**Open-access RT-LAMP enzyme production: HIV-1 RT and Bst-LF**

This protocol describes the production of the open-access RT-LAMP enzymes, **HIV-1 reverse transcriptase (HIV-1 RT)** and **Bst Large Fragment (Bst-LF)**, originally described in the following pre-print: https://www.biorxiv.org/content/10.1101/2020.06.23.166397v2

(*'A rapid, highly sensitive and open-access SARS-CoV-2 detection assay for laboratory and home testing'*).

The enzyme purification protocol was established at the Vienna BioCenter as part of the Vienna COVID-19 Initiative by Irina Grishkovskaya and Benedikt Bauer. Detailed findings covering the RT-LAMP assay establishment and experimental validation can be found in our pre-print. For further information and questions please contact:

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**Addgene expression vectors:**

<https://www.addgene.org/159148/>

<https://www.addgene.org/159149/>

**Protein expression**

BL21(DE3) bacterial cells were transformed with the respective expression plasmids and overnight cultures were grown at 37°C in LB medium at 180 rpm in the presence of ampicillin. Large scale expression was performed in the auto-induction (ZYP5052) medium (see protocol below). The cells were first grown for 5 hours 30 minutes at 37 °C, followed by overnight growth at 18°C. The cells were then harvested by centrifugation (4000 x g, 15 min) and stored at -80°C.

**Purification of Bst-LF**

The cells were resuspended in the lysis buffer (20 mM Tris-HCl pH 7.5, 500 mM NaCl, 0.5 mM TCEP and benzonase). Cell lysis was accomplished using a single cycle in a cell disruptor (Constant systems Ltd), with the pressure set to 1.4 kBar. Lysates were clarified by centrifugation (42.000 x g, 45 min, 4°C), and a two-step purification protocol using an ÄKTA Protein Purification System (GE Healthcare Life Sciences) at 8°C was then followed to obtain the recombinant protein. The supernatant was applied to the His-Trap Crude 5 ml column (GE Healthcare Life Sciences) previously equilibrated with the lysis buffer. The column was washed with 20 column volumes of the lysis buffer, containing 20 mM Imidazole. Then, the protein was eluted using a step gradient of imidazole (equilibration buffer with 250 mM Imidazole and then with 500 mM Imidazole). Fractions were examined by SDS-PAGE for protein content and purity and pooled according to the presence of Bst-LF and diluted with the RQA buffer containing 20 mM Tris-HCl pH 7.5, 0.5 mM TCEP to a final concentration of 50 mM NaCl. Diluted fractions were applied to Resource Q 6 ml ion exchange column (GE Healthcare Life Sciences) pre-equilibrated with the RQA buffer. The protein was then eluted using a linear gradient of 0.05 to 0.74 M NaCl in the RQA buffer. The peak fractions were combined and dialysed overnight against storage buffer 40 mM TrisHCl pH 7.5, 50 mM NaCl, 1 mM DTT, 10% Glycerol, flash frozen in liquid nitrogen and stored at -80°C until use.

**Purification of HIV-1 RT**

The cells were resuspended in the lysis buffer (20 mM Tris-HCl pH 7.5, 500 mM NaCl, 0.5 mM TCEP and benzonase). Cell lysis was accomplished using a single cycle in a cell disruptor (Constant systems Ltd), with the pressure set to 1.5 kBar. Lysates were clarified by centrifugation (42.000 x g, 45 min, 4°C), and a three-step purification protocol using an ÄKTA Protein Purification System (GE Healthcare Life Sciences) at 8°C was then followed to obtain the recombinant protein. The supernatant was applied to the His-Trap Crude 5 ml column (GE Healthcare Life Sciences) previously equilibrated with the lysis buffer. The column was washed with 20 column volumes of the lysis buffer, containing 20 mM Imidazole. Then, the protein was eluted using a step gradient of imidazole (equilibration buffer with 250 mM Imidazole and then with 500 mM Imidazole). Fractions were examined by SDS-PAGE for protein content and purity and pooled according to the presence of HIV-1 RT and diluted with the RSA buffer containing 20 mM Tris-HCl pH 7.5, 0.5 mM TCEP to a final concentration of 50 mM NaCl. Diluted fractions were applied to Resource S 6 ml ion exchange column (GE Healthcare Life Sciences) pre-equilibrated with the RSA buffer. The protein was then eluted using a linear gradient of 0.05 to 0.74 M NaCl in the RQA buffer. The peak fractions were combined and applied to HiLoad 16/600 Superdex 200 pg column (GE Healthcare Life Sciences) equilibrated with the storage buffer 50 mM TrisHCl pH 7.5, 50 mM NaCl, 0.5 mM TCEP, 10% Glycerol. Eluted fractions were analysed by SDS-PAGE, pooled according to the protein purity, concentrated, flash frozen in liquid nitrogen and stored at -80°C until use.

