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about the personal communication reference

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Dear Tigran

I hope that you can publish your cas9 toolkit very soon and make the plasmids available.

I understand that your partner Biotech company do not want to share the manuscript.

You have a very efficient expression cassette integration efficiency when you are using loci specific ends-with the corresponding single Cas9 specific target? Did it depend on expression cassette size. In our end, its worked but not so efficiently.

I would be please to try them. What Cas9 vectors you have , which selection? is it replicative vectors as ours, that you could loos them?

That cleaver to be able to have Golden gate assembly with and without marker using the same Golden gate assembly but using different E coli strain.

Did it work similarly, when you have a golden gate assembly with a marker by transformation into usual E.coli (such as DH5alpha), you could transform your special E.coli strain and then remove the marker?

Deletion efficiency by the classical gene deletion system (Fickers et al, 2003) is gene dependent. Such gene deletion efficiency could be improved using KU70 mutant (Verbeke et al 2003).

"It looks like deletions of some metabolic genes are much difficult to mutate or delete such as MFE2 et EYD1 (Larroude et al, 2020 .Biotechnol Lett . 2020 May;42(5):773-785 and EYL1 and EYL2 (personal communication) which was only possible when the disruption cassette contains a selectable marker."

It was not clear for me if you want to discuss only gene disruption efficiency of expression cassette integration at a specific locus. If I understand correctly what you means if popin of an expression cassette (by GGA with or without a selectable marker) at a specific loci improved by using a single Cas9-gRNA-loci replicative plasmid with a selectable marker?

Yes It could be fine for me.

Let tell me if you want to do a visio which will be easier and faster for the discussion.

Sincerely

Best

Jean-Marc

[Цитируемый текст скрыт]

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