**Gel Purification with Dpn1**

*Walker Orr, 04/24/2023*

**Purpose**

Preparing Inverse PCR fragments for DIMPLE libraries.

**Required Materials**

Kit reagents and supplies from NEB Monarch Gel Purification kit.

Dpn1, 1 uL per sample.

**Sample Workflow**

Total time: 3 hours

Active time: 1.5 hours

|  |  |  |  |
| --- | --- | --- | --- |
| **Time (h)** | **(m)** | **Main steps** | **Prep steps** |
| 0 |  | Treat samples with Dpn1 |  |
|  | 15 |  | Cast gel |
|  | 30 |  |  |
|  | 45 |  |  |
| 1 |  | Add loading dye and load gel. |  |
|  | 15 | Run gel. |  |
|  | 30 |  |  |
|  | 45 |  |  |
| 2 |  |  |  |
|  | 15 | Excise bands. |  |
|  | 30 |  |  |
|  | 45 | Extract DNA. |  |
| 3 |  |  |  |
|  | 15 |  |  |
|  | 30 |  |  |
|  | 45 |  |  |
| 4 |  |  |  |
|  | 15 |  |  |

**General Comments**

|  |  |  |
| --- | --- | --- |
| **Steps** |  | **Helpful Hints** |
| 1. Treat 45 uL PCR products with 1 uL Dpn1. Incubate at 37C for 1 hour. |  | Don’t need to add NEBuffer; it works with the Q5 buffer. |
| 1. Add 6x Loading dye (9 uL). Run on a gel with spacing. 75V for 1 hour. Because you’re purifying, make sure to wash gel tub, gel cassette, and combs thoroughly with water and DI water before using. **Use fresh TAE**. |  | Make sure to cast a gel such that wells can accommodate 55 uL. Gel depth for wide combs should be 0.75 cm. |
| 1. Visualize gel, excise bands, and process with NEB Monarch Gel Purification Kit. Elute in 9 uL 50C (preheated) water and incubate for 3 minutes at 50C before eluting by centrifugation. |  | Remember to add 150 uL water, and to elute in preheated (50C) water. |
| 1. Assess DNA concentration using Qubit HS. |  |  |
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