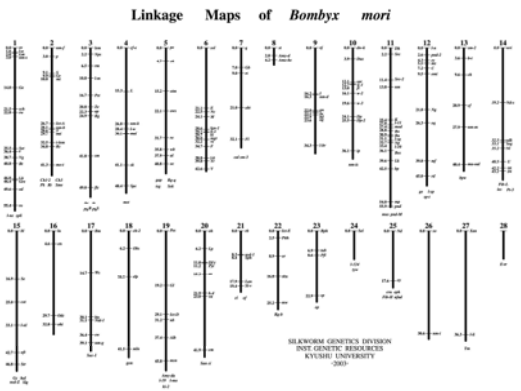
1 2 3 4 5 6 7 8 9 10 11 12

P → F1 → F2 → F3 → F4



National bio-resource project in Japan and development of cryopreservation methods for silkworm resources.

Y. Banno,
Silkworm Genetics Division, Institute of Genetical Resources,
Kyushu University

National Bioresources Project (NBRP)

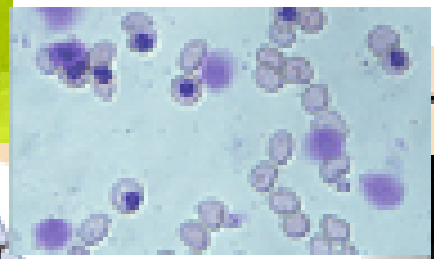
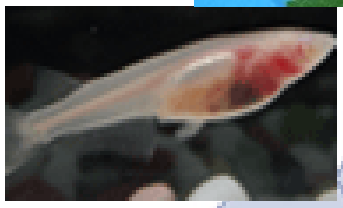
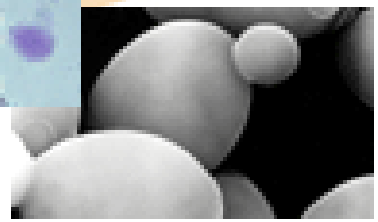
This Project had been started at 2002 supported by the Ministry of Education, Culture, Sports, Science and Technology.

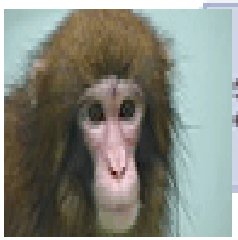
Purpose

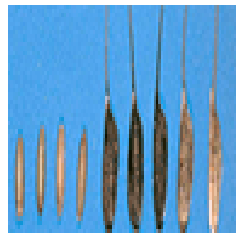
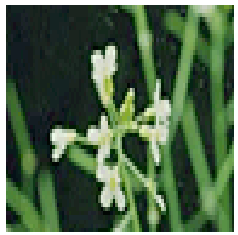
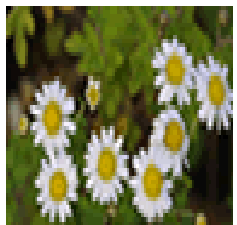
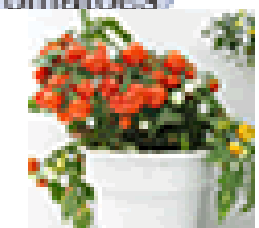
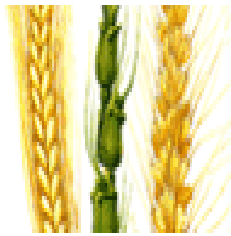
1. Collection
2. Preservation
3. Distribution

for bioresources that are basic materials for life sciences research.





Mice
Silkworms
Cellular slime molds
Pathogenic microorganisms
Medaka
ES Cell
Zebrafish
Rats
Micro-organisms
DNA
stinalis/ onicas
C.elegans
Animal Cells
Bacil subt
Japanese macaques
Core facility upgrading program
E.coli
X.tropicalis
Yeast
Drosophila
Algae
Wheat
Rice
Arabidopsis
Barley
Morning Glory
Lotus/ Glycine
Chrysanthemum
Tomatoes

Participant organizations for silkworm

- Kyushu University (core)

  Silkworm strains (*B. mori and B. mandarina*)
- University of Tokyo (Sub. 1)

  Genome resources
- Shinshu University (Sub. 2)

  Wild silkworms
- National Institute of Agrobiological Sciences (Sub. 3)

  Transgenic silkworms

Available resources

<Domesticated silkworm>

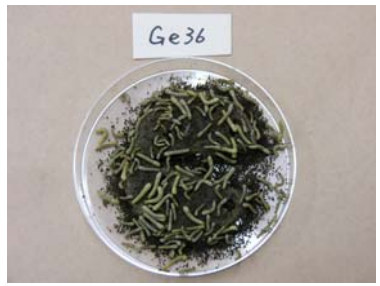
- Mutant strains 500
- Improvement strains for artificial diet 200
- Transgenic strains 100

