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FAQ and Troubleshooting

	Question	Answer
1	Is it possible to get in direct contact with the research team?	Yes, you can arrange for a video call by writing to our service@bpp-peptides.com address.
2	Do you also have protocols for my desired organism?	We can help adapt the standard protocol to your organism and support your project if necessary. This consultation service is free of charge.
3	What media can I use for incubation?	Use any medium suitable for recovery of your organism. Avoid polysaccharides in the medium during the contact / transformation step.
4	What do you know about the mechanism of membrane transport?	The cell-entry mechanism may differ between organisms and is still being investigated. Due to the fact that membrane S-layer and cell wall compositions vary, different strategies may need to be applied. That is why Bioportide variants differ in their cell-penetrating domains. Contact us for help choosing a variant.
5	What kinds of nucleic acids can be transformed or transfected?	RNA and DNA, single stranded and double stranded, short and long sequences, circular and linear as well as PCR products have been successfully tested in different organisms. Contact us if you are looking for data for your organism.
6	Can I always use the same amount of BPP Bioportide™, no matter what type of nucleic acid I use on which microorganism?	We can provide you with a range of nucleic acid concentrations for species and genera for which we have data. You may test concentration down to 1/1000 of your usual concentration. Keep the nucleic acid to BPP Bioportide™ mass ratio at 1:10 by diluting both accordingly. If this does not give the expected result, or if you use short RNA (20–300 nt) or a large plasmid (8–25 kb), use our Molar Ratio Calculator to plan additional conditions. To test lower amounts while keeping 10:1, see the Dilution Series protocol.
7	Can I freeze the whole kit?	Yes, you can store the whole kit at -20 °C or -80 °C for long-term storage. Please handle its components as described in the manual.

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		The freeze-dried BPP Bioportide™ and the 50% glycerol buffer are supplied separately. The dissolved BPP Bioportide™ working solution can be stored at -20 °C and reused.
8	The manual describes a buffer but there is only 50% glycerol as buffer within the kit?	The buffer components are part of the freeze-dried BPP Bioportide™ and dissolve when you add the supplied 50% glycerol.
9	What do you know about cytotoxic effects?	Cytotoxicity depends on the concentration of the BPP Bioportide™ and the cell penetrating domain used. In the tested mammalian cells, concentrations of 0,6-3 µg/ml were compatible with cell viability. In the tested Gram-positive bacteria, tolerated concentrations were lower. To find a suitable BPP Bioportide™ concentration for your organism, refer to our "Dilution Series" protocol (see Download section).
10	The proposed protocol in the manual deviates from the standard protocol that I use in my laboratory. What should I do?	The protocols are examples based on published methods: a reference list is available. They show where to bring the BPP Bioportide™ - nucleic acid complex in contact with your cells. Adapt the other steps to your standard protocol. If you need help, please use our free consultation service.

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Frequent troubles

Order of importance	Question	Answer
I have no colonies on my plate – part 1	I followed the protocol with all precautions and worked under sterile conditions. The cells appeared viable when I plated them.	Please rule out the usual sources of error in the transformation of your specific organism. For BPP Bioportides™, check the following: 1) Verify that the BPP Bioportide™ to nucleic acid ratio is appropriate. 2) The BPP Bioportide™ variant may not be compatible with your organism's cell envelope. Please contact the BPP team. We offer existing customers additional BPP Bioportide™ variants free of charge. 3) Many BPP Bioportides™ are able to transform *E. coli*. Test the BPP Bioportide™ variant in question with a standard *E. coli* strain and plasmid (e.g. pUC19) using our *E. coli* protocol. If the E. coli test succeeds, another BPP Bioportide™ variant may work better for your organism. If it fails, contact the BPP team for advice.
I have no cells on my plate – part 2	I have used the kit as instructed. After adding the BPP Bioportide™– nucleic acid complex, the cell suspension became more turbid before plating.	Check cell viability. The BPP Bioportide™ at this concentration may be toxic to your organism. If viability is reduced, test lower BPP Bioportide™ concentrations as described in document "Dilution Series".
I have no colonies on my plate – part 3	My cells looked healthy and the BPP Bioportide™ transformed *E. coli* in the control test. I used a replicative plasmid and things do not work out.	Confirm that the plasmid replicates in your strain and that its selection marker works. If both are confirmed, your organism may have initiated defense mechanisms after transformation. As your nucleic acid does not integrate, it becomes vulnerable to degradation within the cell. Check whether the plasmid's methylation pattern is compatible with your host.