Ethoscopes: an open platform for high-throughput *ethomics*

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Authors' contributions: QG, LGR and GFG designed the platform; QG and LGR wrote the software; QG and EJB performed the experiments; ARJ contributed the optomotor module; ASF contributed the AGO module; all authors contributed the manuscript.

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Abstract

We present ethoscopes, machines for high-throughput ethomics in *Drosophila* and other animals. Ethoscopes present four unique features: they provide a software and hardware solution that is reproducible and easily scalable; they perform not just real-time tracking, but faithful real-time profiling of behaviour using a supervised machine learning algorithm; they can stimulate flies in a feedback-loop mode; they are highly customisable and open source. Ethoscopes can be easily built using 3D printing technology and they rely on Raspberry Pi and Arduino to provide affordable and flexible hardware.

Introduction

Understanding how behaviour is encoded in the brain is one of the ultimate goals of neuroscience. In particular, much of modern neurobiology focuses on finding the genes and the neuronal circuits underlying simple and complex behaviours alike, aiming to describe and ultimately understand how the brain processes sensory inputs into motor outputs. For many years, starting from Seymour Benzer's seminal work[1], the fruit fly *Drosophila melanogaster* has been considered the model organism of choice to dissect the genetics of behaviour. In the past decade, *Drosophila* has also emerged as an excellent model for studying not only the genes, but the neuronal circuitry of behaviour too: the combination of a rapidly delineating connectome together with an unrivalled repertoire of genetics tools has established fruit flies as the most promising animal model to study neuronal circuits. Optogenetics, thermogenetics, a genome wide collection of RNAi lines, and a plethora of crafted and carefully described GAL4 lines constitute an unprecedented arsenal for neurobiologists interested in studying the neuronal circuitry underpinning behaviour. The limiting factor for *ethomics* - the high-throughput approach to behavioural studies - is therefore not the availability of genetic tools, but the access to an objective, reproducible and scalable system to detect and categorise behaviour. In the past few years, several computational approaches were introduced to address this limitation: some specifically dedicated to a subset of behaviours, like sleep[2–4], and others designed to be more versatile[5–8]. However, while computer-assisted analysis of behaviour has the potential of revolutionising the field, adoption and throughput of currently available techniques are limited by several factors: among others, the scope and versatility of the software itself and the requirement for a non standardised hardware-setup, with relevant problems of cost, footprint, and scalability. Here we present a tool aimed at solving these issues, providing an affordable and versatile complete suite to study *ethomics*: ethoscopes.

Results

Principle and scope

An ethoscope is a self-contained machine able to either record or detect in real-time the activity of fruit flies (and potentially other animals) using computerised video-tracking. It relies on an independent small single-board computer (Raspberry Pi[9]) and a high-definition camera (Raspberry Pi camera) to capture and process infrared-illuminated video up to a resolution of 1920x1080, at 30 frames per second (**Fig. 1a**). Ethoscopes are assembled in a 3D-printed case measuring approximately 10x13x19 cm (**Fig. 1b**). Although we recommend 3D printed assembly for research-grade use, we also provide detailed instruction to build a fully functional ethoscope out of LEGO bricks (**Fig. 1c**, - LEGOscope **Supplementary Material S1**) or out of folded cardboard (**Fig. 1d**, - PAPERscope **Supplementary Material S2**). These latter two options are mainly aimed at education and outreach but they may also be adopted in a real laboratory environment. The technical drawings required to 3D-print and assemble an ethoscope, along with its software (Python code on a Linux instance) are released under the open source GPLv3 license and freely available on the ethoscope website (https://lab.gilest.ro/ethoscope). The combination of consumer-grade electronics, 3D printing and free open source software results into a total cost of about £80 for each machine. Limited cost, along with the fact that each ethoscope relies on its own computing power, allows for easy scaling of the entire platform. Assembly of ethoscopes require very little technical skills and can be accomplished in few minutes. Detailed and up-to-date instructions for assembly are also found on the website. All software is also provided as burnable images, so that the end user can easily transfer the entire operating system either on SD cards to fit inside each Raspberry Pi, or on a CD to work as controlling unit ("the hub" in **Fig. 2**).

Usage scenario

In a typical usage scenario, several ethoscopes are placed in a climatic controlled chamber, each powered through a USB cable; ethoscopes connect via WIFI to a computer acting as data collecting station ("the hub" in **Fig. 2**) and are controlled remotely via a graphical web interface (**Fig. 2** and **Supplementary Video S1**). If the hub is connected to the internet, the entire platform will receive automatic software updates from a central GIT repository. Raspberry Pis are powerful quad-core micro computers and they do generate some heat at heavy load. For this reason, the use of a cooled incubator is a requirement. In our laboratory, we run about 80 ethoscopes daily and we employ modified commercial wine-coolers as climatic chambers, for they are small and inexpensive (details of the modifications are available upon request). Flies to be tracked are loaded into a behavioural arena that slides and locks inside the lower part of the ethoscope (**Fig. 1a**). Alike the rest of the machine, arenas are also 3D-printed and the design of the arena to be employed depends on the nature of the experiment: some examples of arenas developed in our laboratory are provided in **Fig. 3** and span arenas adopted for long term sleep experiments that may be lasting weeks (**Fig. 3a-c,f**) or short term assays such as decision making (**Fig. 3d**) and courtship (**Fig. 3e,g**). Arenas feature three fixed recognition marks on the corners (red circles on **Fig. 3a**) which are used by ethoscope to align and register the regions of interests for tracking. When starting an experiment, the experimenter can decide whether the activity of the animals should be tracked in real-time or whether the ethoscope should record a video to be analysed at a later time, with the ethoscope software or with another software of choice. In real-time tracking mode, ethoscopes will detect and record the position and angle of each animal with a variable frame rate of 1-4 frames per second (**Fig. 2A-b**).

Validation of real-time tracking and behavioural profiling

The ethoscope software is modular in design, meaning that many parts can be replaced as needed. The tracking module is one of the parts that the end users may want to ultimately adapt to their needs. Currently, we provide two tracking options: a foreground extraction model (default option) and an experimental tracking module based on haar-cascades[10], suitable for tracking of multiple animals in the same region of interest. To validate the accuracy of the default tracking mode, we recorded 2736 hours of video and asked three independent experienced fly researchers to manually annotate the position of flies in 1413 frames. We then compared the manually annotated positions to the positions detected by ethoscopes, and found that the median distance between the two was 0.3 mm, corresponding to a tenth of a fly body length. In no cases, did the error exceed one body length (2.5mm). To enrich the capabilities of ethoscopes, we also implemented a real-time behavioural annotator. We created a ground-truth of 1297 videos, each lasting 10 seconds and each manually annotated by at least three experienced fly researchers (**Fig. 4c**, annotation labels were: “walking”, “micro-movement” or “immobile”). Random forest variable importance[11] was used to screen for possible predictors of mobility in a supervised manner and the two highest-ranking features - maximal velocity and cumulative walked distance - were selected for further analysis (**Fig. 4d,e**). Conveniently, ROC analysis showed that maximal velocity alone appeared to serve as faithful predictor of behaviour (**Fig. 4d,e**). Therefore, not only can ethoscopes annotate the position of flies, but also detect in real-time when an animal is immobile, performing a micro-movement (such as grooming or eating), or walking, with an accuracy of 94.3% for micro-movement detection and 99.0% for walking detection.

Real-time feedback-loops on single animals

The ability to recognize simple behaviour in real-time opens a new perspective: animal -dependent feedback-loop stimuli delivered upon behavioural trigger. Interfering with behaviour through external stimuli is an important tool for neuroscientists as it pushes beyond description and allows for more manipulative analysis. In principle, feedback loops can be used for multiple purposes: to reinforce learning, to sleep deprive animals, to stimulate or silence circuits using optogenetics, to study operant conditioning, *etc*. Systems operating feedback-loop stimuli on fruit flies were proposed before and already proved to be useful, but they are not easily compatible with a high-throughput approach and are focused on very specific usage[12,13]. We therefore designed ethoscopes so that they could be extended with modules that seamlessly connect with the machine and react to real-time analysis to trigger an action whenever a condition is satisfied. Figure 5 shows three examples of such modules: an air/gas/odour (AGO) delivery module (**Fig. 5a,b**), a rotational module capable of variable intensity(**Fig. 5d,e**), and an opto-motor module combining optogenetic stimulation and motor disturbance (**Fig. 5g,h**). Modules plug into the bottom part of the machine and are configured through the graphical web-interface, where the experimenter can set the trigger conditions that will activate the stimulus. A trigger can be a combinatorial ensemble of position, time, and behaviour (e.g. "*micro-movement for at least 20 seconds within 5mm from the food*" or "*immobile for at least 5 minutes anywhere*"). As proof of principle, we provide representative evidence of how single flies react to three different stimuli: a 5 seconds delivery of CO_2, triggered by crossing of the midline tube (**Fig. 5c**); a 2 seconds fast rotation of the tube, triggered by 20 seconds of immobility (**Fig. 5f**); a 5 seconds opto-stimulation on "moon-walker"[14] receptive flies, manually and automatically triggered (**Supplementary Video S2**). In general, flies responses to stimuli will obviously depend on the experimental paradigm of choice. On the ethoscope website, we provide detailed instruction on how to build all three modules and a description of the API needed to interface any new custom module to the ethoscope platform. Ultimately, we expect and encourage users to build modules based on their own scientific needs. One unprecedented strength of the feedback-loop module system is the ability to interact with single flies rather than with the entire population, which provides, among other things, the option of performing proper yoked treatments.

Discussion

Ethoscopes emerge from the maker culture to combine three of the most revolutionary innovations of the last decades - 3D printing, small single-board computers and machine learning - into a novel paradigm for behavioural researchers. They were designed to be easy to build, inexpensive, compatible with high-throughput research, and able to generate reproducible results. Moreover, in their LEGO and paper versions, they can serve as excellent tool for education and citizens science. Ethoscopes rely heavily on Raspberry Pis, the third best-selling computer of all time, currently running at their 3rd hardware version. We expect Raspberry Pis to continue in their evolution, and we therefore expect ethoscopes' computing power to grow accordingly. A standardised, plug-and-play, inexpensive tool for behavioural analysis – like ethoscopes are – can be instrumental for future development of the behavioural field, similarly to how activity monitors have been instrumental for the success of circadian biology.

Methods

All methods are available on the ethoscope website https://lab.gilest.ro/ethoscope

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Figure Legends

Figure 1 | The ethoscope. (**a**) Exploded drawing of a prototypical ethoscope. The machine is composed of two main parts: an upper case containing the Raspberry Pi and its camera, and a lower case providing infrared light (IR) and support for the experimental arena. The two cases are separated by spacers maintaining a fix focal distance (140mm). (**b**) A rendered drawing of the assembled model showing actual size. The arena slides in place through sliding guides and locks into position. A webGL interactive 3D model is available as **Supplementary Material 1**. (**c**) The LEGOscope, a version of the ethoscope entirely built using LEGO bricks. A detailed instruction manual is provided in **Supplementary Material 2**. (**d**) A paper and cardboard version of the ethoscope, assembled using 220gsm paper and 1mm grayboard. Blueprints are provided in **Supplementary Material 3**. In all cases, ethoscopes must be powered with a 5Vdc input using a common USB micro cable either connected to the main or to a portable power-pack. Cables not shown for sake of simplicity.

Figure 2 | The ethoscope platform. (**a**) A diagram of the typical setup. Ethoscopes, powered through a USB adapter, are connected in an intranet mesh through an Access Point (AP) or a WI-FI router. A server computer in the network acts as hub, downloading data from ethoscopes and serving a web-based user interface (UI). Ethoscopes can be controlled through the web-UI, either locally or remotely. (**b**) Screenshot of the homepage of the web-UI, showing a list of running machines and some associated experimental metadata (e.g. username and location). (**c**) Screenshot of an ethoscope control page on the web-UI, providing metadata about the experiment and a real-time updated snapshot from the ethoscope point of view.

Figure 3 | Custom behavioural arenas. (**a-g**) Versatility of use with custom behavioural arenas. Examples of 7 different behavioural arenas developed in our laboratory. (**a**) Sleep arena. Most commonly used arena for sleep studies, lodging 20 individual tubes. (**b**) Long tubes arena. Houses 13cm tubes and can be used for odour delivery studies or, more generally, for behaviours where longer walking is required. (**c**) Food bullet arena. Animals are placed directly on the arena and food can be replaced by pushing in a new bullet. Does not require glass tubes. (**d**) Decision making arena. Can be used to study simple decision making behaviours. (**e**) Square wells arena. Can be used for courtship assay or to record activity in a bi-dimensional environment. (**f,g**) Conceptually analogs to **a.** and i**.**, but designed to work in high-resolution (full-HD) settings. Note that all arenas are marked with three visible reference points (indicated by a red circle in **a.**) that are used by the ethoscope to automatically align images for tracking, providing a degree of physical flexibility.

