

Information Infrastructure(s):  
Boundaries, Ecologies, Multiplicity

Edited by

Alessandro Mongili and Giuseppina Pellegrino

Foreword by Geoffrey C. Bowker

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**P U B L I S H I N G**

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## CHAPTER SIX

# GIVE US A PROTOCOL AND WE WILL RISE A LAB: THE SHAPING OF INFRA-STRUCTURING OBJECTS

STEFANO CRABU

*What can be studied is always a relationship or an infinite regress of relationships. Never a "thing".  
(Bateson 1978, 249)*

### **1. Mapping the problem**

The attention paid by Science and Technology Studies (STS) to the relationship between human actors and technological artifacts leads us to consider social practices and situated settings of interaction as an emerging outcome of a network of socio-material relationships (Law 1994; Mol 2002; Barad 2003; Orlikowski 2007). As a whole, these relationships involve heterogeneous processes of knowledge constructions, scientific facts, routines and technologies. Particularly, in its ecological acceptance (Star and Greisemer 1989; Collins and Yearly 1992; Fujimura 1995; Suchman 2000), this perspective illustrates how social contexts are generated and permanently reshaped through complex socio-material relationships that involve classifications, conventions and standards, which are sometimes invisible to the same human actors (Mongili 2007).

As pointed out in a seminal article by Star and Ruhleder (1996), infrastructures as well as technical objects are based on specific relational ecologies and are built around particular works and social practices. The definition of infrastructure later proposed by Star (1999) seems particularly evocative and permits us to clarify the analytical position, inspired by the ecological perspective, which will be adopted in this

chapter.

“[...] infrastructure is a fundamentally relational concept, becoming real infrastructure in relation to organised practices (see also Jewett and Kling, 1991). So, within a given cultural context, the cook considers the water system as working infrastructure integral to making dinner.” (Star 1999, 380).

Following the suggestion of Star, no material entity or technical object exists for itself in the form of an immanent social fact or fixed context containing entities and actions. On the other hand, infrastructures – and in a broader sense “technologically dense environments” (Bruni 2005; Bruni *et al.* 2013) – represent a relational concept since they only occur within a plot of ecologies of action. As a result, an object becomes sociologically relevant as long as it can be considered a manifestation of organizational properties rather than something purified and separated from its context of creation and use (Star and Greisemer 1989; Star and Ruhleder 1996; Star 2010).

This debate has been particularly relevant for those scholars interested in the sociomaterial processes of production and sharing of scientific knowledge, who set themselves in line with a tradition of thought that has superseded the standard positivistic view that used to assign sociological immunity to scientific knowledge (Latour and Woolgar 1979; Knorr-Cetina 1981). In this respect, far from being a mere objective representation of nature, scientific knowledge has been described as an outcome of the cooperation between human actors, material contexts and interdependent technical entities in a relationship of mutual configuration (Latour 1990; Rabinow 1996; Cambrosio and Keating 1998; Keating and Cambrosio 2003, 2012).

Starting from the previously mentioned theoretical assumptions and based on the data collected during an ethnographic research carried out in a biomedical research centre in Northern Italy, this chapter explores in depth the processes of creation, use and *mise-en-contexte* (Latour 1992, 89) of a specific technoscientific object. Research on infrastructures and technological artifacts in general has traditionally focused on the work of coordination and cooperation made possible by the classifications, standards and protocols incorporated therein (Star and Griesemer 1989; Bowker and Star 1999). In this respect, infrastructures were analysed by looking at the people who took them for granted or the practices of design and development.

In this contribution, however, we will focus on one particular artifact, whose infra-structuring character emerges from the situated activities of

use and *mise-en-contexte*. One of the most interesting aspects, as will be shown, lies in the fact that the use and *mise-en-contexte* involve the construction and manipulation of the artifact itself. We will see how a professional community requires not only the skills to use artifacts, but also the knowledge necessary to manipulate and recreate them in everyday activities. In particular, this chapter will focus on the protocols adopted by molecular biology laboratories, which seem to be artifacts of particular relevance for the implementation and positive results of experimental practices. It will be shown how this specific component of laboratories may represent an emblematic case study, which can help thematise the infra-structuring role of technical objects as relational entities supporting the production of scientific knowledge and cooperation between different human subjects.

Assuming this case study as a starting point, we will discuss a number of theoretical issues that will be used to propose and investigate the notion of an *infra-structuring object* as a conceptual device useful for studying the processes of production and sharing of expert knowledge. In particular, assuming the concept of an *infra-structuring object*, we would like to stress how the relationship of mutual generation between technologies, human subjects and contexts implies the creation and management of particular objects that can incorporate and make transparent several elements, such as standards, regulations, pedagogies, routines, conventions and power relationships. The prefix “infra” refers to the possibility that these elements might become transparent (but not less important) during the implementation of work or experimental practices, since they are used in a routine, “natural” and consistent way within the context (except when there are unexpected ruptures), whereas the suffix “structuring” focuses on how objects are allowed to shape and re-shape the local setting, as well as how the ongoing practices may open up new potential uses of such objects. In line with an ecological perspective, the suffix “structuring” serves the need to emphasize the level of “naturalisation” (Bowker and Star 1999) rather than “stabilization” (Bijker 1995), evoking a semantic field that is akin to “plasticity” and the opposite of “rigidity”, which is rather suggested by the concept of “structural”.

