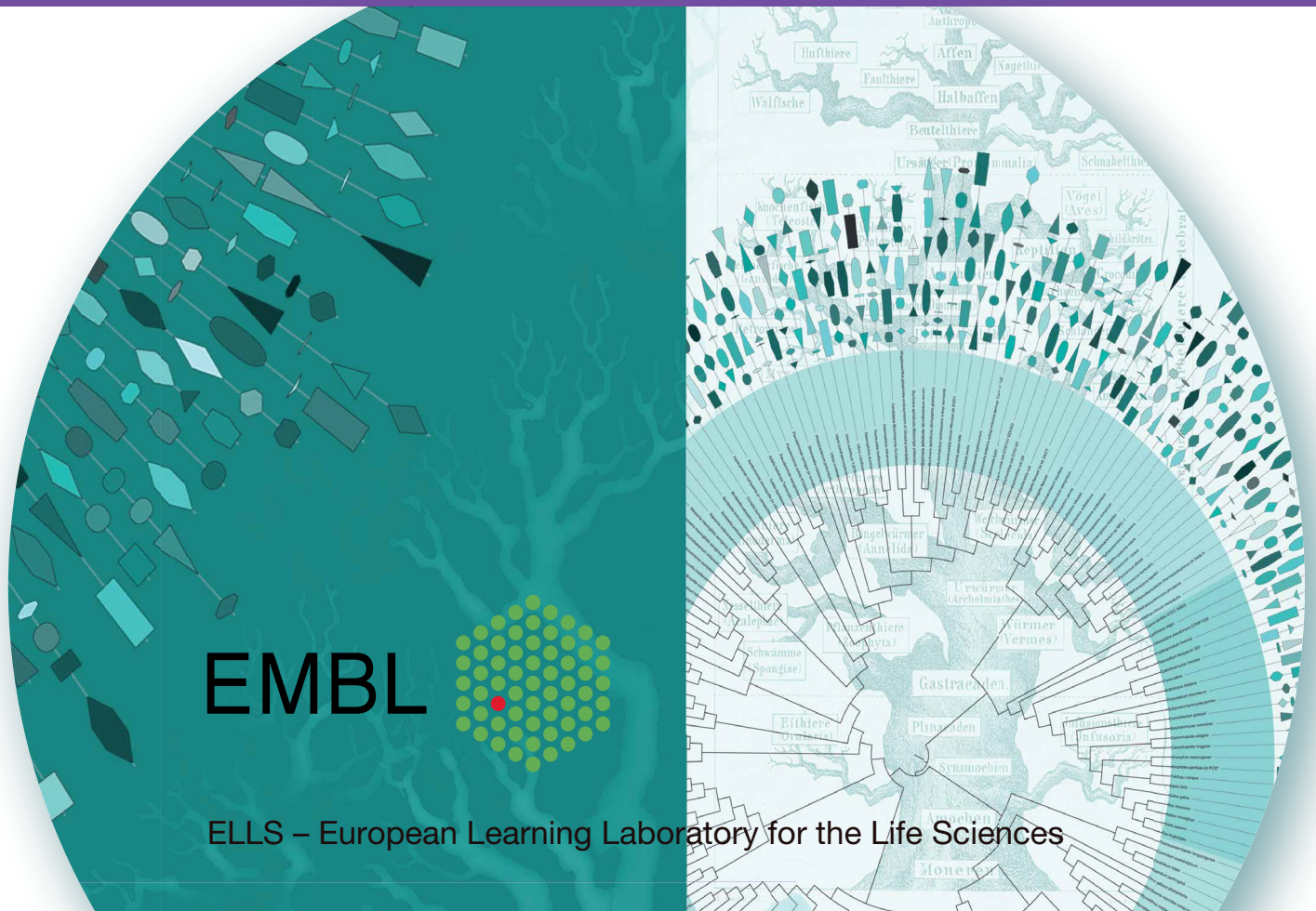
DNA Barcoding

ELLS Troubleshooting Guide



Teacher's Guide

Version 2.0

Experimental Troubleshooting

Version 2.0

Experimental Troubleshooting	
No DNA obtained	**Sample collection (all protocols)** → DNA of starting material already degraded → In case starting material has been repeatedly freeze-thawed, repeat DNA barcoding using fresh starting material instead. **DNA extraction (Spin-column protocol only)** → Insufficient binding of lysate to spin column membrane → Ensure lysate volume prior to adding Buffer AW1 is accurately determined. **DNA extraction (Spin-column protocol only)** → Insufficient cell lysis → Ensure sample is properly homogenised. Ensure sample is incubated sufficiently in the presence of lysis buffer AP1, at the appropriate temperature. **DNA extraction (Spin-column protocol only)** → DNA degraded during DNA extraction process → Ensure the centrifugation step prior to loading lysate onto spin column is carried out. **DNA extraction (Spin-column protocol only)** → DNA lost during elution step → Ensure elution buffer is placed on the centre of the membrane when eluting DNA from spin column. **DNA extraction (Direct PCR protocol only)** → Insufficient cell lysis → Ensure sample is properly homogenised. Ensure sample is sufficiently incubated in the presence of the extraction buffer. **PCR amplification (all protocols)** → No primer added to PCR reaction → Repeat PCR using correct amount of forward and reverse amplification primer. **PCR amplification (all protocols)** → No DNA template added to PCR reaction → Repeat PCR using correct amount of DNA template. **PCR amplification (all protocols)** → Incorrect thermal cycling protocol used → Repeat PCR using correct cycling protocol. **PCR amplification (all protocols)** → Insufficient amount of PCR product loaded on gel → Repeat gel electrophoresis loading larger amount of PCR product. **PCR amplification (Direct PCR protocol only)** → No "Additives Mix" added to PCR reaction → Repeat PCR using correct amount of "Additives Mix".