Figure 4 | Tracking and validation of behavioural classification. (**a**) In real-time tracking mode, ethoscopes record Cartesian coordinates (*x*,*y*) of each animal relative to their ROI (region of interest), along with the numbers describing an ellipsis circumscribing the animal (*w*,*h*,*φ*). (**b**) A screenshot of the data table recorded by ethoscope, showing four data points for a single fly. (**c**) To build a statistical model of activity, we used ethoscopes to record offline 2736 hours of video (144 hours x 19 flies) at resolution of 1280x960pixels and frame rate of 25FPS. Video fragments of the duration of 10 seconds were sampled every hour for all 19 animals and then scored by at least three experienced fly researchers in a randomised order. Consensual annotations – with a margin greater than 0.5 – were kept, resulting in a ground truth of 1297 video fragments (116 ambiguous annotations were excluded using this latter criteria). Scorers manually annotated both the position of the animal in the tube and the perceived behavioural state (i.e. immobile, micro-moving or walking). Ethoscope video-tracking were run independently on the whole video resampled at 1-5FPS, all realistic frame rates for real-time analysis. (**d**) Performance of maximal velocity and cumulative walked distance as features for 1297 annotated videos of behaviours. (**e**) Marginal distribution of maximal velocity for each behaviour, showing the thresholds used to detect movement (dotted line) and walking (dashed line). (**f**) ROC curves for movement detection. A conservative threshold of 0.36mm/s, indicated by a red dot on the curve, yields a sensitivity (TPR) of 91.26% and specificity (1-FPR) of 99.65%. (**g**) ROC curve for walking detection. A threshold of 0.9mm/s (red dot on the curve) results in sensitivity and specificity of 99.34 and 99.39, respectively.

Figure 5 | Versatility of use with behavioural feedback-loop modules. (**a**) Diagram and (**b**) detail of the air/gas/odour (AGO)-delivery module. Two independent air flows (blue and purple in the drawing) are fed into the module using external sources. The module features 10 LEGO valves, each independently controlled through a servo motor. The motor switches the air source on the valve, selecting which source will be relayed to the tube containing the fly. Available positions are: blue source, purple source, and closed. (**c**) Representative response of three flies subjected to CO_2 administration using the AGO module. CO_2 release lasts 5 seconds (gray bar) and it is triggered by midline crossing (red dot). The blue line indicates the fly position in the tube over the 150 second period. (**d**) Model and (**e**) detail of the rotational module. The module employs a servo motor to turn the tube hosting the fly. Direction, speed, duration and angle of the rotation can be modulated to change the quality of the stimulus. (**f**) Representative response of three flies upon stimulation using the rotational module shown in (**d,e**). Rotation of the tube is triggered by 20 consecutive seconds of immobility (dashed line) and it is followed by 5 seconds of masking during which tracking is suspended to avoid motion artefacts (cyan area). The bottom panel shows traces of a dead fly. (**g**) Model of the optomotor module, able to simultaneously stimulate single flies with motion and light. (**h**) Detailed view of the optomotor principle. Light is directed into the tube using optical fiber. **Supplementary Videos 2** shows the optomotor module in action.

Supplementary material 1 | Interactive 3D rendering of the assembled ethoscope – requires webGL capable browser (e.g. Google Chrome)

Supplementary material 2 | Instruction booklet for building a LEGOscope

Supplementary material 3 | Instruction booklet for building a PAPERscope

Supplementary Video S1 | An overview of how the ethoscope platform works

Supplementary Video S2 | The optogenetics component of the optomotor module in action. Moonwalking flies (VT50660-Gal4 :: UAS-CsChrimson) are illuminated for 5-7 seconds using a red LED (630nm) through an optical fibre. Illumination is either manually triggered (first part of the video), or triggered by the fly position.

Supplementary videos available at https://lab.gilest.ro/papers/ethoscopes-an-open-platform-for-high-throughput-ethomics/

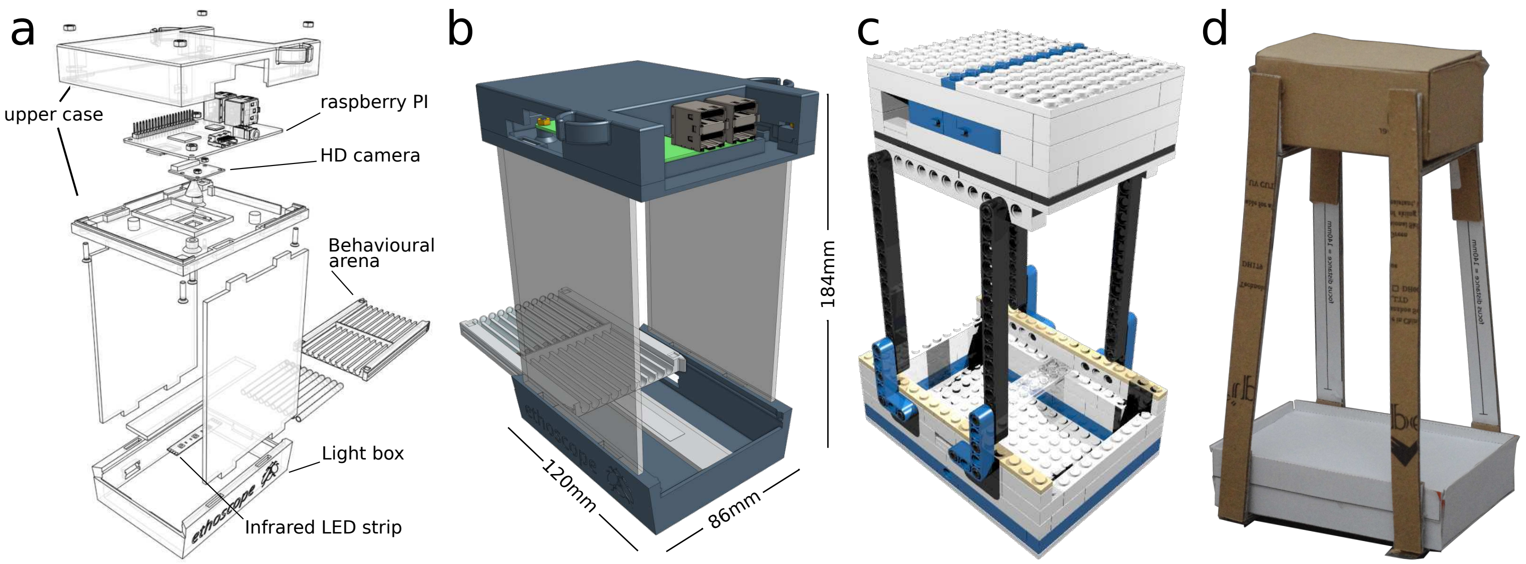

Figure 1

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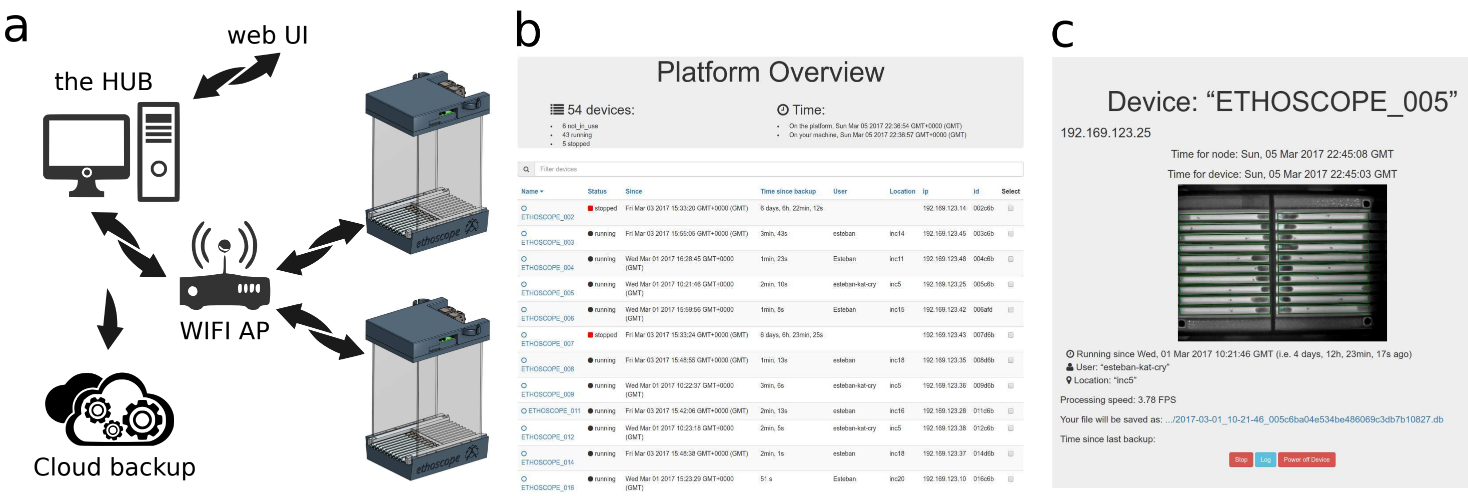

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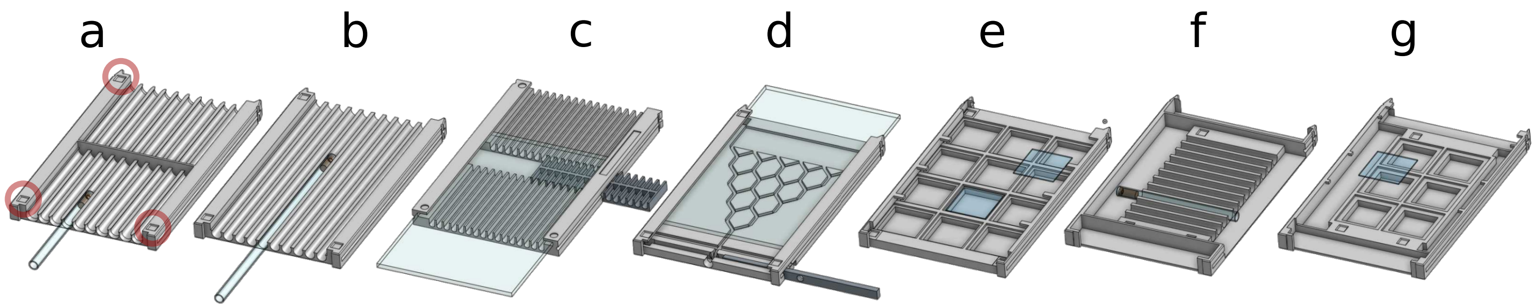

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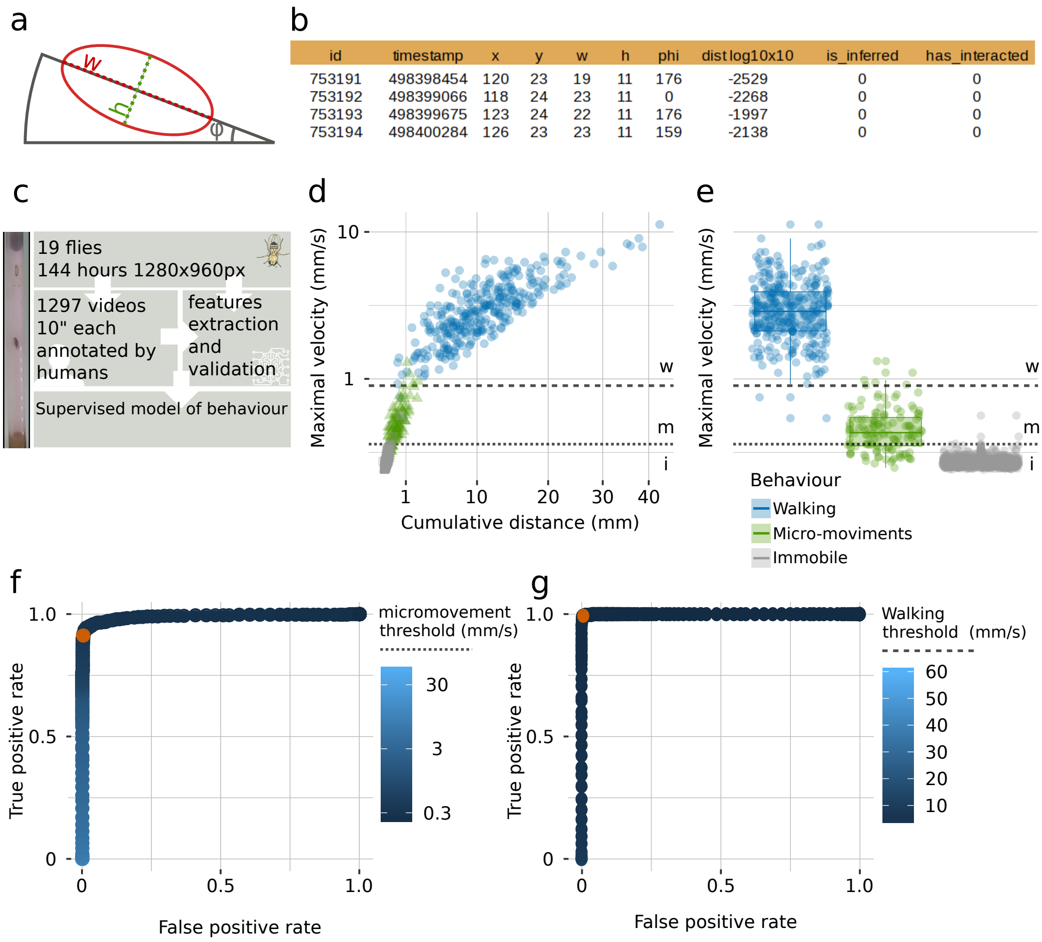

