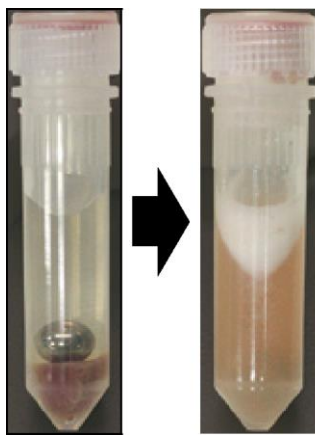
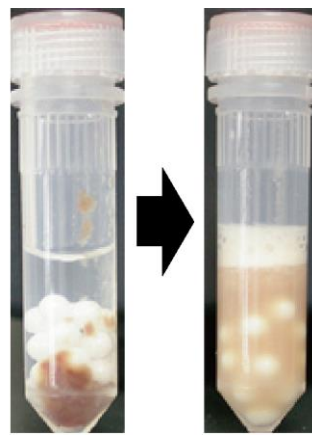


<Beads Crusher μ T-12 Crush Data>



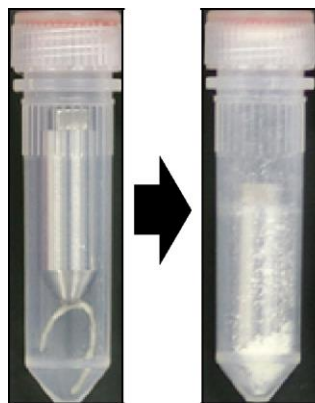
■ Porcine myocardiums

2.0ml screw cap micro tube
100 mg of thawing porcine myocardium
 Φ 5mm stainless bead x1pc
Solvent: 1.0ml
(0.5ml is better)
3200 r/min
30 sec



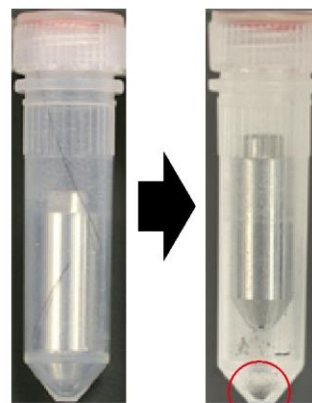
■ Porcine liver

2.0ml screw cap micro tube
100 mg of thawing porcine liver
 Φ 3mm zirconia bead x15pcs
(or Φ 5mm stainless bead x1pc)
Solvent: 0.5ml
3200 r/min
30 sec



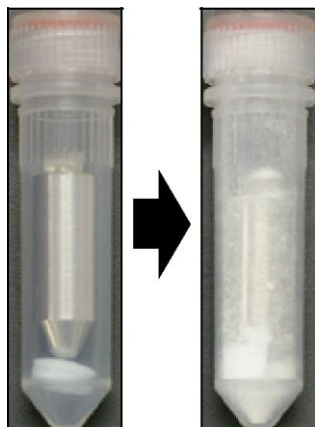
■ Human hand nail

2.0ml screw cap micro tube
Fingernails (adult male)
Metal crusher x1pc
No solvent
2500 r/min
10-30 sec



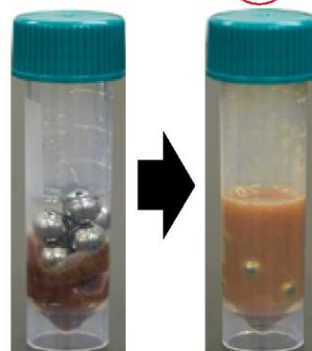
■ Human hair

2.0ml screw cap micro tube
A hair of an adult male (10cm)
Metal crusher x1pc
No solvent
2500 r/min
20 sec



