Certificate of Analysis

GeneArt® CRISPR Nuclease OFP Reporter Kit

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pCRISPR CMV hCas9 OFP Vectors

The parental supercoiled plasmid is qualified by restriction digest to confirm its identity prior to linearization. Restriction digests must demonstrate the correct banding pattern when electrophoresed on an agarose gel. The table below lists the restriction enzymes and the expected fragments.

Restriction Enzyme	Expected Fragments (bp)
BaeI	9195, 691
XbaI, PmeI	9130, 756

Note: Some of the restriction sites used to qualify the parent vector may no longer be present in the linearized, adapted vector.

Cloning Efficiency

Each lot of linearized pCRISPR CMV hCas9 OFP vector is tested in a cloning reaction with a ds control oligo under conditions described in the accompanying manual. 10 transformants are picked and analyzed by Sequencing using the U6 forward primer. The following results must be obtained:

1. >50 colonies on transformation plate for pCRISPR CMV hCas9 OFP and ds control oligo ligation when ligation performed for 2hrs
2. < 10 colonies on transformation plates of vector only ligation.

ds Control Oligo

The ds control oligo is qualified by use in a ligation reaction with pCRISPR CMV hCa9 OFP as described in the accompanying manual.

T4 DNA Ligase and 5X DNA Ligase Buffer

T4 DNA Ligase and 5X DNA Ligase Buffer are functionally qualified and must meet the following specifications:

1. A Refractive Index of 1.400 and 1.410

2. A Unit assay to determine 1 unit/µl

3. Endonuclease Assay − On 1µg of ΦX174 RF DNA, < 10% conversion from Form I to Form II and no conversion to Form III must be obtained after a 2−hour incubation at 22°C with 10 units of enzyme

4. Exonuclease Assay − With 1 pmol of the relevant substrate the following criteria must be obtained after a 1−hour incubation: 3' ss slope, 3' ds slope, 5' ds slope % released: <0.3% of termini released per unit of enzyme.

If the 3' ds and 3' ss substrates are combined in one assay, slope % released: <0.15% of termini released per unit of enzyme.

5. Ligation Efficiency − Lambda DNA/*Hind* III fragments must be > 98% ligated after 1−hour incubation at 22° C. Lambda DNA/*Hae* III fragments must be > 95% ligated after 1−hour incubation at 22° C.

6. Ligation/Recut − Lambda/*Hind* III fragments and Lambda DNA/*Hae* III fragments must be > 95% ligated after 1−hour incubation at 22° C. Of the ligated fragments, 100% must be cleaved (recut) by the appropriate enzyme.

Sequencing Primers

Sequencing primers are lot−qualified by DNA sequencing experiments using the dideoxy chain termination technique.

Each primer must yield > 250 bp of quality sequence from a supercoiled plasmid template using standard sequencing conditions.

For Research Use Only. Not for use in diagnostic procedures. If you have any further questions about this Certificate of Analysis, please contact Technical Services at 1−800−955−6288 (US and Canada) or 1−760−603−7200, x2 (all other countries).

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