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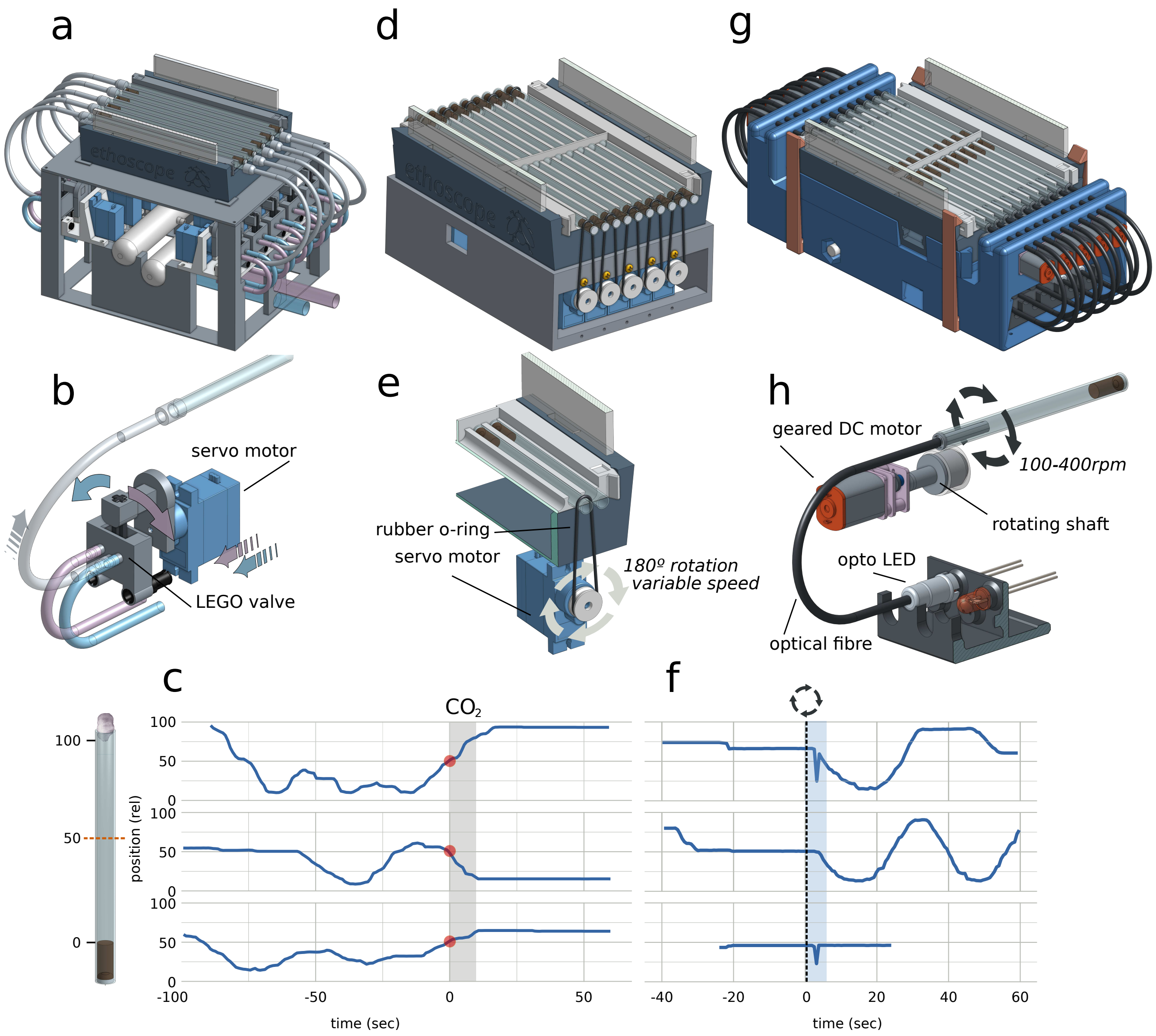

Figure 5

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ethoscope

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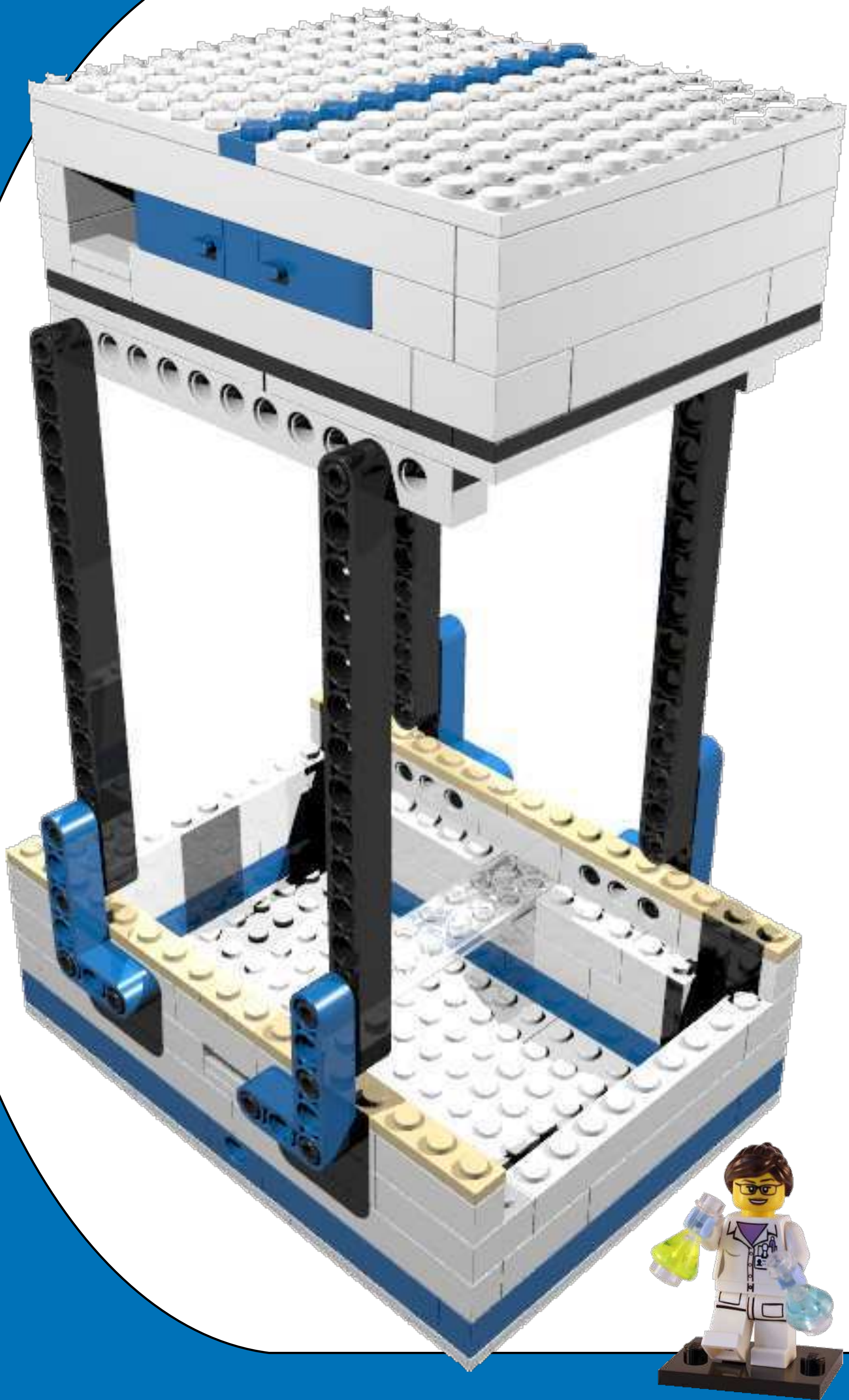

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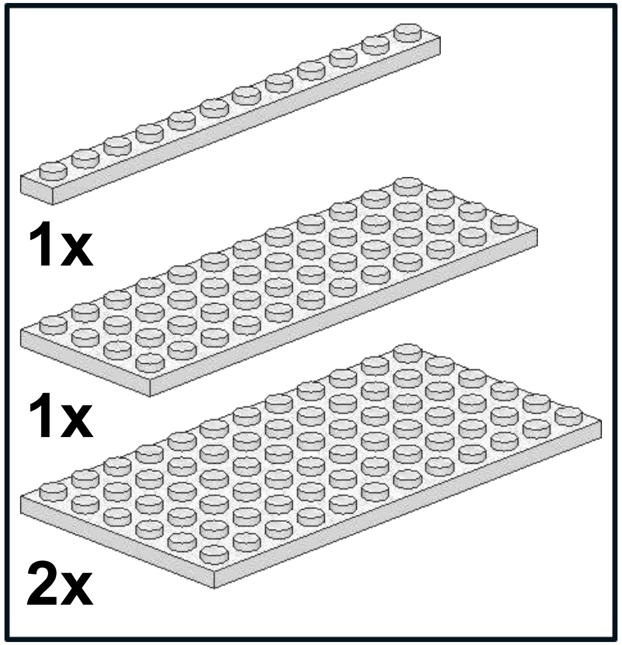

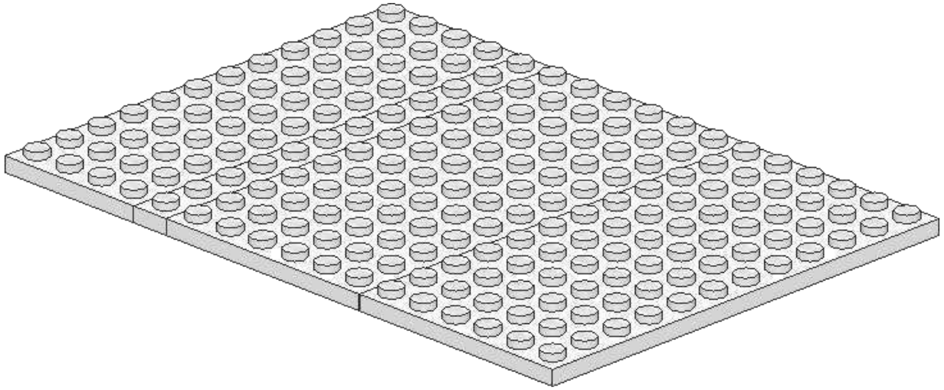

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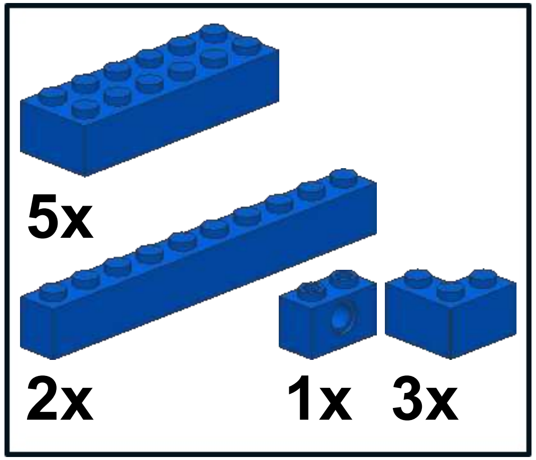

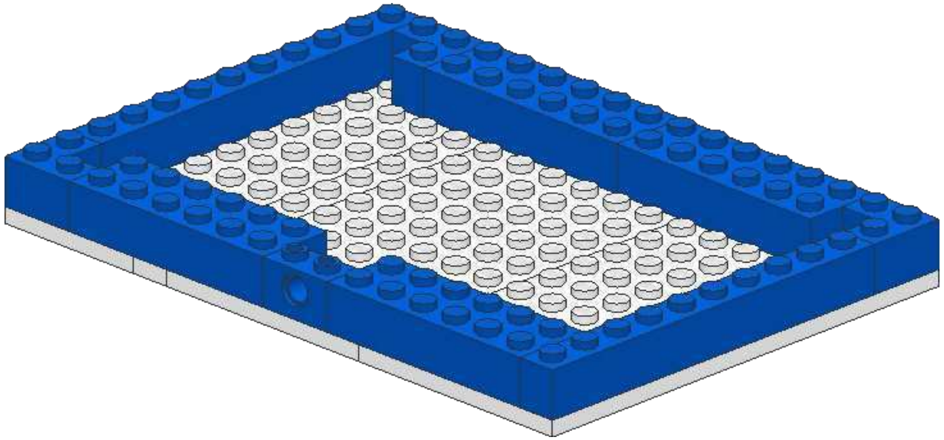

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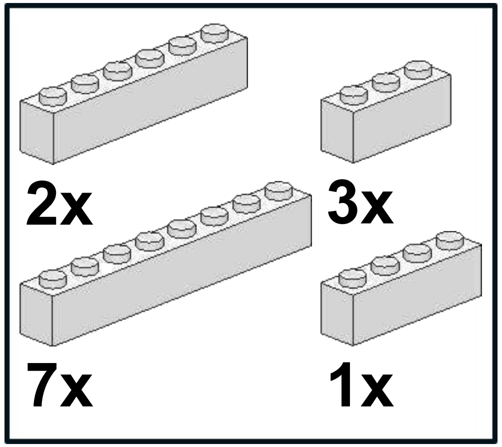