<Wild silkworm

- *Antheraea yamamai* 5
- *Antheraea pernyi*, *Samia cynthia* 1

<Genome>

- 220,000 clone (DNA / cDNA)
  from 50 libraries



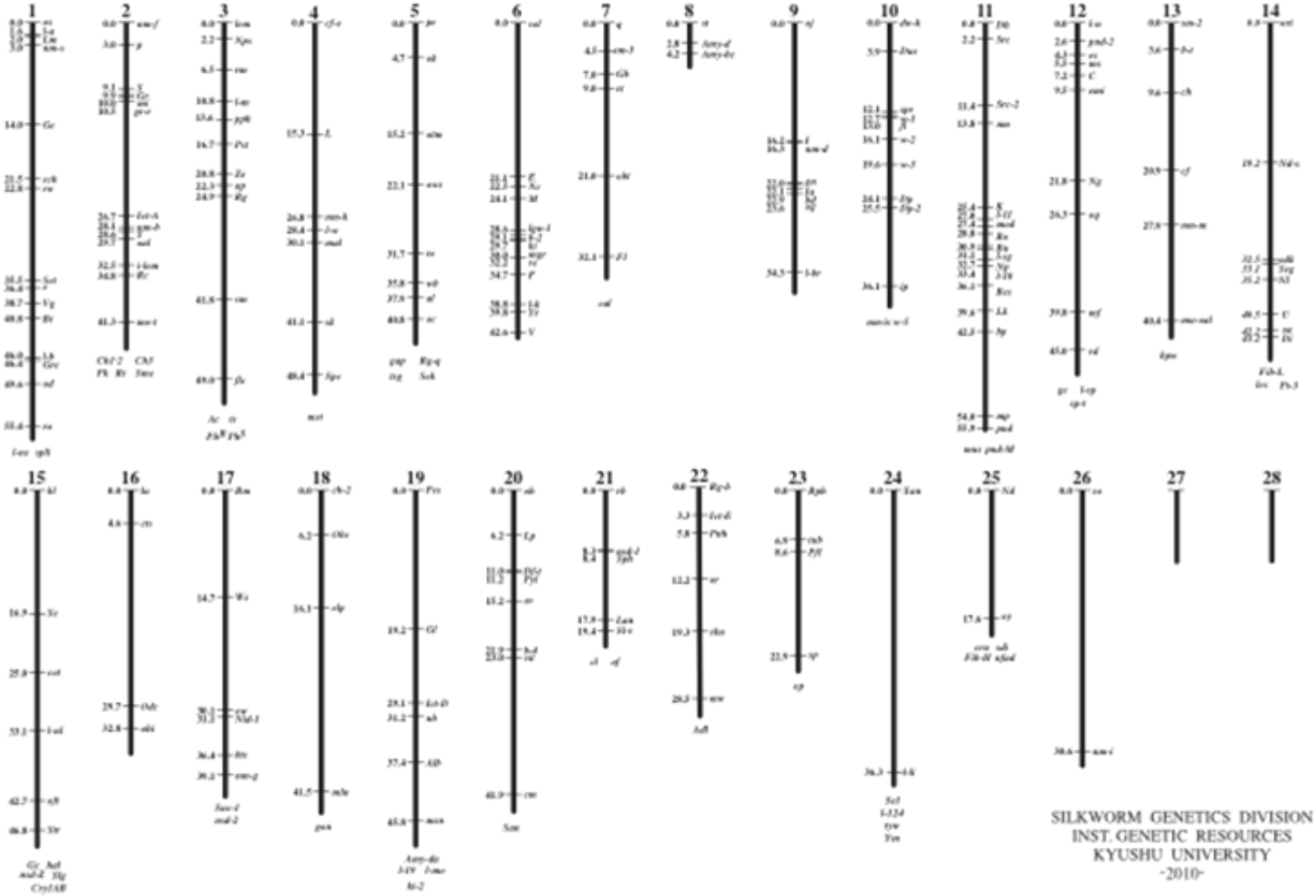
Egg

larva

pupa
(cocoon)

moth

Linkage Maps of *Bombyx mori*



SILKWORM GENETICS DIVISION
INST. GENETIC RESOURCES
KYUSHU UNIVERSITY
-2010-

You can get eggs year-round

Before this Project (normal)	□	□	□	□	□	□	□	□	□	□	□	□
Acid treatment*	□	□	□	□	□	□	□	□	□	□	□	□
Artificial* hibernation	□	□	□	□	□	□	□	□	□	□	□	□
After this Project	□	□	□	□	□	□	□	□	□	□	□	□

- □Distribution is okay .

When we use the egg laid in June*

MTA is used for distribution in NBRP .

MTA :maternal transfer agreement.

<Aim of MTA>

- Protection of the intellectual property rights

  of developer of strains.

→ The usage of the strains is limited in only purpose agreed in MTA.

MATERIAL TRANSFER AGREEMENT
(FOR DISTRIBUTION)

RECIPIENT
Recipient Staff:
Recipient
Organization:_____
Address:

This Material Transfer Agreement sets forth the terms and conditions under which Institute of Genetic Resources, Kyushu University (hereinafter referred to as 'IGRK') will provide with the RECIPENT, and the RECIPIENT will receive, the biological material specified as _____and/or it's derivatives (hereinafter referred to as the 'BIOLOGICAL RESOURCE') in response to the RECIPIENT's request, and with which the RECIPIENT staff and organization agree before the RECIPIENT receives the BIOLOGICAL RESOURCE:

The IGRK is engaged in collecting, maintaining, storing, multiplying and distributing the biological resources, in order to contribute to the Japanese and international research community for the development of the research and utilization in the field of life sciences.

(a) The RECIPIENT shall use the BIOLOGICAL RESOURCE for the following specific purpose:

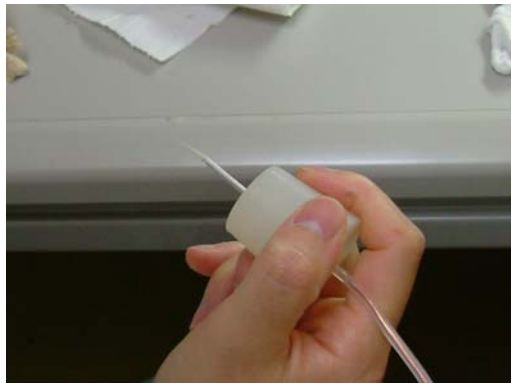
_____.

