BASIC DNA Assembly Practical: Evaluation of Parallel Biopart Assembly Standards.

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Introduction

DNA Assebly is key in genetic engineering, genome editing or automation. Restriction enyzme based methods were introduced in 1990, with significant challenges in residual DNA fragments after digestionEllis et al., 2011, flexibiliy of target DNA sequences. Subsequent variants such as the Gibson Assembly (isothermal) or Golden Gate (multipart, Type II restriction enyzmes) Storch et al., 2015, were still limited. The Biopart Assembly Standard for Idempotent CLoning (BASIC) eliminates scars and specificity issues via othogonal linkers, which do not interfere with the DNA sequence, leading to scar-less cloningStorch et al., 2017.

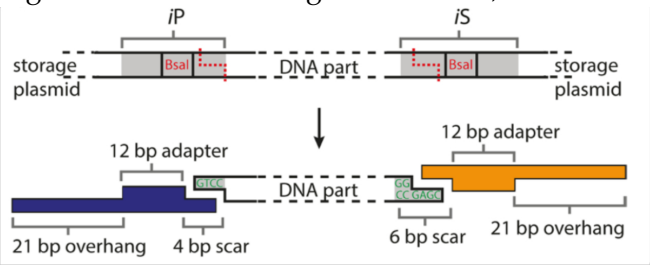

Figure 1. Linker and Adaptors: Modular linkers consists of a 37bp linker section and 12bp long adapter section, designed as a DNA part internal prefix (iP) and suffix (iS). The 4bp BasI annealing region (CTCG, GGAC) is located at the 5 prime end of the linker section (CTCG, GGAC). The linker anneals at the 5 prime end with the DNA parts and at 3 prime orthogonally with eachother to form the reulting 45 bp long final linker. Figure created with Biorender.com with adoption from from Storch et al., 2015

The linkers are reusable and rendering the assemly method as an automated high throughput mehtod Storch et al., 2017; Storch et al., 2015.

In this practical we are using two neutral linkers (L1, L2) and two iternal linker containing methylated prefix and suffix (LMiP,LMiS)**Figure 2.**.

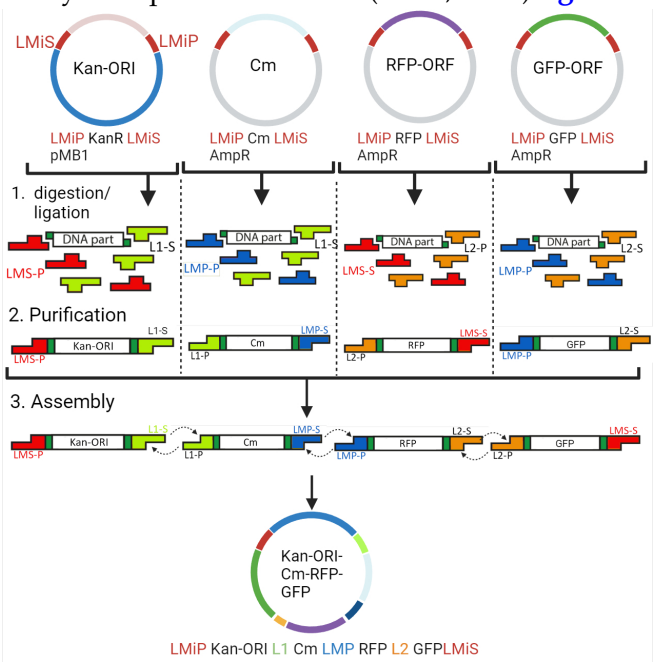

Figure 2. BASIC Assembley Steps: Figure outlines digestion, purification, and annealing steps. Digestion and purification reactions are performed in seperate wells. Assembly is performed in one mixture. Figure created with Biorender.com with adoption from Storch et al., 2015

Neutral linkers as a DNA part prefix (L1P, L2P) and suffix (L1S, L2S). The methylated linkers are designed as 2x2 set of Linkers for the Methylation prefix and suffix (2xLMP,2xLMS), binding to the DNA parts. Each pair of LMP,LMS is further comprised of a orthogonal prefixes (-P) and suffixes (-S) which bind to eachother LMP-S, LMP-P and LMS-S,

LMS-P. The adaptors are methylated at the 5 prime end for the prefix (LMP-P-A) and suffix (LMS-S-A) linkers. These methylations serve as a protection from DNA Ligase in the digestoin-ligations steps. Our Objective is to evalutate the accuracy of a four part BASIC assembly process, consisting of Canamicin resistant DNA part (Kan-Ori); a Chloramphenicol resistance casette (CaR) and two flourecence casstes (RFP, and GFP) **Figure 2.**. First the DNA part containing plasmids are digested in four parallel reactions where DNA ligase cuts the plasmids at the BSaI site within the LMiP/S linkers **Figure 2.**.

Results

The DNA parts concentrations were 200 ng/uL and 50 ng/uL for Kan-Ori. Linker ligation (20 °C) and restiction digestion (37 °C) were performed by a PCR machine running for 20 cycles. We then purified the DNA parts using magnetic beads and a 96 well plate with integrated magenets **Figure 2.** (step2). A subsequent purification step using magneting attraction is performed to remove loose linkers and parts smaller than 100bp before the reaction wells are taken off the magnet to allow for linker and DNA part annealing (Step 2). To check for successfull linear DNA parts, we run an gel-electrophoresis, prior to completing the assembly in a final assembly mixture of the four DNA parts and NEB CuSmart Buffer (10x). The assembly is also performed by the PCR machine at 50 °C for 45 minutes. The final plasmid is then transformed into E.coli, which inturn is then plated on two LB agar plates, one KanR-CamR and KanR only. ligated and digested.