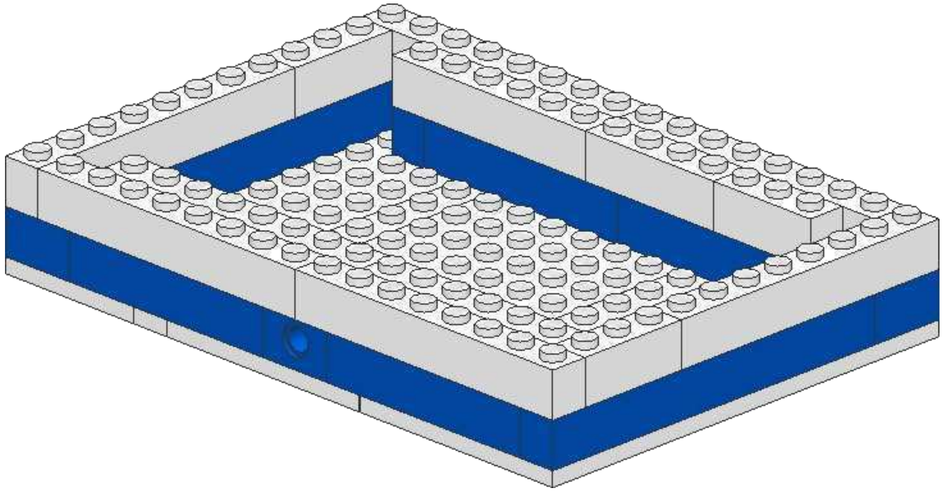

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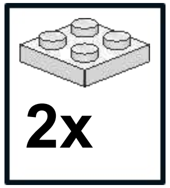

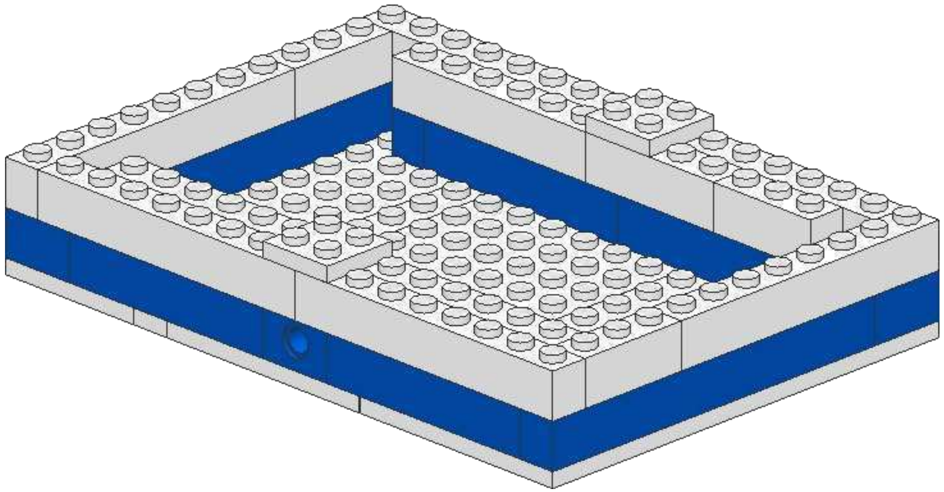

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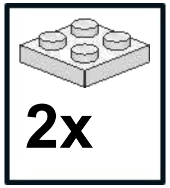

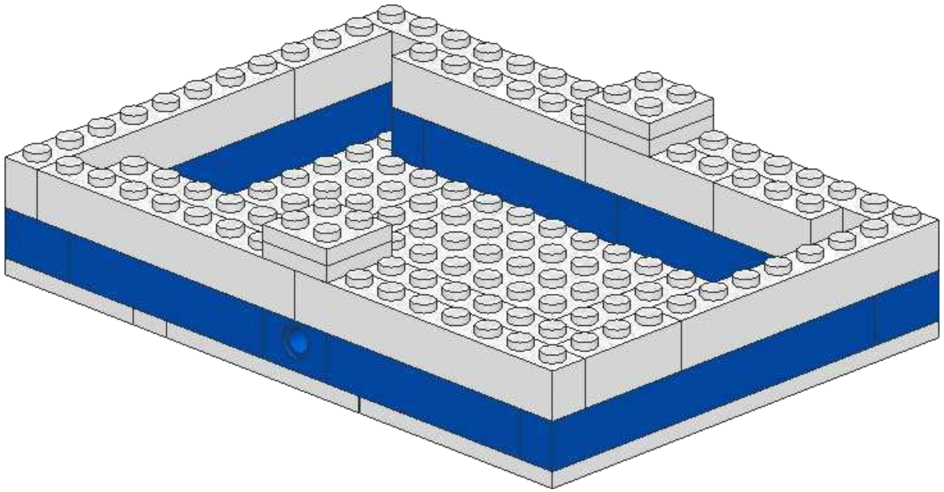

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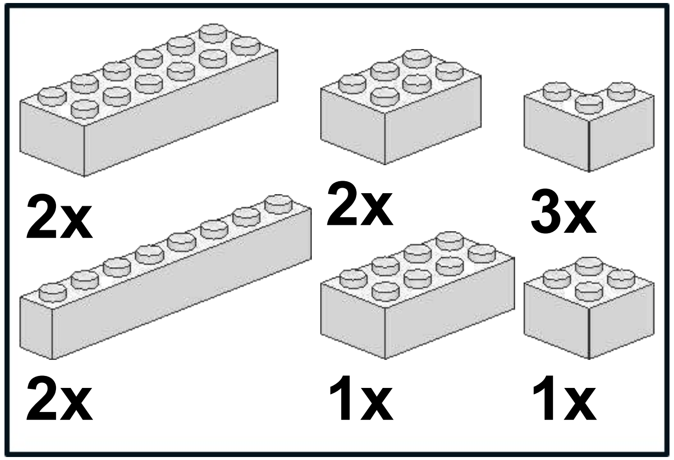

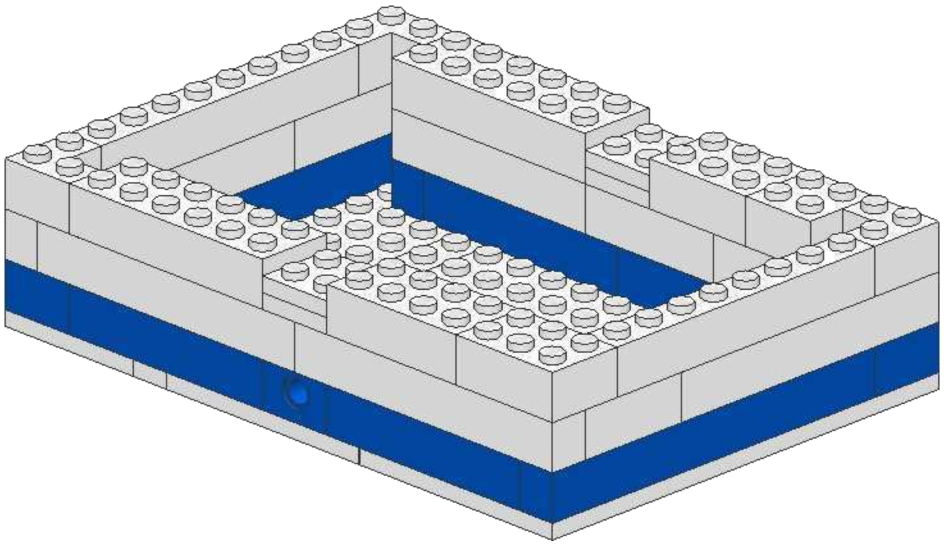

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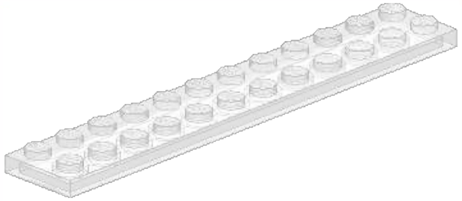

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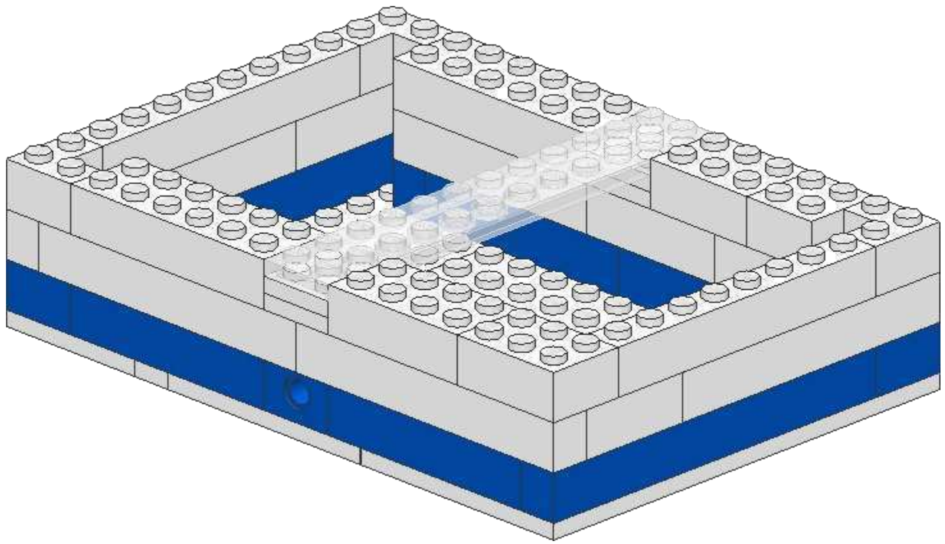

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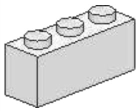

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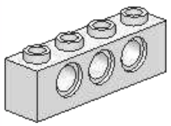

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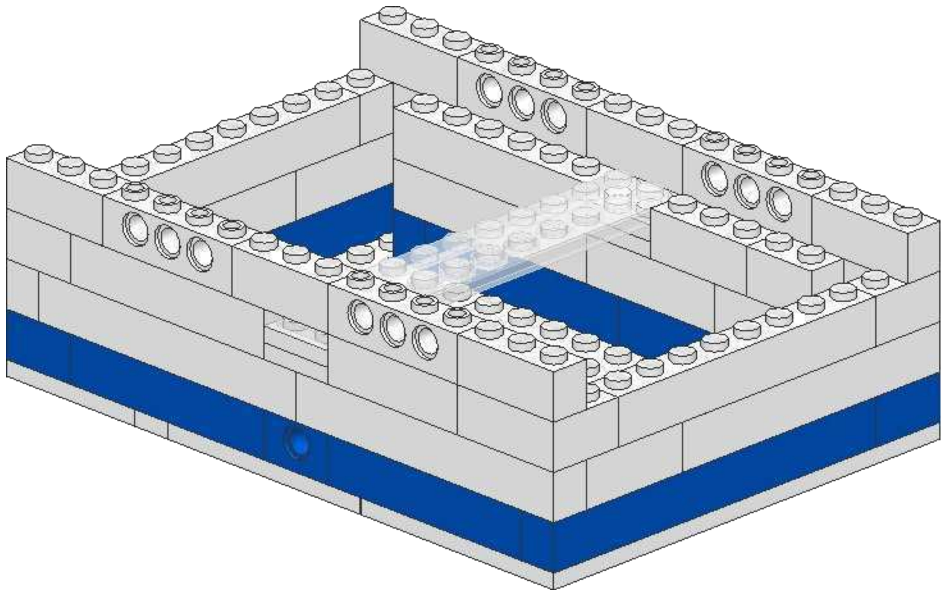

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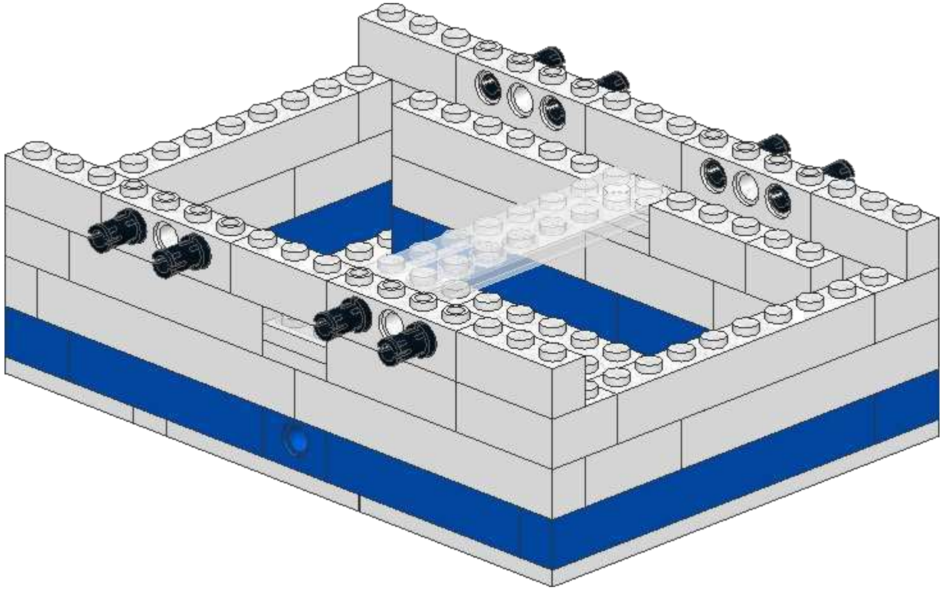