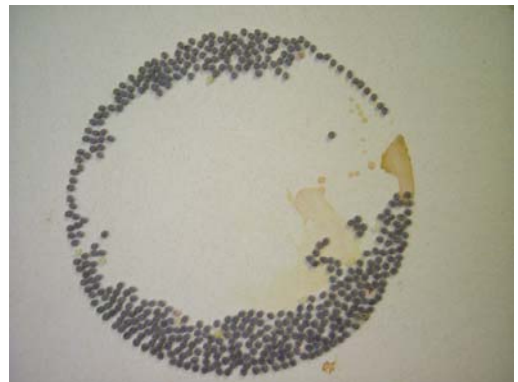
Long- term preservation
□frozen sperm□









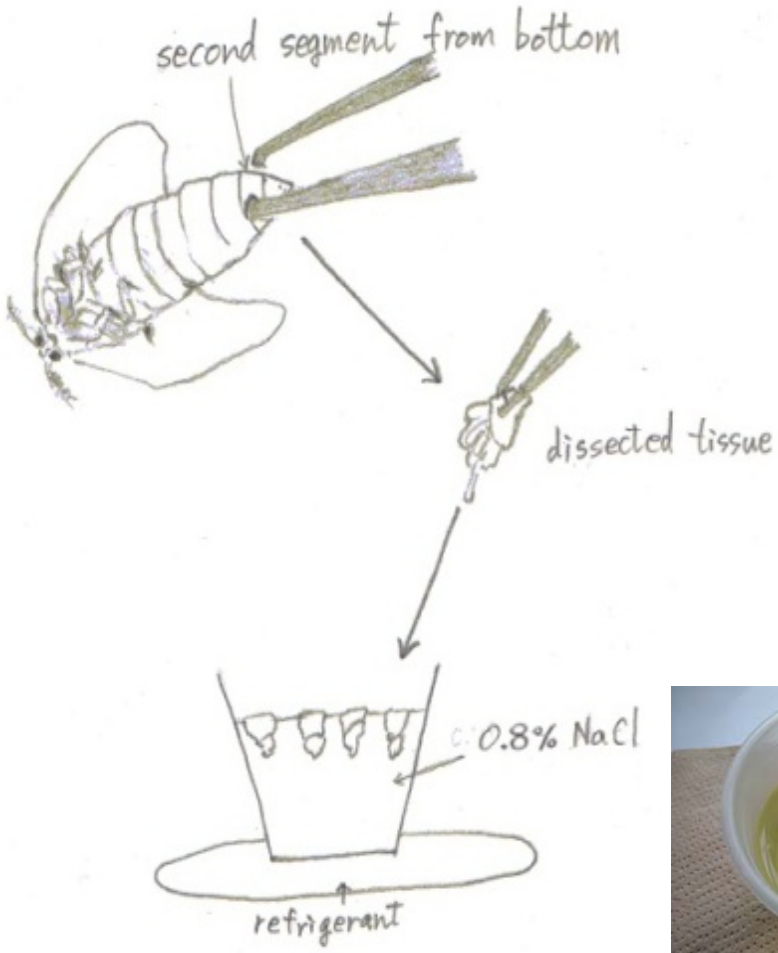


1st STEP

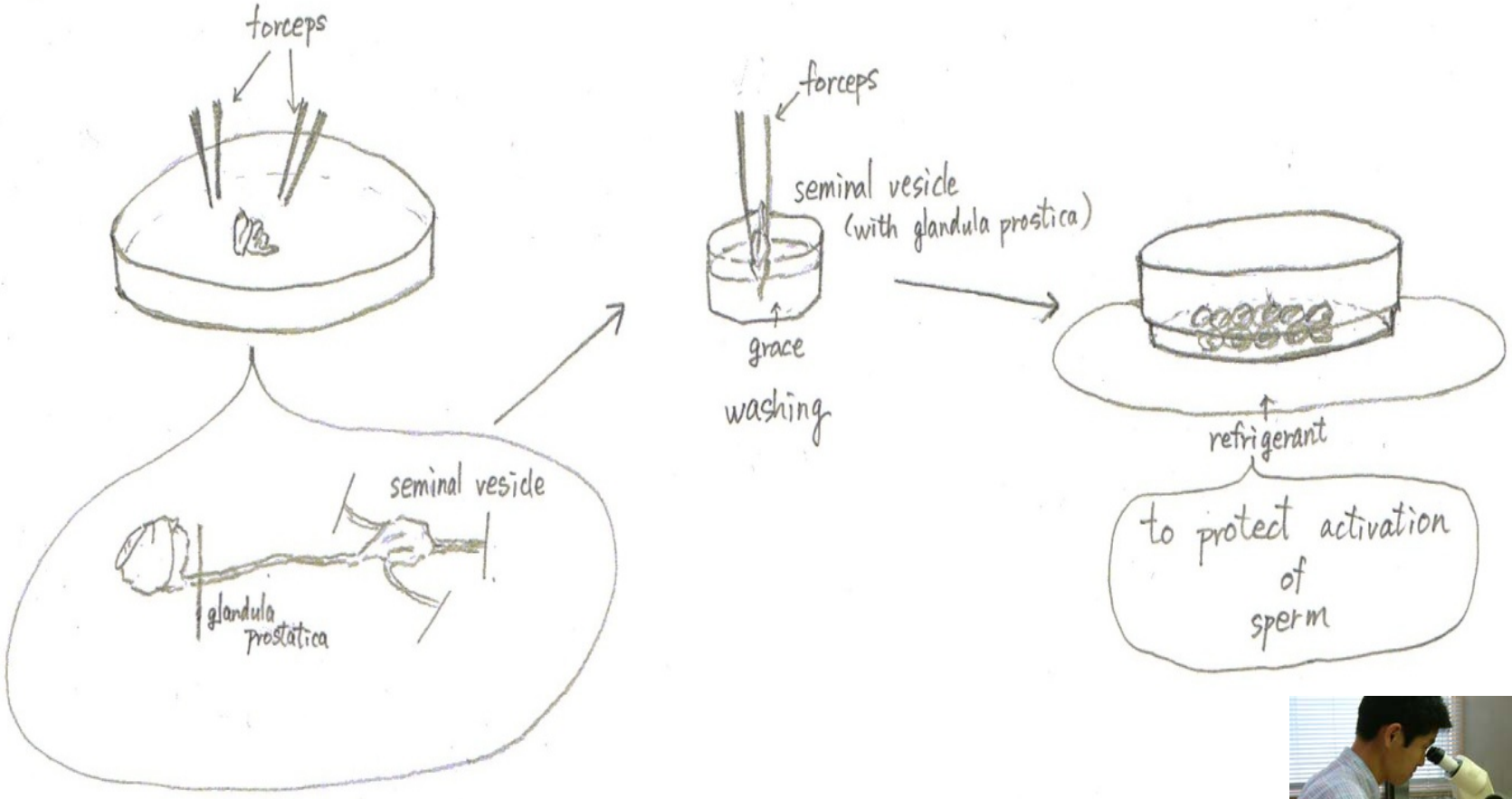
Collection of the sperm from male seminal vesicle of moths.

