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**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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## 29 **Abstract**

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

37

## 38 **Introduction**

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

## 60 **Results**

### 61 **Principle and scope**

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

81

### 82 **Usage scenario**

83 In a typical usage scenario, several ethoscopes are placed in a climatic controlled chamber, each  
84 powered through a USB cable; ethoscopes connect via WIFI to a computer acting as data collecting  
85 station (“the hub” in **Fig. 2**) and are controlled remotely via a graphical web interface (**Fig. 2** and  
86 **Supplementary Video S1**). If the hub is connected to the internet, the entire platform will receive  
87 automatic software updates from a central GIT repository. Raspberry Pis are powerful quad-core micro  
88 computers and they do generate some heat at heavy load. For this reason, the use of a cooled incubator  
89 is a requirement. In our laboratory, we run about 80 ethoscopes daily and we employ modified  
90 commercial wine-coolers as climatic chambers, for they are small and inexpensive (details of the

91 modifications are available upon request). Flies to be tracked are loaded into a behavioural arena that  
92 slides and locks inside the lower part of the ethoscope (**Fig. 1a**). Alike the rest of the machine, arenas  
93 are also 3D-printed and the design of the arena to be employed depends on the nature of the  
94 experiment: some examples of arenas developed in our laboratory are provided in **Fig. 3** and span  
95 arenas adopted for long term sleep experiments that may be lasting weeks (**Fig. 3a-c,f**) or short term  
96 assays such as decision making (**Fig. 3d**) and courtship (**Fig. 3e,g**). Arenas feature three fixed  
97 recognition marks on the corners (red circles on **Fig. 3a**) which are used by ethoscope to align and  
98 register the regions of interests for tracking. When starting an experiment, the experimenter can decide  
99 whether the activity of the animals should be tracked in real-time or whether the ethoscope should  
100 record a video to be analysed at a later time, with the ethoscope software or with another software of  
101 choice. In real-time tracking mode, ethoscopes will detect and record the position and angle of each  
102 animal with a variable frame rate of 1-4 frames per second (**Fig. 2A-b**).

103

#### 104 **Validation of real-time tracking and behavioural profiling**

105 The ethoscope software is modular in design, meaning that many parts can be replaced as needed. The  
106 tracking module is one of the parts that the end users may want to ultimately adapt to their needs.  
107 Currently, we provide two tracking options: a foreground extraction model (default option) and an  
108 experimental tracking module based on haar-cascades<sup>10</sup>, suitable for tracking of multiple animals in the  
109 same region of interest. To validate the accuracy of the default tracking mode, we recorded 2736 hours  
110 of video and asked three independent experienced fly researchers to manually annotate the position of  
111 flies in 1413 frames. We then compared the manually annotated positions to the positions detected by  
112 ethoscopes, and found that the median distance between the two was 0.3 mm, corresponding to a tenth  
113 of a fly body length. In no cases, did the error exceed one body length (2.5mm). To enrich the  
114 capabilities of ethoscopes, we also implemented a real-time behavioural annotator. We created a  
115 ground-truth of 1297 videos, each lasting 10 seconds and each manually annotated by at least three  
116 experienced fly researchers (**Fig. 4c**, annotation labels were: “walking”, “micro-movement” or  
117 “immobile”). Random forest variable importance<sup>11</sup> was used to screen for possible predictors of  
118 mobility in a supervised manner and the two highest-ranking features - maximal velocity and  
119 cumulative walked distance - were selected for further analysis (**Fig. 4d,e**). Conveniently, ROC  
120 analysis showed that maximal velocity alone appeared to serve as faithful predictor of behaviour (**Fig.**  
121 **4d,e**). Therefore, not only can ethoscopes annotate the position of flies, but also detect in real-time

122 when an animal is immobile, performing a micro-movement (such as grooming or eating), or walking,  
123 with an accuracy of 94.3% for micro-movement detection and 99.0% for walking detection.

124

### 125 **Real-time feedback-loops on single animals**

126 The ability to recognize simple behaviour in real-time opens a new perspective: animal -dependent  
127 feedback-loop stimuli delivered upon behavioural trigger. Interfering with behaviour through external  
128 stimuli is an important tool for neuroscientists as it pushes beyond description and allows for more  
129 manipulative analysis. In principle, feedback loops can be used for multiple purposes: to reinforce  
130 learning, to sleep deprive animals, to stimulate or silence circuits using optogenetics, to study operant  
131 conditioning, *etc.* Systems operating feedback-loop stimuli on fruit flies were proposed before and  
132 already proved to be useful, but they are not easily compatible with a high-throughput approach and are  
133 focused on very specific usage<sup>12,13</sup>. We therefore designed ethoscopes so that they could be extended  
134 with modules that seamlessly connect with the machine and react to real-time analysis to trigger an  
135 action whenever a condition is satisfied. Figure 5 shows three examples of such modules: an  
136 air/gas/odour (AGO) delivery module (**Fig. 5a,b**), a rotational module capable of variable intensity(**Fig.**  
137 **5d,e**), and an opto-motor module combining optogenetic stimulation and motor disturbance (**Fig. 5g,h**).  
138 Modules plug into the bottom part of the machine and are configured through the graphical web-  
139 interface, where the experimenter can set the trigger conditions that will activate the stimulus. A trigger  
140 can be a combinatorial ensemble of position, time, and behaviour (e.g. “*micro-movement for at least 20*  
141 *seconds within 5mm from the food*” or “*immobile for at least 5 minutes anywhere*”). As proof of  
142 principle, we provide representative evidence of how single flies react to three different stimuli: a 5  
143 seconds delivery of CO<sub>2</sub>, triggered by crossing of the midline tube (**Fig. 5c**); a 2 seconds fast rotation of  
144 the tube, triggered by 20 seconds of immobility (**Fig. 5f**); a 5 seconds opto-stimulation on “moon-  
145 walker”<sup>14</sup> receptive flies, manually and automatically triggered (**Supplementary Video S2**). In general,  
146 flies responses to stimuli will obviously depend on the experimental paradigm of choice. On the  
147 ethoscope website, we provide detailed instruction on how to build all three modules and a description  
148 of the API needed to interface any new custom module to the ethoscope platform. Ultimately, we  
149 expect and encourage users to build modules based on their own scientific needs. One unprecedented  
150 strength of the feedback-loop module system is the ability to interact with single flies rather than with  
151 the entire population, which provides, among other things, the option of performing proper yoked  
152 treatments.

153

## 154 **Discussion**

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

166

## 167 **Methods**

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

169

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## 181 **Figure Legends**

182

183 **Figure 1** | The ethoscope. **(a)** Exploded drawing of a prototypical ethoscope. The machine is composed  
184 of two main parts: an upper case containing the Raspberry Pi and its camera, and a lower case  
185 providing infrared light (IR) and support for the experimental arena. The two cases are separated by  
186 spacers maintaining a fix focal distance (140mm). **(b)** A rendered drawing of the assembled model  
187 showing actual size. The arena slides in place through sliding guides and locks into position. A WebGL  
188 interactive 3D model is available as **Supplementary Material 1**. **(c)** The LEGOScope, a version of the  
189 ethoscope entirely built using LEGO bricks. A detailed instruction manual is provided in  
190 **Supplementary Material 2**. **(d)** A paper and cardboard version of the ethoscope, assembled using  
191 220gsm paper and 1mm grayboard. Blueprints are provided in **Supplementary Material 3**. In all  
192 cases, ethoscopes must be powered with a 5Vdc input using a common USB micro cable either  
193 connected to the main or to a portable power-pack. Cables not shown for sake of simplicity.

194

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

203

204 **Figure 3** | Custom behavioural arenas. **(a-g)** Versatility of use with custom behavioural arenas.  
205 Examples of 7 different behavioural arenas developed in our laboratory. **(a)** Sleep arena. Most  
206 commonly used arena for sleep studies, lodging 20 individual tubes. **(b)** Long tubes arena. Houses  
207 13cm tubes and can be used for odour delivery studies or, more generally, for behaviours where longer  
208 walking is required. **(c)** Food bullet arena. Animals are placed directly on the arena and food can be  
209 replaced by pushing in a new bullet. Does not require glass tubes. **(d)** Decision making arena. Can be  
210 used to study simple decision making behaviours. **(e)** Square wells arena. Can be used for courtship  
211 assay or to record activity in a bi-dimensional environment. **(f,g)** Conceptually analogs to **a.** and **i.**, but

212 designed to work in high-resolution (full-HD) settings. Note that all arenas are marked with three  
213 visible reference points (indicated by a red circle in **a.**) that are used by the ethoscope to automatically  
214 align images for tracking, providing a degree of physical flexibility.

215

216 **Figure 4** | Tracking and validation of behavioural classification. **(a)** In real-time tracking mode,  
217 ethoscopes record Cartesian coordinates ( $x,y$ ) of each animal relative to their ROI (region of interest),  
218 along with the numbers describing an ellipsis circumscribing the animal ( $w,h,\phi$ ). **(b)** A screenshot of the  
219 data table recorded by ethoscope, showing four data points for a single fly. **(c)** To build a statistical  
220 model of activity, we used ethoscopes to record offline 2736 hours of video (144 hours x 19 flies) at  
221 resolution of 1280x960pixels and frame rate of 25FPS. Video fragments of the duration of 10 seconds  
222 were sampled every hour for all 19 animals and then scored by at least three experienced fly  
223 researchers in a randomised order. Consensual annotations – with a margin greater than 0.5 – were  
224 kept, resulting in a ground truth of 1297 video fragments (116 ambiguous annotations were excluded  
225 using this latter criteria). Scorers manually annotated both the position of the animal in the tube and the  
226 perceived behavioural state (i.e. immobile, micro-moving or walking). Ethoscope video-tracking were  
227 run independently on the whole video resampled at 1-5FPS, all realistic frame rates for real-time  
228 analysis. **(d)** Performance of maximal velocity and cumulative walked distance as features for 1297  
229 annotated videos of behaviours. **(e)** Marginal distribution of maximal velocity for each behaviour,  
230 showing the thresholds used to detect movement (dotted line) and walking (dashed line). **(f)** ROC  
231 curves for movement detection. A conservative threshold of 0.36mm/s, indicated by a red dot on the  
232 curve, yields a sensitivity (TPR) of 91.26% and specificity (1-FPR) of 99.65%. **(g)** ROC curve for  
233 walking detection. A threshold of 0.9mm/s (red dot on the curve) results in sensitivity and specificity of  
234 99.34 and 99.39, respectively.

235

236 **Figure 5** | Versatility of use with behavioural feedback-loop modules. **(a)** Diagram and **(b)** detail of the  
237 air/gas/odour (AGO)-delivery module. Two independent air flows (blue and purple in the drawing) are  
238 fed into the module using external sources. The module features 10 LEGO valves, each independently  
239 controlled through a servo motor. The motor switches the air source on the valve, selecting which  
240 source will be relayed to the tube containing the fly. Available positions are: blue source, purple source,  
241 and closed. **(c)** Representative response of three flies subjected to CO<sub>2</sub> administration using the AGO  
242 module. CO<sub>2</sub> release lasts 5 seconds (gray bar) and it is triggered by midline crossing (red dot). The

243 blue line indicates the fly position in the tube over the 150 second period. **(d)** Model and **(e)** detail of  
244 the rotational module. The module employs a servo motor to turn the tube hosting the fly. Direction,  
245 speed, duration and angle of the rotation can be modulated to change the quality of the stimulus. **(f)**  
246 Representative response of three flies upon stimulation using the rotational module shown in **(d,e)**.  
247 Rotation of the tube is triggered by 20 consecutive seconds of immobility (dashed line) and it is  
248 followed by 5 seconds of masking during which tracking is suspended to avoid motion artefacts (cyan  
249 area). The bottom panel shows traces of a dead fly. **(g)** Model of the optomotor module, able to  
250 simultaneously stimulate single flies with motion and light. **(h)** Detailed view of the optomotor  
251 principle. Light is directed into the tube using optical fiber. **Supplementary Videos 2** shows the  
252 optomotor module in action.