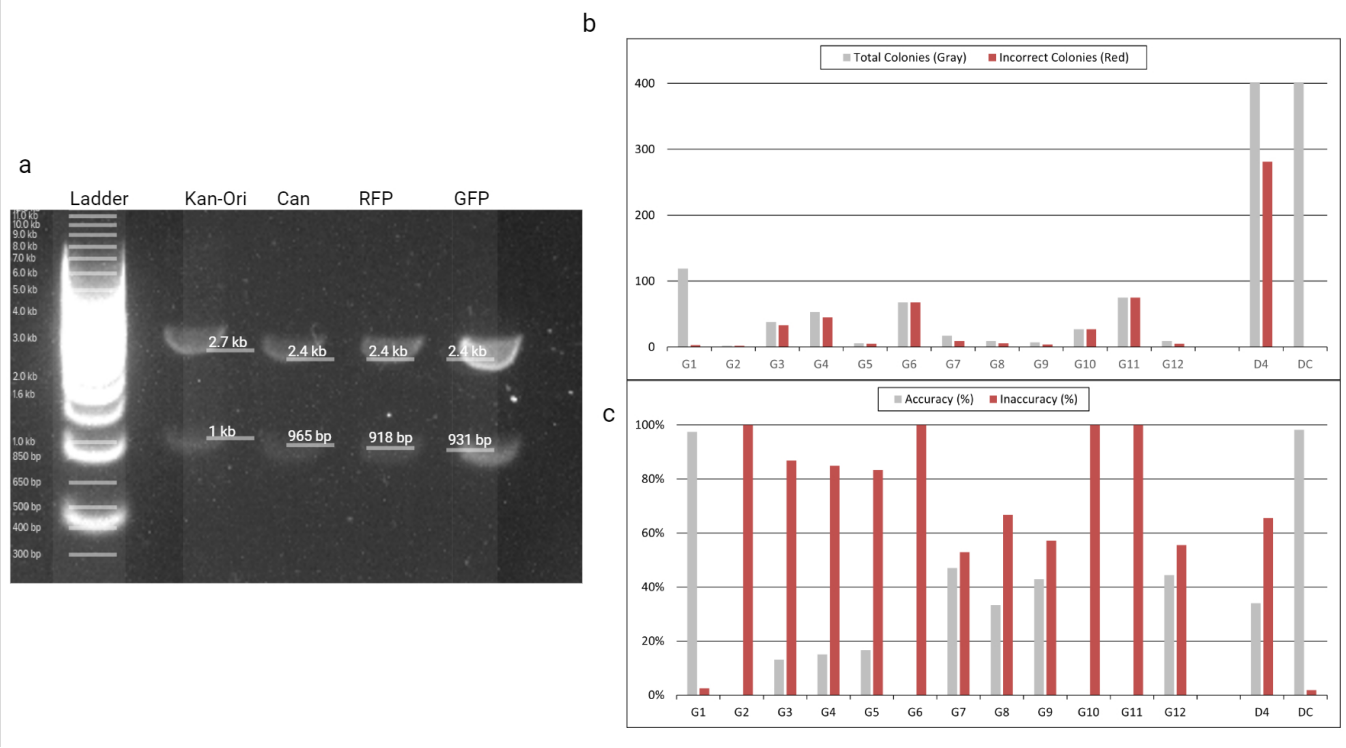

Figure 3. Results. (a) The gel electrophoresis shows successfull signals at the expected range 0.5kb - 3kb. The image contains an overlay of the in silico digestion in benchlinq and the original. We can see that the predicted sized match the theoretical perfectly. (b) Total colony count, showing number of incorrect colonies (red), which is the amount of colonies showing the wrong flourecence profile (green or orange) and correct colleonies (grey). Wells G1-G12 are individual group experiments, D4 is the total ± SEM (c) Accuracy of the assembly, by incorrect collonies divided by correct collonies Wells G1-G12 are individual groups, D4 is the total group accuracy and DC is the control. 65.58% ± 0.88% (D4) and 1.8% ± 6.2% (DC) Figure created with GIMP and Biorender.com with adoption from from Storch et al., 2015.

Figure 2. (a) is showing a successfull digestion for all plasmids. The virtual digestions where layed over the original gel image, perfectly aligning in silico and in vivo outcomes. Part b shows a high number of incorrect flourecence profiles accross all groups exept of of G1, which is very close to the control. This results indicates a low accuracy (c) as seen in the diagram below. The total incauracy is 65.58 % ± 0.88% (D4) and 1.8% ± 6.2% (DC).

Conclusion

The high incauracy can be explained by several factors. misalignment of DNA Parts and linker lead to failed assembly. Another possible explanation the fact that the concentration of the DNA fragments varied between the groups, which is plausable since there was no step to verify or check for that concentration. Therefore if the concentrations were indeed too low it would lead to insufficient ligation. This is likely what caused the high inacuracy since the variations between the groups also indicates a fluctuating accuracy. Another likely event could be contamination in the reaction mix leading to DNA ligase not digesting the BsaI site, this is however unlikely since most groups had a positive gel electrophoresis signal. Implementing multiple negative controls testing for diferent assebly steps would also optimise the accuracy. Comparing the results to the control, which had a control step for the DNA fragments purity Storch et al., 2015, (Supplemental Material), we can conclude that considering the absense of essential control steps the BASIC assembly method is highly accurate and fast when looking at the control.

References

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