1)Take out the male internal reproductive system by dissections.
(important tissues are glandula prostatica and seminal vesicle).

2) Soak the dissected tissues to small beaker filled with physiological solution (0.80% NaCl). Usually 30-40 individuals stoked.

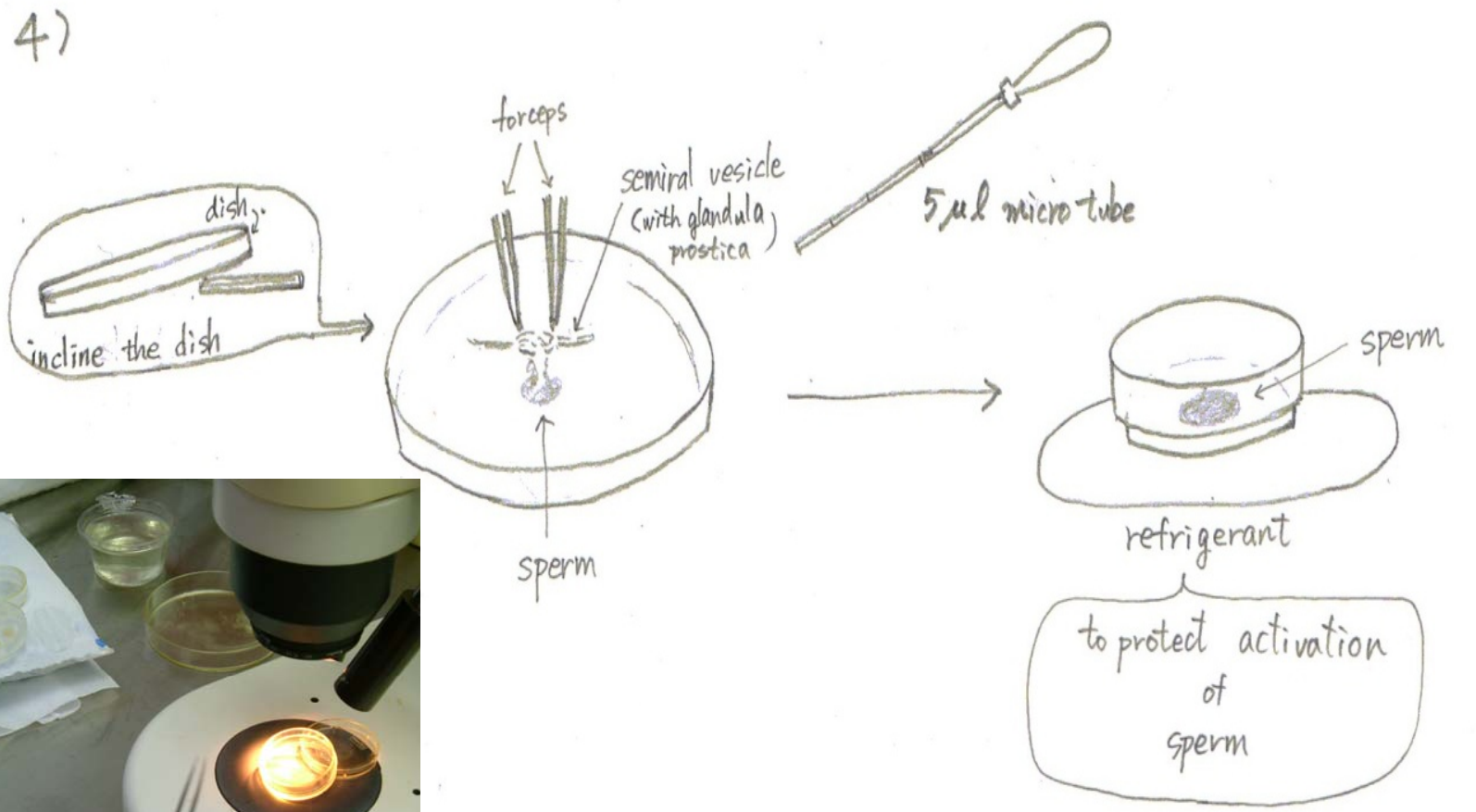


3) Collect the seminal vesicle(with glandula prostatica) by forceps.
Be avoid contamination of other tissues such as fat body and some tiny granules.
Move the dissected seminal vesicle(with glandula prostatica) to cooled small dish.



4) Transfer one seminal vesicle(with glandula prostatica) on small cooled dish.

Rupture seminal vesicle(with glandula prostatica) by forceps so that the sperm go out. Repeat this procedure until you get enough volume (65-80μℓ).