## 2. Investigating protocols: research context and method

The term *protocol* can be traced back to the mediaeval Latin word *protocollum* and refers to the first sheet (*proto*) glued (*collum*) to the front page of the official records of a transaction (see Oxford English Dictionary). This document was used to specify the total value of a

transaction and could also include the name of the notary who drew up the papyrus. From an etymological point of view, the term “protocol” shows an interesting material-discursive juxtaposition, denoting a document that is ancillary to a “main text”, which is likewise necessary and contextual to the former. The juxtaposition of the technology of writing and its medium (papyrus) can be considered a precursor of modularity. The two texts could not be separated: the *protocol* was only meaningful in relation to the accompanying main text. In general, the artifact “papyrus” could circulate between a number of different contexts and places thanks to the presence of the *protocol* on the front page.

Following a longitudinal perspective, the protocols have survived to the present day, pervading the clinical world and life sciences<sup>1</sup>. Within the field of biomedicine, they accompany and regulate the experimental practices of every researcher and express the formal image of the expected outcome of the standardization and dissemination of best practices. In clinical and experimental contexts, we may find different types of protocols aimed at regulating the use of personal protective equipment, access to laboratories, extraction of human DNA, culture of cell populations, animal testing and other activities relating to diagnostic practices. As far as molecular biology is concerned, the protocol describes the set of instructions, methods, “ingredients”<sup>2</sup>, materials and procedures that make up an experiment.

In spite of the key role played by this object within laboratories and clinical areas, STS have paid little attention to the devious processes of development and situated use of these protocols. Whilst deeply involved in the investigation of research activities within a number of laboratories operating in various fields of natural sciences – such as biochemistry (Latour and Woolgar 1979; Knorr-Cetina 1981), neuroscience (Lynch 1985) or physics (Collins 1975; Collins and Harrison 1975) – works falling within the domain of *Laboratory Studies*, both classical and more recent ones (Latour 1983; Scott 1991; Knorr-Cetina 1995; Neresini 2008; Doing 2008; Viteritti 2012), have so far omitted a detailed analysis of the processes of protocol construction in relation to scientific practices.

Major contributions to the study of protocols come from the field of *Sociology of Health and Illness*, which focuses on the relationship between medical knowledge and materiality within clinical practice (Löwy 1995;

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<sup>1</sup> See Lynch (2002) for a detailed discussion on the different types of protocols from an ethnomethodological perspective.

<sup>2</sup> Based on my fieldwork, the vast majority of practitioners consider laboratory protocols as a sort of cookbook, and the laboratory is described as if it were a domestic kitchen.

Timmermans and Berg 1997, 2003; Berg 1998). These last contributions, however, fail to point out the protocol in its processual and relational components. The above authors rather preferred to adopt an analytical approach that only considers the protocol as an organizational device consisting of formal elements aimed at regulating and standardizing medical activities.

In light of these considerations, it should be noted how the protocol, even in its formal aspects, displays a relational nature that might be worth investigating. This artifact, as shown later in this chapter, becomes the generator of a heterogeneous techno-scientific context where data is to be produced by all actors in a relatively unambiguous way. Within the context of molecular biology, the need to explain natural phenomena in formal terms – in order to make knowledge available to the relevant scientific community – reveals a very strong tension between local knowledge, tacit knowledge and public knowledge (Knorr-Cetina 1981). Along with the implementation of information infrastructures (Star 1999), this tension was resolved through the systematic and consistent use of protocols, which can lead to the standardization of methods and the construction of knowledge and data that can be made publicly available. However, even in this case, the implementation of protocols may be a complex and difficult task since they require articulation in a local setting and always in different ways (Lynch 2002). For this reason, it would be interesting to investigate the ecology of actions oriented towards the construction, implementation and situated use of protocols, rather than simply focusing on the protocol in itself.

This contribution will focus on two particular types of protocols: the first describes how molecular biology articulates cell culture activities, whereas the second relates to the activities of purification of the plasmid DNA produced within the laboratory. The following considerations are based on a broader five-month ethnographic research that was carried out within a leading institute of molecular oncology located in Northern Italy. The empirical material discussed here was collected during a 12-week ethnographic observation (Silverman 1997) of a team of molecular biologists. At the time of the research, the team was in the process of forming an independent research unit consisting of three junior researchers assisted by a senior biologist, a laboratory technician and occasionally other practitioners with suitable experience in laboratory activities<sup>3</sup>. From a methodological point of view, great attention has been devoted to

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<sup>3</sup> For the purpose of privacy and confidentiality, practitioners are hereinafter referred to by fictitious names. All names and sensitive information were also removed from the empirical material used in this chapter.

everyday activities conducted by the three junior researchers. By following a team of novice researchers, it became possible to investigate the activities of construction and use of protocols along with the learning practices of scientific work. More precisely, some ethnographers believe that investigating a team of novices may be a good strategy for becoming aware of a number of problems, doubts and difficulties typical of those who, lacking an in-depth knowledge of a specific practice, do not necessarily take existing data for granted (Schwartz and Jacobs 1979; Angrosino 2005). Novices are defined as such since they need to follow an apprenticeship scheme, which allows ethnographers to collect valuable data they could not normally access. To some extent, it will be the novice – in the guise of the ethnographer – who will reasonably ask questions to experienced researchers and stimulate detailed and valuable reports. By implicitly delegating the task of asking questions, the ethnographer may obtain detailed information on experimental practices without the need to disrupt everyday activities within the laboratory. On the other hand, following the senior biologist and laboratory technician presents the ethnographer with an opportunity to observe the styles and practices for the transmission of expert knowledge, which will then be stratified within the protocol.

