**CRISPR - INJECTION REQUEST**

**Scientist:** Mobile Phone# (in case of “emergency”):

**Group:**  **Date:**

**Project Name:**

**Name & MGI-ID** ([http://www.informatics.jax.org](http://www.informatics.jax.org/)) **of targeted gene:**

**Description of mutation:**

**Background info and Rational:**

|  |  |
| --- | --- |
| **Name of the mutation:** |  |

|  |  |
| --- | --- |
| mark |  |
|  | **InDel (insertion, deletion)** |
|  | **Knock in, repair** |

**Intended mutation:**

|  |  |
| --- | --- |
| mark |  |
|  | **Cytoplasmic** |
|  | **Pronuclear** |

**Injection type:**

|  |  |
| --- | --- |
|  |  |

**Result of *in-vitro* Test of sgRNA:**

A quality control should be introduced for the sgRNAs that are used for CRISPR/Cas-mediated editing in injected mouse zygotes. For this, a PCR-amplified DNA fragment containing the sgRNA sequence should be incubated in vitro with the sgRNA and Cas9 protein (see attached document). In this assay, a > 70% cleavage of the DNA fragment indicates that the sgRNA should also be effective for CRISPR/Cas-mediated editing in injected zygotes.

The picture of a gel providing this information should be included in the injection requests (see attached example).

Injection Mix [~ 20-30 µl]:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Component** |  | **Name** | **Size** | **Conc. in final mix** |
| **Cas9 mRNA** | Source |  |  |  ng/µl |
| **Cas9 protein** | Source |  |  | ng/µl |
|  |  |  |  | ng/µl |
| **sgRNA 1** |  |  |  |  ng/µl |
| **sgRNA 2** |  |  |  |  ng/µl |
|  |  |  |  | ng/µl |
| **Repair templ. 1** | Plasmid or oligo? |  |  |  ng/µl |
| **Repair templ. 2** | Plasmid or oligo? |  |  |  ng/µl |
|  |  |  |  |  |
|  |  |  |  |  |

**Requested background of zygotes:**

Please keep in mind that the area you are designing your guides against may NOT be the actual sequence of the mouse strain! Especially with custom strains.

|  |  |
| --- | --- |
| **C57BL/6J** (inbred) |  |
| **B6CBAF2** (hybrid) |  |
| **Custom zygotes** |  |
|  |  |

**Remarks:**

**Date: Approved by (M.Busslinger)**