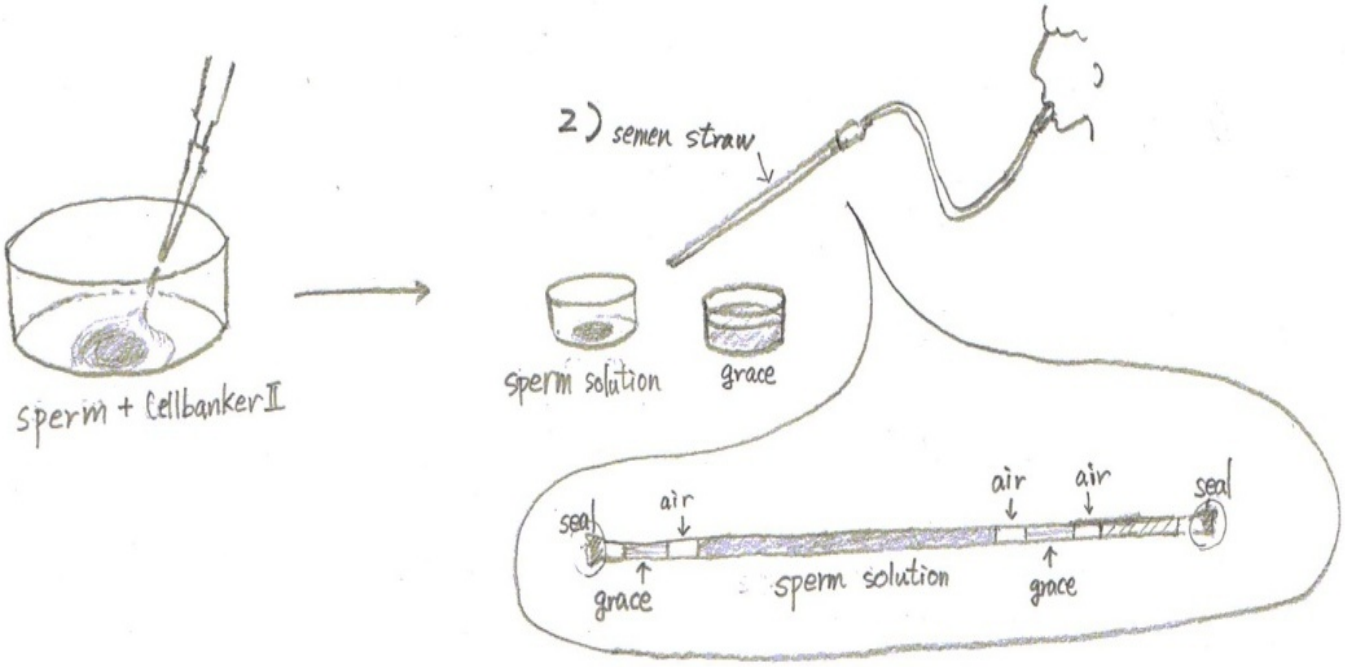
2nd STEP

Freezing process (cryopreservation) of the sperm.

1)Add the same volume of Cell banker 2 (cryoprotectant chemical) to the collected sperm.

2) Draw up the sperm solution in a straw (detail show in Fig.).

3)Storage at -80□ for 15 seconds, and keep it in -196□(liquid N2) until use.