The ethnographic fieldnotes, including the dialogues intercourse among actors, have been fully transcribed in digital format. The coding of the transcribed material was carried out through a qualitative analysis software, following the principles of constructivist grounded theory (Charmaz 2006, 2009). More precisely, the adoption of a Grounded Theory-driven process of data coding initially enabled the emergence of descriptive labels. At a later time, this process was accompanied by the development of more theoretical labels oriented by an ecological approach in order to consider all the elements involved in the creation, use and contextualization of the protocols.

### **3. Protocols in action: the shaping of an infra-structuring object**

Starting from two emblematic ethnographic accounts, we will now examine the ecology of actions oriented towards the construction and *mise-en-contexte* of two different protocols. In the first account, the protocol relating to the culture of cell lines was “hand-built” by the practitioners themselves, whereas in the second account, the practitioners were involved in the “personalisation” and contextualisation of a protocol supplied to the laboratory by a Biotech Company operating on a global



scale. This last protocol relates to the purification of the plasmid DNA.

### 3.1 Cell culture and the handmade protocol

In this section we will see biologists getting involved in the development of an experimental method aimed at identifying the sequence of actions necessary to ensure that an immortal cell line – known as HeLa<sup>4</sup> – reproduces itself in a suitable environment. These cells offer an experimental biological model, which will be used for the implementation of biomedical research activities within the laboratory, such as cytotoxicity testing of molecules that are potentially therapeutic for human beings. The results should be published in specialised international journals. For this reason, it is extremely important that the practice of cell culture is suitably learned by all staff members.

“At 10:20 AM, Luigi, the senior biologist, interrupts the three young biologists, who were busy writing some notes, and says: "If you write the protocols in pdf format, preferably in English, they can become part of the laboratory internal documentation, which can then be used as reference by other practitioners and foreigners who will join the lab in the future". Luigi then starts describing in great detail the set of activities to be carried out. Chiara, Pippo and Leila – the three young biologists – carefully take notes using pen and paper. After a few minutes, Luigi stops and says: "Let's start the test, come on! Ask Colombo for the cells. He will lend us a flask. If you experience any difficulties, you can find me at the director's office. After a few minutes I follow Chiara along the corridor and, somehow irritated, she says: "When Luigi speaks and gives instructions... I mean, it is something that requires concentration. He says things in a rush, without giving you the time to... What are you doing? You have to take note immediately so that you can start working and testing. Under the bio-safety cabinet, I say. Because you will have to write a description of the methods. In other words the protocol needs to be accurate.”

This episode shows how the protocol goes through a number of steps during its development, before it can reach a suitable configuration for circulation within the laboratory itself. As in the situation described above, the process involves moving from one text to another, from oral exchanges to written drafts (Figure 6-1), until an object is developed that will become

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<sup>4</sup> HeLa cells consist of a type of immortal cell line widely used in cancer research. See Landecker (2007) and Skloot (2010) for further details about the generation of this cell line. For a discussion on the technoscientific implications of HeLa cell line, refer to Casati *et al.* (2012).

collectively relevant when it is shared.

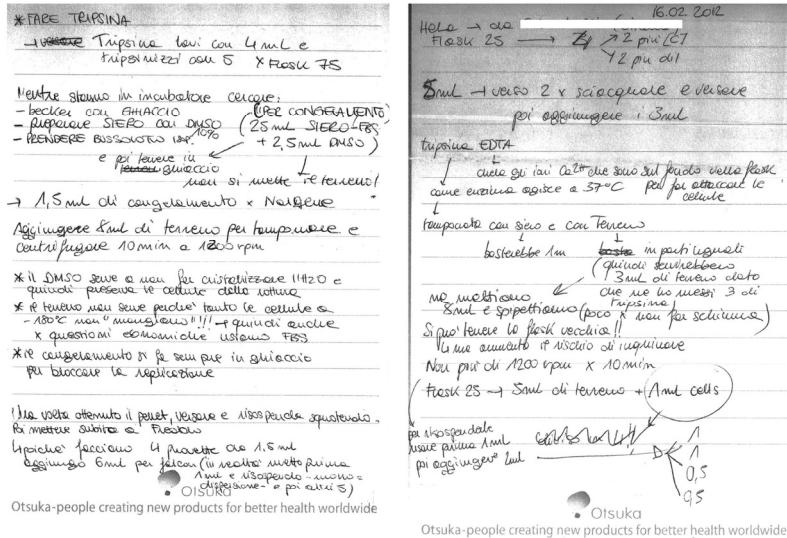


Fig. 6-1: laboratory handwritten draft.

Based on the description provided by Luigi, the three junior biologists share ideas and compare their notes for consistency, before starting the experimental activity under the biosafety cabinet with the help of additional artifacts and technologies.

In their rough draft notes, as shown in Figure 6-1, the junior biologists write down any information they may find relevant for the construction of the final protocol. These notes also include a number of particularly colourful expressions coming directly from the jargon of experimental practice, for example: “agitare un po’ la fiaschetta” (lightly shake the flask), “spipettare almeno dieci volte per staccare bene le cellule” (pipette at least 10 times to detach the cells), “dare colpetti al fondo della fiaschetta” (tap the bottom of the flask), etc. These expressions will then be removed from the finalised protocol, which will be prepared in electronic format, printed and distributed in electrostatic copy. The construction of the protocol requires ongoing negotiation and careful consideration on the part of Chiara, Pippo and Leila during the first experimental practices. This stage basically includes the initial testing of the protocol for usability, where an attempt is made to align, in a

meaningful way, orality (oral description), writing (notes), technologies (biosafety cabinets, pipettes, centrifuges, reagents) and biological entities (cells).