**Protocol for large scale expression with Auto-induction Media ZYP5052**

Based on the method propose by F. William Studier in 2005. (Protein Expression and Purification 41 (2005) 207–234)

Vectors: any vector with a T7lac promoter (e.g. pET series)

Strains: Any E.coli strain with the lactose transport system (Bl21 series)

Solutions to prepare in advanced: (Use de-ionized distilled water for all solutions. Autoclave solutions for 15 min unless specified otherwise)

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| **ZY** |
| 10 g or any tryptic digest of casein, e.g. tryptone  5 g yeast extract  1000 ml water |

|  |  |
| --- | --- |
| **20xP**  (1 X P = 100 mM PO4, 25 mM (NH4)2SO4) | |
| To make 100 ml | To make 1 liter |
| 90 ml water | 900 ml water |
| 6.6 g (NH4)2SO4 | 66 g (NH4)2SO4= 0.5 M |
| 13.6 g KH2PO4 | 136 g KH2PO4 = 1 M |
| 14.2 g Na2HPO4 | 142 g Na2HPO4 = 1 M |
| Add in sequence in beaker, stir until all dissolved | |
| pH of 20-fold dilution in water should be ~6.75 | |

|  |  |
| --- | --- |
| **50x5052**  (1 X 5052 = 0.5 % glycerol, 0.05% glucose, 0.2% alpha-lactose) | |
| To make 100 ml | To make 1 liter |
| 25 g glycerol (weigh in beaker) | 250 g glycerol (weigh in beaker) |
| 73 ml water | 730 ml water |
| 2.5 g glucose | 25 g glucose |
| 10 g a-lactose | 100 g a-lactose |
| Add in sequence in beaker, stir until all dissolved | |
| Lactose is slow to dissolve. It may take two hours or more at room temperature, can speed up by heating in microwave oven | |

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| **1 M MgSO4** (100 ml) |
| 24.65 g MgSO4-7H2O |
| water to make 100 ml |

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| --- | --- | --- | --- |
| **1000X Trace Metals Mix** (50 ml; slight precipitate forms with time)  All stock solutions of metals except 0.1 M FeCl3-6H3O are autoclaved. Store at room temperature | | | |
| **Chemical** | **Volume** | **MW** | **Final [ ]** |
| H2O( steril) | ml |  |  |
| 0.1 M FeCl3-6H3O (dissolved in 0.1 M HCl, 100 fold dilution of conc. HCl) | 25 ml | 270.30 | 50 uM Fe |
| 0.25 M CaCl2 | 4 ml | 93.0 | 20 uM Ca |
| 0.25 M MnCl3 | 2 ml | 197.91 | 10 uM Mn |
| 0.25 M ZnSO4 | 2 ml | 287.56 | 10 uM Zn |
| 0.1 M CoCl2 | 1 ml | 237.95 | 2 uM Co |
| 0.25 M CuCl2 | 0.4 ml | 170.486 | 2 uM Cu |
| 0.1 M NiCl2 | 1 ml | 237.72 | 2 uM Ni |
| 0.25 M Na2MoO4 | 0.4 ml | 241.98 | 2 uM Mo |
| 0.1 M Na2SeO3 | 1 ml | 263.03 | 2 uM Se |
| 0.25 M H3BO3 | 0.4 ml | 61.83 | 2 uM B |

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| **Recipe for 1l of ZYP5052** | |
| **Solution** | **Volume** |
| 20X P | 50 ml |
| 50X 5052 | 20 ml |
| 1000X MgSO4 | 1 ml |
| 1000X Trace Metal | 1 ml (optional) |
| ZY | 1l |

- add the appropriated antibiotic\*.

1. Inoculate the media with pre-culture (the ratio media: pre-culture could vary 1000:1 to100:1)
2. Grow the culture using the following expression protocol: 37 degrees, 5.30-6hrs and 180-200 rpm and then 18 degrees, 16hrs (or over night or even over the weekend) and 180-200 rpm

\* Bl21 increases the tolerance to kanamycin in PO4 enriched media, so it is suggested by Studier to use ~ 100 ug/ml or even as high as ~200 ug/ml for ZYP media.