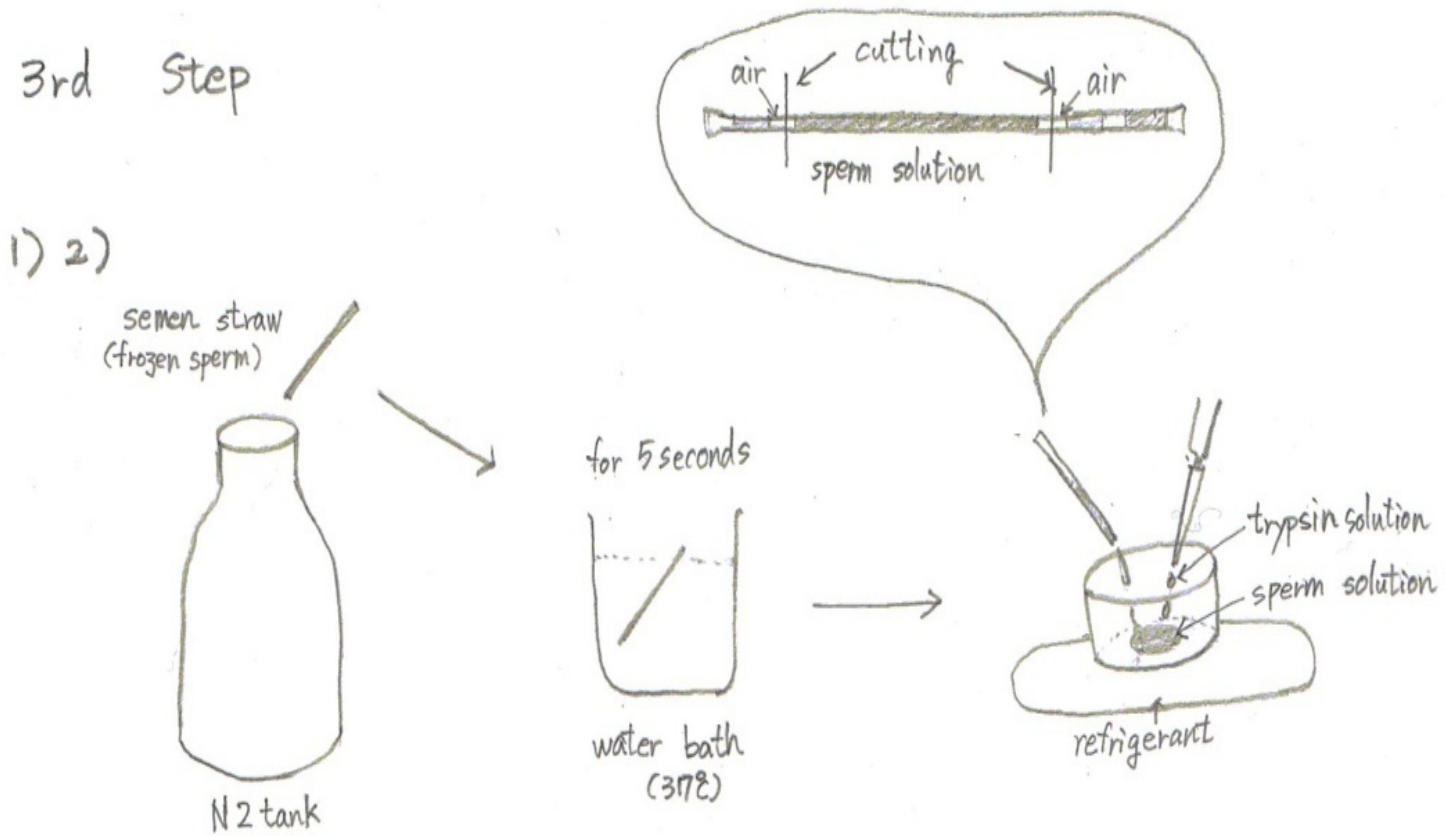


3rd STEP

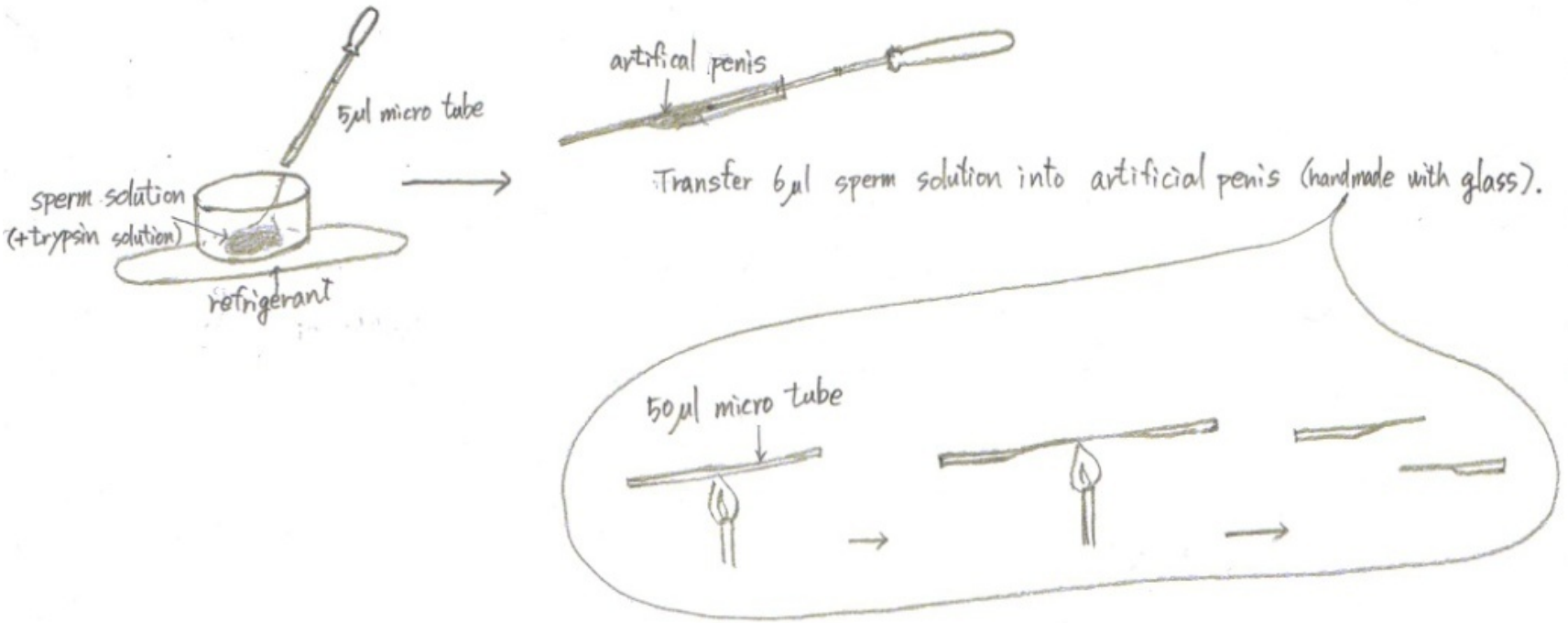
Insemination

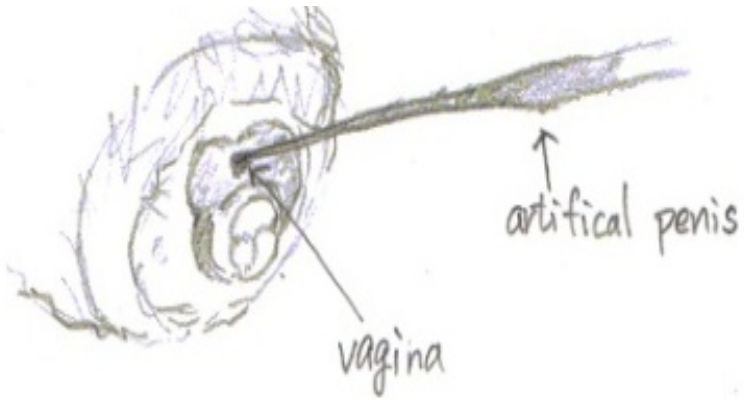
1)Thaw the frozen sperm in water bath (37□) for 5 seconds.

2) Add the trypsin solution and mix the both solutions gently.



3) Transfer 6μℓ sperm solution into artificial penis
(handmade with glass).
4) Inject the sperm to female moth by using the foot switch.