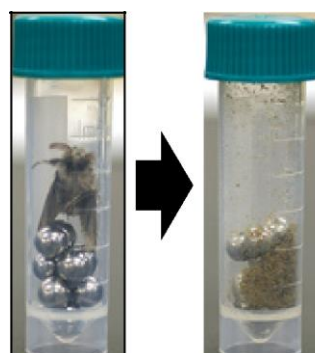
■ Tablet

2.0ml screw cap micro tube
One grain of commercial confectionery
Metal crusher x 1pc
No solvent
2200 r/min
10-15 sec



■ Porcine liver

5.0ml screw cap tube
1g of thawing porcine liver
 Φ 6mm stainless beads x9pc
(or Φ 5mm stainless beads x15pc)
Solvent: 1.0ml
2500 r/min
30 sec



■ Insect (Moth)

5.0ml screw cap tube
One dead moth
 Φ 6mm stainless beads x9pc
(or Φ 5mm stainless beads x15pc)
No solvent
2500 r/min
30 sec



■ Plant roots

5.0ml screw cap tube
0.4g of dried roots of plant
 Φ 10mm stainless bead x2pcs
No solvent
2500 r/min
30 sec for 1-2 times
(May remain some joints or fibers)

※1 rack holder for 5.0 ml screw tube is available.

※There is also heat protection holder of 3 tubes for 2.0 ml (TH-0203) in addition to a normal holder of 6 tubes (TH-0206) .

※The data above is performed without freezing a sample.

※When performing freezed crush, metal tube (2.0ml only) is recommended in order to prevent tube breakage.

※When using a metal crusher, the experimental conditions must be limited; the number of tubes up to a total of 6, shaking speed up to 2500 r/min, operation time up to 1 min (Use 3 tube holder (TH-0203) for cooling).

※Please be careful about a liquid leak or breakage when operating this unit, and use our recommended tubes to avoid these (ex. Watson Co. Ltd., 1392-200 for 2.0 ml tube).

<Additional Crush Data 2011/04/06>

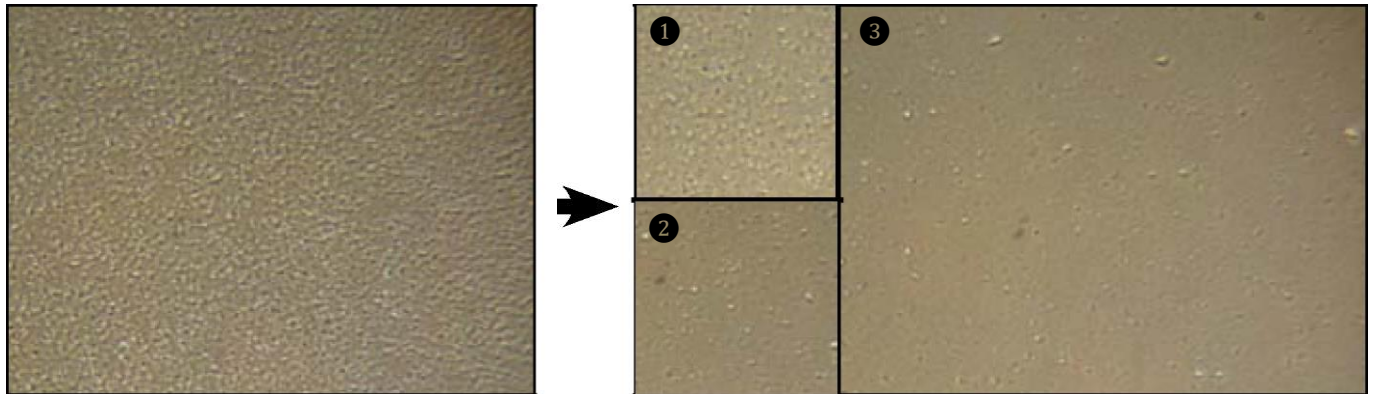
■ *Escherichia coli*

Conditions

2.0 ml screw cap microtube (Watson Co.Ltd. 1392-200), average diameter Φ 0.2mm zirconia beads (approx. volume: 1.0ml), 1.0ml of overnight cultured *E.coli* (HB101) and solvent (Refer to below).

