

Accura™ High-Fidelity Polymerase and Expresso® Protein Expression System: Achieve 30 minute PCR for Expression Cloning Workflows

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Accura™ High Fidelity polymerase is a new proofreading enzyme developed for expression cloning applications. Lucigen has devised a rapid PCR protocol in which Accura High Fidelity Polymerase can produce a cloning-ready 700 base pair (bp) target gene in as little as 30 minutes of thermal cycling. In conjunction with Lucigen's enzyme-free Expresso® cloning system, the gene of interest is cloned directly into an Expresso vector with no additional PCR cleanup steps or ligation. The entire protocol takes 2 hours from PCR amplification to transformation of the expression construct. The rhamnose promoter-based one-host system allows recombinant protein expression to occur the day after cloning, providing users a powerful and robust tool to go from template to protein in only 24 hours. Here, the gene target encodes a blue colored protein that is easily detected one day after transformation on media containing rhamnose. The presence of blue colonies indicates successful PCR amplification and expression of functional protein.

Amplification of PCR products for expression cloning requires the use of costly high fidelity DNA polymerases. Lucigen offers Accura High Fidelity Polymerase as an economical solution for high-fidelity PCR performance at a lower cost. The polymerase consists of a novel fusion protein that is highly efficient, rapid, and maintains exonuclease activity that is essential for proofreading functionality. Using a novel expression cloning fidelity assay that mimics actual user conditions, the fidelity of Accura Polymerase is much higher than the non-proofreading polymerase Taq and equivalent to that of other well-known enzymes (Fig. 1)

FAST PROTOCOL WITH ACCURA AND EXPRESSO:

Here we describe an easy-to-follow 2 hour procedure that utilizes Accura High Fidelity Polymerase and the Expresso expression system to achieve PCR amplification, cloning, and transformation. First, the 700 bp PCR product, the same as the gene above, was amplified using a rapid PCR protocol based on Accura High Fidelity Polymerase in only 30 minutes of thermal cycling (Fig 2).

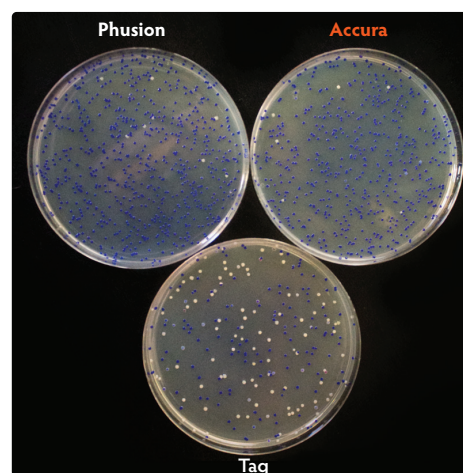
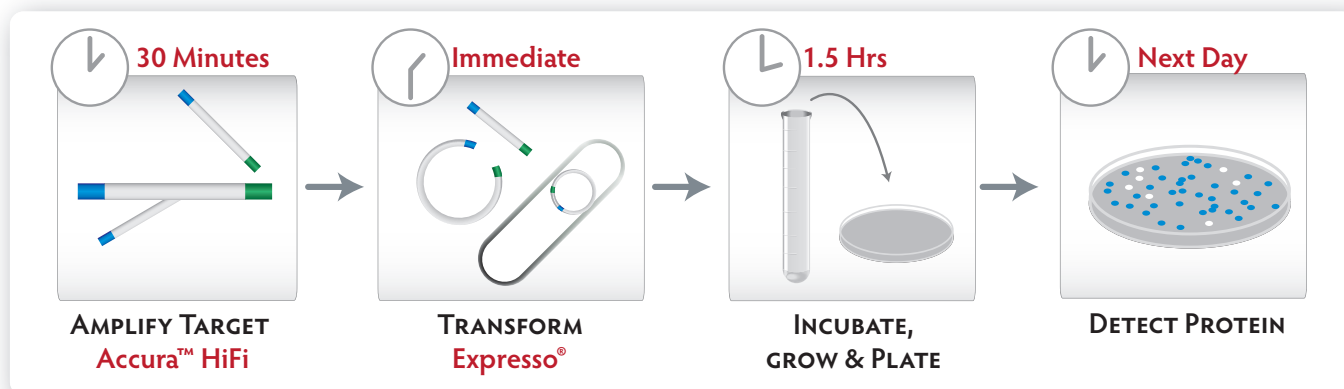


Figure 1. Expression cloning fidelity assay comparing Accura High Fidelity DNA polymerase with Taq and Phusion polymerases. A 700 bp gene encoding a blue colored protein (DNA 2.0, Menlo Park, CA) was amplified using manufacturer's recommended conditions for Accura High-Fidelity DNA Polymerase, Phusion High-Fidelity DNA polymerase (Thermo-Fisher), and non-proofreading EconoTaq® (Lucigen). The amplicons were then cloned using the Expresso® Rhamnose Cloning and Expression system (Lucigen). Blue colonies represent functionally accurate amplification of the gene of interest, while white colonies represent non-functional genes with sequence errors due to low-fidelity amplification. Some white colonies may also be due to empty vector.