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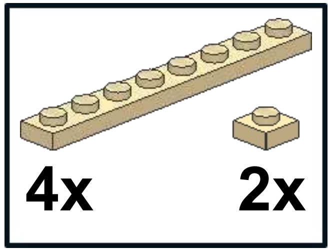

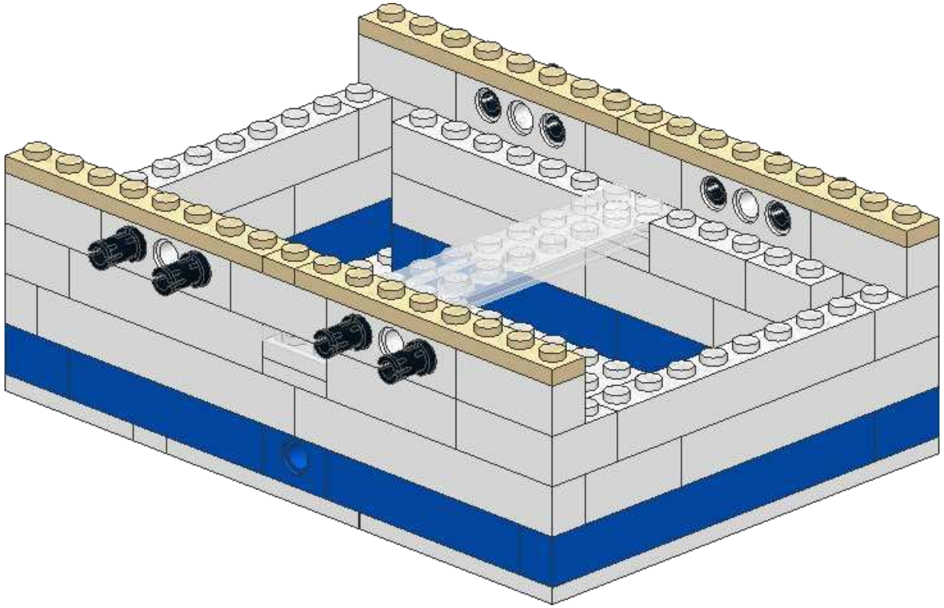

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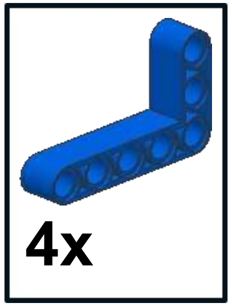

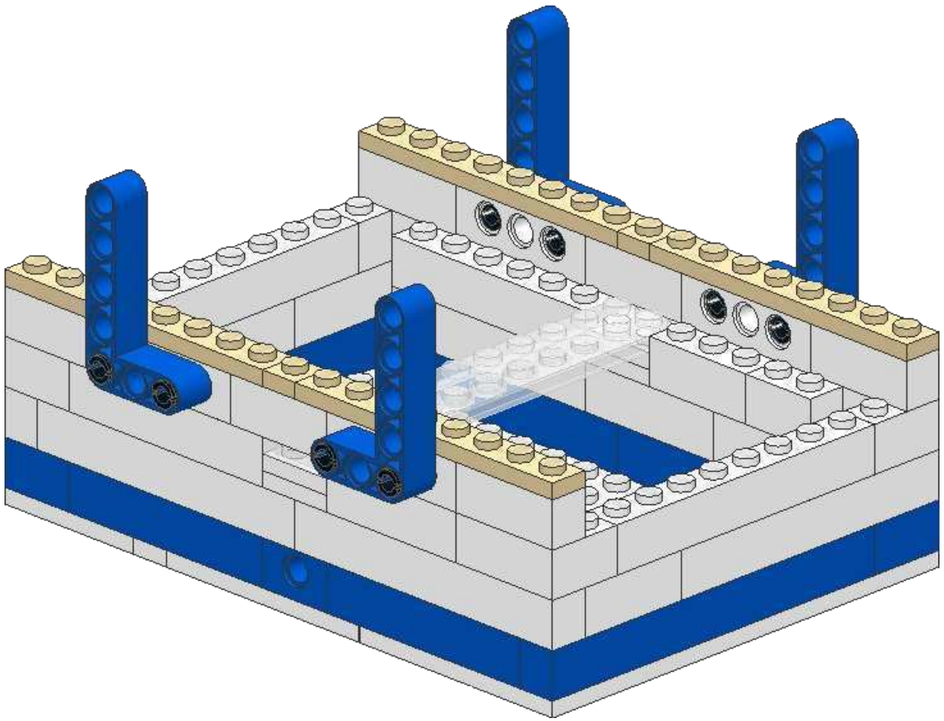

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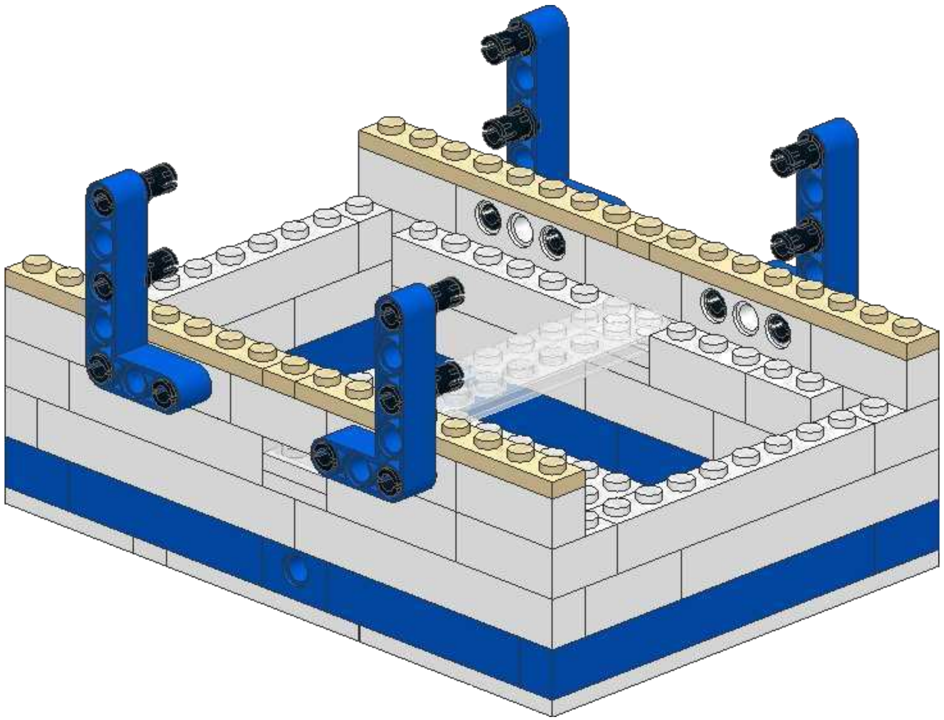

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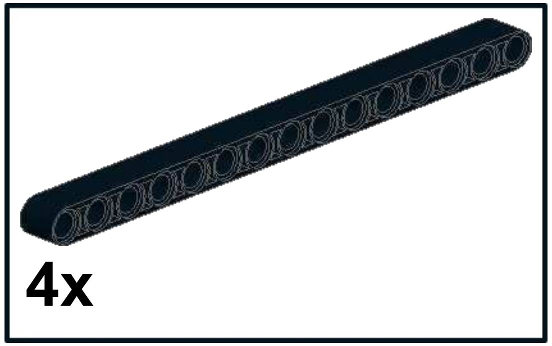

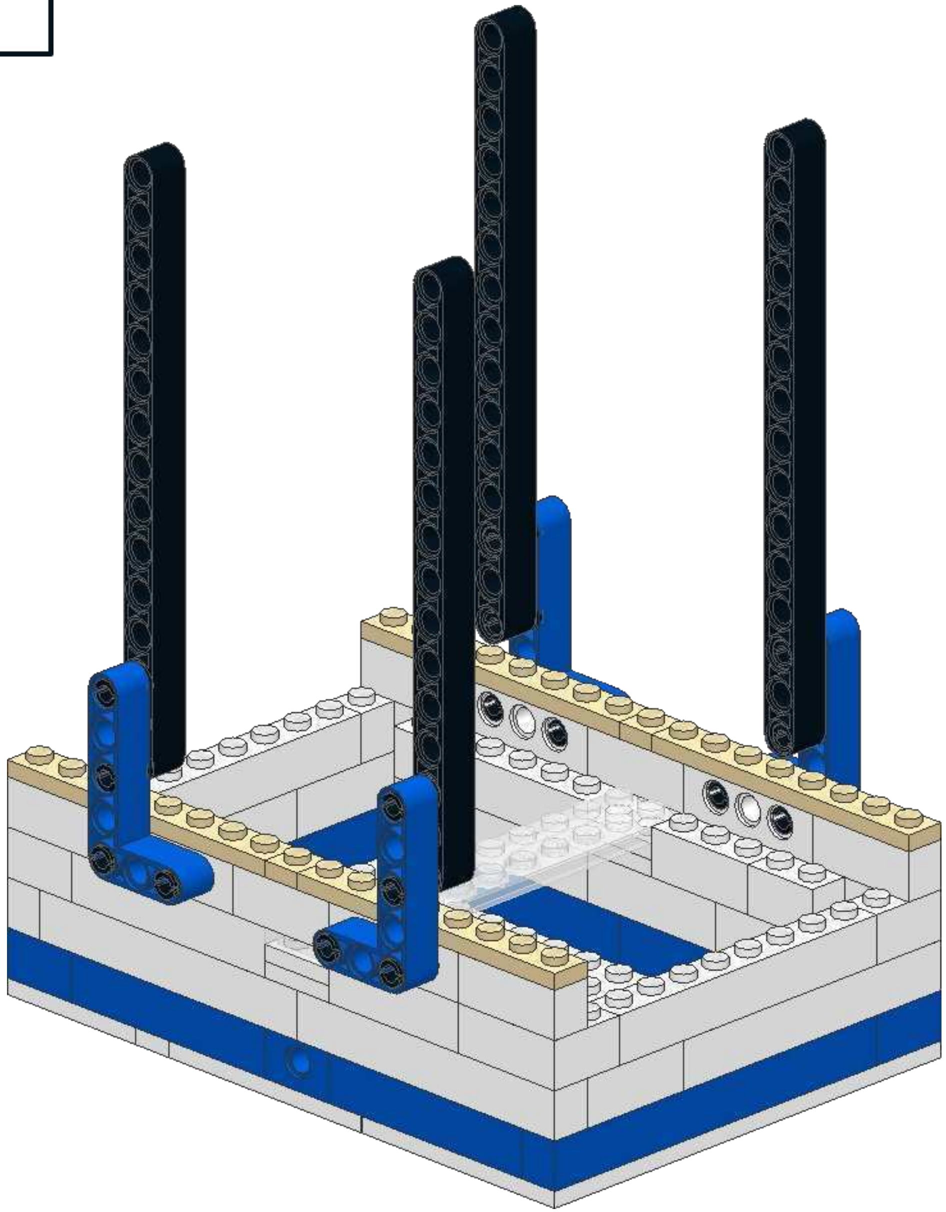

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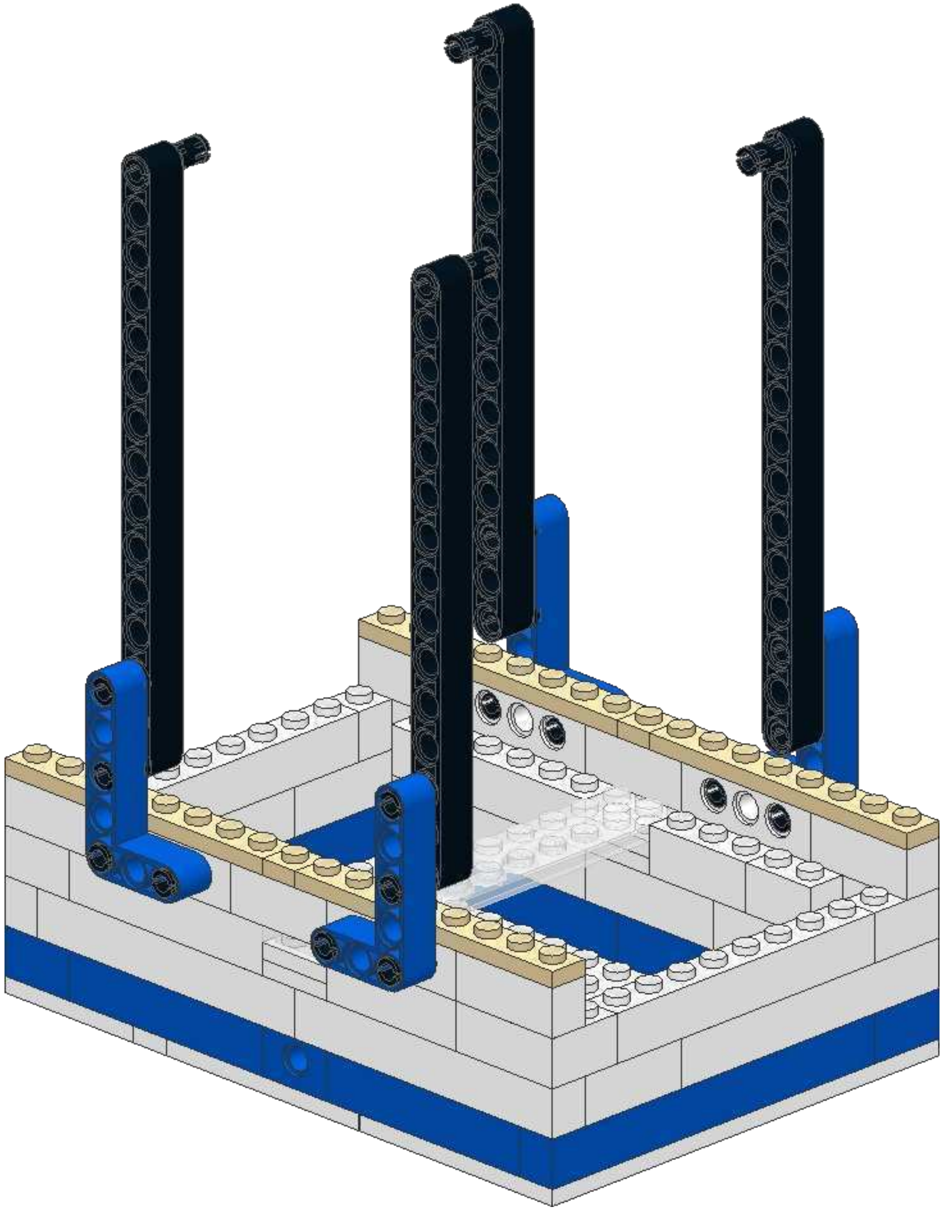

