



HYBRID LAB NETWORK

Hybrid Lab LTTA 2 **PART A: Return to** **Dilmun (revisited)**



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Hybrid Lab Network LTTA 2: Return to Dilmun revisited

Summary

Hybrid Lab Network's 2nd LTTA (Learning Teaching and Training Activity) will revisit Return to Dilmun, a work of art made by Günter Seyfried, Roland van Dierendonck, Federico Mufatto and Hansjörg Petschko, conceived in the Open Wet Lab of the Waag in 2017.

Return to Dilmun uses the CRISPR Cas9 technique *in vitro*, to codify the image of an idol of early agricultural society into DNA, showing the continuity between early breeding and cultivation of species and those contemporary techniques and technologies.

Join us for a 5-day workshop wherein we re-make the artwork by re-doing the *in vitro* and *in silico* protocols and in addition discuss its artistic and cultural significance, the ethics of these type of artistic research practices and scientific experiments, how to turn an experimental artistic gene editing protocol in a meaningful learning-teaching-training module and whether it is important we imagine more of these types of artworks and their making.

All times are CET (Porto-1, Helsinki +1)

Program

Day 1 | Thursday 5 November | Artistic and scientific context of the technique used and starting hands-on: Clean your Lab!

10.00 Introduction into LTTA 2 and the results of LTTA 1 CRISPR Creations

10.30 Introduction into the artwork Return to Dilmun | Federico Mufatto, Guenter Seyfried, Roland van Dierendonck

11.30 Introduction in CRISPR Cas 9 and its successors | José Bessa

12.00 Introduction into the protocol of Return to Dilmun | José, Guenter, Roland

12.30 Lunch

13.30 First *in vitro* activities: Laboratory good practices to work with RNA | José, Guenter, Roland, Marta and Kas

16.00 wrap of the day

Day 2 | Friday 6 November | Ethics and CRISPR Arts

10.00 Continuation of Laboratorial good practices to work with RNA (2h30min)

12.30 Lunch

13.30 Presentation of works of art and design, using CRISPR technique | Lucas Evers

14.00 Reaction of ethical and moral complexities and the notion of risk and contamination found in the presented works | Agnieszka Wołodźko

14.30 Discussion: Why more CRISPR art?

15:00 Speculation of new works of CRISPR art?

16.00 wrap of the day

Day 3 | Saturday 7 November | Return to Dilmun *in silico* and how to understand its full protocol as STEAM education

10.00 Return to Dilmun *in silico* | José, Guenter, Roland

Class 1 From DNA to Protein (1 hour)

Class 2 Using DNA as a storage for binary language information to encode a RGB image. (30 min)

Class 3 Design of sgRNAs (1 hour)

12.30 Lunch

13.30 Conversation and discussion about how the making of Return to Dilmun in its entirety can serve as a model to teach genetic engineering as art making and a creative process and in reverse teach this type of art making a way to understand implications of genetic engineering beyond its scientific application | moderated by Laura Beloff

15.00 *In silico* workshop designing sgRNAs

Go through protocols for Monday (sgRNA synthesis, Cas9 cutting)

16.00 wrap of the day

Day 4 | Sunday 8 November | Day of rest

Day 5 | Monday 9 November | Cleavage day

10.00 *In vitro* synthesis of sgRNAs and cut (Full day in the lab) | José, Guenter, Roland

In vitro synthesis of the sgRNAs (Kit protocol)

11.30 Purify the synthesized RNA using a Kit (Kit protocol)

12.30 Lunch

13.30 Make the CRISPR/Cas9 reaction

16.00 wrap of the day

Day 6 | Tuesday 10 November | Conclusions

- 10.00 **Results on gel** | José, Guenter, Roland
Class 4 Mutate the bull's head *in silico*
- 11.00 **Interpret the results and discussion**
- 11.30 ***In silico*: Cut in the 9kb sequence, simulating modification**
- 12.30 Lunch
- 13.30 **Concluding discussion about Intellectual Output / Imagining and speculating new gene editing works of art, why we need them and what experimental designs are required to make them** | moderated by Lucas Evers
- 15.00 ***In silico*: decoding the altered DNA sequence to image** | José, Roland, Guenter
- 16.00 wrap of the day
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Detailed Agenda

Day 1 | Thursday 5 November | Artistic and scientific context of the technique used and starting hands-on: Clean your Lab!