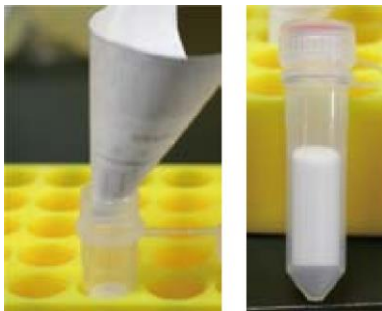
Crushing rate was checked at 3200 r/min for 60 sec (①), 120 sec (②) and 180 sec (③).

→Crushing rate is 50% for 60 sec, and 90% or more for 180 sec.



[Details of a procedure]

- 1) Culture *E.coli* for overnight and harvest 1 ml of cultured cells by centrifuge.
- 2) Add 1ml of PBS containing 0.1% final concentration of Triton X100 and resuspend it.
- 3) Transfer suspension cells to 2.0ml tube with approx. 1ml of beads, and add PBS to full (※).



Images show way to filling of beads into screw cap micro tube. It is easy and convenient to fill beads by using paper.

(※) Although crushing strengths declines, it can reduce bubbling if the inside of a tube is made full.

- 4) Crush at 3200 r/min.

<Freezed Crush Data against Mouse Joint Region, 2011/02/21>

■ Mouse forefoot joint part

2.0ml screw cap micro tube (Watson co. Ltd. 1392-200), metal crusher, liquid nitrogen freezing, 2500 r/min.
Sample was crushed to powder state in 45 sec.

[Details of a procedure]

1) "Holder for 3 tubes for heat protection TH-0203" was pre-chilled in freezer at -20 °C for 30-60 min.



2) Remove the unnecessary meat of the circumference of a joint beforehand (left). Cleave off the joint region (middle), and cleave to approx. 5mm length so that it might be easy to crush (right). Transfer them to a 2.0 ml screw micro tube.

※50-100 μl of sample volume is better for freezed crush. The freezed crush became difficult against too much sample.



3) If the sample of a mouse joint region and a metal crusher are put together in the same tube, a sample will stick and it will induce crushing lack since the mouse joint region contains plenty of fluid. In order to avoid this, freeze samples and metal crusher separately. After putting sample and metal crusher into each tubes and closing a cap loosely, freeze the whole tube by liquid nitrogen.
※Don't close tightly in order to open a cap easily after freezing. Liquid nitrogen does not enter in a tube.



4) Pull the tubes up from liquid nitrogen and open the caps quickly. Transfer the metal crusher to the tube with sample. Close the cap tightly and freeze the tube again in liquid nitrogen.

5) Set the freezed sample tube to the pre-chilled "Holder TH-0203" quickly.

6) Set "Holder TH-0203" with sample tube to the arm of main unit quickly.
Set the same holder on the other side to balance as well. Run the crushes for 45 sec at 2500 r/min.



The mouse joint sample was crushed to powder state.

※ Although there were no tube damages by liquid nitrogen freezing at this time, please pay sufficient attention in the experiment.