The use of pen and paper, and eventually a word processing tool, shows how the process of protocol construction takes place through the mediation of different technological genealogies, which should be aligned, layered and made mutually compatible. These technologies enable the senior researcher to translate the smooth, approximate and narrative description of experimental activities into usable documentation. Junior researchers, for their part, have the opportunity to learn specific relational skills that allow them to note down in a didactic and sufficiently clear way the basic concepts for the design and implementation of experimental activities: technologies to be used, places to cross, reagents and time scales. The translation of the oral description into written notes, and eventually a computer-processed protocol, should be accurate, concise and consistent. The finalised and ready-to-use protocol (Figure 6-2) must be capable of circulating within the laboratory and the drafter should have in mind different recipients and users other than himself. In this respect, the style and register of the finalised protocol will be aimed at a greater formality, behind which lie routine, skills and knowledge taken for granted and treated as common heritage by the professional community.

Preparazione terreno DMEM (500ml) :

- FBS 10% (50ml) → NB: sarebbe opportuno togliere i 50ml di DMEM e portare a volume con FBS (il DMEM prelevato può essere conservato a parte in falcos)
- PENSTREP 100X (5ml)
- PLASMOCIN (25µl)

Aliquota di cellule fornita dal gruppo \_\_\_\_\_, O2 :

**HeLa – conservate in DMSO (flask25)**

**PASSAGGIO CELLULE**

Prima di iniziare:

- Guardare al microscopio lo stato delle cellule

Poiché le cellule sono a confluenza, si è pensato di:

- Dividere le cellule in 4 falcos (due con una C) (raggiare e due più diluite)

Per prima cosa, si elimina il terreno per rovesciamento in un becker contenente in volume minimo di candeggina.

Dopo che le cellule vengono **risospacciate**, prelevati 5ml di trypsina, versati 2ml per effettuare un lavaggio del terreno residuo (poi eliminato per rovesciamento) e infine messi 3ml e lasciati ad incubare a 37°C per circa 5 minuti.

La trypsina, infatti, è un enzima che agisce a 37°C e viene aggiunto insieme all'EDTA il quale svolge la funzione di **chelatare** per gli ioni  $Ca^{2+}$  che sono sul fondo delle falcos e favoriscono l'attacco delle cellule alla superficie.

Passati i 5 minuti, vanno tirate fuori dalle incubatrici e si sbatte per bene la falcos per favorire il completo distacco delle cellule; si tampona la trypsina con siero (basterebbe 1ml) e/o terreno in parti uguali al volume di trypsina aggiunto (quindi 3ml di terreno). Mettiamo 8ml di terreno e **assettiamo** con **aliquota** almeno una decina di volte facendo attenzione a non fare bolle (queste provocano la rottura delle cellule) e si trasferisce in falcos da 15ml.

Centrifugare per 10minuti a 1200rpm.

Eliminare poi il **supernatante** per rovesciamento, lasciando una piccola parte del volume di **supernatante**; ciò che resta del **supernatante** è utile alla **risospaccatura** dei pallidi di cellule (sbattere delicatamente la falcos). Alla **risospaccatura**, vengono aggiunti 3ml di terreno fresco e si **spipetta** con pipetta da 2ml; viene prelevato tutto e distribuito aggiungendo a 5ml di terreno 1ml in due falcos 75 e 75ml nelle altre due falcos 75. Abbiamo così anche le falcos (anche se facendo

5ml di terreno fresco	5ml di terreno fresco
1ml di cellule	0,5ml di cellule

Dopo un week end lasciate ad incubare, al microscopio ottico si può osservare la situazione seguente: **separata in mezzo**. In tutte e tre le falcos c'è stata adesione a bassa intensità, ma nella falcos delle cellule recuperate dalla sospensione ce ne sono meno.

**CONGELAMENTO**

Prima di iniziare:

- Preparare due becker con del ghiaccio
- Il congelamento si fa sempre in ghiaccio per bloccare la replicazione delle cellule.
- Preparare "TERRENO DI CONGELAMENTO": FBS +10 %DMSO
- DMSO serve a non far cristallizzare l'H<sub>2</sub>O e quindi preserva le cellule dalla rottura
- NB: tenere a 4°C e poi in ghiaccio mentre si lavora sotto capotti!

Eliminare il terreno per rovesciamento.

Aggiungere 2ml di TRIPSINA (4ml per falcos75) per lavare e disperderlo su tutta la superficie. Rimuovere subito.

Aggiungere altri 3ml di TRIPSINA (5ml in falcos 75). Disperderla bene su tutta la superficie (con delicatezza per evitare che il distacco avvenga a gruppi). Quindi incubare per 10min a 37°C.

Prelevare le falcos e scuoterle dando dei piccoli colpi lateralmente.

Aggiungere terreno: 8ml in falcos 25 e 11ml in falcos 75. **Sopiettare** bene almeno 10 volte lavando la superficie dove erano **adese** le cellule, cercando di ottenere una sospensione di singole cellule.

Trasferire 2ml di sospensione cellulare in falcos (una per ogni falcos) e centrifugare 10min a 1200rpm.

Dopo la centrifuga si vede sul fondo il pallido di cellule (bianco), e il **supernatante** viene eliminato per rovesciamento lasciando una piccola parte di volume al fine di non rischiare di perdere le cellule e per favorire l'immediata **risospaccatura**. La falcos viene messa subito in ghiaccio.

Il congelamento delle cellule va fatto in crioviali (NALGENE) contenenti 1,5ml di cellule rissosate in

Fig. 6-2: ready-to-use protocol.

The three junior researchers actively cooperate and share ideas during experimental practice. They discuss and negotiate in order to identify the best procedure to be used for the development of a “scientifically sound and functional” method. This becomes clear when the three inexperienced biologists are assisted by Gina, an expert laboratory technician.

