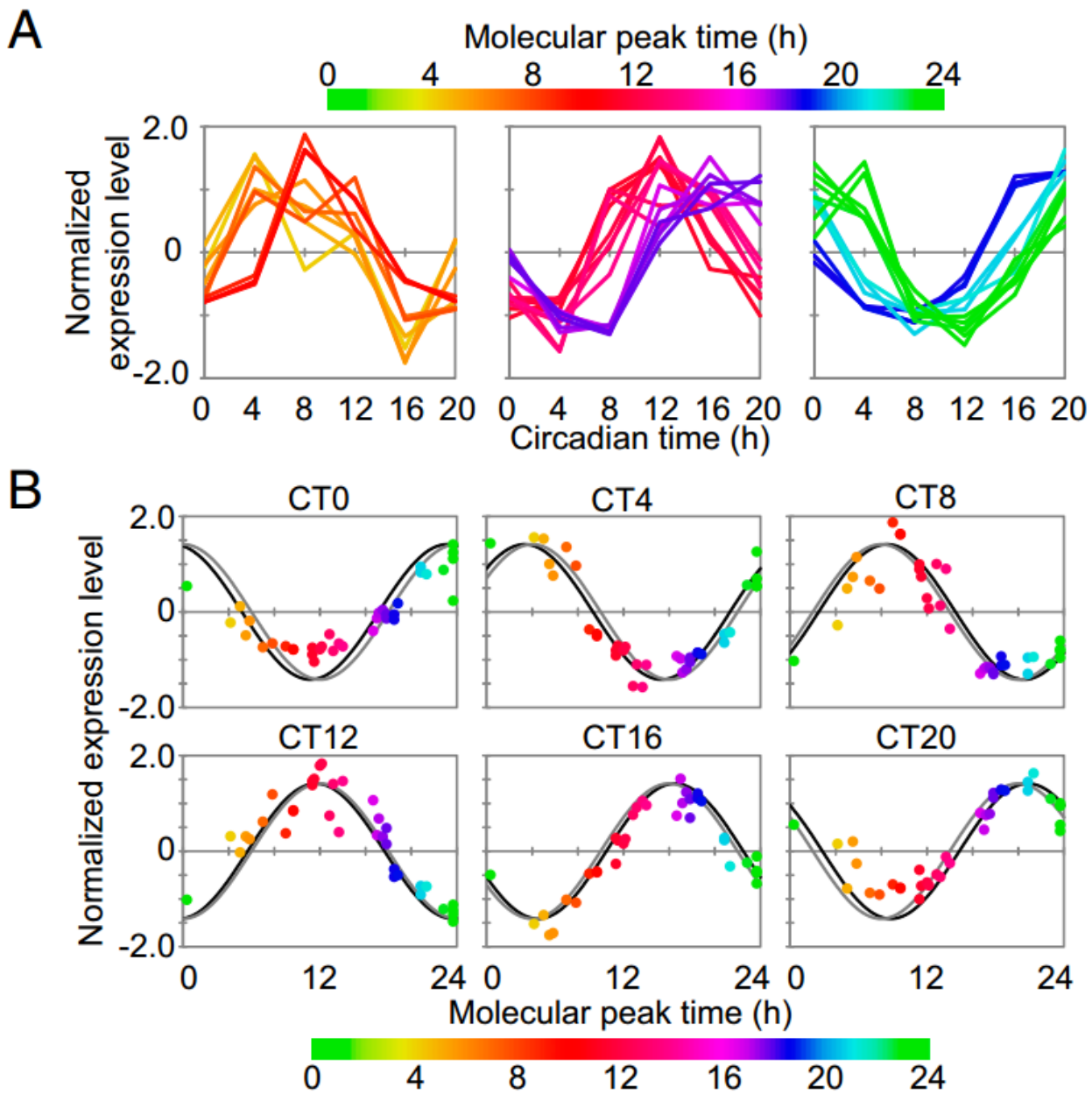
Yields of 120 MS-QBiC peptides synthesized in the PURE system.



Comparison of the quantified values between two biological replicates.




A
BHLHE40 (DEC1)
BMAL1 (ARNTL)
CLOCK
CRY1
CRY2
CKIδ
CKIε
DBP
TEF
NFIL3 (E4BP4)
NR1D1 (REV-ERBα)
NR1D2 (REV-ERBβ)
PER1
PER2
PER3
RORγ
Copies per cell (×1,000)
Relative expression of mRNA (AU)
Circadian time (h)
B
Delay between mRNA and protein peaks (h)
-4 -2 0 2 4 6 8 10
BHLHE40 (DEC1)
BMAL1 (ARNTL)
CLOCK
CRY1
CRY2
DBP
TEF
NFIL3 (E4BP4)
NR1D1 (REV-ERBα)
NR1D2 (REV-ERBβ)
PER1
PER2
PER3
RORγ


A
Molecular peak time (h)
0 4 8 12 16 20 24
Normalized expression level
2.0
0
-2.0
0 4 8 12 16 20
Circadian time (h)
B
CT0
CT4
CT8
CT12
CT16
CT20
Normalized expression level
2.0
0
-2.0
0 12 24
Molecular peak time (h)
0 4 8 12 16 20 24

BT Detection in Wild-Type

- To validate the molecular timetable based on the protein-level expression profiles, mice livers were further collected at CT4 and CT16 with two biological replicates. The quantified values of 36 peptides from livers were applied to BT estimation using the molecular timetable method .
- The livers at CT4 were estimated to be 3.7 and 3.3 h and those at CT16 to be 17 and 16.5 h, respectively, which were close to the actual circadian phase

BT Detection in Clock Mutant Mice

- Livers from One knockout (KO) or double knockout (DKO) mice (Bmal1KO, Per1/2 DKO, and Cry1/2 DKO), which were previously shown to have deficiency in the circadian rhythmicity ,were collected at CT4 and CT16.
- Quantified values of 16 proteins based on the quantification of 47 peptides showed a markedly different behavior compared with those of the wild-type mice .

In Bmal1 KO mice, the absence of CLOCK/BMAL1 heterodimer represses the E/E’-Box promoters and then activates the RRE promoters. In Per1/2 DKO and Cry1/2 DKO mice, the absence of PER1 and PER2 or CRY1 and CRY2 activates the E/E’-Box promoters and in turn activates the D-Box promoters whereas the RRE promoters are suppressed to some extent.

创新点：根据自己研究需要，将多种技术相结合进行使用。

问题：验证分子计时器的准确性时，取时间点样不够多。

谢谢！