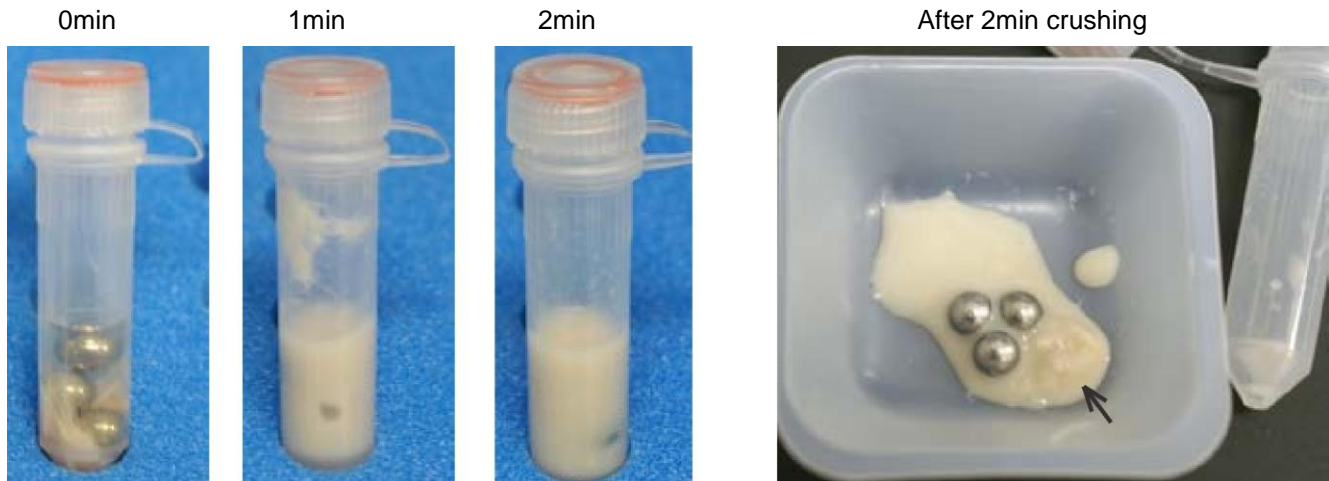
The same tube as this experiment (Watson Co. Ltd. 1392-200) is recommended.

<Additional Crush Data 2011/04/18>

■ Mouse skin (with hair)

2.0 ml screw cap micro tube (Watson Co.Ltd. 1392-200), diameter Φ 5mm SUS beads x3pcs, 100mg of mouse skin and PBS buffer.

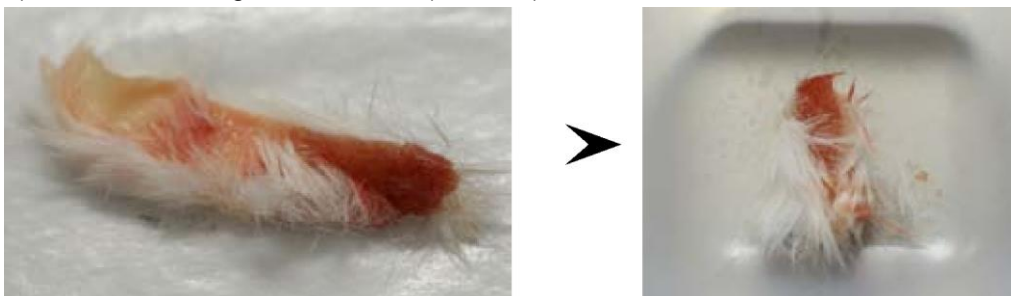
Skin parts were crushed at 3200 r/min in 1 min, and hair was crushed in 2 min.



※In case of sample with hair, the hair often remain after crushing

[Details of a procedure]

1) Cleave off 100mg of mouse skin (with hair)



2) Put 100 mg of mouse skin, 3 pieces of Φ 5mm SUS beads and 500 ul of PBS buffer into 2.0 ml screw cap micro tube (Watson Co. Ltd.1392-200).

3) Run crush at 3200 r/min.

[Additional entry]

※ The skin can be increased up to 200mg per 2ml tube. It may be necessary to set the time longer slightly for crushing in that case.

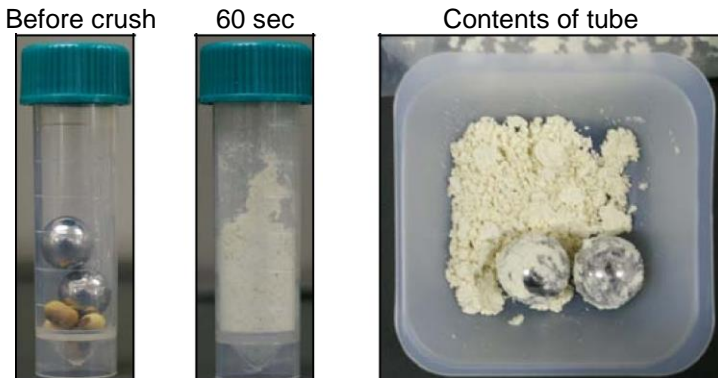
※※ Although it is possible to crush by using metal crusher in the same procedure (2500 r/min, 1-2min), the Φ 5mm SUS beads is recommended in this experiment since metal crusher has much calorific values and a tube is easy to get warm.