253

254

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

257

258 **Supplementary material 2** | Instruction booklet for building a LEGOScope

259

260 **Supplementary material 3** | Instruction booklet for building a PAPERscope

261

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

263

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

268

269 Supplementary videos available at [https://lab.gilest.ro/papers/ethoscopes-an-open-platform-for-high-](https://lab.gilest.ro/papers/ethoscopes-an-open-platform-for-high-throughput-ethomics/)  
270 [throughput-ethomics/](https://lab.gilest.ro/papers/ethoscopes-an-open-platform-for-high-throughput-ethomics/)

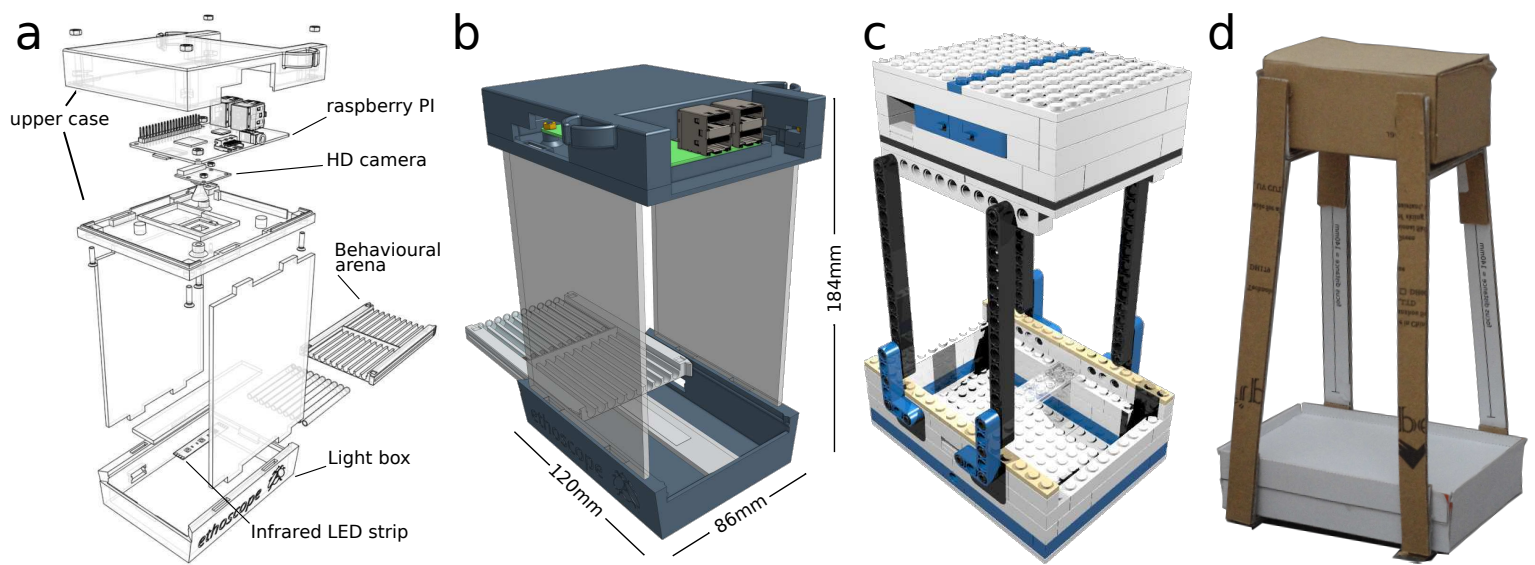


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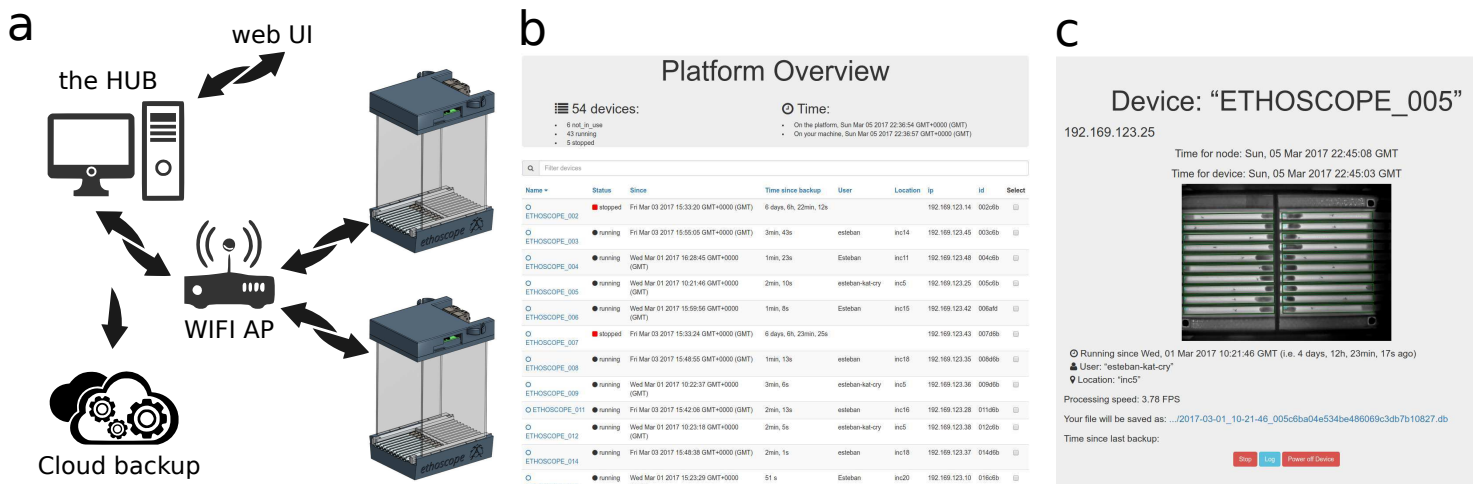


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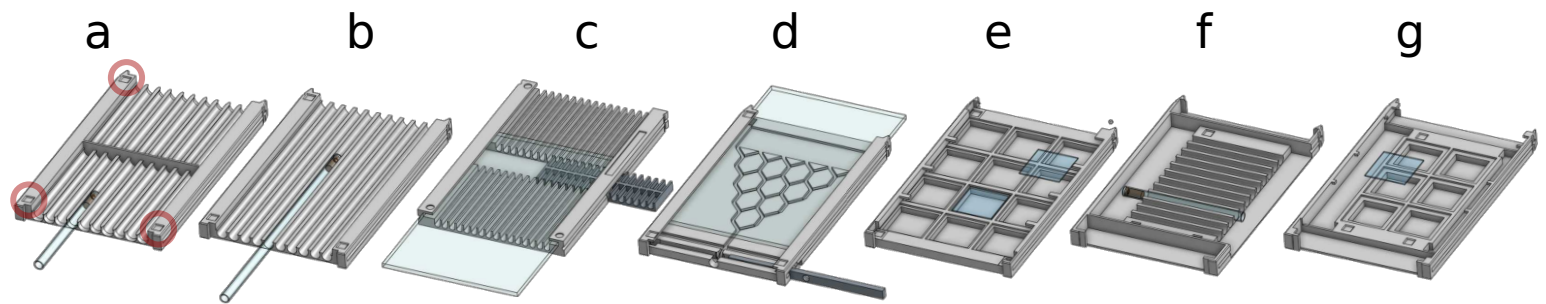


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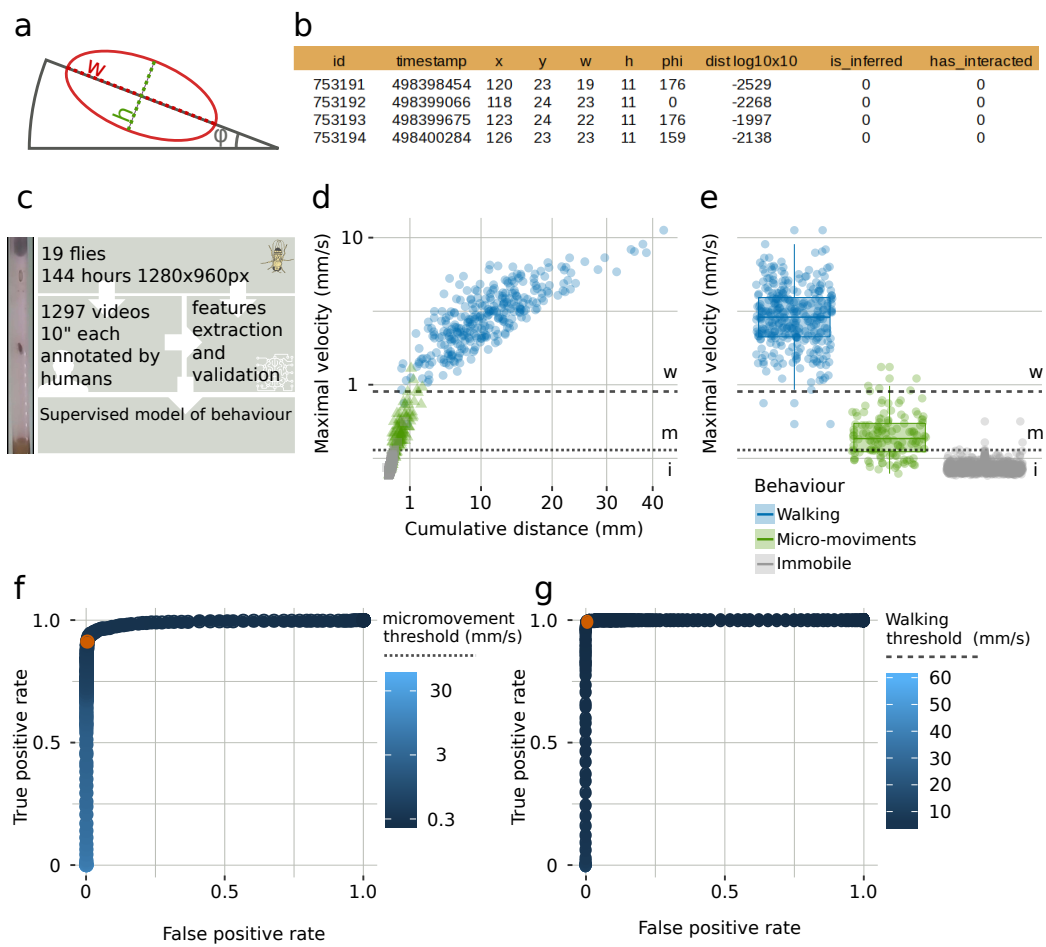


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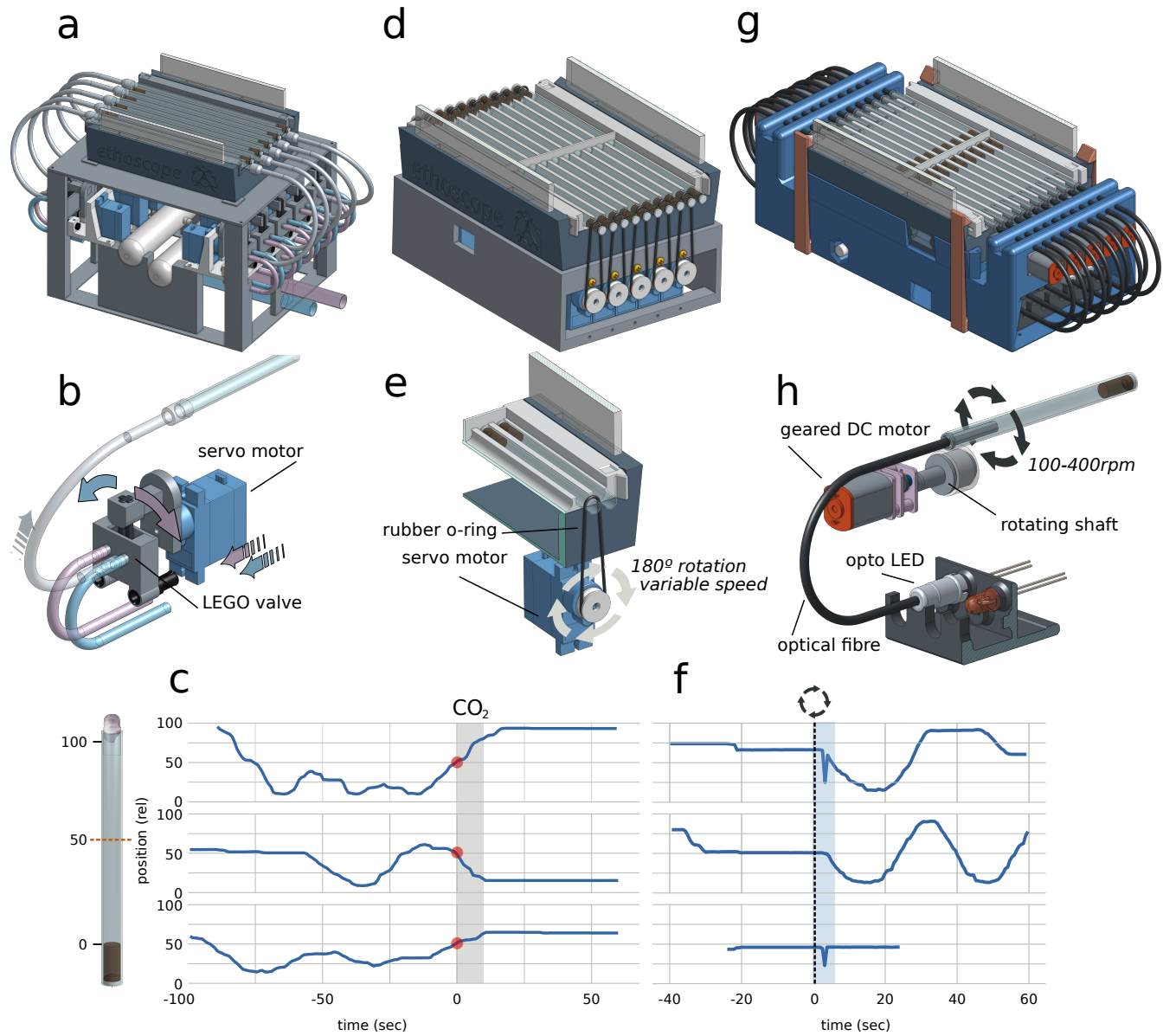


