repliQa HiFi Assembly Mix FAQ

What are the minimum and maximum fragment sizes that can be assembled?

Quantabio has effectively assembled fragments down to 116 bp (70 bp insert and two 23 bp overlaps). When attempting to clone small fragments, we recommend using 2- to 5-fold molar excess of fragments. Regarding maximum sizes, there should be no limit on what can be assembled. The only limitation is from the challenge of pipetting large assemblies without causing damage or shearing.

Why are plots of the number of colonies important?

The number of colonies is indicative of transformation efficiency, which in turn is indicative of reaction assembly efficiency. The high efficiency, reliability, and consistency of the repliQa HiFi Assembly Mix improves the likelihood of obtaining correct assemblies in situations where alternative kits or approaches fail.

Can the reaction time be reduced to 15 minutes rather than 1 hour?

For reactions using three or fewer fragments, the incubation time can be shortened to 15 minutes if necessary.

When should the DpnI be used?

If plasmid DNA is used as PCR template for generation of fragments for assembly, it is recommended to treat the PCR reactions with DpnI prior to the assembly reaction to eliminate residual methylated plasmid that could give rise to colonies lacking the assembled product of interest.

How many fragments can be assembled in a single reaction?

Quantabio routinely generates assemblies of up to six DNA fragments, though the repliQa HiFi Assembly Mix has been successfully used for more complex assemblies.

How is the pmol input of DNA fragment amounts calculated?

Calculate the number of pmols of each fragment using the following formula:

pmoles = (weight in ng) x 1000/(bp x 662).

At what concentration is the repliQa HiFi Assembly Mix supplied?

The repliQa HiFi Assembly Mix comes with a 10X reaction buffer containing dNTPs, magnesium, and cofactors. This 10X concentration allows for use of higher volumes of nucleic acids and flexibility in design of assembly reactions compared to alternative kits or approaches.

What E.*coli* strains are compatible with the repliQa assembly mix?

Common E.*coli* cloning strains and derivatives are compatible with the repliQa HiFi Assembly Mix.

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Is an E.*coli* strain supplied with the repliQa HiFi Assembly Mix?

The repliQa HiFi Assembly Mix does not include competent E.*coli*. Most common E.*coli* cloning strains are compatible with the kit and are available from numerous commercial sources.

What is the recommended amount of sequence overlap?

The repliQa HiFi Assembly Mix is optimized for assembly of fragments with overlap regions between 15 – 60 bp.

Is it necessary to purify the PCR fragments that will be used in the assembly reaction?

Purification of PCR product using a spin column-based cleanup or other method is not required but is highly recommended to achieve highest efficiency fragment assembly.

What are the advantages of the repliQa HiFi Assembly Mix for cloning?

The repliQa HiFi Assembly Mix allows simultaneous cloning of multiple insert fragments into vectors without creating scars. In contrast to traditional cloning, there is no requirement for restriction endonucleases and unique restriction sites.

What incubation temperature should be used for the assembly reaction?

The repliQa HiFi Assembly Mix is optimized for assembly at 50°C. We do not recommend performing the reactions below 48°C or above 52°C.

Is there a preferred method for transforming E.*coli* with the assembled DNA?

The products of the repliQa HiFi Assembly reaction can be introduced into E.*coli* either by chemical transformation or electroporation. If electroporation is to be used for transforming cells, we recommend first diluting the assembly reaction 1:5 in high purity water.