<Additional Crush Data 2011/04/28>

■ Soybeans (dry seed)

5.0 ml screw cap tube (Watson Co.Ltd. 2332-105), diameter Φ 10mm SUS beads x2pcs and soybean (dry seed)
(No solvent buffer).

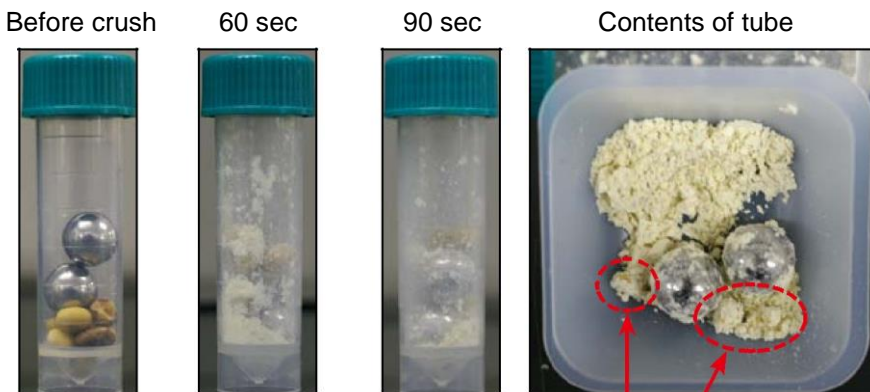
[Result] All 5 grains (about 450 mg) of dried soybeans were crushed at 2000 r/min in 60 sec (※1).



2 pieces of Φ 10mm SUS beads, 2000 r/min, 60sec

(※1) Basically speed limit is 2500 r/min for 5.0 ml tube. However, please use 2000 r/min since the mass of two 10mm SUS beads has large system load and the tube breakages probability increases.

[Reference 1.] 90% of 10grains (about 850mg) of dried soybeans were crushed at 2000 r/min in 90 sec (※2).

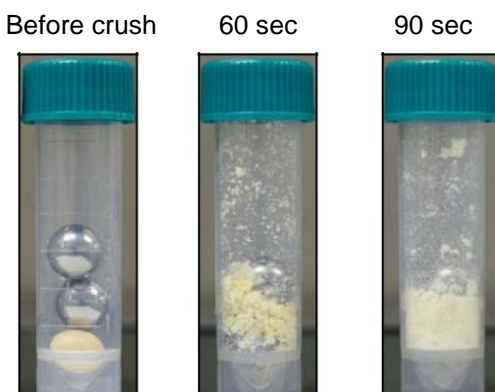


2 pieces of Φ 10mm SUS beads, 2000 r/min, 60sec and 90 sec

Although most became powder, a small amount of large fragment is still remained.

(※2) Although up to 1g of sample can be crushed for 5.0ml tube, a breakage rate did not become 90% or more in this experimental conditions (bead material, size, number) even if extends processing time 90 sec or more. The power of crush might become weaker since the powders interfere in crushing as a cushion. An improvement is expected by changing beads size and numbers or reducing sample volumes.

[Reference 2.] A large grain (about 400mg) of dried soybean was crushed at 2000 r/min in 90 sec.