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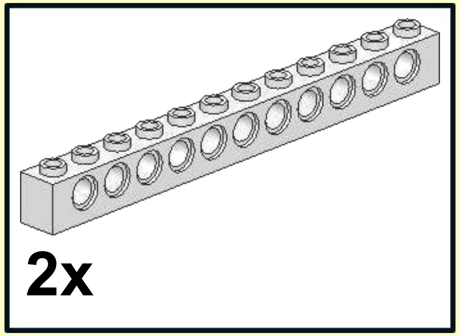

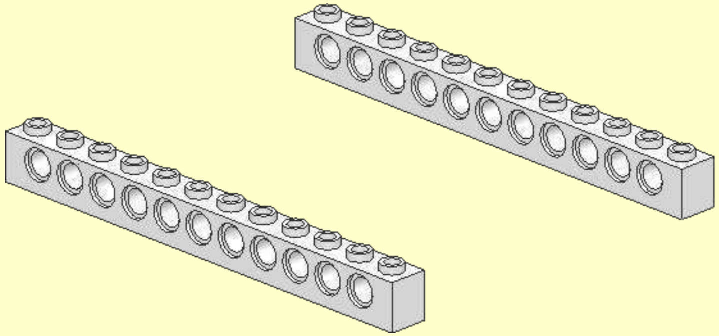

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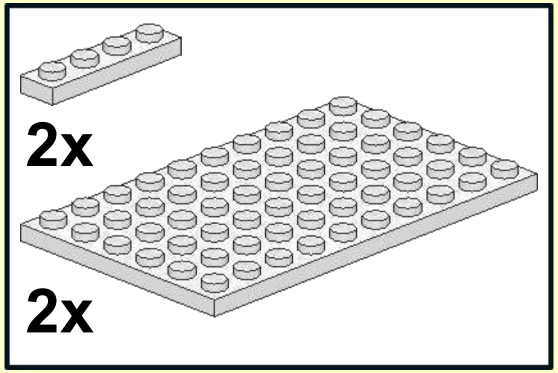

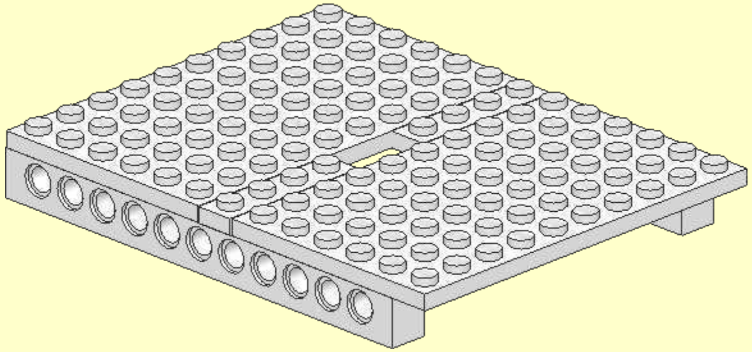

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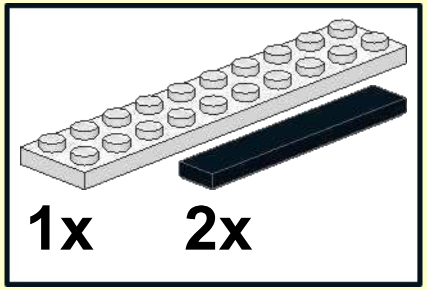

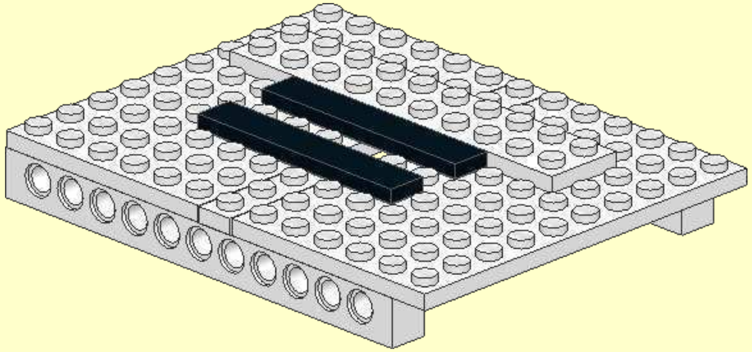

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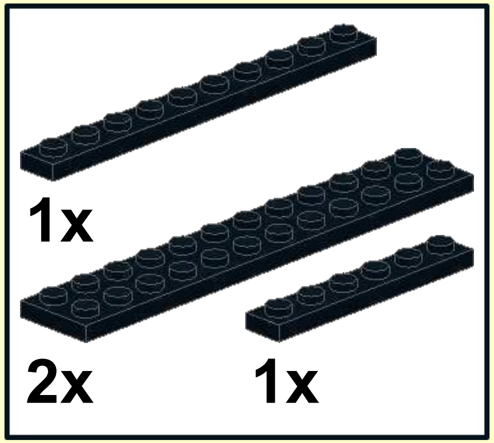

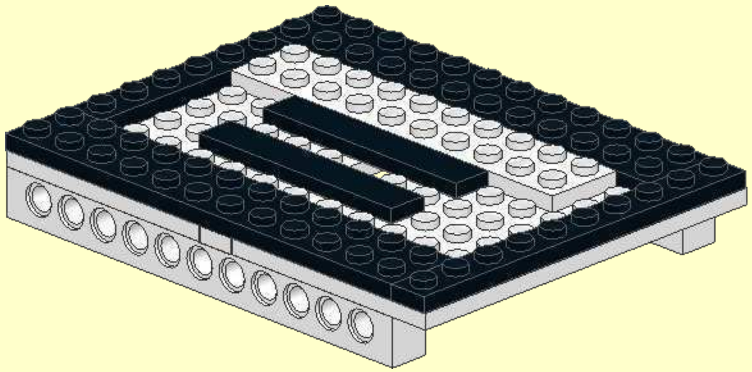

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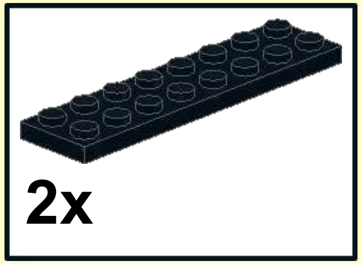

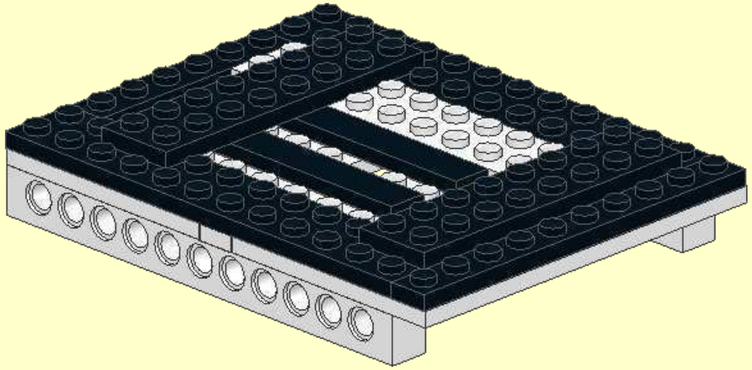

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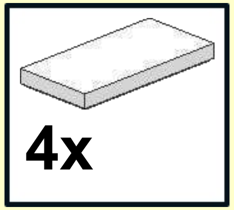

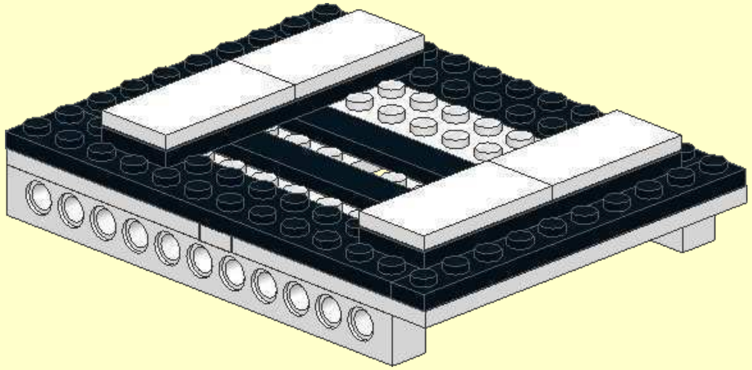

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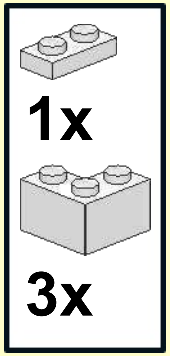

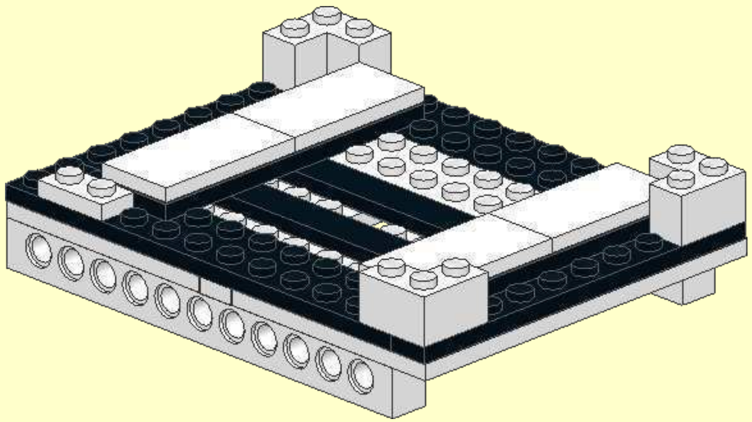

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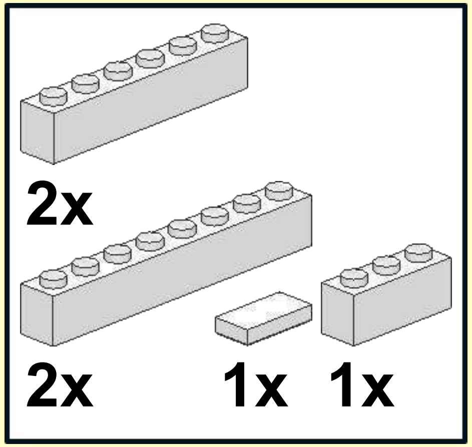

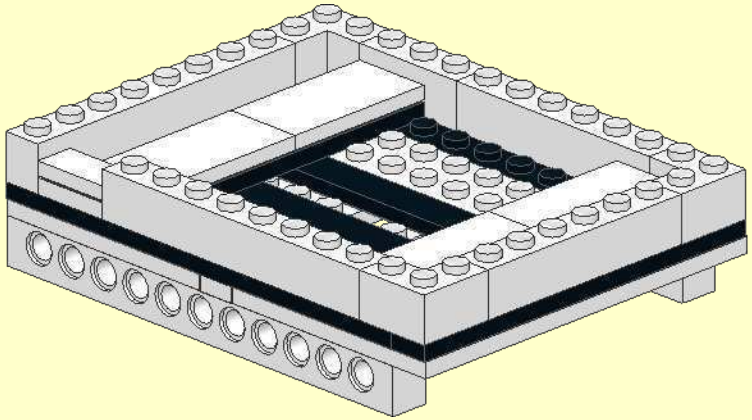

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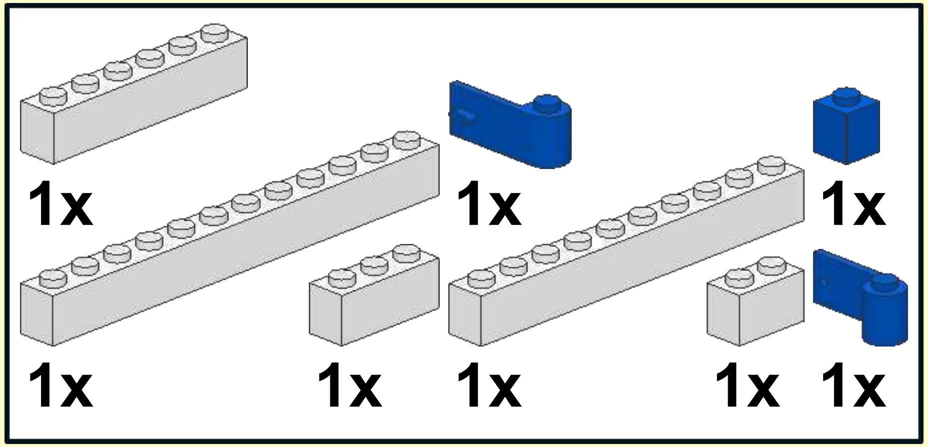