Low DNA yield and/or quality	**Sample collection (all protocols)** → Insufficient starting material → Repeat DNA extraction using larger amount of starting material. Ensure your plant tissue is fresh (or has been stored at -20°C after picking it fresh). In the case of hard material (e.g. seeds), disrupt the plant tissue using pestle and mortar. **DNA extraction (Spin-column protocol only)** → Insufficient binding of lysate to spin column membrane → Ensure lysate volume prior to adding Buffer AW1 is accurately determined. **DNA extraction (Spin-column protocol only)** → Insufficient cell lysis → Ensure sample is properly homogenised. Ensure sample is incubated sufficiently in the presence of lysis buffer AP1, at the appropriate temperature. **DNA extraction (Spin-column protocol only)** → DNA degraded during DNA extraction process → Ensure the centrifugation step prior to loading lysate onto spin column is carried out. **DNA extraction (Direct PCR protocol only)** → Insufficient cell lysis → Ensure sample is properly homogenised. Ensure sample is sufficiently incubated in the presence of the extraction buffer. **PCR amplification (all protocols)** → Insufficient amount of primer added to PCR reaction → Repeat PCR using correct amount of forward and reverse amplification primer. **PCR amplification (all protocols)** → Insufficient amount of DNA template added to PCR reaction → Repeat PCR using correct amount of DNA template. **PCR amplification (all protocols)** → Incorrect thermal cycling protocol used → Repeat PCR using correct cycling protocol. **PCR amplification (all protocols)** → Insufficient amount of PCR product loaded on gel → Repeat gel electrophoresis loading larger amount of PCR product. **PCR amplification (Direct PCR protocol only)** → No "Additives Mix" added to PCR reaction → Repeat PCR using correct amount of "Additives Mix".

Experimental Troubleshooting, contd.