**Leila:** “Gina, how long do you leave HeLa cells in the incubator? Ten minutes?”

**Gina:** “What? Ten minutes is too long. Come on, that is way too long.”

**Leila:** “The other cells, I used to incubate them for five minutes.”

**Gina:** “Do you mean exactly 5 minutes? Well, roughly... I never take the time. At least that's how I do it... in a while you are going to take them off and observe them through the microscope to make sure that everything is OK. My dear, you just have to get used to it. I have been working on cells and toxicity testing of drugs for many years. And in that case you have to be extremely careful. You cannot make mistakes when preparing toxicity bioassays [assessment experiments]. But do not worry, you are just starting out” [laughter] While we are waiting for the incubator, I'll go get a becher with some ice. Working on cells requires low temperatures, but you know that, don't you? And I cannot see any ice round here!”

**Leila:** “Well, I don't know. But Luigi told me that I should follow you and do as you do. He always tells me that I should do as Gina does.”

**Gina:** “Well, everyone knows that. And now you know it too.” [laughter]

Based on the above excerpt, we are able to highlight the key role played by discursive practices in the process of protocol configuration. As in other productive organisations, even in the world of science, narratives should be treated as a work tool (Gherardi 2012). The words of Gina, a particularly influential practitioner within the laboratory, transmit local knowledge that resists formalisation, while denoting technical mastery and professional community membership. It is a narrative knowledge (Bruner 1990) that supports both the construction of the protocol and the learning of scientific work. While hearing these stories, apprentices endorse them, experience them first-hand and begin to learn in a competent way the meaning of “being an expert biologist”. In this case we are not in front of an emic representation of science as knowledge oriented by the principle of falsifiability (Popper 1992). The words of Gina rather show a sort of narrative knowledge (Bruner 1990) oriented by the principle of plausibility, which contributes to the structuring of the experimental style and skills of junior researchers. These skills will be gradually acquired by apprentices and will become part of the protocol, thus becoming (inter)textual, invisible and indisputable, as long as that protocol is needed to keep the cell line alive.

A second remark concerns the microphysics of power (Foucault 1972) that lies under the process of protocol configuration. In this case, what confers power and authority to Gina's utterances is not so much her formal status, but rather her experience and the ability to convey professional knowledge that requires considerable communication skills. These attributes subvert the formal hierarchical order to the extent that it is Luigi himself, a senior researcher, who asks the apprentices to scrupulously observe Gina's instructions. Despite Gina's formal educational background, which is definitely less qualified than the apprentices, she is able to manage the knowledge and ability to mediate between technologies, biological entities and human subjects, and this confers her a key role within the laboratory. The practices of expert knowledge transmission, in this case, show how power is not distributed in a dichotomous and formal way, which introduces a clear distinction between dominated and dominants. Power is not localised, unilaterally exercised and discretionarily held by someone. Power, as a relational concept, is rather exercised through a changing, ubiquitous and widespread relational ecology. During this first ethnographic account, the construction of a ready-to-use protocol called for an ecology of actions that might have been particularly relevant for the learning activities of scientific work. At this stage, we are able to identify a number of features that can help clarify the nature of the protocol as an infra-structuring object.

First of all, the setup of methods highlighted its "structuring" dimension. As a result, the laboratory has been processually reconfigured, not only as a place of knowledge production, but also as a pedagogical educational setting (Fenwick and Edwards 2012; Viteritti 2012), where knowledge acquisition and sharing take place in a considerably different way as compared to higher education systems. In this respect, the protocol triggered a number of subjectivation processes that can affect the identity development of the actors involved. Gina is not simply a lab technician, she is a "teacher" and custodian of expert knowledge. Novices, on the other hand, will learn new skills and techniques and will reconfigure their identity towards the profile of the "professional biologist".

In addition, the method setup and development ensure incorporation into the protocol of the (inter)textual dimensions (knowledge, pedagogies, power relationships, routines and conventions) which will, through use, assume an invisible and transparent cogency.

### **3.2 DNA purification and profaned protocol**

"Gina is preparing the list of orders. I was surprised by the cost of some products. Gina explains to me: "KITs are very expensive. Some of them

may cost up to 10,000 EUR. They should bear the CE marking, should be standardized and so on... I mean, for certain things you would only use KITS now. As explained by the directors, you need to specify what KIT you use every time you publish an article.”

In the last few decades, scientific laboratories have increasingly been using standardized instruments called KITS. They include a number of heterogeneous elements (reagents, solutions, buffers, pipettes, instructions for use, and so on) with nomenclature and standard features for the optimisation of experimental practice. These biotechnological entities are produced by Biotech Company and are always accompanied by a protocol containing the relevant operating instructions. By way of introduction, a KIT can therefore be understood as a tool that incorporates a reified and standardized form of knowledge. In this section, we will see what happens when experimental activities require the use of a KIT and a protocol that is produced by an external organisation.

In this case, biologists will be engaged in the *mise-en-contexte* of a standardized method for the purification of the plasmid DNA<sup>5</sup> previously produced *in vitro* within the laboratory itself. Once ready to use, the KIT protocol raises questions typical of technologies that require articulation within a local setting (Latour 1993). Following the ecology of actions that are put in place “to accommodate” the KIT within the laboratory will help us better understand the infra-structuring nature of protocols.

“Pippo is carefully writing down some notes on the KIT protocol. He lays down his pen on the counter and says to me: “I have added Luigi’s instructions. Look, there are two techniques to purify DNA. The double centrifuge, also included in the KIT, and the other one with the sterile gauze. Here we use the one with the sterile gauze. Our centrifuge does not seem to be suitable for this KIT. That’s how we do it in our lab. I mean, if you have laboratory skills, you can also do it your way. But only because you have experience. For instance, Luigi, who has ten years’ laboratory experience, may slightly deviate from the KIT protocol.”