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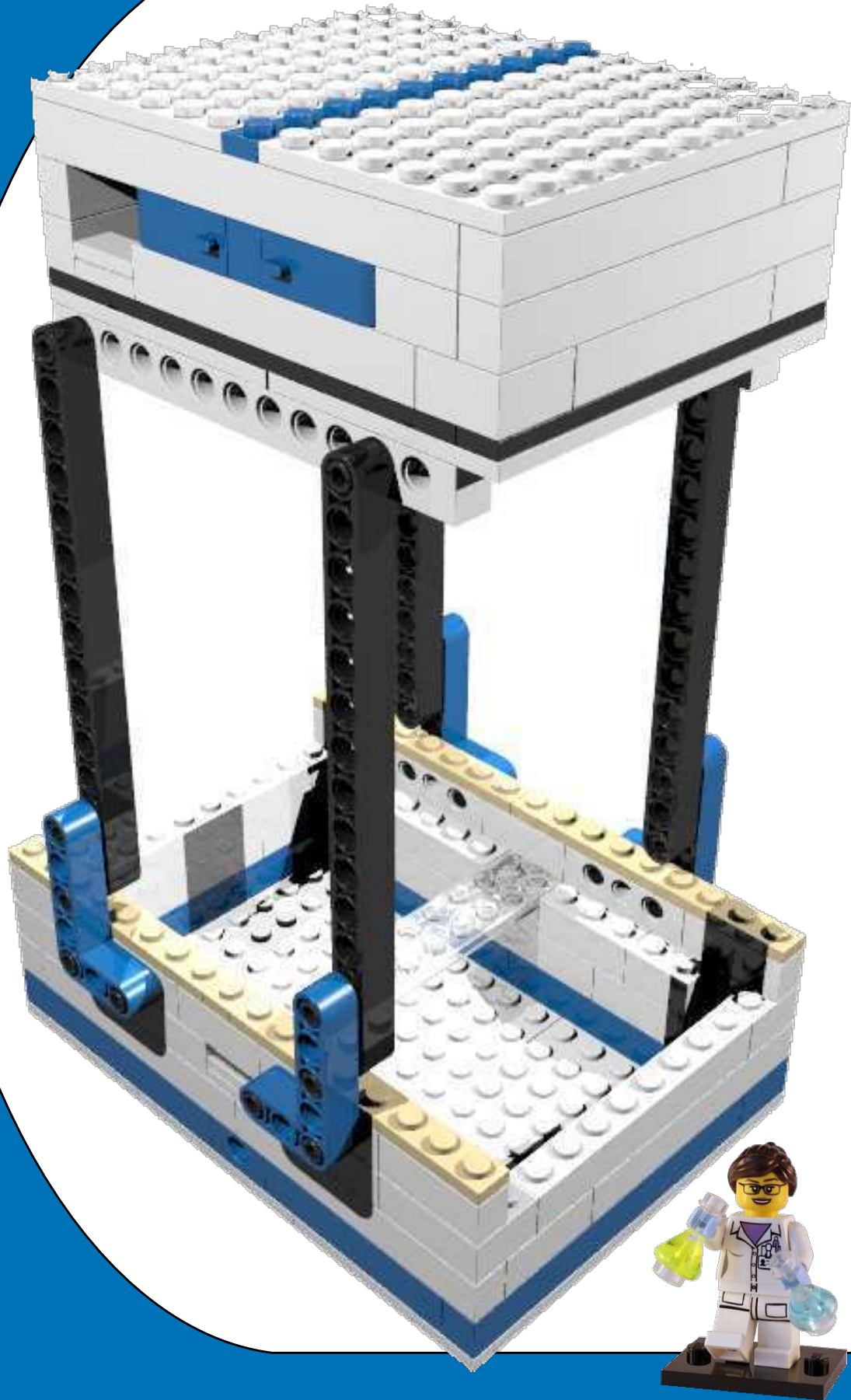


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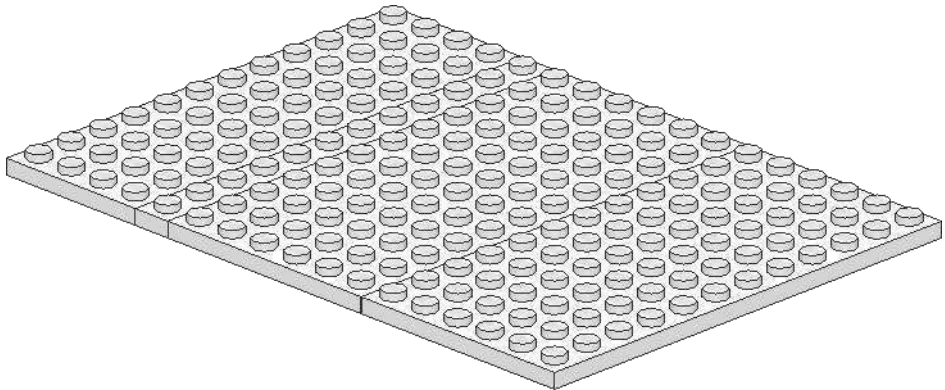
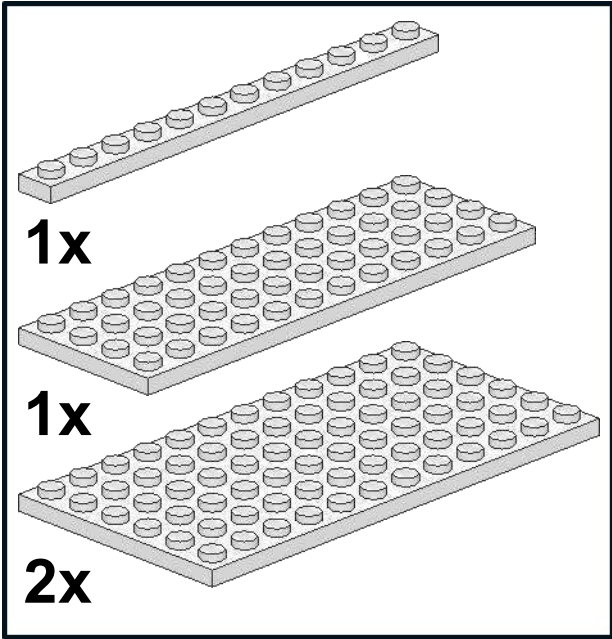
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ethoscope

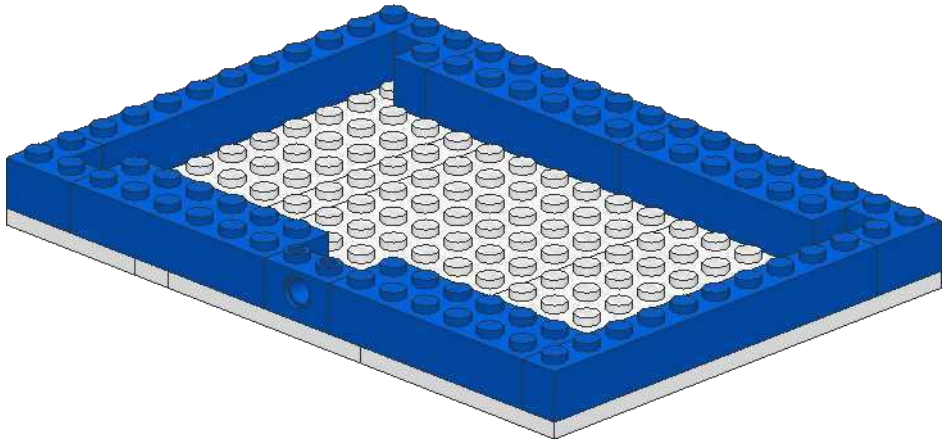
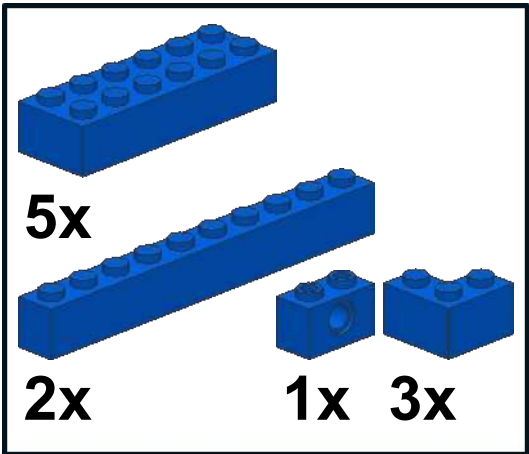
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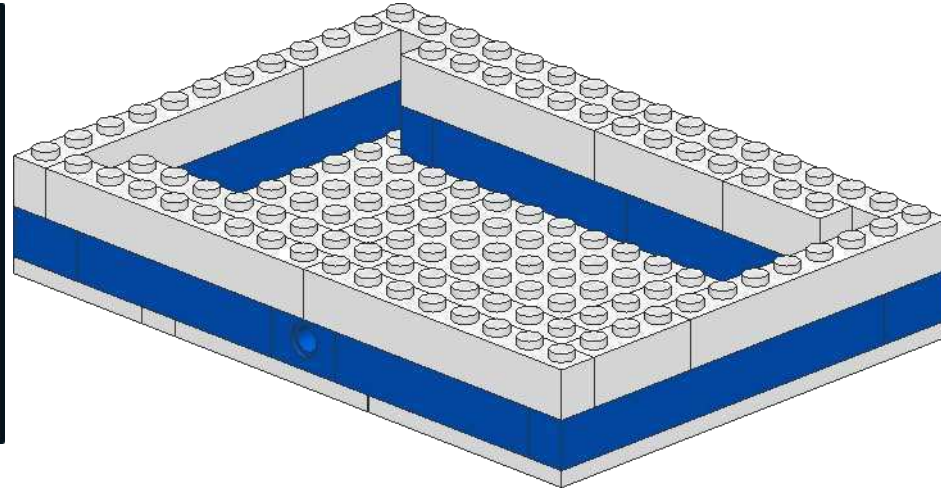
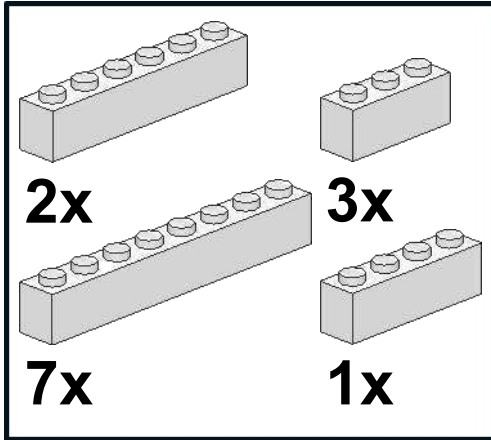
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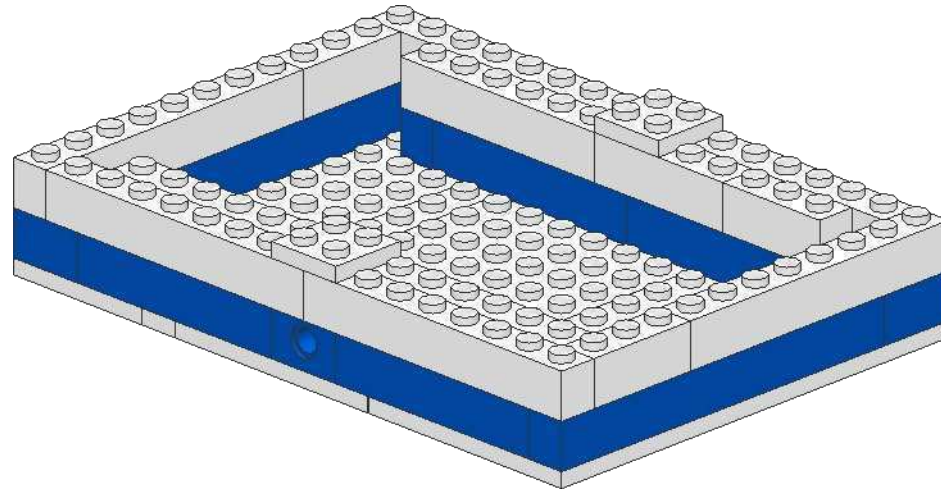
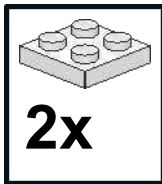
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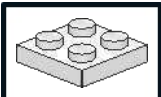
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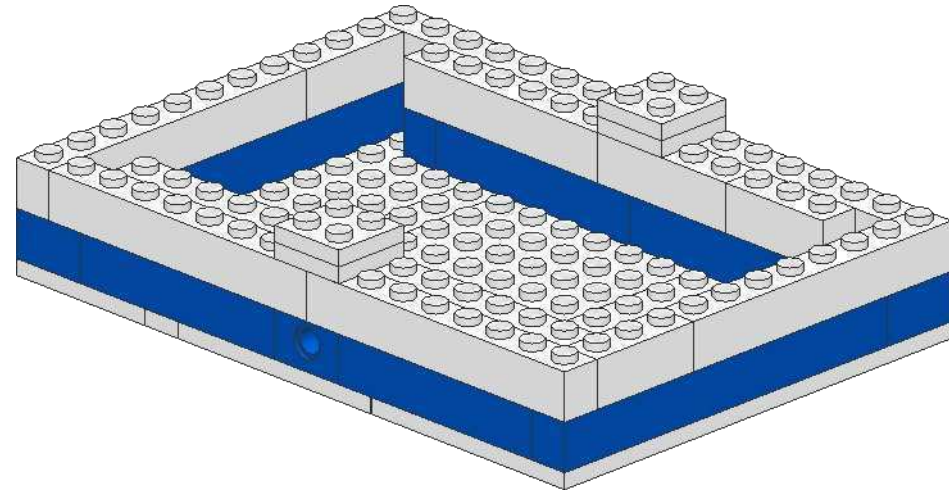
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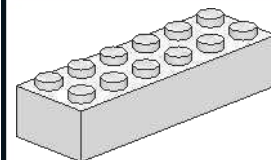
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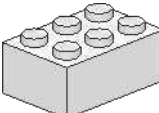
**2x**