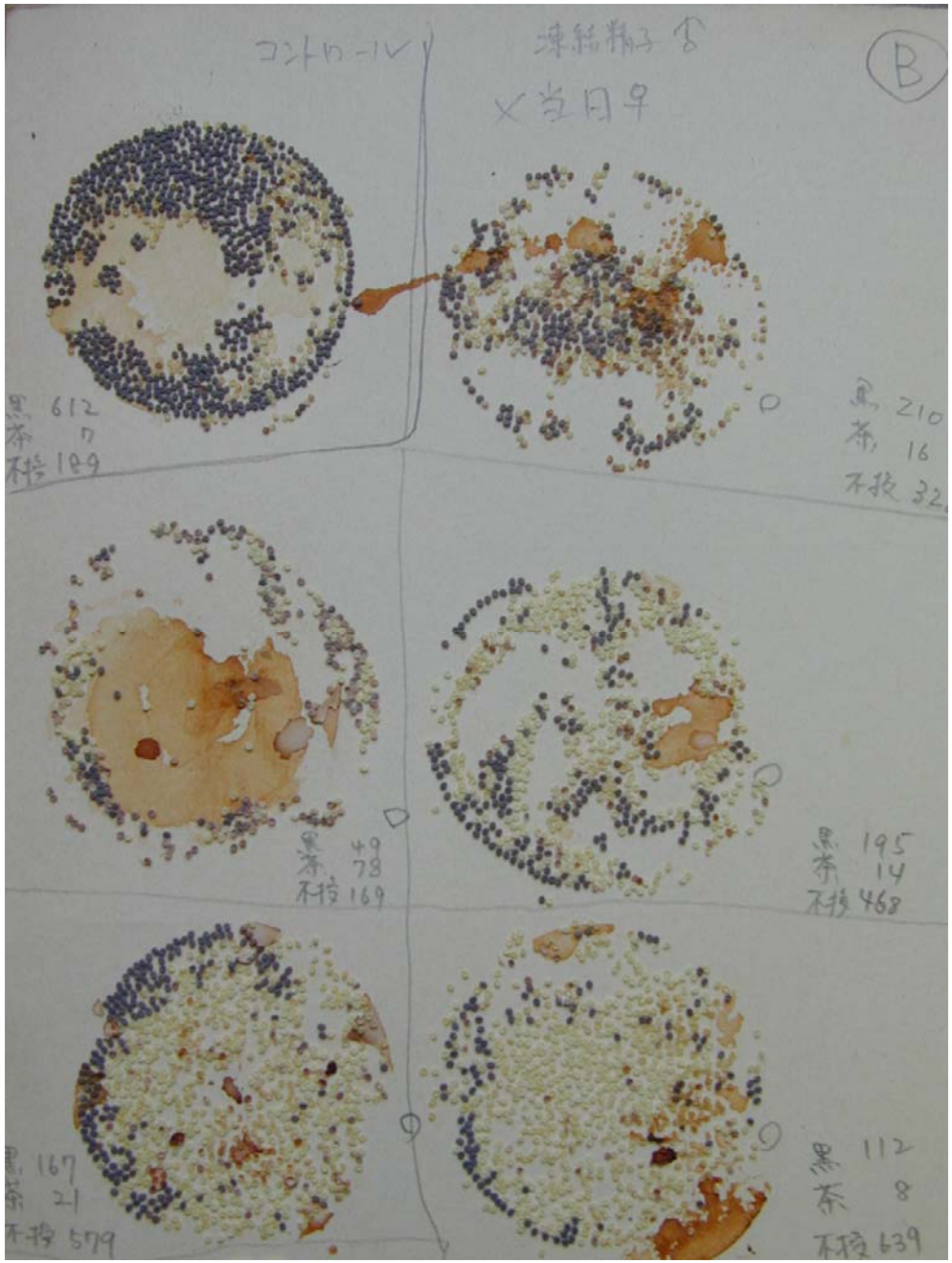




Instruments and chemicals for artificial insemination

Stereoscopic microscope
Clean bench (not necessary)
Forceps
Scissor
Small dish
Refrigerant
N2 tank

Grace's medium
Physiological solution (0.75% NaCl)
DMSO(Dimethyl sulfoxide) → Adjust 10% with Grace's medium
Liquid nitrogen
Trypsin → Adjust the concentration to 0.3μg/ml with Grace's medium if available unit is 5000U trypsin.
Semen straw 0.25ml
Micro tube 50μℓ for artificial penis
Micro tube 5μℓ for semen tranfer