The situation just presented suggests very clearly how a standardized protocol requires a process of translation within local settings (Latour

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<sup>5</sup> In molecular biology, the use of plasmids (small DNA molecules) is very popular since it allows biologists to multiply specific genes of interest by means of bacterial colonies. Purification of plasmid DNA is an operation of genetic manipulation that is rather important in biomedical research, and the ability to perform it further, strengthens the sense of belonging to the professional community of molecular biologists.

1987, 1993). In this case, it is necessary to transpose other artifacts, procedures, conventions and standards from one context to another through local adaptations. Adaptation processes are complex and operate at different levels. They can be mainly found in the official protocol. This text is transformed and customised (Figure 6-3) by junior biologists in relation to their situated learning trajectory and any technologies they use that are not covered by the official document. This happens because the laboratory, which was expected and inscribed in the KIT by Biotech Company, does not match the experimental setting at Pippo’s disposal. Through its situated articulation, the official protocol is corrected and amended based on local technologies (e.g. the centrifuge), conventions and situated experimental practices.

13. Precipitate DNA by adding A. 3.5 ml or B. 10.5 ml (0.7 volume) room-temperature isopropanol to the eluted DNA. Mix and centrifuge immediately at  $\geq 15,000 \times g$  for 30 min at 4°C. Carefully decant the supernatant.
 

*(MIX OMOE 110000 rpm)*

*NOTE: se si usano tubi con tappo a vite, si deve lasciare riposare il campione per 10 minuti prima di centrifugare.*

*ATTENZIONE → OMI SPEED 10000 TIME 60 MINUTI B. 10.5 ML DUTCE TEMP. -10 20 +10 20*

*minimize salt precipitation, overheating of the sample. tubes can be used for anal pellets have a glassy oily, salt-containing pellets inside of the tube before isopropanol pellets are also re should be taken when START*
14. Wash DNA pellet with A. 2 ml or B. 5 ml of room-temperature 70% ethanol, and centrifuge at  $\geq 15,000 \times g$  for 10 min. Carefully decant the supernatant without disturbing the pellet.
 

*Alternatively, disposable conical-bottom centrifuge tubes can be used for centrifugation at 5000 x g for 60 min at 4°C. The 70% ethanol removes precipitated salt and replaces isopropanol with the more volatile ethanol, making the DNA easier to redissolve.*
15. Air-dry the pellet for 5–10 min, and redissolve the DNA in a suitable volume of buffer (e.g., TE buffer, pH 8.0, or 10 mM Tris-Cl, pH 8.5).
 

*Redissolve the DNA pellet by rinsing the walls to recover all the DNA, especially if glass tubes have been used. Pipetting the DNA up and down to promote resuspension may cause shearing and should be avoided. Overdrying the pellet will make the DNA difficult to redissolve. DNA dissolves best under slightly alkaline conditions; it does not easily dissolve in acidic buffers.*

*SOLUZIONE DEI BUFFERS → PASTA DI Glicerolo + acqua = PASTA DI acqua + PASTA DI acqua = PASTA DI acqua + PASTA DI acqua*

*→ Rimozione delle endotossine (purificazione del DNA plasmidico)*

*USARE QUESTA PASTA: acqua + glicerolo + acqua*

**Procedures for Endotoxin Removal**

**During the final stage of DNA preparation**

*Note: The procedure described below was performed on plasmid DNA produced in E. coli DH5α cells.*

- Losses of up to 50% of the DNA are expected.
- Use of a DNA concentration above the recommended 1 mg/ml reduce the efficiency of the procedure.

**1.** Pipette 500 μl of the DNA solution into a sterile microcentrifuge tube.

**2.** Add 50 μl of the 3 M sodium acetate solution to the DNA sample. Allow to sit at room temperature for 10 minutes.

**3.** Incubate on ice for 30 minutes.

**4.** Add 100 μl of cold 100% ethanol.

**5.** Mix thoroughly and incubate on ice for 10 minutes. *The solution should be light blue and clear.*

**6.** Incubate the tube at 27°C for 30 to 60 minutes or until the phases separate.

**7.** Spin for 5 minutes at 3000x g in the microfuge. The upper phase is colorless and microcentrifuged. The lower phase is blue.

**During an earlier stage of DNA preparation**

*This procedure is based on the alkaline lysis of E. coli DH5α cells. The endotoxins are removed immediately after alkaline cell lysis, neutralization, and a clarification step. The resulting high salt solution is suitable for the endotoxin removal step. The procedure used is 'endotoxin free' conditions. The plasmids used in other sterile and disposable, or H2O2-treated. The buffers are prepared with endotoxin free water.*

1. Add the Endotoxin Removal Solution (0.2x volume) to the cell, create DNA solution.
2. Incubate on ice and mix occasionally by inversion to obtain an homogeneous, clear blue solution
3. Incubate at 37°C for 20 to 30 minutes until the phases separation is obvious.
4. Spin for 5 minutes at low speed (3000 x g) at room temperature.
5. Transfer the upper aqueous phase to an endotoxin free container.
6. Proceed with the DNA purification by any method. Use endotoxin-free buffers and containers.

Fig. 6-3: additional text used to contextualize the kit.