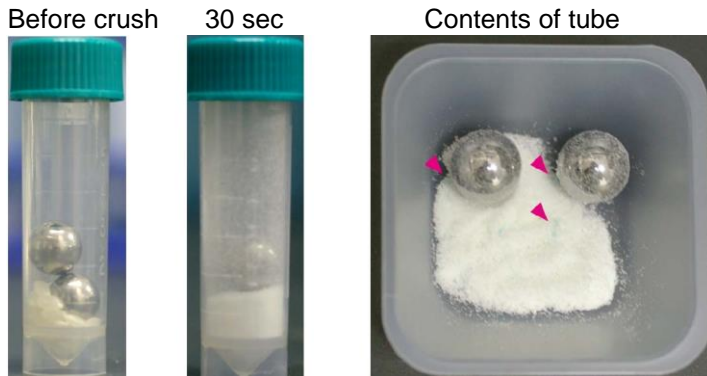
2 pieces of Φ 10mm SUS beads, 2000 r/min, 60sec and 90 sec

<Additional Crush Data 2012/04/18>

■ Raw rice

5.0 ml screw cap tube (Watson Co.Ltd. 2332-105), diameter Φ 10mm SUS beads x2pcs and raw rice (No solvent buffer).

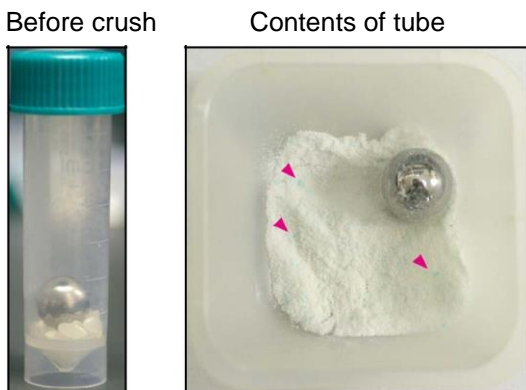
[Result 1.] All 30 grains (about 550mg) of raw rice were crushed at 2000 r/min in 30 sec (※1). (Φ 10mm SUS beads x2pcs)



2 pieces of Φ 10mm SUS beads, 2000 r/min, 30sec

(※1) Basically speed limit is 2500 r/min for 5.0 ml tube. However, please use 2000 r/min since the mass of two 10mm SUS beads has large system load and the tube breakages probability increases. After 30 sec crushing, rice was crushed as uniform. In this experiment, small amount of small particle from green cap was observed shown in arrow. When there are many particles, please set speed lower and reduce number of beads.

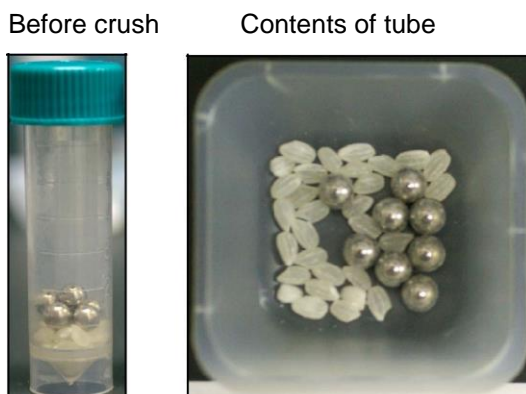
[Result 2.] All 30 grains (about 550mg) of raw rice were crushed at 2500 r/min in 30 sec (※2). (Φ 10mm SUS bead x1pc)



One Φ 10mm SUS beads, 2500 r/min, 30sec

(※2) After 30 sec crushing, rice was crushed as uniform. In this experiment, small amount of small particle from green cap was observed shown in arrow.

[Reference 1.] All 30 grains (about 550mg) of raw rice were NOT crushed at 2500 r/min in 60 sec (※1). (Φ 5mm SUS beads x8pcs) (※3).



8 pieces of Φ 5mm SUS beads, 2500 r/min, 60sec

(※3) In case of using 8 pieces of Φ 5mm SUS beads at 2500 r/min for 60sec, all grains of raw rice were NOT crushed.

In case of using 4 pieces of Φ 6mm SUS beads at 2500 r/min for 60sec, all grains of raw rice were NOT crushed as well (data not shown).

For crushing raw rice with using 5.0 ml tube, Φ 10mm SUS beads is recommended.