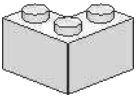
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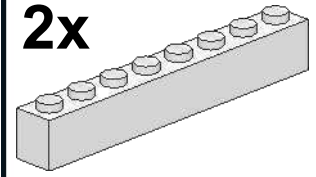
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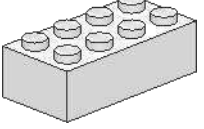
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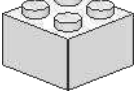
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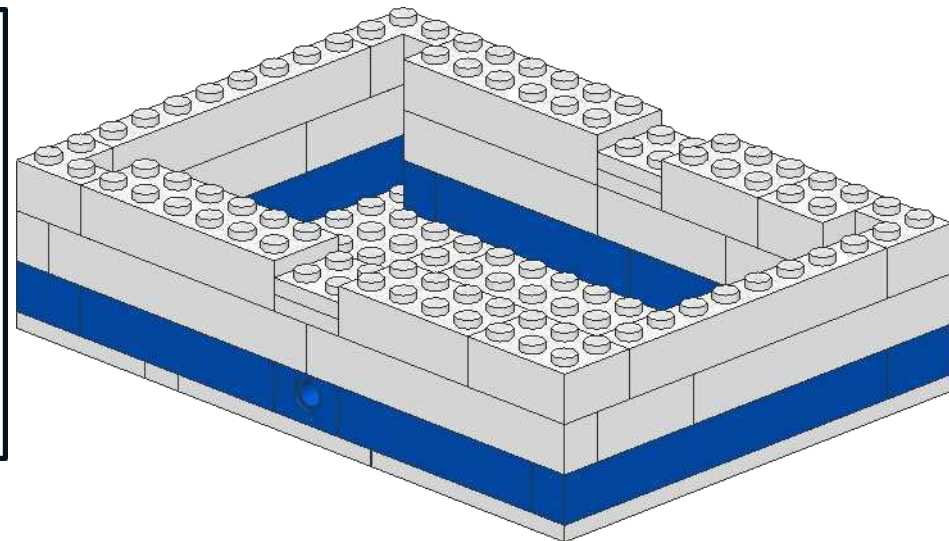
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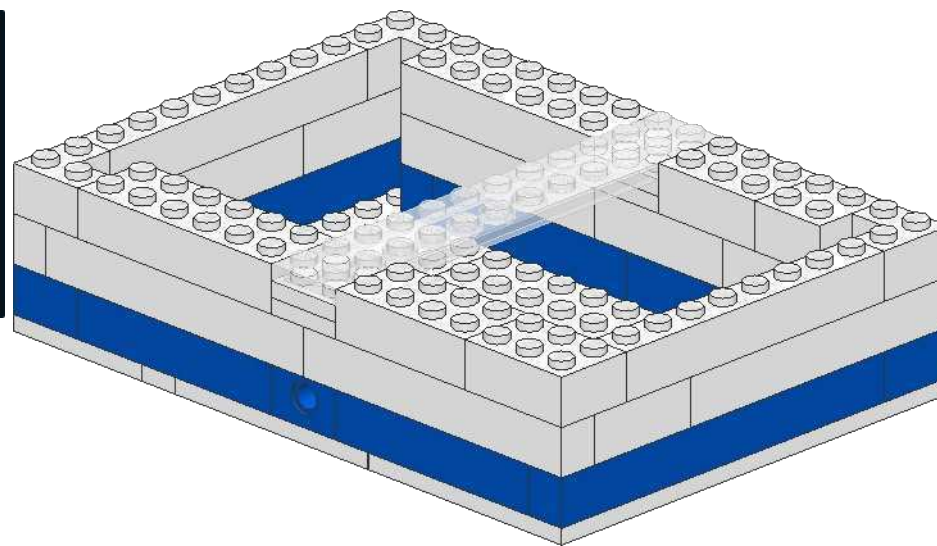
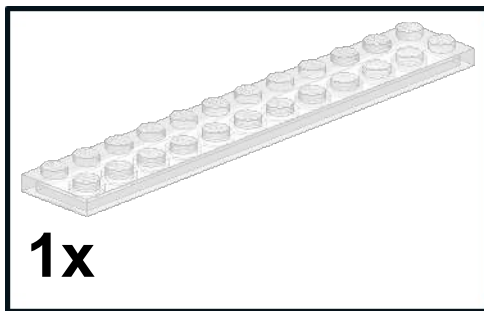
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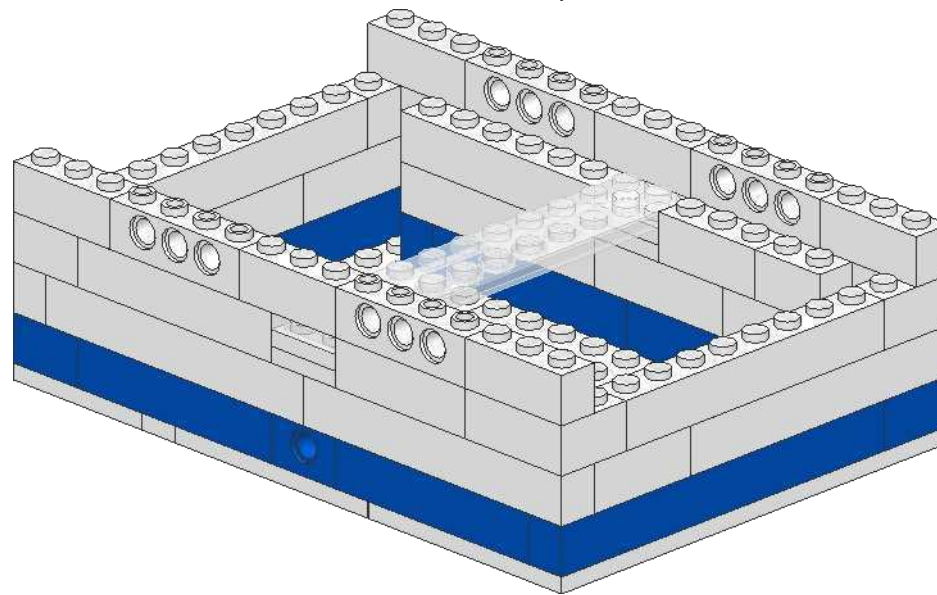
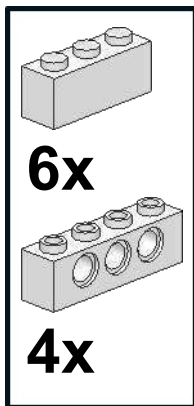
**1x**



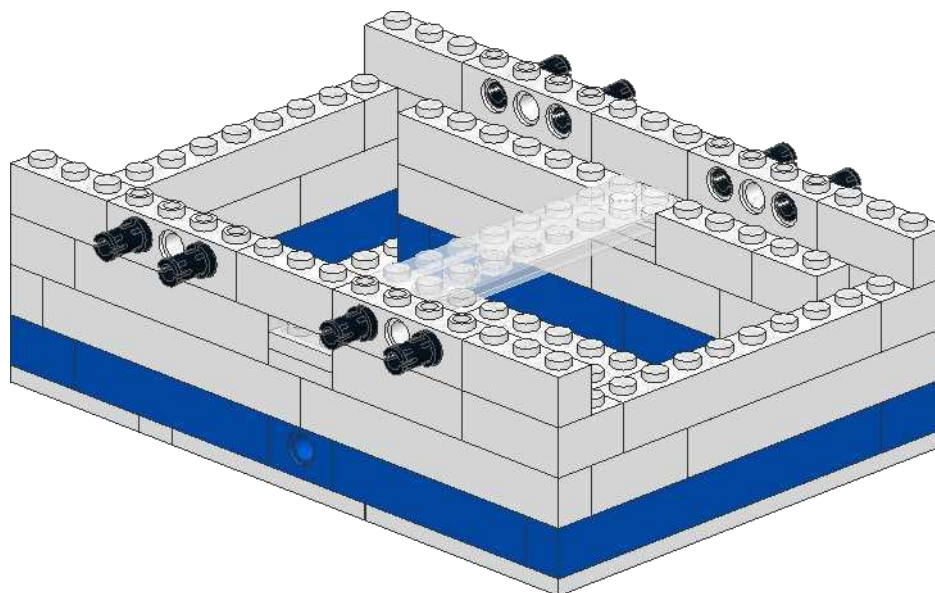
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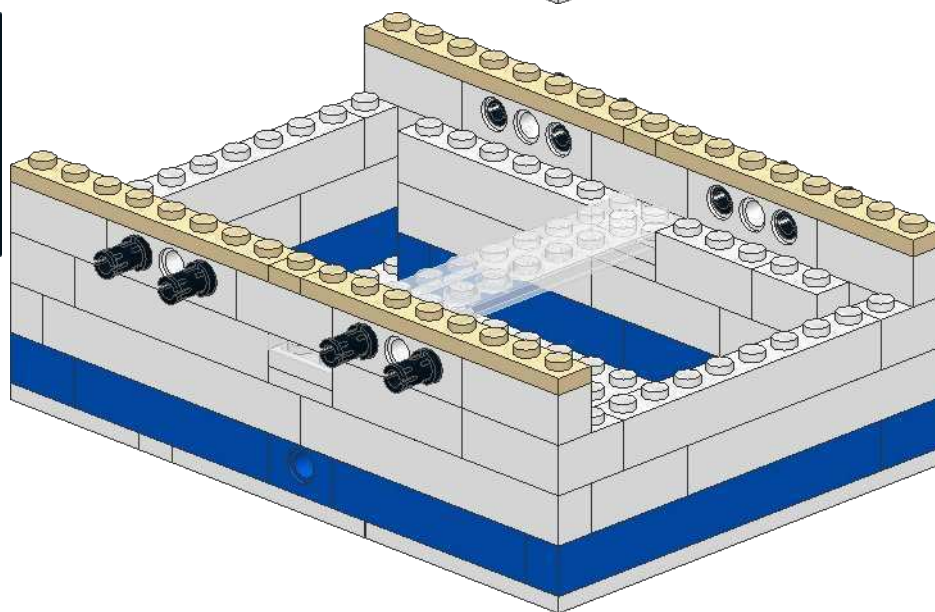
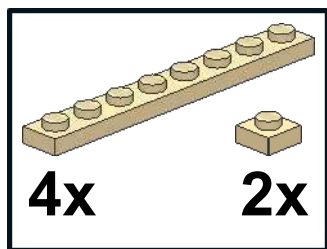
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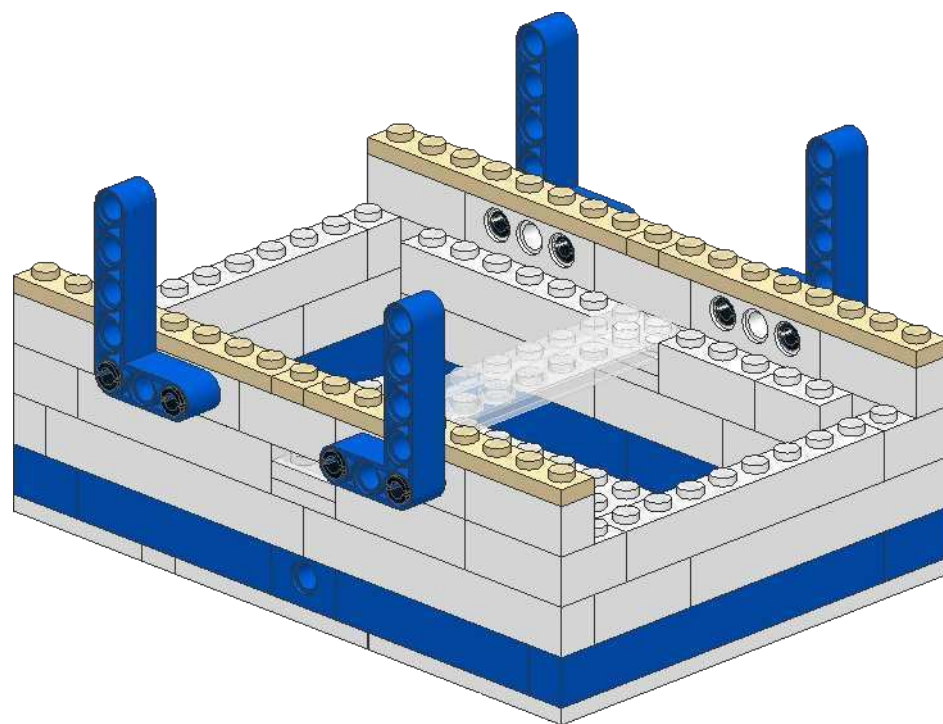
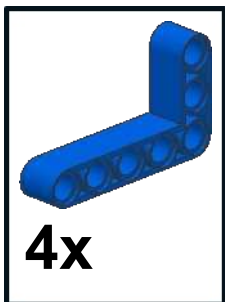
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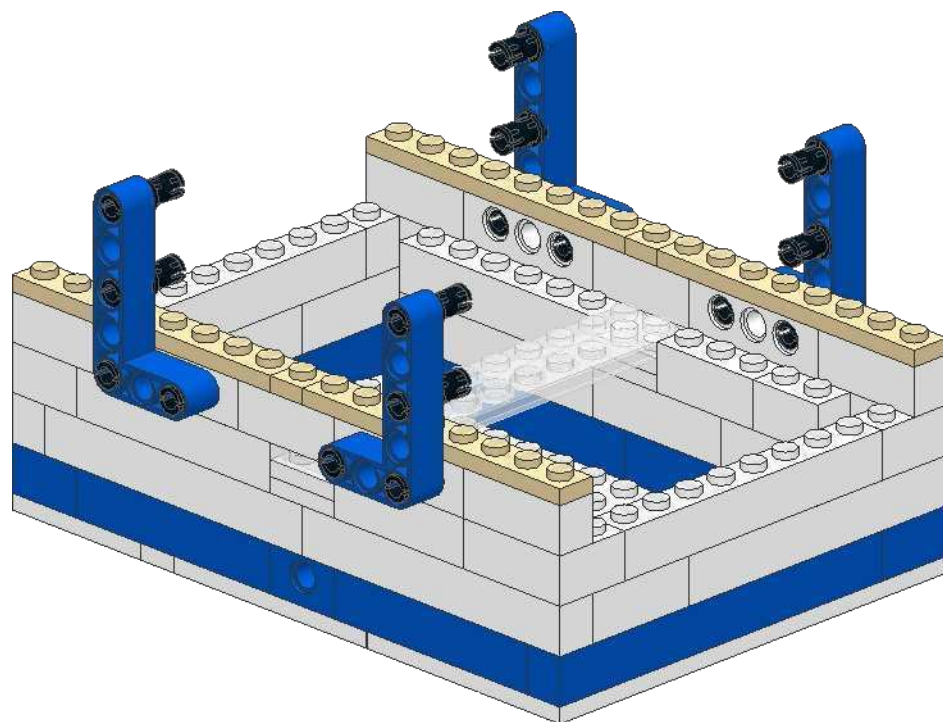
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11