Moreover, it may happen that the official protocol is not sufficiently clear and requires additional interpretation. In this case, junior researchers, assisted by an expert biologist, refer to other texts (Fig. 6-3) – often found on the web – that will complement the official method included in the KIT. These activities of textual stratification, as in the case of the *handmade* protocol, are closely intertwined with learning practices. Junior researchers working on the *mise-en-contexte* of the KIT acquire new techniques and skills together with the ability to carry out protocol usability testing. In this case it seems appropriate to use the image of “flirt” (Bruni 2011) as a metaphor for the interpretation of the relationship between human subjects and heterogeneous materials. Through the situated use of KITs, biologists establish an endless game of sociomaterial relationships so that the KIT and method can interact with the local experimental setting. This triggers an ecology of actions showing the attempt to redefine and re-contextualize

official practices. Through the metaphor of “flirt” a set of local knowledge, conventions, habits and uses (Knorr-Cetina 1981) are juxtaposed and layered as (inter)texts in the official protocol. Through the daily use of the KIT, this knowledge becomes routine, ends up being taken for granted and is eventually naturalized (Latour 1993), if only temporarily.

At this stage, it would be interesting to further investigate what happens within the context of a laboratory when working with the KIT and its protocol.

“While giving instructions to Chiara and Pippo, Pamela (senior biologist) says: “You see, we work more and more often with KITs. Before we used to do everything by hand. We used to prepare the KIT ourselves. And then, you know, there was no official document or hardly any. All you had was your own notes. But it was by oral transmission. You had to work side by side with someone more experienced than you and you just had to get used to it. Now, you see, everything is standardized and you end up losing a number of skills and abilities. The funny thing, though, is that the KIT always shows the word “PROB”<sup>6</sup> in plain view, and that’s how they cover their back. But why? Because, as I usually remind young people, you also need to be able to operate the KIT. I mean, there is a set of margins to be taken into account when using it.”

Pamela’s words suggest how the process of KIT articulation results not only in a transformation of the official methods, but also in the reconfiguration of the local experimental setting. This is possible because the KIT incorporates knowledge, standards and conventions that may affect established practices or make them obsolete and transform the way of learning and teaching scientific research practices. The *mise-en-contexte* and naturalisation of a KIT protocol can therefore be interpreted as the emerging outcome of the conflict between formal requirements and situativity. This conflict finally requires a process of articulation involving the structuring of local settings. Within the situated setting, the initial object is modified and the process stimulates the transformation of the setting itself.

Focusing on the *mise-en-contexte* of the protocol we were able to analyse the ecology of relationships that come to be established between actors (not only humans) shaping experimental practices. However, another important point needs to be made. Thanks to their incorporation of

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<sup>6</sup> The “PROB” marking indicates that analyses performed using a KIT are probabilistic and are always subject to a margin of error. This allows Biotech Company to decline any responsibilities towards individual laboratories in case of error.



standards – officially recognised by the international scientific community – the KIT protocols always bear a “solid” component (e.g. the standards relating to reagents). This component never dissolves within a plurality of contexts. This feature enables the production of scientific data that can be circulated on a global basis through publications in international journals. However, further adjustments are always possible, and sometimes even necessary, in order to keep the dialogue between ready-to-use methods and local settings alive.

“Gina is explaining to Giovanna (a trainee) the need to constantly update the laboratory logbook. With an assertive tone, Gina says: “The logbook should document everything, especially when there is something new. This is because the operators in charge of the experiment can clearly see what you did. For instance, it may happen that one of the reagents included in the KIT might have been replaced and you wouldn’t even realize because you know the protocol by heart and nobody noted this down in the laboratory logbook. We have already experienced similar situations in the past.”

This conversation reveals the involvement of practitioners in an endless process of maintenance of the protocol in relation to its context of use. Once again, this can be achieved through the (inter)textual juxtaposition and the introduction of other objects (e.g. the laboratory logbook), the emergence of which was stimulated by the method included in the KIT. In this respect, the activity of *mise-en-contexte* should be interpreted as an outcome rather than a precondition dictated by the alleged cogency of the organisational structures within the laboratory. The relevant protocol enables a broader techno-scientific project requiring continuous adaptation between protocol and context, and between pre-inscription and use.

In this section, the analysis of the processes of *mise-en-contexte* allowed us to better understand the key role played by infra-structuring objects in shaping the relationship between human actors, technologies and experimental contexts. We have seen how the protocol may lead to the creation of new texts that will be circulated within the laboratory. At the same time, the protocol has come to incorporate a heterogeneous set of conventions, routines, habits and uses that were stimulated by the relationship between KIT components, human subjects and local setting.

#### 4. The protocol is not only a data-shaping device...

Based on some distinct ethnographic accounts, we have seen how the protocols, unlike their apparent rigidity, may be open to potentially new and subversive uses of the action plans included therein through their situated use (Suchman 1987). Similarly, when methods are locally constructed and/or articulated, they enter daily practices, becoming part of the techno-scientific background to whose generation they contributed. Having (inter)textually incorporated procedures, classifications, standards, technical convergences, organisational processes and power relationships, protocols may be considered socio-material entities that turn scientific research practices and their organisation into a visible and justifiable disciplinary regime of truth. In this way, scientific knowledge can be communicated to the whole scientific community.

Both ethnographic events show a common tendency to consider protocols as infra-structuring objects. This suggests the focus should be on the ecology of actions that come to be established between knowledge, scientific practices, human subjects and technologies. On the basis of the above considerations, we are now able to identify more precisely the infra-structuring nature of protocols.