FAST PROTEIN DETECTION WORKFLOW: ACCURA™ AND EXPRESSO®



Instantaneous, directional, and enzyme-free cloning for recombinant protein expression employed Lucigen's Expresso® cloning system. The PCR product was cloned directly into the pre-processed vector without additional steps of restriction digestion and DNA clean-up. Ligase-free Expresso® cloning depends on the 18 base sequences at both ends of the target gene that are added during PCR, enabling recombination of the PCR product and vector *in vivo* without residual cloning scars. Additionally, the one-host Expresso Rhamnose system allows cloning of the PCR product for protein production in 24 hours.

The 700 bp PCR product was cloned directly into the Expresso Rhamnose C-His vector immediately following amplification. Because the gene encodes a blue colored protein, it is conveniently detected on plates containing rhamnose the day following transformation. The presence of blue colonies demonstrates the presence of functional and active protein (Fig. 3). This system provides users with a powerful tool to obtain a clone of interest in less than 24 hours. This rapid procedure does result in a slightly higher level of white colonies than the normal 2.5 hour amplification protocol.

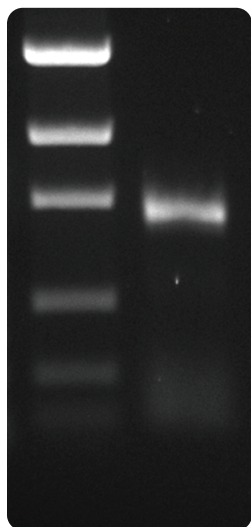


Figure 2. Amplification of a 700 bp product in 30 minutes of thermal cycling using Accura High Fidelity polymerase. The product can be directly cloned into an Expresso vector without additional purification, restriction digestion or ligation steps. See text for details.

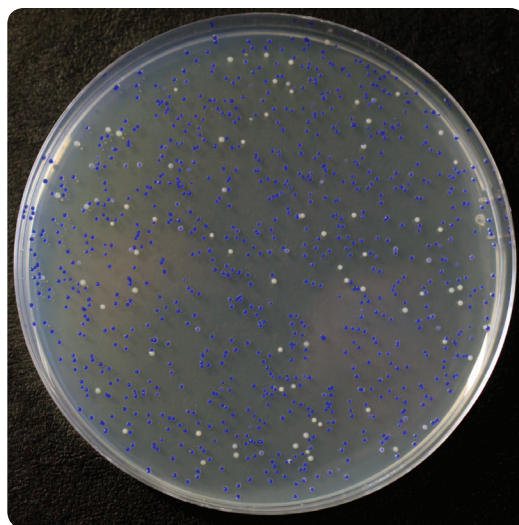


Figure 3. Blue colonies are detected on a selective plate containing rhamnose. The presence of blue colonies indicates functionally accurate amplification of the gene of interest.

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MATERIALS AND METHODS

- Accura™ High Fidelity DNA Polymerase [Lucigen, cat. 30010-1](#)
- 2X Accura HF Buffer [Lucigen, cat. 30010-1](#)
- 2.5mM dNTPs [Lucigen, cat. 30030-1](#)
- Primers (Forward and Reverse)
- Thermal cycler with a heated lid
- Expresso® Rhamnose Cloning and Expression System, C-His (other Expresso Systems available) [Lucigen, cat. 49012-1](#)
- *E. coli*® 10G Chemically Competent Cells [Lucigen, cat. 60106-1](#)
- Recovery Media [Lucigen, cat. 80026-1](#)

STEP 1: AMPLIFY

ACCURA: 30 MINUTES

Reaction conditions for PCR

Reagent	50 µL Rxn	Final concentration
2X Accura HF Buffer	25 µL	1X
2.5 mM dNTPs	4 µL	200 µM
100 µM Forward primer*	0.5 µL	1 µM
100 µM Reverse primer*	0.5 µL	1 µM
Template	1 µL	
2U/µL Accura High Fidelity Polymerase	0.5 µL	0.02 U/µL
Water	18.5	

*Primers were designed according to Expresso Rhamnose Cloning Expression System Manual

Cycling protocol

Step	Temp.	Time	Cycles
Initial denature	94 °C	1 min	1
Denature	94 °C	5 s	25
Anneal	60 °C	5 s	
Extension	72 °C	5 s	
Final Extension	72 °C	1 min	1
Hold	4 °C	-	1

STEP 2: MIX

EXPRESSO: INSTANTANEOUS

1. Pre-chill culture tubes on ice.
2. Assemble the following mix in the culture tube:

Component	Volume
<i>E. coli</i> ® 10G Chemically Competent Cells	40 µL
12.5ng/ µL pRham C-His Kan Vector	2 µL
PCR product	1 µL

STEP 3: TRANSFORM

OVERNIGHT

1. Incubate on ice for 30 minutes.
2. Heat shock in water bath at 42°C for 30 seconds.
3. Add 960 µL recovery media
4. Outgrow, shaking at 37°C
5. Plate selective media containing 0.2% rhamnose Incubate overnight at 37°C
6. Visualize colonies on plate, detect blue protein.

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- routine

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We are proud to offer:

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- Ready-to-use cloning systems appropriate for your most challenging target
- Quality tested DNA polymerases and master mixes for PCR, as well as high-purity enzymes for sequencing and cloning at prices you can afford



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