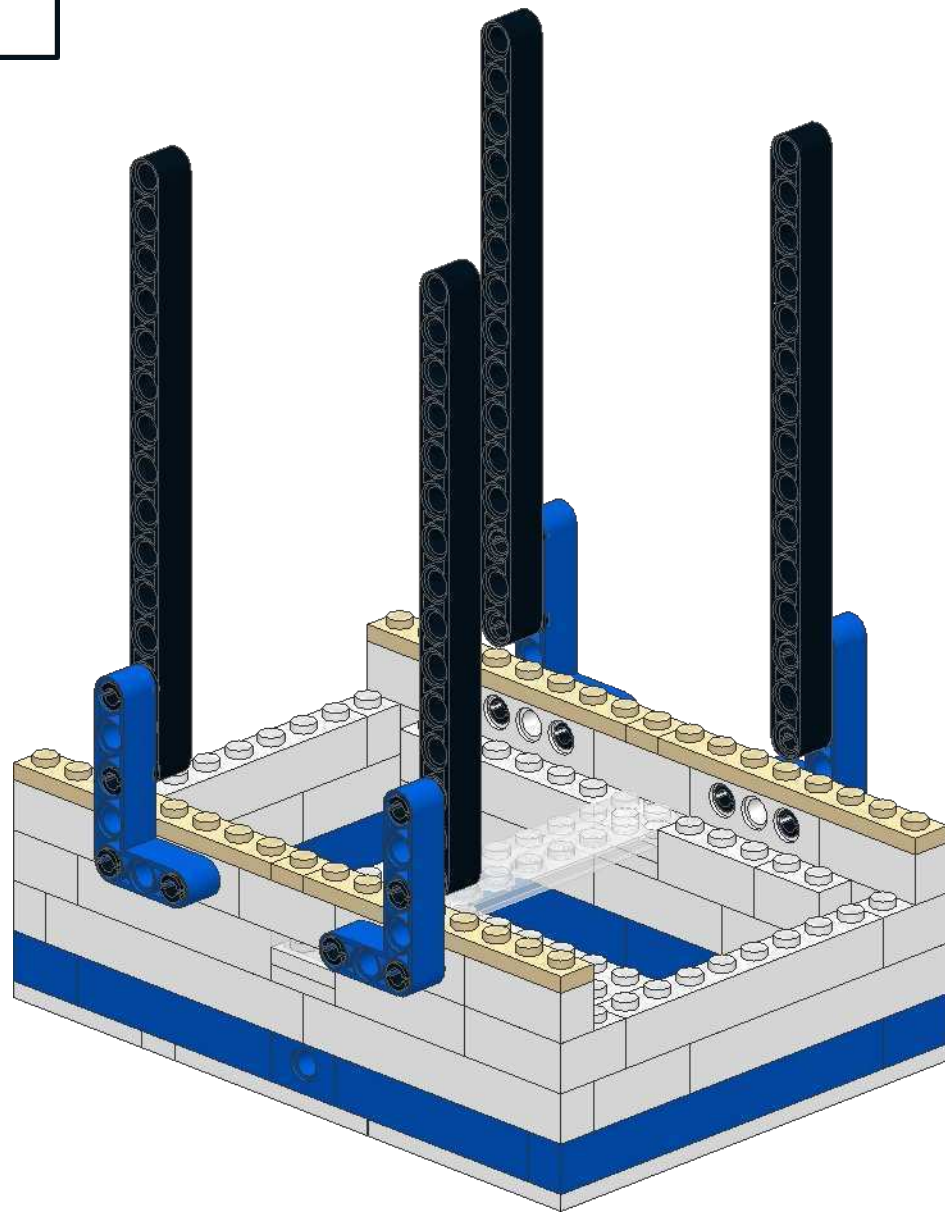
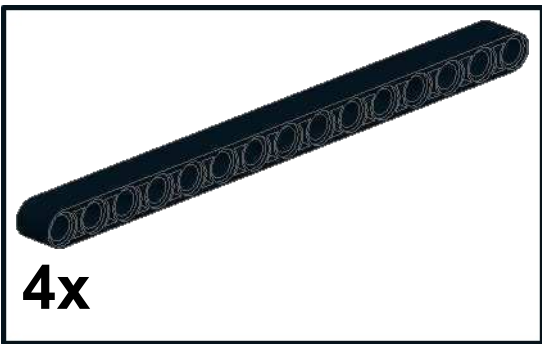


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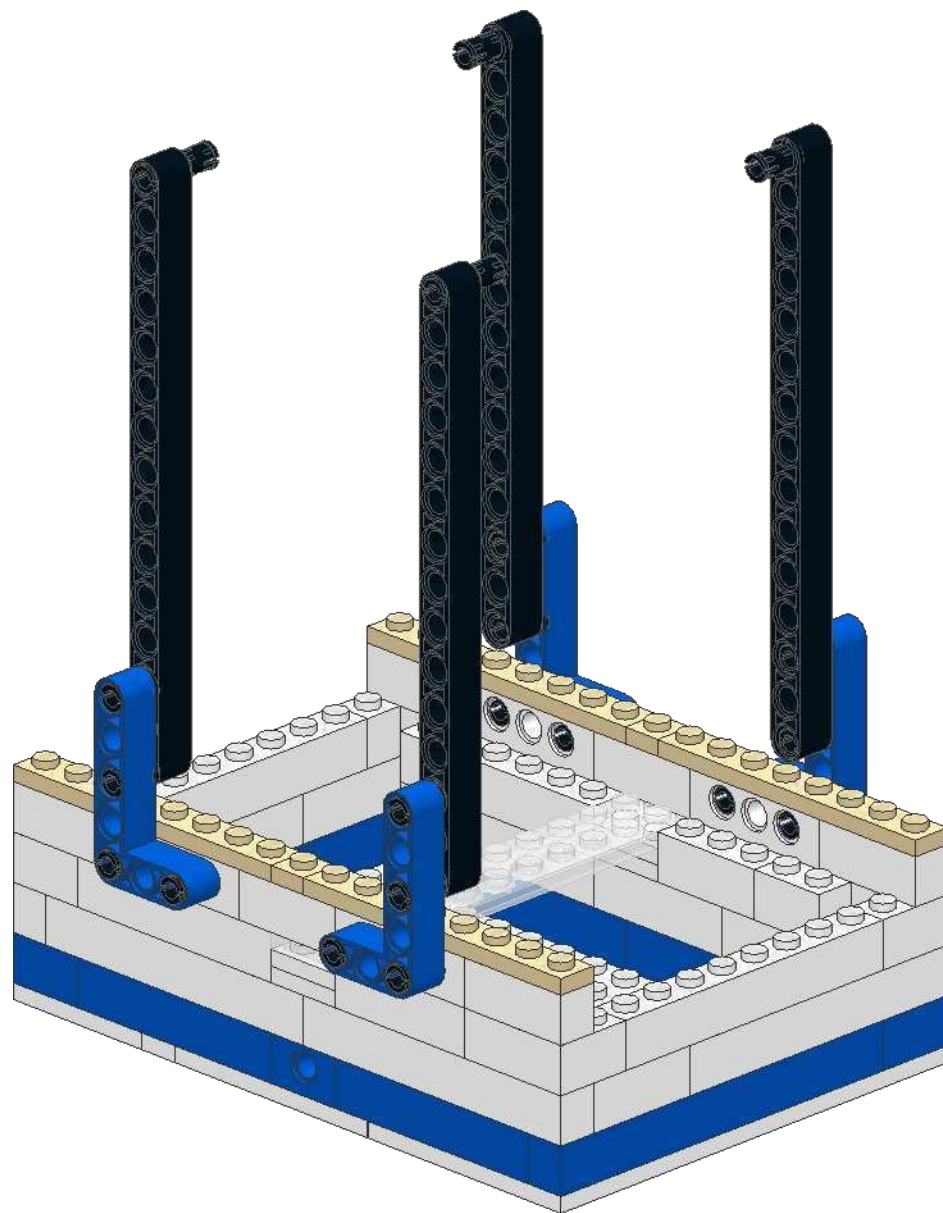