コントロール
凍結精子 5
×当日子
B
黒 612
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黒 210
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茶 14
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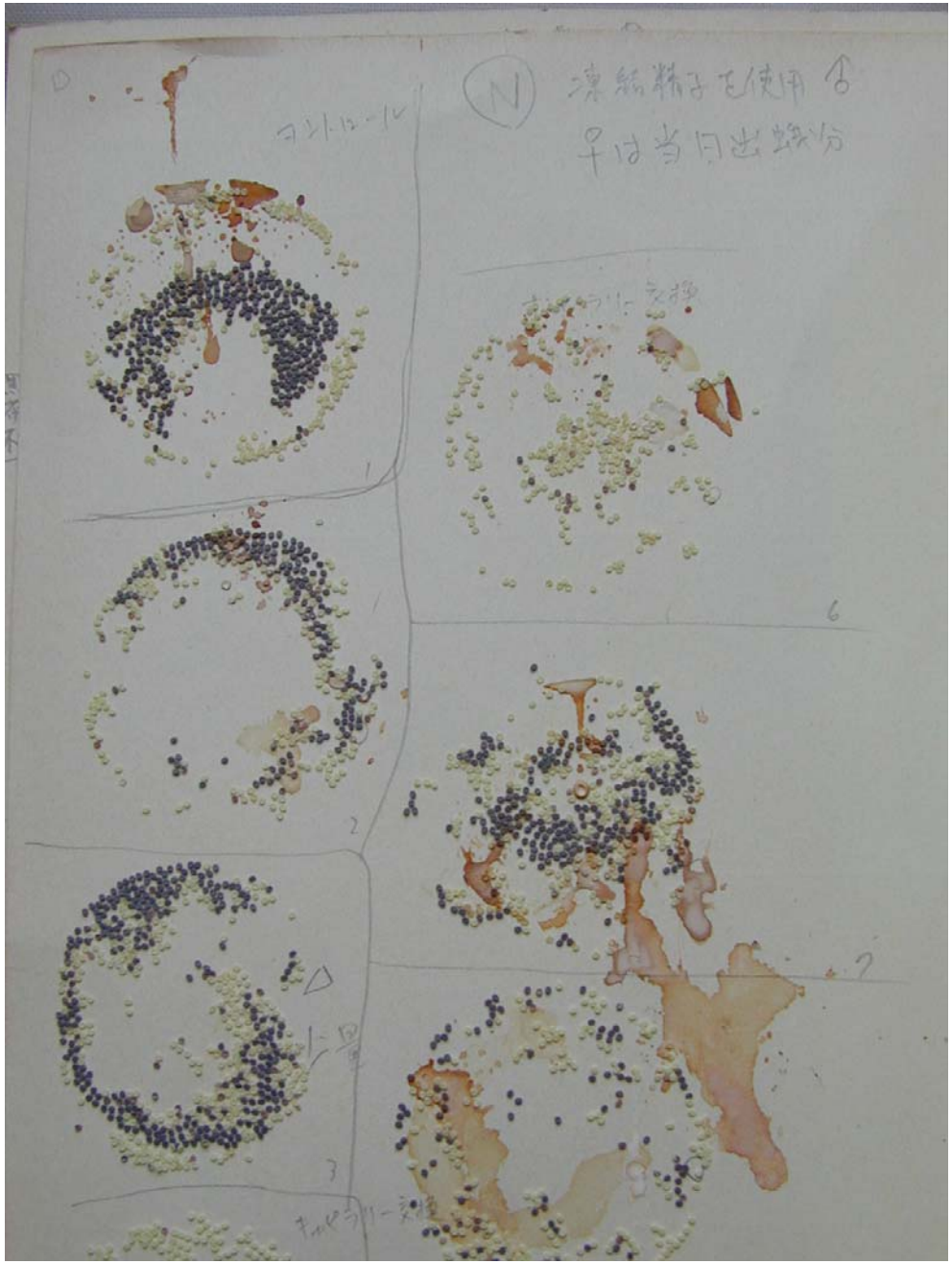
黒 167
茶 21
不投 579
黒 112
茶 8
不投 639


D
コントロール
N
凍結粉末を使用
午は当日出勤分
1
2
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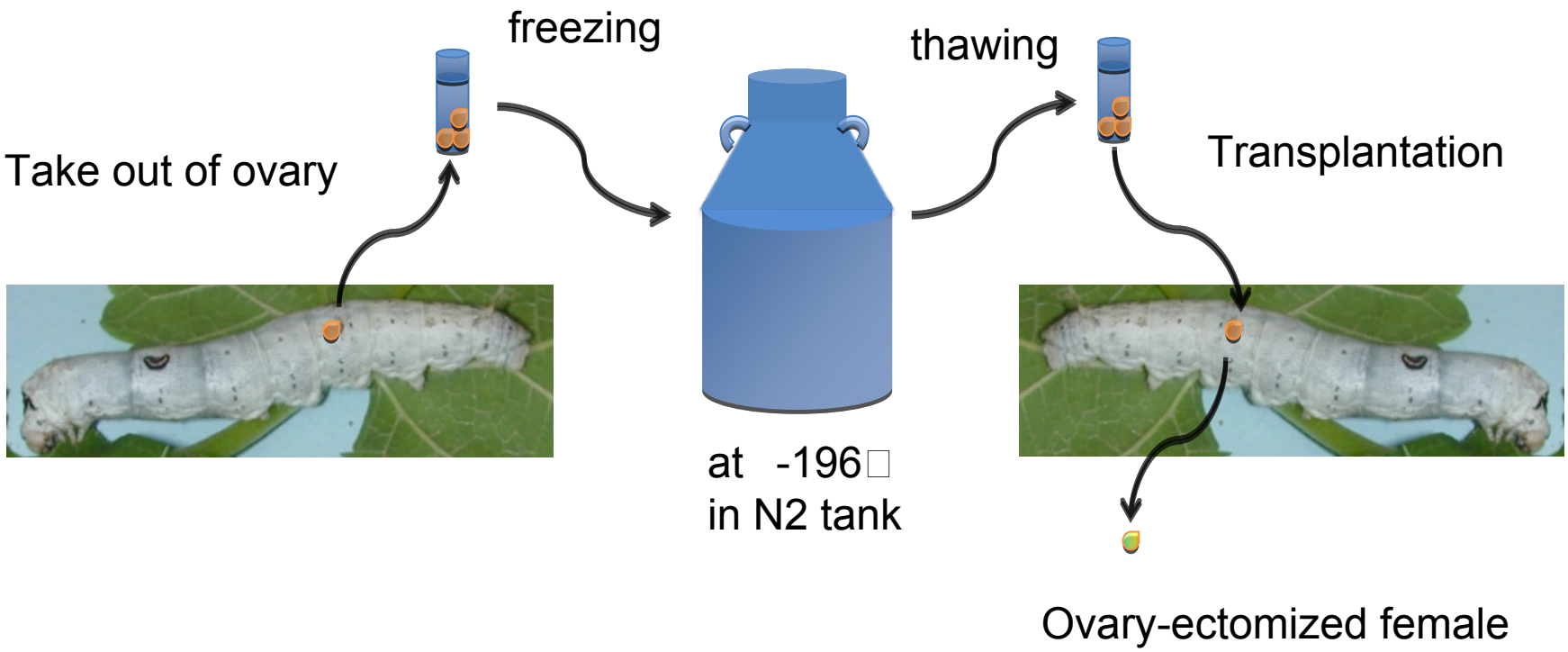
6
7

Long- term preservation
□use of frozen ovaries□



	Success rate (Ave.)	Difference between strains
Sperm	30%	Wide Many strain are in 0%
ovary	30%	Smaller than sperm
testis	10%	To evaluate, we need more experiments.