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No band on gel	See solutions for "No DNA"
Faint band on gel	See solutions for "Low DNA yield and/or quality"
Poor sequencing quality	**Sample collection (all protocols)** → Insufficient starting material → Repeat DNA extraction using larger amount of starting material. Ensure your plant tissue is fresh (or has been stored at -20°C after picking it fresh). In the case of hard material (e.g. seeds), disrupt the plant tissue using pestle and mortar. **DNA extraction (Spin-column protocol only)** → Insufficient binding of lysate to spin column membrane → Ensure lysate volume prior to adding Buffer AW1 is accurately determined. **DNA extraction (Spin-column protocol only)** → Insufficient cell lysis → Ensure sample is properly homogenised. Ensure sample is incubated sufficiently in the presence of lysis buffer AP1, at the appropriate temperature. **DNA extraction (Spin-column protocol only)** → DNA degraded during DNA extraction process → Ensure the centrifugation step prior to loading lysate onto spin column is carried out. **DNA extraction (Direct PCR protocol only)** → Insufficient cell lysis → Ensure sample is properly homogenised. Ensure sample is sufficiently incubated in the presence of the extraction buffer. **PCR amplification (all protocols)** → Insufficient amount of primer added to PCR reaction → Repeat PCR using correct amount of forward and reverse amplification primer. **PCR amplification (all protocols)** → Insufficient amount of DNA template added to PCR reaction → Repeat PCR using correct amount of DNA template. **PCR amplification (all protocols)** → Incorrect thermal cycling protocol used → Repeat PCR using correct cycling protocol. **PCR amplification (all protocols)** → Insufficient amount of PCR product loaded on gel → Repeat gel electrophoresis loading larger amount of PCR product. **PCR amplification (Direct PCR protocol only)** → No "Additives Mix" added to PCR reaction → Repeat PCR using correct amount of "Additives Mix". **PCR clean-up (all protocols)** → Insufficient clean-up, leading to PCR reagents interfering with sequencing reaction → Repeat PCR clean-up
I have an issue which is not described here	Please describe your issue online via the interactive Troubleshooting Guide or write us an email at emblog-ells@embl.org

Sequence Analysis Troubleshooting

Version 2.0

Sequence Analysis Troubleshooting	
Issue with number of sequences returned	I did not get any sequences back → No sequence analysis possible → For further solutions see "Experimental troubleshooting" > "No DNA obtained" and "Poor sequencing quality" I only got one out of two sequences back → Use single seq. read for further analysis → For further solutions see "Experimental troubleshooting" > "Poor sequencing quality"
Issue with sequence coverage (length) and/or quality	My sequence(s) are short and don't cover the whole barcode → Use short sequence(s) for further analysis → For further solutions see "Experimental troubleshooting" > "Poor sequencing quality" My sequence(s) have poor quality (as seen in chromatogram) → Only use sequence(s) with reliable chromatogram(s) → For further solutions see "Experimental troubleshooting" > "Poor sequencing quality"
Issue with contig assembly	Contig assembly is impossible → Use single seq. read for further analysis → For further solutions see "Experimental troubleshooting" > "Poor sequencing quality"
Issue with ENA search	ENA search results are unclear or not as expected → See solutions at "ENA Search Troubleshooting"
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ENA Search Troubleshooting

Version 2.0

ENA Search Troubleshooting	
Incorrect organism identification	Single species incorrectly identified Sequence information insufficient for database Ensure contig is assembled correctly To improve sequence coverage and quality see solutions to "Experimental Troubleshooting" > "Poor sequence quality" No ENA entry for organism No identification via ENA possible at the moment. Come back later to check for new entries. Use phenotypic characteristics of organism for species identification. Expected species is only identified to genus level Sequence information insufficient for database Ensure contig is assembled correctly To improve sequence coverage and quality see solutions to "Experimental Troubleshooting" > "Poor sequence quality" No ENA entry for organism No identification via ENA possible at the moment. Come back later to check for new entries. Use phenotypic characteristics of organism for species identification.
Multiple top hits (entries with identical ENA values)	Unable to distinguish between different species of the same genus Sequence information insufficient for database Ensure contig is assembled correctly To improve sequence coverage and quality see solutions to "Experimental Troubleshooting" > "Poor sequence quality" No ENA entry for organism No identification via ENA possible at the moment. Come back later to check for new entries. Use phenotypic characteristics of organism for species identification. Unable to distinguish between different genera Sequence information insufficient for database Ensure contig is assembled correctly To improve sequence coverage and quality see solutions to "Experimental Troubleshooting" > "Poor sequence quality" No ENA entry for organism No identification via ENA possible at the moment. Come back later to check for new entries. Use phenotypic characteristics of organism for species identification.
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