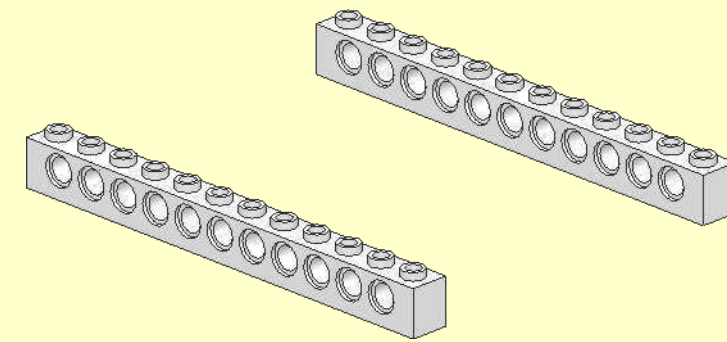
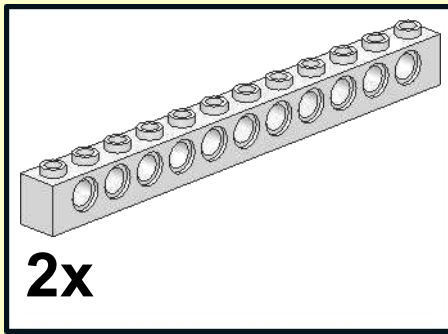
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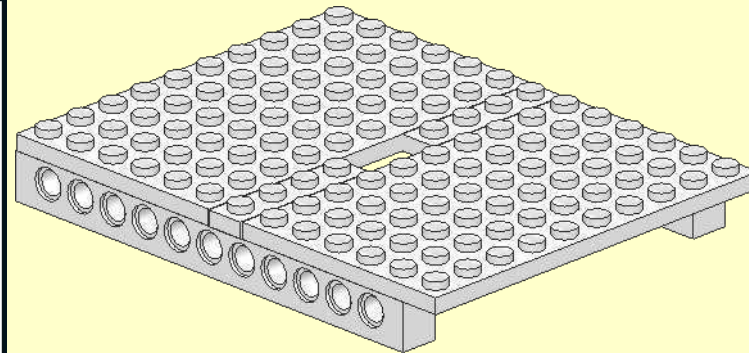
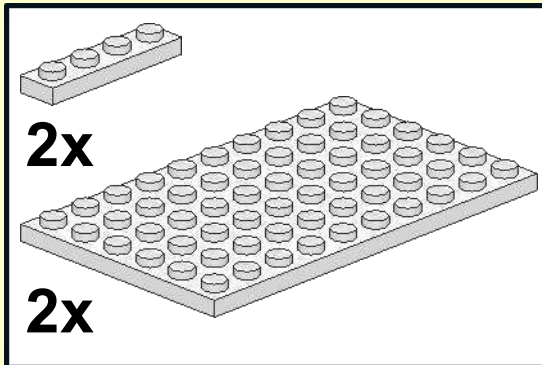
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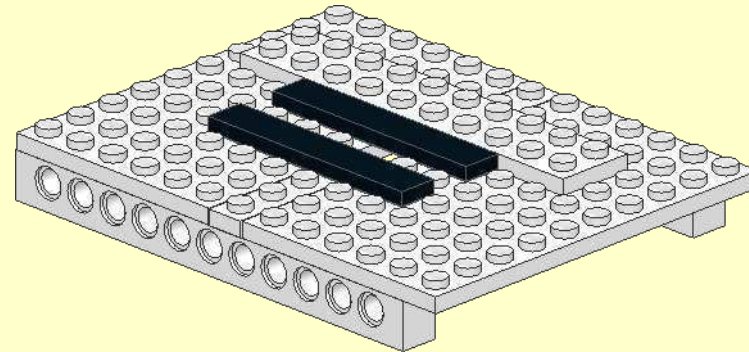
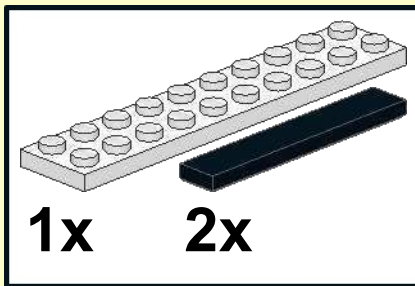
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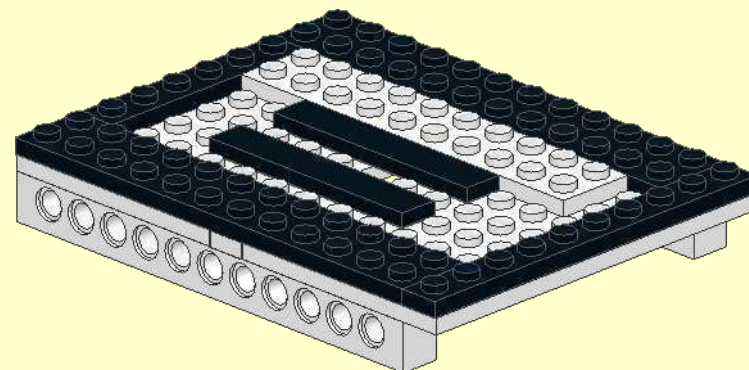
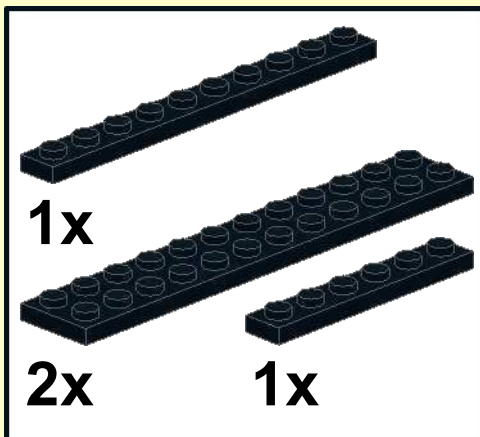
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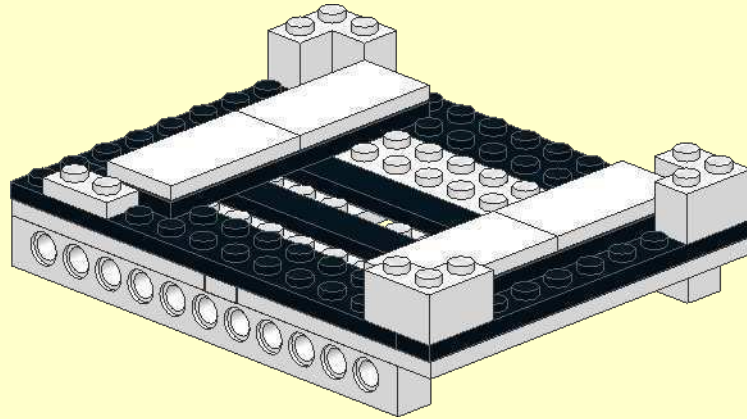
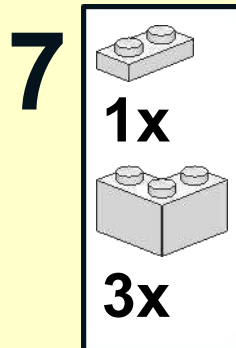
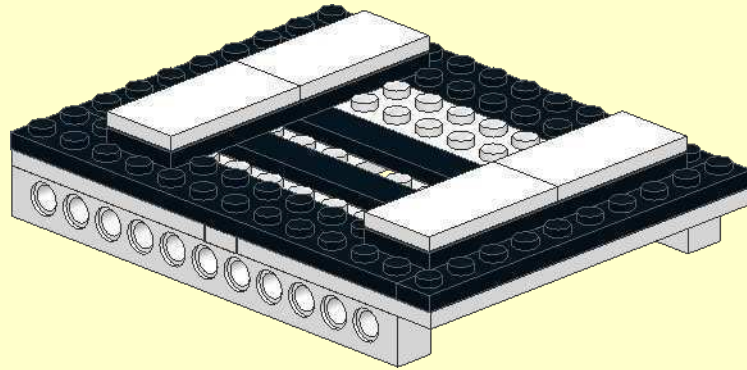
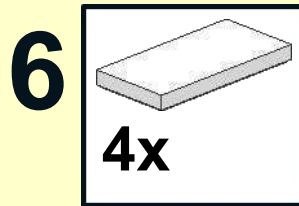
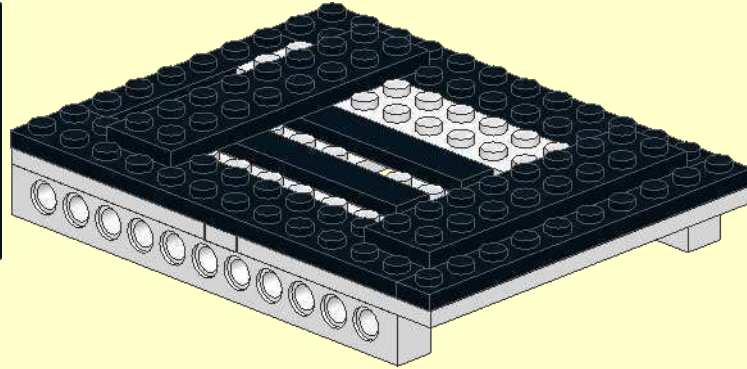
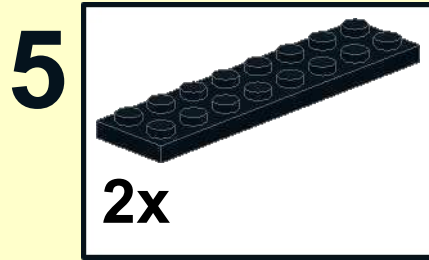


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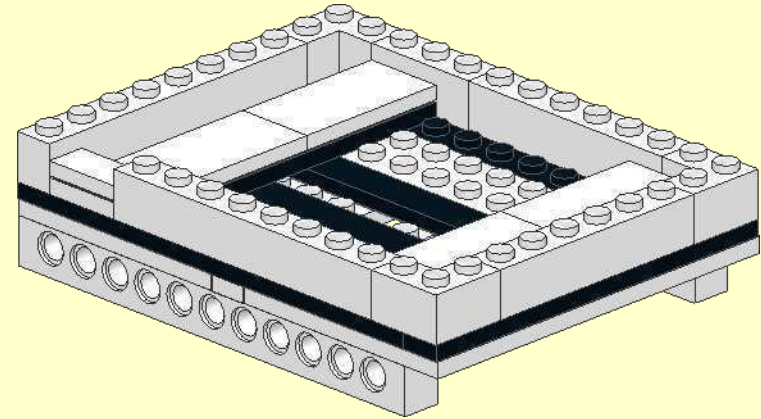
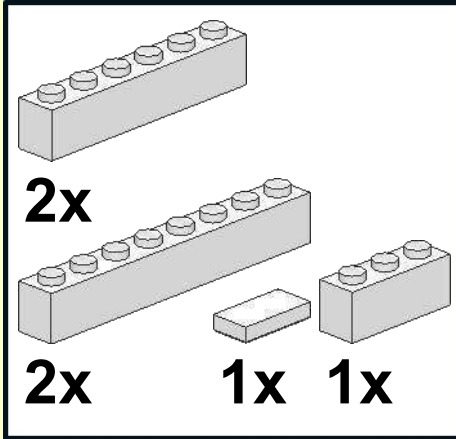


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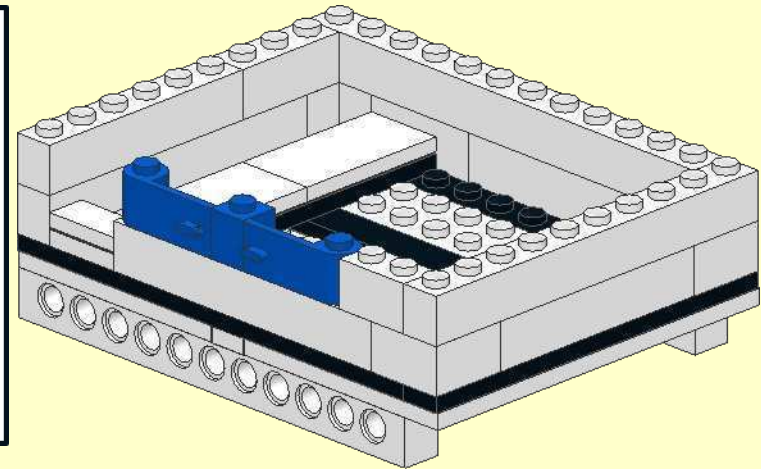
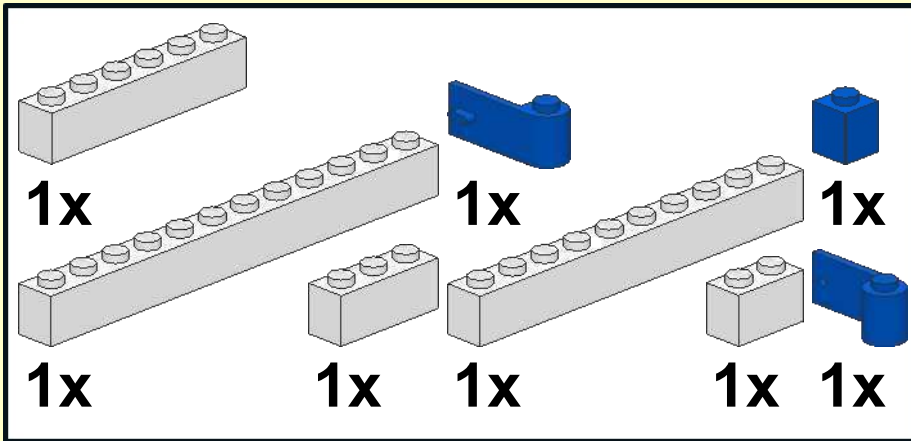