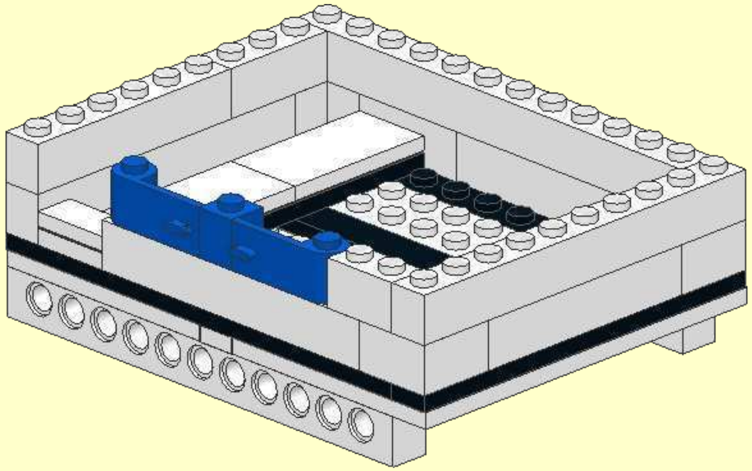

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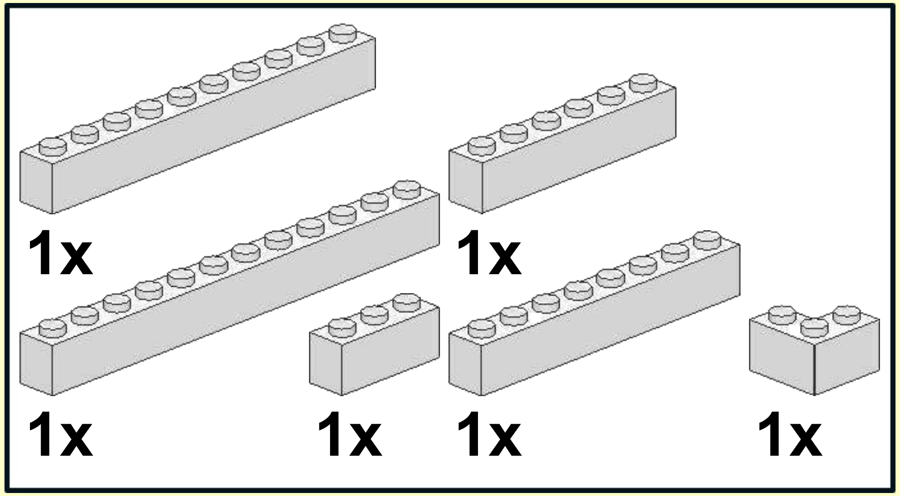

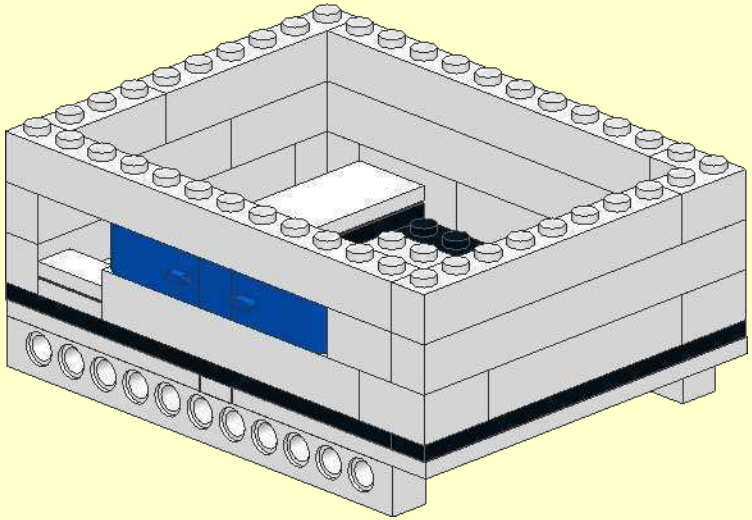

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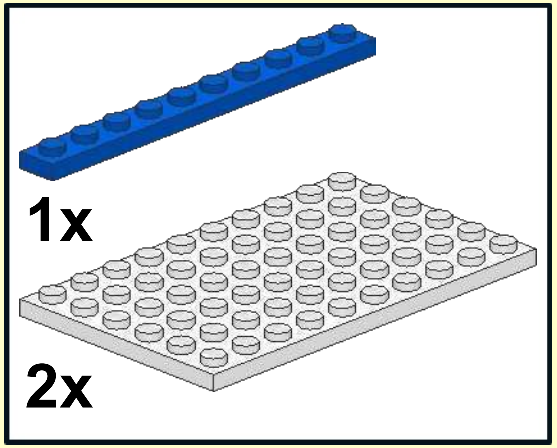

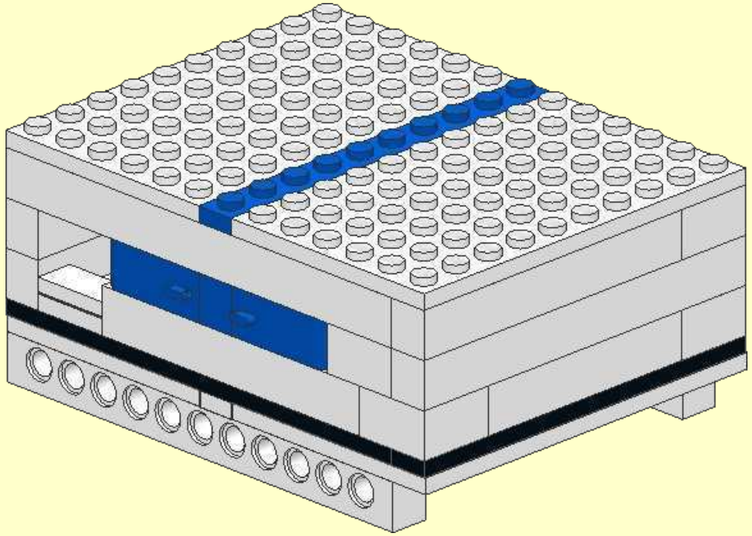

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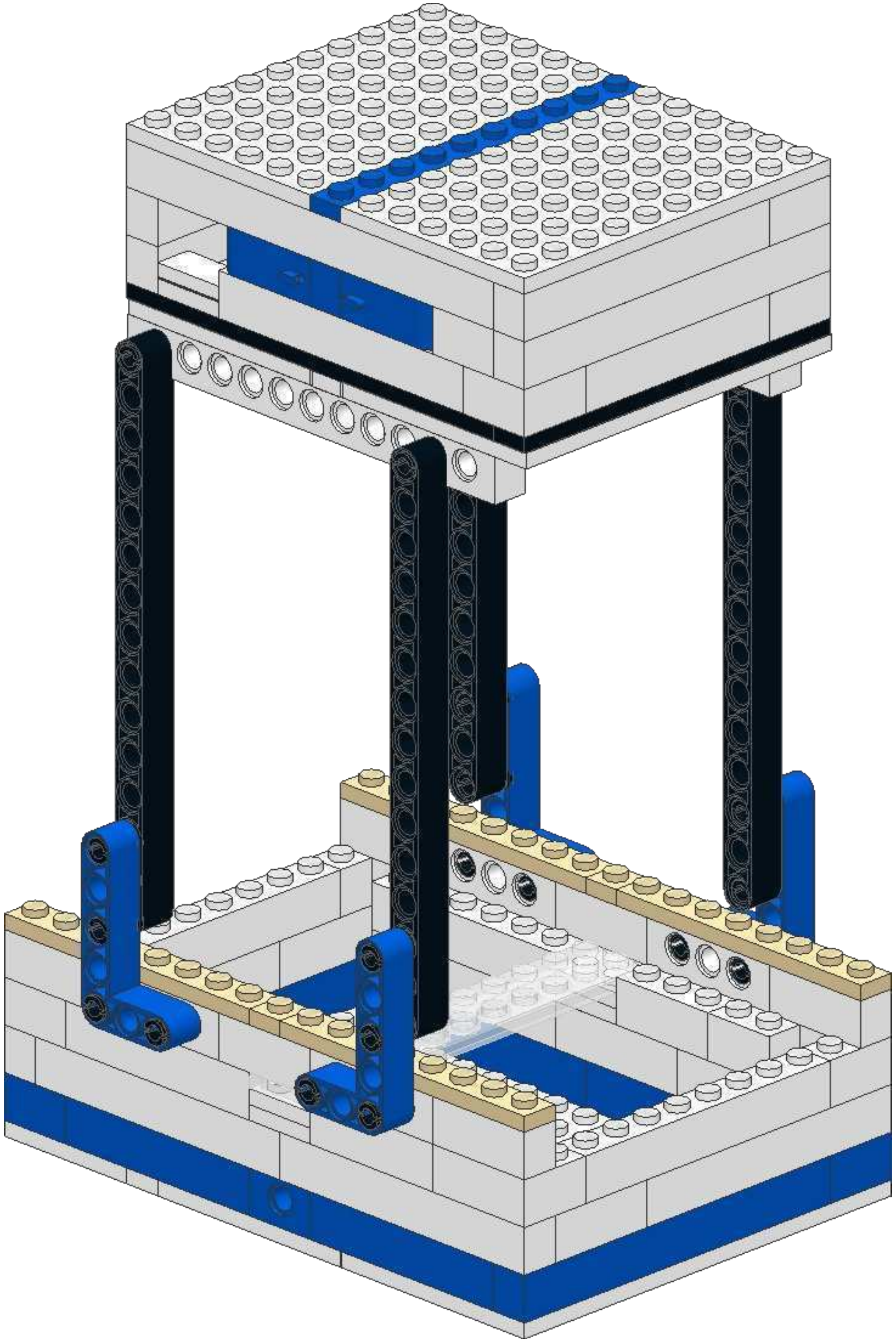