Materials needed:

- Nuclease + RNase free water
- Normal water
- A DNA sample
- A RNA sample
- Nuclease + RNase free plastic consumables: pipet tips, tubes
- Gloves
- 70% Ethanol
- 10% Bleach

Protocols needed:

- A) DNA and RNA degradation

10.00 Introduction into LTTA 2 and the results of LTTA 1 CRISPR Creations

10.30 Introduction into the artwork Return to Dilmun | Federico Mufatto, Guenter Seyfried, Roland Dierendonck

11.30 Introduction in CRISPR Cas 9 and its successors | José Bessa

12.00 Introduction into the protocol of Return to Dilmun | José, Guenter, Roland

12.30 Lunch

13.30 First *in vitro* activities: Good lab Practices | José, Roland, Guenter, Marta, Kas

- 1) **Short introduction (30min):** DNA vs RNA, two molecules very similar, but with very different roles in the cell, that can explain their sensitivity for degradation. One stores information long term (DNA), the other transiently makes it available (RNA) | José Bessa
- 2) **Short explanation of the experiment that we will perform (10min)** | José Bessa
- 3) **Introduction to basic safety measures in the lab** (Gloves, lab coat, no food, don't smell reagents, bio-waste, dangerous materials. etc) (20min) | Kas
- 4) **Incubation (might be the full length of the practical class): Lets incubate clean and "dirty" water with a solution of DNA and another with RNA** | Marta
- 5) **Explain the principle of electrophoresis and how we will observe DNA and RNA using this method. What to expect from the experiment and how to interpret the results** | Kas
- 6) **Explaining laboratorial good practices to work with RNA (30 min).** (Clean the bench, Clean solutions. Freezing all reagents. Gloves, etc.) | Gunter and Roland
- 7) **Walkthrough of the degradation experiment**
DNA and RNA in Dirty and Clean water
Explain the ladder as molecular weight of DNA/RNA and results

16.00 wrap of the day

Day 2 | Friday 6 November | Ethics and CRISPR Arts

Materials needed:

- Nuclease + RNase free water
- DNA solution (100ug/ml)
- RNA solution (50ug/ml)
- 'Dirty' water or tap water
- Nuclease + RNase free plastic consumables: pipet tips, tubes
- Gloves
- 70% Ethanol
- 10% Bleach
- Gel box + comb
- Agarose
- Gel dye (e.g. SYBR green)
- Molecular weight Ladder
- Loading dye
- TAE 1X

Protocols needed:

- A) DNA and RNA degradation
- B) Gel Electrophoresis

10.00 **Continuation of Laboratorial good practices to work with RNA**
Friday Morning

- 1) Practice with working clean by doing the experiment using protocol **A) DNA and RNA degradation**
- 2) **Gel preparation using protocol B) Gel Electrophoresis** (20 min)
- 3) **Gel load and run** (30-45min) in a gel these two conditions to see the differential effect in DNA and RNA of clean and dirty water.
- 4) **Explain the ladder as molecular weight of DNA/RNA and results**
- 5) **Overview of Monday's protocol.** (30 minutes) | Gunter and Roland
 - a. Start by explaining the overall objective of the experiment (demonstrate the targeted and specific double strand break mediated by CRISPR/Cas9)
 - b. *in vitro* synthesis of sgRNAs
 - c. Run sgRNA in a gel to confirm synthesis
 - d. Purify the sgRNA using a RNA column
 - e. Run the purified sgRNA in a gel to confirm recovery of RNA and quantification
 - f. Make the CRISPR cas9 reaction
 - g. Run the reaction
 - h. Interpret the results

12.30 Lunch

13.30 Presentation of works of art and design, using CRISPR technique | Lucas Evers

14.00 Reaction of ethical and moral complexities and the notion of risk and contamination found in the presented works | Agnieszka Wołodźko

14.30 Discussion: Why more CRISPR art?

15:00 Speculation of new works of CRISPR art?

16.00 wrap of the day

Day 3 | Saturday 7 November | Return to Dilmun *in silico* and how to understand its full protocol as STEAM education

Materials needed:

- benchling

10.00 **Return to Dilmun *in silico*** | José, Guenter, Roland

Class 1 From DNA to Protein | José Bessa

- 1) Theoretical introduction to the genetic information and activation. What is stored in the DNA and how information flows from the DNA to RNA to Proteins, the effectors of the cells
- 2) Use of a genomic sequence (DNA) to transcribe into RNA (sum of exons and conversion to ribonucleotidic sequence) using *in silico* approach
- 3) Translate the RNA code into a protein using an *in silico* approach
- 4) Discuss the effect of the protein at the cellular and organism level (The sequence we will use must encode a practical example of protein functionality)

- 5) Going beyond the given example. Explore databases to be able to use any gene in the genome of any sequenced organism

Class 2 Using DNA as a storage for binary language information to encode a RGB image | Roland and Günther

Feedback from Gunter and Roland. Basics on the algorithm? Binary information/RGB information. Maybe giving examples of different sequences and how they can translate into different images. The potential of information storage? Analogies with evolution by random mutagenesis and natural selection? How nature shapes form to reach a function. Link to the selfish gene maybe?