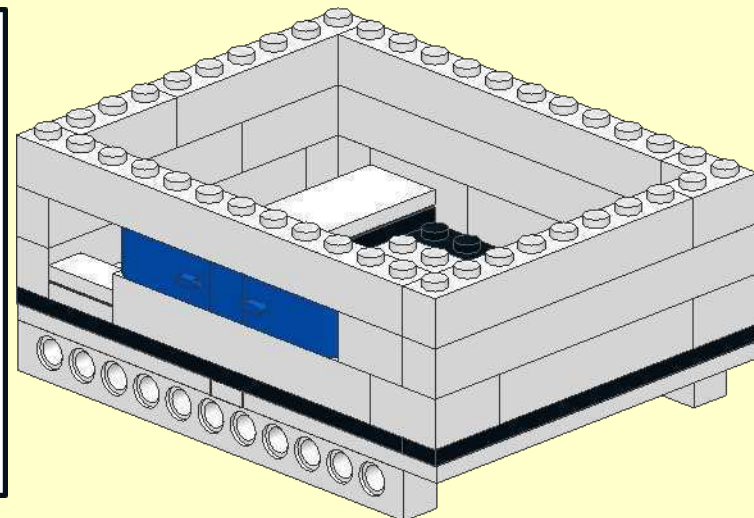
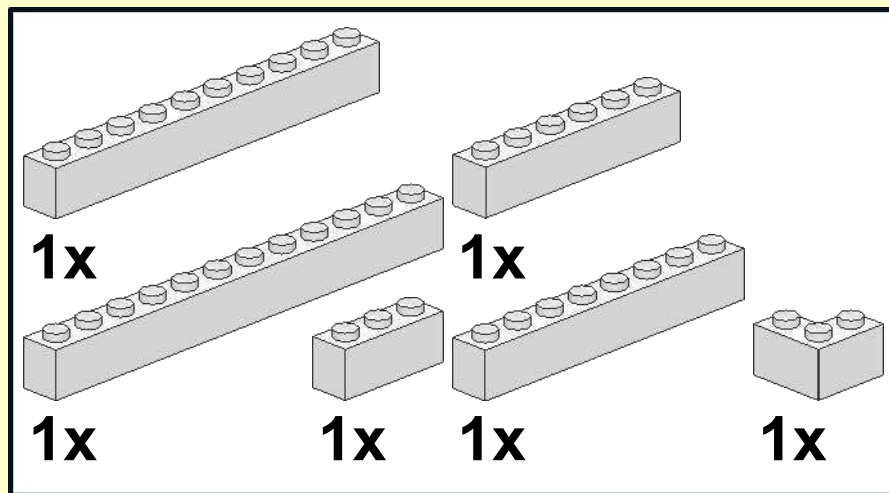
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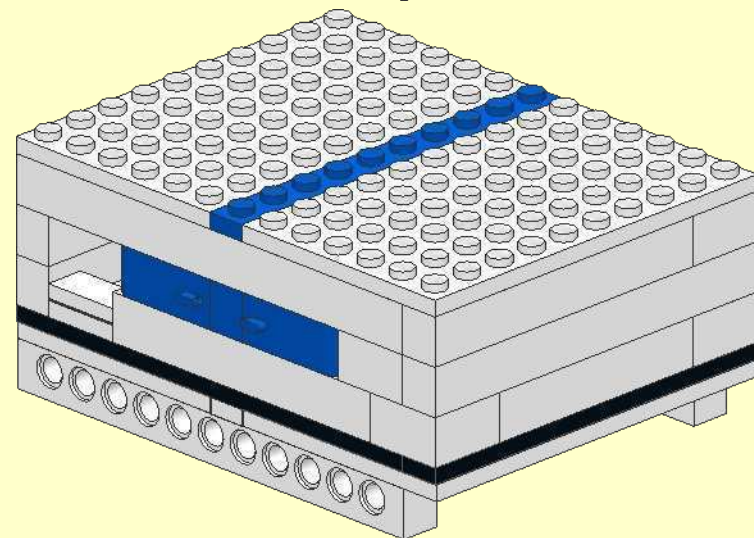
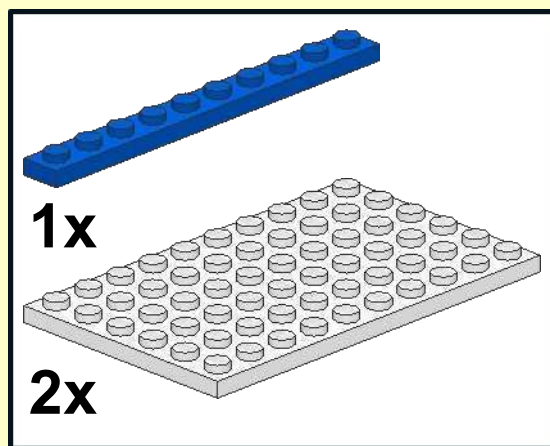
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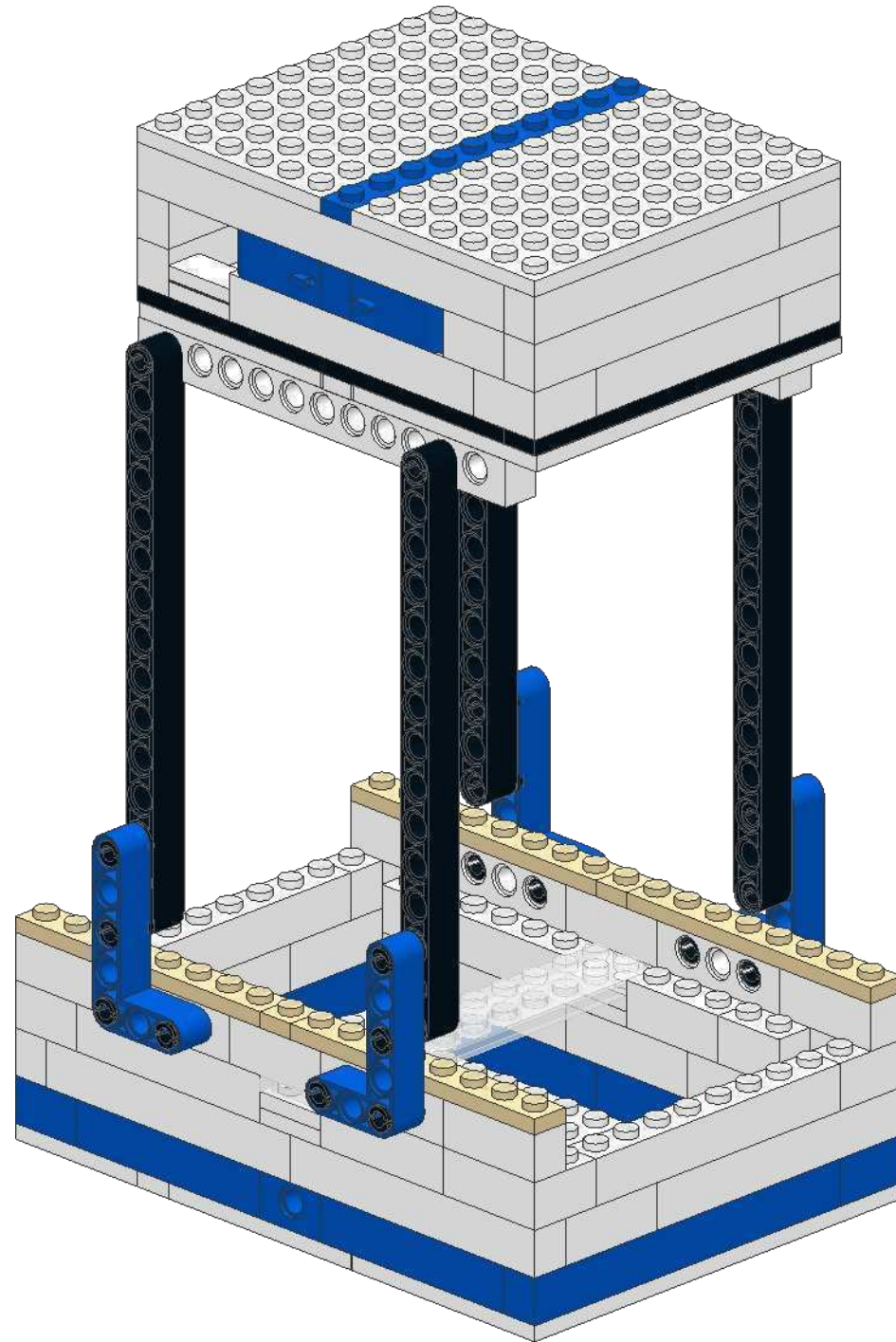
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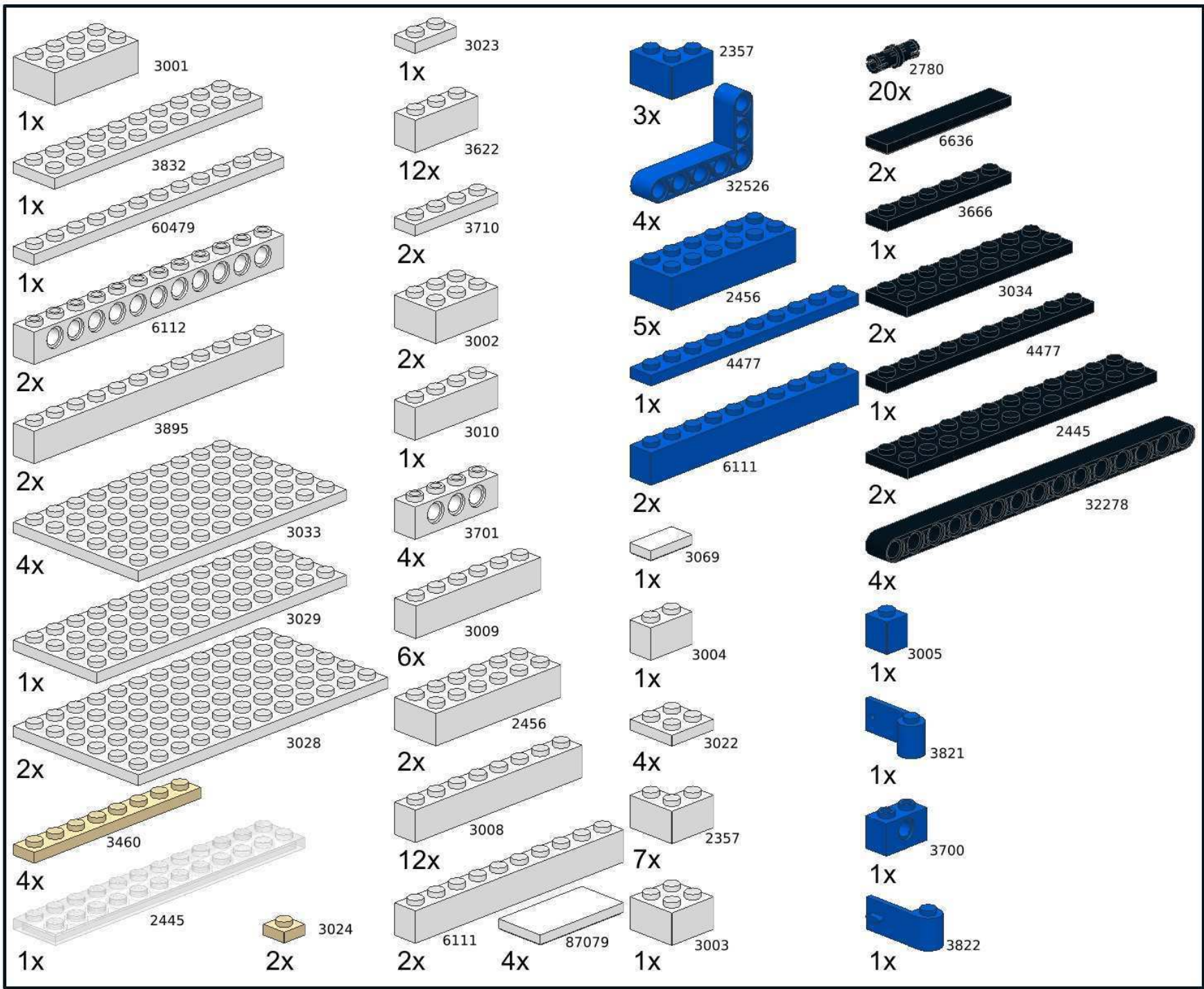