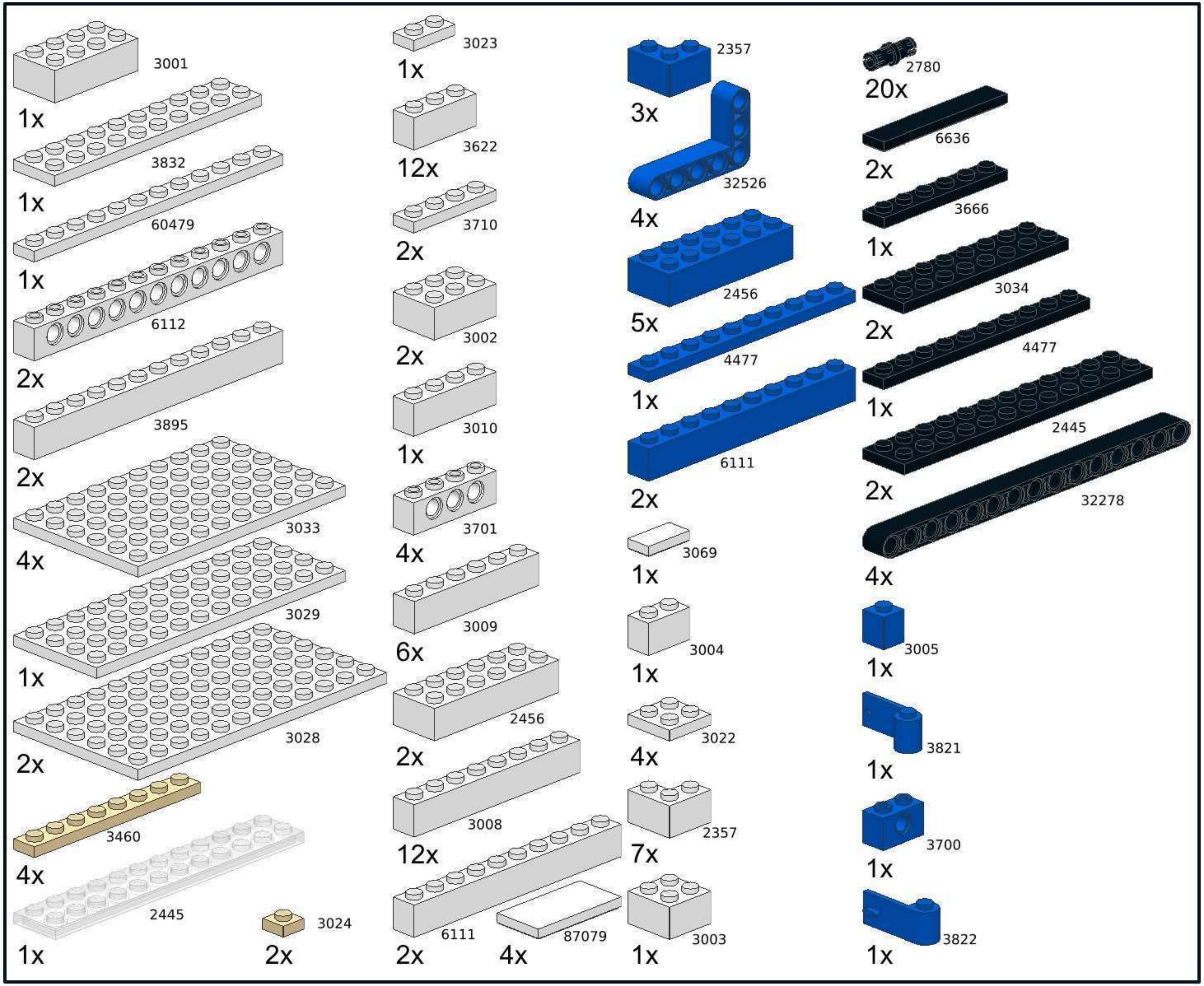
3001
1x
3832
1x
60479
1x
6112
2x
3895
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3460
4x
2445
1x
3024
2x
3023
1x
3622
12x
3710
2x
3002
2x
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3701
4x
3009
6x
2456
2x
3008
12x
6111
2x
87079
4x
2357
3x
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5x
4477
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3069
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3004
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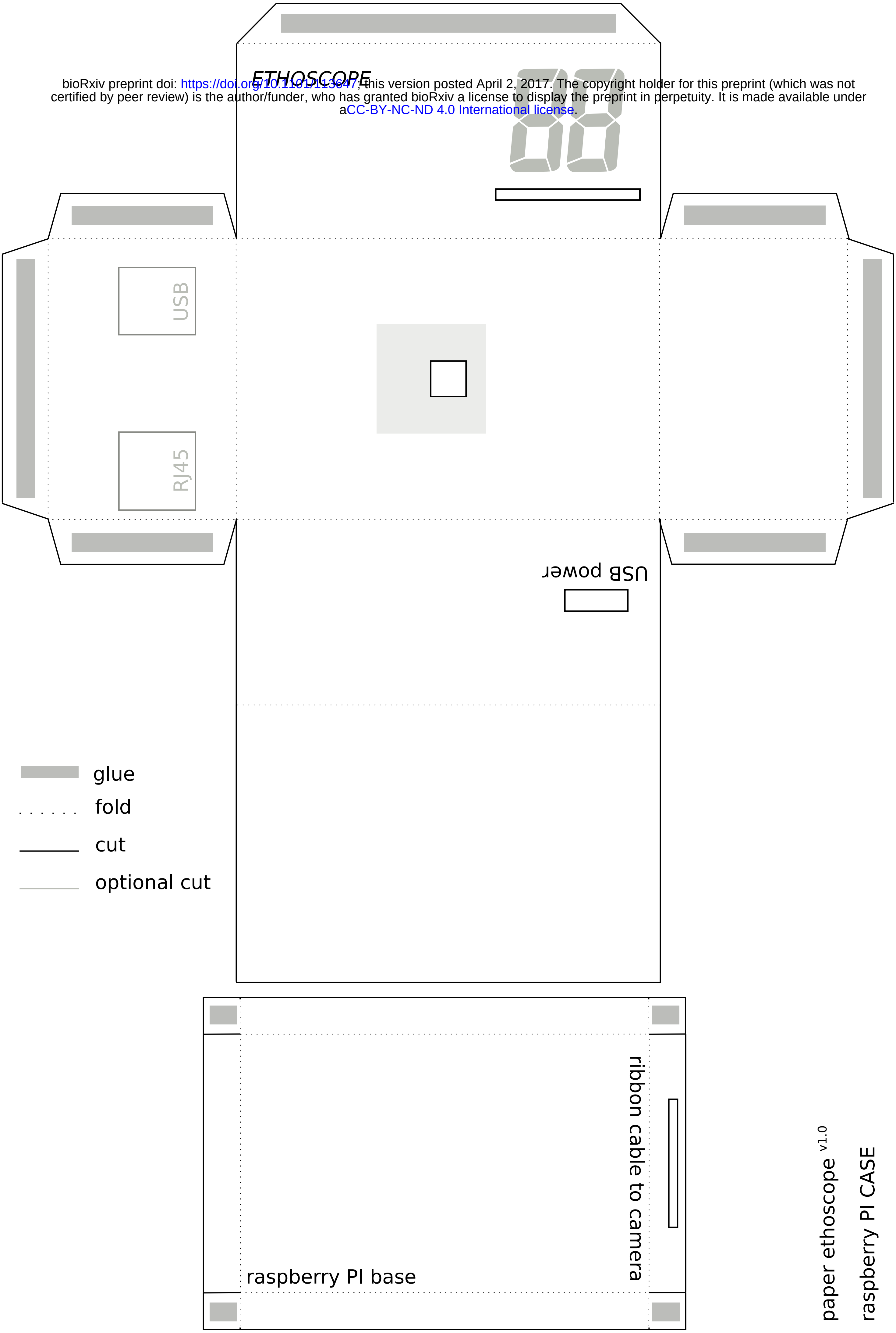
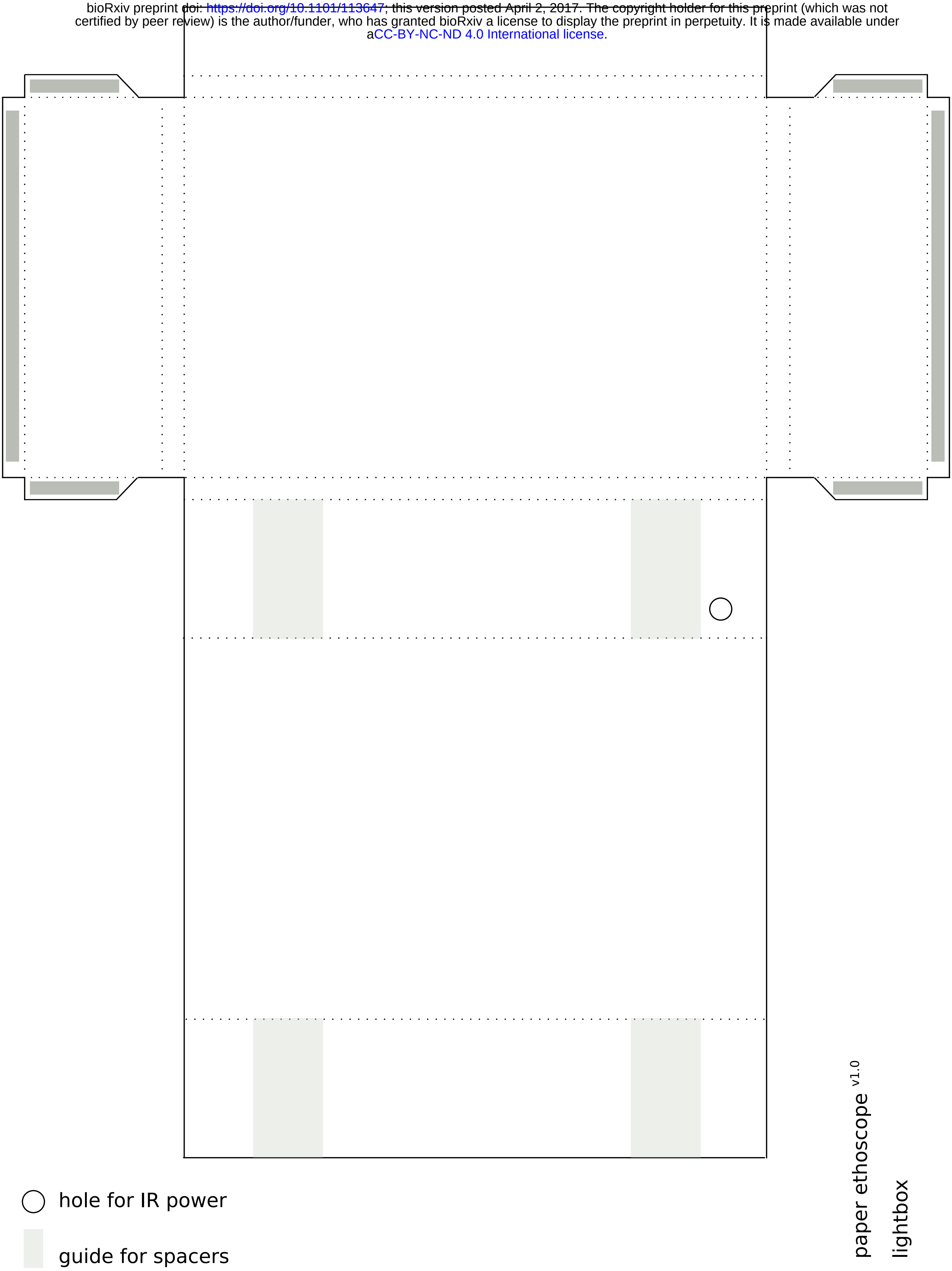
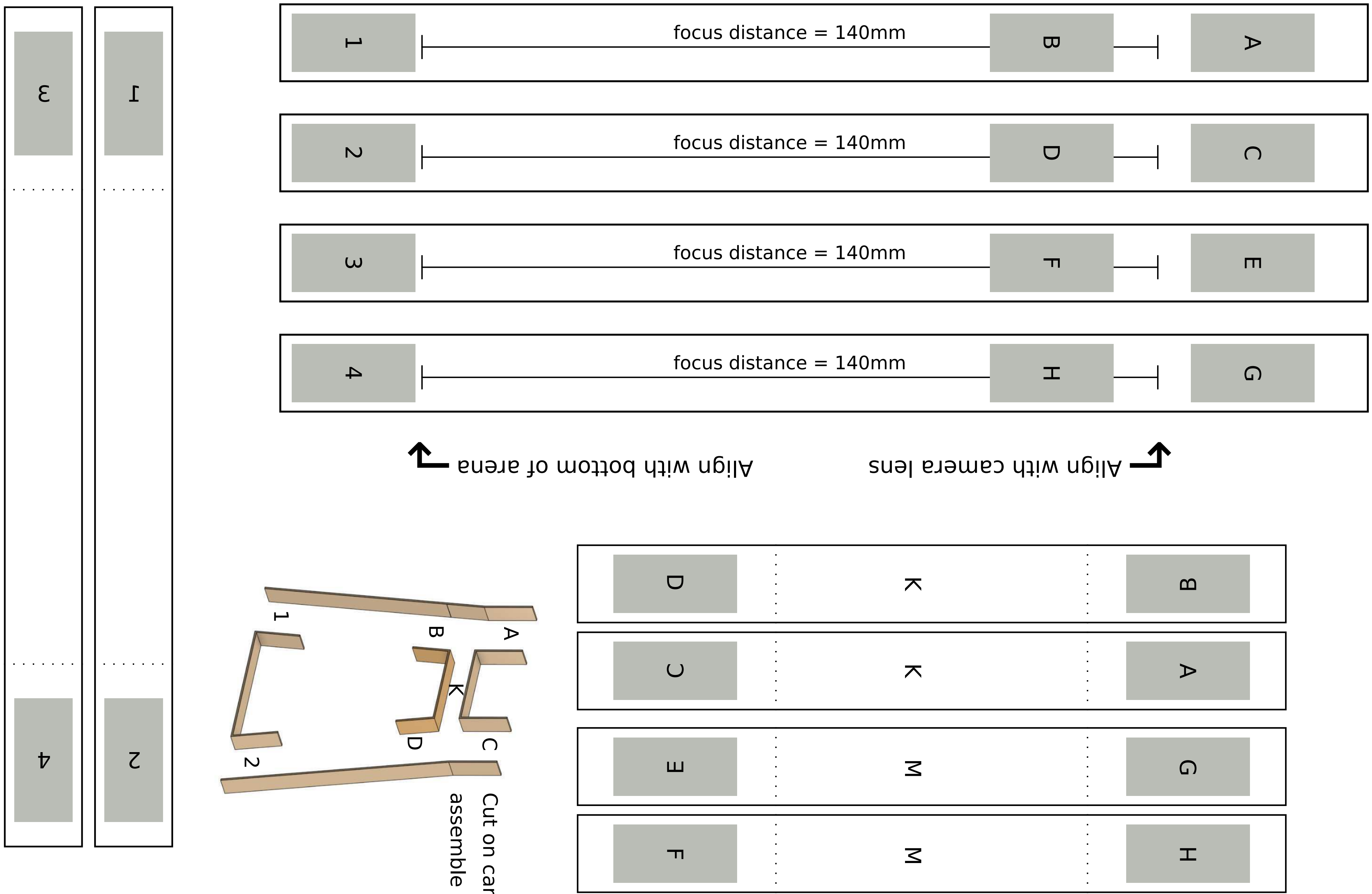

ETHOSCOPE
USB
RJ45
USB power
glue
fold
cut
optional cut
ribbon cable to camera
raspberry PI base
paper ethoscope v1.0
raspberry PI CASE


hole for IR power
guide for spacers
paper ethoscope v1.0
lightbox



Cut on cardboard, assemble using hotglue

paper ethoscope v1.0

spacers