A first dimension of the protocol that allows us to conceptualise it as an infra-structuring object lies in its modular character. In particular, this refers to the “infra-” dimension of the object. We have seen how the protocol incorporates a number of different elements in a stratified way. The concept of modularity is particularly relevant for the identification of the different layers forming the infra-structuring object, including certifications (e.g. nomenclature of reagents and biological entities), standards (e.g. reaction time, concentration of reagents, solutions and buffers), pedagogies, technical negotiations and different technological genealogies (written notes, digitisation, computer processing), which become a transparent and naturalised routine through use. In this case, the most prominent feature is the fact that the infra-structuring object cannot be modified in full following its construction. Each change may involve a specific level and should be integrated with other levels through little adjustments. Moreover, given their stratified nature, infra-structuring objects are never created *ex novo*, but rely on an existing infrastructural basis (e.g. the centrifuge available in the laboratory), which shapes their qualities and attributes.

A second feature, which is closely connected to the “structuring” dimension, is the ability of infra-structuring objects to transpose artifacts, information, standards and conventions from one context to another.

Practitioners attempt to contextualise these elements by “flirting” (Bruni 2011) with the protocol. To do so, it becomes necessary to develop organisational routines and innovate experimental practices through the cooperation between human subject, technological devices and biological entities (e.g. cells, plasmids and DNA). This characteristic contributes to the generation of the techno-scientific context, which should not be treated as a pre-existing and unchangeable social fact.

Finally, the third feature that allows us to establish a connection between the two dimensions described above refers to learning and knowledge. As we have seen through the analysis of scientific laboratory protocols, infra-structuring objects are complex and heterogeneous sets of elements, which, once naturalised, become part of the current scientific practices now taken for granted and institutionalised. As a result, the ability to use protocols allows novices to become part of an institutionally recognised professional community. This latter feature, being based on work practices reproduced and supported collectively, is the main carrier of the socialisation processes of novices. The protocol can therefore be considered an anchor for learning and is able to transform the laboratory into a teaching setting. Ultimately, this aspect implies the ability to manage the different knowledge regimes incorporated into infra-structuring objects. The creation and *mise-en-contexte* of protocols is therefore intertwined with a set of situated abilities (Suchman 1987) that exceed the formal and paradigmatic level. These skills allow actors to carry out their daily activities and are particularly relevant in the socialisation processes of novices.

The above considerations show the ambivalent nature of protocols, which cannot simply be considered as devices to “extract order out of disorder” (Latour and Woolgar 1976, 36-37) and produce scientific data. The interpretative lens offered by the ecological approach shows a much more complex set of elements, which can guide us through a broader final analysis of the concept of infra-structuring objects.

Skeptical observers may question if the protocol can be considered, more simply, just a boundary object (Star and Griesemer 1989; Star 2010). Star and Griesemer developed their concept to take into account the coordination and management of work through multiple and divergent actors. More precisely, the contribution of Star and Griesemer is, therefore, related to the problem of how members of different social worlds interact, and they argue that a boundary object can facilitate the multiple translation of knowledge to arrange a settlement between multiple social worlds. My work, in contrast, did not limit its focus on cooperation between actors from different social worlds. An infra-structuring object differs from a

boundary object, because it is used by scientists of the same professional community to define a space of technical work. In this sense, the concept proposed here is less abstract, and it helps to understand how a technoscientific context becomes less ambiguous and less amorphous. In this sense, an infra-structuring object does not serve only as a communication interface between different social worlds, but rather as a device of dialogue between human actors who have to share the same line of work and resources (e.g. materials, roles, skills and tools).

### 5. ... it is an infra-structuring object!

Following the daily activities of creation and contextualisation of the protocols used in molecular biology, the aim of this chapter was to show how the infra-structuring object is the emerging outcome of an ecology of actions in the form of written notes, narratives, scientific experimentation and so on. Of particular interest is the type of ecology implemented for/by the *infra-structuring object* itself. In this respect, what is more interesting is not so much the purposeful evolution of these objects, but rather the ongoing naturalisation of conventions, routines, practices and knowledge through/in the protocol. Ultimately, the infra-structuring object accompanies human subjects into everyday practices, proving to be an individual and collective resource allowing the interaction between knowledge, artifacts and technologies within a space that covers the molecular (e.g. *in vitro* molecular activities) and global levels (e.g. publication of results in international journals).

In light of these considerations, we are able to identify three main features that could allow the extensive use of the concept of an infra-structuring object as a conceptual device useful for the study of the contemporary processes of knowledge creation:

1. **Heterogeneity:** the infra-structuring object is the multidimensional and reticulated expression of a set of heterogeneous elements. These elements include discursive and non-discursive practices, narratives, technologies, biological entities, conventions, routines, standards and classifications;
2. **Situativity:** the infra-structuring object is sufficiently plastic to undergo modifications, specific transformations and local articulations. From an analytical perspective, local articulations activate situated relationships of power between the actors involved in the management, sharing and

acquisition of a set of abilities oriented towards the use and *mise-en-contexte* of the object itself;

3. **Generativity**: the infra-structuring object includes a number of sufficiently solid components that can generate transformations of the local settings of use. These elements also enable the mediation and interaction between different practices distributed on a molecular, local and global level.

As a result, infra-structuring objects manifest a hybrid character, which allows for the joint analysis of human actors, technologies, practices of knowledge production and power relationships that hold these elements together. In this respect, infra-structuring objects stimulate the combined action of human subjects, technologies and knowledge. In particular, they support collective action, allowing the interaction between human subjects and other technological objects. From a theoretical point of view, this concept consents to focus on the practices of creation and use of specific technological artifacts in order to identify the ecology of actions needed to build and use them.

Finally, infra-structuring objects also grant mediation between the material and social dimension. Potentially, any configuration between social and material dimensions could be naturalised. However, this naturalisation always represents a plastic outcome that is open to change.

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