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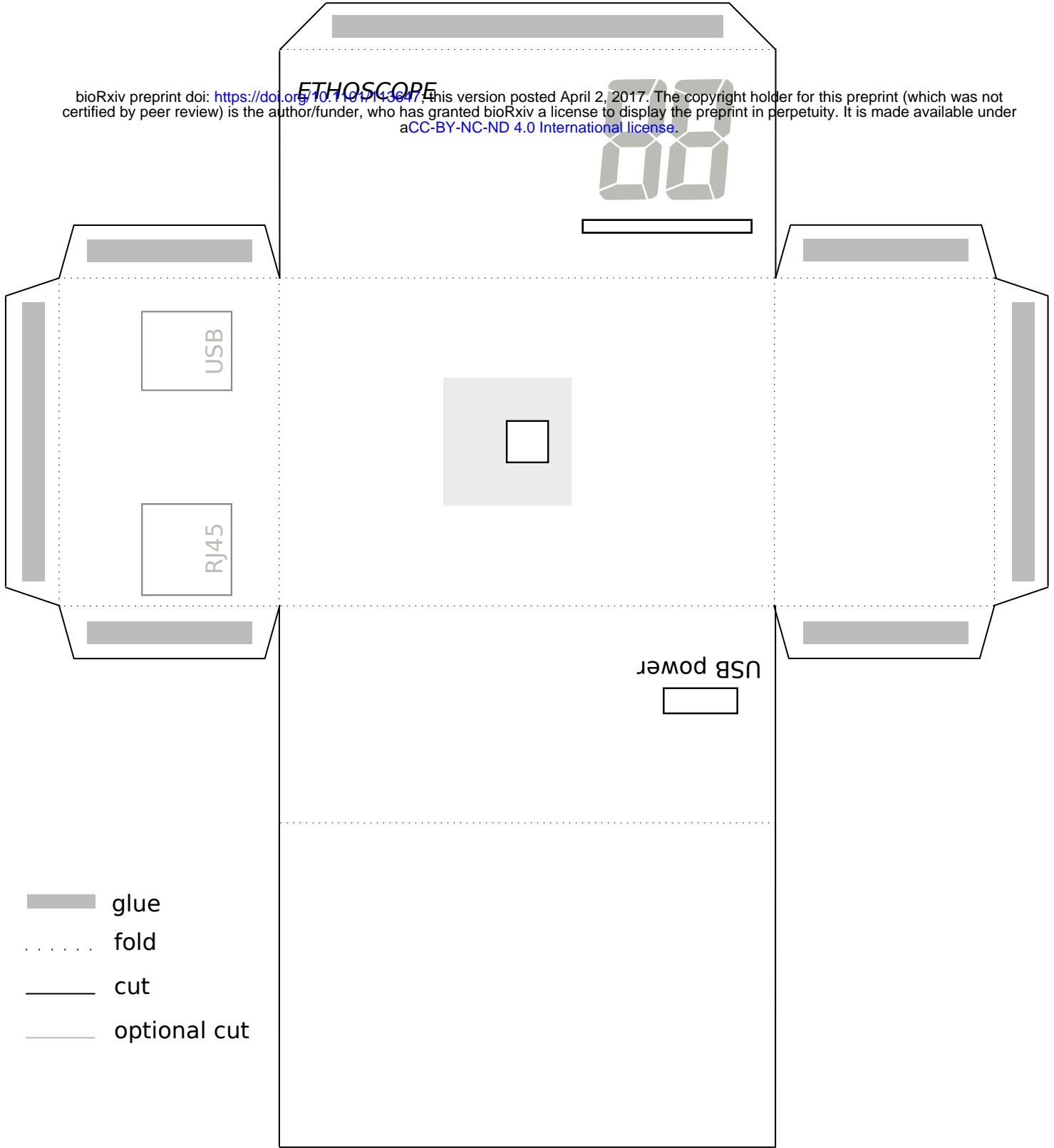






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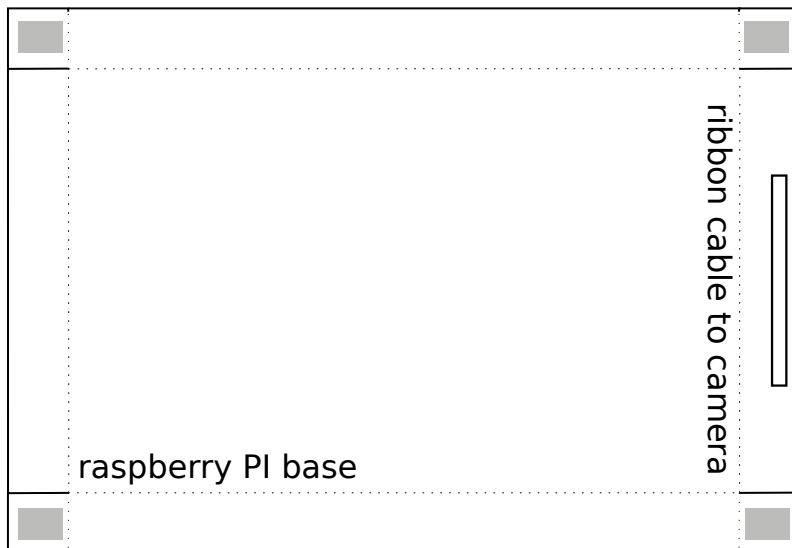


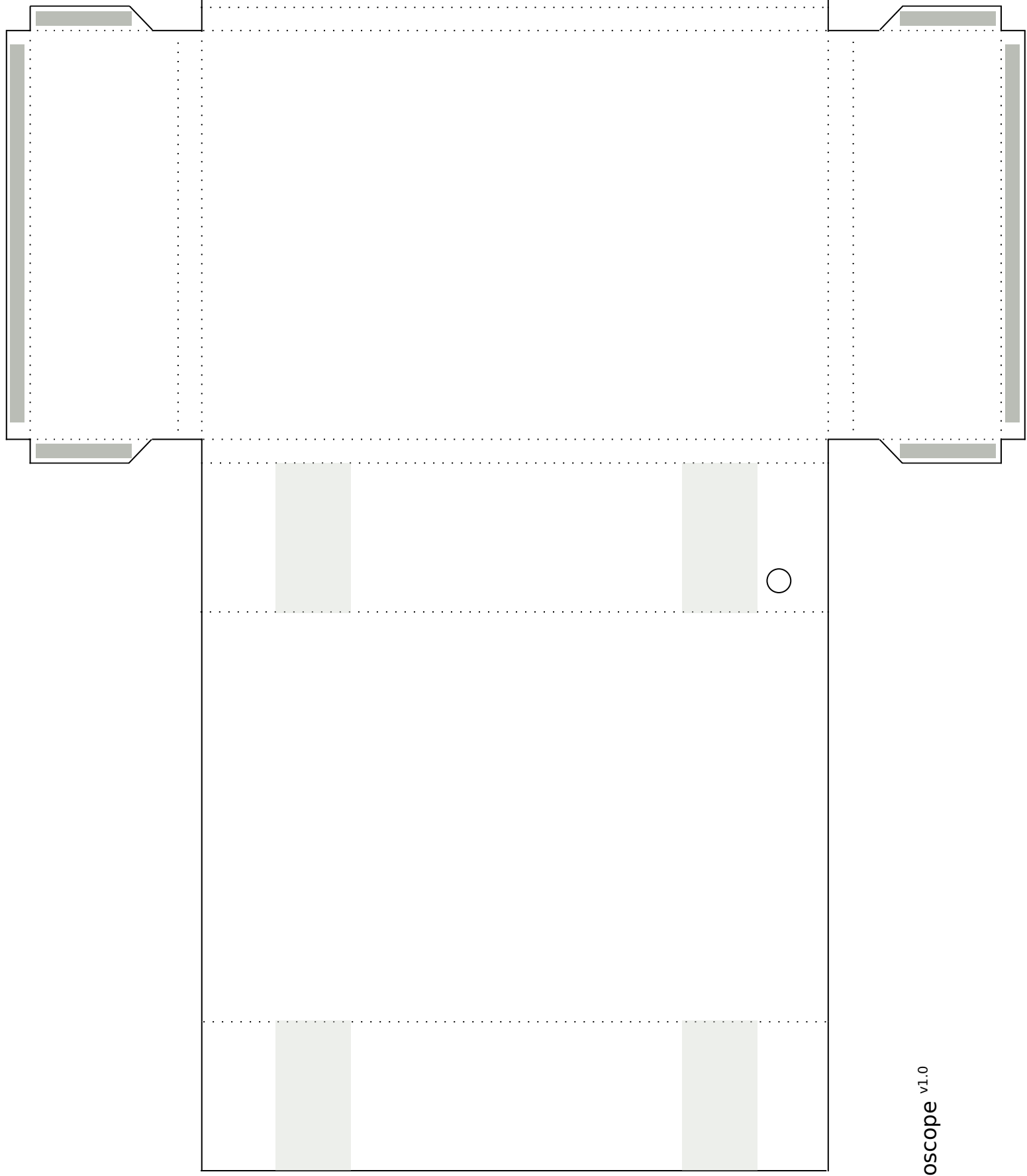






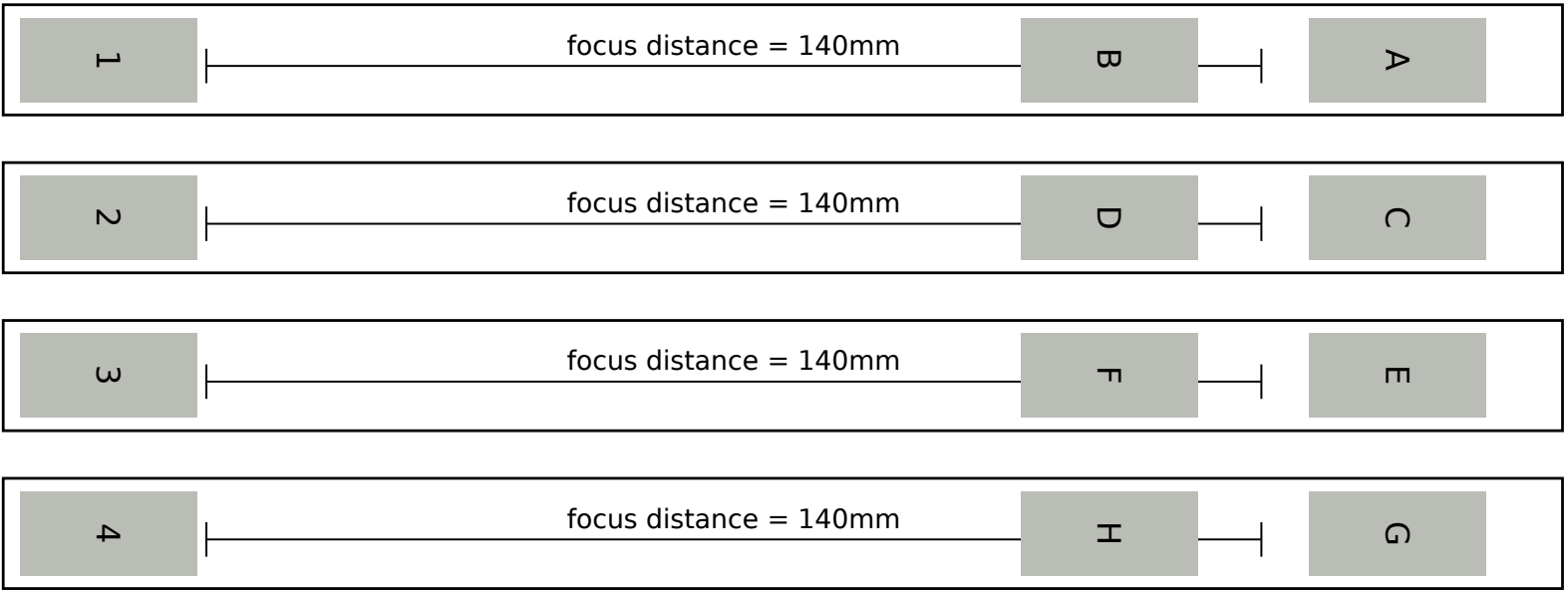
-  glue
-  fold
-  cut
-  optional cut





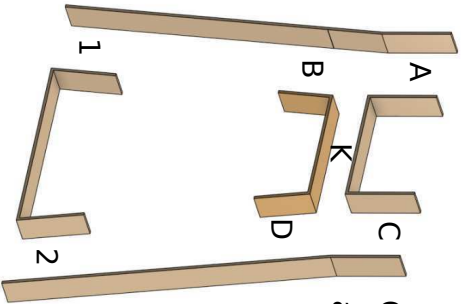
- hole for IR power
- guide for spacers

paper ethoscope v1.0  
lightbox



Align with camera lens →

Align with bottom of arena ↙



Cut on cardboard,  
assemble using hotglue