Class 3 Design of sgRNAs | José Bessa

- 1) Basics of CRISPR/Cas9. Randomness in the repair of breaks
- 2) Basics of sgRNA design
- 3) Exploring the <https://www.crisprscan.org/>
- 4) Oligo design to incorporate the sgRNA information and the ability to transcribe
- 5) Using the basics to design sgRNAs and oligos to mutate the "red" fragment of the bull's head (some of the solutions will be the same we have found in the design)

(In silico workshop | José Bessa: 4 hours)

- how to design sgRNAs: theory
- Design them for the region of interest (Red part of sequence)
- Compare people's solutions to our solution of sgRNA

12.30 Lunch

13.30 Conversation and discussion about how the making of Return to Dilmun in its entirety can serve as a model to teach genetic engineering as art making and a creative process and in reverse teach this type of art making a way to understand implications of genetic engineering beyond its scientific application | moderated by Laura Beloff

15.00 Continuation of in silico workshop | José, Guenter, Roland

Go through protocols for Monday

- 0) Start by explaining the overall objective of the experiment (demonstrate the targeted and specific double strand break mediated by CRISPR/Cas9)
- 1) *in vitro* synthesis of sgRNAs
- 2) Run sgRNA in a gel to confirm synthesis
- 3) Purify the sgRNA using a RNA column
- 4) Run the purified sgRNA in a gel to confirm recovery of RNA and quantification
- 5) Make the CRISPR cas9 reaction
- 6) Run the reaction
- 7) Interpret the results

16.00 wrap of the day

Day 4 | Sunday 8 November | Day of rest

Materials needed:

- bed

Day 5 | Monday 9 November | Cleavage day

Materials needed:

- Nuclease + RNase free water
- Nuclease + RNase free plastic consumables: pipet tips, tubes
- Gloves
- 70% Ethanol
- 10% Bleach
- Gel box + comb
- Agarose
- Gel dye (e.g. SYBR green)
- Molecular weight Ladder
- Loading dye
- TAE 1X
- sgRNA synthesis kit
- Spin columns for RNA cleanup

Protocols needed:

- B) Gel Electrophoresis
- C) sgRNA synthesis
- D) RNA purification with spin column
- E) CRISPR Cas9 - *In vitro* cleavage of target DNA

10.00 Go through protocols for Today (final time)

- 0) Start by explaining the overall objective of the experiment (demonstrate the targeted and specific double strand break mediated by CRISPR/Cas9)
- 1) *in vitro* synthesis of sgRNAs
- 2) Run sgRNA in a gel to confirm synthesis
- 3) Purify the sgRNA using a RNA column
- 4) Run the purified sgRNA in a gel to confirm recovery of RNA and quantification
- 5) Make the CRISPR cas9 reaction
- 6) Run the reaction
- 7) Interpret the results

10.15 *In vitro* synthesis of sgRNAs and cut | José, Guenter, Roland

- 1) *In vitro* synthesis of the sgRNAs using the Kit using protocol [C\) sgRNA synthesis \(1h\)](#)
- 2) Run the synthesized sgRNAs in a gel to determine if it worked (30min)

11.45 Purify the synthesized RNA using protocol D) RNA purification with spin column (30 to 60min, to explain the principle)

12.15 Run the synthesized purified product in a gel to see if the purification worked. Also, to allow a rough quantification of the sgRNA by intensity of fluorescence of the bands. (60min to explain the quantification principle)

12.30 Lunch

13.30 Cas9 cutting

- 1) Make the CRISPR/Cas9 reaction using protocol E) CRISPR Cas9 - In vitro cleavage of target DNA. (Here it would be interesting to use DNase as a non-target specific control, to compare what is a targeted break vs a non-targeted break) (2h)
- 2) Run the *in vitro* digestion in a gel. (30min)
- 3) Interpret the results and discuss (1h)

16.00 wrap of the day

Day 6 | Tuesday 10 November | Conclusions

10.00 Continuation of vitro and in silico activities | José, Guenter, Roland

- Load on gel to check results

Class 4 Mutate the bull's head?

- 1) Select the region to mutate
- 2) Design oligos to mutate
- 3) simulate the mutation by changing the sequence following the rules of randomness
- 4) Send sequence to Gunter and Roland for the image translation
In silico: (In silico: Cut in the 9kb sequence, simulating modification)

12.30 Lunch

13.30 Concluding discussion about Intellectual Output / Imagining and speculating new gene editing works of art, why we need them and what experimental designs are required to make them | moderated by Lucas Evers

15.00 In silico: decoding the altered DNA sequence to image | José, Roland, Guenter

16.00 wrap of the day