High Fidelity PCR Amplification for Cloning

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For most cloning experiments involving PCR amplifications (***except colony screening after ligation)***, you are strongly encouraged to use the high fidelity PCR amplification procedure. We are currently using NEB's **Phusion™ High-Fidelity PCR Kit** (Cat# F-553), which contains:

- Phusion™ High-Fidelity DNA Polymerase (2 units/ul)
- Phusion™ GC Reaction Buffer (5X)
- Phusion™ HF Reaction Buffer (5X)
- Deoxynucleotide Solution Mix (or dNTPs) (10 mM)
- DMSO (100 %)
- $MgCl_2$ solution (50 mM)

Thus, all reagents except primers and template DNA are provided. This kit will be shared by all lab members. So, **it's critical to follow the protocol and avoid any potential contamination**! Please read the **Note** section carefully prior to your experiments.

PCR Reaction Mix

Per 20ul Reaction (for large scale amplification, you can set up **4-5 x 20ul** reactions)

5x HF Buffer (NEB)	4.0ul
dNTPs (10mM each)	0.6ul
DMSO	1.0ul
$MgCl_2$ (50mM)	1.0ul
Primer #1 (330ng/ul)	0.4ul
Primer #2 (330ng/ul)	0.4ul
ddH_2O	q.s to 20ul
Phusion DNA polymerase (NEB)	0.2ul
Template DNA (plasmid)*	(10-200ng)*
	20.0ul

* ***Use approximately 0.5-2ul miniprep DNA per 20ul reaction.***

PCR Cycling Program (on Hybaid OmnE with "block" control)

96°C X45"	X 1 cycle
92°C X 20"	
55°C X 30"	X 10-15 cycles (up to 35 cycles)
70°C X 20-60'' (or **2-4kb/min**)	
70°C X 5′	X 1 cycle

Check 5-10ul of PCR product on a 0.8% agarose gel.

NOTE:

1) Lower cycle numbers are preferred because of lower mutation rate; but as to Phusion DNA polymerase, **using 25-35 cycles** is still acceptable because of its high fidelity. Thus, use more cycles (e.g., 25-35cycles) if low 10-15 cycles do not provide satisfactory amplification.
2) To obtain larger quantity of DNA, one may set up 3 to 5 reactions (20 ul each);
1) To amplify a fragment from a RT-cDNA library, one may have to use touchdown PCR or gradient PCR protocol.
2) Use 15-30s/kb for extension times. Do not exceed 1min/kb.
3) **Phusion may produce blunt-end DNA products**
4) Spin all tubes gently before opening to improve recovery (the first time)
5) Reactions should be set up on ice.
6) Add polymerase last to avoid primer degradation.
7) **HF buffer is preferred for most templates**. But for difficult or GC-rich templates, you can try GC buffer. The error rate in HF buffer is lower than that in GC buffer. Only use GC buffer when amplification with HF buffer does not provide satisfactory results.
8) Initial denaturation time can be extended to 3min for difficult DNA templates.